

The influence of tree species on ant communities: A comparison of fast-growing broadleaf forests and oak forests

Investigating the species composition of ants within different tree species stands in Scania (Skåne)

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Beer Jan Adriaan Verburg of Glencoe

Swedish University of Agricultural Sciences Faculty of Forest Science Southern Swedish Forest Research Centre Alnarp 2025 "I'd rather be a forest than a street"

- Simon & Garfunkel

Abstract

Forestry is an important part of the Swedish economy, and fast-growing broadleaf (FGB) plantations are likely to increase their contribution due to the increasing interest in bioenergy. However, these plantations might not be as valuable for natural assets like biodiversity and support fewer forest specialist species. Ants play major roles in ecosystems and can serve as bioindicators. In this study, I investigate differences in ant community makeup between FGB (*Betula pendula, Populus tremula* \times *P. tremuloides* and *Populus* sp.) stands and oak (*Quercus robur*) stands with longer rotation in Scania. I expect more forest specialist (forest guild) species in oak stands compared to FGB stands.

My results showed (A) no difference between the total number of forest specialist species found and whether a stand was an oak stand or an FGB stand. However, using abundance data from vacuum and bait samples, I found (B) significantly different ant communities in oak stands compared to FGB stands. I also found (C) that a larger dominance of forest specialist species at each sample was seen in oak stands compared to FGB stands, and that the proportion of forest specialised species between oak and FGB stands not homogenous is.

Secondly, I tested whether results from vacuum sampling were different from bait sampling. The only significant difference was that the vacuum sampling process took considerably more time to carry out.

I advise that further research replace vacuum sampling with pitfall traps or more extensive free catch searches. My conclusions partly substantiate the consensus that *Quercus* is a highly valuable genus, with *Quercus* forests providing the most species-rich forest type. And I affirm the value of *Quercus robur* by finding a stronger forest specialist species presence than in FGB forest stands. It would, nevertheless, be worthwhile to broaden this research by including multiple stands across Sweden, natural reserves, and coniferous stands. This is to provide a comprehensive overview of the impacts on ant communities.

Keywords: Ant communities, Fast growing broadleaves, Oak (*Quercus robur*) forests, Temperate forests, Ant diversity, Biodiversity, Formicidae, Sweden, Scania.

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Abbreviations

Abbreviation	Description
SLU	Swedish University of Agricultural Sciences
SCB	Statistiska centralbyrån (Swedish agency for statistics)
FGB	Fast-growing broadleaves/broadleaf
WUR	Wageningen University and Research
EU	European union
GHG	Greenhouse gas(es)

1. Introduction

1.1 Swedish forests

Forestry is an important part of the Swedish economy. The forest industry makes up a considerable part of the exported goods of Sweden. The SCB (Swedish agency for statistics) writes that, in the first half of 2021, the export of forest industry products represents 10.2% of the total export value for that same period (SCB, 2021).

Sweden has diverse geographical and meteorological conditions due to its size and shape and diverse topography over its large latitudinal extent. Thus, different growing conditions occur throughout the country. Nevertheless, two main tree species remain dominant in both northern Sweden as southern Sweden. In northern Sweden (Norrland) there are mainly Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) H. Karst.) plantations, with the dominant choice being Scots pine (Nilsson et al., 2020). While in southern Sweden (Götaland), Norway spruce (*P. abies*) is the dominant species for plantations, surpassing Scots pine in standing volume and regeneration species choice (Nilsson et al., 2020), ("Skogsstyrelsens," 2024). Furthermore, 24% of the standing volume in Götaland consists of broadleaf species, of which 11% is birch (*Betula* spp.) (Nilsson et al., 2020), compared to only about 16% broadleaves in Norrland, most of which are birch, which have not intentionally been planted but establish naturally within other tree species' plantation (*Betula* spp.) (Nilsson et al., 2020).

Much of southern Sweden and almost all of Scania (Skåne) has historically been rich in deciduous forest with *Tilia, Quercus,* and *Alnus* interspersed with *Betula* and *Pinus* (2000 – 1500 years before present day). Later, beech (*Fagus sylvatica* L.) forests became more prevalent (1000 – 500 before the present day) (Björse & Bradshaw, 1998). However, this was drastically changed by both natural and cultural influences (Björse & Bradshaw, 1998). In the present day, forest cover consists mainly of Scots pine (*P. sylvestris*) and Norway spruce (*P. abies*) dominated plantations (Björse & Bradshaw, 1998).

Not only are some natural forests gone and others replaced by plantations, but climate change model (dynamic vegetation model (LPJ-GUESS)) estimates the possibility that within 300 years we will experience a drastic decrease in Norway spruce (*P. abies*) in southern Sweden, and a shift towards a temperate climate favouring broadleaf trees like oak (*Quercus* spp.) and beech (*F. sylvatica*) (Hickler et al., 2011). These changes are projected to be mainly driven by a warmer climate that could result in longer growing seasons and milder winters (Hickler et al., 2011).

1.2 The potential problem

1.2.1 Fast-growing broadleaves for climate change

Climate change is commonly understood as a (mostly) human-induced event, with human-caused emissions of greenhouse gases (GHG) being the main contributor (Calvin et al., 2023). Climate change has major negative implications for both nature, biodiversity, and society (Calvin et al., 2023). Pongsiri and Roman (2007) note that shifts in biodiversity may impact the ability of natural systems "to provide clean water, energy, food, recreation, and other services that contribute to human well-being". In addition, Pongsiri and Roman (2007) note the changes in biodiversity possibly impacting the transmission of vector-borne diseases. Kar et al. (2022) discuss the predation of ants on tick eggs; thus, by shifting biodiversity composition (i.e. changing the diversity of ant communities), pest species might lose natural predators, and their numbers could increase, resulting in transmissions of vector-borne diseases increasing.

The European Union (EU) has set up multiple goals concerning the mitigation of the release of GHG, which is crucial to combat climate change. In their energy policy they describe an aim to achieve a 45% share of energy to be produced by sustainable sources by 2030. Defining sustainable sources as "solar power, wind, ocean and hydropower, biomass and biofuels." (Energy Policy: General Principles | Fact Sheets on the European Union | European Parliament, 2024). Additionally, Sweden aims to decrease its emissions in the Effort Sharing Regulation (ESR) sectors by 63% by 2030 compared to 1990 (Ministry of the Environment, 2020). These ambitious aspirations are supported by more concrete actions, promising "verified emission reductions through investment in [...] bioenergy with carbon capture and storage." They also emphasise that "In the future, the demand for bioenergy is expecting to continue to be important so that the emission targets can be met." (Ministry of the Environment, 2020). To mitigate the effects and further escalation of anthropocentrically induced climate change, fast-growing broadleaves (FGB) might be part of an effective measure. Thus, we can expect a – national and international – increase in demand for forest-based bioenergy (Bouget et al., 2012).

Fast-growing broadleaves (FGB) are a category of latifolious tree species, consisting of a multitude of deciduous tree species, with different definitions not always including the same species (Garfield & Brukas, 2024; Hjelm & Rytter, 2018). Delineation is difficult due to the ambiguous understanding of what FGB are exactly. In short, they are a group of species that grow faster than the average species. But how much faster growth makes a species an FGB is unclear. This growth is also dependent on the site conditions (Ekö et al., 2008) and genetic material (Resende et al., 2012). Klasnja et al. (2008) note that poplars

(*Populus* spp.), willows (*Salix* spp.), and black locusts (*Robinia pseudoacacia* L.) are FGB, while Garfield and Brukas (2024) mention birch (*Betula* spp.) and poplar (*Populus* spp.) species. Other species like elm (*Ulmus* spp.), ash-leaved maple (*Acer negundo* L.) (Tsaralunga et al., 2021), and Hybrid aspen (*Populus tremula* L. × *Populus tremuloides* Michx.) are all noted, as is, again, poplar (*Populus* sp.). These are all considered FGB by different scientific literature (Hjelm & Rytter, 2018).

In this paper, I will mainly consider birch (*B. pendula*), hybrid aspen (*P. tremula* \times *P. tremuloides*), and poplar (*Populus* sp.) when talking about FGB. As these species grow on the investigated stands – stands previously considered FGB stands (Andersson, Petersson, & Holmström, 2024; Oestereich, 2024).

Fast-growing broadleaves have relatively short rotation ages for Swedish forestry, typically between 20-50 years (Birch [*Betula pendula* and *B. pubescens*] [Valkonen & Valsta, 2001], hybrid aspen [*P. tremula* × *P. tremuloides*] [Tullus et al., 2011], and poplar [*Populus* spp.] [Stener & Westin, 2017]). These tree species can accumulate biomass quickly over this period. While mean annual increment (MAI) is highly dependent on the site conditions (Ekö et al., 2008), an estimation for poplar (*Populus* spp.) and hybrid aspen is given by Stener and Westin (2017), finding a "mean annual increment (MAI) of up to 25 m³ of stem wood ha⁻¹ and year⁻¹". These traits make them suitable for the production of bioenergy. Tullus et al. (2011) specifically noting the suitability of hybrid aspen for bioenergy production. Therefore, in southernmost Sweden, Scania, we can expect an increase in the planting of FGB.

Hybrid aspen might be as profitable as Norway spruce (*P. abies*), or even more profitable, when adaptive management strategies aiming to strengthen resistance and resilience to climate change are taken into account (Subramanian et al., 2015). Xu and Mola-Yudego (2020) find an increase in poplar (*Populus* sp.) and hybrid aspen (*P. tremula* \times *P. tremuloides*) plantations in Sweden. However, this is offset by a decrease in Willow (*Salix* sp.) plantations, which Xu & Mola-Yudego (2020) explain by changes in policy frameworks after 1996 and an increase in cereal prices after 2007. This correlates with an expected increase in interest in bioenergy, where Hybrid aspen (*P. tremula* \times *P. tremula* \times *P. tremuloides*) plantation numbers can be expected to keep rising, as they are very suitable for bioenergy (Tullus et al., 2011).

Furthermore, in addition to FGB, native slow-growing broadleaves remain important for the forest industry. "In the future, [...] wood products can also replace fossil-based materials in construction, [...]" writes the Swedish Ministry of Environment (Ministry of the Environment, 2020). Oak (*Quercus* spp.) species are one of the wood products that could contribute to this goal. Both for quality timber and other natural resources, as for carbon sequestration.

1.2.2 The dilemma with fast-growing broadleaves

Some forests hold more natural values compared to others. A shift towards fastgrowing broadleaf plantations on agricultural sites will appear to bring more forest to the landscape, but in effect, this might mean little in terms of increasing habitats for animal communities.

Fast-growing broadleaf forest plantations are different from conventional broadleaf (plantation) forests. Within these FGB stands, much more disturbance is experienced due to a shorter rotation age, which often has severe impacts on biodiversity (Bouget et al., 2012). Additionally, non-native species like hybrid aspen might have undesired effects of which we are unaware (Castro-Díez et al., 2019). Castro-Díez et al. (2019) note the complexity of the synergies and trade-offs that non-native tree species bring. Hybrid aspen is mainly planted on forest land or old agricultural lands, with the highest production on fertile sites (Tullus et al., 2011). An increase in the planting of *Populus* species may increase or decrease biodiversity, depending on the site specifics (Tullus et al., 2011).

Alternatively, there are other tree species with longer rotation ages, that can provide sustainable resources and sequestrate carbon. Oak (*Quercus* spp.), with a rotation age often between 100 and 150 years (Carbonnier, 1975, as cited in Drössler et al., 2012; *Forest Management - Harvesting*, 2025) is known for high quality timber. Additionally, oak (*Quercus* spp.) stands might provide more niches for forest specialist species than FGB trees. According to Jonsell et al. (1998), in Sweden the genus *Quercus* is the most other species supporting tree genus, supporting 202 species, containing 26% of all the red listed insects and 37% of all red listed saproxylic species.

Invertebrate biodiversity shifts (changes in community composition and evenness) due to differing forest management (Niemela, 1997). Niemela (1997) claims that clearcut forestry may lead to a higher local diversity (α -diversity) because forest generalists remain, and numerous open-land species can also settle into this system. This at the cost of losing forest specialists, resulting in a decline in diversity at the landscape level. Ants, as one dominant forest invertebrate group, might undergo this noted effect (Niemela, 1997).

Ants serve many ecosystem functions within forests and are considered indicators of a healthy natural environment. Andersen et al. (2002) discusses the benefits of invertebrate inventories as human induced land-change indicators. They investigate a simplified ant inventory aimed to give a bioindication to landmanagers, covering almost all the important findings that a more intense survey would reap. Ants are understood as ecosystem engineers (Rocha et al., 2024; Sanders & Van Veen, 2011) and may influence many other arthropods directly or indirectly. Sanders and Van Veen (2011) found that ant species have major effects on grassland food webs. Other ant species (*Formica rufa* group) are considered keystone species in boreal forests by Finér et al. (2012) due to the nutrients in and around ant nests. Specifically, ant nests of *Formica sensu stricto* are found to have higher available phosphorus levels for plants than the surrounding area (Frouz et al., 2005). In a meta-study by Farji-Brener and Werenkraut (2017), they found that ant nests have a large role in influencing soil fertility and flora structure. Additionally, they note the key role ants play in soil disturbance. Green islands (of living birches) around ant nests in damaged birch stands caused by ant predation (Niemelä & Laine, 1986) are another example of the substantial impact ants have on a natural system.

In short, ant communities give a good indication of the natural system and the changes occurring. They play a major role in ecosystems, food webs, and specific species are in some systems keystone species and ecosystem engineers.

1.3 Objectives

As seen above, forestry is an important aspect of the Swedish economy and will likely play a role in the upcoming actions to limit climate change. Interest in FGB may very well increase, however, oak remains an alternative as it is native and can be used for high quality timber, additionally it is known to have high biodiversity values. To ascertain the trade-offs it is evidently important to determine the differences in ant species richness between oak-dominated stands and FGB-dominated forestry stands.

I will investigate 6 small forest stands in Scania: 3 forest stands representing FGB stands, of which;

- 1 silver birch (B. pendula; hereafter referred to as "birch") stand,

- 1 poplar (Populus sp.; hereafter referred to as "poplar") stand and,

- 1 hybrid aspen (*P. tremula* × *P. tremuloides*; hereafter referred to as "hybrid aspen") stand.

And 3 pedunculate oak (Q. robur; hereafter referred to as "oak") stands.

Additionally, I will evaluate different inventory methods to provide recommendations for future ant inventories, as these will remain important given the growing interest in FGB and biodiversity conservation.

Due to time constraints, 3 species were chosen to represent FGB, aiming to represent the variety possible within this category. Consequently, my study design compared oak to FGB, thus, oak should also be represented by 3 stands.

I expect that in stands with native longer rotation tree species like oak, I find more niche forest ant species (forest guild) as compared to shorter rotation stands with FGB, where instead open-land species (open (land) guild) are expected to be more prevalent. One reason is that *Quercus* is the most species-rich genus in Sweden (Jonsell et al., 1998). Secondly, Grevé et al. (2018) note how stands with oak (or pine) show a higher abundance and species richness. Just as Seifert (2017) notes, oak being the most species-rich supporting forest type. However, no comparison is made between the FGB stands in my study.

1.4 Scope and delimitations

My study focuses on the effects of forestry stands – with different broadleaf tree species – on ant communities. Even though it aims to understand a broader pattern, it is limited to six production forest stands in Scania, Sweden. The stands are located close to each other and represent only a limited area within Scania. Of those, 3 are oak stands and 3 are FGB stands. Furthermore, ant species were categorised into 3 guilds. The generalist guild species are left out of intra-guild analyses, as defining these can be difficult. Delineation of what is considered a forest, or an open land specialised species is rather difficult. By adding a third category of generalist species I circumvent this difficulty; now certain species' position that would have been uncertain (whether they would be a forest or open land species) in a two-category system are considered generalist.

My study is limited to ants collected via 4 different sampling methods; bait, vacuum, centre collection and free collection (explained further in the methodology). Additionally, data was only collected from April till July of the years 2024 and 2025, which does not fully represent seasonal variation. My study only considered morphological traits for ant identification.

2. Methodology and methods

2.1 Location

Six stands were investigated, one stand for each FGB species and 3 stands for oak. The stands are found south-east, just outside of, Malmö, following the E65 out from Malmö for approximately 5 - 10km (Specific coordinates for each investigated stand can be found in table 2, in Appendix 1.).

In figure 2 these stands are labelled by their Latin binomials. Noting behind the *Q*. *robur* whether these are stand numbers 1, 2 or 3.



Figure 1. The spatial relation of the investigated forest stands The reference map on the right indicates the general location of study sites in southern Sweden.

The birch stand was approximately 0.5 hectares, and the birch trees were around 31 years old. With former agricultural land use (Andersson, n.d. *unpublished*). The hybrid aspen stand was approximately 3.4 hectares, and the hybrid aspen trees were around 28 years old. With former agricultural land use (Andersson, n.d. *unpublished*). The poplar stand was approximately 5.1 hectares, and the poplar trees were around 15 years old. With former forest land use (Andersson, n.d. *unpublished*). The oak trees in oak stands 1, 2 and 3 were around 30 years old. With former agricultural land use (Brunet, 2007). Oak 1 is approximately 4 hectares, oak 2 is 5.5 and oak 3 approximately 14 hectares.

2.2 Sample collection

The FGB samples were collected by Emil Andersson as part of his research (Andersson, n.d., *unpublished*) while the 3 oak stand samples were collected jointly me and my supervisor, Emil Andersson.

2.2.1 Method of collection

Transect lines were laid crossing the stands, from corner to corner. In situations without clear corners, the transect lines were laid so that they covered both the edge and the centre of the stand. Ten points were put onto each line, from beginning to end, equally spaced. One transect line was assigned to the bait samples, the other transect line to the vacuum samples (below follows a clear description of the different sampling methods). Where the lines crossed, the 20-minute centre search was performed. In case of the oak, a 10-minute search by two persons, this is susceptible to inter-observer bias, partly due to different skill levels in ant inventory. For semi-randomisation, a stick was thrown backwards over the shoulder along the transect line from the predetermined sample point to have an exact, semi-random sample location. Free collection was performed throughout the entire stand with no clear temporal or spatial boundaries (except being within the designated stand).

Vacuum collection

To collect the vacuum samples -10 samples per stand (only 5 in the birch stand due to its limiting size) – an inverted leaf blower was utilized. A sock was inserted and kept in place to collect the leaf litter and dirt. The leaf blower was turned on for approximately 10 seconds on one side of the sampler, then another 10 seconds on the direct opposite side of the sampler.

Bait collection

To collect the baits – 10 samples per stand – three different food types were placed on a square green paper (approximately 15 by 15 cm) mounted onto the ground. Namely, fine peanut butter, liquid honey (from a squeeze bottle) and (canned) tuna. Approximately one tablespoon's worth of each. The baits were left for three or more hours. The ants collected were killed and stored using an alcohol solution of approximately 70–90% ethanol.

20- minute centre search

Within each stand, 20 minutes were spent searching for ants in a circle (with a radius of approximately 10 meters). If the sampling was done by 2 persons, only 10 minutes of searching was performed. The search included but was not limited to lifting logs and stones, breaking twigs, searching trees, removing earth mounds, mossy patches and grass mounds. It can be challenging to compare these findings across stands, since these are more qualitative indications of the ant communities and species present. The data from this collection method may only provide an indication of the species present and cannot be used for abundance comparisons (the counts of each species found are not dependent on their actual abundance but

on the number of species collected by the sampler). Ants were killed and stored using an alcohol solution of approximately 70–90% ethanol.

Free collection

Free collection was performed throughout the entire plot with no clear temporal boundary. Any ants were collected during the preparation of laying out the bait samples, with enough distance from the chosen sample locations to prevent interruption. It can be challenging to compare these findings across stands, since these are more qualitative indications of the ant communities and species present. The data from this collection method may only provide an indication of the species present and cannot be used for abundance comparisons (the counts of each species found are not dependent on their actual abundance but on the number of species collected by the sampler). Ants were killed and stored using an alcohol solution of approximately 70–90% ethanol.

2.2.2 Date of collection

The first samples to collected date around the end of May (2024), these include the vacuumed birch samples and the baited birch samples. Later that year, around mid-June (2024), the hybrid aspen bait samples were collected. Then, in early July (2024), the bait poplar samples, and vacuumed poplar and hybrid aspen samples were collected. The free collection took place over a period of days ranging from the end of May to the beginning of July 2024 for all FGB samples. The oak samples were collected at the end of April in the year 2025 (Table 1.).

Collection method	Stand	Date of collection
Vacuum	Poplar (Populus sp.)	03-July-2024
Vacuum	Hybrid aspen (<i>P. tremula</i> \times <i>P.</i>	01-July-2024
	tremuloides)	
Vacuum	Birch (Betula pendula)	05-May-2024
Vacuum	Oak (Quercus robur)	28-April-2025
Bait	Poplar	05-July-2024
Bait	Hybrid aspen	17-June-2024
Bait	Birch	24-May-2024
Bait	Oak 1	28-April-2025
Bait	Oak 2 & 3	29-April-2025
20-minute centre search	Poplar	05-July-2024
20-minute centre search	Hybrid aspen	17-June-2024
20-minute centre search	Birch	24-May-2024
20-minute centre search	Oak 1, 2 & 3	29-April-2025
Free collection	Poplar	Between 24-May-2024 and
		06-July-2024
Free collection	Hybrid aspen	Between 24-May-2024 and
		06-July-2024
Free collection	Birch	Between 24-May-2024 and
		06-July-2024
Free collection	Oak 1, 2 & 3	Between 28-April-2025 and
		29-April-2025

Table 1. The differing dates on which certain samples were collected.

2.3 Sample processing

The FGB samples were mostly collected and sorted by Emil Andersson (60% of the samples) as part of his research. I processed the Vacuumed samples previously collected by Emil Andersson (about 40% of the samples from the 3 FGB stands).

The oak stand samples were processed in collaboration by the author and Emil Andersson.

Vacuum sample processing

The vacuum samples were kept in the freezer for the duration of time between collection and sorting. Processing the samples was done per sample, where the individual sample was first divided into three categories: fine material (<1.98mm), medium fine material (1.98< 5.66 mm) and coarse material (> 5.66 mm). These were separated using sifts (or similar equipment with a mesh size of 1.4 mm- 1.4 mm for the smaller one (Sagitta, n.d.-b) and 4-4mm for the larger one (Sagitta, n.d.-a)).

Every category was carefully examined, collecting any invertebrates and storing these, separating ants into a different tube. The invertebrates (including ants) were collected in a ~95% ethanol solution.

When sorting the oak stand samples, the smallest category was somewhat disregarded as no ants so far were found in this category. However, a general search was nevertheless performed of the material in this size category.

After all samples were sorted through, the ants were identified using external morphological characteristics. Traits such as antenna structure, presence or absence of propodeum spines and presence of a postpetiole. The primary key for identification is provided by Douwes (2012, pp. 56–177) in *Steklar: Myror-getingar*. In case of ambiguity or uncertainty, two additional keys were used to provide insights into the morphological traits of certain ant species (Lebas et al., 2019, pp. 56–103; Seifert, 2018, pp. 79–143).

Bait sample processing, Free sample processing & 20-minute centre search

The collected ants were identified using external morphological characteristics. The primary key for identification is provided by Douwes (2012, pp. 56–177) in *Steklar: Myror-getingar*. In case of ambiguity or uncertainty, two additional keys were used to provide insights into the morphological traits of certain ant species (Lebas et al., 2019, pp. 56–103; Seifert, 2018, pp. 79–143).

2.4 Data analyses

If red listed species would be found, special mention and consideration would be given to these findings. The findings will be related to the red listed species list (Ahrné et al., 2020; *Artfakta Från SLU Artdatabanken*, 2016).

To robustly test the data, multiple statistical tests were performed using Rstudio and Excell for testing and graphical representations. For the guild results, a Chi-square test of homogeneity was carried out. The forest guild holds more forest-specialised species, while the open land guild holds more open-habitatspecialised species. Generalists were left out, as defining these can be difficult; they now served as a buffer zone instead of a clear divide between what was considered a forest species and an open-land species.

The data was formatted in rows for either FGB or oak. With columns for forest species present and open-land species present. This test includes data from all collection methods.

To test the ant communities a permutation analyses of variance was chosen because it is designed for multivariate ecological data. It's suited for this data because it has multiple ant species' abundance and different treatments. Additionally, nonparametric tests are less influenced by outliers. The PERMANOVA test to investigate ant-communities is from the vegan package.

For the first PERMANOVA test, I utilise the abundance data per species per sample (this can be called the count data). Each sample has its row in my permutational analysis of variance. Rows with 0 abundance (samples where no ants were found) were deleted. This test only includes data from vacuum and bait samples. For the results, I used an additive (as no significant interaction was found) Adonis2 test with a Bray-Curtis index.

The second PERMANOVA test looks at the abundance of ant species by counting the presence of each ant species over all the samples from a certain stand and sampling method. Eventually, analysing abundance data of ant species of the entire stand. Thus, abundance is here classified as the frequency a particular ant species occurred in the samples. This test only includes data from vacuum and bait samples. For the results, I used an additive (as no significant interaction was found) Adonis2 test with a Bray-Curtis index.

To further test differences between an FGB stand and oak stand, I used a Chisquare test of homogeneity per guild. The data was formatted into two different tables. One showing the sum of species present per sample for forest species, the other for open species. This test only includes data from vacuum and bait samples.

For the sampling effect on ant communities, I performed a PERMANOVA on the abundance by presence or absence (the same data format as for the second PERMANOVA test described above).

The following test concerned the sampling time between bait sampling and vacuum sampling. The collection time for vacuum samples was estimated to be around 30 minutes per stand. For the collection time of bait samples, more accurate data were available for most stands and were estimated (average from the available data) for the stands where it was not available. A one-sided paired t-test was performed.

Lastly, an ANOVA was used to test the sorting time of the FGB vacuum samples.

2.5 Literature review

The literature search that was conducted for the Introduction and Discussion aims to put this research into a broader perspective. The main literature used and searched for was written in the English language. Google Scholar and Scopus were utilized for searching literature. Search queries using terms like species' Latin binomials, common names, geographical delimitations like Scania, Sweden and Skåne. A part of the body of literature used in this paper comes from snowball sampling (or reference chaining).

3. Results

3.1 Species conservation status

The found ant species were not mentioned on the Swedish species red list. Nor had they a conservation status (*Artfakta Från SLU Artdatabanken*, 2016) (Table 3, Appendix 1).



3.2 Guild results

Figure 2. The number of species of each specialisation guild, shown per tree species stand investigated.

All stands have either more forest species or an equal amount of forest species as open-land species (Figure 3). There are more forest species on average in the oak stands. However, a Chi-square test showed no significant difference in total species difference between the FGB stands and the oak stands across all sampling methods, χ^2 (1, N = 41) = 0, *p*-value > 0.05.

Each FGB stand has a unique ant species presence-absence composition (Figure 4). Nevertheless, some species are present within all 3 FGB stands. I note that all three stands had *Formica fusca* present, as well as *Myrmica ruginodis* and *Lasius platythorax* (Figure 4). Within the radar graphs (Figure 4, 5 & 6) connecting lines between ant species that are near to each other have no

implication, they merely show whether a species was present or absent in the stand(s).



Figure 3. Radar graphs for each investigated fast-growing broadleaf (FGB) species (A) Populus tremula x P. tremuloides, B) Betula pendula and C) Populus sp.), the lines conveying whether an ant species was present or absent on each stand.

Every oak stand is different in ant community composition (figure 5). Interestingly, over all three oak stands *Formica fusca, Myrmica ruginodis, Lasius platythorax* and *L. flavus* were present.



Figure 4. Radar graphs for each investigated oak stand (A) Quercus robur 1, B) Q. robur 2 and C) Q. robur 3), the lines conveying whether an ant species was present or absent on each stand

Lastly, the combined results of species present between FGB and oak stands shows the same trend as previously noted (Figure 6). From *Myrmica rubra* clockwise till and including *Formica cunicularia* are considered open-land specialised species. While from *Lasius fuliginosus* counterclockwise till and including *F*. *rufa* are considered forest-specialised species. Performing a qualitative analysis by counting the amount of presence per category and comparing this to each other, the following is found. The oak category dominated the



Figure 5. A radar graph combining the results from the fast-growing broadleaf (FGB) species stands and oak (Quercus robur) stands, the lines conveying the sum of whether an ant species was present or absent at each stand.

forest specialised species side by 3. This number can be calculated by looking at the difference of each ant species between FGB and oak. (For *L. fuliginosus* both oak and FGB found this species in 2 out of 3 stands. For *L. platythorax* and *M. ruginodis* this is 3 out of 3 for both oak and FGB. However, *Leptothorax acervorum*, *F. polyctena* and *F. rufa* were found in 2 out of 3 oak stands and only in 1 out of 3 FGB stands. Thus, resulting in a dominance of forest specialised ant species of 3 for oak).

Then, for the open-land specialized species, FGB and oak have different ant species presence. However, neither FGB nor oak shows a dominance in open land specialised species combined (for FGB compared to oak: +2 *Myrmica rubra*, +1 *M. lobicornis*, -1 *L. niger*, -2 *L. flavus* = 0).

Ant species *M. schencki, F. cunicularia* and *F. sanguinea* were not found but are shown in the radar graphs. These species were expected to be present according to data from a larger study (Andersson, n.d., *unpublished*) and were surprisingly not found (Larger depictions of the tree species specific radar graphs can be found in Appendix 3).

3.3 Community results

3.3.1 Abundance by count data

Seeing a (statistically insignificant) trend in the last paragraph, I wanted to further investigate the difference between FGB and oak stands by including abundance (only represented by bait and vacuum samples). Thus, after the permutational analysis of variance, I found a significant difference in ant communities for tree species, explaining 11% ($R^2 = 0.11$), F(3, 77) = 3.69, p < 0.001. And whether the stand was FGB or oak, explaining 0.5% ($R^2 = 0.05$), F(1, 81) = 4.32, p < 0.01. The sampling method factor was overdispersed, and thus, I did not include the results of the PERMANOVA test for this variable.

3.3.2 Abundance by presence or absence

Seeing a trend in the last PERMANOVA test, I want to analyse the ant community difference using a different definition of abundance. I expected a difference between ant communities in FGB stands and in oak stands (Figure 7).

To test equal dispersion for my permutational analysis of variance I ran a dispersion test on a Bray-Curtis index and the tested factor. Whether the factor



was tree-species, the sampling method or, FGB or oak stand. I did not find overdispersion (on all three dispersion tests, p > 0.05).

Figure 6. The ant community differences. A) depicting the Multivariate dispersion by FGB or by no FGB stand and B) Multivariate dispersion by tree species (Betula pendula, Populus sp., Populus tremula x P. tremuloides and Quercus robur).

The assumption tests were followed by a PERMANOVA test. I found a significant difference in ant communities depending on whether the stand was classified by us as FGB or oak stand, explaining 43% of the variation ($R^2 = 0.43$), F(1, 10) = 7.66, p < 0.01. Furthermore, I found a significant effect of the stand tree species on the ant communities, explaining 63% of the variation ($R^2 = 0.63$), F(3, 8) = 4.54, p < 0.001. I followed these results by performing a pairwise Adonis2 test. The ant communities in oak stands were shown to be significantly different from ant communities in both Hybrid aspen, explaining 41% ($R^2 = 0.41$), F(1, 7) = 4.15, p < 0.05. And in poplar, explaining 55% ($R^2 = 0.55$), F(1, 7) = 7.37, p < 0.05. No significant results were found comparing birch with oak, nor comparing the FGB stands to each other.

3.4 Homogeneity results

Oak stands have a higher forest species presence per sample than FGB stands (Figure 8A), and FGB stands have a higher open land species presence per sample than the oak stands (Figure 8B).

I used a Chi-square test of homogeneity per guild. The test revealed significant difference in the distribution of forest-specialised ant species whether a stand was FGB or oak χ^2 (2, N = 115) = 16.71, *p*-value < 0.001. This indicates that the

proportion of forest species presence is not homogenous between FGB and oak stands (Figure 8A).



Figure 7. The visual representation of the results of a Chi-square test of association. A) Depicting the proportion of forest ant species count per sample by FGB or no FGB and B) showing the proportion of open-land ant species count per sample by FGB or no FGB.

However, no significant difference was found between the presence of openland specialised ant species and whether the stand was FGB or an oak stand (Figure 8B). This second test violated the Chi-square assumptions of the expected counts and was thus followed by a Fisher's exact test, p > 0.05.

3.5 Sampling method results

3.5.1 Sorting bias

No clear trend in difference of ant communities is visible (figure 9). Testing the sampling method effect on the Ant-communities using PERMANOVA test I found an insignificant difference, F(1, 10) =0.96, p > 0.05. Additionally, the assumptions were not met due to overdispersion in the abundance by count data test, they were met in the abundance by presence or absence.



Figure 8. The ant community differences, the Multivariate dispersion by sampling method.



Figure 9. The average sample sorting time in minutes per tree species per sampling method (either vacuum or bait).

Time differences between bait sample data acquisition and vacuum sample data acquisition differ (Figure 10). Vacuum samples simply required an extra step after field acquisition that took considerable time, before being able to deliver data by ant identification. For the test and graph, the collection time for the vacuum sample was estimated to be around 30 minutes per stand. For the collection time of the bait samples, more accurate data were available for most stands and were favourably estimated (average from the available data) for the stands where it was not available. Performing a paired t-test with a one-sided alternative hypothesis, believing sorting time to be greater for vacuum samples than for bait samples, I found a significant difference, t(5) = 2.83, p < 0.05. Vacuum samples taking longer to sort than bait samples (mean difference = 550.67).

3.5.2 FGB vacuum sample sorting impact



Correlation between sorting time and weight by tree species

Figure 10. The sorting time of vacuum samples in minutes over the weight of the sample in grams. The graph shows 3 estimated lines, one for each FGB species.

All tree species-specific vacuum samples required more time as weight increased, *Betula pendula* vacuum samples took the least amount of time per weight, followed by *Populus tremula x P. tremuloides* and lastly, *Populus* sp. (figure 11).

By utilising an additive ANOVA model (as no significant interactions were found), I can show a significant result for the effect of added weight on the FGB vacuumed sample's sorting time. The overall model was significant, F(3, 21) = 14.37, p < 0.001, indicating that the predictors explained a substantial part of the variance in sorting time ($R^2 = 0.627$, *adjusted* $R^2 = 0.626$).

For every additional gram, sorting time increases by about 13 seconds (0.22 minutes), regardless of tree species ($\beta = 0.223$, SE = 0.055, t = 4.06, p < 0.001). On average, hybrid aspen samples take ~67.6 minutes longer to sort than birch samples at the same weight ($\beta = 67.62$, SE = 18.52, t = 3.65, p < 0.01). Poplar samples take ~92.2 minutes longer to sort than birch at the same weight ($\beta = 92.19$, SE = 17.57, t = 5.25, p < 0.001). Oak sorting time was decidedly not analysed this way, as it was sorted on a later date, by multiple people, convoluting the data.

4. Discussion

I discuss the results briefly and relate them to the scientific field. Then I critically review the methodology and limitations of this study, ending with an ethical recommendation for this scientific field.

Ant community differences are apparent between FGB and stands according to my permutation analyses of variance. To further investigate these differences, a Chi-square test on the vacuum and bait samples was conducted. These results revealed that there was a significant difference between the presence of forestspecialised ant species per sample and whether a stand was growing with FGB or with oak.

Overall, the mean number of forest species was higher in oak stands compared to FGB stands; however, I found no significant difference (Chi^2) from comparing the number of species found per sample (for both specialization) between FGB stands and oak stands.

Grevé et al. (2018) studied forest management effects on ant communities in temperate forests and found that tree species selection of proportionally more oak (and pine) had the largest impact on species richness and abundance, consistent with what Jonsell et al. (1998) note about the immense species richness the genus *Quercus* supports in Sweden. My findings add to the understanding of the *Quercus* genus on ant communities, now being able to state that forest specialists have a larger presence per sample in oak stands in comparison to FGB stands.

Statistical testing of differences between the data from the bait samples and vacuum samples did not reveal significant results. Furthermore, vacuum samples required far more time than bait samples. The increase in sorting time depended on the stand structure and weight of the vacuum sample. Important to note is that the time between deploying the baits and collecting the bait samples was not incorporated into the comparison.

Concerning sampling evaluation, I cannot statistically confirm that vacuum and bait sampling are complementary in their results. However, multiple sources advocate the possible complementary properties of sampling methods (King & Porter, 2005; Romero & Jaffe, 1989). Véle et al. (2009) claim that pitfall traps in combination with bait and excavation are necessary to find all ant species in their investigated stands, thus also advocating a combination of methods.

4.1 Critical evaluation of results and methodology

4.1.1 Critical review of the Methodology

Firstly, the data were collected from stands close to each other, this limited sampling area must be taken into consideration. Both geographical and weather conditions will differ along different coordinates within Scania. This research has not investigated whether this would have an effect, and the possible effect could influence the results.

Secondly, flaws in the sampling methods can be identified. The *bait* samples can be influenced by other animals. The bait samples are subject to local ant communities, possibly misrepresenting the stand's ant community makeup. This was combated with the use of semi-random transects with 10 plots each. Additionally, different ant species may influence the abundance of other ant species at the bait samples. Lester et al. (2010) write that a higher abundance of dominant ant species might result in lower species richness, as these "dominant competitors govern resource use". In situ observations revealed this dominance in my study. At sample 9 (bait sample in *Q. robur* 1.), two *L. platythorax* workers (who had the dominance in individuals at this bait sample) were observed engaging in physical restraint of a *Myrmica sp.* worker, pulling at its limbs in what appeared to be interspecific aggression.

The *vacuum* samples are a very small-scale collection, where finding multiple species in a single sample often did not occur. Additionally, abundance data may be skewed due to possible ant nests collected with the vacuum sampling method. Thus, any conclusion based on abundance by count data should be interpreted with caution. Therefore, another abundance – by presence or absence – was calculated to provide a better representation of actual ant abundance. Both the *20-minute centre search* and the *free collection* methods are highly unreliable for abundance data due to not being intended for this purpose. These methods are reasonably reliable for species richness. Salata et al. (2020) find that hand collecting methods have considerably different results than pitfall trap methods. The results of these sampling methods not only depend on the explanatory variables but also on the skill of the sampler. Furthermore, some species are visually more detectable (*Formica fusca* moves fast and is reasonably large; *Tetramorium caespitum* is much smaller and slower). Thus, interpreting the complete species richness results should be done prudently.

By combining four different sampling methods – two quantitative and two nonquantitative – I aimed to assess species richness as accurately as possible. Combining multiple methods is considered more effective for estimating species richness (King & Porter, 2005) as individual methods can be complementary. Romero and Jaffe (1989) note that combining is most efficient, however, they advocate for pitfall traps combined with an 8-hour-long, free search.

Another consideration is the date and time of collection. The samples from the oak stands are collected almost a year later, but 2 to 3 months earlier in the annual seasonal cycle compared to the FGB stands (Table 1).

Ants are ectotherms and are affected by temperature (Bujan et al., 2020; Fellers, 1989; Greenaway, 1981).

Different species are most active during different times of the year (Fellers, 1989), and the activity of some ant species is even influenced by time of day (Greenaway, 1981). However, they were all collected in the time of year when ants are generally known to be more active. As even nuptial flights can already occur from these dates (Stukalyu et al., 2022).

Nevertheless, this could lead the results to be influenced by their collection date, that day's temperature and, the different ant species present, as well as their net reaction to said combination of factors.

Furthermore, identifying ant species solely based on morphological traits has its caveats. Ng'endo et al. (2013) advocate a "pluralistic approach using several methods to understand the taxonomy," especially when identifying complex lineages. They combine morphological identification and mitochondrial DNA sequencing. DNA barcoding is mainly advertised for cryptic species (Paknia et al., 2015) that pose challenges when using morphological traits. However, it is a reasonable delimitation to solely identify ants based on morphological traits for this bachelor's project due to time constraints and resource usage. Consequently, identification is subjected to possible errors in the identification key or human errors and biases. To aim for a reasonably high accuracy, multiple keys were used for more cryptic species. Still, the results should be interpreted with caution.

4.1.2 Limitations for interpreting the results

Within ant ecology it is complicated what is considered the unit of interest. This influences the possible interpretations of the results and limits the conclusions that can be made. Should one consider a colony as a single unit (a genetical unit) or is a single individual considered a unit, as the impact on the ecosystem could depends on the abundance of individuals.

This dilemma is one of the reasons for very nuanced conclusions. These nuances should not be taken lightly.

Secondly, abundance can be estimated by different factors. Romero and Jaffe (1989) claim that abundance is better represented by the number of samples where

the species is present than by the number of individuals per sample. This was done in the second PERMANOVA test in Abundance by presence or absence.

4.2 Alternative explanations

The results can depend on many other explanatory factors that were not accounted for in isolation or in combination with whether the stands were FGB or were oak planted stands.

It is possible that the different ant communities were found due to the different dates of sampling, as discussed above and shown in Table 1.

Secondly, another variable could have more explanatory power. No tests were done exploring the impacts of the distance of the sample from the forest edge, size of the stand, age of the stand, previous land use, size of the stands, time since the last disturbance, understory microclimate and other variables. Microclimate for example, is shown to influence ant communities in east-Mediterranean pine forests (Izhaki et al., 2009), however, microclimate is often also influenced by the canopy species. Stand size could influence the abundance

of certain species due to the resulting core and edge areas. Smaller stands have more edge zones relative to their core habitats than larger stands.

Stand proximity to the other investigated stands could have influenced the data, mainly for the 3 oak stands. However, the ant communities between the 3 oak stands located close to each other shows relatively large variation (Figure 6).

Important to note is that so far, the stands were considered a valid representation of *oak* stands or *FGB* stands. However, it is possible that these stands were not a fair representation and only held ant communities correlated with their specific conditions, not with whichever tree species was present. Additionally, the influence of neighbouring land use was not considered in my study. Thus, the results may reflect only site-specific differences in ant communities and influence of specific neighbouring land use, not or less so tree species-associated differences.

4.3 Ethical considerations

For any research to be considered ethical by a utilitarian view, the scientific relevance must outweigh the negative impacts of the research on animal harm and on the ecosystem. An extended version of these arguments can be found in Appendix 4, An ethical essay for ant considerations.

This research aspires to be relevant. This research might improve future consideration for ant communities on a larger scale when planting certain tree species. Additionally, the data used in this paper will be used in another scientific research (Andersson, n.d., *unpublished*) by this thesis projects' supervisor Emil Andersson. Furthermore, this research was conducted following the current Swedish regulations (Ethical Approval, 2024) and with the permission of the landowner.

The killing of ants was performed by freezing or drowning in an alcohol solution of around 70% ethanol. A different method could have been more ethical. Gilbertson and Wyatt (2016) write that immediately drowning an invertebrate in an alcohol solution of 70-95% is considered unethical, instead, immobilization in 5% before utilizing the alcohol solution of 70-95% is seen as more acceptable (Gilbertson & Wyatt, 2016).

With respect to ecosystem impacts, removing only a fraction of living ants from the ecosystem will likely only have a small impact, assuming most colonies hold multiple thousands of individuals.

We should consider whether no ethical oversight concerning invertebrates is preferable. Brunt et al. (2022) note that a lack of oversight on invertebrate science lowers the public's trust in scientists. Secondly, Cammaerts (2020) argues that invertebrates should be considered as sentient beings; others argue again for ethical considerations (and possible legal protection) for invertebrates, ants included (De Souza Valente, 2024).

Sentience is a difficult concept to clearly define and consequently measure. Is it a scale or is it a clearly delineated set of characteristics? Dictionary definitions remain vague.

"The quality of being able to experience feelings" (Cambridge Dictionary, 2025)

With concepts as *experience* and *feelings* that require further definition to be of proper use. Are *feelings* merely physical or rather emotional or both. Is experience needed to be vivid, or can it be bland. These questions show the difficulty of answering whether it is a true statement that ants are sentient. However, in my opinion we should show consideration to animals when they share enough traits we commonly associate with sentience. Additionally, we should show consideration to species that are in the (still) grey area of sentience and where no definitive statement can be made for these species not being sentient.

5. Conclusion

I will summarize the main findings and answer my research question. Followed by considering the implications of these conclusions and finishing with recommendations for future research. Then I will discuss the sampling method results and give recommendations concerning vacuum sampling.

From bait and vacuum data, I notice that a higher number of forest species per sample is seen in oak stands compared to FGB stands, their proportions significantly different. Furthermore, I can state that between the ant community composition in oak stands, and the ant community composition in FGB stands, a significant difference in species presence and relative abundance exists.

I can state that there is a higher presence of forest species per sample for oak stands, confirming my hypothesis. I can, however, not conclusively state openland guild difference in proportion between either FGB or oak. Furthermore, I see a trend in the forest species presence being higher in oak stands compared to FGB stands, then again, I cannot statistically demonstrate this.

This implies that forest owners and landscape decision makers should be aware of the consequences of their actions in the forestry industry. Different tree species will probably have an impact on the present ant communities, and as previously mentioned, the role of ants can be crucial as they are often ecosystem engineers and keystone species of different habitats (Rocha et al., 2024; Sanders & Van Veen, 2011).

With an expected increase in FGB plantations, differing ant communities will be present within these forests compared to oak forests. With this change in the tree species makeup of the landscape alongside climate change, we might see biodiversity shifts. By shifting the ant species composition within forests, other species might flourish, unchecked by their natural predators.

Oak (*Quercus* spp.) species will hopefully retain most of their economic value. As the industry must shift to more sustainable resources, bioenergy is not the only domain of interest when it comes to wood products (Ministry of the Environment, 2020). The findings of my study, alongside the findings of Grevé et al. (2018) and Jonsell et al. (1998), point to *Quercus* being a very important genus for natural values. This natural importance might optimistically aid in the advocacy for retaining oak in the forestry sector, possibly even expanding its utilization in the forestry sector.

I suggest further research investigating the implications on ant communities of fast-growing broadleaves compared to native slower slower-growing tree species. It could be interesting to include other Swedish native tree species, like beech (*Fagus sylvatica*) in further ant community studies. Additionally, it might be relevant to include coniferous plantations into the investigation as well, as these

are quite prominent in the forestry industry. Another direction is comparing production oak stands to nature reserves, including oak trees, to investigate specific differences in ant communities.

Lastly, this research was limited to southern Sweden, Scania. National scale research would deliver more knowledge and could further the scientific understanding significantly.

Regarding the collection methods, vacuum samples did not yield significantly different results than bait samples, but they cost far more in terms of time.

I do not recommend performing vacuum sampling in addition to bait sampling and free search. Alternatively, I suggest replacing vacuum sampling with pitfall traps, at least in spruce forests, where they are shown to be most effective (Véle et al., 2009). Alternatively, a more intense free search might be beneficial in savannas (Romero & Jaffe, 1989).

To conclusively decide whether vacuum sampling is a viable myrmecological sampling method, future research could focus on different landscape types, different periods in the annual cycle, and different geographical locations.

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Appendix 1

Table 2. The centre coordinates of each investigated stand.

Stand	Coordinates
Betula pendula	
Populus sp.	
Populus tremula L. × Populus	
tremuloides	
Quercus robur 1	
Q. robur 2	
Q. robur 3	

Due to uncertainty surrounding coordinate permissions, they have been removed from the document. Please contact the author at <u>bevg0001@stud.slu.se</u> or his supervisor at <u>emil.andersson@slu.se</u> to request the specific coordinates.

Species Latin binomial	Conservation status according to Artfakta Från SLU Artdatabanken (2016)
Myrmica rubra	No mention
Myrmica schencki	No mention
Myrmica ruginodis	No mention
Myrmica lobicornis	No mention
Lasius niger	No mention
Lasius flavus	No mention
Lasius umbratus	No mention
Lasius platythorax	No mention
Lasius fuliginosus	No mention
Formica fusca	No mention
Formica rufa	No mention
Formica polyctena	No mention
Leptothorax acervorum	No mention

Table 3. Conservation status of found ant species according to (Artfakta Från SLU Artdatabanken, 2016)

Appendix 2



— Populus sp

Figure 12. Radar graph for the investigated fast-growing broadleaf (FGB) species Populus tremula x P. tremuloides. the lines conveying whether an ant species was present or absent on the stand.

Figure 13. Radar graph for the investigated fast-growing broadleaf (FGB) species Betula pendula . the lines conveying whether an ant species was present or absent on the stand





Figure 16. Radar graph for the investigated oak stand; Quercus robur 1. the lines conveying whether an ant species was present or absent on the stand.



Figure 15. Radar graph for the investigated oak stand; Quercus robur 2. the lines conveying whether an ant species was present or absent on the stand.



Figure 14. Radar graph for the investigated oak stand; Quercus robur 3. the lines conveying whether an ant species was present or absent on the stand.

Appendix 3

Appendix 2 has a collection of images from the sample collection and sorting process for visual representation of the methods applied and actions performed.



Figure 21. The information cards placed attached to the bait samples to inform recreating citizens.



Figure 19. The tools used for sorting the vacuum samples. Storage vials, tweezers, permanent markers and a scissor.



Figure 20. A bait sample, with from the top left corner to the bottom right corner, peanut butter, honey and tuna.



Figure 18. A previously collected vacuum sample just before sorting.



Figure 17. An ant under the microscope for morphological identification.

Appendix 4

An ethical essay for ant considerations

It is of the utmost importance to consider the ethical considerations of any scientific research. This research *has* harmed animals. Invertebrates were killed by the hundreds when collecting for later identification. Ants were removed from their natural habitat, leaving their colonies with less workers and sometimes even without a queen and/or brood.

Two main ethical concerns can be identified, the first is the harming of a being, the second is the removal of actors from a connected system known as the ecosystem, impacting many other cycles and interactions.

Let us start by removing the worst argument. The search for knowledge in the form of science does not excuse us to do harm. It is a slippery slope that should not be explored. Instead, the scientific content must prove to be of high enough relevance to justify harm. This is hard to evaluate, however, not impossible. The main two obstacles are identifying the relevance of the research and identifying the heaviness of the harm (note that this uses a utilitarian perspective on ethics). Troubling is the researcher's bias towards believing their research is relevant and important. As the author of both this ethical essay and this scientific paper I will distance myself from trying to prove the relevance of my research. I shall merely give some facts and leave the judgement to the reader. I will, however, introduce multiple arguments in favour of invertebrate considerations.

The data used in this paper, for which animals are killed, will be used in another scientific research (Andersson, n.d., *unpublished*) by this thesis projects' supervisor, Emil Andersson.

The killing was performed by freezing or drowning in an alcohol solution of around 70% ethanol. Gilbertson and Wyatt (2016) evaluated euthanasia techniques for land snails and concluded that immediately drowning in an alcohol solution of 70-95% is unethical, instead lobbying for immobilizing in a 5% alcohol solution after which placing them in a stronger alcohol solution. Their results came from external reactions of the animal to the 'treatment'.

This research (together with many others) might improve future consideration for ant communities on a larger scale when planting certain tree species. This impact is non-measurable.

Firstly, Brunt et al. (2022) note that a lack of oversight on invertebrate science lowers the public's trust in scientists. A non-animal centered (political philosophy) argument to show ethical consideration for invertebrates in science. Secondly, Cammaerts (2020) argues in her commentary on Mikhalevich & Powell (Mikhalevich & Powell, 2020) on Invertebrate Minds that invertebrates should be considered as sentient beings, just like vertebrates are. Others argue again for ethical considerations (and possible legal protection) for invertebrates, ants included (De Souza Valente, 2024). Considering invertebrates, especially social ones in our case, as sentient would bring many complications to this research.

I thus suggest ethical oversight be prevalent and an ethical review to be mandatory before starting any research concerning invertebrates and possible harm. The ethical board should include invertebrate considerations as soon as possible.

I can only guess at the ecosystem impact of removing ants. Previously, we have seen that ants can bet both ecosystem engineers and keystone species (Rocha et al., 2024; Sanders & Van Veen, 2011). However, it is unknown what the effect is of removing only a fraction of living ants from the ecosystem. Assuming most colonies hold multiple thousand individuals, these fractions taken out of the system will likely only have a small impact.

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