# Dissolved organic nutrients dominate melting surface ice of the Dark Zone (Greenland Ice Sheet)

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- 15 Abstract. Glaciers and ice sheets host abundant and dynamic communities of microorganisms on the ice surface 16 (supraglacial environments). Recently, it has been shown that Streptophyte glacier algae blooming on the surface ice 17 of the south-west coast of the Greenland Ice Sheet are a significant contributor to the 15-year marked decrease in 18 albedo. Currently, little is known about the constraints, such as nutrient availability, on this large-scale algal bloom. 19 In this study, we investigate the relative abundances of dissolved inorganic and dissolved organic macronutrients (N 20 and P) in these darkening surface ice environments. Three distinct ice surfaces, with low, medium and high visible 21 impurity loadings, supraglacial stream water and cryoconite hole water were sampled. Our results show a clear 22 dominance of the organic phase in all ice surface samples containing low, medium and high visible impurity 23 loadings, with 93% of the total dissolved nitrogen and 67% of the total dissolved phosphorus in the organic phase. 24 Mean concentrations in low, medium and high visible impurity surface ice environments are 0.91  $\mu$ M, 0.62  $\mu$ M and 25 1.0 µM for dissolved inorganic nitrogen (DIN), 5.1 µM, 11 µM and 14 µM for dissolved organic nitrogen (DON), 26 0.03 µM, 0.07 µM and 0.05 µM for dissolved inorganic phosphorus (DIP) and 0.10 µM, 0.15 µM and 0.12 µM
- 27 dissolved organic phosphorus (DOP) respectively. DON concentrations in all three surface ice samples are

- 28 significantly higher than DON concentrations in supraglacial streams and cryoconite hole water (0  $\mu$ M and 0.7  $\mu$ M,
- respectively). DOP concentrations are higher in all three surface ice samples compared to supraglacial streams and
- 30 cryoconite hole water (0.07 µM for both). Dissolved organic carbon (DOC) concentrations increase with the amount
- 31 of visible impurities present (low: 83 μM, medium: 173 μM and high: 242 μM) and are elevated compared to
- 32 supraglacial streams and cryoconite hole water (30 µM and 50 µM, respectively). We speculate that the architecture
- 33 of the weathering crust, which impacts on water flow paths and storage in the melting surface ice, and/or the
- 34 production of extracellular polymeric substances (EPS), containing both N and P in conjunction with C, is
- responsible for the temporary retention of DON and DOP in the melting surface ice. The usual presence of
- 36 measurable DIP and DIN, principally as NH<sub>4</sub><sup>+</sup>, in the melting surface ice environments, suggests that factors other
- 37 than macronutrient limitation are controlling the extent and magnitude of the glacier algae.

### 39 1. Introduction

- 40 There has been a significant increase in the net mass loss of the Greenland Ice Sheet (GrIS) during the past two
- 41 decades (Rignot and Kanagaratnam, 2006; Rignot et al., 2011; Shepherd et al., 2012), from 34 Gt yr<sup>-1</sup> to 215 Gt yr<sup>-1</sup>
- 42 between 1992 and 2011 respectively (Sasgen et al., 2012). Surface melt is the primary driver for the increase in ice
- 43 mass loss ( $\sim$ 68%) since 2009, with the remaining ( $\sim$ 32%) coming from solid ice discharge or calving (Enderlin et
- 44 al., 2014). There are two major reasons for this marked increase in surface melting. First, the extent of bare, melting
- 45 surface ice increased, on average, by 7158 km<sup>2</sup> per year from 2000 to 2014 (Enderlin et al., 2014;Shimada et al.,
- 46 2016). Second, the albedo of bare surface ice areas declined between 2000 and 2012, with south-west Greenland
- 47 exhibiting the greatest decrease of up to 18% (Box et al., 2012). A persistent Dark Zone in this region, some 20-30
- 48 km inland and ~50 km wide, has reoccurred annually since at least 2001 (Wientjes and Oerlemans, 2010;Box et al.,
- 49 2012;Stroeve et al., 2013;Tedstone et al., 2017). There is significant variability in the annual extent of the Dark Zone
- 50 (Shimada et al., 2016), which may be the result of both inter-annual climatic variability and factors associated with
- 51 the ice surface, such as melt-out of ancient Holocene dust particles (Wientjes et al., 2011;Tedstone et al., 2017).
- 52 Both snow and bare ice albedo are reduced by light absorbing impurities (LAIs), of both biological and
- 53 mineralogical origin (Gardner and Sharp, 2010), which include atmospheric dust and black carbon, cryoconite, and
- 54 particulates within the meteoric ice that melt out during the ablation season (Warren and Wiscombe, 1980;Warren,
- 55 1984;Warren and Wiscombe, 1985;Gardner and Sharp, 2010;Wientjes et al., 2012;Cook et al., 2016a). The
- 56 importance of biological LAI, particularly Streptophyte glacier algae, which bloom in surface ice environments
- 57 during summer ablation seasons, as a factor in albedo decline has been identified in recent years (Yallop et al.,
- 58 2012). The effect has become known as "bioalbedo", which is derived from the original term "biological albedo
- 59 reduction" (Kohshima et al., 1993;Cook et al., 2017a). Bioalbedo is attributed to a combination of the high
- abundance of cells that grow during the bloom (up to  $\sim 10^4$  cells ml<sup>-1</sup> surface ice) and the heavily pigmented nature
- of ice algal cells, which include a unique dark UV-VIS absorbing pigment, purpurogallin, that provides photo-
- 62 protection from the extreme solar radiation in supraglacial environments (Remias et al., 2012; Williamson et al.,

63 2018). Tedstone et al., (2017) concluded that ice algal blooms are the main factor responsible for inter-annual

64 variability in the extent, magnitude and duration of the Dark Zone, which seem to be regulated by climatic drivers,

65 including the June-July-August sensible heat flux anomaly and the timing of snow-line retreat. The spatial extent of

ice algal blooms may also be linked to the availability of mineralogic LAIs, such as late Holocene dust particles

67 melting out of the meteoric ice (Wientjes et al., 2012). However, the linkage between particles and algae is not

68 presently understood (Tedstone et al., 2017).

69 C, N and P are essential for all living organisms, providing the basis for cellular mass and all metabolic activity

70 (Redfield et al., 1963;Hessen et al., 2013). Carbon is usually in ready supply in surface ice environments, both from

71 the atmosphere and from bubbles trapped in snow and ice, and so nitrogen and phosphorus are more likely the

72 limiting factors for growth and activity of microorganisms (Stibal et al., 2009;Lutz et al., 2017). Bioavailable forms

of N are less readily available, being largely confined to  $NO_3^-$  and  $NH_4^+$  in dry and wet deposition from the

atmosphere (Wolff, 2013), and from snow- and ice-melt (Telling et al., 2011). Dissolved inorganic phosphorous

75 (DIP) is typically the least available nutrient in supraglacial environments, since it is a largely rock-derived and is

only released by chemical weathering or bio-mining (Stibal et al., 2008b; Stibal et al., 2009). P sources in remote

77 glaciated environments, such as the Dark Zone, are largely confined to the small quantities of particulates deposited

78 from the atmosphere and the melt out of debris in snow and ice (Wientjes and Oerlemans, 2010).

79 The presence of such large-scale algal blooms in the Dark Zone might suggest that these environments are nutrient-

80 rich. This would contrast with the current literature, which suggests that supraglacial environments in the Dark

81 Zone, similar to those found in Svalbard, the margins of the Greenland Ice Sheet and Antarctica, are extremely

82 oligotrophic (Stibal et al., 2008b; Stibal et al., 2009; Telling et al., 2011; Telling et al., 2012; Bagshaw et al.,

83 2013;Hawkings et al., 2016;Wadham et al., 2016). Mean dissolved inorganic nitrogen (DIN) concentrations in

**84** Greenland ice are ~1.4  $\mu$ M, with NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> composing 0.97  $\mu$ M and 0.39  $\mu$ M, respectively (Wolff, 2013).

85 There are relatively few measurements of nutrient concentrations in the surface ice environments of the Dark Zone

86 (Telling et al., 2012; Wadham et al., 2016), but the average  $NO_3^-$  concentration in surface ice along the K Transect

87 east of Kangerlussuaq, which passes through the Dark Zone, has been reported to be  $0.6 \pm 0.1 \mu$ M between 17-79

88 km from the ice sheet margin (Telling et al., 2012), while DIP concentrations were below the detection limit,

89 0.33 µM P (Telling et al., 2012). DIN concentrations in snow sampled before the start of the ablation season at the

90 margin of the GrIS had higher concentrations, with an average of 1.4  $\mu$ M (Telling et al., 2012), similar to those of

91 Wolff (2013). Hence, there is no real evidence that neither N nor P concentrations in snow and ice sampled in the

92 vicinity of the Dark Zone are higher than for average Greenland ice. The relatively low concentrations of

93 macronutrient in the snow and ice of the SW Greenland Ice Sheet means that algal blooms are likely to rapidly

94 sequester N and P from snow and ice melt, particularly as the blooms reach their zenith at the height of the ablation

95 season. For example, NPP (Net Primary Production) values in the wet, melting surface ice (also called rotten ice, or

96 the weathering crust) during blooms range from  $21 - 100 \mu$ mol C l<sup>-1</sup> day<sup>-1</sup> (Chandler et al., 2015; Williamson et al.,

97 2018). Should the mean DIN concentration of the ice melt be 1.4  $\mu$ M, this implies a C:N molar ratio of 15 – 71 if all

98 the DIN is sequestered into new organic matter and no other sources of DIN are present. There is no readily

99 available C:N ratio of glacier algae in the literature, but typical C:N ratios of sea ice algae are in the range of 12-46

100 (Niemi and Michel, 2015). It is even more difficult to find C:N:P ratios of glacier algae, but should the C:P ratio be

101 in the region of 100:1 to 1000:1, the P demand will be  $0.02 - 1 \ \mu M$ .

Blooms in other aquatic ecosystems are associated with efficient recycling of nutrients when new sources of N and P
are in scarce supply, often with a balance between nutrient uptake and remineralization (Dodds, 1993), allowing
nutrient accumulation in biomass over time. This balance does not appear to arise in the surface ice environments of
other High Arctic and polar glaciers studied to date. These are predominantly in cryoconite holes, which are waterfilled cylindrical holes with an organic-rich basal sediment in the ice surface that host a range of microbes, including
cyanobacteria (Christner et al., 2003;Anesio and Laybourn-Parry, 2012;Telling et al., 2012). Dissolved
macronutrients tend to become concentrated in organic phases (Stibal et al., 2008b;Telling et al., 2014), suggesting

an imbalance in the uptake and remineralization of dissolved inorganic nutrients in cryoconite hole environments.

110 Indeed, the only ratio of primary production to remineralization measured in the Dark Zone is 30:1 (Nicholes et al.,

111 2019). To date, dissolved organic nitrogen (DON) concentrations in the Dark Zone have only been reported in two

studies (Telling et al., 2012;Wadham et al., 2016), but neither focus on ice populated by Streptophyte glacier algae.

**113** Telling et al., (2012) reported a near 1:1 relationship between  $NO_3^-$  and total dissolved nitrogen (TDN), suggesting

that DON comprised only a small portion of the TDN pool in snow and ice samples. By contrast, Wadham et al.,

115 (2016) suggested mineralization of organic matter by microbial activity, either within the cryoconite holes

themselves or in debris- and cryoconite-rich "dirty" surface ice contributed to DON concentrations that could reach

117 0.7 µM and 3.0 µM, respectively. No dissolved organic phosphorous (DOP) concentrations in the surface ice

118 environments in the Dark Zone have been reported to date.

119 Several studies have noted the heterogeneity in the spatial distribution of glacier algae in the melting surface ice of

the Dark Zone (Yallop et al., 2012; Williamson et al., 2018). This heterogeneity occurs on length scales of cm to 10s

of m (Yallop et al., 2012). This might well signify that macronutrient concentrations are also variable on this scale,
 yet no studies to date have examined variability on these length scales. We contend that it is important to determine

123 the concentrations and relative proportions of dissolved inorganic and organic nutrients in melting surface ice

124 environments of Dark Zone, particularly during Streptophyte glacier algae blooms, since a knowledge of both DIN,

125 DON, DIP and DOP may be crucial to better understand how glacier algae and bacteria can retain, utilize and

126 recycle their limited nutrients to sustain the large-scale blooms observed in this region of the Greenland Ice Sheet.

127 The aims and objectives of this study, therefore, are threefold. First, we aim to quantify dissolved nutrient

128 concentrations in the supraglacial environments of the Dark Zone during the peak ablation season. Second, we

129 determine the relative abundance of dissolved inorganic and organic nutrients during the peak ablation season when

130 microbial recycling is likely to have the greatest influence on the dissolved inorganic and organic ratios. Finally, we

131 investigate if there are systematic changes in the relative proportions of dissolved macronutrients during increased

132 colonization of melting surface ice, which might shed light on the limiting nutrient on algal blooms.

133

134 2. Methods

### 135 2.1 Field Site and Sampling

136

137 camp was located approximately 30 km inland from the ice margin, near to the 'S6' weather station on the Ktransect (Fig 1; 67°04'43.3" N, 49°20'29.7" W). Samples were collected from a designated area of approximately 138 139  $500 \times 500$  m, which included surface ice, supraglacial stream and cryoconite hole habitats. Sampling occurred at 140 intervals of approximately three days from 15<sup>th</sup> of July to 14<sup>th</sup> of August 2016. A categorical sampling strategy was 141 employed, given the evident spatial heterogeneity apparent in ice algal distributions. Five different habitats were 142 sampled; melting surface ice with three differing amounts of visible impurities, referred to here as surface ice with 143 "low" (n=19), "medium" (n=19), and "high" (n=19) visible impurities) (Fig. 2) (Yallop et al., 2012). Water from 144 supraglacial streams (n=10) and cryoconite holes (n=14) was randomly collected, both to act as a comparison with 145 the melting surface ice and to examine how dissolved nutrients were transported through the weathering crust, which 146 is the melting layer of surface ice that has a different physical architecture to the underlying ice (Fig. 2). Surface ice 147 habitats were sampled from a  $1 \times 1$  m area chosen at random, from which the top ~2 cm of ice was removed using a

A field camp was established within the Dark Zone, adjacent to Kangerlussuaq, during the summer of 2016. The

- 148 pre-cleaned ice saw.
- 149 Samples from all five categories were collected for the analysis of dissolved inorganic and organic nutrients and
- 150 dissolved organic carbon (DOC). Algal cell abundances were determined on surface ice samples only. Ice collected
- 151 for nutrient analysis and algal cell abundance was placed into a clean/sterile Whirl-pak<sup>TM</sup> bag, while that collected
- 152 for DOC analysis was transferred into a glass jar that was first rinsed three times with sample. Ice samples were left
- to melt overnight in the lab tent, typically taking 4-5 h. Supraglacial stream water samples for nutrient analysis were
- 154 collected using high-density polyethylene plastic bottles (Nalgene<sup>TM</sup>), whereas those for DOC analysis were
- 155 collected in glass jars. Both sampling containers were rinsed three times with sample prior to collection. Cryoconite
- 156 hole water used for nutrient and DOC analysis was collected using a large pipette and transferred into a Nalgene<sup>TM</sup>
- bottle or glass jar, respectively. The large pipette and collection vessels were rinsed three times with sample prior to
- 158 collection. All high-density polyethylene plastic bottles (Nalgene<sup>TM</sup>) for nutrient samples were acid washed in  $\sim 10\%$
- 159 HCl solution prior to first use and all glass jars for DOC samples were furnaced at 500°C for four hours prior to first
- 160 use.
- 161 Some 15 ml of the homogenised, unfiltered ice melt and water samples were subsampled and fixed using 25%
- 162 glutaraldehyde at 2% final concentration for quantifying algal cell abundance. These fixed samples were stored
- 163 outside in the dark at ambient ice sheet temperatures. Ice melt and water samples for nutrient analysis were filtered
- 164 through a 25 mm, 0.22 μm cellulose nitrate inline syringe filter (Whatman<sup>TM</sup>) and stored in high density
- polyethylene plastic bottles (Nalgene<sup>TM</sup>; 30mL). The bottles were immediately frozen and stored at a temperature of
- 166 -20°C, using a Waeco 32L Freezer. Ice melt and water samples for DOC analysis were filtered using a glass
- 167 filtration column and a furnaced 47 mm, 0.7 µm GF/F. The filtration column was washed three times with sample
- 168 water prior to collection of the filtrate. The filtrate was stored in pre-furnaced amber glass vials and acidified with
- 169 100  $\mu$ L of 1M HCl. They were chilled to a temperature of ~3°C by storing the samples in a box at ambient air
- 170 temperature. The samples were maintained at this temperature during transport and in storage at the LowTex

- 171 Laboratory at the University of Bristol. Nutrient samples were thawed immediately prior to analysis using a  $\sim 40^{\circ}$ C
- 172 hot water bath. Procedural blanks (n=9) were collected over the course of the sampling season, by processing
- 173 deionised water in place of sample.

### 174 2.2 Analytical Methods

175 Algal cell abundance was quantified using a Fuchs-Rosenthal haemocytometer (Lancing, UK) on a Leica DM 2000

176 epifluorescence microscope with attached MC120 HD microscope camera (Leica, Germany). For samples

177 containing sufficient cell abundance, a minimum of 300 cells were counted to ensure adequate assessment of

- assemblage diversity (Williamson et al., 2018).
- 179 DIN species include NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> and were quantified as follows. First, NH<sub>4</sub><sup>+</sup> was quantified
- 180 spectrophotometrically using a Lachat QuickChem<sup>®</sup> 8500 Series 2 Flow Injector Analyzer (FIA; QuickChem<sup>®</sup>
- 181 Method 31-107-06-1-I). Measurements were based on a phenolate-hypochlorite alkaline reaction method measured
- at 630nm (Solorzano, 1969). The limit of detection (LoD) was 0.62 µM, determined by dividing the standard
- deviation of the response of the calibration curve by the slope of the calibration curve, then multiplying the result by
- 184 3 (Shrivastava and Gupta, 2011). Precision was  $\pm 2.1\%$ , and accuracy was  $\pm 8.5\%$ , as determined from comparison
- $\label{eq:2.1} \text{ with a gravimetrically diluted 1000 mg $L^{-1}$ NH_4^+-N$ certified stock standards to a concentration of 1.1 $\mu$M$. (Sigma$
- **186** TraceCERT<sup>®</sup>). Second,  $NO_2^-$  and total oxidised nitrogen (TON) ( $NO_2^- + NO_3^-$ ) were quantified
- 187 spectrophotometrically using a Gallery Plus Automated Photometric Analyzer (Thermo Fisher Scientific, UK). This
- 188 combination of analysis allows the original  $NO_3^-$  concentration to be determined by subtracting  $NO_2^-$  from TON.
- 189 TDN is the sum of DIN and DON, and was determined by digesting the samples with a potassium persulfate,
- sodium hydroxide and boric acid reagent and autoclaving at 121°C for 30 minutes (Grasshoff et al., 1999). This
- 191 process causes the oxidation of organic nitrogen compounds, which can then be measured as TON as above.
- 192 Purification of the potassium persulfate was conducted via recrystallisation in order to remove any N contamination.
- 193 Measurements were based on the hydrazine-sulfanilamide reaction method measured at 540nm. DON was then
- 194 estimated by subtracting DIN from TDN (i.e. DON= TDN-DIN). LoD were 0.14 μM (NO<sub>2</sub><sup>-</sup>), 0.64 μM (TON) and
- 195 0.87  $\mu$ M (TDN/DON). Precision was ±0.87% (NO<sub>2</sub><sup>-</sup>), ±1.17% (NO<sub>3</sub><sup>-</sup>) and ±0.63% (TDN/DON), and accuracy was -
- 4.04% (NO<sub>2</sub><sup>-</sup>), -8.07% (NO<sub>3</sub><sup>-</sup>) and -5.7% (TDN/DON), as determined from comparison with gravimetrically diluted
- 197 1000 mg L<sup>-1</sup> NO<sub>2</sub> $^{-N}$  and NO<sub>3</sub> $^{-N}$  certified stock standards to a concentration of 0.71  $\mu$ M (NO<sub>2</sub><sup>-)</sup>, 1.4  $\mu$ M (NO<sub>3</sub><sup>-)</sup> and
- **198** 7.1  $\mu$ M (TDN/DON) (Sigma TraceCERT<sup>®</sup>).
- 199 TDP (total dissolved phosphorus) is the sum of DIP (principally  $PO_4^{3-}$ ) and DOP. The same persulfate digestion
- 200 method described for TDN was used to measure TDP as  $PO_4^{3-}$ .  $PO_4^{3-}$  in both undigested and the digested samples
- 201 was quantified using a Lachat QuickChem<sup>®</sup> 8500 Series 2 Flow Injector Analyzer (FIA; QuickChem<sup>®</sup> Method 31-
- 202 115-01-1-I) using the molybdenum blue method measured at 880nm. DOP was determined by the subtraction of DIP
- 203 in the undigested sample from the TDP in the digested sample (i.e. DOP = TDP-DIP). The LoD was 0.02  $\mu$ M (PO<sub>4</sub><sup>3-</sup>
- and TDP/DOP). Precision was  $\pm 1.6\%$  (PO<sub>4</sub><sup>3-</sup>) and  $\pm 3.1\%$  (TDP/DOP), and accuracy was  $\pm 2.3\%$  (PO<sub>4</sub><sup>3-</sup>) and  $\pm 5.0\%$

- 205 (TDP/DOP), as determined from comparison with gravimetrically diluted 1000 mg L<sup>-1</sup> PO<sub>4</sub>-P certified stock 206 standards to a concentration of 0.65  $\mu$ M (Sigma TraceCERT<sup>®</sup>).
- 207 DOC concentrations were quantified using a Shimadzu TOC-L Organic Carbon Analyzer, with a high sensitivity
- 208 catalyst. Non-purgeable organic carbon (NPOC) was measured after acidification of samples with HCl and catalytic
- 209 combustion (680°C) of dissolved organic carbon to carbon dioxide, which was then measured by infrared
- absorption. The LoD was 9.5  $\mu$ M. Precision was  $\pm 2.4\%$  and accuracy was -5.9%, as determined from comparison
- 211 with gravimetrically diluted 1000 mg L<sup>-1</sup> TOC certified stock standards to a concentration of 83.3 µM (Sigma
- 212 TraceCERT<sup>®</sup>).

### 213 2.3 Data Analysis

- All measurements below the LoD were considered to be 0 for all statistical analyses. All DIN, DON, DIP, DOP and
- 215 DOC data were water blank-corrected using values from the respective field procedural blanks (Table 1).
- 216 Additionally, all blank corrected values that were negative were assumed to be 0 for all statistical analyses.
- 217 Statistical analysis was performed in RStudio v.1.1.414 (RStudio, Inc 2018). Identification of statistical differences
- 218 between the nutrient content, DOC concentration and algal cell abundance in different habitats was achieved using
- 219 1-way analysis of variance (ANOVA) or t-test comparisons, with post-hoc Tukey HSD analysis applied to all
- 220 significant ANOVA results. Pearson's product-moment correlations were used to identify correlations between
- 221 DON, DOC and algal cell abundance. Homogeneity of variance and normality of distribution were tested prior to all
- 222 parametric analyses, and model assumptions were verified by examination of model criticism plots.
- 223

### 224 3. Results

### 225 3.1 Dissolved nutrient concentrations in surface ice with differing levels of visible impurities

- 226 Supraglacial environments are extremely oligotrophic, making measurements of dissolved nutrients difficult.
- 227 Dissolved nutrient concentrations reported in previous studies of supraglacial environments are typically below or
- just above instrument limit of detections. Some 54 DON, 41 DIN, 74 DOP, 40 DIP and 59 DOC samples out of a
- total of 81 samples for all five supraglacial habitats had concentrations above the LoD.
- 230 Dissolved organic concentrations were significantly higher than dissolved inorganic concentrations for nitrogen and
- phosphorus. Some 93% of the TDN was in the form of DON and about 67% of TDP was present in the form of DOP
- in all three surface ice habitats. Mean DON concentrations for the three surface ice habitats range from  $5.1-14.0 \,\mu$ M,
- while those for DIN range from  $0.62-1.0 \,\mu\text{M}$  (Fig. 3, Table 1). Overall, mean DON concentrations for the three ice
- surface habitats, were significantly higher ( $F_{1,71}$ =12.4, p<0.0001) than mean DIN concentrations. Similarly, DOP
- 235 concentrations were usually at least twice those of DIP concentrations for the three ice surface habitats, with mean
- values ranging from 0.10-0.15 μM and 0.03-0.07 μM respectively (Fig. 4, Table 1). T-tests revealed significant

- 237 differences between DON and DIN in all three surface ice habitats (low:  $t_{36}=3.6$ , p<0.001, medium:  $t_{36}=5.3$ ,
- 238 p<0.0001, high:  $t_{36}=7.4$ , p<0.0001, (Fig. 3) and DOP concentrations as significantly higher than DIP concentrations
- 239 for all three surface ice habitats (low:  $t_{36}=3.1$ , p<0.01, medium:  $t_{36}=2.1$ , p<0.05, high:  $t_{36}=3.7$ , p<0.001) (Fig. 4).
- 240 DON and DOC concentrations in the three surface ice habitats showed clear trends with increasing visible impurities
- 241 (Fig. 3 & 5). DON concentrations increased significantly from low to medium and low to high visible impurity
- loadings (F<sub>4,71</sub>=19.8, p<0.05, F<sub>4,71</sub>=19.8, p<0.001, respectively), while DOC concentrations increased significantly in
- ice with high and low visible impurity loading ( $F_{4,74}=6.8$ , p<0.01).

### 244 3.2 Links between algal abundance and dissolved organic nutrients

- Algal cell abundance, which ranged from 90 cells ml<sup>-1</sup> to  $0.98 \times 10^4$  cells ml<sup>-1</sup>, increased significantly with the
- amount of visible impurities seen on the ice surface, as shown in Figure 6 ( $F_{2,54}$ =26.1, p<0.0001). No determination
- 247 of the mineralogic composition of the visible impurities was conducted. A Pearson's product-moment correlation
- 248 was undertaken to illustrate the relationship between average algal abundance and average DOC and DON
- 249 concentrations, as DOC and DON concentrations also increased with the amount of visible impurities present.
- 250 Correlations between average algal cell counts versus both DON and DOC surface ice concentrations were
- significant ( $t_3=3.5$ , p<0.05, r=0.9 and  $t_3=5.4$ , p<0.01, r=0.95, respectively). Comparison of DOP surface ice
- concentrations and algal cell counts were not significant.
- 253 Dissolved organic nutrient ratios were assessed to investigate the presence of a limiting nutrient. Molar DON:DOP
- ratios, ranging from 49 to 120, were elevated for all three surface ice environments compared to the 16:1 Redfield
- 255 Ratio, and DOC:DOP ratios for all three surface ice habitats, which ranged from 800 to 2000, were considerably
- higher, as much as ~19 times the Redfield ratio, 106:1 (Table 1). DOC:DON ratios, which ranged from 16 to 17,
- were, on average, twice the balanced 6.6:1 ratio (Table 1). DON:DOP and DOC:DOP ratios also increased with the
- amount of visible impurities present, while DOC:DON ratios remain relatively constant for the three surface ice
- habitats (Table 1).

### 260 **3.3** Low transport of dissolved organic nutrients within the water table

- 261 Mean DON and DOP concentrations were significantly lower in supraglacial streams (0 µM and 0.07 µM,
- respectively) and cryoconite hole water (0.7 µM and 0.07 µM, respectively) compared to low, medium and high
- visible impurity ice. All DON concentrations for cryoconite hole and supraglacial stream water were below the LoD
- 264 (Fig. 3). DIN concentrations were relatively constant over all supraglacial habitats with mean concentrations ranging
- from 0.62  $\mu$ M to 1.0  $\mu$ M. DOP concentrations in supraglacial stream (0.07  $\pm$  0.03  $\mu$ M) and cryoconite hole water
- 266  $(0.07 \pm 0.02 \ \mu\text{M} \ \mu\text{M})$  were not significantly different from DIP concentrations  $(0.01 \pm 0.01 \ \mu\text{M} \text{ and } 0.06 \pm 0.02 \ \mu\text{M},$
- respectively). DIP concentrations in low  $(0.03 \pm 0.02 \,\mu\text{M})$ , medium  $(0.07 \pm 0.02 \,\mu\text{M})$  and high  $(0.05 \pm 0.01 \,\mu\text{M})$
- 268 visible impurity ice were only slightly elevated compared to supraglacial streams, whereas cryoconite hole water
- 269 concentrations were comparable to the three surface ice habitats. Mean DOC concentrations in supraglacial stream
- and cryoconite hole water (30 µM and 50 µM, respectively) were significantly lower than ice with high visible
- 271 impurities ( $F_{4,74}$ =6.8, p<0.001, in both cases) (Fig. 5).

#### 273 4. Discussion

#### 274 4.1 Dominance of dissolved organic over dissolved inorganic phases in melting ice surface environments

275 Dissolved organic nutrients (DON and DOP) dominate dissolved inorganic nutrients (DIN and DIP) in the melting 276 surface ice environments of this region of the Dark Zone (Fig. 3 & 4), in contrast with the dominance of DIN in 277 studies of fresh snow or ice cores from the GrIS (Telling et al., 2012; Wolff, 2013), which has a mean concentration 278 of 1.4  $\mu$ M. Further, DIN also dominates on the margins of the ice sheet, where Telling et al., (2012) found DIN 279 concentrations in snow to be  $1.4\pm0.2 \mu$ M, but DON concentrations to be non-detectable. Wadham et al., (2016) 280 reported relatively similar DIN (1.3  $\mu$ M) and DON (~1.6  $\mu$ M, assuming DON = TDN-DIN in their tabulated data) 281 concentrations in a small number (n = 7) surface, debris-rich ice in the Dark Zone of the GrIS during the main 282 ablation season., but these values were thought likely to be associated with dispersed cryoconite, the dark organic-283 rich sediment that accumulates in the bottom of cryoconite holes and larger supraglacial water bodies. Otherwise, 284 DON was not measurable in snow and surface ice, prior to melting. In summary, this suggests that potential input of 285 dissolved N-species to supraglacial environments from fresh snow and melting meteoric ice are dominated by DIN, 286 rather than DON. There is too little data on DIP and DOP to be confident that the this is also the case for P species. 287 By contrast, dissolved N-species in the melting ice surface of the Dark Zone shifts to a domination of DON during 288 the peak ablation season (July and August), when blooming of glacier algae occurs. We therefore hypothesise that 289 the algae present in these blooms drive the shift in nutrients during the peak ablation season from the dissolved 290

# inorganic phase to the dissolved organic phase.

#### 291 4.2 Association of dissolved organic nutrients and algal abundance

292 Figure 6 shows that algal abundance increases in the ice with low, medium and high visible impurities. The

- 293 blooming of the algal cells is also associated with trapping of other mineral particulates at the surface. Yallop et al.,
- 294 (2012) reported a 3:2 mineral particle to algal cell ratio for surface ice collected in the Dark Zone, although these
- 295 particles have only a minor impact on the albedo reduction at the surface (Cook et al., 2019). It is clear from Fig. 3
- 296 that the mean DON concentration increases from low to high visible impurities, consistent with DON formation
- 297 being linked to glacier algae blooms. This is most likely due to a combination of extracellular exudation of
- 298 polymeric substances and the decomposition of glacier algal cells within the supraglacial system. Concentrations of
- 299  $NO_3^-$  and  $NO_2^-$  are zero (Table 1), and  $NH_4^+$  is the only measurable DIN species (mean values range from 0.6 to 1
- 300  $\mu$ M). The absence of measurable NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> is consistent with the uptake of these species by glacier algae, and
- 301 the emergence of NH<sub>4</sub><sup>+</sup> as the dominant DIN species is consistent with heterotrophic remineralization of organic
- 302 matter (Telling et al., 2012). We note that the mass of N held in the microbial biomass is likely increasing over time,
- 303 since the sum of the mean DIN and DON concentrations (1.0 µM and 0.0 µM respectively) in the supraglacial
- 304 stream water, which is the ultimate sink of macronutrients from the melting ice surface, is less than the average DIN

305 concentration of the melting ice (1.4 µM) (Telling et al., 2012;Wolff, 2013;Wadham et al., 2016). The only

306 measurable DIN species in supraglacial meltwater is  $NH_4^+$ , which points to ammonification being an important

307 process in terms of N dynamics and loss of labile N from the melting surface ice. Previous studies of the relative

308 rates of primary production and bacterial production in both the margins and the Dark Zone have produced ratios of

309 30:1 (Yallop et al., 2012; Nicholes et al., 2019). The dominance of dissolved organic nutrients and NH<sub>4</sub><sup>+</sup> in surface

310 ice environments documented here, in combination with reduced secondary production relative to net primary

311 production in the same environments, indicates an inefficiency in the microbial loop for remineralization of organic

312 nutrient N-stocks (Fig. 7).

320

313 There is less data in the literature on the relative abundance of DIP and DOP in snow and ice, but here we show that 314 there are similarities between the accumulation of dissolved N into DON and P into DOP species (Fig. 3 & 4). Mean

315 DOP concentrations in the five sampled environments are higher than mean DIP. This is consistent with uptake of P

316 by glacier algae and subsequent recycling of P into organic forms. An interesting observation is that there is usually

317 some measurable DIP found in the particulate-rich environments that were sampled (surface ice and cryoconite hole

318 water), whereas the mean DIP in the supraglacial stream water is the lowest value recorded and below the limit of

319 detection. This suggests two things. First, that particulates are the source of DIP, and second, that export of P from

the melting surface ice is largely by DOP. We noted above that particulates are associated with glacier algae in the 321 melting surface ice, and it appears that as algal blooms develop, more particulates become trapped in the surface

322 layer (Yallop et al., 2012). It may well be that there is an "inorganic symbiosis" between the glacier algae and the

323 trapped particulates, which provide a P source for algal growth.

324 Our results on the dominance of DON and DOP are consistent with findings of previous studies in polar glacier

325 surface aquatic environments (Stibal et al., 2008a;Stibal et al., 2008b;Stibal et al., 2009;Wadham et al., 2016). For

326 example, Stibal et al., (2008) reported that DON (~72%) and DOP (~89%) in waters in cryoconite holes on a

327 Svalbard glacier dominated the total dissolved N and P pools. Wadham et al., (2016) found elevated DON

328 concentrations in water in cryoconite holes and debris-rich surface ice in the Dark Zone, suggesting either

329 mineralization of organic matter by microbial activity or leaching of allochthonous organic matter in debris. These

330 observations suggest that conversion of dissolved inorganic to dissolved organic nutrients by microbial communities

331 in melting surface ice environments may be a common process on glacier surfaces

#### 332 4.3 Retention of nutrients at ice sheet surface

333 The low concentration of DIN, DIP, DON and DOP in the supraglacial meltwaters relative to the melting surface ice

334 suggests that macronutrients are retained in these surface environments. Melting ice surfaces in the Dark Zone often

335 have a veneer of low density, wet porous ice, which may reach depths of 1-2 m, known as the "weathering crust"

336 (LaChapelle, 1959; Müller and Keeler, 1969; Munro, 1990; Irvine-Fynn et al., 2012). The intense short wave radiation

337 during summer often causes internal melt along ice crystal boundaries, resulting in a surface ice layer with

338 heterogeneous thickness, density, porosity and water content (Müller and Keeler, 1969; Cook et al., 2016b; Christner

et al., 2018). The porous nature of the weathering crust allows flow paths to form through the water table that exists 339

- 340 within the surface ice (Irvine-Fynn et al., 2012;Cook et al., 2016b;Rassner et al., 2016;Christner et al., 2018), which
- 341 act as important links between different supraglacial environments and are believed to transport microbes and
- 342 nutrients via subsurface flow (Irvine-Fynn et al., 2012;Hoffman et al., 2014;Karlstrom et al., 2014;Cook et al.,
- 343 2016b). Water is often in temporary storage in the weathering crust (Irvine-Fynn et al., 2012), particularly at depth
- 344 where connectivity of flow paths can be low. It follows that the first explanation for retention of dissolved organic
- 345 nutrients in the weathering crust is that they accumulate in water stored in the weathering crust.
- 346 DOC concentrations in supraglacial stream water were lower than the DOC in all surface ice habitats, particularly
- 347 surface ice with high visible impurities (Fig. 5). This suggests a second possible mechanism of retention of DON
- 348 and DOP in the weathering crust, via the production of extracellular polymeric substances (EPS). Algae and bacteria
- 349 produce EPS which can alter the physical and chemical environment around their cells (Stibal et al.,
- 350 2012a; Angelaalincy et al., 2017). For example, it has been shown that EPS are used by cyanobacteria in cryoconite
- boles to bind mineral particles together creating the cryoconite granules at the bottom of the hole (Stibal et al.,
- 352 2012b;Yallop et al., 2012;Musilova et al., 2016). EPS is often colliodal (here, operationally defined as passing
- 353 through 0.4  $\mu$ m, but not 0.02  $\mu$ mfilter membranes) (Raiswell et al., 2018), and when analysed from filtered (through
- 354 0.4 µm membranes), melted surface ice samples will be in the dissolved organic fraction (Pereira et al.,
- 355 2009;Hodson et al., 2010). The chemical composition of EPS exuded by glacier algae is unknown. We note that the
- EPS of bacteria living in sewage sludge can have a molar C:N:P ratios that approaches 100:101:14 (Guibaud et al.,
- 357 2008), in order to illustrate that EPS can contain N and P. It is likely that the EPS of glacier algae contains relatively
- 358 more C than N and P, given the depauporate nature of the melting ice surface. EPS certainly seem to be associated
- 359 with the binding and retention of particulates in the weatheirng crust, and it follows at least some of the DON and
- 360 DOP may also be associated with this EPS.
- 361 These two mechanisms of retention of dissolved organic nutrient in the weathering crust, either in temporarily stored
- water or as EPS, mean that DOC, DON and DOP storage in the weathering crust is transitory, and given the
- 363 dynamic response of the weathering crust to climatic perturbations, it is very likely that export of these species from
- the weathering crust will be pulsed, rather than constant. For example, large melt events, accompanying summer
- 365 storms, may result in wholescale melting of the weathering crust (Tedstone et al., In Review), and export of a
- 366 significant quantities of the dissolved organic phases contained within them. By contrast, that stored in the
- 367 weathering crust towards the end of the ablation season, when the crust is freezing and water flow paths are closing,
- 368 may be retained in the frozen ice surface overwinter. For example, Musilova et al., (2017) reported that at the
- 369 margin of the GrIS, DOC remaining in surface ice at the end of the ablation season likely froze over winter and was
- 370 released the following ablation season through ice melt.
- 371

### 372 4.4 Stoichiometry of different supraglacial environments

373 DOC:DON:DOP ratios in melted surface ice samples may provide information on whether N or P is the limiting
 374 nutrient within supraglacial environments in the Dark Zone. For example, Table 1 shows that DON:DOP ratios

375 increase systematically, from 49, 78 to 120, for low, medium and high impurity surface ice environments 376 respectively, as do DOC:DOP ratios (800, 1200, 2000). By contrast, DOC:DON ratios remain relatively stable for 377 the surface ice habitats (16, 16 and 17 respectively). This could indicate that P is limiting for the glacier algal 378 community. However, this does not quite tie in with the DIP data presented in Fig. 4, which shows that measurable, 379 if low, concentrations of P are usually present in the melting surface ice. Rather, NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> are below detection, presumably as a result of uptake by phototrophs, and NH<sub>4</sub><sup>+</sup> is the only measurable DIN species, presumably as a 380 381 result of heterotrophic activity. Phototrophs preferentially utilize both NH4<sup>+</sup> and NO3<sup>-</sup>, and the presence of both DIN 382 and DIP in melting surface ice environments, irrespective of visible particulate loading, and therefore of algal cell 383 abundance, suggests that a factor other than macronutrient concentration is limiting algal growth. Table 1 shows that 384 mean NH<sub>4</sub><sup>+</sup> concentrations in the melting surface ice are in the range of  $0.6 - 1.0 \mu$ M. We noted above that there is 385 no readily available C:N ratio of glacier algae in the literature, but typical C:N ratios of sea ice algae are in the range 386 of 12-46 (Niemi and Michel, 2015). This implies that somewhere in the range of  $7.2 - 26 \,\mu\text{M}$  of C could be 387 additionally fixed, if all the N was taken up by phototrophs with this range of C:N ratios. We also noted that it is 388 even more difficult to find C:N:P ratios of glacier algae, but should the C:P ratio be in the region of 100:1 to 1000:1, 389 then P demand will be  $0.007 - 0.46 \mu$ M. Table 1 shows that the mean concentration of DIP in melting surface ice is 390 in the range of 0.03 to 0.05  $\mu$ M, which suggests that P is not a limiting macronutrient on primary production. The 391 systematic change in DON:DOP and DOC:DOP ratios with increasing visible impurities, a proxy for algal cell 392 abundance, could be driven by the amount of P per cell that is potentially available at the high light intensity of the 393 ablation season (> 1500  $\mu$ mol photons m<sup>2</sup> s<sup>-1</sup>). The DIP content of the surface ice is relatively constant (Table 1) 394 given the much larger change in cell abundance as the visible impurities increase. The combination of lower P 395 availability at high light intensity results in an increase in the C:P ratio of phototrophs in other aquatic environments 396 (Hessen et al., 2013). It is plausible that this too happens with glacier algae, and that subsequent decomposition 397 products and EPS will likewise have higher DOC:DOP ratios as a consequence.

398

### 399 5. Conclusion

400 We conclude that DIN and DON concentrations in the melting surface ice of the Dark Zone on the GrIS are 401 markedly different from those documented in ice cores to date. Wolff et al., 2013 reported DIN, principally in the 402 form of NO3<sup>-</sup>, dominating the initial composition of ice melt, yet in the present study, DON dominates the melting 403 surface ice environments which host blooming glacier algae. Furthermore, DIN in these environments is exclusively 404 present as  $NH_4^+$ , and  $NO_3^-$  is below the detection limit (0.64  $\mu$ M). There is relatively little data on the P content of 405 Greenland ice, but we find that DOP dominates DIP in melting surface ice habitats, although DIP is usually present 406 in measurable quantities (LoD =  $0.02 \mu$ M). The presence of both NH<sub>4</sub><sup>+</sup> and DIP, even in heavily colonised melting 407 surface ice, suggests that factors other than macronutrient limitation control the blooms. We speculate that dissolved 408 macronutrients are held in the melting surface ice because of the architecture of the weathering crust, and/or because 409 EPS is retained within the melting ice latticework. The former controls the hydrology and the connectivity of water

410	flow paths and water storag	e in the surface ice	, and the latter may	y be involved with th	e retention of particulates in
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411 the surface. There is currently no data on C:N:P ratios of EPS exuded by glacier algae, but EPS of other autotrophs

412 does contain both N and P in association with C. DOC:DON ratios are relatively constant in melting surface ice, but

413 DOC:DOP ratios increase markedly with increasing algal cell counts. This may be attributable to the increasing cell

to DIP ratio, which, at high light intensity, increases the C:P ratio of autotrophs in other freshwater environments

415 (Hessen et al., 2013). This could be seen as a beneficial adaption to algal life in melting ice surfaces, where P

416 sources are limited, since blooms are not so dependent on P as a consequence.

417

### 418 Data Availability

All data will be made available upon acceptance and publication of the article. Data will be inputted into an openaccess file.

421

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426

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435

### 436 Author contribution

437 MT, AA and MY conceived and designed the study. AH, CW, MT, AA, AT, JM, JC and the Black & Bloom group

438 collected the samples. CW provided algal counts for the mid to late ablation periods. AH conducted all the nutrient

439	analysis and was aided by FS in the instrument maintenance and data analysis. AH wrote the paper with inputs from
440	MT, CW, AT and AA. All authors reviewed the final manuscript.

### 442 Competing Interests

- 443 The authors declare they have no conflicts of interest.
- 444

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450 Figure 01. Map showing location of Camp BLACK & BLOOM 2016 (67°04'43.3"N, 49°20'29.7"W).

451 Background image sourced from Sentinel 2, taken on 26/7/2016.

452



(d)

(e)



453

454 Figure 02: The five supraglacial habitats sampled: (a) ice with low visible impurities, (b) ice with medium
455 visible impurities, (c) ice with high visible impurities, (d) supraglacial stream, (e) cryoconite hole.



456

Figure 03: Dissolved Organic Nitrogen (DON) and Dissolved Inorganic Nitrogen (DIN) concentrations for all
surface habitats (mean ± SE, n=19 for L,M,H, n=10 for S and n=14 for C). L- ice with low visible impurities,
M- ice with medium visible impurities, H- ice with high visible impurities, S- supraglacial stream water and Ccryoconite hole water. LoD line depicts the limit of detection of the instrument. *Uppercase letters* denote

461 homogeneous subsets derived from post-hoc TukeyHSD analysis on a significant 1-way ANOVA in relation to

dissolved nitrogen phase. *Lowercase letters* denote T-test comparisons in relation to habitat type.



463

464 Figure 04: Dissolved Organic Phosphorus (DOP) and Dissolved Inorganic Phosphorus (DIP) concentrations for

465 all surface ice habitats (mean  $\pm$  SE, n=19 for L,M,H, n=10 for S and n=14 for C). L- ice with low visible

466 impurities, **M**- ice with medium visible impurities, **H**- ice with high visible impurities, **S**- supraglacial stream

467 water and C- cryoconite hole water. LoD line depicts the limit of detection of the instrument. *Lowercase letters* 

468 denote T-test comparisons in relation to habitat type.



Figure 05: Dissolved Organic Carbon (DOC) concentrations for all five surface habitats (mean ± SE, n=19 for
L,M,H, n=10 for S and n=14 for C). L- ice with low visible impurities, M- ice with medium visible impurities,
H- ice with high visible impurities, S- supraglacial stream water and C- cryoconite hole water. LoD line depicts
the limit of detection of the instrument. *Uppercase letters* denote homogeneous subsets derived from post-hoc
TukeyHSD analysis on a significant 1-way ANOVA in relation to habitat type.





Figure 06: Algal cell abundance in ice surface ice habitats (mean ± SE, n=19 for each habitat). L- ice with low
visible impurities, M- ice with medium visible impurities and H- ice with high visible impurities. *Uppercase letters* denote homogeneous subsets derived from post-hoc TukeyHSD analysis on a significant 1-way ANOVA
in relation to habitat type.





482

Ablation Season Progression

- 483 Figure 07: Conceptual diagram of the supraglacial environment in the Dark Zone of the GrIS. Black dashed
- 484 lines represent nutrient inputs to all supraglacial environments. Green lines represent hypothesized nutrient
- 485 inputs utilized by ice algal blooms. Arrow thickness represents relative nutrient concentration.

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- 666 Table 01: Summary statistics for dissolved macronutrient (N and P) and DOC concentrations in the five
- 667 supraglacial habitats. DON, DIP, DOP and DOC denote Dissolved Organic Nitrogen, Dissolved Inorganic
- 668 Phosphorus, Dissolved Organic Phosphorus and Dissolved Organic Carbon respectively.

 $\mu$ M; nutrient ratios are in  $\mu$ M/ $\mu$ M.

	Ice Habitat			Supraglacial Stream	Cryoconite Hole	Field Blank
	Low	Medium	High			
$NH_4^+$	0.91±0.26	0.62±0.21	1.0±0.31	1.0±0.38	0.87±0.25	0.80±0.32
	0-3.8	0-2.9	0-4.3	0-3.1	0-2.7	0-2.6
NO <sub>2</sub> <sup>-</sup>	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	0	0	0	0	0	0
NO <sub>3</sub> -	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	0	0	0	0	0	0
DON	5.1±1.1	11±2.0	14±1.7	0.00±0.00	0.70±0.32	0±0
	0-10	0-40	3.2-27	0-0.82	0-3.2	0
DIP	0.03±0.02	0.07±0.02	0.05±0.01	0.01±0.01	0.06±0.02	0.00±0.00
	0-0.27	0-0.44	0-0.20	0-0.04	0-0.23	0
DOP	0.10±0.02	0.15±0.02	0.12±0.01	0.07±0.03	0.07±0.02	0.00±0.00
	0-0.27	0-0.48	0-0.25	0-29	0-0.22	0-0.04
DOC	83±24	173±30	242±44	30±13	50±33	12±7.7
	0-350	29-451	0-636	0-84	0-435	0-35
DON:DOP	49.3	78.9	116.8	0.00	9.4	Na
DOC:DOP	797.8	1166.2	2013.3	455.3	671.3	Na
DOC:DON	16.2	15.6	17.2	Na	71.3	Na
DIN:DIP	27.2	8.4	19.6	74.1	15.5	Na
Sample Size (n)	19	19	19	10	14	9