Sewage outburst triggers *Trichodesmium* bloom and enhance N$_2$ fixation rates

Eyal Rahav$^1$ & Edo Bar-Zeev$^2$

The southeastern Mediterranean Sea (SEMS) is a warm and sunlit marine environment with low ambient N concentration, thus considered ideal for diazotrophy by autotrophic diazotrophs such as *Trichodesmium*. Despite the favorable conditions, N$_2$ fixation rates are often low and *Trichodesmium* has hardly been spotted in the SEMS. This study reports on the occurrence of a *Trichodesmium* bloom in the SEMS which was ascribed to *T. erythraeum* according to DNA fingerprinting of the *nifH* gene. We found that this bloom (1407 ± 983 cells L$^{-1}$) was triggered by an intense outburst of raw sewage that supplied high concentrations of N, P and dissolved organic carbon (DOC), which resulted in low N:P (~12:1) and exceptionally high C:P (~1340:1) ratios. We surmise that these conditions provided favorable conditions for *Trichodesmium* bloom to form via mixotrophic metabolism. As a result, a fourfold increase in N$_2$ fixation was recorded, which contributed ~70% to new primary production and spur a sharp increase in phytoplankton activity and biomass. The conclusions of this study point on a new paradigm for bloom-forming *T. erythraeum* which is tightly linked to anthropogenic sources and prompt microbial productivity in oligotrophic marine environments such as the SEMS.

Coastal environments are routinely exposed to runoffs from anthropogenic sources, including municipal, industrial and agricultural waste$^1,2$. These abrupt runoffs are often untreated and contain pathogens, organic and inorganic nutrients, heavy metals and detergents and are thus considered major threats to marine ecosystems$^3,4$. Such discharges were previously shown to alter food-web dynamics in coastal environments$^5,6$. For example, sewage outbursts introduce nutrients that can stimulate bottom-up effects$^7$ and shift the phytoplankton community structure to form large blooms of toxic dinoflagellates or other harmful algal species$^8,10$.

The surface water of the southeastern Mediterranean Sea (SEMS) is a warm, sunlit marine environment with ultra-oligotrophic conditions, resulting in low bacterial and phytoplankton biomass and low productivity$^{11,13}$. Phytoplankton productivity is considered either N-limited$^{14}$ or N and P co-limited$^{15,16}$, whereas bacteria are either P-limited$^{17}$ or C-limited$^{14}$. These conditions are supposedly ideal for diazotrophy to occur by autotrophic diazotrophs that can fix CO$_2$ and N$_2$ at high rates reaching 640 pg C cell$^{-1}$ d$^{-1}$ and 29 pg N cell$^{-1}$ d$^{-1}$, respectively, thereby prompt the microbial food web$^{28}$. The formation of *Trichodesmium* blooms are often hindered by the availability of trace metals (mainly Fe) and other nutrients (mainly P)$^{20,30}$, water temperature$^{31}$, light availability$^{32,33}$, high O$_2$ levels$^{34}$, as well as other physiochemical water characteristics$^{35}$. However, the conditions restricting the formation of *Trichodesmium* spp. blooms in the SEMS, which is potentially ideal for diazotrophy, are currently unknown$^{18}$.

In this study, we focused on the possible links between sewage outburst and the development of *Trichodesmium* bloom and corresponding microbial community structure. To this end, we followed a *Trichodesmium erythraeum* bloom in the coastal water of the SEMS which was triggered by an outburst of municipal sewage. During this event, we followed the temporal dynamics of *T. erythraeum* and N$_2$ fixation, as well as picophytoplankton,

---

1National Institute of Oceanography, Israel Oceanographic and Limnological Research, Haifa, 31080, Israel. 2The Jacob Blaustein Institutes for Desert Research, Zuckerberg Institute for Water Research (ZIWR), Ben-Gurion University of the Negev, Sede Boqer Campus, Beer Sheva, 84990, Israel. Correspondence and requests for materials should be addressed to E.R. (email: eyal.rahav@ocean.org.il) or E.B.-Z. (email: barzeeve@bgu.ac.il)
diatoms, dinoflagellates, and heterotrophic bacteria. Our results point on the possible impacts of sewage outbursts that flow into ultra-oligotrophic marine environments such as the SEMS.

Material and Methods

Study site and sample collection. Seawater samples (~20 L) were collected from the surface water (~0.5 m) of the southeastern Mediterranean coastline (32°49′34″N, 34°57′20″E) during winter time. Up to ten measurements were carried out; (i) two-three prior the sewage outburst, (ii) five during the event, and (iii) two immediately after the sewage outburst had ended. Seawater was sampled for inorganic nutrients, dissolved organic carbon, chlorophyll-a (as an algae proxy), picophytoplankton abundance including Synechococcus + Prochlorococcus (collectively referred to as autotrophic cyanobacteria) and picoeukaryotes, heterotrophic bacterial abundance and microphytoplankton abundance (diatoms and dinoflagellates). Furthermore, water samples were collected for Trichodesmium spp. abundance, diazotrophic diversity (nifH gene analysis), primary production, bacterial production and N₂ fixation measurements.

Inorganic nutrients and dissolved organic carbon (DOC). Water samples for NO₃ + NO₂, PO₄ and Si(OH)₄ concentrations were collected in 15 mL acid-washed plastic scintillation vials and placed immediately in a −20 °C freezer until analysis. Inorganic nutrient values were determined using the segmented flow Seal Analytical AA-3 system [42]. Samples for DOC concentrations (40 mL) were collected in septum-cap glass vials and were acidified with concentrated (32%) hydrochloric acid (HCl) at a ratio of 1:1000 and stored in the dark at 4 °C until analysis. Samples were analyzed on a Shimadzu TOCV analyzer with a precision of 2 µmol L⁻¹.

Chlorophyll-a (Chl-a). Seawater samples (300 mL) were filtered through a Whatman GF/F filter (~0.7 µm nominal pore size) and kept at −20 °C in the dark. Filters were extracted overnight in acetone (90%) and determined by the non-acidification method [37] using a Turner Designs (Trilogy) fluorometer with 436-nm excitation and 680-nm emission filters.

Picophytoplankton abundance. Water samples (1.7 mL) were fixed with 50% glutaraldehyde (Sigma-Aldrich G7651), snap-frozen in liquid nitrogen and stored at −80 °C. Picophytoplankton abundance was determined based on the orange auto-fluorescence of phycoerythrin and the red auto-fluorescence of chl-a [38]. Heterotrophic bacterial abundance was measured by staining the sample with 1 µL of SYBR green (Applied Biosystems cat #S32717) followed by a 10-min incubation in the dark. All samples were analyzed by an Attune Acoustic Focusing Flow Cytometer (Applied Biosystems) equipped with 488-nm and 405-nm lasers. For the size standard, 1-µm beads (Polysciences) were used.

Trichodesmium, diatom and dinoflagellate abundance. Cell densities were determined after concentrating 1–5 L of surface seawater and counting three subsamples. Cell numbers were estimated using a Wedgwick–Rafter Cell (550) and an epi-fluorescence light microscope (Olympus BH–2) using 20–40× magnification.

Extraction and sequencing of the nifH gene. Samples (1–5 L) were filtered through 0.2-µm Supor filters (PALL Corp.) and placed in a sterile DNAse/Rnase Free Whirl-Pak bag. The samples were snap-frozen in liquid nitrogen and stored at −80 °C. DNA was extracted using the phenol-chloroform method according to Man-Aharonovich [39]. Nitrogenase Fe protein transcripts (nifH) were amplified using a nested PCR strategy [39]. A paired-end sequencing of DNA was performed on an Illumina MiSeq platform at the Research and Testing Laboratories (Lubbock, TX, USA).

Sequencing analysis. Merged Illumina reads were quality filtered and analyzed with the Quantitative Insights Into Microbial Ecology (QIIME) pipeline [40]. The remaining reads were binned into operational taxonomic units (OTUs) and defined at 97% similarity using the UCLUST algorithm [41]. Taxonomy was assigned with BLAST and a database of nifH sequences from Heller et al. [42]. Phylogenetic trees were generated with FastTree in QIIME [43] and visualized with the Interactive Tree of Life (IToL) and Topiary Explorer v1.0 packages.

Primary production (PP). Photosynthetic carbon fixation rates were estimated using ¹⁴C incorporation [44]. Water samples were analyzed in triplicates with dark and zero-time controls. Samples (50 mL) collected at each time point were added to polycarbonate bottles (Nalgene) containing 5 µCi of NaH¹⁴CO₃ (Perkin Elmer, specific activity 56 mCi mmol¹⁻¹) and incubated for 4 h under ambient natural illumination and temperature. To determine the quantity of the added radioactive, 50 µL of each sample was immediately mixed with 50 µL of ethanol-amine and stored for analysis. The incubations were terminated by filtering the spiked seawater through GF/F filters (~0.7 µm nominal pore size) at low pressure (~50 mmHg). The filters were incubated overnight in 5-mL scintillation vials containing 50 µL of 32% HCl in order to remove excess ¹⁴C-bicarbonate. After adding 5 µL of scintillation cocktail (Ultima-Gold) to each vial, the radioactivity was measured using a TRI-CARB 2100 TR (Packard) liquid scintillation counter.

Bacterial production (BP). Rates were estimated using the [4,5-³H]-leucine incorporation method [45]. Three aliquots (1.7 mL each) from each water sample were incubated with 100 nmol of leucine L⁻¹ (Amersham, specific activity 160 Ci mmol⁻¹) for 4 h at ambient temperature in the dark. Samples treated with trichloroacetic acid (TCA) were used as a control. The incubations were terminated with 100 µL of TCA (100%), followed by micro-centrifugation. After adding 1 mL of scintillation cocktail (Ultima-Gold) to each vial, the samples were
counted using a TRI-CARB 2100 TR (Packard) liquid scintillation counter. A conversion factor of 3 kg C per mole leucine incorporated was used, assuming an isotopic dilution of 2.046.

**Dinitrogen (N\(_2\)) fixation.** Rates were measured in triplicates using the \(^{15}\)N\(_2\)-enriched seawater protocol. \(^{15}\)N\(_2\)-enriched seawater was prepared by injecting 1:100 (vol:vol) \(^{15}\)N\(_2\) gas (99%) into degassed (MiniModule G543) and filtered (Polycarbonate 0.2 \(\mu\)m) seawater collected at the study site. The enriched seawater stock was shaken vigorously in order to completely dissolve the \(^{15}\)N\(_2\) gas, and aliquots (225 mL) were then added to triplicate experimental Nalgene bottles (4.6 L). Following 24 h of incubations under ambient light and temperature conditions, the samples were filtered through pre-combusted (450 °C, 4.5 h) GF/F filters and dried in an oven at 60 °C overnight. The samples were then analyzed using a CE Instruments NC2500 elemental analyzer interfaced to a Thermo-Finningan Delta Plus XP isotope ratio mass spectrometer (IRMS). For isotope ratio mass spectrometry, a standard curve to determine N mass was performed with each sample run.

**Statistical analyses.** Data are displayed as averages; error bars signify one standard deviation (n = 3–5). The relationships between the different environmental and physiological variables were determined with a Pearson correlation test (P < 0.05). All tests were performed using the XLSTAT software.

**Results and Discussion**

Untreated sewage outbursts into the SEMS coastal environment occur a few times every winter, usually due to deficiencies in the sewage network and/or overloading of the sewage drainage system by storm-water runoffs (Fig. 1A). The introduction of high loads of organic matter and nutrients into oligotrophic coastal environments such as the SEMS may often result in the formation of phytoplankton blooms (Figs 1B,C and 2). The formation of these blooms may alter microbial food-web dynamics and release different harmful toxins, as well as hinder the performance of large-scale seawater desalination and power plants.

**Environmental conditions of the study site during wintertime.** The coastal water prior to the outburst event exhibited typical SEMS wintertime characteristics (Table 1), with cold (18.3 ± 0.7 °C), windswept (10 ± 3 knots) and well-oxidized (190 ± 10 \(\mu\)M) water. Inorganic nutrient levels, namely NO\(_x\) + NO\(_3\), PO\(_4\) and Si(OH)\(_4\), were low (close to detection limit for P and up to ~2 \(\mu\)M) as well as dissolved organic carbon (DOC, 10 ± 5 \(\mu\)M). These physicochemical conditions resulted in low phytoplankton biomass (0.32 ± 0.05 \(\mu\)g chl-a L\(^{-1}\)), which is typical for this region. *Synechococcus* were the most abundant phototroph (3.9 ± 1.2 cells \(\times\) 10\(^7\) L\(^{-1}\)), whereas diatom (116 ± 43 cells L\(^{-1}\), mostly *Chaetoceros* spp.) and dinoflagellate (34 ± 13 cells L\(^{-1}\), mostly *Ceratium* spp.) abundances were 5–6 orders of magnitude lower (Fig. 2A,B). At this time, *Trichodesmium* spp. could not be detected (Fig. 2C). The low phototrophic abundance resulted in scant PP rates (3.3 ± 0.6 \(\mu\)g C L\(^{-1}\) d\(^{-1}\), Fig. 2D). Heterotrophic bacterial abundance was higher by ~tenfold relative to *Synechococcus* (64 ± 14 cells \(\times\) 10\(^7\) L\(^{-1}\), Fig. 2B). However, BP rates were low (0.8 ± 0.2 \(\mu\)g C L\(^{-1}\) d\(^{-1}\)) and constituted ~25% of the PP (Fig. 2D). N\(_2\) fixation rates were also low (0.15 ± 0.02 nmol N L\(^{-1}\) d\(^{-1}\), Fig. 2D) and similar to previously reported values from this study area. We attribute the low N\(_2\) fixation rates to alpha, gamma and delta—proteobacteria diazotrophs as recently reported for the coastal SEMS.
Coastal conditions in the SEMS during a sewage outburst event. During February 2015, raw and untreated domestic sewage was continuously and intensely (thousands of L h\(^{-1}\)) discharged for five days into the coastal water of the SEMS due to various impairments in the municipal drainage system of Haifa, Israel (Fig. 1A). During this period, water temperature and salinity were not affected and remained typical to the season, and only a weak breeze (<4 knots) was recorded throughout the sewage outburst event (Table 1). Concurrently, NO\(_2\) + NO\(_3\) (2.3 ± 0.2 μM) and PO\(_4\) (0.2 ± 0.1 μM) levels increased by twofold and fourfold respectively (Table 1). This change in NO\(_2\) + NO\(_3\) and PO\(_4\) resulted in a reduced N:P ratio (~12), which is lower than the colonial 16:1 Redfield ratio. DOC levels have also increased by 25-fold relative to the ambient pre-sewage conditions (268 ± 56 μM).

**Table 1.** The physiochemical and biological characteristics of the SEMS coastal water during a sewage outburst event and during typical wintertime (ambient) conditions.

| Variable tested | Units | Sewage outburst | Ambient conditions | Sewage: Ambient (ratio) |
|-----------------|-------|-----------------|--------------------|------------------------|
| Temperature     | °C    | 18.1–20.6       | 17.3–19.2          | 1.1                    |
| Wind            | Knots | 0–4             | 7–13               | 0.2                    |
| Salinity        | –     | 39.0–39.4       | 39.0–39.6          | 1.0                    |
| NO\(_2\) + NO\(_3\) | μM    | 2.11–2.50       | 0.64–1.37          | 2.1                    |
| PO\(_4\)        | μM    | 0.14–0.29       | 0.02–0.07          | 4.2                    |
| Si(OH)\(_4\)    | μM    | 0.41–2.48       | 1.12–1.94          | 0.9                    |
| N:P             | Ratio | 8.6–15.1        | 19.6–32.0          | 0.5                    |
| DOC             | μM    | 188–339         | 6–13               | 25                     |
| O\(_2\)         | μM    | 141–160         | 177–202            | 0.8                    |
| Chl-a           | μg L\(^{-1}\) | 0.45–0.59     | 0.23–0.36          | 1.6                    |

**Figure 2.** The temporal distribution of (A) microphytoplankton (B) autotrophic and heterotrophic bacteria, (C) the relative abundance of *T. erythraeum* (from all nifH OTUs) and corresponding microscopical counts, (D) bacterial and primary production (BP and PP, respectively) and N\(_2\) fixation rates. Measurements were taken prior to, during, and after a sewage outburst event at the coastal SEMS during wintertime (January to March 2015). Values presented are averages and their corresponding standard deviations (n = 3–5).
Following the sewage outburst, a large tuff-like *Trichodesmium* spp. bloom developed, reaching densities of 1407 ± 983 trichomes L\(^{-1}\) (Figs 1B and 2C). Genetic fingerprinting of *nifH* indicated that the most abundant diazotrophic specie was *Trichodesmium erythraeum* (*T. erythraeum*), constituting >70% of all the diazotrophic OTUs in the sewage plume (Fig. 2C). Concurrently, the relative OTUs of other diazotrophs, such as heterotrophic proteobacteria (i.e., *Desulfovibrio sp.*), are at the lower end of the values reported from other oceanic systems where *T. erythraeum* monocultures\(^{36}\). Still, it should be noted that not all cells within a *Trichodesmium* filament take an active role in *N\(_2\)* fixation\(^{34}\). Therefore, it is possible that the *T. erythraeum* filaments observed here had fewer *N\(_2\)*-fixing cells (out of all vegetative cells) than in other marine systems, which would result in lower specific *N\(_2\)* fixation rates compared with other marine systems.

Concomitant with the sewage outburst event and the *T. erythraeum* bloom, chl-\(a\) levels positively and linearly increased by 60% (0.51 ± 0.06 μg L\(^{-1}\)) and PP by 230% (7.77 ± 0.97 μg C L\(^{-1}\) d\(^{-1}\)) compared with the ambient concentrations (Tables 1 and 2), indicating a significant increase in phototrophic biomass. Specifically, these changes were even more evident in the increased abundances of diatoms (~fourfold, mostly *Asterionellopsis glacialis* and *Leptocylindrus danicus*) and dinoflagellates (~sevenfold, mostly *Ceratium spp.*) following the development of the *T. erythraeum* bloom (Fig. 2A–C, Table 2). In contrast to the significant rise in microphytoplankton abundance, heterotrophic bacteria only increased by 1.9-fold (Fig. 2B); however, BP rates increased by threefold relative to pre-discharge production rates. This decoupling between the moderate rise in total heterotrophic bacteria and the significant increase in BP rates, along with the high DOC levels that were introduced with the sewage (Table 1), suggests that some phototrophs might also utilize these organic substrates by switching to mixotrophic nutrition\(^{57, 58}\).

**Table 2.** Pearson linear correlation matrix between the different variables tested before, during, and after the *Trichodesmium* bloom in the SEMS from January to March 2015. Values in bold show statistically significant relationships between the variables (alpha = 0.05, n = 9–10). Syn.: *Synechococcus*; Diat.: diatoms; Dino.: dinoflagellates; Tricho.: *Trichodesmium*.

|          | N\(_2\) fix | N-P | DOC | Chl-a | BA | BP | PP | Syn. | Diat. | Dino. | Tricho. |
|----------|-------------|-----|-----|------|----|----|----|------|-------|-------|---------|
| N\(_2\) fix | 1           |     |     |      |    |    |    |      |       |       |         |
| N-P      | −0.67       | 1   |     |      |    |    |    |      |       |       |         |
| DOC      | 0.94        | 0.76| 1   |      |    |    |    |      |       |       |         |
| Chl-a    | 0.92        | −0.87| 0.67| 1   |    |    |    |      |       |       |         |
| BA       | 0.56        | −0.41| 0.83| 0.56| 1  |    |    |      |       |       |         |
| BP       | 0.76        | −0.76| 0.91| 0.74| 0.69| 1  |    |      |       |       |         |
| PP       | 0.94        | −0.84| 0.46| 0.96| 0.51| 0.87| 1  |      |       |       |         |
| Syn.     | 0.92        | −0.57| 0.51| 0.77| 0.66| 0.82| 0.86| 1    |       |       |         |
| Diat.    | 0.76        | −0.37| 0.58| 0.62| 0.68| 0.73| 0.71| 0.78| 1     |       |         |
| Dino.    | 0.67        | −0.30| 0.44| 0.55| 0.35| 0.62| 0.59| 0.68| 0.97| 1     |         |
| Tricho.  | 0.89        | −0.59| 0.92| 0.79| 0.47| 0.62| 0.80| 0.83| 0.58| 0.52| 1      |

The importance of *Trichodesmium* to new production and microbial biomass in the SEMS. *Trichodesmium* blooms are known to release bioavailable nitrogen into the surrounding water, leading to enhanced growth of non-diazotrophic cyanobacteria and microphytoplankton\(^{55}\). Indeed, the appearance of *T. erythraeum* in the SEMS water was positively and significantly coupled with phototrophic biomass (as chl-\(a\) levels positively and linearly increased by 60% (0.51 ± 0.06 μg L\(^{-1}\)) and PP by 230% (7.77 ± 0.97 μg C L\(^{-1}\) d\(^{-1}\)) compared with the ambient concentrations (Tables 1 and 2), indicating a significant increase in phototrophic biomass. Specifically, these changes were even more evident in the increased abundances of diatoms (~fourfold, mostly *Asterionellopsis glacialis* and *Leptocylindrus danicus*) and dinoflagellates (~sevenfold, mostly *Ceratium spp.*) following the development of the *T. erythraeum* bloom (Fig. 2A–C). In contrast to the significant rise in microphytoplankton abundance, heterotrophic bacteria only increased by 1.9-fold (Fig. 2B); however, BP rates increased by threefold relative to pre-discharge production rates. This decoupling between the moderate rise in total heterotrophic bacteria and the significant increase in BP rates, along with the high DOC levels that were introduced with the sewage (Table 1), suggests that some phototrophs might also utilize these organic substrates by switching to mixotrophic nutrition\(^{57, 58}\).
of the southwestern Pacific Ocean around New Caledonia22. Furthermore, large-scale *Trichodesmium* blooms were reported to swiftly diminish via grazing by harpacticoid copepods53, by viral lysis64 or by a genetically controlled programmed cell death65, 66, 67. These swift bloom demise (~50% of biomass loss within 24 h)28 may fuel higher trophic levels and significantly change the microbial community structure.

**Factors controlling the activity and bloom formation of *Trichodesmium* in the SEMS.** *Trichodesmium* growth and activity may be affected by several physiochemical characteristics and currently remain a matter of debate in many oceanic regions27, 52. Environmental factors, such as sea surface temperature, $pCO_2$, irradiance, a quiescent sea state and the availability of nutrients such as P and Fe, have all been suggested as controlling factors for *Trichodesmium* blooms29, 30, 33, 66–78. Currently, the reasons why *Trichodesmium* blooms do not develop in the SEMS, despite being potentially ideal marine environment with beneficial characteristics for diazotrophy, are still unknown. Our findings suggest that apart from the calm sea, sunlit conditions and the somewhat low N:P ratio (12:1), two mechanisms may have been in play and triggered the development of the *T. erythraeum* bloom: (i) The high concentrations of N and P (at a low N:P ratio) that were introduced to the coastal environment with the sewage supplied the initial cellular metabolic needs for the growth and proliferation of *T. erythraeum* cells. (ii) The high DOC concentration that was supplied with the sewage may have provided assimilable carbon source to the coastal environment. A recent study showed that *Trichodesmium*, as other unicellular diazotrophs71–74, can utilize DOC as an available carbon source75 and therefore may provide an alternative energy source to fix $N_2$ and form blooms. Assuming that *T. erythraeum* cells are mixotrophs73, we suggest that *Trichodesmium* blooms could be stimulated by these types of sewage outbursts in the oligotrophic SEMS. Furthermore, during the bloom, the high concentrations of DOC resulted in an exceptionally high C:N:P ratio (~1340:12:1) relative to the measured ambient ratio during the non-bloom period (~233:25:1). We suggest that these conditions may have induced N-limiting yet C-rich conditions that prioritized mixotrophic metabolism by *Trichodesmium*. It should be noted that while Benavides et al.73 suggested that natural *Trichodesmium* populations in oligotrophic environments may use an alternative mixotrophic nutrition and assimilate DOC as a primary C source, such an addition will not necessarily trigger a *Trichodesmium* bloom in the SEMS. In fact, a recent study from the coastal SEMS reported that heterotrophic diazotrophy was stimulated by a DOC + N + P addition, whereas *Trichodesmium* spp. was not observed41. We propose that additional materials such as trace metals and vitamins were introduced with the sewage outburst and played mutual roles in the formation of the *T. erythraeum* bloom that was reported here. Still, the reasons as to why *Trichodesmium* spp. blooms do not develop more often in the SEMS and the Mediterranean Sea (despite being routinely exposed to external organic and inorganic nutrients) remains unknown and merit further research.

**Conclusions**

Our results provide the first recorded observation of a *Trichodesmium* (*T. erythraeum*) bloom in the coastal waters of the SEMS, which was stimulated by a prolonged and intense sewage outburst event. We suggest that the introduction of high amounts of N, P and DOC, as well as the C:N:P ratio measured here, triggered the formation of the *Trichodesmium* bloom. Specifically, assuming that *Trichodesmium* are mixotrophs, the high concentrations of DOC may have provided the energy sources to meet the metabolic requirements for $N_2$ fixation and bloom formation. It should be noted that further and dedicated research is merit to test the metabolism strategies of *Trichodesmium*.

Following the above, our results indicate that anthropogenic contamination such as raw sewage outbursts into oligotrophic environments may not only trigger blooms of *T. erythraeum*, but also shift microbial community structure. It is likely that these sewage outburst events may have great ecological implications via *Trichodesmium* blooms in oligotrophic environments such as the SEMS. Yet, we stress that more controlled experiments and *in situ* monitoring are needed to better understand the dynamics, regulation and formation of *Trichodesmium* blooms in the SEMS.

**References**

1. Kirby, R. R. & Beaugrand, G. Trophic amplification of climate warming. *Proc. Biol. Sci.* 276, 4095–4103 (2009).
2. Yuan, Z., Shi, J., Wu, H., Zhang, L. & Bi, J. Understanding the anthropogenic phosphorus pathway with substance flow analysis at the city level. *J. Environ. Manage.* 92, 2021–2028 (2011).
3. Nixon, S. W. Coastal marine eutrophication: A definition, social causes, and future concerns. *Ophelia* 41, 199–219 (1995).
4. Halpern, B. S. *et al.* A global map of human impact on marine ecosystems. *Science.* 319, 948–952 (2008).
5. Hansson, S. *et al.* The stable nitrogen isotope ratio as a marker of food-web interactions and fish migration. *Ecology* 78, 2249–2257 (1997).
6. Rombouts, I. *et al.* Food web indicators under the Marine Strategy Framework Directive: From complexity to simplicity? *Ecol. Indic.* 29, 246–254 (2013).
7. Davis, J. M., Rosemond, A. D., Eggert, S. L., Cross, W. F. & Wallace, J. B. Long-term nutrient enrichment decouples predator and prey production. *Proc. Natl. Acad. Sci. USA* 107, 121–126 (2010).
8. Michael Beman, J. *et al.* Agricultural runoff fuels large phytoplankton blooms in vulnerable areas of the ocean. *Nature* 434, 211–214 (2005).
9. Anderson, D. M. *et al.* Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the United States. *Harmful Algae* 8, 39–53 (2008).
10. Bauman, A. G., Burt, J., Feary, D., Marquis, E. & Usseglio, P. Tropical harmful algal blooms: An emerging threat to coral reef communities? *Mar. Pollut. Bull.* 60, 2117–2122 (2010).
11. Berman, T., Townsend, D. & Elsayed, S. Optical transparency, chlorophyll and primary productivity in the eastern Mediterranean near the Israeli coast. *Oceanol. Acta* 7, 367–372 (1984).
12. Pulido-Villena, E., Wagener, E. & Guieu, C. Bacterial response to dust pulses in the western Mediterranean: Implications for carbon cycling in the oligotrophic ocean. *Global Biogeochem. Cycles* 22, 1–12 (2008).
13. Rahav, E. *et al.* Uncoupling between dinotrophic fixation and primary productivity in the eastern Mediterranean Sea. *J. Geophys. Res. Biogeosciences* 118, 195–202 (2013).
14. Rahav, E., Giannetto, M. & Bar-Zeev, E. Contribution of mono and polysaccharides to heterotrophic N\(_2\) fixation at the eastern Mediterranean coastline. *Scientific Reports* 6, 27858 (2016).
15. Kress, N. et al. Effect of P and N addition to oligotrophic Eastern Mediterranean waters influenced by near-shore waters: A microcosm experiment. *Deep Res. Part II Top. Stud. Oceanogr.* 52, 3054–3073 (2005).
16. Zohary, T. et al. P-limited bacteria but N and P co-limited phytoplankton in the Eastern Mediterranean—a microcosm experiment. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 52, 3011–3023 (2005).
17. Krom, M. D., Emeis, K.-C. & Van Cappellen, P. Why is the Eastern Mediterranean phosphorus limited? *Prog. Oceanogr.* 85, 236–244 (2010).
18. Berman-Frank, I. & Rahav, E. *In Life in the Mediterranean Sea: A Look at Habitat Changes* 199–226 (Nova Science Publishers, 2012).
19. Man-Aharonovich, D., Kress, N., Bar-Zeev, E., Berman-Frank, I. & Béja, O. Molecular ecology of *nifH* genes and transcripts in the Eastern Mediterranean Sea. *Environ. Microbiol.* 9, 2354–63 (2007).
20. Yogeve, T. et al. Is dinoflagellate fixation significant in the Levantine Basin, East Mediterranean Sea? *Environ. Microbiol.* 13, 854–871 (2011).
21. Ibelo, V., Cantonii, C., Cozzi, S. & Civitarese, G. First basin-wide experimental results on N\(_2\) fixation in the open Mediterranean Sea. *Geophys. Res. Lett.* 37, 1–5 (2010).
22. Bonnet, S. et al. Dynamics of N\(_2\) fixation and fate of diazotroph-derived nitrogen in a low-nutrient, low-chlorophyll ecosystem: results from the VAHINE mesocosm experiment (New Caledonia). *Bioessences* 12, 1957–1962 (2016).
23. Raveh, O., David, N., Rilov, G. & Rahav, E. The temporal dynamics of coastal phytoplankton and bacterioplankton in the Eastern Mediterranean Sea. *PLoS One* 10, 1–23 (2015).
24. Spatarhosis, S., Skiri, N. & Meziti, A. First record of a *Trichodesmium erythraeum* bloom in the Mediterranean Sea. *Can. J. Fish. Aquac. Sci.* 69, 1444–1455 (2012).
25. Drira, Z., Chaari, D., Hamza, A. & Bel Hassan, M. Diazotrophic cyanobacteria signatures and their relationship to hydrographic conditions in the Gulf of Gabes, Tunisia. *J. Mar. Biol. Assoc. United Kingdom* 97, 69–80 (2017).
26. Zehr, J. P. Nitrogen fixation by marine cyanobacteria. *Trends Microbiol.* 19, 162–73 (2011).
27. Bergman, B., Sandh, G., Lin, S., Larsson, J. & Carpenter, E. J. *Trichodesmium* - a widespread marine cyanobacterium with unusual nitrogen fixation properties. *PEM Microbiol. Rev.* 37, 286–302 (2013).
28. Bar-Zeev, E., Avidsky, L., Bide, K. D. & Berman-Frank, I. Programmed cell death in the marine cyanobacterium *Trichodesmium* mediates carbon and nitrogen export. *ISME J.* 7, 2340–2348 (2013).
29. Mills, M. M., Ridame, C., Davey, M., La Rocha, J. & Geider, R. J. Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. *Nature* 429, 292–294 (2004).
30. Sanudo-Wilhelmy, S. et al. Phosphorus limitation of nitrogen fixation by *Trichodesmium* in the central Atlantic Ocean. *Nature* 411, 66–69 (2001).
31. Staal, M., Meysman, F. J. R. & Stal, L. J. Temperature excludes N\(_2\)-fixing heterocystous cyanobacteria in the tropical oceans. *Nature* 425, 504–507 (2003).
32. Capone, D. G., Zehr, J. P., Paerl, H. W., Bergman, B. & Carpenter, E. J. *Trichodesmium*, a globally significant marine cyanobacterium. *Science* 276, 1221–1229 (1997).
33. LaRoche, J. & Breitharth, E. Importance of the diazotrophs as a source of new nitrogen in the ocean. *J. Sea Res.* 53, 67–91 (2005).
34. Berman-Frank, I., Cullen, J. T., Shaked, Y., Sherrell, R. M. & Falkowski, P. G. Iron availability, cellular iron quotas, and nitrogen fixation in. *Trichodesmium*. *Limnol. Oceanogr.* 46, 1249–1260 (2001).
35. Carpenter, E. J. & Price, C. C. Nitrogen fixation, distribution and production of *Oscillatoria (Trichodesmium)* spp. in the western Sargasso and Caribbean seas. *Limnol. Oceanogr.* 22, 60–72 (1977).
36. Kress, N. & Herut, B. Spatial and seasonal evolution of dissolved oxygen and nutrients in the Southern Levantine Basin (Eastern Mediterranean Sea): chemical characterization of the water masses and inferences on the N:P ratios. *Deep. Res. II* 48, 2347–2372 (2001).
37. Welshmeyer, N. A. Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. *Limnol. Oceanogr.* 39, 1985–1992 (1994).
38. Vaulot, D. & Marie, D. Die variabilité de photosynthetique picoplankton dans le equatorial Pacific. *Appl. Environ. Microbiol.* 104, 3297–3310 (1999).
39. Zehr, J. P. & McReynolds, L. A. Use of degenerate oligonucleotides for amplification of the *nifH* gene from the marine cyanobacterium *Trichodesmium thiebautii*. *Appl. Environ. Microbiol.* 55, 2522–2526 (1989).
40. Caporusso, J. G. et al. QHIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7, 335–336 (2010).
41. Edgar, R. C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26, 2460–2461 (2010).
42. Keller, P., Trep, H. I., Turk-Kubo, K. & Zehr, J. P. ARBirator: A software pipeline for on-demand retrieval of auto-curated *nifH* sequences from GenBank. *Bioinformatics* 30, 1–8 (2014).
43. Price, M. N., Dehal, P. S. & Arkin, A. P. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. *Mol. Biol. Evol.* 26, 1641–1650 (2009).
44. Steemanni-Nielson, E. On the determination of the activity for measuring primary production. *J Cont Explor Mar* 18, 117–140 (1952).
45. Simon, M., Alldredge, A. & Azam, F. Bacterial carbon dynamics on marine snow. *Mar. Ecol. Prog. Ser.* 65, 205–211 (1996).
46. Simon, M., Alldredge, A. & Azam, F. Protein-content and protein-synthesis rates of planktonic marine-bacteria. *Mar. Ecol. Prog. Ser.* 51, 201–213 (1989).
47. Mohr, W. & Gredtlopf, T., Wallace, D. W. R. & Laroche, J. Methodological underestimation of oceanic nitrogen fixation rates. *PLoS One* 5, 1–7 (2010).
48. Weissbach, A. et al. Phytolankton allelochemical interactions change microbial food web dynamics. *Limnol. Oceanogr.* 56, 899–909 (2011).
49. Heiser, J. et al. Eutrophication and harmful algal blooms: A scientific consensus. *Harmful Algae* 8, 3–13 (2008).
50. Villacorte, L. O. et al. Seawater reverse osmosis desalination and (harmful) algal blooms. *Mar. Ecol. Prog. Ser.* 185, 47–72 (2014).
51. Capone, D. G. An extensive bloom of the *N\(_2\)*-fixing cyanobacterium, *Trichodesmium erythraeum*, in the central Arabian Sea. *Mar. Ecol. Prog. Ser.* 172, 281–292 (1998).
52. Luo, Y.-W. et al. Database of diazotrophs in global ocean: abundance, biomass and nitrogen fixation rates. *Earth Syst. Sci. Data* 4, 47–72 (2013).
53. Spurig, D. et al. Mechanisms of *Trichodesmium*; bloom demise within the New Caledonia Lagoon during the VAHINE mesocosm experiment. *Bioessences* 13, 4187–4203, doi:10.5194/bg-13-4187-2016 (2016).
54. Chang, J., Chiang, K. P. & Gong, G. C. Seasonal variation and cross-shelf distribution of the nitrogen-fixing cyanobacterium, *Trichodesmium*, in southern East China Sea. *Cont. Shelf Res.* 20, 479–492 (2000).
55. González Taboada, F., González Gil, R., Höfer, J., González, S. & Anadón, R. *Trichodesmium* spp. population structure in the eastern North Atlantic subtropical gyre. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 85, 67–77 (2010).
56. Rodriguez, I. B. & Ho, T.-Y. Did nitrogen fixation pattern of *Trichodesmium* the interactive control of light and Ni. *Sci. Rep.* 4, 4445 (2014).
57. Béja, O. et al. Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. *Science*. 289, 1902–1906 (2000).
60. Rahav, E., Ovadia, G., Paytan, A. & Herut, B. Contribution of airborne microbes to bacterial production and N₂ fixation in seawater upon aerosol deposition. Geophys. Res. Lett. 43, 1–9 (2016).

61. Devassy, V. P., Bhattachari, P. M. A. & Qasim, S. Z. Succession of organisms following BOUM cruise. Proc. Natl. Acad. Sci. USA 110, 8597–8602 (2013).

62. Bonnet, S., Grosso, O. & Moutin, T. Planktonic dinitrogen fixation along a longitudinal gradient across the Mediterranean Sea. Aquat. Microb. Ecol. 52, 2260–2269 (2007).

63. O’Neil, J. M. The colonial cyanobacterium Trichodesmium as a physical and nutritional substrate for the harpacticoid copepod Macrosetella gracilis. J. Plankton Res. 20, 43–59 (1998).

64. Hewson, I., Govil, S. R., Capone, D. G., Carpenter, E. J. & Fuhrman, J. A. Evidence of spatial and temporal variability in the distribution of nitrogen fixation in the North Pacific Ocean. Limnol. Oceanogr. 54, 548–559 (2009).

65. Levitan, O. et al. Combined effects of CO₂ and light on the N₂-fixing cyanobacterium Trichodesmium IMS101: a mechanistic view. Plant Physiol. 154, 346–356 (2010).

66. Moore, M. C. et al. Large-scale distribution of Atlantic nitrogen fixation controlled by iron availability. Nat. Geosci. 2, 867–871 (2009).

67. Hutchins, D. A., Fu, F.-X., Webb, E. A., Walworth, N. & Tagliabue, A. Taxon-specific response of marine nitrogen fixers to elevated carbon dioxide concentrations. Nat. Geosci. 6, 790–795 (2013).

68. Fu, F. X. et al. Differing responses of marine N₂ fixers to warming and consequences for future diazotroph community structure. Aquat. Microb. Ecol. 72, 33–46 (2014).

69. Feng, X. et al. Mixotrophic and photoheterotrophic metabolism in Cyanothece sp. ATCC 51142 under continuous light. Microbiology 156, 2566–2574 (2010).

70. Montesinos, M. L., Herrero, A. & Flores, E. Amino acid transport systems required for diazotrophic growth in the cyanobacterium Trichodesmium IMS101. J. Plankton Res. 20, 43–59 (1998).

71. Benavides, M., Berthelot, H., Duhamel, S., Rainbault, P. & Bonnet, S. Dissolved organic matter uptake by Trichodesmium in the Southwest Pacific. Sci. Rep. 7, 41315 (2017).

Acknowledgements

We would like to thank Galit Ovadia for her assistance in the DNA extractions. This work was supported by grants awarded by the Ministry of National Infrastructure, Energy and Water Resources (grant number 3-11519) to E.R., by the Ministry of Environmental Protection (grant number 145–1–2) to E.R., and by the Israel Water Authority (grant number 4501-284678) to E.B.-Z. and E.R.

Author Contributions

Conceived and designed the experiments: E.R. and E.B.-Z. Performed the samplings: E.R. Analyzed the data: E.R. and E.B.-Z. Contributed reagents/ materials/analysis tools: E.R. Wrote the paper: E.R. and E.B.-Z.

Competing Interests: The authors declare that they have no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s) 2017