

Supplementary Information

Ground squirrel coprolites preserve complex archives of ancient environmental DNA over 700,000 years

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Other Supplementary Information for this manuscript include the following:

Supplementary Data 1-4
Source Data file

Supplementary Notes

Supplementary Note 1. Pacific Northwest Quaternary References

A local reference database was built to aid in the taxonomic identification of organisms that could have reasonably existed in northwestern North America during the Quaternary. To build a list of organisms, we queried species by major taxonomic groups (plants, mammals, birds, reptiles, and amphibians) in GBIF using their drawn polygon tool (Fig. S3). We only retained organisms with occurrence data within the delimited coordinates that were recorded via human or machine observations (other records were excluded to remove zoos, botanical gardens, specimen collections, and other potential non-local occurrence data). Species lists that met those criteria were exported as csv files (Table S2). Other species lists recovered from eFauna-BC (https://ibis.geog.ubc.ca/biodiversity/efauna/mobile_index2.html), and eBird (<https://ebird.org/home>) for Pacific Northwest organisms from Alaska, Yukon, BC, Washington, and Oregon. The concatenated lists were deduplicated based on species ID, and common names were added using taxize (sci2comm, db='ncbi' and 'itis'). An additional species list was recovered from Neotoma (<https://www.neotomadb.org/>) for extinct animals within the same geographic region.

Curated species lists queried on NCBI GenBank using a customized ncbi-NT-DL BioPerl script in 3 iterative batches to recover GenBank files. When no record was found in fetch 1 (recovering RefSeq mitochondrial genomes), that species was passed to the fetch 2 search (non-RefSeq mitogenomes), and finally to the third fetch where all mitochondrial or chloroplast target gene reference data was recovered (cyt-b, d-loop, and COX1 [animals], and trnL, rbcL, matK [plants]). All data was imported into Geneious for visual screening, and all "unverified" and "predicted" sequence data was removed. Sequences were deduplicated and poorly annotated references were removed. Non-native domestic organisms with a high chance of being contaminants were also removed. A refined, single step version of this workflow is available now at <https://github.com/tjmurchie/TackleBox/tree/main/FlyGuide>.

Supplementary Note 2. Comparing methods and optimizing sedaDNA extraction

Rationale

The cold spin, high volume purification technique¹ works well for many environmental sample types containing moderate concentrations of humic substances—structurally complex compounds derived from partially degraded organic matter. Humic acids have molecular characteristics similar to DNA, which results in their frequent co-extraction in purification methods lacking a humic-specific inhibitor removal step (and often even despite including an inhibitor removal step). This co-extraction becomes problematic in subsequent processing because polymerases and other enzymes are sensitive to the presence of humic substances, and a sufficient overabundance of these compounds can inactivate enzyme functionality entirely—enzymes necessary for library conversion and amplification. In samples with especially high humic acid concentrations, such as with some palaeofaeces, preserved soft-tissues (i.e. hides), and organic rich sediments/soils (i.e. peat/gyttja), the cold spin appears to reach a humic removal saturation point. This is especially the case with higher sediment inputs (>200 mg) in humic rich samples, which becomes an issue when trying to maximize sedaDNA recovery and complexity from metagenomically rare organisms by increasing sediment input. It is often desirable to include more sediment to ensure that the full taxonomic range of organisms from a site is detectable given the heterogeneous distribution of eDNA.² If input is limited to <150 mg, one

then is incentivized to include multiple extraction replicates per sample to capture the widest possible range of sedaDNA from a given context, which becomes exorbitantly costly and time consuming.

High sediment input DNA purification kits exist, such as the DNeasy PowerMax Soil Kit (Qiagen), but a limitation of commercial kits in general is that they are designed to maximally purify an extract, with manufacturers assuming the end user is working with relatively abundant, high quality DNA from a modern context. Meaning, DNA loss during inhibitor removal is not a major concern for most genetic end users given the presumed DNA abundance within their samples. The difficulty with ancient environmental DNA is extremely low abundance and low quality DNA, and because most inhibitor removal protocols end up unintentionally removing a large portion of viable DNA (because humic acids have very similar molecular properties), when these approaches are applied to ancient samples, the damaged, highly organo-mineral complexed, and low abundance characteristics of a DNA tends to result in poor recovery.¹

These two interrelated challenges create a balancing act between maximally purifying a sample to have full enzyme functionality, and maximally retaining the full complexity of sedaDNA. At this time, no perfect inhibitor removal technique is available that can fully remove humics without also removing some portion of aDNA. We have theoretically conceptualized this challenge in Fig. S3.

Our goal with the extraction experiments reported here is to try and approach the limit of enzymatically accessible sedaDNA. We do not believe we succeed in achieving this goal. The data reported here walks through a hypothetical improvement to the cold spin purifications using ultra-sonication compared with other methods reported in the literature. Our hope is that either we or another research team can further explore the trends we were seeing in the first main set of experiments to better understand why in the final set, the most promising method failed. And, so that a new sedaDNA extraction method can potentially be developed in the near future to maximize sedaDNA recovery while entirely removing inhibition, regardless of initial sediment input, humic concentration, or mineralogy.

Ultrasonication: freeing ancient DNA from organo-mineral bonds with ultrasonic void collapse—initial experiments.

In Murchie et al.¹ the second most promising inhibitor removal technique identified was ultra-sonication. We were interested in expanding on that result here with the running hypothesis that if sedaDNA is preserved through organo-mineral complexes, and if these complexes (containing humics) are incompletely dissolved during lysis disintegration, that our target sedaDNA may either remain inaccessible for purification, or that DNA bound humic acids would be co-extracted. Ultra-sonication uses high frequency (>20 kHz) sound waves to create acoustic cavitations in liquid which agitates and disassociates chemically bound particles. Sound waves propagate into the liquid media resulting in alternating high-pressure (compression) and low-pressure (rarefaction) cycles. During rarefaction, high-intensity sonic waves create small vacuum bubbles or voids in the liquid, which then collapse violently (cavitation) during compression. We had the idea to apply this to sedaDNA extraction through exposure to research where the technique has been used to rapidly age alcoholic beverages.^{3,4} Ultrasonication is already used widely to intentionally shorten modern DNA fragments for use with massively parallel high-throughput sequencing. DNA tends to have a minimum ultrasonic induced fragmentation size of 250 bp, which happens to be much larger than typical ancient DNA with a mean fragment length distribution of 50 bp.^{5,6} Our hypothesis here was that if we intentionally “damaged” the aDNA lysis and associated organo-mineral complexes a little, we might be able to maximally free all

the bound DNA in solution, and then remove the disassociated humics using the cold spin extraction technique.

In order to test this hypothesis, we proceeded through several iterative experiments to assess the potential of ultra-sonication for sedaDNA extraction. We first acquired a budget ultrasonicator designed for jewellery cleaning and tested to confirm that it would work for the typical plastic microtubes we would float in the ultrasonic bath containing sedaDNA lysing supernatant (Fig. S4). We found that the liquid volume within the microtube, along with the duration of sonication, impacted the fragmentation of DNA ladder. We also found that DNA smaller than 300 bp was seemingly unaffected by sonication. We concluded that 60 minutes and maximal volume (1.5 mL) were best moving forward for subsequent experiments. We also noted that heat was an issue with our budget ultrasonic cleaner (which did not have refrigerant functionality), with the temperature of the water bath having risen from ~4°C to ~50°C in 60 minutes.

We next tested whether glass or plastic would conduct ultrasonic waves better into the microtubes, finding again that the maximal volume of liquid in plastic microtubes fragmented the DNA ladder best (Fig. S5). In parallel, we tested whether the presence of an alcohol as a solvent would help with increasing the rate of agitation/extraction/disintegration. We tested this using wood chips immersed in 8 mL of water with variable concentrations of ethyl alcohol (Fig. S5). We found that alcohol did significantly increase the rate of disintegration.

Using these preliminary experiments, we moved forward to testing on ancient sediments with the following parameters: high lysing volume, full-wave sonication for 60 minutes, be cautious of temperature rise, and to add an alcohol to the lysis buffer to increase the rate of extraction.

Experimental ancient eDNA Set 1

We tested six extraction variants on four coprolite/sediment samples; two that had been inhibited previously using cold spin extractions (with 250 mg inputs), and two that were successfully extracted using the cold spin procedure (Fig. S6). This experiment was intended as a pilot project to assess the viability of the method variants. As such, each method was not run with intra-sample replicates to save on costs/time. For each original sample, a subset was removed and thoroughly homogenized and subdivided into 100 mg portions. These were lysed following the same procedure described in the main text with a custom lysis digestion buffer, bead beating, addition of proteinase K, and overnight incubation at 37°C. The next day, the samples were spun down and the supernatant removed. Thereafter, the supernatant was processed following each of the following protocols. The extracts were compared using the same inhibition spike test as described in the main-text¹ and library adaptable DNA was quantified by bringing the extracts through double-stranded library preparation^{7,8} and quantifying adapter conversion using the short amplification qPCR—all as described in the main-text.

- Sonication and cold spin (method A)
 - We ultra-sonicated the lysing supernatant for 60 minutes using the fullwave setting (40 kHz) in a digital ultrasonic cleaner.
 - The sonicated supernatant was centrifuged for 60 seconds at 16,000 x g, then loaded into the standard high-volume guanidinium binding buffer (13 volumes).
 - Extract was centrifuged overnight at 2500 x g at 4°C, then purified following Dabney et al.⁹ and Murchie et al.¹
- Sonication with alcohol and cold spin (method B)
 - Lysing supernatant (~500 mL) was combined with 750 mL of 100% isopropanol

- Samples were then ultra-sonicated for 60 minutes using the fullwave setting (40 kHz) using a digital ultrasonic cleaner.
 - The sonicated supernatant was centrifuged for 60 seconds at 16,000 x g, then loaded into high-volume guanidinium binding buffer (13 volumes).
 - Extract was centrifuged overnight at 2500 rcf at 4°C, then purified following Dabney et al.⁹ and Murchie et al.¹
- Cold spin (method C)
 - Lysis supernatant was combined with guanidinium binding buffer (13 volumes) then centrifuged overnight at 2500 rcf at 4°C, then purified following Dabney et al.⁹ and Murchie et al.¹
- Standard Dabney (method D)
 - Lysis supernatant was combined with high-volume guanidinium binding buffer then purified following standard Dabney et al.⁹
- InhibitEx Tablet (method E)
 - 1 mL of InhibitEx Buffer was added to supernatants in clean 2 mL tube, incubate for 2 min at room temp, then centrifuge at 5000 x g for 5 min.
 - Transferred supernatant (avoiding pellet) to fresh 2 mL tube and centrifuge again, transferred supernatant to 50 mL falcon where it was combined with high-volume guanidinium binding buffer then purified following Dabney et al.⁹.
 - This approach is a modified silica-spin column version of the technique described by Wang et al.¹⁰
- Linear polyacrylamide (LPA) purification (method F)
 - Technique follows protocol of Maixner et al.¹¹
 - Lysis supernatant was combined with high volume guanidinium binding buffer then purified following standard Dabney et al.⁹
 - “Purified” extract then combined with 50 µL of 0.5% LPA solution and 300 µL of 70% ethanol, then gently mixed. Centrifuge at 16,000 x g for 10 min.
 - Carefully removed supernatant until only a distinct translucent pellet remained at the bottom of the tube.
 - Residual ethanol further evaporated by keeping the tube with the lid open for 1 hr at room temperature under a sterile PCR workstation.
 - Re-dissolve pellet in 50 µl EBT.

qPCR results

Inhibition. The inhibition index¹ is derived from measuring the qPCR amplification curve of a spiked oligo mixed with a sample extract relative to the standard of the same oligo at varying concentrations. The measure helps function as a proxy test as to whether an extract is sufficiently purified to successfully undergo library preparation or not.

Method B (ultra-sonication with isopropanol with the cold spin) consistently outperformed all other methods in terms of inhibition. This was followed by method F (LPA), method C (cold spin), and methods A (Son/CS) and E (InhibitEx). Sample SC-10 has low levels of inhibition (max Inhib. Ind. = 0.3 in method D, Fig. S6). As such, this sample functions well to help understand DNA loss from varying extraction methods. The standard Dabney (method D) has the worst inhibition index across all samples as to be expected as this technique has no inhibition removal, being designed for aDNA extraction from bone.

Total DNA. Method B again has the highest average rank total adapted DNA across extracts, followed by methods C/E (tied for 2nd), method A in 4th, and method F in 5th. Method D

was the worst performing method for library adapting sedaDNA, alongside method F (LPA purifications).

Pre-sequencing result summary. As there are no intra-sample replicates, it is difficult to assess the significance of the differences observed here relative to normal variation within an environmental sample. However, method B consistently has the least inhibition and ranks in the top 3 methods for DNA adaptation, aside from SC-10 (the uninhibited sample) where it ranked 4th.

An important caveat here is that these are total DNA qPCR results. Determining ancient DNA specific DNA retention/adaptation—on-target eukaryotic DNA— is important for robustly characterizing the optimal methodology. As such, these samples were subsequently processed through to both shotgun sequencing and targeted capture enrichment using the PalaeoChip Arctic v1.0 bait-set. Methods utilised here were identical to those described in the main-text.

Shotgun and enriched sequencing results

Extraction variant subsamples were first shotgun sequenced to compare metagenomic recovery (Fig. S7). They were taxonomically assigned with either the full sequencing dataset run through *MegaBLASTn* (and normalized in *MEGAN6* to smallest library) or mapped to organelle genomes of ‘target’ taxa and that subset then used as the input to *BLASTn* (normalized prior to mapping by randomly subsampling to smallest library). *MEGAN6* was used to taxonomically assign reads classified with *BLASTn* and *MegaBLASTn*.

Read counts were generally low for eukaryotes with the shotgun data, although method B again was generally either in 1st or 2nd place for total recovery. To improve our resolution in order to better compare extraction methodology impact on sequencing results, we enriched the libraries with the PalaeoChip bait-set. To metagenomically compare their relative recoveries, these libraries were randomly subsampled to the smallest library (810,000 reads) then mapped to the PalaeoChip bait-set original reference sequences, then taxonomically classified with *BLASTn*, and binned with *MEGAN* (Fig. S8).

Among enriched libraries, the three cold spin extracts (methods A, B, and C) were consistently the best performing, with method B (sonication with isopropanol and the cold spin) on average recovering the most plant and animal DNA (Fig. S8). Method E (InhibitEx buffer) also generally performed well, although it was unable to remove inhibition from the sediment sample (PHP-12) and performed poorly with the uninhibited sample (SC-10). Methods D (Dabney) and F (LPA purification) were consistently the worst methods for recovering eDNA from humic rich samples. Method F (LPA) performed poorly even with the uninhibited samples (SC-10). Rarefaction curves recapitulate the same overall trend (Fig. S9).

There appear to be no changes to rates of deamination or fragmentation for ‘endogenous’ ancient ground squirrel DNA (Fig. S10). When comparing subsample normalized fragment length distributions (FLDs) of reads mapped to the arctic ground squirrel (*Urocitellus parryii*) mitochondrial genome in the singular fecal pellet sample (SC-10) where all 6 methods were able to library adapt DNA, the methods cluster ranked from best to worst were: A/B, E/C/D, and lastly F (Fig. S10). There is no decrease in fragment lengths in the sonicated samples. Rather, there is a very slight shift to the right in the distribution towards larger fragments in the sonicated libraries. The same unexpected pattern is observed in other samples when mapping to *U. parryii* as well as *Plantago*, a plant genus commonly found in these samples.

Taxa identified in these samples (which are retained among replicates), include the following (Fig. S11–S14):

- SC-2 (fecal pellet)
 - *Equus*, *Canis*, *Marmot*
 - *Artemisia*, *Plantago*
- SC-10 (fecal pellet)
 - Multiple others in Marmotini, along with *Bison*, *Equus*, *Canis*, *Capra*, *Mammuthus*, *Dicrostonyx*, *Murinae*, *Myotis*
 - *Artemesia*, *Plantago*, *Poa*
- SC-13 (fecal pellet)
 - *Equus*, *Canis*, *Bos/Bubalus* (likely *Bison* mis-IDs), *marmots*, *Mammuthus*, *Capra*, *Soricidae*
 - *Poa*, *Plantago*, *Carex*
- PHP-12 (sediment)
 - *Mammuthus*, *Lepus*, *Microtus*, *Bovinae*, *Cervinae*, *Alces*, *Lagopus*
 - *Salix*, *Equistum*, *Carex*, *Betula*

These results suggest that ultra-sonication significantly aids in both inhibitor removal and DNA recovery during ancient environmental DNA extraction. The addition of isopropanol during ultra-sonication appears to improve inhibitor removal. The standard cold spin is the third best method following the sonicated variants (especially when reducing the starting input), followed closely by the InhibitEx Buffer. Standard Dabney and LPA purifications are ineffective for environmental DNA extraction. These results appear to show no downsides to ultra-sonication on endogenous eDNA. Although, it is theoretically possible that modern background DNA may be sonicated down to smaller fragments that are then more easily sequenced. These would not retain a deamination signal however, and they are unlikely to sonicate down to the size ranges under consideration here (24-80 bp).

Experimental ancient eDNA Set 2

Based on the promising results from batch 1, we decided to expand on the analysis with extraction replicates (n=3 where sufficient material was available) from 5 new ancient samples (4 coprolites, 1 sediment mixed with coprolite) among 6 method variants (Fig. S15). As with batch 1 for each original sample, a subset was removed and thoroughly homogenized, then subdivided into 100 mg portions for each method and replicate (n=88 with 5 additional extraction negative controls and 1 library negative control). These were lysed following the same procedure described in the main text with a custom lysis digestion buffer, bead beating, addition of proteinase K, and overnight incubation at 37°C. The next day, the samples were spun down and the supernatant removed. Thereafter, the supernatant was processed following each of the following protocols.

- Ultrasonication with Dabney high volume purifications (no cold spin) (method Z)
 - We ultra-sonicated the lysing supernatant for 60 minutes using the fullwave setting (40 kHz) using a digital ultrasonic cleaner.
 - Sonicated supernatant was then centrifuged for 60 seconds at 16,000 x g, and the supernatant was then combined with high-volume guanidinium binding buffer then purified following standard Dabney et al.⁹
- Ultrasonication followed by cold spin with Dabney high volume purifications (method B)
 - We ultra-sonicated the lysing supernatant for 60 minutes using the fullwave setting (40 kHz) using a digital ultrasonic cleaner.

- Sonicated supernatant was then centrifuged for 60 seconds at 16,000 x g, and combined with high-volume guanidinium binding buffer
 - Extract was centrifuged overnight at 2500 x g at 4°C, then purified following Dabney et al.⁹ and Murchie et al.¹
- Cold spin with Dabney high volume purifications (method C)
 - Lysis supernatant was combined with guanidinium binding buffer (13 volumes) then centrifuged overnight at 2500 x g at 4°C, then purified following Dabney et al.⁹ and Murchie et al.¹
- Dabney high volume purifications (method D)
 - Lysis supernatant was combined with high-volume guanidinium binding buffer then purified following standard Dabney et al.⁹
- InhibitEx buffer followed by Dabney high volume purifications (method E)
 - 1 mL of InhibitEx Buffer was added to supernatants in clean 2 mL tube, incubated for 2 min at room temp, then centrifuge at 5000 x g for 5 min.
 - Transferred supernatant (avoiding pellet) to fresh 2 mL tube and centrifuge again, transferred supernatant to 50 mL falcon where it was combined with high-volume guanidinium binding buffer then purified following Dabney et al.⁹
 - This approach is a modified silica-spin column version of the technique described by Wang et al.¹²
- DNeasy PowerSoil Pro Kit (Qiagen) (method X)
 - We followed manufacturer's instructions exactly, eluting in the end in 50 µL EBT (the same ending volume as all other method variants).

qPCR results

Inhibition. Inhibition removal performed as observed previously with the Dabney method (D) where applied to environmental samples without an inhibition removal step. These extracts retained a high inhibitor content (Fig. S15). This was followed by method e (InhibitEx buffer) which also had high Inhibition Indexes in all environmental samples. Methods Z, B, and X had low inhibition overall in all five samples.

Total DNA. Unexpectedly (based on experimental set 1), method B performed very poorly in terms of total DNA recovery post-library preparation (Fig. S16). Enough so, that method B was repeated for samples SC-5, SC-9, and SC-15 to confirm the result. In this follow-up test, (labelled method BB), a single change was made from method B. After sonication, samples were not immediately centrifuged. Instead, the supernatants were immediately pipetted into the binding buffer and processed through the cold spin. We noticed in batch 2 that after the post-ultrasonication room temperature spin, a large pellet had formed, and we were concerned that this pellet contained DNA. This was confirmed with method BB, which performed better in all cases, but not as well as batch 1 had led us to expect. We do not know why method B/BB performed poorly in batch 2.

We hypothesize that either there was too much isopropanol in with the sonication which led to a precipitation of DNA during either the room temperature or cold centrifugation. Or, that because the ultrasonicator we were utilizing is not refrigerated, the high temperatures during operation (exceeding >60°C in batch 2) may have caused significant DNA degradation and loss. We are unaware why these factors did not impact batch 1. The author conducting these experiments (TJM) left his position at McMaster University to work at the Hakai Institute shortly after receiving these results. As such, these experiments could not be followed up to explore further.

Despite the failure of ultrasonication to perform well in terms of total DNA recovery in batch 2, several other notable results can be observed. First, the cold spin consistently outperformed alternative approaches with all samples. In the case of SC-5/9/15 where we re-extracted method B (as BB), method C was qPCR assayed again, resulting in 12 replicates per sample for the total DNA quantification in SC-5/9/15. These consistent results clearly demonstrate that the cold spin inhibitor removal procedure outperforms the DNeasy PowerSoil Pro extraction kit and InhibitEx Buffer in terms of total DNA recovery. This serves as an expansion of Murchie et al.¹ where only the original PowerSoil kit was tested. We can confirm here that the cold spin procedure paired with Dabney high-volume purifications results in the highest recoveries of DNA from both sediments and coprolites. This is further confirmed by batch 1 where aside from the sonication variants, the cold spin procedure was the best technique for recovering ancient DNA while minimizing the carry-over of inhibition.

However, an ongoing limitation of the cold spin procedure is raw sediment input, and samples with especially high humic concentrations. The optimal approach in this case was the reduction of starting material paired with the cold spin, but this is a limitation when one wants to include more starting material to recover rare aeDNA. We intend to keep optimizing the cold spin method and testing the ultrasonication technique to overcome this limitation, and to improve processing times. Determining the cause for the unexpected results of method B (BB) in batch 2 may aid in that optimization goal.

Supplementary Note 3. Macrofossils (main text results continued).

Collectively the 11 samples, contained seeds and fruit from herbaceous forbs (11 taxa), subshrubs (4 taxa), graminoids (3 taxa), tree needles (1 taxa), bryophyte stem (1 taxa), and animal tissues (2 taxa). The subshrubs here are comprised of at least one species of *Artemisia*, including *A. cf. frigida* (prairie sagewort, Asteraceae), and *cf. Phyllodoce empetriformis* (pink mountain heather, Ericaceae). Graminoids refers to grass-like plants in the Cyperaceae, Juncaceae, and Poaceae families and are represented here by *Carex* sp. (sedge), *Juncus* sp. (rush), graminoid stems and leaves, and indeterminate Poaceae florets (grass). Graminoids were recorded in 8 out of 11 samples. The dominant forb types collectively present in these samples are Brassicaceae (mustard family, 8 out of 11 samples) and *Potentilla cf. glaucophylla* (blue-leaved cinquefoil, Rosaceae, 5 out of 11 samples) (Figs. S18–26, see appendix datafile: macrofossils).

The youngest aged sample (SC-12, 765 ±15 cal yr BP) contained 4 types including some vascular plant leaf fragments (Angiosperm), abundant fragments of Cyperaceae (sedges) and graminoid stems, one *Hypnum* sp. stem with leaves (bryophyte, carpet moss), and two fragments of *Picea cf. glauca* needle fragments (white spruce, Pinaceae). No other samples contained identifiable tree or bryophyte material. Collectively, samples aged 30 ka cal yr BP (SC-5, SC-16, SC-17) contained *Allium cf. schoenoprasum var. sibiricum* (giant chives, Amaryllidaceae), Brassicaceae, *Carex*, sp., *Juncus* sp., *Papaver* sp. (Papaveraceae), *Potentilla cf. glaucophylla*, *Ranunculus* sp. (Ranunculaceae), and *Silene* sp. (campion, Caryophyllaceae) macrofossils. Sample SC-5 has an abundance of *Juncus* sp. seeds and is the only sample to contain *cf. Phyllodoce empetriformis* seeds, a subshrub typical of higher elevation and open habitat. Sample SC-17 was the only sample to contain indeterminate bone fragments. Sample SC-9 (80 ka cal yr BP) is dominated by indeterminate vegetation fragments, abundant Brassicaceae and

Chenopodium sp. seeds (goosefoot), and some *Ranunculus* sp. and *Carex* sp. achenes. The oldest aged sample (SC-14, 700 ka cal yr BP) contained the fewest types (3), including abundant Brassicaceae seeds and low-common occurrence of *P. cf. glaucophylla* achenes. The 5 unaged samples (SC-2, SC-6, SC-8, SC-10, SC-11) collectively have 4 unique types represented in multiple samples, including *A. cf. frigida* (2 samples), Coleoptera (2 samples), fungal (3 samples), and Brassicaceae seeds (3 samples).

Supplementary Note 4. aeDNA extraction tests summary (main text results continued).

Following a series of extraction experiments (Supplementary Text, Figs. S4-17), we proceeded with processing the top performing extract replicates from batch 2 and relatively uninhibited extracts from batch 1 (Fig. S1). These were extracted with the sedaDNA cold spin method¹ which generated the highest post-library preparation quantifications across replicates (Fig. S1). Reducing the raw coprolite input to 100 mg (from 200 mg in batch 1) significantly aided with reducing inhibitor co-elution and allowing the library preparation enzymes to regain functionality, resulting in greater library complexity. In Batch 4, we library prepared all successful extracts from SC-14 at both the Hakai Institute and McMaster Ancient DNA Centre, in addition to re-enriching specifically for ground squirrels and Proboscideans.

Supplementary Note 5. Metagenomics *in silico* simulation validations (main text results continued)

To validate the performance of our primary metagenomic classifications (map-filtering to a regional organelle database, *BLASTn* aligning those mapped reads against a 2024 local copy of NCBI-NT, and taxonomically binning with strict *MEGAN7* parameters)¹³⁻¹⁶ we performed *in silico* testing using simulated aeDNA data¹⁷ of species detected in our classifications to assess rates of false positives/negatives, and true positives/negatives. Our simulation data supports the empirical taxonomic identifications of our coprolites, confirming that the *MEGAN* classifications have the lowest rates of false positive classifications among tested classifiers with simulated data (Fig. S27-S31, see the Supplementary Text for further details). This benefit is counter-balanced by strict lowest common ancestor (LCA) parameters, which also result in the highest false negative rates, as relatively few reads pass thresholds required for taxonomic binning. Many reads are also only called at higher taxonomic ranks (e.g. family) to avoid miscalling the genus/species (Fig. S28). When false positives are detected, which are significantly fewer in *MEGAN* than alternative classifiers, they generally occur at lower taxonomic ranks, such as calling the wrong species but the correct genus. These simulations help build confidence that the taxonomic classifications on our coprolite aeDNA libraries are maximally conservative within the limits of current database biases (e.g. inherent incompleteness of genetic databases, erroneous public sequences/taxonomies, over-representation of taxa) and other bioinformatic limitations (e.g. the difficulty of setting parameters to remove false positives while not succumbing to overwhelming false negatives, large computational resources when using the most robust methods, and long (>4 months) classifier run times.⁶

Simulations for assessing true/false positive rates among sample communities with varied taxonomic representations, damage profiles, and taxonomic assignment software add further support for our bioinformatic pipeline approach. Simulations which included bacteria and various eukaryotic taxa did not substantially change the false positive proportions within

samples, though plant reads seemed to have decreased specificity among other eukaryotic reads (e.g., plants reads were more likely to be called to higher-order taxa than animals) (Figs. S27-S31). Simulated results did not show that damage causes increased false positives or partial true positives compared to undamaged reads. Furthermore, the *BLASTn+MEGAN* protocol used for empirical analysis assigned reads more conservatively than any other taxonomic assessment pipeline tested, with the fewest false positives across all simulations and the highest partial true positives and false negative proportions across all simulations. These results lend confidence in the taxa identified in the empirical data presented here, but also indicate that many more reads are sequenced than are assigned a taxonomy with this method. Further optimizations to metagenomic classifiers are needed to improve taxonomic assignment going forward.

Supplementary Note 6. Metagenomics fungal taxa (main text results continued)

The most abundant fungal genus observed was *Pseudogymnoascus* sp.—a psychrophilic fungus today most associated with white-nose syndrome due to *P. destructans*.^{18,19} Whether these reads are best classified as a species of *Pseudogymnoascus*, or if they represent a related fungus without database representation (driven here by an abundance of genetic work on *P. destructans* and a lack of ancient fungal references)²⁰ requires further investigation with a curated fungal database. Other identified taxa include subarctic, soil, and faecal typical fungal clades (Fig. S33). The negative controls and snowshoe hare coprolites show their own distinct fungal signals.

Supplementary Note 7. Genome assembly and phylogenetics (main text results continued)

Lepus. The other primary endogenous faecal producer in this dataset is *Lepus americanus*. This relatively young, 700-year-old sample produced DNA libraries with comparatively low rates of deamination and depurination in the reference mapped reads and has a high on-target signal (Fig. S36). These are all expected characteristics of a sample ~16.5 thousand years younger than the second youngest sample in this data set. Despite the morphology and size, the DNA data make it clear that this faecal pellet originates from snowshoe hare, not caribou. SC-12 sits most closely with a snowshoe hare mitogenome from British Columbia, which is distinct (but still within the same clade) as other snowshoe hare samples from Montana.

Supplementary Note 8. Thermal age (main text results continued).

A regression analysis of the deamination rate (a measure of how quickly the sample deaminated) and age of the sample was significant (regression p-value = 0.005; estimate = $-1.718 \cdot 10^{-10}$), suggesting that older samples appear to have experienced slower rates of deamination. A regression analysis using GISP2 $\delta O^{18\ 21}$ showed similar results, with seemingly colder samples experiencing a greater rate of deamination (regression p-value = 0.002; estimate = $-2.056 \cdot 10^{-6}$). These results at first contradicted the thermal age model of DNA damage which posits that samples from colder climates would be less damaged than those from a warmer climate from the same time period²². A recent study, however, suggests that the deamination rate is partially tied to the amount of depurination due to the former (deamination) being reliant on the latter (depurination) to create single-stranded overhangs which can then be subsequently deaminated²³. Taking this into account, a regression model in the form of *DeaminationRate* =

*Depurination + Age * δO^{18}* explains a greater amount of the variation in the damage data (R^2_{adj} for model with depurination, age and $\delta O^{18} = 0.908$; R^2_{adj} for model with = 0.560; R^2_{adj} for model with $\delta O^{18} = 0.635$) and renders all the variables significant (p-values < 0.05). Specifically, increased depurination and δO^{18} will decrease deamination (Figs. S98-S99) (effect sizes $3.365 \cdot 10^{-4}$ and $8.156 \cdot 10^{-6}$ respectively), whereas increased age will lead to a moderately lower rate (Fig. S84c; effect size = $-7.978 \cdot 10^{-6}$). The interaction term between age and δO^{18} was also significant (p-value = 0.0232; effect size = $-2.04 \cdot 10^{-10}$), suggesting that the thermal age model is still applicable and a better predictor of damage than age alone. This analysis is limited of course by the use of GISP2 δO^{18} data²¹ which lacks the spatial-temporal sensitivity to record continental climate dynamics in Beringia given that GISP2 is derived from North Atlantic Greenland. This dataset may prove useful in future investigations of aeDNA decay kinetics with a more locally sensitive climate proxy measure.

A Sample metadata					Batch 1		Batch 2					Batch 3				Batch 4	
aDNA ID	Site	Midden ID	Age (med. cal. yr BP)	Original Pellet ID	Input (g)	Inhibition Index [†]	Input (g)	Inhibition Index ^{††}	qPCR TQ ^{††}	Shotgun Total Reads	Enrichment Total Reads ^{§§}	Input (g)	Inhibition Index	qPCR TQ	Enrichment Total Reads ^{§§}	Shotgun Total Reads	Enrichment Total Reads ^{§§}
SC-1	Paradise Hill	DF-07-26	700,000 ^{GRT}	AGS*	0.2	1.00	Not processed further after batch 1										
SC-2	Hunker Creek	DF09-HC-30	17,175	AGS	0.2	1.00	0.1 (x6) [§]	0.33	3.2E+09	15,920,534	7,188,659	best repl. batch 2		3.2E+09	1,431,007		
SC-3	Hunker Creek	DF09-HC-29a	17,175	AGS	0.2	1.00						0.1	0.2	1.4E+10	1,867,770		
SC-4	?	DF13-02	?	AGS	0.2	0.40	Not processed further after batch 1										
SC-5	Lower Hunker	SLC21-KL-1D	30,000 ^{DT}	AGS	0.2	0.22						0.1	0.15	7.4E+09	2,024,789		8,506,840
SC-6	Quartz Creek	SC-AGS-01	80,000 ^{SCT}	AGS	0.2	1.00						1/10 dil batch 1 [¶]		2.9E+08	2,571,597		
SC-7	?	DF13-02	?	AGS	0.2	1.00	Not processed further after batch 1										
SC-8	Quartz Creek	SC-AGS-04	80,000 ^{SCT}	AGS	0.2	1.00						1/10 dil batch 1 [¶]		8.3E+08	2,131,519		
SC-9	Canyon Creek	SC-AGS-12	80,000 ^{SCT}	AGS	0.2	0.30						0.1	0.15	2.6E+10	2,697,528		17,185,525
SC-10	Eureka Creek	SC-AGS-15	31,035	AGS	0.2	0.32	0.1 (x6) [§]	0.24	2.2E+09	16,287,382	8,483,803	best repl. batch 2		2.2E+09	1,261,310		9,914,557
SC-11	Eureka Creek	SC-AGS-18	28,660	AGS	0.2	0.66						1/10 dil batch 1 [¶]		4.1E+08	1,875,061		
SC-12	Little Blanche	SC-AGS-21	682	Caribou	0.2	0.08						from batch 1 ^{¶¶}		1.1E+09	2,777,265		
SC-13	Quartz Creek	SC-AGS-22	35,850	AGS	0.2	1.00	0.1 (x6) [§]	0.19	2.7E+09	12,734,374	6,522,710	best repl. batch 2		2.7E+09	1,401,971		7,928,078
SC-14	Gold Run	GZ.07.07	700,000 ^{GRT}	AGS	0.2	0.23						0.1	0.16	1.1E+10	2,006,817	242,855,809	290,600,543
SC-15	Lower Hunker	SLC21-KL-4A	30,000 ^{DT}	AGS	0.2	0.36						0.1	0.19	6.0E+08	2,890,549		9,788,279
SC-16	Treadstone Mine	SLC21-TM-1C	30,000 ^{DT}	AGS	0.2	0.62						1/10 dil batch 1 [¶]		1.8E+09	1,956,241		
SC-17	Lower Hunker	SLC21-KL-8	30,000 ^{DT}	AGS	0.2	0.31						from batch 1		8.9E+08	2,208,612		
Negative controls (x13 sequenced)					N/A	0.08	N/A	0.05	7.8E+04	967,848	22,456	N/A	0.06	7.5E+04	1,085,591	7647	3,572,382

*AGS = arctic ground squirrel (*Urocyon* sp.).

[§](#) Number of total replicate variants for extraction methods comparison. 1 unless indicated.

^{§§}Batch 2 and 3 enriched with PalaeoChip Arctic 1.0 animal and plant baits, batch 4 enriched with Xerinae and Proboscidea baits.

Tephrochronology ages = ^{GRT}Gold Run Tephra; ^{SCT}Sheep Creek Tephra; ^{DT}Dawson Tephra. Other ages based on radiocarbon.

[†]Based on Murchie et al. (2021a): 1 = completely inhibited, 0 = no enzymatic inhibition.

^{††}Best performing extraction replicate variant.

[¶]A 1/10 extract dilution from batch 1; ^{¶¶}a direct extract from batch 1

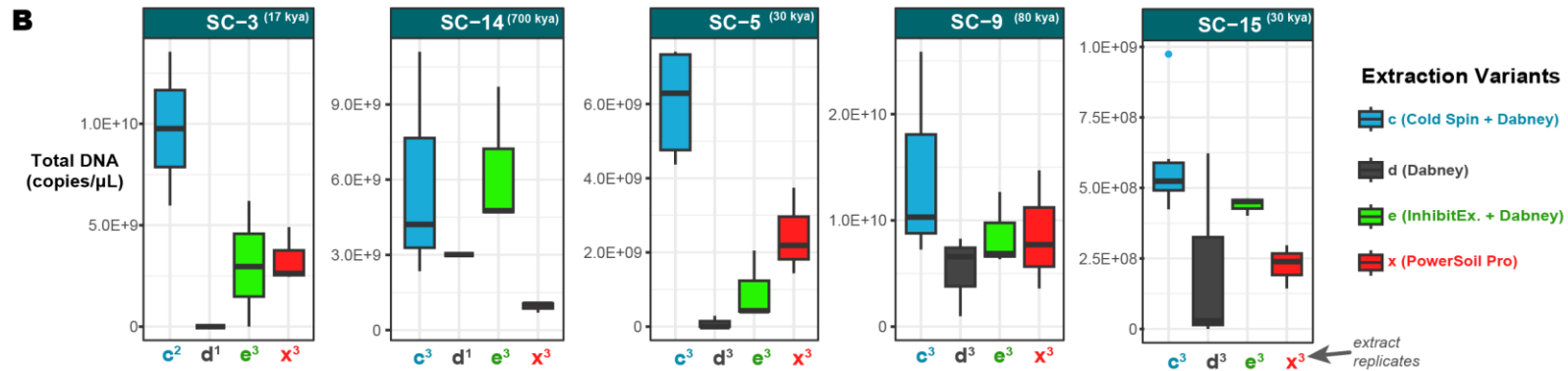


Fig. S1 Summary of (A) sample/midden metadata. (B) Summary of extraction variant testing by total library adapted DNA. Measured by qPCR using IS7/IS8 primers ^{7,8} following library preparation among extraction method variants with replicates. Data here is a subset of Fig. S17.

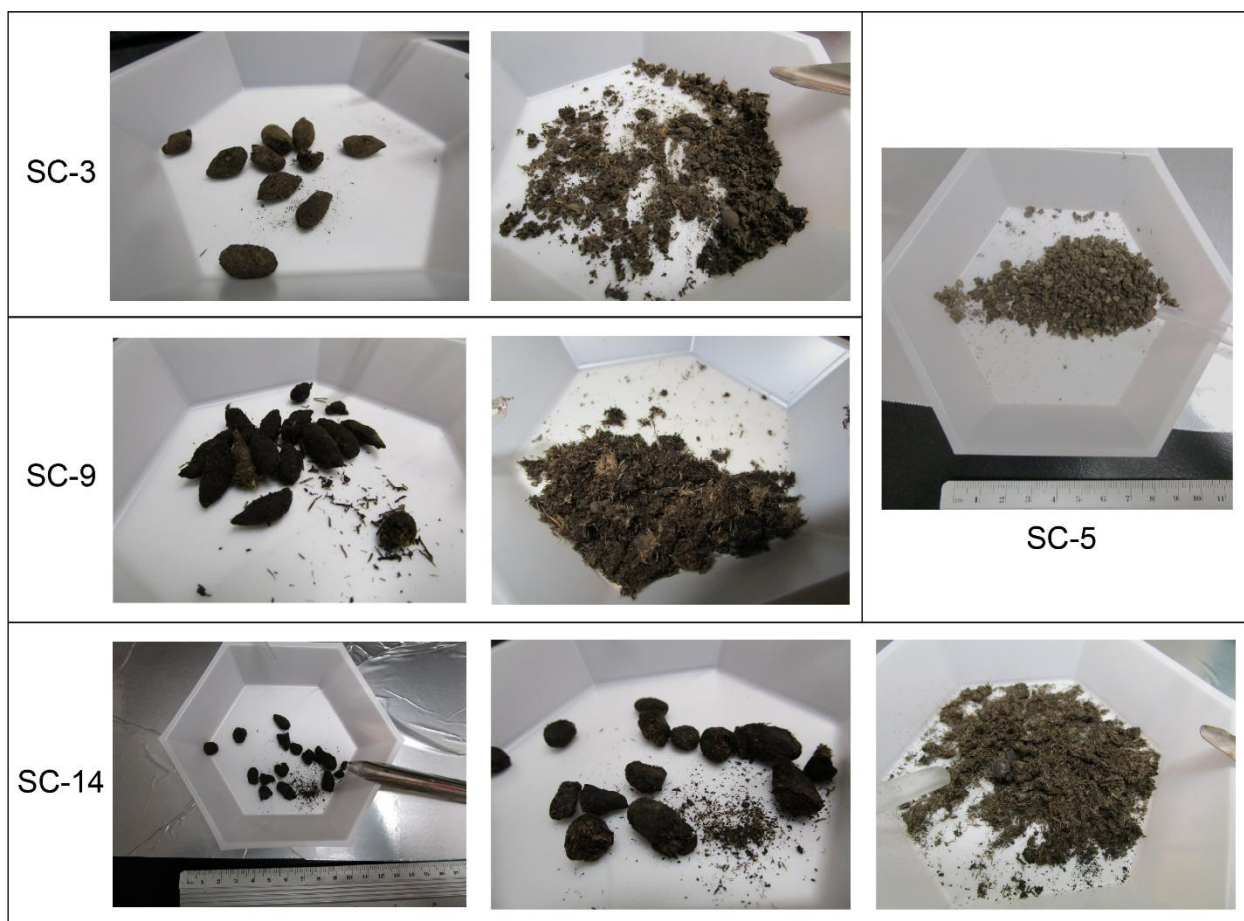


Fig. S2
Example photos of faecal pellets before and after homogenization.

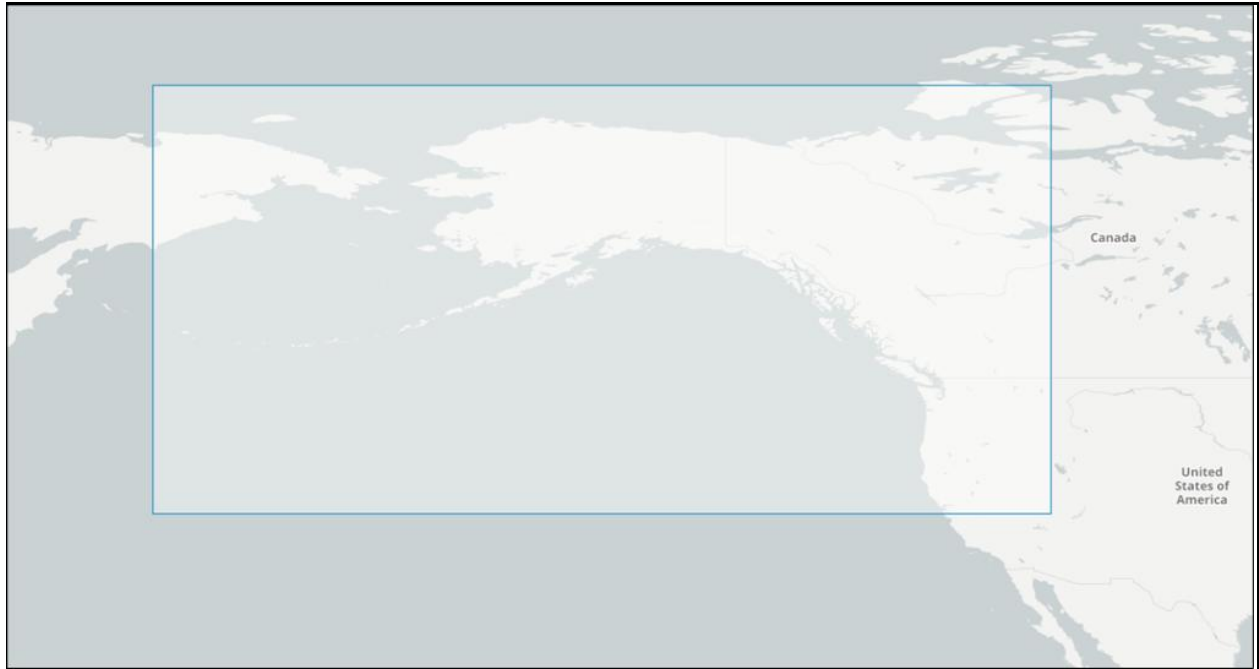


Fig. S3

Geographic coordinate search extent for Pacific NorthWest Quaternary References
(POLYGON((-189.88984 37.35931,-113.35883 37.35931,-113.35883 74.11933,-189.88984
74.11933,-189.88984 37.35931))). GBIF Occurrence Data:

Mammalia: <https://doi.org/10.15468/dl.5kszzd>

Plantae: <https://doi.org/10.15468/dl.tkhs72>

Squamata: <https://doi.org/10.15468/dl.fs3ebk>

Testudines: <https://doi.org/10.15468/dl.8nswgz>

Amphibia: <https://doi.org/10.15468/dl.fckj72>

Theoretical balance between inhibitor co-extraction & DNA retention

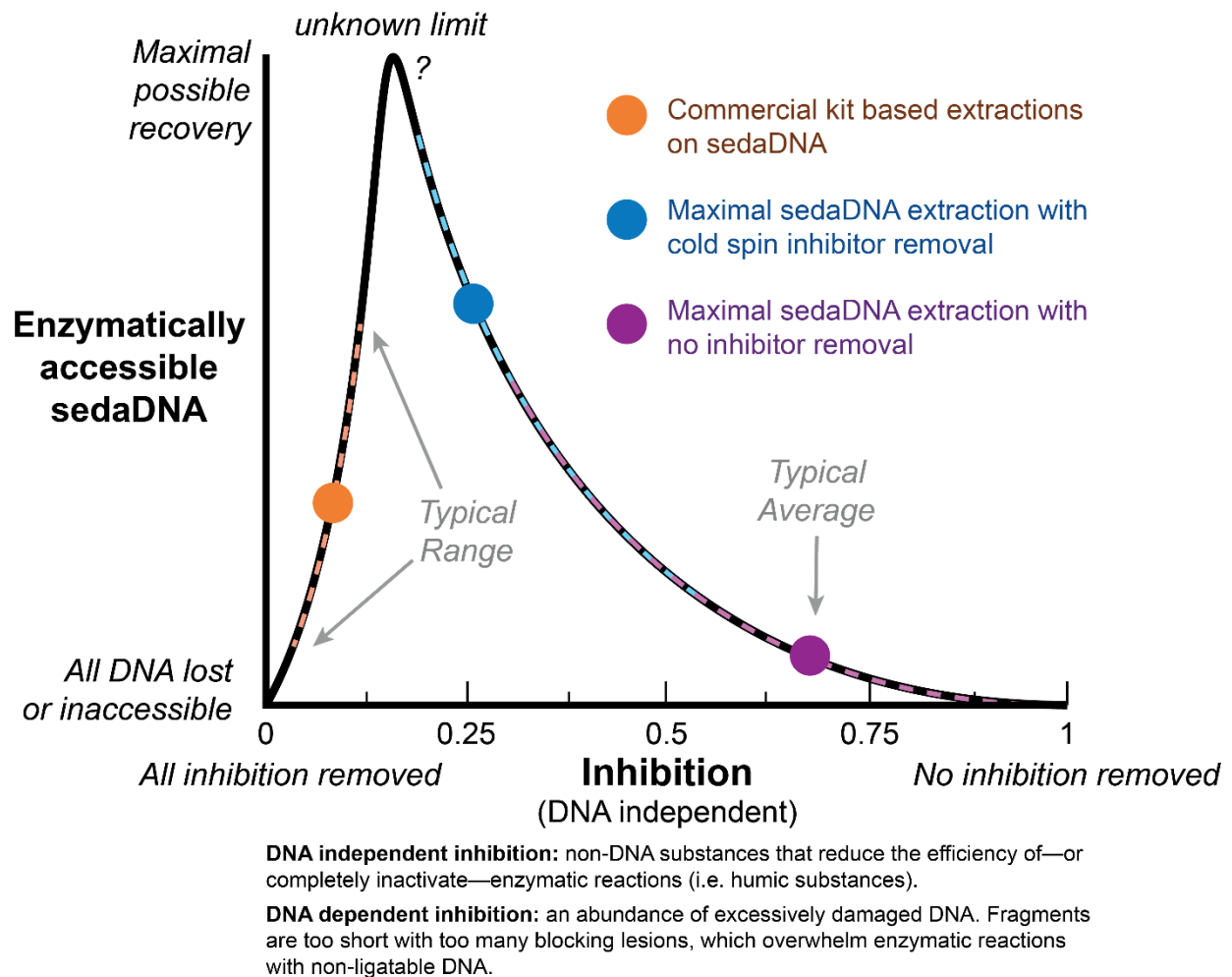


Fig. S4

The hypothesized balance between inhibition and DNA retention with cold spin extracts based on repeated observations of inhibition and DNA recovery. Figure based on anecdotal observation—producing this with data would require a priori knowledge of preserved sedaDNA and humic concentrations, both of which are challenging to accurately measure. Refined from Murchie et al.¹

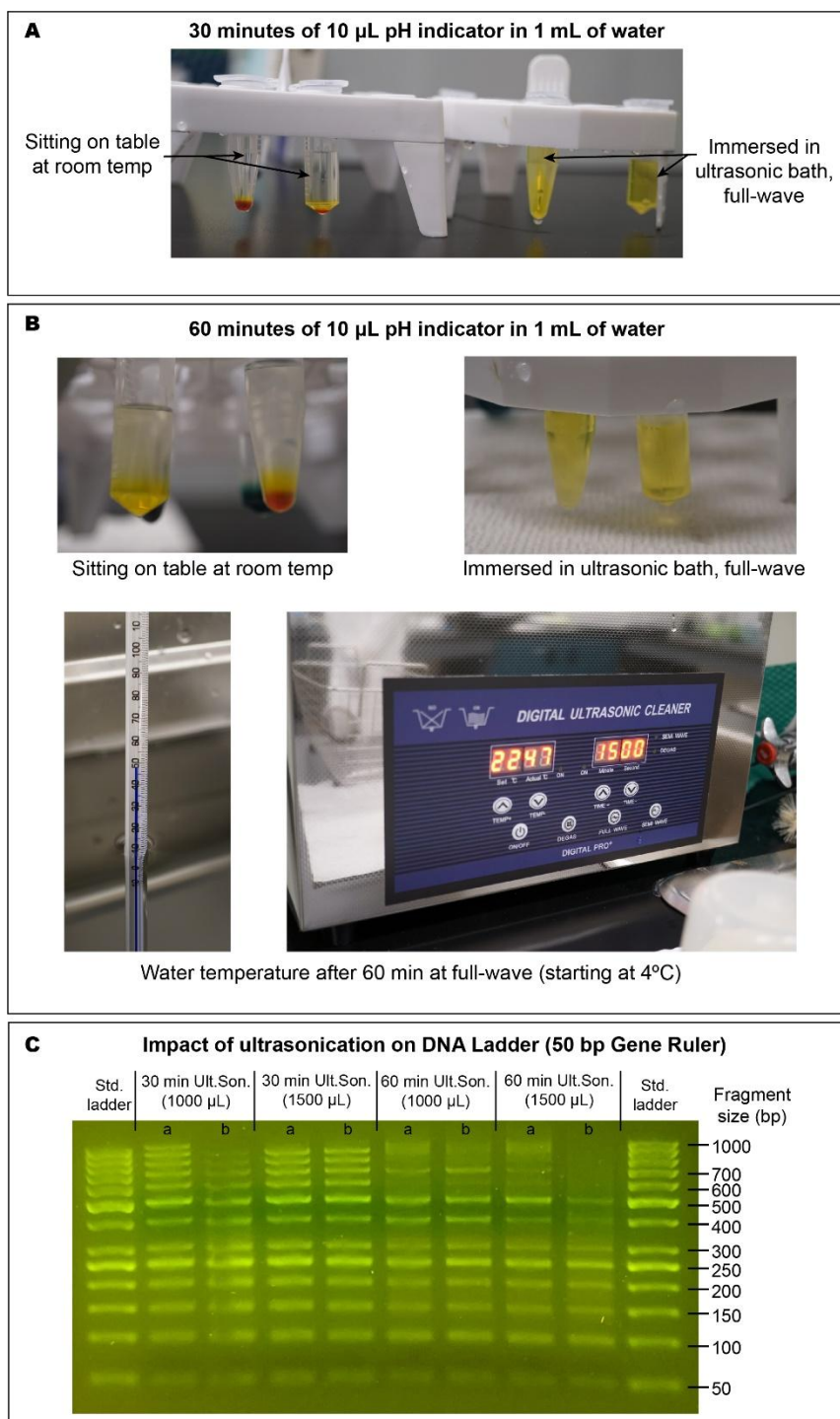


Fig. S5

Tests to confirm that a budget digital ultrasonicator designed for jewelry cleaning can generate liquid particle disruption within a plastic microtube by: **A/B**, visually inferring the dispersal of unmixed pH indicator in water after 30 and 60 minutes. And, **C** observing fragment size breakdown of a synthetic DNA ladder (purified post-sonication with a MinElute PCR purification kit) via agarose gel electrophoresis with variable water volumes and duration of ultra-sonication.

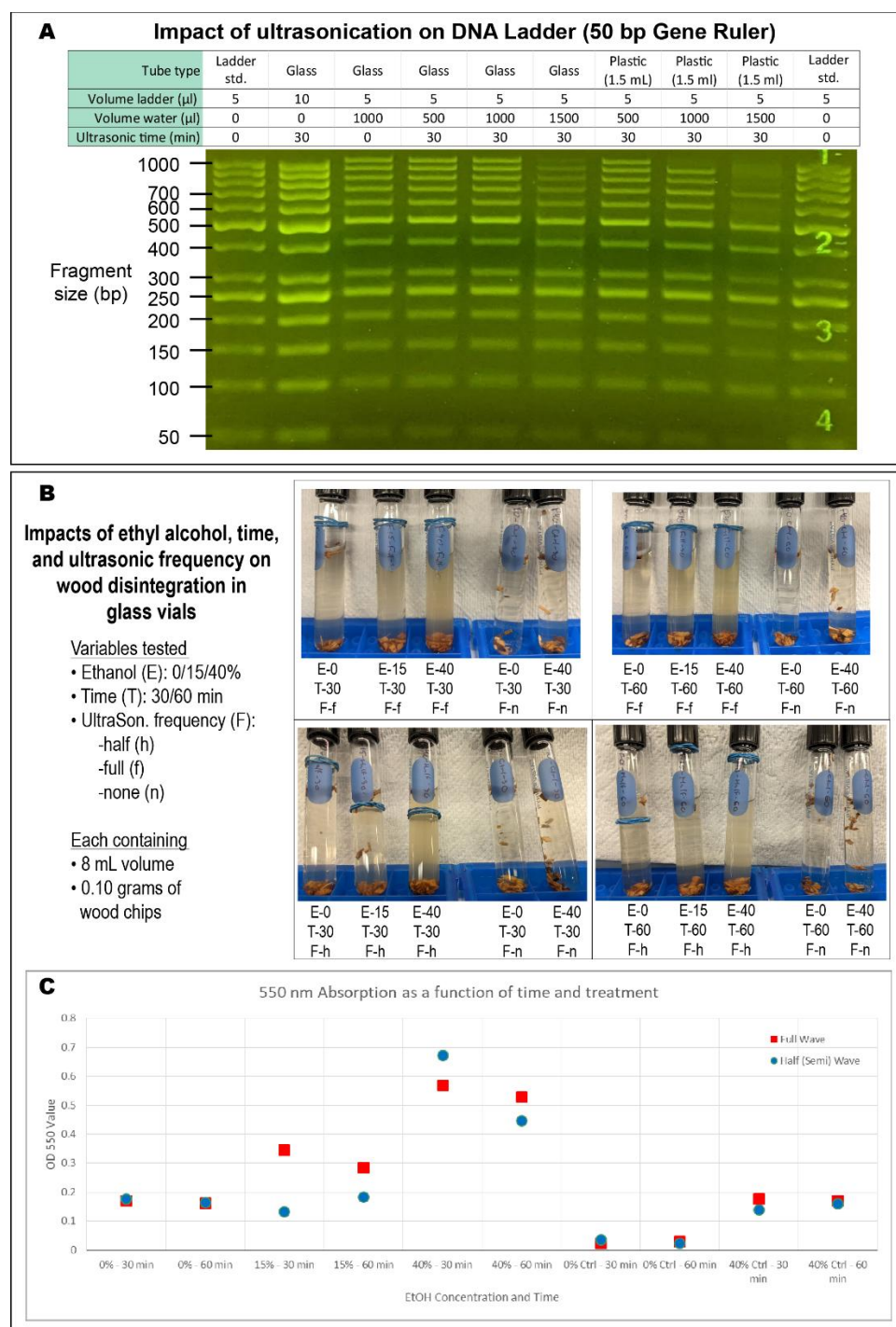


Fig. S6

Tests to confirm optimal ultra-sonication conditions with **A**, glass or plastic tubes at variable volumes; **B** impact of ethyl alcohol, time, and ultrasonic frequency on the disintegration of wood chips; and **C** absorption measures of resultant liquid colouration with spectroscopy.

A	Sample info					Originally inhibited after CS batch 1?	Variables					Summary of extraction variant	Inhibition Index	qPCR concentration Average (pM)
Count	DNA ID	Original ID	Site	Age	Sample Type	Homogenized input (mg)	Cold spin	Ultrasonication (min)	InhibitEX Tablet	LPA				
1	SC-2a	DF-09-HC-30	Hunker Creek (Yukon)	Undated	Arctic ground squirrel fecal pellet	Yes	100	Y	60			Sonication and cold spin	0.5	1,462,000,000
2	SC-2b						100	Y	60 + 750 μ L isoprop.			Sonication with alcohol and cold spin	0.2	2,983,500,000
3	SC-2c						100	Y				Cold spin	0.4	2,017,000,000
4	SC-2d						100					Standard Dabney	1.0	599,550
5	SC-2e						100			Y		InhibitEX Tablet (no CS)	0.3	3,228,500,000
6	SC-2f						100				Y	LCA purification (no CS)	0.5	12,510,000
7	SC-13a	SC-AGS-22	Quartz Creek (Yukon)	Undated	Arctic ground squirrel fecal pellet	Yes	100	Y	60			Sonication and cold spin	0.8	762,200,000
8	SC-13b						100	Y	60 + 750 μ L isoprop.			Sonication with alcohol and cold spin	0.2	2,718,000,000
9	SC-13c						100	Y				Cold spin	0.4	863,000,000
10	SC-13d						100					Standard Dabney	1.0	13,080,000
11	SC-13e						100			Y		InhibitEX Tablet (no CS)	0.4	1,949,500,000
12	SC-13f						100				Y	LCA purification (no CS)	0.3	16,780,000
13	SC-10a	SC-AGS-15	Eureka Creek (Yukon)	Undated	Arctic ground squirrel fecal pellet	No	100	Y	60			Sonication and cold spin	0.2	1,578,500,000
14	SC-10b						100	Y	60 + 750 μ L isoprop.			Sonication with alcohol and cold spin	0.1	889,600,000
15	SC-10c						100	Y				Cold spin	0.2	2,198,000,000
16	SC-10D						100					Standard Dabney	0.3	794,050,000
17	SC-10e						100			Y		InhibitEX Tablet (no CS)	0.3	1,070,500,000
18	SC-10f						100				Y	LCA purification (no CS)	0.1	9,318,000
19	PHP-12a	MM12-119B	Hunker Creek (Yukon)	10,051 cal BP	permafrost silt	No	150	Y	60			Sonication and cold spin	0.6	361,700,000
20	PHP-12b						150	Y	60 + 750 μ L isoprop.			Sonication with alcohol and cold spin	0.1	379,650,000
21	PHP-12c						150	Y				Cold spin	0.2	226,350,000
22	PHP-12d						150					Standard Dabney	1.0	44,285
23	PHP-12e						150			Y		InhibitEX Tablet (no CS)	1.0	474,600
24	PHP-12f						150				Y	LCA purification (no CS)	0.2	76,820
25	Son-EBK1	Negative controls				No	na	Y	60 + 750 μ L isoprop.			Son+isoprop+CS	0.0	249,985
26	Son-EBK2						na				Y	Y	LPA+InhibitEx+Dab	0.1

B	Extract	Ranking in Inhibition index 1 = best, 6 = worst	Ranking in total DNA quantification
SC-2a		4	4
SC-2b		1	2
SC-2c		3	3
SC-2d		6	6
SC-2e		2	1
SC-2f		5	5
SC-13a		5	4
SC-13b		1	1
SC-13c		3	3
SC-13d		6	6
SC-13e		4	2
SC-13f		2	5
SC-10a		3	2
SC-10b		1	4
SC-10c		4	1
SC-10d		6	5
SC-10e		5	3
SC-10f		2	6
PHP-12a		4	2
PHP-12b		1	1
PHP-12c		3	3
PHP-12d		5	6
PHP-12e		5	4
PHP-12f		2	5

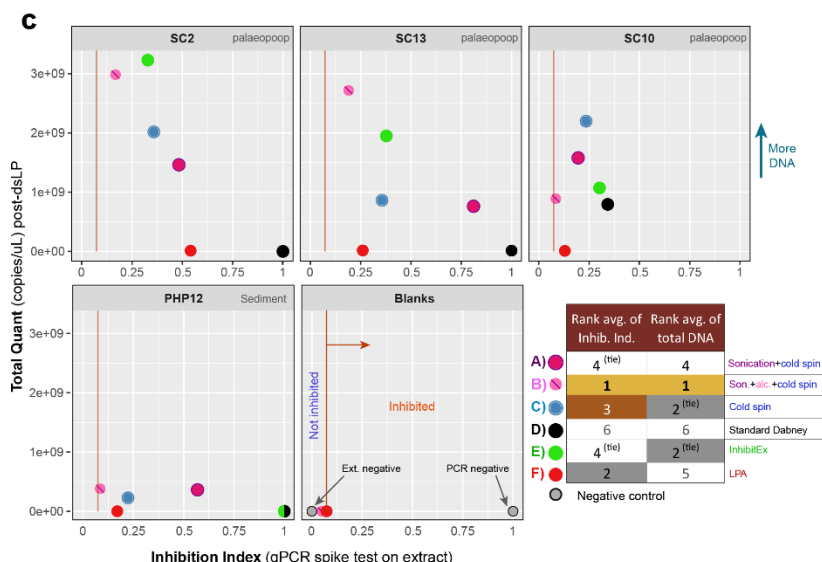


Fig. S7

Ancient eDNA qPCR experimental results. **A** Samples/methods tested in batch 1 and their inhibition/DNA quantifications summary. Inhibition index ranges from 0 (uninhibited) to 1 (completely inhibited). Blanks typically have values < 0.1. qPCR concentration average (pM): Total adapted DNA following library preparation but prior to indexing. **B** Average ranks of inhibition index and short amp total DNA qPCR across method variants. **C** qPCR assay of inhibition (on extracts) on x-axis and total adapted DNA (on libraries) on y-axis. Samples with the best performance are as close to 0 as possible on the x-axis (leftmost) and as high as possible on the y-axis.



Fig. S8

Shotgun megablast metagenomic comparison normalized to the smallest libraries with stacked barcharts of **A** family rank animals, **B** family rank plants, and **C** genus rank eukaryotes normalized prior to map-filtering (randomly subsampled to a depth of 2,000,000 reads each), and metagenomically classified with *BlastN*.

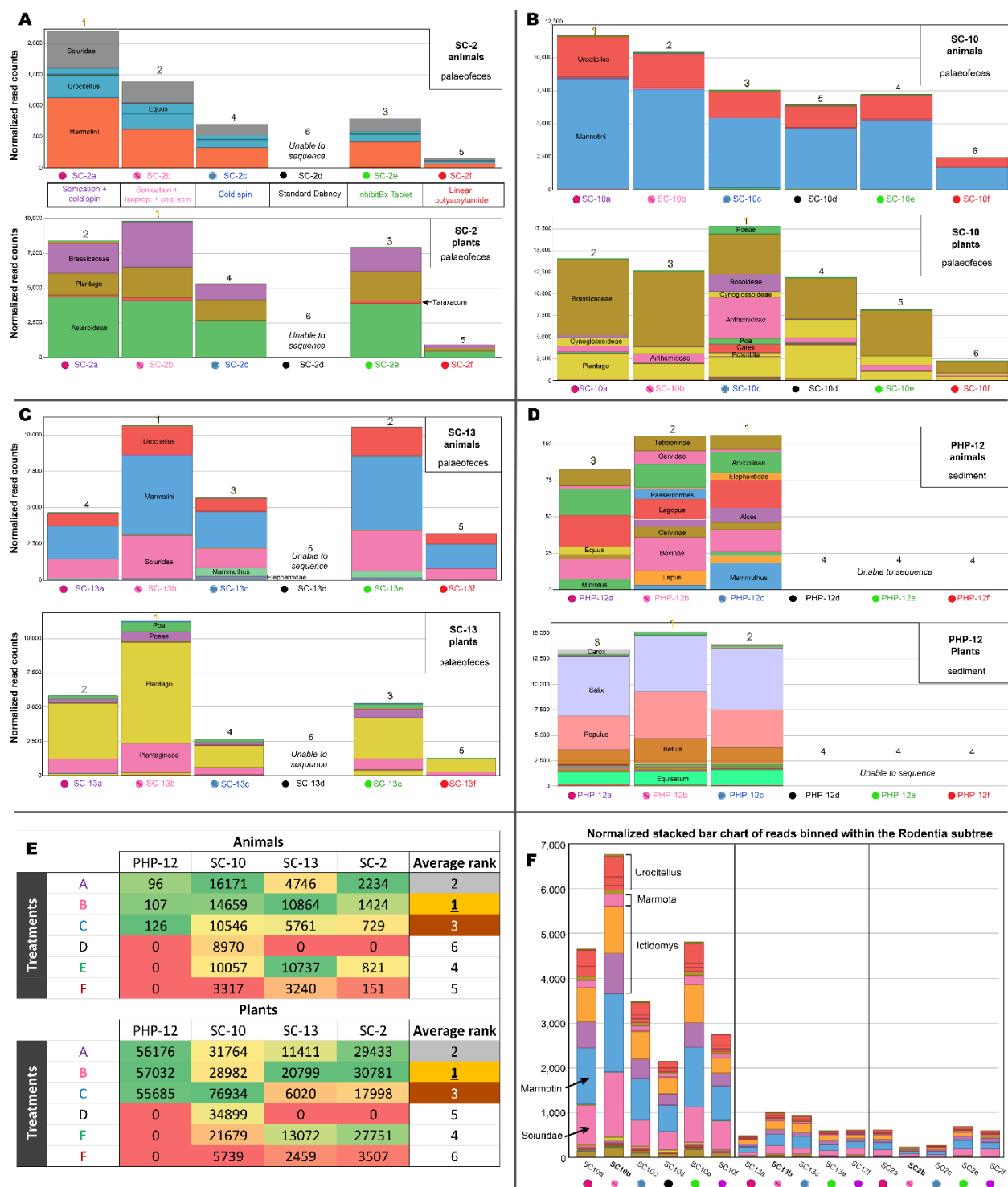


Fig. S9

Pre-subsample normalized metagenomic summary of enriched libraries with **A-D** stacked bar charts; **E** a heatmap of reads assigned to animal and plant subtrees; and **F** reads assigned specifically to Rodentia within the coprolite libraries. All libraries enriched with PalaeoChip baits, subsampled to an equal depth (810,000 reads), map-filtered to bait references, then taxonomically binned with *BlastN* and *MEGAN*.

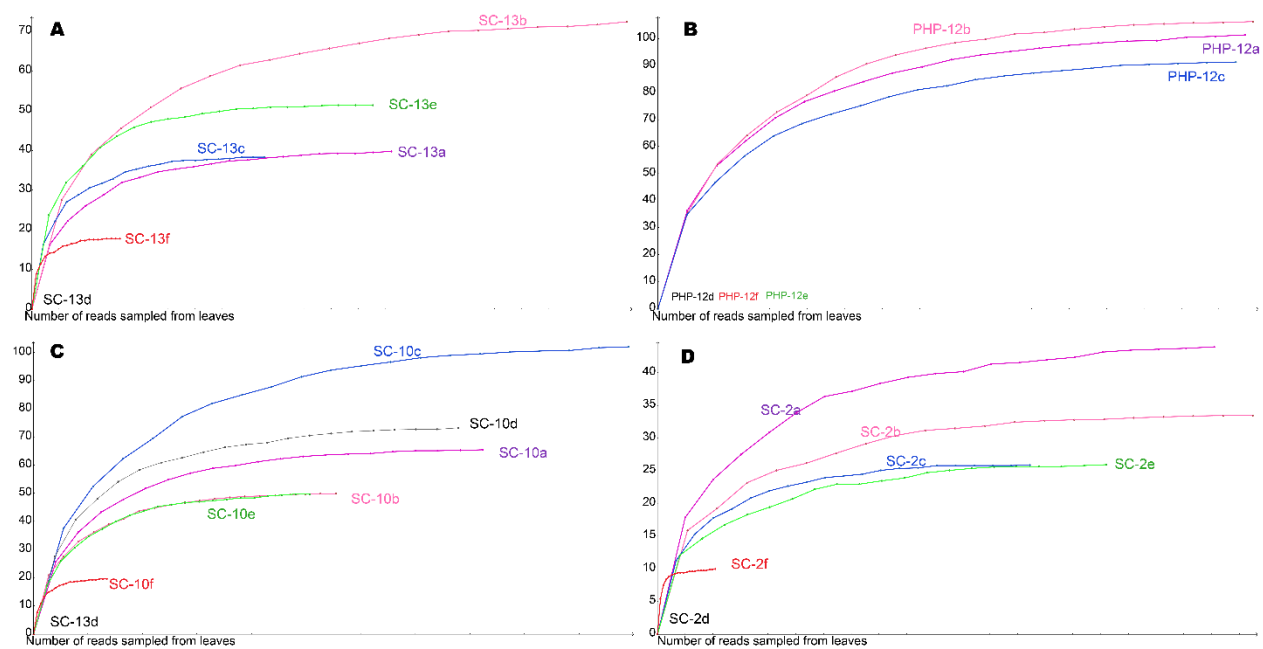


Fig. S10

Rarefaction curves eukaryotic subtree from enriched libraries (subsamped, map-filtered, and *BlastN*).

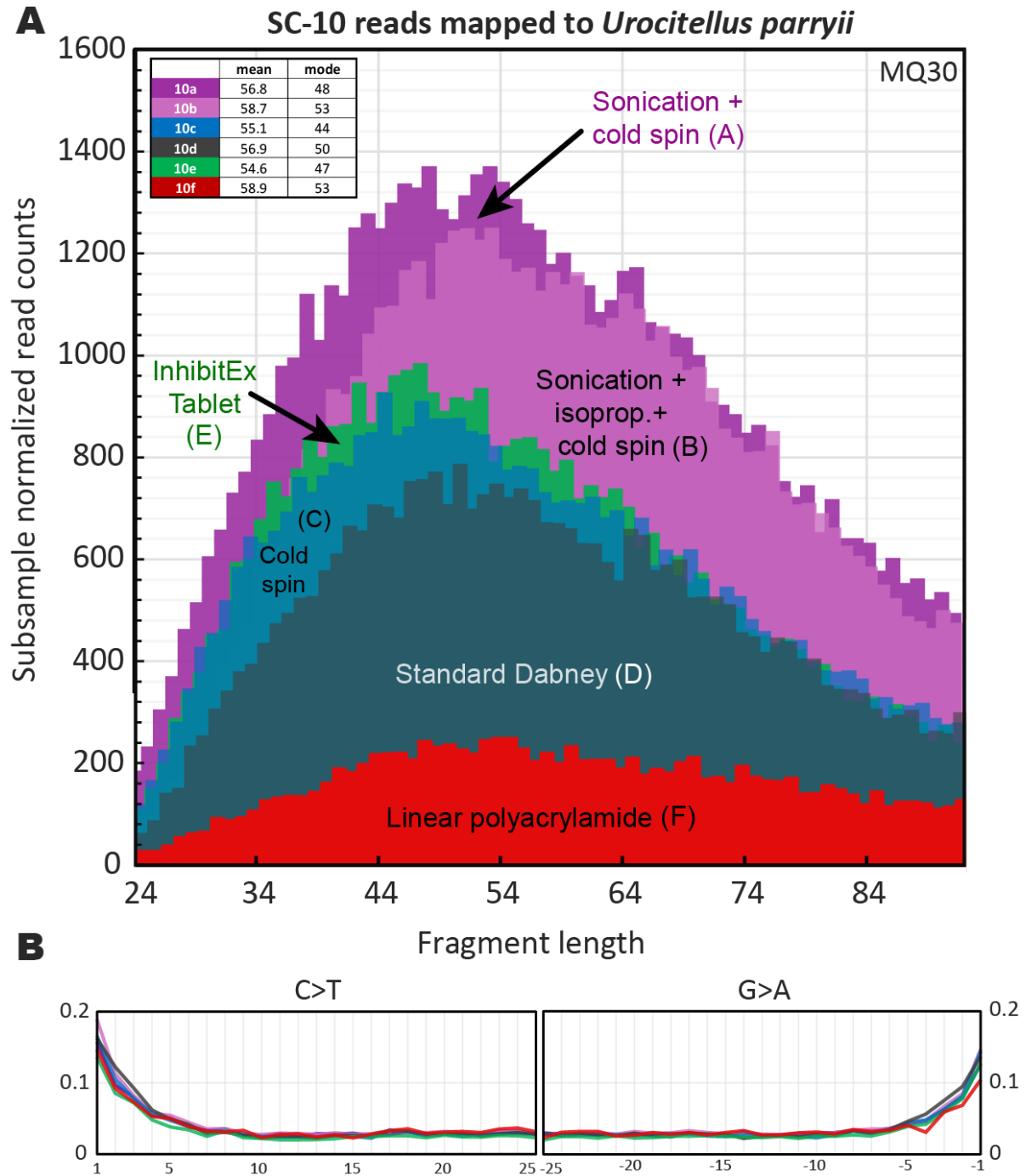


Fig. S11

Comparison of subsample normalized **A** fragment lengths from reads mapped to *U. parryii* in sample SC-10 (the uninhibited sample) with **B** associated *mapDamage* deamination profiles.

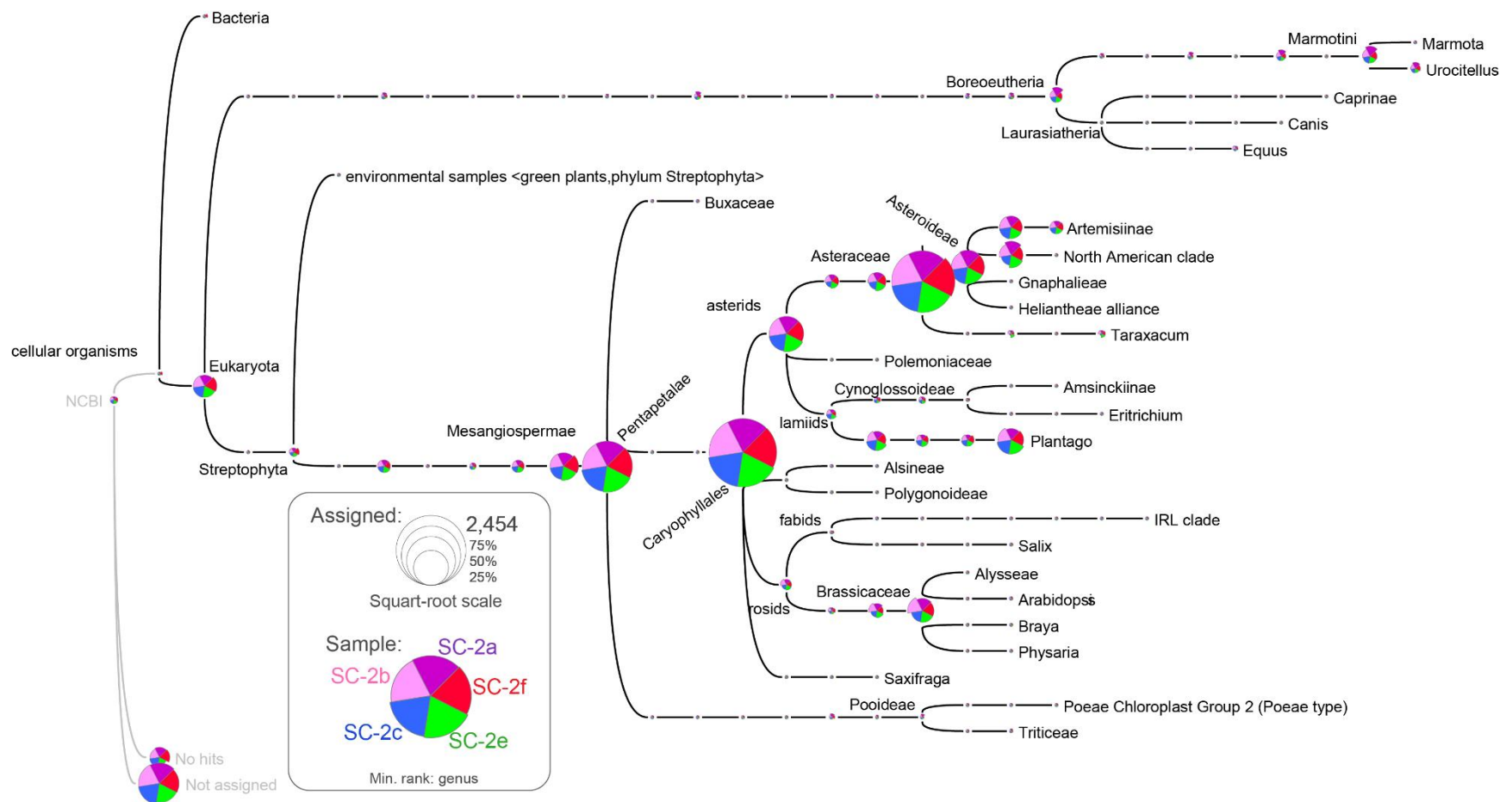


Fig. S12

Metagenomic summary of map-filtered dataset from SC-2 (normalized in MEGAN).

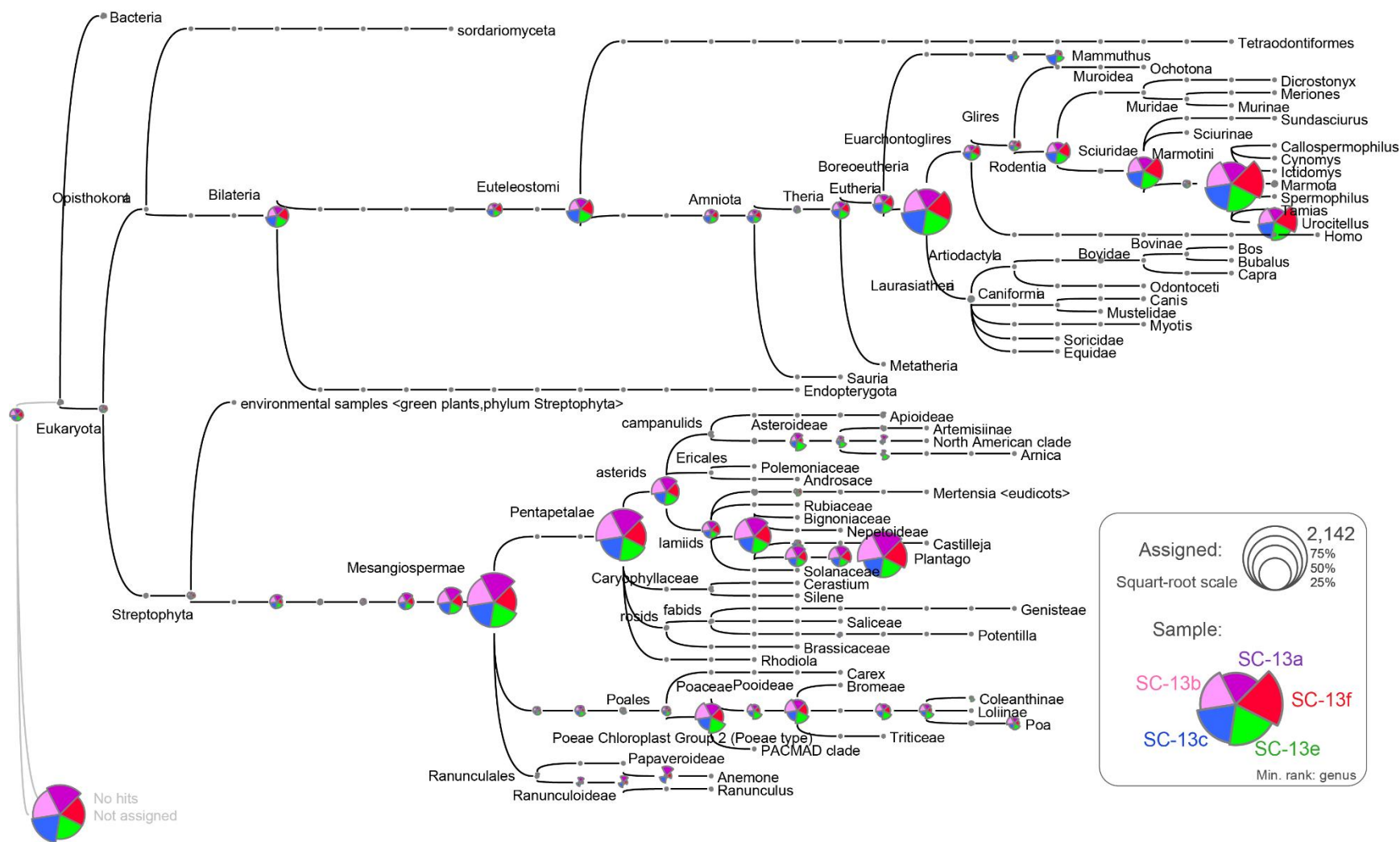


Fig. S14

Metagenomic summary of map-filtered dataset from SC-13 (normalized in MEGAN).

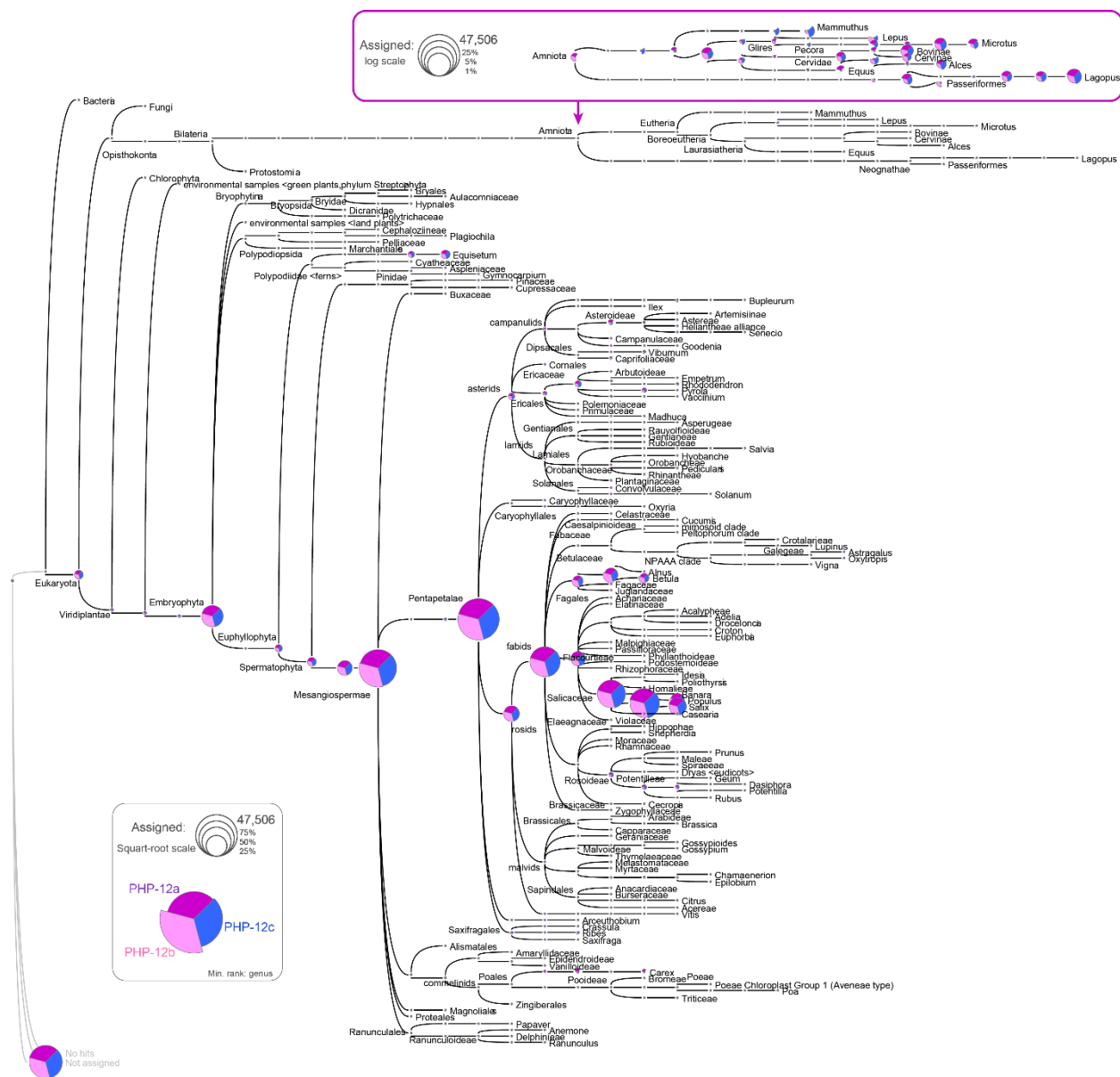


Fig. S15
Metagenomic summary of map-filtered dataset from PHP-12 (normalized in MEGAN).

Sample info							Extraction variants						Extract variant summary	Inhibition Index Avg.	Total DNA (copies/uL) Avg.		Rank
Original ID	SC ID	Sample Type	Site	Age	Prev. Inhibited?	Extract ID	Extraction Replicates	Son. with isoprop.	Cold spin	Dabney	InhibitEX Tablet	PowerSoil					
SLC21-KL-1D	SC-5	AGS coprolite	Lower Hunker	Unknown	No	SC-5z	3*	Y		Y			z (Son. + Dab.)	0.18	5.37E+08		5
						SC-5b	6 [‡]	Y	Y	Y			b (Son. + Cold spin + Dab.)	0.06	1.62E+09		3
						SC-5c	3*		Y	Y			c (Cold spin + Dab.)	0.16	6.05E+09		1
						SC-5d	3			Y			d (Dabney)	0.83	9.66E+07		6
						SC-5e	3			Y	Y		e (Dab. + InhibitEx)	0.40	9.54E+08		4
						SC-5x	3					Y	x (PowerSoil Pro)	0.14	2.46E+09		2
SC-AGS-12	SC-9	AGS coprolite	Canyon Creek	Unknown	No	SC-9z	3*	Y		Y			z (Son. + Dab.)	0.29	5.86E+08		6
						SC-9b	6 [‡]	Y	Y	Y			b (Son. + Cold spin + Dab.)	0.19	7.79E+08		5
						SC-9c	3*		Y	Y			c (Cold spin + Dab.)	0.19	1.20E+10		1
						SC-9d	3			Y			d (Dabney)	0.34	5.28E+09		4
						SC-9e	3			Y	Y		e (Dab. + InhibitEx)	0.39	8.63E+09		3
						SC-9x	3					Y	x (PowerSoil Pro)	0.09	8.67E+09		2
SLC21-KL-4A	SC-15	Sediment with intermixed faecal pellets	Lower Hunker	~30,000 ybp	No	SC-15z	3*	Y		Y			z (Son. + Dab.)	0.19	8.08E+07		6
						SC-15b	6 [‡]	Y	Y	Y			b (Son. + Cold spin + Dab.)	0.05	1.06E+08		5
						SC-15c	3*		Y	Y			c (Cold spin + Dab.)	0.20	5.90E+08		1
						SC-15d	3			Y			d (Dabney)	0.46	2.17E+08		4
						SC-15e	3			Y	Y		e (Dab. + InhibitEx)	0.31	4.38E+08		2
						SC-15x	3					Y	x (PowerSoil Pro)	0.07	2.26E+08		3
DF-09-HC-29a	SC-3	AGS coprolite	Hunker Creek	17,155 ybp	Yes	SC-3b	3	Y	Y	Y			b (Son. + Cold spin + Dab.)	0.08	7.86E+06		4
						SC-3c	2		Y	Y			c (Cold spin + Dab.)	0.24	9.76E+09		1
						SC-3d	1			Y			d (Dabney)	1.00	0.00E+00		5
						SC-3e	3			Y	Y		e (Dab. + InhibitEx)	0.49	3.05E+09		3
						SC-3x	3					Y	x (PowerSoil Pro)	0.15	3.32E+09		2
GZ.07.07	SC-14	AGS coprolite	Gold Run	~700,000 ybp	Yes	SC-14b	3	Y	Y	Y			b (Son. + Cold spin + Dab.)	0.18	3.59E+08		5
						SC-14c	3		Y	Y			c (Cold spin + Dab.)	0.17	5.89E+09		2
						SC-14d	1			Y			d (Dabney)	0.48	3.01E+09		3
						SC-14e	3			Y	Y		e (Dab. + InhibitEx)	0.42	6.38E+09		1
						SC-14x	3					Y	x (PowerSoil Pro)	0.18	9.44E+08		4

Fig. S16

Summary of batch 2 extraction experiments and qPCR results.

*Extraction triplicates, but each quantified twice to pair with method BB. With duplicate qPCR, this resulted in 12 replicates qPCR tested for total DNA.

‡3 replicates for method B, and after poor results, 3 new extracts were made from the original starting material for method BB.

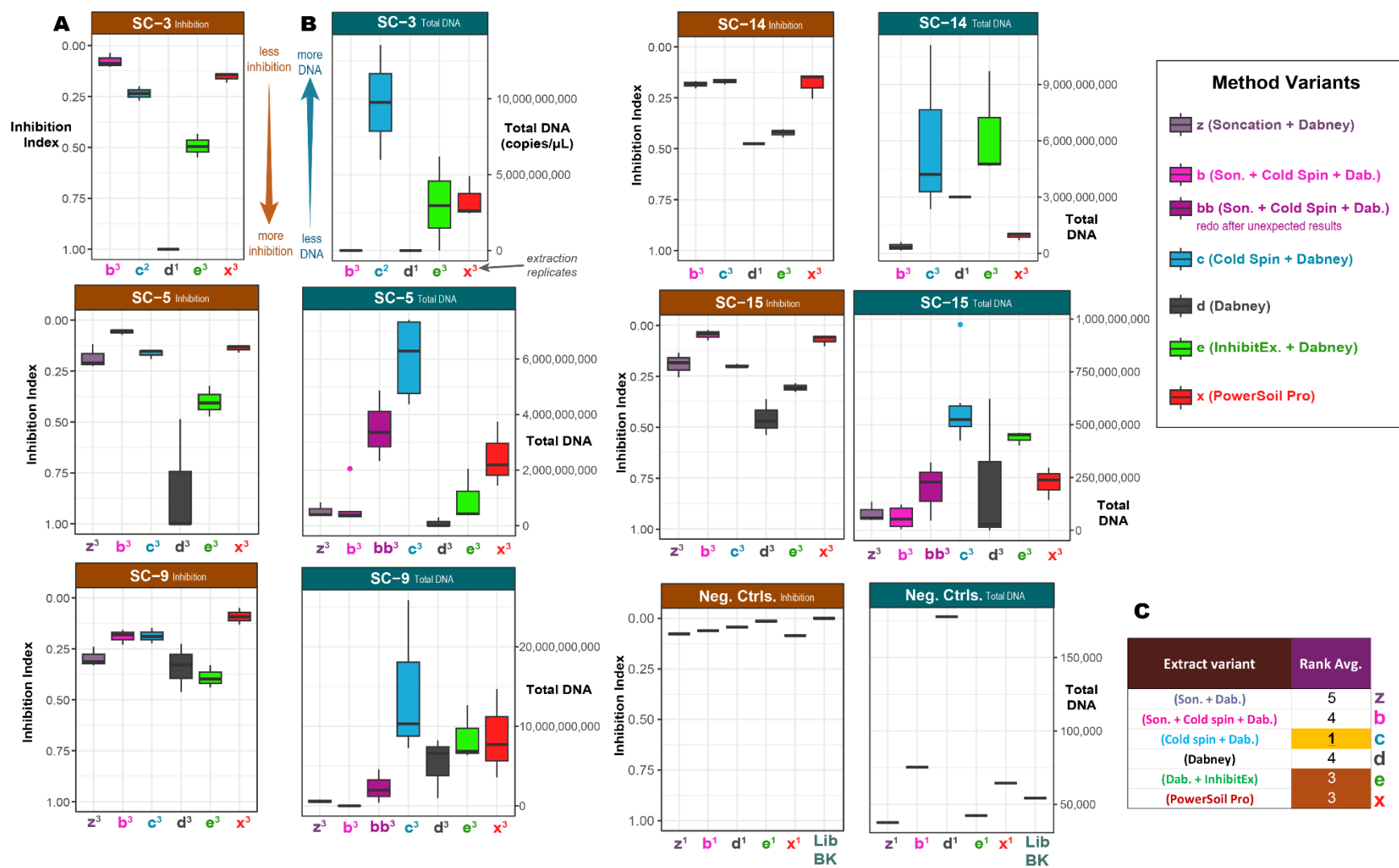


Fig. S17

Summary of batch 2 qPCR results for the **A** Inhibition Index, **B** total DNA quantification, and **C** the rank averages of method variants for total DNA successfully library adapted.



Fig. S18.

Macrofossil microscope photos, 1 of 9.



Fig. S19.

Macrofossil microscope photos, 2 of 9.

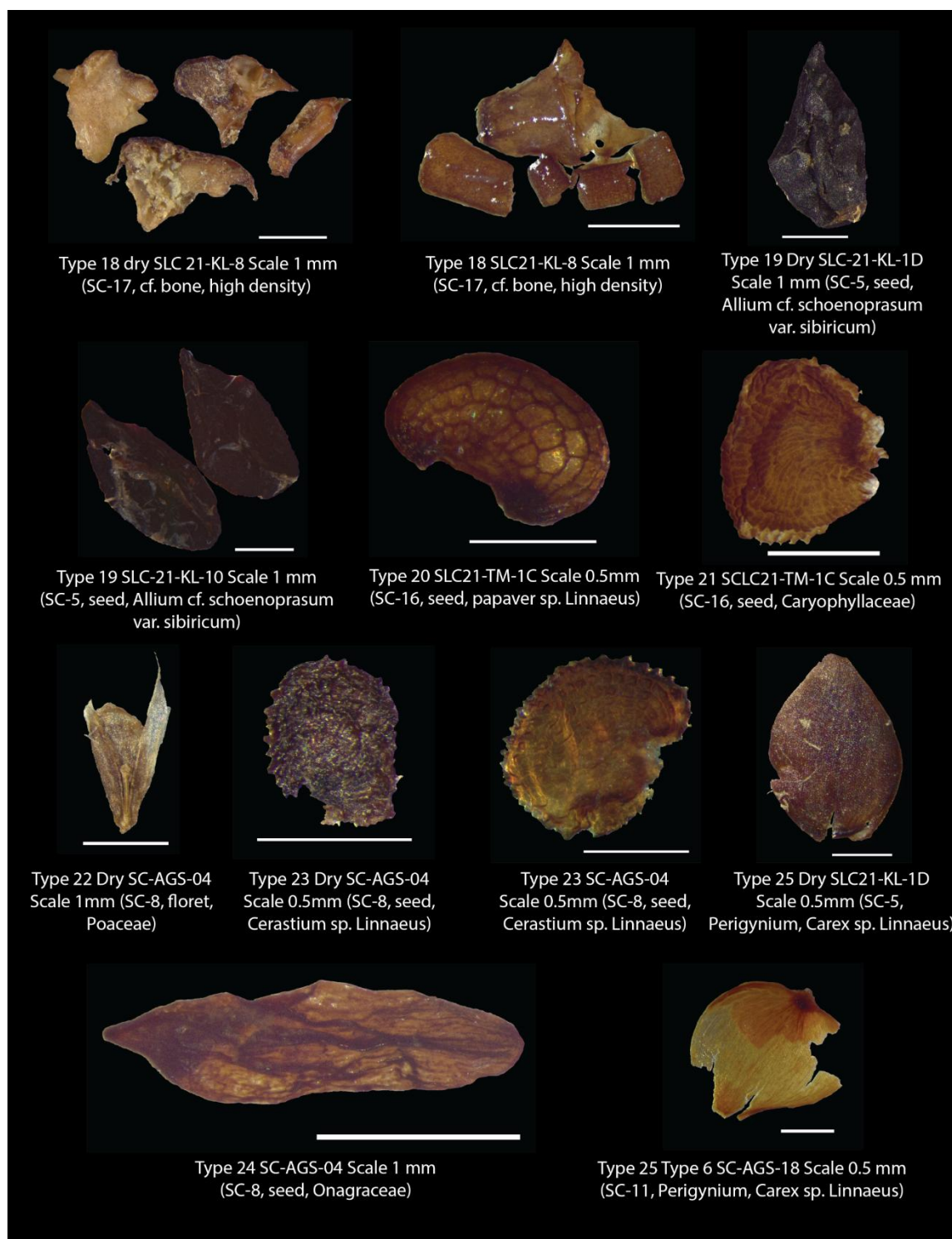


Fig. S20.

Macrofossil microscope photos, 3 of 9.

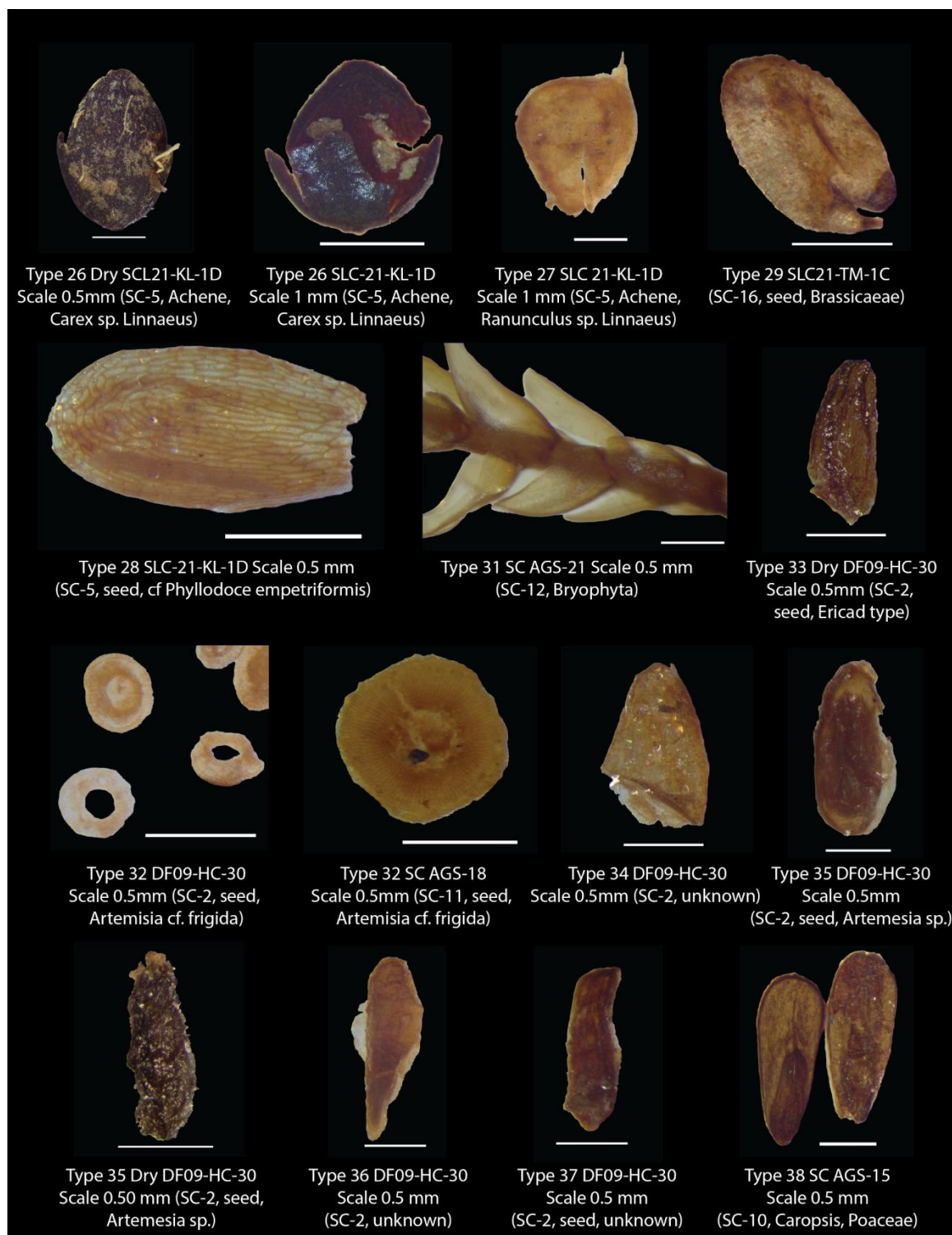


Fig. S21.

Macrofossil microscope photos, 4 of 9.

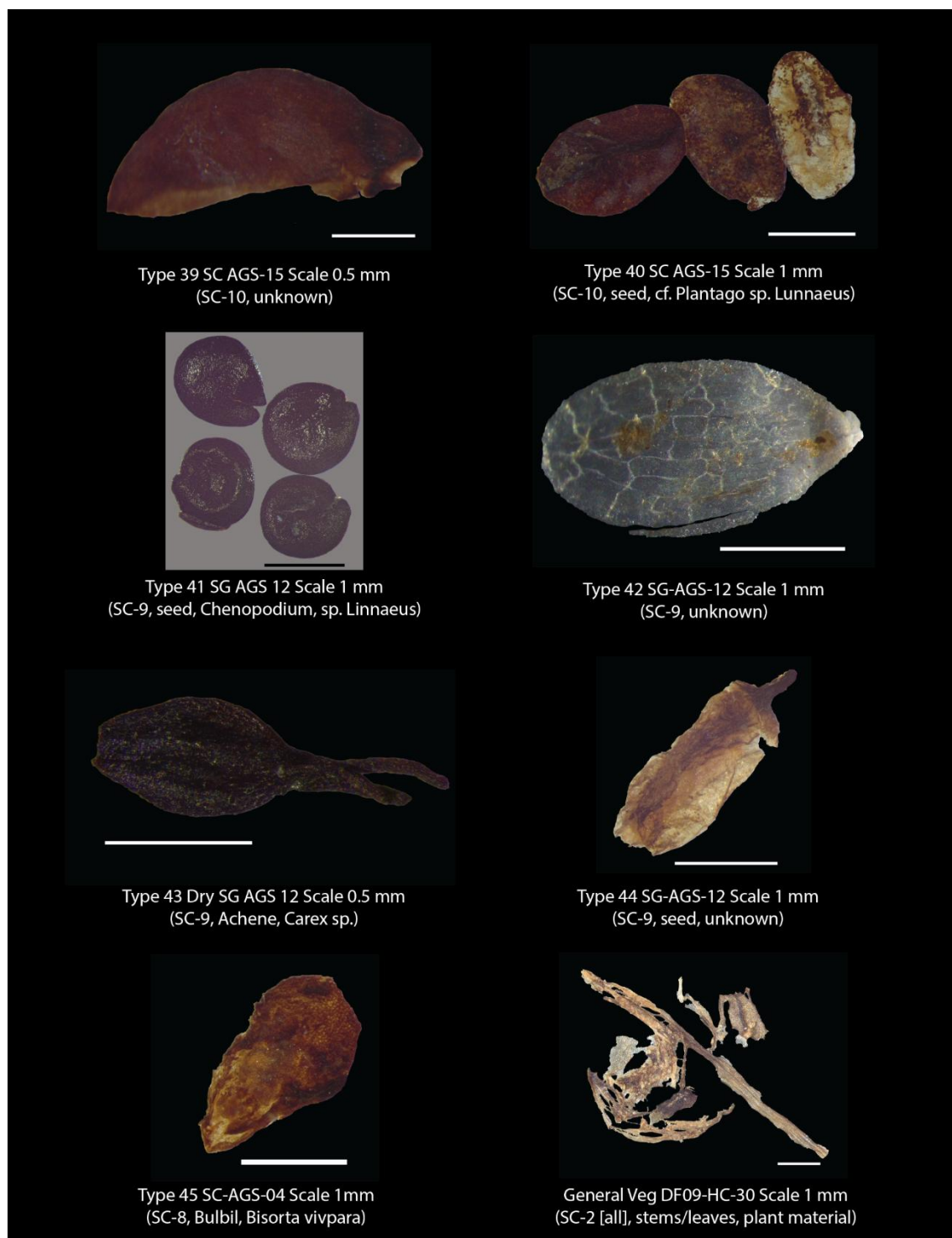


Fig. S22.
Macrofossil microscope photos, 5 of 9.

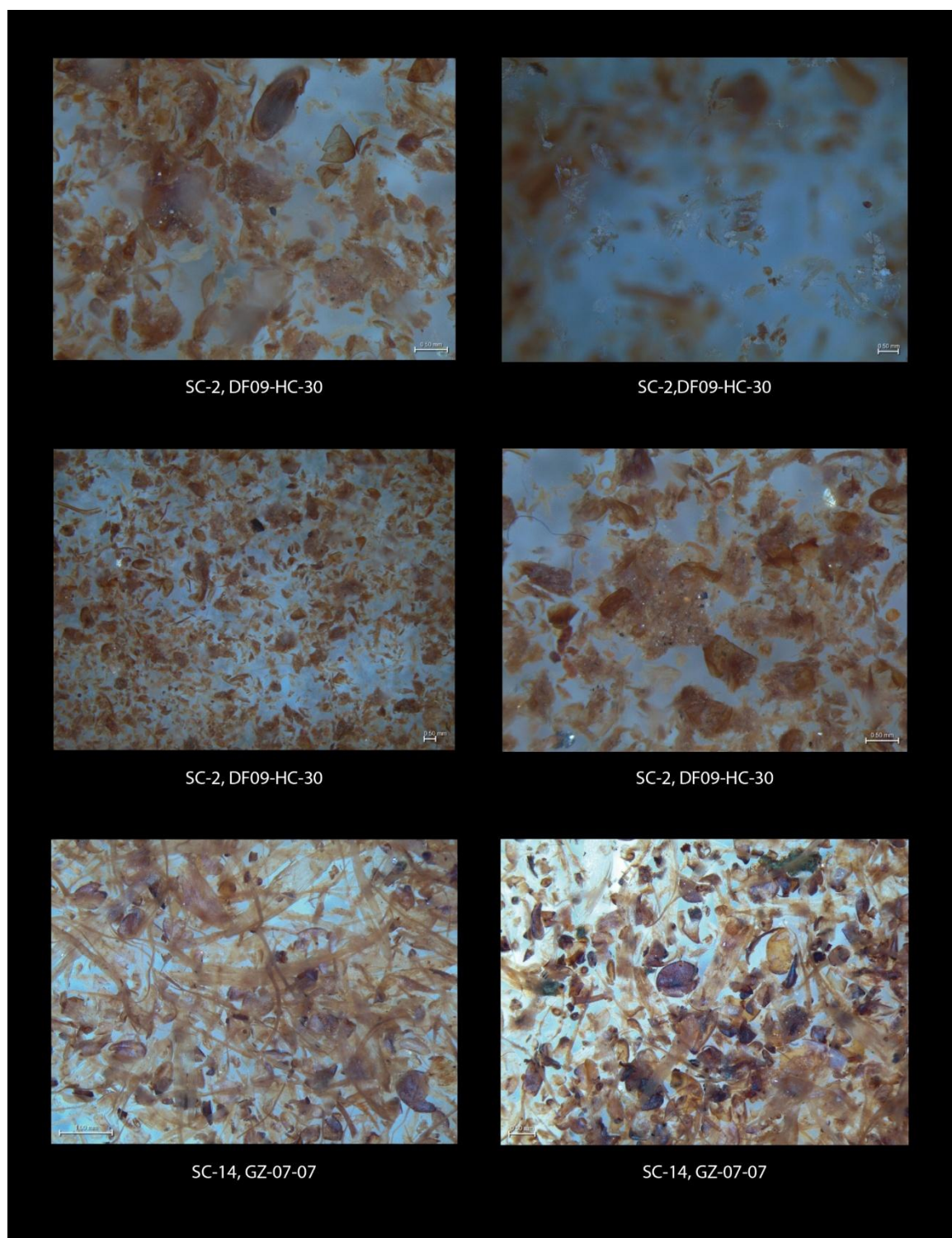


Fig. S23.
Macrofossil microscope photos, 6 of 9.

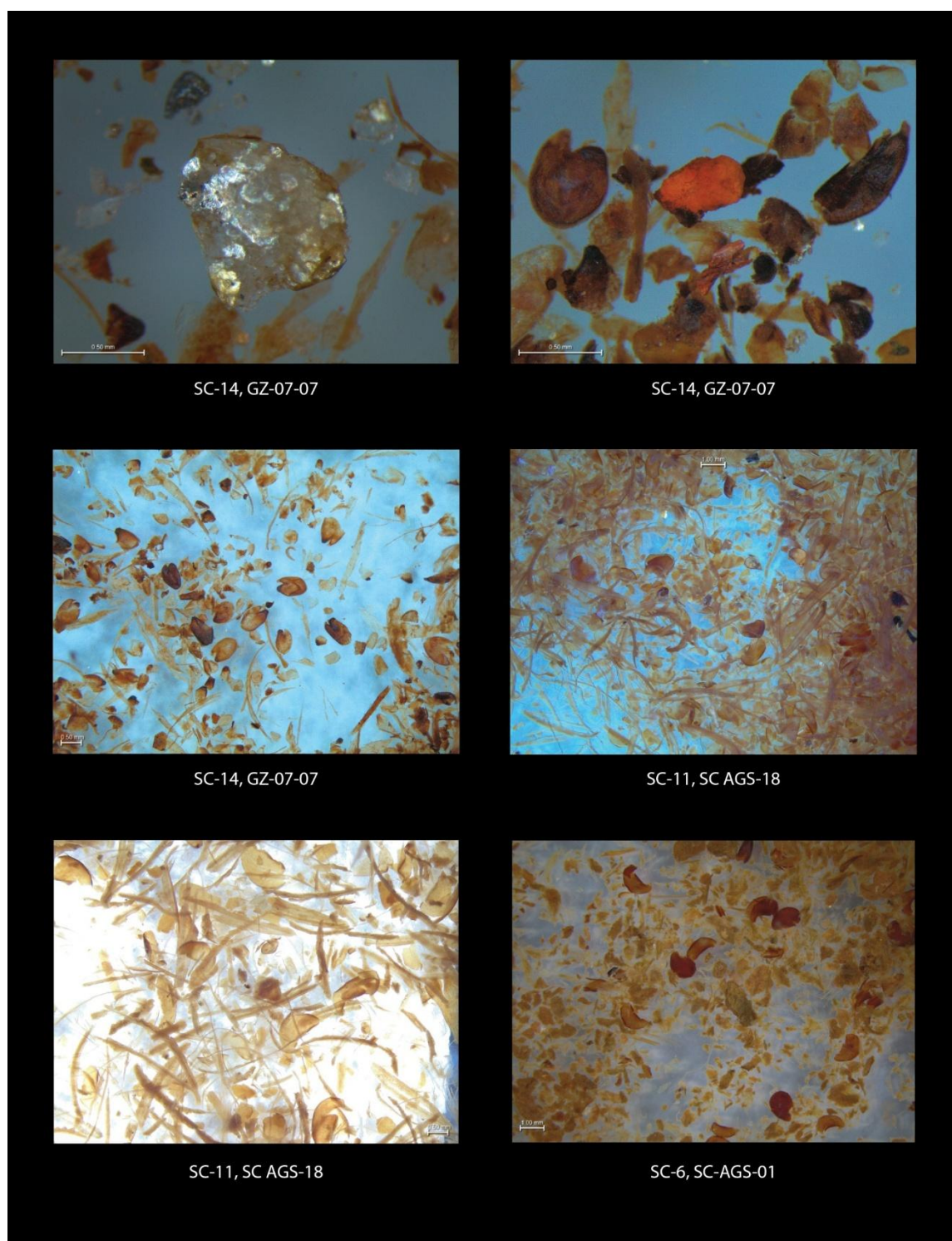


Fig. S24.
Macrofossil microscope photos, 7 of 9.

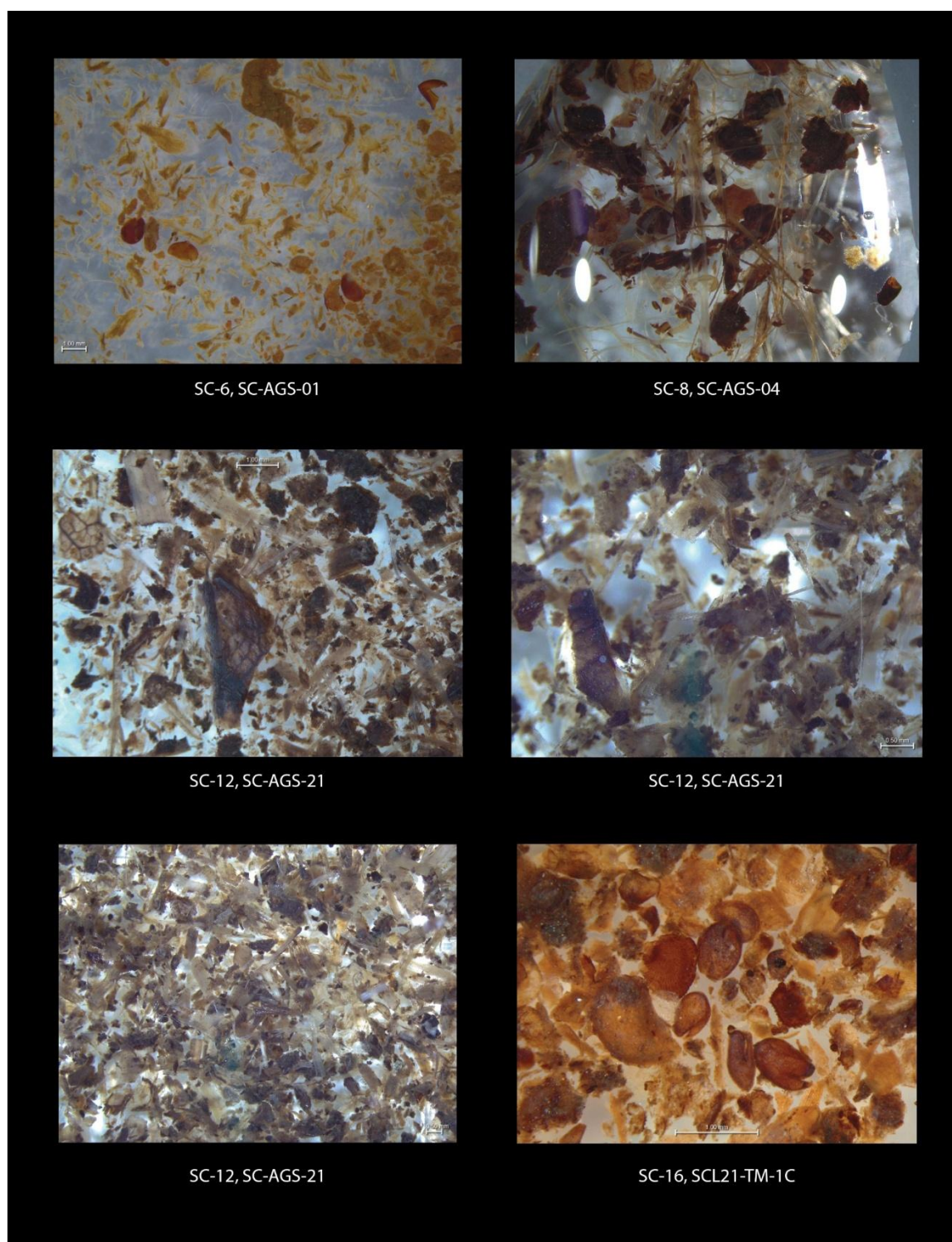


Fig. S25.
Macrofossil microscope photos, 8 of 9.

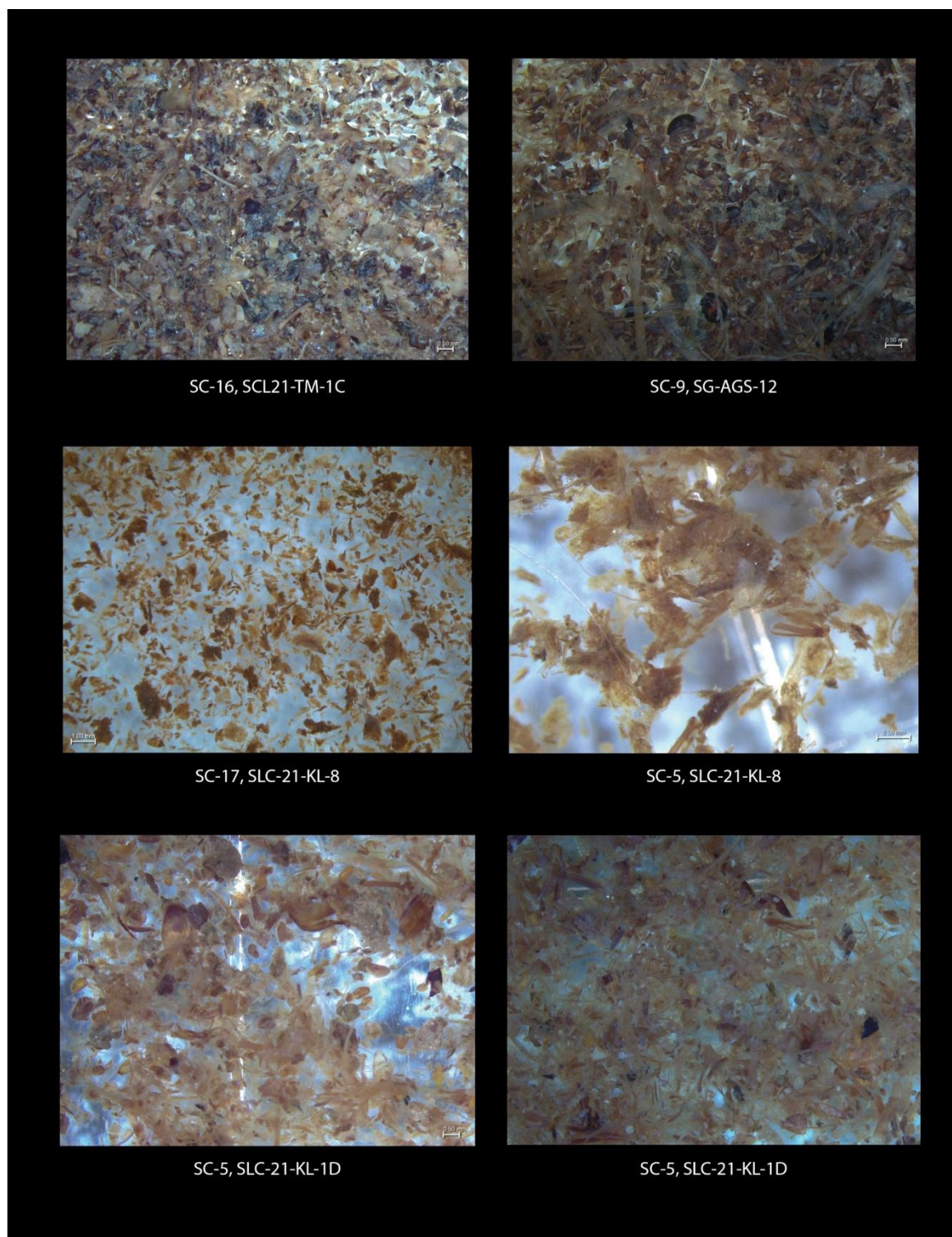


Fig. S26.
Macrofossil microscope photos, 9 of 9.

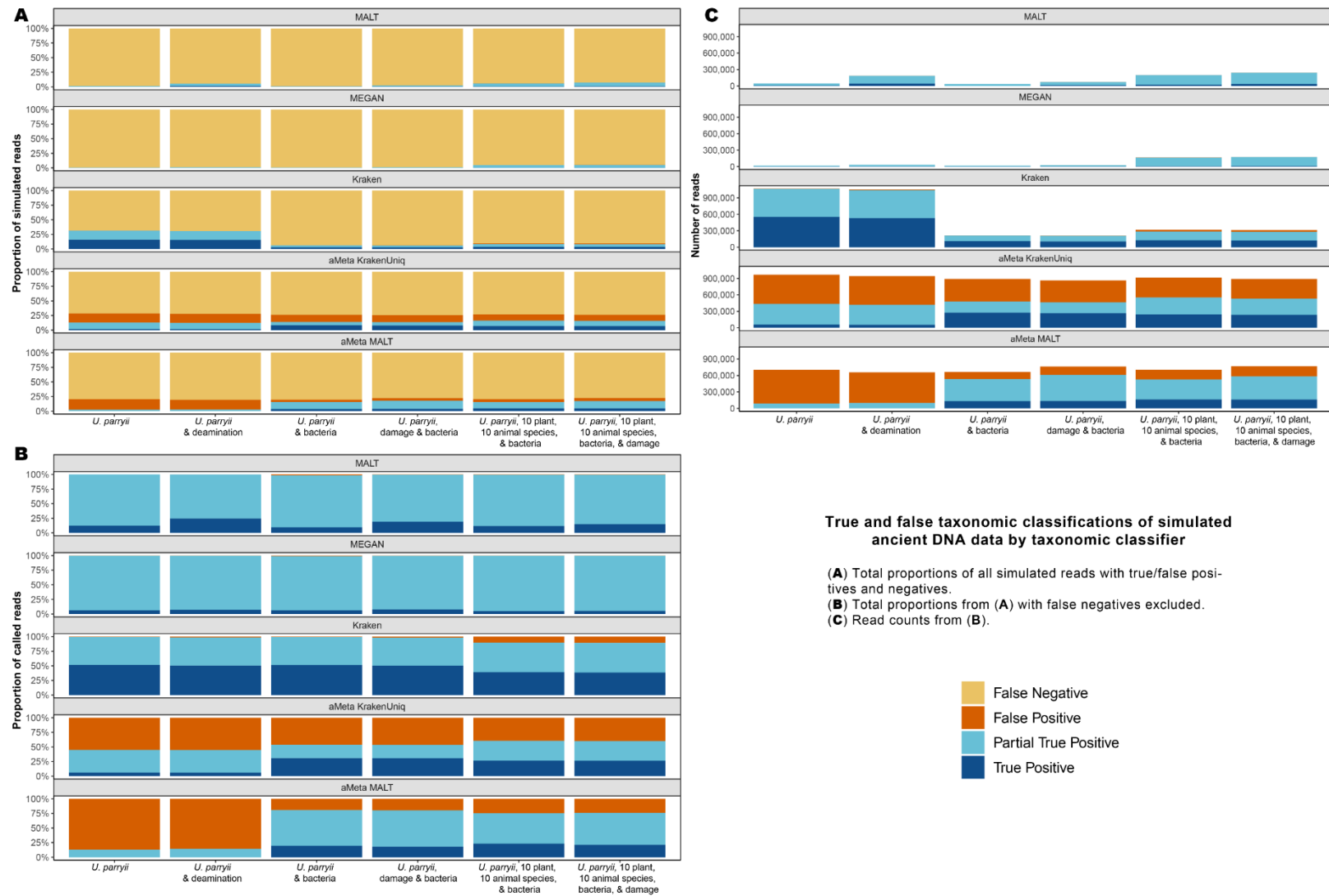


Fig. S27.

True and false taxonomic classifications of simulated ancient DNA data. (A) Total proportions by method including false negatives (unassigned reads). (B) Total proportions from A excluding false negatives. (C) Read counts of true, partial true, and false positives.

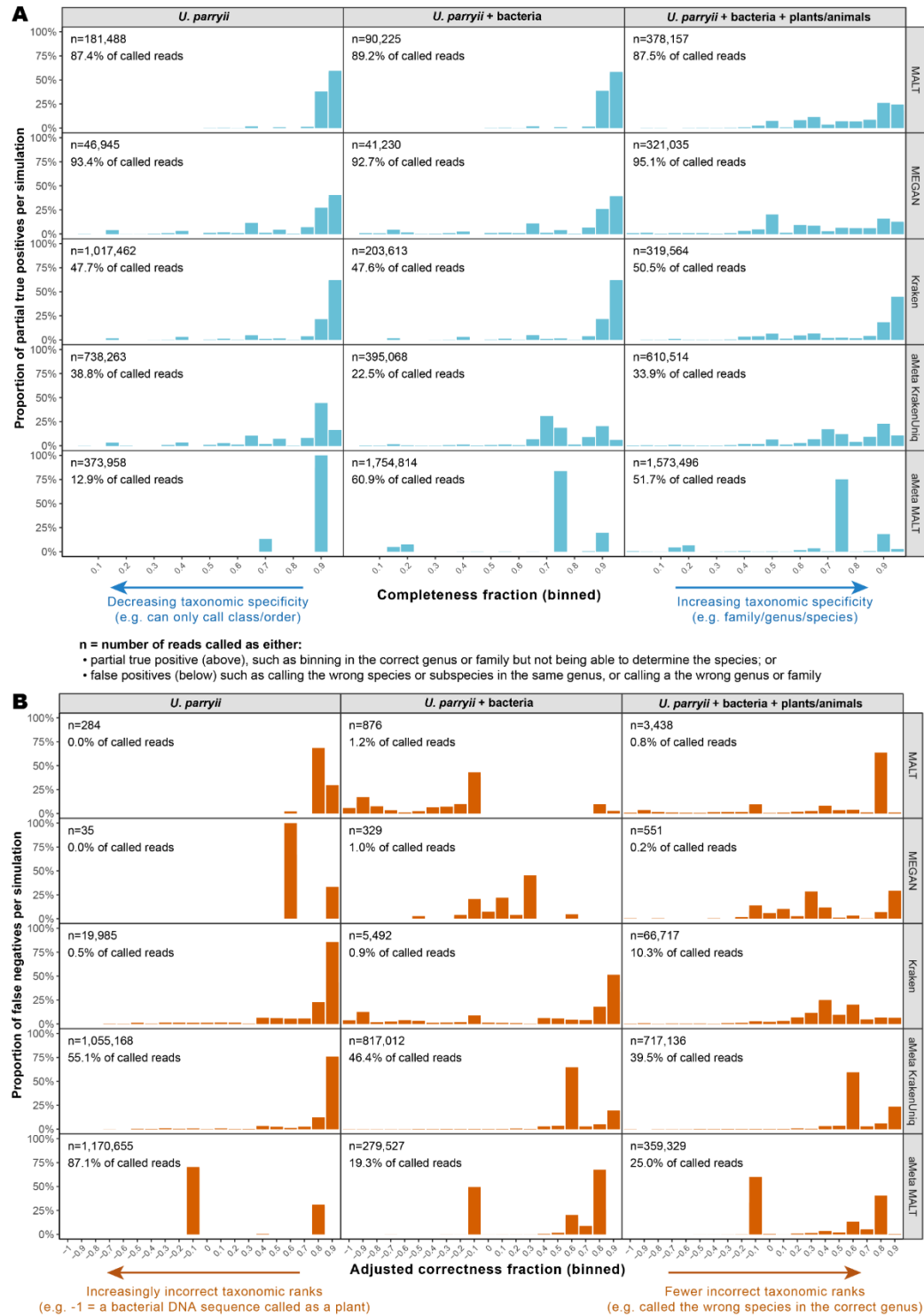


Fig. S28.

Completeness fraction of taxonomically binned reads using simulated data by classifier with (A) partial true positives for proportion of correct assignment, and (B) false positives for quantifying the degree of incorrect (-1) vs correct (1) correct/incorrect the assignment was.

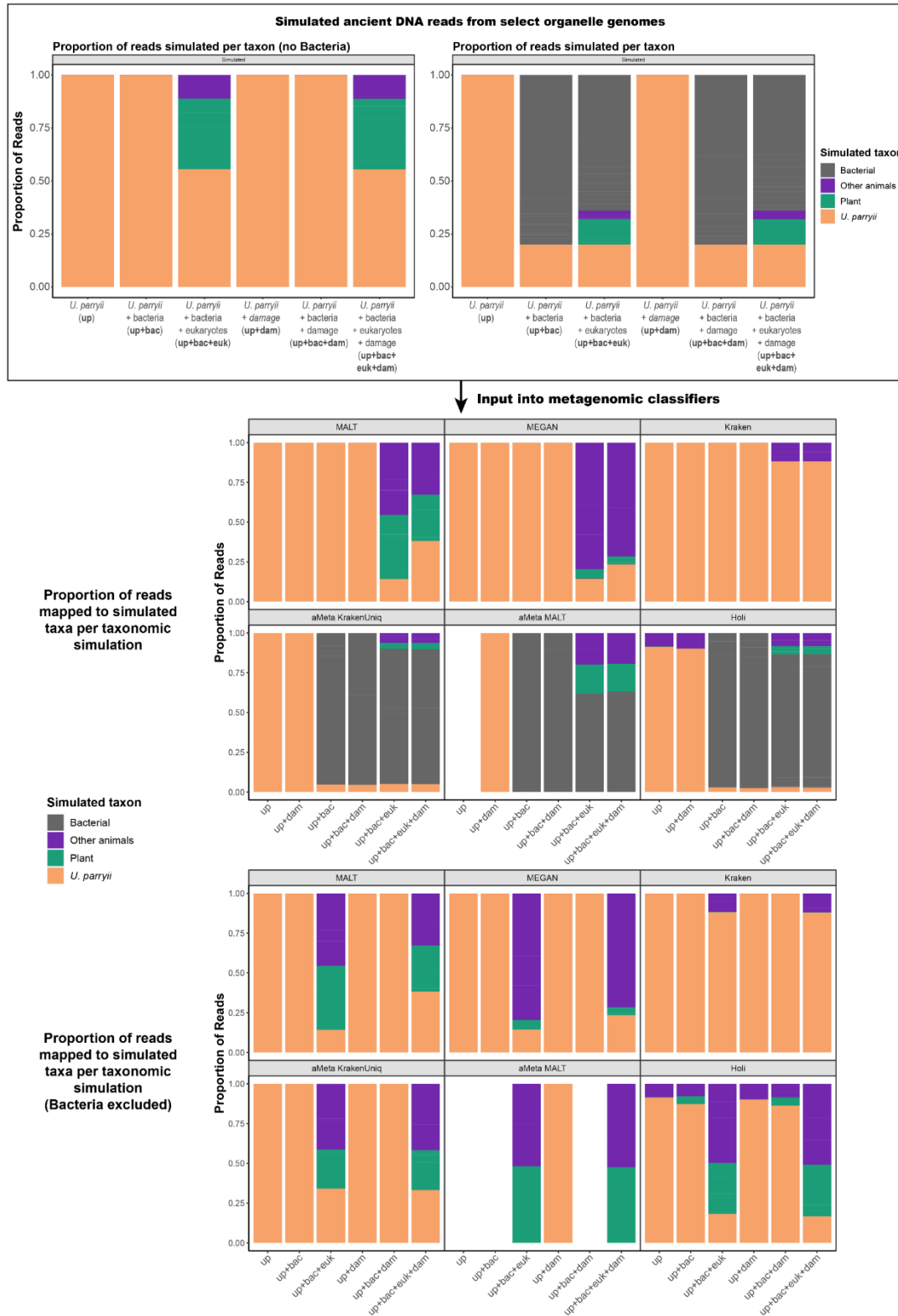


Fig. S29.
Proportion of mapped reads by simulation type/method.

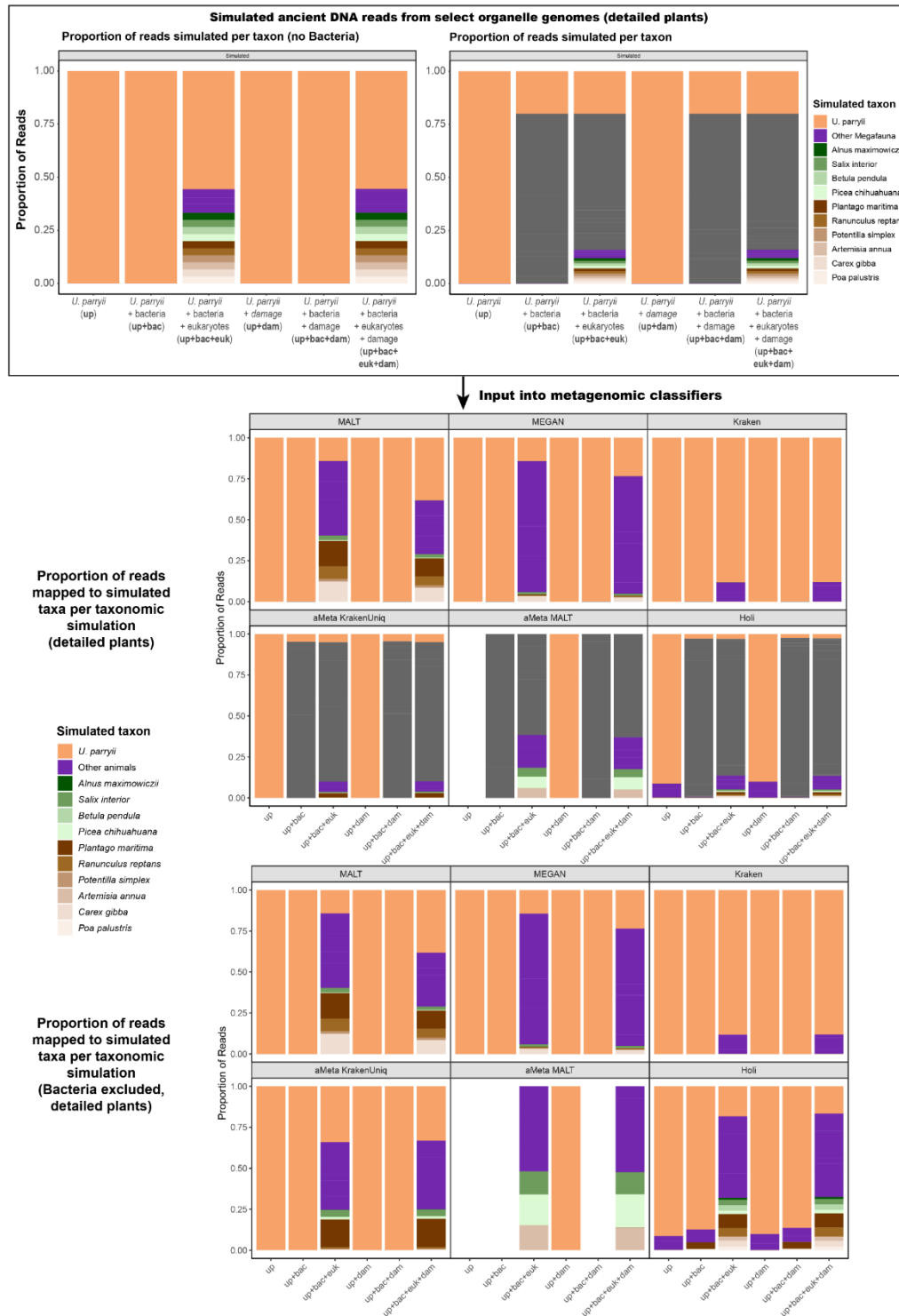


Fig. S30.

Proportion of mapped reads by simulation type/method with an emphasis on plants.

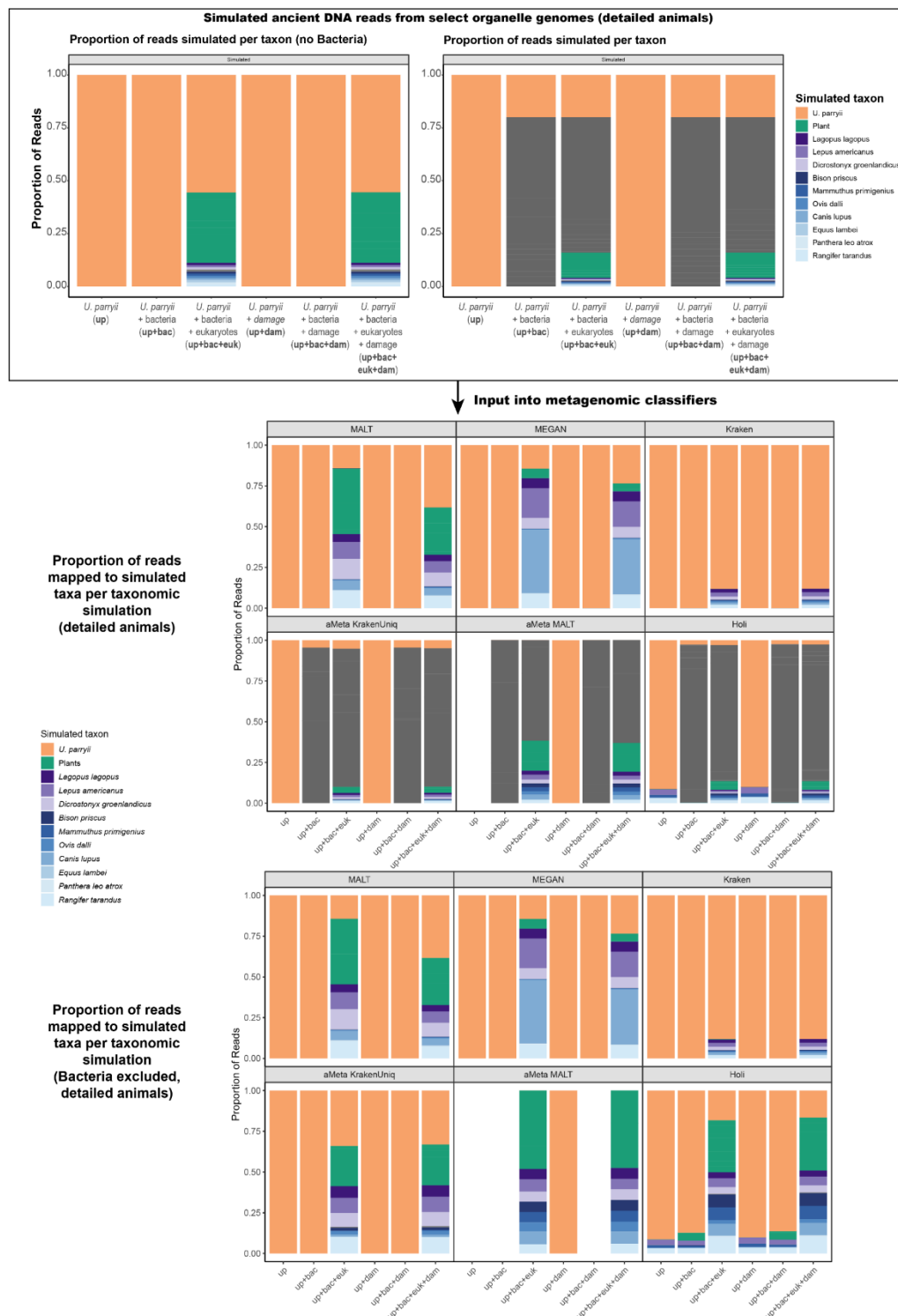


Fig. S31.

Proportion of mapped reads by simulation type/method with an emphasis on animals.

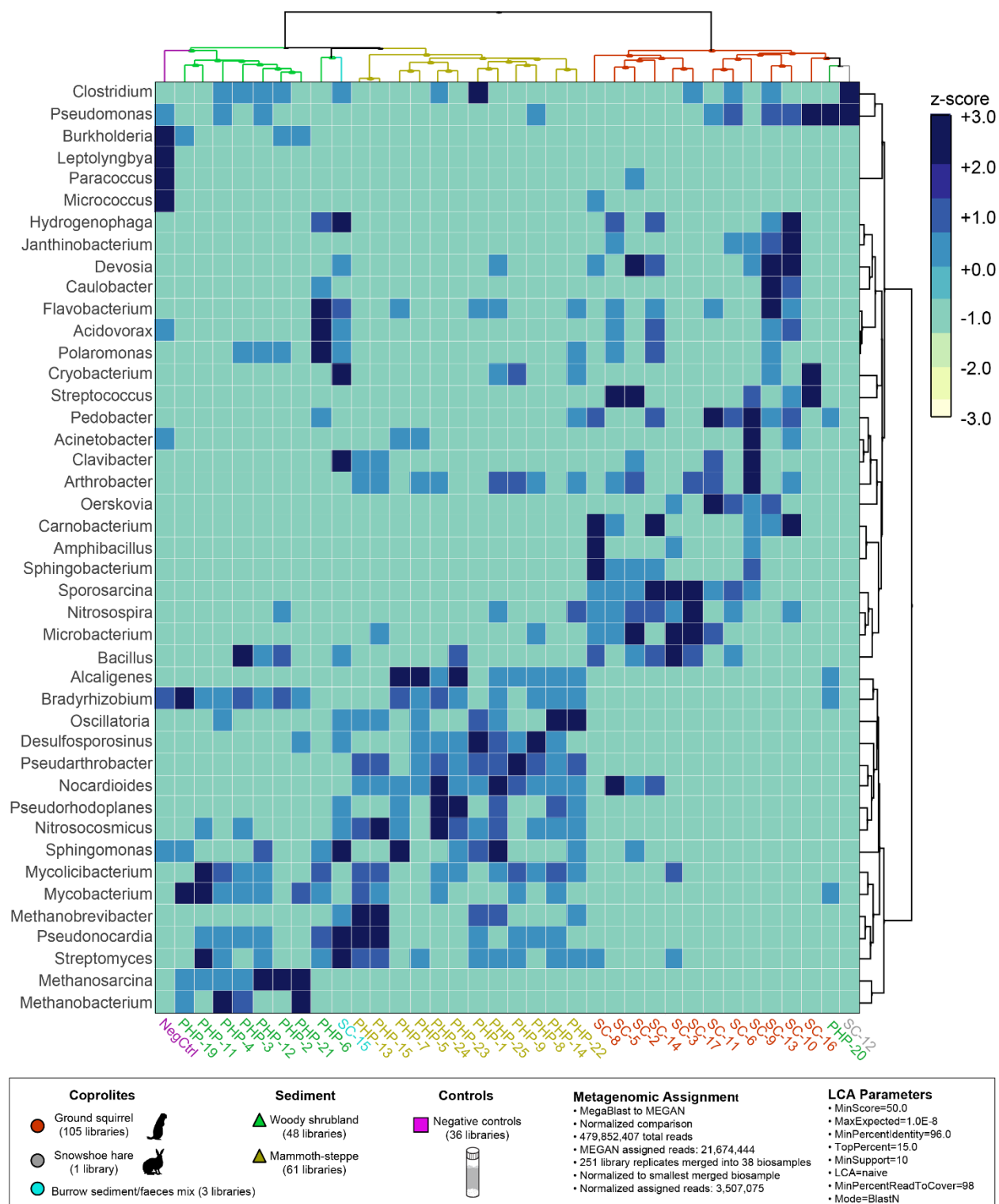


Fig. S32.

Metagenomic classifications of most abundant bacterial genera identified with *MegaBlast* when comparing sediments and coprolites from the Klondike. Taxa and samples hierarchically clustered and samples are coloured based on type.

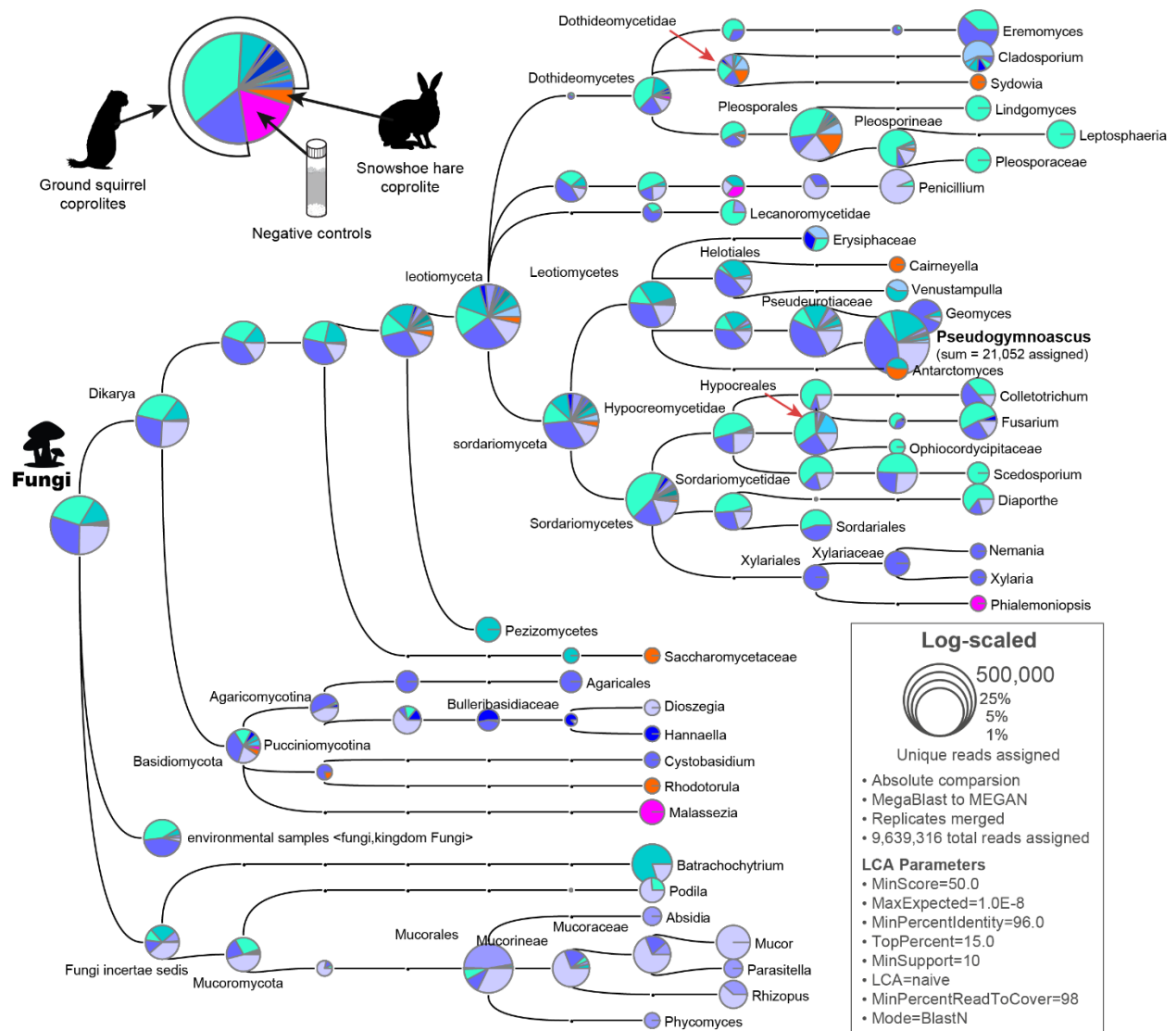


Fig. S33.
Metagenomic classifications of non-target fungi identified with *MegaBlast*.

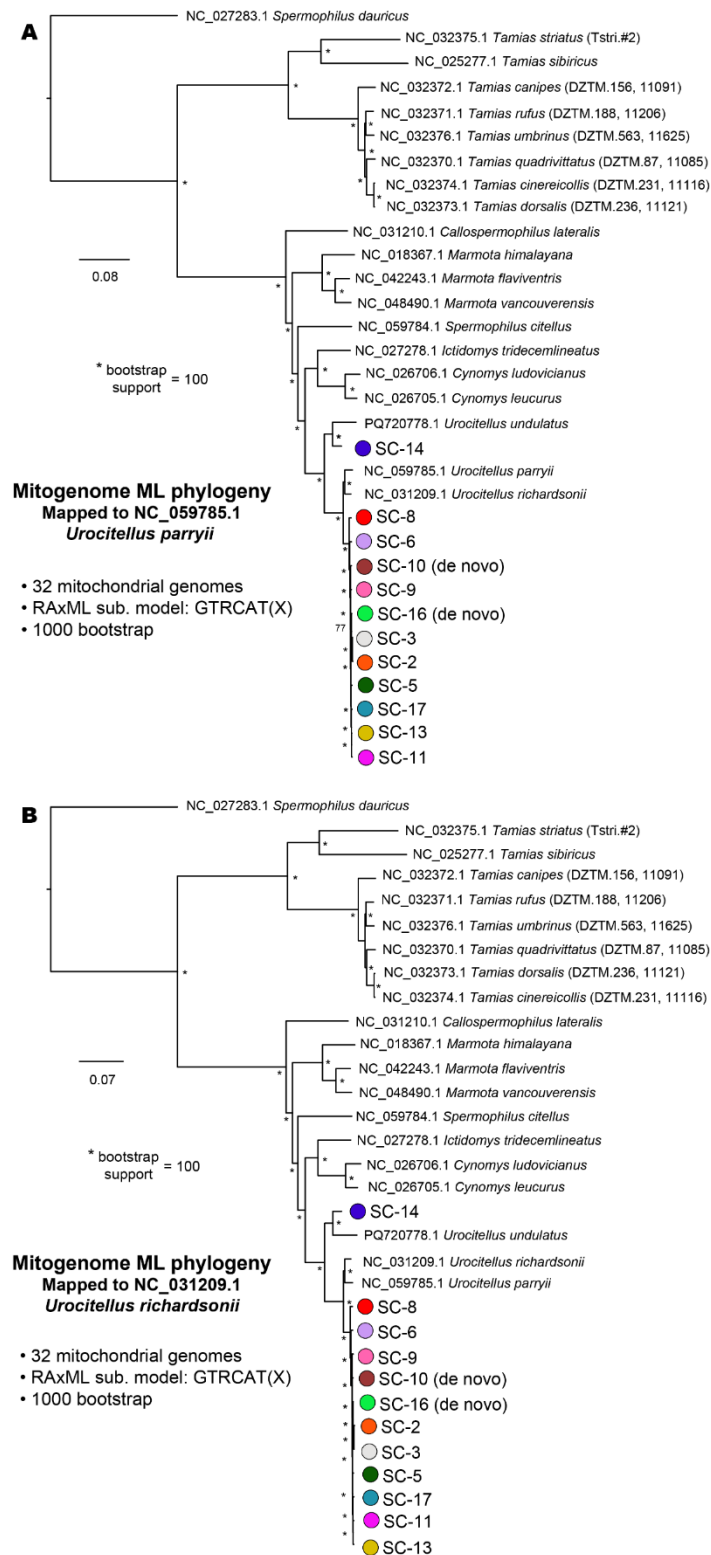


Fig. S34.

Maximum likelihood phylogenies of *Urocitellus* using mitochondrial genomes of all available Marmotini taxa. (A) Coprolite mitogenomes assembled via reference mapping to *U. parryii*, and (B) *U. richardsonii*.

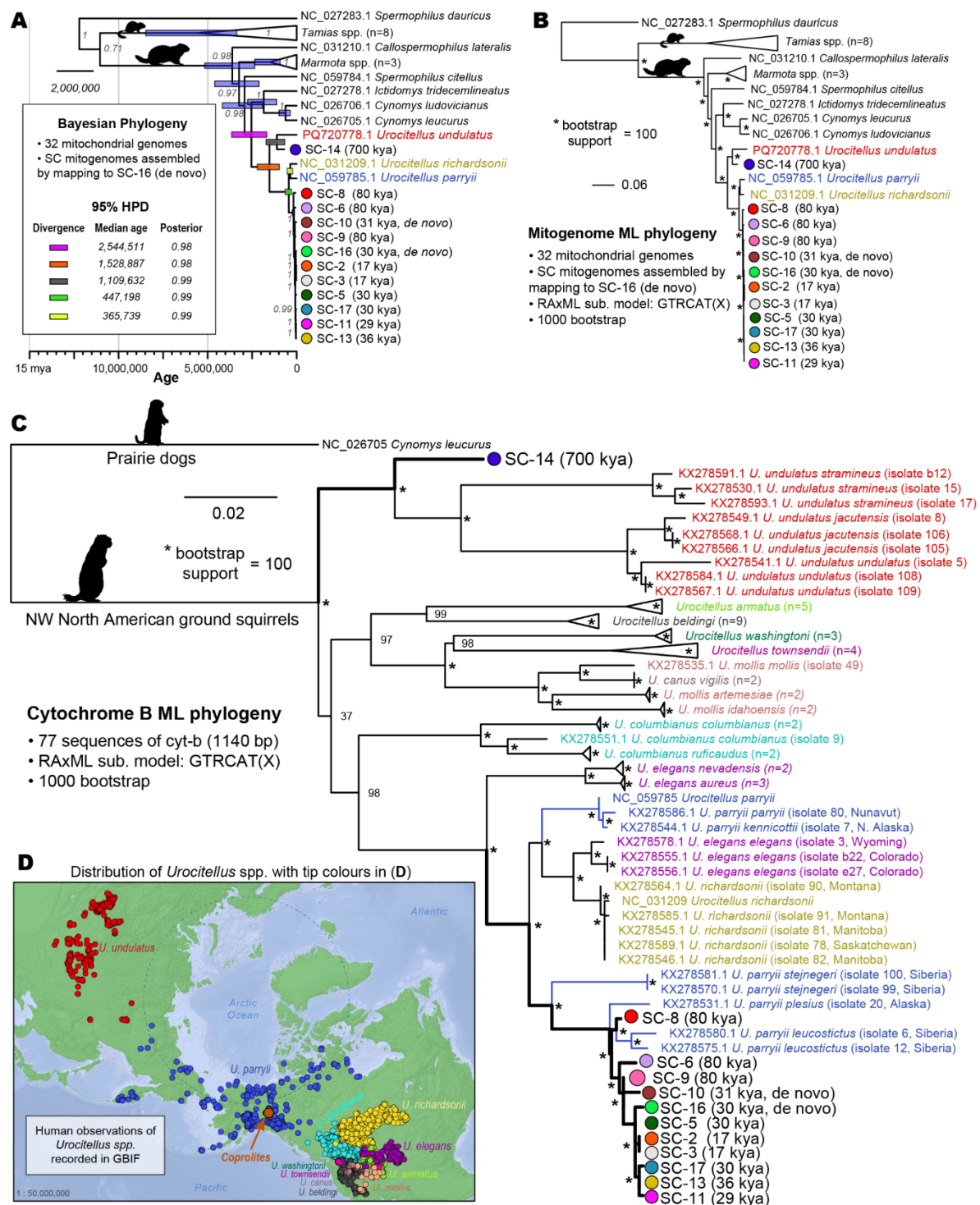


Fig. S35.

Phylogenies of *Urocitellus* using (A) Bayesian and (B) maximum likelihood methods with all available mitochondrial genomes of Marmotini. (C) Maximum likelihood phylogeny of *Urocitellus* using publicly available sequences for cytochrome b. (D) Distribution of *Urocitellus* spp. recorded in GBIF represented as coloured dots that correspond with the tip colours in panel C.

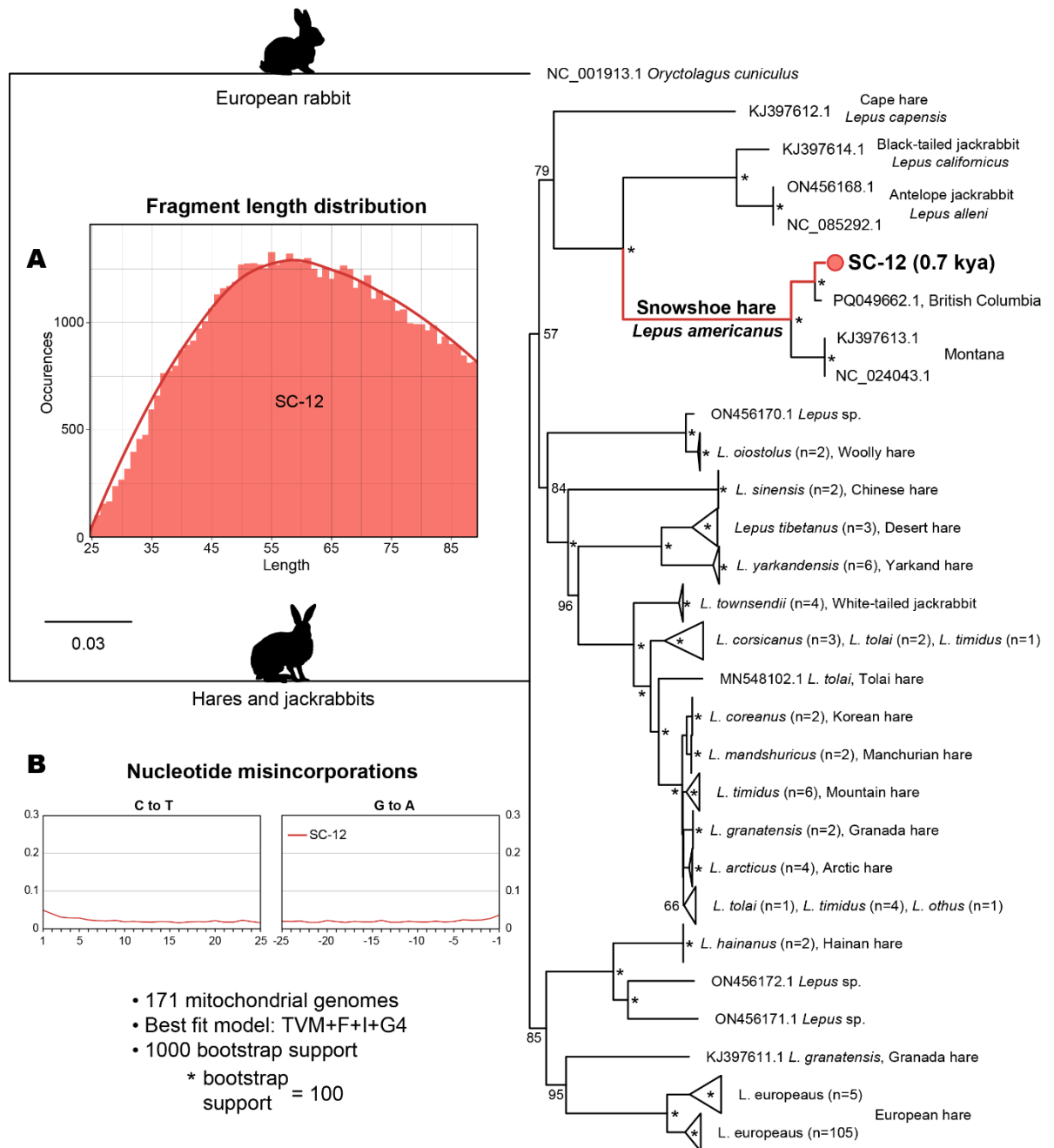


Fig. S36.
Maximum likelihood phylogenies of *Lepus*.

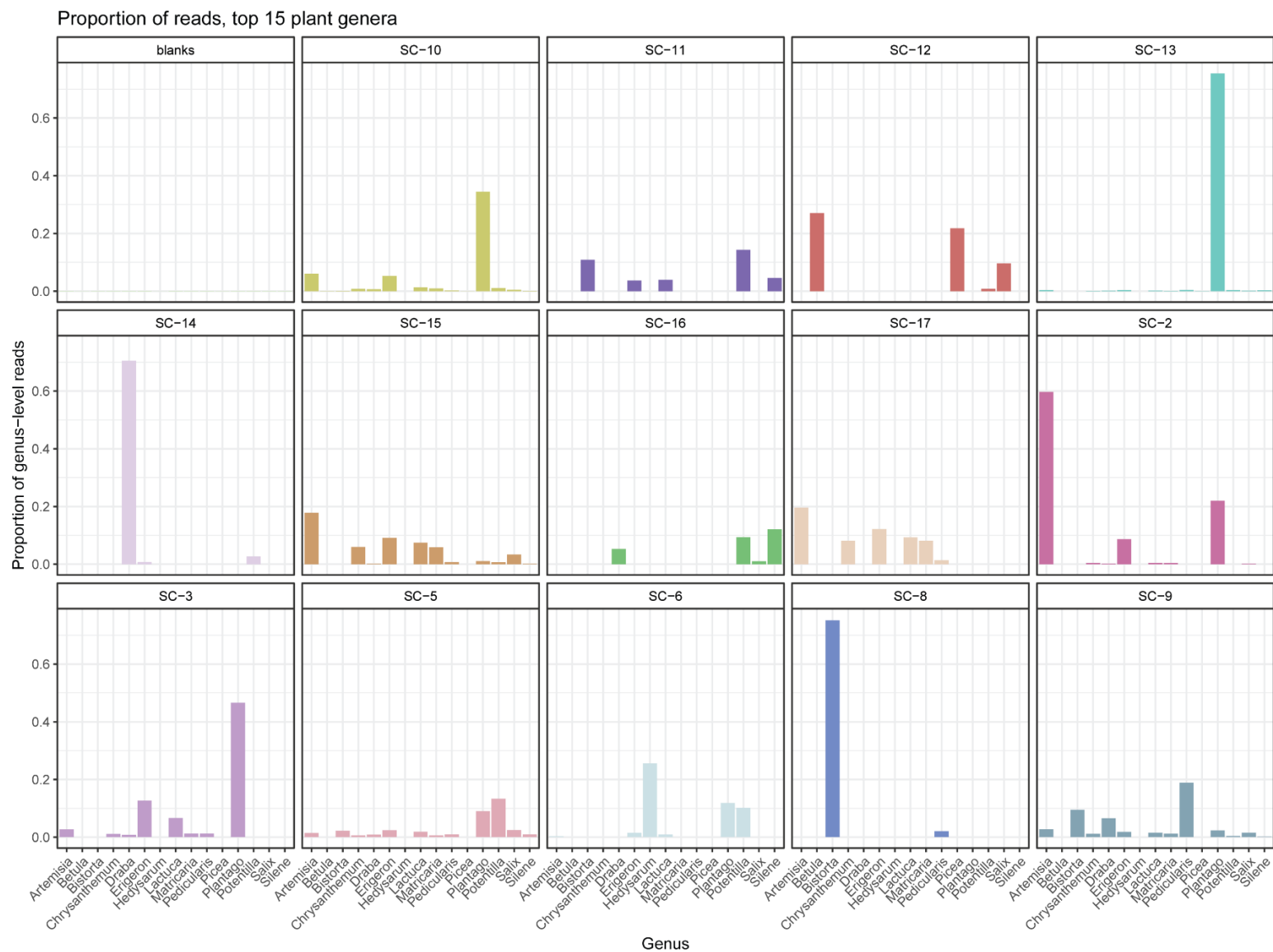


Fig. S37.
Summary of top plant genera with metaDMG values above significance and damage thresholds.

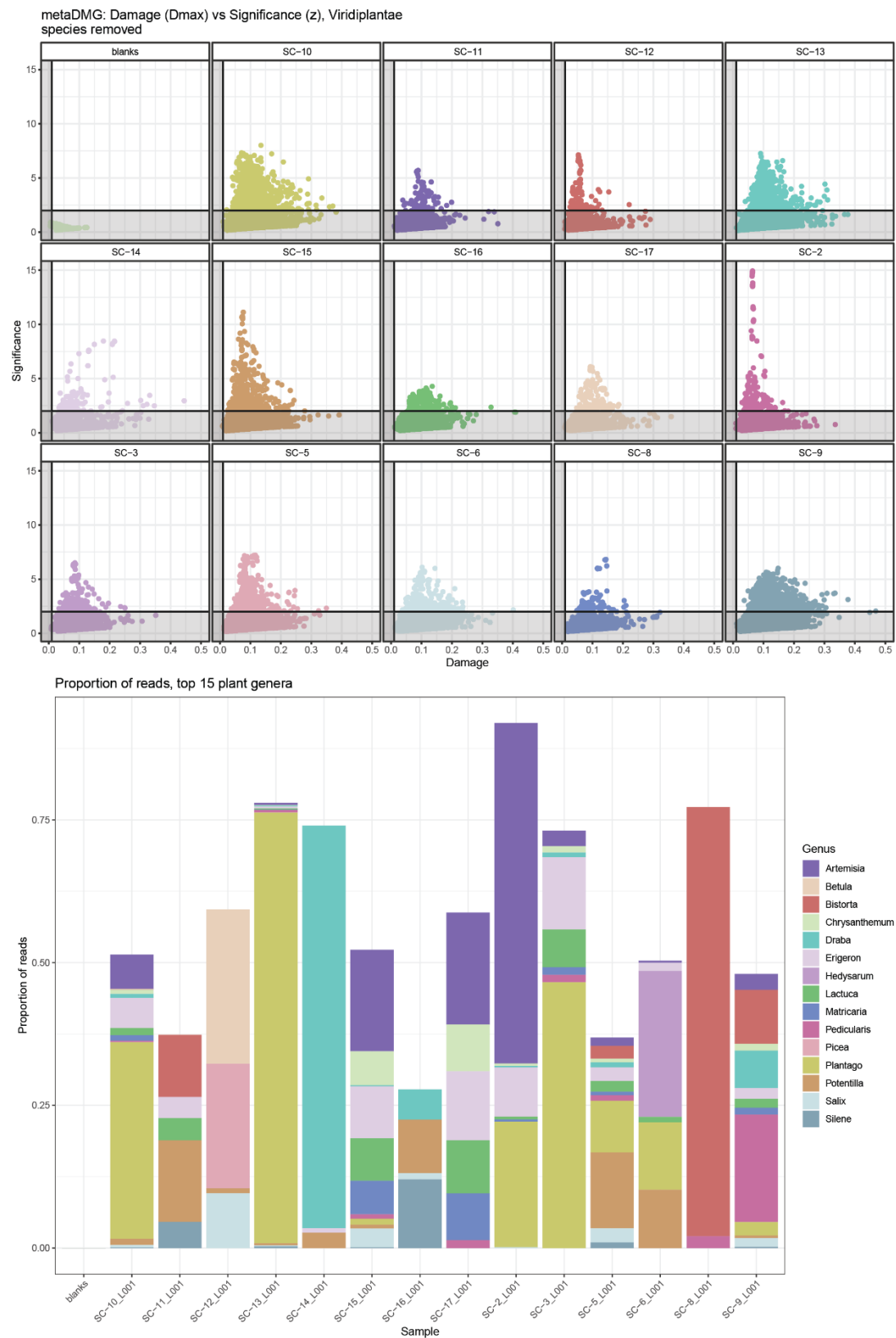


Fig. S38.
Summary of plant metaDMG plots and top taxa in a stacked bar chart.

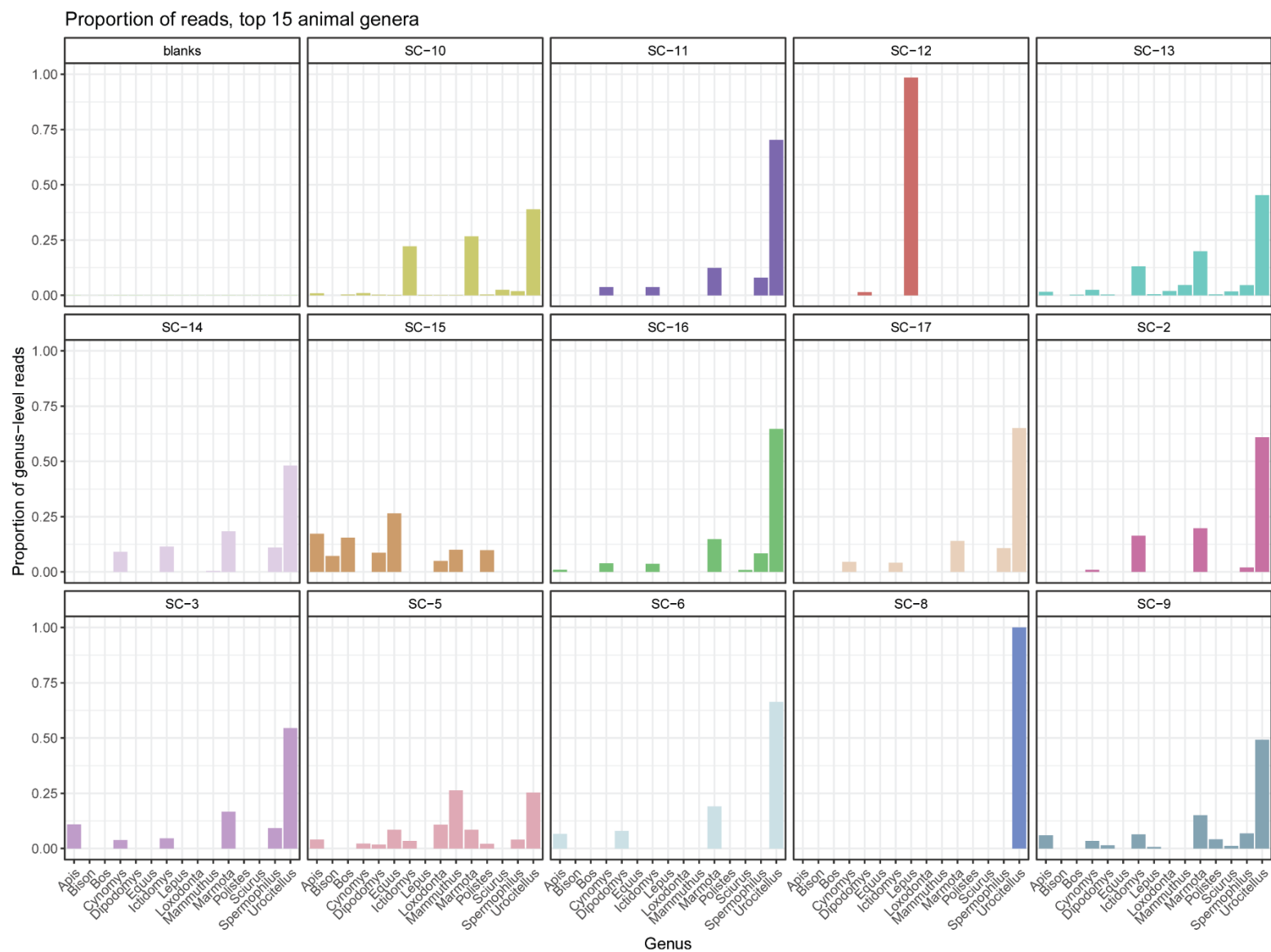


Fig. S39.
Summary of top animal genera with *metaDMG* values above significance and damage thresholds.

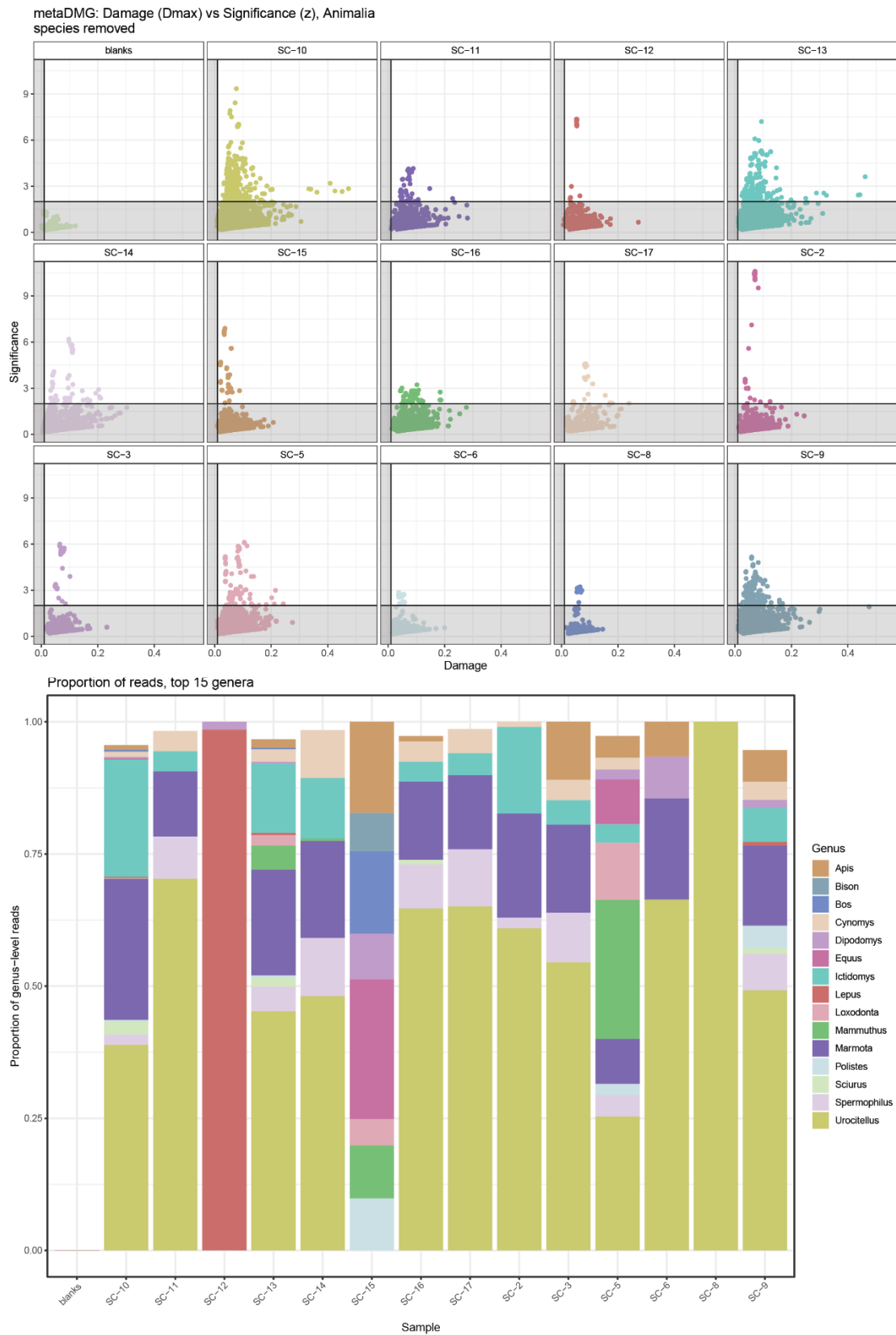


Fig. S40.
Summary of animal *metaDMG* plots and top taxa in a stacked bar chart.

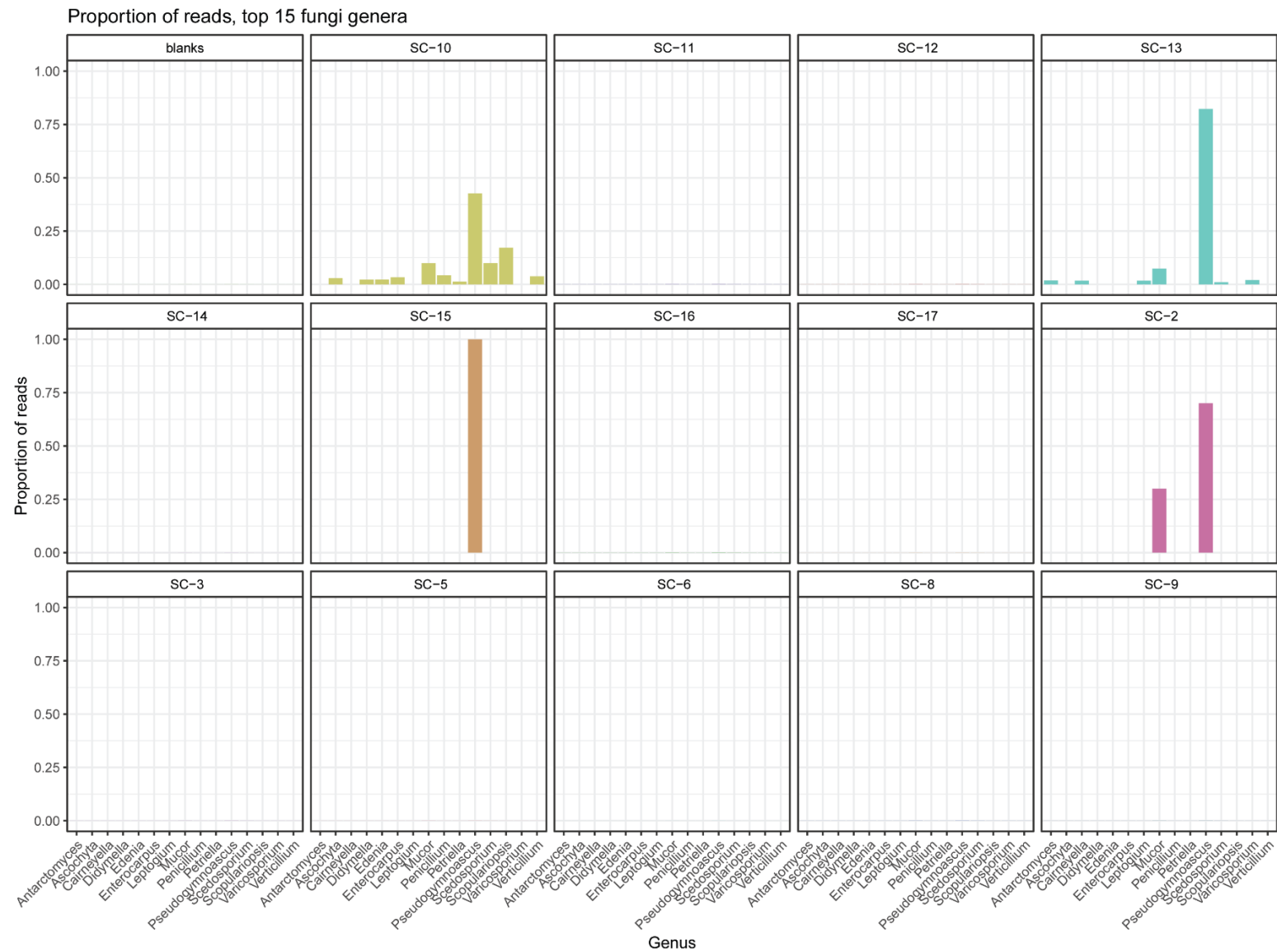


Fig. S41.
Summary of top fungi genera with *metaDMG* values above significance and damage thresholds.

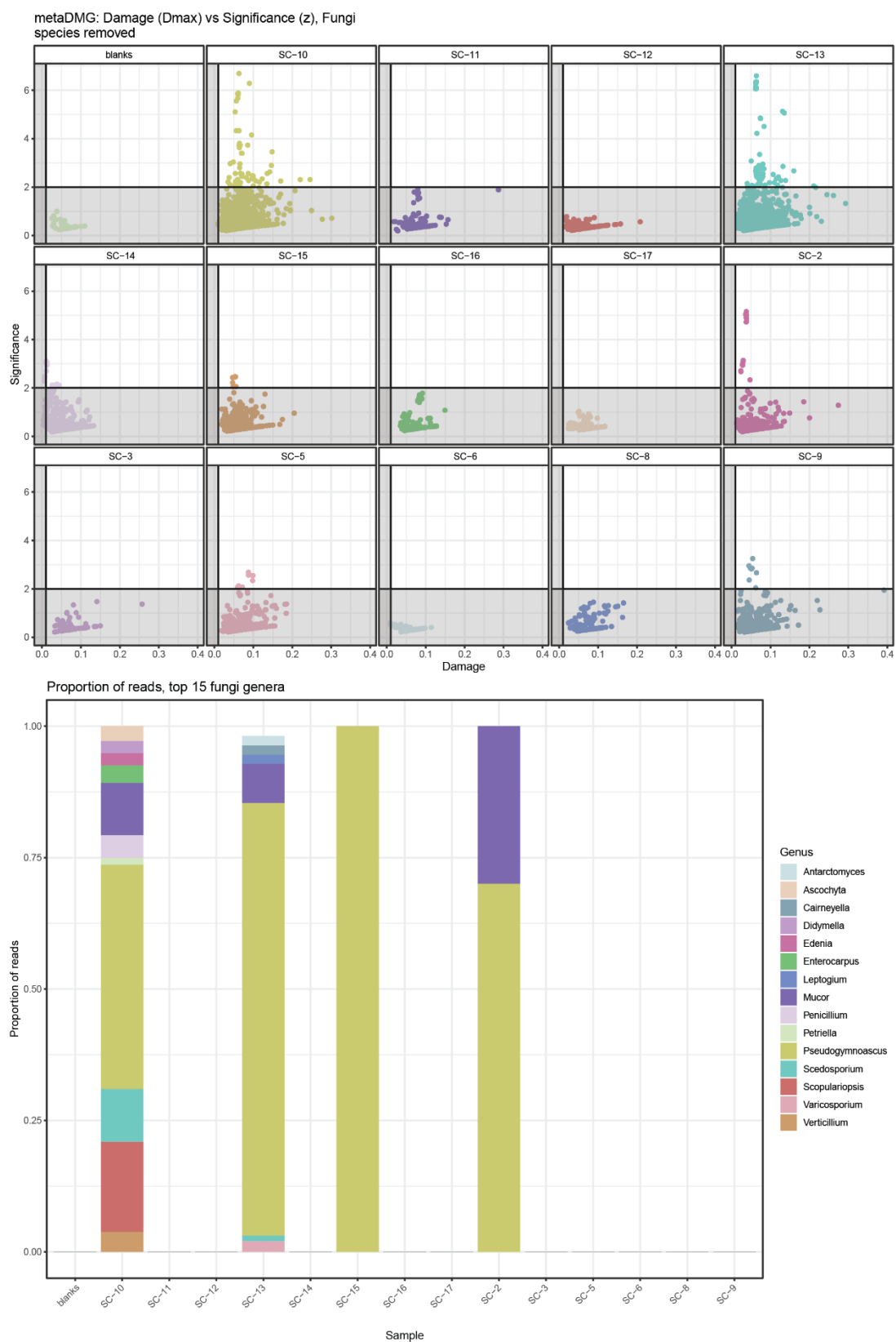


Fig. S42.
Summary of fungi *metaDMG* plots and top taxa in a stacked bar chart.

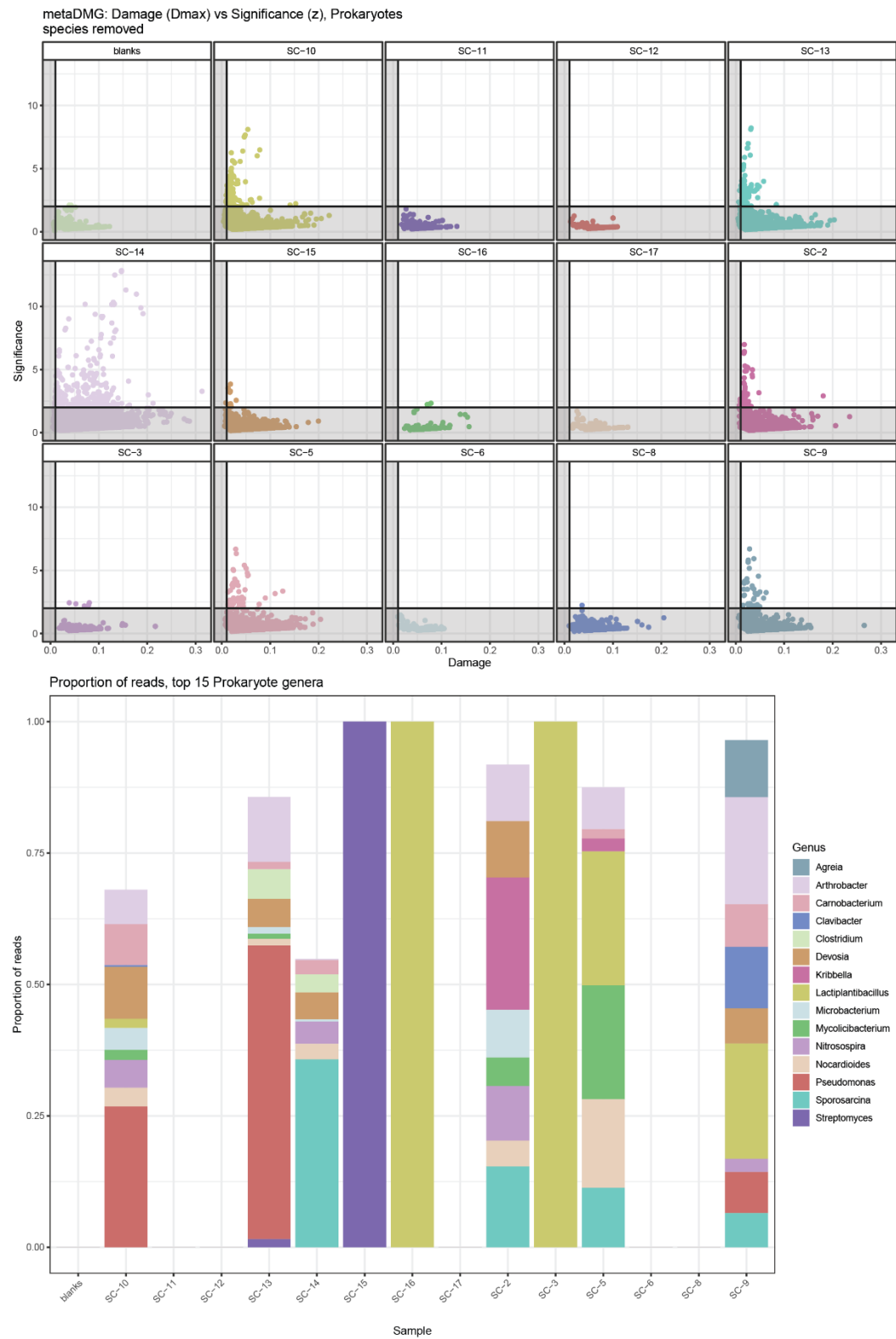


Fig. S44.
Summary of prokaryote *metaDMG* plots and top taxa in a stacked bar chart.

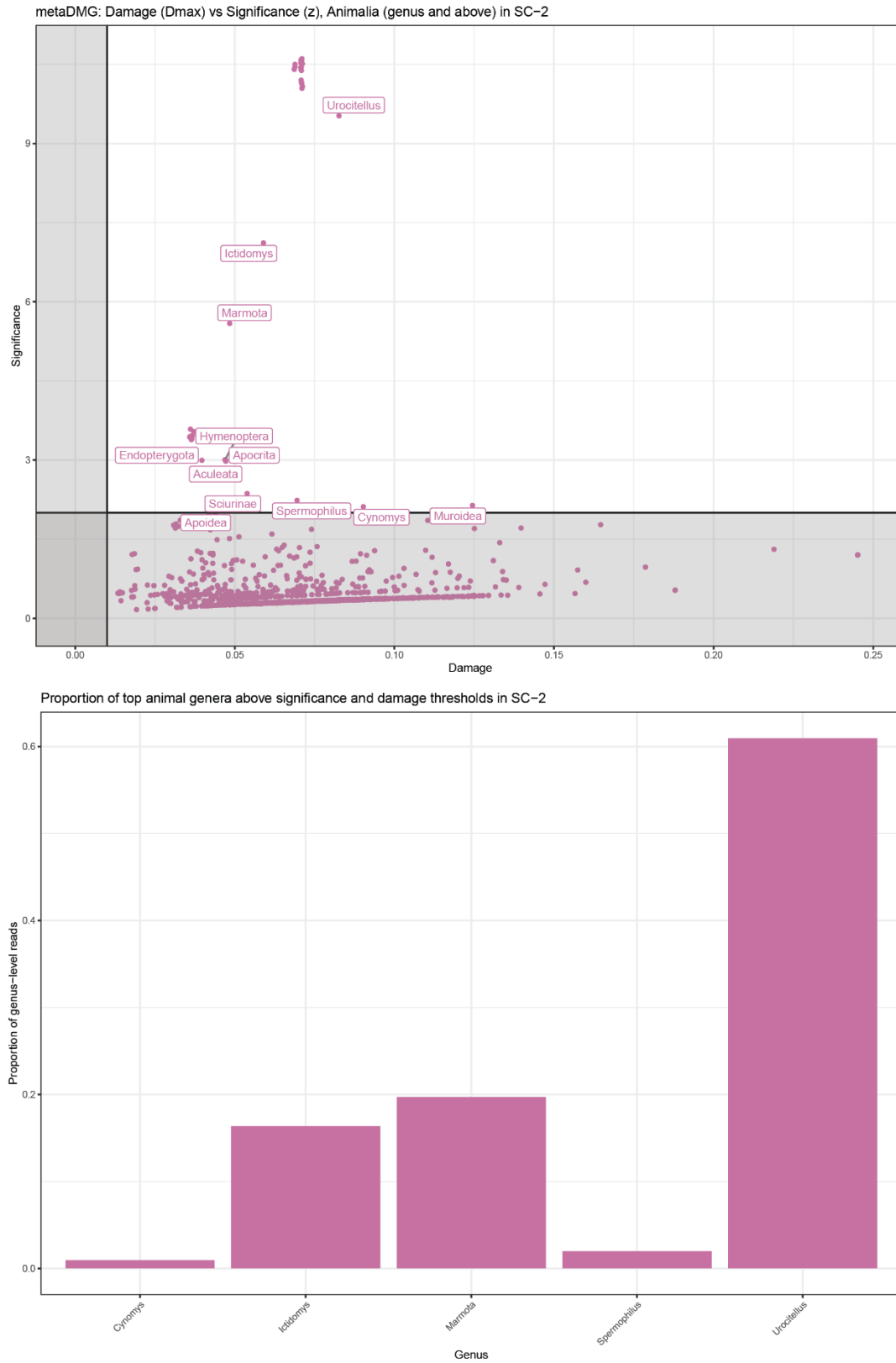


Fig. S46.
SC-2 *metaDMG* plot of animal taxa above significance and damage thresholds with top genera.

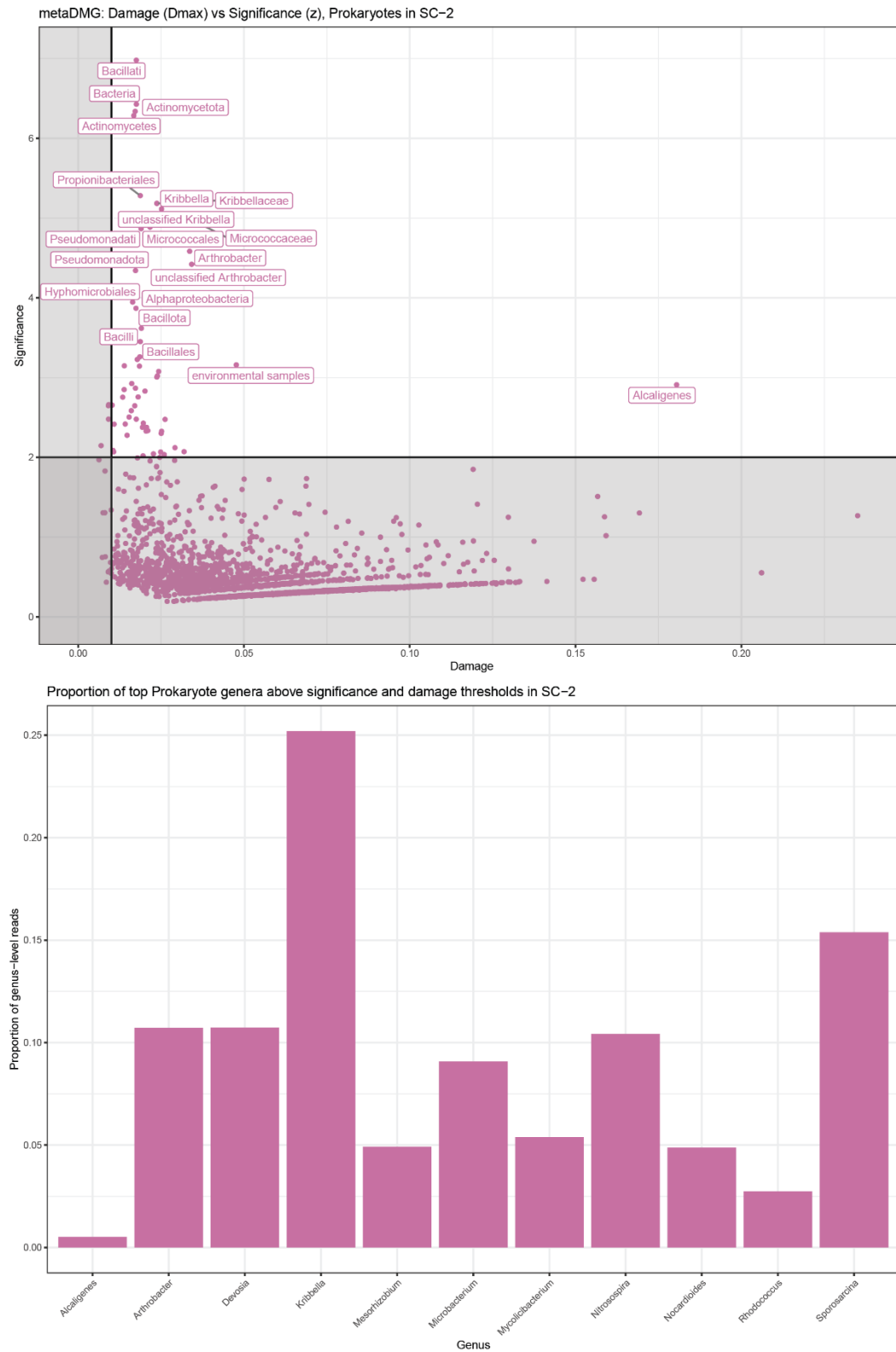


Fig. S47.
SC-2 *metaDMG* plot of prokaryote taxa above significance and damage thresholds with top genera.

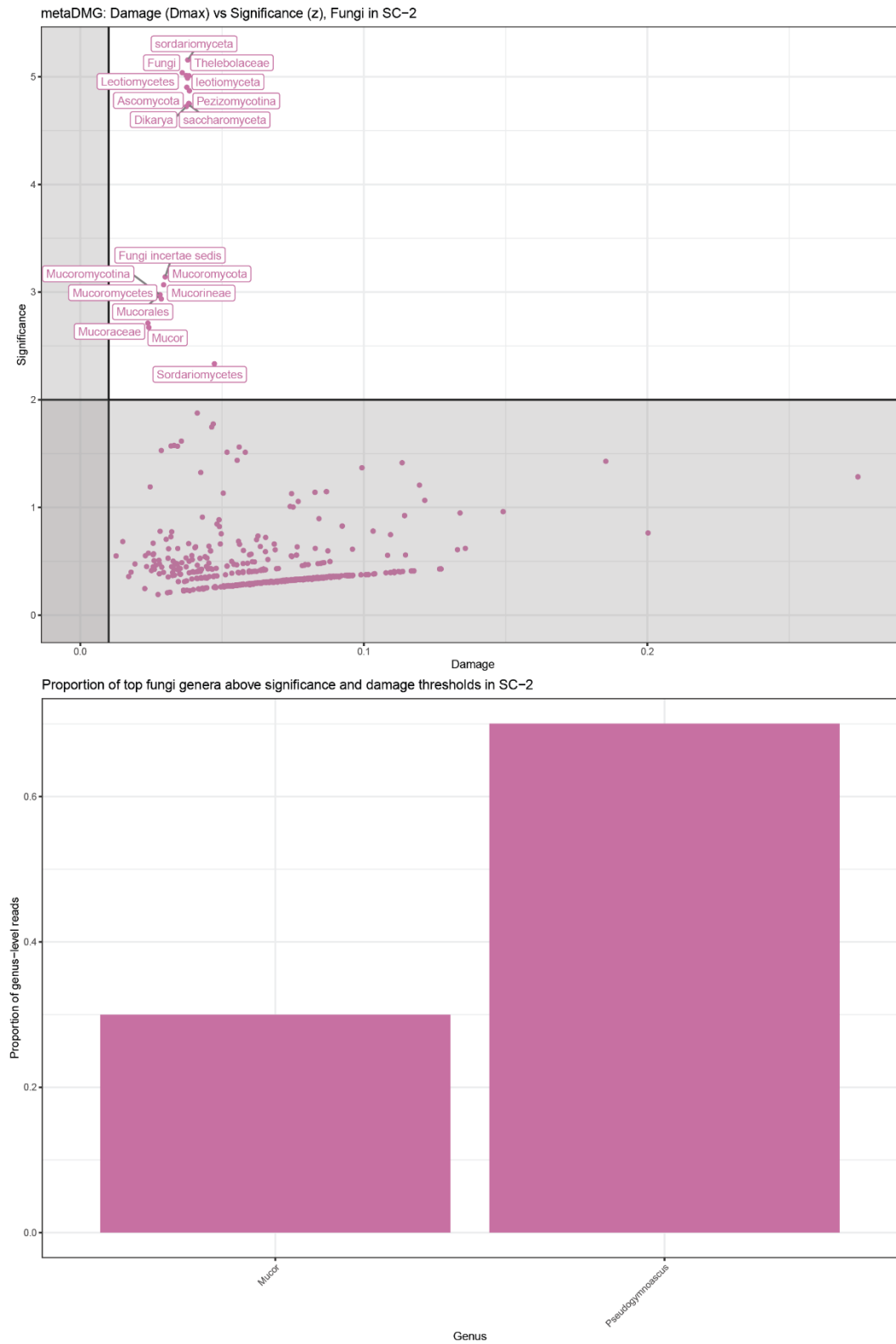


Fig. S48.
SC-2 *metaDMG* plot of fungi taxa above significance and damage thresholds with top genera.



Fig. S49.
SC-3 *metaDMG* plot of plant taxa above significance and damage thresholds with top genera.

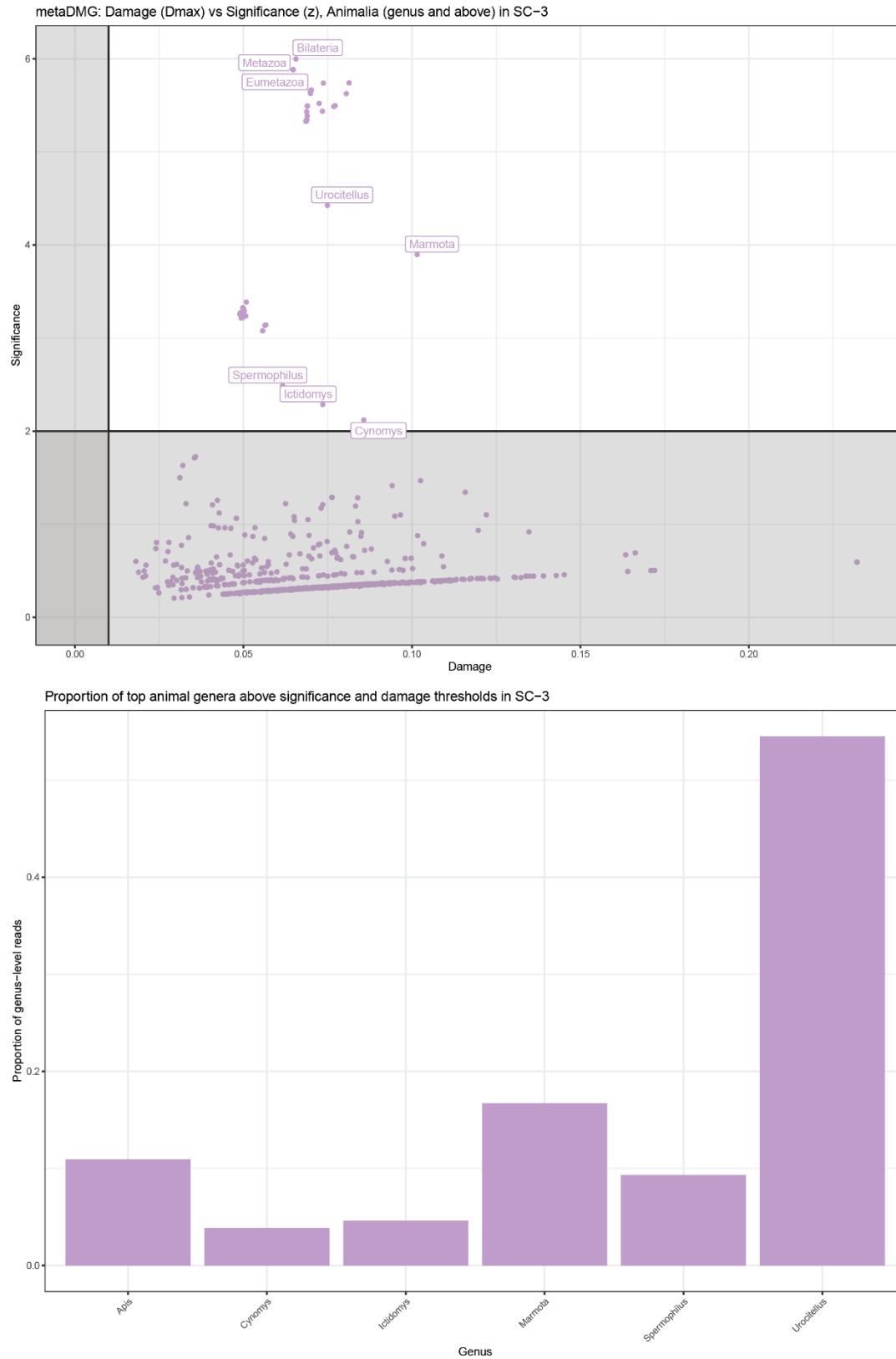


Fig. S50.
SC-3 *metaDMG* plot of animal taxa above significance and damage thresholds with top genera.

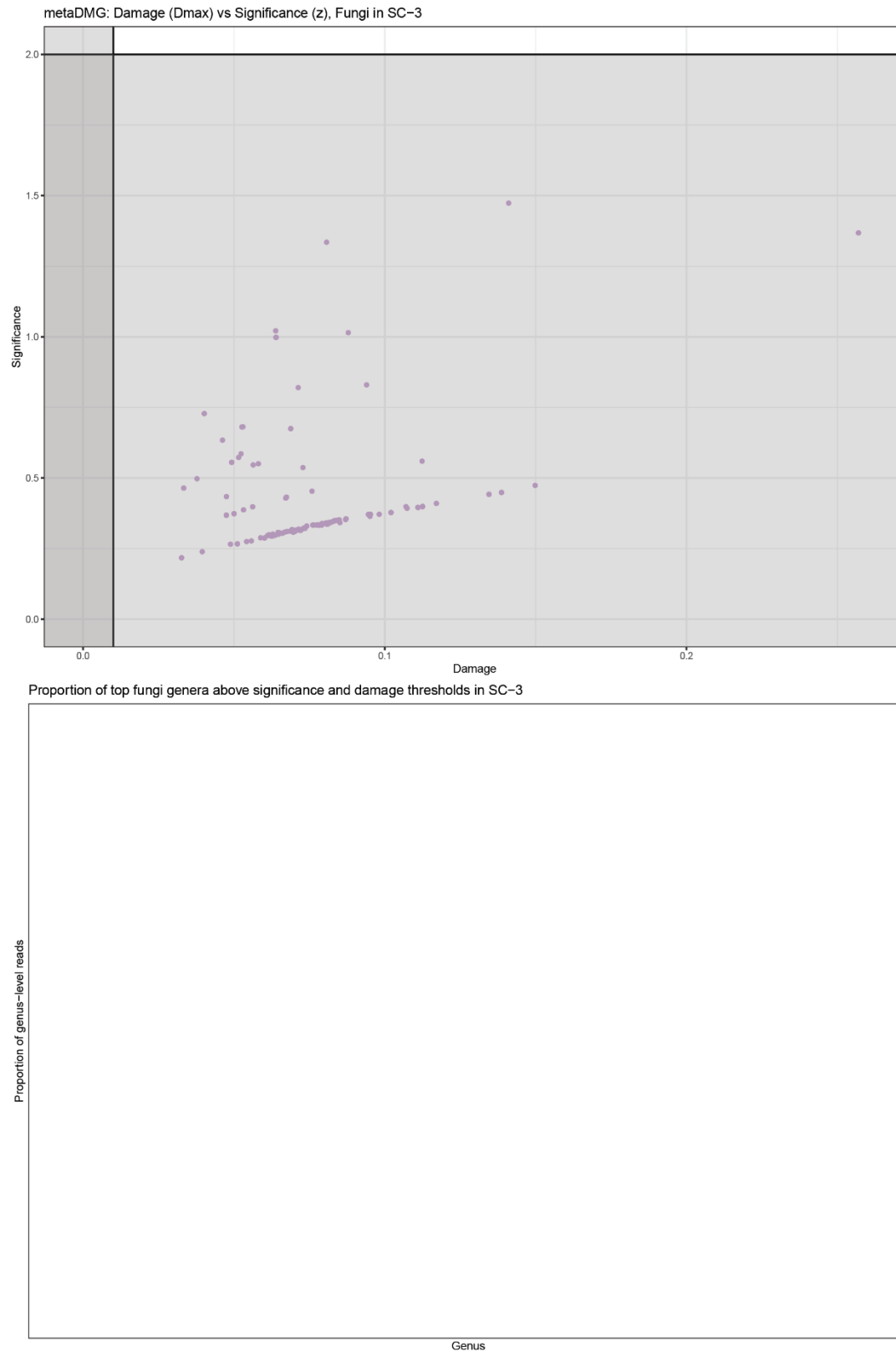


Fig. S51.
SC-3 *metaDMG* plot of fungi taxa above significance and damage thresholds with top genera.

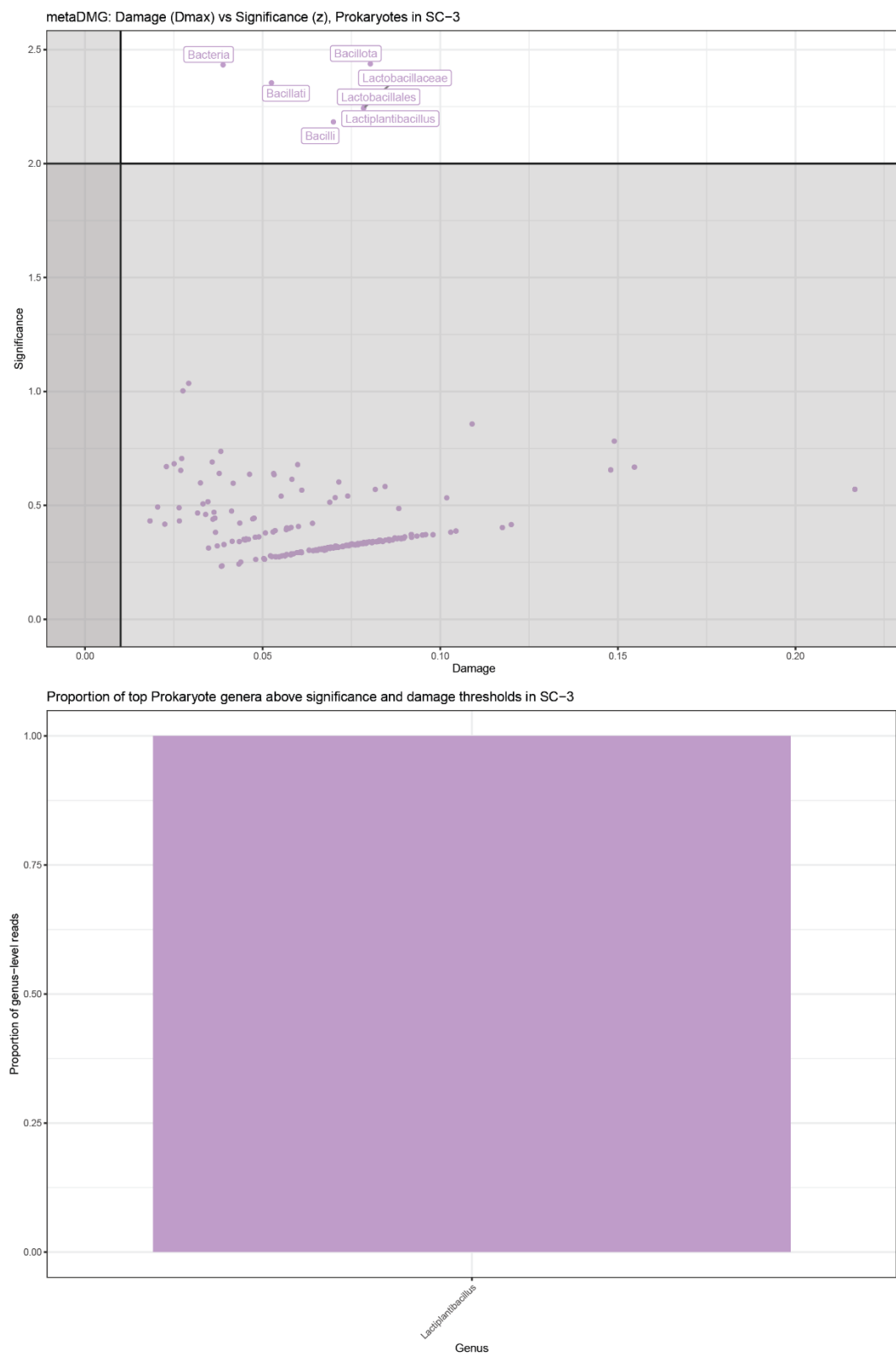


Fig. S52.
SC-3 *metaDMG* plot of prokaryote taxa above significance and damage thresholds with top genera.

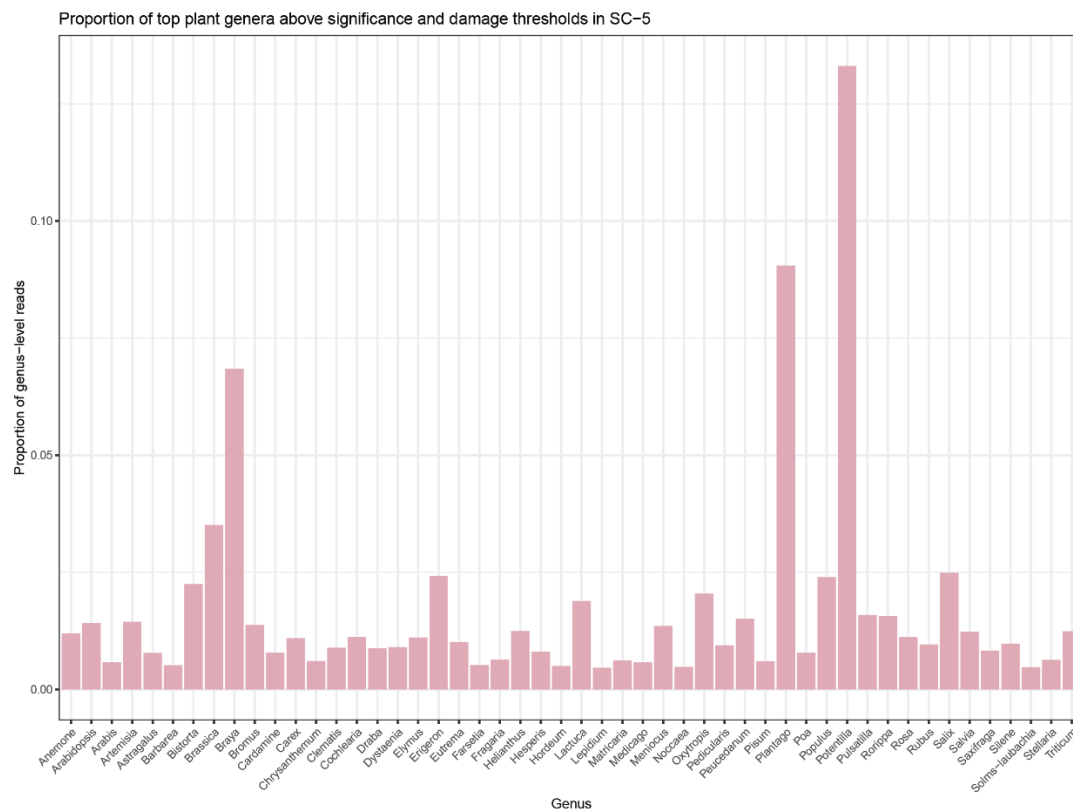
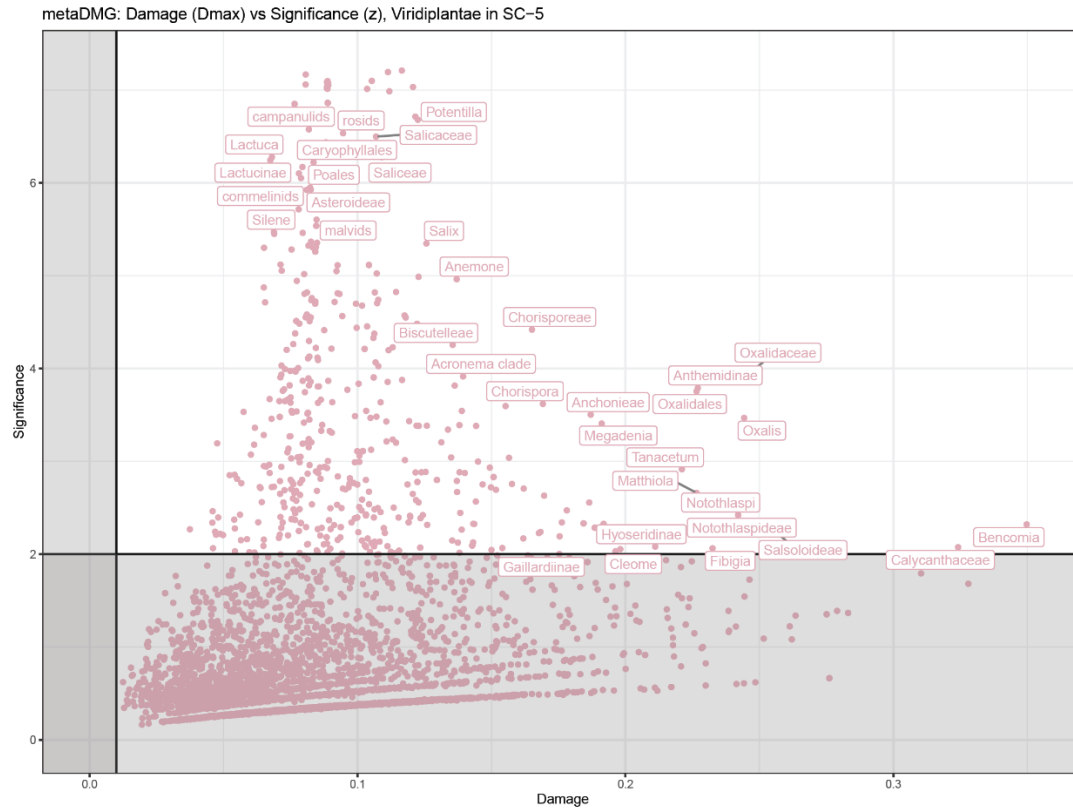


Fig. S53.
SC-5 *metaDMG* plot of plant taxa above significance and damage thresholds with top genera.

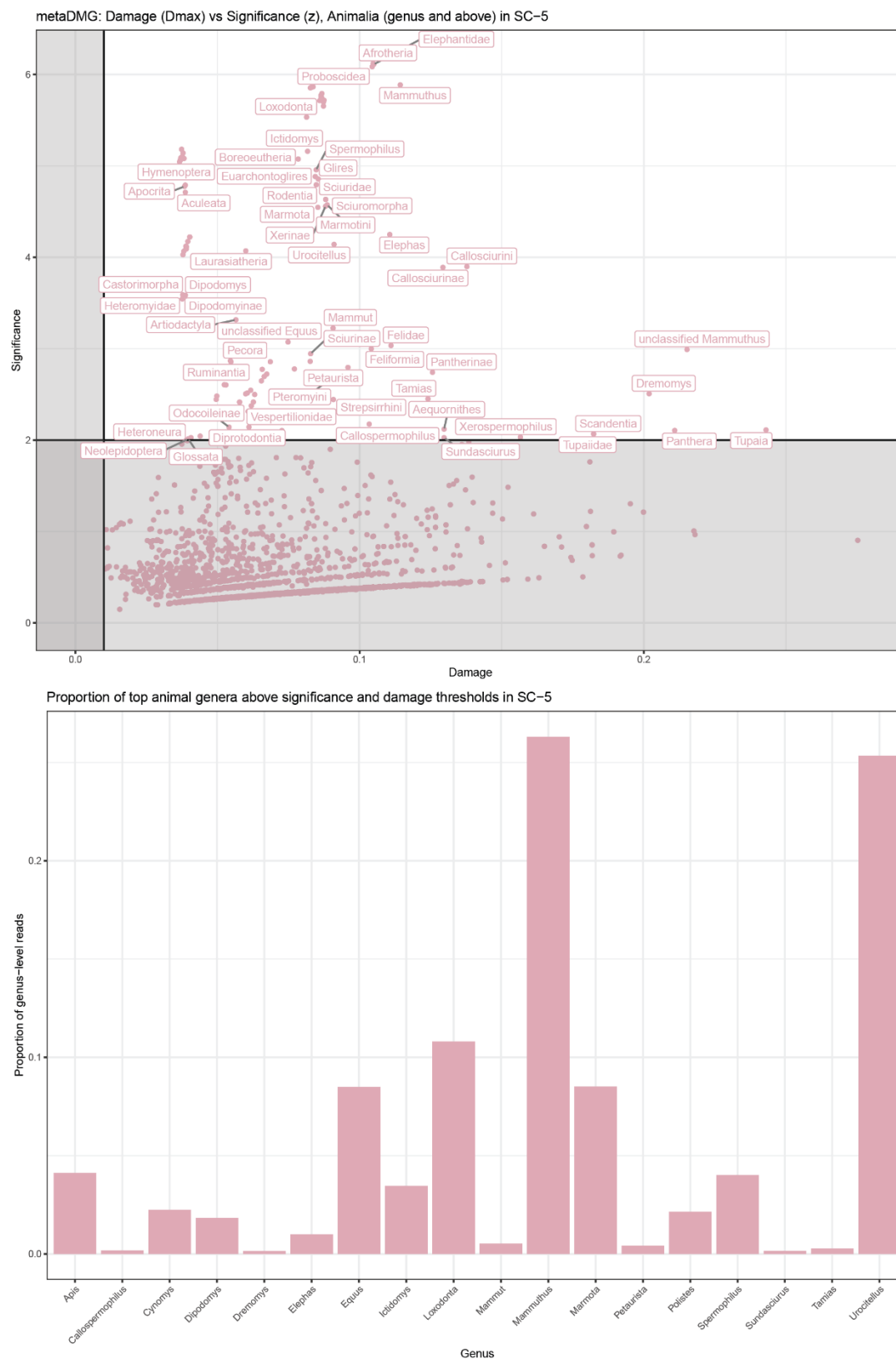
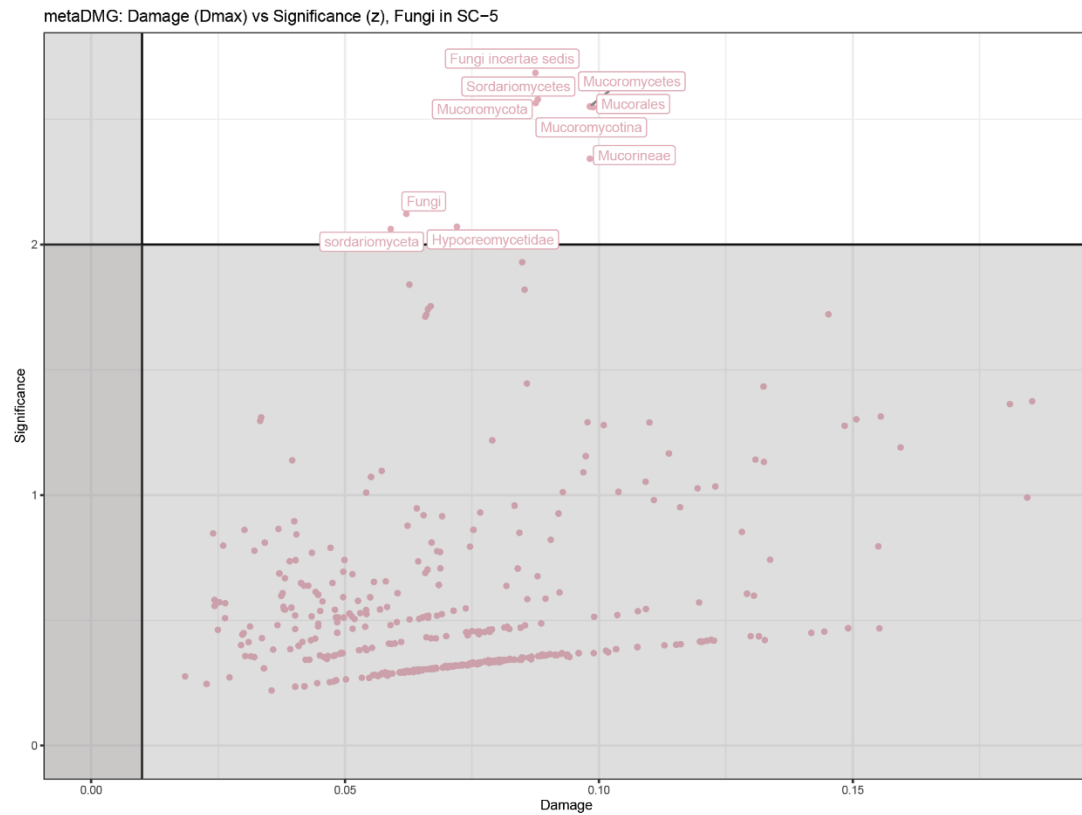


Fig. S54.
SC-5 *metaDMG* plot of animal taxa above significance and damage thresholds with top genera.



Proportion of top fungi genera above significance and damage thresholds in SC-5

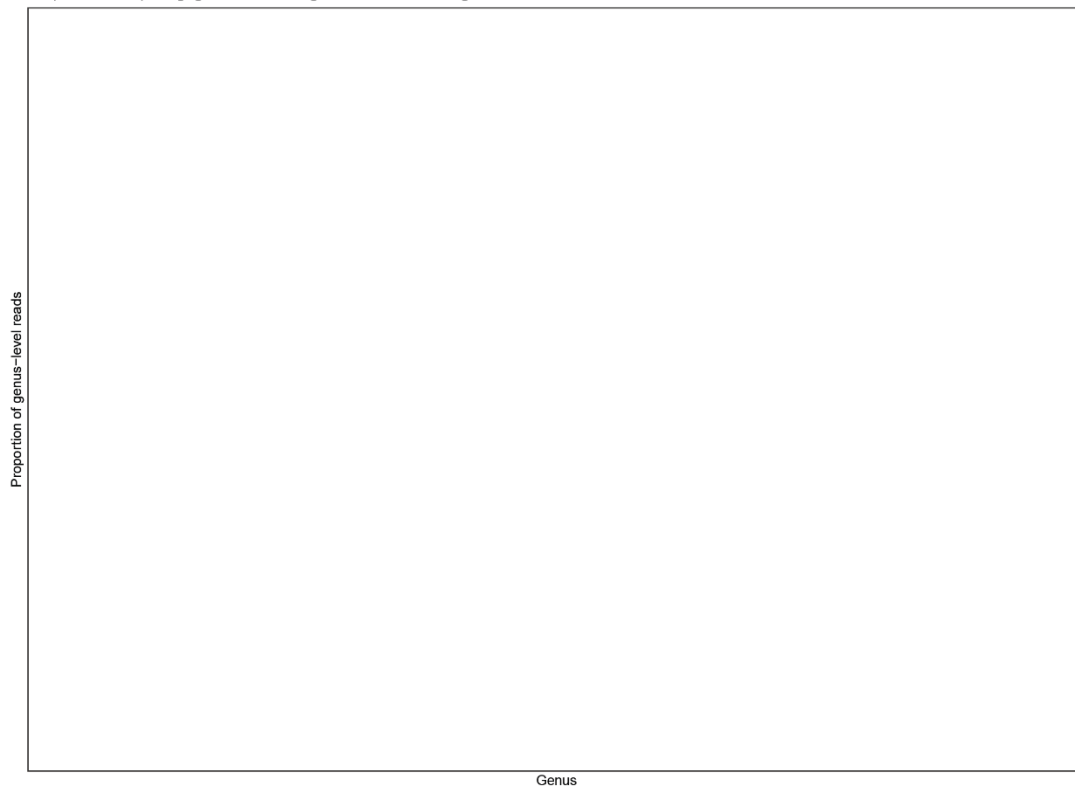


Fig. S55.
SC-5 *metaDMG* plot of fungi taxa above significance and damage thresholds with top genera.

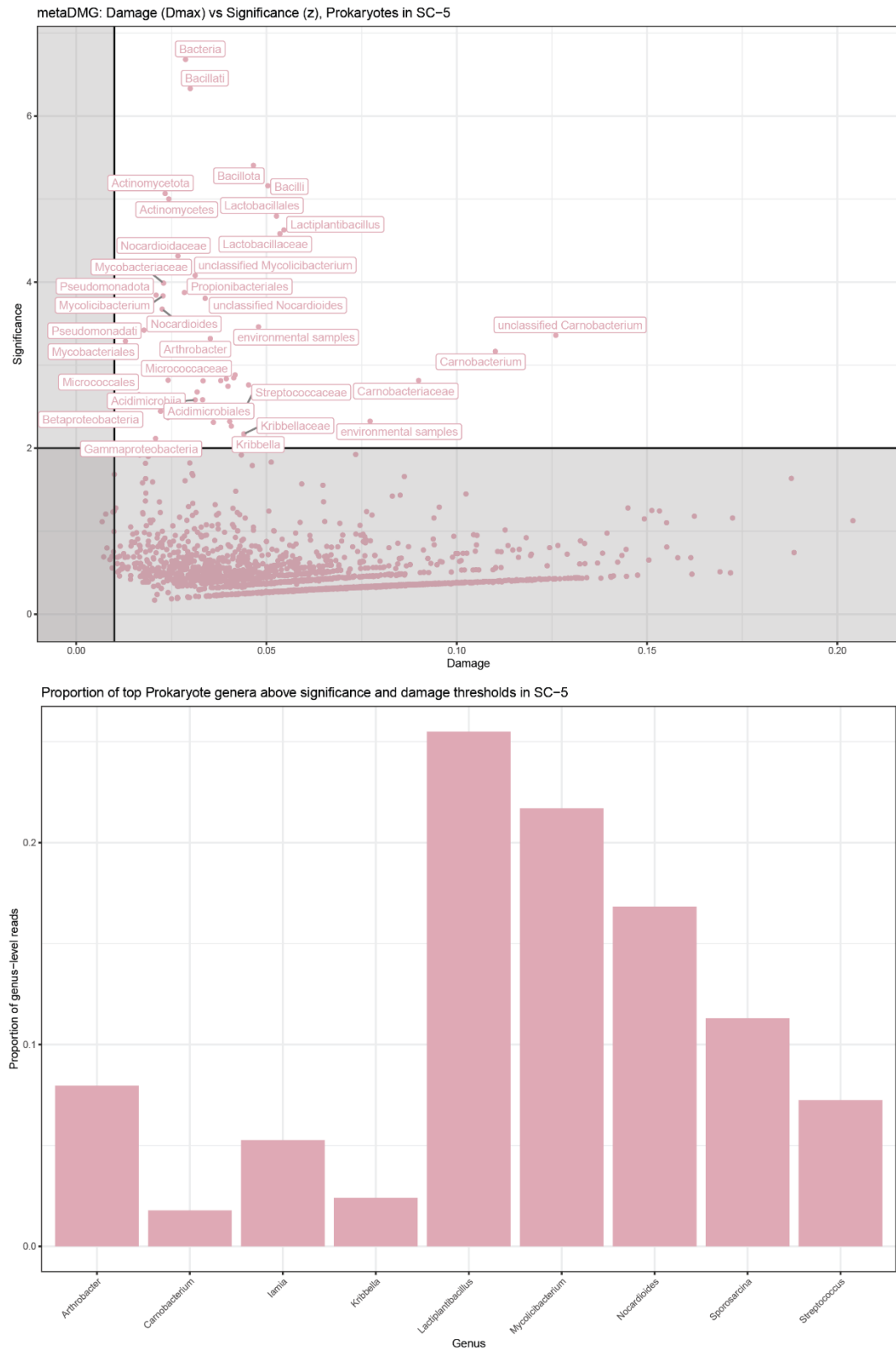


Fig. S56.
SC-5 *metaDMG* plot of prokaryote taxa above significance and damage thresholds with top genera.

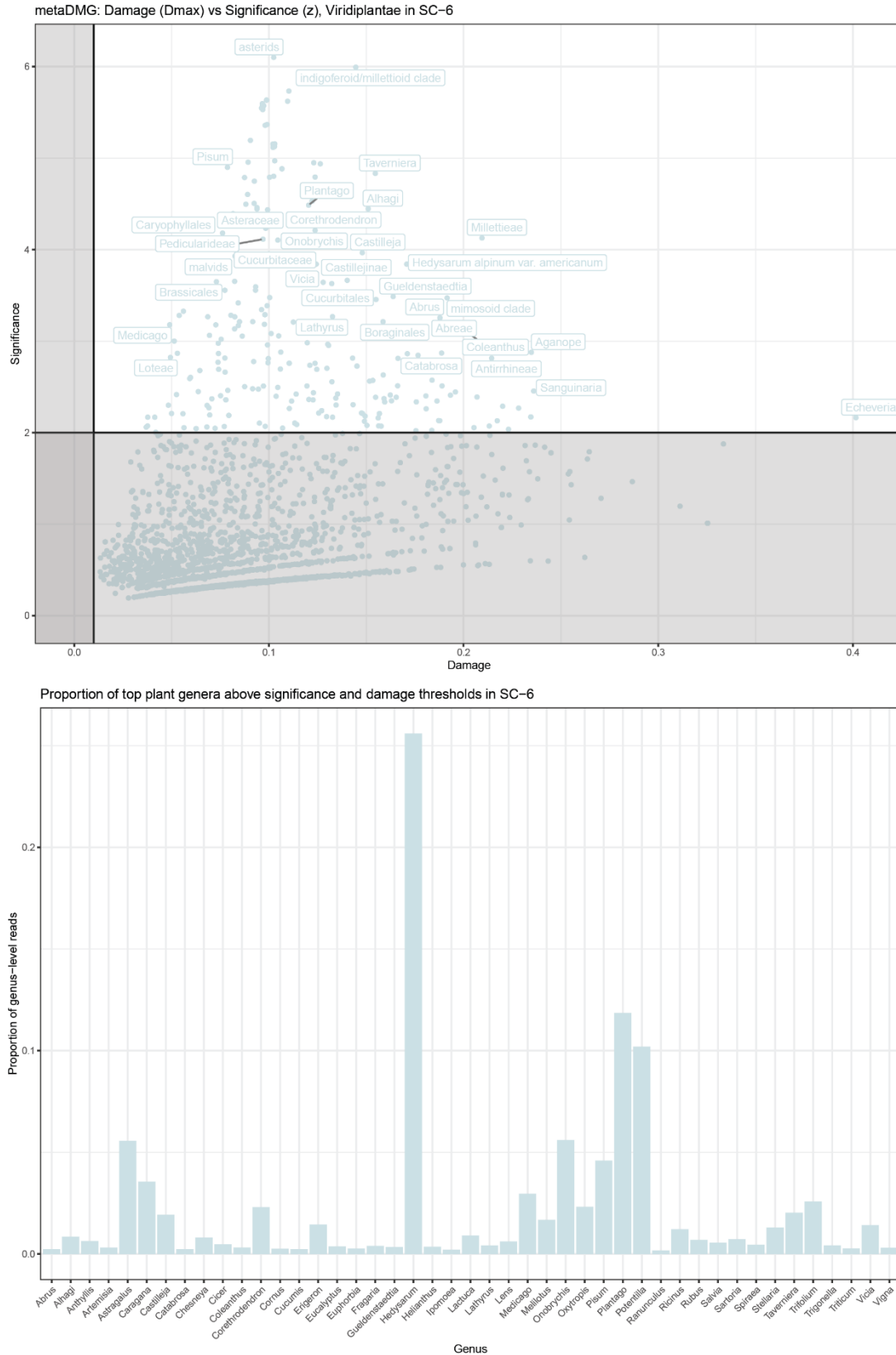


Fig. S57.
SC-6 *metaDMG* plot of plant taxa above significance and damage thresholds with top genera.

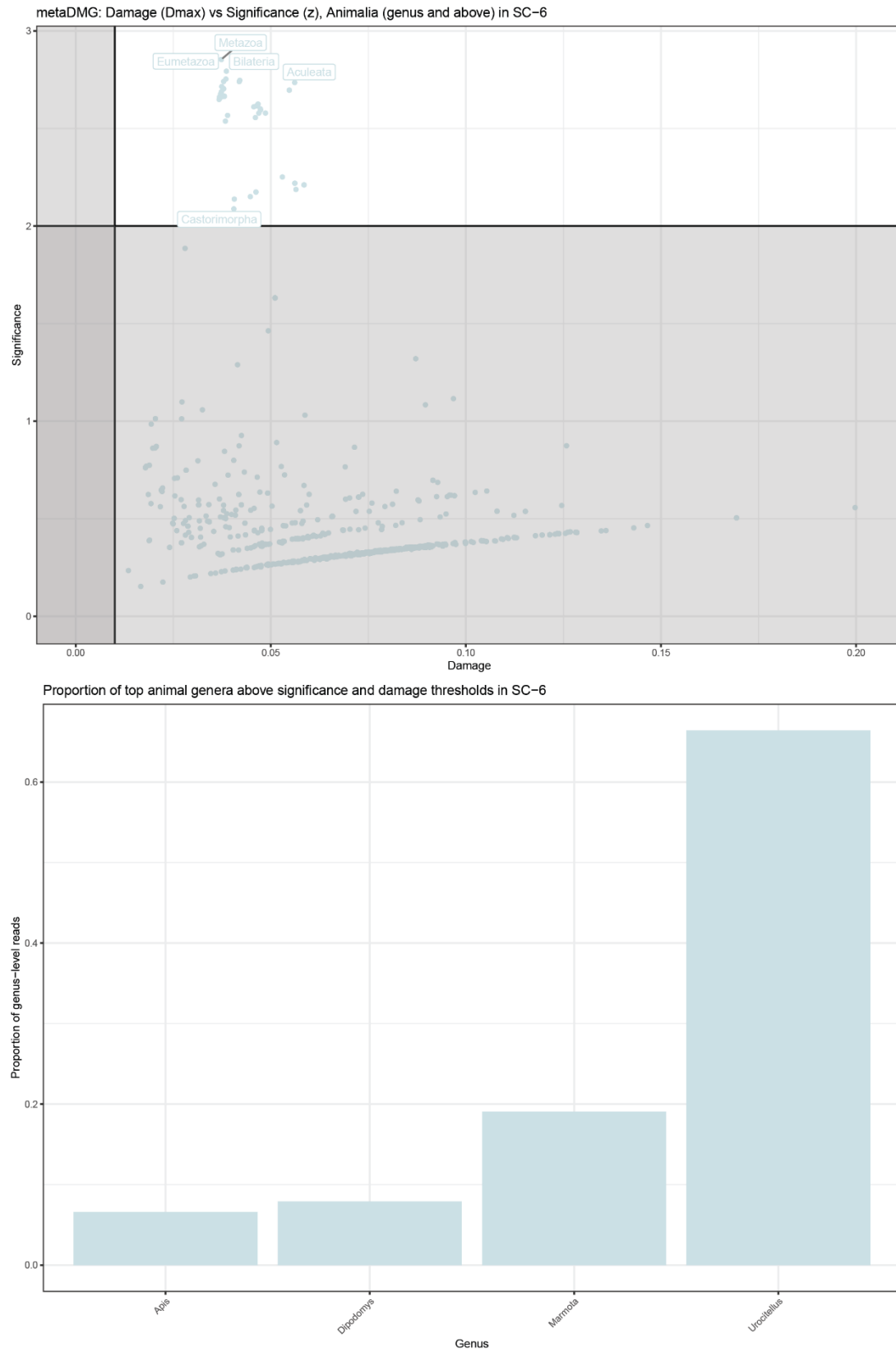
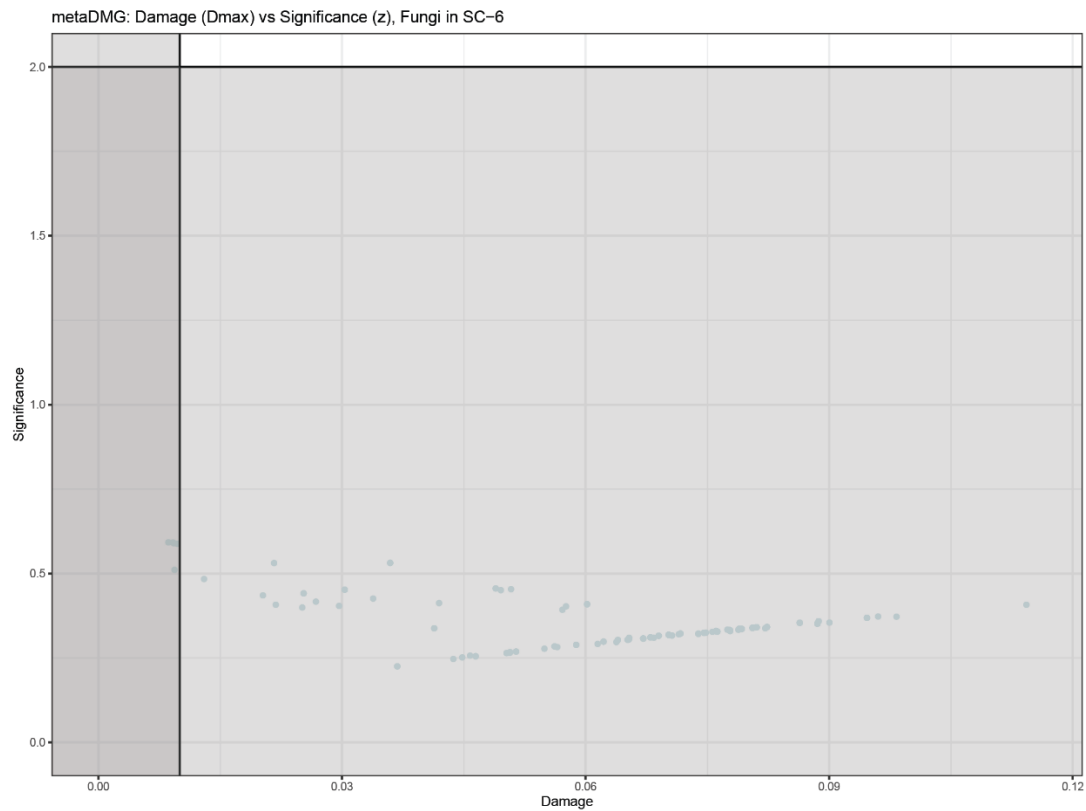


Fig. S58.
SC-6 *metaDMG* plot of animal taxa above significance and damage thresholds with top genera.



Proportion of top fungi genera above significance and damage thresholds in SC-6

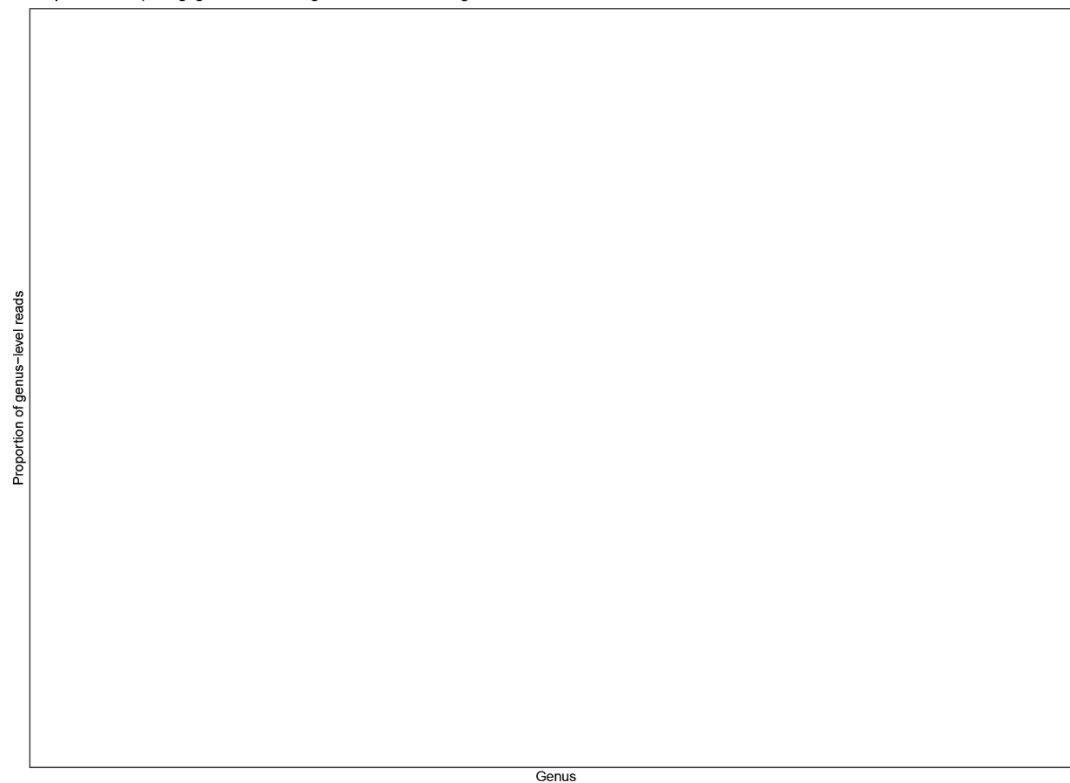
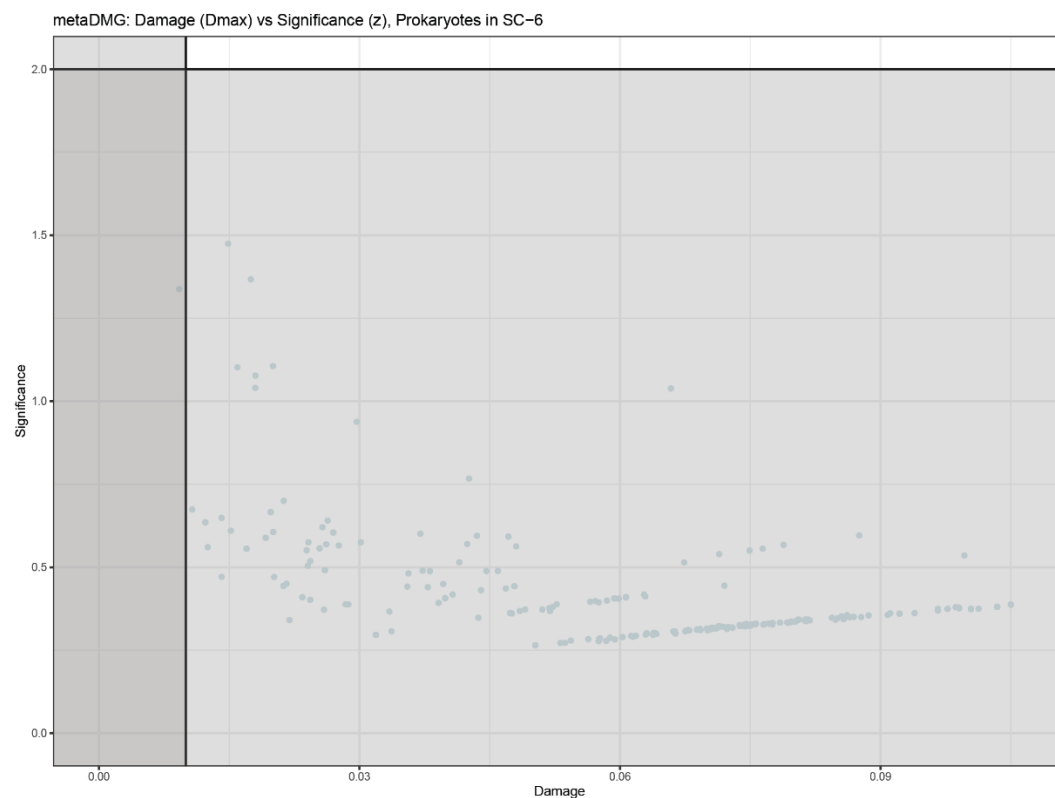


Fig. S59.
SC-6 *metaDMG* plot of fungi taxa above significance and damage thresholds with top genera.



Proportion of top Prokaryote genera above significance and damage thresholds in SC-6

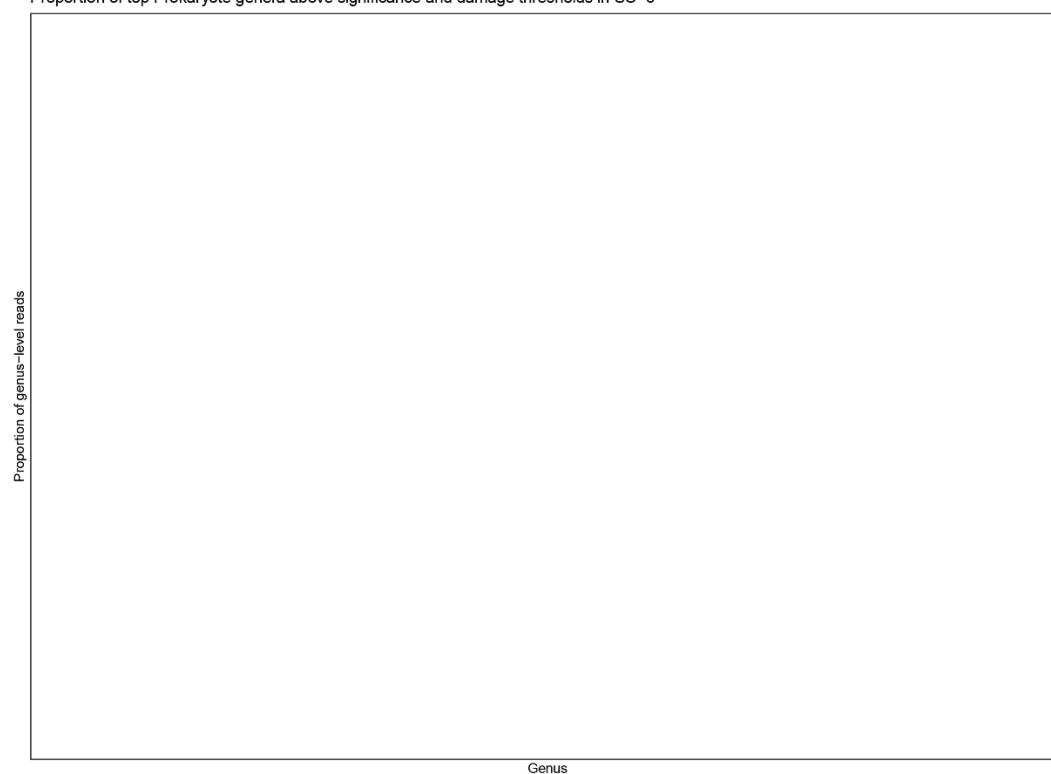


Fig. S60.
SC-6 *metaDMG* plot of prokaryote taxa above significance and damage thresholds with top genera.

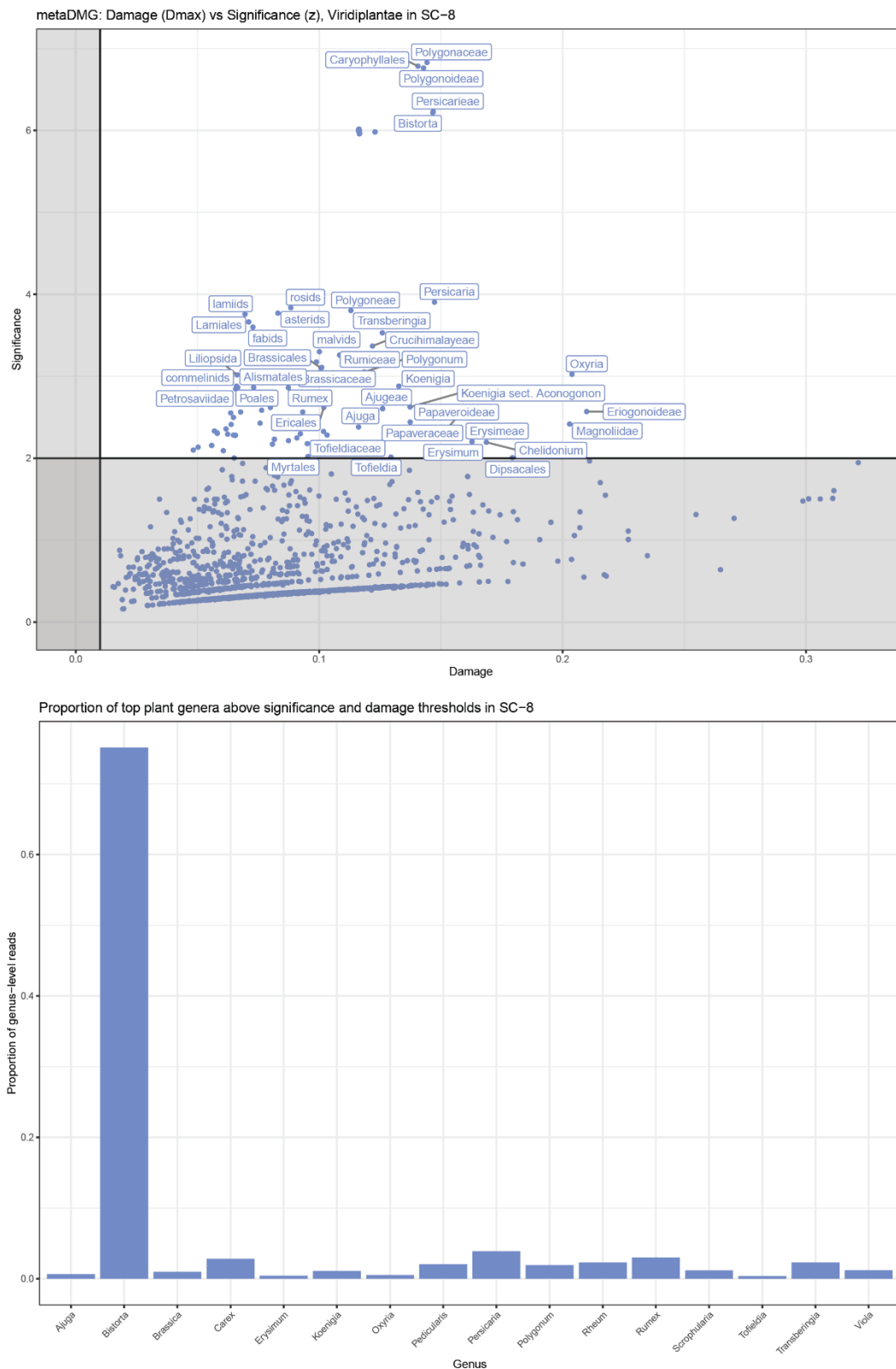


Fig. S61.
SC-8 *metaDMG* plot of plant taxa above significance and damage thresholds with top genera.

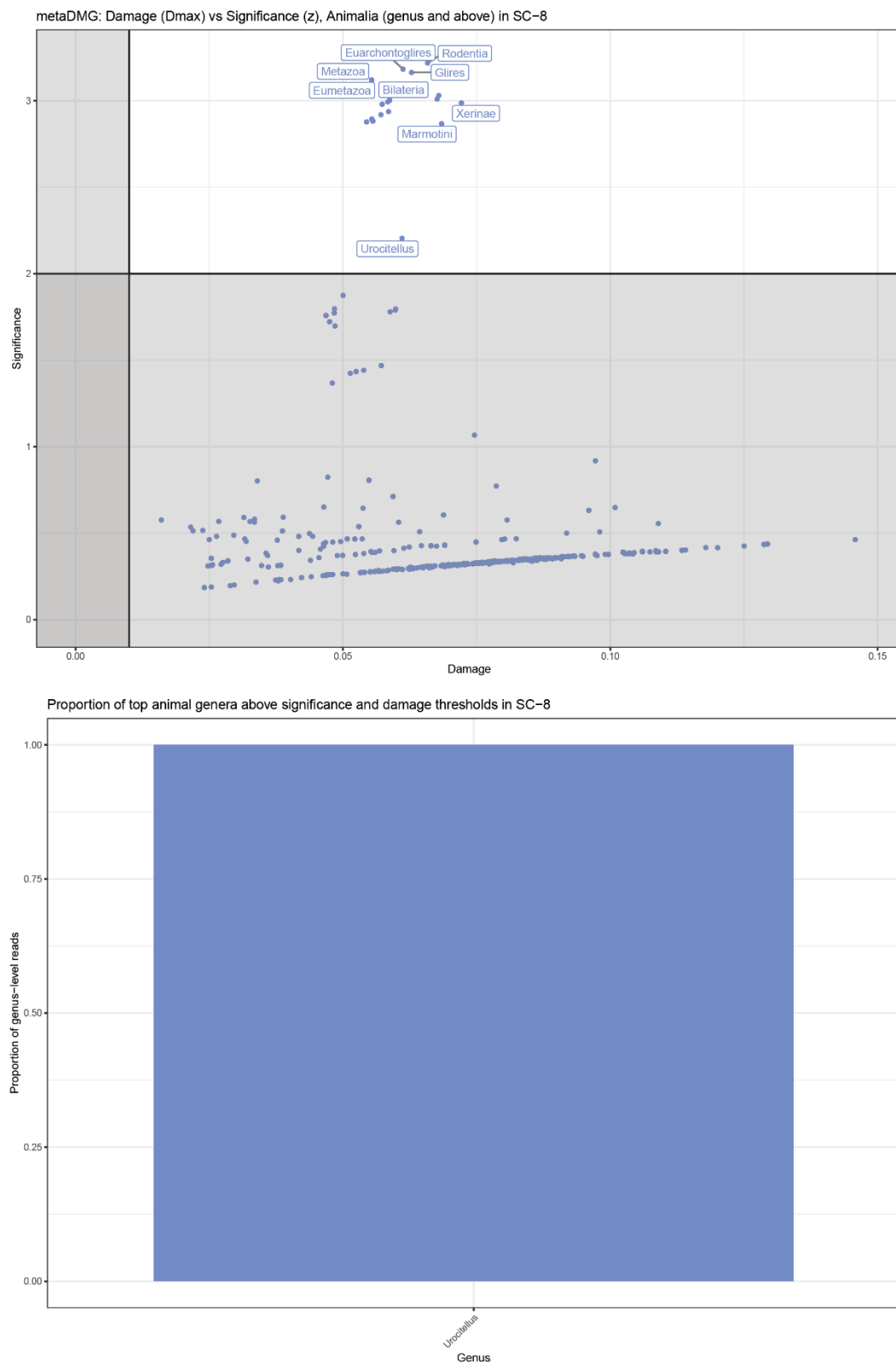


Fig. S62.
SC-8 *metaDMG* plot of animal taxa above significance and damage thresholds with top genera.

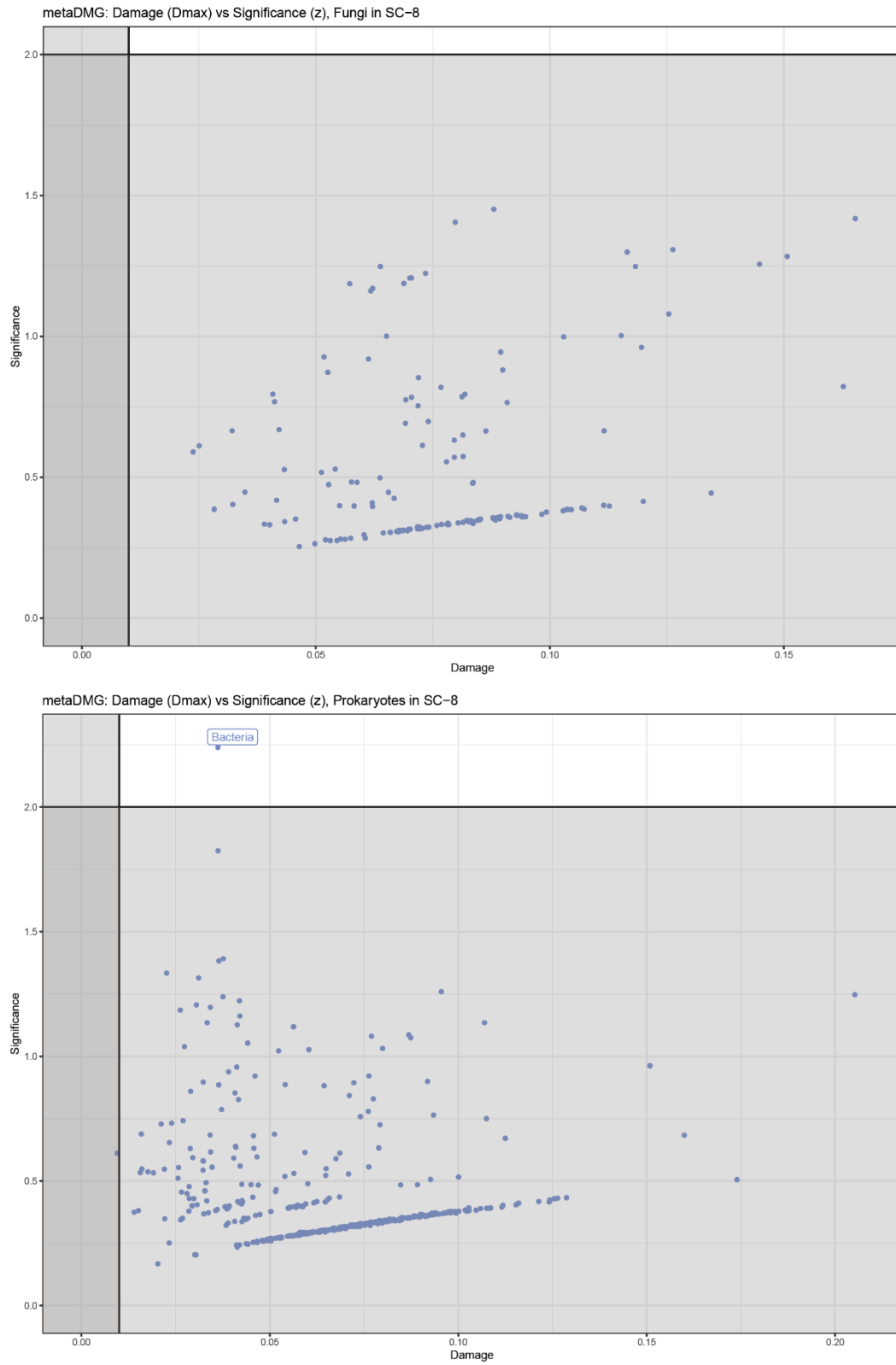


Fig. S63.
SC-8 *metaDMG* plot of fungi and prokaryote taxa above significance and damage thresholds.

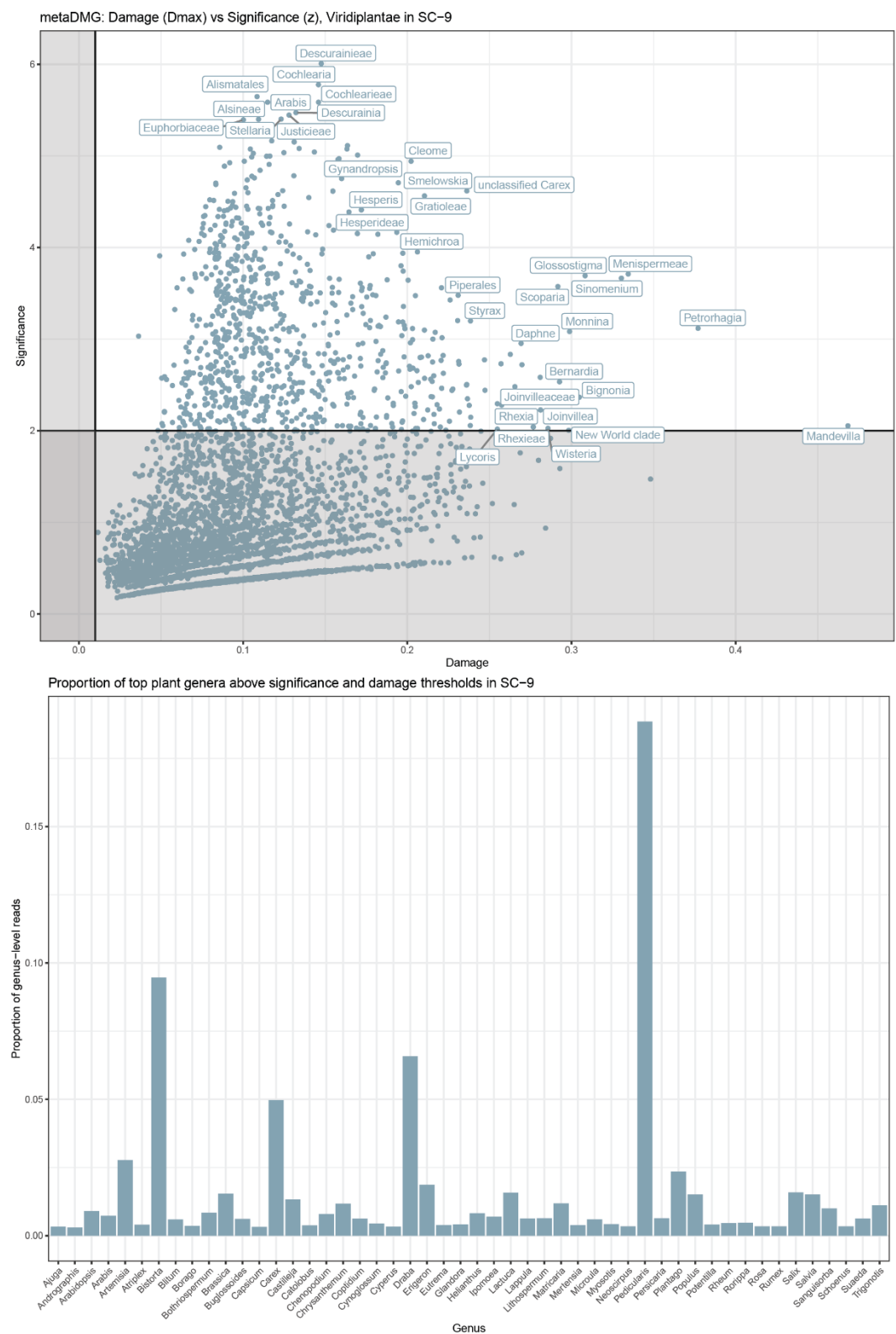


Fig. S64.
SC-9 *metaDMG* plot of plant taxa above significance and damage thresholds with top genera.

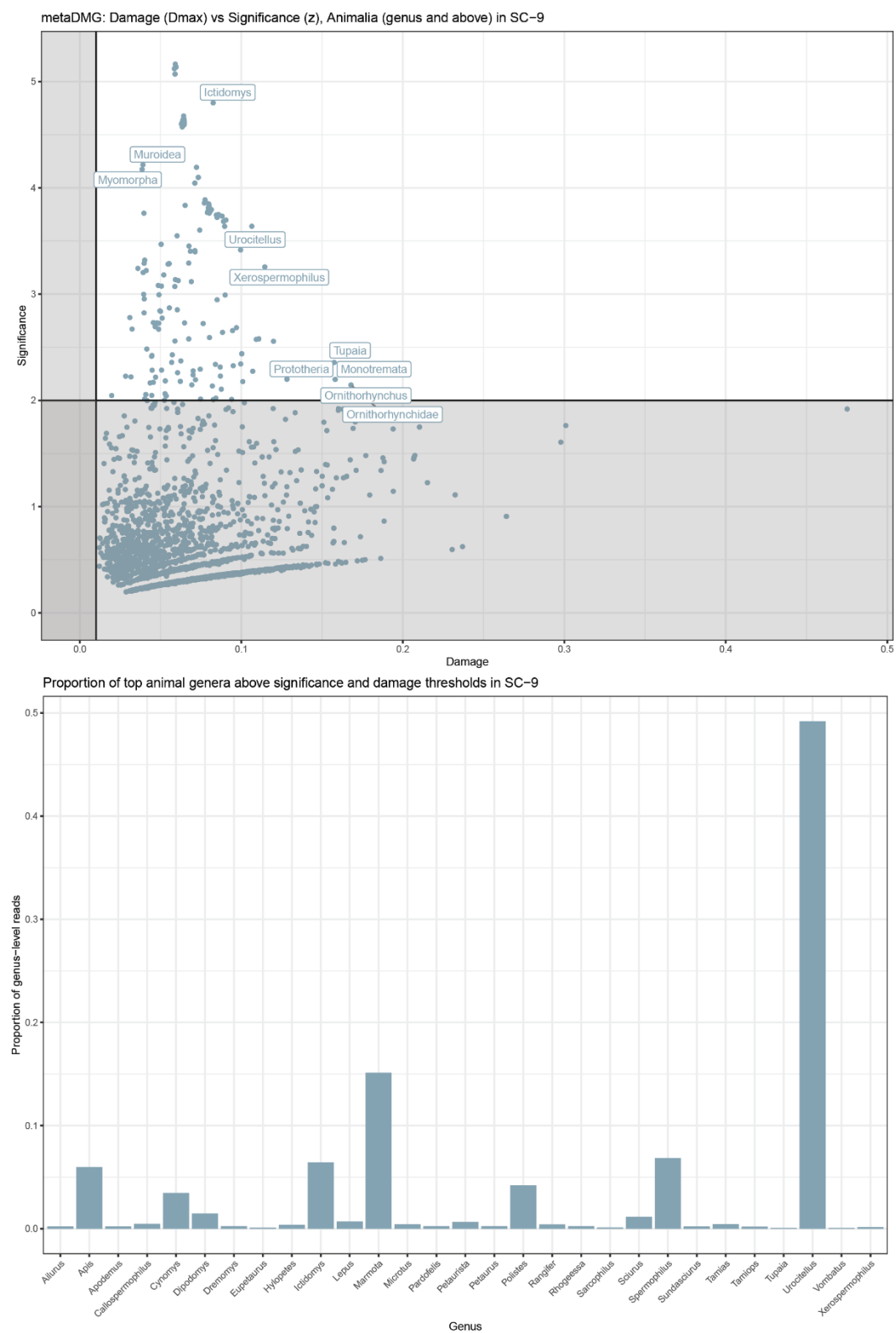
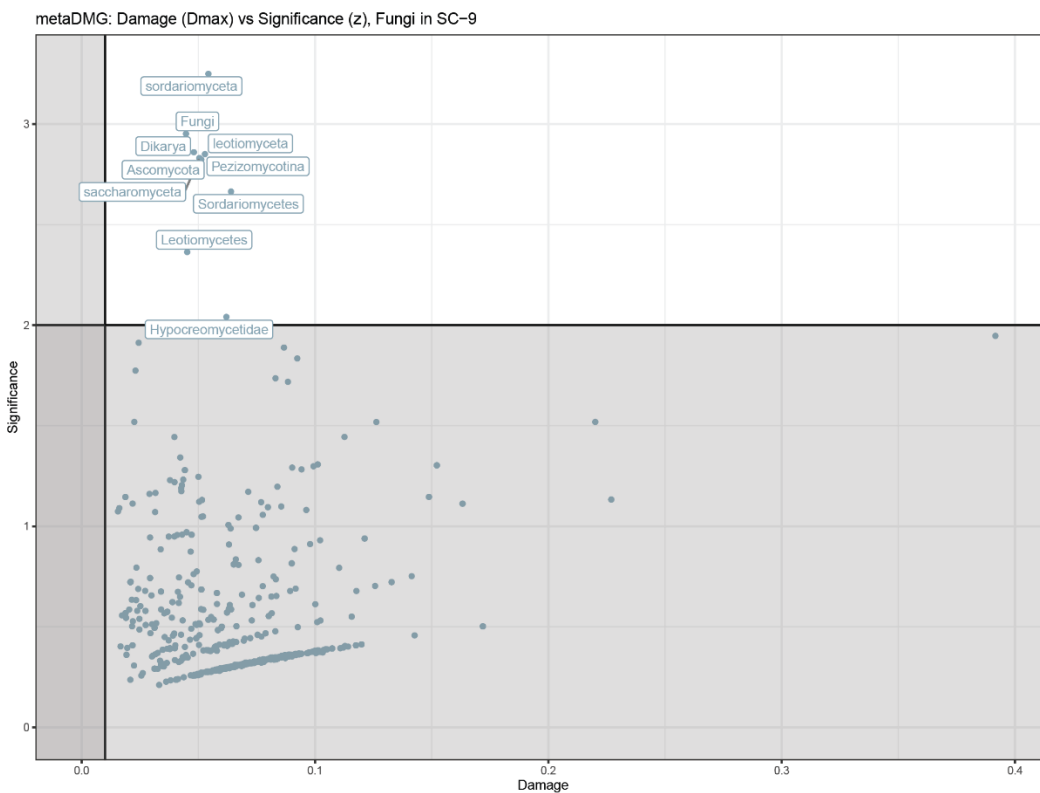


Fig. S65.
SC-9 *metaDMG* plot of animal taxa above significance and damage thresholds with top genera.



Proportion of top fungi genera above significance and damage thresholds in SC-9

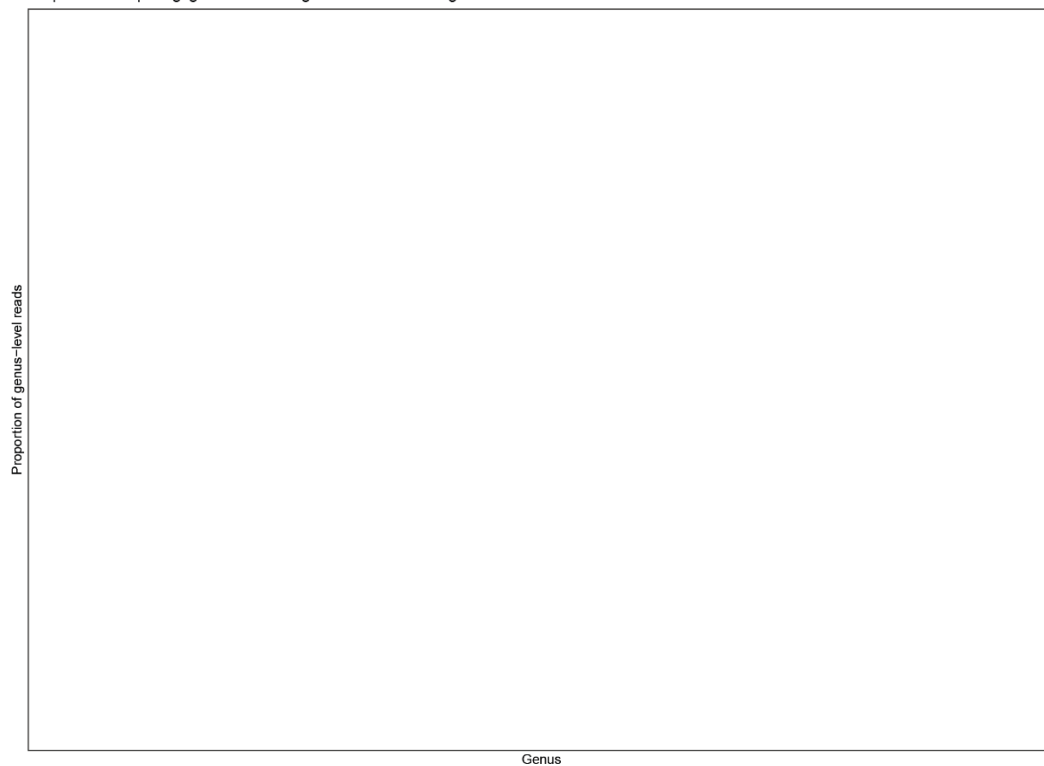


Fig. S66.
SC-9 *metaDMG* plot of fungi taxa above significance and damage thresholds with top genera.

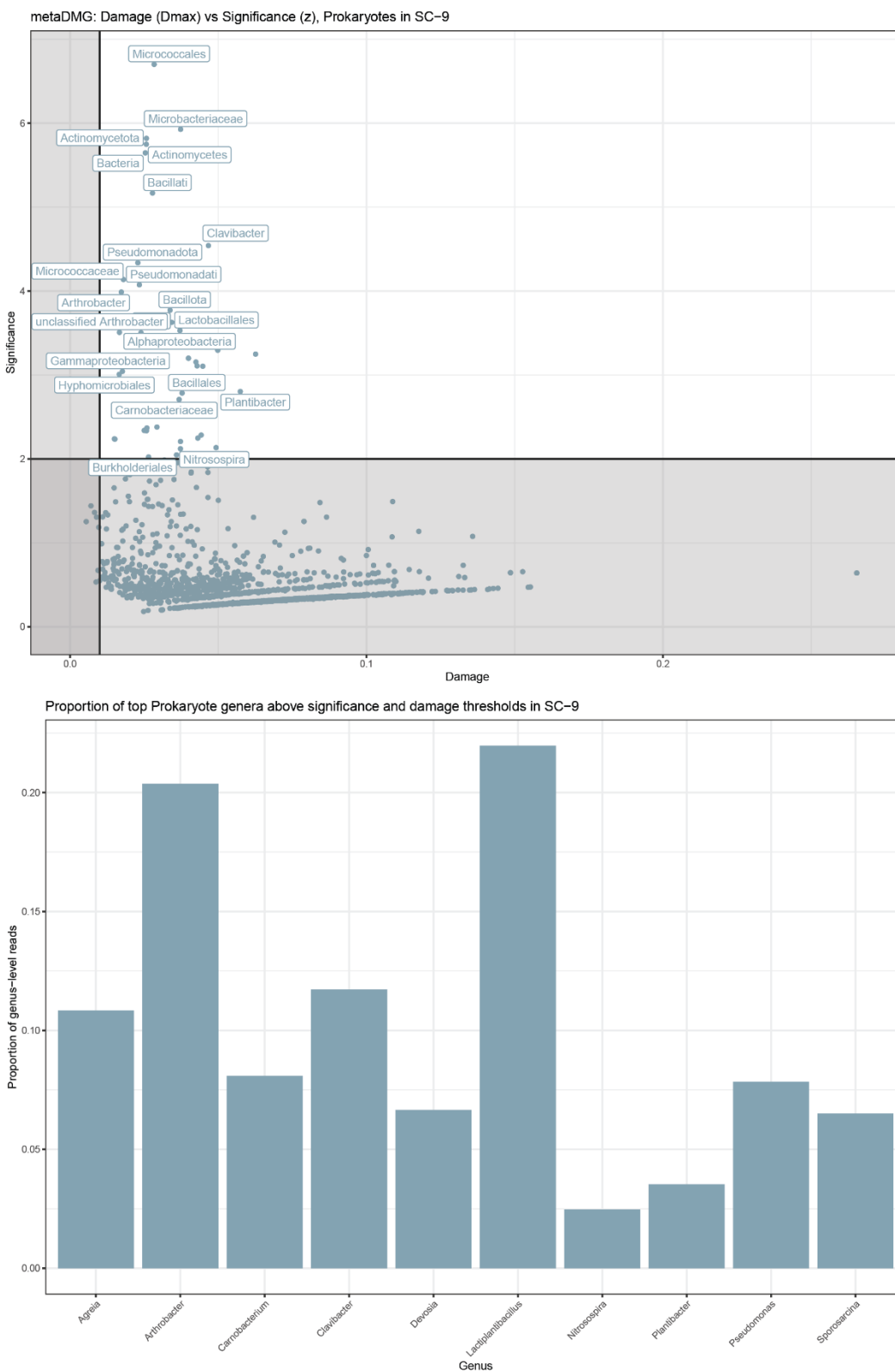


Fig. S67.
SC-9 *metaDMG* plot of prokaryote taxa above significance and damage thresholds with top genera.

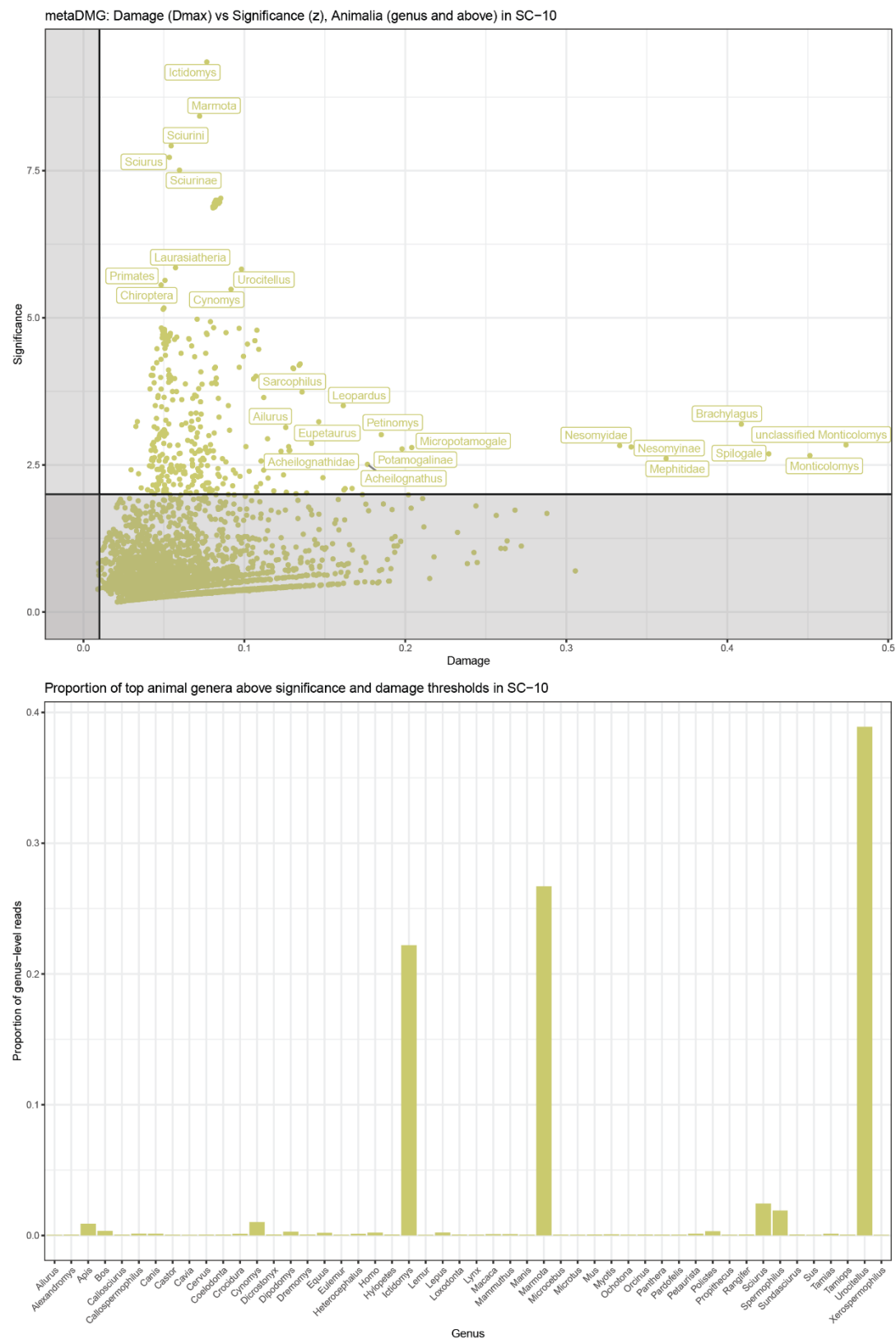


Fig. S69.
SC-10 *metaDMG* plot of animal taxa above significance and damage thresholds with top genera.

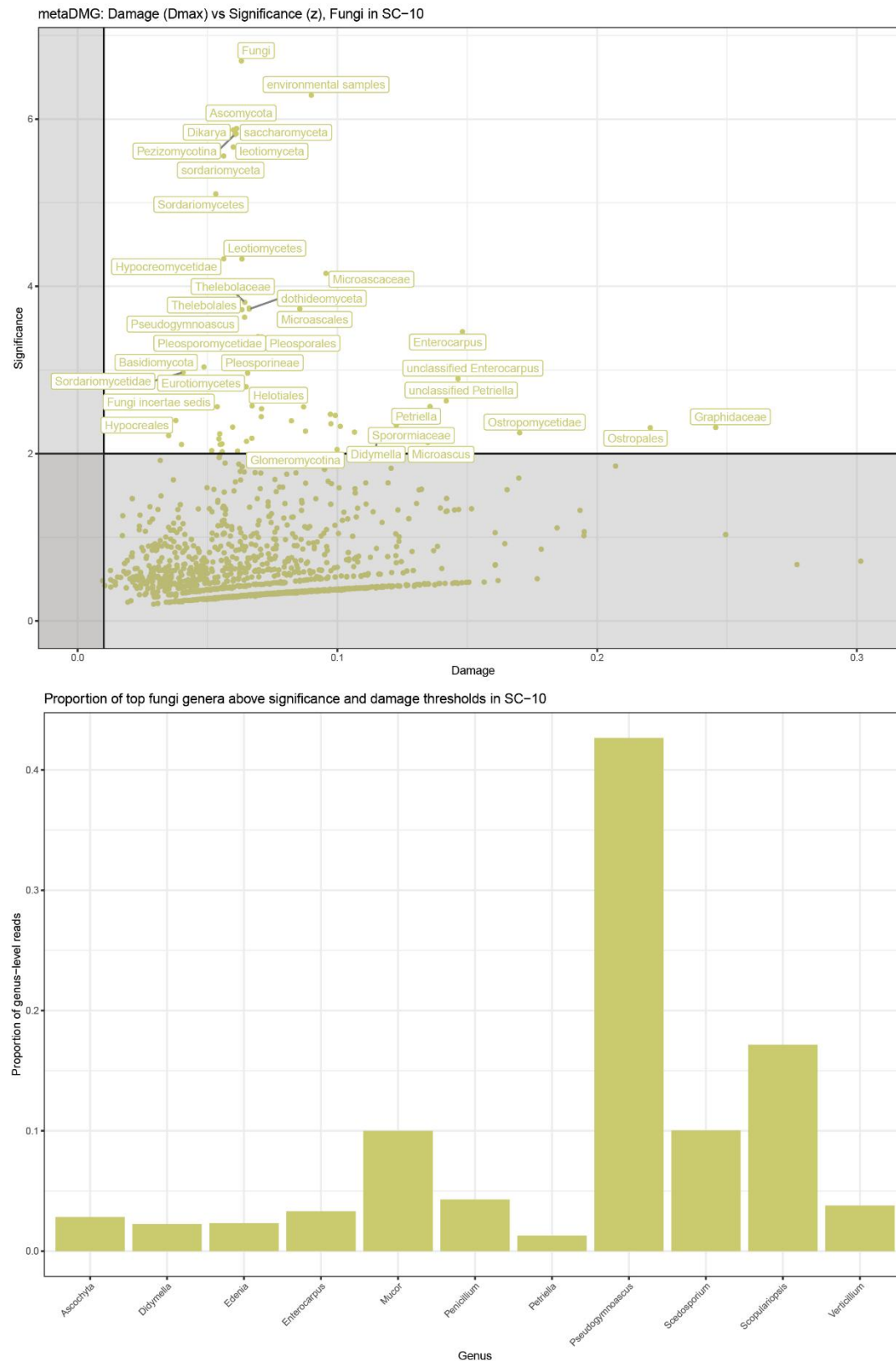


Fig. S70.
SC-10 *metaDMG* plot of fungi taxa above significance and damage thresholds with top genera.

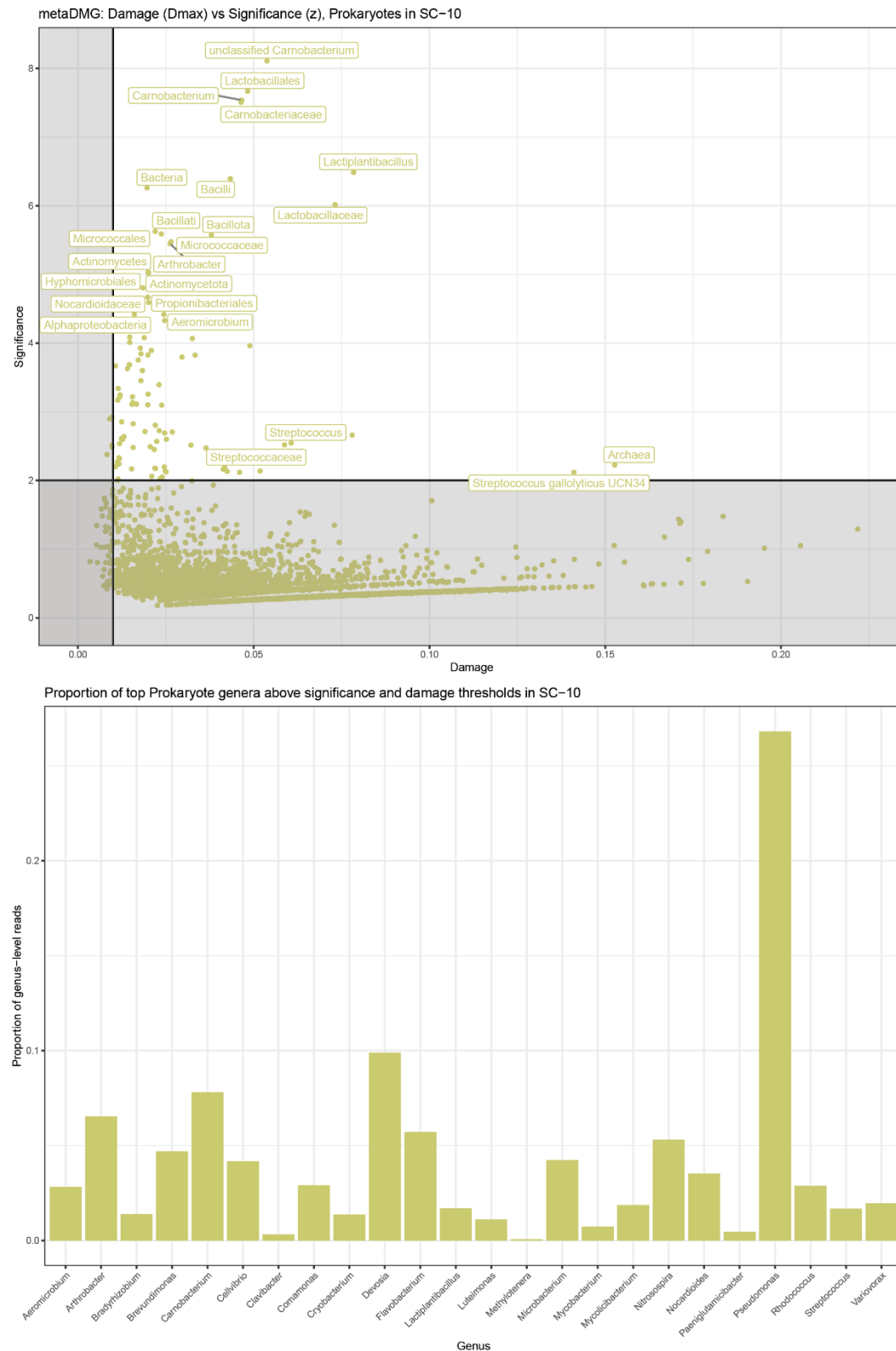


Fig. S71.
SC-10 metaDMG plot of prokaryote taxa above significance and damage thresholds with top genera.

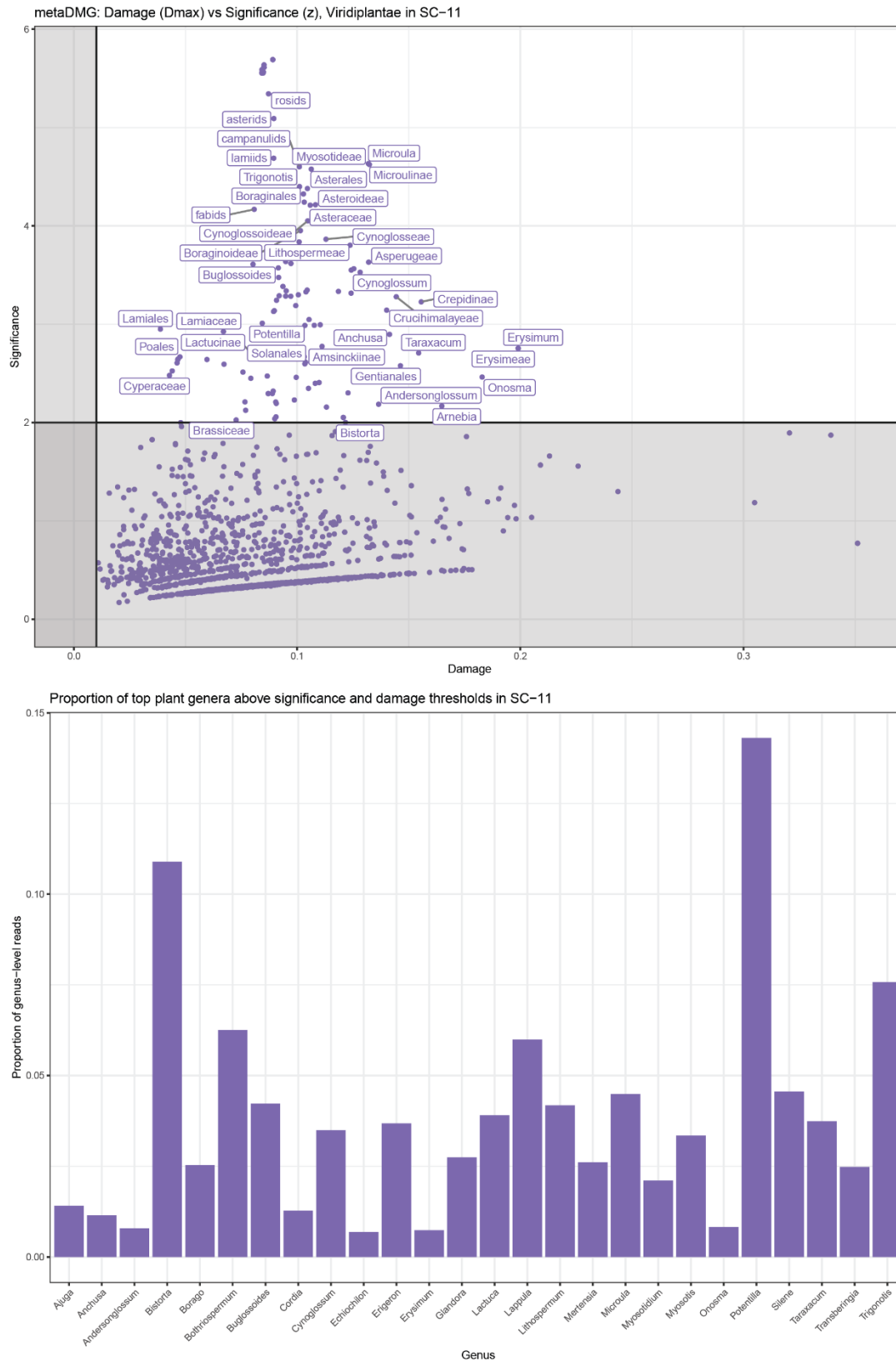


Fig. S72.
SC-11 *metaDMG* plot of plant taxa above significance and damage thresholds with top genera.

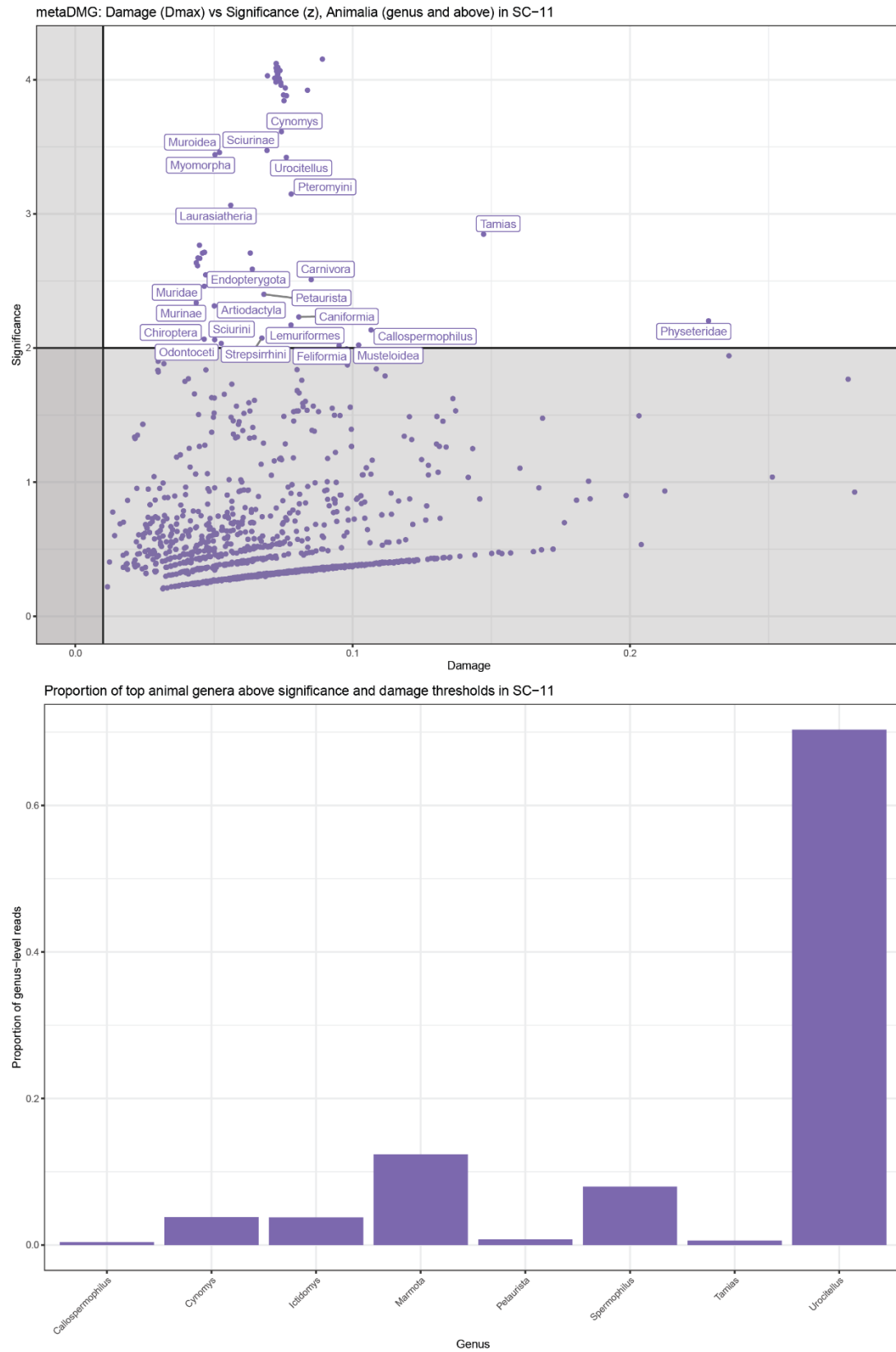


Fig. S73.
SC-11 *metaDMG* plot of animal taxa above significance and damage thresholds with top genera.

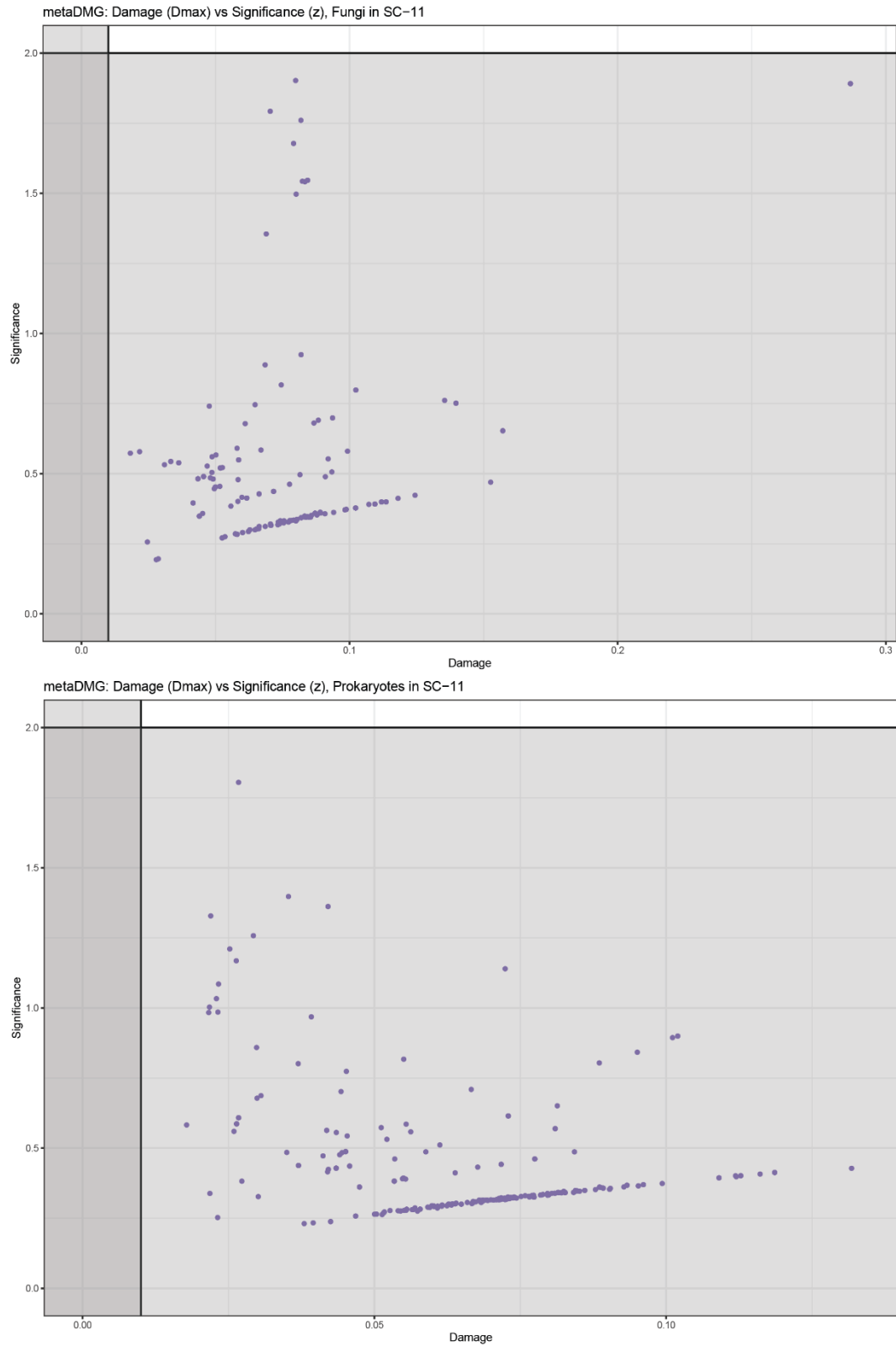


Fig. S74.
SC-11 *metaDMG* plot of fungi and prokaryote taxa above significance and damage thresholds with top genera.

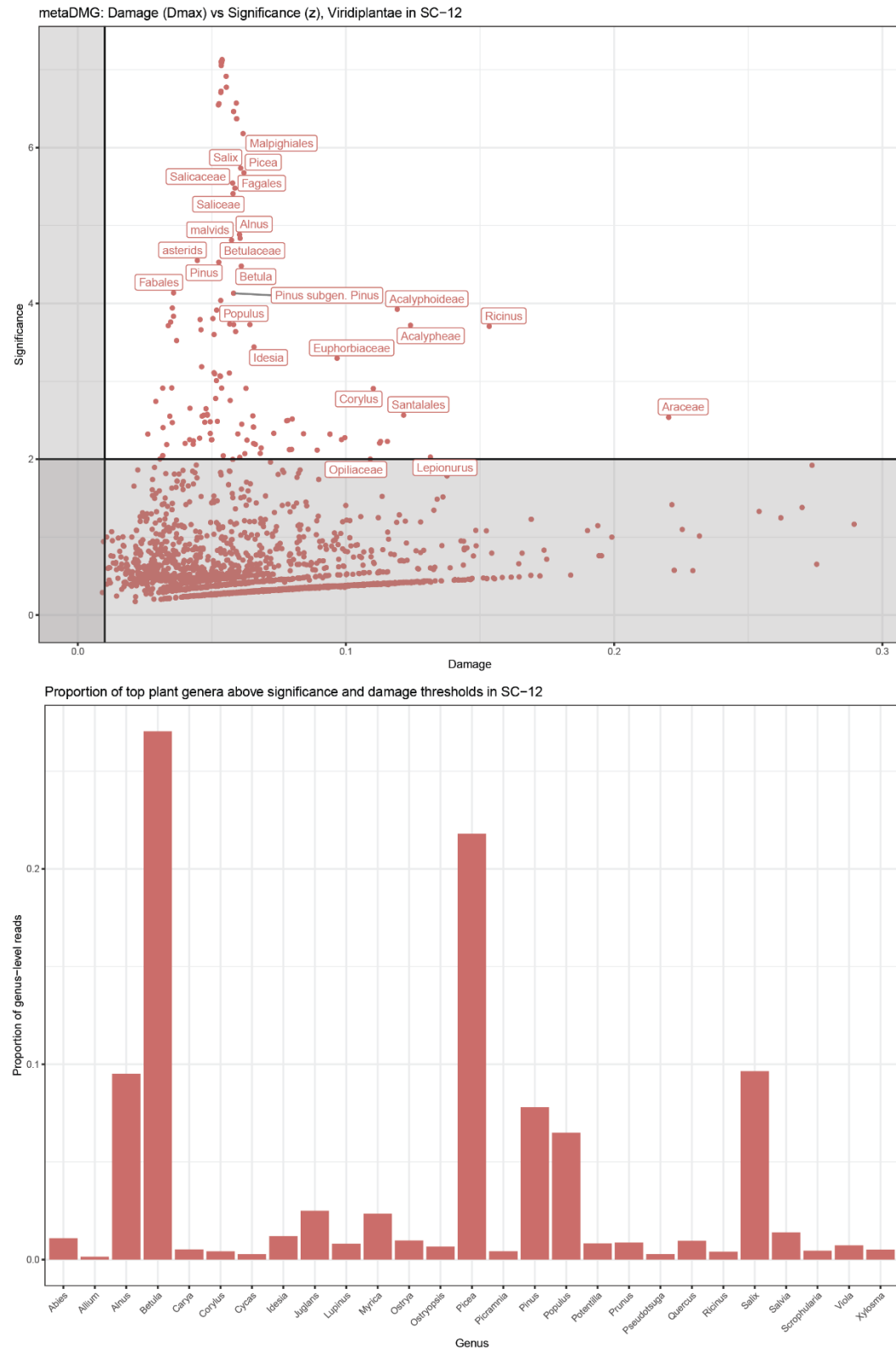


Fig. S75.
SC-12 *metaDMG* plot of plant taxa above significance and damage thresholds with top genera.

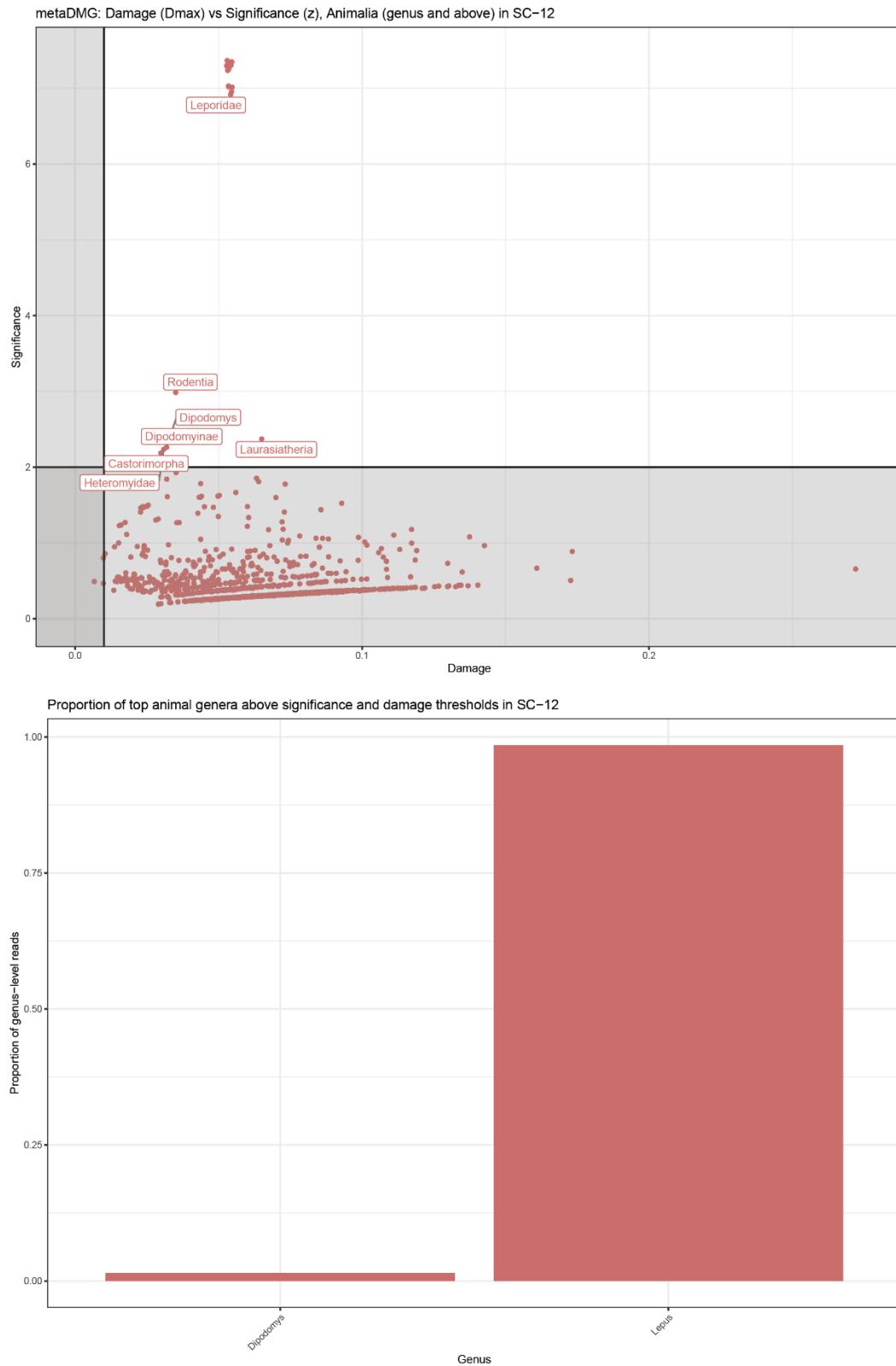


Fig. S76.
SC-12 *metaDMG* plot of animal taxa above significance and damage thresholds with top genera.

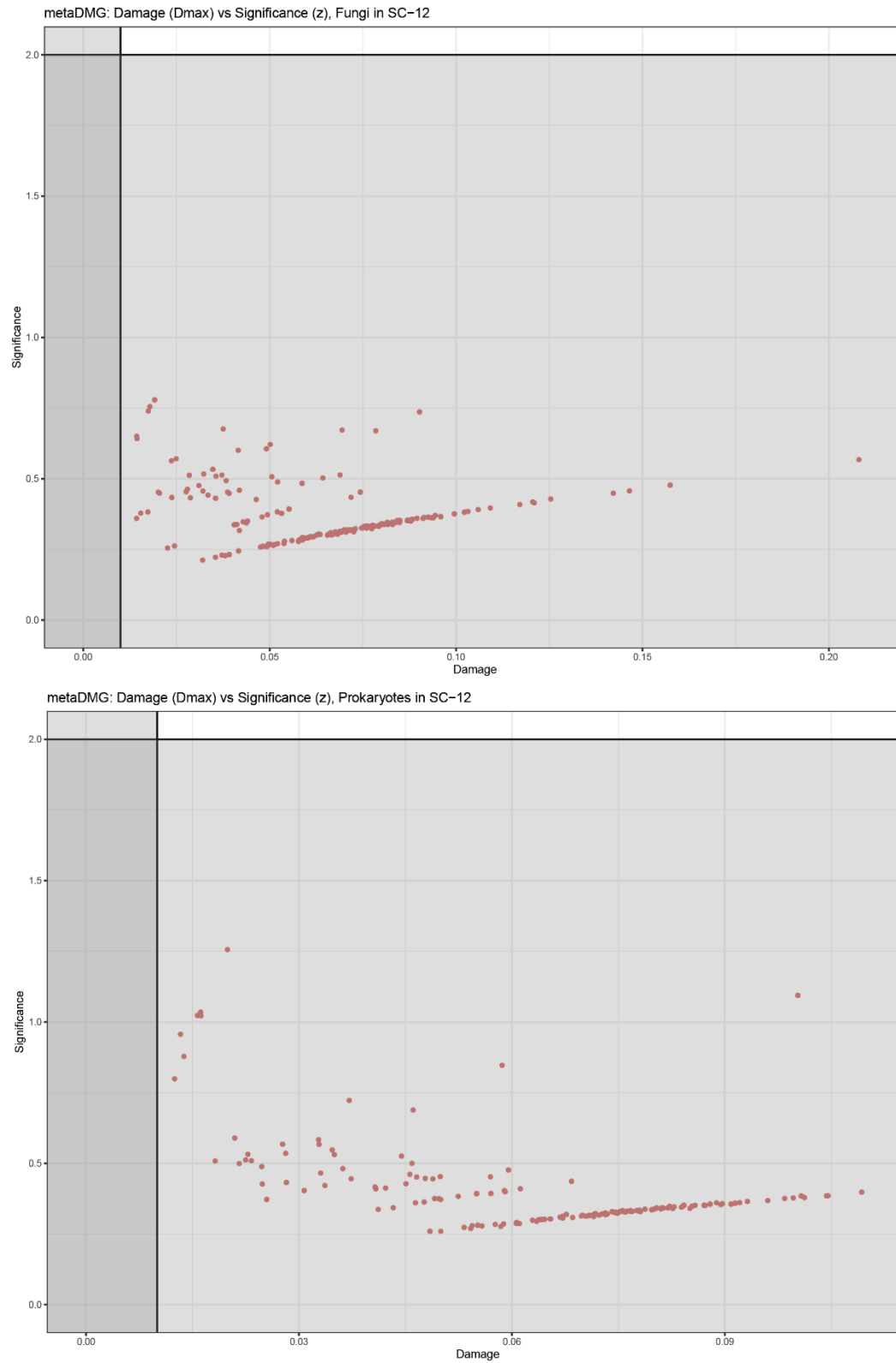


Fig. S77.
SC-12 *metaDMG* plot of fungi and prokaryote taxa above significance and damage thresholds with top genera.

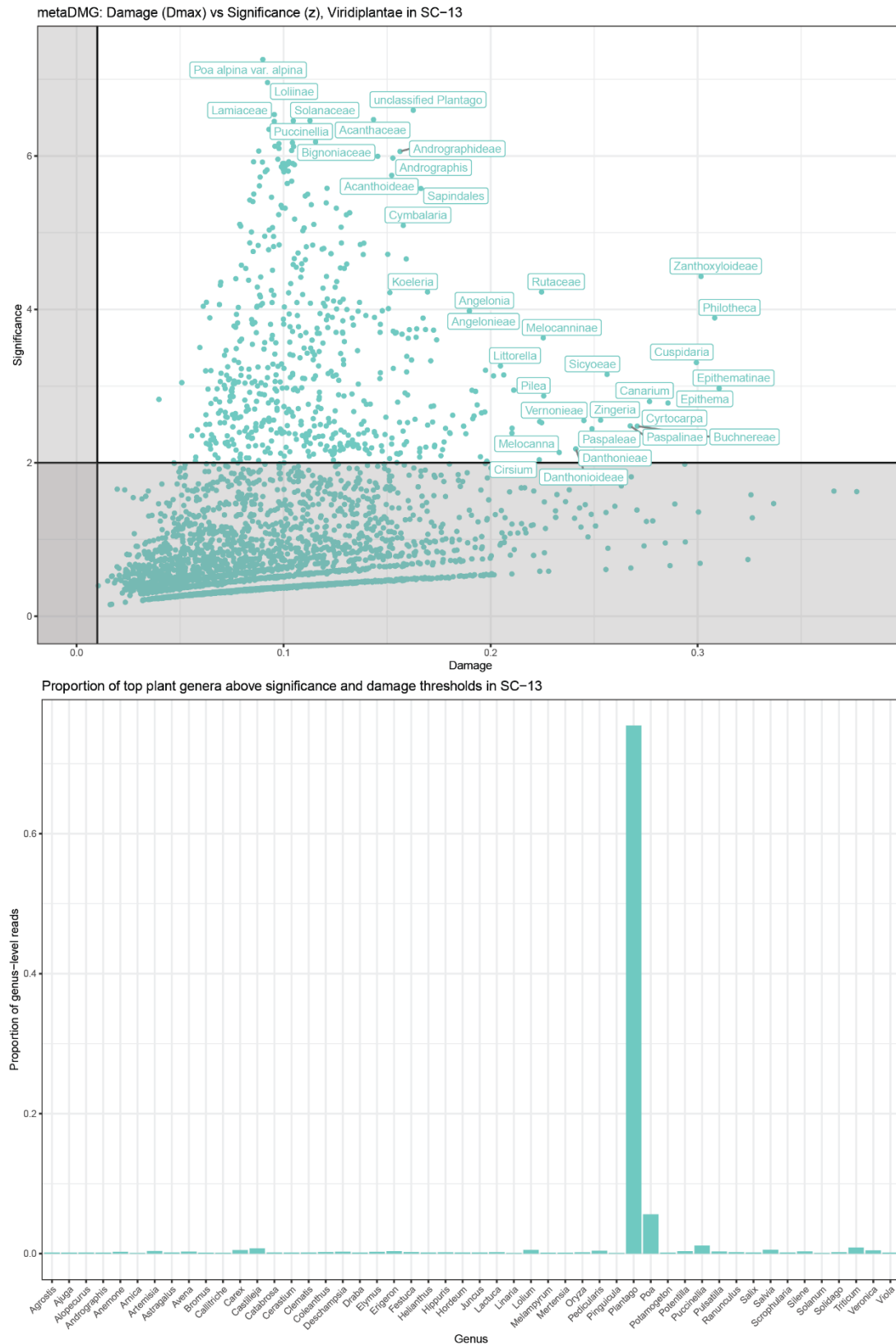


Fig. S78.
SC-13 *metaDMG* plot of plant taxa above significance and damage thresholds with top genera.

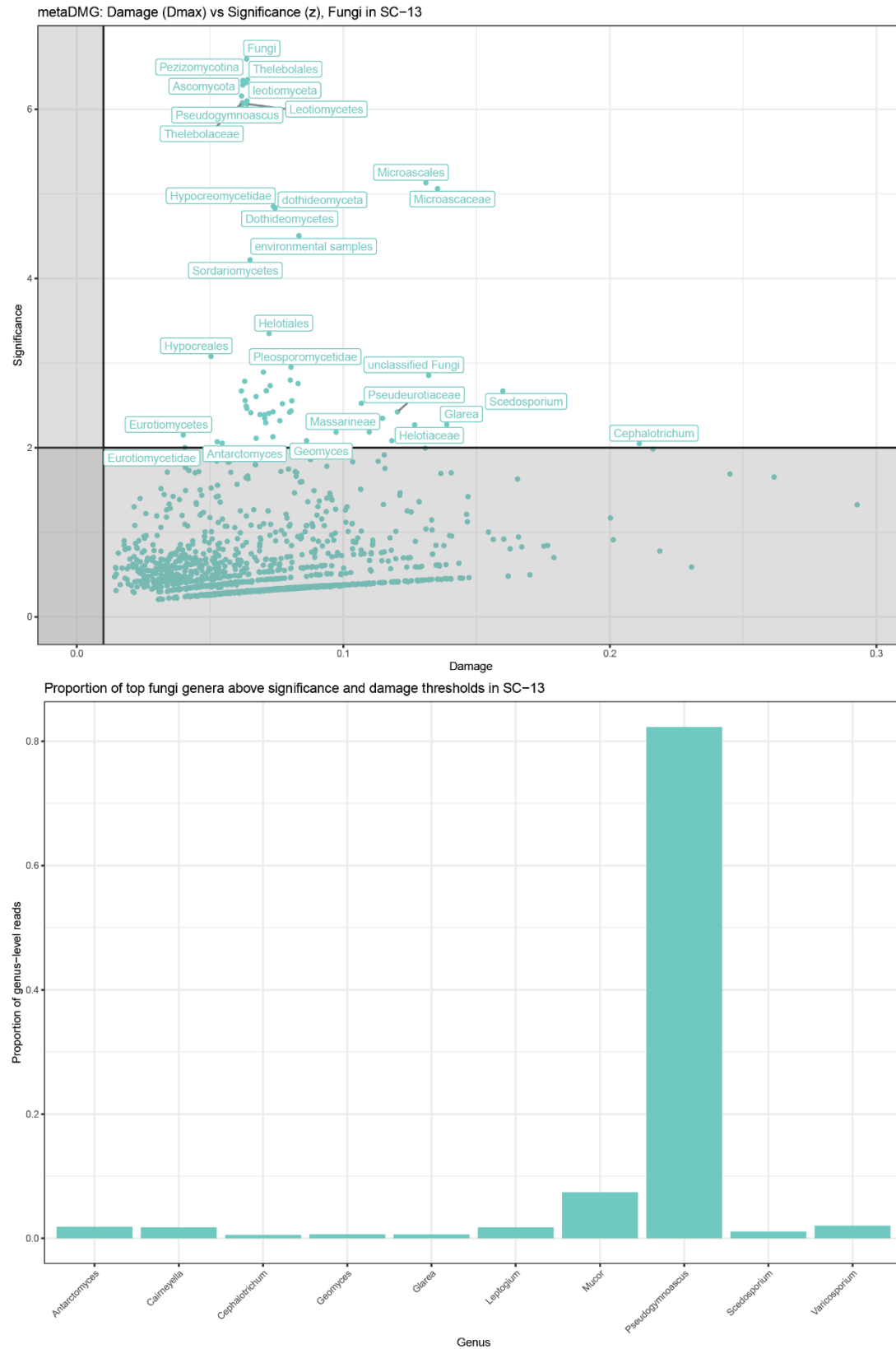


Fig. S80.
SC-13 metaDMG plot of fungi taxa above significance and damage thresholds with top genera.

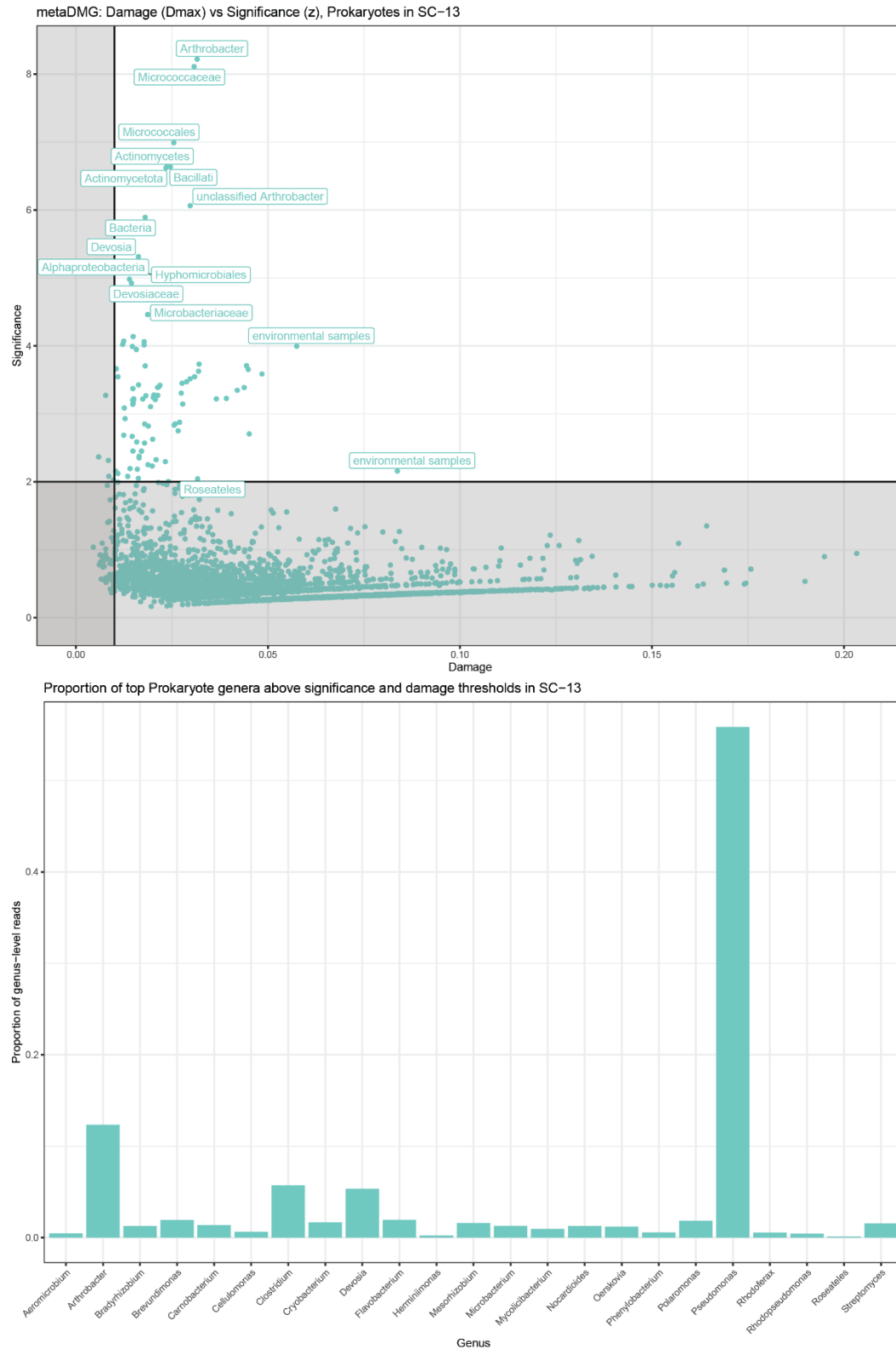


Fig. S81.
SC-13 *metaDMG* plot of prokaryote taxa above significance and damage thresholds with top genera.

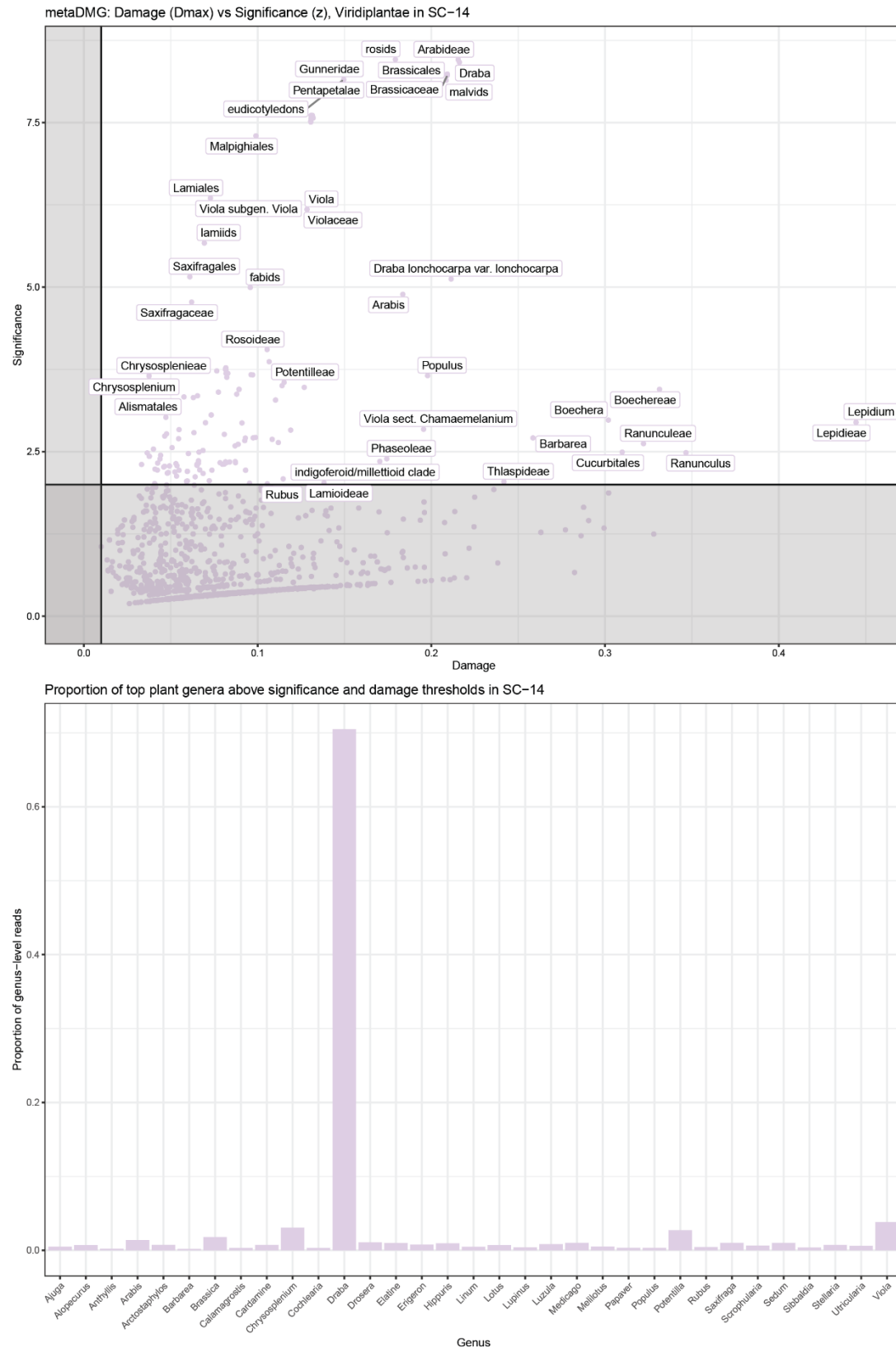


Fig. S82.
SC-14 metaDMG plot of plant taxa above significance and damage thresholds with top genera.

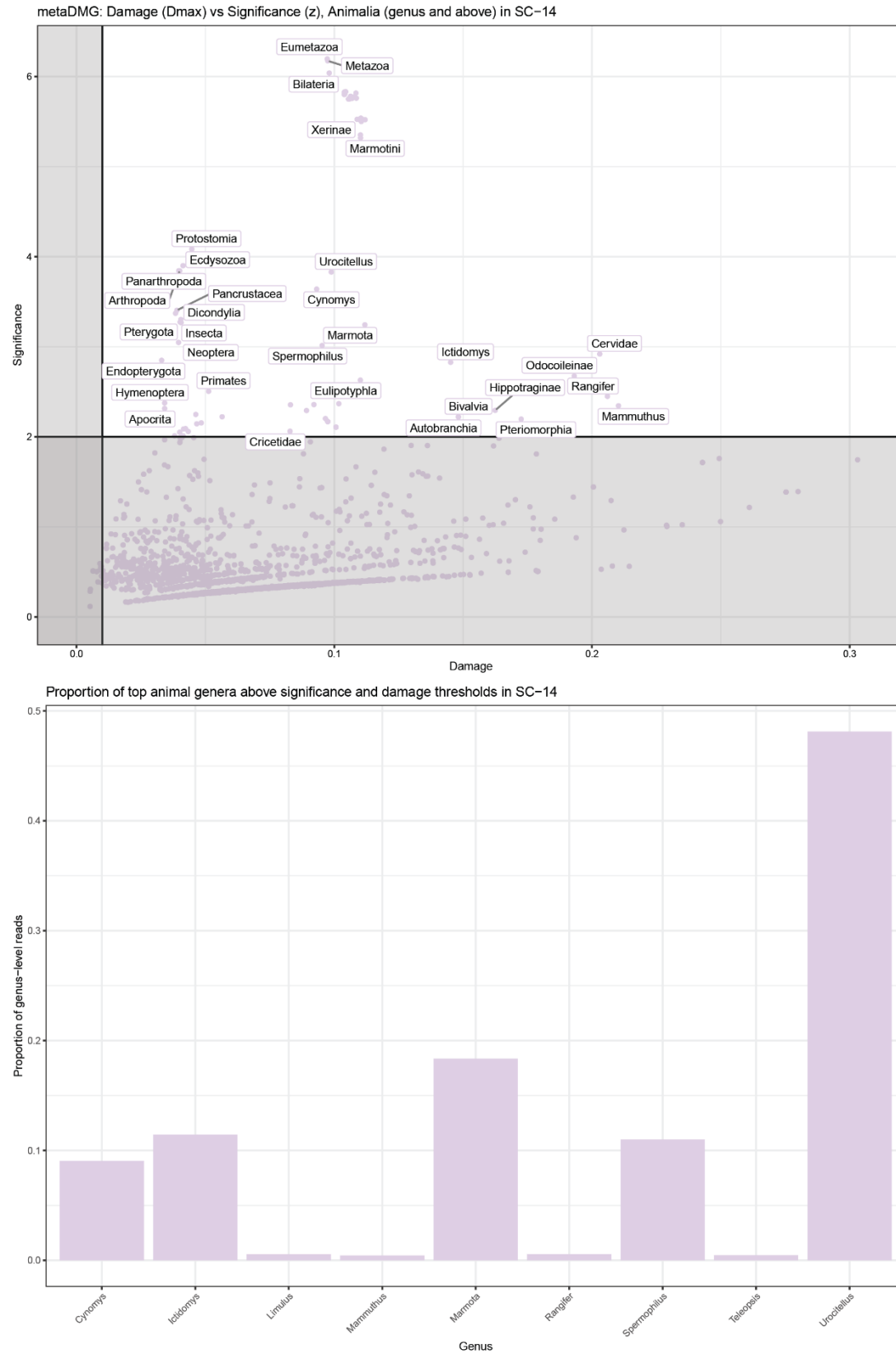
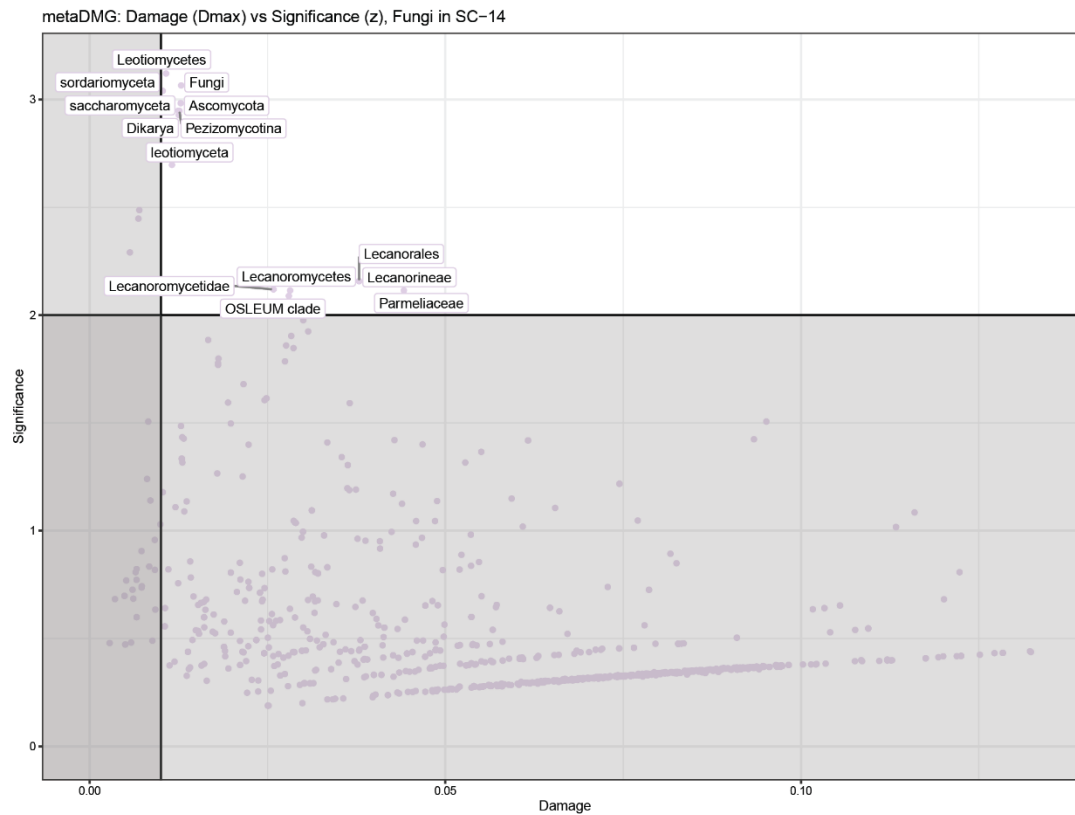


Fig. S83.
SC-14 *metaDMG* plot of animal taxa above significance and damage thresholds with top genera.



Proportion of top fungi genera above significance and damage thresholds in SC-14

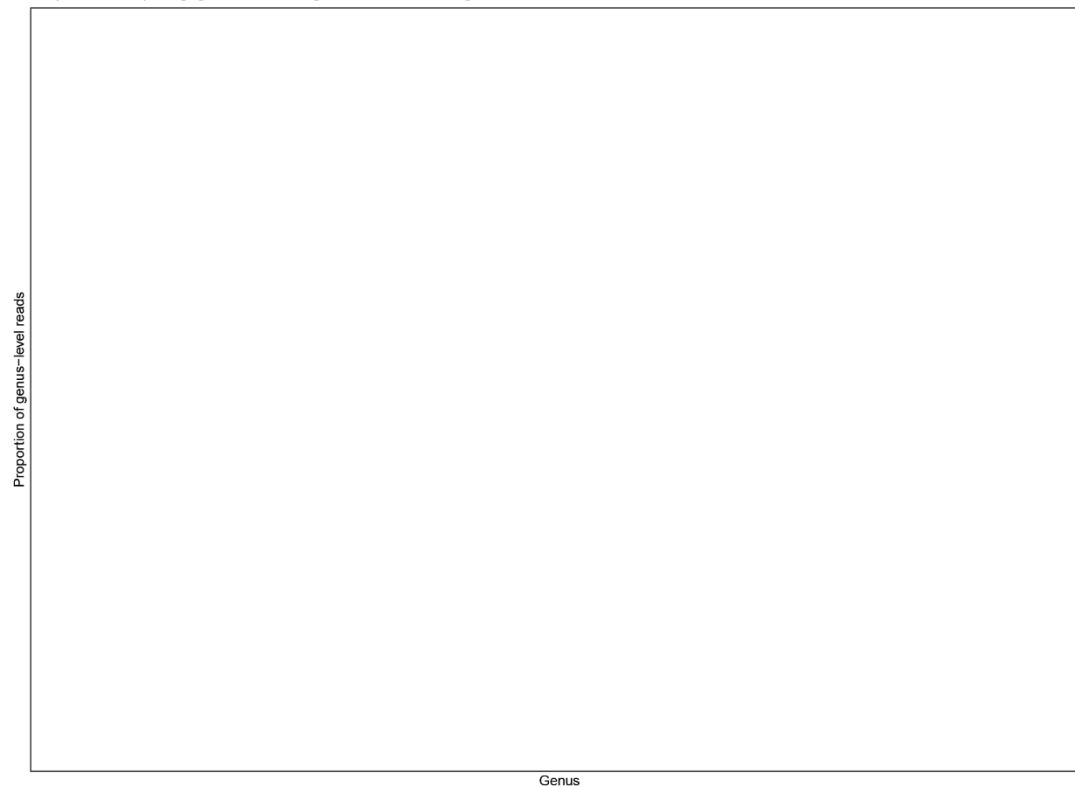


Fig. S84.
SC-14 *metaDMG* plot of fungi taxa above significance and damage thresholds with top genera.

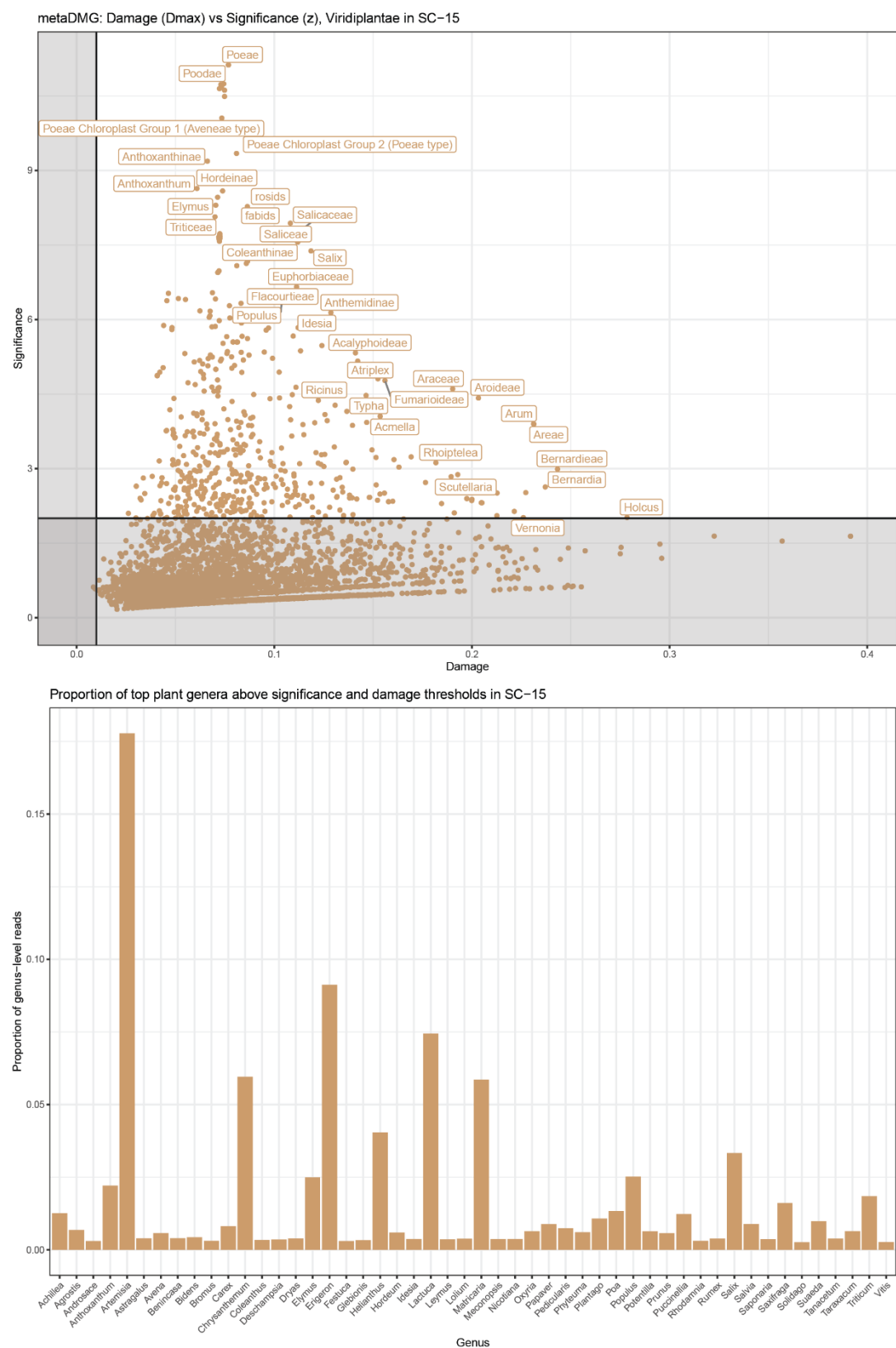


Fig. S86.
SC-15 *metaDMG* plot of plant taxa above significance and damage thresholds with top genera.

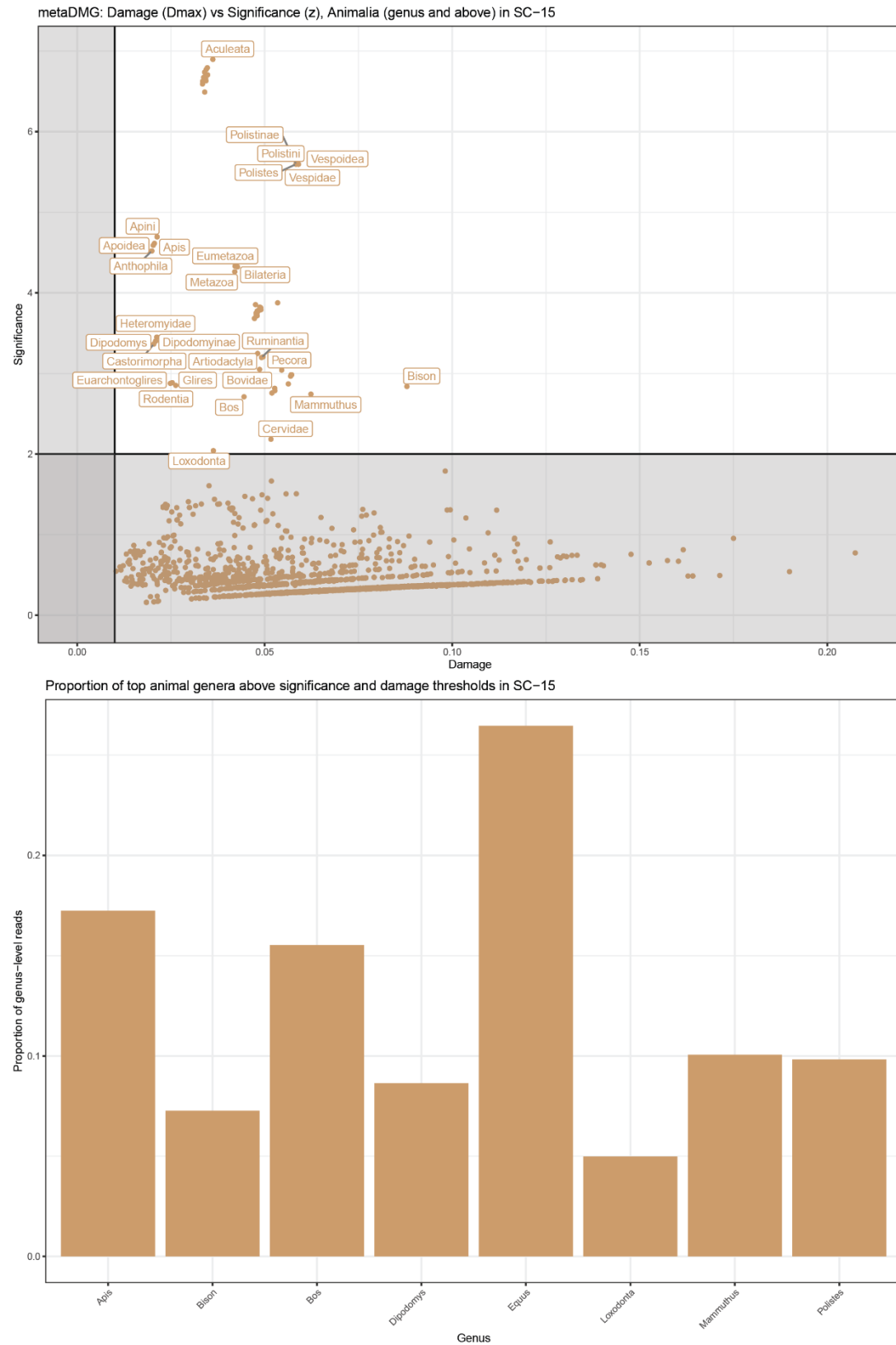


Fig. S87.
SC-15 *metaDMG* plot of animal taxa above significance and damage thresholds with top genera.

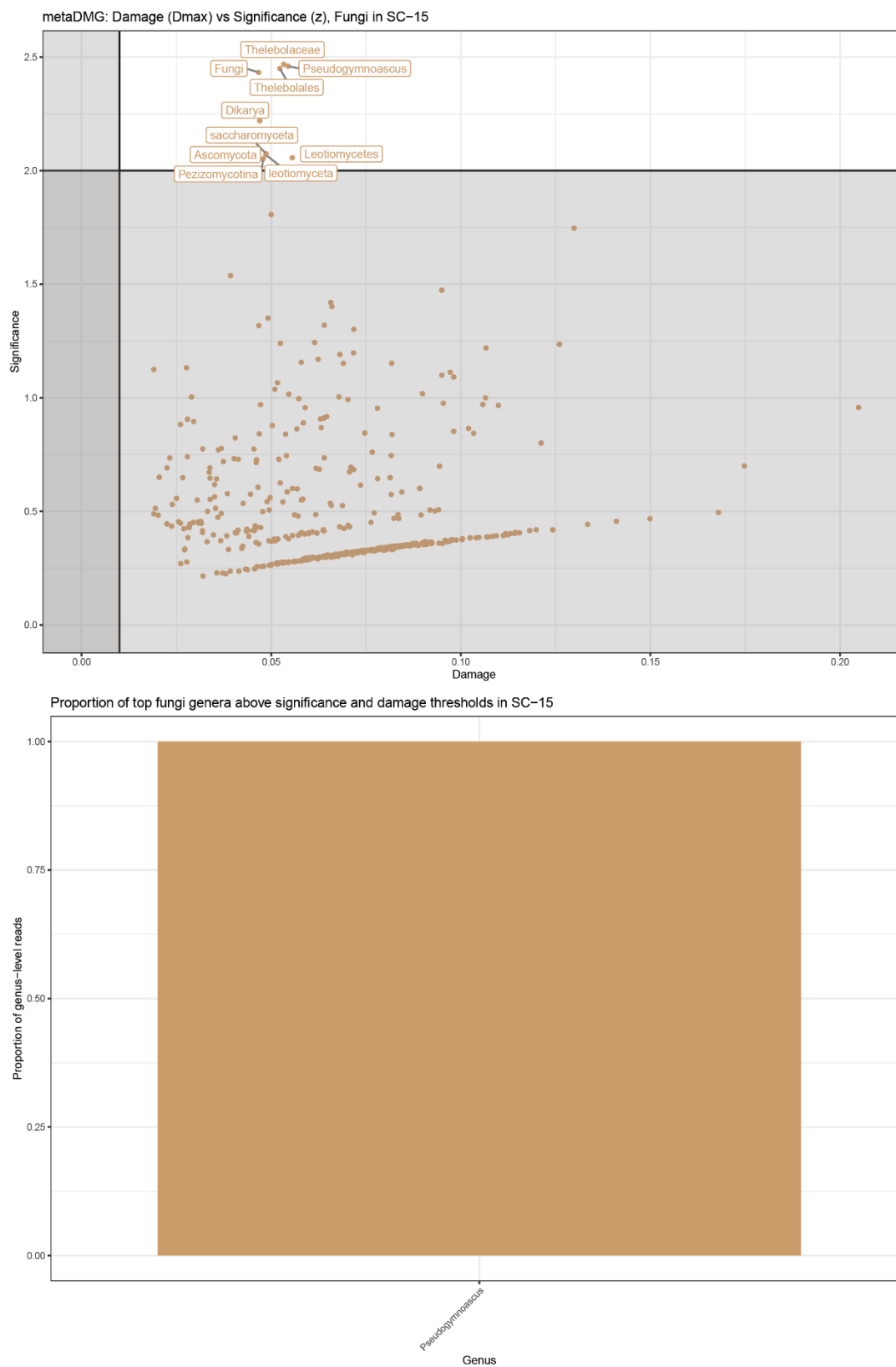


Fig. S88.
SC-15 *metaDMG* plot of fungi taxa above significance and damage thresholds with top genera.

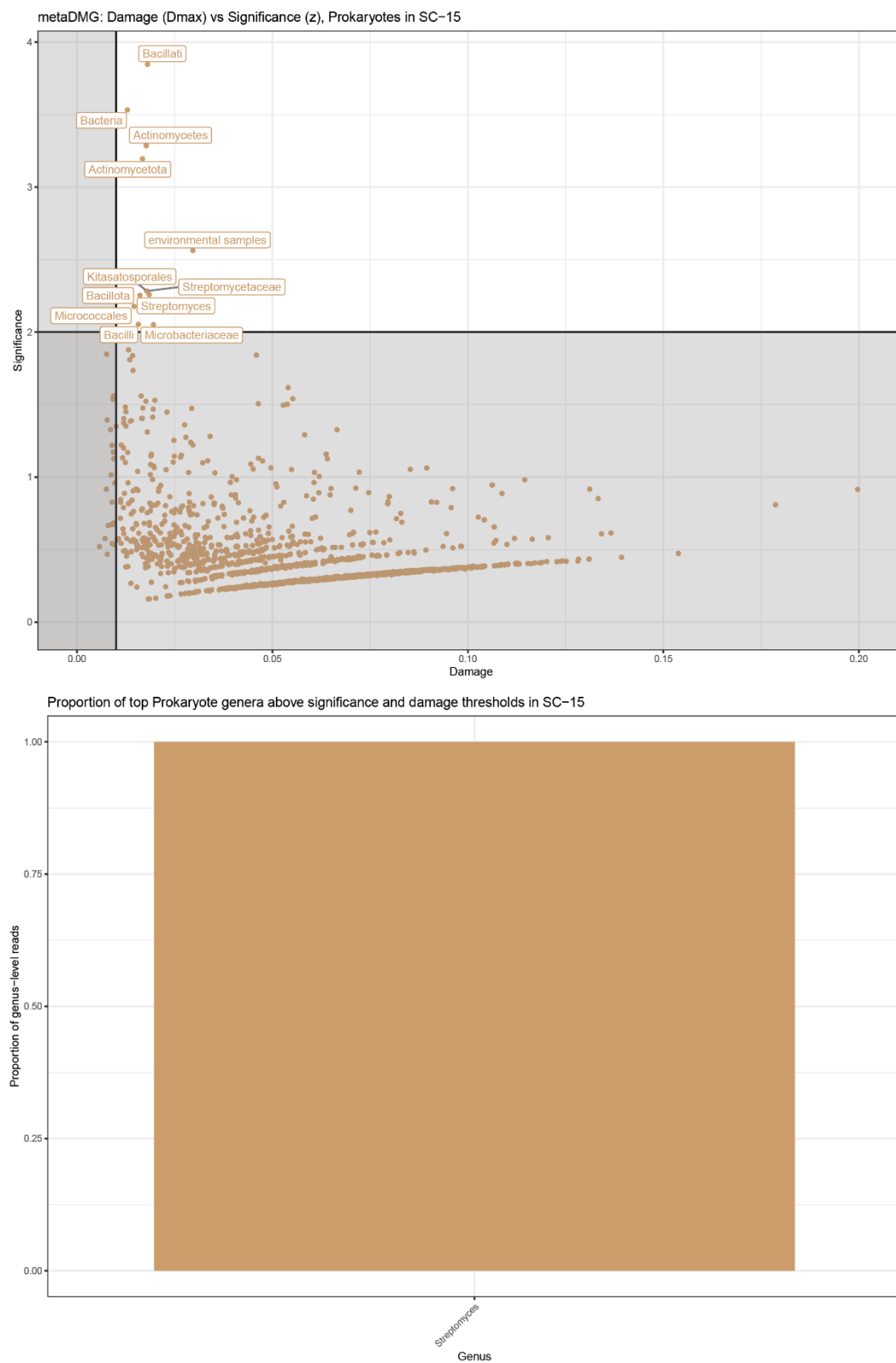


Fig. S89.
SC-15 *metaDMG* plot of prokaryote taxa above significance and damage thresholds with top genera.



Fig. S90.
SC-16 metaDMG plot of plant taxa above significance and damage thresholds with top genera.

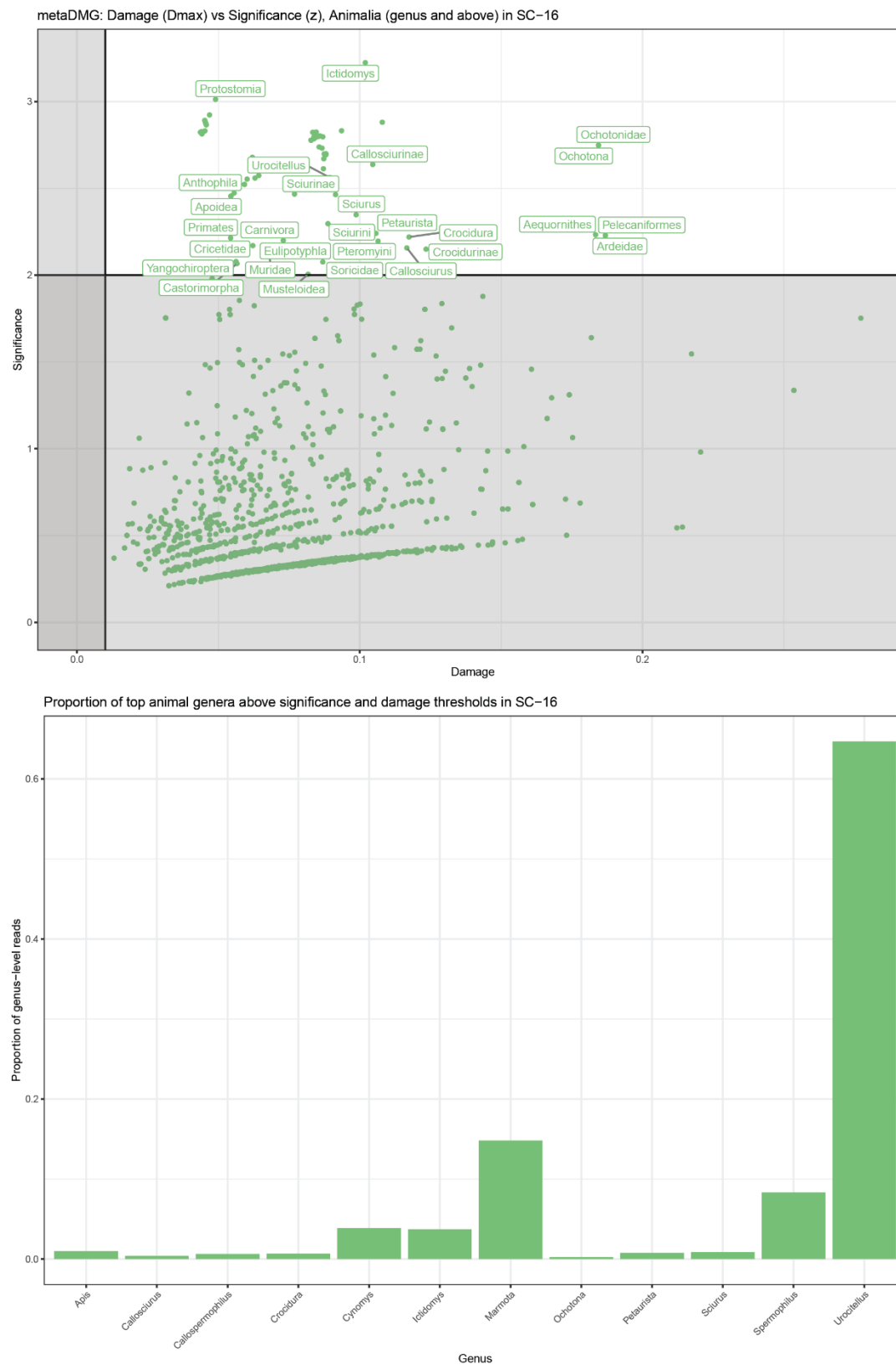
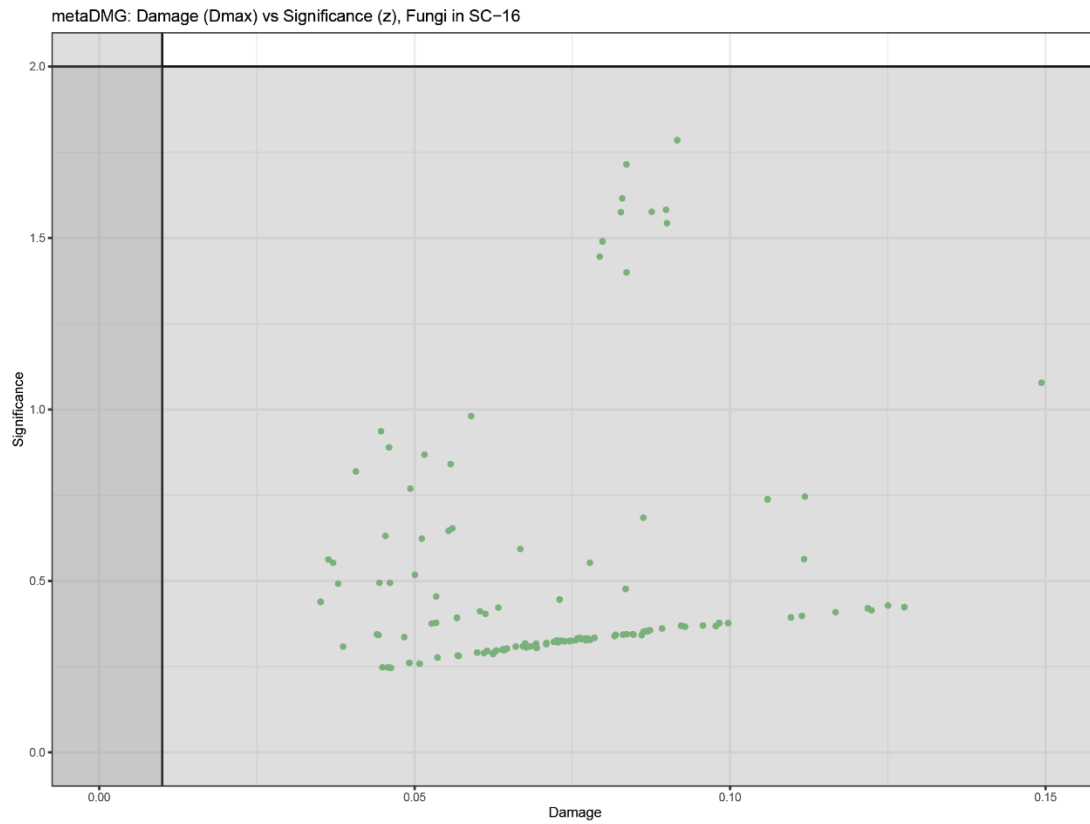


Fig. S91.
SC-16 *metaDMG* plot of animal taxa above significance and damage thresholds with top genera.



Proportion of top fungi genera above significance and damage thresholds in SC-16

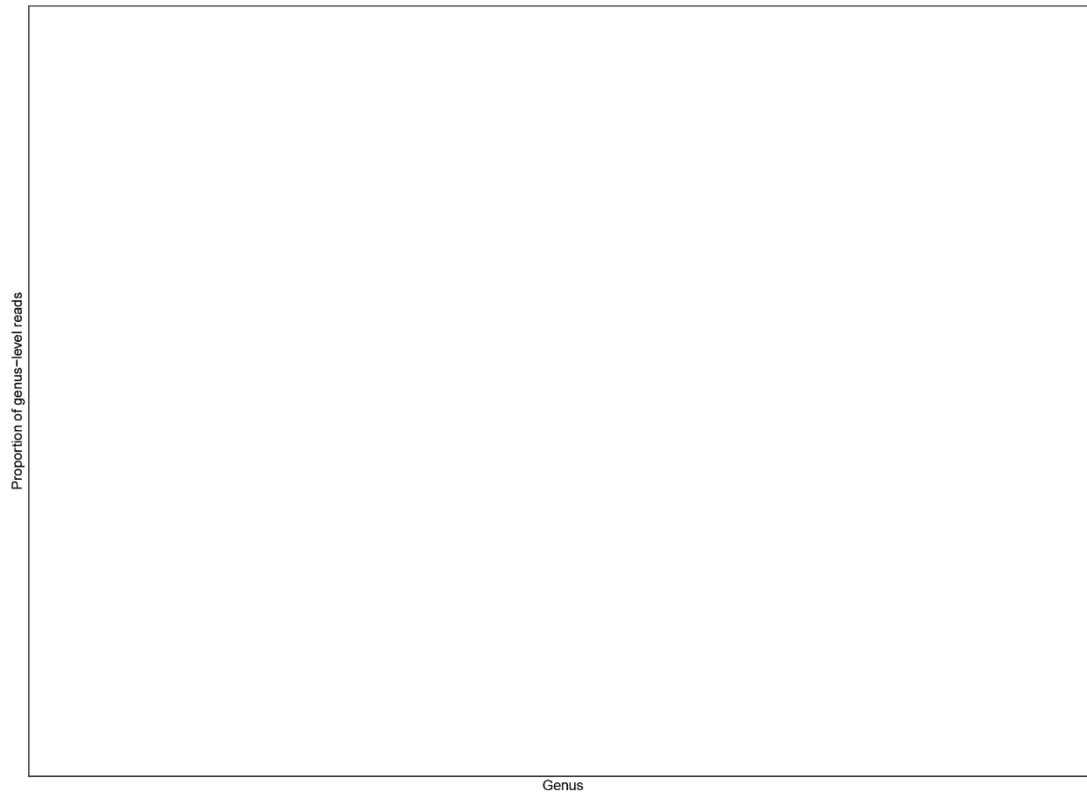


Fig. S92.

SC-16 *metaDMG* plot of fungi taxa above significance and damage thresholds with top genera.

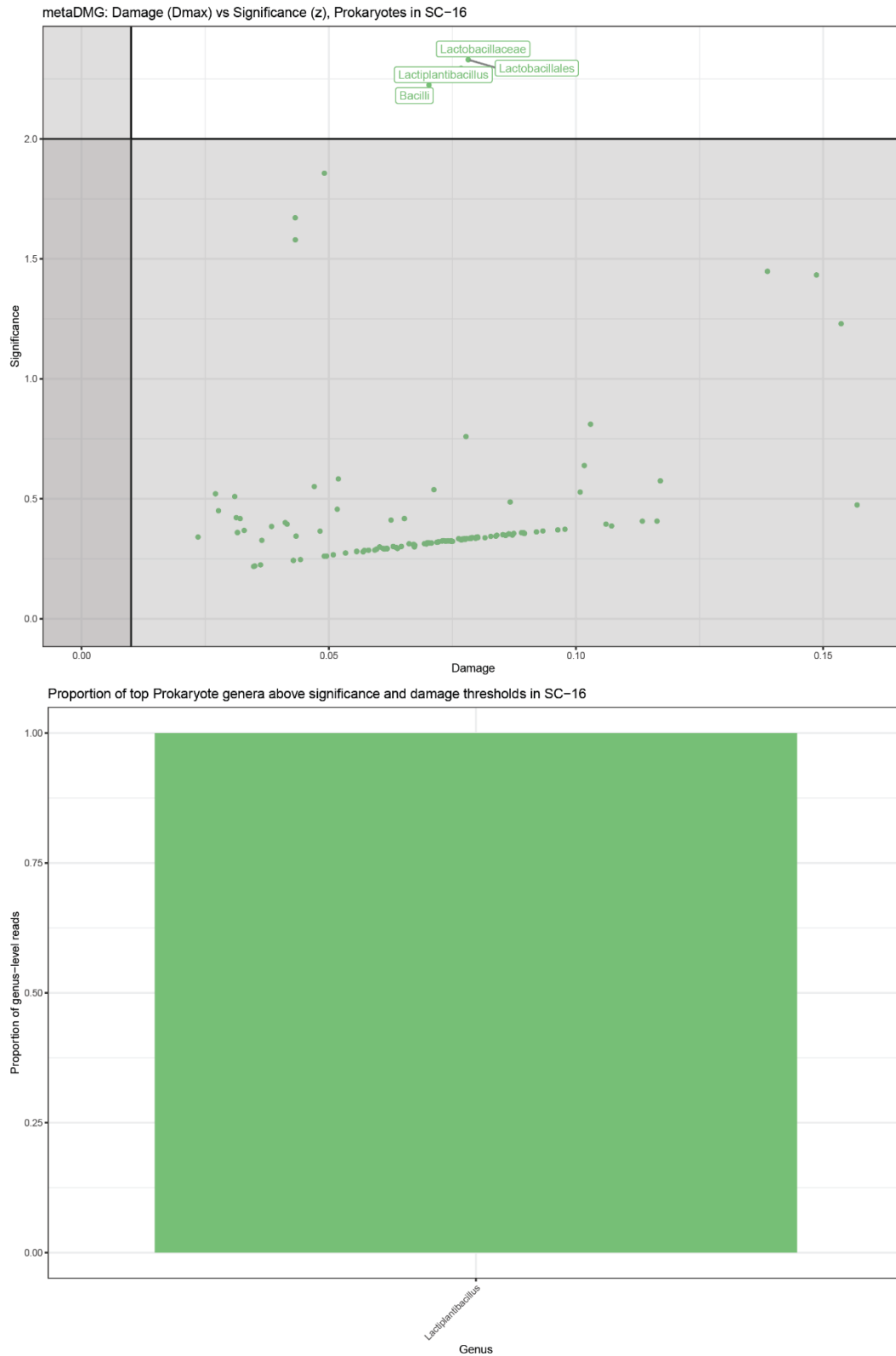


Fig. S93.
SC-16 *metaDMG* plot of prokaryote taxa above significance and damage thresholds with top genera.

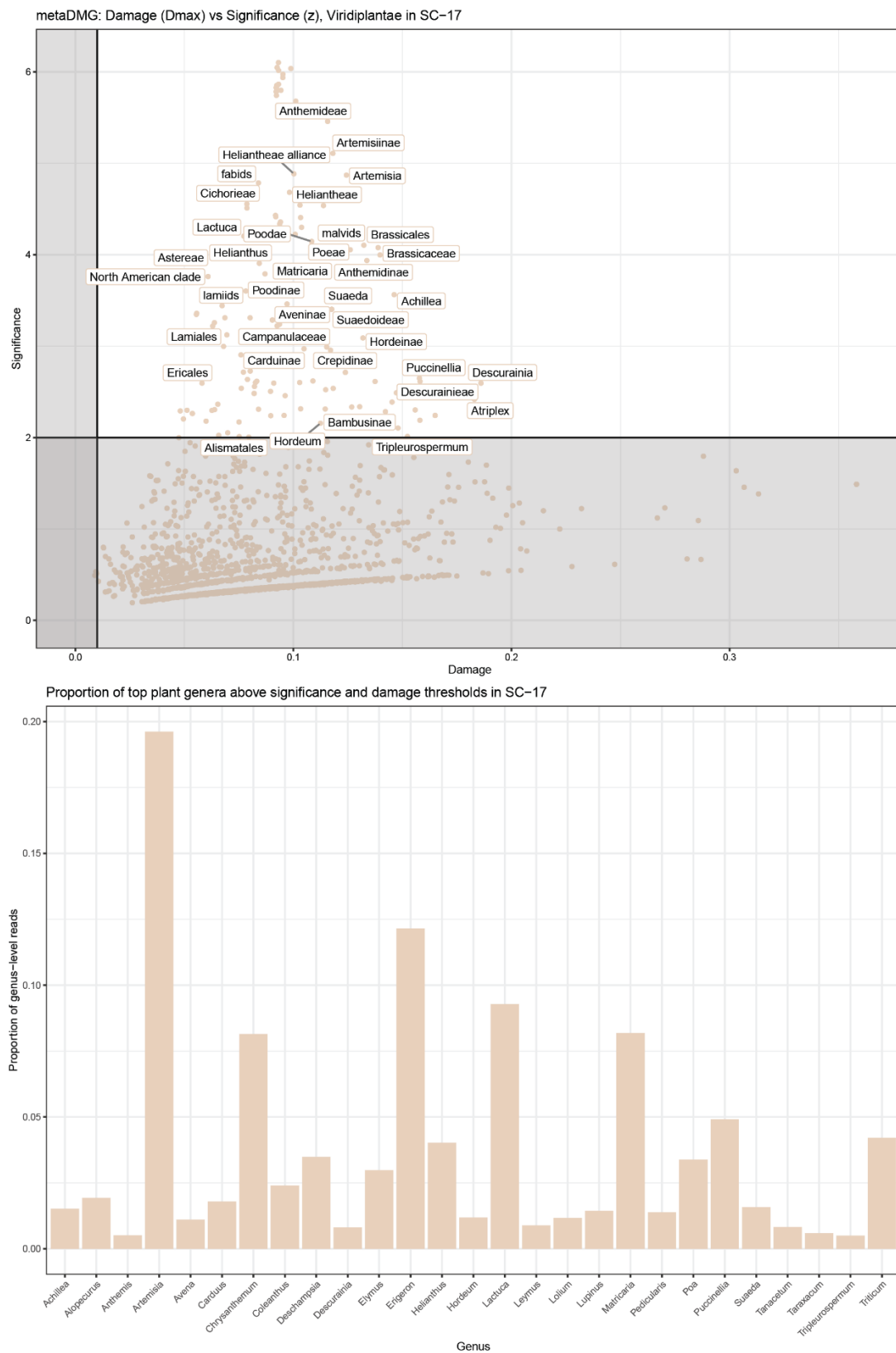


Fig. S94.
SC-17 metaDMG plot of plant taxa above significance and damage thresholds with top genera.

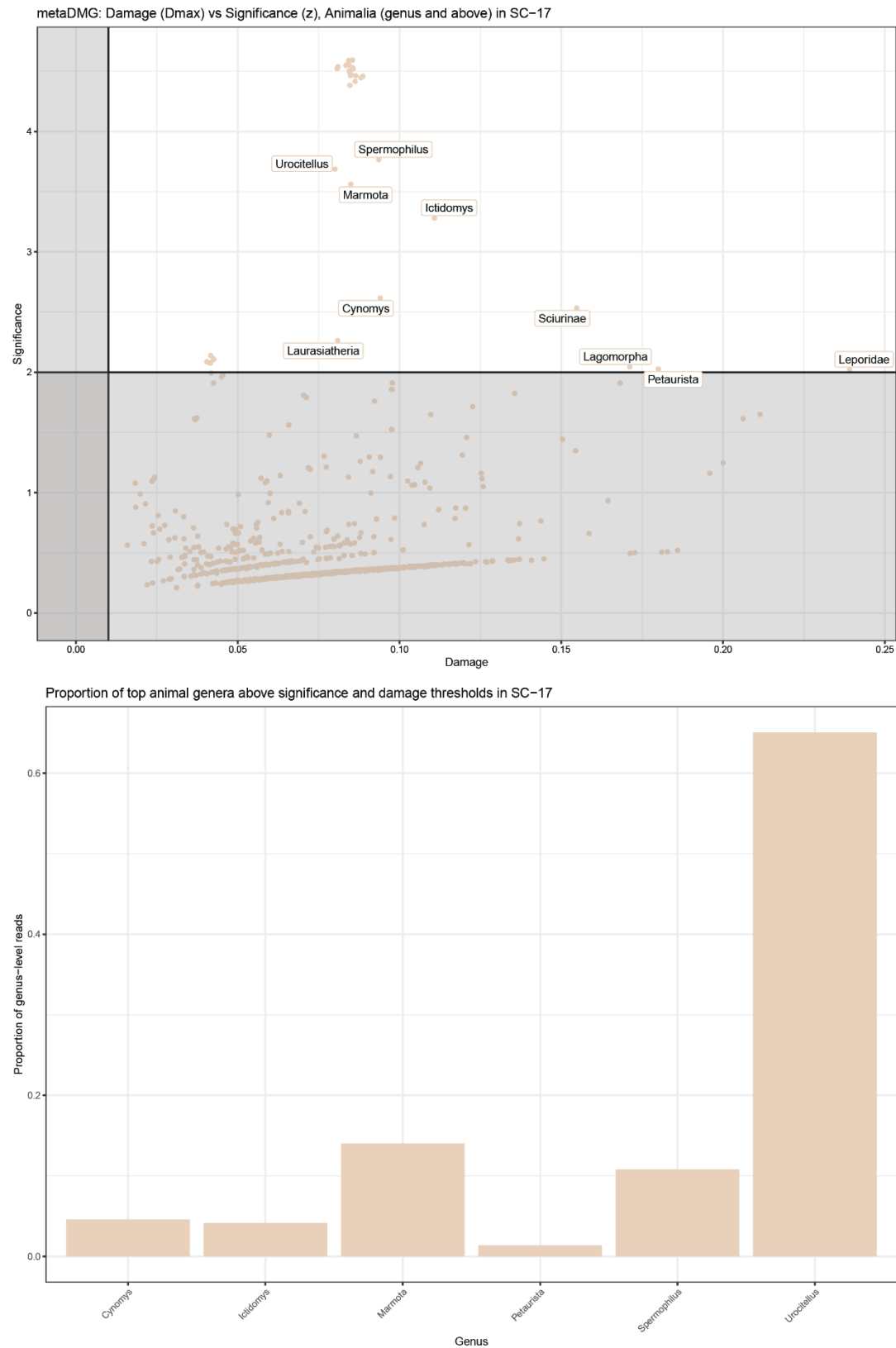
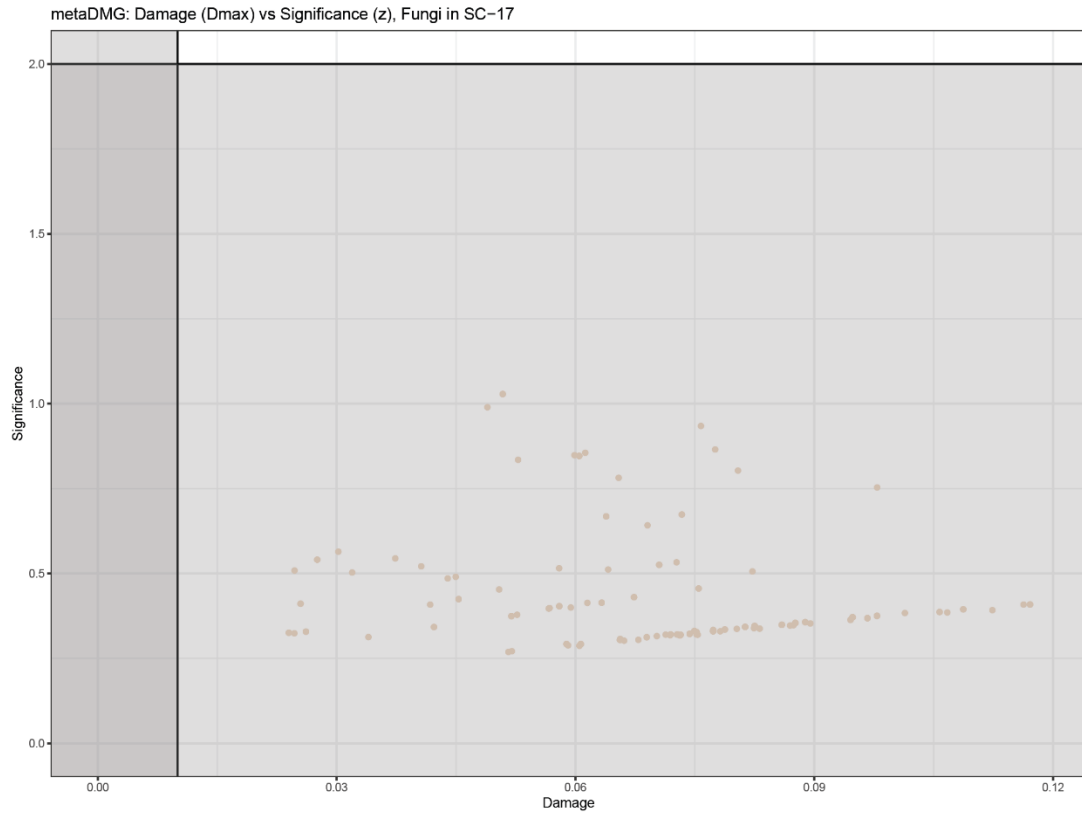


Fig. S95.
SC-17 metaDMG plot of animal taxa above significance and damage thresholds with top genera.



Proportion of top fungi genera above significance and damage thresholds in SC-17

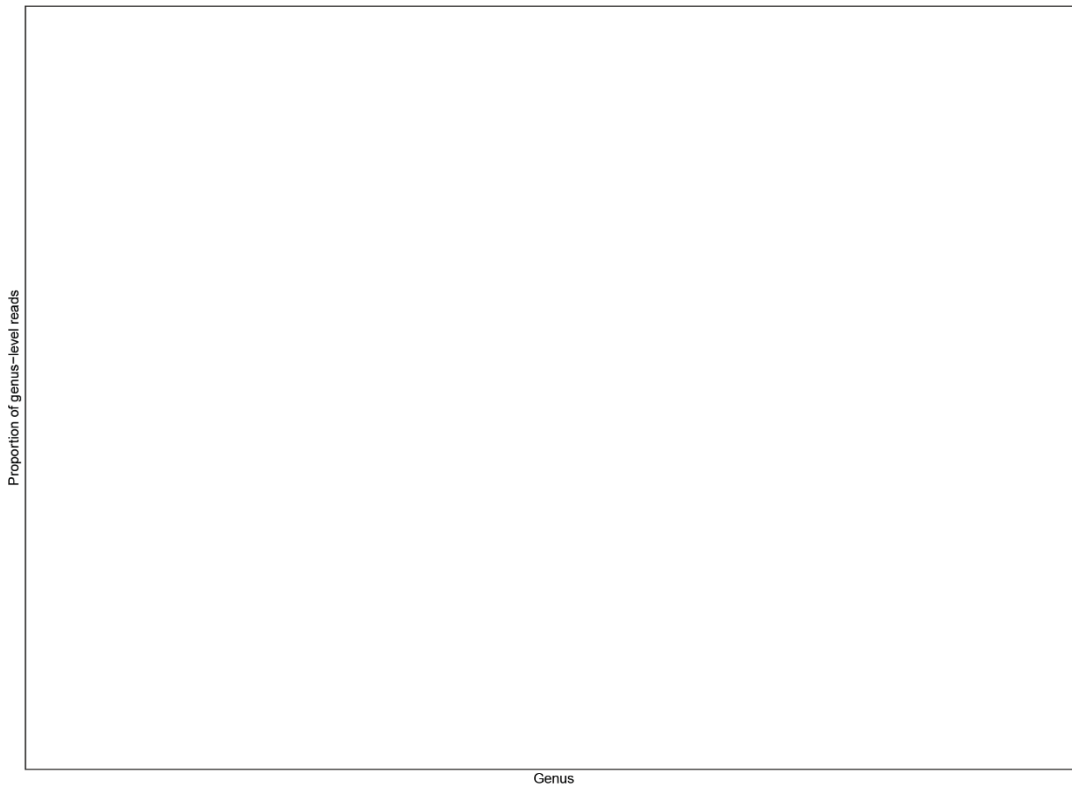
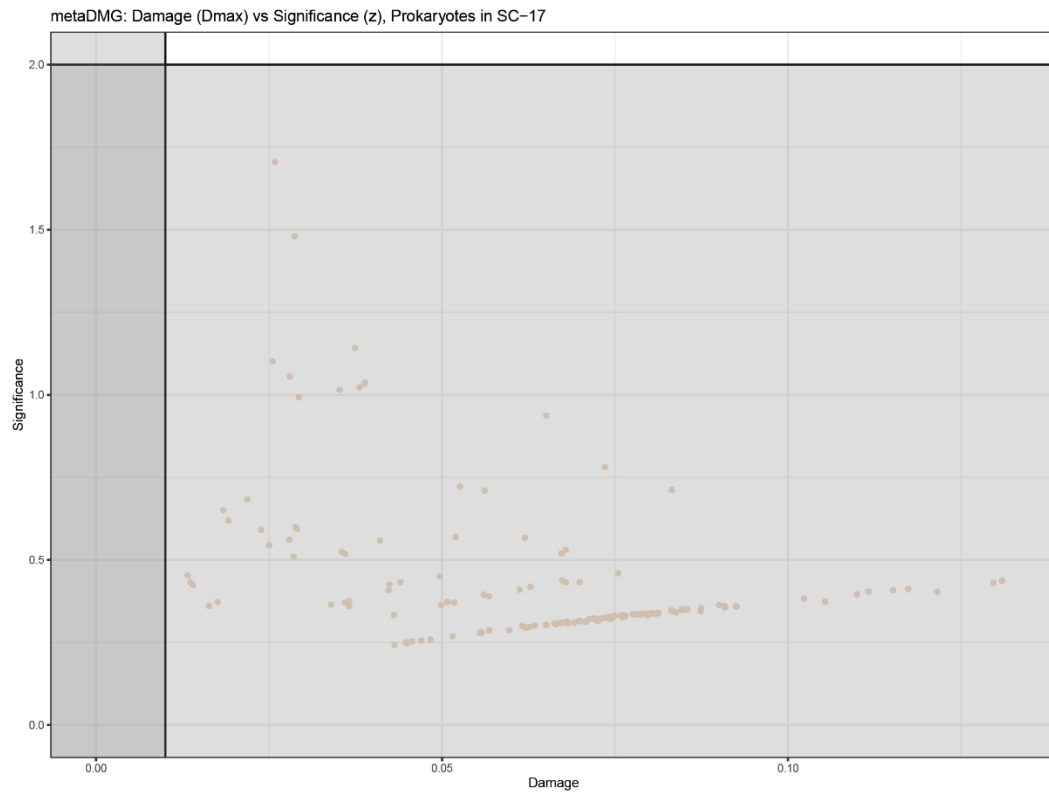


Fig. S96.
SC-17 *metaDMG* plot of fungi taxa above significance and damage thresholds with top genera.



Proportion of top Prokaryote genera above significance and damage thresholds in SC-17

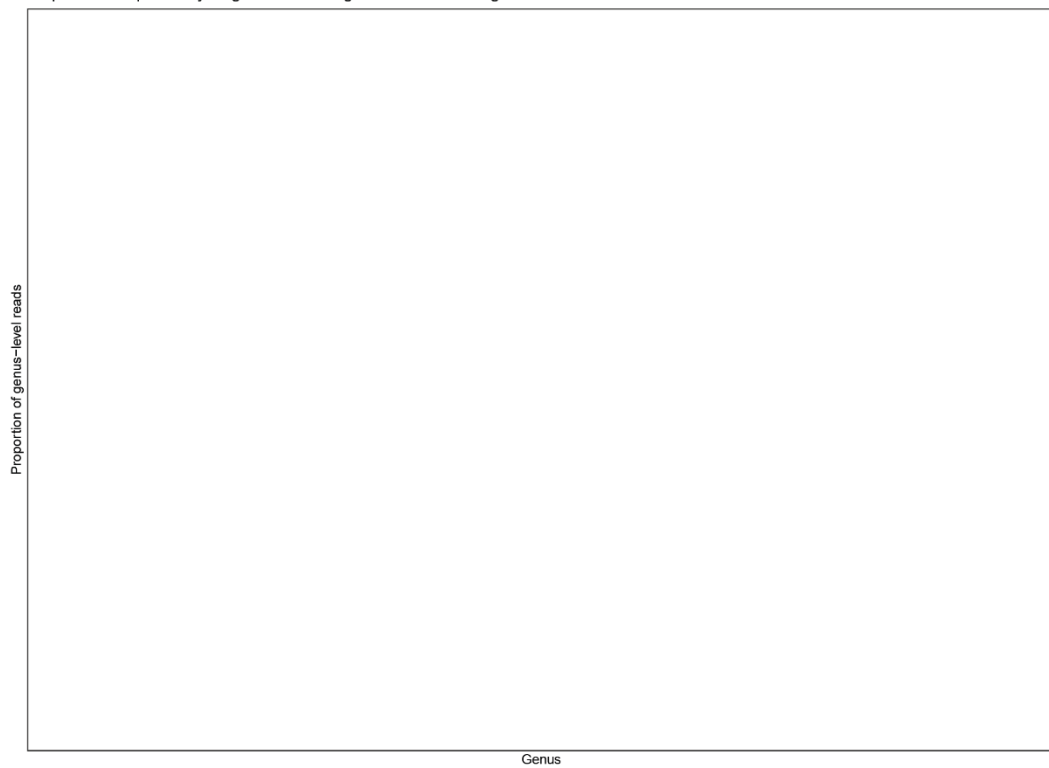


Fig. S97.
SC-17 *metaDMG* plot of prokaryote taxa above significance and damage thresholds with top genera.

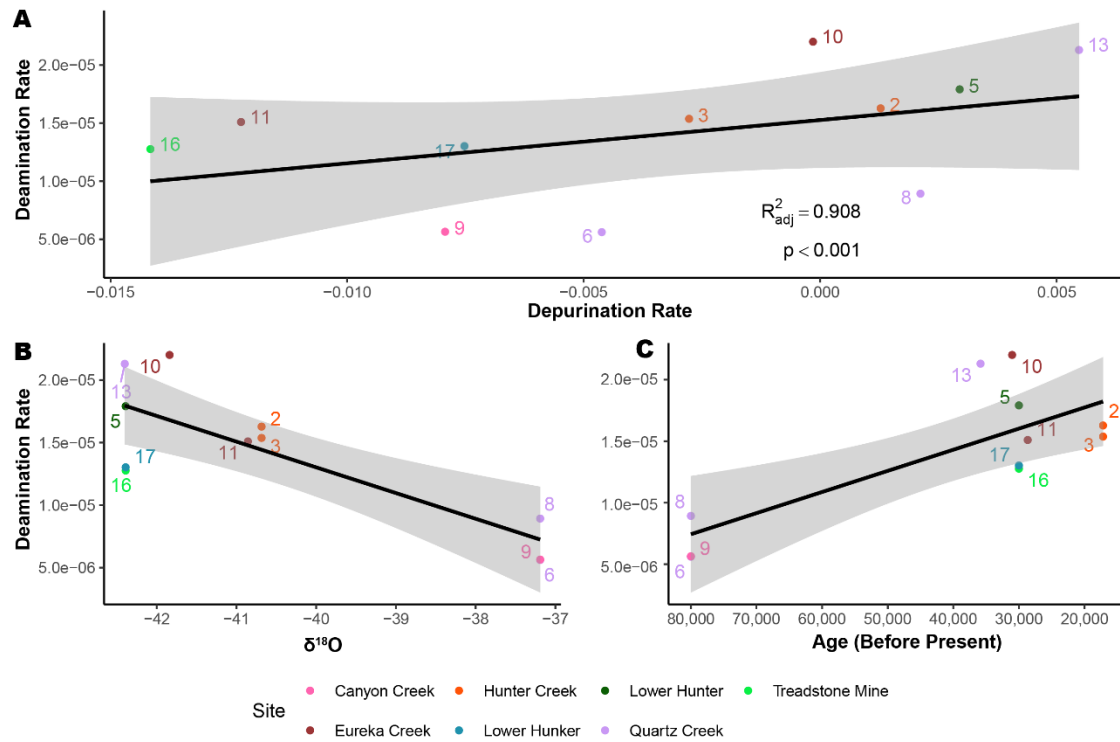


Fig. S98.
Deamination and depurination rates (**A**) plotted against $\delta^{18}O$ (GISP2) (**B**) and age (**C**).

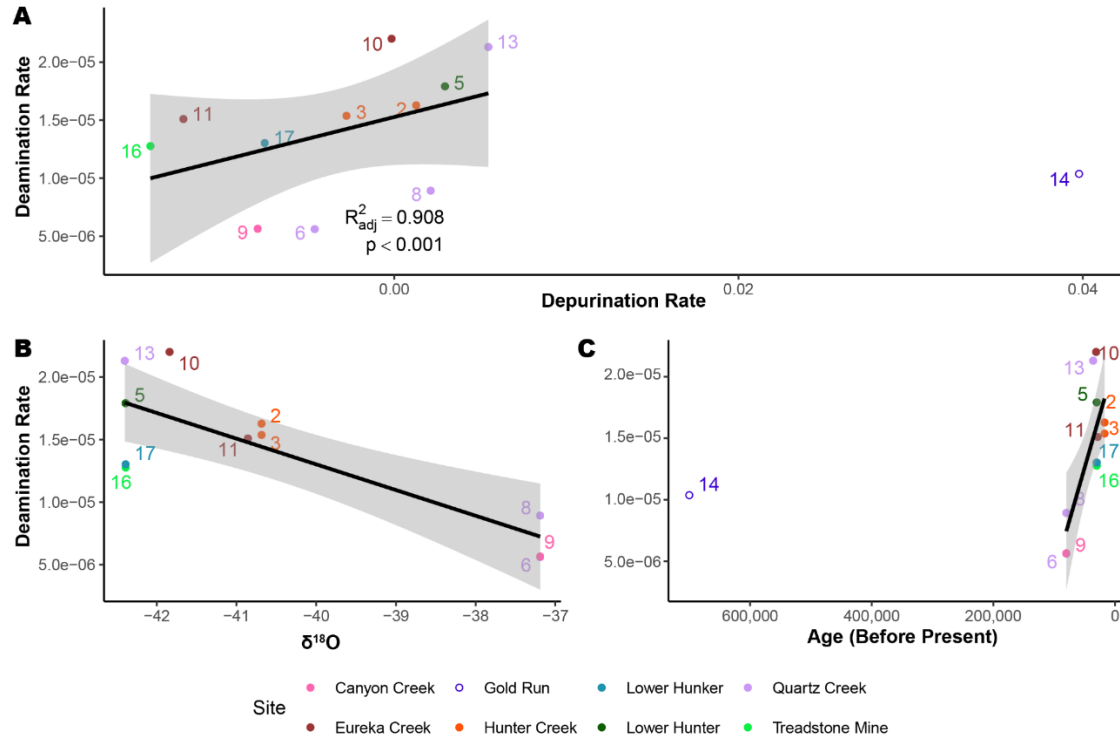


Fig. S99.
Deamination and depurination rates (**A**) plotted against $\delta^{18}O$ (GISP2) (**B**) and age (**C**) with SC-14.

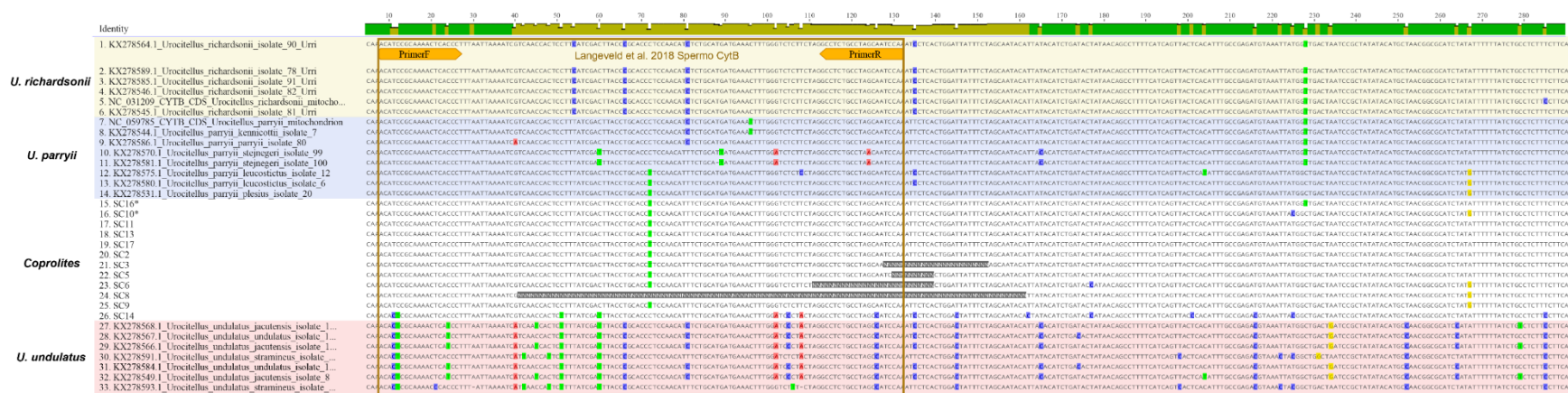
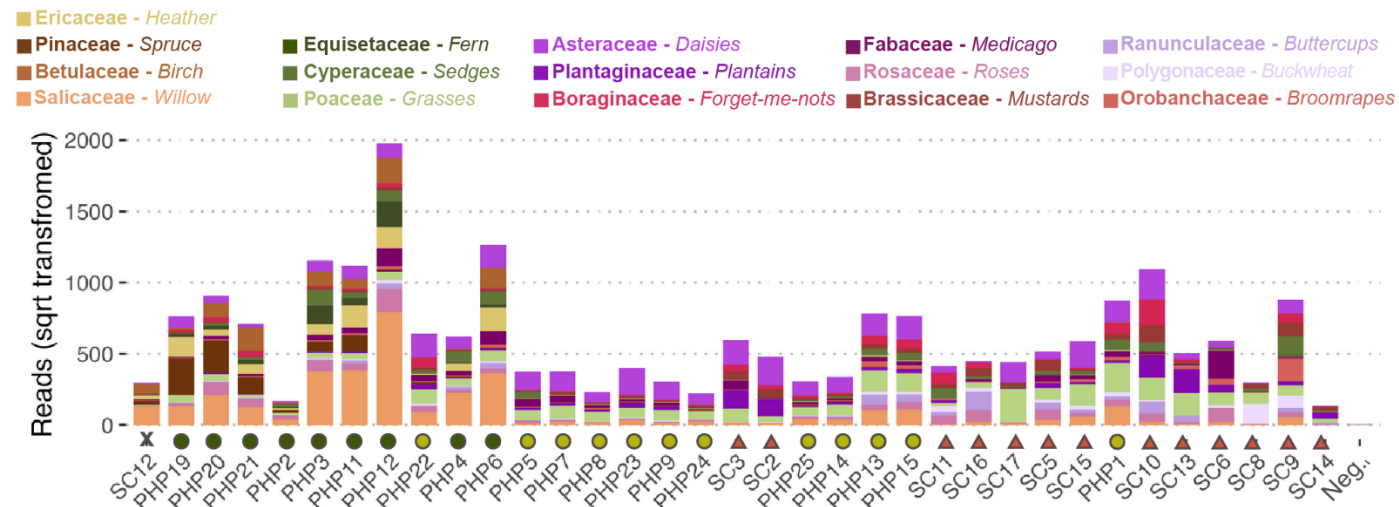


Fig. S100.
Cytochrome-b alignment with primer landing sites from Langveld et al. (2018).

A Plant Families



B Animal Families

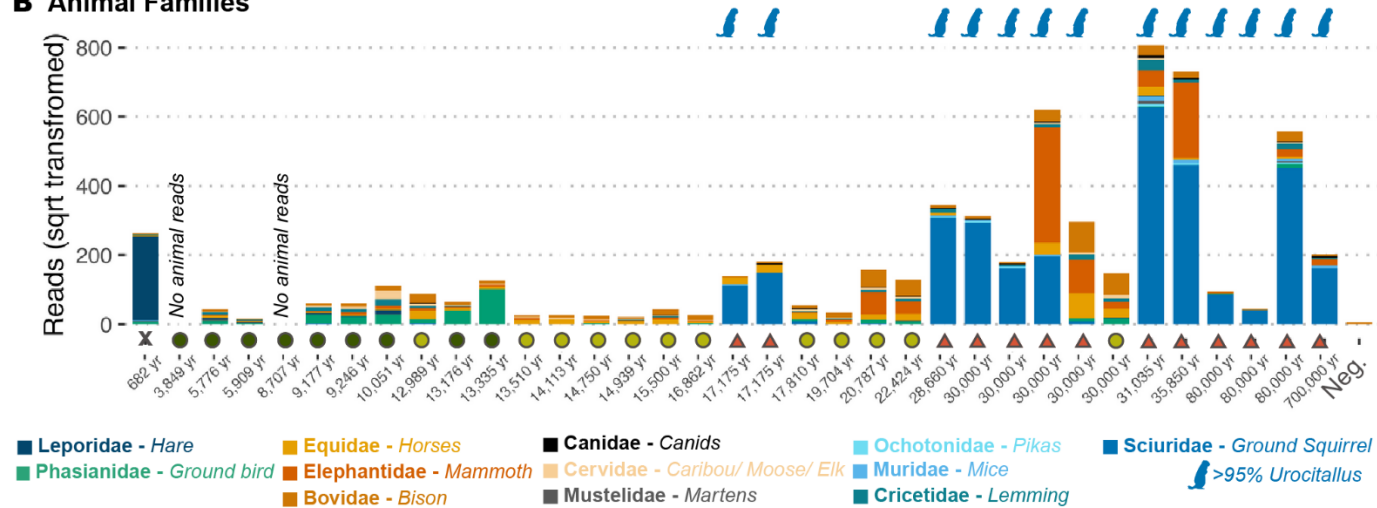


Fig. S101.

Sediment and coprolite aeDNA reconstructed ecology change through the Quaternary. Read counts from Fig. 9.

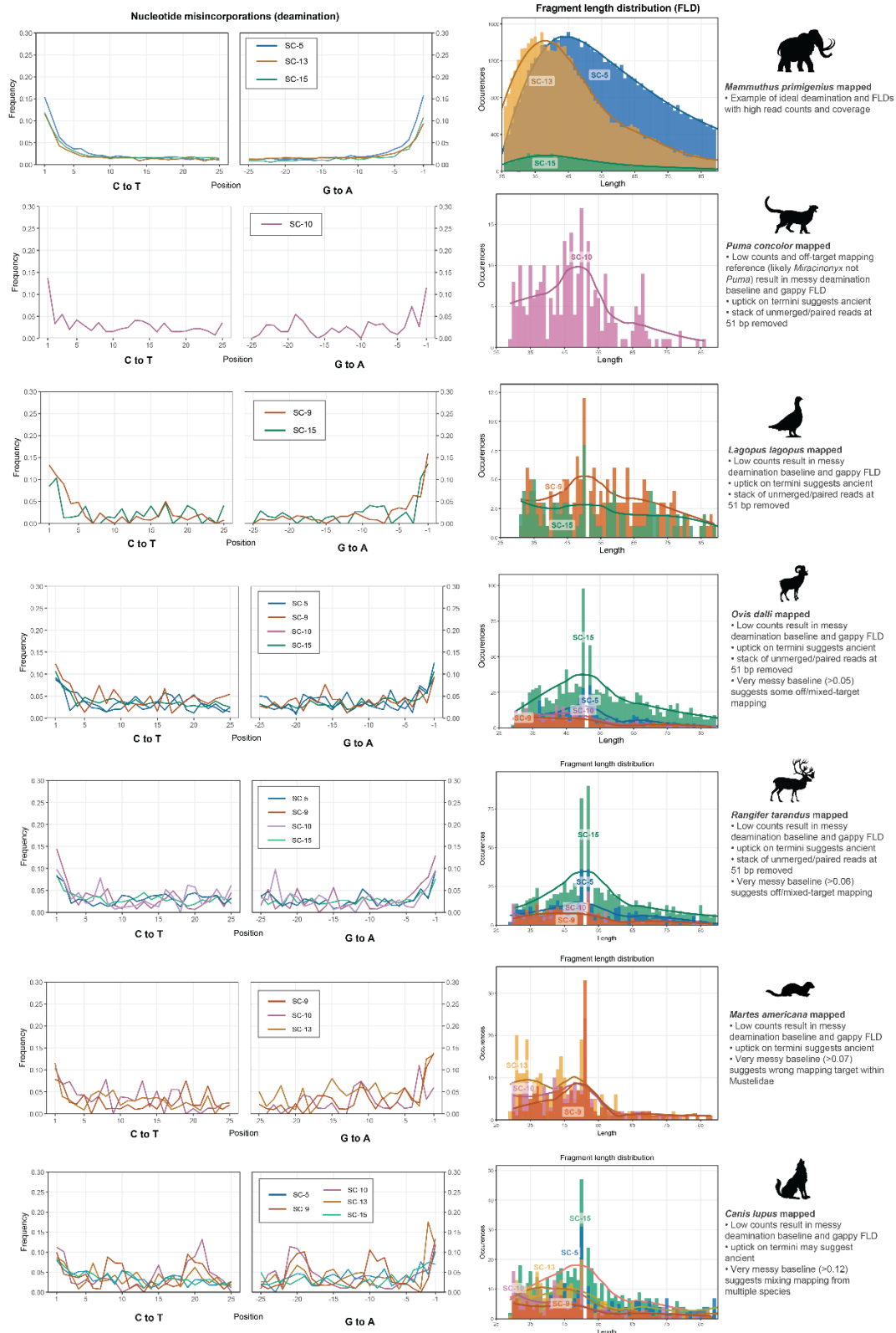


Fig. S102.

Deamination and FLD plots of competitively mapped reads between *Urocitellus parryii* and the low signal targets shown here. Libraries merged by coprolite ID (i.e. SC-3, SC-9).

Table S1. Sample libraries sequenced.

Sample	Type	Sequenced reads	Shotgun/enriched	Enrichment Target
LSC-10a-E2	Coprolite	4,306,624	Enriched	Proboscideans
LSC-10c-E2	Coprolite	4,170,495	Enriched	Proboscideans
LSC-13a-E2	Coprolite	3,744,163	Enriched	Proboscideans
LSC-13c-E2	Coprolite	4,183,915	Enriched	Proboscideans
LSC-15ci-E2	Coprolite	3,802,554	Enriched	Proboscideans
LSC-15cii-E2	Coprolite	3,100,894	Enriched	Proboscideans
LSC-5ci-E2	Coprolite	4,687,293	Enriched	Proboscideans
LSC-5cii-E2	Coprolite	3,819,547	Enriched	Proboscideans
LSC-9ci-E2	Coprolite	4,567,974	Enriched	Proboscideans
LSC-9cii-E2	Coprolite	3,386,763	Enriched	Proboscideans
Li2-SC14-ci-EMp	Coprolite	18,565,383	Enriched	Proboscideans
Li2-SC14-ci-U-EMp	Coprolite	8,749,462	Enriched	Proboscideans
Li2-SC14-cii-EMp	Coprolite	14,094,113	Enriched	Proboscideans
Li2-SC14-cii-U-EMp	Coprolite	14,627,954	Enriched	Proboscideans
Li2-SC14-ciii-EMp	Coprolite	15,213,205	Enriched	Proboscideans
Li2-SC14-ciii-U-EMp	Coprolite	23,496,528	Enriched	Proboscideans
Li2-SC14-di-EMp	Coprolite	15,992,387	Enriched	Proboscideans
Li2-SC14-di-U-EMp	Coprolite	15,733,390	Enriched	Proboscideans
Li2-SC14-ei-EMp	Coprolite	17,335,001	Enriched	Proboscideans
Li2-SC14-ei-U-EMp	Coprolite	14,532,286	Enriched	Proboscideans
Li2-SC14-eii-EMp	Coprolite	16,853,448	Enriched	Proboscideans
Li2-SC14-eii-U-EMp	Coprolite	21,725,635	Enriched	Proboscideans
Li2-SC14-eiii-EMp	Coprolite	19,004,423	Enriched	Proboscideans
Li2-SC14-eiii-U-EMp	Coprolite	18,644,305	Enriched	Proboscideans
Li2-SCLBk-1-U-EMp	Extraction Blank	7,647	Enriched	Proboscideans
LSC14-b1-2-E2	Coprolite	3,439,849	Enriched	Proboscideans
LSC14-b2-2-E2	Coprolite	2,336,197	Enriched	Proboscideans
LSC14-b3-2-E2	Coprolite	2,724,696	Enriched	Proboscideans
LSC14-c1-2-E2	Coprolite	2,198,288	Enriched	Proboscideans
LSC14-c2-2-E2	Coprolite	2,726,231	Enriched	Proboscideans
LSC14-c3-2-E2	Coprolite	2,601,448	Enriched	Proboscideans
LSC14-e1-2-E2	Coprolite	3,178,781	Enriched	Proboscideans
LSC14-e2-2-E2	Coprolite	2,458,850	Enriched	Proboscideans
LSC14-e3-2-E2	Coprolite	3,662,775	Enriched	Proboscideans
SC14-ci-ESq	Coprolite	7,015,895	Enriched	Sciuridae
SC14-ci-U-ESq	Coprolite	5,487,299	Enriched	Sciuridae
SC14-cii-ESq	Coprolite	5,054,529	Enriched	Sciuridae
SC14-cii-U-ESq	Coprolite	5,541,228	Enriched	Sciuridae
SC14-ciii-ESq	Coprolite	6,798,391	Enriched	Sciuridae
SC14-ciii-U-ESq	Coprolite	8,919,837	Enriched	Sciuridae
SC14-di-ESq	Coprolite	6,125,914	Enriched	Sciuridae
SC14-di-U-ESq	Coprolite	6,911,392	Enriched	Sciuridae

SC14-ei-ESq	Coprolite	6,760,434	Enriched	Sciuridae
SC14-ei-U-ESq	Coprolite	6,415,830	Enriched	Sciuridae
SC14-eii-ESq	Coprolite	5,697,537	Enriched	Sciuridae
SC14-eii-U-ESq	Coprolite	6,567,673	Enriched	Sciuridae
SC14-eiii-ESq	Coprolite	7,802,780	Enriched	Sciuridae
SC14-eiii-U-ESq	Coprolite	7,697,248	Enriched	Sciuridae
SCLBk-1-U-ESq	Library Blank	1,651	Enriched	Sciuridae
SC14-ci-Shg	Coprolite	7,274,967	Shotgun	na
SC14-ci-U-Shg	Coprolite	6,186,224	Shotgun	na
SC14-cii-Shg	Coprolite	6,308,038	Shotgun	na
SC14-cii-U-Shg	Coprolite	6,075,283	Shotgun	na
SC14-ciii-Shg	Coprolite	5,766,327	Shotgun	na
SC14-ciii-U-Shg	Coprolite	5,652,527	Shotgun	na
SC14-di-Shg	Coprolite	5,091,107	Shotgun	na
SC14-di-U-Shg	Coprolite	6,795,634	Shotgun	na
SC14-ei-Shg	Coprolite	6,191,627	Shotgun	na
SC14-ei-U-Shg	Coprolite	4,366,404	Shotgun	na
SC14-eii-Shg	Coprolite	5,240,902	Shotgun	na
SC14-eii-U-Shg	Coprolite	6,593,773	Shotgun	na
SC14-eiii-Shg	Coprolite	4,498,967	Shotgun	na
SC14-eiii-U-Shg	Coprolite	6,602,930	Shotgun	na
SCLBk-1-U-Shg	Library Blank	2,581	Shotgun	na
LiB-BK2	Library Blank	9,119	Enriched	PalaeoChip
SC-10c-TCMP2	Coprolite	1,261,310	Enriched	PalaeoChip
SC-11-EAP	Coprolite	1,875,061	Enriched	PalaeoChip
SC-12-EAP	Coprolite	2,777,265	Enriched	PalaeoChip
SC-13c-TCMP2	Coprolite	1,401,971	Enriched	PalaeoChip
SC-14-ci-EAP	Coprolite	2,006,817	Enriched	PalaeoChip
SC-14BK-d-EAP	Extraction Blank	668,532	Enriched	PalaeoChip
SC-15-ciii-EAP	Coprolite	2,890,549	Enriched	PalaeoChip
SC-16-EAP	Coprolite	1,956,241	Enriched	PalaeoChip
SC-17-EAP	Coprolite	2,208,612	Enriched	PalaeoChip
SC-18-BK1-EAP	Extraction Blank	372,347	Enriched	PalaeoChip
SC-19-LBK2-EAP	Library Blank	34,823	Enriched	PalaeoChip
SC-2c-TCMP2	Coprolite	1,431,007	Enriched	PalaeoChip
SC-3-ci-EAP	Coprolite	1,867,770	Enriched	PalaeoChip
SC-5-ciii-EAP	Coprolite	2,024,789	Enriched	PalaeoChip
SC-6-EAP	Coprolite	2,571,597	Enriched	PalaeoChip
SC-8-EAP	Coprolite	2,131,519	Enriched	PalaeoChip
SC-9-ciii-EAP	Coprolite	2,697,528	Enriched	PalaeoChip
SC-EBK-bb-EAP	Extraction Blank	770	Enriched	PalaeoChip
SC-10D-Shg	Coprolite	1,766,168	Shotgun	na
SC-10a-Shg	Coprolite	2,642,744	Shotgun	na
SC-10b-Shg	Coprolite	2,666,318	Shotgun	na
SC-10c-Shg	Coprolite	2,463,532	Shotgun	na
SC-10e-Shg	Coprolite	2,458,064	Shotgun	na

SC-10f-Shg	Coprolite	2,055,983	Shotgun	na
SC-13a-Shg	Coprolite	2,419,761	Shotgun	na
SC-13b-Shg	Coprolite	2,723,140	Shotgun	na
SC-13c-Shg	Coprolite	2,265,782	Shotgun	na
SC-13d-Shg	Coprolite	18	Shotgun	na
SC-13e-Shg	Coprolite	1,887,433	Shotgun	na
SC-13f-Shg	Coprolite	2,116,317	Shotgun	na
SC-2a-Shg	Coprolite	2,240,284	Shotgun	na
SC-2b-Shg	Coprolite	2,360,492	Shotgun	na
SC-2c-Shg	Coprolite	2,410,066	Shotgun	na
SC-2e-Shg	Coprolite	3,855,520	Shotgun	na
SC-2f-Shg	Coprolite	2,968,046	Shotgun	na
Son-EBK1-Shg	Extraction Blank	233,252	Shotgun	na
Son-EBK2-Shg	Extraction Blank	233,839	Shotgun	na
Son-LBK3-Shg	Library Blank	142,841	Shotgun	na
SC-10D-TCMP	Coprolite	1,012,887	Enriched	PalaeoChip
SC-10a-TCMP	Coprolite	1,794,164	Enriched	PalaeoChip
SC-10b-TCMP	Coprolite	2,107,917	Enriched	PalaeoChip
SC-10c-TCMP	Coprolite	1,261,310	Enriched	PalaeoChip
SC-10e-TCMP	Coprolite	1,370,397	Enriched	PalaeoChip
SC-10f-TCMP	Coprolite	937,128	Enriched	PalaeoChip
SC-13a-TCMP	Coprolite	1,466,275	Enriched	PalaeoChip
SC-13b-TCMP	Coprolite	1,478,958	Enriched	PalaeoChip
SC-13c-TCMP	Coprolite	1,401,971	Enriched	PalaeoChip
SC-13d-TCMP	Coprolite	1	Enriched	PalaeoChip
SC-13e-TCMP	Coprolite	1,358,546	Enriched	PalaeoChip
SC-13f-TCMP	Coprolite	816,959	Enriched	PalaeoChip
SC-2a-TCMP	Coprolite	1,391,271	Enriched	PalaeoChip
SC-2b-TCMP	Coprolite	1,093,607	Enriched	PalaeoChip
SC-2c-TCMP	Coprolite	1,431,007	Enriched	PalaeoChip
SC-2e-TCMP	Coprolite	1,742,312	Enriched	PalaeoChip
SC-2f-TCMP	Coprolite	1,530,462	Enriched	PalaeoChip
LSC14-BLK-E2	Extraction Blank	1,534,055	Enriched	Proboscideans
LSC14-bi-E2	Coprolite	2,083,902	Enriched	Proboscideans
LSC14-bii-E2	Coprolite	2,932,984	Enriched	Proboscideans
LSC14-biii-E2	Coprolite	2,806,586	Enriched	Proboscideans
LSC14-ci-E2	Coprolite	4,450,293	Enriched	Proboscideans
LSC14-cii-E2	Coprolite	6,707,930	Enriched	Proboscideans
LSC14-ciii-E2	Coprolite	5,550,709	Enriched	Proboscideans
LSC14-di-E2	Coprolite	7,969,918	Enriched	Proboscideans
LSC14-ei-E2	Coprolite	3,270,197	Enriched	Proboscideans
LSC14-eii-E2	Coprolite	4,700,818	Enriched	Proboscideans
LSC14-eiii-E2	Coprolite	4,140,872	Enriched	Proboscideans
LSC14-xi-E2	Coprolite	3,608,172	Enriched	Proboscideans
LSC14-xii-E2	Coprolite	2,489,938	Enriched	Proboscideans
LSC14-xiii-E2	Coprolite	4,228,634	Enriched	Proboscideans

LSC15ciii-E2	Coprolite	2,884,831	Enriched	Proboscideans
LSC-EBK14d-E2	Extraction Blank	2,029,029	Enriched	Proboscideans
LSC14-c1-E2	Coprolite	3,369,095	Enriched	Proboscideans
LSC14-c2-E2	Coprolite	3,746,403	Enriched	Proboscideans
LSC14-c3-E2	Coprolite	3,227,140	Enriched	Proboscideans
LSC14-d1-E2	Coprolite	2,466,219	Enriched	Proboscideans
LSC14-e1-E2	Coprolite	4,655,646	Enriched	Proboscideans
LSC14-e2-E2	Coprolite	4,271,230	Enriched	Proboscideans
LSC14-e3-E2	Coprolite	3,819,183	Enriched	Proboscideans
LSC14-c1-2-E2-redo	Coprolite	4,076,192	Enriched	Proboscideans
LSC14-c2-2-E2-redo	Coprolite	3,838,942	Enriched	Proboscideans
LSC14-c3-2-E2-redo	Coprolite	3,276,684	Enriched	Proboscideans
LSC-10e-E2	Coprolite	944,472	Enriched	Proboscideans
LSC-10f-E2	Coprolite	492,966	Enriched	Proboscideans
LSC-9ciii-E2	Coprolite	948,115	Enriched	Proboscideans
LSC14-Mix-E2	Coprolite	23,472,159	Enriched	Proboscideans
LSC9-di-E2	Coprolite	748,754	Enriched	Proboscideans
LSC9-dii-E2	Coprolite	656,519	Enriched	Proboscideans
LSC9-ei-E2	Coprolite	521,069	Enriched	Proboscideans
LSC9-eii-E2	Coprolite	1,393,460	Enriched	Proboscideans
LSC9-eiii-E2	Coprolite	3,607,603	Enriched	Proboscideans
LSC9-xii-E2	Coprolite	610,483	Enriched	Proboscideans
LSC9-xiii-E2	Coprolite	744,785	Enriched	Proboscideans
	TOTAL	699,685,819		

Table S2. Sample library replicate counts.

Sample	Shotgun	Enriched	Totals
SC-2	5	6	11
SC-3		1	1
SC-5		3	3
SC-6		1	1
SC-8		1	1
SC-9		11	11
SC-10	6	11	17
SC-11		1	1
SC-12		1	1
SC-13	6	9	15
SC-14	14	62	76
SC-15		4	4
SC-16		1	1
SC-17		1	1
EBK	2	6	8
LBK	2	3	5
Totals	35	122	157

Sample ID	Year Collected	Lab ID	Site	¹⁴ C Lab ID	¹⁴ C Date	Age (cal yr BP) 2 sigma	Median age (cal yr BP)
DF09-HC-29a	2009	SC-3	Hunker Creek	UCIAMS-67157	14,100±40	17,335-17,040	17,175
DF09-HC-30	2009	SC-2	Hunker Creek	*	14,100±40	17,335-17,040	17,175
SC-AGS-01	2012	SC-6	Quartz Creek	UOC-26555	>45,600		
SC-AGS-04	2012	SC-8	Quartz Creek	UOC-26556	>45,600		
SC-AGS-15	2011	SC-10	Eureka Creek	UOC-26557	26,800±140	31,380-30,830	31,035
SC-AGS-18	2011	SC-11	Eureka Creek	UOC-26558	24,400±110	28,950-28,300	28,660
SC-AGS-21	?	SC-12	Little Blanche	UCIAMS-265205	765±15	725-670	680
SC-AGS-22	2011	SC-13	Quartz Creek	UOC-26559	31,500±240	36,290-35,360	35,850

* Sample associated with DF09-HC-29a.

Table S3.

Radiocarbon dating of coprolite samples.

Sample ID	Year Collected	Lab ID	Site	Associated tephra	Age estimate	Presented age (yr BP)
SLC21-KL-1D	2021	SC-5	Hunker Creek (Sailer's Property)	Dawson	29,055-29,470 cal BP	~30,000
SLC21-KL-4A	2021	SC-15	Hunker Creek (Sailer's Property)			
SLC21-TM-1C	2021	SC-16	Eureka Creek			
SLC21-KL-8	2021	SC-17	Hunker Creek (Sailer's Property)			
SC-AGS-12	2018	SC-9	Canyon Creek	Sheep Creek Klondike	77±7 ka	~80,000
GZ.07.07	2007	SC-14	Gold Run	Gold Run	688±44 ka	~700,000

Table S4.

Tephrochronology of coprolite middens associated with volcanic tephra.

Urocitellus species	GBIF Occurance Data
U. armatus	https://doi.org/10.15468/dl.7f6wzx
U. beldingi	https://doi.org/10.15468/dl.wt5j2e
U. brunneus	https://doi.org/10.15468/dl.fuc9f6
U. canus	https://doi.org/10.15468/dl.nvs57u
U. columbianus	https://doi.org/10.15468/dl.bjq3pq
U. elegans	https://doi.org/10.15468/dl.h96ub6
U. endemicus	https://doi.org/10.15468/dl.pswyep
U. mollis	https://doi.org/10.15468/dl.ux27ku
U. parryii	https://doi.org/10.15468/dl.tsrmhy
U. richardsonii	https://doi.org/10.15468/dl.bjq3pq
U. townsendii	https://doi.org/10.15468/dl.98ay9p
U. undulatus	https://doi.org/10.15468/dl.7u83q2
U. washingtoni	https://doi.org/10.15468/dl.3wprha

Table S5.

Urocitellus GBIF occurrence data.

Pacific Northwest Quaternary Panel												Total species targeted	
Summary of publicly available genetic data													
Taxonomic group	Total taxa searched	RefSeq Mito and Plastid Genomes	%	Non-RefSeq Mito and Plastid Genomes	%	Single Gene Refs	%	No genetic data	%	Has genetic data	GBIF DOI		
Extant mammals	276	140	50.7%	29	10.5%	103	37.3%	4	1.4%	98.6%	10.15468/dl.5kszzd	8005	
Extant birds	710	214	30.1%	84	30.4%	401	56.5%	11	1.5%	98.5%	<i>list from eBird and eFauna-BC</i>		
Extant reptiles, amphibians	184	44	23.9%	15	5.4%	120	65.2%	5	2.7%	97.3%	10.15468/dl.fckj72 , 10.15468/dl.8nswgz , 10.15468/dl.fs3ebk		
Extant plants	9642	1185	12.3%	1634	16.9%	6727	69.8%	2847	29.5%	70.5%	10.15468/dl.tkhs72		
Extinct animals	203	38	18.7%	11	5.4%	11	5.4%	143*	70.4%	29.6%			
*This includes many extinct species classifications that are longer accepted and many of which are debatable as to whether they are truly seperate species.													
Palaeo-Chip Arctic v1.0 (designed in ~2017)													
Taxonomic group	Total taxa	Organelle Genome	%	Single Genes	Total species targeted								
Animals	185	111	60.0%	61	33.0%	2262							
Plants	2717			2090	76.9%								

Table S6.

Taxonomic and genetic references of terrestrial taxa within the region of eastern Beringia through the Quaternary. Full references in *fasta* format are located here: <https://doi.org/10.5281/zenodo.16621723>.

Organism	Reference	Variables	SC-2	SC-3	SC-5	SC-6	SC-8	SC-9	SC-10	SC-11	SC-14	SC-16	SC-17	SC-15	SC-12
Urocitellus	<i>Urocitellus richardsonii</i>	Percentage of Covered Bases	88	85	81	82	73	91	96	92	95	91	87		
	<i>Urocitellus richardsonii</i>	Average Coverage	5.9	20.0	21.7	14.2	6.4	79.8	48.1	159.1	376.4	116.5	41.2		
	<i>Urocitellus richardsonii</i>	Number of unresolved Bases	1,962	2,442	2,984	2,824	4,305	1,383	650	1,195	682	1,405	1,975		
	<i>Urocitellus richardsonii</i>	Total Length	16,461	16,461	16,461	16,461	16,461	16,461	16,461	16,461	16,461	16,461	16,461		
	<i>Urocitellus parryii</i>	Percentage of Covered Bases	87	84	81	82	73	90	96	92	95	90	87		
	<i>Urocitellus parryii</i>	Average Coverage	5.8	20.1	21.8	14.3	6.4	80.5	48.1	159.5	371.9	116.8	41.7		
	<i>Urocitellus parryii</i>	Number of unresolved Bases	2,012	2,500	3,032	2,868	4,301	1,522	651	1,279	753	1,485	2,087		
	<i>Urocitellus parryii</i>	Total Length	16,461	16,461	16,461	16,461	16,461	16,461	16,461	16,461	16,461	16,461	16,461		
	SC16 de novo assembly	Percentage of Covered Bases	88	85	81	82	74	91	95	92	94	91	88		
	SC16 de novo assembly	Average Coverage	5.9	20.4	22.1	14.5	6.5	81.4	49.7	163.4	366.7	119.2	42.2		
	SC16 de novo assembly	Number of unresolved Bases	1,699	2,289	2,844	2,680	4,074	1,252	643	1,083	792	1,269	1,830		
	SC16 de novo assembly	Total Length	16,461	16,461	16,461	16,461	16,461	16,461	16,461	16,461	16,461	16,461	16,461		
Equus	Equus lambei	Percentage of RefSeq covered	89.2%		93.4%									96.6%	
	Equus lambei	Average Coverage	11.4		11.5									6.3	
	Equus lambei	Number of unresolved Bases (3x min)	7,119		5,526									2,816	
	Equus lambei	Reads mapped	4,424		4,083									1,915	
	Equus lambei	Total Length	16,661		16,660									16,660	
Bison	Bison priscus	Percentage of RefSeq covered			83.9									97.5	
	Bison priscus	Average Coverage			10.2									5.6	
	Bison priscus	Number of unresolved Bases (3x min)			9,255									3,151	
	Bison priscus	Reads mapped			3,693									3,693	
	Bison priscus	Total Length			16,317									16,318	
Lepus	Lepus americanus	Percentage of RefSeq covered												98.5	
	Lepus americanus	Average Coverage												640.9	
	Lepus americanus	Number of unresolved Bases (3x min)												276	
	Lepus americanus	Reads mapped												196,619	
	Lepus americanus	Total Length												17,045	

Table S7.
Comparison for reference-based assembled samples.

Kingdom	Genus	Species	Accession	Purpose
Animal	Scotoplanthes	sp.	LC416624	True negative and false positive
Animal	Urocitellus	parryii		True vs. false positive and false negative
Animal	Bison	priscus	NC_027233	True vs. false positive and false negative
Animal	Canis	lupus	NC_008092	True vs. false positive and false negative
Animal	Dicrostonyx	groenlandicus	NC_034313	True vs. false positive and false negative
Animal	Equus	lambei	KT168322	True vs. false positive and false negative
Animal	Lagopus	lagopus	NC_035568	True vs. false positive and false negative
Animal	Lepus	americanus	NC_024043	True vs. false positive and false negative
Animal	Mammuthus	primigenius	NC_007596	True vs. false positive and false negative
Animal	Ovis	dalli	NC_039432	True vs. false positive and false negative
Animal	Panthera	leo atrox	OK513020	True vs. false positive and false negative
Animal	Rangifer	tarandus	NC_007703	True vs. false positive and false negative
Plant	Alnus	maximowiczii	NC_036754	True vs. false positive and false negative
Plant	Artemisia	annua	NC_034683	True vs. false positive and false negative
Plant	Betula	pendula	LT996895	True vs. false positive and false negative
Plant	Carex	gibba	NC_061300	True vs. false positive and false negative
Plant	Picea	chihuahuana	NC_039584	True vs. false positive and false negative
Plant	Plantago	maritima	NC_028519	True vs. false positive and false negative
Plant	Poa	palustris	NC_027484	True vs. false positive and false negative
Plant	Pontellia	simplex	NC_087036	True vs. false positive and false negative
Plant	Ranunculus	reptans	NC_036977	True vs. false positive and false negative
Plant	Salix	interior	NC_024681	True vs. false positive and false negative
Bacteria				True negative and false positive

Table S8.

Comparison for reference-based assembled samples. See simulation datafile for additional information on simulated comparisons.

References cited

1. Murchie, T. J. *et al.* Optimizing extraction and targeted capture of ancient environmental DNA for reconstructing past environments using the PalaeoChip Arctic-1.0 bait-set. *Quat. Res.* **99**, 305–328 (2021).
2. Massilani, D. *et al.* Microstratigraphic preservation of ancient faunal and hominin DNA in Pleistocene cave sediments. *Proc. Natl. Acad. Sci. U. S. A.* **119**, (2022).
3. Delgado-González, M. J. *et al.* Study of a laboratory-scaled new method for the accelerated continuous ageing of wine spirits by applying ultrasound energy. *Ultrason. Sonochem.* **36**, 226–235 (2017).
4. Chang, A. C. Study of ultrasonic wave treatments for accelerating the aging process in a rice alcoholic beverage. *Food Chem.* **92**, 337–342 (2005).
5. Dabney, J., Meyer, M. & Pääbo, S. Ancient DNA damage. *Cold Spring Harb. Perspect. Biol.* **5**, (2013).
6. Murchie, T. J., Giguët-Covex, C., Heintzman, P. D., Slon, V. & Wang, Y. Terrestrial Fauna and Hominin DNA from Sedimentary Archives. in *Tracking Environmental Change Using Lake Sediments. Developments in Paleoenvironmental Research*, vol 21. (eds. Capo, E., Barouillet, C. & Smol, J.) 299–378 (Springer, Cham, 2023). doi:10.1007/978-3-031-43799-1_11.
7. Meyer, M. & Kircher, M. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harb. Protoc.* **5**, (2010).
8. Kircher, M., Sawyer, S. & Meyer, M. Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform. *Nucleic Acids Res.* **40**, 1–8 (2012).
9. Dabney, J. *et al.* Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proceedings of the National Academy of Sciences* **110**, 15758–15763 (2013).
10. Wang, Y. *et al.* Late Quaternary dynamics of Arctic biota from ancient environmental genomics. *Nature* 2021 600:7887 **600**, 86–92 (2021).
11. Maixner, F. *et al.* Linear polyacrylamide is highly efficient in precipitating and purifying environmental and ancient DNA. *Methods Ecol. Evol.* **13**, 653–667 (2022).
12. Wang, Y. *et al.* Late Quaternary dynamics of Arctic biota from ancient environmental genomics. *Nature* 2021 600:7887 **600**, 86–92 (2021).
13. Huson, D. H., Auch, A. F., Qi, J. & Schuster, S. C. MEGAN analysis of metagenomic data. *Genome Res.* **17**, 377–386 (2007).
14. Huson, D. H. *et al.* MEGAN Community Edition - Interactive Exploration and Analysis of Large-Scale Microbiome Sequencing Data. *PLoS Comput. Biol.* **12**, (2016).
15. Camacho, C. *et al.* BLAST+: Architecture and applications. *BMC Bioinformatics* **10**, 1–9 (2009).
16. NCBI Resource Coordinators. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res.* **46**, 8–13 (2018).
17. Renaud, G., Hanghøj, K., Willerslev, E. & Orlando, L. gargammel: a sequence simulator for ancient DNA. *Bioinformatics* **33**, 577–579 (2017).
18. Reynolds, H. T., Ingersoll, T. & Barton, H. A. Modeling the environmental growth of *Pseudogymnoascus destructans* and its impact on the white-nose syndrome epidemic. *J. Wildl. Dis.* **51**, 318–331 (2015).

19. Urbina, J., Chestnut, T., Allen, J. M. & Levi, T. Pseudogymnoascus destructans growth in wood, soil and guano substrates. *Sci. Rep.* **11**, 1–11 (2021).
20. Shuey, M. M., Drees, K. P., Lindner, D. L., Keim, P. & Foster, J. T. Highly sensitive quantitative PCR for the detection and differentiation of *Pseudogymnoascus destructans* and other *Pseudogymnoascus* species. *Appl. Environ. Microbiol.* **80**, 1726–1731 (2014).
21. Zielinsk, G. A. & Mershon, G. R. Paleoenvironmental implications of the insoluble microparticle record in the GISP2 (Greenland) ice core during the rapidly changing climate of the Pleistocene-Holocene transition. *Bulletin of the Geological Society of America* **109**, 547–559 (1997).
22. Allentoft, M. E. *et al.* The half-life of DNA in bone: measuring decay kinetics in 158 dated fossils. *Proceedings of the Royal Society B: Biological Sciences* **279**, 4724–4733 (2012).
23. Kistler, L., Ware, R., Smith, O., Collins, M. & Allaby, R. G. A new model for ancient DNA decay based on paleogenomic meta-analysis. *Nucleic Acids Res.* **45**, 6310–6320 (2017).