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Response of fungal and actinobacterial communities to water-level drawdown in boreal peatland sites

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Abstract

We used PCR-DGGE fingerprinting and direct sequencing to analyse the response of fungal and actinobacterial communities to changing hydrological conditions at 3 different sites in a boreal peatland complex in Finland. The experimental design involved a short-term (3 yrs; STD) and a long-term (43 yrs; LTD) water-level drawdown. Correspondence analyses of DGGE bands revealed differences in the communities between natural sites representing the nutrient-rich mesotrophic fen, the nutrient-poorer oligotrophic fen, and the nutrient-poor ombrotrophic bog. Still, most fungi and actinobacteria found in the pristine peatland seemed robust to the environmental variables. Both fungal and actinobacterial diversity was higher in the fens than in the bog. Fungal diversity increased significantly after STD whereas actinobacterial diversity did not respond to hydrology. Both fungal and actinobacterial communities became more similar between peatland types after LTD, which was not apparent after STD. Most sequences clustered equally between the two main fungal phyla Ascomycota and Basidiomycota. Sequencing revealed that basidiomycetes may respond more (either positively or negatively) to hydrological changes than ascomycetes. Overall, our results suggest that fungal responses to water-level drawdown depend on peatland type. Actinobacteria seem to be less sensitive to hydrological changes, although the response of some may similarly depend on peatland type.

Keywords: Microbial community; Climate change; Fungi; Actinobacteria; Drainage; Fen; Bog; DGGE; CA

1. Introduction

Peatlands represent the most important store of terrestrial carbon (C). Despite their importance in the global C cycle, microbial communities in peatlands are still poorly understood. Mapping the diversity of the different microbial groups in peatlands has just begun and the microbes involved in methane cycling, i.e., methanotrophic bacteria (e.g., Dedysh et al., 1998; Morris et al., 2002; Jaatinen et al., 2005; Raghoebarsing et al., 2005) and methanogenic archaea (e.g., Galand et al., 2002; Basiliko et al., 2003; Sizova et al., 2003), have been studied the most extensively. Bacterial diversity of peatlands has been explored with molecular methods in, e.g., *Sphagnum* bogs of New England (Morales et al., 2006), two drained fens in Slovenia (Kraigher et al., 2006), and a *Sphagnum* bog in western Siberia (Dedysh et al., 2006). The molecular studies have broadened our general view of the distribution of microbes in peatlands.

The response of microbial communities to a persistent decrease in the water-level is important to document, since this is the ultimate outcome in peatlands following drainage or climate warming. Comparative analysis of various peatland sites in the UK showed that even a short-term drought can change the microbial community and that any changes are dependent on peatland type and the microbial group (Kim et al. 2008). Peatlands sequester organic matter into the anoxic layer, but most of the C flux takes place in the often relatively thin oxic layers above the water level. Lowering the water level of a northern boreal fen is accompanied with coincided with an increased CO_2 flux from peat to the atmosphere (Jaatinen et al., 2008). In oxic conditions, fungi are considered to be the principal decomposers (e.g., Thormann 2006a,b), although actinobacteria may contribute significantly to the decomposition of organic matter since they are mainly strict aerobes (Goodfellow and Williams, 1983) that can degrade polymers such as lignin, cellulose, pectin, and chitin as well as humic materials (McCarthy, 1987; Pankratov et al., 2006). In Russian peatlands, 30 % of sequenced clones were most similar to Actinobacteria (Dedysh et al., 2006) and certain members of this group have been found to play a leading role in cellulose degradation (Pankratov et al., 2006).

Here, we study the fungal and actinobacterial communities of a boreal peatland complex with polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) fingerprinting. We amplified 18S and 16S ribosomal DNA (rDNA) to study how the communities: i) differ among pristine sites with different vegetation and nutrient levels, and; ii) change following water-level drawdown. The sites covered the common peatland types in boreal Eurasia: two minerotrophic fens and an ombrotrophic bog exhibiting microtopographical variation as hummock, lawn level and hollow surfaces, and included a control (contemporary water-level regime) as well as two levels of hydrological manipulation. Results from our earlier work (Jaatinen et al., 2007) showed how both fungal and actinobacterial phospholipid fatty acid (PLFA) biomarkers responded to water-level drawdown, and fungal PLFAs were positively correlated with potential CO_2 production in these sites, thus supporting our experimental rationale.

2. Materials and methods

2.1. Site description and sampling

Sampling was conducted at Lakkasuo peatland complex in central Finland (61°48´N, 24°19´E, ca. 150 m above sea level) on May 18, 2004. A detailed description of Lakkasuo and the study sites can be found elsewhere (Laine et al., 2004; Jaatinen et al., 2007). The

study sites represented three different nutrient levels along a fen-bog gradient, each with a characteristic vegetation type: two minerotrophic fens; mesotrophic (ME; a relatively nutrient-rich "intermediate fen") and oligotrophic (OL, "poor fen"), and an ombrotrophic bog (OM; nutrient-poor) (hereafter referred to as nutrient levels; see Table 1 for surface peat nutrient concentrations and pH). Minerotrophic sites received ground water and nutrient inputs from the surrounding catchment whereas the ombrotrophic site was "fed" by precipitation and dry deposition only.

Each site (see abbreviations in Table 1) included a pristine control plot (P), a plot with short-term water-level drawdown (STD), and a plot with long-term water-level drawdown (LTD), all of which had uniform vegetation and soil properties before disturbance. The P and STD plots at the OM site included dry hummock (Hu), intermediate lawn (La) and wet hollow (Ho) microforms. The LTD plots were drained by ditching for forestry in 1961 and the STD plots were ditch drained in 2000/2001 to simulate a predicted effect of climate change (Laine et al., 2004). The average water-level drawdown induced in the STD plots was about 10 cm during the growing season of 2003 in all sites, which is close to the estimate for the impact of climate change on water levels in northern peatlands (Roulet et al., 1992). In the LTD plots, the average water levels were about 15 (bog)–30 (fen) cm deeper than in the corresponding pristine sites. Together, these plots formed a successional gradient from a wet pristine peatland towards a dry peatland forest (Laiho et al., 2003). Water levels at the time of sampling are given in Table 1. The upper limit of the anaerobic layer is usually 5-15 cm closer to the surface than water level (Lähde, 1969, 1971).

The vegetation in the pristine ME site was characterized by sedges (*Carex rostrata* Stokes, *C. lasiocarpa* Ehrh.), some herbaceous species (e.g., *Potentilla palustris* Scop. and *Menyanthes trifoliata* L.) and mosses (e.g., *Sphagnum fallax* (Klinggr.) Klinggr., *S. flexuosum* Dozy & Molk., *S. magellanicum* Brid.). The OL site was characterized by *C. lasiocarpa*, some *Betula nana* L., and *S. papillosum* Lindb., *S. fallax*, and *S. flexuosum* in the moss layer. In the OM site, *Eriophorum vaginatum* L., *Andromeda polifolia* L. and *Rubus chamaemorus* L. were the most abundant field layer species, while *S. cuspidatum* Hoffm. was the dominant moss in the Ho, *S. balticum* (Russow) C. Jens. in the La and *S. fuscum* (Schimp.) Klinggr. in the Hu microforms. Some stunted Scots pine (*Pinus sylvestris* L.) occurred on the hummocks. In the STD plots of the ME and OL sites, sedges had suffered while shrubs had flourished together with Scots pine and birch (*Betula pubescens* Ehrh.). The *Sphagnum* carpets showed signs of increased desiccation but had not decreased in cover. In the OM site, the changes in vegetation were small: most obviously, *S. cuspidatum* had decreased in cover and vitality. On the LTD plots, vegetation had changed dramatically. Tree stands had become tall and dense, and consisted of Scots pine in the OM site, and Scots pine with a mixture of birch and spruce (*Picea abies* (L.) Karst.) in the ME and OL sites. Shrubs characterized the field layer in all sites, being most notable at the OM site. Moss layers consisted of *Pleurozium schreberi* Brid., *S. angustifolium* (Russow) C. Jens., *S. russowii* Warnst. and *S. magellanicum*, with some *S. fuscum* on the OM LTD plot. In the OL and especially in the ME LTD plots, there were also surfaces covered mostly with birch leaf litter (i.e., no mosses).

Triplicate peat cores (8 x 8 cm; n = 39) were taken at each plot and sub-sampled by depth: 0-5 (L1), 5-10 (L2), and 20-30 (L4) cm. All samples (n = 117) were fresh frozen and stored at -20 °C prior to molecular analysis.

2.2. DNA extraction, PCR-DGGE fingerprinting and phylogenetic analyses

For peat soil DNA extraction, a modified manufacturer's protocol of the FastPrepTM DNA-kit (Bio101, Vista, CA, USA) and the bead-beating method of Yeates and Gillings (1998) was used as previously described (Jaatinen et al., 2004). We used the oligonucleotide primer set FF390 (with GC-clamp) and FR1 for fungi, which is reported to be suitable for analysing complex fungal communities from various environmental samples in DGGE (Vainio and Hantula, 2000; Pennanen et al., 2001). For the actinobacteria we used the primer set S-C-Act-235-a-S-20 (with GC-clamp) and S-C-Act-878-a-A-19, which amplifies DNA from various pure culture taxa and environmental samples (Stach et al., 2003). The primer set was optimized for DGGE-fingerprinting as in Jaatinen et al. (2008) and PCR amplifications with both primer sets were conducted as previously described (Stach et al., 2003; Jaatinen et al., 2008).

Microbial communities were analysed by DGGE using DCodeTMSystem (Bio-Rad, Hercules, CA, USA) with primer-specific denaturing gradients and running parameters (Vainio and Hantula, 2000; Jaatinen et al., 2008). Control ladders with several known DGGE bands of known mobility in the denaturing gradient gel were included for comparison in the sample runs. The DGGE gel photographs were screened for the presence (1) or absence (0) of fungal and actinobacterial bands using the AlphaImager 2.1 program of the AlphaDigiDoc gel documentation system (Alpha Innotech Corp., California, USA). To verify the presence or absence of a weak DGGE band and to control for possible fusion of closely migrating bands, DGGE gels were run at least twice. DGGE runs were optimized for band isolation and gels were poured on GelBond PAG film (FMC Bioproducts, Rockland, ME, USA) to minimize distortion. DGGE bands representing a unique sequence type (different mobility in DGGE) were selected for sequencing and excised, amplified and run again in DGGE three to five times so that we could be sure about their high quality and the right initial position in a gel. Finally, the sequences were reamplified with 20 or 25 cycles and purified with the QIAquick PCR purification kit (Qiagen GmbH, Hilden, Germany). Fungal PCR products were directly sequenced using a Long Readir 4200DNA Sequencer (LI-COR, Lincoln, Nebr., USA) with the SequiThermEXCEL^{TM}II DNA Sequencing Kit-LC (Epicentre Technologies, Madison, WIS, USA) using infrared dyed primers FR1 and FF390 according to the manufacturer's protocol. Direct sequencing of the partial actinobacterial PCR products was conducted in both directions in a CEQTM8000 capillary electrophoresis device (Beckman Coulter, Fullerton, CA, USA) with the CEQ Dye Terminator Cycle Sequencing kit according to a modified manufacturer's protocol as previously reported (Jaatinen et al., 2008).

Thirty-one fungal and twenty-four actinobacterial DGGE-derived sequences were aligned with those available on the public databases of GenBank/EMBL/DDBJ and RDP-II release 9.44 (Cole et al., 2005) using FastAligner 3.1 in the ARB software package (Ludwig et al., 2004). True chimeric (CHIMERA_CHECK version 2.7 in RDPII) and clearly non-fungal or non-actinobacterial sequences were excluded. Phylogenetic analyses were conducted as previously reported (Jaatinen et al., 2008). All sequence data obtained in this study has been deposited in the GenBank, EMBL and DDBJ databases under Accession Numbers EU527020 to EU527074.

2.3. Community analyses

First, we examined the extent of variation in the binary data of the observed seventy-eight fungal and sixty actinobacterial DGGE bands with detrended correspondence analysis (DCA). According to the gradient lengths obtained using DCA, we applied a unimodal

species response model for both the fungal 18SrDNA and the actinobacterial 16SrDNA derived binary data using correspondence analysis (CA) to detect patterns of variation in the banding patterns, and canonical correspondence analysis (CCA) for evaluating the significance of our explanatory variables (Lepš and Šmilauer, 2003). Rare species were downweighted in analyses. We used Canoco for Windows 4.5 software (ter Braak and Šmilauer, 2002).

We tested whether community structure differed between pristine sites with different nutrient levels (OM, OL, ME) using the binary matrix of DGGE-derived fungal or actinobacterial data as response variables, and binary variables describing the nutrient levels as explanatory variables. A significant canonical axis based on the environmental variables indicates differences in banding patterns among nutrient levels. Significance levels of the canonical axes were determined with Monte Carlo permutation tests. We tested each nutrient level separately for significant community structure changes following water-level drawdown using hydrological status (P, STD, LTD) as explanatory variables. Since we assumed that community structure would vary with depth in the peat profile, we separated this effect by using dummy variables indicating sampling depth as covariates in CCA. Similarly, we tested the effect of depth while using either nutrient level or hydrological status as covariates. We further analyzed the combined effects of depth and nutrient level, as well as depth and hydrological status (within nutrient level). We did this by constructing interaction terms showing both which layer and which site or hydrological status the samples represented, and using them as explanatory variables. The effect of microtopography (Hu, La, Ho) at the OM site was tested by using both sample depth and hydrological status (P vs. STD) as covariates.

Shannon-Weaver diversity indices (Shannon and Weaver, 1963) were calculated for each peat core (i.e., integrating layers L1-L3 per sampling location) as follows:

$$H' = -\sum_{i=1}^{s} pi \ln(pi)$$

where *pi* is the proportion of each DNA band from all observed DGGE bands in a sample core and S the total number of DNA bands in the sample core. The index takes into account both the total number of the DGGE bands and the proportion of each band in the total of each peat core. The diversity indices were subjected to a two-way analysis of variance (ANOVA) with nutrient level and hydrological status as the main effects. Differences between the microforms in the OM P and STD plots were tested with a two-way ANOVA using microform and drainage status as the main effects. Tukey's test ($p < 0.05$) was used for pairwise comparisons. ANOVAs were completed using General Linear Models in the SYSTAT v. 10 package, which can handle unbalanced data; because of the microtopographical variation, we had more observations for OM than for the other sites.

3. Results

3.1. Fungal and actinobacterial sequences

We obtained representatives of four fungal phyla (Fig. 1). The majority of the sequences were split equally between Ascomycota (32 %) and Basidiomycota (32 %). The rest of the sequences belonged to two families (Monoblepharidaceae and Chytridiaceae) of the phylum Chytridiomycota (16 %), phylum Zygomycota (6 %), and to several

uncultured fungal or eukaryotic sequences/clones (13%) distantly related to any known fungus (Fig. 1). Almost all basidiomycete-related sequences clustered within the six different orders of the class Agaricomycetes, which contains 53 % of described basidiomycetes. Ascomycete-related sequences clustered within four different classes of the subphylum Pezizomycotina, and within the order Saccharomycetales.

Over 80% of the actinobacterial DGGE sequences were most similar to uncultured actinobacterium/bacterium clones. All of the sequences were similar to actinobacterial sequences rather than some other group of bacteria, and clustered into two main clades (Fig. 2). Within these two clades, nine subclades of uncultured actinobacterium/bacterium clones were found as well as sequences most similar to *Acidothermus cellulolyticus* Mohagheghi**,** several *Rhodococcus* or *Nocardia* (family Nocardiaceae) strains, and several *Mycobacterium* strains.

3.2. Fungal and actinobacterial communities in pristine sites

Two gradients were distinguished in both the fungal and the actinobacterial community structure according to the CA (Figs. 3 and 4). The first axis corresponds in both cases to the variation in site nutrient level. Also, pH correlated with the main gradient of distribution for the fungal community. The left-hand side of the ordination plots represents the fen sites (ME and OL) while the right-hand side represents the bog site (OM) with all its microforms (Hu, La, Ho). CCA results confirmed that nutrient levels significantly affected ($p=0.002$) both the fungal and the actinobacterial banding patterns. The second axis corresponds to the distance of the sampled layer from water level, or sample depth. In Figs. 3 and 4, the lower part of the ordination diagram represents relatively drier and the upper part relatively wetter sampling locations.

Fungal communities were most different between the mesotrophic fen and the bog. Despite the differences observed between fens and bogs, and between peat layers with different wetness and, possibly, substrate quality, most fungi and actinobacteria found in the pristine peatland seemed robust to the environmental variables we measured. The first two canonical axes explained only 9% and 7% of the variation in the fungal community, and 9% and 8% of the variation in the actinobacterial community, respectively.

Generally, fungal diversity was slightly higher than that of actinobacteria in all sites. Both the fungal and the actinobacterial diversity correlated positively with site nutrient level ($p=0.001$ and $p=0.008$, respectively), being significantly higher in both the fen sites compared to the bog (Table 2). Among the different microforms of the OM site, the fungal diversity in the intermediate La-level was significantly lower than in either the drier Hu- or wetter Ho-surfaces. The actinobacterial diversity did not vary significantly between microforms.

Some fungal sequences seemed to associate with certain nutrient levels, e.g., rj1F-24, -39 and -3 in fens, and rj1F-D in bog, or layers, e.g., rj1F-9, -33 and -47, while others seemed to be more "generalist" (Fig. 3b; Appendix). There was no clear pattern in the obtained actinobacterial sequences in the pristine sites. Although, one actinobacterial sequence (rj1A-A18) showed some preference for the pristine bog site.

3.3. Fungal community following water-level drawdown

Persistent water-level drawdown induced significant changes in the fungal community composition at all sites (p-values for canonical axes < 0.05). Hydrological status alone explained from 7% (OM) to 13% (ME) of the variation in the fungal community, while sampling depth alone explained from 5% (OM) to 10% (OL). The responses to water-

level drawdown were not uniform in all layers, but by accounting for both hydrological status and layer depth using the combined variables, 18% (OM) to 37% (OL) of the variation could be explained. Fungal communities in LTD plots of the different sites seemed to be more similar to each other than those in P plots (Fig. 5; LTD centroids are closer to each other than P centroids).

Fungal diversity responded significantly to hydrological status ($p=0.018$) and increased significantly following STD ($p=0.013$) (Table 2). Differences between P and LTD, and STD and LTD were not significant. In the OM site, the increasing trend generally continued after LTD but not strongly enough to make the site x hydrology interaction significant. Overall, there were slightly more fungal sequences that responded positively (appeared as new compared to P) than negatively (disappeared as compared to P) to STD (Table 3). In contrast, over half of the responses to LTD were negative, as compared to the pristine conditions. The CCA results implied that the responses to both STD and LTD were rather unidirectional in the ME and OM sites; however, in the OL site the community in the STD plot was even more different from that of the LTD plot than was the community of the P plot (Fig. 5).

Many fungal sequences were observed in all sites regardless of the hydrological condition or site, e.g., rj1F-Ftls, -Gtls, -15, -28, -C and -44 (Appendix). Our results indicate that basidiomycetes may be more sensitive (respond positively or negatively) to hydrological changes than ascomycetes (Table 3). Sequence rj1F-33 was the only ascomycete-related sequence that showed a response (positive) to LTD, even though the patterns were not consistent among sites. A sequence similar to genera *Lactarius* and *Russula* (rj1F-47) was observed only from the deepest 20-30 cm layer of the OM site after LTD, whereas it was observed from all layers of OL and from the top 0-10 cm layer of ME sites (Appendix). A sequence rj1F-o became observable after STD but was absent again after LTD. Differently, sequence rj1F-D was observed in all OM plots, but was absent from P plots in the fen sites (Appendix). Some sequences, e.g., rj1F-42 and rj1F-39, occurred after STD only in some specific sites and in the other sites they were present despite the hydrological change. Three of the five Chytridiomycota sequences showed different responses to water-level drawdown depending on nutrient level (Table 3, Appendix).

3.4. Actinobacterial community following water-level drawdown

The effect of water-level drawdown on the actinobacterial banding pattern diminished directly with site nutrient level. In the ME site, the relationship was significant and accounted for 15% of the variation in banding pattern. In the OL site, the effect was only barely significant and accounted for 10% of the variation. Finally, in the OM site, the effect of water-level drawdown on the actinobacterial community was not significant. Similar to the fungal community, the actinobacterial community seemed to be generally more homogeneous across sites in LTD plots (Fig. 5).

Sample depth had a significant effect at all sites and influenced the banding patterns somewhat more than water level in the more nutrient-poor OL (13%) and OM (8%) sites. In the ME site, sample depth and water-level drawdown were equally effective; however, when accounting for the variation in both factors, 20% (OM) to 46% (ME) of the variation in banding pattern could be explained, which reemphasizes the specific relationship between response and layer depth. Actinobacterial diversity did not respond significantly to hydrological status ($p=0.461$) (Table 2).

Sequences rj1A-A3, -B9, -B12, -A16, -B17 and -B26 were ubiquitous. In general, almost equal numbers of the obtained sequences responded positively and negatively to

both STD and LTD (Table 4). Changes in detected sequences after water-level drawdown were mainly co-dependent on nutrient level. For example, sequences rj1A-A8, -B16, -B15, and -B24 showed a negative response to LTD in the ME site only (Table 4). In contrast, sequences rj1A-A7 and B18 were observed in all sites except in OM following LTD. Sequence rj1A-B3 was common in the OL sites and sporadically observed in the ME site following water-level drawdown, and in all hydrological conditions in the OM site.

4. Discussion

4.1. Fungal and actinobacterial sequences

The fungal primers generated a PCR-product of 390 bp and provided relatively high resolution in DGGE for the various fungal taxa, similar to the performance noted by Vainio and Hantula (2000). Since only 32 % of the obtained sequences showed any similarity to ascomycetes, our results do not support the implication based on cultivation and isolation methods that ascomycetes represent over 80% of the fungal species found in peatlands (Thormann et al., 2006b). Both methods have their flaws: the cultivation methods may favor fast-growing species that produce large numbers of spores (Artz et al., 2007) whereas the molecular PCR-DGGE based methods may overestimate species that are present with high DNA concentrations in the samples. Problems with direct sequencing from DGGE arise also when bands are insufficiently resolved, which makes them difficult to separate by excision; this explains why only ca. 40 % of our fungal DGGE bands were successfully sequenced.

Fungal sequences clustered with fungi capable of utilizing a broad range of substrates. Chytrids belonging to genera *Rhizidium*, *Chytriomyces* and *Podochytrium* are known to mineralize chitin (Kiziewicz and Kurzatkowska, 2004). Zygomycetes (genus *Mortierella*) are pioneer saprobes that remain common throughout the entire process of decomposition (Deacon, 1997; Thormann, 2006b). Several mitosporic ascomycetes cover aquatic polysaccharide degraders (e.g., *Tethachaetum*, *Articulospora*) (Chamier and Dixon, 1982), aero-aquatic cellulose degraders (e.g, *Helicodendron*, *Helicoon*) (Fisher et al., 1977; Abdullah and Taj-Aldeen, 1989), woody-litter degraders (*Lulworthia*) (Bucher et al., 2004). Basidiomycetes-related sequences showed similarity to genera containing ectomycorrhizal (ECM) fungi (e.g., *Clavaria*, *Russula*, *Lactarius*), wood-decomposers (*Cymatoderma* and *Panus*) and cellulose-litter decomposers (*Mycena* and *Baeosphora*) (Lindahl and Boberg, 2008). Sequences related to phylum Glomeromycota, arbuscular-mycorrhizal (AM) fungi were not obtained although they form mutualistic associations with fen plants such as sedges (Turner et al., 2000); however, they are probably more common in more nutrient-rich fens (Wolfe et al., 2007).

That most of the obtained actinobacterial sequences did not match any actinobacterial sequences probably reflects an incomplete coverage currently available in public databases. A minority of sequences were similar to members of *Rhodococcus*, *Nocardia* and *Mycobacterium*, which are commonly found in soil and water and include human pathogens, saprophytes (Ryan, 2004) and decomposers able to process complex aromatics (van der Geize and Dijkhuizen, 2004), lignin (Trojanowski et al., 1977; Haider et al., 1978) and complex hydrocarbons such as petroleum distillates (Aislabie et al., 1998).

4.2. Fungal and actinobacterial communities in pristine sites

The result that fungal and actinobacterial communities separated between the two fen sites and the bog site is in line with the vegetation pattern which is more similar between the two fens than between either of the fens and the bog. This reflects ecohydrological and, consequently, physical and chemical characteristics (e.g., pH, substrate quality, gas exchange) of the peatland types (Table 1; Laine et al., 2004; Jaatinen et al., 2007). It seems that fungi in the pristine sites are more affected by moisture or aeration (Lähde, 1969) of the substrate, measured as distance to water level, while actinobacteria respond more to some other depth-related factor, possibly substrate quality, which generally decreases downwards along with the more advanced state of decomposition (Hogg et al., 1992; Hogg; 1993). Microbial diversities were higher in the fens than in the bog, which is a pattern similar to that found for plant species diversity (Laine et al., 1995). This pattern may reflect the variation in substrate quality and heterogeneity: saprobes differ in their ability to utilize different compounds as C sources (Thormann et al., 2001, 2002) and may affiliate with different litter types according to their chemical composition (Thormann, 2004). Plant community composition also has implications for fungal mycorrhizal associations (Natel and Neumann, 1992).

The result that fungal communities were most different between the mesotrophic fen and bog is congruent with the results from Swedish mires (Nilsson et al., 1992) and a Scottish moorland–forest gradient (Anderson et al., 2003), where the site type seemed to be the strongest determinant of fungal community. Despite the detected differences between fens and bogs, we want to emphasize that most of the fungal and actinobacterial sequences found in the pristine peatland were not highly affected by the environmental variables. Thus, it seems that most fungi and actinobacteria in boreal peatlands may be rather resilient to variation in environmental conditions. On the other hand, we must bear in mind that molecular techniques such as DGGE and sequencing are qualitative approaches that reveal only the presence of certain taxa and provide no information on their relative activity rates under the different conditions.

Some sequences were characteristic of fen sites. For example, sequences rj1F-24 and -39, which clustered with novel uncultured fungal sequences, could represent a species characteristic of wet, nutrient-rich mesotrophic fens. In addition, sequence rj1F-3 was most similar to uncultured sequences of Mortierellaceae (mainly soil saprobes; White et al., 2006) and characterized the 5-10 cm layer of the oligotrophic fen.

In contrast, some of the obtained sequences apparently represent species characterizing the nutrient-poor environment of the bog site, e.g., *Helicoon*- and *Tyrannosorus*-related sequence (rj1F-D) seemed typical of the upper layers of the bog site. These fungi include major decomposers of wood and plant litter as well as plant parasites and biotrophs (Goos, 1987). *Helicoon fuscosporum* is known to degrade cellulose (Fisher et al., 1977). Interestingly, two species of *Helicoon* (*H. myosuroides* Voglmayr and *H. dendroides* Voglmayr) have also earlier been suggested to be specialists of raised bogs (Voglmayr, 1997).

Even though the abundance and growth of some mycorrhizae may be limited in soil that is either flooded or too dry (Lodge, 1989), woody plants in wet peatland habitats are commonly mycorrhizal (Glenn et al., 1991; Thormann et al., 1999; Baar et al., 2002). Ericoid (ERM), AM and ECM fungi have been observed in different shrub and tree species from peatlands (Thormann et al., 1999). In our study, ECM fungi related sequence, rj1F-47, was found from the top layers in all our pristine sites, except the wettest hollow surface of the bog site.

Only one actinobacterial sequence showed some preference for the pristine bog site. This sequence was similar to uncultured group of actinobacterium or bacterium sequences, which are only distantly related to the known actinobacterial taxa and thus might represent a novel lineage.

4.3. Fungal community following water-level drawdown

Our results indicate that the fungal community responds to persistent water-level drawdown, especially in fens. Also the sampling depth seems to have a strong effect on the community and its response. These results agree with our earlier observations from the northern boreal fen Suonukkasuo (Jaatinen et al., 2008), and emphasize the interactive roles of water level, site type and depth in shaping the fungal communities. The fungal communities after long-term water-level drawdown become more similar among sites with different nutrient levels than those of the pristine plots, resembling the changes in vegetation pattern. Earlier, Trinder et al. (2008) have suggested that litter type may have a greater impact than water level on peatland fungal communities.

Short-term drainage affects many fungal sequences positively rather than negatively and the effect is opposite after long-term drainage, which calls for some attention. In wet conditions, where oxygen concentration drops rapidly when moving from the peat surface downwards (Boggie, 1977), it is likely that specialist species dominate the microbial community. For each nutrient level, the environmental conditions are further modified by the quality of soil water. Following short-term water-level drawdown, changes in environmental conditions, including litter quality, moisture, pH, temperature and oxygen availability, are still rather small (Jaatinen et al., 2007; Vávřová et al., unpublished), but seemingly enough to allow colonization by more common aerobes. This will first lead to an overlap of communities, and higher overall diversity. Following long-term water-level drawdown, upon establishment of the drier conditions, the environment dramatically differs from the pristine one (Laiho, 2006). Continued gradual replacement of specialists by generalists leads to lowered diversity. Similar patterns of turnover have been observed for vegetation (Laine et al., 1995; Vasander et al., 1997). Short-term here means 3-4 years, during which time the ecosystems we studied were still clearly under transient conditions. Based on vegetation studies, it may take several decades before the ecosystems have reached a new "equilibrium" state relative to the new hydrological conditions (Laine et al., 1995).

Our results suggest that hydrological changes, directly or indirectly via the change in vegetation and litter quality, may have a greater effect on the existence of basidiomycetes than ascomycetes. Only one of the obtained ascomycetes-related fungal sequences showed a response (positive) to long-term water-level drawdown. This sequence related to the Sordariales, which is a diverse group containing lignicolous, herbicolous, coprophilous and soil-dwelling taxa (Huhndorf et al., 2004).

We hesitate to say whether the differences observed in the response patterns among sites imply real differences in all cases, or simply reflect the limited coverage of our sample cores. For instance, Russulaceae are a common fungal group following the drainage of a boreal bog (Salo, 1980). Yet, in our data, a sequence similar to *Russula* and *Lactarius* was observed only from the deepest layer of the bog site after long-term water-level drawdown whereas it was observed from all layers of the oligotrophic fen site and from the top layer of the mesotrophic fen site. On the other hand, different Russulaceae-species may be affected differently depending on the species; one *Russula*-related sequence strongly characterized the driest sampling location and another one the wetter location of the hydrological gradient in a boreal Suonukkasuo fen (Jaatinen et al., 2008).

Notably, some species of *Lactarius* are known to have saprotrophic capabilities and may adopt the decomposing role depending on the ecological niche they live in (Buée et al., 2007).

A sequence related to *Clavulina* appeared after the transient conditions created by STD but disappeared when the drying succession continued. Species of *Clavulina* have been tentatively considered mycorrhizal, but a radiocarbon study demonstrated that some species of this genus are also saprobic (Hobbie et al., 2002). In addition, water-level drawdown may not affect at all, or affect positively some sequences representing basidiomycetes: e.g., a sequence belonging to the wood-decomposing genera *Cymatoderma* and *Panus* in the OL fen site and in the hummock-surfaces of the OM sites.

Chytridiomycota (or chytrids) seem to respond differently to water-level drawdown depending on the nutrient level. Chytrids thrive in aquatic environments and are found commonly in terrestrial habitats where a periodic film of surface water is available for dissemination of zoospores (Shearer et al., 2007). Many chytrids can survive periods of desiccation by the formation of resistant spores (Shearer et al., 2007). Thus, at least some of the obtained chytrid sequences might survive the moderate water-level drawdown but die when conditions become too dry for too long.

Overall, our results confirm the findings of a previous PLFA study and show that the fungal community response to changes in hydrology is dependent on the type of peatland (Jaatinen et al., 2007).

4.4. Actinobacterial community following water-level drawdown

Effect of water-level drawdown on actinobacteria only seemed apparent in the most nutrient-rich fen site and diminished as sites became more nutrient poor. Also, similar to fungi, the actinobacterial community seems to become more similar across sites after LTD. Sample depth seems to be a more important determinant of community structure at all sites and more influential than water level in the more nutrient-poor fen and bog sites. The results echo a trend observed in a northern boreal fen (Suonukkasuo) where the actinobacterial community response was more dependent on depth than water level and in contrast to the response of the fungal community (Jaatinen et al., 2008).

Several sequences might relate to generalist species, since they were observed throughout the peatland sites regardless of depth, nutrient level and hydrological conditions. These sequences were similar to *Mycobacterium avium* subsp. *avium* Chester, uncultured bacterium clones and *Acidothermus cellulolyticus* Mohagheghi. A sequence similar to rj1A-B17 was also found also in the Suonukkasuo fen but not at the wettest location of the hydrological gradient (Jaatinen et al., 2008).

Our results would indicate that the occurrence of some actinobacteria after water-level drawdown, either short- or long-term, is codependent on nutrient level. For example, LTD seems to negatively affect sequence rj1A-A8 (related to cellulolytic and xylanolytic *Cellulolomonas* species: e.g., Elberson et al., 2000; Rivas, 2004) only in the nutrient-rich fen. In contrast, LTD seems to negatively affect sequences rj1A-A7 and -B18 only in the bog. Thus, another *Mycobacterium*-related sequence may represent a species for which drying created a positive effect only in a certain type of peatland.

Some of our results showed a similar trend to the PLFA study from the same site indicating that at least some actinobacteria would have negative effects after water-level drawdown in the ME fen but positive in the OM bog (Jaatinen et al., 2008). Most of the actinobacterial DGGE sequences (over 70%) were similar to unknown actinobacterial or bacterial sequences deriving from various different environments and showed a random distribution among hydrologically different sites. Their roles remain unclear.

4.6 Conclusions

Our study obtained molecular data of fungi and actinobacteria from a complex peatland ecosystem with three site types differing in their vegetation (nutrient levels) and wetness. Our pristine plots formed an ecohydrological gradient from a wet, nutrient-rich mesotrophic fen to a nutrient-poor bog. The drained plots represented different stages of a secondary succession leading to more forest-like ecosystems. We detected sequences related to various fungal decomposers capable of using a broad range of substrates. Fens had more diverse fungal and actinobacterial communities compared to bogs. Fungal diversity increased following STD irrespective of site nutrient level. Furthermore, water-level drawdown also was associated with a change in the fungal sequences detected. In contrast, sampling depth seemed to have a greater effect on the actinobacterial communities, especially in nutrient-poor sites. However, changes in communities were neither drastic nor clear and it appears that the distribution of most sequences could be random and independent of the prevailing environmental conditions and this result reflects the dominance of generalists capable of utilizing various substrates.

Basidiomycetes seemed to be more responsive to water-level drawdown than ascomycetes. The response of each putative fungus was often different after short- and long-term water-level drawdown and/or dependent on peatland type. Overall, different fungi may have different strategies to cope with hydrological changes caused by climate change or forestry drainage and these strategies are at least partly dependent on peatland type. Actinobacteria in general were less sensitive to hydrological changes, although the response of some actinobacteria to water-level drawdown may similarly depend on peatland type. Generally, the differences between peatland types in both fungal and actinobacterial communities diminished after long-term water-level drawdown. This result is most likely connected to vegetation, which has become more similar (dominated by Scots pine) over the four decades.

The water-level drawdown in our sites was achieved by ditching and was thus faster than would likely happen with a gradual change in climate. While we assume that the general change patterns in the ecosystem structure would be similar, the timescale of change could be different according to a more gradual drying.

Our study has broadened the picture of fungal and actinobacterial community structure in natural and manipulated boreal peatland sites. To study the system further, a comprehensive clone library from the collection of peat soils should be completed. Also, using RNA as a molecular marker could open a window into the relative activity levels of different microbial species in the peatland community.

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Tables

Table 1. Average water-levels, peat element concentrations and pH (6 mL fresh peat in water; 1:3; vol:vol) with standard errors (SE) for each plot at the time of sampling (May 2004). Nitrogen concentration was measured with a LECO CHN-1000 analyzer, the other element concentrations with an ICP emission spectrometer after dry ashing.

Plot	WL (cm)	Layer	N	P	K	Ca	pH
P ME	0	L1	2.11 (0.21)	851 (74)	1021 (190)	5910 (804)	5.9 (0.18)
		L2	2.38 (0.02)	814 (17)	786 (64)	6410 (703)	5.7 (0.24)
		L4	2.61 (0.15)	558 (43)	96 (2)	6277 (433)	5.3 (0.02)
STD ME	-15	L1	2.08 (0.15)	933 (39)	1015 (90)	8317 (1230)	5.4 (0.22)
		L2	2.93 (0.10)	1110 (68)	620 (71)	5797 (282)	5.5 (0.13)
		L4	2.40 (0.03)	553 (23)	89 (14)	6370 (297)	5.0 (0.06)
LTD ME	-15	L1	1.52 (0.11)	704 (68)	851 (208)	3177 (352)	3.8 (0.06)
		L2	2.04 (0.34)	766 (116)	322 (17)	3247 (1012)	3.8 (0.13)
		L4	2.52 (0.21)	540 (40)	40 (3)	5127 (767)	4.5 (0.01)
P OL	-11	L1	1.23 (0.03)	338 (24)	958 (139)	5407 (122)	4.6 (0.05)
		L2	1.56 (0.12)	474 (36)	314 (37)	4423 (405)	4.6 (0.08)
		L4	2.54 (0.19)	806 (76)	57 (14)	3813 (128)	4.8 (0.01)
STD OL	-30	L1	1.36 (0.12)	401 (32)	1156 (214)	6830 (1203)	4.9 (0.30)
		L2	1.93 (0.11)	781 (46)	401 (47)	5237 (997)	4.9 (0.25)
		L4	2.54 (0.05)	806 (14)	50 (10)	3830 (101)	4.9 (0.05)
LTD OL	-30	L1	2.21 (0.20)	1330 (119)	734 (196)	3720 (465)	3.9 (0.07)
		L2	2.97 (0.04)	1243 (38)	189 (42)	2473 (238)	4.2 (0.12)
		L4	2.42 (0.05)	762 (19)	30 (0)	3183 (74)	4.6 (0.09)
P OM Hu	-22	L1	0.92 (0.07)	236 (27)	2017 (286)	1500 (32)	4.0 (0.09)
		L2	0.84 (0.08)	213 (18)	830 (215)	1327 (41)	3.8 (0.03)
		L4	1.10 (0.05)	276 (23)	161 (11)	1263 (43)	3.6 (0.03)
STD OM Hu	-24	L1	0.92 (0.01)	278 (41)	1305 (192)	1593 (149)	4.0 (0.14)
		L2	0.96 (0.01)	248 (27)	795 (59)	1247 (15)	3.6 (0.04)
		L4	1.18 (0.03)	280 (28)	222 (27)	1323 (98)	3.6 (0.02)
P OM La	-6	L1	0.87 (0.03)	170 (27)	1917 (406)	1180 (59)	4.4 (0.08)
		L2	0.93 (0.11)	200 (19)	609 (88)	1203 (58)	3.9 (0.08)
		L4	1.08 (0.07)	223 (13)	94 (10)	981 (80)	3.7 (0.06)
STD OM La	-15	L1	1.09 (0.13)	286 (35)	1152 (254)	1180 (49)	3.8 (0.09)
		L2	1.11 (0.09)	278 (55)	464 (28)	1233 (110)	3.7 (0.01)
		L4	1.18 (0.02)	240 (19)	101 (25)	998 (119)	3.8 (0.07)
P OM Ho	-3	L1	0.83 (0.03)	189 (16)	659 (318)	798 (18)	4.0 (0.11)
		L2	1.05 (0.06)	190 (28)	205 (68)	731 (8)	4.0 (0.04)
		L4	1.27 (0.04)	183 (12)	81 (14)	622 (55)	3.9 (0.06)
STD OM Ho	-10	L1	1.09 (0.12)	297 (32)	601 (82)	1023 (9)	4.0 (0.04)
		L2	1.20 (0.10)	246 (49)	216 (64)	825 (10)	4.1 (0.06)
		L4	1.36 (0.12)	228 (33)	57 (3)	721 (129)	3.9 (0.12)
LTD OM	-15	L1	1.48 (0.06)	814 (58)	791 (66)	2923 (156)	3.7 (0.15)
		L2	1.02 (0.05)	461 (38)	393 (59)	2197 (202)	3.6 (0.02)
		L4	1.13 (0.05)	260 (12)	52 (4)	1147 (208)	3.6 (0.01)

Abbreviations: P, pristine; STD, short-term drainage; LTD, long-term drainage, ME, mesotrophic; OL, oligotrophic; OM, ombrotrophic; Hu, hummock; La, Lawn; Ho, hollow. WL, water-level. L1, 0-5 cm; L2, 5-10 cm; L4, 20-30 cm.

Table 2. Shannon-Weaver diversity indices (*H´)* for the fungal (Fung) and actinobacterial (Act) DGGE bands. Average of 3 sample cores per plot, standard error of mean in parentheses.

			Hydrological status		
			P	STD	LTD
Fung	Nutrient level	ME	3.56 (0.09)	3.75 (0.07)	3.51 (0.07)
		OL	3.28 (0.11)	3.68 (0.15)	3.49 (0.14)
		OM	3.12 (0.08)	3.27 (0.08)	3.42 (0.07)
	Microforms	OM Hu	3.12 (0.04)	3.34 (0.03)	
		OM La	2.90 (0.08)	3.21 (0.06)	3.42 (0.07)
		OM Ho	3.44 (0.03)*	3.44 (0.14)	
Act	Nutrient level	ME	3.18 (0.08)	2.82 (0.38)	2.83 (0.15)
		OL	3.16 (0.07)	3.31 (0.09)	3.44 (0.09)
		OM	2.70 (0.09)	2.95(0.07)	3.19 (0.09)
	Microforms	OM Hu	2.82 (0.16)	3.11 (0.13)	
		OM La	2.68 (0.23)	2.93 (0.13)	3.19 (0.09)
		OM Ho	2.66 (0.13)	2.82 (0.07)	

* One sample core from P OM Ho was excluded from the index-calculations because its value was an outlier. See footnote of Table 1 for abbreviations.

Table 3. Fungal derived 18SrDNA sequences that showed either positive (+) or negative (-) response to water-level drawdown.

		ME		OL		OM	
Sequence	Taxonomic affiliation	STD	LTD	STD	LTD	STD	LTD
rj1F-q	*Rhizidium* (C)	-	+		-	-	-
rj1F-9	*Chytriomyces* (C)					+	
rj1F-30	*Monoblepharella* (C)				-	+	
rj1F-3	uncultured fungus (Z)	+	-		-	+	-
rj1F-35	uncultured fungus (Z)	-	+			+	
rj1F-45	*Ascocalyx abietina* (A)					+/-	+
rj1F-33	Sordariales (A)		-				
rj1F-47	*Lactarius*/*Russula* (B)					-	+
rj1F-o	*Clavulina* (B)		-			+/-	-
rj1F-D	*Helicoon/Tyrannosorus* (B)	+	-	+			
rj1F-x	uncultured Boletaceae (B)		-		-	-	-
rj1F-s	uncultured fungus (B)				-	-	-
rj1F-42	*Cymatoderma/Panus* (B)			+		+	
rj1F-39	uncultured Boletaceae (B)					+	
rj1F-26	*Mycena*/*Baeospora* (B)					-	
rj1F-50	uncultured soil fungus (B)	+			-	+/-	+
rj1F-18	uncultured fungus (novel)					+	
rj1F-24	uncultured fungus (novel)			+	-		

Abbreviations: B, Basidiomycota; A, Ascomycota; Z, Zygomycota; C, Chytridiomycota; See footnote of Table 1 for abbreviations

Table 4. Actinobacterial derived 16SrDNA sequences that showed either positive (+) or negative (-) response to water-level drawdown.

		ME		OL		OM	
Sequence	Taxonomic affiliation	STD	LTD	STD	LTD	STD	LTD
rj1A-B3	*Mycobacterium*	+				-	+
rj1A-A2	*Mycobacterium*	-	+	+		+	
rj1A-A8	uncultured *Cellulomonas*		-	-		+	
rj1A-A7	uncultured bacterium	+				+	-
rj1A-A21	uncultured bacterium	-	+			+	
rj1A-B18	uncultured bacterium	-	+	-	+	+	-
rj1A-A18	uncultured bacterium		+	+	-		-
rj1A-B16	uncultured bacterium		-			+	
rj1A-B15	uncultured bacterium		-			-	+
rj1A-B24	uncultured bacterium		-			+/-	+
rj1A-A20	uncultured bacterium					+	
rj1A-A13	uncultured bacterium					+	
rj1A-B23	uncultured bacterium					-	+
rj1A-B25	uncultured bacterium			-	+		-

See abbreviations in Table 1.

Figure legends

Fig. 1. ARB generated phylogenetic tree of fungal partial 18SrDNA sequences. Scale bar represents 10% dissimilarity.

Fig. 2. ARB generated phylogenetic tree of actinobacterial partial 16SrDNA sequences. Scale bar represents 10% dissimilarity.

Fig. 3. Correspondence analysis (CA) of the fungal partial DGGE-derived 18SrDNA bands from the three pristine plots with different nutrient levels. a. The relation of the environmental variables to the 18SrDNA banding patterns. Centroids are shown for dummy variables and arrows represent continuous variables. Nutrient levels: ME, mesotrophic; OL, oligotrophic; OM, ombrotrophic. Microforms: Hu, hummock; La, lawn; Ho, hollow. Sampled layers: L1 = 0–5 cm, L2 = 5–10 cm, L4 = 20–30 cm from the peat surface. Depth, sampling depth (cm from the peat surface; increases downwards); WL, depth of the water level (cm from the mire surface) at the time of sampling; Dist to WL; distance from the sampled layers to the water level. b. DGGE-derived partial 18SrDNA bands responsible for the separation of the samples. Included in the plot are such bands of whose variation axes 1 and 2 explained at least 10%.

Fig. 4. Correspondence analysis (CA) of the actinobacterial partial DGGE-derived 16SrDNA bands from the three pristine sites with different nutrient levels. a. The relation of the environmental variables to the 16SrDNA banding patterns. Centroids are shown for dummy variables and arrows represent continuous variables. b. DGGE-derived partial 16SrDNA bands responsible for the separation of the samples. Included in the plot are such bands of whose variation axes 1 and 2 explained at least 10%. See abbreviations in the legend of Fig 3.

Fig. 5. Differences between the plots representing different nutrient levels and hydrological conditions based on a. CA of the fungal partial DGGE-derived 18SrDNA bands, b. CA of the actinobacterial partial DGGE-derived 16SrDNA bands. Dummy variables indicating the layers sampled were used as covariates. Nutrient levels: ME, mesotrophic; OL, oligotrophic; OM, ombrotrophic. Hydrological conditions: P, pristine; STD, short-term drainage; LTD, long-term drainage.

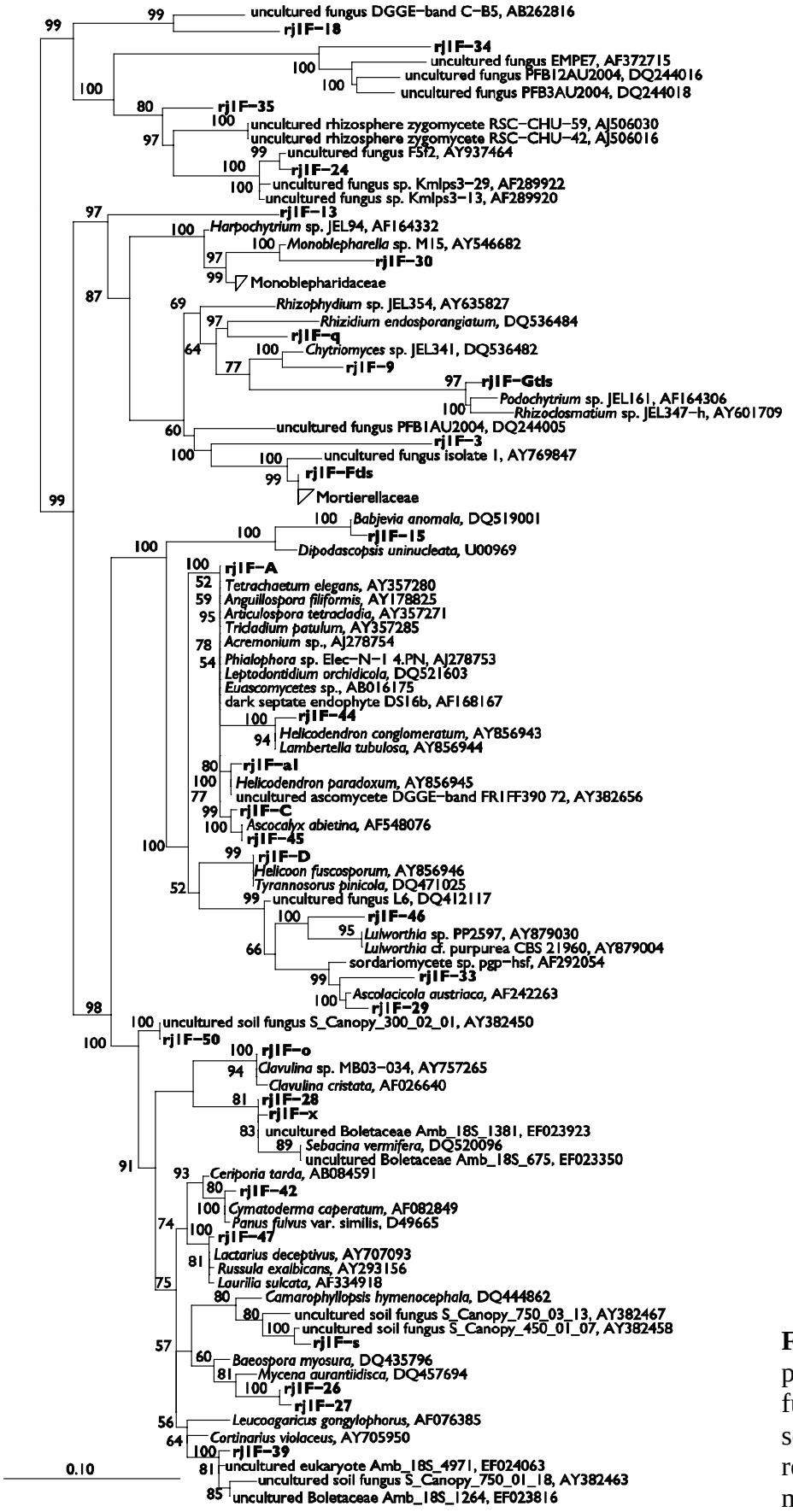

Fig. 1. ARB generated phylogenetic tree of fungal partial 18SrDNA sequences. Scale bar represents 10% dissimilarity.

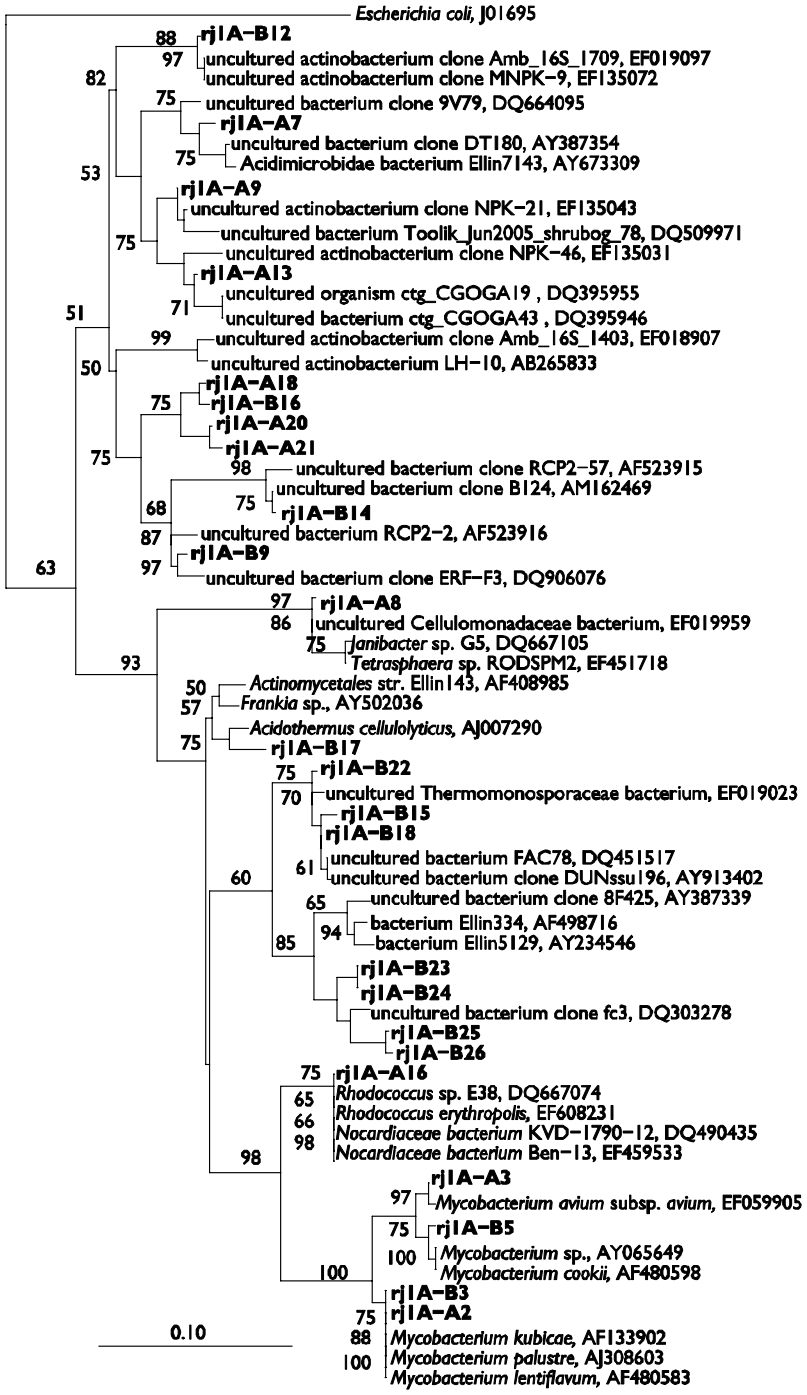

Fig. 2. ARB generated phylogenetic tree of actinobacterial partial 16SrDNA sequences. Scale bar represents 10% dissimilarity.

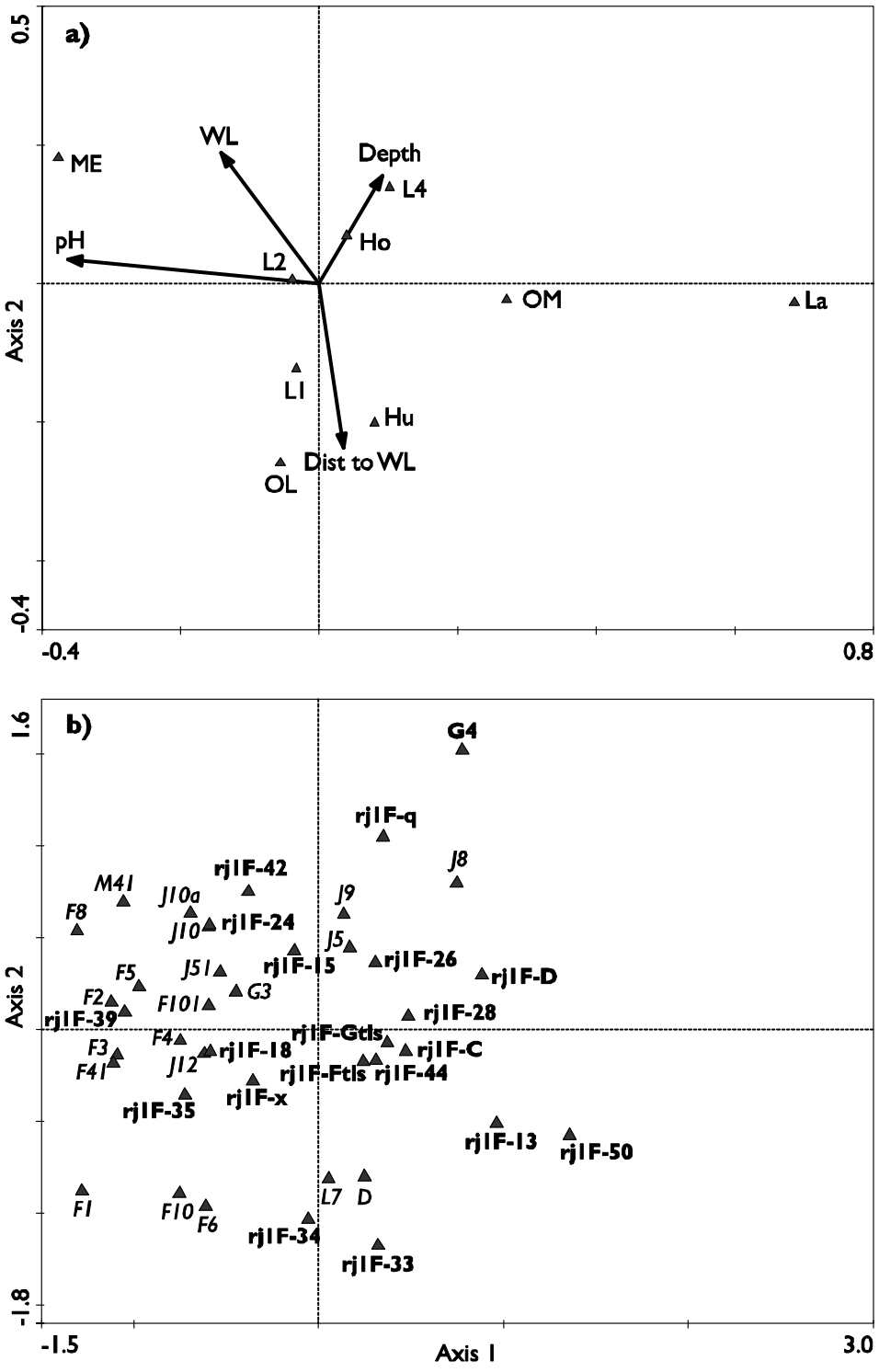

Fig. 3. Correspondence analysis (CA) of the fungal partial DGGE-derived 18SrDNA bands from the three pristine plots with different nutrient levels. a. The relation of the environmental variables to the 18SrDNA banding patterns. Centroids are shown for dummy variables and arrows represent continuous variables. Nutrient levels: ME, mesotrophic; OL, oligotrophic; OM, ombrotrophic. Microforms: Hu, hummock; La, lawn; Ho, hollow. Sampled layers: L1 = 0–5 cm, L2 = 5–10 cm, L4 = 20–30 cm from the peat surface. Depth, sampling depth (cm from the peat surface; increases downwards); WL, depth of the water level (cm from the mire surface) at the time of sampling; Dist to WL; distance from the sampled layers to the water level. b. DGGE-derived partial 18SrDNA bands responsible for the separation of the samples. Included in the plot are such bands of whose variation axes 1 and 2 explained at least 10%.

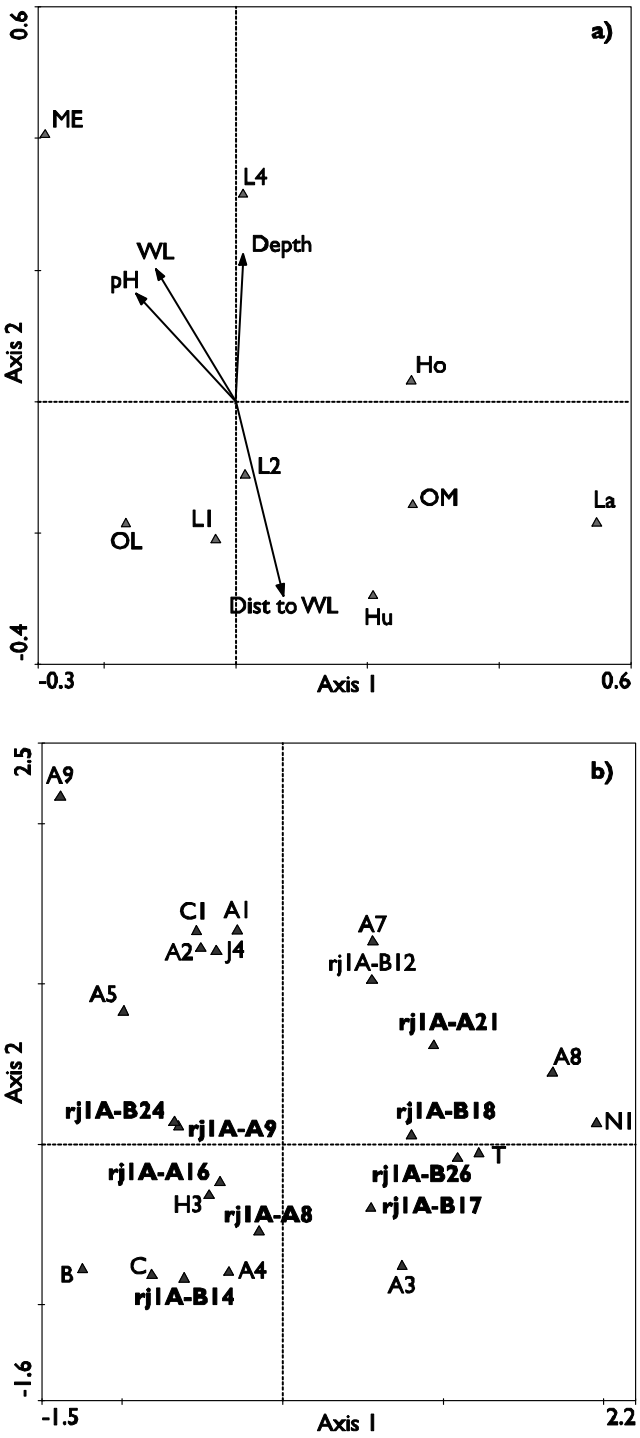

Fig. 4. Correspondence analysis (CA) of the actinobacterial partial DGGE-derived 16SrDNA bands from the three pristine sites with different nutrient levels. a. The relation of the environmental variables to the 16SrDNA banding patterns. Centroids are shown for dummy variables and arrows represent continuous variables. b. DGGE-derived partial 16SrDNA bands responsible for the separation of the samples. Included in the plot are such bands of whose variation axes 1 and 2 explained at least 10%. See abbreviations in the legend of Fig 3.

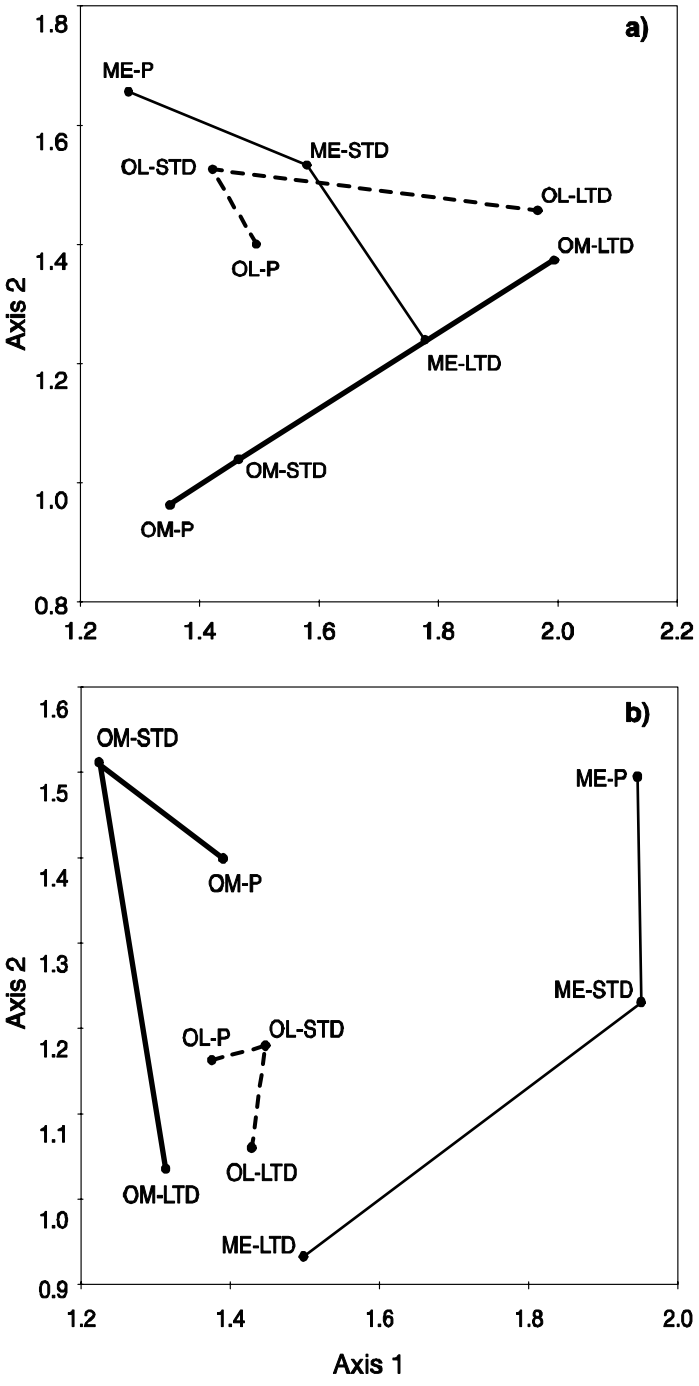

Fig. 5. Differences between the plots representing different nutrient levels and hydrological conditions based on a. CA of the fungal partial DGGE-derived 18SrDNA bands, b. CA of the actinobacterial partial DGGE-derived 16SrDNA bands. Dummy variables indicating the layers sampled were used as covariates. Nutrient levels: ME, mesotrophic; OL, oligotrophic; OM, ombrotrophic. Hydrological conditions: P, pristine; STD, short-term drainage; LTD, long-term drainage.

Appendix A.

Fungal sequences (prefix rj1F-)

Sample			3	9	Ftls	Gtls	13	15	18	q	s	24	26	28	29	30	x	33	34	35	o	39	A	42	C	44	45	46	47	D	50
P	ME	L1		1	3	3		1	3		1	1		1	2		3	1	2	1		2	2	2	3	3					
P	ME	L2			2	1		3	2	1	2	1		1		1	1			1		2	1	2	2	2	1	1	1		
P	ME	L4			3	2		3	2	2	2	2	2	1	2		2			1	2	1	1	2	3	3		1			
STD	ME	L1	1	2	3	2		2	2			1	1	3			3	2	2		1		3	1	3	2	2		2	1	1
STD	ME	L2		1	3	1		3	1		1	1	1	2	1	2	2	1	2		1			1	3	3			1		1
STD	ME	L4		1	3	2		1						1		1	1	1				2	1		2	1	1	2	1		
LTD	ME	L1			3	3		1	1		1		3	2	1				1			1		1	3	3	1	1	2		2
LTD	ME	L2		1	3	2		2	2	2			2	2	2	1			1	2		2	1	1	3	2	1	1	1		1
LTD	ME	L4			3	2		1				1	1	2	2				1	1			1		3	2	1	1			
P	OL	L1	1	1	2	2		1		1			1	1	2				1		1	1	1		3	1	1	1	1		
P	OL	L2		1	3	2	1	2	1	2	2		2	1	1	1	1		3		1	1	1		3	2	1	2	1		
P	OL	L4			2										1				2	1					2	2	1				1
STD	OL	L1	1	1	3	2		1	1				1	1					1		1	1	1		3	3	1	2	1	1	
STD	OL	L2		1	3		1	1	3	2	1	2	3	3		1		1	2	1	3		2	1	3	3	1	1		1	1
STD	OL	L4	2		2	1			1	1		1	1	2	2	2	2	1	2			1	2		3	2		1		2	1
LTD	OL	L1			3	2		1	1				2	1	1			1	2		1				3	3		1	3		
LTD	OL	L2			2	1	1							1					1	1	1	1			3	1	2	3	1		
LTD	OL	L4		1	2	1		2						1	1				3		1		2	2	3			1	1	2	
P	OM Hu	L1			2	2		1	1		1			1	1	1	2	1	2	2	1		1		3	2			1	1	
P	OM Hu	L2			3	3		1						1					1	1	1				3	2				1	
P	OM Hu	L4			2	3	1	2		1			1	2		1							1		2			1			
P	OM La	L1		2	2	2				2				1									1		3	2	1		1	2	
P	OM La	L2		1	3	3		1		1	1		1	2										1	3	3	2		1		2
P	OM La	L4			3	2	1	1					2	1	1		1		2				2		3	2					
P	OM Ho	L1			2	2	1	2	1	1			1	1			1	1		1			1	1	2	2	1			1	1
P	OM Ho	L2			1	2		1		1	2		1		1		1		1				2		2	2	1	1		1	
P	OM Ho	L4			1	1		1		1					1									1	1						
STD	OM Hu	L1	1	3	3	3	1				1		1	3					1	2		1		1	2	2				2	1
STD	OM Hu	L2		1	3	3		1							1		2	1	1	1					3	3					
STD	OM Hu	L4			2	3	1	1	1				1	2		1	1			1		1	2	1	3	3	1	1	1		
STD	OM La	L1		1	3	3		2	1	1				1		1			1	1	1				3	3	1			1	
STD	OM La	L2			3	3		3	1	1				1	1	2			1	1					3		1				1
STD	OM La	L4			3	3		1	1					2	1				1				1	1	1	2					
STD	OM Ho	L1			3	3		1		1	1		3	3	1			1	3	2				1	3	2		3		1	
STD	OM Ho	L2		1	3	2	1	1	2	2				2		2			3	1		1		1	2	3					
STD	OM Ho	L4	1		1	1					1		1			1	1														
LTD	OM	L1		1	3	3	1	2						1		1			1			1	1	1	2	1	2				
LTD	OM	L2		2	3	3	2	1					1		3							1	2	2	1	2	2	1		1	
LTD	OM	L4			1	2	1	1	1				1					1	1	1					2	1	1	1	1		1

Actinobacterial sequences (prefix rj1A-)

Sample			B3	A2	A3	A7	A8	A9	B9	A13	B12	A16	B14	B16	B15	B17	A18	B18	A20	A21	B23	B24	B25	B26
P	ME	L1			2		1	2	2	2		2	1			2		2	2			2		1
P	ME	L2		1	3		1	2	2	1	3	3		1	1	1					1	3		
P	ME	L4			3			2	2	1	1	2		1						1	1	1		
STD	ME	L1	1		3	1	1	3	3	2	2	3		1		2						1		1
STD	ME	L2			2		2	2	2		1	2	2	2	1				1		1	2		
STD	ME	L4			2		1				1	2				1								
LTD	ME	L1			2	1			2	2		1	1			2	1			1	1			2
LTD	ME	L2	2		3				1		1					1		1	3					
LTD	ME	L4		1	3			1	1	1		3												
P	OL	L1	2		2		1	1	2	2		2	1			2								
P	OL	L2	1		3	1	2	3	3	2		3				2		1	1	1	1	2	1	
P	OL	L4	1		3	1			1	1	1	1	1						1	2	1			1
STD	OL	L1	2		3				3	3	3	3				2						2		1
STD	OL	L2	3		3	1		1	3	2	1	3				2	1		1		1	2		1
STD	OL	L4	2	1	3	1		1	1	3		2	1							1	1			1
LTD	OL	L1	3	1	3		1		3	2		1		1		3			2		1		1	2
LTD	OL	L2	2	2	3			1		1	2	2	1		1	2		1	2			3		2
LTD	OL	L4	2	1	3	1	2	2	1	3	2	2						3	1	1				2
P	OM Hu	L1			3			1	3			2	1			1	1					1	1	1
P	OM Hu	L2	1		2				1			1	1	1		2				1	1			2
P	OM Hu	L4					2		1		1	2			1	2	1		2					1
P	OM La	L1			1				2			1				2								
P	OM La	L2			2				2	2		1			1	2	1	2						2
P	OM La	L4			2						1	2				1					1			
P	OM Ho	L1		1	2				3	1	2					1	1	1	1	1				
P	OM Ho	L2		1	1				1		1					2		1						1
P	OM Ho	L4	1		3	1				1		2	1	2	1	1				1	2			
STD	OM Hu	L1		2	2		1		3		2	1	1			1	1			1				3
STD	OM Hu	L2	2		2				3	1		1	1	1	1	3	1							2
STD	OM Hu	L4			2	1	2	1		1	1		1	1		1		1	1	2				
STD	OM La	L1			2	1	1		2		1					2								2
STD	OM La	L2		1	3		1		3	1	1	2				2	1	3	1	1	1	1		3
STD	OM La	L4			2	1				1				1						1	1			
STD	OM Ho	L1			2				3	3		1	1	3		3	1			1				1
STD	OM Ho	L2			2	1			1	1	1					2		1	1	2				
STD	OM Ho	L4		2	3	2	1	1				2				3	1		1	2	1	1		
LTD	OM	L1	2		3				3	1		1	1	1		2			2	1			1	1
LTD	OM	L2		1	3		1	2	3	3	1	2		1	2	3			3	1			1	2
LTD	OM	L4	1	1	2						1		2			2					1	1		