New Phytologist

Research

Soil fungal community assembly in a primary successional glacier forefront ecosystem as inferred from rDNA sequence analyses

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Received: *15 January 2003*
Accepted: *4 March 2003*

doi: 10.1046/j.1469-8137.2003.00767.x

Summary

• Soil fungal community assembly in nonvegetated areas on the forefront of a receding glacier was analyzed by cloning of the PCR-amplified partial small subunit (18S) of the ribosomal RNA genes (rDNA) from soil DNA samples.

• Fungal sequences obtained from areas adjacent to the present glacier terminus (young substrate) represented three fungal phyla, whereas those obtained adjacent to the terminal moraine (old substrate) were distributed among Ascomycetes and Hymenomycetes. The cloned sequences from both substrates represented mainly filamentous ascomycetes or basidiomycetes with a likely affinity to Agaricales. Unexpected biotrophic fungi with affinities to Taphrinomycetes, Urediniomycetes (the rust fungi) and Ustilaginomycetes (the smut fungi) plus an unknown, likely chytridiomycetous group were detected exclusively in the young substrates.

• These observations of biotrophic fungi are attributed to an aerially deposited, dormant spore bank, which may also be present in the older substrate but is masked by larger active mycelial biomass.

• This study underlines the importance of stochastic events and airborne spore deposition in the assembly of early fungal communities.

Key words: fungi, fungal community structure, glacier foreland, primary succession, soil, environmental DNA.

Introduction

Theories of primary succession were developed by plant ecologists. Clements *et al.* (1926), for example, viewed the ecosystem as a superorganism whose development was determined and directional. The ecosystems, according to Clements, develop towards a more or less fixed point of climax determined by local edaphic or climatic conditions. The determined successional development of plant communities was criticized by Clements' contemporaries (Gleason, 1926) who suggested that the succession in plant communities was largely driven by stochastic processes involving competition among resident species, and availability or dispersal of propagules.

The emphasis on autotrophic succession and the community assembly of plants has dominated successional ecology. Although complex feedback mechanisms between the autotrophic and below-ground heterotrophic communities exist, plant communities and their development may largely control the structure of soil-inhabiting food webs (Bever *et al.*, 1997; Wardle, 2002). Both the quantity and quality of the organic matter produced by the vegetation affect the biomass and community composition of the microbial primary and secondary consumers (Bradley & Fyles, 1995; Grayston & Campbell, 1996; Bardgett & Shine, 1999; Priha *et al.*, 1999). However, in primary successional ecosystems, defined as void of vegetation in their initial stages, the community assembly preceding autotrophs and their local carbon fixation is comprised of heterotrophs, which feed on allochthonous deposited organic material (Hodkinson *et al.*, 2001; Hodkinson *et al.*, 2002). This deposition and turnover of the organic matter may be instrumental in facilitating the later vegetation assembly.

Can such airborne deposition of organic matter adequately support a heterotrophic food web? As exemplified in the few

existing studies, the carbon deposition can be substantial in nonvegetated areas. Schlesinger *et al.* (1998) estimated that the organic carbon accumulated at a rate of 450–1120 kg ha^{-1} yr^{-1} during the first 110 yr after eruption of the volcano Krakatau. However, estimates for annual carbon deposition vary wildly. Fahnenstock *et al.* (2000) recorded carbon deposition up to 1430 kg ha^{-1} on snow patches in Alaskan tussock tundra, whereas the total organic detritus input at sites located on Mount St. Helens after its eruption was estimated to range between 0.31 and 0.93 kg ha^{-1} (Edwards & Sugg, 1993). Although some estimates indicate that deposition of organic matter is fairly limited, it may be sufficient to allow establishment of consumer and decomposer food webs on nutrient limited sites such as primary succesional systems (Hodkinson *et al.*, 2002). Furthermore, the deposition may not be spatially uniform but accumulates in select sites, which may serve as safe sites similar to those characterized by Jumpponen *et al.* (1999b).

In a recent review Hodkinson *et al.* (2002) suggested that aerial deposition of highly dispersive invertebrates and allochthonous organic detritus provide the initial energy and nutrients for heterotrophic community assembly in the absence of autotrophic plants. In the present study, the soil fungal community assembly on a receding glacier forefront was examined by analysis of polymerase chain reaction (PCR) amplified partial small subunit (18S) sequences of the ribosomal RNA gene (rDNA). It was hypothesized that the early communities on young substrate would differ from those present in the more developed old substrates adjacent to the terminal moraine. Furthermore, the early communities were hypothesized to contain mainly saprotrophic Ascomycetes and Basidiomycetes, whereas the communities in older substrates would include fungi associated with living plants – particularly symbiotic mycorrhizal fungi residing in plant roots. Although the life histories or the nutritional modes of the fungi detected in this study remain uncertain, detection of strictly or mainly biotrophic taxa indicate that the fungal communities sampled in the youngest substrates were mainly comprised of fungi in the dormant, aerially deposited spore bank.

Materials and Methods

Study site

Lyman Glacier (48°10′52″ N, 120°53′87″ W) is located in the Glacier Peak Wilderness Area in the North Cascade Mountains (Washington, USA). The site has been utilized in several studies on early plant community assembly in recently deglaciated substrate (Jumpponen *et al.*, 1998; Jumpponen *et al.*, 1999b). Similarly, it has been a focus of studies aiming to examine fungal community assembly in such an environment (Jumpponen *et al.*, 1999a; Jumpponen *et al.*, 2002).

The elevation of the present glacier terminus is about 1800 m. The deglaciated forefront is approximately 1000 m long over an elevation drop of only 60 m with no distinctive recessional moraines (Cázares, 1992; Jumpponen *et al.*, 1998). The glacier has receded steadily since the 1890s, thereby opening the forefront to colonization by plants and fungi. Periodic photographs and snow survey data have allowed reconstruction of the glacier retreat over the last century (Jumpponen *et al.*, 1998).

Sampling and DNA extraction

Nonvegetated sites adjacent to the glacier terminus (hereafter young soil substrate) and similar nonvegetated sites adjacent to the terminal moraine (hereafter old soil substrate) located on the deglaciated areas were sampled in August of 2001. Nine *c.* 200 ml soil samples were collected from each of the two areas, homogenized and mixed manually in plastic bags. Samples were stored on ice or frozen (–20°C) until returned to the laboratory. Approximately 0.25 g of soil was transferred to the extraction buffer; DNA was extracted following the protocol provided by the extraction kit manufacturer (UltraClean Soil; Molecular Biology Laboratories Inc., Carlstad, CA, USA). Extracted DNA was frozen (–20°C) until further processing.

PCR amplification of the fungal DNA

A partial sequence of the 18S of the fungal rDNA was amplified in 50 µl PCR mixtures containing final concentrations or absolute amounts of reagents as follows: 400 nM of each of the forward and reverse primers (nu-SSU-0817–5′- and nu-SSU-1536–3′ – Borneman & Hartin, 2000), 2 µl of the extracted template DNA, 200 µM of each deoxynucleotide triphosphate, 2.5 mM $MgCl_2$, 0.5 units of Taq DNA polymerase (Promega, Madison, WI, USA), and 5 µl of manufacturer's PCR buffer. The PCR cycle parameters consisted of an initial denaturation at 94°C for 3 min, then 40 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 3 min, followed by a final extension step at 72°C for 10 min. Long extension steps were used to minimize potential formation of chimeric sequences. The PCR reactions were performed in a Hybaid OmniCycler (Hybaid Ltd, Middlesex, UK). Possible PCR amplification of airborne and/or reagent contaminants was determined using two different controls: first a blank sample run through the extraction protocol simultaneously with the actual samples; second a negative PCR control in which the template DNA was replaced with ddH_2O.

Small-subunit rDNA clone library construction and analysis

Primers specific to fungi and stringent PCR conditions resulted in amplicons of the expected size (*c.* 780 bp) when the PCR products were visualized on 1.5% agarose gels. The mixed populations of PCR products were ligated into a

Table 1 BLAST analyses of the fungal soil clones obtained from the receding forefront of Lyman glacier, North Cascade Mountains (Washington, USA)

Clone	GenBank Match Taxon	Similarity	Score (Expected value)
Young 01–04 [AY168884][b]	*Oidiodendron tenuissimum* [AB015787] (Onygenales)	99%	1449 (0.0)
Young 01–07 [AY168885][b]	*Cladophialophora devriesii* [CDE232947] (Chaetothyriales)	98%	1340 (0.0)
Young 01–08 [AY168886][b]	*Donadinia* sp. *mh669* [AF104342] (Pezizales)	99%	1421 (0.0)
Young 02–04 [AY168887][c]	*Baeomyces rufus* [AF085471] (Lecanorales)	98%	1342 (0.0)
Young 02–07 [AY168888][c]	*Sympodiomycopsis paphiopedili* [D14006] (Microstromatales)	95%	1189 (0.0)
Young 03–03 [AY168889][c]	*Sympodiomycopsis paphiopedili* [D14006] (Microstromatales)	96%	1213 (0.0)
Young 03–06 [AY168890][b]	*Baeomyces rufus* [AF085471] (Lecanorales)	98%	1334 (0.0)
Young 03–08 [AY168891][c]	*Taphrina deformans* [U00971] (Taphrinales)	98%	1302 (0.0)
Young 03–18 [AY168892][a]	*Penicillium namyslowskii* [AB028190] (Eurotiales)	99%	1415 (0.0)
Young 04–05 [AY168893][b]	*Dark septate endophyte DS16b* [AF168167] (Unknown)	99%	1417 (0.0)
Young 05–13 [AY168896][b]	*Oidiodendron tenuissimum* [AB015787] (Onygenales)	99%	1378 (0.0)
Young 05–16 [AY168897][b]	*Neocallimastix frontalis* [X80341] (Neocallimasticales)	95%	1065 (0.0)
Young 06–02 [AY168898][c]	*Camptobasidium hydrophilum* [U75449] (Uredinales)	96%	1108 (0.0)
Young 06–04 [AY168899][b]	*Neocallimastix frontalis* [X80341] (Neocallimasticales)	94%	1112 (0.0)
Young 07–10 [AY168900][b]	*Laccaria pumila* [AF287838] (Agaricales)	98%	1390 (0.0)
Young 07–12 [AY168901][b]	*Peziza griseo-rosea* [AF133150] (Pezizales)	99%	1419 (0.0)
Young 07–13 [AY168902][b]	*Strobiloscypha keliae* [AF006310] (Pezizales)	98%	1392 (0.0)
Young 08–11 [AY168903][c]	*Laccaria pumila* [AF287838] (Agaricales)	96%	1257 (0.0)
Young 08–16 [AY168904][b]	*Panellus stipticus* [AF026589] (Agaricales)	98%	1360 (0.0)
Old 20–06 [AY168907][b]	*Tricholoma myomyces* [AF287841] (Agaricales)	98%	1342 (0.0)
Old 21–14 [AY168908][c]	*Dark septate endophyte DS16b* [AF168167] (Unknown)	95%	1152 (0.0)
Old 21–15 [AY168909][b]	*Russula compacta* [AF026582] (Agaricales)	98%	1360 (0.0)
Old 26–16 [AY168913][c]	*Hygrophorus sordidus* [AF287834] (Agaricales)	95%	1148 (0.0)
Old 26–05 [AY168910][c]	*Hypomyces chrysospermus* [M89993] (Hypocreales)	97%	1289 (0.0)
Old 27–14 [AY168916][b]	*Thelephora* sp. [AF026627] (Thelephorales)	99%	1070 (0.0)
Old 31–03 [AY168917][a]	*Trichophaea hybrida* [U53390] (Pezizales)	99%	1272 (0.0)
Old 31–18 [AY168918][b]	*Donadinia* sp. *mh669* [AF104342] (Pezizales)	96%	1199 (0.0)
Old 33–04 [AY168919][c]	*Hypomyces chrysospermus* [AB027339] (Hypocreales)	96%	1180 (0.0)
Old 33–19 [AY168920][c]	*Hypomyces chrysospermus* [AB027339] (Hypocreales)	96%	1231 (0.0)

[a]sequence not determined chimeric in the Chimera Check; [b]sequence potentially chimeric but the score low in the Chimera Check; [c]sequence potentially chimeric and the score high in the Chimera Check. Expected value 0.0 indicates $E = 10^{-180}$.

linearized pGEM-T vector (Promega). The circularized plasmids were transformed into competent JM109 cells (Promega) by heat shock and the putative positive transformants were identified by α-complementation (Sambrook, 1989).

Twenty putatively positive transformants from each clone library were randomly sampled and presence of the target insert confirmed by PCR amplification in 15 µl reaction volumes under the same reaction conditions as described above. To select different plasmids for sequencing, these PCR products were digested with endonucleases (*Hin*fI, *Alu*I; New England BioLabs, Beverly, MA, USA) and resolved on 3% agarose gels (Gardes & Bruns, 1996). The approach using PCR and restriction fragment length polymorphisms (RFLP), in addition to allowing efficient screening of clone libraries, enabled selection of different RFLP phenotypes for sequencing. An approximately 780 bp sequence was obtained from each RFLP phenotype in all clone libraries by use of fluorescent dideoxy-terminators (ABI Prism® BigDye™; Applied Biosystems, Foster City, CA, USA) and an automated ABI Prism® 3700 DNA Analyzer (Applied Biosystems) at the DNA Sequencing and Genotyping Facility at Kansas State University (GenBank accession numbers AY168884–AY168920). Vector contamination was removed with the automated vector trimming function in Sequencher (GeneCodes, Ann Arbor, Michigan). The similarities to existing rDNA sequences in the GenBank database (Tables 1 and 2) were determined at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/– Altschul *et al.*, 1997) by standard nucleotide BLAST (version 2.2.1) without limiting queries and Sequence Match (version 2.7) at the Ribosomal Database Project (RDP – http://rdp.cme.msu.edu/html/– Maidak *et al.*, 1999).

Thirty-seven unique clones were identified in the RFLP analyses and sequenced (Table 1). Three of these were unalignable with fungal sequences along their full length because of a potential nonfungal chimeric contaminant at the 5′-end (Young_05–12) or 3′-end (Old_26–06 and Old_26–19) of the obtained sequence. Five of the cloned sequences contained large insertions, which showed partial similarities to *Homo sapiens, Drosophila melanogaster* and ericoid mycorrhizal fungi (Table 2). Although similar insertions have been observed in the rDNA of, for example, Helotiales, Lecanorales

Table 2 Clones with large insertions deemed possibly chimeric and excluded from further analyses. The full-length, cloned sequences were first used to query GenBank by BLAST. The intron position was identified by alignment against other fungi and used in a separate BLAST query

Clone	GenBank Match Taxon	Intron Match Taxon	Score (Expected value)
Young 05–09 [AY168894]	*Capronia dactylotricha* [AJ232943] (Chaetothyriales)	*Homo sapiens* [AC007297]*	607 (10^{-170})
Young 05–12 [AY168895]	*Holwaya mucida* [AF292091] (Helotiales)	*Drosophila melanogaster* [AY051570]*	672 (0.0)
Old 19–05 [AY168905]	*Connersia rilstonii* [L76626] (Eurotiales)	*Beauveria bassiana* [AF464942]*	676 (0.0)
Old 19–14 [AY168906]	*Monascus purpureus* [M83260] (Eurotiales)	*Barmaelia oxyacanthae* [AF064048]*	618 (10^{-174})
Old 26–06 [AY168911]	*Uncultured eukaryote clone* [AY082984]	*Drosophila melanogaster* [AE003683]*	1205 (0.0)
Old 26–19 [AY168913]	*Rhizoctonia solani* [D85636] (Ceratobasidiales)	*Drosophila melanogaster* [AE003683]*	1209 (0.0)
Old 27–04 [AY168914]	*Amylocarpus encephaloides* [U45438] (Helotiales)	*Unidentified ericoid mycorrhiza* [AF158828]	533 (10^{-148})
Old 27–13 [AY168915]	*Amylocarpus encephaloides* [U45438] (Helotiales)	*Unidentified ericoid mycorrhiza* [AF158828]	494 (10^{-136})

The asterisk indicates a partial < 50 bp match for the intron search. Expected value 0.0 indicates E = 10^{-180}.

and Onygenales (Gargas *et al.*, 1995; Holst-Jensen *et al.*, 1999; Borneman & Hartin, 2000; Perotto *et al.*, 2000), these sequences were omitted because true insertions and chimeric PCR products could not be identified reliably. The remaining 29 environmental sequences and 127 sequences from GenBank were aligned in 784 positions using Sequencher (GeneCodes Inc., Ann Arbor, MI, USA) and manually adjusted to maximize conservation. Regions adjacent to the priming sites were omitted due to high frequency of ambiguous sites. Three highly variable regions were observed within the sequenced region (positions 230–272, 545–587 and 710–722 in the alignment). Exclusion of these regions did not affect the topologies obtained in subsequent neighbour joining (NJ) analyses and they were therefore included in the analyses.

The taxonomic relationships among the fungal sequences were inferred by NJ analyses in PAUP* (Swofford, 2001). A chytridiomycetous taxon (*Monoblepharis hypogyna*) was selected for the outgroup. Although selection of a chytridiomycetous taxon may result in unreliable placement of the basal taxa, this was considered preferable to an outgroup outside Kingdom Fungi. Such outgroups would have reduced the terminal branch lengths and possibly resulted in midpoint rooting. Data matrices were left uncorrected, rates for variable sites were assumed equal and no sites were assumed invariable. Sites with missing data, ambiguous nucleotides or gaps, were randomly distributed among taxa. The robustness of the inferred NJ topologies was tested by one thousand bootstrap replicates. A partial heuristic search (with > 80×10^{-6} rearrangements) was also conducted. The number of equiparsimonious trees was expected to be high and the maximum number of saved trees was limited to one thousand. The placement of the environmental clones in the consensus (strict and 50% majority rule) trees largely agreed with the NJ topologies. Accordingly, only the more expedient and complete NJ analyses are presented.

Detection and analysis of chimeric sequences

Chimeric sequences may be frequent in environmental samples with diverse, mixed populations of competing templates (Kopczynski *et al.*, 1994; von Wintzingerode *et al.*, 1997). Minimization of the chimeric amplification was attempted by relatively long (3 min) extension times. To control for the occurrence of possibly chimeric sequences, all sequenced clones were analyzed by the Chimera Check program of the RDP database (version 2.7; Maidak *et al.*, 1999). The sequences were classified in three categories: first not chimeric, if Chimera Check did not indicate that the sequence was possibly obtained from more than one organism; second possibly chimeric, if Chimera Check indicated possible origin from more than one organism but the scores were low (< 20); and third likely chimeric, if Chimera Check indicated possible origin from more than one organism and the scores were high (> 20). The sequences with

Table 3 Clone occurrences (number of samples with a given clone) and average frequencies (proportion of the clone in the clone libraries) among all clone libraries within one sampling area. Occurrences were inferred from RFLP phenotype frequencies in the clone libraries

Clone	GenBank taxon affinity	Occurrence	Average frequency (%)
Young 01–04	*Oidiodendron tenuissimum*	3	18
Young 01–07	*Cladophialophora devriesii*	3	8
Young 01–08	*Donadinia* sp.	5	16
Young 02–04	*Baeomyces rufus*	1	6
Young 02–07	*Sympodiomycopsis paphiopedili*	1	2
Young 03–03	*Sympodiomycopsis paphiopedili*	1	2
Young 03–06	*Baeomyces rufus*	2	5
Young 03–08	*Taphrina deformans*	1	2
Young 03–18	*Penicillium namyslowskii*	2	5
Young 04–05	*Dark septate endophyte DS16b*	1	6
Young 05–09	*Capronia dactylotricha*	1	1
Young 05–12	*Holwaya mucida*	1	1
Young 05–13	*Oidiodendron tenuissimum*	1	1
Young 05–16	*Neocallimastix frontalis*	1	1
Young 06–02	*Camptobasidium hydrophilum*	1	2
Young 06–04	*Neocallimastix frontalis*	1	3
Young 07–10	*Laccaria pumila*	1	4
Young 07–12	*Peziza griseo-rosea*	1	4
Young 07–13	*Strobiloscypha keliae*	1	4
Young 08–11	*Laccaria pumila*	1	3
Young 08–16	*Panellus stipticus*	1	7
Old 20–06	*Tricholoma myomyces*	1	13
Old 21–14	*Dark septate endophyte DS16b*	1	11
Old 21–15	*Russula compacta*	1	2
Old 26–05	*Hypomyces chrysospermus*	1	10
Old 26–06	*Uncultured eukaryote clone*	2	3
Old 26–16	*Hygrophorus sordidus*	1	1
Old 26–19	*Rhizoctonia solani*	1	1
Old 27–04	*Amylocarpus encephaloides*	1	6
Old 27–13	*Amylocarpus encephaloides*	1	2
Old 27–14	*Thelephora* sp.	1	3
Old 31–03	*Trichophaea hybrida*	1	11
Old 31–18	*Donadinia* sp.	1	2
Old 33–04	*Hypomyces chrysospermus*	1	10
Old 33–19	*Hypomyces chrysospermus*	1	3

large insertions (Table 2) were invariably identified as possibly or likely chimeric.

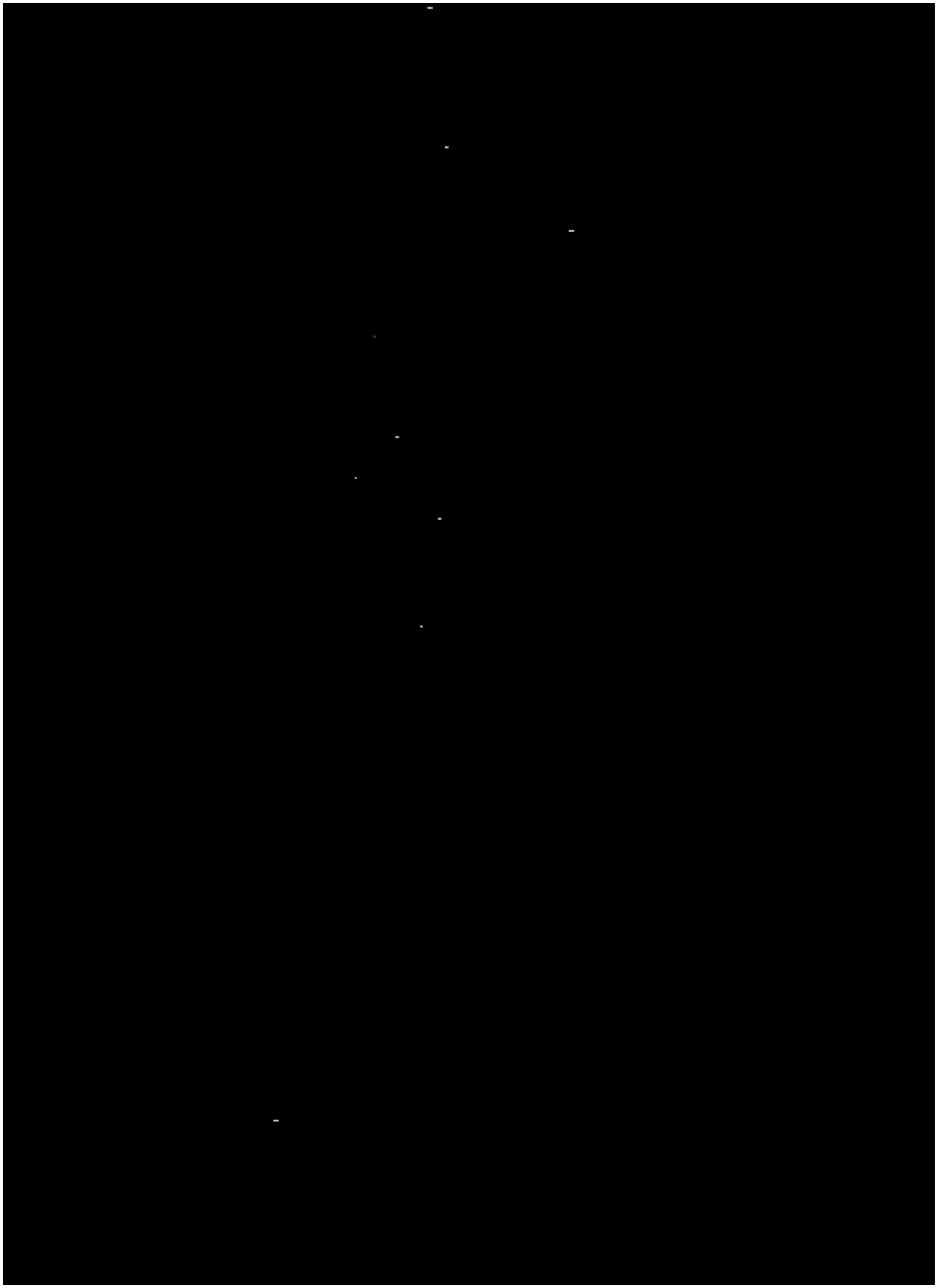
To test the effects of the chimeric sequences on the placement of the environmental clones in the obtained NJ topologies, the data were reanalyzed after exclusion of data upstream and downstream of the most commonly encountered chimera break points (positions 1–290 and 550–784 in the alignment). The obtained topologies were compared to those obtained without data exclusion.

Results

Fungal community assembly

In this study, PCR-amplification and subsequent rDNA analyses were attempted from a total of 18 samples. In spite of repeated attempts, two of the included samples failed to produce visible amplicons and were omitted from the analyses, leaving eight samples for both young and old soil substrates. A total of 320 rDNA clones in the 16 libraries were screened and 37 unique RFLP phenotypes identified and sequenced. A majority of RFLP types occurred only in one sample (Table 3) suggesting substantial spatial heterogeneity. Samples from the young substrate contained organisms from three fungal phyla and tended to represent greater diversity than the older substrates as measured by pairwise genetic distances (average, maximum and minimum pairwise distances were 0.0764 and 0.0685, 0.14143 and 0.10627, 0.00137 and 0.00145 for young and old soil substrates, respectively). The average pairwise distances, however, were not significantly different as determined by one-tailed heteroscedastic *t*-test for two populations ($P = 0.126$),

Both BLAST (Tables 1 and 2) and NJ analyses (Fig. 1) placed the cloned environmental sequences into the Kingdom Fungi. The sequences represented Ascomycota, Basidiomycota and Chytridiomycota; no definitive zygomycetous sequences



were identified. Overall, the cloned sequences represented various groups of fungi and samples obtained from young and old substrates showed little overlap.

Samples originating from close to the glacier terminus contained representatives of three unexpected fungal taxa, that is biotrophic taxa whose occurrence was considered unlikely in the absence of any hosts. First a single clone (Young_06–02) showed a well-supported affinity to *Camptobasidium hydrophilum* (Urediniomycetes, the biotrophic rust fungi). BLAST searches similarly placed this clone among the teliosporic rust fungi (96% similar to *C. hydrophilum*). Second a well-supported group (clones Young_02–03 and Young_03–03) was placed in a clade containing *Exobasidium vaccinii* and *Sympodiomycopsis paphropedili* (Ustilaginomycetes, the biotrophic smut fungi). The two clones most resembled *S. paphropedili* in BLAST analyses (Table 1). Third a single clone (Young_03–08) grouped with Taphrinomycetes with high bootstrap support (biotrophic prototunicate ascomycetes). This clone was congruently identified in the BLAST analyses and found to be 98% similar to *Taphrina deformans* (Table 1). Additionally, a unique, well-supported, potentially chytridiomycetous group was detected exclusively in young substrates and placed basal to zygomycetes in the NJ analyses (clones Young_05–16 and Young_06–04). BLAST searches found the two clones from two different samples related to the chytridiomycete *Neocallimastix frontalis* (95% similarity). Similar biotrophic or chytridiomycetous groups were absent in the clone libraries from older soil substrates. Three clones Old_26–05, Old_33–04 and Old_33–19) from two samples, however, formed a unique cluster placed as a sister group to species representing Ophiostomatales (Sordariomycetes).

Other clones from both young and old substrates were placed among Hymenomycetes and filamentous ascomycetes. Clones obtained from young substrates included taxa with likely affinities within the ascomycetous Eurotiomycetes, Chaetothyriomycetes, Lecanoromycetes, Leotiomycetes (including Onygenales) and basidiomycetous Agaricales (Fig. 1 and Table 1). Clones from older substrates represented taxa with likely affinities within ascomycetous Sordariomycetes and basidiomycetous Agaricales (Fig. 1 and Table 1). Various taxa of Pezizomycetes were represented in the clone libraries from both young and old soil substrates. Two general points are noteworthy. First, clones from both young and old substrates grouped with ectomycorrhizal basidiomycetes. One clone from young substrate was grouped with *Laccaria pumila* (Young_07–10) by both BLAST (99% similarity) and NJ analyses (82% bootstrap support). Similarly, one clone (Old_21–15) from the old substrate was placed within Russulaceae and one (Old_27–14) grouped with *Thelephora* sp. in both analyses. Second, two clones (Young_02–04 and Young_03–06) from young substrate were grouped with lichenized taxa within Lecanoromycetes. No lecanoromycetous clones were detected in the older substrates.

Detection and importance of chimeric sequences

A majority of the environmental sequences were determined to be possibly chimeric or likely chimeric by the Chimera Check of the RDP (Table 1). Five clones contained large insertions, which may or may not represent true chimeras but were omitted from the analyses regardless. Three additional clones were unalignable because of possible chimeric fragments belonging to a phylum other than fungi. To test the possible effect of chimeras on the placement of the remaining environmental clones, sequence data potentially obtained from another organism were omitted at most frequently observed chimera break points. When chimeric upstream data were excluded (positions 1–290 in this alignment), five rearrangements of the environmental clones were observed. First, clone Young_08–11 was not placed basal to Hymenomycetes but showed a marginally supported affinity to an agaric, *Laccaria pumila*. Second, instead of a well-supported affinity with pezizalean *Pulvinula archeri*, clone Old_21–14 grouped with another clone with a likely affinity to Russulaceae (Old_21–15). The affinity of this cluster to Russulaceae was not supported in the bootstrap analyses. Third, clones Young_02–04 and Young_03–06 were placed as a well-supported group of their own and not a sister group with an affinity to Lecanoromycetes (Fig. 1). Fourth, clone Young_07–10 was still nested within Agaricales, but did not group with *L. pumila* with high bootstrap support. Fifth, the group of three clones (Old_26–05, Old_33–04 and Old_33–19), although still within Sordariomycetes, was not placed as a sister group to Ophiostomatales. When the downstream chimeric region (positions 550–784 in this alignment) was excluded, similar rearrangements were less frequent. As with the upstream chimeric exclusion, clones Young_02–04 and Young_03–06 were still placed as a sister group with affinity to Lecanoromycetes but this placement was not supported by bootstrap analyses. The bootstrap support of various groups varied but no significant repositioning of the environmental clones occurred like in the upstream exclusion.

Discussion

Fungal community assembly in the primary successional glacier foreland soil

Fungal PCR-amplicons were successfully obtained from environmental soil samples collected at the forefront of a

Fig. 1 Neigbor joining phylogram showing the placement of 29 environmental clones (bold) from the forefront of a receding glacier. Numbers above the clades indicate bootstrap support. Asterisks indicate clones whose placement changed when data were reanalyzed after omission of questionable data: * = placement similar but lacked support; ** = radical change in placement in the reanalyses.

receding glacier. The two controls, one that was included in the DNA extraction and another from which the template was omitted in the PCR amplification, remained free from visible PCR products indicating that the clone libraries indeed represented fungi obtained from soil samples. A large proportion of the sequences was determined to be chimeric by the Chimera Check software of the RDP. Analyses conducted after exclusion of the sequence data potentially obtained from another target organism confirmed that the placement of most cloned sequences was insensitive to exclusion of the potentially chimeric data. In other words, the placement of a majority of the cloned sequences was similar whether or not the data identified as possibly chimeric were included in the analyses.

Thirty-seven unique RFLP phenotypes were identified and sequenced from 16 environmental samples. A majority of these clones was present in only one soil sample. The observed low similarity among samples emphasizes the high heterogeneity in soil-inhabiting fungal communities and underlines the difficulty of making general conclusions about factors governing fungal occurrence in an ecosystem scale. Samples from the young substrate tended to represent greater diversity than the older substrates as indicated by exclusive detection of basal asco- and basidiomycetes. Clones obtained from young substrates contained taxa from three of the four main phyla in the Kingdom Fungi: Ascomycota, Basidiomycota and Chytridiomycota; no zygomycetous taxa were observed. The samples collected adjacent to the terminal moraine and therefore representing older substrate contained exclusively higher fungi, filamentous ascomycetes and hymenomycetous basidiomycetes. These results are likely due to the dormant spore bank in the young substrate, whereas the fungal communities in the older substrate were mainly comprised of active biotrophic and saptrotrophic mycelium.

Early community assembly by airborne heterotrophs has been proposed to precede assembly of autotrophic communities in primary successional environments (Hodkinson *et al.*, 2002). It was therefore hypothesized that diverse saprotrophic fungal communities would dominate in the young substrates. Unexpectedly, the clone libraries contained mycorrhizal, parasitic or pathogenic ascomycetes and basidiomycetes unlikely to be able to sustain considerable growth in the absence of autotrophic hosts. Most surprisingly, three biotrophic clades – the Taphrinomycetes (Ascomycota), Urediniomycetes (Basidiomycota) and Ustilaginomycetes (Basidiomycota) – were detected adjacent to the present terminus of Lyman Glacier. It is unlikely that the detected biotrophic fungi were present in soil as active mycelium or unicellular, yeast-like colonies. Although Ustilaginomycetes may have brief saprobic periods after basidiospore germination and Taphrinomycetes may have a budding unicellular stage after ascospore release, the three taxa depend largely on their host plants and have limited growth in soil substrate in the absence of their hosts (Alexopoulos *et al.*, 1996). Similarly, potentially mycorrhizal fungi were detected although no susceptible hosts were in the early successional areas. Taken together, data presented here suggest that early community assembly has a significant component of aerially deposited, dormant spore bank in soil. This conclusion corroborates earlier experiments conducted at the Lyman Glacier study site. The fungal biomass in soil was estimated to be very low in the early successional communities but significantly increased with time since deglaciation (Ohtonen *et al.*, 1999). Focusing on ectomycorrhizal fungi, Jumpponen *et al.* (2002) concluded that fungi and their propagules were sparse in the young soil substrates but their numbers increased with time since deglaciation. The few resident spores in the soil spore bank detected in the present study may not be adequate for establishment of a functional mycorrhizal symbiosis due to unpredictable germination of the spores in the spore bank. It is important to note that the areas near the glacier terminus are completely void of any mycorrhizal plants.

The fourth surprising group was identified as chytridiomycete *Neocallimastix frontalis* (95% similarity) in BLAST analyses and placed among Zygomycota and Chytridiomycota in the NJ analyses without a well-supported affinity to either. The true affinity of these clones remains unclear. The two obtained clones may represent a new higher level taxon (class or order) as has been proposed in a study that assessed fungal communities in plant roots by random sequencing of PCR amplicons (Vandenkoornhuyse *et al.*, 2002). More likely, the two clones emphasize the limited taxon sampling of lower fungi in GenBank.

The unexpected, biotrophic groups (Taphrinomycetes, Urediniomycetes and Ustilaginomycetes) observed in the young substrate were absent in the clone libraries from older soil substrate. By contrast to the dormant soil fungi in the spore bank in young soil substrates, many of the fungi observed in the older soil substrates likely represent active soil mycelium. For instance, mycorrhizal fungi (clones with well-supported affinities to Russulaceae and Thelephoraceae) likely match macrofungi collected in earlier surveys of ectomycorrhizal fungi in the Lyman Glacier study site (Jumpponen *et al.*, 1999a; Jumpponen *et al.*, 2002). The inability to detect the biotrophic Ascomycetes and Basidiomycetes in the older substrates does not necessarily indicate their absence in the soil. Ohtonen *et al.* (1999) observed increasing fungal biomass based on estimated hyphal length and measured fungus-specific phopholipid fatty acids with increasing age of the soil substrate. Accordingly, it is most likely that the members of Taphrinomycetes, Urediniomycetes and Ustilaginomycetes are not exclusive to young substrate on this glacier forefront but, rather, that the resident, dormant sporebank is masked by more abundant, actively growing fungal mycelium.

Additional basidiomycetes likely representing ectomycorrhizal taxa were detected in both young and old substrates. Clones with high similarities to *Thelephora* sp. and *Inocybe geophylla* were detected in BLAST, the affinity to *Thelephora*

sp. was also supported in NJ analyses. One clone originating from a sample collected adjacent to the glacier terminus was placed in Tricholomataceae and found 99% similar to *Laccaria pumila*. *Laccaria pumila* is closely related to *L.* cf. *montana*, which has been frequently observed in previous sporocarp surveys on this site (Jumpponen *et al.*, 1999a; Jumpponen *et al.*, 2002). Nonetheless, it is likely that the observed fungi in the older substrates are active, whereas the fungi detected in the young substrate, void of any adjacent vegetation, represent a resident dormant spore bank.

Other clones from both young and old substrates were placed among basidiomycetous Hymenomycetes and filamentous ascomycetes. The nutritional modes of these taxa remain unclear as they may be saproptrophic or symbiotically associated with plant host mycorrhizas. For example, several taxa belonging to Pezizomycetes were detected in both old and young substrates. These likely form symbiotic associations with mycorrhizal plants as well as occur as free-living saprotrophs (Danielson, 1984; Egger & Paden, 1986a; Egger & Paden, 1986b).

The effect of chimeric sequences on placement of environmental clones

The majority of the cloned sequences determined to be possibly or likely chimeric were congruently placed, whether or not the upstream or downstream chimeric regions were excluded. However, several clones were placed on different phyla in the analyses after exclusion of the chimeric data indicating their likely chimeric origin. For example, dramatic rearrangements such as those of clone Young_08–11 (placed basal to Hymenomycetes in the analyses with full data set, but showed a marginally supported affinity to *L. pumila* when upstream chimeric data were excluded) and Old_21–14 (grouped with pezizalean *P. archeri* in the analysis of the full data set, but was nested within Agaricales when upstream chimeric data were excluded) clearly show sequence data originating from more than just one organism. Other changes among the analyses of the different data sets were rather minor and involved loss of bootstrap support but similar placement of the environmental clones. Determining whether these clones represent truly chimeric sequences is difficult at best. For instance, clones Young_02–04 and Young_03–06 (affinity to Lecanoromycetes was not supported in the bootstrap analyses when the upstream or downstream data were excluded) or clones Old_26–05, Old_33–04 and Old_33–19 (placement as sister group to Ophiostomatales was not supported in the bootstrap analyses when the upstream or downstream data were excluded) are unlikely true chimeras for two reasons. First, closely similar sequences were obtained from more than just one sample. It is unlikely that similar sequences would be obtained by random chance of pooling DNA from different sources. Second, the placement of these clones was insensitive to the exclusion of the possibly chimeric data although the support for their placement was reduced because of lesser available data. To avoid errors due to inclusion of chimeric data or false exclusion of nonchimeric data requires specific effort in order to confirm the correct assessment of the community structure.

It was fortunate that these analyses detected obligate biotrophs whose biological activity is unlikely in the absence of their autotrophic hosts and thus indicated the presence of a soil-borne, dormant spore bank. In other words, the studies presented here did not confirm the original hypotheses but provided other valuable insights into the initial fungal community assembly in the absence of established vegetation. The resident spore bank and its biotrophic fungi underline the importance of aerial deposition and stochastic events in early community assembly. Although providing a unique view into the early fungal communities, these observations also outline the shortcomings of the environmental DNA analyses. First, it can be difficult to infer the function of the detected organisms because gross taxonomic resolution on the level of family or order is often inadequate for that task. Second, the conclusions may be shadowed by the uncertainties of the data quality. Chimeric sequences are difficult to detect and present detection tools only indicate need for reanalyses of the data. In this study, several sequences were confirmed to be chimeric based on the inconsistent placement of the cloned sequences when some (potentially chimeric) data were omitted. In light of these limitations environmental rDNA analyses will be most valuable when combined with traditional microscopic and culture-based techniques.

Acknowledgements

This work was supported by Kansas State University BRIEF program and National Science Foundation EPSCoR Grant no. 9874732 with matching support from the State of Kansas. I am grateful to Dr Franceco T. Gentili, Nicolo Gentili, Anna Jumpponen and Dr James M. Trappe for their assistance during sample collection, transport and preparation in August 2001; to Emily L. King and Justin Trowbridge for their assistance in clone library screening and plasmid preparation. Drs Charles L. Kramer, Marc Rehfuss and James M. Trappe provided helpful comments on early drafts of this manuscript. Anna Jumpponen edited and revised the manuscript.

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