

**The effect of red wood ants on the ecology of an associated isopod:
a study of spatial demography and behaviour**

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Introduction

Symbioses are one of the most important processes in nature and cover all long-term biological interactions in the natural world (Paracer & Ahmadjian, 2000). They can be divided into three main groups: commensalism, mutualism and parasitism. In commensalism, one species benefits from the other one without getting benefited or harmed. The interaction where both species benefit from each other is called mutualism. And the last group is parasitism, whereby one species harms the other one and benefits from it. The most specialized symbioses are the ones that are most well-known. The mutualism between corals and zooxanthellae is one of these specialized symbioses that are thoroughly studied (Brown, 1997; Muscatine & Porter, 1977; Pratchett et al., 2008). These associations are between species that co-occur together for a long period. But there are also less strong **associations** found between species, millipedes for instance can live in association with birds. Different millipede species can be found in high abundances in bird nests of different species (Tajovsky et al., 2001). These millipede species are not exclusively found within bird's nests but are able to colonize it depending on the type of nest, ecological characters and geographic range of the millipede species (Tajovsky et al., 2001). Mites (Uropodina) are also loosely associated with the common mole (Napierała et al., 2016). The two most common mites species in the mole nests can also be found in nests of other mammals, so there is no exclusive association with these mites and moles (Kurek et al., 2020; Napierała et al., 2016). The presence of ant species sheltering in cavities of tank bromeliads is another facultative association or non-specific interaction between the *Bromeliaceae* species and the ant species (Blüthgen et al., 2000; Talaga et al., 2015).

Eusocial insects (e.g. ants, termites, some wasp and bee lineages) form interesting study species for symbioses and associations in general. Their nests not only harbour obligate associates, i.e. those species who depend on social insects for their existence, but also facultative associates, which are normally not associated with social insects (Parmentier et al., 2014). Obligate associates have their complete life cycle, or part of it, associated with social insects. They can be host specific or targeting multiple unrelated host species. Associates can originate from multiple groups, such as bacteria, fungi, plants, vertebrates and arthropods, but this term refers to arthropods in this thesis. These associated arthropods are known as melittophiles/bee guests (Fig. 1A), sphecophiles/wasp guests (Fig. 1B), termitophiles/termite guests and myrmecophiles/ant guests. The latter two, termites and ants, harbour the greatest diversity of guests (Parmentier, 2020a). For instance, the amount of myrmecophile species is estimated to be between 10 and 100 thousand (Parmentier, 2020a). Several causes can explain why termites and ants have a higher diversity of guests (Parmentier, 2020a). First of all, the density of stable, long lasting ant and termite nests is often much larger per area (Parmentier, 2020). Next to that, their nests contain more organic material than those of wasps and bees, attracting more guests (Parmentier, 2020a). The fact that ants and termites are also less effective at defending their nest and detecting intruders, has also a big part in explaining why they have a much higher diversity in their nests than wasps and bees (Parmentier, 2020a).

A multitude of free-living insects have made the transition to becoming obligate associates of social insects (Howard et al., 1980; Kistner, 1979, 1982). This depends on pre-adaptive traits the free-living, not associated arthropod possess (Parker, 2016). Features such as small body sizes or possessing defensive strategies will make the free-living organisms able to evolve to obligate associates, with being facultatively associated as an intermediate evolutionary step (Parker, 2016).

The presence of associates of social insects can already be traced back to as early as 99 million years ago (Cai et al., 2017; Zhou et al., 2019). Although on the one hand, **social insect nests** are heavily protected fortresses and are hostile for intruders, e.g. a multitude of workers are equipped with powerful mandibles, venom and/or other chemical weapons to defend their nests, on the other hand, the nests as a habitat have many benefits. First of all, the nests are homeostatic with regulated temperature and humidity (Hughes et al., 2008; Kronauer & Pierce, 2011). Secondly, they are resource-rich environments with a multitude of resources in each nest that can be exploited by associates, such as adult workers, brood, retrieved or cultivated food and nutrient-rich debris (Parmentier, 2020a).

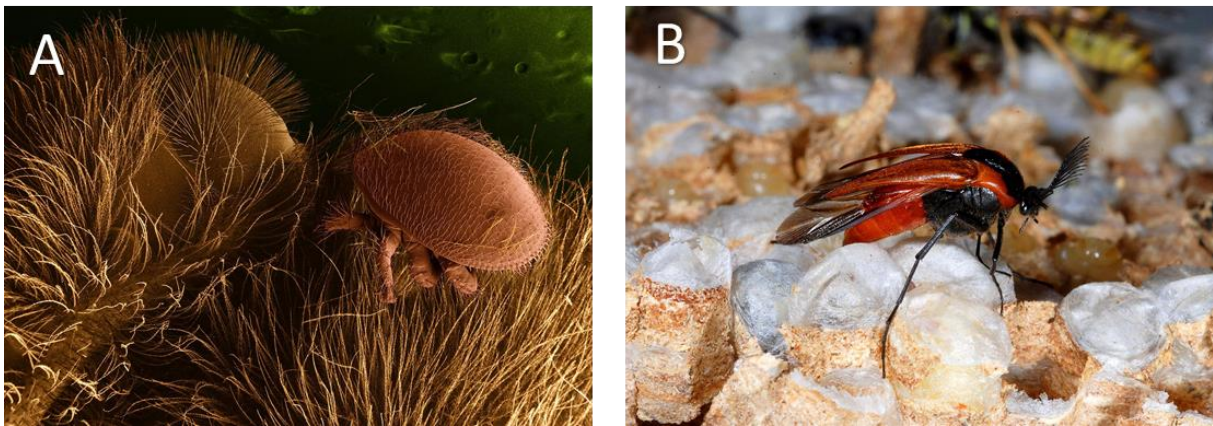


Fig. 1 A) The notorious parasitic melittophile, *Varroa destructor*, on a honey bee. They form a major threat for apiculture by feeding on honey bee fat body tissue and hereby killing entire bee colonies (Ramsey et al., 2019; Rosenkranz et al., 2010). B) The sphecophile *Metoecus paradoxus* in a *Vespula vulgaris* nest. They consume the larvae of their host, *Vespula vulgaris*, during their larval stage (Parmentier, 2020a). Photo by A) Eric Courtesy & Christopher Pooley Erbe - https://fr.wikipedia.org/wiki/Varroa_destructor. B) Tom Wenseleers - <https://bio.kuleuven.be/ento/index.htm>.

Ant nests are very heterogeneous, encompassing earth nests, tree nests, organic mounds and temporary nests. These different micro-niches support a large variety of associated myrmecophiles (Kronauer & Pierce, 2011; Parmentier et al., 2020b). For example, weaver ants construct nests in trees by weaving leaves together using silk of their larvae (Hughes et al., 2008). Some parasitic wasps are associated with them (Pérez-Lachaud & Lachaud, 2014). In contrast to weaver ants, *Lasius flavus* builds their nests underground (Boer, 2015). These ants regurgitate food in the mouth of the myrmecophilous beetle *Claviger testaceus* (Cammaerts, 1992). But some of the most fascinating nests are those from army ants. They do not build a permanent nest but make nests out of their own workers. This is because their cycle alternates between a two-week of a nomadic phase, used to migrate to new hunting grounds, and a three-week stationary phase (Parmentier, 2020a). The army ant *Eciton burchellii* for example has more than 300 associates (Rettenmeyer et al., 2011). Feeding ecology can also differ between ant species. Some ant species live from the prey they catch; others, from honeydew from aphids (Boer, 2015). While leafcutter ants only feed on fungi that they cultivate in ventilated chambers of the nest (Saverschek & Roces, 2011), they also carefully select plant-material as basis to grow these fungi on (Saverschek & Roces, 2011). Not surprisingly, this ecological diversity of host species supports a high myrmecophile diversity, allowing to exploit multiple resources in the host nests.

Many associates prey on the brood of the ants, such as the larvae of the hoverfly genus *Microdon* (Parmentier, 2020a; von Beeren et al., 2011). Furthermore, some guests steal food intended for the colony by begging for food at workers by tapping the mouthparts of an ant. The beetle *Amphotis marginata* shows this behaviour in *Lasius fuliginosus* nests (Parmentier et al., 2021). And others may feed on organic material in the nest such as nest material of red wood ants (Parmentier et al., 2016a). But myrmecophiles can also feed on other myrmecophiles, as demonstrated in two myrmecophilous spiders that prey on other small myrmecophiles (Parmentier et al., 2016a). While some myrmecophiles are host-specific, others are host-generalists. One example of each are two ant cricket species found on the Nansei Islands, Japan (Komatsu et al., 2009). Unlike the host-generalist cricket, the host-specific cricket cannot survive without their one host species and even gets attacked by other ant species (Komatsu et al., 2009).

Being attacked by one or multiple ant species is what myrmecophiles try to avoid. Therefore, they are adapted chemically, morphologically and/or behavioural. The odour of myrmecophiles can be an important factor for them to avoid being assaulted by the ants. The recognition cues on the cuticle or so called **cuticular hydrocarbons** (CHCs) are the components that ants can smell and detect. The same chemicals are also used as nest-mate recognition cues for ants (van Zweden & d’Ettorre, 2010). CHCs are distributed in the colony through trophallaxis (=food/fluid transfer, mostly mouth to mouth) and grooming. The postpharyngeal gland stores these CHCs obtained by grooming, mixes them and re-distributes them (Soroker et al., 1994). But not all CHCs are used to recognize nest-mates, for instance straight-chained alkanes are used to prevent desiccation and are not used as recognition cues (Kleeberg et al., 2017). Because CHCs play an important role in nest-mate recognition, they are also used to detect intruders. A mismatch with the chemical profile of the colony will lead to an attack (van Zweden & d’Ettorre, 2010). Myrmecophiles can have a number of strategies to avoid this. For instance, they could actively produce the same CHCs as the ants (chemical mimicry) (Parmentier et al., 2017a). Another strategy is chemical camouflage, hereby an animal rubs the scent of the predator or another scent from the environment on its body to avoid being detected as prey or intruder. A myrmecophilous cricket does this by rubbing themselves against workers and hereby obtaining the smell of the colony (Akino, 2008). Some myrmecophiles also don’t limit themselves to one strategy, just as the caterpillar of *Monochroa rebeli*, a myrmecophilous butterfly, that uses both of the strategies described above (Akino et al., 1999). They first emit recognition pheromones of their host and later they acquire CHCs within the ant nest (Akino et al., 1999). Another strategy amongst myrmecophiles is chemical insignificance. Here chemical recognition cues are suppressed, making them undetectable for the ants (Parmentier, 2017a). Related to that is chemical transparency, where only the CHCs that are not used for nest-mate recognition remain (Kleeberg et al., 2017).

Morphological adaptations can also help protect the myrmecophiles. Myrmecophiles are usually considerably smaller than their hosts (Hughes et al., 2008). Other morphological adaptations are reduced body parts that protrude or hidden body parts under tough armour (Kronauer & Pierce, 2011; Thomas et al., 2005). A recurring body shape is a smooth teardrop shaped or limuloid body form (Fig. 2A), which makes it difficult for the ants to grab onto with their mandibles (Kronauer & Pierce, 2011). Within the same species, plasticity of morphology can also occur depending on the host species. This is the case for *Platyarthrus hoffmannseggi* (Fig. 4A), a myrmecophilous isopod, whose head size varies depending on the size of its host species (Parmentier et al., 2017b). Another example are the eggs of *Clytra quadripunctata* that have the same shape as the nest material of the red wood ants. These eggs are carried into the nest by the ants. The larvae (Fig. 2B) will then build their own protective case using their own excreta and earth (Parmentier et al., 2014). But the most extreme morphological adaptation is the Wasmannian mimicry or ant-mimicking body forms (Fig. 2C), which can be found in some myrmecophilous rove beetles who strikingly resemble army ants (Maruyama & Parker, 2017).

Lastly, **behavioural adaptations** can also keep them alive in this otherwise hostile environment. Because unspecialized or generalists are recognized by their host species they will provoke aggression from the ants (Parmentier et al., 2016b). To limit aggressive reactions they avoid as many interactions with the ants as possible (Parmentier et al., 2018). They will resort to simple behavioural strategies such as hiding to avoid their aggressive host (Parmentier et al., 2018; von Beeren et al., 2011). The unspecialized parasitic rove beetle *Thiasophila angulata* feeds on ant brood and prey collected by the ants (Parmentier et al., 2016; Zagaja et al., 2017). When the beetle comes in contact with red wood ants it showed more frequently hiding behaviour than when ants were absent (Parmentier et al., 2018). The beetle hereby balances out the trade-off between the benefit of being protected while hiding against to cost of not being able to forage (Cooper Jr & Frederick, 2007; Jennions et al., 2003; Martín & Ló, 1999; Parmentier et al., 2018). Within the same genus myrmecophiles can show different behaviours, such as the myrmecophilous beetle genus, *Pella*. While *P. laticollis* ducks down and presents its abdomen tip with appeasing chemicals to *Lasius fuliginosus* ants, *P. cognata* and *P. funesta* avoid close contact with the ants by swift movement and secreting repelling chemicals (Stoeffler et al., 2011). Behavioural traits from myrmecophiles may also be not that different from behaviour shown in closely related species who are not associated with ants. This is because modifications in expressions of behaviours already present evolves easier than novel behaviours (Milbrath et al., 1993; Sloggett et al., 1998). In ladybirds the obligate associate of red wood ants *Coccinella magnifica* shows similar behaviour with ants as the non-associated *Coccinella septempunctata* (Sloggett et al., 1998). On red wood ant trails, both show ducking behaviour to avoid having damaged appendages. But on aphid tended colonies *C. magnifica* shows way more frequently running away behaviour than *C. septempunctata*. The later one resorts more to letting itself fall from the branch so it can quickly escape attacks (Sloggett et al., 1998). Others, like some ant crickets, beg for food (Fig. 2D) and will receive it via trophallaxis from their host (Komatsu et al., 2009). Also *Amphotis marginata* beetles show this begging behaviour, by mimicking a hungry worker ant and caressing the mouthparts of an ant. The beetle seems to do this only during night while during the day being in shelters nearby the ants foraging trail. This is probably to avoid being visually spotted by the ants (Hölldobler & Kwapich, 2017). Others go as far as mimicking the sound of their hosts queen, such as the parasitic butterfly *Maculinea rebeli*, hereby achieving a high status in the nest and receiving extra treatment from the worker ants (Barbero et al., 2009).

Specialist species will have a high degree of **host specialization** and their adaptations will be far more advanced and in tune with their host, whereas generalist will have adaptations that will allow them to have different host species. Host specific myrmecophiles will often possess conspicuous adaptations in chemical, morphological and behavioural traits (von Beeren et al., 2018). For instance the specialist parasites of red wood ants chemically mimic their host which is very specific for their host. Generalists on the other hand use chemical insignificance strategy or are not even showing chemical adaptations but behavioural adaptations instead (Parmentier et al., 2017a). The generalist army ant associated rove beetles show no apparent anatomical modification compared to non-ant associated beetles (Maruyama & Parker, 2017; von Beeren et al., 2018). In contrast to the host-specific rove beetles who go as far as having Wasmannian mimicry and morphologically resembling their host (Maruyama & Parker, 2017). The difference with closely related species can be striking with host-specific ant crickets showing begging behaviour and approach their host while generalist ant crickets avoid contact with the ants (Komatsu et al., 2009). Because of this it is expected that facultative associates do not possess advanced adaptations, but rather rely on pre-adaptive traits that might be fine-tuned.



Fig. 2 A) *Atelura formicaria* with limuloid body shape in *Formica fusca* nest. B) *Clytra quadripunctata* larvae with protective case in *F. rufa* nest. C) *Ectophya* rove beetle showing Wasmannian mimicry. D) The ant cricket *Myrmecophilus albicinctus* receiving food via trophallaxis from its host *Anoplolepis gracilipes*. Photo by A) Philipp Hönle - <https://twitter.com/myrmecoPhil>. B) Ross Piper - <https://www.rosspiper.net/2018/01/03/mega-colonies/>. C) Munetoshi Maruyama & Joseph Parker - (Maruyama & Parker, 2017). D) Taku Shimada - <http://www.antroom.jp/>.

One of the most iconic group of ants in Europe are the **Eurasian red wood ants**. Six morphological similar species in the genus *Formica* are grouped as Eurasian red wood ants being *F. polyctena*, *F. rufa*, *F. lugubris*, *F. paralugubris*, *F. aquilonia*, and *F. pratensis* (Goropashnaya et al., 2004; Parmentier et al., 2014). Their dome-shaped mounds can be found in both temperate and boreal forests as well as in heath lands across Eurasia (Parmentier et al., 2014). To maintain and expand their nests, huge amounts of organic material is brought to the nests which alters the soil chemical properties (Jílková et al., 2011) and has an effect on the vegetation near the nest (Wardle et al., 2011).

Special about red wood ants is that they not only defend their nests, but also extranidal food sources and their **territory** (Savolainen & Vepsäläinen, 1989). Estimated sizes of territories range from 272 m² to 1616 m² (Skinner, 1980). They navigate through their territory from their nest to a resource by use of foraging trails or ant highways (Buhl et al., 2009). With these trails they reduce the cost of retrieving a resource by reducing energy and time (Denny et al., 2001).

Red wood ants are important ecosystem engineers, in which they on the one hand control communities as a predator and superior competitor, but on the other hand they attract a range of associated species that benefit from the ants as well as their resource rich nests.

As an important **predator** (Fig. 3A) red wood ants have an effect on invertebrate communities around them (Styrsky & Eubanks, 2007). They are opportunistic predators and prey on different arthropod species depending on their availability. Flies and aphids make up a major part of the prey (Domisch et al., 2009; Rosengren et al., 1979; Sörensen & Schmidt, 1987), but as said before depending on the availability other species might be a major part of the prey (Domisch et al., 2016). During one foraging season a large nest can collect 6 to 8 million invertebrates as prey, not surprisingly they shape insect communities nearby (Domisch et al., 2016). On canopy-invertebrate communities they have a large effect affecting most invertebrate groups negatively (Punttila et al., 2004). They reduce significantly the amount of herbivorous invertebrates in nearby trees (Punttila et al., 2004). The abundance of ticks also decreases near red wood ant nests due to predation and the repellent effect of formic acid (Zingg et al., 2018). Apart from catching their prey, red wood ants also scavenge to bring back home animal proteins needed as food for their larvae.

Red wood ant also have an effect on the invertebrate community within their territory by being strongly competitive. They do this with interference **competition**, whereby they attack other species over food sources, and exploitation competition, hereby the red wood ants deplete resources so there is less available for other species. Carabid beetles species richness and abundance for instance is affected by the presence of red wood ants (Reznikova & Dorosheva, 2004). They are both generalist predators with comparable size living in forest habitats and compete with each other over resources. Habitats with high density of red wood ants show often lower amounts of carabids and a reduced species richness, primary due to interference competition (Reznikova & Dorosheva, 2004). Hereby the ants alter the spatial distribution of carabid beetles (Johansson & Gibb, 2016). At high ant densities they just kill carabid beetles without consuming them (Johansson & Gibb, 2016). Not only invertebrates compete with red wood ants but also birds. The reproductive success of treecreepers declines significantly in red wood ant territories due to exploitation competition. The high predation pressure of the red wood ants on invertebrates lowers the abundance of invertebrates used food for bird chicks (Haemig, 1992; Jäntti et al., 2007).

A large part of the red wood ant diet, next to prey, consists of honeydew collected from **aphids**. There is a trophobiosis between both parties, or food-for-protection mutualism, whereby the ants protect the aphids against enemies and, in return, they give honeydew to the ants (Fig. 3B & C).

Next to aphids, 125 obligate arthropod **myrmecophiles** have been found in association with red wood ants, most of which are mites and beetles (Parmentier et al., 2014). Because red wood ants can form large colonies, they can support a huge amount of myrmecophiles (Kronauer & Pierce, 2011; Päävinen et al., 2004; Parmentier et al., 2020b). This big diversity in large colonies is likely caused by an increase in ecological niche and greater longevity of these large colonies (Stockan & Robinson, 2006).



Fig. 3 A) Foraging *Formica polyctena* worker retrieving a spider. B) *F. polyctena* foraging trail. C) Aphid colony tended by *F. polyctena* on oak.

One group associated with red wood ants are isopods. ***Platyarthrus hoffmannseggii* Brandt (1833)** is an obligate myrmecophile found in red wood ants' nests as well as in other ant nests (Parmentier et al., 2017b) (Fig. 4A). Having lost both eyesight and colour (Parmentier et al., 2014) suggests that *P. hoffmannseggii* is perfectly adapted to underground living in ant nests. Next to that, their very low amounts of CHCs allow them to avoid being detected by the ants (Parmentier et al., 2017a). Stable carbon and nitrogen analysis has shown that *P. hoffmannseggii* might be a specialized brood parasite (Parmentier et al., 2016a).

Another isopod that is associated with red wood ants is *Porcellio scaber* Latreille (1804) or the common rough woodlouse (Fig. 4B). They can be very numerous and even outnumber other nest associates species (Robinson & Robinson, 2013). Because *P. scaber* is common without the presence of ants (De Smedt et al., 2020) it can be classified as a facultative associate (Parmentier et al., 2014). As *P. scaber* is a drought resistant isopod species it can profit from the higher temperatures, which could be found at the forest edge, to reproduce faster (De Smedt et al., 2018). That is why they are abundant in numbers at the forest edge, which is also the place where red wood ant nests can be found. As detritus feeders, i.e. feeding on dead organic material from plant as animal sources, red wood ant mounds are a perfect feeding place for them. Previous inventory of nest associates in red wood ant mounds showed that almost all nests contain *P. scaber* individuals (Robinson & Robinson, 2013). Pregnant females as well as juveniles were found suggesting that *P. scaber* can breed in red wood ant nests (Robinson & Robinson, 2013). Stable carbon and nitrogen analysis of *P. scaber* individuals found in the nests also confirm that they feed on the organic nest material (Parmentier et al., 2016a). It also possesses some adaptations to avoid and withstand attacks by the ants. They carry extremely low amounts of CHCs, making them chemical insignificant and therefore difficult to detect (Parmentier et al., 2017a). Furthermore they also have a hard exoskeleton which they can use to hide under while being attacked. Although they seem to have adaptations to live with the ants they are also a food source for red wood ants (Loones et al., 2008). Several studies have done toxicological research on *P. scaber* (Bayley & Baatrup, 1996; Dallinger & Prosi, 1988; Fischer et al., 1997). The spatial demography of *P. scaber* in a with heavy metal polluted area as well as the behavioural effects of heavy metals on *P. scaber* have been studied (Bayley & Baatrup, 1996; Jones & Hopkin, 1998). But a limited amount of similar studies have been done on the effect of biotic stress, especially stress imposed by ants on *P. scaber* or closely related species, have been done (Castillo & Kight, 2005).

The main objective is to shed light on the paradox of *P. scaber* being abundant around red wood ant nests while being an important food source of them. More specifically, I will study how red wood ants influence

- i) the spatial distribution of *P. scaber* in and around the nests
- ii) the effect of red wood ants on isopod weight, gender ratio, pregnancy ratio, and degree of injury along a spatial gradient away from the nest
- iii) the behaviour of *P. scaber* and other isopod species along a gradient of ant association.



Fig. 4 A) *Platyarthrus hoffmannseggii*. B) *Porcellio scaber*. Photo by A) Andy Murray - https://en.wikipedia.org/wiki/Platyarthrus_hoffmannseggii B) Gert Arijs - <https://www.spinicornis.be/porcellio-scaber>.

Objectives

The overall aim of this thesis is to examine how a stressful ant environment affects the distribution and life history of the isopod *P. scaber*. This was done by looking at the spatial demography of *P. scaber* around the nests, scoring life history parameters around the nest and with behavioural experiments in the lab.

Spatial demography of *P. scaber*

In this part the spatial demography of *Porcellio scaber* Latreille (1804) around active red wood ant nests (*Formica polyctena* Förster (1850) & *Formica rufa* Linnaeus (1761)) will be assessed. Hereby information about *P. scaber* individuals on 1, 5 and 10 meters from the nest as well as in the nest was determined. Information about the dry weight, age class, gender, pregnancy and amount of antennae was determined for each individual.

individual dry weight

As general myrmecophiles are typically smaller than their host species, it is expected that closer to the nest the individuals will be smaller and therefore lighter (Hughes et al., 2008; Parker, 2016). Smaller myrmecophiles will have the advantage of being detected less by the ant workers (Barclay & Brigham, 1991; Blanckenhorn, 2000; Parker, 2016). So *P. scaber* individuals will probably also benefit from being smaller at high ant densities and closer to the nests where there is a higher density of ant trails, meaning more high ant densities areas. Bigger individuals will probably avoid high densities of ants and will be found further away from the nest.

Total biomass per pitfall

The biomass per pitfall is expected to be having the same trend as the amount of adults found per pitfall. Because of their small size the sum of the weights of juveniles will probably not contribute to changes from the expected pattern.

Abundance of adults per pitfall

It is expected that at high ant densities and closer to the nests that there will be less *P. scaber* adults. This is because their expected size disadvantage will probably drive them away from the nests to lower ant density places. Avoiding behaviour is considered as a simple behaviour yet very efficient and widespread strategy to avoid attacks of enemies (Parmentier et al., 2018). For a facultative generalist, like *P. scaber*, it is expected that they will probably show this behaviour.

Proportion of juvenile individuals

Because juveniles are smaller, and will probably be detected less (Barclay & Brigham, 1991; Blanckenhorn, 2000; Parker, 2016), they are most likely not going to show as much avoiding behaviour as the adults. That is why it is expected that the proportion of juveniles will increase when the abundance of adults decreases, this being at high ant density and closer to the nest.

Proportion of females

Because females do not possess other obvious features, that help them avoid or defending an attack, than males we expect no changes in sex-ratio with changing sampling distance and ant density.

Proportion of pregnant females

Because pregnant females carry their eggs with them in a marsupial pouch (Sutton, 1980) it is expected that they are less mobile and agile and less able to fend of possible ant attacks. Hereby we hypothesis that the proportion of pregnant females will decrease closer to the nests and with increasing ant densities.

Proportion of missing antennae

Each isopod has two antennae on its head, which cannot be shielded when being attacked. Hereby the antennae are still in reach for the ants. Because the ants could rip of antennae from *P. scaber* (Kronauer & Pierce, 2011; Liang & Silverman, 2000) we hypothesis that individuals closer to the nest and at higher ant densities will have a higher probability of having missing antennae.

Behavioural experiments

In the behavioural experiments the goal is to understand the behavioural changes of both *P. scaber* and ants when they encounter each other. The same experiment will be done with four other isopod species, the obligate myrmecophilous isopod *Platyarthrus hoffmannseggii* Brandt (1833) and three isopod species that are common in Flanders, but are thought not to be associated with red wood ants: *Oniscus asellus* Linnaeus (1758), *Philoscia muscorum* Scopoli (1763) and *Armadillidium vulgare* Latreille (1804) (Fig. 5). Hereby the initial behaviour of the isopod will be scored, the behaviour of the ants when encountering an isopod showing that behaviour and the behaviour the isopod shows after the interaction.

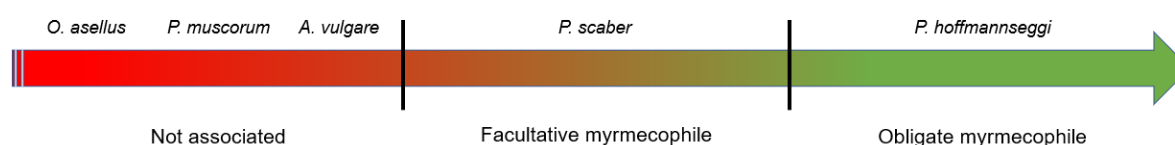


Fig. 5 The five used isopod species in the behavioural experiments ordered along a degree of association with red wood ants from left to right: not associated, facultative myrmecophile and obligate myrmecophile.

Total aggression

Overall it is expected that there will be a big difference in ant aggression depending on the species. *Platyarthrus hoffmannseggii* is expected to face way less aggressive interactions than the other four species due to their obvious lifestyle (Parmentier et al., 2017b). Hence *P. scaber* its abundance around red wood ants (Robinson & Robinson, 2013) it is expected that it will encounter less aggressive interactions than the three species that are thought not to be associated with red wood ants.

Effect initial behaviour isopod on ant aggression

It is expected that the initial behaviour of isopods will also have an effect on ant aggression. Overall it is hypothesized that running as an initial behaviour, from each isopod species, will provoke most aggressive reactions compared to the other behaviours. This is because fast-moving individuals are more efficiently detected in general (Dorosheva et al., 2011; Parmentier et al., 2018)

The reaction of the isopod on the behaviour of the ants

Aggressive behaviour from the ants will alter the behaviour of the isopod. Depending on the species these changes will be different. First, *P. hoffmannseggii* will probably encounter such a low degree of aggression, patterns in behavioural patterns will not be present. *Porcellio scaber* and *O. asellus* will probably have similar defensive behaviours, not moving and running, based on previous research (Pekár et al., 2016). This is because they both possess a type of defensive behaviour called clinging (Pekár et al., 2016). Hereby the individual presses its body against the ground and resorts to its hard exoskeleton to protect the vulnerable parts of its body such as the legs and ventral side. *Philoscia muscorum* cannot hide its legs under the exoskeleton, because of that they will have to run when attacked. Lastly, *A. vulgare* which is the only one of the five species that is capable roll itself into a ball will probably use this to protect itself (Pekár et al., 2016).

Material and methods

Sample collection

Spatial demography of *P. scaber*

To have a better understanding on how red wood ants influences *P. scaber* the spatial demography was assessed of these isopods. During the summer months of 2020, red wood ant nests were identified as sampling sites (Fig. 6 & 7). Of the 20 selected nests 18 of them were inhabited by one of the two red wood ant species used in this research, being *Formica polyctena* and *Formica rufa*. The remaining two nests were formerly inhabited by red wood ants, but are now abandoned. All selected nests were located in the province of West-Flanders in the North-West of Belgium, more specific in Beisbroek (Bruges), Snellegem and Vleteren (Fig. 6 & 7). The inhabited nests were grouped into seven different site categories. This division was done based on similarities in the environments and proximity (Appendix 1 & Fig. 7).

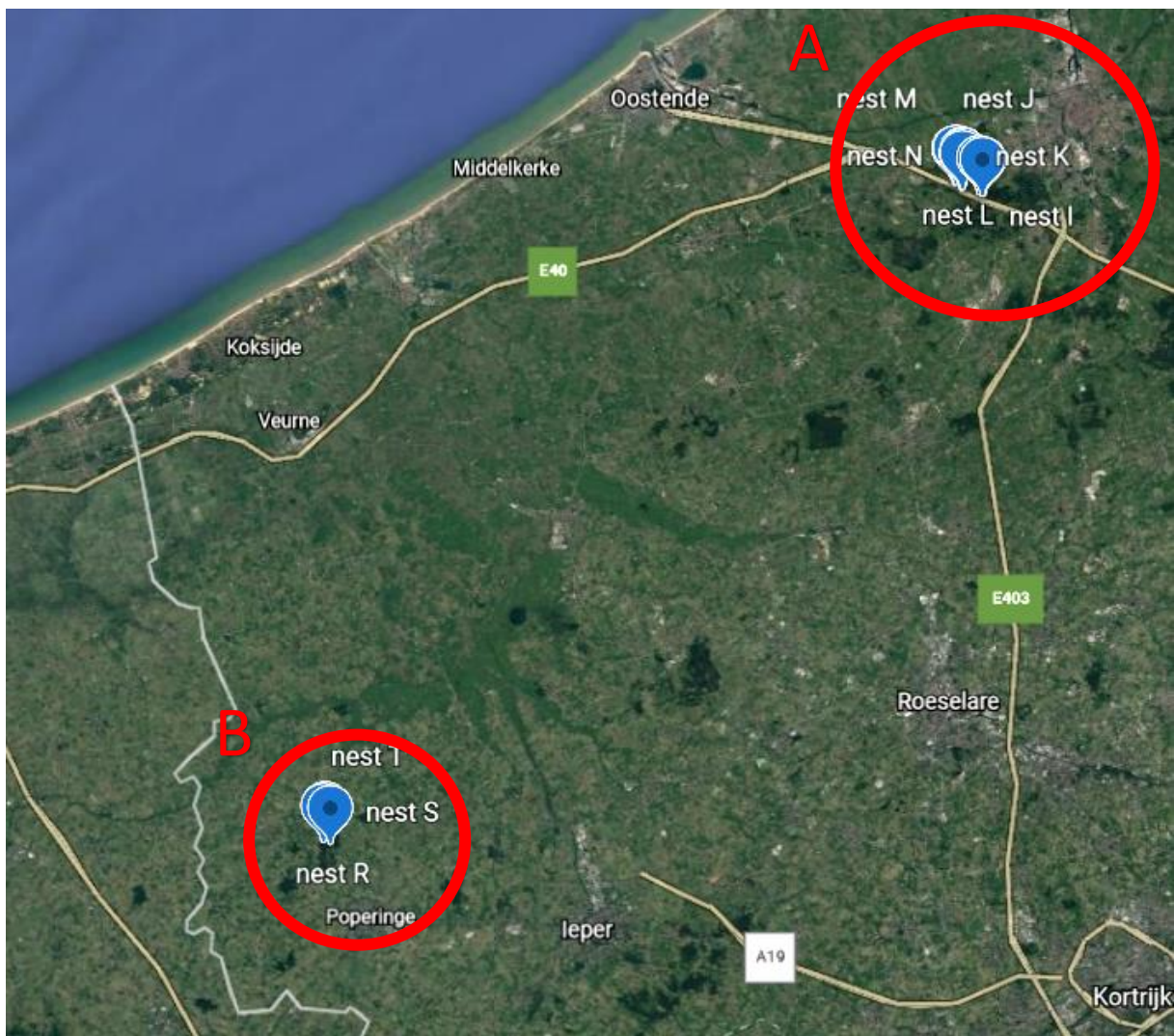
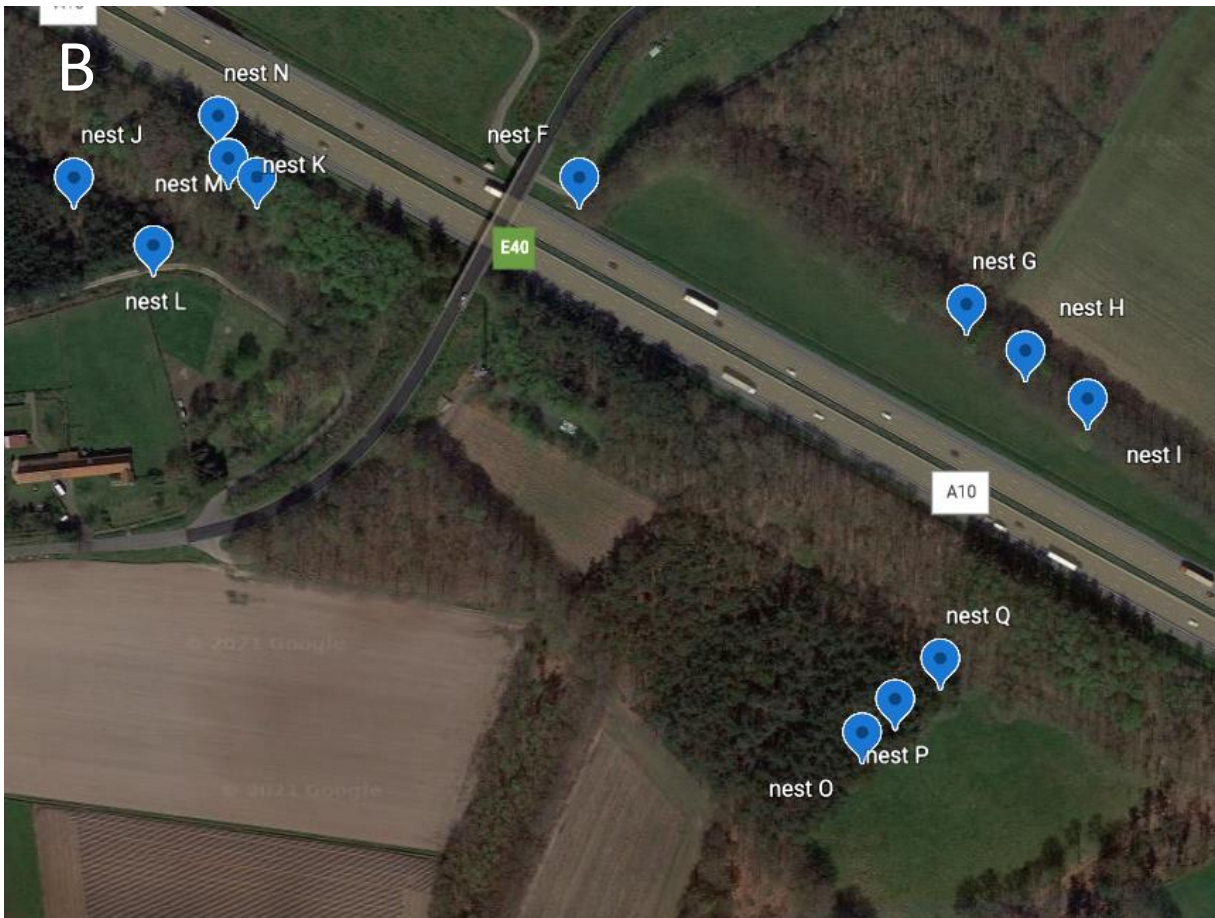
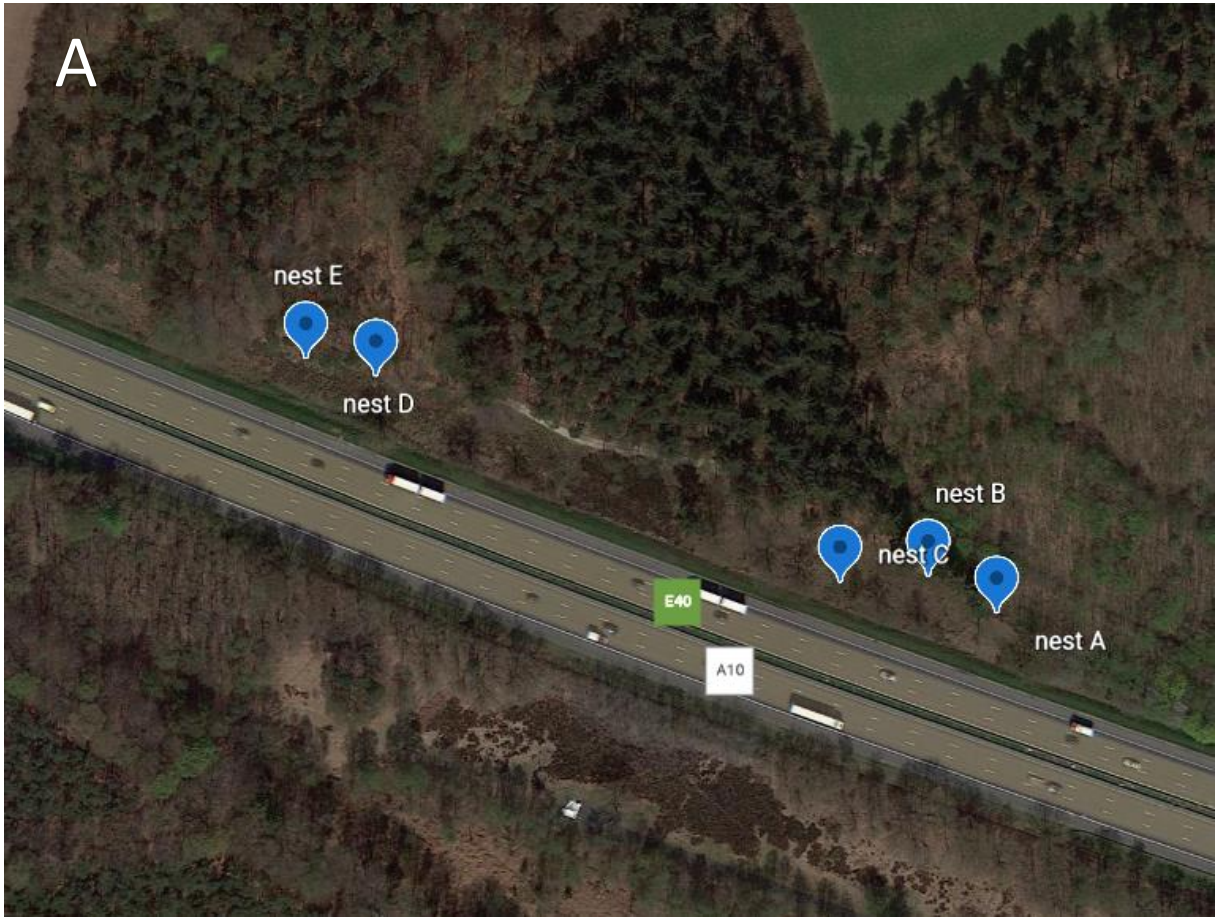


Fig. 6 All selected nests in the province of West-Flanders in the North-West of Belgium, more specific in Beisbroek (Bruges), Snellegem, both in circle A, and Vleteren in circle B.



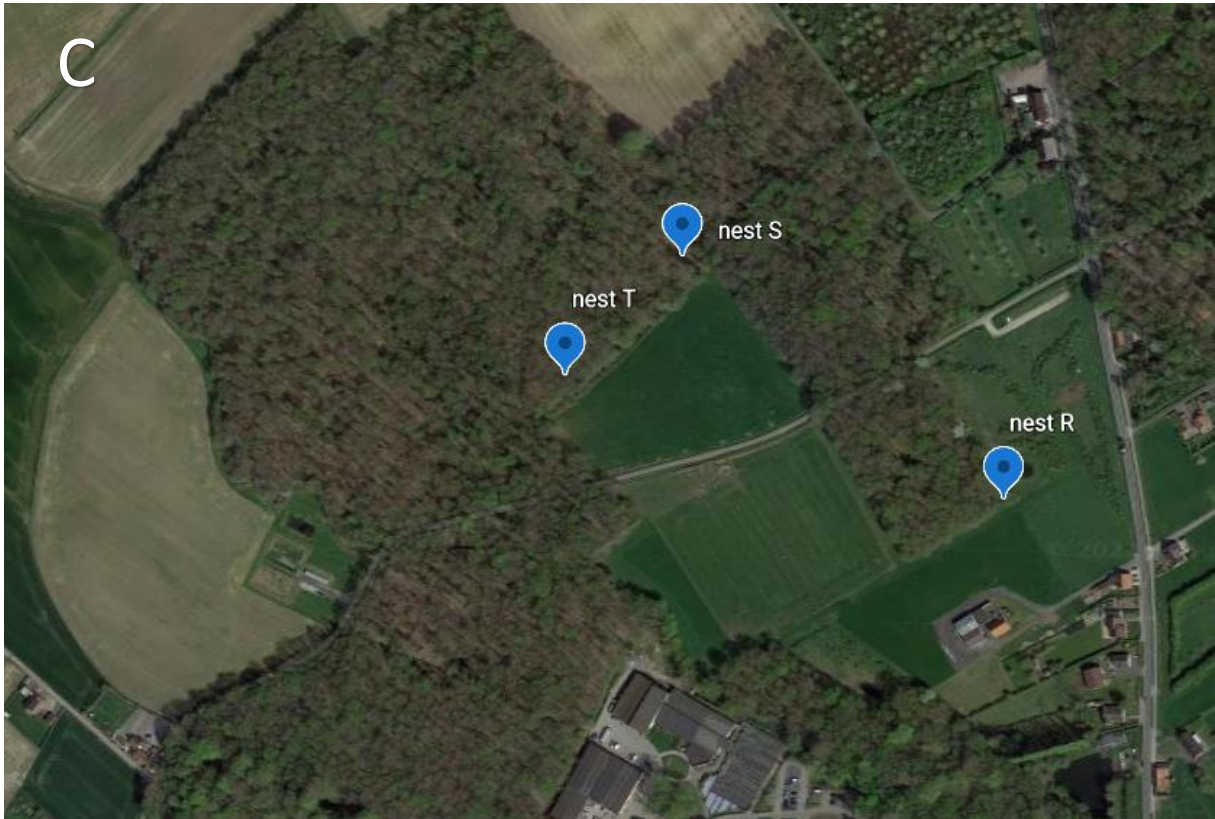


Fig. 7 All sampled nests A) Site 1 (nests A-E) in Beisbroek (Bruges). B) Site 2 (nests F-I) in Beisbroek (Bruges), site 3 (nest J), 4 (nest L), 6 (nests N & M) and 7 (nests P & Q) in Snellegem. C) Site 5 (nests R-T) in Vleteren.

Each nest was then sampled at three distances outside the nest (1, 5 and 10 meter) and also inside the nest. Three pitfalls were installed for each distance outside the nest (Fig. 8). They were positioned as much as possible parallel to the forest edge to minimize the influence of the edge effect on the results (De Smedt et al., 2018).

Forest edge

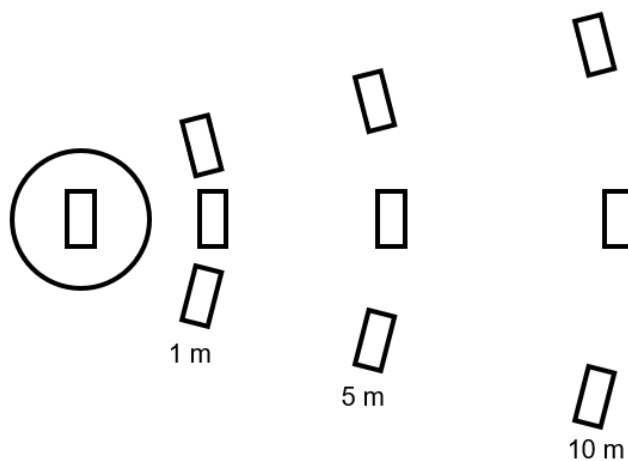


Fig. 8 Top view of red wood ant nest (circle) and pitfalls (rectangles) on 1, 5 and 10 meters from the nest and one pitfall in the nest.

Cuboid pitfalls (25 cmx7.5 cmx8 cm) containing an approximate 1 cm bottom layer of plaster were used to catch the isopods. To prevent isopods and other caught arthropods to die from desiccation the plaster was moisturized. Debris of the surrounding also got into the pitfalls due to the wind and passing ants, creating extra humidity and places to hide for the caught arthropods. By collecting alive isopods, we avoided collecting dead isopods that were brought into the pitfall by foraging workers. All pitfalls outside of the nests were then dug into the ground with the long side of the pitfall parallel towards the nest and the top part of the pitfall level with the ground. Thereafter an elevated roof was applied on the pitfalls. The elevated roof prevents the pitfall of flooding and killing all caught arthropods after rainfall, but it also leaves a slit big enough for arthropods to get caught by the pitfall (Fig.9A). The pitfalls inside the nest were dug into the nest and covered again with nest material (Fig.9B). A roof, kept in place by rubber bands, was also used to prevent nest material to fill the pitfalls.

The pitfalls outside the nest were emptied after one week. The ones inside the nests, however, were emptied every one to two days, because they rapidly fill with nest material pushed inside by the ants. All arthropods that were caught, except ants, were stored per pitfall on 70% ethanol. Only the isopods were used in this research, the other arthropods were stored for later research by Dr. Thomas Parmentier. Although the ants were not collected, they were counted in the pitfalls outside the nests. These numbers were used to determine if the pitfall was placed on an ant trail.



Fig. 9 A) Pitfall with roof near red wood ant nests. B) Pitfall being installed in a red wood ant nest.

Behavioural experiments

The aim of the behavioural experiments is to see if *P. scaber* reacts differently when faced with ants and if ants react differently towards different isopod species. For this experiment, 20 individuals of five isopod species were collected: *O. asellus*, *P. muscorum*, *A. vulgare*, *P. scaber* and *P. hoffmannseggii*. All individuals that were collected had no prior contact with red wood ants, as well as the *P. hoffmannseggii* individuals who were collected in a *Lasius flavus* nest.

Red wood ants (*Formica polyctena*) were collected from Beisbroek from nest F-I (Fig. 7B).

Data collection

Spatial demography of *P. scaber*

Gender, state of pregnancy and number of antennae was determined for each of the 7881 collected specimens of *P. scaber* using a stereomicroscope (Kyowa optical model SD-2P). Gender of *P. scaber* and woodlice in general can be determined by looking at the presence of external male genitalia used for sperm transfer (Sutton, 1980) (Fig. 10A). Because this external male genitalia develops when they are adult, we used the size/weight of when there were as much males as individuals without external male genitalia as a cut-off value for determining age (juveniles < 0.0013 g). When pregnant the female will develop a brood pouch or a so-called marsupium holding the eggs (Fig. 10B). This micro-aquarium allows the embryonic development without an external water-source and can be easily spotted at the ventral side of the female woodlice (Sutton, 1980). Isopods possess two antennae but because *P. scaber* is not able to hide its antennae under its hard exoskeleton, the ants could attack those leading to a loss of one or two antennae.

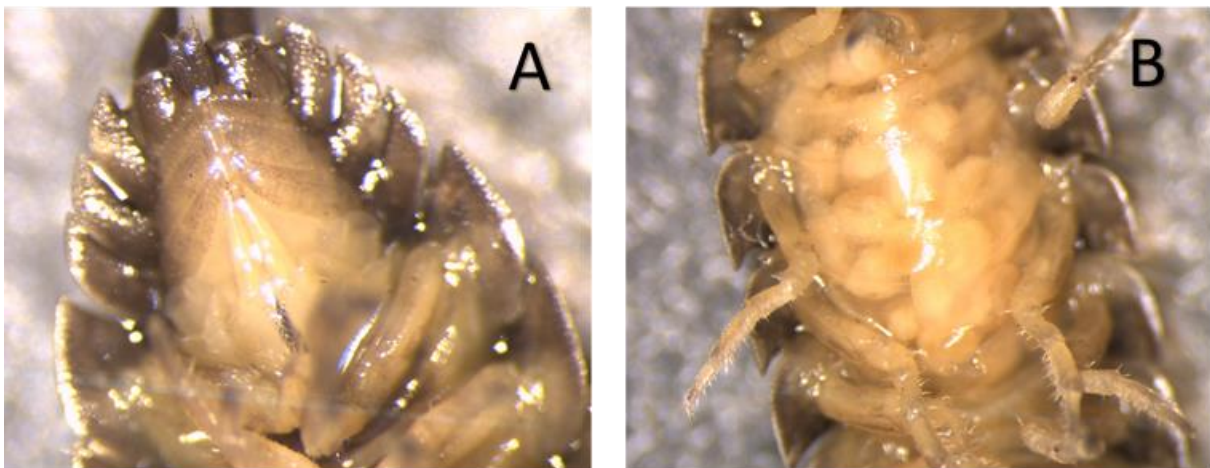


Fig. 10 A) External male genitalia of *P. scaber* . B) Marsupium of *P. scaber* female with eggs inside.

In addition, every individual was also weighed as a proxy for its size. Thereby isopods were placed in an oven on 60 degrees for 2 hours up to maximum 24 hours. Because after 2 hours of drying the weight did not change anymore the isopods could be left in the oven for longer (Appendix 2). The dry weight was measured using a microbalance (Brand: OHAUS; accuracy: 0.1 milligram). Pregnant females were also weighed in the same way. Because the brood pouch consists largely of liquid, it only added a minimum to no additional weight. Therefore, no penalty was added for pregnancy.

Behavioural experiments

The behavioural experiments were conducted in plastic cylindrical containers (diameter = 6.5 cm; height = 7 cm) that represents an arena. The arena had a moisturized plaster bottom of +- 1 cm and the inner side of the arena was coated with fluon so that the ants and isopods could not escape.

Seven medium sized ants were put into the arenas to acclimatize for 1 hour. The choice for medium sized ants was based on previous research that demonstrated that smaller red wood ant workers react more aggressively (Parmentier et al., 2015). By leaving out the smallest and biggest workers the variation in aggression of the ants would be minimized. Seven ants were selected because at this ant density ant-free spaces still exist, while still maintaining a high density of ants sufficient enough to score the desired number of interactions.

After the acclimatization period of the ants, an isopod was introduced into each of the two arenas under a camera. Ten seconds following the introduction of the isopod, the behaviours were filmed for ten minutes. The videos were analysed later, frame per frame, with the program “VirtualDub”. During the video-analysis the initial behaviour of the isopod was accounted (Table 1), the behaviour of the ant while encountering (=an ant’s antennae cross the body of the isopod or is in very close proximity of it) the isopod (Table 2) as well as the reaction of the isopod following the interaction with the ant (Table 3). For each individual the first 20 interactions were recorded/assessed.

For each of the five selected isopod species, we repeated the behavioural trial with 18-20 unique individuals. Each time another individual was used for the experiment new ants in the arena were also used. These ants also had an acclimatization period in the arenas.

Table 1 The initial behaviour of the isopod prior to ant contact.

Initial behaviour isopod	Description
Not moving	The isopod was not moving.
Walking	The isopod was moving at a slow to moderate speed.
Running	The isopod was running.
Ball	The isopod rolled itself up in a perfect ball or stayed in a ball, only <i>A. vulgare</i> is morphologically able to do this.

Table 2 The reaction of the ant when encountering an isopod, with in green the passive behaviours of the ants and in red the aggressive ones.

Behaviour ant	Description
Ignored	There was no change in ant behaviour when it encountered the isopod. This also included minor movements with the antennae in the direction of the isopod when the ant was stationary.
Attention	When an ant encountered an isopod it stopped its initial behaviour. Unaggressive movement of the ant’s head in the direction of the isopod as well as movement of the antennae above the isopod without touching it were included in this behaviour.
Investigated	The ant noticed the isopod and started investigating it by touching the isopod with its antennae multiple times.
Mandibles	Upon contact the ant opened its mandibles, slightly or completely, and moved its head in an aggressive way in the direction of the isopod.
Lashing out	When an ant encountered an isopod it attempted to bite it by moving in its direction, this without mandibles touching the isopod.
Chasing	The ant lashed out multiple times at the isopod.
Biting	The ant bit with its mandibles the isopod.
Acid spraying	The ant moved its abdomen forward under his thorax and sprayed acid towards the isopod.

Table 3 The behavioural reaction of the isopod when encountering an ant.

Reaction isopod	Description
Not moving	The isopod was not moving.
Short stop	The isopod stopped its movement shortly when encountering an ant before it moved again.
Walking	The isopod was moving at a slow to moderate speed.
Running	The isopod was running.
Ball	The isopod rolled itself up in a perfect ball or stayed in a ball, only <i>A. vulgare</i> is morphologically able to do this.

Data-analyses

Spatial demography of *P. scaber*

For ant density the amount of ants per pitfall was fourth-root transformed to use it in the spatial demography models.

individual dry weight

The weight of adult *P. scaber* individuals around red wood ant nests (not including individuals caught in the nest) was modelled using a LMM (= linear mixed model) using the packages lme4 and lmerTest in R 4.0.3. The full model included distance from the nest, gender, ant density and the interactions between those 3 variables as fixed effects. As random effects site, nest and pitfall was chosen. After eliminating the non-significant fixed factors the normality of the model residuals was checked. Because normality was not met the logarithm of the weight of the isopods was modelled with the same fixed and random factors described above. A post-hoc test was then conducted using the package emmeans, multcomp and multcompView.

Total biomass per pitfall

The total biomass per pitfall was modelled using modelled using a LMM (= linear mixed model) using the packages lme4 and lmerTest. The used fixed effects were the sampling distance from the nest, ant density and the interaction between those two. Site and nest were selected as random effects. Because normality was not met, the total biomass was fourth-root transformed and modelled with the same fixed and random factors as described above.

Abundance of adults per pitfall

Similar statistics are used as in the individual dry weight model. The full model included distance from the nest, the ant density and the interactions between those two variables as fixed effects. As random effects site and nest were selected. Because normality was not met the amount of adults was fourth-root transformed and was modelled with the same fixed and random factors described above.

Proportion of juveniles, females, pregnant females and individuals with missing antennae

The effect of the predictors distance, ant density and the interaction was assessed on i) proportion of juveniles ii) the proportion of females iii) the proportion of pregnant females, iv) the proportion of individuals with missing antennae. Here we ran four binomial GLMM models (= generalized linear mixed model) using the packages lme4 and lmerTest in R 4.0.3. The full model included sampling distance from the nest, ant density and the interactions between those two variables as fixed effects. As random effects site and nest was chosen. Overdispersion was checked by using the DHARMA package and a post-hoc test was then conducted using the packages emmeans, multcomp and multcompView.

Behavioural experiments

Total aggressivity

The proportion of aggressive reaction on the five species was modelled using a binomial GLMM model (= generalized linear mixed model). The full model included the species as fixed effect and the individual as random effect. Overdispersion was checked by using the DHARMA package and a post-hoc test was then conducted using the packages emmeans, multcomp and multcompView.

Effect initial behaviour isopod on ants aggression

Per species a binomial GLMM (=generalized linear mixed model) was modelled using the packages lme4 and lmerTest in R 4.0.3. The full model included the initial behaviour of the isopod as a fixed effect and the ID of the isopod as a random effect. Overdispersion was checked by using the DHARMA package and a post-hoc test was conducted using the packages emmeans, multcomp and multcompView.

The reaction of the isopod on the behaviour of the ants

Per species a multinomial model was used to see what effect the behaviour of the ants had on the behaviour of the isopod after the interaction, hereby the nnet package was used. Finally, the lsmeans package was used to determine if the change in behaviour of the isopod with and without aggression was significant.

Results

Porcellio scaber is abundant around red wood ant nests, we collected 7881 specimens of *P. scaber*. In comparison with other isopod species who were only at low densities found around the nests. In 11 of the 18 sampled nests *P. scaber* individuals were even found within the nests. A total of 80 individuals were found there and in some nests they were even more abundant than some obligate myrmecophiles.

Spatial demography of *P. scaber*

individual dry weight

There was a clear pattern showing with increasing ant density, hereby the dry weight of the isopods decreases irrespective of the gender of the isopod (Chisq = 7.5, Df = 1, $p = 0.006$) (Fig. 11). The dry weight of a *P. scaber* individual was lower close to the nest than away from the nest, but there was an interaction effect with gender (Chisq = 13.8, Df = 2, $p = 0.001$) (Fig. 11). In females there was a significant difference between the individuals caught at 1 meter, who weighed less than the ones at 5 and 10 meters ($p < 0.001$) (Fig. 11). The latter two were not significantly different from each other ($p = 0.909$). Males at 10 meters had a significant larger dry weight than the males caught at 1 meter ($p = 0.002$) (Fig. 11). The males caught at 5 meters distance from the nests didn't have a significant weight difference compared with the ones caught at 1 meter ($p = 0.053$), which was borderline insignificant, and 10 meters ($p = 0.353$) (Fig. 11).

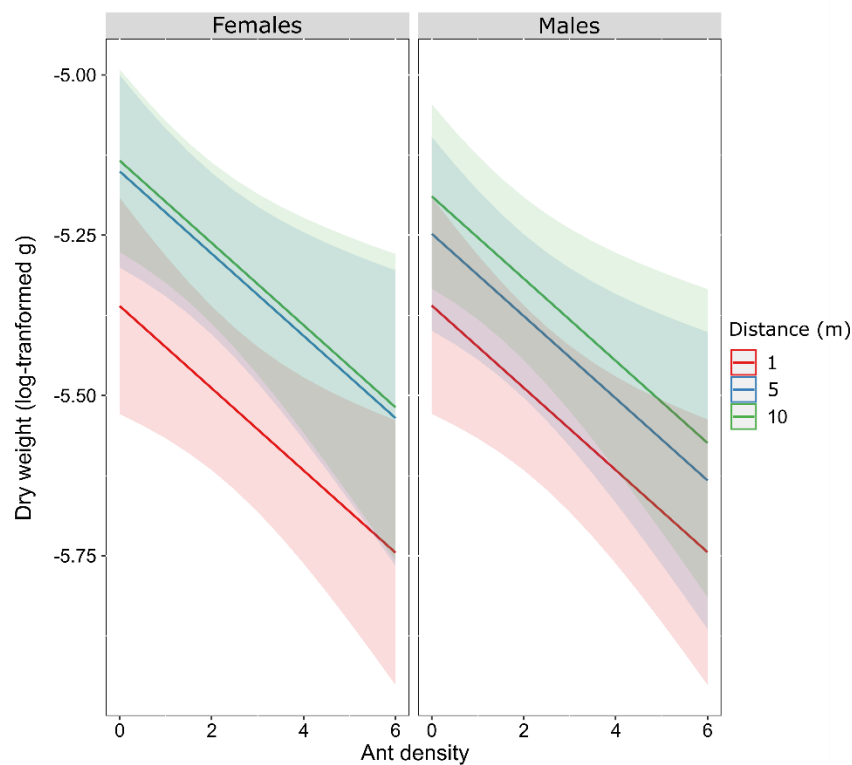


Fig. 11 The effect plot displays the effect of the ant density (fourth-root transformed) and sampling distance from the nest on the dry weight of adult, female and male, *P. scaber* individuals (log transformed).

Total biomass per pitfall

The interaction effect between ant density and sampling distance was significant (Chisq = 18.9, Df = 2, $p < 0.001$) (Fig. 12). At 1 meter, ant density had the most distinct effect on the total biomass, with a decrease in biomass with increasing ant density (Fig. 12). At 5 and 10 meters the effect of ant density is less pronounced in comparison with the total biomass at 1 meter (Fig. 12). At high ant densities a lower total biomass was found at 1 meter than at 5 and 10 meter, while at lower ant densities the difference between the sampling distances was not as pronounced (Fig. 12).

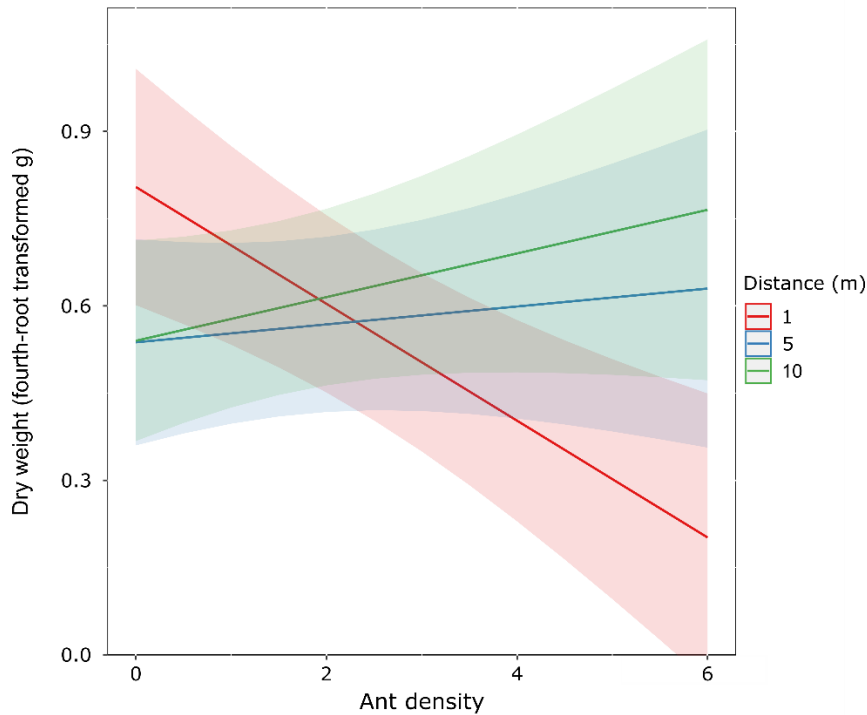


Fig. 12 The effect plot displays the effect of the ant density (fourth-root transformed) and sampling distance from the nest on the total biomass in dry weight of *P. scaber*, both juvenile as adults, individuals per pitfall (fourth-root transformed).

Abundance of adults per pitfall

There was a significant interaction between distance and ant density (Chisq = 9.1, Df = 2, p = 0.011) influencing the abundance of adults (Fig. 13). At 1 meter there was a clear decrease of abundance with increasing ant density (Fig. 13), while at 5 and 10 meters no such a remarkable changes were noticed with changing ant densities (Fig. 13). At high ant densities at 1 meter the abundance is lower than at the other distances, on lower ant density the difference in abundance between the different sampling distances is less (Fig. 13).

Note that 71 adults were found in the nests, but they were not included in the model.

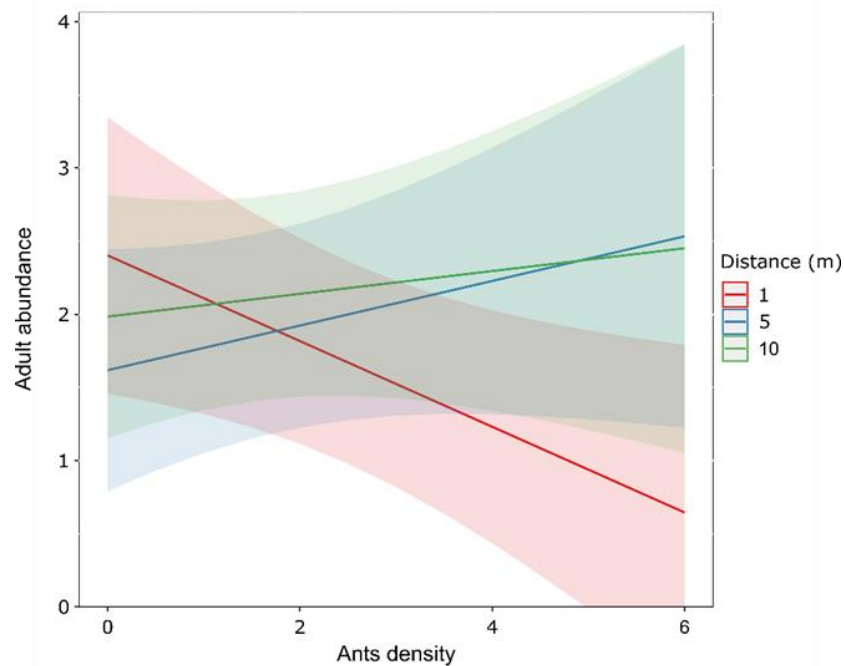


Fig. 13 The effect plot displays the effect of the ant density (fourth-root transformed) and sampling distance from the nest on the abundance of adult *P. scaber* individuals per pitfall (fourth-root transformed).

Proportion of juvenile individuals

There was a significant interaction effect between sampling distance and ant density (Chisq = 6.5, Df = 2, $p = 0.038$) (Fig. 14). At 1 meter the most distinct pattern was found with a large increase in the proportion of juveniles with increasing ant density (Fig. 14). While at 10 meters the proportion of juveniles stayed stable with increasing ant density and in 5 meters we observed a small decrease in the proportion of juveniles with increasing ant density (Fig. 14).

Not included in the model were the nine juveniles found in the nests.

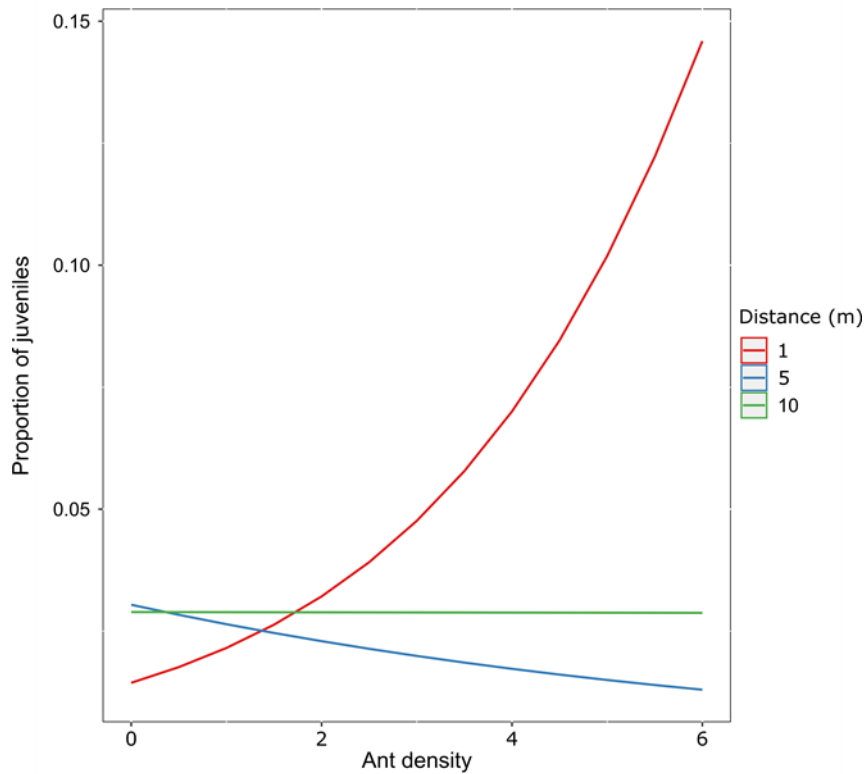


Fig. 14 The effect plot displays the effect of the ant density (fourth-root transformed) and sampling distance from the nest on the abundance of juvenile *P. scaber* individuals per pitfall (fourth-root transformed).

Proportion of females

In the adult individuals the proportion of females increased significantly further away from the nests (Chisq = 8.0, Df = 2, $p = 0.018$) (Fig. 15). But only the difference between 1 meter and 10 meter was significant where 60.4% of all caught adults were female at 1 meter, and 64.3% of them were females at 10 meter ($p = 0.023$) (Fig. 15).

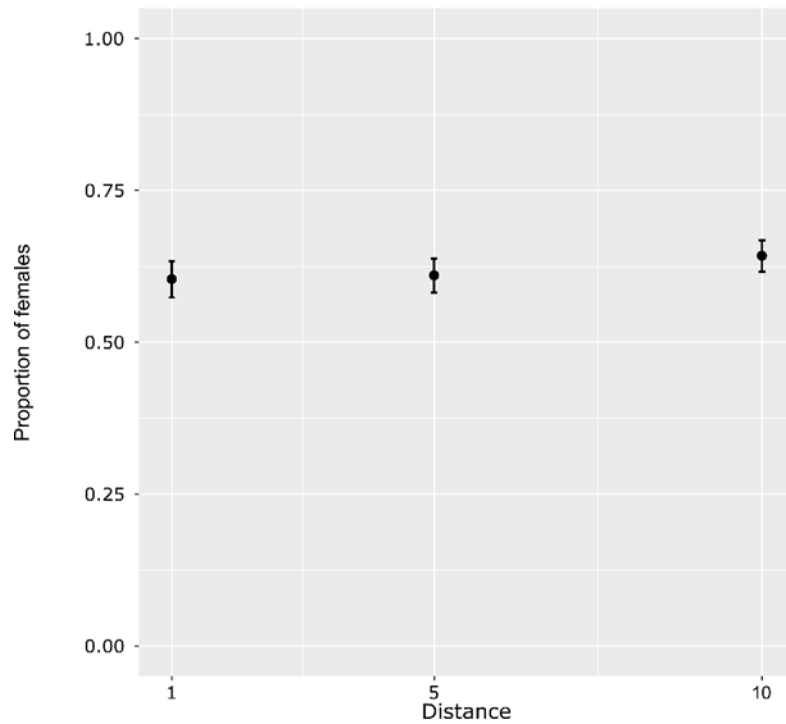


Fig. 15 The effect plot displays the effect of sampling distance from the nest on the proportion of female *P. scaber* individuals in the adults.

Proportion of pregnant females

The proportion of pregnant females increased significantly with increasing ant density independent of the sampling distance (Chisq = 6.7, Df = 1, p = 0.011) (Fig. 16). With increasing sampling distance the proportion of pregnant females also increased significantly (Chisq = 10.1, Df = 2, p = 0.006) (Fig. 16). At 10 meters there was a significant higher pregnancy proportion than at 1 meter (p = 0.0048), while the pregnancy proportion at 5 meter did not differ from the other sampling distances.

In the nests 12 pregnant females were found, but they were not included in the model.

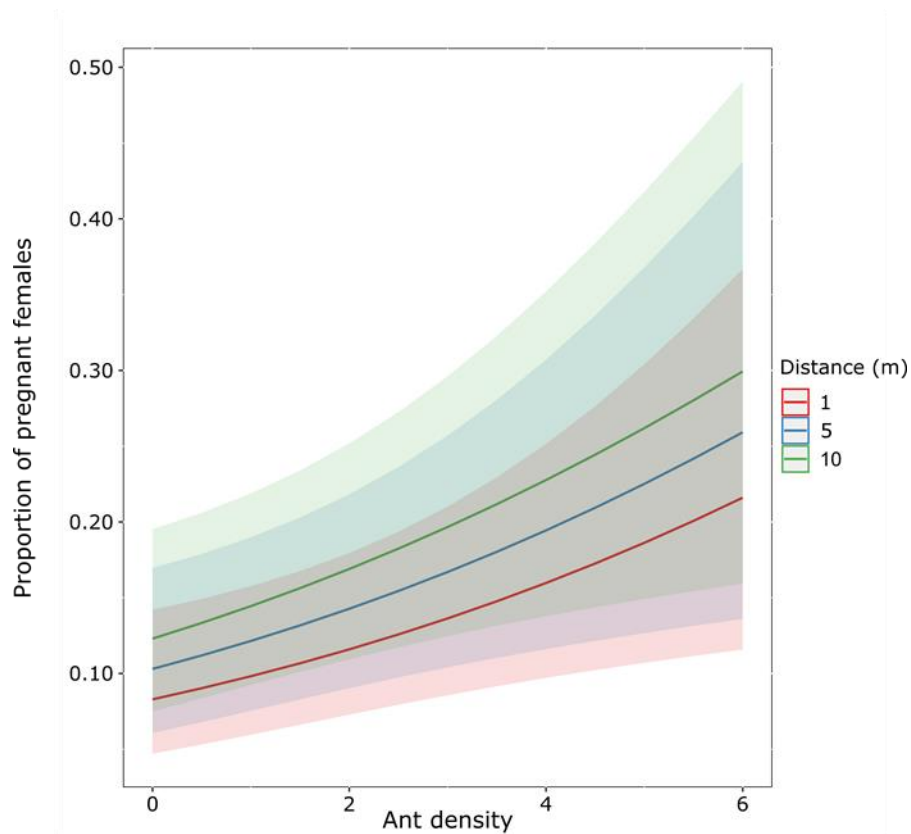


Fig. 16 The effect plot displays the effect of the ant density (fourth-root transformed) and sampling distance from the nest on the proportion of pregnant female *P. scaber* individuals.

Proportion of individuals with missing antennae

There was a significant increase in the amount of isopods missing one or two antennae with increasing ant densities (Chisq = 4.4, Df = 1, $p = 0.036$) (Fig. 17). There was also an extremely significant difference between all three sample distances (Chisq = 38.0, Df = 2, $p < 0.001$), with the most damaged individuals at 1 meter, less at 5 meters and even less at 10 meters ($p_{1/5} = 0.003$; $p_{1/10} < .001$; $p_{5/10} = 0.001$) (Fig. 17).

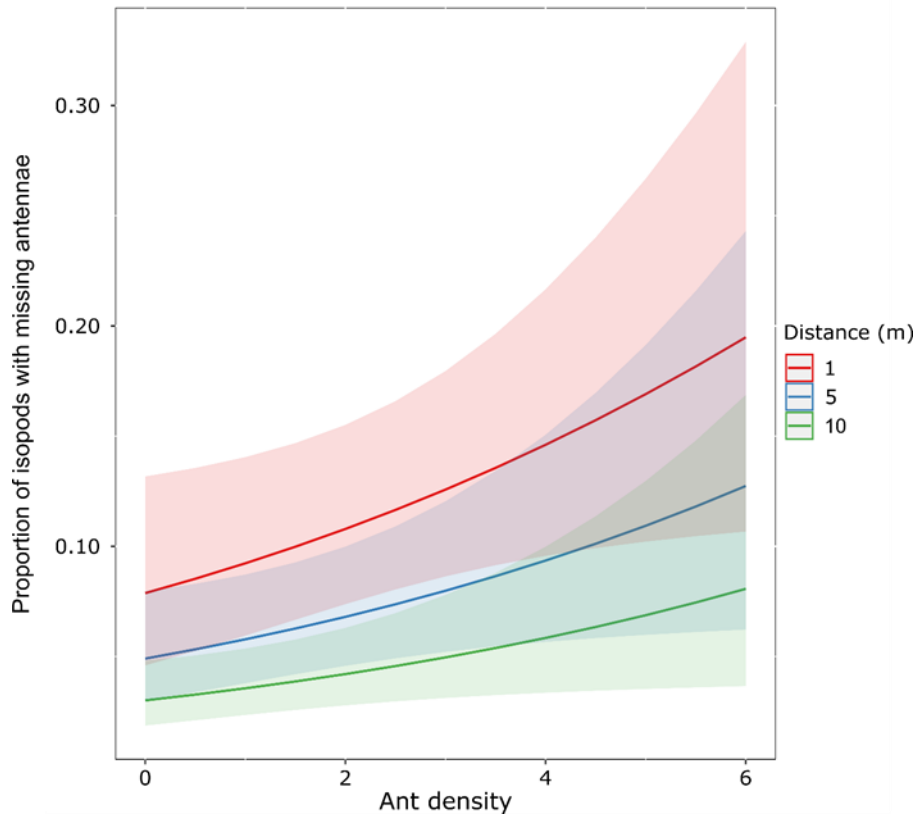


Fig. 17 The effect plot displays the effect of the ant density (fourth-root transformed) and sampling distance from the nest on the proportion of *P. scaber* individuals missing antennae.

Behavioural experiments

Total aggressivity

When the aggressivity towards the five different isopod species was assessed without taking initial behaviour into account, there was a significance difference in aggressivity of the ants towards the different isopod species (Chisq = 119.8, Df = 4, $p < 0.001$) (A = *P. hoffmanseggi*, B = *O. asellus* & *P. scaber*, C = *A. vulgare* & *P. muscorum*) (Fig. 18). The obligate myrmecophilous isopod *P. hoffmanseggi* provoked significantly less aggressive reactions from the ants than the other four species ($p < 0.001$) (Fig. 18). *Oniscus asellus* and *P. scaber* were aggressively approached a similar amount of times ($p = 1$), but significantly less than *A. vulgare* and *P. muscorum* ($p < 0.001$) who were approached aggressively a similar amount of times ($p = 0.960$) (Fig. 18).

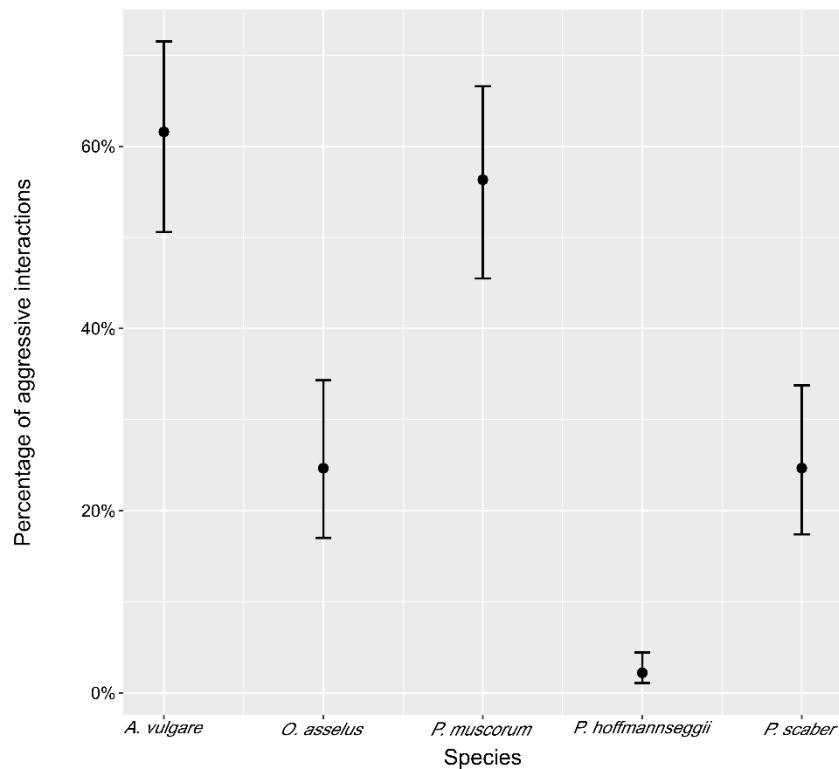


Fig. 18 The effect plot displays the percentage of aggressive interactions the five isopod species (*A. vulgare*, *O. asellus*, *P. muscorum*, *P. hoffmanseggi* and *P. scaber*) endured during the behavioural experiments.

Due to the extremely low amount of aggressive interaction (12 out of the 400 scored interactions) with *P. hoffmanseggi* they were not included in following results.

Effect initial behaviour isopod on ants aggression

Porcellio scaber had significantly more aggressive interactions when it had running as initial behaviour (61%) while not moving and walking had much lower percentage of aggressivity, 10.9% and 17% (Chisq = 63.7, Df = 2, $p < 0.001$) (Fig. 19). *Oniscus asellus* on the other hand, who had similar levels of aggressive interactions in total, had a difference in aggressive response from the ants in both not moving (8.6%), walking (47.5%) and running (76.1%) (Chisq = 79.649, Df = 2, $p < 0.001$) (Fig. 19). *Armadillidium vulgare*, which was the only one who could fold itself in a ball, showed just as the previous two species the highest aggressivity rate when running (76.6%), but there was no significant difference between walking or running (73.6%) ($p = 0.985$) (Fig. 19). The difference between running/walking and not moving (32.9%) was significant (Fig. 19). Being in a ball as initial behaviour had no significant difference with all the other behaviours (Fig. 19). Unlike the others, *P. muscorum* did not have any significant difference in aggressivity shown towards itself with changing initial behaviour (Chisq = 2.7, Df = 2, $p = 0.262$) (Fig. 19).

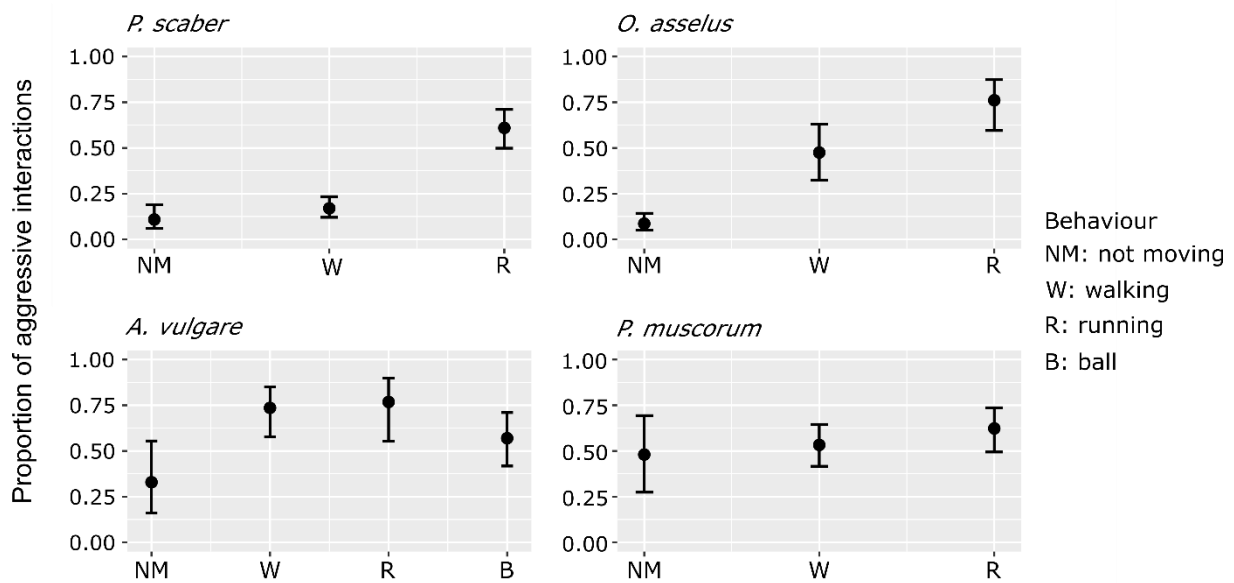


Fig. 19 The effect plot displays the proportion of aggressive interactions the four isopod species (Top left: *P. scaber*; Top right: *O. asellus*; Bottom left: *A. vulgare*; Bottom right: *P. muscorum*) endured depending on their initial behaviour.

The reaction of the isopod on the behaviour of the ants

Porcellio scaber individuals ran away significantly more when approached in an aggressive way by an ant than when approached more passively ($p < 0.001$). Walking and short stopping on the other hand were done significantly less when *P. scaber* was approached aggressively ($p = 0.053$ & $p = 0.003$) (Fig. 20).

Oniscus asseilus, like *P. scaber*, resorted more to running when approached in an aggressive way by an ant ($p < 0.001$). Not moving as a behavioural reaction, in the contrary to running, was used less when facing aggressive ants ($p < 0.001$) (Fig. 20). Different to *P. scaber*, *O. asseilus* mostly did not move when approached passively, while this behaviour was shown less with *P. scaber*.

Armadillidium vulgare did not have any significant change in behaviour when approached differently by the ants (Chisq = 7.7, Df = 4, $p = 0.104$). In both cases being in a ball was the most used strategy (Fig. 20).

Unlike *A. vulgare*, *P. muscorum* did have significant changes in behaviour (Chisq = 88.2, Df = 3, $p < 0.001$). *Philoscia muscorum*, just as *P. scaber* and *O. asseilus*, showed more running behaviour when aggressively approached ($p < 0.001$). This was at a decrease of walking when aggressively approached ($p < 0.001$) (Fig. 20).

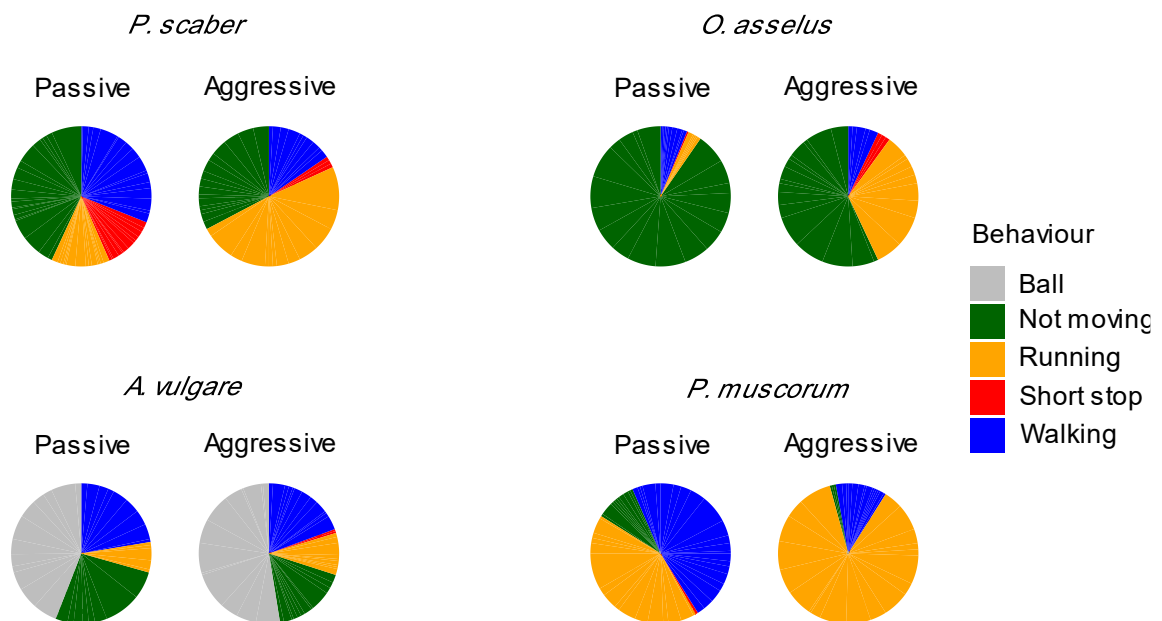


Fig. 20 The plot displays the behavioural changes of the four isopod species (Top left: *P. scaber*; Top right: *O. asseilus*; Bottom left: *A. vulgare*; Bottom right: *P. muscorum*) depending on passive or aggressive approached by the ants.

Discussion

Porcellio scaber was found around red wood ant nests and even within the nests. This is paradoxical due to the fact that they make up a part of the diet of red wood ants (Loones et al., 2008). Apart from *P. hoffmanseggi*, an obligate myrmecophilous isopod, *P. scaber* seems to be the only isopod species in association with the sampled red wood ant colonies. This research provides new insights into the association between red wood ants and a facultative associated isopod, *P. scaber*. Overall, our research demonstrated that the ants do have an influence on *P. scaber*'s spatial distribution as well as on their life history traits.

Ant density and proximity to the nest strongly influenced the size of *P. scaber* individuals. They were generally smaller at higher ant densities and closer to the nests. Myrmecophiles in general have a size that does not exceed the size of their hosts' workers and are mostly smaller than those workers (Hughes et al., 2008; Parker, 2016). A smaller size may help them to avoid detection by ants' workers (Parker, 2016). A smaller size may help to avoid detection by ants' workers (Parker, 2016), this has been shown also with for example bats, which have problems successfully detecting small prey (Barclay & Brigham, 1991; Blanckenhorn, 2000). The smaller size together with the low CHCs might lower the detection rate drastically. Being small might also help them against becoming subdued by groups of ant workers when being detected (Parker, 2016). Because of that, it is most likely that bigger *P. scaber* individuals avoid contact with the ants and move further away from the ant nests, where the ant trail density is higher, and therefore, avoiding higher ant densities.

The abundance of *P. scaber* adults found around red wood ant nests stayed fairly constant at 5 and 10 meters from the nest with changing ant density, while at 1 meter, *P. scaber* abundance decreased with increasing ant density. Most likely, the density of ant trails at different distances from the nest influenced the abundance of *P. scaber*. Prudent behaviour is a simple behaviour yet very efficient and wide-spread strategy to avoid attacks of enemies (Parmentier et al., 2018). As a facultative generalist, *P. scaber* does not possess specialist traits such as chemical mimicry and morphological adaptations, and that is why they probably resort to avoiding behaviour in combination with being chemical insignificant (Parmentier, 2017a) and having a hard exoskeleton. When combining these results with the size of individuals at 1 meter at high ant densities, only smaller individuals were found. Closer to the nests and at higher ant densities, smaller individuals will probably be less noticed by the ants. Hypothetically smaller individuals will then resort less to avoiding behaviour, in the contrary to larger individuals. Avoidance behaviour of unpreferred circumstances by *P. scaber* is not new, it was already recorded in a toxicological experiment (Zidar et al., 2019). The total biomass of all *P. scaber* individuals per pitfall, which included juveniles, had the same pattern as the abundance of adult individuals. The total biomass on 1 meter also decreased with increasing ant densities, while on 5 and 10 meters no remarkable changes were noticed with changing ant densities. This was expected because the weight of juveniles can be perceived as negligible.

Similarly, the proportion of juveniles at 5 and 10 meters was more or less the same with changing ant density. On the other hand, at 1 meter, there was an increase in the proportion of juveniles with increasing ant density. This was just the opposite pattern observed in adults, matching the previous findings. Juveniles have a very small size and will likely be noticed even less, having fewer problems with higher ant densities. At the same time, adults, who are bigger, will avoid those high ant density areas. The presence of a multitude of small arthropod species in red wood ants nests also suggests that small size is an advantage (Robinson & Robinson, 2013).

The proportion of pregnant females increases further away from the nests, as expected. However, our hypothesis was that pregnant females would avoid being around ants because of their reduced mobility and agility and therefore, the high density of ant trails closer to the nests would probably be not ideal for them. Contradictory to our hypothesis, is that proportionally more pregnant females were found at high ant densities. A possible explanation is that further away from the nests, pregnant isopods use the ant trails as an enemy safe space. They would be next to ant trails and when predators such as *Dysdera crocata*, a specialist woodlice preying spider, or less efficient predators (Pekár et al., 2016) come close the pregnant isopods will resort to fleeing behaviour (Seidl et al., 2012) to an ant trail. Here the ants will attack the predator of the isopod and the isopod will rely on its low CHC amounts and its hard exoskeleton to avoid and/or survive attacks from the ants. Although the isopod would prefer a lower ant density, it is a temporary safer space. Hypothetically, when the danger is gone, it would position itself back next to the ant trail. However, we were unable to observe that due to our sampling method that made the isopods unable to escape the pitfalls and hereby were also not able to leave the ant trail.

A relatively high proportion of isopods missed one or both of their antennae, which is likely caused by aggressive red wood ants *Porcellio scaber* is not able to hide its antennae under its hard exoskeleton so they are exposed to an attack. The higher the ant density, the more likely the isopod has encountered an ant attack and lost one or two of its antennae (Kronauer & Pierce, 2011; Liang & Silverman, 2000) Also, closer to the nests, proportionally more mutilated isopods were found. However, closer to the nest does not automatically imply that there will be a higher ant than further away from the nest. The main difference is that closer to the nests a higher density of ant trails will be found. So also at 1 meter, pitfalls were installed next to ant trails and will consequently have lower ant densities. To reach a low ant density place at 1 meter the individually probably had to cross multiple ant trails in this high ant trail density space. While further away from the nests, at 5 and 10 meters, the density of ant trails is much smaller and hereby the chance that an isopod has crossed one or multiple ant trail decreases.

Although living with the ants has apparently negative effects on *P. scaber*, there are also positive effects. It has been shown that there was an increased mortality of *P. scaber* with red wood ants (Parmentier et al., 2016c). But the red wood ant nests might still be sources rather than sinks when the benefits of the thermoregulated, moist and protected nests with resources outweigh the negative effects (Hughes et al., 2008; Kronauer & Pierce, 2011; Parmentier et al., 2016c). Because of the presence of pregnant individuals in the nests, it is likely that there are no major negative effects and that they can complete their complete lifecycle in the nests. But when these positive effects outweigh the negative effects is not yet clear.

The significant difference in sex-ratio between 1 and 10 meters is minimal. Only a slight increase in the abundance of females was found at 10 meters in comparison to the isopods caught at 1 meter. This slight difference is possibly of minimal biological relevance.

Out of the behavioural experiments, there was a clear difference in attack rate by the ants found between three groups. Firstly, the obligate myrmecophile *P. hoffmannseggi* was attacked the least, as expected from an obligate myrmecophile living inside the nests (Kronauer & Pierce, 2011). Secondly, *P. scaber* and *O. asselus* were attacked more than *P. hoffmannseggi* but less than *A. vulgare* and *P. muscorum*. From *P. scaber*, this was expected because it was already defined as a facultative associate (Parmentier et al., 2014) while *O. asselus* was not. Although both are drought resistant species (De Smedt et al., 2016), *O. asselus* was not abundant around the red wood ant nests. While looking at the initial behaviour of both *P. scaber* and *O. asselus*, a plausible explanation of why *O. asselus* was not found around red wood ant nests was found. Although they were both attacked a similar number of times, the attack rate on *O. asselus* increased when it was in motion, while *P. scaber*'s attack rate increased only when it was running. For *O. asselus*, avoiding attacks by not moving also means that it cannot forage, search for a mate and reproduce (Cooper Jr & Frederick, 2007; Jennions et al., 2003; Martín & Ló, 1999; Parmentier et al., 2018). This would be a huge trade-off where the costs would be too high, and it would explain why *O. asselus* is not found in big numbers around red wood ant nests as seen in *P. scaber*. On top of that, even though *O. asselus* is a drought resistant species, it is still way less resistant than *P. scaber*, and this might also explain the difference in abundance of the two species found around the nests (De Smedt et al., 2016). Both *A. vulgaris* and *P. muscorum* had a high attack rate from the ants and that is probably why they are not associated with red wood ants. When looking at all four isopod species who are not obligatory associated with red wood ants, the main trend is that running provokes the most attacks from the ants. This is because fast-moving individuals are more efficiently detected by red wood ants (Dorosheva et al., 2011; Parmentier et al., 2018).

While running is counteractive for many species, *P. scaber* mainly resorted to running when attacked by the ants. This flight reaction can explain multiple findings of the spatial demography of *P. scaber* in the field. First of all, bigger individuals will probably be noticed more by the ants and will consequently have a bigger chance of being attacked. When this happens, they will likely run away to an area where they encounter fewer ants. At the same time, smaller individuals will hypothetically encounter fewer aggressive interactions and hereby have fewer flight reactions. The abundance change of adults at 1 meter with increased ant density can also be explained by this running behaviour.

Oniscus asselus, just as *P. scaber*, also had an increased running behaviour when aggressively approached. *Philosia muscorum* also used running in most of its reactions after an aggressive encounter with an ant. This is as expected because *P. muscorum* is unable to tuck its legs under its body. Hereby *P. muscorum* only has running as an option to avoid being killed. On the other hand, *A. vulgare* did not show changes in behaviour when being aggressively or passively approached by the ants. In both cases, they were rolled up in a ball most of the time. This defensive behaviour is an evidence that that *A. vulgare* is actually not able to live with the ants.

Because three out of the four non-obligatory associated isopod species resorted to running when attacked, it seems that *P. scaber* has not developed special adaptations to deal with the ants. It rather uses its hard exoskeleton for protection and its low CHC amounts to avoid being detected. These pre-adaptive traits also allow *P. scaber* to be a guest in other social insect nests, such as in honey bee-nests (Kärcher & Ratnieks, 2010). Because of the relatively low attack rate on *P. scaber* and the morphological protection it has against those attacks, combined with the avoidance behaviour to more suitable places, it is likely that a low number of individuals are killed by the ants. Hereby, we hypothesise that red wood ants scavenge most of the *P. scaber* individuals they bring to the nest. Dead *P. scaber* individuals have a penetrant smell making them more likely to be detected than the alive individuals who are almost odourless.

Future research

Due to delayed and/or failed experiments together with being too ambitious, we were not able to conduct all experiments we envisioned. Hereunder, three experiments, that can be conducted in the future, are described with their expected outcome.

The pregnancy duration and the number of offspring of *P. scaber* females with and without ants were going to be tested. An incubation period of the females was performed to avoid using individuals who were already in an early stage of pregnancy prior to collecting them in the field. A sudden death of a large number of females during this incubation period made it impossible to continue the experiment. Collecting new individuals and putting them in an incubation period would have delayed the experiment considerably. The aim of this experiment was to put together individuals of both genders and have different groups with ants and control groups without ants. Based on previous research with a closely related species (Castillo & Kight, 2005), the duration of the pregnancy was expected not to change in the group with the ants. However, since previous research on another isopod species showed that some species under stress release their offspring earlier (Castillo & Kight, 2005), we wanted to test it for *P. scaber*. Additionally, it was also expected that the group with the ants would have fewer offspring due to the stress-induced oocyte loss (Warburg, 1994).

Combined with that experiment, the survival of the four non-obligatory associated isopod species used in the behavioural experiments with and without ants would have been tested. The survival of the species was going to be checked every two days. Due to delay with other experiments, we were not able to conduct this experiment. Here it was expected that *P. scaber* would have the highest number of surviving individuals in the groups exposed to the ants while the other three species would have a decrease in survival. In hindsight with the results from the behavioural experiments, *A. vulgare* and *P. muscorum* individuals would probably not last long with the ants due to the high attack rate they endured, *O. asselus*, on the other hand, *O. asselus*, would probably survive longer than those two, because of the lower attack rate. Their survival would probably be similar to *P. scaber* because both species preferred to running when attacked, leading to more attacks.

A final experiment was planned, where dead and alive *P. scaber* individuals were going to be presented to the nests. This would give support our hypothesis that most *P. scaber* individuals brought to the nests were scavenged. However, due to unpredictable weather and lots of precipitation the last months, we were not able to carry it out.

Conclusion

Overall this study could link behavioural experiments with the spatial demography of *P. scaber* around red wood ants in the field.

Porcellio scaber was aggressively approached by the ants less than other species, and a relatively low amount of times. But when it was aggressively approached it had flight behaviour. This avoidance behaviour was probably the explanation for some of the spatial demography results. As with increasing isopods the detection rate likely increases, and consequently the attack rate, bigger individuals and adults will more show this flight reaction. This would explain their spatial distribution, while smaller individuals and juveniles probably would endure less attacks. Although living with the ants has apparently negative effects on *P. scaber*, there are also positive effects.

The red wood ant nests might still be sources rather than sinks when the benefits of the thermoregulated, moist and protected nests with resources outweigh the negative effects. Because of the presence of pregnant individuals in the nests, it is likely that there are no major negative effects and that they can complete their lifecycle in the nests. But when these positive effects outweigh the negative effects is not yet clear.

Comparing the results of the behavioural experiments of *P. scaber* with the other isopod species, it is likely that *P. scaber* has not developed special adaptations to deal with the ants. It rather uses its hard exoskeleton for protection and its low CHC amounts to avoid being detected. Because of the relatively low attack rate on *P. scaber* and its morphological protection against those attacks, combined with the avoidance behaviour to more suitable places, it is likely that a low number of individuals are killed by the ants. Hereby, we hypothesise that red wood ants scavenge most of the *P. scaber* individuals they bring to the nest. Dead *P. scaber* individuals have a penetrant smell making them more likely to be detected than the alive individuals who are almost odourless.

Further studies in this field would help to clarify when negative effects outweigh the positive effects. This would give us a better understanding about the ecology of facultative associates of ants.

Summary

Background: Red wood ant nests form an important micro-habitat for many associated species. One facultatively associated species, that can be found at high abundances around and in the nests, is the rough isopod *Porcellio scaber*. Paradoxically to their high abundance is that they are part of the ant's diet.

We examined how a stressful ant environment affects the distribution and life history of *P. scaber*. This was done by looking at their spatial demography around the nests, scoring life history parameters around the nest and with behavioural experiments in the lab.

Results: We found that at 1 meter from the nests increasing ant densities had a negative effect on the abundance of adult individuals as well as the total biomass. Moreover, we found that large isopods and less isopods with missing antennae were found further away from the nests and at high ant densities. The opposite pattern was found in the proportion of juveniles. Additionally, the proportion of pregnant females increased further away from the nests and at higher ant densities.

In the behavioural experiments *P. scaber* provoked lower levels of aggression and were mostly ignored compared to other abundant terrestrial isopod species. *Porcellio scaber* likely stays undetected by a low amount of chemical cues as demonstrated before, which the other species probably do not possess. Additionally, they also show avoiding behaviour, which might explain the spatial demography of the species.

Conclusion: *Porcellio scaber* has not developed advanced adaptations to deal with the ants. It likely stays undetected by a low amount of chemical cues. When discovered, it may make use of its hard exoskeleton or show avoidance behaviour to less stressful places. It is likely that a low number of individuals are killed by the ants. Although living with the ants has negative effects, there are also positive effects, in the form of protection against enemies and resource availability. It is yet not clear when the positive effects outweigh the negative.

Samenvatting

Achtergrond: Rode bosmier nesten zijn een belangrijk micro-habitat voor veel geassocieerde soorten van rode bosmieren. Een facultatief geassocieerde soort, die in grote aantallen te vinden is rond en in de nesten, is de ruwe pissebed *Porcellio scaber*. Paradoxaal aan de grote aantallen is dat de ruwe pissebed deel uitmaakt van het dieet van rode bosmieren.

Wij onderzochten de invloed van een stressvolle omgeving, gecreëerd door de mieren, op de ruimtelijke demografie rond de nesten, kijkend naar de levensgeschiedenis rond de nesten. Dit werd gecombineerd met gedragsexperimenten in het labo.

Resultaten: Wij vonden dat er op één meter van de nesten een toename was in het aantal mieren. Dit had een negatief effect op het aantal volwassene *P. scaber* individuen en de totale biomassa ervan. Bovendien werden grotere pissebedden en individuen zonder beschadigde voelsprieten proportioneel meer gevonden verder van het nest en bij lagere mieren aantallen. Daarnaast werden er proportioneel meer zwangere individuen gevonden verder van het nest en bij hogere mieren aantallen.

In de gedragsexperimenten zagen we dat *P. scaber* minder agressief werd benaderd door de mieren dan andere abundante pissebedden. Dit komt waarschijnlijk doordat ze een lage hoeveelheid chemische signalen hebben en hierdoor minder worden gedetecteerd, terwijl de andere soorten dit waarschijnlijk niet hebben. Bovendien vertoont *P. scaber* ontwijkingsgedrag die waarschijnlijk hun ruimtelijke demografie verklaart.

Conclusie: *Porcellio scaber* heeft geen geavanceerde adaptaties ontwikkeld om met mieren te leven. Vooral hun lage chemische signalen samen met hun hard exoskelet en ontwijkingsgedrag naar minder stressvolle plaatsen helpen hun waarschijnlijk om samen te leven met mieren. Het is aannemelijk dat lage aantallen *P. scaber* individuen worden gedood door de mieren. Niet tegenstaande dat de mieren *P. scaber* negatief beïnvloeden, zijn er ook een aantal positieve punten aan leven met deze mieren, zoals bescherming tegen vijanden en de aanwezigheid van voldoende voedsel. Het is nog niet duidelijk wanneer de positieve effecten opwegen tegen de negatieve.

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Appendix 1

Table 4 Information about coordinates, city and habitat of each sampled inhabited nests to justify the choice of site.

Site	Nest	Coordinates	City	Habitat
1	A	51,1703421 ° N 3,1567128 ° E	Bruges	Heath land, receiving a lot of sunlight
1	B	51,170473 ° N 3,1565637 ° E	Bruges	Heath land, receiving a lot of sunlight
1	C	51,170473 ° N 3,1565637 ° E	Bruges	Heath land, receiving a lot of sunlight
1	D	51,1709161 ° N 3,154444 ° E	Bruges	Heath land, receiving a lot of sunlight
1	E	51,1709161 ° N 3,154444 ° E	Bruges	Heath land, receiving a lot of sunlight
2	F	51,1753562 ° N 3,1399624 ° E	Bruges	Next to a grassland area under big oak trees
2	G	51,1747689 ° N 3,1426264 ° E	Bruges	Next to a grassland area under big oak trees
2	H	51,1745227 ° N 3,1427744 ° E	Bruges	Next to a grassland area under big oak trees
2	I	51,1745227 ° N 3,1427744 ° E	Bruges	Next to a grassland area under big oak trees
3	J	51,1753173 ° N 3,1363078 ° E	Snellegem	Coniferous forest, receiving less sunlight
4	L	51,1750187 ° N 3,1370439 ° E	Snellegem	Open grassland area
5	R	50,8853419 ° N 2,6972826 ° E	Vleteren	At the forest edge of deciduous forest
5	S	50,8853419 ° N 2,6972826 ° E	Vleteren	At the forest edge of deciduous forest
5	T	50,8853419 ° N 2,6972826 ° E	Vleteren	At the forest edge of deciduous forest
6	N	51,1754484 ° N 3,1373572 ° E	Snellegem	Under vegetation, low sunlight receiving
6	M	51,1754001 ° N 3,137338 ° E	Snellegem	Under vegetation, low sunlight receiving
7	P	51,1733449 ° N 3,1419038 ° E	Snellegem	At the edge of a coniferous forest with a pasture
7	Q	51,1733449 ° N 3,1419038 ° E	Snellegem	At the edge of a coniferous forest with a pasture

Appendix 2

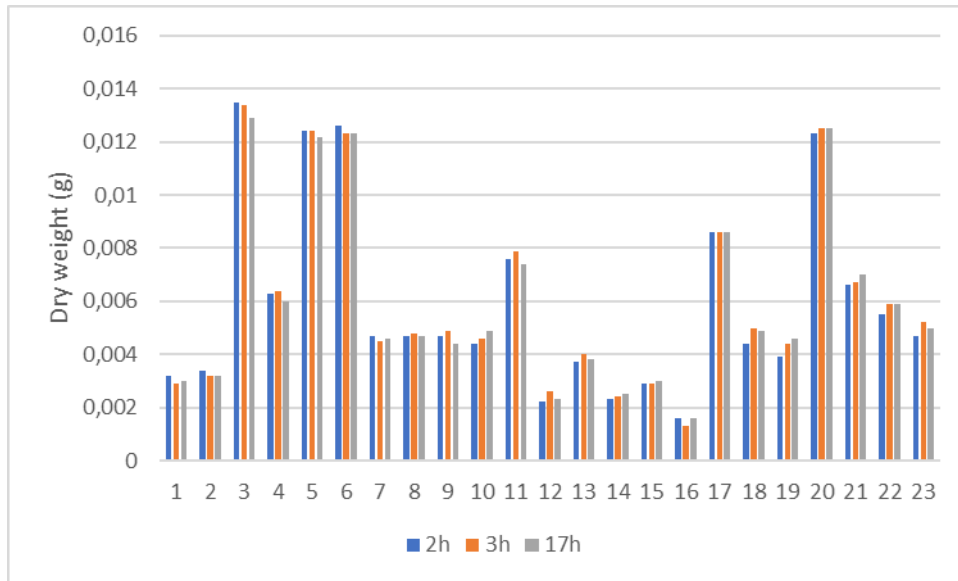


Fig. 21 The dry weight of 23 individuals at three different timepoint (2, 3 and 17 hours).