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ORIGINAL CONTRIBUTION

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Aphid cards – Useful model for assessing predation rates or bias prone nonsense?

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Abstract

Predation on pest organisms is an essential ecosystem function supporting yields in modern agriculture. However, assessing predation rates is intricate, and they can rarely be linked directly to predator densities or functions. We tested whether sentinel prey aphid cards are useful tools to assess predation rates in the field. Therefore, we looked at aphid cards of different sizes on the ground level as well as within the vegetation. Additionally, by trapping ground-dwelling predators, we examined whether obtained predation rates could be linked to predator densities and traits. Predation rates recorded with aphid cards were independent of aphid card size. However, predation rates on the ground level were three times higher than within the vegetation. We found both predatory carabid activity densities as well as community weighted mean body size to be good predictors for predation rates. Predation rates obtained from aphid cards are stable over card type and related to predator assemblages. Aphid cards, therefore, are a useful, efficient method for rapidly assessing the ecosystem function predation. Their use might especially be recommended for assessments on the ground level and when time and resource limitations rule out more elaborate sentinel prey methods using exclosures with living prey animals.

KEYWORDS
carabid beetles, ecosystem services, ground-dwelling predators, methods, natural pest control, sentinel prey

1 | INTRODUCTION

Pests remain a severe burden for modern agriculture and with climate change additionally pressuring crops, enhancing natural pest control is essential both to control pests and to limit pesticide use and their negative impact on the environment (Deutsch et al., 2018; Savary et al., 2019). While good standardized methods exist for measuring pest and predator densities, assessing the actual pest control function remains intricate and several studies used different techniques yielding contradicting results (see e.g., Greenop et al. (2019), Zaller, Moser, Drapela, and Frank (2009) and Rusch, Birkhofer, Bommarco, Smith, and Ekbom (2015)). Apart from very time and labour consuming exclusion experiments, sentinel or artificial prey experiments have been proposed and performed to assess predation rates but so far, no standardized methods and protocols exist (Birkhofer et al., 2017; Lövei & Ferrante, 2017).

The use of sentinel prey cards with dead aphids ('aphid cards') for recording invertebrate predation via prey removal gained popularity over the last years (Holland, McHugh, Moreby, & Jeanneret, 2017; Jeanneret et al., 2017; Winqvist et al., 2011; Ximenez-Embun, Zaviezo, & Grez, 2014). Apart from the use of artificial plasticine caterpillars, it is the suggested method for assessing predation rates in the Rapid Ecosystem Function Assessment (REFA) and is therefore on the way of becoming a standard method for recording predation rates (Meyer, Koch, & Weisser, 2015). Conveniently, aphids are actual agricultural pests, have a suitable size for most predators in agroecosystems and in contrast to plasticine caterpillars, they are more realistic. In previous studies, carabid and staphylinid beetles as well as spiders were identified as aphid predators under field conditions (Symondson, Sunderland, & Greenstone, 2002; Thies et al., 2011).

In theory, two main factors drive predation on sentinel prey cards: (a) detection, as the cards must be found by predators and (b) consumption, as not all predators consume the same amount of prey or the same prey types. It could be assumed that detection is facilitated by placing a larger quantity of smaller cards (and therefore covering more area) instead of fewer, larger ones. For consumption, assumptions are more intricate: If detected, predation on larger sentinel prey cards could be higher due to high consumption rates of certain predators (and because not all of the smaller cards were detected). However, larger sentinel prey cards are more efficient in manufacturing and handling. Larger cards are (a) faster assembled than a comparable amount of smaller ones, (b) require less storage space and (c) handling time during fieldwork is reduced due to the lower number of cards in total. However, it is currently unknown whether predation rates are biased by sentinel prey card size, and if resulting predation rates are comparable.

Sentinel prey cards have been used both on the ground and within the vegetation—but rarely within the same study (but see Ximenez-Embun et al. (2014)). Therefore, little is known about whether and how much predation rates differ between the vegetation level and the ground level. As agricultural pests develop, disperse and forage on different vegetation levels and are therefore potentially also affected by different predator guilds (Dainese, Schneider, Krauss, & Steffan-Dewenter, 2017), assessing predation rates on different levels could unravel important information about pest control. However, it remains unclear whether the same methods are suitable for assessing predation rates on ground and the vegetation level.

Recorded predation rates do not necessarily represent the pest control function as all methods for recording predation rates introduce some levels of artificiality into the system and might, on the one hand, exclude potential predators or on the other hand ease predation as prey items are gathered at one spot. Predation rates which are recorded with one type of sentinel prey allow to estimate potential pest control on the field level on the actual mix of different crop pests. Ideally, predation rates can be linked to predator assemblage characteristics such as density or body size. Densities of predators are the major driver of predation rates as with an increasing number of individuals, predation benefits from both additives but also synergistic effects due to species-specific preferences and traits (Gagic et al., 2015). This relation is generally accepted in agroecological studies dealing with pest control services but has rarely been shown. Menalled, Lee, and Landis (1999) and Greenop et al. (2019) showed that predation rates depended on activity densities of ground beetles but Rusch et al. (2015) did not observe this dependence in an exclusion experiment.

Another trait of ground-dwelling predator assemblages that is associated with predation rates is body size. Prey consumption is a function of body size with larger individuals consuming a higher prey biomass to keep their metabolic equilibrium (Reichle, 1968). On the contrary, Rusch et al. (2015) found that predation rates decreased with increasing body size in ground-dwelling predator assemblages indicating assemblages dominated by larger species to be less efficient in biocontrol. As species differ in many aspects apart from body size and have a species-specific set of traits and prey preferences, prey consumption might not only be dependent on body size.

In this study, we investigate the effects of card size and positioning of the sentinel prey card within the field on the resulting predation rates. We also examined whether observed predation rates could be linked to actual ground-dwelling predator assemblage characteristics on the commonly used example of carabid beetles. Concluding, we give recommendations for the use of aphid cards for predation rate monitoring. We followed three main research questions:

- How does card size and positioning affect predation rates drawn from aphid cards?
- Can the obtained predation rates be linked to activity densities of carabid beetles?
- Does carabid beetle community weighted mean body size explain the obtained predation rates?

2 | MATERIALS AND METHODS

2.1 | Study design

We selected 10 plots within five large winter wheat fields (two plots per field) around the agricultural estate ‘Gut Gieshügel’ (Germany, Bavaria, Gerbrunn) near the university campus of the University of Würzburg. All plots (including their subplots) had minimum distances of 75 m to the closest neighbouring plot (mean: 99.4 ± 4.9 m) and of 60 m to the closest field edge (mean: 78.5 ± 6 m). We conducted the experiment on one farm to homogenize landscape composition around the plots and therefore standardize the potential impact of landscape-level effects on predation rates. All winter wheat fields were sown with the same cultivar and managed by the same farmer in the same way to avoid management bias. At each plot, six subplots containing different treatments (see below) were established circularly around a central pitfall trap with distances between subplots and to the central pitfall trap being approximately 5 m (Figure 1).

2.2 | Data collection

To test for the effects of positioning and card size, we manufactured six different types of aphid cards. Cards which were placed on the ground level were folded up like a gable (with the aphids on one of

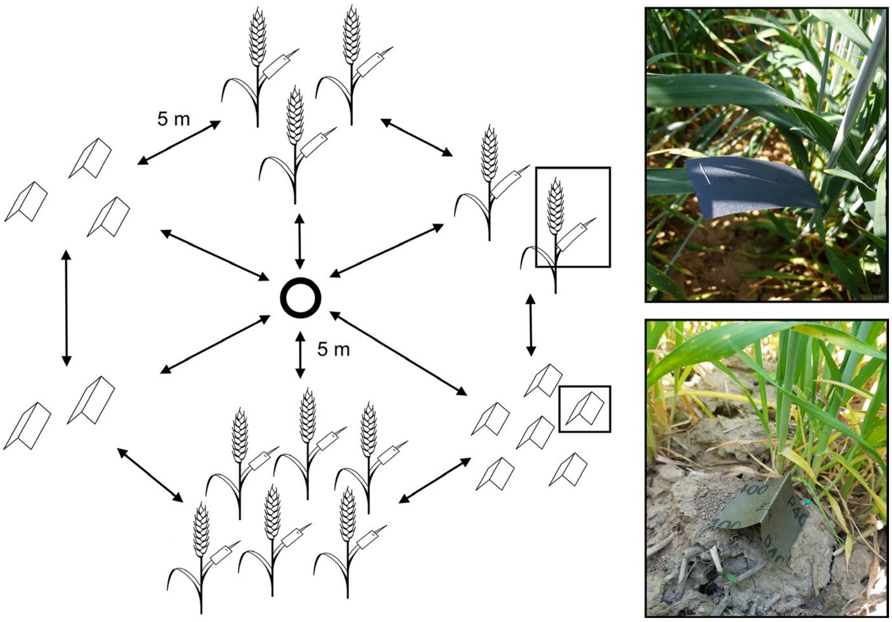


FIGURE 1 Schematic representation of predation rate assessment on each plot. Each plot had six subplots with one of the six combinations of the two positions (ground level and vegetation level) and the three card sizes (small, medium and large) on each subplot. Pictures show an aphid card (large) applied to a wheat plant and an aphid card (small) set up on the ground in the field. The central open circle represents the pitfall trap position, all arrows indicate distances of approximately 5 m [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Different types of aphid cards used

Position	Ground			Vegetation		
Card size	Small	Medium	Large	Small	Medium	Large
Width [cm]	6	6	6	3	3	3
Length [cm]	4	7	10	4	7	10
#Aphids	3	6	9	3	6	9
#Per subplot	6	3	2	6	3	2

the interior sides) and were therefore twice as broad as the cards which were placed within the vegetation. The sides containing the aphids were of same size in both designs. For both positionings, we made three different types of aphid cards containing different numbers of aphids: three (small), six (medium) and nine (large). At each subplot, we randomly placed cards of one type with a total of 18 aphids per subplot with distances of 0.5 m between the cards on the same subplot (Table 1 & Figure 1).

Aphid cards were manufactured out of conventional sand paper (grain-size 400). Adult aphids (Grain Aphid, *Sitobion avenae* (Fabricius, 1775)) were glued alive onto the cards using a brush and placed centrally on the cards (1.5 cm from the lateral edges of the cards and 1 cm from each other and the front/hind edges). As glue, we used the water-soluble polysaccharide and food supplement astragalus (Tragant-powder 1 g in 100 ml H_2O) to avoid chemical evaporations from conventional glues which could attract or deter predators (Birkhofer et al., 2017). After gluing, the aphid cards were immediately frozen at −20°C and stored until use (not longer than 3 days).

In the field, aphid cards on the ground level were fixed with a pin punched through the side containing no aphids as described by Winqvist et al. (2011), the cards within the vegetation were stapled to a wheat leaf in approximately 70 cm height as described in Jeanneret et al. (2017) (Figure 1).

Sentinel prey cards were exposed over three sampling intervals from the end of May to the end of June 2018, which corresponded to the period of milk ripening in winter wheat in the region. In this period, wheat is very vulnerable for pests and therefore pest control is essential. After 24 hr of exposure in the field, sentinel prey cards were collected and remaining aphids were counted.

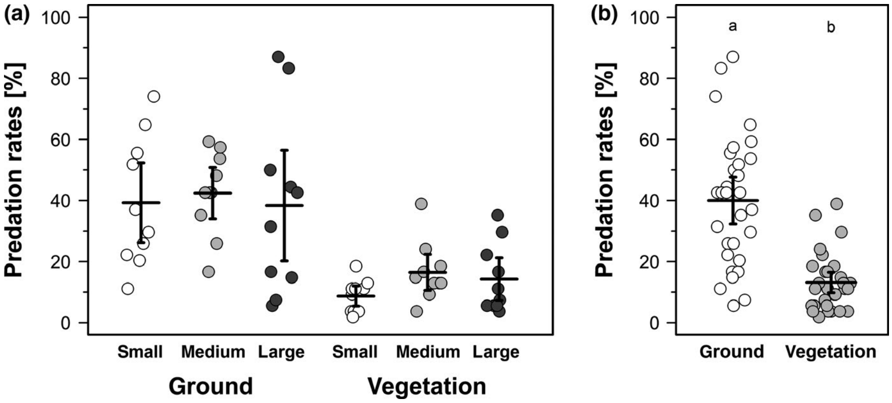
Simultaneously, we recorded the ground-dwelling predator potential on each plot using a conventional pitfall trap (height: 9 cm, diameter: 7.5 cm, transparent) filled with 200 ml oversaturated salt-water (200 g NaCl per 1l H_2O). Pitfall traps were activated on the same day the sentinel prey cards were placed and were active for 7 days.

Carabid beetles (Carabidae) were sorted from the trap samples, counted and identified following Müller-Motzfeld (2006). Carabid beetle life-history traits (diet and mean body size) were obtained from the online database 'carabids.org' (Homburg, Homburg, Schäfer, Schuldt, & Assmann, 2014). Staphylinid beetles and spiders were also counted, however as initial analyses showed no relations to predation rates, results are not shown.

2.3 | Statistical analyses

All statistical analyses were performed in R 3.5.2 for Windows (R Development Core Team, 2018), using the packages 'lme4' (Bates,

FIGURE 2 Predation rates (mean ± 95% CI) obtained from aphid cards over (a) different positions and card sizes as well as (b) only between the two tested positions. Different letters above indicate significant differences ($p < .05$; for statistics see results section)



Machler, Bolker, & Walker, 2015), 'lmerTest' (Kuznetsova, Brockhoff, & Christensen, 2017) and 'MuMIn' (Barton, 2018).

Instead of using crossed random intercepts for plot and interval, we pooled data over all three sampling intervals as otherwise, models were overparameterized. In a linear mixed-effects model ('lmer') we calculated effects of aphid card 'position' and 'card size' (as well as their interaction) on predation rates (aphids gone/aphids total) using 'plot ID' as random intercept.

To assess whether predation rates could be linked to predator assemblage characteristics, we calculated predation rates on the ground level over all card sizes (as there were no significant differences between card sizes) and intervals. To assess whether predation rates could be linked to ground-dwelling predator assemblage characteristics, we calculated separate linear mixed-effects models ('lmer') relating the response predation rates to the two mainly found predictors in previous studies: 'carabid beetle activity density' and 'carabid beetle community weighted mean body size' (again including 'plot ID' as random intercept). For these models, we excluded species that are known to be granivorous as they should not be involved in pest control (Table S1). Carabid beetle activity densities were square-root transformed to improve normality.

Model results were obtained using the command 'ANOVA' (type II sums of squares, Kenward–Roger approximation of denominator degrees of freedom) from the package 'lmerTest'. All models were checked carefully and met the required assumptions on residual and variance distributions. R^2-values (marginal) were obtained from the 'r.squaredGLMM' function from the package 'MuMIn'.

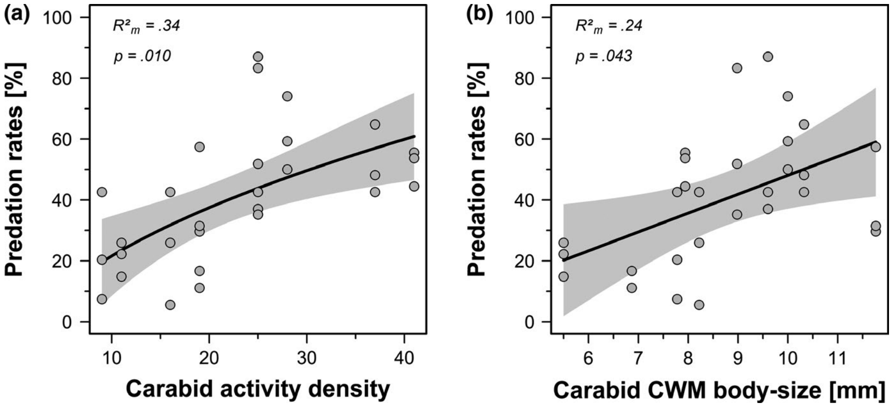
3 | RESULTS

In total, we used 3,240 aphids on 660 sentinel prey cards (360 small, 180 medium and 120 large). Predation was found in 43% of all aphid cards (38.3% in small, 49.4% in medium and 45.8% in large) after 24 hr of exposure.

Predation rates did not vary significantly between aphid cards of different sizes (LMER: $F_{2,45} = 0.80$, $p = .454$; Figure 2a). Positioning of the aphid cards, however, was an important predictor for predation rates with mean predation rates on the ground level (40.0 ± 3.9%) being three times higher than those within the vegetation (13.1 ± 1.7%; LMER: $F_{1,45} = 57.8$, $p < .001$; Figure 2a,b). Predation rates on the ground were marginally positively correlated with those within the vegetation (LMER: $F_{1,25} = 4.01$, $p = .056$; Figure S1). We did not find a significant interaction effect between aphid card size and positioning of the aphid card (LMER: $F_{2,45} = 0.30$, $p = .744$).

Over the three intervals, we collected a total of 258 carabid beetles from 22 species (thereof 230 individuals from 18 species were predatory or omnivorous; Table S1). Predation rates were increasing with increasing predatory and omnivorous carabid beetle activity density (LMER: $F_{1,8} = 11.13$, $p = .010$; Figure 3a). Moreover, also a larger community weighted mean body size of predatory

FIGURE 3 Predation rates in relation to (a) carabid beetle activity densities and (b) carabid beetle community weighted mean body size. Model predictions with 95% confidence interval. For statistics see results section



and omnivorous carabid beetles resulted in higher predation rates (LMER: $F_{1,8} = 5.75$, $p = .043$; Figure 3b).

4 | DISCUSSION

Predation rates obtained from aphid cards were independent of card size but differed between cards positioned on the ground and within the vegetation with mean predation on the ground level being three times higher than within the vegetation. Predation rates were positively related to activity density as well as community weighted mean body size of predatory carabid beetles.

In our study, predation rates obtained from aphid cards were independent from card size. This means, larger, more rapidly manufactured and more easily transported, and therefore, more efficient cards can be used instead of a large number of small cards. The use of large aphid cards results in a drastic reduction in labour and field work time and makes their use more convenient. This result also indicates that results obtained from aphid cards can potentially be compared between studies even if aphid cards used differed in card size (i.e., the total number of aphids used). As medium-sized aphid cards featuring six aphids per card had the lowest number of 0% or 100% predation extremes, we would recommend their use as they seem to resemble the optimal compromise between handling time efficiency and reduction in outliers due to non-detection or maximum predation.

In contrast to the difference in predation rates between the ground and the vegetation levels observed in our study, Ximenez-Embun et al. (2014) found similar predation rates on aphid cards placed on the ground level and 30 cm above ground within the vegetation. However, in the study of Ximenez-Embun et al. (2014), aphid cards were regularly replaced every 3 hr. Many ground-dwelling predators are rather generalistic and complementary in the function they deliver (Roubinet et al., 2018). We suspect that predators that are not that generalistic (like many vegetation dwelling predators such as syrphid larvae, lacewings and their larvae and coccinelid beetles) rather reject the dead and continuously desiccating aphids. Not all ground-dwelling predators, however, are climbing up into the vegetation. This combined with the lower number of vegetation level predators that potentially accept the sentinel prey offered, explain the lower predation rates within the vegetation level. Aphid cards are therefore recommended when the aim is to investigate ground-dwelling predator potential as many of the contemplable species are rather generalistic (e.g., many carabid beetles). In studies specifically aiming at aphid pest control (which typically takes place within the vegetation), we recommend using more 'realistic' sentinel prey methods such as inoculating plants with living aphids in exclusion experiments as performed by Martin, Reineking, Seo, and Steffan-Dewenter (2013), Rusch, Bommarco, Jonsson, Smith, and Ekbom (2013) or Karp et al. (2016). Such methods, however, have the disadvantage that preparation and observation in the field is more time and labour consuming, and they limit the amount of additional work that can be done simultaneously.

A demonstration of the ability of sentinel prey cards to reflect natural pest control is to link measured predation rates to predator assemblages or their pest control function. In our study, we could link the predation rates measured with aphid cards to two predictors investigated in previous studies: the activity densities and the community weighted mean body sizes of predatory carabid beetles (as a commonly used example for ground-dwelling predators). This link, however, has not been found consistently in previous studies. While some found density effects on predation rates (Greenop et al., 2019; Menalled et al., 1999) or pest control (Zaller et al., 2009), other studies did not find these activity density driven effects (Rusch et al., 2015). However, trait composition within assemblages might differ between studies and relations between activity densities, and predation functions could be disguised by functional limitations of the species present in the assemblages.

Functional traits of animal ecosystem service providers have been shown to be better predictors for the ecosystem functions provided than richness or densities of these organisms (Gagic et al., 2015). Apart from diet, body size should be the main trait influencing pest control in predators as larger individuals generally need a higher food intake to maintain activity. Surprisingly, Rusch et al. (2015) found the exact opposite relation for ground-dwelling carabid beetles and spiders combined (although not significant for carabid beetles alone). In contrast, we found predation rates increasing with mean predatory carabid beetle body size as it would be suspected.

In both analyses, we limited the data used to non-exclusively granivorous carabid beetles as we assumed that only species consuming animal prey would contribute to the predation function of the assemblage. Including all carabid beetle species might alter results depending on the distributions of species and traits in the specific assemblage compositions of different studies. Relationships for predatory species might be disguised by the presence of granivorous species and the resulting trait shifts in the assemblages. However, filtering of species affords prior sophisticated knowledge about their life-history traits, which is not yet available for most regions in the world.

Aphid cards are a simple, cost-effective way to measure predation rates. Other methods such as the use of living organisms in exclosure experiments are undoubtedly more elaborate but also much more time consuming, costly and labour intensive. With a magnitude of sentinel prey methods and organisms of different sizes falling into different predator feeding spectra (including e.g., aphids (Gardiner et al., 2009; Karp et al., 2016; Ximenez-Embun et al., 2014), corn earworm eggs (Meehan, Werling, Landis, & Gratton, 2012), fall armyworm larvae (Meehan et al., 2012), wax moth larvae (Meehan et al., 2012; Zirbel, Bassett, Grman, & Brudvig, 2017), cabbage moth larvae (Ferrante, Barone, & Lövei, 2017), ladybird eggs (Schneider, Krauss, & Steffan-Dewenter, 2013), onion fly pupae (Menalled et al., 1999), earthworms (Tschumi, Ekroos, Hjort, Smith, & Birkhofer, 2018), mealworms (Tschumi et al., 2018) as well as artificial plasticine caterpillars (Howe, Lövei, & Nachman, 2009; Howe, Nachman, & Lövei, 2015; Lemessa, Hambäck, & Hylander, 2015) proposed, comparability between studies is questionable.

While these differences in methods can be useful to answer specific questions in specific environments (Birkhofer et al., 2017; Macfadyen, Davies, & Zalucki, 2015), this generally highlights the need for a unified and standardized design to record predation rates under the REFA regime to allow comparability in large scale assessments. While plasticine caterpillars are handy and convenient to use, it remains unclear whether predation rates measured in this very artificial system designed for bird caused predation related to actual pest control services (see e.g., Jeanneret et al. (2017) for comparison with other sentinel preys). How predation rates obtained from different methods and different sentinel prey types actually relate to each other is largely unknown and requires a deeper investigation. In cases where the aim is to study specific crop systems, living sentinel prey of the specific crop pests will undoubtedly always remain the best method for obtaining trustworthy pest control information. With more basic research being needed to unravel the relations between methods, aphid cards are however a good simple alternative to record predation rates of generalist ground-dwelling predators in the field.

5 | CONCLUSIONS

Aphid cards are very efficient in the sense of how much investment goes into their use: They are easily made from cheap and readily available materials, can be stored in the freezer and are easily transported and applied to the sites of interest, with handling time during fieldwork being minimized. In terms of REFA, that is a good method to assess predation rates quickly and over large spatial scales and designs, and they resemble more realistic prey than plasticine caterpillars. However, these advantages come with drawbacks: the use of dead aphids might not relate directly to the pest control function provided by natural enemies against living aphid pests (but might still be more realistic than the use of artificial plasticine caterpillars). If resources and time are not limited, we would recommend using sentinel prey methods involving living prey items that ideally resemble the actual pest species in the crop of interest. But as we could show that predation rates obtained from aphid cards are stable and can be linked to ground-dwelling predators—which are one of the most important predatory guilds in agroecosystems—our results suggest that their use can be recommended to get a quick pest control assessment within the REFA framework.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

FAB designed the experiment, AK conducted the field work, FAB identified carabid beetles, FAB and AK analysed data and FAB wrote the first draft of the manuscript, all authors interpreted the results, revised the manuscript and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data available via the Dryad Digital Repository https://doi.org/10.5061/dryad.644cp2q (Boetzl, Konle & Krauss, 2019)

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SUPPORTING INFORMATION

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