Patient propagules: Do long-term soil archives preserve legacy fungal and bacterial communities?

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ABSTRACT

Soil is a complex and diverse matrix, populated by billions of microorganisms per cm^3. We assessed the detection capacity of high-throughput next generation sequencing tool (Illumina MiSeq) of microbial ITS (internal transcribed spacer) and 16S rRNA genes in soil across time, and identified taxa resistant to temporal archiving. Results showed a decrease in fungal and bacterial OTU richness with time in deciduous forest and Populus *soils. Effects of sampling site and DNA extraction also influenced richness variation. Future research is aimed at 1) identifying factors in soil samples that impact preservation, 2) developing storage methods that may better preserve microbial DNA and 3) testing the viability of propagules through direct isolation.*

INTRODUCTION

Soils harbor rich and diverse microbial communities whose DNA can variably persist in the environment. Soils at the KBS LTER (Kellogg Biological Station Long-Term Ecological Research) site have been sampled, air-dried and archived for more than 20 years. It is unknown how well this process preserves microbial DNA. Here, we test whether soil archives can be used to reconstruct historic fungal and bacterial communities at these sites. Fungal and bacterial communities in the *Populus* and Deciduous Forest treatments of the KBS LTER main cropping system were reconstructed across a 20 year time span from soil archives. Our **goal** was to assess whether soil archives can be used to understand microbial communities of past years, and to identify microbial taxa that appear most sensitive to temporal archiving.

MATERIAL AND METHODS

- In 2015-16, fresh soils were sampled (n=3) from 3 plots of the *Populus* (**T5R**) and Deciduous Forest (**DF**) treatments of the KBS LTER main cropping system.
- Archived soils were sampled from the same 3 plots representing > 20 year time frame: 2015, 2014, 2010, 2005, 2000, 1995.
- Soil DNA was extracted with the PowerSoil DNA Kit (Qiagen) on a KingFisher robot. Fungal ITS (ITS1F-ITS4) and bacterial 16S (V4 - 515F-806R) were amplified. Amplicons libraries were prepared according Lundberg et al. (2013) and sequenced on a MiSeq Illumina platform.
- Sequences were demultiplexed (QIIME), quality filtered and trimmed (USEARCH), clustered (UPARSE) in OTUs at 97% sequence similarity, and taxonomically classified using RDP Classifier using UNITE (Fungi) and Greengenes (Bacteria) databases.
- Data analysis was performed in R (R Core Team 2017).

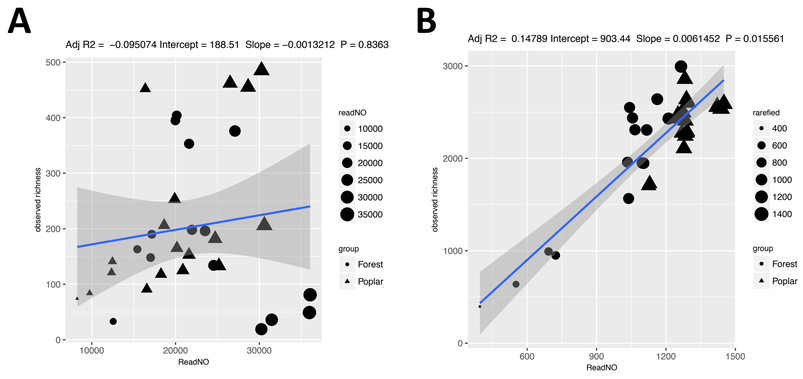
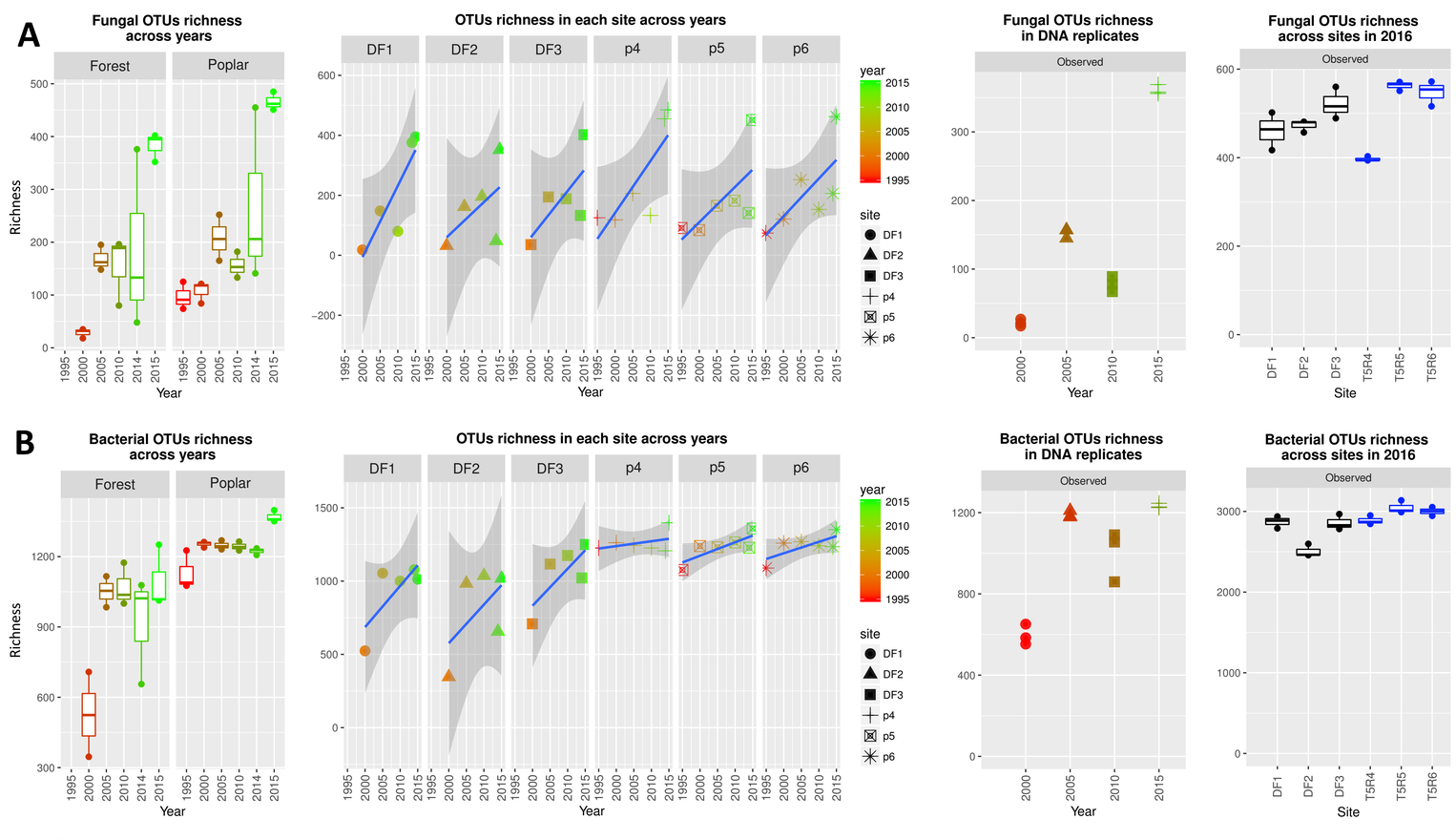


Fig. 1 Modelling species richness before rarefaction for Fungi (**A**) and Bacteria (**B**).



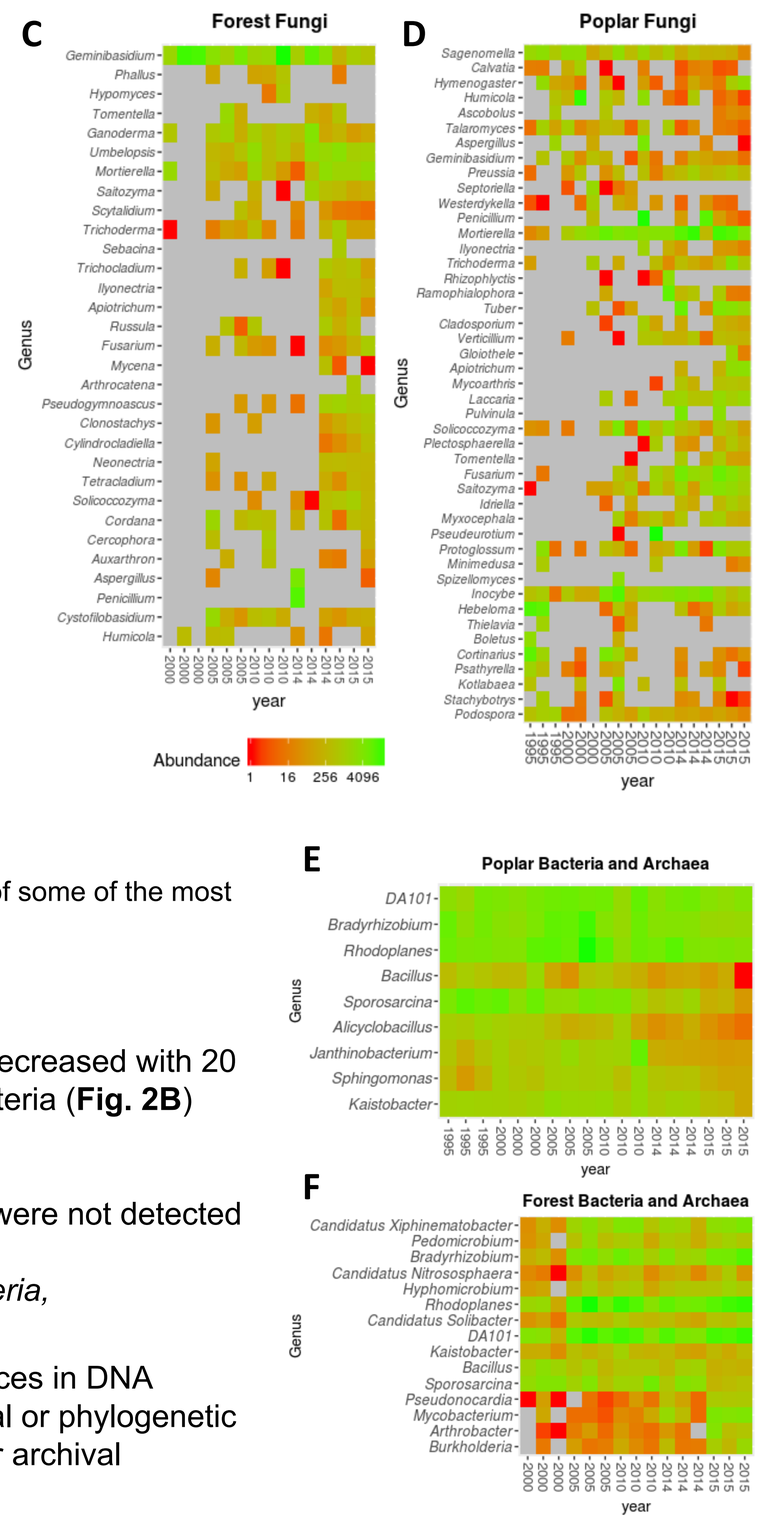


Fig. 2 Species richness across years, sites and DNA replicates of forest and *Populus* soils for (**A**) Fungi and (**B**) Bacteria. Heatmaps of some of the most variable (OTU sd>100 reads) fungal (**C, D**) and bacterial (**E, F**) genera in the forest and *Populus* soils.

RESULTS

- OTU richness was associated with sequencing depth of Bacteria but not Fungi (**Fig. 1**).
- Impacts of soil archiving was observed on both fungal and bacterial richness (**Fig 2**). Richness of Fungi (**Fig. 2A**) decreased with 20 years storage time form 404 to 19 in the deciduous forest and from 485 to 74 in the *Populus* soils. Richness of Bacteria (**Fig. 2B**) decreased from 1261 to 346 in the deciduous forest and from 1398 to 1076 in the *Populus* soils.
- Both sampling site and DNA extraction replicate showed effects on OTU richness.
- Few fungal taxa were consistently detected in each of the sampled years (e.g., *Geminibasidium*, *Inocybe*), others were not detected (e.g., *Ilyonectria*, *Laccaria*) after 2 to 5 years of storage.
- Bacterial read abundance was less variable across time than that of Fungi. However some genera (e.g., *Burkholderia, Mycobacterium*) were less detectable in older soils.

CONCLUSION Utility of soil archives for reconstructing past microbial communities is limited due to apparent differences in DNA degradation across different fungal and bacterial taxa. Tolerance to storage appears to be independent from ecological or phylogenetic fungal or bacterial group. Future work is needed to assess whether propagules in soil archives are active and whether archival procedures can be improved to preserve microbial community DNA.

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