

Trophic cascades in a complex terrestrial community

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ABSTRACT To test for direct and indirect effects of a top predator on three lower trophic levels, we conducted two multiyear predator addition experiments in a tropical wet forest. Periodic additions of a top predator (predatory clerid beetle) to a wet forest understory shrub caused a reduction in the predatory beetle's prey (a predatory ant), increased herbivory, and reduced leaf area of the plant. These effects occurred whether beetles were added to naturally occurring shrubs or to reproductive fragments, suggesting fitness effects of top predators through three trophic levels. A correlational study showed that trophic effects of top predators also cascaded to nearby conspecifics in the forest understory. We use trends from understory plant surveys to suggest mechanisms by which these cascades could ultimately affect species diversity in the local plant community.

Top-down trophic cascade models predict that ecological systems with even numbers of trophic levels will have low plant biomass and that systems with odd numbers will have high plant biomass (1–3). Top-down effects may also alter species diversity (4, 5). Although tests in aquatic systems often support these models (6), the occurrence of top-down trophic cascades in terrestrial systems is debatable because terrestrial communities might be more complex and, thus, have a greater capacity for buffering and compensation among species (7, 8). New evidence shows that top predators in three-level terrestrial systems can have a positive effect on plant biomass (9–14). However, current examples of top-down trophic cascades in four-trophic level terrestrial communities are complicated by the fact that the top predator feeds on both predators and herbivores in the system (15–19). In multiyear experiments, we added a fourth trophic level to a terrestrial community and examined the effects of these top predators on plant biomass. We also measured top-down effects on nearby conspecifics in the understory and examined the relationship between herbivory levels and survivorship of established understory plants in the local community.

Our study in a tropical wet forest system, involving a clerid beetle that feeds on predatory ants but not on the ants' herbivorous prey, tested for cascading effects through four trophic levels. *Piper cenocladum* C. DC. (family Piperaceae), a small rain forest understory shrub, is associated with three consumer groups (Fig. 1). Organisms on the second trophic level include dozens of species of arthropod herbivores (ref. 20; L.A.D. and D.K.L., unpublished observations); scavenging, predatory, and parasitic arthropods comprise the third trophic level, especially *Pheidole bicornis* ants (Formicidae: Myrmicinae), which occupy the stem and hollow petioles; and top predators include *Tarsobaenus letourneauae* (Coleoptera: Cleridae; equivalent to *Phyllobaenus* sp., W. Barr, unpublished manuscript), which feeds in its larval stage on *Pheidole bicornis* ant brood inside the petioles (fourth trophic level; ref. 21). In

a previous 18-month experiment, when predatory beetles were added periodically to *P. cenocladum* fragments, their ant prey were reduced 5-fold, average herbivory to foliage was increased nearly 3-fold, and shrub leaf area was reduced by nearly half (22). Here, we present two complementary tests of top-down forces that compare top predator effects on reproductive fragments and on established shrubs.

METHODS

Field experiments were conducted at the La Selva Biological Station, Heredia Province, Costa Rica, at 10°25' N, 84°5' W and ≈100 m elevation. This lowland rain forest reserve receives a mean annual precipitation of ≈4000 mm and has a mean temperature of 26°C. *P. cenocladum*, a tropical shrub with large leaves and branches and a primary stem up to 4 m tall, occurs commonly in understory patches on relatively swampy soils. Over 90% of the *P. cenocladum* shrubs in this reserve are inhabited by ants, with *Pheidole bicornis* living in the petiole chambers and stems of all the plants in most patches (23). Most of the plants that are not inhabited by ants have been invaded by the specialist ant predator *T. letourneauae* as either small, newly established individuals [seedlings and small fragments (leaves or twigs) that have broken off a larger plant and rooted] or as fully established plants. Therefore, we tested the effects of top-down forces on *P. cenocladum* and its trophic web associates by using two separate experiments within the forest reserve: one with plant fragments and one with naturally occurring plants.

The long-term fragments experiment, initiated in July 1994, used 360 *P. cenocladum* cuttings, each with three leaves, established as 10 transplants per plot in 24 10 m × 10 m plots. This experiment was designed to detect indirect effects of top predators on *P. cenocladum* fragments, which, as vegetative ramets of established plants, constitute the major form of reproduction for this shrub at La Selva (24). Experimental plots were distributed among areas with different levels of canopy cover (70–96% cover depending on the proximity of tree falls and the particular species composition of trees overhead) and on both inceptisols and ultisols, which represent the richest and poorest soils, respectively, that support natural stands of *P. cenocladum* at La Selva (22). Top predators were introduced into half of the plants and levels of ants, herbivory, and leaf area were compared over a 3-year period. The second experiment, established in mid-April 1996, used 80 naturally occurring plants, 35–140 cm tall, on ultisol soils to measure the effects of predatory beetles over 1.5 years. Canopy cover for these individual shrubs ranged from 94% to 98%.

Top predator treatments were applied to half of the plants (randomly selected) in each experiment by collecting early instars outside the experiment and manually transferring the *Tarsobaenus* beetle larva to a petiole chamber of the treatment plants. Manual transfer of early instars poses some risk to the larvae, which normally enter the petiole after eclosing from an egg deposited by the adult on the exterior of the petiole. To

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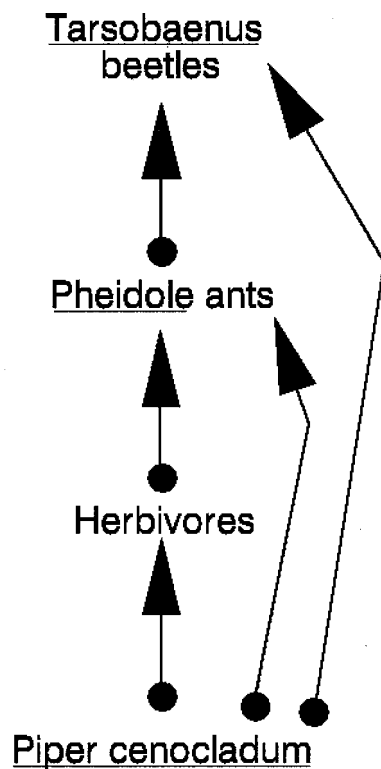


FIG. 1. Trophic structure of the target arthropods associated with *P. cenocladum* shrubs. Arrows, flow of energy; ●, negative effect caused by consumption. Arrows from the plants to both the ants and beetle larvae are present because both the ants and beetles eat food bodies produced by the plant.

increase the survival rate of transferred larvae, which can be vulnerable to ant defensive behaviors, we weakened the ant colony with 0.2 ml of very dilute insecticide (one to two drops of 0.85 mg/liter Diazinon wettable powder in distilled water) introduced into each petiole of the shrub 2–4 weeks before beetle larvae were introduced in December 1994 and June 1996 for the two experiments. Here, we analyze data for control plants in the cuttings experiment that were also treated with the insecticide because these were the only control plants for which we collected data over the entire sampling period. In a previous, shorter study (22), we reported data for unmanipulated controls because the effects of insecticide on ants, percentage herbivory, and leaf area were not significant over the long term (Table 1). Although standard statistical criteria indicated no significant differences between the two controls, there did seem to be a slight effect of insecticides on plant size (Table 1), but this effect was much smaller than the eventual effects of the beetles (22). Our insecticide treatments were

even less effective at killing ant colonies when they were added to shrubs. In fact, identical applications of insecticides that were concentrated by more than 4 orders of magnitude did not kill the ant colonies in our preliminary tests with *P. cenocladum* shrubs outside the experiment, and the only way to maintain depressed colony numbers in those shrubs was to continually (i.e., weekly or monthly) apply the insecticides (L.A.D. & D.K.L., unpublished observations). Because the insecticides did not significantly affect ants or other trophic levels over the long term (ref. 22; L.A.D. & D.K.L., unpublished observations), an unmanipulated control was used for the experiment with naturally occurring shrubs. In both experiments, we reintroduced new beetle larvae to one petiole per treatment plant every 3–4 months because early instars take 3–4 months to leave the plant as adult beetles. These subsequent beetle introductions did not require application of insecticides.

To monitor the effects of predatory beetles on herbivory and plant size, we tagged each cutting and shrub with a unique number. At this time, none of the fragments had new growth, so ant density, leaf area, and herbivory were initially zero. Ant density was still zero in the new growth even 2 months after the initial tagging because new leaves emerge from what will become petiole chambers for ants; thus, several months of growth must occur for the new leaves to house ants. Initial ant density in established shrubs was estimated from subsamples (two to three petiole chambers), and only plants with ants in each chamber sampled were retained in the experiment. Starting in January 1995 and June 1996 in the respective experiments, before predatory beetle treatments were applied, each petiole chamber on every plant was gently pried open to detect the presence or absence of ants, and all leaves were measured along with the area of leaf tissue lost to herbivores. Potential leaf area was estimated by measuring the area of entire leaves, filling in leaf margins when there was herbivore damage, and counting the number of 0.23-cm² grid squares on a transparent thermoplastic overlay. Percentage herbivory, which we used for analyses, was calculated as leaf area eaten divided by potential leaf area. Standing leaf area was calculated as potential leaf area minus leaf area removed by herbivores. These measurements were repeated at intervals of 2–5 months and again in July 1997, when both experiments were terminated.

We examined the effects of top predator manipulation on ant density in the plants, the percentage leaf area eaten by arthropod herbivores, and the average leaf area per plant over time by using multivariate ANOVA (each sampling date was a separate dependent variable) followed by profile analysis, comparing the last sampling date with the first. All *F* values reported are Wilks' λ for the profile analysis. These analyses were performed only with data for the new growth from fragments, thus representing the response to experimental conditions. All analyses in this paper were performed with SAS (version 8.06).

Table 1. Results of multivariate and univariate ANOVAs comparing insecticide-treated and unmanipulated controls

	Multivariate ANOVA	Sampling date					
		1	2	3	4	5	6
Ants, %	$F_{6,24} = 1.4$ $P = 0.2$	No variance	$F_{1,26} = 8.9$ $P = 0.007$	$F_{1,26} = 3.1$ $P = 0.09$	$F_{1,26} = 0.05$ $P = 0.8$	$F_{1,26} = 2.2$ $P = 0.2$	$F_{1,26} = 0.06$ $P = 0.8$
Herbivory, %	$F_{6,21} = 1.7$ $P = 0.2$	$F_{1,26} = 1.8$ $P = 0.2$	$F_{1,26} = 0.5$ $P = 0.5$	$F_{1,26} = 1.9$ $P = 0.2$	$F_{1,26} = 0.8$ $P = 0.4$	$F_{1,26} = 0.5$ $P = 0.5$	$F_{1,26} = 3.1$ $P = 0.09$
Leaf area, cm ²	$F_{6,21} = 1.9$ $P = 0.1$	$F_{1,26} = 0.2$ $P = 0.6$	$F_{1,26} = 8.8$ $P = 0.006$	$F_{1,26} = 3.5$ $P = 0.07$	$F_{1,26} = 6.0$ $P = 0.02$	$F_{1,26} = 6.0$ $P = 0.02$	$F_{1,26} = 5.3$ $P = 0.03$

The multivariate ANOVA was performed by using separate sampling dates (for the first 18 months) as unique dependent variables and the three levels of the predator treatment (control, insecticide control, and beetles) as independent variables. The *P* values reported are from a contrast between the insecticide control and the unmanipulated control. The ANOVAs were conducted for each sampling date with a contrast between insecticide and unmanipulated controls. To adjust for multiple hypothesis tests, a critical α of 0.002 was used (using a standard Bonferroni correction). No differences were significant. Profile analysis that was performed by comparing the first two sampling dates and using the same contrast yielded similar results.

In an associated correlational study conducted in June and July 1995, we examined herbivory levels on *P. cenocladum* that harbored naturally occurring top predators. To test whether herbivore damage was greater on shrubs containing top predators than on shrubs without beetles but exposed to the same conditions, we categorized damage (high, at least one leaf with >50% of the leaf area removed; or low, all leaves having less than 50% damage) on *P. cenocladum* shrubs containing beetles ($n = 145$), on their nearest neighbor conspecific without beetles ($n = 145$) and a plant in the next nearest patch without a beetle ($n = 145$). Beetle-containing shrubs were located by examining all individuals found within 40 m of all the trails at La Selva where *P. cenocladum* occurs (≈ 30 km of trails). The distance from each shrub with a beetle larva to its nearest neighbor (without a beetle) was measured. To test the additional hypothesis that herbivore damage is reduced as distance increases from the beetle-containing plant, we used logistic regression, with herbivory levels as the dependent variable and distance from the beetle-containing plant as a predictor variable.

We also include data from an ancillary experiment to test for an association between herbivory rates and survival rates of understory plants. All understory plants between 20 cm and 2 m in height in 16.2 m \times 4 m survey plots (8 demarcated within all top predator treatment plots of the fragments experiment and 8 in sites at least 75 m from beetle additions) were tagged and identified in July, 1994. Presence or absence of each individual and the maximum level of herbivory (the proportion of leaf lost on the most damaged leaf of each plant was estimated visually) were recorded before beetles were applied to treatment plants and at 8, 12, 16, 20, and 24 months after beetles were added to plants surrounding half the survey plots. Survivorship was monitored for all plants tagged in the initial sample. To test whether herbivore damage can determine survivorship of established understory plants, the frequency of plants that lived for 2 years vs. those that died during the experiment was compared for plants with high initial herbivory (at least one leaf with >50% of leaf tissue lost) and low initial herbivory (0–5%) by using the Fisher's exact test. Finally, we compared herbivore damage levels (frequency of high and low damage) after 1 year on understory plants in survey plots within the *P. cenocladum* beetle addition plots with damage on understory plants in survey plots away from beetle addition plots, also by using Fisher's Exact test.

RESULTS AND DISCUSSION

Cascading effects of top predators were evident through three trophic levels in experiments with transplanted *P. cenocladum* fragments and with naturally occurring, established shrubs, but the effects occurred more slowly in fragments as they moved through the trophic structure from beetles to ants to herbivores to plants. Direct effects of beetles on ants were shown by the development of 2.2 times lower ant densities (a significantly lower percentage of ant-colonized petiole chambers) in new growth from beetle-treated fragments as compared with new growth from control fragments that had been treated only with insecticides (Fig. 2A; $F_{1,16} = 5.5$, $P = 0.032$). The differences in ant density between plants with and without beetles were consistent for the two controls, which was also true for percentage herbivory and leaf area. When top predators were applied to naturally occurring shrubs, ants were reduced significantly compared with control plants ($F_{1,75} = 46.0$, $P < 0.0001$), and the pattern persisted throughout the experiment. On the last sample date, ant density was reduced by a factor of 1.7 on shrubs with top predators compared with control shrubs (Fig. 3A).

Indirect effects of top predators on herbivory levels were detectable in less than 1 year. Fragments experienced up to 2.4 times as much herbivory (Fig. 2B, $F_{1,11} = 9.4$, $P = 0.011$) when they were inoculated with predatory beetles. Stronger effects

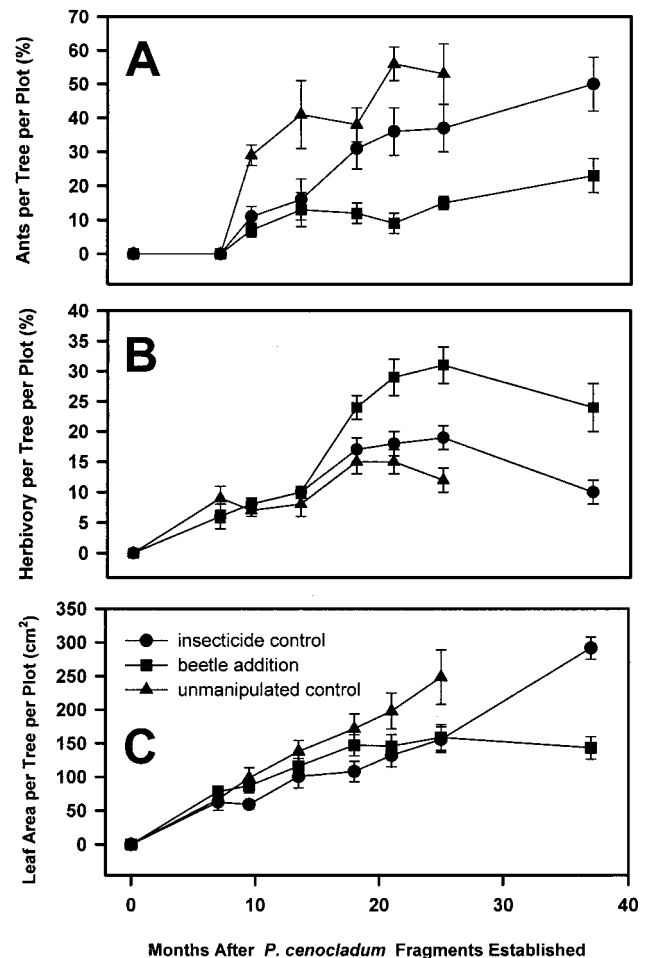


Fig. 2. (A) Mean density of ants (percentage of petiole chambers containing ants per transplanted *P. cenocladum* fragment per 10 m \times 10 m plot). (B) Mean folivory (leaf area eaten on all leaves of 10 transplanted fragments per plot). (C) Mean leaf area per shrub per plot. Measurements were made on new plant growth before (0–8 months) and after (10–37 months) beetle additions to 120 of 240 plants. Newly produced petioles did not have ants on the first sampling dates. Error bars, SE. The legend in C applies to A–C.

of top predators on herbivory in standing shrubs (Fig. 3B, $F_{1,75} = 23.4$, $P < 0.0001$) were shown, possibly because of both early effects of insecticide application on ant colonies in the fragment controls and potentially higher herbivore pressure in low-light environments (22). Established shrubs with beetles had 3 times greater herbivory than control shrubs (Fig. 3B).

A reduction in the total leaf area of *P. cenocladum* fragments because of predatory beetles took longer to accrue (Fig. 2C, $F_{1,11} = 5.5$, $P = 0.038$), with significant effects detectable only in the last sample when leaf area of control plants was twice as great as for plants with beetles. Again, the contrast was more rapid in naturally occurring shrubs with unmanipulated ant colonies (Fig. 3C, $F_{1,77} = 26.4$, $P < 0.0001$), with leaf area increasing by a factor of 2.0 within a year of beetle additions. These responses of 2–3 times greater ant density, lower herbivory, and greater total leaf area per shrub were consistent for control fragments and shrubs, as compared with fragments and shrubs with top predators. Thus, the top predator additions in our complex terrestrial study site caused changes predicted by top-down trophic cascades hypotheses (1–3).

P. cenocladum fitness could, therefore, be affected by this cascade in a number of ways. If the ant colony in an established plant is reduced in number by predation, then fragments from that individual would likely have more herbivore damage and less leaf tissue than fragments with undisturbed ant colonies.

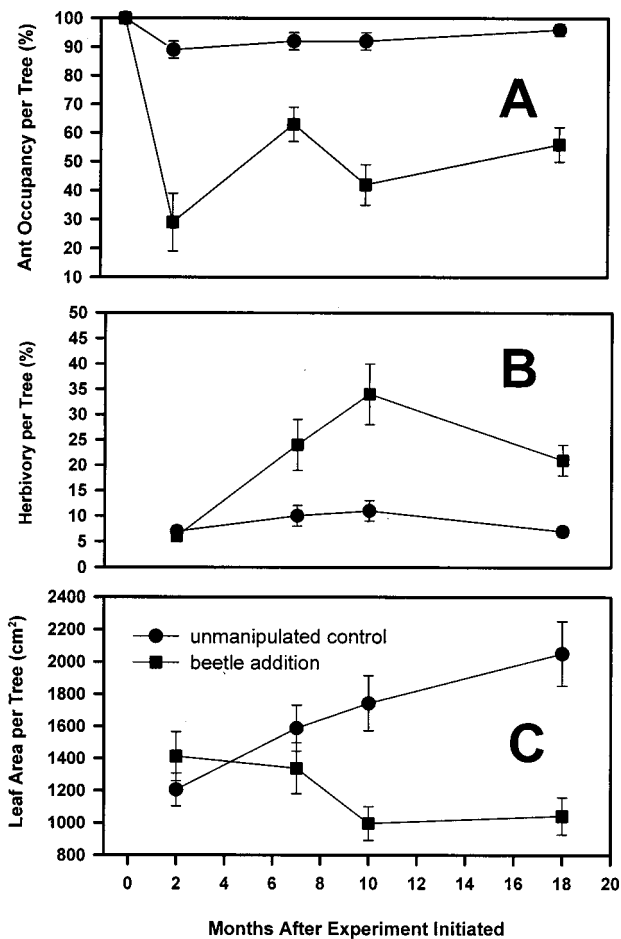


FIG. 3. (A) Mean density of ants (percentage of petiole chambers per plant containing ants). (B) Mean folivory (leaf area eaten on all leaves per established *P. cenocladum* shrub). (C) Mean leaf area per shrub. Initial measurements were taken before (0–2 months) and after (7–18 months) beetles were added to petiole chambers on 40 of 80 plants. Measurements were made on all leaves until the final sample, which was estimated from a sample of four leaves per shrub. Error bars, SE. The legend in C applies to A–C.

Damaged fragments may not establish successfully (25). Likewise, once a fragment is created, it may be crucial to the plant that ants colonize the petiole chambers before beetles; otherwise, the plants will lose too much leaf tissue in the early stages of rooting.

In the associated nearest-neighbor study, we found that *P. cenocladum* shrubs found containing *Tarsobaenus* beetles had the highest frequency of severe herbivore damage (61% of 145 shrubs), followed by their nearest neighbors (41% of 145 shrubs had high herbivory) and then plants from patches without beetles (21% of 145 shrubs with high herbivory) ($\chi^2 = 12.78$, $df = 2$, $P = 0.0017$). In addition, distance from a beetle-containing shrub was a significant predictor of herbivory levels on locally occurring *P. cenocladum* ($\chi^2 = 11.18$, $df = 1$, $P = 0.0008$; Fig. 4), with *P. cenocladum* shrubs near beetle containing individuals suffering higher levels of herbivory than those further away. Thus, the effect of beetles also cascaded to nearby individuals.

If the survival or reproductive success of *P. cenocladum* in the forest understory affects other plant species through competitive or positive interactions, then effects of top predators potentially extend to the plant community through plant-plant interactions. Insect-plant interactions within the understory are also possible: given the high diversity of the wet forest understory (113 species of plants in 47 families were

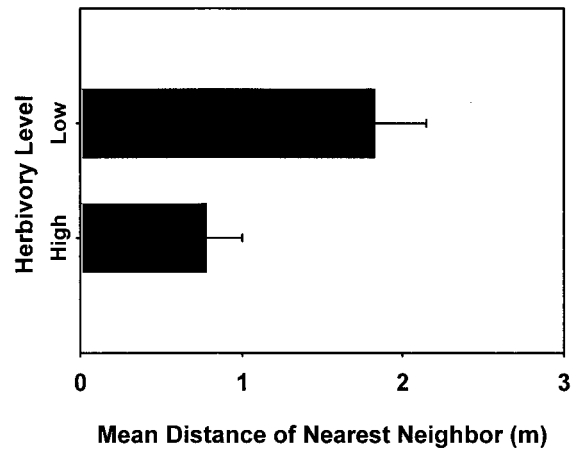


FIG. 4. Mean (bars, SE) distance from a naturally occurring *P. cenocladum* plant containing a *Tarsobaenus* beetle (top predator) for the nearest neighbor *P. cenocladum* (without a beetle) having high vs. low herbivore damage.

identified in our understory survey plots), other species may also serve as hosts to the herbivores released by the presence of top predators. Indeed, most of the lepidopteran larvae that eat *P. cenocladum* are generalists on plants in the Piperaceae (geometrids, limacodids, and hesperiids) as well as plants in several other families (apatelodids) (24). Thus, the effect of beetles could potentially cascade to the local plant community via species that are alternative hosts of the generalist herbivores. Our survey data support this notion, with 53% of understory plants exhibiting high herbivory in plots with beetle-additions vs. 25% of understory plants in areas with no top predators added. Overall, we did not find a significant effect of initially high herbivory rates on the survivorship of understory plants (53% of 87 plants with high herbivory died compared with 47% of 76 plants with low herbivory; Fisher's exact test, $P > 0.05$). Herbivory may be a stronger determinant of seedling survival than of established plants (20 cm to 2 m tall) that were surveyed in our experiment. When only plants less than 60 cm tall were used in the analysis, 60% of plants ($n = 40$) with high herbivore damage died compared with 40% of those with low herbivory ($n = 41$; Fisher's exact test, $P = 0.07$).

In conclusion, top-down trophic cascades occurred in a species-rich, terrestrial community with four trophic levels, despite predictions that such cascades could not occur in such complex systems (7). A specialist top predator caused cascading effects through three trophic levels, resulting in fewer predators, greater herbivory, and smaller plants with less leaf area. These effects cascade to other plants of the same species in the understory vegetation and, possibly, to different species of plants that share the same herbivores. Such a four-trophic level cascade could ultimately alter community variables such as plant species richness.

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