Fungal communities from geothermal soils in Yellowstone National Park

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Abstract

Geothermal soils offer unique insight into the way extreme environmental factors shape communities of organisms. However, little is known about the fungi growing in these environments and in particular how localized steep abiotic gradients affect fungal diversity. We used metabarcoding to characterize soil fungi surrounding a hot spring-fed thermal creek with water up to ~85 ºC and pH ~10 in Yellowstone National Park. No soil variable we measured determined fungal community composition. However, soils with pH >8 had lower fungal richness and different fungal assemblages when compared to less extreme soils. Saprotrophic fungi community profile followed more closely overall community patterns while ectomycorrhizal fungi did not, highlighting potential differences in the factors that structure these different fungal trophic guilds. In addition, *in vitro* growth experiments in four target fungal species revealed a wide range of tolerances to pH levels but not to heat. Overall, our results documenting fungal communities within a few hundred meters suggest stronger statistical power and wider sampling are needed to untangle so many co-varying environmental factors affecting such diverse species communities.

Keywords

Soil, pH, hot spring, geothermal activity, mycorrhizal, saprobe, *Pisolithus*, *Agaricus*, *Fusarium*

Introduction

Soil abiotic factors are known to strongly influence global fungal diversity [1], with soil moisture and chemistry as key drivers of large-scale fungal richness and composition [2]. However, little is known about whether these same drivers function to structure fungal communities at a scale of <1,000 m, especially in environments with dramatic small-scale soil abiotic gradients. Geothermal areas are ideal systems to investigate such effects. Thermal sites

are geologic units characterized by hot geothermal water, steam, and gases such as carbon dioxide, sulfur dioxide, and hydrogen chloride. They are also notable for increased ambient soil temperature and landscape level thermal features such as hot springs and fumaroles. Thermal areas are by definition hot but can vary greatly in temperature ranging from mildly hot (~30˚C) to boiling point. In addition, geothermal water can cover the range of the pH scale [3]. Thermal soils can therefore display strong edaphic gradients within localized areas, with high variation in soil chemistry, moisture, and temperature.

Thermal areas and hot springs in particular are known for hosting diverse and specialized bacterial and archaeal communities, composed of thermophilic lineages, including novel ones, that evolved to tolerate these extreme environmental conditions [4-7]. However, much less is known about eukaryotic communities from thermal areas [8]. For the most part, members of Eukarya lack thermally stable membranes and are much less resilient to high temperatures compared to Archaea and Bacteria [9]. While the vast majority of Fungi fit this pattern, a very small number of thermophilic fungi have been documented. Thermophily evolved independently multiple times across the fungal tree of life [10] and a few species are able to withstand temperatures up to ~60 ºC [11]. Thermophilic fungi have been found in a wide variety of habitats responding to a variety of different environmental pressures and can also occur outside of thermal areas [12]. Additionally, fungi are well known to withstand a range of other extreme abiotic factors including broad pH ranges [13]. Several fungal species withstand 5-9 pH unit differences even when originating from non-extreme habitats [14-16], thus indicating that perhaps at the local scale, pH does not contribute strongly to structuring fungal communities. The rarity of thermophily in fungi, as opposed to tolerance of other environmental factors, make it logical to hypothesize that in thermal areas, soil temperature is likely the main factor driving

fungal richness and composition, while other parameters such as pH are expected to be less relevant for structuring fungal diversity.

Little is known about the soil fungi from Yellowstone National Park (YNP)'s thermal habitats which are famous for extremophile research. Available studies have been either based on fungal culturing from soil (known to detect only a small portion of fungal diversity) or used low-resolution molecular approaches that do not allow to fully document fungal species diversity [17-20]. Here we report on the effects of steep, localized soil abiotic gradients on fungi from an alkaline thermal area in YNP. We expected geothermal water to form strong abiotic gradients forming in surrounding soils, directly impacting soil fungal diversity. Specifically, we hypothesized that more extreme soil conditions (with higher temperature and pH) will host differentiated and depauperate fungal communities relative to less extreme conditions. Given the low fungal tolerance to high temperatures, we predicted temperature to be the main factor affecting fungal diversity in this site. We used amplicon sequencing of the fungal ITS rRNA gene to characterize the fungal communities in soils across a gradient surrounding a hot spring-fed thermal creek with water up to ~85 ºC and pH ~10. We found soil temperature was much lower than that of nearby boiling thermal water and covaried with moisture and pH. Our data indicated that no single variable was a determining factor driving fungal community composition. However, we found the highest pH (>8) hosted lower fungal richness when compared to less extreme soils. We also found saprotrophic fungi matched the pattern in the overall fungal community much more closely than mycorrhizal (tree-associated) fungi. In addition, we conducted *in vitro* growth assays testing for temperature and pH tolerance in four target fungal species (three saprobes and one mycorrhizal species) from Rabbit Creek. Although

none of our target fungi were found to be thermophilic, they strongly differed in the ability to tolerate high or low soil pH.

Methods

Sampling site and sample processing

We sampled soils around Rabbit Creek, located in the Yellowstone National Park Lower Geyser Basin which is a thermal area characterized by numerous thermal features. Rabbit Creek flows directly out of a set of hot springs (Table S1, Figure S1). Water temperatures in Rabbit Creek start at approximately 84 °C at the main hot spring source and cool to 30 °C downstream. The water stays consistently alkaline at approximately pH 10. Site vegetation consisted of *Pinus contorta* forest with herbaceous plants including the heat tolerant hot springs panic grass, *Dichanthelium lanuginosum* (see Stout and Al□Niemi [21] for a complete geothermal plant survey of Yellowstone National Park).

In September 2018, we collected a total of 70 soil cores (2.5 × 10 cm) along 14 transects in the Rabbit Creek area. For each 20 m transect we sampled a total of five cores with one core every 5 m. We sampled eight transects perpendicular to Rabbit Creek (T2-T8) which were 150 m apart along the creek. We also collected six transects perpendicular to three nearby hot springs (two transects per hot spring, T1, T9-T13) and an additional transect (T14) away from surface water and in the pine forest (Table S1).

We measured the soil temperature 10 cm deep next to each soil core and homogenized the soil in each core before flash freezing and storing approximately 2 g of soil. We saved the remaining soil for chemical analyses. All cores were processed within 24 h of collection. Soil chemistry and moisture analyses were conducted at the Environmental Analytical Laboratory (Land Resources

and Environmental Sciences, Montana State University). See Tables S1 and S2 for complete list of measurements.

DNA extraction and library preparation for sequencing

We extracted genomic DNA from soil with the QIAGEN DNA Power Soil Pro kit using 0.5 g of soil instead of the recommended 0.25 g. For library preparation, we amplified the ITS1 region using indexed primers ITS1F [22] and ITS2 [23] in the first round of PCR , followed by a second PCR to attach the 8 bp barcodes and Illumina adapters, using Hi-Fidelity Phusion DNA Polymerase for both reactions. Between each amplification step the product was cleaned from reagents and primers using the NGS SPRI Bead clean-up kit (ABM). Negative PCR controls were sequenced along with the experimental samples [24]. We included a synthetic mock community for ITS1 as a positive control to recover tag-switching among samples [25]. Amplicons of equimolar concentrations were pooled from each of 71 samples. The libraries were sent for paired 250-nucleotide reads on the Illumina MiSeq sequencing platform at the UC Davis DNA Sequencing Facility (Davis, California).

Sequence processing

Raw sequence reads were demultiplexed at the UC Davis DNA Sequencing Facility (Davis, California). We used the QIIME2 software package [26], and a modified QIIME2 pipeline for fungal ITS by Nguyen [27] with some additional modifications. We quality filtered the demultiplexed raw sequences ($q > 30$) setting the truncation of sequences 240 bp for forward and 200 bp for reverse sequences, and denoised the raw reads with DADA2 [28]. After quality filtering, we *de novo* clustered sequences into operational taxonomic unit (OTU) with

VSEARCH at a 97% sequence similarity followed by a re-clustering step with UCLUST at 97% to ensure the best recovery of our OTUs according to our input mock community [24]. We then excluded all sequences that were ≤ 85% coverage in the BLAST search since this primer set amplifies sequences that only partially match to a small part of the ITS gene. We used the UNITE database v8, [29, 30] for chimera checking, OTU clustering, and assigning taxonomy. And finally, we rarefied each sample to a sampling depth of 2831 sequences.

Initial ordination analyses of the OTU community matrix led us to exclude 30 samples as they clustered due to partial, very short sequences spanning only ~20 nucleotides at the beginning of the gene making them unidentifiable. In short, the non-metric dimensional scaling (NMDS) ordination including all remaining samples clustered in three groups (Figure S2) separated by pH but with high environmental variable overlap (Figure S3). A species indicator analysis showed the group with pH>8 clustered due to shared unidentified sequences (Table S3) and in fact, the ITS1 sequences for the OTUs in this group could not be identified by performing additional BLAST searches. We therefore excluded cores S5, S6, S7, S8, S9, S60, S63, S64, S67, and S69 from subsequent analyses. These cores were sampled near the three pools and the forest transect away from surface water (Table S1 and S2). After filtering, we used 40 cores to perform all further analyses. All scripts and data tables are available online at https://github.com/abazzical/YellowstoneFungi.

Soil Chemistry and Fungal Community Analyses

We performed Principal Coordinates Analysis (PCoA) to assess chemistry and community similarities across soil cores using the vegan package [31, 32] and Non-Metric Dimensional Scaling (NMDS) based on Bray-Curtis distances implemented in R [33]. Because our data had

no multivariate normality and unequal variances, we tested if the clusters identified were significantly different with the *adonis* function in the *vegan* package in R. We performed an indicator species analysis to determine if specific OTUs reflected soil abiotic parameters using the *indicspecies* package (ver. 1.7.8) [34] in R with 9999 permutations. We produced a species accumulation curve using the *specaccum* function estimating the mean curve and its standard deviation from 100 random permutations of the species accumulation data.

We categorized fungi into different trophic guilds using FUNGuild [35]. We further used the ectomycorrhizal (EM) OTUs and saprotrophic OTUs for additional NMDS and MANOVA analyses to test if specific guilds drive the patterns found in the total community. We excluded core S42 from EM fungi NMDS as it contained only one taxon, the orchid associated *Ceratobasidium* that was unique to that core.

To test for significant differences in the number of OTUs per core between the clusters identified in the NMDS analysis of the communities, we performed three Welch's T-tests (with a Bonferroni correction p=0.01667) for the whole data set, EM OTUs and for saprotrophic OTUs.

Fungal culture isolation and growth assays

We obtained pure cultures for four species growing at the Rabbit Creek site. We cultured the saprotophic *Agaricus campestris* and ectomycorrhizal *Pisolithus tinctorius* from fruitbodies on Potato Dextrose Agar (PDA) and Modified Melin-Norkans (MMN, [36]) media respectively. We also isolated cultures of potential plant pathogens *Fusarium oxysporum* and *F. avenae* from soil by plating serial soil dilutions in PDA. For all four species, we measured mycelial radial expansion at 30, 35, and 40˚C and pH 4, 7, and 9 separately. Each culture was grown in triplicate and measured at 2-4 day intervals. *Fusarium* and *Agaricus* cultures were grown on PDA and

Pisolithus was grown on MMN media. The growth in pH was calculated as the average of four radius measurements per colony for each time point. The temperature measurements were made by calculating the area of the fungal colony on the agar plate by importing the drawn outline of the fungus into ImageJ [37, 38].

We obtained culture identifications by sequencing the ITS rRNA gene. In short, we extracted genomic DNA using the Extract-N-Amp Sigma kit and amplified the ITS region with primers ITS1F [22] and ITS4 [23]. The PCR product was Sanger sequenced at GenScript and the sequences were checked and trimmed for low quality bases in the software FinchTV (http://www.geospiza.com/finchtv/). To identify the fungi, we used the BLAST tool in the NCBI database. Sequences are deposited in GenBank (MW471687 - MW472279).

Results

Soil abiotic variation

Soils in the Rabbit Creek area showed wide variation in temperature, moisture content, and chemistry (Fig. 1). Despite the creek's very high temperature water sources (84ºC), soil temperature was relatively low and varied between 10 ºC and 31ºC. Soil pH and moisture content varied broadly, with pH ranging from 4 to 10 and moisture from 10% to 100%. Temperature, pH, and moisture were correlated as soil cores collected closest to the creek were wetter, hotter, and more alkaline. The remaining soil chemical parameters also varied considerably (Table S1 and S2). Manganese (Mn), cobalt (Co), and cadmium (Cd) were highly correlated and inversely proportional to pH, temperature, and moisture. Total Nitrogen (N), total Carbon (C), and zinc (Zn) were orthogonal to these other parameters.

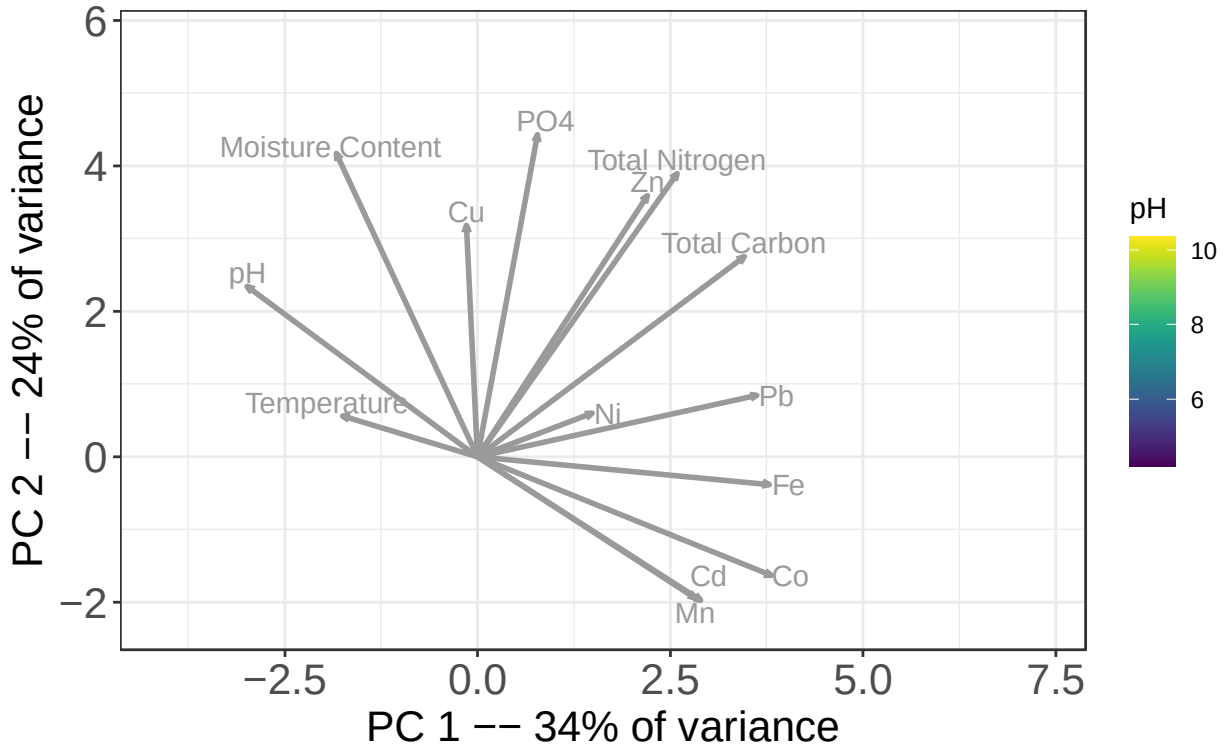

Figure 1 – Rabbit Creek soil chemistry variation. Principal components analysis on abiotic parameters measured across soil samples. Colors reflect the pH level measured for each soil core.

Overall soil fungal community composition

We found a total of 593 fungal OTUs at Rabbit Creek and were able to obtain taxonomic identifications other than "fungi" for close to 65% of them. This thermal site was heavily dominated by members of Ascomycota (38.4%) and Basidiomycota (18.7%) but also included Chytridiomycota, Rozellomycota, Mucoromycota, Glomeromycota, Kickxellomycota, Mortierellomycota, and Calcarisporiellomycota. We detected a few fungi known for thriving at high temperatures (>30 ºC), but not resilient enough to be considered heat tolerant fungi (with the ability to grow at 40 ˚C [39]). These included *Exophiala opportunistica*, a black yeast that also withstands high moisture and alkalinity and is commonly found in dishwashers [40, 41], *Waitea circinata*, a grass pathogen with optimal growth at 25-30 ºC [42], *Umbelopsis vinacea*, a soil fungus that remains active up to 37 ºC [43], *Gaertneriomyces semiglobifer*, a chytrid that

also growth up to 37 ºC [44], and *Fusarium kerasplasticarum*, an opportunistic animal pathogen that can also grow up to 37 ºC. In addition, we found one unidentified species of *Talaromyces*, a genus that includes thermophilic and thermotolerant species [45]. Notably, we recovered a number of cold-adapted fungi in our survey, including *Extremus aquaticus*, *Elasticomyces elasticus*, *Endophoma alongata*, *Alternaria chlamydosporigena*, *Mrakiella aquatica*, *Mrakia frigida*, *Tausonia pamirica*, *T. pulullans*, *Naganishia adeliensis*, and *Udeniomyces megalosporus*. Many of these species occur in Polar regions and have documented optimal growth temperatures <20 ºC [46-51].

Our species accumulation curve does not plateau (Figure S4), suggesting that community diversity is not saturated with our sampling.

Community clustering and indicator species analysis

We found fungal community differences across soil cores were not significantly different. Although the soil pH values mapped to the community NMDS analysis showed a pattern of samples clustering by pH, the two clusters of pH > and < 8 were not statistically significant (Figure 2A, S3A). A set of 42 OTUs was present only at pH >8 (Figure 2B).

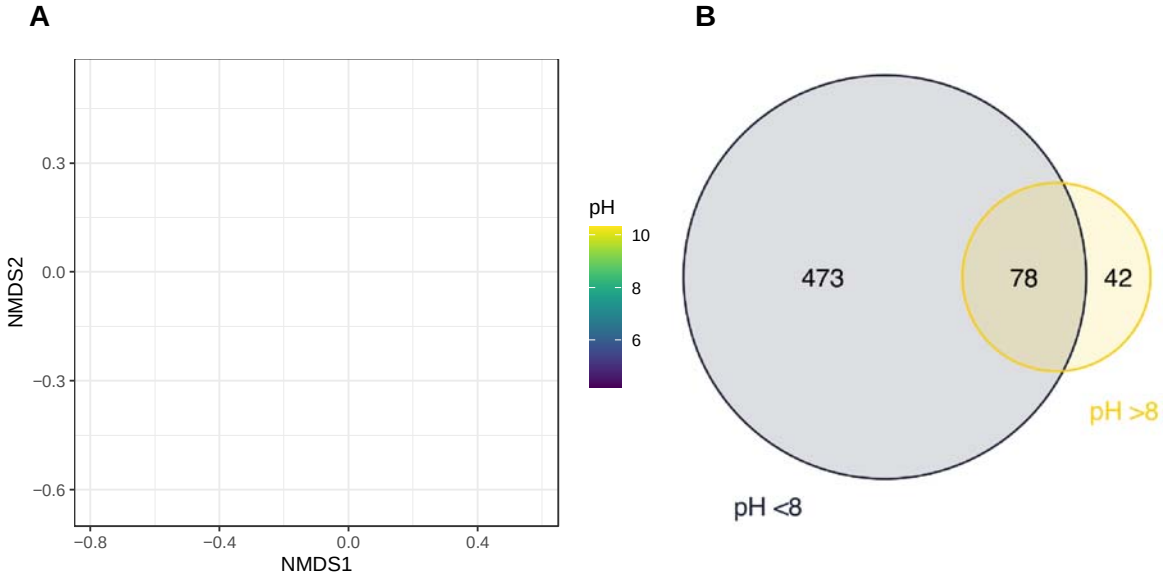

Figure 2 – Fungal community composition across soil cores collected in Rabbit Creek. (A) Non-Metric Dimensional Scaling across cores with respective pH measurements >8 (yellow to light green) and <8 (green – dark blue) (stress = 0.1577); (B) Venn diagram depicting fungal OTU overlap between the two clusters matching cores with pH measurements >8 (yellow) and <8 (dark blue).

The indicator species analysis on the two NMDS clusters detected 33 species associated with pH >8 and seven species associated with pH <8 (Table 1 and Table S3). 51% of taxa from the group pH >8 and all of the taxa from the group pH <8 belonged to the phylum Ascomycota. Three OTUs not identified to species were part of Glomeromycotina and six in Basidiomycota (Table S3).

Table 1 Indicator species identified to species for clusters of cores with pH >8 and <8. The complete list including unidentified taxa can be found in Table S3.

Cluster	Phylum	Class	Order	Species
pH >8	Ascomycota	Dothideomycetes	Pleosporales	*Septoriella phragmitis*
	Ascomycota	Sordariomycetes	Hypocreales	*Acremonium exuviarum*
	Ascomycota	Sordariomycetes	Hypocreales	*Acremonium tubakii*
	Ascomycota	Sordariomycetes	Hypocreales	*Fusarium keratoplasticum*
	Ascomycota	Sordariomycetes	Hypocreales	*Metarhizium pemphigi*

	Ascomycota	Sordariomycetes	Hypocreales	*Purpureocillium lavendulum*
	Ascomycota	Sordariomycetes	Hypocreales	*Sarocladium bactrocephalum*
	Ascomycota	Sordariomycetes	Sordariales	*Schizothecium miniglutinans*
	Basidiomycota	Agaricomycetes	Agaricales	*Agaricus gastronevadensis*
	Basidiomycota	Agaricomycetes	Agaricales	*Coprinus cordisporus*
	Basidiomycota	Agaricomycetes	Agaricales	*Simocybe centunculus*
	Basidiomycota	Agaricostilbomycetes	Agaricostilbales	*Kondoa miscanthi*
	Basidiomycota	Tremellomycetes	Cystofilobasidiales	*Udeniomyces megalosporus*
	Basidiomycota	Ustilaginomycetes	Ustilaginales	*Ustilago nunavutica*
pH <8	Ascomycota	Dothideomycetes	Capnodiales	*Elasticomyces elasticus*
	Ascomycota	Eurotiomycetes	Eurotiales	*Penicillium nodositatum*
	Ascomycota	Leotiomycetes	Helotiales	*Infundichalara minuta*
	Ascomycota	Leotiomycetes	Helotiales	*Phialocephala fortinii*

Indicator species for cores with pH >8 included *Acremonium exuviarum* and *Acremonium tubakii* and closely related *Sarocladium bactrocephalum* [52], which belong to the Emericellopsis clade characterized by alkalitolerant fungi [53]. We also identified the plant-associated and psychrophilic fungus *Udeniomyces megalosporus* as an indicatory species for cores with pH >8 [54]. For cores with pH <8, we found indicator species *Elasticomyces elasticus* reported as being able to grow at 0 as well as at 25 °C [55], and *Infundichalara minuta* also recorded from Pb-Zn mining sites with pH 7.3, although no other information is available for pH tolerance of these species [56].

Saprotrophic taxa follow overall fungal community clustering and pH gradient

Although not significant, we found that saprotrophic fungi but not ectomycorrhizal (EM) fungi closely followed the overall community pattern regarding pH (Figure 2). EM fungi (Figure 3A & B) showed much less separation between cores of higher and lower pH compared to saprotrophic taxa (Figure 3C & D). We found an almost complete EM OTU overlap between cores with high and low pH and only a single OTU unique to pH >8 cores while six out of 26 saprotrophic fungi

were unique to the pH >8 cores (Figure 3). A total of 234 (out of 593) OTUs were classified into trophic guilds, of which 22.2% (52) OTUs were mycorrhizal (including 16.7% (39) ectomycorrhizal (EM) fungi), 34.6% (81) saprotrophic, 9.8% (23) plant pathogens, 15% (35) animal pathogens, and 6.8% (16) dung-associated fungi (Table S4).

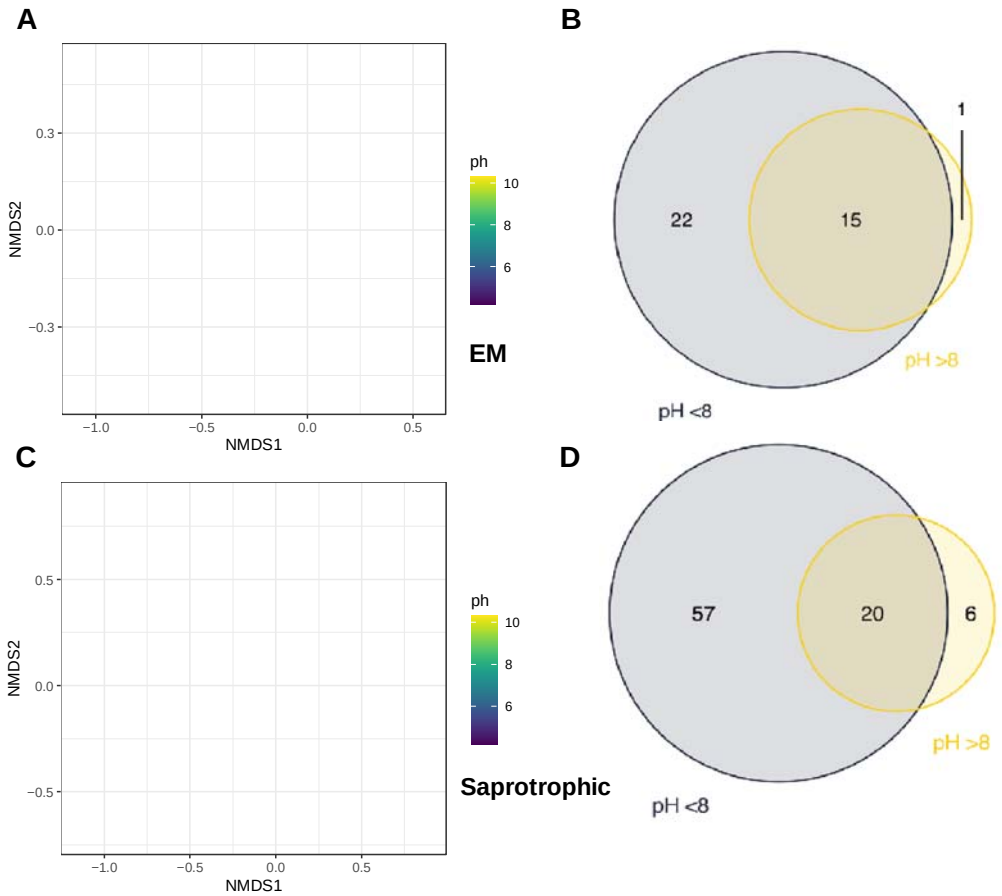

Figure 3 – EM and saprotrophic fungal community composition and OTU overlap. (A) EM fungi Non-Metric Dimensional Scaling across soil samples (stress = 0.205) with color scale of pH >8 (yellow to light green) and pH <8 (green – dark blue). (B) Venn diagram depicting EM fungal OTU overlap between soil cores with pH measurements >8 (yellow) and <8 (dark blue). (C) Saprotrophic fungi Non-Metric Dimensional Scaling across soil samples (stress = 0.196) with color scale of pH >8 (yellow to light green) and pH <8 (green – dark blue). (D) Venn diagram depicting saprotrophic fungal OTU overlap between soil cores with pH measurements >8 (yellow) and <8 (dark blue).

Saprotrophic OTU numbers decrease in more alkaline soils but EM OTU numbers do not

We found the environmental change from pH <8 to pH >8 reduced the number of total soil fungi and saprotrophic fungi, but not EM fungi. Soils with pH > 8 to host lower OTU richness (31 OTUs) compared to soils with pH <8 (40 OTUs, Figure 4, Welch's T-test: t = -3.0974, df = 30.356, p-value = 0.00418) . Saprotrophic OTUs followed a similar pattern (Welch's T-test: t = -3.1753, df = 17.662, p-value = 0.005335), with a mean of 6 OTUs in soils with pH >8 and a mean of 10 OTUs in soils with pH <8. EM fungi did not show a significant difference in numbers of OTUs across soils with pH > and < 8 (Welch's T-test: t = 1.5064, df = 12.7, p-value = 0.1564).

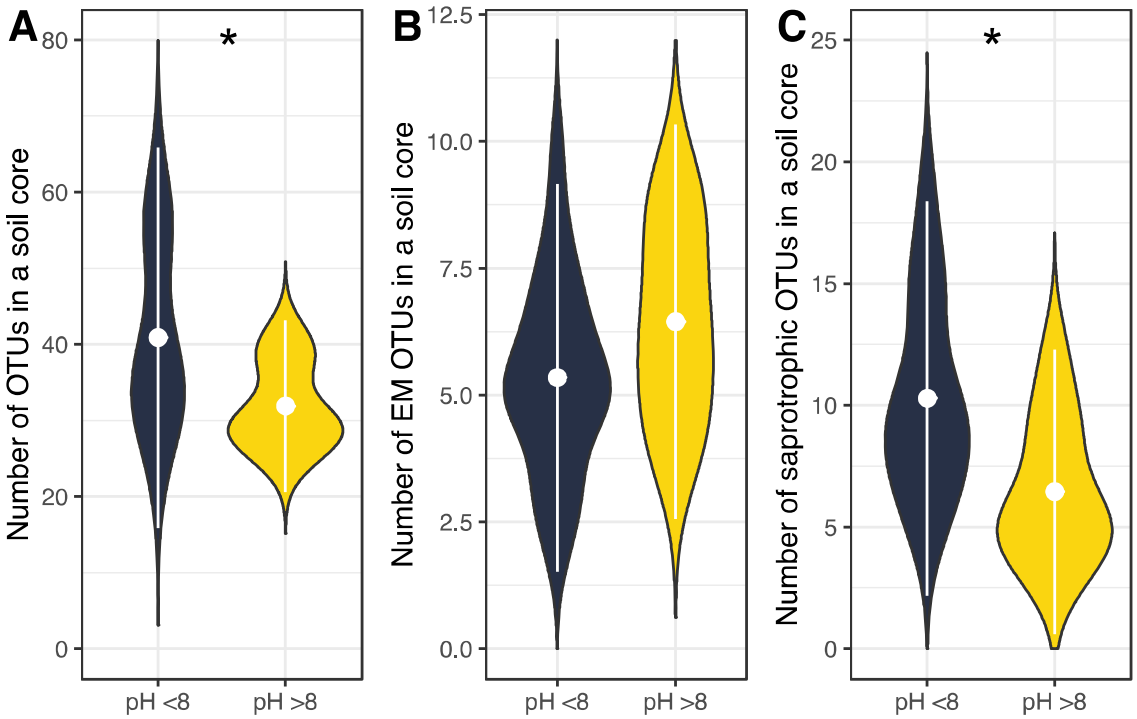

Figure 4 – Number of fungal OTUs detected in soil samples with pH <8 (dark blue) and >8 (yellow). (A) Number fungal OTUs; (B) Number of EM fungal OTUs; (C) Number of

saprotrophic fungal OTUs. Note the scale bar differences. Asterisks (*) indicate significance between two groups.

Effect of temperature and pH on four fungi from Yellowstone National Park soils

The four fungal species tested for temperature and pH tolerance lacked thermal tolerance but had the ability to tolerate a wide range of pH (Figures 5 and 6). Notably, all four species performed well at pH 7. Both *Fusarium oxysporum* and *F. avenae* grew equally well at pH 9 and pH 7 showing less growth at pH 4. Both *Agaricus campestris* and *Pisolithus tinctorius* grew best at pH 7, however, *P. tinctorius* was the only fungus with no growth at pH 9 (Figure 5). All four species grew well at 30 ºC but very poorly at 40 ºC (Figure 6). *P. tinctorius* and *F. oxysporum* grew equally well at 30 ˚C and 35 ˚C, while *A. campestris* and *F. avenae* grew better at 30 ˚C than 35 ˚C.

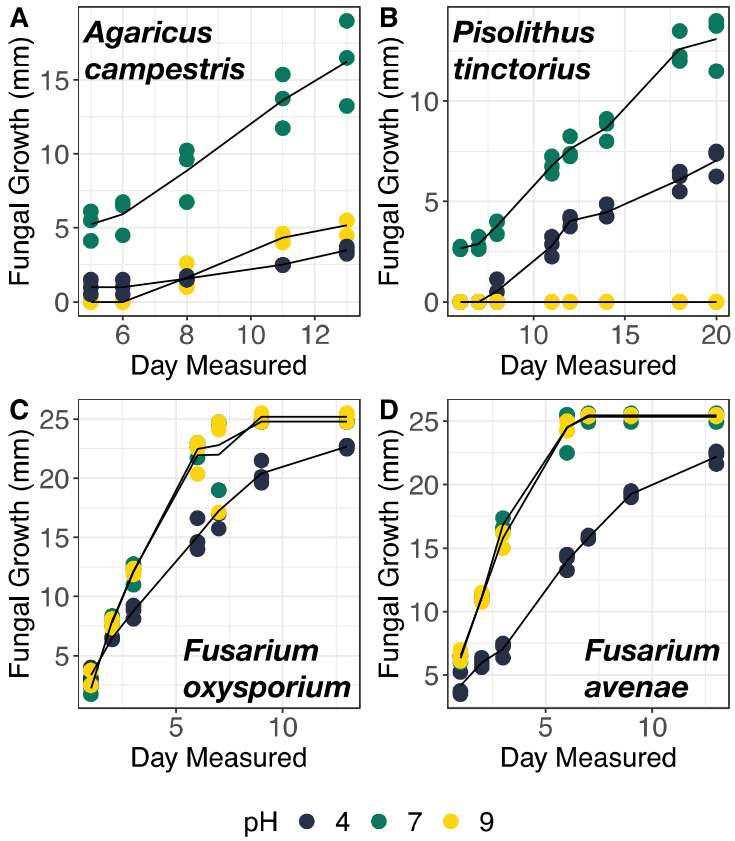

Figure 5 – Mycelial growth (average of 4 radius measurement per fungal thallus) at pH 4 (dark blue), pH 7 (green), and pH 9 (yellow) in *Agaricus campestris* (A), *Pisolithus tinctorius* (B), *Fusarium oxysporum* (C), and *Fusarium avenae* (D).

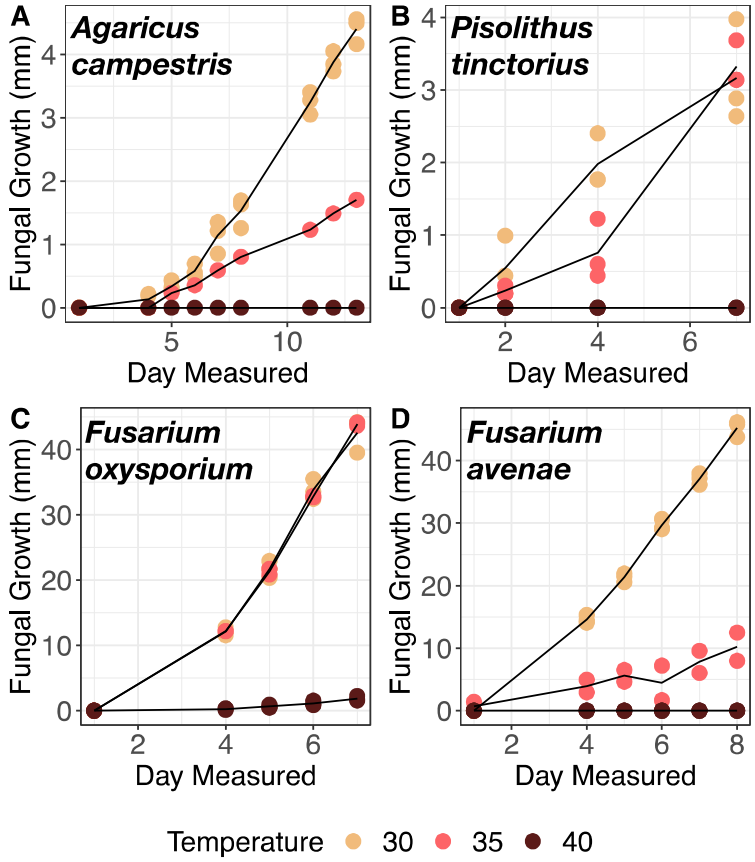

Figure 6 – Mycelial growth (fungus growth area mm^3) at 30ºC (peach), 35ºC (pink), and 40ºC (burgundy) in *Agaricus campestris* (A), *Pisolithus tinctorius* (B), *Fusarium oxysporum* (C), and *Fusarium avenae* (D).

Discussion

We investigated soil fungal communities from a geothermal area in Yellowstone National Park and found that although no single soil variable explained or structured fungal community composition, higher soil pH hosted lower fungal OUT richness. Saprotrophic fungi followed more closely the observed community trends rather than ectomycorrhizal fungi. Notably, we did not detect any thermophilic fungi in Rabbit Creek. In contrast, we detected several cold-adapted species previously recorded in the region. Specifically, we found the psychrophilic

basidiomycete yeast *Guehomyces pulullans* [57] and a few cold adapted Ascomycete yeasts identified in Vu, Groenewald, Szöke, Cardinali, Eberhardt, Stielow, de Vries, Verkleij, Crous, Boekhout and Robert [58], including *Naganishia adeliensis* and *Udenomyces megalosporus* (=*U. pyricola* in Vu et al 2016). We also found the psychrophiles *Elasticomyces elasticus* and *Mrakia* sp., which had been detected in previous soil surveys in both West Yellowstone and Hyalite near Cooke City, Montana [59]. Additionally, our *Tausonia pamirica* OTU matched 100% to the sequence of the type specimen (Genbank # NR_154490), a known psychrotolerant yeast from Antarctica [60]. The occurrence of these species is most likely unrelated to the thermal nature of Rabbit Creek and rather may reflect the regional climate characterized by severe cold winters.

We were surprised by the absence of thermophilic fungi and the presence of cold-adapted lineages in a thermal area and that none of the fungi we isolated in culture were able to grow at temperatures higher than 35˚C. Soil temperature is thought to be the most significant factor influencing bacterial and archaeal species composition in geothermally heated environments [21, 61, 62]. Thermophilic fungi are mostly known from studies on plant-associated fungi from geothermal environments [63] and surveys such as the YNP metagenome project [64]. There is also experimental evidence documenting previously unknown thermophilic and acidophilic fungal species from hot spring habitats (Yamazaki, Toyama and Nakagiri [65]. However, the soil temperature of our sampled cores was relatively low, ranging from 10 ºC to 31ºC, which is well within the range of known temperature tolerance of most fungi including the detected psychrophilic species [57, 58] and the fungi tested in this study (Fig. 6). It is important to note that our findings do not imply the absence of heat-adapted fungi in Yellowstone, as other thermal

areas characterized by warmer soils may well host thermophilic fungi of unknown taxonomic identity.

Conditions in highly acidic or alkaline environments often co-vary with other environmentally extreme variables such as elevated salt concentrations or temperatures (Orwa, Mugambi, Wekesa, & Mwirichia, 2020), and in our case, soil moisture content. It is known that edaphic parameters structure the communities of soil fungi and variation in chemistry (including pH) affects fungal diversity on global, continental and local scales [1, 2]. Individual fungi appear to have a wider pH tolerance range compared to bacteria [66], however there are several examples of fungi (including *P. tinctorus* which was studied here) being able to withstand both low and high pH levels [14]. Studies on acid- or alkaline-loving fungi have recorded new species and have expanded our knowledge of the possible habitat types for commonly found fungal species [13]. In addition, research on fungi associated with hot spring sediment across the globe has documented the phylum Ascomycota as dominant in these habitats. These included newly discovered species [65] and common members of widespread genera, such as *Aspergillus*, *Penicillium*, and *Cladosporium*. Mycorrhizal species belonging to Ascomycota have also been detected soda lake (pH range) habitats [67]. We found the Rabbit Creek soils with higher pH are inhabited by many members of the Ascomycota lineage, which includes several alkalitolerant fungi such as *Acremonium* from the Emericellopsis clade [53].

In conclusion, we found thermophilic fungi to be absent from the soils surrounding thermal water in Rabbit Creek. We also found no statistical support suggesting soil variables determined the structure of fungal community composition, but both overall and saprotrophic fungal richness

decreased with higher pH values. Further identifying the abiotic variables driving the fungal community assemblage of these soils will require more statistical power to untangle the weight of several co-varying factors. Further research on thermal areas characterized by hot soils is however needed, as it will shed light on the effects of high soil temperature on the ecology and evolution of fungi.

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