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ORIGINAL ARTICLE

Microbial diversity in alpine tundra soils correlates with snow cover dynamics

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The temporal and spatial snow cover dynamics is the primary factor controlling the plant communities' composition and biogeochemical cycles in arctic and alpine tundra. However, the relationships between the distribution of snow and the diversity of soil microbial communities remain largely unexplored. Over a period of 2 years, we monitored soil microbial communities at three sites, including contiguous alpine meadows of late and early snowmelt locations (LSM and ESM, respectively). Bacterial and fungal communities were characterized by using molecular fingerprinting and cloning/sequencing of microbial ribosomal DNA extracted from the soil. Herein, we show that the spatial and temporal distribution of snow strongly correlates with microbial community composition. High seasonal contrast in ESM is associated with marked seasonal shifts for bacterial communities; whereas less contrasted seasons because of long-lasting snowpack in LSM is associated with increased fungal diversity. Finally, our results indicate that, similar to plant communities, microbial communities exhibit important shifts in composition at two extremes of the snow cover gradient. However, winter conditions lead to the convergence of microbial communities independently of snow cover presence. This study provides new insights into the distribution of microbial communities in alpine tundra in relation to snow cover dynamics, and may be helpful in predicting the future of microbial communities and biogeochemical cycles in arctic and alpine tundra in the context of a warmer climate.

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Introduction

Seasonally snow-covered soils account for 20% of the global land surface (Beniston *et al.*, 1996). It is largely assumed that these soils sequester large amounts of organic carbon (Davidson and Janssens, 2006), and that the mineralization of this carbon stock is of increasing concern in a warmer climate (Hobbie *et al.*, 2000; Oechel *et al.*, 2000; Melillo *et al.*, 2002). In arctic and alpine tundra, the duration of snow cover has dramatic impacts on ecosystem structure and functioning (Fisk *et al.*, 1998; Walker, 2000; Welker *et al.*, 2000; Edwards *et al.*, 2007). The high topographic complexity found in alpine tundra triggers strong landscape-scale snow-cover gradients, which in the short term strongly affects local climatic conditions. In the long term, it leads to striking differences in plant cover and ecosystem processes (Billings, 1973; Bowman *et al.*, 1993; Körner, 1999; Choler, 2005). Thus, alpine tundra offers ecologically relevant opportunities to assess the impact of snow on local climatic conditions and ecosystem processes (O'lear and Seastedt, 1994; Litaor *et al.*, 2001; Choler, 2005). Several studies have suggested that many key drivers of soil organic matter mineralization, such as soil temperature, soil moisture, and litter quantity and quality, vary in a conserved manner along snow cover gradients in alpine landscapes (Fisk *et al.*, 1998; Hobbie *et al.*, 2000). Concomitantly, other studies highlighted the seasonal shift of microbial communities and activities in dry alpine tundra (Lipson *et al.*, 1999; Schadt *et al.*, 2003; Lipson and Schmidt, 2004; Schmidt *et al.*, 2007). Given that increasing temperatures will influence the snow cover dynamics in the alpine tundra (Marshall *et al.*, 2008), mineralization processes and associated microbial communities will most likely be affected by these changes as well. However, alpine microbial communities are not well known, and only a few comparative studies of microbial community

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dynamics in relation to snow cover patterns have been reported (Zak and Kling, 2006; Björk *et al.*, 2008).

In this study, our main objective was to test for spatial (that is, plant cover and soil characteristics) and temporal co-variations between soil bacterial and fungal communities, and snow cover dynamics in alpine tundra. We compared two contrasted conditions in alpine tundra, namely early snowmelt (ESM) and late snowmelt (LSM) locations, for 2 years. The phylogenetic structure of bacterial and fungal communities was first assessed using single-strand conformation polymorphism (SSCP) (Stach *et al.*, 2001; Zinger *et al.*, 2007, 2008) and were further characterized by cloning/sequencing. The molecular diversity in microbial communities was examined at four different sampling periods: (i) May, in the presence of late winter snowpack in LSM or immediately after thawing in ESM; (ii) June, corresponding to snowmelt in LSM locations and the greening phase for ESM; (iii) August, when there is a peak of standing biomass; and (iv) October, during litterfall and just before the early snowfalls (Figure 1a).

Materials and methods

Sample collection and soil characterization

The study site was located in the Grand Galibier massif (French southwestern Alps, 45°0.05′N, 06° 0.38′E) on an east-facing slope. Microbial communities were studied in three sites (ESM A: 45° 1′48.47″N 6°13′50.14″W, B: 45°1′52.78″N 6°13′ 26.88″W, C: 45°1′54.35″N 6°13′19.32″W; LSM A: 45°1′48.34″N 6°13′50.20″W, B: 45°1′52.79″N 6°13′ 22.85″W, C: 45°1′53.67″N 6°13′26.73″W) each comprising neighboring LSM and ESM locations. For each site, the locations stand a few meters away (5–10 m) and the sites are separated by 200–500 m. The surface of each location is comprised between 50 and 100 m^2. Plant coverage and soil parameters are same among sites for a given location (ESM or LSM). Five spatial replicates for each plot at each date were collected from the top 10 cm of soil and sieved (2 mm). During the first year of the survey (2005–2006), only site B was sampled on 24 June, 10 August and 10 October 2005, and 3 May 2006. Sites A, B and C were monitored during the second year (2006–2007), and were sampled on 30 July and 2 October 2006, and 18 May 2007 (Figure 1a). Late-winter snow cover consisted of 1–2.5 m depth in LSM locations. Soil organic matter content was determined by loss on ignition (Schulte and Hopkins, 1996) in soil sampled in September. Soil texture was determined using standard methods by the Institute National de la Recherche Agronomique (Laboratoire d'Analyses des Sols, Arras, France). For each spatial replicate ($n=5$), 5 g of soil were mixed in 15 ml of distilled water to determine the pH. Differences of pH ($P<0.05$) between each point were determined by Tukey's test with the R software (The R Development Core Team, 2007).

CE-SSCP analysis of microbial diversity

Three replicates of soil DNA extraction were carried out for each sample with the Power Soil Extraction

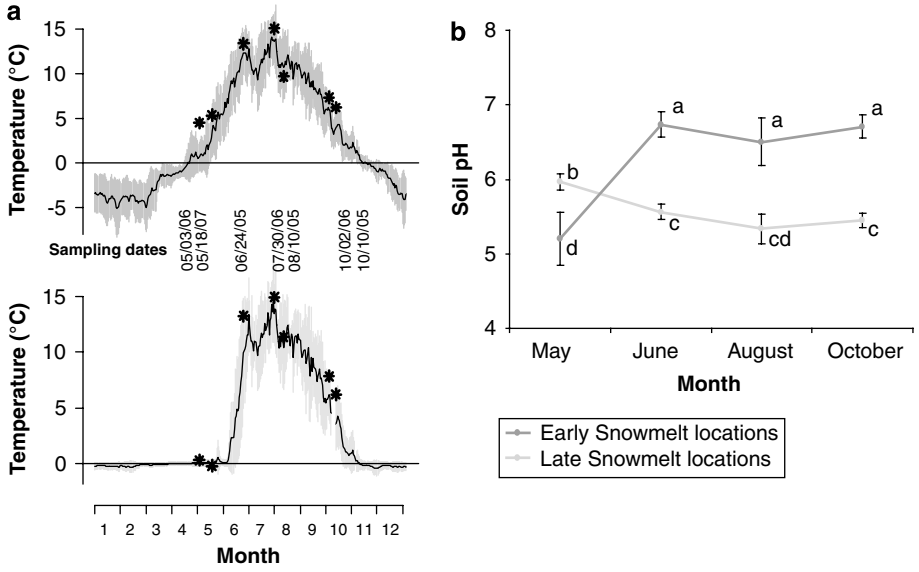

Figure 1 Temperature and pH in ESM (dark gray) and LSM (light gray). (**a**) Yearly course of daily mean (± s.e.) soil temperature at 5 cm belowground. Data are averaged over the period 1999–2007 and were recorded in two or three different sites depending on the year. Snowmelts occurred around 1 May in ESM and 40 days later (mid-June) in LSM. Daily mean soil temperatures corresponding to sampling dates, shown by stars, point out the typical climate conditions that occurred during the survey in both ESM and LSM locations. (**b**) Mean soil pH measured from June 2005 to May 2006 in the site B ($n=5$). Error bars indicate ± s.d. Both studied locations are significantly different throughout the year, and each of them revealed a significant shift of pH in May (indicated by lower-case letters, $P<0.05$).

Kit (MO BIO Laboratories, Ozyme, St Quentin en Yvelines, France) according to the manufacturer's instructions. To limit the effects of soil spatial heterogeneity, 15 DNA extracts obtained from the five spatial replicates per location and date were pooled, rendering one DNA pool per location per date. This sampling and pooling strategy is in accordance with recent reports (Schwarzenbach *et al.*, 2007; Yergeau *et al.*, 2007a, b), and have been validated for fungal communities (June 2005 to May 2006, Zinger, unpublished data). The V3 region of 16S rRNA gene was used as the bacterial-specific marker using the primers W49 (5′-ACGGTCCAGA CTCCTACGGG-3′) and W104-FAM (5′-TTACCG CGGCTGCTGGCAC-3′) (Delbes *et al.*, 1998), whereas the ITS1 region, amplified with the primers ITS5 (5′-GGAAGTAAAAGTCGTAACAACG-3′) and ITS2-FAM (5′-GCTGCGTTCTTCATCGATGC-3′) (White *et al.*, 1990), was used as a fungal marker. PCRs (25 μl) were set up as follows: 2.5 mM of $MgCl_2$, 1 U of AmpliTaq Gold polymerase (Applied Biosystems, Courtaboeuf, France), 1 × of buffer provided by the manufacturer, 20 g l^{-1} of bovine serum albumin, 0.1 mM of each dNTP, 0.2 μM of each primer and 10 ng of DNA template. A 9700 dual 96-well sample block (Applied Biosystems) was used for thermocycling, with an initial denaturation at 95 °C for 10 min, 30 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 15 s and extension at 72 °C for 15 s, and a final elongation at 72 °C for 7 min. The amplicons of each sample were then submitted to CE-SSCP as described earlier (Zinger *et al.*, 2007, 2008). The profiles obtained from CE-SSCP were normalized and compared by constructing dendrograms from Edwards' distance and Neighbor-Joining, with 1000 bootstrap replications. These analyses were carried out with the R software (R Development Core Team, 2007).

Clone library construction and analysis

Clone libraries were constructed for the samples from the site B (2005–2006). Bacteria communities were monitored using the 16S rRNA genes, amplified with 63F (5′-CAGGCCTAACACATGC AAGTC-3′) (Marchesi *et al.*, 1998) and Com2-ph (5′-CCGTCAATTCCTTTGAGTTT-3′) (Schmalenberger *et al.*, 2001). The 28S rRNA genes were amplified for fungal communities with U1 (5′-GTGA AATTGTTGAAAGGGAA-3′) (Sandhu *et al.*, 1995) and with nLSU1221R (5′-CTAGATGAACYAA-CACCTT-3′) (Schadt *et al.*, 2003). PCRs were carried out with 2.5 mM $MgCl_2$, 0.1 mM each ddNTP, 0.4 μM (bacteria) or 0.2 μM (fungi) each primer, 1 U AmpliTaq Gold polymerase, 1 × of buffer provided by the manufacturer, 20 g l^{-1} of bovine serum albumin and 10 ng of DNA of each location pool as a template. PCR was carried out as follows: initial denaturation at 95 °C for 10 min, 25 (bacteria) or 30 (fungi) cycles at 95 °C for 30 s, 54 °C (bacteria) or 53 °C (fungi) for 30 s and 72 °C for 1 min and 30 s, and final elongation at 72 °C for 15 min (bacteria) or 7 min (fungi). Eight independent PCR amplifications were carried out on each sample, pooled and cloned using a TOPO TA PCR 4.1 cloning kit (Invitrogen SARL, Molecular Probes, Cergy Pontoise, France). The titers of ligation were between 25 and 446 c.f.u. ng^{-1} of soil DNA. The transformation and sequencing were carried out at the Centre National de Séquençage (Genoscope, Evry, France). Approximately 350–380 sequences per library were obtained. Clones were identified using Ribosomal Database Project's Classifier (Cole *et al.*, 2003) for bacteria and BLAST (Altschul *et al.*, 1997) for fungi. Bellerophon (Huber *et al.*, 2004) was used to identify chimerical sequences. A multiple alignment for each kingdom was carried out with ClustalW (Chenna *et al.*, 2003) and cleaned by removing nucleotide positions with more than 30% of gaps and sequences smaller than 400 bp. After this cleaning step, 2226 sequences with 499 nucleotide positions for bacteria (GenBank accession nos. FJ568339–FJ570564) and 2559 sequences with 617 nucleotide positions for fungi (GenBank accession nos. FJ568339–FJ570564) were included in the phylotype composition and diversity analysis. We used pairwise distances and complete linkage method to cluster 700 randomly sampled DNA sequences of bacteria or of fungi. Sequences were then pooled according to different similarity thresholds (from 70 to 100%). For each sequence similarity level, we calculated the converse of the Simpson index to estimate the evenness of the profile of operational taxonomic unit (OTU) abundances (Smith and Wilson, 1996). The procedure was repeated 1000 times. All computations were carried out using the R software (The R Development Core Team, 2007).

Results

Characterization of ESM and LSM locations

The temperature of soil from ESM and LSM locations was determined for 7 years. ESM locations are characterized by shallow or inconsistent winter snow cover, leading to long periods of soil freezing (Figure 1a). In contrast, LSM locations exhibit long-lasting, deep and insulating snowpack almost 8 months per year, which leads to a fairly constant winter soil temperature around 0 °C (Figure 1a). In almost all the cases, the soil temperature during sampling was comprised between the usual temperatures for the season (Figure 1a). The contrasting snow cover environments are associated with marked variations in plant communities (Table 1) (Choler, 2005). LSM are dominated by low-stature species, such as *Carex foetida* (Cyperaceae) and *Salix herbacea* (Salicaceae), which must cope with a shorter growing season. Plant cover in ESM locations is more discontinuous and dominated by *Kobresia myosuroides* (Cyperaceae), a stress-tolerant turf graminoid, and *Dryas octopetala* (Rosaceae), a dwarf shrub. The upper soil layer in ESM locations

Table 1 Characteristics of LSM and ESM locations

	LSM situation	*ESM situation*
Plant cover		
Dominant species	*Carex foetida All.* *Alchemilla pentaphyllea L.* *Salix herbacea L.* *Alopecurus alpinus Vill.*	*Kobresia myosuroides* *Dryas octopetala* *Carex curvula All. subsp. rosae* *Gilomen*
Soil characteristics		
Soil classification	Stagnogley enriched in clay	Alpine Ranker
% Organic matter (top soil 10 cm)	8.7 ± 2.5	15.7 ± 4.7
Grain size analysis		
Clay (<2 μm)	34.6 ± 2.6	9.7 ± 0.5
Silt (2–50 μm)	59.0 ± 3.5	41.4 ± 1.0
Sand (50–2000 μm)	6.5 ± 1.7	48.6 ± 1.2

Abbreviations: ESM, early snowmelt; LSM, late snowmelt.
Values presented here are mean ± s.d.

has a higher soil organic matter content than that in LSM locations, but the carbon stock is lower due to shallower soils (Table 1). Soil pH is stable and higher in ESM throughout the year, except in winter when ESM soils become more acidic than LSM soils (Figure 1b).

Effects of snow cover patterns on temporal microbial community structure revealed by molecular profiling

The microbial communities were monitored from August 2006 to May 2007 at three sites, each including ESM and LSM locations. The structure of the microbial communities was assessed using capillary electrophoresis-based SSCP (CE-SSCP) by amplifying the V3 region of *ssu* gene using PCR for bacteria and the ITS1 (internal-transcribed spacer 1) for fungi. Distance trees based on the SSCP profiles revealed a significant difference within bacterial and fungal communities between ESM and LSM. This pronounced difference was noticed for all study sites and sampling dates (Figure 2a). The similar pattern for the three sampling sites indicates

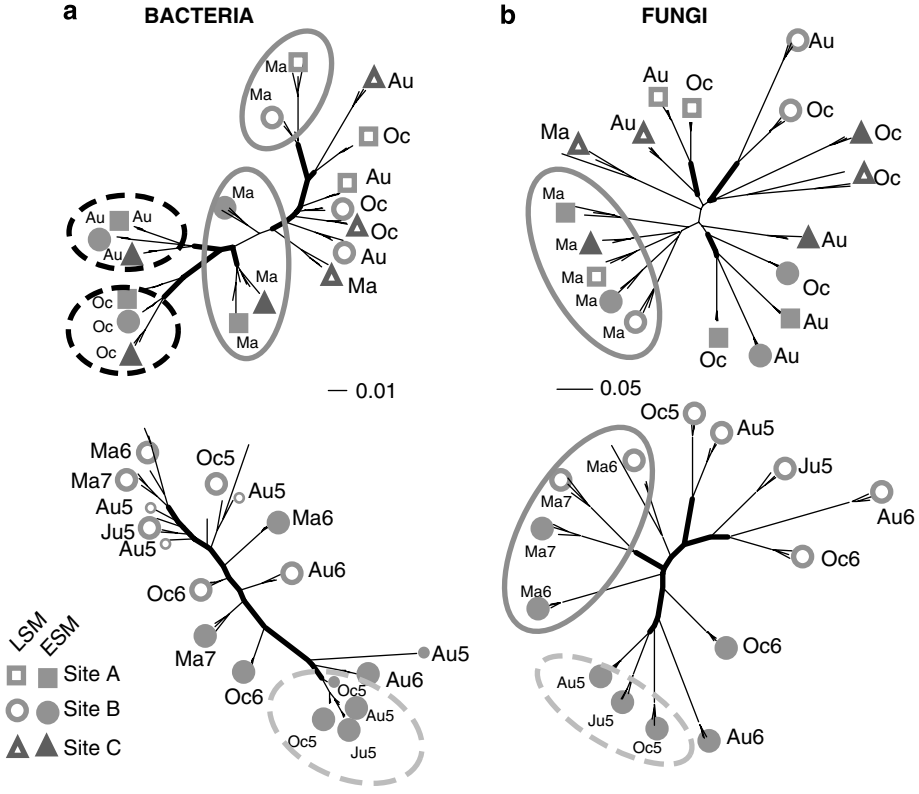

Figure 2 Seasonal variations of bacterial and fungal communities assessed by CE-SSCP. The molecular profile of fungal and bacterial communities was obtained as described in Material and methods, using one DNA pool per each location/site/date. PCRs were carried out by triplicate to limit the influence of PCR biases. Clustering of molecular profiles: (**a**) between three sites from August 2006 to May 2007, (**b**) in the site B during 2 years from June 2005 to May 2007. The ESM locations are in filled symbols and LSM in open symbols, squares indicate Site A, circles Site B, diamond Site C; June, Ju; August; Au; October, Oc; and May, Ma; 2005, 5; 2006, 6; and 2007, 7. Small symbols indicate samples grouping at atypical positions. Molecular fingerprints were compared by computing bootstrapped dendrograms. Thick lines indicate branches supported by a bootstrap value >500. The ovals show the relevant groupings: thick dark-gray lines: May samples post-winter convergence; dark-grey-dashed lines: monthly grouping of ESM sites; light-grey dashed lines: yearly grouping in ESM.

that the observed differences are not because of local conditions but is rather inherent to each location. During the growing season, ESM bacterial fingerprints from the three study sites were consistently grouped according to sampling dates. In contrast, the only grouping for LSM bacteria was found in the May samples. Fungal communities at each location did not display identical seasonal variation for all studied sites. Interestingly, the least distance between microbial communities indigenous to ESM and LSM was observed in May. Although this convergence was strong for fungi, it was less pronounced for bacteria. The same results were found for the three sites, indicating that the shift of microbial communities in May is a general feature of these two habitats. Microbial communities were also followed over 2 years (from June 2005 to May 2007) at one site, always including both studied locations. Similar to data presented in Figure 2a, microbial communities were strongly different between the two locations (Figure 2b). During the growing season, however, microbial communities were not clustered by season, but by year with the exception of May. The aforementioned convergence of microbial communities in May was thus also found to be consistent over 2 years (Figure 2b).

Temporal fluctuations of microbial phylotype composition along the snow cover gradient

Clone libraries were constructed from samples of one site from June 2005 to May 2006. These libraries comprised of small subunit ribosomal DNA (for bacteria), and large subunit ribosomal DNA (for fungi), and consisted in ~350 sequences/library. These sequences were taxonomically assigned using Ribosomal Database Project's Classifier (Cole *et al.*, 2003) for bacteria and BLAST (Basic Local Alignment Search Tool) (Altschul *et al.*, 1997) for fungi. As illustrated in Figure 3, ESM bacterial clone libraries were dominated by the phyla *Acidobacteria* (22 ± 11%), *Actinobacteria* (18 ± 3%) and *Alphaproteobacteria* (19 ± 4%). In contrast, LSM bacterial sequences were by far dominated by *Acidobacteria* (42 ± 3%) throughout the year, whereas *Actinobacteria* (6% ± 4) were less abundant. For fungi, ESM communities were dominated by *Agaricomycotina* (41 ± 14%), whereas LSM fungal communities

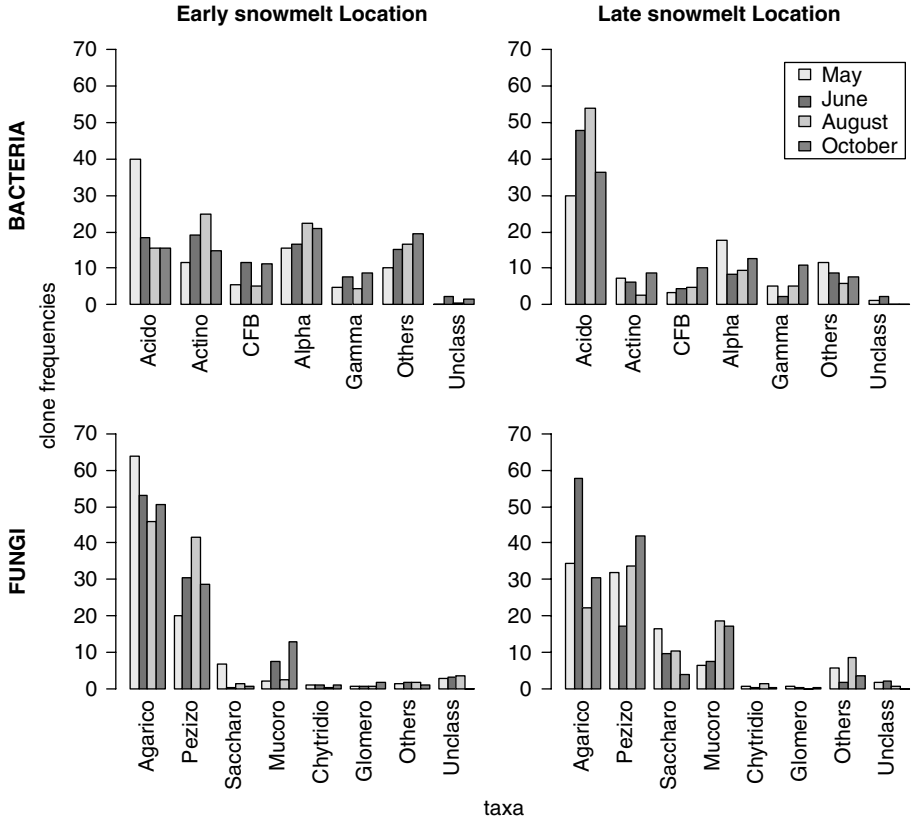

Figure 3 Frequencies of major microbial groups in clone libraries from LSM and ESM. Samples were collected in June, August, October 2005 and May 2006. These libraries consisted of ~350 clones of bacterial 16S rRNA gene or of fungal 28S rRNA gene per sample. For bacteria: Acido, *Acidobacteria*; Actino, *Actinobacteria*; CFB, the *Cytophaga-Flavobacterium* lineage of the *Bacteroidetes*; Alpha and Gamma, α and γ subgroups of *Proteobacteria*; Others: other minor bacterial divisions. For fungi: Basidiomycota are mainly represented by Agarico, *Agaricomycotina*. Ascomycota: Pezizo, *Pezizomycotina*; Saccharo, *Saccharomycotina*. Zygomycota are represented by Mucoro, *Mucoromycotina*; Glomero, *Glomeromycotina*; Chytridio, *Chytridiomycotina*. Others: other minor fungal groups. For the whole figure, Unclass represents unclassified sequences.

appeared more diversified and characterized by the presence of *Saccharomycotina* and *Mucoromycotina*. Furthermore, several seasonal fluctuations in phylotype abundance were observed. For example, *Agaricomycotina* in the LSM location exhibited a sharp increase in June. *Pezizomycotina* was in lower abundance in May in ESM location and in June in LSM location. In October, we noticed an increase of the *Cytophaga-Flavobacterium* lineage of the *Bacteroidetes* (CFB), *Gammaproteobacteria* and *Mucoromycotina* in both locations. In May, we found a noticeable burst of *Acidobacteria* and *Saccharomycotina* and of other minor groups (data not shown) in ESM location that reached the same proportions as those in LSM location.

Temporal and spatial behavior of microbial diversity
The diversity index of microbial communities estimated from clone libraries revealed that bacterial diversity was not different between ESM and LSM locations (Figure 4). Nevertheless, the diversity of ESM bacterial communities was higher in June. In contrast, LSM bacterial diversity was found to be stable throughout all seasons. The diversity of bacterial communities strongly decreased in May, whatever the location. Interestingly, the diversity of fungal communities was noticeably higher in LSM location, particularly in August. In parallel, ESM fungal diversity was found slightly enhanced during June and August. Seasonal variations in fungal and bacterial diversities were thus found to be different between the two studied locations.

Discussion

Alpine tundra is strongly heterogeneous because of the fine-scale variations in topography that lead to differential snow cover. Consequently, plant cover and soil quality in such meadows are highly variable (O'lear and Seastedt, 1994; Litaor *et al.*, 2001; Choler, 2005). Our study locations reflected these variations, having noticeable shifts of snow accumulation (Figure 1a), plant cover, soil organic matter content, soil pH and texture (Table 1, Figure 1b). The results obtained from this study confirmed these trends at the microbiological level. Indeed, the comparison of SSCP profiles revealed pronounced differences between bacterial and fungal communities from ESM and LSM locations, independent of season (Figure 2). These differences were consistent for all study sites (Figure 2a) and throughout the 2-year monitoring (Figure 2b). Thus, these results show a clear distinction between microbial profiles over short distances (5–10 m) at the two extremes of the snow cover gradient, which clearly mirror the surrounding edaphoclimatic conditions as well as the variation in the overlying plant community composition (Table 1, Figure 1).

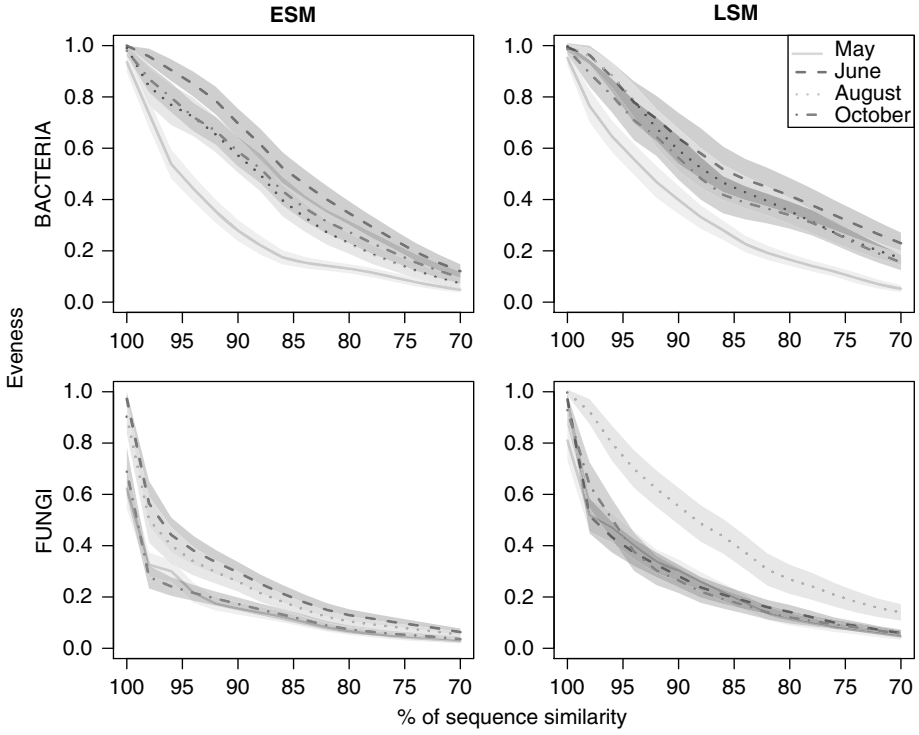

Figure 4 Variations in molecular evenness of bacterial and fungal communities in ESM and LSM, according to the similarity level between sequences. Evenness was estimated from the sequence distance matrix, with 700 resampling by calculating the inverse of the Simpson index and weighted by the library sizes. Each color corresponds to one date sampling and light-coloured areas represent s.d. values. A full colour version of this figure is available at *The ISME Journal* online.

We found that ESM bacterial communities were correlated with growing season progress (Figure 2a). Earlier work indicated that plant cover has a two-fold effect on microbial communities of Antarctic soils. First, when compared with bare soils, the plant cover increases bacterial diversity (Yergeau *et al.*, 2007b). Second, the structure of microbial communities varies with plant cover (Yergeau *et al.*, 2007a). Indeed, root development modifies soil structure, root turnover and litterfall influence carbon input in soil. Moreover, root exudates are composed of various C-containing components (Bais *et al.*, 2006), the quality and quantity of which have been reported to be temporally variable (Farrar *et al.*, 2003). Seasonal variations in bacterial communities may be thus linked to plant development and concomitant rhizodeposition, as shown in greenhouse plants (Butler *et al.*, 2003; Mougel *et al.*, 2006). The bacterial communities of ESM locations seem to be synchronized, probably by the slow growing status of *K. myosuroides* and *D. octopetala*. In contrast, LSM bacteria profiles obtained from August and October samples were separated by small distances (Figure 2a), suggesting the presence of the same phylogenetic groups during the growing season. Conversely, fungal communities either in ESM or in LSM did not show identical seasonal variation whatever the site (Figure 2a), suggesting that the temporal dynamics of these organisms are more sensitive to local, site-specific conditions.

Interestingly, the fewest differences between LSM and ESM microbial communities were always observed in late winter (Figure 2), although this convergence was less pronounced for bacteria. Although seasonal changes in microbial succession have already been described (Schadt *et al.*, 2003; Lipson and Schmidt, 2004; Schmidt *et al.*, 2007; Björk *et al.*, 2008), the convergence of late-winter microbial communities from two contrasting conditions has never been reported. This result suggests that partially identical phylogenetic groups are dominant in both locations at the end of winter. The analysis of the microbial phylotypes shed some light on the basis of this convergence (Figure 3), especially for bacteria. Actually, in late winter, the dominant bacterial phyla in both ESM and LSM are *Acidobacteria* and *Alphaproteobacteria*. Although this convergence was concomitant with soil pH variations and cold temperatures, our data do not allow us to determine what factors are responsible for this winter effect.

These data shown in Figure 2b also provide insights regarding the inter-annual succession of microbial communities. Within each location, microbial communities tended not to be clustered by season, but instead by year, with the exception of winter. The source of this inter-annual variability suggests the existence of a bank of microbial strains in soil represented by only a few individuals (the 'rare biosphere'), similar to the situation that occurs in seawater (Sogin *et al.*, 2006). These yearly changes may arise through the recruitment of functional equivalent strains into the rare biosphere.

The spatial and temporal behavior of microbial communities was further confirmed by DNA sequencing from site B samples (Figure 3), which shed light on ESM and LSM functioning. Bacterial communities in ESM location were dominated throughout the year by the phyla *Acidobacteria* and *Actinobacteria*, which are known for their capacity to degrade recalcitrant substrates (Crawford, 1978; Falcon *et al.*, 1995), as well as by *Alphaproteobacteria* that are often found in rhizosphere (Fierer *et al.*, 2007). In contrast, LSM bacterial communities were by far dominated by *Acidobacteria* throughout the year. This phylum has been found to be well represented in low pH soils (Sait *et al.*, 2006), which may explain their dominance in fairly acidic soils such as in LSM (Figure 1b). Fungal communities in ESM location were dominated by *Agaricomycotina*, with numerous sequences belonging to the genera *Inocybe* and *Russula* (data not shown) (these were earlier reported as *D. octopetala* ectomycorrhiza (Gardes and Dahlberg, 1996)). In contrast, LSM fungal communities appeared more diversified. Thus, in ESM locations, the dominance of symbiotic associations with plants and bacterial species capable of degrading recalcitrant organic matter correlates well with the low fertility observed in ESM locations (Chapman *et al.*, 2006). Environmental conditions seem to promote fungal diversity in LSM location and in *Acidobacteria*. This is possibly caused by higher resource availability (Waldrop *et al.*, 2006) and soil pH (Table 1) for *Acidobacteria* (Lauber *et al.*, 2008).

The phylotype composition of microbial communities was also found to be variable throughout the year, confirming our earlier results (Figure 3). Several microbial groups were indeed found to be linked to growing season (*Alphaproteobacteria*), whereas others, earlier described for their ability to degrade recalcitrant substrates (Cottrell and Kirchman, 2000), emerged during litterfall in ESM as well as in LSM locations (*Gammaproteobacteria*). Late-winter communities were also composed of similar microbial groups.

To further characterize the impact of snow cover patterns on microbes, we estimated bacterial and fungal diversities from clone libraries (Figure 4). Interestingly, bacterial diversity was not influenced by the topographical location. Although bacterial diversity has been described as being strongly influenced by soil pH (Fierer and Jackson, 2006), the range of pH in the studied soils is too small to observe this effect. Bacterial diversity was also found slightly variable across seasons. Nevertheless, ESM bacterial communities were more diversified during the greening phase in June, indicating that plant development promotes bacterial diversity in

such meadows. In agreement with our earlier results, LSM bacterial diversity was stable because of the constant dominance of *Acidobacteria*, implying that this phylum displayed a constant diversity across seasons. However, bacterial diversity dramatically decreased in both locations at the end of winter, suggesting that few bacterial strains are cold tolerant. In contrast, location had a strong impact on the diversity of fungal communities. Fungal diversity was noticeably enhanced in LSM location, particularly at the peak of plant biomass in August. This pattern may be explained by an increase in the nutrient availability due to (i) high root turn over of fast growing plants that are dominant in LSM locations, and (ii) the advanced state of mineralization processes at the end of winter (Bardgett *et al.*, 2005). In contrast, ESM fungal diversity, dominated by ectomycorrhizal fungi, was found lower. Indeed, the ability of this fungal group to switch between saprobic and symbiotic lifestyles (Read and Perez-Moreno, 2003; Martin *et al.*, 2008) may thus allow them to be represented throughout time, independent of resource availability. However, this diversity was enhanced during growing season, possibly in relation to increased root exudation (Bais *et al.*, 2006). These findings highlight different responses between fungal and bacterial diversity along the snow cover gradient.

Conclusion

This paper provides evidence that snow cover dynamics and microbial community composition are strongly interrelated in alpine tundra. Alpine tundra exhibit mosaic of plant communities in relation to fine-scale topographical variations (Körner, 1999). Here, we showed that this strong heterogeneity also occurs at the microbial level. However, further larger-scale surveys are needed to extend this conclusion to other alpine habitats. Moreover, we observed seasonal variations in microbial phylotype composition at each location at the phylum or at sub-phylum levels. This seasonal pattern confirms earlier findings (Schadt *et al.*, 2003; Lipson and Schmidt, 2004; Björk *et al.*, 2008). In contrast to these studies, however, we found that the spatial variations are stronger than the seasonal variations.

This study also reveals that bacterial communities are particularly structured in ESM locations, which show high amplitude of seasonality and limited nutrient availability. In contrast, fungal communities are more stimulated in LSM locations that display weak seasonality and higher nutrient availability. The difference in the response between bacteria and fungi supports the earlier observations of Lauber *et al.* (2008) and may result from their morphological and physiological characteristics, which may be more or less favorable in a given environment (for example, unique cell vs mycelium, enzymatic capabilities, and so on), or from positive or negative interactions between these organisms (Johansson *et al.*, 2004; de Boer *et al.*, 2005; Mille-Lindblom *et al.*, 2006). Moreover, the synchronizing effect of winter can be similar to other extreme events (for example, drought, water logging, and so on). These results may thus be useful to predict microbial successions in the framework of longer time scale studies with varied seasonal or anthropogenic stresses; however, further works are needed to understand the impact of such selective events on ecosystem functioning.

These outcomes also call for a more thorough consideration of snow cover gradients in any attempt to model the carbon cycle of alpine tundra in the context of global change. Considering our results, the change of snow cover dynamics in alpine tundra will have profound impacts on microbial communities. For example, a reduction in snowpack could result in a loss of fungal diversity, as we observed between LSM and ESM locations. Microbes are largely implied in driving large-scale biogeochemical processes. Thus, the question can be raised about the functional importance of the biogeochemical cycles of spatial and seasonal variations of alpine microbial communities, especially under climate change.

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