



Deciphering the microbial ecology of nitrogen and sulfur cycling in sediments of the non-perennial Mühlbach stream

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Introduction

In CAMPOS, the Mühlbach is investigated as a first-order intermittent artificial stream, heavily affected by agricultural nitrogen input. Unlike other perennial lower-order streams (e.g., the Schönbrunnen), Mühlbach is primarily governed by exfiltration, and the interface between surface water and groundwater is subject to pronounced seasonal oxidation and reduction. Preliminary data indicate high sulfide (up to 1 mM) and ammonia (up to 0.5 mM) concentrations in recurrent exfiltrating groundwater, whereas surface water is fed also by the south-western spring during the sampling season. While nitrification can oxidize ammonia and thus contribute to stream nitrate loading, sulfur species driven denitrification represents a possibly important nitrogen sink. To tackle this, PacBio full length 16S rRNA amplicon sequencing of sediment microbiota was interpreted with PICRUSt2, to enable a high resolution prediction of microbial functionalities related to the local hydrological conditions.







imicrobiia	Holophagae	Subgroup 17
obacteriia	Ignavibacteria	Subgroup 18
nobacteria	JG30-KF-CM66	Subgroup 22
aproteobacteria	KD4-96	Subgroup 25
nicenantia	MB-A2-108	Subgroup 5
erolineae	Microgenomatia	Subgroup 6
eliae	NC10	Subgroup 9
lli	Negativicutes	Thermoanaerobaculia
eroidia	Nitrospira	Thermoleophilia
tocatellia (Subgroup 4)	OM190	unclassified_Acidobacteria
ipylobacteria	P9X2b3D02	unclassified_Gemmatimonadetes
tridia	Parcubacteria	unclassified_Planctomycetes
abacteriia	Phycisphaerae	unclassified_Proteobacteria
alococcoidia	Pla4 lineage	vadinHA49
aproteobacteria	Planctomycetacia	Verrucomicrobiae
rse others	S0134 terrestrial group	
maproteobacteria	Spirochaetia	

Fig. 1, 2 (left and up): Class which contributes >2% to the relative abundance according to the ASVs based taxonomy

Sulfur-oxidizing populations



Fig. 7 (left): Bacterial classes which contributed >2% of S-cycling enzymes according to the ASVs. *Gammaproteobacteria* were dominant populations carrying sulfur-oxidizing enzymes. Distinct microbial community patterns were again suggested for ditch S2.



are depicted separately here. *Gammaproteobacteria* and *Deltaproteobacteria* were dominant microbial populations in all sampling locations. All samples were collected in March,

MU1_5a MU1_5a MU1_5b MU1_5c MU1_5c MU1_5c MU2_5b MU2_5b S2M1_55 S2M1_55 MD1_5b MD1_5b MD1_5b MD1_5b MD1_55 MD1_555

Spatially explicit abundance of S- and N-cycling Enzymes



2000 - 20

Fig. 3, 4 (left): Distinct functional enzyme abundance patterns were predicted for samples from ditch S2. EC was a significant factor driving these distinctions, interpreted as a proxy for distinct water sources. The high concentrations of sulfate and nitrate in upstream waters were among the factors significantly affecting enzyme prediction patterns.

Fig. 5, 6 (bottom): Functional enzyme predictions indicated a marked enrichment of enzymes indicative of sulfur-driven nitrate reduction processes in ditch S2.



MU1_55 MU1_55 MU1_55 MU1_55 MU1_55 MU1_55 MU1_55 MU2_55 MU2_55 MU2_55 S2M1_55 S2M1_55 S2M1_55 S2M1_55 S2M1_55 S2M1_55 MU2_156 MD1_55 MD1_55 MD1_55 MD1_55 MD1_55 MD1_55 MD1_155 MD1_55





Fig. 8 (left top): Main bacterial genera contributing to S-oxidizing enzyme abundances predicted from ASVs. The genus *Sulfurifustis* was more important in upstream samples, whereas the genera *Sideroxydans* and *Thioalkalispira* were more abundant in down-stream samples. Unclassified taxa (*SC-I-84* and *TRA3-20*) were also important in samples from ditch S2. The genus *Thiobacillus* was always the major S-oxidizer, except for samples from ditch S2, where other taxa are also major contributors.

Fig. 9 (left): Community composition for capacities predicted for S-oxidizing *Gammaproteobacteria*.



MU1_56 -MU1_55 -MU1_55 -MU1_55 -MU1_156 -MU1_156 -MU1_156 -MU2_55 -S2M1_55 -S2M1_55 -S2M1_55 -S2M1_55 -S2M1_55 -MD1_55 -MD1_55 -MD1_56 -

5000 -

2500



Summary

- We present a first targeted dissection of microbial S- and N-cycling potentials in sediments of the Mühlbach first-order stream.
- A considerable abundance and diversity of bacteria with capacities for autotrophic S-oxidation were discovered.
- Especially in ditch S2 (upstream of W2), a high predicted abundance nitrate-reduction capacities was indicative of a possible hot-spot of chemolithoautotrophic nitrate reduction.
- Potentials for nitrification will be further elucidated to generate a first comprehensive understanding of microbial N- and S-cycling capacities in sediments of this non-perennial first-order stream.

