Fear of Predation Slows Plant-Litter Decomposition

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most likely caused by the pulsed nature of our sulfide supply. This may have led to short periods of exposure of *Zostera* to toxic sulfide levels.

Coastal ecosystems, and seagrass meadows in particular, are currently declining at an alarming and increasing rate worldwide, leading to loss of biodiversity (*1*). Extensive restoration efforts have had little success so far (<30%), despite their extremely high costs (±$100,000 per hectare) (*23*). Similar to the function of mycorrhizae, pollinators, or seed dispersers in terrestrial systems (*24*–*26*), our findings indicate that restoration efforts should not only focus on environmental stressors such as eutrophication, sediment runoff, or high salinity as a cause of decline but should also consider internal ecological interactions, such as the presence and vigor of symbiotic or mutualistic relations. Breakdown of symbiotic interactions can affect ecosystem functioning, with bleaching events in coral reefs as a clear example (*27*). Similar to the well-known symbiosis between corals and their unicellular algal endosymbionts (*28*), we conclude that symbioses, rather than one defining species, forms the foundation of seagrass ecosystems.

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Supplementary Materials

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Materials and Methods
Supplementary Text
Figs. S1 to S4
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Fear of Predation Slows Plant-Litter Decomposition

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Aboveground consumers are believed to affect ecosystem functioning by regulating the quantity and quality of plant litter entering the soil. We uncovered a pathway whereby terrestrial predators regulate ecosystem processes via indirect control over soil community function. Grasshopper herbivores stressed by spider predators have a higher body carbon-to-nitrogen ratio than do grasshoppers raised without spiders. This change in elemental content does not slow grasshopper decomposition but perturbs belowground community function, decelerating the subsequent decomposition of plant litter. This legacy effect of predation on soil community function appears to be regulated by the amount of herbivore protein entering the soil.

The quantity and quality of detrital inputs to soil regulate the rate at which microbial communities perform ecosystem processes such as decomposition, nitrogen (N) mineralization, and carbon (C) sequestration (*1*, *2*). Because uneaten plant litter makes up the majority of detritus (*3*), it is assumed that these belowground ecosystem processes are only marginally influenced by biomass inputs from higher trophic levels in aboveground food webs, such as herbivores themselves (*4*). We provide evidence here, however, that predators may influence the decomposition of plant litter via a legacy effect of predation risk. Specifically, a physiological stress response to the risk of predation changes the elemental content of herbivore biomass. In turn, the decomposition of these stressed herbivores alters the function of belowground communities, leading to an overall decrease in the decomposition of plant litter.

Our work addresses whether food web structure (especially the existence of predators) influences ecosystem functioning via changes in the nutritional contents of prey (*5*, *6*). The prevailing view is that food web structure does not influence prey body C-to-N (C:N) contents, because to survive and reproduce, prey must maintian relatively constant body C:N ratios (*7*). However, this view assumes that predator effects on prey are entirely consumptive (*5*). Instead the presence of predators generates fear, leading to physiological stress responses in prey, such as elevated metabolism and the synthesis of heat shock proteins (*8*). Together, these stress responses increase basal energy demands (*9*–*12*) that, in nutrient-limited systems, reduce the energy available for the competing demands of production (that is, reproduction and growth) (*13*). Thus, to meet heightened maintenance-energy demands, stressed herbivores divert energy from production, as well as increase their consumption of energy-rich carbohydrates (*12*). Given that the amount of energy used for production correlates positively with N demand, and that herbivores have limited ability to store excess nutrients, stressed herbivores should also excrete more N (*8*, *14*). N excretion is further enhanced because chronically heightened stress hormone levels increase the breakdown of body proteins to produce glucose (*15*). Ultimately, prey stressed by predation risk should increase their body C:N ratio (*8*), and this is observed in field and laboratory experiments (*12*, *16*).

In this study we asked whether predators can regulate plant-litter decomposition through

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indirect influences on the elemental stoichiometry of their prey. Our working hypothesis is that the C:N content (quality) of herbivore biomass entering soils as detritus induces changes in how soil communities process other resource inputs such as plant litter. Such legacy effects of resource quality have been observed in litter-decomposition studies (*17*). Moreover, simple organic compounds may prime soil communities in ways that enhance the decomposition of more-complex organic compounds (*18*).

Using a combination of laboratory and field experiments, our work built on examinations of food web effects on prey physiological stress and elemental stoichiometry in a well-studied grassland ecosystem including the spider predator *Pisaurina mira*, a dominant grasshopper herbivore (*Melanoplus femurrubrum*), and a variety of grasses and forbs (*12*). We reared grasshoppers in the field with the risk of spider predation (stress treatment) and without (control) (*19*). The body C:N content of stressed grasshoppers (mean ± SE, 4.00 ± 0.03; N = 11.62 ± 0.12%) was significantly higher (Wilcoxon signed-rank test $z = -2.023$; $P < 0.05$) than that of nonstressed grasshoppers (3.85 ± 0.04; N = 12.11 ± 0.16%). We then used carcasses of these grasshoppers in laboratory microcosm experiments to test whether the difference in body elemental composition altered the decomposition of the grasshoppers and subsequent plant litter inputs. We added a small amount (3.5 mg) of either stressed or nonstressed grasshopper biomass to microcosms containing soil collected from our grassland ecosystem. Carbon mineralization rates of grasshopper biomass were monitored until rates did not differ from reference microcosms containing only soil. At this time (42 days), there was little difference in cumulative C mineralization (that is, the total amount of C respired as CO_2 across the entire incubation) between stressed and stress-free grasshopper treatments (Fig. 1A). This was not unexpected, given that both stressed and stress-free grasshoppers represent high-quality resources to belowground communities. More interesting was how small elemental changes in these inputs might affect the subsequent functioning of soil communities, especially the decomposition of lower-quality substrates such as plant litter (*18*)

To test the functional implications of slight nutrient differences in high-quality resource inputs, we added grass litter (500 mg) to the microcosm soils previously amended with stressed or stress-free grasshopper carcasses and then measured C mineralization. After 118 days, mineralization of the grass litter in soils previously amended with stressed grasshoppers was 62% lower ($F_{1,4}$ = 13.9, $P < 0.05$) than that of the same litter in soils amended with nonstressed grasshoppers (Fig. 1B). Thus, the small input of herbivore biomass (~140 times less than the added litter mass), coupled with a 4% difference in the C:N ratio between stressed and nonstressed grasshopper carcasses, caused a threefold difference in the mineralization of plant-litter inputs. These results suggest a causative link between predation-induced changes in prey body chemistry and altered soil community function. The most plausible explanation for why such a small shift in prey C:N ratio might

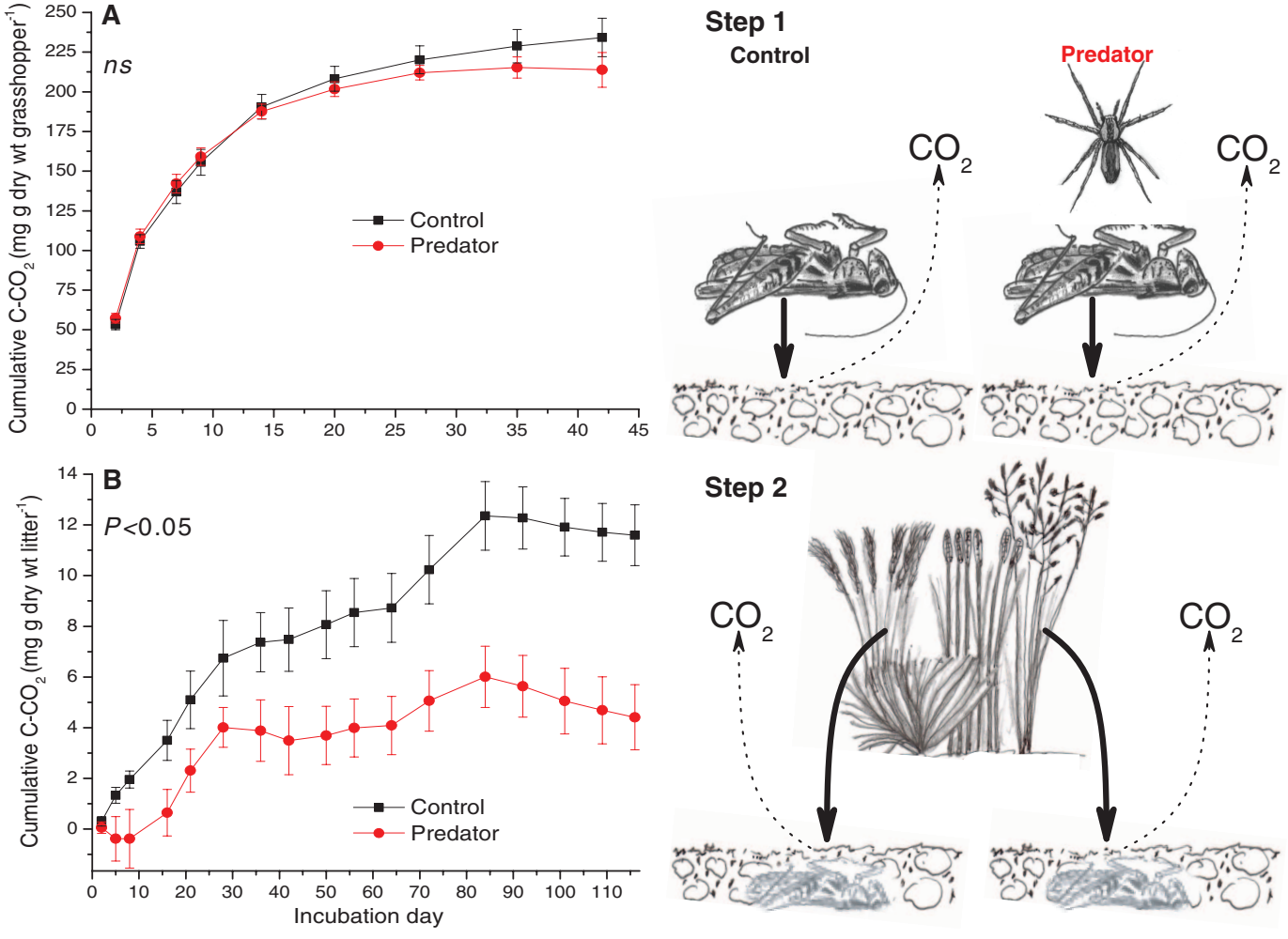

Fig. 1. Cumulative C mineralization (mean ± 1 SE, n = 5 microcosms) during decomposition of (**A**) nonstressed grasshoppers versus those stressed by predators (step 1); and (**B**) grass litter added to the same microcosms (step 2) after the completion of the grasshopper decomposition experiment shown in step 1. Although control and stressed grasshoppers were mineralized at similar rates ($P > 0.05$), the addition of grasshopper carcasses reared with disarmed predators led to subsequent reductions in plant-litter decomposition rates ($P < 0.05$). Rates are differences from microcosms not amended with grasshoppers, so cumulative values can be negative.

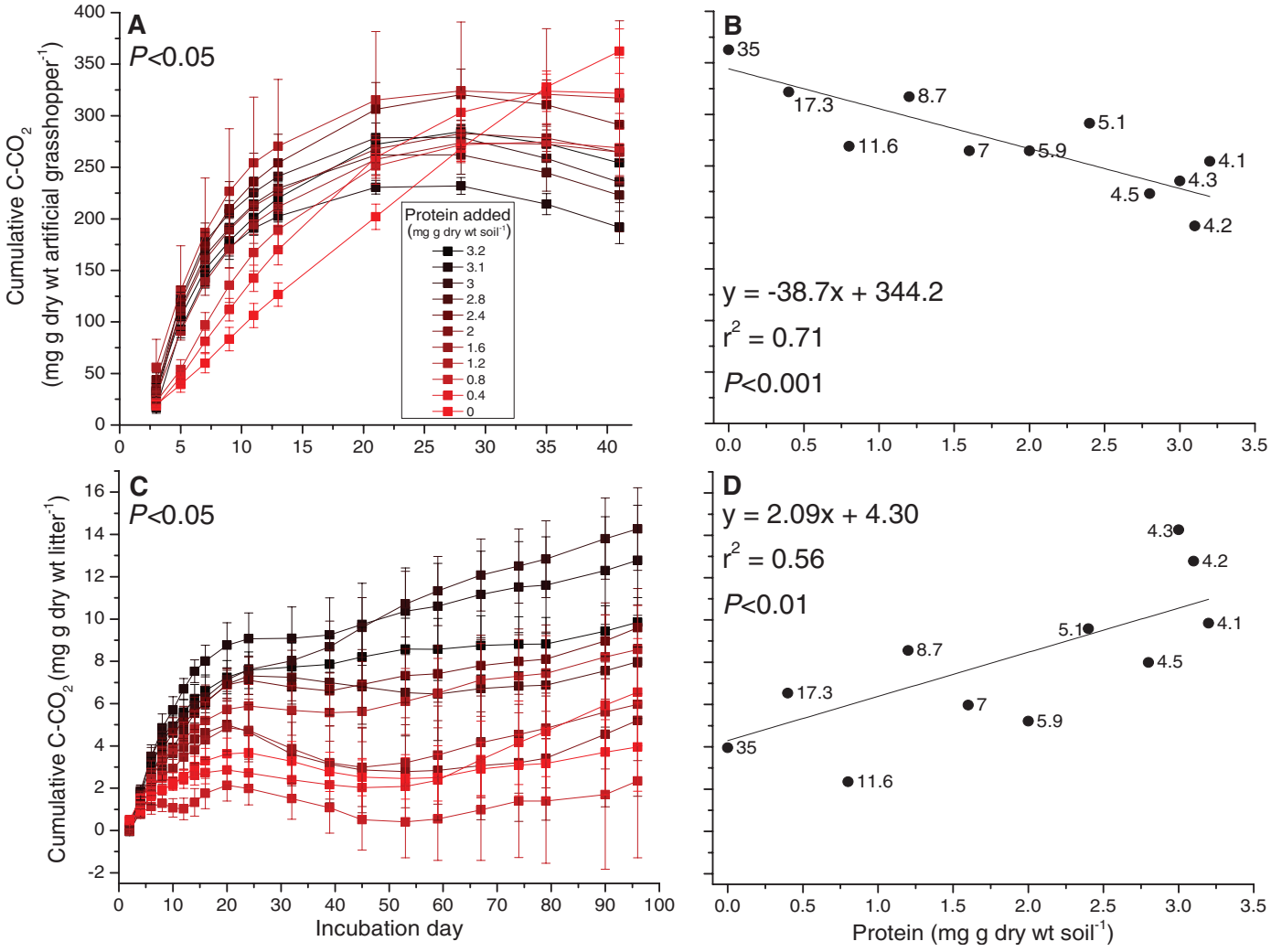

Fig. 2. (**A**) Cumulative C mineralization (mean ± 1 SE, n = 6 microcosms) during decomposition of artificial grasshoppers with varying C:N ratios and protein contents and (**B**) the relationship between the amount of protein added and the cumulative mineralization associated with these artificial grasshoppers. (**C**) The cumulative C mineralization (mean ± 1 SE) of grass litter added to the same microcosms after completion of artificial grasshopper decomposition and (**D**) the relationship between the amount of protein added and subsequent grass-litter mineralization. Rates are differences from microcosms not amended with artificial grasshoppers, so cumulative values can be negative [as in (C)]. The C:N ratios associated with the different artificial grasshoppers are shown next to their respective points.

translate to such a large shift in litter mineralization rates is that small increases in N availability can prime the activities of decomposer microorganisms and hence accelerate litter mineralization (*20*). We next examined whether the observed effects on litter mineralization were probably attributable to the variation in the C:N content of prey tissue being linked to N availability and hence priming of microbial activity.

To test whether prey body C:N content influenced plant-litter decomposition, we again used a laboratory microcosm approach, but this time manipulated C:N ratios by creating "artificial grasshoppers" (*19*). To simulate grasshopper tissue, we used 4-mg organic matter mixtures of 20% chitin and varying proportions of carbohydrates (0 to 80%) and proteins (0 to 80%), generating C:N ratios that spanned more than the observed variation. Carbon mineralization of the artificial grasshoppers was measured (for 40 days)

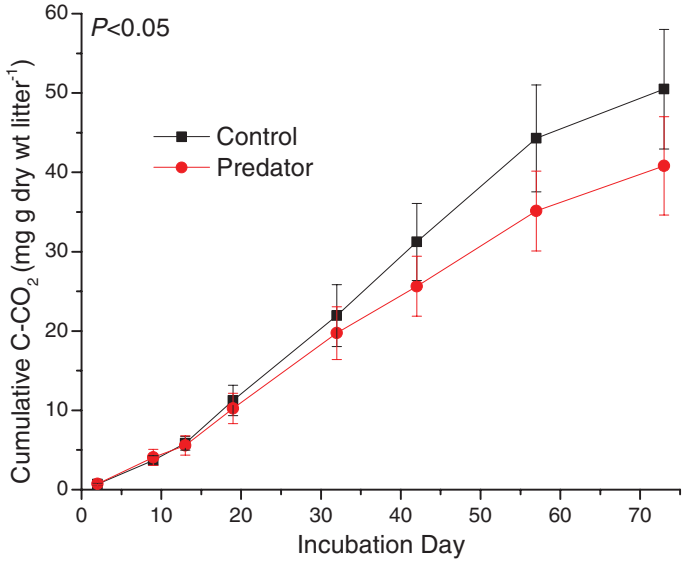

Fig. 3. Cumulative C mineralization (mean ± 1 SE, n = 7 field plots) of ^{13}C-labeled grass litter decomposed in blocked field plots, first amended with control grasshoppers or those stressed by predator presence.

until it approximated rates in reference microcosms containing only soil. Cumulative C mineralization varied by up to twofold across the artificial grasshopper treatments ($F_{10, 55} = 2.15$, $P < 0.05$; Fig. 2A). Notable, given the broad range in C:N across treatments, is the positive relationship between the C:N of artificial grasshoppers and the cumulative C-mineralization, as well as the negative relationship between mineralization and amount of protein (Fig. 2B). These observations are most likely explained by higher growth efficiencies of soil organisms. This might be expected because organisms consuming resource inputs with lower C:N ratios and consequently higher protein levels will favor production over waste respiration and hence reduce total C mineralization (*20*).

The availability of protein N is essential both for microbial production and for facilitating the decomposition of organic matter, because it is used to produce extracellular enzymes that catalyze the degradation of complex C compounds (*20*–*22*). It then follows that the carcasses of stressed grasshoppers, which have higher biomass C:N, probably because of lower body protein levels (*23*), provide less available N and thus should retard plant-litter decomposition. Further support for this interpretation came when we added grass litter (500 mg) to the microcosms previously amended with artificial grasshoppers. Across 96 days, the mineralization rates of grass litter diverged by as much as sixfold ($F_{10, 55} = 2.34$, $P < 0.05$; Fig. 2C), despite the only twofold difference in the cumulative mineralization of artificial grasshoppers. These results mirror, qualitatively, our first experiment with real grasshoppers, in which lower available N led to reductions in the decomposition of plant litter (Fig. 2, C and D). These results also show that varying C:N ratios only partially explain altered plant-litter mineralization. Specifically, the protein content of artificial grasshoppers, for which the C:N ratio is a common but indirect index (*23*), has over twice the power [coefficient of determination-explained variance (R^2) = 0.56, $F_{1,10} = 13.5$, $P < 0.01$; Fig. 2D] of the C:N ratio ($R^2 = 0.23$, $F_{1,10} = 4.0$, $P = 0.08$) to explain plant-litter decomposition rates. This is probably because the C:N ratio is influenced by both labile and recalcitrant N-bearing compounds. Consequently, a small difference in the C:N ratio may reflect much larger variation in protein N content. Together, the laboratory experiments reveal a potentially important general mechanism (*8*) by which predators regulate soil ecosystem processes through stress-induced changes to herbivore nutrient content. It remained uncertain whether this mechanism explains variation in belowground community function in nature.

To test for predator-induced regulation of decomposition variation in mineralization rates under natural conditions, we added intact carcasses of grasshoppers reared either with predation risk (stress treatment) or without (control) to field plots. After 40 days, we added ^{13}C-labeled grass litter (550 g m^{-2}) to the same plots and measured ^{13}C mineralization in situ, using cavity ring-down spectroscopy, a highly sensitive form of laser absorption spectrometry that quantifies the stable isotope composition of C in CO_2 (*19*). Using ^{13}C labeling meant that we could separate the contribution of mineralization of the added litter from total soil respiration. After 73 days, mineralization of the grass litter, in plots amended with stressed grasshoppers, was 19% lower ($F_{1,6} = 9.06$, $P < 0.05$) than in plots that received stress-free grasshoppers (Fig. 3). This mechanism is not mutually exclusive of other mechanisms that regulate soil communities (*4*, *21*, *24*). Nonetheless, in our experiments, the effect of predation on litter decomposition had a measureable impact through changes in the nutrient content of herbivore carcasses. These results highlight the potential for this mechanism to influence decay rates of organic matter inputs and hence ecosystem C and N cycling.

A key remaining question is whether predator-induced changes persist when multiple aboveground and belowground pathways act simultaneously. We examined this question by rearing grasshoppers in field plots where predation risk was absent (no spiders) or present (spiders with glued mouthparts) (*19*). Toward the end of the growing season and 1 month after the grasshopper adults had reproduced, died, and been allowed to decompose, we transferred surface soil from the field plots to laboratory microcosms. We found no difference in soil pH between risk and risk-free field mesocosms ($F_{1,6} = 0.761$, $P = 0.417$). To test for legacy effects of fear, we then added grass litter to the microcosms and measured C mineralization for 93 days. Cumulative mineralization of grass litter, amended to soil communities developed under the predator treatment, was ~200% lower than litter mineralization from communities developed without spider predators ($F_{1,6} = 6.23$, $P < 0.05$; Fig. 4). Collectively, our experiments suggest that cascading effects of predation risk are measurable on litter decomposition in the field and laboratory and occur through predator-induced changes in prey chemical composition.

Traditional concepts of trophic pyramids in ecosystems highlight the idea that inputs of plant-derived materials to soils are more important for regulating belowground processes than are inputs from other trophic levels, because plant inputs are dominant (*4*). Accordingly, predators are presumed to regulate ecosystem processes mainly by altering the quality and quantity of plant-derived materials entering belowground systems, through the control of herbivore density (that is, through trophic cascades) and/or by altering herbivore foraging behavior (*4*, *5*). Our work instead suggests that predators can regulate ecosystem processes more directly through stress-induced changes in the chemical composition of prey body tissue.We find that small additions of high-quality herbivore biomass influence the decomposition of much larger inputs of recalcitrant plant litter, with effects lasting for at least the duration of a normal growing season (80 to 110 days). Indeed, we show that predator-induced changes in the nutritional composition of herbivore biomass dramatically slow the decomposition of plant litter through legacy effects on soil communities. Our work suggests that the mechanism governing these effects is the amount of animal protein that enters the soil. Our work adds to the body of recent work (*5*, *25*) showing that predators exert top-down control, through multiple mechanisms, on ecosystem processes. Evaluating the importance of these newly identified roles of predators in ecosystems is made all the more urgent because we are losing them from ecosystems at disproportionately higher rates than other species (*25*, *26*).

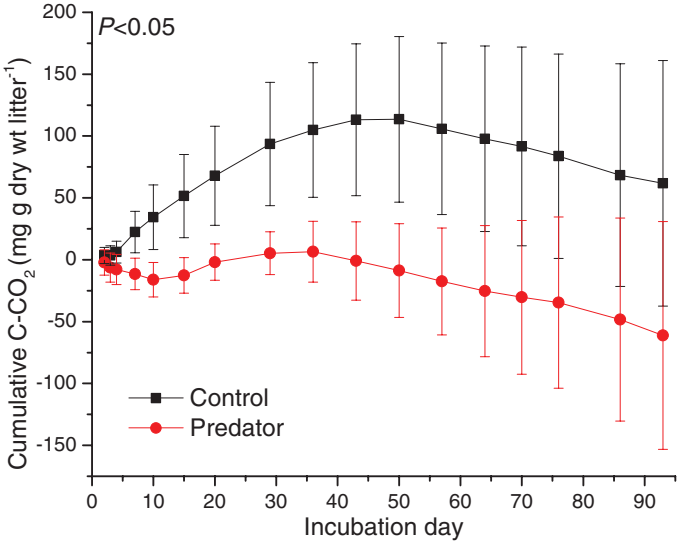

Fig. 4. Cumulative C mineralization (mean ± 1 SE, $n = 7$ microcosms) of grass litter on soil collected from blocked field plots with or without predation risk. Rates are differences from reference soils, so cumulative values can be negative.

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Supplementary Materials

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Continental-Scale Effects of Nutrient Pollution on Stream Ecosystem Functioning

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Excessive nutrient loading is a major threat to aquatic ecosystems worldwide that leads to profound changes in aquatic biodiversity and biogeochemical processes. Systematic quantitative assessment of functional ecosystem measures for river networks is, however, lacking, especially at continental scales. Here, we narrow this gap by means of a pan-European field experiment on a fundamental ecosystem process—leaf-litter breakdown—in 100 streams across a greater than 1000-fold nutrient gradient. Dramatically slowed breakdown at both extremes of the gradient indicated strong nutrient limitation in unaffected systems, potential for strong stimulation in moderately altered systems, and inhibition in highly polluted streams. This large-scale response pattern emphasizes the need to complement established structural approaches (such as water chemistry, hydrogeomorphology, and biological diversity metrics) with functional measures (such as litter-breakdown rate, whole-system metabolism, and nutrient spiraling) for assessing ecosystem health.

Nutrient enrichment from organic inputs and agricultural run-off is placing the world's vulnerable fresh waters in a precarious position (*1*–*4*). Far-reaching environmental legislation has been introduced to redress human impacts on aquatic communities (*5*, *6*), yet the consequences of nutrient loading for stream ecosystem functioning remain poorly understood (*4*, *7*, *8*). This is worrying because key ecosystem services (such as maintenance of viable fisheries as a provisioning service, and organic matter decomposition as a supporting service) ultimately depend on ecosystem processes, such as leaf-litter breakdown and other processes involved in nutrient cycling (*3*, *9*).

Many aquatic ecosystems are supported by plant litter inputs (*10*–*12*). This includes streams, where terrestrial leaf breakdown—which is driven by resource quality; the abundance, diversity, and activity of consumers; and environmental factors—is a key ecosystem process (*10*, *13*, *14*). Moderate nutrient enrichment of streams can accelerate breakdown by stimulating microbial conditioning and invertebrate consumption (*15*, *16*). However, a wide range of responses along nutrient gradients has been reported in field studies, suggesting environmental drivers beyond elevated nutrient supply. For instance, wastewater discharge can induce anoxia, mobilize heavy metals, and physically smother benthic organisms (*17*, *18*). Litter breakdown by invertebrates (*19*) appears especially sensitive to nutrient pollution relative to that mediated by microbes (*20*) and, because invertebrates often attain their highest densities in moderately enriched streams, a hump-shaped breakdown rate response might be expected along long nutrient gradients (*5*).

We hypothesized that breakdown rates are constrained by microbial nutrient limitation at the low end of nutrient pollution gradients and by the effects of environmental degradation on invertebrates at the high end. Most studies, however, have been unable to detect this pattern because they have been conducted over relatively short nutrient gradients and small spatial scales (*5*, *7*). Here, we report a field experiment in 100 European streams spanning 1000-fold differences in nutrient concentrations, as proxy measures of nutrient loading by direct and indirect inputs (*21*). The validity of this approach is highlighted by the positive relationship between biochemical oxygen demand (BOD_5) and nutrient concentrations in more than 8000 European streams, and the comparable frequency distributions of nutrient concentrations between these and our sites (fig.

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