Indirect, tri-trophic effects of fear on biodiversity

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To my father, who was always proud of me

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Original publications and manuscripts

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JAE designed the experiment and methodology. All authors improved and broadened the experiment methodology; CMF and JAE collected all data. CMF and JAE performed the analyses, JAE and MD contributed with analysis tools. All authors contributed equally to the drafts and final version for publication.

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Chapter II *So many choices, so little time: food preference and movement vary with landscape of fear*

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All authors conceived and designed the experiment. CMF and MD collected all data. CMF performed the analyses with feedback from JAE and MD. All authors contributed equally to the drafts and final version for publication.

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Chapter III   *Effects of the invasive raccoon on rodent foraging behaviour*

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CMF, MD, CS and MR conceived and designed the experiment methodology, with support by JAE. CMF, CS, MD and MR conducted the field work. CMF, CS and MR analysed data from camera traps, CMF performed the analyses with feedback from JAE and MD. CMF, JAE and MD wrote the first draft, which was further edited by CS and MR. Manuscript was finalized by CMF, JAE and MD.

Manuscript for submission

I Abstract

Predator-forager interactions are a major factor in evolutionary adaptation of many species, as predators need to gain energy by consuming prey species, and foragers needs to avoid the worst fate of mortality while still consuming resources for energetic gains. In this evolutionary arms race, the foragers have constantly evolved anti-predator behaviours (e.g. foraging activity changes). To describe all these complex changes, researchers developed the framework of the landscape of fear, that is, the spatio-temporal variation of perceived predation risk. This concept simplifies all the involved ecological processes into one framework, by integrating animal biology and distribution with habitat characteristics. Researchers can then evaluate the perception of predation risk in prey species, what are the behavioural responses of the prey and, therefore, understand the cascading effects of landscapes of fear at the resource levels (tri-trophic effects). Although tri-trophic effects are well studied at the predator-prey interaction level, little is known on how the forager-resource interactions are part of the overall cascading effects of landscapes of fear, despite the changes of forager feeding behaviour - that occur with perceived predation risk - affecting directly the level of the resources.

This thesis aimed to evaluate the cascading effects of the landscape of fear on biodiversity of resources, and how the feeding behaviour and movement of foragers shaped the final resource species composition (potential coexistence mechanisms). We studied the changes caused by landscapes of fear on wild and captive rodent communities and evaluated: the cascading effects of different landscapes of fear on a tri-trophic system (I), the effects of fear on a forager's movement patterns and dietary preferences (II) and cascading effects of different types of predation risk (terrestrial versus avian, III).

In Chapter I, we applied a novel measure to evaluate the cascading effects of fear at the level of resources, by quantifying the diversity of resources left after the foragers gave-up on

foraging (diversity at the giving-up density). We tested the measure at different spatial levels (local and regional) and observed that with decreased perceived predation risk, the density and biodiversity of resources also decreased. Foragers left a very dissimilar community of resources based on perceived risk and resources functional traits, and therefore acted as an equalising mechanism.

In Chapter II, we wanted to understand further the decision-making processes of rodents in different landscapes of fear, namely, in which resource species rodents decided to forage on (based on three functional traits: size, nutrients and shape) and how they moved depending on perceived predation risk. In safe landscapes, individuals increased their feeding activity and movements and despite the increased costs, they visited more often patches that were further away from their central-place. Despite a preference for the bigger resources regardless of risk, when perceived predation risk was low, individuals changed their preference to fat-rich resources.

In Chapter III, we evaluated the cascading effects of two different types of predation risk in rodents: terrestrial (raccoon) versus avian predation risk. Raccoon presence or absence did not alter the rodents feeding behaviour in different landscapes of fear. Rodent's showed risk avoidance behaviours towards avian predators (spatial risk avoidance), but not towards raccoons (lack of temporal risk avoidance).

By analysing the effects of fear in tri-trophic systems, we were able to deepen the knowledge of how non-consumptive effects of predators affect the behaviour of foragers, and quantitatively measure the cascading effects at the level of resources with a novel measure. Foragers are at the core of the ecological processes and responses to the landscape of fear, acting as variable coexistence agents for resource species depending on perceived predation risk. This newly found measures and knowledge can be applied to more trophic chains, and inform researchers on biodiversity patterns originating from landscapes of fear.

II Zusammenfassung

Die Wechselwirkungen zwischen Raubtier und Beute sind ein wichtiger Faktor in der Evolution der Tierwelt, da sich die Raubtiere anpassen müssen, um ihre Beute besser jagen zu können und die Beutetiere vermeiden müssen, gefressen zu werden, während sie immer noch genügend Ressourcen für ihre täglichen Bedürfnisse verbrauchen. In diesem ständigen Kampf müssen die Beutetiere ihr Verhalten ständig ändern, da sie die Anwesenheit von Raubtieren fürchten. Die Landschaft der Angst ist ein Rahmen, der alle ökologischen Prozesse beschreibt, die ablaufen, wenn die Tiere das Raubtierrisiko auf unterschiedliche Weise wahrnehmen. In Angstlandschaften reichen die indirekten Auswirkungen der Angst vor einem Raubtier aus, um eine Vielzahl von Reaktionen bei den Beutetieren hervorzurufen und folglich die Art und Weise zu beeinflussen, in der die Beutetiere Naturgutstype fressen (tritrophe Effekte).

Während die Interaktionen zwischen Raubtieren und Beutetieren gut erforscht sind, fehlt es an Wissen darüber, wie die Landschaft der Angst die Interaktionen zwischen Beutetieren und Naturgutstype beeinflussen kann (z. B. Pflanzenfresser, die Pflanzen fressen). In dieser Arbeit untersuchten wir die Kaskadeneffekte (d.h. Domino Effekte), die Beutetiere auf Naturgutstype haben, wenn sie verschiedene Prädationsrisiken wahrnehmen. Insbesondere wollten wir untersuchen, wie die Beutetiere entscheiden, was sie fressen und wohin sie sich bewegen, wie sich diese Veränderungen auf die biologische Vielfalt der Ressourcen auswirken können und welche Folgen dies für die Evolution der Ressourcenarten hat.

Für alle unsere Studien haben wir Nagetiere als Modellarten verwendet. Wir entwickelten ein neues Maß zur Quantifizierung der Auswirkungen von Angst auf die biologische Vielfalt von Ressourcen und testeten es erfolgreich an wilden Nagetierpopulationen. Wir konnten beobachten, dass die Nagetiere unterschiedliche Samenarten und -mengen fressen, je nachdem, wie sie das Raubtierrisiko einschätzen und abhängig von den Eigenschaften der

Samen und der Art der vorhandenen Raubtiere (terrestrische oder aviäre Fleischfresser). Wir konnten diese Veränderungen quantifizieren und Vorhersagen darüber machen, wie sich der Wettbewerb zwischen den Samen um das Wachstum verändern würde (Koexistenzmechanismen). Mit diesem Wissen haben wir den Rahmen der Angstlandschaft um die komplexen Wechselwirkungen zwischen Beute und Ressourcen erweitert und können unsere Erkenntnisse auch dazu nutzen, um zu verstehen, wie weitere Tierarten die biologische Vielfalt anderer Arten verändern, indem wir einfach verstehen, wie ängstlich sie sind.

III General introduction

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Predation risk and fear in prey species

The evolution of animals is constantly shaped by predator-prey interactions in a constant arms race, as the fitness of both predator and prey species depend on whether they can feed and avoid becoming food themselves (Kappeler 2021). The risks are higher for prey animals, as the failure to avoid predators results in life termination and loss of fitness, while predators can try to hunt again if the prey escapes. Therefore, prey animals have evolved a set of anti-predator strategies to avoid the worst faith of mortality, from changes in morphology for blending in the environment (Stevens and Merilaita 2009), mechanical and chemical protection (Dugatkin 2020), to changes in behaviour that might include, for example, vigilance (Hunter and Skinner 1998, Treves 2000) or permanence in or closer to safe habitats (Lind and Cresswell 2006, Bhattacharyya et al. 2015).

There is a wide array of behavioural anti-predator strategies dependant on the prey's biology (Dugatkin 2020, Kappeler 2021), but all these tactics require trade-offs from the prey (therein foragers), as they must decide how much time and effort they can allocate to anti-predator behaviours or other activities, such as feeding on resources, mating, etc. (Lima and Dill 1990). These changes in behaviour particularly affect the feeding activities of foragers, as it is a crucial process to obtain energy for all the other ecological processes of the foragers. Just the presence of a predator in a habitat can cause disturbances in a forager's behaviour (non-consumptive effects) and based on this predation risk, animals must decide when to eat to temporally avoid predators (Lima and Bednekoff 1999), where to eat to avoid the riskier habitats (Mayor et al. 2009), and what resources they should eat to maximise their gains (Verdolin 2006). However, predation risk (i.e. likelihood that a forager is killed by a predator) cannot be directly measured by foragers, instead, they have an innate fear of possible predation risk that they measure with multiple cues, that is, perceived predation risk. Direct cues are those produced by a predator and sensed by foragers, such as visual, olfactory, auditory or tactile, while indirect cues can be any environmental feature that can correlate

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with potential risk or prevent predation (examples of direct and indirect cues comparison studies: Thorson et al. 1998, Orrock et al. 2004, Mella et al. 2014).

The multiple cues of potential predation risk and their importance vary greatly according to the predator and forager biology, as well the physical characteristics of the landscapes in which both predator and foragers inhabit. Furthermore, both perceived and real risk can vary in space and time (Palmer et al. 2022), as both predators and foragers move throughout the landscapes and experience temporal changes (e.g. season). The responses of animals to all these processes depends on the spatio-temporal variation in perceived predation risk by foragers – the landscape of fear (Gaynor et al. 2019). The term was first coined by Laundré et al. (2001), as they observed the changes in elk and bison behaviour caused by the re-introduction of wolves as an apex predator in Yellowstone National Park, USA. Just the novel presence of wolves caused changes in the foraging and reproductive behaviour of the big herbivores, as their perception of predation risk changed. The big herbivores had to allocate more time to vigilance, changed their distribution patterns and, consequently, lowered foraging time and resource consumption. This enabled for the plant community to recover in open habitats, which in its turn allowed some animal species to recover in numbers, such as the beaver (Ripple and Beschta 2012). The landscape of fear as an ecological process can create behaviourally mediated trophic cascades, as the changes in behaviour of foragers caused by perceived predation risk shape the interactions among trophic chains and can alter ecosystems (Matassa and Trussell 2011, Smith and Schmitz 2016, Mills et al. 2018). In the last decade, studies on natural or manipulated landscapes of fear allowed researchers to understand better the complex changes of behaviourally mediated trophic cascades as both top-down and bottom-up effects (Gaynor et al. 2021, Monk and Schmitz 2022, Palmer et al. 2022), as the landscape of fear is an ecological process that is both mediated and mediates several others (for a detailed framework, see Gaynor et al. 2019).

Measures of perceived predation fear

Before researchers can evaluate the impacts of the landscape of fear, it's necessary to actually measure the variation of perceived predation risk first. We cannot evaluate directly the mental state of animals to know exactly how are they perceiving risk and feel fear. Stress hormones measurements are difficult to study in the wild, as it's unknown if they relate to fear of a predator or other stressors (intra-guild/species competition, environmental conditions, human presence/handling, etc.; Clinchy et al. 2013). Instead, we must rely on indirect measures that can relate directly to the trade-off of avoiding predation with other activities. These are usually measured together with foraging activities, as it was mentioned before, this is a crucial activity that animals cannot avoid.

It is therefore important to understand how animals make decisions before and during foraging (when, where and what to eat). The most used and cited models on how animals forage are the optimal foraging theory (Pyke 1984, Stephens and Krebs 1986) and marginal value theorem (Charnov 1976). In the optimal foraging theory, its assumed that an animal tries to maximise its metabolic gains of foraging in the least amount of time possible (Stephens and Krebs 1986, Pyke 2019). Given a choice, the forager will decide to forage on the most profitable resources, which might be the resource with the most nutritional value and beneficial handling or consumption time, before moving to least profitable resources (Charnov 1976, Sih and Christensen 2001). The marginal value theorem models how much time an animal should spend in a patch before moving on to the next one (Krebs et al. 1974, Charnov 1976). The principle is simple: as the animal spends more time foraging in a patch, it depletes more and more resources, to a point where it's spending more time and energy looking for resources rather than actively feeding. At a certain threshold (optimal patch time), the animal must then make a decision to move to another patch (for model details and assumptions see Pyke, 2019). The model also takes into account the quality of the patch (the higher the patch quality, the more time a forager spends in there) as well the travel time to

other patches (higher travel costs between patches increases the time a forager spends in one patch). Therefore, there are lots of costs involved in the foraging decision of animals, that further impacts how they move in the landscape (Louzao et al. 2014).

The costs of movement are extensively described in the area of energy landscapes, that is, the movement of animals is explained by the varying costs of travel within a landscape with different environmental characteristics (Wilson et al. 2012, Shepard et al. 2013). As with optimal foraging theory, animal movement is predicted based on the minimization of costs of travel in relation to potential gains, including gains from foraging behaviours, and reducing the predation avoidance costs (Gallagher et al. 2017). Like the landscape of fear, habitat characteristics, the animal biology and distribution impact the decisions on how, where and when to move, and generate different behavioural responses from the animal. Animals also do not exist continuously in space, but live in a defined home range that might incorporate their preferred resources, therefore, animals have the additional cost of travelling between their living central-place to any possible foraging place. The model of optimal foraging theory can then be expanded with the central-place foraging theory (Orians and Pearson 1979, Wetterer 1989, Olsson and Bolin 2014), which describes the decision making process of going foraging when travelling costs are involved. The model makes the assumption that the probability that an animal will forage on a patch decreases with the distance to their central-place, unless the distant patches are of high quality, and therefore the gains of foraging surpass the costs of travelling (Schoener 1979, Fryxell 1999, Rosenberg and McKelvey 1999).

Knowing how animals decide to go forage, how they spend their time in a patch, as well the costs of travelling to and among patches, we can now start to include the perceived predation risk into the equation of the optimal foraging theory (Brown 1988, Brown and Kotler 2004). As previously mentioned, predation risk can have great costs for the foragers (Lima and Dill 1990), as animals must make a wide range of decisions to avoid the risk of mortality,

with the worst scenario being the failure to avoiding predation, and therefore the termination of the animal and any future gains.

When going into a patch, foragers must decide how long they will stay and feed on the resources based on the costs of foraging, the costs of perceived predation risk, and the costs of missed opportunities (i.e. activities that may increase the individual's fitness that cannot be done at the same time as foraging, for example, mate finding). When the cost of these three components surpass the gains of foraging, then the forager will give-up, stop feeding and leave the patch. Therefore, if one can experimentally manipulate the costs of foraging and missed opportunities, the harvest rate of a patch will be determined by the inverse of perceived predation risk. This method of measuring perceived predation risk was first proposed by Joel S. Brown (see Brown 1988), and makes use of a defined volume of a substrate and quantity of resources that the forage can deplete at a linear rate depending on how they perceive predation risk costs – giving-up density (GUD). In simpler terms, GUD is defined by the density of resources left in a patch once the forager decides to give-up on foraging, and with foraging and missed opportunity costs accounted for, the point where the forager gives-up is determined by perceived predation risk. As foragers perceive a patch as safer, their patch residency (time spent in a patch) increases and GUD decreases as the potential gains of feeding overcome the costs of potential predation. GUD has been widely used for many species to measure the landscape of fear (e.g. Jacob and Brown 2000, Altendorf et al. 2001, Petty and Grossman 2010, McMahon et al. 2018, Abdulwahab et al. 2019), as the optimal foraging theory and marginal value theorem that GUD is based on spans across many animal taxa.

With this measure of perceived predation risk, we can further understand the animal responses to landscapes of fear, specifically how much it can impact the spatio-temporal distribution of predators and foragers, as well the impactful chances of anti-predator behaviour. For example, we can see changes in the movement patterns of animals faced

with landscapes of fear. Movement increases the likelihood that a forager is seen by predators (overlap of energy landscape and the landscape of fear, see Gallagher et al. 2017), and travelling becomes especially risky and costly the more an animal moves away from it's central-place location (Vásquez 1994, Russell et al. 2007), unless the patches provide high quality resources (Bakker et al. 2005, Nilsson et al. 2020). GUD can then be used to analyse the interaction of movement costs with perceived predation risk (e.g. Eccard and Liesenjohann 2014), and in the given example, we would observe that patches further away from the central-place location would have a higher GUD than patches closer to the central-place, unless the resources were manipulated to be of higher profitability, then GUD would decrease only in the highly profitable patches further away.

Despite the many advantages of this simple method, GUD also has some caveats such as difficulty with producing standardized results when dealing with group foraging (e.g. Carthey and Banks 2015), individuals with different energetic states (e.g. starved animals will present lower GUDs despite predation costs; Sánchez et al. 2008) or personalities (e.g. bolder individuals might forage more on riskier patches; Dammhahn et al. 2022), as well problems with non-target species when applying experimental patches in the wild. Researchers must be aware of potential problems with GUD before any experiment and how to solve them (for a complete guide see Bedoya-Perez et al. 2013). Furthermore, there are problems in standardizing the GUD measure in different taxa due to the different types of food resources each forager species consumes (e.g. different nutrients, plant defences, etc.), as well with the different forager behaviours (e.g. scatter-hoarding). This often limits GUD to use one single type of resources within a defined substrate, which might not represent the natural landscape, in which a forager has a diverse community of resources to explore. Additionally, this limitation confined most studies using GUDs to predator-forager interactions, although there is the potential to extend the method to analyse tri-trophic effects with the addition of

the forager-resource interactions and understand in detail the cascading effects of landscapes of fear to the species biodiversity.

Coexistence in landscapes of fear

As previously mentioned, foragers make decisions under the optimal foraging theory assumptions, those are, that individuals maximise gains in the least amount of time possible and often forage on the most profitable resource. However, what is defined as the most profitable resource can vary among taxa, for example, profitability can be defined by how easily available a resource is, if it's easily handled and/or consumed by the forager, or even its nutritional content (Kappeler 2021, King and Marshall 2022). All of these characteristics of resources define the energetic gains for the forager, but are often also the functional traits for the resource species, that is, diverse characteristics that are expressed phenotypically and are relevant for the fitness of species (Violle et al. 2007). As the forager depletes different resources unevenly based on functional traits, not only the biodiversity of resources left in the landscape will change, but the competition advantage among resource species based on their functional traits can disappear, with foragers serving as coexistence mechanisms (HilleRisLambers et al. 2012, Kraft et al. 2015, Stump and Chesson 2017).

The coexistence theory was first developed by Peter Chesson and was further refined in several other publications by the author and colleagues (notable examples of publications: Chesson and Warner 1981, Chesson 1994, 2000, 2003). It describes how biodiversity of species is maintained through coexistence despite competition among species. While there is a lot of mathematical background to Chesson's coexistence theory, this thesis will focus on the qualitative resource niche responses to differential feeding from foragers (for a more detailed review on coexistence theory, see Barabás et al. 2018). Simplified, there are two mechanisms that promote coexistence and maintenance of biodiversity: equalising and

stabilising. In equalising mechanisms, the average fitness differences are reduced, that is, it lowers the degree of how one competitor is superior to another. An example from forager-resources interaction: in a community of seed bearing plants, the bigger and most nutritious seeds are most often the most competitive in terms of germination (e.g. Ignace and Chesson 2014, Lebrija-Trejos et al. 2016) and therefore have a higher fitness than plants with smaller and less nutritious seeds. However, larger and nutritious functional traits of seeds can also be preferred by granivore foragers, as predicted in the optimal foraging theory, since these seeds allow foragers to maximise their gains in the shortest period of time (Dylewski et al. 2020). With the time allocation constrains of perceived predation risk, foragers might only forage and possibly deplete these seeds, giving an opportunity for the smaller and less nutritious seeds to germinate (Reader 1993, Stump and Chesson 2017). This allows for coexistence of a diverse plant community, as the predation from granivores becomes an equalising mechanism. In stabilising mechanisms, the niche differences among different competitors becomes stable, as the intra-species competition overcomes inter-species competition and niche overlap is reduced. Unlike equalising mechanisms, which not all competitor species can have a positive growth rate, in stabilising mechanisms the competitors can present a positive growth rate. Another example from forager-resource interactions come from scatter-hoarding species (Lichti et al. 2017). Similarly to the example above, scatter-hoarders often remove the bigger seeds first, but instead of consuming them at the spot, they disperse the seeds to a caching location for later consumption. As not every seed that is cached is consumed (e.g. not found by the granivore in the future), these larger seeds might germinate in a newer location with more suitable conditions than seeds from the same species that were not dispersed or were consumed on the spot (Longland et al. 2001). Both mechanisms reduce the competitive advantages among species and often occur concurrently (Levine et al. 2017, Barabás et al. 2018).

The coexistence of species can therefore be maintained by variation of foraging behaviours. However, predation risk also affects foraging behaviours, therefore impacting the resulting biodiversity of resource species community and coexistence mechanisms as well (Germain et al. 2013). For example, in places where new predators where introduced, non-consumptive effects caused forager species to overlap their habitats and diets, ultimately destabilising the coexistence of forager species and even driving some populations to extinction (Pringle et al. 2019). Perceived predation risk might also promote coexistence of species, with an example being the famous Yellowstone National Park case studied by Laundré et al. (2001). When wolves were introduced, non-consumptive effects also caused elks to change their habitat and foraging distribution, alleviating the pressure of overgrazing in plants in open habitats where elks were more visible to wolf predation (Ripple and Beschta 2004). The recovery of vegetation allowed for other species to re-colonize and/or feed on resources without the competitive pressure of elks (Ripple and Beschta 2012).

In summary, no process exists on its own and we need to integrate the entirety of animal behaviours and distributions, demographic processes of both animals and resources, as well the coexistence mechanisms into the landscape of fear framework. Only then can we understand better all the possible cascading effects of fear.

Rodents as a model organisms

Observational studies have always been important to define the landscape of fear, especially in natural systems, and often make use of well defined predator-forager interactions, such as the relations between large mammalian carnivores and herbivores. Whoever, there are limitations to how much the landscape of fear can be manipulated in these systems, moreover large herbivores have few predators so the results might not be generalized for other animal interactions. Another group of model organisms widely used in

studies of landscape of fear are small rodents, as these present a wide range of foraging behaviours while being prey to many different types of animals. Furthermore, it is easy to manipulate or control for their perceived predation risk in both natural and captive conditions. In this thesis, we will mainly focus on ground-dwelling rodents from Europe, although the results from these species are similar to other rodents species or even taxa, even if species present different biologies (Zanette and Clinchy 2019).

Small rodents perceive both direct and indirect cues when evaluating predation risk. Direct cues come mostly from terrestrial carnivores (e.g. family Carnivora) in the form of olfactory cues (e.g. faeces or shed fur). Other types of cues from predators directly sensed by the rodents also affect foraging behaviour and/or trigger anti-predator behaviours based on the rodents senses (for a comprehensive review see Bedoya-Pérez et al. 2019). Even ungulate carcasses may generate a landscape of fear for rodents (Frank et al. 2020). When faced with direct cues from terrestrial predators, rodents often lower their foraging activity and change their time allocation at the short-term temporal scale (e.g. Eccard et al. 2008, Kotler et al. 2010, Moll et al. 2020). However, the greatest perceived threat for rodents comes from avian predators, for which they have no direct cues to assess their presence/absence. Instead, rodents will evaluate possible avian risk through the indirect cues of cover (e.g. vegetation height), with more open and/or exposed habitats being perceived as riskier than denser and/or covered habitats (e.g. Jacob and Brown 2000, Pusenius and Schmidt 2002, Yadok et al. 2019, Dammhahn et al. 2022). Microhabitats can further change these patterns of safety and create variation in what can seemingly seem homogenous habitats at a larger spatial scale (Brown and Kotler 2004). This pattern will occur regardless of possible avian presence or its density, since this threat is seemingly omnipresent for rodents (Mohr et al. 2003). Snakes also pose a threat to rodents, and although their presence can be directly sensed by rodents, the habitat cover is also the most important factor for rodents to perceive possible snake predation risk (Bouskila 1995).

There have been multiple studies evaluating the effects of each predator type in the foraging behaviour of rodents (notable examples: Jacob and Brown 2000, Orrock et al. 2004, Eccard et al. 2008, Liesenjohann and Eccard 2008, Fanson 2010, Sivy et al. 2011, Bleicher et al. 2019). As previously mentioned, avian predators are perceived by rodents as omnipresent, so habitat and vegetation cover is a strong predictor of perceived predation risk small ground-dwelling rodent species. If terrestrial predators are suddenly present, besides the time allocation strategy, rodents can also choose less dense or covered patched where they can avoid an ambush (e.g. Bouskila 1995, Korpimaki et al. 1996), or change their habitat residency based on each predator's active time. For example, in a study of Jacob and Brown (2000), weasels were more active during daytime, while owls hunted at night. Therefore, during the day, voles foraged more on habitats with mowed grass (less cover, weasels are visible), while at night they foraged more on habitats with unmowed grass (less cover, voles are more hidden from avian predators once more). Furthermore, other environmental factors might interaction with perception of risk in rodents. For example, moon phases change the lighting available during the night, possibly making rodents more or less visible in open habitats (Kotler et al. 2002, 2010, Mandelik et al. 2003). The presence of artificial lights also affect the foraging behaviour of rodents, with individuals being more visible to predators under night lights (Rotics et al. 2011, Hoffmann et al. 2018, 2019, Zhang et al. 2020).

As all other animals, rodents also behave according to the optimal foraging theory and marginal value theorem, with the resource species profitability being chosen for consumption based on their functional traits, with the bigger and/or most caloric food items being often chosen in ground-dwelling rodent species in Europe (Kelrick et al. 1986, Fischer and Türke 2016, Wang and Corlett 2017, Mortelliti et al. 2019). If the rodent species also scatter-hoard, the shape and size of the food item will also influence the choice of rodents, as some food

items are easier to transport and, therefore, require less energetic costs regardless of the food energetic gains (Kelrick et al. 1986, Muñoz and Bonal 2008, Muñoz et al. 2012).

Given their food preference, rodent seed predation can also act as coexistence mechanism and shape the biodiversity of the landscape (Stump and Chesson 2017). By acting as a coexistence mechanism through foraging, rodents have the potential to affect future seed germination and change the composition of vegetation re-growth in landscapes (Mills et al. 2018). Even though rodents are widely used as GUD experiment models, there is no quantitative way of measuring the cascading effects of fear to resource communities, as studies on GUD are standardized with one or few food items solely to measure perceived predation risk.

Research objective and thesis structure

This thesis makes an approach to integrate all of current theories of landscape of fear, optimal and central-place foraging, as well coexistence theory into empirical studies on a tri-trophic system, thus expanding analyses of cascading effects of predator-forager into forager-resource interactions. The latter interactions are less studied in the ecology of fear area, although there is still much to be known on how the presence of predators can ultimately affect the biodiversity of resource communities. Furthermore, it is still fairly unknown if functional trait preference is affected by perceived predation risk, or if it is a fixed behaviour of an animal species regardless of fear.

In Chapter I, we evaluate how cascading effects of the landscape of fear affect the biodiversity of a resources community. As previously mentioned, there is no quantitative measure for all the behaviourally-mediated cascading effects of fear, since GUD mostly measures the harvest rate of food items in relation to perceived predation risk. However, it is possible to use more food items in a GUD study (e.g. Garb et al. 2000, Abu Baker and Brown

2012, Abdulwahab et al. 2019) and expand the giving-up behaviour using a community of resources to measure the harvest rate of each food item and its final diversity - diversity at the giving-up density (DivGUD, see Eccard et al. 2022). In simple terms, DivGUD is the diversity of the resource species community once the forager decides to quit foraging and uses diversity indeces as a measure unit (Whittaker 1960, 1972, Hill 1973). Under different perceived predation risk levels, DivGUD can quantify both the density of each resource left by the forager, as well the final resource species composition. Furthermore, like all the diversity indeces, the DivGUD can be applied at different spatial scales to evaluate the impact of foragers at different movement distances or distributions, from a more local scale (alpha diversity) to a regional scale (gamma diversity), with the dissimilarity among both (that is, the dissimilarity among resource species community) being calculated with the beta diversity (Whittaker 1972, Jost 2006). At first, the measurement was tested with rats (*Rattus norvegicus*) on artificial food patches (plant seeds mixed in a sand substrate) in risk-uniform landscapes (Eccard et al. 2022). Patch residency decreased the diversity of the resource species community left, with harvest rates differing among seeds depending on their functional traits (bigger and most caloric seeds were harvested at faster rates). We then tested the DivGUD measure in wild conditions in Chapter I, by setting artificial food patches in different landscapes of fear. We expected that DivGUD would perform similarly to GUD (decreased density and diversity of resources left with lower perceived predation risk), but would also provide further information on the dissimilarity of resource species community left in the landscape and if functional traits were relevant for the forager (potential for coexistence mechanisms) instead of harvest rates being completely random (i.e. forager's would take the first encountered seeds in an opportunistic way).

Due to the nature of the experiment, in Chapter I it would not be possible to fully understand the impact of each functional trait of the resource species for the decision-making process of the foragers, as the artificial food trays were left in the wild under no constant

surveillance. So, in Chapter II we moved our experiment to captive conditions, where we analysed the importance of each seed functional traits not only for the optimal foraging of rodents, but evaluated if it interacted with varying perceived predation risk. Since the captive rodents were monitored for the entire duration of the experiment, we could calculate the harvest curves for each resource species, but also the movement patterns of individuals throughout the entire experiment. We expected that foragers would be more active, move more and consume more seeds in safer conditions, but also show dietary preference for one specific functional trait as they perceive the predation risk as lower and feel safe enough to make a choice, contrary to opportunistically take a random resource from the patch. Furthermore, foragers should forage in the patches closer to their safe shelter (central-place foraging), as these patches provide more energetic benefits (less energetic cost of movement) with little predation risk costs.

While Chapter I and II evaluates the cascading effects of a tri-trophic chain, natural systems are often more complex than predator-forager-resources, with foragers not only having multiple types of predators, but predators themselves can be prey to other predators (e.g. mesocarnivores). In Chapter III, we analysed the cascading effects of both a mesocarnivore (raccoons) and rodents landscapes of fear. The raccoons activity was manipulated with artificial food patches, where raccoons were attracted to a location to forage, then repelled from it with wolf urine (potential direct cue of predation risk). At the same time, we analysed if rodents foraging behaviour was affected by the changes in raccoon activity, by analysing the GUD and DivGUD left from artificial food patches. While avian predation risk is described as the most impactful to rodent's behaviour, we expected that the increasing presence of a terrestrial predator would drastically change the rodents foraging activity.

In the end of this work, we hope to evaluate the cascading effects of fear not only qualitatively, but also quantitatively with the new DivGUD measure and understand how

different landscapes of fear affect coexistence mechanisms, by studying the several

interactions of different types of predator presence, forager behaviour and the resource

species community.

Chapter I

Forager-mediated cascading effects on food resource species diversity

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Abstract

Perceived predation risk varies in space and time. Foraging in this landscape of fear alters forager-resource interactions via cascading non-consumptive effects. Estimating these indirect effects is difficult in natural systems. Here we applied a novel measure to quantify the diversity at giving-up density that allows to test how spatial variation in perceived predation risk modifies diversity of multi-species resources at local and regional spatial levels. Furthermore, we evaluated whether the non-consumptive effects on resource species diversity can be explained by preferences of foragers for specific functional traits and by the forager species richness.

We exposed rodents of a natural community to artificial food patches, each containing an initial multi-species resource community of 8 species (10 items each) mixed in sand. We sampled 35 landscapes, each containing seven patches in a spatial array, to disentangle effects at local (patch) and landscape levels. We used vegetation height as a proxy for perceived predation risk. After a period of three nights, we counted how many and which resource species were left in each patch to measure giving-up density and resource diversity at the local (alpha-diversity) and the regional level (gamma-diversity and beta-diversity). Furthermore, we used wildlife cameras to identify foragers and assess their species richness.

With increasing vegetation height, i.e. decreasing perceived predation risk, giving-up density, local alpha- and regional gamma-diversity decreased, and patches became less similar within a landscape (beta-diversity increased). Foragers consumed more of the bigger and most caloric resources. The higher forager species richness, the lower giving-up density, alpha and gamma-diversity. Overall, spatial variation of perceived predation risk of foragers had measurable cascading effects on local and regional resource species biodiversity, independent of the forager species. Thus, non-consumptive predation effects modify forager-resource interactions and might act as an equalising mechanism for species coexistence.

Introduction

Complex trophic interactions shape the evolution of plants and animals (Estes et al., 2013; Karban, 2011). In this evolutionary arms race, prey species evolved a set of anti-predation strategies such as morphological features (Eklöv & Jonsson, 2007), physiological responses (Boudreau et al., 2019), and behavioural changes such as, for example, reduction of plasticity (Pessarrodona et al., 2019) or the avoidance of predation risk in space and time (Lima & Bednekoff, 1999; Lima & Dill, 1990). Non-consumptive effects cause complex changes in trophic chains, often in the form of top-down effects (Mitchell & Harborne, 2020). The behavioural responses of prey can be mapped into a landscape of fear, which is defined as the spatiotemporal variation in perceived predation risk by the forager (Gaynor et al., 2019; Laundré et al., 2001, 2014), which affect the distribution of multi-species resources in a landscape (Monk & Schmitz, 2022). The presence of a predator can be evident and perceived directly via sight or smell (Pustilnik et al., 2021; Saavedra & Amo, 2020), or just inferred indirectly by the forager through environmental conditions, such as habitat cover (Wagnon et al., 2020) or variable visibility conditions (Ranåker et al., 2012). Thus, even if no predator is present, foragers perceive predation risk.

Many studies on landscapes of fear focus on predator-forager interactions and study how the presence/absence of predators can change the morphology, physiology, ecology and behaviour of their prey (Smith et al., 2019). This system can be widened to include forager-resources interactions into a tri-trophic system, that is, the interactions among predator-forager-resources (Price et al., 1980). These systems allow to study behaviourally-mediated trophic cascades of perceived predation risk by the foragers (Smith & Schmitz, 2016), with

the non-consumptive effects of predators affecting the forager's level, and consequently changing the population dynamics and multi-species interactions at the lower trophic level of the resources (Matassa & Trussell, 2011; Mills et al., 2018; Wirsing et al., 2020). The main aim of our study was to zoom in on the consequences of forager-resource interactions and illuminate how variation in perceived predation risk of foragers have cascading effects on the biodiversity of resource communities at different spatial scales.

Perceived predation risk is often measured using giving-up density (GUD), which quantifies the resource density left in a patch when the forager decided to quit harvesting (Brown, 1988; Brown & Mitchell, 1989). Since GUD is a measure that depends directly on the forager's feeding behaviour under varying perceived predation risk, it became an established method to quantify landscapes of fear (Gaynor et al., 2019; Jacob & Brown, 2000; Van der Merwe & Brown, 2008). Experiments using GUD typically make use of food mixed with a substrate, to force the forager to actively search for food in a patch with diminishing returns (e.g. rodents digging in sand trays to find seeds; Brown, 1988 and Orrock et al., 2004). If a forager perceives the predation risk as higher, it will quit harvesting sooner as the costs of searching for food surpass the metabolic gains of moving and foraging, the missed opportunity costs and the predation costs, which results in higher density of resources left behind when leaving the patch (the GUD). Under the assumption of metabolic and missed opportunity costs being stable, GUD reflects the costs of perceived predation for the forager. Experiments usually make use of a single or few food species (e.g. Brown & Mitchell, 1989) and have a limited ability to assess top-down effects of landscape of fear on the biodiversity of a resource species community.

Using multiple resource species in forager harvesting experiments can, however, further inform of whether variation in foraging can act as a coexistence mechanisms at the resource species level (Garb et al., 2000). Combined with diversity indexes, this approach can illuminate whether and how predation risk effects in prey foraging are a biotic filtering

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mechanism for biodiversity at the resource level. In this study, we applied a novel measure, the diversity at the giving-up density (DivGUD; Eccard et al., 2022) and provided a resource species community to foragers and, similarly to GUD, quantified the diversity of resource species left behind in a patch by the foragers. The DivGUD approach can also be used to provide information at different spatial sampling scales since it is measured using classical diversity indices (Whittaker, 1972) on different spatial scales (Figure 1). Species diversity at the local level (foraging patch) is alpha diversity (α-DivGUD) and is driven by forager-specific behaviour and their individual interactions with the patch at the microhabitat level. When the scale is expanded to contain the cumulative information of several foraging patches, gamma diversity (γ-DivGUD) at a regional level (foraging landscape) can be assessed. At a landscape, differences in species composition among local patches can be assessed as the beta diversity (β-DivGUD).

Changes in DivGUD might occur due to dietary preferences of the forager, as predicted by optimal foraging theory (Stephens & Krebs 1986), as resource species present functional traits (i.e. characteristics that may increase the individual's fitness or performance; McGill et al., 2006) which can also be beneficial for the forager's energy intake. Therefore, foragers are expected to select resources based on expected energetic gains, and change the final relative abundance and species richness of the resource community in a functional-trait-dependent way (Eccard et al., 2022). The relevant functional traits of resource species may include morphological traits (e.g. seed size and presence of a husk; Lichti et al., 2017), and physiological traits that increase the competitive capability of resources (e.g. plant nutrients and energy storage correlated to development and growth; Salgado-Luarte & Gianoli, 2012). Differential feeding by foragers also acts as a biotic filter for resources, creating further variation in population dynamics of the resource species, and contributes further to resource species coexistence as an equalising mechanism (Chesson, 2000; Larios et al., 2017). We expect perceived predation risk to modify the strength of coexistence mechanisms. Under

elevated risk, the foragers ought to consume resources that provide them with the most energetic intake, thus reducing the abundance of resource species that might have a competitive advantage over others (Kotler & Holt, 1989; Stump & Chesson, 2017). Alternatively, when perceived risk is high, the foragers might be less selective when feeding as they spend less time in the food patch (Eccard et al., 2022).

Spatial scales			True diversity
Ecological	Foraging	Sampling	Formula
Local	Patch	Single seed tray	$\alpha = \exp(\sum_n^i p_i \ln p_i)$
Regional	Landscape	Seed tray array	$\gamma = \exp(\sum_n^i p_i \ln p_i)$
Regional variation	Between-patch variaton	Seed community dissimilarity	$\beta = \gamma/\overline{\alpha}$

Figure 1 – Layout of the experimental design, with the different spatial scales and respective study level, as well as the true diversities used for each sampling level based on Shannon's entropy and Whittaker's beta diversity; Jost, 2006). Each spatial scale is highlighted with a bold outline in the sketch of the experimental outline.

We used ground-dwelling rodents as a study system, whose perception of predation risk is often related to how exposed they are in their surrounding habitat. While they react to olfactory cues of terrestrial carnivores (Eccard et al., 2008; Moll et al., 2020), vegetation cover is their main proxy for the omnipresent and less predictable avian predation risk (Kotler, 1992; Yadok et al., 2019), which translates into a landscape of fear mapped in experiments (e.g. Dammhahn et al., 2022; Eccard et al., 2022; Eccard & Liesenjohann, 2014). Since small rodents are both primary consumers of resources and prey to several secondary consumers, they serve as a suitable connector in a tri-trophic system model. Small rodents are also important predators of seeds, often shaping plant coexistence in ecosystems (Dylewski et al., 2020; Garb et al., 2000), especially due to their preferences for larger and most caloric seeds (Chang & Zhang, 2014; Mortelliti et al., 2019; Wang & Chen, 2009).

Here we investigated the cascading effects of the landscape of fear on food resource species diversity, using different vegetation heights as a proxy for perceived predation risk of small rodent foragers (Dammhahn et al. 2022). We provided a resource species community of seeds with different functional traits (size, caloric content, and husk) in discrete food patches to wild foragers. We assessed GUD and DivGUD on the resource level at two different foraging scales (patches – α-diversity, and landscapes – γ-diversity and β-diversity; Eccard et al. 2022) to test the following predictions:

(i) With with increasing vegetation height, i.e. decreasing perceived predation risk, both α-diversity and γ-diversity of food resource species would decrease, as the foragers stay longer in the patch and target single – highly rewarding - food species.

(ii) We expected β-diversity of food resource species to increase with vegetation height, as microhabitat heterogeneity should increase with vegetation height which might impact the presence and foraging behaviour of rodents.

(iii) The removal of each resource species should not be at random under varying risk, but related to how much it is preferred by the forager due to size and nutritional value, with bigger and/or most nutritious seeds being removed first, independent of vegetation height (perceived risk).

(iv) To account for the confounding effects of working with a whole community of wild rodents, we also assessed how forager species richness in the landscape can affect GUD and DivGUD. Assuming that different co-occurring rodent species experience similar predation risk and have similar food preference, we expected them to react similarly to perceived predation risk and thus effects of vegetation height on diversity measures being independent of the identity or diversity of forager species.

Materials and Methods

Study site

We conducted a landscape-wide experiment at the Ecological Research Station Gülpe, in Brandenburg, Germany (52°44'00.1"N 12°12'41.7"E). The study area is characterized by a mixture of grassland and extensively used grasslands (Burkart et al., 2003). Meadows are mowed twice a year, so small to medium (2-50 cm) grass species are dominant, representing around 80% of the area. Riparian corridors with shrub and reeds (50-280 cm) cover around 20% of the area. The area harbours a diverse community of small rodents (Kath, 2012), with the possible occurrence of four murine species (*Apodemus agrarius*, *A. flavicollis*, *Micromys minutus*, *Mus musculus*) and four vole species (*Arvicola terrestris*, *Microtus agrestris*, *M. arvalis*, *Myodes glareolus*).

Experimental set-up

The sampling was done in autumn, for three consecutive nights, in a total of 35 locations (September 2017: 8 locations; December 2018: 17 locations; December 2019: 10 locations)

with different vegetation heights (our proxy for perceived predation risk). We chose locations based on their accessibility to pathways, their independence from each other (inter-landscape distances: median = 227 m, min-max range: 25 m to 630 m), and also aimed to fill a gradient between 5 cm to 200 cm of vegetation height. We performed the experiment in autumn to avoid an over-abundance of natural resources, which would change the metabolic gains and costs, as well as to reduce missed opportunity costs as rodents do not breed after September in the sampled area (Niethammer & Krapp, 1978). Both these costs would create a confounding effect for GUD/DivGUD analyses. Within sampling years, we set up all locations simultaneously to avoid confounding effects of weather and lunar cycles (Kotler et al., 2010; Wróbel & Bogdziewicz, 2015).

At each of the 35 locations, we placed an array of seven foraging patches, that were hexagonally distributed with one patch in the centre, and separated by 6 m between patches (Figure 1). The spatial coverage of the patches at each location was chosen to reflect home range sizes of the naturally occurring rodent species, which are reduced in size during late autumn/winter (Baláž & Ambros, 2012; Briner et al., 2005; Yletyinen & Norrdahl, 2008), and to ensure a variety of microhabitats in each location. Each array of patches covered an area of 113 m^2, and therein will be referred to as a landscape. We measured the vegetation height in each patch at four random points up to one meter from the patch, and averaged within each patch for patch-level analyses and across the landscape for landscape-wide analyses. Vegetation height (varying from 2 cm to 271 cm) was used as a continuous variable, or, for similarity analysis, converted into three categories, by pooling all the average vegetation heights and using the first and third quantiles as thresholds (Low: ≤ 15 cm, n = 13; Medium: > 15 cm and ≤ 52 cm, n = 15; High: > 52 cm, n = 7). Due to the managed grassland nature of the sampled area, vegetation density (sampled as proportion in 1 m^2) was highly correlated to vegetation height (Kendall's correlation: r_T = 0.65, p < 0.001), therefore, vegetation height could serve as a good proxy for both vegetation cover and density.

Each patch consisted of a plastic tray with 400 ml of fine sand ($\varnothing$ = 14.5 cm, depth = 4 cm), mixed with seeds of eight different plant (resource) species: sunflower, kardi, wheat, hemp, flaxseed, millet, canary seed, and sesame (Table 1). Each patch contained ten seed items of each species, i.e. an initial total of 80 seed items per patch was provided. A protective cover was sheltering the sand from rain, small enough as to provide sufficient shelter from mild rain, but not from predators for the foragers. The patches and covers were set-up before early afternoon, and were monitored for foraging activity at every dawn over three consecutive days, by checking the patches for signs of digging in the sand, droppings, and empty seed husks.

We obtained information on the diversity of foragers for the sampling period of 2018 and 2019, by setting-up wildlife cameras pointed directly at the tray to identify the forager's species and their activity before the third night of the experiment. We placed two cameras per visited landscape, by randomly choosing two of the foraged patches. Landscapes with no visits were not surveyed with cameras due to logistic constrains.

At the end of the third night, the trays were collected and dried in an incubator at 60°C to filter the sand easily and recover all remaining intact seeds. The final number of seeds of each provided resource species was counted for each patch. We did not include in the final datasets five patches that were evidently affected by human error (e.g. had complete misses of single resource species, but counts of other species were within normal range), as well two patches not found at the end of the experiment. Furthermore, we also removed data of seven more patches (63-270 cm) that were completely depleted by the foragers, as the GUD/DivGUD measures cannot be calculated from them.

Table 1 – Additional information on the plant species used as resources in the experiment. Mass per seed item of each species was obtained by weighting 100 seeds and dividing it by 100. Energetic content is based on the package information, or when this information was missing, on external sources (Reference). Cal/item was calculated based on these information

Seed	Species	Mass per item (mg)	Kcal/100g	Cal/item	Reference
Sunflower	*Helianthus annuus*	38.8	679	263	U.S. Department of Agriculture (2021)
Kardi	*Carthamus tinctorius*	35.2	517	182	U.S. Department of Agriculture (2021)
Wheat	*Triticum aestivum*	39.4	326	128	Package
Hemp	*Cannabis sativa*	11.9	461	55	Package
Flaxseed	*Linum usitatissimum*	7.0	538	38	Package
Millet	*Pennisetum glaucum*	6.1	384	23	Package
Canary seed	*Phalaris canariensis*	4.6	399	18	CSDCS (2016)
Sesame	*Sesamum indicum*	3.7	600	22	Package

Data analyses

We analysed our data at the patch level (n = 231 patches) and at the landscape level (n = 35 landscapes; see Figure 1). At the patch level, we first tested whether the probability of a patch being used (yes/no) was explained by the patch vegetation height, using a generalized linear mixed effects model (GLMM) with a binomial error distribution. In this and subsequent models, we normalised the vegetation height variable with a natural logarithm transformation. The landscape identity was used as a random factor (random intercept), to account for potential non-independence of patches within a landscape, due for example, by the same

foraging individual. Furthermore, we also included year as a fixed effect to control for potential differences among years in this and subsequent models.

We calculated GUD by summing the counts of seeds left in the patch and dividing it by the initial 0.4 liters of sand (seed/L) and α-DivGUD using the formula given in Figure 1. All diversity indices were expressed as true diversities (i.e. effective number of species; Hill, 1973) and calculated based on the exponential of Shannon's entropy (Jost, 2006) using the vegan package in R (Oksanen et al., 2020). We chose to use Shannon's entropy due to its sensitivity to diversity changes and because it is known to be accurate in cases of complete sampling, even though it may weight rarer species disproportionally high (Nagendra, 2002). We used a linear mixed effects models (LMM) to test the effect of patch vegetation height on GUD or α-DivGUD, respectively, with landscape identification included as a random factor. GUD was log transformed.

At the landscape level, we summed the species-specific seed counts from all the patches of each landscape, and averaged the vegetation heights of each patch over the landscape (landscape vegetation height). We calculated average GUD across the landscape and γ-DivGUD based on the cumulative seed counts. To obtain a landscape mean α-diversity ($\overline{\alpha}$-DivGUD) we averaged across all α-DivGUDs of the seven patches. To evaluate the dissimilarity of resource species communities within landscapes, we calculated β-DivGUD for each landscape by dividing the γ-DivGUD by $\overline{\alpha}$-DivGUD (Witthaker, 1972).

We analysed the differences of resource community composition using analysis of similarity and visualised it with non-metric multidimensional scaling (NMDS) plots. The dissimilarity of resource diversity in the different vegetation categories was calculated with the adonis function in the vegan package in R with 999 permutations. The NMDS plots were also generated using the vegan package, using the dissimilarity matrices calculated previously with the metaDMS function.

The photos from the wildlife cameras were analysed using the software Digikam, and for each photo we labelled the forager's species and the landscape name. We exported all relevant metadata using EXIFTOOL and managed the photo database in EXCEL. The final database contained only photos with the presence of foragers, from which the species could be clearly identified. Eight landscapes with no activity recorded were excluded, as well as nine landscapes where the seed tray was not visible for the entire time due to external conditions or logistical problems (e.g. strong flash, rain droplets, etc.).

We first tested whether forager species richness (as number of rodent species observed per landscape) varied with the landscape vegetation height and/or sampling year, using a generalized linear model (GLM) with a Poisson error distribution. Second, we built a linear regression model to test if variation in GUD was predicted by the landscape vegetation height and/or forager's species richness, we used similarly structured models to predict variation for each DivGUD spatial level ($\bar{\alpha}$-DivGUD, γ-DivGUD and β-DivGUD). We checked if adding the forager's species richness improved the model, using the Akaike's Information Criterion (AIC). We also evaluated potential effects of spatial autocorrelation on forager species richness with the Moran's I index (Moran, 1950) with the ape package (Paradis & Schliep, 2019).

All analyses were done in R 4.0.4 (R Core Team, 2021). If not specified otherwise, all analyses were run with the lm4 package (Bates et al., 2015). The accepted significance level was set to $\alpha < 0.05$.

Results

At the patch level, the probability of a patch being used increased with vegetation height and between 2017-2018 (Table 2, Figure 2). In eight landscapes foragers never visited a single patch (vegetation height: 2-27 cm). These landscapes had scarce vegetation cover

and no recent signs of forager presence (e.g. faecal pellets) could be found. With absent foragers, we cannot measure GUD/DivGUD, therefore we removed these landscapes from subsequent analyses. The new datasets included 27 landscapes with a total of 177 patches, in which at least one patch was foraged (i.e. forager presence was confirmed).

In subsequent models, the inclusion of year as a variable did not improve the models nor was it significant, so this variable was dropped. At the patch level, both GUD (Figure 3A) and α-DivGUD (Figure 3B) decreased with average vegetation height (Table 2). Similarly, at the landscape level all GUD (Figure 3C) and DivGUD (Figure 3D, 3E) measures decreased with average landscape vegetation height (Table 2), except for β-DivGUD (Figure 3F) that scaled positively with landscape vegetation height.
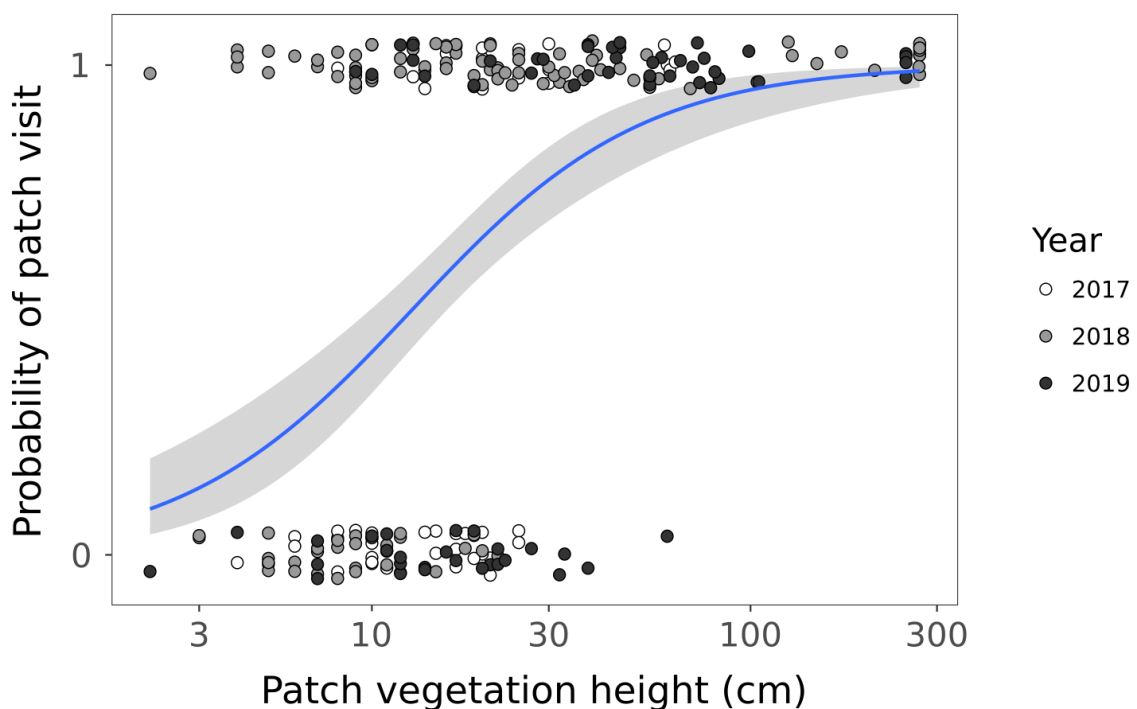
Figure 2 – Probability that a forager visited (1) or did not visit (0) a foraging patch in relation to the average vegetation height (logged) at the patch level among the sampled years. The blue trend line and its 95%-confidence intervals (grey) are based on a logistic regression without the landscape as random effect.

| | Patch-level | | | | | | Landscape-level | | | | | | | |
| | Probability of patch visit (0 / 1) | | Logged giving-up density (GUD, item/L) | | True alpha diversity (α-DivGUD) | | Logged mean giving-up density (mean GUD) | | Mean true alpha diversity (ᾱ-DivGUD) | | True gamma diversity (γ-DivGUD) | | Beta diversity (β-DivGUD) | |
	$β ± SE$	p	$β ± SE$	p	$β ± SE$	p	$β ± SE$	p	$β ± SE$	p	$β ± SE$	p	$β ± SE$	p
Vegetation model														
Intercept	-7.69 ± 2.23		5.99 ± 0.44		8.86 ± 0.82		6.64 ± 0.58		10.02 ± 1.22		10.47 ± 1.02		0.66 ± 0.25	
Logged average vegetation height (cm)	**2.30 ± 0.65**	**<0.001**	**-0.74 ± 0.12**	**<0.001**	**-1.24 ± 0.23**	**<0.001**	**-0.84 ± 0.17**	**<0.001**	**-1.56 ± 0.35**	**<0.001**	**-1.24 ± 0.29**	**<0.001**	**0.24 ± 0.07**	**0.003**
Year (2017-2019)														
2018	**3.74 ± 1.56**	**0.016**												

	Patch-level						Landscape-level							
(cont)	Probability of patch visit (0 / 1)		Logged giving-up density (GUD, item/L)		True alpha diversity (α-DivGUD)		Logged mean giving-up density (mean GUD)		Mean true alpha diversity ($\bar{\alpha}$-DivGUD)		True gamma diversity (γ-DivGUD)		Beta diversity (β-DivGUD)	
	β ± SE	p	β ± SE	p	β ± SE	p	β ± SE	p	β ± SE	p	β ± SE	p	β ± SE	p
2019	0.85 ± 1.65	0.605												
Chisq (Df)	7.07 (2)		31.93 (1)		26.30 (1)									
R^2 marginal	0.42		0.28		0.24									
R^2 conditional	0.82		0.67		0.67									
Random effect variance	0.76		0.75		2.73									
Fstat (df)							25.90 (1,25)		20.38 (1,25)		18.64 (1,25)		11.28 (1,25)	
multi R^2							0.51		0.45		0.43		0.31	
adj R^2							0.49		0.43		0.40		0.28	
Vegetation + Forager model														
Intercept							7.08 ± 0.66		11.57 ± 1.15		10.92 ± 1.40		0.35 ± 0.38	
Logged average vegetation height (cm)							**-0.75 ± 0.20**	**0.004**	**-1.71 ± 0.34**	**<0.001**	-0.92 ± 0.42	0.053	**0.35 ± 0.11**	**0.011**

Forager's species richness				**-0.39 ± 0.15** (**0.024**)	**-0.59 ± 0.25** (**0.044**)	**-0.76 ± 0.31** (**0.035**)	-0.03 ± 0.08 (0.716)
Fstat (df)				15.27 (2,10)	20.39 (2,10)	7.71 (2,10)	5.09 (2,10)
multi R^2				0.75	0.80	0.61	0.50
adj R^2				0.70	0.76	0.53	0.41
Model selection							
Inclusion of year: ΔAIC (p)	**-3.07 (0.029)**	1.93 (0.355)	3.23 (0.680)	2.51 (0.062)	-1.97 (0.422)	2.57 (0.061)	-3.37 (0.765)
Inclusion of forager's species richness: ΔAIC (p)				**-5.02 (0.023)**	-3.53 (0.054)	**-4.07 (0.035)**	1.82 (0.716)
Inclusion of forager's species richness plus year: ΔAIC (p)				0.81 (0.175)	1.72 (0.666)	1.16 (0.458)	1.98 (0.899)

Shown are the estimated effects (*β*), their standard errors (SE), the p-value as obtained through the R package lmerTest (Kuznetsova et al 2017), R^2 based on the fixed factors (R^2 marginal) and based on fixed and random factors (R^2 conditional), Chi-square (Chisq) or F-statistic (Fstat) with degrees of freedom (df), as well the multiple R^2 (multi R^2) and adjusted R^2 (adj R^2), and change in Akaike's Information Criterion (ΔAIC). The sample size of the 'Vegetation model' was adjusted to be comparable with the 'Vegetation + Forager model' during model selection with the forager's species richness variable. All significant relations are shown in bold.
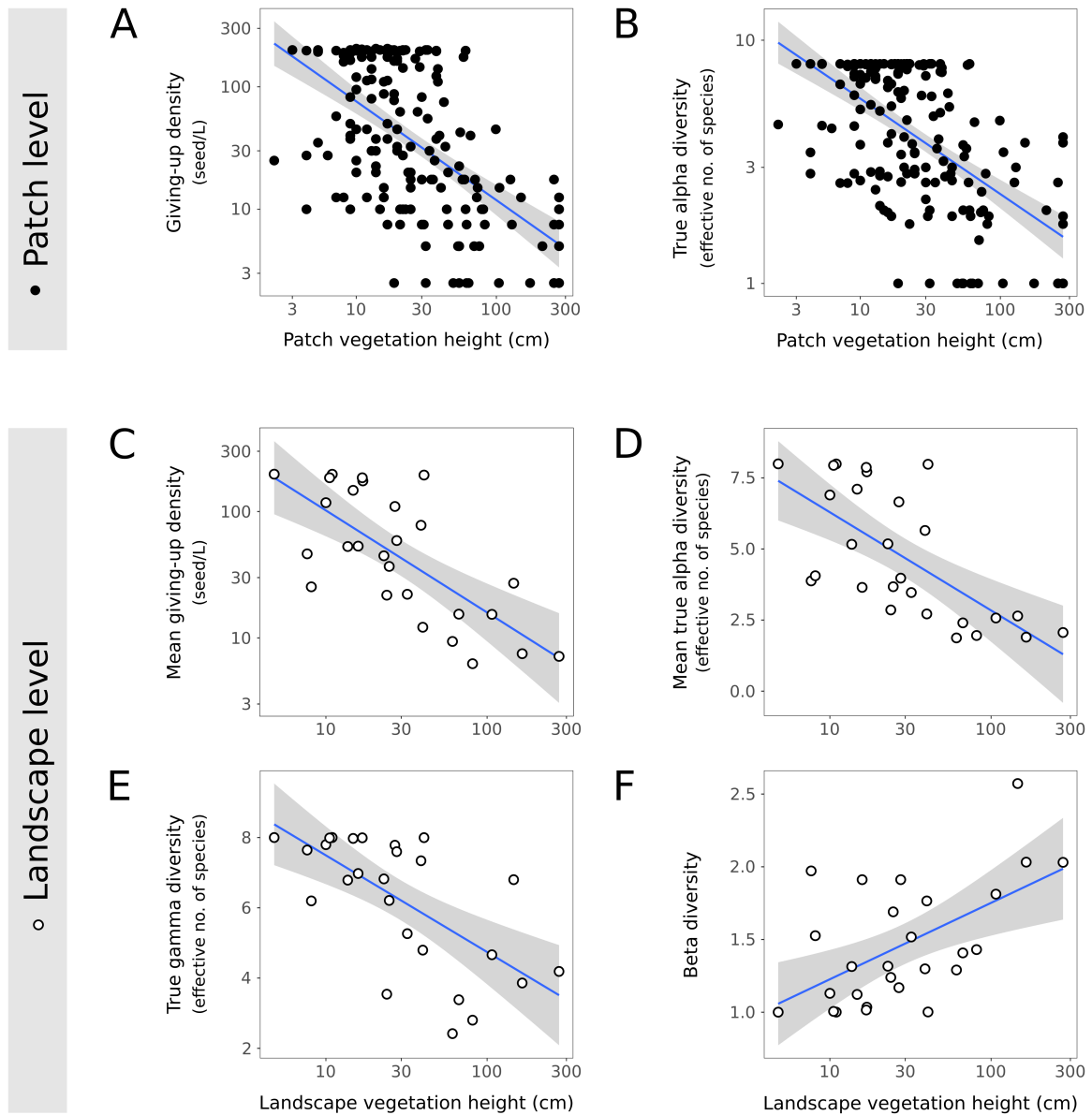
Figure 3 – Patch level – relation between average vegetation height at a foraging patch and: A) giving-up density (logged) and B) local diversity at the giving-up density (α-DivGUD). Landscape level – relation between the average vegetation height (logged) and: C) mean giving-up density (logged) D) the mean local diversity at the giving-up density ($\bar{\alpha}$-DivGUD), E) the regional diversity at the giving-up density (γ-DivGUD), and F) regional variation ratio (β-DivGUD). The blue trend lines and their 95%-confidence intervals (grey) are based on linear models, without the landscape as random effect in the 'patch level'.

Remaining resource species communities were dissimilar among categories of vegetation height, (Analysis of similarity: R^2 = 0.39, p = 0.001). Graphical inspection of the NMDS plot (Figure 4) suggests that landscapes in the high vegetation height category ('High': > 52 cm) showed a different resource composition from other vegetation height categories (pairwise comparisons; 'Low' – 'High': R^2 = 0.52, p = 0.002; 'Medium' – 'High': R^2 = 0.39, p = 0.001), while the low and medium vegetation height categories overlapped ('Low' – 'Medium': R^2 = 0.02, p = 0.636). In the high vegetation category, foragers left over a higher proportion of small and less caloric seeds (Appendix Figure 1).
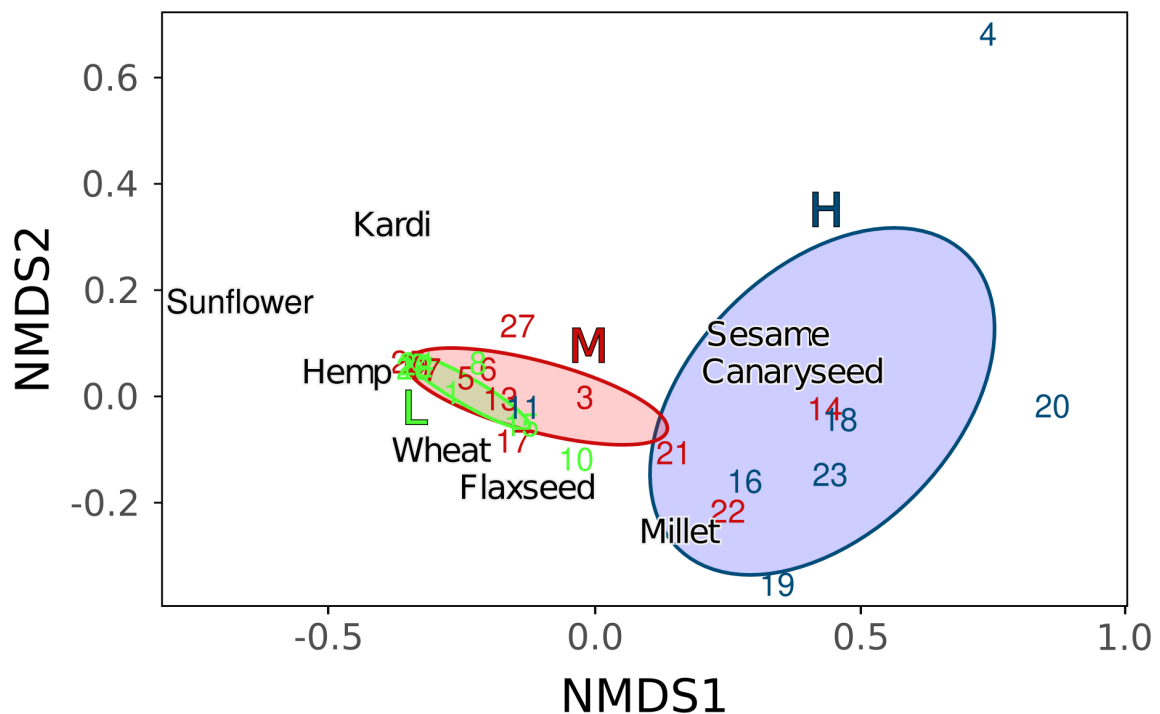
Figure 4 – Non-metric multidimensional scaling (NMDS) of the final resource species' seeds community (true gamma diversity) left over in 27 landscapes (numbers) divided in three categories of vegetation height: Low: up to 15 cm ("L", green); Medium: from 15 to 52 cm ("M", red); High: more than 52 cm ("H", blue). The axes (NMDS1 and NMDS2) can be related to functional traits of the eight seed species (see Table 1). We used a three dimensions model when generating the plot, as this model converged and presented a low stress value (0.03).

The camera surveillance in 2018 and 2019 yielded a total of 1,246 photos, and we identified four rodent taxa that foraged in 19 patches of 13 landscapes (out of 35 patches and 19 landscapes kept under surveillance). The most common species was the yellow-necked mouse (*Apodemus flavicollis*, nine landscapes), followed by *Microtus* spp. (seven landscapes), bank vole (*Myodes glareolus*, six landscapes) and harvest mouse (*Micromys minutus*, three landscapes). *Microtus* voles are difficult to separate into species based on wildlife camera pictures and both common voles (*Microtus arvalis*) and field voles (*M. agrestis*) were previously recorded to be present in the study area (Kath, 2012). Recorded activity (minimum seconds spent on a landscape) was higher for medium to high vegetation heights (Appendix Figure 2). We did not record any other non-rodent taxa foraging in our seed trays.

Forager species richness (number of species per landscape) did not vary with average vegetation height (β = 0.21 ± 0.24, p = 0.372, residual deviance = 7.41 on 11 df), nor between years (β = -0.48 ± 0.45, p = 0.287, residual deviance = 7.01 on 11 df). GUD and $\bar{\alpha}$-DivGUD decreased with an increase of forager's species richness and average vegetation height in the data from 2018-2019 (Table 2, Figure 5). γ-DivGUD decreased significantly with an increase of forager's species richness, with a decreasing trend when average vegetation height increased. β-DivGUD increased significantly with increased average vegetation height, with forager's species richness having no effect. All models were improved by the inclusion of forager species richness, except for β-DivGUD. Forager species richness was not spatially autocorrelated (I_{2018} = 0.12, p = 0.106; I_{2019} = -0.17, p = 0.512).
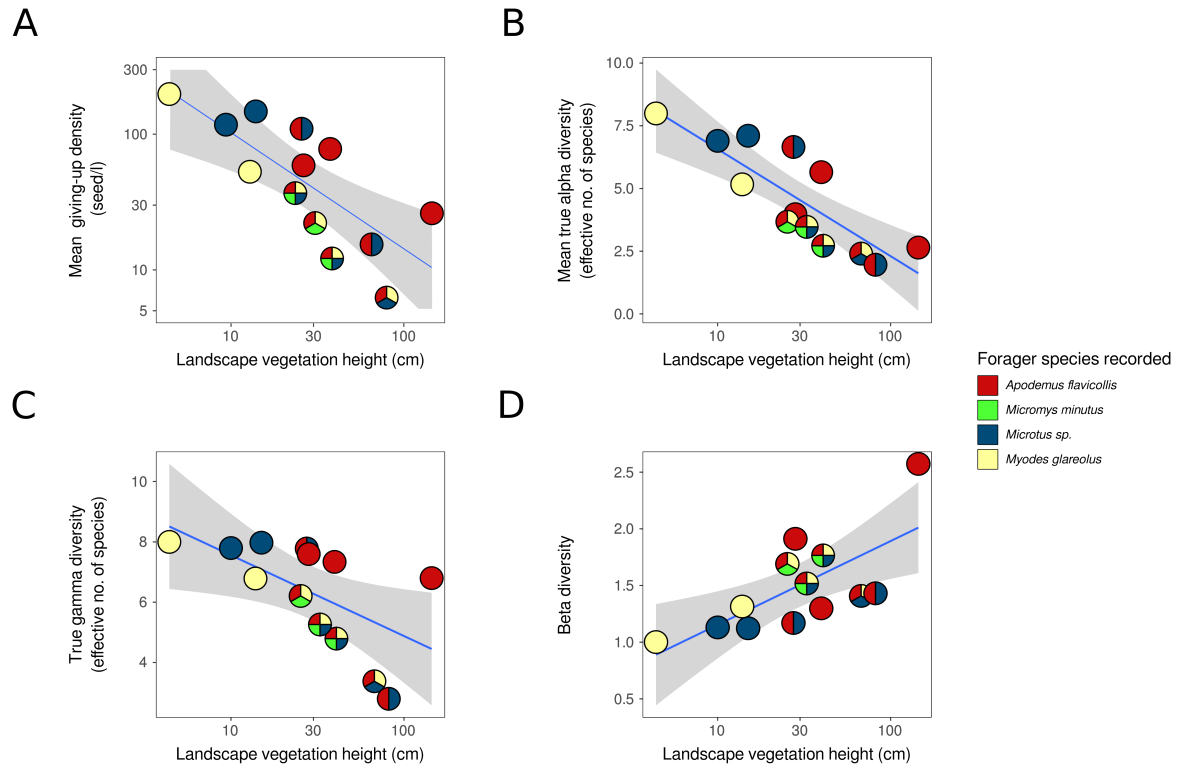
Figure 5 – Relation between the average vegetation height (logged) and composition of the forager community, and: A) the mean giving-up density (logged); B) the mean local diversity at the giving-up density ($\bar{\alpha}$-DivGUD); C) the regional diversity at the giving-up density (γ-DivGUD); D) regional variation ratio (β-DivGUD). The blue trend lines and their 95%-confidence intervals (grey) are based on linear models, without the landscape random effect in the 'patch level'.

Discussion

Top-down effects of predators cascade down to the primary resource level and, thus, shape complex processes in ecosystems. Here, we showed under natural conditions that foragers adjust their foraging behaviour to the protective cover of vegetation height, with consequences on the biodiversity of the resource species community (prediction i).

At the foraging patch level, the higher the vegetation height, the more resources were exploited by foragers, resulting in lower densities of food when quitting the patch (GUD). This

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finding was expected based on previous GUD studies, but necessary to confirm that in our experimental landscape srodents indeed perceived higher predation risk in short vegetation and, thus, variation in vegetation height maps a landscape of fear. The exploitation pattern at the patch level follows the prediction of the patch-use model (Brown, 1988, 1992) as demonstrated many times, particularly using vegetation height and cover as proxies for perceived predation risk (e.g. Jacob & Brown, 2000; Yadok et al. 2019). Some of the landscapes with very short vegetation were not visited at all. In these cases, it remains to be disentangled whether foragers were completely absent from these areas (which could also be due to high perceived predation risk) or did not visit the food patches with low vegetation because it was too risky to forage in these patches.

In addition to GUD, we could also show that the diversity of resource species left in a patch also decreased with decreasing perceived predation risk. Thus, foragers feeding for longer, more often or more efficiently in a patch, reduced not only the amount of food left behind but also the local biodiversity (α-DivGUD). The same pattern occurred at the regional level, with both density and biodiversity (γ-DivGUD) being lower in landscapes perceived as safer by the foragers. These changes in biodiversity are direct measures of the cascading effects of a forager's landscape of fear and connect variation in the foragers' feeding behaviour to changes in ultimate resource species composition.

Contrary to α-DivGUD and γ-DivGUD, regional variation between patches (β-DivGUD) increased with the decrease of perceived predation risk (prediction ii). This pattern was expected, as habitats with higher vegetation heights can also present a greater variety of natural plant diversity, creating the potential for microhabitat effects of variation that may influence foraging (Orrock et al., 2004; Thompson, 1982). In some of these foraging landscapes, we observed that one or two patches were barely used, while the remaining patches were almost depleted. This might have happened because the vegetation cover at the patch level could be variable, even though maximum vegetation height was still very

high. In the patches with high vegetation height, the habitats were not managed as the other grassland areas, which might create further variation in vegetation cover and further influence predation risk (Hinkelman et al., 2012). However, even at more homogenous vegetation height distributions, patches might not have been exploited equally across landscapes (increased β-DivGUD), as the smaller seeds are difficult to find in the sand and foragers might give-up searching at different density and diversity of small seeds. This exploitation pattern can be further observed in the analyses of the final composition of the resource community in higher vegetation heights (prediction iii), as the smaller and less caloric resources (i.e. millet, canary seed and sesame) were left behind by the foragers at very different densities among foraging patches, and thus increasing the regional variation of resources. Rodents are known to have a preference for larger and/or more nutritious seeds (Fischer & Türke, 2016; Wang & Yang, 2014, Eccard et al., 2022), and it is likely that our foragers extracted those resources first in all foraging patches, rather than randomly selecting seeds, especially at high perceived risk. In the landscapes with high perceived risk, the foragers might limit their time feeding on those seeds, despite potential higher handling time with larger seeds (Kelrick et al., 1986), and also evenly forage on the patches (low β-DivGUD). However, in our study we cannot differentiate the effects of size and energetic content, as most of the larger seeds also contain more calories, and are also encountered first due to their size. Because we can only assess intact seeds left in the tray, we also cannot take into account the feeding strategies of the rodents, namely, if they scatter-hoard the seeds or not, which can change their preference to lighter seeds that are easier to transport (Muñoz & Bonal, 2008). Independently of which characteristic is favoured the most, we could still observe that the rodents forage differently based on the functional traits of the seeds.

Size and energetic content are functional traits that give seeds a competitive advantage at germination and growth (Lichti et al., 2017, Salgado-Luarte & Gianoli, 2012), but at the same

time these characteristics also make these seeds more profitable food sources for foragers and, thus, increase predation by granivores. Functional-trait dependent seed predation might act as an equalising effect for species coexistences, that is, it levels the competition among plant species by allowing seeds with lower germination rates to grow in the absence of the more competitive seeds (Larios et al., 2017, Stump & Chesson, 2017). Our results suggest that this coexistence mechanism is likely at play because the most removed seeds had functional traits advantageous for germination, but were also more attractive for rodent consumption. The created dissimilarity in diversity patterns can also act as a stabilising mechanism of species coexistence: with different abundances of resource species left in different landscapes, there is an increase of intra-specific competition rather than inter-specific, as same species have to compete for the same niche. Experiments using DivGUD can provide more insights into these coexistence mechanisms, while also informing on non-consumptive cascading effects of perceived predation risk in foragers. This measurement can also be used to understand possible bottom-up feedbacks, as the resource species biodiversity left behind by foragers can eventually shape the growth and diversity of the vegetation (Riginos & Grace, 2008).

Forager species richness did not vary with vegetation height; therefore, our results are not simply driven by variation in the forager community composition. Furthermore, despite conducting the experiment across three years, we did not detect much evidence for annual variation. Only the number of visits at patches differed between years, which was likely due to differences in sampled vegetation heights (in 2017 we could not sample patches higher than 70 cm). This indicates that neither environmental factors nor population density variation among years affected our results.

But - in addition to vegetation height - the number of forager species present had an effect on GUD and DivGUD (both $\bar{\alpha}$ and γ). This pattern was contrary to our expectations (prediction iv), as all rodent species were expected to react in a similar manner in safer

landscapes (i.e. feed on the same resources), or that inter-species interactions would exclude less competitive species from feeding, and thus not have an additional effect on the resources left. It is possible that some species have a greater effect on GUD/DivGUD, as species might have different foraging strategies (Thompson, 1982), activity patterns (Kołakowski et al., 2018) or learning behaviour (Haupt et al., 2010). In our data, minimum recorded activity of rodents occurred mostly in medium and high vegetation height categories (Figure 5, Appendix Figure 2), likely due to microhabitat heterogeneity. The yellow-necked mouse (*A. flavicollis*) was most frequently recorded in the foraging patches, especially at medium vegetation height, an expected result given dominant habitat presence and behaviour in relation to others species (Grüm & Bujalska, 2000; Hille & Mortelliti, 2010).

The unpredictability of foragers is common in experiments done in the wild, since there is variation in the diversity of species, their respective abundances, potential among-individual variation in states (e.g. starved individuals), age or experience (Bedoya-Perez et al., 2013). Despite our artificial setting of equally profitable patches, these factors may have created some variation in the final giving-up densities and biodiversity of resources. However, our results consistently indicated the importance of perceived predation risk, since we found similar effects of vegetation height on the resource diversity (DivGUD). Future experiments should take into account the variation in the forager's species community, and how each species contributes to changes in DivGUD across landscapes of fear, further linking behavioural ecology with community ecology across trophic levels.

Conclusions

Foraging under perceived risk has cascading effects on resource species diversity at local and regional spatial scale, which can be measured via diversity at giving-up density (DivGUD; Eccard et al. 2022). Thus, non-consumptive predation effects can promote

resource species coexistence in the landscape of fear of a forager with just the perceived predation risk shaping the forager-resources interactions. Combining several food resource species of different functional traits into experimental assemblages provided a first glimpse into how perceived risk during foraging might modify coexistence mechanisms at the local and regional spatial scale. We hope that this experimental approach can pave the way to further studies on possible bottom-up effects, such as the growth of plant species caused by differential feeding and scatter-hoarding behaviour of rodents. The changes in biodiversity occurring with the variation of fear become important when dealing with anthropogenic impacts or species reintroductions, which can further cascade through trophic networks or generate bottom-up effects into other trophic levels.

Chapter II

So many choices, so little time: food preference and movement vary with the landscape of fear

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Abstract

Spatial and temporal variation in perceived predation risk is an important determinant of movement and foraging activity of animals. Foraging in this landscape of fear, individuals need to decide where and when to move, and what resources to choose. Foraging theory predicts the outcome of these decisions based on energetic trade-offs, but complex interactions between perceived predation risk and preferences of foragers for certain functional traits of their resources are rarely considered.

Here, we studied the interactive effects of perceived predation risk on food trait preferences and foraging behaviour in bank voles (*Myodes glareolus*) in experimental landscapes. Individuals (n = 19) were subjected for periods of 24h to two extreme, risk-uniform landscapes (either risky or safe), containing 25 discrete food patches, filled with seeds of four plant species in even amounts. Seeds varied in functional traits: size, nutrients, and shape. We evaluated whether and how risk modifies forager preference for functional traits. We also investigated whether perceived risk and distance from shelter affected giving-up density, time in patches, and number of patch visits.

In safe landscapes, individuals increased time spent in patches, lowered giving-up density and visited distant patches more often compared to risky landscapes. Individuals preferred bigger seeds independent of risk, but in the safe treatment they preferred fat-rich over carb-rich seeds. Thus, higher densities of resource levels remained in risky landscapes, while in safe landscapes resource density was lower and less diverse due to selective foraging. Our results suggest that the interaction of perceived risk and dietary preference adds an additional layer to the cascading effects of a landscape of fear which affects biodiversity at resource level.

Keywords: giving-up density, foraging behaviour, functional traits, *Myodes glareolus*, perceived predation risk

Introduction

Foraging is a crucial activity for animals and incurs a variety of decisions (Stephens 2008): where to forage, how to balance metabolic costs, missed opportunity costs and energetic gains of foraging or staying in each patch (e.g. Brown 1988, Sih 1994, Stephens et al. 2007)? Furthermore, predation risk is an important determinant of foraging decisions, with foragers adjusting their activities to the spatiotemporal variation of their perception of predation risk, i.e., landscape of fear (Laundré et al. 2014, Gaynor et al. 2019). While direct cues from predators can be a strong indicator of mortality risk (Sivy et al. 2011, Mayer et al. 2020), prey species often rely on indirect cues to map their landscape of fear, such as ground cover or illumination (e.g. Orrock et al. 2004, Mella et al. 2014). Based on their landscape of fear, individuals must decide on how much time they allocate to feeding or to risk avoidance (Altendorf et al. 2001, Brown and Kotler 2004), therefore, there is a trade-off between the gains of foraging (Charnov 1976, Stephens and Krebs 1986) against the risk of mortality (Lima and Dill 1990).

Important determinants of energy gain for the forager are functional traits of the resources that are consumed, that is, the characteristics which increase the fitness of resource species (Adler et al. 2013). These often entail attributes that are preferred by foragers (Lantová and Lanta 2009, Salgado-Luarte and Gianoli 2012), especially under optimal foraging theory (i.e. foragers will maximize energetic gains with the more profitable resources in the least time possible; Charnov 1976, Sih and Christensen 2001). For example, in plants one common functional trait is energy reserves, and bigger plant seeds have higher germination rates compared to smaller seeds (Jakobsson and Eriksson 2000). Meanwhile, bigger seeds are also preferred by foragers as they contain more energy per item (Gómez 2004), even though bigger seeds are also more difficult or time consuming to handle and/or transport (Chang and Zhang 2014, Boone and Mortelliti 2019), therefore impacting the final energetic gain or possibly increasing mortality risk by predation with increased patch residency (Lima 1985,

Newman et al. 1988). The nutrient values of each food item might also affect the forager's behaviour, with individuals preferring items that are more caloric (Gerber et al. 2004). Therefore, functional traits of resources and dietary preferences of foragers are often interlinked, but as perceived predation risk also affects the forager's behaviour, it can alter the choice for certain resource traits. Ultimately, the landscape of fear generates behavioural-mediated cascading effects that alter the final composition of communities of resource species (Eccard et al. 2022), and foragers can affect the coexistence of plant species (Garb et al. 2000, Stump and Chesson 2017, Ferreira et al. 2022). To better understand the complex demographic and biodiversity effects of foraging in different landscapes of fear - especially at the community level of the resources - it becomes important to understand forager's dietary preferences for specific functional traits, and how perceived predation risk modifies these preferences.

A forager also has to decide when and where to forage, and this decision is based on the balance between energy gains of feeding against possible mortality risks (Mitchell and Lima 2002). Metabolic cost of movement itself, either of foraging behaviour or the risk during movement and/or feeding, also contribute to the outcome of this decision. Animal movement is then directly tied with energy landscapes, that is, the metabolic costs of movement for animals, both in space and in time, based on the physical properties of the habitats (Gallagher et al. 2017, Masello et al. 2021). Animals tend to choose pathways that minimize metabolic costs, while maximizing the energy gain (e.g. moving to high quality food patches; Wilson et al. 2012), which also includes minimizing the costs of potential predation risk (e.g. movement can make the prey itself more visible to predators; Turcotte and Desrochers 2003, Ciuti et al. 2012). The risk while travelling in a matrix between food patches can affect the duration of travel and, therefore, energetic gains of foraging (Eccard et al. 2020). Thus, movement is affected by three components: potential energy gain of foraging, energetic losses through movement (and foraging) itself, and potential energy loss through predation

risk avoidance. To both maximize gain and minimize risk, the forager could forage closer to its shelter or refuge (i.e. safe place to rest), as described in the central-place foraging theory (foragers first exploit patches near the central location, except if distant patches have high quality and profitable resources; Orians and Pearson 1979, Schoener 1979, Fryxell 1999, Bakker et al. 2005, Nilsson et al. 2020).

Basically, the act of foraging involves many decisions for animals simultaneously, concerning risk taking, nutritional value of resources, and movement. To better understand the process of decision making when foraging in landscapes of fear and biodiverse resources, we need to consider all these processes together. Therefore, we aimed to test the behavioural-mediated cascading effects on the resource levels caused by differential feeding under different landscapes of fear, and further study how movement and foraging activity are affected by perceived predation risk levels. We use a ground-dwelling rodent as the study species (bank vole, *Myodes glareolus*). Rodents are widely used in studies regarding landscapes of fear, as one can manipulate their perception of fear easily though manipulating the cover (rodents decrease their foraging activity in low to no cover; e.g. Kotler 1992, Eccard and Liesenjohann 2014, Dammhahn et al. 2022). Furthermore, illumination can also be used to manipulate perceived risk, as rodents decrease their activity under light to avoid being highly visible to avian predators (e.g. Clarke et al. 1983, Kotler et al. 1991, Rotics et al. 2011, Hoffmann et al. 2018, 2019), with bank voles being a suitable species for light manipulation experiments as they present polyphasic activity patterns (Ylönen et al. 1988, Galsworthy et al. 2005, Halle 2006). Perceived predation risk can also be quantified using the giving-up density (GUD), that is, the density of food resources left in a patch after the individual decides to stop foraging due to decreased gains over the increasing predation risk (Brown 1988). Like most seed-eating rodents, bank voles also show preference for specific food functional traits, with size and/or high energetic content being the most preferable traits (Eccard and Ylönen 2006, Fischer and Türke 2016, Fischer et al. 2017, Ellingsen et al.

2017). This is in concordance with other rodent species, which also show preference for larger and/or nutrient-rich seeds (Kelrick et al. 1986, Wang and Corlett 2017, Boone and Mortelliti 2019, Mortelliti et al. 2019, Hou et al. 2021), with fat-content being the most preferred nutrient. Rodents can also show preference for certain seed shapes as, together with size, some seeds might be more easily handled and transported than others (Muñoz et al. 2012). Under varying predation risk, we expect foragers pursue an optimal foraging strategy, with the effects possibly being more visible in high perceived risk, as foragers spent less time in the patch (lower patch residency) and might try to maximize gains by foraging on the most profitable (bigger and/or most caloric seeds) or easier to handle resources (e.g. circle shaped seeds). However, it is still difficult to disentangle which of these three functional traits actually influences foraging dietary preferences and if perceived predation risk affects these choices, especially choice between size and nutrient-value, as these functional traits are often correlated (Wang and Yang 2014).

Under indoor captive conditions, we introduced bank voles to two risk uniform landscapes of fear (safe versus risky) with discrete patches of uniform initial resource diversities. We hypothesized that:

i) Perceived predation risk differences can be confirmed by changes in foraging behaviour, as foragers consume less resources, but will also move less and spend less time foraging in a risky landscape compared to a safe landscape;

ii) Under different perceived predation risk treatments, we expect an interaction of perceived risk and forager's dietary preference for certain functional traits. As foragers become more active in safer conditions, they prioritize one functional trait over others.

We further monitored the spatial distribution of foraging effort, assuming that animals would forage on patches closer to their shelter (central-place foraging theory; Orians and

Pearson 1979, Schoener 1979, Fryxell 1999), and assumed that this concentration of foraging effort would be higher under risky conditions (Eccard and Liesenjohann 2008).

Material and Methods

Animal housing and experimental design

We captured 19 individuals (13 females and 6 males) from wild bank vole populations near Potsdam, Germany (52°26'17.4"N 13°00'22.4"E) in October 2018. We used Ugglan live traps (Grahnab Sweden, Special No. 2, with shrew exit) baited the traps with rolled oats and apples. Upon capture, we weighed, sexed and identified age and reproductive status. Lactating or pregnant females were immediately released at the capture site. The 19 individuals were transferred to the housing room at the laboratory of the Animal Ecology group of the University of Potsdam, and kept singly in standard makrolon cages (Ehret GmbH Germany, Type III: 42 cm × 27 cm × 16 cm), with bedding (wood shaving and hay). Food pellets (ssniff® NM, ssniff® R/M-H Ered II) and water were provided *ad libitum*. The room was kept at ca. 20ºC and the photoperiod was adjusted to seasonal day length.

The experiments were done between April and July 2019, with the second treatment being done with at least two weeks of separation in between, and occurred in indoor arenas (therein landscapes) of 2.5 m x 3.0 m, surrounded by 0.7 m tall galvanized metal fences. Each individual was placed singly in one arena and four arenas were run in parallel in the same room, with two of them running under light and the other two running under dark with a thick curtain dividing both sides. In the centre of each arena, we put a small wooden nest box and a water bottle providing individuals full access to a safe shelter and water *ad libitum* during the duration of the experiment. Each landscape had a grid of 25 seed trays (therein patches, round plastic bowls of 13 cm diameter and 4.5 cm height) separated by 50 cm (Figure 1a). Each patch was filled with 0.5 l of sand and seeds from four plant species with

different functional traits (Table 1), with seven individual seeds each (total of 28 seeds in each patch). Seed quantity was chosen to ensure that individuals would have their minimum food intake needs (average 2.5 g per day; Peacock and Speakman 2001, Eccard and Ylönen 2006), and even if foraging activity was lower than expected, this seed quantity would still enable us to quantify the diminishing returns of GUD. We video surveyed the whole landscape continuously during the entire experiment, using an analogue HD Dome Camera (ABUS HDCC31500 720p) with a top view of the landscape. Prior to our experiment, all individuals experienced the arenas and set-up of patches (containing only millet seeds) twice, once for four days ca. 2 weeks before the experiment and once for three days just before the experiment. Furthermore, all individuals were familiarized with the four seed types in their home cages over one week before the experiment. At the beginning of our experiment, all remaining sand or seeds were removed from the area and the shelter to avoid spillover from the previous experiment.

Each individual was tested for 24h once in each of two different risk uniform levels (landscapes of fear): risky – the perceived predation risk was high, the individuals were kept under light with no cover; and safe – the perceived predation risk was low, the individuals were kept under dark with a net cover (mesh size 1.5 cm x 1.5 cm) over the patches to simulate the effect of vegetation cover brushing on animal's fur. We know from previous experiments that illuminated open areas are perceived as risky (Hoffmann et al. 2019) and areas under net cover are perceived as safe by voles (Eccard and Liesenjohann 2008, Eccard et al. 2020). A net cover also allowed us to record and track the animals position. By providing extreme levels of uniform risk, we could assess the effects of fear in movement and dietary preference of foragers. We varied the order of risk treatments (therein "order") across individuals, so that the first treatment was either risky or safe and the second treatment would be the opposite. Due to logistic constrains, four individuals only underwent one of the

treatments (two individuals safe, two individuals risky). These individuals were also included in the analyses.
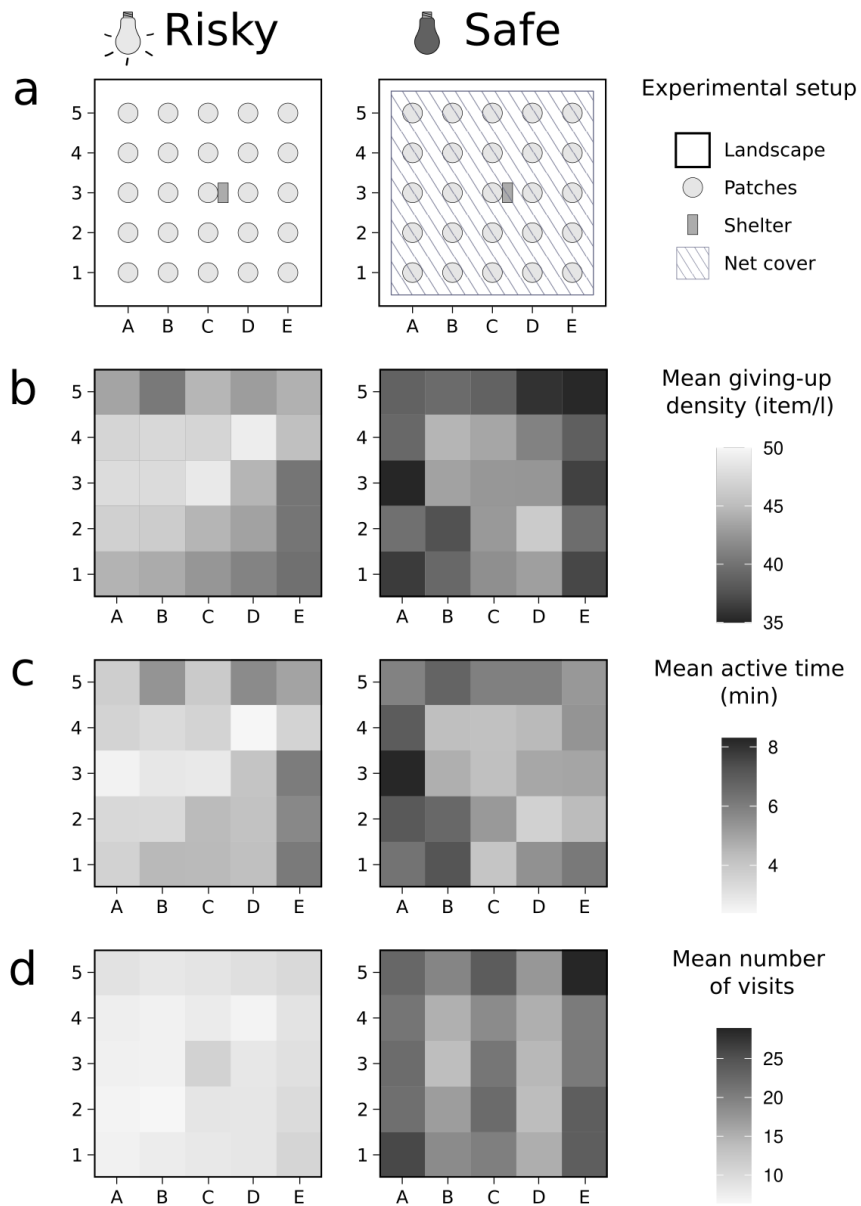

Figure 1 – a) Experimental setup by perceived predation risk treatment (risky – no cover, under light; safe – cover, under dark), with the 25 patches spaces by 50 cm both horizontally (A-E) and vertically (1-5) in the landscape, and with shelter and water in the center (position C3). Greyscale heat-maps of: b) mean giving-up density (GUD), c) mean time in patch, and d) mean number of visits at each patch across all 19 animals.

Table 1 – Characteristics of the seeds of four plant species used as resources in the experiment. Nutritional values were obtained from the package nutritional tables. Mass per seed item of each species was obtained by weighting 100 seeds and dividing it by 100. Seeds were grouped into pairs based on similarity in functional traits, indicated by similar font (bold or non-bold text).

Plant species		Husked	Mass per seed (mg)	Calories per seed (J)
Wheat	*Triticum aestivum*	**No**	**39.4**	**536.8**
Hemp	*Cannabis sativa*	Yes	**11.9**	**229.7**
Flaxseed	*Linum usitatissimum*	Yes	7.0	158.2
Millet	*Pennisetum glaucum*	**No**	6.1	91.1

(cont)		Calories per 100g (J)	Fat (%)	Total carbohydrates (%)
Wheat	*Triticum aestivum*	1362.5	1.8	**60.0**
Hemp	*Cannabis sativa*	**1930.3**	**32.0**	22.0
Flaxseed	*Linum usitatissimum*	**2259.4**	**42.5**	2.0
Millet	*Pennisetum glaucum*	1493.9	3.9	**69.0**

(cont)		Seed pairs		
		Size	Nutrients	Shape
Wheat	*Triticum aestivum*	**Big**	**Carb-rich**	Oval
Hemp	*Cannabis sativa*	**Big**	Fat-rich	**Circular**
Flaxseed	*Linum usitatissimum*	Small	Fat-rich	Oval
Millet	*Pennisetum glaucum*	Small	**Carb-rich**	**Circular**

After 24h, the individuals were removed and transferred back to their home cages. We collected the trays, sieved the sand, and counted the remaining seeds that were still intact (i.e. uneaten). We calculated the overall GUD (across all seed types, i.e. food items), and the

seed specific GUD by dividing the total number of seeds left in each patch by the 0.5 l of sand. Two patches were removed from the dataset as the contents of the trays were accidentally mixed-up. Seven patches showed signs of human error (more than seven seeds for each species in the final count), however, we kept them in our analyses as we investigate giving-up densities, which should be independent of initial fillings.

Data and statistical analyses

Tracking of individual movement was done via the AnimalTracker plugin for ImageJ (Gulyás et al. 2016), using the recorded videos of the experiments (for details, see Appendix A). Using AnimalTracker, we quantified for each active bout (i.e. animal leaving the shelter), the individual's total active time in the arena (time spent outside the shelter, in sec), the cumulative time spent in each patch (measure of patch residency, in sec), and the cumulative distance travelled (in cm). Due to the automatic tracking methodology of AnimalTracker (i.e. blob detection), the time and distance were counted as soon as the entire head of the animal was visible outside the shelter or inside the patch. Time and distance travelled were later converted and are reported as min and m for easier interpretation. Since cumulative time and distance travelled in a patch were highly correlated (Kendall's correlation coefficients (tau) = 0.79, $p < 0.001$), we only used cumulative time in a patch for subsequent analyses. We then calculated the number of unique visits per patch, with each visit either being the individual moving from one patch to another, or if the individual left the patch for ≥ 5 sec and returned. This interval was chosen to differentiate events where individuals returned to the shelter (e.g. for caching seeds) from events where individuals very briefly exited the patch, as most of the latter events occurred within five seconds. Repeated visits gave us a spatial measure of risk perception, in which animals would only visit patches repeatedly if they perceived the energetic gains (profitability of a patch) as higher and predation risk as lower.

We built linear mixed effects models (LMM) with dependent variables GUD, cumulative time spent in a patch, and number of patch visits. We square-rooted the cumulative time and number of visits variables to normalize them. Although number of visits is a count variable, the final number of patch visits had a large range (min-max range: 0 - 56 visits), so it was treated as a continuous variable. We used treatment (risky or safe), order (1st or 2nd), and distance of patch to shelter as fixed effects, as well as all two-way interactions among these three fixed effects. Models included patch and individual identity as random effects (random intercepts) to control for repeated measures, spatial correlation as well possible individual variation. If patch identity explained zero variance, it was dropped from the model. Before running our models, we visually inspected variation across experimental days (four animals tested simultaneously) and across arenas (arenas being used repeatedly). We also used these as random effects in preliminary analyses. Since these variances appeared homogeneous, we decided not to include them as extra random effects in the model to avoid overfitting models.

The importance of functional traits was investigated by grouping the seeds from the four plant species into pairs that shared at least one functional trait of the seeds (size, nutrient and shape - Table 1). By dividing the seeds according to their properties (big or small, carb-rich or fat-rich, circular of oval), we could distinguish and disentangle which functional traits were important under different risk conditions, rather than knowing which plant species seed would possibly drive the harvest curves. We used a Poisson log-linked GLMM to test if the total amount of seeds eaten (dependent variable) varied among seed pairs. Each functional trait grouping was analysed in a separate model (3 models). We also included in the model the independent variable interaction of seed pairs with treatment or order. We removed interactions from the model if they were non-significant, but always kept treatment, order and trait difference as fixed factors. Models included patch and animal ID as random effects (random intercepts) to control for non-independence among seeds within the same tray, and

differences among individual foragers. If patch ID explained zero variance, it was dropped from the model. If any of the interactions were significant, we did a simple post-hoc comparison using the least-square means, comparing among factor levels within levels of the respective other factor. To investigate harvesting dynamics of single seed types, we plotted seed specific harvesting functions using polynomial regression fittings of seeds by time spend in a patch.

All analyses were done in R 4.0.4 (R Core Team 2021), and run using the lm4 package (Bates et al. 2015) and the lsmeans package (Lenth, 2016). For each model, we tested if the removal of different fixed effects improved the model fit based on the Akaike information criterion (AIC), starting with the least supported interactions, and report the most parsimonious model. Model fit was evaluated based on residual distribution using qqplots. P-values for LMM and GLMM models were obtained through the R package lmerTest (Kuznetsova et al. 2017). The accepted significance level was set to $\alpha < 0.05$. To correct for repeated testing in the seed pair analyses, we adjusted the significance level with a Bonferroni correction to $\alpha = 0.016$.

Ethics statement

Animal capture, housing and experiment were done under the permission of the "*Landesamt für Umwelt Brandenburg*" (reference number: AZ: N 1 0424), "*Landesamt für Arbeitsschutz, Verbraucherschutz und Gesundheit Brandenburg* (LAVG)" (reference number: AZ: 2347-46-2018) and "*Landeshauptstadt Potsdam, Veterinärwesen und Lebensmittelüberwachung*" (reference number: AZ 386-1-). The experiment was conducted in accordance with all applicable international, national and/or institutional guidelines for the use of animals.

Results

Over a 24-hr period, individuals travelled 512.49 m ± 326.15 (mean ± standard deviation) and were active for 212 min ± 104. In the safe treatment, GUD was lower and cumulative time spent in a patch was higher compared to the risky treatment (Table 2, Figure 1b-c, Figure 3a,c). On average, individuals moved 744.88 m ± 237.59 in the safe treatment and 280.10 m ± 219.52 in the risky treatment. When subjected to the experiment a second time, GUD was higher and cumulative time spent in a patch was lower (Figure 3b,d), but there were no differences in the number of patch visits (Figure 3f). GUD decreased and cumulative time spent in a patch increased in patches further away from the shelter (Table 2, Figure 1b-c, Figure 2a-d). In the safe treatment the number of patch visits increased with distance from the shelter (Table 2, Figure 1d, Figure 3e), but not in the risky treatment.

Bigger seeds were eaten more than smaller seeds, which were left uneaten more often in the patch (z-ratio = 15.26; $p < 0.001$; Table 3, Figure 4a-b) regardless of risk treatment. There were tendencies for interactions between seed nutrients and risk treatments, as well as between test order and seed shape (Table 3). In the safe treatment, individuals ate more fat-rich seeds than carb-rich seeds, leaving the latter in higher densities in the patch, but there was no effect of nutrients in the risky treatment (Figure 4c-d, Table 4). Individuals ate more seeds in the first run (Table 2-3), and preferred circular seeds over oval in the first run (Figure 4e-f, Table 4). Seed specific harvesting curves (Figure 5) suggest that animals reached meaningful GUDs (no further depletion) only in the safe treatment, since an asymptote was reached for each seed type. The bigger seeds (wheat and hemp) reached its value earlier (visual inspection) and were depleted to lower asymptotes than the smaller seeds. Meanwhile, in the risky treatment animals stayed shorter, and asymptotes were not reached for any of the seed types.

Table 2 – Results of final LMMs for the dependent variables of giving-up density (GUD), cumulative time spent in a patch, and number of patch visits. The models included the individual identity as a random effect and some models also included patch identity as a random effect. Categories are given in brackets and compared to reference levels. Shown are estimated fixed effects (β), their standard errors (SE), p-values (p), Chi-square (Chisq) with degrees of freedom (df), the R^2 based on the fixed factors (R^2 marginal) and based on fixed and random factors (R^2 conditional), as well variance and standard deviation (SD) of each random effect. All significant relationships are shown in bold font.

	Giving-up density (item/l)		Time in a patch (square-root transformed, min)		Number of patch visits (square-root transformed)	
	$\beta \pm$ SE	p	$\beta \pm$ SE	p	$\beta \pm$ SE	p
Intercept	47.16 ± 1.72		1.60 ± 0.14		2.83 ± 0.24	
Treatment (Safe)	**-5.16 ± 0.78**	**<0.001**	**0.49 ± 0.06**	**<0.001**	**0.91 ± 0.15**	**<0.001**
Order (2nd)	**3.89 ± 0.78**	**<0.001**	**-0.39 ± 0.06**	**<0.001**	-	
Distance	**-0.05 ± 0.02**	**<0.001**	**0.003 ± 0.001**	**<0.001**	-0.001 ± 0.002	0.75
Treatment*Distance	-		-		**0.007 ± 0.001**	**<0.001**
Subset analyses — Distance (Safe)	-		-		**$6.83 \times 10^{-3} \pm 2.19 \times 10^{-3}$**	**0.005**
Subset analyses — Distance (Risky)	-		-		$-5.13 \times 10^{-4} \pm 1.25 \times 10^{-3}$	0.69
Chisq (Df)	42.60 (1)		66.30 (1)		1.62 (1)	
R^2 marginal	0.08		0.11		0.37	
R^2 conditional	0.27		0.32		0.69	
Patch identity — Variance	-		-		0.05	
Patch identity — SD	-		-		0.74	
Individual identity — Variance	30.01		0.20		0.55	
Individual identity — SD	5.48		0.45		0.74	
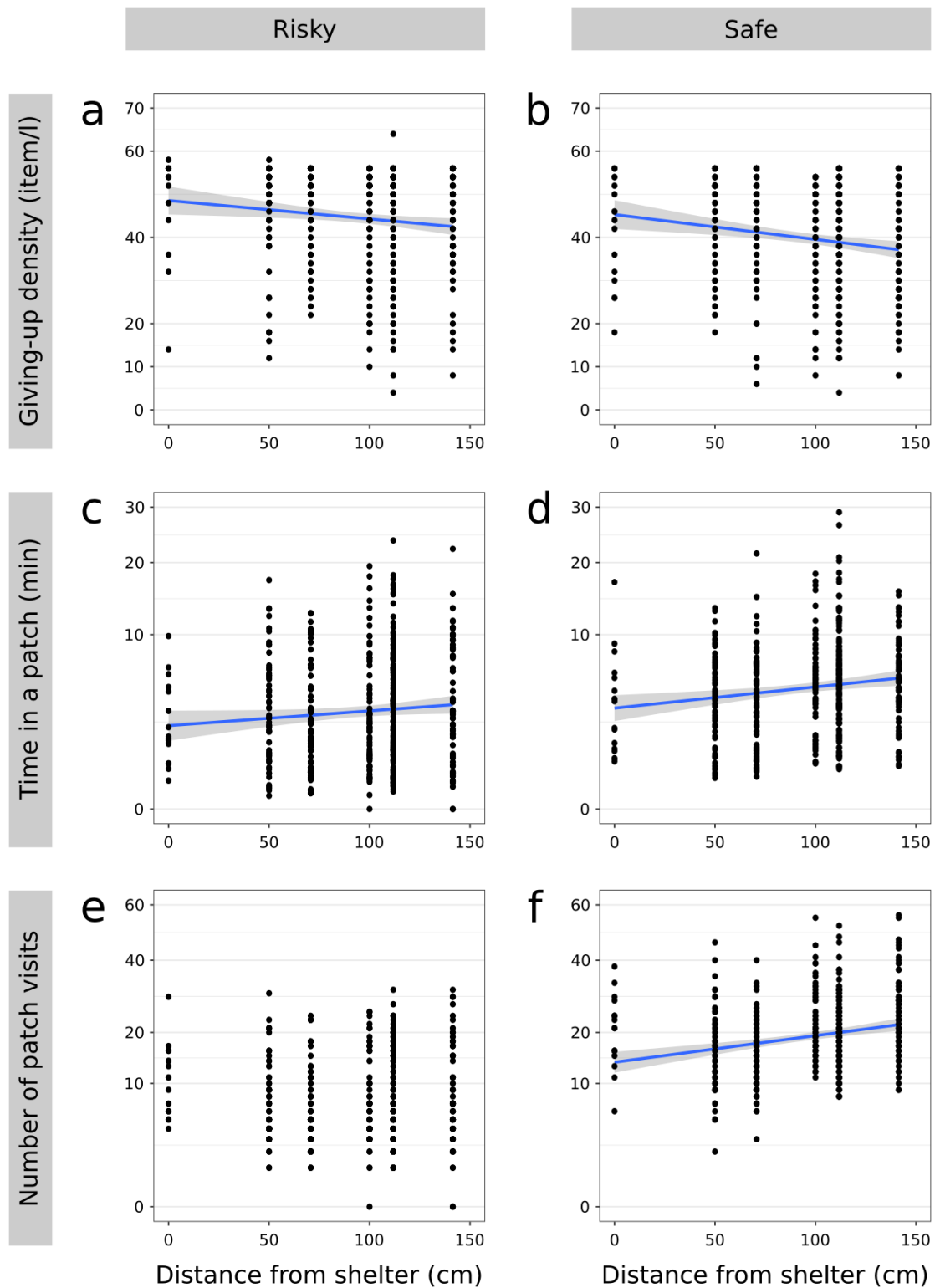
Figure 2 – Relation between a,b) giving-up density, c,d) cumulative time spent in a patch (square-rooted), and e,f) total number of patch visits (square-rooted) to the distance from the shelter, between perceived predation risk treatments (risky and safe). The blue trend lines and their 95% confidence intervals (grey) are based on the significant linear models, without random effects for illustration only.
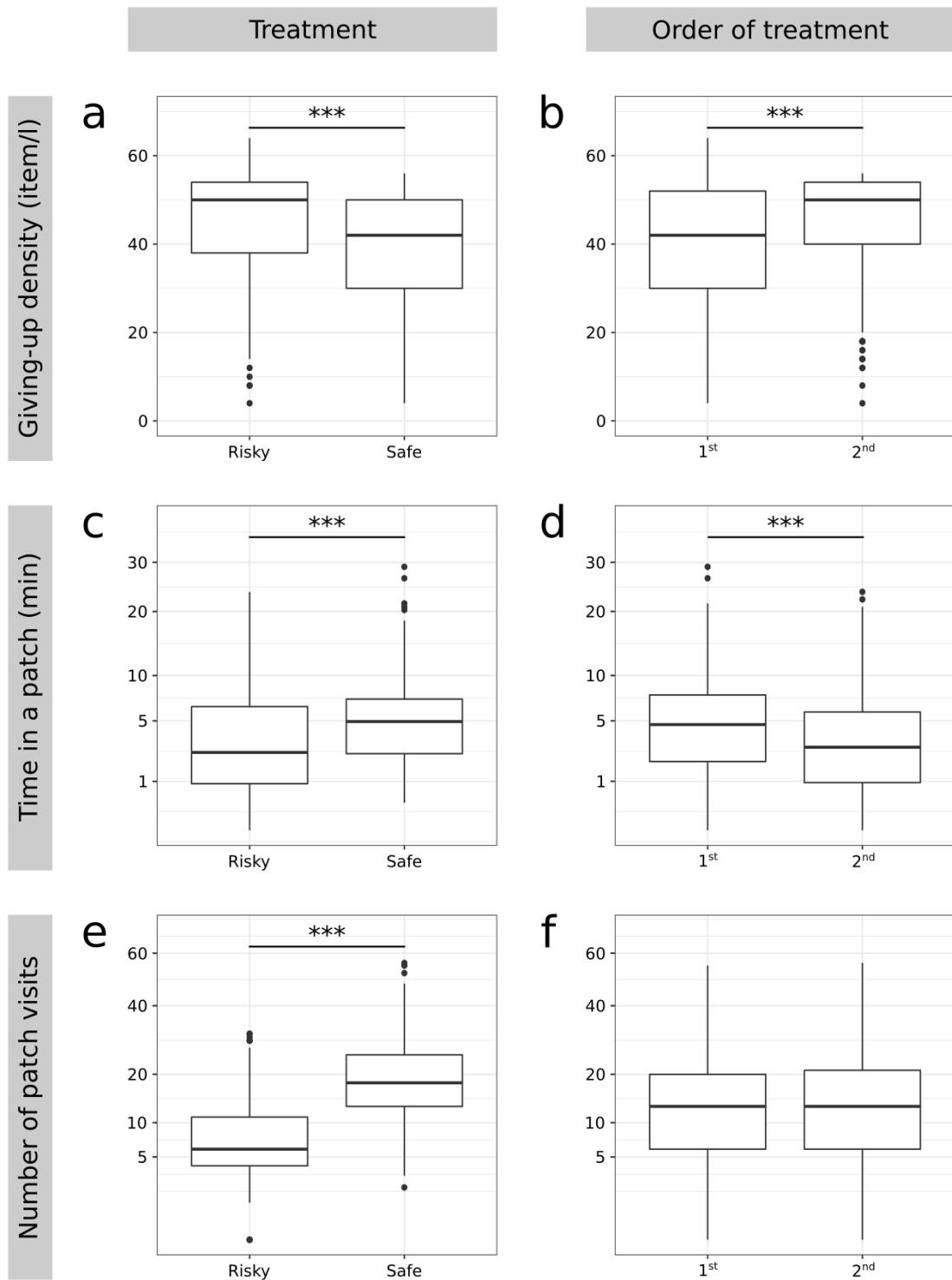
Figure 3 – Differences in giving-up density, cumulative time spent in a patch (square-rooted), and total number of patch visits (square-rooted) between perceived predation risk treatments (risky and safe; a, c, e), and between first and second treatment experienced by an individual (order of treatments was randomized; 1st and 2nd; b, d, f). Significant differences are shown with *** (*p*-value < 0.001) above box-plots.

Table 3 – Results of final GLMMs on the number of seeds eaten within seed pairs differing in levels of one functional trait: size (big and small), nutrients (carb-rich and fat-rich) or shape (oval or circular). Interactions of seed pairs with perceived predation risk treatment (risky and safe) or randomized order of treatment that individuals underwent in the experiment (1st and 2nd) were also included in the model. The models included patch and individual identity as random effects. Shown are estimated fixed effects (β), their standard errors (SE) and p-values (p). All significant relationships are shown in bold font ($\alpha < 0.05$) and underlined if still significant with Bonferroni correction ($\alpha < 0.016$).

	Size		Nutrients		Shape	
	$\beta \pm SE$	p	$\beta \pm SE$	p	$\beta \pm SE$	p
Intercept	1.26 ± 0.11		1.09 ± 0.12		0.99 ± 0.11	
Trait	**-0.43 ± 0.03** (Small)	**<0.001**	-0.04 ± 0.04 (Carb-rich)	0.36	**0.15 ± 0.03** (Circular)	**<0.001**
Treatment	**0.45 ± 0.03** (Safe)	**<0.001**	**0.51 ± 0.04** (Safe)	**<0.001**	**0.45 ± 0.03** (Safe)	**<0.001**
Order	**-0.35 ± 0.03** (2nd)	**<0.001**	**-0.35 ± 0.04** (2nd)	**<0.001**	**-0.29 ± 0.04** (2nd)	**<0.001**
Trait * Treatment	-		**-0.12 ± 0.05** (Carb-rich * Safe)	**0.02**	-	
Trait * Order	-		-		**-0.11 ± 0.05** (Circular * 2nd)	**0.04**

Figure 4 – Differences in total number of seeds eaten (forager's 'Behavior') or left in the patch (seed community 'Biodiversity' after forager's give-up) for each pair of seeds sharing one functional trait (a) size, (b) nutrient-content, and (c) shape between perceived predation risk treatments (risky versus safe, a and b) and between the first or second treatment experienced by an individual (order of risk treatments was randomized, 1st and 2nd in c). Dotted lines represent individual responses. Different letters indicate significant group differences based on least-square means (adjusted with the Tukey method).

Table 4 – Post-hoc pairwise comparison of the significant interactions of the generalized linear mixed model (GLMM) with the total number of seeds eaten (dependent variable) and interactions of seed pairs (by functional trait) with treatment (safe versus risky) or order (1st or 2nd; i.e. randomized order of treatment that individuals underwent in the experiment). Shown are estimated comparison effect (β), their standard errors (SE), z-score (z-ratio), and the p-value adjusted with the Tukey method. Effect sizes are reported in a log scale. First column shows the reference pair in all analyses. All significant comparisons are shown in bold.

Seed pair (Nutrients) - Treatment interactions		β ± SE	z-ratio	p
Carb-rich - Risky	Fat-rich - Risky	0.04 ± 0.04	0.92	0.80
Carb-rich - Safe	Fat-rich - Safe	0.16 ± 0.03	4.60	<0.001

Carb-rich - Safe	Carb-rich - Risky	-0.39 ± 0.04	**-9.47**	<0.001
Fat-rich - Safe	Fat-rich - Risky	-0.51 ± 0.04	-12.85	<0.001
Seed pair (Shape) - Order interactions		**β ± SE**	**z-ratio**	***p***
Circular - 1st	Oval - 1st	-0.15 ± 0.03	-4.77	<0.001
Circular - 2nd	Oval - 2nd	-0.04 ± 0.04	-0.98	0.76
Oval - 2nd	Oval - 1st	0.29 ± 0.04	6.96	<0.001
Circular - 2nd	Circular - 1st	0.40 ± 0.04	**9.98**	<0.001
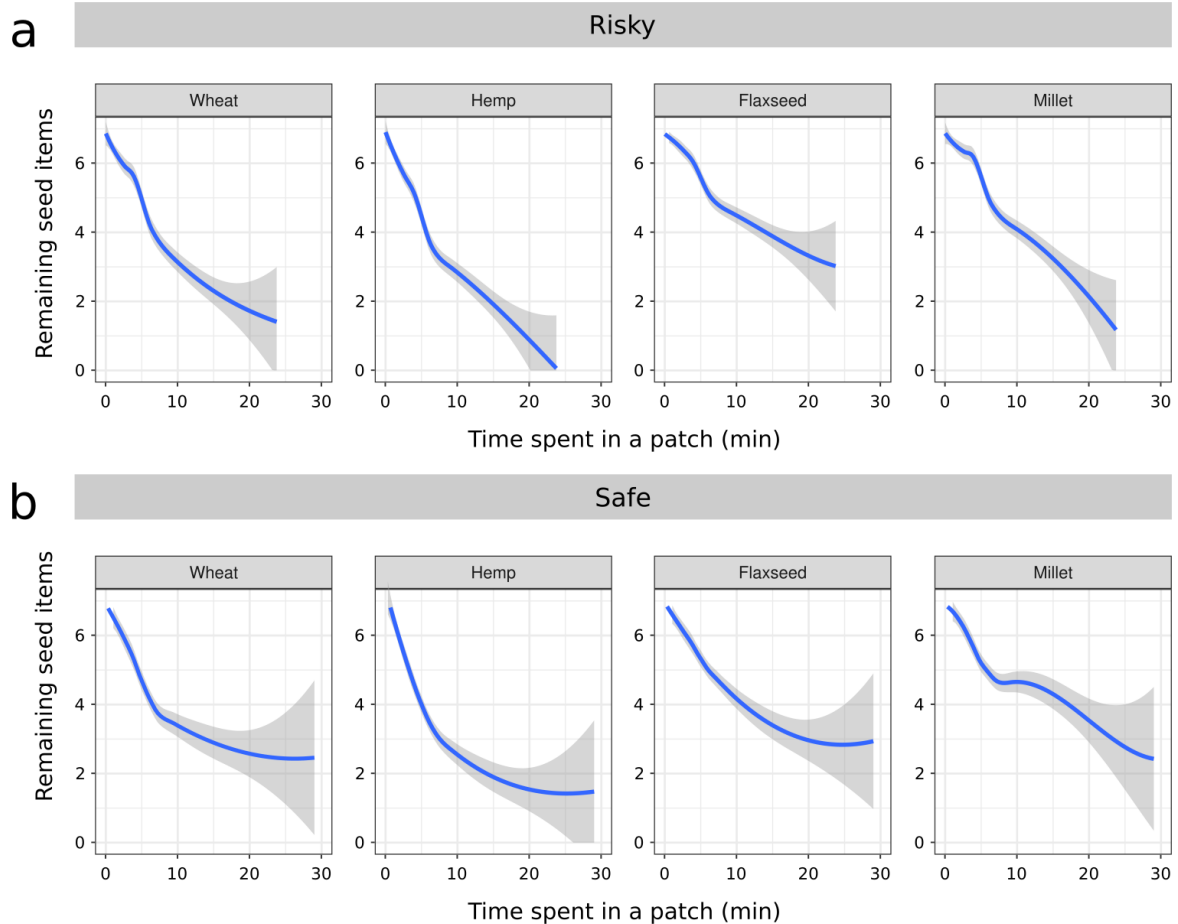
Figure 5 - Harvest curves per seed of four plant species (wheat, hemp, flaxseed and millet, blue lines), showing the remaining seed items and cumulative time spent in a patch between (perceived) predation risk treatments (risky, a, and safe, b). The harvest curves (blue lines) and their 95%-confidence intervals (grey) are based on polynomial regression fittings.

Discussion

As predicted, voles in a high predation risk treatment were less active and consumed less resources (higher GUD) and than when subjected to lower risk. These patterns are in line with other studies showing higher GUD and less time spent in risky landscapes compared to safe ones (e.g. Kotler 1992, Orrock et al. 2004, Eccard et al. 2022). Individuals also visited patches less often in the risky treatment, further confirming the pattern that individuals maximized their gains through foraging by minimizing their foraging activity and repeated movements and, therefore, lowering the risk of becoming prey (Masello et al. 2017, Dammhahn et al. 2022). In contrast to our prediction for a central place forager, we observed that GUD decreased and cumulative time in a patch increased with higher distance from the shelter, and number of patch visits increased with distance in the safe treatment only. Given that all patches had the same initial gains, this result was unexpected within the context of central-place foraging theory, as there were no higher quality patches that would justify the repeated travel costs to the most distant patches (Bakker et al. 2005, Nilsson et al. 2020). However, these observations may be due to the small dimensions and fencing of the arena, as foraging closer to the high metal fence could have been perceived as safer than the more exposed patches in the centre. We observed in some situations that, when leaving the shelter, bank voles would immediately move to the edges of the arena, and then use that location to go into the patches. While the patches were still separated from the metal fence, future studies should separate patches further from the limits of the arena so the foragers can choose solely to move within the patch array, or increase the size of arenas (e.g. Dammhahn et al. 2022). Albeit the experimental arenas were small and might not fully allow voles to express variation in foraging movement or represent their energy landscapes, we could demonstrate differential movement patterns between safe and risky treatments. Additionally, the safer areas accidentally created in our design (heterogeneous perceived predation risk) further illustrated the decision-making process of foragers, as this area took priority in their foraging effort distribution despite the increased travelling costs for the forager.

We observed that preference for some functional traits of plant seeds changed depending on perceived risk, even though foragers seemed to prefer larger seeds independently of the risk

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treatment. This result is in concordance with previous studies, that showed bank voles (Fischer et al. 2017, Ellingsen et al. 2017) and other rodents (e.g. Wang and Chen 2009, Mortelliti et al. 2019) forage on larger seeds. Under high perceived risk, it is expected that by foraging for shorter periods of time, the individual can be less selective of which seeds they remove, therefore, not express their actual dietary preference (Eccard et al. 2022). When placed in a substrate, it is possible that voles consume the bigger seeds first not by dietary preference but by opportunistic chance, as these larger seeds are not only more visible to the voles (Garb et al. 2000) but also often rise quicker to the top than smaller seeds when the substrate is moved by the digging action of the vole (Gajjar et al. 2021). Therefore, the foraging period is reduced substantially regardless of risk merely by the higher visibility of the seeds. However, bigger seeds also have proportionally more nutrient content per seed than smaller ones, making them more profitable food (Wang and Yang 2014, Wang and Corlett 2017, Hou et al. 2021), and this preference might therefore not be affected by varying perceived predation risk (Sivy et al. 2011). A preference for maximizing energetic gains is also evident by the removal of the larger and most nutritious seeds first, while smaller and less nutritious seeds are left in higher densities in the patch, or consumed only after the most preferable seeds are depleted in safer landscapes (Figure 4-5; see also Eccard et al. 2022, Ferreira et al. 2022).

In the safe treatment, we found that the foragers expressed a preference for fat-rich seeds, which also had more caloric content proportionally to the seed size, which was not expressed in the risky treatment. A possible preference for fat-rich seeds in safer landscapes would allow us to disentangle the importance of two functional traits that are often linked together: size and fat-content (Wang and Yang 2014). Studies on bank voles have previously shown their preference for fat-rich seeds (Ellingsen et al. 2017, Fischer et al. 2017), especially in pregnant females requiring more nutritional rich food (Eccard and Ylönen 2006), but this preference is also seen in other rodents (Wang and Chen 2012, Boone and Mortelliti 2019). If significant, preference of fat-rich seeds in safe landscapes could be actual nutrition preference acting, as foragers might feel safe enough to spend more time choosing among seeds and acting on their actual dietary preference, rather than seizing a fast opportunity with the bigger and most visible seeds. This interaction of

nutrient-type with perceived predation risk treatment also demonstrates that risk landscapes can also affect Diversity at GUD (Eccard et al 2022), as in safer landscapes the carb-rich seeds are left in higher densities once the forager gives-up on feeding. Further, food preference should be taken into account when testing landscapes of fear using artificially provided food sources, as the food item nutritional values can drive part of the results (McMahon et al. 2018). More nutrient types should be investigated in the future, as well as the presence of husk in seeds, as in our study husk, high protein and fibre content were traits only present in the seeds with already high fat content and thus we could not disentangle them.

The third functional trait studied – shape – did not seem to be preferred by foragers among or within treatments. However, foragers seemed to consume more of the circular seeds when tested the first time, while later they showed either no preference for any functional traits and overall ate less seeds than the first time. The seed's shape is a functional trait that is less studied in foragers preference, even though it can influence handling and transportation, two behaviours that are part of the decision making of foraging (Kelrick et al. 1986, Muñoz et al. 2012). The possible preference for circular-shaped seeds in our study could have been driven by hemp, a large and fat-rich seed that was always depleted at faster rates regardless of treatment (Figure 5). In comparison to the bigger wheat seed, hemp has a circular shape, relatively smaller mass and is much more caloric in proportion to its size (Table 1), which makes it a seed that is highly nutritious, but also easier to both handle and transport. Other studies have also shown that smaller seeds with high nutritional values can be preferred by foragers due to its lightweight and easiness to transport rather than overall size (Muñoz and Bonal 2008, Fischer and Türke 2016), further, a simpler or more circular seed shape can also be preferred due to easier manipulation (Kelrick et al. 1986, Muñoz et al. 2012).

The preference for shape could also be misleading in our results, as overall, individuals showed higher GUD and less active time when submitted to the experiment a second time, regardless of risk treatment. Given its artificial setting, as well as familiarity with previous feeding experiments, it is possible that individuals got habituated to the experiment (Martin and Réale 2008). One component that we also did not analyse was individual personality, which is known to also

influence both foraging behaviour under varying predation risk (Mazza et al. 2019, Dammhahn et al. 2022), but also food preference (Boone et al. 2022). Results within individual show that there might be some individually based variation (Figure 4a-f), as some individuals constantly foraged more than others, although its relation to perceived predation risk and/or seed functional traits was not analysed.

Conclusion

Variation in perceived predation risk impacts the ecology of prey species, affecting their foraging behaviour in terms of amount of energy consumed and movement pathways. Prey species navigate in these landscapes of fear carefully to avoid mortality, while still trying to obtain enough resources to survive in the least amount of foraging time. In this study, we observed that the landscape of fear not only affects the amount of food consumed and the active time foraging, but also affects the diet preference of foragers beyond a species' standard preference for certain functional traits. Individuals minimized potential mortality costs by prioritizing safer areas in the landscape, despite moving longer distances, while maximizing the energetic gains by foraging selectively on resources within and among perceived predation risk treatments. Under safer conditions, animals selected seeds for their larger size and fat content, while under risky conditions only a size selection was observed. Thus, risk while foraging affects the remaining seed community diversity. Understanding the variation of behaviours within landscapes of fear helps us to understand the complexity of the non-consumptive effects of perceived predation risk, and further illuminate the cascading effects of fear on biodiversity.

Acknowledgements

Chapter III

Fear or disgust? Effects of the invasive raccoon on rodent foraging behaviour

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Abstract

Raccoons (*Procyon lotor*) are an invasive species in Europe, and their opportunistic feeding habits can be harmful for the native wildlife. When they prey on animals, raccoons most feed on birds and eggs, but their diet can partially comprise of rodents. Although rodents can avoid predation by raccoons, it is unknown if this invasive species has any non-consumptive effects on rodent behaviour. Raccoons are known to create a landscape of disgust in their native habitats, with birds and rodents actively avoiding their latrines to prevent infections from roundworms.

We studied how rodent foraging behaviour was affected by raccoon presence, by manipulating the landscape of fear of both species. Raccoons were gradually attracted to feeding locations with pet food, and then expected to be repelled with wolf urine. Simultaneously, we used resource-diverse artificial food patches for rodents to evaluate the impacts of varying raccoon presence on rodent's behaviour (using giving-up density and diversity at the giving-up density). Vegetation height was used as a proxy for avian predation risk.

The density and diversity of resources decreased with increasing vegetation height and increased rodent habituation, but did not vary with increased or decreased raccoon activity. Rodents showed no temporal risk avoidance with the presence of raccoons, and we observed instances of concurrent feeding activity of rodents and raccoons. While rodents did not perceive raccoons as potential predators, we also did not observe a landscape of disgust in which rodents avoided this invasive species, and further studies on the potential of disease transmission to native rodents should be investigated further.

Introduction

The increase of human activity all around the globe is the leading cause of biodiversity loss (Johnson et al. 2017), and the introduction of invasive species to already impacted habitats is contributing to this effect. Invasive species cause disruption throughout trophic chains, either by competing with, feeding on, or spreading diseases to the native species (Lockwood et al. 2013). The raccoon (*Procyon lotor*) is a mesocarnivore native to North and Central America, but

introduced in the 1900s in Europe in fur farms, and both escapes and released occurred at the 1930s in Germany (Müller-Using 1959). Now, raccoons are now present in central, eastern and southern Europe (Salgado 2018). In their native range, their omnivorous diet ranges from vegetables, fruits, insects, aquatic wildlife, small wildlife (birds and small mammals), eggs and carcasses (Lotze and Anderson 1979). Just like their native counterparts, invasive raccoons are opportunistic and omnivorous, and often impact the native wildlife (Bartoszewicz et al. 2008, Oe et al. 2020). In Europe, the biggest concern is the damage by raccoons to ground- and cavity-breeding birds (Fischer 2016), since the bird's nests are easily accessible for the dexterous raccoons. Further conservation concerns of raccoons include predation on hibernating bats (Cichocki et al. 2021).

Although rodent predation is not common, as healthy and agile rodents can easily escape raccoons (Mumford and Whitaker 1982), they still are part of the raccoon's diet as prey, with one study in Europe reporting rodents as 34% of the consumed biomass in faeces (Bartoszewicz et al. 2008). It is possible, therefore, that raccoon presence in habitats may generate a landscape of fear in rodents, that is, the spatio-temporal variation of perceived predation risk. Furthermore, in their natural range raccoons are host to roundworms that can then spread and cause disease in birds and small mammals foraging in raccoon faeces for leftover seeds (LoGiudice and Ostfeld 2002, LoGiudice 2003). As foragers try to avoid the possibility of being infected by the parasites, the raccoon presence may also create a prey avoidance as part of a landscape of disgust (Weinstein et al. 2018, Doherty and Ruehle 2020). It was observed that, in the native range of the raccoon, granivorous foragers might avoid raccoon latrines due to the possibility of roundworms (*Baylisascariasis* infection), although the raccoon non-consumptive effect due to possible predation could also not be disentangled (Weinstein et al. 2018). In it's non-native regions, it is also unknown what are the non-consumptive effects of this invasive species for potential prey animals.

Rodents are a good model species for studies on landscapes of fear, since they are prey to many species, rodents starkly change their foraging behaviour according to perceived predation risk using different cues (e.g. olfactory cues and/or vegetation cover). When faced with terrestrial predators, rodents often show temporal risk allocation, either by avoiding the times when predators

are active or changing their foraging behaviour when they smell the presence of a predator (Eccard et al. 2008, Moll et al. 2020). However, they show higher risk avoidance to avian predators, as rodents cannot predict the presence or absence by olfactory cues, but rather show risk avoidance by foraging less in low vegetation cover habitats (e.g. Dammhahn et al. 2022). However, it still remains understudied how the invasive raccoon might affect the landscape of fear of native rodent species, or if they also display the same landscape of disgust that North America rodents do.

In this study, we report our findings on changes of rodent foraging behaviour impacted by raccoon presence. Both raccoon and rodents were subjected to species specific landscapes of fear, and we investigated if rodent foraging and temporal activity were affected by changes in raccoon presence. We used artificial food patches and measured the giving-up density (GUD, i.e. total number of remaining seeds left divided by the total amount of sand; Brown 1988) as well the diversity at the giving-up density (DivGUD, i.e. remaining Shannon's alpha entropy at each seed tray; Eccard et al. 2022) to evaluate the perceived predation risk of rodents faced with different raccoon levels of activity. We expected that if raccoons were attracted to certain areas, the rodent activity would drop during the night and we would observe higher GUD and DivGUD, but as raccoon were repelled and their activity dropped, rodents should sharply decrease the GUD and DivGUD to make up the lost energetic costs of missed opportunities (Eccard et al. 2008). Furthermore, we predicted that rodents would shift their temporal activity to avoid the nocturnal raccoons.

Materials and Methods

We performed the experiment in the Uckermark district, Brandenburg, Germany in August 2020. The area is comprised by mostly agricultural landscapes, with some wetlands also being present. Raccoon presence and activity range were previously assessed through capture and GPS- and radio-tracking, and for experimental manipulation we chose three locations that were repeatedly visited by several GPS- and radio-collared raccoons (unpublished data). In each location, two sites were setup 200 meters apart from each other but in the same habitat type, one receiving the

'feeding' and one the 'control' treatment level. For the 'control' treatment level, the site remained unchanged. In the 'feeding' treatment, raccoons were attracted to dog food to concentrate their activity, and then wolf urine was sprayed in the feeding areas to create a higher perceived predation risk and repel them. Racoons are known to avoid areas with large carnivores (Suraci et al. 2016). Both areas, control and feeding site, were equipped with three wildlife cameras (CuddeLink Black Flash G-5079, Cuddeback, U.S.A.) to record raccoon activity. The experimental period (1 month) was divided in four rounds to manipulate landscapes of fear of both raccoons and rodents: 1st round – the raccoon were not fed (control round); 2nd round – raccoons were fed, low raccoon activity observed; 3rd round – raccoons were still fed, high raccoon activity was observed; 4th round – wolf urine was sprayed in the raccoon feeding areas, raccoons were expected to be repelled.

At each site, we set-up two arrays of seed trays for wild rodents (n = 12 in total). For the raccoon 'control' treatment, we wanted to establish a baseline for standard rodent behaviour. Each array of seed trays had a total of seven seed trays spaced by 6 m in a hexagonally pattern with a tray in the centre (methods mimic those in Ferreira et al. 2022). Each seed tray consisted of a plastic tray with 500ml of fine sand ($\varnothing$ = 14.5 cm, depth = 4 cm) mixed with a total of 80 seeds of seeds from eight different plant species with 10 items each (sunflower, kardi, wheat, hemp, flaxseed, millet, canary seed, and sesame).

In each experimental round, all arrays of seed trays were set-up simultaneously for three consecutive nights. We monitored the species visiting of seed trays by setting up two cameras per array of seed trays, at randomly selected seed trays. The cameras took up to three consecutive photos with no delay in between, to maximize rodent activity recording and species identification. The average vegetation height was measured around each seed tray during the first round. After the third night of each round, seed trays were collected and dried in an incubator at 60ºC to sieve all remaining intact seeds, and then we calculated GUD and DivGUD. We removed 13 trays with zero seeds left, as no GUD/DivGUD could be calculated. The photos from the rodent experiment were analysed to identify rodents and terrestrial carnivore species visiting the seed trays and activity density was assessed for species with more than 100 photo records in total using the

camtrapR package (Niedballa et al. 2016). We also calculated the diurnality index (proposed by Halle 1995) using the recorded counts from the species appearing in the seed tray cameras for all treatments and rounds. For raccoon activity, we further included the photos taken from the cameras at the raccoon's experimental sites. We did a linear mixed model to test if GUD and DivGUD (dependant variables) changed with average vegetation height and the interaction of raccoon treatment with mean-centred experimental day (based on absolute number of days since beginning of experiment). Location was included as a random factor. GUD was log transformed and DivGUD was square rooted. All analyses were done in R 4.0.4 (R Core Team 2021).

Results and Discussion

A total of 12,334 photo were taken of at least six rodent species were recorded in the cameras pointed at the seed trays (*Apodemus agrarius*, n = 5,327; *A. flavicollis/sylvaticus*, n = 4,338; *Micromys minutus*, n = 5; *Microtus spp.*, n = 34; *Myodes glareolus*, n = 2,308; *Rattus spp.*, n = 23) as well three carnivore species (*Martes martes*, n = 10; *Mustela nivalis*, n = 102; *Procyon lotor*, n = 87). Based on camera trap photos alone, it is not possible to identify any *Microtus* species, nor distinguish between *A. flavicollis* and *A. sylvaticus* reliably. Since the cameras pointed directly to the seed tray, we could also not get an entire body view of *Rattus spp.* to identify the species, but given rarity of *R. rattus* in NE Germany as well the data from the raccoons site's cameras, it was most likely *R. norvegicus*. The presence of *M. martes* and *M. nivalis* was only found in the 'control' site cameras.

Both GUD and DivGUD decreased with increasing average vegetation height and with the number of days since the beginning of the experiment regardless of treatment (Table 1). It is very likely that the rodents got habituated to the seed trays, and foraged more often with each passing round as they expected high quality artificial food patches to appear each time in all sites. There was seemingly no effect of raccoon presence or foraging activity in the overall resource consumption of seeds by rodents (Figure 1).

Patterns of activity density and diurnality indices also did not change with raccoon activity (Figure 2), and *A. agrarius* as well *A. flavicollis/sylvaticus* individuals showed nocturnal activity pattern, and *M. glareolus* individuals alternated between diurnal and nocturnal activity patterns. While *Apodemus spp.* species are typically nocturnal (Greenwood 1978, Łopucki and Kiersztyn 2020), *M. glareolus* are mostly diurnal, especially when the larger *Apodemus spp.* are present (Andrzejewski and Olszewski 1963). It is possible that *M. glareolus* actively avoided periods of time where *Apodemus spp.* would be actively foraging in the trays or, in the 'control' treatment, avoid possible diurnal predators such as *M. nivalis*. However, none of the rodents avoided the seed trays during periods of raccoon activity in the 'feeding' treatment. In fact, in the raccoon site cameras, there were 11 recorded instances of raccoons and rodents feeding together at a distance of a body length of a raccoon (*A. flavicollis/sylvaticus* = 8; *M. glareolus* = 2; *Rattus norvegicus* = 1).

Table 1 – Results of the linear mixed model on the relation between average vegetation height, raccoon treatment ('feeding' versus 'control') and mean-centred experimental day ('days') with the dependent variables of giving-up density (GUD) and local diversity at the giving-up density (α-DivGUD).

		Giving-up density (item/l)		True alpha diversity (α-DivGUD)	
		β ± SE	p	*β ± SE*	p
Intercept		3.88 ± 0.41		2.33 ± 0.20	
Treatment ('Feeding')		-0.59 ± 0.57	0.35	-0.26 ± 0.28	0.4
Days		**-0.10 ± 0.01**	**<0.001**	**-0.05 ± 0.005**	**<0.001**
Average vegetation height (cm)		**-0.01 ± 0.001**	**<0.001**	**-0.003 ± 0.001**	**<0.001**
Treatment*Days		**0.04 ± 0.01**	**0.01**	**0.02 ± 0.01**	**0.01**
Subset analyses	Days ('Feeding')	**-0.06 ± 0.01**	**<0.001**	**-0.03 ± 0.005**	**<0.001**
	Days ('Control')	**-0.01 ± 0.01**	**<0.001**	**-0.05 ± 0.005**	**<0.001**
R^2 marginal		0.28		0.27	
R^2 conditional		0.58		0.60	
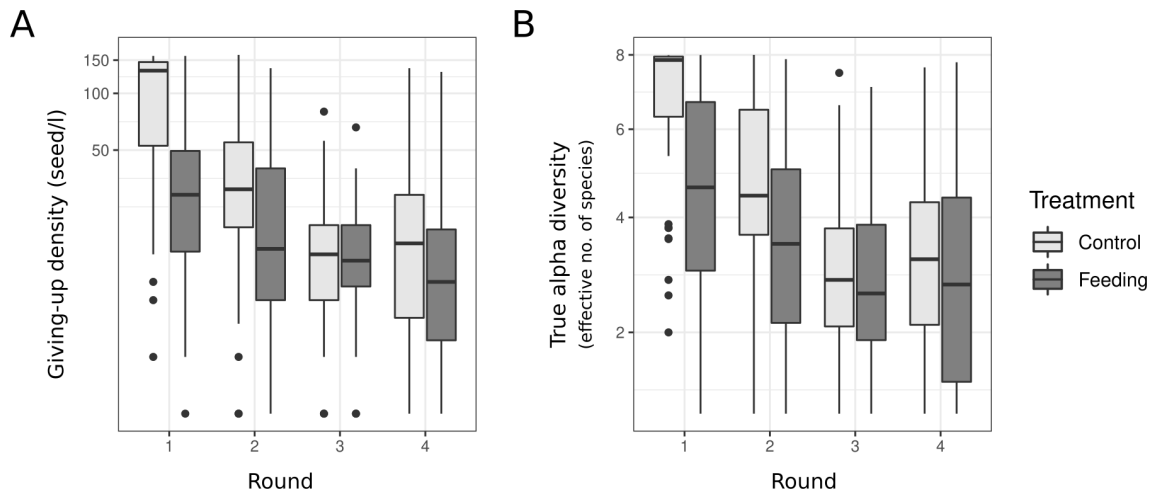
Figure 1 – Changes in giving-up density (GUD, a) and true alpha diversity (DivGUD, b) in the seed trays for wild rodents among experimental rounds in each raccoon landscape of fear treatment.
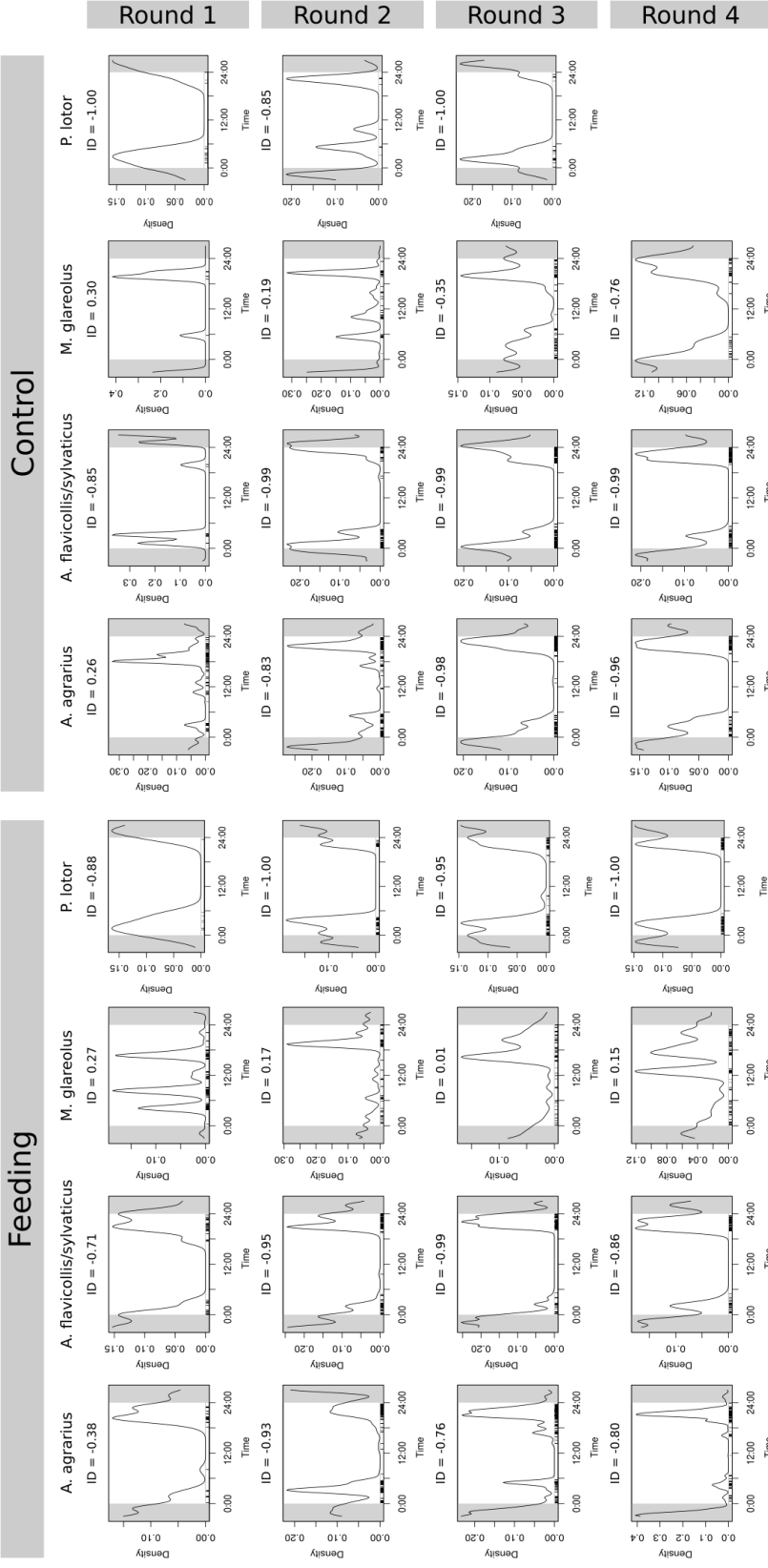
Figure 2 – Activity densities for three rodent species (*Apodemus agrarius*, *A. flavicollis/sylvaticus*, and *Myodes glareolus*) and raccoons (*Procyon lotor*) among treatments and experimental rounds. Diurnality indexes are shown above (negative values represent mostly nocturnal activity, while positive values represent mostly diurnal).

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In our study, rodents did not seem to respond to raccoon presence in the same way they respond to other terrestrial predators (temporal-risk allocation). Avian predation (approximated by vegetation height) still seems to be the highest factor in anti-predator behaviour of ground-dwelling rodents. The native rodents might not recognize the raccoon as a threat, as raccoons were only recently introduced. Together with low mortality risk, the rodents might not have learned that these invasive mesocarnivores are a possible mortality (albeit low) risk that they should avoid (see Bedoya-Pérez et al. 2019 and references therein for fear learning mechanisms). Furthermore, given that raccoon predation risk might be low, the costs of anti-predator behaviour of the rodents might not benefit the costs of not foraging in the landscape (Brown 1988). The lack of a response might also indicate that rodents are not acting on a landscape of disgust. It is known that raccoons in Europe also host to parasites (Popiołek et al. 2011, Karamon et al. 2014, Al-Sabi et al. 2015), including the roundworm *Baylisascaris procyonis*, that causes Baylisascariasis infections in American rodents. It is still fairly unknown if invasive raccoons can transmit parasites and diseases to native European rodent species (Beltrán-Beck et al. 2012). In Japan, there are documented cases of captive raccoons causing outbreaks of Baylisascariasis in domesticated rabbits (Kazacos et al. 1983, Sato et al. 2003), however wild ranging raccoons have not caused any documented outbreaks in wildlife so far.

Rodents can also become a new vector of parasites and diseases originally present in raccoons, and then affect other natural predators or humans in contact with rodents. There are rare documented human cases of Baylisascariasis infections in Europe, with cases restricted to humans taking care of raccoons in captivity/as pets (Küchle et al. 1993, Conraths et al. 1996). As the raccoon distribution range expands in Europe, and its contact with both wildlife and humans increases, it becomes increasingly important to monitor the effects of raccoon's presence.

IV General discussion

The spatio-temporal variation of perceived predation risk (landscape of fear) has many behaviourally-mediated cascading effects on trophic chains, spanning throughout predator-forager-resources interactions (Gaynor et al. 2019, Palmer et al. 2022). It affects not only the distribution and foraging behaviour of predators and foragers, but also changes the coexistence mechanisms occurring among resource species (Stump and Chesson 2017) as the foragers shape their movement and activity patterns (Eccard and Liesenjohann 2014, Gallagher et al. 2017), as well dietary preferences (Eccard et al. 2022) to maximise their energetic gains while avoiding the worst fate of mortality. The variation of landscapes of fear can also affect bottom-up effects, as the resource species biodiversity is shaped by action of foragers (Ripple and Beschta 2012, Mills et al. 2018). While the effects of fear are widespread, the research is often disconnected, focusing on certain responses to landscapes of fear with little empirical research integrating all processes and responses. In this thesis, we integrated all current theories into empirical experiments, and thus evaluated not only the qualitative responses to the landscape of fear, but also measured quantitatively the cascading effects in a tri-trophic chain. We assessed how feeding behaviour and movement of foragers shaped the final resource community (changes in coexistence mechanisms). By connecting all theories, we took a step forward to fully measure the cascading effects of landscapes of fear and expand the framework of the landscape of fear to include responses and consequences of forager-resources interactions changed by perceived risk.

Throughout the thesis, we found that biodiversity of resource species left in the landscapes depends greatly on the variation of perceived predation risk, as indicated by the biodiversity of resources left in the patch once the forager decided to quit (DivGUD). As perceived predation risk was lower (higher vegetation height, existence of cover or under dark conditions), not only did the density of resources decreased (lower GUD), but also the diversity of the resource species community (DivGUD, results from Chapter I and III). While past studies required for the standardization of the GUD method to account for all potential

confounding effects (e.g. differences in resource functional traits affecting the way a forager harvested the resources), the use of a single or few food items might no longer be required when measuring perceived predation risk in both wild and captive conditions. The DivGUD-measure overcame a limitation of the GUD measure by providing a direct measure of cascading effects at the level of a diverse community of resources while still measuring perceived predation risk at the forager level. Despite being tested with a wild and diverse community of foragers and at different spatial scales, the DivGUD measure still managed to provide the same measure of perceived predation risk as GUD. In Chapter I, we could see that both alpha (α-DivGUD) and gamma-diversity (γ-DivGUD) were lower with decrease of perceived predation risk, meaning that variation in landscapes of fear affects biodiversity at both local and regional scales. However, the decrease of resource species diversity was different among local and regional scales, as the beta-diversity (β-DivGUD) increased with the decrease of perceived predation risk. The β-DivGUD measures the regional variation among landscapes, that is, how dissimilar the resource species community was between patches after the forager's stopped foraging. The dissimilarity among local and regional resource species communities shows us that further variation of external factors can affect the responses to the landscape of fear. For example, potential microhabitat variation within the landscapes of fear can further create slight variations in the perceived risk of foragers (Thompson 1982, Orrock et al. 2004), especially in unmanaged grassland areas where vegetation might be more heterogeneous (Hinkelman et al. 2012).

However, the changes in β-DivGUD may also happen due to uneven harvest rates of different resource species. In fact, another advantage of DivGUD is that we can measure the differences in harvest rates of each resource species, which are in its turn cause by changes in the forager's feeding behaviour as predicted with optimal foraging theory (i.e. forager needs to decide which resource species gives the most energy in the least amount of time). The most profitable resources can often be differentiated by their functional traits, which in

the case of our thesis were plant seeds-specific functional traits: size, nutritional value and shape. In Chapter I, dissimilarity of resource species community was observed at different categories of vegetation height (proxy of perceived predation risk, with higher vegetation heights equating to lower perceived risk). At lower and medium vegetation heights, forager's exploited mostly the bigger and most nutritious seeds (e.g. sunflower and kardi seeds), while in the higher vegetation height most seeds were exploited, leaving behind the smaller and less nutritious seeds (e.g. millet, canary and sesame seeds). The smaller and less nutritious seeds were left at very different species richness and abundance levels in the patches with the higher vegetation heights, therefore, β-DivGUD might have increased with these stark dissimilarities of the seed community among patches.

In Chapter II, it was also observed that forager's depleted more of the bigger and most nutritious seeds. The preference for certain seed functional traits has been previously observed in rodents, with studies often finding that foragers have a preference for the bigger and/or most nutritious seeds (Wang and Chen 2009, Wang and Yang 2014, Fischer and Türke 2016, Fischer et al. 2017, Wang and Corlett 2017, Ellingsen et al. 2017, Mortelliti et al. 2019, Hou et al. 2021, Eccard et al. 2022). However, it's often very difficult to disentangle the effects of each functional trait (Wang and Chen 2009, Wang and Yang 2014), but it's also understudied if perceived predation risk changes the diet preference of foragers. In a study by Sivy et al. (2011), they could not find an effect of perceived predation risk on seed preference in rodents, even though there is the potential for forager's dietary change due to changing predation risk (example in anthropods: McMahon et al. 2018), or even due to other factors affecting the individual state of the forager (e.g. pregnancy in voles; Eccard and Ylönen 2006). We found that while individuals foraged more on the bigger seeds regardless of perceived predation risk, there was a possible shift to fat-rich seeds in safer conditions. With detailed recording, we could observe that forager's spent more time foraging in safer landscapes, so it's very likely that they can actively choose the seeds that get them the most

energetic gains even though they spend more time digging (metabolic energetic cost). In the Chapter II study, we could also ensure that all patches had equal initial gains, individual internal state was similar at the start (forager's were fed in a previous experiment and all stashed/previous food was removed) and no other external factors were present (e.g. no pregnant individuals), so that the changes in seed preference were found to be only due to perceived risk changes, which would be difficult to experiment in wild conditions. While the preference for bigger seeds matches optimal foraging theory (maximum gains with a bigger seed in less time), it might occur due to opportunity rather than choice (Garb et al. 2000), as bigger seeds will be very visible in a tray for a small forager. Furthermore, due to the physical properties of the sandy substrate, as foragers dig the vibrations will move the bigger seeds more quickly to the top of the substrate compared to the smaller seeds (Gajjar et al. 2021). True preference might occur only in safer conditions as the foragers are likely to perceive the costs of risks as lower and spend more time focused on feeding. Then, foragers had a tendency to eat more of the fat-rich seeds, that would enable them to not only optimize gains despite diminishing returns of continuously digging, as these seeds also provided them with more calories proportionally to seed size, or be beneficial for foragers to gain fat reserves for other activities (e.g. pregnancy; Eccard and Ylönen 2006).

Changes in dietary preference have even more cascading effects at the resource trophic level, as both size and nutritional value are important functional traits linked to germination success (Salgado-Luarte and Gianoli 2012, Lichti et al. 2017). By feeding more on the most competitive plant species' seeds, especially in landscapes perceived as safe, foragers can change the patterns of germination (Riginos and Grace 2008, Mills et al. 2018) and act as a coexistence mechanism in plant communities (Stump and Chesson 2017, Larios et al. 2017). The removal of the superior competitors by foragers reduces the average fitness differences within a resource community and acts as a equalising mechanism. This is especially evident in landscapes with high perceived predation risk, where only the least competitive plant

seeds were left in the landscape and could have germinated. Even at lower risk landscapes we could possibly observe other coexistence mechanisms at play. As forager's spend less time in these risky landscapes, even though the most competitive plant seeds were the first to be removed, the foragers gave-up faster and still left some of these seeds. The final abundances of plant seeds will then vary and may increase intra-specific competition within the most competitive plant species (stabilising mechanism).

Scatter-hoarding may also provide further stabilising effects, as the most competitive plant seed are harvested and cached for future consumption. If the forager does not consume all cached seeds, these competitive resource species might germinate and, thus, reduce niche overlap with the least competitive species. However, we did not analyse the possible effects of scatter-hoarding behaviour in this thesis, even though in Chapter I we observed some seeds that were consumed outside the patch at a short distance (unpublished data). Scatter hoarding involves further costs of transportation and movement, as foragers need to not only carry a food items outside a patch, but might also need to return several times to the patch to obtain more food items. In Chapter II, we did observe that foragers repeatedly visited patches more often in the safe landscapes, with some individuals caching seeds in their shelter (unpublished data). We also analysed the energetic cost of moving throughout the landscape (energy landscapes) and its interaction with perceived predation risk and observed that in risky landscapes, foragers moved less and visited patches less often, possibly to minimize the costs of movement and time spent in a landscape that might come with a higher risk of mortality.

In Chapter II, foragers were also expected to forage closer to their central-place to further minimize travel costs, however, we did not observe that in our study. Foragers consumed more resources (lower GUD) and had increased activity time in patches further away from their central location. In the safe landscapes only, the foragers also increased the number of visits in the most distant patches. These results are contrary to our expectations, as the

foragers should have foraged more often and for longer periods of time in closer patches to minimize the travel costs and maximise the gains of foraging, especially since all patches had equal initial gains that do not justify travelling further away from a central location. This might have been a consequence of our experimental setup, as the metal fences enclosing our landscape might have acted as a potential safe zone for foragers. Individuals moving next to the fence would feel safer as one side could be concealed from potential avian predators. Therefore, the patches that were closer to the metal fence were foraged for longer periods of time and, in safe landscapes, these patches were even visited more often as the repeated costs of travel are now reduced from the safer area to the foraging patch. Despite the design flaw, we could observe that, during the decision making process of foragers, individuals take priority in reducing potential predation risk despite potential increased travel costs. This is expected, as predation leads to the ultimate cost of mortality.

Different types of predation might also change the decision making process of foragers, which in the case of rodents comes from either terrestrial (e.g. carnivorous mammals, direct cues can be present) or avian predators (only indirect cues). In Chapter III, we evaluated the effects of a carnivorous mammal (raccoon) and avian predators (approximated by average vegetation height) in the foraging activity of rodents. Rodents did not change their diurnality patterns with changes in raccoon activity, but rather showed temporal avoidance with other rodents species (e.g. the smaller bank vole, *Myodes glareolus*, possibly avoided the larger *Apodemus spp.*) or even terrestrial carnivores with a diet rich in rodents (e.g. weasels and martens). We found that avian predation still takes priority when rodents decide to forage on landscapes of fear, as the decrease of vegetation height caused an increase of GUD and DivGUD. This result was expected, since rodents have no direct cues of avian presence, so potential avian predation is seemingly omnipresent for them. Furthermore, as an invasive species that feeds mostly on easy-to-get food such as vegetables, fruits and bird eggs, raccoons might not be perceived as a threat to rodents. Due to low predation risk together

with lack of predatory behaviour of raccoons towards rodents, it is possible that the wild community of rodents in our study are habituated to raccoons and do not perceive them as a potential risk, unlike other terrestrial species that might create an innate fear response (Bedoya-Pérez et al. 2019). This was further confirmed by video recordings of raccoons and rodents foraging in relative proximity. Foragers also took the opportunity to forage on the highly nutritious food items within our artificial food patches (in relation to wild resource communities, the artificial food items were highly nutritious, see Stiegler et al. 2021) and as time passed from the beginning of the experiment, the foragers got habituated to the artificial food patches and GUD and DivGUD decreased further with each experimental round.

Responses to the landscape of fear also varied further with other factors, such as foragers species richness or individual personality. In Chapter I and III, we worked with wild communities of rodents (i.e. unpredictable), and while we could estimate the diversity of rodent species visiting our seed trays, we could not assess species-specific abundances, individual sate (e.g. starved individuals) or experience and even personality. These are known factors that influence the responses to varied perceived predation risk (Bedoya-Perez et al. 2013) and might have resulted in increased variance in our results. Contrary to our expectations, in Chapter I the increase of foragers species richness had an additional effect on GUD and DivGUD. While rodents perceive landscapes of fear and forage optimally in similar patterns, the different behaviour or activity patterns of species (Thompson 1982, Haupt et al. 2010, Kołakowski et al. 2018) might have an additional effect on GUD and DivGUD, together with individually-based behaviours. In Chapter III, foragers got habituated to the artificial seed trays, while in Chapter II individuals foraged less resources when submitted to the experiment a second time regardless of perceived risk treatment (risky or safe). We also observed individually-based variation in total of seeds eaten from our experiment in Chapter II, which hints at personality based variation influencing how and for how much individuals forage (Mazza et al. 2019, Dammhahn et al. 2022). The individual

behaviour might also influence the foragers choice for certain functional traits (Boone et al. 2022). While size and nutritional values of seed are the most preferred functional traits by foraging rodents, the shape of the seed might also play a role on how rodents forage, as some seeds might be easier to transport and handle than others (Kelrick et al. 1986, Muñoz and Bonal 2008, Muñoz et al. 2012, Fischer and Türke 2016). In our study, we only observed a preference for circular seeds as an interaction with order of treatment, as individuals foraged more on the circular seeds when tested for the first time. Further studies will be necessary in the future to evaluate all these external factors, and thus deepen our knowledge on variation in responses to the landscape of fear.

Outlook and conclusions

Only by analysing the landscape of fear processes at its entirety could we truly understand the decision making process of foragers. The non-consumptive effects of predators took a greater toll in foragers trade-off decisions, with priority given to safety over travel, food searching and/or diet preference costs. Rather than obtaining the most profitable food items, foragers seemed to have an opportunistic foraging behaviour regardless of perceived predation risk, with the most visible food items being taken first. Only when foragers perceived the landscape as safer did we observe a possible diet preference, with foragers choosing the fat-rich food items that would enable them to obtain more energetic gains. These foragers decisions and changes in behaviour due to fear of predation have consequences at the resource species level, with the foragers acting as a coexistence mechanism that shapes the biodiversity of resources. However, we could only discern these patterns by quantitatively analysing the cascading effects of fear using the measure of DivGUD with a diverse community of resources. Not only DivGUD allowed us to measure the perceived predation risk similarly to GUD in wild conditions, but we could analyse further the foragers diet preferences and harvest patterns, as well the final composition of the resource

community. We could observe that varied perceived predation risk interacts with the microhabitats, foragers species richness, anti-predation behaviours and individual variation (e.g. personality) to further create spatial variation of the resource community left at the patch and landscape levels after foragers give up on feeding.

Changes on biodiversity caused by the landscape of fear can be beneficial, such as in the case of foragers acting as equalising effects to maintain the coexistence and biodiversity of resource species, even though it might also generate stark changes in habitat composition, especially when the foragers are large herbivores (e.g. Riginos and Grace 2008, Ripple and Beschta 2012). Furthermore, anthropogenic effects might also affect the landscape of fear further, with human activity being a cause of perceived predation risk even when no mortality is involved (Rösner et al. 2014, Shamoon et al. 2018). However, the landscape of fear can also be used to the benefit of anthropogenic activities without further harming nature, for example, to repel pest species from crops or urbanized areas (Mahlaba et al. 2017, Krijger et al. 2017), serve as a non-lethal method of repelling predators from cattle (Gaynor et al. 2021), or even aid conservation measures to promote biodiversity at all trophic levels (Kuijper et al. 2013).

Future studies should also incorporate both the density and diversity of foragers species richness into the analyses, as well possible effects of personality. The combination of live-trapping with capture-mark-recapture, personality tests and continuous video-recording are possible methods to incorporate all the possible internal factors of foragers to further understand the complexity of responses to the landscape of fear.

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VI Declaration of authorship and VII Acknowledgements

VI Declaration of authorship

I hereby declare that the thesis submitted, titled "Indirect, tri-trophic effect of fear on biodiversity" is my own work. All direct or indirect sources used are given as references. All contributions of co-authorship are acknowledged.

This thesis has not been submitted to another university of institute for acquiring a higher degree.

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VII Acknowledgements

VIII Appendix

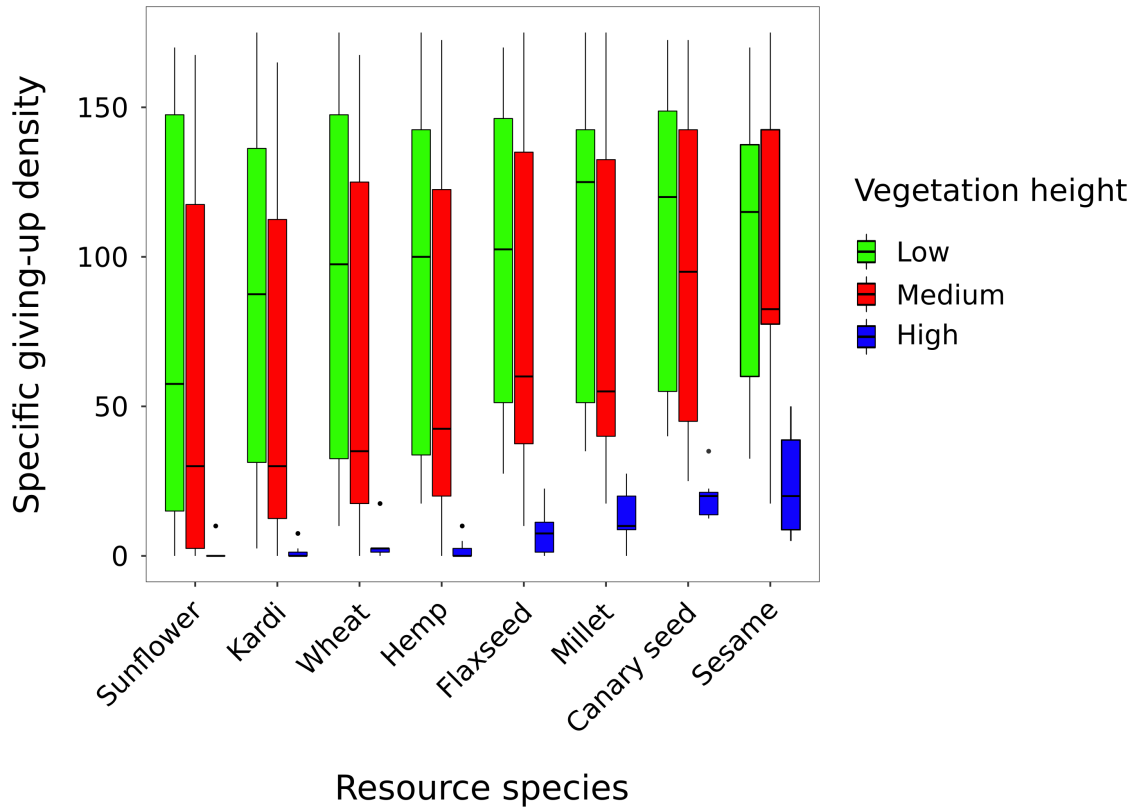**Figure A1** - Total quantity of each resource species left by foragers, at all foraging landscapes, in three vegetation height categories: low: < 15 cm (green); medium: ≥15 and ≤ 52 cm (red); high: > 52 cm (blue). Seeds species are sorted by size.
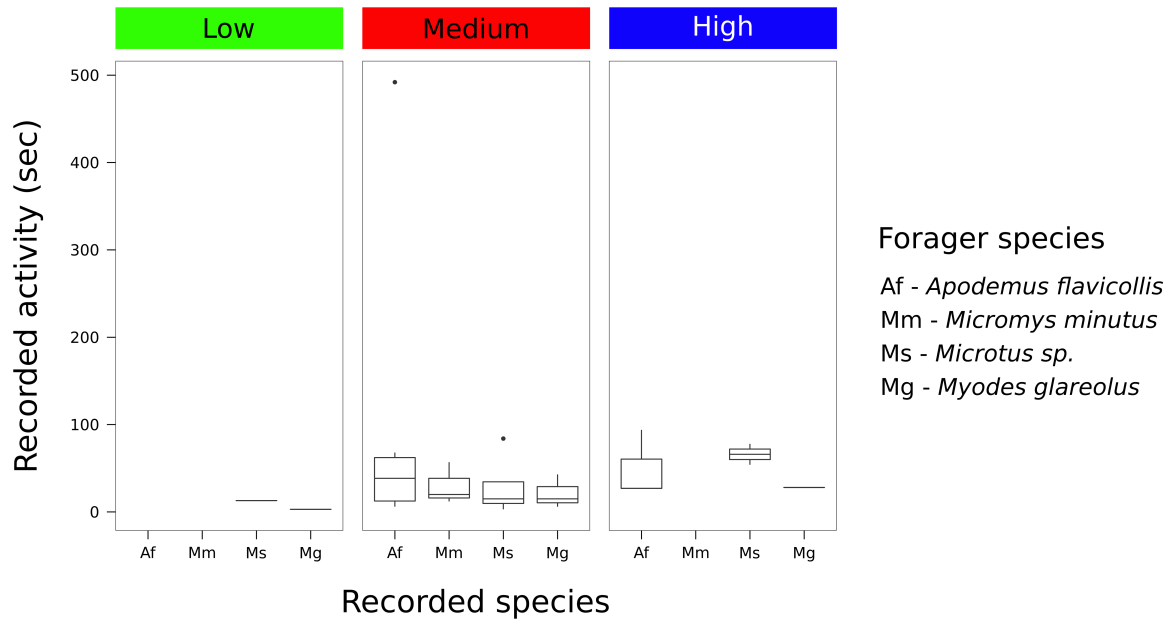
Forager species

Af - *Apodemus flavicollis*
Mm - *Micromys minutus*
Ms - *Microtus sp.*
Mg - *Myodes glareolus*

Figure A2 - Minimum activity recorded by the camera traps for different forager species in three vegetation height categories: low: < 15 cm (green); medium: ≥15 and ≤ 52 cm (red); high: > 52 cm (blue). Activity was measured through photos taken by the camera traps, each photo consisted of one second of activity. Minimum recorded activity was summed for each landscape and species.

Appendix chapter II - Forager-mediated cascading effects on food resource species diversity

Appendix A - AnimalTracker analyses

The plugin AnimalTracker (Gulyás et al. 2016) makes use of AVI video files in ImageJ to track animals in the landscape. Even though our videos spanned for the whole experiment duration of 24h, the whole file was not analysed due to technological constrains. Since the animals were not active for the entirety of the period, we first divided the videos into smaller video files with the activity bouts. Furthermore, elements in the video could interfere with automatic tracking, so we created a opaque white mask to overlay any element that was not supposed to be tracked (such as the outside of the arena, the water bottle bubbles created by the rodents drinking, and time stamp from the video). This mask was applied to the video using a ffmpeg script (Tomar 2006), also used to convert the video file from MP4 to AVI. The video resolution was also reduced to half (from 1280x720 px to 640x360 px, keeping the frame rate of 25 fps), as tests indicated that this would accelerate the automatic tracking analyses without compromised the quality of the tracking under any conditions. For the safe treatment, we increased the contrast by 0.4x and brightness by 0.15% in ffmpeg to make it easier to track the animal under dark. Following the AnimalTracker instructions, we used the Background substractor with three frames where the animal was either hidden or in different parts of the arena. We used a threshold of 20 for all videos, and the post-processing options were done in the following order: Erode (default parameters), Dilate (default parameters), Close (default parameters), Size filter (10-250). After the automatic tracking was done, we would overview the video to check for errors in tracking. If the program stopped tracking the animal for any reason (could not pick up the animal again or tracked another blob in the arena), we saved the correct part of the tracking and re-did the automatic tracking from the frame where the problem started to occur. Furthermore, we error proofed our final tracking

output by tracking sudden changes in coordinates, including: unnatural jumps in coordinates from one frame to another, coordinates that aren't supposed to exist during a time period (e.g. coordinates outside of the shelter range when the animal was inside the shelter), or repeated coordinates over a spam of several frames that could indicate a wrongful tracking of a non-animal blob. If the animal was present, we corrected the coordinates manually or re-did the video analyses to ensure the correct tracking, or if the coordinates were deleted if the animal was not present (thus preventing false tracking points). To calculate the number of visits per patch, we used a raster from the experimental layout from AnimalTracker's 'Zone Designer' module for each experiment run, created a shapefile in qGIS (QGIS.org 2021) and checked which tracking points were within each seed tray.