

Trophic cascade alters ecosystem carbon exchange

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Trophic cascades—the indirect effects of carnivores on plants mediated by herbivores—are common across ecosystems, but their influence on biogeochemical cycles, particularly the terrestrial carbon cycle, are largely unexplored. Here, using a ¹³C pulse-chase experiment, we demonstrate how trophic structure influences ecosystem carbon dynamics in a meadow system. By manipulating the presence of herbivores and predators, we show that even without an initial change in total plant or herbivore biomass, the cascading effects of predators in this system begin to affect carbon cycling through enhanced carbon fixation by plants. Prolonged cascading effects on plant biomass lead to slowing of carbon loss via ecosystem respiration and reallocation of carbon among plant aboveground and belowground tissues. Consequently, up to 1.4-fold more carbon is retained in plant biomass when carnivores are present compared with when they are absent, owing primarily to greater carbon storage in grass and belowground plant biomass driven largely by predator nonconsumptive (fear) effects on herbivores. Our data highlight the influence that the mere presence of predators, as opposed to direct consumption of herbivores, can have on carbon uptake, allocation, and retention in terrestrial ecosystems.

experimental ecosystem ecology | animal-mediated carbon cycling | carbon tracer experiment | carbon retention

Trophic downgrading—the disproportionate loss of species occupying top trophic levels of ecosystems—is a symptom of global biodiversity decline (1). Cutting short trophic chains in ecosystems causes significant changes in plant community biomass, composition, and diversity (2). These changes come about because loss of carnivores leads to increased impacts of herbivores on plant biomass through changes in herbivore density and foraging strategies (3).

It is becoming increasingly recognized that the cascading effects of carnivores may affect ecosystem carbon dynamics as well. By altering the impact of herbivores on plants, carnivores may indirectly regulate the amount and type of plant biomass available for photosynthetic carbon fixation and storage (3–5). Moreover, herbivory can trigger physiological adjustments in the remaining damaged plants, including reduction in photosynthetic rates and increased respiration (6–8). Accordingly, we hypothesized that carnivores should increase plant community carbon fixation and reduce respiration, thereby increasing carbon retention, by causing herbivores to reduce their foraging impacts on plants. We tested this hypothesis with a ¹³CO₂ pulse-chase field experiment in a grassland ecosystem in northeastern Connecticut.

Using established methods to discern indirect effects of carnivores on plants in ecosystems (9), we applied three experimental treatments in replicated 0.25-m² fine-mesh enclosures (Fig. S1): (i) plants only (control), (ii) plants and herbivores (+ herbivore), and (iii) plants, herbivores, and carnivores (+ carnivore). The first treatment served as a control for animal effects, the + herbivore treatment allowed for measurement of the direct effects of herbivores on plants, and the + carnivore treatment allowed for measurement of the indirect effects of carnivores on plants. All herbivore and carnivore species within the enclosures were removed before stocking by carefully sorting through the live vegetation. Natural field densities of the dominant species existing within the ecosystem were maintained. These included grasses and

perennial herbs that grew naturally within the enclosure plots, along with the generalist grasshopper herbivore *Melanoplus femurrubrum* and the sit-and-wait hunting spider carnivore *Pisaurina mira*, both of which were stocked into the enclosures at their natural field densities for that time in the field season. The grasshopper species consumes both grasses and herbs. The hunting spider species is known to consume *M. femurrubrum* grasshoppers within the study ecosystem and induce behavioral and physiological changes in the grasshopper, thereby altering the nature and level of this herbivore's impact on plants (9, 10). These effects in turn may control the rate of ecosystem processes, such as nutrient cycling and litter decomposition (9–11).

Expanding on previous work, we evaluated whether the carnivore in this system also could indirectly control ecosystem carbon exchange by regulating the amount of carbon fixed by the plant community, the aboveground and belowground allocation of this carbon, and ecosystem respiration.

Results

At 21 d after the field enclosures were stocked with herbivores and carnivores, each enclosure was pulse-labeled with ¹³CO₂, immediately after which plant community uptake of ¹³C was measured (Fig. S2). Of note, no significant effect ($F_{2,22} = 2.24$; $P = 0.13$) of treatment on total plant community biomass was seen at this time (Fig. 1A). However, significantly less of the ¹³C label was fixed by the plant community in the + herbivore treatment than in the control or + carnivore treatment, whether evaluated spatially (i.e., ¹³C fixed-m⁻²; $F_{2,22} = 7.15$; $P < 0.01$; Fig. 1B) or per unit of plant biomass (i.e., ¹³C fixed-plant biomass C⁻¹; $F_{2,22} = 3.69$; $P < 0.05$; Fig. 1C). The experiment thus produced the classic biomass pattern associated with a trophic cascade, but for fixation of recent photosynthate, with 33% less ¹³C in the + herbivore treatment than in the control treatment and the + carnivore treatment mitigating the decline in fixed carbon caused by herbivores.

Total respiration of the ¹³C label was measured repeatedly throughout the remainder of the growing season. Although respiration of total ¹³CO₂ did not differ among the three treatments across time ($F_{2,10} = 0.38$; $P = 0.69$; Fig. 2A), treatment did significantly affect the amount of ¹³C respired when scaled by amount of ¹³C originally fixed by the plant community ($F_{2,10} = 4.73$; $P < 0.05$; Fig. 2B). Over the course of the growing season, 9.3% more fixed ¹³C was respired in the + herbivore treatment than in the control and + carnivore treatments. Because less ¹³C was initially fixed in the + herbivore treatment, a greater proportion of fixed ¹³C was lost via respiration in that treatment compared with the other two treatments (Fig. 2B).

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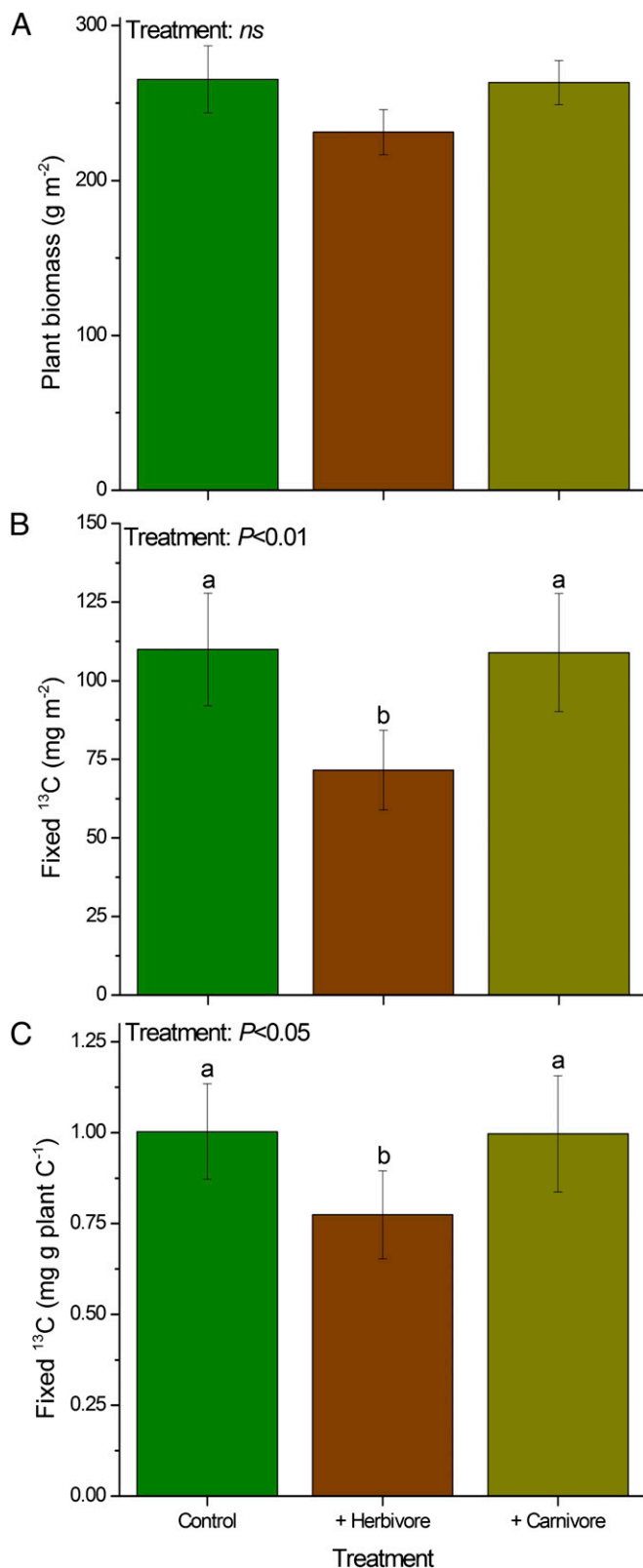


Fig. 1. Total plant biomass (g m^{-2}) immediately before the pulse-chase experiment (A), amount of ^{13}C fixed- m^{-2} (B), and amount of ^{13}C fixed-g of plant biomass C^{-1} (C); $n = 12$ in all instances. There was no treatment effect on total plant biomass even though the treatments had been in place for 21 d before ^{13}C labeling. However, a treatment effect was observed for the amount of ^{13}C fixed on both an areal basis (B) and a plant biomass basis (C). Letters indicate Tukey honest significant differences between treatments. Data are mean ± 1 SE.

The combination of increased ^{13}C fixation and a decreased proportion of that ^{13}C respired in the presence of carnivores indicates that the total amount of ^{13}C stored in plants was greater in the + carnivore treatment than in either the + herbivore treatment (1.4-fold greater) or the control treatment (1.2-fold greater) ($F_{2,4} = 10.26$; $P < 0.05$; Fig. 3A). Moreover, there was decreased variation in carbon storage among replicates in the + carnivore treatment compared with the + herbivore and control treatments, providing empirical support for the idea that the presence of carnivores leads to more similar ecosystem process rates (12).

Differences in total ^{13}C retention among the three treatments were associated with differences in belowground as opposed to aboveground allocation (belowground: $F_{2,4} = 18.68$; $P < 0.01$; aboveground: $F_{2,4} = 1.17$; $P = 0.40$), with the greatest belowground storage seen in the + carnivore treatment. Moreover,

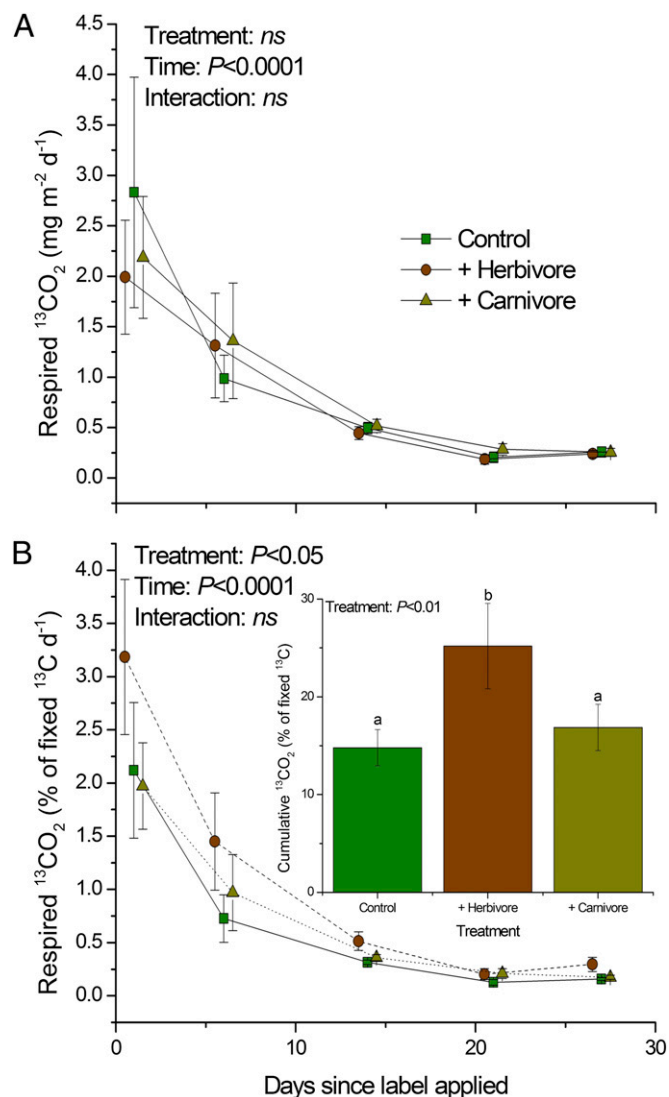


Fig. 2. Respiration of $^{13}\text{CO}_2$ ($n = 6$) across the experiment showing total respired $^{13}\text{CO}_2\text{-m}^{-2}$ (A) and respired $^{13}\text{CO}_2$ as a proportion of fixed ^{13}C (B). Total respired $^{13}\text{CO}_2$ did not differ across treatments (A), but because treatments in which only herbivores were present fixed less ^{13}C , a greater proportion of this fixed C was lost from these treatments (B). (B, Inset) Approximately 25% on average was respired, compared with $\sim 15\%$ in the plant-only treatment and $\sim 17\%$ in the carnivore treatment. Letters indicate Tukey honest significant differences between treatments. Data are mean ± 1 SE.

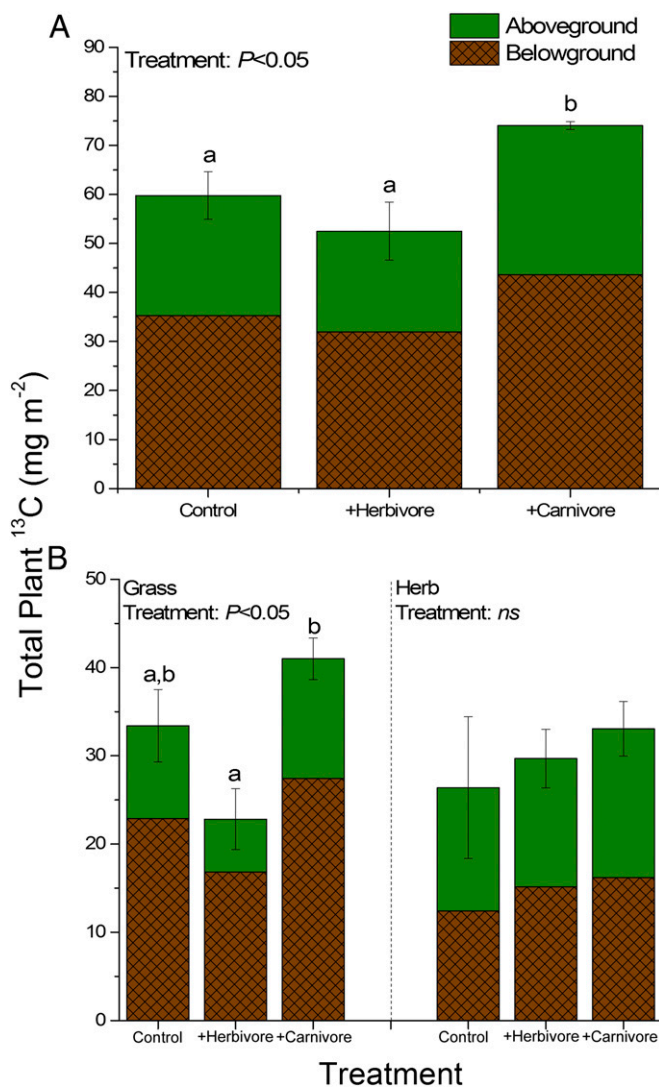


Fig. 3. Recovery in total plant biomass (A) and in either grass or herb (i.e., *Solidago*) biomass (B) of $^{13}\text{C}\cdot\text{m}^{-2}$ at the end of the 27-d experiment ($n = 3$). The amount of ^{13}C (mean) recovered in aboveground and belowground plant biomass is indicated as green and brown portions of each bar, respectively. Letters indicate Tukey honest significant differences between treatments. Data are mean ± 1 SE for total recoveries.

total (aboveground and belowground) ^{13}C stored in grass biomass ($F_{2,4} = 7.23$; $P < 0.05$), but not in *Solidago* ($F_{2,4} = 0.78$; $P = 0.52$) was greatest in the presence of the carnivore (Fig. 3B), indicating that carnivores increase the potential for grasses to act as a carbon sink.

All of the spiders and grasshoppers added to the experimental treatments were recovered at the end of the experiment. No significant differences in the amount of grasshopper biomass ($F_{1,2} = 0.88$; $P = 0.45$) or the amount of ^{13}C label incorporated into grasshopper biomass ($F_{1,2} = 3.3$; $P = 0.21$) were seen between the + herbivore and the + carnivore treatments (Fig. 4).

Discussion

Our study reveals that cascading effects of carnivores can influence carbon dynamics in terrestrial ecosystems, and that these cascading effects manifest through a previously unreported pathway involving changes in plant carbon uptake (i.e., carbon fixation) and ecosystem respiration, resulting in increased carbon storage in the presence of carnivores (Fig. S3). Reductions in ^{13}C fixation and

increases in the proportion of ^{13}C respired are indicative of the physiological responses of plants to herbivory (6–8). Our data demonstrate that these physiological responses translate to the ecosystem level, because the effect of carnivores on herbivores leads to greater retention of carbon in ecosystems by essentially slowing down carbon turnover in the plant community.

A difference in carbon dynamics between the + herbivore and the + carnivore treatments was seen even though there was no significant difference in total grasshopper biomass in the two treatments (Fig. 4). The lack of a net biomass effect on the grasshopper population is not an artifact of the comparatively short duration of the field experiment (40 d of a 100-d growing season), in which the predators might not yet have consumed the prey; this lack of net biomass effect has also been observed in longer-term experiments during which this spider predator consumed prey (10). This spider species normally causes compensatory, rather than additive, mortality on the grasshopper prey populations owing to its sit-and-wait hunting strategy. With compensatory mortality, predators only consume prey that would otherwise have died because of unfavorable abiotic conditions during life cycle development or through intraspecific competition. Thus, we would expect no net effect of this predator species on the biomass of the prey populations over the course of the growing season.

Because the spider did not affect grasshopper abundance, the differences in carbon uptake, release, and allocation among the treatments were likely related to altered grasshopper feeding pressure. That is, the cascading effects of the spider species on plants were mediated by a nonconsumptive, rather than a consumptive, predator effect on the herbivores (3, 13). In support of this inference, the indirect effect of the spider species on our study ecosystem was mediated largely by reductions in grasshopper daily feeding times and per capita foraging rates in favor of increased vigilance (14), along with shifts in grasshopper foraging pressure from grasses to the herb *Solidago* (9, 10, 14). This foraging shift in response to perceived predation risk could explain why grasses, but not *Solidago*, became a carbon sink in the presence of carnivores. The higher level of fixed ^{13}C found in grass roots in the + carnivore treatment compared with the + herbivore treatment is consistent with the expectation that plants alter aboveground-belowground carbon allocation in response to foliar herbivory, with low levels of herbivory (i.e., the + carnivore treatment) promoting allocation belowground and higher levels (i.e., the + herbivore treatment) reducing it (15, 16). This stimulation of belowground allocation with low levels of herbivory also may explain the greater ^{13}C retention in the + carnivore

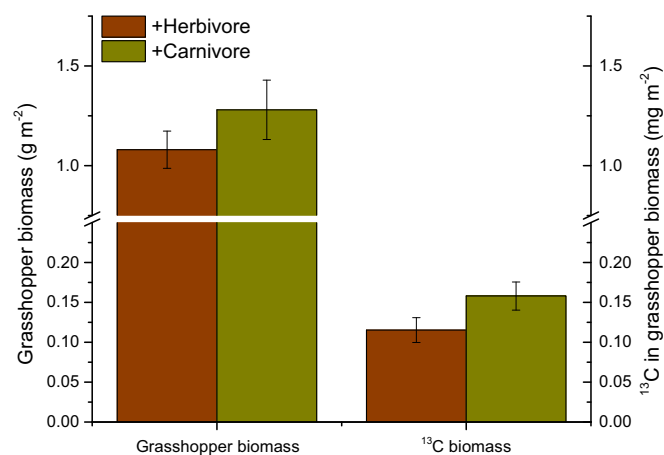


Fig. 4. Total grasshopper biomass and grasshopper biomass derived from fixed ^{13}C at 27 d after the initial pulse-labeling event. No significant treatment differences were noted for either total biomass or ^{13}C biomass.

treatment compared with the control treatment (Fig. 3A), in which there was no herbivory. Greater allocation of ^{13}C belowground likely stimulates such processes as root exudation, with exudates being a dominant precursor of stable soil organic carbon (17, 18). Thus, the carnivore-mediated effect on belowground allocation that we observed (Fig. 3) has the potential to translate into greater long-term carbon storage in terrestrial ecosystems.

The cascading effects of carnivores are known to be important to carbon dynamics through carnivore-induced changes in autotroph community abundance and species composition (5, 19, 20). Our research shows that carnivores also can influence carbon dynamics by causing differential herbivory on different plant species coupled with plant physiological responses. In addition, our results indicate that the changes in prey foraging behavior driving changes in carbon uptake, allocation, and retention are related not to a net consumptive effect of predators on herbivores—grasshopper biomass was unaffected by predator presence—but rather largely to a nonconsumptive fear effect.

We previously reported that spider carnivores in our ecosystem can directly and indirectly alter the nutritional and elemental balance (higher C:N ratios) of herbivore and plant organic matter (9, 11). These chemical changes in turn cause a threefold reduction in the decomposition rate of organic matter entering the soil (and hence microbial respiration of carbon) compared with that seen in the absence of carnivores, because the elemental changes likely starve microbes of essential protein-N for enzyme synthesis. Accordingly, predators may enhance the retention time of carbon within detrital pools. Those findings, combined with the data on plant carbon fixation and retention presented herein, suggest that carnivores, via trophic cascades, influence multiple pathways of carbon flux that collectively enhance or maintain ecosystem carbon storage. The implications of our results for ecosystem carbon balance could be significant given the ubiquity of trophic cascades (1, 21), as well as the increasing loss of top carnivores from ecosystems worldwide (1).

Materials and Methods

Experimental Setup. We placed 0.25-m²-basal area, 1-m-high cylindrical enclosures over growing meadow vegetation in the Yale Myers Research Forest in northeastern Connecticut. We arrayed the enclosures in three blocks of 12 cages each. We randomly assigned enclosures within each block to one

of three treatments that manipulated the trophic structure of the aboveground food chain. We captured second instar *M. femurrubrum* grasshopper nymphs and randomly assigned six individuals to each of the enclosures containing two- or three-level food chains. One day later, we added one adult *P. mira* spider to the designated three-level enclosures.

$^{13}\text{C}_2$ Pulse-Chase. The use of $^{13}\text{C}_2$ pulse-chase techniques enables quantification of the fate of recent photosynthetically fixed carbon with a level of mechanistic insight not afforded by methods that do not separately resolve lighter and heavier isotopes (22, 23). We tracked the fate of plant carbon into aboveground and belowground plant tissues and then back to the atmosphere through respiration. We infused $^{13}\text{C}_2$ by covering all 12 enclosures in each block (36 total) with 0.25-m³ Plexiglas chambers (Fig. S1) and then injecting ~0.27 g of 99 atom% $^{13}\text{C}_2$ into each chamber. By adding 99 atom% ^{13}C , we were able to introduce a minimal amount of CO_2 into the system, reducing the risk of altering plant carbon partitioning, while still detecting changes in the ^{13}C content of respiration and plant tissues.

The amount of ^{13}C fixed by the plants was determined by taking foliar clip samples immediately after the labeling event. These clip samples were then oven-dried at 65 °C, and ^{13}C was determined via isotope ratio mass spectroscopy (IRMS). The amount of ^{13}C fixed was then scaled to total plant biomass via allometric relationships between percentage cover and biomass (Fig. S2). After the pulse-labeling event (specifically, at 1, 6, 14, 21, and 27 d after labeling), respiration of the ^{13}C label was tracked with a Picarro G1101-I gas analyzer, which uses cavity ring-down spectroscopy to enable high-precision $\delta^{13}\text{C}$ measurements. These measurements were conducted at night, to avoid photosynthesis, by placing the Plexiglas chambers over 6 of the 12 mesocosms in each block. (The method was too time-consuming to allow us to do all 36 enclosures.)

We had planned to harvest the six mesocosms per block on which we followed respiration after 27 d, but were able to harvest only three per block before a hurricane hit the field site. We removed all of the foliage and also the roots to a depth of 10 cm. Plant material was air-dried and ball-milled to a fine powder, followed by measurement of ^{13}C content in the tissues by IRMS. The amounts of ^{13}C fixed, respired, and contained in aboveground and belowground plant biomass were derived using standard isotopic mixing models (24). All data analysis was done using linear mixed-effects models. Further details on the methods are provided in *SI Materials and Methods*.

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