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SUPPLEMENTARY INFORMATION

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Relic DNA is abundant in soil and obscures estimates of soil microbial diversity

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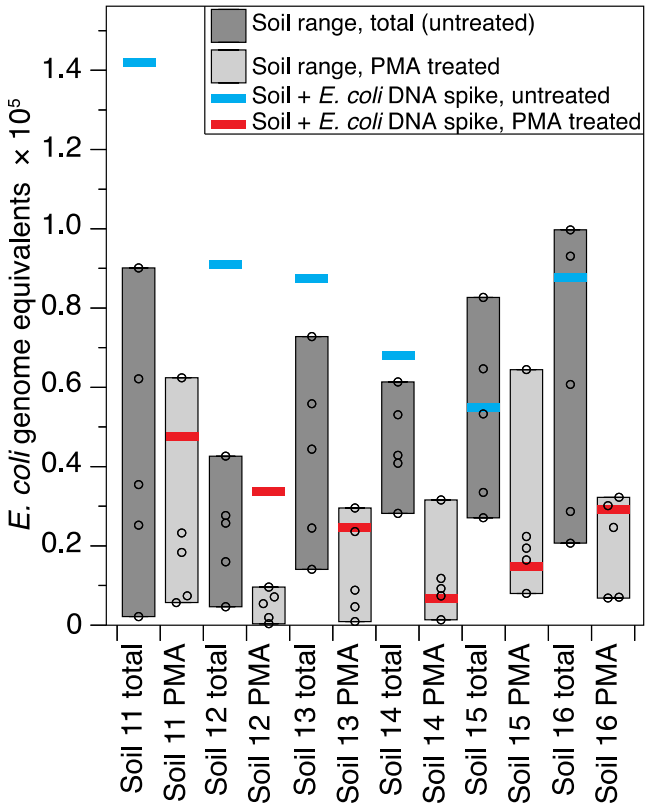
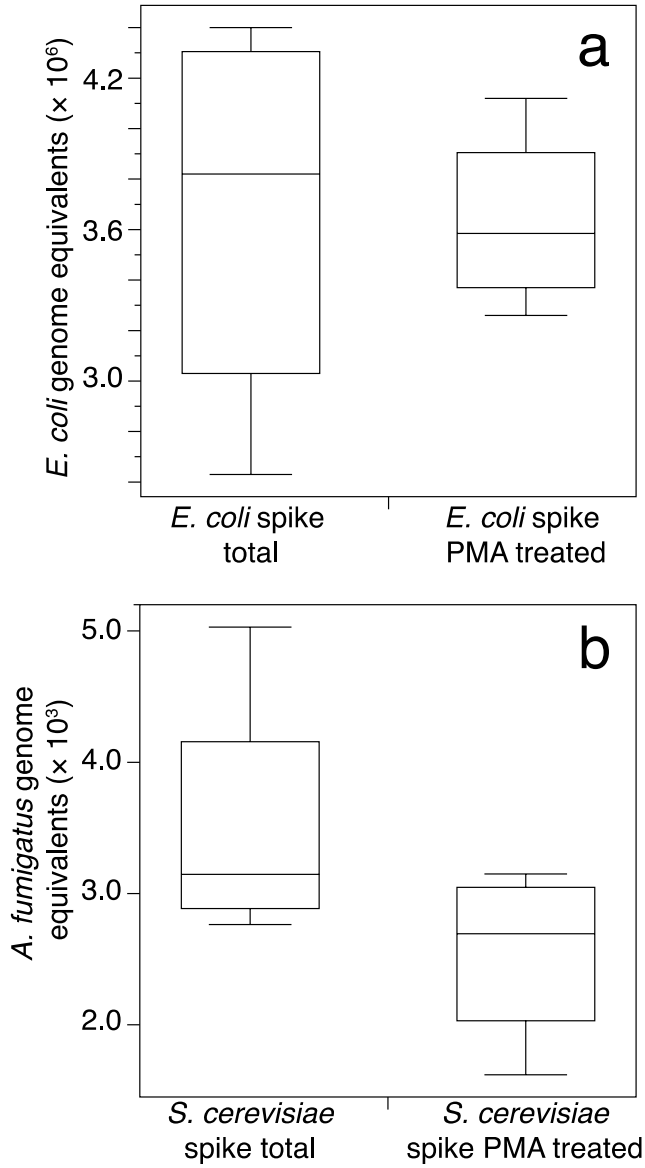
Supplementary Table 1: List of soil sample locations, ecosystem descriptions, edaphic characteristics, mean community dissimilarity after relic DNA removal, mean percent relic DNA for each soil, mean richness, and mean 16S and ITS amplicon abundances for both untreated and PMA treated soils.

Supplementary Dataset 1: *i)* Full dataset for Fig. 1; *ii)* mean % of each taxa per soil, per treatment; and *iii)* amplicon copies per replicate per gram of soil.

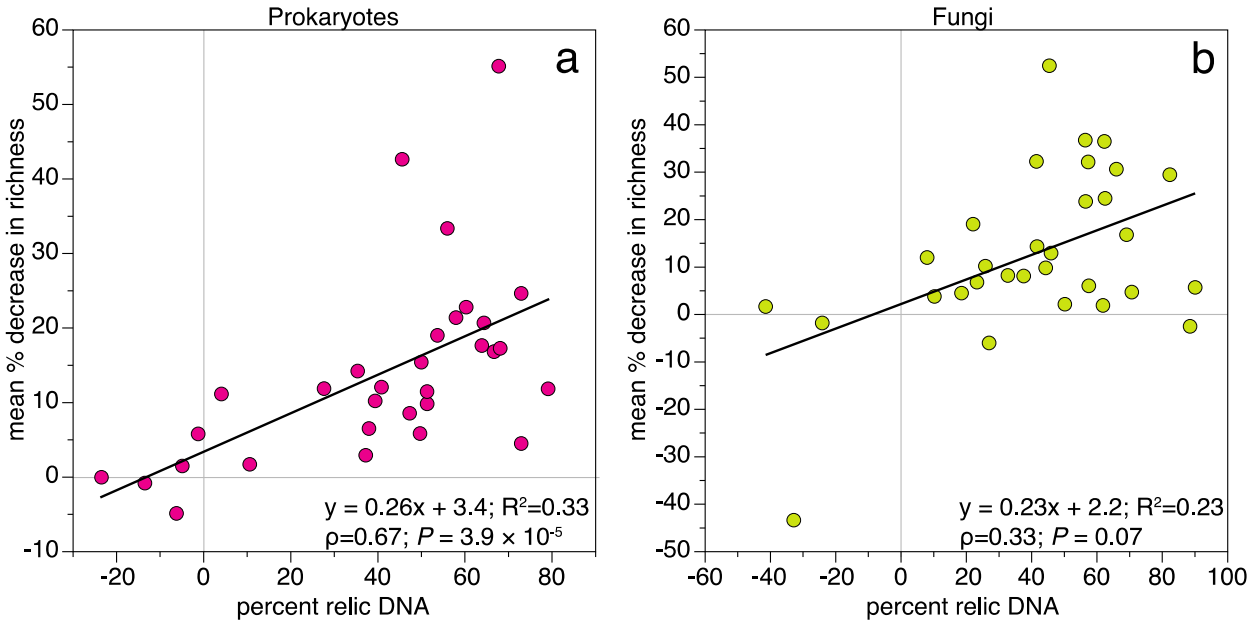
Supplementary Figures:



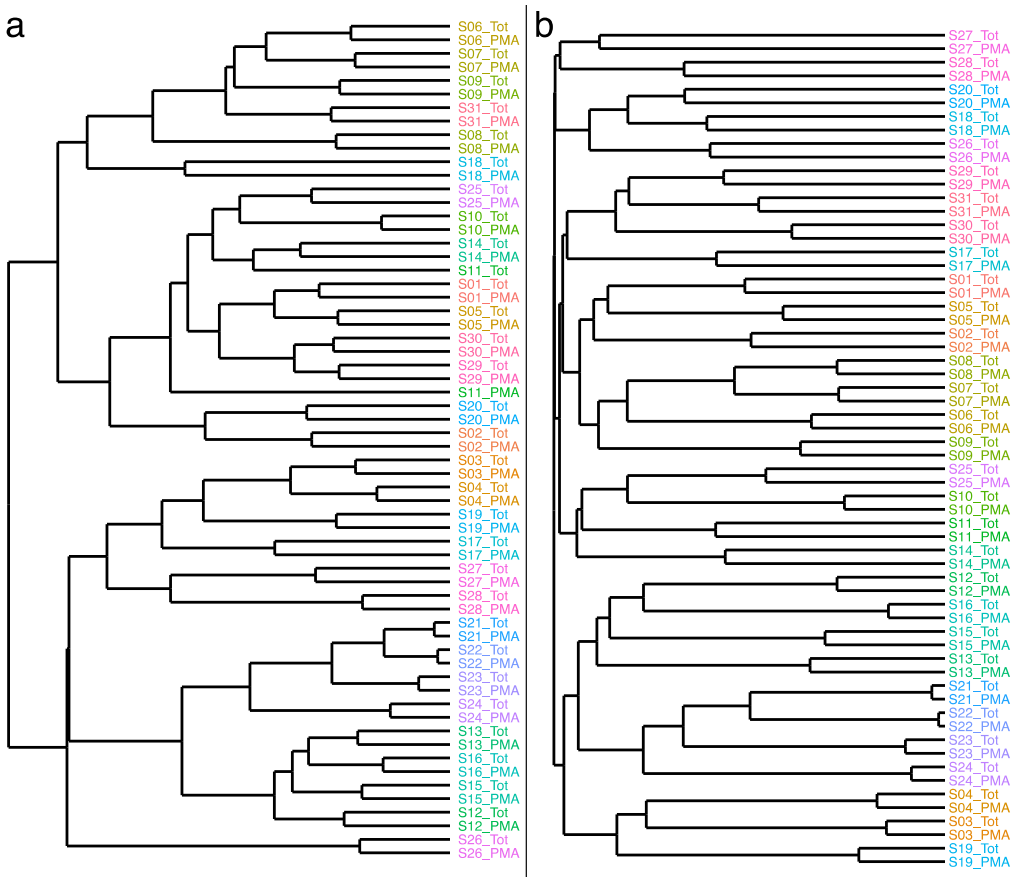
Supplementary Figure 1: PMA effectively removed experimentally added naked DNA from soil. Dark and light shaded boxes show the range of 16S rRNA gene copies detected from DNA extracts of untreated or PMA treated soils, respectively. Open circles in shaded boxes are individual data points. Blue bars represent the abundance of 16S rRNA gene copies quantified in soil spiked with ~8.8 × 10^4 copies of purified *E. coli* genomic DNA (i.e. – extracellular DNA). Red bars represent the abundance of 16S rRNA gene copies quantified in soil spiked with ~8.8 × 10^4 copies of purified *E. coli* genomic DNA and treated with 40 µM PMA as described for soils in Methods. PMA did not remove all of the spiked DNA in Soil 12, which also contained the largest percent of extracellular DNA (Fig. 1), suggesting additional PMA was necessary to remove the spiked DNA. In some soils we did not recover the full spike in the untreated sample (for example, blue bars in soils 14 and 15 are < 8.8 × 10^4 copies). This may be the result of the spiked DNA adsorbing to soil particles, damaging it or sequestering it, making it unamplifiable. However, in these same soils, spiked DNA was completely removed after PMA treatment (red bars fall within light shaded region after PMA treatment).



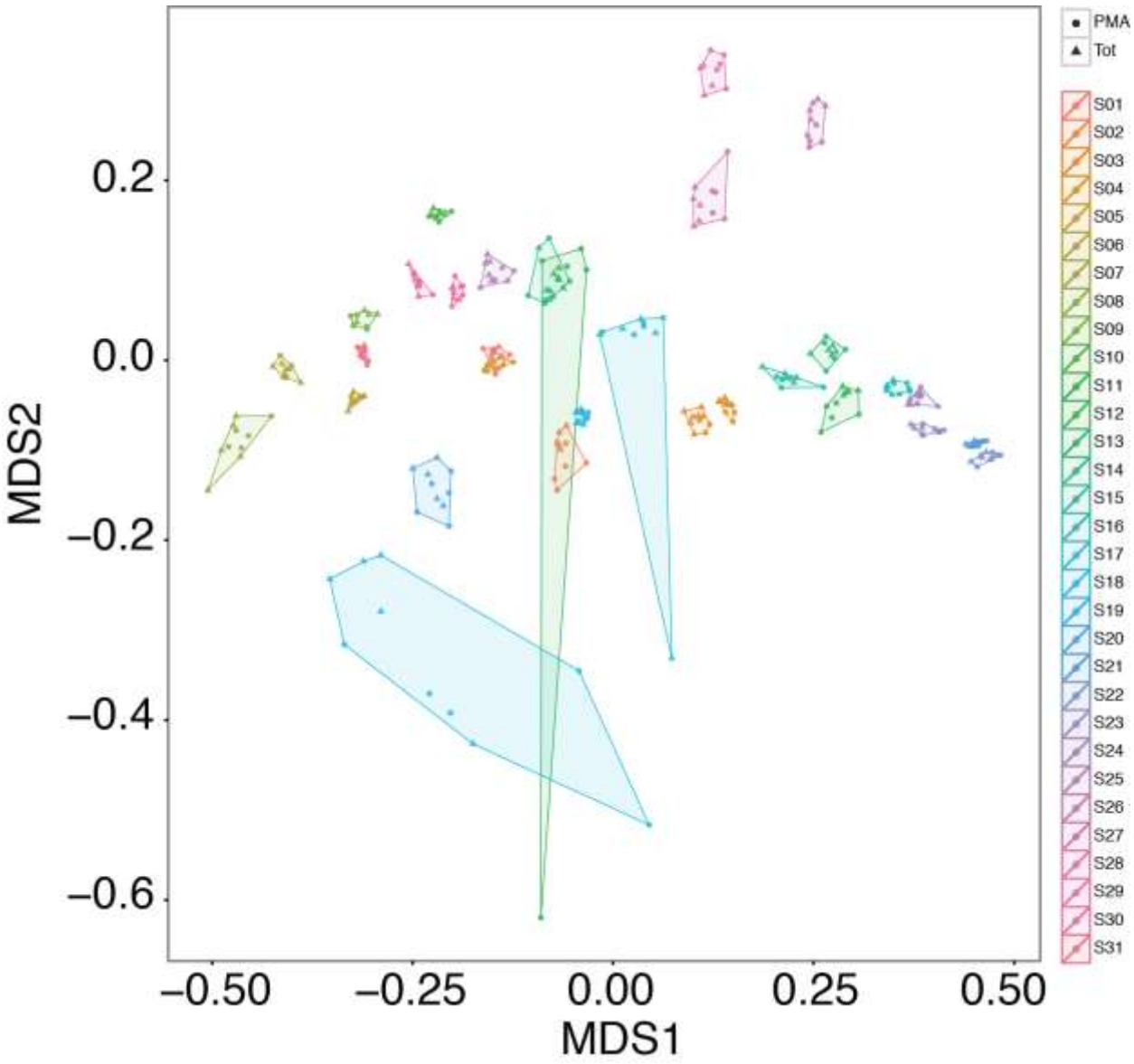
Supplementary Figure 2: PMA did not penetrate live *E. coli* (a) or *S. cerevisiae* (b) cells. Sterile PBS was spiked with equal volumes of exponentially growing *E. coli* and *S. cerevisiae* cell cultures. Tubes were either treated with 40 µM PMA (n=5) or left untreated ("total"; n=5) as described in the Methods section. The abundance of 16S rRNA gene copies or ITS amplicon copies were indistinguishable to untreated controls (two-tailed *t* test P=0.96 and P=0.22, respectively), illustrating PMA did not significantly penetrate live bacterial or fungal cells.



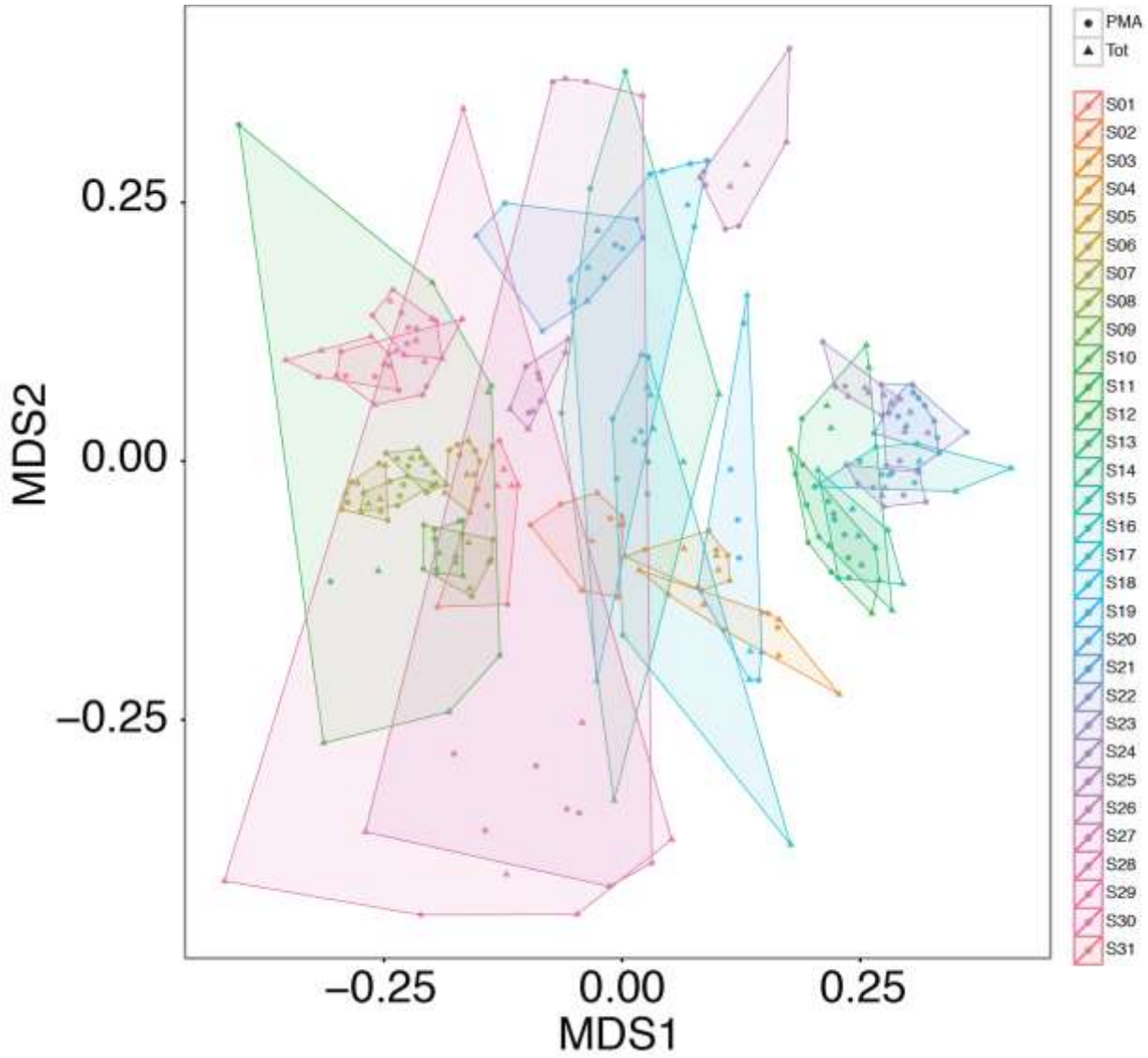
Supplementary Figure 3: The percent of 16S rRNA genes or ITS amplicons in the relic DNA pool is positively correlated with the richness decrease after relic DNA removal, but this correlation is only significant for prokaryotes. Points are the mean percent relic DNA and mean reduction in richness after relic DNA removal, taken from Fig. 1. a) prokaryotes, b) fungi. Linear regressions, formulas, Spearman's ρ and P value are shown.



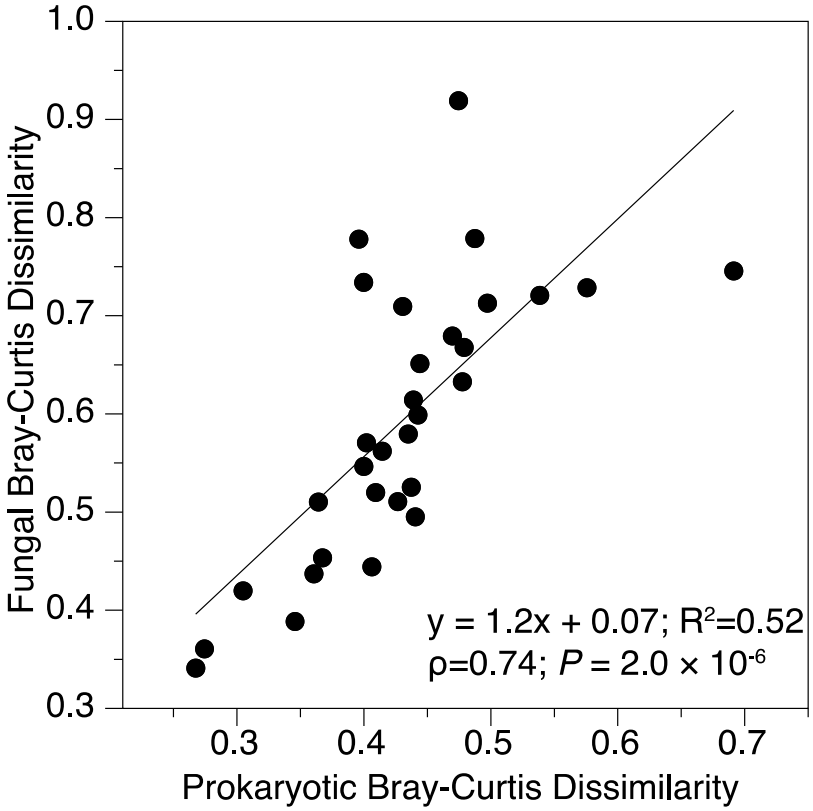
Supplementary Figure 4: Differences in community composition between individual soil types is greater than the effect of relic DNA removal for a given soil. Dendrogram of prokaryotic (a) and fungal (b) community composition for all 31 soils. Labels presented as “soil number_treatment” where ‘PMA’ denotes PMA treated samples, and ‘Tot’ denotes untreated samples.



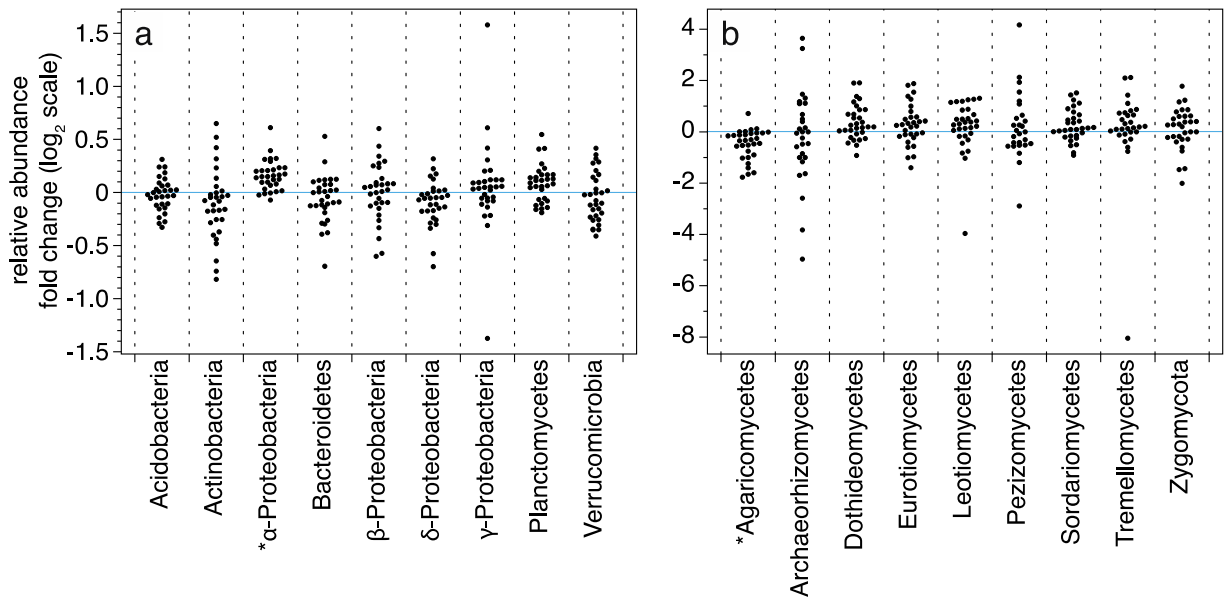
Supplementary Figure 5: Differences in prokaryotic community composition between individual soil types is greater than the effect of relic DNA removal for a given soil. Multidimensional scaling plot of prokaryotic community composition for all 31 soils. Hulls connect the outermost points for each soil.



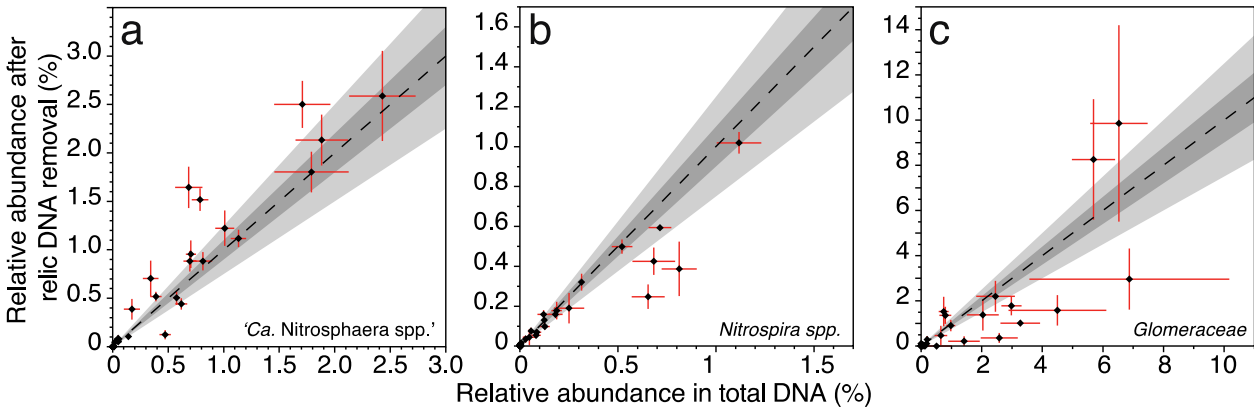
Supplementary Figure 6: Differences in fungal community composition between individual soil types is greater than the effect of relic DNA removal for a given soil. Multidimensional scaling plot of fungal community composition for all 31 soils. Hulls connect the outermost points for each soil.



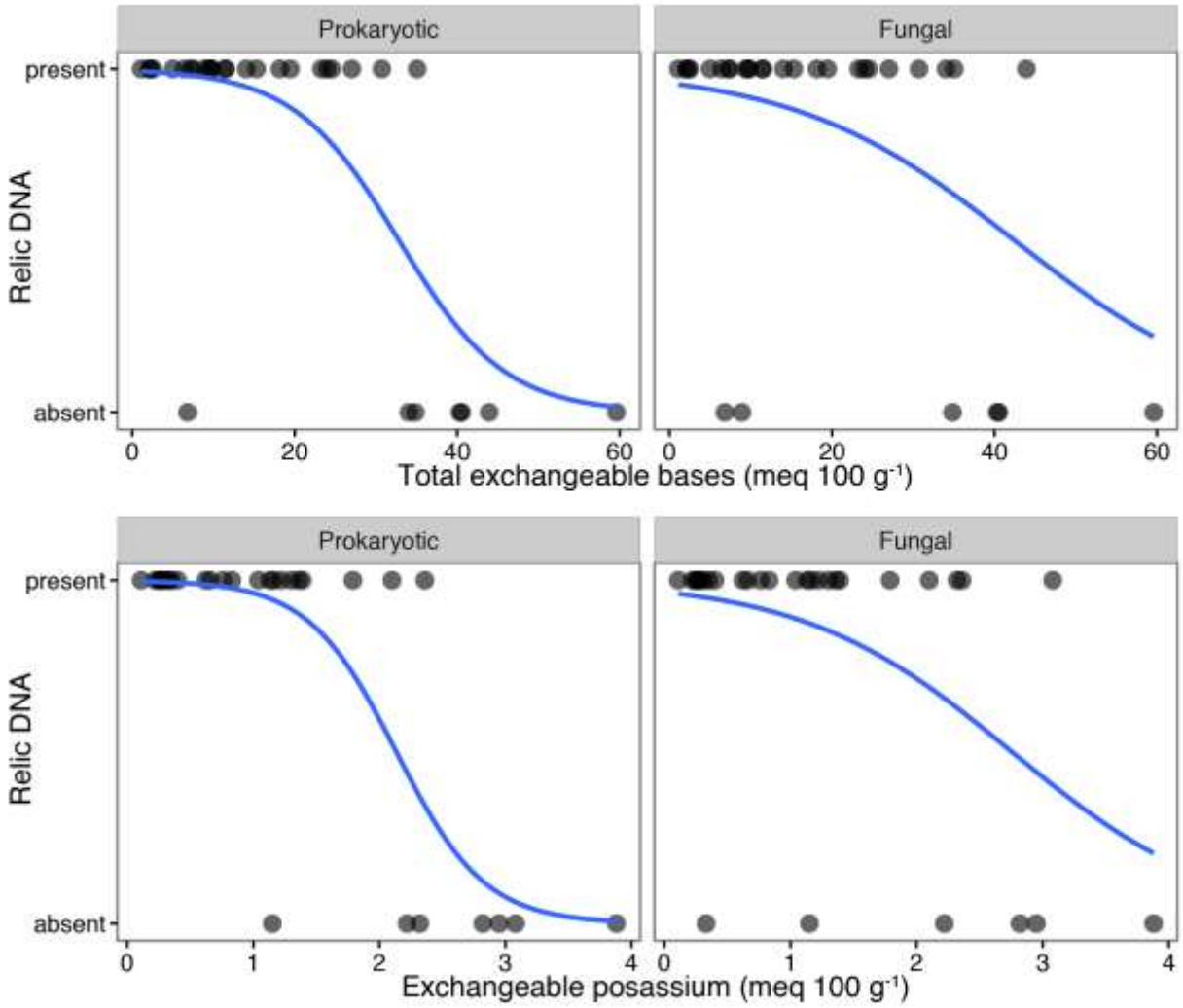
Supplementary Figure 7: Mean dissimilarity in prokaryotic communities after relic DNA removal is correlated with the associated dissimilarity in fungal communities. Points are the mean dissimilarity of prokaryotic and fungal communities in each soil after relic DNA removal, relative to total DNA pools. See Supplementary Table 1 for mean Bray-Curtis dissimilarity values for each soil.



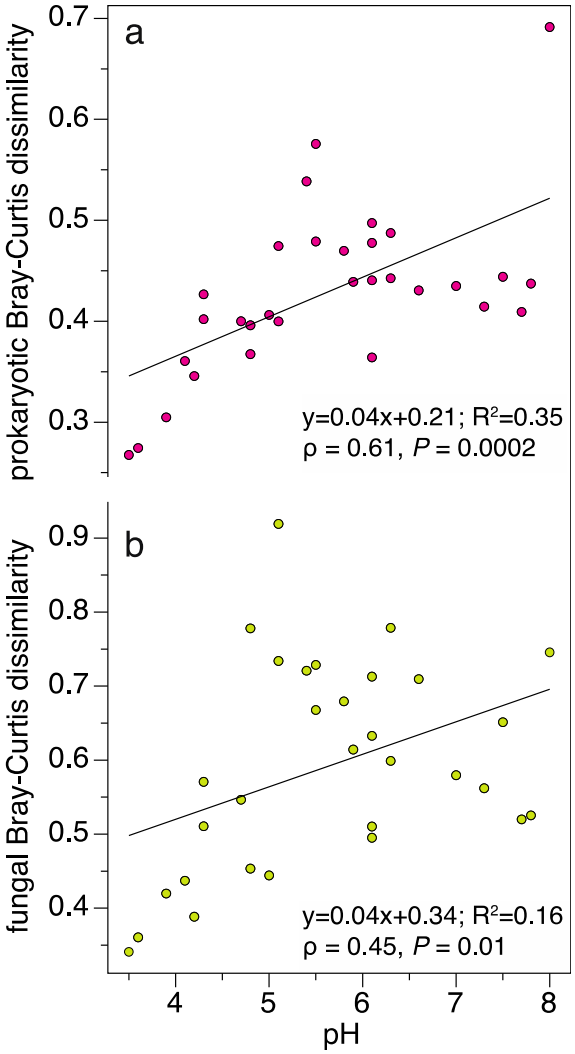
Supplementary Figure 8: Fold changes in the estimated relative abundances of individual prokaryotic (a) and fungal (b) taxa after removal of relic DNA across all soils. Only major taxonomic groups comprising ≥ 5% of the total prokaryotic community or ≥ 3% of the total fungal community are shown. Points are the $\log_2$ fold change in estimated mean relative abundances after relic DNA removal, relative to the abundance in untreated samples. Points are not shown for some fungal taxa because they were not detected in one of the two conditions, preventing calculation of fold change. See Supplementary Dataset 1 for raw data. *Significant difference in relative abundance after relic DNA removal, relative to untreated samples (two-tailed Mann-Whitney U $P \leq 0.05$).



Supplementary Figure 9: The relative abundances of prokaryotic nitrifiers and arbuscular mycorrhizal fungi change after relic DNA removal in some soils. Relative abundance of ammonia oxidizing archaea (a) or of nitrite oxidizing bacteria (b) before and after relic DNA removal (mean ± SE). c) Relative abundance of *Glomeraceae* (mycorrhizal fungi) before and after relic DNA removal (mean ± SE). In all plots, dashed line represents no change in relative abundance (1:1 line). Dark grey shaded region represents ±10% change; light grey shaded region represents ±25% change. Red error bars represent ± SE.



Supplementary Figure 10: Logistic regressions fitting the presence of relic DNA to total exchangeable base cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) and exchangeable K^+. Relic DNA was considered present it comprised ≥20% (prokaryotic) or ≥27% (fungal) of the total DNA pool. Logistic regressions are shown in blue. Associated *P* values are provided in Table 1.



Supplementary Figure 11: Soil pH is correlated with the difference in community composition after relic DNA removal. Points show the mean dissimilarity in soil microbial communities for prokaryotes (a) and fungi (b) after relic DNA removal, relative to total DNA. Linear regressions, formulas, Spearman's ρ and *P* values are shown.