Habitat and season determine the interplay between DOM pool and bacteria in subarctic freshwaters

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Abstract

Carbon in lakes is a complex mixture of terrestrial carbon from the catchment and algal carbon from the in-lake production. Both of them serve as substrates for bacterial growth, but their composition and availability differ. Here we show how terrestrial and algal carbon compounds are linked to the bacterial metabolism and community composition (BCC) in three different habitats of subarctic freshwaters. We measured dissolved organic matter quality indices including different components of algal and terrestrial carbon together with bacterial metabolism and BCC. The samples were collected from 1) lake inlets representing habitats influenced by allochthonous carbon arriving to lakes, 2) lake outlets i.e. habitats integrating carbon from the in-lake algal production and 3) ponds that contain carbon with a mixed signature of terrestrial and algal compounds. Terrestrial drainage and associated nutrients and humic carbon compounds supported higher bacteria production but lower bacterial diversity than carbon from the algal production. There was a high variation in BCC which was best explained by the habitat-specific concentrations of nutrients, dissolved organic carbon, fulvic acids and proteins. The results also show strong variation related to pool size and seasonality, and emphasize the winter period that has previously gained little attention in aquatic studies.

INTRODUCTION

Dissolved organic matter (DOM) in surface waters is a complex mixture of humic substances, carbohydrates, carboxylic acids, amino acids and nutrients. These compounds originate from terrestrial and aquatic production, and they are a major energy source for the aquatic food webs. The main energy source for the food webs in transparent lakes (dissolved organic carbon; DOC < 5 mg L^{-1}) is assumed to be DOM produced by autotrophic phytoplankton (1, 2), but some additional energy comes from the terrestrial fraction of DOM (3, 4, 5). The availability of different fractions of DOM to bacteria differs tremendously with amino acids being readily uptaken by most bacteria, while the recalcitrant compounds in humic substances, such as lignin, can be degraded only by more specialized groups (6). The DOM quality, or proportions of different fractions of DOM may also vary depending on the type of the water body and the location within it (7, 8, 5). Further, the vegetation in the catchment has a prominent impact as the DOM from catchment with coniferous forest has been shown to support higher bacterial production than DOM from bog area (7, 9). Within the water column autochthonous amino acid-like DOM has been reported to dominate in the euphotic mixed layer whereas in the deeper layers humic-like DOM is overrepresented (10). Less is known about the horizontal and habitat-specific variations in organic carbon bioavailability.

The variation in carbon quality shapes the bacteria residing in lakes. It has been shown that bacterial community composition (BCC) and metabolism are linked to

carbon source (11, 12, 13, 14, 15, 16, 17) and to the quality of the carbon within different sources (18, 19, 20). Further, it has been shown that the composition of bacterial community plays a significant role in the rate of carbon mineralization (21), and while the bacteria are processing DOM, some compounds are produced while others get degraded (22, 23). Thus, the bacteria are influenced by the DOM milieu but also contribute to defining the quality and quantity of carbon in lakes. Another factor that needs to be taken into account especially at high latitudes is seasonality, which adds up to the changes in quantity and quality of DOM (24, 25). Seasonal changes in solar radiation, runoff, primary production and water chemistry all influence DOM properties (8). For example, DOM spectral slope distributions have been shown to differ between summer and winter (10) and under the ice DOM has been shown to have higher presence of terrestrially derived carbon (26).

DOM characteristics should also be influenced by lake morphometry, although this has received little attention. It is well known that morphometry creates differences in habitats and influences photo exposure, residence time, velocity, primary production and species composition, all of which contribute to defining DOM. For example, the size of the water body has been shown to influence the bacterial diversity (27). Similarly, the vertical location in the water column plays a critical role as photochemical processes in shallow euphotic zones make DOM more bioavailable to bacteria compared to DOM in dark (28). The efficiency of DOM transformations drops when the residence time increases, suggesting that the reactivity of organic matter is reduced as it ages (29). Thus, it can be expected

that DOM varies between different habitats of the lake and also between water bodies of different sizes, resulting in variation in microbial community compositions and microbial processes between these habitats.

Our objective was to test hypothesis that habitat-specific characteristics influence bacterial metabolism and BCC by regulating the organic matter quality. Because aquatic DOM quality (i.e. composition) reflects the dynamic interplay between DOM sources and biogeochemical reactions, we hypothesized that the DOM biogeochemistry and bioavailability have variation based on seasonality and habitat within a water body. To test this, water samples representing four different seasons were collected from nine locations in six subarctic Finnish water bodies. These included i) lake inlets representing habitats that should be influenced by allochthonous light-exposed carbon arriving to lakes, ii) lake outlets i.e. habitats that integrate carbon from in-lake algal production including euphotic and aphotic depths and iii) ponds that should contain carbon with a fast renewal time and a mixed signature of terrestrial and algal compounds. The bacterial metabolism and community composition were analyzed in relation to DOM quality (carbon compounds, spectrophotometric properties, nutrients etc.) and physical attributes of the habitats.

MATERIALS AND METHODS

Study site and sampling

We sampled three ponds, three lake inlets and three lake outlets in the Kilpisjärvi region, subarctic Finnish Lapland (69ºN, 20ºE). The sites were located between 473 and 850 m

a.s.l. in the subarctic landscape where treeline of mountain birch (*Betula pubescens* subsp. *Czerepanovii*) is at 600 m a.s.l (Table 1). All sites were sampled five times in 2011; in February (winter), in early May (spring), in mid-June just after the ice break up (ice break up), in late July (summer) and in early October (fall). Ponds were sampled in the middle of the pond and the lakes were sampled from near the inlet and outlet rivers. Samples were collected with a 2 L Limnos water sampler as integrated samples from the first meter of the water column. Water temperature was measured in the field with YSI Professional Plus (Yellow Springs, OH, USA). Total phosphorus (TP) and nitrogen (TN) concentrations were analysed from sieved (50 µm) water using standard methods (http://www.sfs.fi/). For the determination of chlorophyll a (Chl-*a*) concentrations, 1-2 L were filtered onto GF/F filters. Samples were collected in duplicate and stored at -80^{0}C until fluorometric analysis according to Nusch (30). Dissolved organic carbon (DOC) concentration was analysed from water filtered through 0.2 µm prerinsed cellulose acetate filters using Shimadzu TOC-5000A carbon analyser.

Quality measurements of carbon

A set of indicators for the quality of carbon was measured using spectrophotometric and spectrofluorometric methods. All the measurements were carried out for water that had been filtered through a 0.2 µm prerinsed cellulose acetate filter and stored in the dark at + 4°C. Scanning of absorption coefficient at 320 nm (a320), specific UV-absorbance index (SUVA) and the spectral slope (S289) was performed in a dual-beam mode with Cary 100 UV-Vis spectrophotometer (Agilent) using a 10-cm quartz cuvette. Samples were corrected against MilliQ water. Absorption coefficient at 320 nm (a_{320}) was measured as indicator of

coloured dissolved organic carbon (CDOM) concentration. Values were calculated from absorbance measurements (A_λ) at 320 using $a_\lambda = 2.303/L \times A$, where L is the length of the cuvette in meters (31). SUVA, which is an indicator of the share of terrestrially derived organic carbon (32, 33), was calculated from DOC normalized absorbance at the wavelength 254 nm with higher values indicating a higher share of terrestrial carbon compounds in the sample (34). S289, indicating the amount of carbon compounds likely related to autochthonous production (35), was calculated from the spectrophotometric measurements. For the calculation an absorption slope was calculated for the 20 nm interval between 279-299 nm. Algal derived carbon has a maximum at 289 nm, thus the higher the S289 values the bigger is the share of carbon compounds from autochthonous production (35). There are some environmental factors that could have compromised the fluorometric measurements, most important such factors being iron and pH. According to previous measurements of the lakes in the area the iron concentration is low (mean of 37 lakes 0.24 mg L^{-1}) (36) and not likely to cause a bias. Also, the pH was stable within the samples (6.5 ± 0.5) and should not interfere with the measurements. Thus, we are confident that our measurements were correct and reliably showing the true variation in carbon quality.

Composition of different humic, fulvic and protein-like carbon compounds was identified with excitation-emission matrixes (EEM) using a spectrofluorometer Cary eclipse (Agilent). They were measured across excitation (220-450 nm) and emission (240-600 nm) wavelengths with 5 and 2 nm increments, respectively. EEMs were corrected for inner filter effect (37), machine specific biases, background scattering (38) and were standardized to Raman units (R.U.) (39). Raman and Rayleigh scattering were removed

using the DOMfluor 1.7 toolbox in MATLAB 2008b (MathWorks, Natick, MA, USA) as recommended in Stedmon and Bro (40). The obtained EEMs were inserted to the parallel factor analysis (PARAFAC) model based on samples collected from > 100 lakes from boreal, subarctic and arctic lakes from Finland, Canada and Greenland (data not shown). The model was used to identify and calculate intensities of all main carbon components in the sample. Five different components (C1-C4, C6) identified from the EEMs were highly correlated with each other (correlation coefficients for all pairs > 0.87, $p < 0.0001$) and were pooled for the analyses as terrestrial humic-like compounds, while the component C5 was considered as a fulvic acid and the component C7 as protein, according to Fellman *et al*. (41) (Supplementary Fig. 1). The compounds C1-C4 and C6 are widespread terrestrial humic-like components originating e.g. from forest streams and wetlands (41, 42, 43, 44). C5 have been associated with irradiated DOM that has been microbially degraded (43). C7 resembles amino acid-like tryptophan found commonly in different freshwater environments (41).

Bacterial metabolism analyses

Bacteria production (BP) was measured using ^{3}H-leucine (specific activity 73 Ci mmol^{-1}) incorporation with a centrifugation method (45). Incubations were started within 2-6 hours after sampling using a leucine concentration of 30 nM and incubation time of 3 h according to the saturation curves in Roiha *et al*. (20). Incubations were conducted in dark in a constant temperature of 6.4±0.5 °C which deviated from the in-situ field temperatures 5.1 ± 2.1°C. TCA was added to terminate incubation (TCA; 5 % final concentration) after which the samples were stored at -20°C until centrifuging and radioassaying according to

Smith and Azam (45). Bacterial respiration was measured as oxygen (O_2) consumption using fibre-optic O_2 mini-sensors (Fibox 3, PreSens Precision Sensing GmbH, Regensburg, Germany) (46). Filtered (3 µm) water samples were incubated in top-filled 500 ml Erlenmeyer vials closed with airtight silicone stopper. Samples were incubated as above but in a water bath to further reduce temperature variability as this infers with O_2 sensor reading. The incubations were let to stabilize for few hours before the first sensor reading. Over the first five days O_2 concentrations were measured 1-2 times a day while the last measurement was taken in the beginning of the next sampling trip (total incubation time 4-6 weeks). BR rates were calculated from the linear slope of O_2 consumption that was converted to carbon units using respiratory quotient (RQ) of 1.0. To estimate actual bacteria metabolism in the sampled sites, the BP and BR values were corrected for *in-situ* temperatures with Q_{10} values according to Berggren *et al*. (47). Such corrections were not applied when the aim was to measure temperature-independent bacteria control. Bacterial growth efficiency (BGE), i.e. bacterial production (BP) per unit of assimilated carbon was calculated using equation 1.

$$(1)\ BGE = BP/(BP + BR)$$

Bacterial community analyses

Unfiltered water samples for DNA extraction were frozen within 2-4 hours of sampling. 300 ml subsample of the frozen water was freeze dried with an Alpha 1-4 LD plus (Christ, Osterode, Germany). DNA extraction, PCR (primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3'); 48) and 454-pyrosequencing were

performed as described in Peura *et al*. (49). The amplicon processing, including quality trimming and noise and chimera removal was done as outlined in Schloss *et al*. (50) using mothur (51). The sequences were assigned into operational taxonomic units (OTUs) using 97 % sequence similarity cutoff, loosely corresponding to bacterial species and OTUs were classified using taxonomic framework for freshwater bacteria introduced by Newton *et al*. (52). Two samples with likely fecal contamination were removed from the sample set. Contamination was likely caused by lowered water level during samplings. Prior to further analysis, the sequence data was resampled to smallest sample size (1153 sequences per sample) using perl script daisychopper.pl (available at http://www.genomics.ceh.ac.uk/GeneSwytch/Tools. html; 53). The sequences are available at the NCBI Sequence Read Archive under project number PRNA244724.

Statistical analysis

Differences in environmental and temperature-corrected bacterial metabolism variables between seasons and habitats were tested using a 2-way ANOVA. Season and habitat were considered as fixed factors in the analysis. Normality and homogeneity of variance were checked with visual examination of residuals (54). Square root transformations were applied to TN and Chl-a, logarithmic (base 10) transformations to a320, fulvic acids, BP and BR, and inverse (x^{-1}) transformation to S289 to achieve ANOVA assumptions. When a factor was significant, *a posteriori* multiple comparison test (Tukey-Kramer) was carried out to identify differences.

Statistical testing of the impact of season, habitat and their interaction to the bacterial community and environmental data structure was done using a Permutational Multivariate

analysis of variance (PERMANOVA; 55) with 999 permutations. Multiple regression analyses were used to identify which environmental variables (TP, TN, Chl-a, DOC, SUVA, S289, humic acids, fulvic acids and proteins) best explained the changes in bacterial metabolism (BP, BR, and BGE). The absorption coefficient a320 was omitted from the model due to its high Pearson correlation with DOC (r = 0.85) and humic acids (r = 0.96). Best model (using forward procedure) was selected according to the lowest value of AICc index. Regression equations were produced with all the dataset and separately for each habitat (pond, inlet, outlet). For the statistical testing of the BCC, all OTUs with more than 100 sequences in the total data were retained in the analysis. Bacterial data were square root transformed prior to generating a resemblance matrix of Bray-Curtis similarities. Environmental data were normalised and Euclidian distances were used to generate resemblance matrix. Pairwise permutation *t*-tests were performed on the factors that were identified as significant in PERMANOVA to identify differences among levels. The effects of season and habitat on BCC were visualized with a Principal Coordinates Analysis (PCO). A similarity percentage analysis (SIMPER) was used to assess the percentage contribution of each OTU to the observed dissimilarities among habitats (pond, inlet, outlet).

Spearman's rank correlations were used to examine relationships between the resemblance matrices of BCC and environmental variables to identify the environmental variables (alone or in subset) that explain best the observed patterns of BCC (BIO-ENV analyses, PRIMER). For this analysis, OTU and environmental variable matrices were constructed using Bray-Curtis dissimilarity (square-root transformed) and Euclidean distances respectively (see 56, 57). Diversity indices and relationships between BCC and carbon components were analysed with Spearman's rank correlation in R (58). Shannon

index was used to evaluate the evenness of the community, that is, how evenly the observations were distributed among OTUs (59). To measure the species richness, or the number of different OTUs in samples, we used inverse Simpson's index (60). The software JMP (JMP®, Version 10.0. SAS Institute Inc., Cary, NC, 1989-2012) was used for all univariate tests while PRIMER+PERMANOVA (version 6.1.6; 61, 55) was used for multivariate analyses. A threshold of significance of 0.05 was adopted for all statistical tests.

RESULTS

Environmental variables

Many of the environmental variables had variation based on both, the season and habitat (Table 2, Supplementary Table 1, Supplementary Fig. 2 and 3). The most drastic seasonal variation was seen in temperature which was close to zero in winter while the summer maximum was about 15°C. Total phosphorus (TP) had its maximum in the spring and in the ponds. Also total nitrogen (TN), DOC and proteins had the highest values in ponds, but the difference between ponds and other habitats was significant only in samples from under the ice (winter and spring). The indicator of algal production (S289) was always highest in the outlets but these values were significantly different only from ponds and only in winter and spring. Chlorophyll a (Chl-a), another indicator of algal carbon, was low in all samples (< 1 μg L^{-1}) and no differences between seasons or habitats were detected. Fulvic acids (indicator for microbially degraded DOC) had some habitat and seasonal variation that was expressed with ponds having the smallest amount of these compounds in winter. There were no significant differences in the indicator of the total amount of coloured DOM (CDOM; absorption coefficient a320) or in the fluorescence of humic-like compounds

(indicator of the share of terrestrial carbon in the CDOM), though those were the lowest in the outlets. Several variables in the total dataset were highly correlated with each other, with highest correlations (all $p < 0.0001$) observed between TN and TP (Pearson's correlation $r = 0.88$), DOC and humic-like substances ($r = 0.81$), DOC and TP ($r = 0.68$) and DOC and TN ($r = 0.61$).

According to PERMANOVA, there was a difference in environmental variables according to seasons (Pseudo-$F_{4,21} = 3.92$, $p < 0.001$) with all pairwise comparisons, except for winter – spring, ice breakup – summer and ice breakup – fall, suggesting different conditions ($p < 0.05$ for all). The data structure was also different between habitats (Pseudo-$F_{2,21} = 6.22$, $p < 0.001$) with the ponds being distinct from the inlets (Permutation pairwise test, $t = 2.22$, $p = 0.005$) and outlets ($t = 3.21$, $p < 0.001$) while inlets and outlets were similar.

Bacterial metabolism

Bacterial metabolism exhibited large seasonal variation (bacterial production (BP): $F_{4,29} = 8.23$, $p < 0.0001$; bacterial respiration (BR): $F_{4,27} = 3.75$, $p = 0.0150$; bacterial growth efficiency (BGR): $F_{4,27} = 18.71$, $p < 0.0001$) (Fig. 1) and there was also marked variation between the habitats for BP and BGE (Fig. 2). Highest BP values were measured for the ponds (4.5 μg C L^{-1} d^{-1} ± 3.9) and inlets (1.5 μg C L^{-1} d^{-1} ± 0.8) during the ice breakup while the maximum BP in the outlets (1.0 μg C L^{-1} d^{-1} ± 0.5) was reached in summer. In all habitats the BP was lowest in fall with values < 1 μg C L^{-1} d^{-1}. BR followed a different seasonal pattern, with the highest values measured in the ponds in the spring (20.7 μg C L^{-1} d^{-1} ± 4.4) and the lowest in the inlets in the summer (3.2 μg C L^{-1} d^{-1} ± 1.2). BGE was rather low and the maximum values, 20-39 %, were reached in the summer. There was also

293 variation between the habitats, with the ponds and inlets providing an environment that
294 allowed for higher BGE than that of the outlets (Fig. 2).
295 Multiple regression models were constructed to assess the importance of each variable that
296 was confirmed to have significant impact on the BP, BR and BGE. The models explained up
297 to 62% of the variance in BP, 87% in BR and 26% in BGE (Table 3). Overall, TN explained
298 the largest share of the bacterial metabolism (on average 45 %), but there was a lot of
299 variation between sites and processes. The highest explanatory degree was acquired for
300 the BR in ponds, where concentrations of TN and Chl-a explained 66 and 21 % of the
301 variation, respectively. When models selected algal carbon variables (i.e. S289 and Chl-a)
302 their negative coefficients showed that they were negatively linked to bacterial
303 metabolism. Models for all data and for specific habitats retained nearly the same variables,
304 however, for certain habitat – bacterial variable pairs the model could not produce any
305 significant explanatory factors. This was most likely due to the low number of observations
306 on which these data sets were based.
307
308 *Bacterial community and interactions with the environment*
309 There was a clear change in the community structure along the season (Pseudo-$F_{2,22}$ =3.64,
310 $p < 0.001$) with all season pairs except for winter-spring, spring-fall and summer-fall being
311 different from each other (Supplementary Fig. 3). Also the communities residing in the
312 habitats were different from each other (Pseudo-$F_{2,22}$ =5.76, $p < 0.001$). The pond
313 communities were more similar to the inlet (pair-wise test $t = 1.77$, $p = 0.019$) than to the
314 outlet communities ($t = 3.76$, $p < 0.001$), but also the inlet and outlet communities were
315 distinct from each other ($t = 1.61$, $p = 0.037$). The BIO-ENV analyses suggested that the

316 environmental variables that best explained the OTU distribution among habitats were TP,
317 DOC, fulvic acids and proteins (Table 4). The proteins represent the readily available,
318 amino acid-like fraction of DOM and they were the carbon compounds that alone best
319 captured most of the variability. The Spearman correlations further suggested connections
320 between certain bacterial groups and carbon fractions (Fig. 3). For example, most OTUs
321 associated with flavobacterial tribe Flavo-A3 were positively correlated with humic
322 fraction and SUVA-index. Both of these are indicators of the share of terrestrial DOC. Also
323 all OTUs associated with betaproteobacterial tribe Janb had positive correlation with SUVA.
324 The indicator for algal carbon (S289) had correlations for example to alphaproteobacterial
325 lineage LD12, betaproteobacterial LD28 and verrucomicrobial LD19. The protein fraction
326 appeared to favor only a few OTUs and all of the protein correlations were weak.
327 According to the SIMPER analysis, the difference in the BCC between habitats was caused
328 primarily by the different abundance distribution of OTUs 10973, 10878, 10854, 10771,
329 10891, 10100 and 10977. Also, the conformation of the community was distinct between
330 ponds and outlets with ponds having few very abundant OTUs, whereas the outlets were
331 harboring many small ones (Fig. 4). The ponds were more abundant especially with taxa
332 such as *Betaproteobacteria* (tribes PnecC (OTU 10973) and Lhab-A2 (OTU10878)) and
333 *Bacteroidetes* (clade bacIII-A (OTU 10854)) than inlets and outlets. Correspondingly, the
334 inlets and outlets had a higher abundance of *Actinobacteria* (tribe Myco (OTU 10771) and
335 clade acI-A (OTU 10977)), *Verrucomicrobia* (OTU 10891) and *Alphaproteobacteria* (tribe
336 LD12 (OTU 10100)). A detailed analysis of BCC revealed that the ponds and outlets had
337 rather distinct communities while the inlet community was more of a mixture of the two
338 former ones (Fig. 4). The diversity of bacterial communities was affected by habitat, but not

339 by season. According to Shannon index the communities in inlets and outlets had more
340 even communities than ponds ($\chi^2 = 13.99$, $p < 0.001$; Supplementary Table 2) and also the
341 species richness (Inverse Simpson index) was higher in inlets and outlets than in ponds (χ^2
342 $= 11.97$, $p < 0.005$).
343

DISCUSSION

Interaction between DOM quality, seasonality and habitats

The results strongly suggest that crude quantity measurements of DOC are not sufficient to demonstrate the seasonal and spatial variation in organic carbon in freshwaters, but also the quality of carbon should be taken into account. Consistent with earlier reports from the area (26, 20), the total concentration of DOC was not connected to seasonality. How ever, some of the CDOM fractions (S289, fulvic acids and proteins) did exhibit seasonal variation, which is consistent with earlier observations on fulvic acids (62, 25). We also observed seasonal variation in total phosphorus and nitrogen in ponds and in inlets, but not in outlets. The lack of variation in the outlets is in accordance with the observations of Forsström *et al*. (63) from similar environment.

Seasonal changes in carbon compounds were most pronounced in the ponds, where also the concentration was highest. Under the ice samples from ponds were especially rich with amino acids, which are often considered as an indicator of the labile fraction of DOM and can therefore be used as a predictor of DOM availability (64). This fraction has been suggested to originate from autochthonous production (65), but it can also be produced by bacterial degradation (23). Here the indicator of autochthonous production, S289, was lower in under the ice samples from ponds suggesting that the increased proportion of amino acid fraction during ice cover could originate from bacterial degradation. Thus, here the protein fraction might not predict as much the availability of the carbon, but rather the degradation rate (66).

Consistent with our hypothesis, carbon in the lake outlets was characterized by fraction, which is coming from within lake production. This observation has earlier been supported by Jonsson *et al*. (25), who suggested higher impact of phytoplankton to the carbon in lake outlets than in inlets. Also the concentration of amino acids was higher in the outlets than in the inlets. In lakes the main producers of amino acids are phytoplankton (67, 65), supporting the importance of primary production to the DOM pool in outlets. Conversely, humic substances were more typical for the ponds and inlets. The humic fraction could originate either from the terrestrial production, or from *in situ* production by microbes (68), but based on the low values of S289 it can be assumed that the contribution of fulvic and humic compounds from autochthonous production was minor (35). Thus, it seems that for these variables the volume of the pool was influencing the DOM quality with smallest and fast renewing pond waters showing the highest seasonality and terrestrial impact.

Season and habitat control the bacterial metabolism in subarctic waters

Our analyses suggest that community composition and metabolic activity of subarctic aquatic bacteria is a result of a complex interplay between the community and physical and chemical variables determining the environment. In high latitude ecosystems seasonal changes are major determinants of their physico-chemical environment (69). One of the most notable determinant is temperature, but water as a habitat levels out much of the seasonal temperature variation due to its heat absorbing capacity, which, in turn, is mainly regulated by

water volume. This could be seen in this study, with the ponds having the lowest winter and highest summer temperatures. Also the rate of temperature change followed the size of the water mass. The impact of temperature to the plankton metabolism is known to be more linear in low temperatures, where it usually decreases the metabolic rates (70, 71) and also the rate of bacterial carbon degradation (72). Thus, the low temperature combined with typically low nutrient and carbon concentrations of the harsh environment at higher latitudes usually results in slow bacterial metabolism (73). Our results are well in accordance with this notion as the BP was indeed higher in the ice breakup and summer samples than in under the ice or autumn samples, and BGE peaked in summer during the maximal temperatures.

Higher concentrations of nutrients, humic acids and proteins in ponds supported the highest BP and BGE, while the algal carbon was the greatest contributor to the secondary production in lake outlets. We did not see any link between crude DOC concentration and BP, which is controversial to some earlier studies (74, 20). In contrast, the humic fraction of DOM had a positive impact on BP. Many compounds in the humic fraction of DOC are regarded as calcitrant to bacterial degradation (6) and are reported to support less BP than the non-humic fraction of DOC (75). However, humic compounds are also highly sensitive to photodegradation (28, 43, 76), which generates products that enhance bacterial metabolism (77). The occurrence of humic-like substances was highest during the ice break up in June when also the intensity of solar radiation increased in the water column after the dark winter and was at its annual maximum. Thus, the

413 photodegradation of humic compounds was likely contributing to the increased
414 BP and more so in the shallow ponds and inlets than in the outlets. Further, the
415 potential of the humic compounds for supporting growth was likely also nutrient
416 regulated as indicated by high correlation between nitrogen and phosphorus,
417 DOC and humic compounds. Also the multiple linear regression models indicated
418 that the strongest controlling factor over bacterial metabolism was total nitrogen
419 and total phosphorus concentrations. Also previous studies have suggested
420 phosphorus alone (78, 79), or together with nitrogen (80) to be the limiting
421 factor for bacterial metabolism. In accordance with our results, the availability
422 and quality of organic carbon and the availability of inorganic P and N have been
423 suggested to be key limiting factors of BGE (7, 81).
424 Models also suggested that BP and BGE had a negative relationship to S289,
425 which is the descriptor of autochthonous primary production and BP was higher
426 in the ponds and inlets. In oligotrophic lakes autochthonous production often
427 dominates over bacterial production (5, 82) and primary production is thought to
428 support BP (83, 84). This has been suggested to lead to higher BP in outlets than
429 in inlets (85). One reason for opposite trends in our study and for the negative
430 relationship between the S289 and BP and BGE could be the seasonal effect. Most
431 studies are concentrated in open water season (e.g. 82, 85) whereas very little
432 information exists for winter season. We could see a clear seasonal impact on
433 bacterial production with highest values measured during the open water season.
434 For S289, the pattern especially in the inlets was opposite and it was exhibiting
435 the highest values in under the ice samples, possibly reflecting convective

influence from perennial benthic algae that dominate the overall algal biomass in shallow arctic waters (86, 87). Thus, in order to fully understand the interaction between autochthonous carbon and BP more efforts should be addressed to include also the winter season to sampling schemes.

Implications of carbon quality, season and habitat to bacterial community composition

The combination of molecular microbiology and chemical analyses enabled us to link certain bacterial tribes to carbon fractions across habitats. Our environmental data corroborates the experimental results that members of tribe Lhab would seem to have a preference to algal carbon over terrestrial carbon (16). Another interesting link was seen between two indicators of terrestrial carbon (humic fraction and SUVA) and OTUs associated with flavobacterial tribe Flavo-A3. Bacteria associated with this group have been previously suggested to benefit from phytoplankton exudates (88), which is opposite to what was observed here. However, in a review study 30 % of the previous occurrences of tribe Flavo-A3 were from soil habitats (52), suggesting that Flavo-A3 consists of at least two groups of bacteria with very distinct environmental preferences. Another group in the bacterial community that was associated with terrestrial carbon was tribe Janb. *Janthinobacterium*, the representative genus of tribe Janb, is described as soil bacterium (52). Thus, both Flavo-A3 and Janb could be transient members of the lake community and may originate from the catchment area. There were also groups that were associated only with algal carbon. These

459 included, for example, alphaproteobacterial tribe LD12. This tribe is a sister
460 group of highly abundant marine cluster SAR11 and has been described as typical
461 for freshwater habitats (89). The previous reports suggest that the members of
462 tribe LD12 are poor competitors and their abundance has previously been
463 reported to be negatively correlated with phytoplankton (90). How ever, it has
464 been show that generally there is a lot of variation in substrate and
465 environmental preferences within bacterial tribes (80) and even within species
466 (91, 92) and further, for LD12 specifically it has been suggested that this tribe has
467 wide variations in environmental preferences across lakes (90). Thus, it is not
468 surprising that we see variation in preferences between the members of same
469 tribe residing in different habitats.
470
471 While there were indications of certain substrate preferences for bacterial OTUs,
472 the overall composition of bacterial community was controlled by season and
473 habitat. One factor that can be assumed to influence BCC is temperature. While
474 the data for under the ice BCC of freshwater lakes is scarce, it is known that there
475 is a wide variation in bacterial adaption to extreme temperatures and certain
476 bacteria are better adapted to lower temperatures or to substantial temperature
477 changes (93). This was likely a factor in the organization of winter vs. summer
478 communities in these systems. Another factor likely contributing to differences in
479 seasonal communities was the quality and availability of substrates and
480 nutrients. It has been established that BCC will change depending on the DOC
481 source (e.g. 12, 14, 15) and quality (20). Especially in the ponds carbon quality

was very different during different seasons and likely one of the most important factors contributing to variation in BCC.

Within habitats, pond community assembly was less even and there were less species than in other habitats. This is well in accordance with previous report showing that bacterial diversity increases with lake size (27). Also the observation of difference in composition between the inlet and outlet communities is corroborated by earlier results (85). The variables best explaining differences in OTU distributions between habitats included TP, DOC, fulvic acids and proteins. As stated before, phosphorus is a typical limiting source for bacteria (78, 79), explaining the strong impact.

To conclude, our results show that pond DOM contains the best combination of carbon compounds and nutrients to support BP and stimulate BGE. Further, there are indications of distinct preferences for terrestrial vs. algal carbon among certain bacterial tribes found in subarctic waters. Our study also demonstrates how the spatial variability of DOM in subarctic waters is tightly connected to season and habitat and within those, temperature and the size of the pool are major determinants creating variation beyond what is seen within season or habitat specific studies.

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775 **Legends**

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777 **TABLE 1** Physical characteristics of the sampled lakes and ponds.

778

779 **TABLE 2** Mean values of temperature, total phosphorus (TP), total nitrogen (TN),
780 chlorophyll-a (chl-a), dissolved organic carbon (DOC), specific UV-absorbance index
781 ($SUVA_{254}$), absorption at 320 nm (a_{320}), spectral slope at 289 nm (S289) and fluorescence
782 intensity of humic, fulvic and protein compounds of DOC in Raman units (R.U). Data are
783 shown for five seasons in 2011: winter (W), spring (S), ice break (I), summer (Su), and Fall
784 (F).

785

786 **TABLE 3** Results of different multiple linear regression models (based on lowest AICc) to
787 estimate a) bacterial production (BP), b) bacteria respiration (BR) and c) bacteria growth
788 efficiency (BGE) for all data and for the three studied habitats (pond, inlet, outlet)
789 separately. Total phosphorus (TP), humic acids (Humic), total nitrogen (TN), spectral slope
790 at 289 nm (S289) and chlorohyll-a (Chl-a) were the variables used in the regression models
791 (only significant values are listed). ns: not significant. Partial R^2 below each regression
792 coefficient, N = number of data included, total R^2 (adjusted R^2), small sample size–corrected
793 Aikaike Information Criterion Index (AICc) and root mean square errors (RMSE) are
794 shown.

795

796 **TABLE 4** Combinations of environmental variables (TP, TN, DOC, Chl-a, S289, SUVA, humic,
797 fulvic and protein), taken *k* at a time, giving the four best variables alone and the largest

rank correlation ρ_s between OTU and environmental variable similarity matrices; **bold** indicates the best combination overall.

FIG 1 Bacterial metabolism measured as bacterial production and respiration (μgC L^{-1} d^{-1}) and bacterial growth efficiency (BGE) in different seasons in subarctic Kilpisjärvi waters. W = winter, S = spring, I = ice breakup, Su = summer and F = fall. The letters next to the symbols indicate statistical differences between seasons. Note logarithmic scale on y-axis on the left side.

FIG 2 Average values ± SE of a) bacteria production (μgC L^{-1} d^{-1}) and b) bacteria growth efficiency (BGE) between subarctic ponds, inlets and outlets. The letters above the bars indicate statistical differences between sites.

FIG 3 Heatmap visualizing the Spearman correlations between abundances of OTUs and concentrations of different fractions of CDOM.

FIG 4 Ternary plot showing the distribution of OTUs between the habitats in the dataset. Axes represent the pond, inlet and outlet and the percentage of reads associated with each environment. The size of the symbol indicates number of reads associated with each OTU and taxonomic affiliations are indicated by colors. All OTUs with at least 20 reads are included into the plot.

Supplementary TABLE 1 Summary of ANOVAs showing the effects of Habitat (Ha), Season

(Se) and crossed factors (Ha x Se) on a) temperature, b) total phosphorus (TP), c) total nitrogen (sqrt TN), d) chlorophyll-a (sqrt chl-a), e) dissolved organic carbon (DOC), f) specific UV-absorbance index ($SUVA_{254}$), g) absorption at 320 nm (log a_{320}), h) spectral slope at 289 nm (x^{-1} S289) and fluorescence intensity of i) humic, j) fulvic (log) and k) protein compounds of DOC. Significant values are shown bold.

Supplementary FIG 1 Fluorescence signatures of components C1-C7 identified from the subarctic PARAFAC model. Components 1-4 and 6 (C1-C4 and C6) were combined to represent terrestrial humic-like components whereas component C5 was identified as fulvic microbial component and a commonly found component C7 as a protein-like (Tryptophan) component. Identification is based on Fellman *et al*. 2010 and refs therein.

Supplementary FIG 2 Variation in a) dissolved organic carbon (DOC) and b) total phosphorus concentration between habitats and c) in total phosphorus between seasons. The letters above the bars indicate statistical difference between values. Error bars represent standard error. W = winter, S = spring, I = ice breakup, Su = summer, F = fall.

Supplementary FIG 3 Variation in the environmental variables between seasons and habitats. a) Temperature, b) total nitrogen (TN), c) fulvic acids, d) proteins, e) spectral slope at 289 nm (S289). The letters above the bars indicate statistical difference between values. Error bars represent standard error. W = winter, S = spring, I = ice breakup, Su = summer, F = fall.

844 **Supplementary FIG 4** Principal Coordinate Analysis (PCoA) showing a) the OTU variability
845 between seasons and b) between habitats.

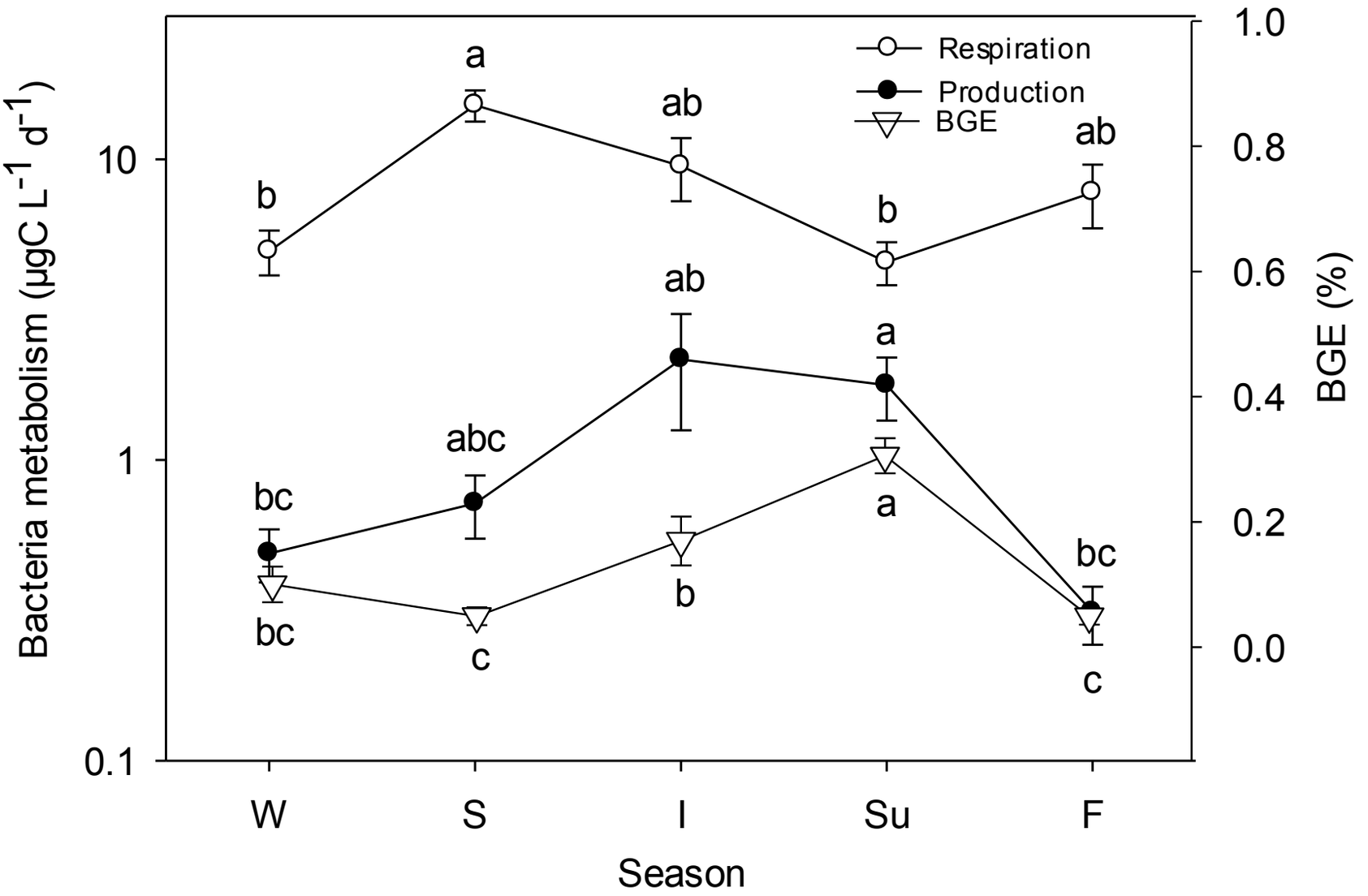


FIG 1 Bacterial metabolism measured as bacterial production and respiration (µg C L^{-1} d^{-1}) and bacterial growth efficiency (BGE) in different seasons in subarctic Kilpisjärvi waters. W=winter, S=spring, I=ice breakup, Su=summer and F=fall. The letters next to the symbols indicate statistical differences between seasons. Note logarithmic scale on the y-axis on the left side.

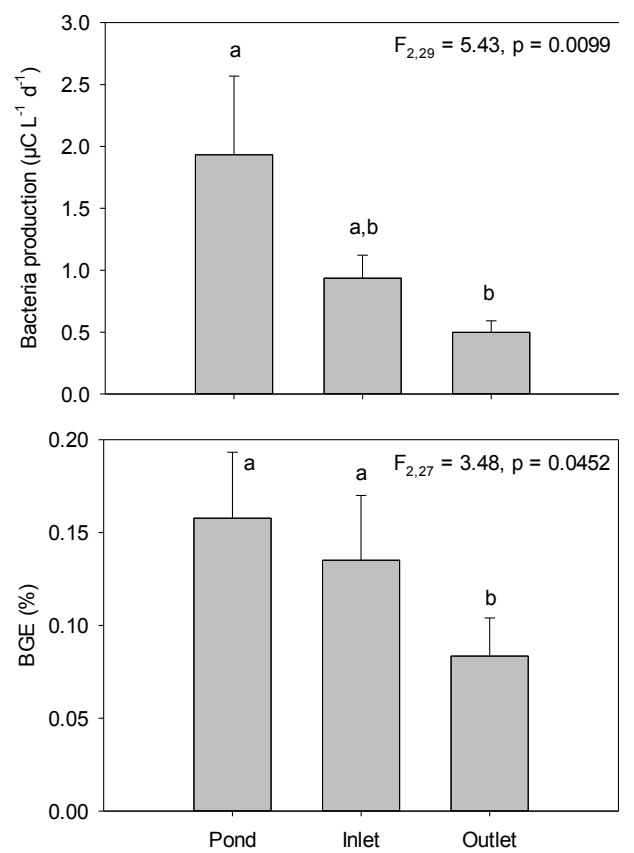


FIG 2 Average values ±SE of a)bacterial production (µg C L^{-1} d^{-1}) and b) bacterial growth efficiency (BGE) between subarctic ponds, inlets and outlets. The letters above the bars indicate statistical differences between sites.

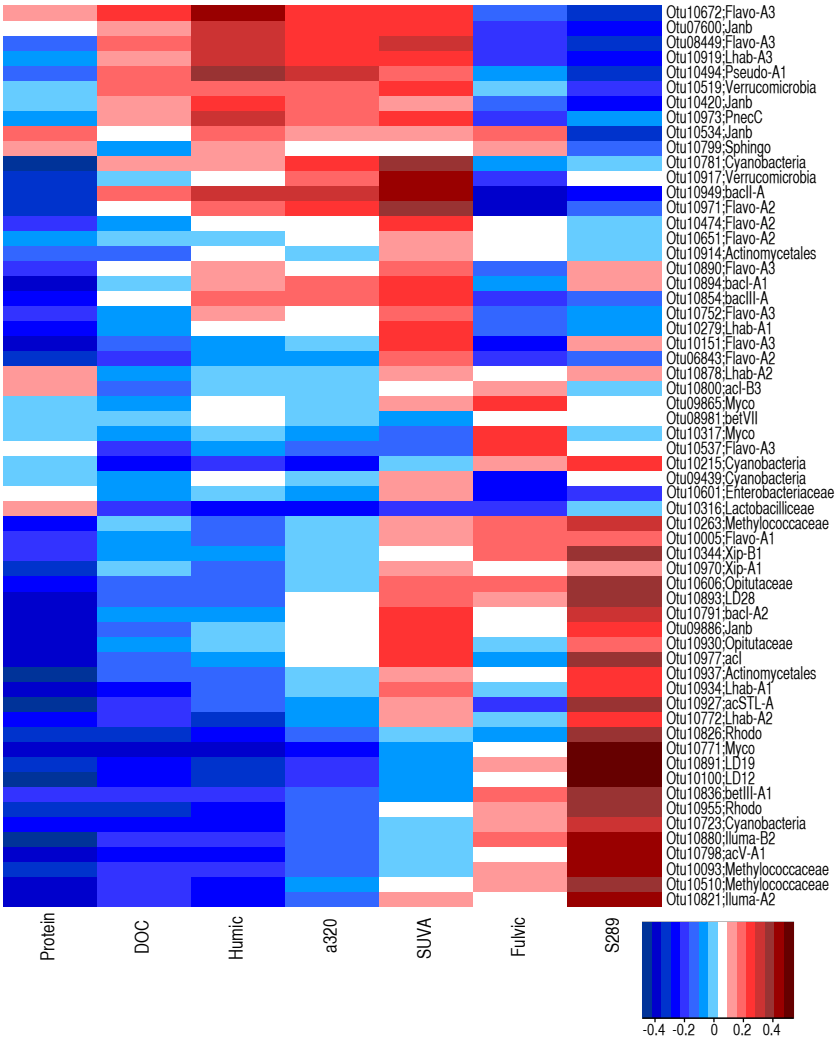


FIG 3 Heatmap visualizing the Spearman correlations between abundances of OTUs and concentrations of different fractions of CDOM.

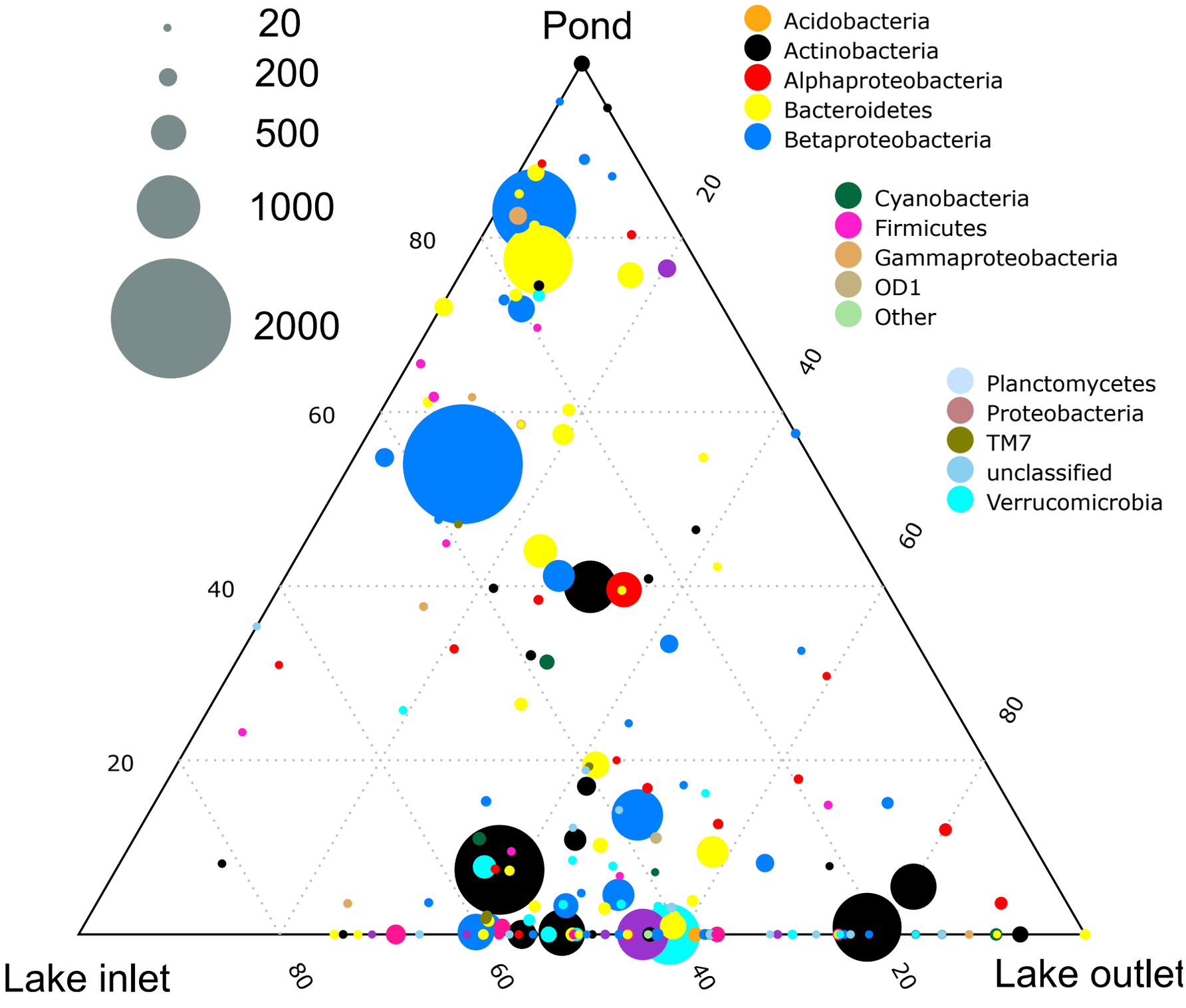
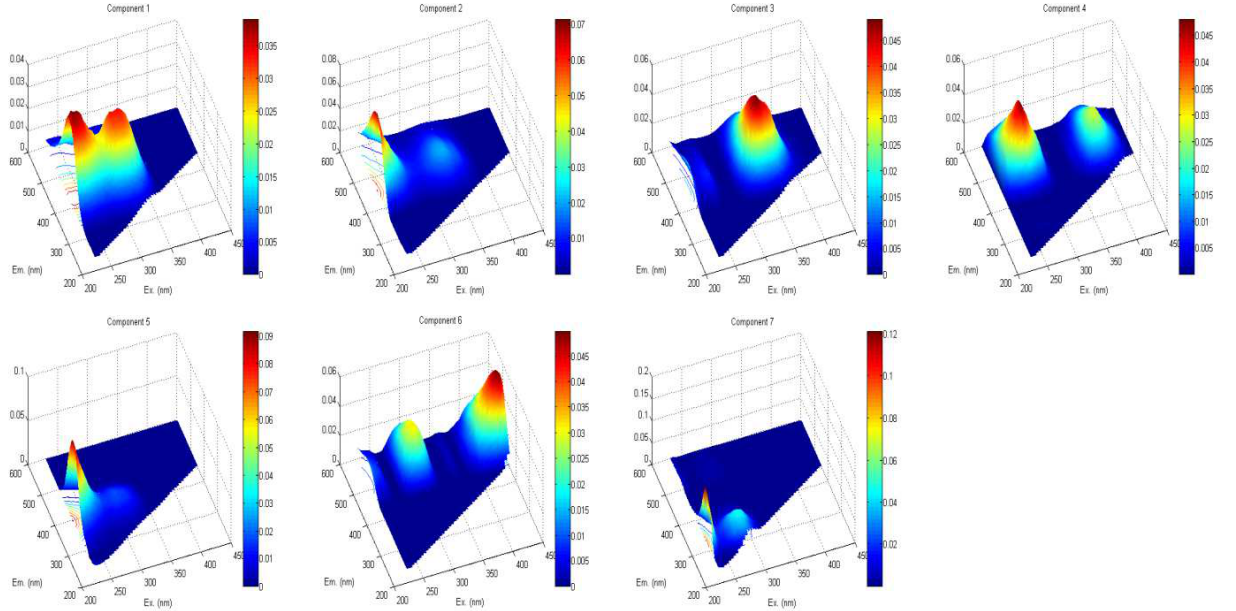
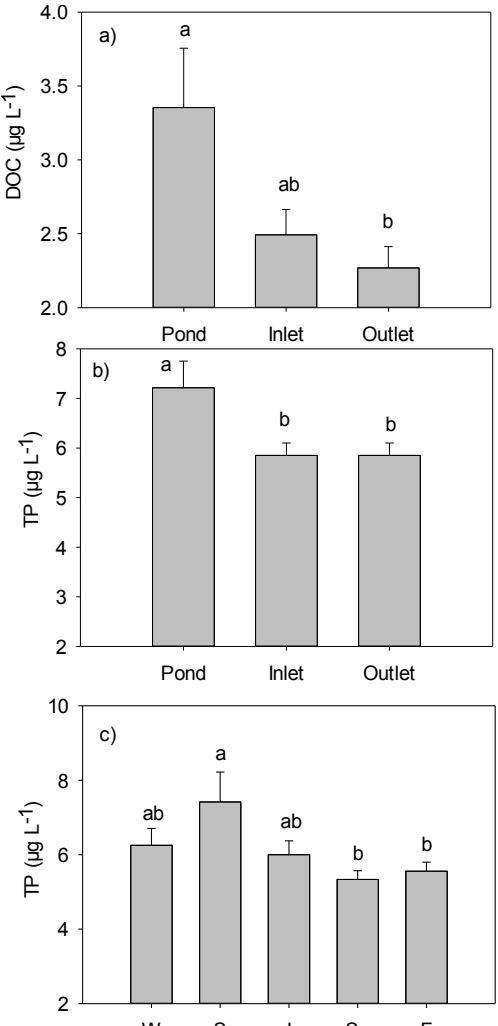


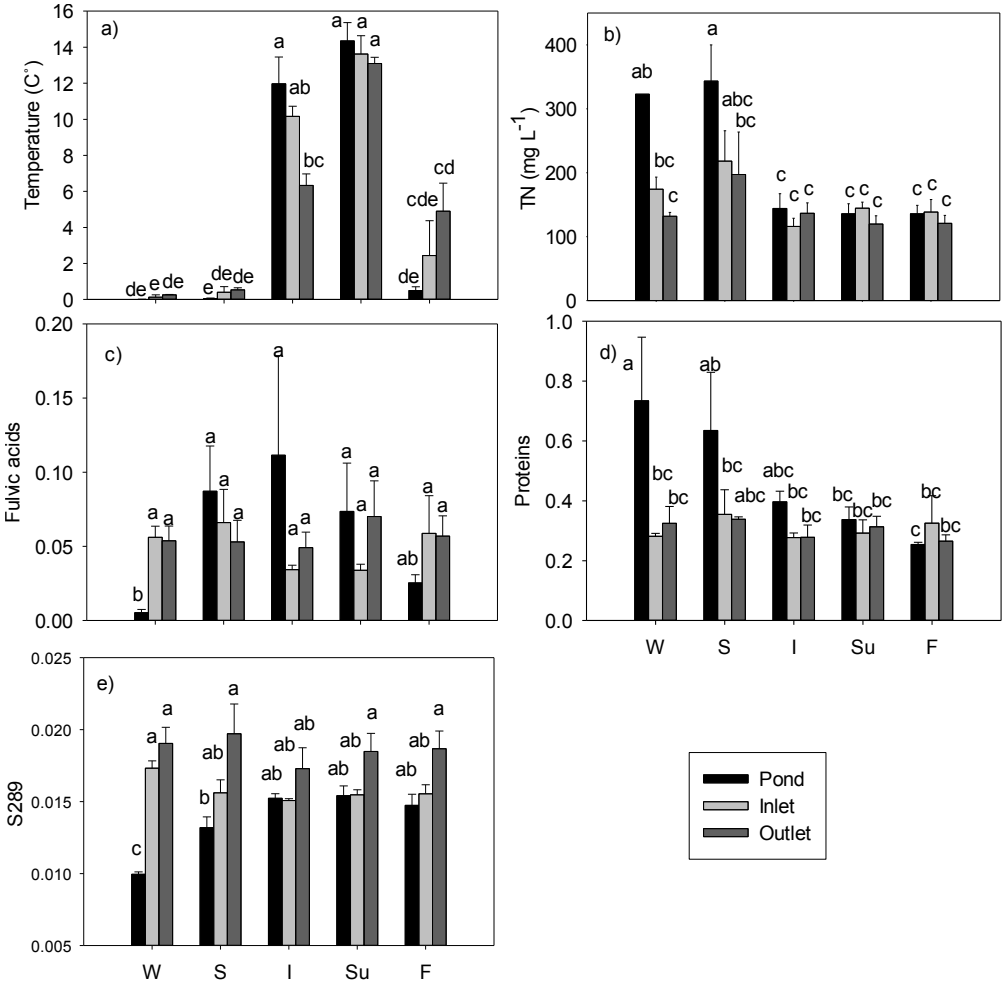
FIG 4 Ternary plot showing the distribution of OTUs between the habitats in the dataset. Axes represent ponds, inlets and outlets and the percentage of reads associated with each environment. The size of the symbol indicates number of reads associated with each OTU and taxonomic affiliations are indicated by colors. All OTUs with at least 20 reads are included into the plot.



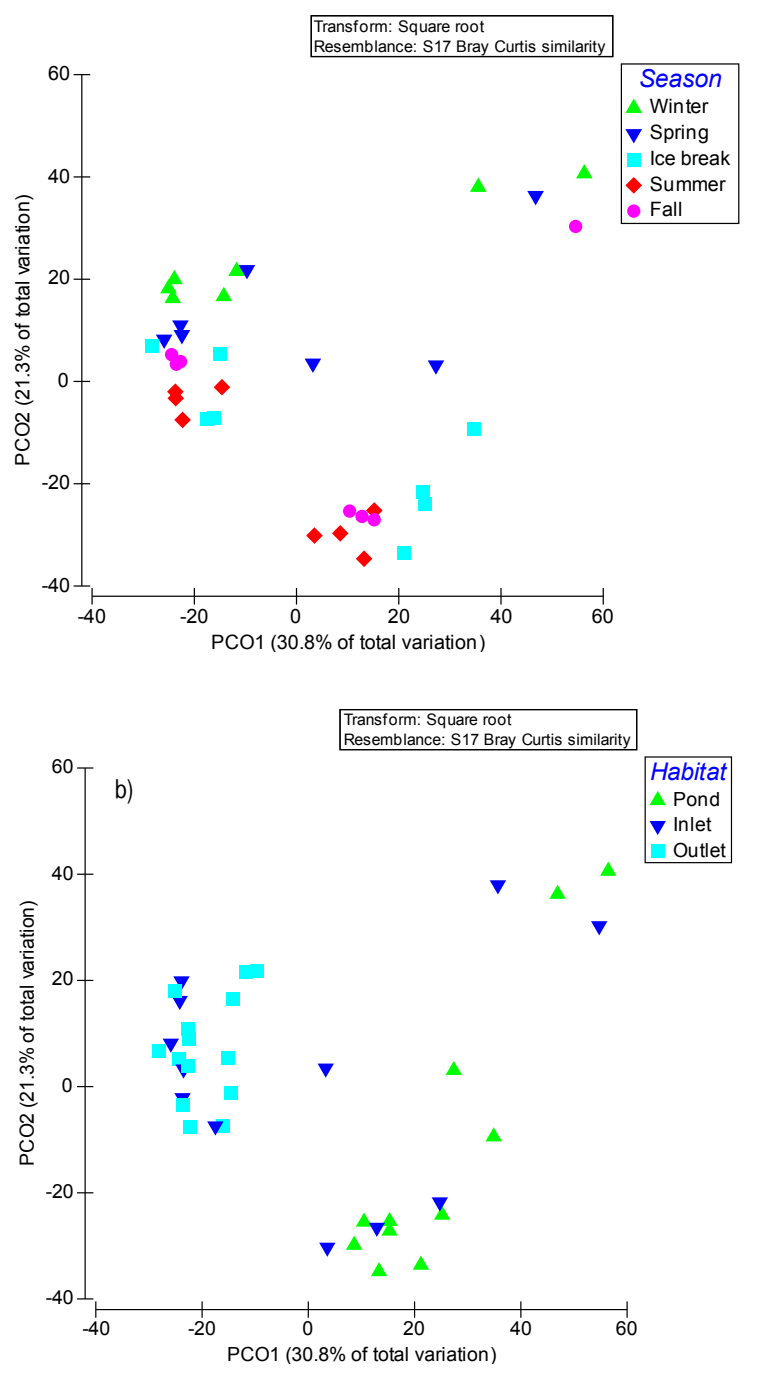
Supplementary Fig 1 Fluorescence signatures of components C1-C7 identified from the subarctic PARAFAC model. Components C1-C4 and C6 were combined to represent terrestrial humic-like component whereas component C5 was identified as fulvic microbial component and a commonly found component C7 as protein-like (tryptophan) component. Identification is based on Fellman et al. 2010 and references therein.



Supplementary Fig 2 Variation in a) dissolved organic carbon (DOC) and b) total phosphorus concentration between habitats and c) in total phosphorus between seasons. The letter above the bars indicate statistical difference between values. Error bars represent standard error. W=winter, S=spring, I=ice breakup, Su=summer, F=fall.



Supplementary Fig 3 VAriation in the environmental variables between seasons and habitats. a) Temperature, b) total nitrogen (TN), c) fulvic acids, d) proteins and e) spectral slope at 289 nm (S289). The letters above the bars indicate statistical difference between values. Error bars represent standard error. W=winter, S=spring, I=ice breakup, Su=summer, F=fall.



Supplementary Fig 4 Principal Coordinate Analysis (PCoA) showing a) the OTU variability between seasons and b) between habitats.

Table 1. Physical characteristics of the sampled lakes and ponds.

	Area (ha)	Catchment (ha)	Depth (m)	Altitude (m)
Pond 1 (Saana 15)	0.7	27	7.5	850
Pond 2 (Saana 11)	0.8	39	2.0	710
Pond 3 (Saana 12)	1.3	-	2.0	710
Lake 1 (Saanajärvi)	70	461	24.0	679
Lake 2 (Tsâhkaljärvi)	113	3396	18.0	559
Lake 3 (Kilpisjärvi)	3370	27100	57.0	473

Table 2. Mean values of temperature, total phosphorus (TP), total nitrogen (TN), chlorophyll-a (chl-a), dissolved organic carbon (DOC), specific UV-absorbance index ($SUVA_{254}$), absorption at 320 nm (a_{320}), spectral slope at 289 nm (S289) and fluorescence intensity of humic, fulvic and protein compounds of DOC in Raman units (R.U). Data are shown for five seasons in 2011: winter (W), spring (S), ice break (I), summer (Su), and Fall (F).

Site	Season	Temp (°C)	TP ($\mu g\ L^{-1}$)	TN ($\mu g\ L^{-1}$)	Chl-a ($\mu g\ L^{-1}$)	DOC ($mg\ L^{-1}$)	a_{320}	$SUVA_{254}$ ($mgC\ L^{-1}m^{-1}$)	S289	Humic (R.U.)	Fulvic (R.U.)	Protein (R.U.)
Pond	W	0.02	8.0	350	0.51	3.9	19.8	3.8	0.0099	0.7031	0.0075	0.7342
Inlet	W	0.13	6.0	174	0.11	2.8	6.6	2.6	0.0173	0.6848	0.0562	0.2815
Outlet	W	0.26	5.3	132	0.09	2.3	4.7	2.4	0.0190	0.4871	0.0538	0.3247
Pond	S	0.05	10.0	381	0.22	4.6	9.1	1.7	0.0132	0.9431	0.0872	0.6349
Inlet	S	0.40	6.6	218	0.16	2.6	6.5	2.6	0.0156	0.6465	0.0660	0.3547
Outlet	S	0.54	5.7	198	0.19	2.2	4.9	2.4	0.0197	0.4904	0.0531	0.3384
Pond	I	11.97	6.7	144	0.18	2.8	6.9	2.5	0.0152	0.7339	0.1115	0.3966
Inlet	I	10.17	5.7	116	0.29	2.5	7.7	3.2	0.0151	0.8036	0.0344	0.2771
Outlet	I	6.33	5.7	137	0.34	2.3	5.9	2.7	0.0173	0.5803	0.0491	0.2783
Pond	Su	14.35	5.7	136	0.16	2.9	7.4	2.6	0.0154	0.8631	0.0736	0.3374
Inlet	Su	13.62	5.3	145	0.20	2.6	6.7	2.7	0.0155	0.7303	0.0339	0.2924
Outlet	Su	13.10	5.0	120	0.19	2.4	5.1	2.3	0.0185	0.4702	0.0702	0.3134
Pond	F	0.50	6.0	136	0.25	2.5	6.9	2.7	0.0147	0.6996	0.0354	0.2542
Inlet	F	2.44	5.7	139	0.22	2.0	5.8	3.0	0.0155	0.6121	0.0588	0.3253
Outlet	F	4.90	5.0	121	0.54	2.2	6.2	2.4	0.0187	0.4333	0.0569	0.2652

Table 3. Results of different multiple linear regression models (based on lowest AICc) to estimate a) bacterial production (BP), b) bacteria respiration (BR) and c) bacteria growth efficiency (BGE) for all data and for the three studied habitats (pond, inlet, outlet) separately. Total phosphorus (TP), humic acids (Humic), total nitrogen (TN), spectral slope at 289 nm (S289) and chlorohyll-a (Chl-a) were the variables used in the regression models (only significant values are listed). ns: not significant. Partial R^2 below each regression coefficient, N = number of data included, total R^2 (adjusted R^2), small sample size–corrected Aikaike Information Criterion Index (AICc) and root mean square errors (RMSE) are shown.

	Intercept	TP	Humic	TN	S289	Chl-a	N	R^2 (adj. R^2)	AICc	RMSE
a) BP										
All data	-1.00	0.24	0.86	ns	ns	ns	42	0.26 (0.22)	105.65	0.816
Partial R^2		0.19	0.07							
Pond	-	ns	ns	ns	ns	ns	13	-	-	-
Partial R^2										
Inlet	4.77	ns	ns	0.007	-320.31	ns	15	0.62 (0.56)	30.12	0.495
Partial R^2				0.38	0.24					
Outlet	-	ns	ns	ns	ns	ns	14	-	-	-
Partial R^2										
b) BR										
All data	-2.45	ns	ns	0.078	ns	ns	39	0.36 (0.22)	105.65	0.816
Partial R^2				0.36						
Pond	-4.80	ns	ns	0.12	ns	-24.77	13	0.87 (0.84)	80.23	4.47
Partial R^2				0.66		0.21				
Inlet	-	ns	ns	ns	ns	ns	14	-	-	-
Partial R^2										
Outlet	-7.16	ns	ns	0.11	ns	ns	13	0.80 (0.79)	75.67	3.46
Partial R^2				0.80						
c) BGE										
All data	0.63	ns	ns	-00006	-22.10	-0.18	39	0.26 (0.20)	-52.48	0.11
Partial R^2				0.07	0.11	0.08				
Pond	-	ns	ns	ns	ns	ns	13	-	-	-
Partial R^2										
Inlet	-	ns	ns	ns	ns	ns	14	-	-	-
Partial R^2										
Outlet	-	ns	ns	ns	ns	ns	13	-	-	-
Partial R^2										

Table 4**.** Combinations of environmental variables (TP, TN, DOC, Chl-a, S289, SUVA, humic, fulvic and protein), taken *k* at a time, giving the four best variables alone and the largest rank correlation ρ_s between OTU and environmental variable similarity matrices; **bold** indicates the best combination overall.

k	Best variable combinations (ρ_s)			
1	Protein (0.42)	DOC (0.38)	TN (0.35)	TP (0.34)
3	TP, fulvic, protein (0.54)			
4	**TP, DOC, fulvic, protein (0.57)**	TP, humic, fulvic, protein (0.55)	TP, S289, fulvic, protein (0.54)	
5	TP, DOC, S289, fulvic, protein (0.56)	TP, DOC, humic, fulvic, protein (0.56)	TP, DOC, SUVA, fulvic, protein (0.55)	TP, S289, humic, fulvic, protein (0.57)

Supplementary table 1. Summary of ANOVAs showing the effects of Habitat (Ha), Season (Se) and crossed factors (Ha x Se) on a) temperature, b) total phosphorus (TP), c) total nitrogen (sqrt TN), d) chlorophyll-a (sqrt chl-a), e) dissolved organic carbon (DOC), f) specific UV-absorbance index ($SUVA_{254}$), g) absorption at 320 nm (log a_{320}), h) spectral slope at 289 nm (x^{-1} S289) and fluorescence intensity of i) humic, j) fulvic (log) and k) protein compounds of DOC. Significant values are shown bold.

Source of variation	df	MS	F	p-value	Source of variation	df	MS	F	p-value
a) Temperature					b) TP				
Ha	2	0.56	0.24	0.7904	Ha	2	14.15	12.88	**<0.0001**
Se	4	320.0	135.36	**<0.0001**	Se	4	6.12	5.57	**0.0019**
HaXSe	8	10.10	4.24	**0.0018**	HaXSe	8	2.10	1.91	0.0970
Residual	29				Residual	29			
C. Total	43				C. Totakl	43			
c) TN (sqrt)					d) Chl-a (sqrt)				
Ha	2	32.73	10.83	**0.0003**	Ha	2	0.027	0.87	0.4312
Se	4	38.75	12.83	**<0.0001**	Se	4	0.030	0.94	0.4530
HaXSe	8	8.83	2.92	**0.0160**	HaXSe	8	0.038	1.18	0.3430
Residual	29				Residual	29			
C. Total	43				C. Total	43			
e) DOC					f) a_{320} (log)				
Ha	2	4.97	5.10	**0.0127**	Ha	2	1.04	2.90	0.0722
Se	4	1.26	1.30	0.2950	Se	4	0.11	0.30	0.8731
HaXSe	8	0.86	0.88	0.5453	HaXSe	8	0.13	0.37	0.9277
Residual	29				Residual	27			
C. Total	43				C. Total	41			
g) SUVA					h) S289 (x^{-1})				
Ha	2	0.50	1.36	0.2736	Ha	2	1421	50.17	**<0.0001**
Se	4	0.47	1.29	0.2987	Se	4	100.9	3.56	**0.0186**
HaXSe	8	0.48	1.31	0.2801	HaXSe	8	244.1	8.62	**<0.0001**
Residual	27				Residual	27			
C. Total	41				C. Total	41			

Source of variation	df	MS	F	p-value	Source of variation	df	MS	F	p-value
i) Humic					j) Fulvic (log)				
Ha	2	0.290	2.29	0.1204	Ha	2	0.655	2.30	0.1200
Se	4	0.023	0.18	0.9464	Se	4	1.008	3.54	0.0190
HaXSe	8	0.014	0.11	0.9984	HaXSe	8	1.355	4.76	**0.0010**
Residual	27				Residual	27			
C. Total	41				C. Total	41			
k) Proteins									
Ha	2	0.120	9.87	**0.0006**					
Se	4	0.048	3.36	**0.0118**					
HaXSe	8	0.033	2.62	**0.0289**					
Residual	27								
C. Total	41								

Supplementary Table 2. Shannon and Inverse Simpson indices for different habitats.

Habitat	Shannon (± std error)	Inverse Simpson (± std error)
Pond	2.1 ± 0.8	6.2 ± 4.2
Inlet	2.9 ± 0.9	16.4 ± 10.0
Outlet	3.3. ± 0.4	18.6 ± 7.9