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ORIGINAL ARTICLE

High specificity but contrasting biodiversity of *Sphagnum*-associated bacterial and plant communities in bog ecosystems independent of the geographical region

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Mosses represent ecological niches that harbor a hitherto largely uncharacterized microbial diversity. To investigate which factors affect the biodiversity of bryophyte-associated bacteria, we analyzed the bacterial communities associated with two moss species, which exhibit different ecological behaviors and importance in bog ecosystems, *Sphagnum magellanicum* and *Sphagnum fallax*, from six temperate and boreal bogs in Germany and Norway. Furthermore, their surrounding plant communities were studied. Molecular analysis of bacterial communities was determined by single-strand conformation polymorphism (SSCP) analysis using eubacterial and genus-specific primers for the dominant genera *Burkholderia* and *Serratia* as well as by sequence analysis of a *Burkholderia* 16S rRNA gene clone library. Plant communities were analyzed by monitoring the abundance and composition of bryophyte and vascular plant species, and by determining ecological indicator values. Interestingly, we found a high degree of host specificity for associated bacterial and plant communities of both *Sphagnum* species independent of the geographical region. Calculation of diversity indices on the basis of SSCP gels showed that the *S. fallax*-associated communities displayed a statistically significant higher degree of diversity than those associated with *S. magellanicum*. In contrast, analyses of plant communities of *Sphagnum*-specific habitats resulted in a higher diversity of *S. magellanicum*-specific habitats for all six sites. The higher content of nutrients in the *S. fallax*-associated ecosystems can explain higher diversity of microorganisms.
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Introduction

Bog ecosystems belong to the oldest vegetation forms with more or less constant conditions for thousands of years. The bryophyte genus *Sphagnum*, consisting of approximately 300 different species, is worldwide distributed and a dominant component of the mire and bog vegetation (Daniels and Eddy, 1985). During bog formation, the lower parts of the *Sphagnum* plants die off and build deep layers of dead plant material, the so-called peat. *Sphagnum* bogs are unique habitats for numerous plant and animal species, and their environmental and ecological significance is immense because of the well-recognized role in the global carbon budget and emission of the greenhouse gas methane (Succow and Joosten, 2001; Raghoebarsing *et al.*, 2005). High acidity (pH 3.5–5.0), low temperature, water saturation together with oxygen deficiency and extremely low concentration of mineral nutrients are characteristic for these extreme habitats. Furthermore, *Sphagnum* leafs are highly specialized: they form a special tissue of living, chlorophyll-containing chlorocytes and dead, cell content-free hyalocytes, which are responsible for the huge potential to store water. Due to their morphology and ecology, *Sphagnum* plants are

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unique host plants for microorganisms. So far, only the microbial populations involved in CH_4 cycling, that is methanotrophic bacteria (Dedysh *et al*., 1998, 2001; Dedysh, 2002; Morris *et al*., 2002; Raghoebarsing *et al*., 2005; Merila *et al*., 2006) and methanogenic archaea (Horn *et al*., 2003; Sizova *et al*., 2003; Kotsyurbenko *et al*., 2004) have attracted research interest. Studies of Mitchell *et al*. (2003) analyzed the bacterial abundance and activity in water samples collected from aquatic *Sphagnum* habitats, while in other studies the bacterial community at the oxic and anoxic regions of *Sphagnum* peat bogs was investigated (Morales *et al*., 2006). However, the composition of bacterial communities associated with living *Sphagnum* plants remains largely unknown.

Several studies have identified different biotic and abiotic factors influencing the structural and functional diversity of bacterial communities associated with different crop plants (Berg *et al*., 2002, 2006; Kowalchuk *et al*., 2002; Garbeva *et al*., 2004; Costa *et al*., 2006). Evidence has also been presented that the structure of rhizosphere microbial communities is influenced by the plant species, because of differences in root exudation and rhizodeposition (Brimecombe *et al*., 2001; Smalla *et al*., 2001; De Boer *et al*., 2006). Host specificity is also known for the microbial communities living in the phyllosphere (Yang *et al*., 2000). However, Costa *et al*. (2006) showed that the impact of soil type is higher than those of the plant species, as analyzed for different crops at different sites in Germany. Bezemer *et al*. (2006) found interaction between the vegetation and terrestrial microbial communities. Additionally, biogeographical aspects and endemicity of microorganisms (see review in Ramette and Tiedje, 2007) can influence biodiversity of plant-associated bacteria. The extent to which plants are associated with specific bacterial communities in their natural habitats in different geographical regions, especially in the case of bryophytes, is still unclear.

The objective of this work was to analyze the associated bacteria of two different *Sphagnum* species, *Sphagnum magellanicum* and *Sphagnum fallax* (class *Sphagnopsida*, family *Sphagnaceae*), which were isolated from six bog ecosystems at different sites in the Northeast of Germany and Southwest of Norway, and to determine the extent to which the bryophyte species, the diversity of the surrounding plant community and the geographical region influence biodiversity. *S. magellanicum* BRID. (section *Sphagnum*) is typical for strong acidic, oligotrophic and ombrotrophic habitats, whereas *S. fallax* H. KLINGGR. (section *Cuspidata*) grows in weakly acid, more mesotrophic situations influenced by minerotrophic groundwater. Both bryophytes colonize extreme and specific habitats, and they belong to the typical and very important vegetation in peat bogs (Daniels and Eddy, 1985; Nordbakken *et al*., 2003). The selection of species and sampling locations were designed to ensure (i) bryophyte species difference, (ii) ecological difference (oligotrophic and mesotrophic mires) and (iii) geographical difference (Germany/Norway). Molecular tools were used for investigating the biodiversity of the bacterial populations associated with the two *Sphagnum* species. Particular interest was layed on the analysis of members of the genus *Burkholderia* and *Serratia*, which belong to the dominant bacteria associated with *Sphagnum* (Opelt and Berg, 2004; Belova *et al*., 2006).

Materials and methods

Sampling and isolation of the bacterial fraction

Adult gametophytes of both bryophytes *S. magellanicum* and *S. fallax* were sampled in three different natural habitats in the Northeast of Germany and Southwest of Norway (Table 1). The bryophyte samples were collected in August and September in 2004. We have chosen two common European *Sphagnum* species growing in light and wet mires,

Table 1 Sampling locations

Country, province	*Abbreviation of locality*	*District, locality, coordinates*	*Name/habitat*	*Altitude over sea level*
Norway, Hordaland province	N1	Etne district, 8 km east of Etne; 59°39′4″ N, 5°48″ E	Bjørkenes peat bog	30 m
	N2	Etne district, 5 km northeast of Fjæra; 59°43′48″ N, 6°28′36″ E	Sørdalen peat bog	350 m
	N3	Odda district, 10 km northwest Røldal; 59°53′24″ N, 6°38′52″ E	Seljestad peat bog	650 m
Germany, Mecklenburg-Western Pomerania	G1	County of Güstrow, Niegleve; 53°48′30″ N, 12°21′25″ E	Schlichtes Moor	30 m
	G2	County of Nordvorpommern, Sanitz; 54°07′30″ N, 12°25′45″ E	Dänschenburger Moor	40 m
	G3	County of Bad Doberan, Graal Müritz; 54°16′15″ N, 12°17′30″ E	Großes Ribnitzer Moor	1 m

Table 2 Some ecological characters of the habitats

Location[a]	Sphagnum-*species*	*Abbreviation of collecting point*[a]	*Moisture value*[b]		*pH value*[b]		*Nutrient value*[b]	
			Sampling site	*Literature*[c]	*Sampling site*	*Literature*[c]	*Sampling site*	*Literature*[c]
N1		N1SM	7.4		1.3		1.4	
N2		N2SM	8.0		1.4		1.2	
N3	*Sphagnum magellanicum*	N3SM	7.8	7.8	1.7	1.7	1.6	1.5
G1		G1SM	7.7		1.6		1.0	
G2		G2SM	8.0		1.4		1.3	
G3		G3SM	8.1		1.7		1.6	
N1		N1SF	7.4		2.8		2.8	
N2		N2SF	7.5		2.4		2.2	
N3	*Sphagnum fallax*	N3SF	7.9	8.1	3.0	2.6	3.1	2.0
G1		G1SF	7.9		2.6		1.8	
G2		G2SF	7.8		2.3		2.0	
G3		G3SF	8.0		3.0		2.0	

[a]Letters represent the locations and microhabitats: G, Germany; N, Norway; SF, *Sphagnum fallax*; SM, *Sphagnum magellanicum*; Arabic numerals represent the sampling site (1–3).
[b]Average indicator values by Ellenberg *et al.* (1991) calculated on the basis of species lists of every collecting point. The figures indicate ecological gradients (moisture: 1 = extremely dry, 9 = wet; reaction: 1 = extremely acidic, 9 = calcareous; nutrient content: 1 = extremely nutrient poor, 9 = extremely nutrient rich).
[c]Average indicator values by Ellenberg *et al.* (1991) calculated on the basis of 560 vegetation relevés of *S. fallax* communities and 465 vegetation relevés of *S. magellanicum* communities in Northeast Germany and Scandinavia after Timmermann (2001) and Dierßen (1996).

but in different ecological situations (Table 2). The ecological characters of the different habitats were characterized by different abiotic conditions, especially with regard to moisture, soil pH and nutrient content.

From each of the six investigated *Sphagnum* bogs, six (three from *S. magellanicum* and three from *S. fallax*), different composite samples (consisting of five single samples of approximately 20 *Sphagnum* plantlets) were taken and analyzed separately. The living green parts of the gametophytes were placed into sterile petri dishes and transported to the laboratory, and then 5 g were transferred to a sterile stomacher bag. Extraction of the moss-associated bacteria from the gametophytes was carried out as described by Opelt and Berg (2004).

Scanning electron microscopy
Gametophytes were fixed in glutaraldehydephosphate buffer (2%; 0.1 M; pH 7.2) and post fixed in 2% glutaraldehyde buffer. After removal of the fixative by a wash in phosphate buffer, samples were dehydrated, critical point dried and coated with gold before undergoing scanning electron microscopy (Carl Zeiss, Oberkochen, Germany).

Total-community DNA isolation
The total-community DNA of bacterial cell consortia was extracted as described by Martin-Laurent *et al.* (2001). For mechanical lysis, samples were homogenized in a FastPrep Instrument (Qbiogene, BIO101, Carlsbad, CA, USA) for 30 s at speed 5.0 m/sec. The extracted DNA was purified by using the Geneclean Spin kit (Qbiogene) according to the manufacturer's protocol.

Molecular analysis by SSCP
Fingerprinting of the moss-associated bacterial communities by single-strand conformation polymorphism (SSCP) was carried out as described by Schwieger and Tebbe (1998). Briefly, 16S rRNA fragments were amplified by PCR using bacterial DNA isolated from moss tissue as the template, with the eubacterial primer pair Unibac-II-515F/Unibac-II-927rP along with specific primers for *Serratia* (Lieber *et al.*, 2003). For the analysis of the *Burkholderia* sp community a nested PCR was performed. In a first PCR, the eubacterial primer pair Eub1/Eub2 (Lane, 1991) was used in a 20 μl reaction mixture as follows: 95°C for 5 min, followed by 10 cycles of 95°C for 30 s, 52°C for 30 s and 72°C for 1 min 40 s, followed by 20 cycles of 95°C for 30 s, and 72°C for 1 min 30 s + 10 s/cycle with a 72°C final extension step for 5 min before holding at 15°C when the reaction was terminated. The second PCR was performed in a 20 μl reaction mixture using 1 μl of the product from the first PCR and the *Burkholderia*-specific primer pair BKH143Fw (5′-TGGGGGATAGCYCGGCG-3′) and BKH1434Rw (5′-TGCGGTTAGRCTASCYACT-3′) (Schönmann *et al.*, unpublished). The *Burkholderia*-specific primers were designed using the phylogenetic software package ARB with probe design and probe match tools (Ludwig *et al.*, 2004; www.arb-home.de) under Linux Suse 8.2 with the ARB 16S rRNA database (ssu_jan04_full.arb), which was updated with the available sequences found in the hierarchy browser of the ribosomal database project (rdp)

(http://rdp.cme.msu.edu/index.jsp) (Release 9.27, 25. April 2005, 136 355 16S rRNA sequences). Only sequences over 1200 bp length belonging to the path: class *Proteobacteria*/order *Burkholderiales*/family *Burkholderiaceae*/genus *Burkholderia* (May 2004: 0/525/0) out of Bergey's Taxonomy were chosen. Parameters for the *Burkholderia*-specific PCR were as follows: 94°C for 4 min, followed by 40 cycles of 94°C for 40 s, 52°C for 40 s and 72°C for 1 min 30 s, with a 72°C final extension step for 10 min. After the second PCR, we obtained DNA fragments of 1300 bp. The third PCR was performed to obtain smaller fragments for the analysis with the SSCP. For this PCR, the eubacterial primer pair Unibac-II-515F/Unibac-II-927rP (Lieber *et al.*, 2003) was used. For the 60 μl reaction mixture, 6 μl from a 1:50 dilution of the second PCR product were used as the template and we obtained DNA fragments of 412 bp. The amplicons were separated by using the TGGE Maxi system (Biometra, Göttingen, Germany) at 400 V and 26°C for 26 h using 8% (wt/vol) acryl amide gels. Silver staining was used to detect DNA in SSCP gels (Bassam *et al.*, 1991).

Construction of a Burkholderia *clone library*

16S rRNA gene fragments of *Burkholderia* sp were amplified by using the *Burkholderia*-specific forward primer BKH143Fw and the reverse primer BKH1434Rw (Schönmann *et al.*, unpublished). A nested PCR was performed using *EX* Taq polymerase (Takara Biomedicals, Otsu, Shiga, Japan) and the eubacterial primers 616 V and 630R (Brosius *et al.*, 1981) in a first amplification round, followed by a second PCR using the *Burkholderia*-specific primers BKH143Fw and BKH1434Rw. Amplification reactions were set up in a total volume of 50 μl reaction mix containing community DNA in concentrations ranging from 25 to 130 ng/μl. Reaction mixtures contained 25 μM primers, 2 mM $MgCl_2$ (Promega, Madison, WI, USA) and 1.5 U of Ex Taq/Taq polymerase. Thermal cycling was carried out as follows: initial denaturation at 94°C for 4 min, followed by 40 cycles of denaturation at 94°C for 40 s, annealing at 52°C for 40 s and elongation at 72°C for 1 min 30 s. Cycling was completed by a final elongation at 72°C for 10 min. The PCR amplification products were separated on 1.0% agarose gels and the bands with the expected sizes were excised and the DNA was purified using the QIAquick Gel Extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The amplicons were ligated into the cloning vector pCR 2.1 (Invitrogen Corp., Carlsbad, Canada) and transformed into *Escherichia coli*. Cloning, restriction enzyme analysis, and transformation of *E. coli* XL1 blue were performed essentially as described previously (Sambrook *et al.*, 1989). For phylogenetic analyses, 16S rRNA sequences of closely related organisms were collected at the public database rdp (http://rdp.cme.msu.edu). The 16S rRNA moss clone sequences were added to the alignment of about 200 closely related small-subunit rRNA sequences by using the alignment tool of the ARB program package (O Strunk and W Ludwig, http://www.arb-home.de). Alignments were refined by visual inspection. Phylogenetic analyses were performed using neighbor joining.

Computer-assisted cluster analysis and calculation of diversity indices

Computer-assisted evaluation of bacterial community profiles obtained by SSCP was performed by using the GelCompar program (Applied Maths, Kortrijk, Belgium). To compare the community fingerprints, the SSCP gels were scanned transmissively (Epson perfection 4990 Photo, Nagano, Japan) to obtain digitized gel images. The gels were normalized, which made it possible to compare band pattern of different gels, and the background was subtracted (details at the manufacturer manual). The Pearson's correlation index (r) for each pair of lanes within a gel was calculated as a measure of similarity between the community fingerprints. Finally, cluster analysis was performed by applying the unweighted pair group method using average linkages (UPGMA) to the matrix of similarities obtained. To assess differences in the diversity of the various bacterial communities, the diversity index H′ was calculated according to Shannon and Weaver (1999). H′ was calculated according to the equation $H' = -\Sigma_{i=1}^{S} [(ni/N) \ln(ni/N)]$ where ni is the relative abundance of a single taxon (a single SSCP band), N is the total abundance of all taxa, and S is the total number of abundant species. The relative abundance of the SSCP bands was calculated using the GelCompar program. For this, the different peaks of a densitometric curve of each SSCP lane were analyzed. For each SSCP pattern, the maximum peak value was 255 (100%) and only peaks (bands) that showed a value more than 5% were included in the calculation. Statistically significant differences were determined by the Mann–Whitney U-test ($P \leqslant 0.05$).

Habitat characterization by the analysis of the composition of the plant vegetation

In each of the 12 bryophyte collecting points, we recorded all bryophyte and vascular plant species (lichens did not occur, fungi were ignored as well as microscopic plants) in a square of 2×2 meter around the sampling point. Plant abundance was estimated by Braun–Blanquet approach with a conventional scale first published by Reichelt and Wilmans (1973). The collected data were analyzed by the unweighted TWINSPAN ordination analysis (Hill, 1979). TWINSPAN is a common numerical classification method by hierarchical division specifically developed for vegetation data. The program is based on the concept that a group of ecological

similar sample plots will have preferential species. Species and relevés are sorted depending on the maximum likelihood based on a reciprocal averaging ordination, refined by a discriminant function analysis (Hill, 1979). For ecological characterization of the *Sphagnum* habitats, we used indicator values by Ellenberg *et al*. (1991). These are empirical developed ordinal values of environmental variables along ecological gradients of light, temperature, continentality, soil moisture, soil pH, fertility and salinity. They have been widely used and have been tested, improved and verified by measurements for central European vascular plants and bryophytes during a period of many decades (Diekmann, 2003).

For multivariate analysis of the vegetation data, we used the indirect unimodal correspondence analysis (CA) of the software package Canoco 4.5 (Lepš and Šmilauer, 2003). Indicator values are given as supplementary environmental data. Species nomenclature was followed according to Ellenberg *et al*. (1991).

Results

Microscopic observation of surfaces of gametophytes
Impressions of the specific microenvironment were obtained by analyzing gametophytes by electron microscopy (Figure 1). Figures 1a and b show that the surfaces of leaves from *S. fallax* and *S. magellanicum*, respectively, are colonized by bacteria. The *Sphagnum* tissue forms a regular pattern of alternating green (chlorocytes) containing chloroplasts and hyaline cells, which appear to shape a very specialized microenvironment for the bacteria. The hyaline cells with large pores, normally filled with water, are dead at maturity and now serve to provide water. In general, both *Sphagnum* species showed similar surface structures and they appear to form similar microhabitat structures for bacteria.

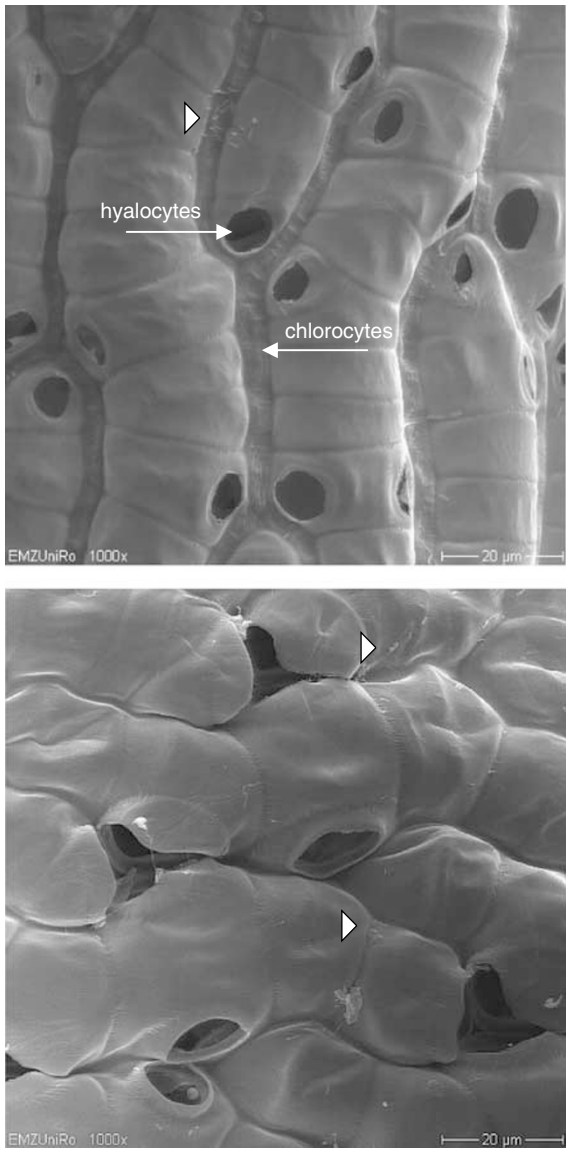


Figure 1 Scanning electron micrographs of the surface of gametophytes of *S. fallax* (**a**) and *S. magellanicum* (**b**). Triangles indicate the microorganisms on the bryophyte surface.

Molecular fingerprinting and diversity of Sphagnum*-associated bacterial communities*
SSCP gels showed that the diversity of 16S rRNA fragments amplified from *Sphagnum* community DNA is high. For both bryophyte species we found between 30 and 50 bands for the total bacterial and the *Burkholderia*-specific community patterns. About 20–30 bands were found for the *Serratia*-specific SSCP patterns. For eubacterial as well as group-specific community patterns, dominant bands were detectable.

To analyze the bryophyte specificity of the bacterial community composition, Pearson's correlation coefficients were compared by UPGMA. The eubacterial community patterns of *S. magellanicum* from different geographical sites formed one cluster and the patterns of *S. fallax* formed another cluster at similarity of 15% (Figure 2). These two clusters indicate that the *Sphagnum*-associated eubacterial communities are specific for the moss species, irrespective of the sampling site. The similarity between the associated bacterial communities of *S. fallax* from German and Norwegian sites was up to 70%, whereas the similarity between the associated bacterial communities of *S. magellanicum* from both sites was up to 65%. In contrast to the high plant specificity, no influence of the geographical site could be detected. With regard to the *Serratia*- and *Burkholderia*-specific SSCP patterns also a high degree of similarity between *S. magellanicum*-associated bacterial communities from different geographical regions was observed (Figures 3 and 4). Likewise, the similarities of *S. fallax*-associated communities of the genera *Serratia* and *Burkholderia* from different sites was very high. The patterns obtained from *S. magellanicum* and

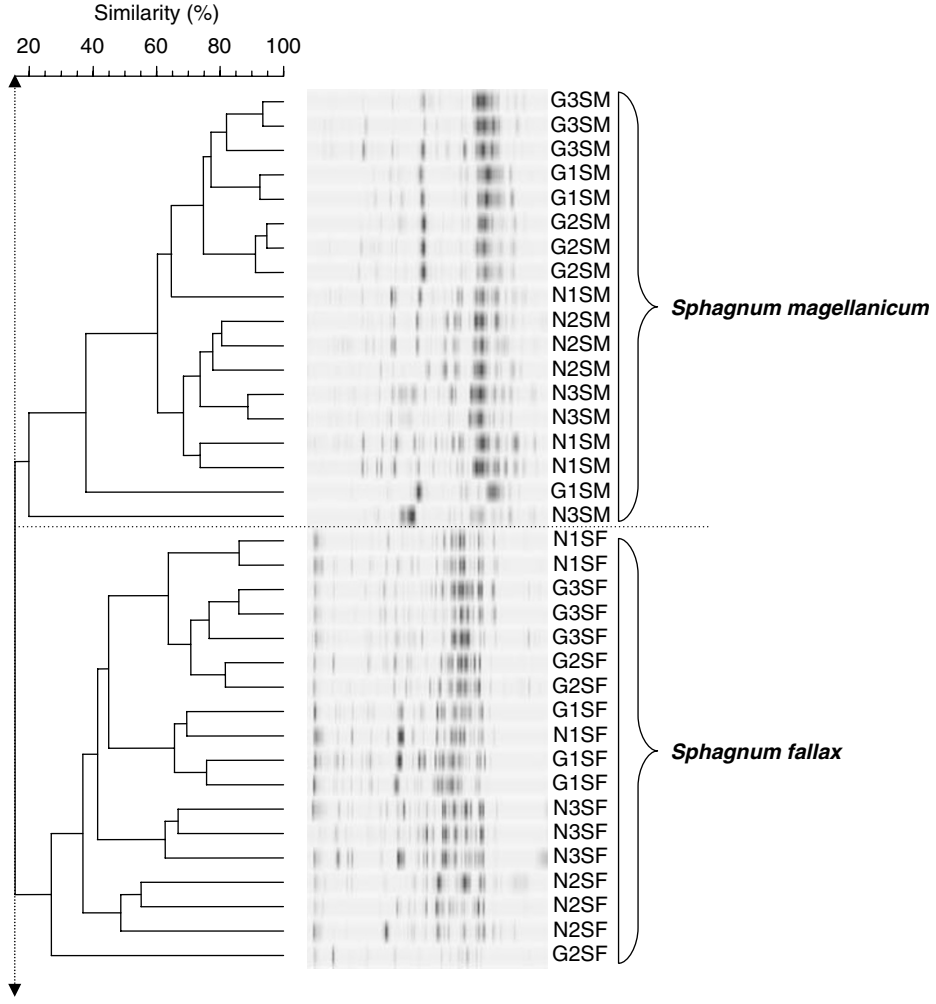


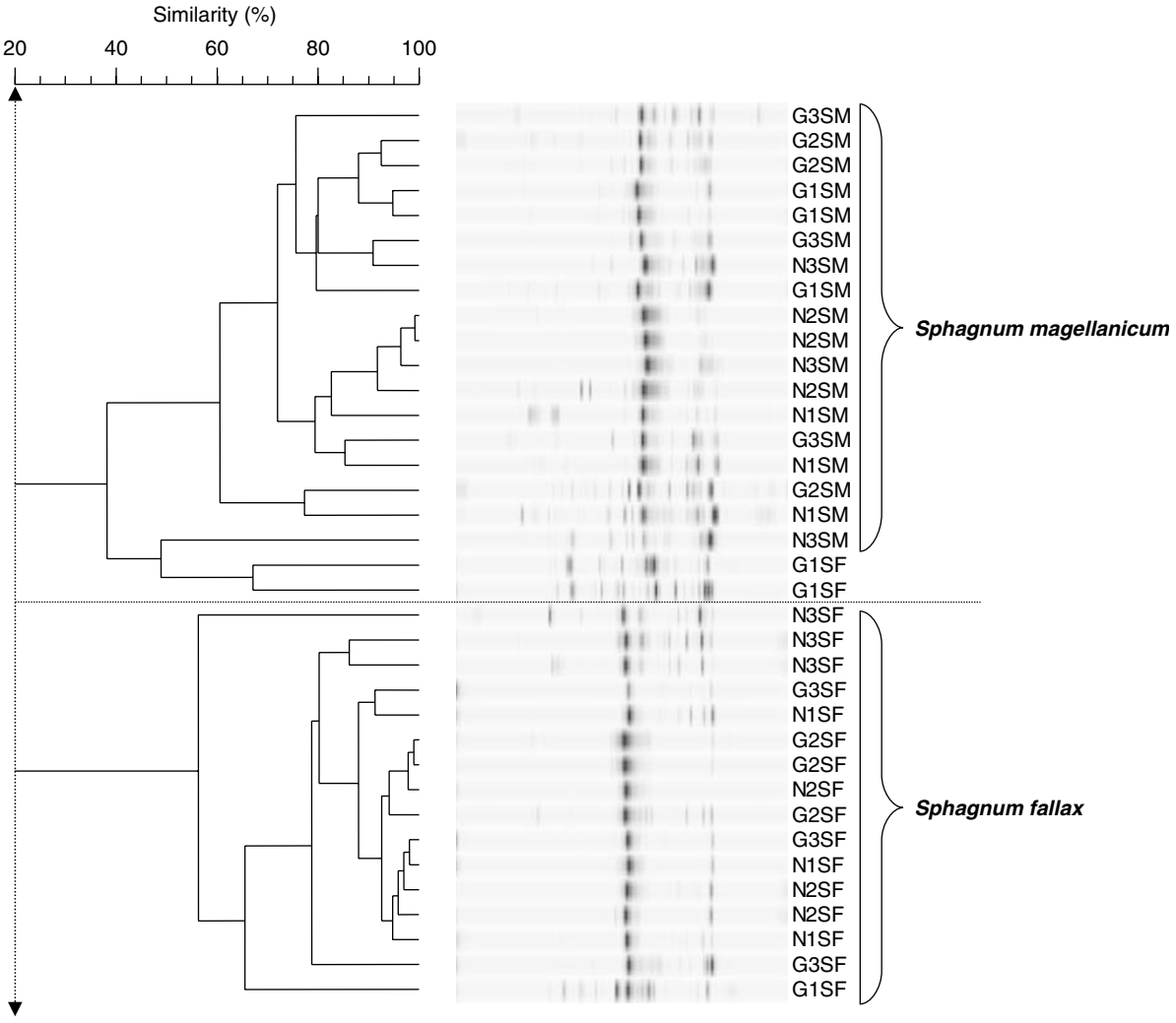
Figure 2 Dendrogram based on amplified 16S rRNA fragments of *S. magellanicum*-associated bacterial communities from different sites in Germany (G1-3SM) and Norway (N1-3SM) and from *S. fallax* from different sites in Germany (G1-3SF) and Norway (N1-3SF) obtained by using eubacterial primers and separated by SSCP. The patterns obtained were grouped by UPGMA. Double-headed vertical arrows indicate the similarity for the groupings. SM = *Sphagnum magellanicum*; SF = *Sphagnum fallax*; SSCP = single-strand conformation polymorphism.

S. fallax, respectively, formed one cluster at a similarity level of 20% for *Serratia* and 24% for *Burkholderia* (Figures 3 and 4). For both bryophytes we found that the *Serratia*- or *Burkholderia*-specific SSCP patterns of one bryophyte and one sampling site is not so often clustered together than for the eubacterial communities. However, for *Serratia*-specific communities of *S. fallax* from different geographical sites, the similarity was up to 95% (Figure 3). The geographical site had a greater effect on the *Burkholderia*-specific SSCP patterns of associated bacterial communities in case of *S. fallax* than *S. magellanicum*. In fact, at a similarity level of 52%, the patterns of associated *Burkholderia*-specific communities of *S. fallax* from the German and Norwegian sites, respectively, formed only one cluster (Figure 4).

The Shannon diversity index H′ was calculated for the bacterial communities from all sampling locations (Table 3). Diversity indices of $H' = 3.24$ and $H' = 3.25$ on average were calculated for the eubacterial communities associated with *S. fallax* in Germany and Norway, whereas the indices for *S. magellanicum* were significantly lower ($H' = 2.69$ and $H' = 2.91$). In contrast to the specificity for both *Sphagnum* species, there was no statistically significant difference between the different geographical sites.

Analysis of a 16S rRNA gene library of moss-associated Burkholderia *species*

The 16S rRNA genes of members of the genus *Burkholderia* associated with the mosses were PCR amplified and cloned as described in Materials and methods. Altogether, 38 clones were sequenced and compared to sequences deposited in GenBank. Sequences were designated as follows: M1 for *S. fallax* (Dänschenburger Moor, Germany), M2 for *S. magellanicum* (Sørdalen peat mire, Norway) and



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M3 for *S. magellanicum* (Seljestad peat mire, Norway). Analysis of the sequence polymorphism of the 16S rRNA genes was used to infer the phylogenetic positions of the sequences (Figure 5). Examination of the phylogram revealed that the sequences formed distinct phylogenetic clusters.

A large group of clones formed a cluster with the novel species *Burkholderia bryophila* (strains 1S18, A5, 1A11 and S512), which was isolated from *Sphagnum rubellum* (Vandamme *et al.*, in press) and *Burkholderia* sp strain Y, which was recently isolated from a *Sphagnum* peat bog in Estonia (Belova *et al.*, 2006). Furthermore, two sequences from *Burkholderia* sp isolates from woodland (WR19X and RW36) and from *Burkholderia* sp SB1, F4W and F4, all of which were isolated in the course of the analysis of the microbial community associated with *Sphagnum* peat bogs by Belova *et al.* (2006), were located within the same cluster. Four sequences (M2-K4, M2-K14, M3-K2 and M3-K6) clustered with the heavy metal resistant *Burkholderia* sp strain DM-Ni1, which was isolated from a moderate acid mine drainage (DQ419958), *Burkholderia phenazinium* and *Burkholderia megapolitana*, which was recently isolated from *Aulacomnium palustre*, grown in the natural reserve 'Ribnitzer Großes Moor' (Mecklenburg-Pommerania, Germany). In conclusion, this entire branch contains mainly strains associated with the peat of acidic *Sphagnum* bogs and acid mine drainage. Another large cluster forms a separate branch that is most closely related to *B. cepacia* (92–98% similarity). Among these sequences, M1-K13 and M2-K81 showed high degrees of similarities (98%) to the 2,4-dichlorophenoxyacetic acid degrading strain *Burkholderia* sp Ff54 and *B. cepacia* 383, an isolate from forest soil in Trinidad (Stanier *et al.*, 1966). A single sequence, M1-K7, showed 92% similarity to *Burkholderia* sp

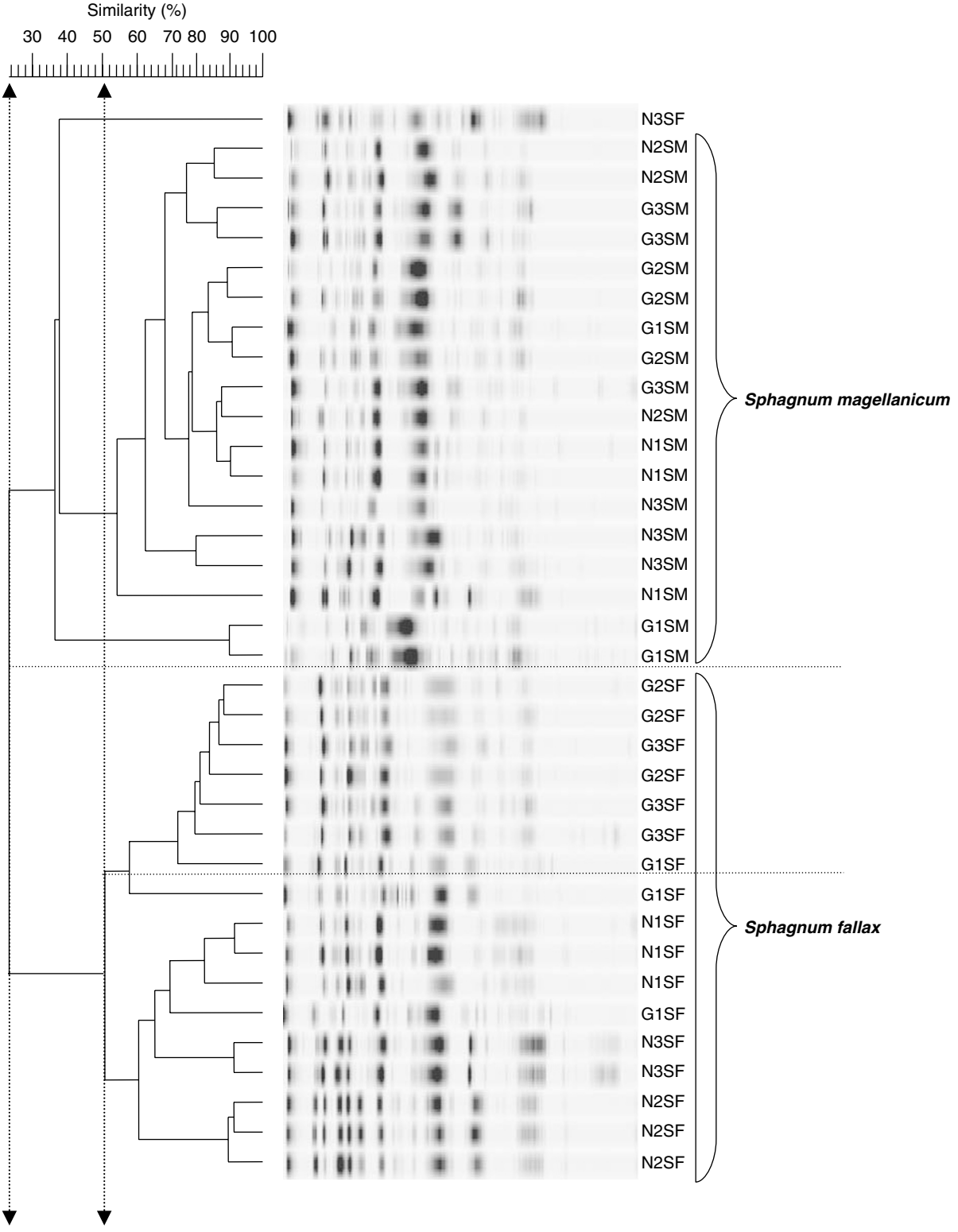

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Table 3 Biodiversity indices for eubacterial SSCP patterns and vascular plant and bryophyte community calculated using the Shannon diversity index

Sample[a]	*Eubacterial communities*				*Vascular plant and bryophyte communities*			
	Shannon diversity index, H′	*s.d.*	*Average content of Shannon diversity index*	*s.d.*	*Shannon diversity index, H′*	*s.d.*	*Average content of Shannon diversity index*	*s.d.*
G1SF	3.49	0.028			1.60	0.119		
G2SF	3.00	0.059	3.24	0.198	0.80	0.137	1.16	0.404
G3SF	3.23	0.135			1.08	0.094		
N1SF	3.29	0.164			1.13	0.120		
N2SF	3.21	0.027	3.25	0.034	0.95	0.063	1.32	0.506
N3SF	3.26	0.036			1.90	0.103		
G1SM	2.88	0.023			1.82	0.108		
G2SM	2.53	0.090	2.69	0.142	1.57	0.119	1.56	0.256
G3SM	2.66	0.182			1.31	0.070		
N1SM	3.11	0.035			1.72	0.096		
N2SM	2.89	0.272	2.91	0.149	2.00	0.089	1.92	0.170
N3SM	2.75	0.137			2.04	0.092		

Abbreviation: s.d., standard deviation.
[a]Letters represent the locations and microhabitats: G, Germany; N, Norway; SF, *Sphagnum fallax*; SM, *Sphagnum magellanicum*; Arabic numerals represent the sampling site (1–3).

strain SB6, which was isolated from Belova *et al*. (2006) in a tundra bog (Vorkuta) and clustered together with *Burkholderia* sp strain DM5 isolated from a moderate acid mine drainage.

Habitat characterization and composition of the different plant communities

The different *Sphagnum* locations in Germany and Norway were selected with respect to abiotic conditions including moisture, soil pH and nutrient content. The occurrence and abundance of bryophyte and vascular plant species in the 12 sampling squares is shown in Table 4. Ten species correlated to *S. magellanicum*, and four species correlated to *S. fallax* were detected. Several bryophyte or vascular plant species were found only once. Our data provide strong evidence that different groups of species prefer the *S. magellanicum* and the *S. fallax* habitats. The occurrences of the different plant species were evaluated by the TWINSPAN analysis. The ordination of the species list by TWINSPAN analysis is provided in Figure 6. Regardless of the composition of the plant communities, all *S. magellanicum* sampling sites and all locations of *S. fallax* showed a high level of similarity, respectively. It is evident that the habitat, as characterized by the specific abiotic conditions, including pH and nutrient availability, has a higher influence for the bryophyte and vascular plant species composition than the geographical distance.

The ecological characteristics of the macrohabitats were determined by the aid of ecological indicator values (Table 2). The results were in good agreement with common geobotanical knowledge about the habitat preferences of both *Sphagnum* species. For better evidence, we further checked synoptic data from 465 vegetation relevés of the typical *S. fallax* community from Northeast Germany (Timmermann, 2001) and 104 relevés from Scandinavia (Dierßen, 1996) as well as 382 vegetation relevés of the *S. magellanicum* community from Northeast Germany and 83 relevés from Scandinavia by the same authors (Table 2). With regard to the different abiotic conditions, the habitats of both *Sphagnum* mosses are highly specific. The habitats of *S. magellanicum* were characterized by an extremely acidic pH value (indicator value 1.3–1.7), and an extremely low nutrient content (indicator nutrient value 1.0–1.6). However, the locations of *S. fallax* showed a higher nutrient content (indicator nutrient value 1.8–2.8) and pH value (indicator value 2.3–3.0) than those of *S. magellanicum* (Table 2). A CA of the sampling sites and selected species revealed statistically significant differences (Figure 7). Indicator values are given as supplementary environmental data. The first and second axes explain 39.2% and 14.8% variability of the data set, respectively. The correlation coefficient of the pH value is 0.9364, and of the nutrient value 0.8492. Interestingly, the same differences between both *Sphagnum* species were found in the Western Alps by measurements in the field too (Miserere *et al*., 2003).

The average species richness was much higher in the *S. magellanicum* habitats (9–19 species, average 11.7) than in *S. fallax* habitats (4–17 species, average 8.0) (Table 4). This was confirmed by Shannon diversity index, which was based on the plant communities of all sampling sites (Table 3). For statistical significance ($\alpha = 0.01$) of these results, 291 relevés of *S. fallax* community and 122 relevés of the *S. magellanicum* community from Northeast

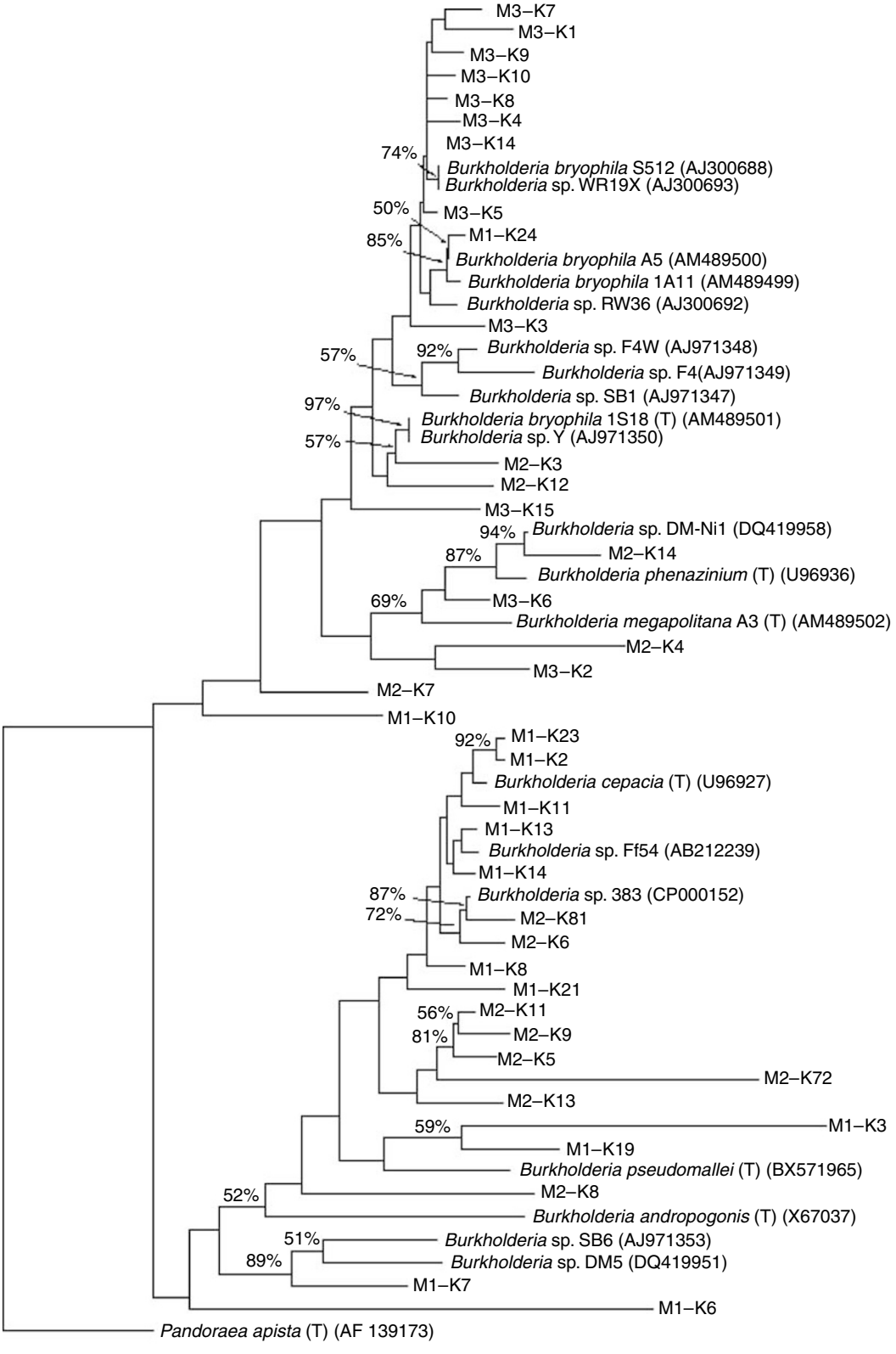

Figure 5 16S rRNA gene-based neighbor joining tree showing the phylogenetic relationship of 38 peat bog clones (M1, M2 and M3) to the nearest phylogenetic neighbors within the genus *Burkholderia*. *Pandorea apista* typestrain (AF139173) was used as outgroup in this analysis. Bootstrap values for 1000 replications superior to 50% are indicated. Type strains are depicted as T. The scale bar represents 0.1 substitution per nucleotide position.

Table 4 Vegetation relevés (bryophyte and vascular plant species) of the 12 sampling squares

Sampling site[a]	*N1SM*	*N2SM*	*N3SM*	*G1SM*	*G2SM*	*G3SM*	*N1SF*	*N2SF*	*N3SF*	*G1SF*	*G2SF*	*G3SF*
Surface area (m^2)	4	4	4	4	4	4	4	4	4	4	4	4
Number of species	9	11	19	11	10	10	5	8	17	8	4	6
Species correlated to *Sphagnum magellanicum*												
Sphagnum magellanicum	2b	3	4	3	4	4	—	—	—	—	—	—
Sphagnum capillifolium	1	2a	1	3	—	—	—	—	—	—	—	—
Sphagnum papillosum	3	2a	3	—	—	—	—	—	—	—	—	—
Calluna vulgaris	2a	—	—	1	2a	—	—	—	—	—	—	—
Drosera rotundifolia	1	—	1	1	2m	1	—	—	—	—	—	—
Eriophorum vaginatum	2a	2a	2a	2a	3	1	—	—	—	2b	—	—
Andromeda polifolia	—	1	1	1	1	+	—	—	—	+	—	—
Erica tetralix	—	2a	—	—	—	2a	—	—	—	—	—	—
Pinus sylvestris	—	—	—	—	+	+	—	—	—	—	—	—
Vaccinium uliginosum	1	—	2a	—	+	—	—	—	+	—	—	—
Polytrichum commune	2m	—	1	—	—	—	—	2a	—	—	—	—
Species correlated to *Sphagnum fallax*												
Sphagnum fallax	—	—	—	—	—	—	5	5	4	4	5	4
Eriophorum angustifolium	—	—	—	—	—	—	—	—	1	2a	1	2b
Carex echinata	—	—	—	—	—	—	2a	1	2a	—	—	—
Molinia caerulea	—	—	—	—	—	2a	3	2a	—	—	3	2a
Betula pubescens	—	—	+	—	—	r	+	r	4	4	—	4
Atlantic or nordic species only occur in Norway												
Carex pauciflora	—	2m	1	—	—	—	—	—	—	—	—	—
Empetrum hermaphroditum	—	—	+	—	—	—	—	—	—	—	—	—
Vaccinium microcarpum	—	1	1	—	—	—	—	—	—	—	—	—
Narthecium ossifragum	1	2b	—	—	—	—	—	—	—	—	—	—
Sphagnum lindbergii	—	—	+	—	—	—	—	—	—	—	—	—
Rubus chamaemorus	—	—	1	—	—	—	—	—	—	—	—	—
Salix glauca	—	—	—	—	—	—	—	—	2a	—	—	—
Cornus suecica	—	—	—	—	—	—	—	—	1	—	—	—
Accompanied species												
Carex nigra	—	—	1	—	—	—	—	+	—	—	—	2m
Aulacomnium palustre	—	—	—	1	1	2m	—	—	—	2a	—	—
Vaccinium oxycoccus	—	—	—	+	+	+	—	—	—	—	—	1
Calliergon stramineum	—	—	—	1	—	—	—	—	1	—	—	—

Species found only once: *Sphagnum majus* 1, *Sphagnum tenellum* 1 (N2SM), *Mylia anomala r*, *Sphagnum flexuosum* +, *Trichophorum cespitosum* 2a, *Carex limosa* 1 (N3SM), *Sphagnum angustifolium* 2a, *Polytrichum strictum* 2a (G1SM), *Sphagnum rubellum* 2b (G2SM), *Sphagnum palustre* 3, *Carex canescens* 2b (G1SF), *Sphagnum cuspidatum* 1 (G2SF), *Juncus effusus* 2b (N1SF), *Juncus squarrosus* +, *Juncus filiformis* 1 (N2SF), *Potentilla palustris r*, *Equisetum sylvaticum* 2a, *Viola palustris* 2a, *Potentilla erecta* +, *Calamagrostis canescens* 2a, *Sphagnum teres* 1, *Sphagnum subsecundum* 1 (N3SF).
Species nomenclature followed Ellenberg *et al.* (1991). Abundance scale after Reichelt and Wilmanns (1973).
[a]Letters represent the locations and microhabitats: G, Germany; N, Norway; SF, *Sphagnum fallax*; SM, *Sphagnum magellanicum*; Arabic numerals represent the sampling site (1–3).

Germany using data by Timmermann (2001) were checked. For an average relevé area of $4\,m^2$, we calculated an average species number of 8.6 for the *S. fallax* community and 12.9 for the *S. magellanicum* community.

Discussion

In this study we were able to demonstrate that the bacterial communities associated with bryophytes of the genus *Sphagnum* show a high degree of host specificity independent of the geographical region, where they occurred: in temperate bogs in Germany or in boreal bogs in Norway. The same was found to be true for the surrounding plant communities, which were highly specific for each *Sphagnum* species independent of the site. On the other hand, biodiversity, which was analyzed for the bacterial as well as plant communities, was different: the *S. fallax*-associated bacterial communities displayed a statistically significant higher degree of diversity than those associated with *S. magellanicum*. In contrast, analyses of plant communities of *Sphagnum*-specific habitats resulted in a higher diversity of *S. magellanicum*-specific habitats for all six sites.

Although an impact of the plant species on their associated bacteria could be clearly shown, other factors such as soil quality, climate and geographical zones may also influence the bacterial community structure (see review in Garbeva *et al.*, 2004; De Boer *et al.*, 2006). Over the past few years, the role of biogeography for prokaryotic diversity was subject

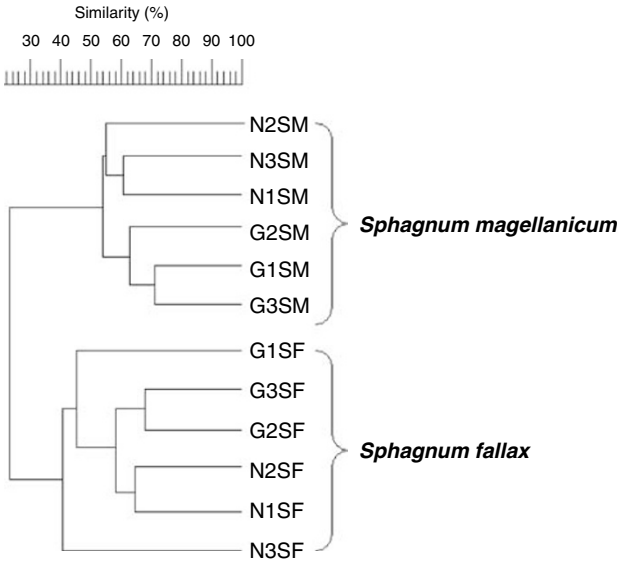

Figure 6 Dendrogram based on the composition of different plant communities analyzed by recording all bryophyte and vascular plant species around the different *S. magellanicum* sampling points in Germany (G1-3SM) and Norway (N1-3SM), and the different *S. fallax* sampling points in Germany (G1-3SF) and Norway (N1-3SF) by TWINSPAN analysis. SM = *Sphagnum magellanicum*; SF = *Sphagnum fallax*.

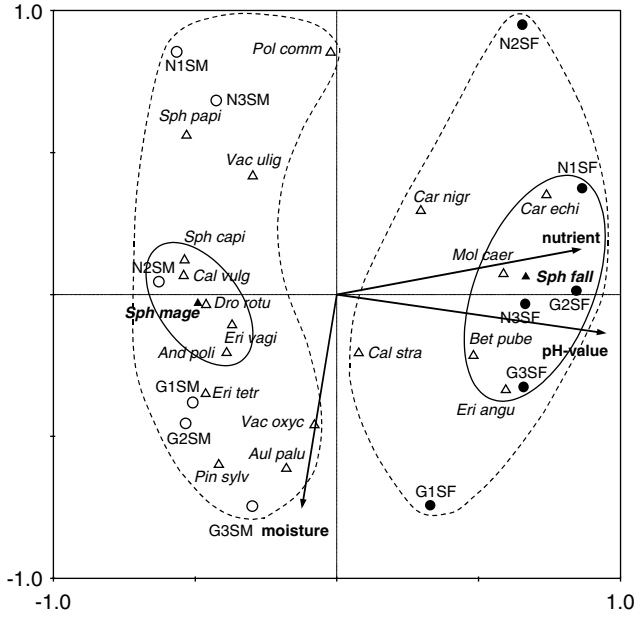

Figure 7 Correspondence analysis biplot (indirect unimodal gradient analysis) of sampling sites and selected species of Table 4. The first and second axes explain 39.2% and 14.8% variability of the data set, respectively. Correlation coefficient of pH value is 0.9364, of nutrient value 0.8492 regarding the first axis. Dashed line indicates the variability of the two communities, and full line the high correlated species. G = Germany, N = Norway, SF = *Sphagnum fallax*, SM = *Sphagnum magellanicum*; Arabic numerals represent the sampling site (1–3). Species names are given in a 3 + 4 abbreviation manner.

to debates (see review in Ramette and Tiedje, 2007). For example, strong endemicity of fluorescent *Pseudomonas* genotypes, which were isolated from soil samples from 10 sites on four continents, was observed, suggesting that these heterotrophic soil bacteria are not globally mixed (Cho and Tiedje, 2000). In this light, the high similarity of *Sphagnum*-associated bacteria independent of the location was unexpected. In fact, we were unable to detect a statistically significant difference between bacterial communities analyzed from Germany and Norway. Furthermore, some of the 16S rRNA gene sequences in our *Burkholderia* clone library showed a very high similarity with sequences of strains obtained from a *Sphagnum* bog in Estonia (Belova *et al.*, 2006). Taken into consideration that, at the genus and species resolution levels, the prokaryotes tend to be cosmopolitan (Ramette and Tiedje, 2007), this result is especially interesting. Intriguingly, *Burkholderia* strains isolated from German and Norwegian *Sphagnum* bogs showed very similar BOX-PCR patterns (Opelt *et al.*, 2007). The long history of the vegetation type can be one reason for specificity. Bog ecosystems belong to the oldest vegetation type of Europe, for example the Dänschenburger Moor investigated in this study (G2) is about 8000 years old (Precker and Krbetschek, 1996). In contrast to most vegetation types, the *Sphagnum* bog vegetation belongs to the azonal vegetation with vast distribution areas. This means that the composition of plant species is similar over large distances, and under the condition of water surplus, the bog vegetation can establish itself in different humid climates. Moreover, there might be a very close interaction between *Sphagnum* mosses and their associated bacteria. Bacteria on and in *Sphagnum* may be important to survive in a harsh environment; they fix nitrogen, solubilize phosphate and show plant growth promoting abilities (unpublished results).

A high microbial diversity and complexity was determined for the bacterial communities in the phyllosphere of both moss species. In a previous study it was also shown that these microbial consortia are highly active (Opelt *et al.*, 2007). This is similar to the so-called rhizosphere effect, which describes the phenomenon that due to the roots exudates, the activity of the microorganisms in the rhizosphere is greatly enhanced relative to those of communities inhabiting other plant microenvironments or bulk soil (Brimecombe *et al.*, 2001). However, *Sphagnum* mosses have no root, no rhizosphere and to transportation system through the plants. They live on peat, which is a substrate made up of partially rotted remains of themselves. However, in their phyllosphere, they are heavily colonized by microorganisms. Like the rhizosphere of higher plants, which is responsible for pathogens defence against soil-borne pathogens (Cook *et al.*, 1995), the phyllosphere of *Sphagnum* plants shows a high antagonistic potential against pathogenic fungi (Opelt *et al.*, 2007).

Interestingly, *Sphagnum* mosses are ectohydric and depend on high atmospheric humidity and wet conditions for growth and survival. They have the

ability to release hydrogen (H^+) ions in exchange for dissolved cations (Andrus, 1986), and have a high capacity for cation exchange, with more cation-binding sites per unit area of cell wall than any other plant (Gignac, 1987). This process alters the pH of *Sphagnum* surroundings and acidifies the *Sphagnum* habitat. The plant also modifies its environment considerably by accumulating peat and hence raising the vegetation surface. *Sphagnum* has furthermore the ability to absorb large amounts of water, usually 10–20 times its dry weight (Andrus, 1986). In conclusion, *Sphagnum* gametophytes can absorb water and dissolved minerals over their surfaces and this may be one reason for bacterial colonization. Also, the specialized leaf structure of content cavernous hyalocytes, as shown in Figure 1, may present an extraordinary habitat for microorganisms. For the ecosystem *Sphagnum* bog high bacterial abundances were also shown in earlier studies (Dedysh, 2002; Mitchell *et al.*, 2003; Morales *et al.*, 2006). Despite of high acidity and an extremely low nutrient content, *Sphagnum* bogs are colonized by large numbers of diverse microorganisms. Previous work suggested that dissolved organic carbon rather than pH affects bacterial abundance (Fisher *et al.*, 1997).

Biodiversity in bacterial as well as plant communities were calculated using Shannon's diversity index (Shannon and Weaver, 1949), an index to measure diversity or entropy in ecosystems. For plants in Germany as well as Norway, the *S. magellanicum*-associated communities show a significant higher diversity than *S. fallax*-associated plant communities. In contrast, for the eubacterial communities associated with *S. fallax*, a higher diversity was observed than for *S. magellanicum*. Again, this could be detected for the German as well as Norwegian sites. In contrast to *S. magellanicum*, *S. fallax* grows in weakly acid, some more mesotrophic situations. Higher content of nutrients and less acidity could explain the higher diversity of bacteria in this microenvironment. Natural habitats below pH 5 are considered extreme acidic environments, which are inhabited by acidophilic microorganisms. Acidophiles have evolved a variety of specialized mechanisms to maintain their internal cellular pH at a constant level (usually 7.2), and therefore they are highly specialized bacteria.

In conclusion, due to their phylogenetical age and their interesting morphology and ecology, *Sphagnum* plantlets are interesting objects to study microbial ecology. Here, we could demonstrate a high specificity and biodiversity for *Sphagnum*-associated communities.

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