

Past and potential future effects of habitat fragmentation on structure and stability of plant–pollinator and host–parasitoid networks

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Habitat fragmentation is a primary threat to biodiversity, but how it affects the structure and stability of ecological networks is poorly understood. Here, we studied plant–pollinator and host–parasitoid networks on 32 calcareous grassland fragments covering a size gradient of several orders of magnitude and with amounts of additional habitat availability in the surrounding landscape that varied independent of fragment size. We find that additive and interactive effects of habitat fragmentation at local (fragment size) and landscape scales (1,750 m radius) directly shape species communities by altering the number of interacting species and, indirectly, their body size composition. These, in turn, affect plant–pollinator, but not host–parasitoid, network structure: the nestedness and modularity of plant–pollinator networks increase with pollinator body size. Moreover, pollinator richness increases modularity. In contrast, the modularity of host–parasitoid networks decreases with host richness, whereas neither parasitoid richness nor body size affects network structure. Simulating species coextinctions also reveals that the structure–stability relationship depends on species' sensitivity to coextinctions and their capacity for adaptive partner switches, which differ between mutualistic and antagonistic interaction partners. While plant–pollinator communities may cope with future habitat fragmentation by responding to species loss with opportunistic partner switches, past effects of fragmentation on the current structure of host–parasitoid networks may strongly affect their robustness to coextinctions under future habitat fragmentation.

Habitat fragmentation is a primary threat to biodiversity and ecosystem functioning^{1,2}. Although ecologists have well-established knowledge of how habitat fragmentation affects biodiversity patterns in species richness, less is known about fragmentation effects on the structure and stability of complex ecological networks that emerge from the interactions between plants and their pollinators or hosts and their parasitoids^{3,4}.

Much research has focused on the effects of habitat fragmentation on numeric responses of plants and pollinators^{5–8} and of hosts and parasitoids^{9–11}. These studies show that the two main processes associated with fragmentation (that is, local reduction in habitat extent and increasing spatial isolation of the remaining habitat patches¹) differ between plant–pollinator and host–parasitoid communities. Host–parasitoid communities comprising Hymenoptera (bees and wasps) and their natural enemies are mainly structured by processes at local scales; for example, host availability and habitat structure^{10,12,13}. In contrast, pollinators are highly variable in scale responses to landscape structure ranging from a few hundred metres to several kilometres¹⁴. Pollinator richness on fragments is therefore not only a function of fragment size, but also strongly depends on the amount of additional habitat in the surrounding landscape and matrix permeability^{6,15}. Moreover, this can result in interactive effects of local- and landscape-scale habitat availability on species communities¹⁶.

Species responses to habitat fragmentation are strongly related to their mobility, of which body size is a good proxy in insects^{14,17}. Pollinator body size correlates positively with the spatial scale that affects their responses to landscape structure^{6,14,18,19}. Metapopulation dynamics allows mobile species to persist even under suboptimal

local habitat conditions (for example, on small fragments) as long as immigration rates are high²⁰. Likewise, greater insect richness in less fragmented landscapes is often driven by increases in small-sized species^{17,21}, which may result in a decrease in average body size in species-rich communities.

Habitat fragmentation alters the structure of ecological networks through changes in species richness and the functional composition (for example, body size) of communities, including behavioural responses such as partner switches^{4,22–24}. In plant–pollinator and host–parasitoid networks, two structural properties are particularly important. A nested interaction structure reflects the prevalence of specialist species that mainly interact with species of a core group of generalists. A modular interaction structure results from the grouping of closely interacting species into compartments that are only loosely connected to each other^{25–28}. Both nestedness and modularity change with the number of interacting species, as well as their functional composition^{4,27,29}. Network nestedness increases with the preferential attachment of rare species that interact with abundant generalists³⁰. Loss of rare species with habitat fragmentation should therefore reduce network nestedness²². Likewise, a higher number of interacting species can increase the modularity of plant–pollinator networks²⁷. However, because opportunistic attachment of species to ecological networks has also been observed³¹, generalizations regarding network assembly or disassembly towards specific topologies are not yet resolved. Further modification of network nestedness and modularity is expected as a result of shifts in functional composition, comprising changes in the body size of consumer communities; for example, due to size-specific trait matching between plants and pollinators or hosts and their parasitoids^{32,33}.

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By changing the number of interacting species and their functional composition, habitat fragmentation can thus have cascading effects on network structure^{4,22}. If and how these effects also alter network stability in the event of potential future species loss (for example, as may happen under further habitat fragmentation) remains poorly understood. Theory predicts that current network structure determines network stability following future perturbations²⁵. In mutualistic communities (for example, plant–pollinator communities), facilitative effects among species at the same trophic level can outweigh competition, resulting in nested interaction networks^{34,35}. Greater nestedness may thereby enhance plant–pollinator network stability following future disturbance^{26,35,36}, whereas greater modularity inhibits facilitative effects and destabilizes mutualistic networks²⁵. In contrast, antagonistic networks (for example, host–parasitoid networks) are strongly structured by exploitative competition between consumers, favouring species segregation into network modules²⁵. Therefore, host–parasitoid networks should be most stable when they are highly modular^{37,38}. Despite these theoretical expectations, recent work highlights that ecological networks exerting high levels of interaction generalization and flexibility are significantly more robust to species loss than might be expected from their structural properties. In particular, species in plant–pollinator networks show low sensitivity to the loss of their interaction partners³⁹ and high potential for adaptive switches to persisting partners^{31,40}. In contrast, the high specialization of host–parasitoid interaction networks may impede adaptive responses to species loss, suggesting a strong link between current network structure and stability under future habitat fragmentation. However, little is known about the roles of species sensitivity and adaptive partner switches for the structure–stability relationship in ecological networks under habitat fragmentation.

Here, we use empirical data from 32 plant–pollinator and 32 host–parasitoid networks and simultaneously explore the responses of mutualistic and antagonistic interaction networks to habitat fragmentation. We study species communities of calcareous grasslands—a highly biodiverse habitat type that has become increasingly fragmented during the past half-century (on average, the fragments in our study region had experienced around a 50% loss in area since the 1960s⁴¹). Our study design covers a fragment size gradient of several orders of magnitude, with amounts of additional habitat availability in the surrounding landscape that vary independent of the fragment size, allowing the identification of additive and interactive effects of local- and landscape-scale habitat loss on interaction networks. First, we investigate how past effects of habitat fragmentation shape current patterns in the richness and body size composition of plant–pollinator and host–parasitoid communities, including interactive effects of local- and landscape-scale habitat availability. Second, we relate these patterns to the structure of the resulting ecological networks, focusing on nestedness and modularity. Third, we ask how current network structure influences network stability under future habitat fragmentation. To this end, we simulate species coextinctions under future habitat loss, considering variation in species' sensitivity to interaction loss and their capacity for adaptive partner switches.

In the studied communities, we find that past habitat fragmentation shapes current patterns in richness and, indirectly, the body size composition of consumers, which in turn shape the structure of their ecological networks. Our simulations suggest that the current structure of plant–pollinator and host–parasitoid networks affects their response to coextinctions under species loss from potential future habitat fragmentation, but with contrasting patterns for the different interaction types. While modularity always stabilizes host–parasitoid networks, the positive effects of nestedness are limited to simulations with high pollinator sensitivity to coextinctions and no potential for adaptive partner switches. In contrast, low-to-medium sensitivity of pollinator species to the loss of their interaction

partners and the possibility of rewiring interactions stabilizes plant–pollinator networks against potential future extinctions, regardless of current degrees of network nestedness.

Results

We observed 5,553 plant–pollinator interaction events among 101 plant species and 138 pollinator species during our surveys on the 32 calcareous grassland fragments. On average, each pollination network included interactions among 21 ± 6 plant and 28 ± 10 pollinator species (mean \pm s.d. throughout). Trap nest samples yielded 1,730 host–parasitoid interaction events, involving 39 host species and 22 parasitoid species (8 ± 3 host and 6 ± 2 parasitoid species per network) (Fig. 1). Likewise, nestedness and modularity of the corresponding interaction networks varied substantially (see Supplementary Table 1 and Supplementary Fig. 1 for details).

Past effects of habitat fragmentation on current network structure.

We used structural equation models (SEM) to investigate how habitat fragmentation affected species numbers and the average body size of higher-trophic-level species in the plant–pollinator and host–parasitoid networks, as well as cascading effects on network structure and eventual stability (Fig. 2). We simplified the SEMs to retain only statistically significant pathways ($P < 0.05$), except for lower-order effects of fragment size and additional habitat in the surrounding landscape in the case of a significant interaction. All final, simplified SEMs were well justified by test statistics (Supplementary Table 2), supporting our expectation that habitat fragmentation affects network structure and, consequently, stability indirectly by modifying species numbers and functional composition. Both plant–pollinator and host–parasitoid communities responded to habitat amount at local and landscape scales, albeit with differing effects on network structure. The nestedness and modularity of plant–pollinator networks increased with body size and species richness of consumers (that is, pollinators), but were unrelated to plant richness. In contrast, the modularity of host–parasitoid networks decreased with host richness, but was unrelated to parasitoid richness.

In detail, we found that fragment size and the amount of additional habitat in a 1,750 m radius (the scale at which richness responses of species groups to additional habitat at landscape scales were generally strongest; see Methods) in the surrounding landscape had additive and interactive effects on plant and pollinator richness, respectively (Fig. 2b), and an interactive effect on host richness, but no direct effects on parasitoid richness (Fig. 2c). Specifically, plant richness increased with fragment size and decreased with higher amounts of habitat at landscape scales (Fig. 2b). High amounts of additional habitat in a 1,750 m radius reduced pollinator richness on small fragments, but less so on large fragments (Figs. 2b and 3a). This interactive effect of local- and landscape-scale habitat amount was even more pronounced for host richness, which decreased on small fragments with increasing additional habitat amount within a 1,750 m radius, but increased on large fragments (Figs. 2c and 3b). While we found no effects of plant richness on pollinator richness, parasitoid richness was related positively to host richness (Fig. 2c). In addition to numerical responses, the average pollinator body size decreased in more species-rich pollinator communities, suggesting that richness gains on larger fragments were mainly driven by small species (Fig. 2b and Supplementary Fig. 2a). In turn, pollinator body size and species richness were the main determinants of plant–pollinator network structure: networks dominated by large pollinators were more nested (Fig. 2b and Supplementary Fig. 2b) and modular. In addition, high pollinator richness increased network modularity (Fig. 2b). We found no analogous relationship for parasitoid body size or parasitoid richness and the structure of host–parasitoid networks. However, the modularity of host–parasitoid networks decreased with host richness (Fig. 2c).

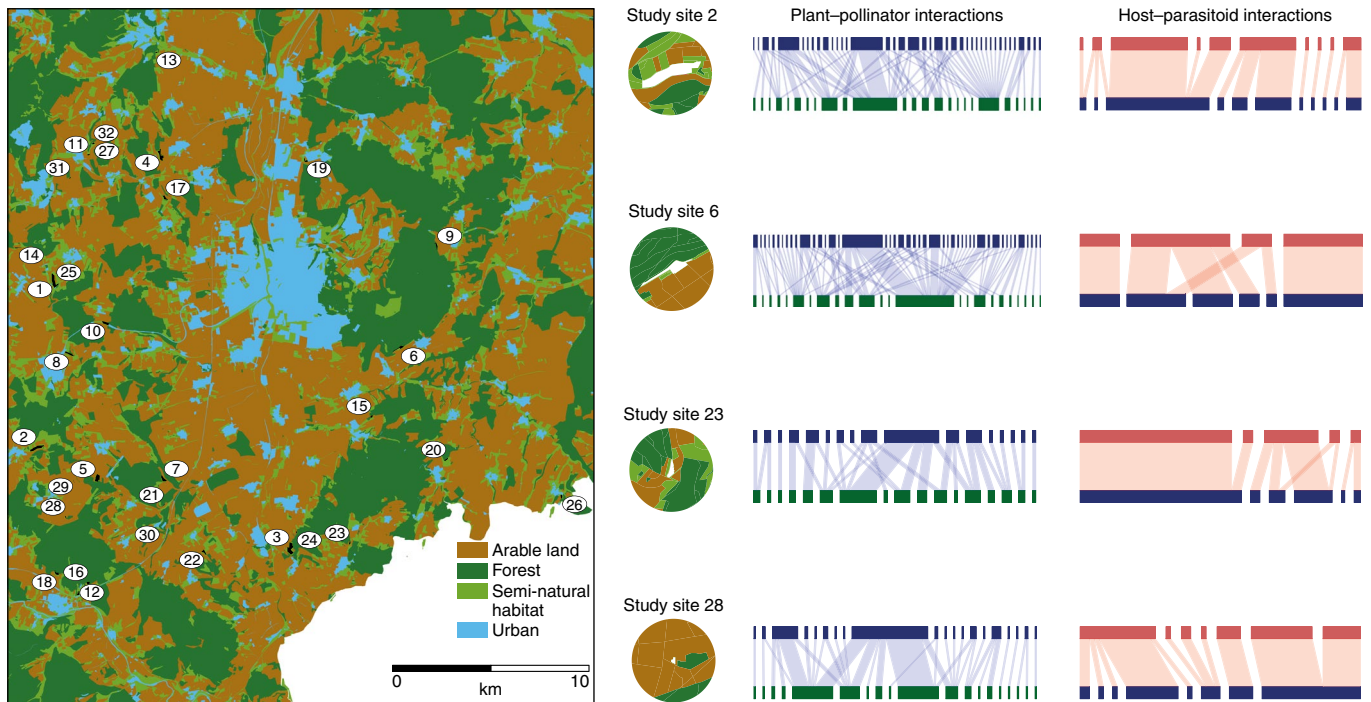


Fig. 1 | Map of the study area, and examples of study landscapes and associated plant-pollinator and host-parasitoid interaction networks. Interaction networks were studied on 32 calcareous grassland fragments (fragment size: 314–51,395 m²). Fragments were selected so that the amount of additional habitat in the surrounding landscape varied independent of fragment size, ranging from complex landscapes with a high proportion of semi-natural habitats to simple landscapes dominated by arable fields and forest. The example landscapes shown (calcareous grasslands in white, with a landscape buffer of 500 m radius) include: a large fragment in a complex landscape (site 2), a large fragment in a simple landscape (site 6), a small fragment in a complex landscape (site 23) and a small fragment in a simple landscape (site 28). Interaction networks are shown with plants and hosts scaled in proportion to their interactions with pollinators and parasitoids. Lines connecting trophic levels indicate pairwise interactions, with the line width proportional to the interaction frequency. Green, plants; blue, pollinators and hosts; red, parasitoids.

Effects of current network structure on network robustness under future habitat fragmentation. We used a simulation approach based on secondary extinctions of pollinators and parasitoids as a consequence of the sequential loss of their plant or host partners to assess variation in the robustness of interaction networks to species loss under potential future habitat fragmentation. First, we identified the sequence of plant and host extinctions from changes in their abundance as observed along the fragment size gradient. Second, we varied pollinator or parasitoid sensitivity to interaction loss by simulating coextinctions beyond different thresholds of interaction loss (75, 50 or 25% decreases in the total interaction frequency). Furthermore, we accounted for the possibility of adaptive partner switches by comparing simulations that allowed pollinators or parasitoids to reallocate 50% of their potentially lost interactions to remaining partners versus simulations without any potential to rewire. We found that network structure has contrasting effects on network robustness, depending on species' sensitivity to coextinction and their rewiring capacity (Fig. 4). High nestedness of plant-pollinator networks could enhance network robustness to simulated coextinctions; however, this positive effect was limited to the assumption of high pollinator sensitivity to the loss of plant partners (coextinctions already occur at 25% interaction loss) and no capacity to reallocate lost interactions to remaining partners (Fig. 4). In contrast, high modularity destabilized plant-pollinator networks under the assumption that pollinator coextinction occurred after species had lost >75% of their interactions and were able to rewire (Fig. 4). We found no relationship between plant-pollinator network robustness and structure for the other simulations (Fig. 4). In contrast with plant-pollinator networks, the nestedness of host-parasitoid interaction networks did not affect their

robustness under any coextinction simulation. However, across all simulations, we found strong positive effects of network modularity on host-parasitoid robustness to coextinctions, regardless of the assumed parasitoid sensitivity and potential for adaptive host switches (Fig. 4).

Discussion

We show that habitat fragmentation, involving independent reductions in local fragment size and habitat availability at landscape scales, has cascading effects on the structure and stability of ecological networks. In particular, habitat fragmentation alters network structure through changes in the number of interacting species and concurrent shifts in body size composition. Whether and how changes in nestedness or modularity in turn affect network robustness to simulated species extinctions under future habitat fragmentation depends on species' sensitivity to coextinctions and their capacity for adaptive partner switches, with contrasting outcomes for plant-pollinator and host-parasitoid communities.

We found that plant and pollinator but not host and parasitoid communities were generally more species rich on larger fragments of calcareous grasslands than on small fragments. In addition, high amounts of additional habitat in the surrounding landscape reduced the species richness of pollinators and hosts on small fragments but not on large fragments. As a result, the species-fragment area relationship for pollinators and hosts was strongest given much additional habitat at landscape scales. These findings suggest that the relevance of small habitat fragments for mobile species such as pollinators or bee and wasp host species of parasitoids diminishes when additional habitats and resources in the surrounding landscape are accessible. Local- and landscape-scale effects shape insect

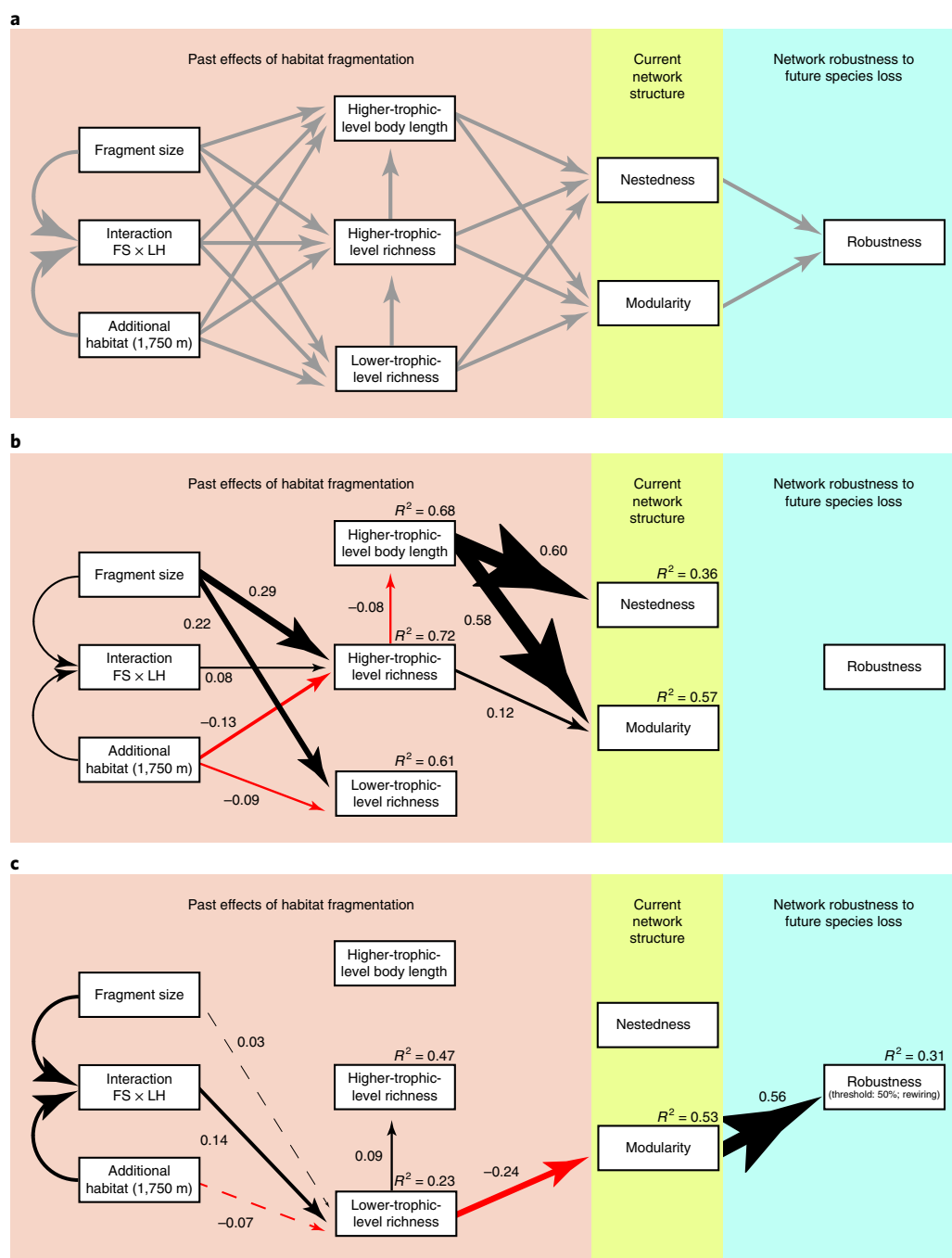


Fig. 2 | SEMs of the effects of habitat fragmentation on the structure and stability of plant-pollinator and host-parasitoid interaction networks. **a**, All hypothesized pathways included in the SEMs before model simplification. Past effects of habitat fragmentation alter the size of calcareous grassland fragments and the amount of additional habitat in the surrounding landscape within a 1,750 m radius. The additive and interactive effects of habitat amount at the local (FS, fragment size) and landscape scale (LH, additional habitat in the surrounding landscape) determine the species richness of interacting communities on the fragments, as well as the body size of consumers. These, in turn, shape the current network structure (nestedness and modularity; all network metrics relative to null expectations). The current network structure relates to the robustness of interaction networks to future species loss, as may result from further habitat loss of the highly threatened calcareous grasslands. **b,c**, SEMs for plant-pollinator (**b**) and host-parasitoid interactions (**c**) after model simplification. Only statistically significant pathways have been retained (solid arrows; $P < 0.05$), except for lower-order effects of fragment size and additional habitat within 1,750 m in the case of a significant interaction (dashed arrows). Arrows are scaled to standardized path coefficients, thus corresponding to relative effect strengths. R^2 values indicate the explained proportion of variance in the response variables. Colours indicate effect directions (red, negative; black, positive). See Supplementary Table 2 for model fits of all SEMs and Fig. 3 for a visualization of the interactive effects of fragment size and additional habitat within a 1,750 m radius. Note that the past effects of habitat fragmentation on current network structure remain the same irrespective of the type of simulation used to estimate network robustness under species loss from potential future habitat fragmentation; however, effects of current network structure on robustness depend on the assumed sensitivity of species to coextinction and their rewiring capacity. For the plant-pollinator networks, the presented SEM refers to network robustness based on a threshold of 75 or 50% interaction loss before coextinction or any simulation (75, 50 or 25% thresholds) without rewiring. For the host-parasitoid networks, the results from the coextinction simulation based on a 50% threshold and rewiring are shown. The structure-stability relationships for all simulations are shown in Fig. 4.

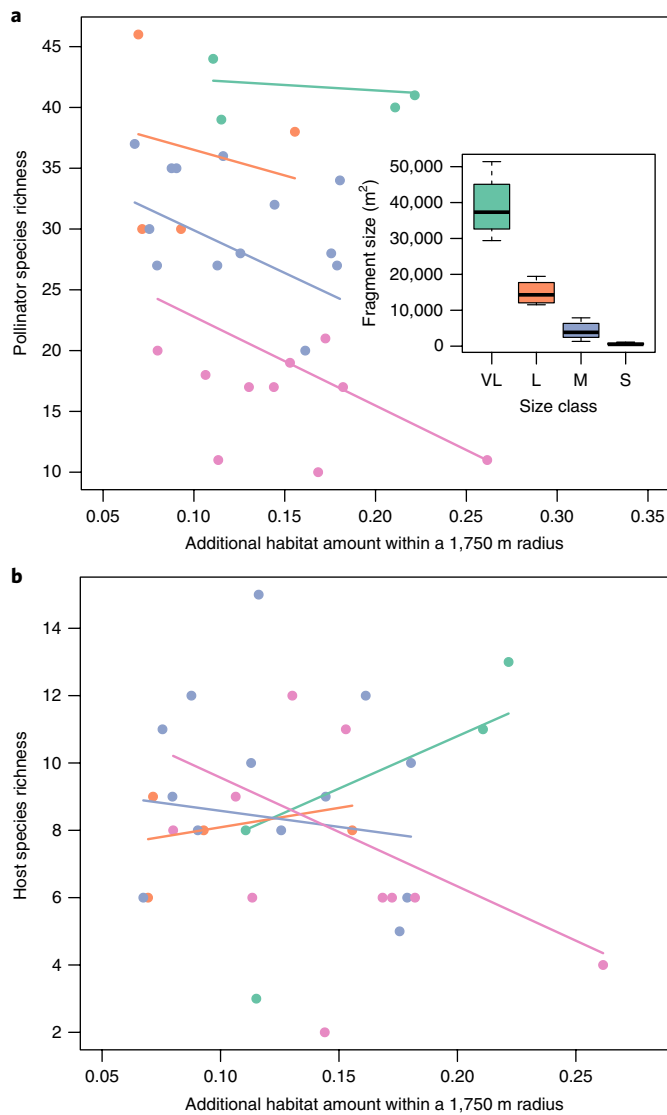


Fig. 3 | Interactive effects of fragment size and the proportion of additional semi-natural habitats within a 1,750 m radius on the species richness of pollinators and hosts. For visualization purposes, fragments were grouped into four size classes (L, large (orange); M, medium (blue); S, small (pink); VL, very large (green)). **a**, A high amount of additional habitat at the landscape scale reduces pollinator richness on small fragments, but these negative effects vanish with increasing fragment size. **b**, Likewise, a high amount of additional habitat reduces host richness on small fragments, but has a positive effect on host richness on very large fragments. Inset, distribution of fragment sizes across size classes. Boxplots represent the median (black bars), the 25–75% intervals (box edges) and the 1.5 interquartile range (whiskers).

communities in fragmented landscapes^{15,17}. In particular, enhanced connectivity in structurally diverse landscapes rescues habitat specialists from extinction and increases resource availability for generalist species⁴². Pollinators preying on crop pests (for example, aphidophagous hoverflies) can persist in agricultural landscapes that are unsuitable for bee species⁴³. Species persistence is therefore not solely determined by local fragment area but by the total meta-population capacity of the landscape, which is driven by habitat amount and spatial configuration⁴⁴. In addition, we found that host extinctions could trigger bottom-up trophic cascades⁴⁵, in line with the generally strong association between host and parasitoid richness⁴⁶ and the high specialization of host–parasitoid interactions

that makes them particularly sensitive to perturbations^{42,47}. Hence, although parasitoids were not directly affected by local- or landscape-scale habitat amount, negative effects of fragmentation on their hosts may result in their eventual extinction.

Body size strongly influenced plant–pollinator network structure. The average pollinator body length decreased in more species-rich pollinator communities (Supplementary Fig. 2a), suggesting that gains in pollinator richness on larger grassland fragments were mainly driven by small pollinators with limited dispersal abilities¹⁴, which particularly rely on locally available habitats and resources^{5,21,48}. However, increasing pollinator richness did not result in greater nestedness; instead, large pollinators in particular appeared to promote interaction nestedness (Supplementary Fig. 2b). Additional analyses showed that larger pollinators generally had more plant partners and fewer specialized interactions than small pollinators (Supplementary Fig. 3). Size matching between plants and pollinators could be a functional explanation, whereby larger pollinators with longer proboscises can exploit a greater variety of flower resources than small pollinators with short proboscises³². In turn, large pollinators may have promoted the integration of specialist plants into networks, increasing nestedness and modularity^{26,27}. Generalizing pollinator responses to land-use change from functional traits has proven difficult⁴⁹. Body size remains among the most promising traits for predicting trait-based responses of network structure to environmental perturbations and subsequent effects on ecosystem functioning^{49–51}. Our study demonstrates that understanding network responses to environmental perturbations benefits from including functional traits in analyses.

The numeric responses of pollinators and hosts to fragmentation affected network modularity, but with contrasting effect directions. Higher pollinator richness increased the modularity of plant–pollinator networks. A modular network structure can result from convergent traits of interacting species²⁷, as exemplified in the case of size matching between plants and their pollinators³², or from overlapping phenophases⁵². Habitat fragmentation can cause functional homogenization of pollinator communities (for example, variation in proboscis length)⁵³. Functional impoverishment, which can be further coupled with phenological shifts or shortened phenologies⁵⁴, may thus explain changes in the topology of ecological networks and ecosystem functioning^{4,11,51}, and remains an important future research area. Habitat fragmentation also reduced host species richness. However, in contrast with pollinators, host richness reduced modularity. Hence, host–parasitoid networks were more modular (and thereby increasingly robust to simulated coextinctions) when strongly affected by habitat fragmentation. Here, it should be noted that our standardized measures of network structure detect changes in interaction patterns that go beyond mere changes in network size⁵⁵. Our results therefore suggest that the modularity of host–parasitoid networks increases more gradually than would be expected from concurrent increases in network size on habitats that are less affected by habitat fragmentation. The fact that unstandardized modularity does not increase linearly with host richness, but saturates quickly, supports this assumption (Supplementary Fig. 4). However, nonlinear relationships were not the focus of our study. In addition, because a lack of standardization can result in failure to isolate pure network patterns (that is, third-order patterns⁵⁵) from those that follow from mere changes in network size (first- or second-order patterns), we decided to standardize all network metrics.

Calcareous grassland fragments need constant management, such as grazing or mowing, to avoid succession of woody species and losses in threatened plant diversity⁵⁶. Owing to agricultural intensification and abandonment, the calcareous grasslands in our study region underwent severe declines in habitat extent during the second half of the twentieth century, with an average decline of 50% in area since the 1960s⁴¹. If this trend continues, they are likely to suffer from future fragmentation that further diminishes habitat

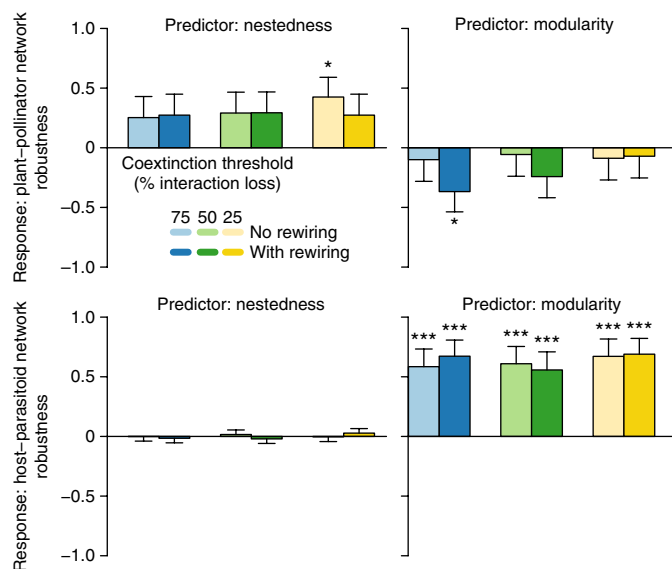


Fig. 4 | The effects of current network structure on network robustness to simulated future species extinctions depend on species sensitivity to coextinction and rewiring capacity, and differ between plant–pollinator and host–parasitoid networks. Thresholds for coextinction were 75% (blue), 50% (green) and 25% (yellow) interaction loss. Rewiring to persisting partners was either allowed (dark colours) or not (light colours). Standardized regression coefficients are shown (* $P < 0.05$; *** $P < 0.001$). Errors bars represent 1 s.e.m.

quantity and quality, which is expected to threaten associated insect communities and biotic interactions^{42,57}. We therefore investigated whether and how the current network structure predicts the robustness of plant–pollinator and host–parasitoid networks to species losses under future habitat fragmentation. Nestedness has been proposed as a crucial determinant of stability in mutualistic networks^{26,35,36}. Our simulations of bottom-up-driven coextinction in plant–pollinator networks support this hypothesis under the assumption that pollinators are highly sensitive to the loss of their plant partners and have poor capacity for adaptive partner switches (Fig. 4). Coextinctions are likely to occur long before species have lost all interaction partners; for example, because population sizes have fallen below critical thresholds^{47,58}. Hence, at least for some pollinator species, high sensitivity to the loss of their plant partners may be reasonably assumed, which is corroborated by studies highlighting the importance of partner fidelity in ecological networks^{59,60}. Conversely, recent studies suggest that interaction turnover and partner switches are widespread events in plant–pollinator networks; for example, when comparing assembly across years^{61,62} or following habitat restoration³¹. This high degree of interaction flexibility may buffer plant–pollinator networks against species loss^{40,63}. In fact, long-term studies suggest that opportunistic rather than preferential attachment may govern the assembly of plant–pollinator networks, stabilizing them in dynamic environments^{31,62}. Under these circumstances, current network structure will be a poor predictor of network stability under future species loss, as also suggested by the lack of a corresponding relationship between nestedness and plant–pollinator network robustness in our simulations that included adaptive partner switches. Interestingly, one of our simulations also indicated that high modularity may destabilize plant–pollinator networks, as predicted by ecological theory²⁵. In contrast, greater modularity should enhance the stability of antagonistic interaction networks^{38,64}. We found strong support for this hypothesis: modularity always enhanced host–parasitoid network robustness regardless of the assumed species sensitivity or rewiring

capacity, contrasting the effects of nestedness and modularity on plant–pollinator robustness that were limited to few specific simulations. Host–parasitoid interactions are generally more specialized than plant–pollinator interactions, making parasitoids highly sensitive to host extinctions⁴⁷. Host specificity varies with factors such as the relative fitness of parasitoids on different hosts and the phylogenetic spectrum of hosts^{65,66}, and the resulting coevolution may constrain species roles in antagonistic networks more strongly than in mutualistic networks⁵⁹. Our study shows that as a consequence, the fragmentation of calcareous grasslands fundamentally differently affects mutualistic and antagonistic communities: while plant–pollinator communities may cope with future habitat fragmentation by responding to species losses with opportunistic partner switches, past effects of fragmentation on host–parasitoid communities and their current network structure may strongly affect network robustness to coextinctions under future habitat fragmentation.

In conclusion, by affecting the number of interacting species and their functional composition, habitat fragmentation can have cascading effects on the structure and stability of ecological networks. Notwithstanding, our simulations suggest that these effects depend on species' sensitivity to coextinctions and their potential for adaptive partner switches, and that their pathways can differ substantially between plant–pollinator and host–parasitoid communities. Little is known about how the adaptive capacities of species to habitat fragmentation affect the structure and stability of ecological networks in real-world ecosystems, and eventually ecosystem functioning. Yet, shifts and losses of species interactions can be early signs of future extinction and ecosystem function debts⁶⁷. Habitat fragmentation is continuing at alarming rates globally, threatening even formerly remote regions⁶⁸. A better understanding of the consequences for ecological networks and ecosystem functioning is urgently needed.

Methods

Study region and site characteristics. The study was conducted in 2004 in the Leine-Bergland around the city of Göttingen in Lower Saxony, Germany. The study region covers an area of approximately 2,000 km² and is mostly dominated by intensively managed arable land (approximately 40%) and forest (approximately 35%). Our study region included a total of 285 calcareous grassland fragments; however, these covered only 0.3% of the area. Calcareous grasslands are of outstanding conservation value and support by far the highest levels of biodiversity among the habitat types of our study region per unit area⁶⁹. These grasslands are highly fragmented and sharply delimited from the surrounding agricultural matrix. The calcareous grasslands in our study region are believed to have reached their maximum distribution in the nineteenth century, and underwent severe declines in habitat extent during the second half of the twentieth century, with an average decline of around 50% in area since the 1960s⁴¹. The primary reasons are agricultural intensification and abandonment, in particular because their constant management by grazing or mowing has become economically unattractive³⁶. We selected 32 out of the 285 calcareous grasslands in our study region that covered the full gradient of habitat fragmentation typical of the region (Fig. 1). The area (fragment size) of the calcareous grassland fragments at the time of sampling was measured with a differential GPS GEOMeter 12L (GEOsat) and ranged from 314–51,395 m². In addition, we determined the percentage cover of semi-natural habitats (species-rich grasslands, gardens, hedgerows, calcareous grasslands, orchard meadows and fen; excluding the focal fragments) at 12 spatial scales from 250–3,000 m radius surrounding the centre of the selected fragments, using the geographic information systems ArcView 3.2 (ESRI Geoinformatik). Notably, fragment size and the amount of additional habitat in the surrounding landscape were statistically independent, allowing us to differentiate between the effects of habitat fragmentation at local and landscape scales (Supplementary Table 3). Moreover, the amount of additional habitat in the surrounding landscape strongly correlated with other measures of habitat isolation; for example, the habitat amount within a 1,750 m radius correlated negatively with the distance to the next calcareous grassland fragment (Pearson's coefficient of correlation, $r = -0.53$, $P = 0.002$) and positively with Hanski's connectivity index ($r = 0.67$; $P < 0.001$). For more information on the study sites, see ref. 21.

Sampling of plant–pollinator and host–parasitoid interactions. Flower visitors (wild bees and hoverflies; assumed to be pollinators of visited plants) were sampled via 5 min transect walks 6 times from April to September 2004 within a 4 m corridor. To achieve adequate sample sizes for the differently sized grassland fragments, we conducted 4 of the 5 min transects (total = 20 min) in 11 small fragments (314–1,133 m²), 8 of the 5 min transects (total = 40 min) in 13

medium fragments (1,326–7,887 m²) and 12 of the 5 min transects (total = 60 min) in 8 large fragments (11,528–51,395 m²). Data from the 5 min transects of all 6 sampling events were pooled per grassland fragment. Hence, the total sampling effort was 22 h for all small fragments, 52 h for medium fragments and 48 h for large fragments (122 sampling hours for all sites in total). Pollinators were either identified on the wing or caught with a net and identified in the laboratory. The visited plant species was recorded for each pollinator individual. Resource availability was quantified after each pollinator sampling date by determining all plant species in flower within the sampled area and estimating their percentage floral cover. Flower diversity ranged from 23–47 (mean 35) flowering species per site. Some pollinator individuals could not be identified to species level and were identified to genus level (*Heringia*, *Paragus* and *Pipizella* species) or to species groups (*Bombus terrestris/lucorum*, *Cheilosia albitarsis/ranunculi*, *Eumerus strigatus/sogdianus*, *Melanostoma mellinum/scalare*, *Pipiza bimaculata/noctiluca* and *Platycheirus scutatus/splendidus*). All study sites were sampled in a randomized sequence between 9:00 and 18:00 on sunny days with little wind.

Parasitoids/parasites and hosts were sampled using trap nests at the same sites. Trap nests provide standardized nesting sites for naturally occurring bee and wasp communities and are a well-established method of studying the structure of host–parasitoid interaction networks¹³. Trap nests consisted of bundles of reed internodes of the common reed *Phragmites australis* (approximately 150–180 reed internodes of 2–10 mm diameter in plastic tubes of 10 cm diameter per trap nest) exposed at a height of 100–120 cm. Depending on the fragment size, 4–6 wooden posts with 2 trap nests each were used: 4 posts (8 trap nests) in 11 small fragments; 5 posts (10 trap nests) in 13 medium fragments; and 6 posts (12 trap nests) in 8 large fragments. The trap nests were spread regularly over the study sites and exposed at the beginning of the flowering period (mid-April) until autumn (beginning of October). Afterwards, trap nests were stored in a climate chamber at 4°C and the occupied reed internodes were opened. For each nest, the numbers of brood cells and parasitized cells were recorded. We identified host and parasitoid identities to genus or species level as far as possible using larvae and nest characteristics. Because *Osmia rufa* overwinter as adults, these cocoons were opened to check for parasitoids. All other nests were stored separated in test tubes closed with a wad of cotton wool. Tubes were exposed to room temperature (around 20°C) to end diapause. Reared adults were identified to species level. When the whole brood of a nest was lost to parasitism and no adult could be reared, the host genus was determined from nesting characteristics. These nests were only used as additional species if no other species of the same genus was found at the same site.

The body sizes of bees, hoverflies and parasitoids/parasites were derived from the literature^{69–75} and public databases (www.wildbienen.de). For comparability among taxa, we always used the body length of species as the size measurement, taking mean values when ranges were given and averaging between the sexes.

Adequate sampling of species interactions is a considerable challenge for all ecological network studies, and in particular for those conducted along environmental gradients⁴. We therefore estimated the sampling completeness across networks and its variation along the fragment size gradient to exclude potential sampling bias that might affect the calculated network metrics. The sampling completeness of each network was estimated by dividing the observed richness of pairwise interactions (links) by the estimated link richness^{67,77}. The estimated link richness was approximated using the Chao1 estimator of asymptotic richness⁷⁸. Here, links between species pairs form the equivalent of 'species' and interaction frequencies form the equivalent of 'abundances'⁷⁶. The estimated sampling completeness was 50 ± 10% (mean ± s.d.) for plant–pollinator interactions and 80 ± 16% for host–parasitoid interactions. Notably, some authors have argued that asymptotic diversity estimators overestimate the total number of possible pairwise interactions^{79,80} and that the estimation does not allow differentiation between missing links due to under-sampling and forbidden links from phenological or morphological mismatches among species. In fact, such mismatches may explain up to 80% of unobserved interactions⁸¹, and a large number of unobserved links in our study that covered a full season may thus be explained. Moreover, our estimates of sampling completeness agree with those of other plant–pollinator network studies (57% in ref. ⁸²; 55% after 171 sampling hours in ref. ⁷⁷). Most importantly, sampling completeness was not related to the fragment size (plant–pollinator: slope of linear regression, $\beta_{\text{size}} = -0.013$, $P = 0.282$, coefficient of determination, $R^2 = 0.04$; host–parasitoid: $\beta_{\text{size}} = -0.007$, $P = 0.686$, $R^2 = 0.01$; Supplementary Fig. 5). These findings suggest that even if some links remained unobserved (Supplementary Fig. 6), no systematic bias in sampling completeness affected the network metrics along the fragmentation gradient.

Network structure and simulations of species coextinctions. Using the data from the plant–pollinator and host–parasitoid surveys, we assembled 64 quantitative interaction networks; that is, one for each interaction type on the 32 calcareous grassland fragments. Interaction frequencies between species were assumed to be surrogates of functional dependencies between species^{83,84}. For each network, we calculated quantitative measures of network nestedness and modularity based on the weighted nestedness metric WNODF and the QuanBiMo algorithm, respectively^{85,86}. We used a simulation approach to quantify how changes in the current structure of interaction networks influence their stability in response to future

habitat fragmentation. As stability measures, we calculated network robustness of interaction networks as the area under the secondary extinction curve from simulations of secondary extinctions of higher-trophic-level species (pollinators and parasitoids) as a consequence of the sequential loss of species at the lower trophic level (plants and hosts)^{87,88}. Assuming there are bottom-up effects of species loss at the lower trophic levels on interaction networks is justified by the fact that plant–pollinator and host–parasitoid interactions are strongly driven by bottom-up effects of resources on consumers^{45,89–91}, and recent studies have shown that animals in mutualistic networks are probably more sensitive to the loss of their plant partners under climate change than vice versa³⁹. Furthermore, declines in plants and hosts are a primary driver of pollinator and parasitoid declines, respectively^{45,47,89,92}. We used network robustness as a measure of the tolerance of interaction networks to future species extinctions on the grassland fragments, as could result from future habitat loss and degradation³⁶ (Fig. 2a). Following this rationale, we determined the extinction order of plants and hosts from changes in their abundance along the fragmentation gradient. Hence, in our simulations on species coextinctions, we first removed the plant or host species experiencing the largest decline from large to small fragments, followed by the species with the second largest decline, until the last species with the least negative response (or strongest positive response) (see ref. ³⁹ for similar simulations of coextinctions in ecological networks under climate change). Plant species abundances were inferred from cover estimates collected after the transect walks and converted to count data by multiplying percentage values by 100. For host abundances, we used the total number of brood cells per species and fragment, irrespective of parasitism occurrence. Hence, both plant and host species abundance can be considered estimates of resource availability that were collected independent of species interactions. We then used a model-based approach to these multivariate abundance data; that is, we fitted a generalized linear model with negative binomial error distribution to each species (function 'manyglm' in the R package 'mvabund'⁹³). We subsequently determined the extinction order of plants or hosts from the model coefficients, with species ordered from the most negative to the most positive coefficient (see Supplementary Data 1 for a compilation of coefficients and extinction sequence positions for all plant and host species, and Supplementary Fig. 7 for a visualization of example responses of species along the fragmentation gradient).

In the first study describing the calculation of network robustness, secondary extinctions were assumed when species had lost all interactions with their partners⁸⁸. However, high numbers of functional extinctions suggest that coextinctions probably already occur at much lower thresholds of interaction loss⁸⁸. We therefore simulated extinctions when species had lost at least 75, 50 or 25% of their interactions, referring to increasing levels of species' sensitivity to interaction loss³⁹. In addition, species may respond to the extinction of their partner(s) by reallocating part of their lost interactions to persisting partners (constrained rewiring^{4,40}). We included the capacity of pollinators or parasitoids for adaptive partner switches in our simulations by allowing them to reallocate 50% of their otherwise completely lost interactions among their remaining plant or host partners, whereby interactions were spread proportionally to the total interaction frequencies of the persisting partners³⁹. We only simulated constrained rewiring and thus assumed that species would not interact with new partners. Although this assumption is conservative in that it prevents introducing new pairwise interactions or species into the networks, we considered it most suitable for our simulations. First, assuming only constrained rewiring precludes introducing 'forbidden links' that result from simulating impossible interactions because of, for example, phenological or morphological mismatches between species⁸¹. Second, rewiring may be generally constrained to persisting partners in specialized trophic networks such as host–parasitoid communities⁸⁴.

The absolute values of almost all network metrics depend to at least some degree on network size⁹⁴. To directly compare the networks along the fragmentation gradient, we therefore standardized all metrics relative to a null expectation^{22,25,86,95}. The standardized metrics can then be interpreted as the extent to which the network structure differs from random for a given network size^{4,86}. Standardized metrics were calculated as

$$\text{metric}_z = \frac{\text{metric}_{\text{obs}} - \text{metric}_{\text{null}}}{\sigma_{\text{metric}_{\text{null}}}}$$

Separate null distributions were created for each metric using Patefield's algorithm, which shuffles species interactions while fixing the marginal totals of the interaction matrix (1,000 replicate randomized networks). The standardized metrics were used for all network analyses.

SEMs of past and future effects of habitat fragmentation on interaction networks. We used generalized multilevel path analysis—a method recently introduced as 'piecewise SEM'^{96,97}—to infer causal relationships between past effects of habitat fragmentation on the studied species communities and subsequent effects on their current network structure, and to link variation in network structure to the network robustness under potential future habitat loss (Fig. 2a). This method allows for constructing and testing complex causal models even with relatively low sample sizes^{97,98} and non-Gaussian distributions. The overall fit

of a piecewise SEM is tested using Shipley's d-separation test, which assesses the validity of independence claims (that is, the null probabilities of those paths that were not included in the SEM)⁹⁶. The combined probabilities are compared with a chi-squared distribution with $2k$ degrees of freedom, where k is the number of independence claims (for details, see ref. ⁹⁶). The SEM is rejected if the resulting C value (formula in ref. ⁹⁶) is unlikely to have occurred by chance ($P < 0.05$). Hence, given $P > 0.05$, the model is considered an appropriate fit to the data.

Predictors of habitat fragmentation included the size of the calcareous grassland fragments and the amount of additional habitat in the surrounding landscape. For simplicity and because of statistical restrictions imposed by sample size, we did not include all landscape scales (250–3,000 m; see Methods). Instead, we identified the most relevant scale from comparisons of effect sizes of additional habitat amount at different spatial scales for predicting the species richness of the studied communities (generalized linear models with Poisson distribution). Comparisons of effect sizes indicated that plant, host and parasitoid richnesses were not significantly related to the additional habitat amount at any spatial scale. In contrast, pollinator richness increased with additional habitat amount within a 250 m radius and decreased with additional habitat amount at spatial scales of between 1,500 and 2,000 m radius (Supplementary Fig. 8). As fragmentation effects at relatively small spatial scales were positively correlated with fragment size (Supplementary Table 3), we focused our analysis only on landscape effects at the scale with the highest predictive power, which was the surrounding landscape within a 1,750 m radius. Hence, we included in the SEMs the additive and interactive effects of fragment size and additional habitat amount within 1,750 m on species richness at lower and higher trophic levels, and on the mean body size of consumers at the higher trophic level within each community. Models with species richness as a response were fitted with Poisson distribution. We assumed that the species richness of consumers would affect their body size composition and thus the mean body size. We then included all possible pathways from species richness and body size to network structure; that is, nestedness and modularity. Finally, we included the effects of current network structure on the robustness of networks under future habitat fragmentation. Because our simulations of coextinctions resulted in six simulations to estimate robustness per interaction type (three extinction thresholds, each with and without rewiring), a total of 12 SEMs were fitted (see Supplementary Data 2 for a compilation of all data used for the SEMs). Note that the type of simulation used to estimate network robustness only affects the pathways from network structure (not the pathways included under 'past effects of habitat fragmentation'; Fig. 2a) and the overall SEM fit. We stepwise deleted non-significant pathways until only significant pathways ($P < 0.05$) or non-significant lower-order effects of significant interactions remained. As a measure of the explained proportion in each response variable, we calculated variance-function-based R^2 -values⁹⁹. All statistical analyses were conducted using R version 3.4.3 (ref. ¹⁰⁰) and the dedicated packages mvabund version 3.12.3 (ref. ⁹³), bipartite version 2.08 (ref. ¹⁰¹), piecewiseSEM version 1.2.1 (ref. ⁹⁷) and rsq version 1.0.1 (ref. ¹⁰²).

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Data availability. Model coefficients of changes in plant and host species abundances, from the largest to the smallest calcareous grassland fragments, and corresponding extinction sequence positions are provided in Supplementary Data 1. The study site and network data used for the SEMs are provided in Supplementary Data 2, including the fragment size, amount of additional semi-natural habitat in the surrounding landscape, species richness estimates, average pollinator and parasitoid body size, raw and standardized network metrics (nestedness and modularity), and standardized network robustness estimates from coextinction simulations. The plant–pollinator and host–parasitoid network matrices are available upon request from the corresponding author.

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Author contributions

I.G., B.J., I.S.-D., T.T. and F.J. conceived the study. I.S.-D. obtained the funding and designed the field study. B.J. and F.J. conducted the field work and compiled the data.

I.G. analysed the data and prepared the manuscript. All authors discussed the results and contributed to revisions of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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State explicitly what error bars represent (e.g. SD, SE, CI)

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Software and code

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Data collection	No software was used for data collection.
Data analysis	All statistical analyses were conducted using R ver. 3.3.2 and dedicated packages 'bipartite' ver. 2.07, 'lavaan' ver. 0.5-22 and 'mgcv' 1.8-16. The R-code and associated data are available upon request.

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Model coefficients of changes in plant and host species abundances from the largest to the smallest calcareous grassland fragments and corresponding extinction sequence positions are provided in Supplementary Data 1. The study site and network data used for the structural equation models (SEMs) are provided in

Supplementary Data 2, including fragment size, amount of additional semi-natural habitat in the surrounding landscape, species richness estimates, average pollinator and parasitoid body size, raw and standardized network metrics (nestedness, modularity), and standardized network robustness estimates from coextinction simulations. The plant-pollinator and host-parasitoid network matrices are available upon request from the corresponding author.

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Ecological, evolutionary & environmental sciences study design

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Study description	Field study of plant-pollinator and host-parasitoid interactions on calcareous grasslands, conducted along a fragmentation gradient.
Research sample	32 calcareous grassland fragments were selected. On each fragment, plant-pollinator interactions comprising bees, hoverflies and plants were sampled by direct observation and sweep netting. Bees/wasps and their parasitoids (parasites) were sampled using trap nests on the same fragments.
Sampling strategy	The number of selected calcareous grasslands was based on prior work and long-standing experience from studies on the same study sites (e.g., Krauss et al. 2003 Journal of Biogeography, Jauker et al. 2013, Landscape Ecology). The 32 selected fragments were further selected for independence between fragment size and surrounding landscape context. The number of fragments (32) exceeds typical numbers of other studies on the effects of habitat fragmentation on ecological networks. The sample effort for plant-pollinator and host-parasitoid interactions was based on previous studies on the same study sites, and furthermore increased with increasing fragment size.
Data collection	Birgit Jauker and Frank Jauker collected the interaction data. Plant-pollinator interactions were sampled directly in the field. Host-parasitoid interactions were derived from rearing samples of the trap nests.
Timing and spatial scale	Flower visitors (wild bees and hoverflies; assumed to be pollinators of visited plants) were sampled via five-minute-transect walks six times from April to September 2004 within a 4 m corridor. To achieve adequate sample sizes for the differently sized grassland fragments, we conducted four of the 5-min-transects (total = 20 min) in eleven small fragments (314–1,133 m ²), eight 5-min-transects (total = 40 min) in 13 medium fragments (1326–7887 m ²), and twelve 5-min-transects (total = 60 min) in eight large fragments (11,528–51,395 m ²). Data from the 5-min-transects of all six sampling events were pooled per grassland fragment. Hence, the total sampling effort for all small fragments was 22 hours, for medium fragments 52 hours and for large fragments 48 hours (122 sampling hours for all sites in total). Parasitoids/parasites and hosts were sampled using trap nests at the same sites. Trap nests provide standardized nesting sites for naturally occurring bee and wasp communities and are a well-established method to study the structure of host-parasitoid interaction networks. Trap nests consisted of bundles of reed internodes of common reed <i>Phragmites australis</i> (approximately 150–180 reed internodes of 2–10 mm diameter in plastic tubes of 10 cm diameter per trap nest) exposed at a height of 100–120 cm. Depending on the fragment size, 4–6 wooden posts with 2 trap nests each were used: 4 posts (8 trap nests) in 11 small fragments, 5 posts (10 trap nests) in 13 medium fragments, 6 posts (12 trap nests) in eight large fragments. The trap nests were spread regularly over the study sites and exposed at the beginning of the flowering period (mid-April) until autumn (beginning October).
Data exclusions	No data were excluded.
Reproducibility	The field study was so far not repeated.
Randomization	All study sites were sampled in a randomized sequence between 0900 and 1800 hours on sunny days with little wind.
Blinding	No blinding was conducted.
Did the study involve field work?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

Field work, collection and transport

Field conditions	The study was conducted in 2004 in the Leine-Bergland around the city of Göttingen in Lower Saxony, Germany. The study region covers an area of approximately 2000 km ² and is mostly dominated by intensively managed arable land (approx. 40%) and forest (approx. 35%). For this study, 32 out of 285 calcareous grassland fragments were selected to cover a size gradient from large to small fragment and at the same time an independent gradient of additional habitat amount in the surrounding landscape. All study sites were sampled in a randomized sequence between 0900 and 1800 hours on sunny days with little wind.
Location	The study was conducted in 2004 in the Leine-Bergland around the city of Göttingen in Lower Saxony, Germany (51°31'03N, 9°54'38E).

Access and import/export	The grasslands were readily accessible. No samples were exported. Sampling permits were obtained from the nature protection authority of the district of Goettingen.
Disturbance	The study sites and organisms were not disturbed by any other means than the sampling, which was restricted to 4 m wide transects.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Unique biological materials
<input checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants

Methods

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Animals and other organisms

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Laboratory animals	NA
Wild animals	Bees, wasps, hoverflies and parasitoids were sampled with sweep nets and trap nests as described above. The insects were identified and released in the field, if possible. Otherwise, they were killed with ethyl acetate for later identification in the laboratory.
Field-collected samples	Trap nests were stored in a climate chamber at 4°C and occupied reed internodes were opened. For each nest, the number of brood cells and number of parasitized cells were recorded. We identified host and parasitoid identities to genus or species level as far as possible using larvae and nest characteristics. Because <i>Osmia rufa</i> overwinter as adults, these cocoons were opened to check for parasitoids. All other nests were stored separated in test tubes closed with a wad of cotton wool. Tubes were exposed to room temperature (ca. 20°C) to end diapause. Reared adults were identified to species level.