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Chemical and photophysiological impact of terrestrially-derived dissolved organic matter on nitrate uptake in the coastal western Arctic

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Abstract

The Arctic is warming at a rate nearly twice the global average, leading to thawing permafrost, increased coastal erosion, and enhanced delivery of riverine terrestrially-derived dissolved organic matter (tDOM) to coastal waters. This humic-rich tDOM has the ability to attenuate light required for photosynthesis and stimulate heterotrophic growth by supplying a source of labile organic carbon. Due to tDOM's high carbon to nitrogen (C : N) ratio, additional nitrogen is required for microorganisms to utilize this excess carbon for growth, thus exacerbating competition between autotrophs and heterotrophs for limiting nutrients and potentially reducing primary production. The effect of Arctic tDOM additions on nitrate uptake by two microplankton size fractions in the coastal Chukchi Sea was quantified using ^{15}N tracer methods. To assess the biogeochemical vs. spectral impacts of tDOM, the uptake incubations were amended with either tDOM or light attenuating films that mimic light absorption by the tDOM. Nitrate uptake and primary production rates in the larger, predominantly phytoplankton, size fraction generally decreased with increasing tDOM additions. The change in light attenuation alone accounted for a $\sim$ 50% reduction in nitrate uptake. Responses in the smaller size fraction varied seasonally with tDOM additions stimulating uptake in spring and suppressing it in summer. The largest variation in summer nitrate uptake can be explained by the shared effect of biogeochemistry and light attenuation. Therefore, large increases in tDOM delivery currently occurring and predicted to increase in the coastal Arctic, could reduce primary production, broadly impact nitrogen and carbon cycling, and affect higher trophic levels.

Rising Arctic atmospheric temperatures are fueling changes on land that include thawing of permafrost, increased coastal erosion, and rising river discharge (McClelland et al. 2006; Froese et al. 2008; Schuur et al. 2008; Simmonds and Keay 2009). The resulting terrestrial runoff is capable of changing both the biogeochemistry and the light field within coastal waters. Our understanding of the interactions between terrestrially-derived dissolved organic matter (tDOM), nutrients, primary and secondary production, and food webs in the coastal Arctic are still rudimentary largely due to logistical difficulties associated with working in this remote region. The impacts of releasing vast amounts of carbon-rich organic material stored in frozen Arctic soils and peatlands to the ocean is of particular concern for Arctic aquatic ecosystems. As much as 50% of the world's terrestrial organic carbon pool is stored in the northern hemisphere as permafrost (Tarnocai et al. 2009). Uncertainties of the impacts of this material on nearshore and coastal microbial communities are a serious impediment to predicting the impacts of the changing climate on Arctic food webs and biogeochemical cycles (Thingstad et al. 2008).

Currently, primary productivity is expected to increase across the Arctic, especially in coastal regions, due to increased light penetration associated with sea ice loss

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(Arrigo et al. 2008; Pabi et al. 2008; Arrigo and Van Dijken 2015). The expansion of open water areas earlier in the season will coincide, however, with increased riverine discharge of humic-rich tDOM from thawing permafrost, which has the potential to reduce light availability and primary production in coastal regions. Arctic tDOM is often dark in color because of its high humic content and the large fraction of chromophoric DOM (CDOM). Technically, CDOM refers to the light-absorbing fraction of DOM present in water (Blough and Del Vecchio 2002), while humic substances are operationally defined as the fraction of DOM that is extractable from natural waters using specific resins (Aiken 1985). Although light absorption by CDOM in the UV-A and UV-B ranges can limit the amount of damaging radiation reaching microorganisms, CDOM light absorption also ranges into the visible spectrum, decreasing photosynthetically available radiation (PAR) and negatively impacting primary production (Arrigo and Brown 1996; Schindler et al. 1996; Vincent et al. 1998; Blough and Del Vecchio 2002; Thrane et al. 2014; Seekell et al. 2015). There is currently no one accepted method for isolating the CDOM fraction of tDOM, however, humic acids have been shown to correlate with CDOM absorbance and thus isolated tDOM (humic acids) are a meaningful proxy for the CDOM fraction of the DOM pool (Boyle et al. 2009; Osburn and Stedmon 2011; Spencer et al. 2012).

In addition to attenuating light, tDOM is composed of a complex mixture of dissolved organic carbon (DOC), nitrogen (DON), and phosphorus (DOP) compounds, which may be bioavailable, refractory, or even toxic to receiving aquatic microbial communities (Steinberg et al. 2008). Thus, tDOM can impact the carbon budget by reducing photosynthetic carbon fixation and by providing an organic carbon source for heterotrophic growth or respiration. Further, Arctic tDOM generally has a high molar carbon to nitrogen ratio (C : N) of 24–57 (Lara et al. 1998; Lobbes et al. 2000; Amon and Meon 2004). The unbalanced stoichiometric nature of the tDOM, relative to microbial biomass (C : N of 5–7; Fukuda et al. 1998), suggests that exogenous nitrogen will be required for heterotrophic and mixotrophic microorganisms to take full advantage of the labile DOC fraction. In the seasonally nitrogen-limited western Arctic (Baer 2013; Codispoti et al. 2013; Mills et al. 2015), this need for additional nitrogen could increase competition between autotrophs and heterotrophs for available inorganic nitrogen.

This study investigates how increases in Arctic tDOM discharge could impact the competition between autotrophs and heterotrophs for available nitrate (NO_3^-) in the coastal Chukchi Sea. We used standard isotope tracer techniques paired with the addition of light attenuating film or isolated tDOM to (1) determine how additions of humic-rich tDOM impact the rate at which NO_3^- is taken up by two different microbial size fractions, and (2) tease apart the biogeochemical and photophysiological impacts of tDOM on NO_3^- assimilation by primary producers. We found that increases in tDOM discharge from Arctic rivers will likely shift the balance of NO_3^- assimilation toward heterotrophs via a reduction in autotrophic uptake in the coastal Arctic.

Materials and methods

Site descriptions

Coastal seawater samples were collected at 71° 20′ 40″ N, 156° 41′ 25″ W, 2.5 km northwest of Barrow, Alaska, in the Chukchi Sea. Experiments were conducted in spring (23–25 April 2010 and 26 April–2 May 2011), summer (25–28 August 2010 and 15–20 August 2011), and winter (26–30 January 2011 and 16–21 January 2012). During winter and spring, the sample site was covered by landfast ice and the water samples collected below the ice were −1.8°C. During the summer, the site was in open water and water temperature was 6 ± 0°C in 2010 and 5.1 ± 0.7°C in 2011. Each of the sampling efforts (within a single season and year) included two or three trips to the sampling site. For the purposes of this article, the data collected within a single season and year were averaged to provide a more robust statistical assessment. Sample bottles were filled with seawater, sealed, placed in coolers, surrounded by ambient seawater, and transported to the laboratory within 1–2 h of collection to minimize temperature fluctuations. Temperature was monitored at 60-s intervals using a HOBO TidbiT v2 water temperature data logger (Onset Computer Corporation, Bourne, Massachusetts). Temperatures within the experimental bottles were maintained within 0.3°C of the ambient temperature for the duration of the experiment. A full description of site characteristics can be found in Baer (2013).

tDOM sources and preparation

Due to logistical constraints, two different sources of tDOM were used in this study (Nordic and Barrow). Commercially available Nordic Lake reference material (#1R105H), purchased from the International Humic Substance Society (IHSS), was used in spring and summer of 2010 because a local source had not yet been isolated. A single large volume sample of local (Barrow) tDOM was collected and isolated in the summer of 2010 and then used in all subsequent field trips. Both Nordic and Barrow tDOM were used in January and April 2011, to determine if the microbial response was similar. The Barrow tDOM was the sole tDOM source added in the spring and summer of 2011 and winter of 2012.

To prepare the isolated Barrow tDOM, water was collected from a thermokarst that contained permafrost meltwater that was actively flowing from a deep fissure into the mouth of the Meade River, Alaska (70° 54′ 39.132″ N, 156° 7′ 25.878′ W) on 29 August 2010. The salinity was 4, temperature was 7°C, the pH was 6.5, and the water was a dark tea color at the time of collection. A total of 100 L of thermokarst water was collected into acid washed (10% HCl) high-

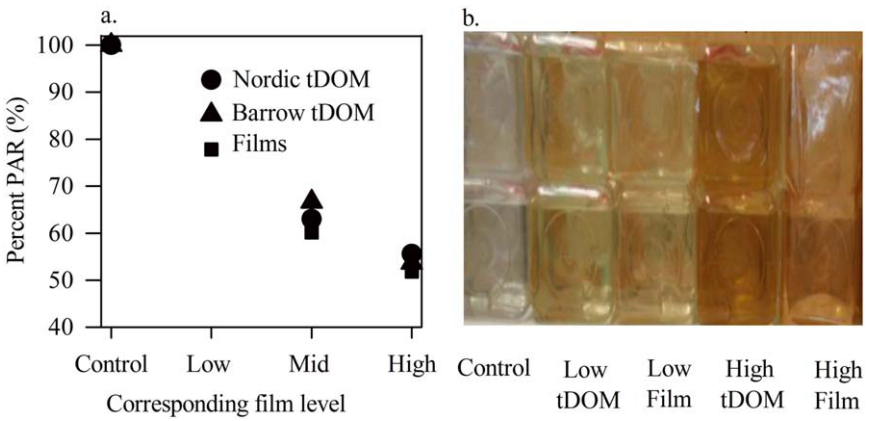

Fig. 1. Comparison of the light attenuating properties of the custom-matched GamColor films, Nordic tDOM, and Barrow tDOM in their ability to (**a**) decrease the ambient PAR associated with low, mid, and high film, Nordic tDOM, and Barrow tDOM, presented as a percent. Low, mid, and high additions were 75 μmol C L^{-1}, 300 μmol C L^{-1}, and 585 μmol C L^{-1} for the Nordic tDOM additions and 95 μmol C L^{-1}, 420 μmol C L^{-1}, and 800 μmol C L^{-1} for the Barrow tDOM additions, respectively; and (**b**) a photograph of the incubation bottles including the no-amendment control, low Barrow tDOM, low film, high Barrow tDOM, and high film treatments.

density polyethylene (HDPE) carboys and transported back to the Barrow Arctic Research Center (BARC) where it was sequentially filtered through 5 μm pre-rinsed polycarbonate filters, combusted (450°C for 4 h) GF/F filters (nominal pore size of 0.7 μm, henceforth referred to as 0.7 μm), and 0.2 μm pre-rinsed Supor® (PALL Corp) filters. Once filtered, the pH of the tundra water was reduced to 2 using a 1% HCl solution. The 0.2 μm filtered and acidified samples were stored at 4°C and shipped to the Virginia Institute of Marine Science in freeze safes to maintain temperature. The total transport time of these samples was approximately 20 h. Temperatures were monitored during transport at 60-s intervals with a HOBO TidbiT v2 water temperature data logger and did not increase more than 0.7°C during transport.

Once back in Virginia, the tDOM was isolated using Supelite™ DAX-8 resin as described in Aiken (Aiken 1985) for Amberlite XAD-8. To determine the optimal isolation procedure for this project, the recovery of the thermokarst DOC using DAX resin was compared to extractions of the same material using PPL solid phase extraction, a commonly used high recovery extraction method (Dittmar et al. 2008). The DAX isolation approach was chosen for this study because the PPL resin retained 48% of the total DOC while the DAX resins retained 61% of the same DOC pool. The DAX recovery was also similar to Arctic river DOC recoveries using tangential flow ultrafiltration (62%) (Benner et al. 2005).

The salinity of both the Nordic tDOM and Barrow tDOM stocks were adjusted to 30 using artificial seawater brine, and filtered through pre-rinsed 0.2 μm Supor® (PALL Corp) filters to make the final tDOM stocks. The artificial seawater brine was made by adding baked (500°C for 4 h) sodium chloride, magnesium sulfate, and sodium bicarbonate to Milli-Q water (DOC < 2 μmol C L^{-1}). This was done to mimic the physical dynamics of humic-rich riverine DOM mixing with marine waters, to ensure that the resulting organic precipitates were removed before being added to experiments, and to prevent large salinity changes within the uptake incubations. Reported DOM recoveries, nutrient concentrations, and elemental ratios are for the final salt-adjusted stocks.

Nitrate and bicarbonate uptake

During each field effort, seawater samples were incubated in 2 L acid-washed polyethylene terephthalate glycol (PETG) bottles for 24 h in temperature controlled chambers at light and temperature levels mimicking in situ conditions (Baer 2013). All treatments were inoculated with tracer level (9% ± 6% of ambient NO_3^- concentrations) ^{15}N-labeled potassium nitrate ($K^{15}NO_3$; 98%). Isotope was purchased from Cambridge Isotope Laboratories, Andover, Massachusetts. Treatments were amended with either (1) spectrally relevant GamColor films (GAM Products, Los Angeles, California), custom-matched to the spectral properties of the isolated tDOM additions, (2) Nordic tDOM, (3) Barrow tDOM, or (4) a no addition control. The film and tDOM additions were made based on their reduction of PAR (Fig. 1a). Low film or tDOM levels reduced PAR by 15% ± 7% and high film or tDOM levels reduced PAR by 46% ± 2%. The Nordic tDOM attenuated more light per μmol C than the Barrow tDOM source. Nordic and Barrow tDOM additions were normalized to the reduction in PAR of the tDOM, compared to the films, and thus have different DOC concentrations. The DOC additions for the low and high Nordic tDOM concentrations were 75 μmol C L^{-1} and 585 μmol C L^{-1}, respectively. The DOC additions for the low and high Barrow tDOM concentrations were 100 μmol C L^{-1} and 810 μmol C L^{-1}, respectively. Bottles designated for the low film treatments were wrapped in one layer of GamColor film 440, and the high film treatments were wrapped in one layer of GamColor film #440 and one layer of #455 (Fig. 1b). An additional mid-

concentration Nordic tDOM treatment was performed only in summer 2010. The DOC addition for the mid-Nordic tDOM treatment was 333 μmol C L^{-1} and the corresponding mid-film treatments were wrapped in four layers of Gam-Color film #440. Film treatments were not conducted in winter (January 2011 or 2012) because samples were incubated in the dark due to the lack of natural ambient light sources.

Due to sampling limitations, the impact of elevated tDOM on primary production was only assessed in summer 2011. Changes in primary production were determined by inoculating treatments containing low or high film and low or high Barrow tDOM with tracer levels (8% of ambient HCO_3^- concentrations) of ^{13}C-labeled bicarbonate ($H^{13}CO_3^-$; 99%) from Cambridge Isotope Laboratories, Andover, Massachusetts.

At the end of each incubation, duplicate subsamples from every treatment bottle were filtered separately to determine uptake by two different size fractions. The ^{15}N or ^{13}C incorporation into the larger size fraction was determined by filtering whole water onto a 5 μm (in spring 2010 only) or 3 μm Sterlitech^{TM} silver filter (Sterlitech Corp). The second whole water subsample was similarly filtered onto a 0.2 μm Sterlitech silver (in spring 2010 only) or a 0.7 μm precombusted (450°C for 2 h) Whatman GF/F filter. Uptake rates in the smaller (0.2–5 μm or 0.7–3 μm) size fraction was estimated by subtracting the rates measured from the larger pore size filters (5 μm or 3 μm) from those measured on the smaller pore size filters (0.2 μm or 0.7 μm). The change in smaller filters was made following spring 2010 because very slow sample flow rates through the 0.2 μm silver filters could allow the sample to warm during filtration and thus artificially change the rates observed. The larger (0.7 μm) filters allowed samples to filter more quickly and thus provide more accurate rates, particularly in higher biomass seasons (i.e., summer). The change from 5 μm to 3 μm was made to maximize separation of phytoplankton from bacteria.

Filters were stored frozen at −20°C until being thawed and dried overnight at 40°C. Once dried, the samples were analyzed on a Europa Geo 20-20 isotope ratio mass spectrometer with an Automated Nitrogen and Carbon Analyzer for Solids and Liquids (ANCA-SL) front end. Absolute nitrate uptake rates were calculated according to methods described in Dugdale and Goering (1967), and absolute bicarbonate uptake rates were calculated following methods described in Hama et al. (1983). Absolute uptake rates are normalized using particulate nitrogen concentrations to remove variance associated with differences in biomass between treatments and seasons. Nitrate uptake rates were not corrected for isotope dilution due to the high ambient nitrate concentrations observed in winter and spring.

Analytical methods

Ambient concentrations of chlorophyll *a* (Chl *a*) were assessed via acetone extraction according to Arar and Collins (1997). Bacterial abundance was measured from whole water samples fixed in the field with paraformaldehyde at final concentration of 0.2% w/v and stored frozen at −80°C until analysis. Samples were stained using SYBR Green (Life Technologies, Grand Island, New York) and analyzed on a FACScalibur flow cytometer (Becton Dickinson, San Jose, California). Reference beads (Spherotech, Fluorescent Yellow Particles, 1.7–2.2 μm) were used as a quality control measure. FlowJo software (Treestar, San Carlos, California) was used to process the data. Concentrations of ammonium (NH_4^+), NO_3^-, nitrite (NO_2^-), phosphate (PO_4^{3-}), silicate (Si), total dissolved N (TDN), DOC, and DON were measured at the start of each set of incubations. Concentration of NH_4^+ was analyzed in triplicate using the colorometric phenol-hypochlorite method (Koroleff 1983). A Lachat QuikChem 8500 autoanalyzer was used to measure concentrations of NO_3^-, NO_2^-, PO_4^{3-}, and Si (Parsons et al. 1984). Concentrations of TDN and DOC were measured in triplicate by high temperature combustion on a Shimadzu TOC-V TNM (Hansell 1993; Sharp et al. 2002; Sharp et al. 2004); analytical accuracy was assessed through the inclusion of deep-sea and low-carbon reference water samples from the University of Miami consensus reference material program (Hansell 2005). Concentrations of DON were determined as the difference between TDN and combined NH_4^+ and NO_x^-; the errors from the TDN, NH_4^+, and NO_x^- measurements were propagated to provide a standard deviation for DON. Spectral comparisons were made using a Perkin Elmer Lambda 25 UV/VIS Spectrometer and reductions in PAR were determined using a Biospherical Instruments QSL-100 PAR meter.

Statistical analyses

Nitrate uptake data were analyzed using one-way analysis of variance (ANOVA). A generalization of the two sample Welch (1951) test for many samples was used to account for the unequal sample sizes and heteroscedasticity between groups. Differences were considered significant at a p-value < 0.05. Pairwise comparisons were run with a Bonferroni correction to control the family wise Type I error rate. Variation partitioning analyses were performed on the spring and summer samples using the varpart function in the vegan: community ecology package (Oksanen et al. 2016). The variation partitioning analysis allowed us to determine what proportion of the variation could be attributed to the individual or combined manipulated variable (i.e., changes in PAR with DOC as a covariable, additions of DOC with PAR as a covariable, or their shared impact). The ANOVA and variation partitioning analyses were performed using the open-source statistical software program R, version 3.3.2 and Rstudio version 1.0.44 for Mac (R Core Team 2016).

A principal component analysis (PCA) of physical (temperature, salinity, PAR, and ice thickness), chemical (NH_4^+,NO_3^-, DON, DOC, PO_4^{3-}, and Si), and biological (Chl *a* and bacterial abundance) variables was also performed for all samples and years (Table 1). The PCA analysis was

Table 1. Seasonal averages of biogeochemical parameters at coastal sites near Barrow, Alaska. A full survey of this system can be found in Baer (2013). Data represent the mean ± one standard deviation ($n = 6$). NA, not assessed; BD, below detection (< 0.03 μmol N L^{-1}).

	Chl *a* (μg L^{-1})	Bacterial abundance (10^8 cells L^{-1})	NH_4^+ (μmol N L^{-1})	NO_3^- (μmol N L^{-1})	DON (μmol N L^{-1})	PO_4^{3-} (μmol P L^{-1})	Si (μmol Si L^{-1})	DOC (μmol C L^{-1})
Winter	0.02 ± 0.01	2.86 ± 0.28	1.57 ± 1.25	8.00 ± 1.51	3.7 ± 0.9	1.32 ± 0.05	24.23 ± 5.93	79 ± 6
Spring	0.55 ± 0.61	2.08 ± 1.41	0.78 ± 0.12	7.68 ± 2.51	4.8 ± 0.5	1.06 ± 0.12	22.36 ± 16.41	79 ± 16
Summer	0.63 ± 0.16	14.19 ± 13.02	0.59 ± 0.30	0.25 ± 0.13	6.6 ± 0.8	0.56 ± 0.05	5.80 ± 0.93	92 ± 6
Nordic stock	NA	NA	BD	BD	396 ± 8	0.05 ± 0.00	NA	16,675 ± 28
Barrow stock	NA	NA	0.80 ± 0.52	BD	600 ± 14	0.08 ± 0.01	NA	15,430 ± 31

accomplished in Primer 7 (Clarke and Gorley 2015). Variables were first normalized then analyzed to identify the variables that may have influenced nitrate uptake rates over the three sampling seasons. The normalization process enables the meaningful comparison of environmental data on different scales with indiscriminate origins. To do this, the values for each variable have their mean subtracted and are divided by their standard deviation. This process makes it possible to derive meaning from the Euclidean distances between samples. A vector plot was generated using the base variable function in Primer 7. The respective vectors indicate the relative magnitude of the coefficients (Eigenvectors) for each variable in principle components (PCs) 1 and 2.

Results

tDOM sources

Two different tDOM sources were compared during this study—commercially available Nordic humic acids (Nordic tDOM) and a local source (Barrow tDOM), which was isolated from a thermokarst near the Meade River, Alaska. The isolation procedure that was used retained a large portion of the total Barrow thermokarst DOC (61%) and DON (56%) pools. The C : N ratio of the Barrow thermokarst DOM and isolated tDOM fraction was 24 and 26, respectively. The Nordic source had a higher C : N ratio of 42. All were within the range (C : N of 24–57) of other Arctic observations (Lara et al. 1998; Lobbes et al. 2000; Amon and Meon 2004).

Site characteristics

Concentrations of Chl *a* and bacterial abundance were highest in summer and lowest, in winter (Table 1). Organic substrate concentrations (i.e., DOC and DON) followed the same trend with the highest concentrations occurring in summer. Inorganic nutrients (i.e., NH_4^+, NO_3^-, PO_4^{3-}, and Si) were highest in winter with decreasing concentrations in spring reaching their lowest concentration in summer (Table 1). The opposing trend between organic and inorganic substrates was expected in Arctic samples as inorganic nutrients stocks accumulate in winter when phytoplankton growth is limited and decrease through summer as phytoplankton (Chl *a*) concentrations increase. For a more detailed description of the seasonal physical and chemical characteristics at these sites, please see our companion study Baer (2013).

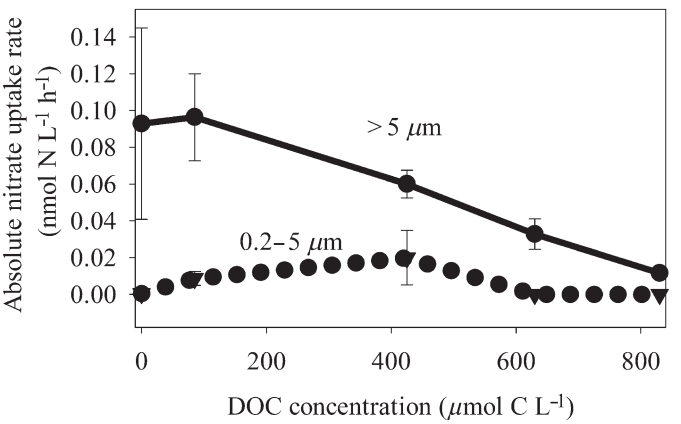

Fig. 2. Changes in absolute nitrate uptake rates with increasing DOC concentrations of Nordic tDOM for April 2010, in the > 5 μm (large phytoplankton; solid line) and 0.2–5 μm (small phytoplankton, bacteria, and archaea; dotted line) size fractions. Rates represent the mean ± one half of the range ($n = 2$).

Size fractions

For all seasons, the large size fraction was composed primarily of larger phytoplankton as opposed to detrital particulate organic matter. The small size fraction was composed of bacteria, archaea, and some small phytoplankton. Approximately 57% of bacterial cells were retained on the 0.7 μm filters (reported in companion study Baer 2013). The presence of small phytoplankton and archaea was confirmed through DNA analysis (described in companion study Connelly et al. 2014).

Nitrate uptake

In spring 2010, the ambient NO_3^- concentration was 5.91 ± 1.25 μmol N L^{-1}, the light level was ~ 5 μmol quanta m^{-2} s^{-1} and the ice thickness was ~ 0.6 m. Nordic tDOM was added at increasing DOC concentrations to Chukchi Sea water amended with tracer additions of $^{15}NO_3^-$. In the large size fraction, the absolute NO_3^- uptake rates decreased with

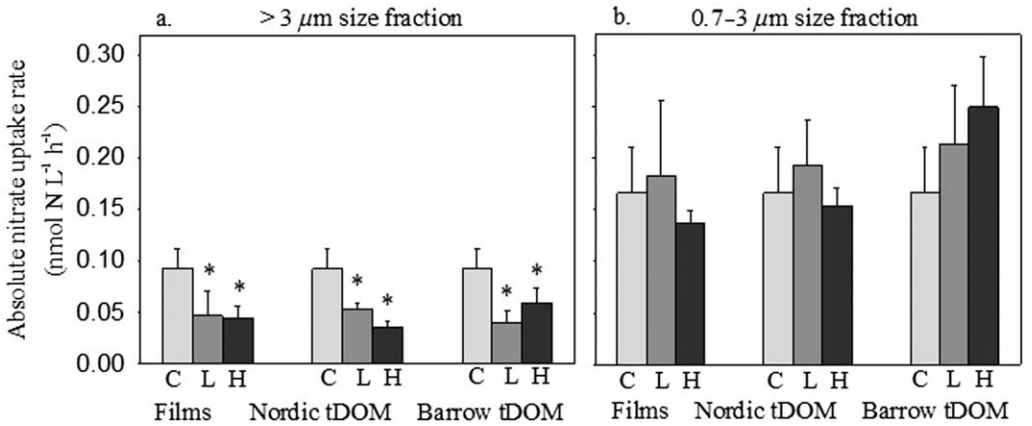

Fig. 3. Absolute nitrate uptake rates for April 2011 in the (**a**) larger size fraction and (**b**) small size fraction in no-amendment controls (C) and with light attenuating films, Nordic tDOM or Barrow tDOM additions at low (L), and high (H) treatment levels. Rates are the mean ± one standard deviation of replicate incubations ($n = 4$) for the > 3 μm and 0.7–3 μm size fractions. Asterisks denote a significant ($p < 0.01$) reduction in absolute nitrate uptake compared to the control.

Table 2. Variation partitioning of absolute nitrate uptake for large and small size fractions in both spring and summer explained by manipulated variables (reduction in PAR, DOC addition, and the their combined impact). Light attenuation (%) is the variation explained by a reduction in PAR with DOC addition as a covariable. DOC addition (%) is the variation explained by DOC addition with reduction in PAR as a covariable. Combined (%) is shared variation explained by both the reduction in PAR and the addition of DOC (attribution of variation cannot be determined due to correlation between the variables). The % of total variance is the proportion of variation between samples that can explained by a reduction in PAR, the addition of DOC, and the shared variation.

Size faction	Season	Light attenuation (%)	DOC addition (%)	Combined (%)	% of total variance
Large	Spring	17.1	0.0	0.0	17.1
Small	Spring	0.0	4.1	0.0	4.1
Large	Summer	6.6	7.6	14.0	28.2
Small	Summer	3.9	2.3	7.6	13.8

increasing tDOM (Fig. 2). In the small size fraction, NO_3^- uptake rates increased in the Nordic tDOM treatments with DOC additions ≤ 425 μmol C L^{-1} but were unchanged in the treatments with DOC additions ≥ 630 μmol C L^{-1}.

Therefore, Nordic tDOM additions at all concentrations suppressed NO_3^- uptake by larger phytoplankton, but enhanced NO_3^- uptake by small cells at lower doses.

An important question from the spring 2010 study was whether light absorption by the CDOM fraction limited light availability for photosynthesis, thus inhibiting NO_3^- uptake by phytoplankton, or alternatively, if the suppression of phytoplankton NO_3^- uptake resulted from a physiological response to the complex chemistry of this tDOM source. To address these questions in the following seasons, light attenuating films were used that mimicked the spectral properties of the tDOM without changing the chemistry within the incubation. The low and high level light attenuating film designations correspond to similar absorbance properties of the low and high tDOM additions, which produced ~ 15% and 46% reductions in PAR for the low and high films, respectively (Fig. 1).

In spring 2011, the ambient NO_3^- concentration was higher (9.46 ± 1.69 μmol N L^{-1}), and the light level were similar (~ 5 μmol quanta m^{-2} s^{-1}) to spring 2010 observations. The ice thickness was ~ 1.4 m. In the large size fraction, the low film, low Nordic, and low Barrow tDOM treatments all had similar, significant ($p < 0.005$) reductions (43–56%) in NO_3^- uptake compared to controls (Fig. 3a). There was little change in NO_3^- uptake rates between the low (49%) and high (52%) film treatments compared to the control, suggesting a threshold for the impact of light attenuation. Similar to spring 2010, Nordic tDOM suppressed NO_3^- uptake in proportion to the amount of tDOM added, resulting in ~ 62% ($p < 0.001$) reduction in NO_3^- uptake rate in the high Nordic tDOM treatments compared to the controls (Fig. 3a). The high Barrow tDOM treatments showed a 36% ($p < 0.05$) reduction in NO_3^- uptake compared to the controls. In the small size fraction, the films and tDOM additions had no statistically significant effect on NO_3^- uptake (Fig. 3b). Also, the spring NO_3^- uptake rates in the control treatments were two-fold higher in the smaller size fraction than in the larger size fraction (Fig. 3); in spring 2010, the reverse was observed. The variation partitioning analysis revealed that 17.1% of the variation associated with nitrate uptake by the large size fraction could be explained by the

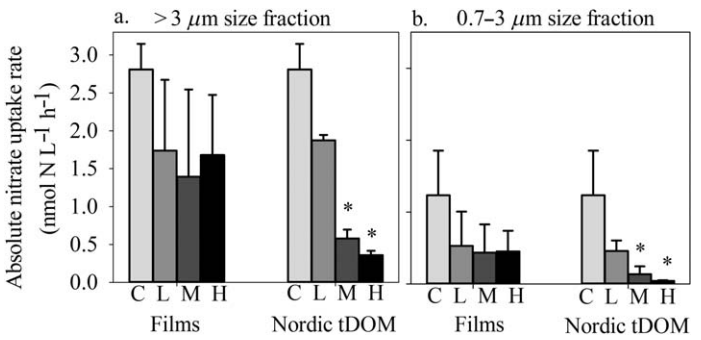

Fig. 4. Absolute nitrate uptake rates for August 2010 in the (**a**) larger size fraction and (**b**) small size fraction in no-amendment controls (C) and with light attenuating films or Nordic tDOM additions at low (L), medium (M), and high (H) treatment levels. Rates shown are the mean ± one standard deviation of replicate incubations ($n = 4$). The asterisks denote a significant ($p < 0.05$) reduction in nitrate uptake compared to the control.

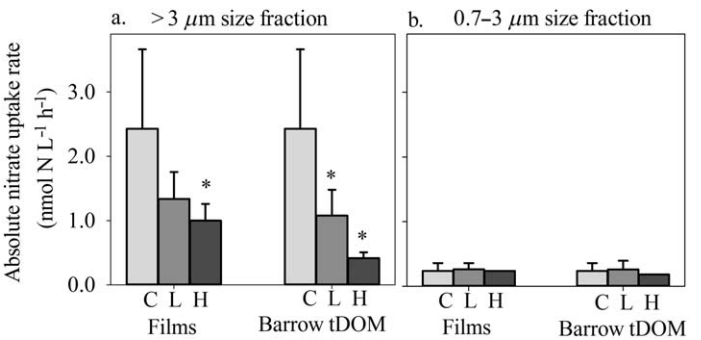

Fig. 5. Absolute nitrate uptake rates for August 2011 in the (**a**) larger size fraction and (**b**) small size fraction in no-amendment controls (C) and with light attenuating films or Barrow tDOM additions at low (L) and high (H) treatment levels. Rates shown represent the mean ± one standard deviation of replicate incubations ($n = 4$). The asterisks denote a significant ($p < 0.01$) reduction in nitrate uptake compared to the control.

reduction in PAR (Table 2). The addition of DOC associated with the tDOM additions did not explain any of the variation for the large size fraction in spring. The opposite was true for the small size fraction where PAR did not explain any of the variation but the addition of DOC explained 4.1% of the variation for that fraction. There was no shared impact of PAR reduction and DOC addition in either size fraction in spring (Table 2).

In summer 2010, the NO_3^- concentration was much lower (0.14 μmol N L^{-1}) than spring, light was 10-fold higher ($\sim$ 50 μmol quanta m^{-2} s^{-1}) and the sampling sites were ice-free. Uptake rates for both size fractions were higher in summer than any other season, and NO_3^- uptake by the larger size fraction remained higher than the smaller size fraction in all treatments. In the large size fraction, although not significant, on average the films reduced NO_3^- uptake by $\sim$ 43% (Fig. 4a). Uptake rates by both size fractions were significantly reduced in the mid ($p < 0.005$) and high ($p < 0.001$) level Nordic tDOM treatments (Fig. 4).

In summer 2011, the NO_3^- concentration was again much lower than spring (0.37 μmol N L^{-1}). In the large size fraction, the light attenuating films significantly ($p < 0.005$) suppressed NO_3^- uptake when the high films were used (48% reduction in PAR) and when both low and high concentrations of Barrow tDOM were added ($p < 0.01$; Fig. 5a). No significant change in NO_3^- uptake was observed in the small size fractions (Fig. 5b). We also note that the NO_3^- uptake rates, for both size fractions, were higher in summer than in any other season. The total variation explained by the tDOM and film manipulations was larger in summer than spring for both size fractions accounting for 28.2% of the variation in the larger size fraction and 13.8% of the variation for the smaller size fraction (Table 2). The shared effect of the reduction in PAR and DOC addition explained the largest portion (14% and 7.6%) of the total variation for the larger and

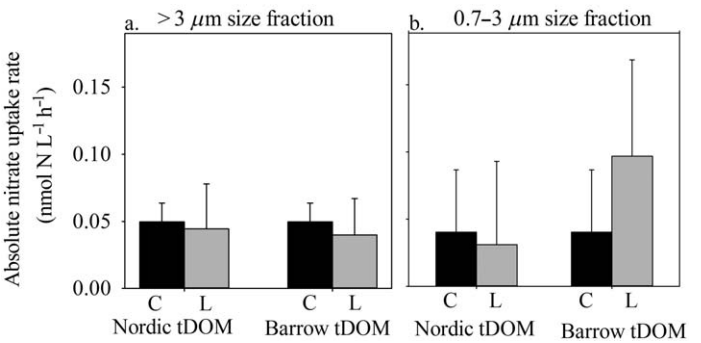

Fig. 6. Absolute nitrate uptake rates for January 2011 in the (**a**) larger size fraction and (**b**) small size fraction in no-amendment controls (C) and with Nordic or Barrow tDOM additions at low (L) treatment levels. Rates represent the mean ± one standard deviation ($n = 6$). No significant change in the rate of uptake was observed for either source.

smaller size fractions, respectively. This analysis shows that the combined photochemical properties of tDOM were more influential over summer nitrate uptake than the reduction in PAR or addition of DOC alone.

Enrichment studies were also performed in winter (January 2011 and 2012) before the first seasonal sunrise. All winter incubations were conducted in near darkness (< 0.3 μmol quanta m^{-2} s^{-1}) so film treatments were not performed. The average ice thickness in both 2011 and 2012 was $\sim$ 0.9 m. Nitrate uptake rates were low in all winter treatments, and tDOM additions did not significantly alter NO_3^- uptake on the time scale tested in the large or small size fractions (Fig. 6).

Looking across all sampling events, the PCA revealed that 97% of the variance is explained by five components and approximately 72% of the variance can be attributed to two components, with PC1 accounting for 55.4% of the variance and PC2 accounting for 16.7% of the variance (Table 3). The

Table 3. Results of the principle component analysis (PCA) performed using physical, chemical, and biological variables for all samples and seasons. Eigen values, % contribution to the total variation and Eigenvectors for each of five principle components (PCs) and twelve environmental variables. Abbreviations include PAR (photosynthetically available radiation), DOC (dissolved organic carbon), DON (dissolved organic nitrogen), NH_4^+ (ammonium), NO_3^- (nitrate), PO_4^{3-} (Phosphate), Si (silicate), Chl *a* (chlorophyll *a*), and BA (bacterial abundance).

	PC1	PC2	PC3	PC4	PC5
Eigen values	6.65	2.01	1.41	1.25	0.34
% variation	55.4	16.7	11.7	10.4	2.8
Eigenvectors					
Temperature	−0.376	0.048	−0.047	0.102	−0.032
PAR	−0.193	−0.013	0.683	−0.159	−0.137
Salinity	0.297	0.131	−0.359	0.326	0.199
Ice thickness	0.326	−0.149	0.353	−0.125	−0.194
DOC	−0.047	−0.682	−0.141	0.072	−0.077
DON	−0.070	−0.681	−0.127	−0.021	−0.020
NH_4^+	0.128	0.098	−0.358	−0.688	−0.510
NO_3^-	0.371	−0.083	0.150	−0.070	0.224
PO_4^{3-}	0.379	0.006	−0.094	−0.057	0.032
Si	0.347	−0.107	0.256	−0.131	0.355
Chl *a*	−0.377	0.018	0.010	0.019	0.075
BA	−0.244	−0.025	−0.127	−0.581	0.674

samples grouped in two distinct areas with respect to PC1 (X-axis), with winter and spring samples grouping together and summer samples grouping separately (Fig. 7). Using the Eigenvectors as a guide, this separation displayed by PC1 appears to be strongly influenced by seasonal differences with the variables temperature, Chl *a*, PAR, and bacterial abundance grouping apart from a variable cluster that includes inorganic nutrients (NO_3^-, PO_4^{3-}, and Si), salinity, and ice thickness. The divergence associated with PC2 appears to be most strongly influenced by the presence of DOC and DON, specifically the tDOM addition treatments (Fig. 7). The other dominant variable associated with PC2 was NH_4^+, which did not correlate with DOC and DON. Although the impact of light attenuation and tDOM additions on NH_4^+ uptake was not assessed in this study, PC2 shows that reduced forms of nitrogen were important in structuring the response of this microbial community.

Primary productivity

Though not the focus on the current study, the direct impact of the light attenuating films and Barrow tDOM on primary production (i.e., bicarbonate uptake) was assessed in the summer of 2011 (Fig. 8). In the large size fraction, changes in bicarbonate uptake mimicked the observed decreases in NO_3^- uptake (Fig. 5a). Bicarbonate uptake rates in the larger size fraction decreased by approximately 80% in low film, high film, and low Barrow tDOM treatments and by 93% in the high Barrow tDOM treatment. In the small size fraction, a substantial (66–88%) decrease in bicarbonate uptake rates also occurred with both film and tDOM additions (Fig. 8b).

Discussion

This project explored the effects of increased tDOM on coastal Arctic microorganisms over three different seasons. Here, we highlight the effect of tDOM on NO_3^- uptake, provide preliminary evidence for a similar effect on primary production, and discuss implications for the future productivity of the coastal Arctic and beyond.

tDOM effects on NO_3^- uptake and primary production

The tDOM source, its concentration, and the time of year, each play a role in determining the impact of tDOM on NO_3^- uptake on relatively short (24 h) time scales. Here, we provide data indicating that the delivery of tDOM to coastal waters will decrease NO_3^- utilization by larger phytoplankton and potentially increase competition between large phytoplankton and bacteria/picophytoplankton for available nitrogen. A study by Li et al (2009) predicts that smaller cells will be favored through the reduction of larger competitors under future climate conditions, but attribute this to terrestrially-based freshwater incursion, not specifically the tDOM that it carries. Therefore, the concurrent increases in freshwater discharge combined with the subsequent release of vast amounts of tDOM will likely significantly impact marine Arctic biota. If smaller microorganisms continue to use NO_3^- at their current rate, or if the non-significant positive trends observed in spring 2010 and 2011 (Figs. 2, 3) are indicative of potential increases in NO_3^- uptake by smaller microorganisms, the competition between large phytoplankton and smaller bacteria and picophytoplankton may be trophically disruptive. The observed reductions in NO_3^- uptake by larger phytoplankton also translated into a reduction in primary production. Though limited in number, these data show reduced bicarbonate utilization in the presence of elevated tDOM, provide further evidence that changing terrestrial carbon inputs will likely have multifaceted consequences for larger primary producers in coastal ecosystems. The implied changes to the phytoplankton community will negatively impact some grazers while others may benefit from the shift away from larger phytoplankton, toward smaller cells. For example, filter feeding gelatinous zooplankton including larvaceans may benefit (Eisner et al. 2014). In either case, expected shifts from current larger phytoplankton-dominated conditions to smaller picophytoplankton/bacteria-dominated communities will likely impact the community composition of and energy transfer pathways to higher trophic levels in the coastal Arctic.

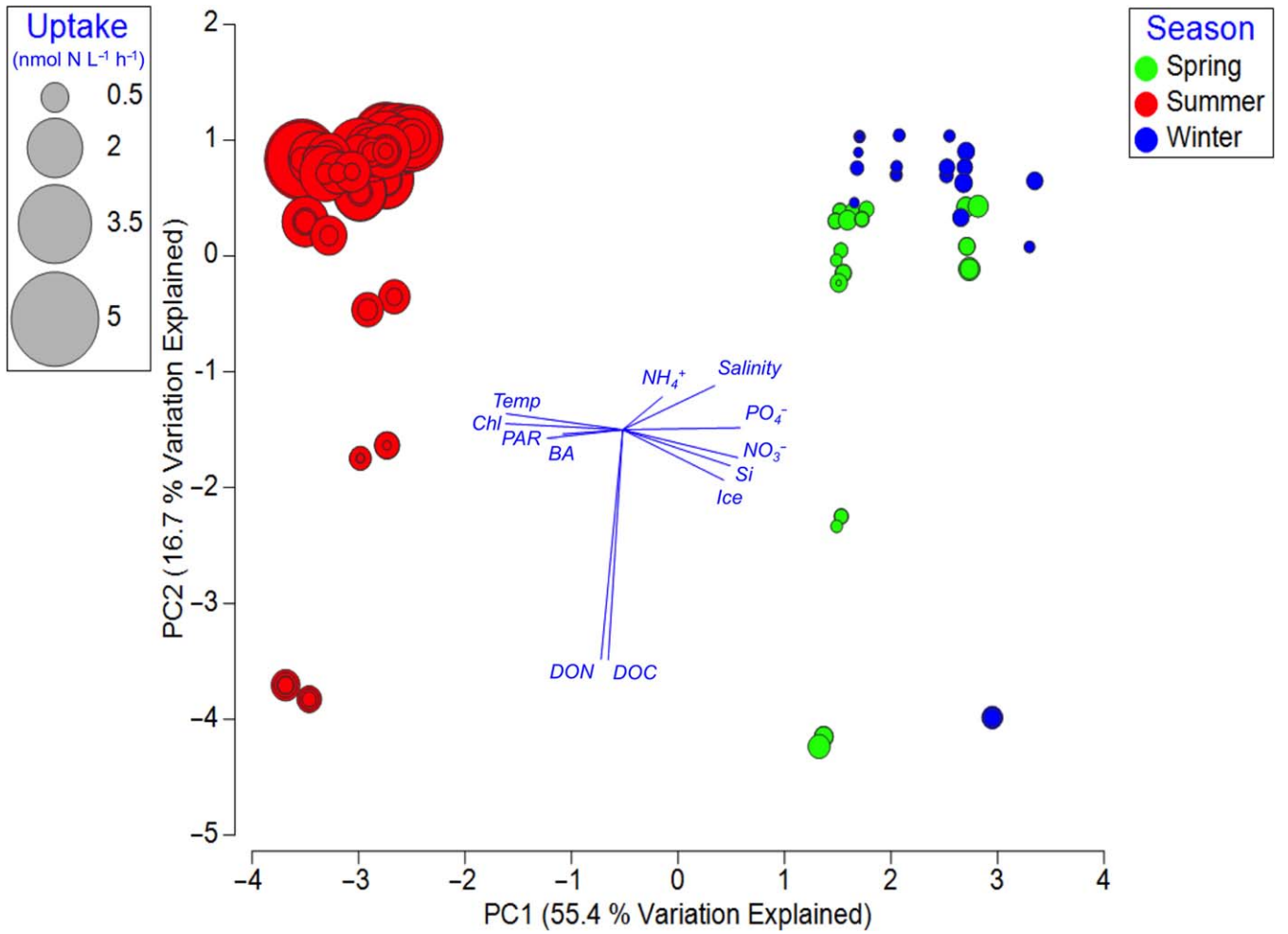

Fig. 7. PCA of the physical, chemical, and biological variables for all samples and seasons. An Eigenvector plot of the variables is overlaid showing the relative contribution to PCs. The magnitude of the corresponding nitrate uptake rates for each sample is overlaid as a bubble plot and color coded by season: spring (green), summer (red), and winter (blue). Abbreviations include Temp (temperature), Ice (ice cover), PAR (photosynthetically available radiation), DOC (dissolved organic carbon), DON (dissolved organic nitrogen), NH_4^+ (ammonium), NO_3^- (nitrate), PO_4^{3-} (phosphate), Si (silicate), Chl *a* (chlorophyll *a*), and BA (bacterial abundance).

The only significant decrease in NO_3^- uptake in the smaller size fraction was observed in the summer of 2010 when uptake rates significantly decreased in the mid ($p < 0.005$) and high ($p < 0.001$) Nordic tDOM treatments (Fig. 4). There was no significant change in the uptake rates of NO_3^- in the smaller size fraction in the summer of 2011 when the local, Barrow tDOM source was added (Fig. 5). While it is interesting that significant reductions in NO_3^- uptake were not observed in both summer seasons (2010 and 2011), we note that this is a comparison of two different tDOM sources, which may have contributed to this variance in observed response between years. Further, in the field trips where both Nordic and Barrow tDOM were compared directly (April 2011and January 2011) those treatments containing Barrow tDOM had higher NO_3^- uptake rates than those containing Nordic tDOM. The distinction in response to the Nordic and Barrow tDOM treatments is likely due to the chemical composition of the sources. The microbial community may find local sources of DOM more labile than sources to which they have not previously been exposed.

Seasonal differences in the general magnitude of NO_3^- uptake by the smaller size fraction are likely due to observed seasonal shifts in heterotrophic microbial activity and community composition (Nikrad et al. 2012; Connelly et al. 2014). DNA-stable isotope probing (DNA-SIP) of these communities conducted as part of this study (Connelly et al. 2014), showed no NO_3^- incorporation into bacterial or archaeal DNA during the summer, but ^{15}N incorporation was detectable in both bacterial and archaeal DNA in winter. The highest bacterial abundance and production rates in this area were associated with warmer seawater temperatures and

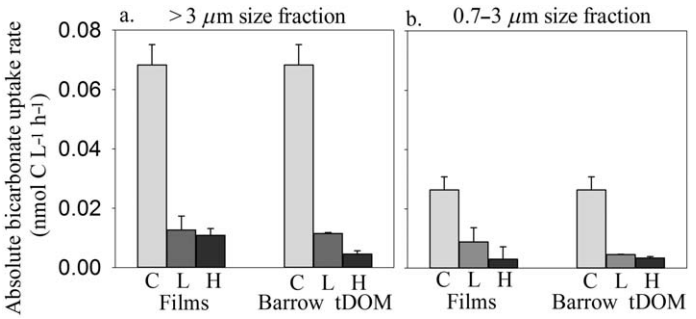

Fig. 8. Absolute bicarbonate uptake rates for August 2011 in the (**a**) larger size fraction and (**b**) small size fraction in no-amendment controls (C) and with light attenuating films or Barrow tDOM additions at low (L) and high (H) treatment levels. Rates represent the mean ± one half of the range ($n = 2$).

increased DOM in summer, but with higher dissolved inorganic nutrients in winter (T. L. Connelly pers. comm.). Thus, our summer NO_3^- uptake rates in the smaller size fraction were more likely associated with mixotrophic picophytoplankton populations than with bacteria or archaea.

This study focused on NO_3^- because it is the common currency for new production in the Arctic. Although NO_3^- is seasonally the dominant source of available nitrogen, it is generally utilized at lower rates than reduced forms of nitrogen (e.g., NH_4^+ or amino acids) by both large and small size fractions of coastal Arctic microbial communities (Baer 2013). Ambient DON concentrations were also 43% higher in summer than spring (Table 1). This additional DON from primary production and the ambient and regenerated NH_4^+ may have provided bioavailable reduced nitrogen that was preferentially used by the heterotrophic summer community instead of or before the NO_3^- supplied with the tracer in the current and DNA-SIP studies. This supplementation of their nitrogen demand with available NH_4^+ and DON could have thus contributed to the lack of a statistically significant increase in NO_3^- uptake by the smaller size fraction.

Implications for the Arctic and beyond

The Arctic shelves are shallow, wide, and some of the most productive waters on Earth (McClelland et al. 2012). The Arctic Ocean is also strongly influenced by terrestrial freshwater inputs, receiving > 10% of the global riverine discharge, which delivers 18 Tg C yr^{-1} to 36 Tg C yr^{-1} to the Arctic shelves and the greater Arctic basin (Dittmar and Kattner 2003; Raymond et al. 2007; Holmes et al. 2012; McClelland et al. 2012). Riverine flow peaks between May and June with DOC concentrations in some rivers reaching > 1000 μmol C L^{-1} (Raymond et al. 2007; Holmes et al. 2008; Stedmon et al. 2011; Holmes et al. 2012; McClelland et al. 2014). More than half of the DOC introduced via rivers is removed over the shelves, based on a decay constant of 0.24 ± 0.07 yr^{-1} calculated by Letscher et al. (2011) for the Eurasian shelves. Therefore, it is clear that riverine inputs to the coastal Arctic are seasonal and important to the ecosystem (McClelland et al. 2012). However, little is known about the changes in the distribution and concentration of DOC in coastal areas during peak flow periods due to the dangerous nature of sampling at that time. Based on the high DOC concentrations within Arctic rivers and the large volumes of water released (Amon et al. 2012; Holmes et al. 2012; Holmes et al. 2013; McClelland et al. 2014), it is understood that large pulsed increases in DOC occur annually in these coastal regions. One recent study conducted in the coastal Alaskan Beaufort found DOC concentrations in June that ranged from ~ 100 μmol C L^{-1} to 400 μmol C L^{-1} with an average concentration of 200 ± 63 ($n = 43$) (Dunton and Crump 2014). The observed DOC concentration ranges validate the ecological significance of our low level additions and show that even higher concentrations are likely in near coastal locations. Our study mimics these large changes in DOC dynamics. In addition to the expected increases in the concentration, volume or frequency of exposure to tDOM-rich river water, our study shows that large changes in DOC dynamics, predicted in future climate change scenarios, could significantly impact large primary producers in coastal Arctic ecosystems. To estimate the impact that these pulses of DOM will have on coastal production, we must quantify the discharge of DOM-rich freshwater from small rivers, thermokarsts and eroded coasts and better constrain DOM dynamics within coastal areas during seasonal freshet. Only then will we be able to extrapolate these observed reductions in NO_3^- utilization and primary production to the broader shelf area.

Increasing tDOM inputs via rivers is not limited to the Arctic. Long-term (~ 10–30 yr) records of riverine DOC have documented a significant increase in DOC concentrations in rivers in Europe (Hejzlar et al. 2003), Scandinavia (Forsberg and Petersen 1990; Andersson et al. 1991; Hejzlar et al. 2003; Erlandsson et al. 2008), the UK (Freeman et al. 2001; Worrall and Burt 2004; Worrall and Burt 2007), and North America (Driscoll et al. 2003; Stoddard et al. 2003; Balch et al. 2016). The increase in DOC concentrations suggests that an increase in turnover of terrestrial carbon reserves, particularly in peat soils (Aitkenhead et al. 1999), is mobilizing DOC on a large scale in northern European and North American lakes and rivers (Freeman et al. 2004; Worrall and Burt 2007). Furthermore, a five-fold decrease in primary production, measured as carbon fixation by phytoplankton, in the Gulf of Maine over the last decade is most closely correlated to increased light absorption by CDOM (Balch et al. 2012; Balch et al. 2016).

While few marine studies have observed the link between increased DOC and decreased primary production, several freshwater studies have made similar observations (e.g., Thrane et al. 2014; Sanders et al. 2015; Seekell et al. 2015*a*,*b*). For example, one study of the Arctic and boreal lakes found that there was a positive relationship between

primary production and DOC at concentrations less than 400 μmol C L^{-1}, but that primary production was negatively impacted by DOC at concentrations above 400 μmol C L^{-1} (Seekell et al. 2015). These results are similar to the observations we made in spring 2010 for NO_3^- uptake (Fig. 2). Identifying the contributing factors (e.g., light attenuation, DOC concentration, or the combination) and the thresholds at which primary production is impacted in marine systems is paramount for the accuracy of climate and ecosystem models in the future.

Marine microorganisms mediate productivity and carbon flow in all marine systems (Arrigo 2005; Suttle 2005; Falkowski et al. 2008). In Arctic seas, bacterial production is typically controlled by the bottom-up supply of carbon (Garneau et al. 2008), while primary production is typically controlled by the environmental forcings of nitrogen supply and light (Tremblay and Gagnon 2009). The release of labile (Vonk et al. 2013), carbon-rich (Lara et al. 1998; Lobbes et al. 2000; Amon and Meon 2004) DOM to the coastal ocean will thus have profound implications for global biogeochemical cycles as well as energy transfer to higher trophic levels via its impact on microorganisms at the base of the food web (Garneau et al. 2006; Vallières et al. 2008).

Conclusions

The DOM entering the coastal Arctic Ocean from terrestrial sources is compositionally carbon-rich and nitrogen-poor. We found that additions of tDOM, isolated from thermokarsts fed by thawing permafrost, negatively impact NO_3^- utilization and primary production by reducing light needed for photosynthesis and that there is an additive effect that we attribute to the geochemical composition of the tDOM. Although a general reduction in PAR could easily be added to current Arctic models, the impact of tDOM is more complex than light attenuation alone. Changes in the magnitude and composition of tDOM could shift the coastal communities toward picoplankton and bacterioplankton and away from larger phytoplankton critical to the coastal Arctic food web and to global carbon and nitrogen cycles. The results of this study propose a scenario of change for the coastal Arctic with the largest impact coming at the expense of the larger coastal primary producers. In a changing Arctic, primary production will be dependent not only on the availability of nitrogen but the community's ability to utilize it. Insights are urgently needed for predicting trophic level impacts and obtaining accurate global carbon, nitrogen and phosphorus cycle estimates.

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Conflict of Interest

None declared.

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