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Nutrient Loading through Submarine Groundwater Discharge and Phytoplankton Growth in Monterey Bay, CA

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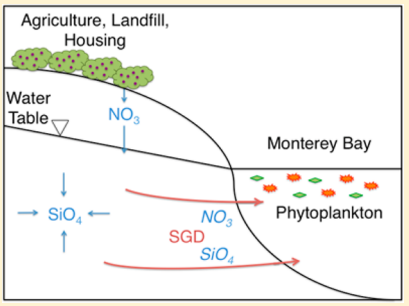
Supporting Information

ABSTRACT: We quantified groundwater discharge and associated nutrient fluxes to Monterey Bay, California, during the wet and dry seasons using excess ^{224}Ra as a tracer. Bioassay incubation experiments were conducted to document the response of bloom-forming phytoplankton to submarine groundwater discharge (SGD) input. Our data indicate that the high nutrient content (nitrate and silica) in groundwater can stimulate the growth of bloom-forming phytoplankton. The elevated concentrations of nitrate in groundwater around Monterey Bay are consistent with agriculture, landfill, and rural housing, which are the primary land-uses in the area surrounding the study site. These findings indicate that SGD acts as a continual source of nutrients that can feed bloom-forming phytoplankton at our study site, constituting a nonpoint source of anthropogenic nutrients to Monterey Bay.



INTRODUCTION

Immense quantities of nutrients are injected into the euphotic zone in eastern boundary upwelling systems, resulting in high levels of primary productivity.[1,2] Our study region, Monterey Bay, California (Supporting Information Figure 1), lies in the California Current System, the eastern boundary upwelling system of the North Pacific. Climatological conditions in outer Monterey Bay show seasonally modulated upwelling and associated high productivity between approximately March and November.[3] Regional wind driven upwelling exhibits strong variability on not only seasonal, but also interannual and intraseasonal time scales. Interannual variability is linked to larger scale phenomena, such as El Niño.[4,5] Intraseasonal variability occurs through alternation of upwelling and relaxation/downwelling on time scales of days to weeks,[6−8] with consequences for phytoplankton bloom ecology.[9−11] The advective supply of nutrients to Monterey Bay from regional upwelling originates at upwelling centers north and south of the bay.[7,8] Upwelling can also occur within the bay, in response to diurnal sea-breeze forcing.[12] In addition to wind-forced upwelling, internal oscillations over Monterey Canyon can force nutrient fluxs from the canyon onto the shelf.[13] Blooms of algae in general and particularly of harmful algal species in Monterey Bay have been directly linked to canyon upwelling and regional wind-driven upwelling.[11,14]

The purpose of this study is to examine the potential role of submarine groundwater discharge (SGD) in affecting phytoplankton blooms in Monterey Bay. Extensive agriculture is prevalent near the bay's coast, and nutrients from fertilizers and other land based sources (sewage, landfills) may affect coastal phytoplankton ecology through land-sea nutrient fluxes, by surface or submarine transport pathways.[15] Nitrate is the primary limiting nutrient in this environment and during rain-induced land flushing events, the supply of nitrate from local rivers can exceed that from upwelling.[16−18] For example, a harmful algal bloom that caused mass stranding of seabirds in Monterey Bay started as a small but intense bloom near the outlet of a river, days after the first land flush of the rainy season.[19] These observations suggested a role of land-derived nutrients in bloom inception. Statistical description of exceptionally dense dinoflagellate "red tide" blooms, detectable by remote sensing, shows maximum frequency and intensity in near coastal waters of Northern Monterey Bay (NMB).[20] Alternative hypotheses for the near-coastal blooms include oceanographic forcing of nutrient fluxes into near-coastal habitat and near-coastal convergence in which motile phytoplankton accumulate.[9,20,21] Atmospheric deposition is a relatively small contributor to the bay's nutrient budget during most of the year.[17] One additional source of nutrients that has

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not been quantified is that associated with SGD, particularly along the northern margin of the bay where these blooms occur repeatedly. Indications of ecological impacts from land drainage motivate better understanding of terrestrial nutrient sources from both surface land drainage and SGD. Two primary aquifers (Aromas Sands and Purisima) and a more limited alluvial aquifer outcrop within NMB, and may act as preferential flow paths for SGD into the bay.[22] We characterize the nutrient composition of SGD flowing into NMB and phytoplankton responses to SGD amendments in incubation experiments to develop a better understanding of SGD and its potential influences on phytoplankton ecology in Monterey Bay.

SGD is a mix of fresh groundwater and seawater that has circulated through the coastal aquifer because of tide and wave action before discharging to the ocean.[23] SGD can account for a considerable fraction of nutrient loads to water bodies.[24−28] While upwelling and stream flows occur during defined and restricted times of the year, SGD may be a consistent source of nutrients to NMB throughout the year because of slow aquifer response and relatively consistent tidal and wave pumping.[23] Radium isotopes are often used as effective natural tracers of SGD because of their enrichment in brackish and saline coastal groundwater compared to receiving seawater and their relatively well-constrained behavior after discharge.[29] Specifically, ^{224}Ra with a half-life of ∼3.5 days is useful to quantify SGD in coastal areas where water residence time is short because of extensive wave action and mixing.

MATERIALS AND METHODS

Study Area, Sampling Procedures, and Sample Analyses. Sunset State Beach (36° 52.790′ N, 121° 49.685′ W) is located in NMB, well within the area of bloom formation (map in Supporting Information Figure 1). Land use in the area surrounding Sunset State Beach is largely agricultural with a year-round growing season because of central California's Mediterranean climate and irrigation in the dry season. Rural residential units with septic tanks and a landfill are also located near the study site.

Discrete seawater and groundwater samples were collected twice, at the end of the wet season (May 2012) and at the end of the dry season (October 2012). Groundwater samples were collected on the beach using temporary well points installed to a depth that allowed sampling of the coastal unconfined aquifer. Samples were also collected farther inland from established nested monitoring wells screened in the upper Aromas, lower Aromas, and the alluvial aquifers.[30] No samples were collected from the Purisima aquifer, which is not thought to contribute to SGD as much as the Aromas aquifer because of its formation properties.[30] Surface seawater samples were collected from the surf-zone and along transects extending off-shore perpendicular to the beach (up to 3.1 km).

Large volume water samples (80−120 L for seawater and 13−120 L for groundwater) were collected using either submersible pumps or buckets (for surface water). Water samples were passed through columns containing MnO_2-impregnated acrylic fiber at a rate of <1.5 L min^{-1} for collection of Ra isotopes.[31] Samples were analyzed at the University of California Santa Cruz (UCSC) on a radium delayed coincidence counter (RaDeCC) for measurement of ^{224}Ra activities.[32] Samples were analyzed on the RadeCC again 3−5 weeks after collection for ^{224}Ra to correct for ^{224}Ra that is produced from ^{228}Th decay.[31] Standards were run on a monthly basis as part of the quality control for maintenance of the instrument. Analytical error of the instrument was calculated and is typically <10%.[33]

Brackish and saline groundwater discharge (for our purposes salinity ≥ 5) was calculated using a well-established mass balance model:[34−36] $SGD = (Ra_{box} - Ra_{off})(V/R)(1/Ra_{gw})$. Groundwater radium activity (Ra_{gw}) was calculated from an average of the saline and brackish groundwater samples, and ocean Ra activity (Ra_{box}) was calculated from a weighted average of the near shore and transect samples, where surf-zone samples were averaged first, the result of which was averaged with the transect values. This prevents artificially high SGD fluxes due to preferential sampling of the surf zone. The residence time of water in the coastal area (R) studied (8 days) was taken from the literature.[37] Ra_{off} is the activity of radium offshore. Discharge (SGD) was normalized per meter of shoreline. Thus, a volumetric unit (V) of the area studied was defined by the length of the transect (3.1 km), 1 m of shoreline, and depth of the thermocline in the near shore (4 m),[4,20] which confines the majority of phytoplankton to the mixed layer. A deeper mixed layer, as occurs further offshore, would increase the calculated SGD (as more SGD would be needed to balance more radium in the larger mixed layer), hence our calculations are conservative.

For nutrient analysis, water was collected in 500 mL HPDE acid-cleaned sample rinsed bottles and stored on ice in a cooler until filtering (within 12 h). Forty milliliter aliquots were filtered (0.45 μm) into acid-cleaned centrifuge tubes and frozen until analysis. Nitrate, silica, and soluble reactive phosphate were measured by colorimetric methods on a flow injection auto analyzer (FIA, Lachat Instruments Model QuickChem 8000).

Bioassay-Incubation Experiments. Two bioassay incubation experiments were conducted to determine if SGD can support bloom-forming species of Monterey Bay. The first incubation experiment was conducted in June 2011 (hereafter referred to as EX1), and the second experiment was conducted in November 2012 (hereafter referred to as EX2). Bay water for both experiments was collected from within NMB (collection locations shows on Supporting Information Figure 1); water was collected so as to avoid ongoing blooms.

Groundwater for the incubations was collected from the upper Aromas Sand Aquifer (same well as for Ra) the day before each experiment. Groundwater was collected via a pumping system installed in the monitoring well into acid-cleaned carboys, and kept in the dark during transport. The groundwater was filtered through a 0.2 μm cartridge into an acid-cleaned container and stored at 8 °C until use in the experiments the next day. Aliquots were taken for nutrient concentrations of nitrate, ammonium, soluble reactive phosphate, and soluble reactive silica.

NMB water was filtered through 105 μm mesh to remove zooplankton grazers into 20 L acid-cleaned sample-rinsed carboys, which were kept in the dark during transport to the UCSC. Aliquots were taken for nutrient concentrations of nitrate, ammonium, soluble reactive phosphate, and soluble reactive silica prior to amendments (baseline) and processed as previously described. The water was distributed randomly into acid-cleaned sample-rinsed 500 mL clear polycarbonate bottles to which nutrients or groundwater was added (see below). Three bottles were processed from each treatment immediately following additions (time zero, t_0) for the following analyses: chlorophyll a, nutrient concentrations, flow cytometry, and

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phytoplankton species abundance. Remaining bottles were placed into a flow-through tank at Long Marine Lab through which Monterey Bay water was continuously pumped to maintain surface ocean temperature. The tank was covered with shading (50% attenuated irradiance) material to simulate mixed layer light levels. Three bottles from each treatment were removed from the tank and processed for chlorophyll *a*, nutrient concentrations, flow cytometry, and species abundance after 24, 48, and 72 h, for a total of 12 bottles per treatment and control for each experiment.

Treatments included a control (no additions), additions of nitrate (target concentration = 30 μM for both experiments), ammonium (10 μM for both experiments), urea (5 μM, EX1 only), phosphate (1 μM for both experiments), silica (20 μM, EX 2 only), dissolved organic phosphorus (5 μM, EX1 only), iron (5 μM, EX1 only), vitamin B12 (100 pM, EX1 only), and a combination of nitrate and silica (30 and 20 μM, respectively, EX2 only). Amendments were based on nutrient concentrations in upwelled water in Monterey Bay.[3] Groundwater additions were 50%, 20%, and 10% by volume (EX1) and 10%, 5%, and 1% by volume (EX2). We deliberately added more groundwater (as a percent contribution) to the treatment bottles than would be found naturally in the bay to elicit a response that could be observed in 3 days.

Chlorophyll *a*. Samples for chlorophyll *a* were collected by filtering 200 mL aliquots under gentle vacuum onto Whatman GF/F filters and stored frozen until analyzed. Filters were extracted for 16 h in 90% acetone at 6 °C in the dark. Fluorescence was measured with a Turner Fluorometer (Turner Designs 10-AU-005 CE). Groundwater treatment samples were multiplied by a coefficient to account for dilution of the original seawater volume used in the groundwater treatments.

Flow Cytometry. Flow cytometry samples (1.5 mL) were collected and fixed with glutaraldahyde to a final concentration of 0.1%, and stored at −80 °C until analysis on a Cytopeia Influx flow cytometer. FlowJo software was used for data analysis (TreeStar Inc.). Cells were classified as *Synechococcus* or Picoeukaryotes based on size and on autofluorescence characteristics. Total cell counts were normalized to sample volume.

Phytoplankton Abundance. Samples for determining phytoplankton abundances were collected by fixing 50 mL aliquots with formalin to a final concentration of 0.4% and stored in the dark at 6 °C. Phytoplankton (≥15 μm) were enumerated according to previously established methods,[38] using Utermöhl settling chambers for 24 h. Counts were completed on an Olympus IX 70 Inverted microscope equipped with epi-fluorescence, with 400 cells counted from each sample at 200× magnification.

RESULTS AND DISCUSSION

SGD Nutrient Fluxes. Ra^{224} activity (Supporting Information Tables 1 and 2) was highest in saline/brackish (here defined as salinity ≥5) groundwater (wet season, 138 ± 12 dpm 100 L^{-1}; dry season, 138 ± 32 dpm 100 L^{-1}), followed by fresh (here salinity <5) groundwater (wet season, 12 ± 2 dpm 100 L^{-1}; dry season, 18 ± 2 dpm 100 L^{-1}), then coastal seawater within the NMB (wet season, 2.3 ± 2.6 dpm 100 L^{-1}; dry season, 3.8 ± 4.5 dpm 100 L^{-1}), and the lowest activities were seen in off-shore water (wet season, 0.17 dpm 100 L^{-1}; dry season, 0.69 dpm 100 L^{-1}). ^{228}Th activity was typically less than 5% of ^{224}Ra. These averages represent weighted averages of the near shore and transect samples, where surf-zone samples were averaged first, the result of which was averaged with the transect values. The same weighted average was used for nutrient concentrations following. Brackish/saline groundwater was statistically different from fresh groundwater and seawater using ANOVA ($F = 25.85$, $p \leq 0.01$) and Tukey's ($p \leq 0.01$). Using the same analysis, Ra^{224} activities in fresh groundwater and seawater were not statistically different. Differences between seasons for each water type were also not statistically different. These results indicate ^{224}Ra is a good tracer of brackish/saline groundwater at this site. Brackish/saline groundwater is likely to be the dominant type of SGD at this site because of the lack of fresh water near the shoreline. SGD calculated using the mass balance box model in the wet season is 24 ± 19 m^3 m^{-1} day^{-1} (17 ± 13 L m^{-1} min^{-1}); the SGD in the dry season is calculated to be 35 ± 22 m^3 m^{-1} day^{-1} (24 ± 15 L m^{-1} min^{-1}). Errors were determined following the general rule for error propagation,[39] and are based on natural sample variability, which was greater than analytical error. These fluxes are consistent with fluxes observed in other beaches of similar geology in central California.[25,40] Although the dry season SGD flux is slightly higher than the wet season flux, wet and dry season SGD values are within error relative to each other. This is consistent with our hypothesis that submarine groundwater discharges continuously throughout the year. The persistent SGD flux during the late summer and early fall (when inputs from other nutrient sources are generally lower compared to other times of the year) suggests that SGD nutrient flux may be particularly important for sustaining the phytoplankton because its relative contribution to the total nutrient pool is higher at that time.

In the wet season, nitrate, silica, and phosphate concentrations (Supporting Information Tables 1 and 2) were highest in the fresh groundwater (330 ± 320, 350 ± 14, and 7.5 ± 5.2 μM, respectively), were lower in the saline/brackish groundwater (110 ± 68, 96 ± 38, and 2.2 ± 0.3 μM, respectively), and lowest in the coastal NMB water (3.6 ± 1.6, 3.8 ± 1.2, and 0.8 ± 0.1 μM, respectively). Offshore concentrations were higher than those in NMB (12.8, 15.9, and 1.09 μM, respectively), most-likely due to upwelling of nutrient-rich water further offshore, which is common in May.[3]

In the dry season, nitrate, silica, and phosphate concentrations were highest in the fresh groundwater (200 ± 100, 280 ± 20, and 3.9 ± 1.2 μM, respectively), lower in the saline/brackish groundwater (110 ± 24, 106 ± 47, and 2.4 ± 0.4 μM, respectively), and lowest in seawater of NMB (4.1 ± 1.9, 6.3 ± 1.9, and 0.7 ± 0.1 μM, respectively). Offshore values (outside the bloom area) were similar to those in NMB waters for phosphate (0.7 μM) and lower for nitrate and silica (1.1 and 5.7 μM). These data suggest there was no offshore source (such as upwelling) during this sampling period. There was no significant difference across seasons and water types for nutrient concentrations using ANOVA ($p \geq 0.05$) except for silica in the wet season when the fresh groundwater was statistically different from other water types.

Multiplying the SGD volume flux of each season by the corresponding saline/brackish groundwater end-member nutrient concentrations yields estimated nutrient fluxes. Nitrate fluxes were similar for the wet season (2.6 mol m^{-1} day^{-1}) and dry season (3.9 mol m^{-1} day^{-1}). Silica fluxes were higher in the wet season (8.3 mol m^{-1} day^{-1}) than the dry season (3.7 mol m^{-1} day^{-1}) because of the higher silica concentration in the groundwater in the wet season. Phosphate fluxes were similar in

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the wet season (0.05 mol m^{-1} day^{-1}) and dry season (0.08 mol m^{-1} day^{-1}). Overall it appears nutrient fluxes are relatively consistent across seasons. The nitrate and silica fluxes are higher than the nearby San Francisco Bay (≤0.7 mol m^{-1} day^{-1} nitrate and 0.25−0.5 mol m^{-1} day^{-1} silica). However, these differences may be due to less wave activity, and therein less SGD volume flux in San Francisco Bay since it is more protected than Monterey Bay.[27] SGD-associated nutrient fluxes at Stinson Beach, a beach ∼6 miles north of San Francisco, has similar nutrient fluxes (1.4−2.4 mol m^{-1} day^{-1} nitrate, 3.3−5.4 mol m^{-1} day^{-1} silica, and 0.08−0.14 mol m^{-1} day^{-1} phosphate) to Monterey Bay.[40]

A persistent flux of nutrients through SGD contributes to an environment conducive to phytoplankton growth. Specifically, the coupled nitrate and silica SDG fluxes in a ratio of near 1:1, provide optimal conditions for diatom-based blooms, consistent with the observed repeated occurrences of *Pseudo-nitzschia* blooms in NMB.[41,42] SGD phosphate fluxes are below the Redfield ratio (required, N/P 16:1; observed, N/P ∼30−75:1). Previous studies have shown that nitrogen is the limiting nutrient in NMB and phosphate is likely supplied through efficient recycling by phytoplankton and utilization of organic phosphorus compounds within this region.[21,42,43]

Incubation Experiment Results. *Chlorophyll a.* For EX1, the control showed little change in chlorophyll *a* over the duration of the experiment: phosphate, iron, vitamin B12, dissolved organic phosphorus, and the 50% groundwater treatments did not differ significantly from the control (Figure 1) using ANOVA (EX1, $F = 35.7$, $p \leq 0.01$) and Tukey's ($p \leq 0.05$, here and hereafter). The 10% groundwater treatment showed the most significant increase in chlorophyll *a* with time, followed by the 20% groundwater treatment. Additions of nitrogen (nitrate, ammonium) also resulted in statistically significant chlorophyll *a* increases, although not as much as the groundwater treatments.

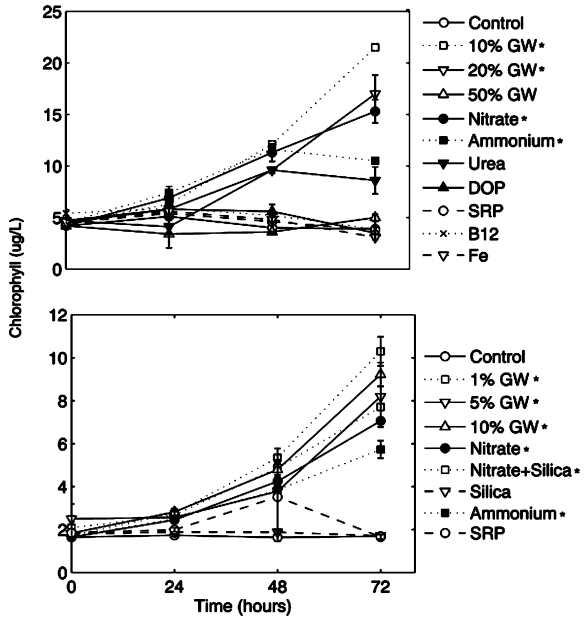

Figure 1. Chlorophyll at each time point for each treatment for EX1 (top) and EX2 (bottom). In EX1 the 10% groundwater treatment showed the most growth, followed by 20% groundwater, nitrate, ammonium, and urea treatments. In EX2, the nitrate plus silicate treatment showed the most growth, followed by the 10% groundwater, 5% groundwater, 1% groundwater, nitrate, and ammonium treatments. Treatments statistically different from the control are denoted with an asterisk.

For EX2 (EX2 $F = 47.55$, $p \leq 0.01$), the control showed no change in chlorophyll *a* concentration with time and the sole amendments with phosphate or silicate did not differ significantly from the control (Figure 1). The combined nitrate+silica treatment showed the most increase in chlorophyll *a* with time, followed by the 10% groundwater, 5% groundwater, 1% groundwater, and nitrogen (nitrate and ammonium) treatments in that order (Figure 1).

The nitrate + silica treatment group in EX2 was designed to simulate the nutrient combinations in groundwater that we suspected spurred growth observed in the groundwater treatments of EX1. Indeed the growth response to the nitrate + silica addition (even more than the growth with the groundwater treatments) in EX2 supports our hypothesis that it was the combination of these two nutrients together which spurred the growth observed in the groundwater treatments. The diatom dominance in many of the blooms in NMB also points to a need for both silica and nitrogen to sustain these blooms.

Nutrients. Initial nutrient concentrations in groundwater and ocean water were similar for ammonium for both EX1 and EX2 (shown in table form Supporting Information Table 3). More ammonium was present in the groundwater (0.9 μM) in EX1 than the ocean water (0.6 μM), while more ammonium was present in the ocean water (1.0 μM) than the groundwater (0.5 μM) for EX2. Groundwater phosphate (EX1, 3.7 μM; EX2, 1.0 μM) for both experiments was higher than the ocean water (EX1, 0.5 μM; EX2, 0.3 μM). Nitrate and silica concentrations were much higher in the groundwater (EX1, 1186.6 and 750.1 μM, respectively; EX2, 629.1 and 459.1 μM, respectively) than the ocean water (EX1, 2.6 and 3.5 μM; EX2, 0.7 and 2.7 μM) for both experiments. Relatively low concentrations of ammonium (e.g., similar to seawater) suggest groundwater is not an important source of nitrogen in the form of ammonium to NMB. In contrast, nitrate concentrations in groundwater are orders of magnitude higher than in seawater. Our incubation experiments indicate that to maximize algal growth (chlorophyll *a*) in the water collected from NMB both nitrate and silica are needed, consistent with the expectation that diatoms grow at a molar ratio of ∼1:1 N/Si.[44]

Changes in concentration of phosphate, silica, nitrate, and ammonium over the 3 day incubation are shown in Figure 2. Values were calculated by subtracting the initial nutrient concentration of each treatment (Supporting Information Table 4) from the final nutrient concentrations. Negative values indicate a decrease in concentration (nutrient drawdown assumed to be uptake by phytoplankton), while positive values indicate an increase in nutrient concentration in the bottles (due to excretion, cell lysis, or microbial or chemical transformations). Negative values extending beyond the dotted line in Figure 2 indicate nutrient drawdown in excess of the concentrations present in the ocean water used for the experiment, indicating phytoplankton utilized nutrients provided by either the groundwater or nutrient additions.

Phosphate concentration decreased for all treatments in EX1. All decreases in phosphate concentration for EX1 were statistically significant using the two-sample *t* test ($p \leq 0.05$) where initial phosphate concentrations of each treatment group were tested against final phosphate concentrations of the same treatment, except for the decrease observed in the phosphate

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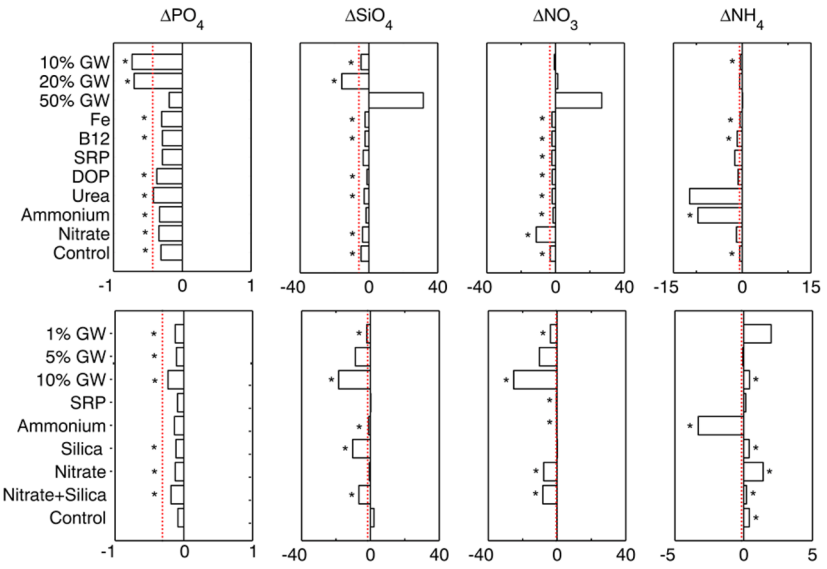

Figure 2. Change in phosphate, silicate, and nitrate concentration in the water of each treatment over each 3 days for with EX1 (top row) and EX2 (bottom row). Negative values indicate uptake by phytoplankton. The dotted line indicates the portion of uptake that could have been supplied by pretreatment Monterey Bay water. Negative values beyond this line indicate use of nutrients supplied by treatments. Drawdown of nitrate and silicate in EX2 correspond to the greatest chlorophyll increases observed. Units are in μM. An asterisk denotes the change is statistically significant.

and 50% groundwater treatments, both containing much higher phosphate than the other treatments and thus the fraction utilized ($\pm$0.4 and 0.2 μM) is very small with respect to phosphate decreases in other treatments, which were all larger (Figure 2) and similar to the analytical error ($\pm$0.2 and $\pm$0.1 μM). Only the 10% groundwater and 20% groundwater treatment groups showed a decrease in phosphate concentration beyond what could have been supplied by the ocean water, indicating phytoplankton drawdown of phosphate supplied by the groundwater. The phosphate concentration similarly decreased for all treatments in EX2. These decreases for EX2 were statistically significant, except for the decrease observed in the control and the treatment receiving high phosphate addition (again likely because of error in the latter case). Unlike EX1, none of the treatment groups showed a decrease in phosphate concentration beyond what could have been supplied by the ocean water. These results are consistent with the phytoplankton community in NMB being phosphate replete. However, when nitrate and silica are provided and growth is extensive phosphate may become limiting. Phosphate from SGD is then utilized and may enable further drawdown of nitrate and silica.

The silica concentration decreased for nearly all of the treatment groups in EX1, with a decrease of 4.6 $\pm$ 0.7 μM for the control and ranged between 16 $\pm$ 3 μM for the 20% groundwater treatment (largest decrease observed) and 1 $\pm$ 1 μM for the phosphate treatment (smallest decrease observed). All of these decreases were statistically significant, except for the decrease observed in the ammonium and the phosphate treatments. Only the 20% groundwater treatment group showed a decrease in concentration beyond what could have been supplied by the ocean water. The 50% groundwater treatment showed an increase of 30 $\pm$ 20 μM; we attribute this to experimental error as there was no other source of silica in the treatment bottles; a 2% error in the amount of groundwater added to the bottles of the treatment groups would account for the increase seen, which was not statistically significant. The initial silica concentrations of this treatment group (416.6, 359.4, and 348.0 μM) have a range larger than all other treatment groups (<5 μM silica) which also stems from analytical error in the amount of groundwater added to this treatment. This could result in a calculated increase which is not real (final silica concentrations for this treatment 414.2, 398.7, and 406.0 μM).

In EX2, the 10% groundwater treatment showed the largest decrease in silica concentration, 18 $\pm$ 4 μM, and the nitrate treatment showed the smallest decrease, 0.6 $\pm$ 0.3 μM. The 10% groundwater, 5% groundwater, 1% groundwater, nitrate+silica, and silica treatment groups showed a decrease in silica concentration beyond what could have been supplied by the ocean water, indicating phytoplankton utilized silica supplied by the groundwater or nutrient additions. The decrease observed in these treatments, as well as the ammonium treatment, were statistically significant. The control and SRP treatments showed increases of 2 $\pm$ 3 and 0 $\pm$ 1 μM, respectively, neither of which were statistically significant at the $p \leq 0.05$ level. These results suggest that when nitrate is available silica is drawn down and groundwater becomes an important source of silica.

In EX1, the nitrate treatment showed the largest decrease in nitrate concentration, 11.2 $\pm$ 0.6 μM, and the 10% groundwater treatment showed the smallest decrease, 0 $\pm$ 2 μM. The control showed a decrease of 3.1 $\pm$ 0.1 μM. All of these decreases were statistically significant. Only the nitrate treatment group showed a decrease in nitrate concentration beyond what could have been supplied by the ocean water, indicating that nitrate could be limiting growth as previously reported for Monterey Bay.[16,21] Nitrate in the 20% and 50% groundwater treatments increased (1 $\pm$ 2 and 30 $\pm$ 40 μM, respectively). However, the increase observed in the 20% and 50% and the small change in 10% groundwater treatments were likely due to analytical errors associated with respect to adding the groundwater, similar to the silicate increases observed in EX1. Initial and finale ranges for the 20% treatment (228.9–231.7 and 228.3–234.2 μM, respectively) show that this initial range is encompassed in the final range of almost 5 μM. Similarly for the 50% treatment, the initial (507.8–556.0 μM) and final

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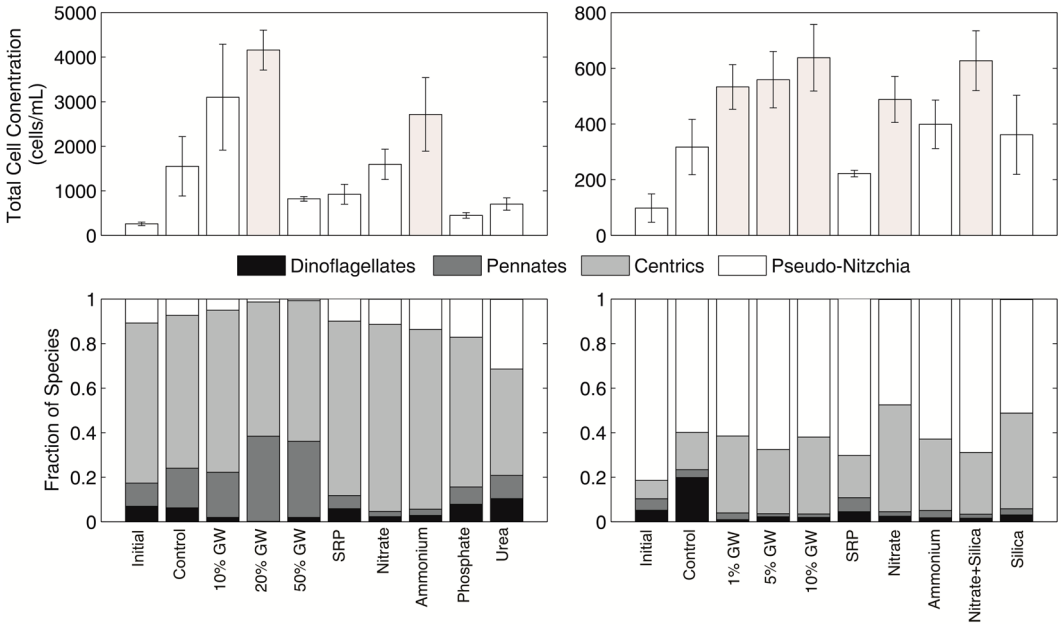

Figure 3. Cell abundances for diatoms (pennates, centrics, and *Pseudo-nitzschia*) and dinoflagellates for EX1 (top left) and EX2 (top right), and diatom and dinoflagellate relative cell abundances for EX1 (bottom left) and EX2 (bottom right). *Pseudo-nitzschia*, a dominate bloom forming species in Monterey Bay, responded well to groundwater treatments (statistically significant against initial using ANOVA and Tukey's $p \leq 0.05$) in EX2 and the silicate plus nitrate treatment. While the cell abundances in EX2 followed the trends seen in chlorophyll. Shaded column in total cell concentration denote statistical significance (ANOVA and Tukey's $p \leq 0.05$) from the initial. See Supporting Information Figure 2 for significance of each group from the initial.

(504.9−598.8 μM) concentration ranges overlap with a maximum range in the final treatment >90 μM. Error associated with analyzing very high nitrate concentrations (5% error above 300 μM) can also account for these observed increases, which were not statistically significant.

In EX2, the 10% groundwater treatment showed the largest decrease for nitrate, 25 ± 9 μM, and the ammonium treatment showed the smallest decrease (0.22 ± 0.05 μM). The control decreased 0.05 ± 0.19 μM. The 10% groundwater, 5% groundwater, 1% groundwater, nitrate, and nitrate + silica treatments all decreased more than could be accounted for by ocean water, indicating phytoplankton utilized nitrate supplied by the groundwater and nutrient additions. All of the decreases in nitrate were statistically significant except for the 5% groundwater treatment.

Ammonium decreased in all treatments for EX1, except for the 50% groundwater treatment, which showed a nonsignificant increase. Change in concentration of ammonium was statistically significant for the control, ammonium, vitamin B12, iron, and 10% groundwater treatments. In EX2, decreases in ammonium were observed only in the ammonium and 5% groundwater treatments. All other treatments showed an increase in ammonium concentration. Changes observed in the control, nitrate + silica, nitrate, silica, ammonium, and 10% groundwater treatments were significant. Changes observed in ammonium are likely due to the combined effects of excretion, cell lysis, and consumption of ammonium by phytoplankton during growth.

Overall, phytoplankton in our experiments were nitrogen limited and nitrate (but generally not ammonium) supplied by the treatments, either as nutrient additions or from groundwater, was utilized by the residing phytoplankton and supported growth. The largest decreases in nitrate were observed when silica was also provided and both nutrients were drawn down simultaneously, suggesting colimitation of Si and N for the diatom population that is utilizing the nitrate. Like nitrate, silica concentrations often decreased beyond what could have been supplied by ocean water, indicating that SGD is likely supplementing the biological nutrient demand, particularly during blooms of diatoms.

N/Si drawdown ratios observed in the 1% groundwater, 5% groundwater, 10% groundwater, and nitrate + silica treatment groups of EX2 are very similar to Redfield ratios expected for diatoms (1:1). Ammonium and nitrate treatment groups (which had similar silica concentrations to those in Monterey Bay water) exhibited an N/Si drawdown ratio higher than that expected for diatoms. The lower silica concentrations in Monterey Bay water and these treatments likely limited the growth of diatoms in these treatment groups. Interestingly the ratio of N/Si in groundwater is very similar to the ratio required by diatoms, and to the N/Si drawdown ratios observed in the 1% groundwater, 5% groundwater, 10% groundwater, and nitrate+silica treatment groups. SGD at our study site provides nitrogen and silica in optimal ratios for Monterey Bay sourced diatoms used in our experiments, whereas ocean water from Monterey Bay was depleted in silica relative to nitrogen, potentially limiting the growth of bloom-forming diatoms.[42]

Phytoplankton Abundance. Phytoplankton abundance of the most common groups *Pseudo-nitzschia*, dinoflagellates, centric diatoms, and pennate diatoms (other than *Pseudo-nitzschia*) in our incubation experiments are shown in Figure 3 (concentration data and statistical significance for each group show in Supporting Information Figure 2). *Pseudo-nitzschia* is of particular interest to this study due to its tendency to form blooms in Monterey Bay, and is therefore given its own category. The increase in total cell abundances of these phytoplankton groups observed in both EX1 and EX2 is consistent with the chlorophyll *a* results (although more

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robustly for EX2). The response of the phytoplankton to the groundwater (except for 50% groundwater) and groundwater-like (nitrate+silica) treatments suggests that the phytoplankton concentration increased more when both high concentrations of nitrate and silica were provided. This is supported by the statistically significant increases in total cell concentrations (Figure 3) with the nitrate and silica containing treatments. Phytoplankton growth was also seen in treatments receiving only nitrogen, but with a muted response compared to treatments with both nitrogen and silica. While the nitrate + silica treatment is statistically different from the nitrate treatments, the other nitrogen and silica containing treatments (groundwater treatments) were not statistically different from the nitrate treatment.

Phytoplankton relative abundance present in Monterey Bay (and in our baseline incubation water) was different in EX1 and EX2, with centric diatoms more dominant in EX1 and *Pseudo-nitzschia* more dominant in EX2. This is probably due to seasonal changes in phytoplankton abundance within Monterey Bay. However, despite the differences in initial phytoplankton composition, both experiments showed a shift toward increased abundance of diatoms with time, although not all diatoms increases are not statistically significant (Supporting Information Figure 2). The shifts observed are consistent with the nutrient drawdown results that indicate growth of diatoms (e.g., drawdown of both nitrate and silica coupled with increasing chlorophyll *a*). Interestingly, in EX2 all of the nitrate and silica containing treatments showed a significant increase in *Pseudo-nitzschia* with time, while other centric diatoms only significantly increase in the nitrate treatment (Supporting Information Figure 2). This supports that the total cell concentration increase for the nitrate and silica containing treatments are due to an increase in *Pseudo-nitzschia* while the increase in the nitrate only treatment was due to increases in centric diatoms (Figure 3).

Flow Cytometry. Flow cytometry results are shown in Supporting Information (Figure 3). For both EX1 and EX2 the increases in chlorophyll *a* observed did not correspond with increases in picoplankton concentrations, indicating *Synechococcus* and Picoeukaryotes were not responsible for the chlorophyll *a* increases observed in the nitrogen and groundwater treatments. In contrast, these species showed the most positive response to ammonium, urea, and phosphate additions, which are less enriched in groundwater and thus SGD is not expected to increase the growth of these nonbloom-forming taxa.

SGD Fluxes and Phytoplankton Demand. The growth of bloom forming phytoplankton *Pseudo-nitzschia* is enhanced by nitrate and silica addition as shown by our bioassay incubation experiments. These nutrients are elevated in groundwater compared to seawater at Sunset State Beach, located in the heart of NMB. The extremely high levels of nitrate in the fresh groundwater (up to 1186.6 μM), prior to its dilution by low nutrient seawater forming the brackish to saline groundwater, suggest an anthropogenic source of nitrate. This is further supported by the presence of agriculture and extensive urban/suburban development on the land above the aquifer from which the groundwater was collected. The elevated silica concentrations in groundwater are likely due to dissolution of aquifer rock material and are typical of many groundwater samples.[45] Radium mass balance models indicate submarine groundwater is consistently discharging in this area throughout the year, providing nitrate and silicate to NMB, even when other nutrient sources (upwelling and rivers) are at a minimum.

To put this study into context, we performed a scaling analysis to estimate the distance from shore that SGD could substantially influence NMB. For this analysis we used the 1% groundwater treatment as our benchmark, as it represents the most conservative addition of both nitrate and silica to still elicit a positive growth response from phytoplankton. The initial concentrations of nitrate and silica in the 1% groundwater treatments were 7.0 (7.0×10^{-3} mol m^{-3}) and 7.7 μM (7.7×10^{-3} mol m^{-3}). Dividing the SGD nutrient fluxes (2.6–3.9 mol m^{-1} day^{-1} nitrate and 3.7–8.3 mol m^{3} day^{-1} silica) by the depth of the mixed layer (4 m) and the nutrient concentrations, yields the distance from shore SGD increases the nutrient concentrations to the 1% treatment level. This yields distances of 90–140 m for nitrate and 120–270 m for silica, at least for our study site.

The implication of the scaling analysis is that the influence of SGD is limited to very close to shore, a spatial scale smaller than would be detectable by satellite remote sensing. We suggest that there are three possible ways that SGD could impact phytoplankton ecology in NMB. The first is that nutrients sourced from SGD maintain a seed population of phytoplankton which bloom when supplemented by other nutrient sources such as pumping of deep nutrient-rich water from Monterey Canyon, inflow of a wind-driven upwelling filament, or a flush of nutrients from runoff from the first seasonal rains. A second possibility is that some (spatially restricted) parts of NMB mix less (have longer residence times) or experience more focused flow of SGD (higher discharge), which would contribute greater nutrient inputs relatively to the volume of water affected. A third possibility is that while not providing enough nutrients to initiate a bloom, SGD can spur growth of diatoms (including *Pseudo-nitzschia*, which need not bloom to result in negative impacts), as observed in the incubation experiments. Regardless of the mechanism, our results are consistent with the hypothesis that SGD can contribute to the persistent formation and maintenance of algal blooms within the NMB by providing a persistent source of nutrients throughout the year.

■ ASSOCIATED CONTENT

Supporting Information

Map of the sampling sites overlaid on a land use map, cell concentrations of each of the major groups of diatoms and dinoflagellates, cell concentrations of *Synechococcus* and Picoeukaryotes in each experiment, water quality data used for the box models, nutrient concentrations for groundwater and bay water used in each experiment, and initial nutrient concentrations after treatment additions. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b00909.

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Notes

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