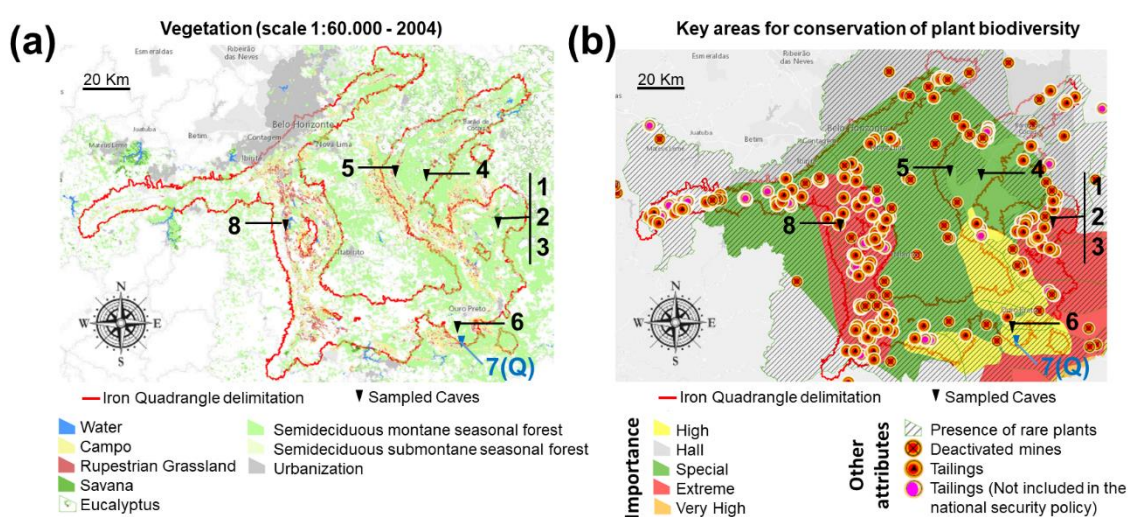


## 16S rRNA gene amplicon sequencing data of the Iron Quadrangle ferruginous caves (Brazil) shows the importance of conserving this singular and threatened geosystem

**Short title:** 16S rRNA gene amplicon sequencing of ferruginous caves

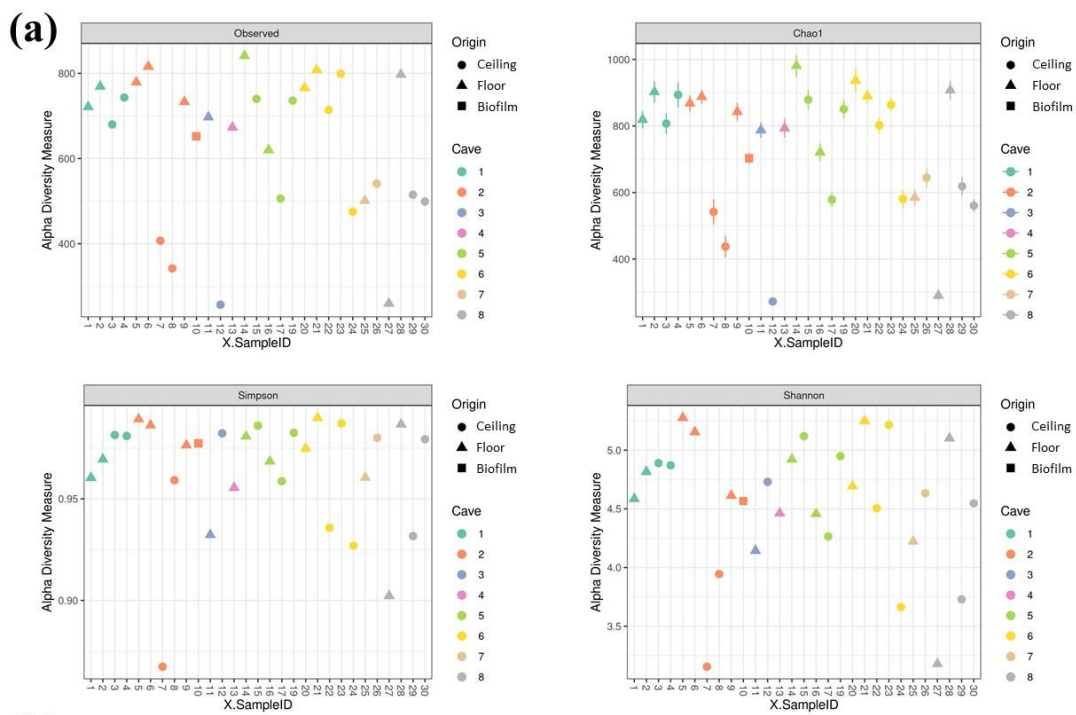
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**Fig. S1**



**Supplementary Figure S1. Maps characterizing the iron quadrilateral region.** (a) Map showing predominant vegetation types. (b) Map highlighting key areas for biodiversity conservation. Both maps were obtained from “Instituto Pristino: atlas digital geoambiental. WebGis system (Web Geographical Information System) of free access to the environmental database”. Available in < <https://institutopristino.org.br/atlas/>>. Accessed in: 15/jun/2020.

Fig. S2



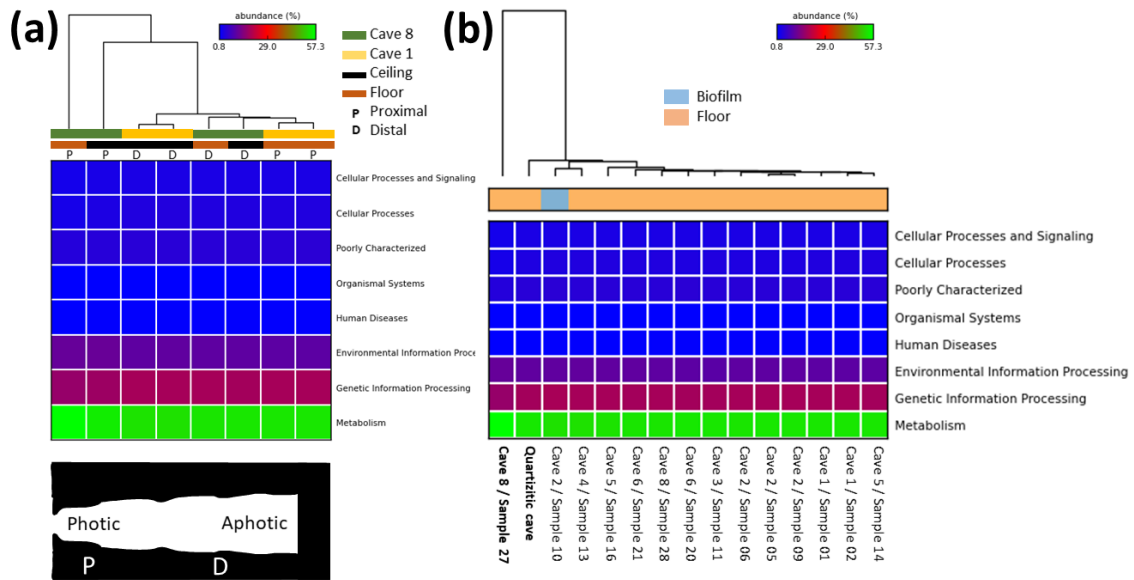
(b)

	Ceiling vs Floor	Observed OTUs	Shannon	Simpson	chao1	Faith PD
Cave 1		1.0000000	0.1213350	0.1213350	1.0000000	0.4385780
Cave 2		0.0832650	0.0832650	0.0832650	0.0832650	0.0832650
Cave 3				See the note		
Cave 4				See the note		
Cave 5		0.5637030	1.0000000	0.5637030	0.5637030	0.5637030
Cave 6		0.3742590	0.2482130	0.2482130	0.0832650	0.5637030
Cave 7				See the note		
Cave 8		1.0000000	1.0000000	1.0000000	1.0000000	1.0000000
All caves (ceiling vs floor)		0.1159645	0.6032529	0.9732701	0.1568890	0.1324335
All caves (cave X vs cave Y)		0.4704925	0.6725018	0.9181045	0.2759770	0.3971378

Note: It requires at least two samples within a group to calculate the distance within a group, but there is only one sample in a group

**Supplementary Figure S2. Diversity indices.** (a) Assessment of diversity indices for the characterization of the prokaryotic community associated to cave samples. (b) Assessment of diversity indices for the comparison between ceiling and floor cave samples. Welch's  $t$ -test ( $p < 0.05$ ).

**Fig S3.**



**Supplementary Figure S3. Functional metabolic prediction.** (a) Analysis of the functional diversity of cave 8 samples compared to cave 1 samples. The samples (both floor and ceiling) collected near the photic zone in cave 8, significantly differed from other samples, underlining the effects of anthropogenic activity on natural microbiome. (b) Comparative analysis of functional diversity in all floor samples with the biofilm hanging from the ceiling sample. It is observed that the floor samples obtained in the region closest to the entrance of cave 8 depart from the others, reiterating the results presented in (a). It is also observed that the biofilm sample is functionally very close to the floor samples, contrasting the fact that it is physically attached to the cave 2 ceiling. Colors vary according to the relative abundance of taxa as detailed in legend of the figure.

**Fig. S4**



**Supplementary Figure S4. Plant root system throughout the structure of the cavities.**  
Credits Dr. Flávio Fonseca do Carmo.