Supplemental Online Materials

for

Culturing captures members of the soil rare biosphere

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Supplemental Methods

The study site was an apple orchard in southern Wisconsin (42°58'N, 89°28'W). Soil cores 10 cm in diameter and 5 cm deep were collected under the north and south sides of eight trees at four time points: 08 and 27 May 2008, and 01 and 22 May 2009. Each was homogenized in its collection bag.

Five-gram soil subsamples were prepared for plating as described previously (Gilbert *et al.*, 1996). Briefly, 5 g of soil were diluted with 25 ml of sterile H_2O, shaken for 1 minute, and then sonicated for 30 seconds in a bath sonicator. Soil was allowed to settle, and then 1/10 dilutions were made. For each core, we plated duplicate series of 100 µl of 10^{-3}, 10^{-4}, and 10^{-5} dilutions on rhizosphere isolation medium (Buyer, 1995) modified by replacing nystatin with cycloheximide (100 µg/ml) to inhibit fungal growth. Plates were incubated at ambient temperature for 6 days before colonies were scraped, and then the plates diluted from north and south cores were pooled within a site (either 8, 10, or 12 plates pooled total per sample, as the 10^{-3} dilution was often overgrown and excluded). Next, each pool of colonies was homogenized by mixing with a spatula and vortexing. The pooled samples were stored in 10% glycerol at -80º C prior to DNA extraction with GenElute Bacterial Genomic DNA kit (Sigma-

Aldrich, St. Louis, MO) protocol for Gram-positive bacteria. For the culture-independent description of bacterial communities, genomic DNA was isolated directly from the soil within 6 hours of collection using the Power Soil kit (MoBio, Carlsbad, CA) with published modifications (Cadillo-Quiroz *et al.*, 2006).

Barcoded-16S gene amplification was performed on 64 samples (8 sites x 4 time points x 2 approaches, culture-based and culture-independent) by the University of Colorado-Boulder pyrosequencing lab, and submitted for pyrosequencing on a 454 Life Sciences Genome Sequencer FLX (Roche Diagnostics, Branford, CT) at Eugencore, South Carolina, using prokaryotic 16S rRNA gene primers 515/806 to target the V3-V4 variable regions. Of 64 total samples, we failed to obtain sequence from six samples, which were omitted from analysis. Sequence quality control was performed using the QIIME v 1.2 default parameters, which included a minimum quality score of 25, a minimum sequence length of 200, a maximum length of 1000, and no ambiguous bases permitted, and no primer mismatches (Caporaso *et al.*, 2010b). Operational taxonomic units (OTUs) were assigned based on 97% sequence similarity using uclust (Edgar, 2010), and the most abundant sequence within each OTU was used as the representative for alignment and taxonomic assignment. Alignments were performed using PyNAST (Caporaso *et al.*, 2010a) and assignments made using the RDP Classifier (Wang *et al.*, 2007) with 0.8 minimum confidence and 0.001 minimum e-values.

There were no global differences in the composition of bacterial communities across sites or time points, as assessed by analysis of similarity of ranked Bray-Curtis distances in the Primer-e V6 software ($p = 0.87$ and 0.33). Heatmap and Venn analyses were performed in the R environment for statistical computing (R Development Core Team).

To assess the reproducibility of community structure across GenElute and Power Soil DNA extraction methods, we built clone libraries containing 16S rRNA genes amplified from two samples, for a total of four libraries (two samples and two extraction methods each). 16S rRNA genes were amplified from soil metagenomic DNA using primers 27f and 1492r pooled from 4-6 separate 50-µL PCR reactions and purified using the Promega Wizard kit.

Purified PCR products were then dialyzed against dH_20, on membrane filters (Millipore VSWP02500) for 1 hour at room temperature. We ligated approximately 90 ng insert DNA into 50 µg of pGEM T easy vector (Promega) overnight at 4^oC. Following ligation, 1 µl of the ligation reactions was mixed with 50 µl of electro-competent *E. coli* DH5α cells, incubated on ice for 20 min, transferred to a 1-mm electroporation cuvette, and pulsed at 2500 V, 4.8 – 5.8-ms time constant. The cells recovered in SOC media for 1 hour at 37 ^oC with shaking before plating on LB medium amended with 50 µg/mL ampicillin plus ChromoMax™ IPTG (isopropyl thiogalactoside)/ X-GAL (5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside) for blue/white colony selection (Fisher). After overnight growth, white colonies were picked with toothpicks and patched on LB medium amended with ampicillin and grown overnight. Cells were transferred to 96-well plates and pelleted by centrifuging at 14,000 G for 15 minutes. The culture medium was discarded and replaced with 100 µl sterile water. The plate was heated to 95^oC for 10 min to lyse the cells. One µl of the cell suspension was applied to the PCR reaction using primers M13f and 357r. BigDye sequencing reactions were performed on successful amplifications. Reactions were analyzed by the University of Wisconsin-Madison Biotechnology Center on a 3730 xL DNA Analyzer (Applied Biosystems).

We identified chimeras from aligned sequences with Mallard (Ashelford *et al.*, 2006),

and removed them after confirmation of low quality with BLAST. The remaining analyses were performed in the mothur environment (Schloss *et al.*, 2009). Sequences were aligned against mothur's greengenes reference collection and screened for the same start and end position. After quality control, 417 sequences remained that were used to create a PHYLIP distance matrix (Felsenstein, 1993). This matrix was applied to the neighbor-joining algorithm of clearcut (Evans *et al.*, 2006). We used unweighted UniFrac algorithm (Lozupone and Knight, 2005) to test for differences in structure among groups. We detected no differences between the GenElute and the Power Soil extraction methods (both UniFrac $p > 0.01$). This result was confirmed using the S-Libshuff algorithm (Schloss *et al.*, 2004), applied to the PHYLIP distance matrix (S-Libshuff $p \geq 0.01$).

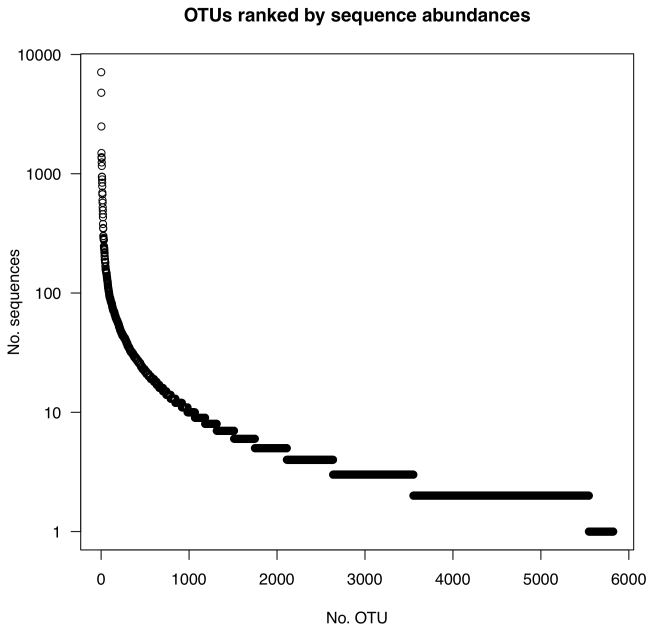
Supplemental Table

Supplemental Table 1. Summary of sequencing effort and number of OTUs detected in culture-based, culture-independent, and the total dataset. Note that the total is not necessarily the sum of the culture-based and culture-independent (*e.g.,* some OTUs were shared across both communities).

	Culture-based	Culture-independent	Total
No. samples	32	26	58
No. sequences	37645	46347	83992
No. OTUs	1377	4898	5822
No. OTUs, singletons removed	1054	4356	4957
Max. no. sequences per sample	1764	3294	83992
Avg. no. sequences per sample	1176.4	1782.6	1448.1
Min. no. sequences per sample	471	626	471
Max. no. OTUs per sample	253	1410	1410
Avg. no. OTUs per sample	168.8	820.8	461.1
Min no. OTUs per sample	92	379	92
Max no. OTUs per sample, singletons removed	221	1358	1358
Avg. no. OTUs per sample, singletons removed	158.8	800.0	446.2
Min no. OTUs per sample, singletons removed	84	366	84

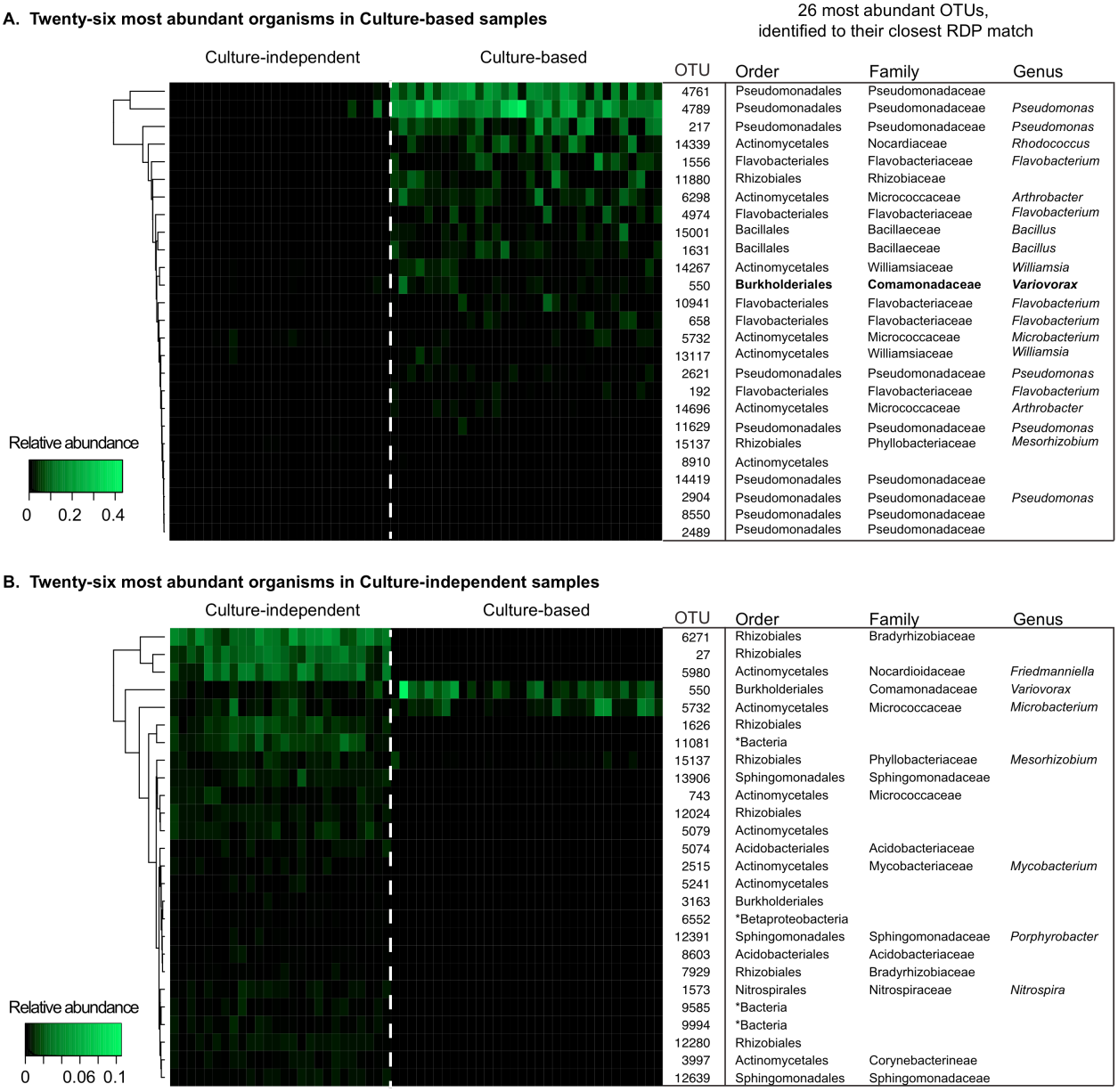
Supplemental Figures

Supplemental Figure 1



Supplemental Figure 1. Rank abundance distribution of OTUs from singleton-omitted, rarefied dataset from soil.

Supplemental Figure 2.

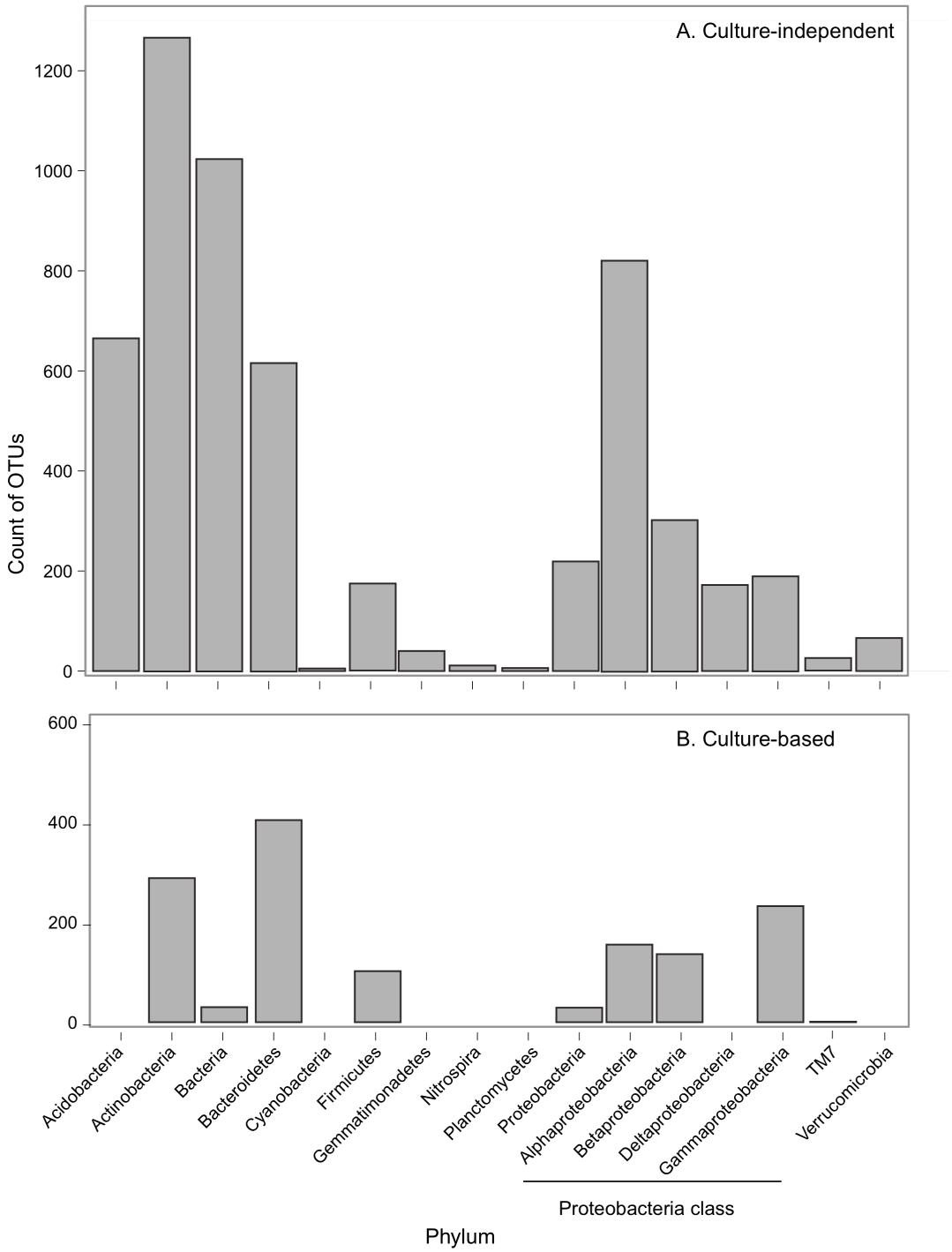


OTU	Order	Family	Genus
4761	Pseudomonadales	Pseudomonadaceae	
4789	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
217	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
14339	Actinomycetales	Nocardiaceae	*Rhodococcus*
1556	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*
11880	Rhizobiales	Rhizobiaceae	
6298	Actinomycetales	Micrococcaceae	*Arthrobacter*
4974	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*
15001	Bacillales	Bacillaeceae	*Bacillus*
1631	Bacillales	Bacillaeceae	*Bacillus*
14267	Actinomycetales	Williamsiaceae	*Williamsia*
550	**Burkholderiales**	**Comamonadaceae**	***Variovorax***
10941	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*
658	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*
5732	Actinomycetales	Micrococcaceae	*Microbacterium*
13117	Actinomycetales	Williamsiaceae	*Williamsia*
2621	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
192	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*
14696	Actinomycetales	Micrococcaceae	*Arthrobacter*
11629	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
15137	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*
8910	Actinomycetales		
14419	Pseudomonadales	Pseudomonadaceae	
2904	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
8550	Pseudomonadales	Pseudomonadaceae	
2489	Pseudomonadales	Pseudomonadaceae	

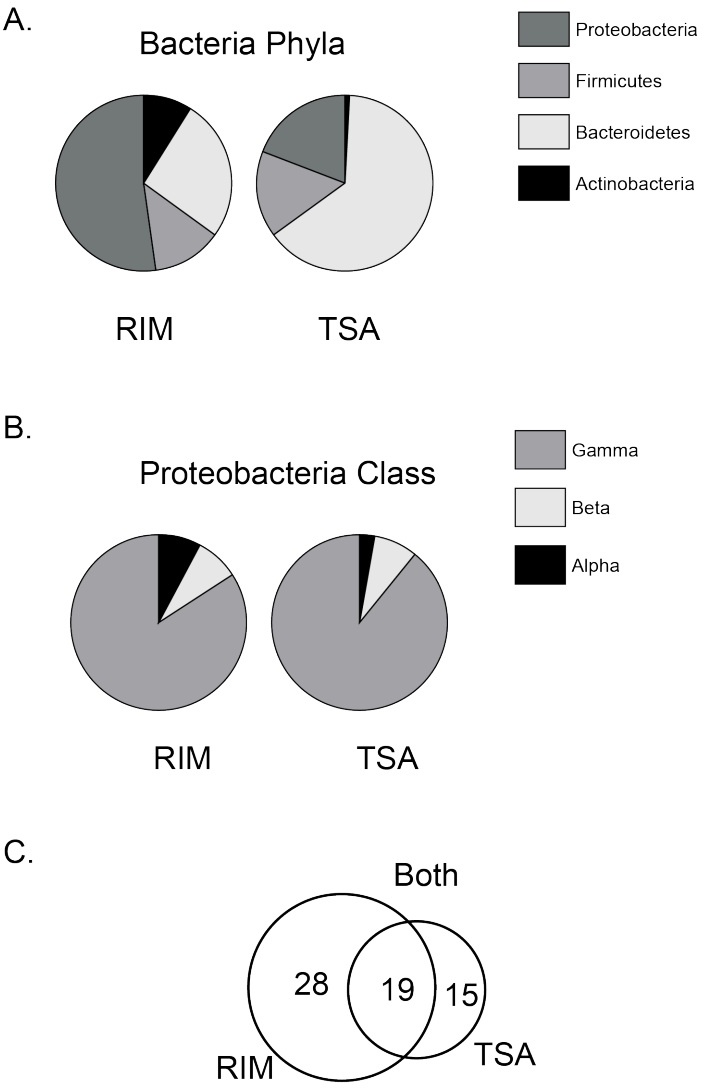
OTU	Order	Family	Genus
6271	Rhizobiales	Bradyrhizobiaceae	
27	Rhizobiales		
5980	Actinomycetales	Nocardioidaceae	*Friedmanniella*
550	Burkholderiales	Comamonadaceae	*Variovorax*
5732	Actinomycetales	Micrococcaceae	*Microbacterium*
1626	Rhizobiales		
11081	*Bacteria		
15137	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*
13906	Sphingomonadales	Sphingomonadaceae	
743	Actinomycetales	Micrococcaceae	
12024	Rhizobiales		
5079	Actinomycetales		
5074	Acidobacteriales	Acidobacteriaceae	
2515	Actinomycetales	Mycobacteriaceae	*Mycobacterium*
5241	Actinomycetales		
3163	Burkholderiales		
6552	*Betaproteobacteria		
12391	Sphingomonadales	Sphingomonadaceae	*Porphyrobacter*
8603	Acidobacteriales	Acidobacteriaceae	
7929	Rhizobiales	Bradyrhizobiaceae	
1573	Nitrospirales	Nitrospiraceae	*Nitrospira*
9585	*Bacteria		
9994	*Bacteria		
12280	Rhizobiales		
3997	Actinomycetales	Corynebacterineae	
12639	Sphingomonadales	Sphingomonadaceae	

Supplemental Figure 2. Heatmap visualizations of the 26 most abundant OTUs within the (A) culture-based and (B) culture-independent communities, and organized within each map by similar response patterns. Columns are communities, and rows are OTUs. Please note

differences in scale of relative abundances for A and B. *Variovorax* (OTU 550) was the only abundant OTU shared across both cultured-based and culture-independent communities. OTUs marked with an asterisk could not be resolved to the Order level.



100 **Supplemental Figure 3**. Frequency of OTUs across Bacteria phyla and Proteobacteria class for (a) culture-independent and (b) culture-based communities.



Supplemental Figure 4. Comparison of taxa detected in rhizosphere isolation medium (RIM) and 0.1x tryptic soy agar (TSA) at the (a) phylum level and (b) Proteobacteria class. (c) Venn analysis of OTUs detected on each type of medium. OTU assignments were made with Ribosomal Database Project assignment of full-length 16S RNA gene sequences from clone libraries of metagenomic DNA extracted from a pool of isolates for each culturing media. RIM was ultimately selected for cultivation because it inhibited the growth of filamentous *Bacillus mycoides*, which often obscured quantification of smaller, non-filamentous colonies on TSA.

Supplemental References

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