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Effects of Experimental Seaweed Deposition on Lizard and Ant Predation in an Island Food Web

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The effect of environmental change on ecosystems is mediated by species interactions. Environmental change may remove or add species and shift life-history events, altering which species interact at a given time. However, environmental change may also reconfigure multispecies interactions when both species composition and phenology remain intact. In a Caribbean island system, a major manifestation of environmental change is seaweed deposition, which has been linked to eutrophication, overfishing, and hurricanes. Here, we show in a whole-island field experiment that without seaweed two predators—lizards and ants—had a substantially greater-than-additive effect on herbivory. When seaweed was added to mimic deposition by hurricanes, no interactive predator effect occurred. Thus environmental change can substantially restructure food-web interactions, complicating efforts to predict anthropogenic changes in ecosystem processes.

Global environmental change is expected to have a profound impact on the structure and function of ecological communities by changing interactions between their component species. Range shifts, extinctions, and species introductions change community composition, deleting some interactions and adding others (1, 2). In addition, changes in the seasonal timing of migration and other life-history events can produce phenological mismatches, which can affect communities even when species composition is unchanged (1–3). The absence of alterations in species composition and phenology, however, does not necessarily mean that ecosystems will remain unaltered (4): Environmental change can also influence ecosystem processes by reconfiguring interactions in communities whose species lists remain intact (5–9).

An important aspect of global environmental change is the mobilization and transport of resources between ecosystems. Seaweed deposition in particular is likely to become a more common feature in shoreline ecosystems as anthropogenic effects (such as overfishing and eutrophication) facilitate a shift toward algae-dominated marine ecosystems (10). Furthermore, intense storms, which are associated with the deposition of large amounts of seaweed (11, 12), have increased in frequency—a trend that is expected to continue with increasing global warming (13). Such pulsed inputs of external resources can increase prey availability, “subsidizing” in situ predators (14–17) and altering predator effects on lower trophic levels (12, 16, 18–21).

We used a whole-island field experiment in the Bahamas to probe how major seasonal pulses of seaweed deposition—mimicking what occurs in an active storm year (22)—influence the effects of multiple predators on herbivory in a terrestrial food web. Twelve small islands (one is shown in Fig. 1A), six with lizards and six naturally lizard-free, were used in the experiment. Seaweed was added or removed from islands in a

crossed design; each combination of seaweed and lizard presence or absence was represented by three islands. Seaweed was manipulated in October and December of 2008, coinciding with that season when large storms are most likely to cause natural deposition events. On seaweed-addition islands, 0.4 to 1.4 kg/m² of seaweed was distributed patchily throughout each island, mimicking what occurs after a large storm (22). The removal treatment maintained seaweed at a level near zero, which is consistent with natural levels of about half of the islands before manipulation. Ant exclusions were established on branches of four (three in one case) *Conocarpus erectus* plants on each island; ants were excluded with a sticky resin, which lizards were able to bypass by crossing a narrow gap between wire mesh cones (Fig. 1B). Each of the four predator treatments in the experiment—(i) ants and lizards absent; (ii) ants present and lizards absent; (iii) ants absent and lizards present; and (iv) ants and lizards present—was represented by 12 branches in the absence of seaweed subsidy and 11 or 12 branches in the presence of seaweed subsidy.

Anolis sagrei was the only lizard species on the lizards-present islands. Previous experiments in this ecosystem demonstrated that *A. sagrei* reduced herbivory on *C. erectus* (23, 24), one of the most common plants in shoreline habitats. Extrafloral nectaries on *C. erectus* foliage attract mutualistic ants, which can also decrease herbivory (25). The possibility that these two predators interactively affect lower trophic levels (26) had not been explored in previous studies.

Figure 1C shows a working model of the main food web components in our system. Seaweed deposits support an abundance of detritivores, which attract both ants and lizards (12). The most common herbivorous arthropods include Coleoptera, Lepidoptera, and Hemiptera (12). Previous studies in this system indicate that a shift in predator foraging behavior after two closely spaced pulses of seaweed is associated with increased herbivory on *C. erectus* (12), suggesting that the subsidies

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interfere with herbivore suppression by predators (27).

The present study shows that seaweed subsidies diminish the effect of predators on *C. erectus* by eliminating the greater-than-additive effect of

ants and lizards on herbivory found in the absence of seaweed subsidies. Specifically, without seaweed the combined effect of ants and lizards on herbivory was more than three times greater than expected on the basis of the sum of their individual

effects (Fig. 2, left). In other words, the difference between the treatment with both predators present and the no-predators treatment was three times greater than the difference between the ants/no-lizards treatment and the no-predators treatment plus the difference between the lizards/no-ants treatment and the no-predators treatment. In contrast, in the presence of seaweed subsidies there was no interactive effect of ants and lizards on herbivory (Fig. 2, right). Ant abundance increased on islands with seaweed subsidies—the (least-squares) mean ant abundance on seaweed-addition islands was higher than that on seaweed-removal islands—but was not affected by lizards (Fig. 3A). In contrast to ants, lizards did not increase in overall abundance on subsidized islands (Fig. 3B).

We suggest that the synergistic effect of lizards and ants on herbivory in the absence of seaweed subsidies derives from the fact that these two predators are active at different times of day. The only lizard species present, *A. sagrei*, is diurnal. In contrast, the dominant ant species (comprising 88% of all pan-trapped ants), *Camponotus tortuganus*, is nocturnal. This temporal partitioning of foraging activity may create a dilemma for herbivores: They can avoid *A. sagrei* by feeding at night, and they can avoid *C. tortuganus* by feeding during the day, but they cannot simultaneously avoid both types of predators. This hypothesis is analo-

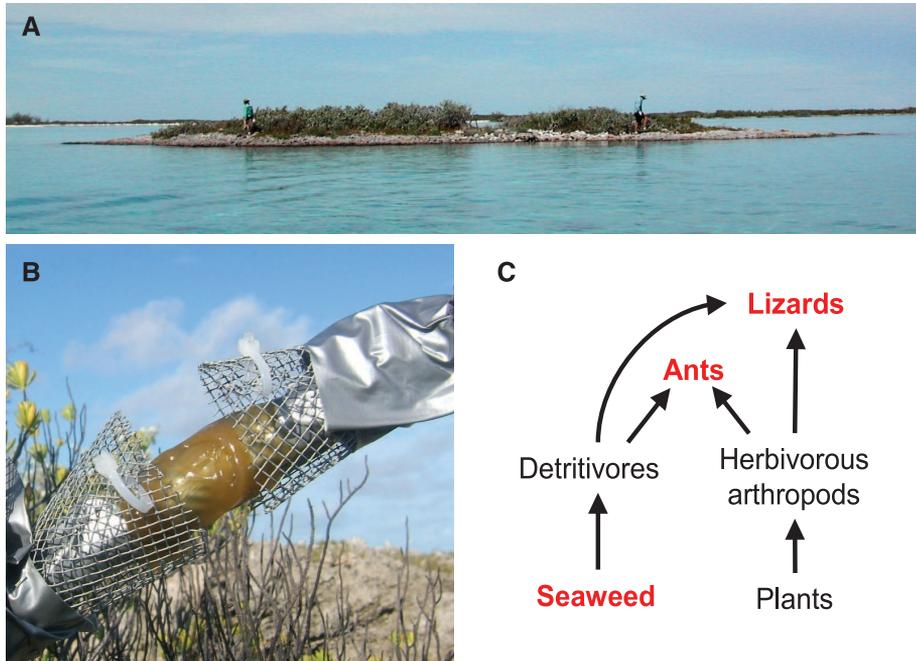


Fig. 1. Study system. (A) One of the twelve experimental islands. (B) An ant-exclusion treatment on one of the experimental plants. The wire mesh cones on either side allow lizards to pass over the sticky resin barrier. (C) Working hypothesis of food-web relationships on the small islands used in this experiment. Red text denotes the components that were manipulated in this study.

Fig. 2. Effects of seaweed, lizards, and ants on herbivory in *C. erectus*. (A) Leaf damage on *C. erectus* in four predator treatments on seaweed-removal and seaweed-addition islands. Data are mean \pm SE; $n = 11$ branches for the seaweed addition–lizards treatments and 12 branches for all other treatments. The interactive effect of lizards and ants on *C. erectus* leaf damage differed between seaweed treatments [mixed model analysis of covariance (ANCOVA); seaweed* lizard*ant interaction: $F_{1,39} = 6.0$, $P = 0.02$]. On seaweed-removal islands, there was a greater-than-additive effect of ants and lizards on herbivory (mixed model ANCOVA; lizard*ant interaction on seaweed-removal islands: $F_{1,20} = 11.5$, $P = 0.003$), but there was no such interactive effect on seaweed-addition islands (mixed model ANCOVA; lizard*ant interaction on seaweed-addition islands: $F_{1,19} = 0.16$, $P = 0.7$). (B) Predator effects on leaf damage. Effect sizes were calculated as \ln (leaf damage with predators/leaf damage without predators) by using least-squares means from the mixed model ANCOVA described in (22).

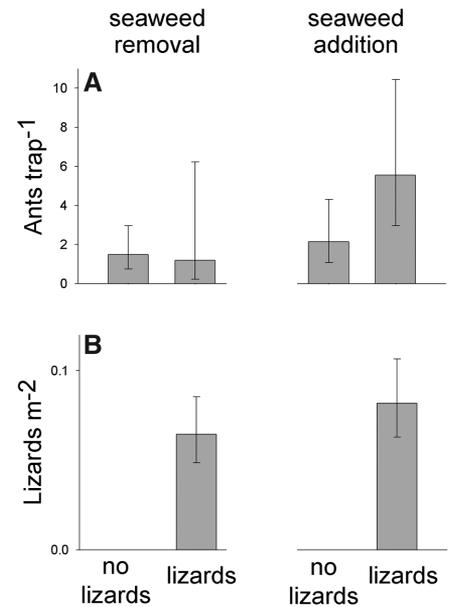
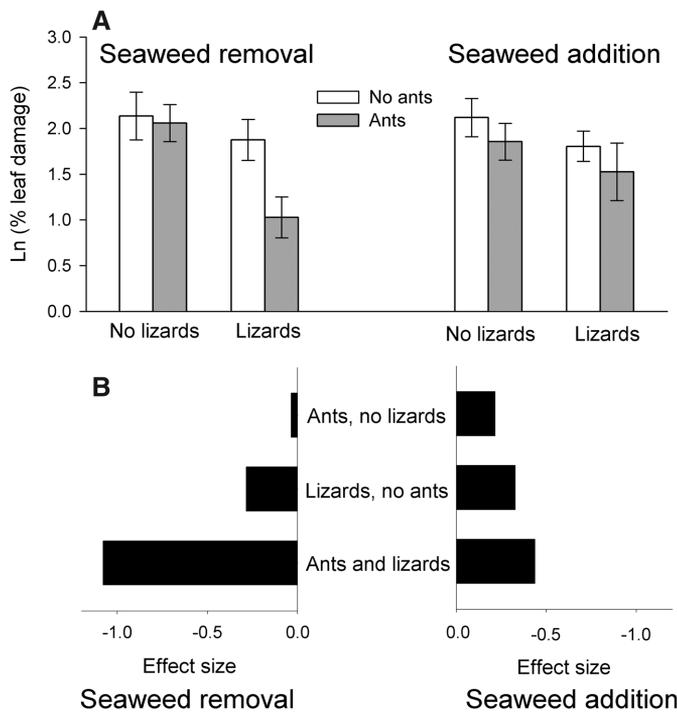


Fig. 3. Effects of seaweed on ant and lizard abundance. (A) Ant abundance in pan traps on experimental islands. Ant abundance was higher on seaweed-addition islands than seaweed-removal islands (ANCOVA; $F_{1,7} = 7.7$, $P = 0.03$); there was no difference between lizard and no-lizard islands [analysis of variance (ANOVA); $F_{1,7} = 0.15$, $P = 0.71$]. (B) Lizard abundance on experimental islands. There was no effect of seaweed on lizard density (ANOVA; $F_{1,4} = 0.38$, $P = 0.60$). Data are mean \pm SE of log-transformed abundances; $n = 3$ islands for each treatment combination.

gous to what has been observed when predators exhibit spatial complementarity in foraging: Prey seeking to escape one predator enter habitats in which they are more vulnerable to the other predator (26). In addition to eliminating prey refuges, temporal partitioning may reduce the frequency of antagonistic interactions between predators, which probably explains why lizards did not affect ant abundance in our study.

When seaweed subsidies were added to the system, the greater-than-additive effect of ants and lizards on herbivory was not present. We suggest that the seaweed caused both ants and lizards to spend more time on the ground foraging for detritivores associated with seaweed deposits, reducing their combined impact on herbivory. This hypothesis is consistent with the results of an earlier study, in which seaweed was added or removed from shoreline plots on large islands (12). Despite higher abundances of both ants and lizards in seaweed-addition plots, *C. erectus* sustained higher levels of herbivory. An analysis of carbon-stable-isotope signatures indicated that lizard diets contained more marine-derived prey in seaweed-addition plots, and ants were observed foraging for detritivores in the seaweed deposits, suggesting that shifts in predator foraging behavior were responsible for the observed increases in herbivory (12). In the current experiment, the increase in ant density in pan traps may have been caused by a shift in foraging patterns, although an increase in the overall abundance of ants resulting from increased food supply may have also been contributory. Although lizard density increased in response to seaweed subsidies in mainland plots (12), we observed no such increase in the current study. We suggest that the absence of a numerical response in lizards on small islands was caused by (i) the inability of lizards to immigrate to the experimental sites from surround-

ing areas (as they could in the previous, mainland-plot experiment) and (ii) the lack of reproductive activity during much of the study period (12). Because emigration and reproductive lags influence the timing of predator responses to subsidy, we expect the long-term impact of pulsed seaweed subsidies on predator effects to depend on the frequency of pulses and degree of habitat isolation (15, 16). Seaweed did not appear to cause a reduction in the effects of either ants or lizards by themselves (Fig. 2B), suggesting that the interactive effect of these two predators is more sensitive to subsidy than their individual effects.

Predicting the effects of environmental change on ecosystems is an important challenge. There is increasing recognition that species interactions strongly influence how environmental change affects ecosystem processes, complicating efforts to make reliable forecasts (28, 29). Our results show that large seaweed-deposition events affect the structure and function of an ecological community by reconfiguring the effects of multiple predators on lower trophic levels. This suggests that predictions that are based on single-species responses or pairwise interactions may not adequately represent community responses to environmental perturbations. Experiments such as the one we report here, conducted at a spatial scale large enough to capture community-wide dynamics, are particularly relevant for conservation and management decisions in the face of ever-increasing anthropogenic disturbances.

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Materials and Methods

SOM Text

Figs. S1 to S3

References

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Metagenomic Discovery of Biomass-Degrading Genes and Genomes from Cow Rumen

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The paucity of enzymes that efficiently deconstruct plant polysaccharides represents a major bottleneck for industrial-scale conversion of cellulose biomass into biofuels. Cow rumen microbes specialize in degradation of cellulose plant material, but most members of this complex community resist cultivation. To characterize biomass-degrading genes and genomes, we sequenced and analyzed 268 gigabases of metagenomic DNA from microbes adherent to plant fiber incubated in cow rumen. From these data, we identified 27,755 putative carbohydrate-active genes and expressed 90 candidate proteins, of which 57% were enzymatically active against cellulosic substrates. We also assembled 15 uncultured microbial genomes, which were validated by complementary methods including single-cell genome sequencing. These data sets provide a substantially expanded catalog of genes and genomes participating in the deconstruction of cellulosic biomass.

Biofuels derived from lignocellulosic plant material represent an important renewable energy alternative to transportation fossil

fuels (1, 2). A major obstacle to industrial-scale production of fuel from lignocellulose lies in the inefficient deconstruction of plant material, owing

to the recalcitrant nature of the substrate toward enzymatic breakdown and the relatively low activity of currently available hydrolytic enzymes. Although the success of protein engineering to improve the performance of existing lignocellulose-degrading enzymes has been limited (3), retrieving enzymes from naturally evolved biomass-degrading microbial communities offers a promising strategy for the identification of new lignocellulolytic enzymes with potentially improved activities (4).

Metagenomics, the direct analysis of DNA from environmental samples, represents a strategy for discovering diverse enzymes encoded in nature (5, 6). Although metagenomics has been used

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Supporting Online Materials

Materials and Methods

Study site and experimental design

This study was conducted on 12 small islands (areas 194 – 1747 m²) in the Exuma island chain in the central Bahamas. Six of these islands are located in the vicinity of Georgetown, Great Exuma (23.5° N, 75.8° W), and the other six are located 100 km to the northwest, near Staniel Cay. Brown anoles (*Anolis sagrei*) are among the most common vertebrate predators in the Bahamas and were present on six of the experimental islands; five of these lizard populations were the result of experimental introductions while the sixth was the result of natural colonization. Four of the islands with lizards were near Georgetown, while the remaining two were near Staniel Cay. The six islands without lizards had been lizard-free for at least 15 years at the time of the study. Islands with and without lizards did not differ in area (*t*-test: $P=0.76$).

The 12 islands in the experiment were divided into three blocks of four islands. Each block contained two islands with lizards and two islands without lizards. Given that constraint, blocks were chosen on the basis of location and island area. Seaweed addition and removal treatments were assigned randomly within each block such that each of the four seaweed/lizard combinations was represented by one island. Seaweed was manipulated in October 2008 and again in December of the same year, coinciding with the season when large storms are most likely to cause natural deposition events. There was enough natural variation in the amount of seaweed deposited on the study islands (coefficient of variation = 1.5 in October 2008, prior to manipulation) that we opted to use seaweed removals rather than unmanipulated controls in order to ensure that amounts of seaweed differed substantially between treatments. On seaweed-addition islands 0.4 – 1.4 kg/m² of seaweed was added during each session, an amount corresponding to deposition events associated with large storms (Figure S1), and within the range of natural variation observed on shoreline plots on larger islands (6 out of 29 plots had > 1 kg/m² seaweed at some point between November 2007 and October 2010 [unpublished data]). Seaweed was distributed patchily throughout seaweed-addition islands, mimicking what occurs after a large storm. Seaweed was removed from seaweed-removal islands during each session, bringing them down to the same level as about half the islands before manipulation.

Ant exclusions were conducted on four *Conocarpus erectus* plants on each island except one, which had only three experimental plants. *Conocarpus erectus* was one of the most common plant species on the study islands. The nocturnal *Camponotus tortuganus* is the most common ant species on *C. erectus* plants at our study sites (SI). Two branches were selected on each plant, one of which was randomly chosen for ant-exclusion while the other served as a control. Ants were excluded using Tree Tanglefoot® (The Tanglefoot Company) applied on top of a band of duct tape wrapped around the branch to protect the plant from the resin; control branches had duct tape but no tanglefoot applied on top of the tape. Branches in both ant-exclusion and control treatments had two cones of wire mesh hardware cloth installed on either end of the duct tape (Fig. 1B). These cones extended towards each other but did not meet, allowing lizards but not ants to cross from cone to cone and bypass the Tanglefoot® used in the ant-exclusion treatment. Trials

conducted with field-captured lizards confirmed that they easily crossed over the Tanglefoot barriers. Twenty of the 47 plants used in the experiment had three experimental branches and featured a cage-control treatment – duct tape without tanglefoot and without lizard cones – in addition to the ant-exclusion and control treatments. Analyses of leaf damage on plants with all three treatments indicated that the lizard cones did not affect leaf damage (Figure S2).

Data collection

Lizard density was measured on islands where they were present in October 2008, prior to the experiment, and again in May 2009. Lizard abundance was estimated using multiple-mark-recapture censuses (S2), and density was expressed as lizards per m² of vegetated area.

A sample of ten randomly-selected leaves was collected from each branch in October 2008 and another sample was collected in May 2009. Trichome density was estimated by examining the surface of each leaf at 50X magnification and giving it a score from 0 – 3 (0: < 1 mm⁻², 1: ~125 mm⁻², 2: ~250 mm⁻², 3: >375 mm⁻²). Each sample of leaves was pressed and photographed, and the digital images were analyzed for leaf damage (S3). Experimental plants grew little during the course of the experiment, which took place mainly during the dry season, when plant growth is typically low and ant activity is typically high.

Ant abundance was monitored on experimental islands in October 2008 and May 2009 using pan traps. The traps consisted of a plastic bowl covered with ½ inch hardware cloth (to keep hermit crabs out) and filled with water containing a small amount of detergent; traps were set for one 24 h period on each island. Pan traps were distributed systematically on each island by placing them at regular intervals along a transect stretching the length of the island. Ants were identified to genus or species and densities were calculated on a per trap basis. Herbivore abundance was measured in October 2008 and May 2009 using aerial sticky traps. On each island six traps were tied to *C. erectus* branches approximately 1 m off the ground. After 24 h, traps were removed and insects were identified to Order. Lepidoptera, Hemiptera (Auchenorrhyncha), and Coleoptera were considered to be herbivores (most Coleoptera were in the family Chrysomelidae – predatory taxa were rare) and densities were calculated on a per trap basis.

Statistical analyses

Leaf damage was analyzed using a mixed model ANCOVA in which island and plant were random factors. In this model plants are nested within islands and branches are nested within plants, thereby accounting for the lack of independence between samples taken from plants on the same island and from branches on the same plant. Seaweed treatment, lizard presence/absence, and block were used as island-level fixed effects. Ant treatment was used as a branch-level fixed effect and average trichome density was used as a branch-level covariate, as trichomes are associated with lower leaf damage (S1, S4, S5). Trichome densities were centered using a z-transformation to facilitate the interpretation of model parameters. Leaf damage data collected prior to the initiation of seaweed and ant treatments was used as a covariate in preliminary models, but was dropped as it was not significant ($P>0.5$). Leaf damage was log-transformed to meet model assumptions and make appropriate tests for interactive effects of predators (S6).

Preliminary models included interactions between all fixed effects except block. Non-significant interactions between fixed effects were removed from the final model (S7). In addition to ant, lizard, and seaweed effects (and their interactions), the final model included a seaweed*ant*trichome interaction ($P=0.02$). Fitting a model without interactions between trichomes and other fixed effects yields results similar to those described in the text (e.g. seaweed*lizard*ant interaction: $P=0.045$; lizard*ant interaction on seaweed-removal islands: $P=0.003$; lizard*ant interaction on seaweed-addition islands: $P=0.98$).

The effect of seaweed on ant, lizard, and herbivore densities was analyzed using ANCOVA with seaweed treatment and block as factors and abundance prior to the initiation of treatments as a covariate (this covariate was removed if it was not significant at $P=0.05$). The covariate was retained in the model for ant density ($P=0.002$), but not in the models for lizard and herbivore density ($P=0.19$ and $P=0.98$, respectively). A similar analysis was used to analyze the effect of lizards on ant and herbivore densities, but the covariate was dropped, as lizards were not manipulated in this study. All densities were log-transformed to meet model assumptions.

Analyses were conducted using the `lm` and `lme` functions in R (S8, S9). All hypotheses were tested using two-tailed tests.

Supporting Text

Effects of seaweed and lizards on herbivores

There was no effect of seaweed or lizards on herbivore abundance on experimental islands (ANOVA: $F_{1,7}=0.003$, $p=0.96$, and $F_{1,7}=1.6$, $p=0.24$, respectively), and no evidence for a seaweed*lizard interaction (ANOVA: $F_{1,6}=1.1$, $p=0.34$) (Figure S3). In our previous study of shoreline plots, seaweed deposition increased herbivore abundance (*SI*). We suggest that this effect was not observed in the current study in part because herbivores cannot migrate to small islands as readily as shoreline plots, limiting the possibility of an aggregative response.

While herbivory tended to be lower on islands with lizards (Figure 2), there was no indication that lizards reduced herbivore density. We suggest two reasons for the discrepancy between these two results. First, the effects of predators on herbivory may be mediated by changes in herbivore traits, rather than changes in herbivore density. If this were the case, predator effects on herbivory would not be associated with changes in herbivore abundance. Second, there are important differences between our measurements of herbivore abundance and our measurements of herbivory. Sticky traps were set for a 24-hour period so do not capture the folivorous life stages of some herbivores (e.g. Lepidoptera), while leaf damage was a cumulative measure of herbivory over the duration of the experiment, thereby including all folivory that did not result in leaf removal or abscission. We did not measure herbivore abundance on individual experimental branches, so it was not possible to test for the effect of ants or the interactive effect of lizards and ants on herbivore abundance.

Figure S1. Seaweed deposition after large storms. **A,B.** Seaweed deposited on small islands near Staniel Cay following Hurricane Frances in 2004. **C.** Seaweed deposited on the island of Great Exuma following Tropical Storm Noel in 2007. Arrows indicate piles of seaweed.

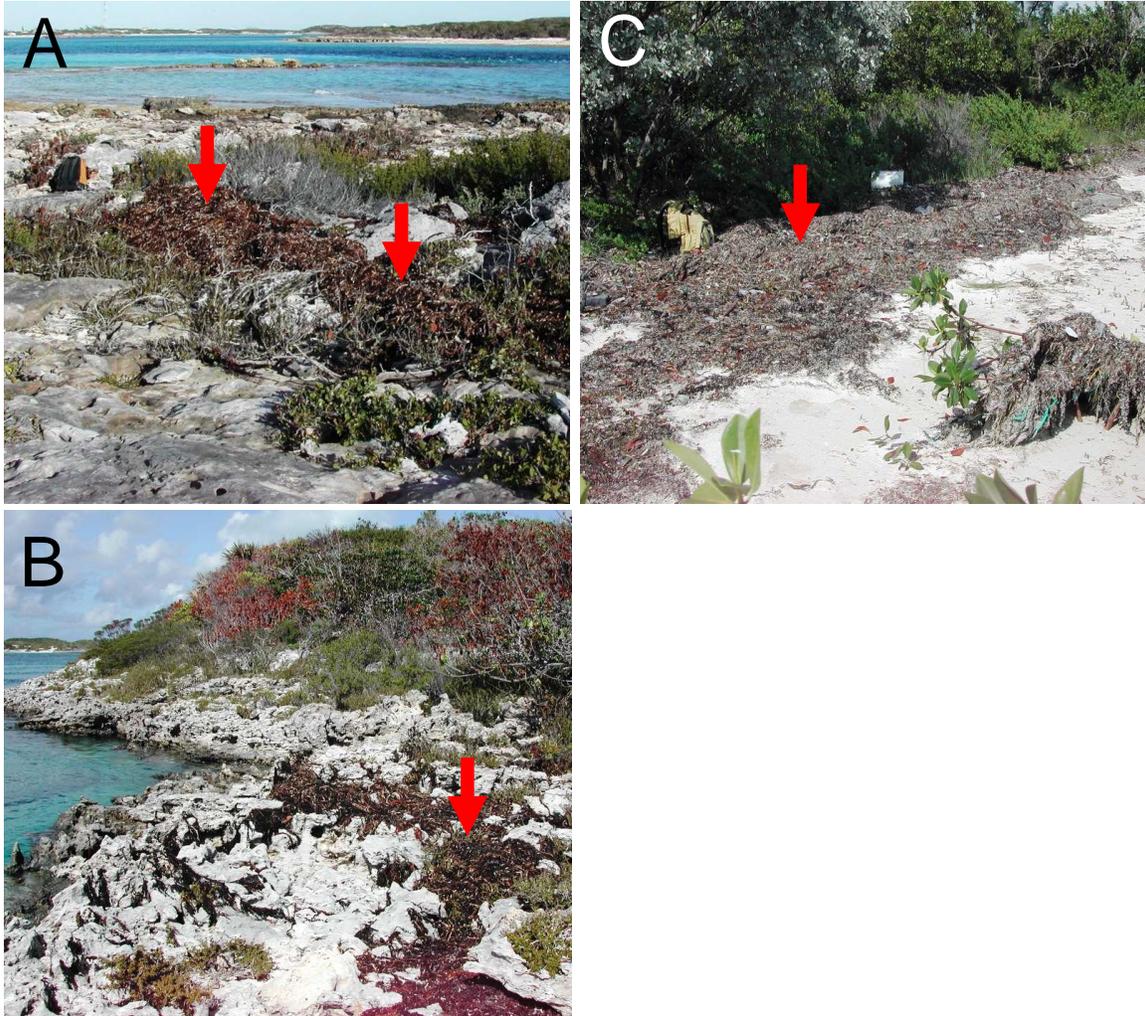


Figure S2. Testing for cage effects. Leaf damage on twenty plants with three treatments: (i) cage controls – duct tape alone wrapped around the branch, (ii) ant controls – duct tape wrapped around the branch, cones allowing lizards to pass over, no tanglefoot on duct tape, (iii) ant exclusions – duct tape wrapped around the branch, cones allowing lizards to pass over, tanglefoot applied on top of duct tape. Data are mean \pm SE. There was a significant effect of ant treatment (mixed model ANCOVA: $F_{2,37}=4.2$, $P=0.02$), with the ant-exclusion treatment having higher leaf damage than the cage control (mixed model ANCOVA: $t_{37}=2.8$, $P=0.007$) and tending to have higher leaf damage than the control treatment (mixed model ANCOVA: $t_{37}=2.0$, $P=0.06$). However, there was no difference between controls and cage controls (mixed model ANCOVA: $t_{37}=0.86$, $P=0.39$).

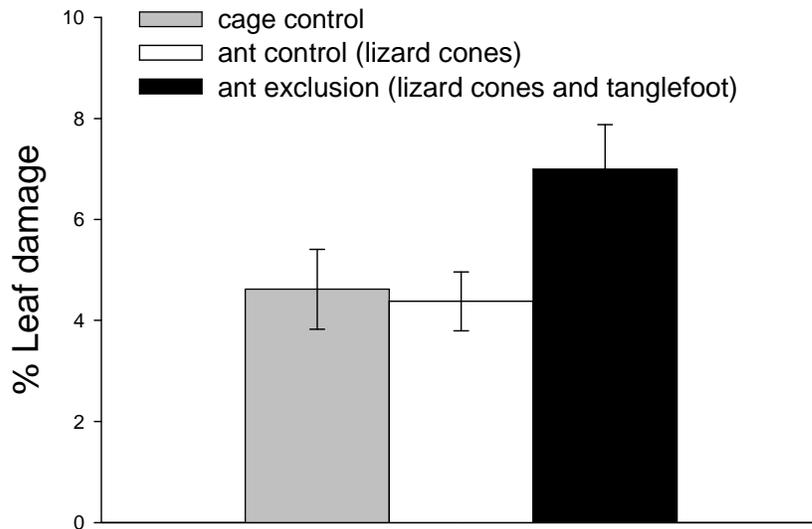
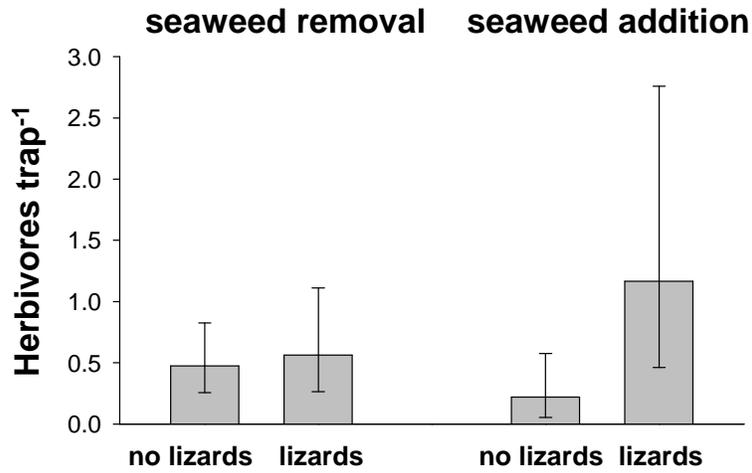


Figure S3. Herbivore abundance on experimental islands. Data are mean \pm SE of log-transformed abundances; n=3 islands for each treatment combination.



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