Supporting Information

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SI Materials and Methods

Experimental Design. We placed 0.25-m^2-basal area, 1-m-high enclosures over growing meadow vegetation in the Yale Myers Research Forest in northeastern Connecticut. The design of these enclosures has been described previously (1) and is shown in Fig. S1. We arrayed the enclosures in three blocks of 12 cages each across the meadow, placed on similar vegetation. We randomly assigned enclosures within each block to one of three treatments that manipulated the trophic structure of the aboveground food chain: one trophic level, containing plants only; two trophic levels, containing plants and herbivores; and three trophic levels, containing plants, herbivores, and carnivores. We captured second instar *Melanpolus femurrubrum* grasshopper nymphs and randomly assigned six individuals to each of the enclosures containing two- or three-trophic level food chains. One day later, we added one adult *Pisaurina mira* spider to the designated three-trophic level enclosures. Cages were monitored up until the pulse-chase experiment to maintain the desired trophic structure. Herbivores or predators were removed from cages if found in the incorrect treatment.

^{13}C Pulse-Chase and ^{13}C Analyses. The use of $^{13}CO_2$ 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 resolve lighter and heavier isotopes separately (2, 3). We tracked the fate of plant carbon into aboveground and belowground plant tissues and then back to the atmosphere through respiration. We infused $^{13}CO_2$ by covering all 12 enclosures in each block (36 total) with 0.25-m^3 Plexiglas chambers (Fig. S1) and then injecting ~0.27 g of 99 atom% $^{13}CO_2$ into each chamber. The Plexiglass chambers were designed to fit over the enclosures and were sealed to the soil surface using a rubber-lined wooden base (Fig. S1). The chambers were fitted with 1/4" bulkhead fittings that enabled us to infuse $^{13}CO_2$ label into the chambers and to monitor CO_2 concentrations and ecosystem respiration. The label was introduced to the chambers by connecting a sealed vial to one of the bulkhead fittings containing sodium carbonate (99 atom% ^{13}C). Then excess HCl to produce CO_2 from the carbonate was added slowly to the sealed vial via a syringe. 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.

Immediately after labeling, the amount of ^{13}C fixed by the plants was determined by obtaining foliar clip samples from the dominant plants in the enclosures. The clip samples were oven-dried (at 65 °C), and ^{13}C was measured by continuous-flow isotope ratio mass spectroscopy (IRMS; DELTA V Plus Advantage; Thermo Scientific). The amount of ^{13}C fixed was then scaled to the total plant biomass via allometric relationships between percent cover and biomass (Fig. S2). For this, we first determined the percent cover for all of the enclosures, then, using nine enclosures (one of each treatment across the three blocks) harvested at 2 d after the pulse-labeling event, we determined the allometric relationship between percent cover and plant biomass for the two dominant plant species (i.e., grasses and perennial herb *Solidago*) in the enclosures (Fig. S2). We used these allometric relationships to estimate the total plant biomass in the remaining enclosures.

For 27 d after the pulse-labeling event (specifically, at 1, 6, 14, 21, and 27 d after labeling), we tracked respiration of the ^{13}C label using a Picarro G1101-I gas analyzer, which uses cavity ring-down spectroscopy technology to enable high-precision $\delta^{13}C$ measurements. These measurements were performed at night, to avoid photosynthesis, by placing the Plexiglas chambers over 6 of the 12 enclosures in each block. The chambers were of a dynamic open-chamber design, and CO_2 concentrations in individual chambers were monitored for 30 min at a time. CO_2 concentrations in the chamber were corrected for atmospheric CO_2 concentrations and isotope mixing equations (see below) were used to estimate the amount of ^{13}C label respired.

We had planned to harvest the six mesocosms per block in 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 depth of 10 cm. Plant material was air-dried and ball-milled to a fine powder, followed by measurement of ^{13}C by IRMS. The amounts of ^{13}C fixed, respired, and contained in aboveground and belowground plant biomass were derived using standard isotopic mixing models (4). The amount of C derived from recently fixed ^{13}C was calculated as the atom% excess in that pool (4). Specifically, the mass of ^{13}C label fixed by the plants and found in the other C pools was determined by subtracting the atom% ^{13}C values of unlabeled materials from labeled, correcting for the natural abundance ^{13}C of C in a given pool. The atom% excess of a given pool was then multiplied by the total C in that pool, giving the mass of ^{13}C label. Along with the ^{13}C-labeled enclosures, we also monitored three natural abundance chambers (one in each block; all were carnivore treatments) during respiration measurements. A total of nine natural abundance chambers (all three treatments in each block) were harvested at 27 d after the pulse-labeling event. These natural abundance controls were used to constrain the isotope mixing models.

Measurements of labeled plant carbon uptake (i.e., carbon fixation) and ecosystem respiration revealed significant relationships between estimated and actual net primary production (NPP) across treatments, with the highest NPP seen in the presence of carnivores (Fig. S3).

Statistical Analyses. All data were analyzed using linear mixed-effects models, with block treated as a random effect (5). If necessary, data were log-transformed to meet assumptions of homogeneity. All analyses were conducted using the freeware statistical package R (http://www.r-project.org/). Statistical significance was defined as $P < 0.05$, and marginal significance was defined as $P < 0.10$.

1. Schmitz OJ (2008) Effects of predator hunting mode on grassland ecosystem function. *Science* 319(5865):952–954.
2. Bradford MA, et al. (2007) Carbon dynamics in a model grassland with functionally different soil communities. *Funct Ecol* 21(4):690–697.
3. Strickland MS, et al. (2010) Rates of in situ carbon mineralization in relation to land-use, microbial community and edaphic characteristics. *Soil Biol Biochem* 42:260–269.
4. Fry B (2006) *Stable Isotope Ecology* (Springer, New York).
5. Pinheiro JC, Bates DM (2000) *Mixed-Effects Models in S and S-PLUS* (Springer, New York).

Fig. S1. The enclosures and Plexiglas chambers used in this study. Note that the plexglass chambers were sealed to the soil surface using the wooden rubber-lined frames inserted into the soil (~6 cm) seen around the bases of the enclosure cages.

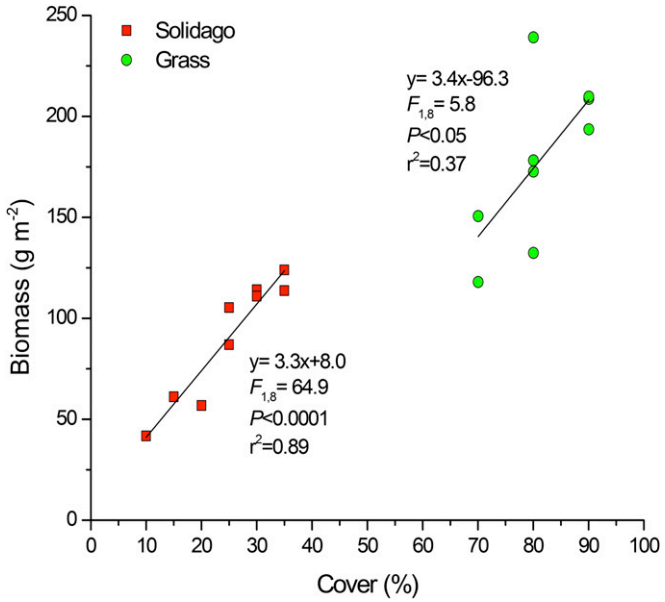

Fig. S2. Allometric relationships between percentage cover and biomass (g m^{-2}) for *Solidago* (herb) and *Poa* (grass) species. These estimates were used to determine the amount of ^{13}C fixed in each enclosure.

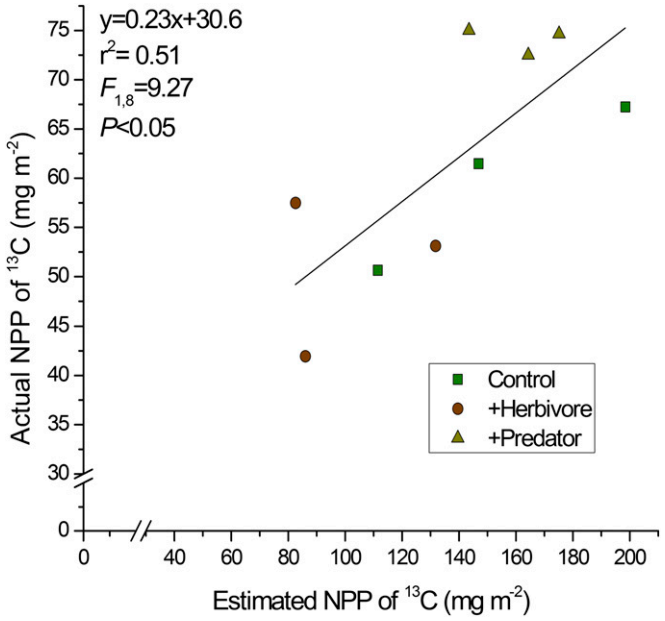

Fig. S3. Actual versus predicted NPP of ^{13}C. Predicted NPP was derived from the amount of ^{13}C initially fixed minus the proportion of that label respired. Actual NPP was determined using the measurements described for the data shown in Fig. 3. Note that this was not a 1:1 relationship, with the actual NPP being lower than that estimated from fixation and respiration. This is likely because we did not measure additional losses of the ^{13}C label into the soil as root exudates, mycorrhizal carbon demand, inputs below 10 cm, and so on. Future work is needed to explore how trophic cascades influence the fate of carbon in these pools; nonetheless, this relationship does demonstrate the potential for using initial fixation and respiration to estimate relative treatment effects on plant carbon storage of recent photosynthate.