



# Concentrations and Uptake of Dissolved Organic Phosphorus Compounds in the Baltic Sea

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The dissolved organic phosphorus (DOP) pool in marine waters contains a variety of different compounds. Knowledge of the distribution and utilization of DOP by phyto- and bacterioplankton is limited, but critical to our understanding of the marine phosphorus cycle. In the Baltic Sea, detailed information about the composition of DOP and its turnover is lacking. This study reports the concentrations and uptake rates of DOP compounds, namely, adenosine triphosphate (dATP), deoxyribonucleic acid (dDNA), and phospholipids (dPL), in the Baltic Proper and in Finnish coastal waters in the summers of 2011 and 2012. Both areas differed in their dissolved inorganic phosphorus (DIP) concentrations (0.16 and 0.02–0.04  $\mu\text{M}$ ), in the C:P (123–178) and N:P (18–27) ratios, and in abundances of filamentous cyanobacteria and of autotrophic and heterotrophic picoplankton. The mean concentrations of dATP-P, dDNA-P, and dPL-P were 4.3–6.4, 0.05–0.12, and 1.9–6.8 nM, respectively, together contributing between 2.4 and 5.2% of the total DOP concentration. The concentrations of the compounds varied between and within the investigated regions and the distribution patterns of the individual components are not linked to each other. DIP was taken up at rates of 10.1–380.8 nM d<sup>-1</sup>. dATP-P and dDNA-P were consumed simultaneously with DIP at rates of 6.9–24.1 and 0.09–0.19 nM d<sup>-1</sup>, respectively, with the main proportion taken up by the size fraction <3  $\mu\text{m}$  and with DIP to be the dominant source. Groups of hydrographical and biological parameters were identified in the multiple regression analysis to impact the concentrations and uptake rates. It points to the complexity of the regulation. Our results indicate that the investigated DOP compounds, particularly dATP-P, can make significant contributions to the P nutrition of microorganisms and their use seems to be not intertwined. Therefore, more detailed knowledge of all DOP components including variation of concentrations and the utilization is required to understand the roles of DOP in marine ecosystems.

**Keywords:** phosphorus cycle, Baltic Sea, dissolved organic phosphorus (DOP), DOP compounds, adenosine triphosphate, DNA, phospholipids, phosphorus uptake rates

## INTRODUCTION

In the marine phosphorus (P) cycle, dissolved inorganic phosphorus (DIP), particulate inorganic (PIP) and organic phosphorus (POP) and dissolved organic phosphorus (DOP) are the main P pools. The transformations between the various pools have been described in several studies, e.g., Jauzein et al. (2010), White et al. (2012), Karl (2014), however, a complete understanding of the P cycle is lacking. DIP is consumed by phyto- and bacterioplankton and incorporated into biomass, forming POP, and following cell death and remineralization is partly released as DOP into the surrounding water. DOP acts as an additional P source to microbial organisms, especially when it dominates the dissolved P pool, as observed in oligotrophic regions of the ocean (Cavender-Bares et al., 2001) and in marine ecosystems where DIP is seasonally depleted (Mather et al., 2008; Lomas et al., 2010).

Dissolved organic phosphorus concentrations are reported to range between 0.06 and 0.54  $\mu\text{M}$  in ocean surface waters (Karl and Björkman, 2015), but can be elevated in coastal waters due to enhanced productivity, as reported by Lin et al. (2012) for the St. Louis estuary (Gulf of Mexico), where DOP levels ranged from 0.16 to 0.67  $\mu\text{M}$ . In the Baltic Sea, investigations of DOP are sparse and mostly limited to measurements of concentrations, with few studies on the bioavailable fraction. Concentrations of DOP in coastal regions of the Baltic Sea reached up to 0.98  $\mu\text{M}$ , while in the central Baltic Sea the observed concentration range was 0.2–0.52  $\mu\text{M}$  (Hoikkala et al., 2015). The DOP pool has been characterized in terms of its molecular size and compound groups. DOP includes low and high molecular weight compounds (LMW-DOP < 10 kDa and HMW-DOP > 10 kDa) (Ridal and Moore, 1990; Clark et al., 1999; Young and Ingall, 2010). The DOP fraction < 10 kDa comprises 50–80% of the DOP pool in open ocean surface waters (Benitez-Nelson, 2000) and 54–76% in the Bay of Tokyo (Suzumura et al., 1998). Concerning compound groups, the DOP pool includes phosphoesters (mono- and di-esters) and phosphonates (Kolowith et al., 2001; Monbet et al., 2009; Repeta et al., 2016). The majority of the DOP pool consists of phosphoesters (Young and Ingall, 2010) including sugar phosphates, vitamins, nucleotides such as adenosine mono- and tri-phosphate (AMP, ATP) and nucleic acids, i.e., deoxyribonucleic (DNA) and ribonucleic acid (RNA) (Karl and Björkman, 2015). All these substrates are assumed to be bioavailable to the microbial community after enzymatic degradation. Phospholipids (PL) are hydrophobic compounds assumed to have a relatively low bioavailability (Björkman and Karl, 1994; Suzumura and Ingall, 2004). Phosphonates are considered to form part of the recalcitrant DOP fraction (Suzumura et al., 1998). However, several bacteria and cyanobacteria are reported to be genetically equipped for phosphonate utilization (Dyhrman et al., 2006; Feingersch et al., 2012; Repeta et al., 2016). Siuda and Chrost (2001) showed that P is hydrolyzed through enzymatic processes from DOP compounds to various degrees, with the highest proportion of P released from nucleotides.

ATP has been used as a proxy for the labile DOP fraction (Casey et al., 2009), with radioactive [ $^{33}\text{P}$ ]-labeled ATP used to quantify labile DOP uptake by organisms. The uptake of ATP is influenced by nutrient conditions, besides other factors like temperature. However, Casey et al. (2009) and Michelou et al. (2011) observed taxon-specific differences in the degree of ATP uptake under identical environmental conditions. In the North Pacific subtropical gyre, the ATP uptake is realized by heterotrophic bacteria rather than by pigmented picoplankton (Björkman et al., 2012). Accordingly, there seems to be a broad range of utilization of DOP compounds by microorganisms in marine waters.

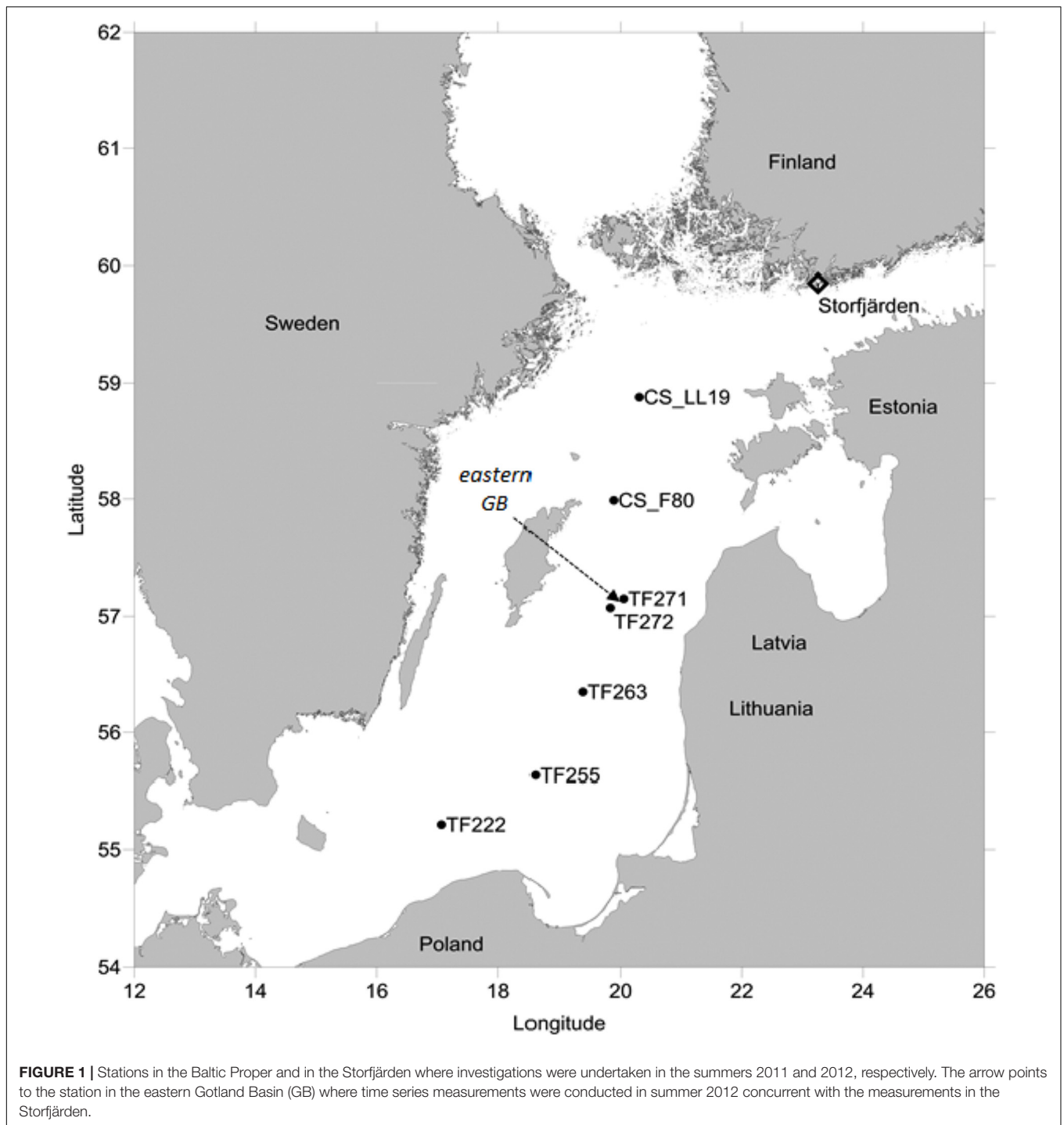
In semi-enclosed waters such as the Baltic Sea, knowledge of the DOP composition and turnover of specific DOP components is still lacking. However, determining the DOP composition is the first step toward understanding the dynamics of the DOP pool and its functions, which is the basis for a comprehensive understanding of the P cycle. For this purpose, we studied the concentrations of the DOP compounds ATP, PL, and DNA, as well as their spatial and temporal variations and the utilization of ATP and DNA in relation to DIP, in the summer season, when DOP usually forms the main dissolved P source for microorganisms (Nausch et al., 2004, 2008). We aimed to understand the contribution of these compounds to the DOP pool and to the P nutrition of phyto- and bacterioplankton in the Baltic Sea.

## MATERIALS AND METHODS

### Study Sites and Hydrographical Conditions

Dissolved organic phosphorus compounds in the central Baltic Sea were investigated during a cruise with RV ELISABETH-MANN-BORGESE along a north-south transect through the Baltic Proper (**Figure 1**) from 25 to 28 July 2011. Weak to moderate winds were observed, varying from the south-southwest to the west-northwest directions. The surface temperature of  $16.5 \pm 0.5^\circ\text{C}$  and salinity of  $7.10 \pm 0.07$  at the southern stations (TF 222–TF 263) differed from those at more northern stations (TF272-CS\_LL19), which had an observed temperature of  $17.6 \pm 0.4^\circ\text{C}$  and a salinity of  $6.92 \pm 0.21$ , indicating warmer water with a slightly lower salinity (**Figure 1** and **Table 1**).

Time series data were collected in the eastern Gotland Basin (GB) and in Storfjärden (northwestern Gulf of Finland) (**Figure 1**). In the eastern GB (station TF 271:  $57^\circ 19.2' \text{N}$ ,  $20^\circ 30.0' \text{E}$ ), sampling was conducted onboard RV METEOR from 7 to 20 July 2012, with a water temperature of  $15.1 \pm 0.9^\circ\text{C}$  and a salinity of  $7.12 \pm 0.03$  in the upper mixed surface layer. In Storfjärden, the study was undertaken between 19 June and 24 July 2012 in the proximity of the Tvärminne Zoological Station ( $59^\circ 51.5' \text{N}$ ,  $23^\circ 15.5' \text{E}$ ). The station was influenced by wind-driven upwelling events of varying intensities that caused variations in water temperatures, which ranged from 8.1 to  $15.9^\circ\text{C}$  (mean  $11.9 \pm 2.1^\circ\text{C}$ ) and with salinities ranging between 5.46 and 5.97 (Paul et al., 2015).



## Water Sampling

### Central Baltic Sea

On-board the research vessels, water was sampled using a rosette sampler (HydroBios) equipped with 5-L free flow bottles from 1 and 10 m depth to cover the surface mixed layer above the thermocline. The combined Seabird SBE911 probe was equipped with sensors for conductivity, temperature, depth, and chlorophyll fluorescence. Water from the bottles was collected in

pre-cleaned (HCl- and deionized water) polyethylene containers that had been pre-rinsed with sample water. From the containers, subsamples were taken for each parameter and for uptake rate measurements immediately after sampling.

### Storjården

For the DOP, other P-fractions, chlorophyll and picoplankton analyses, samples were collected every second day over a depth of

**TABLE 1** | Wind, hydrographical, and biological conditions in both sub-regions of the transect in the Eastern Gotland Basin (GB) and in the Storfjärden.

	Transect 2011		Eastern GB	Storfjärden
	Southern stations	Northern stations	2012	2012
Wind speed (m s <sup>-1</sup> )	4.42 ± 1.36 (n = 3)	3.03 ± 0.83 (n = 3)	5.1 ± 1.9 (n = 10)	3.2 ± 0.80 (n = 30)
T (°C)	16.5 ± 0.5 (n = 6)	17.6 ± 0.4 (n = 8)	15.1 ± 0.9 (n = 22)	11.9 ± 2.1 (n = 16)
Salinity	7.10 ± 0.07 (n = 6)	6.92 ± 0.21 (n = 8)	7.12 ± 0.03 (n = 22)	5.72 ± 0.13 (n = 16)
PC (μM)	33.6 ± 7.3 (n = 6)	44.0 ± 7.8 (n = 7)	28.1 ± 2.6 (n = 22)	28.5 ± 5.9 (n = 15)
PN (μM)	5.3 ± 0.7 (n = 6)	6.8 ± 0.9 (n = 8)	3.7 ± 0.48 (n = 22)	4.1 ± 0.8 (n = 15)
PP (μM)	0.23 ± 0.02 (n = 6)	0.25 ± 0.03 (n = 8)	0.21 ± 0.03 (n = 22)	0.22 ± 0.03 (n = 15)
C:N ratio	6.3 ± 0.7 (n = 6)	6.6 ± 1.2 (n = 7)	7.6 ± 0.5 (n = 22)	6.9 ± 0.5 (n = 15)
C:P ratio	145 ± 29 (n = 5)	178 ± 26 (n = 8)	138 ± 21 (n = 22)	123 ± 30 (n = 15)
N:P ratio	23 ± 2.6 (n = 6)	27 ± 4.4 (n = 8)	18 ± 3 (n = 22)	19.6 ± 3.6 (n = 14)
Chla (μg l <sup>-1</sup> )	2.96 ± 0.65 (n = 6)	3.78 ± 0.33 (n = 8)	3.11 ± 0.40 (n = 19)	2.81 ± 1.18 (n = 16)
Total filamentous cyanobacteria (×10 <sup>4</sup> units l <sup>-1</sup> )	4.1 ± 1.4 (n = 6)	3.2 ± 2.4 (n = 8)	12.8 ± 4.8 (n = 8)	7.5 ± 6.8 (n = 10)
<i>Dolichospermum</i> sp. (×10 <sup>4</sup> units l <sup>-1</sup> )	0.5 ± 0.6	0.2 ± 0.2	6.8 ± 4.2	0.1 ± 0.2
<i>Aphanizomenon</i> sp. (×10 <sup>4</sup> units l <sup>-1</sup> )	3.4 ± 1.6	3.1 ± 1.9	4.5 ± 1.5	2.4 ± 3.4
<i>Pseudanabaena</i> sp. (×10 <sup>4</sup> units l <sup>-1</sup> )	0	0	0	5.3 ± 4.0
<i>Nodularia spumigena</i> (×10 <sup>4</sup> units l <sup>-1</sup> )	0.2 ± 0.1	0.2 ± 0.3	1.5 ± 2.2	0
Autotrophic picoorganisms (×10 <sup>8</sup> cells l <sup>-1</sup> )	3.1 ± 0.7 (n = 6)	2.4 ± 0.4 (n = 8)	n.d.	1.6 ± 0.1 (n = 16)
Heterotrophic bacteria (×10 <sup>9</sup> cells l <sup>-1</sup> )	4.2 ± 0.4 (n = 6)	4.1 ± 0.6 (n = 8)	n.d.	3.2 ± 0.4 (n = 14)

Wind data for Finland (Helsinki-Malmi) and for the Gotland Basin were obtained from [www.eurometeo.com](http://www.eurometeo.com). Means and standard deviations are given for the respective study period (n.d., not detected; n, number of data).

17 m using a depth-integrating water sampler (IWS HydroBios) that was lowered slowly on a cable by hand from a small boat (see Nausch et al., 2016 for further details). For the DOP compounds, samples were taken every fourth day.

## Analytical Methods

### Chlorophyll *a* and Particulate Carbon

Water (300–800 ml) for Chlorophyll *a* (Chla) analysis was filtered (200 mbar pressure difference) through glass fiber filters (GF/F, Whatman, diameter 25 mm) with a nominal pore size of 0.7 μm. The filters were flash frozen in liquid nitrogen and stored at –80°C until extraction with 96% ethanol for at least 3 h (Wasmund et al., 2006). Chla fluorescence was measured with a TURNER fluorometer (10-AU-005) at 450 nm excitation and 670 nm emission (Welschmeyer, 1994). The Chla extraction procedure for the Storfjärden samples was different. For these samples, the GF/F filters were extracted with 90% acetone after homogenization in a cell mill for 5 min (Paul et al., 2015).

According to Wasmund et al. (2006), both methods provide comparable results.

Particulate carbon (PC) was analyzed from 300 to 800 ml samples filtered (200 mbar pressure difference) onto pre-combusted (450°C, 6 h) GF/F filters (Whatman, diameter 25 mm) and stored frozen at –20°C until analysis. Samples from the cruises were measured by flash combustion using a Carlo Erba EA 1108 at 1020°C in the IOW and using a EuroEA elemental analyzer coupled with a Conflo II interface to a Finnigan Delta<sup>plus</sup> mass spectrometer for samples from Storfjärden.

### Filamentous Cyanobacteria and Picoplankton

Water samples for the microscopic analyses were preserved with acetic Lugol (KI/I2) (1% v/v final concentration) and settled in an Utermöhl sedimentation chamber for 24 h. The species composition and abundance were assessed with an inverted microscope (ZEISS or Leica) at 100× magnification. Filaments were enumerated as units; one unit corresponds to a filament length of 100 μm.

Picoplankton was counted using a FACS Calibur flow cytometer (BD Biosciences) equipped with a 488 nm laser. The detection of autotrophic picoplankton was based on their autofluorescence and side-scatter properties, whereas bacteria were stained with SYBRGreen I (Molecular Probes) before counting. SYBR green fluorescence was plotted versus the side-scatter traits. Populations were clustered using the software program “Cell Quest Pro” (Gasol and Del Giorgio, 2000).

### Inorganic and Organic Phosphorus

Dissolved inorganic phosphorus was determined colorimetrically in an autoanalyzer (Alliance Instruments) according to Grasshoff et al. (1983) onboard the research vessels during the cruises. The detection limit was 0.02  $\mu\text{M}$ . During the Storfjärden time-series, we used a segmented continuous-flow analyzer coupled with a liquid-waveguide capillary flow-cell (LWCC) of 2 m length to determine DIP with a detection limit of 0.8 nM (Patey et al., 2008). These measurements were conducted at the Tvärminne Zoological Station.

Total phosphorus (TP) and dissolved phosphorus (DP) were analyzed from 40 ml unfiltered and filtered (pre-combusted GF/F-filters, 450°C, 6 h) water samples, respectively, stored at  $-20^{\circ}\text{C}$  until further treatment. During the analysis, TP and DP samples were oxidized to DIP in a laboratory microwave equipped with 40 Teflon tubular vessels in an alkaline medium (Grasshoff et al., 1983). The resulting DIP concentration was measured in a 10 cm cuvette with a precision of 0.01  $\mu\text{M}$ . DOP was calculated as the difference between the DP and DIP concentrations.

Particulate phosphorus (PP) was calculated as the difference between TP and DP. The microwave digestion does not discriminate between organic and inorganic P because all P is transformed into DIP. However, due to the correlation with Chla, we assumed that PP consisted predominantly of POP.

### DOP Compounds

The samples for DOP compound analysis were filtered through pre-combusted (6 h, 450°C) GF/F filters, followed by filtration through 0.2  $\mu\text{m}$  cellulose acetate filters (Sartorius Stedim). Subsamples of the filtrate were prepared for storage according to the specific method used for each compound. The subsamples were analyzed as described by Unger et al. (2013); each compound is described below by its P content.

#### *Dissolved adenosine triphosphate (dATP)*

Dissolved adenosine triphosphate (dATP) was determined according to the method of Björkman and Karl (2001), which was adapted by Unger et al. (2013) to Baltic Sea conditions. A  $\text{Mg}(\text{OH})_2$  precipitate was prepared by the addition of 1 M NaOH (0.5% v/v) to 200 ml of the filtered seawater samples. The precipitate was concentrated by centrifugation at 1680 g for 1.5 h. The resulting pellet was dissolved by dropwise addition of 5 M HCl; the pH was adjusted to 7.2 using TRIS buffer (pH 7.4, 20 mM). The dATP concentrations were detected by bioluminescence flash analysis in a Sirius luminometer (Bertold) after addition of firefly lantern extract (FLE-250, Sigma-Aldrich) with a detection limit of 2.5 pM. A standard series

of adenosine 5'-triphosphate disodium salt hydrate (Sigma-Aldrich) was applied to each sample set. The P content of the dATP (ATP-P) was calculated by assuming that 1 mol ATP was equivalent to 3 moles P.

#### *Dissolved phospholipids (dPL)*

The concentration of phosphate bound to dPL (dPL-P) was analyzed according to Suzumura and Ingall (2004). To do so, 400 ml of the filtered water was stored at  $-20^{\circ}\text{C}$ . Prior to analysis, samples were thawed and extracted twice with 100 ml of chloroform. The chloroform phase was concentrated in a rotary evaporator, transferred into microwave tubes and completely evaporated in a  $60^{\circ}\text{C}$  water bath. After addition of 20 ml of deionized water, the samples were digested in a microwave in alkaline medium, as done for the TP and DP analyses. A set of standard concentrations of L-phosphatidyl-DL-glycerol sodium salt (Sigma Aldrich) prepared in Baltic Sea water and chloroform as blank were analyzed in the same way as the samples. The detection limit of the concentrated samples was 0.8 nM.

#### *Dissolved DNA (dDNA)*

The concentration of dDNA was measured according to Karl and Bailiff (1989). A total of 200 ml of the filtered sample was gently mixed with equivalent amounts of ethylene-diamine-tetraacetic acid (EDTA, 0.1 M, pH 9.3, Merck) plus 4 ml of cetyltrimethyl-ammonium bromide (CTAB, Sigma-Aldrich) and stored at  $-20^{\circ}\text{C}$  for at least 24 h. After thawing the samples, the precipitate was filtered onto pre-combusted (450°C, 6 h) GF/F filters (25 mm diameter) and stored at  $-80^{\circ}\text{C}$ . DNA was measured using a fluorescence-spectrophotometer (excitation 404 nm, emission 502 nm) with a detection limit of 20 ng. DNA standards (Sigma Aldrich D3779) were prepared in seawater and processed as described above. The P content of the DNA was calculated by multiplying the measured DNA concentration by 2.06 nmol  $\text{P}^* \mu\text{g dDNA}^{-1}$  detected by alkaline digestion of DNA-sodium salt from calf thymus (SIGMA-ALDRICH) (Trinkler, 2009).

#### *Uptake rates of DIP, ATP, and DNA*

To measure the DIP uptake rates,  $[^{33}\text{P}]\text{PO}_4$  (specific activity:  $\sim 110 \text{ TBq mmol}^{-1}$ ) was added to 50 ml of water sample at a final concentration of 50 pM. Isotope-labeled samples were incubated under laboratory light conditions and *in situ* temperatures. For the controls, formaldehyde was added at a final concentration of 1% before radiotracer addition. During the incubation period of 2–2.5 h, 5 ml subsamples were harvested every 30 min. and amended with 100  $\mu\text{M}$  non-radioactive  $\text{PO}_4$  to halt the  $[^{33}\text{P}]\text{PO}_4$  incorporation (Thingstad et al., 1993), which was followed by immediate filtration on polycarbonate filters (Millipore, 0.2 and 3  $\mu\text{m}$  pore size) that had been pre-soaked with 1 mM  $\text{KH}_2\text{PO}_4$ .

The  $[^{33}\text{P}]\text{PO}_4$  uptake was measured in a Perkin Elmer liquid scintillation counter using IrgaSafe as scintillation cocktail. The  $[^{33}\text{P}]\text{PO}_4$  uptake rate ( $\% \text{ h}^{-1}$ ) is the consumed fraction from the added  $[^{33}\text{P}]\text{PO}_4$  per time calculated from the linear slope of increasing counts on the filters (Nausch et al., 2012). The gross uptake rate was calculated from the  $[^{33}\text{P}]\text{PO}_4$  uptake rate and the *in situ* DIP concentration. Turnover times ( $T_t$ ) were calculated by the equation  $T_t = t / -\ln(1-r)$ , where  $t$  = incubation time,

$r$  = fraction of added [ $^{33}\text{P}$ ]PO $_4$  consumed after the incubation time (Thingstad et al., 1993).

Filters of 0.2  $\mu\text{m}$  filters and 3  $\mu\text{m}$  pore size were used to discriminate the uptake rates of the total community ( $>0.2 \mu\text{m}$ ) and the size fractions  $>3 \mu\text{m}$ , which mostly contained phytoplankton and particle associated bacteria. The uptake into the size fraction  $<3 \mu\text{m}$ , which contained autotrophic and heterotrophic picoplankton, was calculated by the difference of the radioactivity on the 0.2 and 3  $\mu\text{m}$  filters.

The method for ATP uptake measurements was derived from the protocol of Ammerman (1993) developed for the detection of ATP hydrolysis and the uptake of hydrolysed P. Here only the uptake of hydrolysed ATP were measured by addition of [ $^{33}\text{P}$ ]ATP (specific activity: 111 TBq mmol $^{-1}$ ) to 10 ml samples and to formaldehyde-treated (1%) controls at a final concentration of 50 pM. The samples were incubated in the dark at *in situ* temperature for 1 h. The uptake was halted by addition of 200  $\mu\text{l}$  of a cold 20 mM ATP solution to the samples, which were then processed and calculated as described for the PO $_4$  uptake measurements.

To measure DNA uptake rates, radiolabeled DNA was prepared using the Amersham Nick Translation Kit (GE Healthcare Life Sciences). According to the manufacturer instructions, 3.7 MBq of [ $^{33}\text{P}$ ]dCTP (specific activity  $> 111$  TBq mmol $^{-1}$ ) was paired with 1  $\mu\text{g}$  DNA (Lovdal et al., 2007). The specific activity of the produced [ $^{33}\text{P}$ ]DNA was about  $2 \times 10^7$  cpm  $\mu\text{g}^{-1}$ . About 0.3  $\mu\text{g L}^{-1}$  [ $^{33}\text{P}$ ]DNA (0.6 nM nucleotide P) were added to the samples and handled as described for the [ $^{33}\text{P}$ ]ATP uptake rate measurements.

## Statistical Analysis

Spearman rank correlations, ANOVA and *t*-tests were performed using the online program <http://www.socscistatistics.com/tests/studentttest/Default.aspx>. The *t*-test was applied to check whether the differences between the northern and the southern part of the transect were significant. ANOVA was used to test whether the differences in the turnover times of DIP, dATP-P, and dDNA-P in each part of the transect was significant. The correlation of the DOP compounds with *Chla* or with other parameters was examined using the Spearman rank correlation. The multiple regression analysis, performed with Excel 2013, was used to determine which factors influenced the variations in DOP and in the measured compounds. The significance level of  $p < 0.05$  was used in all applications. Additionally, principal component analysis (PCA), performed with the computer program SPSS statistics 22, was applied to identify the parameter groups influencing the DOP compound distribution patterns.

## RESULTS

### Transect in 2011

#### DOP Concentrations and Compounds

There were no significant differences in the TP, DOP, and DIP concentrations between the southern and the northern stations of the transect through the central Baltic Sea (Tables 2, 3). DIP

concentrations of 0.02 and 0.03  $\mu\text{M}$  were near the detection limit of the analytical method, and  $>88\%$  of the DP pool was DOP. The DOP concentrations varied marginally between the stations (Figure 2A), with a maximum difference of 0.05  $\mu\text{M}$  between the lowest and the highest value. Multiple regression analysis showed that none of the single parameters, e.g., *Chla*, PC concentrations, C:P ratios, temperature or bacteria abundances, had a significant influence on the DOP concentrations (Table 5 and Supplementary Figure 1). Together, the single parameters explained 99% of the variation, and the sum of the parameters PP, DIP, POC, PON, the C:N ratio and the C:P ratio explained approximately 88% of the variation.

The dATP-P, dPL-P, and dDNA-P concentrations were in the nanomolar range (Figure 3 and Table 2) and together constituted only 2.4–5.2% (mean 4.1%,  $n = 14$ ) of DOP. The other  $> 95\%$  of the DOP remained unidentified in our study (Table 2). The dATP-P concentrations varied between 2.6 and 7.4 nM along the whole transect, and there were no significant differences between the southern and the northern parts of the transect (Tables 2, 3). However, the dATP-P concentration range was greater at the northern than at the southern stations, with both lower and higher concentrations (Figure 3A).

Among the three analyzed DOP compounds, dPL-P had the highest concentrations ranging between 2.1 and 10.6 nM along the whole transect (Figure 3B). The concentrations of dDNA-P (0.04–0.07 nM) were two orders of magnitude lower than those of the other compounds (Figure 3C). Both dPL-P and dDNA-P showed slightly higher concentrations at the southern stations than at the northern stations. However, in the *t*-test, the differences between the two parts of the transect were significant only for dDNA-P (Table 3). Although dATP-P and dPL-P were not related to any other of the investigated parameters in the Spearman rank correlation test (Table 4), dDNA-P was inversely related to DIP, PON and the C:P ratio. As observed for DOP, the multiple regression analysis (Table 5 and Supplementary Figure 1) did not indicate that any other single parameter had a significant influence on the variations in the three DOP compounds. However, together they explained up to 91% of the dDNA-P variation, 98% of the dATP-P variation and 78% of the dPL-P variation. The sum of the *Chla*, PP, DIP, PC, PN, C:N ratio and C:P ratio could explain 89% of the dDNA-P variation, 27% of the dATP-P variation and 41% of the dPL-P variation.

#### Phytoplankton and Bacteria

The average *Chla* concentration at the three southern stations,  $3.0 \pm 0.7 \mu\text{g l}^{-1}$  ( $n = 6$ ), was significantly lower than that at the northern stations,  $3.8 \pm 0.3 \mu\text{g l}^{-1}$  ( $n = 8$ ) (Tables 1, 3). The abundances of filamentous cyanobacteria were comparable for both areas, and picoplankton counts were significantly higher in the southern than in the northern area; therefore, these components do not explain the observed difference in *Chla* concentrations (Tables 1, 3). The diazotrophic filamentous cyanobacteria were numerically dominated by *Aphanizomenon* sp. along the whole transect, representing 73–96% of the total counts. *Nodularia spumigena* contributed 2.2–11.0% of the total counts and was abundant in observable numbers only at the

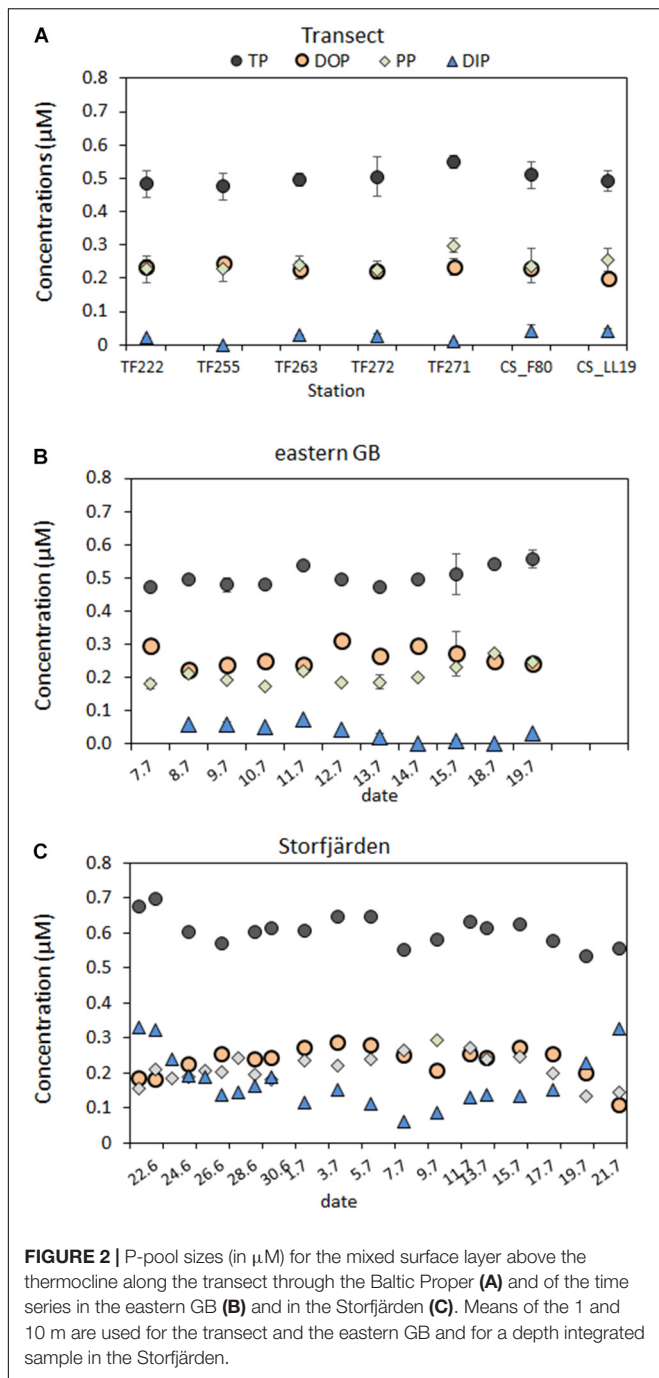
**TABLE 2** | Mean concentrations and standard deviation of P-pool sizes, its contribution to total dissolved phosphorus (DP), and the contribution of single components (dATP-P, dPL-P, dDNA-P) to the DOP pool in three studies conducted in the Baltic Sea (n, number of data).

	Transect 2011		Gotland Basin	Storfjärden
	Southern stations	Northern stations	2012	2012
TP ( $\mu\text{M}$ )	0.48 $\pm$ 0.02 (n = 6)	0.51 $\pm$ 0.03 (n = 8)	0.50 $\pm$ 0.04 (n = 22)	0.62 $\pm$ 0.06 (n = 16)
DP ( $\mu\text{M}$ )	0.25 $\pm$ 0.01 (n = 6)	0.25 $\pm$ 0.02 (n = 8)	0.29 $\pm$ 0.02 (n = 22)	0.40 $\pm$ 0.06 (n = 16)
DOP ( $\mu\text{M}$ )	0.23 $\pm$ 0.01 (n = 6)	0.22 $\pm$ 0.02 (n = 8)	0.26 $\pm$ 0.03 (n = 22)	0.24 $\pm$ 0.03 (n = 16)
DIP ( $\mu\text{M}$ )	0.02 $\pm$ 0.01 (n = 6)	0.03 $\pm$ 0.02 (n = 8)	0.03 $\pm$ 0.03 (n = 22)	0.16 $\pm$ 0.07 (n = 16)
DIP (%)	6.4 $\pm$ 5.4	11.4 $\pm$ 6.5	12.7 $\pm$ 10.6	39.0 $\pm$ 12.2
DOP (%)	93.5 $\pm$ 5.3	88.6 $\pm$ 6.5	83.7 $\pm$ 10.6	61.0 $\pm$ 12.2
dATP-P (nM)	5.0 $\pm$ 0.9 (n = 6)	4.8 $\pm$ 1.6 (n = 8)	4.3 $\pm$ 1.7 (n = 22)	6.4 $\pm$ 3.4 (n = 8)
dPL-P (nM)	6.8 $\pm$ 3.3 (n = 5)	6.7 $\pm$ 2.1 (n = 8)	1.9 $\pm$ 1.9 (n = 20)	2.2 $\pm$ 1.4 (n = 8)
dDNA-P (nM)	0.06 $\pm$ 0.01 (n = 6)	0.05 $\pm$ 0.01 (n = 8)	0.12 $\pm$ 0.04 (n = 22)	0.10 $\pm$ 0.02 (n = 7)
dATP-P (%)	2.1 $\pm$ 0.4	2.2 $\pm$ 0.7	1.7 $\pm$ 0.8	2.2 $\pm$ 1.4
dPL-P (%)	2.9 $\pm$ 1.4	3.0 $\pm$ 1.1	0.7 $\pm$ 0.7	0.9 $\pm$ 0.6
dDNA-P (%)	0.03 $\pm$ 0.01	0.02 $\pm$ 0.01	0.05 $\pm$ 0.02	0.04 $\pm$ 0.01
Unidentified DOP (%)	95.0 $\pm$ 1.4	94.8 $\pm$ 1.2	97.6 $\pm$ 0.8	96.9 $\pm$ 1.1

**TABLE 3** | Results of the *t*-test to validate if the differences between the southern and the northern stations of the transect investigated in summer 2011 were significant at a level  $p < 0.05$ .

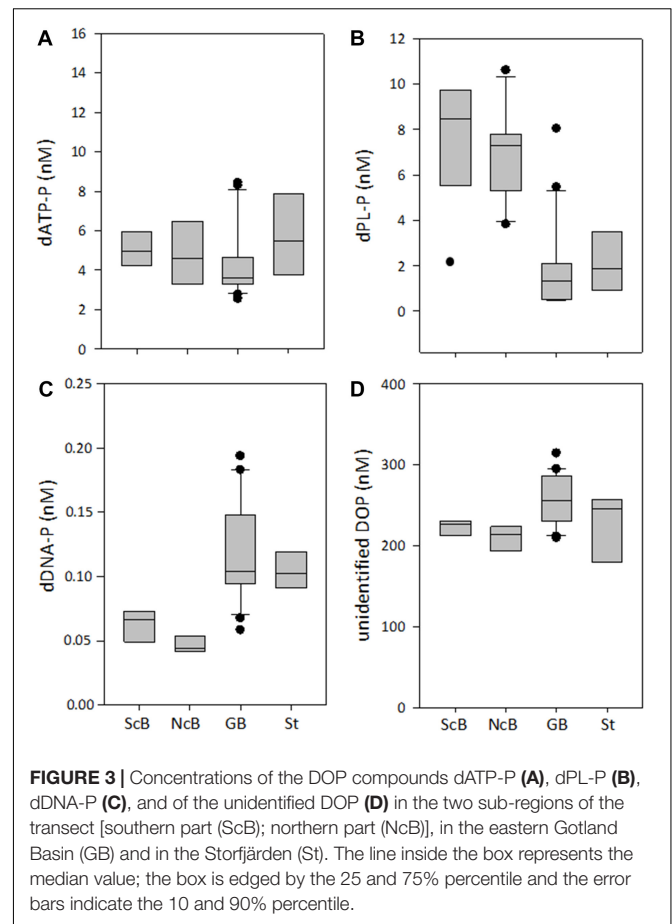
Parameter	t-Value	p	n 1	n 2	
T ( $^{\circ}\text{C}$ )	-3.91	0.001	s	6	8
Salinity	2.07	0.030	s	6	8
Chla	-2.24	0.022	s	6	8
Heterotrophic bacteria ( $\text{cells l}^{-1}$ )	0.05	0.479	n.s.	6	8
Autotrophic picoplankton ( $\text{cells l}^{-1}$ )	2.39	0.017	s	6	8
Filamentous cyanobacteria ( $\text{units l}^{-1}$ )	0.76	0.232	n.s.	6	8
PC ( $\mu\text{M}$ )	-2.47	0.016	s	6	7
PN ( $\mu\text{M}$ )	-3.27	0.004	s	6	8
PP ( $\mu\text{M}$ )	-1.00	0.112	n.s.	6	8
Particulate C:N ratio	-0.53	0.303	n.s.	6	7
Particulate C:P ratio	-2.14	0.027	s	6	8
Particulate N:P ratio	-1.96	0.037	s	6	8
TP ( $\mu\text{M}$ )	-1.31	0.107	n.s.	6	8
DOP ( $\mu\text{M}$ )	1.44	0.087	n.s.	6	8
DIP ( $\mu\text{M}$ )	-1.28	0.089	n.s.	6	8
dATP-P (nM)	0.27	0.395	n.s.	6	8
dPL-P (nM)	0.05	0.481	n.s.	5	8
dDNA-P (nM)	2.27	0.021	s	6	8
DIP uptake ( $\text{nMd}^{-1}$ )	-4.386	0.0002	s	6	8
dATP-P uptake ( $\text{nMd}^{-1}$ )	-2.862	0.008	s	6	8
dDNA-P uptake ( $\text{nMd}^{-1}$ )	-2.49	0.015	s	6	8
DIP turnover time (h)	12.88	<0.0001	s	6	7
dATP-P turnover time (h)	3.81	0.001	s	6	7
dDNA-P turnover time (h)	5.11	0.0002	s	6	7

*n1* and *n2* are the number of values for the southern and the northern stations, respectively (s., significant; n.s., not significant).



northernmost station CS\_LL19. Apart from that, the abundances of *N. spumigena* did not differ between the southern and northern parts of the transect (Table 1).

The Chla concentrations correlated positively with PC and PN along the transect but not with PP (Table 4). The average PC concentrations were  $33.6 \pm 7.3 \mu\text{M}$  in the southern and  $44.0 \pm 7.8 \mu\text{M}$  in the northern part of the transect. PN concentrations of  $5.3 \pm 0.7$  and  $6.8 \pm 0.9 \mu\text{M}$  were observed in the southern and the northern parts, respectively (Table 1). The PC and PN concentrations correlated with each other (Table 4),



**TABLE 4** | Relationships between DOP, dATP-P and Chla and other parameters that were significant in the Spearman Rank correlation test.

		Parameter	R	p	n
Transect	Chla ( $\mu\text{g l}^{-1}$ )	T ( $^{\circ}\text{C}$ )	0.703	0.007	13
	Chla ( $\mu\text{g l}^{-1}$ )	PC ( $\mu\text{M}$ )	0.637	0.019	13
	Chla ( $\mu\text{g l}^{-1}$ )	PN ( $\mu\text{M}$ )	0.674	0.008	14
	Chla ( $\mu\text{g l}^{-1}$ )	C:P ratio	0.632	0.02	13
	PC ( $\mu\text{M}$ )	PN ( $\mu\text{M}$ )	0.769	0.002	13
Eastern GB	DOP ( $\mu\text{M}$ )	T ( $^{\circ}\text{C}$ )	0.470	0.02	22
	DOP ( $\mu\text{M}$ )	DIP ( $\mu\text{M}$ )	-0.900	0.001	22
	dATP-P (nM)	dDNA-P (nM)	0.486	0.01	22
Storfjärden	DOP ( $\mu\text{M}$ )	DIP ( $\mu\text{M}$ )	-0.522	0.04	16
	Chla ( $\mu\text{g l}^{-1}$ )	T ( $^{\circ}\text{C}$ )	0.824	<0.001	16
	Chla ( $\mu\text{g l}^{-1}$ )	Salinity	0.631	<0.009	16
	Chla ( $\mu\text{g l}^{-1}$ )	DIP ( $\mu\text{M}$ )	-0.770	<0.001	16
	Chla ( $\mu\text{g l}^{-1}$ )	PP ( $\mu\text{M}$ )	0.676	<0.006	16

resulting in similar C:N ratios of  $6.3 \pm 0.7$  and  $6.6 \pm 1.2$  in both parts of the transect (Table 1). The average C:P ratios of the northern stations were significantly higher than those of the southern stations ( $178 \pm 26$  and  $145 \pm 29$ , respectively) and reached values  $> 200$  at three stations in the 1 m but not in 10 m depth.



**TABLE 5** | Coefficients of determination (CoD) to describe the impact of parameter groups on concentrations of DOP, dATP-P, dPL-P, and dDNA-P.

	Parameter groups	DOP ( $\mu\text{M}$ )	dATP-P (nM)	dPL-P (nM)	dDNA-P
Transect	(DOP), T ( $^{\circ}\text{C}$ ), salinity, Chla	0.991	0.981	0.792	0.916
	Heterotrophic bacteria, autotrophic picoplankton	( $n = 14$ )	( $n = 14$ )	( $n = 14$ )	( $n = 14$ )
	PP, DIP, PC, PN, C:N ratio, C:P ratio				
	<i>Dolichospermum</i> sp.	0.134	0.234	0.178	0.309
	<i>Aphanizomenon</i> sp.	( $n = 13$ )	( $n = 13$ )	( $n = 13$ )	( $n = 13$ )
Eastern GB	<i>Nodularia spumigena</i>				
	(DOP), T ( $^{\circ}\text{C}$ ), salinity, Chla	0.879*	0.815	0.795	0.780
	PP, DIP*, PC, PN, C:N ratio, C:P ratio	( $n = 16$ )	( $n = 16$ )	( $n = 16$ )	( $n = 16$ )
	<i>Dolichospermum</i> sp.*	0.195	0.377	0.058	0.932*
	<i>Aphanizomenon</i> sp.*	( $n = 8$ )	( $n = 8$ )	( $n = 8$ )	( $n = 8$ )
Storfjärden	<i>Nodularia spumigena</i> *				
	(DOP), T ( $^{\circ}\text{C}$ ), salinity, Chla	0.929	1	1	1
	PP, DIP, PC, PN, C:N ratio, C:P ratio	( $n = 14$ )	( $n = 7$ )	( $n = 7$ )	( $n = 6$ )
	Heterotrophic bacteria Autotrophic picoplankton				
	PC, PN, C:N ratio, C:P ratio	n.d	0.611	0.513	0.866
			( $n = 7$ )	( $n = 7$ )	( $n = 7$ )
	T ( $^{\circ}\text{C}$ ), salinity, Chla, PP, DIP	n.d	0.997	0.46	0.69
			( $n = 7$ )	( $n = 8$ )	( $n = 7$ )
	T ( $^{\circ}\text{C}$ ), salinity, Chla, PP, DIP	n.d	0.997	0.460	0.690
			( $n = 7$ )	( $n = 7$ )	( $n = 7$ )
Heterotrophic bacteria Autotrophic picoplankton	n.d	0.408	0.038	0.622	
		( $n = 7$ )	( $n = 7$ )	( $n = 6$ )	
<i>Dolichospermum</i> sp.*	0.13	0.999*	0.647	1*	
<i>Aphanizomenon</i> sp.*	( $n = 10$ )	( $n = 5$ )	( $n = 5$ )	( $n = 4$ )	
<i>Pseudanabaena</i> sp.*					

The higher the value the stronger the impact. It has been calculated in the multiple regression analysis for DOP, and for each component. DOP as an influencing factor was included only for the calculation of the compounds. The calculations were performed with subgroups of factor when the CoD = 1 by including all parameters. Significant relationships of a single parameter with DOP or a compound are marked with \*.

### Uptake Rates of DIP, dATP-P, and dDNA-P

The turnover times of DIP ( $T_{tDIP}$ ), dATP-P ( $T_{tdATP-P}$ ), and dDNA-P ( $T_{tdDNA-P}$ ) were the longest at the southern stations of the transect (Figures 5A–C and Table 6). At these stations, the  $T_{tDIP}$  and  $T_{tdATP-P}$  were comparable (17 and 15 h, respectively), while the  $T_{tdDNA-P}$  was as high as 107 h. At the northern stations, the turnover times differed from each other (DIP < dATP-P < dDNA-P) (ANOVA:  $F = 51.14$ ;  $p < 0.0001$ ), and the turnover times of dDNA-P (average 22 h) were considerably shorter than those at the southern stations (Table 3).

The uptake rates show that DIP was always the preferred P source, followed by dATP-P (Figures 5F–H and Table 6). The uptake rates differed between the two parts of the transect by a factor of 8 for DIP and by a factor of approximately 3 and 2 for dATP-P and dDNA-P, respectively; the differences were significant in the  $t$ -test for DIP, but not for dATP-P and dDNA-P (Table 3). The P uptake from DIP, dATP-P, and dDNA-P resulted in a total P uptake of  $37.3 \pm 14.4 \text{ nM d}^{-1}$  in the southern part and  $248.5 \pm 118.2 \text{ nM d}^{-1}$  which is about 30 and 11%, respectively, more than from DIP alone. The contribution of dDNA-P was generally small (maximum  $0.38 \text{ nM d}^{-1}$ ).

More than two-thirds of the DIP and each DOP compound were taken up by the size-fraction <  $3 \mu\text{m}$  (Table 6).

Multiple regression analysis including subgroup analysis (Table 7) revealed that the sum of the temperature, DOP, DIP,

Chla, PC, PN, PP, C:N ratio, and C:P ratio could explain 95.4–97.7% of the variation. Among these parameters, the temperature and DIP concentrations had the strongest effects on the dATP-P and dDNA-P-uptake rates (Supplementary Figure 2). The effects of the autotrophic picoplankton and heterotrophic bacteria seemed to be of minor importance.

### Time Series Measurements in 2012 DOP Concentration and Compounds

Dissolved organic phosphorus concentrations of  $0.26 \pm 0.03 \mu\text{M}$  in the Eastern Gotland Basin were relatively consistent during the investigation period (Figure 2B) and contributed to ~84% of the DP (Table 2). The DIP concentrations were  $0.06$ – $0.08 \mu\text{M}$  during the first 6 days and  $0.02$ – $0.04 \mu\text{M}$  after day 6. The average DIP concentration for the whole period was  $0.04 \pm 0.03 \mu\text{M}$  (Figure 2B). DOP was positively correlated with temperature and inversely correlated with DIP (Table 4). In the multiple regression analysis, 85% of the DOP variation could be explained by the sum of Chla, DIP, PP, PC, PN, and the C:N and C:P ratios and ~19% could be explained by the filamentous cyanobacteria (Table 5). The highest  $t$ -value was obtained for DIP, indicating that DIP might have the strongest effect.

Together, the three DOP compounds (dATP-P, dPL-P, and dDNA-P) constituted just  $2.4 \pm 0.9\%$  of the total DOP pool (Table 2). The dATP-P concentrations ranged between 2.5 and

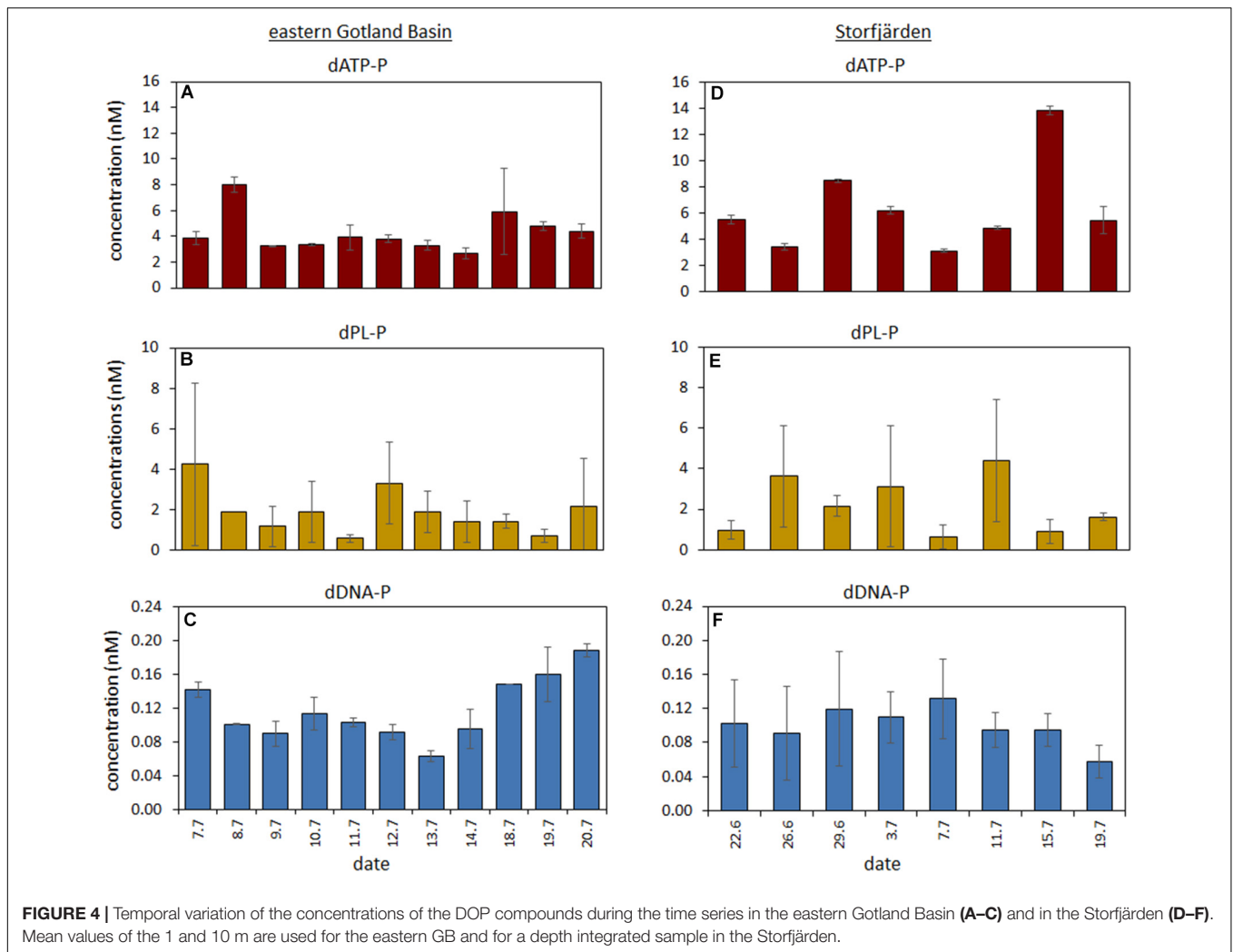
**TABLE 6 |** Mean values and standard deviation of turnover times, uptake rates of DIP, dATP-P, and dDNA-P and its distribution into size fractions.

	Transect		Storfjärden
	Southern stations	Northern stations	
		<b>DIP uptake</b>	
Turnover times (h)	16.9 ± 3.0 (n = 6)	2.4 ± 0.4 (n = 7)	118.3 ± 79.1 (n = 15)
Total uptake (nM d <sup>-1</sup> )	28.8 ± 14.8 (n = 6)	229.5 ± 97.5 (n = 7)	40.7 ± 19.7 (n = 15)
Portion < 3 μm (%)	78.9 ± 0.5	76.4 ± 8.3	71.5 ± 18.2
Portion > 3 μm (%)	21.1 ± 0.5	23.6 ± 8.2	28.5 ± 18.2
		<b>ATP uptake</b>	
Turnover times (h)	15.2 ± 3.5 (n = 6)	6.8 ± 4.3 (n = 7)	20.9 ± 15.5 (n = 12)
Total uptake (nM d <sup>-1</sup> )	8.4 ± 1.7 (n = 6)	24.1 ± 24.3 (n = 7)	6.9 ± 2.6 (n = 6)
Portion < 3 μm (%)	81.6 ± 3.3	76.4 ± 3.8	93.1 ± 4.4
Portion > 3 μm (%)	18.4 ± 3.3	23.6 ± 3.8	6.9 ± 4.4
		<b>DNA uptake</b>	
Turnover times (h)	107.4 ± 44.3 (n = 6)	21.9 ± 4.9 (n = 7)	n.d.
Total uptake (nM d <sup>-1</sup> )	0.09 ± 0.02 (n = 6)	0.19 ± 0.10 (n = 7)	n.d.
Portion < 3 μm (%)	77.1 ± 6.2	69.7 ± 8.7	n.d.
Portion > 3 μm (%)	22.9 ± 6.2	30.3 ± 8.7	n.d.

**TABLE 7 |** Coefficients of determination (CoD) to describe the impact of parameter groups on uptake rates of DIP, dATP-P, and dDNA-P.

	Factors included in the calculation	DIP uptake	dATP-P uptake	dDNA-P uptake
Transect	DOP, T (°C)*, salinity, Chla	0.955	0.954	0.977*
	PP, DIP, PC, PN, C:N ratio, C:P ratio	(n = 13)	(n = 13)	(n = 13)
	Heterotrophic bacteria	0.183	0.066	0.033
	Autotrophic picoplankton	(n = 13)	(n = 13)	(n = 13)
	<i>Dolichospermum</i> sp.	0.166	0.179	0.108
	<i>Aphanizomenon</i> sp.	(n = 12)	(n = 13)	(n = 12)
	<i>Nodularia spumigena</i>			
Storfjärden	DOP, T (°C), salinity	0.264 (n = 15)	0.881 (n = 5)	n.d.
	Chla*, PP*, DIP*	0.335 (n = 15)	0.999* (n = 5)	n.d.
	PC, PN, C:N ratio, C:P ratio	0.401 (n = 15)	1 (n = 5)	n.d.
	PC, PN	n.d.	0.821 (n = 5)	n.d.
	C:N ratio, C:P ratio	n.d.	0.825 (n = 5)	n.d.
	Heterotrophic bacteria	0.293	0.927*	n.d.
	Autotrophic picoplankton*	(n = 15)	(n = 5)	
	<i>Dolichospermum</i> sp.	0.528		
	<i>Aphanizomenon</i> sp.	(n = 10)	n.d.	n.d.
	<i>Pseudanabaena</i> sp.			

The higher the value the stronger the impact. It has been calculated in the multiple regression analysis for the uptake of DIP, and for each component. The calculations were performed with subgroups of factor when the CoD = 1 by including all parameters. Significant relationships of a single parameter with the uptake rates are indicated by \*.

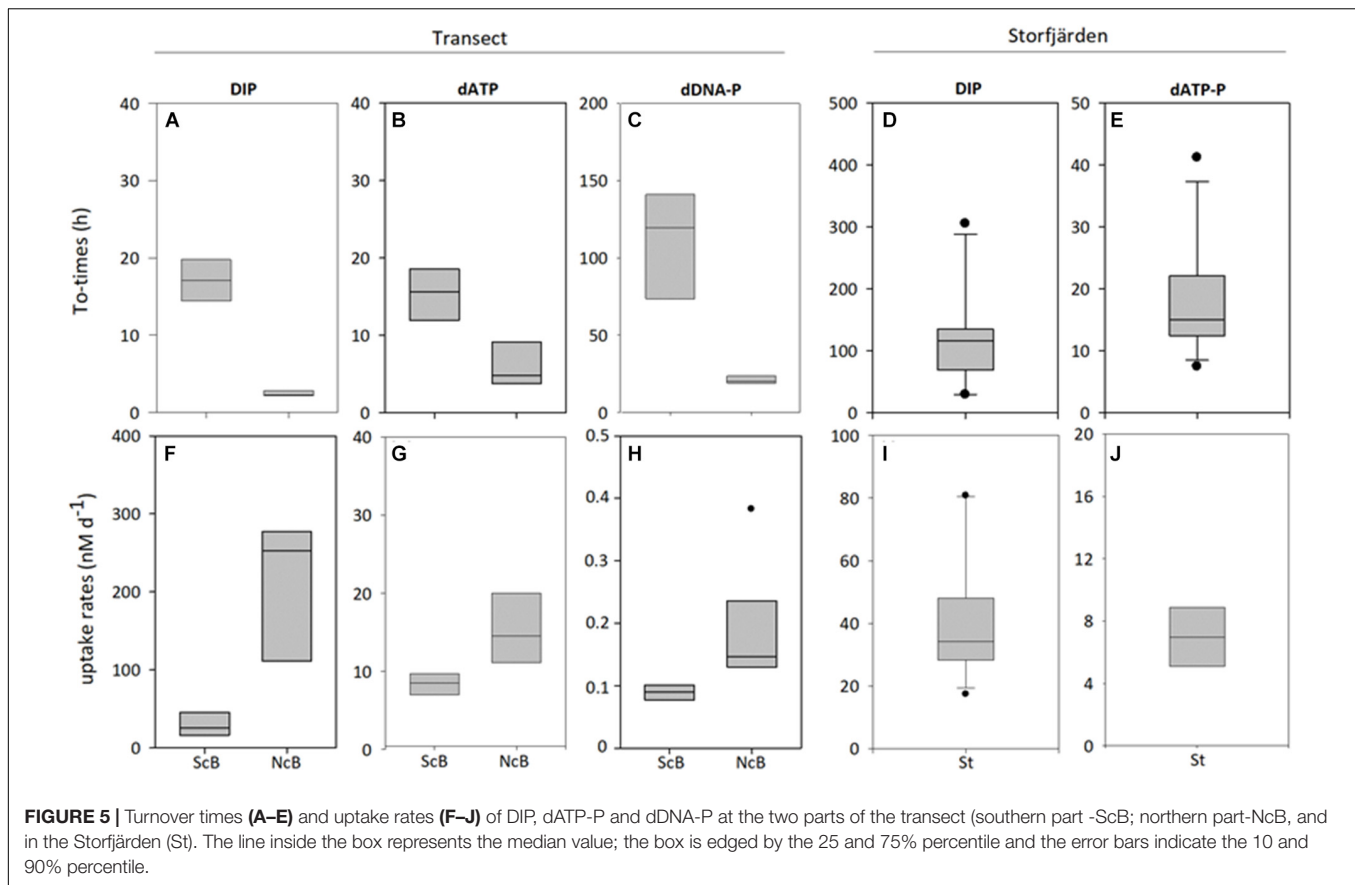


8.4 nM (mean:  $4.3 \pm 1.7$  nM) (Figure 3A and Table 2). The dPL-P concentrations ranged between 0.4 and 8.0 nM (mean  $1.9 \pm 1.9$  nM), but the values of 5.5 and 8.0 nM were outside the 95 percentile and could be outliers (Figure 3B). The average dDNA-P concentrations of  $0.12 \pm 0.04$  nM (Figure 3C) were again the lowest among the three investigated compounds.

The temporal dynamics of the three compounds differed (Figure 4). Nevertheless, the Spearman rank correlation indicated a similarity between dATP-P and dDNA-P concentrations (Table 4), as both compounds had high concentrations at the beginning and at the end of the investigation period. The multiple regression (Table 5 and Supplementary Figure 1) analysis again showed that no single parameter alone could explain the variation in each of the three components. All parameters together could explain 98% of the variation in dATP-P, 79% of the variation in dPL-P and 92% of the variation in dDNA-P, and 78–81% of the variations could be explained by the sum of the hydrographical and biological parameters (Table 5). The variation in the *t*-values (Supplementary Figure 1) was within a small range, so it was difficult to identify a dominating factor.

The averaged TP concentrations ( $0.61 \pm 0.06$   $\mu$ M) sampled in Stor fjärden exceeded those measured in parallel in the eastern GB ( $0.50 \pm 0.04$   $\mu$ M) (Table 2). The average DIP concentration of  $0.16 \pm 0.07$   $\mu$ M accounted for 39% of DP, whereas the average DOP concentration of  $0.24 \pm 0.03$   $\mu$ M accounted for 61% of DP. DOP increased from 0.18  $\mu$ M on day 0 to 0.29  $\mu$ M on day 11 and thereafter decreased to 0.11  $\mu$ M (Figure 2C), thus correlating inversely with DIP (Table 4). Approximately 93% of the DOP variation could be explained by the sum of all 11 parameters, including temperature, salinity, DIP, Chl*a*, particulate matter and its ratios, in the multiple regression analysis and was most strongly influenced by temperature, heterotrophic bacteria and PC (Table 5).

The dATP-P concentration of  $6.4 \pm 3.4$  nM was the highest among the three measured compounds and accounted for  $2.2 \pm 1.4\%$  of DOP (Table 2). The dPL-P and dDNA-P concentrations of  $2.2 \pm 1.4$  and  $0.10 \pm 0.02$  nM, respectively, accounted for  $0.9 \pm 0.6$  and  $0.04 \pm 0.01\%$  of DOP, respectively. No correlation was detected between the DOP compounds and any other parameter. The multiple regression analysis showed strong influences of filamentous cyanobacteria, especially on



variations in dATP-P and dDNA-P (Table 5). However, the variations in the DOP components can also be explained by other parameter combinations, as evident by the sum of temperature, salinity, Chl $a$ , DIP, and PP explaining nearly 100% of the dATP-P variation, 46% of the dPL-P variation and 69% of the dDNA-P variation. Within this parameter set, the  $t$ -values (Supplementary Figure 1) indicated that PP and DIP had the strongest influences on the variation in dATP-P, whereas no single parameter had a greater influence than the others for dPL-P and dDNA-P.

### Phytoplankton

The Chl $a$  concentrations in Storfjärden were comparable to those in the eastern GB (Table 1). However, the phytoplankton composition differed between both stations due to the occurrence of filamentous cyanobacteria. The species *Dolichospermum* sp., *Aphanizomenon* sp. were abundant in both regions of investigation, but their abundances differed. *N. spumigena* occurred only in low abundances in the eastern GB, while *Pseudanabaena* sp. was found only in Storfjärden where it was the predominant filamentous species (Table 1).

The Chl $a$  concentrations in Storfjärden varied synchronously with changes in temperature, salinity and DIP, as indicated by significant Spearman rank correlations (Table 4); the PC and PN concentrations ( $28.5 \pm 5.9$  and  $4.1 \pm 0.8 \mu\text{M}$ , respectively)

did not correlate with Chl $a$ . The C:P ratios ranged from 91 to 128, except for during the first 8 days, when the C:P ratios were between 146 and 190 (Table 1).

Similar PC and PN concentrations and a similar range of C:P ratios were observed in the eastern GB (Table 1). Thus, both stations seemed to have a comparable particulate matter biogeochemistry.

### Uptake Rates of DIP, dATP-P, and dDNA-P

In Storfjärden, the average  $Tt_{DIP}$  was  $118 \pm 79$  h (Figure 5D and Table 6) and decreased to approximately 30 h during 2 days in the middle of the time series when DIP concentrations decreased to  $0.06 \mu\text{M}$  (Figure 2A); the  $Tt_{dATP-P}$  of  $21 \pm 16$  h (Figure 5E) was significantly shorter than that of DIP. Approximately 50% DIP was uptaken by the size fraction  $< 3 \mu\text{m}$ . During the other times, on average, 71.5% DIP was uptaken the size fraction  $< 3 \mu\text{m}$  at a rate of  $40.7 \pm 19.7 \text{ nM d}^{-1}$  (Table 6 and Figure 5I). This size fraction realized  $93.1 \pm 4.4\%$  of the dATP-P uptake (Table 6 and Figure 5J). Both the DIP and dATP-P uptake ( $6.9 \pm 2.6 \text{ nM d}^{-1}$ ) together resulted in a total uptake of 23.7–59.0  $\text{nM d}^{-1}$  P which averaged 17% more than by the use of DIP alone.

In the multiple regression analysis (Table 7) for the uptake rates in Storfjärden, groups of 2–4 parameters had differing impacts on the uptake of DIP and dATP-P. Chl $a$ , PP, and DIP seemed to have a strong influence ( $\sim 100\%$ ) on the uptake of

dATP-P, but the influence of this group on the uptake of DIP was only 33.5%. In addition, other parameter combinations, e.g., DOP, temperature, and salinity (88%) or bacteria and autotrophic picoplankton (99%) could explain the uptake of dATP-P to a high degree (88%).

## DISCUSSION

### Concentrations of DOP and DOP Compounds

The overall mean DOP concentration of 0.23  $\mu\text{M}$  detected in the Baltic Sea has also been reported in other oceanic regions, including coastal waters (Björkman and Karl, 2003; Lomas et al., 2010; Karl and Björkman, 2015), suggesting that DOP is a relatively constant pool in marine ecosystems. However, DOP contains a number of different compounds that are unknown, how significant they are and how they behave. Knowledge of the individual components is necessary to understand the marine DOP pool. This study provides warranted insight into the internal dynamic of the DOP pool in the Baltic Sea.

Our study shows that DOP compounds, namely, dATP, dDNA and dPL, comprised a small share of the total DOP pool in the Baltic Sea. Nevertheless, they made a significant contribution to the nutrition of microorganisms, with differences in the contribution of dATP-P and dDNA-P. The individual components were not obviously regulated by single factors but were rather controlled by the simultaneous action of sets of factors.

The mean dATP-P concentrations of 4.3–6.4 nM in our study are comparable with those reported for the productive Brainsfield Strait (Antarctica) (Nawrocki and Karl, 1989), whereas they are an order of magnitude higher than those reported for the oligotrophic North Pacific (0.2–0.8 nM) (Karl and Björkman, 2015) where higher concentrations in summer than in winter occurred (Björkman and Karl, 2005). Thus, it seems that the dATP-P concentrations increase when the productivity of the water is higher, evident in the seasonal variation as well as in ecosystems of different eutrophication levels. The multiple regression analysis showed that the combination of several parameters could explain the distribution of all three compounds to a high degree in the Baltic Sea. The sum of temperature, DIP, Chla, particulate matter and their ratios and the abundance of heterotrophic bacteria (Table 5) seemed to control the dATP-P concentrations in all three regions. Within this parameter group, Chla, heterotrophic bacteria and the C:N ratio had the strongest effect on the dATP-P concentrations along the transect but not in the eastern GB and Storfjärden. In Storfjärden, DIP and PP were chief among these factors. Here, filamentous cyanobacteria had much stronger effects on dATP-P (Table 5), which contrasted among species. *Dolichospermum* sp. had a positive relation with dATP-P, while *Aphanizomenon* sp. and *Pseudanabaena* sp. had a negative relation with dATP-P. This different relation of the individual cyanobacteria species to dATP-P was also observed for DOP compounds dPL-P and dDNA-P especially in the time series studies (Supplementary Figure 1) where it can be assumed that the plankton community remained relatively unchanged. It

indicates that the single cyanobacteria species obviously behaved differently in its impact on the DOP compounds. We observed a negative relation between cyanobacteria and the C:N ratio along the transect. This could mean that nitrogen fixation (reducing the C:N ratios) and dATP-P concentrations are related to each other. The dATP-P concentration seemed to be higher when the nitrogen fixation was high. So it may be that the release of fixed nitrogen (Gilbert and Bronk, 1994; Mulholland et al., 2004; Wannicke et al., 2009) was accompanied by dATP-P release. The dATP-P release interacted with its utilization by heterotrophic bacteria, as deduced from their negative relationship to dATP-P. However, such relationships were not observed in the eastern GB. We have no explanation for this observation. Under marginal nitrogen fixation at sufficient nitrate concentrations in Storfjärden (Paul et al., 2016), the abundance of the cyanobacteria became more significant as a regulatory factor. Release of dATP-P from growing cyanobacteria seems to be possible as found for other organisms. So, diatoms that form blooms in spring, have been found to release dATP-P (Riemann (1979). In culture experiments, Mempin et al. (2013) found that heterotrophic bacteria were able to release dATP during growth. Alternatively, Nawrocki and Karl (1989) suggested grazing by micro and macrozooplankton is the dominant dATP source in Antarctic waters. Viral lysis is general accepted to be a source for dissolved organic matter (DOM) (Tuomi and Kuuppo, 1999; Middelboe and Jørgensen, 2006; Anderson et al., 2012) including DOP. To assess the significance of viral lysis on dATP-release we made the assumption that virus induced mortality is 12–18% (Weinbauer et al., 2003) and that the death cells release their total ATP content of  $0.5\text{--}6.9 \times 10^{-9} \mu\text{g cell}^{-1}$  ( $3\text{--}40 \text{amol P cell}^{-1}$ ) (Hamilton and Holm-Hansen, 1967). Thus bacteria abundances of  $4.2 \times 10^9 \text{ cells L}^{-1}$  estimated in the central Baltic Sea (Table 2) could release about 2–25 nM P. It becomes evident that viral lysis can significantly contribute to the release of dATP-P in the Baltic Sea although this value is overestimated, since it is unlikely that the entire ATP is released simultaneously and parts of the released dATP can be consumed immediately as found for DOM (Anderson et al., 2012).

The concentrations of dPL-P (1.7–6.8 nM) were similar to those observed in other semi-enclosed systems, such as in Tokyo Bay (Suzumura and Ingall, 2004). Within this concentration range, differences were observed between studies performed along the transect in 2011 and in the eastern GB in 2012, despite similar hydrographical and biogeochemical conditions. Both investigation sites were located in the central Baltic Sea and had similar low DIP concentrations (0–0.06  $\mu\text{M}$  along the transect, 0–0.08  $\mu\text{M}$  in the eastern GB); therefore, it can be assumed that DOP is used as a P source by phyto- and bacterio-plankton. The only significant difference between the two studies was the abundance of filamentous cyanobacteria (Table 1). The negative relationship between dPL-P and the species of filamentous cyanobacteria in the multiple regression (Table 5) and in the PCA analysis (Supplementary Figure 3) indicated that the cyanobacteria were possibly consumers of dPL-P in our study. Filamentous cyanobacteria are known to express a high amount of alkaline phosphatase activity under DIP-depleted (<0.05  $\mu\text{M}$ ) conditions (Endres et al., 2013; Unger et al., 2013)

and can constitute the major fraction (42%) of the total activity in summer when filamentous cyanobacteria occurred in high abundances while the contribution of bacterial APA is rather low (3% in the size fraction  $<0.8 \mu\text{m}$ ) (Nausch et al., 2004). In a certain proportion of phospholipids, P is coupled via ester linkage to the lipid part of the molecule. This portion can be degraded via phosphodiesterase and phosphatase (Liu et al., 1998) and is therefore bioavailable. Therefore, it is conceivable that the higher abundance of cyanobacteria was related to the lower dPL-P concentration in the eastern GB in 2012. The eastern GB and Storfjärden investigated in the same year, had similar dPL-P concentrations (Figures 3, 4) despite their differences in DIP concentrations. This indicates that DIP availability did not influence the level of dPL-P concentrations. It can be deduced from the multiple regression analysis that the combination of hydrographic and stock parameters obviously had a stronger effect on dPL-P than the filamentous cyanobacteria. In the PCA analysis, no factor was shown to have a significant influence on dPL-P concentrations (Supplementary Figure 4). Goutx et al. (2009) assumed eukaryotic picophytoplankton and zooplankton were sources of dPL in the Mediterranean Sea. In Storfjärden, we could not find any relationship between eukaryotic nano-phytoplankton and the dPL-P concentrations (data not shown). Zooplankton were not considered in our study.

Using the method of Karl and Bailiff (1989) for determination the Baltic Sea dDNA-P concentrations of 0.05–0.12 nM were extremely low compared to other oceanic regions, e.g., Karl and Bailiff (1989), and Weinbauer et al. (1995). To better compare our results with previous results, we applied the factor 2.06 nmol P per  $\mu\text{g}$  DNA (Trinkler, 2009) to convert the data reported by these authors into [nM] dDNA-P. The dDNA-P concentrations were then 2.1–43.4 nM in the coastal regions of Hawaii (Karl and Bailiff, 1989), 4–31 nM in Tampa Bay (Gulf of Mexico), approximately 2.5 nM in the surface layer of the HOT station, and 21–26 nM in the Northern Adriatic Sea (Weinbauer et al., 1995). Thus, marine eutrophic waters seemingly have higher dDNA-P concentrations than oligotrophic regions. Furthermore, phytoplankton seems the main regulator of seasonal and diel variations (Paul et al., 1988). In contrast to the dPL-P concentrations, higher dDNA-P concentrations occurred in the Storfjärden and the eastern GB than in the transect (Table 2) indicating that probably the cyanobacteria had a higher release than uptake of dDNA-P inversely to dPL-P. This interpretation is not supported by earlier observations in experiments with *N. spumigena*, namely, that no release of dDNA-P after uptake occurred in contrast to dATP-P and dPL-P (Unger et al., 2013). Note however that in our field experiments, *N. spumigena* occurred only in marginal abundances and *Aphanizomenon* sp., was the dominant species. It cannot be excluded that *Aphanizomenon* sp. uses different DOP compounds than *N. spumigena*.

Our results confirm that the regulation of DOP constituents is very complex as they are constantly subject to uptake and release. The concentrations seem to be predominantly affected by the abundance or nitrogen fixing activity of filamentous cyanobacteria. Single species can have contrasting effects that can

interact with heterotrophic bacteria as evident for dATP-P under DIP-depleted conditions.

## Uptake and Turnover Times of DIP and DOP Compounds

The mean DIP uptake rates of 28.8–229.5  $\text{nM d}^{-1}$  measured in the Baltic Sea in this study are comparable to those reported for the Atlantic Ocean (Casey et al., 2009; Lomas et al., 2010; Sohm and Capone, 2010), but with higher minimum values. Calculated from depth-integrated data, DIP uptake rates of 1.4–8.2  $\text{nM d}^{-1}$  in the oligotrophic subtropical North and Southeastern Pacific (Björkman et al., 2012; Duhamel et al., 2017) were lower than those in the Baltic Sea. The mean dATP-P uptake rates in the Baltic Sea (6.9–24.11  $\text{nM d}^{-1}$ ) exceeded those estimated in the Pacific and Atlantic Ocean (Casey et al., 2009; Lomas et al., 2010; Björkman et al., 2018) by a factor of 10–220, probably due to the higher plankton production in the Baltic Sea. Nausch et al. (2004) reported that >90% of the hydrolysed P were immediately taken up in summer while only about 20% were used in spring (Nausch et al., 2004). Thus, the importance of dATP-P for the nutrition of phyto- and bacterio-plankton in summer might be high in the Baltic Sea, while the P-uptake from dDNA might be relatively low (about 100 times lower than that of dATP-P). However, for the DNA uptake in the open ocean there is little information available. Phyto- and bacterio-plankton develop probably a higher affinity to all three forms (dATP-P, dDNA-P, and DIP) simultaneously when DIP is depleted (Lovdal et al., 2007); it can explain why the uptake rates of dATP-P and dDNA-P along the transect in our study showed the same trend as the uptake of DIP. Hence, the organisms seemingly developed a strategy to acquire P from all or from many of the DOP compounds; the efficiency of P delivery varies depending on the bioavailability. According to the multiple regression analysis (Table 7), all three uptake rates along the transect were regulated by a combination of hydrographical and biological factors. Among these factors, the temperature and DIP concentration had the strongest effects (Table 7). Temperature and DIP can directly affect the uptake rates, while the influence of other factors (e.g., C:P ratios) is indirectly via the P deficiency of organisms. Lovdal et al. (2007) estimated that, under P deficiency, the 0.2–1  $\mu\text{m}$  size fraction is the first to develop a higher affinity for DIP, dATP-P, and dDNA-P. Subsequently, as the P limitation increases, the dATP-P and dDNA-P affinity of the larger size fraction increases too.

The use of dATP-P as a P source persists, even under DIP-replete conditions in Storfjärden. However, within the DIP range of 0.06–0.32  $\mu\text{M}$ , the uptake of dATP-P decreased with increasing DIP concentrations (negative *t*-values in Supplementary Figure 2) indicating the lower importance of the dATP-P for the plankton nutrition when more DIP is available. PP and Chla were also strongly related with the dATP uptake, negatively with Chla and positively with PP and with autotrophic picoplankton (Table 7). It could be interpreted that more dATP-P can be released at increasing cellular P and that filamentous cyanobacteria which might account for a high Chla proportion also uses organic P in parallel with DIP

under DIP depleted conditions. The simultaneous positive and negative relation of the individual parameters to the dATP-P uptake shows the complexity of the regulation of a single DOP component. The size fractionation of the uptake rates of dATP-P and dDNA-P (Table 6) along the transect and the Storfjärden (Table 6) reflects the findings by Lovdal et al. (2007). The proportion of dATP-P and dDNA-P taken up by the <3 and >3  $\mu\text{m}$  size fractions shifted to the >3  $\mu\text{m}$  size fraction under P-limitation. This trend was not very strong. Further studies should divide phytoplankton into several other size classes to elucidate differences.

Turnover times of DIP ( $T_{DIP}$ ) are often used as indicators of the P deficiency or the P demand of organisms (Sohm and Capone, 2010), especially when DIP concentrations are near the detection limit (Moutin et al., 2008). In our study, we observed a relationship between the  $T_{DIP}$  and DIP concentrations in Storfjärden, where a DIP gradient occurred. No relationship was found along the transect, where all DIP concentrations ranged between 0.01 and 0.06  $\mu\text{M}$ . These observations are comparable with findings of Tanaka et al. (2006), who found a linear relationship only for DIP concentrations > 0.1  $\mu\text{M}$ . In previous studies performed in the Baltic Sea the  $T_{DIP}$  shortened from 179 to 1 h when the DIP concentrations declined from 0.1 to 0.01  $\mu\text{M}$  (Nausch et al., 2004). It seems that DIP concentrations have a high impact on turnover times which is visible when a gradient in concentrations (Supplementary Figure 6 and Figure 2C) occurs. At low DIP concentrations (Figure 2A) increases in biomass (Sohm and Capone, 2010) or the induction of a higher P demand, e.g., by a nitrogen and carbon surplus (Tanaka et al., 2006; Lovdal et al., 2007), could induce shortening of the DIP turnover times. Along the transect, small but significant increases in the temperature, biomass parameters (Chl*a*, PC, PN) and particulate C:P ratios (Table 3) were monitored; these factors could be the driving factors underlying shorter  $T_{DIP}$  in the northern stations compared to the southern stations and an indication for a strengthening of P limitation. Van Den Broeck et al. (2004) reported that “long DIP turnover times are a result of enhanced concentrations and slow assimilation rates, while short turnover times are due to low DIP concentrations and more rapid fluxes.” We can now specify that turnover times are related to the ratio between concentrations of DIP and DOP compounds and respective uptake rates, and vice versa, the uptake rates are related to the ratio between turnover times and concentrations (Supplementary Figure 5). These findings can also be applied to the uptake of dATP-P and dDNA-P.

In the Baltic Sea, the dATP-P and dDNA-P pools showed longer turnover times when concentrations were higher, as is the case in other regions of the ocean (Nawrocki and Karl, 1989; Björkman and Karl, 2005; Björkman et al., 2012) and longer turnover times were associated with lower uptake rates (Table 6). That is not the case with DIP when the Storfjärden and the transect were compared. The mean DIP uptake rate in Storfjärden was lower than that in the northern part congruent with the longer turnover times. However, the DIP uptake in the Storfjärden exceeded the uptake in the southern part despite sevenfold longer turnover times (Table 6). So it seems that the long DIP turnover in the Storfjärden were compensated by the

DIP concentrations to a certain degree resulting in higher uptake rates than expected from the turnover times.

One question that must be answered is whether differences in substrate concentrations caused the differences in the turnover times of DIP, dATP-P, and dDNA-P in our study. The shorter  $T_{dATP-P}$  than  $T_{DIP}$  in Storfjärden seems to be due to the relatively low dATP-concentrations in this area. A similar finding was reported for the Southeast subtropical Pacific Ocean (Duhamel et al., 2017). However, this relationship between substrates and turnover times was not observed along the transect. Possibly, the affinity of organisms to DIP (Lovdal et al., 2007) increased more than the affinity of organisms to dATP-P under P-depleted conditions such as those observed.

The relationship between turnover times and P concentrations cannot be applied when dDNA-P and dATP-P are considered. The  $T_{dDNA-P}$  was longer than the  $T_{dATP-P}$  despite much lower DNA concentrations. The reason for this can be found in the complex DNA structure requires a set of different enzymes for degradation and P release. Siuda and Güde (1996) therefore assumed enzymatic hydrolysis is the rate limiting step in DNA conversion. Lovdal et al. (2007), however, considered the diffusive transport of DNA to the cells as the limiting step for the lower intake. Our investigations show that the relation between concentrations and turnover times is valid only for a limiting number of situations; many other factors can influence the turnover times.

The  $T_{DIP}$  values in the Baltic Sea were within the range of those reported in other regions of the ocean, e.g., the North Subtropical Pacific Gyre, Southeast Pacific Gyre (Moutin et al., 2008), and the tropical North Atlantic (Sohm and Capone, 2010).  $T_{dATP-P}$  values of 1–2 days were reported for the HOT-station in the North Pacific Subtropical Gyre (Björkman and Karl, 2005). The Storfjärden characterized by upwelling of cold water from below the thermocline had similar  $T_{dATP-P}$  as observed in the eastern GB in spring 2001 (Nausch et al., 2004).  $T_{dATP-P}$  of about 1 day or longer do not represent a typical summer situation. In summer, the  $T_{dATP-P}$  can be less than 1 day, which emphasizes the importance of dATP-P for the nutrition of organisms in the Baltic Sea in this season.

## CONCLUSION

Our study shows that the dissolved DOP compounds (dATP-P, dPL-P, dDNA-P) are significant P sources for the nutrition of phyto- and bacterio-plankton, despite their low concentrations. DIP remained the dominant P source in the Baltic Sea under both DIP depleted and replete conditions. The variations in the dATP-P, dPL-P, and dDNA-P concentrations and how they are influenced by different combinations of underlying factors imply that these DOP compounds are regulated independently of each other. The observed filamentous cyanobacteria seemed to possess regulatory functions. However, the DOP functioning could not be clarified completely in this study due to the complexity of interactions. Therefore, further detailed knowledge of the DOP pool, including the fraction that was not identified in our study, is necessary to explain its ecological function in marine ecosystems.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2018.00386/full#supplementary-material>

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