Macroalgal terpenes function as allelopathic agents against reef corals

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Edited* by Jerrold Meinwald, Cornell University, Ithaca, NY, and approved September 19, 2011 (received for review May 29, 2011)

During recent decades, many tropical reefs have transitioned from coral to macroalgal dominance. These community shifts increase the frequency of algal–coral interactions and may suppress coral recovery following both anthropogenic and natural disturbance. However, the extent to which macroalgae damage corals directly, the mechanisms involved, and the species specificity of algal–coral interactions remain uncertain. Here, we conducted field experiments demonstrating that numerous macroalgae directly damage corals by transfer of hydrophobic allelochemicals present on algal surfaces. These hydrophobic compounds caused bleaching, decreased photosynthesis, and occasionally death of corals in 79% of the 24 interactions assayed (three corals and eight algae). Coral damage generally was limited to sites of algal contact, but algae were unaffected by contact with corals. Artificial mimics for shading and abrasion produced no impact on corals, and effects of hydrophobic surface extracts from macroalgae paralleled effects of whole algae; both findings suggest that local effects are generated by allelochemical rather than physical mechanisms. Rankings of macroalgae from most to least allelopathic were similar across the three coral genera tested. However, corals varied markedly in susceptibility to allelopathic algae, with globally declining corals such as *Acropora* more strongly affected. Bioassay-guided fractionation of extracts from two allelopathic algae led to identification of two loliolide derivatives from the red alga *Galaxaura filamentosa* and two acetylated diterpenes from the green alga *Chlorodesmis fastigiata* as potent allelochemicals. Our results highlight a newly demonstrated but potentially widespread competitive mechanism to help explain the lack of coral recovery on many present-day reefs.

allelopathy | chemical ecology | competition | phase shift

Corals are structurally complex foundation species that generate and maintain tropical reef biodiversity. However, the direct and interactive effects of climate-induced coral bleaching (1, 2), ocean acidification (2, 3), coral disease (4), coastal overfishing, and eutrophication (5–8) have led to coral decline over wide areas. On many reefs, dramatic declines in coral cover have co-occurred with significant increases in fleshy macroalgae (9–11). Once established, macroalgae can inhibit coral recruitment and decrease herbivore grazing, producing negative feedbacks that reinforce phase shifts and further diminish reef function (12–14). Thus, local (e.g., overfishing) and global (e.g., climate) stresses may interact in complex ways to suppress coral cover, promote algal proliferation, and compromise reef resilience; such complexities provide both challenges and opportunities for managing these dynamic ecosystems (11, 13).

As corals decline and macroalgae proliferate, the frequency of algal–coral interactions will increase, potentially affecting the survivorship, growth, and reproduction of remnant adult corals and new coral recruits (12, 13). However, the consequences of and mechanisms driving most algal–coral interactions remain poorly understood. Recent field studies suggest that macroalgae may damage corals by (*i*) shading and abrasion (15), (*ii*) vectoring of coral disease (16), (*iii*) release of water-soluble compounds that stimulate harmful, coral-associated microbes (17), or (*iv*) transfer of hydrophobic allelochemicals by direct contact (18). However, for most of these studies, it is unclear whether the findings are particular to the macroalgal and coral species tested or are common to algal–coral interactions in general and could thus transform the way ecologists and resource managers view processes driving phase-shifts on coral reefs. Despite recent studies demonstrating the potential importance of chemically mediated algal–coral competition (17–19) and its increasing impact as the result of ocean acidification (20), no algal compounds mediating these interactions have been identified.

Here we assessed the role of seaweed allelopathy in algal–coral interactions across three abundant shallow-water corals contacting eight common macroalgae. We monitored effects of macroalgae on coral bleaching using photographic image analysis and in situ pulse-amplitude–modulated (PAM) fluorometry. To examine the probable mechanism producing the field patterns we observed, we assessed the effects of hydrophobic chemistry from whole-algal extracts and from surface-only extracts of chemically active macroalgae on each coral. For two of the most damaging macroalgae, we used a bioassay-guided fractionation approach to isolate and identify four surface-associated compounds that are allelopathic to corals. Our results indicate that numerous macroalgae harm a diverse array of corals using allelochemicals and that harmful macroalgae contain multiple hydrophobic compounds that fulfill this allelopathic role.

Results

Algal–Coral Competition. When placed in contact with eight common macroalgae for 20 d, all three corals experienced bleaching and suppression of photosynthetic efficiency due to contact with some macroalgae (Fig. 1 *A*–*F*). Visual bleaching and photosynthetic efficiency were correlated for all three corals ($r = -0.80$ to -0.96, $P < 0.001$ for all comparisons) (Fig. S1); thus, PAM fluorometry measurements are indicative of visual bleaching but are less subjective (17, 18, 21, 22). The most resistant coral was *Montipora digitata*; for this coral, *Dictyota bartayresiana*, *Galaxaura filamentosa*, and *Chlorodesmis fastigiata* caused significant bleaching and suppression of photosynthetic efficiency, whereas *Liagora* sp. and *Padina boryana* caused no significant bleaching and a mild suppression of photosynthetic efficiency (Fig. 1 *A* and *D*). In contrast, contact with *Amphiroa crassa*, *Sargassum polycystum*, or *Turbinaria conoides* had no significant effect on *M. digitata*. Regardless of algal species, significant bleaching occurred only in areas of direct contact and never on the far sides of *M. digitata* (5–10 mm away from algal contact) (Kruskal–Wallis ANOVA, $P \geq 0.18$).

The corals *Acropora millepora* and *Pocillopora damicornis* were more susceptible to algal damage. For *A. millepora*, all macroalgae except *S. polycystum* and *T. conoides* bleached corals or suppressed photosynthetic efficiency (Fig. 1 *B* and *E*); for

Author contributions: D.B.R. and M.E.H. designed research; D.B.R., E.P.S., S.E., J.K., and M.E.H. performed research; D.B.R. analyzed data; and D.B.R. and M.E.H. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

Freely available online through the PNAS open access option.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1108628108/-/DCSupplemental.

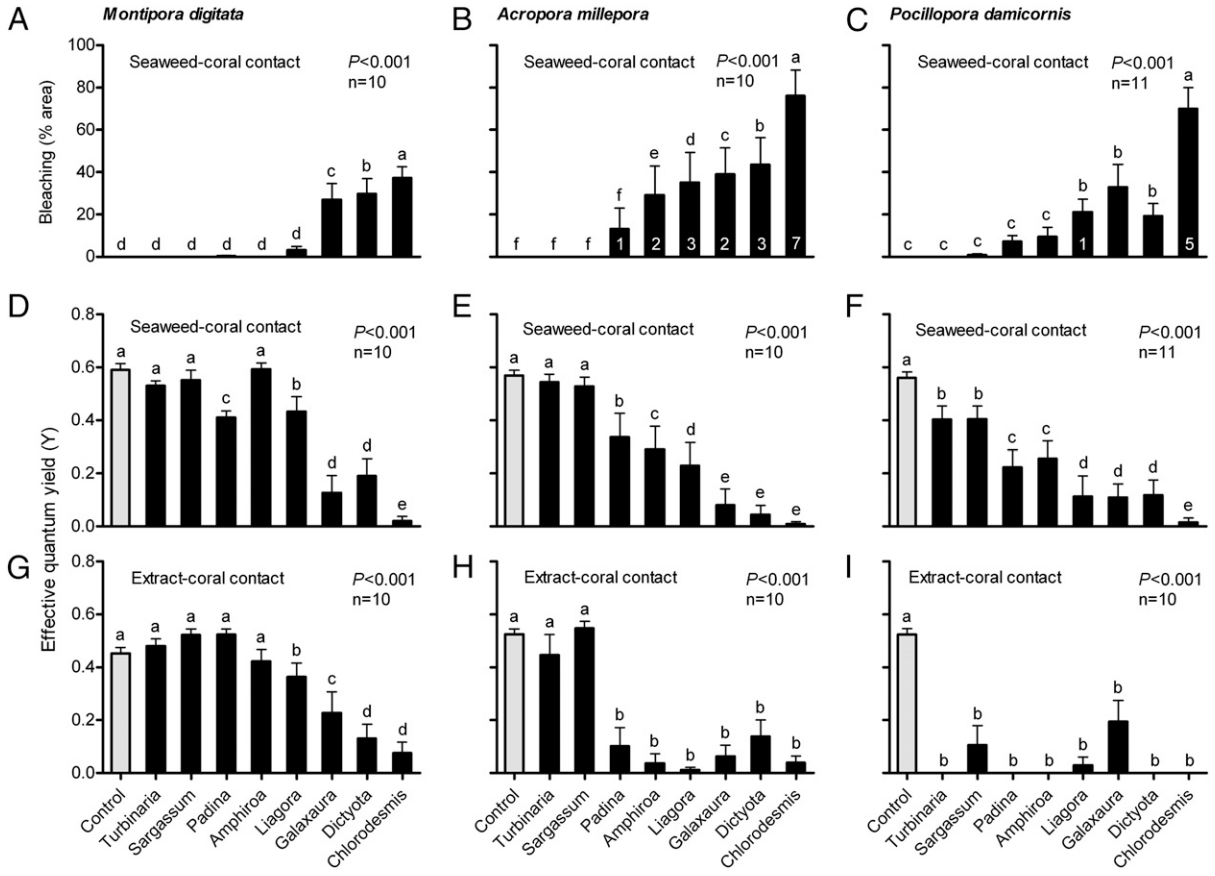

Fig. 1. Effects of macroalgae and algal extracts on corals. (*A*–*C*) Coral bleaching (2D percentage of area; mean ± SE) and (*D*–*I*) effective quantum yield (*Y*; mean ± SE) of three species of corals when in contact with macroalgae for 20 d (*A*–*F*) or in contact with gel strips containing hydrophobic extracts from the same algae for 24 h (*G*–*I*), relative to controls (n = 10–11). Analyzed by Kruskal–Wallis ANOVA on ranks. Letters above bars indicate significant groupings by post hoc Student–Newman–Kuels tests. Numbers inset within bars indicate number of replicates experiencing 100% mortality.

P. damicornis, four of the eight macroalgae caused significant bleaching, but all eight suppressed photosynthetic efficiency (Fig. 1 *C* and *F*). Several macroalgae caused mortality of some *A. millepora* and *P. damicornis* replicates (Fig. 1 *B* and *C*). However, only *C. fastigiata* caused significant whole-replicate mortality of *A. millepora* (Fisher's exact test, $P = 0.003$) and *P. damicornis* ($P = 0.035$). Only *C. fastigiata* caused bleaching on the far sides of *A. millepora* (Kruskal–Wallis ANOVA, $P = 0.021$) or *P. damicornis* ($P = 0.042$), 5–10 mm away from contact. In contrast, corals did not damage macroalgae. Macroalgae in contact with corals experienced no significant bleaching or suppression of photosynthesis relative to controls lacking coral contact (Fig. S2).

Elucidation of Competitive Mechanisms. Algal effects on corals were largely localized to areas of direct contact. These effects could result from shading, abrasion, or transfer of hydrophobic allelochemicals upon contact. When inert plastic models mimicking bladed algae such as *P. boryana* and filamentous species such as *C. fastigiata* were put in contact with *A. millepora* (the most sensitive coral, $n = 10$) for 16 d in the field, mimics produced neither coral bleaching (*Padina* and *Chlorodesmis* mimics: 0 ± 0% bleached) nor suppression of photosynthetic efficiency [*Padina* mimic: effective quantum yield (Y) = 0.639 ± 0.013; *Chlorodesmis* mimic: Y = 0.648 ± 0.017], relative to controls lacking a mimic (Y = 0.630 ± 0.014; ANOVA; bleaching: $F = 1.000$, $P > 0.999$; effective quantum yield: $F = 0.295$, $P = 0.747$). In contrast, the alga *C. fastigiata* significantly suppressed *A. millepora* photosynthesis after only 2 d, and five of the eight macroalgae suppressed the coral after only 10 d (Fig. S3), suggesting that allelopathy, rather than shading or abrasion, damaged corals in our field assays (Fig. 1).

Consistent with an allelopathic mechanism, hydrophobic algal extracts placed in contact with corals at natural volumetric concentration for 24 h produced effects (Fig. 1 *G*–*I*) that paralleled or exceeded effects of macroalgal contact after 20 d (Fig. 1 *D*–*F*). Control gels lacking algal extract had no detectable effect on corals (Fig. 1 *G*–*I*). Macroalgae that did not bleach corals in the field also had no effect in assays using their extracts. *P. boryana* was unusual in that it suppressed photosynthetic efficiency of *M. digitata* during 20-d assays using algal thalli, but its extract was not allelopathic over 24 h. Its allelopathic compounds may be unstable or may take longer than 24 h to affect this coral, or it may stress corals mildly through nonchemical mechanisms.

When deployed at natural concentration for 24 h, hydrophobic extracts from only algal surfaces (Fig. 2) produced effects that mirrored effects of algal thalli and of hydrophobic extracts from whole-algal tissues (Fig. 1), indicating that hydrophobic compounds occur on algal surfaces at concentrations sufficient to cause coral bleaching and mortality. This assertion could be in error if surface extraction caused cell lysis and extracted internal compounds, but microscopic evaluations of surface-extracted *C. fastigiata* and *G. filamentosa* (the most allelopathic algae) indicated that cell lysis did not occur (Table S1).

Isolation of Allelochemicals. Using bioassay-guided chromatographic separations of *G. filamentosa* and *C. fastigiata* crude extracts (15–24 g of extract), we purified and identified four allelopathic compounds—the degraded sesquiterpenes 6-hydroxy-isololiolide and iso-

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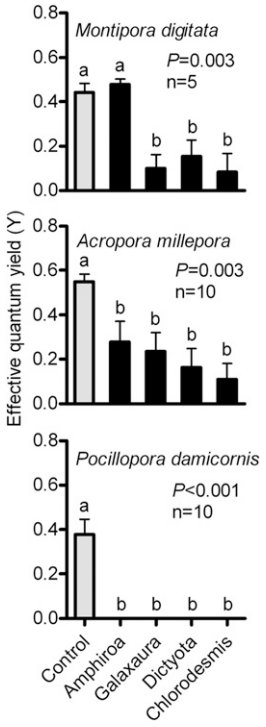

Fig. 2. Effects of surface-bound algal extracts on corals. Effective quantum yield (*Y*; mean ± SE) of three coral species when in direct contact for 24 h with gel strips containing hydrophobic extracts from the surfaces of macroalgae, relative to controls (n = 5–10). Analysis and symbols as in Fig. 1.

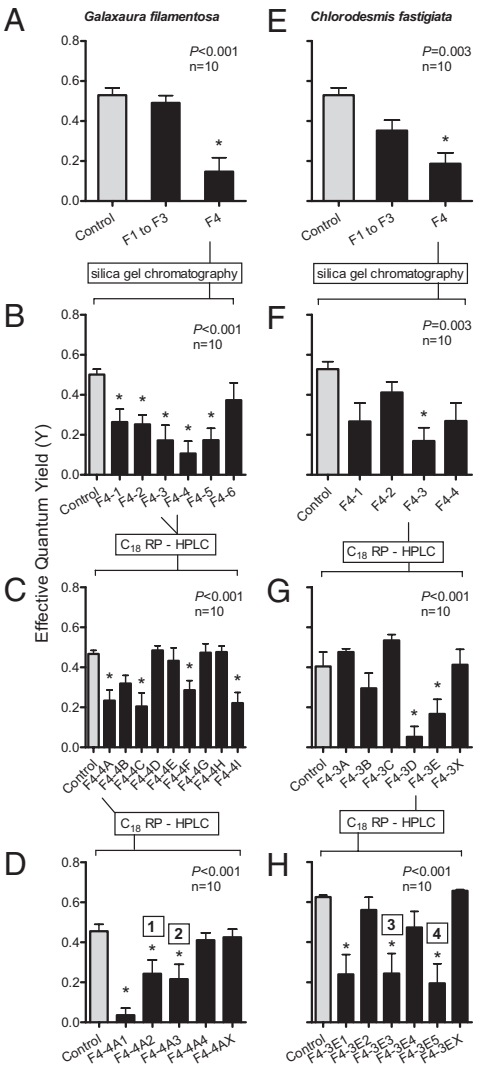

Fig. 3. Effects of *G. filamentosa* and *C. fastigiata* extract fractions on corals. Effective quantum yield (*Y*; mean ± SE) of the coral *Acropora millepora* when in contact with extract fractions from *G. filamentosa* (*A–D*) or *C. fastigiata* (*E–H*) for 24 h (n = 10 per fraction), relative to controls. Methanol-soluble crude extracts of both algal species were fractionated by HP20ss reversed-phase chromatography before initial bioassay (*A* and *E*). Vertical lines indicate path and boxes indicate method of subsequent extract fractionation based on bioactivity. Asterisks indicate significant ($P < 0.05$) differences between fractions and controls by posthoc Student Newman–Kuels tests. Bioassays led to the isolation of allelopathic compounds 1 and 2 (Fig. 4) from *G. filamentosa* and compounds 3 and 4 (Fig. 4) from *C. fastigiata*.

loliolide from *G. filamentosa* (Figs. 3 *A–D* and 4, compounds 1 and 2) and the acetylated diterpenes (*E*)-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)but-2-ene-1,4-diyl diacetate and (1E, 3E)-2-((3*E*,7*E*)-12-formyl-4,8-dimethyl-10-oxotrideca-3,7,12-trienyl)buta-1,3-diene-1,4-diyl diacetate from *C. fastigiata* (Figs. 3 *E–H* and 4, compounds 3 and 4). Compounds 1–4 were isolated and bioactive at 0.032–0.12 μg/g of algal dry mass, indicating strong potency. Despite these minute concentrations, compounds 1-4 were detected in the surface extracts of their associated algae (Figs. S4 and S5). Compound 3 occurred in *C. fastigiata* surface extracts (n = 3) at 0.43–7% of its abundance in whole-algal extracts (n = 3); compounds 1, 2, and 4 were detected in low concentrations in both surface (Figs. S4 and S5) and whole-algal crude extracts (n = 3) of *G. filamentosa* and *C. fastigiata*, respectively, but internal vs. external concentrations could not be compared rigorously because of their minor abundance relative to other molecules. Additional allelopathic compounds were present in both macroalgae (Fig. 3). We attempted to identify these molecules but failed to do so because of low yield or degradation following purification.

Discussion

Direct contact between eight common macroalgae and three genera of common corals caused visible coral bleaching in 50%, suppression of coral photosynthetic efficiency in 79%, and complete death of some coral replicates in 33% of the 24 algal–coral interactions examined (Fig. 1). In contrast, none of the macroalgae we tested bleached or experienced suppressed photosynthesis as the result of coral contact (Fig. S2). Field patterns of coral damage were reproduced or exceeded in 96% of our bioassays using hydrophobic whole-tissue extracts and hydrophobic surface extracts from these macroalgae; we detected no negative effects of shading or abrasion using inert algal mimics. Additionally, larger and more abrasive macroalgae such as *T. conoides* and *S. polycystum* that should have produced the greatest abrasion and shading had no detectable effect (vs. *M. digitata* and *A. millepora*) or minimal effect (vs. *P. damicornis*) on corals (Fig. 1). In contrast, some soft, nonabrasive algae with allelopathic extracts (e.g., *D. bartayresiana* and *C. fastigiata*) rapidly bleached and sometimes killed corals within only 2–10 d (Fig. S3). Our findings show that allelopathic rather than physical mechanisms mediate these interactions.

A previous study discovered similar patterns for these macroalgae and the coral *Porites cylindrica* in Fiji (18), but no allelopathic compounds were identified, and it was not possible to assess whether that single coral species was typical or unusual relative to other corals. The present more inclusive study demonstrates that chemical mediation of algal–coral competition is common, and although the magnitude of algal effects varies

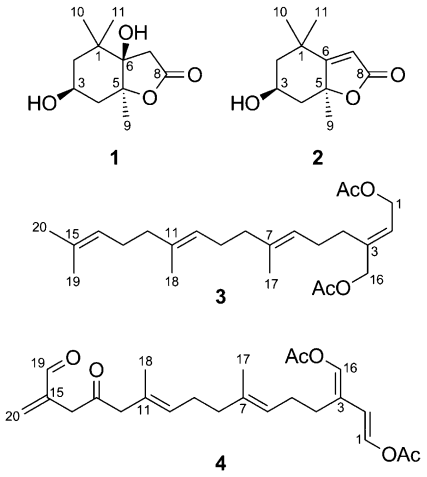

Fig. 4. Allelopathic compounds isolated from *G. filamentosa* (compounds 1 and 2) and *C. fastigiata* (compounds 3 and 4). Assigned carbon positions are noted for each molecule, and corresponding NMR spectroscopic data are available in Tables S2 and S3.

among corals, some macroalgae (*D. bartayresiana, G. filamentosa, C. fastigiata, Lobophora variegata, Halimeda opuntia*) are chemically damaging to most corals (Fig. 1) (18), and others (*T. conoides* and *S. polycystum*) are more chemically benign. These results suggest that macroalgal allelopathy against corals may be common on degraded reefs dominated by certain macroalgae and point to a potentially widespread mechanism to help explain why corals fail to recover on many reefs with abundant macroalgae (11, 13).

For two of the most allelopathic macroalgae, we isolated multiple allelopathic compounds but were able to identify only two from each species (Figs. 3 and 4). These isoprenoid natural products were known previously from macroalgae (23–27); here we elucidate their ecological role. Isolated allelopathic isoprenoids were effective at yields of only 0.032–0.12 μg/g of algal dry mass, suggesting that only minute quantities on algal surfaces can damage corals. The hydrophobicity of these allelochemicals likely makes them efficient surface-mediated toxins because their water solubility is very low, allowing these compounds to be retained at algal–coral interfaces. Allelopathic interactions documented among other reef species also occur as the result of transfer of compounds by contact rather than dissolution through the water (18, 22, 28–30), indicating that in general allelopathic compounds may be hydrophobic rather than hydrophilic in benthic marine systems.

Coral species varied in susceptibility to macroalgal allelopathy (Fig. 1) (18). *A. millepora* and *P. damicornis* were more sensitive to allelopathic damage than *M. digitata* (Fig. 1) or *P. cylindrica* (18). Moreover, mortality occurred only for some replicates of *A. millepora* (23%) and *Pocillopora damicornis* (8%) and never for *M. digitata* or *P. cylindrica* (Fig. 1) (18). Despite some inconsistencies associated with taxonomic relatedness (see responses of *A. millepora* vs. *M. digitata*, both in the family Acroporidae, to algal contact), these differences in coral sensitivity to algal allelopathy parallel the differing tolerances of the genera *Acropora* and *Porites* to climate-induced bleaching and mirror the high extinction risk of *Acropora* and stability of *Porites* at a global scale (31). Therefore, differential effects of macroalgae on corals may reinforce trajectories of coral decline initially produced by large-scale disturbance.

Macroalgae such as *S. polycystum*, *T. conoides*, and *P. boryana* that commonly bloom following herbivore removal (7, 9, 32) did not damage corals in our assays. Because we used only one thallus per coral, it is possible that higher densities of these macroalgae or longer contact durations could damage corals (7). In contrast, macroalgae such as *D. bartayresiana* (Fig. 1) or *L. variegata* (18) that also commonly bloom following herbivore exclusion (9, 33, 34) had large allelopathic effects on corals and could suppress coral resilience (11, 13). However, not all species in these genera are allelopathic (15), making generalizations based on relatedness or functional group alone problematic. Interestingly, other strongly allelopathic macroalgae such as *C. fastigiata*, *G. filamentosa*, *Liagora* sp. (Fig. 1), or *Ochtodes secundaramea* (18) rarely proliferate following reef disturbance. Differential susceptibility to disturbance (herbivory, hydrodynamics, and other factors) or differential competitive ability may explain in part why some chemically damaging macroalgae rarely bloom on declining reefs (18, 33, 35).

Recent laboratory studies demonstrated that macroalgae near but not in contact with corals triggered coral mortality and suggested that algae release water-soluble compounds that kill corals indirectly by stimulation of harmful coral-associated microbes (17). Field studies indicate that benthic algae release hydrophilic molecules, such as dissolved organic carbon (DOC), capable of fueling these interactions (36, 37). However, in our field assays, where advection would disperse and dilute such compounds rapidly, we did not detect macroalgal effects beyond sites of direct contact for either *M. digitata* (Fig. 1) or *P. cylindrica* (18), as would be expected for interactions involving hydrophilic molecules. We detected algal effects beyond areas of direct contact in the more chemically sensitive corals (*A. millepora* and *P. damicornis*) when contacted by *C. fastigiata*, but the allelopathic effects of this alga and the alga *G. filamentosa* were traced from methanol-soluble crude extracts (containing both hydrophobic and hydrophilic molecules) to potent hydrophobic compounds that would be dispersed minimally by water (Fig. 3). Water-soluble compounds within the polar fractions (F1–F3) of *C. fastigiata* and *G. filamentosa* crude extracts were not active in bioassays (Fig. 3 *A* and *E*). Thus, in our field studies most algal damage to corals appeared to be caused by hydrophobic molecules transferred by direct contact, suggesting that water-soluble compounds such as DOC need not be involved in these contact allelochemical interactions (although water-soluble compounds might be involved under some conditions) (37). Whether the hydrophobic compounds and extracts we detected poison corals directly or indirectly by altering microbial communities (16, 17) was not assessed.

Algal–coral interactions may have been rare on pristine or "pre-human" reefs where herbivores limited macroalgae to spatial refuges (5, 6); thus, factors historically selecting for macroalgal allelopathy against corals remain unclear. Given that macroalgae share a long evolutionary history with microbial pathogens (38), that the isoprenoid compounds we identified are found in several green (23–25), red (Figs. 3 and 4), and brown macroalgae (26, 27), and that some of these compounds suppress marine microbes (24), it is plausible that these compounds evolved independently in multiple algal lineages as defenses against microbes. These natural products may damage corals fortuitously and thus provide an advantage for macroalgae on present-day reefs.

Recent analyses suggest that marine protected areas may promote local processes, such as herbivory and coral recruitment (39, 40), that limit macroalgal proliferation and promote coral recovery, thereby bolstering coral resilience to large-scale disturbance (7, 8, 11, 41, 42). However, because several allelopathic macroalgae (e.g., *Dictyota*, *Chlorodesmis*, *Galaxaura, Lobophora, Halimeda, Ochtodes*) also contain toxins that deter some herbivores (43), developing effective marine reserves may require protecting a diverse herbivore guild that includes species that consume chemically defended macroalgae (18, 33, 43). Establishing no-take reserves or fishing bans that protect herbivores capable of consuming chemically rich macroalgae may minimize allelopathic effects of algae on corals.

We show that numerous common macroalgae damage a variety of corals using surface-associated allelochemicals transferred

by algal–coral contact; these interactions will be especially detrimental to the recovery of small remnant coral colonies or to the survivorship of small juvenile corals encountering a high ratio of algal contact per unit area. Such interactions also may contribute to the algal suppression of coral fecundity and recruitment documented in previous investigations (12, 19, 44) and may become increasingly damaging to corals as oceans acidify (20). If so, chemically mediated algal–coral competition may play a critical and increasing role in both the degradation of coral reefs and the formation of negative feedbacks limiting reef recovery (12, 13). Understanding which macroalgae most harm corals and what processes limit these macroalgae may allow more proactive management that increases coral resilience to the many stresses impacting tropical reefs (11, 13).

Materials and Methods

Experimental Design and Study Organisms. In July 2008, we collected branches of the corals *M. digitata*, *A. millepora*, and *P. damicornis* from colonies on Votua Reef, Viti Levu, Fiji (18°13.049′S, 177°42.968′E) and epoxied them (Emerkit) individually into small cement cones. Corals then were transplanted onto reef flat coral racks (1 m deep at low tide) and given 7 wk to acclimate before experiments. We embedded 4-cm nails on opposite sides of the surface of each cone so that a three-stranded rope holding an alga could be slipped over each nail head to hold the alga in contact with the coral. Control corals received a rope but lacked macroalgae. Control algae were deployed in ropes on cement cones but lacked a coral. In our algal–coral contact experiments we used representative-sized individuals of macroalgae that (*i*) were common around our site, (*ii*) were observed in contact with corals, (*iii*) represented a range of taxonomic and morphological forms, and (*iv*) were used in a previous study with *P. cylindrica* from this site with the same experimental design (18), thus making possible contrasts across four coral genera. Whole thalli were used to avoid stress compounds that might be released from clipped macroalgae. The procedures produced algal–coral contacts representative of interactions we observed in the field.

To simulate the effects of macroalgae on remnant adult coral colonies or juvenile corals recruiting to adult populations, we used individuals (6–8 cm in length) of each coral species in our experiments. We chose the branching corals *M. digitata*, *A. millepora*, and *P. damicornis* because they (*i*) are common at our study sites, (*ii*) are three of the dominant corals on reef flats in Fiji, (*iii*) could be fragmented with minimal damage to the host colony, and (*iv*) possess a range of life-history traits, including brooding vs. broadcast reproduction.

We interspersed treatment and control replicates (n = 12 for each species) haphazardly (15 cm apart in all directions) across five racks of metal mesh into which the bases of the cones could be placed. Racks were secured 3–4 m apart on a coral-dominated reef flat in Votua Village's no-take marine reserve. At low tide, corals in these racks were at ∼1-m depth. We caged racks with metal screen (1-cm^2 grid) to exclude large herbivores and brushed the cages every 2 d to remove fouling organisms. During routine maintenance, we replaced macroalgae lost to wave action (an infrequent occurrence). After 2, 10, and 20 d, we assessed the effects of algal contact on coral bleaching, relative to controls. Any bleaching on corals was photographed, and the 2D percentage of the area bleached in each replicate was quantified using ImageJ (v1.40; National Institutes of Health) photoanalysis software. Because visual assessments can be subjective (17, 18, 21, 22), we also assessed coral bleaching and algal photosynthesis using in situ PAM fluorometry (Walz). Fluorometry measurements were taken on treatment corals at the most damaged location of algal–coral contact. To assess effects on coral tissues only millimeters away from affected tissues but not in direct contact with macroalgae, we also took samples at the same height on the opposite side of the coral branch. Fluorometry measurements of treatment algae were taken at the site of greatest coral contact. Control corals and algae were sampled in the same manner as treatments.

PAM Fluorometry Measurements. PAM fluorometry was used in situ to assess the effects of macroalgae and their extracts on coral photosynthetic efficiency and bleaching and the effects of corals on algae (measured as effective quantum yield). PAM fluorometry provides an additional measure of bleaching compared with visual assessments alone, which can be subjective (17, 18, 21, 22). Values for healthy corals typically range from 0.5 to 0.7 (21); values of ∼0.0–0.25 indicate severe bleaching and mortality (17, 18, 22).

Ideally, researchers use dark-adapted corals to minimize variance in yield values associated with nonphotochemical processes, such as UV irradiance and water temperature (21). However, we conducted our study in situ on light-adapted corals because of logistical constraints. So that readings for a treatment would not be confounded by variance in environmental parameters, we took all readings between 0900–1400 h and interspersed readings of all treatments through time. Low variance among replicates that were interspersed through time (Fig. 1), significant correlations between fluorometric and visual assessments of bleaching for all corals (Fig. S1), and our demonstration of large and significant differences among treatments indicate that treatment effects overwhelmed whatever uncontrolled variance occurred from not using dark-adapted corals.

Algal Mimic Bioassay. We used inert algal mimics to assess effects of algal abrasion and shading in the absence of chemical effects. To mimic *Padina boryana* (a broad, foliose alga with high potential to shade), we constructed opaque foliose mimics from black plastic sheeting and grouped them with cable-tie "holdfasts." A filamentous mimic of *C. fastigiata* (the most allelopathic alga) was created by cutting 60 loops of Dacron line (White River Fly Shop) into filaments and grouping them with cable-tie "holdfasts". Mimics of *S. polycystum* and *T. conoides* were not created, because these algae had no effect during our 20-d field-contact assays. Algal mimics (n = 10 per treatment) were inserted into segments of three-stranded rope and attached to fragments of *A. millepora* (a coral species that showed high sensitivity to direct macroalgal contact) (Fig. 1*B*) on racks at Votua Reef, Fiji. We also deployed control corals (n = 10) with rope segments lacking an algal mimic. We assessed the effects of algal mimics versus controls on coral bleaching and photosynthesis after 16 d. We chose a 16-d duration because live macroalgae had shown strong effects after only 2–10 d (Fig. S2).

Allelochemical Bioassays. We exhaustively extracted whole tissues (20 mL displacement volume) of each alga with methanol and filtered and dried each extract in vacuo. We then partitioned each extract between water and ethyl acetate three times, yielding an ethyl acetate-soluble fraction which was dried in vacuo and stored at −5 °C for 2–3 d until assayed.

For bioassays, we resuspended the ethyl acetate-soluble extract of each alga in methanol and added it at natural volumetric concentration to Phytagel (Sigma-Aldrich) squares (1 cm^2) that were formed on window screen (29). Controls contained methanol but no extract. In the field, we applied each gel square (n = 10 for each treatment) around a coral branch (fragmented as described above) and secured the square with a cable tie. After 24 h, we removed each gel and took a PAM fluorometry reading beneath its center.

To assess whether allelochemicals were on algal surfaces at concentrations that produced the effects we observed in bioassays of whole-algal tissue extracts, we also extracted hydrophobic compounds from only the surfaces of three strongly allelopathic macroalgae and one less allelopathic alga (i.e., it affected some corals but not others) using the "hexane dip" method (45). Each alga (20-mL displacement volume) was spun in a salad spinner to remove excess water and extracted with hexanes for 30 s while vortexing (45). We then dried each extract in vacuo, resuspended it in hexanes, and added it at natural volumetric concentration to Phytagel strips. Controls contained hexanes but lacked algal extract. Treatment and control gels (n = 10 per extract per coral species, except for *Montipora*, n = 5) were deployed and assayed as described above.

Allelochemical Isolation. Methanol extracts of *C. fastigiata* and *G. filamentosa* were separated by reversed-phase Diaion HP20ss (Supelco Analytical) chromatography into four fractions using aqueous methanol and acetone. Based on field-bioassay activity, the least polar fraction from each species was separated further with silica gel chromatography, eluting with hexanes/ethyl acetate and methanol. Active fractions from each species then were separated by two rounds of reversed-phase HPLC (Alltech Altima C_{18}, 250 × 10 mm, 5 μm) using a gradient of 70% methanol [aqueous (aq)] to 100% methanol over 60 min and then isocratic 50% methanol (aq) over 35 min to yield allelopathic compounds 1 and 2 (Fig. 4) from *G. filamentosa*. A gradient of 95% methanol (aq) to 100% methanol over 46 min followed by isocratic 100% methanol over 35 min yielded allelopathic compounds 3 and 4 (Fig. 4) from *C. fastigiata*.

Structure Determination. Compounds 1–4 were isolated in total quantities of 5–18 μg. ^{1}H NMR spectral data and high-resolution mass spectral (HRMS) data of allelopathic compounds isolated from *G. filamentosa* did not match any known natural products from that genus. For compound 1, high-resolution electron spray ionization mass spectrometry (HR-ESI-MS) [M + Na] *m/z* equaled 237.1114 (calculated for $C_{11}H_{18}O_4Na$, 237.1103); for compound 2, HR-ESI-MS [M + Na] *m/z* equaled 219.1021 (calculated for $C_{11}H_{16}O_3Na$, 219.0997). However, 1D and 2D NMR spectral data for compounds 1 and 2, recorded on a Varian 800 MHz NMR spectrometer (Agilent Technologies), were compared with and matched with spectral data from compounds isolated from the brown alga *Undaria pinnatifida* (27). ^{1}H and ^{13}C NMR spectral

data for compounds 1 and 2 are reported in Table S2, with ^{13}C NMR assignments based on heteronuclear single-quantum correlation spectroscopy and heteronuclear multiple bond correlation data. Carbon positions are noted in Fig. 4.

^{1}H NMR spectral and HRMS data for allelopathic compounds 3 and 4 (Fig. 4) isolated from *C. fastigiata* were compared with and matched with previously reported spectral data from *C. fastigiata* (23, 24). For compound 3, HR-ESI-MS [M + Na] *m/z* equaled 413.2657 (calculated for $C_{24}H_{38}O_4Na$, 413.2667); for compound 4, HR-ESI-MS $[M + H]^+$ *m/z* equaled 417.2425 (calculated for $C_{24}H_{33}O_6$, 417.2277). ^{1}H NMR values for compounds 3 and 4 are reported in Table S3; carbon positions are noted in Fig. 4.

Statistical Analyses. Coral response data from our competition and allelochemical bioassays violated parametric assumptions and so were evaluated using Kruskal–Wallis ANOVA on ranks. Algal response data from our competition bioassays were analyzed by one-factor ANOVA or by Kruskal–Wallis ANOVA on ranks if parametric assumptions were violated. If some replicates lost algae or were missed when scoring our 20-d competition study, we randomly excluded replicates from other treatments and controls (~1–2) to equalize sample sizes and allow the use of more powerful post hoc tests that require balanced sample sizes. We analyzed the algal mimic assay results with one-factor ANOVA. Differences among subgroups were analyzed for all ANOVA using Student–Newman–Kuels multiple comparisons tests. Relationships between photosynthetic efficiency and coral bleaching were analyzed via Pearson's correlation coefficients. Binomial coral mortality data were analyzed by Fisher's exact tests. Epifluorescent microscopy data were analyzed by Mann–Whitney *U* rank sum tests.

ACKNOWLEDGMENTS. We thank the Fijian government for collection and research permits, the Votua Village elders for granting local research permissions, the University of the South Pacific for logistical support, and P. Skelton (University of the South Pacific) for identifying macroalgae. V. Bonito, G. Fraser, M. Sharma, R. Kumar, P. Prisad, K. Poulson-Ellestad, and M. Teasdale provided valuable field and laboratory assistance. Support was provided by National Institutes of Health Research Grant U01-TW007401, National Science Foundation Research Grant OCE 0929119, National Science Foundation Integrative Graduate Education and Research Traineeship Grant DGE-0114400, and the Teasley Endowment to the Georgia Institute of Technology.

1. Hoegh-Guldberg O, et al. (2007) Coral reefs under rapid climate change and ocean acidification. *Science* 318:1737–1742.
2. Baker AC, Glynn PW, Riegl B (2008) Climate change and coral reef bleaching: An ecological assessment of long-term impacts, recovery trends and future outlook. *Estuar Coast Shelf Sci* 80:435–471.
3. De'ath G, Lough JM, Fabricius KE (2009) Declining coral calcification on the Great Barrier Reef. *Science* 323:116–119.
4. Bruno JF, et al. (2007) Thermal stress and coral cover as drivers of coral disease outbreaks. *PLoS Biol* 5:1220–1227.
5. Jackson JBC, et al. (2001) Historical overfishing and the recent collapse of coastal ecosystems. *Science* 293:629–637.
6. Pandolfi JM, et al. (2003) Global trajectories of the long-term decline of coral reef ecosystems. *Science* 301:955–958.
7. Hughes TP, et al. (2007) Phase shifts, herbivory, and the resilience of coral reefs to climate change. *Curr Biol* 17:360–365.
8. Carilli JE, Norris RD, Black BA, Walsh SM, McField M (2009) Local stressors reduce coral resilience to bleaching. *PLoS ONE* 4:e6324.
9. Hughes TP (1994) Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. *Science* 265:1547–1551.
10. Bruno JF, Sweatman H, Precht WF, Selig ER, Schutte VGW (2009) Assessing evidence of phase shifts from coral to macroalgal dominance on coral reefs. *Ecology* 90: 1478–1484.
11. Hughes TP, Graham NAJ, Jackson JBC, Mumby PJ, Steneck RS (2010) Rising to the challenge of sustaining coral reef resilience. *Trends Ecol Evol* 25:633–642.
12. Birrell CL, McCook LJ, Willis BL, Diaz-Pulido GA (2008) Effects of benthic algae on the replenishment of corals and the implications for the resilience of coral reefs. *Oceanogr Mar Biol Ann Rev* 46:25–63.
13. Mumby PJ, Steneck RS (2008) Coral reef management and conservation in light of rapidly evolving ecological paradigms. *Trends Ecol Evol* 23:555–563.
14. Hoey AS, Bellwood DR (2011) Suppression of herbivory by macroalgal density: A critical feedback on coral reefs? *Ecol Lett* 14:267–273.
15. Box SJ, Mumby PJ (2007) Effect of macroalgal competition on growth and survival of juvenile Caribbean corals. *Mar Ecol Prog Ser* 342:139–149.
16. Nugues MM, Smith GW, Hooidonk RJ, Seabra MI, Bak RPM (2004) Algal contact as a trigger for coral disease. *Ecol Lett* 7:919–923.
17. Smith JE, et al. (2006) Indirect effects of algae on coral: Algae-mediated, microbe-induced coral mortality. *Ecol Lett* 9:835–845.
18. Rasher DB, Hay ME (2010) Chemically rich seaweeds poison corals when not controlled by herbivores. *Proc Natl Acad Sci USA* 107:9683–9688.
19. Diaz-Pulido G, Harii S, McCook LJ, Hoegh-Guldberg O (2010) The impact of benthic algae on the settlement of a reef-building coral. *Coral Reefs* 29:203–208.
20. Diaz-Pulido G, Gouezo M, Tilbrook B, Dove S, Anthony KRN (2011) High CO_2 enhances the competitive strength of seaweeds over corals. *Ecol Lett* 14:156–162.
21. Fitt WK, Brown BE, Warner ME, Dunne RP (2001) Coral bleaching: Interpretation of thermal tolerance limits and thermal thresholds in tropical corals. *Coral Reefs* 20: 51–65.
22. Pawlik JR, Steindler L, Henkel TP, Beer S, Ilan M (2007) Chemical warfare on coral reefs: Sponge metabolites differentially affect coral symbiosis *in situ*. *Limnol Oceanogr* 52:907–911.
23. Wells RJ, Barrow KD (1979) Acyclic diterpenes containing 3 enol acetate groups from the green alga *Chlorodesmis fastigiata*. *Experientia* 35:1544–1545.
24. Paul V, Fenical W (1985) Diterpenoid metabolites from Pacific marine algae of the order Caulerpales (Chlorophyta). *Phytochem* 24:2239–2243.
25. Handley JT, Blackman AJ (2005) Secondary metabolites from the marine alga *Caulerpa brownii* (Chlorophyta). *Aust J Chem* 58:39–46.
26. Kuniyoshi M (1985) Germination inhibitors from the brown alga *Sargassum crassifolium* (Phaeophyta, Sargassaceae). *Bot Mar* 28:501–503.
27. Kimura J, Maki N (2002) New loliolide derivatives from the brown alga *Undaria pinnatifida*. *J Nat Prod* 65:57–58.
28. de Nys R, Coll JC, Price IR (1991) Chemically mediated interactions between the red alga *Plocamium hamatum* (Rhodophyta) and the octocoral *Sinularia cruciata* (Alcyonacea). *Mar Biol* 108:315–320.
29. Thacker RW, Becerro MA, Lumbang WA, Paul VJ (1998) Allelopathic interactions between sponges on a tropical reef. *Ecology* 79:1740–1750.
30. Kubanek J, et al. (2002) Multiple defensive roles for triterpene glycosides from two Caribbean sponges. *Oecologia* 131:125–136.
31. Carpenter KE, et al. (2008) One-third of reef-building corals face elevated extinction risk from climate change and local impacts. *Science* 321:560–563.
32. Lewis SM (1986) The role of herbivorous fishes in the organization of a Caribbean reef community. *Ecol Monogr* 56:183–200.
33. Burkepile DE, Hay ME (2008) Herbivore species richness and feeding complementarity affect community structure and function on a coral reef. *Proc Natl Acad Sci USA* 105: 16201–16206.
34. Sotka EE, Hay ME (2009) Effects of herbivores, nutrient enrichment, and their interactions on macroalgal proliferation and coral growth. *Coral Reefs* 28:555–568.
35. Hoey AS, Bellwood DR (2009) Limited functional redundancy in a high diversity system: Single species dominates key ecological process on coral reefs. *Ecosystems* 12:1316–1328.
36. Haas AF, et al. (2010) Organic matter release by coral reef associated benthic algae in the northern Red Sea. *J Exp Mar Biol Ecol* 389:53–60.
37. Hauri C, Fabricius KE, Schaffelke B, Humphrey C (2010) Chemical and physical environmental conditions underneath mat- and canopy-forming macroalgae, and their effects on understory corals. *PLoS ONE* 5:e12685.
38. Goecke F, Labes A, Wiese J, Imhoff JF (2010) Chemical interactions between marine macroalgae and bacteria. *Mar Ecol Prog Ser* 409:267–299.
39. Mumby PJ, et al. (2006) Fishing, trophic cascades, and the process of grazing on coral reefs. *Science* 311:98–101.
40. Mumby PJ, et al. (2007) Trophic cascade facilitates coral recruitment in a marine reserve. *Proc Natl Acad Sci USA* 104:8362–8367.
41. Mumby PJ, Harborne AR (2010) Marine reserves enhance the recovery of corals on Caribbean reefs. *PLoS ONE* 5:e8657.
42. Selig ER, Bruno JF (2010) A global analysis of the effectiveness of marine protected areas in preventing coral loss. *PLoS ONE* 5:e9278.
43. Schupp PJ, Paul VJ (1994) Calcium carbonate and secondary metabolites in tropical seaweeds—Variable effects on herbivorous fishes. *Ecology* 75:1172–1185.
44. Foster NL, Box SJ, Mumby PJ (2008) Competitive effects of macroalgae on the fecundity of the reef-building coral *Montastraea annularis*. *Mar Ecol Prog Ser* 367: 143–152.
45. Nylund GM, Gribben PE, de Nys R, Steinberg PD, Pavia H (2007) Surface chemistry versus whole-cell extracts: Antifouling tests with seaweed metabolites. *Mar Ecol Prog Ser* 329:73–78.