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Supplementary Material

Bacterial response to permafrost derived organic matter input in an Arctic fjord

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1. Statistical justification of the study design:

The experimental design was chosen based on our hypothesis that the increased organic matter (tDOM) input would provide a potential source of organic matter for fjord microbial communities. tDOM is assumed to stimulate growth of some bacterial groups that are initially low in abundance but able to respond fast and increase in abundance over time. Accordingly there are two factors to investigate, first whether tDOM has an effect on bacterial community composition and second, how does the community change over time. Based on results from previous studies (Herlemann *et al.*, 2014, 2017; Blanchet *et al.*, 2017; Sipler *et al.*, 2017; Traving *et al.*, 2017) an effect of tDOM on community composition was likely, however the majority of these studies (with the exception of Sipler et al. 2017) had a low sampling resolution during the first 8-10 days and as a consequence it was only possible to identify changes, but not how these changes developed over time. Our aim was to follow the changes and possible increase of bacterial groups over time due to increased tDOM concentrations. This could best be achieved by using an experimental design with high sampling resolution during the first 8-10 days. These dense sampling designs have been documented to better determine the correct levels of non-sampled points when compared to replicate sampling in gene expression studies (Sefer *et al.*, 2016). Furthermore, by using this type of sampling design chances for making a type-1-error (not finding a pattern that is there) are higher than the chances for making a type-2-error (finding a pattern that is not there). We tested the statistical probability for the observed increase in *Glaciecola* abundance in the tDOM treatment by randomizing our data and only in 0.14% of all simulations (n=10000) was the modelled linear regression statistically as good as for our data (p=0.0004005) (Figure S3).

The results can also be analyzed by applying regression analysis comparing treatment and control incubations, with time as the continuous variable and community composition as the response variable. Regression analysis and analysis of variance (ANOVA) share the same underlying mathematical model (the general linear model), but regression analysis can be more powerful and does not require replication (Cottingham *et al.*, 2005; Lennon, 2011). The regression analysis shows that not only all individual incubations with tDOM have a higher abundance of *Glaciecola*, but also and more importantly that the increase in abundance is much faster when tDOM was added, with a significant difference between the slopes (p= 0.00002144). Additionally, since the regression analysis is based on individual samples and not subsampling from one experimental incubation, our study provides evidence of community change due to tDOM addition in 7 individual incubations compared to 2 or 3 replicated incubations of which subsamples were taken over time (Herlemann *et al.*, 2014, 2017; Blanchet *et al.*, 2017; Sipler *et al.*, 2017; Traving *et al.*, 2017).

2. Supplementary Figures:

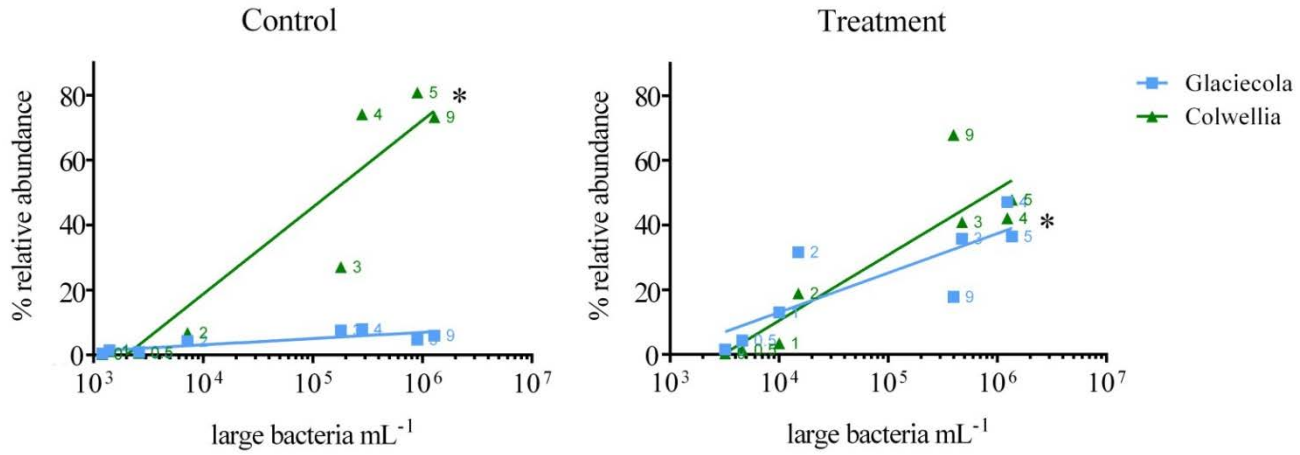

Figure S1: Relative abundance of *Glaciecola* and *Colwellia* from sequencing data plotted against the abundance of large bacteria obtained from flow cytometry for both control and tDOM treatment incubations. Black asterisk indicates significant correlation between relative abundance and large bacteria. The data is normally distributed (Shapiro-Wilk) and correlation was tested with Pearson's correlation coefficient (control: *Glaciecola*: r= 0.4451, p=0.2692; *Colwellia*: r= 0.8292, p=0.0109; treatment: *Glaciecola*: r=0.7667, p=0.0265; *Colwellia*: r=0.6614, p=0.0741).

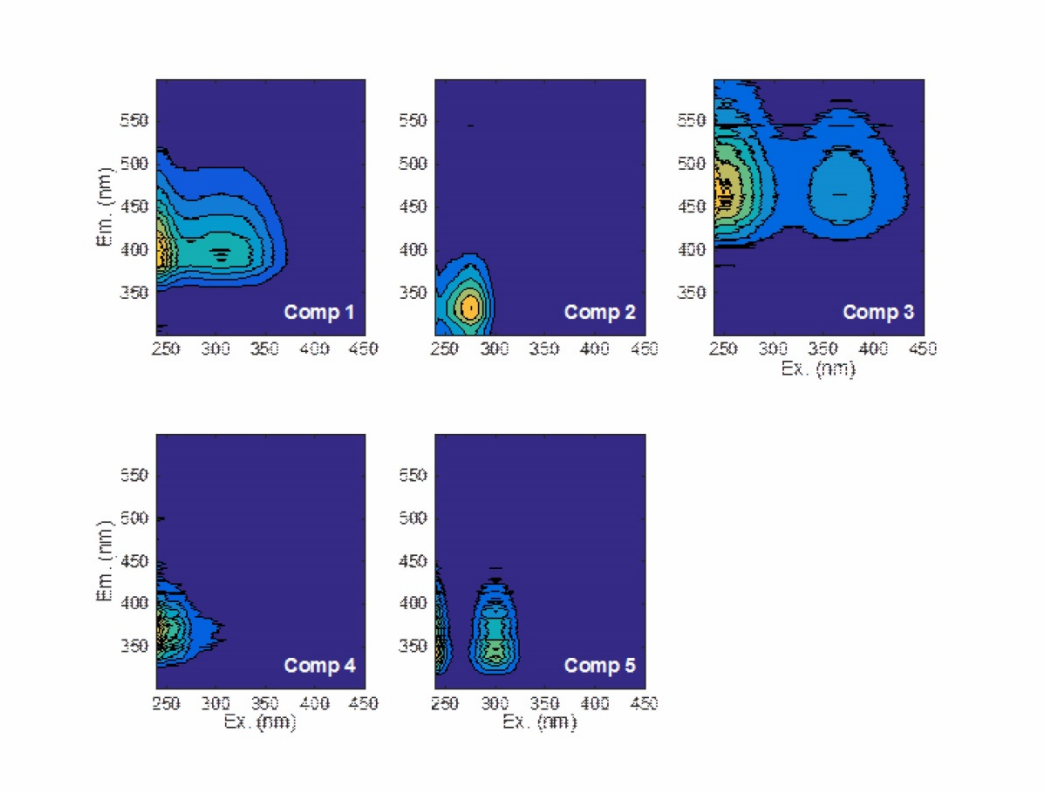

Figure S2: The contour plots show the spectral characteristics of each of the five fluorescence components 1–5 (C1-C5). C1 and C3 are characterized as humic-like fluorescent components and C2, C4 and C5 are characterized as amino-like fluorescent components as in (Stedmon and Markager, 2005).

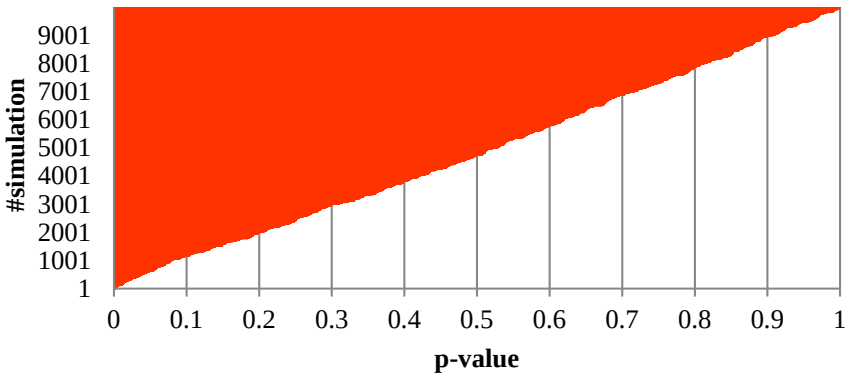

Figure S3: Modelled statistical significance values for linear regression analysis of simulations (n=10000) of randomized *Glaciecola* relative abundance data.

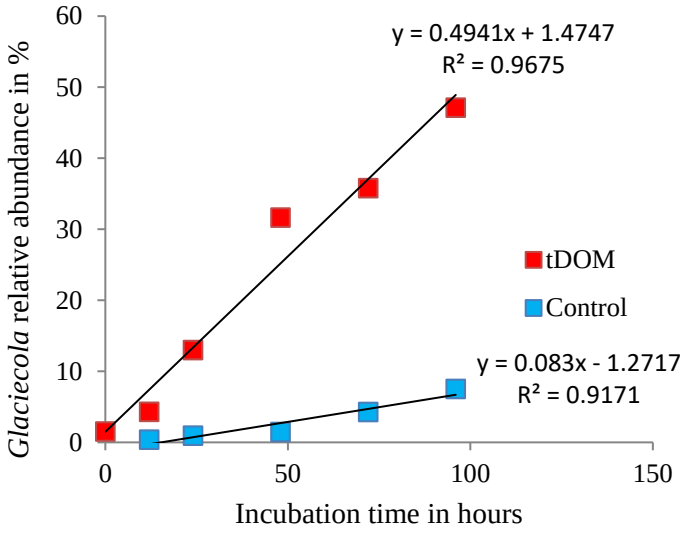

Figure S4: Linear regression analysis of the increase of *Glaciecola* relative abundance until the highest abundance measured at day 4.

3. Supplementary Tables:

Table S1: The averaged ± SD (n=8) fluorescent intensity (Raman units) of five fluorescence components of the tDOM-solution and control (0.2 filtered fjord water) illustrated in Figure S2. C1 and C3 are characterized as humic-like fluorescent components and C2, C4 and C5 are characterized as amino-like fluorescent components as in (Stedmon and Markager, 2005).

		tDOM		Control	
		Avg.	SD	Avg.	SD
Humic	C1	0.111	0.004	0.051	0.003
Humic	C3	0.064	0.001	0.028	0.002
Amino	C2	0.081	0.010	0.071	0.004
Amino	C4	0.104	0.007	0.001	0.000
Amino	C5	0.052	0.024	0.063	0.012

4. References:

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