Molecular assessment of food web dynamics identifies critical periods for managing resilience in biological pest control

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Abstract

Food webs are not static over time, but our knowledge on their dynamics is extremely scarce due to methodological challenges. These have imposed significant limitations on our ability to mechanistically understand how temporal changes affect trophic networks. Here, we address this gap using high-throughput molecular diagnostics to measure the season-wide dynamics of trophic interactions between invertebrate generalist predators, pest and alternative prey in replicated cereal fields across two years. We used the level of food web specialisation as a proxy for predator redundancy in pest control, and hypothesized that it would hit its minimum, and invertebrate diversity its maximum, at the middle point of the season. Additionally, alternative prey availability was indirectly increased by adding manure to half of each field, to test if this would reduce specialisation. In line with our predictions, it showed an inverse bell-shaped curve over the season, while prey, but not predator, diversity showed an opposite trend. No significant effects of fertilization were found on food web specialisation. Our findings identify early and late season as the times when generalist predators are behaviourally most constrained, pin-pointing these periods as the ones with the lowest redundancy in pest control. Hence, molecular trophic analyses provide unique insights into the temporal dynamics of food webs and their properties. This allows the generation of temporal roadmaps for when management interventions are expected to be most effective.

Introduction

Biological communities are defined by the changes that occur over time and space in response to environmental conditions, resources and interactions between species. Many of those interactions are antagonistic, and often of a predator-prey nature (Schmitz 2017). These interactions occur at an individual level, and are at least partially responsible for changes in population range, abundance, community structure, evolution and behaviour (Schmitz et al. 1997, Schmitz et al. 2004, Kishida et al. 2010). Even though population regulation responses often are studied from the perspective of mortality and recruitment from reproduction that occur over time, this is not necessarily what occurs on short time scales. Recruitment can occur in the form of migration or behavioural adaptation, such as when dietary niche overlap increases. Both of which are mechanisms that would result in a greater number of individuals, and species diversity, involved in the trophic interactions (Birkhofer et al. 2015).

Moreover, when induced across spatial and temporal scales, predator-prey interactions create a constant feedback loop (Webster 2003, Dingemanse & Wolf 2013), that can be split into periods when predators are either behaviourally-free or behaviourally-constrained (Barbier & Loreau 2019). Behaviour-constrained periods are akin to bottom-up regulation from the perspective of the forces that drive behaviour (as opposed to community structure). More specifically, bottom-up and behaviourally constrained phases occur when the resources available are limiting and restricting consumer populations on the successive trophic levels (Hunter & Price 1992). Behaviourally, this limitation can be both because resources and food sources are not present in sufficient amounts, but also because high intra- and inter-species competition limits predators' access to resources (Oksanen 1988). The latter of which forces species to adapt their niches to avoid other
competitors by changing their behaviour, meaning that it causes species to reduce their dietary overlap, effectively increasing the network specialisation within a food web (H2' – network level specialisation in Blüthgen et al. 2006). Conversely, behaviour-free periods occur when there is plenty of a resource, which in turn releases consumers and predators from competition due to high food availability. This enables consumers to share niches, and mean that consumers can reduce their specialisation on specific diet items. Which, in turn effectively increase the redundancy of top-down regulation of prey by predators (Lawton & Brown 1993, Loreau 2004, Biggs et al. 2020).

However, the empirical study of behaviour-constrained and behaviour-free periods in real-world food webs, requires a great deal of sampling effort in order to represent the systems accurately (De Barba et al. 2014), especially when dealing with generalists. Such temporally highly resolved food webs require a standardized, large-scale assessment of many feeding links between multiple prey and predator species, an endeavour challenged by the difficulties inherent to identifying prey remains at high specificity and sensitivity (Nielsen et al. 2018). Molecular methods have revolutionised the study of trophic interactions (Symondson 2002), as they allow examining trophic links at great accuracy, many of which are intractable by other methodologies, and, importantly, to scale up a standardized analysis of diet samples allowing to measure the food of thousands of individual consumers (Deagle et al. 2023). In fact, we are now at a point when it is not so much the ability to describe food webs, but rather of how much temporal resolution the data should have, in order to answer specific questions (Pringle & Hutchinson 2020).

It has also been pointed out that there is a lack of understanding on the temporal dynamics of food webs (Isbell et al. 2018), due to past research focusing on limited time windows (McMeans et al. 2015, Roubinet et al. 2018), and of empirical studies on how biological control is built up and regulated throughout an entire growing season (Welch & Harwood 2014, Cohen & Crowder 2017, Roubinet et al. 2018, Staudacher et al. 2018, Barbier & Loreau 2019). Both relate to the temporal resolution of data, allowing the detection of changes over time in food webs.

These food web temporal dynamics are especially relevant for conservation biological control (CBC), as we still lack a solid understanding of which mechanisms drive its efficacy and robustness. Being linked to natural enemy diversity (Crowder et al. 2010), the more species contributing to CBC (higher redundancy) the higher the likelihood that this service is retained if one species disappears (insurance hypothesis, Yachi & Loreau 1999). The other half of the equation, are the prey. Increasing their overall abundance should reduce competition, which in turn reduce constraints on predator behaviour and dietary niches and decreases specialisation. In order to assess how and when this occurs under natural conditions, we conducted a study to record the food web dynamics and behaviour-constrained and behaviour-free period shifting in barley (Hordeum vulgare L.) fields. We did this by sampling repeatedly every other week across the full growing season of the cereal, during two consecutive years, and by examining invertebrate food webs, with generalist predators (beetles and spiders), non-pest (earthworms and springtails) and pest prey (aphids). Each field also had half of its area fertilized with manure, to create a difference in baseline primary production, and induce a resource release by increasing non-pest prey availability and thereby reducing specialisation. The use of high-throughput molecular gut content analysis (MGCA) allowed us to examine the diets of the several thousand generalist predators, and generate a unique time-series of empirically established and replicated trophic networks required to see how food web specialisation fluctuates over time.

Using this setup, and taking the functional link between diversity and CBC, we have derived three hypotheses for our study. First, we predict that, after an initial predator establishment phase with high specialisation when they are exploring available niche space, network specialisation will decrease and reach its minimum in the middle point of season, then increase towards the end as the cereals quickly ripen and dry. (H1, Fig. 1). Second, after the initial migration into the fields as the vegetation grows, invertebrate diversity will increase and reach its maximum also during mid-season, then decrease (H2, Fig. 1). Third, we predict food web specialisation should be lowest and invertebrate diversity highest with the organic fertilization treatment (H3). Through these hypotheses, we aim to address the knowledge gaps on the temporal dynamics of regulation phases, as well as niche overlap of generalist predators and the build-up of biological control.
build-up over time.

**Figure 1** – Predictions of network specialization (full blue line), invertebrate community diversity (dashed brown line) and vulnerable periods for biological control (shaded areas), throughout the season (months) and sampling sessions (numbers) in relation to barley growth stages.

**Materials and Methods**

**Field Sampling**

The study was conducted in Kematen in Tirol, near Innsbruck, Austria, where spring barley (*Hordeum vulgare* L.) was grown in three fields each year (2020 and 2021, respectively), for a total of six different fields. In each field we split the area in half, fertilizing one side with cattle manure and the other remaining unfertilized, as a control. The manure was applied independently by the respective owners of each field, at a rate of 1.5 metric tons (1 500kg) per hectare, using manure spreaders. Four 5x5m plots per treatment were delimited to carry out all sampling (Fig. 2), with no barriers.

Sampling was conducted every two weeks, starting on the 21st of April and ending on the 14th of July in 2020, and starting on the 3rd of May and ending on the 12th of July in 2021, resulting in seven sampling time points for the first year and six for the second. Dry pitfall traps with wood chips, were active for a single day to catch live predators, for the molecular gut content analysis (Staudacher *et al.* 2016). The taxa collected were ground beetles (Carabidae), rove beetles (Staphylinidae) and spiders (Araneae). Wet pitfall traps, with a teaspoon of salt and a drop of detergent per litre of water, were active for four days to sample the soil surface dwelling community (see supplementary tables 1 to 3 for list of taxa obtained from pitfall traps). A total of 168 wet pitfall trap samples were obtained in 2020 (4 sampling plots x 2 treatments x 3 fields x 7 sampling sessions) and 144 for 2021 (4 x 2 x 3 x 6 sampling sessions), from which Shannon-Weaver’s diversity was calculated for the predator and prey communities. Additionally, transects were carried out on the sides of the plots, collecting 30 individual barley plant tillers to count the number of aphids per tiller.
Figure 2 – Schematic of sampling plot distribution within fields (not to scale), and sampling methods within plots. One half of each field was fertilized with bovine cattle manure at rate of 1.5t/ha, and the other half left untreated. Wet and dry pitfall traps were used to assess the community and predator diet respectively, while plant collect transects were done to determine the number of aphids present in the fields.

Molecular gut content analysis

A total of 2404 ground beetles, 913 rove beetles and 567 spiders were captured in 2020, and 1977 ground beetles, 891 rove beetles and 250 spiders in 2021. The beetles’ gut content and the spiders’ full bodies were extracted with a BioSprint 96 DNA Blood Kit (Qiagen, Hilden, Germany) on a QIAGEN Biosprint96® workstation for automated DNA extraction, following the manufacturer’s recommendations. After extraction, three different multiplex-PCR assays were run per sample. The first assay targeted several prey taxa (assay in Rennstam Rubbmark et al. 2019), the second targeted generalist and specialist predators (primers from Sint et al. 2014 and Staudacher et al. 2016). Lastly the third assay identified the genus of beetles consumed as intraguild prey, from a selected set of taxa consisting of Bembidion spp., Harpalus spp., Poecilus spp., Pterostichus spp., Philonthus carbonarius and Philonthus cognatus (see supplementary tables 4 to 6 for details of all three multiplex-PCR assays). Following the multiplex-PCRs, the samples were screened using capillary electrophoresis on an QIAxcel Advanced system and the ScreenGel software, with a DNA Screening Kit (2400) using a 15-3k base pair alignment marker, following the manufacturer’s recommendations. The screening profile on ScreenGel was set to the standard AM320 method, with the relative fluorescence units (RFU) detection threshold defined at 0.07, as opposed to the default 0.1, to account for the fact the samples were extracted from gut content and thus partially digested.

Data analysis

All data analysis was carried out using R 4.1.2 and RStudio 2023.06.0+421, with the packages bipartite (Blüthgen et al., 2006) to calculate food web network level specialisation ($H^2$, an index bound between 0, for absolute generalization, and 1, for absolute specialisation) and for randomized food web generation, vegan (Oksanen et al. 2022) for Shannon-Weaver’s diversity calculation, lme4 (Bates et al. 2015) for linear mixed effects model creation, tidyverse (Wickham et al. 2019) for data formatting and ggplot2 (Wickham 2016) for graphic creation. The diet for each species was averaged across all individuals of that species captured in each unique combination of sampling plot, field and sampling session. Prey diversity was calculated
for Symphypleona and Arthropleona springtails (Collembola), aphids, *Oulema melanopus*, earthworms and gastropods collected. Predator diversity was calculated for all 38 and 30 species present in the regurgitate list for 2020 and 2021, respectively (see supplementary tables 7 and 8 for list of taxa).

The null models were obtained by randomly recombining data points 99,999 times, generating as many random food webs, calculating specialisation for each one, then calculating Cohen’s $d$ standardized effect size (SES) for the difference between the random and the real food webs’ specialisation. This was done to provide with a robust measure of how much more, or less, specialised the real food webs were than random, as opposed to simply analysing the real specialisation without a reference value.

Three main linear mixed effects models were carried out, to test the relationship of various variables. The first model analysed how predator diversity changed across treatments and over the sampling sessions, with the sampling field as a random factor, to account for the variability in location. The second model was identical, but for prey diversity instead. The third model analysed how specialisation, as the standardized effect size described above, changed across treatments and sessions once again. As with the previous two models, the random effect was field. The stepwise model selection was done by selecting the lowest Akaike Information Criterion (AIC), as reported by the `lmer` function of the `lme4` package, and the significance of the variables and interaction terms was assessed through Chi-squared ($\chi^2$) tests using the `anova` function of base R.

**Results**

*Food web specialisation over time*

The food web network specialisation was greater than expected at random (Fig. 3), indicating that, even though the predators analysed were generalists, there was a degree of selection of prey. Additionally, while specialisation was not significantly different in the fertilization treatment when compared to the control, in both years ($2020 - \chi^2 = 0.305$, df = 1, N = 165, p = 0.581; $2021 - \chi^2 = 1.249$, df = 1, N = 130, p = 0.264), it varied over time ($2020 - \chi^2 = 22.146$, df = 6, N = 165, p = 0.001; $2021 - \chi^2 = 11.715$, df = 5, N = 130, p = 0.039), with a clear valley during the stem elongation phase on the 4th sampling session, meaning the predator community in general became less selective, and had greater overlap in their diet. This decrease towards the middle of the season was more pronounced in 2020, but was still present in 2021, where both the initial decrease and the increase towards the end were smoother but visible (Fig. 3).
Figure 3 – Cohen’s d standardized effect size of the network level specialisation (H2’) between real and random food webs over time (every two weeks, between the 21st of April and the 14th of July in 2020, and between the 3rd of May and the 12th of July in 2021), for both fertilized (circles, full lines and darker shading) and unfertilized (triangles, dashed lines and lighter shading) treatments, in 2020 and 2021, with loess smoothing (default “loess” method in ggplot2) and one standard error.

Diversity of predators and prey over time

Predator diversity was different across treatments and session together for 2020, being higher in the control treatment, (treatment – $\chi^2 = 5.658$, df= 1, N= 168, p= 0.017; session – $\chi^2 = 18.723$, df= 6, N= 168, p= 0.005; Fig. 4), but only session for 2021 (treatment – $\chi^2 = 0.275$, df= 1, N= 144, p= 0.602; session – $\chi^2 = 25.769$, df= 6, N= 144, p<< 0.001; Fig. 4). For prey diversity in 2020 both treatment and session had an effect, being higher in the control (treatment – $\chi^2 = 11.154$, df= 1, N= 168, p< 0.001; session – $\chi^2 = 33.807$, df= 6, N= 168, p<< 0.001; Fig. 4), and in 2021, once again, only session had an effect (treatment – $\chi^2 = 3.035$, df= 1, N= 168, p= 0.082; session – $\chi^2 = 53.307$, df= 5, N= 144, p<< 0.001; Fig. 4).
Discussion

Using high-throughput molecular diagnostics we were able to track the temporal dynamics within replicated invertebrate terrestrial food webs, providing unique insights into how the diet of generalist arthropod predators changes across the growing season. As these trophic data have been collected under field conditions, they reflect the natural food web dynamics which is relevant to both fundamental and applied food web ecology. With this data we found support for our first hypothesis, proposing that food web specialisation undergoes seasonal shifts decreasing towards mid-season and rising again towards crop ripening. This pattern was more pronounced in 2020, but was still observable in 2021, and is likely a direct effect of the prey available to predators rather than a change in the predator community. Regarding predator diversity, it was overall lower
in the fertilized treatment, and decreased slightly as the season progressed in 2020, while the opposite was true for 2021, with no effect of fertilization. Prey diversity showed a peak during mid-season for both years, and was overall lower with fertilization in 2020. These patterns only partially support our second hypothesis on invertebrate diversity being highest during the middle of the season, as this only occurred for prey. Lastly, fertilization had no effect on network specialisation, but decreased predator and prey diversity, the latter only for 2020, partially disproving our third hypothesis. Regarding the shifts of consumers’ behaviour, there was a shift in early tillering from behaviour-constrained (high specialisation) to free (low specialisation) until late stem extension, then from free to constrained during stem extension and ear forming for both years, with 2021 alone having an additional shift at the end during grain filling.

Food web specialisation and diversity

Owing to the functional link between network specialisation and invertebrate diversity, we believe the initial values of the former were mainly due to the lower prey diversity. The latter can be attributed to the relatively sparse vegetation cover in the fields (Rouabah et al. 2015, Beaumelle et al. 2021), as it was during the tillering stage, only a few weeks after the barley had been sown and the crop had not yet grown tall. Then, as the crop grew taller during stem extension, prey diversity increased, enabling a niche expansion of the predators, and specialisation decreased towards the middle of the season, reaching its lowest value, while prey diversity reached its highest. From the 4th sampling session onwards, or late stem extension and the following growth stages, prey diversity decreased and specialisation increased for both years, which is consistent with the ripening and drying of the crop. The link to predator diversity is less clear as, unlike for prey, the trend differed between years, decreasing throughout the season in 2020, but increasing in 2021.

Contrary to our expectations, manure fertilization appeared to have no effect on food web specialisation in both years. However, even though we could not detect the behavioural release we expected, we have previously shown intraguild predation to be reduced and the abundances of springtails increased with manure fertilization, demonstrating an expected numerical effect of manure on both predator interference and detritivorous prey (data presented in Leote et al., under review). This shows that fertilization can have immediate effects on specific aspects of the trophic network, and boost the densities of detritivores even when applied in the short-term. However, we also expected this short-term application of manure to increase not just detritivores but other invertebrate diversity, and found the opposite to be true. This is in contrast to other studies, which investigated the long-term effect of organic fertilization on invertebrates and found that it increased general invertebrate diversity (Birkhofer et al. 2008, Winqvist et al. 2011, Kremen & Merenlender 2018, Liang et al. 2022). The most likely explanation to these contrasting findings is that invertebrate communities take a longer time to adapt than that of the duration of our study. Furthermore, differences in the spreading and quantity of the manure also come into play. In our study, the manure was spread quite finely in a thin, homogenous layer over the soil, as opposed to large pats, which provide an attractive microhabitat for invertebrates such as earthworms, springtails, ground and rove beetles (PNBL, ORR and MT, personal observations).

Implications for biological control management

The observed differences in food web dynamics have distinct implications regarding CBC potential by generalist arthropod predators. Fagan et al. (2002) defend that generalist predators may prevent an invasion or delay the exponential growth of pests, if predator density is sufficiently high. Raymond et al. (2015) argue that, for aphids specifically, due to the damage they can cause early in the season, CBC should be strengthened during that period when pest density is lower. Following these arguments, and expanding upon them based on the points made by Snyder (2019) regarding complementarity and interference, we argue the system should be managed to allow generalist predators to overlap their diets and reduce competition during the early period. To achieve this, non-pest prey availability should be increased and predator interference should be decreased, which we have demonstrated recently (Leote et al., under review). That, along with low food web specialisation, should increase predation pressure on pests, by virtue of a greater abundance and richness of generalist predator species contributing to pest suppression.
Nonetheless, there are other treatments that can be applied as an alternative, or along with manure, to induce both greater diversity and lower specialisation. These include increasing habitat structure, through inter-cropping, cover crops or reduced tillage to increase ground cover. This boosts invertebrate diversity and reduces predator interference, reducing behavioural constraints of predators, allowing them to be more effective biological control agents, as shown by studies (Janssen et al. 2007) and extensive reviews (Gontijo 2019, Snyder 2019). Most important, however, is the timing of such actions, as indicated by our results.

Conclusions

To conclude, we have found that food web specialisation fluctuated over time, almost mirroring prey, but not predators’ diversity, implying that communities are highly plastic, and that redundancy was modulated within the available diversity. However, the fertilization effects were not consistent across years, and depending on the year the predator community had different constraints at different time points. While in this study we looked mainly at the diversity of available prey and fertilization, there can also be other factors. Njue et al. (2021) found that drought affected aphid consumption by carabids and wolf spiders in barley, while Liu et al. (2018) and Beaumelle et al. (2021) found that increased vegetation structure and surrounding crops at the landscape scale play a role on biological control and predator communities. As such, organic fertilizers are only a part of the bigger picture for managing fields to increase CBC.

Such findings bring further relevance to the need to generate data sets that allow the understanding of these complex temporal dynamics of empirical food webs. This knowledge is crucial if we are to move forward, not just in the testing of models and assumptions of theoretical food webs, but also with regard to applying ecological findings to practice such as the optimization of management of pests within CBC strategies. By tracking of food web changes over time and addressing the general lack of empirical studies, which deal with temporal dynamics in real-world food webs (McMeans et al. 2015, Isbell et al. 2018, Roubinet et al. 2018), we have taken a first step towards generating the datasets needed to bridge empirical and theoretical food web science (Pereira et al. 2010, Hooper et al. 2012, Pimm et al. 2014), with direct implications for biological control.

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Author contributions

PNBL and ORRR carried out the field work, PNBL carried out the lab work, PNBL and ORRR conducted the data analysis, PNBL wrote the first draft, ORRR and MT designed the study and procured the funding, all authors contributed to the revisions.

References


Data Accessibility and Benefit-Sharing

Data accessibility
The authors agree to archive the data used to generate the results presented here, and respective metadata, in a publicly accessible repository upon acceptance of the article and provide a DOI.

Benefit-sharing
Benefits from this research accrue from the sharing of our data and results on public databases as described above, with no financial benefits resulting from publication.