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ORIGINAL ARTICLE

Molecular Community Analysis of Arbuscular Mycorrhizal Fungi in Roots of Geothermal Soils in Yellowstone National Park (USA)

Susann Appoloni · Ylva Lekberg · Michael T. Tercek · Catherine A. Zabinski · Dirk Redecker

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Abstract To better understand adaptation of plants and their mycorrhizae to extreme environmental conditions, we analyzed the composition of communities of arbuscular mycorrhizal fungi (AMF) in roots from geothermal sites in Yellowstone National Park (YNP), USA. Arbuscular mycorrhizal fungi were identified using molecular methods including seven specific primer pairs for regions of the ribosomal DNA that amplify different subgroups of AMF. Roots of *Dichanthelium lanuginosum*, a grass only occurring in geothermal areas, were sampled along with thermal and nonthermal *Agrostis scabra* and control plants growing outside the thermally influenced sites. In addition, root samples of *Agrostis stolonifera* from geothermal areas of Iceland were analyzed to identify possible common mycosymbionts between these geographically isolated locations. In YNP, 16 ribosomal DNA phylotypes belonging to the genera *Archaeospora*, *Glomus*, *Paraglomus*, *Scutellospora*, and *Acaulospora* were detected. Eight of these phylotypes could be assigned to known morphospecies, two others have been reported previously in molecular studies from different environments, and six were new to science. The most diverse and abundant lineage was *Glomus* group A, with the most frequent phylotype corresponding to *Glomus intraradices*. Five of the seven phylotypes detected in a preliminary sampling in a geothermal area in Iceland were also found in YNP. Nonthermal vegetation was dominated by a high diversity of *Glomus* group A phylotypes while nonthermal plants were not. Using multivariate analyses, a subset of three phylotypes were determined to be associated with geothermal conditions in the field sites analyzed. In conclusion, AMF communities in geothermal soils are distinct in their composition, including both unique phylotypes and generalist fungi that occur across a broad range of environmental conditions.

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S. Appoloni · D. Redecker (✉)
Institute of Botany, University of Basel,
Hebelstrasse 1,
4056 Basel, Switzerland
e-mail: dirk.redecker@unibas.ch

Y. Lekberg · M. T. Tercek · C. A. Zabinski
Department of Land Resources and Environmental Sciences,
Montana State University Bozeman,
821 Leon Johnson Hall,
Bozeman, MT 59717, USA

Present address:
S. Appoloni
Biolytix AG,
Witterswil 4108, Switzerland

Present address:
Y. Lekberg
Department of Mycology, University of Copenhagen,
Copenhagen, Denmark

Present address:
M. T. Tercek
Walking Shadow Ecology,
Gardiner, MT 59030, USA

Introduction

Volcanic activity in Yellowstone National Park (YNP) in the Northwestern USA has caused the formation of geysers, thermal springs, mud pots, and steam vents (fumaroles). Microbes from aquatic geothermal environments have been characterized extensively, including cyanobacteria, green sulfur bacteria, and archaea (e.g., [20]). Soils that occur in

these geothermally active areas often show an elevated temperature and altered chemistry, with elevated levels of manganese, iron, aluminum, arsenic, and sulfide [14, 32]. Plant growth is often sparse in these areas and restricted to a few species. The heat-resistant grass *Dichanthelium lanuginosum* (Schmoll) Spellenb. (*Dichanthelium acuminatum* (Sw.) Gould and Clark; hot springs panicgrass, Poaceae) occurs exclusively in geothermal areas within YNP and can grow in soil temperatures reaching 57°C [38]. A second grass, *Agrostis scabra* Willd., (rough bentgrass, Poaceae) occurs in geothermal as well as nonthermal sites. It has been suggested that thermal and nonthermal *A. scabra* are reproductively isolated and represent different species [42].

Several studies have addressed possible mechanisms that enable plants to survive in geothermal areas, including altered root morphology, expression of heat-shock proteins [37], and use of water and steam resources to ameliorate heat stress [11]. Furthermore, growth within moss mats has been observed in some geothermal sites where the moss may ameliorate harsh environmental conditions for plants at some stages of their life cycle [43].

Besides the plants' tolerance and avoidance strategies, interactions with symbiotic fungi may confer increased fitness in geothermal environments. Fungal endophytes from the ascomycete genus *Curvularia* infected with a mycovirus confer significantly higher temperature tolerance to *Dichanthelium* in YNP [22, 30] by an unknown mechanism. Plants in geothermal areas are also consistently colonized by arbuscular mycorrhizal fungi (AMF), opening the possibility that this symbiosis may play a role in plant survival under these extreme conditions [3].

Arbuscular mycorrhiza is a mutualistic symbiosis formed between the majority of land plants and fungi from the phylum Glomeromycota. Many plants depend to a large extent on this interaction for their mineral nutrition. The host plants provide carbohydrates to the fungi, while the fungi increase the uptake of phosphorus and other nutrients of poor mobility [8] and improve resistance against certain pathogens [2]. The mycorrhizal symbiosis is of particular importance under nutrient-limiting conditions [35]. Fungal morphospecies and isolates may differ considerably in their ability to alleviate stress factors, such as high Al^{3+} concentrations and low pH [4]. If environmental conditions favor certain symbiont combinations, then specialized AMF communities would be expected. The distinct chemical and physical conditions in geothermal areas [3] may result in unique AMF communities. One objective of this research was to characterize AMF community composition in geothermal areas within YNP.

AMF have traditionally been classified and identified based on microscopic characteristics of spores. Currently, approximately 200 morphospecies are distinguished (http://www.amf-phylogeny.com). The task of identifying spores based on their morphology is notoriously difficult, and similar spore types can be formed by fungi that are distantly related [29]. In addition, spore formation is not always correlated with the extent of root colonization, and thus, evaluations of AMF communities based on spores alone may not accurately reflect the AMF community that is interacting with plants. To address this potential problem, molecular techniques that allow identification of AMF within roots were developed during the last decade, targeting the nuclear-encoded ribosomal genes [27]. Field studies based on molecular markers have provided numerous new insights into the dynamics of AMF communities and have presented evidence for host preference and host specificity [13], effects of season and plant host development stage [16], as well as an influence of agriculture and ecosystem type [15]. These studies have also shown that while some AMF species appear to be rather limited in their distribution, others are generalists and surprisingly widespread [25].

In this study, we used molecular methods to analyze AMF communities in geothermal areas in YNP to study the influence of extreme environmental factors on these communities. The steep edaphic gradients in geothermal areas of YNP provided an intriguing environment to study the response of AMF communities to environmental factors. To exclude possible geographic effects, we also sampled and analyzed a geothermal area in Iceland.

As nothing was previously known about AMF species occurring under these conditions, we expected two alternative scenarios: (1) unique AMF phylotypes specialized for geothermal conditions and not occurring in other environments; (2) AMF phylotypes with wide tolerances also occurring in other environments.

Methods

Description of Geothermal Sites, Host Plants, and Sampling Procedures

We sampled from three geothermal areas and their surrounding transition zones in YNP: Rabbit Creek (RC) in the Midway Geyser Basin (44°31′ N 110°49′ W) and two geothermal areas located 50 m apart in the Upper Geyser Basin in the Lone Star Geyser Area (LG 1 and 2, respectively, 44°34′ N 110°48′ W). For comparison, eight glomeromycotan rDNA sequences from *D. lanuginosum* roots and spores from a third site in the Lone Star Geyser Basin (LG3, Lekberg et al. unpublished; 44°26′ N 110°49′ W) were included in the phylogenetic trees. These

sequences were obtained using the same molecular methods as the ones described here.

At each site, *D. lanuginosum* was growing in the warmest areas with thermal *A. scabra*. The nonthermal *A. scabra* was found in geothermally influenced, but lower-temperature soils ("transition zone") together with other nonthermal plant species. The geothermal environment and transition zone at RC were previously characterized by Bunn and Zabinski [3] where soil temperatures of 31–42°C and soil pH values of 3.4–4.8 were reported. Furthermore, chemical profiles of geothermal and adjacent transition area soils were analyzed and appeared to differ significantly from each other. Iron was four times higher in geothermal soils compared to transition area soils, whereas Zn and Mn were much lower. *Dichanthelium* plants growing in the geothermal area were heavily colonized (averaging 53% colonized root length) throughout the summer, suggesting that this was an appropriate site for our study.

Field sites at LG1 and LG2 are located approximately 11 km from RC. Plants at LG1 grow on the moss *Racomitrium canescens*, but this is lacking at LG2. Both soils are acidic with pH around 4 at LG1 and 4.8 at LG2.

Sample collection is summarized in Supp. Table 1. At all sampling dates, the temperatures at a depth of 2 cm in thermal areas were substantially higher (averages of 34.9° C, 45°C, 41.8°C, 41.9°C) than in transition areas in RC (averages 25°C, 23.9°C) or nonthermal control plants in LG1 and LG2 (25°C and 27°C).

We collected samples in September 2003, October 2004, and June 2005 from RC, and in May 2005 from LG1 and LG2.

Whole plants were excavated, roots were stored at 4°C, washed in the laboratory at Montana State University within 24 h, and divided into aliquots of approximately 100 mg. After transport to Switzerland, they were frozen in liquid nitrogen and stored at −80°C, or they were lyophilized before transport.

In addition to the collections in geothermal and transition zones, we also collected mixed-species root cores (depth: approximately 5 cm) from nonthermal adjacent areas in LG1 and LG2, which contained *A. scabra* and other plant species that were not determined ("control plants").

In August 2005, roots of *Agrostis stolonifera* (creeping bentgrass, Poaceae) growing in a geothermal site at Ölkelduhals (Iceland) were sampled. Soil pH was 4–4.5 and soil temperature ranged from 37–41°C in thermal soils. The plants that were sampled in Iceland were washed and frozen at −80°C within 3 days.

A total of 37 root samples from 33 plants from YNP, and three plants from Iceland were analyzed. Internal root colonization was estimated qualitatively after staining subsamples of the roots with trypan blue according to standard procedures [19].

Molecular Identification

DNA Extraction

Aliquots of 100 mg roots were ground in liquid nitrogen with mortar and pestle or with a pellet pestle within the 1.5-ml tube. DNA was extracted from roots with a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA was eluted in one step in 30 µl elution buffer. Purified DNA was diluted 1:10 and 1:100 in Tris ethylene diamine tetraacetic acid (TE) buffer and used as template for the first polymerase chain reaction (PCR) of the nested procedure.

Polymerase Chain Reaction

PCR was performed in a nested procedure [15] using Taq polymerase from Amersham (Basel, Switzerland), 2 mM $MgCl_2$ 0.5 µm primers, and 0.25 mM of each desoxynucleotide phosphate. The first round of amplification was performed using universal eukaryote primers NS5 and ITS4 [46]. The cycling parameters were: 3 min at 95°C, followed by 30 cycles of 30 s at 95°C, 30 s of 51°C, and 2 min at 72°C. The program was concluded by a final extension phase of 10 min at 72°C. PCR products were diluted 1:100 in TE buffer and used as template in the second round. Eight separate PCR reactions were performed using the following primer pairs [15, 40]: GLOM1310/ITS4i, ARCH1311AB/ITS4i, PARA1313/ITS4i, ACAU1661/ITS4i, NS5/GLOM5.8R, GIGA1313/GIGA5.8R, GLOM1310/GLOM5.8R, and LETC1677/ITS4i.

Primer sequences and target AMF clades were: ACAU1661 (TGA GAC TCT CGG ATC GGG, Acaulosporaceae), ARCH1311AB (equimolar mixture of TGC TAA ATA GCT AGG CTG C and TGC TAA ATA GCC AGG CTG T; *Archaeospora* or *Paraglomus*), GIGA1313 (CTA AAT AGT CAG GCT AWT CTT, Gigasporaceae), GLOM1310 (AGC TAG GYC TAA CAT TGT TA, *Glomus* group A), LETC1677 (CGG TGA GTA GCA ATA TTC G, *Glomus* group B), PARA1313 (CTA AAT AGC CAG GCT GTT CTC, *Paraglomus*), GIGA5.8R (ACT GAC CCT CAA GCA KGT, Gigasporaceae), GLOM5.8R (TCC GTT GTT GAA AGT GAT, *Glomus* group A), ITS4i (TTG ATA TGC TTA AGT TCA GCG).

The PCR parameters for the second round were: 3 min at 95°C, followed by 30 cycles of 45 s at 95°C, 50 s of 61°C, and 1.5 min at 72°C. The program was concluded by a final extension phase of 10 min at 72°C. A "hot start" at 61°C was performed manually to prevent nonspecific amplification. In order to check the success of amplification, PCR products from the second round of nested PCR were run on agarose gels (2:1% NuSieve/

SeaKem, FMC, Rockland, ME, USA) in Tris or Acetate buffer at 90 V for 45 min.

Cloning and Sequencing

PCR products were cloned into a pGEM-T vector (Promega/Catalys, Wallisellen, Switzerland). Inserts from ten clones per ligation were reamplified, digested with *Hin*fI and *Mbo*I, and run on agarose gels. These enzymes have recognition sequences of four nucleotides and have proven to be useful in the gene region of the Glomeromycota used here [40]. Restriction fragment patterns were compared to a database based on Good-Enough RFLP Matcher [7]. Representatives of restriction types newly found in the respective sample were, then, sequenced in both directions. PCR products to be sequenced were purified using a High Pure Kit from Hoffman LaRoche (Basel, Switzerland). A BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) was used for labeling. Samples were run on an ABI 310 capillary sequencer. Sequences were deposited in the European Molecular Biology Laboratory database under the accession numbers AM942452 to AM942537 and AM905249 to AM905256.

Sequence Analysis

Sequences were aligned to previously published sequences in PAUP*4b10 [39]. The glomeromycotan origin of the sequences was verified by Basic Local Alignment and Search Tool [1] and by phylogenetic analysis of an alignment of 5.8S subunit sequences [28]. Separate ITS alignments were used for *Archaeospora, Paraglomus, Acaulospora* and *Glomus* group A [34]. In addition, an alignment of the partial 3′ end of the 18S rDNA small subunit was compiled for the 18S sequences amplified with GIGA5.8R, GLOM5.8R, GLOM1310, PARA1313, and ARCH1311AB. Representative sequences of each sequence type were checked manually for possible chimaeras, which were excluded from the analysis.

Phylogenetic trees were primarily obtained by distance analysis using the neighbor joining algorithm in PAUP* using the Kimura two-parameter model and a gamma shape parameter = 0.5. Neighbor-joining bootstrap analyses were performed with 1,000 replications. Results were verified by performing maximum likelihood analyses based on parameters estimated in Modeltest 3.5 [26].

Definition of Sequence Phylotypes

Single morphospecies and even spores of Glomeromycota contain multiple minor variants of rDNA sequences. It is not possible to assign a single sequence to a certain species or a fungal isolate. Sequence phylotypes were, therefore, defined in a conservative manner as consistently separated monophyletic groups in the phylogenetic trees. Only those clades were used that were supported by neighbor joining bootstrap analyses and also present in the respective maximum likelihood tree. The sequence phylotypes were designated after the major AM fungal clade in which they were placed, followed by a numerical index (x in the following examples) identifying the type: *Glomus* group A (GLOM-Ax, [34]), *Glomus* group B (GLOM-Bx), Acaulosporaceae (ACAU-x), Gigasporaceae (GIGA-x), *Paraglomus* (PARA-x), *Archaeospora* sensu lato (ARCH-x [24]). Numbering is consistent with prior studies conducted in the laboratory in Basel (e.g., [15]).

Data Analyses

The presence or absence of each AMF phylotype in each root sample was used to construct a sampling effort curve in EstimateS [6]. In order to investigate the influence of environmental factors (site, temperature, and host plant) on the distribution of AMF phylotypes, ordination analyses were conducted in CANOCO for Windows v. 4.5 [41] using the presence or absence data for each root sample. Environmental variables (site, host plant, sampling date) were coded by dummy variables (1/0) with the exception of temperature. Initial Detrended Correspondence Analysis suggested a unimodal character of the data response to the sample origin (the lengths of gradients were >4); therefore, the Canonical Correspondence Analysis (CCA) was used. The analyses were conducted using the following settings: focus on interspecies distances, biplot scaling, no transformation, downweighting of rare species.

Results

AM Fungal Lineages and Their Frequency

PCR products of glomeromycotan fungi were obtained from 29 out of 36 root samples. With the exception of *Glomus* group B (primer LETC1677), all glomeromycotan lineages targeted by the primer pairs were detected, including *Glomus* group A, Acaulosporaceae, Gigasporaceae, *Archaeospora*, and *Paraglomus*. Mostly in the absence of the target taxa, some primers amplified sequences of Ascomycota or (to a much smaller extent) Basidiomycota. This was particularly the case with primers ARCH1311AB, PARA1313, and ACAU1661.

Based on phylogenetic analyses of the five different datasets (Figs. 1, 2, and 3, Figs. S1 and S2), 18 phylotypes were defined, eight of which could be reliably assigned to known morphospecies from other environments. The largest diversity was found in *Glomus* group A (ten

phylotypes). Representatives of this group were present in 76% of all positive samples. Three phylotypes from *Glomus* group A could be assigned to known species: *Glomus intraradices* (GLOM-A1), *Glomus aureum* (GLOM-A11) and *Glomus diaphanum* (GLOM-A13). Because only ITS sequences were available from *G. diaphanum*, this relationship could only be demonstrated using the *Glomus* group A ITS dataset (Fig. S1). Phylogenetic analysis of 5.8S subunit/ITS2, as well as characteristic sequence motifs in the ITS1 region (not shown), were used to confirm some clades receiving only weak support by analysis of the 18S subunit (e.g., GLOM-A1, GLOM-A7 a, b, c in Fig. 1).

One of the two phylotypes from the Acaulosporaceae, ACAU-6, was found frequently and showed a close phylogenetic relationship to *Acaulospora morrowiae*, whereas ACAU-7 was only detected once (Fig. S2). The *Archaeospora* phylotype ARCH-5 was found consistently in geothermal environments in YNP. This phylotype belongs to a major clade within *Archaeospora* as defined by Morton and Redecker [24] that is only known from environmental sequences. It is very distantly related to known morphospecies (Fig. 2). In a recent revision, the genus *Archaeospora* was divided into the two genera *Ambispora* and *Archaeospora* [36, 45]. According to this nomenclature, the clade containing ARCH-5 would constitute an unknown glomeromycotan genus.

Three phylotypes of *Paraglomus* were distinguished (Fig. 3), two of which fell within the morphospecies *Paraglomus occultum* (PARA-2, PARA-3). The third (PARA-1) corresponds to the newly described species *Paraglomus laccatum* [31] and has been previously detected in a number of environments (e.g., [15]). Only one phylotype (GIGA-4) of *Scutellospora* (Gigasporaceae) was found, which was closely related to *S. pellucida* (not shown). The most frequently found phylotype was GLOM-A1 (*G. intraradices*), found in 41.7% of the samples, followed by GLOM-A11, GLOM-A26, and PARA-1 (all 19.7%) and GLOM-A-13, ARCH-5, and ACAU-6 (all 16.7%). In all, we found six new phylotypes in YNP that were distinct from known sequences. Whereas some new phylotypes appear to be rare and were only found once (GLOM-A24, ACAU7), others were found more than once (GLOM-A27, GLOM-A7a) and two were detected regularly (ARCH-5 and GLOM-A26).

In the preliminary sampling in Iceland, seven phylotypes were detected, five of which were also found in YNP (GLOM-A1, PARA-1, GLOM-A7a, GLOM-A7b, ACAU-6). GLOM-A7a is currently only known from Iceland and YNP, whereas GLOM-A7b has been detected within roots in molecular field studies from other habitats [33, 40].

Root samples harbored between zero and five phylotypes, with many root samples from the hottest soils being apparently nonmycorrhizal. The sampling effort curve (Fig. S3) showed that in the case of *Dichanthelium* and the Rabbit Creek field site, the major proportion of the phylotype diversity was characterized. In nonthermal *A. scabra*, fewer plants were sampled, but the curve had a similar trajectory as in *Dichanthelium*. The phylotype richness in thermal *A. scabra* clearly shows the tendency to stabilize on a much lower level than for *D. lanuginosum* and non-geothermal *A. scabra*. The sampling in Iceland as well as LG1 and LG2 (not shown) was not exhaustive and provided only a first sample of phylotypes occurring in these sites. However, the purpose of analyzing these samples was to obtain a first insight into the phylotypes occurring across sites, and this analysis indicated that there were a number of phylotypes occurring in more than one site.

AMF Community Structure in Geothermal and Non-geothermal Samples

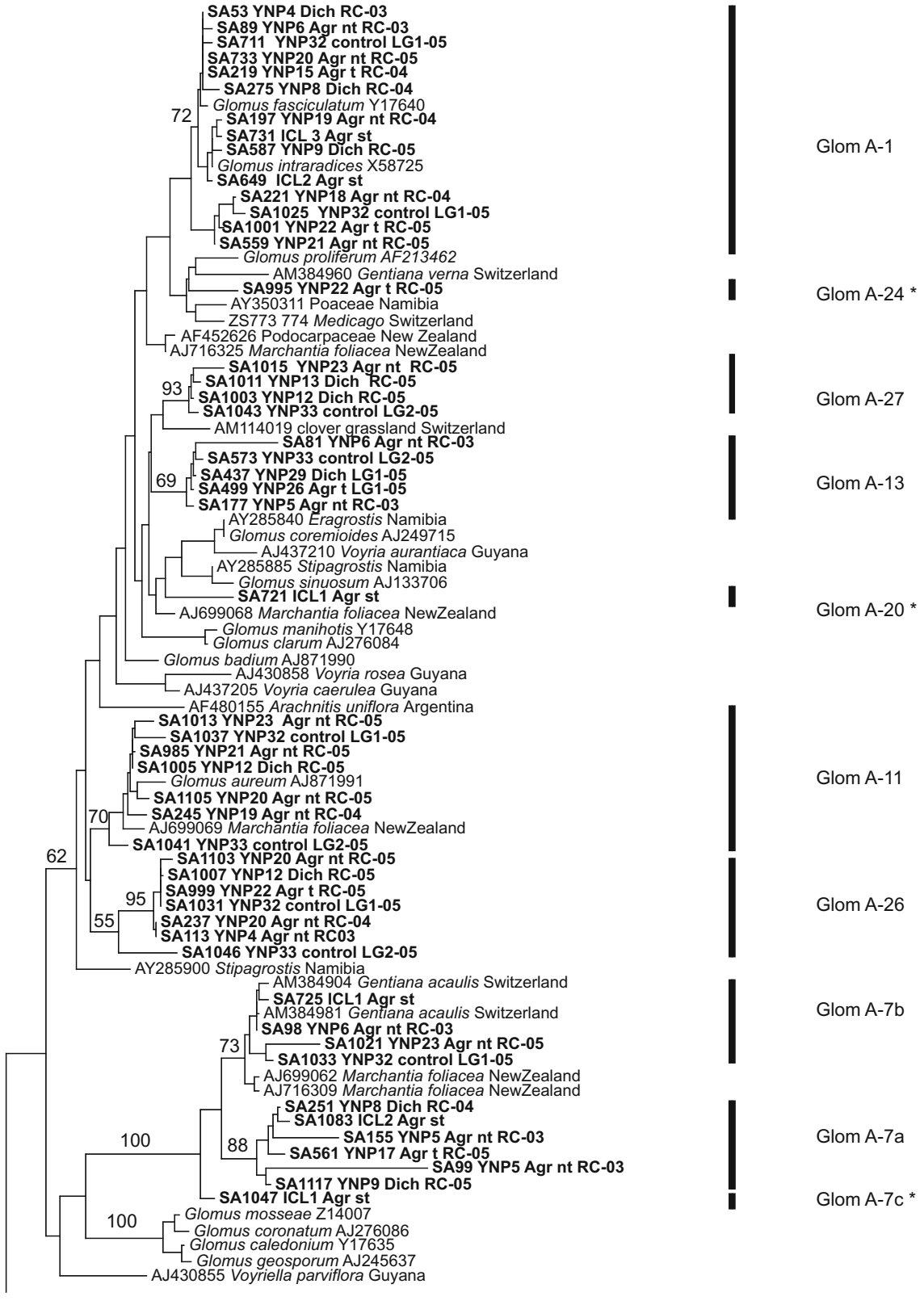
A group of three phylotypes was consistently and exclusively found in geothermal samples, i.e., in *D. lanuginosum* and thermal *A. scabra* but never in nonthermal *A. scabra* and control plants: ARCH-5, ACAU-6, and PARA-3. Two other phylotypes (ACAU-7 and GIGA-4) were only detected once in thermal samples; therefore, the distribution of these phylotypes cannot be addressed reliably.

Multivariate analyses were conducted on the whole dataset as well as on a subset containing only samples from the more exhaustively sampled Rabbit Creek field site. In the species–environmental variable biplots based on these analyses, the group of three geothermal-associated AMF phylotypes was associated with the temperature gradient and the vectors of the host plants *D. lanuginosum* and thermal *A. scabra*.

As there was an apparent trend for the absence of AMF colonization in root samples from higher temperatures, a new category "NoSpecies" was created for some analyses to visualize these samples in the biplots. The introduction of this category did not affect the conclusions about the grouping of the three geothermal-associated phylotypes.

With only temperature and host plants included as environmental factors in an analysis of the complete dataset (Fig. 4), three distinct groups of AMF emerged that were associated with *A. stolonifera* from Iceland, thermal *Agrostis* or *Dichanthelium*, and nonthermal *A. scabra* or control plants, respectively. A similar separation of two groups comprising thermal *A. scabra* or *Dichanthelium* and nonthermal *A. scabra* or control plants was obtained by a parallel analysis of the well-sampled Rabbit Creek site only (Fig. S4).

Interestingly, in the field sites in YNP studied here, *Glomus* group A phylotypes, did not associate with thermal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SA89 YNP6 Agr nt RC-03
SA711 YNP32 control LG1-05
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SA219 YNP15 Agr t RC-04
SA275 YNP8 Dich RC-04
Glomus fasciculatum Y17640
72
SA197 YNP19 Agr nt RC-04
SA731 ICL 3 Agr st
SA587 YNP9 Dich RC-05
Glomus intraradices X58725
SA649 ICL2 Agr st
SA221 YNP18 Agr nt RC-04
SA1025 YNP32 control LG1-05
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SA559 YNP21 Agr nt RC-05
Glom A-1
Glomus proliferum AF213462
AM384960 Gentiana verna Switzerland
SA995 YNP22 Agr t RC-05
AY350311 Poaceae Namibia
ZS773 774 Medicago Switzerland
Glom A-24 *
AF452626 Podocarpaceae New Zealand
AJ716325 Marchantia foliacea NewZealand
SA1015 YNP23 Agr nt RC-05
SA1011 YNP13 Dich RC-05
93
SA1003 YNP12 Dich RC-05
SA1043 YNP33 control LG2-05
Glom A-27
AM114019 clover grassland Switzerland
SA81 YNP6 Agr nt RC-03
SA573 YNP33 control LG2-05
SA437 YNP29 Dich LG1-05
69
SA499 YNP26 Agr t LG1-05
SA177 YNP5 Agr nt RC-03
Glom A-13
AY285840 Eragrostis Namibia
Glomus coremioides AJ249715
AJ437210 Voyria aurantiaca Guyana
AY285885 Stipagrostis Namibia
Glomus sinuosum AJ133706
SA721 ICL1 Agr st
AJ699068 Marchantia foliacea NewZealand
Glom A-20 *
Glomus manihotis Y17648
Glomus clarum AJ276084
Glomus badium AJ871990
AJ430858 Voyria rosea Guyana
AJ437205 Voyria caerulea Guyana
AF480155 Arachnitis uniflora Argentina
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SA1037 YNP32 control LG1-05
SA985 YNP21 Agr nt RC-05
SA1005 YNP12 Dich RC-05
Glomus aureum AJ871991
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SA245 YNP19 Agr nt RC-04
AJ699069 Marchantia foliacea NewZealand
SA1041 YNP33 control LG2-05
Glom A-11
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SA1103 YNP20 Agr nt RC-05
SA1007 YNP12 Dich RC-05
SA999 YNP22 Agr t RC-05
95
SA1031 YNP32 control LG1-05
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SA237 YNP20 Agr nt RC-04
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SA1046 YNP33 control LG2-05
Glom A-26
AY285900 Stipagrostis Namibia
AM384904 Gentiana acaulis Switzerland
SA725 ICL1 Agr st
AM384981 Gentiana acaulis Switzerland
SA98 YNP6 Agr nt RC-03
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SA1021 YNP23 Agr nt RC-05
SA1033 YNP32 control LG1-05
Glom A-7b
AJ699062 Marchantia foliacea NewZealand
AJ716309 Marchantia foliacea NewZealand
SA251 YNP8 Dich RC-04
SA1083 ICL2 Agr st
100
88
SA155 YNP5 Agr nt RC-03
SA561 YNP17 Agr t RC-05
SA99 YNP5 Agr nt RC-03
SA1117 YNP9 Dich RC-05
Glom A-7a
SA1047 ICL1 Agr st
Glom A-7c *
100
Glomus mosseae Z14007
Glomus coronatum AJ276086
Glomus caledonium Y17635
Glomus geosporum AJ245637
AJ430855 Voyriella parviflora Guyana
0.005 substitutions/site

◀ **Figure 1** Phylogenetic tree of *Glomus* group A based on partial 18S rDNA. A tree comprising all glomeromycotan lineages was obtained by neighbor joining, only the *Glomus* group A clade is shown here for clarity. *Numbers above branches* denote bootstrap values (percent) from 1,000 replications. Sequences obtained in the present study are shown in *boldface*. They are labelled with the internal sequence identification number (e.g. SA53), the sample code (*YNPx* for Yellowstone, *ICLx* for Iceland, see Table S1), an abbreviation of the host plant (*Dich* for *D. lanuginosum*, *Agr t* for thermal *A. scabra*, *Agr nt* for nonthermal *A. scabra*, control for unidentified nonthermal control plants, and *Agr st* for *A. stolonifera*), the location (RC, LG1, LG2, Iceland, see text) and the sampling date (*03*=Sept 2003, *04*=Oct 2004, *05*=May–June 2005). Sequence phylotypes labelled with a *star* (*) were only found in one root sample. Sequences from the databases are labelled as follows: accession number, host plant, and country of origin for root-derived sequences; fungal species and accession number for spore-derived sequences

Agrostis or *Dichanthelium* in the biplots but with nonthermal samples. A striking example were the samples from LG1 and LG2, where only one phylotype from *Glomus* group A (GLOM-A13) was detected, whereas the control samples taken nearby from nonthermal plants contained a rich variety of phylotypes from this clade. Overall, GLOM-A phylotypes were significantly more diverse in nonthermal plants in YNP (average: 2.90 phylotypes per sample) than in thermal plants (1.13; P=0.0002, t-test). Furthermore, GLOM-A phylotypes were significantly more diverse compared with all other phylotypes in nonthermal plants (0.50; P<0.0001, t-test). There was no significant difference in phylotype richness between *Glomus* group A and other phylotypes in thermal plants.

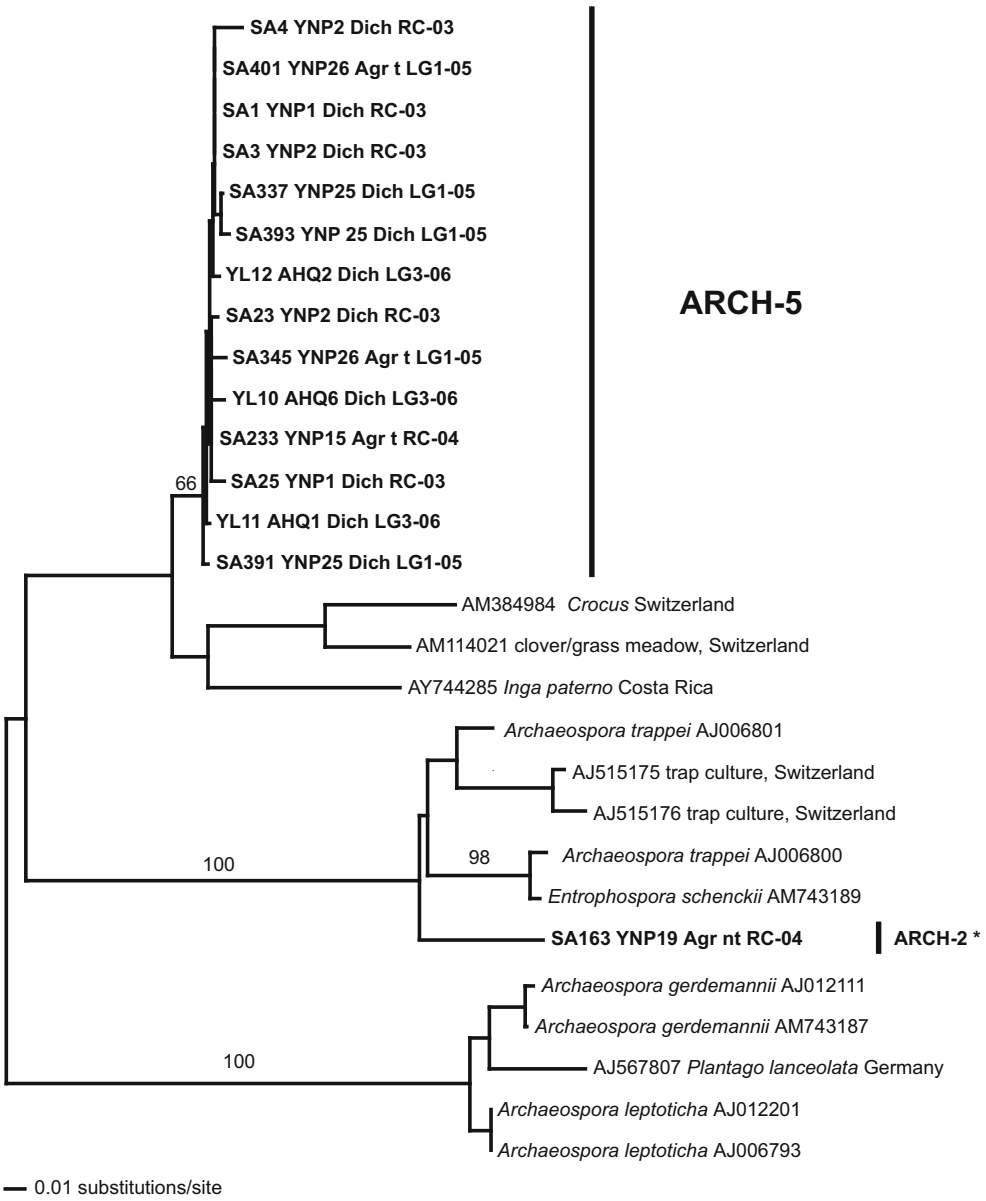

Figure 2 Phylogenetic tree of *Archaeospora* based on 5.8S rDNA and ITS2. The tree was obtained by neighbor joining. *Numbers above branches* denote bootstrap values from 1,000 replications. For an explanation of the label details, see Fig. 1. For comparison, some sequences from another study are included, which originate from another field site LG3 in YNP (Lekberg et al. unpublished)

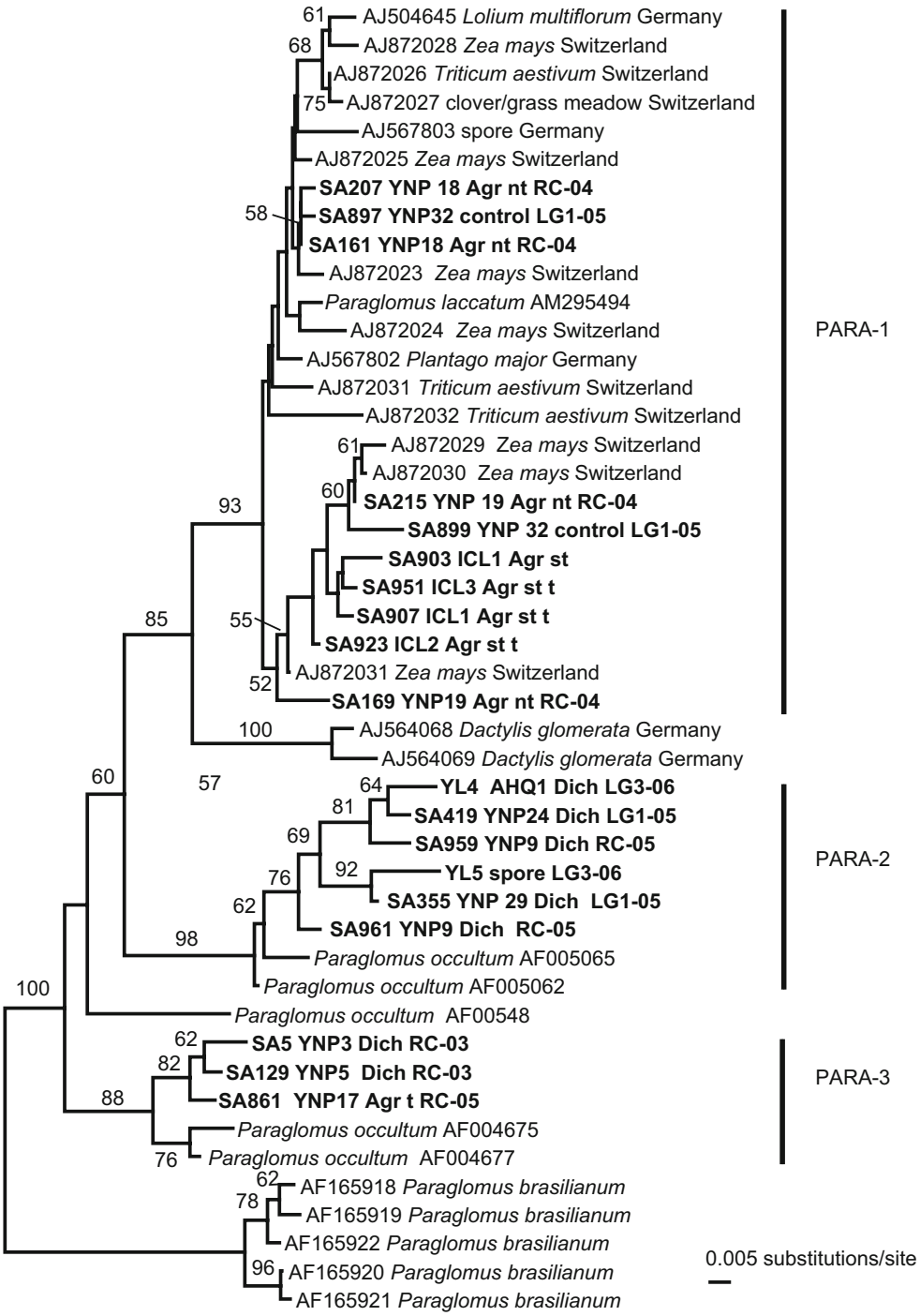

Figure 3 Phylogenetic tree of *Paraglomus* based on 5.8S rDNA and ITS2. The tree was obtained by neighbor joining. *Numbers above branches* denote bootstrap values from 1,000 replications. For an explanation of the label details, see Fig. 1. For comparison, some sequences from another study are included, which originate from another field site LG3 in YNP (Lekberg et al. unpublished)

Discussion

This is the first study using molecular methods to characterize AMF community composition in geothermal soils. Previous studies have addressed effects of soil warming on AMF in greenhouse experiments [12] or root organ cultures [9], but those studies focused on lower soil temperature than was observed here. Tropical soils may also often reach temperatures above 40°C [10], but to our knowledge, the effect of this warming on AMF communities has not been addressed. It should be emphasized, however, that in a field study as the one presented here, the temperature factor cannot be separated from the possible influence of the host plants, which occur within specific temperature ranges and the unique soil chemistry of the geothermal soils.

Whether all phylotypes we identified correspond to species and whether biological species concepts can be

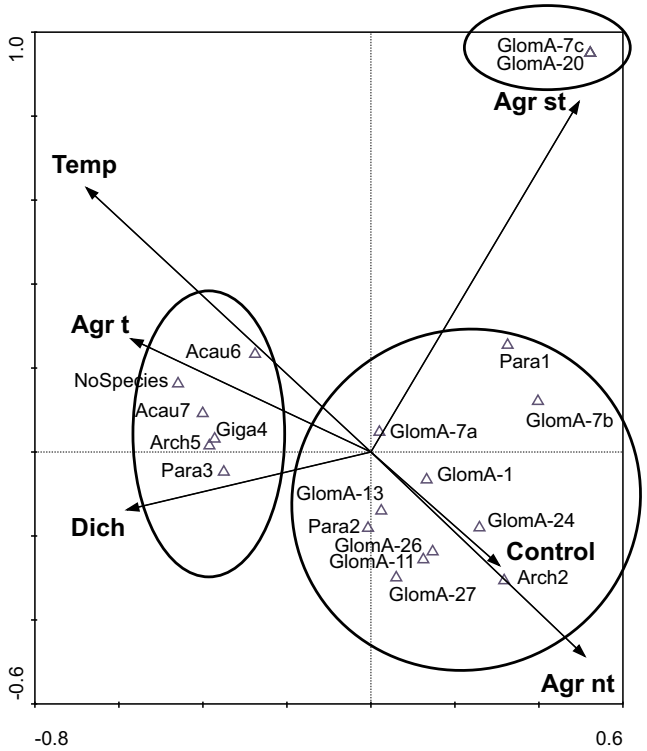

Figure 4 Species-environmental variables biplot (*arrows* = environmental variables, *triangles* = species = AMF phylotypes) showing a CCA analysis of the complete dataset with environmental variables restricted to temperature and host plants. The *x-axis* represents the first canonical axis, the *y-axis* represents the second canonical axis. *Dich* = *D. lanuginosum*, *Agr t* = thermal *A. scabra*, *Agr nt* = nonthermal *A. scabra*, *Control* = nonthermal control plants, *Agr st* = *A. stolonifera*, and *Temp* = Temperature. This biplot shows the distinct cluster of AMF phylotypes that associates with thermal plant species (Dich and Agr t) and is positively correlated with the temperature gradient. *Ellipses* were drawn around this cluster and the groups of phylotypes associated with YNP nonthermal samples and *A. stolonifera* to highlight them

applied in the putatively asexual Glomeromycota remains to be addressed. Nonetheless, we detected a considerable proportion of AMF phylotypes in YNP that have been found elsewhere. In particular, the ubiquitous generalist *G. intraradices* has been found in an astonishingly broad range of environments, including arable soils in Switzerland, Germany [15] and Zimbabwe [21], high montane meadows in the Swiss Alps [40] and in *Prunus africana* roots from Ethiopia [47]. In fact, the GLOM-A1 phylotypes detected in YNP fall within the range of ITS sequence variation of a single spore of a *G. intraradices* isolate from Switzerland [17]! This range served as a molecular working species definition here (Fig. S1). The fact that this fungus has now been detected in an environment as extreme as thermal soils of YNP has interesting implications for its biology. Koch et al. [18] found that even using highly polymorphic genetic markers, isolates of this species from Switzerland and Canada were surprisingly similar, supporting its status as a widespread generalist.

Multivariate analyses suggested that although generalists such as *G. intraradices* may occur in thermal as well as nonthermal soils, the composition of AMF communities in *D. lanuginosum* and thermal *A. scabra* in geothermal environments was distinct from *A. scabra* and control plants in nonthermal environments. Out of the 16 phylotypes detected in YNP, three (ARCH-5, ACAU-6, and PARA-3) showed a clear tendency to occur predominantly at higher soil temperatures and in association with thermal *A. scabra* and *D. lanuginosum*.

The phylotype ARCH-5 has not been described outside YNP and belongs to a third major lineage within the genus without any described morphospecies. Members of this clade have been detected in Podocarpaceae roots [33] as a rare phylotype in a clover-grass meadow in Switzerland [15] and abundantly in roots of submerged water plants from oligotrophic lakes (I. Paradi, personal communication). Interestingly, ACAU-6 (*A. morrowiae*) was found in both YNP and Iceland. This species was previously reported as an efficient plant growth promoter in acidic and high aluminum soils [5], which may explain its occurrence in the hot, acidic soils of geothermal areas. PARA-3 falls within the morphological range of *P. occultum* (together with PARA-2) but can be distinguished genetically and, as demonstrated here, also ecologically as they showed different distribution patterns (Fig. 4). *P. occultum* was reported to be associated with *G. diaphanum* (GLOM-A13) in acid mine spoils in West Virginia [23], but it is uncertain if that *Paraglomus* isolate corresponded to either PARA-2 or PARA-3 found here. In YNP, only PARA-2 once co-occurred in the same sample together with GLOM-A13, which was found in six samples. As *Paraglomus* species form small, hyaline spores devoid of any prominent morphological characters, the possibility of cryptic species within this morphospecies appears plausible. Generally, the data available on the distribution of *P. occultum* and *A. morrowiae* also allow for the possibility that the occurrence of ACAU-6 and PARA-3 in geothermal soils can be attributed, in part, to their ability to tolerate low pH or the unique soil chemistry.

In many higher temperature samples, no phylotypes could be detected, resulting in the "NoSpecies" phylotype category strongly associated with temperature. This apparent absence of AMF was not due to PCR failure by inhibitor compounds as nontarget fungi were amplified from most of these samples. Samples were only taken from green, living plants and although microscopic examination revealed some degraded roots in the samples, intact roots devoid of AMF colonization were also present. We have observed that root mortality increases with soil temperature (unpublished data), which presents two possible explanations for the increased "NoSpecies" abundance in higher temperature soils: (1) plant roots may have a greater heat tolerance than AMF, or (2)

decreased host quality by heat stress may cause lower colonization by the fungus. These two possibilities are currently being investigated under controlled greenhouse conditions using AMF isolates from YNP.

There is a complex relationship in the field between temperature and root growth. Soil temperatures in thermal areas increase with depth, and they fluctuate both diurnally and seasonally. The values for soil temperature were the temperature at the time we sampled and are, therefore, snapshots at a given time. In most field sites, there was a clear zonation from high-temperature areas where only *D. lanuginosum* grew, followed by areas with thermal *A. scabra* and *D. lanuginosum*, and a transition to more diverse meadow communities with nonthermal *A. scabra*. The latter grass could also be termed "thermal-influenced" due to the spatial proximity of the geothermal areas. In all sites, the difference between the average temperatures of thermal vs. nonthermal or control plants was at least 9.5°C. Based on this, we believe we correctly identified ecologically relevant habitats in the field sites.

As thermal and nonthermal *A. scabra* may constitute different species [42], the possible effects of host preference on AMF communities cannot be separated from the influence of temperature in our study because different plant species occurred at specific temperature ranges. This is inevitable in a field study of this kind. Host preference is relatively low in the AM symbiosis but has been reported in the field [13, 44]. In contrast to nonthermal *A. scabra* and *D. lanuginosum*, thermal *A. scabra* appears to be an annual [42]. This may explain the lower AMF richness in its roots because there is less time for the establishment of a complex AMF community. However, AMF taxa detected in thermal *A. scabra* generally represent a subset of those found in *D. lanuginosum*, and AMF in nonthermal *A. scabra* were more similar to control samples taken outside the geothermally influenced areas. Therefore, we consider the patterns we observed to be primarily due to habitat influence. In addition to temperature, Bunn and Zabinski [3] reported drastic differences in the soil chemical environment between thermal and adjacent thermally influenced soils. Unfortunately, soil chemical properties were not recorded in our samples and based on this, we cannot separate the effect of temperature from other variables that may change among the sample sites. This does not, however, influence our conclusion that the AMF communities appear to differ between different habitats.

The occurrence of distinct AMF in geothermal environmental soils does not provide evidence of a positive effect on the fitness of the plants by these fungi. Recently, a positive effect on *Dichanthelium* heat resistance by a different, unexpected group of fungi was reported, namely by ascomycete endophytes of the genus *Curvularia* [30]. This effect was attributed to a virus present in some of the *Curvularia* strains [22]. We detected *Curvularia* as nontarget sequences in ten of our root samples (interestingly, not only in *D. lanuginosum*), but it was impossible to compare them to the *Curvularia* endophytes reported by Redman et al. [30] as the authors did not provide the respective sequences in the database. Nevertheless, these findings suggest the possibility that a wide range of symbiotic interactions may be involved in heat tolerance of plants in YNP.

In summary, we detected the presence of both generalist and potential specialist AMF in geothermal areas of YNP. The communities observed appeared to be different from those located in nonthermal areas, but whether or not this was caused by temperature or other edaphic factors remains uncertain.

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