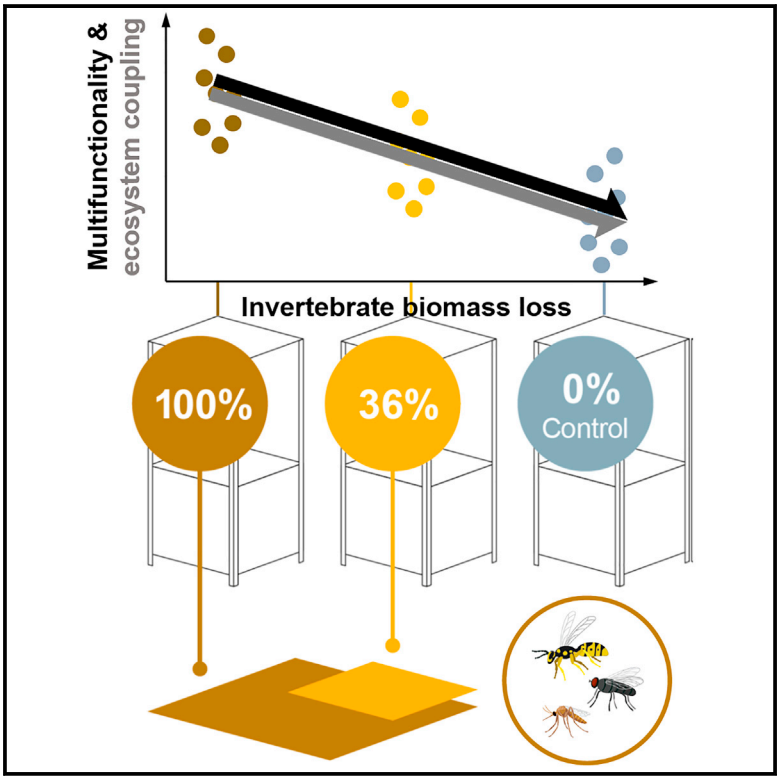
Report

Current Biology

Ecosystem consequences of invertebrate decline

Graphical abstract



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In brief

Eisenhauer et al. test the ecosystem consequences of invertebrate loss in experimental grassland mesocosms. They find that invertebrate biomass loss decreases ecosystem multifunctionality and coupling, indicating that invertebrate loss threatens the integrity of grasslands by decoupling ecosystem processes and decreasing ecosystem-service supply.

Highlights

- We test the ecosystem consequences of simulated invertebrate decline in an Ecotron
- Loss of invertebrate biomass decreases ecosystem multifunctionality
- Invertebrate loss reduces aboveground pest control and belowground decomposition
- Ecosystem functions become decoupled with a lower biomass of invertebrates

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Report

Ecosystem consequences of invertebrate decline

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SUMMARY

Human activities cause substantial changes in biodiversity.[1,2] Despite ongoing concern about the implications of invertebrate decline,[3–7] few empirical studies have examined the ecosystem consequences of invertebrate biomass loss. Here, we test the responses of six ecosystem services informed by 30 above- and belowground ecosystem variables to three levels of aboveground (i.e., vegetation associated) invertebrate community biomass (100%, 36%, and 0% of ambient biomass) in experimental grassland mesocosms in a controlled Ecotron facility. In line with recent reports on invertebrate biomass loss over the last decade, our 36% biomass treatment also represented a decrease in invertebrate abundance (−70%) and richness (−44%). Moreover, we simulated the pronounced change in invertebrate biomass and turnover in community composition across the season. We found that the loss of invertebrate biomass decreases ecosystem multifunctionality, including two critical ecosystem services, aboveground pest control and belowground decomposition, while harvested plant biomass increases, likely because less energy was channeled up the food chain. Moreover, communities and ecosystem functions become decoupled with a lower biomass of invertebrates. Our study shows that invertebrate loss threatens the integrity of grasslands by decoupling ecosystem processes and decreasing ecosystem-service supply.

RESULTS AND DISCUSSION

Anthropogenic environmental changes threaten biodiversity and the integrity of ecosystems worldwide.[1,2] One particularly troubling trend is the global decline of invertebrate abundance, biomass, and diversity during the last decades,[3–7] which also attracted political and public attention.[8,9] Despite limited and biased data on invertebrate-diversity trends,[10–12] decline in this diversity appears to be driven by land-use change, landscape simplification, and elevated urbanization, including habitat loss and chemical pollution.[4–6] Terrestrial invertebrates at higher trophic levels may be particularly vulnerable to environmental changes[3,13] and thus disappear at unprecedented rates.[3–7]

Invertebrates represent ~75% of all species described on Earth[14] and are a fundamental part of ecosystems.[15,16] They provide manifold critical ecosystem functions and services, such as pollination,[17] decomposition,[11,18] and natural pest control.[14,19,20] These functions and services are indispensable for many ecosystems upon which humans depend.[14,20] Accordingly, invertebrates have been referred to as "the little things that run the world."[21] As

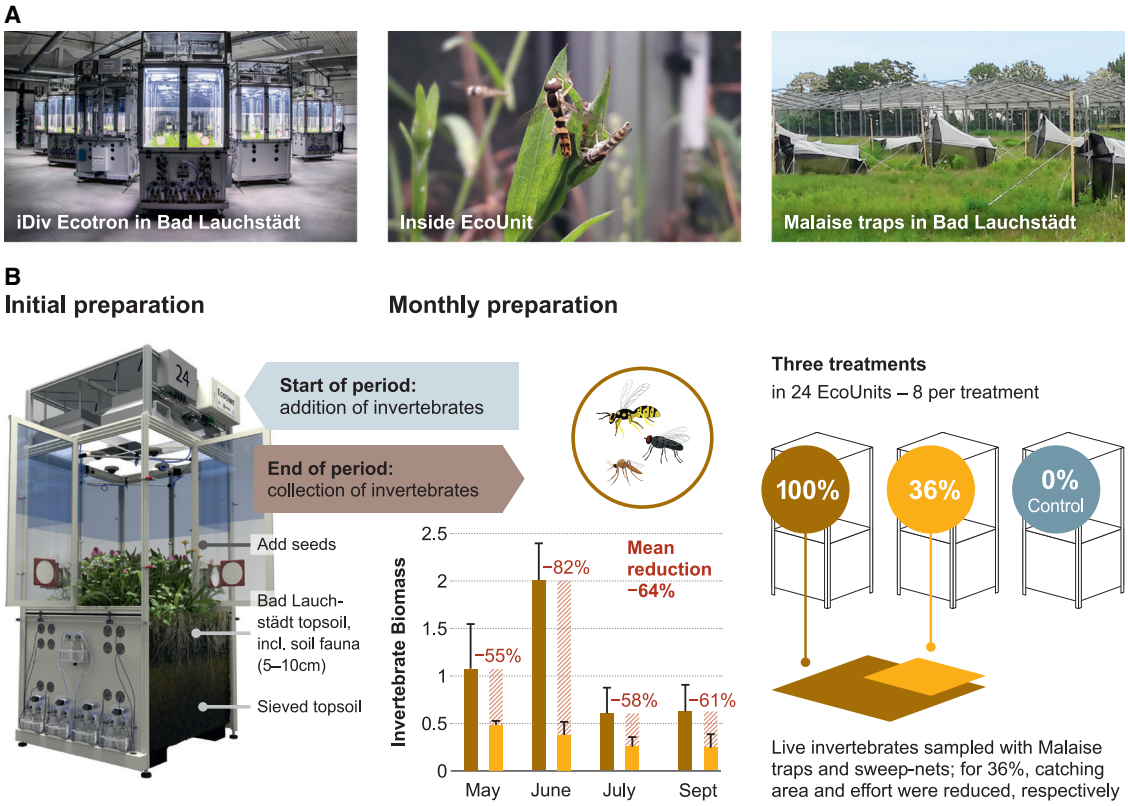

Figure 1. Design of and impressions from the invertebrate decline experiment in the iDiv Ecotron

(A) From left to right: photos of the iDiv Ecotron facility with 24 EcoUnits, photo of some hoverflies inside an exemplary EcoUnit during the experiment, and photo of the setup of Malaise traps at the field site of the research station in Bad Lauchstädt to catch invertebrates for experimental treatments.

(B) Technical sketch of an EcoUnit, i.e., experimental unit,[31] of the iDiv Ecotron with information on the setup of the experiment. Biomass of aboveground invertebrates added to each of the treatments to simulate species turnover across the seasons (see also Tables S1–S6). Briefly, the belowground part was filled with a sieved topsoil (80%) and sand (20%) mixture. To inoculate soil organisms, an additional 20 kg of topsoil from the site where invertebrates were sampled was added to each EcoUnit. In each of the EcoUnits, a standardized plant community was grown from seeds, comprising three grass and nine herb species representative of a tall oatgrass (Arrhenatherion elatioris) meadow. We collected flying invertebrates alive with Malaise traps and sweep-net sampling at the Research Station in Bad Lauchstädt, Germany, from May until September 2018. We simulated the natural phenological turnover of aboveground invertebrate communities by exchanging invertebrate communities three times (June, July, and September; see also Figure S1) and established three treatments: 100% invertebrates, 36% invertebrates (simulating the dramatic invertebrate biomass decline across German grasslands over the last decade, as reported in Seibold et al.[5]), and a 0% invertebrate treatment where we did not add any aboveground invertebrates (shown are means with SEs).

such, multitrophic diversity and interactions among trophic levels of invertebrate communities determine the simultaneous supply of multiple ecosystem functions, i.e., ecosystem multifunctionality.[22,23] So far, however, measuring the potential effect of invertebrate loss has proven difficult because targeted experiments that manipulate the diversity and/or biomass of invertebrate communities under controlled conditions have focused on the consequences of changes in diversity per se, often at a particular trophic level[24,25] with heavy emphasis on primary producers.[24,26–28] However, real-world biodiversity change is often non-random and with non-parallel changes in functional diversity.[12,29] We lack experimental evidence testing the ecosystem consequences of declining invertebrate biomass in naturally complex multi-trophic communities.[11,30]

Here, we report on the first study on multitrophic invertebrate decline effects[4,5] on entire experimental ecosystems, considering their community changes across the season in extensively used hay meadows. We set up grassland ecosystems in 24 independent chambers (called EcoUnits) of the iDiv Ecotron facility (Figure 1A), which was specifically designed to study the effects of multitrophic biodiversity and aboveground-belowground interactions on multiple ecosystem functions.[31] We established three aboveground vegetation-associated invertebrate treatment levels, using a combination of different sampling methods (Malaise traps[4] and sweep-net sampling[5]; Figure 1A): 100% invertebrates, 36% invertebrates (simulating the dramatic invertebrate biomass decline across German grasslands over the last decade, as reported in Seibold et al.[5]), and a 0% invertebrate treatment, where we did not add any aboveground invertebrates (Figure 1B). The decline of aboveground invertebrate biomass by −64% coincided with a −44% and −70% reduction in invertebrate richness and abundance, respectively, which also reflects observations in Seibold et al.[5] This design is in line with space-for-time substitutions to explore the consequences of invertebrate decline.[32] The experiment ran from May until November 2018, and we simulated the natural phenological turnover of aboveground invertebrate communities by exchanging invertebrate communities three times (June, July, and September; Figure 1B; see STAR Methods for details). All plant

and invertebrate species were taken from the same adjacent extensively used hay meadow of the iDiv Ecotron (seeds for plants were bought, but based on knowledge we had from the field site), and the experimental soil was inoculated with topsoil from that same field site, to account for the life-history traits of species and consider their trophic and non-trophic interactions. In addition to detailed assessments of plant abundance and plant phenology[33] and bacterial diversity on leaves and flowers,[34] we assessed 30 different above- and belowground, abiotic and biotic ecosystem variables, representing six crucial ecosystem services[27] provided by multitrophic biodiversity, such as natural pest control (above- and belowground), decomposition (12 assessment campaigns over time), plant diversity, aboveground plant biomass production, and soil-aggregate stability[22,35,36] (Table 1; Figure 2). Over the course of the experiment, we observed that simulated declines in invertebrate biomass were associated with aphid outbreaks. Therefore, in addition to testing for experimental treatment effects, we also evaluated the influence of aphid biomass as a covariate, since aphids represent important pests across many ecosystems,[37] and pest outbreaks are a widespread consequence of biodiversity loss at higher trophic levels[3,19] with significant cascading effects on crop production and other ecosystem services[20] (see STAR Methods for details).

We assessed average ecosystem-service multifunctionality (hereafter ecosystem multifunctionality for brevity),[35,38] defined as the simultaneous supply of multiple ecosystem services[39] (Figure 2A). Further, to assess whether high ecosystem multifunctionality was achieved by multiple functions performing at high (or low) levels, we counted the number of functions performing above a given threshold using the multiple-thresholds approach[38] (Figures 2B and 2C). This was done by quantifying the number of services, with the service value exceeding a given threshold. The thresholds are varied at 1% intervals along a gradient from 5% to 99% of the maximum observed stability of the function.[38] We found that average ecosystem multifunctionality decreases significantly with decreasing biomass of invertebrates (Table 1; Figures 2A–2C), indicating that a loss of aboveground invertebrate biomass[4–7] threatens ecosystem functioning with consequences for human needs.[39] This effect remained significant when comparing only the 100% with the 36% invertebrate treatments (i.e., excluding the 0% treatment; Table 1). The multiple-thresholds analysis largely confirms these results and indicates that effects of invertebrate loss are most pronounced at thresholds >80% (Figures 2A and 2B).

Most of the individual ecosystem services tend to decrease with decreasing invertebrate biomass (Figures 2D–2I), with statistically significant effects on aboveground pest control (Figure 2E) and decomposition (Figure 2F). For information on explained variance, see R^2 values; the percentage of explained variance varied across ecosystem functions (Figure 2). When comparing only 100% with 36% invertebrate biomass treatments, belowground pest control also decreases marginally non-significantly (Table 1). These results provide experimental evidence of the significant role of complex aboveground invertebrate communities in natural pest control[19,20] and decomposition,[11,18] which has been suggested by observational studies and exclusion experiments, respectively. By contrast, aboveground plant biomass increases in the reduced invertebrate biomass treatments (Figure 2G), suggesting that more aboveground plant biomass is consumed and energy is channeled up from plants to higher trophic levels in intact invertebrate communities.[36,40] Again, these findings are robust across sensitivity analyses testing effects of aphids as well as excluding the 0% invertebrate treatment (Table 1). By contrast, root biomass tends to decrease with decreasing invertebrate biomass, indicating that invertebrate decline may shift above- versus belowground biomass allocation and decrease plant inputs into the soil.[41] Most of the additional abiotic and biotic ecosystem variables are not significantly affected by invertebrate biomass decrease, with the exception of the density of Collembola and aphids, as well as plant tissue nitrogen and potassium concentrations (all significant or marginally non-significant increases with decreasing invertebrate biomass). Moreover, plant tissue carbon concentration and mean nitrogen concentration in throughfall significantly decrease with decreasing invertebrate biomass (Table 1), indicating altered quality of resource inputs into the soil fueling soil biological activity.[42]

Next, we sought to assess whether the ecosystem consequences of invertebrate decline responded in concert. Therefore, we used an integrated system-level perspective called ecosystem coupling, which measures the degree of correlation among all above- and belowground animal, plant, and microbial groups, and also correlations of these taxa with variables linked to the biogeochemical cycling of elements.[43,44] In addition to total ecosystem coupling, we separately assessed the coupling of the biotic (biotic coupling) and abiotic variables (biogeochemical coupling) (STAR Methods; Figures 3A–3C). Similarly, we assessed the overall strength of ecosystem correlations (ecosystem network strength) as well as the correlation strength of biotic (biotic network strength) and abiotic variables (biogeochemical network strength) based on the proportion of significant links in the network (Figures 3D–3F). We found that the observed total ecosystem, biotic, and biogeochemical coupling in the 100% invertebrate treatment are all significantly higher than in a null model, generated after 100 random permutations of the same dataset. Ecosystem and biotic coupling are also higher than in the random model in the 36% invertebrate treatment, but this is not the case for biogeochemical networks, which become consistently decoupled (i.e., not different than by chance) in response to invertebrate biomass loss. Results based on the proportion of significant links in the network fully support these findings. Taken together, these results indicate that decreasing aboveground invertebrate biomass reduces the coupling of biotic and biogeochemical properties of ecosystems (Figures 3G–3I), which may threaten species diversity, as well as animal, plant, and microbial nutrition.[45,46] Given that a complexity of ecological networks is an inherent characteristic of healthy ecosystems,[47–49] our results strongly suggest that with a decline of aboveground invertebrates, belowground biotic networks may also become dismantled.[44]

The results of our Ecotron experiment provide empirical evidence for causal relationships between previously observed multitrophic invertebrate decline[4–7] and significant changes in the functioning and the supply of multiple ecosystem services in grasslands.[22] Notably, we observed close coupling between many above- and belowground ecosystem variables in the presence of an intact invertebrate community (Figures 3G–3I), and the decoupling of ecological networks with aboveground invertebrate loss may have caused significant reductions in the key belowground process of decomposition (Figure 2F). Given that

Table 1. Effects of invertebrate loss on ecosystem services as well as biotic and abiotic ecosystem variables

	Model 1			Model 2						Model 3		
	Invertebrate treatment			Aphid biomass (log)			Invertebrate treatment (residuals)			Invertebrate treatment (100% versus 36%)		
Dependent variables	$F_{1,22}$	p		$F_{1,21}$	p		$F_{1,21}$	p		$F_{1,14}$	p	
Multifunctionality	5.43[a]	0.029[a]	↓	N/A	N/A		N/A	N/A		5.99[a]	0.028[a]	↓
Ecosystem services												
Plant diversity	1.69	0.207		0.22	0.643		1.39	0.251		0.26	0.616	
Plant biomass	9.73[a]	0.005[a]	↑	1.01	0.325		8.37[a]	0.009[a]	↑	5.42[a]	0.035[a]	↑
Aboveground pest control	4.33[a]	0.049[a]	↓	N/A	N/A		N/A	N/A		5.26[a]	0.038[a]	↓
Belowground pest control	1.32	0.263		0.07	0.790		1.23	0.280		3.69[b]	0.075[b]	↓
Decomposition	10.29[a]	0.004[a]	↓	4.18[b]	0.054[b]	↑	6.64[a]	0.018[a]	↓	4.49[b]	0.053[b]	↓
Soil aggregate stability	2.10	0.161		0.02	0.891		2.67	0.117		0.15	0.705	
Biotic variables												
Root biomass	3.24[b]	0.086[b]	↓	8.06[a]	0.010[a]	↑	0.78	0.388		1.51	0.240	
Active microbial biomass	0.55	0.465		0.49	0.491		0.24	0.631		0.86	0.370	
Biomass bacteria	0.73	0.402		0.48	0.494		0.37	0.549		1.60	0.227	
Biomass fungi (log)	0.42	0.523		0.10	0.758		0.70	0.411		0.54	0.475	
Plant-feeding nematodes (log)	1.32	0.263		0.07	0.790		1.23	0.280		3.69[b]	0.075[b]	↑
Bacteria-feeding nematodes (log)	0.18	0.674		0.04	0.837		0.30	0.589		0.93	0.351	
Fungi-feeding nematodes (log)	0.22	0.642		0.61	0.444		0.75	0.398		0.72	0.410	
Predaceous nematodes	1.69	0.206		1.06	0.315		0.89	0.355		0.80	0.386	
Collembola (log)	3.13[b]	0.091[b]	↑	12.51[a]	0.002[a]	↓	0.47	0.502		11.58[a]	0.004[a]	↑
Mites (log)	1.00	0.329		0.21	0.650		0.75	0.398		0.50	0.492	
Aphid biomass (log)	4.33[a]	0.049[a]	↑	N/A	N/A		N/A	N/A		5.26[a]	0.038[a]	↑
Abiotic variables												
Soil water content (%)	0.11	0.746		0.01	0.941		0.10	0.754		0.40	0.536	
Total SOC (%; log)	0.36	0.556		3.50[b]	0.075[b]	↓	0.02	0.886		1.61	0.226	
Total soil nitrogen (%; log)	0.94	0.344		0.38	0.547		0.58	0.453		1.57	0.231	
Soil nitrate (SW; %; log)	0.00	0.960		0.01	0.939		0.00	0.984		1.35	0.264	
Soil ammonium (SW; %; log)	0.82	0.375		0.02	0.896		1.07	0.313		0.13	0.723	
Soil phosphate (SW; %; log)	0.27	0.608		0.89	0.356		0.99	0.331		0.01	0.930	
Soil sulfate (SW; %; log)	0.20	0.658		0.44	0.512		0.04	0.853		0.31	0.585	
Mean throughfall N (%)	1.31	0.265		1.54	0.228		0.48	0.496		8.46[a]	0.011[a]	↓
Total throughfall N content	1.27	0.271		4.47[a]	0.047[a]	↑	0.13	0.724		10.05[a]	0.007[a]	↓
Plant carbon (%)	4.40[a]	0.048[a]	↓	0.03	0.873		4.84[a]	0.039[a]	↓	2.07	0.172	
Plant nitrogen (%)	9.35[a]	0.006[a]	↑	8.60[a]	0.008[a]	↓	4.87[a]	0.039[a]	↑	5.20[a]	0.039[a]	↑
Plant phosphorous (mg/kg)	0.09	0.768		0.71	0.407		0.00	0.960		0.27	0.615	
Plant potassium (mg/kg)	3.15[b]	0.090[b]	↑	0.12	0.738		3.07[b]	0.094[b]	↑	1.00	0.335	

SOC, soil organic carbon; SW, nutrient concentrations in soil water; N, nitrogen. Models 1 and 2 show results of linear models (type I sum of squares) on the effect of invertebrate treatment (100%, 36%, and 0 invertebrates; linear) on ecosystem services as well as biotic and abiotic ecosystem variables. In model 2, we included the (log10) aphid biomass (as a covariate) before the invertebrate treatment in a sequential analysis (residuals), to account for the potential influence of aphids. In model 3, we used one-way ANOVAs to examine the effect of invertebrate treatment (100% versus 36% invertebrates, i.e., excluding the 0% [control] treatment) on ecosystem variables.

[a]Significant ($p < 0.05$) relationship; direction of the relationship is indicated by arrow

[b]Marginally significant ($p < 0.1$) relationship; direction of the relationship is indicated by arrow

our manipulation of invertebrate communities was mostly based on vegetation-associated insects,[4,5] and only few decomposers were added (see STAR Methods and supplemental information for details), we speculate that changes in belowground decomposition were likely mediated via changes in plant-community composition and root inputs into the soil,[42] such as indicated by a marginally non-significant decrease of standing root biomass with invertebrate decline. Indeed, we found some support for this assumption, as belowground decomposition correlated significantly positively with grass biomass at the first sampling

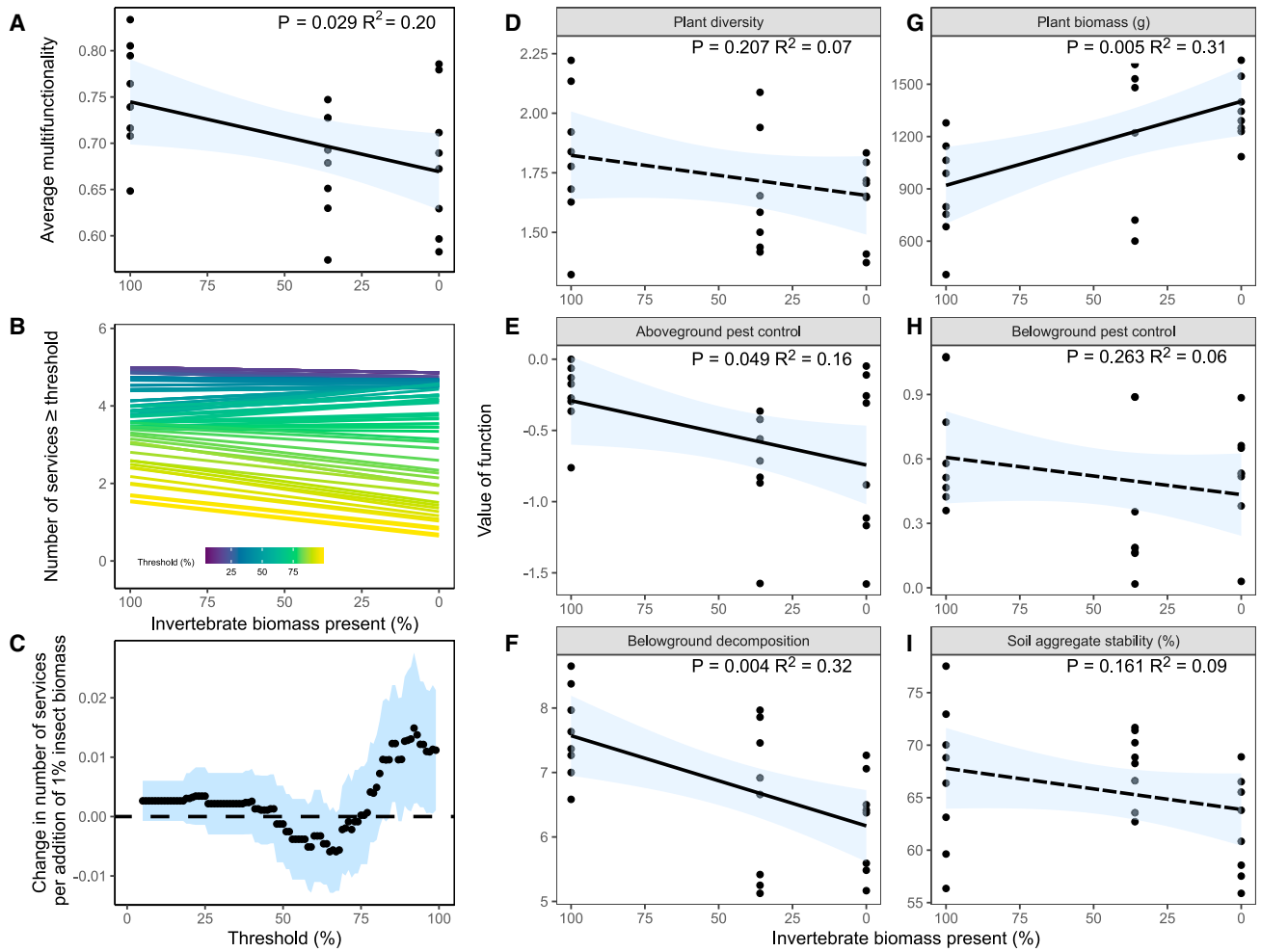

Figure 2. Effects of invertebrate loss on ecosystem services and multifunctionality

(A and B) Invertebrate-treatment effects on (A) average ecosystem service multifunctionality and (B) the number of ecosystem services above a threshold for multiple different threshold values. Colors indicate different thresholds as shown in the legend with cooler colors denoting lower thresholds and warmer colors denoting higher thresholds.

(C) Corresponding relationship between threshold value and slope of the relationship between the invertebrate treatment and the number of ecosystem services reaching a threshold.

(D–I) Invertebrate-treatment effects on individual ecosystem services, including plant diversity (Shannon diversity index), plant aboveground biomass (g per EcoUnit), aboveground pest control (aphid biomass was log10 transformed to improve distribution of variances, then multiplied by −1), belowground pest control (density of plant-feeding nematodes was log10 transformed to improve distribution of variances, then multiplied by −1), average belowground decomposition (number of decomposed baits per strip, based on 12 2-week bait-lamina measurements), and soil-aggregate stability (water-stable aggregates > 250 μm [%]). Points are jittered fitted values, and shading indicates 95% confidence intervals.

campaign (R^2 = 0.23, p = 0.018), while there were no significant relationships with herb and legume biomass, respectively (R^2 < 0.06, p > 0.1). This observation highlights the significance of above-belowground interactions for the functioning of terrestrial ecosystems[42,50] and suggests that aboveground invertebrate decline can have far-reaching ecosystem consequences.[51] The observed significant changes in belowground decomposition are likely to alter nutrient and carbon dynamics, which may have cascading long-term effects on plant-nutrient supply and productivity, as well as soil carbon storage,[18,42] and thus soil health.[52] Long-term studies are urgently needed to fully appreciate the ecosystem consequences of invertebrate decline.

We chose to follow the extreme examples of local invertebrate biomass loss of ~75% across roughly three decades of monitoring in 63 nature protection areas in Germany[4] and the ~67% invertebrate biomass loss during the last decade recorded in 150 grasslands across Germany.[5] The realized ~64% loss of invertebrate biomass simulated in our experiment is closer to the observations of Seibold et al.[5] and may reflect more recent changes in invertebrate communities across a larger geographical region. However, there may be high variability in the temporal trends of invertebrate biomass (less significant declines and even some increases) across ecosystem types and locations.[5,6] Further, the species composition in our treatments may not be representative of changes in invertebrate communities that are happening across

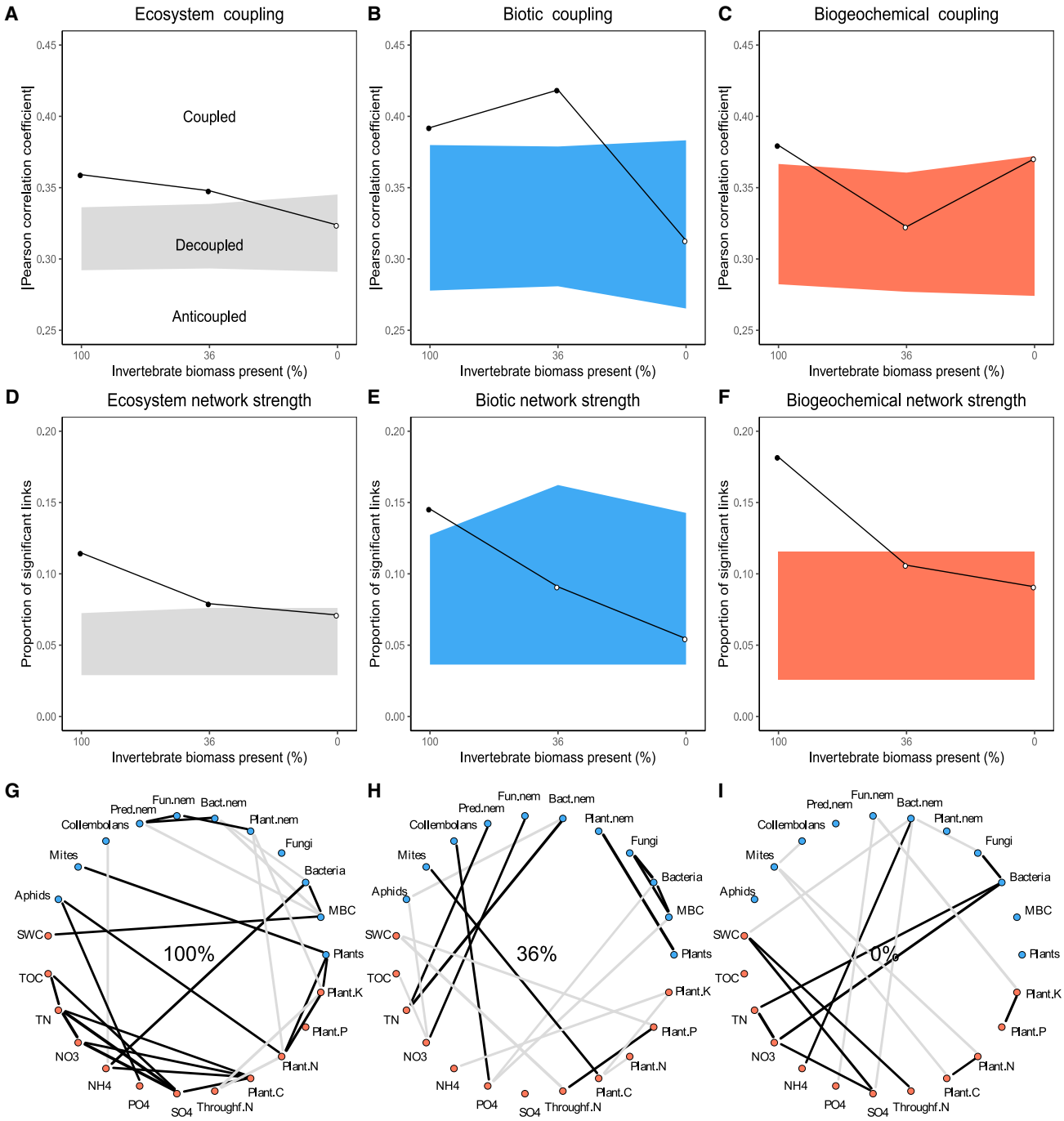

Figure 3. Effects of invertebrate loss on ecosystem coupling

(A–C) Network coupling as compared to a 95% envelope representing the null model (see main text for details; filled dots mean that the value observed is greater than the null model at $p < 0.05$).

(D–F) Ecosystem network strength as compared to a 95% envelope representing the null model.

(G–I) Complete networks considering the biotic and biogeochemical variables (positive and negative links are coded as dark and light gray, respectively). For full list and names of ecosystem variables, see Table 1.

time.[32] Future studies may want to reconstruct invertebrate communities based on community-level data from previous monitoring events, if the respective species can still be found. Such work could also try to differentiate the roles of invertebrate abundance, biomass, and richness driving multiple ecosystem functions and services, e.g., in *in situ* exclosures, as well as simultaneous changes in plant and soil communities. However, there may be substantial challenges in treating a high number of invertebrate

species for repeated additions at the right development stage and in a way that maintains their health. Further, the combination of individuals from different locations may miss important local adaptations based on genetic and epigenetic differences. Additionally, aphid populations colonized the EcoUnits after establishment, potentially due to cross-contamination, but they represented an opportunity to test natural pest control in our experimental setup.[19,20] These aphids occurred in all EcoUnits, so based on experience from previous studies (e.g., Eisenhauer and Scheu and Thakur et al.[53,54]), we applied pesticides that are typically used to control aphids in intensively managed agricultural systems after the first plant biomass harvest to manage this population (see STAR Methods for details), monitored aphid populations, and considered those in sensitivity analyses that did not change any of our main findings and conclusions (Table 1). This suggests that our results on the consequences of invertebrate decline are robust to substantial environmental fluctuations.

Conclusions

Here, we report experimental evidence that the loss of invertebrates threatens the integrity of grasslands by decoupling communities and ecosystem processes and deteriorating ecosystem services. These findings imply that the current invertebrate decline will lead to the reduction of aboveground ecosystem services like natural pest control.[20] The resulting decoupling of above-belowground processes[44] may also reduce belowground decomposition processes. The decrease in ecosystem-service multifunctionality may be a significant consequence of the decoupling of ecological communities from their biotic and abiotic environment with invertebrate loss.[44,55] Because these connections can result from both direct and indirect, positive or negative interactions,[56] future research should address how these interactions change with invertebrate loss. We note that we are reporting only short-term ecosystem effects, and effects of biodiversity loss might even strengthen over time in experiments.[57,58] In our study, we detected results of multiple ecosystem functions in one growing season under one set of environmental conditions, including ecosystem functions that may only change after multiple years of treatment, such as plant diversity and soil aggregate stability. A global synthesis showed that although species may appear functionally redundant when one function is considered under one set of environmental conditions, many species are needed to maintain multiple functions at multiple times and places in a changing world.[59] Therefore, we speculate that the 10%–15% decrease in ecosystem functioning reported in our experiment likely reflects a very conservative estimate of potential effects of invertebrate decline on ecosystem stability, as a longer experiment under a broader array of conditions would likely show more influences of species declines,[57–59] calling for future experiments at larger spatial and temporal scales. However, ecosystem effects of invertebrate decline may also depend on other factors, such as network structure, community composition, plant stoichiometry, fertility, and climate. While the findings of our study are alarming, evidence of increasing freshwater insect populations indicates that ecosystem recovery after changes in legislation (e.g., Clean Water Act) may be successful in favoring invertebrate diversity[6] and thus turning the tide toward safeguarding multitrophic invertebrate communities and thereby ecosystem multifunctionality.[22,24] However, invertebrate species may be disappearing at unprecedented rates[1] and partly unidentified,[11] necessitating immediate protection measures. Early in the UN Decade on Restoration, the study calls for tailored conservation actions to maintain and restore invertebrate biodiversity,[14] as well as the many ecosystem functions driven by this essential part of global biodiversity.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2023.09.012.

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The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Insecticide (Karate Zeon; Raiffeisen)	N/A	N/A
MacFadyen extractor	N/A	N/A
Formaldehyde solution	N/A	N/A
Ethanol	N/A	N/A
Deposited data		
Raw data and code used to perform analyses of this paper	This paper	iDiv data repository (DOI TBD)
Software and algorithms		
R software (version 4.1.1)	The R Foundation / R Development Core Team	https://www.r-project.org
R package multifunc (version 0.9.3)	Byrnes et al.[38]	https://cran.r-project.org/web/packages/multifunc/index.html
Other		
iDiv Ecotron (EcoUnits: 1.55 m × 1.55 m × 3.20 m)	Schmidt et al.[31]	N/A
Malaise traps	Hallmann et al.[4]	N/A
Insect rearing cages (30 × 30 × 30 cm, BugDorm)	N/A	N/A
Bait lamina strips (1 mm × 6 mm x 120 mm)	Terra Protecta GmbH, Berlin, Germany	N/A
Litter traps made of PVC pipes (2 cm in height, 14 cm diameter; bottom covered with 325 μm mesh size)	N/A	N/A
Suction cup (length 5 cm, 2 cm in diameter)	UGT Müncheberg, Germany	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and code should be directed to and will be fulfilled by the lead contact, Nico Eisenhauer (nico.eisenhauer@idiv.de).

Materials availability

This study did not generate new unique reagents.

Data and code availability

All data and code are available through the iDiv data repository: https://doi.org/10.25829/idiv.3557-dcq3v4.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Plants

In each of the EcoUnits, a standardized plant community was grown from seeds, comprising three grass species (*Arrhenatherum elatius* (L.), P. Beauv. ex J. Presl & C. Presl, *Phleum pratense* L., and *Dactylis glomerata* L.), eight predominantly insect-pollinated herbs (*Centaurea jacea* L. s. l., *Lotus corniculatus* L., *Medicago lupulina* L., *Scorzoneroides autumnalis* (L.) Moench, *Trifolium pratense*

L., *Achillea millefolium* L., *Knautia arvensis* (L.) Coult., and *Bellis perennis* L.), and one primarily anemochorous species (*Plantago lanceolata* L.) representative for a tall oatgrass (*Arrhenatherion elatioris*) meadow. The seed material was obtained from Rieger Hofmann GmbH, Blaufelden-Raboldshausen, Germany. Details on plant species selection (typical grassland species, high germinability, flower development in the first year) and planting procedure can be found in Ulrich et al.[33]

Animals

We collected flying invertebrates alive with Malaise traps (similar to those used by Hallmann et al.[4]; mostly flying insects) and sweep-net sampling (in line with Seibold et al.[5]; including also non-flying invertebrates) at the Research Station in Bad Lauchstädt, Germany, from May until September 2018 (Figure 1C), to include a wide range of functionally important invertebrates.[11] Invertebrates were collected in two different quantities by modifying the catching area of the traps (100% and 36%) (Figure 1C; Tables S1 and S2), the number of catching days (24 h with the 36% traps, 2 × 24h with the 100% traps), as well as by the number of net sweeps (each time 2x and 8x, respectively). For the 0% invertebrate biomass treatment, no invertebrates were collected. To collect invertebrates alive, sampling bottles were replaced by insect rearing cages (30 × 30 × 30 cm, BugDorm) suited with wadded-up moistened paper towels to provide humidity and shelter. Invertebrates from sweep net catches were transferred to EcoUnits at the same time as invertebrates from Malaise traps. The gender of collected animals was not assessed.

METHOD DETAILS

Design and setup of the experiment

The experiment was carried out at the iDiv Ecotron[31] at the research station of the Helmholtz-Centre for Environmental Research (UFZ) in Bad Lauchstädt, Germany (51° 22′ 60N, 11° 50′ 60E, 118 m a.s.l.). It is located in the Central German dry area (Querfurter Platte) with a mean annual temperature of 8.9°C (1896-2013) as well as a mean annual precipitation of 489 mm (1896-2013) (see Ulrich et al.[33] and references therein). We established grassland plant communities with 12 different plant species in the 24 independent, identical experimental units (EcoUnits; 1.55 m × 1.55 m × 3.20 m (L × W × H)) of the iDiv Ecotron (Figures 1A and 1B) with controlled environmental conditions such as light, air, and soil temperature, and irrigation. For the experiment, the belowground part was filled with a sieved (15 mm mesh size) topsoil (80%) and sand (20%) mixture (provided by LAV Technische Dienste GmbH & Co.KG, Erdwerk Kulkwitz). To inoculate soil organisms, an additional 20 kg of topsoil from the site where invertebrates were sampled (research station described above) was added to each EcoUnit. With the soil inoculum, we introduced a common and naturally diverse decomposer community (as also reflected by our data on Collembola, mites, bacteria, fungi, and nematodes; see below). This standardized community was not part of the aboveground invertebrate treatment, but served as a response variable as affected by aboveground invertebrate decline.

Environmental conditions were standardized: day:night cycle was set to 12:8 h; 6 L of deionized water were irrigated per day per EcoUnit; air temperature was on average 24°C during daytime within the vegetation layer and 19°C during nighttime. The soil temperature at a depth of 9 cm below the soil surface was nearly constant at 18°C.

We collected invertebrates at ~ monthly intervals (May 23/24 [for details, see Table S3], June 19/20 [for details, see Table S4], July 24/25 [for details, see Table S5], September 11/12 [for details, see Table S6]; see Figure 1 for total invertebrate biomass data and supplemental information (Tables S1 and S2) for total and taxon-specific densities as well as invertebrate richness; biomass data reported in Figure 1 are based on three additional sampling campaigns per invertebrate treatment that we realized each time, because the biomass and community composition of the invertebrates added to the EcoUnits could not be determined, while maintaining the health of the animals) and transferred them to EcoUnits within one day after collection (for time of all experimental events, see Figure S1 in supplemental information). We chose to establish the 36% biomass treatment through a decreased sampling area (Malaise traps) and effort (sweep net catches), because it allowed us to capture the most dominant species (which are also likely to resist any environmental stressors) while carefully handling the captured invertebrates. We intended to collect these invertebrates and release them into the experimental units with minimal delay and without having to handle each specimen individually, which almost certainly would have enhanced unintended mortality. Also, given that it is not straightforward to predict which species will decrease or disappear with environmental change, we think that the random approach based on sampling intensity is a robust manipulation resulting in a more representative, and less arbitrary community (in fact, this random approach is also the rationale behind the design of most biodiversity experiments). While we intended to simulate the dramatic invertebrate biomass decline across German grasslands over the last decade of between 60 and 70%,[5,7] the exact percentage reduction was impossible to predict prior to the experiment. However, we ran several test trials on the number of catching days and sweeps to optimize our approach. We note that the reduction in invertebrate biomass ranged between -54% and -78% across the four sampling rounds. While these differences might have caused changes in the effect size of the invertebrate treatment over time, we are not able to test for these, because of few times-series datasets and potential lag effects. Before adding new invertebrate communities, the previous communities were removed with a suction sampler (i.e., modified vacuum cleaner; Bosch Industriestaubsauger GAS 25). Collected invertebrates were transferred into 70% Ethanol, before they were weighed, counted, and identified to broad taxonomic groups (June 14/15, July 19/20, August 23/24, November 8/9).[34] This was done to simulate natural turnover of invertebrate communities across the season. Details on the added invertebrates are provided in Supplementary Information. We maintained standardized temperature and precipitation regimes that supported invertebrate communities across the experimental duration (see above), while future work might also simulate climatic fluctuations across seasons.

Even though the establishment of the invertebrate treatments was successful, we observed aphid infestation (Aphidina) across all EcoUnits, which increased in severity from week 5 to week 18.[34] As aphids may represent a confounding driver of the treatment and

to support the interpretation of our results, we assessed patterns in aphid biomass between the treatments (see Table 1 for sensitivity analyses). We found that aphids had high biomass in the 36%- and 0%-treatments (Table 1). After the first plant biomass harvest (27 to 31 August 2018, see below), we applied a standard insecticide (Karate Zeon; Raiffeisen) that is typically used for the control of biting and sucking insects for a wide range of plant communities. Notably, we treated all EcoUnits (including the 100%, 36%, and 0% treatments) in the same way. As typical for temperate grasslands in the region, we performed two plant biomass harvests. Prior to the first harvest, we removed the invertebrate community, according to our approach to simulate community turnover across the growing season. After the first harvest, we then applied the insecticide according to the manufacturer's instructions (Karate Zeon; Raiffeisen) across all EcoUnits. This approach was taken, given that the invertebrate communities had been removed, and any potential aphids residing close to the soil surface could be treated with a low amount of an insecticide that is commonly used in agricultural landscapes in the region. After 14 days, the insecticide is typically no longer detectable (https://www.syngenta.de/sites/g/files/zhg146/f/karate_zeon_sicherheitsdatenblatt.pdf?token=1614877131), and we introduced the new invertebrate communities to the EcoUnits (September 11/12).

Biotic and abiotic ecosystem variables

Plant biomass and diversity

Simulating common management in Central European mesophilous grassland, the meadows were mown twice throughout our experiment. We determined plant aboveground biomass, focal plant species richness, and Shannon diversity of the plant community at the EcoUnit level just before mowing. The first harvest took place from 27 to 31 August 2018. One-quarter of the harvested biomass was sorted into the twelve common grassland species sown, dead organic matter, and rest (unidentifiable plant parts and non-sown species). During the experiment, we continuously weeded the plant communities, to keep the proportion of non-target plant species low. Given that the EcoUnits were visited regularly for the many sampling campaigns (see Figure S1 in supplemental information), the number and biomass of non-target plants was low and was not recorded. All aboveground biomass per EcoUnit was dried at 40°C in a drying oven for 48 h and then weighed. The second and final harvest took place from 12 to 15 November 2018, following the same protocol as for the first harvest.

Plant tissue nutrient concentrations

For the analysis of plant tissue nutrient concentrations of focal plant species, randomly-selected leaves were pooled per species and EcoUnit. Leaf carbon concentration (C%) and leaf nitrogen concentration (N%) were measured using 0.5 g of dried (at least 72 h at 60°C), milled (mixer mill NN 400, Retsch) leaf tissue weighed into tin capsules. The elementary analyses were carried out by SYNLAB Analytics & Services Germany GmbH. Phosphorous and potassium were analyzed using DIN EN ISO 17294-2:2017-01 in mg/kg. The analysis for nitrogen and carbon followed DUMAS, DIN EN ISO 16634:2016-11 and was measured in percentage terms.

Standing root biomass

At the end of the experiment, three cores with a diameter of 3.3 cm and a depth of 60 cm were taken in each EcoUnit and pooled together for measuring standing root biomass. Roots were washed, oven dried at 70°C for 48 h, and weighed.

Soil microbial properties

The soil for microbial, nematode, aggregate stability, soil nutrient and pH analyses was obtained by sampling 12 soil cores per EcoUnit (2 cm diameter and 10 cm depth) in November 2018. The soil samples were pooled per EcoUnit in plastic bags, carefully but thoroughly homogenized, and stored at 4°C until further processing. Before measurement of soil microbial parameters, soil sub-samples were sieved (2 mm) to remove roots.[35] Soil microbial biomass C and respiration of approximately 5 g soil (fresh weight) was measured using an O_2-microcompensation apparatus.[60] The microbial respiratory response was measured at hourly intervals for 24 h at 20°C. Soil respiration ($\mu l\ O_2\ h^{-1}\ g^{-1}$ soil dry weight) was determined without addition of substrate and measured as the mean of the O_2 consumption rates of hours 14 to 24 after the start of the measurements. Substrate-induced respiration was calculated from the respiratory response to D-glucose for 10 h at 20°C.[35] Glucose was added according to preliminary studies to saturate the catabolic enzymes of microorganisms (4 mg g^{-1} dry weight dissolved in 400 μL deionized water). The mean of the lowest three readings within the first 10 h (between the initial peak caused by disturbing the soil and the peak caused by microbial growth) was taken as maximum initial respiratory response (MIRR; $\mu l\ O_2\ g^{-1}$ soil dry weight h^{-1}) and microbial biomass ($\mu g\ C\ g^{-1}$ soil dry weight) was calculated as 38 × MIRR.[61] To assess microbial community structure, phospholipid fatty acid (PLFA) analysis was performed, according to the protocol from Frostegård et al.[62] PLFAs are commonly used to assess soil microbial communities based on the biomass of main microbial groups, such as gram-positive bacteria, gram-negative bacteria, and fungi. Fatty acid biomarkers were converted to biomasses (ng g^{-1} dry soil), and the total biomass of bacteria as well as total biomass of fungi were determined.

Soil carbon and nitrogen analyses

Soil sampled were dried at 60°C for 72 h, ground with a ball mill, subsequently dried for another 24 h, and transferred into tin capsules for C and N analyses.[63] Analyses were performed using an elemental analyzer (Vario EL II, Elementar Analysensysteme GmbH, Hanau, Germany). C and N concentrations are given as relative mass proportion of the element (in %) per sample mass.

Soil aggregate stability

To determine the resistance of soil aggregates against water as a disintegrating force, we applied an approach modified from Kemper and Rosenau.[64] The resulting index represents the percentage of water-stable aggregates with a diameter smaller than 4 mm. Dry soil (4.0 g, measured in duplicates) was placed onto small sieves with a mesh size of 250 μm, capillarily re-wetted with deionized water prior, and then placed in a sieving machine (Agrisearch Equipment, Eijkelkamp, Giesbeek, Netherlands), where the samples were agitated for 3 min. The re-wetting and agitation of the tested soil aggregates causes the compression of entrapped air inside of them resulting in a process called slaking, which is a function of re-wetting intensity, volume of entrapped air, and aggregate

shear-strength.[65] This process leads to a separation into water-stable and water-unstable fraction with a size >250 μm. Additionally, debris (i.e., coarse matter) had to be separated from the water-stable fraction to correctly determine the water-stable aggregates (WSA) fraction of the sample: %WSA = (water-stable fraction - coarse matter) / (4.0 g - coarse matter).

Belowground decomposition

Decomposition was assessed based on soil invertebrate feeding activity using the bait lamina test (Terra Protecta GmbH, Berlin, Germany) as a commonly-used rapid ecosystem function assessment method.[66,67] The bait strips are made of PVC (1 mm × 6 mm x 120 mm) and have 16 holes (1.5 mm diameter). Original sticks were ordered from Terra Protecta and filled with an artificial organic bait substrate, which was prepared according to the recommendations of Terra Protecta, consisting of 70% cellulose powder, 27% wheat bran, and 3% activated carbon. The bait substrate is primarily consumed by mites, collembolans, millipedes, and earthworms, whereas microbial activity plays a minor role in bait substrate loss (see Siebert et al.[68] and references therein). The bait lamina strips were inserted vertically into the soil with the uppermost hole just beneath the soil surface. A steel knife was used to make a slot in the soil into which the strips were carefully inserted. Five strips placed at a distance of >30 cm from one another were used per plot to account for potential spatial heterogeneity. After two weeks of exposure, the bait lamina strips were removed from the soil, directly evaluated at the iDiv Ecotron, and replaced by a new bait strip. Each hole was carefully inspected and rated as 0 (no invertebrate feeding activity), 0.5 (intermediate feeding activity) or 1 (high invertebrate feeding activity). Soil invertebrate feeding activity can therefore range from 0 (no feeding activity) to 16 (maximum feeding activity) per strip. Mean bait consumption of the five strips was calculated per plot prior to statistical analysis. Over the duration of the experiment, we conducted a total of 12 bait lamina assessments (summing up to a total of 1440 bait lamina strips), providing a comprehensive picture of belowground decomposition. For this study, we used mean decomposition activity across all time points per EcoUnit for statistical analyses.

Soil nematodes

Nematode extraction was conducted with a modified Baermann method.[69] Approximately 25 g of fresh soil per plot were transferred to plastic vessels with a milk filter and a fine gauze (250 μm) at the bottom and placed in water-filled funnels. More water was added to saturate the soil samples and to ensure a connected water column throughout the sample and the funnel. Hence, nematodes migrated from the soil through the milk filter and the gauze into the water column and gravitationally settled at the bottom of a closed tube connected to the funnel. After 72 h at 20°C, the nematodes were transferred to a 4% formaldehyde solution. All nematodes per sample were counted at 100x magnification using a Leica DMI 4000B light microscope. Identification was conducted at 400x magnification. For identification, sediment material from the bottom of each sample vial was extracted with a 2 mL plastic pipette and examined in temporary mounted microscope slides. At least 100 well-preserved specimens (if available in the sample) were randomly selected and identified to genus (adults and most of the juveniles) or family level (juveniles). Nematode taxa were then arranged into trophic groups (bacteria-, fungal- and plant-feeders, omnivores and predators).[70]

Soil microarthropods

For soil mesofauna extraction, four soil cores (with 5 cm diameter and 10 cm depth) were taken from each EcoUnit in November 2018. The soil cores were separated into two intact partial cores, each 5 cm of height, and the mesofauna was extracted in a MacFadyen extractor[71] by heating the samples from 25°C to 50°C for seven days. After seven days, the extracted animals were transferred to ethanol (70%) and stored. The mesofauna was counted with a microscope and assigned to taxonomic groups, according to Crotty and Shepherd[72] as well as Schäfer and Brohmer.[73] The animals were mainly identified at the level of orders and families, while we only used data on total Collembola and oribatid mite densities for this study.

Throughfall nitrogen content

To assess total nitrogen content and concentration (%) of invertebrate and plant throughfall, we collected throughfall samples on a weekly basis using litter traps made of PVC pipes (2 cm in height, 14 cm diameter; bottom covered with 325 μm mesh size; until the first plant biomass harvest). All collected throughfall samples were sorted into invertebrate frass, carcasses or plant leaves, and pooled by EcoUnit. Then, all samples were dried at 60°C for 48 h, weighted, milled (using Retsch MM 400, Retsch GmbH, Haan Germany), and again dried at 60°C for another 24 h. Finally, 1 mg per sample (leaf, carcass, and frass throughfall) was weighed into tin capsules and used for the nitrogen measurements using an Isotope Ratio Mass Spectrometer (Thermofisher Scientific, Flash 2000, Conflo IV, delta V advantage, Bremen Germany). In case the total sample weight was lower than 1 mg, the entire sample was measured into the tin capsule and used for the nitrogen measurement. However, as the insecticide application in September contaminated throughfall samples with trace elements containing sulfur, which distorted N concentration measurements, we excluded all data on throughfall N concentration from samples collected after insecticide application from further analyses. To quantify throughfall N concentration of both insect and plant throughfall, we calculated the mean nitrogen concentration (%) over all throughfall samples per EcoUnit across all time points. Moreover, we calculated the mean throughfall nitrogen concentration relative to the total throughfall biomass per EcoUnit.

Soil water sampling and chemical analyses

In all EcoUnits, one suction cup (length 5 cm, 2 cm in diameter; from UGT Müncheberg, Germany) was installed. Soil water was sampled from June until October 2018 in fortnightly periods. The sampling bottles were evacuated to a negative pressure of -30 kPa, so that the suction pressure was approximately 5 kPa above the actual soil water tension. Thus, only the soil leachate was cumulatively collected. Soil water was analyzed to obtain concentration of dissolved organic carbon (highTOC, sum parameter analyzer, Elementar Analysensysteme GmbH, Hanau, Germany), total bound nitrogen (TN-100, a1 enviroscienes Düsseldorf, Germany), as well as of inorganic nitrogen NH^{4+} (ICS-5000, Thermo Fisher Scientific GmbH, Dreieich) and NO^{3-} (Dionex DX-500, Thermo Fisher Scientific GmbH, Dreieich). All samples were stored at 4°C until measurements within two weeks after sampling.

QUANTIFICATION AND STATISTICAL ANALYSIS

Ecosystem services

Based on the 30 ecosystem functions measured (Table 1), we defined six ecosystem services: plant diversity, aboveground plant biomass, aboveground pest control, belowground pest control, decomposition, soil aggregate stability. We note that we are here studying the immediate ecosystem consequences of above- and belowground ecosystem services to invertebrate decline. Such short-term declines (e.g., a drop in a single year due to climate anomalies), such as the ones manipulated in our study, are very common and their consequences need to be studied. We present a comprehensive set of ecosystem functions and services, some of which may need months to years to change, such as plant diversity and soil aggregate stability. This approach provides a lot of nuance to our assessment, because we do not only study functions and services that are directly related to the activity of aboveground invertebrates and change immediately. Aboveground pest control was calculated as -1*log scaled aphid biomass. Although the control of aphid populations is commonly used as an indicator of top-down control, we note that there could have also been bottom-up effects of plant biomass production on aphid populations. Belowground pest control was calculated as -1*log scaled plant-feeding nematode density. We selected these variables, because they represent highly complementary above-belowground ecosystem services.

Coupling

We developed a quantitative index of the degree of ecosystem-level coupling (*sensu* Ochoa-Hueso et al.[49]) of eleven groups of organisms and twelve biogeochemical properties, based on the mean of Pearson correlation coefficients of all potential pairwise comparisons of all variables in absolute value. Coupling was calculated at the experimental treatment level (n = 8). The average coupling within each treatment was then compared against randomly generated null models derived from our dataset based on 100 permutations ($p < 0.05$, two-tailed). The null model comparisons were performed to assess three coupling states[49] (see below) and to compare with previous work on ecosystem coupling. We also calculated coupling separately for the biotic portion of our dataset by only considering correlations between pairs of the eleven groups of organisms evaluated (*biotic coupling*), and for the abiotic portion of the dataset (*biogeochemical coupling*). We also measured coupling based on the proportion of significant correlations ($p < 0.05$) to total pairwise correlations, and again compared our expectations against a randomly generated null model following the same procedure as previously described. Within this framework, ecosystems can be found in three coupling states[49]: (i) coupled, when measured coupling observations are above the 95% quantile of random observations; (ii) decoupled, when observations fall within the 5%–95% quantile envelope; and (iii) anti-coupled, when measured coupling observations are below the 5% quantile of random observations. The reader should note that the assessed soil invertebrate community reflects a very powerful combination of common bioindicators, and provides a good overview of belowground responses to aboveground stressors, covering major gradients in body size, behavior, physiology, and trophic position.

Statistical and multifunctionality analyses

We used linear models to test the effect of invertebrate treatment (100%, 36%, and 0% invertebrates; as linear term) on ecosystem services as well as biotic and abiotic ecosystem variables. For the data that was not normally distributed, we log(10)-transformed them prior to analysis, to meet the requirements of normal distribution and homoscedasticity of residuals (Table 1). For aphid biomass, we used log10 (x+1) transformation to avoid the log10(x) approaching negative infinity, as the x approaches zero. Firstly, we only included invertebrate treatment as the fixed term. Then**,** we included the (log10) aphid biomass (as a covariate), fitted before the invertebrate treatment to test the aphid effect in a sequential analysis (type I sum of squares[74]). As an additional sensitivity analysis, we used one-way ANOVAs to test whether there is any difference between the 36% invertebrate treatment and the 100% invertebrate treatment on ecosystem variables. For the six ecosystem services (plant diversity [Shannon diversity], aboveground plant biomass, aboveground pest control, belowground pest control, decomposition, soil aggregate stability), we calculated average multifunctionality by averaging the standardized ecosystem services. Here, we show results including the aboveground plant biomass data in August (peak time of the aboveground plant biomass). If we use the total of the aboveground plant biomass considering also the second harvest at the end of the experiment, the multifunctionality using an averaging approach decreases even more significantly with decreasing of the invertebrate biomass ($F_{1,22} = 7.67$, $p = 0.011$). We also used the multifunctional threshold method[38] to quantify the number of services with the service value exceeding a given threshold, where thresholds are varied along a gradient from 5% to 99% of the maximum observed stability of the function. Here, we explored threshold values between 5% and 99% at 1%-intervals. This method allowed us to determine whether more invertebrates support more services. We examined the relationships of invertebrate treatment (100%, 36%, and 0% invertebrates as linear term) with the number of services above a threshold by fitting general linear models. Separate models were fitted for each of the threshold levels and the slope, and associated 95% confidence intervals were recorded. All statistical analyses and data processing were done using R software (version 4.1.1), including the package *multifunc* for the multifunctionality calculation.[38] Moreover, we analyzed relationships between the biomass of grasses, herbs, and legumes, respectively, assessed at the first biomass harvest in August with mean decomposition rates of the two bait lamina campaigns performed in August using correlation analyses. The first harvest was chosen, because of overall higher plant biomass production and two bait lamina assessments in the same month, which was not available for the second harvest.