

# Supplementary Information

## **Altitudinal patterns of diversity and functional traits of metabolically active microorganisms in stream biofilms**

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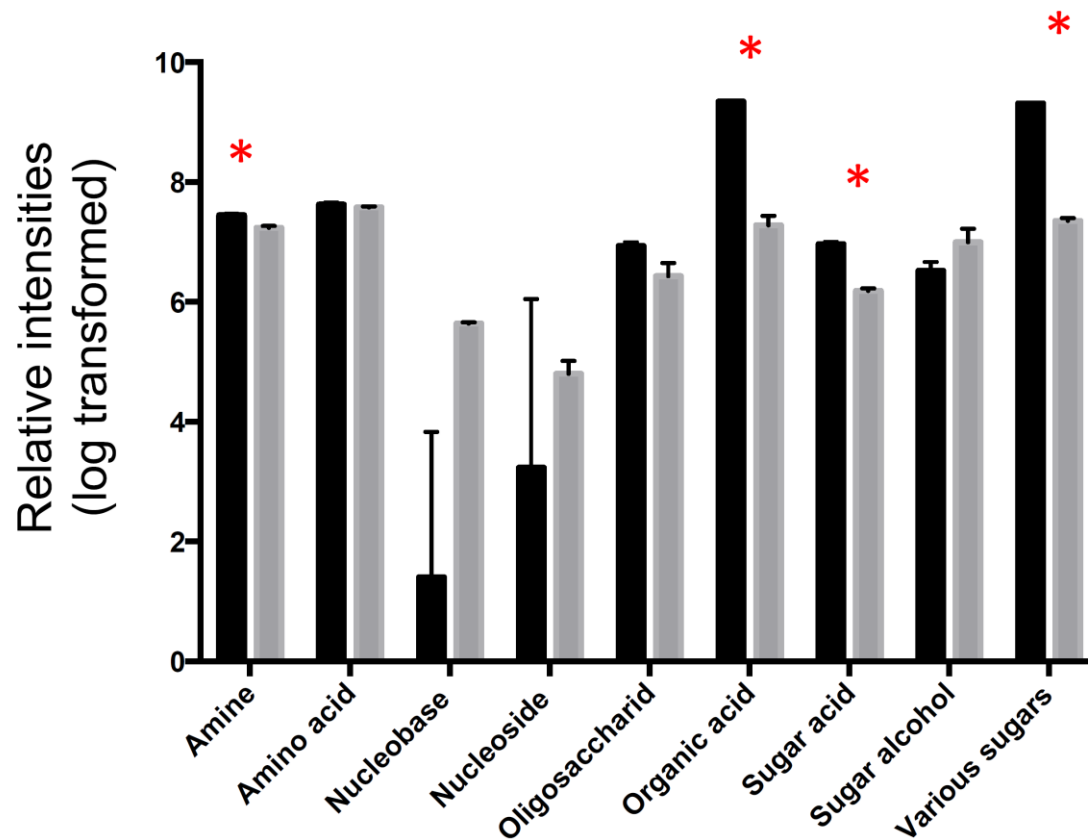
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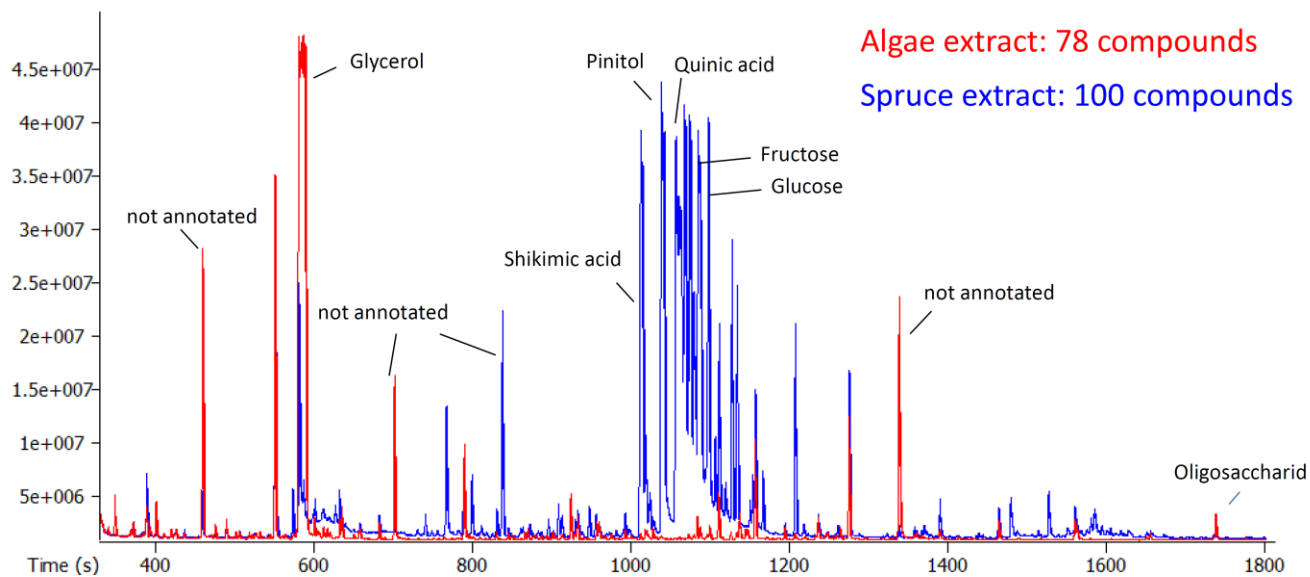
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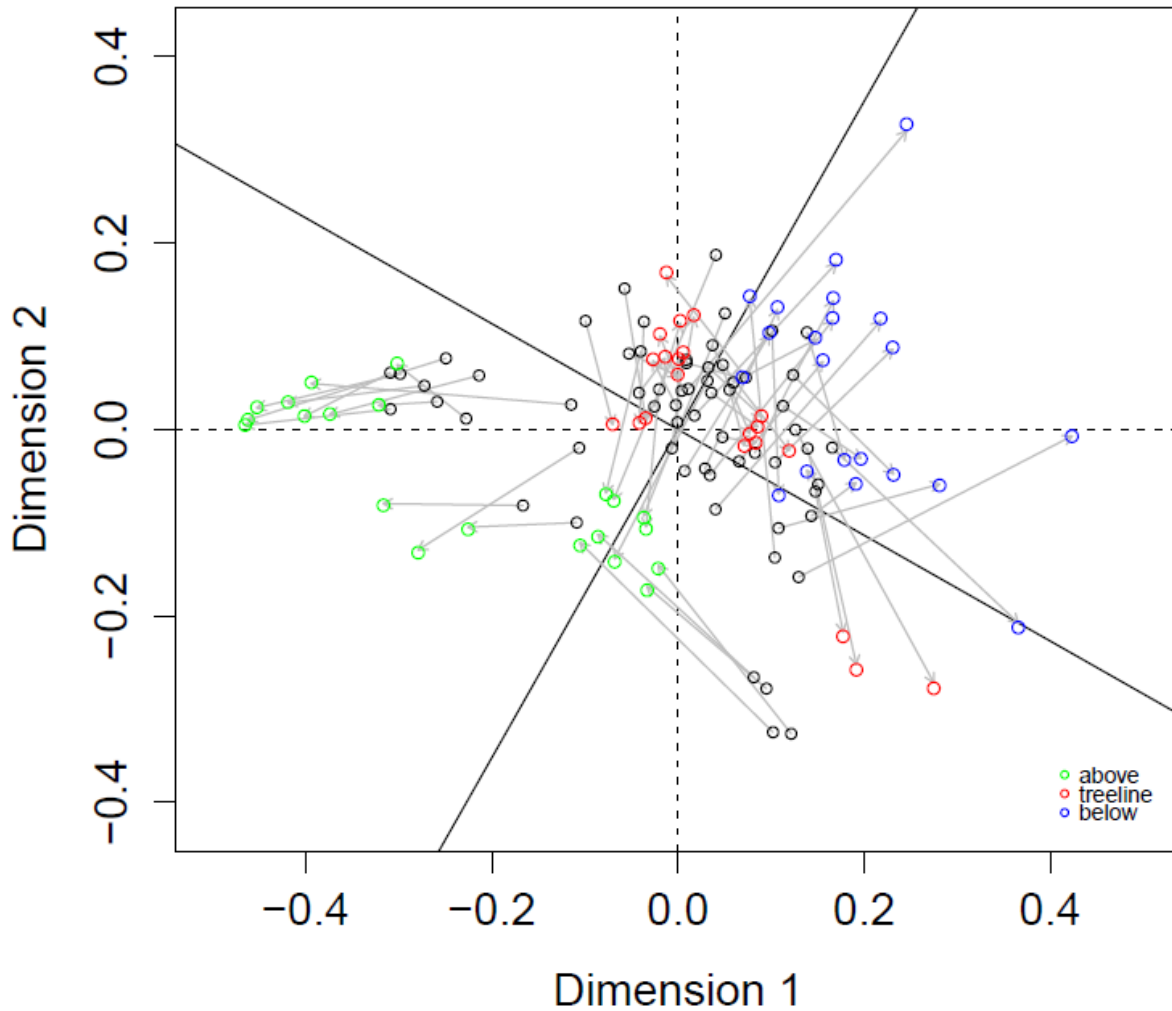
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**Figure 1.** Chemical composition of the model allochthonous (spruce) and autochthonous (algae) carbon sources. Black bars represent spruce treatment and grey bars represent algae treatment. Red asterisks describe significant differences ( $P < 0.05$ ) between spruce and algae substrate.



**Figure 2.** Chromatogram of the spruce and algal extract displaying a strong difference in their chemical composition. Red line = algal extract, blue line = spruce extract. Both extracts were measured at the same concentration in triplicates, while only one representative chromatogram is shown here.



**Figure 3.** Procrustes rotation showing the associations between biofilm bacterial community and metabolic orthologue similarity based on the relative abundance-based Horn distance for all samples. In the procrustes analysis, two ordinations (here nMDS using the metaMDS function in R's vegan package) are scaled and rotated in a way to optimally preserve the structure of the two ordinations. The rotation is indicated by the secondary axis, while an arrow links the same sample in the two ordinations. Black circles depict the patterns of community composition while color-coded circles indicate the functional composition based on the PICRUST analysis. The altitudinal gradient can be observed in both ordinations, however is even more pronounced in the nMDS based on metabolic functional orthologues.

**Table 1. Environmental characteristics of the study streams at the start (grey fields) and at the end (white fields) of the experiment.** 1, 2 and 3 in site description refers to above, around and below treeline, respectively.

Sites	Altitude (m.a.s.)	Temperature (°C)		Conductivity ( $\mu\text{S cm}^{-1}$ )		pH		Nitrate ( $\mu\text{g l}^{-1}$ )		DOC (ppb)		Cell counts ( $\times 10^6$ cells $\text{cm}^{-2}$ )
OTB1	1850	9.4	8.1	10.8	46.6	6.6	7.6	34	144	720	293	10.8 $\pm$ 2.3
OTB2	1651	10.1	10.9	45.0	50.6	7.3	7.6	164	147	615	552	22.6 $\pm$ 5.0
OTB3	1214	11.1	11.3	46.1	52.3	7.4	7.5	197	206	511	549	13.2 $\pm$ 2.6
SRB1	2062	12.1	14.5	16.2	24.0	7.0	7.4	122	85	396	395	4.2 $\pm$ 4.0
SRB2	1656	4.8	6.3	48.7	52.8	7.0	7.4	294	240	268	150	7.4 $\pm$ 2.6
SRB3	1114	10.9	9.9	45.4	50.7	7.2	7.5	382	379	521	458	8.1 $\pm$ 2.2
RB1	2275	2.5	15.8	3.9	5.9	5.5	6.8	132	120	286	395	5.2 $\pm$ 1.7
RB2	1670	8.8	12.7	14.8	18.0	6.7	7.3	171	161	511	447	5.0 $\pm$ 0.7
RB3	1130	13.8	15.7	30.3	37.0	7.0	7.6	224	217	849	865	21.4 $\pm$ 3.2

## Detailed description of the metabolic multifunctionality analysis

We assessed metabolic functional traits based on 16S rRNA marker genes using ‘Phylogenetic Investigation of Communities by Reconstruction of Unobserved States’, PICRUSt, (Langille *et al.*, 2013), which uses the sequenced relatives and ancestral state reconstruction for microorganisms for which no genome sequence is available. For this, the 16S rRNA sequences are first aligned with the Greengenes database currently including more than 200,000 bacterial sequences and 2,590 fully sequenced genomes. The PICRUSt algorithm then predicts the metagenome, which is adjusted for 16S rRNA gene copy number and OTU abundance to produce the KEGG (Kyoto Encyclopedia of Genes and Genomes) functions of the community. Based on 140 orthologues related to carbohydrate and amino acid metabolism we assessed metabolic multifunctionality and by relating multifunctionality to diversity we estimate multifunctional redundancy (Miki *et al.*, 2013). Metabolic orthologue diversity was computed as Shannon diversity, which represents the potential range of metabolic functions of the community accounting for gene copy number. Multifunctional redundancy was derived from a power-law function ( $MF = cOR^\alpha$ ) describing the orthologue accumulation curve, which is the graph of the orthologue number (i.e., MF) as a function of rarefied OTU richness in the community (Miki *et al.*, 2013). The coefficient  $\alpha$  serves as a multifunctional redundancy index with lower values indicating larger multifunctional redundancy (Miki *et al.*, 2013).

**Model summary and confident intervals for non-linear regressions between rarified species diversity (Shannon Index) and multifunctionality (metabolic orthologue diversity (Shannon Index)).**

**above treeline**

formula: multifunctionality ~ b \* rarified species diversity^z

parameters:	estimate	confident intervals		std. error	t value	Pr(> t )
		2.5%	97.5%			
b	4.3475	4.3267	4.3684	0.0099	436.498	< 2e-16 ***
z	0.0046	0.0036	0.0056	0.0004	9.864	6.56e-09 ***

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 residual standard error: 0.0045 on 19 degrees of freedom  
 number of iterations to convergence: 14  
 achieved convergence tolerance: 7.785e-07

**around treeline**

formula: multifunctionality ~ b \* rarified species diversity^z

parameters:	estimate	confident intervals		std. error	t value	Pr(> t )
		2.5%	97.5%			
b	4.3585	4.3316	4.3856	0.0129	337.966	< 2e-16 ***
z	0.0041	0.0030	0.0052	0.0005	7.568	3.78e-07 ***

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 residual standard error: 0.0033 on 19 degrees of freedom  
 number of iterations to convergence: 22  
 achieved convergence tolerance: 1.257e-06

**below treeline**

formula: multifunctionality ~ b \* rarified species diversity^z

parameters:	estimate	confident intervals		std. error	t value	Pr(> t )
		2.5%	97.5%			
b	4.3488	4.3097	4.3883	0.0188	231.780	2e-16 ***
z	0.0045	0.0030	0.0061	0.0007	6.033	8.37e-06 ***

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 residual standard error: 0.0031 on 19 degrees of freedom  
 number of iterations to convergence: 28  
 achieved convergence tolerance: 1.018e-06

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

## References

1. Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA *et al.* (2013). Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotech* **31**: 814-821.
2. Miki T, Yokokawa T, Matsui K. (2014). Biodiversity and multifunctionality in a microbial community: a novel theoretical approach to quantify functional redundancy. *Proc R Soc B* **281**: 20132498.