Whole-community cryopreservation reaches the phylogenetic blind spots of culture collections

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Presented by Sara Pipponzi and Lars Möller

ABSTRACT

Culture collections hold some **600,000** strains, but set against environmental sequence data that is genus-level coverage of **76%** of bacteria, **50%** of fungi and **33%** of protists. What falls outside is not a random sample. We asked whether cryopreserving a whole soil community reaches red-zone taxa, those held in no collection, that isolation has not. Two soils were cryopreserved under four protocols against a 4 °C control, held for a year at −196 °C, and profiled by rRNA after 24 h resuscitation. Cryopreservation recovered **60 of 61** red-zone bacterial classes and **23 of 41** fungal classes. For bacteria it also restructured them: two in five classes shifted in abundance, while no fungal class did. The fungal shortfall is not a failure to bank material, 39 of 41 classes returned as DNA, but a failure to resume activity within the window allowed. Whole-community cryopreservation is complementary to isolate-based biobanking, not a replacement.

INTRODUCTION

Coverage follows culturability.

The bacterial wedge is broadly green: Pseudomonadota, Actinomycetota and Bacillota are the lineages that grow on a plate, and they are the lineages collections hold. The protist wedge is red almost throughout. Fungi sit between the two, pale rather than green. The gap is phylogenetic, not statistical. Whole clades are absent together, because what excludes one genus excludes its neighbours: obligate biotrophy, unculturability, a host that cannot be brought into the lab.

The question.

Can whole-community cryopreservation reach red-zone taxa that isolation cannot? And if it reaches them, does it return them unchanged?

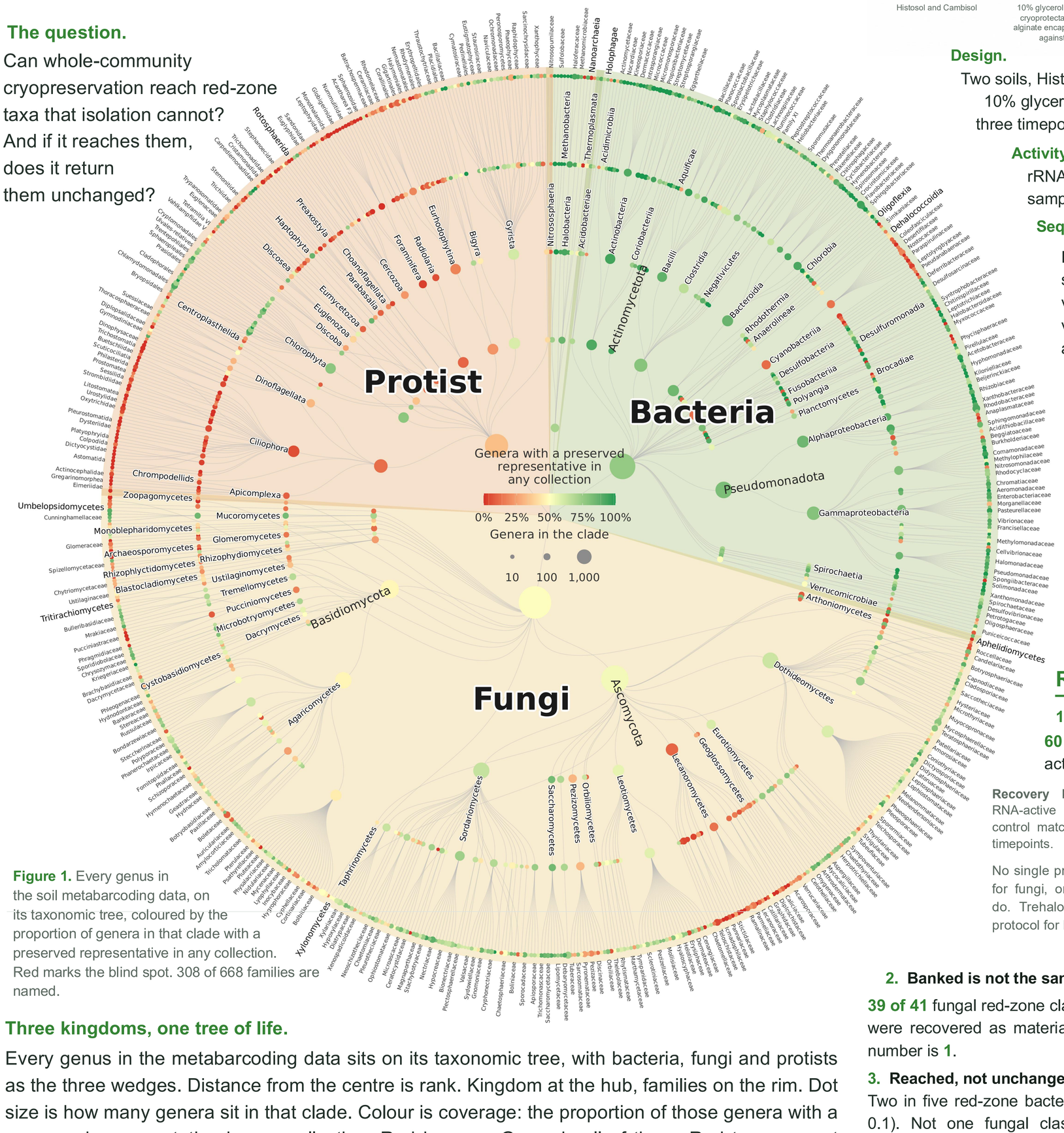
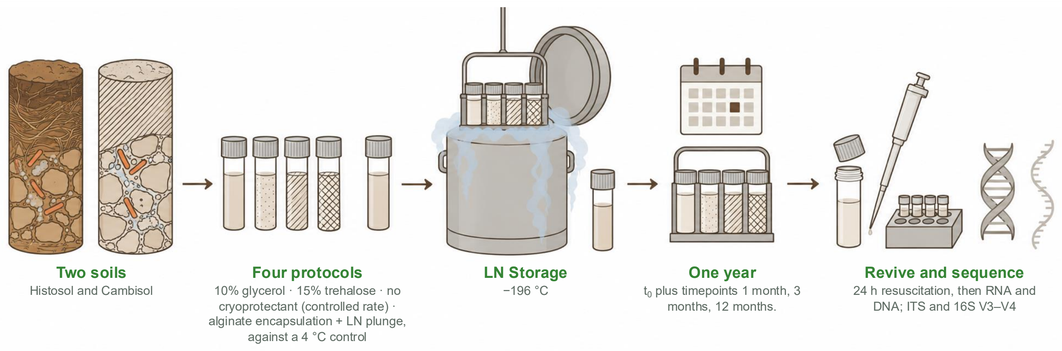


Figure 1. Every genus in the soil metabarcoding data, on its taxonomic tree, coloured by the proportion of genera in that clade with a preserved representative in any collection. Red marks the blind spot. 308 of 668 families are named.

Three kingdoms, one tree of life.

Every genus in the metabarcoding data sits on its taxonomic tree, with bacteria, fungi and protists as the three wedges. Distance from the centre is rank. Kingdom at the hub, families on the rim. Dot size is how many genera sit in that clade. Colour is coverage: the proportion of those genera with a preserved representative in any collection. Red is none. Green is all of them. Red taxa are not scattered at random across the tree, it collects in whole branches.

METHODS



Design.

Two soils, Histosol and Cambisol. Four cryoprotocols against a 4 °C wet-storage control: 10% glycerol, trehalose, no cryoprotectant, alginate encapsulation. Sampled at t_0 and three timepoints across one year at −196 °C, then 24 h resuscitation before extraction.

Activity.

rRNA, reverse-transcribed, as a metabolic-activity proxy. DNA from the same samples separates *present* from *active*.

Sequencing and taxonomy.

ITS and 16S V3–V4, ASVs by DADA2. ITS against UNITE sh_general_release_dynamic_s_all 04.04.2024; 16S against SILVA nr99 v138.1. Agglomerated to class for both kingdoms; ITS also to genus, viable there because 70.5% of ITS ASVs carry a genus assignment against 33.7% for 16S.

Red-zone scoring.

Every genus is scored against the Global Catalogue of Microorganisms: A genus keeps its own score where GCM has one; where it does not, Scores are applied per kingdom, because neither amplicon is single-kingdom: the 16S set also carries Archaea and the ITS set carries green algae.

Statistics.

MaAsLin3 on TSS+LOG counts, ~ Group_time + (1|soil), reference 4 °C at t_0. Abundance and prevalence models, Benjamini–Hochberg corrected. Bacteria and fungi were fitted at the same rank with the same formula so the two kingdoms are comparable. Scoring, detection and models re-run end to end on 2026-09-03.

RESULTS

1. The red zone is reached.

60 of 61 red-zone bacterial classes and **23 of 41** fungal classes were RNA-active after cryopreservation.

Recovery by protocol. Classes RNA-active in that protocol, t_1–t_3; control matched to the same three timepoints.

No single protocol beats the fridge for fungi, only the four combined do. Trehalose is the best single protocol for bacteria.

PROTOCOL	COOLING RATE	BACTERIA	FUNGI
Trehalose	−1°C / min	95%	34%
Encapsulation	−100°C / min	92%	37%
Glycerol	−1°C / min	84%	39%
No cryoprotectant	−1°C / min	84%	34%
4 °C control	-	82%	44%
All four combined		**98%**	**56%**

2. Banked is not the same as awake.

39 of 41 fungal red-zone classes came back as DNA. Only **23** came back as RNA. **17** fungal classes were recovered as material but never resumed activity within the 24 h window. For bacteria that number is **1**.

3. Reached, not unchanged.

Two in five red-zone bacterial classes shifted in abundance after cryopreservation (**23 of 58**, q < 0.1). Not one fungal class did. 17 classes up, 6 down. Strongest: Vampirivibrionia (+6.9), Acidimicrobiia (+5.8), Bacilli (+4.6), *Gammaproteobacteria* (+0.7, q = 0.003). Down: Longimicrobia (−2.6), Kiritimatiellae (−1.7). Fungi were tested at the same rank with the same model and returned nothing: minimum q = 1.00, and fewer small p-values than chance alone would give.

“Whole classes are missing from every collection. Microbiome preservation brought most of them back."

DISCUSSION

Cryopreservation reaches the phylogenetic red zone that isolation cannot. After a year at −196 °C, 60 of 61 red-zone bacterial classes and 23 of 41 fungal classes were RNA-active, taxa held in no collection anywhere. **Whole-community cryopreservation is complementary to isolate-based biobanking, not a replacement.**

The fungal shortfall is a **limit on recovery time** rather than on banking: 39 of 41 classes returned as DNA and only 23 as RNA, and we hypothesise the 24 h resuscitation window sets that boundary. **Recovery is also not neutral.** Two in five red-zone bacterial classes shifted in abundance while no fungal class did: spore-forming Bacilli (+4.6) and fast-growing Gammaproteobacteria (+0.7) increased, the slow-growing Longimicrobia (−2.6) and Kiritimatiellae (−1.7) declined, consistent with a pulse disturbance **favouring the taxa that multiply fastest into vacated niche space**. **RNA remains a proxy for metabolic activity rather than growth,** and a cryopreserved community is not a catalogued strain: it cannot be distributed or typed as an isolate can, and **red zone means held in no GCM collection rather than never cryopreserved.**

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