

# Identifying Sources of Nitrogen to Hanalei Bay, Kauai, Utilizing the Nitrogen Isotope Signature of Macroalgae

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Sewage effluent, storm runoff, discharge from polluted rivers, and inputs of groundwater have all been suggested as potential sources of land derived nutrients into Hanalei Bay, Kauai. We determined the nitrogen isotopic signatures ( $\delta^{15}\text{N}$ ) of different nitrate sources to Hanalei Bay along with the isotopic signature recorded by 11 species of macroalgal collected in the Bay. The macroalgae integrate the isotopic signatures of the nitrate sources over time, thus these data along with the nitrate to dissolved inorganic phosphate molar ratios (N:P) of the macroalgae were used to determine the major nitrate source to the bay ecosystem and which of the macro-nutrients is limiting algae growth, respectively. Relatively low  $\delta^{15}\text{N}$  values (average  $-0.5\text{‰}$ ) were observed in all algae collected throughout the Bay; implicating fertilizer, rather than domestic sewage, as an important external source of nitrogen to the coastal water around Hanalei. The N:P ratio in the algae compared to the ratio in the Bay waters imply that the Hanalei Bay coastal ecosystem is nitrogen limited and thus, increased nitrogen input may potentially impact this coastal ecosystem and specifically the coral reefs in the Bay. Identifying the major source of nutrient loading to the Bay is important for risk assessment and potential remediation plans.

## Introduction

Nutrient loading in several coastal systems, including reefs in Japan, Australia, Hawaii, and the Caribbean, has been cited as a main cause of degrading reef health (1–4). Nutrient loading is a consequence of runoff from roads, fertilized pasture and agriculture land, polluted rivers and groundwater, and improper sewage treatment and disposal. When nutrients such as nitrogen (N) and phosphorus (P) enter reef ecosystems in excessive amounts a phase shift can be seen where the abundance of corals decreases while the population of large, fleshy macroalgae increases (5, 6). Consequently, the algae can deprive the naturally occurring organisms of needed nutrients, compete for substrate, and block out light needed to stimulate the growth of the coral reefs.

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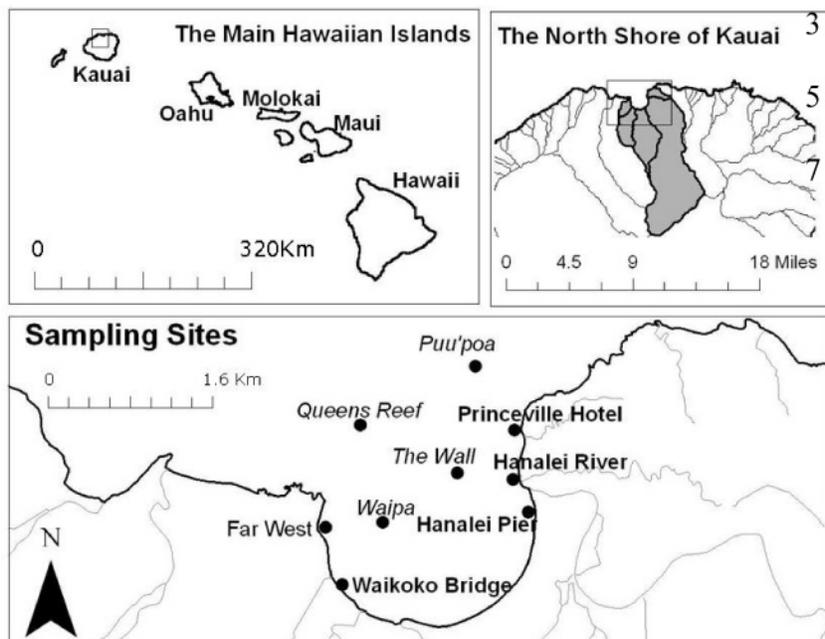
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The reef ecosystems in the Hawaiian Islands have been subject to anthropogenic impacts due to increasing density of population (7). Land-use practices, such as agriculture and increasing urban development, has been reported as a main factor in alteration of coral reef ecosystems in Hawaii due in part to nutrient loading (8). In some locations, such as Kaneohe Bay, this change has been documented since the 1970s (6, 9, 10). However, on other Hawaiian reefs, including Hanalei Bay, nutrient loading is assumed to be a more recent phenomenon and the impact of this nutrient loading has not yet been assessed. Because of scarce monitoring, the source and fate of land based nutrient loading is not well understood. For instance, there is no quantitative estimate for the total nutrient loading and a lack of appreciation of whether these nutrients are removed from the bay by currents or are utilized by organisms within the bay waters. This study attempts to trace the source and flow of nutrients in Hanalei Bay with specific emphasis on N.

The stable isotopes of N can be used to investigate nutrient sources, utilization, and cycling (2–4, 11). Terrestrial nutrient sources can be identified by examining the isotopic signatures of marine organisms that utilize them, including macroalgae, because the signatures of the tissue reflect the source and availability of nutrients over time (3, 12, 13). Certain marine organisms, such as macroalgae, take nutrients directly from the water column, and thus they provide a reliable insight on the long-term uptake and cycling of nutrients in an aquatic system as they integrate the signature of the source over their lifetime. By measuring  $\delta^{15}\text{N}$  as well as the N:P ratio in macroalgae and comparing these ratios to source signatures, the sources of N to the ecosystem can potentially be determined (2, 14, 15).

While certain organisms, such as bacteria and phytoplankton, preferentially utilize  $^{14}\text{N}$  over  $^{15}\text{N}$ , there is no evidence which suggests that macroalgae fractionate N in a similar manner. That is, the  $\delta^{15}\text{N}$  of the macroalgae will generally reflect the  $\delta^{15}\text{N}$  of the source, with relatively little alteration of the original signal (11). Due to the relatively slow turnover time of N within macroalgae, their isotopic composition reflects sources of N integrated over a period of weeks (14). When compared to potential sources of N to a coastal ecosystem (e.g., N associated with open seawater, rivers, and groundwater), the results can be used to map the effect of land-derived nutrients (assuming the isotopic signatures of these sources are distinct) and their cycling within the system. A compilation of the isotopic signature of potential sources of N by Kendall and McDonnell (1998) indicates that, in general, it should be possible to distinguish between various input sources of N to aquatic systems (16).

Aquatic systems impacted by sewage typically show enrichment in  $^{15}\text{N}$  relative to  $^{14}\text{N}$  (2, 15, 17, 18); sewage signatures range between +10 and +20‰, whereas soil N is typically between +2 and +4‰ but could be as low as 0‰ (16). In contrast, artificial fertilizers typically have a much lighter isotopic signature than sewage. Most fertilizers are made by the fixation of atmospheric  $\text{N}_2$ , which has a  $\delta^{15}\text{N}$  value of 0‰, and during the process of fixation the  $^{14}\text{N}$  is preferentially incorporated into the fertilizer resulting in a slightly lower than atmospheric (negative) isotopic signature (16, 19). Thus, systems affected by a fertilizer input of N are expected to exhibit  $\delta^{15}\text{N}$  values between  $-4$  and  $+4\text{‰}$ , with most fertilizers falling around 0‰. In general, if the N is efficiently utilized by coastal organisms, specifically macroalgae, these signatures will be recorded in the algae which utilize the N (16, 20).



**FIGURE 1.** Map of the sampling sites in their geographic context. Each small rectangle indicates the area enlarged in the next panel. In the upper right panel, the shaded area indicates watersheds draining into Hanalei Bay. In the bottom panel, offshore sites are indicated with italics. All five transect locations included three, 60 m transects. Four offshore locations were used. No sampling was possible along the center shoreline due to a lack of rocky substrate and no macroalgal growth.

The N:P ratio of the algae may also contribute to our understanding of nutrient dynamics in aquatic ecosystems and specifically shed light on the relative availability of nutrients compared to the demand (21). For example, in Kanahoe Bay on Oahu, the total nitrogen (TN) to total phosphorus (TP) ratio is approximately 4:1 in the water column, while the TN:TP in macroalgal tissue is 44:1 (22). This has been interpreted as suggesting that P is available in excess relative to the amount demanded by the macroalgae (e.g., the algae are N limited). The C:N ratios in phytoplankton in the water column can also shed light on N availability with high ratios, compared to the Redfield ratio of 6.6, generally indicative of low N availability. In this work, 11 macroalgae species were collected throughout Hanalei Bay and analyzed for their  $\delta^{15}\text{N}$  and C:N:P ratios to shed light on the sources and availability of N to Hanalei Bay. We also determined  $\delta^{15}\text{N}$  signature of potential N sources (fertilizer, soil, offshore seawater, and sewage effluent) and collected suspended particulate matter for determination of its isotopic signature and C:N ratio.

## Materials and Methods

**Site Description.** Hanalei Bay is broad open embayment (~3 km wide, 10 m average depth) located at the north shore of the island of Kaua'i, Hawaii (Figure 1). The greater Hanalei Bay watershed extends from the top of Mount Wai'ale'ale to the coral reefs off Pu'u Po'a and Makahoa Points. The Hanalei Bay watershed includes the Hanalei River, Waioli, Waipa, and Waikoko watersheds. Each freshwater segment drains to an estuary before discharging into Hanalei Bay. The Hanalei River watershed is the largest watershed in the Hanalei Bay system, making up 73.2% of the drainage area, and the major river draining into the Bay is the Hanalei River. Much of the rainfall in the area occurs during the wet season from November through April. The Hanalei headwaters receive intermittent but extremely heavy rainfall (~1150 cm year<sup>-1</sup>), and the land use in the lower part of this watershed is mostly agriculture. Hanalei Valley farmers produce over 75% of the state's taro (see Supporting Information for land use map of the watershed). Hanalei town, the only major urban site in

the watershed is a rural village surrounded by wetlands. Hanalei lacks centralized wastewater collection and treatment and is served by cesspools (225), septic systems (75), and two package treatment plants with injection wells. Shallow groundwater is polluted by these cesspools and septic systems and flows directly to Hanalei Bay and River. At Hanalei there are specific recognized land-use practices that may serve as sources of nutrient subsidies to the Bay, including urban (sewage), golf course (fertilizer and gray water), and agriculture (fertilizers from taro fields) activities (23, 24).

**Sample Collection.** The majority of samples for this study were collected in June 2005. Five locations throughout the Bay were examined to account for spatial variability and enable identification of potential point-source inputs of nutrients into the water. Three, 60 m transects were conducted at each of the five sites, and algae were collected along these transects at 15 m intervals resulting in five collection points along the 60 m line. In addition, algae were collected at four separate sites offshore in the middle of the bay (Figure 1). No macroalgae were available to sample at some locations of the Bay due to lack of rocky substrate.

All samples were collected at low tide, and the maximum water depth that any given transect reached was roughly 2 m. The transects conducted by the Waikoko Bridge were only 45 m in length (and thus only four points were sampled along these transects) due to high surf and deep waters. Eleven separate species of algae were chosen based on their regular and widespread distribution throughout the bay at various depths: *Sphacelara furcigera*, cyanophyta (blue green algae), *Dictyota acutiloba*, *Dictyosphaeria Versluisii*, *Halimeda opuntia*, *Jania* sp., *Microdictyon japonicum*, *Neomeris annulata*, *Padina* sp., and *Symploca* sp. Tissue nutrient content (specifically N and P) vary between species due to physiologic and morphologic variation among the various macroalgae (22). Therefore, each species was analyzed separately to control for differences in nutrient processing. In addition, to investigate potential seasonal variability, samples of *Sphacelara furcigera* were also collected at the Hanalei Pier on June 2005 and August 2006 and *Dicty-*

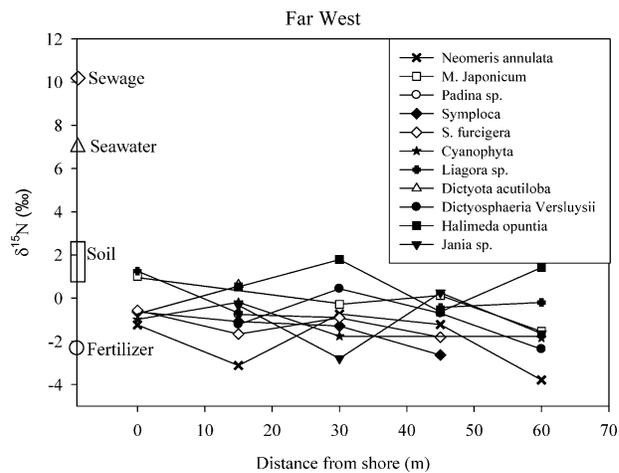
*osphaeria versluysii* samples were collected off Princeville Hotel in June 2005 and February 2007.

Macroalgae were bagged, identified, and labeled, and frozen for transportation to the mainland. Fertilizers used in taro fields and golf courses in the watershed (one commercial supplier provides all fertilizers used) and sewage samples from cesspools and a sewage treatment facility were sampled as well to represent the terrestrial end members. Several soil samples from various parts of the watershed including samples representing the upper part of the watershed at elevations above any human activity were also obtained. Additionally, water samples were taken along each transect, at both the 0 m and the 60/45 m points and from the Hanalei river upland and at the mouth. Approximately 100 mL of this water was filtered through a 0.2  $\mu\text{m}$  filter, bottled, and frozen for later nutrient and dissolved nitrate isotope analyses and 450 mL was filtered through pre-ashed glass fiber filters for particulate organic matter (POC) isotope and C:N ratio analysis. Temperature and salinity of the water at each site was determined in the field using a YSI 85/50 probe.

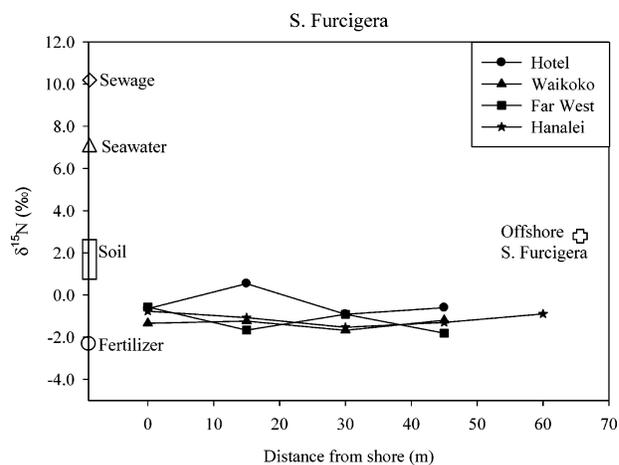
**Sample Preparation and Analyses.** The macroalgae were dried in ovens at 50 °C to a constant weight, ground, and homogenized using a mortar and pestle. About 2 mg of the ground samples were placed in small silver capsules and sent for mass spectrometric analysis. For C isotopes, samples were acidified to remove carbonate. The POC filters were also placed into tin capsules for isotopic and C:N ratio analysis. Isotope analyses were conducted at the UC Davis Stable Isotope facility using a Europa Hydra 20/20 mass spectrometer with a precision of 0.3‰ for  $\delta^{15}\text{N}$ . Phosphorus concentrations in the macroalgae were determined using a Hitachi U-2001 spectrophotometer after digestion as described in Parkinson and Allen (25). To obtain a sample representative of the water leachable nitrate from soils, 10 g of dried soil was suspended in 100 mL of deionizer water and shaken overnight followed by filtration of the sample and analysis of the extract (water) for nutrient content and the nitrate isotopic signature; this was done three times to compare the isotopic signature of the initial nitrate leached to that of subsequent leaching (26). Nutrient (nitrate, nitrite, soluble reactive phosphate, and ammonium) concentrations of the water samples (and soil extracts) were determined using a nutrient flow through auto analyzer at Oregon State University (an Alpkem RFA 300). Nitrogen isotopes of dissolved nitrate in water samples were determined using the nitrifying bacteria method at the U.S. Geological Survey, Menlo Park, CA (27). All samples were calibrated and adjusted for exchange and fractionation against the international nitrate isotopic USGS 34 and USGS 35 (28). Reference standards were run before, after, and at regular intervals between samples. Analytical precision measured from multiple determinations on standards was approximately 0.2‰ for  $\delta^{15}\text{N}$ . Density and salinity of the water samples were obtained using a densimeter (all data are given as Supporting Information).

## Results

The  $\delta^{15}\text{N}$  of the various macroalgae are shown in Figures 2 and 3 (and they are given in the Supporting Information). At any given location, there was no consistent difference in  $\delta^{15}\text{N}$  among the different species (Figure 2). Across all species,  $\delta^{15}\text{N}$  generally ranged between  $-4$  and  $+4$ ‰,  $\pm 0.5$ ‰ at all sites. No systematic variability (gradient) with distance from shore was detected for any of the species examined and at any given site (Figures 2 and 3). Algae collected at a distance slightly further from shore potentially influenced more by open seawater N sources had an average  $\delta^{15}\text{N}$  of  $+2$ ‰ (slightly higher than the Bay samples). The *Sphacelara furcigera* samples collected at the Hanalei Pier in June 2005 and August 2006 and the *Dictyosphaeria versluysii* samples collected off



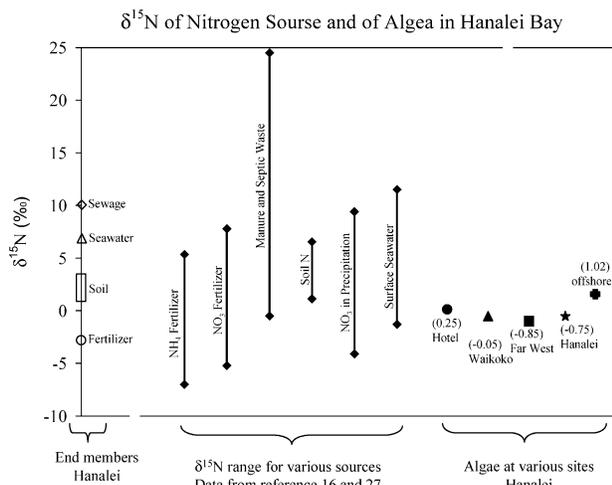
**FIGURE 2.**  $\delta^{15}\text{N}$  ratios of algae (all species) at various distances from shore at the Far West transect. Error bars of 0.3‰ are represented by the size of the symbol. End member  $\delta^{15}\text{N}$  ratios are plotted on the y axis and labeled accordingly.



**FIGURE 3.**  $\delta^{15}\text{N}$  of *S. furcigera* with distance from shore at the various sites. Because no significant variation by species exists, one species (*S. furcigera*) is used as an example to represent all of the species at the different locations. No significant difference is observed between the various inshore sites (Hotels, Waikoko, Far West, Hanalei) yet offshore ( $\sim 1000$  m) values (open cross symbol in the figure) are significantly higher than those of inshore samples.

Princeville Hotel in June 2005 and February 2007 did not show substantial seasonal differences (4.49 vs 3.61‰ and 2.04 vs 2.62‰ respectively). Fertilizer used in the watershed ( $n = 4$ ) and sewage (cesspool sludge and wastewater samples,  $n = 3$ ) had average  $\delta^{15}\text{N}$  of  $-2.1$  and  $+10.1$ ‰, respectively, while our off shore surface seawater samples ( $n = 3$ ) had a nitrate  $\delta^{15}\text{N}$  of  $+7$ ‰. The soil extractable nitrate  $\delta^{15}\text{N}$  ( $n = 5$ ) ranged from  $+0.75$  to  $+4.21$ ‰ (average  $+2.3$ ‰). These end member isotopic signatures are also shown in Figures 2 and 3. The isotopic composition of leachable nitrate from soil increased slightly with repeated extractions (from  $+0.75$  to  $2.18$ ‰ in three consecutive leaches).

Because of the lack of systematic differences between species (or temporally), in Figure 4 we show the average  $\delta^{15}\text{N}$  value of all species collected at each site. Thus, the differences in  $\delta^{15}\text{N}$  between locations were evaluated using the average values of all species sampled at each location. No statistically significant differences (Supporting Information Table 1) were found in  $\delta^{15}\text{N}$  at all of the inshore transects (considering an error of 0.5‰). There was, however, a significant difference ( $p < 0.01$ ) between the inshore macroalgae samples and the  $\delta^{15}\text{N}$  values of algae collected offshore. Samples taken from



**FIGURE 4.** Average  $\delta^{15}\text{N}$  of all species at each site (average value in parenthesis). The comparison between the average of all inshore values (Hotels, Waikoko, Far West, Hanalei) and the average of offshore values is statistically significant ( $z = 2.5$ ,  $p < 0.01$ ). The  $\delta^{15}\text{N}$  range of various N sources compiled in reference (16) and of reported values for seawater from reference (27) are also shown along with the  $\delta^{15}\text{N}$  of end members measured in this study. All data are given in Supporting Information Table 2.

offshore locations, on average, had more positive  $\delta^{15}\text{N}$  (+2‰) than inshore samples (average  $-0.5\text{‰}$ ) (Figures 3 and 4). The isotopic signature of potential sources of N to Hanalei Bay and ranges of various sources compiled from the literature (16, 27) are also shown in Figure 4 for comparison.

To determine whether the macroalgae are nitrogen limited in the bay the N:P in the algae was calculated (Figure 5) and compared to N:P ratios present in the water column. The average N:P of dissolved inorganic nutrients in Hanalei Bay at the time of our sampling was 5.9 (data given in Table 1 of the Supporting Information) while the ratio for all algae species while variable is substantially higher (Figure 5). In Figure 6 we show the C:N ratio of the macroalgae and of particulate organic matter in the bay collected at various sites. The dashed line represents the C:N Redfield ratio of 6.6.

## Discussion

No species, site, or temporal variability in macroalgae  $\delta^{15}\text{N}$ , and no gradient (with distance from shore) were observed in our data from Hanalei Bay (Figures 2–4) suggesting that Hanalei is a very well-mixed bay and that no point-source identification of nutrient flow into the bay is possible without further testing. Given the shape and size of the bay, in addition to the heavy current flow and large-wave potential during certain months of the year, this is not surprising. In fact, the observation that the bay is well-mixed has profound implications for any anthropogenic nutrients or other pollutants that flow into Hanalei waters. Specifically, any point source pollution will be mixed and diluted into the whole bay, thus reducing localized impact. However, if the pollution is considerable such that dilution is not enough to reduce the risk then the whole bay may be impacted.

Overall, the macroalgae samples in our study had relatively low  $\delta^{15}\text{N}$  when compared to other studies in similar reef environments. As noted, most ratios fell between  $-4$  and  $+4\text{‰}$  (SI Table 1, Figure 4), while the overall average (including all species from all bay sites) was  $-0.5\text{‰}$ . While there may be a range in  $\delta^{15}\text{N}$  of macroalgae due to species specific nutrient uptake and processing, this study does not show significant species-dependent variation in  $\delta^{15}\text{N}$  ratios. Therefore, the average of all species can be used as representative of the N source to the algae in the bay (Figure

4). Similar work on macroalgae from other anthropogenically impacted coastal environments typically report much higher  $\delta^{15}\text{N}$  which were interpreted as sewage pollution inputs. For example, Costanzo et al., (2004) compared the  $\delta^{15}\text{N}$  in macroalgae between 1998 and 2003 in Moreton Bay, Australia (2). They discovered  $\delta^{15}\text{N}$  of up to  $+9\text{‰}$  in macroalgae in 1998 compared to  $+2\text{‰}$  in macroalgae in low nutrient environments. In 2003 these values were reduced to  $+7\text{‰}$  and they concluded that the reduction in  $\delta^{15}\text{N}$  was due in large part to the greater investments in sewage treatment infrastructure. Similarly, Umezawa et al., (2002) tracked land-derived N by observing  $\delta^{15}\text{N}$  in macroalgae from the reefs of the Ryukyu Islands in Japan (3). They noticed values of up to  $+8\text{‰}$  along the shoreline and approximately  $+2\text{‰}$  along the reef crest further away from shore. They concluded that land-derived N (specifically from sewage) led to greater  $\delta^{15}\text{N}$  along the shoreline and that more pristine signatures were seen as distance from shore increased. Studies in other settings have used  $\delta^{15}\text{N}$  measurements in seagrasses (14) estuarine (15) or bay (29) algae and in coral reefs (30) to infer nutrient loading from sewage and terrestrial sources. Our data does not show high isotope ratios, as seen in our sewage impacted end member samples, or an off shore gradient in isotopic signatures, suggesting that sewage is not an important source of N to the algae in Hanalei Bay, or that if it does enter the bay, it is mixed out and diluted such that the signature of this source is lost.

While it can be difficult to determine differences between sources with very similar  $\delta^{15}\text{N}$  (26), the suggested sources of nutrients into Hanalei Bay (e.g., fertilizers and septic waste) have distinct isotopic signatures and thus can be easily differentiated and identified (Figures 2–4). In this work, the  $\delta^{15}\text{N}$  obtained for the fertilizer used commercially in all taro and golf course applications in Hanalei (16-16-16, Brewer Environmental, Hawaii) was  $-2.1\text{‰}$  while the cesspool, wastewater and other sewage impacted sample we analyzed had an average value of  $+10.1\text{‰}$ . These ratios are consistent with Kendall's (1998) data compilation (16) (Figure 4). Another source of N to Hanalei Bay could be nitrate leached from soil by rain. This natural soil nitrate may enter the bay through rivers or runoff. Our water extraction of local soil samples had an average  $\delta^{15}\text{N}$  of  $2.3\text{‰}$ , consistent with reported values for leachable nitrate in soils from Hawaii (between 0 and  $5\text{‰}$ ) (31–33). Repeated leaching of the soil slightly increased the isotopic signature from 0.75 to  $2.18\text{‰}$  (see Supporting Information). The typical N isotopic signature of seawater nitrate, another potential N source to marine algae, is expected to be about  $+5.5\text{‰}$  for deep water nitrate and it may become more enriched (e.g.,  $>5\text{‰}$ ) in surface waters due to the preferential uptake of  $^{14}\text{N}$  nitrate over  $^{15}\text{N}$  nitrate by marine phytoplankton (34). Indeed N isotope analyses of dissolved nitrate in the surface water in Hanalei Bay gave values close to  $+7\text{‰}$ , similar to other reported surface seawater values (27).

The macroalgae  $\delta^{15}\text{N}$  values, averaging  $-0.5\text{‰}$ , fall within the range that could be expected for locations heavily impacted by fertilizer nitrate. The algae used in this study do not fix di-nitrogen thus N-fixation is not considered important here. The difference between the average algae and the fertilizer signatures ( $-0.5\text{‰}$  vs  $-2.1\text{‰}$ ) could be explained by some denitrification modifying the fertilizer nitrate isotopic signature in the groundwater before discharge. Utilization of small amounts of nitrate from additional sources with a more enriched signature (sewage and seawater N) by the algae resulting in a mixed signal which is proportional to the relative contribution of the various sources can also explain the offset.

Although it is not trivial to distinguish between the isotopic signatures of fertilizer and the possible range of N from local soils, we suggest that fertilizer input through rivers, runoff,

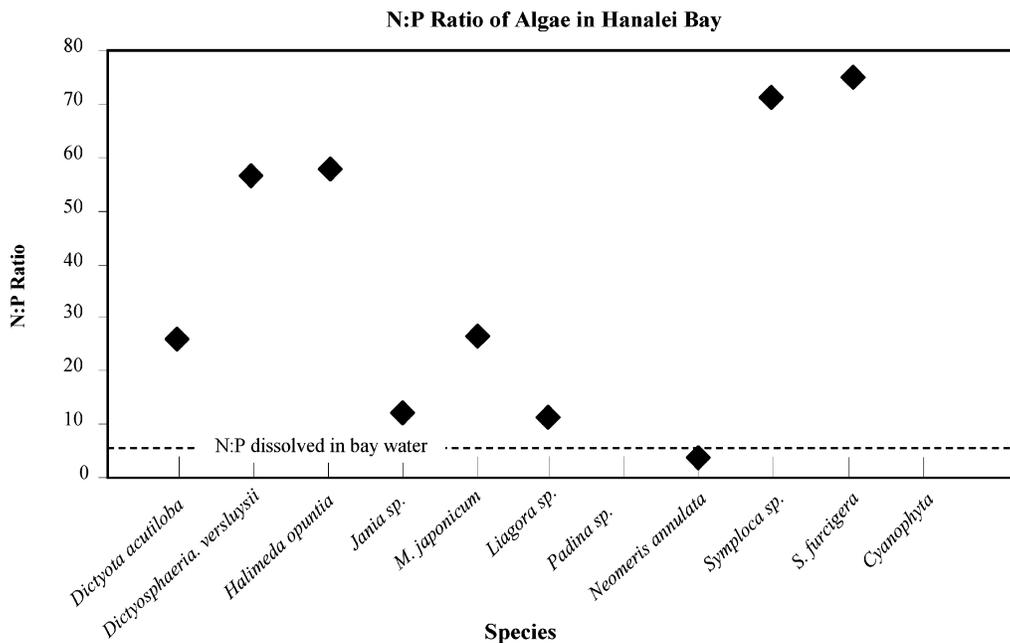


FIGURE 5. N:P ratio by species averaged over all sites. The solid line (5.9) represents the average ratio of nitrate to soluble reactive phosphate dissolved in the Hanalei Bay water. Note that two species were not analyzed for N:P values due to a lack of sufficient sample mass to complete a phosphorus digest.

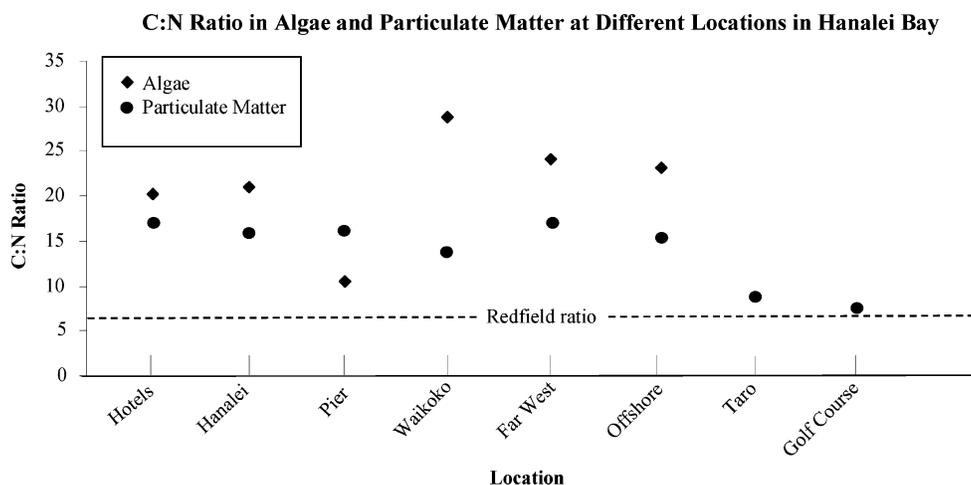


FIGURE 6. C:N Ratio of particulate organic matter and algae in Hanalei Bay. The dashed line represents the Redfield ratio (6.6).

and groundwater is the major source of N to the bay waters. Indeed, the average algae  $\delta^{15}\text{N}$  value ( $-0.5\text{‰}$ ) is lower than any soil leached in our study (lowest value  $0.75\text{‰}$ ) and the soil leached nitrate becomes even more enriched with repeated leaching. A soil sample from a taro field where fertilizer was applied interestingly gave a leachable nitrate isotopic signature of  $-0.56\text{‰}$ , which was similar to the value observed in the algae and lower than other soil samples. Moreover, in recent years, agricultural production and tourist attractions have led to an overall increase in the use of fertilizer in Hanalei and surrounding areas. In fact, much of the area surrounding Hanalei Bay is comprised of a large golf course (property of the Princeville Hotel) and farms producing taro (see Supporting Information). Nutrient monitoring of river waters in the Hanalei watershed show that nitrate levels in the river at locations/elevation where taro fields and development are not present is quite low ( $\sim 0.25 \mu\text{mol kg}^{-1}$ ) representing concentrations expected from leaching of the natural soil (e.g., nonanthropogenic nitrate contribution), while down stream, after the river has encountered taro agriculture and residential development, a 6-fold increase in the nitrate concentrations to at least

$1.5 \mu\text{mol kg}^{-1}$  (and at times substantially higher) is observed. This suggests that the land use practices result in nitrate amendment (C. Berg, personal communication). Indeed, previous studies (23) concluded that due to fertilization of taro ponds, or lo'i, N loads in return waters (discharge from the fields) were 4–40 times higher when compared to inflow waters (before interacting with the fertilizer). This is consistent with our interpretation of the macroalgae isotope data. Thus, we conclude that fertilizer is indeed the source of much of the external N used by the macroalgae in Hanalei Bay.

While no significant difference in  $\delta^{15}\text{N}$  at any of the inshore sites was observed, the most noticeable distinction is that the offshore average of the different species sampled is significantly higher in  $\delta^{15}\text{N}$ . The N isotopic signature at the offshore sites may be elevated compared to the bay as a result of additional contribution from open seawater nitrate which is characterized by  $\delta^{15}\text{N}$  values of around  $+5\text{‰}$  or above (average  $+7\text{‰}$  in our study), thus potentially elevating the isotopic signature of algae offshore. However, the  $\delta^{15}\text{N}$  in the macroalgae at the offshore sites is still quite low. While offshore values are more positive than inshore values, even the offshore average  $\delta^{15}\text{N}$  is only  $+2\text{‰}$ . It is probable that

the influence of the fertilizer N is still evident at these sites about 1000 m off shore.

The potential impact of anthropogenic nutrient loading on aquatic ecosystems is more pronounced when the added nutrient is limiting growth. In other words, if the algae do not utilize the added nutrients because another parameter is limiting their growth then the impact from that specific nutrient loading is not expected to be great. Accordingly, N:P ratios in algae and seawater were assessed. Large variations in N/P ratios were found between the different species, ranging between 4:1 and 78:1. This variability was expected, and reflects the differential metabolic needs of each particular species. Previous research suggests that the average N:P ratio for macroalgae is approximately 40:1 (20), and the values obtained in this study are generally consistent with the expected N:P ratios for each species (20, 35). High N:P ratios in macroalgae tissue combined with low N concentrations in the water column has been previously suggested as a possible indicator of N limitation (20). Accordingly, the N:P ratio available in the water column was compared to the ratio in the algae (Figure 5). As noted, the N:P ratio in the bay water column (e.g., the dissolved inorganic N to soluble reactive phosphorus molar ratio), averaged over all of the sites, was 5.9 (e.g., lower than the Redfield ratio of 16). Because the ratio of N:P in the water column was significantly less than the N:P ratio in each macroalgae species (with the exception of *N. annulata*), this suggests that nitrogen is most likely the limiting macronutrient for macroalgae productivity (20).

The C:N ratio of particulate organic matter (POM) (Figure 6) in the bay, representing phytoplankton, also supports N limitation. This ratio at various sites ranges from 10 to 30, always higher than the Redfield ratio of 6:6 which is expected to be the C:N ratio for non N-limited average marine phytoplankton. While we cannot exclude some contribution from terrestrial organic matter with higher C:N to the particulate matter samples, samples from pools and ponds in the taro field and golf course (Figure 6) which should be more impacted by terrestrial matter have, in fact, a lower C:N ratio than the POM in the bay indicating that terrestrial influence could be neglected. These results are consistent with the N:P ratio also suggesting N limitation in the bay. The C:N ratio in the macroalgae was also higher than the Redfield ratio, although it is not clear if such ratios are indicative of N-limitation as they are in phytoplankton. As stated above the C:N of particulate matter in the golf course ditches and taro fields are closer to the Redfield most likely because of the higher nitrate concentrations in these waters (no N limitation). Based on these results, it is probable that all excess anthropogenic N input into the bay is utilized immediately for macroalgae (and other primary producers) growth. This implies that the macroalgae might be growing as fast as the nutrients can be supplied, and such macroalgae growth could possibly have an alarming impact on the surrounding reef environment if input will increase in the future. This evidence, combined with the implications that fertilizer runoff is flowing into the bay in large amounts, suggests that the reef in Hanalei Bay could be in crisis if nutrient loads increase in the future. The suggestion that increased fertilizer use and subsequent fertilizer-rich runoff and groundwater input to the bay may result in a large-scale impact on the nutrient dynamics in the bay and possibly on the surrounding reef environment has severe implications for the impact of future increased agriculture and tourism development in the region and in Hawaii in general.

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### Supporting Information Available

Three tables with all of the data analyzed and one figure of a land use map of Hanalei Bay watershed. This material is available free of charge via the Internet at <http://pubs.acs.org>.

### Literature Cited

- Hoegh-Guldberg, O. Climate change, coral bleaching, and the future of the world's coral reefs. *Mar. Freshwater Res.* **1999**, *50*, 839–866.
- Costanzo, S. D.; Udy, J.; Longstaff, B.; Jones, A. Using nitrogen stable isotope ratios ( $\delta^{15}\text{N}$ ) to determine the effectiveness of sewage upgrades: changes in the extent of sewage plumes over four years in Moreton Bay, Australia. *Mar. Pollut. Bull.* **2002**, *51*, 212–217.
- Umezawa, Y.; Miyajima, T.; Yamamuro, M.; Kayanne, H.; Koike, I. Fine scale mapping of land-derived nitrogen in coral reefs by  $\delta^{15}\text{N}$  in macroalgae. *Limnol. Oceanogr.* **2002**, *47*, 1405–1416.
- Cole, Marci L.; Valiela, I.; Kroeger, K. D.; Tomasky, G. L.; Cebrian, J.; Wigand, C.; McKinney, R. A.; Grady, S. P.; Carvalho da Silva, M. H. Assessment of a  $^{15}\text{N}$  Method to indicate anthropogenic eutrophication in aquatic ecosystems. *J. Environ. Qual.* **2004**, *33*, 124–132.
- McCook, L. J. Macroalgae, nutrients and phase shifts on coral reefs: scientific issues and management consequences for the Great Barrier Reef. *Coral Reefs.* **1999**, *18* (4), 357–367.
- Stimson, J.; Larned, S.; Conklin, E. Effects of herbivory, nutrient levels, and introduced algae on the distribution and abundance of the invasive macroalga *Dictyosphaeria cavernosa* in Kaneohe Bay, Hawaii. *Coral Reefs.* **2001**, *19* (4), 343–357.
- Laws, E. A. Human impacts on fluxes of nutrients and sediment in waimanalo stream, O'ahu, Hawaiian Islands. *Pac. Sci.* **2003**, *57* (2), 119–140.
- Fong, P.; Boyer, K. E.; Zedler, J. B. Developing an indicator of nutrient enrichment in coastal estuaries and lagoons using tissue nitrogen content of the opportunistic algae, *Enteromorpha intestinalis*. *Exp. Mar. Biol. Ecol.* **1998**, *231*, 63–79.
- Smith, J.; Smith, C.; Hunter, C. An experimental analysis of the effects of herbivory and nutrient enrichment on benthic community dynamics on a Hawaiian reef. *Coral Reefs.* **2001**, *19* (4), 332–342.
- Garrison, G. H.; Glenn, C. R.; McMurtry, G. H. Measurement of submarine groundwater discharge in Kahana Bay, O'ahu, Hawai'i. *Limnol. Oceanogr.* **2003**, 920–928.
- Cohen, R. A.; Fong, P. Experimental evidence supports the use of  $\delta^{15}\text{N}$  content of the opportunistic green macroalga *Enteromorpha intestinalis* (chlorophyta) to determine nitrogen sources to estuaries. *J. Phycol.* **2005**, *41*, 287–293.
- Heikoop, J. M.; Risk, M. J.; Lazier, A. V.; Edinger, E. N.; Jompa, J. Nitrogen-15 signals of anthropogenic nutrient loading in reef corals. *Mar. Pollut. Bull.* **2000**, *40*, 628–636.
- Swart, P. K.; Saied, A.; Lamb, K. Temporal and spatial variation in the delta N-15 and delta C-13 of coral tissue and zooxanthellae in *Montastraea faveolata* collected from the Florida reef tract. *Limnol. Oceanogr.* **2005**, *50*, 1049–1058.
- Fourqurean, J. W.; Moore, T. O.; Fry, B.; Hollibaugh, J. T. Spatial and temporal variation in C:N:P ratios, delta N-15 and delta C-13 of eelgrass *Zostera marina* as indicators of ecosystem processes, Tomales Bay, California, U.S.A. *Mar. Ecol.: Prog. Ser.* **1997**, *157*, 147–157.
- McClelland, J. W.; Valiela, I. Linking nitrogen in estuarine producers to land-derived sources. *Limnol. Oceanogr.* **1998**, *43*, 577–585.
- Kendall, C. Tracing Nitrogen Sources and Cycling in Catchments. In *Isotope Tracers in Catchment Hydrology*; Elsevier: Amsterdam, 1998; pp 519–576.
- Arawena, R.; Evans, M. L.; Cherry, J. A. Stable isotopes of oxygen and nitrogen in source identification of nitrate from septic systems. *Ground Water* **1993**, *31*, 180–186.
- Macko, S. A.; Ostrom, N. E.; Lajtha, K.; Michener, R. H. *Stable Isotopes in Ecology and Environmental Science*; Blackwell Scientific Publications: Oxford, 1994.

- (19) Macko, S. A.; Ostrom N. E. Pollution Studies Using Stable Isotopes. In *Stable Isotopes in Ecology and Environmental Science*; Macko, S. A., Ostrom, N. E., Lajtha, K., Michener, R.H., Eds; Blackwell Scientific Publications: Oxford, 1994; pp 45–62.
- (20) Larned, S. T. Nitrogen- versus phosphorus-limited growth and sources of nutrients for coral reef macroalgae. *Mar. Biol.* **1998**, *132*, 409–421.
- (21) Fourqurean, J. W.; Zieman J. C. Nutrient content of the seagrass *Thalassia testudinum* reveals regional patterns of relative availability of nitrogen and phosphorus in the Florida Keys USA. *Biogeochemistry* **2002**, *61*, 229–245.
- (22) Fong, P.; Kamer, K.; Boyer, K. E.; Boyle, K. A. Nutrient content of macroalgae with differing morphologies may indicate sources of nutrients for tropical marine systems. *Mar. Ecol.: Prog. Ser.* **2001**, *220*, 137–152.
- (23) Berg N.; McGurk B.; Calhoun R. S. *Hydrology and Land Use Effects on the Hanalei National Wildlife Refuge, Kaua'i, Hawai'i*; USDA Forest Service, Interagency Agreement 14-48-0001-94588, Final Report; U.S. Department of Agriculture: Washington, DC, 1997.
- (24) Schueler T. R.; Holland H. K. *The Practice of Watershed Protection*; Center for Watershed Protection: Ellicott City, MD, 2000.
- (25) Parkinson, J. A.; Allen, S. E. A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. *Community Soil Sci. Plant Anal.* **1975**, *6*, 1–11.
- (26) Feigin, A.; Kohl, D. H.; Shearer, G.; Commoner, B. Variation in natural nitrogen-15 abundance in nitrate mineralized during incubations of several Illinois soils. *Soil Sci. Soc. Am. J.* **1974**, *38*, 90–95.
- (27) Wankel, S. D.; Kendall, C.; Francis, C. A.; Paytan, A. Nitrogen sources and cycling in the San Francisco Bay Estuary: A nitrate dual isotopic composition approach. *Limnol. Oceanogr.* **2006**, *51*, 1654–1664.
- (28) Bohlke, J. K.; Mroczkowski, S. J.; Coplen, T. B. Oxygen isotopes in nitrate: new reference materials for O-18 : O-17 : O-16 measurements and observations on nitrate-water equilibration. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 1835–1846.
- (29) Rogers, K. M. Stable carbon and nitrogen isotope signatures indicate recovery of marine biota from sewage pollution at Moa Point, New Zealand. *Mar. Pollut. Bull.* **2003**, *46*, 821–827.
- (30) Sammarco, P. W.; Risk, M. J.; Schwarcz, H. P.; Heikoop, J. M. Cross-continental shelf trends in coral delta N-15 on the Great Barrier Reef: further consideration of the reef nutrient paradox. *Mar. Ecol.: Prog. Ser.* **1999**, *180*, 131–138.
- (31) Vitousek, P. M.; Walker, L. R.; Whiteaker, L. D.; Matson, P. A. Nutrient limitation to plant growth during primary succession in Hawaii. Volcanoes National Park. *Biogeochemistry.* **1993**, *23*, 197–215.
- (32) Vitousek, P. M.; Turner, D. R.; Kitayama, K. Foliar nutrients during long-term soil development in Hawaiian montane rain forest. *Ecology.* **1995**, *76*, 712–720.
- (33) Martinelli, L. A.; Piccolo, M. C.; Townsend, A. R.; Vitousek, P. M. Nitrogen stable isotope composition of leaves and soil: tropical versus temperate forests. *Biogeochemistry.* **1999**, *46*, 45–65.
- (34) Granger, J.; Sigman, D. M.; Needoba, J. A.; Harrison, P. J. Coupled nitrogen and oxygen isotope fractionation of nitrate during assimilation by cultures of marine phytoplankton. *Limnol. Oceanogr.* **2004**, *49*, 1763–1773.
- (35) Atkinson, M. J.; Smith, S. V. C:N:P ratios in benthic marine plants. *Limnol. Oceanogr.* **1983**, *28*, 568–574.

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