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Dose-dependent regulation of microbial activity on sinking particles by polyunsaturated aldehydes: Implications for the carbon cycle

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Diatoms and other phytoplankton play a crucial role in the global carbon cycle, fixing CO_2 into organic carbon, which may then be exported to depth via sinking particles. The molecular diversity of this organic carbon is vast and many highly bioactive molecules have been identified. Polyunsaturated aldehydes (PUAs) are bioactive on various levels of the marine food web, and yet the potential for these molecules to affect the fate of organic carbon produced by diatoms remains an open question. In this study, the effects of PUAs on the natural microbial assemblages associated with sinking particles were investigated. Sinking particles were collected from 150 m in the water column and exposed to varying concentrations of PUAs in dark incubations over 24 h. PUA doses ranging from 1 to 10 μM stimulated respiration, organic matter hydrolysis, and cell growth by bacteria associated with sinking particles. PUA dosages near 100 μM appeared to be toxic, resulting in decreased bacterial cell abundance and metabolism, as well as pronounced shifts in bacterial community composition. Sinking particles were hot spots for PUA production that contained concentrations within the stimulatory micromolar range in contrast to previously reported picomolar concentrations of these compounds in bulk seawater. This suggests PUAs produced in situ stimulate the remineralization of phytoplankton-derived sinking organic matter, decreasing carbon export efficiency, and shoaling the average depths of nutrient regeneration. Our results are consistent with a "bioactivity hypothesis" for explaining variations in carbon export efficiency in the oceans.

polyunsaturated aldehydes | sinking particles | particle-associated bacteria | marine carbon cycle | bioactivity hypothesis

Planktonic microbes in the world's oceans play a major role in the global carbon cycle. Through photosynthesis, phytoplankton convert carbon dioxide into particulate organic carbon (POC), which then has the potential to sink to the deep sea. This process is opposed by zooplankton and heterotrophic bacteria, which, as agents of respiration, degrade organic matter and convert it back into carbon dioxide. In addition to respiring POC, heterotrophic bacteria that are associated with sinking POC use membrane-bound ectohydrolytic enzymes that affect the disaggregation of POC into smaller nonsinking particles and dissolved organic carbon (DOC) (1). Despite the long-recognized role of particle-associated bacteria (2), the relationships between the activities of these bacteria, particle properties, and the time and depth scales of sinking POC degradation, disaggregation, and respiration remain poorly constrained. Although the molecular-level composition of sinking POC has been used to identify its phytoplanktonic sources and to constrain the timescales of its degradation by heterotrophic bacteria (3–5), none of these studies has accounted for the potential impacts of bioactive molecules within sinking POC on the activities of particle-associated bacteria.

Diatoms are key members of the phytoplanktonic communities across the world's ocean and are known to produce a large diversity of organic molecules, including many that are bioactive. Polyunsaturated aldehydes (PUAs) have received particular interest. The stage for discovery of these molecules was set in the 1990s, when a group of researchers advanced the "paradox of diatom-copepod interactions"—the observation that copepods, which prey on diatoms, exhibited decreased reproductive success when exclusively fed diatoms (6–8). Miralto et al. (9) later purified PUAs from diatom cultures and observed arrested embryogenesis of copepod eggs that were exposed to these compounds. PUA production is now a well-characterized stress surveillance response to wounding during grazing and to nutrient depletion, both of which are bloom termination mechanisms (10, 11).

It has been proposed that PUAs also mediate phytoplankton bloom dynamics by impacting other members of the marine planktonic community, aside from zooplankton. In culture conditions, many eukaryotic phytoplankton experience a decrease in growth rate when exposed to PUAs (12, 13). Isolated bacterial strains demonstrate a varied response to PUAs, whereby diatom-associated isolates are generally unaffected, whereas other strains exhibit either dose-dependent decreases or increases in growth rate in response to PUAs (14). Although many culture studies have been conducted on zooplankton, phytoplankton, and bacterial isolates—all important players in the microbial loop—there have been few attempts to study the impact of PUAs on these trophic levels in natural marine ecosystems under in situ condition (15, 16). Surveys of water column concentrations of PUAs suggest that concentrations are generally much lower than levels required to elicit responses in phytoplankton, zooplankton,

Significance

Phytoplankton live in the sunlit surface waters of the ocean, and through photosynthesis they convert atmospherically derived carbon dioxide into their biomass. A fraction of this biomass sinks into the darker depths where it is colonized by bacteria that turn it back into carbon dioxide through respiration. Thus, phytoplankton–bacteria interactions effectively transport carbon dioxide from the atmosphere deep into the ocean. We discovered that the biomass of some phytoplankton contains bioactive molecules that stimulate these associated bacteria, resulting in respiration of phytoplankton biomass at shallower depths. Given that the ocean mixes gradually over time, carbon dioxide released by bacteria at shallower depths returns to the surface more quickly and thereby is "sequestered" from the atmosphere for a shorter duration.

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or bacteria (17, 18). Consequently, the impact of PUAs on the marine carbon cycle remains an open question.

Results

Exposure to PUAs Affects Changes in the Rates of Sinking POC Remineralization. We tested the linkages between PUAs and the rate of organic matter remineralization by particle-associated bacteria at six stations across the North Atlantic Ocean (Fig. S1 and Table S1): three stations in the Sargasso Sea (SS), two in the temperate western North Atlantic (TWNA), and one in the Subarctic North Atlantic (SANA). Our experimental methods centered on collecting sinking particles, incubating particles in the presence of exogenous PUAs (mixture of heptadienal, octadienal, and decadienal) at a range of concentrations, and assessing changes in organic matter respiration, hydrolytic enzyme activity, bacterial cell abundance, bacterial production rates, and bacterial community structure. The absolute values of these parameters varied considerably between stations (Fig. S2). To remove between-stations variability, we divided the average values of the PUA-amended treatments by the average of the no-amendment controls from each corresponding station. These control-normalized data showed strikingly similar responses by particle-associated bacteria to PUA treatments across this ocean basin (Figs. 1 and 2). We then asked whether there were differences between the controls and the incubations amended with different concentrations of PUAs using a series of Wilcoxon ranked-sum statistical tests.

In general, the addition of exogenous PUAs at lower concentrations led to stimulated rates of bacterial organic matter remineralization. The average respiration rates in the 1 and 10 μM treatments were approximately double that observed in the control treatment (Fig. 1). The average respiration rate in the 100 μM treatment did not differ from the control. Enzyme activity assays revealed enhanced alkaline phosphatase (APase) and lipase activity compared with the control over the same stimulatory range of concentrations observed for respiration rates (Fig. 2). Average APase activity in the 1 and 10 μM treatments was quadruple that of the controls. The average lipase activity was one-and-a-half times that of the control in the 1 and 10 μM treatments. Lipase activity was significantly lower than the control in the 100 μM treatments. Peptidase activity was significantly lower than the control in the 10 and 100 μM treatments. However, α-glucosidase activity did not significantly deviate from the control in any of the treatments.

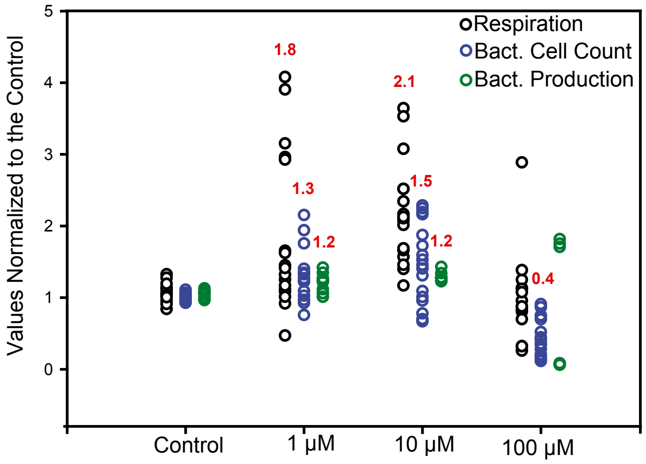

Fig. 1. Average effects of PUA treatments on respiration (n = 18), production (n = 9), and abundance (n = 17) of particle-associated cells, presented as the normalized ratio to the no-amendment control incubations. Data are derived from triplicate incubations at six stations. To isolate and compare the effects of the amendments across stations, results from the incubations at each station were normalized by dividing by the average value of the no-amendment control. With the between-station variability removed, differences between treatments and the control were identified using Wilcoxon statistical tests. The red values above the data report the average values for each treatment across all experiments and denote statistical difference from the control, $P < 0.05$ (Wilcoxon rank sum).

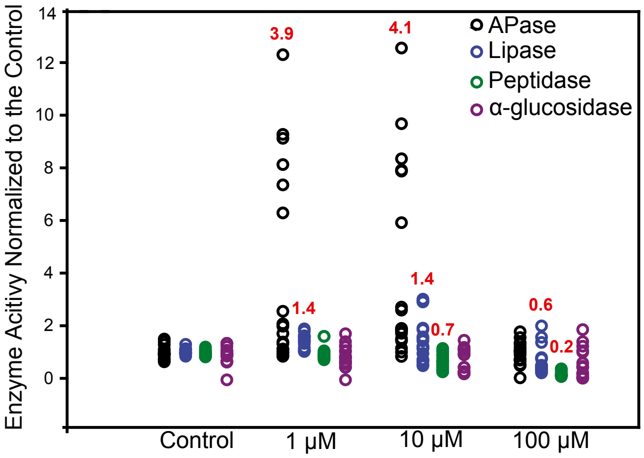

Fig. 2. Average effects of PUA amendments on APase, lipase, aminopeptidase, and α-glucosidase activity (n = 18 for all), presented as the normalized ratio to the no-amendment control incubations. Statistical analyses conducted as described for Fig. 1. The red values above the data report the average values for each treatment across all experiments and denote statistical difference from the control, $P < 0.05$ (Wilcoxon rank sum).

The changes in the rates of organic matter remineralization in response to PUAs were reflected in the growth of particle-associated bacteria. Bacterial cell abundances in the 10 μM treatments were ∼50% greater than in the controls (Fig. 1), whereas bacterial production was about 20% higher (Fig. 1). Similar responses in these signals, although of lesser magnitude, were observed in the 1 μM PUA treatments, hinting at a dose-dependent growth response to PUAs. In contrast to the 1 and 10 μM treatments, bacterial cell abundance was significantly lower than the control in the 100 μM PUA treatments, pointing to an inhibitory threshold between 10 and 100 μM. The average bacterial production rates were also generally lower in the 100 μM treatment compared with the control, but this effect was not statistically significant because of geographic variability; the two TWNA sites showed almost complete inhibition of bacterial production, whereas SS3 showed stimulation (Fig. S2).

Sinking Particles are Hot Spots for PUA Production. Sinking particles were also collected for PUA analysis by high-performance liquid chromatography–UV–multistage mass spectrometry (HPLC-UV-MS^n). Decadienal was clearly observed in the sinking particles at TWNA2 before incubation (i.e., t = initial; Fig. S3); based on POC content of these sinking particles and a previously published relationship between POC and volume of diatom-derived marine snow particles (19), the in situ concentration of decadienal within sinking particles was estimated to be 26 μM (Fig. 3 and Table S2). This concentration was comparable to the stimulatory range of concentrations observed in our incubation experiments. Additionally, significant production of PUAs of multiple chain lengths was observed in the no-amendment controls after 24 h of incubation (i.e., t = final) in all three sampling regions (Fig. 3 and Table S2); heptadienal, octadienal, and decadienal reached concentrations as high as 10.4, 12.9, and 34.0 μM, respectively.

Shift in Bacterial Community upon Exposure to PUAs. Changes in the community structure of particle-associated bacteria were assessed by using automated ribosomal intergenic spacer analysis (ARISA)

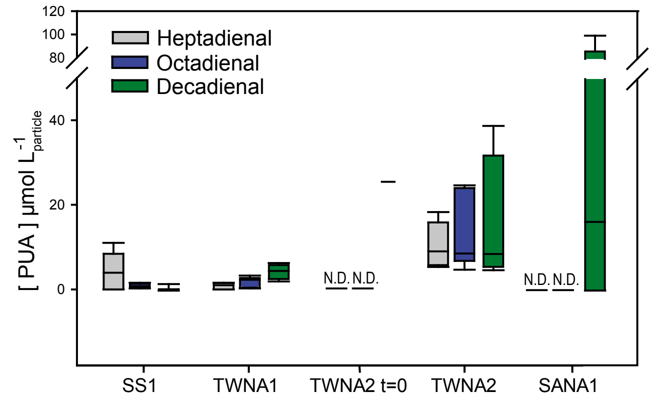

Fig. 3. Box plots displaying the average concentration, range of concentrations, and SD from the mean of heptadienal (gray), octadienal (blue), and decadienal (green) [in micromoles per liter $_{\text{particle}}$] for the t = final control treatments from incubation experiments at SS1, TWNA1, TWNA2, and SANA1 and the t = initial from TWNA2 (n = 3 for all). These values were estimated from the PUA concentration within the incubations, the POC content of the trap material, and the relationship between POC and volume of diatom-derived marine sinking particulate matter published by Brzezinski et al. (19). N.D., not determined.

at one station in the TWNA (TWNA1) and at one station in the Sargasso Sea (SS3). The presence/absence of each operational taxonomic unit (OTU) identified in the various treatments was analyzed with multidimensional scaling to describe the variations in community structure. The particle-associated bacteria communities from the control, 1 μM, and 10 μM treatments formed two distinct clusters based on geographic location (Fig. 4), indicative of negligible impacts on bacterial community structure at these low PUA concentrations. In contrast, the 100 μM treatments led to dramatic changes in community structure vs. the other treatments.

An ARISA clone library database was used to assign a putative identity to each OTU, and we calculated the relative abundance of the following bacterial phyla/classes: Actinobacteria, Bacteroidetes, Cyanobacteria, Deferribacteres, Firmicutes, α-Proteobacteria, β-Proteobacteria, δ-Proteobacteria, Ɛ-Proteobacteria, and γ-Proteobacteria (Fig. S4). The effects of PUAs on individual clades were assessed using Spearman's rank correlations. Abundances of most groups did not show a significant correlation with the amount of PUA added, which includes noted particle specialists (e.g., Bacteriodetes and Firmicutes) (20, 21). At the same time, γ-Proteobacteria were significantly negatively correlated with PUA concentrations and Actinobacteria were significantly positively correlated (Table S3).

Discussion

Doses of PUAs ranging from 1 to 10 μM stimulated organic matter respiration on sinking particles (Fig. 1). Enhanced organic matter respiration in these treatments supported bacterial growth, as suggested by parallel increases in bacterial cell abundance and bacterial production (Fig. 1). Stimulatory concentrations of 1–10 μM agree with data reported for two cultured bacterial strains, *Eudora adriatica* and *Alteromonas hispanica*, which showed enhanced growth rates when exposed to PUA concentrations as low as 13 μM (14). Ribalet et al. (14) conducted incubations that suggested that the bioactivity of PUAs is derived specifically from their combination of carbonyl group and double bonds, precluding the potential for PUAs to be used as a food source.

If PUAs were a consistent food source, then a relationship between the PUA consumption ($[\text{PUA}]_{t=\text{initial}} - [\text{PUA}]_{t=\text{final}}$) and oxygen consumption by respiration ($[\text{O}_2]_{t=\text{initial}} - [\text{O}_2]_{t=\text{final}}$) would be expected. This comparison was made for the 1–10 μM treatments in experiments conducted at TWNA1, TWNA2, and SS3 where $[\text{PUA}]_{t=\text{initial}}$ and $[\text{PUA}]_{t=\text{final}}$ data were available (Table S4). In three of the six comparisons, the consumption of oxygen was greater than the drawdown in PUAs, yielding strong evidence in those instances that the stimulation of respiration was not driven solely by respiration of the PUA amendments themselves. Indeed, in the 10 μM experiments at SS3, and in most of the control experiments across the study (Fig. 3), net PUA production was observed, which further suggests that PUAs are not a readily accessible food source. The remaining two incubations where oxygen consumption was less than PUA drawdown were from TWNA1; this does not necessarily contradict the results from the other four incubations because PUAs could have been partially degraded, which would remove them from our analytical window without incurring stoichiometric oxygen consumption. The inconsistent relationship between respiration and PUA consumption bolsters our interpretation that the stimulatory effect of PUAs was not simply the result of direct respiration of these molecules.

Our data and subsequent calculations suggest that PUA concentrations in environmental samples of sinking particles were in the low micromolar range and comparable to the stimulatory range in the incubation experiments (Fig. 3 and Table S2). The concentrations of PUAs within sinking particles were calculated using a previously published POC–volume relationship for diatom-derived marine snow particles (19), and thus there are considerable uncertainties in these concentrations. However, dissolved concentrations of PUAs from phytoplankton in North Atlantic seawater were recently determined to be generally less than 1 pM (18). Because the concentrations we observed in sinking particles are orders of magnitude higher, we propose that sinking particles are hot spots for PUA production. This idea is supported both by the direct observation of decadienal in native (i.e., t = initial) particles from TWNA, as well as the accumulation of PUAs to micromolar concentrations within particles from four of the six no-amendment control treatments (t = final; Fig. 3).

The accumulation of PUAs in the incubations also suggests that PUAs continue to be produced as particles descend into the mesopelagic. It will be important to quantify PUAs on particles at different depths (vs. only 150 m in this study) because the accumulation of PUAs to concentrations above ∼10 μM during transit could potentially result in a transition between stimulatory and

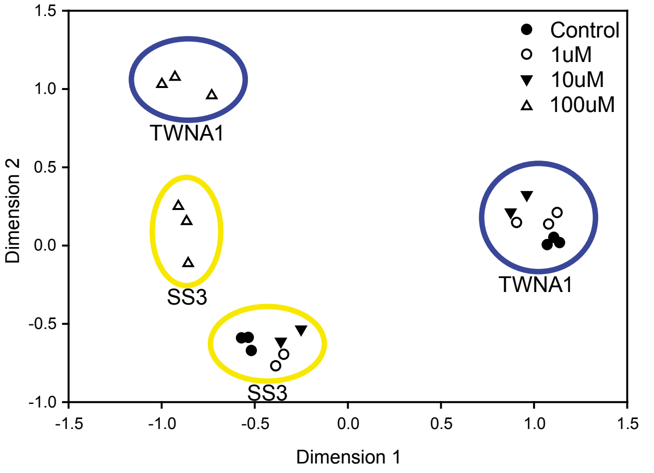

Fig. 4. Multidimensional scaling analysis of community structures determined using presence/absence of OTUs identified by ARISA. Data points within blue and yellow circles are from experiments TWNA1 and SS3, respectively. Solid black circles are control treatments (n = 6). Open circles are 1 μM PUA treatments (n = 5). Solid black triangles are 10 μM treatments (n = 4). Open triangles are 100 μM treatments (n = 6).

toxic effects on bacteria associated with the particles at particular depth horizons (Fig. 1). For example, a recent large-scale study of a diatom bloom in the North Atlantic concluded that export efficiency was low at depths above 100 m (22), but then increased substantially at depths below 100 m (23); these results are consistent with our data suggesting that PUA dynamics could have complex and potentially contrasting effects on the export depths and the biogeochemical fate of POC sinking through the water column.

Export of POC to depth via sinking particles represents a globally significant carbon sink. The observed stimulatory concentrations of PUAs within sinking particles could affect biogeochemical cycling by decreasing POC export efficiency (Fig. 5). Direct PUA-enhanced respiration of sinking POC would accelerate the transfer of carbon from the organic carbon pool to the dissolved inorganic carbon pool; to a first approximation, the shallower this occurs, the shorter the timescales of carbon sequestration from the atmosphere (24). By increasing the hydrolysis of POC, stimulatory concentrations of PUAs could also cause additional transfer of carbon from the POC pool into the DOC pool through disaggregation or dissolution. Disaggregation leads to decreased sinking speeds and dissolution to greater rates of microbial utilization (25, 26). All of these PUA-induced changes cumulatively lead to shallower remineralization depths causing the release of CO_2 in waters that are more likely to be mixed to the surface and reequilibrate with the atmosphere on shorter timescales, thus attenuating carbon sequestration in the deep sea.

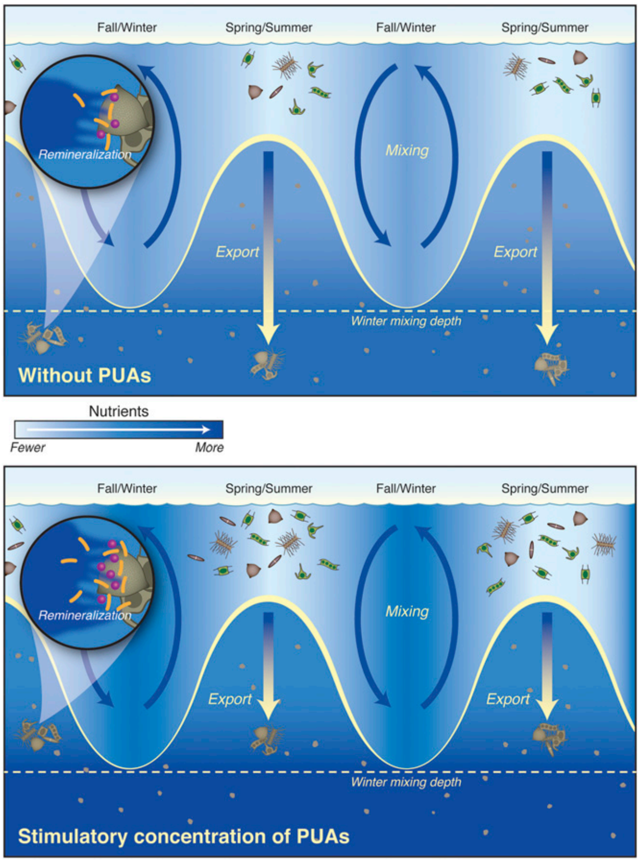

Fig. 5. Conceptualization of the impact of polyunsaturated aldehydes (PUAs) on seasonal organic carbon export from diatom blooms and subsequent remineralization of organic matter by particle-associated bacteria. Stimulatory concentrations of PUAs lead to enhanced regeneration of nutrients above the winter mixing depth.

The shoaling of remineralization depths by PUAs would also affect greater release of inorganic nutrients from sinking particles in shallower waters (Fig. 5). The depth of mixing is 100–450 m in the North Atlantic during the winter (27), and thus nutrients released from sinking particles above this depth during spring diatom blooms have the potential to fuel primary productivity in the subsequent spring. APase activity quadrupled in our incubation experiments suggesting PUAs led to enhanced release rates of dissolved inorganic phosphorus (Fig. 2). Enhanced lipase activity directed toward phospholipids may also affect the liberation of dissolved phosphorus and other nutrients (28). Phosphorus can be a limiting nutrient in regions of the North Atlantic (29). The C:P ratios of exported particles in these regions can be high, which could also point toward enhanced release of phosphorus (30, 31). Furthermore, physiological studies with diatoms in cultures and in mesocosm experiments have shown that PUA production increases under phosphorus-depleted conditions (32, 33). Thus, there appear to be feedbacks between PUA production and organic phosphorus remineralization, potentially leading to the release of more phosphorus above the winter mixed layer, and thereby affecting greater rates of primary production on interannual and basinwide scales.

Although PUAs may stimulate the recycling of phosphorous, the decreases in peptidase activity (Fig. 2) might indicate a decrease in biogenic Si remineralization on particles because diatom frustules are covered by glycoproteins that biochemically protect frustules from contact with seawater that is undersaturated in dissolved silica. Thus, decreased peptidase activity directed toward these glycoproteins could result in lower dissolution of biogenic silica (BSi) in the euphotic zone (34, 35), a process that supports ~60% of global BSi production (36). Preferential recycling of phosphorous over silica could play a role in phytoplankton community succession; in the North Atlantic, diatom blooms are followed by blooms of coccolithophores, which do not have a silica requirement (37). It should be noted that the link between peptidase activity and BSi dissolution does not appear to be universal across all diatom-associated strains of bacteria (38). Thus, the impact of PUAs on BSi regeneration and the potential link with enhanced organic phosphorus cycling remain to be fully elucidated.

The variable response of different enzyme activities to different PUA concentrations might belie a connection between PUAs and the biochemical composition of sinking particles. Although the ectoenzymatic hydrolysis measurements were based on a few well-established model substrates routinely used in microbial ecology since the 1980s (39), they likely do not encapsulate the complete enzymatic response to the molecularly diverse organic matter in sinking particles. Future studies could focus on how the addition of PUAs to sinking particles alters the biochemical composition of POC and DOC, as well as the exchange of classes of biochemicals between the various particulate and dissolved pools. However, our current data clearly show enhanced rates of respiration when particles were amended with ecologically relevant concentrations of PUAs. Notably, respiration represents the ultimate utilization and complete remineralization of organic matter to CO_2 regardless of the biochemical composition of the particles or the enzyme activities of particle-associated bacteria.

Our finding that the addition of high doses of PUAs significantly altered the community structure of particle-associated bacteria suggests that PUAs could play a role in bacterial community succession on sinking particles as PUA levels accumulate. Indeed, 100 μM PUA treatments exhibited dramatic shifts in community structure combined with significant decreases in bacterial cell abundance (Figs. 1 and 4). In contrast, lower levels of PUA amendments in the 1–10 μM range stimulated bacterial metabolic activity (cell abundance, production, and APase activity) (Figs. 1 and 2), while affecting much more subtle shifts in resident bacterial community, which included phyla and classes known to contain particle specializers (Firmicutes and Bacteriodetes) (Fig. 4 and Fig. S4).

By using Spearman's rank correlations to assess the effects of PUAs on individual bacterial clades, we were able to identify one PUA-sensitive clade and one PUA-tolerant clade. The negative correlation between PUA addition and the relative abundance of γ-Proteobacteria (Table S3), suggests that this group is PUA-sensitive. This is consistent with a report that γ-Proteobacteria became dominant in the surface community in the North Sea only after a bloom of potentially PUA-rich diatoms subsided (40). By contrast, the relative abundance of Actinobacteria was positively correlated with PUA addition, and this clade was previously shown to be strongly correlated with diatom pigments in the Sargasso Sea (41). We found no other correlations between PUA amendments and any of the other clades we were able to resolve with ARISA. Ribalet et al. (14) noted that bacterial isolates within the same genera had the potential to respond differently to PUAs, which suggests that PUA sensitivity is not strictly defined by taxonomic position.

In contrast to our 100 μM results, recent work by Paul et al. (42) concluded that PUAs do not impact the structure of free-living bacterial communities. However, these investigators examined the effects of individual PUAs at nanomolar concentrations, and it is possible that nanomolar concentrations simply do not affect community structure, whereas micromolar concentrations do. In addition, it should be noted that because particle-associated and free-living bacterial communities are distinct (20, 43), their corresponding stimulatory and inhibitory concentration ranges are also likely to be different (14, 44). Alternatively, multiple chain lengths of PUAs together are often more potent than the same chain lengths separately, as has been observed for the metabolic activity of coastal free-living bacteria (16). Diatoms often release multiple chain lengths of PUAs at once (45), and the accumulation of heptadienal, octadienal, and decadienal in many of our no-amendment controls suggest that this is the case on sinking particles across the North Atlantic (Fig. 3).

The mechanisms by which PUAs stimulate or inhibit marine bacteria are not known. It has been proposed that PUAs stimulate bacteria by acting as growth cofactors (14). It is also possible that PUAs released from diatoms in sinking particles function as cues for the presence of a larger pool of labile organic matter, which bacteria respond to with enhanced enzymatic and catabolic activity. We speculate that PUAs might be bona fide signals that diatoms secrete upon cell death to stimulate remineralization by bacteria, and thereby increase the likelihood that nutrients such as P are retained in surface water for the rest of the population to use (Fig. 5). On the other hand, PUAs are known Michael acceptors, which are highly reactive toward $-NH_2$ and/or $-SH$ groups, with the potential to cause unspecific damage inside bacterial cells (46); Michael reactions could explain PUAs' inhibitory effects at high concentrations. Clearly, additional work is necessary to understand the cellular and molecular bases for the impact of PUAs on marine bacteria in sinking particles.

Conclusions

This is one of the first reports showing that a specific class of bioactive molecules from phytoplankton impacts the activity and community structure of natural bacterial communities associated with sinking particles. We observed consistent dose-dependent bioactivity of PUAs in six iterations of the same incubation experiment across three different regions of the North Atlantic. Higher respiration rates, APase activity, lipase activity, and bacterial growth were observed over a stimulatory range of PUA exposure (1–10 μM) and for generally similar bacterial communities. PUAs at higher concentrations tended to have inhibitory effects and induced dramatic shifts in the bacterial community structure, demonstrating that PUAs may play a role in bacterial community succession on sinking particles. Decadienal concentrations comparable to the observed stimulatory range were observed within sinking particles collected in the TWNA and PUAs accumulated in incubations at other locations, suggesting that sinking particles are hot spots for PUA production.

Overall, the data are consistent with the hypothesis that PUAs in sinking particles affect an increase in remineralization of sinking particles, which results in a concomitant decrease in the efficiency of POC export from surface waters. This could in turn lead to retention of phosphorus and other nutrients in shallower waters, potentially fueling increased primary productivity on interannual timescales (Fig. 5). Although PUAs are only a very small component of the organic carbon in sinking particles (Table S2), their bioactivity exerts a disproportionate influence on the fate of this carbon in the mesopelagic zone. Our results support a broad-reaching "bioactivity hypothesis," which states that the bioactivity of the organic matter itself, through its ability to stimulate or inhibit particle-associated bacteria, affects POC export in much the same way that mineral protection and ballasting affect the efficiency of POC export (47–49). Testing this hypothesis will involve spatially comprehensive field-based research focused on numerous molecular targets, efforts that must ultimately go far beyond our current study.

Materials and Methods

A more detailed explanation of our methods can be found in *SI Materials and Methods*. Six iterations of the experiment were conducted overall: SS1, SS2, SS3, TWNA 1, TWNA2, and SANA1 (Fig. S1 and Table S1). Sinking particles were collected using unpoisoned surface-tethered net traps deployed at 150 m for 24 h. Before setting up the incubations, the trap material was diluted by 2-fold (SS2, SS3, TWNA 1, TWNA 2, and SANA 1) or 15-fold (SS1) with 0.2-μm filtered seawater such that the microbial communities within the incubations were dominated by particle-associated microbes (~99%). Experiments were conducted in triplicate at each station by incubating the diluted trap material with amendments of varying concentrations of PUAs (0, 1, 10, and 100 μM) in the dark for 24 h at in situ temperature.

The incubations were conducted in biological oxygen demand bottles equipped with oxygen optode minisensors (PreSens), which allowed us to monitor the drawdown of O_2 during the incubation period and in turn calculate the respiration rate (50). At the end of the incubations, enzymatic activity of APase, lipase, α-glucosidase, and aminopeptidase were determined for each triplicate by measuring the hydrolysis product of commonly used fluorogenic substrates (39). Bacterial cell abundance was determined using flow cytometry (51). Bacterial production rates within triplicate treatments were determined by tracing the uptake of tritiated leucine using the standard microcentrifuge method (52).

A 20-mL sample of each triplicate was taken for PUA derivatization and extraction at sea. For experiments SS3, TWNA1, TWNA2, and SANA1, t = initial samples were also extracted. PUAs were quantified using HPLC coupled with UV-visible spectroscopy. Atmospheric pressure chemical ionization MS^n was used to verify that the peaks detected by UV-visible spectroscopy were indeed the molecules of interest (Fig. S5) (53).

Samples for bacterial community structure analysis were taken at TWNA1 and SS3 by filtering 50 mL of each incubation onto a 0.2-μm-pore size 25-mm Durapore filter, which were then frozen at −80 °C. Back in the laboratory, the DNA samples were extracted (54), amplified with 6-FAM–labeled primers for the 16S–23S intergenic spacer (ITS) region, and community structure was parameterized using ARISA (55).

Wilcoxon rank-sum tests and Spearman rank-correlation tests were used to evaluate the statistical significance ($P < 0.05$) of experimental data, and were performed with STATISTICA software.

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