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Effects of submarine groundwater discharge on bacterial growth efficiency in coastal Hawaiian waters

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ABSTRACT: An unresolved question in microbial oceanography is to what extent do heterotrophic bacteria serve as a carbon (C) and nutrient source for higher trophic levels in food webs. Coastal bacterial growth efficiency (BGE) studies addressing this question have focused largely on river-dominated estuaries, but submarine groundwater discharge (SGD) is also an important freshwater, nutrient, and organic matter source to coastal waters, and its effect on BGE is unknown. We assessed BGE, cell abundance, growth rates, production, respiration, and dissolved organic carbon (DOC) bioavailability in surface waters inside and outside of SGD plumes at 4 sites (2 leeward and 2 windward) on Hawaiʻi Island. SGD effects on bacterial dynamics were greatest within windward SGD plumes, where discharge rates were highest. SGD effects were minimal within leeward plumes as their values were comparable to those in nearby ocean waters. In windward SGD plumes, BGE and cell abundance were lowest, but bacterial growth rates and DOC bioavailability were highest. Bacterial cell abundance was also inversely related to salinity, suggesting that either SGD diluted marine bacterial cells or that it had lower abundances compared to marine waters. Uncoupling of bacterial production and respiration may explain the inverse patterns observed with BGE and growth rates. Overall, low BGE (8 to 20%) in these coastal waters, especially those with high SGD, suggest that bacteria transfer only a small fraction of their consumed C to the next trophic level, and are possibly a source of CO_2 to the atmosphere.

KEY WORDS: Bacterial cell abundance · Bacterial growth efficiency · Bacterial growth rates · Bacterial production · Bacterial respiration · Hawaiʻi · Submarine groundwater discharge

INTRODUCTION

The idea that microorganisms play an important role in marine food webs was first suggested by Pomeroy (1974), and our understanding of their role has been evolving ever since. Microbes are no longer thought of as just decomposers in the marine ecosystem, but as major consumers and respirers of dissolved organic matter (DOM) which funnel carbon (C) and nutrients back into the food web, supporting higher trophic levels (Pomeroy 1974, Azam et al. 1983, Cole et al. 1988, Lee et al. 2009, Atwood et al. 2012). However, it is debated whether these bacteria are primarily a source of organic C to higher trophic levels, or sinks, remineralizing the organic C and releasing CO_2 into the water. Identifying these patterns and variations of microbial processes is fundamental to understanding C cycling. Bacterial metabolism indices such as bacterial growth efficiency (BGE) allow us to analyze the amount of C taken in by bacteria and determine how much is being used for biomass production that can be cycled into the food web ver-

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sus the amount being lost through respiration. Specifically, BGE is the ratio of the C converted into biomass relative to the bacterial C demand (BCD), measured as the sum of bacterial production (BP) and respiration (BR). Studies conducted in temperate regions have found that BGE varies over time and space, depending on temperature, primary productivity, and the quality and quantity of DOM available in the system (del Giorgio & Cole 1998, Lemée et al. 2002, Pradeep Ram et al. 2003, Apple & del Giorgio 2007). However, few studies have examined bacterial metabolism in the tropics, as well as how bacterial abundance, growth, production, and respiration vary and influence BGE (Lee et al. 2009).

Tropical coastal waters are often net heterotrophic, where respiration is greater than primary production (Pradeep Ram et al. 2003, Johnson & Wiegner 2013). These conditions are thought to be largely driven by heterotrophic bacteria whose BGE is low and respiration is fueled by allochthonous DOM inputs (Lee et al. 2009, Duarte et al. 2013, Ducklow & Doney 2013, Williams et al. 2013). However, the fate of allochthonous C inputs to coastal systems is not well known (Pradeep Ram et al. 2003). Studies that have examined BGE and allochthonous organic C inputs into coastal systems have primarily been conducted in temperate estuaries with rivers (Apple & del Giorgio 2007), with only a few conducted in the tropics (Pradeep Ram et al. 2003, Lee et al. 2009), and to our knowledge, none in areas with submarine groundwater discharge (SGD).

SGD can be a significant source of freshwater entering the coastal environment. In the Atlantic Ocean, SGD is similar in volume to the riverine flux (Moore et al. 2008). Additionally, because SGD often has higher nutrient concentrations than river water, its nutrient inputs to the ocean can rival, and in certain regions substantially exceed, those from rivers (Slomp & Van Cappellen 2004, Paytan et al. 2006, Longnecker & Kujawinski 2011). For example, in coastal bays in New England, SGD delivers 2 to 32 times more nitrogen (N) than rivers (Valiela et al. 1990). In regions where the substrate is highly permeable and has reduced capabilities of river formation — such as in the Hawaiian Islands, particularly on the dry leeward sides — SGD is the dominant freshwater and nutrient source to the ocean (Paytan et al. 2006, Street et al. 2008). While nutrient inputs from SGD have been well studied in Hawai'i and elsewhere (Gobler & Boneillo 2003, ArandaCirerol et al. 2006, Street et al. 2008, Knee et al. 2010), few studies have examined inputs of allochthonous DOM from SGD (Tedetti et al. 2011, Nelson et al. 2015), and none have examined its fate in coastal waters. SGD can have variable concentrations of DOM based on flow rate, seawater mixing, microbial activity, and land use (Longnecker & Kujawinski 2011). However, there is limited information on DOM sources, chemical composition, and concentrations in SGD in many places, especially in the tropics (Longnecker & Kujawinski 2011). Likewise, very little is known about the quality and fate of organic C in SGD plumes anywhere (Johnson & Wiegner 2013).

Recent studies on the leeward side of Hawai'i Island have observed heterotrophy and non-conservative mixing of DOM and nutrients in SGD plumes (Johnson & Wiegner 2013). These results suggest that SGD-derived DOM and nutrients were being consumed, as respiration was greater than primary production in these SGD plumes (Johnson & Wiegner 2013). In other locations, SGD nutrients have been shown to cause macroalgal and phytoplankton blooms, some of which have been ecologically and economically devastating (Gobler & Sañudo-Wilhelmy 2001, Smith et al. 2005, Hu et al. 2006). While the response of primary producers has begun to be documented in coastal waters with SGD (Troccoli-Ghinaglia et al. 2010, Johnson & Wiegner 2013), little is known about the effects of these nutrients and DOM on bacteria within the surface waters, where SGD accumulates. With increasing coastal development, spread of invasive N_2-fixing plants, and climate change, there is a concern about enhanced nutrient loading to SGD and its potential negative effects on coastal water quality, such as eutrophication and macroalgae blooms (Johnson et al. 2008, Street et al. 2008, Knee et al. 2010, Engott 2011, Dudley et al. 2014). Understanding the biological impacts of SGD on coastal waters can help in determining how these waters may be affected by changes in nutrient and DOM loads and whether these cause a shift in microbial community structure and function (Valiela et al. 1990, Paytan et al. 2006).

The objective of this study was to determine how microorganisms respond to SGD and the fate of the allochthonous DOM from SGD in tropical coastal waters. We examined bacterial metabolism in surface waters both inside and outside the influence of SGD plumes at 4 sites on Hawai'i Island, specifically, the response of bacterial cell abundance, growth rates, BP, BR, and BGE. Windward (wet) and leeward (dry) sides of the island were compared to see how differences in climate patterns affect this response. The windward side of Hawai'i Island is greatly understudied with regards to SGD, even though there is greater annual rainfall there than on the

leeward side and it has one of the greatest basal aquifers in the Hawaiian Islands (M&E Pacific 1980, Giambelluca et al. 2013). Based on the climate, high SGD, and previous observations of stronger heterotrophy inside of SGD plumes (Johnson & Wiegner 2013), it was hypothesized that the windward SGD plumes will have higher BR, and therefore lower BGE, than leeward plumes. Our bacterial metabolism measurements will help determine how SGD affects bacterial communities and the fate of C in these coastal waters. With this knowledge, we will have a better understanding of the oceanic C budget in tropical coastal waters, as well as how it might respond to global climate change and other anthropogenic influences.

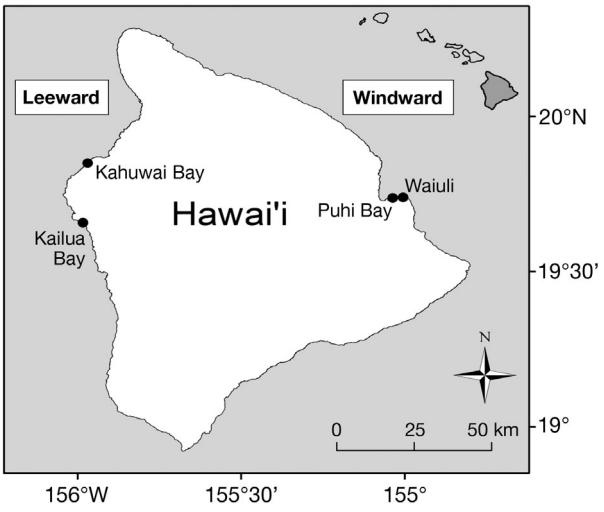

Fig. 1. Sampling sites on Hawai'i Island, HI, USA. Windward (wet) sites are Waiuli and Puhi Bay; leeward (dry) sites are Kailua and Kahuwai Bays

MATERIALS AND METHODS

Study sites

Water for this study was collected from 4 nearshore sites with known SGD on Hawai'i Island from August to November 2012 (Fig. 1, Table 1). Two sites, Waiuli Beach Park and Puhi Bay, are located on the windward (east) side along the Keaukaha coast in Hilo, which receives ~330 cm of rainfall annually (Giambelluca et al. 2013). Surface runoff and SGD are frequent on this side of the island; however, SGD is the only freshwater source along the studied stretch of coastline (M&E Pacific 1980), as there are no rivers. The other 2 sites, Kailua Bay and Kahuwai Bay, are located on the leeward (west) coast, which is arid and receives ~55 cm of rainfall annually (Giambelluca et al. 2013). The young permeable basaltic rock here allows for rainfall to reach the ocean through unconfined aquifers (Oki 1999, Street et al. 2008).

Sampling

High-resolution spatial salinity mapping was conducted using a YSI 6600 V2 multi-parameter sonde interfaced with a YSI 650 MDS data logger and Gar-

Table 1. Characteristics of windward (wet; Waiuli and Puhi Bay) and leeward (dry; Kailua Bay and Kahuwai Bay) watersheds on Hawai'i Island, HI, USA. Watersheds were delineated using ArcGIS 10.1™ software (ESRI) and Hawai'i 10 m digital elevation model (DEM) (NOAA). SGD: sub-marine groundwater discharge

Side	Site	Latitude	Longitude	Watershed area (km^2)	Plume area (m^2)	SGD ($m^3\ d^{-1}$)	Population (no. of people)	Land use (% of total area)				
								Urban	Forest	Agri-culture	Range-land	Barren
Windward	Waiuli	19.735° N	155.014° W	2889	110 000[a]	11 089[b]	108	5	29	4	62	0
	Puhi	19.732° N	155.046° W	1787	1 100 000[a]	110 089[b], 380 000[c]	142	32	51	0	0	17
Leeward	Kailua	19.639° N	155.997° W	1094	4400[d], 7900[d], 89 000[d]	529[d], 879[d], 8989[d], 8700[e]	1384	25	0	0	75	0
	Kahuwai	19.832° N	155.987° W	53 491	55 000[d]	5589[d]	697	0	19	4	52	25

[a]Plume area estimated by aerial thermal infrared images (Fischer et al. 1966)
[b]SGD calculated from equation in Johnson (2008) relating SGD to plume area ($y = 0.10x + 88.5$). This equation was derived from aerial thermal infrared imagery which determined plume area, and from SGD quantified using ^{222}Rn, ^{224}Ra, salinity, and tidal heights in a mass balance model
[c]SGD estimated using rainfall, a contingency equation, and Darcy's Law (M&E Pacific 1980)
[d]Plume area estimated by aerial thermal infrared imagery (Johnson 2008). For Kailua, estimates for 3 areas are provided. SGD was calculated using the equation in footnote b (above)
[e]SGD calculated using ^{222}Rn, ^{224}Ra, salinity, and tidal heights in a mass balance model (Peterson et al. 2009)

min eTrex GPS unit to delineate the SGD plumes at each site (Fig. 2). The sonde was towed by a kayak first along the coastline to define a boundary and then within each bay in a grid-like fashion, while surface water salinity and GPS coordinates were recorded every 3 s. Data were uploaded to YSI's Ecowatch software and imported into Golden Software's SURFER 9, where each sampling event was mapped using Kriging interpolation.

Salinity data from the sonde were also used to determine 3 SGD in-plume (salinity < 32) and 3 SGD out-plume (salinity > 32) stations at each site. These values were based on salinity maximums at the windward sites. Water samples were collected at each of the 6 stations, using 10 l acid-washed high-density polyethylene carboys, at low tide conditions when SGD influence is greatest (Peterson et al. 2009). Water samples were also collected at the surface, where SGD accumulates due to density stratification. Samples were collected at each site once a month for 3 mo from August to November 2012 when the bays receive the least amount of wave action and mixing, as well as when the lowest tides of the year occur. This is the time of year when effects of SGD on microbial processes should be greatest and easiest to detect. Water samples were insulated with towels, away from direct sunlight to maintain a stable temperature while being transported to the laboratory for further analysis.

Laboratory analyses

Water samples taken from each of the carboys were used to assess BR and BP, as well as nutrient concentrations. Prior to water filtration, an unfiltered sample was fixed in gluteradehyde (0.5% final concentration in sample) and stored at –80°C until analysis to determine the initial *in situ* bacterial and phytoplankton cell abundances. Additionally, salinity was measured using the YSI multi-parameter sonde.

Bacterial respiration

A total of 1 l of water from each station was filtered through pre-combusted (6 h at 500°C) GF/F filters (Whatman) to remove particles and bacterial grazers (Lee et al. 2009) into acid-washed, pre-combusted glass flasks. Bacterial cell abundance differences before and after filtration revealed that 62 ± 8.5% (mean ± SD) of the total bacterial population was removed during filtration. Water was then siphoned into 3 acid-washed, pre-combusted 60 ml borosilicate biological oxygen demand (BOD) bottles (Wheaton). The initial dissolved oxygen (DO) concentration was measured in each BOD bottle before being incubated for 48 h in a dark cooler filled with sample water, following methods of Lee et al. (2009). Bottles were kept at constant temperature (22 ± 1°C) between the average temperatures of SGD (~20°C) and ocean water (24 to 28°C) in Hawai'i (Johnson et al. 2008) in order to be able to isolate effects of water chemistry on bacterial metabolism. Final DO concentrations were then measured after the 48 h incubation in order to detect a DO concentration decrease, as shorter incubations did not produce detectable decreases. DO measurements were made using a Hach® LBOD101 luminescence DO (LDO) probe (ASTM D888-05 method C). The LDO probe provides more precise DO measurements (SE; ±0.5 µmol O_2 l^{-1}) than the standard Winkler titration method (±2.0 µmol O_2 l^{-1}) (Johnson & Wiegner 2013). The rate of DO decrease over time was calculated from the difference between the final and initial concentrations, and then dividing that difference by the incubation time. A respiratory quotient of 1.0 was used to convert this DO decrease to BR in C units (Pradeep Ram et al. 2003, Farjalla et al. 2006, Lee et al. 2009).

Bacterial cell abundance, growth, and production

To determine BP, growth rates, and DOC bioavailability, the remaining filtered water, not allocated to the BOD bottles, was incubated in the dark in acid-washed, pre-combusted glass 1 l flasks in the same cooler with the BOD bottles. Every 12 h for 48 h, water from each flask (1 flask station^{-1}) was sampled for bacterial cell abundance and nutrient concentrations. Water for bacterial cell abundances was preserved in glutaraldehyde (0.5%) and stored at –80°C until analysis. Samples for nutrient analysis were filtered with pre-combusted GF/F filters and stored frozen until analysis (~1 to 4 wk).

Thawed samples for bacterial cell abundance, high/low nucleic acid content (HNA/LNA), equivalent spherical diameter (ESD,) as well as phytoplankton cell abundance were analyzed on an Accuri C6 flow cytometer, calibrated with Invitrogen Countbrite counting and Spherotech 8-peak validation beads. Cell counting and size precision for this instrument are <3 and 6% coefficient of variation, respectively. Prior to bacteria cell counts, the thawed samples were run for 120 s with 1 wash between them for phytoplankton cell counts on the red chan-

nel of the flow cytometer. Samples were then stained with Invitrogen SYTO BC green nucleic acid stain and run for 20 s, with 2 washes between samples for bacterial cell counts. The natural log of bacterial cell abundance was plotted using the least-squared regression method to determine an assumed *in situ* bacterial specific growth rate (μ) (Lee et al. 2009).

BP was then calculated by multiplying the bacterial growth rate and *in situ* bacterial cell abundance. Average BP (BP_{avg}) after filtration and during the incubation was calculated using the bacterial growth rate multiplied by the geometric mean of the bacterial cell abundance measurements during the incubation (Briand et al. 2004, Lee et al. 2009). Bacterial biovolume (x) was calculated from ESD, and then converted to bacterial protein content (BPC; fg protein cell^{-1}) using the equation $BPC = 88.6x^{0.59}$ (Simon & Azam 1989). These values were converted to C equivalents (bacterial C content, BCC; fg C cell^{-1}) using a conversion factor (0.86 fg C fg protein^{-1}; Simon & Azam 1989). This BCC was used to convert BP measured in cell abundance to C equivalents. BGE was then calculated using the following equation: $BGE\ (\%) = BP_{avg} / (BP_{avg} + BR) \times 100$ (Lemée et al. 2002, Pradeep Ram et al. 2003, Briand et al. 2004, Lee et al. 2009).

Nutrient analyses

Water samples were analyzed for dissolved organic C (DOC), total dissolved N (TDN), and total dissolved phosphorus (TDP), as well as for $NO_3^- + NO_2^-$, PO_4^{3-}, H_4SiO_4, and NH_4^+ at the University of Hawai'i Analytical Laboratory. DOC (EPA 415.1, Detection Limit [DL]: 10 μmol l^{-1}) and TDN (ASTM D5176, DL: 5 μmol l^{-1}) were analyzed on a Shimazdu TOC-V CSH, TNM-1 analyzer following recommendations of Sharp et al. (2002). Concentrations of $NO_3^- + NO_2^-$ (USEPA 353.4, DL: 0.1 μmol l^{-1}), PO_4^{3-} (USEPA 365.5, DL: 0.1 μmol l^{-1}), H_4SiO_4 (USEPA 366, DL: 1 μmol l^{-1}), NH_4^+ (USGS I-2525, DL: 1.0 μmol l^{-1}), and TDP (USGS 4650-03, DL: 0.5 μmol l^{-1}) were determined using a Technicon Pulse II AutoAnalyzer. Dissolved organic N (DON) was calculated from the differences between TDN and dissolved inorganic N (DIN = $NO_3^- + NO_2^- + NH_4^+$), while dissolved organic P (DOP) was calculated from the difference between TDP and PO_4^{3-}.

DOC bioavailability was calculated as C consumption relative to initial DOC concentration in the water used for bacterial cell abundance, growth, and production measurements for each of the 12 sampling dates (Apple & del Giorgio 2007). C consumption was calculated as BP_{avg} + BR over the 48 h incubations.

Statistical analyses

Bacterial cell abundance, growth rate, respiration, production, C content, BGE, and nutrient concentrations were checked for normality using Shapiro-Wilk test and were log, square root, or rank transformed when needed to meet assumption for parametric analyses. A 2-way analysis of variance (ANOVA) and Tukey's test were run for each parameter to test for differences inside and outside the SGD plumes, between sides of the island, and interactions among these factors ($\alpha < 0.05$). Regressions and correlations were also performed to examine relationships and associations, respectively, among parameters. Data were analyzed in the statistical program R (version 3.0.2).

RESULTS

Hydrography

Surface water salinity determined from discrete samples was significantly lower within SGD plumes ($p < 0.001$), with salinities ~10 psu lower in the windward plumes than in the leeward ones (Table 2). Surface water salinities outside the SGD plumes were similar between windward and leeward sites, at ~34 psu (Table 2). Mapping revealed that surface water salinity and temperature fluctuated over a wider range at the windward than at the leeward sites (Fig. 2). Salinities ranged from 5.83 to 34.65 psu at the windward sites, and 26.80 to 35.57 psu at the leeward ones. Temperatures ranged from 18.55 to 26.14°C at the windward, and 22.28 to 26.64°C at the leeward sites.

Nutrients

Inorganic nutrient concentrations were higher inside than outside SGD plumes across all sites (Table 2). $NO_3^- + NO_2^-$, PO_4^{3-}, and H_4SiO_4 concentrations were ~6, 13, and 10 times higher within the SGD plumes than outside of them, respectively, across sites (Fig. 3a,b,c). H_4SiO_4 concentrations were ~2 times higher at the windward sites compared to the leeward ones (Fig. 3c). $NO_3^- + NO_2^-$ and PO_4^{3-}

Table 2. Mean ± SD salinity (psu) and nutrient concentrations (µmol l^{-1}) of surface water samples collected inside and outside of submarine groundwater discharge (SGD) plumes at windward (wet; Waiuli and Puhi Bay) and leeward (dry; Kailua Bay and Kahuwai Bay) sites on Hawai'i Island, HI, USA. ND: non-detectable. TDN: total dissolved nitrogen; TDP: total dissolved phosphorus; DOC: dissolved organic carbon; DON: dissolved organic nitrogen; DOP: dissolved organic phosphorus. Detection limits can be found in the 'Materials and methods'

Side	Site	n	Salinity	$NO_3^- + NO_2^-$	PO_4^{3-}	H_4SiO_4	NH_4^+	TDN	TDP	DOC	DON	DOP
In-plume												
Windward	Waiuli	9	20.35 ± 4.39	11.9 ± 4.1	0.6 ± 0.5	191 ± 62	0.6 ± 0.3	16 ± 4	0.6 ± 0.5	117 ± 105	4 ± 2	0.1 ± 0.1
	Puhi	9	18.93 ± 3.43	19.2 ± 5.4	0.8 ± 0.5	280 ± 67	0.8 ± 0.4	22 ± 4	0.7 ± 0.6	37 ± 16	2 ± 2	0.1 ± 0.2
Leeward	Kailua	9	30.15 ± 0.88	16.0 ± 4.8	0.7 ± 0.5	121 ± 40	ND < 1.0	21 ± 5	0.7 ± 0.3	77 ± 49	4 ± 3	0.1 ± 0.1
	Kahuwai	9	30.21 ± 0.99	30.4 ± 7.7	0.5 ± 0.5	143 ± 36	ND < 1.0	41 ± 9	0.4 ± 0.2	253 ± 199	8 ± 5	ND
Average		36	24.91 ± 6.03	19.0 ± 8.7	0.7 ± 0.5	184 ± 82	0.6 ± 0.3	24 ± 11	0.6 ± 0.4	117 ± 134	5 ± 4	0.1 ± 0.1
Out-plume												
Windward	Waiuli	9	33.26 ± 1.04	2.5 ± 0.7	ND < 0.1	25 ± 13	0.7 ± 0.5	8 ± 1	0.3 ± 0.2	161 ± 165	5 ± 1	0.2 ± 0.2
	Puhi	9	33.83 ± 0.67	2.2 ± 0.9	ND < 0.1	23 ± 25	ND < 1.0	7 ± 4	ND < 0.5	77 ± 20	4 ± 4	ND
Leeward	Kailua	9	34.18 ± 0.91	5.5 ± 2.8	0.1 ± 0.0	28 ± 20	ND < 1.0	13 ± 4	0.4 ± 0.6	95 ± 36	7 ± 4	0.4 ± 0.6
	Kahuwai	9	35.49 ± 0.16	1.2 ± 0.9	ND < 0.1	3 ± 5	ND < 1.0	8 ± 1	ND < 0.5	239 ± 218	6 ± 1	ND
Average		36	34.19 ± 1.11	2.9 ± 2.2	0.1 ± 0.0	19 ± 20	0.6 ± 0.2	9 ± 4	0.3 ± 0.3	143 ± 147	6 ± 3	0.3 ± 0.3

concentrations were similar between windward and leeward sites (Fig. 3a,b).

In comparison to inorganic nutrients, DOC and DON concentrations were significantly higher outside of the SGD plumes than within them (Fig. 3d, Table 2; DON $p = 0.017$). Overall, DOC and DON concentrations were both ~2 times higher at the leeward sites than the windward ones (Fig. 3d, Table 2; DON $p < 0.001$). However, the DOC:DON ratio in and out of the SGD plumes and between sides of the island was similar. DOP concentrations were low and frequently below detection limits (Table 2).

Bacterial and phytoplankton cell abundances

In situ bacterial cell abundance ranged from 0.58×10^5 to 10.9×10^5 cells ml^{-1} across sites. Bacterial cell abundance within the windward SGD plumes was 6 times lower than outside of them, while at the leeward sites, bacterial cell abundance was similar inside and outside the SGD plumes (Fig. 4a). In addition, bacterial cell abundance at the windward sites was significantly different from those at the leeward sites (Fig. 4a). Across sites, bacterial cell abundance had a significant and positive relationship with salinity (Fig. 5). However, when examining windward and leeward sites separately, bacterial cell abundance and salinity had a stronger relationship at the windward sites ($R^2 = 0.89$, $p < 0.001$) than when the analysis included both sides of the island. There was no relationship between these parameters at the leeward sites.

The percent of HNA bacteria also had a significant interaction between inside and outside the SGD plumes, as well as between the windward and leeward sides of the island. At the windward sites, the percent of HNA bacteria was similar inside and outside the SGD plumes. However, the percent of HNA bacteria inside the leeward SGD plumes was 1.4 times higher than outside of them, and it was also higher than values inside and outside of the windward plumes (Fig. 6). The percent of HNA bacteria was also significantly and positively correlated with TDN concentrations ($r = 0.52$, $p < 0.001$).

Phytoplankton cell abundance was significantly lower inside than outside the SGD plumes at both the windward and leeward sites ($p < 0.001$ and 0.012, respectively; Table 3). Phytoplankton cell abundances were similar inside the plumes on both sides of the island. In contrast, phytoplankton cell abundances were 3 times higher outside the SGD plume on the windward side than on the leeward side ($p < 0.001$). Phytoplankton cell abundance was also positively correlated with bacterial cell abundance (Fig. 7a) and salinity ($r = 0.34$, $p = 0.003$), and negatively correlated with bacterial growth rates (Fig. 7b) and $NO_3^- + NO_2^-$ concentrations ($r = -0.42$, $p < 0.001$).

Bacterial growth rates

Across sites, bacterial growth rates ranged from 0.014 to 0.096 h^{-1}. Growth rates within the windward SGD plumes were 2 times higher than rates outside of them (Fig. 4b). Growth rates within the windward SGD plumes were also significantly higher than ones both inside and outside of the leeward SGD plumes (Fig. 4b). Bacterial growth rates within and outside of the leeward SGD plumes were similar.

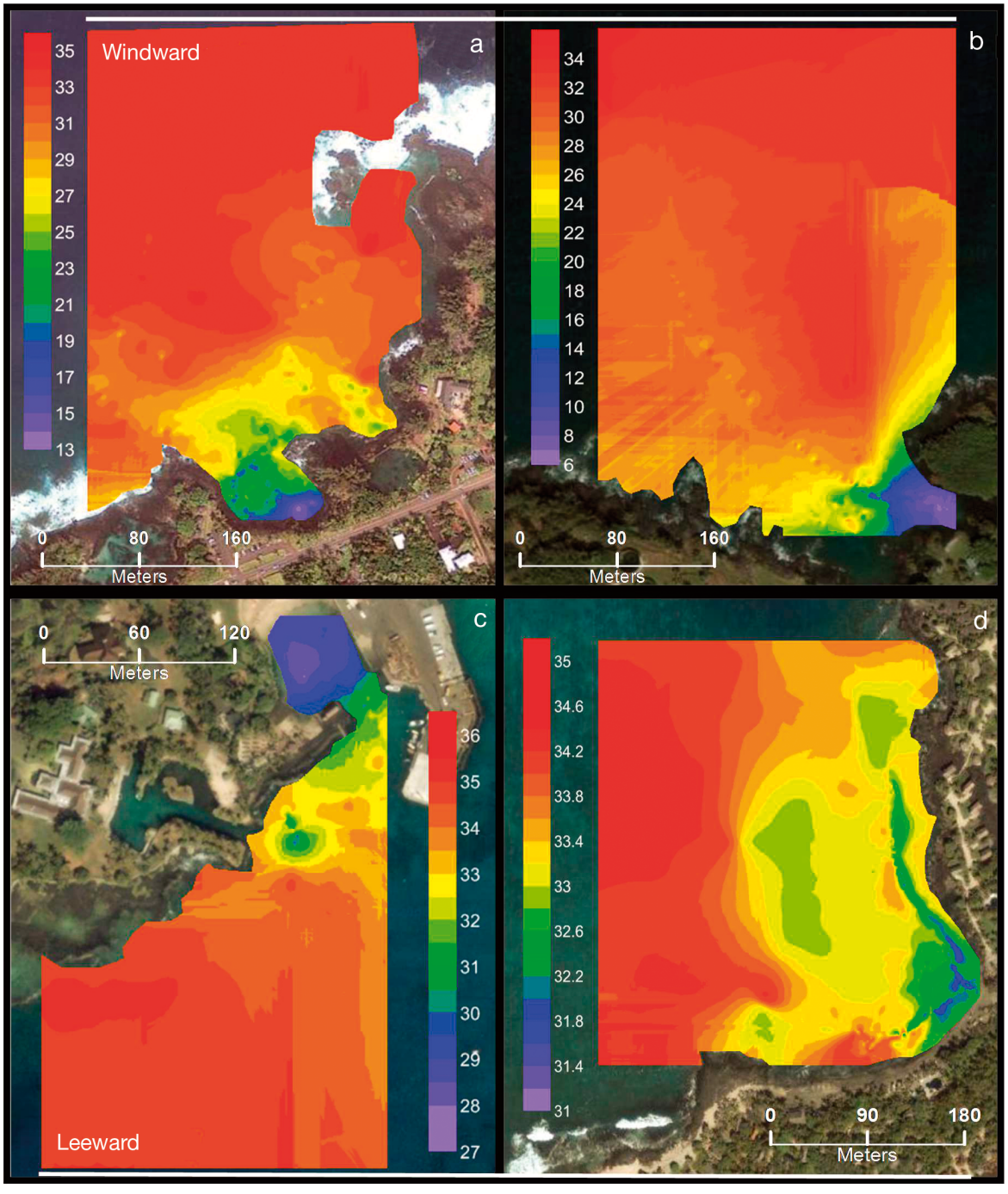

Fig. 2. High-resolution salinity (psu) maps of submarine groundwater discharge (SGD) plumes at windward (wet) sites (a) Waiuli Beach Park, (b) Puhi Bay, and at leeward (dry) sites (c) Kailua Bay, and (d) Kahuwai Bay, Hawai'i Island, HI, USA

BCC, BP, and BR

BCC in our study ranged from 8.56 to 12.60 fg C cell^{-1} across sites and was significantly higher inside the SGD plumes than outside of them ($p = 0.025$) (Table 3).

BP also varied across sites, from 3.50×10^{-3} to 33.60×10^{-3} µmol C l^{-1} h^{-1} (Table 3). BP within windward SGD plumes was 2 times lower than outside the plumes, and 2 times lower than values at the leeward sites (Fig. 4c). BP inside and outside of the leeward SGD plumes were similar.

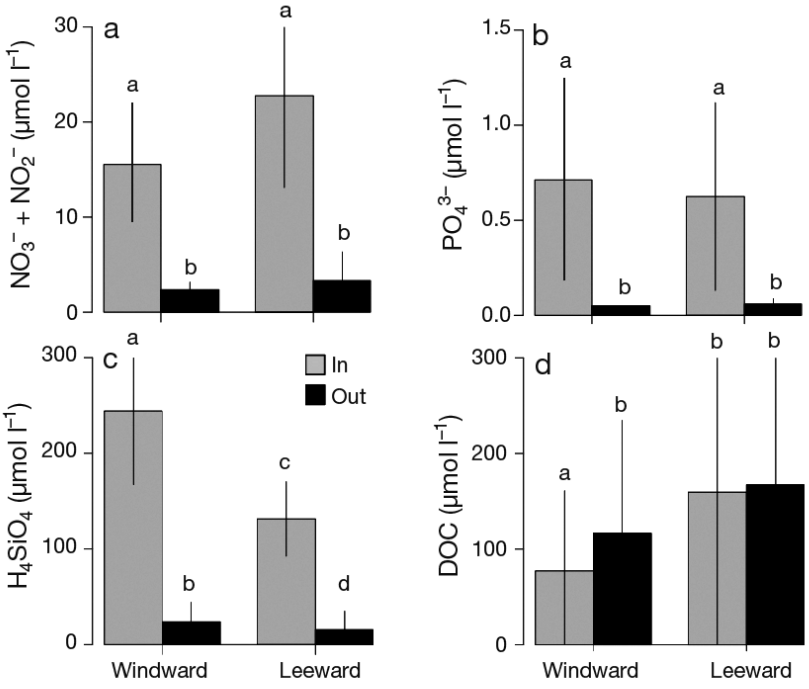

Fig. 3. Mean (±SD) (a) $NO_3^- + NO_2^-$, (b) PO_4^{3-}, (c) H_4SiO_4, and (d) dissolved organic carbon (DOC) concentrations inside and outside of the submarine groundwater discharge (SGD) plumes at windward (wet; Waiuli and Puhi Bay) and leeward (dry; Kailua Bay and Kahuwai Bay) sites on Hawai'i Island, HI, USA. Letters above bars indicate significant differences (Tukey's test, $\alpha < 0.05$)

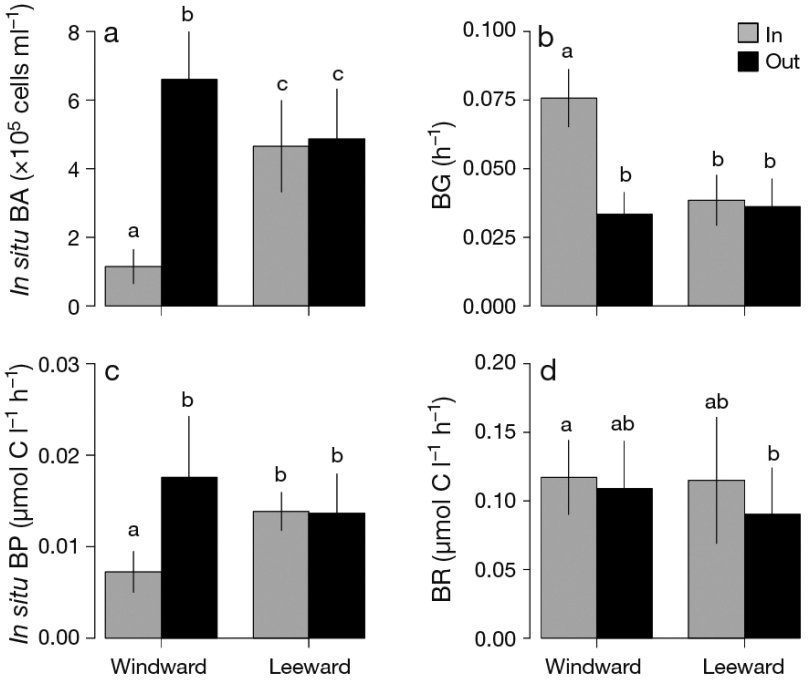

Fig. 4. Mean (±SD) (a) *in situ* bacterial cell abundance (BA), (b) bacterial growth (BG), (c) *in situ* bacterial production (BP), and (d) bacterial respiration (BR) inside and outside of the submarine groundwater discharge (SGD) plumes at windward (wet; Waiuli and Puhi Bay) and leeward (dry; Kailua Bay and Kahuwai Bay) sites on Hawai'i Island, HI, USA. Letters above bars indicate significant differences (Tukey's test, $\alpha < 0.05$)

Conversely, BR was significantly higher within SGD plumes than outside of them, and it was similar between sides of the island (Fig. 4d). Across all sites, BR ranged from 0.046 to 0.226 µmol C l^{-1} h^{-1} (Table 3).

DOC bioavailability

DOC bioavailability was significantly different inside and outside the SGD plumes ($p = 0.002$), as well as between windward and leeward sites ($p < 0.001$). DOC bioavailability was higher within the SGD plumes (10.5 ± 7.40%) compared to outside of them (6.16 ± 3.24%), and higher at the windward sites (10.9 ± 6.94%) than at the leeward ones (5.77 ± 3.64%).

BGE

BGE ranged from 4 to 39% during the incubations (Table 3). At the windward sites, BGE inside and outside of the SGD plumes were similar to one another, and they were also similar to BGE outside of the leeward sites' plumes (Fig. 8). In contrast, BGE within the windward sites' SGD plumes was 1.5 times lower than inside the leeward sites' plumes (Fig. 8). Overall, BP explained 53% ($p < 0.001$) of the variability in BGE, while BR explained 15% ($p < 0.001$)

DISCUSSION

Effects of SGD on coastal water quality

SGD is an important freshwater and nutrient source to coastal waters, especially those without rivers (Valiela et al. 1990, Paytan et al. 2006, Street et al. 2008). At these locations, SGD creates plumes generally characterized by low temperatures and salinities, as well as high inorganic nutrient concentrations at the surface

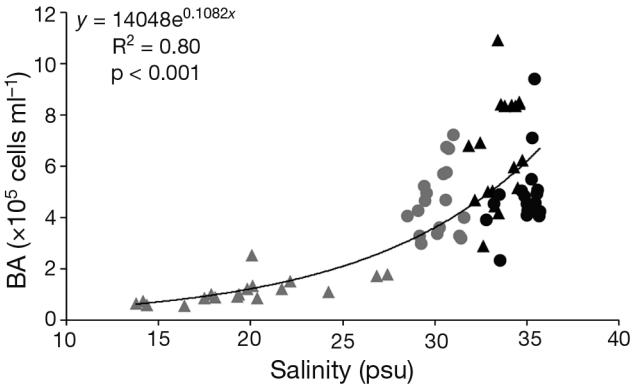

Fig. 5. Relationship between *in situ* bacterial cell abundance (BA) and salinity across all sites at Hawai'i Island, HI, USA. Triangles: windward (wet) sites; circles: leeward (dry) sites. Grey symbols: inside the submarine groundwater discharge (SGD) plume; black symbols: outside the plume

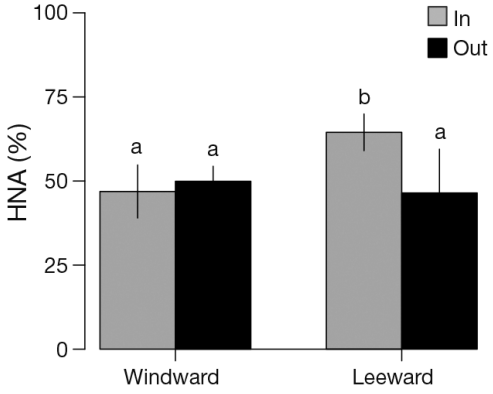

Fig. 6. Mean (±SD) percent bacteria with high nucleic acid content (HNA) from total bacterial cell abundance inside and outside of the submarine groundwater discharge (SGD) plumes at windward (wet; Waiuli and Puhi Bay) and leeward (dry; Kailua Bay and Kahuwai Bay) sites on Hawai'i Island, HI, USA. Letters above bars indicate significant differences (ANOVA, $\alpha < 0.05$)

compared to the surrounding waters and those below the surface (Valiela et al. 1990, Paytan et al. 2006, Street et al. 2008). SGD plumes at all our sites had these characteristics, but values differed significantly between windward and leeward sides of the island. Windward sites had a greater range of temperature, salinity, and silica concentrations. Surface water salinity mapping (Fig. 2), as well as estimates of SGD and plume areas from previous studies substantiate that the windward sites have more SGD (Table 1). Differences in SGD may be attributed to variations in microclimate, bedrock structure, and topography on each side of the island (Oki 1999, Street et al. 2008, Peterson et al. 2009). Nutrient concentrations were higher within the SGD plumes on both sides of the island and fell within the lower range of other SGD studies (Valiela et al. 1990, Street et al. 2008, Knee et al. 2010). In contrast, organic matter concentrations (DOC, DON, DOP) were slightly lower within the SGD plumes than in the surrounding seawater, similar to previous observations (Beck et al. 2007, Nelson et al. 2015). Our results, and those from other studies, suggest that SGD may not be a significant source of DOM to coastal waters.

Table 3. Mean ± SD bacterial cell abundance (BA), estimate spherical diameter (ESD), bacterial growth (BG), bacterial carbon content (BCC), *in situ* bacterial production (BP), bacterial respiration (BR), bacterial growth efficiency (BGE), and phytoplankton cell abundance (PA) of surface water samples collected inside and outside of submarine groundwater discharge (SGD) plumes at windward (wet; Waiuli and Puhi Bay) and leeward (dry; Kailua Bay and Kahuwai Bay) sites on Hawai'i Island, HI, USA

Side	Site	n	BA ($\times10^5$ cells ml^{-1})	ESD (µm)	BG (h^{-1})	BCC (fg C cell^{-1})	BP ($\times10^{-3}$ µmol C l^{-1} h^{-1})	BR (µmol C l^{-1} h^{-1})	BGE (%)	PA ($\times10^3$ cells ml^{-1})
In-plume										
Windward	Waiuli	9	1.20 ± 0.39	0.40 ± 0.01	0.081 ± 0.009	10.30 ± 0.57	8.15 ± 1.90	0.120 ± 0.032	12 ± 5	5.38 ± 5.11
	Puhi	9	1.09 ± 0.61	0.35 ± 0.02	0.070 ± 0.009	10.66 ± 1.33	6.33 ± 2.26	0.115 ± 0.023	8 ± 4	4.41 ± 2.37
Leeward	Kailua	9	4.25 ± 1.09	0.35 ± 0.02	0.042 ± 0.009	9.98 ± 0.63	14.36 ± 1.99	0.096 ± 0.027	19 ± 6	3.02 ± 0.73
	Kahuwai	9	5.06 ± 1.49	0.38 ± 0.01	0.035 ± 0.008	9.64 ± 0.23	13.34 ± 1.16	0.134 ± 0.054	14 ± 9	4.97 ± 2.57
Average		36	2.90 ± 2.04	0.37 ± 0.03	0.057 ± 0.021	10.15 ± 0.05	10.55 ± 3.97	0.116 ± 0.037	14 ± 7	4.44 ± 3.13
Out-plume										
Windward	Waiuli	9	7.19 ± 2.07	0.38 ± 0.02	0.031 ± 0.007	9.52 ± 0.67	17.68 ± 7.34	0.114 ± 0.046	12 ± 3	29.71 ± 23.86
	Puhi	9	6.02 ± 2.04	0.33 ± 0.02	0.036 ± 0.008	10.06 ± 1.38	17.47 ± 6.36	0.104 ± 0.019	14 ± 7	22.73 ± 6.97
Leeward	Kailua	9	4.27 ± 0.82	0.35 ± 0.02	0.041 ± 0.010	9.75 ± 0.57	14.51 ± 5.15	0.074 ± 0.034	18 ± 4	5.87 ± 2.21
	Kahuwai	9	5.46 ± 1.74	0.38 ± 0.02	0.031 ± 0.008	9.55 ± 0.27	12.79 ± 3.33	0.106 ± 0.026	10 ± 3	10.19 ± 6.35
Average		36	5.74 ± 1.98	0.36 ± 0.03	0.035 ± 0.009	9.72 ± 0.83	15.61 ± 5.87	0.100 ± 0.035	14 ± 5	17.13 ± 15.66

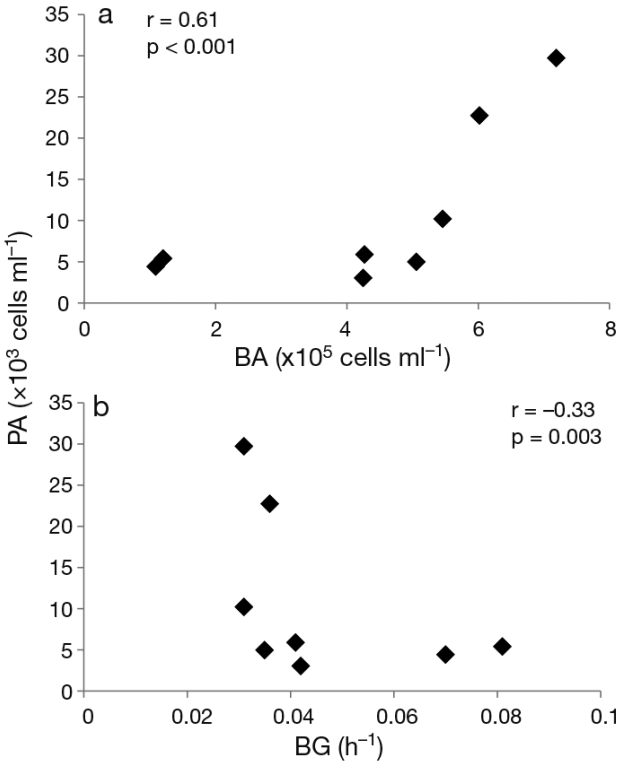

Fig. 7. Correlations between mean phytoplankton cell abundance (PA) and (a) bacterial cell abundance (BA) and (b) bacterial growth rate (BG) in surface waters collected inside and outside of submarine groundwater discharge (SGD) plumes at windward (wet; Waiuli and Puhi Bay) and leeward (dry; Kailua Bay and Kahuwai Bay) sites on Hawai'i Island, HI, USA

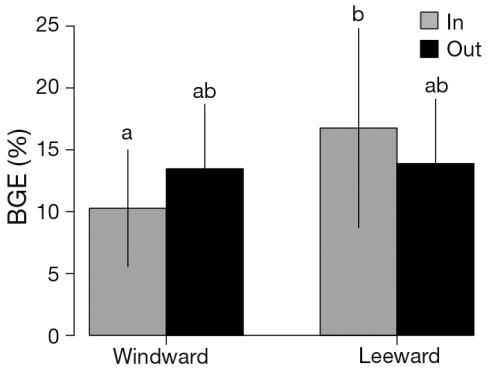

Fig. 8. Mean (±SD) bacterial growth efficiency (BGE) inside and outside of the submarine groundwater discharge (SGD) plumes at windward (Waiuli and Puhi Bay) and leeward (Kailua Bay and Kahuwai Bay) sites on Hawai'i Island, HI, USA. Letters above bars indicate significant differences (ANOVA, $\alpha < 0.05$)

Bacteria cell abundance

Variation in typical bacterial cell abundances for eutrophic lagoons and estuaries (10^7 cells ml^{-1}), coastal zones (10^6 cells ml^{-1}), and the open ocean (10^5 cells ml^{-1}) are driven by bottom-up factors such as DOM and nutrient concentrations, as well as top-down factors such as zooplankton grazing and viral lysis (Ducklow 1992, Pace & Cole 1994, Li 1998). Bacterial cell abundance across our sites ranged from less than average open ocean values to typical abundances found in coastal waters (Ducklow 1992). The lowest cell abundances were found inside the windward plumes compared to all the stations outside of these plumes and those at the leeward sites. This trend is unusual for near shore areas with higher nutrient concentrations (Lee & Bong 2008). Other studies have shown that oceanographic and physical forces, like mixing of water masses, seasonal weather patterns, water residence time, or aquifer recharge, may also affect bacterial cell abundances (Troccoli-Ghinaglia et al. 2010, Walker 2012). Although not documented for SGD, a decrease in bacteria and phytoplankton cell abundances has been observed with high river discharge during storms, and is referred to as a washout effect (Cox et al. 2006, Williams et al. 2008, Wiegner et al. 2012). A strong positive relationship between bacterial cell abundances and salinity at these sites suggests that SGD washed out or diluted marine bacterial cell abundances, or that SGD has lower bacterial cell abundances compared to marine waters (Fig. 5). These explanations are likely as the observed relationship was driven by dynamics on the windward side where SGD is high (Fig. 5).

At the leeward sites, bacterial cell abundances both within and outside of the SGD plume were similar to open ocean values and those found in Hawai'i's oligotrophic waters (Stn ALOHA: 4.80×10^5 cells ml^{-1}; Li 1998), but lower than those measured outside of the windward SGD plume. Observed differences in bacterial cell abundance between the 2 sides of Hawai'i Island may be explained by differences in SGD, as indicated by the spatial distribution in salinity (Fig. 2). Higher salinities inside the leeward SGD plumes suggest mixing of groundwater and ocean water may be occurring within the subterranean estuary, whereas the lower salinities at the windward sites suggest that the majority of this mixing is occurring offshore (Paytan et al. 2006). It is these hydrologic processes that are most likely affecting variation in cell abundances (Oki 1999, Troccoli-Ghinaglia et al. 2010).

Bacterial communities may also respond to varying salinities and nutrient concentrations either by physiological adaptation or by changes in community composition (Rheinheimer 1997, Bouvier & del Gior-

gio 2002, Lemée et al. 2002, Abed et al. 2007). Examining the cellular nucleic acid content of bacteria using fluorescence signatures on a flow cytometer can be a valuable tool for distinguishing the assemblages and/or physiological status of populations (Nishimura et al. 2005). In our study, bacteria with HNA content comprised approximately half of the total bacteria at the windward sites and outside the plume at the leeward sites (Fig. 6). This pattern is similar to that observed outside Kiholo Bay on Hawai'i Island and in surface waters of the Mediterranean Sea (Scharek & Latasa 2007, Walker 2012). At the leeward sites, HNA bacteria made up 64% of the total bacteria within the SGD plumes, higher than outside of them and similar to inside the SGD plume at Kiholo Bay (Walker 2012). Other studies have found that the percent of HNA bacteria varies depending on the environment, increasing along a gradient from open ocean to eutrophic bays (Nishimura et al. 2005). In our study, the percent of HNA bacteria was significantly correlated with TDN concentrations. The higher percentage of HNA bacteria within the leeward plumes could be an indication of a more eutrophic environment, potentially from more development and a larger population compared to the windward sites (Table 1). Additionally, BGE was higher within the leeward plumes, suggesting that these conditions facilitated more effective use of resources towards bacterial cell production (del Giorgio & Cole 1998), and/or that this HNA bacterial community comprised more efficient bacteria compared to those at our other sites.

Bacterial growth rates

In contrast to bacterial cell abundance patterns, bacterial growth rates were twice as high within the windward SGD plumes compared to outside of them. Similarly, a study in the tropical waters of Goa, southwest India, found bacterial growth rates to be 2 times higher at an estuarine site compared to a coastal one; however, estuary bacterial growth rates in that study were 2 times lower than our average growth rate across sites (Pradeep Ram et al. 2003).

Top-down factors such as protozoan grazing and viral lysis are important controls of bacterial growth rates (Pace & Cole 1994, Pradeep Ram et al. 2003, Tsai et al. 2013). In our experiments, we aimed to reduce bacterial grazing pressure by filtering water prior to incubations (Pradeep Ram et al. 2003, Lee et al. 2009, Tsai et al. 2013), and we assume that it was reduced substantially and equally across our experiments as filtering decreased bacterial cell abundance by 62%. However, bacterial cell accumulation rates were higher in the windward SGD plumes compared to waters outside of them, and on the leeward side of the island. High SGD on the windward side of the island may have decreased bacterivore abundances and grazing rates, allowing for higher bacterial growth rates. Similar patterns have been observed in grazer dilution experiments where prey growth rates increase due to their reduced mortality from predators (Landry & Hassett 1982). Likewise, abundance of free viruses, which pass through filters, could have been substantially reduced from SGD dilution, allowing for higher bacterial growth rates in these high SGD environments.

Bottom-up factors such as DOM quantity and quality also affect bacterial growth (Middelboe & Søndergaard 1993, Pace & Cole 1994, Pradeep Ram et al. 2003). In our study, the lack of correlation between bacterial growth rates and DOC concentrations suggests that quality rather than quantity is limiting their growth. Most DOC in the marine environment supporting heterotrophic bacteria is released by phytoplankton during photosynthesis or grazing by zooplankton (Azam et al. 1983, Middelboe & Søndergaard 1993, Strom et al. 1997, Nagata 2000, Lee & Bong 2008), and is considered to be highly labile (Søndergaard & Middelboe 1995). However, in our study, the windward SGD plumes had the highest bacterial growth rates and DOC bioavailability, and the lowest phytoplankton cell abundances and DOC concentrations compared to surrounding coastal waters and those on leeward side of the island (Tables 2 & 3). Photochemical reactions, which can change DOC quality, may explain differences in DOC bioavailability, and thus, bacterial growth rates on each side of the island. Studies have shown that fresh algal-derived DOC becomes less bioavailable upon exposure to sunlight, decreasing bacterial growth rates and BP, while bioavailability of terrigenous DOC from rivers is enhanced following sunlight exposure with increased rates of bacterial growth and respiration (Benner & Biddanda 1998, Moran & Covert 2003, Smith & Benner 2005). The inverse correlation between bacterial growth rates and phytoplankton cell abundances suggests that these processes may be occurring in the SGD plumes (Fig. 7b).

Bacterial production

Higher bacterial growth rates within windward SGD plumes, however, did not result in higher BP. The low bacterial cell abundance in these oligotro-

phic waters could also be the reason for the overall low *in situ* BP measurements compared to other studies. BP calculated using initial *in situ* cell abundance (unfiltered) ranged from 3.53×10^{-3} to 33.70×10^{-3} µmol C l^{-1} h^{-1}, which is lower than values reported for the tropics (Pradeep Ram et al. 2003, Lee & Bong 2008, Lee et al. 2009), but similar to water column measurements in Japan (10 m) and the northwest Mediterranean Sea (0 to 130 m) (Lee et al. 2002, Lemée et al. 2002).

The use of various conversion factors and methods has led to a wide range of values reported for BP (Farjalla et al. 2006). Bacterial incorporation of labeled leucine provides instantaneous BP measurements, while our method provides bacterial biomass increases throughout the growth phase in cultures (0 to 48 h) (Farjalla et al. 2006). These measurements of bacterial biomass increase can be strongly dependent on the conversion factor used. Conversion factors for both BCC (5.9 to 47.9 fg C $cell^{-1}$) and biovolume-to-biomass (105 to 560 fg C $µm^{-3}$) can vary greatly, and thus affect BP values (Fukuda et al. 1998, Farjalla et al. 2006). Since bacterial biovolume and C content are known to vary across trophic gradients, we converted biovolume measured on the flow cytometer to BCC for each sample (Simon & Azam 1989, Fukuda et al. 1998, Lee & Bong 2008). In our study, bacterial cell biovolume varied across sites and BCC was significantly higher within than outside SGD plumes, indicating variation within a short distance and the necessity of measuring these variables at different locations. BCC in our study were lower than average coastal waters (15.7 to 47.9 fg C $cell^{-1}$), even those in tropical waters like Malaysia (13.9 to 32.8 fg C $cell^{-1}$) (Fukuda et al. 1998, Lee & Bong 2008). Our BCC were more comparable to those measured in oligotrophic waters and at Stn ALOHA (avg. 10 fg C $cell^{-1}$), the North Subtropical Pacific (avg. 12.8 fg C $cell^{-1}$), and other oceanic regions (5.9 to 23.5 fg C $cell^{-1}$) (Christian & Karl 1994, Fukuda et al. 1998). Coastal waters in the Hawaiian Archipelago, specifically those on the leeward side of Hawai'i Island, may not be like other tropical coastal waters, but more of a continuation of the oligotrophic open ocean waters that surround them due to their location in the middle of the Pacific Ocean.

Bacterial respiration

BR is primarily regulated by temperature, but substrate quality can also be important (Rivkin & Legendre 2001, Apple et al. 2006, Lee et al. 2009). In our study, BR was similar among sites (0.11 ± 0.04 µmol C l^{-1} h^{-1}), which contrasted with our hypothesis that BR would be higher inside the windward SGD plume. Overall, our rates were low compared to other coastal and estuarine sites in the tropics (Pradeep Ram et al. 2003, Lee & Bong 2008, Lee et al. 2009), but fall within the range of temperate coastal sites (Lee et al. 2002, Lemée et al. 2002). Our low BR rates could be a result of reducing large free-living and particle-bound bacterial cell abundance during pre-filtration, which may have changed community growth and respiration dynamics. Additionally, our low BR rates may reflect lower temperatures (22°C) in both Hawaiian waters and in our incubations compared to those from other tropical sites (28 to 32°C) (Pradeep Ram et al. 2003, Lee & Bong 2008, Lee et al. 2009). To place our BR rates within the context of other tropical studies, we standardized our rates to 30°C, using a Q_{10} of 2.1 (Raymond & Bauer 2000, del Giorgio & Davis 2003). Although our rates increased by 50%, they still fell within the lower range of most tropical coastal studies and were most comparable to a tropical lagoon in Brazil (Pradeep Ram et al. 2003, Farjalla et al. 2006, Lee & Bong 2008, Lee et al. 2009).

BGE

BGE ultimately determines whether bacteria are a source or a sink of organic C to the marine food web; the lower the value, the more organic C is remineralized and released as CO_2 rather than incorporated into new bacterial biomass. BGE ranges from 1% in oligotrophic to 50% in eutrophic waters (del Giorgio & Cole 1998), and our values fall within this range (4 to 39%). In our study, BP was calculated from cellular abundance and growth rates measured in the same flasks as BR (Briand et al. 2004). This allowed for all processes that govern BGE to be determined under the same conditions, but we do recognize that pre-filtration, the long incubation period, and the subset of bacteria in the incubations could have affected our BGE estimates. Our low BGE is characteristic of oligotrophic waters, where maintenance energy requirements are a significant fraction of energy flow in microbial assemblages (del Giorgio & Cole 1998, Biddanda et al. 2001, Alonso-Sáez et al. 2007, Carlson et al. 2007, Lønborg et al. 2011). BGE has been found to greatly increase over an incubation period. Therefore, we calculated BGE using the geometric mean of bacterial cell abundance during our experiments and found BGE to be fairly similar across sites,

and only differed between inside the SGD plumes at the windward and leeward sites. As hypothesized, BGE was generally lower inside the windward SGD plume, but bacterial growth rates were higher, suggesting that bacteria maximized growth at the expense of efficiency (del Giorgio & Cole 1998).

Many factors affect BGE, such as the quantity and quality of organic matter, and they vary over time and space resulting in temporal and spatial changes in BGE (del Giorgio & Cole 1998, Lemée et al. 2002, Briand et al. 2004). Although BGE is calculated from both BP and BR, BP is often more dynamic and the dominant factor determining BGE (del Giorgio & Cole 2000, Lemée et al. 2002, Pradeep Ram et al. 2003, Lee et al. 2009). We found this to be true in our study, where BP had a larger coefficient of variation than BR, and BP explained 53% of the variability in BGE, while BR only explained 15%. BP and BR are not always coupled because different variables can affect them differentially (Apple & del Giorgio 2007, Lee et al. 2009). Bacteria can also disassociate catabolic and anabolic processes to cope with environmental conditions, maximizing growth at the expense of efficiency (Middelboe & Søndergaard 1993, del Giorgio & Cole 1998, Pradeep Ram et al. 2003). Thus, uncoupling of BP and BR may explain why bacterial growth rates were high and BGE was low within the windward SGD plumes.

CONCLUSIONS

The degree to which heterotrophic bacteria are a C and nutrient source to higher trophic levels in marine food webs is not well known, especially in SGD-dominated coastal waters. We found that bacterial dynamics were affected by SGD, especially in areas with high discharge rates. Here, BGE and cell abundance were lowest, but bacterial growth rates and DOC bioavailability were highest. Uncoupling of BP and BR may explain the inverse patterns observed with BGE and growth rates. The low BGE (8 to 20%) in these coastal waters suggests that bacteria transfer only a small fraction of their consumed C to the next trophic level, and are possibly a source of CO_2 to the atmosphere. Our results provide insight into SGD's contribution to, and effects on, coastal and global C budgets. These findings are important as SGD is ubiquitous, occurring along the world's continental margins and islands, and it is anticipated that development, spread of invasive alien vegetation, and climate change will reduce the quality and quantity of SGD (Engott 2011, Dudley et al. 2014).

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