



Alkaline phosphatase activity in the subtropical ocean: insights from nutrient, dust and trace metal addition experiments

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Phosphorus is an essential nutrient for all life on earth. In the ocean, the most bioavailable form of phosphorus is inorganic phosphate, but in the extensive subtropical gyres, phosphate concentrations can be chronically low and limit primary productivity and nitrogen fixation. In these regions, organisms produce hydrolytic enzymes, such as alkaline phosphatase (AP), that enable them to utilize the more replete dissolved organic phosphorus (DOP) pool to meet their cellular phosphorus demands. In this study, we synthesized data from 14 published studies and present our own findings from two research cruises (D326 and D361) in the eastern subtropical Atlantic to explore the relationship between AP activity (APA) and nutrients, Saharan dust and trace metals. We found that below a threshold phosphate concentration of ~30 nM, APA increased with an inverse hyperbolic relationship with phosphate concentration. Meanwhile, DOP concentrations decreased with enhanced APA, indicating utilization of the DOP pool. We found APA rates were significantly higher in the subtropical Atlantic compared to the subtropical Pacific Ocean, even over the same low phosphate concentration range (0–50 nM). While the phosphate concentration may have a first order control on the APA rates, we speculate that other factors influence this basin scale contrast. Using bioassay experiments, we show that the addition of Saharan dust and zinc significantly increased the rate of APA. To our knowledge, our results are the first direct field-based evidence that APA is limited by zinc in the subtropical ocean. Further work is required to explore the relationship between trace metals such as iron and zinc, which are co-factors of phosphohydrolytic enzymes, specifically PhoX and PhoA, respectively, and APA in the ocean.

Keywords: subtropical North Atlantic, alkaline phosphatase, iron, phosphate limitation, zinc

INTRODUCTION

Phosphorus is an essential macronutrient for all life on earth due to its role in key cellular components such as genetic biomolecules (DNA and RNA), energy transfer molecules (ATP) and cell structure (phospholipids) (Karl and Björkman, 2002). The inorganic form, phosphate, is the most biologically available form of phosphorus in the ocean but its concentration can be chronically low in tropical and subtropical ocean regions (Wu et al., 2000; Cavender-Bares et al., 2001). In contrast, the concentration of dissolved organic phosphorus (DOP) can be 5–10 times higher than phosphate (Mather et al., 2008). DOP is a complex array of molecules consisting of phosphomonesters, phosphodiesters, phosphonates and polyphosphates (Kolowitz et al., 2001) although the variability in the contribution of each of these components to DOP is currently unknown, mostly due to the technical difficulty in analysing the different components of DOP (Karl and Yanagi, 1997).

Phytoplankton can assimilate phosphate directly via a high affinity uptake pathway, whereas DOP assimilation requires enzyme-mediated hydrolysis to cleave the phosphate molecule from the organic moiety prior to assimilation. Thus, phytoplankton have a strong metabolic preference for the assimilation of phosphate over DOP to meet their cellular phosphorus demands. However, when phosphate is in short supply, many marine organisms, including the marine filamentous diazotroph, *Trichodesmium* (Sohm et al., 2008; Orcutt et al., 2013), cyanobacteria such as *Synechococcus* (Tetu et al., 2009) and *Prochlorococcus* (Kathuria and Martiny, 2011), some species of dinoflagellate (Dyhrman and Palenik, 1999; Lin et al., 2012), coccolithophores (Jakuba et al., 2008), diatoms (Dyhrman and Ruttenger, 2006) and bacteria (Huang and Hong, 1999) are known to synthesize hydrolytic enzymes in order to access the DOP pool. Since the late 1980s, studies on phosphohydrolytic enzymes have largely focused on the activity of alkaline phosphatase (AP; Sebastián et al., 2004a,b; Sohm and Capone, 2006; Mather et al., 2008;

Sohm et al., 2008; Duhamel et al., 2010, 2011; Lomas et al., 2010; Orchard et al., 2010; Suzumura et al., 2012; McLaughlin et al., 2013; Lin et al., 2013; Sato et al., 2013; Wurl et al., 2013; Martin et al., 2014; Reynolds et al., 2014), a group of metalloenzymes that hydrolyse phosphomonoester bonds (Cembella et al., 1984a,b; Hoppe, 2003). As phosphate esters account for up to 70% of the DOP pool (Karl and Yanagi, 1997; Kolowitz et al., 2001; Karl and Björkman, 2002), AP plays a significant role in the cycling and metabolism of phosphorus in the ocean.

Alkaline phosphatase activity (APA) is induced at low phosphate concentrations and regulated by the balance between internal cellular phosphate stores and the supply of phosphate (Cembella et al., 1984a,b; Dyhrman and Ruttenberg, 2006). Studies from a range of marine environments indicate thresholds in phosphate concentration, below which APA is induced. For example, the rate of APA is reported to increase when the concentration of phosphate is less than 10 nM in the Sargasso Sea (Lomas et al., 2010), ~20 nM in the subtropical Pacific (Suzumura et al., 2012) and ~100 nM in the northwest African upwelling region (Sebastián et al., 2004b). These “tipping points” or thresholds may provide valuable information on the phosphate concentration considered to limit or stress the extant phytoplankton community in the open ocean. In addition to this inducible behavior, relatively high rates of APA have been measured in phosphate-replete systems (Sebastián et al., 2004a,b; Dyhrman and Ruttenberg, 2006; Sato et al., 2013), demonstrating the constitutive behavior of AP, which may be indicative of bacterial regeneration of organic phosphorus or demand for easily accessible carbon (Nausch and Nausch, 2004; Neddermann and Nausch, 2004; Luo et al., 2011). The combination of inducible and constitutive behavior of AP means that its relationship with phosphate may be more complex when considered across a spectrum of marine environments, even when considering only the open ocean and excluding coastal regions.

Quantifying the rate of APA involves incubating seawater samples (either filtered or unfiltered) with a synthetic substrate, e.g., 4-methylumbelliferyl-phosphate (MUF-P) or 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP), that upon hydrolysis of the phosphomonoester bond, releases a soluble fluorescent compound, in this case, methylumbelliferone (Perry, 1972; Ammerman, 1993; Hoppe, 2003). Another technique, called enzyme labeled fluorescence (ELF-97, Dyhrman and Palenik, 1997; Hynes et al., 2009), allows the phosphorus status of individual cells to be assessed through use of a substrate that precipitates an insoluble green product at the site of hydrolysis which is commonly located in the periplasm (Wanner, 1996), but APA rates cannot be quantified using this method. More recently, the presence and expression of the genes encoding for AP, specifically *phoA*, *phoX*, and *phoD*, have been studied in key cyanobacteria including *Prochlorococcus* (Scanlan et al., 2009; Kathuria and Martiny, 2011; Reistetter et al., 2013), *Synechococcus* (Scanlan et al., 2009; Tetu et al., 2009), *Trichodesmium* (Orchard et al., 2009) and diatoms and other eukaryotes (Dyhrman et al., 2012; Lin et al., 2013), providing valuable insight into the distribution and regulation of this enzyme group.

In the subtropical North Atlantic, surface ocean phosphate concentrations are persistently low (Cavender-Bares et al., 2001;

Mather et al., 2008; Sohм et al., 2008; Lomas et al., 2010; Orchard et al., 2010). Of the 450 Tg of dust delivered to the ocean per year, 43% is deposited on the surface waters of the subtropical North Atlantic alone, resulting in elevated surface ocean iron concentrations (0.2–1.2 nM; Bergquist and Boyle, 2006; Moore et al., 2009; Rijkenberg et al., 2012; Schlosser et al., 2014) relative to other ocean basins (typically <0.4 nM, Boyle et al., 2005; Brown et al., 2005; Blain et al., 2008). Nitrogen fixation is co-limited by iron and phosphate (Mills et al., 2004) and thus, while elevated iron from dust deposition stimulates nitrogen fixation (Mills et al., 2004; Moore et al., 2009; Langlois et al., 2012), phosphate is drawn down to chronically low levels. Bioassay experiments have shown that phosphate limits both nitrogen fixation (Mills et al., 2004) and primary productivity (Moore et al., 2008) in the subtropical Atlantic.

To meet their phosphorus demands in the phosphate-deplete subtropical North Atlantic, phytoplankton and nitrogen fixing organisms (Sohm et al., 2008; Orchard et al., 2010; McLaughlin et al., 2013; Lin et al., 2013; Orcutt et al., 2013) utilize the labile and semi-labile components of the DOP pool. The drawdown of DOP is suggested by the lower DOP concentration and higher APA activity in the phosphate-deplete subtropical North Atlantic compared to the phosphate-replete subtropical South Atlantic where DOP concentrations are elevated and APA almost immeasurable (Mather et al., 2008). There is also a zonal contrast, with higher APA in the western subtropical Atlantic than the eastern subtropical Atlantic (Lomas et al., 2010; Wurl et al., 2013; Reynolds et al., 2014), suggesting that phosphate limitation is more prevalent in the western subtropical Atlantic. These north-south and east-west basin scale contrasts in phosphorus dynamics are partially constrained by the spatial extent of wet and dry dust deposition (Moore et al., 2006; Schlosser et al., 2014) and hence magnitude and distribution of nitrogen fixation, as well as physical transfer of nutrients via nutrient streams (Williams et al., 2011). However, APA may also be co-limited by trace metals.

The potential for iron to limit nitrogen fixation is due to iron being a co-factor of the enzyme responsible for nitrogen fixation, nitrogenase. In a similar manner, AP is a metalloenzyme. The AP, PhoA, requires both zinc and magnesium (Coleman, 1992). Both culture studies (Cox and Saito, 2013) and ship based observations (Shaked et al., 2006; Jakuba et al., 2008) demonstrate the potential for co-limitation of phosphorus metabolism by zinc, but direct studies, especially in low phosphate regions, are scarce (Jakuba et al., 2008). Recently, the AP PhoX, in the bacteria *Pseudomonas fluorescens*, has been found to contain iron (Yong et al., 2014). If the iron content of PhoX in *P. fluorescens* is representative of PhoX in other organisms, then this raises the possibility that iron may directly impact the ability of organisms to access labile fractions of DOP via AP in the ocean (Moore, 2014; Yong et al., 2014). Thus, while the impact of dust deposition on iron biogeochemistry (Sarhou et al., 2003; Rijkenberg et al., 2008, 2012; Mahowald et al., 2009; Baker et al., 2013; Ussher et al., 2013) and nitrogen fixation (Mills et al., 2004; Moore et al., 2009; Langlois et al., 2012) has been well studied in the subtropical Atlantic, the relationship between dust deposition, trace metals and phosphorus biogeochemistry is still poorly understood.

In this study, we synthesized data collated from 14 studies to explore the relationship between phosphate, DOP and rates of APA across the subtropical Atlantic and Pacific Oceans. In addition, we present new observations from a series of *in-situ* and bioassay experiments designed to investigate the impact of dust, iron, nutrients and zinc on APA in the subtropical eastern Atlantic Ocean.

MATERIALS AND METHODS

SYNTHESIS OF DATA ON PHOSPHATE CONCENTRATION AND APA

Data on the phosphate concentration, rates of APA and chlorophyll *a* concentration (or chlorophyll *a* normalized APA) were collated from 14 studies (Table 1). The database includes 807 paired data points for phosphate and APA, 486 paired data points for phosphate and chlorophyll *a* normalized APA and 475 paired data points for DOP and APA between 0 and 300 m in the Atlantic and Pacific Oceans (Table 1). Zero values were excluded from the data set. Data was obtained from tables in the original manuscript or directly from the author. Data was grouped by phosphate into 5 nM bins starting from 0 to 5 nM in order to estimate the mean \pm standard error (SE) rate of APA and variability at low phosphate concentrations (<200 nM). To estimate the phosphate concentration below which the rate of APA increases significantly (herein, the threshold phosphate concentration), we compared the APA rate in each 5 nM phosphate bin where phosphate was <100 nM with the APA rate between 100 and 200 nM using a *t*-test and identified the threshold phosphate concentration as the highest phosphate concentration when $p < 0.05$.

Studies reporting rates of APA are often difficult to compare due to the use of different substrate concentrations and different substrates (Duhamel et al., 2010). In this study, we have included data resulting from the use of both a high single

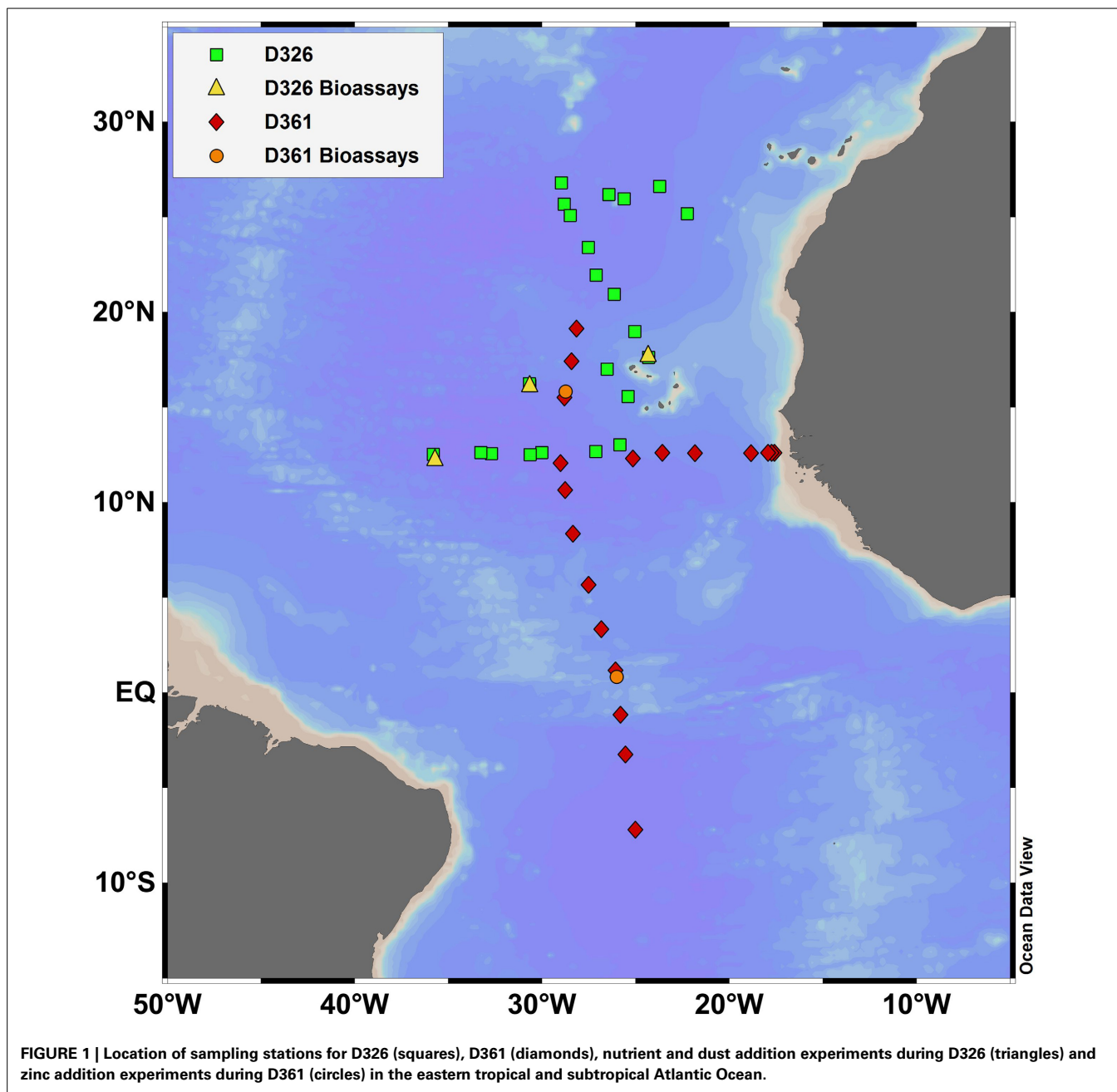
substrate concentration (10–20 μ M MUF-P) and a range of substrate concentrations (50–1000 nM) because a recent study found that the two methods can yield consistent maximum rates of enzyme activity (V_{max}) in open ocean environments (Suzumura et al., 2012). Two substrates have been used in studies included here, specifically 4-methylumbelliferyl phosphate (MUF-P) was used in 10 studies and 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP) was used in 3 studies (Table 1). It has been suggested that DiFMUP is less sensitive to changes in pH than MUF-P and also that the different substrates are accessible to different enzymes. We statistically compared the rates of APA produced using two different substrates to quantitatively assess the impact of substrate choice on the rate of APA using *t*-tests (Supplementary Table 1).

FIELD CAMPAIGNS

Sampling was conducted onboard the RRS *Discovery* during two research cruises in 2008 (D326: 5th January to 5th February 2008, SOLAS) and 2011 (D361: 7th February to 19th March 2011, GA06 GEOTRACES) in the eastern subtropical Atlantic (Figure 1, Table 2). Methods used to collect and analyse seawater samples for dissolved iron, aluminum and chlorophyll *a* are described by Rijkenberg et al. (2012) for D326 and Schlosser et al. (2014) for D361. Whole water rates of nitrogen fixation were determined using the $^{15}\text{N}_2$ technique, involving the addition of 4 ml of $^{15}\text{N}_2$ gas (99%, Cambridge) to 4 L of unfiltered seawater and determination of the ^{15}N content of particulate nitrogen (see methods in Schlosser et al., 2014). Nanomolar concentrations of nitrate and phosphate were determined using standard colorimetric methods using segmented flow analysis coupled to a 2 m liquid waveguide (Patey et al., 2008). Seawater for nutrient and rate measurements was collected using 20 L Niskin bottles attached to a stainless steel

Table 1 | Summary of location, range in phosphate concentration (nM) and rates of alkaline phosphatase activity (APA, nM d⁻¹) for published studies used in the data synthesis. The substrate (and supplier when reported) used in the study, 4-methylumbelliferyl phosphate (MUF-P), 4-methylumbelliferyl phosphate (MUP) or 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP) are noted.

Study region	Substrate (supplier)	Phosphate concentration range (nM)	APA (nM d ⁻¹)	Reference
Sargasso Sea	DiFMUP (Invitrogen)	0.5–10.6	19–45	Orchard et al., 2010
Atlantic Meridional Transect	MUF-P (Sigma Aldrich)	5–430	0.9–41	Mather et al., 2008
(Sub)-tropical North Atlantic	MUF-P	25–59	6–184	Sohm et al., 2008
(Sub)-tropical North Atlantic	MUF-P	14–71	4–31	Sohm and Capone, 2006
Bermuda Atlantic Time Series	DiFMUP (Invitrogen)	1–159	0.05–329	Lomas et al., 2010
(Sub)-tropical North Atlantic	MUF-P	5–72	5–148	Wurl et al., 2013
Sargasso Sea	MUF-P (2012 data only)	1–692	0.01–116	Martin et al., 2014
(Sub)-tropical Northeast Atlantic	MUF-P (Sigma Aldrich)	6–926	0.1–122	Reynolds et al., 2014
(Sub)-tropical Northeast Atlantic	MUF-P (Sigma Aldrich)	3–98	0.2–28	This study
Hawaii Ocean Time series, subtropical North Pacific	DiFMUP (Molecular Probes)	13–59	9–31	Duhamel et al., 2010
North and South subtropical Pacific	MUF-P(Sigma Aldrich)	12–117	0.3–9	Duhamel et al., 2011
subtropical Northwestern Pacific	MUF-P(Sigma Aldrich)	8–116	5–18	Suzumura et al., 2012
Subtropical North Pacific	MUF-P	31–71	0.5–19	Sohm et al., 2008
North and South subtropical Pacific	MUP (Invitrogen)	3–141	2–90	Sato et al., 2013



rosette. Seawater used for nutrient, dust, and zinc addition experiments was collected using the trace metal clean towed fish at ~ 3 m depth as previously described (Rijkenberg et al., 2012; Schlosser et al., 2014). Sampling and nutrient additions were performed under strict trace metal clean protocols in an over-pressurized clean air container onboard the ship.

Alkaline phosphatase activity

Whole water rates of APA were measured during D326 and D361 using the fluorimetric substrate MUF-P (Ammerman, 1993) following protocols reported in Sohm et al. (2008) and Reynolds et al. (2014). Briefly, 250 ml of unfiltered seawater was incubated in a polycarbonate bottle after the addition of MUF-P substrate

at a concentration of 200 nM, or at a range of concentrations between 200 and 1000 nM. Bottles were placed in on-deck incubators, which were cooled with surface seawater and adjusted to *in-situ* light intensity. Fluorescence was measured at the start of the incubation and approximately every 2 h for up to 24 h using a Turner 10 AU field fluorometer fitted with a long wavelength oil lab filter kit (10–302R).

Nutrient and dust addition experiments (D326)

Nutrient and dust addition experiments were conducted at 4 stations in the eastern subtropical gyre during D326 (Figure 1) with APA measurements made on 3 experiments (herein B2–B4, Table 3). Unfiltered seawater was collected in 20 L carboys from

Table 2 | Range in surface ocean properties, concentrations of inorganic and organic nutrients (nM), trace metals (nM), chlorophyll *a* ($\mu\text{g L}^{-1}$), rates of alkaline phosphatase activity (APA, nM d^{-1}) and nitrogen fixation (nM d^{-1}) during cruises D326 and D361.

Parameter	D326	D361
Dates	5th January to 5th February 2008	7th February to 19th March 2011
SST ($^{\circ}\text{C}$)	21.05–25.01	22.93–28.69
Chlorophyll <i>a</i> ($\mu\text{g L}^{-1}$)	0.13–0.32	0.05–0.33
Phosphate (nM)	3–98	12–99
Nitrate (nM)	6–70	1–49
Iron (nM)	0.07–0.37	0.02–0.96
Aluminum (nM)	10–47	10–64
DOP (nM)	75–186	71–192
APA (nM d^{-1})	0.2–28	0.7–20
APA ($\text{nmol } \mu\text{g chl d}^{-1}$)	1–101	1–144
Surface nitrogen fixation rate (nM d^{-1})	0.06–25	0.6–5.8

the towed fish. Initial concentrations and rate measurements were made on unfiltered seawater before the addition of nutrients. Nutrient addition experiments involved the single addition of nitrogenous nutrients ($1 \mu\text{M}$ of ammonium plus $1 \mu\text{M}$ of nitrate), phosphate ($0.2 \mu\text{M}$) or iron (2 nM of iron in dilute hydrochloric acid) or a combination of two or three nutrients. In parallel, 2 mg per L of dust (D1) or 2 mg per L of dust treated with sulphuric acid to simulate atmospheric processing (D2) was added to incubation bottles (Xylouri, 2009). A control was conducted in parallel to quantify the influence of the added nutrients or dust on the rates of APA and biomass relative to containment. Dust dissolution experiments conducted in parallel found that the addition of D1 and D2 would result in a release of $<0.2 \text{ nM}$ and $<1 \text{ nM}$ of dissolved iron (Xylouri, 2009). Bottles were incubated for 48 h ($T_{48\text{h}}$) at 20% surface light and cooled with surface seawater. At $T_{48\text{h}}$, a suite of samples were taken for analysis of chlorophyll *a*, Fv:Fm ratio, rates of nitrogen fixation and rates of APA (sampled from only one triplicate incubation bottle). Here, we report the volumetric and chlorophyll *a* normalized rates of APA only. All incubations were conducted in triplicate. However, the rates of APA were determined using seawater from one replicate carboy only, meaning that statistical analysis between the control and treatments and between treatments were not possible. Here, we focus on the relative magnitude of the response and do not attempt to make any statistical comparison between treatments within an experiment. However, the change in the rate of APA after the addition of nutrients and trace metals was consistent between experiments (see Section Nutrient, iron, and dust bioassays), implying that our results were reproducible.

Zinc addition experiments (D361)

To investigate the potential for zinc to limit the rate of APA, zinc addition experiments were performed at 1°N (Zn1) and at $\sim 16^{\circ}\text{N}$ (Zn2) in the eastern tropical and subtropical Atlantic during D361. Unfiltered seawater was collected from the towed fish into 10 L carboys and incubated for 48 h ($T_{48\text{h}}$) at 20% surface

Table 3 | Date, location and initial concentrations (\pm standard deviation) of inorganic and organic nutrients (nM), trace metals (nM), surface ocean rates of alkaline phosphatase activity (APA, nM d^{-1}) and nitrogen fixation (nM d^{-1}) at the start (T_0) of bioassays conducted in the eastern subtropical Atlantic during D326 (Spring 2008).

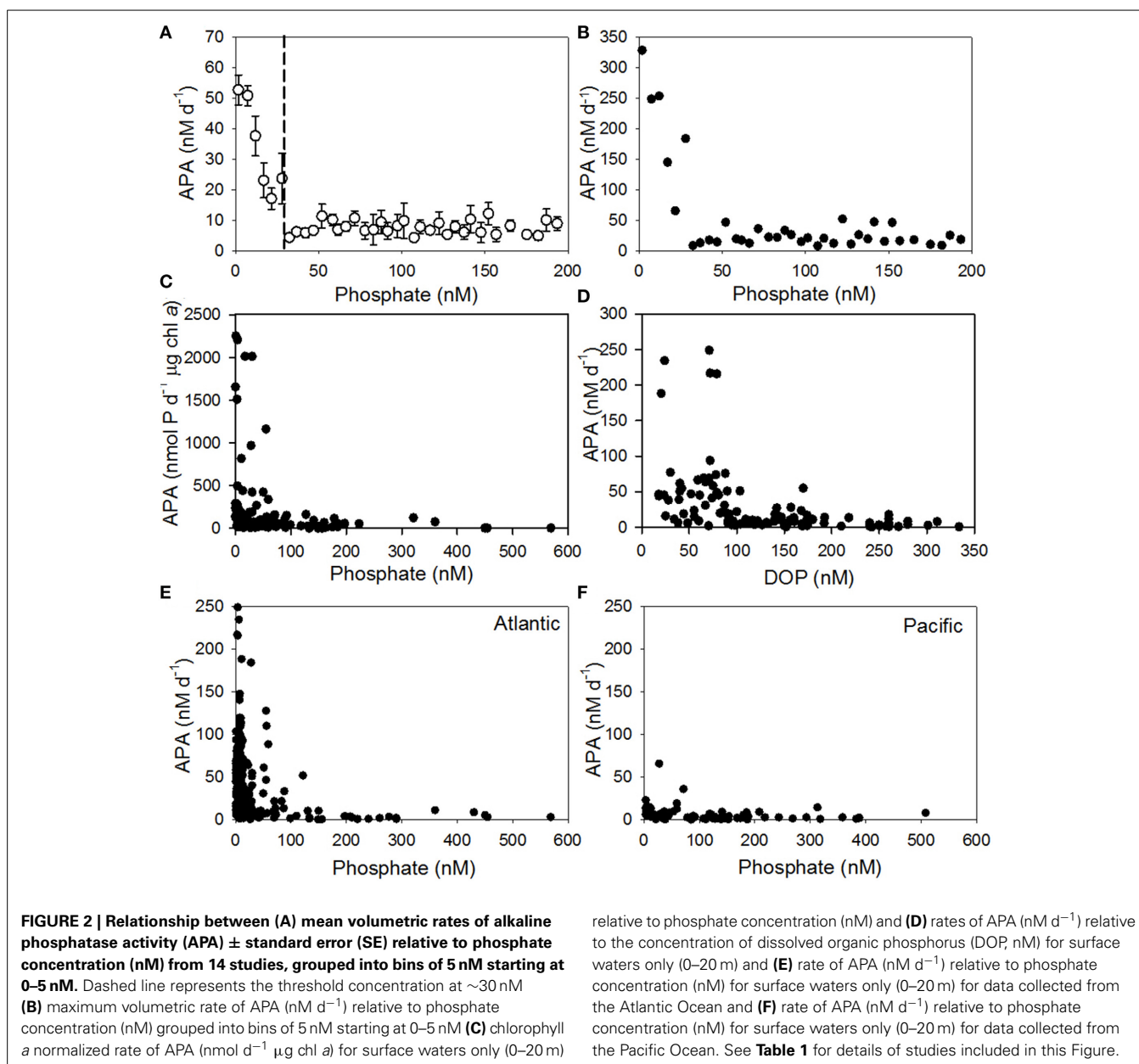
Parameter	B2	B3	B4
Date	15th January 2008	21st January 2008	25th January 2008
Location	$17.7^{\circ}\text{N}, 24.3^{\circ}\text{W}$	$12.3^{\circ}\text{N}, 35.8^{\circ}\text{W}$	$16.1^{\circ}\text{N}, 30.6^{\circ}\text{W}$
SST ($^{\circ}\text{C}$)	22.33	24.89	23.45
Chlorophyll <i>a</i> ($\mu\text{g L}^{-1}$)	0.31	0.19	0.13
Phosphate (nM)	85 ± 1	14 ± 1	12 ± 2
Nitrate (nM)	19 ± 2	12 ± 1	6 ± 1
N:P	~ 0.2	~ 0.9	~ 0.5
Iron (nM)	0.09 ± 0.03	0.23 ± 0.01	0.21 ± 0.002
Aluminum (nM)	10 ± 2	27 ± 1	26 ± 2
DOP (nM)	124	147	152
APA (nM d^{-1})	3.48	8.48	14.01
Surface nitrogen fixation rate (nM d^{-1})	0.1 ± 0.1	3.0 ± 0.3	25 ± 0.5

light after the addition of 1.3 or 1.9 nM of zinc chloride in Zn1 and Zn2, respectively. Control incubations (no zinc addition) were conducted in parallel. All incubations were performed in triplicate. At $T_{48\text{h}}$, a suite of samples were taken for analysis of phosphate, chlorophyll *a*, zinc and rates of APA. The concentration of zinc was determined using flow injection with fluorimetric detection as previously described (Wyatt et al., 2014). The rate of APA was determined in triplicate using methods described above except that a single substrate addition of 100 nM MUF-P was used. The rates of APA were measured within 8 h of adding the MUF-P substrate. Pairwise comparisons between controls and treatments were performed using a *t*-test and significance is reported where $p < 0.05$.

RESULTS

SYNTHESIS OF DATA ON PHOSPHATE CONCENTRATION AND APA

Laboratory and field studies show that the rate of APA increases with decreasing phosphate concentration, suggesting that APA is regulated by the supply of phosphate (Cembella et al., 1984a,b; Mather et al., 2008; Lomas et al., 2010; Duhamel et al., 2011; Suzumura et al., 2012). We collated data from 14 studies (Table 1) with phosphate ranging from 0.5 to 926 nM and the rate of APA ranging from 0.01 to 329 nM d^{-1} for the entire data set (Supplementary Figure 1). Our analysis supports the general observation of an inverse hyperbolic relationship between the concentration of phosphate and rate of APA (Supplementary Figure 1). When grouped into 5 nM phosphate bins, the mean (\pm SE) rate of APA in the lowest phosphate bins of 0–5 nM and 5–10 nM were $53 \pm 5 \text{ nM d}^{-1}$ ($n = 121$, from 0.5 to 329 nM d^{-1}) and $51 \pm 3 \text{ nM d}^{-1}$ ($n = 154$, from 0.8 to 249 nM d^{-1} , Figure 2A). A 2–3-fold increase in phosphate (10–15 nM)



reduced the mean rate of APA to 38 ± 7 nM d⁻¹ ($n = 72$, ranging from 2 to 254 nM d⁻¹) and a 3–4-fold increase in phosphate (15–20 nM) more than halved the rate of APA to 23 ± 6 nM d⁻¹ ($n = 33$, from 0.9 to 145 nM d⁻¹), highlighting the sensitivity of AP to increasing phosphate concentrations even at nanomolar phosphate concentrations. From our analysis, the rate of APA in the 25–30 nM phosphate bin was significantly higher than the rate of APA in the 100–200 nM phosphate bin ($p = 0.042$). This implies that the threshold phosphate concentration was \sim 30 nM (**Figure 2A**, dashed line). Below this concentration, the mean rate (**Figure 2A**) and maximum rate (**Figure 2B**) of APA increased above a background APA rate observed at phosphate concentrations greater than 50 nM. The relationship between phosphate and APA is the same when the volumetric rate of APA is normalized to chlorophyll *a* (**Figure 2C**).

The increase in the rate of APA at low phosphate concentrations clearly has an impact on the concentration of DOP, which decreases as APA increases (**Figure 2D**). The mean rate of APA (\pm SE) when DOP < 100 nM is 52 ± 8 nM d⁻¹ ($n = 54$) compared to 7 ± 1 nM d⁻¹ ($n = 120$) when DOP is > 100 nM.

We compared the rates of APA resulting from the use of two substrates, MUF-P and DiFMUP (Supplementary Table 1). When the rate of APA is compared for the entire data set, the Atlantic ocean only or when phosphate is < 100 nM, the rate of APA generated using DiFMUP is significantly higher ($p < 0.001$) than the rate of APA generated using MUF-P (Supplementary Table 1). However, due to the location of the studies that used DiFMUP, the phosphate concentration was also significantly lower ($p < 0.001$) in studies that used DiFMUP compared to those that used MUF-P

(Supplementary Table 1). Comparison of the rate of APA using data collected in the Pacific Ocean only showed that there was no significant difference in rates of APA generated using DiFMUP or MUF-P, despite phosphate being significantly lower ($p = 0.009$) in studies using DiFMUP. In light of the sensitivity of APA rates to the concentration of phosphate and the significant differences in the phosphate concentration between studies that used DiFMUP versus those that used MUF-P, we cannot verify if the use of different substrates has an impact on the resulting rates of APA and thus, we consider the patterns shown in our data synthesis to be a reflection of the response of APA to environmental conditions and not the use of different substrates. Further assays using different substrates in the same region would be required to investigate the influence of substrate on the accuracy of rates of APA.

We compared the surface ocean (0–20 m) phosphate and rates of APA between the subtropical Atlantic and subtropical Pacific (Figures 2E,F, respectively). The mean (\pm SE) phosphate concentration in the subtropical Atlantic (45 ± 5 nM, $n = 299$) was half that observed in the subtropical Pacific (108 ± 11 nM, $n = 88$). The mean (\pm SE) DOP concentration was also lower in the subtropical Atlantic (131 ± 7 nM, $n = 141$) relative to the subtropical Pacific (229 ± 52 , $n = 60$). The volumetric rate of APA was significantly higher ($p < 0.001$) in the subtropical Atlantic (39 ± 2 nM d⁻¹, ranging from 0.4 to 249 nM d⁻¹, $n = 299$) relative to the subtropical Pacific (6 ± 1 nM d⁻¹, ranging from 0.2 to 90 nM d⁻¹, $n = 88$). The biomass corrected rate of APA was also significantly higher in the subtropical Atlantic (692 ± 44 nmol P d⁻¹ μ g chl *a*) compared to the subtropical Pacific (91 ± 22 nmol P d⁻¹ μ g chl *a*, $p < 0.001$), implying that the extant phytoplankton population were more phosphate stressed in the subtropical Atlantic relative to the subtropical Pacific. Overall, the higher mean rate of APA in the subtropical Atlantic relative to the subtropical Pacific, is to a first order, due to the higher phosphate concentration in the subtropical Pacific. However, when the rate of APA is compared over the same relatively low phosphate concentration range (0–50 nM), the mean rate (\pm SE) of APA in the subtropical Atlantic (43 ± 3 nM d⁻¹, $n = 242$) is more than 4-fold higher than in the subtropical Pacific (9 ± 2 nM d⁻¹, $n = 35$), implying other factors are controlling the rate of APA in the subtropical Pacific. A striking feature of the subtropical North Atlantic is that it receives 43% of global dust deposition, whereas the North and South Pacific Oceans receive only 15 and 6% respectively (Jickells et al., 2005). To explore this further, we examined the impact of dust on the rate of APA.

IMPACT OF NUTRIENTS, IRON, AND DUST ON APA FROM BIOASSAY EXPERIMENTS

Hydrography and characteristics of the subtropical Atlantic

The eastern subtropical Atlantic, the region of study on both D326 and D361, is a warm, permanently stratified, oligotrophic region (Table 2; Rijkenberg et al., 2012; Reynolds et al., 2014). Surface phosphate concentrations were below 100 nM, excluding the northwest African upwelling region, during both field campaigns (Table 2). Surface iron concentrations were highly variable (Table 2) due to a large atmospheric dust event during D326 (Rijkenberg et al., 2012) and wet deposition in the inter-tropical convergence zone (ITCZ) during D361 (Schlosser et al., 2014).

Dust deposition event and APA

During D326, there was a strong atmospheric dust event between 17th and 19th January 2008 and 25th to 1st January 2008, which resulted a significant increase in the surface concentration of iron (from 0.05 ± 0.02 nM to 0.15 ± 0.02 nM) and phosphate (from 30 ± 20 nM to 80 ± 20 nM, Rijkenberg et al., 2012). Volumetric and chlorophyll normalized rates of APA for the entire D326 cruise ranged from 0.2 nM d⁻¹ to 28 nM d⁻¹ and 1 to 101 nmol P d⁻¹ μ g chl *a* (Tables 1, 2). The rate of APA decreased from 6.1 ± 2.1 nM d⁻¹ (or 39 ± 14 nmol d⁻¹ μ g chl *a*) to 3.9 ± 0.1 nM d⁻¹ (or 19.2 ± 0.4 nmol d⁻¹ μ g chl *a*) after the dust event. The relationship between dust and APA was explored further in a series of bioassays.

Nutrient, iron, and dust bioassays

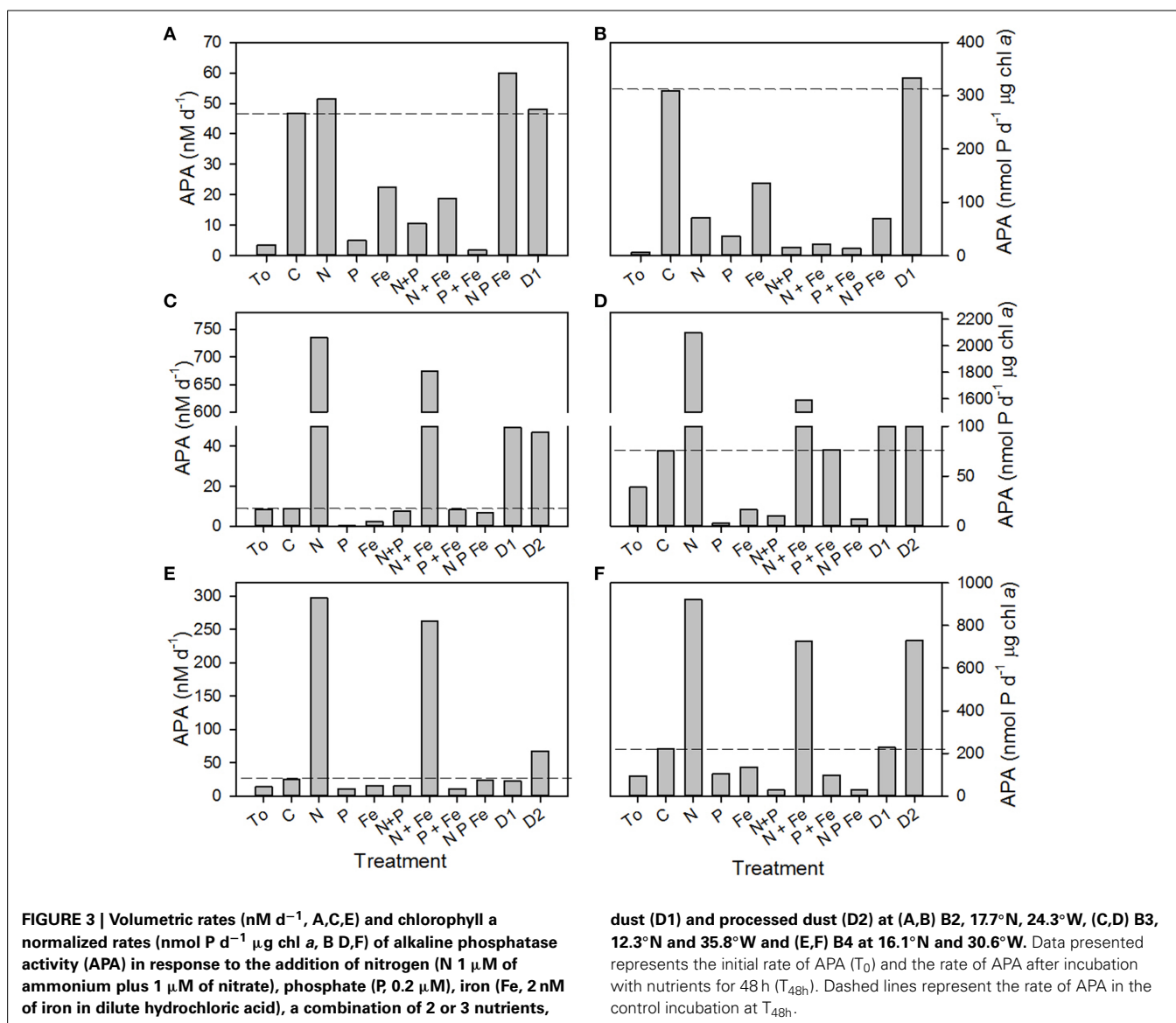
The rate of APA was measured during nutrient, trace metal and dust addition experiments conducted during D326. B2 was performed using seawater that was relatively phosphate-replete and iron-deplete with high biomass and low nitrogen fixation rates (Table 3). In contrast, the B3 and B4 were performed using seawater that was relatively phosphate-deplete and iron-replete with low biomass and enhanced rates of nitrogen fixation (Table 3).

The rate of APA in the control relative to APA at T_0 increased >10-fold in B2, remained unchanged in B3 and doubled in B4 after the 48 h incubation period (Figures 3A,C,E, respectively). The reasons for such a large change in the control in B2 are unclear, especially as the phosphate concentration at T_0 was ~ 80 nM, above the 30 nM threshold previously discussed. Thus, changes in the rates of APA will be compared to the control at T_{48h} and not the rate of APA at T_0 . Although there was a large increase in the rate of APA in the control in B2, a response to the addition of nutrients and dust relative to the control was still noticeable and indeed, a clear pattern emerged in the response to nutrients and dust in all three experiments.

Firstly, the rate of APA decreased after the addition of phosphate alone or in combination with nitrogen and/or iron, demonstrating the repressible behavior of AP with increasing phosphate concentrations (Figures 3A,C,E; Sebastián et al., 2004a,b). The addition of iron alone decreased the rate of APA relative to the control. Secondly, the rate of APA increased only slightly in B2 but by two orders of magnitude in B3 and B4 after the addition of nitrogen alone or with iron, probably because the system was shifted toward phosphate limitation. Lastly, the rate of AP increased 6-fold in B3 (Figure 3C) after the addition of D1 and 6-fold in B3 (Figure 3C) and 2-fold in B4 (Figure 3E) after the addition of D2. In B2, the addition of D1 and D2 increased the rate of APA slightly above that observed in the control (Figure 3A). When normalized to the concentration of chlorophyll *a* at T_{48h} , the same pattern was observed as phosphate (and iron alone) repressed the rate of APA, whilst nitrogen, dust and processed dust enhanced the rate of APA (Figures 3B,D,F).

Impact of zinc on APA

As zinc is a co-factor of some AP, such as PhoA, we explored the influence of zinc on the rate of APA. SST was $\sim 5^\circ\text{C}$ warmer at sampling site of Zn1 compared to Zn2 (Table 4). The concentration of phosphate was >10-fold higher at the start (T_0)



of Zn1 ($\sim 43 \text{ nM}$) compared to Zn2 ($\sim 3 \text{ nM}$) but the DOP and nitrate plus nitrite concentrations were similar (Table 4). The concentration of iron and zinc, and the integrated rates of nitrogen fixation were 2-fold higher in surface waters in the subtropical North Atlantic (Zn2) compared to close to the equator (Zn1; Table 4).

The rate of APA at T_0 of Zn1 was below our limits of detection ($\sim 0.5 \text{ nM d}^{-1}$). After 48 h ($T_{48\text{h}}$), the volumetric and chlorophyll *a* normalized rate of APA was measurable in both the control and treatment incubations and were almost 3-fold higher in the treatment relative to the control ($p = 0.045$ and $p = 0.049$, respectively, Figures 4A,C). Between T_0 and $T_{48\text{h}}$ during Zn2, the volumetric and chlorophyll *a* normalized rate of APA increased 3-fold and 6-fold respectively in the control and 6-fold and 8-fold respectively in the treatment (Figures 4B,D). The increase in the rate of APA in the added zinc treatment was significantly higher than observed in the control for both volumetric and chlorophyll *a*

normalized rates ($p < 0.001$, Figures 4B,D). The concentration of phosphate remained relatively constant throughout Zn1 but the zinc concentration decreased from $1.66 \pm 0.02 \text{ nM}$ to $0.6 \pm 0.1 \text{ nM}$ by $T_{48\text{h}}$. The phosphate concentration was not measured during Zn2 but the zinc concentration decreased from 2.1 ± 0.04 to $1.7 \pm 0.2 \text{ nM}$ by $T_{48\text{h}}$. These experiments demonstrate that the addition of zinc stimulated APA, despite the zinc concentration being almost 2-fold higher at Zn2 compared to Zn1 at T_0 (Table 4). However, a zinc concentration of 0.3 nM is considered to be fairly low and therefore we speculate that APA may have been limited by the availability of dissolved zinc in our study area of the tropical and subtropical Northeast Atlantic Ocean.

DISCUSSION

Results from our data synthesis, *in-situ* observations and bioassay experiments highlight the sensitivity of APA to the concentration of phosphate. We estimate that the threshold phosphate

Table 4 | Date, location and initial concentrations (\pm standard deviation) of inorganic and organic nutrients (nM), trace metals (nM) and rates of alkaline phosphatase activity (APA, nM d⁻¹) and nitrogen fixation (nM d⁻¹) at the start (T₀) of zinc addition experiments conducted in the eastern subtropical Atlantic during D361 (Spring 2011).

Parameter	Zn 1	Zn 2
Date	1st March 2011	13th March 2011
Location	0.83°N, 25.23°W	15.81°N, 28.73°W
SST (°C)	28.3	23.8
Chlorophyll a ($\mu\text{g L}^{-1}$)	0.110	0.110
Phosphate (nM)	43	3
Nitrate + nitrite (nM)*	13	14
Zinc (nM)	0.16 \pm 0.01	0.30 \pm 0.01
Iron (nM)*	0.08 \pm 0.01	0.16 \pm 0.01
DOP (nM)*	172 \pm 10	158 \pm 8
APA (nM d ⁻¹)	No measurable activity	9.0 \pm 0.9
Integrated nitrogen fixation rate ($\mu\text{mol m}^{-2} \text{d}^{-1}$)* ^a	88	142

*Based on nearest station or FISH data.

^aFrom Schlosser et al. (2014).

concentration required to induce APA is \sim 30 nM, below which the rate of APA increases significantly. As the concentration of phosphate decreased from 15 to 20 nM to 0 to 5 nM, we observed a 50% increase in the rate of APA. After a dust deposition event, where the phosphate concentration increased from \sim 30 to 80 nM and thus above the 30 nM threshold, we observed a decline in the rate of APA. Bioassay experiments revealed that the addition of phosphate alone or in combination with nitrogen and/or iron repressed APA in bioassay experiments. Collectively, our findings support the use of APA as an indicator for phosphate stress, provides useful constraints on rates of APA relative to the phosphate concentration in open ocean and provides insight into the dynamics of phosphate limitation on a broad basin scale.

The impact of APA on DOP is clearly seen from our data synthesis, where the concentration of DOP decreases at elevated rates of APA, supporting meridional observations from the subtropical Atlantic (Mather et al., 2008). To maintain surface ocean concentrations, DOP must either be regenerated locally (McLaughlin et al., 2013) or be advected in from regions where there is net production of DOP (Lomas et al., 2010; Reynolds et al., 2014), such as the flanks of gyres or adjacent upwelling regions where there is enhanced productivity.

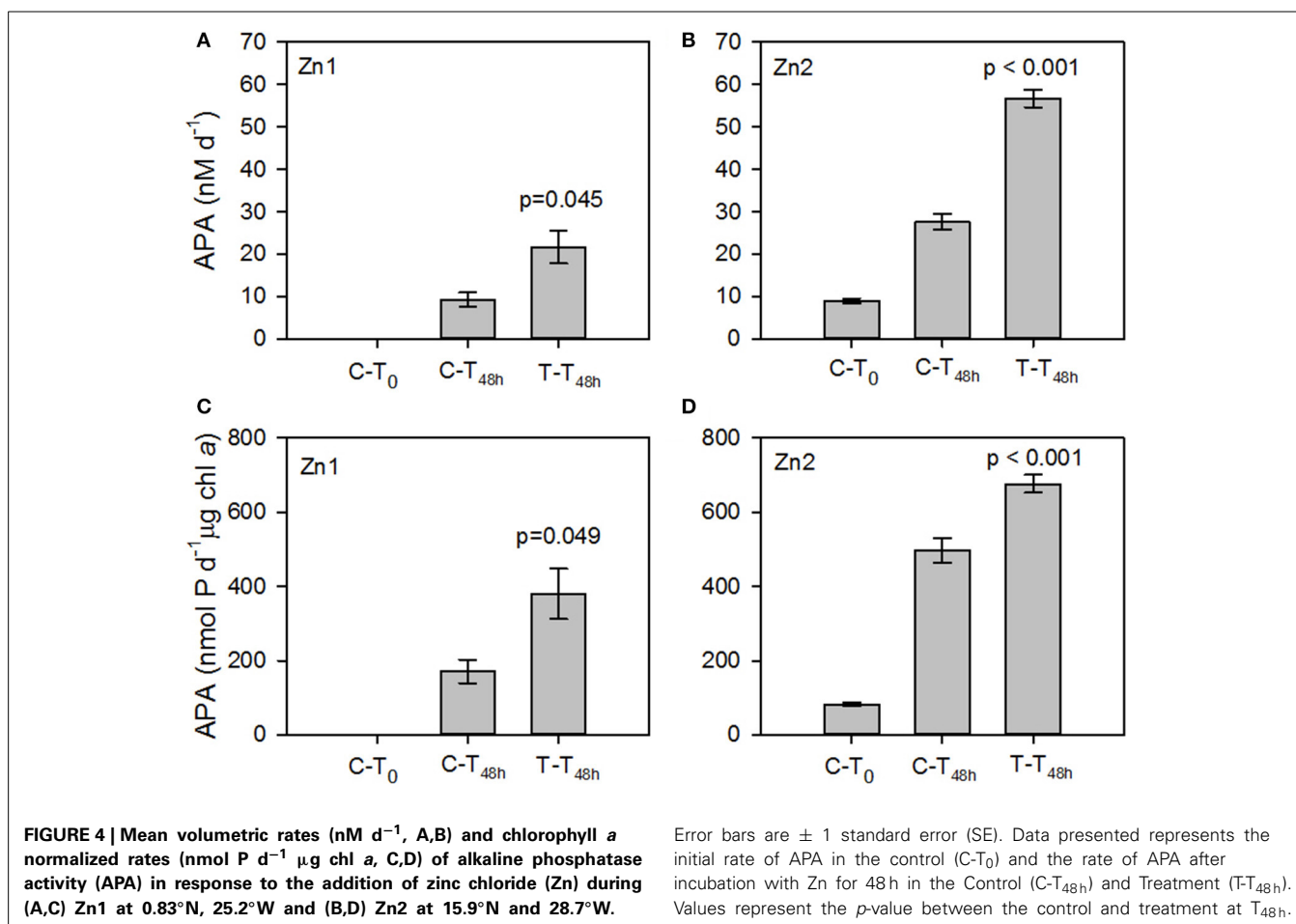
In contrast to our *in-situ* observations following a dust deposition event, the rate of APA increased after the addition of dust in the bioassay experiments. The addition of dust has been known to increase the rate of nitrogen fixation in bioassays (Mills et al., 2004; Langlois et al., 2012) and increase the abundance and enhance colony formation of nitrogen fixing organisms (Langlois et al., 2012). Enhanced nitrogen fixation would certainly stimulate the rate of APA, especially if the diazotroph community included *Trichodesmium*, which is known to intensively use AP to access labile DOP compounds to sustain its cellular phosphorus demand (Sohm et al., 2008; Orcutt et al., 2013). However, there

was no measurable increase in the rate of nitrogen fixation after the addition of D1 or D2 during these bioassays (Moore, personal communication). Either the rates of nitrogen fixation sustained were sufficient to drawdown phosphate and stimulate APA within the 48 h incubation period, or other factors induced APA.

The increase in APA may be due to subtle differences in nutrient stocks. We added 2 mg of dust per L of seawater during the dust addition experiments. Assuming that dust contains 0.1% phosphorus and 0.12% nitrogen (Guieu et al., 2002; Mills et al., 2004), we estimate that 65 nmol phosphorus and 170 nmol nitrogen was added per L to the incubation bottles. The N:P ratio at the start of the experiments was 0.2, 0.9, and 0.5 (Table 3) for B2–B4 respectively. After the addition of dust and dissolution of nitrogen and P, the N:P ratio increased in all incubations to 1.3, 2.3, and 2.3 in B2–B4, respectively. Although these ratios are much lower than the Redfield ratio of 16N:1P (Redfield et al., 1963), this subtle increase in the amount of nitrogen relative to phosphorus may have stimulated the rate of APA. Indeed, Ruttenburg and Dyhrman (2012) hypothesize that APA is sensitive to the N:P ratio and not just the phosphate concentration.

We found that the rate of APA was significantly higher in the subtropical Atlantic Ocean relative to the subtropical Pacific Ocean. To a first order, this is probably due to the phosphate concentration being higher in the subtropical Pacific (108 ± 11 nM) compared to the subtropical Atlantic (45 ± 5 nM) in studies used in the data synthesis exercise here. However, APA rates were significantly higher in the subtropical Atlantic even over the same relatively low concentration range of phosphate (0–50 nM), implying that factors other than the phosphate concentration influenced APA. As previously mentioned, one of the most striking differences between the subtropical North Atlantic and subtropical Pacific is that the Atlantic receives almost half the global supply of atmospheric dust (Jickells et al., 2005; Mahowald et al., 2009), causing surface ocean iron concentrations to be elevated in the Atlantic relative to the Pacific Ocean. As a consequence, areal rates of nitrogen fixation are higher in the subtropical North Atlantic ($250 \mu\text{mol N m}^{-2} \text{d}^{-1}$; Capone et al., 2005) relative to the North Pacific ($30\text{--}120 \mu\text{mol N m}^{-2} \text{d}^{-1}$; Dore et al., 2002) and South Pacific ($30\text{--}70 \mu\text{mol N m}^{-2} \text{d}^{-1}$; Raimbault and Garcia, 2008), where nitrogen fixation is considered to be iron limited (Sohm et al., 2011). Thus, fertilization of surface waters with nitrogen via nitrogen fixation forces the system toward phosphate limitation and organisms produce AP to meet their metabolic phosphorus demands. In contrast, nitrogen fixation is lower in the iron-deplete subtropical Pacific, allowing phosphate concentrations to be relatively high, thus repressing APA (Duhamel et al., 2010, 2011). However, this narrative does not explain why AP is lower in the subtropical Pacific relative to the subtropical Atlantic Ocean even over the same phosphate concentration range. A second but more speculative argument is based on the trace metal requirement of phosphatase enzymes, specifically PhoX, which requires iron (Yong et al., 2014) and PhoA, which requires zinc (Coleman, 1992), both of which are lower in the subtropical Pacific compared to the subtropical Atlantic Ocean.

The addition of dust in bioassays stimulated the rate of APA. While the phosphorus demands of nitrogen fixers, or subtle



differences in nutrient stoichiometry may have induced APA, the dissolution of iron from dust may have had a direct impact, as some AP such as PhoX, contain iron (Yong et al., 2014). However, the addition of iron alone in bioassays caused the rate of APA to decrease relative to the control rather than increase. The subtropical Atlantic is considered to be iron-replete and thus iron may not constrain the activity of PhoX in this region. However, further investigation is required to examine the relationship between iron and PhoX in the ocean (Moore, 2014).

There is ongoing debate on the potential for zinc to limit the synthesis of AP, such as PhoA, which contains at least two zinc ions in the three metal binding sites available (Coleman, 1992; Jakuba et al., 2008, 2012; Cox and Saito, 2013). We observed a significant increase in the rate of APA 48 h after the addition of ~1.5 nM of zinc in two experiments conducted near the equator and at ~16°N in the eastern subtropical Atlantic. At each station, the surface ocean concentration of zinc was relatively low (0.16 and 0.30 nM; Table 4) and a similar significant increase in the rate of APA was observed at both stations. Our results imply that the concentration of zinc in surface waters in the subtropical Atlantic limits the activity of AP.

Previously, culture based experiments have shown the potential for zinc limitation of APA in *Emiliania huxleyi* (Shaked et al., 2006; Jakuba et al., 2008) and *Synechococcus* (Cox and Saito,

2013). In the latter study, the concentration of an APA-related protein was 5-times higher when *Synechococcus* was grown under phosphate deplete, zinc replete conditions compared to phosphate and zinc replete conditions (Cox and Saito, 2013). These culture-based studies have provided quantitative constraints on the zinc demand of key phytoplankton in the ocean, but evidence of zinc limitation from the subtropical ocean is sparse. On a basin scale, the regression slope between zinc and phosphate is much higher in the Atlantic (593–7870) relative to the Pacific Ocean (251–373; Jakuba et al., 2008 and references therein), implying a higher apparent uptake of zinc relative to phosphate in the Atlantic relative to the Pacific, possibly to meet the zinc demand for APA (or PhoA), thus driving the system toward zinc limitation. To our knowledge, results from this study provide the first direct evidence for limitation of APA by zinc.

CONCLUSIONS

Our data synthesis, *in-situ* observations and bioassay experiments demonstrates that (a) the relationship between APA and phosphate is consistent across subtropical regions, (b) the rate of APA increases significantly below a threshold phosphate concentration of 30 nM (c) enhanced APA decreases the concentration of DOP, (d) APA is repressed by the addition of phosphate and induced

by the addition of nitrogen, (e) the addition of dust enhances APA, (f) the rate of APA is higher in the subtropical Atlantic relative to the subtropical Pacific, possibly due to co-limitation of APA by trace metals such as iron and zinc, and (g) there is direct evidence for limitation of APA by zinc. This information should help further our basin scale understanding of phosphate cycling and limitation. However, while the patterns are consistent, the accuracy of current estimates of AP as well as our understanding of the role of phytoplankton groups may be uncertain. Using bioinformatics, a recent study found that 41% of AP is in the cytoplasm of a cell rather than the periplasm (Luo et al., 2009). A consequence of this is that current techniques used to measure APA using MUF-P may underestimate the activity as MUF-P cannot be transported across the cell membrane and therefore measures extracellular APA only (Luo et al., 2009; White, 2009). In addition, the ubiquitous cyanobacterium, *Trichodesmium* are known to contribute significantly to water column APA (81–90%; Sohm et al., 2008; Orcutt et al., 2013). However, there is evidence that epibiotic bacteria living on *Trichodesmium* colonies use quorum sensing to regulate APA and thus influence the phosphorus available to *Trichodesmium* (van Mooy et al., 2012). This complicates our interpretation of AP as it is not only regulated by the extent of nutrient limitation for the broad phytoplankton community but is dependent upon microbe-host interactions.

Our understanding of AP has improved significantly over the past decade due to the increase in number of studies focused on quantifying the rate of AP and its role in the cycling of phosphorus in the ocean (Sebastián et al., 2004a,b; Sohm and Capone, 2006; Mather et al., 2008; Sohm et al., 2008; Duhamel et al., 2010, 2011; Lomas et al., 2010; Orchard et al., 2010; Suzumura et al., 2012; McLaughlin et al., 2013; Sato et al., 2013; Wurl et al., 2013; Martin et al., 2014; Reynolds et al., 2014) and the detection of genes and proteins associated with the enzyme (Orchard et al., 2009; Scanlan et al., 2009; Tetu et al., 2009; Kathuria and Martiny, 2011; Cox and Saito, 2013; Reistetter et al., 2013). However, further work is required to understand the role of trace metals, particularly zinc and iron, in regulating the rate of APA and constraining the activity of different phosphatases, such as PhoA and PhoX, which have different trace metal requirements.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://www.frontiersin.org/journal/10.3389/fmars.2014.00073/abstract>

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