







RESEARCH ARTICLE

Diversified cropping strengthens herbivore regulation by providing seasonal resource continuity to predators

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Funding information

Sveriges Lantbruksuniversitet

Handling Editor: Maria Fernanda Peñaflor

Abstract

1. Agricultural practices shape arthropod communities in arable fields, consequently influencing their interactions and the resulting ecosystem services, in particular pest regulation. Predatory arthropods play a pivotal role by preying on herbivores, soil fauna, and on other predators. However, the intricate mechanisms through which agricultural practices shape the dietary preferences of predators, and regulate herbivore populations remain complex and inadequately understood.
2. We assessed how fertilisation with organic fertiliser and extending crop rotations with perennial ley affected predation pressure across prey taxa. We mapped predator and prey trophic linkages with molecular analysis of carabid predator gut contents, and measured densities and taxonomic richness of predators, herbivores, and soil fauna in 19 cereal fields during three samplings across the growing season.
3. We derived two food web structure metrics: prey vulnerability that is the average number of predators feeding on a selected prey, and predator trophic redundancy, that is dietary overlap. Prey vulnerability was compared among soil fauna, herbivores, and other predator species (that is interspecific intraguild predation) over the growing season, and across treatments. The mechanistic underpinnings of observed shifts in vulnerability of herbivorous prey at different crop stages were identified using information criteria to select among candidate variables related to the richness, density and interaction structure of the different guilds during both the current, and the previous crop stages.
4. Agricultural diversification via organic fertilisation combined with perennial ley in the crop rotation decreased the vulnerability of both intraguild prey and soil fauna prey, and stabilised herbivore vulnerability. Mechanistically, the vulnerability of herbivorous prey at crop ripening emerges from the combination of predator richness and trophic redundancy during this sampling round, rather than from carryover effects from previous crop stages.

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5. *Synthesis and applications*: Our results suggest that locally provided resource continuity through diversified cropping practices bolster biological pest regulation, thus underline the importance of lesser disturbance in arable ecosystems for the provision of ecosystem services. Enhanced predator species richness together with availability of alternative prey through the season underpins this enhanced pest regulation.

KEYWORDS

biological pest control, crop protection, crop rotation, fertiliser, food web, intraguild predation, perennials, vulnerability

1 | INTRODUCTION

Diversification of agricultural practices in the crop field, such as adding organic fertiliser and diversifying crop rotations, affects communities of arthropods and the ecosystem services they provide (Kremen, 2005; Tamburini et al., 2020). The key agricultural ecosystem service of biological pest regulation is driven by trophic interactions between predator and prey, where the community of interacting arthropods is sensitive to management-induced changes. However, the outcome of different farming practices on predator diversity, prey availability, trophic interactions, and the resulting strength of pest regulation are poorly understood (Tsiafouli et al., 2015).

Species rich predator communities can promote biological pest regulation (Letourneau et al., 2009). Much of this evidence, however, stems from short-term cage experiments with single herbivore species in predator species poor communities (Tscharrntke et al., 2008), insufficiently reflecting the rich and complex interactions in open arable fields under contrasting practices. We know that predator richness is higher in field conditions under combined organic fertilisation and perennial leys compared with mineral fertilised field (Heinen et al., 2023), but without understanding predator-prey interactions, predator richness effects are hard to predict. Performing an analysis of predators' diets in fields with contrasting management, and hence arthropod assemblages, can shed light on the role of predator diversity and interactions on herbivore regulation in open arable fields.

Predator-prey interactions fluctuate during a cropping season (Roubinet et al., 2017) partly due to variations in prey-resources over time (Schellhorn et al., 2015). Analyses of predatory arthropod diets often focus on herbivorous prey due to the interest in pest management. However, alternative prey, notably soil fauna, has been suggested to indirectly enhance herbivore regulation by allowing early colonisation of fields and subsequent maintenance of predator communities in periods with fewer herbivores (Aguilera et al., 2021; Agustí et al., 2003; Settle et al., 1996). An assessment of predator communities under diversified management with organic fertiliser and perennial leys shows that early-season build-up of the predator community is likely bolstered by abundant soil prey (Heinen et al., 2023). However, whether the consumption of soil prey

is purely supportive of, or distracts predators from consuming herbivorous prey remains unclear.

Intraguild predation among predators can also weaken herbivore population regulation if predators interfere with each other (Snyder et al., 2022). Intraguild predation is ubiquitous in agroecosystems, but whether it stabilises predator communities over time (Rosenheim & Harmon, 2006; Uiterwaal et al., 2023) or releases herbivores from predation pressure and weakens herbivore regulation remains unclear (Finke & Denno, 2004; Halaj & Wise, 2002). Specifically, the interplay between species rich arthropod communities and the potential increase in predator-predator interference with its resulting consequences for the regulation of herbivore populations in large-scale field settings needs further study.

Empirical information on who eats whom has demonstrated the importance of predators for regulation of herbivorous arthropods (Bellone et al., 2023; Han et al., 2022), but the structural properties of the network of interactions established between predators and prey are poorly resolved (Macfadyen et al., 2009; Thébault & Fontaine, 2010; Tylanakis et al., 2007). Metrics such as the vulnerability of a prey taxon, that is the average number of predators feeding on it, describe feeding interactions from the prey's perspective, and indicates the level of prey regulation (Bersier et al., 2002; Gagic et al., 2011). Further, from a predator's perspective, reduced dietary overlap with other predators, that is trophic dissimilarity, can increase predation pressure as interference and specialisation of predators on specific prey are reduced (Dainese et al., 2017). To reconcile these pieces of information, we need to explore well-resolved food webs in time and across ecosystems under contrasting management to assess their impact on ecosystem functioning (Duffy et al., 2007; Lohaus et al., 2013). Metabarcoding of gut contents now allows for the simultaneous detection of a multitude of prey items, and the characterisation of trophic interactions in complex field settings (Yang et al., 2021).

Here, we examine how shifts over time in food web structure and community composition affect prey vulnerabilities, and how this depends on diversified crop rotation and fertilisation. We characterise the links between dietary overlap of carabid predators and the vulnerability of prey in trophic interaction networks linking above- and below-ground communities. Specifically, we ask (1) how the vulnerability of prey groups of soil fauna, herbivores, and intraguild prey

change in time, and in relation to each other (2). How agricultural diversification through organic fertilisation, or through the inclusion of perennial crops in the rotation, affects prey vulnerability in time, and (3) how dietary overlap in the network structure, predator richness and the density of prey taxa affect herbivore prey vulnerability in crop fields across the summer growing season.

Since the soil fauna offers a prey resource throughout the cropping cycle, we *a priori* expected higher predation on soil fauna early in the season, when the herbivores are not yet present, rather than late in the season. Over the season, we expected diet preferences to change towards herbivores, as herbivores colonise the crop and grow until the crop ripens. In more diversified fields, we expected a greater dependency on soil fauna and an increase in herbivore consumption over the season as a result of higher predator richness and reduced dietary overlap with complementary resource use. Consequently, we expected higher herbivore vulnerability with diverse predator communities. Because predator communities build-up over time, we predicted carryover effects between time steps, with early-present carabid communities providing augmented herbivore regulation at later steps.

2 | MATERIALS AND METHODS

We assessed regulation of herbivores in two steps. First, we sampled the realised trophic interactions between predators and their prey through metabarcoding of predator gut contents. Second, we characterised predator and prey population densities of soil fauna, herbivore, and intraguild prey, sampled with soil extraction, sweep netting, and pitfall trapping. With these data, we investigated the vulnerability of soil fauna, herbivores and intraguild prey in time, and across diversification treatments, including as predictors predator species richness, population densities and network metrics describing predation redundancy. This study did not require ethical approval, licences or permits to conduct fieldwork.

2.1 | Field selection and diversification treatments

We selected nineteen conventionally farmed fields sown with spring cereals (oats or barley) in Halland county, south-western Sweden in 2020 (Figure A1). Fields were diversified or not via inclusion of perennial crop species mixes in their crop rotations, and via amendments with organic fertilisers in previous years (Table A3). Hence, treatments included fields that only received mineral fertiliser and were rotated with only annual crops (FminRa; $n=6$), fields treated with organic fertilisers rotated with annual crops (ForgRa; $n=7$), and fields treated with organic fertiliser where crop rotation included 3 years of perennial leys (ForgRI, $n=6$). To capture longer-term legacy effects and not only immediate pre-crop effects, we selected fields where the farmers had implemented all treatments for a minimum of 6 years. All fields had been cropped with ley at least 3 years before sampling. To ensure a balanced landscape composition across

treatments (Table A1), we calculated the proportion of arable land and forest cover based on digital land cover maps (Terrängkartan, Lantmäteriet, 2018 & IACS).

At each study site, a 25×50m sampling area was established in which no insecticides were applied. Herbicides and fungicides were applied according to the individual farmer's decisions. The sampling areas were placed either at the border or inside the field according to farmer's preferences, but the placement was balanced across treatments (Table A3). Sampling took place along three transects at 8, 12 and 17m within the sampling area to avoid the effect of insecticide spray drift (Figure A1).

We sampled during three crop stages: early tillering approximately 20 days after sowing, heading stage 50 days after sowing, and early ripening of the crop 80 days after sowing (Table A2). These sampling events will henceforth be referred to as "sampling rounds". Because they were evenly spaced in time, we treated sampling round as a continuous variable in all analyses.

2.2 | Data collection

2.2.1 | Trophic interactions

We sampled gut contents of carabid predators to identify trophic links between predators and prey below- and above-ground. Four dry pitfall cups, approximately 3.5m apart, were placed in each of the three transects. Plastic cups (12 cm diameter, 12 cm height) were dug into the soil, filled with 200 mL of wood chips, and protected from sunlight and rainfall using a plastic roof (Figure A1). Pitfalls were open for 24 h during each sampling round. Each collected specimen was placed headfirst into an Eppendorf tube, and stored in a cooling box until regurgitation in the lab. A maximum of 100 individuals per site and time was processed (mean ± SE of individuals per site: Sampling round 1: 56.315 ± 7.366 , Sampling round 2: 78.157 ± 5.959 ; Sampling round 3: 69.421 ± 6.940).

In a clean workspace, tubes with carabids were dipped into hot water for 1–2 s to provoke carabid regurgitation. After regurgitation, live carabids were released from the tube, identified to species and released into their natural habitat. Then, 200 µL lysis buffer was added to tubes containing the regurgitate. The lysis buffer mix contained 5 mL of proteinase-K and 200 mL of ATL buffer. Samples were then homogenised and frozen at –20 degrees until molecular analysis. In total, 3670 samples were analysed (Table A4).

To obtain highly resolved data on all consumed prey items in the predators' diet, gut content samples were analysed through DNA metabarcoding. Initially, DNA was extracted by direct incubation of the samples followed by purification with SPRI beads (Vesterinen et al., 2016). Each extraction batch included a negative control sample to measure the purity of reagents and the level of cross-contamination. Then, DNA was amplified using the primers fwhF2 + fwhR2n targeting DNA barcode region in the arthropod mitochondrial cytochrome oxidase subunit I (COI) gene region (Vamos et al., 2017). The choice of primers was based on a pre-run with a

subset of our samples and multiple primer pairs. A blank PCR control was added to each PCR batch. All samples were amplified as two technical replicates, in a 10 µL PCR reactions consisting of: 5 microliters of 2× MyTaq HS Red Mix (Bioline, UK), 2.4 microliters of H₂O, 150 nM of each primer (two forward and two reverse primer versions, total primer concentration 600 nM), and 2 microliters of DNA extract per each sample according to Kankaanpää et al. (2020). Thermal cycling conditions were as follows: 95°C for 1 min, 5 cycles of 95°C for 60s, 90s in 45°C and 90s in 72°C, then 35 cycles of 60s in 95°C, 90s in 50°C, and 90s in 72°C, ending with 7 min in 72°C.

The NGS-library preparation followed Vesterinen et al. (2018) with minor modifications as presented in the appendix. All samples, including call controls, were then pooled and purified using magnetic beads (Vesterinen et al., 2018). Sequencing was performed through an Illumina NovaSeq6000 SP Flowcell v1.5 PE 2×150 run (Illumina Inc., San Diego, California, USA) at the Turku Centre for Biotechnology, Turku, Finland. The first sequencing run produced too few reads, and the run was repeated after further purification of the library pool to remove the left-over adapters and other potential non-target DNA fragments. Data from both runs were then used for the bioinformatics steps, which closely followed Kaunisto et al., 2020. In short, the reads were merged, trimmed for primers, dereplicated into unique haplotypes, denoised into sequence variants (ZOTUs), assigned to taxa and finally mapped to a zotutable. Detailed information on all steps is available in the appendix.

2.2.2 | Construction of the trophic networks

To characterise the predator–prey interaction networks, we used the predator gut content analysis to construct networks between carabid predator species and prey genera. To achieve comparable resolution among networks, we did not sample carabids proportional to their abundance in the field but rather assessed the diet of individuals per species. Reflecting this choice, we decided to use binary networks to avoid confounding predators' diet preference with their actual capacity to regulate herbivore populations. Moreover, the interpretation of interaction structure indices is easier for the unweighted case (Bersier et al., 2002).

A separate network was built for each crop field and sampling round. Prey was added at the genus level, reflecting high confidence in taxonomic assignments of DNA sequences at this level. Feeding interactions were encoded in a network composed of s taxa, and can be represented as a predation matrix α composed of prey species (k) as rows and predator species (j) as columns ($s = k + j$).

2.2.3 | Community densities and taxonomic richness

To characterise the density and taxonomic richness of predator and prey communities in the fields during the three sampling rounds, we used a separate set of samples that were previously published

(Heinen et al., 2023). We used a combination of soil core extraction, sweep netting, and pitfall trapping to obtain densities of soil fauna, herbivores, and activity-density of carabids. The first sampling round for sweep netting was cancelled due to limitations for sampling with this technique at the tillering stage of the crop. From the samples, we estimated taxonomic richness measured as herbivore genera, and carabid species richness. Densities of soil fauna, herbivores and predators were analysed as the sum of individuals caught per field and sampling round. The sampling effort was the same in every field at each occasion. Species and genus richnesses were calculated as the number of species or genera per field and sampling round. See Appendix Section 2 for a detailed description of sampling methods, sampling times, genera included, and their classification into prey groups.

2.2.4 | Vulnerability

To detect changes in predators prey choices, we estimated the vulnerability of prey (V_k) as the average number of predators per prey item in the network, summed across all observed trophic links (Bersier et al., 2002; Williams & Martinez, 2000):

$$V_k = \frac{1}{l/s} \sum_{j=1}^s a_{kj},$$

where, l is the number of realised links, s is the number of species, j is the number of predators, and k is the number of prey. Element a_{kj} thus represents the link between predator species j and prey species k .

Vulnerability was calculated separately for soil fauna, herbivores, and intraguild prey, based on the subnetworks of the respective species group (Table A11), hence generating one value of vulnerability per site and prey group. Note that metabarcoding cannot resolve cannibalism because the prey and the predator will share the same sequence of the marker gene region. Thus, in analysing intraguild prey, we here only refer to interspecific intraguild predation.

2.2.5 | Trophic redundancy

As a measure of trophic redundancy, we calculated the mean number of shared partners among predator species as a matrix of the numbers of prey species shared by each pair of predator species. We used the full network including all prey species in each field, as we assumed complementary in prey choice among the prey items, potentially affecting herbivore vulnerability. Calculations were implemented in the 'grouplevel' function of the package 'bipartite' (Roberts & Stone, 1990; Stone & Roberts, 1992). To address potential mathematical linkages between prey vulnerability and predators' trophic redundancy; we explored their relationship in both, our empirical and in random networks of similar range of number of species and connectance. By doing so, we show that there is no mathematical relationship between the studied metrics (Appendix Section A.4.3).

2.3 | Analyses

2.3.1 | Vulnerability in time and across treatments

We first analysed changes in vulnerability of focal prey groups in time, that is between sampling rounds. In a second model, we analysed vulnerability in time depending on management contexts. We separated these analyses because our replication was not sufficient to allow for an analysis of a three-way interaction with sampling, management, and prey group. This was a reflection of the trade-off between sampling intensively in each field for a proper network mapping, and the number of field replicates.

To model vulnerability of the different prey groups along time, we used prey group, sampling round, and their interaction as fixed factors. To model shifts in vulnerability between management contexts, that is different combinations of organic fertiliser addition and crop rotations, we analysed three models with either herbivore, soil fauna or intraguild prey vulnerability (V_H ; V_{SF} ; V_{IGP}) as response variables. The interaction of management and sampling round were included as fixed effects in a mixed model. To account for repeated measures of the same fields in each season, we added field identity as a random effect in both models (Table A5).

2.3.2 | Determinants of herbivore vulnerability

To form a more mechanistic understanding of herbivore regulation by predator communities, we used both network and community density information to investigate how arthropod communities drive herbivore vulnerability. Comparing metrics from both community and trophic interaction sampling, we identify whether interaction structures or community characteristics best explain herbivore vulnerability. In particular, we investigated how herbivore vulnerability during the three sampling periods was affected by trophic network redundancy, predator richness, and community densities across fields, taking into account both the same sampling period (t) and the previous sampling period ($t - 1$; Table A1). Hence, we did not include crop management context in this analysis, and focussed on overall patterns between the availability and diversity of predators and prey in fields. Note that predator richness estimated from wet pitfall and gut contents trapping are both present in the full model. This is to allow the model selection process to identify the most relevant measure.

We started from a maximal set of potential predictors building two separate linear mixed effect models for the second and third sampling round (Table A5). No herbivore sampling was performed during the first sampling round. Hence, the density of herbivores during the first sampling round was not included in the set of predictors. We assumed that the density estimates for herbivores from sweep netting and soil fauna from soil cores would reflect the overall availability of prey, with the density of predators from wet pitfall catches reflecting both the overall density of predators and the availability of intraguild prey. Of the metrics derived from gut content

sampling, predator richness will reflect the complexity of predator communities directly involved in trophic interactions, whereas redundancy in prey use by predators will reflect the strength of predation pressure on each prey. As effects can occur both for the same time step t and with a lag from the previous time step $t - 1$, we included as predictors of herbivore vulnerability, both the current time point (t) and the previous time step ($t - 1$).

Starting from an initial model including all predictor variables, we used a backward elimination approach to arrive at a final reduced model. We used the model selection function “dredge” from the “MuMin” package (version 1.43.17) to drop independent terms based on AICc. To avoid multicollinearity, we tested for correlation among predictor variables and excluded variables with $r > 0.66$ prior to model assembly (see Figure A4).

2.3.3 | Community and diet turnover in the networks

To address the importance of predator species identity on the predator overall communities' diet preferences across time, we examined the changes in overall beta diversity of predator communities engaged in trophic networks, as well as the beta diversity of their diet, that is dietary changes in prey use for each individual predator present in both time steps. Hence, beta diversity was assessed within the same field from first to second, and from second to third sampling round.

Specifically, we calculated a , b , and c components, with a being the number of species shared in both sampling rounds, b being the number of species exclusive to time t , and c the number of species exclusive to $t - 1$. Overall beta diversity (Koleff et al., 2003) was calculated as the sum of B_{div} and B_{rich} (Legendre, 2014; Noreika et al., 2019):

$$B_{div} = 2 * \min((b, c)) / ((a + b + c))$$

$$B_{rich} = |(b - c)| / ((a + b + c))$$

where B_{div} describes changes in diversity and B_{rich} in richness components (Carvalho et al., 2012) of communities across the three sampling rounds. Predator or diet beta or diet diversity in time as a function of management context was tested using two models with the package ‘lme4’.

2.3.4 | Validation of model assumptions

Model assumptions and model fit were validated by testing for over- and underdispersion. Model residuals were visually inspected using diagnostics of scaled residuals simulated from the model fit. The plots of observed versus predicted residuals were examined for systematic deviations and homoscedasticity. Parameter significance was tested using type II-Wald chi-square tests for models without interaction terms, and type III-Wald chi-square tests for models

containing interactions. Estimated marginal means were obtained using a Tukey *post-hoc* test within the “emmeans” package version 1.7.2 (V. Lenth, 2022).

All data were analysed using R version 4.1.1 (R Core Team, 2020) and packages ‘glmmTMB’ version 1.1.2.9000 (Brooks et al., 2017), ‘stats’ version 4.1.1, ‘DHARMA’ version 0.4.5 (Hartig, 2021), ‘car’ version 3.0–12 (Fox & Weisberg, 2019).

3 | RESULTS

3.1 | Vulnerability in time and across treatments

The vulnerability of soil fauna decreased over time (Table A6c), whereas the vulnerability of both herbivores and intraguild prey did not change with time (Figure 1, Table A6a). *Post-hoc* comparisons of mean vulnerability of the different prey groups revealed similar vulnerability of soil fauna (mean ± SE: 1.55 ± 0.075) and herbivores (mean ± SE: 1.48 ± 0.075), whereas the vulnerability of intraguild prey was higher (mean ± SE: 1.96 ± 0.075, Table A6b).

The vulnerability of herbivores increased over the growing season for fields receiving mineral (Figure 2a, Table A7a,c). Relative to that, herbivore vulnerability remained stable for fields receiving organic fertiliser, both in rotation with annual crops or perennial leys (Figure 2a, Table A7a). *Post-hoc* comparisons revealed

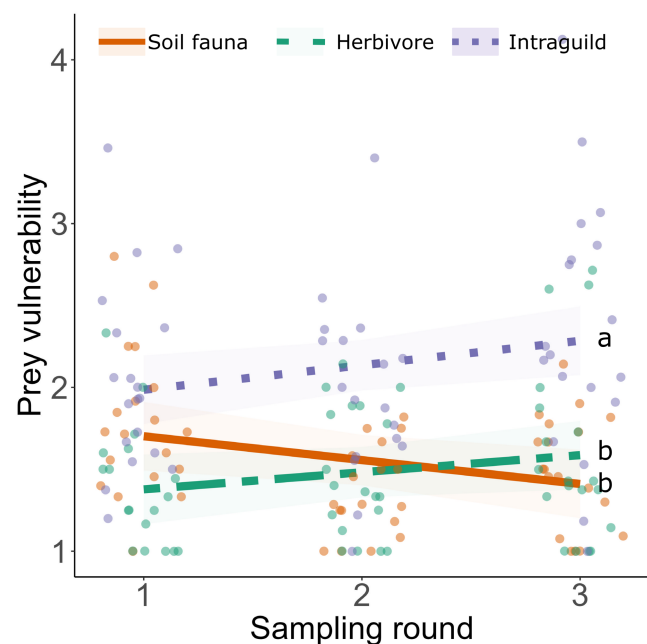


FIGURE 1 Model predictions showing changes in the vulnerability of herbivorous (H, green, dashed line), intra guild (IGP, purple, dotted line), and soil fauna prey (SF, orange, solid line) in time expressed as sampling rounds 1, 2, and 3. Shaded areas represent the 95% confidence intervals. Dots represent raw data. Letters indicate significant differences ($p < 0.05$) in estimated marginal means. Significances of slopes relative to zero can be found in Tables A6–A8c.

similar mean vulnerability of herbivorous prey across treatments (Table A7b).

The vulnerability of soil fauna decreased in time for fields under combined organic fertilisation and perennial leys in the rotation (Figure 2b, Table A8c), and remained stable for fields receiving mineral fertiliser or organic fertiliser with annual crop rotations (Figure 2b, Table A8a). The mean vulnerability of the soil fauna (Table A8b) was similar across treatments.

Treatments showed differing trajectories in time for the vulnerability of intraguild prey. Both treatments having annual crop rotations with mineral or organic fertiliser increased over time, whereas for the most diversified fields under combined treatment of organic fertiliser and perennial leys in the rotation, the vulnerability of intraguild prey decreased in time (Figure 2c, Table A9a,c).

3.2 | Determinants of herbivore vulnerability

Herbivore vulnerability during sampling round t was determined by the state of the network and community during the same time step (t), whereas there were no carryover effects from time point $t-1$. The final selected model of mid-season herbivore vulnerability included soil fauna density, and the predator richness observed in networks, that is from gut contents sampling, during mid-season (Figure 3a,b). The final model of late-season herbivore vulnerability included predator richness in the network (i.e. from gut contents sampling), and the mean number of partners shared among predators during the late season (Figure 3c,d). All predictors had a positive effect on herbivore vulnerability (Figure 3).

While we find a positive relationship between vulnerability and trophic redundancy in our empirical networks (Figure 3), we do not find such relationship in random networks built with a null model encompassing a similar range and size of connectedness to that of our empirical networks (Figures A5 and A6).

3.3 | Predator community and diet turnover in networks

Predator species had an average beta diversity of $69.9\% \pm 10.9$ from early to mid, and $73.8\% \pm 11.87$ from the mid to the late sampling round. In both cases, most of this beta diversity was attributed to turnover of species with an average of $45\% \pm 22$, and to lesser extent to richness components with an average of $24.3\% \pm 21$. Overall beta diversity was higher in fields under mineral fertilisation and annual crop rotation, from early to mid-sampling round (Table A10, Figure A3a,b).

For the fraction of predators present in both sampling rounds (on average $28.57\% \pm 11.26$ of the entire predator community), predators' diet had an average beta diversity of $84.5\% \pm 13.7$ consistently throughout time. These changes were to equal parts explained by the changes in diet breadth (richness of preys consumed) ($45.4\% \pm 26.7$), and the turnover of interactions ($39.1\% \pm 24.9$). Treatments did not explain any further variation in the data (Figure A3c).

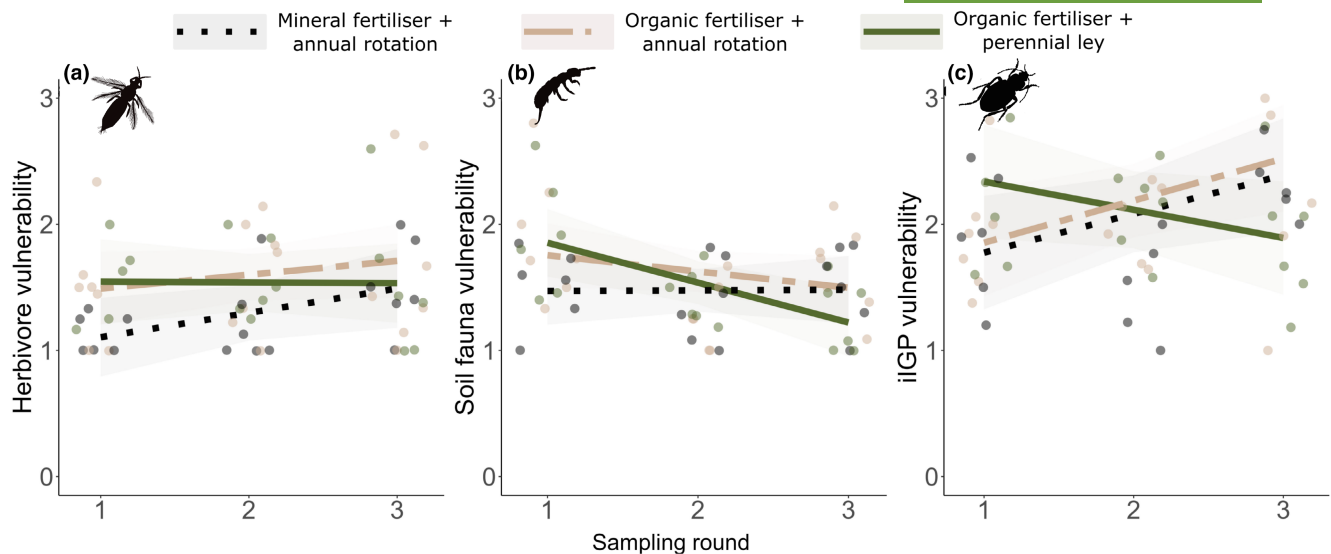


FIGURE 2 Model predictions for changes in vulnerability of herbivorous (a), soil fauna (b) and interspecific intraguild (IGP) (c), prey in time (sampling round 1–3) and among treatments of fields with mineral (FminRa, grey, dotted) or organic fertiliser with annual crop rotations (ForgRa, orange, dashed), and fields with organic fertiliser with perennial leys in crop rotation (ForgRI, green, solid). Shaded areas represent 95% confidence interval. Dots show the raw data. Significances of slopes relative to zero can be found in [Tables A7c–A9c](#).

4 | DISCUSSION

Mapping arthropod predator–prey food webs in open crop fields along the crop-growing season, we confirm that predator species richness enhances herbivorous pest regulation. Changes in predators' diet preferences across diversification treatments and over time, reflects changes in alternative prey. In small scale experiments with few species, it has been suggested that predator species richness increases herbivore regulation (Crowder & Jabbour, 2014). We extend this knowledge by showing that predator richness enhances herbivore regulation also in rich and structurally complex food webs in open fields. This occurs despite counteracting forces such as intraguild predation and access to alternative prey.

Against our expectation, it was trophic redundancy, and not complementarity, which best predicted herbivore vulnerability. Redundancy in resource use is common among generalist consumers (Wirta et al., 2015). Redundancy can insure the stability of functional responses when selected predator species are lost (Feit et al., 2019), especially in disturbed ecosystems such as arable fields. As natural communities are often characterised by an uneven abundance distributions among species, consumption redundancy among common prey items will be accentuated as compared with complementary resource use in experimental evenly distributed communities (Winfree et al., 2018). While we were unable to fully standardise the differences in abundances of predators across times and fields, we analysed binary networks in order to not confound diet preferences of predators with their capacity to regulate herbivores. This approach does not account for the abundance-evenness of predator communities that can affect herbivore regulation (Crowder et al., 2010), and we acknowledge the limitation of our study in addressing the full capacity of predators to regulate herbivores. However, it is worth

noting that while we included predator abundance as predictor in the model, it did not emerge as a good determinant of herbivore vulnerability.

Previous characterisations of predator and soil fauna communities suggested that early season availability of soil prey enhanced predator abundance (Heinen et al., 2023). We found that assumption to be supported by high soil fauna vulnerability, illustrating its frequent predation, early in the season. Hence, we demonstrate that soil prey is indeed a pivotal resource for predators early in the crop-growing season (Costamagna et al., 2015). Additionally, we found intraguild prey to be a major part of the predators' diet consistently throughout the entire season. Intraguild predation is widespread in generalist predator communities (Rosenheim & Harmon, 2006). Gut content analysis of spiders showed similarly high levels of intraguild predation alongside the usage of other complementary resources (Saqib et al., 2021). Examining the food web in its full complexity and across the season we found that intraguild predation likely dampens herbivore regulation, given the two-fold higher vulnerability of intraguild prey compared with that of soil fauna and herbivorous prey. However, contrasting consumption of intraguild prey across cropping practices highlight the potential of diversified management to mitigate potential constraints on herbivore regulation due to other trophic interactions.

Predator diet preferences differed across treatments. Vulnerability of both alternative prey resources, that is soil fauna and intraguild prey, decreased over time in diversified fields under combined organic fertilisation and perennial ley in the rotation. In contrast, intraguild prey vulnerability increased over time, and the vulnerability of soil fauna remained stable for fields with annual and either organic or mineral fertiliser. The difference in trajectory over time depending on cropping practice suggests that in the most

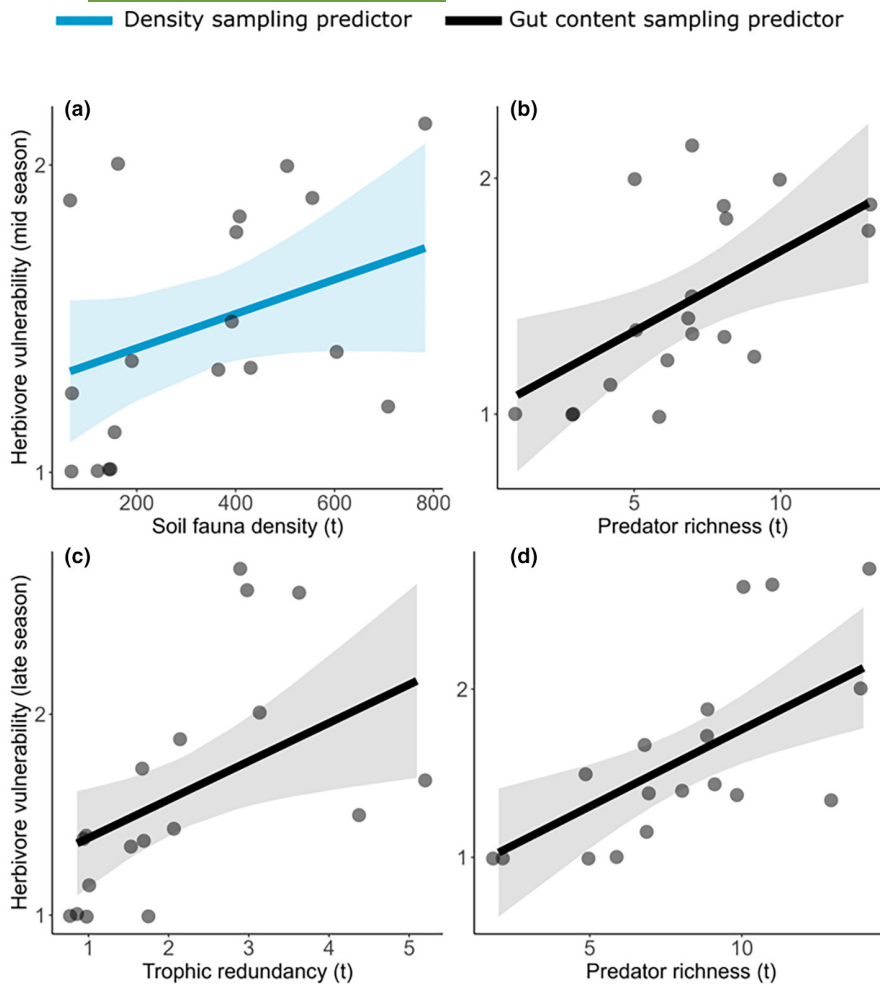


FIGURE 3 Vulnerability of herbivores during mid (a and b) and late (c and d) season plotted against predictors selected by model reduction. Shown are the effects of the four predictors retained in the final model: the soil fauna density (a, blue), predator richness in networks (b, d), trophic redundancy, that is mean shared partners (c). All predictors retained in the model represent the state of the community or network at time t . Shaded areas represent the 95% confidence interval. Blue colour indicates predictors derived from community density sampling, grey colour from gut content sampling.

diversified fields, the potential interference of alternative prey with target herbivorous prey is relaxed. This has applied relevance in the light of the efforts to reduce pesticide use, and to stabilise biological pest regulation through the provisioning of alternative prey (Jacquet et al., 2022). It has been suggested that regulation of herbivores on crops is more efficient if resource continuity is provided to the predators and other service providing organisms (Schellhorn et al., 2015; Vasseur et al., 2013). One such mechanism is that predator diet changes from a main dependence on soil fauna and intraguild prey towards herbivorous prey when the latter become more abundant in the crop as the growing season progresses, a potentially ubiquitous process that, however, has not often been demonstrated in detail (Settle et al., 1996).

Interestingly, abundance of soil fauna predicted herbivore vulnerability mid-season. This confirms that soil fauna supports herbivore regulation by acting as an added reliable prey resource (Riggi & Bommarco, 2019). We propose that abundant soil fauna attracts predators into the field, thereby increasing predation pressure on all prey, including herbivores. Contrary to our initial hypothesis, we found little evidence for lagged effects in explaining herbivore vulnerability (Costamagna et al., 2015). The lack of such effects can be explained through high predator community turnover between seasons.

Predator turnover was highest in fields with mineral fertiliser and annual rotation, where disturbances might have hindered communities to build up within the crop field. In contrast, diversified fields might provide greater local stability in predator communities over time (Schellhorn et al., 2015), resulting in lower turnover. Additionally, the diet turnover was high for the small fraction of predators shared in both time steps, suggesting that individual predators will switch diet with the availability of new prey across the crop-growing season (Hsu et al., 2021). However, given the high turnover in predator communities, we want to highlight that species richness is the key element to enhance herbivore regulation. This underlines the merit of examining pest regulation across various, diverse communities of predators across time, thus potentially circumventing sampling effects as seen in experimental communities with very few species.

5 | CONCLUSIONS

Our study provides detailed insights into the seasonal changes in predator communities' diets and its implication for pest regulation, emphasising the importance of multiple complementary prey resources and intraguild predation for a full understanding of the complex ecosystem service of pest regulation.

We provide evidence that the diversified farming practices, including crop rotation and fertilisation with organic manure, increases resource continuity to predators over the crop-growing season. This fosters predator richness, which enhanced herbivore regulation in rich and complex food webs in open fields. Further, predator communities assembled under diversified management engaged less in intraguild predation at times of peak herbivore densities, thus potentially stabilising biological regulation of herbivores in these fields. Combined diversification practices, and especially the inclusion of perennials in diverse crop rotations, shows promise in enhancing and stabilising predator and prey communities and their contribution to pest control in agricultural fields.

AUTHOR CONTRIBUTIONS

Janina Heinen and Riccardo Bommarco planned and designed the experiment. Janina Heinen and Gerard Malsher conducted fieldwork. Eero Vesterinen performed genetic analysis and bioinformatics work. Janina Heinen, Virginia Domínguez-García, Guillermo Aguilera and Ignasi Bartomeus conceptualised the analysis. Janina Heinen analysed the data with supervision from Virginia Domínguez-García, Tomas Roslin and Ignasi Bartomeus and led the writing of the manuscript. All authors contributed to the manuscript preparation.

ACKNOWLEDGEMENTS

We gratefully thank all field assistants for help in collecting and processing samples, and all farmers for providing access to their fields. We thank Sara Bergström-Nilsson at The Rural Economy and Agricultural Societies for assistance in finding field sites. The project was funded by a senior career funding from the Swedish University of Agricultural Sciences to Riccardo Bommarco.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data is available through The Swedish National Database (SND) at <https://doi.org/10.5878/7qn6-ec60> (Heinen & Bommarco, 2024).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table A1. Test statistics with χ^2 -value, degrees of freedom, p-value for the difference of arable- and forest land cover among treatments. Statistically significant differences ($p < 0.05$) are presented in italic.

Table A2. Sampling dates for regurgitate, independent carabid, independent soil fauna and independent herbivore sampling during sampling round I, II and III.

Table A3. Additional information on field sites 1 to 19.

Table A4. Number of regurgitate samples per sampling round I–III for the fields 1–19 and the respective management context of fields receiving either mineral (FminRa) or organic fertiliser with annual crop rotations (ForgRa) or fields receiving a combination of organic fertiliser and perennial ley in the crop rotation (ForgRI).

Table A5. Response and predictor variables used in linear models to assess: vulnerability in time (A), vulnerability in time and management context (B), beta diversity changes between time points (C) and predicting herbivore vulnerability (D).

Table A6a. Parameter estimates for the model on changes in vulnerability of soil fauna (SF), herbivores (H) and interspecific intraguild prey (IGP) in time including the interaction effects between prey type (SF, H, IGP) and time (Analysis A, Table A5).

Table A6b. Estimated marginal means (a) for the model on changes in vulnerability of soil fauna (SF), herbivores (H) and interspecific intraguild prey (IGP) in time including the interaction effects between prey type (SF, H, IGP) and time (Analysis A, Table A5) and its contrasts (b) across treatments.

Table A6c. ANOVA tables presenting chi-squared values, degrees of freedom and p-values for the model evaluating the slopes across

time for herbivore, IGP and soil fauna vulnerability in addition to the relative increase or decreases to each other, as presented in analysis.

Table A7a. Parameter estimates for the model on changes in vulnerability of herbivores in time and in interaction with treatments (analysis B in Table A5): FminRa, ForgRa and ForgRI.

Table A7b. Estimated marginal means (a) for the model on changes in vulnerability of herbivores in time and in interaction with treatments (analysis B in Table A5): FminRa, ForgRa and ForgRI, and its contrasts (b) across treatments.

Table A7c. ANOVA tables presenting chi-squared values, degrees of freedom and p-values for the model evaluating the slopes across time for each treatments (mineral fertiliser with annual crop rotation (FminRa), organic fertiliser with annual crop rotation (ForgRa) and combined organic fertiliser with perennial ley in the rotation (ForgRI)) across time for the vulnerability of herbivores in addition to the relative slopes across treatments as presented in analysis II.

Table A8a. Parameter estimates for the model on changes in vulnerability of soil fauna in time and in interaction with treatments: FminRa, ForgRa and ForgRI (Analysis B, Table A5).

Table A8b. Estimated marginal means (a) for the model on changes in vulnerability of soil fauna in time and in interaction with treatments: FminRa, ForgRa and ForgRI (Analysis B, Table A5), and its contrasts (b) across treatments.

Table A8c. ANOVA tables presenting chi-squared values, degrees of freedom and p-values for the model evaluating the slopes across time for each treatments (mineral fertiliser with annual crop rotation (FminRa), organic fertiliser with annual crop rotation (ForgRa) and combined organic fertiliser with perennial ley in the rotation (ForgRI)) across time for the vulnerability of soil fauna in addition to the relative slopes across treatments as presented in analysis II.

Table A9a. Parameter estimates for the model on changes in vulnerability of IGP prey in time and in interaction with treatments: FminRa, ForgRa and ForgRI (Analysis B, Table A5).

Table A9b. Estimated marginal means (a) for the model on changes in vulnerability of IGP prey in time and in interaction with treatments: FminRa, ForgRa and ForgRI (Analysis B, Table A5), and its contrasts (b) across treatments.

Table A9c. ANOVA tables presenting chi-squared values, degrees of freedom and p-values for the model evaluating the slopes across time for each treatments (mineral fertiliser with annual crop rotation (FminRa), organic fertiliser with annual crop rotation (ForgRa) and combined organic fertiliser with perennial ley in the rotation (ForgRI)) across time for the vulnerability of intraguild prey in addition to the relative slopes across treatments as presented in analysis II.

Table A10. Parameter estimates for the model depicting predator community changes within fields from first to second sampling round and second to third sampling round for the three treatments: mineral fertiliser with annual crops in rotation (FminRa, Intercept), organic fertiliser with annual crops in rotation (ForgRa) and organic fertiliser with perennial ley in the rotation (ForgRI), analysis C, Table A5.

Table A11. List of included prey items (genus resolution) classified into the three groups of soil fauna, herbivores and intraspecific

intraguild prey (IGP) used in network analysis assessing the vulnerability of the different prey groups.

Table A12. Primers used in the analysis.

Table A13. Number of original reads from the sequencing and after each bioinformatic step.

Figure A1. Experimental sites located in south-western Sweden (upper panel) and experimental set up at each field (lower panel), including dry pitfall trap locations for live catching of carabids for regurgitation (1–12), wet pitfall sampling locations for independent carabid sampling (a–h), sample locations for independent soil mesofauna densities (I–IV) and sweep netting transects for independent herbivore sampling (dashed lines) within the 25×50m sampling area.

Figure A2. Independent sampling of densities that is, individuals per field and sampling round (a–c) and taxonomic richness i.e. species/genera per field and sampling round (d, e) of carabids, herbivore and soil fauna communities for the three treatments of mineral (grey) or organic (orange) fertiliser with annual crop rotations and organic fertiliser with perennial ley in the rotation (green).

Figure A3a. Overall beta diversity depicting predator community changes within fields from first to second sampling round (a) and second to third sampling round (b) for the three treatments: mineral fertiliser with annual crops in rotation (FminRa, grey), organic fertiliser with annual crops in rotation (ForgRa, orange) and organic fertiliser with perennial ley in the rotation (ForgRI, green). Beta diversity decomposed into its turnover and richness components can be found in Figure A3b.

Figure A3b. Beta diversity decomposed into richness and turnover components within fields between first and second and second and

third sampling round for the three treatments of mineral fertiliser annual crop rotations (FminRa, grey), organic fertiliser with annual crop rotations (ForgRa, orange) as well as organic fertiliser with perennial leys (green).

Figure A3c. Overall beta diversity for each predators diet present in both sampling sessions, and for the three treatments of mineral fertiliser annual crop rotations (FminRa, grey), organic fertiliser with annual crop rotations (ForgRa, orange) as well as organic fertiliser with perennial leys (green) (a), and its partitioning into richness (b) and turnover components (c).

Figure A4. Correlation plots for predictor variables used in the model for predicting herbivore vulnerability at the second (a) and third (b) sampling round (Table A5, analysis D).

Figure A5. Empirical versus randomised values of lower level species vulnerability (left) and carabid trophic redundancy (right).

Figure A6. Vulnerability versus trophic redundancy in 1000 random networks created with the null model that keeps fixed the number of species and links, with $n_{low}=47$, $n_{high}=7$ and average connectance=0.22 (the average values of those that we find in our empirical networks).

How to cite this article: Heinen, J., Domínguez-García, V., Aguilera, G., Malsher, G., Vesterinen, E., Roslin, T., Bommarco, R., & Bartomeus, I. (2024). Diversified cropping strengthens herbivore regulation by providing seasonal resource continuity to predators. *Journal of Applied Ecology*, 61, 1829–1840. <https://doi.org/10.1111/1365-2664.14674>