Supplemental Material

Cyanobacterial contribution to travertine deposition, Hoyoux, Belgium

## Supplements to:

## Cyanobacterial contribution to travertine deposition

## in the Hoyoux river system, Belgium

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**Supplemental Figures and Tables** 

**Suppl. Figure 1**: Variations of the partial pressure of  $CO_2$  (p $CO_2$ , ppm), CH<sub>4</sub> and N<sub>2</sub>O concentrations (nmol L<sup>-1</sup>) in the Hoyoux and Triffoy in February and October 2014. Horizontal dotted lines indicate atmospheric equilibrium, 400 ppm, 2 nmol L<sup>-1</sup> and 10 nmol L<sup>-1</sup> for  $CO_2$ , CH<sub>4</sub> and N<sub>2</sub>O, respectively.



**Suppl. Figure 2**: Schematic view of a longitudinal profile of a travertine cascades in the Hoyoux River. Indicated are the different types of samples: (a) In the nearly laminar flow over the tops of travertine barriers, we observed poorly calcified flat mats; (b) in moderate flow between barriers and cascades, we collected oncoids and submersed encrusted wooden branches; (c) in cascades, on sites receiving high water impact, we encountered compact crusts with smooth surface of shiny appearance, blue-green to steel-blue in color which covered the travertine surface or encrusted fixed branches.



**Suppl. Fig. 3.** Microorganisms participating in Travertine deposition. (a) *Vaucheria* sp., filament tips (damaged filaments in background). (b) Detail: Diatom *Gyrosigma* sp. and *Vaucheria* sp. (c) The rhodophyte *Batrachospermum* sp., chantransia growth stage. (d) An oomycetous fungus entrapped in the travertine crust with germinating spores. (e) Clusters of settled baeocytes (B) of pleurocapsalean cyanobacteria (possibly *Xenococcus*). (f) The pleurocapsalean cyanobacterium *Hyella fontana* Huber et Jadin (arrow) surrounded by recently released baeocytes (B). (g) *Lyngbya* sp. extracted from carbonate crust. (h) *Xenococcus* sp. (arrow) (i) *Leptolyngbya* sp. The scale in i. is valid for b-i.



**Suppl. Fig. 4:** X-ray elemental analyses and mappings of *in situ* travertine with cyanobacteria and mineral encrustation. (a) The area subject to analysis (outlined by white frame in Fig. 9a, and mapped in Fig. 9d). (b) Elemental composition targeting the trichome of *Phormidium incrustatum* in a. (c) Target is carbonate cluster in a. (d) Target is abandoned *Phormidium* sheath in a. (e) Target is entrapped microorganisms in a.



**Suppl. Fig. 5:** Neighbor-joining tree based on 454 bp of the 16S rRNA gene including environmental sequences and isolates from this study (**bold**), the most closely related BLAST hits as well as tufa-related sequences from Europe (*italics*). Kimura correction for multiple mutations with ratio calculated from data, 500 bootstraps.



**Suppl. Fig. 6:** Neighbor-joining tree based on ITS sequences of environmental sequences and isolates (**bold**) and the most closely related BLAST hits. Kimura correction for multiple mutations, 500 bootstraps. No 'tufa' associated ITS sequences are available in public databases. Therefore, the sequences from the Hoyoux system of this study form a distinct cluster.

**Suppl. Table 1:** Amplified 16S rRNA and ITS sequences from environmental samples (MOD) and isolates. The length of the fragment, the 1<sup>st</sup> (closest) cultured and uncultured hit in the GenBank database obtained by a BLAST search are indicated, as well as the accession number.

Sequence	Sampling	fragment	# bp	1st uncultered hit,	1st cultured hit,	GenBank
	site			accession # and	accession # and	Accession #
				pairwise identity	pairwise identity	
MOD 14	Site 3	16S rRNA	1403	Uncult. cyanobacterium clone WB 18.8	Oscillatoria limosa SAG 42.87	KR002126
	Triffoy			GQ324964	KM019961	
	June			99.9%	98.8%	
MOD 14	Site 3	ITS	531	-	Phormidium autumnale PO12	KR002126
	Triffoy				JQ347240	
	June				91.6%	
MOD 14	Site 3	16S rRNA	1159	Uncult. cyanobacterium clone WB 18.8	Phormidium autumnale PO12	KR002127
surface	Triffoy			GQ324964	JQ712612	
	June			99.8%	98.9%	
MOD 14	Site 3	ITS	531	-	Phormidium autumnale PO12	KR002127
surface	Triffoy				JQ347240	
	June				91.5%	
MOD 15	Site 2	16S rRNA	1144	Uncult. cyanobacterium clone WB 18.8	Phormidium cf. subfuscum I-Roc	KR002128
	Hoyoux			GQ324964	EU196634	
	June			99.2%	98.7%	
MOD 15	Site 2	ITS	531	-	Phormidium autumnale PO12	KR002128
	Hoyoux				JQ347240	
	June				90.3%	
MOD 15	Site 2	16S rRNA	1161	Uncult. cyanobacterium clone WB 18.8	Phormidium autumnale PO12	KR002129
surface	Hoyoux			GQ324964	JQ712612	
	June			99.5%	98.9%	
MOD 15	Site 2	ITS	543	-	Phormidium autumnale PO12	KR002129
surface	Hoyoux				JQ347240	
	June				91.0%	
MOD 21	Site 3	16S rRNA	1315	Uncult. cyanobacterium clone WB 18.8	Oscillatoria limosa SAG 42.87	KR002130
	Triffoy			GQ324964	KM019961	
	August			99.8%	99.1%	
MOD 21	Site 3	ITS	531	-	Phormidium autumnale PO12	KR002130
	Triffoy				JQ347240	
	August				91.9%	

Isolate 2a	Site 4 Hoyoux February	16S rRNA	1070	-	Phormidium autumnale CYN79 JQ687337 99.4%	KR002120
Isolate 2a	Site 4 Hoyoux February	ITS	534	Oscillatoriales cyanobacterium Fil.1SE EF061076 93.6%	Phormidium autumnale PO19 JQ347235 89.2%	KR002120
Isolate 3	Site 3 Triffoy February	16S rRNA	1403	Uncult. cyanobacterium clone AM-20-10 FJ866615.1 99.1%	<i>Oscillatoria limosa</i> SAG 42.87 KM019961 99.1%	KR002121
Isolate 3	Site 3 Triffoy February	ITS	547	-	<i>Oscillatoria</i> sp. PCC 9240 EF061079 90.5%	KR002121
Isolate 4	Site 2 Hoyoux February	16S rRNA	1403	Uncult. cyanobacterium clone AM-20-10 FJ866615.1 99.3%	<i>Oscillatoria limosa</i> SAG 42.87 KM019961 99.2%	KR002122
Isolate 4	Site 2 Hoyoux February	ITS	543	-	Phormidium autumnale PO12 JQ347240 90.7%	KR002122
Isolate 5	Site 3 Triffoy February	16S rRNA	1116	-	Phormidium autumnale LCR-CYANT10 - KM052841 99.7%	n.a.
Isolate 6	Site 3 Triffoy February	16S rRNA	1403	Uncult. <i>Phormidium</i> sp. AB820727 99.4%	Cf. <i>Tychonema</i> sp. SAG 2388 KF417637 99.3%	KR002123
Isolate 6	Site 3 Triffoy February	ITS	560	-	Phormidium autumnale CYN48 GU018024.1 93%	KR002123
Isolate 7	Site 2 Hoyoux February	16S rRNA	706	Uncult. <i>Phormidium</i> sp. AB820727 99.6%	Phormidium cf. uncinatum JX088095 99.6%	KR002124
Isolate 8	Site 3 Triffoy February	16S rRNA	1403	Uncult. cyanobacterium clone WB18.8 GQ324964 99.8%	<i>Oscillatoria limosa</i> SAG 42.87 KM019961 98.8%	KR002125
Isolate 8	Site 3 Triffoy February	ITS	552	-	<i>Phormidium autumnale</i> PO12 JQ347240 90.7	KR002125