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Cultivable Microbial Community Structure Along a Light Gradient in a Tropical Volcaniclastic Cave

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for the degree of
Bachelor of Science in Biology

Advisor:
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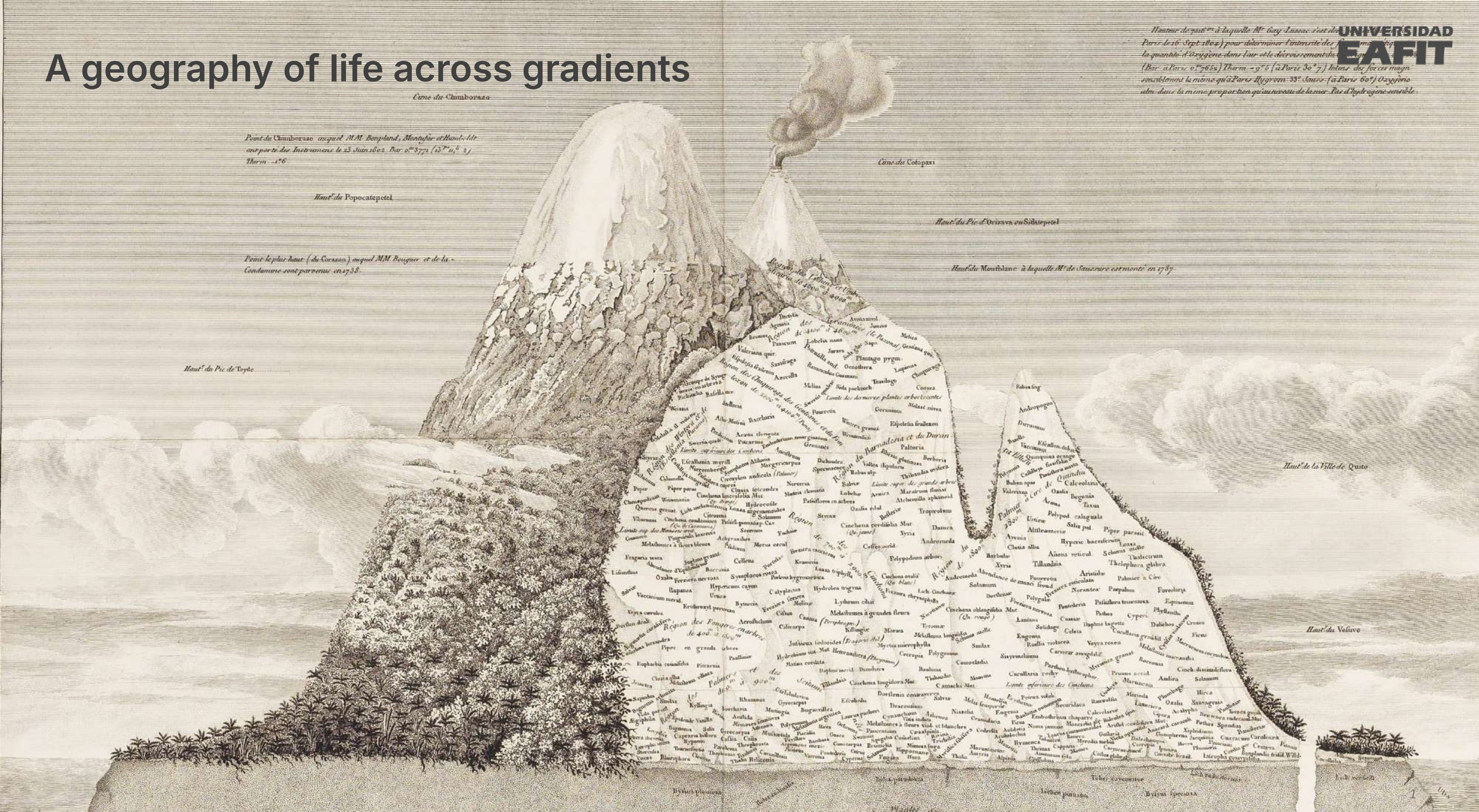
Field support:
Rodrigo Echeverri

Universidad EAFIT
May 2026

A geography of life across gradients



Humboldt & Bonpland (1805); Nottingham et al. *Ecology* 2018;99:2455–2466.

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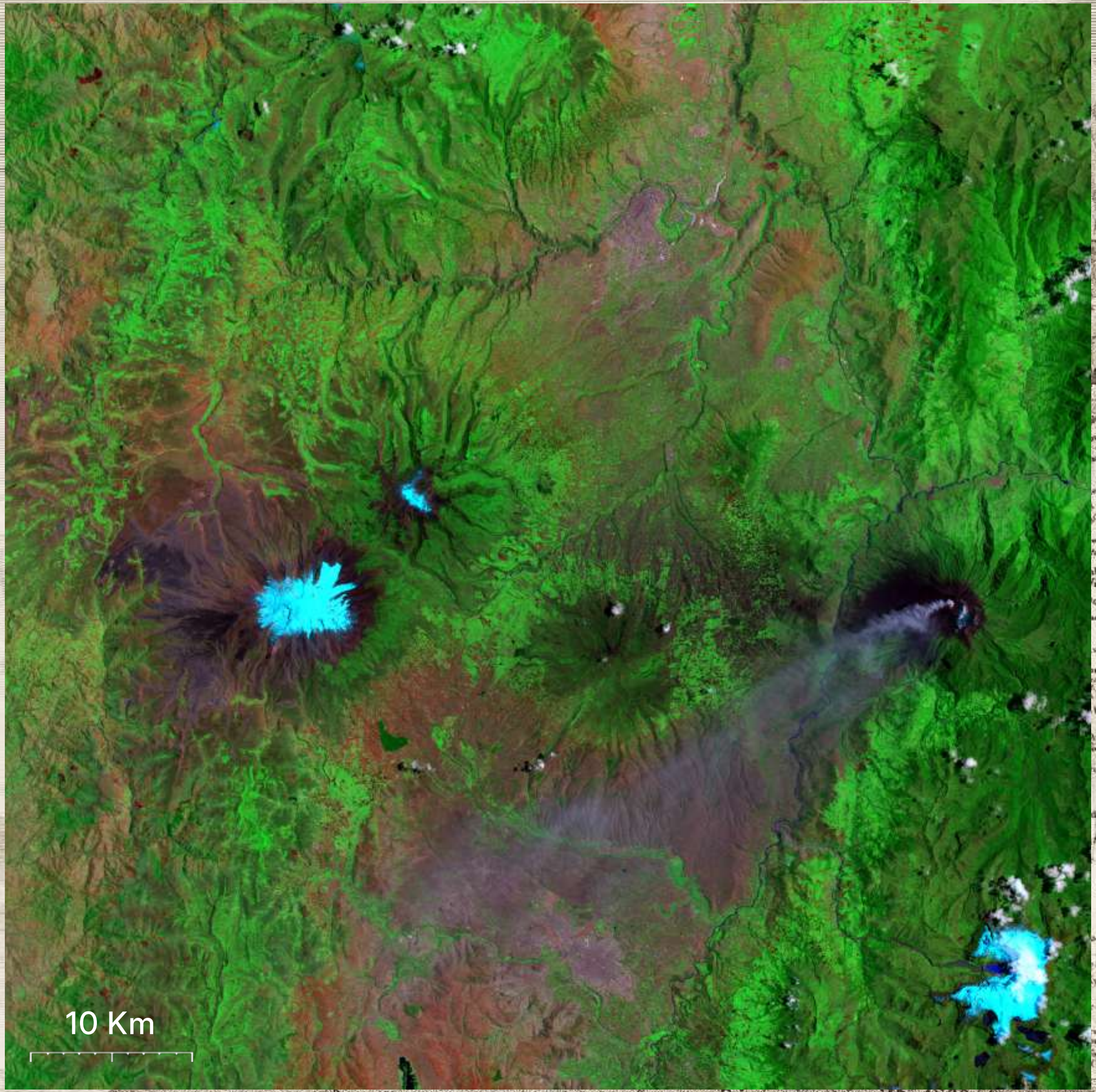
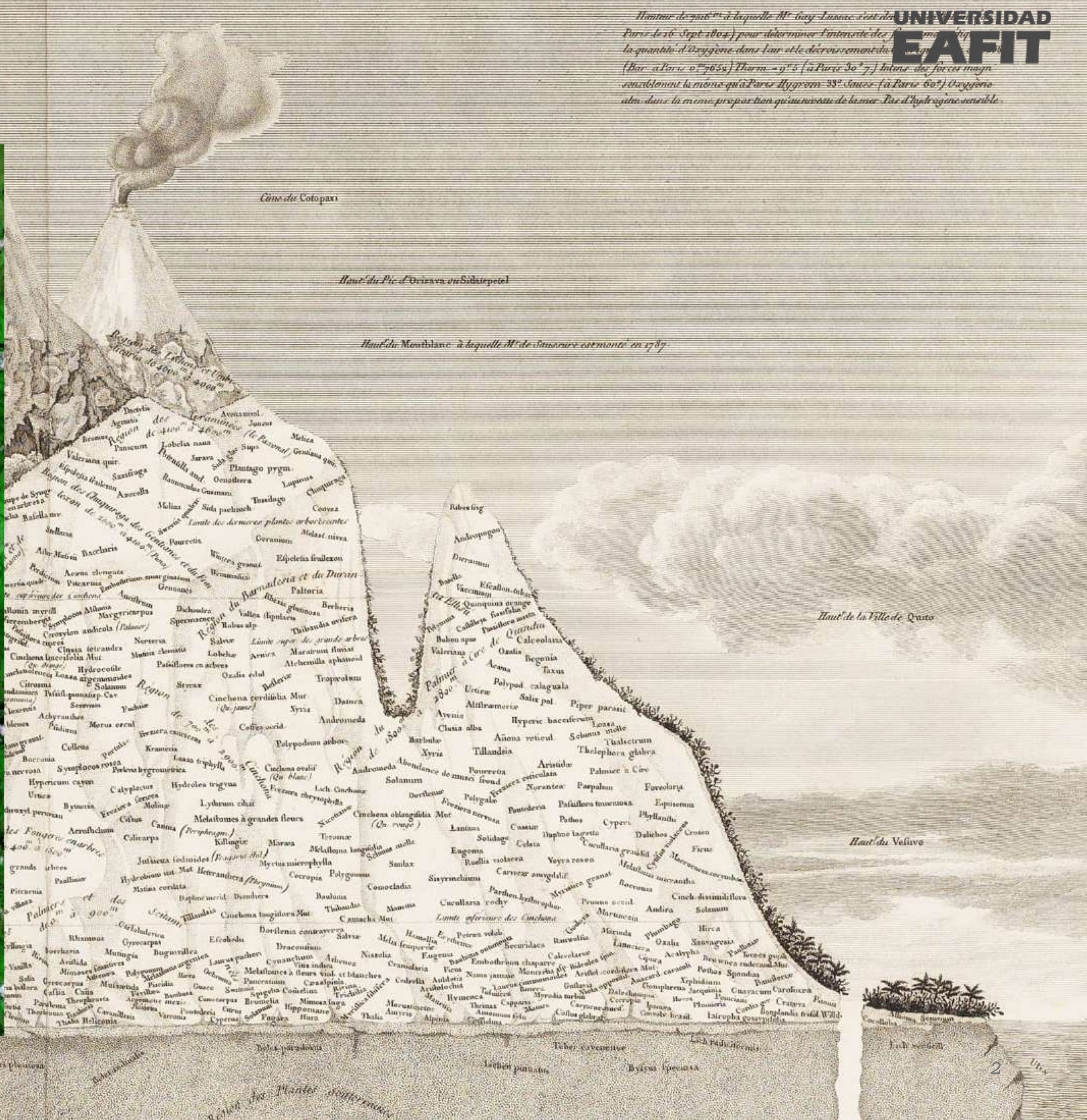
A geography of life across gradients



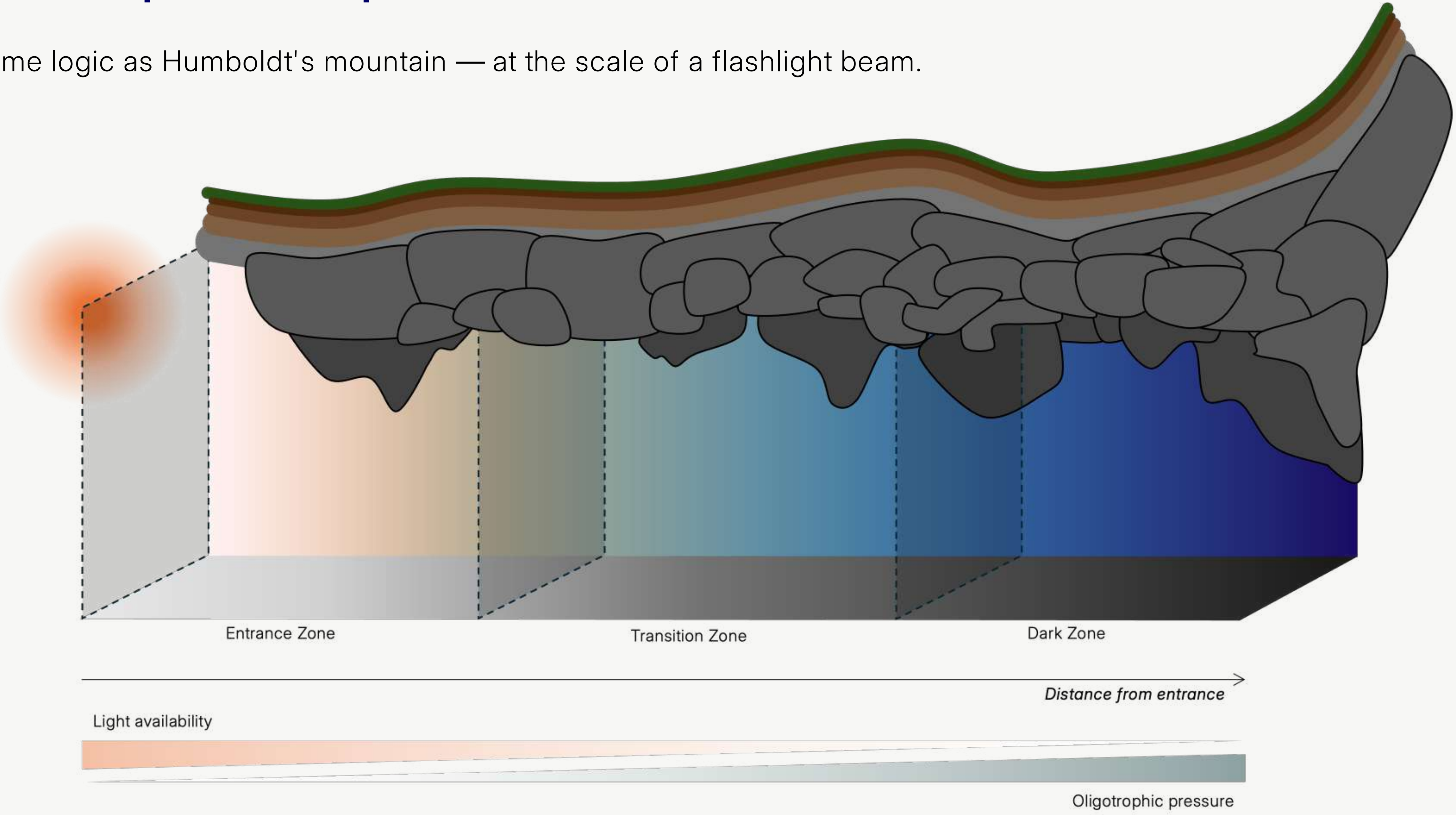
NASA image created by Jesse Allen, Earth Observatory, based on data provided by the Landsat 7 science team and the University of Maryland's Global Land Cover Facility



Humboldt & Bonpland (1805); Nottingham et al. *Ecology* 2018;99:2455–2466.

Caves compress sharp environmental transitions into meters

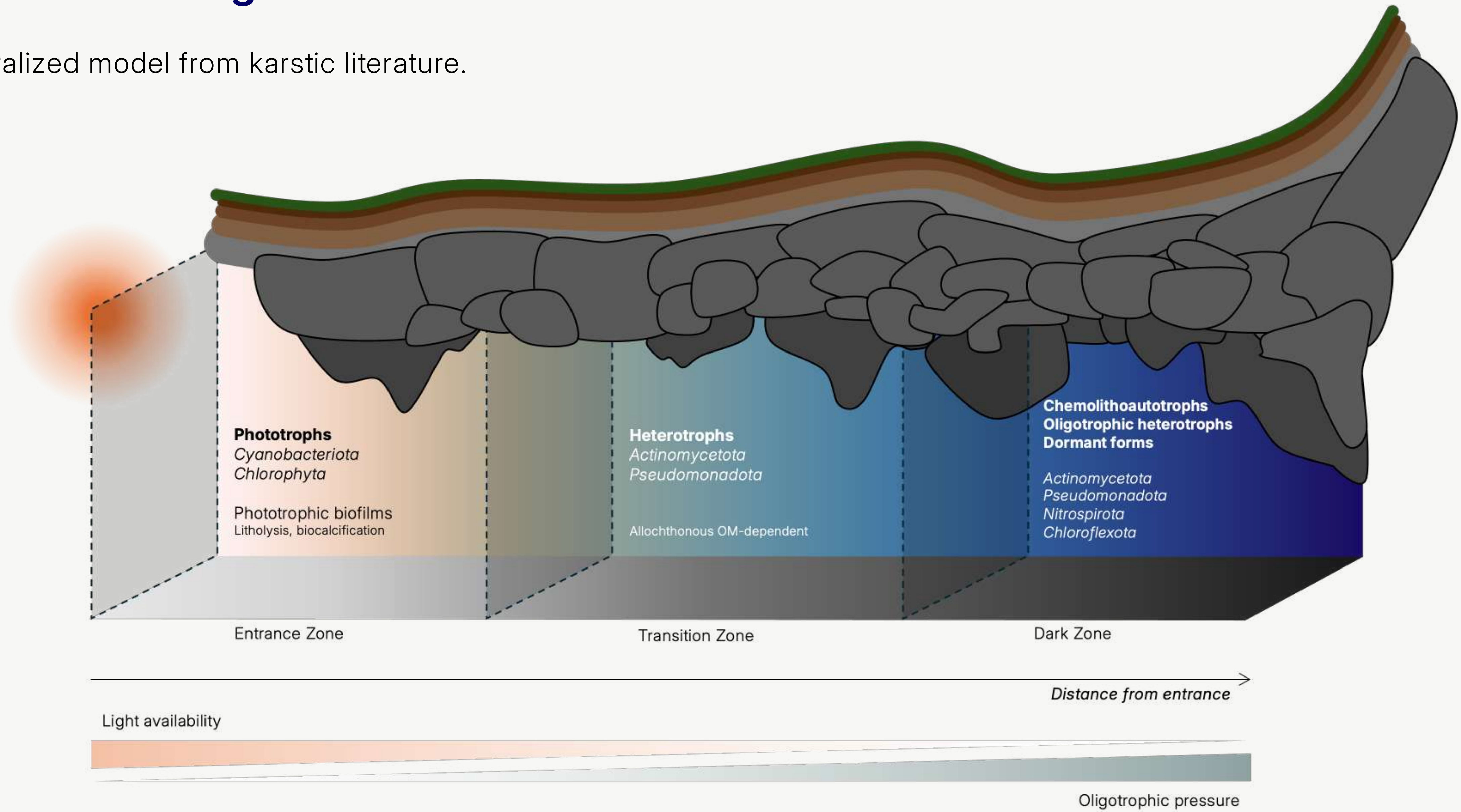
The same logic as Humboldt's mountain — at the scale of a flashlight beam.



Howarth. *Annu Rev Entomol* 1983;28:365–389; Culver & Pipan. *The Biology of Caves and Other Subterranean Habitats* (Oxford Univ. Press, 2019).

Environmental gradients structure cave microbial communities

Generalized model from karstic literature.



Northup & Lavoie. *Geomicrobiol J* 2001;18:199–222; Barton & Northup. *J Cave Karst Stud* 2007;69:163–178; Hershey & Barton. In: *Cave Ecology* (Springer, 2018):69–90.

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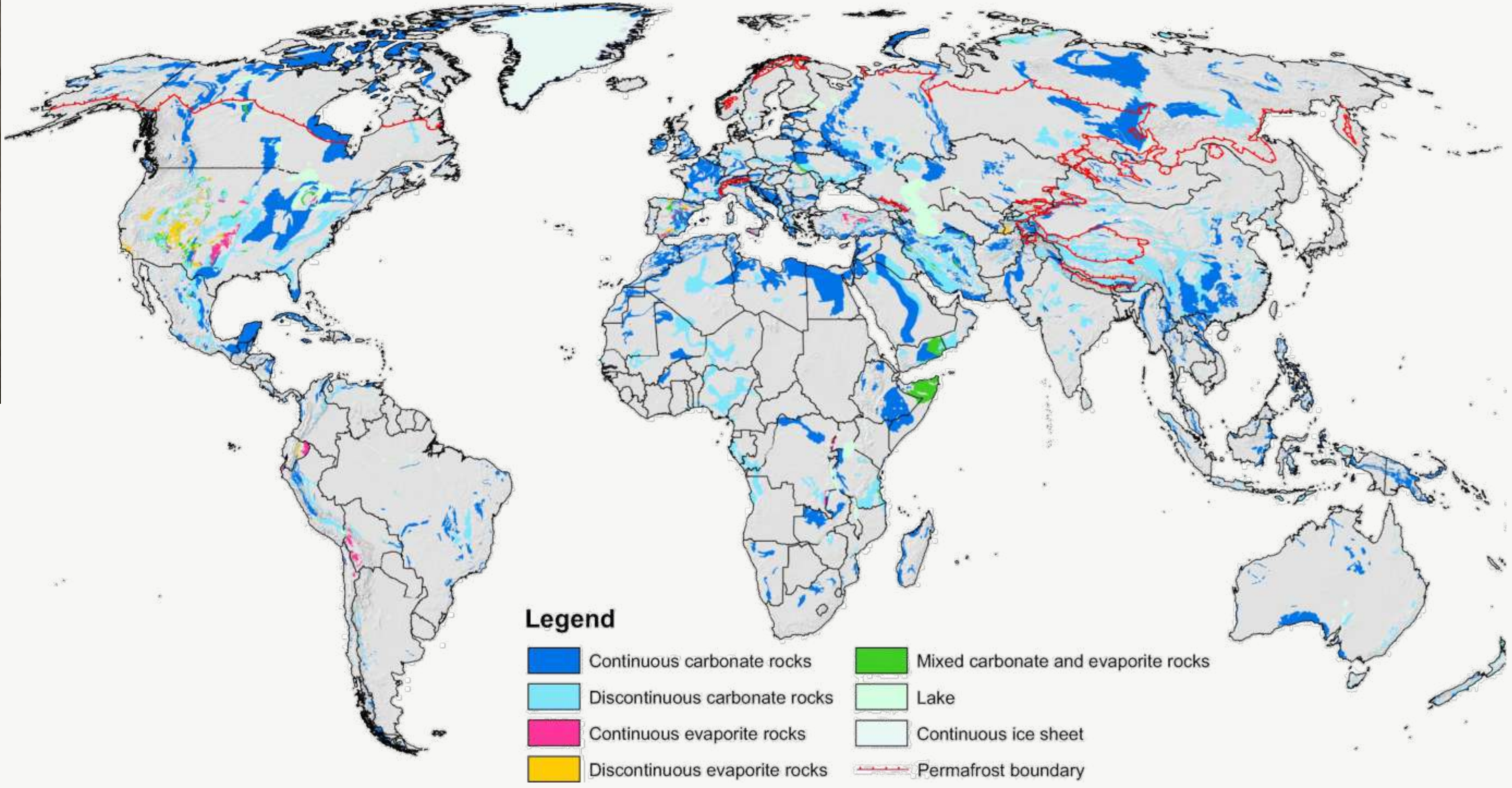
Cave microbiology has a geological blind spot

Source: Nicolas Pinel

Source: Dave Bunell, CC BY-SA 4.0

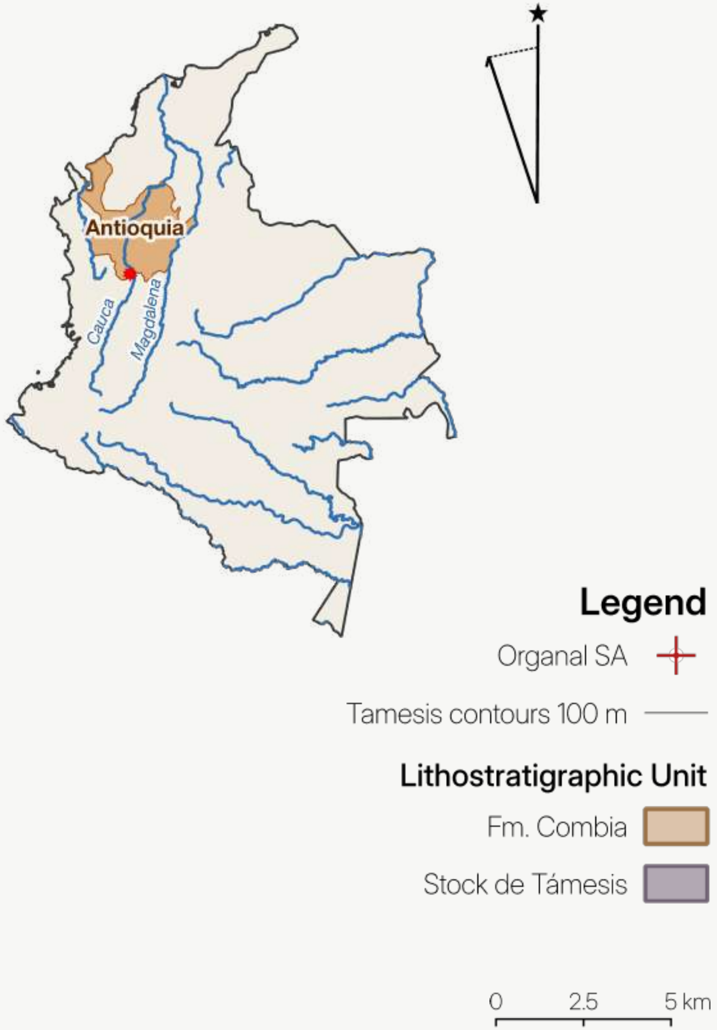


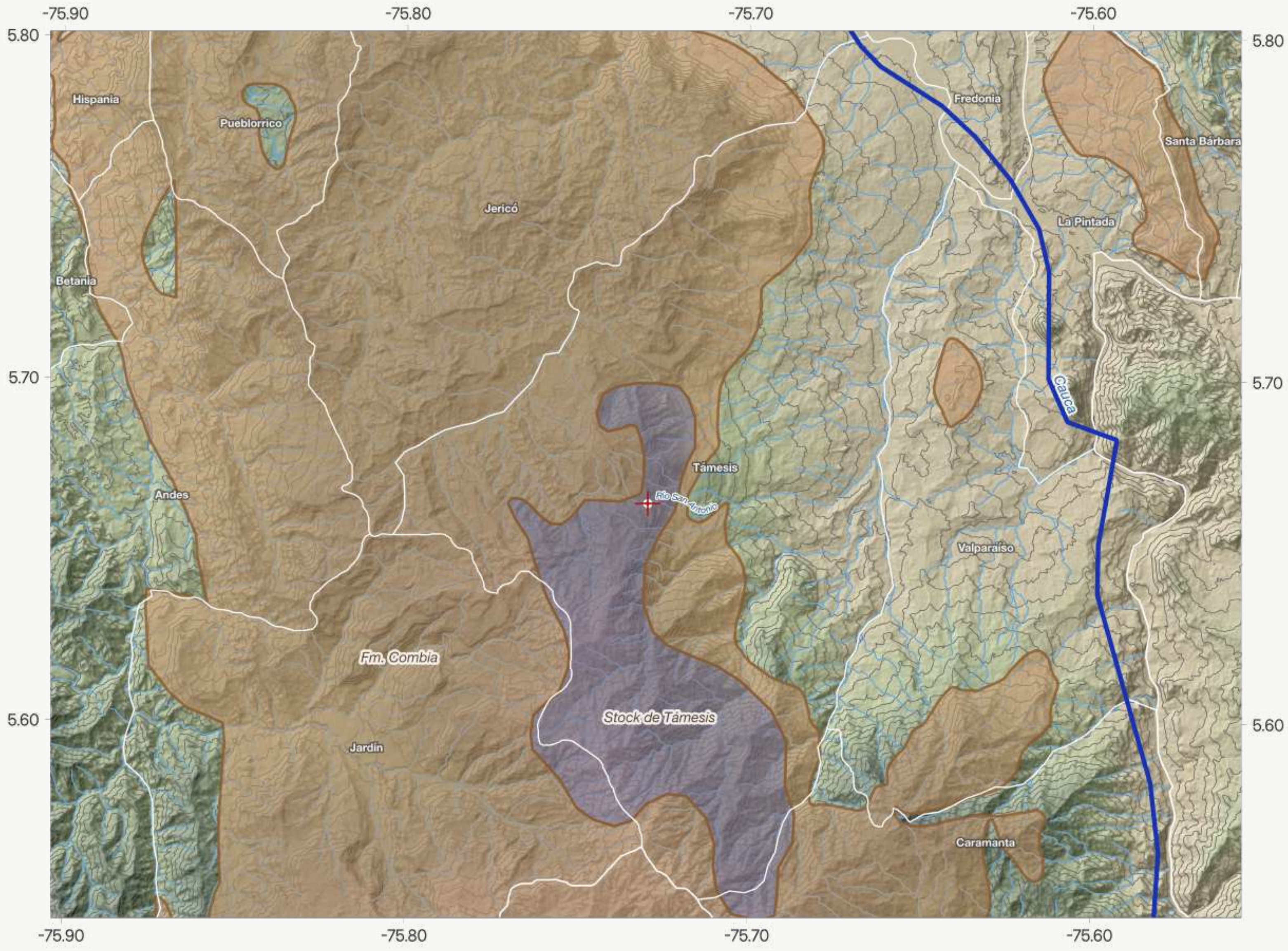
Source: Goldscheider et al. *Hydrogeol J* 2020;28:1661–1677. Modified.

Northup & Lavoie. *Geomicrobiol J* 2001;18:199–222; Barton & Northup. *J Cave Karst Stud* 2007;69:163–178; Hershey & Barton. In: *Cave Ecology* (Springer, 2018):69–90.


Organal San Antonio
Támesis, Colombia
Tropical Andes, ~2,350 m.a.s.l.

Geological context





S. Correa-Gallego. Terrain: Copernicus DEM GLO-30 (ESA, 2022). Boundaries: Natural Earth (2023); IGAC (2023).
Geology: Atlas Geológico de Colombia (SGC, 2023). Projected: MAGNA-SIRGAS / EPSG:9377.

Weber et al. In: *Geology of Colombia* Vol. 3 (SGC, 2020):355–394; Cano Alzate & Rentería Cárdenas (B.Sc. thesis, Univ. EAFIT, 2020).

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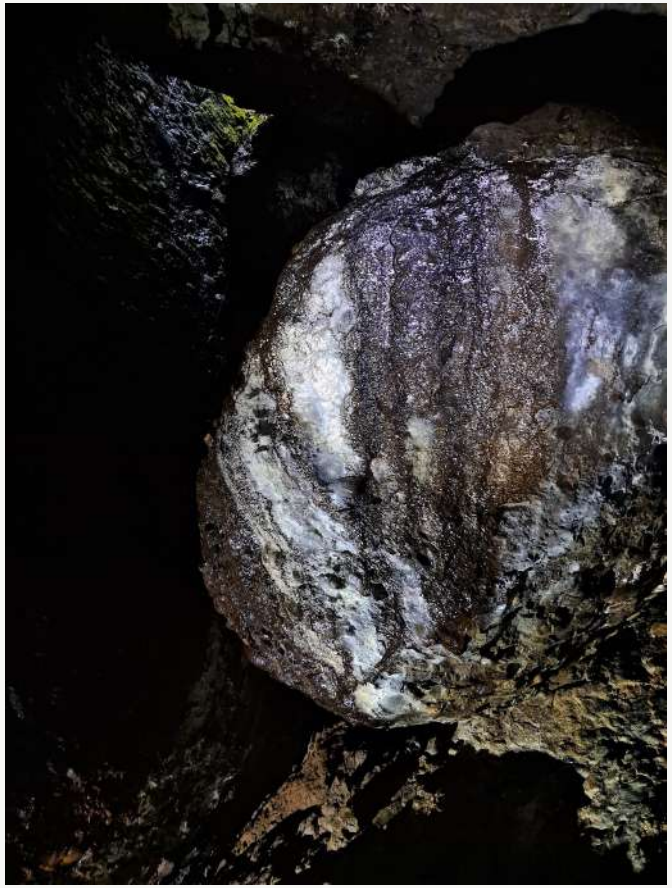
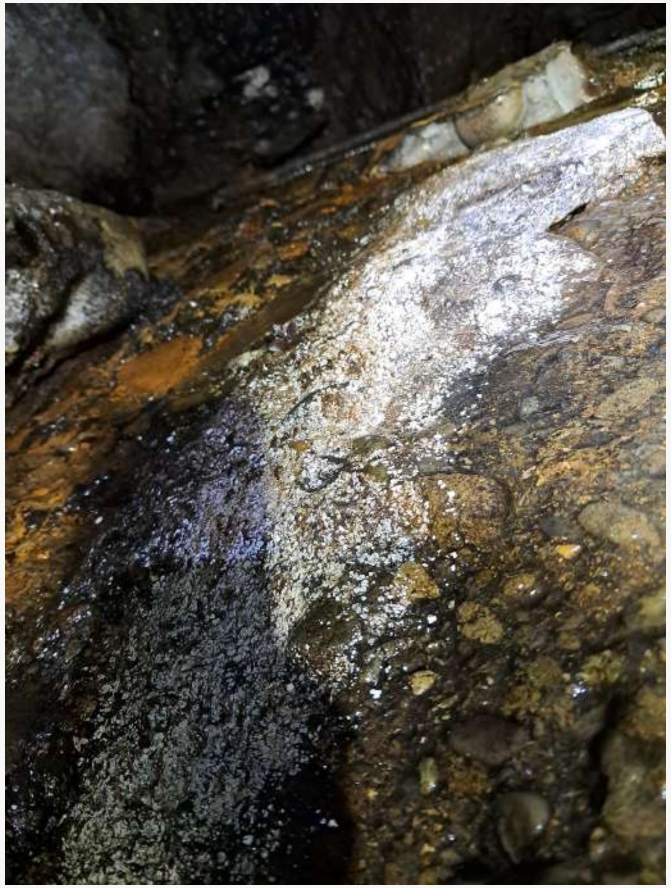
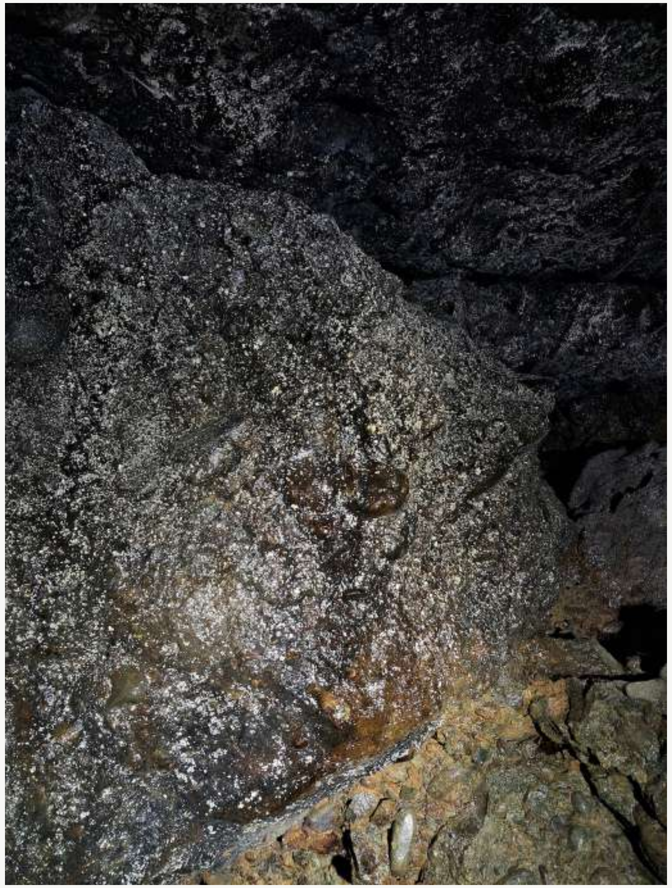
Visible microbial colonization varies along the gradient







Distance from entrance

Light availability

Oligotrophic pressure

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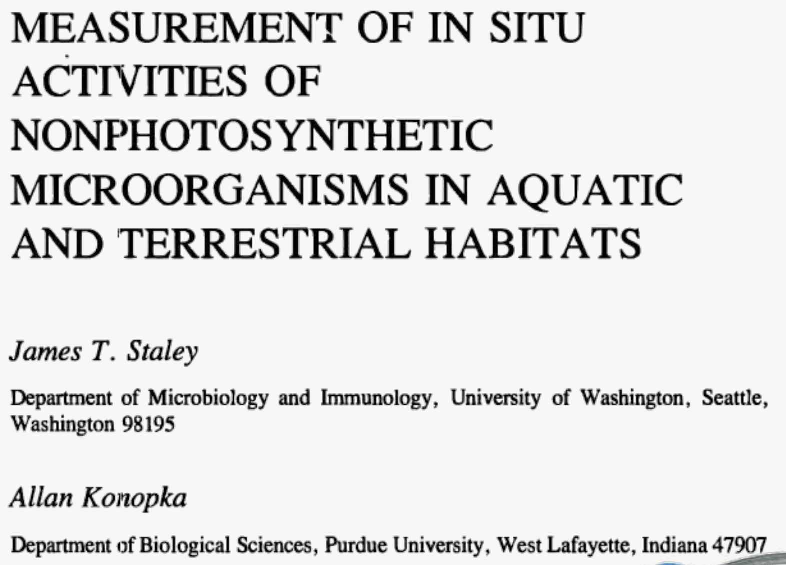
Why cultivation? A complementary window into community structure

What cultivation accesses:



MEASUREMENT OF IN SITU ACTIVITIES OF NONPHOTOSYNTHETIC MICROORGANISMS IN AQUATIC AND TERRESTRIAL HABITATS

James T. Staley

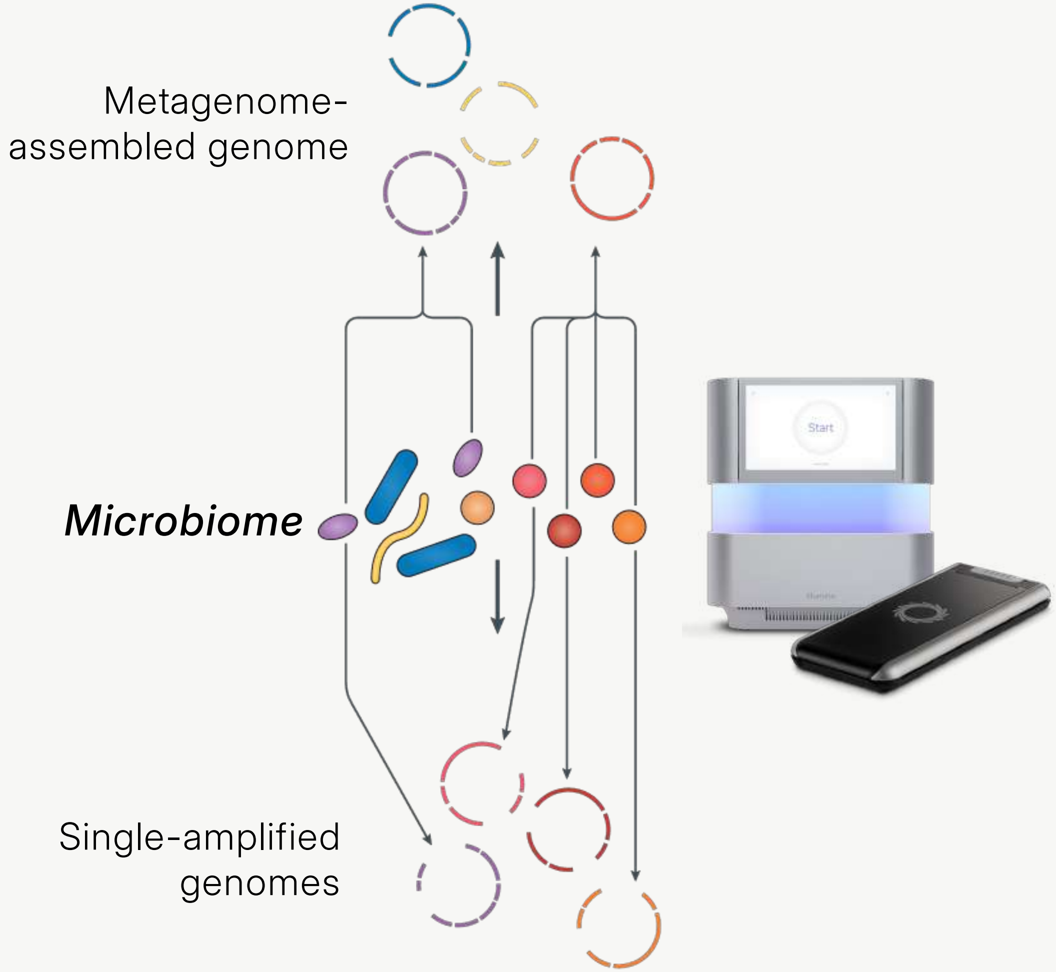
Department of Microbiology and Immunology, University of Washington, Seattle, Washington 98195

Allan Konopka

Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907

Estimated culturability in oligotrophic cave sediments: ***0.02–1%*** *of total cell counts.*

What molecular surveys can access:



Source: Hatzenpichler et al. *Nature Reviews Microbiology* 2020;**18:**241–256. Modified.

Staley & Konopka. *Annu Rev Microbiol* 1985;39:321–346; Barton. *J Cave Karst Stud* 2006;68:43–54; Shade et al. *Environ Microbiol* 2012;14:2247–2252.

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How is the cultivable microbial community structured along a light-defined ecological gradient in a tropical volcaniclastic cave?

To test whether sector identity is associated with differences in cultivable density, local morphotype-cluster diversity, and morphotype-level community composition.

(i) How much life? — cultivable density

(ii) What kinds? — local morphotype-cluster diversity

(iii) How organized? — compositional turnover across sectors

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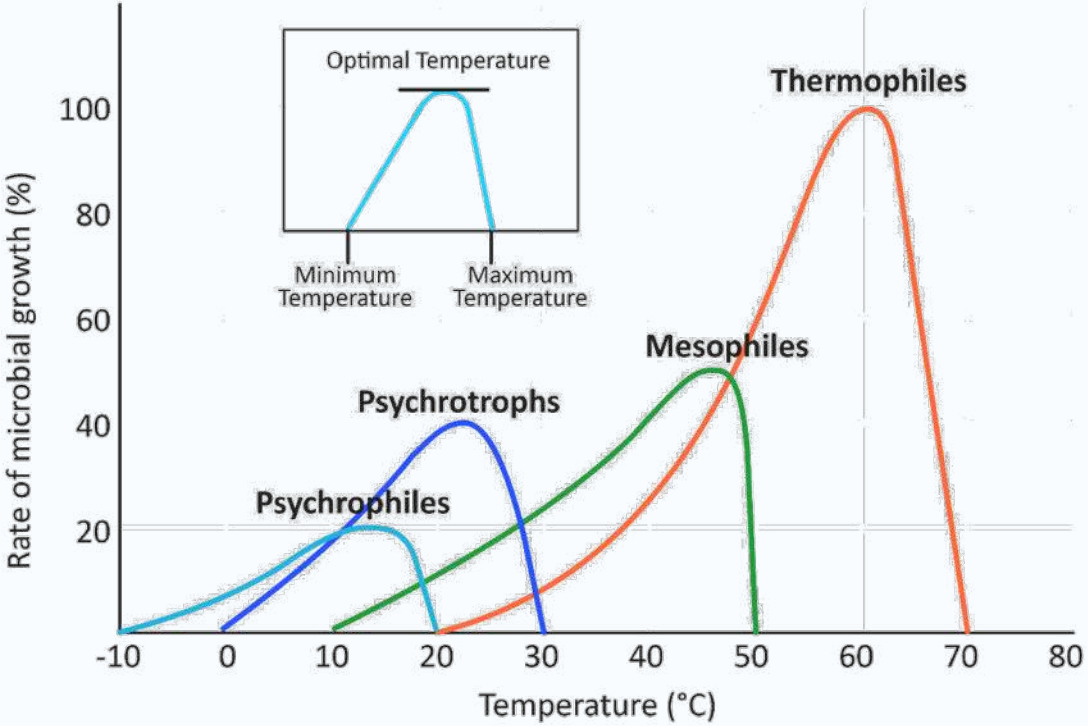
Cultivation under cave-mimetic conditions

15 °C incubation

Total darkness



Source: Akindolire et al. *Renewable and Sustainable Energy Reviews* 2022;**161**:112394.

25% R2A agar + 0.1 g/L $NaHCO_3$

Sterile cave stream water as diluent

Yeast extract	0.50	g
Proteose peptone (Difco no. 3)	0.50	g
Casamino acids	0.50	g
Glucose	0.50	g
Starch (soluble)	0.50	g
Na-pyruvate	0.30	g
K_2HPO_4	0.30	g
$MgSO_4 \times 7\ H_2O$	0.05	g
Agar	15.00	g
Distilled water	1000.00	ml

Reasoner & Geldreich. *Appl Environ Microbiol* 1985;49:1–7; Barton & Jurado. *Microbe* 2007;2:132–138.

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From sediment to community structure

Morphotype classification

Abundance matrix

1. Color and pigmentation
2. Colony shape
3. Elevation
4. Margin
5. Size
6. Surface texture
7. Transparency

Reasoner & Geldreich. *Appl Environ Microbiol* 1985;49:1–7; Haldeman & Amy. *Appl Environ Microbiol* 1993;59:933–935.

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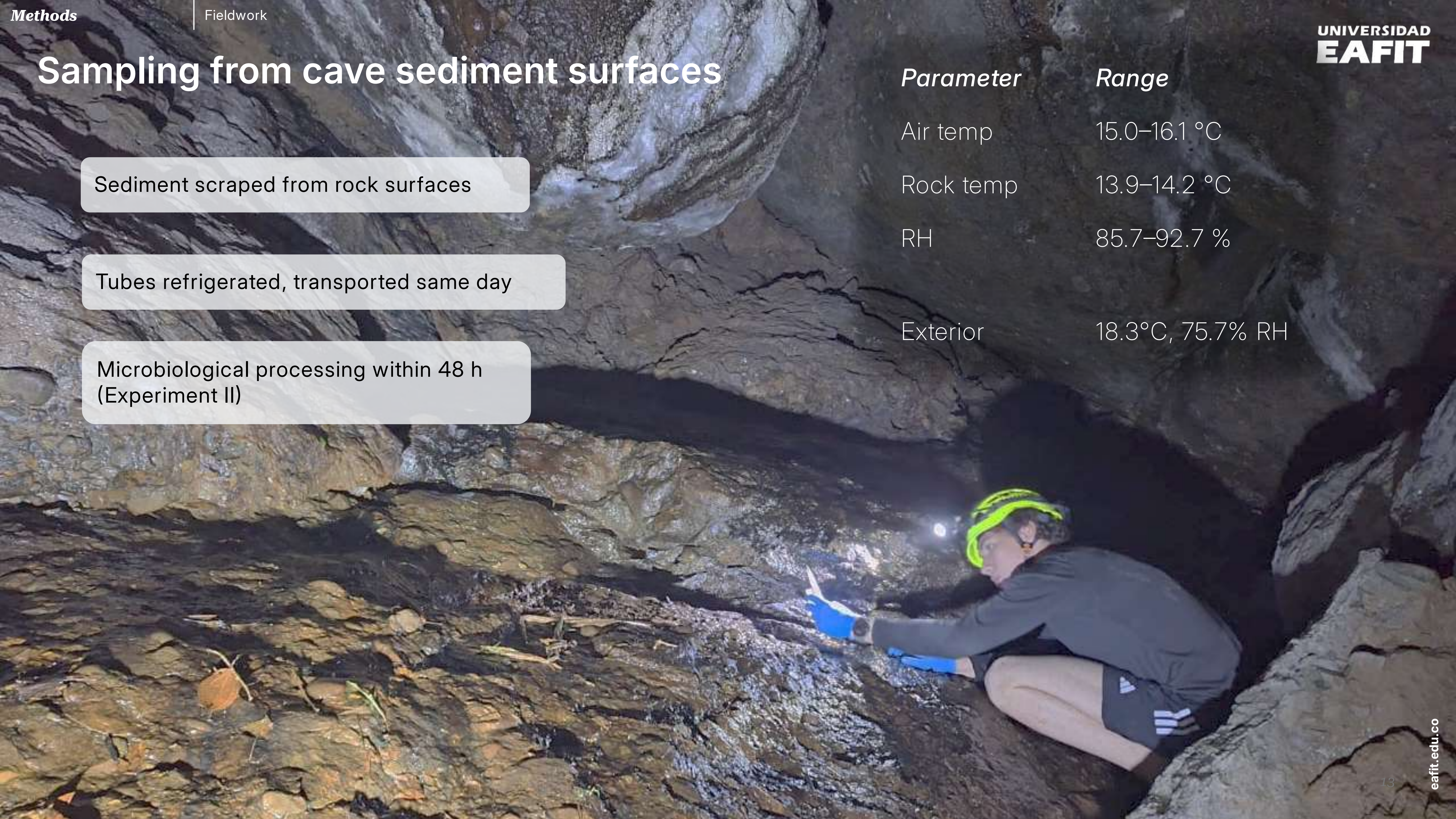
Sampling from cave sediment surfaces



- Sediment scraped from rock surfaces
- Tubes refrigerated, transported same day
- Microbiological processing within 48 h (Experiment II)

Parameter	*Range*
Air temp	15.0–16.1 °C
Rock temp	13.9–14.2 °C
RH	85.7–92.7 %
Exterior	18.3°C, 75.7% RH

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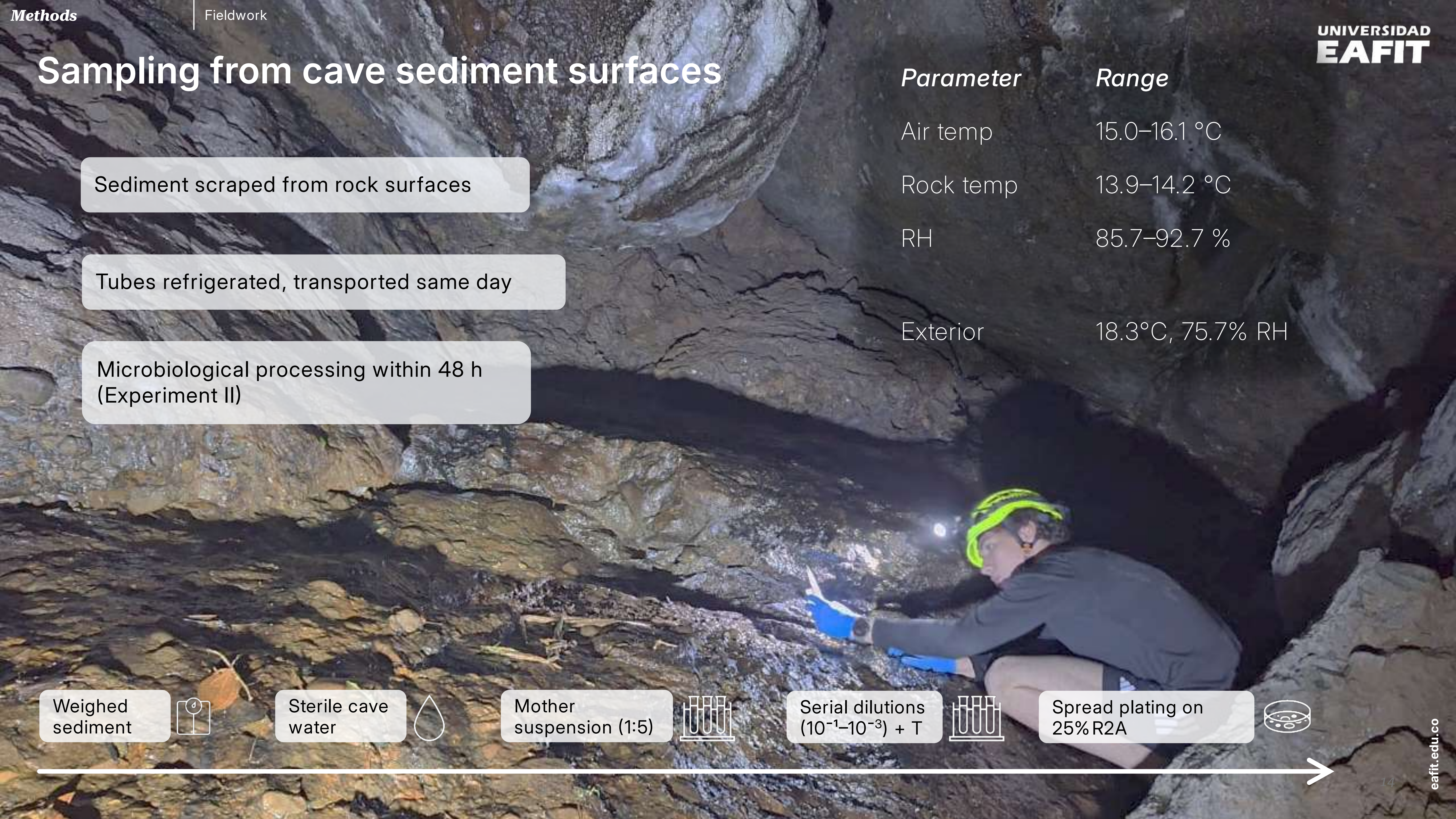
Sampling from cave sediment surfaces



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RH	85.7–92.7 %
Exterior	18.3°C, 75.7% RH

Weighed sediment → Sterile cave water → Mother suspension (1:5) → Serial dilutions (10^{-1}–10^{-3}) + T → Spread plating on 25% R2A

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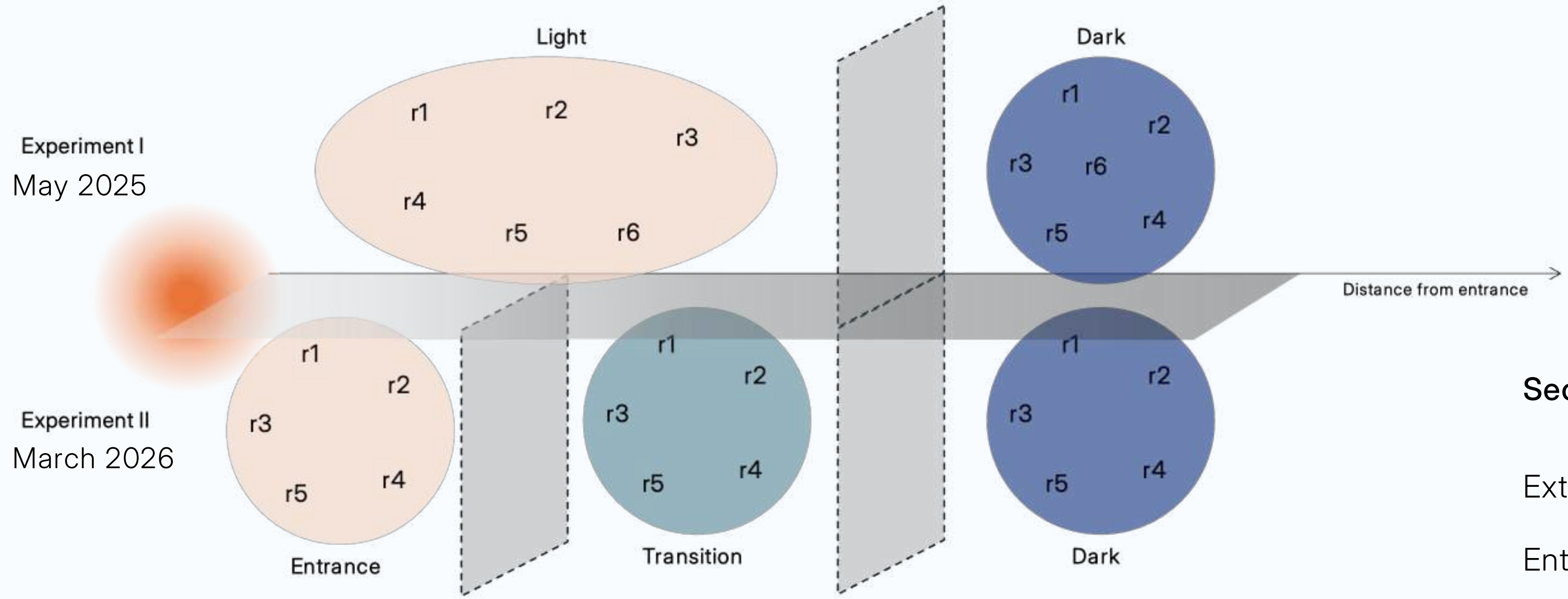
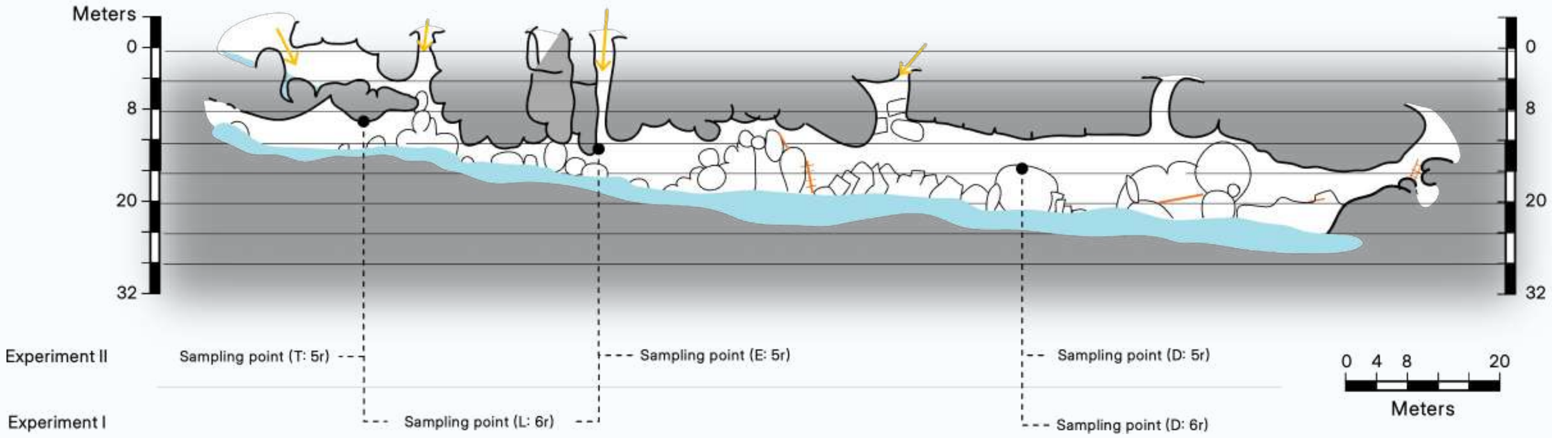
Two connected experimental designs





Modified from the work of Juliana Rincón, 2017.

Sector	Air temp (°C)	RH (%)	Rock temp (°C)
Exterior	18.3	75.7	14.8
Entrance	15.0	92.7	14.2
Transition	16.1	87.5	14.1
Dark	15.2	92.0	13.9

pH of wet cave sediment: 4.40
pH of filtered cave stream water: 5.74

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Analytical levels

0.

1.

Density

$\log_{10}$(CFU g^{-1} field-moist sediment); ANOVA + η^2

2.

Local diversity

α Morphotype-cluster richness, Shannon H′, Simpson 1−D

3.

Spatial structure

β Bray–Curtis on Hellinger-transformed counts; PERMANOVA + betadisper

4.

Complementary approaches

Observation of field samples under the microscope to describe possible heterotrophic microbial taxa.

Organism-level preliminary characterization: Gram, KOH 3% string test, catalase, light microscopy

R v4.5.2. Statistical analyses: vegan v2.7-3; iNEXT v3.0.2; effectsize v1.0.2

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Analytical framework

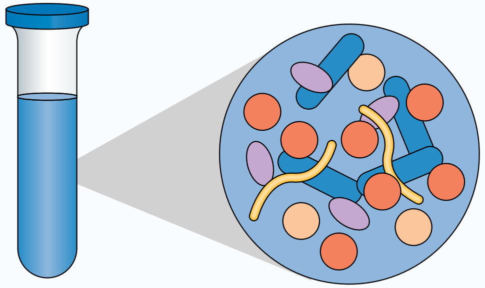
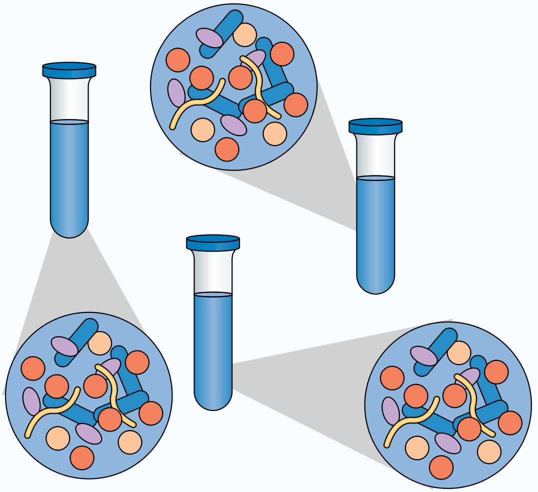


Illustration from
Nat. Rev. Microbiology 2020;**18:**241–256.

α diversity
(Within)

Observed richness	Shannon entropy	Simpson complement
S	$H' = -\Sigma\, p_i \ln(p_i)$	$1 - D = 1 - \Sigma\, p_i^2$



β diversity
(Between)

Hellinger

$$y'_{ij} = \sqrt{\frac{y_{ij}}{y_{i\cdot}}}$$

Bray-Curtis

PERMANOVA

R v4.5.2. Statistical analyses: vegan v2.7-3; iNEXT v3.0.2; effectsize v1.0.2

Legendre & Gallagher. *Oecologia* 2001;129:271–280; Anderson. *Austral Ecol* 2001;26:32–46; Anderson. *Biometrics* 2006;62:245–253.

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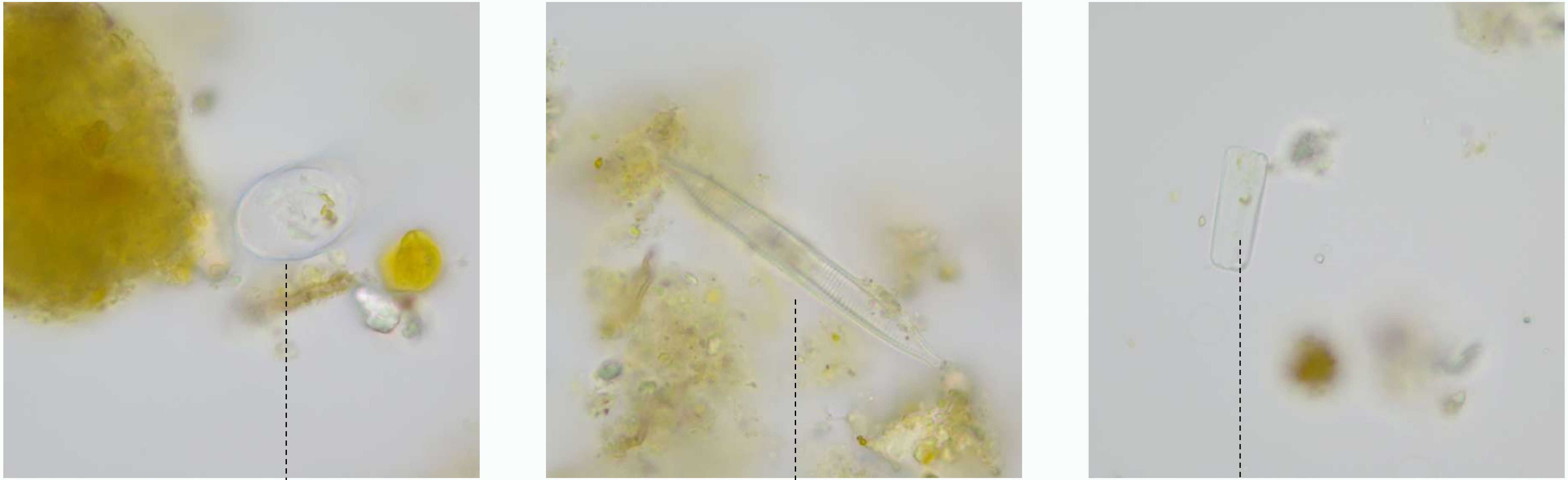
Sediment microhabitats along the cave gradient

Hydrated rock corridor near external light incidence. Diatom frustules and ciliate- like eukaryotes observed in wet mounts from entrance sector.



Ciliate-like

Diatom frustule

20 µm

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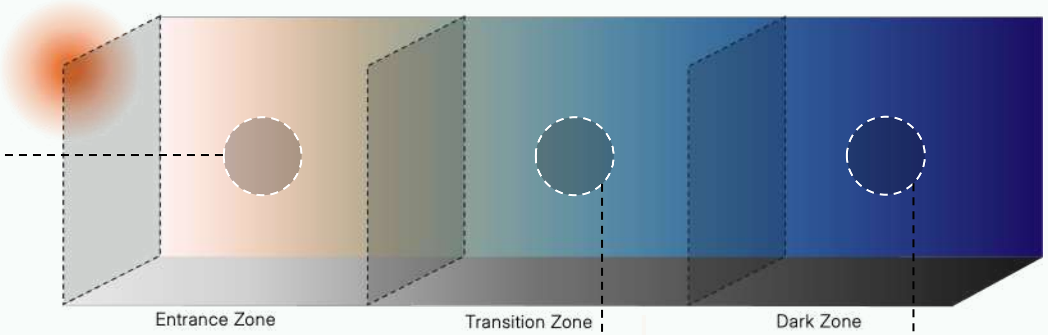
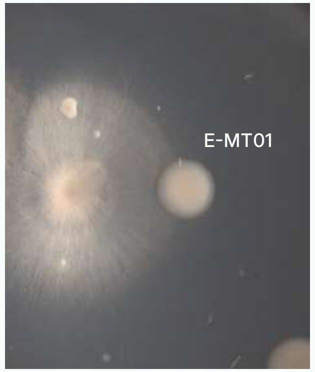
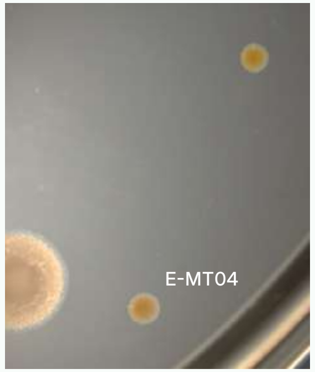
Colony morphotypes across the light gradient

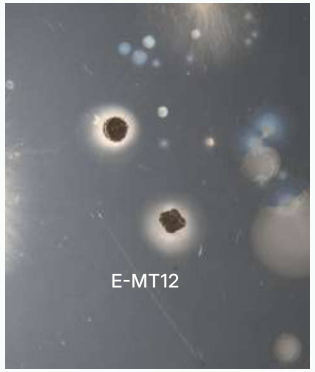
Forty-two zone-specific morphotypes recovered in Experiment II.



Entrance

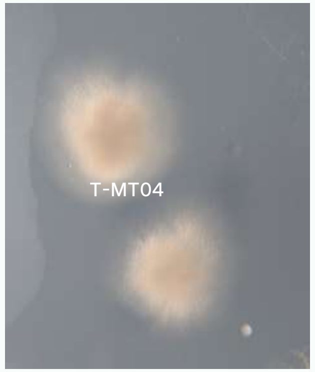


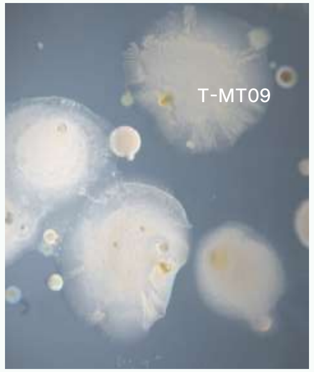




15 morphotypes

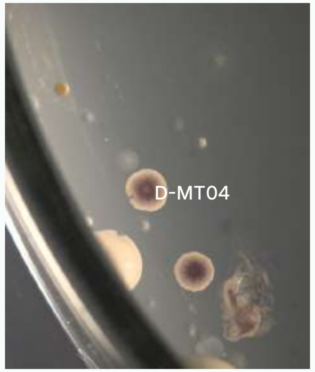
Transition





14 morphotypes

Dark



13 morphotypes

5 mm

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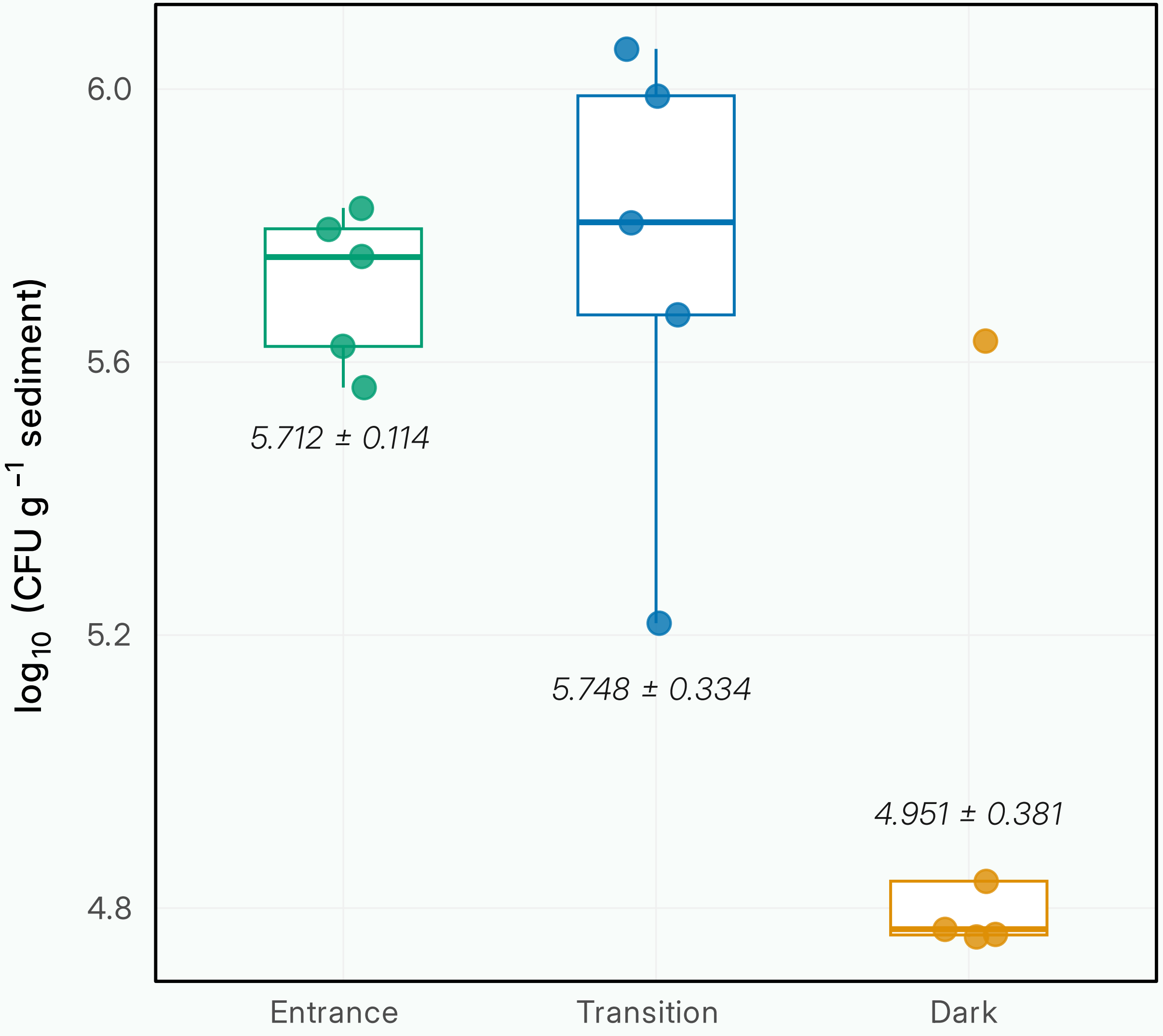
Cultivable density declines sharply in the Dark sector



Ligth	*Transition*	*Dark*
~5.15 × 10^5 CFU/g	~5.60 × 10^5 CFU/g	~8.94 × 10^4 CFU/g

ANOVA

$$F_{2,12} = 11.27$$

p = 0.0018; η^2 = 0.652
Kruskal-Wallis: H = 7.58, p = 0.023

Pairwise comparisons (Welch t, BH-adjusted):

E vs. T:

p = 0.829; Hedges' g = −0.144 (95% CI: −1.381 to 1.102)

E vs. D:

p = 0.013; Hedges' g = 2.707 (95% CI: 0.871 to 4.467)

T vs. D:

p = 0.013; Hedges' g = 2.224 (95% CI: 0.548 to 3.824)

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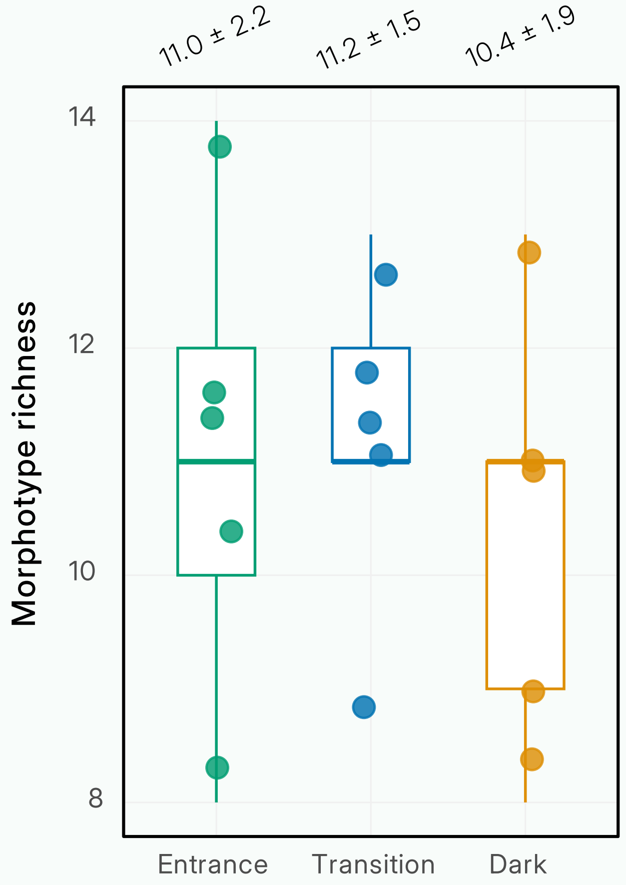
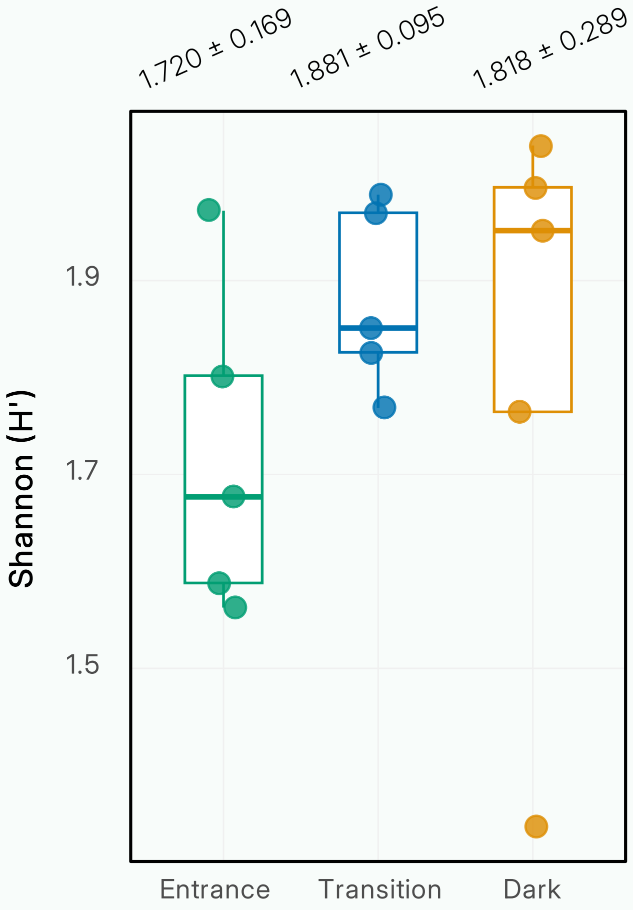
Local diversity is conserved across sectors



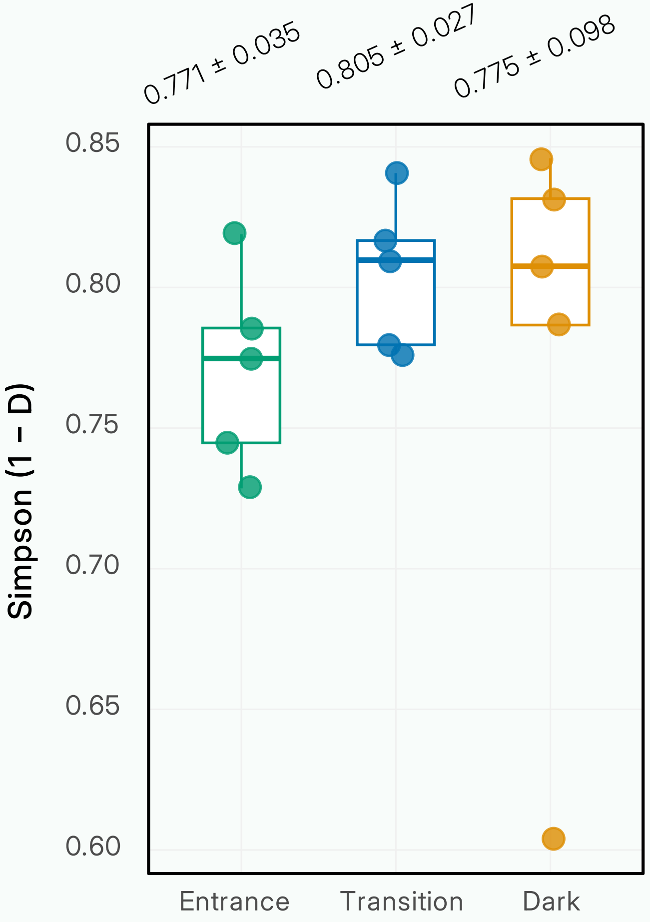
F = 0.236

p = 0.793; Kruskal–Wallis p = 0.764



F = 0.810

p = 0.468; Kruskal–Wallis p = 0.336

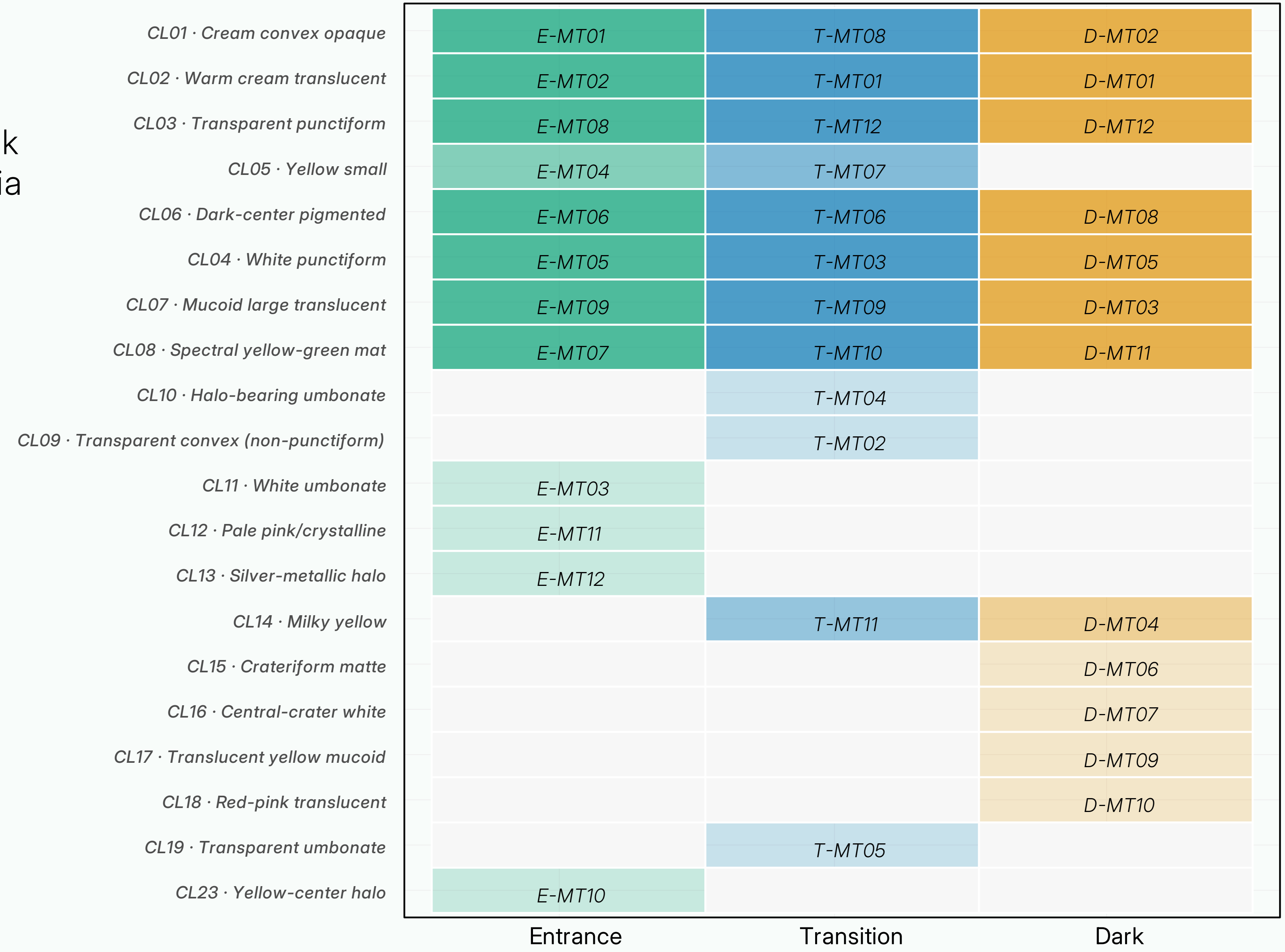


H = 2.16

p = 0.340; non-parametric test prioritized

A 23-cluster phenotypic crosswalk reconciles morphotypes across sectors

42 zone-specific morphotype codes unified to **23** operational clusters via diagnostic crosswalk based on 10 macroscopic criteria



Shared core:
7 cross-sector clusters

Zone-restricted:
Entrance: 4 clusters (~5.6%)
Transition: 3 clusters (~26.9%)
Dark: 4 clusters (~22.4%)

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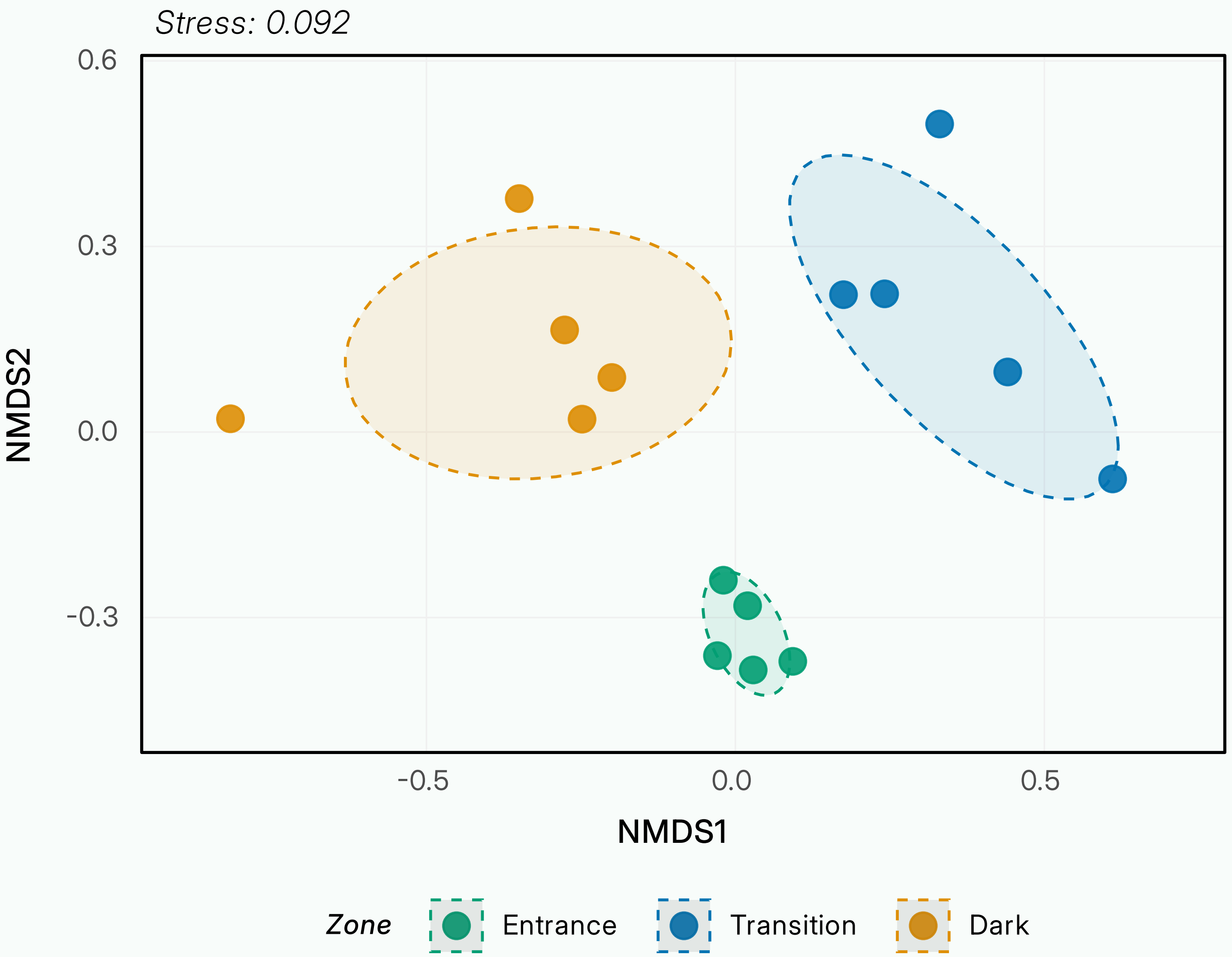
Cultivable communities partition strongly by cave sector



PERMANOVA

$R^2 = 0.573$

$F_{2,12} = 8.06$; $p = 0.001$; 999 permutations

Betadisper

$p = 0.334$

balanced multivariate dispersion
F = 1.257

Pairwise PERMANOVA (BH-adjusted)

Contrast	*R²*	*p (adj)*
Entrance vs. Transition	0.509	0.016
Entrance vs. Dark	0.539	0.016
Transition vs. Dark	0.466	0.016

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The story of the gradient

Cultivable density declines ~6× in the Dark sector

How much life:
cultivable biomass

Local α-diversity is conserved across sectors

What kinds: morphotype composition and diversity

Composition partitions strongly by sector

How structured spatially: beta-diversity and zonation

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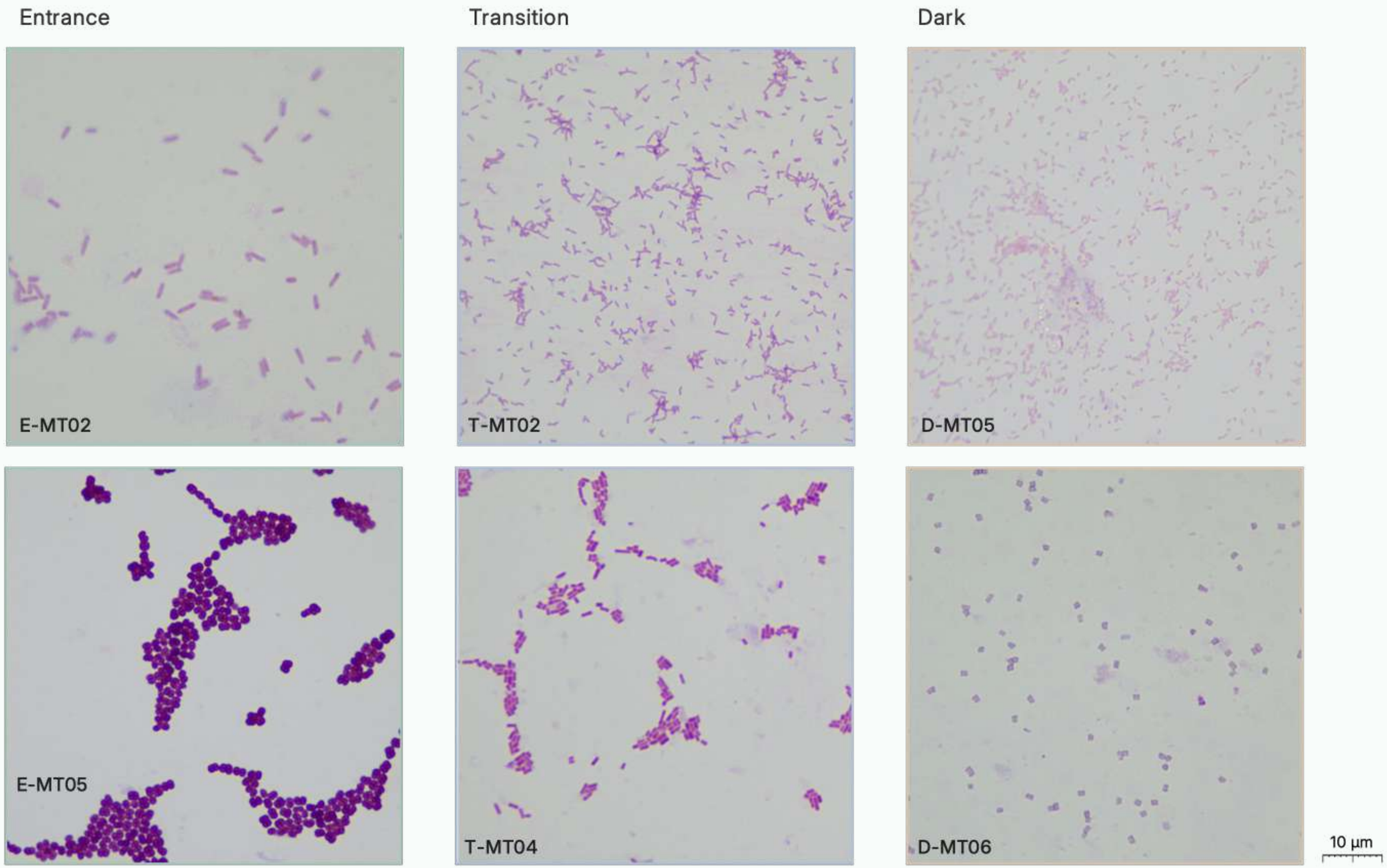
Preliminary phenotypic characterization at the cellular level



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The story of the gradient

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How structured spatially: beta-diversity and zonation

Phenotypic crosswalk groups cellularly distinct organisms — morphotype resolution is coarser than taxonomic resolution

Who are these organisms: preliminary characterization

Acknowledgements

To all the mentors, colleagues, family, and friends who shaped my scientific training and way of thinking.

"In the fields of observation, chance favours only the prepared mind."

Louis Pasteur
Lecture, University of Lille (1854)

Supplementary material

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The organal: a pseudokarstic cave type endemic to the Colombian Andes

Feature	Karstic cave	Lava tube	Organal (pseudokarst)
Speleogenesis	Carbonate dissolution	Lava flow dynamics	Gravitational accumulation of pyroclastic blocks
Substrate	Limestone / dolomite	Basaltic lava	Volcaniclastic breccia, agglomerate
Speleothems	Yes (stalactites, etc.)	Lavacicles, lava shelves	None
pH buffering	Carbonate dissolution — near neutral	Lower — basaltic	Acid — pH 4.40 sediment
Hydrological connectivity	High — continuous	Variable	Seepage through block matrix
Literature coverage	Dominant in microbiology	Growing since 2000s	This study: first microbiological investigation

Cano Alzate & Rentería Cárdenas (B.Sc., Univ. EAFIT, 2020); Halliday. *J Cave Karst Stud* 2007;69:114–133.

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Experiment I informed Experiment II

What was observed	*Why it matters*	*What was corrected*
Incubation at ambient laboratory	*May shift selective window*	15°C controlled incubator
Two categories (Light and Dark)	*Full gradient not represented*	Three categories (Entrance, Transition, Dark)
Sediment volume estimated	*Reduces quantitative precision*	Sediment mass weighed gravimetrically
PERMANOVA confounded by significant betadisper	*Cannot attribute compositional differences to centroid displacement*	5 independent biological replicates per sector; betadisper resolved

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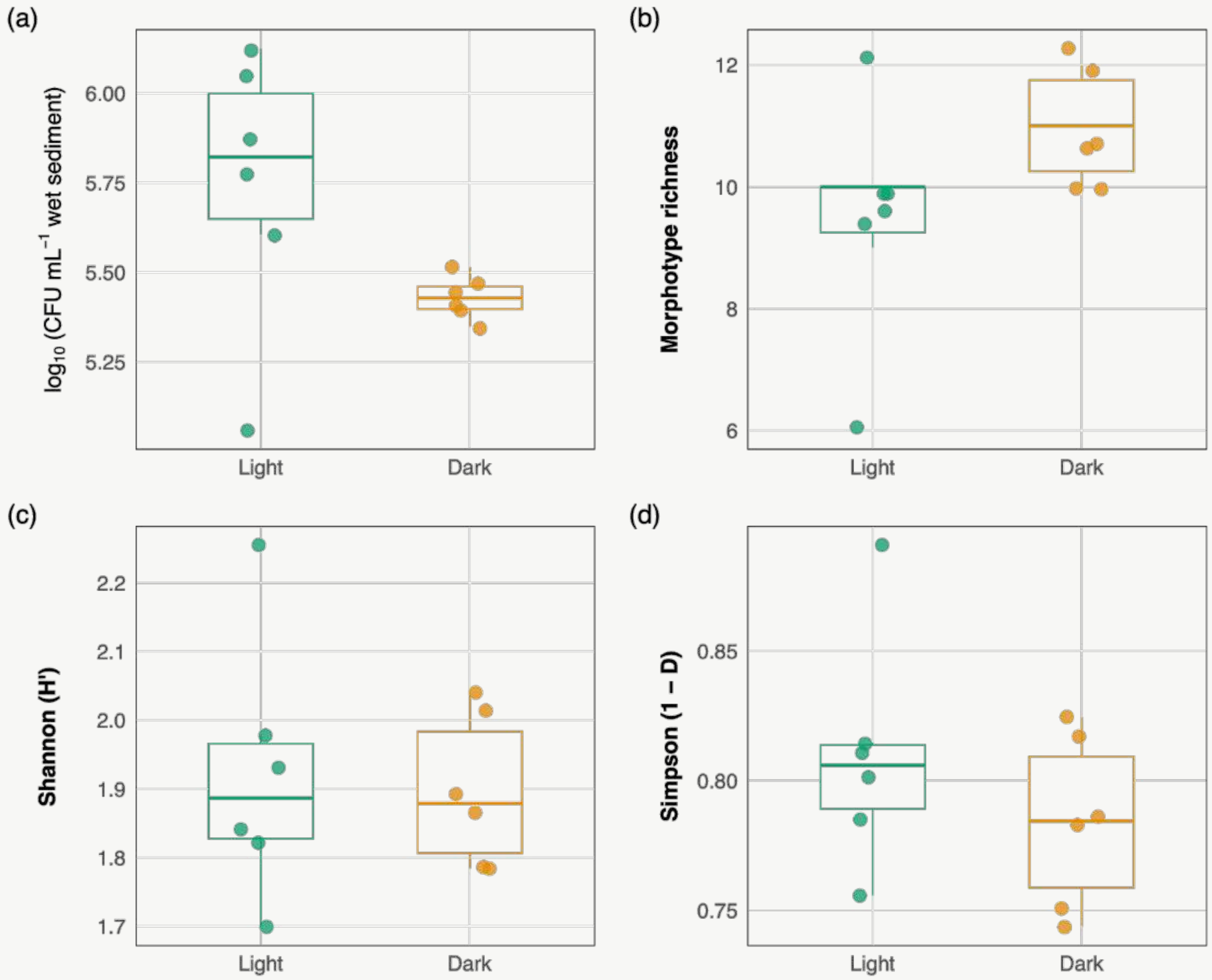
Experiment I — statistical results



Light vs. Dark, n = 6 per zone, 12 total. Single 19 morphotypes described; 14 in community matrix.

CFU

Zone	*n*	*Mean log_{10}*	*SD*
Light	6	5.748	0.382
Dark	6	5.430	0.058

Alpha diversity:
Richness: Welch p = 0.134, Wilcoxon p = 0.111, g = −0.978

Shannon: Welch p = 0.797, Wilcoxon p = 1.000, g = 0.154

Simpson: Welch p = 0.292, Wilcoxon p = 0.471, g = 0.646

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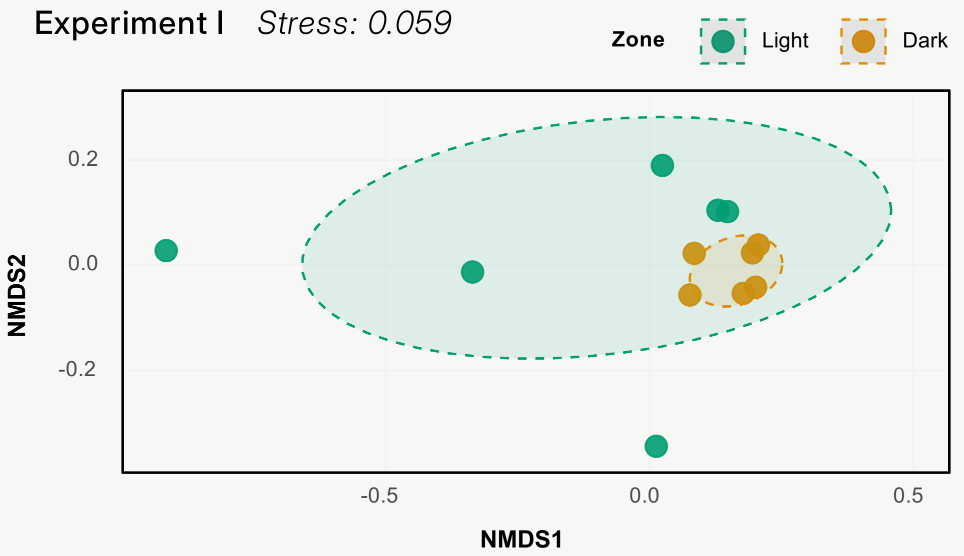
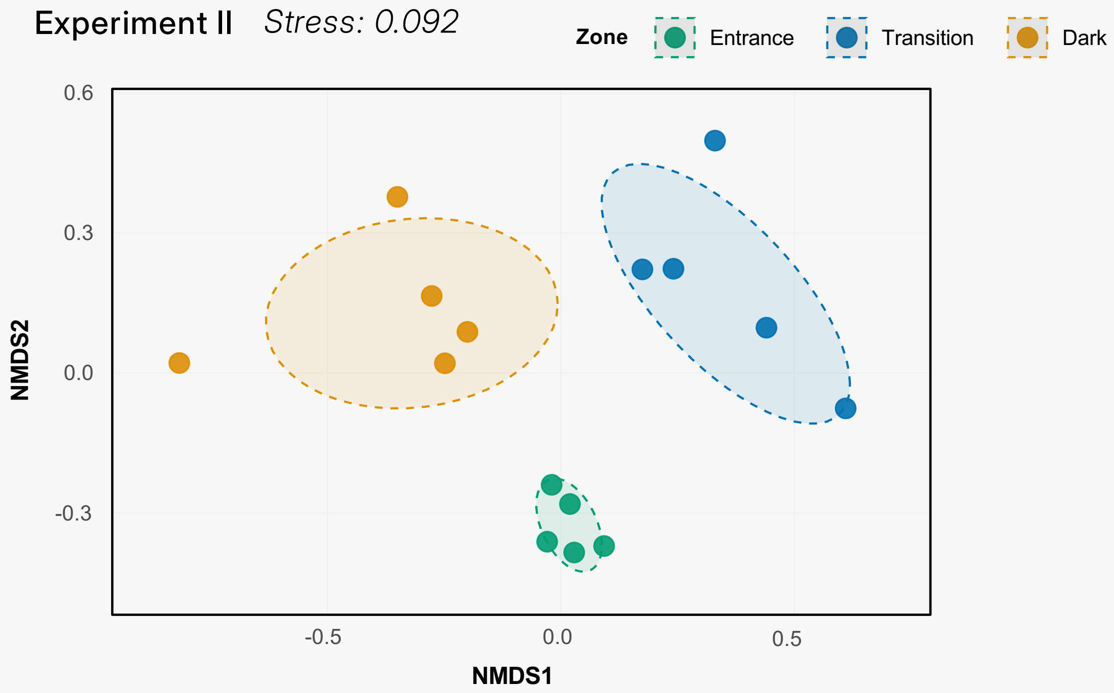
Betadisper: pilot vs. main experiment

	Experiment I	Experiment II
Design	*Light vs. Dark (binary)*	*Entrance / Transition / Dark*
n =	*12 (6 per zone)*	*15 (5 per sector)*
Morphotype units	*14 morphotypes*	*23 phenotypic clusters*
PERMANOVA R^2	*0.227*	*0.573*
PERMANOVA F	*2.942*	*8.064*
PERMANOVA p	*0.021*	*0.001*
Betadisper F	*5.389*	*1.257*
Betadisper p (permutation)	*0.021*	*0.334*





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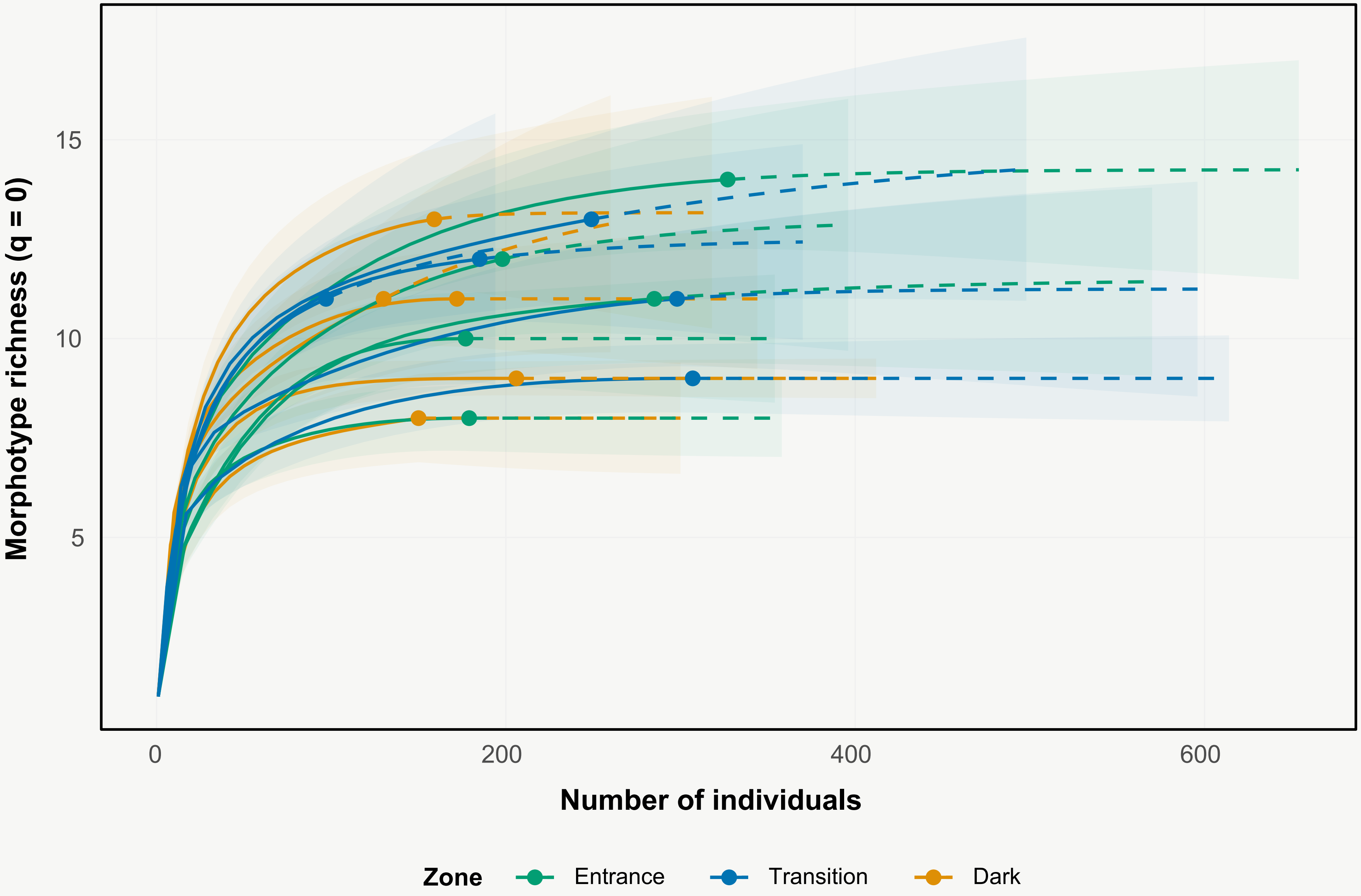
Rarefaction curves confirm morphotype sampling adequacy — Experiment II



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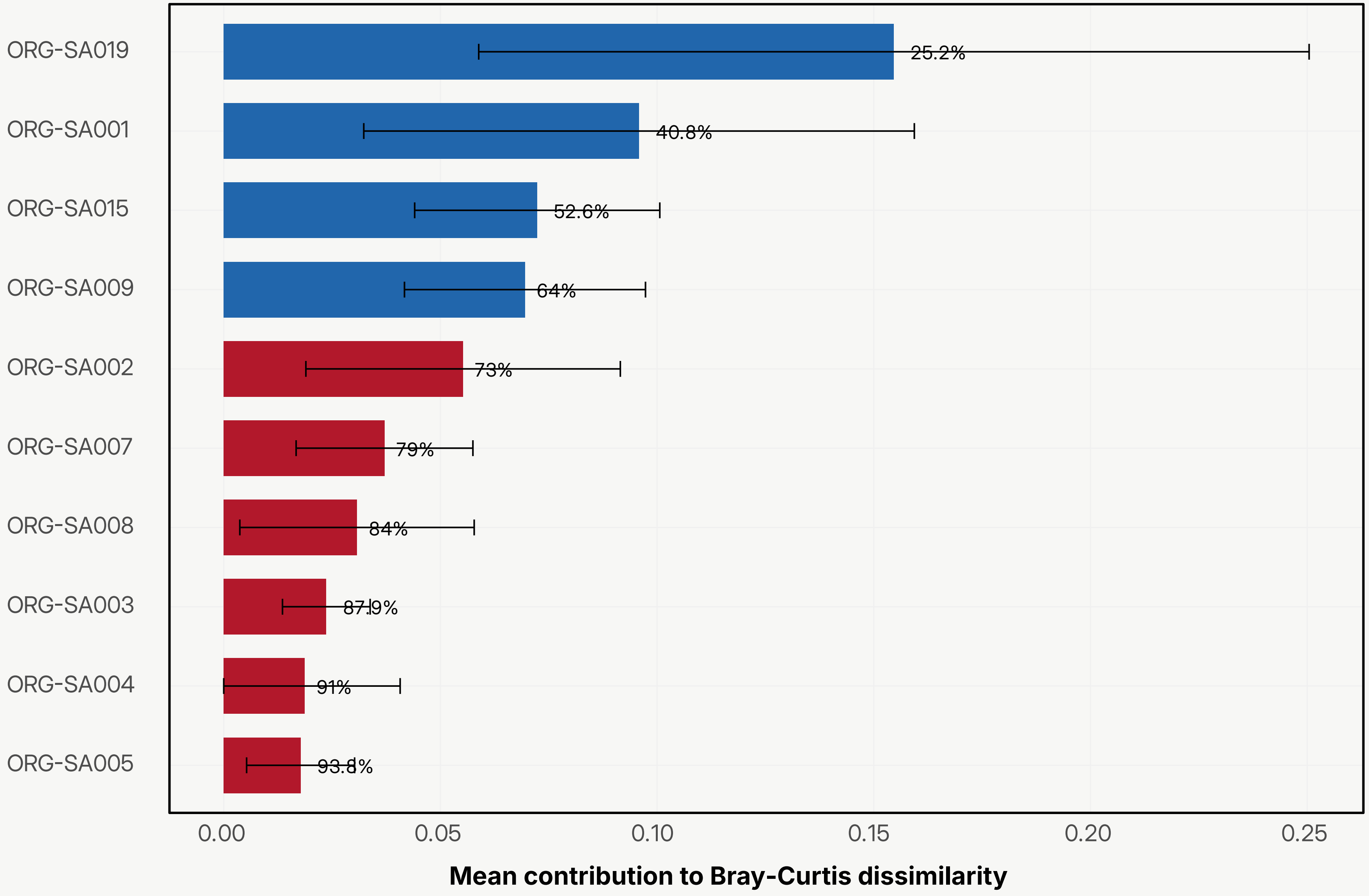
SIMPER: phenotypic clusters driving between-sector dissimilarity



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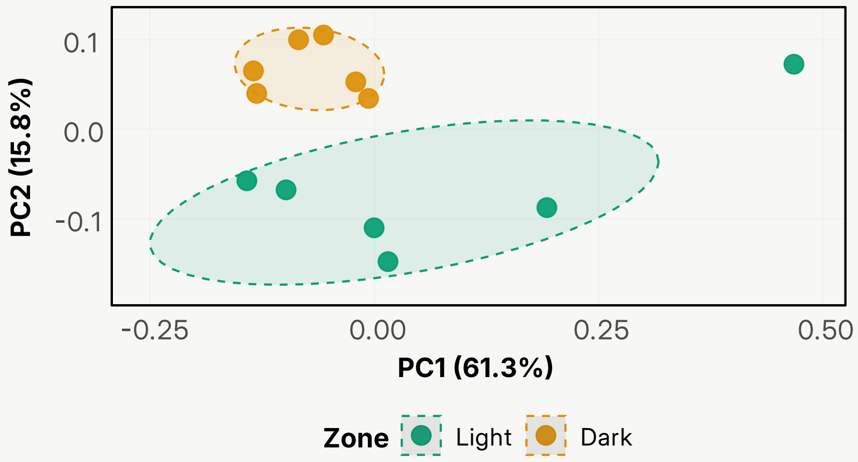
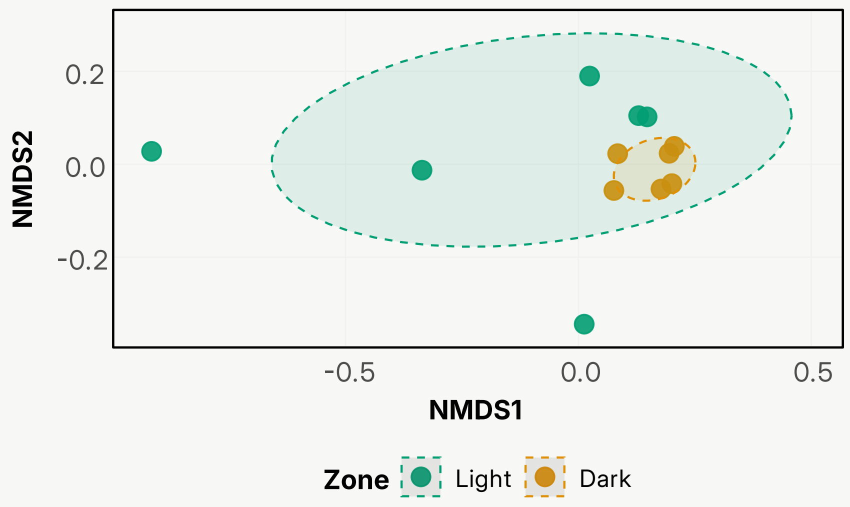
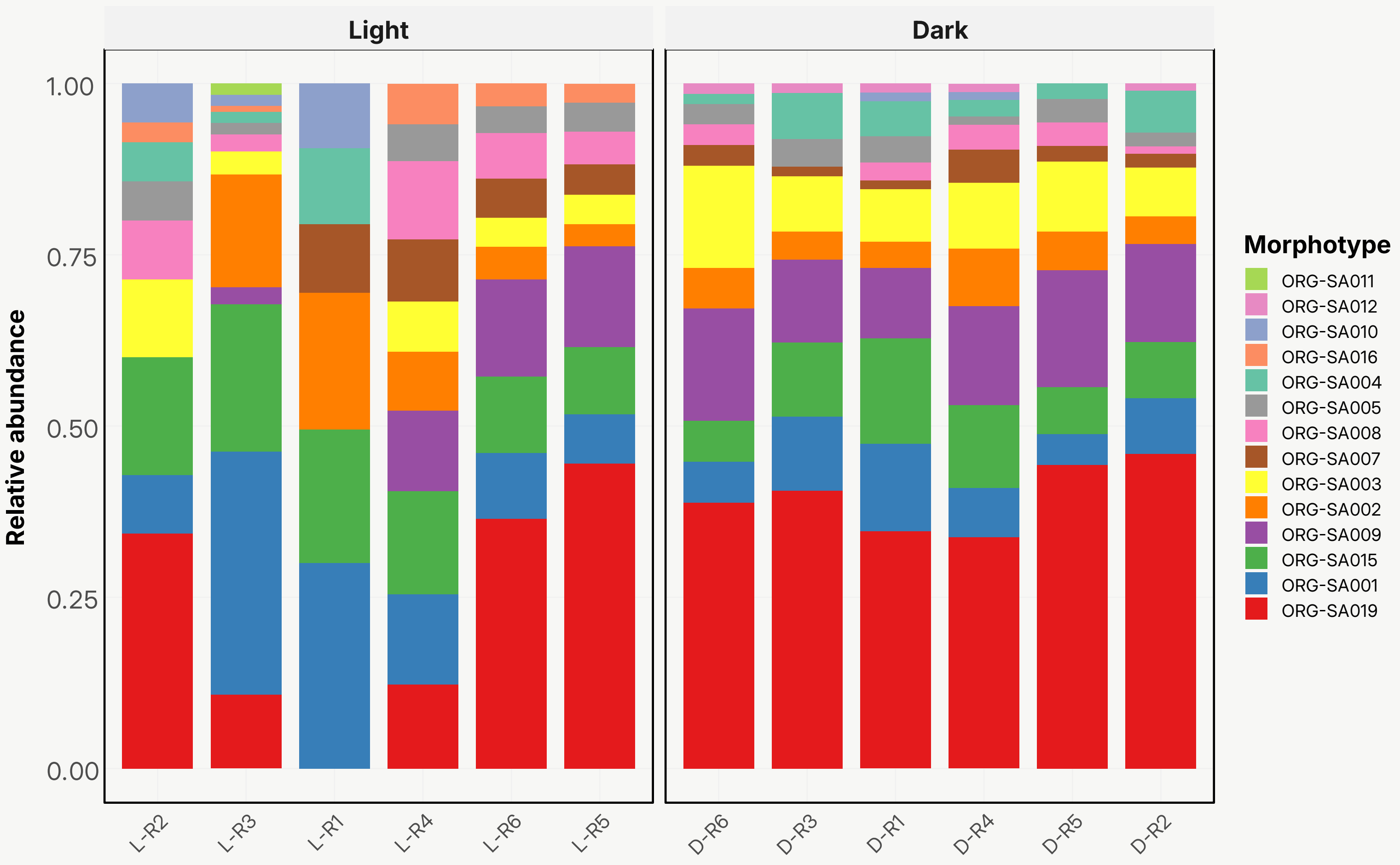
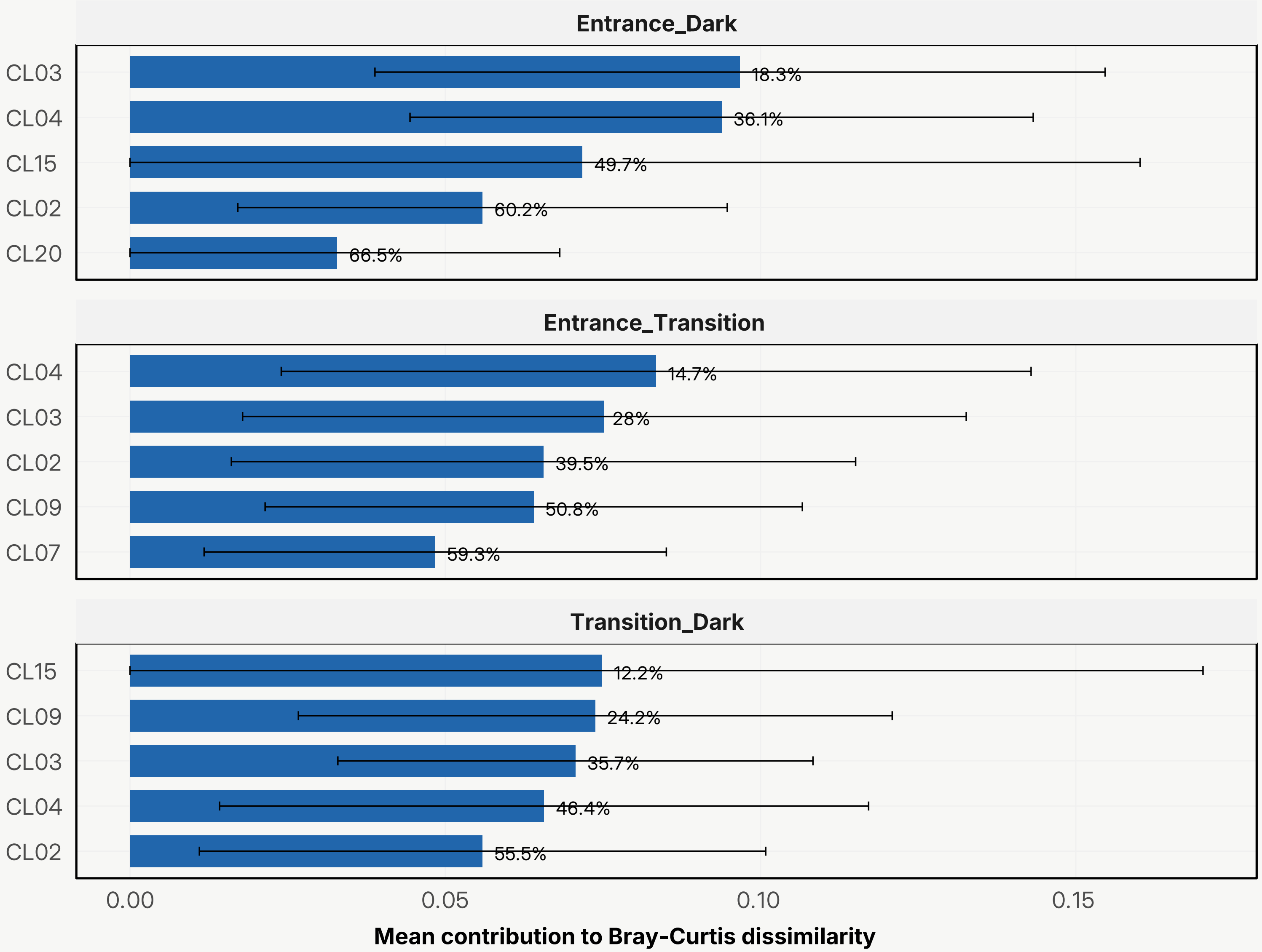
Rank-abundance curves (i)





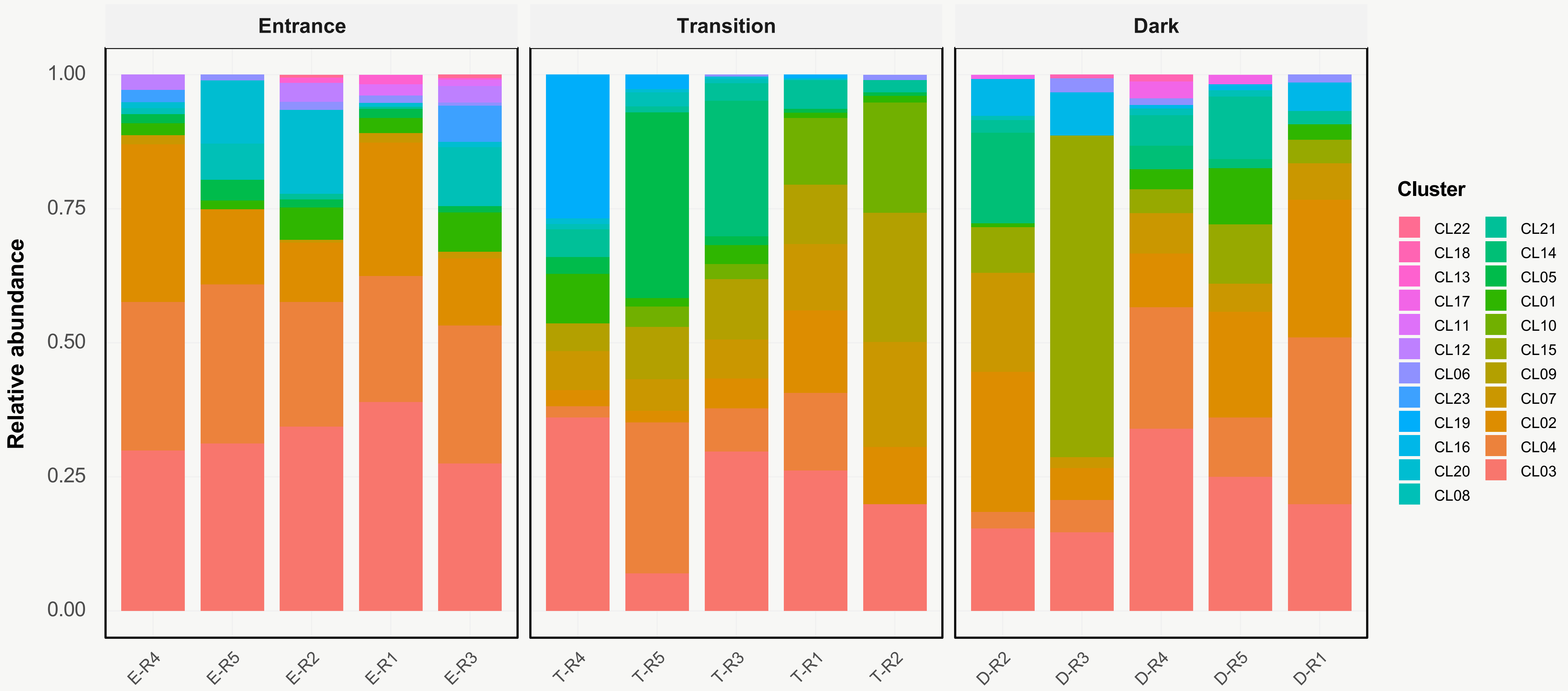


SIMPER: phenotypic clusters driving between-sector dissimilarity



SIMPER is used here as a descriptive tool only. Per-cluster p-values indicate which contributions are statistically distinguishable from zero but do not constitute inferential evidence for ecological mechanism. Inferential conclusions rest on PERMANOVA and betadisper.

Rank-abundance curves confirm comparable evenness across sectors



Full crosswalk

Cluster	Cluster name	Conf.	Entrance	Transition	Dark	Water	Diagnostic features
CL01	Cream convex opaque	High	E-MT01	T-MT08	D-MT02	C-MT01	White-cream, circular convex, entire margin, opaque, smooth glistening, 1–4 mm
CL02	Warm cream translucent	High	E-MT02	T-MT01	D-MT01	—	Warm cream, circular convex, entire, semi-translucent with milky interior, smooth glistening, 3–6 mm
CL03	Transparent punctiform	High	E-MT08	T-MT12	D-MT12	—	Transparent to near-clear, circular convex punctiform, entire, smooth glistening, 0.5–1 mm, water-drop appearance
CL04	White punctiform	Moderate	E-MT05	T-MT03	D-MT05	C-MT05	White opaque, punctiform convex, entire, smooth glistening, 0.5–1 mm
CL05	Yellow small	High	E-MT04	T-MT07	—	C-MT02	Yellow, circular convex, entire, smooth glistening, opaque, approximately 1 mm
CL06	Dark-center pigmented	High	E-MT06	T-MT06	D-MT08	C-MT04	Dark purple-black center with pale or grey border, low elevation, matte, opaque, 1–2 mm
CL07	Mucoid large translucent	Moderate	E-MT09	T-MT09	D-MT03	—	Large, mucoid, translucent, irregular colony shape, convex to deformed, glistening, 5–10 mm
CL08	Spectral yellow-green mat	Moderate	E-MT07	T-MT10	D-MT11	C-MT03	Flat spreading, translucent yellow-green, radial or diffuse margin, uniform pigment, glistening, 7–20 mm
CL09	Transparent convex non-punctiform	Low	—	T-MT02	—	—	Transparent, circular convex, entire, smooth glistening, 0.5–1 mm; retained separately from CL03 because of non-punctiform appearance
CL10	Halo-bearing umbonate	Moderate	—	T-MT04	—	—	Pale white center with clear halo, umbonate, slightly irregular margin, matte, 2–3 mm
CL11	White umbonate	Low	E-MT03	—	—	—	White-cream, circular umbonate, entire, smooth glistening, approximately 2 mm, opaque
CL12	Pale pink/crystalline	Low	E-MT11	—	—	—	White-cream with faint pink tone, circular convex, semi-translucent crystalline, 1.5–4 mm
CL13	Silver-metallic halo	Low	E-MT12	—	—	—	White to silver center, translucent halo, umbonate, low luster, 1–2 mm
CL14	Milky yellow	Low	—	T-MT11	D-MT04	—	Pale milky yellow, circular convex, translucent to semi-translucent, approximately 2–3 mm
CL15	Crateriform matte	Low	—	—	D-MT06	—	White-cream, crateriform, slightly wrinkled, diffuse irregular margin, dry matte, approximately 1.5 mm
CL16	Central-crater white	Low	—	—	D-MT07	—	White, elevated with subtle central crater, entire to subtly irregular margin, matte, approximately 2 mm
CL17	Translucent yellow mucoid	Low	—	—	D-MT09	—	Translucent yellow, circular convex, mucoid, 6–8 mm
CL18	Red-pink translucent	Low	—	—	D-MT10	—	Pale translucent red-pink, circular convex, mucoid, liquid-like deformation, 6–8 mm
CL19	Transparent umbonate	Low	—	T-MT05	—	—	Transparent, punctiform umbonate with central tip, entire, smooth glistening, approximately 1 mm
CL20	Fungal rhizoid (Penicillium-like)	High	E-MT14	T-MT14	—	—	Pale cream, rhizoid with abundant hyphae, matte fungal texture, 10–50 mm
CL21	Fungal pubescent	Moderate	E-MT13	T-MT13	D-MT13	C-MT06	White, pubescent or hairy, elevated, matte opaque, radial margin, 10–22 mm
CL22	Fungal large asymmetric	Low	E-MT15	—	—	—	Very pale white, flat, very large, matte, asymmetric radial growth, 40–50 mm
CL23	Yellow-center halo	Low	E-MT10	—	—	—	Bright yellow umbonate center with translucent halo and pigment gradient, 2 mm center up to 10 mm halo

Code	Color / Pigment	Shape / Elevation	Margin	Size (mm)	Texture / Surface	Transparency	Type
C-MT01	White-cream	Circular, elevated	Entire, regular	3–4	Smooth, low luster	Opaque	Bact.
C-MT02	Yellow	Circular, convex	Entire, slightly diffuse	1–2	Smooth, glistening	Translucent	Bact.
C-MT03	Spectral yellow	Flat, spreading	Radial, diffuse	~10	Glistening, irregular	Translucent	Bact.
C-MT04	Purple-lilac	Circular, convex	Entire, regular	1–2	Matte, smooth	Opaque	Bact.
C-MT05	Pale cream, punctiform	Circular, convex	Entire, irregular	0.5–1	Low luster	Opaque	Bact.
C-MT06	White, pubescent	Convex, pubescent	Entire, radial	~10	Matte, hairy	Opaque	Fungal?

Fresh-water dilution	Colony count	CFU mL^{-1}
Undiluted	292	2.92×10^3
1:10	21	2.10×10^3
1:100	8	8.00×10^3

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Within-sector variability

$$m_{\text{aliquot}} = m_{\text{tube}} \times \frac{V_{\text{aliquot}}}{V_{\text{tube}}}$$

$$\text{CFU per unit substrate} = \frac{N}{D_{\text{serial}} \times V_{\text{plated}} \times A}$$

Table S10. Aliquot-level gravimetric normalization used for cultivable density estimates in Experiment II. m_{tube} is the measured field-moist sediment mass in each microtube. m_{aliquot} is the estimated field-moist mass of the 200 µL sediment aliquot used to prepare the mother suspension.

Selected plate	Zone	Rep.	m_{tube} (g)	m_{aliquot} (g)	Dilution	Count	$\log_{10}$(CFU g^{-1})
OSA-E1-D2	E	1	1.85	0.2467	10^{-2}	154	5.795
OSA-E2-D2	E	2	1.59	0.2120	10^{-2}	89	5.623
OSA-E3-D2	E	3	1.89	0.2520	10^{-2}	143	5.754
OSA-E4-D2	E	4	1.87	0.2493	10^{-2}	167	5.826
OSA-E5-D2	E	5	1.56	0.2080	10^{-2}	76	5.563
OSA-T1-D2	T	1	1.25	0.1667	10^{-2}	163	5.990
OSA-T2-D2	T	2	1.27	0.1693	10^{-2}	194	6.059
OSA-T3-D2	T	3	1.61	0.2147	10^{-2}	137	5.805
OSA-T4-D2	T	4	1.50	0.2000	10^{-2}	33	5.217
OSA-T5-D2	T	5	1.51	0.2013	10^{-2}	94	5.669
OSA-D1-D2	D	1	1.67	0.2227	10^{-2}	95	5.630
OSA-D2-D1	D	2	1.64	0.2187	10^{-1}	126	4.761
OSA-D3-D1	D	3	1.65	0.2200	10^{-1}	152	4.839
OSA-D4-D1	D	4	1.57	0.2093	10^{-1}	123	4.769
OSA-D5-D1	D	5	1.82	0.2427	10^{-1}	139	4.758