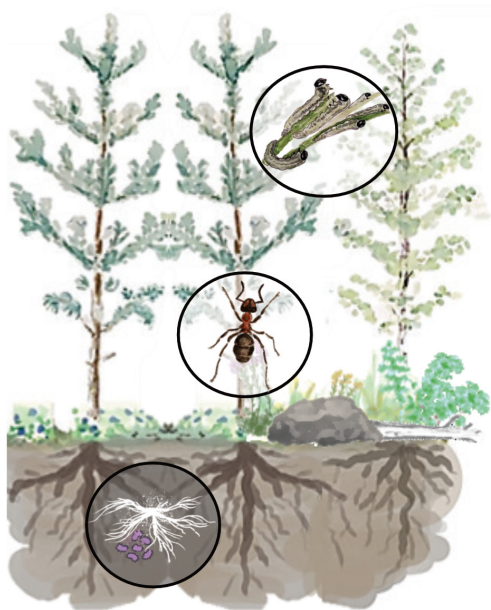




DOCTORAL THESIS NO. 2026:48
FACULTY OF FOREST SCIENCES

Effects of tree species composition on arthropod interactions and on soil microbiomes

FREDRIKA WRETHLING



Effects of tree species composition on arthropod interactions and on soil microbiomes

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SWEDISH UNIVERSITY
OF AGRICULTURAL
SCIENCES

DOCTORAL THESIS

Uppsala 2026

Acta Universitatis Agriculturae Sueciae
2026:48

Cover: Illustration of European sawfly larvae (*Neodiprion sertifer*), wood ant (*Formica sp.*), and soil microbiome (bacteria and fungi) in Scots pine forests. Illustrated by Fredrika Wrethling.

ISSN 1652-6880

ISBN (print version) 978-91-8124-265-2

ISBN (electronic version) 978-91-8124-295-9

<https://doi.org/10.54612/a.2o1doct8td>

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Print: SLU Grafisk service, Uppsala 2026

Effects of tree species composition on arthropod interactions and on soil microbiomes

Abstract

Natural disturbances are predicted to be more severe with future climatic extremes, especially in the boreal zone, where the low tree diversity may worsen the scale of damage. Boreal forests are widely managed for production of tree resources, often resulting in forests dominated by one tree species. Mixed forests are suggested to be less susceptible to biotic disturbance from insect herbivores and pathogens, through bottom-up and top-down effects on the organisms' populations. However, supporting evidence of the positive relationships between plant diversity and top-down effects have been found in agricultural systems but its relation to tree diversity remains inconsistent and context dependent in forests. Thus, investigating tree species compositions in boreal forests as a measure to decrease damage from biotic disturbance is important. In this thesis, I investigate the effect of tree species composition on the insect herbivore the European pine sawfly (*Neodiprion sertifer*), tree-dwelling arthropod community and soil microbiome all related to Scots pine (*Pinus sylvestris*), the most common tree species in Sweden. By using field experiments measuring habitat characteristics and manipulating tree species composition, I explore how tree species diversity affects predation pressure, abundance and richness of the arthropod community, and the community composition of the soil microbiome compared to pure pine stands. I found that (i) predation pressure of sawfly larvae was not affected by tree diversity but by local habitat heterogeneity, (ii) predation patterns from birds and arthropods on prey with different traits differed depending on habitat characteristics (iii) the arthropod community composition was not affected by tree species composition, and lastly (iv) tree species effect in the soil microbiome depend on fungal functional group and tend to depend on spatial scale. I conclude that tree species composition may have limited effects on top-down control on insect herbivores and community composition of arthropod predators, while local habitat heterogeneity may be of more importance in this study system, and that relationships detected at a local scale may be obscured at stand level as for the soil microbiomes.

Keywords: tree species composition, habitat heterogeneity, predator-prey interactions, tree diversity, pre-commercial thinning, arthropod community, *Pinus sylvestris*, *Neodiprion sertifer*, soil microbiome

Effekter av trädslagsblandning på leddjurens interaktioner och på markmikrobiom

Abstract

Naturliga störningar förutspås bli allvarigare med framtida klimatextremer, särskilt i den boreala zonen där den låga trädslagsdiversiteten kan förvärra skadornas omfattning. Boreala skogar brukas i stor utsträckning för produktion av skogsråvara, vilket ofta resulterar i att skogar domineras av ett trädslag. Blandskogar föreslås vara mindre känsliga för biotiska störningar från växtätande insekter och patogener, genom värdväxt- (bottom-up) och fiende- (top-down-) effekter på organismernas populationer. Det vetenskapliga stödet för relationen mellan växtblandning och top-down effekter är dock tydligare för jordbrukssystem medan resultat för trädslagsblandning i skogar är motsägelsefulla och kontextberoende. Det är därför viktigt att undersöka om trädslagsblandning kan vara en åtgärd för att minska skador från biotiska störningar i boreala skogar. I denna avhandling undersöker jag effekter av trädslagsblandning på insekten röd tallstekel (*Neodiprion sertifer*), på trädlevande leddjurssamhällen och på markmikrobiom som alla är knutna till tall (*Pinus sylvestris*) som är Sveriges vanligaste trädslag. Jag har använt mig av fältexperiment där jag mätt olika habitatfaktorer och manipulerat trädslagsblandning för att undersöka hur diversitet av trädslag påverkar predationstryck, leddjurssamhällets storlek och artrikedom samt sammansättning av markmikrobiomens samhällen jämfört med rena tallskogar. Jag fann att (i) predationstrycket på tallstekelns larver inte påverkades av trädslagsdiversitet utan av småskalig habitatheterogenitet, (ii) predationsmönstren från fåglar och leddjur på byten med olika egenskaper skilde sig åt beroende på habitategenskaper, (iii) leddjurssamhällets artrikedom och mängd påverkades inte av förändrad trädslagsblandning, och slutligen (iv) effekter av blandskog på markmikrobiom berodde på svampars funktionella grupper och tenderade att bero på rumslig skala. Jag drar slutsatsen att blandbestånd verkar ha begränsad effekt på predationstrycket på växtätande insekter och begränsad effekt på samhällen av rovlevande leddjur, medan småskalig habitatheterogenitet kan vara viktigare i detta studiesystem, samt att samband som syns på en lokal nivå kan vara dold på beståndsnivå som för markmikrobiom.

Keywords: trädslagsblandning, habitatheterogenitet, interaktioner mellan predatorer och bytesdjur, träddiversitet, röjning, leddjurssamhälle, *Pinus sylvestris*, *Neodiprion sertifer*, markmikrobiom

Dedication

To my children, who light up my way.

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List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I. Wrethling F*, Björkman C, & Klapwijk MJ (2026). Local heterogeneity an important driver for predation pressure of a defoliating insect in boreal forests. *Forest Ecology and Management*, 609, 123637.
<https://doi.org/10.1016/j.foreco.2026.123637>
- II. Klapwijk MJ*, Wrethling F, & Björkman C (2026). Predation rates on real and artificial prey in the context of tree diversity. *Frontiers in Ecology and Evolution*, 14, 1794147.
<https://doi.org/10.3389/fevo.2026.1794147>
- III. Wrethling F*, Björkman C, Klapwijk MJ. (2026). Tree species composition effects on arthropod communities on Scots pines (*Pinus sylvestris*) in managed boreal forest. (Manuscript)
- IV. Wrethling F*, Ranheim Sveen T, Björkman C, Klapwijk MJ, Bahram M (2026). Functional microbial groups differ in their initial response to pre-commercial forest thinning. (Manuscript)

Paper I and II are open access publications (CC BY 4.0).

*Corresponding author

The contribution of Fredrika Wrethling to the papers included in this thesis was as follows:

- I. Developed research question and experimental design with MJK and CB. Performed data collection and assisted with statistical analyses. Led the writing of the paper with contributions from co-authors. First author of the paper.
- II. Developed research question and experimental design with MJK and CB. Performed data collection and contributed to the writing of the paper as co-author.
- III. Developed research question and experimental design with MJK and CB. Leading experimental setup with Sveaskog, partial data collection and management of field assistants. Performed statistical analyses. Led the writing of the paper with contributions from co-authors. First author of the paper.
- IV. Developed research question and experimental design with MJK and CB. Partial data collection and management of field assistants. Contributed to the statistical analysis that was performed by TRS. Led the writing of the paper with contributions from the co-authors TRS, MB, MJK and CB. First author of the paper.

Abbreviations

PCT	Pre-commercial thinning
EcM	Ectomycorrhiza
C:N ratio	Carbon:Nitrogen ratio

AI declaration

The generative AI tools ChatGPT and Claude have been used as tools for learning and development of my own skills. I have used it for improving the readability of my own texts, as a search engine, and in learning and problem-solving code language in RStudio. All outputs have been treated as examples and have been critically questioned and carefully revised.

1. Introduction

Forests are a fundamental part of terrestrial ecosystems, regulating ecosystem processes, sustain biodiversity, and provide resources on which societies depend. The latter has resulted in extensive, and sometimes intensive, managed forests, often dominated by a single tree species (pure forest stands). Pure stands may be more vulnerable to natural disturbances, which are expected to become more severe and frequent with climate change (Seidl et al., 2017, Sturrock et al., 2011, Hartmann et al., 2022, Pureswaran et al., 2018). Moving away from pure stand to mixed stands is suggested to increase forests' ability to withstand biotic stressors such as insect pests and pathogens. However, evidence supporting beneficial outcomes from mixed stands on pests and pathogens, are inconsistent and the outcome may depend on several factors whose strength may vary depending on the type of environment they are measured in (Jactel et al., 2021, Huang et al., 2026).

Aboveground, two hypotheses are proposed to explain reduced insect damage in mixed plant systems: 1) resource concentration, and 2) increased top-down control by natural enemies (Root, 1973). However, in forests the evidence of the enemies hypothesis is inconsistent, and the outcome may be shaped by multiple factors acting together (Tylianakis and Romo, 2010). Therefore, experimental tests of the enemies hypothesis in boreal forests have recently been identified as priority for future research (Vázquez-González et al., 2024). Belowground, mixed stands can reduce transmission between susceptible host trees from soil pathogens, called the dilution effect (Keesing et al., 2006), similar to the resource concentration hypothesis by Root (1973). Mixed stands have been found to reduce pathogen damage, however the evidence is not universal (Field et al., 2025). Moreover, little is known about how tree species composition directly affects soil microbiomes, as such effects are often confounded with soil chemical properties (Tedersoo et al., 2016, Field et al., 2025).

Biotic disturbances are a natural part of the forest ecosystems, but climate change is expected to lead to stronger effects of natural disturbances, and especially in boreal forests (Seidl et al., 2017). To expand our knowledge of how to manage forests to mitigate negative consequences of climate change on natural disturbances, it is important to understand how our choices in management, such as tree species composition, affect trophic interactions and shape communities important for tree health, and in turn forest health

(see box 1: Tree and forest health). In this thesis, I explore the importance of tree species composition on top-down effects from natural enemies, structure of trophic levels of tree-dwelling arthropods, and community composition of soil microbiome in semi-natural boreal forests.

Box 1. Tree and forest health

Tree health is often evaluated by observing species-specific appearance of individual trees, by inspecting e.g. the crown, leaf, growth, and colour. Tree health is strongly shaped by the environment and by the interactions with damage-inducing biotic agents, such as herbivores or pathogens. (Witzell et al., 2022)

Defining **forest health** is, however, subjective and depend on the context. The context is often based on an ecological perspective that emphasize resilience and ecosystem functioning, or on a forest management perspective that emphasize productivity, or on both. The role of trees in ecosystem functioning and biodiversity is irreplaceable, as well as providing resources for human needs. Keeping forests healthy is essential but the multiple processes that keep forests healthy are highly complex, and difficult to understand. (Witzell et al., 2022)

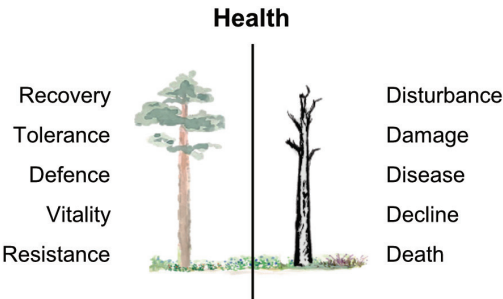


Figure 1. Conceptual image of the balance of supporting (left) and weakening (right) processes for health. Adapted illustration from Witzell, J., Tapia, C.R., Aldea, J., and Löf, M. (2022). Chapter 3 - Forest diversity and productivity: Implications for forest health in future climates, in *Forest Microbiology*, eds. F.O. Asiegbu & A. Kovalchuk. Academic Press), 49–59. Redrawn by Fredrika Wrethling.

The predation pressure of the European pine sawfly was studied in **paper I** and **II**, while considering both tree species composition and local habitat characteristics. The direct effect of tree species composition on herbivore and predator arthropod community is studied in **paper III**. In **paper IV**, I extend the focus to belowground microbial communities, testing how altering tree species composition affects soil microbiomes, as a complementary and more explorative part of the thesis. Improved knowledge of how tree species compositions affect trophic interactions between herbivores and their natural enemies may help explain the inconsistent evidence for top-down control in boreal forests. Insect herbivores and soil pathogens co-occur in the same local habitat, and extending similar question belowground may help broaden the knowledge of how tree species composition structures the soil communities also relevant to tree health.

1.1 Boreal forests

The boreal forest is the second largest terrestrial biome covering almost a third of the world's forest area. Biotic disturbances are a natural part of the boreal forest ecosystems, but the effect from climate change on natural disturbances is expected to be stronger in the boreal zone (Seidl et al., 2017). Boreal forests are expected to be more exposed to climate change as warming amplifies at higher latitudes, and historically climate boundaries limited population growth and survival of insect pests and pathogens (Venäläinen et al., 2020). Tree species diversity is relatively low in boreal forests compared to temperate and tropical forests, and which means that damage of one species will affect large forests areas (Gauthier et al., 2015). Moreover, approximately two thirds of boreal forests are managed; supplying 37% of global wood production (Gauthier et al., 2015, Girona et al., 2023). Forest management has a large effect on tree species composition and can further lower tree diversity through management practices. In Fennoscandia, forestry is dominated by even-aged rotation management based on regeneration, thinning, and clear-cut harvesting (Figure 2), often resulting in stands dominated by Scots pine (*Pinus sylvestris*) or Norway spruce (*Picea abies*) (Kuuluvainen et al., 2012). This forest management system has been economically successful by sustaining high and predictable timber harvests, with greatly increasing timber volumes since the early twentieth century.

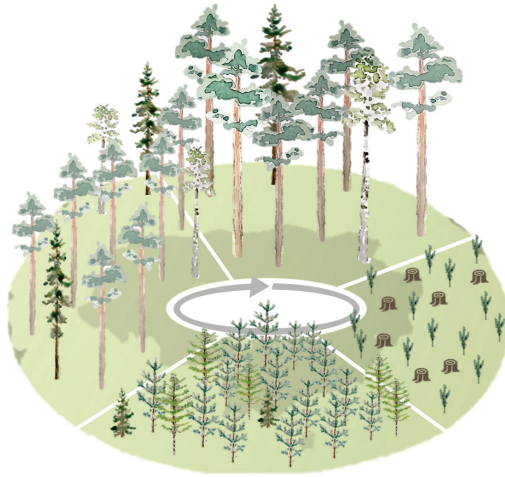


Figure 2. Illustration of even-aged rotation forestry in Fennoscandia, with regeneration, pre-commercial thinning, commercial thinning, and clear-cut harvest. Illustrations by Fredrika Wrethling, inspired by similar illustrations used in various forestry sources.

1.2 Tree diversity and insect herbivores

Species usually are considered pests when too many of them affect our shared interest in certain plants so called outbreaks. Outbreaks occur when populations rapidly increase and escape regulation by a combination of bottom-up (resources) and top-down (natural enemies) forces (Hunter and Price, 1992, Hairston et al., 1960). Plant diversity is one aspect of bottom-up effects that shapes arthropod communities and interactions between trophic levels (Siemann, 1998, Haddad et al., 2009, Schuldt et al., 2019). The landmark study by Root (1973) reported two foundational hypotheses: 1) insect herbivores are more likely to colonize and reproduce where their host plant is abundant (resource concentration hypothesis), and 2) that natural enemies have a positive relationship with diverse habitats that result in higher top-down control of pests compared to simple habitats (enemies hypothesis). Studies have observed lower insect pest damage in both agricultural and forest systems with higher plant diversity (Vehviläinen et al., 2006, Jactel et al., 2005, Jactel and Brockerhoff, 2007, Wetzal et al., 2016). Although, support for these hypotheses have mostly been found in agricultural systems and grasslands (Staab and Schuldt, 2020), patterns in forests are less clear (Tahvanainen and Root, 1972, Price et al., 1980, Andow, 1991, Landis et al.,

2000, Gurr et al., 2003, Letourneau et al., 2011, Hatt et al., 2018). Mixed stands have been found to have less damage from insects compared to pure stands, and that this effect is driven by host dilution and apparency (Jactel and Brockerhoff, 2007, Jactel et al., 2021), consistent with the resource concentration hypothesis (Root, 1973). However, this effect seems to mostly apply to specialist herbivores, whereas generalists show weaker and sometimes opposite responses (Jactel et al., 2005). None of these hypotheses have been shown to be universally applicable, not least in forest systems (Griffin et al., 2013, Iverson et al., 2014, Staab and Schuldt, 2020, Stemmelen et al., 2022). In this thesis, I focus on the natural enemies hypothesis. Both a review by Staab & Schuldt (2020) and a meta-analysis by Stemmelen et al. (2022) did not confirm that tree diversity results in higher predation pressure in temperate and boreal forests and therefore would be plausible mechanism for the observed lower damage.

1.3 Tree diversity and soil microbiomes

Extending belowground, mixed stands also tend to suffer less pathogen damage compared to pure stands (Jactel et al., 2017, Pautasso et al., 2005, Field et al., 2025). Although, this relationship is most consistent for root associated pathogens, where admixing of non-host trees reduces transmission between hosts (Pautasso et al., 2005, Jactel et al., 2017). The overall positive pattern of mixed stands compared to pure stands is largely based on studies from temperate forests, and with contrasting results in the boreal forest as no consistent effect of tree diversity on pathogen damage was observed (Field et al., 2025). However, tree species identity has been shown to influence soil pH and C:N ratio (Augusto et al., 2002, Dawud et al., 2016). Both pH and C:N ratio have strong effects on microbial diversity and community composition, and thus coniferous and deciduous trees can harbour distinct soil microbial communities (Bahram et al., 2020). Hence, mixing trees can lead to changes in belowground processes of nutrient cycling, carbon turnover, decomposition, and plant health (Romero et al., 2024, Urbanová et al., 2015).

1.4 Sources of context-dependency

1.4.1 Highly controlled vs. field experiments

Several important studies testing the ecological relationship between tree diversity and pest insects has been made in stands similar to plantations, and these studies has shown the ecological importance of tree species on pest insects using highly controlled experiments. (Castagneyrol et al., 2013, Castagneyrol et al., 2014, Verheyen et al., 2016, Vázquez-González et al., 2024). However, plantations often lack the established forest-floor and forest structures found in semi-natural forests(Figure 3) and studies in semi-natural forests have large amounts of unexplained variation that could be attributed to forest heterogeneity (Huston, 1997, Nadrowski et al., 2010). Thus, the unexplained variation from forest heterogeneity may be a reason to the inconsistency in results when testing the enemies hypothesis in mixed compared to pure stands. Belowground, tree diversity effects on soil microbiomes are often confounded with soil chemical properties (Tedersoo et al., 2016), and less is known about direct tree species composition effects on soil microbial communities (Tedersoo et al., 2016, Field et al., 2025). Thus, understanding the relationship between tree species composition and trophic interactions is complicated, because of confounding with other forest factors.

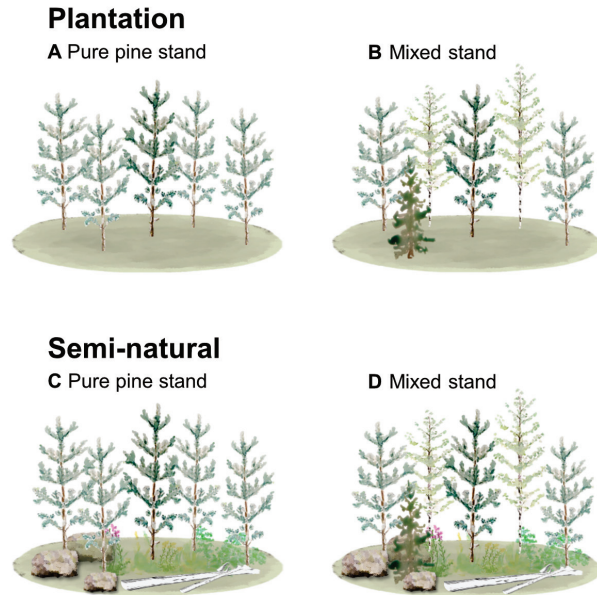


Figure 3. Illustration showing conceptual image of the differences between plantation and semi-natural forests in relation to forest heterogeneity beyond tree diversity. Pure pine stands (A and C) and mixed stand (B and D) are illustrated without and with established forest-floor and habitat structures. Illustration by Fredrika Wrethling.

1.4.2 Habitat heterogeneity

Plant diversity is expected to provide a wider range of niches increasing arthropod diversity (Pickett and Cadenasso, 1995, Stein et al., 2014, Thomsen et al., 2022, Sola and Griffin, 2025). This is consistent with the habitat heterogeneity hypothesis (MacArthur and MacArthur, 1961), which suggests that structurally complex habitats increase niche diversity and thus species diversity. However, tree diversity is one aspect of heterogeneity and other forest factors may be important in shaping arthropod communities. Understory vegetation can affect associated arthropod communities by effects on microclimate and microhabitat (Aude and Lawesson, 1998, Kovács et al., 2017, Latimer and Zuckerberg, 2017), and other structures such as dead wood and boulders can increase availability of shelter and affect movement of natural enemies (Matevski et al., 2021, Terlau et al., 2023, Kollberg et al., 2014). Moreover, tree species composition can affect the understory through changes in light, water and nutrient availability (Barbier

et al., 2008, Tinya et al., 2009, Márialigeti et al., 2016, Braziunas et al., 2024). This type of forest heterogeneity can still be complex in forests with low tree diversity (Staab and Schuldt, 2020), and contribute with confounding sources when investigating effects of tree diversity.

1.4.3 Scale-dependence

Forests are commonly classified as mixed or pure at the stand level, convenient for forest management. However, organisms as small as arthropods and soil microbes experience their environment at a much finer scale. For example, local forest heterogeneity can cause top-down regulation of insect populations to vary greatly at local scales (Bellone et al., 2020), and soil microbial communities are spatially structured at scales of one to a few metres depending on the organism group (Ettema and Wardle, 2002). Consequently, patterns may be masked as a result of diversity metrics used, measurements, and spatial scale, and depending on the organisms studied.

2. Aim of the thesis

The overall aim of this thesis is to investigate how the choice of tree species composition influence top-down effects on insect pests aboveground, and structure the soil microbiome belowground. The thesis focuses on predation pressure on the European pine sawfly (*Neodiprion sertifer*), tree-dwelling arthropods, and soil microbial communities beneath pines across two spatial scales, stand level and local tree level.

The overarching questions addressed in this thesis were:

- I. Does predation pressure on insect herbivores depend on tree species composition or local habitat heterogeneity (**paper I**)?
- II. Does the relationship between habitat characteristics and top-down effects depend on what kind of prey is present (**paper II**)?
- III. How do direct effects of tree species composition affect the composition of arthropod herbivores and predators (**paper III**)?
- IV. How does direct tree species composition affect soil microbial community structure (**paper IV**)?

3. Study system

In this thesis, managed forests dominated by Scots pine (*Pinus sylvestris* L.) provide the setting for the studies. Scots pine is the most common tree species in Sweden, one of the most commercially and culturally important tree species in northern Europe (Durrant et al., 2016), and an important tree species for both arthropod communities aboveground and soil microbial communities belowground. I used the larvae of the European pine sawfly to assess predation pressure, collected tree-dwelling arthropods on pines to investigate arthropod community structures, and sampled the soil microbiome beneath the pines to explore soil microbial community structures.

3.1 European pine sawfly

As study species, to assess predation pressure, I used the specialist herbivore European pine sawfly (*Neodiprion sertifer* Geoffr.; Hymenoptera: Diprionidae). The biology and life cycle (Figure 4) of sawflies are well understood. The sawfly larvae feed on pine needles and have irregular outbreaks that sometimes are widespread (Larsson and Tenow, 1984). Severe defoliation from sawflies can reduce Scots pine growth with economic consequences (Lyytikäinen-Saarenmaa and Tomppo, 2002). This sawfly is native to Eurasia, and introduced in North America (Olofsson, 1987). Eggs are placed in a single batch on the needles of a current year shoot from August to September. The eggs hibernate until May the following year (Pschorn-Walcher, 1991). The larvae feed on needles in groups with on average 70 individuals (Gur'yanova, 2006). Larvae feed from May to late June, then they molt into fourth (for males), and fifth (for females) instar followed by a final molt to prepupa. Following this, they drop from the branch into the topsoil and find a suitable place to spin cocoons (Wallace and Sullivan, 1963). After summer diapause the prepupa pupate in the cocoon. Adults emerge between August and September.

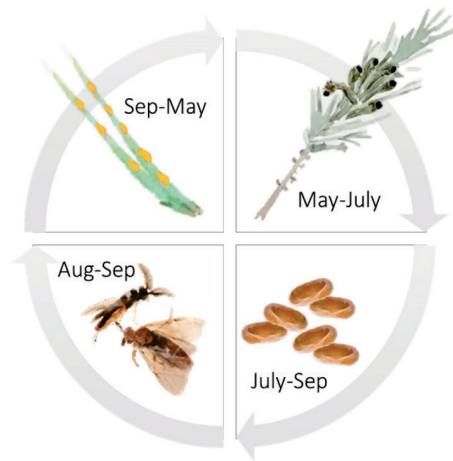


Figure 4. Illustration of the lifecycle of the European pine sawfly (*Neodiprion sertifer*). Illustration by Fredrika Wrethling.

3.2 Trophic interactions

Bottom-up and top-down effects from natural enemies are important for sawfly population regulation. Several enemies predate on sawflies in its different life stages (Figure 5). The most important predators on sawfly larvae are several species of ants, spiders and parasitoids (Olofsson, 1987, Olofsson, 1992, Lindstedt et al., 2006, Kaitaniemi et al., 2007) that forage/predate in the crown of pine trees. Sawfly larvae employ collective defence behaviour when threatened, by flicking their heads and tails and sequester resin acid from the mouthparts. Thus, they are thought to be unpalatable for birds and other predators. During the pupal stage the most important natural enemies are ground dwelling arthropods and small mammals (Hanski and Parviainen, 1985, Olofsson, 1987).

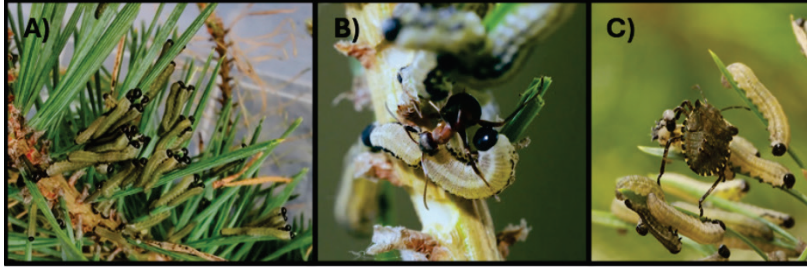


Figure 5. (A) Photo of reared sawfly larvae (*Neodiprion sertifer*), (B-C) predation attempts by natural enemies. Photo A by Fredrika Wrethling and photo B-C by Hasse Andersson.

3.3 Soil microbiome

Scots pine is also associated with a rich community of soil bacteria and mycorrhizal, saprotrophic, and pathogenic fungi that drive plant-soil feedback belowground. Scots pine is an obligate host for ectomycorrhizal fungi, which exchange soil nutrients for carbon-rich sugars produced by the pine's photosynthesis (Smith and Read, 2008). Saprotrophic fungi are decomposers of litter and dead wood (Lindahl et al., 2007), and pathogenic fungi can infect plant tissue and may reduce tree growth and survival (Fisher et al., 2012). Soil bacteria contribute to nitrogen fixation, (Lladó et al., 2017) which is a limited resource in boreal forests. While tree species identity is known to shape soil microbial communities, less is known about how altering tree species composition affects soil microbiomes (Prescott and Grayston, 2013, Otsing et al., 2021).

4. Methods

To answer the questions in this thesis I used two different field-based experimental designs. Field experiments were performed between established mixed species stands compared to pure pine stands (**paper I and II**), and mixed species plots and pure pine plots was created within established forest stands (**paper III and IV**). All stands were semi-natural forests and dominated by Scots pine, with natural regeneration of silver birch and Norway spruce.

4.1 Between stand experiments

To study the effect of tree species composition on predation pressure of sawfly (**paper I**) and predation patterns of sawfly and plasticine models (**paper II**) field experiments in twelve forest stands divided at two localities in Uppland (Figure 6), Sweden, were established (locality 1: 59°54'34"N, 18°15'56"E, and locality 2: 60°11'45"N, 17°53'46"E). The forests were semi-natural stands, 6-15 years old, and a tree height between 2-6 m. The stands were either dominated by over 80 % Scots pine trees (pure pine stands) or consisted of 30-50 % Scots pine with admixing of silver birch and Norway spruce (mixed stands). At both localities mixed and pure pine stands were represented equally.

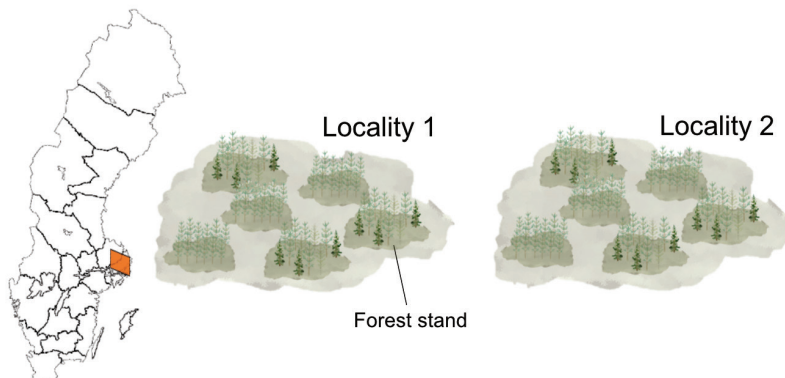


Figure 6. Illustration of location of experimental site in Sweden (orange square), and conceptual image showing comparison between established forests stands, with mixed or pure pine stand type. Illustration by Fredrika Wrethling.

4.1.1 Predation (paper I and II)

To study the effect of tree species composition and local habitat characteristics on predation pressure and predation patterns I deployed 1,500 plasticine models and reared over 4,000 sawfly larvae on pine trees in both mixed and pure pine stands. Sawfly eggs used in **paper I** and **II** was collected near Ramnäs in South-central Sweden (59°46'39"N, 16°05'49"E) from a natural ongoing outbreak. The aim of **paper I** was to study top-down effects on sawfly larval survival. The sawfly larvae were reared on branches of pine trees in all twelve forest stands, once every week for three weeks I counted survived larvae and added a new batch to reset the group size. Ant presence on the branches was noted, and local habitat measurements was made for tree and understory characteristics, deadwood and boulders. The measurements were made in a circular plot with a radius of 5.64 m (total basal area of 100 m²) around all experimental pine trees. In **paper II** the aim was to study the effect of habitat characteristics on predation patterns on different prey types. Groups of sawfly larvae, groups of ten plasticine models, and five solitaire plasticine models were placed together on pine trees but on different branches in all twelve forests (Figure 7). The sawfly larvae are defended gregarious specialist herbivores, the plasticine models represented undefended prey, as group prey or solitaire prey. Survival of sawfly larvae was measured as in **paper I**, and attack marks on the model prey was determined using a guide (Low et al., 2014). Using plasticine models as an artificial prey is an established method of assessing relative difference in attack rates in studies with different habitats or treatments (Howe et al., 2009, Lövei and Ferrante, 2017). Measurements of habitat characteristics was made in a circular plot with a radius of 5.64 m (total basal area of 100 m²) around all experimental pine trees.



Figure 7. (A, B) Construction of the single (A) and grouped plasticine (B) models. (C) Placing of the single plasticine model in the pine tree. (D) Placement of the grouped plasticine model in the pine tree. Figure reproduced from **paper II** (Klapwijk, M. J., Wrethling, F., & Björkman, C. (2026). Predation rates on real and artificial prey in the context of tree diversity. *Frontiers in Ecology and Evolution*, 14, 1794147).

4.2 Within stand experiments

To study the effect of tree species composition on arthropod community structure (**paper III**) and soil microbial community structure (**paper IV**) field experiments in ten forest stands in central Sweden were established along a wide north-south gradient (Figure 8). The northernmost stand was located near Kungsberg (60°46'01.6"N 16°23'12.5"E) and the southernmost stand was located near Riddarhyttan (59°47'43.0"N 15°29'06.1"E). The forests were semi-natural stands, approximately 12 years old, with a tree height between 3.25 to 6.5 m. The dominant tree species in the stands were Scots pine (42-65%) and silver birch (25-31%). The high birch amount allowed us to create two plots of a 50/50 pine-birch mix, and two pure pine (>90 %) plots. All plots were 50 x 50 m in size and was created using pre-commercial thinning executed by the same team. With this design the original conditions are kept in each plot and only tree species composition is manipulated. This allowed a direct effect of tree species composition to be tested with more control of confounding factors.

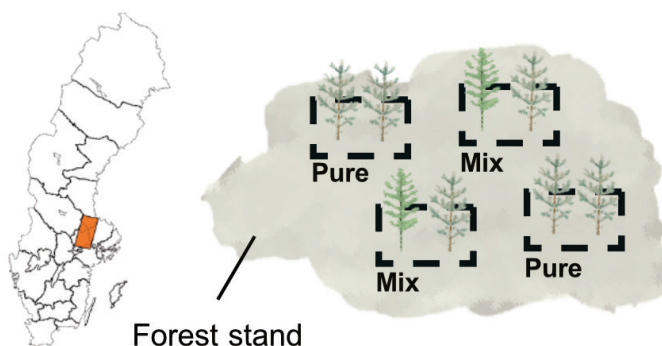


Figure 8. Illustration of location of forest stands in Sweden (orange square), and conceptual image showing the experimental design of diverse and pure pine plots implemented by Sveaskog. Illustration by Fredrika Wrethling.

4.2.1 Community composition (paper III and IV)

The aim of **paper III** was to study the effect of tree species composition on the structure of tree-dwelling arthropod communities (Figure 9, left figure). To accomplish this, the beating tray method was used to collect arthropod communities on pine trees (Speight, 2005). First, arthropod communities in the experimentally created plots in all ten stands was sampled on 160 pines. Then I deployed plasticine models on half of the same pines (80 pines) in five of the ten stands and measured attack rate of 240 models for three weeks. After model retrieval, the same 80 pines were sampled once more with the beating tray method. The individuals of the arthropod collection were counted, functional groups (herbivore/predator) and taxonomic order or family was determined. In **paper IV**, the aim was to test tree species composition on soil microbial communities and their functional groups (Figure 9, right figure). Soil samples were collected beneath 160 pines in both mixed and pure pine plots in the ten stands. Soil cores were collected at five points 1-1.5 m around each pine and then mixed to the final sample. A subset of 48 soil samples from six of the forest stands was sent for DNA extraction and sequencing to obtain the soil microbial communities and analysis of soil chemical characteristics. The fungal sequences were classified to functional groups using the FungalTraits database at the genus level (Pölme et al., 2020).

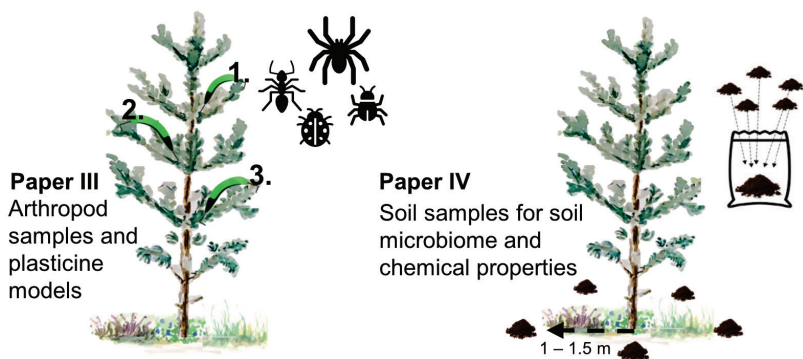


Figure 9. Conceptual image showing the data collection of attack on plasticine models and sampling of arthropod communities on Scots pine (**paper III**), and sampling of soil microbiome and soil chemical properties (**paper IV**). Illustration by Fredrika Wrethling.

5. Results and discussion

This thesis explored the effects of tree species composition and habitat heterogeneity on top-down control of the European pine sawfly (*Neodiprion sertifer*) as well as the structure of arthropod and soil microbial communities in semi-natural boreal forests. In this study system, I found no evidence that predation pressure on sawfly larvae was higher in mixed stands at stand level, instead habitat heterogeneity at the local tree level, independent of tree diversity, was a better predictor for higher predation pressure (**paper I**). Moreover, ant presence and community composition of predatory arthropods did not differ between mixed and pure pine stands (**paper I and III**), while ants were positively related to local characteristics of the herbaceous layer (**paper I**). In addition, the comparison of prey traits of sawfly larvae and plasticine models were important in explaining the relationship between habitat structure and attack rate (**paper II**). Belowground, soil pathogens responded to tree species composition at stand level, while responses from saprotrophs and ectomycorrhizal fungi differed depending on tree characteristics and spatial scales (**paper IV**). The novelty of this thesis is that I test the effect of measured local heterogeneity and stand level tree diversity on predation pressure, and subsequently test direct effects of tree diversity on sampled arthropod and soil microbiome communities with an experimental design that controls more for confounding factors.

5.1 Effects of tree species composition

5.1.1 Predation pressure

In **paper I**, I found no evidence of predation pressure of sawfly larvae being higher in the mixed compared to the pure pine stands. The results are consistent with Bellone et al. (2020), as both our studies did not find an effect of tree diversity in the same study system. Although, higher predation rate of sawfly by ants have been found in pine stands mixed with birch by Kaitaniemi et al. (2007), but the importance of the local heterogeneity found in our study, independent of tree species diversity, may suggest that other habitat structures can override tree diversity. This further supports the importance of habitat heterogeneity and the inconsistency in the enemies hypothesis discussed by Staab and Schuldt (2020) and Stemmelen et al.

(2022). The result from **paper II** shows that the effect of habitat characteristics on relative predation rate differed between defended sawfly larvae and plasticine models, arranged as groups or as solitaires. Specifically, ants were positively related to predation only on sawfly larvae, while the solitaire models had slightly more bird marks than grouped, and the grouped models had more arthropod marks than the solitaire models. These results indicate that different predators that are favoured by different habitat characteristics plausibly attack different prey types. This variability in responses may be a contributing factor to the inconsistency in results when testing the enemies hypothesis (Stemmelen et al., 2022). Thus, the predator assembly around a prey may be an important factor when evaluating the enemies hypothesis.

5.1.2 Arthropod predator community

In **paper III**, I designed an experimental study to test the direct effect of tree species composition and control more for this unexplained variation. I found that the abundance and richness of predatory arthropods on the focal pines was similar between mixed and pure pine plots within the stands. This again supports the findings in **paper I** and may indicate that other heterogeneity factors, independent of tree diversity, drive predator assembly on pine trees (Figure 10). It is also possible that the community of predators need more time to respond to the altered tree species composition, and responses may differ depending on tree maturity and stand age. Moreover, tree-dwelling arthropods do not represent the whole arthropod community, and we did not sample for example the ground-dwelling arthropod community. However, other studies of ground-dwelling arthropods have also found communities to be similar between mixed and pure stands in similar settings but slightly different tree species combinations (Oxbrough et al., 2012, Vehviläinen et al., 2008).

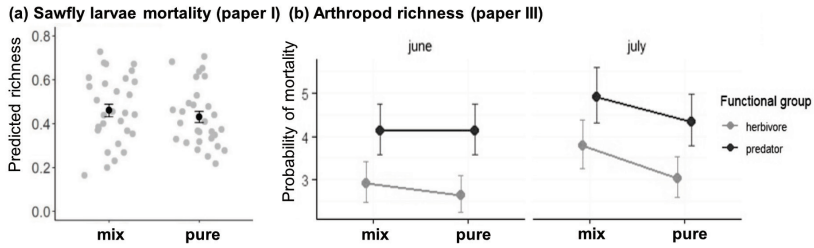


Figure 10. (a) Probability of larval mortality in mixed and pure pine stands, number of observations = 59. Grey dots represent individual trees. Black circles $\pm$ SE indicate model-predicted means. Larval mortality did not significantly differ significantly between forest types (**paper I**). (b) Arthropod richness (tax: order) per treatment plot, sampled in June and July for 5 forest stands (**paper III**). Herbivores (light grey) and predators (dark grey). Points show predicted values with error bars giving 95% confidence intervals. Herbivore and predator richness did not differ between mixed and pure pine plots. Left figure reproduced from **paper I** (Wrethling, F., Björkman, C., & Klapwijk, M. J. (2026). Local heterogeneity an important driver for predation pressure of a defoliating insect in boreal forests. *Forest Ecology and Management*, 609, 123637) and right figure reproduced from **paper III** (manuscript).

5.2 Local habitat heterogeneity

In **paper I**, I found that local understory density, herbaceous layer characteristics and boulders was a better predictor of higher predation pressure with both direct and indirect effects, independent of tree diversity (Figure 11). The findings in **paper I** extends the finding by Bellone et al. (2020) by measuring heterogeneity at local tree level beyond stand level characteristics. The findings in **paper III** further support the findings in **paper I** and shows that tree species composition may not be as important as other forest characteristics defining the local habitat heterogeneity. A plausible explanation of the importance of the local habitat heterogeneity may be that effects of the herbaceous layer, understory density, and boulders represent different microhabitats with associated microclimate important for predator activity (Terlau et al., 2023, Matevski et al., 2021, Langellotto and Denno, 2004).

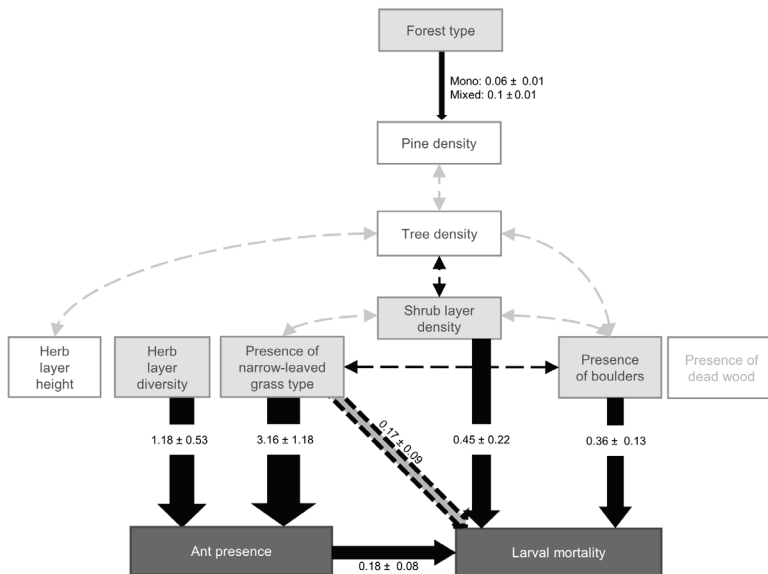


Figure 11. The black directed arrows represent direct significant effects at $P < 0.05$, the grey directed arrow with dashed lines represents marginally significant effects. The numbers show the estimate and standard error. The dashed double headed arrows represent significant covariances, the black and grey colours indicate positive and negative covariances, respectively. Figure reproduced from **paper I** (Wrethling, F., Björkman, C., & Klapwijk, M. J. (2026). Local heterogeneity an important driver for predation pressure of a defoliating insect in boreal forests. *Forest Ecology and Management*, 609, 123637).

The contribution of habitat characteristics, other than tree diversity, to predator activity is further supported by the findings in **paper II**. Here, ants were positively related to understory diversity and were only important for predation on sawfly larvae. Tree density was negatively related to predation rate of both solitary models and sawfly larvae, but the opposite was found for grouped models. All predators are unlikely to respond in the same way to habitat characteristics, just as they have been shown to respond differently to tree diversity in previous studies (Kaitaniemi et al., 2007, Klapwijk and Björkman, 2018, Bellone et al., 2020). Mixed stands effects on the predator communities effectiveness might depend on predator species being complementary or interfering to each other (Klapwijk, 2019). Intraguild predation and competition can be strong amongst arthropod predators, especially for ants and spiders (Mooney, 2006, Halaj et al., 1997, Hodge,

1999). Moreover, spiders are affected by apex predators such as birds (Kozlov et al., 2015, Gunnarsson, 1996, Gunnarsson and Hake, 1999), and in this study system the presence of birds as shown by the attack marks on the plasticine models in **paper II and III**. The role of local habitat heterogeneity may provide structural complexity that benefits avoidance of intraguild predation and predator-predator competition and thus favouring co-existence, as predicted by the habitat heterogeneity hypothesis (MacArthur and MacArthur, 1961).

Altogether, local habitat heterogeneity resulted in higher predation pressure independent of tree diversity (**paper I**), predation patterns differed depending on prey type and habitat characteristics, suggesting that predator assembly around prey may differ (**paper II**), and that arthropod predator communities did not differ in diverse compared to pure pine plots (**paper III**) indicates that tree species composition may be less important for favouring predation pressure on insect herbivores. Thus, these papers support that the enemies hypothesis may be determined by several interacting factors (Tylianakis and Romo, 2010).

5.3 Scale-dependence

In **paper I**, the fine-scale habitat characteristics measured were found to be more important than stand-level tree diversity, and we found that the local habitat heterogeneity did not differ between mixed and pure pine stands. Extending this belowground, in **paper IV**, different responses were found depending on the functional group of soil fungal microbiome and the responses tended to be different depending on spatial scale. Soil pathogen abundance decreased with removal of birches in the pure pine plots, while no response of tree species composition was detected for ectomycorrhizal fungi or saprotrophic fungi at the plot level. However, ectomycorrhizal fungal richness and saprotrophic fungi tended to respond to heterospecific neighbours around the focal pines. An explanation may be that stand-level categorisation based on the averaged tree species composition may sometimes be a poor fit for local reality. Moreover, the local proportion of birch around the focal pines measured in **paper III**, did not always match the plot level categorization of diverse or pure pine. Thus, investigating several spatial scales simultaneously can be necessary, otherwise effects of tree species composition can be obscured at the stand level.

6. Conclusions and outlook

This thesis contributes to the existing evidence that altering tree species composition to strengthen resistance to biotic agents are not straight forward. I found no evidence that tree diversity increased predation pressure or abundance and richness of arthropod predator communities (**paper I**, and **III**). Instead, fine-scale habitat characteristics, independent of tree diversity, predicted higher predation pressure (**paper I**). How predation patterns by birds and arthropods are affected by habitat characteristics differ depending on prey type (**paper II**). In addition, responses to tree species composition differ depending functional group and at spatial scale (**paper IV**).

Altogether, it indicates that fine-scale habitat heterogeneity is more important for top-down control of insect herbivores, and that broad stand classifications can obscure ecologically important variation occurring at finer spatial scales. This emphasizes the complexity in semi-natural systems, and to understand bottom-up and top-down effects future studies needs to address habitat heterogeneity effects at multiple trophic levels and across spatial scales, as discussed by Hunter and Price (1992) and Vidal and Murphy (2018). Following this, interdisciplinary studies with this approach help to identify methods relevant to forest management become more important. Focus needs to be on the reduction of damage from synergistic effects from both abiotic and biotic disturbances.

For forest management today, the choice of tree species composition is an important tool that can be used in a variable way, and mixed stands by itself have other important benefits (Huuskonen et al., 2021). Moreover, diversifying different habitat characteristics other than tree species diversity, across spatial scales, may be a complementary tool to strengthen the top-down control of insect herbivores. Finding a balance between the multitude of factors affecting the outcome of natural disturbances, together with the many societal goals expected from forests will remain a challenge.

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Popular science summary

The boreal zone stretches across the northern hemisphere and is both a globally important ecosystem and an important resource for societies. Large parts of the boreal forests are managed to meet societal demand for wood resources. Sweden is one of the largest exporters of timber and pulpwood in the world, and over 80 percent of the productive forest land in Sweden are managed for this purpose. The productive forests are often managed as even-aged forest stands often dominated by a single tree species. However, in the future, these forests may be more susceptible to natural disturbances such as fires, storms, and outbreaks of insect pests and disease-causing fungi, as climate change is expected to intensify these disturbances in the boreal zone. Mixed forests have been suggested as a measure to counteract this risk. Several scientific studies have observed that mixing different plant species in agricultural systems increases the pressure exerted on herbivorous insects by predators. In forests, however, it is more difficult to see effects of tree diversity, as much depends on other conditions that are difficult to separate to see what affects what. In this thesis, I have studied if mixed stands increase the mortality of herbivorous insects by favouring the abundance and diversity of arthropod predators. I used the European pine sawfly (*Neodiprion sertifer*), a pest that can cause severe damage to pine forests, as my study insect, and I additionally used artificial larvae made from clay. I have also studied how microscopic soil bacteria and fungi beneath pine trees are affected by tree species composition. I have mainly focused on different groups of soil fungi that contribute to decomposition, live in symbiosis with trees, or cause damage to trees. With this thesis, I have shown that mixed stands do not increase the richness and abundance of arthropod predators, or the mortality of pine sawfly larvae. However, I found that a varied habitat at a small spatial scale increases larval mortality regardless of forest type, and that the influence of different predators differs depending on the habitat and on the traits of pine sawfly larvae compared with artificial larvae. I also show that mixed stands affect microscopic fungi differently depending on which fungal group they belong to and which spatial scale is considered. These results contribute to a better understanding of the complexity of different forest types and show that increased mortality of herbivorous insects depends on several different factors, among which tree species composition is not necessarily the most critical.

Populärvetenskaplig sammanfattning

Det boreala barrskogsbältet sträcker sig över norra halvklotet och är både ett globalt viktigt ekosystem och en viktig resurs för samhällen. Stora delar av det boreala barrskogsbältet brukas för att möta samhällets efterfrågan på skogsråvara. Sverige är en av världens största exportörer av timmer och massaved, och över 80 procent av den produktiva skogsmarken brukas för detta ändamål. De produktiva skogarna sköts ofta som likåldriga bestånd dominerade av ett trädslag. I framtiden kan dessa skogar dock vara mer känsliga för naturliga störningar som bränder, stormar och utbrott av skadeinsekter och sjukdomsframkallande svampar, eftersom klimatförändringarna väntas förstärka styrkan på dessa störningar i den boreala zonen. Blandskog har föreslagits som en åtgärd för att motverka risken för stora störningar. Flera vetenskapliga studier har observerat att en inblandning av olika växterarter i jordbrukssystem ökar trycket från rovdjur på växtätande insekter. Men det är svårare att se en tydlig effekt av blandskog, eftersom flera faktorer påverkar och att urskilja vad som påverkar vad är svårt. I den här avhandlingen har jag undersökt om blandskog ökar dödligheten hos växtätande insekter genom att gynna mängden och mångfalden av rovlevande leddjur. Som studieinsekt använde jag röd tallstekel (*Neodiprion sertifer*), en skadegörare som kan orsaka stora skador i tallskog, och jag använde mig också av konstgjorda larver av lera. Jag har också undersökt hur mikroskopiska bakterier och svampar i marken under tallar påverkas av trädslagsblandning. Jag har främst fokuserat på olika grupper av marksvampar som bidrar till nedbrytning, lever i symbios med träd eller orsakar skador på träd. Med den här avhandlingen har jag visat att blandbestånd inte ökar mångfalden och mängden av rovlevande leddjur, och inte heller dödligheten hos tallstekellarver. Däremot fann jag att en variationsrik livsmiljö på en småskalig nivå ökade larvdödligheten oavsett skogstyp, och att olika rovdjurs påverkan skiljer sig åt beroende på livsmiljön och egenskaper hos tallstekellarver jämfört med konstgjorda larver. Jag visar också att trädslagsblandning påverkar mikroskopiska svampar olika beroende på vilken svampgrupp de tillhör och vilken rumslig skala som betraktas. Dessa resultat bidrar till en bättre förståelse för komplexiteten hos olika skogstyper och visar att ökad dödlighet av växtätande insekter beror på flera olika faktorer, där trädslagsblandning inte nödvändigtvis är den mest avgörande faktorn.

Acknowledgements

I am sincerely grateful to my supervisor team, thank you for your guidance, encouragement, and enthusiasm of the captivating world of (insect) ecology. Thank you for your belief in me and for all support to completing this thesis. I have learned so much from you and your abilities have complemented each other in the best possible way.

Maartje, I have truly enjoyed these years, and I am very grateful for everything that you have taught me. I am also grateful that you bared with me through two kids and my move to another city. I am very happy that we got to know each other outside of work too. I have really enjoyed your company. Thank you!

Christer, thank you for all your wisdom, knowledge and creativity. Your ability of listening and guidance through the tough thoughts that arise as part of the PhD process as been essential for me. I regret that I did not ask you more questions!

Special thanks to **Tord** who supported me through my whim to learn about microbes, they are truly fascinating. I have really appreciated our lunch meetings and discussions.

Also, special thanks to **Mo** who supported me through the microbes too. Without you it was not possible. I am very grateful for your support.

I also want to thank everyone in the Forest Entomology unit and other colleagues at the department for creating such a friendly atmosphere.

Special thanks to **Michelle**, **Stephanie**, **Danja** and **Amelia** for your friendship. I was very lucky to meet you all.

Many thanks to everyone who helped me with the field work. Thank you **Sammy** for helping me with all the “snitsling” of the forest stands. What a workout, I had fun! Special thanks to everyone involved 2021, when all my sawfly larvae died of the virus, and I was to sick from my pregnancy to work in the field. Thank you: **Stephanie**, **Helena H**, **Matilda**, **Oliver**, **Bernabé**, **Kaspar**, and **Erik**. You made this thesis possible.

Special thanks to my friend **Emilio**, I truly enjoyed our time in the field and all the memories that it created. Thank you for letting me crash in your home when I needed it, and all the fun times outside of work!

Many thanks to my family members, extended family, friends and neighbours for all assistance, wonderful dinners, and talks that have helped me with the work-life balance. I am especially thankful to my sister **Andrea**, how has continuously helped with childcare and dog walks the last half year. This would not be without you.

Finally, my love to **Nils**, for your endless support and patience. Thank you for all encouragement and for being such a great parent to our children. I am deeply grateful of all that you have handled to make it possible for me to work with this thesis.



Local heterogeneity an important driver for predation pressure of a defoliating insect in boreal forests

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ARTICLE INFO

Keywords:

Boreal forests
Insect herbivores
Local heterogeneity
Mixed forests
Natural enemies
Predator–prey interactions

ABSTRACT

Plant diversity is suggested to favour predation pressure on pest insect populations across agriculture and forestry. Although plant diversity is often correlated with predator diversity, its relationship with predation pressure remains inconsistent. Plant diversity and predator activity are affected by biotic and abiotic habitat factors. Thus, habitat heterogeneity may serve as a stronger predictor of predation pressure, however, its effect on the regulation of trophic interactions, such as predation, remains unclear. In this study, we investigated the influence of local habitat heterogeneity and tree species diversity on predation on *Neodiprion sertifer* larvae feeding on *Pinus sylvestris*. We measured tree and understory diversity and structure, presence of dead wood, boulders, and ants to examine their relationships with arthropod predation pressure on *N. sertifer* larvae. Notably, both predation rates and ant presence did not differ between mixed and monoculture stands. Local heterogeneity directly increased ant presence, which in turn increased larval predation. Furthermore, shrub density and boulders had additional direct positive effects on larval predation. Our results show that within-stand heterogeneity factors (shrubs, boulders, herb layer characteristics) may be a stronger determinant for predation on *N. sertifer* than tree species diversity. These findings indicate that plant diversity effects on predator–prey interactions are strongly dependent on the context of the forests where they are measured. To mitigate pest insect populations, forest management should, therefore, not only consider increasing the number of tree species but also include other aspects of local heterogeneity.

1. Introduction

Plant diversity is considered a key factor influencing ecosystem processes (Joern and Laws, 2013) that determines community assembly and biodiversity at higher trophic levels (Siemann, 1998; Haddad et al., 2009; Schuldt et al., 2019). Plant diversity is shaped by environmental conditions such as water and nutrient availability, climate and weather conditions, and soil properties such as coarseness, acidity, and decomposition (Zhang et al., 2014; Ma et al., 2024). The combined abiotic and biotic variables related to plant diversity and vegetation structure determine environmental heterogeneity (Stein and Kreft, 2015). Conversely, shifts in plant diversity will change vegetation structure. Thus, niche diversity for arthropod herbivores as well as predators is expected to be affected by changes in plant diversity (Pickett and Cadenasso, 1995; Stein et al., 2014; Thomsen et al., 2022; Sola and Griffin, 2025) according to the habitat heterogeneity hypothesis (MacArthur and MacArthur, 1961).

The majority of the pioneering studies on the effect of plant diversity

on the diversity and biomass of arthropod herbivores and ... enemies has been conducted in experimental grasslands (Siemann et al., 1998; Tilman, 1999; Haddad et al., 2009; Scherber et al., 2010; Borer et al., 2015; Hertzog et al., 2017; Ebeling et al., 2018; Ebeling et al., 2020). Research on the relationship between plant diversity and natural enemy pressure (the natural enemies hypothesis; Root, 1973) has primarily focused on agricultural and forestry systems (Cardinale et al., 2003). The few studies exploring the broader role of environmental heterogeneity in community assembly and diversity at higher trophic levels for agriculture and forestry are inconclusive. Therefore, the effect of plant diversity and heterogeneity on higher trophic levels remains ambiguous.

Root (1973) posited that structurally complex habitats contain more niches for arthropod predators and this is underlying the increased abundance and effectiveness of natural enemies in these habitats. Thus, tree diversity, through the increased number of niches, is expected to lead to increased predation pressure, both directly and indirectly through associated habitat heterogeneity (MacArthur and MacArthur, 1961).

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<https://doi.org/10.1016/j.foreco.2026.123637>

Received 21 November 2025; Received in revised form 17 February 2026; Accepted 18 February 2026

Available online 21 February 2026

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Increased predation pressure is thought to be a contributing factor to the reduced levels of endemic damage in agricultural and forestry systems that utilize higher plant diversity compared to monocultures (Goodman, 1975; Jactel et al., 2005; Vehviläinen et al., 2006; Jactel and Brockerhoff, 2007; Wetzel et al., 2016). However, studies comparing predation pressure in simple versus diverse habitats are showing trends in opposite directions (Griffin et al., 2013; Iverson et al., 2014; Staab and Schultdt, 2020; Stemmelen et al., 2022; Vázquez-González et al., 2024). An increase in arthropod diversity is frequently observed in habitats with high diversity; however, predation pressure on the target species or model larvae appears to be context-dependent (Bellone et al., 2020; Anttonen et al., 2023). A recent global dataset study found a slight positive effect of tree diversity on bird, but not on arthropod, predation pressure (Vázquez-González et al., 2024). Moreover, a review by Staab and Schultdt (2020) and meta-analysis by Stemmelen et al. (2022) failed to establish a consistent positive correlation between tree diversity and increased predation pressure. In tropical forest the correlation with tree diversity was present and positive but in both temperate and boreal forest no correlation was found. Context-dependence, as discussed by Catford et al. (2022), is often used to explain inconsistencies in observed patterns. These inconsistencies could be related to variations in habitat heterogeneity among different types of forests and different locations. Thus, unmeasured confounding variables may covary with tree diversity and independently affect predation pressure.

Forestry systems exhibit high local variability due to differences in understory structure and diversity, as well as an increased presence of abiotic structures such as streams and stones (Naiman et al., 1993; Gilliam, 2007; Barbier et al., 2008; Stein et al., 2014). The combination of these biotic and abiotic structures leads to a higher diversity in microhabitats and microclimates, thereby increasing the niche diversity for plants, herbivores, and predators. For example, differences in light, water, and nutrient availability associated with specific tree species can influence the composition of the understory trees and vegetation (Barbier et al., 2008; Tinya et al., 2009; Márialigeti et al., 2016; Braziunas et al., 2024). The understory affects microclimates and microhabitats, thereby changing the associated arthropod communities (Aude and Lawesson, 1998; Kovács et al., 2017; Latimer and Zuckerberg, 2017). Additionally, biotic and abiotic structures other than plants may influence predation pressure. Dead wood and other abiotic structures affect microhabitats, which, in turn, influence predator activity through changes in prey availability, shelter provision, or microclimate modification (Kollberg et al., 2014; Matevski et al., 2021; Terlau et al., 2023). These factors can influence arthropod predator–prey interactions in ways that cannot be solely attributed to tree diversity. Hence, it is necessary to examine whether habitat heterogeneity influences these interactions regardless of tree diversity.

In this study, we focus on multiple habitat factors that define the context of boreal forestry systems. Specifically, we investigated the effect of tree diversity in combination with variables affecting local heterogeneity to place predation of insect herbivores in the context of local habitat heterogeneity. We quantified factors that determine local heterogeneity in both mixed and monoculture forest stands. Our study system included the insect herbivore *Neodiprion sertifer* (Geoffroy; Hymenoptera; Diprionidae). We selected mixed and monoculture stands of Scots pine (*Pinus sylvestris* L.) and measured understory diversity and structure, dead wood, and the presence of boulders to examine their direct and indirect relationships with arthropod predation pressure on *N. sertifer* larvae around the selected experimental trees. Previous studies on the predation pressure on *N. sertifer* have found that predation is affected by habitat heterogeneity rather than tree diversity (Bellone et al., 2020), which offers a strong framework for examining habitat heterogeneity effects. We addressed the following research questions: (1) Does tree diversity at the stand or tree level contribute to the predation rate? (2) What factors of heterogeneity contribute significantly to predation rates? (3) Are there indirect effects of tree diversity and other local heterogeneity factors on predation rates?

2. Materials and methods

2.1. Study site

This study was conducted from May to July 2019 in 12 forest stands near Uppsala, central Sweden (see Appendix S1, Fig. 1 for details). We identified six forest stands dominated by > 80 % Scots pine (monoculture forests) and six forest stands with 30–50 % Scots pine primarily mixed with silver birch and Norway spruce (mixed forests) (Table 1). Detailed information on the study species and site is provided in Appendix S1: Sections 1–2. The selected forest stands were characterised as semi-natural forests, where Scots pine (*Pinus sylvestris* L.) was the dominant species and Norway spruce (*Picea abies* (L.) H. Karst.) was common, and silver birch (*Betula pendula* Roth) was the most common deciduous species. The shrub layer included regenerating Norway spruce, silver birch, and Scots pine. These sites have well-developed herbal layers, often dominated by bracken, grass, and ericaceous dwarf shrubs. All forest stands exceeded 1 hectare in size, with tree height ranging from 2 to 6 m (mean height = 3.54 m, SD = 1.12 m) and age ranging from 6 to 15 years. Five areas within each forest stand were randomly selected using a grid system (cells: 30 × 30 m), resulting in five cells per stand. Within each grid cell, one Scots pine was randomly selected as an experimental tree, resulting in 5 experimental trees per stand and 60 experimental trees in total. All selected experimental trees were 2–6 m high (mean height = 3.22 m, SD = 0.74 m). The distances between the experimental trees within the stands was at minimum 20 m, and at maximum 330 m.

2.2. *Neodiprion sertifer*

In this study, we used a gregarious specialist herbivore (*Neodiprion sertifer*) as a study system, the species feeds predominantly on Scots pine in Sweden. The population dynamics of this species show irregular outbreaks that can be widespread (Larsson and Tenow, 1984). The European pine sawfly (*N. sertifer*) is native to Eurasia, and occurs as a non-native species in North America (Olofsson, 1987). From August to September eggs are laid in a single batch on the needles of a current year shoot, where they hibernate until May the following year (Pschorn-Walcher, 1991). After hatching the larvae feed in colonies on mainly on 1-year-old or older needles until males and females molt into fourth and fifth instar, respectively, followed by a final molt into a prepupa. Then they drop from the tree and find a suitable place in the topsoil to spin cocoons. *Neodiprion sertifer* egg batches were collected from the Ramnäs area (Västmanland County, Sweden), ~100 km west of the experimental sites, where larval densities were sufficiently high (see Appendix S1, Section 2 for details). Larvae were placed on experimental trees at second instar, to avoid the high mortality common in first instars (Larsson et al., 1986). Second-instar larvae were ~6 mm long, increasing to 20–25 mm by the fourth instar. At each experimental tree, one group of 20–30 *N. sertifer* larvae was placed on a healthy branch above 1.5 m from the ground. In total 4060 larvae were used. The addition of larval groups to branches is an adequate and established method for assessing the predation rate of *N. sertifer*, as the larvae have a sedentary nature (Griffiths, 1959; Larsson et al., 1986; Björkman et al., 1997; Kaitaniemi et al., 2007; Kollberg et al., 2015; Bellone et al., 2020). Thus, the missing larvae can be assumed to be lost due to predation. The larvae were exposed in the field to predation for 4 weeks. Once a week, the remaining larvae were counted to estimate the predation rate, and new larvae with similar instars were added to maintain the groups at ~25 individuals. Based on previous experience, groups of fewer than 20 individuals show increased larval mortality compared to groups over 20 individuals (M.J. Klapwijk pers. Obs.). We summarised the three weekly counts of disappeared larvae to obtain the total predation rate per experimental tree.

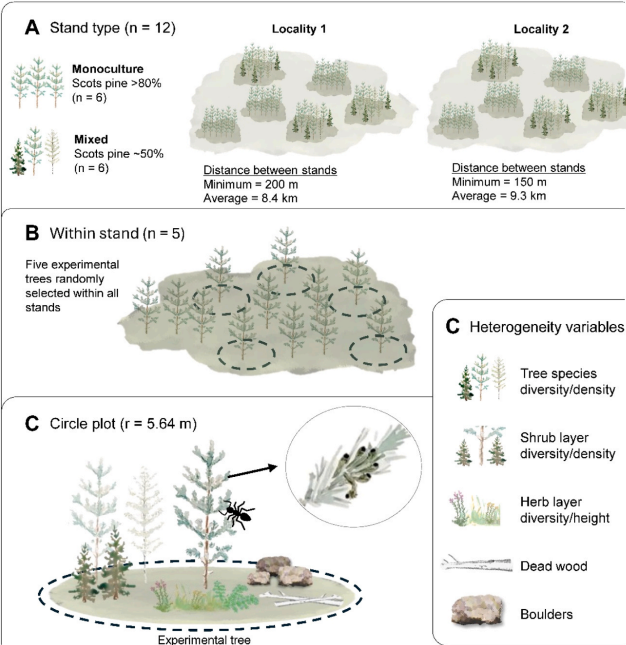


Fig. 1. Descriptive figure of experimental setting, (A) of the 12 selected forest stands divided at two localities, (B) selection of five Scots pine trees within each stand, (C) and local heterogeneity factors measured around each of the five experimental trees. In total 60 experimental trees.

Table 1
Geographic coordinates (latitude and longitude) and tree species composition (%) of the forest stands included in the study. Forest stand names (A–L) correspond to those shown in Fig. 1. Data were provided by the forest owner.

Site	Stand name	Lat (°N)	Lon (°E)	Scots pine (%)	Spruce (%)	Birch (%)	Other (%)
Knutby	A	59.87871	18.22079	57	37	6	0
Knutby	B	59.97528	18.30783	95	0	5	0
Knutby	C	59.99786	18.30211	100	0	0	0
Knutby	D	59.99785	18.29824	60	7	33	0
Knutby	E	59.94806	18.21683	37	57	7	0
Knutby	F	59.99905	18.17804	100	0	0	0
Österbybruk	G	60.20977	17.92461	39	56	5	0
Österbybruk	H	60.21069	17.92723	39	56	5	0
Österbybruk	I	60.28299	17.98033	100	0	0	0
Österbybruk	J	60.27006	18.04239	96	1	3	0
Österbybruk	K	60.18753	17.77672	48	39	13	0
Österbybruk	L	60.15029	17.84135	41	59	0	0

2.3. Natural enemies

In this study system, the dominant generalist predators on the larvae are several species of ants (Olofsson, 1987; Olofsson, 1992; Codella and Raffa, 1995; Lindstedt et al., 2006; Kaitaniemi et al., 2007). Thus, the presence or absence of ants was estimated by repeated observations of ants on the experimental trees with larvae. The ants observed on the host trees belonged to the species *Formica rufa*, *F. polyctena*, and *F. fusca*. *Formica rufa* and *F. polyctena* mounds are built from conifer litter mixed with soil (Kristiansen and Amelung, 2001), while *F. fusca* nest are found in woody debris and under stones (Goertz and Hoch, 2013). Ant foraging is common in the canopy since their major food source is ant-tended arboreal aphid colonies (Riihimäki et al., 2005). Other natural enemies include spiders and parasitoids, however, their abundance have been found to be negatively associated with the presence of ants

(Nordkvist et al., 2021). Bird predation of *N. sertifer* larvae is rare, most likely because of reduced palatability as the larvae sequester resin acids (Lindstedt et al., 2006). For more details see Appendix S1: Sections 1–3.

2.4. Local heterogeneity

The local heterogeneity factors were measured in a circular plot around each tree with a radius of 5.64 m (total basal area of 100 m²). Local heterogeneity was measured by assessing tree and shrub diversity and density, herb layer diversity, height and type, and the presence or absence of dead wood and boulders (Fig. 1, Table 2). The structure and diversity of vegetation in forests can affect arthropod predator-prey interactions by modifying microclimate and microhabitat availability, and by altering predator foraging efficiency (Langellotto and Denno, 2004). Here, trees > 2 m were classified as the tree layer, and naturally

Table 2
Detailed information of local heterogeneity measurements.

	Measurement	Method/Tool	Details
Stand level			
Forest type	Mix and mono.	Identification	Data from forest owner. Mixed: 30 %–50 % pines and monoculture: > 80 % pines, 12 forest stands in total.
Local heterogeneity			
Tree layer	Experimental tree	Grid cell	Haphazardly selecting five experimental trees within five randomly selected grid cells in each forest stand. In total 60 experimental trees.
	Diversity and density	Counting nr. of trees	The total nr. was transformed to stems per m ² . Diversity was transformed to proportions of pine, spruce and birch.
Shrub layer	Diversity and density	Counting nr. of shrubs	The total nr. was transformed to stems per m ² . Diversity was transformed to proportions of pine, spruce and birch.
Herb layer	Cover	Visual estimate	Assessing the cover (%) of predominating species
	Diversity	Calculation	Transforming cover of species into presence/absence and subsequently summing the different species found per experimental tree.
	Mean height	Height measurements	Four random measurements around experimental tree (2 m from stem).
	Type	Site classification	Site classification system adapted to the study site (Hågglund and Lundmark (1977)).
Dead wood	Presence or absence	Visual estimate	Fine woody debris > 10 cm diameter (Oxbrough et al., 2010).
Boulders	Presence or absence	Visual estimate	Boulders between 0.25 and 1 m in diameter.

regenerated pine, birch and spruce between 0.5 and 2 m as the shrub layer. All tree and shrub species were counted in the circle plots. Herb layer height was measured around the experimental tree, and herb-layer diversity was calculated as the number of dominant species present in the circle plot. Herb-layer type was classified using Hågglund and Lundmark (1977), which classifies sites along a fertility gradient from poor to high. In addition to vegetation structure and diversity, we included dead wood and boulders. Dead wood increases structural complexity and offers shelter and microhabitats (Gunnarsson et al., 2004), as well as breeding material for several forest species (Stokland et al., 2012). Moreover, fine woody debris can have a positive impact on forest associated spider communities (Castro and Wise, 2009; Oxbrough et al., 2010). Dead wood, in the form of fine woody debris larger than 10 cm diameter (Oxbrough et al., 2010) were classified as present or absent in the circle plot. High amounts of boulders are common in the study area and contribute to abiotic local heterogeneity. Boulders were defined as larger than 0.25 m and smaller than 1 m in diameter and was classified as present or absent in the circle plot. For more details of local heterogeneity variables see Appendix S1: Section 4.

2.5. Statistical analysis

Statistical analysis was performed using R version 4.3.0. First, the correlations between individual forest characteristics were calculated using a spearman rank correlation test using the function `cor.test::stat` to assess the level of potential co-linearity between the variables. Next, the effect of stand type (mixed or monoculture) on *N. sertifer* predation rates was tested using a generalised linear mixed model (glmmTMB package;

Brooks et al., 2017) with a beta-binomial error distribution. Stand type was the explanatory variable and stand ID was included as a random effect. Next, logistic regression was used to test whether ant presence or absence differed between stand types using the same fixed and random effects. Furthermore, a piecewise structural equation model (SEM; piecewiseSEM package; Lefcheck et al., 2019) was applied to analyse complex relationships, including indirect effects on predation rates (Lefcheck, 2016). SEM (Structural Equation Model) was performed using generalised linear mixed models (glmmTMB package; Brooks et al., 2017). The response variables included predated larvae (over-dispersed count data; negative binomial) and ant presence (binomial), with local heterogeneity factors as explanatory variables and stand ID as a random effect (Table 2). Following the directed separation test, significant direct relationships were added to the model, either as direct relationships or covariance structures, which led to the final model (Appendix S2: Table S2). The goodness of fit for the SEM was tested using Fisher’s C test, and the model was deemed appropriate if $p > 0.05$. Model adequacy for each model in the SEM was assessed using diagnostics based on the visual assessment of residuals, Kolmogorov–Smirnov test, and Levene’s test using the R package DHARMa version 0.4.6 (Hartig, 2018), prior to inclusion in the SEM.

3. Results

The predation rate of *N. sertifer* and the presence or absence of ants did not differ between the mixed and monoculture stands (Fig. 2a–b, see Appendix S1: Table S1 for details).

3.1. Description of forest type characteristics

There were no significant differences in herb layer diversity or height between stand types (Fig. 2c–d, see Appendix S1: Table S2 for details). Tree and shrub densities did not differ, whereas pine density was significantly higher in the monoculture stands (Fig. 3a). Narrow-leaved grass, dead wood, and boulders did not differ significantly between the stand types (Fig. 3b). The correlations between the individual forest characteristics were rather weak with the highest significant correlation between tree density and shrub density ($r = 0.37$; Table 3.)

3.2. The structural equation model

Piecewise SEM satisfied the goodness-of-fit criteria (Fisher’s C = 50.59, $p = 0.80$; $df = 60$). In the SEM the most noticeable result is the lack of a direct effect of forest type on any of the heterogeneity factors (Fig. 4b). The presence of ants increased the predation rate of *N. sertifer* larvae (0.18 ± 0.08) and had a strong positive relationship with herb-layer diversity (1.18 ± 0.53) and narrow-leaved grass type (3.16 ± 1.18). Narrow-leaved grass type (0.17 ± 0.09), shrub density (0.45 ± 0.22), and the presence of boulders (0.36 ± 0.13) increased the predation rate; however, the effect of narrow-leaved grass type was marginally significant. Presence of boulders covaried positively with narrow-leaved grass type, while shrub density covaried negatively with both presence of boulders and the narrow-leaved grass type (Fig. 4b, see Appendix S2: Table S2 for details). Tree density covaried negatively with presence of boulders, herb layer height and pine density, and covaried positively with shrub density (Fig. 4b, see Appendix S2: Table S2 for details). Presence of dead wood was the only variable without any effects or covariances (Fig. 4b).

4. Discussion

We found that abiotic (boulders) and biotic structures (shrub density and herb layer characteristics) affected the predation rate directly and indirectly, independent of tree diversity. Our findings are consistent with Bellone et al. (2020) as both studies found no differences in predation rates between monoculture and mixed stands, however, our

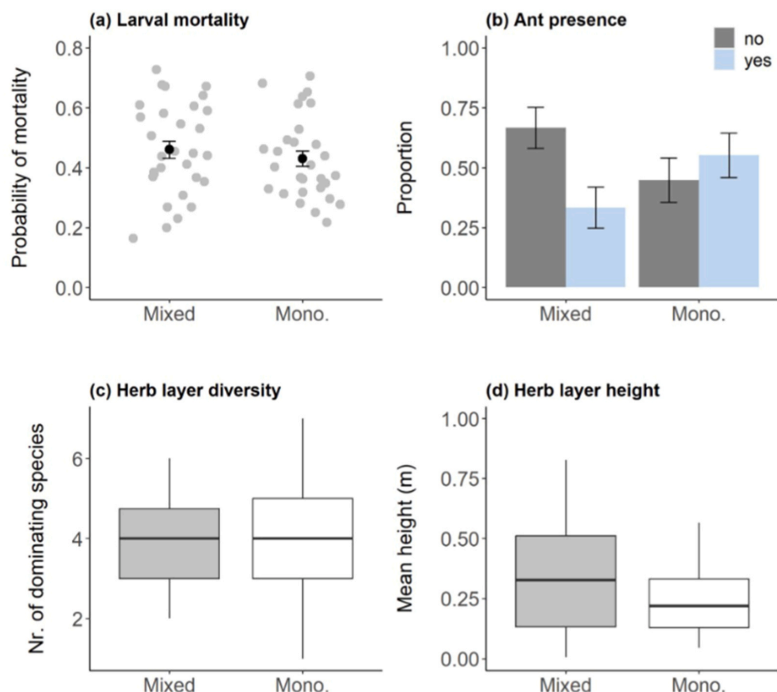


Fig. 2. (a) Probability of larval mortality in mixed and monoculture stands, number of observations = 59. Grey dots represent individual trees. Black circles $\pm$ SE indicate model-predicted means. Larval mortality did not differ significantly between forest types (GLMM, see Appendix S1: Table S1 for details). (b) Proportion of stands with ants present (blue) or absent (grey) in mixed and monoculture stands. Bars show mean $\pm$ SE, number of observations = 59. Ant presence did not differ significantly between forest types (GLMM, see Appendix S1: Tables S2-S3 for details). (c) Descriptive plot of herb layer height (m) between mixed (grey) and monoculture (white) stands. (d) Descriptive plot of herb layer diversity measured as number of dominating species between mixed (grey) and monoculture (white) stands. Between-stand comparisons of heterogeneity factors were included in the SEM, which revealed no significant effects of stand type on herb layer height, or herb layer diversity (see Appendix S2: Table S2 for details).

analysis extends this by considering within stand heterogeneity beyond tree layer characteristics. Although we expected a relationship between tree diversity and stand heterogeneity, the absence of such a relationship indicated that the local heterogeneity in our experimental forest stands was independent of tree diversity.

Predation patterns can depend on tree species diversity (Kemp and Simmons, 1978) and local tree density, which affects different types of predators (Vehviläinen et al., 2008; Bellone et al., 2020). Kaitaniemi et al. (2007) found higher predation of *N. sertifer* in mixed versus monoculture stands in Finland. Moreover, wider patterns show reduced pest insect damage (Jactel et al., 2005), and increased natural enemy abundance and species richness (Staab and Schuldt, 2020) in mixed forests compared to those of monoculture forests. These observations in the mixed forest stands partially support the hypothesis of natural enemies. However, our findings align with those of previous reviews that found no consistent relationship between tree diversity and higher predation rates (Staab and Schuldt, 2020; Stemmelen et al., 2022), indicating that other heterogeneity factors contribute to the observed patterns. Our findings demonstrate the quantifiable influence of local habitat heterogeneity on the contribution of various habitat factors to predation pressure through direct and indirect pathways. This supports the habitat heterogeneity hypothesis, suggesting that habitat complexity may more effectively account for variation in predation pressure. Boreal forests are relatively species-poor and typically dominated by a few tree species compared with temperate and tropical forests. Forestry can

further simplify stand structure, which may increase the relative importance of local heterogeneity.

We examined the relationship between local heterogeneity and predation rates, based on previous research findings. We found that the presence of ants on the host tree led to a higher predation rate of *N. sertifer* (Fig. 4b). Conversely, ant presence was positively influenced by herb layer diversity and narrow-leaved grass type, but not by tree diversity (Fig. 3b). This finding aligns with previous research indicating that ants are not influenced by tree diversity (Vehviläinen et al., 2008). Local herb diversity presumably increases the food resources for ants, such as seeds and prey (Gorb and Gorb, 1999; Heinken and Winkler, 2009; Haddad et al., 2011; Wardle et al., 2011; Zhao et al., 2019). Ant species that require open and sunlit habitats are often found in young forests (Niemelä et al., 1996), where herb layer types such as grass are more common (Hägglund and Lundmark, 1977). Shrub density, narrow-leaved grass type, and the presence of boulders increased the predation rate. Considering these effects it is conceivable that these variables may represent different types of local microhabitats and associated microclimates that affect movement and predation rates by arthropods (Terlau et al., 2023), and small mammalian predators (Kollberg et al., 2014). Furthermore, increased light availability and herb layer complexity promote the predation rates on model caterpillars (Matevski et al., 2021), and the presence of habitat structures can increase predation on cocoons by small mammals (Bellone et al., 2017). Studies on the effects of boulders on microhabitats, microclimates, and

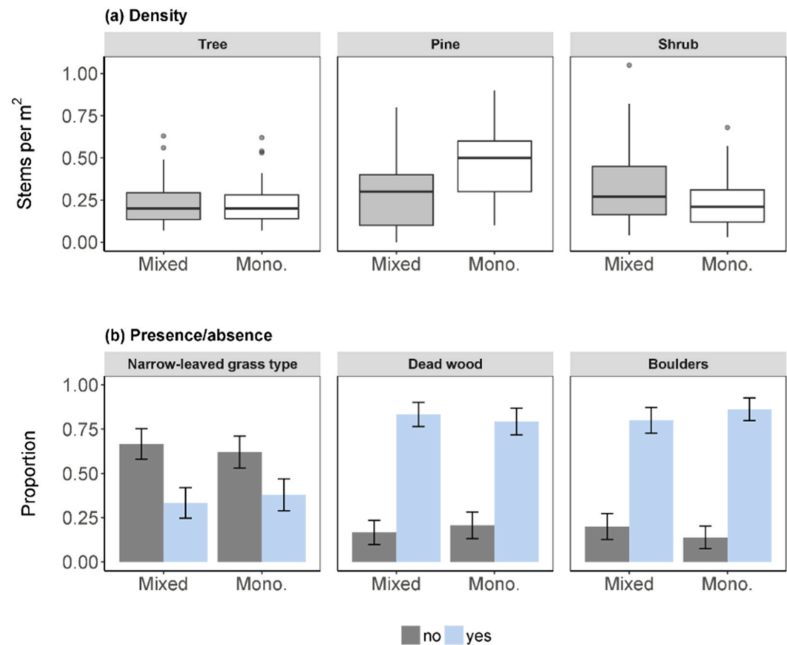


Fig. 3. (a) Descriptive plot of tree density, pine density, and shrub density between mixed (grey) and monoculture (white) stands. Between-stand comparisons of heterogeneity factors were included in the SEM, which revealed significant effect at $P < 0.05$ of stand type on pine density, but no significant effect of stand type on tree density, or shrub density (see [Appendix S2: Table S2](#) for details) (b) Descriptive plot of presence (blue) or absence (grey) of narrow-leaved grass type, dead wood, and boulders between mixed and monoculture stands. Between-stand comparisons of heterogeneity factors were included in the SEM, which revealed no significant effect of stand type on narrow-leaved grass type, dead wood, or boulders (see [Appendix S2: Table S2](#) for details).

Table 3
Mean and standard deviation (SD) of heterogeneity variables and Spearman rank correlations among continuous and ordinal heterogeneity variables included in the SEM. Asterisks show significant correlations (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Spearman rank correlation matrix							
Heterogeneity variables	Mean	SD					
1. Herb layer height	0.30	0.21	1				
2. Herb layer diversity	3.97	1.11	-0.1	2			
3. Tree density	0.24	0.14	-0.16	-0.1	3		
4. Pine density	0.08	0.06	-0.16	0.09	0.33*	4	
5. Shrub density	0.29	0.22	0.18	0.07	0.37**	0.19	5

arthropod activity are rare. The few existing studies on this subject have speculated that boulders influence arthropod activity by providing shelter (Nordlander et al., 2023), increasing soil temperature and moisture content (Saini and MacLean, 1967) and stabilising soil temperature (Shoo et al., 2010). These interactions may help explain the higher predation rate associated with understory characteristics and boulders in our study system, indicating that local heterogeneity affect predation pressure more strongly than tree species diversity.

We conducted a well-replicated but local study to determine whether sources other than tree diversity, such as predation rates, are important for species interactions. Although our experimental approach enabled direct measurement of habitat heterogeneity factors and predation rates using live larvae, the 3-week field exposure offered only a snapshot of predation dynamics. Furthermore, because we used young stands in our study, the effects of tree diversity on heterogeneity and predation pressure may appear when the stands are older. This highlights the importance of context when interpreting divergent results from studies

investigating the natural enemy hypothesis at local and global scales. In conclusion, we found that in semi-natural forests, herb layer characteristics, shrub density, and boulders override the effects of tree diversity. Consistent with the habitat heterogeneity hypothesis, our results suggest that environmental heterogeneity, even independent of tree diversity, influences ecological interactions such as predation. This indicates that both biotic and abiotic structural diversity should be taken into account when studying ecological interactions, such as predation. Forest management should consider within stand heterogeneity in addition to tree diversity to provide structural and biological diversity for a wide range of natural enemies to control different types of pest insects. Two recent reviews on tree diversity and predation pressure concluded that a positive relationship is not apparent, particularly in temperate and boreal forests (Staab and Schuldt, 2020; Stemmelen et al., 2022). The small effect sizes often found in studies predominantly focusing on tree diversity may indicate unaccounted variations in habitat heterogeneity. Future research should focus on identifying the

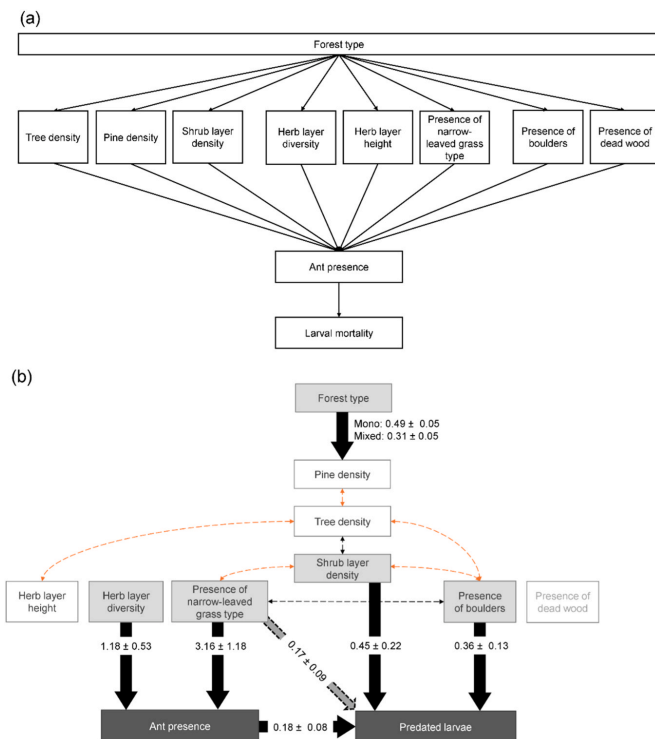


Fig. 4. (a) Initial model with all the tested relationships between forest type, heterogeneity factors, ant presence, and predation rate (see Appendix S2: Table S1). (b) The black directed arrows represent direct significant effects at $P < 0.05$, the grey directed arrow with dashed lines represents marginally significant effects (see Appendix S2: Table S2 for details). The numbers show the estimate and standard error. The dashed double headed arrows represent significant covariances, the black and orange colours indicate positive and negative covariances, respectively. Global goodness-of-fit: Fisher's C = 50.588 with p-value = 0.802 and on 60 ° of freedom.

specific habitat components that enhance predation pressure across different forest types to improve understanding of the influence of biotic and abiotic heterogeneity interaction on trophic interactions.

Author contributions

FW, MJK, and CB conceived the ideas and designed methodology; FW collected the data; and MJK and FW analysed the data; FW and MJK conceptualised and wrote the first version of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

CRediT authorship contribution statement

Fredrika Wrethling: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation. **Christer Björkman:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Klapwijk Maartje:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no competing financial interests

or personal relationships that may have influenced the work reported in this study.

Acknowledgments

Special thanks go to Emilio Otero for field assistance and the forest company BillerudKorsnäs, who provided information on the forest stands. This study was supported by the holistic management of emerging forest pests and diseases (HOMED) project, which received funding from the European Union's Horizon 2020 Research and Innovation Program under grant 771271.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.foreco.2026.123637](https://doi.org/10.1016/j.foreco.2026.123637).

Data availability

The data that support the findings of this study are available in "Dryad". See attached file step for DOI link, either in the manuscript or in "Dryad DOI link-document".

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Supplementary material for:

Local heterogeneity an important driver for predation pressure of a defoliating insect in boreal
forests

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Forest Ecology and Management

Supporting information for material and method

Section 1: study site

In total we used twelve forest stands, six stands at each locality (Figure 1). Locality (1) is located 40 km East of Uppsala and the central point is 59°54'34"N, 18°15'56"E. Locality (2) is located 45 km North of Uppsala with the central point 60°11'45"N, 17°53'46"E. The distance between the two nearest stands between locality 1 and 2 was 25 km. For the first locality, the minimum distance between the two nearest stands was 200 m, and the average distance between stands was 8.4 km. For the second locality, the minimum distance between the two nearest stands was 150 m, and the average distance between stands was 9.3 km. At both localities mixed and monoculture stands were represented equally, resulting in six monoculture and six mixed stands in total. The stands are used primarily for timber harvest.

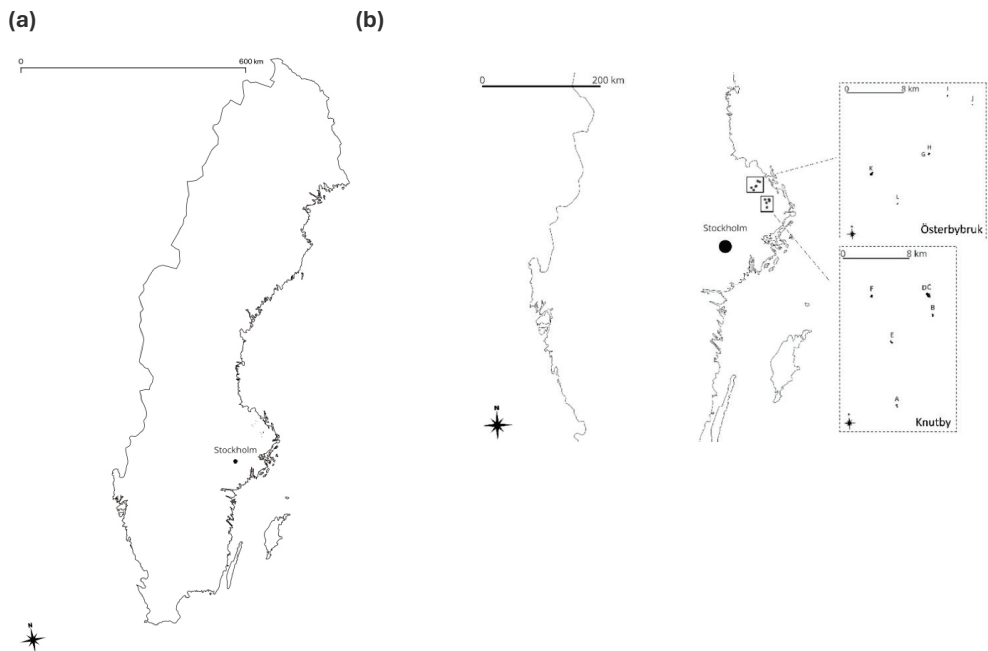


FIGURE 1. Location of study sites in central Sweden. (a) Overview map of Sweden showing the position of Stockholm. (b) Enlarged map show the locations of the study sites Österbybruk and Knutby. Black dots represent forest stands included in the study (letters correspond to forest stand ID).

Section 2: study species Neodiprion sertifer

The European pine sawfly (*N. sertifer*) is a widespread species native to Eurasia, and has been introduced in North America (Olofsson, 1987). From August to September eggs are laid in a single batch on the needles of a current year shoot, where they hibernate until May the following year (Pschorn-Walcher, 1991). After hatching the larvae feed in colonies on the needles until males and females molt into fourth and fifth instar, respectively, followed by a final molt into a prepupa. Then they drop from the tree and find a suitable place in the topsoil to spin cocoons. After summer diapause the prepupa pupate in the cocoons and develop until adult emergence between August and September. The most important predators are several species of ants, spiders and parasitoids (Olofsson, 1987; Olofsson, 1992; Lindstedt et al., 2006; Kaitaniemi et al., 2007). Bird predation of larvae is rare as the larvae are thought to be unpalatable for birds because of the sequestration of resin acid, which is used as defense. The larvae feed in groups with on average 70 individuals (Gur'yanova, 2006), and employ collective defense behaviour when threatened, by flicking up their heads and tails and regurgitating a resin bubble from the mouthparts. In the pupal stage the most important natural enemies are ground dwelling insects and small mammals (Hanski et al., 1985; Olofsson, 1987).

Section 3: rearing of larvae

Branches with egg batches were collected from a natural population with high densities, reared outside at the Department of [Anonymous]. We reared the egg batches after hatching until 2nd instar larvae on the collected branches to avoid early instar mortality affecting the experiment which can be high in first instar larvae (Larsson et al., 1986). Second-instar larvae were ~6 mm long, increasing to 20-25 mm by the fourth instar. We used in total 4,060 larvae in this experiment. *Neodiprion sertifer* larvae are sedentary, and once established they remain on the same branch (Kaitaniemi et al., 2007). Adding groups of larvae on branches is an adequate and established method to assess predation rate of *N. sertifer* (Griffiths, 1959; Larsson et al., 1986; Björkman et al., 1997; Kaitaniemi et al., 2007; Kollberg et al., 2015; Bellone et al., 2020). Thus, missing larvae can be assumed to be lost due to predation.

Section 4: heterogeneity measurements

Local heterogeneity factors were measured in a circle plot around each tree, with a radius of 5.64 m (total basal area of 100 m²). Local heterogeneity was measured in terms of tree- and shrub diversity and density, herb layer diversity, height and type, presence or absence of dead wood and boulders. Local tree diversity and density were measured by counting the number of tree species (>2 m) within the circle plot. For local tree diversity, we transformed the number of different tree species to proportions of pine, spruce, and birch in the circle plot. The total number of trees in the circle plot was transformed to stems per m². The shrub

layer consisted of smaller (0.5-2 m) naturally regenerated pine, birch and spruce trees. The total number of shrubs in the circle plot was transformed to stems per m². Herb layer vegetation cover was estimated by assessing the coverage in percent of the predominant species within the circle plot. Herb layer diversity was then determined by transforming the coverage data into presence or absence of that dominant species and subsequently summing the different species found per experimental tree. Mean height of the herb layer was determined by four random height measurements within a 2 m distance around each experimental tree. To assess herb layer type, we used a site classification system with a flow chart adapted to the study site from Hägglund & Lundmark (1977). The different herb layer types are indicators of the forest fertility conditions and can be placed on a scale from poor to good fertility. The herb layer types included were: dwarf-shrub type (defined by species from the *Ericaceae* family), narrow-leaved grass type (mostly defined by *Festuca ovina*, *Avenella flexuosa*, together with *Pteridium aquilinum*, and relative nutrient-demanding low-growing herbs), broad-leaved grass type (mostly defined by *Deschampsia cespitosa*, together with the fern *Pteridium aquilinum* and nutrient-demanding tall-growing herbs), and herb type (defined by the quantity of tall- and low growing herbs typical for the study site). Dead wood, in the form of fine woody debris larger than 10 cm diameter (Oxbrough et al., 2010), and boulders were classified as presence or absence in the circle plot. Boulders were defined as larger than 0.25 m and smaller than 1 m in diameter.

Table S1: Summary table for Figure 2a

Table S1a shows the result of generalized linear mixed model (glmmTMB) predicting probability of larval predation between forest types (2 levels; mixed and monoculture) using beta-binomial error distribution with a logit link function. The grouping of the stands was included as a random effect in the model. The table shows model estimates, the standard error of the mean (SE), z-value, and the p-value for the fixed predictor and the variance and the standard deviation of the random effects. Total number of observations: 59.

Fixed effect	Estimate	SE	z-value	p-value
Intercept	-0.164	0.105	-1.559	0.119
Forest type: mono.	-0.108	0.150	-0.720	0.472
Random effect	Variance	Std. Dev		
Site identity (Intercept)	1.134e-09	3.367e-05		

Table S2: Summary table for Figure 2b

Shows the result of generalized linear mixed model (glmmTMB) predicting presence/absence of ants between forest types (2 levels; mixed and monoculture) and using beta-binomial error distribution with a logit link function. The grouping of the stands was included as a random effect in the model. Table shows model estimates, the standard error of the mean (SE), z-value, and the p-value for the fixed predictor and the variance and the standard deviation of the random effects. Total number of observations: 59.

Fixed effect	Estimate	SE	z-value	p-value
Intercept	-0.808	0.552	-1.462	0.144
Forest type: mono.	1.036	0.768	1.350	0.177
Random effect	Variance	Std. deviation		
Site identity (Intercept)	0.6804	0.8249		

Table S3: Summary table for Figure 2b

Shows counts of presence/absence, total count, probability of presence/absence, and the standard error. Total number of observations: 59.

Type	Ant presence	Count	Total	Probability	SE
Mixed	no	20	30	0.667	0.0861
Mixed	yes	10	30	0.333	0.0861
Mono.	no	13	29	0.448	0.0923
Mono.	yes	16	29	0.552	0.0923

Structural equation modeling (SEM), also called ‘confirmatory path analysis’, is an effective method for analysis of complex networks with multiple factors and effects by allowing simultaneous determination of all potential relationships (Lefcheck et al., 2016). The models are evaluated individually (locally) and simultaneously (globally). By evaluating the models individually the SEM allows for a lower sample size than the traditional way of modelling structural equation analysis. The SEM analysis allows to assess indirect effects on the predation rates of *N. sertifer*. Using piecewiseSEM allowed us to build the structural equation model with generalized linear mixed model using the `glmmTMB()` function from the `glmmTMB` package (Brooks et al., 2017).

For larval mortality we used a negative binomial distribution. We used the sum of the number of predated larvae over proportion predated, as we added new larvae weekly in groups where they had disappeared. Binomial distributions were used for ant presence, narrow-leaved grass type, presence of dead wood, and presence of boulders. Lognormal distributions were used for herb layer diversity, and herb layer height. Gaussian distributions were used for tree density, shrub density, and pine density. For each model the grouping of the stands was included as a random variable. We assessed all individual models included in SEM separately. Hereafter, we employed a piecewise structural equation model (SEM), constructed from the individual generalised linear mixed models (piecewiseSEM package; (Lefcheck et al., 2019).

Table S1. Path model structure for figure 4a using PieceWise SEM, direct relationships

Call:*

	Response									
	Larval mort.	Ant s	NL - grass	HL- height	HL- div	P/A- DW	P/A- B	T- den.	P- den.	S- den.
Explanatory										
Larval mortality										
Ants	x									
Forest type			x	x	x	x	x	x	x	x
Narrow-leaved grass type		x								
Herb layer height		x								
Herb layer diversity		x								
Presence/absence dead wood		x								
Presence/absence boulders		x								
Tree density		x								
Shrub density		x								
Pine density		x								

*the cells with X are the explanatory variables used to model the response variable.

AIC

603.179

Tests of directed separation:

Independ.Claim	Test.Type	DF	Crit.Value	P.Value
Ants ~ Forest type + ...	anova	1	2.174	0.140
Larval mortality ~ Forest type + ...	anova	1	0.943	0.332
Narrow-leaved grass type ~ Herb layer height + ...	coef	59	-1.486	0.137
Herb layer diversity ~ Herb layer height + ...	coef	59	0.020	0.984
Presence/absence dead wood ~ Herb layer height + ...	coef	59	-0.725	0.468
Herb layer height ~ Presence/absence boulders + ...	coef	59	-1.694	0.090
Tree density ~ Herb layer height + ...	coef	59	-2.225	0.026
Herb layer height ~ Pine density + ...	coef	59	0.548	0.584
Herb layer height ~ Shrub density + ...	coef	59	0.630	0.529
Larval mortality ~ Herb layer height + ...	coef	59	0.381	0.703
Herb layer diversity ~ Narrow-leaved grass type + ...	coef	59	-0.597	0.551
Presence/absence dead wood ~ Narrow-leaved grass type + ...	coef	59	-1.052	0.293
Presence/absence boulders ~ Narrow-leaved grass type + ...	coef	59	1.779	0.075
Tree density ~ Narrow-leaved grass type + ...	coef	59	-1.519	0.129
Pine density ~ Narrow-leaved grass type + ...	coef	59	-1.655	0.098
Shrub density ~ Narrow-leaved grass type + ...	coef	59	-3.053	0.002
Larval mortality ~ Narrow-leaved grass type + ...	coef	59	2.076	0.038
Presence/absence dead wood ~ Herb layer diversity + ...	coef	59	0.980	0.327
Herb layer diversity ~ Presence/absence boulders + ...	coef	59	-1.020	0.308
Tree density ~ Herb layer diversity + ...	coef	59	0.098	0.922
Pine density ~ Herb layer diversity + ...	coef	59	0.624	0.532
Herb layer diversity ~ Shrub density + ...	coef	59	0.442	0.659
Larval mortality ~ Herb layer diversity + ...	coef	59	-0.311	0.756
Presence/absence boulders ~ Presence/absence dead wood + ...	coef	59	0.086	0.932
Tree density ~ Presence/absence dead wood + ...	coef	59	-0.936	0.350

Pine density ~ Presence/absence dead wood + ...	coef	59	-1.066	0.286
Shrub density ~ Presence/absence dead wood+ ...	coef	59	-0.634	0.526
Larval mortality ~ Presence/absence dead wood + ...	coef	59	-1.380	0.168
Tree density ~ Presence/absence boulders + ...	coef	59	-2.644	0.008
Presence/absence boulders ~ Pine density + ...	coef	59	-1.365	0.172
Shrub density ~ Presence/absence boulders + ...	coef	59	-4.336	0.000
Larval mortality ~ Presence/absence boulders + ...	coef	59	2.646	0.008
Pine density ~ Tree density + ...	coef	59	-2.976	0.003
Shrub density ~ Tree density + ...	coef	59	3.071	0.002
Larval mortality ~ Tree density + ...	coef	59	-0.772	0.440
Shrub density ~ Pine density + ...	coef	59	2.532	0.011
Larval mortality ~ Pine density + ...	coef	59	-0.452	0.652
Larval mortality ~ Shrub density + ...	coef	59	-0.392	0.695

* Bold text shows significant and marginally significant values

Global goodness-of-fit:

Fisher's C = 161.298 with P-value = 0 and on 76 degrees of freedom

Coefficients

Response	Predictor	Estimate	Std.Error	DF	Crit.Value	P.Value	Std.Estimate
Larval mortality	Ants	0.194	0.088	59	2.196	0.028	-
Ants	Herb layer height	3.202	2.799	59	1.144	0.253	0.218
Ants	Narrow-leaved grass type	2.757	2.757	59	2.121	0.034	0.440
Ants	Herb layer diversity	1.377	0.640	59	2.150	0.032	0.507
Ants	Presence/absence dead wood	-1.035	1.296	59	-0.799	0.424	-0.135
Ants	Presence/absence boulders	-0.184	1.554	59	-0.119	0.906	-0.023
Ants	Tree density	-0.645	4.193	59	0.154	0.877	0.03
Ants	Pine density	-9.044	8.768	59	-1.032	0.302	-0.168
Ants	Shrub density	-2.383	2.557	59	-0.932	0.352	-0.171
Herb layer height	Forest type	-	-	1	0.073	0.787	
Herb layer height	Forest type = mix	-1.301	0.198	Inf	-6.579	<0.001	
Herb layer height	Forest type = mono	-1.231	0.199	Inf	-6.195	<0.001	
Narrow-leaved grass type	Forest type			1	0.066	0.798	
Narrow-leaved grass type	Forest type = mix	-0.828	0.598	Inf	-1.383	0.167	
Narrow-leaved grass type	Forest type = mono	-0.612	0.603	Inf	-1.016	0.310	
Herb layer diversity	Forest type			1	0.038	0.846	
Herb layer diversity	Forest type = mix	1.353	0.082	Inf	16.523	<0.001	
Herb layer diversity	Forest type = mono	1.374	0.081	Inf	17.054	<0.001	
Presence/absence dead wood	Forest type			1	0.163	0.687	
Presence/absence dead wood	Forest type = mix	2.204	1.214	Inf	1.815	0.070	

Presence/absence dead wood	Forest type = mono	2.838	1.432	Inf	1.982	0.048
Presence/absence boulders	Forest type			1	0.628	0.428
Presence/absence boulders	Forest type = mix	1.951	1.036	Inf	1.883	0.060
Presence/absence boulders	Forest type = mono	3.302	1.653	Inf	1.998	0.046
Tree density	Forest type			1	0.002	0.969
Tree density	Forest type = mix	0.241	0.041	55	5.912	<0.001
Tree density	Forest type = mono	0.243	0.041	55	5.999	<0.001
Pine density	Forest type			1	7.999	0.005
Pine density	Forest type = mix	0.062	0.010	55	6.159	<0.001
Pine density	Forest type = mono	0.103	0.010	55	10.040	<0.001
Shrub density	Forest type			1	1.623	0.203
Shrub density	Forest type = mix	0.236	0.057	55	4.167	<0.001
Shrub density	Forest type = mono	0.338	0.056	55	6.000	<0.001

* Bold text shows significant values

Individual R-squared:

Response	Method	Marginal	Conditional
Larval mortality	none	0.09	0.12
Ants	none	0.42	0.70
Herb layer height	none	0.00	0.44
Narrow-leaved grass type	none	0.00	0.23
Herb layer diversity	none	0.00	0.03
Presence/absence dead wood	none	0.01	0.56
Presence/absence boulders	none	0.06	0.55
Tree density	none	0.00	0.37
Pine density	none	0.14	0.17
Shrub density	none	0.06	0.34

Table S2. Path model structure for figure 4b using PieceWise SEM, final model

Call*:	Response									
	Larval	Ant	NL -	HL-	HL-	P/A-	P/A-	T-	P-	S-
	mort.	s	grass	height	div	DW	B	den.	den.	den.
Explanatory										
Larval mortality										
Ant presence	x									
Forest type			x	x	x	x	x	x	x	x
Narrow-leaved grass type	x	x								
Herb layer height			x							
Herb layer diversity			x							
Presence/absence dead wood										
Presence/absence boulders	x									
Tree density										
Shrub density	x									
Pine density										
Covar	Tree density			~~	Herb layer height					
	Shrub density			~~	Narrow-leaved grass type					
	Tree density			~~	Presence/absence boulders					
	Shrub density			~~	Presence/absence boulders					
	Pine density			~~	Tree density					
	Shrub density			~~	Tree density					
	Shrub density			~~	Pine density					
	Herb layer height			~~	Presence/absence boulders					
	Presence/absence boulders			~~	Narrow-leaved grass type					
	Pine density			~~	Narrow-leaved grass type					

* the cells with (x) are the explanatory variables used to model the response variable.

AIC

590.163

Tests of directed separation:

Independ.Claim	Test.Type	DF	Crit.Value	P.Value
Larval mortality ~ Forest type + ...	anova	1	0.643	0.423
Ant presence ~ Forest type + ...	anova	1	2.341	0.126
Larval mortality ~ Herb layer height + ...	coef	59	1.478	0.140
Herb layer diversity ~ Larval mortality + ...	coef	59	-0.627	0.531
Presence/absence dead wood ~ Larval mortality + ...	coef	59	-0.492	0.623
Tree density ~ Larval mortality + ...	coef	59	-0.584	0.560
Pine density ~ Larval mortality + ...	coef	59	-0.248	0.804
Narrow-leaved grass type ~ Herb layer height + ...	coef	59	-1.486	0.137
Herb layer diversity ~ Narrow-leaved grass type + ...	coef	59	-0.597	0.551
Presence/absence dead wood ~ Narrow-leaved grass type + ...	coef	59	-1.052	0.293
Tree density ~ Narrow-leaved grass type + ...	coef	59	-1.519	0.129
Herb layer diversity ~ Presence/absence boulders + ...	coef	59	-1.020	0.308
Presence/absence dead wood ~ Presence/absence boulders + ...	coef	59	0.066	0.948
Presence/absence boulders ~ Pine density + ...	coef	59	-1.365	0.172
Ant presence ~ Presence/absence boulders + ...	coef	59	0.020	0.984
Herb layer height ~ Shrub density + ...	coef	59	0.630	0.529
Herb layer diversity ~ Shrub density + ...	coef	59	0.442	0.659
Presence/absence dead wood ~ Shrub density + ...	coef	59	-0.521	0.603
Ant presence ~ Shrub density + ...	coef	59	-0.564	0.573
Herb layer diversity ~ Herb layer height + ...	coef	59	0.020	0.984
Presence/absence dead wood ~ Herb layer height + ...	coef	59	-0.725	0.468
Herb layer height ~ Pine density + ...	coef	59	0.547	0.584
Presence/absence dead wood ~ Herb layer diversity + ...	coef	59	0.980	0.327
Herb layer diversity ~ Tree density + ...	coef	59	-0.292	0.771
Pine density ~ Herb layer diversity + ...	coef	59	0.624	0.532
Tree density ~ Presence/absence dead wood + ...	coef	59	-0.936	0.350
Pine density ~ Presence/absence dead wood + ...	coef	59	-1.066	0.286
Ant presence ~ Presence/absence dead wood + ...	coef	59	-0.305	0.760
Ant presence ~ Tree density + ...	coef	59	-0.093	0.926
Ant presence ~ Pine density + ...	coef	59	-1.233	0.218

Global goodness-of-fit:

Fisher's C = 50.588 with P-value = 0.802 and on 60 degrees of freedom

Coefficients

Response	Predictor	Estimate	Std.Error	DF	Crit.Value	P.Value	Std.Estimate
Larval mortality	Ant presence	0.182	0.083	59	2.178	0.029	-
Larval mortality	Narrow-leaved grass type	0.172	0.091	59	1.900	0.057	-
Larval mortality	Presence/absence boulders	0.363	0.127	59	2.863	0.004	-
Larval mortality	Shrub density	0.455	0.224	59	2.034	0.042	-
Ant presence	Narrow-leaved grass type	3.159	1.183	59	2.670	0.008	0.544
Ant presence	Herb layer diversity	1.178	0.532	59	2.214	0.027	0.468
Ant presence	Herb layer height	3.774	2.314	59	1.631	0.103	0.277
Herb layer height	Forest type	-	-	1	0.073	0.787	-
Herb layer height	Forest type = mix	-1.301	0.198	Inf	-6.579	>0.001	-
Herb layer height	Forest type = mono	-1.231	0.199	Inf	-6.195	>0.001	-
Narrow-leaved grass type	Forest type	-	-	1	0.066	0.798	-
Narrow-leaved grass type	Forest type = mix	-0.828	0.598	Inf	-1.383	0.167	-
Narrow-leaved grass type	Forest type = mono	-0.612	0.603	Inf	-1.016	0.310	-
Herb layer diversity	Forest type	-	-	1	0.038	0.846	-
Herb layer diversity	Forest type = mix	1.353	0.082	Inf	16.523	>0.001	-
Herb layer diversity	Forest type = mono	1.374	0.081	Inf	17.054	>0.001	-
Presence/absence dead wood	Forest type	-	-	1	0.163	0.687	-
Presence/absence dead wood	Forest type = mix	2.204	1.214	Inf	1.815	0.070	-
Presence/absence dead wood	Forest type = mono	2.838	1.432	Inf	1.982	0.048	-

Presence/absence boulders	Forest type	-	-	1	0.628	0.428	-
Presence/absence boulders	Forest type = mix	1.951	1.036	Inf	1.883	0.060	-
Presence/absence boulders	Forest type = mono	3.302	1.653	Inf	1.998	0.046	-
Tree density	Forest type	-	-	1	0.002	0.969	-
Tree density	Forest type = mix	0.241	0.041	55	5.912	>0.001	-
Tree density	Forest type = mono	0.243	0.041	55	5.999	>0.001	-
Pine density	Forest type	-	-	1	7.999	0.047	-
Pine density	Forest type = mix	0.062	0.010	55	6.160	>0.001	-
Pine density	Forest type = mono	0.103	0.010	55	10.040	>0.001	-
Shrub density	Forest type	-	-	1	1.623	0.203	-
Shrub density	Forest type = mix	0.236	0.057	55	4.167	>0.001	-
Shrub density	Forest type = mono	0.338	0.056	55	6.000	>0.001	-
~~Tree density	~~Herb layer height	-0.311	-	59	-2.444	0.009	-0.311
~~Shrub density	~~Narrow-leaved grass type	-0.346	-	59	-2.764	0.004	-0.346
~~Tree density	~~Presence/absence boulders	-0.362		59	-2.908	0.003	-0.362
~~Shrub density	~~Presence/absence boulders	-0.466		59	-3.942	>0.001	-0.466
~~Pine density	~~Tree density	0.331		59	2.626	0.006	0.331
~~Shrub density	~~Tree density	0.368		59	2.959	0.002	0.368
~~Shrub density	~~Pine density	0.314		59	2.476	0.008	0.314
~~Herb layer height	~~Presence/absence boulders	-0.123		59	-0.924	0.180	-0.123
~~Presence/absence boulders	~~Narrow-leaved grass type	0.267		59	2.076	0.021	0.267
~~Pine density	~~Narrow-leaved grass type	-0.213		59	-1.630	0.054	-0.213

* Bold numbers show significant values.

Individual R-squared:

Response	Method	Marginal	Conditional
Larval mortality	none	0.26	NA
Ants	none	0.38	0.63
Herb layer height	none	0.00	0.44
Narrow-leaved grass type	none	0.00	0.23
Herb layer diversity	none	0.00	0.03
Presence/absence dead wood	none	0.01	0.56
Presence/absence boulders	none	0.06	0.55
Tree density	none	0.00	0.37
Shrub density	none	0.14	0.17
Pine density	none	0.06	0.34

Table S4: Summary table for Figure 3b

Shows counts of presence/absence, total count, probability of presence/absence, and the standard error.
Total number of observations: 59.

Type	Variable	Presence	Count	Total	Probability	SE
Mixed	Narrow-leaved grass type	no	20	30	0.667	0.086
Mixed	Narrow -leaved grass type	yes	10	30	0.333	0.086
Mixed	Presence/absence dead wood	no	5	30	0.167	0.068
Mixed	Presence/absence dead wood	yes	25	30	0.833	0.068
Mixed	Presence/absence boulders	no	6	30	0.200	0.073
Mixed	Presence/absence boulders	yes	24	30	0.800	0.073
Mono.	Narrow -leaved grass type	no	18	29	0.621	0.090
Mono.	Narrow -leaved grass type	yes	11	29	0.379	0.090
Mono.	Presence/absence dead wood	no	6	29	0.207	0.075
Mono.	Presence/absence dead wood	yes	23	29	0.793	0.075
Mono.	Presence/absence boulders	no	4	29	0.138	0.064
Mono.	Presence/absence boulders	yes	25	29	0.862	0.064

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OPEN ACCESS

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RECEIVED 22 January 2026

REVISED 22 June 2026

ACCEPTED 26 June 2026

PUBLISHED 29 July 2026

CITATION

Klapwijk MJ, Wrethling F
and Björkman C (2026) Predation
rates on real and artificial prey
in the context of tree diversity.
Front. Ecol. Evol. 14:1794147.
doi: 10.3389/fevo.2026.1794147

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Predation rates on real and artificial prey in the context of tree diversity

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Introduction: Research on the relationship between tree diversity and herbivore predation mortality suggests that the effect of tree diversity on predation rates is context-dependent. Prey traits, such as the type of defense, can affect the predator community associated with a prey, with consequences for the effects of tree diversity on the predation rates of different insect herbivores in forest ecosystems.

Methods: In this study, we set out to investigate the predation rates for larvae of the pine sawfly, *Neodiprion sertifer* (Geoffroy) (Hymenoptera; Dirpionidae), in mixed and monoculture stands in Sweden, together with plasticine models placed individually and in groups, to assess the relationship between habitat variables and attack rates. We measured habitat variables such as understory richness and tree diversity, and we divided the stands into categories with respect to the abundance of the focal tree species, *Pinus sylvestris*. We also noted the presence of ants in the experimental trees, as ants are a common predator of *N. sertifer*.

Results: A qualitative comparison showed that the relationship between attack, or predation rates, and habitat characteristics differs by prey type and that only the predation of *N. sertifer* larvae showed an increase with ant presence.

Conclusion: Our study indicates a possibility for a response of predators to prey traits, confounding the effects of tree diversity on the predation rates. Hence, future studies should further investigate predator community assembly across different prey types to better contextualize the potential for application of the enemies hypothesis.

KEYWORDS

associational effects, community assembly, forest heterogeneity, predator-prey interaction, trait-based ecology

Introduction

The seminal article by Richard B. Root (1973) elegantly showed the difference in the diversity of arthropod communities associated with cabbage in a single-species system compared with a multispecies system. Root proposed the “enemies hypothesis” as a potential explanation for the lower damage in more diverse systems. In both agricultural and forestry systems, research over the last decades has been directed toward understanding whether or not the “enemies hypothesis” is a useful ecological concept for pest management (e.g., Cardinale et al., 2003; Barbosa et al., 2009 and references therein; Griffin et al., 2013; Staab and Schuldt, 2020). The enemies hypothesis assumes that increased niche availability in more complex habitats (MacArthur and MacArthur, 1961) is expected to increase the

presence of both prey and predators. The increased diversity in the predator community is assumed to provide a more stable predation pressure and the switching of predators to the most abundant prey (Yachi and Loreau, 1999).

To discern overarching patterns in the relationship between plant diversity and predation pressure, researchers have synthesized findings from studies of both types of ecological systems using review articles and meta-analyses. A recent review by Staab and Schuldt (2020) suggested that increasing diversity in forest stands does not always lead to increased predator diversity. Using a meta-analysis, Stemmel et al. (2022) found a significant relationship between tree diversity and natural enemy diversity and abundance; however, no relationship was found with predation pressure. The meta-analysis by Griffin et al. (2013) did find that predator diversity increases prey suppression. However, in their meta-analysis, they did not explore the relationship between plant richness and predator host suppression. Nevertheless, the plethora of research in both agricultural and forest ecosystems appears to show that whether or not the “enemies hypothesis” applies, it is context-dependent and could be determined by several factors (Tylianakis and Romo, 2010).

Predation pressure is determined, in part, by the ratio of predators to prey within a system. High predator competition can lead to interference and/or intraguild predation between predators with a shared prey (Roubinet et al., 2015; Laubmeier et al., 2020). Variability in predator traits in the community, such as body size and hunting strategy, is considered to contribute to increased predation with increased predator richness (Jonsson et al., 2017). Other mechanisms, including preemptive resource depletion, complementarity, or redundancy, might affect the efficacy of the combined predator guild (Dainese et al., 2017; Klapwijk, 2019) in increasing diversity. Extrinsic factors, such as temperature, that affect predation have been widely discussed (Laubmeier et al., 2020; Staab and Schuldt, 2020) and have been found to determine the outcome of vegetation richness on predation pressure. From a prey perspective, a recent theoretical study found that prey defense phenotype can mediate the effect of multiple predators in a tri-trophic food web (Guariento et al., 2022). Staab and Schuldt (2020) observed that the majority of research related to tree diversity and the enemies hypothesis has been conducted with a focus on generalist predators. From a pest management perspective, this is logical, as generalist predators are thought to be crucial for population regulation at endemic levels (Hanski et al., 1991).

However, not all generalist predators will (be able to) attack every herbivore or, in the case of pest management, every pest herbivore (Nedvéd, 2026). Hypothetically, a parallel can be drawn with plant-herbivore interactions. Similarly, plants have different communities of herbivores associated with them, which are determined by plant traits (Jung et al., 2010; Novotny et al., 2010; Van der Plas et al., 2012; Forister et al., 2015). Although the underlying mechanisms will be different, it could be hypothesized that the community of predators associated with a herbivore species is determined by the specific traits of that herbivore. From this, it follows that increases in tree diversity might specifically affect the richness of the community of predators for the target prey. We propose this as a potential explanation for the ambiguous direction of the accumulated effects of plant diversity on predation pressure.

Following the argument above, in this article, we aimed to begin understanding the importance of herbivore traits in the relationship between predation rate and habitat characteristics.

Herein, we ask the following questions:

1. What are the qualitative differences in the patterns of predation rates between real and artificial prey?
2. How does forest type affect the patterns of predation rates on the real and artificial prey?
3. How does the presence of ants (*Formica* spp.), a commonly occurring predator, interact with forest heterogeneity, and how does this affect predation rates?

We answer these questions by using an experimental approach in existing forest stands that have been allocated by the forest owner as mixed or monoculture stands. We used *Neodiprion sertifer* (Geoffroy) (Hymenoptera; Diprionidae) as a study system in relation to the focal tree species Scots pine (*Pinus sylvestris* L.). *N. sertifer* is a species known for its gregarious feeding and effective group defense (Björkman et al., 1997). In addition, we used plasticine models to assess predation rates on non-defended solitary and gregarious prey items. We did not sample the predator community. However, as ants are an important predator of *N. sertifer* larvae (Lindstedt et al., 2006), we made observations of their presence or absence in the experimental trees.

Materials and methods

Study site and experimental design

The study was carried out during the growing season of 2019 at two localities in the vicinity of Uppsala, Central Sweden. The first site (central point; 59°54'34" N, 18°15'56" E) is located 40 km east of Uppsala. The other site (central point; 60°11'45" N, 17°53'46" E) is located 45 km north of Uppsala. These forest areas are characterized by semi-natural forests, where Scots pine (*P. sylvestris* L.) and Norway spruce [*Picea abies* (L.) H. Karst] are the dominant tree species. Silver birch (*Betula pubescens* Ehrh.) is the most common deciduous tree species.

The forest stands are owned by Billerudkorsnäs AB. We identified 12 suitable forest stands, six per locality. We divided the stands into monoculture and diverse forest stands based on the pine proportion using the data provided by the forest owner. The monoculture stands were dominated by at least 80% Scots pine. In the diverse stands, Scots pine represented between 30% and 50% of the tree cover, with the other trees present in the mixture being spruce and birch. All stands were larger than 1 ha, had an average height between 2 and 4 m, and were between 6 and 15 years old.

There were five areas within the stands randomly selected based on a grid system (cells, 30 m × 30 m). Within the selected grid cell, a host tree was randomly selected, five for each stand, for a total of 60 host trees. The average height of the selected experimental trees was 3.22 ± 0.73 m, with a minimum tree height of 1.73 m and a maximum tree height of 5.70 m. The majority of the trees were approximately 3 m high.

Study species

For this study, we used the European sawfly, *N. sertifer*, a gregarious specialist herbivore of pine species (*Pinus* spp.). *N. sertifer* is a widespread species native to Eurasia that also occurs as an introduced species in North America (Olofsson, 1987). The species exhibits outbreak dynamics, which are sometimes widespread (Larsson and Tenow, 1984). From August to September, eggs are laid in a single batch on the needles of a current-year shoot, where they hibernate until May of the following year (Pschorn-Walcher, 1991). The larvae feed in colonies on the needles until the males and females molt into the fourth and fifth instars, respectively. After the final molting into the pre-pupa stage, they drop from the tree, disperse, and spin cocoons in the topsoil. The adults emerge from August to September. For the experiment, a total of 4,060 larvae were used, placing out one group consisting of 20–30 larvae on a suitable branch at ~1.5 m height on each host tree. To avoid early-instar mortality (associated with resin acids) and to ensure establishment of the group, we used second-instar larvae (Larsson et al., 1986). This is because the mortality due to bottom-up effects can be neglected after establishment of the larvae (Larsson et al., 2000). When the larvae reached the fourth instar, all groups were covered with a fine-meshed net to prevent dispersal. The groups were collected when all of the larvae had spun cocoons.

Predator associations and predation rates

The predator community of *N. sertifer* consists of both generalist and specialist natural enemies. Specialist predators are parasitoids active during the egg and larval stages (Olofsson, 1987). Generalist predators are primarily ants (*Formica* spp.) (Olofsson, 1987, 1992; Lindstedt et al., 2006) during the larval stage and small mammals during the cocoon stage (Holling, 1959; Hanski and Parviainen, 1985; Olofsson, 1987). Other than predation mortality, sawfly larvae may die from the nuclear polyhedrosis virus (NPV) (Olofsson, 1987). Larvae infected with this virus are easily identified, and none were observed during the study. Bird predation is rare in *N. sertifer* and is most likely related to low palatability as a result of resin sequestration by the larvae (Lindstedt et al., 2006). The sedentary nature of *N. sertifer* larval groups means that, once a

group is established, it remains on the same branch; disappeared larvae can be assumed to be lost to predation (Griffiths, 1959; Larsson et al., 1986; Björkman et al., 1997; Gur'yanova, 2006; Kaitaniemi et al., 2007; Kollberg et al., 2015; Bellone et al., 2020). The larvae were exposed in the field for 4 weeks in total. The groups were checked once a week, and when needed, larvae were added to maintain the group size at 20–30 individuals. The sum of all disappeared larvae is considered the predation rate. The presence or absence of ants was determined through repeated observations of ants on the experimental trees containing larvae. The ants found on the host trees comprised the species *Formica rufa*, *Formica polyctena*, and *Formica fusca*. The observations were repeated each week, and absence/presence was determined from the collated data of all observations.

Attack rates: plasticine models

When the sawfly larvae reached the fourth instar, we placed out 1,500 plasticine model larvae (dummy caterpillars). We designed the models to mimic the size and shape of typical insect larvae or caterpillars. Each plasticine model measured approximately 2 cm in length and 0.3–0.5 cm in diameter, consistent with those reported by earlier studies (reviewed by Lövei and Ferrante, 2017), with median lengths of 2.5 cm and diameters of 0.35 cm. The models were made by shaping plasticine clay (500 g, in medium green; Smeedi Plus, Viborg, Denmark) around a copper wire (Figures 1A, B), which was then used to secure them to the tree (Figures 1C, D). The grouped models were created in the same way, with the models organized in groups of five using the copper wire. Each host tree had 10 plasticine models placed together in a group, and the group was left out for a duration of 3 weeks (this was a compromise based on the limitations of the logistics of the fieldwork). We also placed out five plasticine models as solitaires at each selected host tree (five host trees in 12 stands). The models were placed individually on branches of the same whorl, all at approximately the same height in the tree at ~1.5 m. These solitary models were replaced with new ones every week for a duration of 3 weeks. The plasticine models were assessed for attack marks, and a model was scored as attacked or not based on the presence of a mark. If a model was attacked multiple times, this was counted as one attacked model. The use of plasticine models has



FIGURE 1
(A, B) Construction of the single (A) and grouped plasticine (B) models. (C) Placing of the single plasticine model in the pine tree. (D) Placement of the grouped plasticine model in the pine tree.

become a common method for obtaining an indication of predation rate (Birkhofer et al., 2017), as working with real prey can be notoriously difficult. Using this method, we could estimate predation rates for groups of models and solitary models.

Habitat variables

As previously described, the selected stands were divided into monoculture or mixed stands based on the proportion of pine present in the stand, providing the variable “stand diversity” with two levels: mixed and monoculture stands. We measured habitat heterogeneity around each host tree, with a radius of 5.64 m ($\approx 100 \text{ m}^2$). Tree density was defined as the total number of tree stems per square meter. Within stands, individual trees were divided into high-, medium-, or low-density pines, which were calculated based on the number of pines as a proportion of the total number of trees present. Understory richness is the count of different species of plants within the radius around the focal tree. Means and variance for each variable described above are depicted in Figures 2A–D.

Statistical analysis

All analyses were conducted using R (R Core Team, 2025). The specific packages used were glmmTMB (Brooks et al., 2017), car (Fox and Weisberg, 2010), piecewise SEM (Lefcheck and Freckleton, 2016), and performance (Lüdtke et al., 2021). For the plotting of data, the packages ggplot2 (Wickham and Chang, 2016) and ggeffects (Lüdtke, 2018) were used.

Additive and interactive effects on predation/attack rates

We used individual generalized linear models to quantify the additive and interactive effects of habitat variables or ant presence/absence and the predation/attack rates (in this model, including ant

presence/absence as a response variable) separately. The first model was run with a binomial error structure (link = logit), while the other models were run with either a Poisson error structure (link = log; glmmTMB) or a negative binomial error structure (link = log; nbinom1; glmmTMB). The models were evaluated using a Wald type II ANOVA test (Anova; car-package) (Fox and Weisberg, 2010). In these models, “tree” is the level of replication, and “stand identity” was included as a random effect. The habitat variables were used as response variables. As tree density (all trees per square meter) was positively correlated with the number of pines, the use of pine proportion divided into categories (i.e., low, medium, or high) avoided collinearity in the model. All models were checked for adherence to model assumptions.

Direct and indirect relationships affecting predation/attack rates

To assess the factors influencing attack and predation rates in different habitats, we used piecewise structural equation modeling (the piecewiseSEM package) (Lefcheck and Freckleton, 2016). This is a useful method when working with more complicated networks where multiple factors and effects are included, as it allows for the determination of all possible relationships simultaneously (Lefcheck and Freckleton, 2016). Piecewise SEM uses individual generalized linear models. These models are individually evaluated, which allows for a smaller sample size compared with the more “traditional” structural equation models. The models are evaluated individually (local evaluation) and simultaneously (global evaluation). This type of analysis is also called “confirmatory path analysis,” but has been referred to as “structural equation modeling” (Lefcheck and Freckleton, 2016). The initial structural equation model is represented in Figure 3. The individual response variables assessed in separate models included the number of predated larvae, the number of attacked single models, and the number of attack models in a group using a Poisson distribution. The presence/

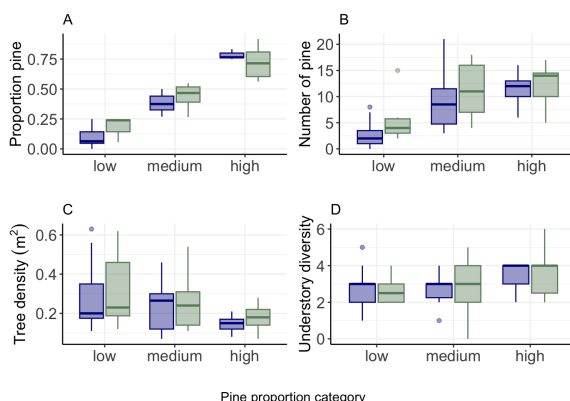
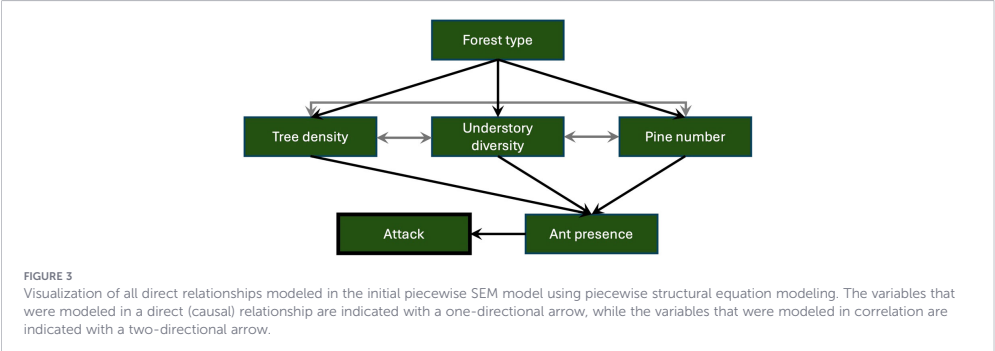


FIGURE 2
Visualization of the difference between the habitat variables in the mixed (dark blue) and monoculture (dark green) stands and grouped by the proportion of pine around the experimental tree. (A) Proportion of pine. (B) Number of pines. (C) Tree density per square meter. (D) Understory richness.



absence of ants was modeled using a binomial distribution (link = logit; logistic regression). Forest type is a categorical variable with two levels (i.e., monoculture and mixed stands), number of pines is the count of pines within the radius around the focal tree (link = log; modeled with a negative binomial distribution because of overdispersion), tree density is the number of trees (disregarding tree species) per square meter, and understory richness was modeled with a Poisson distribution due to the count of the understory plant species.

We used a test of directed separation and parameter significance to develop the final structural equation model, using a methodology analogous to model simplification to the minimum adequate model (Crawley, 2012, p. 391). We used generalized linear mixed models (glmmTMB; package: glmmTMB) with stand identity as a random variable. In addition, potential correlations between the habitat variables were modeled. Non-significant relationships were removed from the individual models, following which we assessed Fisher's *C* and its associated *p*-value, i.e., the test of directed separation. This test examines the assumption that all variables are conditionally independent, which means that there are no significant direct relationships left out of the model (Shipley, 2000, cited from Lefcheck and Freckleton, 2016). Fisher's *C* and its associated significance indicate the model's fit. The higher the value of Fisher's *C* and the further the *p*-value is removed from significance, the better the piecewise SEM fit of the data.

Results

Summary statistics

The variables relevant for the analyses are summarized in Table 1 and visualized in Figures 1A–D. We placed out 4,060 *N. sertifer* larvae, of which 1,845 were predated. We also placed out 900 single plasticine models, on which 75 had 108 marks. These could be divided into 40 bird marks and 35 arthropod marks. One group of 10 plasticine larvae (600 models) was placed out on all 60 trees. In total, we observed 121 marks on 62 models in the groups, and these could be divided into five bird marks and 55 arthropod marks. Each model with marks was counted as one observation, and multiple

marks on one model were disregarded in the analyses. The bird and arthropod data were pooled in the analyses due to the low individual sample sizes. In 27 of the 60 focal trees, ants were observed. The probability of observing ants in trees was slightly higher in mixed stands, where the number of pines around the focal tree was low (Table 2; Figure 4). The probability of ants did not differ between forest types or pine number (levels: high, medium, and low).

Additive and interactive effects on the predation/attack rates

Generalized linear mixed models with the number of predated larvae as the response variable showed a significant positive effect of the presence of ants on predation rates (model A in Table 3 and Figure 5A), but no significant effect of forest type or the proportion of pine trees (levels: high, medium, and low) around the focal tree. Neither attacks on single-placed plasticine models nor attacks on

TABLE 1 Summary statistics for the monoculture and diverse forest stands.

Variables	Monoculture Count	Diverse Count
No. of stands	6	6
No. of experimental trees	30	30
Ant presence	17	10
Larval predation	922	923
Single model attack	51	34
Group model attack	25	37
	Mean ± SE	Mean ± SE
Tree density (trees/m ²)	0.24 ± 0.02	0.24 ± 0.02
No. of pines	10.57 ± 0.67	6.23 ± 0.7
Categories of pine presence		
Low	5.67 ± 0.16	2.53 ± 0.08
Medium	11.15 ± 0.18	9.20 ± 0.19
High	12.55 ± 0.12	11.40 ± 0.12
Understory richness	3.06 ± 0.17	2.93 ± 0.12

TABLE 2 Results of the generalized linear mixed logistic regression for ant presence.

Ant presence/absence	χ^2	df	Pr(> χ^2)
Forest type	2.46	1	0.12
No. of pines	2.20	2	0.33
Random effects			
Groups	Name	Variance	SD
Stand identity (12)	(Intercept)	1.06	1.03
No. of observations: 60			

The model was run with a binomial error structure (glmmTMB) and was evaluated using a Wald type II ANOVA test (Anova; car-package) (Fox and Weisberg, 2010).

plasticine models in groups were significantly affected by any of the variables (Table 3, models B and C; Figures 5B, C).

Attack/predation rates and habitat heterogeneity

In the piecewise SEM for ant presence, direct effects were found between forest type and the number of pine trees (mixed = 1.752 ± 0.159 , mono = 2.380 ± 0.129 , $p = 0.002$), and between understory richness and ant presence (est $\pm$ SE = 0.73 ± 0.35 , std.est = 0.404 , $p = 0.036$; Fisher's $C = 6.453$, $p = 0.168$, $df = 4$). Correlations were found between the number of pine trees and the occurrence of ants ($r = -0.289$, $p = 0.013$), and the number of pine trees and tree density (trees per square meter; $r = 0.313$, $p = 0.008$) (Figure 6A). A higher number of pine trees in monoculture stands, combined with the negative correlation with ant presence, indirectly indicated a negative effect of monoculture stands on ant presence. The positive relationship between ant presence and predated larvae (0.28 ± 0.06 , std.est = 0.440 , $p < 0.001$) (Figure 6B) indirectly indicated a negative effect of monocultures on predation (Fisher's $C = 9.995$, $p = 0.441$, $df = 10$). This effect was absent in the single models (Fisher's $C = 17.812$, $p = 0.122$, $df = 12$) and the group models (Fisher's $C = 14.202$, $p = 0.288$, $df = 12$). Tree density had a direct effect on predated larvae (-0.48 ± 0.23 , std.est = -0.212 , $p = 0.039$) (Figure 6B), attacked single models (-2.07 ± 0.99 , std.est = -0.268 , $p = 0.037$) (Figure 6C), and attacked group models (2.72 ± 0.85 , std.est = 0.314 , $p = 0.001$) (Figure 6D). As tree density was positively correlated with the number of pines around the focal tree, and the number of pines was higher in monocultures, the models gave an indirect indication of a negative effect on single-model attacks and larval predation in monocultures but an indication of a positive effect of monocultures on group-model attacks. The full model results can be found in the Supplementary Material.

Discussion

In this study, we attempted to estimate the importance of habitat on the predation of a defended prey, *N. sertifer* larvae, and a gregarious and solitary undefended “prey” (the plasticine single and group models). The different prey types involved in the experiment differ in their defensive characteristics and in the

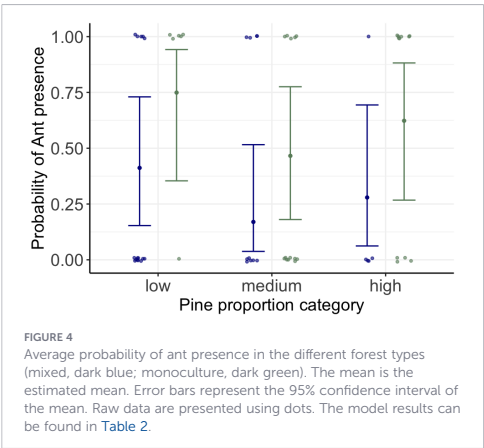


FIGURE 4 Average probability of ant presence in the different forest types (mixed, dark blue; monoculture, dark green). The mean is the estimated mean. Error bars represent the 95% confidence interval of the mean. Raw data are presented using dots. The model results can be found in Table 2.

olfactory and visual cues they provide to predators. The attack rates on solitary models were negatively affected by tree density, while the opposite was found for the group models. Only the gregarious defended larvae showed a direct relationship with the presence of ants, which does not appear to be a determinant of the attack rates on any of the plasticine models.

The glmm showed that forest type and the proportion of pine (divided into categories as shown in Figure 2A) did not affect predation/attack rates, individually (additive) or in interaction. In addition, in these models, only the larval predation rates were higher when ants were present. The piecewise SEM revealed the variables affecting ant presence, which showed a positive relationship with understory richness and a negative correlation with the number of pines around the focal trees. In the piecewise SEM, tree density was the main explanatory variable for the predation/attack rates, which were positively correlated with the number of pines around the focal trees. In turn, the number of pines was determined by forest type (i.e., higher in monoculture stands). The strength of using both approaches was that the different models provided insights into the relationship between tree diversity and predation, with the potential to elucidate both additive and interactive effects (glmm) and direct and indirect relationships (piecewise SEM).

Ants are important generalist predators in many (forest) habitats (Dejean et al., 2025). Studies have shown that ants do not respond to tree diversity in temperate forests (Greve et al., 2018). In our study, we found a slightly higher presence of ants in the monoculture stands; however, this difference was not significant. Kaitaniemi et al. (2007) found lower survival rates of *N. sertifer* in mixed stands due to the presence of ants. However, no difference was found in parasitism rates for the same species (Klapwijk and Bjorkman, 2018). More mobile and specialized predators may not benefit in the same way from habitat diversity as less mobile generalist predators do (Bellone et al., 2020). Thus, the type of predator will affect the way total predation rates are impacted.

Total recruitment over one generation will depend on mortality rates during different life stages. Small mammals are important predators during the cocoon stage of *N. sertifer*, and these predators

TABLE 3 Results of the generalized linear mixed-effects model for the number of predated larvae, the number of attacked single models, and the number of attacked models in a group.

χ^2	df	Pr(χ^2)		Est.	SE	
Predated larvae (model A)						
				(Intercept)	3.21	±0.12
Forest type	0.18	1	0.67			
Pine class	2.26	2	0.32			
Ant presence	7.75	1	<0.0001	ants present	0.26	±0.09
Random effects						
Groups	Name	Variance	SD			
Stand identity (12)	(Intercept)	0.01	0.10			
No. of observations: 60						
Dispersion parameter for nbinom1 family (): 2.08						
Attacked single models (model B)						
Forest type	0.24	1	0.62			
Pine class	0.15	2	0.93			
Ant presence	0.35	1	0.55			
Random effects						
Groups	Name	Variance	SD			
Stand identity (12)	(Intercept)	0.0002	0.015			
No. of observations: 60						
Attacked group models (model C)						
Forest type	0.41	1	0.52			
Pine class	0.28	2	0.87			
Ant presence	0.03	1	0.87			
Random effects						
Groups	Name	Variance	SD			
Stand identity (12)	(Intercept)	<0.001	<0.001			
No. of observations: 60						
Dispersion parameter for nbinom1 family (): 1.93						

Models A and C were fitted using a negative binomial error structure, and model B was run with a Poisson error structure (glmmTMB) and evaluated using a Wald type II ANOVA test (Anova; car-package) (Fox and Weisberg, 2010). Estimated values were calculated based on a treatment contrast, meaning that the (intercept) in the model is the estimate for a mixed forest stand. All other estimates were calculated based on their deviation from the (intercept).

have been found to be more active in habitats that provide more shelter (Kollberg et al., 2014; Bellone et al., 2017), often but not necessarily associated with higher tree diversity. Although the attack rates were affected by tree density, the attacks on model larvae were not found to be significantly related to the presence of ants.

The indirect relationships revealed by the structural equation model showed that, for the attack/predation on single models and the larvae, the more diverse stands are beneficiary; however, the monocultures appeared to benefit predation on group models. These differences can point to different predators attacking the groups in denser stands and the models and larvae in the less dense stands. For the *N. sertifer* larvae, the attacks were negatively related to tree densities, as were single models; however, grouped models were more often attacked at higher tree density (Figure 6). The models placed in a group showed primarily arthropod marks (55 arthropod and five bird marks), while single-placed models showed

approximately equal numbers of bird and arthropod marks (35 arthropod and 40 bird marks). Unfortunately, the attack rates were generally low and did not allow for statistical analyses that would compare patterns for birds and arthropods. However, plasticine models have been successfully used to assess both bird (Muiruri et al., 2016) and arthropod predation rates (e.g., Zvereva et al., 2020; Nordkvist et al., 2023). This result for *N. sertifer* larvae is surprising as, in a previous study, it was found that larvae in pine trees surrounded by denser vegetation incur higher mortality by generalist natural enemies (Bellone et al., 2020).

In several studies investigating the enemies hypothesis, ants were found to play an important role as generalist predators (Kaitaniemi et al., 2007; Moreira et al., 2012; Leles et al., 2017). In our study, the mortality of larvae was related to the presence of ants in the experimental tree. However, for the models (single and grouped) in the same tree, no significant effect was found. The relatively low number of arthropod marks on the single models could be an

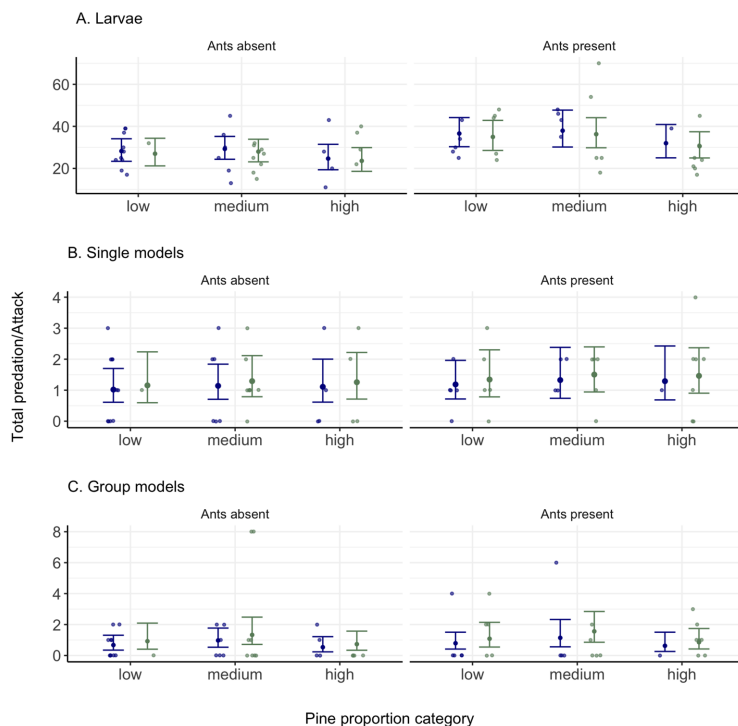


FIGURE 5

Average number of attacks per stand in each pine proportion category. The panels of the plots show the averages over pine categories around the focal tree: "low," "medium," and "high" when ants were observed to be present in the focal tree (right-hand panel) and when no ants were observed (left-hand panel). Dark blue bars represent the mixed stands, while dark green bars represent the monoculture stands. (A) Larval predation = predation rate of *Neodiprion sertifer* larvae. (B) Model attack = attack of single plasticine models. (C) Group attack = plasticine models placed in a group. The mean is the estimated mean. Error bars represent the 95% confidence interval of the mean. Raw data are presented using dots. The model results can be found in Table 3.

explanation for this finding, but the majority of the marks on the grouped models were from arthropods. One explanation could be that different predators attack the defended (*N. sertifer* larvae) and undefended (grouped models) prey. We do not have specific information on the type of arthropod marks on the models. Ferrante et al. (2014) found that the majority of the attacks on the models in their study came from carabid beetles, with only a small number coming from ants. Zvereva and Kozlov (2023) find that ants appear to investigate the artificial prey but do not attack or leave bite marks on the model. Taken together, these studies provide an explanation for the lack of a relationship between attack rates and ant presence, as plasticine models are avoided by ants and carabid beetles dominate the attacks. By showing different patterns in the piecewise SEM model, we believe that it is plausible that different predators have been responding to different types of prey.

Interference or other intraguild interactions will affect the performance of the generalist predator group. It has been found that the presence of ants in the predator guild reduces prey suppression, whereas the presence of different types of spiders

increases suppression additively (Sanders et al., 2011). Ants actively interact with other species in the predator community. Thus, although they are important generalist predators, overall, the presence of ants could flatten out the effect of tree diversity on predation. The effectiveness of predator guilds in suppressing prey depends on their functional complementarity. This means that, if the predator community increases in diversity with tree diversity, as shown by Stemmelen et al. (2022), the activity/prey suppression is expected to increase only if the diversity leads to complementarity rather than interference (Klapwijk, 2019). Key predators, such as ants, may be dominant in the habitat and negatively affect herbivore abundance through predatory or non-predatory effects. They may also affect the presence of other predators, such as spiders (Moreira et al., 2012). Staab and Schuldt (2020) concluded that several factors, including latitude, identity effects, intraguild predation, and the functional and phylogenetic components of biodiversity, may confound the predictions of the enemies hypothesis. In our study, an additional component is highlighted, namely, the community of predators associated with prey.

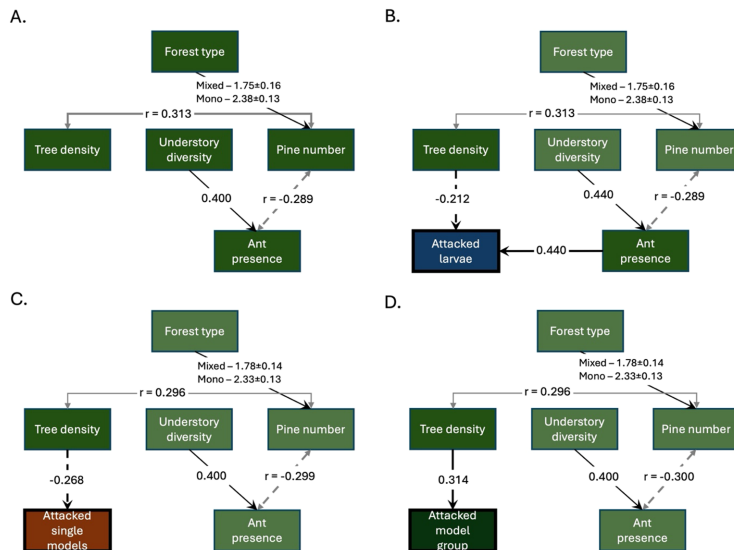


FIGURE 6

Graphical representation of the results for the path analyses of ant presence (A), the number of predated *Neodiprion sertifer* larvae (B), the number of attacked single models (C), and the number of attacked larvae in a group (D). In the model, standardized estimates are given for the continuous variables and estimates $\pm$ SE for the categorical variables. Only the significant causal relationships are represented in the model using black directional arrows. Significant co-varying relationships are represented by gray lines with bidirectional arrows. Positive variables are denoted by a solid line, while negative relationships are represented by a dashed line. The detailed model composition and results can be found in the [Supplementary Material](#).

Bird predation appears to be associated with increased diversity in a number of studies (Castagneyrol et al., 2017; Vázquez-González et al., 2024). However, other studies have found predation by birds to be unaffected (Leles et al., 2017) or decreased (Yang et al., 2018). Interestingly, Vázquez-González et al. (2024) found no relationship between tree diversity and arthropod predation, whereas both Leles et al. (2017) and Yang et al. (2018) found a positive relationship. All three studies used model larvae (dummy caterpillars) but were conducted in distinctly different forest habitats. This confirms that context is important when investigating the effects of tree diversity on predation rates. Prey traits are not often considered as part of the context, although it is known that the defense of many insect larvae could deter predation. Examples of defense include chemical defense, such as the ingestion and sequestration of plant toxins, and morphological and behavioral defense (Greeney et al., 2012). However, the studies directly investigating the effects of tree diversity on predation rates are few and skewed toward temperate or boreal insects (see the meta-analysis literature compiled by Stemmelen et al., 2022). No discernible differences in response based on prey traits could be extracted from these studies.

In our study system, bird predation was negligible for larvae of *N. sertifer* (but see Lindstedt et al., 2011), and known predators include ants, spiders, omnivorous true bugs, and parasitoids. However, bird predation was higher on the single model larvae compared with grouped model larvae. Small mammals, carabid beetles, and other ground-dwelling predators are important during

the cocoon stage, and eggs are subject to egg parasitoids and also, at high densities, bird predation. Thus, even in one herbivore species during its different life stages, different predators cause mortality, and not all of these predators will respond similarly to tree diversity, as shown in previous studies (Kaitaniemi et al., 2007; Klapwijk and Björkman, 2018; Bellone et al., 2020).

One obvious caveat of our study is that we used both real prey and artificial prey. Although the use of artificial prey is widely accepted as a good method to assess predation rates (Birkhofer et al., 2017), it has been found that patterns of predation are similar, but that the actual attack rates may differ (Ferrante et al., 2014; Lövei and Ferrante, 2017). We recommend exploring the relationships subject to this study using different types of real, living prey. However, we do acknowledge that, in the majority of natural systems, this is complicated and labor-intensive. In our study, we did not compare predation pressures directly, but compared the patterns in predation. By doing so, we avoided any differences in the proportion of predation/attack rates for artificial and real prey. By counting only the attacks and not the number of marks on the artificial prey, we avoided an overestimation of the attacks, as real prey can only be successfully attacked once. However, by replacing the removed sawfly larvae and not counting the weekly attacks on the grouped artificial prey or replacing the attacked grouped artificial prey, we might have positively biased the effect of the presence of ants on the sawfly larvae compared with the artificial larvae.

As a last consideration, our experimental sites comprise young pine semi-natural forests prior to pre-commercial thinning, with

the average height of the pine trees varying from 2 to 4 m. The interactions between natural enemies and their prey are affected not only by tree diversity but also by the heterogeneity in the forest stands (Wrethling et al., 2026). Some of the biotic heterogeneity, such as understory composition, changes with the maturation of the forest stand (Barsotti et al., 2026). One might expect understory vegetation to have a smaller effect on predation rates in mature stands compared with younger stands, and for parasitism rates to become more important. Little is known about predation of herbivorous larvae in the canopy of mature stands. However, it has been found that predatory arthropods are less abundant in the canopy than in the understory (Aikens et al., 2013; Sivault et al., 2024) and that ant exclusion does not affect herbivore damage, but that vertebrate predator exclusion does result in higher arthropod herbivore damage (Sivault et al., 2024). Therefore, the predation mortality in the canopy of mature trees may be driven primarily by insectivorous birds (Zvereva et al., 2020). For our study, this could mean higher survival rates in the canopy for *N. sertifer* larvae, as arthropod predators are thought to play a larger role in larval mortality compared with insectivorous birds (Šmits et al., 1996).

Conclusion

Our study indicates that there is a possibility for “prey”-specific response of generalist predators. As not all predators respond similarly to factors of habitat heterogeneity in their environment, different predator assemblages could result in different responses to tree diversity and other associated habitat heterogeneity (Jakel and Roth, 2004; Jouveau et al., 2019). Thus far, it appears that investigations into the link between predator community diversity and predation rates have focused on predator traits (Jonsson et al., 2017) and that prey traits as drivers of community assembly and predation rates have remained underemphasized (but see Tylanakis and Romo, 2010). We believe that our results indicate that there may be different underlying mechanisms for the attack rates in the undefended plasticine models and the defended larvae. Another crucial aspect to acknowledge is that arthropod pests will have different predators that are important throughout their life cycle. To understand the mechanisms underlying the relationship between predator abundance and diversity, predation pressure, and population regulation, we need to focus on the predator community associated with herbivores throughout their life cycles, particularly in perennial systems, such as forest ecosystems.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The manuscript presents research on animals that does not require ethical approval.

Author contributions

MK: Writing – original draft, Conceptualization, Funding acquisition, Writing – review & editing, Formal analysis, Supervision. FW: Investigation, Methodology, Writing – review & editing. CB: Funding acquisition, Supervision, Writing – review & editing.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This study was supported by the holistic management of emerging forest pests and diseases (HOMED) project, which received funding from the European Union’s Horizon 2020 Research and Innovation Program under grant 771271.

Acknowledgments

The authors thank the reviewers for their comments on the manuscript. The field assistance provided by Emilio Otero Johansson was greatly appreciated. The authors thank the forest company BillerudKorsnäs, which provided information on the forest stands.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2026.1794147/full#supplementary-material>

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Supplementary material for:
Predation rates on real and artificial prey in the context of tree diversity

Maartje J. Klapwijk, Fredrika Wrethling, Christer Björkman

Frontiers in Ecology and Evolution

Online appendix 1

The individual models were linear mixed models (glmmTMB; glmmTMB package). In each model the random variable stand is included. In addition, an optimisation function was included; control=glmmTMBControl(optimizer=optim, optArgs=list(method="BFGS")) to overcome problems with convergence.

Table S1 - Output of the structural equation model for ant presence

Call:

	Response		Explanatory
Direct	Ant presence/absence	~	Understory diversity
	Ant presence/absence	~	Understory diversity
	Number of Pine	~	Forest type
	Tree density	~	Forest type
	Understory density	~	Forest type
Covariance	Number of Pine	~~	Tree density
	Understory diversity	~~	Tree density
	Number of Pine	~~	Understory diversity
	Number of Pine	~~	Ant presence/absence

AIC

586.79

Tests of directed separation

Independence Claim	Test type	DF	Crit.Value	P.Value
Ant presence/absence ~ Forest type + ...	anova	1	1.997	0.158
Ant presence/absence ~ Tree density + ...	coef	60	-1.146	0.252

Global goodness-of-fit:

Chi-Squared = 3.495 with P-value = 0.174 and on 2 degrees of freedom

Fisher's C = 6.453 with P-value = 0.168 and on 4 degrees of freedom

Coefficients:

	Response	Predictor	Estimate	Std.Error	DF	Crit.Value	P.Value	Std.Estimate
Direct	Ant presence	Understory diversity	0.737	0.352	60	2.091	0.036	0.400
	Number of Pine	Forest type			1	9.983	0.002	-
		mixed	1.752	0.159				-
		monoculture	2.380	0.129				-
	Tree density	Forest type			1	0.004	0.948	-
	Understory diversity	Forest type			1	0.089	0.766	-
Covariance	Number of Pine	Tree density	0.313		60	2.491	0.008	0.313
	Understory diversity	Tree density			60	-0.002	0.499	
	Number of Pine	Understory diversity			60	0.814	0.209	
	Number of Pine	Ant presence	-0.289		60	-2.278	0.013	-0.289

Individual R-squared:

Response	method	Marginal	Conditional
Ant presence	none	0.14	0.34
Number of Pine	none	0.19	0.25
Tree density	none	0.00	0.36
Understory diversity	none	0.00	0.00

Table S2 - Structural Equation Model of *N. sertifer* larvae

Call:

	Response	Explanatory
Direct	Attack Larvae	Ant presence + Tree density
	Ant presence/absence ~	Understory diversity
	Number of Pine ~	Forest type
	Tree density ~	Forest type
	Understory diversity ~	Forest type
Covariance	Number of Pine ~~	Tree density
	Understory diversity ~~	Tree density
	Number of Pine ~~	Understory diversity
	Number of Pine ~~	Ant presence/absence

AIC

1088.29

Tests of directed separation:

Independence Claim	Test Type	DF	Crit.Value	P-Value
ants.no ~ type + ...	anova	1	2.00	0.16
total_loss ~ type + ...	anova	1	0.36	0.55
ants.no ~ tree_density + ...	coef	60	-1.15	0.25
total_loss ~ total_div + ...	coef	60	0.06	0.95
total_loss ~ nr_pine + ...	coef	60	0.98	0.33

Global goodness-of-fit:

Chi-Squared = 4.84 with P-value = 0.436 and on 5 degrees of freedom

Fisher's C = 9.999 with P-value = 0.441 and on 10 degrees of freedom

Coefficients:

	Response	Predictor	Estimate	Std.Error	DF	Crit.Value	P.Value	Std.Estimate
Direct	Larvae attack	Ant presence	0.277	0.055	60	5.067	0.000	0.440
	Larvae attack	Tree density	-0.476	0.230	60	-2.066	0.039	-0.212
	Ant presence	Understory diversity	0.737	0.352	60	2.091	0.037	0.400
	nr_pine	Forest type			1	9.983	0.002	
		mixed	1.752	0.160				
		mono	2.380	0.129				
	Tree density	Forest type			1	0.004	0.948	
	Understory diversity	Forest type			1	0.089	0.766	
Covariance	Number of Pine	Tree density	0.313		60	2.491	0.008	0.313
	Understory diversity	Tree density			60	-0.002	0.499	
	Number of Pine	Understory diversity	0.107		60	0.814	0.210	
	Ant presence	Number of Pine	-0.289		60	-2.278	0.013	-0.289

Individual R-squared:

Response	method	Marginal	Conditional
Larvae attack	none	0.29	0.65
Ant presence	none	0.14	0.34
Number of Pine	none	0.19	0.25
Tree density	none	0.00	0.36
Understory diversity	none	0.00	0.00

Table S3 - Structural Equation Model of single model larvae

Call:

	Response		Explanatory
Direct	Single models		Tree density
	Ant presence/absence	~	Understory diversity
	Number of Pine	~	Forest type
	Tree density	~	Forest type
	Understory density	~	Forest type
Covariance	Number of Pine	~~	Tree density
	Understory diversity	~~	Tree density
	Number of Pine	~~	Understory diversity
	Number of Pine	~~	Ant presence/absence

AIC

816.34

Tests of directed separation:

Independence Claim	Test Type	DF	Crit.Value	P-Value
Single models ~ Forest type + ...	anova	1	0.64	0.42
Ant presence ~ Forest type + ...	anova	1	1.99	0.16
Ant presence ~ Tree density + ...	coef	60	-1.15	0.25
Single models ~ Understory diversity + ...	coef	60	-1.58	0.11
Single models ~ Number of Pine + ...	coef	60	-0.81	0.42
Ant presence ~ Single models	coef	60	1.37	0.17

Global goodness-of-fit:

Chi-Squared = 8.7 with P-value = 0.191 and on 6 degrees of freedom

Fisher's C = 17.812 with P-value = 0.122 and on 12 degrees of freedom

Coefficients:

	Response	Predictor	Estimate	Std.Error	DF	Crit.Value	P-Value	Std.Estimate
Direct	Single models	Tree density	-2.073	0.9994	60	-2.074	0.038	-0.268
	Ant presence	Understory diversity	0.737	0.3524	60	2.091	0.037	0.400
	Number of Pine	Forest type			1	8.247	0.004	
		mixed	1.7758	0.1415				
		mono	2.3296	0.1317				
	Tree density	Forest Type			1	0.004	0.948	
Covariance	Understory diversity	Forest type			1	0.089	0.766	
	Number of Pine	Tree density	0.296	-	60	2.339	0.011	0.296
	Understory diversity	Tree density	-0.0002	-	60	-0.002	0.499	
	Number of Pine	Understory diversity	0.088	-	60	0.667	0.254	
	Ant presence	Number of Pine	-0.299	-	60	-2.372	0.011	-0.299

Individual R-squared:

Response	method	Marginal	Conditional
Single models	none	0.13	0.13
Ant presence	none	0.14	0.34
Number of Pine	none	0.28	0.59
Tree density	none	0.00	0.36
Understory diversity	none	0.00	0.00

Table S4 - Structural Equation Model of grouped model larvae

Call:

	Response	Explanatory
Direct	Grouped models	Tree density
	Ant presence/absence ~	Understory diversity
	Number of Pine ~	Forest type
	Tree density ~	Forest type
	Understory diversity ~	Forest type
Covariance	Number of Pine ~~	Tree density
	Understory diversity ~~	Tree density
	Number of Pine ~~	Understory diversity
	Number of Pine ~~	Ant presence/absence

AIC

844.19

Tests of directed separation:

Independence Claim	Test Type	DF	Crit.Value	P - Value
Grouped models ~ Forest type + ...	anova	1	2.56	0.11
Ant presence ~ Forest type + ...	anova	1	2.00	0.16
Ant presence ~ Tree density + ...	coef	60	-1.15	0.25
Grouped models ~ Understory diversity + ...	coef	60	-0.53	0.60
Grouped models ~ Number of Pine + ...	coef	60	0.65	0.51
Ant presence ~ Grouped models + ...	coef	60	0.50	0.62

Global goodness-of-fit:

Chi-Squared = 6.539 with P-value = 0.366 and on 6 degrees of freedom

Fisher's C = 14.202 with P-value = 0.288 and on 12 degrees of freedom

Coefficients:

	Response	Predictor	Estimate	Std.Error	DF	Crit.Value	P-Value	Std.Estimate
Direct	Grouped models	Tree density	2.719	0.850	60	3.200	0.001	0.314
	Ant presence	Understory diversity	0.737	0.352	60	2.091	0.037	0.400
	Number of Pine	Forest type	-	-	1	8.247	0.004	
		mixed	1.776	0.142				
		mono	2.330	0.132				
	Tree density	Forest type	-	-	1	0.004	0.948	
	Understory diversity	Forest type	-	-	1	0.089	0.766	
Covariance	Number of Pine	Tree density	0.296	-	60	2.340	0.011	0.296
	Understory diversity	Tree density	-	-	60	-0.002	0.499	
	Number of Pine	Understory diversity	-	-	60	0.667	0.254	
	Ant presence	Number of Pine	-0.300	-	60	-2.372	0.011	-0.300

Individual R-squared:

Response	method	Marginal	Conditional
Grouped models	none	0.17	0.23
Ant presence	none	0.14	0.34
Number of Pine	none	0.28	0.59
Tree density	none	0.00	0.36
Understory diversity	none	0.00	0.00

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DOCTORAL THESIS NO. 2026:48

Mixed forests are thought to be less susceptible to biotic disturbance by favouring natural enemies and top-down control on insect herbivores. This thesis examines how tree species composition affects predation pressure, arthropod communities, and soil microbiomes. Soil microbiomes responded directly to tree species composition, with effects varying between functional groups and spatial scales. Mixed forests showed no higher arthropod abundance, richness, or top-down control than pure pine forests. Instead, local habitat heterogeneity increased predation pressure, independent of stand type.

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ISSN 1652-6880

ISBN (print version) 978-91-8124-265-2

ISBN (electronic version) 978-91-8124-295-9