



**HAL**  
open science

# Alpine soil bacterial community and environmental filters

Bahar Shahnava

► **To cite this version:**

Bahar Shahnava. Alpine soil bacterial community and environmental filters. Other [q-bio.OT]. Université Joseph-Fourier - Grenoble I, 2009. English. NNT: . tel-00515414

**HAL Id: tel-00515414**

**<https://theses.hal.science/tel-00515414>**

Submitted on 6 Sep 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# THÈSE

Pour l'obtention du titre de  
l'Université Joseph-Fourier - Grenoble 1

École Doctorale : Chimie et Sciences du Vivant  
Spécialité : Biodiversité, Écologie, Environnement

## Communautés bactériennes de sols alpins et filtres environnementaux

Par

**Bahar SHAHNAVAZ**

Soutenue devant jury le 25 Septembre 2009



Composition du jury

Dr. Thierry HEULIN  
Dr. Christian JEANTHON  
Dr. Sylvie NAZARET  
Dr. Jean MARTIN  
Dr. Yves JOUANNEAU  
Dr. Roberto GEREMIA

Rapporteur  
Rapporteur  
Examineur  
Examineur  
Président du jury  
Directeur de thèse

Thèse préparée au sien du Laboratoire d'Ecologie Alpine (LECA, UMR UJF- CNRS 5553)



## **THÈSE**

Pour l'obtention du titre de  
**Docteur de l'Université de Grenoble**

**École Doctorale : Chimie et Sciences du Vivant**  
**Spécialité : Biodiversité, Écologie, Environnement**

# **Communautés bactériennes de sols alpins et filtres environnementaux**

**Bahar SHAHNAVAZ**

Directeur :

**Roberto GEREMIA**

Soutenue devant jury le 25 Septembre 2009

Composition du jury

Dr. Thierry HEULIN  
Dr. Christian JEANTHON  
Dr. Sylvie NAZARET  
Dr. Jean Manuel MARTINS  
Dr. Yves JOUANNEAU  
Dr. Roberto GEREMIA

Rapporteur  
Rapporteur  
Examineur  
Examineur  
Président du jury  
Directeur



# Remerciements

Je voudrais remercier les membres du jury, Messieurs et Mesdames Thierry Heulin, Christian Jeanthoneulin, Jean Manuel Martins, Sylvie Nazaret et Yves Jouanneau pour avoir accepté de juger mon travail de thèse.

Cette thèse n'aurait pas vu le jour sans la confiance et les conseils avisés de mon directeur de recherche, Roberto Geremia, Je voudrais le remercier pour cela ainsi que pour son soutien tout au long de ces années.

Ma reconnaissance s'adresse aussi à Philippe Choler d'avoir toujours été disponible à répondre à mes questions. C'était grâce à lui que j'ai fait la connaissance avec l'écosystème alpin.

Je remercie également mes collègues Armelle, Sylvie et Tarafa qui m'ont encouragée à continuer et finir cette recherche. Je n'oublierai jamais les beaux jours que j'ai passés à leurs côtés.

Je tiens également à remercier très chaleureusement à Lucie pour ses remarques pertinentes.

Je remercie tous les professeurs, chercheurs et post-docs particulièrement Luicle Sage, Sebastien Lavergne, Jérôme Gury, Patrick Ravanel, Pierre Taberlet, Irène Till-Bottraud, Christiane Gallet, David Lejon et Bello pour leurs discussions scientifiques qui m'ont guidé au cours de mon travail, et toujours dans une ambiance bien sympathique.

Je tiens à remercier tous mes collègues du LECA. C'est avec joie que j'ai partagé ces quatre années avec eux. Que tous mes collègues, Florence, Annelaure, Rodolph, Angélique, Mathieu, Ozgur, Asam, Claire, Carole, Christian, Margot, Alice et Olivier. LECA me manquera.

Merci à toutes l'équipes TDE/PEX et GPB. Cette thèse est effectuée en collaboration avec trois.

Je remercie à mes chers amis iraniens particulièrement Shokooh, Shadi et Maryam pour tous les fantastiques moments passés ensemble et de m'avoir encouragé à continuer mon chemin. Je vais également remercier à deux mes amies iranien au LECA Hamid et saeid pour leurs qualités humaines extraordinaires.

Je ne saurais jamais exprimer ma reconnaissance à mon époux Mohsen, qui m'a accompagné pendant les douze ans de notre vie commune. Son amour et son aides me encourage tout ces années. Je remercie ma chère fille, Negar qui a pu supporter notre séparation et m'a exprimé ses chagrins avec ses dessins.

Mes derniers remerciements, mais pas les moindres vont à mes parents qui ont été mes meilleurs enseignants, et auxquels je dois tout ce que j'ai actuellement.

## Sommaire

<b>CHAPITRE 1 : Introduction</b>	<b>9</b>
<i>Background and objective of the thesis</i>	11
<i>Thesis content and structure</i>	13
<i>The challenge of environmental microbiology</i>	14
<i>Microbial ecology history</i>	14
<i>Bacteria</i>	14
<i>Bacterial genetic diversity</i>	14
<i>Bacterial roles in nature</i>	15
<b>Bacterial Diversity</b>	<b>16</b>
<i>Importance of bacterial diversity in ecosystem</i>	16
<i>Factors important on bacterial diversity</i>	17
<i>Abiotic factors</i>	17
<i>Biotic factors</i>	17
<i>Measurement of bacterial diversity</i>	18
<i>Measurements of taxonomic distinctness</i>	19
<i>The species equal to OUT</i>	21
<i>How to assess microbial diversity?</i>	21
<i>Traditional methods</i>	21
<i>Culture-independent method</i>	22
<b>Importance of soil</b>	<b>29</b>
<i>The spatial distribution patterns of micro-organisms</i>	29
<i>The temporal distribution patterns of micro-organisms</i>	30
<b>Alpine ecosystem</b>	<b>32</b>
<i>Definition</i>	32
<i>Heterogeny of the alpine stage</i>	32
<i>The history of bacterial characterization in artico-alpine</i>	34
<i>Phenotypic characterization and adaptation to cold condition</i>	34
<i>Bacterial diversity in cold environment</i>	35
<i>Bacterial diversity and structure based on culture</i>	35
<i>Bacterial diversity and structure based on method molecular</i>	36
<b>CHAPITRE 2: Effet des régimes d'enneigement sur la diversité et relative abondance de la communauté bactérienne des sols alpins</b>	<b>39</b>
<i>Contexte général</i>	41
<i>Objectifs de l'étude</i>	42

<i>Article I: Microbial diversity in alpine tundra soils correlates with snow cover dynamics</i>	44
<i>Principaux résultats et discussion</i>	55

**CHAPITRE 3: Effet des régimes d'enneigement sur la structure phylogénétique des communautés bactériennes des sols alpins** \_\_ 59

<i>Contexte général</i>	61
<i>Objectifs de l'étude</i>	64
<i>Article II: Snow cover dynamics and phylogenetic structure of bacterial communities in alpine tundra soils</i>	67
<i>Abstract</i>	68
<i>Introduction</i>	69
<i>Material and Methods</i>	71
<i>Study site, sample collection and bacterial ssu sequences</i>	71
<i>Sequence data treatment</i>	71
<i>Statistical Analysis</i>	72
<i>Results</i>	74
<i>Comparison of bacterial communities using taxonomic information</i>	74
<i>Comparison of bacterial communities using phylogenetic information</i>	74
<i>Distribution of bacterial groups at finer taxonomic scale</i>	75
<i>Discussion</i>	76
<i>Conclusion</i>	79
<i>Acknowledgements</i>	80
<i>References</i>	81
<i>Principaux résultats et discussion</i>	96

**CHAPITRE 4 : Effet des régimes d'enneigement sur la richesse des espèces bactériennes cultivables des sols alpins** \_\_ 99

<i>Contexte général</i>	101
<i>Objectifs de l'étude</i>	102
<i>Article III: Phylogenetic diversity of cultivable bacteria in soils of temperate alpine tundra</i>	103
<i>Abstract</i>	104
<i>Introduction</i>	105
<i>Material and Method</i>	107
<i>Site sampling description and sample collection</i>	107
<i>Culture media</i>	107
<i>Isolation of bacterial strains</i>	107
<i>PCR amplification and sequencing of 16S rRNA</i>	108



<i>Phylogenetic analysis and sequence comparison</i>	108
<b>Results</b>	110
<i>Counts and phenotypic characterization of the isolates</i>	110
<i>Phylogenetic analysis of isolated strains</i>	110
<i>Comparison with ESM and LSM ssu libraries</i>	111
<i>Seasonal and spatial distribution</i>	111
<i>Discussion</i>	112
<i>Conclusion</i>	114
<i>Acknowledgements</i>	115
<i>References</i>	121
<b>Principaux résultats et discussion</b>	139
<i>L'effet le enneigement sur la richesse, abondance et composition des bactéries cultivable</i>	139
<i>Comparaison des résultats obtenus par approches classiques et moléculaires</i>	140
<b>CHAPITRE 5: Synthèse et perspectives</b>	<b>143</b>
<i>The limits of molecular approaches</i>	145
<i>DNA extraction and PCR</i>	145
<i>Molecular profiling method: CE-SSCP</i>	146
<i>Random sequencing of ssu libraries</i>	146
<i>Importance of traditional and cultural methods</i>	147
<i>Link between bacterial phylogenetic and function</i>	148
<i>Bacterial assemblage and filtering factors</i>	149
<i>Link between snow cover and bacterial activity</i>	150
<i>Bacteriobiogeography</i>	151
<i>Conclusion</i>	153
<b>Bibliographie</b>	<b>155</b>
<b>Annexe</b>	<b>171</b>



# **Chapitre 1:**

## **Introduction**



## Background and objective of the thesis

Nearly 40% of terrestrial ecosystems are covered with snow during the winter that is mostly marked in Alpine and Arctic ecosystems. These soils sequester large amounts of organic carbon (Davidson and Janssens, 2006). In these ecosystems, low temperature leads to reduced plant-litter decomposition, generating an imbalance between C input and output (mineralization) that leads to the accumulation of organic matter. In the context of climate change this C pool is vulnerable.

Actually, the increase temperature will lead to reduction of snow cover and increase of soil temperature. The soil micro-organisms are involved in the degradation of plant organic matter. The increase of temperature may result in an increase of C mineralization (CO<sub>2</sub> release) and result in a positive feedback to global warming. The understanding of microbial communities from these habitats is essential to build predictions about its role.

Alpines tundra soils belong to snow-covered ecosystems showing a strong spatial heterogeneity. The topography of these environments creates gradients of snow cover. The extremes of this gradient can be used to study the impact of snow-cover. The convexities lead to an inconsistent or inexistent snow cover (Early Snow Melt locations, ESM) while the snow accumulates in the concavities, leading to longer snow cover (Late Snow Melt locations LSM). The ESM soil is frozen during winter and suffers from freeze/thaw cycles in late autumn and early spring, the adapted plants are slow growing and stress-resistant. The LSM soil is kept around 0°C during winter, but plant productive season is reduced, leading to fast-growing plants. So, beside of soil characterisation, plant strategy growth and their diversity is different between two locations.

The studies on spatial and seasonal variations of micro-organisms in snow covered habitats are scarce. Recent studies on an alpine meadow using molecular markers, showed temporal variations on bacterial and fungal communities (Lipson et al., 2002; Lipson and Schmidt, 2004; Schadt et al., 2003). The spatial and temporal variations of microbial communities were found using phospho-lipid fatty acid analysis (PLFA) (Bjork et al., 2008) and molecular markers (Wallenstein et al., 2007). These pioneering studies suggest that fungal microflora dominates during winter under the snow and would be replaced by bacterial microflora during the summer. These investigations also suggested that a grand part of litter decomposition occurred under snow during winter. However, effect of snow cover dynamic on microbial diversity, composition and structure remain mystery. In addition, the seasonal and altitudinal

differences of bacterial communities were studied using a phylogenetic structure approach (Lipson, 2007).

In macro-organisms biology, the phylogenetic information has been used to examine the ecological mechanisms on phylogenetic structure (Webb et al., 2002). These methods analyse the phylogenetic between two species on different environments at different times and can be used to compare environmental properties. There are a few reports concerning bacterial communities, actually the biotic and abiotic factors impacted on clustering or overdispersion of bacterial community (Horner-Devine and Bohannan, 2006; Bryant et al., 2008; Newton et al., 2007). To date, there is no report on this subject for alpine soil.

The classical approach of cultivation, though limited to assess diversity, was successfully used in soils from Siberia, Colorado Mountains, Arctic/Antarctic soils and also permafrost samples (Shi et al., 1997; Lipson and Schmidt, 2004, Bai et al., 2006, Zhang et al., 2007, Steven et al., 2007, Hansen et al., 2008). These successes encouraged us to isolate alpine bacterial strains.

**The mains goal of this study was:**

- Characterize the phylogenetic diversity of bacterial communities in two ESM and LSM as well as seasonality to better characterize the effect of snow cover on bacterial diversity
- Characterize the spatial (soil properties, plant composition species) and seasonal variation for bacterial phylogenetic structure at different taxonomic scale to assess the environmental filters
- Characterize of cultivated bacteria in alpine soil and structure phylogenetic of this community
- Comparison of the bacterial composition and structure obtained by molecular and culture methods

## Thesis content and structure

This thesis is a part of the study of spatial and temporal distribution of micro-organisms in alpine tundra habitats. Indeed, the precise idea was characterization the microbial communities of the alpine soils by using molecular and traditional approaches.

When I arrived at the laboratory (October 2005), the activity of group Microbiology was started in the team PEX (Perturbations Environnementales et Xénobiotiques) with the work of Alice Durand (characterization of fungi communities in alpine soil) and Lucie Zinger (optimization of molecular fingerprinting methods). The spatial and seasonal variation of bacterial communities in alpine soil was defined as subject of my thesis.

This work was performed in the frame of the Microalpes project (ANR Blan 2006) in collaboration with Philippe Choler and Florence Baptist of equip TDE (Traits fonctionnels végétaux et Dynamique des Ecosystèmes alpins) who focused on carbon cycling flow in ESM and LSM. We first studied the spatial and seasonal of microbial communities in the two alpine habitats by molecular fingerprinting (L. Zinger). I did all the work concerning molecular characterization of bacterial communities. The results were published in the *ISME Journal*, **Zinger et al 2009; chapter II**.

A detailed analysis of phylogenetic groups and dynamics of the phylogenetic structure of bacterial communities allowed identifying two environmental filters in alpine habitats understanding filter events in alpine soil. This study is submitted in *Environmental Microbiology*, **Shahnavaz et al, submitted; chapter III**.

A characterization of cultivated bacteria in alpine soil and comparison between results obtained by molecular and traditional methods is the subject of **chapter IV**. Unfortunately, I could not use the same soil that we used for clones library (when I arrived at laboratory, sampling of soil was made, the soil were saved in 4°C during months, so we hypothesized saving in cold temperature can affect on bacterial communities). The results of this study are in preparation for publication.

Finally, a second study from the collaborative Microalpes, concerning the effect of tannins and freezing in ESM soils, was published in the *Environmental Microbiology*, **Baptist et al., 2008; Annex**.

# The challenge of environmental microbiology

## Microbial ecology history

Microbiology is a young science that started after discovery of bacteria by Antony van Leeuwenhoek (1632-1723). In the 18<sup>th</sup> century that was named the "Golden age of microbiology", this knowledge was developed with Louis Pasteur, Joseph Lister and Robert Koch studies. The first study of microbial ecology started with Serge Winogradsky (1893) that investigated the process of nitrification. Afterward, Martinus Willem Beijerinck discovered nitrogen fixation. As of 1950, increasing of awareness public health and environmental pollution led to development of microbial ecology; therefore this branch of microbiology is very young.

## Bacteria

Bacteria are a lineage of prokaryotic (cells without a membrane-bound nucleus) (Hayward, 2006), they display a small volume (1.5-2.5 mm<sup>3</sup>) (Beveridge, 2001). Other characteristic is the size of ribosome, which is smaller than the eukaryotic one (70S versus 80S). Bacteria are sexless and so a biological species concept should be adapted. The standard for species definition is DNA-DNA hybridisation, but it only applies to cultured species. The most used way to define a bacterial species relies on the similarity of the gene coding for the 16S rRNA. Strains sharing more than 97% of similarity belong to the same species. This criterion applies for uncultured strains.

## Bacterial genetic diversity

The micro-organisms (particularly bacteria) display variation in their metabolic properties, cellular structure and phenotype characteristics (Ochman et al., 2000). These characteristics allow bacteria to adapt to almost all habitats (Pace, 1997; Hayward, 2006). The functional diversity relies on the plasticity of their genomes. Actually, besides natural mutations, bacteria can acquire genes by taking up DNA from the environment or by exchange with other



bacterial species by horizontal gene transfer (HGT). HGT leads to incorporate genetic material from another organism without being its descendant.

### **Bacterial roles in nature**

They inhabit the nutrient-rich environment being sea, ocean, lake, soil, even in extreme ecosystems (spring hot). In fact, they are able to survive in outer environmental limits of life (Horner-Devine et al., 2004). They are most abundant in soil. Besides the pathogenic bacteria (affecting animal and plant health), bacteria play a major role in ecosystem functioning (Fenchel, 2007), by their implication in the biogeochemical cycles of N and C.

The nitrogen cycle provides an example of the importance of bacterial processes (Kowalchuk and Stephen, 2001; Burris, 2004; Oren, 2008). Bacteria are the only organisms able to carry out this biochemical process known as nitrogen fixation, conversion of  $N_2$  to ammonia, indispensable for life. The best examples of nitrogen fixation bacteria are *Rhizobiales* that form nodules in leguminous plant (Dowine, 2007) or *Frankia sp* that fixes nitrogen in nodules of non leguminous plants (Kohls et al., 1994). They are also involved in organic nitrogen mineralization (Stevenson, 1999). The bacteria are vital for another process, which makes the life possible on earth: the cycle of carbon (Hogberg et al., 2001). Besides  $CO_2$  fixation by *Cyanobacteria*, they are responsible for breaking up complex matter such as cellulose and lignin (Embley and Stackebrandt, 1994; Lynd et al., 2002) from plant or animal dead material through the secretion of specific enzymes. For example the members of phylum *Actinobacteria* such as *Arthrobacter*, *Rhodococcus* and *Micrococcus* have a high potential to degrade complex and recalcitrant matter. It has been documented that carbon stock in soil regulate by size and activity of soil microbial community via mineralization (respiration) and immobilization of soil organic carbon (Zhu and Miller, 2003).

The metabolism and genetic diversity abilities had been paid attention on bacterial for bioremediation, particularly for oil spills, pesticides, and other toxic materials (Bentahir et al., 2000; Rothschild and Mancinelli, 2001; Margesin, 2007).

***Bacteria are unique because of their diversity, role in environment cycles and abundance. The better understanding of ecological processor such as global warming and pollution requires to deeply understanding of bacterial behaviours.***

## Bacterial Diversity

### Importance of bacterial diversity in ecosystem

Bacteria are the most abundant micro-organisms in soil and inhabit nearly every environment. They have existed for more than three billion years. The total number of bacteria may be as high as  $10^8$  -  $10^9$  bacteria,  $10^7$  -  $10^8$  *Actinobacteria* and  $10^5$  -  $10^6$  fungi cells per gram soil (Islam and Wright, 2006). Bacterial cells contain 350-550 Pg of carbon (1 Pg = 10<sup>15</sup> g), up to 60-100% of the total carbon found in plants, as well as large amounts of nitrogen and phosphorous (Whitman et al., 1998). So, they are not only everywhere; they are also very abundant (Table 1.1).

Habitat	abundance(cells/cm <sup>-3</sup> )	diversity(genom equivalent)
forest soil	$4.8 \times 10^9$	6000
forest soil (cultivated isolates)	$1.4 \times 10^7$	35
pasture soil	$1.8 \times 10^{10}$	3500-8800
arable soil	$2.1 \times 10^{10}$	140-350
pristine marine sediment	$3.1 \times 10^9$	11400
marin fish-farm sediment	$7.7 \times 10^9$	50

TABLE 1.1 – Bacterial abundance and diversity. Abundance was determined by fluorescence microscopy; diversity was estimated from the reassociation rate of DNA isolated from samples of the habitats indicated (Horner-Devine et al., 2004).

It is important to study microbial diversity to understand the link between diversity and community structure and function. The phylogenetic position of a bacterial species gives hints about its function. Bacterial diversity helps to increase soil quality and fertility. The diversity of bacteria in soil is important to the maintenance of good soil health because they are involved in many soil processes (Borneman et al., 1996). Species diversity can increase ecosystem stability through the ability of species to respond differently to perturbation in the soil environment (Sturz and Christie, 2003). Actually, natural stresses probably submit bacteria to cyclic "mass extinctions". The survival of ecosystems suggests that the functional redundancy of bacteria is high. The human activity (pollution, agriculture, global change) could affect microbial diversity, and therefore above and below-ground ecosystem functioning. In this context, the study of bacterial diversity gives hints about the impact of stresses.

## Factors important on bacterial diversity

Soil bacterial diversity is influenced by several biotic and abiotic factors (Schmidt, 2006), but, it remains unclear whether there are general patterns of bacterial diversity. Some studies suggest that patterns of bacterial diversity are determined by a few of environmental variables.

### Abiotic factors

Fierer and Jackson (2006) reported that soil pH is an important factor for bacterial diversity. Other scientists demonstrated that pH have influence particularly on bacterial abundance and diversity (Lauber et al., 2008). However, other soil properties such as salinity have a strong correlation with bacterial diversity (Lozupone and Knight, 2007). Bacterial communities are also strongly influenced by soil temperature. Based to the growth temperature classification (Morita, 1975), the microbes classed to three main groups: Psychrophiles, Mesophile and Thermophiles. This range of temperature not only influences microbial growth, but also microbial diversity. For example, in a study on hot spring bacterial communities, colony forming units (CFU) of bacterial vary in temperature range (Abou-Shanab, 2007). Soil texture is another factor that effects on bacterial diversity and composition. Sessitsch et al (Sessitsch et al., 2001) and Lauber et al (Lauber et al., 2008) showed that the bacterial diversity and composition was affected by particle size. However, several investigations insisted that abiotic factors such as pH and organic matter (OM) did not display a significant role on community structure (Johnson et al., 2003) and the soil type itself is primary determinant on bacterial composition (Girvan et al., 2003). This indication is supported by Dalmastrri et al investigated diversity of *Burkholderia cepacia* populations associated with the *Zea mays* root system. They showed that soil type was a main factor affect on diversity of maize root-associated *Burkholderia cepacia* (Dalmastrri et al., 1999).

### Biotic factors

One of most important biotic factors is presence or absence of covering plants. Numerous studies demonstrated that presences of plant can impact bacterial diversity and communities. For example Yergeau et al showed that bacterial diversity is higher in vegetated soil than in

bare soil (Yergeau et al., 2007a). Singh et al (2009) showed that the bare soil was dominated by Firmicutes, while soils with different plant species were mainly dominated by Acidobacteria. The microbial structure varies with plant cover (Yergeau et al., 2007b) or between bulk and rhizospheric soils (Kielak et al., 2008). Indeed, plants strongly affect microbial structure and activity (Grayston and Campbell, 1996; Grayston et al., 1998; Bais et al., 2006; Yergeau et al., 2007ab). A few reports suggest that the *Proteobacteria* and *Acidobacteria* dominate in rhizosphere locations (Singh et al., 2007).

Numerous studies demonstrated that microbial community is affected by released carbon sources such as sugar, amino acid, etc from root plants (Maloney et al., 1997; Miethling et al., 2000). The quality and quantity of this carbon can largely change by plant development (Mougel et al., 2006), crop species (Grayston and Campbell, 1996; Maloney et al., 1997; Miethling et al., 2000) and then can affect microbial structure and composition. Griffiths et al (2006) found that the upper organic layer is dominated by *a-Proteobacteria* and the mineral layer by *Acidobacteria*.

This question "Is plant species the most important factor or not?" remains open. For example, Miethling (Miethling et al., 2000) showed that plant species has great impact on microbial structure in the rhizosphere, while Ulrich and Becker (2006) showed that the effect of the plant was negligible. Plant will impact on nutrient cycle, particularly for nitrogen which is a limiting growth factor (Hobbie, 1992; Menyailo et al., 2002). Association between plant and microbes has been known for certain genera of *Proteobacteria* (*Rhizobiales*), *Actinobacteria* (*Frankia*). Other important biotic factor is organic matter that has a great role for microbial growth. Quality and quantity of these matters directly depend of plant species and their phenology. The variety of organic matter compound released by plant depends on plant type and age. Root-exudation can increase or decrease nutrient pool soil with change in chemical or biological properties (Bais et al., 2006). So up to now, the most important factor's influences on bacterial diversity remain mysterious.

### **Measurement of bacterial diversity**

Bacterial diversity refers to diversity within a given community ( $\alpha$ -diversity) and partition of biological diversity along an environmental gradient ( $\beta$ -diversity).  $\alpha$ -diversity can characterize the total number of species in a given environment (Richness), relative abundance of the species (evenness). In other hand,  $\alpha$ -diversity is diversity within a single community and a

manner to understand total diversity in different communities. For example, forest soil contains more diverse micro-organisms than agricultural soil, and the soils of different altitudes contain more or less diverse micro-organism. In these cases, we use  $\alpha$ -diversity measurement that contains qualitative or quantitative measures and are the powerful tool (Table 1.2). In most of the microbial studies, because of poorly defined species level, OTUs (Operational Taxonomic Unit) was defined as a species. Two of the microbial studies most popular formula for species diversity in ecology are Simpson index (Simpson, 1949), and Shannon index (Shannon, 1948). These indices are based on richness and evenness. It should be noted that a high Shannon index value equates higher diversity, whereas a low value correlates to a lower diversity. This relation is inversely defined for Simpson index.

$\beta$ -diversity measures the difference among two or more assemblage at regional scales. For example, we would know in a regional assemblage, how much diversity change over season or space. This type of measure can refer to ecological fundamental questions that whether the micro-organisms have biogeography or they are cosmopolitans because of small size and facilitation distribution, "*Everything is everywhere, but, the environment select*" (Baas Becking 1934). This diversity can show that how ecological process lead to clustering or overdispersion and can structure communities (more information in part of Measurements of taxonomic distinctness).

These two types of diversity can be measure in different ways as illustrated in Table (1.2) (see more information in Lozupone and Knight, 2008). The qualitative measures are based on present/absent of species, while quantitative measures comprise the abundance of species.

	$\alpha$ -diversity	$\beta$ -diversity
qualitative	Chao 1 ACE Rarefaction Phylogenetic diversity	Unweighted UNIFRAC Taxonomic similarity
quantitative	Shannon index Simpson index	weighted UNIFRAC Qst

TABLE 1.2 – Diversity indices usually used for bacterial diversity estimation

## Measurements of taxonomic distinctness

Despite the existence of bacteria from million years ago, their abundance and importance in ecosystems processes, the investigations of their diversity patterns are recent. This diversity is considered as very important in agriculture, industry and nutrient cycles. It is suggested that bacteria show similar patterns in taxonomic diversity compared to macro-organism (Horner-Devine et al., 2004). However, most of these studies are focused on bacterial diversity and composition but not on their phylogenetic structure. Taxonomic distinctness shows that the species in a community tend to be closely related (phylogenetically clustering) or distinctly related (phylogenetically overdispersion). Based on Darwin's idea closely related taxa are more inclined to interact with each other than distantly related taxa. Now, it is suggested that the closely related taxa tend to be more similar from an ecological point of view than distantly related taxa (Harvey and Pagel, 1991). For example, occurrence of rainforest tree species is more closely related than by chance and this phylogenetic clustering in a given ecosystem indicates the selective filters (e.g., environmental conditions) (Webb et al., 2002). For bacteria, this tendency to be cluster has been observed in several studies (Horner-Devine and Bohannan, 2006; Newton et al., 2007; Bryant et al., 2008).

For example, Bryant et al (2008) showed that at all altitude *Acidobacteria* tended to be more phylogenetically clustered. In the same way, the studies of Horner-Devine and Bohannan (2006) confirmed that bacterial communities in a wide range of environments tended to be phylogenetically more closely related than expected by chance. The factors that lead to phylogenetic clustering of bacteria are not precise. The abiotic filtering may be a force in the clustering of bacteria communities such as altitude (Bryant et al., 2008), pH (Newton et al., 2007), soil deepness and total organic carbon (Horner-Devine and Bohannan, 2006). Phylogenetic overdispersion can be explained by two biotic interactions: competition (Webb et al., 2000) or facilitation (Valiente- Banuet and Verdu, 2007). Competition refers to this hypothesis that a strong competition occurring between closely related taxa that occurs in same ecological niches leads to a phylogenetically dispersed community and facilitation explains by this assumption that microhabitats created by facilitator species permit the adaptation of other species in a given environment. There are two commons metrics to measure phylogenetic structure. The net relatedness index (NRI) is a mean distance between two species randomly in a community and nearest taxon index (NTI) define mean distance between each species and its nearest neighbour species (Webb et al., 2002). These two metric can not show abundance of species in a given community. NRI focus on higher taxonomic level, while NTI focus on lower taxonomic levels.

## **The species equal to OUT**

According to the definition proposed by Ernst Mayr (Mayr, 1982), a species is produced from interbreeding two organism capable to fertilize. This concept is still commonly used to define a species in higher organisms, but it is clearly inapplicable to bacteria, due to the lack of true sexuality. The development of molecular biology allowed circumventing these problems. The generalization of the analysis of the rDNA has prompted many scientists to question on the correspondence between a DNA sequence encoding the small subunit ribosomal and bacterial species (Fox et al., 1992; Stackebrandt and Goebel, 1994). Thus, a correlation between the percentages of identities between 16S rRNA and the percentages of re-association DNA / DNA was researched. In general, the rDNA sequences with identities below 97% correspond to different species. To overcome the difficulties of defining a species, many authors use the concept of Operational Taxonomic Unit (OTU). OTU can be determined as a group of sequences with 99% (Kroes et al., 1999) or 97% (McCaig et al., 1999) similarity.

## **How to assess microbial diversity?**

Many different methods and approaches have been applied to gain access to micro-organisms residing in soil and to assess the microbial diversity. The methods to measure microbial diversity can be divided into two groups: (i) traditional methods and (ii) molecular methods.

### **Traditional methods**

In the past, microbial diversity was quantified by plate counting methods and isolation of microbial cells followed by identification by a variety of methods, addressing only the cultivable diversity. Methods for microbial characterization can be based on isolation and cultivation followed by tests such as colony morphology, pigment production, cellular morphology and size, oxygen tolerance, carbon utilization, pH range, temperature optimum range, enzyme pattern (Dworkin, 2007). However, in a given sample soil, approximately 99% of bacteria observed under a microscope were not cultivable under standard conditions (Torsvik et al., 1990). Figure 1.1 represents an example from the bacterial division in soil that are cultivated or not cultivated. This problem is related to the lack of knowledge of the real conditions under which most bacteria are growing in their natural habitat. To overcome this

problem there has been recent attempts to develop new culture media to maximize the recovery of diverse microbial groups (Janssen et al., 2002; Sait et al., 2002; Gish et al., 2005). Certain traditional methods allowed to knowledge on microbial biomass, such as MPN (most probability number), chloroform fumigation/extraction method and enumeration by specific dye binding to DNA (DAPI: 4',6-diamidino-2-phenylindole).

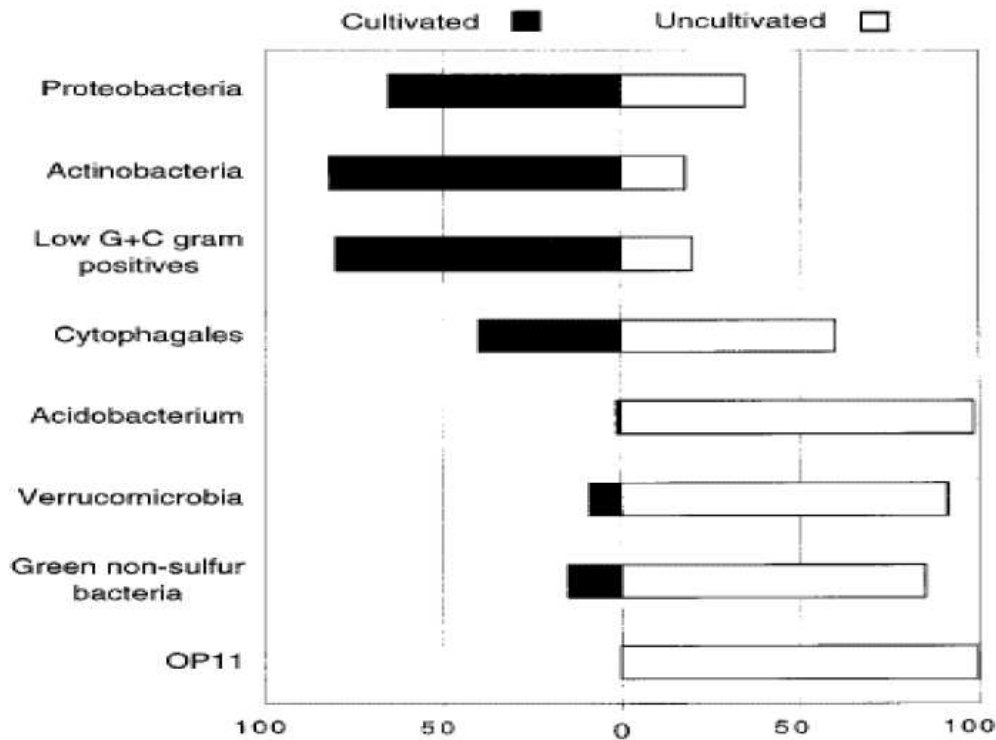


FIGURE 1.1 – Relative representation in selected cosmopolitan bacterial divisions of 16S rRNA sequences from cultivated and uncultivated organisms. Results were compiled from 5224 and 2918 sequences from cultivated and uncultivated organisms, respectively (Hugenholtz et al., 1998).

### Culture-independent method

Discovery and culture of novel micro-organisms were the major pattern of microbiology since Koch. Nonetheless, uncultured microbes were a challenge to allow a whole microbial population and diversity view. Some years later, Woese et al (Woese and Fox, 1977), recognized the 16S rRNA as a useful and powerful molecular tool for phylogenetic purposes, and built the first 16S rRNA based trees of life. They presented that all of living systems could place in one of the lineages: (i) the eubacteria, containing all typical bacteria; (ii) archaeobacteria, comprising methanogenic bacteria and (iii) eukaryotes. Now, the 16S rRNA



has been shown to be a powerful phylogenetic tool due to the following properties (Stackebrandt, 2001):

- It is a universal molecule, which can be used in prokaryotic cells.
- Its size is adequate to the present molecular methods (1500 nucleotides).
- It contains sufficient conserved and variable regions (Figure 1.2).

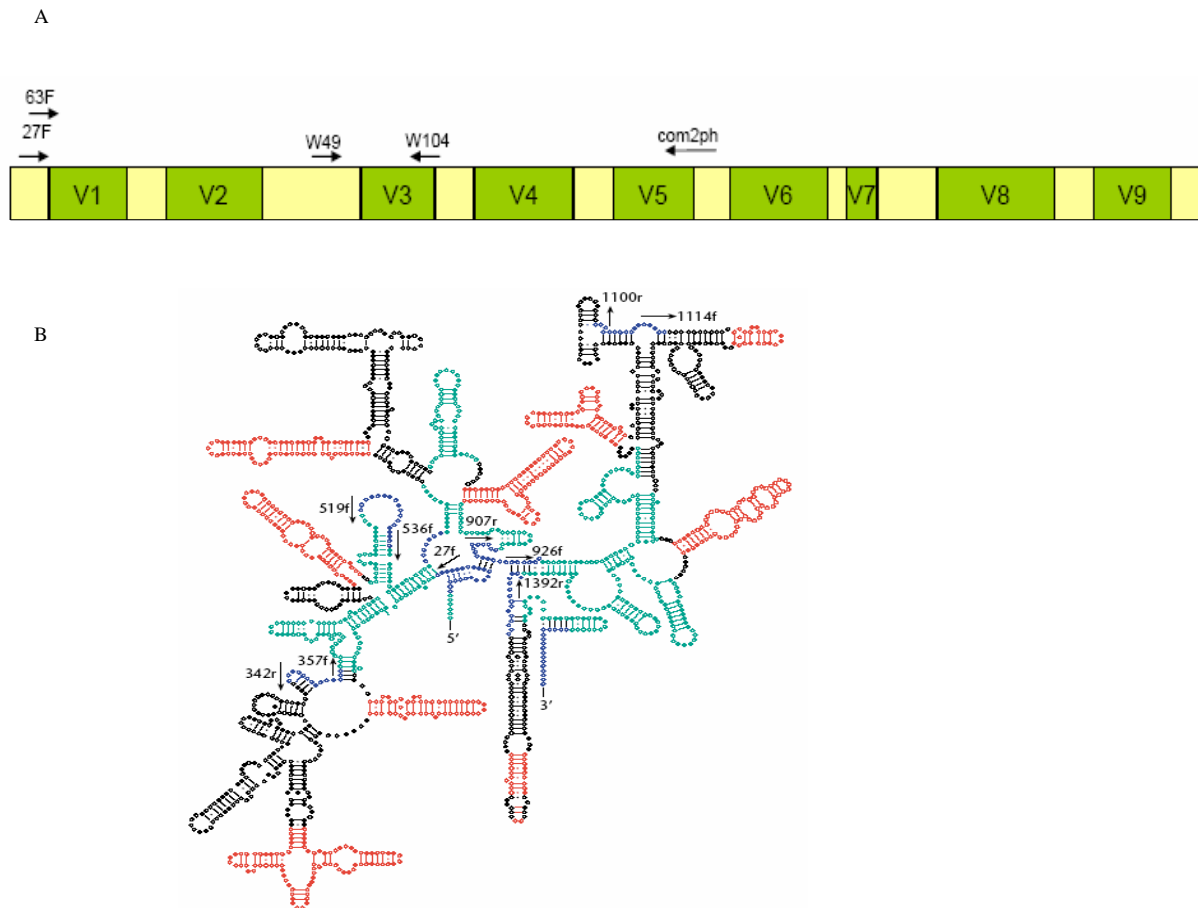


FIGURE 1.2 – 16S rRNA molecule based on the *E. coli*. A: linear structure, highly variable regions are green and conserved region are yellow. B: secondary structure, highly variable regions are red; highly conservative stretches are green. Binding sites of primers used in PCR amplification of the rRNA gene are blue, with the direction of amplification indicated by arrows. The other nucleotides are black ( Stackebrandt, 2001).

The number of 16S RNA gene sequences published in GenBank have increased from environment samples since 1997 (Rappé and Giovannoni, 2003). Culture-independent techniques for community analysis became widely applied methods, which are based on extraction of nucleic acids from soil. These methods became a valid support to traditional techniques, especially tools including the sequencing of 16S rRNA genes (Amann et al.,

1995). Molecular methods can overcome problems associated with cultivation of bacteria from natural samples. They give an overview of microbial diversity and help to monitor alterations in microbial composition after stresses or changes in environmental conditions through the use, for instance, genetic fingerprints. However, the approach of determining microbial diversity by traditional cultivation techniques should not be neglected because cultivable bacteria may have an ecological significance in soil (Bakken, 1997) and their physiology can easily be characterized. Now, the current bacterial classification is based on the phylogenetical relation among micro-organisms described by 16S rRNA trees. Using 16S

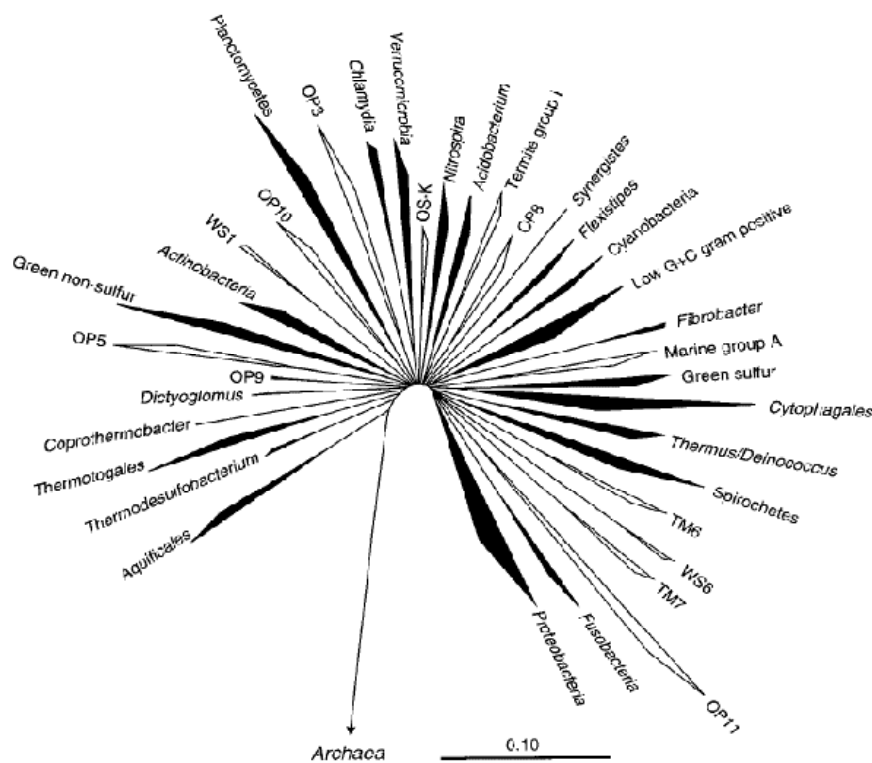


FIGURE 1.3 – Cultivated and uncultivated bacterial divisions were presented by Hugenholtz et al in 1998. Divisions which are cultivated are shown in black; divisions represented only by environmental sequences are shown in outline.

rRNA, Hugenholtz (Hugenholtz et al., 1998) explained up to divisions within the bacteria domain (Figure 1.3).

For comprehensive information on the genetic structure of community, polymerase chain reaction (PCR)-based fingerprinting techniques, such as Temperature Gradient Gel

Electrophoresis (TGGE), Denaturing Gradient Gel Electrophoresis (DGGE), Amplified Ribosomal DNA Restriction Analysis (ARDRA), Single Strand Conformation Polymorphism (SSCP), Amplified Fragment- Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Terminal Restriction Fragment Length Polymorphism (TRFLP) analysis, have been developed (Figure 1.4) (Torsvik and Ovreas, 2002). The use of cloning / sequencing of molecular markers allow a more detailed analysis of microbial communities. Briefly, the steps of cloning allow isolating each fragment before sequencing. This method provides access not only to the composition but also to the richness and diversity of microbial communities. Unfortunately, the microbial diversity is too vast and needs many clones (Curtis et al., 2002). However, like all techniques, they may be limited by many biases, particularly in combination with environment samples. For example, it has been recommended to extract of soil, immediately after sampling because of impact of keeping condition on microbial composition.

The soil compounds such as humic acid can inhibit of the PCR condition. The PCR itself can be a source of errors and bias (number of amplification cycles, chimeric formation, error of DNA polymerase, primers specificity) (Wintzingerode et al., 1997; Prosser, 2000; Qiu et al., 2001; Anderson et al., 2003). Due to the cost of cloning/sequencing methods, new method such as pyrosequencing has been paid attention (Sogin et al., 2006; Huber et al., 2007), although analysing the many sequences produced by pyrosequencing is not easy. The advantages and disadvantages of these methods are illustrated in Table 1.3.

TABLE 1.3 – The advantages and disadvantage of molecular and traditional method using to microbial diversity and composition (Lee et al., 1996; Muyzer et al., 1999; Tiedje et al., 1999; Torsvik and Ovreas 2002; Kirk et al., 2004)

Type of method	Method	Advantages	Disadvantages
Traditional methods	Culture (MPN, enumeration, isolation)	Basic information, inexpensive	Low, growth medium selection, inability to culture of certain group, difficult for large number samples
	Microscopic (DAPI, Gram reaction)	fast, inexpensive	lack of knowledge on active microbial, difficult for large number samples
	Metabolic (Test Biochimic, PLFA)	fast, inexpensive	lack of knowledge on active microbial, difficult for large number samples
	Cloning/sequencing	Complet information on microbial population	Low, expensive, PCR biases, PCR biases dependent on lysing and extraction method
Molecular methods	Electrophoretic analysis: DGGE/TGGE, ARDRA, SSCP	Large number of samples, reproducible, rapid, inexpensive, detect structure change in microbial community	Dependent on lysing and extraction method, PCR biases, interpretation is difficult, no formation at species-level
	Enzymatic digestion	Large number of samples, automated, highly Reproducible	Dependent on lysing and extraction method, PCR biases, choice of restriction enzyme influence on community
	Pyrosequencing	Large number of samples, not depend to DNA, extraction or PCR	Difficult for analysis

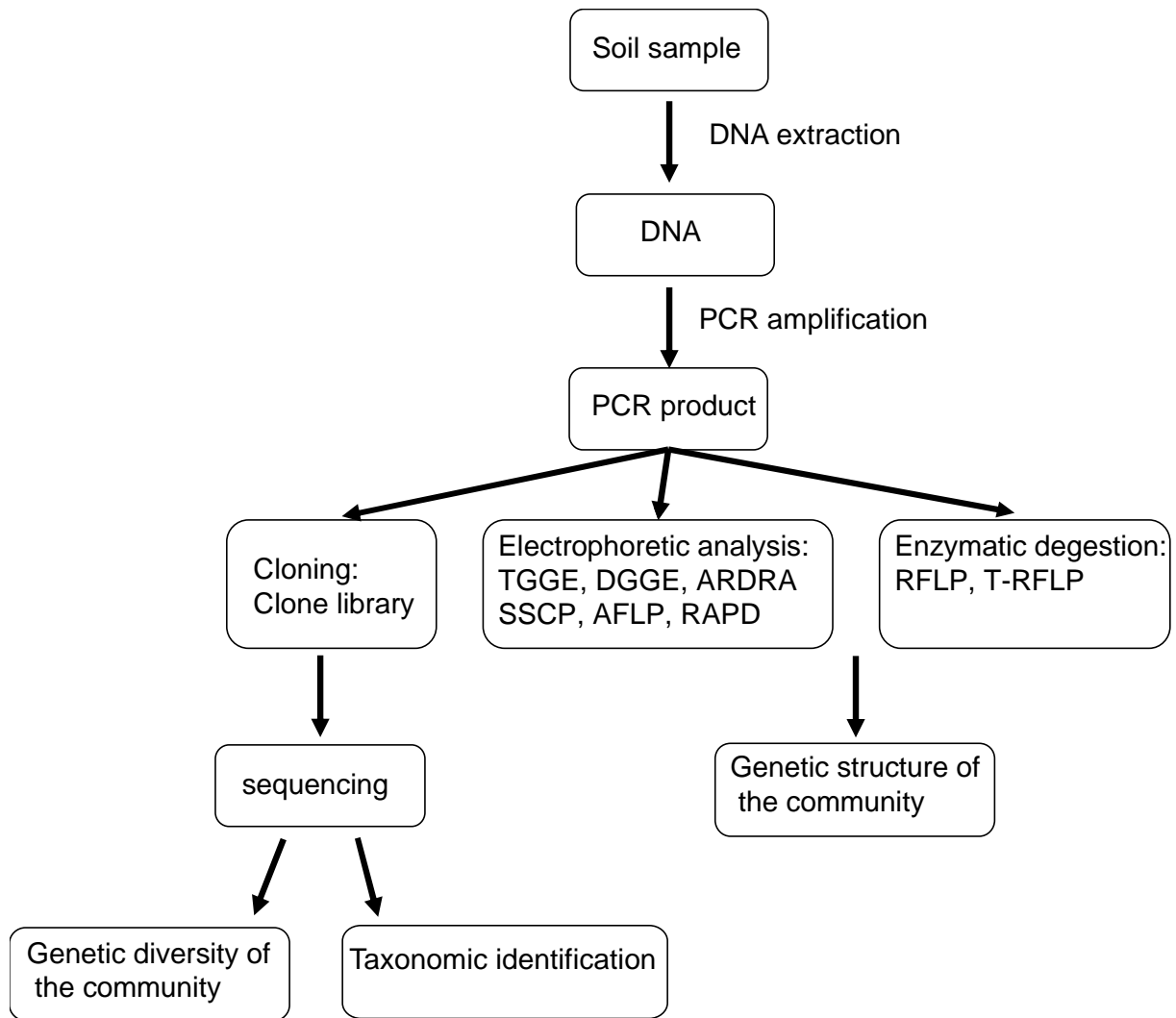


FIGURE 1.4 – Schematic representation of different molecular approaches for assessing the genetic diversity and structure of soil bacterial communities.

*The methods of analysis of microbial communities are diverse and each of them includes both advantages and disadvantages. None of them is complete alone. Based on used methods, collected informations could be different. So, better understanding of microbial communities will request to combine the different technical approaches.*

## Importance of soil

The soil plays an important role in biogeochemical cycles (including carbon and nitrogen). Recycling of organic matter (OM), decomposition of recalcitrant matter occurs in the soil. The mineralization of this matter is a source of nutrients for plants. Soil is the most complex and heterogenic habitat (Young and Crawford, 2004) dominated by a solid phase.

Some characteristics of soil refer to bedrock. The physical and chemical properties of soil constitute a complex environment and heterogeneous for investigation. For example, the rate and structure of pores result in complex distribution of water availability and gradient in resource that can potentially vary, soil to soil or even within a soil sample in a small scale (Young and Crawford, 2004). However, it seems that a main part of this heterogeneity is dependent on micro-organisms and plants that live in soil (Crawford et al., 2005). Furthermore, not only spatial scale in soil will be important to understand soil ecology (Ettema and Wardle, 2002), but also change in time scale hours to days, season or year can be an important factor for soil structure (Bardgett et al., 2005). However, soil constitutes a highly fragmented habitat due to the spatial heterogeneity of organic matter content, soil texture, pH, moisture, temperature, and other factors. While, the prokaryotes are presented in other ecosystems such as marine environment (Azam and Malfatti, 2007), environment extreme (Rothschild and Mancinelli, 2001) (including extreme environmental condition such as temperature, oxygen pressure, salinity, pH, osmotic, desiccation and radiation), they are especially abundant and diverse in soil ecosystems (Torsvik and Ovreas, 2002).

## The spatial distribution patterns of micro-organisms

The pioneer idea on micro-biogeographical pattern (geographical distributions of micro-organisms over the Earth in both space and time) refer to decade 1930-1940, when Baas Becking (1934) hypothesised that *Everything is everywhere, but, the environment selects*. This hypothesis can be consider the first idea about micro-biogeography and microbial distribution pattern. This hypothesis argues that microbial species are likely present in all of environment, but the local environmental conditions can promote different species. In other words, the soils with similar characteristic harbour similar bacterial communities regardless of geographic distance. Some recent studies (Finlay and Clarke, 1999) on eukaryotes supported this

hypothesis (Fig 1.5-B). Numerous studies showed that environment heterogeneity can be very important for microbial communities. For example, pH is the best predictor for soil bacteria in continental-scale pattern (Fierer and Jackson, 2006), shift in Bacterioplankton correspond to salinity (Crump et al., 2004), and change in hot spring *Cyanobacteria* is related to temperature (Ward et al., 1998).

In contrast, some other investigations showed that some bacteria exhibit biogeographical patterns (Cho and Tiedje, 2000; Whitaker et al., 2003) and it is believed that spatial variability in microbial communities is a product of historical events or "dispersal limitation". In assumption, geographic distance is the best predictor for genetic divergence between communities and closed habitats display similar microbial communities. The best example is the work of Whitaker (Whitaker et al., 2003) that showed the genetic distance between *Sulfolobus* (archaea) strains isolated from hot springs was explained by geographic distance. Nevertheless, these two hypotheses represent opposite extremes, but this is not true considering these two processes independently. In other example, Cho and Tiedje (Cho and Tiedje, 2000) analysed 258 of *Pseudomonas sp* from different continent with similarity in soil pH and moisture. They used different molecular methods with different resolutions. Finally, they found that there was a statistically significant association between genotype and geographical distance (Fig 1.5-A).

Three phenomena are considered as responsible for bio-geographical pattern: **Dispersal**, **Speciation** and **Extinction** (Horner-Devine et al., 2004). Bacteria disperse a lot due to atmosphere and water movement, their small size and spore formation. The dispersal itself will not alter biogeographical patterns unless be accompanied with favourable conditions in new environment. Microbes that display to survive at a wide range of environment are more able to colonize than the microbes that can grow in specific conditions such as pathogens, species-specific *mycorrhizae* and *rhizobiales*. It can be speculated that microbes speciation rates are high due to short generation time, high level of horizontal gene transfer and large population sizes. It is confirmed that speciation rates in controlled conditions laboratory are very rapid (Rainey et al., 2000; Elena and Lenski, 2003), but whether *in situ* condition can be as fast as, is not clear. The large size of microbial populations on Earth implies that rates of extinction should be minor. Some of the traits of microbial populations such as dormant cells that allow them to survive in harsh condition decrease the risks of extinction. Of course, if dispersal rates over large distance are high, extinction at local environment increases because of reduced chances of change at local scale (Horner-Devine et al., 2004).

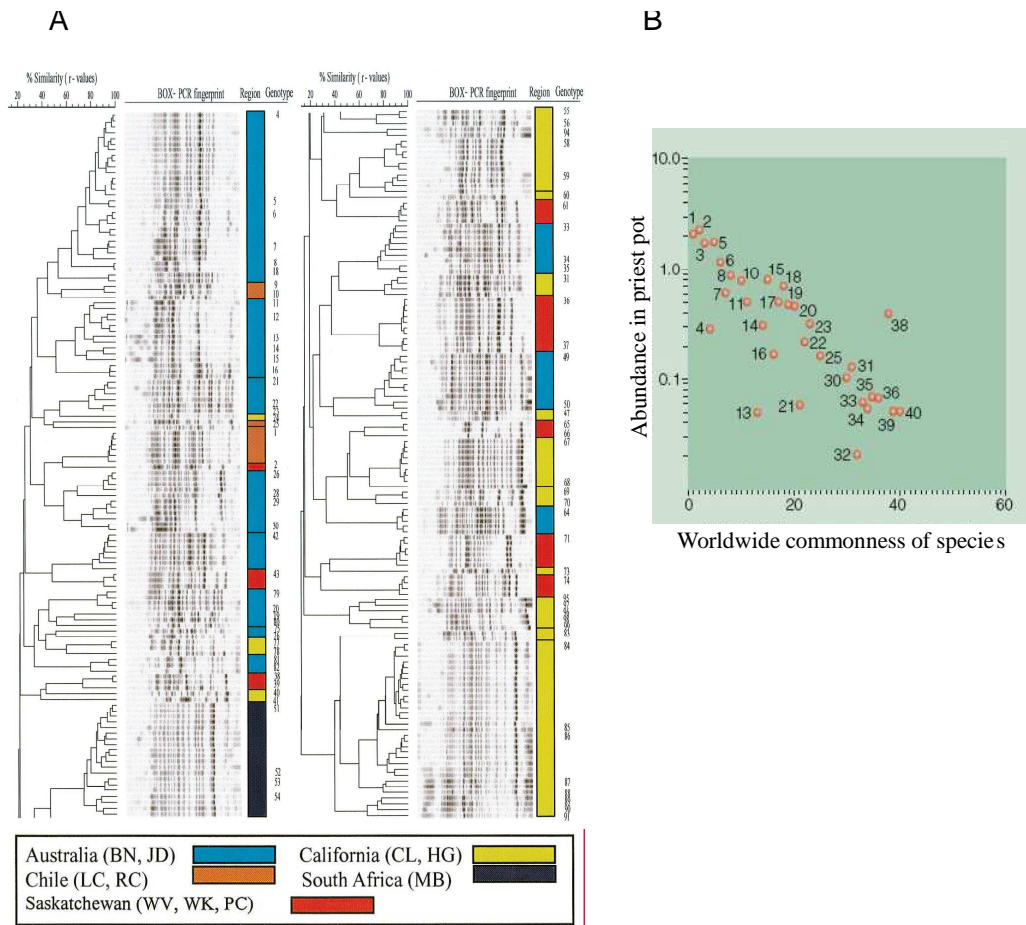


FIGURE 1.5 – Two different experiments on biogeographical pattern for micro-organism. A: Pattern biogeographical of *Pseudomonas* bacteria in Cho and Tiedje study’s that showed a significant relation between genotype and geographical distance. B: The relative abundance of *Paraphysomonas* species in priest pot in study’s Finlay was similar to the global one.

## The temporal distribution patterns of micro-organisms

Although, micro-biogeography principally focuses on spatial distribution, changes over time can be very important. These temporal variations can occur in a short time (hours to day, season), or long time scale (thousands years). Change over time leads to shifts in nutrient availability and resource, consequently microbial biomass, diversity and composition (Bardgett et al., 1999). For example, snow/thaw and wet/dry in short time can be source of changes in microbial communities, or seasonal variation as well as documented in alpine ecosystem (Lipson and Schmidt, 2004). As shown in Figure 1.6, the senescence of plant and cooler temperature in autumn lead to microbial community able to degrade more recalcitrant compounds like polyphenols found in litter plant. The highest levels of microbial biomass are



under snowpack in winter condition and these communities are more adapted to cold temperature. The recalcitrant material organic promote decomposers, particularly fungi.

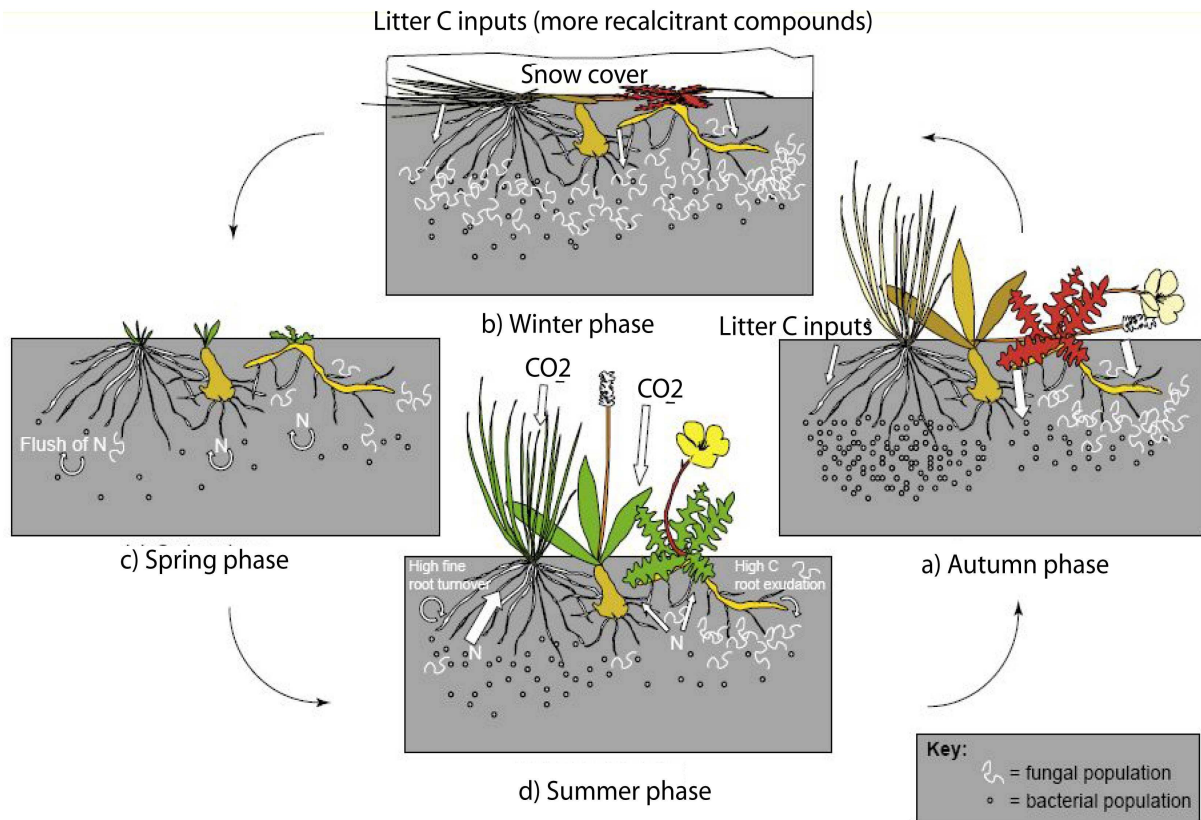


FIGURE 1.6 – Seasonal dynamics of alpine plant and microbial interaction. (a): In autumn, cooler temperature and senescent plant provide soil carbon input for microbial communities. (b): Under snow cover, carbon and nitrogen from plant are consumed and mineralized by microbes. More difficult degraded recalcitrant leads to the increase of fungal species during winter. (c): Snow melt leads to a rapid change in C available for microbes and release of N for beginning of plant growth season (microbial crash), concomitant of condition anaerobes because of waterlogged conditions. (d): During summer, root exudation leads to the dominance of bacterial communities, more adapted and competitive to dissolved nutrients and promote bacterial diversity.

As winter passes to spring, snow melt and warmer temperature cause a turnover of microbial composition and a higher availability of nitrogen for beginning of plant growth in early summer. During summer, the microbial composition is fast-growth and adapts to root exudates (Bardgett et al., 2005).

*Thus, not only bacterial diversity (metabolic/ genetic), biotic and abiotic environmental factors can impact these communities, but also other processes such as distance and time scale can be important in structuration. Further studies to better understand of bacterial diversity and functioning will be require.*

## Alpine ecosystem

### Definition

The Earth is a tempered planet. Cold habitats are mainly located in the Arctic and the Antarctic, in high-mountains and in deep oceans as well (Margesin, 2007). Term "alpine" was usually used to define the mountainous chain in the north of Italy, now it is generally accepted as a term for a high altitude belt (about 1,800-2,500 m above sea level) above treeline. These ecosystems display cold temperature, high solar radiation, exposed to wind and poor in nutrient (Körner, 1999). Compared to the Arctic, the Alpine region is characterized by higher maximum temperature; precipitation and humidity; intensity of solar radiation, similar or lower minimum temperature, lower atmospheric pressure, less organic matter and frequent freeze/thaw events (Nemergut et al., 2005; Margesin, 2007).

### Heterogeneity of the alpine stage

Already, physical gradients that influence on biological gradient were classified based on different scales: macro and mesotopography. The variation of the relief on a scale of kilometric that namely macrotopography, can be origin of strong variation in solar radiation, precipitation, wind speed (Billings, 1973). The typical opposition between the south faces (adret) and the north faces (ubac) illustrates very well this phenomenon (Figure 1.7-A). In addition, the mesotopography that defines a variation of the topography on lower distances in 100m (Billings, 1973) is responsible for the distribution of soil moisture and the origin of important differences of snow-cover between convex (thermic or in this thesis Early Snow Melt location) and concave zones (snowbeds or Late Snow Melt location) (Figure 1.7-B). Mesotopography controls snowpack accumulation and locally controls the length of plant growing season. Persistence of snow-cover leads to shorter growingseason. Thus, these variations in topography, often characterized as the snow cover gradient, have a considerable impact on soil and climatic conditions (Liator et al., 2001) on the composition of plant communities (Körner, 1999) and ecosystem processes (Fisk et al., 1998; Hobbie et al., 2000). Already, physical gradients that influence biological gradient were classified based on different scales: macro and meso-topography. The variation on a scale of kilometric that

namely macrotopography, can be origin of strong variation in solar radiation, precipitation, wind speed (Billings, 1973). The typical opposition between the south faces (adret) and the north faces (ubac) illustrates very well this phenomenon (Figure 1.7-A).

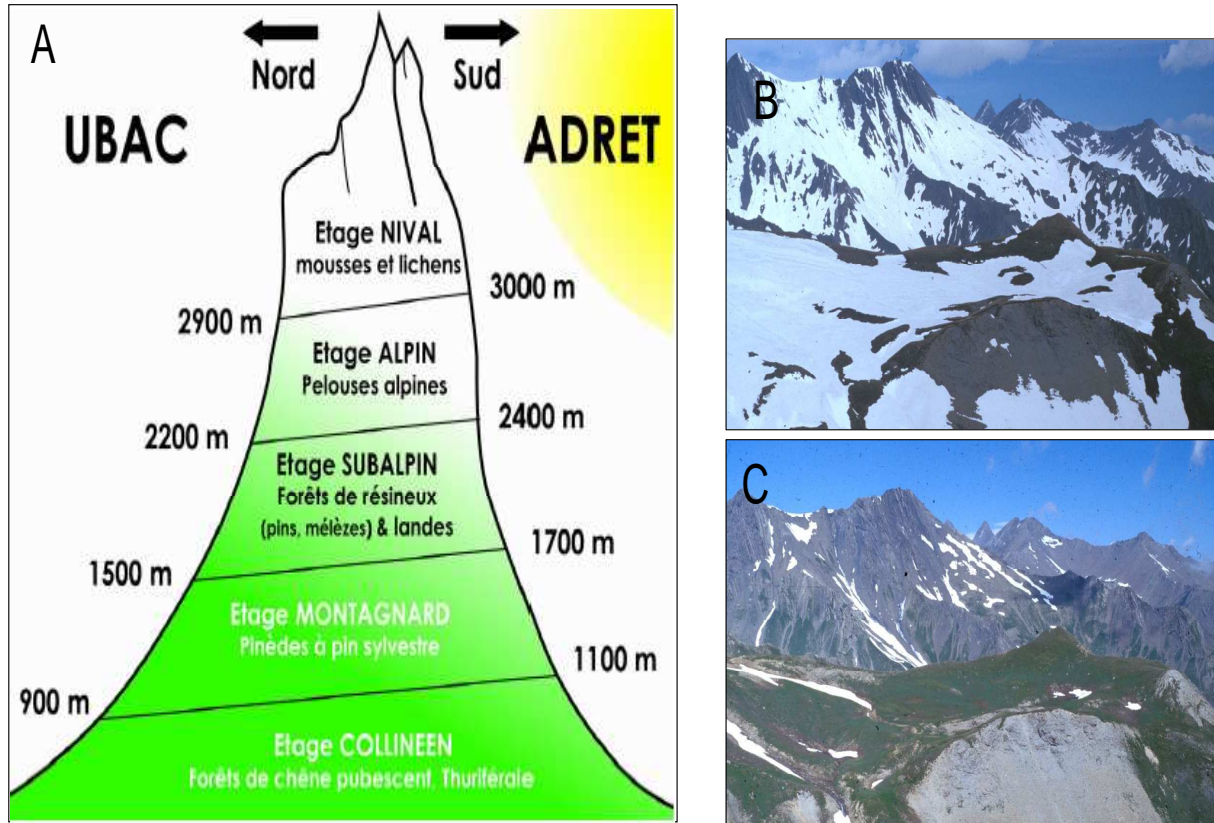


FIGURE 1.7 – A: Schematic representation of the stages of vegetation in the Alps. B: pictures of winter and C: summer in Alpine French. Source: Station Alpine Joseph

In addition, the meso-topography that defines a variation of the topography on lower distances (Billings, 1973) is responsible for the distribution of soil moisture and the origin of important differences of snow-cover between convex (thermic or in this thesis Early Snow Melt location) and concave zones (snowbeds or Late Snow Melt location) (Figure 1.7-B). Meso-topography controls snow-pack accumulation and locally controls the length of plant growing season. Persistence of snow-cover leads to shorter growing season. Thus, these variations in topography, often characterized as the snow cover gradient, have a considerable impact on soil and climatic conditions (Liator et al., 2001) on the composition of plant communities (Körner, 1999) and ecosystem processes (Fisk et al., 1998; Hobbie et al., 2000).

Due to low temperature in these ecosystems, the micro-organisms activity and soil organic matter degradation is reduced (Davidson and Janssens, 2006). Consequently, there is a carbon

imbalance between input (via photosynthesis) and output (via respiration). So, these ecosystems can consider such as stock carbon organic. This pool of carbon is vulnerable to global warming, but their fate remains undefined because of the complexity of the processes of degradation of organic matter (Hobbie et al., 2000). Finally, the microbial composition and their behaviour in these systems remain poorly studied.

## The history of bacterial characterization in artico-alpine

The initial objectives of early studies in permafrost microbiology were to determine if viable micro-organisms could be isolated. Majority of these investigations were reported in permafrost from antarctic and arctic sites. These investigations led to numerous and varieties of viable microorganism from different cold ecosystems.

Russian scientists reported  $10^2 - 10^8$  cell/g of viable micro-organisms from Siberian permafrost (Gilichinsky, 1992). The bacterial counts in different cold ecosystems are illustrated in Table 1.4.

Region	CFU (cell/g dry soil/ ice or ml of water)	References
High Canadian Arctic permafrost	$10^1 - 10^3$	Steven et al., (2007)
Antarctic		
Qinghia-Tibet plateau permafrost	$0 - 10^7$	Zhang et al., (2007)
Siberian permafrost	$0 - 10^8$	Gilichinsky (1992)
Alpine permafrost from Tianshan Mountains	$10^5 - 10^6$	Bai et al., (2006)
High Arctic permafrost soil from Spitsbergen, Northern Norway	$10^5 - 10^6$	Hansen et al., (2007)
Greenland Glacier ice/permafrost	$10^2$	Miteva et al., (2004)
Siberian permafrost	$10^2 - 10^8$	Rivkina et al., (1998)
South Island of New Zealand	$10^6$	Foght et al., (2004)

TABLE 1.4 – Enumeration of bacterial population from permafrost and alpine tundra

## Phenotypic characterization and adaptation to cold condition

Various microscopic observations demonstrated a wide range of morphologies including rod and cocci. Some of these investigations showed that majority of these isolates were rod (Shi et al., 1997; Bai et al., 2006; Zhang et al., 2007).

Gram staining cell wall showed characteristics of both Gram-positive and Gram-negative by different reports (Table 1.5) on gram behaviour from arctic and alpine tundra (Shi et al., 1997; Bai et al., 2006; Zhang et al., 2007; Hansen et al., 2007). The Russian scientists reported that bacteria showed the highest viability in cold ecosystem, maybe because of surface capsular layers of both gram-negative and gram-positive bacteria (Soina et al., 1994).

The first question after the presence of bacteria in these extreme ecosystems is that "Do these micro-organisms are metabolically active?" Although, the definitive answer is still unclear, the current experiments and observations demonstrated that there is the metabolic activity at a very low level. These metabolic activities provide the functions for survival that including repairing of DNA damage and maintenance of membrane integrity (Shi et al., 1997). Certain studies showed that bacteria trend to form the pigmented colony that suggests playing a role in life adaptation to low temperature to environmental stress (Foght et al., 2004; Miteva et al., 2004; Mueller et al., 2005). Other physical characteristics such as spore formation and enzymatic activity at low temperatures can allow surviving in harsh condition (Groudieva et al., 2004).

Region	Gram-negative(%)	Gram-positive(%)	References
Qinghia-Tibet plateau permafrost	57.6	42.4	Zhang et al., (2007)
Siberian Permafrost	55	45	Shi et al., (1997)
Alpine permafrost from Tianshan Mountains	39.5	60.4	Bai et al., (2006)
High Arctic permafrost soil from Spitsbergen,Northern Norway	15	85	Hansen et al.,(2007)

TABLE 1.5 – Percents of Gram behaviour bacteria from permafrost and alpine tundra

## Bacterial diversity in cold environment

There are a few studies on the bacterial diversity of Arctic and Antarctic regions. These results can be interpreted differently based on the used methods.

## **Bacterial diversity and structure based on culture**

The analyses of bacterial communities and diversity in these ecosystems were first assessed through culture dependent methods. Bacterial community structure using different media and temperatures have been studied in some of these ecosystems (Foght et al., 2004; Miteva et al., 2004; Mueller et al., 2005). These cultivable bacteria isolates, identified by 16S rRNA gene sequencing, belonged to the *Actinobacteria*, *Firmicutes* and *Proteobacteria* (Shi et al., 1997; Bakermans et al., 2003; Bai et al., 2006; Vishnivetskaya et al., 2006; Hansen et al., 2007; Steven et al., 2007; Zhang et al., 2007) and Bacteroidetes (Bai et al., 2006; Vishnivetskaya et al., 2006; Hansen et al., 2007; Zhang et al., 2007). They showed a wide variety of bacteria including *Bacillus*, *Arthrobacter*, *Micrococcus*, *Pseudomonas*, *Flavobacterium*, *Cytophaga*, *Acinetobacter*, *Streptomyces* and *Rhodococcus* from world wild permafrosts (Vishnivetskaya et al., 2006).

## **Bacterial diversity and structure based on method molecular**

The use of cultural-independent methods provided a global view of diversity and bacterial community structure. A useful method to study communities utilizes 16S rRNA gene clones libraries constructed from extracted DNA. These libraries can determine bacterial diversity and structure that is essential to understanding the role of these bacteria in nature and their impact on biogeochemical cycles.

Some investigations reported the abundance or presence of bacterial phyla. For example, Hensen and colleagues reported the presence of *Thermomicrobia*, *Mollicutes*, *Spirochaetes*, *Acidobacteria*, *Bacteroidetes*, *Verrucomicrobia*, *Proteobacteria* and *Planctomycetacia* in a high Arctic permafrost soil from Spitsbergen, Northern Norway (Hansen et al., 2007) or the wet meadow in the Niwot Ridge after snow melt was dominated by *Acidobacteria*, followed by the  $\beta$ - and  $\alpha$ -*Proteobacteria* (Costello and Schmidt, 2006). Some of these investigations reported that a large proportion of bacterial communities in cold ecosystems were affiliated to *Acidobacteria* (Zhou et al., 1997; Saul et al., 2005; Smith et al., 2006).

Already, some studies reported the seasonal variations of such bacterial communities (Zogg et al., 1997; Bossio et al., 1998; Bardgett et al., 1999). They showed a significant temporal change in microbial composition. Shortly after, Lipson et al in alpine dry meadow in the Niwot Ridge demonstrated changes in soil microbial biomass across seasons (Lipson et al., 2002). They concluded that these microbial communities were affected by changes in

temperature and nutrient availability and therefore vary seasonally. This hypothesis led to several experiences in dry alpine meadows.

In 2002, they showed that the winter microbial communities had higher cellulase activity than spring or summer communities and were more adapted to cold temperature, whereas the summer microbial communities were related to rhizodeposition. Until 2004, all of these investigations explained microbial communities did not include more detailed information on seasonal variation or specific phyla.

In 2004, Lipson and Schmidt used 16S rRNA for determining seasonal variation in dry alpine meadows. Their studies demonstrated that *Acidobacteria*, *α-Proteobacteria* and *Verrucomicrobia* were more frequent than other throughout the year (Figure 1.8-A).

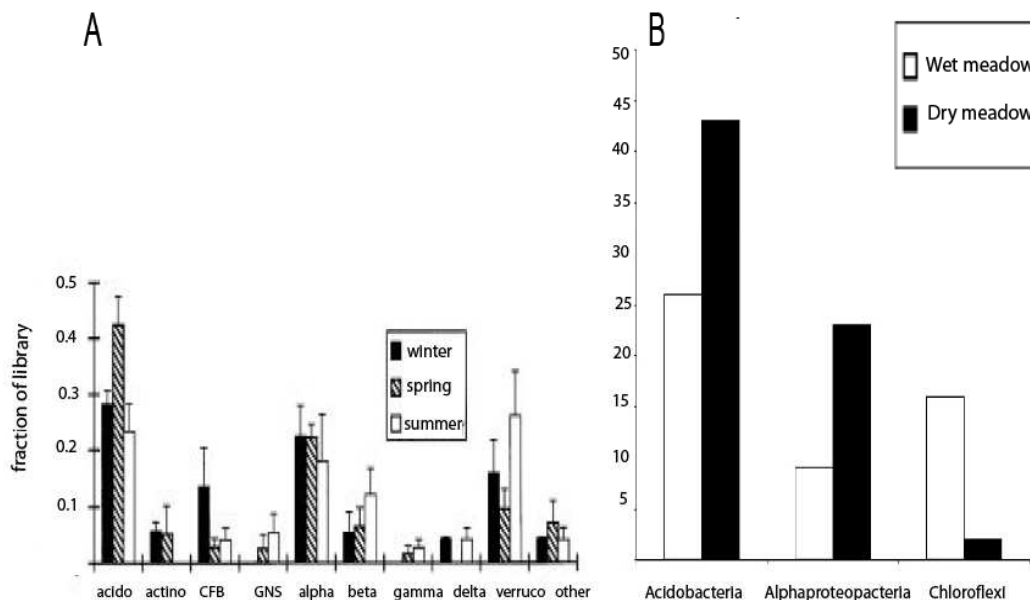


FIGURE 1.8 – A: Frequency of bacterial group in a dry alpine meadow using 16S RNA (Lipson and Schmidt, 2004) from winter, spring and summer samples. This analysis is based on 185 sequences from spring (snowmelt), summer (growth vegetation) and winter (snowpack). B: A comparison of frequency of major bacterial groups in wet meadow and dry meadow during spring (Nemergut et al., 2005).

However, the other bacterial groups displayed seasonal variations. For example *Acidobacteria* were more abundant after snowmelt that can be explained by anaerobic conditions. The high frequencies of *Verrocomicrobia* in summer can be related to plant root activity. The highest abundance of *Actinobacteria* and *Bacteroidetes* was detected in winter, and these groups are known to degrade material complex (Cottrell and Kirchman, 2000). Instead, they failed to find significant variations for *α-Proteobacteria*. They showed that microscopic count of total

bacterial in summer was significantly more elevated than that in winter and bacteria were also more active.

Nemergut (Nemergut et al., 2005) showed that in Niwot Ridge, topography play an important role in bacterial structure and composition (Figure 1.8-B). She emphasized that *Acidobacteria*,  *$\alpha$ -Proteobacteria* and *Chloroflexi* changed between dry (dominated by *Kobresia myosurides*) and wet (dominated by *Carex scopulorum*) meadows. Of course, this comparison was done for only spring season (snow melt) and this proportion related to bacterial groups may change over time for each meadow.

The results obtained by different studies were different. The investigations of David Wallenstein in Toolik Lake Long-Term Ecological Research (LTER) site demonstrated a seasonal and spatial variation in bacterial composition (Wallenstein et al., 2007). They showed that bacterial composition changes over time (prefreeze and post-thaw) and over space (the plant Tussock and Shrub). They resumed that the variation which can be attributed to plant composition can be more important than seasonal change. By contrasted, similar study was assessed in midi-alpine using PLFA by Bjork that showed change over time in the soil microbial community was more significant than plant community (Lipson and Schmidt, 2004; Bjork et al., 2008). Now, it is accepted that in alpine ecosystem, the bacterial compositions change over time and space. This question of seasonal variation is more important or spatial change will be open and response is mysterious.

*The snow covered ecosystems are a unique opportunity for investigations of microbial communities. In recent decades, in spite of developing of molecular methods, our knowledge on bacterial diversity, structure phylogenetic and functional remains weak. Available information on cold ecosystems is restricted to permafrost ecosystems that are not similar to tempered alpine tundra. In particular, at cultivated bacteria domain in alpine environment is very rare information.*



## **Chapitre 2**

# **Effet des régimes d'enneigement sur la diversité et relative abondance de la communauté bactérienne des sols alpins**



## Contexte général

Une grande part des écosystèmes terrestres est recouverte de neige durant l'hiver. L'enneigement le plus marqué concerne les écosystèmes arctiques et alpins. Une grande quantité du carbone organique terrestre est séquestrée dans les sols de ces milieux enneigés via une activité réduite des micro-organismes du sol (Davidson and Janssens, 2006). Ces sols sont ainsi considérés comme très vulnérables au changement climatique (Hobbie et al., 2000). En effet, l'augmentation de la température pourrait entraîner une reprise de l'activité microbienne, et donc, une augmentation de la minéralisation du carbone organique. Ce processus est accompagné d'une plus grande libération de CO<sub>2</sub> par respiration de la microflore du sol. Cette reprise de l'activité microbienne conduirait ainsi à une diminution nette du pool de carbone dans les écosystèmes froids.

Les écosystèmes alpins présentent une forte hétérogénéité spatiale liée à une topographie, une exposition, et une roche mère différentes. Cette hétérogénéité abiotique peut donner lieu à des changements du cortège floristiques à petite échelle. Aux deux extrêmes du gradient topographique, on distingue les écosystèmes thermiques (crête) et nivaux (combe à neige). Bien que ces deux localisations soient séparées par environ 20 m, ils diffèrent fortement par la durée de la couverture neigeuse en hiver en raison des effets topographiques. Les écosystèmes thermiques présentent un faible enneigement ou un manteau neigeux instable et sont soumis à des phénomènes de gel/dégel. Un faible enneigement entraîne une saison de végétation assez longue, promouvant des espèces végétales stress tolérantes à litière récalcitrante et d'autant plus difficile à dégrader à cause de températures hivernales basses (Thèse Baptist. F). En revanche, les systèmes nivaux sont protégés de la rigueur de l'hiver grâce à un épais manteau neigeux qui maintient la température de sol autour de 0°C pendant l'hiver. Cet enneigement plus long diminue la durée de la saison de végétation, favorisant des espèces végétales à croissance rapide dont les litières sont plus faciles à dégrader. Cette différence d'enneigement entre les deux écosystèmes, pourtant spatialement très proches, semble induire des différences de comportement vis-à-vis de leur cycle du carbone.

Malgré une prise de conscience croissante de l'importance des micro-organismes dans les cycles biogéochimiques et dans un contexte de réchauffement global, la composition et la dynamique des communautés microbiennes alpines restent mal connues. Néanmoins, la dynamique saisonnière des communautés bactériennes et fongiques a été caractérisée dans les écosystèmes thermiques couverts par *Kobresia myosuroides* (Schadt et al, 2003; Lipson and

Schmidt, 2004). De même, les effets du régime d'enneigement sur la composition des communautés bactériennes et fongiques ont déjà été appréhendés par utilisation de PLFA (PhosphoLipidic Fatty Acids, Bjork et al, 2008), mais leur diversité et leur composition demeurent bien mal caractérisées.

## Objectifs de l'étude

Notre objectif a été de tester les variations spatiales et temporelles des bactéries et des champignons dans des écosystèmes alpins dans les régimes d'enneigement sont contrastés. Le site d'étude était composé de deux types écosystèmes alpins (Figure 2.1): un domaine thermique (crête) et un domaine nival (combe à neige). L'étude a été menée sur 3 répliques de sites combe/crêtes (sites A, B et C). Les prélèvements de sol ont été répétés 5 fois. Afin suivre la dynamique saisonnière de la microflore du sol, les prélèvements ont été réalisés dans les conditions suivantes : au milieu de la saison végétative (Août), en fin de saison de végétation (Octobre), sous la couverture neigeuse en combe en conditions de gel/dégel en crête (Mai), à la fonte des neiges en combe et en début de saison de végétation en crête (Juin).

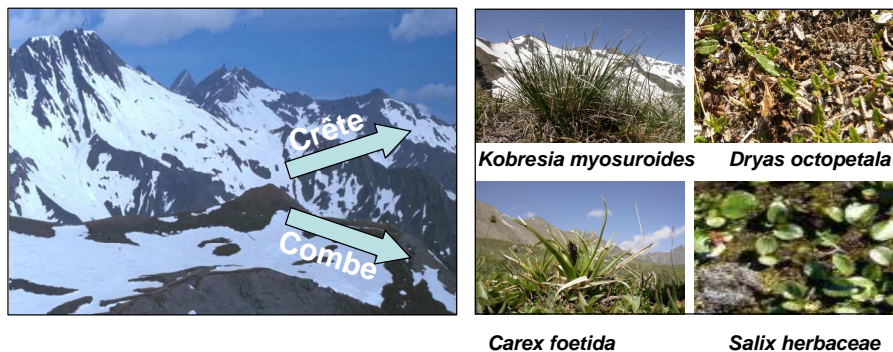


FIGURE 2.1 – Les deux types d'écosystème étudiés présentent des régimes d'enneigement différents, et par conséquent sont dominés par des espèces végétales différentes.

La structure des communautés bactériennes (en rapport à ma thèse) et des communautés de champignons (Thèse Zinger. L) ont été évaluées à l'aide d'une technique d'empreintes moléculaires (SSCP, Single Strand Conformation Polymorphism) pendant un an dans les trois sites, et sur deux années dans le site B. Les communautés de la première année sur le site B ont été caractérisées par clonage / séquençage. La diversité moléculaire des communautés

microbiennes a été examinée au cours de l'année comme précédemment mentionné (**Article I**).

Par ailleurs, une étude destinée à tester l'impact de la qualité de la litière et de la température sur les communautés microbiennes en situation thermique (crête) a été effectué en collaboration avec l'équipe de MICROALPES en 2005 (**Annexe**).

## **Article I: Microbial diversity in alpine tundra soils correlates with snow cover dynamics**

Lucie Zinger, **Bahar Shahnava**z, Florence Baptist, Roberto A. Geremia, Philippe Choler

*ISME Journal* (2009) doi:10.1038/ismej.2009.20: 10p

## ORIGINAL ARTICLE

# Microbial diversity in alpine tundra soils correlates with snow cover dynamics

Lucie Zinger<sup>1</sup>, Bahar Shahnava<sup>1</sup>, Florence Baptist<sup>1</sup>, Roberto A Geremia<sup>1</sup> and Philippe Choler<sup>1,2,3</sup>

<sup>1</sup>Laboratoire d'Ecologie Alpine, CNRS UMR 5553, Université de Grenoble, BP 53, Grenoble Cedex, France;

<sup>2</sup>Station Alpine J Fourier CNRS UMS 2925, Université de Grenoble, Grenoble, France and <sup>3</sup>CSIRO Marine and Atmospheric Research, Canberra, Australia

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.

*The ISME Journal* (2009) 0, 000–000; doi:10.1038/ismej.2009.20

**Subject Category:** microbial ecology and functional diversity of natural habitats

**Keywords:** alpine tundra; microbial diversity; carbon cycle; global change

## 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 (Olear 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

Correspondence: RA Geremia, Laboratoire d'Ecologie Alpine UJF/CNRS, Université de Grenoble, 2233, rue de la Piscine, BP 53 Bat D Biologie, Grenoble F-38041, France.

E-mail: roberto.geremia@ujf-grenoble.fr

Received 5 January 2009; revised 12 February 2009; accepted 12 February 2009

dynamics in relation to snow cover patterns have been reported (Zak and Kling, 2006; Bjork *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 for snowcover 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

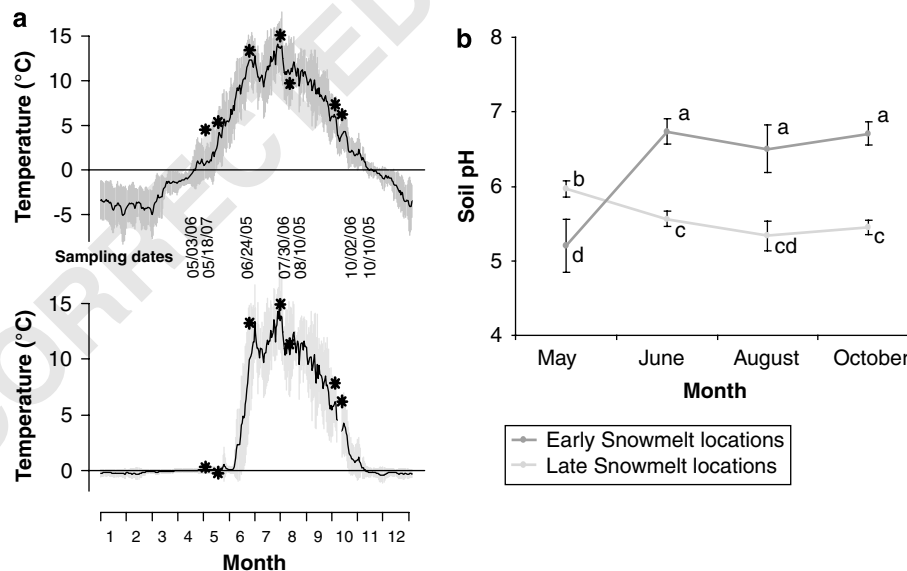
### Sample collection and soil characterization

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<sup>2</sup>. 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 ( $\pm$  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  $\pm$  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'-ACGGTCCA-GACTCCTACGGG-3') and W104-FAM (5'-TT ACCGCGGCTGCTGGCAC-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  $\mu$ l) were set up as follows: 2.5 mM of MgCl<sub>2</sub>, 1 U of AmpliTaq Gold polymerase (Applied Biosystems, Courtaboeuf, France), 1  $\times$  of buffer provided by the manufacturer, 20 gl<sup>-1</sup> of bovine serum albumin, 0.1 mM of each dNTP, 0.2  $\mu$ M 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'-CAGGCCTAACACATGCAAGTC-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'-CTAGATGAACYAACACCTT-3') (Schadt *et al.*, 2003). PCRs were carried out with 2.5 mM MgCl<sub>2</sub>, 0.1 mM each ddNTP, 0.4  $\mu$ M (bacteria) or 0.2  $\mu$ M (fungi) each primer, 1 U AmpliTaq Gold polymerase, 1  $\times$  of buffer provided by the manufacturer, 20 gl<sup>-1</sup> 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 elonga-

tion 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<sup>-1</sup> 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 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

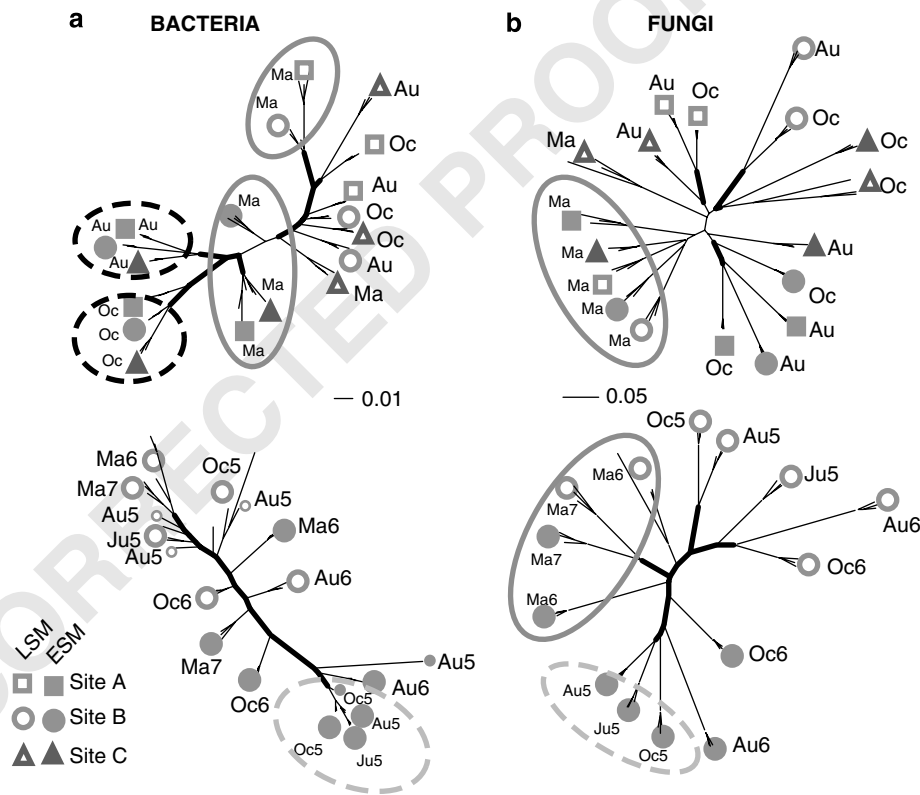
**Table 1** Characteristics of LSM and ESM locations

	LSM situation	ESM situation
<i>Plant cover</i>		
Dominant species	<i>Carex foetida</i> All. <i>Alchemilla pentaphyllea</i> L. <i>Salix herbacea</i> L. <i>Alopecurus alpinus</i> Vill.	<i>Kobresia myosuroides</i> <i>Dryas octopetala</i> <i>Carex curvula</i> All. <i>subsp. rosae</i> Gilomen
<i>Soil characteristics</i>		
Soil classification	Stagnogley enriched in clay	Alpine Ranker
% Organic matter (top soil 10 cm)	8.7 ± 2.5	15.7 ± 4.7
<i>Grain size analysis</i>		
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



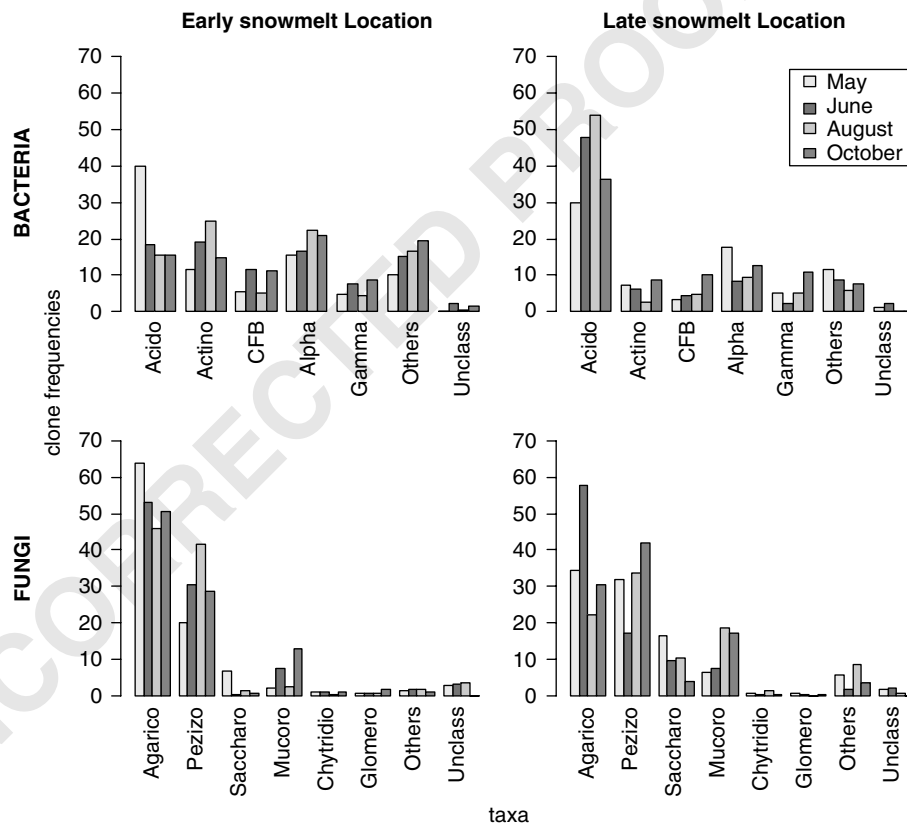
**Figure 2** Seasonal variations of bacterial and fungal communities assessed by CE-SSCP. The molecular profile of fungal and bacterial communities was obtained as described in Material and methods, using one DNA pool per each location/site/date. PCRs were carried out by triplicate to limit the influence 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-grey 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 micro-

bial 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 small 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, *Cytophaga-Flexibacter-Bacteroides*; Alpha and Gamma,  $\alpha$  and  $\gamma$  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 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

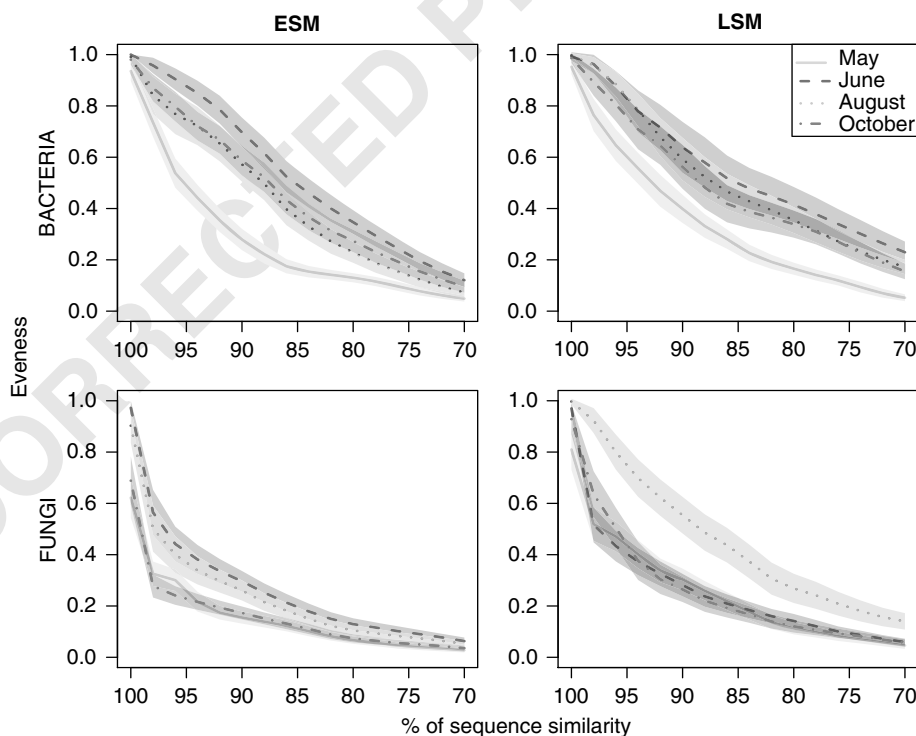
The diversity index of microbial communities estimated from clone libraries revealed that bacterial diversity was not different between ESM and LSM locations (Figure 4). Nevertheless, the diversity of ESM bacterial communities was higher in June. In contrast, LSM bacterial diversity was found to be stable 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 (Olear 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).

We found that ESM bacterial communities were correlated with growing season progress (Figure 2a).



**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.

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; Mougél *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; Bjork *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). As fungi are mostly aerobic, their diversity decreased during snowmelt when soils were waterlogged. 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; Bjork *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.

### Acknowledgements

We thank Pierre Taberlet, Jean-Marc Bonneville, Jérôme Gury, David Lejon (LECA) and Richard Bardgett (Lancaster) for reading the paper, helpful suggestions and discussions, Armelle Monier for technical assistance; and Alice Roy and the Centre National de Séquençage (Genoscope, Evry) for sequencing the libraries; and Serge Aubert and the staff of Station Alpine J Fourier for providing logistic facilities during the field work. The study was supported by the ANR-06-BLAN-0301 'Micro-alpes' project.

### References

- Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W *et al.* (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**: 3389–3402.
- Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu Rev Plant Biol* **57**: 233–266.
- Bardgett RD, Bowman WD, Kaufmann R, Schmidt SK. (2005). A temporal approach to linking aboveground and belowground ecology. *TRENDS Ecol Evol* **20**: 634–641.
- Beniston M, Fox DG, Adhikary S, Andressen R, Guisan A, Holten J *et al.* (1996). The impacts of climate change on mountain regions. *Second Assessment Report of the Intergovernmental Panel on Climate Change (IPCC)*, Chapter 5. Cambridge University Press. pp 191–213.

- Billings WD. (1973). Arctic and alpine vegetations: similarities, differences, and susceptibility to disturbance. *BioScience* **23**: 697–704.
- Bjork RG, Bjorkman MP, Andersson MX, Klemedtsson L. (2008). Temporal variation in soil microbial communities in alpine tundra. *Soil Biol Biochem* **40**: 266–268.
- Bowman WD, Theodose TA, Schardt JC, Conant RT. (1993). Constraints of nutrient availability on primary production in two alpine tundra communities. *Ecology* **74**: 2085–2097.
- Butler JL, Williams MA, Bottomley PJ, Myrold DD. (2003). Microbial community dynamics associated with rhizosphere carbon flow. *Appl Environ Microb* **69**: 6793–6800.
- Chapman SK, Langley JA, Hart SC, Koch GW. (2006). Plants actively control nitrogen cycling: uncorking the microbial bottleneck. *New Phytol* **169**: 27–34.
- Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG *et al.* (2003). Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Res* **31**: 3497–3500.
- Choler P. (2005). Consistent shifts in alpine plant traits along a mesotopographical gradient. *Arct Antarct Alp Res* **37**: 444–453.
- Cole JR, Chai B, Marsh TL, Farris RJ, Wang Q, Kulam SA *et al.* (2003). The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. *Nucleic Acids Res* **31**: 442–443.
- Cottrell MT, Kirchman DL. (2000). Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. *Appl Environ Microb* **66**: 1692–1697.
- Crawford DL. (1978). Lignocellulose decomposition by selected *Streptomyces* strains. *Appl Environ Microb* **35**: 1041–1045.
- Davidson EA, Janssens IA. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* **440**: 165–173.
- de Boer W, Folman LB, Summerbell RC, Boddy L. (2005). Living in a fungal world: impact of fungi on soil bacterial niche development. *FEMS Microbiol Rev* **29**: 795–811.
- Delbes C, Godon JJ, Moletta R. (1998). 16S rDNA sequence diversity of a culture-accessible part of an anaerobic digester bacterial community. *Environ Microbiol* **4**: 267–275.
- Edwards AC, Scalenghe R, Freppaz M. (2007). Changes in the seasonal snow cover of alpine regions and its effect on soil processes: a review. *Quatern Int* **162**: 172–181.
- Falcon MA, Rodriguez A, Carnicero A, Regalado V, Perestelo F, Milstein O *et al.* (1995). Isolation of microorganisms with lignin transformation potential from soil of Tenerife island. *Soil Biol Biochem* **27**: 121–126.
- Farrar J, Hawes M, Jones D, Lindow S. (2003). How roots control the flux of carbon to the rhizosphere. *Ecology* **84**: 827–837.
- Fierer N, Bradford MA, Jackson RB. (2007). Toward an ecological classification of soil bacteria. *Ecology* **88**: 1354–1364.
- Fierer N, Jackson RB. (2006). The diversity and biogeography of soil bacterial communities. *Proc Natl Acad Sci USA* **103**: 626–631.
- Fisk MC, Schmidt SK, Seastedt TR. (1998). Topographic patterns of above- and below-ground production and nitrogen cycling in alpine tundra. *Ecology* **79**: 2253–2266.
- Gardes M, Dahlberg A. (1996). Mycorrhizal diversity in arctic and alpine tundra: an open question. *New Phytol* **133**: 147–157.
- Hobbie SE, Schimel JP, Trumbore SE, Randerson JR. (2000). Controls over carbon storage and turnover in high-latitude soils. *Glob Change Biol* **6**: 196–210.
- Huber T, Faulkner G, Hugenholtz P. (2004). Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. *Bioinformatics* **20**: 2317–2319.
- Johansson JF, Paul LR, Finlay RD. (2004). Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiol Ecol* **48**: 1–13.
- Körner C. (1999). *Alpine Plant Life*. Springer Verlag: Berlin.
- Lauber CL, Strickland MS, Bradford MA, Fierer N. (2008). The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biol Biochem* **40**: 2407–2415.
- Lipson DA, Schmidt SK. (2004). Seasonal changes in an alpine soil bacterial community in the Colorado Rocky Mountains. *Appl Environ Microb* **70**: 2867–2879.
- Lipson DA, Schmidt SK, Monson RK. (1999). Links between microbial population dynamics and nitrogen availability in an alpine ecosystem. *Ecology* **80**: 1623–1631.
- Litaor MI, Seastedt TR, Walker DA. (2001). Spatial analysis of selected soil attributes across an alpine topographic/snow gradient. *Landscape Ecol* **17**: 71–85.
- Marchesi JR, Sato T, Weightman AJ, Martin TA, Fry JC, Hiom SJ *et al.* (1998). Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Appl Environ Microbiol* **64**: 795–799.
- Marshall JD, Blair JM, Peters DPC, Okin G, Rango A, Williams M. (2008). Predicting and understanding ecosystem responses to climate change at continental scales. *Front Ecol Environ* **6**: 273–280.
- Martin F, Aerts A, Ahren D, Brun A, Danchin EGJ, Duchaussoy F *et al.* (2008). The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. *Nature* **452**: 88–92.
- Melillo JM, Steudler PA, Aber JD, Newkirk K, Lux H, Bowles FP *et al.* (2002). Soil warming and carbon-cycle feedbacks to the climate system. *Science* **298**: 2173–2176.
- Mille-Lindblom C, Fischer H, Tranvik LJ. (2006). Antagonism between bacteria and fungi: substrate competition and a possible tradeoff between fungal growth and tolerance towards bacteria. *Oikos* **113**: 233–242.
- Mougel C, Offre P, Ranjard L, Corberand T, Gamalero E, Robin C *et al.* (2006). Dynamic of the genetic structure of bacterial and fungal communities at different developmental stages of *Medicago truncatula* Gaertn. cv. Jemalong line J5. *New Phytol* **170**: 165–175.
- Oechel WC, Vourlitis GL, Hastings SJ, Zulueta RC, Hinzman L, Kane D. (2000). Acclimation of ecosystem CO<sub>2</sub> exchange in the Alaskan Arctic in response to decadal climate warming. *Nature* **406**: 978–981.
- Olear HA, Seastedt TR. (1994). Landscape patterns of litter decomposition in alpine tundra. *Oecologia* **99**: 95–101.
- Read DJ, Perez-Moreno J. (2003). Mycorrhizas and nutrient cycling in ecosystems—a journey towards relevance? *New Phytol* **157**: 475–492.

- Sait M, Davis KER, Janssen PH. (2006). Effect of pH on isolation and distribution of members of subdivision 1 of the phylum *Acidobacteria* occurring in soil. *Appl Environ Microb* **72**: 1852–1857.
- Sandhu GS, Kline BC, Stockman L, Roberts GD. (1995). Molecular probes for diagnosis of fungal-infections. *J Clin Microbiol* **33**: 2913–2919.
- Schadt CW, Martin AP, Lipson DA, Schmidt SK. (2003). Seasonal dynamics of previously unknown fungal lineages in tundra soils. *Science* **301**: 1359–1361.
- Schmalenberger A, Schwieger F, Tebbe CC. (2001). Effect of primers hybridizing to different evolutionarily conserved regions of the small-subunit rRNA gene in PCR-based microbial community analyses and genetic profiling. *Appl Environ Microb* **67**: 3557–3563.
- Schmidt SK, Costello EK, Nemergut DR, Cleveland CC, Reed SC, Weintraub MN et al. (2007). Biogeochemical consequences of rapid microbial turnover and seasonal succession in soil. *Ecology* **88**: 1379–1385.
- Schulte EE, Hopkins GG. (1996). Estimation of organic matter by weight loss-on-ignition. In: Magdoff FR et al. (eds). *Soil Organic Matter: Analysis and Interpretation*. SSSA spec. Publ. 46. SSSA: Madison, WI. pp 21–31.
- Schwarzenbach K, Enkerli J, Widmer F. (2007). Objective criteria to assess representativity of soil fungal community profiles. *J Microbiol Meth* **68**: 358–366.
- Smith B, Wilson JB. (1996). A consumer's guide to evenness indices. *Oikos* **76**: 70–82.
- Sogin ML, Morrison HG, Huber JA, Mark Welch D, Huse SM, Neal PR et al. (2006). Microbial diversity in the deep sea and the underexplored 'rare biosphere'. *Proc Natl Acad Sci USA* **103**: 12115–12120.
- Stach JEM, Bathe S, Clapp JP, Burns RG. (2001). PCR-SSCP comparison of 16S rDNA sequence diversity in soil DNA obtained using different isolation and purification methods. *FEMS Microbiol Ecol* **36**: 139–151.
- The R Development Core Team (2007). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing: Wien.
- Waldrop MP, Zak DR, Blackwood CB, Curtis CD, Tilman D. (2006). Resource availability controls fungal diversity across a plant diversity gradient. *Ecol Lett* **9**: 1127–1135.
- Walker DA. (2000). Hierarchical subdivision of Arctic tundra based on vegetation response to climate, parent material and topography. *Global Change Biol* **6**: 19–34.
- Welker JM, Fahnestock JT, Jones MH. (2000). Annual CO<sub>2</sub> flux in dry and moist arctic tundra: field responses to increases in summer temperatures and winter snow depth. *Climatic Change* **44**: 139–150.
- White TJ, Bruns T, Lee S, Taylor J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Shinsky JJ, White TJ (eds). *PCR Protocols: A Guide to Methods and Applications*. Academic Press: San Diego. pp 315–322.
- Yergeau E, Bokhorst S, Huiskes AHL, Boschker HTS, Aerts R, Kowalchuk GA. (2007a). Size and structure of bacterial, fungal and nematode communities along an Antarctic environmental gradient. *FEMS Microbiol Ecol* **59**: 436–451.
- Yergeau E, Newsham KK, Pearce DA, Kowalchuk GA. (2007b). Patterns of bacterial diversity across a range of Antarctic terrestrial habitats. *Environ Microbiol* **9**: 2670–2682.
- Zak DR, Kling GW. (2006). Microbial community composition and function across an arctic tundra landscape. *Ecology* **87**: 1659–1670.
- Zinger L, Gury J, Alibeu O, Rioux D, Gielly L, Sage L et al. (2008). CE-SSCP and CE-FLA, simple and high-throughput alternatives for fungal diversity studies. *J Microbiol Meth* **72**: 42–53.
- Zinger L, Gury J, Giraud F, Krivobok S, Gielly L, Taberlet P et al. (2007). Improvements of polymerase chain reaction and capillary electrophoresis single-strand conformation polymorphism methods in microbial ecology: toward a high-throughput method for microbial diversity studies in soil. *Microb Ecol* **54**: 203–216.



## Principaux résultats et discussion

La CE-SSCP est une technique d'empreinte moléculaire utilisée pour l'étude des communautés microbiennes ; elle repose en effet sur la séparation des fragments d'ADN selon leur taille mais aussi selon leur séquence. La CE-SSCP étant optimale pour des brins d'ADN de petite taille, nous avons choisi en conséquence des marqueurs moléculaires adaptés à savoir : la région V3 (bactéries) de l'ADNr 16S. Cette approche permet de prendre en compte (ou d'approcher) la structure des communautés microbiennes et est adaptée pour une grande variété microbienne.

Un outil informatique permet de récupérer les données numériques des profils SSCP préalablement normalisés avec le logiciel Scilab. Enfin, la normalisation des données nous permet de calculer une matrice de distances entre les différents profils et de construire un dendrogramme par Neighbor-Joining. La robustesse du dendrogramme est testée par bootstrapping (1000 retirages). Cette méthode a été développée avec le logiciel R (avec l'aide de P. Choler). Les résultats obtenus par SSCP pour les champignons et les bactéries, montrent une variation saisonnière des communautés dans chaque type d'écosystème (Article I). Ce résultat s'applique également aux *Acidobactéries* et *Actinobactéries*, qui ont été suivies en parallèle à l'aide d'amorces spécifiques (Figure 2.2). Le V3 de gènes de l'ARNr 16S a été amplifiée avec des amorces PA (Barn et al., 1999) et W104-FAM (Delbes et al., 1998) pour *Acidobactéries* et F243 et R513-FAM (Heuer et al., 1997) pour *Actinobactéries*.

La répartition et la dynamique des *Acidobactéries* sont similaires à celle de l'ensemble des bactéries ce qui s'explique par leur forte abondance dans les sols étudiés (Article I). Les communautés bactériennes sont bien groupées en crête surtout pendant la saison de végétation ce qui confirme l'effet de la composition des communautés végétales sur la structure des communautés bactériennes.

L'effet de la composition et de la diversité des plantes sur la structure des micro-organismes a été démontré par plusieurs études (Bardgett et al., 1999; Smalla et al., 2001; Zak et al., 2003), notamment dans les écosystèmes arctiques (Yergeau et al., 2007a et b). Les plantes peuvent structurer les communautés bactériennes de différentes façons : par leur exsudats racinaires, et par la qualité ou la quantité de leur litière. De plus, la qualité des exsudats racinaires peut varier selon l'espèce végétale (Maloney et al., 1997) et selon le stade de développement de la plante (Mougel et al., 2006). L'effet des plantes n'a pas seulement été observé sur la structure

des communautés bactériennes, mais aussi sur leur diversité pendant la saison de végétation en crête. En effet, la diversité bactérienne en crête était plus élevée pendant le mois de Juin indiquant que le stade de développement des plantes peut impacter la diversité bactérienne. Cependant, en termes de diversité globale, nous n'avons pas observé de différence entre combe et crête. La diversité bactérienne en combe était stable tout au cours de l'année et peut être reliée à l'abondance et la stabilité des *Acidobactéries* au fil des saisons.

Cependant, la diversité des communautés bactériennes diminue fortement en Mai, indépendamment de la localisation des échantillons indiquant qu'une majorité des espèces bactériennes alpines sont sensibles au froid. Bien que le pH du sol ait été rapporté comme étant le facteur structurant principal de la diversité bactérienne (Fierer et Jackson, 2006), la différence de pH entre combe et crête semble trop faible dans notre cas pour observer cet effet.

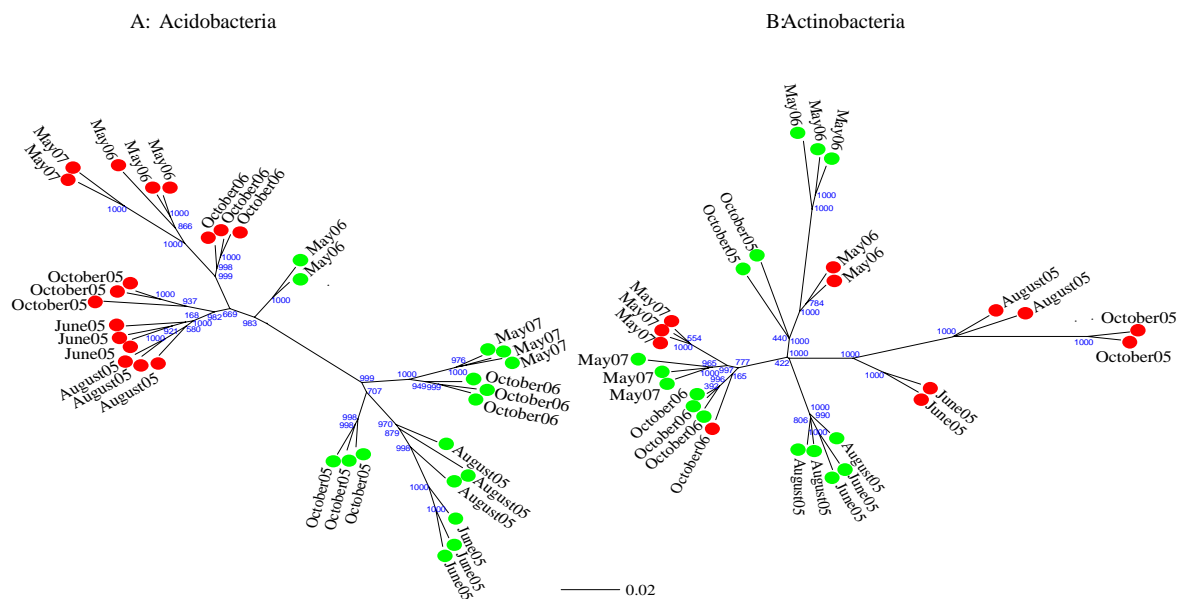


FIGURE 2.2 – Dendrogramme montrant les variations saisonnières des communautés de A) *Acido*- et B) *Actinobactéries* en situations thermiques et nivales. Ces dendrogrammes ont été obtenus à partir des données issues de la CE-SSCP. La distance d'Edwards et le Neighbor-Joining ont été utilisés pour construire les dendrogrammes. Les Valeurs de bootstrap (1000 retirages) sont indiquées par bleu. Les symboles vert et rouges présentent les échantillons correspondant de la crête et de la combe, respectivement.

Nous avons observé que la composition en espèce des communautés bactériennes est différente dans les deux écosystèmes étudiés (combe et crête). Il apparaît notamment que les différences majeures d'abondance relative concernent les *Acidobactéries*, les *Actinobactéries*

et les *a-Protéobactéries*. *Acidobactéries* qui sont notamment plus abondantes en combe, alors que les *Actinobactéries* et  *$\alpha$ -Protéobactéries* sont plus fréquentes en crête. Le pH du sol a été décrit comme étant un facteur structurant majeur de la composition des communautés bactériennes (Fierer et Jackson, 2006). Le pH acide des sols de combe peut donc être responsable de l'abondance des *Acidobactéries*. Dans ce sens, certaines espèces cultivables de *SDI* (*Acidobactéries*) sont favorisées par un milieu de pH ~ 5.5 (Sait et al., 2006) ; valeurs avoisinant le pH des sols de combe, et de crête pendant l'hiver. Ainsi, le changement de pH du sol en situation thermique en hiver concorde avec l'augmentation des *Acidobactéries* en crête, particulièrement le groupe *SDI*. Les différences de composition spécifique entre combe et crête peuvent également être dues à la matière organique (MO). Le sol de crête contient une plus grande quantité de MO ce qui peut favoriser la prolifération du phylum *Actinobactéries* (Article I). En effet, celles ci sont connues pour leur capacité à décomposer les polymères lignocellulosique et d'autres polymères récalcitrants (Crawford, 1978). L'événement majeur dans la dynamique des communautés microbiennes a lieu en hiver. Les changements de pH des sols cette période résulte en une convergence des communautés bactériennes de crêtes et de combe. Une analyse comparative des séquences par RDP (option- Library Compare) montre aussi que les plus faibles différences sont observées entre combe et crête au cours du mois de Mai (Table2.1).

	Acido	Actino	Alpha	Beta	Gamma	Delta	Bacteroidetes
Juin	4E - 14	6.3E - 5				3.4E - 3	1.2E - 3
Août	6E - 14	3E - 13	4E - 5				
Octobre	2E - 9	3.6E - 3		7.6E - 4			
Mai	3.4E - 3						

TABLE 2.1 – P-valeurs obtenues pour différents phyla en utilisant LIBCOMPARE entre les bibliothèques de crête et combe. Acido : *Acidobactéries*, Actino : *Actinobactéries*, Alpha, Beta, Gamma et Delta :  $\alpha$ - $\beta$ - $\gamma$ - et  $\delta$ -*Proteobactéries*.

*Ces résultats montrent que les différences édapho-climatiques et de couvert végétal entre les deux écosystèmes "combe" et "crête" ainsi que les variations saisonnières de ces variables environnementales peuvent fortement influencer la diversité, la composition et la structure des communautés bactériennes.*



**Chapitre 3**

**Effet des régimes d'enneigement sur la  
structure phylogénétique des  
communautés bactériennes des sols  
alpins**



## Contexte général

Aujourd'hui, il est admis que les bactéries jouent un rôle central dans le fonctionnement d'un écosystème et dans les cycles de nutriments. En conséquence, l'impact du réchauffement climatique sur ces organismes est devenu une préoccupation majeure (Monson et al., 2006; Fenchel, 2007). La toundra alpine est caractérisée par des gradients environnementaux de grande amplitude sur des distances courtes (Körner, 1999; Litaor et al., 2002; Edwards et al., 2007), et apparaît sensible au réchauffement climatique (Edwards et al., 2007). Dans le précédent chapitre, nous avons pu voir que l'effet des régimes d'enneigement était déterminant sur la composition des communautés bactériennes à l'échelle des phyla (Chapitre II). Malgré les nombreuses études traitant de la diversité micro-organismes dans l'environnement arctico-alpine (Schadt et al 2003; Lipson and Schmidt, 2004; Yergeau et al., 2007a et b ; Lipson, 2007; Bjork et al., 2008; Bryant et al., 2008), les connaissances sur la structure phylogénétique à une échelle taxonomique fine des communautés bactériennes reste mal décrite dans ces écosystèmes.

Or, l'information phylogénétique permet d'examiner les mécanismes écologiques et évolutifs qui touchent l'intégralité des communautés (Webb et al., 2002; Vamosi et al., 2009). Cette approche est basée sur le fait que la distance phylogénétique entre les deux espèces est une mesure de similarité de l'environnement, en supposant que les caractéristiques écologiques des taxons sont phylogénétiquement conservées. Ainsi, deux espèces phylogénétiquement proches (agrégées) ont tendance à montrer des préférences écologiques similaires (Figure 3.1).

La surdispersion phylogénétique, c'est-à-dire une structure phylogénétiquement très diversifiée (Figure 3.1) peut être expliquée par deux interactions biotiques: la compétition (Webb et al., 2002) ou la facilitation (Valiente-Banuet and Verdu, 2007). La compétition peut en effet se produire entre deux espèces phylogénétiquement proches, occupant des niches écologiques similaires, et résulte à terme à l'exclusion de l'une des espèces. Cette compétition aboutit donc au recrutement d'espèces phylogénétiquement distantes et aux préférences écologiques différentes. La facilitation a lieu lorsque des espèces modifient leur environnement en mettant en place de micro-habitats favorisant l'installation d'espèces phylogénétiquement distantes.

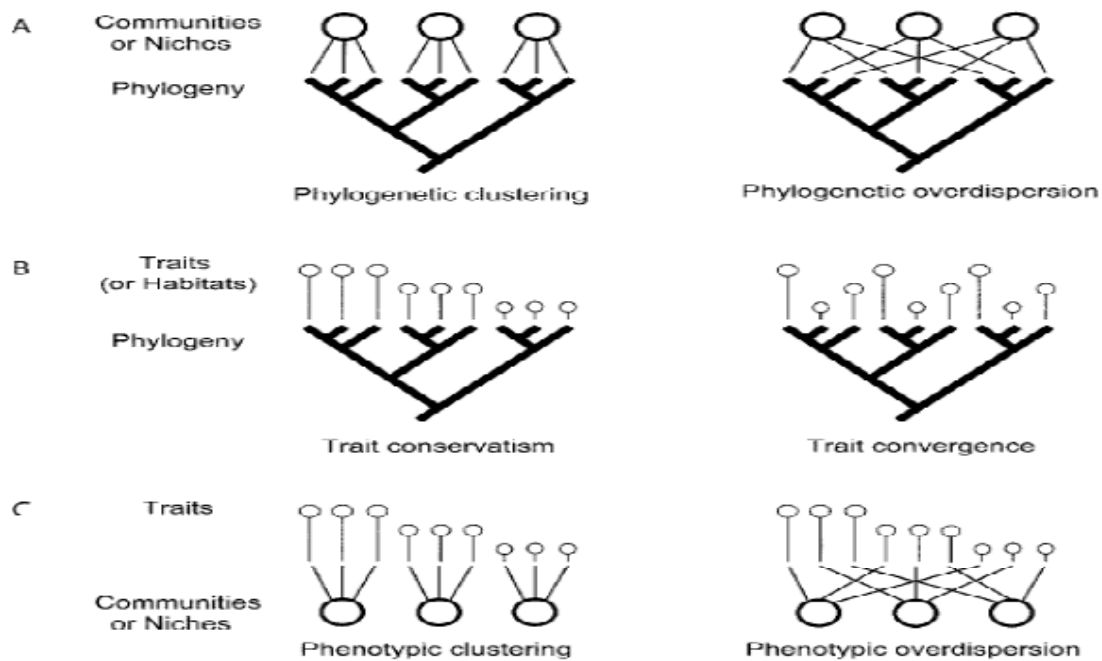


FIGURE 3.1 – Phylogénétique structure des communautés ou des niches peuvent montrer des modes de regroupement (à gauche) ou surdispersion (à droite).

Différents paramètres ont été utilisés pour caractériser la distribution des espèces d'une communauté, y compris le net relatedness index (NRI), le nearest taxon index (NTI, Webb et al., 2002), et la diversité phylogénétique  $\beta$ .

Le NRI correspond à la distance taxonomique moyenne entre des espèces (une espèce avec d'autres espèces au hasard) dans une communauté. Le NTI correspond quant à lui à la distance moyenne entre chaque espèce et son plus proche voisin dans la communauté (cf. Introduction). Les valeurs positives indiquent que la communauté est agrégée, alors que les valeurs négatives indiquent que les membres de la communauté sont surdispersés dans un arbre phylogénétique. Ces méthodes sont basées sur la présence/absence des espèces et non sur les abondances. Par contre le  $\beta$  diversité phylogénétique est basée sur les abondances des l'espèce présentées.

La diversité phylogénétique  $\beta$  mesure la partition de la diversité à travers d'un gradient environnemental en tenant compte des abondances relatives des espèces. La majorité de ce type d'analyse a, à ce jour, été appliqué aux plantes (voir Vamosi et al., 2009). En revanche, peu d'études ont examiné la structure phylogénétique des communautés bactériennes en utilisant les règles mentionnées au-dessous (mais voir Horner-Devine and Bohannan 2006 ;



Taxon	Type d'habitat	Niveau taxonomique	Niveau taxonomique examinée	Localisation	Nombre de sites	Agrégation/ Surdispersion	Référence
Bactéries	FW	Royaume	Oui	Michigan, USA	5	A	Horner-Devine and Bohannon, 2006
Bactéries du sol	Terre	Royaume	Non	Virginia+Delawa, USA	5	A/S	Horner-Devine and Bohannon, 2006
Bactéries ammoniac-oxydantes	Terre	Royaume	Non	Costa Rica	14	A	Horner-Devine and Bohannon, 2006
Bactéries ammoniac-oxydantes	SW	Royaume	Non	Maryland, USA	5	A	Horner-Devine and Bohannon, 2006
Bactéries dénitrifiantes	SW	Royaume	Non	Maryland, USA	5	A/S	Horner-Devine and Bohannon, 2006
Actinobactéries	FW	Royaume	Non	Wisconsin, USA	18	A	Newton et al., 2007
Acidobactéries	Terre	Royaume	Non	Rocky Mountains, Colorado, USA	5	A	Bryant et al., 2008

TABLE 3.1 – Résumé des études bactériennes analysant la structure phylogénétique. FW : eau douce, SW : eau du sol. A : Agrégation, A/S : l'agrégation a été la plus fréquente réponse, mais une surdispersion phylogénétique a aussi été observée (Vamosi et al., 2009)

Newton et al., 2007; Bryant et al., 2008). Ces études ont montré que la structure phylogénétique des communautés bactériennes a tendance à être plus agrégée que ne le prévoirait le hasard au sein d'une vaste gamme d'environnements (Table 3.1). Ces études montrent aussi que certains facteurs biotiques ou abiotiques ne recrutent que certains taxons dans les communautés bactériennes, comme par exemple l'altitude et le pH pour *Acidobactéries* et *acI* d'*Actinobactéries*, respectivement.

Les bactéries offre une occasion unique d'examiner les structure phylogénétique des communautés parce que les données de diversité bactérienne dans différent écosystèmes sont maintenant de plus en plus disponibles et peuvent être plus facilement interprétées par rapport aux communautés de macro-organismes, parce que la majorité de ces méthodes sont basées sur la distance entre les séquences qui sont plus disponibles pour les bactéries. Cependant, nos connaissances sur l'effet des facteurs environnementaux conduisant à une agrégation ou à une surdispersion de la structure phylogénétique des bactéries sont très peu connues.

L'étude de la variation temporelle et spatiale de la structure phylogénétique microbienne peut fournir de nouvelles informations concernant l'impact et l'effet filtrant des changements environnementaux sur les communautés microbiennes et donc sur la biodiversité.

## **Objectifs de l'étude**

Dans l'étude précédente (Chapitre II), nous avons déterminé la diversité  $\alpha$  pour chaque localisation combe/crête et à différentes saisons. Nous avons pu observé que la diversité  $\alpha$ , ainsi que l'abondance relative des grands groupes bactériens et fongiques sont influencée par différents facteurs biotiques et abiotiques, comme la composition de la végétation et le pH de sol. Cependant, nos méthodes ne permettent pas de déterminer l'impact et l'effet filtrant potentiel, de ces facteurs sur la structure phylogénétique des communautés bactériennes.

Les études phylogénétiques ne sont pas toujours suffisantes pour mettre en évidence l'effet de l'environnement sur les communautés bactériennes. Parce que les facteurs qui produisent des patterns phylogénétiques comme agrégation/surdispersion peuvent varier aux différentes échelles taxonomiques qui ne sont pas encore clair pour la communauté des bactéries. Dans plusieurs cas, les mécanismes de compétition entre espèces bactériennes sont mal connus.

Dans cette étude nous avons donc utilisé l'indice NTI qui permet de mettre en évidence l'effet de l'environnement sur l'agrégation et la surdispersion phylogénétique des différents groupes bactériens au cours de la saison dans une situation nivale (combe) et dans une situation

thermique (crête). Nous avons ensuite cherché à déterminer si les variations observées dans l'Article I sont dues à une agrégation des communautés via des événements sélectifs ou à une surdispersion des communautés par facilitation/compétition.

Par ailleurs, les résultats de l'Article I montrent que la variation spatiale, au niveau taxonomique du phylum, est plus importante que les variations saisonnières. Ces résultats ne nous permettent cependant pas de déterminer la façon dont ces variations s'effectuent à une résolution taxonomique fine. Ici, nous avons utilisé la méthode diversité phylogénétique  $\beta$  (cf. Introduction) afin de déterminer les écarts de diversité entre les deux localisations, entre les différentes saisons et l'interaction localisation/ saison à un niveau taxonomique plus fin. Pour cela, nous avons utilisé les séquences qui ont par ailleurs servi au calcul de la diversité et l'abondance relative des bactéries dans le Chapitre II.



## Article II: Snow cover dynamics and phylogenetic structure of bacterial communities in alpine tundra soils

**Bahar Shahnava**<sup>1</sup>, Lucie Zinger<sup>1</sup>, Sebastien Lavergne<sup>1</sup>, Philippe Choler<sup>1,2,3</sup>, Roberto A. Geremia<sup>1\*</sup>

**Submitted in Environmental Microbiology**

<sup>1</sup>Laboratoire d'Ecologie Alpine, CNRS UMR 5553, Université de Grenoble, BP 53, F-38041 Grenoble Cedex 09, France

<sup>2</sup>Station Alpine J. Fourier CNRS UMS 2925, Université de Grenoble, F-38041 Grenoble, France

<sup>3</sup>CSIRO Marine and Atmospheric Research, PO Box 1666, Canberra, ACT, 2601, Australia

\*Corresponding Author : Laboratoire d'Ecologie Alpine (LECA) UJF/CNRS UMR 5553 BP 53; Bât. D, F-38041 Grenoble Cedex 9, France

FAX: +33 (0) 476 51 42 79

Phone: +33 (0) 476 51 41 13

E-mail: [roberto.geremia@ujf-grenoble.fr](mailto:roberto.geremia@ujf-grenoble.fr)

Running title: Bacterial communities of alpine soils

## Abstract

Because of their role in nutrient cycling, soil bacterial communities play an essential role in the response of tundra ecosystems to global warming. The variation in snow cover dynamics in alpine tundra has a profound impact on ecosystem structure and functioning, but its relation with the dynamics of bacterial communities remains unknown. Here, we compared the yearly course of phylogenetic structure of soil bacterial communities sampled from early snowmelt locations (ESM) and late snowmelt locations (LSM) in temperate alpine tundra. The analysis of 1700 *ssu* sequences from both locations and 4 seasons revealed that the overall phylogenetic structure of bacterial communities was strongly associated with temporal change in snow cover. Temporal variations were mainly due to strong environmental filtering, as indicated by the phylogenetic clustering of *Acidobacteria*, *Actinobacteria* and  $\alpha$ -*Proteobacteria* at the end of winter in ESM and of  $\beta$ -  $\gamma$ -*Proteobacteria* and *Bacteroidetes* at both locations at autumn. The drop of soil pH in ESM after soil thawing possibly induces selection of *Acidobacteria SD1*. Autumn litter-fall was associated with the selection of *Pseudomonadales*, *Burkholderiales*, *Flavobacteriales* and *Sphingobacteriales*. Our results show that bacterial communities are highly influenced by seasonal environmental variation, indicating that they will respond strongly to changes in climatic drivers.

## Introduction

The mechanisms driving the assembly of microbial communities are still largely unexplored, despite the central role they play in ecosystem functioning and response to global changes. In particular, alpine ecosystems, which are characterised by abrupt environmental gradients (Körner, 1999; Litaor et al., 2002; Edwards et al., 2007), are predicted to be heavily impacted by climate changes in future decades. Indeed, strong modifications in the dynamics of snow cover and length of the growing season are expected (Beniston, 2005), and these changes could have strong effects on plant and microbial communities (Theurillat and Guisan, 2001; Choler, 2005; Bryant et al., 2008). At the two extreme of the snow cover gradient, alpine tundra from Early and Late Snow Melt locations (hereafter ESM and LSM), though spatially very close, exhibit contrasting plant cover, soil characteristics (including soil carbon stock), and microbial communities (Litaor et al., 2002; Choler, 2005; Edwards et al., 2007; Zinger et al., 2009). Therefore, this gradient offers a unique opportunity to examine how the natural dynamics of snow cover may impact soil microbial communities and soil functioning. This is of particular relevance in the light of the measured and predicted changes of snow regimes in arctic and alpine tundra (IPCC, 2007).

In recent years, phylogenetic information has been increasingly used to infer the ecological and evolutionary mechanisms driving contemporary community assembly (Webb et al., 2002; Vamosi et al., 2009). This approach is based on the rationale that phylogenetic distance between two species or taxa is a measure of ecological similarity, assuming that ecological characteristics of taxa are phylogenetically conserved. Thus, if one assumes that habitat filtering is the major mechanism of community assembly, it is expected that local environmental conditions will only allow the occurrence of related species (or phylotypes) that share specific ecological characteristics, leading to phylogenetic clustering of communities (Webb et al., 2002; Cavender-Bares et al., 2006). However, when resource competition is the dominant mechanism, closely related taxa should tend to mutually exclude each other within local communities, leading to a pattern of phylogenetic overdispersion (Webb et al., 2002; Cavender-Bares et al., 2006).

So far, the assembly rules of microbial communities have been little investigated (but see (Horner-Devine and Bohannan, 2006; Newton et al., 2007; Bryant et al., 2008)). Such studies have usually revealed a significant phylogenetic clustering, suggesting that drastic selective pressures alter the structure of microbial communities. However, more data are needed to

confirm whether phylogenetic clustering is common in microbial communities. Moreover, the study of variation in microbial phylogenetic structure over time and space may provide new insights into the spatial distribution and succession of these communities and how environmental changes may influence their activity.

Here, we studied bacterial communities over the course of one year in ESM and LSM locations. A previous study highlighted the differences between these two communities and their seasonal variations (Zinger et al., 2009). However, the temporal and spatial variations in the bacterial phylogenetic structure in these two communities are poorly understood. First, analysis using Ribosomal Data Project (RDP) tools confirmed the previous structure of the subphylum level. Second, we used two different statistical approaches to assess phylogenetic structure: one based on the computation of the beta (between-locations and between-date) component of phylogenetic diversity (from species abundance data), and one based on the computation of indices depicting the phylogenetic structure of each local community at one given date (from patterns of species presence/absence).



## Material and Methods

### Study site, sample collection and bacterial *ssu* sequences

The study area is located in the Grand Galibier massif (southwestern Alps, France, 45°05' N, 06°37' E). The sampling site displays a topographical gradient comprising two neighbouring habitats: ESM and LSM, located in an area of less than 100 m<sup>2</sup>. In harsh winter conditions, snow-cover in LSM plays a protective role for bacteria communities by keeping the soil temperature around 0°C. Conversely, ESM soils are submitted to freeze-thaw cycles because of intermittent snow coverage. These differences lead to a longer growing season in ESM and result in differences in plant cover and soil characteristics between locations (Choler, 2005). Slow-growing, stress-tolerant plants dominate ESM (*Kobresia myosuroides*, *Dryas octopetala*, *Carex curvula* All. subsp. *rosae*), while LSM is dominated by fast-growing species (*Carex foetida*, *Salix herbacea* L., *Alopecurus alpinus* Vill., *Alchemilla pentaphyllea* L.). Five soil samples from each location were collected in 2005 on June 24<sup>th</sup> (spring), August 10<sup>th</sup> (beginning of growing season) and October 10<sup>th</sup> (after litter-fall), and in 2006 on May 3<sup>rd</sup> (late winter). During the late-winter sampling, the mean soil temperature of ESM rose above 0°C for 10 days, and the mean for LSM was around 0°C. The LSM soil was covered by >2.5 m of snow pack and was waterlogged. The soil samples were immediately transported to the laboratory in sterile conditions and sieved at 2 mm before DNA extraction. The clone libraries' construction has been previously described (Zinger et al., 2009). The accession numbers of the *ssu* sequences analysed in the present study are FJ568339 to FJ570564.

### Sequence data treatment

The chimerical sequences were identified using the Bellerophon program (Huber et al., 2007) and removed from the dataset. The length of the remaining sequences (1077 for LSM and 1311 for ESM, 270 and 320 sequences per location/date) ranged from 400 to 900 bp. The taxonomic assignment of *ssu* sequences was done using the Classifier (Cole et al., 2003) tool from the Ribosomal Database Project (RDP). The closest matching sequences, identified with Match (Cole et al., 2005), were used as references for the phylogenetic analysis. The multiple alignments were performed with 1707 sequences using the ClustalW algorithm (Thompson et

al., 1994) for each phylum. For *Proteobacteria*,  $\alpha$ -*Proteobacteria* were analysed separately from other *Proteobacteria* because of their phylogenetic distance (Kersters et al., 2006). The phylogenetic analysis was performed with MEGA 3.1 (Kumar et al., 2004) by using the distance of Kimura2 to calculate evolutionary distances and Neighbour-Joining to construct phylogenetic dendrograms. The robustness of the phylogenetic tree was evaluated by performing 1000 bootstraps.

## Statistical Analysis

The Classifier tool from RDP allowed the assessment of phylotypes' abundances at different taxonomic levels. From the phylotypes' abundances at the phylum and sub-phylum levels, we analysed seasonal and spatial differences for each of the bacterial taxa with the Pearson's Chi squared test and Library Compare (Cole et al., 2005) from RDP. We also computed a frequency-based distance matrix using Edward's distance and constructed Neighbour-Joining dendrograms, the robustness of which was assessed by performing 1000 bootstraps. The latter data treatment was performed using R software (R Development-Core Team, 2008). To study bacterial phylogenetic structure, we used two different approaches. First, we used the framework provided by Rao's quadratic entropy, which allows the partitioning of phylogenetic diversity into its alpha and beta components (Hardy and Senterre, 2007). We computed the  $Q_{st}$  index ( $\beta$  phylogenetic diversity), which measures how communities are structured, taking into account taxa phylogenetic relationships and their local abundances, following (Villeger and Mouillot, 2008). We computed  $Q_{st}$  between the ESM and LSM sites, between sampling dates, and between dates and sites, in order to estimate the spatial, temporal and spatio-temporal variation, respectively, in communities' phylogenetic structure. Significance of  $Q_{st}$  estimates was assessed by comparing the estimated value to the values obtained by drawing 9,999 permutations of phylotypes across the phylogenetic tree.

Second, we computed for each local community the nearest taxon index (hereafter NTI), which quantifies the degree of phylogenetic clustering of taxa in local communities given their patterns of presence/absence in communities and their phylogenetic relationships. The NTI of each community was defined as  $[-(MNND - MNND_{null}) / SD(MNND_{null})]$ , where MNND is the mean phylogenetic distance of species to their nearest neighbour in the phylogeny (Webb et al., 2002).  $MNND_{null}$  is the mean MNND for the same communities after permuting taxa across the phylogenetic tree 9,999 times, and  $SD(MNND_{null})$  is the

standard deviation of these permuted MNND values. As defined here, a positive NTI indicates phylogenetic clustering within a given community, whereas a negative value indicates phylogenetic overdispersion in the local community (R Development-Core Team, 2008). We chose to use only the NTI index, and not both the NTI and NTR (net relatedness index), as done elsewhere (Webb et al., 2002), because phylogenetic trees were only phenetic.

## Results

### Comparison of bacterial communities using taxonomic information

We have obtained 8 *ssu* libraries, 1 per date and site. The taxonomic affiliation of phylotypes present in ESM and LSM libraries was assessed using Classifier from RDP. The spatial variations of the main phyla are shown in Table 1. Briefly, the most striking differences between ESM and LSM were found in *Acido-* and *Actinobacteria*. The  $\alpha$  and  $\beta$ -*Proteobacteria* were also found to vary between the two locations. These results confirm that the spatial distributions of bacterial groups in LSM and ESM are different at the phylum and sub-phylum levels (data not shown).

In order to validate the seasonal and spatial variations at the phylum and sub-phylum levels, we constructed a Neighbour-Joining tree based on their relative abundance frequencies (Fig. 1). There was a marked effect of location in the clustering of bacterial phyla, except in May (Fig. 1A). At the subphylum level, the largest distance between ESM and LSM was observed in June and August and decreased from October to May (Fig. 1B).

### Comparison of bacterial communities using phylogenetic information

In order to gain insights into the effect of location and seasonal variation on the phylogenetic structure of bacterial communities, we constructed phylogenetic trees for each phylum (see Suppl. Fig. 1, 2, 3, 4 and 5).

The phylogenetic  $\beta$  diversity was computed using the local relative abundance of each phylotype to determine how locations, seasons and the interaction between locations and seasons contribute to the structure and assembly of bacterial communities. As shown in Fig. 2, the estimators of spatial, temporal and spatio-temporal  $\beta$  diversities were always significantly different from the value computed under a null model of phylotype distribution. Phylogenetic  $\beta$  diversity was always found to be much higher between sampling dates than between sampling sites. Spatial and temporal environmental variations seemed to exert a synergistic effect on bacterial community structure, with spatio-temporal variation in phylogenetic composition always being higher than both spatial and temporal variation taken alone.

In the ESM location, bacterial communities showed no significant pattern of phylogenetic clustering during the growing season until October, when *Bacteroidetes* and  $\beta$ - $\gamma$ - $\delta$

*Proteobacteria* were phylogenetically clustered. In May, *Acidobacteria*, *Actinobacteria*, and  $\alpha$ -*Proteobacteria* communities also began to display significant clustering (Fig. 3A). Interestingly, bacterial communities in the LSM location also displayed phylogenetic clustering of  $\beta$ - $\gamma$ - $\delta$  *Proteobacteria*, *Bacteroidetes*, *Acidobacteria* and *Actinobacteria* in October. Also, the *Acidobacteria* community showed a significant phylogenetic clustering all year round (Fig. 3B).

### Distribution of bacterial groups at finer taxonomic scale

We characterised the distribution of the main bacterial orders or suborders in each sample (Fig. 4). In ESM and LSM, the *Acidobacteria* were mainly represented by subdivisions (SD) 1 to 7 and 17. The first striking feature is the spatio-temporal variation of *SDI*, which largely dominated the LSM libraries and, in May and October, the ESM library (Fig. 4A). As shown in Fig. 4B, *Actinobacteria* were represented by ten suborders and was more abundant in ESM in June and August. Interestingly, *Streptosporanginaceae* became more abundant in both locations in October and May.  $\alpha$ -*Proteobacteria* were found to be more abundant in ESM, except in May, and were mostly represented by *Rhizobiales* (Fig. 4C).

The  $\beta$ -*Proteobacteria* were more abundant in ESM than LSM due to an increase of the order *Burkholderiales* in October (Fig. 4D). The  $\gamma$ -*Proteobacteria* did not display a significant difference between the two locations but increased in the October libraries of both locations, mainly due to phylotypes affiliated with *Pseudomonadales* (Fig. 4E). The clustering of these classes during October in both locations (Fig. 3) may correspond to the increasing of *Pseudomonadales* and *Burkholderiales*. The *Bacteroidetes* phylum was more frequent in October in both locations, exemplified by *Flavobacteriales* and *Sphingobacteriales* (Fig. 4F).

## Discussion

The community assembly of bacteria, in particular in alpine tundra, is poorly understood. Previous studies using PLFA have reported similar patterns in alpine tundra habitats (Bjork et al., 2008). However, due to the previously used methodology, the bacterial clades responsible for these seasonal changes remain poorly characterised. The few molecular studies regarding alpine tundra showed seasonal variations in a *K. myosuroides* alpine meadow (Lipson and Schmidt, 2004; Lipson, 2007). In this study we examined the phylogenetic structure of bacterial communities from alpine tundra soils sampled in Early and Late Snow Melting locations, representing the two extremes of an environmental gradient. Our sampling was timed by the natural snow cover dynamics observed in these contrasting locations. Our results showed a significant effect of location (ESM vs. LSM) at the phylum and subphylum levels (Fig. 1), and even at the finer taxonomic level (Fig. 2). Thus, the effect of location on  $\beta$  diversity seems to be related to the plant-growing season. Plants can influence soil bacteria communities via root exudates (Bais et al., 2006), and thus different plant cover may recruit different bacterial phylotypes (Grayston and Campbell, 1996; Maloney et al., 1997). LSM and ESM display different plant species compositions, which can partially explain the observed differences in their bacterial communities. Actually, the correlation between plant species and soil bacterial communities has already been explored (Buyer et al., 2002), even in snow-covered ecosystems (Yergeau et al., 2007a; Yergeau et al., 2007b). However, there are few reports on the co-occurrence of specific bacterial phylotypes and plant species. One interesting finding was the presence of *Acidobacteria* SD6 in ESM (dominated by *K. myosuroides*), which was nearly absent from LSM. Actually, this *Acidobacteria* subdivision was found to be associated with the rhizosphere (Ludwig et al., 1997; Zhou et al., 2003) and in soils covered by *K. myosuroides* (Lipson and Schmidt, 2004). Given the lack of cultivable species of this subdivision, environment studies are useful tools to pinpoint a putative interaction with specific plants. Moreover, ESM is expected to contain more recalcitrant litter than LSM (Choler, 2005), which may drastically reduce the availability of nitrogen for the plant (Baptist et al., 2008), suggesting that this location relies on symbiotic N<sub>2</sub> fixation. This hypothesis is supported by the relative abundance of *Rhizobiales* (Fig 4), which are known for their ability to fix N<sub>2</sub> (Parker, 2007).

However, within bacterial clades, the analysis of phylogenetic  $\beta$  diversity showed that temporal variation in bacterial phylogenetic structure was greater than the variation between

locations (Fig. 2), suggesting that the temporal fluctuations of environmental conditions are a major determinant for the community assembly of bacteria in alpine tundra. Indeed, this was due to a strong phylogenetic clustering of bacterial communities that was pronounced in May for *Acidobacteria* in ESM sites and in October for Bacteroidetes,  $\beta$ - and  $\gamma$ -*Proteobacteria* in both ESM and LSM sites. This strong phylogenetic clustering suggests the existence of drastic environmental filters in determining the function of alpine bacterial communities. As shown elsewhere, the environmental requirements of different bacterial phylotypes may show a phylogenetic signal, causing closely related phylotypes to have similar environmental requirements (Horner-Devine and Bohannan, 2006; Newton et al., 2007; Bryant et al., 2008) and therefore respond similarly to spatio-temporal variation in environmental variables (such as the ones imposed by snow cover dynamics).

The clustering in May occurred concomitantly with the low soil temperature and the drop of ESM's soil pH (see (Zinger et al., 2009)), which was mainly due to the increase of *Acidobacteria SDI* in ESM. Indeed, *Acidobacteria SDI* was found to be more abundant in LSM (pH ~5.5) than in ESM (pH ~ 6.5) during the productive season (Fig. 4A). Interestingly, the decrease of pH in late winter in ESM was concomitant with the increase of *SDI*. *Acidobacteria* were previously found to be more abundant at pH lower than 5 (Lauber et al., 2008), while *Acidobacteria SDI* have been reported to have a preference for acidic pH (Sait et al., 2006). Thus, in a larger context than alpine soils, it is conceivable that *Acidobacteria* abundance in soils with pH<5 is due mainly to SDI. A larger molecular survey is needed to support this hypothesis. The phylogenetic clustering of community *Actinobacteria* in late winter concurs with observations in aquatic ecosystems (Newton et al., 2007). Hence, the pH appears to be a determinant filter of bacterial communities, confirming the observations of Fierer (Fierer and Jackson, 2006) and Lauber (Lauber et al., 2008).

The clustering in October occurred after plant senescence and was concomitant with the increase of *Pseudomonadales*, *Burkholderiales*, *Flavobacteriales* and *Sphingobacteriales* (Figs. 4D, E, F). These orders comprise species that are heterotrophic and are associated with the degradation of complex organic matter and/or are plant or human pathogens (Halden et al., 1999; Cottrell and Kirchman, 2000; Sheng and Gong, 2006; Rein et al., 2007; Manasiev et al., 2008). Since this sample was taken after plant senescence, the bacterial communities thriving on root exudation were probably diminished. Given the ecological characteristics of the bacterial phylotypes and the presence of plant litter, we hypothesise that the observed

patterns resulted from a change in resource input, namely a shift from root exudates to plant litter.



## **Conclusion**

In alpine tundra, the strong mesotopographical gradients trigger important differences in the composition and diversity of plant and microbial communities. Here, we have shown that the soil bacterial communities at the two extremes of a snow cover/mesotopographical gradient display different phylogenetic structures and phylotype abundance. The phylum and subphylum levels of bacterial communities highlighted the effect of snow cover regimes, which was confirmed by  $\beta$  diversity analysis. The seasonal effect strongly impacted bacterial phylogenetic structure. This study found that the bacterial community assembly is strongly dependent on environmental conditions and that changes in nutrient resources coupled with soil temperature and soil pH may constitute strong filters on bacterial communities. However, further research is needed to assess the role of each environmental factor in the assembly of bacterial communities in alpine tundra. Finally, this paper also calls for more attention to the impact of global change in alpine tundra, which may deeply impact soil bacterial communities and thus ecosystem processes.

## Acknowledgements

We thank Armelle Monier for technical assistance, Jérôme Gury for technical and scientific discussions, Alice Roy and the Centre National de Séquençage (Genoscope, Evry) for sequencing the libraries, and Serge Aubert and the staff of Station Alpine J. Fourier for providing logistic facilities during the fieldwork. B. Shahnavaaz was supported by PhD scholarships from the Iranian Ministry of Science, Research, and Technology. The work was funded by the ANR-06-BLAN-0301 "Microalpes" project.

## References

- Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., and Vivanco, J.M. (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. *Ann. Rev. Plant Biol* **57**: 233-266.
- Baptist, F., Zinger, L., Clement, J.C., Gallet, C., Guillemin, R., Martins, J.M.F. et al. (2008) Tannin impacts on microbial diversity and the functioning of alpine soils: a multidisciplinary approach. *Environ Microbiol* **10**: 799-809.
- Beniston, M. (2005) Climatic change and its possible impacts in the alpine region. *Rev géogr alp* **93**: 25-32.
- Bjork, R.G., Bjorkman, M.P., Andersson, M.X., and Klemetsson, L. (2008) Temporal variation in soil microbial communities in Alpine tundra. *Soil Biol Bioch* **40**: 266-268.
- Bryant, J.A., Lamanna, C., Morlon, H., Kerkhoff, A.J., Enquist, B.J., and Green, J.L. (2008) Microbes on mountainsides: Contrasting elevational patterns of bacterial and plant diversity. *P Natl Acad Sci USA* **105**: 11505-11511.
- Buyer, J.S., Roberts, D.P., and Russek-cohen, E. (2002) Soil and plant effects on microbial community structure. *Can J Microbiol* **48**: 955-964
- Cavender-Bares, J., Keen, A., and Miles, B. (2006) Phylogenetic structure of floridian plant communities depends on taxonomic and spatial scale. *Ecology* **87**: S109-S122.
- Choler, P. (2005) Consistent shifts in Alpine plant traits along a mesotopographical gradient. *Arct Antarct Alp Res* **37**: 444-453.
- Cole, J.R., Chai, B., Farris, R.J., Wang, Q., Kulam, S.A., McGarrell, D.M. et al. (2005) The Ribosomal Database Project (RDP-II): sequences and tools for high-throughput rRNA analysis. *Nucl Acids Res* **33**: D294-296.
- Cole, J.R., Chai, B., Marsh, T.L., Farris, R.J., Wang, Q., Kulam, S.A. et al. (2003) The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. *Nucl Acids Res* **31**: 442-443.
- Cottrell, M.T., and Kirchman, D.L. (2000) Natural assemblages of marine proteobacteria and members of the Cytophaga-Flavobacter cluster consuming low- and high-molecular-weight dissolved organic matter. *Appl Environ Microbiol* **66**: 1692-1697.
- Edwards, A.C., Scalenghe, R., and Freppaz, M. (2007) Changes in the seasonal snow cover of alpine regions and its effect on soil processes: A review. *Quater Int* **162**: 172-181.

- Fierer, N., and Jackson, R.B. (2006) The diversity and biogeography of soil bacterial communities. *P Natl Acad Sci USA* **103**: 626-631.
- Grayston, S.J., and Campbell, C.D. (1996) Functional biodiversity of microbial communities in the rhizospheres of hybrid larch (*Larix eurolepis*) and Sitka spruce (*Picea sitchensis*). *Tree Physiol* **16**: 1031-1038.
- Halden, R.U., Tepp, S.M., Halden, B.G., and Dwyer, D.F. (1999) Degradation of 3-phenoxybenzoic acid in soil by *Pseudomonas pseudoalcaligenes* POB310(pPOB) and two modified *Pseudomonas* strains. *Appl Environ Microbiol* **65**: 3354-3359.
- Hardy, O.J., and Senterre, B. (2007) Characterizing the phylogenetic structure of communities by an additive partitioning of phylogenetic diversity. *J Ecol* **95**: 493-506.
- Horner-Devine, M.C., and Bohannan, B.J.M. (2006) Phylogenetic clustering and overdispersion in bacterial communities. *Ecology* **87**: S100-S108
- Huber, J.A., Mark Welch, D., Morrison, H.G., Huse, S.M., Neal, P.R., Butterfield, D.A., and Sogin, M.L. (2007) Microbial population structures in the deep marine biosphere. *Science* **318**: 97-100.
- IPCC (2007) Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. In. Core Writing Team, P., R.K and Reisinger, A. (ed). Geneva, Switzerland: IPCC, p. 104.
- Kerstens, K., De Vos, P., Gillis, M., and Swings, J. (2006) Proteobacteria. *Encyclopedia of Life Sciences*.
- Körner, C. (1999) *Alpine Plant Life*. B 335 erlin: Springer Verlag.
- Kumar, S., Tamura, K., and Nei, M. (2004) MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform* **5**: 150-163.
- Lauber, C.L., Strickland, M.S., Bradford, M.A., and Fierer, N. (2008) The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biol Biochem* **40**: 2407-2415.
- Lipson, D.A. (2007) Relationships between temperature responses and bacterial community structure along seasonal and altitudinal gradients. *FEMS Microbiol Ecol* **59**: 418-427.
- Lipson, D.A., and Schmidt, S.K. (2004) Seasonal changes in an alpine soil bacterial community in the colorado rocky mountains. *Appl Environ Microbiol* **70**: 2867-2879.
- Litaor, M.I., Seastedt, T.R., and Walker, D.A. (2002) Spatial analysis of selected soil attributes across an alpine topographic/snow gradient. *Landscape Ecol* **17**: 71-85.

- Ludwig, W., Bauer, S.H., Bauer, M., Held, I., Kirchhof, G., Schulze, R. et al. (1997) Detection and in situ identification of representatives of a widely distributed new bacterial phylum. *FEMS Microbiol Lett* **153**: 181-190
- Maloney, P.E., vanBruggen, A.H.C., and Hu, S. (1997) Bacterial community structure in relation to the carbon environments in lettuce and tomato rhizospheres and in bulk soil. *Microb Ecol* **34**: 109-117.
- Manasiev, J., Gerginova, M., Yemendzhiev, H., Peneva, N., and Alexieva, Z. (2008) Molecular analysis of phenol-degrading microbial strains. *Z Naturforsch C* **63**: 133-138.
- Newton, R.J., Jones, S.E., Helmus, M.R., and McMahon, K.D. (2007) Phylogenetic ecology of the freshwater Actinobacteria acI lineage. *Appl Environ Microbiol* **73**: 7169-7176.
- Parker, M.A. (2008) Symbiotic Relationships of Legumes and Nodule Bacteria on Barro Colorado Island, Panama: A Review. *Microb Ecol* **55**: 662-672
- R Development Core Team. (2008) R: A Language and Environment 3.5.9 for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.
- Rein, A., Fernqvist, M.M., Mayer, P., Trapp, S., Bittens, M., and Karlson, U.G. (2007) Degradation of PCB congeners by bacterial strains. *Appl Microbiol Biot* **77**: 469-481.
- Sait, M., Davis, K.E., and Janssen, P.H. (2006) Effect of pH on isolation and distribution of members of subdivision 1 of the phylum Acidobacteria occurring in soil. *Appl Environ Microbiol* **72**: 1852-1857
- Sheng, X.F., and Gong, J.X. (2006) Increased degradation of phenanthrene in soil by *Pseudomonas* sp GF3 in the presence of wheat. *Soil Biol Biochem* **38**: 2587-2592.
- Theurillat, J.P., and Guisan, A. (2001) Potential impact of climate change on vegetation in the European Alps: A review. *Climatic Change* **50**: 77-109.
- Thompson, J.D., Higgins, D.G., and Gibson, T.J. (1994) Clustal-W - Improving The Sensitivity Of Progressive Multiple Sequence Alignment Through Sequence Weighting, Position-Specific Gap Penalties And Weight Matrix Choice. *Nucleic Acids Res* **22**: 4673-4680.
- Vamosi, S.M., Heard, S.B., Vamosi, J.C., and Webb, C.O. (2009) Emerging patterns in the comparative analysis of phylogenetic community structure. *Mol Ecol* **18**: 572-592.
- Villegger, S., and Mouillot, D. (2008) Additive partitioning of diversity including species differences: a comment on Hardy and Senterre (2007). *J Ecol* **96**: 845-848.
- Webb, C.O., Ackerly, D.D., McPeck, M.A., and Donoghue, M.J. (2002) Phylogenies and community ecology. *Annu Syst* **33**: 475-505.

Yergeau, E., Newsham, K.K., Pearce, D.A., and Kowalchuk, G.A. (2007a) Patterns of bacterial diversity across a range of Antarctic terrestrial habitats. *Environ Microbiol* **9**: 2670-2682.

Yergeau, E., Bokhorst, S., Huiskes, A.H.L., Boschker, H 383 .T.S., Aerts, R., and Kowalchuk, G.A. (2007b) Size and structure of bacterial, fungal and nematode communities along an Antarctic environmental gradient. *FEMS Microbiol Ecol* **59**: 436-451.

Zhou, J.Z., Xia, B.C., Huang, H.S., Treves, D.S., Hauser, L.J., Mural, R.J. et al. (2003) Bacterial phylogenetic diversity and a novel candidate division of two humid region, sandy surface soils. *Soil Biol Biochem* **35**: 915-924.

Zinger, L., Shahnava, B., Baptist, F., Geremia, R.A., and Choler, P. (2009) Microbial diversity in alpine tundra soils correlates with snow cover dynamics. *ISME J* (doi:10.1038/ismej.2009.20).

**Chapitre 3 : Effet des régimes d'enneigement sur la structure phylogénétique des communautés bactériennes des sols alpins**

---

Table 1

Principal groups of bacteria identified by RDP, relative abundance in ESM and LSM and different significant tested by Library Compare and Chi-Square. The sequences belonging to *Verrucomicrobia*, *Aerolineae*, *Nitrospira*, *Cyanobacteria*, *Deinococci* were rare in our libraries that presented namely "Other".

Phylum bacteria	% in LSM	% in ESM	P by RDP	P by chi-square
<i>Acidobacteria</i>	42 ± 10	22 ± 11	6E – 14	8.75E – 07
<i>Actinobacteria</i>	6 ± 3	17.6 ± 5.8	6E – 14	2.744E – 06
<i>α-Proteobacteria</i>	12 ± 4	19 ± 3	0.00006	0.026
<i>β-Proteobacteria</i>	3.5 ± 0.7	7 ± 3.8	0.0003	0.021
<i>γ-Proteobacteria</i>	5.8 ± 3.7	6 ± 2.07	0.9	0.76
<i>δ-Proteobacteria</i>	1.5 ± 1.3	4 ± 2	0.001	0.03
<i>Bacteroidetes</i>	5.6 ± 3	8.3 ± 3.5	0.008	0.154
<i>Firmicutes</i>	1 ± 0.4	0.9 ± 0.5	0.99	0.8
<i>Gemmatimonadetes</i>	2.4 ± 1.3	3 ± 0.1	0.6	0.5
Other	0.8 ± 1	1 ± 1.07	0.4	0.5
Unclassified bacteria	18.9 ± 4	10 ± 2	NA	0.001

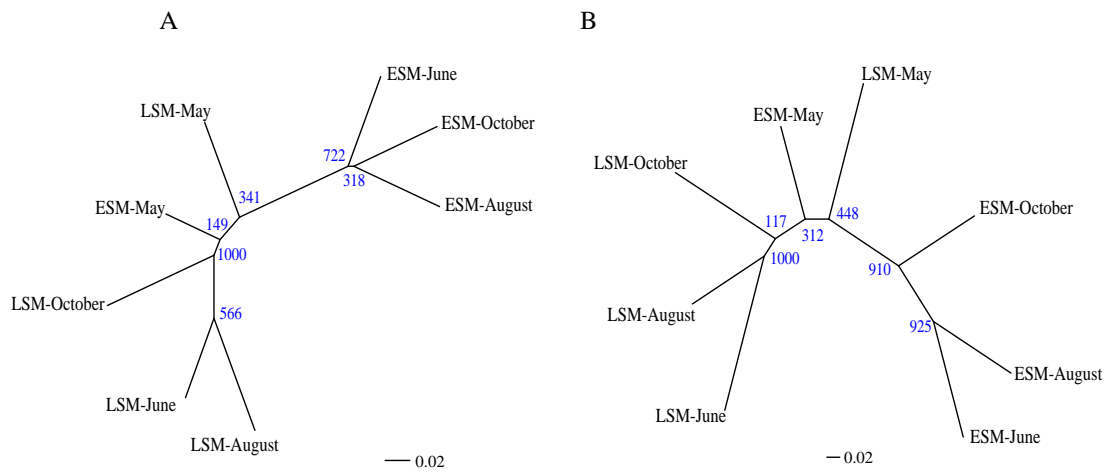


Figure 1. **Spatial and seasonal variation of bacterial community.** The dendrograms are based on phylotype frequencies at the phylum (A) and sub-phylum levels (B). The bootstrap value is shown for each node.



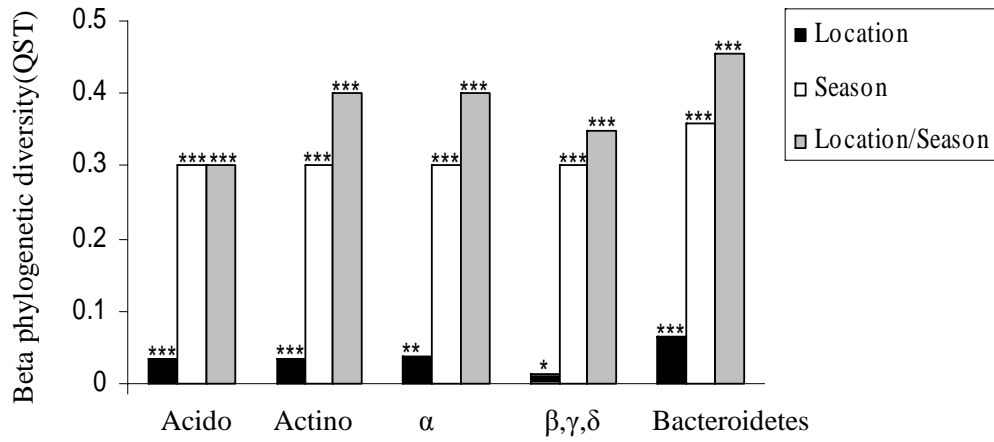


Figure 2.  **$\beta$  phylogenetic diversity between ESM and LSM locations.** Significance of  $Q_{st}$  estimates was assessed by comparison to a null model obtained with 9999 permutations and is indicated as follows: \*\*\*:  $P < 0.001$ , \*\*:  $P < 0.01$ , \*:  $P < 0.05$ .

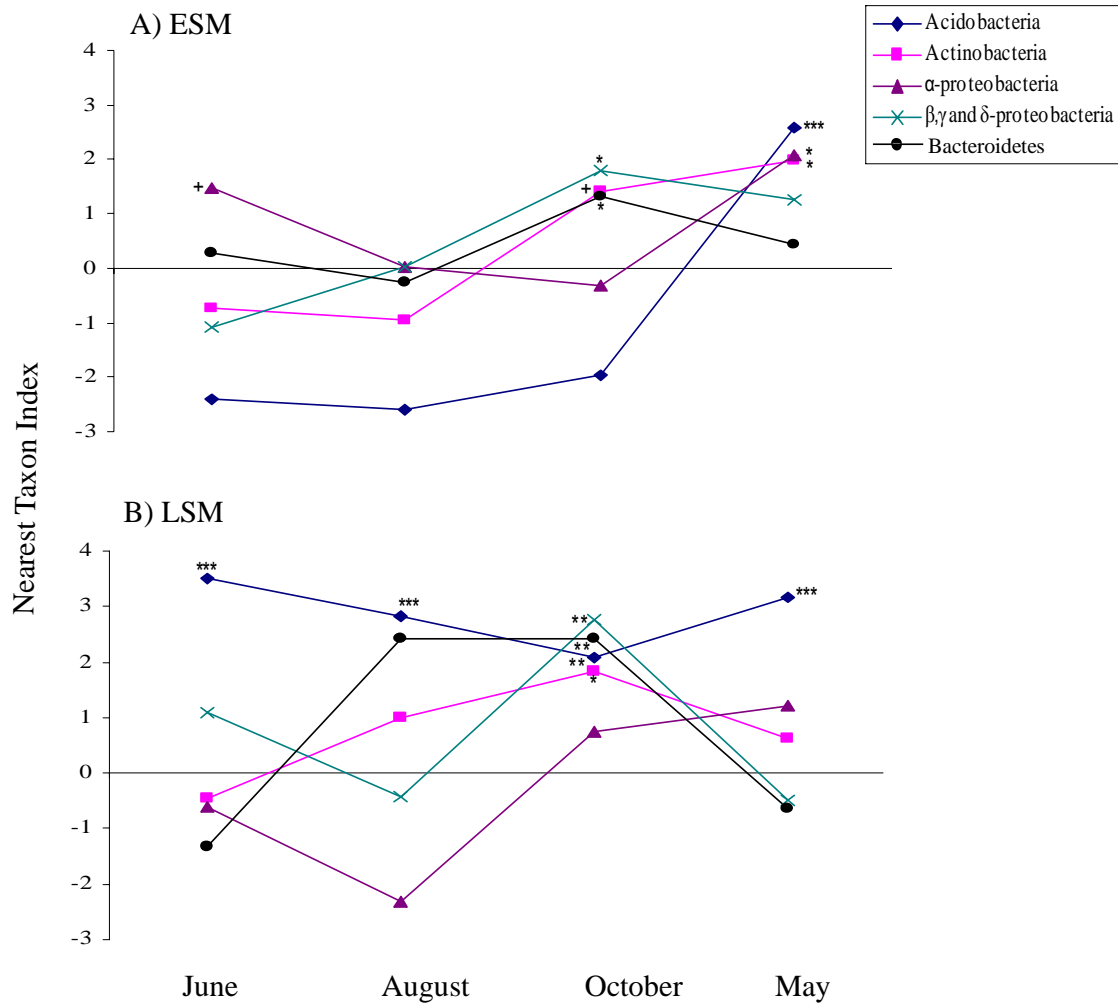


Figure 3. **Seasonal variation in the phylogenetic structure of bacterial communities.** A: ESM and B: LSM. NTI positive index values present phylogenetic clustering, and negative values indicate phylogenetic overdispersion. The significance of NTI estimates is shown as follows:

\*\*\*:  $P < 0.001$ , \*\*:  $P < 0.01$ , \*:  $P < 0.05$ , +:  $P < 0.1$ .

### Chapitre 3 : Effet des régimes d'enneigement sur la structure phylogénétique des communautés bactériennes des sols alpins

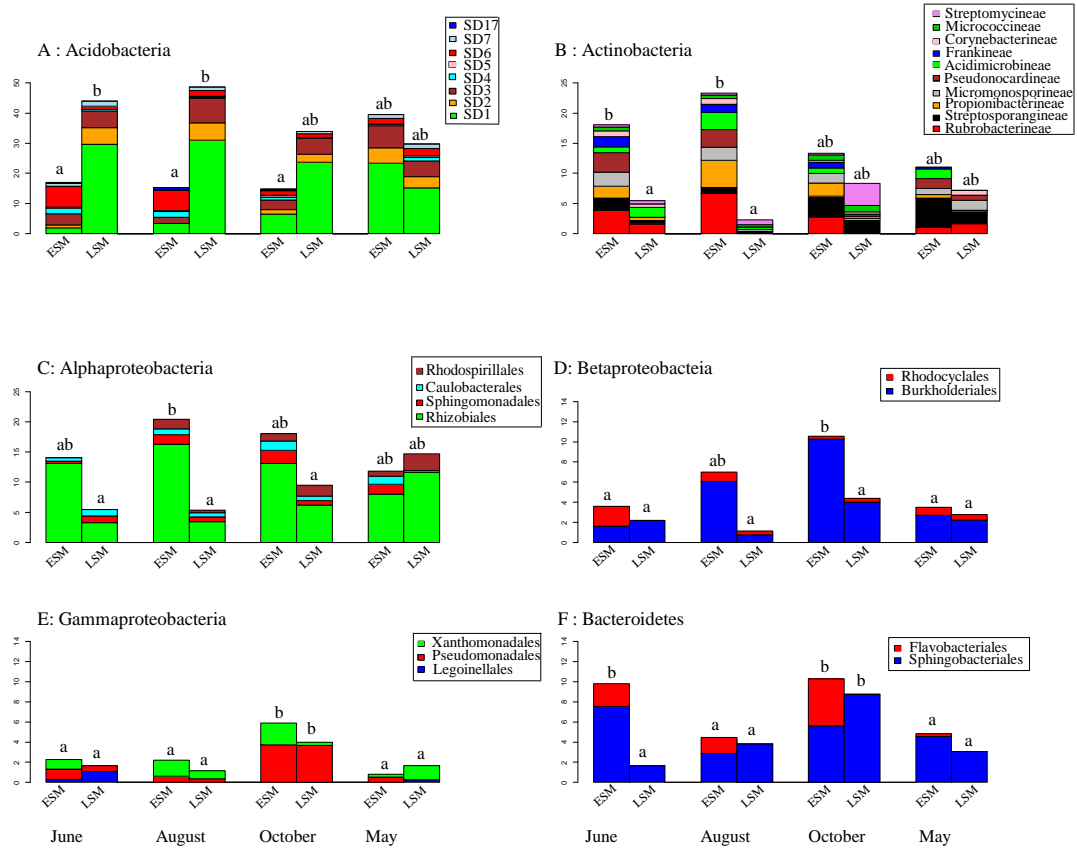
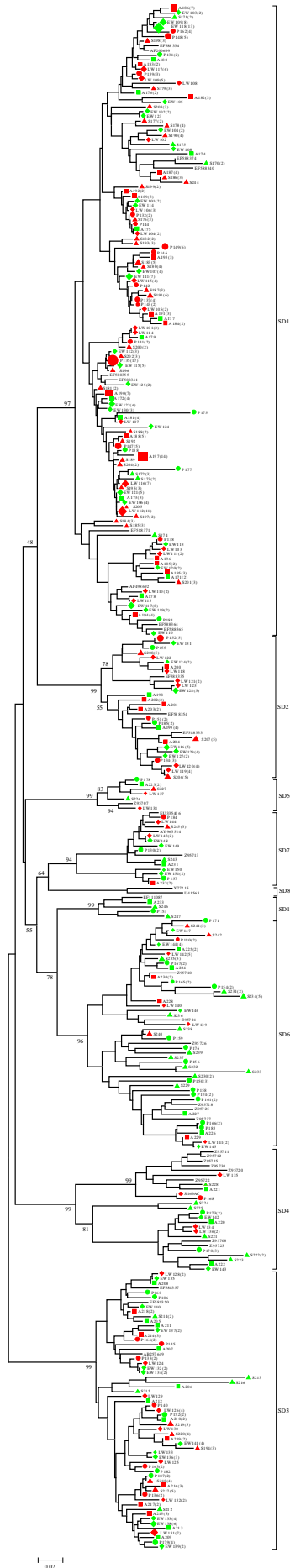


Figure 4. **Relative abundance of bacterial phyla and class.** A: *Acidobacteria*, B: *Actinobacteria*, C:  $\alpha$ -*Proteobacteria*, D:  $\beta$ -*Proteobacteria*, E:  $\gamma$ -*Proteobacteria* and F: *Bacteroidetes*. Significant differences between the two studied locations and seasons are indicated by small letters.

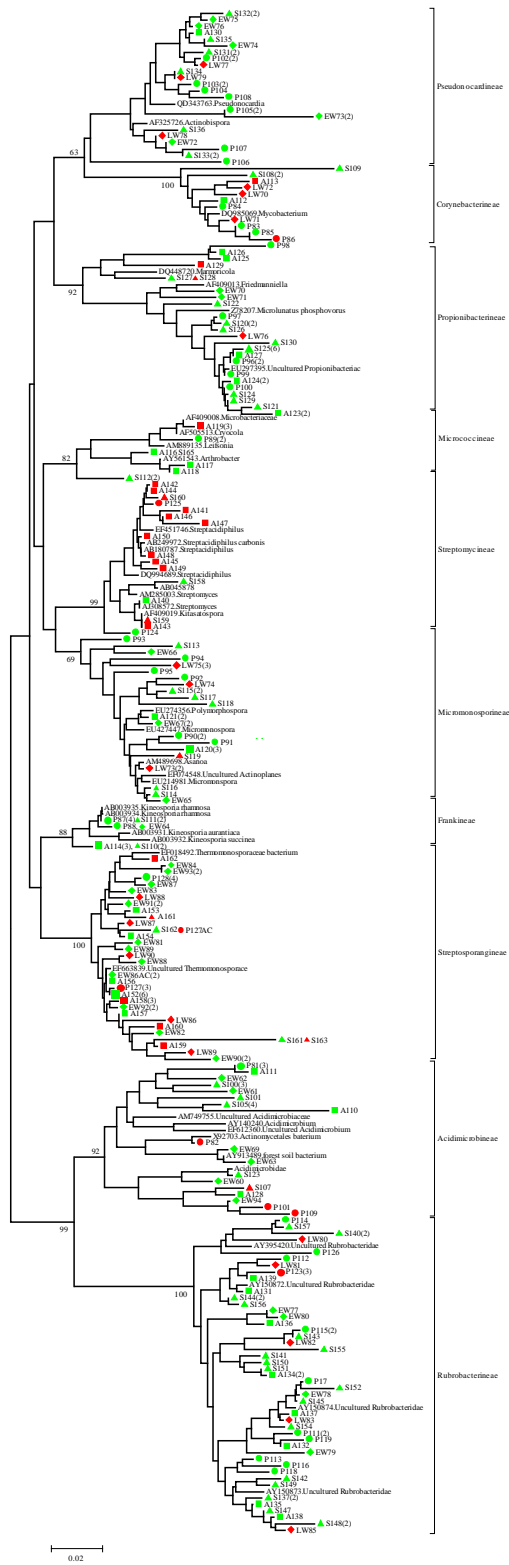
Supplementary Figures shows the NJ phylogenetic tree of *Acidobacteria* (SF 1), *Actinobacteria* (SF2),  *$\alpha$ -Proteobacteria* (SF3),  *$\beta$ - $\gamma$ - $\delta$ -Proteobacteria* (SF 4) and *Bacteroidetes* (SF5).

Spring clones begin with P, summer clones with S, autumn clones with A and late winter clones with EW and LW. ESM and LSM identified green and red, respectively. The different seasons are showed by symbols: Circle: June, Triangle: August, square: October and diamond: May.

### Chapitre 3 : Effet des régimes d'enneigement sur la structure phylogénétique des communautés bactériennes des sols alpins



### Chapitre 3 : Effet des régimes d'enneigement sur la structure phylogénétique des communautés bactériennes des sols alpins

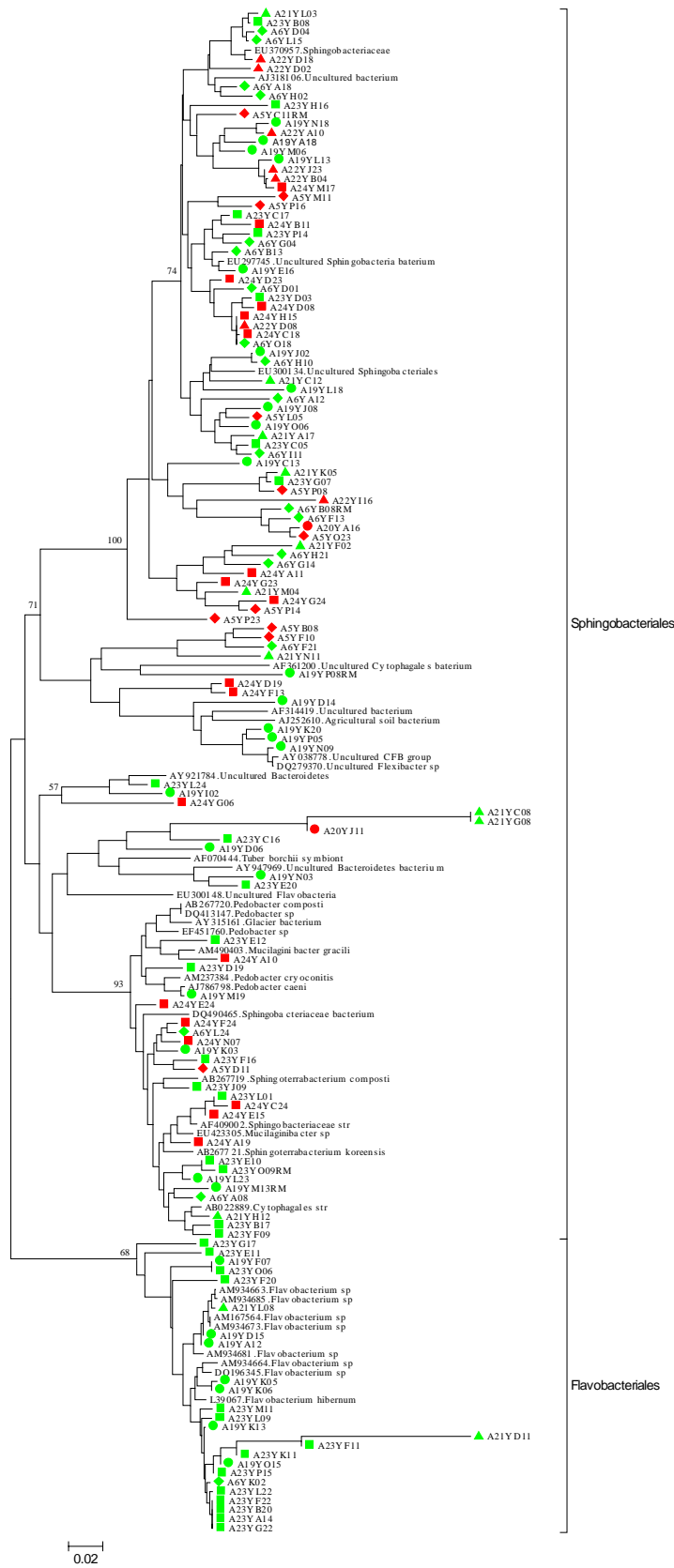








### Chapitre 3 : Effet des régimes d'enneigement sur la structure phylogénétique des communautés bactériennes des sols alpins



## Principaux résultats et discussion

Les dendrogrammes obtenus par distance de Edward (après séquences *ssu*) avec les abondances relatives des différents phyla montrent un pattern similaire à celui observé avec la SSCP bactérienne, indiquant que la résolution de la méthode SSCP se situe au niveau du phylum. En revanche, le dendrogramme obtenu à partir des sous-phyla indique qu'il n'y a pas seulement une convergence pendant l'hiver (Article I) mais aussi pendant le mois d'Octobre.

D'autre part, la plus grande distance entre les communautés bactériennes des deux localisations est obtenue au cours de la saison de végétation, montrant ainsi que les plantes ont non seulement un impact sur la diversité (Article I et II) mais aussi sur la structure phylogénétique des communautés bactériennes (Article II). Toutefois, il existe peu de rapports sur la co-occurrence de certains phylotypes bactériennes avec certaines espèces végétales. Une conclusion intéressante est la présence de *SD6* (*Acidobactéries*) en crête, dominée par *Kobresia myosuroides*, un groupe souvent décrit comme étant associé à la rhizosphère (Ludwig et al., 1997; Lee et al., 2008), et présent dans d'autres sols également couverts par *K. myosuroides* (Lipson and Schmidt, 2004). Malheureusement, aucun des représentants de *SD6* n'a été isolé à ce jour pour vérifier les propriétés morphologiques et le rôle écologique de ce groupe d'*Acidobactéries*.

La comparaison du dendrogramme obtenu par séquences *ssu* et les analyses de diversité phylogénétique  $\beta$  (Fig. 1 et 2 de Article II) ont montré qu'au niveau du phylum à celui de l'ordre, la distribution et l'abondance des groupes bactériens sont influencées par la situation (thermique ou nivale), et donc par la couverture végétale, le pH du sol et la qualité et la quantité des ressources. L'ensemble de ces variables est fortement contrasté entre les deux localisations et associées à la durée du manteau neigeux. De même, bien que notre étude n'ait pas pour but de déterminer l'influence de la structure du sol sur la structure des communautés bactériennes, nos résultats correspondent aux précédentes observations. En effet, l'abondance relative des *Acidobactéries* peut être expliquée par la taille des particules du sol et il a été montré que les petites particules sont riches en *Acidobactéries* (Sessitsch et al., 2001). Dans notre étude, on retrouve également ce type de patron car les sols les plus argileux (combe) sont riches en *Acidobactéries*.

En revanche, à une échelle taxonomique plus fine (espèce), l'effet saison est plus important, montrant ainsi que les espèces au sein d'un même phylum s'adaptent aux conditions edaphoclimatiques. Pendant la saison de végétation les communautés bactériennes ne

montrent pas d'agrégation phylogénétique. Le développement des racines qui peut modifier la structure du sol et les exsudats racinaires - contenant divers éléments carbonés - créent des conditions différentes et pourraient diversifier les ressources en été, résultant en une surdispersion phylogénétique des communautés bactériennes. En Octobre, la distinction des situations thermique et nivale existe encore, mais semble plus faible que pendant la saison de végétation. La convergence pendant le mois d'Octobre peut s'expliquer par la sénescence des plantes à cette période. L'agrégation phylogénétique des bactéries ayant lieu au mois d'Octobre, pendant la sénescence des plantes coïncide avec l'augmentation de bactéries hétérotrophes, connues pour dégrader la matière organique complexe (e.g. *Burkhoderales*, *Pseudomonales*, *Flavobacteriales* et *Sphingobacteriales*) (Halden et al., 1999; Cottrell and Kirchman, 2000; Sheng and Gong, 2006; Rein et al., 2007; Manasiev et al., 2008). Nous avons donc observé que le développement des plantes peut impacter de deux façons les communautés bactériennes. Premièrement, pendant la croissance des plantes qui cause une surdispersion phylogénétique, et deuxièmement, pendant leur sénescence qui conduit à une agrégation phylogénétique.

L'effet de la plante disparaît en hiver (mois de Mai). Il apparaît que durant cette période, les conditions environnementales conduisent à une agrégation, particulièrement en crête. L'agrégation des *Acidobactéries* en Mai en crête se produit avec la diminution du pH du sol. Cependant, les *Acidobactéries*, principalement représenté par *SD1*, en combe dont le pH du sol est constant (~ 5.5) sont regroupées de l'année. Donc, le pH du sol n'est pas seulement un facteur de structuration des communautés bactériennes du sol (Fierer and Jackson, 2006), particulièrement chez les *Acidobactéries* (Lauber et al., 2008) ; il s'agit d'un réel filtre environnemental pour les bactéries, ne favorisant la présence que de certains groupes d'*Actinobactéries* comme *acI* dans les lacs (Newton et al., 2007) et *SD1* dans les sols alpins (Article II).

En écologie des macro-organismes, l'agrégation de la structure phylogénétique a lieu dans des conditions sélectives. Nos résultats présentent deux événements causant l'agrégation phylogénétique des communautés bactériennes : les mois de Mai et d'Octobre, dont l'effet se joue probablement à travers le pH du sol et la sénescence des plantes, respectivement.

*Les régimes d'enneigement n'influencent pas seulement la diversité et la composition des groupes bactériens, ils influencent également la structure phylogénétique des communautés bactériennes à une échelle taxonomique fine.*

## **Chapitre 4**

# **Effet des régimes d'enneigement sur la richesse des espèces bactériennes cultivables des sols alpins**



## Contexte général

La terre contient de nombreux environnements soumis à de basses températures. Plus de 80% de la biosphère est froide en permanence: 90% des océans (qui occupent 71% de la surface de terrestre) sont soumis à des températures inférieures à 5°C. De nombreux environnements aquatiques et terrestres sont également froids, comme les régions polaires (14% de la surface de la terre), les hautes montagnes et certains lacs. Plusieurs organismes sont capables de se développer dans les habitats froids (Margesin, 2007). Les objectifs initiaux des études de la microbiologie du permafrost et des écosystèmes froids ont été de déterminer s'il était possible d'isoler des microorganismes viables.

L'étude des communautés bactériennes par culture sur différents milieux a été menée dans écosystèmes enneigés comme dans les permafrosts du plateau Tibétain, de Sibérie, dans les montagnes Tianshan, à Spitsbergen et dans l'arctique canadien (Shi et al., 1997; Bai et al., 2006; Hansen et al., 2007; Steven et al., 2007; Zhang et al., 2007). Ces bactéries cultivables, identifiées par séquençage des gènes de l'ARNr 16S, appartenait aux phyla *Actinobactéries*, *Firmicutes*, *Proteobactéries* et *Bacteroidetes* (références au-dessus et Bakermans et al., 2003; Vishnivetskaya et al., 2006). Ces différentes études ont montré une grande variété de bactéries, y compris *Bacillus*, *Arthrobacter*, *Micrococcus*, *Pseudomonas*, *Flavobacterium*, *Cytophaga*, *Acinetobacter*, *Streptomyces* et *Rhodococcus*. Les bactéries gram-positives ont été montrées comme étant les plus diversifiées et abondantes, telles que *Arthrobacter*. Toutefois, la majorité de ces isolats ont été signalés dans le permafrost, l'Antarctique et arctiques écosystèmes. Plusieurs caractéristiques (Fong et al., 2001; Kumar et al., 2002; Groudieva et al., 2004; Mueller et al., 2005) permettent de survivre dans les froids habitats. Certain étude montre que grâce au protecteur rôle de manteau neigeux, une importante de la dégradation et de décomposition de ces écosystèmes se produit pendant l'hiver (Schmidt et al., 2007).

Il y a peu de rapports sur les bactéries cultivables du sol de tundra alpine (mais voir Margesin et al., 2003; Lipson and Schmidt, 2004) et il est difficile d'extrapoler les résultats précédemment cités aux écosystèmes alpins. En effet, ces écosystèmes diffèrent des écosystèmes arctiques. Outre les différences de latitude, les sols alpins européens sont régulièrement soumis à des événements de gel-dégel, de grandes fluctuations de température, et de fortes précipitations (2.000 à 3.000 mm par an) (Margesin et al., 2003; Nemergut et al., 2005).

Bien que les approches par culture soient souvent critiquées à cause de leur sélectivité (Prosser, 2002), ce type d'approche reste cependant importante pour mieux connaître la physiologie des micro-organismes dans des buts aussi bien écologiques qu'industriels.

## **Objectifs de l'étude**

Dans cette étude, séquençant l'ARNr 16S des souches isolées, nous avons essayé i) de montrer si la diversité, la composition et l'abondance et la structure phylogénétique des bactéries cultivables peuvent changer dans le temps et l'espace (voir chapitre I pour plus d'informations), ii) de déterminer si certains morpho/phénotypes sont caractéristiques de certaines conditions, iii) de préciser l'impact de la température d'isolement sur la richesse des bactéries cultivables, et iv) et de comparer les résultats obtenus par l'approche moléculaire avec ceux obtenus par des approches de culture. Afin d'établir une étude comparative, les prélèvements de sols sont réalisés dans les conditions suivantes : en milieu de saison végétative (Juillet), en début de la phase de sénescence des plantes (Octobre), et sous la couverture neigeuse en combe et gel/dégel en crête (Mai).

Malheureusement, nous ne pouvions pas utiliser le même sol que celui utilisé pour la bibliothèque de clones (le prélèvement de sol avait été réalisé et conservé à 4°C pendant des mois, nous avons donc émis l'hypothèse d'un effet de la température sur les communautés bactériennes).



## Article III: Phylogenetic diversity of cultivable bacteria in soils of temperate alpine tundra

Bahar Shahnava<sup>1</sup>, Jérôme Gury<sup>4</sup>, Philippe Choler<sup>1,2,3</sup>, Roberto A. Geremia<sup>1\*</sup>,

**In preparation for Research in Microbiology**

<sup>1</sup> Laboratoire d'Ecologie Alpine, CNRS UMR 5553, Université de Grenoble, BP 53, F-38041 Grenoble Cedex 09, France

<sup>2</sup> Station Alpine J. Fourier CNRS UMS 2925, Université de Grenoble, F-38041 Grenoble, France

<sup>3</sup> CSIRO Marine and Atmospheric Research, PO Box 1666, Canberra, ACT, 2601, Australia

<sup>4</sup> Laboratoire d'écologie microbienne, CNRS-UCBL UMR5557, Equipe symbiose actinorhizienne, France

\*Corresponding Author : Laboratoire d'Ecologie Alpine (LECA) UJF/CNRS UMR 5553 BP 53; Bât. D, F-38041 Grenoble Cedex 9, France

FAX: +33 (0) 476 51 42 79

Phone: +33 (0) 476 51 41 13

E-mail: [roberto.geremia@ujf-grenoble.fr](mailto:roberto.geremia@ujf-grenoble.fr)

Key words: alpine France; bacterial diversity; bacteria cultivable; 16S rRNA

## Abstract

The bacteria play important role in the nutrient cycles. However their characterization and role in Alpine soil tundra has been poorly investigated. This study is an attempt to describe the phylogenetic diversity of the cultivated bacterial communities in alpine soil from French alpine. We have isolated 173 strains using different culture media and temperature. Strains growing in 4°C are psychrotrophic. The *ssu* gene was amplified and sequenced for phylogenetic analysis. The most abundant strains are *Arthrobacter*. Our results suggest a spatial distribution of *Arthrobacter*, *Kitasatosporia* and *Zoogloea*. Seasonal variations are in the phylum level. Increase of *Flavobacterium* in October correlates with previous results from clone library. However, most of phylotypes remains uncultured. This study calls for improve cultivation techniques to better understand the ecological role of bacterial communities in alpine ecosystem.

## Introduction

Over the last decades, a large number of studies have used culture-dependence method to characterize the bacterial flora of snow covered ecosystems, e.g in the Arctic permafrost [24, 52, 55], in high elevation grasslands of Central Asia [3, 63], in temperate grasslands from North America [35]. These studies have reported that the most abundance bacterial community found in these extreme environments belongs to *Proteobacteria* (*Alpha*, *Beta* and *Gamma*), gram-positive bacteria (*Actinobacteria*, *Firmicuts*) and *Bacteroidetes* (references above and [2]). Several characteristics such as spore formation, pigmentation [19, 43], activity enzymatic [23] and membrane fluidity [33] allow these bacteria to survive in cold habitats. Other studies have shown that microbes benefit from the insulation of the winter snowpack and that microbial activities of snow covered soils play a significant role in the biogeochemical cycling of these ecosystems (e.g. [7, 51]).

Culture-dependent approaches have often been criticized for their selectivity [59]. However, they remain an essential approach in environmental microbiology [9]. Understanding the physiology and ecology of these bacteria, will require isolation and cultivation methods. In addition, there has been a renewed interest in the study of cold-active enzyme [6, 21, 27], the impact of freeze-thaw on biodegradation and the use of cold-adapted bacteria for bioremediation [37, 38]. Alpine environments are sources of cold-tolerant bacteria that can potentially be important for fundamental and applied researches.

The methods based on direct DNA extraction and 16S rRNA amplification are rapidly replacing traditional methods for understanding composition and structure of bacterial communities. As for culture-dependent approaches, these methods have their own weaknesses: bias in DNA extraction [39, 45, 62], the number copy of PCR amplification such as the choice of primers, the concentration of amplifies DNA, formation of the chimeric molecules [60]. Numerous authors have compared different methods to assess the diversity of bacterial communities [10, 15, 17, 56, 57]. The main conclusion of these studies is that cultivation captures a fraction of total diversity of the sample represented by heterotrophic bacteria. The main reason is that conventional culture methods are designed to isolate heterotrophic bacteria. It was also proposed that bacterial cells can be present in active and quiescent form [25]. The use of a combined strategy culture/molecular was also proposed [45]. Indeed both approaches are complementary, the cultural approach will reveal the

biochemical and physiological capabilities of soil bacteria, while the molecular approach will reveal the comprehensive diversity.

In a previous study, we used 16S rRNA to show that bacterial phylogenetic diversity of alpine tundra soils strongly differed between Early Snow Melt location (ESM) and Late Snow Melt (LSM) location [64]. In this present study, our aim was (i) to characterize the phylogenetic diversity of cultivable bacterial communities and (ii) to compare the obtained results by culture-dependent methods with those obtained by clone library.

## Material and Method

### Site sampling description and sample collection

The study area was located in the alpine tundra of the Grand Galibier massif (south-western alpine, French). Two contrasting sampling locations representing the two extremes of a snow cover / mesotopographical gradient were selected: an Early Snow Melt location (ESM) and a Late Snow Melt (LSM) location. The edapho-climatic characteristics of these two locations have been reported elsewhere [64]. ESM is dominated by the turf graminoid *Kobresia myosuroides* and the dwarf shrub *Dryas octopetala* both of which are slow-growing and stress tolerant plants whereas LSM is dominated by fast-growing plant species like the sedge *Carex fotedia* and the dwarf shrub *Salix herbacea* [11]. Five spatial replicates for each location at each date were collected from the top 10 cm of soil. Soils were sampled in October 2006 (litter-fall), May 2008 (snow-cover for LSM and free-snow for ESM) and July 2008 (growth vegetation).

### Culture media

The culture experiments were designed to select the heterotrophic bacteria in three different media and growing at 4°C and 20°C. The PYG agar medium (0.4% Peptone, 0.2% Yeast extract and 0.4% Glucose) was prepared as previously described [3]. The dTSA, was prepared by 10 fold dilution of Tryptocase Soy Agar. Soil Extract (SE) was prepared as follows: 40 gram of ESM soil were resuspended in 100 ml of distilled water, homogenised by agitation with a stirring bar, autoclaved at 121°C for 60 min; the suspension was filtered through a 0.2- $\mu$ m-pore-size filter, pH was adjusted to 7 and autoclaved at 121°C for 20 min after addition of 1.5% of agar.

### Isolation of bacterial strains

Cultivable heterotrophic bacteria were enumerated by the spread plate method. One gram of each replicate was selected for obtain 5 gram for each sample. 5 gram of dry weight from each soil sample was suspended in 50 ml of sterile buffer (1 g /litter MgSO<sub>4</sub>.7H<sub>2</sub>O, 1 mM

K<sub>2</sub>HPO<sub>4</sub>, pH 7.0) [35] and agitated for 15 min. After preliminary tests, the plates (n=3 for each media/temperature) were inoculated with 10<sup>-3</sup> soil dilution. Colonies were counted after 3 weeks (4°C) or 3 days (20°C) of incubation in aerobic conditions. Selected colonies were purified by streaking on PYG, dTSA and SE at least 3 times. A total of 89, 59 and 25 (Table S1) aerobic colonies from October, May and July were selected randomly from the PYG, dTSA and SE.

### **PCR amplification and sequencing of 16S rRNA**

The selected colonies were grown on PYG, dTSA and SE were suspended in 500 µl sterilized deionised water and frozen during 24h. After thawing, 1 µl of the bacterial suspension was used as the template to amplify the 16S rRNA by bacterial universal primers 27F (5'-GAGTTTGATCMTGGCTCAG- 3') [36] and com2ph (5' CCGTCAATTCCTTTGAGTTT-3') [50], corresponding to positions 8-27 and 907-926 in the 16S rRNA sequence of *Escherichia coli* [8], respectively. PCRs were performed with 2.5 mM MgCl<sub>2</sub>, 0.1 mM each deoxynucleoside triphosphate (dNTP), 0.4 µM each primer, 0.6 U Tag gold polymerase, and serum bovine buffer. The thermal PCR profile was as follows: initial denaturation at 95°C for 10 min followed by 30 cycles of denaturation at 95°C for 30 s, primer annealing at 56°C for 30 s, elongation at 72°C for 1.5 min, and the final elongation step was 7 min at 72°C. Sequencing was carried out at Genome Express, Grenoble (France).

### **Phylogenetic analysis and sequence comparison**

The partial 16S rRNA sequences of the isolates were compared with Ribosomal Database Project (RDP) (<http://rdp.cme.msu.edu>). The EMBL/GenBank accessions of the sequences are FN293176 to FN293348. Multiple sequence alignments were performed using ClustalW [58] and visually checked. The method of Kimura 2-parameter was used to calculate evolutionary distances, phylogenetic dendrograms were constructed by the Neighbor-Joining method and phylogenetic tree were evaluated by performing bootstrap analysis of 1000 data sets using MEGA 3.1 [34]. We assume that *ssu* libraries are representative of the presence of bacteria in soil. To asses the representation of cultivated strains, we created a local database from isolated strains using BioEdit 7.0.0 software (<http://www.mbio.ncsu.edu/BioEdit>) and

then compared the sequences obtained from clones' library against this local database using BLAST [1]. The sequences of *ssu* libraries sharing 97% similarity with the sequences of isolates were assigned to the same and were considered to belong at least to the same genus and cultivable. For ESM and LSM, we randomly selected 50 sequences from available *ssu* clone-libraries [64]-GenBank accession numbers FJ568339- FJ570564 and 25 sequences from isolated collection. For tree construction, we fixed a similarity threshold of 97%.

### **Statistical analysis**

We used ANOVA test using R software [46] to determine whether the number of cell viable bacteria and pigmented colonies were significantly different between the two locations, temperatures and culture media.

## Results

### Counts and phenotypic characterization of the isolates

The bacterial counts were higher in dTSA, and lower in SE, and more abundant at 20°C than at 4°C whatever the media and season for both locations (Fig. 1 and Table 1). The overall percentage of pigmented bacterial colonies was 33%. It was higher for ESM than for LSM (Fig. S1). Interestingly, both CFU and pigmented colonies counts displayed a different repartition according to the season and culture media at the two tested temperatures (Table 1). Indeed, CFU counts between two studied locations are not significantly different at two tested temperature. Pigmentation counts show an effect of temperature between two locations. Effect of season is significant for CUF and pigmentation count at two temperatures. Effect of medium culture is significant at all of condition in particular at 20°C.

Interestingly, the bacterial communities from July developed after 50 days at 4°C, while those of October and May developed after 20 days only and this was the case for both locations (personal observation).

### Phylogenetic analysis of isolated strains

We selected 173 representative colonies for characterization by *ssu* sequencing. The strains were assigned using Seqmatch-option [12] in RDP. Also a phylogenetic tree for isolates strains was constructed (Fig. S2). The cultivated strains belonged to four major lineages of the bacteria kingdom, namely  $\alpha$ - $\beta$ - $\gamma$ -*Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Bacteroidetes* and corresponded to 33 different genera (Fig. 3 and S2). The most abundant and diverse isolates were *Actinobacteria*, with *Arthrobacter* and *Streptomyces* as the dominants genera. The isolates of *Bacteroidetes* belong were related to *Flavobacteria* and *Sphingobacteria*. The  $\gamma$ -*Proteobacteria* strains were mostly related to *Pseudomonas*. For  $\alpha$ - and  $\beta$ -*Proteobacteria*, the isolates were less abundant and related to diverse genera (Fig. S2). For many strains (i.e. *Pseudomonas*, *Arthrobacter*, *Flavobacterium*) the ribotypes were very close, but displayed different morphological characteristics. Interestingly, for many genera (*Arthrobacter*, *Plantibacter*, *Rhodococcus*, *Paenibacillus*, *Caulobacter*, *Sphingomonas*,



*Flavobacterium*, *Pedobacter* and *Pseudomonas*), the best matches in RDP corresponded to bacteria isolated from cold habitats (Fig. S2).

### **Comparison with ESM and LSM *ssu* libraries**

The availability of *ssu* library allowed us to compare the bacterial diversity with those of the cultivated collection. The proportion of cultivated strains sharing similarity with the *ssu* library was assessed by BLAST on the database of *ssu* libraries. Isolates represented 3.5% of clones from the ESM library, and 1% of clones from the LSM library. The phylogenetic diversity of cultivated strains and the *ssu* library were compared in phylogenetic tree. The tree was constructed by random chose of molecular and cultivated sequences (Fig. 2). The phyla *Acidobacteria*,  $\delta$ -*Proteobacteria* and *Gemmatimonadetes* were absent in culture collection. The families can be cultivated were listed in Table S2. We found the genera *Arthrobacter*, *Streptomyces* and *Bacillus* were underrepresented in the *ssu* library.

### **Seasonal and spatial distribution**

The phylogenetic diversity of cultivated strains changed over the sampling times. In both locations, *Actinobacteria*, *Bacteroidetes* and  $\gamma$ -*Proteobacteria* were more abundant in July, October and May, respectively (Fig. 3). At the genus level, isolates related to *Streptomyces* and *Plantibacter* were mostly represented in July-October and July-May (Fig. 3). Spatial variations were observed at genus level; members of *Arthrobacter* were specific to ESM, *Kitasatosporia* and *Zoogloea* was specific to LSM (Fig. 3 and Fig. S2)

## Discussion

Cultivable bacteria represent less than 1% of total bacteria [59]. However, the study of cultivable bacterial diversity is important to assess the impact of anthropic activities or for the search of cold adapted bacteria for biotechnology. The presence culture of bacteria from cold environments like permafrost soils has already been investigated [3, 22, 55, 63]. Reported values ranged from  $10^1 - 10^3$  to  $10^2 - 10^8$  CFU/gr of dry soil. Our estimates ( $10^5 - 10^7$ ) are comparable to those of the permafrost in Tianshan ( $10^5 - 10^6$  [3]) and Spitsbergen Norway ( $10^5 - 10^6$  [24]).

The number of CFU was higher at 20°C than at 4°C (Fig. 1). This phenomenon has also been observed in other cold habitats [18, 47, 52]. Here, the selected isolates growing at 20°C grew also at 4°C, and vice versa (B. Shahnava, unpublished results). Thus, and according to Morita's classification [42], these isolates were psychrotrophic (cold-tolerant) rather than psychrophilic (cold-adapted).

In the present study, we found that one third of isolates formed pigmented colonies. The presence of pigments has been suggested to play a role in adaptation to both low temperature and environmental stress [43]. Similar results were also reported for other cold ecosystems [18, 41]. These results support the importance of pigmentation as a mechanism for bacterial survival in alpine tundra.

Temperature incubation seems to have a stronger impact in bacteria isolation. Interestingly, the CFU counts were higher in ESM than in LSM at 20°C during October; while in the LSM samples of May (covered by snow), CFU were much higher at 20°C than at 4°C, that may be consistent with the protective role of snow cover and microbial activity under the snow, which were reported in previous studies [44, 51].

As in other surveys conducted in cold ecosystems [3, 4, 24, 32, 49, 61], we found that members of the *Actinobacteria* were dominant among cultivable bacteria (Fig. 3). The high G+C content, the specific composition of cell wall and the ability of this group to form dormant cells provide the capability to survive under harsh conditions [61]. Along the same line, the high frequency of isolation of *Arthrobacter* species from cold environments has already been noticed [3, 24, 61]. Species related to genus *Arthrobacter* was reported in polluted site [29, 30]. These species were mainly isolated from ESM that was consistent with higher content of recalcitrant compounds in this location.

The dominance of *Streptomyces* in July (Fig. S2), i.e. peak of the growing season, and October, i.e. leaf senescence and litter fall, can be explained to their association to root plant [14, 48] and the cellulose-degrading capabilities that characterizes members of this genus [60].

In a previous study, we found that the abundance of clones from *Pseudomonadales*, *Burkholderiales*, *Flavobacteriales* and *Sphingobacteriales* increased in October (after litter fall). This increase was concomitant with the clustering of the corresponding phyla or sub-phyla ( $\beta$ -, $\gamma$ -*Proteobacteria* and *Bacteroidetes*). We have suggested that this increase was due to the shift of nutrient source (from root exudates to plant-litter). The increase of *Flavobacterium* and *Pseudomonas* cultivated strains during October at both locations support the previous conclusions. Strains from these genera are capable to degrade complex organic material [13]. Moreover, the closest relatives of some strains are able to degrade complex organic molecules like pollutants (see Fig. S2B).

The increased abundance of members of *Pseudomonas* compared to other members of  $\gamma$ -*Proteobacteria* was consistent with previous results in other cold habitats [3, 24, 40, 63]. Furthermore, the failure to cultivate them during July was previously observed [53].

The culture results correlate with the molecular studies for the above mentioned strains. However, it should be reminded that several phyla are not cultivable using standard methods. Actually the clone libraries show the presence of *Acidobacteria* (ranging between 15 to 54% in our clones library) [64], *Rubrobacterineae* and *Acidimicrobineae* that require special cultivation approaches and exhibit few cultivated strains [16, 26, 28, 31]. Despite of diversity and abundance of *Acidobacteria* in soils [5], even in cold habitats [35, 54], the knowledge on their physiological characteristics and role is very limited.

It should be noted that for cyst-like or sporulating bacteria (i.e. *Arthrobacter*, *Streptomyces* and *Bacillus*) a cultivation methods provides a higher richness, while they are rare in clone libraries. It is possible that the DNA extraction from these strains are less performing leading to an under representation in the clone libraries [39, 20]. The present study demonstrated that each method showed a fraction of bacterial communities in our studies. The amount of match between culture collection and direct PCR amplification depended on many factor such as complexity of environment sample, different between size of 16S RNA and culture. Nevertheless of quality or quantity of overlapping between two methods, the most importance is the presence similar results between two methods.

## Conclusion

The present study provided evidence that majority of bacteria from alpine soil are not cultivable. Unfortunately, none of the culture conditions that were tested allowed us to isolate strains of the most abundant bacteria in these soils, i.e. *Acidobacteria*. This calls for improved cultivation technique or alternative methodologies, i.e. soil metatranscriptomic [9] to understand the ecological role of these bacteria and their potential interest for bioremediation and biotechnological developments.

## **Acknowledgements**

We thank Jean-Marc Bonneville and Lucile Sage for technical and scientific discussion. Serge Aubert and the staff of Station Alpine J. Fourier for providing logistic facilities during the fieldwork. B. Shahnavaaz was supported by PhD scholarships from the Iranian Ministry of Science, Research, and Technology. The work was funded by the ANR-06-BLAN-0301 "Microalpes" project.

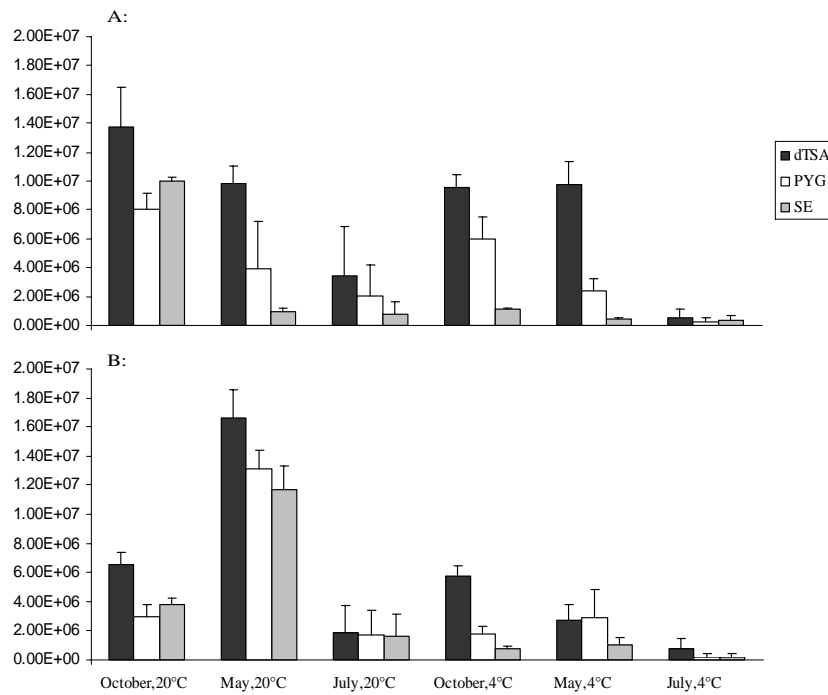
**Chapitre 4 : Effet des régimes d'enneigement sur la richesse des espèces bactériennes cultivables des sols alpins**

Table 1:

**Effect of two different temperatures on cell viable bacteria in different season and location. Mean of count in each temperature for CFU and pigmentation was calculated (n=27).** Different of count and pigmentation were determined by ANOVA test with R software [46]. The significant of count estimates is shown as follow: \*\*\*: P<0.001, \*\*: P<0.01, \*: P<0.05.

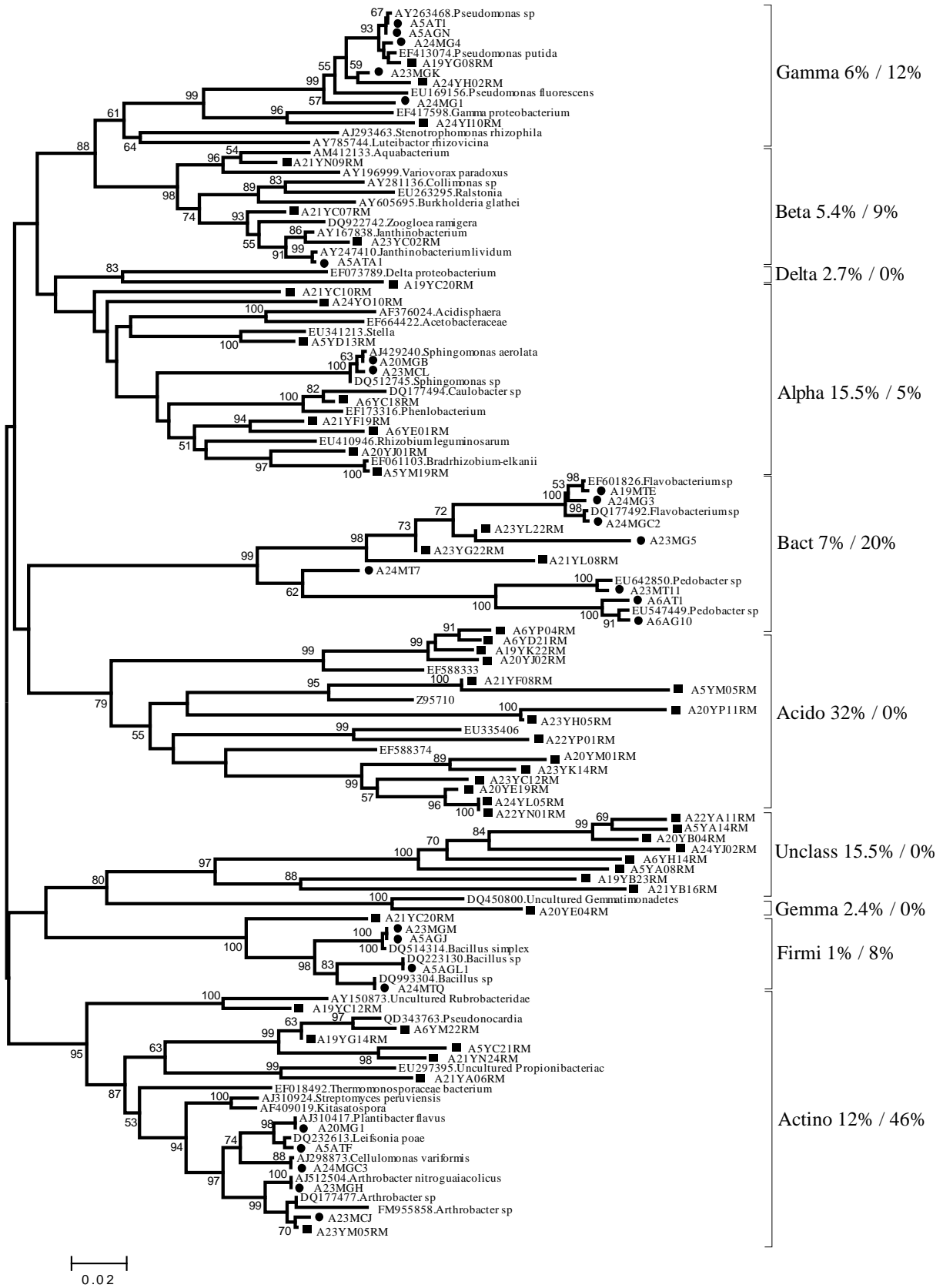
	Df	CFU in different temperature		Pigmentation in different temperature	
		F value (significant value)		F value (significant value)	
		4°C	20°C	4°C	20°C
Location	1	0.73 (0.4)	2.2 (0.14)	0.8 (0.4)	10.05 (0.003**)
Season	2	10.03 (0.0003***)	46.76 (9.78E-11***)	36.4 (2.27E-09***)	34.9 (3.7E-09***)
Medium	2	4.3 (0.02*)	15.8 (1.17E-05***)	4.7 (0.015*)	24.33 (2.06E-07***)
Location/Season	2	0.15 (0.85)	26.11 (9.8E-08***)	3.2 (0.05)	5.11 (0.01*)
Location/Medium	2	0.4 (0.7)	5.8 (0.006**)	5.18 (0.01*)	5.2 (0.009**)
Season/Medium	4	0.4 (0.7)	2.06 (0.1)	2.3 (0.07)	3.3 (0.02*)

## Chapitre 4 : Effet des régimes d'enneigement sur la richesse des espèces bactériennes cultivables des sols alpins



**Figure 1. Number of Cell viable bacteria in A: Early and B: Late Snow Melt locations in different medium and at two different temperatures. dTSA: Diluted Trypto Soy Broth agar, PYG: Peptone yeast glucose agar and SE: soil extracted.**

Chapitre 4 : Effet des régimes d'enneigement sur la richesse des espèces bactériennes cultivables des sols alpins





**Figure 2. Phylogenetic tree of randomly sequences from clone library and isolates collection.**

Black-filled circles indicate isolated bacterial strains. The clones and isolates from ESM are labelled with A19 (June, July), A21 (August), A23 (October) and May (A6). The clones and isolates from LSM are labelled with A20 (June, July), A22 (August), A24 (October) and May (A5). The numbers indicated for each bacterial group showed the percentage of total sequences in clones library and culture, respectively. For example, in Gamma 6% and 12% of sequences were related to clones library and culture, respectively. Acido: *Acidobacteria*; Actino: *Actionbacteria*; Alpha, Beta, Gamma and Delta:  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ -*Proteobacteria*; Bact: *Bacteroides*; Gemma: *Gemmatimonadetes*; Unclass: Unclassified bacteria.

## Chapitre 4 : Effet des régimes d'enneigement sur la richesse des espèces bactériennes cultivables des sols alpins

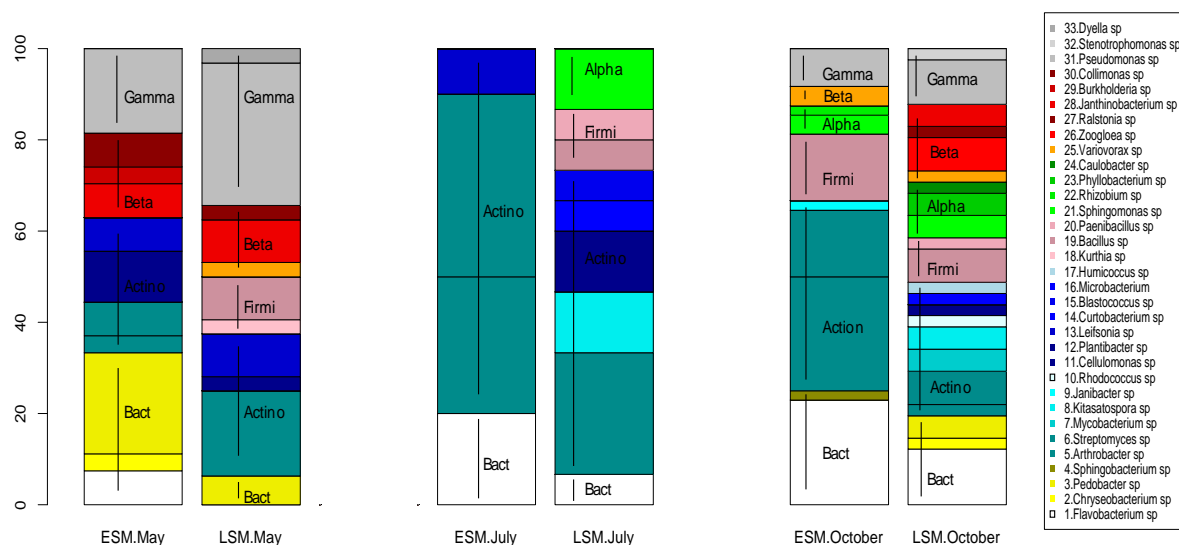


Figure 3. Seasonal and spatial variation of cultivated bacteria in ESM and LSM at genus level. Genera related to  $\alpha$ -,  $\beta$ -,  $\gamma$ -Proteobacteria, Actinobacteria and Bacteroidetes were showed with grey, red, green, blue and yellow. Abbreviations are showed in Figure 2.

## References

- [1] S.F. Altschul, T.L. Madden, A.A. Schaffer, J.H. Zhang, Z. Zhang, W. Miller, D.J. Lipman, Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, *Nucl Acids Res* 25 (1997) 3389-3402.
- [2] P. Amato, R. Hennebelle, O. Magand, M. Sancelme, A.M. Delort, C. Barbante, C. Boutron, C. Ferrari, Bacterial characterization of the snow cover at Spitzberg, Svalbard, *FEMS Microbiol Ecol* 59 (2007) 255-264.
- [3] Y. Bai, D. Yang, J. Wang, S. Xu, X. Wang, L. An, Phylogenetic diversity of culturable bacteria from alpine permafrost in the Tianshan Mountains, northwestern China, *Res Microbiol* 157 (2006) 741-751.
- [4] C. Bakermans, A.I. Tsapin, V. Souza-Egipsy, D.A. Gilichinsky, K.H. Nealson, Reproduction and metabolism at -10 degrees C of bacteria isolated from Siberian permafrost, *Environ Microbiol* 5 (2003) 321-326.
- [5] S.M. Barns, E.C. Cain, L. Sommerville, C.R. Kuske, Acidobacteria phylum sequences in uranium-contaminated subsurface sediments greatly expand the known diversity within the phylum, *Appl Environ Microbiol* 73 (2007) 3113-3116.
- [6] M. Bentahir, G. Feller, M. Aittaleb, J. Lamotte-Brasseur, T. Himri, J.P. Chessa, C. Gerday, Structural, kinetic, and calorimetric characterization of the cold-active phosphoglycerate kinase from the Antarctic *Pseudomonas* sp TACII18, *J BIOL CHEM* 275 (2000) 11147-11153.
- [7] P.D. Brooks, M.W. Williams, S.K. Schmidt, Microbial activity under alpine snowpacks, Niwot Ridge, Colorado, *Biogeochemistry* 32 (1996) 93-113.
- [8] J. Brosius, T.J. Dull, D.D. Sleeter, H.F. Noller, Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*, *J Mol Biol* 148 (1981) 107-127.
- [9] E. Cardenas, J.M. Tiedje, New tools for discovering and characterizing microbial diversity, *Curr Opin Biotechnol* 19 (2008) 544-549.
- [10] D.P. Chandler, S.M. Li, C.M. Spadoni, G.R. Drake, D.L. Balkwill, J.K. Fredrickson, F.J. Brockman, A molecular comparison of culturable aerobic heterotrophic bacteria and 16S rDNA clones derived from a deep subsurface sediment, *FEMS Microbiol Ecol* 23 (1997) 131-144.
- [11] P. Choler, Consistent shifts in Alpine plant traits along a mesotopographical gradient, *Arct Antarct Alp Res Arctic Antarctic And Alpine Research* 37 (2005) 444-453.

- [12] J.R. Cole, B. Chai, T.L. Marsh, R.J. Farris, Q. Wang, S.A. Kulam, S. Chandra, D.M. McGarrell, T.M. Schmidt, G.M. Garrity, J.M. Tiedje, The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy, *Nucl Acids Res* 31 (2003) 442-443.
- [13] M.T. Cottrell, D.L. Kirchman, Natural assemblages of marine proteobacteria and members of the Cytophaga-Flavobacter cluster consuming low- and high-molecular-weight dissolved organic matter, *Appl Environ Microbiol* 66 (2000) 1692-1697.
- [14] J.M. de Araujo, A.C. da Silva, J.L. Azevedo, Isolation of endophytic actinomycetes from roots and leaves of maize (*Zea may L.*), *Braz Arch Biol Techn* 43 (2000) 447-451.
- [15] J. Dunbar, S. Takala, S.M. Barns, J.A. Davis, C.R. Kuske, Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning, *Appl Environ Microbiol* 65 (1999) 1662-1669.
- [16] S.A. Eichorst, J.A. Breznak, T.M. Schmidt, Isolation and characterization of soil bacteria that define *Terriglobus* gen. nov., in the phylum Acidobacteria, *Appl Environ Microbiol* 73 (2007) 2708-2717.
- [17] R.J. Ellis, P. Morgan, A.J. Weightman, J.C. Fry, Cultivation-dependent and -independent approaches for determining bacterial diversity in heavy-metal-contaminated soil, *Appl Environ Microbiol* 69 (2003) 3223-3230.
- [18] J. Foght, J. Aislabie, S. Turner, C.E. Brown, J. Ryburn, D.J. Saul, W. Lawson, Culturable bacteria in subglacial sediments and ice from two Southern Hemisphere glaciers, *Microb Ecol* 47 (2004) 329-340.
- [19] N.J.C. Fong, M.L. Burgess, K.D. Barrow, D.R. Glenn, Carotenoid accumulation in the psychrotrophic bacterium *Arthrobacter agilis* in response to thermal and salt stress, *Appl Microbiol Biot* 56 (2001) 750-756.
- [20] A. Frostegard, S. Courtois, V. Ramisse, S. Clerc, D. Bernillon, F. Le Gall, P. Jeannin, X. Nesme, P. Simonet, Quantification of bias related to the extraction of DNA directly from soils, *Appl Environ Microbiol* 65 (1999) 5409-5420.
- [21] D. Georlette, Z.O. Jonsson, F. Van Petegem, J.P. Chessa, J. Van Beeumen, U. Huscher, C. Gerday, A DNA ligase from the psychrophile *Pseudoalteromonas haloplanktis* gives insights into the adaptation of proteins to low temperatures, *Eur. J. Biochem* 267 (2000) 3502-3512.

- [22] D.A. Gilichinskii, E. A. Vorobyova, L. G. Erokhina, D. G. Fyodorov-Dayvdov and N. R. Chaikovskaya, Long-term Preservation of Microbial Ecosystems in Permafrost, *Adv. Space Res* 12 (1992) 255-263.
- [23] T. Groudieva, M. Kambourova, H. Yusef, M. Royter, R. Grote, H. Trinks, G. Antranikian, Diversity and cold-active hydrolytic enzymes of culturable bacteria associated with Arctic sea ice, Spitzbergen, *Extremophiles* 8 (2004) 475-488.
- [24] A.A. Hansen, R.A. Herbert, K. Mikkelsen, L.L. Jensen, T. Kristoffersen, J.M. Tiedje, B.A. Lomstein, K.W. Finster, Viability, diversity and composition of the bacterial community in a high Arctic permafrost soil from Spitsbergen, Northern Norway, *Environ Microbiol* 9 (2007) 2870-2884.
- [25] T. Hattori, H. Mitsui, H. Haga, N.Wakao, S. Shikano, K. Gorlach, Y. Kasahara, A. ElBeltagy, R. Hattori, Advances in soil microbial ecology and the biodiversity, *Antonie Van Leeuwenhoek International Journal Of General And Molecular Microbiology* 72 (1997) 21-28.
- [26] A.J. Holmes, J. Bowyer, M.P. Holley, M. O'Donoghue, M. Montgomery, M.R. Gillings, Diverse, yet-to-be-cultured members of the Rubrobacter subdivision of the Actinobacteria are widespread in Australian arid soils, *FEMS Microbiol Ecol* 33 (2000) 111-120.
- [27] A. Hoyoux, I. Jennes, P. Dubois, S. Genicot, F. Dubail, J.M. Francois, E. Baise, G. Feller, C. Gerday, Cold-adapted beta-galactosidase from the Antarctic psychrophile *Pseudoalteromonas haloplanktis*, *Appl Environ Microbiol* 67 (2001) 1529-1535.
- [28] P. Hugenholtz, B.M. Goebel, N.R. Pace, Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity, *J Bacteriol* 180 (1998) 4765-4774.
- [29] C. Karigar, A. Mahesh, M. Nagenahalli, D.J. Yun, Phenol degradation by immobilized cells of *Arthrobacter citreus*, *Biodegradation* 17 (2006) 47-55.
- [30] S. Keuth, H.J. Rehm, Biodegradation Of Phenanthrene By *Arthrobacter Polychromogenes* Isolated From A Contaminated Soil, *Appl Microbiol Biot* 34 (1991) 804-808.
- [31] N. Kishimoto, T. Tano, Acidophilic Heterotrophic Bacteria Isolated From Acidic Mine Drainage, Sewage, And Soils, *J. Gen. Appl. Microbiol* 33 (1987) 11-25.
- [32] G.A. Kochkina, N.E. Ivanushkina, S.G. Karasev, E.Y. Gavrish, L.V. Gurina, L.I. Evtushenko, E.V. Spirina, E.A. Vorob'eva, D.A. Gilichinskii, S.M. Ozerskaya, Survival of micromycetes and actinobacteria under conditions of long-term natural cryopreservation, *Microbiology* 70 (2001) 356-364.

- [33] G.S. Kumar, M.V. Jagannadham, M.K. Ray, Low-temperature-induced changes in composition and fluidity of lipopolysaccharides in the antarctic psychrotrophic bacterium *Pseudomonas syringae*, *J Bacteriol* 184 (2002) 6746-6749.
- [34] S. Kumar, K. Tamura, M. Nei, MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment, *Brief Bioinform* 5 (2004) 150-163.
- [35] D.A. Lipson, S.K. Schmidt, Seasonal changes in an alpine soil bacterial community in the colorado rocky mountains, *Appl Environ Microbiol* 70 (2004) 2867-2879.
- [36] J.R. Marchesi, T. Sato, A.J. Weightman, T.A. Martin, J.C. Fry, S.J. Hiom, D. Dymock, W.G. Wade, Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA, *Appl Environ Microbiol* 64 (1998) 795-799.
- [37] R. Margesin, Alpine microorganisms: Useful tools for low-temperature bioremediation, *J Microbiol* 45 (2007) 281-285.
- [38] R. Margesin, F. Schinner, Biodegradation and bioremediation of hydrocarbons in extreme environments, *Appl Microbiol Biot* 56 (2001) 650-663.
- [39] F. Martin-Laurent, L. Philippot, S. Hallet, R. Chaussod, J.C. Germon, G. Soulas, G. Catroux, DNA extraction from soils: Old bias for new microbial diversity analysis methods, *Appl Environ Microbiol* 67 (2001) 2354-2359.
- [40] A.F. Meyer, D.A. Lipson, A.P. Martin, C.W. Schadt, S.K. Schmidt, Molecular and metabolic characterization of cold-tolerant alpine soil *Pseudomonas sensu stricto*, *Appl Environ Microbiol* 70 (2004) 483-489.
- [41] V.I. Miteva, P.P. Sheridan, J.E. Brenchley, Phylogenetic and physiological diversity of microorganisms isolated from a deep Greenland glacier ice core, *Appl Environ Microbiol* 70 (2004) 202-213.
- [42] R.Y. Morita, Psychrophilic Bacteria, *Bacteriological Reviews* 39 (1975) 144-167.
- [43] D.R. Mueller, W.F. Vincent, S. Bonilla, I. Laurion, Extremotrophs, extremophiles and broadband pigmentation strategies in a high arctic ice shelf ecosystem, *FEMS Microbiol Ecol* 53 (2005) 73-87.
- [44] D.R. Nemergut, E.K. Costello, A.F. Meyer, M.Y. Pescador, M.N. Weintraub, S.K. Schmidt, Structure and function of alpine and arctic soil microbial communities, *Res Microbiol* 156 (2005) 775-784.
- [45] J.I. Prosser, Molecular and functional diversity in soil micro-organisms, *Plant Soil* 244 (2002) 9-17.

- [46] R Development Core Team. R: A Language and Environment for Statistical Computing. (2008). <http://www.R-project.org>.
- [47] E. Rivkina, K. Laurinavichius, J. McGrath, J. Tiedje, V. Shcherbakova, D. Gilichinsky, Microbial life in permafrost, *Space Life Sciences: Search For Signatures Of Life, And Space Flight Environmental Effects On The Nervous System* 33 (2004) 1215-1221.
- [48] P. Sardi, M. Saracchi, S. Quaroni, B. Petrolini, G.E. Borgonovi, S. Merli, Isolation Of Endophytic Streptomyces Strains From Surface-Sterilized Roots, *Appl Environ Microbiol* 58 (1992) 2691-2693.
- [49] D.J. Saul, J.M. Aislabie, C.E. Brown, L. Harris, J.M. Foght, Hydrocarbon contamination changes the bacterial diversity of soil from around Scott Base, Antarctica, *FEMS Microbiol Ecol* 53 (2005) 141-155.
- [50] A. Schmalenberger, F. Schwieger, C.C. Tebbe, Effect of primers hybridizing to different evolutionarily conserved regions of the small-subunit rRNA gene in PCR-based microbial community analyses and genetic profiling, *Appl Environ Microbiol* 67 (2001) 3557-3563.
- [51] S.K. Schmidt, E.K. Costello, D.R. Nemergut, C.C. Cleveland, S.C. Reed, M.N. Weintraub, A.F. Meyer, A.M. Martin, Biogeochemical consequences of rapid microbial turnover and seasonal succession in soil, *Ecology* 88 (2007) 1379-1385.
- [52] T. Shi, R.H. Reeves, D.A. Gilichinsky, E.I. Friedmann, Characterization of viable bacteria from Siberian permafrost by 16S rDNA sequencing, *Microb Ecol* 33 (1997) 169-179.
- [53] E. Smit, P. Leeflang, S. Gommans, J. van den Broek, S. van Mil, K. Wernars, Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods, *Appl Environ Microbiol* 67 (2001) 2284-2291.
- [54] J.J. Smith, L.A. Tow, W. Stafford, C. Cary, D.A. Cowan, Bacterial diversity in three different Antarctic Cold Desert mineral soils, *Microb Ecol* 51 (2006) 413-421.
- [55] B. Steven, G. Briggs, C.P. McKay, W.H. Pollard, C.W. Greer, L.G. Whyte, Characterization of the microbial diversity in a permafrost sample from the Canadian high Arctic using culture-dependent and culture-independent methods, *FEMS Microbiol Ecol* 59 (2007) 513-523.
- [56] M.T. Suzuki, M.S. Rappe, Z.W. Haimberger, H. Winfield, N. Adair, J. Strobel, S.J. Giovannoni, Bacterial diversity among small-subunit rRNA gene clones and cellular isolates from the same seawater sample, *Appl Environ Microbiol* 63 (1997) 983-989.

- [57] H. Tamaki, Y. Sekiguchi, S. Hanada, K. Nakamura, N. Nomura, M. Matsumura, Y. Kamagata, Comparative analysis of bacterial diversity in freshwater sediment of a shallow eutrophic lake by molecular and improved cultivation-based techniques, *Appl Environ Microbiol* 71 (2005) 2162-2169.
- [58] J.D. Thompson, D.G. Higgins, T.J. Gibson, Clustal-W - Improving The Sensitivity Of Progressive Multiple Sequence Alignment Through Sequence Weighting, Position-Specific Gap Penalties And Weight Matrix Choice, *Nucl Acids Res* 22 (1994) 4673-4680.
- [59] V. Torsvik, F.L. Daae, R.A. Sandaa, L. Ovreas, Novel techniques for analysing microbial diversity in natural and perturbed environments, *J Biotechnol* 64 (1998) 53-62.
- [60] A. Ulrich, G. Klimke, S. Wirth, Diversity and activity of cellulose-decomposing bacteria, isolated from a sandy and a loamy soil after long-term manure application, *Microb Ecol* 55 (2008) 512-522.
- [61] T.A. Vishnivetskaya, M.A. Petrova, J. Urbance, M. Ponder, C.L. Moyer, D.A. Gilichinsky, J.M. Tiedje, Bacterial community in ancient Siberian permafrost as characterized by culture and culture-independent methods, *Astrobiology* 6 (2006) 400-414.
- [62] F. vonWintzingerode, U.B. Gobel, E. Stackebrandt, Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis, *FEMS Microbiol Rev* 21 (1997) 213-229.
- [63] G.S. Zhang, F.J. Niu, X.J. Ma, W. Liu, M.X. Dong, H.Y. Feng, L.Z. An, G.D. Cheng, Phylogenetic diversity of bacteria isolates from the Qinghai-Tibet Plateau permafrost region, *Can. J. Microbiol* 53 (2007) 1000-1010.
- [64] L. Zinger, B. Shahnavaaz, F. Baptist, R.A. Geremia, P. Choler, Microbial diversity in alpine tundra soils correlates with snow cover dynamics, *ISME J* (doi:10.1038/ismej.2009.20) (2009)



**Chapitre 4 : Effet des régimes d'enneigement sur la richesse des espèces bactériennes cultivables des sols alpins**

**Supplementary Figures and Information**

Table S1

Numbers of isolates in different seasons and locations

Isolate	Division	October		May		July		Medium culture
		ESM	LSM	ESM	LSM	ESM	LSM	
Flavobacterium	Bacteroidetes	11	5	2		2	1	dTSA-PYG
Chryseobacterium	Bacteroidetes		1	1				dTSA
Pedobacter	Bacteroidetes		2	6	2			dTSA-PYG-Soil Extract
Sphingobacterium	Bacteroidetes	1						dTSA
Arthrobacter	Actino	12	1	1	6	3		dTSA-PYG-Soil Extract
Streptomyces	Actino	7	3	2		4	4	dTSA-PYG-Soil Extract
Mycobacterium	Actino		2					dTSA-PYG
Kitasatospora	Actino		2			2		Soil Extract-PYG
Janibacter	Actino	1						PYG
Rhodococcus	Actino		1					dTSA
Cellulomonas	Actino		1					PYG
Plantibacter	Actino			1	1		2	dTSA-PYG-Soil Extract
Leifsonia	Actino			4	3			dTSA-PYG-Soil Extract
Curtobacterium	Actino						1	PYG
Blastococcus	Actino						1	PYG
Microbacterium	Actino		1					PYG
Humicoccus	Actino		1					Dtsa
Kurthia	Firmicutes				1		1	PYG
Bacillus	Firmicutes	7	3		3		1	dTSA-PYG-Soil Extract
Paenibacillus	Firmicutes		1		1			dTSA
Sphingomonas	Alpha	2	2				2	dTSA-PYG-Soil Extract
Rhizobium	Alpha	1						Soil Extract
Phyllobacterium	Alpha		2					dTSA-PYG
Caulobacter	Alpha		1					dTSA
Variovorax	Beta	2	1		1			dTSA-Soil Extract
Zoogloea	Beta		3		1			dTSA-PYG
Ralstonia	Beta		1					dTSA
Janthinobacterium	Beta		2	1	3			PYG-dTSA
Burkholderia	Beta			1				dTSA
Collimonas	Beta			3				dTSA-PYG
Pseudomonas	Gamma	4	4	5	9			dTSA-PYG-Soil Extract
Stenotrophomonas	Gamma		1					dTSA
Dyella	Gamma				1			Soil Extract
<b>Total</b>		<b>48</b>	<b>41</b>	<b>27</b>	<b>32</b>	<b>10</b>	<b>15</b>	

Chapitre 4 : Effet des régimes d'enneigement sur la richesse des espèces bactériennes cultivables des sols alpins

Table S2

The phylum, order and family obtained by clone library method. The phylum and subphylum of this method that were cultivable in comparison with culture method were highlighted by star. *Acidobacteria* and *Gemmatimonadetes* have not shown.

Actino	Alpha	Beta	Gamma	Delta	Bacteroidetes	Firmicutes
Acidimicrobiales	Caulobacterales*	Burkholderiales*	Legoinellales	Bdellovibrionales	Flavobacteriales*	Bacillales*
Actinomycetales*	Rhizobiales*	Rhodocyclales	Pseudomonadales*	Mycoccales	Sphingobacteriales*	Clostridiales
Rubrobacterales	Rhodospirillales		Xanthomonadales*			Incertaedis8
	Sphingomonadales*					Thermoanaerobacteriales
Acidimicrobiaceae	Acetobacteraceae	Alcaligenaceae	Chromatiaceae		Crenotrichaceae	Acidaminococcaceae
Catenulisporaceae	Beijerinckiaceae	Burkholderiaceae*	Coxiellaceae		Cryomorphaceae	Bacillaceae
Cellulomonadaceae	Bradyrhizobiaceae	Comamonadaceae*	Ectothiorhodospiraceae		Flexibacteraceae	Clostridiaceae
Geodermatophilaceae	Caulobacteraceae*	Gallionellaceae	Hahellaceae		Flavobacteriaceae*	Erysipelotrichaceae*
Glycomycetaceae	Hyphomicrobiaceae	Incertaedis5	Halomonadaceae		Porphyromonadaceae	Paenibacillaceae
Gordoniaceae	Incertaedis4	Neisseriaceae	Methylococcaceae		Rikenellaceae	Planococcaceae
Kineosporiaceae	Methylobacteriaceae	Nitrosomonadaceae	Piscirickettsiaceae		Saprosiraceae	Thermoanaerobacteriaceae*
Microbacteriaceae *	Phyllobacteriaceae*	Oxalobacteraceae*	Pseudomonadaceae*		Sphingomonadaceae*	
Micromonosporaceae*	Rhodobacteraceae	Rhodocyclaceae	Saccharosporillaceae			
Mycobacteriaceae*	Rhizobiaceae*		Thiotrichaceae			
Micrococcaceae*	Rhodobiaceae		Xanthomonadaceae*			
Nakamurellaceae	Rhodospirillaceae					
Nocardioideae	Sphingobacteriaceae*					
Pseudonocardioideae						
Patulibacteraceae						
Propionibacteriaceae						
Rubrobacteraceae						
Sanguibacteraceae						
Sporichthyaceae						
Streptosporangiaceae						
Streptomycetaceae*						
Thermomonosporaceae						

Table S3

The bacterial isolates obtained from alpine soils collected on various dates and grown under a variety of conditions.

#### Chapitre 4 : Effet des régimes d'enneigement sur la richesse des espèces bactériennes cultivables des sols alpins

Isolate	colony morphology	Temp (°C)	Medium	Location	Season	Division	Identity	Accession	Similarity (%)
A24MGC2	orange.rond.small.smooth	20	PYG	LSM	October	Bacteroidetes	Flavobacterium	AM167566	95,7
A24MTG	orange.long.smooth	20	dTSA	LSM	October	Bacteroidetes	Flavobacterium	AM177624	94
A24MG510	orange.long.smooth	20	PYG	LSM	October	Bacteroidetes	Flavobacterium	AB183888	98,1
A24MGC1	white.small.star.rough	20	PYG	LSM	October	Actinobacteria	Streptomyces	AJ308571	100
A24MCD	white.rough	20	Soil Extract	LSM	October	Actinobacteria	Streptomyces	AB217604	97,7
A24MG592	violet.smalle.rough	20	PYG	LSM	October	Actinobacteria	Streptomyces	DQ462657	97,8
A24MG23	rose.smooth.small	20	PYG	LSM	October	Actinobacteria	Mycobacterium	DQ372731	96
A24MTL	rose.small.smooth	20	dTSA	LSM	October	Actinobacteria	Mycobacterium	AF408955	97,3
A24MG562	white.smalle.rough	20	PYG	LSM	October	Actinobacteria	Kitasatospora	U93318	99
A24MG561	white.smalle.rough	20	PYG	LSM	October	Actinobacteria	Kitasatospora	U93318	99
A24MTA	orangepale.rond.smooth	20	dTSA	LSM	October	Actinobacteria	Rhodococcus	AY512641	92,5
A24MGC3	yellowgrin.rond.smooth	20	PYG	LSM	October	Actinobacteria	Cellulomonas	AB210980	94,2
A24MG571	yellowgrin.grand.smooth	20	PYG	LSM	October	Actinobacteria	Microbacterium	AJ968705	89,2
A24MTO	white.small.smooth	20	dTSA	LSM	October	Actinobacteria	Humicoccus	DQ321750	90
A24MTM	white.grand.smooth	20	dTSA	LSM	October	Alphaproteobacteria	Phyllobacterium	AJ968698	98,8
A24MGD	white.grand.smooth	20	PYG	LSM	October	Alphaproteobacteria	Phyllobacterium	FJ154092	97
A24MT2	cream.moyenne.rond	20	dTSA	LSM	October	Alphaproteobacteria	Caulobacter	DQ341428	98,2
A24MCF	white.small.cream	20	Soil Extract	LSM	October	Betaproteobacteria	Variovorax	AB196432	90,3
A24MTE1	white.opaque.small.viscous	20	dTSA	LSM	October	Betaproteobacteria	Zoogloea	D14256	94
A24MTE3	white.clear.small	20	dTSA	LSM	October	Betaproteobacteria	Zoogloea	D14256	93
A24MG582	white.opaque.large.viscous	20	PYG	LSM	October	Betaproteobacteria	Zoogloea	X74914	94,4
A24MG105	rosepale.small.smooth	20	PYG	LSM	October	Betaproteobacteria	Ralstonia	AY509958	100
A24MTF	white.cream.medium	20	dTSA	LSM	October	Gammaproteobacteria	Pseudomonas	DQ227338	96,1
A24MG1	white.grand.smooth	20	PYG	LSM	October	Gammaproteobacteria	Pseudomonas	AY785324	97,5
A24MTH	yellow.grand.smooth	20	dTSA	LSM	October	Gammaproteobacteria	Stenotrophomonas	DQ321750	75
A24MTP	white.small.smooth	20	dTSA	LSM	October	Firmicutes	Bacillus	AY660701	94,4
A24MTQ	lemone.grande.smooth	20	dTSA	LSM	October	Firmicutes	Bacillus	AM293001	100
A24MG104	white.small.smooth	20	PYG	LSM	October	Firmicutes	Bacillus	AY660701	90
A24MTD	mocoid.moyenne.yellowgrine	20	dTSA	LSM	October	Firmicutes	Paenibacillus	AB073364	90
A24MT	orange.long.smooth	4	dTSA	LSM	October	Bacteroidetes	Flavobacterium	EU057852	98
A24MG3	yellow.medium.rond	4	PYG	LSM	October	Bacteroidetes	Flavobacterium	AM230488	98
A24MT7	yellow.medium.rond	4	dTSA	LSM	October	Bacteroidetes	Chryseobacterium	DQ301786	92,8
A24MT10	orange.moyenne.cream	4	dTSA	LSM	October	Bacteroidetes	Pedobacter	AB267717	85
A24MT15	yellow.moyenne.cream	4	dTSA	LSM	October	Bacteroidetes	Pedobacter	DQ778037	99
A24MT11	white.rond.smooth	4	dTSA	LSM	October	Actinobacteria	Arthrobacter	AB288059	99,6

#### Chapitre 4 : Effet des régimes d'enneigement sur la richesse des espèces bactériennes cultivables des sols alpins

Isolate	colony morphology	Temp (°C)	Medium	Location	Season	Division	Identity	Accession	Similarity (%)
A24MT8	orange.small.smooth	4	dTSA	LSM	October	Alphaproteobacteria	Sphingomonas	EF103200	95,3
A24MT9	orange.ornd.moyenne.smooth	4	dTSA	LSM	October	Alphaproteobacteria	Sphingomonas	Z23157	98
A24MT6	violet.grande.smooth	4	dTSA	LSM	October	Betaproteobacteria	Janthinobacterium	AF174648	96,6
A24MG5	violet.grande.smooth	4	dTSA	LSM	October	Betaproteobacteria	Janthinobacterium	EF111127	92,1
A24MG4	white.cream.medium	4	PYG	LSM	October	Gammaproteobacteria	Pseudomonas	DQ377743	97
A24MG14	white.rond.smooth	4	dTSA	LSM	October	Gammaproteobacteria	Pseudomonas	AF105388	96
A23MTL	white.small.smooth	20	dTSA	ESM	October	Bacteroidetes	Flavobacterium	AM177621	92,3
A23MTB	orange.moyenne.smooth.long	20	dTSA	ESM	October	Bacteroidetes	Flavobacterium	DQ778312	94
A23MTG	long.yellow.smooth.small	20	dTSA	ESM	October	Bacteroidetes	Flavobacterium	AM177626	95,5
A23MTP	lemone.grande.smooth	20	dTSA	ESM	October	Bacteroidetes	Flavobacterium	AY263479	96,7
A23MTW	long.yellow.smooth.small	20	dTSA	ESM	October	Bacteroidetes	Flavobacterium	AM177621	96,3
A23MT11	yellow.smooth.small	20	dTSA	ESM	October	Bacteroidetes	Flavobacterium	AM167561	94,4
A23MTA	yellow.moyenne.smooth.rond	20	dTSA	ESM	October	Bacteroidetes	Flavobacterium	AM177621	92
A23MTG1	white.small.smooth	20	dTSA	ESM	October	Bacteroidetes	Flavobacterium	AM167561	95,2
A23MTM1	white.small.smooth	20	dTSA	ESM	October	Actinobacteria	Arthrobacter	DQ310475	95,8
A23MCJ	lemon.small.smooth	20	Soil Extract	ESM	October	Actinobacteria	Arthrobacter	EF459536	94
A23MCE	lemon.small.smooth	20	Soil Extract	ESM	October	Actinobacteria	Arthrobacter	AB199328	97
A23MCF	white.small.smooth	20	Soil Extract	ESM	October	Actinobacteria	Arthrobacter	DQ288888	98
A23MGP	white.small.smooth	20	PYG	ESM	October	Actinobacteria	Arthrobacter	DQ288888	97,3
A23MGH	white.small.smooth	20	PYG	ESM	October	Actinobacteria	Arthrobacter	AB288059	97
A23MTB1	white.smooth.small	20	dTSA	ESM	October	Actinobacteria	Arthrobacter	DQ158001	98,4
A23MGJ	white.small.smooth	20	PYG	ESM	October	Actinobacteria	Arthrobacter	AB0985735	97,8
A23MTD	white.small.smooth	20	dTSA	ESM	October	Actinobacteria	Arthrobacter	AB098573	95,6
A23MCG	rouph.gray	20	Soil Extract	ESM	October	Actinobacteria	Streptomyces	AB184682	98,8
A23MTR	white.rouph.small	20	dTSA	ESM	October	Actinobacteria	Streptomyces	AB246922	95,4
A23MTY	gray.rouph	20	dTSA	ESM	October	Actinobacteria	Streptomyces	AB184779	95
A23MTZ	rouph.gray	20	dTSA	ESM	October	Actinobacteria	Streptomyces	AB249976	94,3
A23MCD	rouph.white	20	Soil Extract	ESM	October	Actinobacteria	Streptomyces	DQ026638	97
A23MCC	rouph.graywish	20	Soil Extract	ESM	October	Actinobacteria	Streptomyces	AB184339	97
A23MCM	rouph.gray	20	Soil Extract	ESM	October	Actinobacteria	Streptomyces	AY029699	95
A23MCK	orange.moyenne.smooth.cream	20	Soil Extract	ESM	October	Alphaproteobacteria	Rhizobium	DQ835308	97,8
A23MTA1	mocoid.moyenne.yellowgrine	20	dTSA	ESM	October	Betaproteobacteria	Variovorax	DQ177491	90
A23MTP1	yellowgreen.mocoid	20	dTSA	ESM	October	Betaproteobacteria	Variovorax	AJ785762	90,2
A23MCL	white.small.smooth	20	Soil Extract	ESM	October	Alphaproteobacteria	Sphingomonas	EF103200	96
A23MGK	ceremy.moyenne.smooth	20	dTSA	ESM	October	Gammaproteobacteria	Pseudomonas	AJ012712	100

#### Chapitre 4 : Effet des régimes d'enneigement sur la richesse des espèces bactériennes cultivables des sols alpins

Isolate	colony morphology	Temp (°C)	Medium	Location	Season	Division	Identity	Accession	Similarity (%)
A23MTZ1	cream.small.smooth	20	dTSA	ESM	October	Gammaproteobacteria	Pseudomonas	AY263479	100
A23MTF	Smooth.pink-cream	20	dTSA	ESM	October	Gammaproteobacteria	Pseudomonas	AY263468	98
A23MGC	ceremy.moyenne.smooth	20	PYG	ESM	October	Gammaproteobacteria	Pseudomonas	DQ628969	97,6
A23MGO	white.small.smooth	20	PYG	ESM	October	Firmicutes	Bacillus	DQ514314	96,1
A23MGM	white.small.smooth	20	PYG	ESM	October	Firmicutes	Bacillus	AY572480	100
A23MCB	white.small.smooth	20	Soil Extract	ESM	October	Firmicutes	Bacillus	AJ439078	95
A23MTU1	white.grand.smooth (mocoid)	20	dTSA	ESM	October	Firmicutes	Bacillus	AF501367	98
A23MT11	pink.smooth.small	4	dTSA	ESM	October	Bacteroidetes	Sphingobacterium	AY549545	87
A23MT4 4	yellow.moyenne(rond)mocoid	4	dTSA	ESM	October	Bacteroidetes	Flavobacterium	AM177621	96,7
A23MG7	grand(rond).orange.cream	4	PYG	ESM	October	Bacteroidetes	Flavobacterium	AM110987	92,4
A23MG5	grand(rond).yellow.cream	4	PYG	ESM	October	Bacteroidetes	Flavobacterium	AM177626	89
A23MT9	white.small.smooth	4	dTSA	ESM	October	Actinobacteria	Arthrobacter	AY512631	99
A23MG10	cream.small.smooth	4	PYG	ESM	October	Actinobacteria	Arthrobacter	AJ551151	100
A23MG2	white.smooth.small	4	PYG	ESM	October	Actinobacteria	Arthrobacter	AF197050	90
A23MG1	yellow.grand.mocoid	4	PYG	ESM	October	Actinobacteria	Janibacter	AY522568	95,8
A23MC3	rose.small.smooth	4	Soil Extract	ESM	October	Alphaproteobacteria	Sphingomonas	AF548567	96,7
A23MT12	white.moyenne.smooth	4	dTSA	ESM	October	Firmicutes	Bacillus	AY572480	99,3
A23MT15	white.grand.smooth	4	dTSA	ESM	October	Firmicutes	Bacillus	DQ514315	100
A23MC2	white.smooth.small	4	Soil Extract	ESM	October	Firmicutes	Bacillus	DQ485414	97,5
A5ACP	small.translucent.smooth	20	Soil Extract	LSM	May	Bacteroidetes	Pedobacter	AM491371	90,3
A5AGM	small.yellow.opaque.smooth	20	PYG	LSM	May	Actinobacteria	Arthrobacter	AF134183	88,2
A5ATE	yellow.opaque.smooth	20	dTSA	LSM	May	Actinobacteria	Arthrobacter	AJ810894	88,9
A5ACO	translucent.smooth.yellowwish	20	Soil Extract	LSM	May	Actinobacteria	Arthrobacter	DQ172997	84,3
A5ATB	medium.white.smooth	20	dTSA	LSM	May	Actinobacteria	Arthrobacter	M23411	99,1
A5AGH	medium.Transparent.white.smooth	20	PYG	LSM	May	Actinobacteria	Arthrobacter	DQ288888	96,5
A5ATC1	medium.Translucid.white.smooth	20	dTSA	LSM	May	Actinobacteria	Arthrobacter	DQ288888	98,1
A5AGG	small.yellow.opaque.smooth	20	PYG	LSM	May	Actinobacteria	Plantibacter	DQ339591	86,2
A5ATF	yellowwish.translucent.smooth	20	dTSA	LSM	May	Actinobacteria	Leifsonia	DQ232613	94,9
A5AGK	medium.Translucid.white.smooth	20	PYG	LSM	May	Actinobacteria	Leifsonia	AM410682	91,6
A5AGI	medium.Translucid.white.smooth	20	PYG	LSM	May	Actinobacteria	Leifsonia	AF409008	97
A5ATD	greenwish.smooth	20	dTSA	LSM	May	Betaproteobacteria	Variovorax	EU169153	96,2
A5ATA1	violet.smooth	20	dTSA	LSM	May	Betaproteobacteria	Janthinobacterium	EF672646	96,4
AA5ATF	medium.Translucid.white.smooth	20	dTSA	LSM	May	Betaproteobacteria	Collimonas	AY281147	91,8
A5AGN	Large colony.Translucid.smooth	20	PYG	LSM	May	Gammaproteobacteria	Pseudomonas	AY263472	96,2
A5ATH	Transparent.smooth	20	dTSA	LSM	May	Gammaproteobacteria	Pseudomonas	AB098591	96,2

**Chapitre 4 : Effet des régimes d'enneigement sur la richesse des espèces bactériennes cultivables des sols alpins**

Isolate	colony morphology	Temp (°C)	Medium	Location	Season	Division	Identity	Accession	Similarity (%)
A5ATI	Translucid.smooth	20	dTSA	LSM	May	Gammaproteobacteria	Pseudomonas	AY599715	96,7
A5ACR	medium.white.wrinkly	20	Soil Extract	LSM	May	Gammaproteobacteria	Pseudomonas	AJ551161	95,4
A5AGL	Translucid.smooth	20	PYG	LSM	May	Gammaproteobacteria	Pseudomonas	DQ778036	85,5
A5ACQ	medium.yellow.pale.smooth	20	SoilExtract	LSM	May	Gammaproteobacteria	Dyella	AY785744	95,6
A5AGJ	Large.white.opaque.smooth	20	PYG	LSM	May	Firmicutes	Bacillus	DQ993300	94,5
A5AGK1	Large.white.opaque.smooth	20	PYG	LSM	May	Firmicutes	Bacillus	AY572480	96
A5ATC	Large.yellow	20	dTSA	LSM	May	Firmicutes	Bacillus	EU124559	95,7
A5AGL1	small.white.smooth	20	PYG	LSM	May	Firmicutes	Kurthia	AJ627211	96,4
A5AC12	small.translucent.type smoth	4	Soil Extract	LSM	May	Bacteroidetes	Pedobacter	AJ438170	91,4
A5AC11	medium.bright.violet.smooth	4	Soil Extract	LSM	May	Betaproteobacteria	Janthinobacterium	AF174648	94,6
A5AC14	medium.bright.violetwish.smooth	4	Soil Extract	LSM	May	Betaproteobacteria	Janthinobacterium	DQ151836	90,3
A5AG6	small.white.bright.smooth	4	PYG	LSM	May	Gammaproteobacteria	Pseudomonas	AB098591	95,6
A5AG8	small.yellowwish.bright.smooth	4	PYG	LSM	May	Gammaproteobacteria	Pseudomonas	EU414476	90,9
A5AT1	medium.white.bright.smooth	4	dTSA	LSM	May	Gammaproteobacteria	Pseudomonas	AJ551161	96
A5AT2	small.white.wrinkly spreader	4	dTSA	LSM	May	Gammaproteobacteria	Pseudomonas	AB098591	95,3
A5AT3	medium.white.bright.wrinkly	4	dTSA	LSM	May	Gammaproteobacteria	Pseudomonas	AJ551161	96,9
A6ATF1	grand.orange.smooth	20	dTSA	ESM	May	Bacteroidetes	Flavobacterium	AM177631	92,1
AA6ATF	yellow.medium.smooth	20	dTSA	ESM	May	Bacteroidetes	Chryseobacterium	EF601827	90
A6ATH	small.pale yellow.smooth	20	dTSA	ESM	May	Bacteroidetes	Pedobacter	AJ438170	94,9
A6ATC1	orange.moyenne.smooth	20	dTSA	ESM	May	Bacteroidetes	Pedobacter	AJ786798	91,8
A6ATD	Pale white. Large	20	dTSA	ESM	May	Bacteroidetes	Pedobacter	AJ438170	66,7
A6AGG	pale gray.rough	20	PYG	ESM	May	Actinobacteria	Streptomyces	FJ154606	97
A6AGD1	small.white.rough	20	PYG	ESM	May	Actinobacteria	Streptomyces	AB184289	96,4
A6ATD1	small.smooth.pale yellow	20	dTSA	ESM	May	Actinobacteria	Plantibacter	DQ339591	85
A6AGE	small.white.smooth	20	PYG	ESM	May	Actinobacteria	Leifsonia	AF465411	87,9
A6ATE	small.yellow.smooth	20	dTSA	ESM	May	Actinobacteria	Leifsonia	DQ339591	86,2
A6ATA	small.orange.smooth	20	dTSA	ESM	May	Actinobacteria	Leifsonia	DQ232612	100
A6ACH	Small.Translucid.white.smooth	20	Soil Extract	ESM	May	Actinobacteria	Leifsonia	DQ232613	100
AA6ATA	violet.smooth	20	dTSA	ESM	May	Betaproteobacteria	Janthinobacterium	AJ864846	96,1
A6AGD2	small.white.smooth	20	PYG	ESM	May	Betaproteobacteria	Burkholderia	AJ011508	94,7
A6ATD5	pale yellow.smooth.opaque	20	dTSA	ESM	May	Betaproteobacteria	Collimonas	AY281146	94
A6AGF	medium.white.smooth.opaque	20	PYG	ESM	May	Betaproteobacteria	Collimonas	AY281143	95,3
A6ATC	medium.Yellowwish.smooth.opaque	20	dTSA	ESM	May	Gammaproteobacteria	Pseudomonas	DQ177467	95,1
A6AGB1	medium.Yellowwish.smooth.opaque	20	Soil Extract	ESM	May	Gammaproteobacteria	Pseudomonas	DQ453818	100
A6AGB	medium.yellowwish.smooth.opaque	20	PYG	ESM	May	Gammaproteobacteria	Pseudomonas	DQ536522	95,6

**Chapitre 4 : Effet des régimes d'enneigement sur la richesse des espèces bactériennes cultivables des sols alpins**

Isolate	colony morphology	Temp (°C)	Medium	Location	Season	Division	Identity	Accession	Similarity (%)
A6AT7	small.regular.oragne.smooth	4	dTSA	ESM	May	Bacteroidetes	Flavobacterium	AM177631	93,4
A6AT1	bright.smooth	4	dTSA	ESM	May	Bacteroidetes	Pedobacter	EU169155	92,2
A6AT6	medium.bright	4	dTSA	ESM	May	Bacteroidetes	Pedobacter	AJ786798	92,3
A6AG10	medium.regular.yellow	4	PYG	ESM	May	Bacteroidetes	Pedobacter	AJ438170	95
A6AG9	small.regular.curved.smooth	4	PYG	ESM	May	Actinobacteria	Arthrobacter	AF134183	89
A6AT3	irregular.medium.opaque.rouph	4	dTSA	ESM	May	Betaproteobacteria	Janthinobacterium	EF672646	95
A6AG8	grand.smooth.bright	4	PYG	ESM	May	Gammaproteobacteria	Pseudomonas	DQ011918	95,3
A6AG11	regular.opaque white.bright.rough	4	PYG	ESM	May	Gammaproteobacteria	Pseudomonas	DQ011918	96,7
A20MEG1	white.bright.rough	20	Soil Extract	LSM	July	Actinobacteria	Streptomyces	AB217604	93,7
A20MEG	pale rose.bright.rough	20	Soil Extract	LSM	July	Actinobacteria	Streptomyces	AB217604	94,4
A20MGE	rouph.gray.rough	20	PYG	LSM	July	Actinobacteria	Streptomyces	AB217604	94,7
A20MGG	grand.white.rouph	20	PYG	LSM	July	Actinobacteria	Streptomyces	EU273545	91,1
A20MEE	yellow.bright.rough	20	Soil Extract	LSM	July	Actinobacteria	Kitasatospora	U93324	90,4
A20MGF	medium.rouph.flower shape	20	PYG	LSM	July	Actinobacteria	Kitasatospora	AB278568	88,6
A20ME1	small.smooth.yellow	20	Soil Extract	LSM	July	Actinobacteria	Plantibacter	AF474326	95,4
A20MG1	small.smooth.pale yellow	20	PYG	LSM	July	Actinobacteria	Plantibacter	AF465411	95,5
A20MGC	medium.smooth.yellow	20	PYG	LSM	July	Actinobacteria	Curtobacterium	DQ339588	94
A20MGK	grand.rose.bright.opaque	20	PYG	LSM	July	Actinobacteria	Blastococcus	L40620	80,5
A20MGB	medium.smooth.orange	20	PYG	LSM	July	Alphaproteobacteria	Sphingomonas	DQ166180	93,9
A20MGD	grand.smooth.white	20	PYG	LSM	July	Firmicutes	Bacillus	AF290558	94,6
A20MT1	medium.orange.opaque	4	dTSA	LSM	July	Bacteroidetes	Flavobacterium	EU057852	89,7
A20MT2	medium.orange.opaque	4	dTSA	LSM	July	Alphaproteobacteria	Sphingomonas	EF540484	96,3
A20MT3	grand.pale yellow.bright	4	dTSA	LSM	July	Firmicutes	Paenibacillus	DQ444978	82,1
A19MGK	grand.pale rose.bright.opaque	20	PYG	ESM	July	Actinobacteria	Arthrobacter	AJ551154	95,8
A19MGB	rouph.flower shape	20	PYG	ESM	July	Actinobacteria	Streptomyces	EF494229	91,9
A19MGC	rouph.flower shape	20	PYG	ESM	July	Actinobacteria	Streptomyces	EU285474	95,8
A19MGD	grand.rough.white center	20	PYG	ESM	July	Actinobacteria	Streptomyces	EU196762	91,3
A19MTDJ	grand.rough.gray center	20	dTSA	ESM	July	Actinobacteria	Arthrobacter	DQ177477	95,4
A19MTE	medium.smooth.yellow	20	dTSA	ESM	July	Bacteroidetes	Flavobacterium	AM934671	89,8
A19MTI	grand.rough.gray center	20	dTSA	ESM	July	Actinobacteria	Streptomyces	EU273545	91,1
A19MT1	medium.orange.opaque	4	dTSA	ESM	July	Actinobacteria	Arthrobacter	EF601814	91,9
A19MG2	medium.opaque.yellow	4	PYG	ESM	July	Actinobacteria	Leifsonia	AM889135	97,1
A19MT3	grand.orange.plate	4	dTSA	ESM	July	Bacteroidetes	Flavobacterium	AM177621	94,6



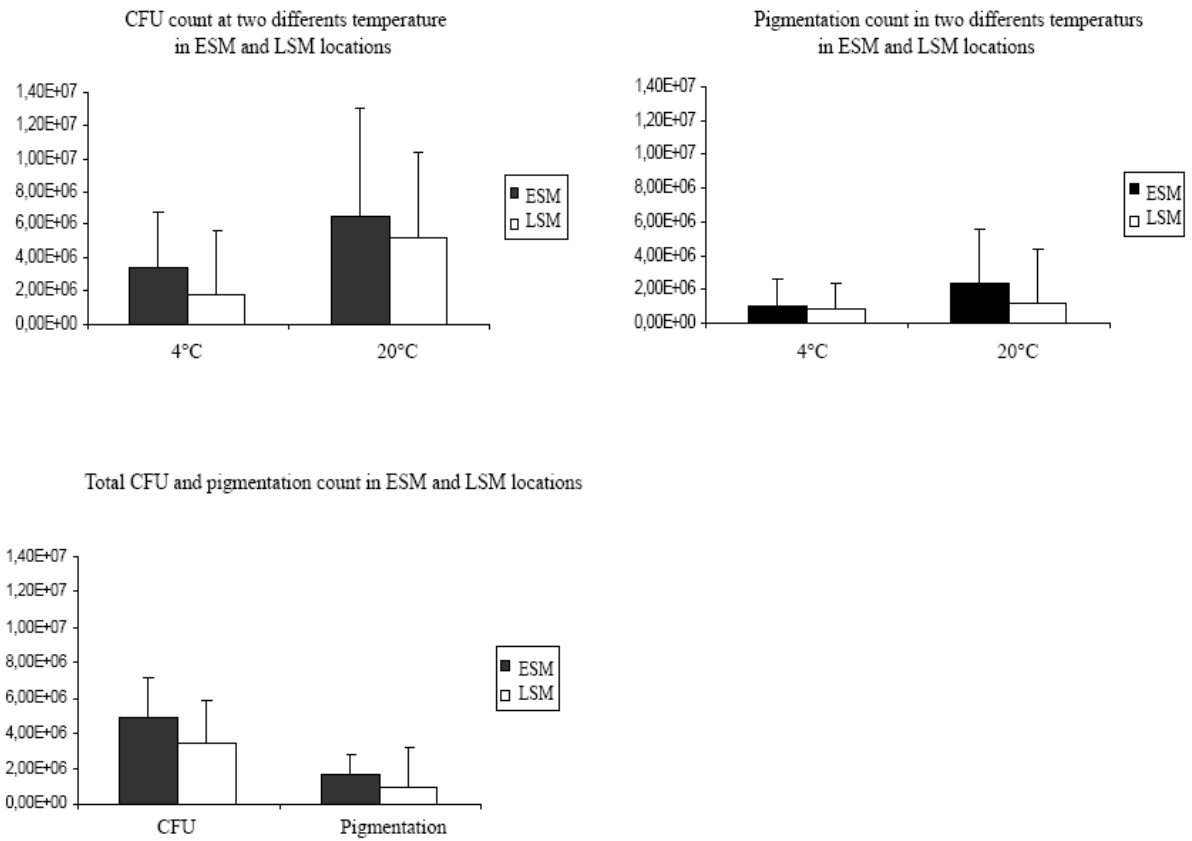


Figure S1. Effect of two different temperatures on cell viable bacteria in different temperatures in ESM and LSM location.

# Chapitre 4 : Effet des régimes d'enneigement sur la richesse des espèces bactériennes cultivables des sols alpins

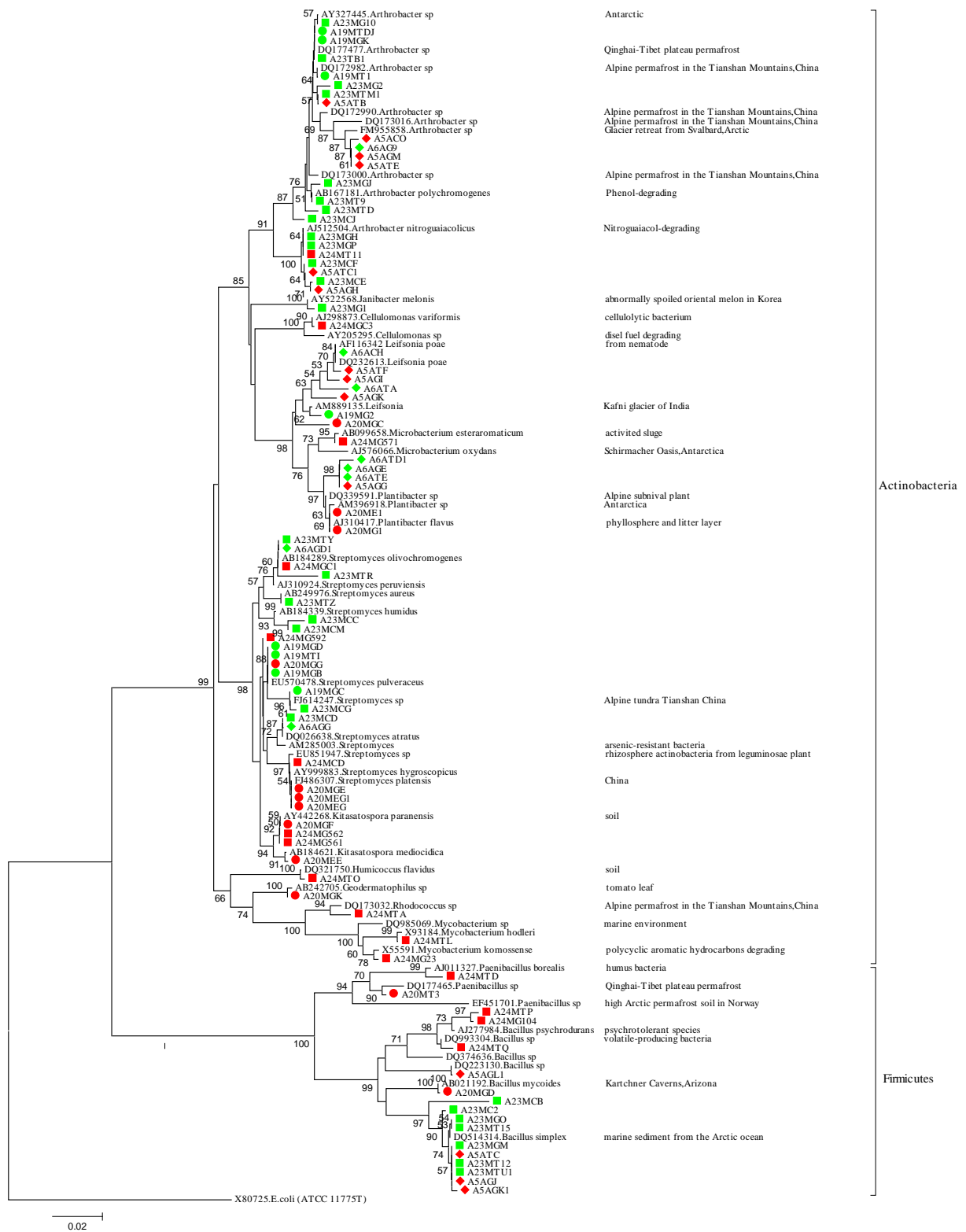




Figure S2. Phylogenetic tree of the 16S rRNA of the A: gram-positive and B: gram-negative isolates from the Grand Galibier massif (alpine, French) using neighbour-joining method. October, May and July isolates were showed with square, diamond and circle, respectively. The ESM and LSM isolated were showed with green and red, respectively. Other sequences are from GenBank. Only > 50% bootstrap values (n=1000 replications) were indicate

## Principaux résultats et discussion

### L'effet le enneigement sur la richesse, abondance et composition des bactéries cultivable

Les dénombrements bactériens et la pigmentation des bactéries sont influencés par la variation saisonnière plutôt que par les régimes enneigement (combe/crête). En revanche, la température d'incubation semble avoir un impact plus fort sur l'isolement de bactéries. Les résultats de culture bactériennes à deux températures : ambiante (20°C) et froide (4°C) indiquent que les bactéries cultivables des sols alpins sont tolérantes au froid plutôt qu'adaptées au froid. Ces résultats sont semblables à ceux rapportés pour d'autres sols alpins (Margesin 2003) ou arctiques (Shi et al., 1997; Foght et al., 2004; Rivkina et al., 2004). Certaines études montrent que les bactéries adaptées au froid sont absentes ou très rares dans les habitats terrestres, même en Antarctique (Shi et al., 1997).

En accord avec d'autres rapports sur les écosystèmes froids (Arctique et Antarctique), nous avons constaté que les membres cultivables des *Actinobactéries* (notamment *Arthrobacter*) sont généralement dominants dans les sols alpins. En fait, certaines caractéristiques spécifiques à ce groupe, comme la composition de la paroi cellulaire, un génome riche en (G + C), et la capacité de former des cellules dormantes permettant de survivre dans des conditions difficiles (Vishnivetskaya et al., 2006) leur permettent de résister.

Les résultats montrent que bien que les morphologies des colonies bactéries soient différentes, surtout au niveau de leur pigmentation, elles sont phylogénétiquement similaires. L'analyse phylogénétique a révélé que certains genres comme *Arthrobacter*, *Bacillus*, *Pseudomonas*, *Collimonas*, *Pedobacter* et *Flavobacterium* ont des aspects morphologiques différents selon les conditions (température d'incubation ou milieu utilisé) mais apparaissent en contre partie phylogénétiquement similaire. En revanche, la température d'incubation peut favoriser la présence des certains groupes des bactéries. Par exemple, les genres *Streptomyces* et *Kitasatosporia* sont plutôt isolés à température ambiante et *Jantnobacterium* a tendance être plus facilement isolé à basse température.

Le régime d'enneigement ne semble pas influencer l'abondance relative des différents phyla bactériens chez les bactéries cultivables, mais agit au niveau du genre. Par exemple, les

membres du genre *Arthrobacter* sont spécifiques aux crêtes, tandis que *Kitasatosporia* et *Zoogloea* sont spécifiques aux combes. Les variations saisonnières ont quant à elles une influence sur la composition et la structure des communautés bactériennes aussi bien au niveau du phylum que de l'espèce. Par exemple, dans les deux locations, les phyla *Actinobactéries*, *Bacteroidetes* et  $\gamma$ -*Proteobactéries* sont successivement dominants en Juillet, Octobre et Mai. Ces différences sont aussi observées au niveau du genre notamment par la dominance des *Streptomyces* et des *Plantibacter* en Juillet et Mai.

Malgré la limitation des méthodes de culture, la présence de certains isolats reflète les conditions édapho-climatiques correspondant à une certaine saison ou à une certaine location. Typiquement, le genre *Arthrobacter* est souvent retrouvé dans des sites pollués (Keuth et al., 1991; Karigar et al., 2006). Le fait de le retrouver abondant en crête peut être expliqué par une plus grande quantité de matière organique récalcitrante dans les sols (Baptist 2008). De plus, les *Streptomyces* sont plus abondants pendant la saison de végétation et la sénescence de la plante, les *Flavobacterium* pendant la sénescence de la plante ; ce qui peut s'expliquer respectivement par l'association ces genres avec les plantes et leur capacité à dégrader la matière organique (Sardi et al., 1992, de Araújo1 et al., 2000).

## **Comparaison des résultats obtenus par approches classiques et moléculaires**

La plus importante conclusion de la comparaison entre les séquences identifiées par clonage/séquençage à partir d'ADN de sol et celles obtenues à partir des isolats est que seule une faible fraction (4.5%) de bactéries de sols alpins est facilement cultivable. Nous n'avons isolé aucune souche appartenant aux groupes des *Acidobactéries* qui représentent pourtant une part importante de la banque de clone (32%), de *Gemmatimonadetes* (2.4% de la banque de clones) et  $\delta$ -*Proteobactéries* (2.7 % de la banque de clones). L'abondance des groupes bactériens est différente entre les deux approches. En absence des groupes précédemment mentionnés, certains phyla sont largement représentés en collection comme les *Actinobactéries*, les *Bacteroidetes*, les *Firmicutes* qui sont 4, 3 et 8 fois respectivement plus abondantes par approche de culture. De façon intéressante l'ensemble de ces groupes appartient aux genres *Arthrobacter*, *Streptomyces*, *Bacillus* qui sont connus pour entrer en phase de dormance et former des spores. Ces résultats peuvent indiquer un biais des méthodes

basées sur l'extraction de l'ADN (Frostegard et al., 1999; Martin-Laurent et al., 2001). Toutefois, les isolats les plus représentés, correspondant aux groupes *Pseudomonadales*, *Burkholderiales*, *Sphingomonadales*, *Micrococcineae*, *Streptomyces*, *Flavobacteriales* et *Sphingobacteriales* sont également plus représentés dans la bibliothèque de clones. Cette étude a montré que chaque méthode ne met en évidence qu'une fraction des communautés bactériennes totales dans les sols étudiés.

*Cette étude montre que les régimes d'enneigements n'ont pas d'effet important sur la composition spécifique des communautés bactériennes cultivables tandis que les variations saisonnières ont un effet significatif sur ces communautés. Une grande part de la bibliothèque de clone des sols alpins demeure non cultivable en raison de l'abondance des Acidobactéries. Cette étude montre aussi l'importance des méthodes de cultures qui permettent de renseigner sur le rôle écologique de certaines espèces.*





# **Chapitre 5**

## **Synthèse et perspectives**



During this thesis, I studied the spatio-temporal dynamics of bacterial communities in the alpine tundra. Using molecular and classical methods, I have shown that bacterial communities are structured by the habitat and the seasons. These conclusions are strongly supported by our results. However, these results should be tempered by the limits of the techniques that have been used.

## **The limits of molecular approaches**

One of the major goals of environmental microbiology is to understand the basis of microbial distribution and role in different habitats. The so-called molecular methods are keys to understand the distribution of micro-organisms. In molecular ecology, these methods are well suited to study a representative number of samples. Though they are powerful, they suffer from several pitfalls that should be mentioned to better interpret the results.

### **DNA extraction and PCR**

The first step in molecular methods is DNA extraction. Though the DNA extraction protocols are optimized to extract a maximum amount of DNA, it is possible that many organisms are recalcitrant to DNA extraction, resulting in an underestimation of the real levels in the community such as recalcitrant organisms or dormant forms (spores).

The second step is the amplification by PCR of the molecular marker. Several issues should be raised here. First, the molecular marker should be universally enough to scan all bacterial taxa. The random sequencing metagenomic approaches revealed that this is not true (Chouari et al., 2005). Second, the PCR is a source of biases. Actually, and besides the artifactual mutations induced by the DNA polymerases, it may amplify preferentially certain targets (Polz and Cavanaugh, 1998) or produce heteroduplexes leading to chimera formation (Thompson et al., 2002). While preferential amplification may bias the representativity of each phylotype, the formation of chimera may artificially increase the richness. Solutions to this issue were proposed (Acinas et al., 2004; Acinas et al., 2005), but they are difficult to implement when dealing with multiple samples. On the other hand, in the emulsion PCR (Kiss et al., 2008), since each target DNA molecule is isolated in the droplet where

amplification occurs, the preferential amplification or chimera formation should be minimised.

## **Molecular profiling method: CE-SSCP**

The CE-SSCP is a molecular profiling method based on the fact that a single base modification can change the conformation of single-strand DNA molecules leading to a different electrophoretic mobility in a non-denaturing gel (Orita et al., 1989). Actually, this method was developed for clinical studies, but it has been used in to study the microbial profile of complex samples (Lee et al., 1996; Delbes et al., 2000; Zumstein et al., 2000; Zinger et al., 2008). This approach was developed in LECA for microbial communities by L. Zinger and J. Gury.

As other signature methods, the number of peaks does not match the real number of species. Indeed, and given the taxonomic resolution of the molecular marker use, many species can be represented in one peak. While in the conditions used here, CE-SSCP is not suited for genotyping, it can be used to estimate the number of species (Curtis et al., 2002). Still, it can be used as a first step to compare different ecological conditions.

The sensibility of CE-SSCP at taxonomic level is poorly studied. Actually, the sensibility is limited both by the resolution of the electrophoresis and the molecular marker. Data from Zumstein et al (2006), suggests that the electrophoretic profiles are saturated at around 35 peaks. This is mainly due to the fact that the fragments display a very limited size polymorphism. Concerning the molecular marker, while the V3 is highly polymorphic, even the sequence does not allow a species resolution. Our results (**Article II**) and recent simulations (Thesis Zinger.L) show that the topology of the tree is very close to the phylum level.

## **Random sequencing of *ssu* libraries**

To cope with taxonomic affiliation of soil bacteria, we have performed random sequencing of *ssu* library. This approach has commonly been used in molecular ecology. The marker used here is the 3' end fragment encompassing 800 bp. Though submitted to the same limitations than a molecular fingerprinting method, the use of a larger fragment provides better resolution.

The common tools for analysing the sequence data are NCBI and RDP that provide the information on hierarchical classification particularly the later that is specific for prokaryotic kingdom. While, this database provides some information on classification of each sequence, in many cases it cannot identify the sequences at species level. Furthermore, a significant amount of the sequences remain unclassified, highlighting bacterial kingdom. So, some the sequences obtained from nature samples contain the unclassified bacteria that can not be identify even at phylum level.

The main drawback of this approach is the limited number of sequences. Actually, 400 sequences/ points is a very modest sequencing effort to apprehend the soil bacterial diversity. A recent survey (Quince et al., 2008), predicted that the deep of sequencing for complex environments like soil might contain at least 140.000, which is beyond present sequencing capabilities. Thus, the present results should be interpreted at a higher taxonomic level than species, or even family.

The new sequencing technology opens a new perspective in molecular ecology. Though the faisability of such sequencing depth is still beyond reality, it will be possible to produce better estimates of diversity, and probably set a higher taxonomic level for comparison. These technologies produce lengths around 250 (Illumina) and 400 bp. The RDP has reported in classification of partial sequences to the genus level for 400-base reads and to the family level for 200-base reads (Wang et al., 2007). These size ranges set the upper level of taxonomic resolution in future experiments using massive sequencing. The question remains about the suitability of data to perform phylogenetic structure analysis.

## Importance of traditional and cultural methods

Numerous studies compared the phylogeny between micro-organisms isolated by traditional culture methods followed by molecular identification (Chandler et al., 1997; Suzuki et al., 1997; Dunbar et al., 1999). These studies demonstrated that culture gives access to different fractions of bacterial communities.

Cultivation of isolated bacteria supplies us with a great amount about the physiological capabilities of bacteria. This information is useful to understand the ecology of the organisms and their environmental role. Although, the molecular methods pay more attention in recently decades because of overcome to access of non-cultivated bacteria, they therefore do not allow to estimate empirically the microbial biomass or function. For example, in our case,

about 30% of the sequences clones library were corresponding to *Acidobacteria* that are not cultivated. Lack of information in physiological properties avoid to precise ecological role of this bacterial group.

Recently, culture strategies have been improved to allow isolation of new bacterial strains (Janssen et al., 2002; Sait et al., 2002; Cardenas and Tiedje, 2008). These reports are improvements of traditional techniques. The modifications include dark incubation, changes in pH, O<sub>2</sub> and CO<sub>2</sub> concentration, use of different incubation temperatures, pre-treatment with biologically active molecules and different carbon sources (composition and concentration). While some of studies suggested that *Acidobacteria* may grow in anaerobic conditions, our attempt to isolate these bacteria failed.

When comparing the results of random sequencing with culture, it was also found that *Arthrobacter* and *Streptomyces* were abundant in culture but rare in clone library. This can be due to spore production. Actually, a similar phenomenon was observed when studying fungal populations (L. Sage, personal communication), when *Penicillium sp* was abundant in culture but very rarely represented in clones library. This difference can be also attributed to the amount of spores produced by this fungal species. In both cases, the high rate of isolation may be due to grow of spores that represent very few of the total biomass.

The functional abilities of isolated strains can be biochemically determined. The isolates of alpine soil ecosystems (especially from early snowmelt location) are phylogenetically close to those of bacterial isolates able to degrade organic matter. These isolates can be tested for their abilities for degrading aromatic compound in order to determine their efficiency for bioremediation of polluted sites. The isolates of cold ecosystems are expected to have cold-resistant enzymes (our isolates are capable to growth in 4°C). These isolates are thus good candidates to use in bioremediation and/or industrial purposes.

### **Link between bacterial phylogenetic and function**

Although, sequencing/cloning of 16S rRNA provide the good information on dynamic of bacterial communities and their richness or diversity, it is not useful to understand the ecological role of bacteria. Different approaches have been proposed to comprehension of microbial function.

The ideal method would be to isolate soil bacteria to get information on their metabolic activity through biochemical studies and genome sequencing. Given the current limitations of

this method, its use remains limited. May be future technological developments would allow to massif isolation of bacteria.

Other approach is the search for functional and phylogenetic genes using DNA microarray. This technique provides useful information on genes function presented in a given sample. Nevertheless, probes are designed on the bases of known genes and preclude gene discovery. Finally, massif sequencing of genomic DNA and RNA open new perspectives in microbial ecology. Using new sequencing technologies, the ability to produce massif amount of sequences at a reasonable cost will boost metagenomic and metatranscriptomatic analysis. The major advantages of the new methods are reduction in cost and making massive gene to discover new microbes and genes, opening the door for the description of biochemical pathways in complex bacterial communities.

## **Bacterial assemblage and filtering factors**

The chapters II and III allow to highlight the strongly different in microbial communities from two ecosystems very close spatially but different in edapho-climatic characteristics. This variation in space produced modifications in the dynamic of snow cover and the strong meso-topographical gradient. Alpine ecosystems display seasonal cycles that are characterize by long-lasting and cold winters, frequent freezing/thawing events, short growing season for plants, and snow cover (Körner, 1999; Litaor et al., 2002; Edwards et al., 2007).

For the macro-organisms, phylogenetic clustering has been interpreted as evidence of habitat filtering, where a group of clustered species share eco-physiological properties allowing their persistence in a given environment (Webb et al., 2002; Cavender-Bares 2004). The pertinence of this statement for micro-organisms, particularity bacteria, remains unknown, mainly because of their ability to gene exchange with other bacterial species via HGT. Our results showed that bacterial communities tend to be more phylogenetically clustered expected by chance consistently with previously reports from a wide range of environments (Horner-Devine and Bohannan, 2006; Newton et al., 2007; Bryant et al., 2008). These reports demonstrated that abiotics and biotics factors such as altitude, pH, and total organic carbon contribute to phylogenetic clustering. Our results support the hypothesis of environmental filtering, more precisely at October and May.

We explain that the low pH in ESM location leads to bacterial clustering. Although, previous studies showed that soil pH structures bacterial communities (Fierer and Jackson 2006,

Lauber et al., 2008), these studies remain at the level of MOTU, without taxonomical assignation. In late winter, other phenomenon such as low soil temperature and low plant productivity may be also important to this effect. Thus, in late winter, the whole environmental conditions recruit few bacteria that are cold tolerant and cluster, but our data is not sufficient to check and deeply understand of this observation.

The snow cover gradient can determine length of season vegetation and consequently in plant composition and diversity (Choler, 2005). In seasonal gradients, the alternance of resources and quality of organic carbon produces microhabitats in the soil upper layer (Kowalchuk 2002). Furthermore, the chemistry of root exudates vary with plant species (Souto et al., 2001) or even during development (Mougel et al., 2006), with the consequent impacts on microbial processes and phylogenetic structure. Thus, it is conceivable that different plant composition mirrors in different microbial population. This correlation was studied using *Salix brachycarpa* and *Kobresia myosuroides* (Schmidt et al., 2000). Recent work in LECA (J. Roy, M1) further supports this correlation. Consequently, the variation between locations can be partially explained by differences in the floristic composition. This effect is supported by the clustering of *α-Proteobacteria* during productive season. This subphylum is classically associated with plants.

## Link between snow cover and bacterial activity

Numerous studies have demonstrated that bacterial activities continue under snow cover. Furthermore, they showed that the litter decomposition rate increase at this time because of the protective role of snow cover against low atmospheric temperatures (Lipson et al., 2002; Hobbie and Chapin, 1996). In this way, in our site, the results obtained by previous studies showed that the decomposition of organic matter was enhanced in late snowmelt locations compared to early snowmelt locations (Thesis of Baptist. F). The decomposition rate during winter was significantly correlated to the winter soil temperature. Frozen soils at early snowmelt locations displayed a reduced decomposition rate.

Our results showed that the bacterial groups known to degrade organic matter were more represented in autumn. However, some of studied suggest that cultivated members of *Acidobacteria* have the decomposition role in nature (Ward et al., 2009), because they posed cellulose degrading enzymes. However, the actual role of each subdivision of this phylum remains open.



The ESM location shows all the characteristics of low fertility. Actually, Baptist (Thesis) showed that the low soil temperature during winter reduce litter decomposition, which coupled with tannin abundance (Baptist et al., 2008, Annex) may limit N availability to plant during the growing season. Accordingly, it has been showed that ESM contained more recalcitrant litter than LSM location (Thesis Baptist. F). Thus, the conditions in ESM promote symbiotic interactions for the atmospheric N<sub>2</sub> fixation by several bacteria species such as *Frankia sp.*, a trait of low fertility soils. This hypothesis has been supported by the abundance of Rhizobiales in ESM location during vegetation growth season. Thus, biological fixation of N<sub>2</sub> may be important for plant development in ESM.

## Bacteriobiogeography

Our data revealed no correlation between the assembly of microbial communities and distance across the landscape, being the effect of habitat on bacterial assembly highlighted in our studies. The availability of sequences for a site in the Rocky Mountain (Colorado, EU) allows a biogeographical comparison. This site is a meadow covered with *K. myosuroides*, thus similar to ESM. Actually, this comparison reveals a significant dissimilarity between bacterial communities (*Acidobacteria* phylum) of Rocky Mountain with our site (Figure 5.1). While same analysis with sequences obtained from South Korea and Germany (rhizospheric soil) showed the ESM sequences are closer from them than from a meadow with the same plant (Figure 5.1).

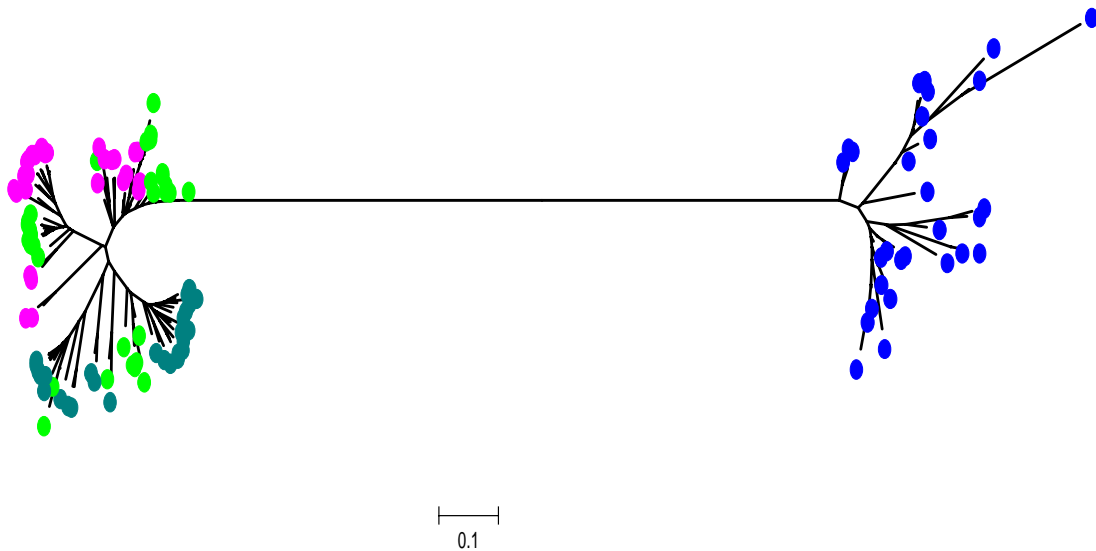


FIGURE 5.1 – Phylogenetic structure of the bacterial species from different sites. The sites of sampling are represented in light green: alpine France, blue: alpine North America, pink: South Korea and green: Germany. The data are from NCBI and this study. The sequences were aligned by ClustalW. The distances were calculated according to Kimura's 2-parameter model. Phylogenetic trees were inferred using the neighbor-joining algorithm and the tree topology was statistically evaluated by 1000 bootstrap resamplings.

These results illustrate why the Baas-Becking hypothesis "*Everything is everywhere, but, the environment selects*" is matter of controversy.

## Conclusion

This study showed that snow cover variations driven by meso-topography is responsible to change of many environmental factors that lead to strong spatial turnover of bacterial communities in terms of composition and structure using culture and molecular methods. From our data is difficult to dissociate the effects of plants and soil characteristics (that result from differential snow cover). This study provides evidence that the habitat has important effects on the pattern bacterial communities' of phylogenetic structure, which depend upon environmental factors and seasonal variation. This thesis will be interest for global richness of cultivated bacteria in alpine tundra and different bacterial fraction that can present using different methods. In global warming context, the changes in composition, structure and assembly of bacterial communities should be taken into account for modelling studies.



# **Bibliographie**



- Abou-Shanab, R.A.I. (2007) Characterization and 16S rDNA Identification of Thermo tolerant Bacteria Isolated from Hot Springs. *J Appl Sci Res* **3**: 994-1000.
- Anderson, I.C., Campbell, C.D., and Prosser, J.I. (2003) Potential bias of fungal 18S rDNA and internal transcribed spacer polymerase chain reaction primers for estimating fungal biodiversity in soil. *Environ Microbiol* **5**: 36-47.
- Acinas, S.G., Sarma-Rupavtarm, R., Klepac-Ceraj, V., and Polz, M.F. (2005) PCR-induced sequence artifacts and bias: Insights from comparison of two 16S rRNA clone libraries constructed from the same sample. *Appl Environ Microbiol* **71**: 8966-8969.
- Acinas, S.G., Klepac-Ceraj, V., Hunt, D.E., Pharino, C., Ceraj, I., Distel, D.L., and Polz, M.F. (2004) Fine-scale phylogenetic architecture of a complex bacterial community. *Nature* **430**: 551-554.
- Amann, R.L., Ludwig, W., and Schleifer, K.H. (1995) Phylogenetic Identification And In-Situ Detection Of Individual Microbial-Cells Without Cultivation. *Microbiol Rev* **59**: 143-169.
- Azam, F., and Malfatti, F. (2007) Microbial structuring of marine ecosystems (vol 5, pg 782-791, 2007). *Nat Rev Microbiol* **5**: 966-U923.
- Bai, Y., Yang, D., Wang, J., Xu, S., Wang, X., and An, L. (2006) Phylogenetic diversity of culturable bacteria from alpine permafrost in the Tianshan Mountains, northwestern China. *Res Microbiol* **157**: 741-751.
- Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., and Vivanco, J.M. (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu Rev Plant Biol* **57**: 233-266.
- Bakermans, C., Tsapin, A.I., Souza-Egipsy, V., Gilichinsky, D.A., and Neilson, K.H. (2003) Reproduction and metabolism at -10 degrees C of bacteria isolated from Siberian permafrost. *Environ Microbiol* **5**: 321-326.
- Bakken, L.R. (1997) Culturable and nonculturable bacteria in soil. Modern soil microbiology (van Elsas JD, Trevors T and Wellington EMH, eds). *Marcel Dekker, New York, NY*: pp. 47-61.
- Baptist, F. (2008) Impact de la durée d'enneigement sur les cycles biogéochimiques dans les écosystèmes alpins. *Thesis*.
- Bardgett, R.D., Lovell, R.D., Hobbs, P.J., and Jarvis, S.C. (1999) Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. *Soil Biol Biochem* **31**: 1021-1030.

- Bardgett, R.D., Bowman, W.D., Kaufmann, R., and Schmidt, S.K. (2005) A temporal approach to linking aboveground and belowground ecology. *Trends Ecol Evol* **20**: 634-641.
- Barns, S.M., Takala, S.L., and Kuske, C.R. (1999) Wide distribution and diversity of members of the bacterial kingdom Acidobacterium in the environment. *Appl Environ Microbiol* **65**: 1731- 1737.
- Bentahir, M., Feller, G., Aittaleb, M., Lamotte-Brasseur, J., Himri, T., Chessa, J.P., and Gerday, C. (2000) Structural, kinetic, and calorimetric characterization of the cold-active phosphoglycerate kinase from the Antarctic *Pseudomonas* sp TACII18. *J Biol Chem* **275**: 11147-11153.
- Beveridge, T.J. (2001) Bacterial Cells. *Encyclopedia of Life Sciences*.
- Billings, W.D. (1973) Arctic And Alpine Vegetations - Similarities, Differences, And Susceptibility To Disturbance. *Bioscience* **23**: 697-704.
- Bjork, R.G., Bjorkman, M.P., Andersson, M.X., and Klemedtsson, L. (2008) Temporal variation in soil microbial communities in Alpine tundra. *Soil Biol Biochem* **40**: 266-268.
- Borneman, J., Skroch, P.W., Osullivan, K.M., Palus, J.A., Rumjanek, N.G., Jansen, J.L. et al. (1996) Molecular microbial diversity of an agricultural soil in Wisconsin. *Appl Environ Microbiol* **62**: 1935-1943.
- Bossio, D.A., Scow, K.M., Gunapala, N., and Graham, K.J. (1998) Determinants of soil microbial communities: Effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. *Microb Ecol* **36**: 1-12.
- Bryant, J.A., Lamanna, C., Morlon, H., Kerkhoff, A.J., Enquist, B.J., and Green, J.L. (2008) Microbes on mountainsides: Contrasting elevational patterns of bacterial and plant diversity. *P NATL ACAD SCI USA* **105**: 11505-11511.
- Burris, R.H. (2004) Nitrogen Fixation. *Encyclopedia of Life Sciences*.
- Buyer, J.S., Roberts, D.P., and Russek-Cohen, E. (2002) Soil and plant effects on microbial community structure. *Can J Microbiol* **48**: 955-964.
- Cardenas, E., and Tiedje, J.M. (2008) New tools for discovering and characterizing microbial diversity. *Curr Opin Biotechnol* **19**: 544-549.
- Chandler, D.P., Li, S.M., Spadoni, C.M., Drake, G.R., Balkwill, D.L., Fredrickson, J.K., and Brockman, F.J. (1997) A molecular comparison of culturable aerobic heterotrophic bacteria and 16S rDNA clones derived from a deep subsurface sediment. *FEMS Microbiol Ecol* **23**: 131-144.



- Cavender-Bares, J., Ackerly, D.D., Baum, D.A., and Bazzaz, F.A. (2004) Phylogenetic overdispersion in Floridian oak communities. *Am Nat* **163**: 823-843.
- Cho, J.C., and Tiedje, J.M. (2000) Biogeography and degree of endemism of fluorescent *Pseudomonas* strains in soil. *Appl Environ Microbiol* **66**: 5448-5456.
- Choler, P. (2005) Consistent shifts in Alpine plant traits along a mesotopographical gradient. *Arct Antarct Alp Res* **37**: 444-453.
- Chouari, R., Le Paslier, D., Daegelen, P., Ginestet, P., Weissenbach, J., and Sghir, A. (2005) Novel predominant archaeal and bacterial groups revealed by molecular analysis of an anaerobic sludge digester. *Environ Microbiol* **7**: 1104-1115.
- Costello, E.K., and Schmidt, S.K. (2006) Microbial diversity in alpine tundra wet meadow soil: novel Chloroflexi from a cold, water-saturated environment. *Environ Microbiol* **8**: 1471-1486.
- Cottrell, M.T., and Kirchman, D.L. (2000) Natural assemblages of marine proteobacteria and members of the Cytophaga-Flavobacter cluster consuming low- and high-molecular-weight dissolved organic matter. *Appl Environ Microbiol* **66**: 1692-1697.
- Crawford, D.L. (1978) Lignocellulose decomposition by selected streptomyces strains. *Appl Environ Microbiol* **35**: 1041-1045.
- Crawford, J.W., Harris, J.A., Ritz, K., and Young, I.M. (2005) Towards an evolutionary ecology of life in soil. *Trends Ecol Evol* **20**: 81-87.
- Crump, B.C., Hopkinson, C.S., Sogin, M.L., and Hobbie, J.E. (2004) Microbial biogeography along an estuarine salinity gradient: Combined influences of bacterial growth and residence time. *Appl Environ Microbiol* **70**: 1494-1505.
- Curtis, T.P., Sloan, W.T., and Scannell, J.W. (2002) Estimating prokaryotic diversity and its limits. *P NATL ACAD SCI USA* **99**: 10494-10499.
- Dalmastri, C., Chiarini, L., Cantale, C., Bevivino, A., and Tabacchioni, S. (1999) Soil type and maize cultivar affect the genetic diversity of maize root-associated *Burkholderia cepacia* populations. *Microb Ecol* **38**: 273-284.
- Davidson, E.A., and Janssens, I.A. (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* **440**: 165-173.
- Delbes, C., Moletta, R., and Godon, J.J. (2000) Monitoring of activity dynamics of an anaerobic digester bacterial community using 16S rRNA polymerase chain reaction - single-strand conformation polymorphism analysis. *Environ Microbiol* **2**: 506-515.

- Delbes, C., Godon, J.J., and Moletta, R. (1998) 16S rDNA sequence diversity of a culture-accessible part of an anaerobic digester bacterial community. *Anaerobe* **4**: 267-275.
- Davis, K.E.R., Joseph, S.J., and Janssen, P.H. (2005) Effects of growth medium, inoculum size, and incubation time on culturability and isolation of soil bacteria. *Appl Environ Microbiol* **71**: 826-834.
- de Araujo, J.M., da Silva, A.C., and Azevedo, J.L. (2000) Isolation of endophytic actinomycetes from roots and leaves of maize (*Zea mays* L.). *Braz Arch Biolo Techn* **43**: 447-451.
- Dunbar, J., Takala, S., Barns, S.M., Davis, J.A., and Kuske, C.R. (1999) Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning. *Appl Environ Microbiol* **65**: 1662-1669.
- Downie, J.A. (2007) Rhizobia. *Encyclopedia of Life Sciences*.
- Dworkin, M., S. Falkow, E. Rosenberg, K.H. Schleifer, E. Stackebrandt (2007) The Prokaryotes. Springer Reference.
- Edwards, A.C., Scalenghe, R., and Freppaz, M. (2007) Changes in the seasonal snow cover of alpine regions and its effect on soil processes: A review. *Quatern Int* **162**: 172-181.
- Elena, S.F., and Lenski, R.E. (2003) Evolution experiments with microorganisms: The dynamics and genetic bases of adaptation. *Nat Rev Genet* **4**: 457-469.
- Embley, T.M., and Stackebrandt, E. (1994) The Molecular Phylogeny And Systematics Of The Actinomycetes. *Ann Rev Microb* **48**: 257-289.
- Ettema, C.H., and Wardle, D.A. (2002) Spatial soil ecology. *Trends Ecol Evol* **17**: 177-183.
- Fenchel, T. (2007) Bacterial Ecology. *Encyclopedia of Life Sciences*.
- Fierer, N., and Jackson, R.B. (2006) The diversity and biogeography of soil bacterial communities. *P NATL ACAD SCI USA* **103**: 626-631.
- Finlay, B.J., and Clarke, K.J. (1999) Ubiquitous dispersal of microbial species. *Nature* **400**: 828-828.
- Fisk, M.C., Schmidt, S.K., and Seastedt, T.R. (1998) Topographic patterns of above- and belowground production and nitrogen cycling in Alpine tundra. *Ecology* **79**: 2253-2266.
- Foght, J., Aislabie, J., Turner, S., Brown, C.E., Ryburn, J., Saul, D.J., and Lawson, W. (2004) Culturable bacteria in subglacial sediments and ice from two Southern Hemisphere glaciers. *Micro Ecol* **47**: 329-340.

- Fong, N.J.C., Burgess, M.L., Barrow, K.D., and Glenn, D.R. (2001) Carotenoid accumulation in the psychrotrophic bacterium *Arthrobacter agilis* in response to thermal and salt stress. *Appl Microbiol Biotech* **56**: 750-756.
- Fox, G.E., Wisotzkey, J.D., and Jurtshuk, P. (1992) How Close Is Close - 16s Ribosomal-Rna Sequence Identity May Not Be Sufficient To Guarantee Species Identity. *Int J Syst Bacteriol* **42**: 166-170.
- Frostegard, A., Courtois, S., Ramisse, V., Clerc, S., Bernillon, D., Le Gall, F. et al. (1999) Quantification of bias related to the extraction of DNA directly from soils. *Appl Environ Microbiol* **65**: 5409-5420.
- Garvish, E., Bollmann, A., Epstein, S., and Lewis, K. (2008) A trap for in situ cultivation of filamentous actinobacteria. *J Microbiol Meth* **72**: 257-262.
- Gish, F., Schubert, K., Bruns, A., Hoffelner, H., and Overmann, J. (2005) Specific detection, isolation, and characterization of selected, previously uncultured members of the freshwater bacterioplankton community. *Appl Environ Microbiol* **71**: 5908-5919.
- Gilichinskii, D.A., E. A. Vorobyova, L. G. Erokhina, D. G. Fyodorov-Dayvdov and N. R. Chaikovskaya (1992) Long-term Preservation of Microbial Ecosystems in Permafrost. *Adv. Space Res* **12**: 255-263.
- Girvan, M.S., Bullimore, J., Pretty, J.N., Osborn, A.M., and Ball, A.S. (2003) Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. *Appl Environ Microbiol* **69**: 1800-1809.
- Grayston, S.J., and Campbell, C.D. (1996) Functional biodiversity of microbial communities in the rhizospheres of hybrid larch (*Larix eurolepis*) and Sitka spruce (*Picea sitchensis*). *Tree Physiol* **16**: 1031-1038.
- Grayston, S.J., Wang, S.Q., Campbell, C.D., and Edwards, A.C. (1998) Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biol Biochem* **30**: 369-378.
- Griffiths, R.I., Bailey, M.J., McNamara, N.P., and Whiteley, A.S. (2006) The functions and components of the Sourhope soil microbiota. *Appl Soil Ecol* **33**: 114-126.
- Groudieva, T., Kambourova, M., Yusef, H., Royter, M., Grote, R., Trinks, H., and Antranikian, G. (2004) Diversity and cold-active hydrolytic enzymes of culturable bacteria associated with Arctic sea ice, Spitzbergen. *Extremophiles* **8**: 475-488.
- Grundmann, L.G., and Gourbiere, F. (1999) A micro-sampling approach to improve the inventory of bacterial diversity in soil. *Appl Soil Ecol* **13**: 123-126.

- Halden, R.U., Tepp, S.M., Halden, B.G., and Dwyer, D.F. (1999) Degradation of 3-phenoxybenzoic acid in soil by *Pseudomonas pseudoalcaligenes* POB310 (pPOB) and two modified *Pseudomonas* strains. *Appl Environ Microbiol* **65**: 3354-3359.
- Hansen, A.A., Herbert, R.A., Mikkelsen, K., Jensen, L.L., Kristoffersen, T., Tiedje, J.M. et al. (2007) Viability, diversity and composition of the bacterial community in a high Arctic permafrost soil from Spitsbergen, Northern Norway. *Environ Microbiol* **9**: 2870-2884.
- Harvey, P.H., and Purvis, A. (1991) Comparative Methods For Explaining Adaptations. *Nature* **351**: 619-624.
- Hayward, C.A. (2006) Microorganisms. *Encyclopedia of Life Sciences*.
- Heuer, H., Krsek, M., Baker, P., Smalla, K., and Wellington, E.M. (1997) Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel electrophoretic separation in denaturing gradients. *Appl Environ Microbiol* **63**: 3233-3241.
- Hobbie, S.E. (1992) Effects Of Plant-Species On Nutrient Cycling. *Trends Ecol Evol* **7**: 336-339.
- Hobbie, S.E., Schimel, J.P., Trumbore, S.E., and Randerson, J.R. (2000) Controls over carbon storage and turnover in high-latitude soils. *Glob Change Biol* **6**: 196-210.
- Hogberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Hogberg, M.N. et al. (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature* **411**: 789-792.
- Horner-Devine, M.C., and Bohannan, B.J.M. (2006) Phylogenetic clustering and overdispersion in bacterial communities. *Ecology* **87**: S100-S108.
- Horner-Devine, M.C., Carney, K.M., and Bohannan, B.J.M. (2004) An ecological perspective on bacterial biodiversity. *P Roy Soc Lond B Bio* **271**: 113-122.
- Huber, J.A., Mark Welch, D., Morrison, H.G., Huse, S.M., Neal, P.R., Butterfield, D.A., and Sogin, M.L. (2007) Microbial population structures in the deep marine biosphere. *Science* **318**: 97-100.
- Hugenholtz, P., Goebel, B.M., and Pace, N.R. (1998) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *J Bacteriol* **180**: 4765-4774.
- Islam, K.R., and S. R. Wright (2006) Micronial Communities, *Encyclopedia of Soil Science*. Second edition Volume 2.
- Janssen, P.H., Yates, P.S., Grinton, B.E., Taylor, P.M., and Sait, M. (2002) Improved culturability of soil bacteria and isolation in pure culture of novel members of the divisions

Acidobacteria, Actinobacteria, Proteobacteria, and Verrucomicrobia. *Appl Environ Microbiol* **68**: 2391-2396.

Johnson, M.J., Lee, K.Y., and Scow, K.M. (2003) DNA fingerprinting reveals links among agricultural crops, soil properties, and the composition of soil microbial communities. *Geoderma* **114**: 279-303.

Karigar, C., Mahesh, A., Nagenahalli, M., and Yun, D.J. (2006) Phenol degradation by immobilized cells of *Arthrobacter citreus*. *Biodegradation* **17**: 47-55.

Keuth, S., and Rehm, H.J. (1991) Biodegradation Of Phenanthrene By *Arthrobacter Polychromogenes* Isolated From A Contaminated Soil. *Appl Microbiol Biot* **34**: 804-808.

Kielak, A., Pijl, A.S., van Veen, J.A., and Kowalchuk, G.A. (2008) Differences in vegetation composition and plant species identity lead to only minor changes in soil-borne microbial communities in a former arable field. *FEMS Microbiol Ecol* **63**: 372-382.

Kirk, J.L., Beaudette, L.A., Hart, M., Moutoglis, P., Khironomos, J.N., Lee, H., and Trevors, J.T. (2004) Methods of studying soil microbial diversity. *J Microbiol Meth* **58**: 169-188.

Kiss, M.M., Ortoleva-Donnelly, L., Beer, N.R., Warner, J., Bailey, C.G., Colston, B.W. et al. (2008) High-Throughput Quantitative Polymerase Chain Reaction in Picoliter Droplets. *Anal Chem* **80**: 8975-8981.

Kohls, S.J., Thimmapuram, J., Buschena, C.A., Paschke, M.W., and Dawson, J.O. (1994) Nodulation Patterns Of Actinorhizal Plants In The Family Rosaceae. *Plant Soil* **162**: 229-239.

Korner, C. (1999) Alpine Plant Life. Berlin: *Springer Verlag*.

Kowalchuk, G.A., Buma, D.S., de Boer, W., Klinkhamer, P.G.L., and van Veen, J.A. (2002) Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. *Anton Leeuw Int J G* **81**: 509-520.

Kowalchuk, G.A., and Stephen, J.R. (2001) Ammonia-oxidizing bacteria: A model for molecular microbial ecology. *Annu Rev Microbiol* **55**: 485-529.

Kroes, I., Lepp, P.W., and Relman, D.A. (1999) Bacterial diversity within the human subgingival crevice. *P NATL ACAD SCI USA* **96**: 14547-14552.

Kumar, G.S., Jagannadham, M.V., and Ray, M.K. (2002) Low-temperature-induced changes in composition and fluidity of lipopolysaccharides in the antarctic psychrotrophic bacterium *Pseudomonas syringae*. *J Bacteriol* **184**: 6746-6749.

- Lauber, C.L., Strickland, M.S., Bradford, M.A., and Fierer, N. (2008) The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biol Biochem* **40**: 2407-2415.
- Lee, S.H., Ka, J.O., and Cho, J.C. (2008) Members of the phylum Acidobacteria are dominant and metabolically active in rhizosphere soil. *FEMS Microbiol Lett.*
- Lee, D.H., Zo, Y.G., and Kim, S.J. (1996) Nonradioactive method to study genetic profiles of natural bacterial communities by PCR-single-strand-conformation polymorphism. *Appl Environ Microb* **62**: 3112-3120.
- Lipson, D.A. (2007) Relationships between temperature responses and bacterial community structure along seasonal and altitudinal gradients. *FEMS Microbiol Ecol* **59**: 418-427.
- Lipson, D.A., and Schmidt, S.K. (2004) Seasonal changes in an alpine soil bacterial community in the colorado rocky mountains. *Appl Environ Microbiol* **70**: 2867-2879.
- Lipson, D.A., Schadt, C.W., and Schmidt, S.K. (2002) Changes in soil microbial community structure and function in an alpine dry meadow following spring snow melt. *Microb Ecol* **43**: 307-314.
- Litaor, M.I., Seastedt, T.R., and Walker, D.A. (2002) Spatial analysis of selected soil attributes across an alpine topographic/snow gradient. *Landscape Ecol* **17**: 71-85.
- Lozupone, C.A., and Knight, R. (2007) Global patterns in bacterial diversity. *P NATL ACAD SCI USA* **104**: 11436-11440.
- Lozupone, C.A., and Knight, R. (2008) Species divergence and the measurement of microbial diversity. *FEMS Microbiol Ecol* **32**: 557-578.
- Ludwig, W., Bauer, S.H., Bauer, M., Held, I., Kirchhof, G., Schulze, R. et al. (1997) Detection and in situ identification of representatives of a widely distributed new bacterial phylum. *FEMS Microbiol Lett* **153**: 181-190.
- Lynd, L.R., Weimer, P.J., van Zyl, W.H., and Pretorius, I.S. (2002) Microbial cellulose utilization: Fundamentals and biotechnology (vol 66, pg 506, 2002). *Microbiol Mol Biol R* **66**: 739-739.
- Maloney, P.E., vanBruggen, A.H.C., and Hu, S. (1997) Bacterial community structure in relation to the carbon environments in lettuce and tomato rhizospheres and in bulk soil. *Microb Ecol* **34**: 109-117.
- Manasiev, J., Gerginova, M., Yemendzhiev, H., Peneva, N., and Alexieva, Z. (2008) Molecular analysis of phenol-degrading microbial strains. *Z Naturforsch A* **63**: 133-138.

- Margesin, R., Labbe, D., Schinner, F., Greer, C.W., and Whyte, L.G. (2003) Characterization of hydrocarbon-degrading microbial populations in contaminated and pristine alpine soils. *Appl Environ Microbiol* **69**: 3085-3092.
- Margesin, R. (2007) Alpine microorganisms: Useful tools for low-temperature bioremediation. *Journal Microbiol* **45**: 281-285.
- Martin-Laurent, F., Philippot, L., Hallet, S., Chaussod, R., Germon, J.C., Soulas, G., and Catroux, G. (2001) DNA extraction from soils: Old bias for new microbial diversity analysis methods. *Appl Environ Microbiol* **67**: 2354-2359.
- Mayr, E. (1982) Biologie is not postage stamp collecting. *Science* **216**: 718-720.
- McCaig, A.E., Glover, L.A., and Prosser, J.I. (1999) Molecular analysis of bacterial community structure and diversity in unimproved and improved upland grass pastures. *Appl Environ Microbiol* **65**: 1721-1730.
- Menyailo, O.V., Hungate, B.A., and Zech, W. (2002) The effect of single tree species on soil microbial activities related to C and N cycling in the Siberian artificial afforestation experiment - Tree species and soil microbial activities. *Plant Soil* **242**: 183-196.
- Miethling, R., Wieland, G., Backhaus, H., and Tebbe, C.C. (2000) Variation of microbial rhizosphere communities in response to crop species, soil origin, and inoculation with *Sinorhizobium meliloti* L33. *Microb Ecol* **40**: 43-56.
- Miteva, V.I., Sheridan, P.P., and Brenchley, J.E. (2004) Phylogenetic and physiological diversity of microorganisms isolated from a deep Greenland glacier ice core. *Appl Environ Microbiol* **70**: 202-213.
- Monson, R.K., Lipson, D.L., Burns, S.P., Turnipseed, A.A., Delany, A.C., Williams, M.W., and Schmidt, S.K. (2006) Winter forest soil respiration controlled by climate and microbial community composition. *Nature* **439**: 711-714.
- Morita, R.Y. (1975) Psychrophilic Bacteria. *Bacteriol Rev* **39**: 144-167.
- Mougel, C., Offre, P., Ranjard, L., Corberand, T., Gamalero, E., Robin, C., and Lemanceau, P. (2006) Dynamic of the genetic structure of bacterial and fungal communities at different developmental stages of *Medicago truncatula* Gaertn. cv. Jemalong line J5. *New Phytol* **170**: 165-175.
- Mueller, D.R., Vincent, W.F., Bonilla, S., and Laurion, I. (2005) Extremotrophs, extremophiles and broadband pigmentation strategies in a high arctic ice shelf ecosystem. *FEMS Microbiol Ecol* **53**: 73-87.
- Muyzer, G., Dewaal, E.C., and Uitterlinden, A.G. (1993) Profiling Of Complex Microbial-Populations By Denaturing Gradient Gel-Electrophoresis Analysis Of Polymerase Chain

Reaction-Amplified Genes-Coding For 16s Ribosomal-RNA. *Appl Environ Microbiol* **59**: 695-700.

Nemergut, D.R., Costello, E.K., Meyer, A.F., Pescador, M.Y., Weintraub, M.N., and Schmidt, S.K. (2005) Structure and function of alpine and arctic soil microbial communities. *Res Microbiol* **156**: 775-784.

Newton, R.J., Jones, S.E., Helmus, M.R., and McMahon, K.D. (2007) Phylogenetic ecology of the freshwater Actinobacteria acI lineage. *Appl Environ Microbiol* **73**: 7169-7176.

Ochman, H., Lawrence, J.G., and Groisman, E.A. (2000) Lateral gene transfer and the nature of bacterial innovation. *Nature* **405**: 299-304.

Oren, A. (2008) Biogeochemical Cycles. *Encyclopedia of Life Sciences*.

Orita, M., Suzuki, Y., Sekiya, T., and Hayashi, K. (1989) Rapid And Sensitive Detection Of Point Mutations And Dna Polymorphisms Using The Polymerase Chain-Reaction. *Genomics* **5**: 874-879.

Pace, N.R. (1997) A molecular view of microbial diversity and the biosphere. *Science* **276**: 734-740.

Polz, M.F., and Cavanaugh, C.M. (1998) Bias in template-to-product ratios in multitemplate PCR. *Appl Environ Microbiol* **64**: 3724-3730.

Prosser, J.I. (2002) Molecular and functional diversity in soil micro-organisms. *Plant Soil* **244** 9-17.

Qiu, X.Y., Wu, L.Y., Huang, H.S., McDonel, P.E., Palumbo, A.V., Tiedje, J.M., and Zhou, J.Z. (2001) Evaluation of PCR-generated chimeras: Mutations, and heteroduplexes with 16S Rrna gene-based cloning. *Appl Environ Microbiol* **67**: 880-887.

Quince, C., Curtis, T. P., and Sloan, W. T (2008) The rational exploration of microbial diversity. *ISME J* **2**: 997-1006

Rainey, P.B., Buckling, A., Kassen, R., and Travisano, M. (2000) The emergence and maintenance of diversity: insights from experimental bacterial populations. *Trends Ecol Evol* **15**: 243- 247.

Rappe, M.S., and Giovannoni, S.J. (2003) The uncultured microbial majority. *Annu Rev Microbiol* **57**: 369-394.



- Rein, A., Fernqvist, M.M., Mayer, P., Trapp, S., Bittens, M., and Karlson, U.G. (2007) Degradation of PCB congeners by bacterial strains. *Appl Microbiol Biot* **77**: 469-481.
- Rivkina, E., Laurinavichius, K., McGrath, J., Tiedje, J., Shcherbakova, V., and Gilichinsky, D. (2004) Microbial life in permafrost. *Adv Space Res* **33**: 1215-1221.
- Rothschild, L.J., and Mancinelli, R.L. (2001) Life in extreme environments. *Nature* **409**: 1092-1101.
- Sait, M., Hugenholtz, P., and Janssen, P.H. (2002) Cultivation of globally distributed soil bacteria from phylogenetic lineages previously only detected in cultivation-independent surveys. *Environ Microbiol* **4**: 654-666.
- Sardi, P., Saracchi, M., Quaroni, S., Petrolini, B., Borgonovi, G.E., and Merli, S. (1992) Isolation Of Endophytic Streptomyces Strains From Surface-Sterilized Roots. *Appl Environ Microbiol* **58**: 2691-2693.
- Saul, D.J., Aislabie, J.M., Brown, C.E., Harris, L., and Foght, J.M. (2005) Hydrocarbon contamination changes the bacterial diversity of soil from around Scott Base, Antarctica. *FEMS Microbiol Ecol* **53**: 141-155.
- Schadt, C.W., Martin, A.P., Lipson, D.A., and Schmidt, S.K. (2003) Seasonal dynamics of previously unknown fungal lineages in tundra soils. *Science* **301**: 1359-1361.
- Schmidt, T.M. (2006) The maturing of microbial ecology. *Int Microbiol* **9**: 217-223.
- Sessitsch, A., Weilharter, A., Gerzabek, M.H., Kirchmann, H., and Kandeler, E. (2001) Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. *Appl Environ Microbiol* **67**: 4215-4224.
- Schmidt, S.K., Costello, E.K., Nemergut, D.R., Cleveland, C.C., Reed, S.C., Weintraub, M.N. et al. (2007) Biogeochemical consequences of rapid microbial turnover and seasonal succession in soil. *Ecology* **88**: 1379-1385.
- Schmidt, S.K., Lipson, D.A., and Raab, T.K. (2000) Effects of willows (*Salix brachycarpa*) on populations of Salicylate-mineralizing microorganisms in alpine soils. *J Chem Ecol* **26**: 2049-2057.
- Shannon, C.E. (1948) A Mathematical Theory Of Communication. *AT T TECH J* **27**: 379-423.
- Sheng, X.F., and Gong, J.X. (2006) Increased degradation of phenanthrene in soil by *Pseudomonas* sp GF3 in the presence of wheat. *Soil Biol Biochem* **38**: 2587-2592.
- Shi, T., Reeves, R.H., Gilichinsky, D.A., and Friedmann, E.I. (1997) Characterization of viable bacteria from Siberian permafrost by 16S rDNA sequencing. *Microb Ecol* **33**: 169-179.

- Simpson, E.H. (1949) Measurement Of Diversity. *Nature* **163**: 688-688.
- Singh, B.K., Dawson, L.A., Macdonald, C.A., and Buckland, S.M. (2009) Impact of biotic and abiotic interaction on soil microbial communities and functions: A field study. *Appl Soil Ecol* **41**: 239-248.
- Singh, B.K., Munro, S., Potts, J.M., and Millard, P. (2007) Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. *Appl Soil Ecol* **36**: 147-155.
- Smalla, K., Wieland, G., Buchner, A., Zock, A., Parzy, J., Kaiser, S. et al. (2001) Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: Plantdependent enrichment and seasonal shifts revealed. *Appl Environ Microbiol* **67**: 4742-4751.
- Smith, J.J., Tow, L.A., Stafford, W., Cary, C., and Cowan, D.A. (2006) Bacterial diversity in three different Antarctic Cold Desert mineral soils. *Microb Ecol* **51**: 413-421.
- Sogin, M.L., Morrison, H.G., Huber, J.A., Mark Welch, D., Huse, S.M., Neal, P.R. et al. (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". *P NATL ACAD SCI USA* **103**: 12115-12120.
- Soina, V.S., Vorobiova, E.A., Zvyagintsev, D.G., and Gilichinsky, D.A. (1994) Preservation Of Cell Structures In Permafrost: A Model For Exobiology. *Adv. Space Res* **15**: 237-242.
- Souto, X. C., Bolaño, J. C., González, L., and Reigosa, M. J. (2001) Allelopathic effects of tree species on some soil microbial populations and herbaceous plants. *Biologia Plantarum* **44**, 269-275.
- Stackebrandt, E. (1994) The Molecular Phylogeny And Systematics Of The Actinomycetes. *Ann Rev Microb* **48**: 257-289.
- Stackebrandt, E. (2001) Phylogeny Based on 16S rRNA/DNA. *Encyclopedia of Life Sciences*.
- Stackebrandt, E., and Goebel, B.M. (1994) A Place For DNA-DNA Reassociation And 16s Ribosomal-Rna Sequence-Analysis In The Present Species Definition In Bacteriology. *Int J Of Syst Bacteriol* **44**: 846-849.
- Stevenson, F.J., M. A. Cole (1999) Cycle of soil: carbon, nitrogen, phosphorus, sulfur, micronutrients. John Wiley and Sons.
- Steven, B., Briggs, G., McKay, C.P., Pollard, W.H., Greer, C.W., and Whyte, L.G. (2007) Characterization of the microbial diversity in a permafrost sample from the Canadian high Arctic using culture-dependent and culture-independent methods. *FEMS Microbiol Ecol* **59**: 513-523.

Sturz, A.V., and Christie, B.R. (2003) Beneficial microbial allelopathies in the root zone: the management of soil quality and plant disease with rhizobacteria. *Soil Till Res* **72**: 107-123.

Suzuki, M.T., Rappe, M.S., Haimberger, Z.W., Winfield, H., Adair, N., Strobel, J., and Giovannoni, S.J. (1997) Bacterial diversity among small-subunit rRNA gene clones and cellular isolates from the same seawater sample. *Appl Environ Microbiol* **63**: 983-989.

Tiedje, J.M., Asuming-Brempong, S., Nusslein, K., Marsh, T.L., and Flynn, S.J. (1999) Opening the black box of soil microbial diversity. *Appl Soil Ecol* **13**: 109-122.

Thompson, J.R., Marcelino, L.A., and Polz, M.F. (2002) Heteroduplexes in mixed-template amplifications: formation, consequence and elimination by 'reconditioning PCR'. *Nucl Acids Res* **30**: 2083-2088.

Torsvik, V., and Ovreas, L. (2002) Microbial diversity and function in soil: from genes to ecosystems. *Curr Opin Microbiol* **5**: 240-245.

Torsvik, V., Goksoyr, J., and Daae, F.L. (1990) High Diversity in DNA of Soil Bacteria. *Appl Environ Microb* **56**: 782-787.

Ulrich, A., and Becker, R. (2006) Soil parent material is a key determinant of the bacterial community structure in arable soils. *FEMS Microbiol Ecol* **56**: 430-443.

Valiente-Banuet, A., and Verdu, M. (2007) Facilitation can increase the phylogenetic diversity of plant communities. *Ecol Lett* **10**: 1029-1036.

Vamosi, S.M., Heard, S.B., Vamosi, J.C., and Webb, C.O. (2009) Emerging patterns in the comparative analysis of phylogenetic community structure. *Mol Ecol* **18**: 572-592.

Vishnivetskaya, T.A., Petrova, M.A., Urbance, J., Ponder, M., Moyer, C.L., Gilichinsky, D.A., and Tiedje, J.M. (2006) Bacterial community in ancient Siberian permafrost as characterized by culture and culture-independent methods. *Astrobiology* **6**: 400-414.

Wallenstein, M.D., McMahon, S., and Schimel, J. (2007) Bacterial and fungal community structure in Arctic tundra tussock and shrub soils. *FEMS Microbiol Ecol* **59**: 428-435.

Ward, D.M., Ferris, M.J., Nold, S.C., and Bateson, M.M. (1998) A natural view of microbial biodiversity within hot spring cyanobacterial mat communities. *Microbiol Mol Biol R* **62**: 1353- 1370.

Wallenstein, M.D., McMahon, S., and Schimel, J. (2007) Bacterial and fungal community structure in Arctic tundra tussock and shrub soils. *FEMS Microbiol Ecol* **59**: 428-435.

- Ward, D.M., Ferris, M.J., Nold, S.C., and Bateson, M.M. (1998) A natural view of microbial biodiversity within hot spring cyanobacterial mat communities. *Microbiol Mol Biol R* **62**: 1353- 1370.
- Yergeau, E., Newsham, K.K., Pearce, D.A., and Kowalchuk, G.A. (2007a) Patterns of bacterial diversity across a range of Antarctic terrestrial habitats. *Environ Microbiol* **9**: 2670-2682.
- Yergeau, E., Bokhorst, S., Huiskes, A.H.L., Boschker, H.T.S., Aerts, R., and Kowalchuk, G.A. (2007b) Size and structure of bacterial, fungal and nematode communities along an Antarctic environmental gradient. *FEMS Microbiol Ecol* **59**: 436-451.
- Young, I.M., and Crawford, J.W. (2004) Interactions and self-organization in the soil-microbe complex. *Science* **304**: 1634-1637.
- Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D., and Tilman, D. (2003) Plant diversity, soil microbial communities, and ecosystem function: Are there any links? *Ecology* **84**: 2042-2050.
- Zhang, G.S., Niu, F.J., Ma, X.J., Liu, W., Dong, M.X., Feng, H.Y. et al. (2007) Phylogenetic diversity of bacteria isolates from the Qinghai-Tibet Plateau permafrost region. *Can J Microbiol* **53**: 1000-1010.
- Zhou, J.Z., Davey, M.E., Figueras, J.B., Rivkina, E., Gilichinsky, D., and Tiedje, J.M. (1997) Phylogenetic diversity of a bacterial community determined from Siberian tundra soil DNA. *Microbiol- UK* **143**: 3913-3919.
- Zhu, Y.G., and Miller, R.M. (2003) Carbon cycling by arbuscular mycorrhizal fungi in soilplant systems. *Trends Plant Sci* **8**: 407-409.
- Zinger, L., Gury, J., Alibeu, O., Rioux, D., Gielly, L., Sage, L. et al. (2008) CE-SSCP and CEFLA, simple and high-throughput alternatives for fungal diversity studies. *J Microbiol Methods* **72**: 42-53.
- Zogg, G.P., Zak, D.R., Ringelberg, D.B., MacDonald, N.W., Pregitzer, K.S., and White, D.C. (1997) Compositional and functional shifts in microbial communities due to soil warming. *Soil Sci Soc Am J* **61**: 475-481.
- Zumstein, E., Moletta, R., and Godon, J.J. (2000) Examination of two years of community dynamics in an anaerobic bioreactor using fluorescence polymerase chain reaction (PCR) singlestrand conformation polymorphism analysis. *Environ Microbiol* **2**: 69-7

# **Annexe**



# Tannin impacts on microbial diversity and the functioning of alpine soils: a multidisciplinary approach

F. Baptist,<sup>1</sup> L. Zinger,<sup>1</sup> J. C. Clement,<sup>1</sup> C. Gallet,<sup>2\*</sup>  
R. Guillemin,<sup>1</sup> J. M. F. Martins,<sup>3</sup> L. Sage,<sup>1</sup>  
B. Shahnavaz,<sup>1</sup> Ph. Choler<sup>1,4</sup> and R. Geremia<sup>1</sup>

<sup>1</sup>Laboratoire d'Ecologie Alpine, CNRS UMR 5553, Université de Grenoble, BP 53, F-38041 Grenoble Cedex 09, France.

<sup>2</sup>Laboratoire d'Ecologie Alpine, CNRS UMR 5553, Université de Savoie, F-73367 Bourget-du-Lac, France.

<sup>3</sup>Laboratoire d'études des Transferts en Hydrologie et Environnement, CNRS UMR 5564 Université de Grenoble, F-38041 Grenoble, France.

<sup>4</sup>Station Alpine J. Fourier, CNRS UMS 2925, Université de Grenoble, F-38041 Grenoble, France.

## Summary

**In alpine ecosystems, tannin-rich-litter decomposition occurs mainly under snow. With global change, variations in snowfall might affect soil temperature and microbial diversity with biogeochemical consequences on ecosystem processes. However, the relationships linking soil temperature and tannin degradation with soil microorganisms and nutrients fluxes remain poorly understood. Here, we combined biogeochemical and molecular profiling approaches to monitor tannin degradation, nutrient cycling and microbial communities (Bacteria, Crenarcheotes, Fungi) in undisturbed wintertime soil cores exposed to low temperature (0°C/–6°C), amended or not with tannins, extracted from *Dryas octopetala*. No toxic effect of tannins on microbial populations was found, indicating that they withstand phenolics from alpine vegetation litter. Additionally at –6°C, higher carbon mineralization, higher protocatechuic acid concentration (intermediary metabolite of tannin catabolism), and changes in fungal phylogenetic composition showed that freezing temperatures may select fungi able to degrade *D. octopetala*'s tannins. In contrast, negative net nitrogen mineralization rates were observed at –6°C possibly due to a more efficient N**

**immobilization by tannins than N production by microbial activities, and suggesting a decoupling between C and N mineralization. Our results confirmed tannins and soil temperatures as relevant controls of microbial catabolism which are crucial for alpine ecosystems functioning and carbon storage.**

## Introduction

In arctic and alpine ecosystems, seasonally snow-covered soils sequester a very large pool of organic carbon, which appears particularly vulnerable in the context of global warming (Hobbie *et al.*, 2000). A positive feed-back between increased soil respiration and rising atmospheric CO<sub>2</sub> has been put forward several times in global carbon balance models (Knorr *et al.*, 2005). However, whether snow-covered ecosystems are carbon sources or sinks is still highly debated (Mack *et al.*, 2004; Knorr *et al.*, 2005). This is partly explained by the incomplete understanding we have of the processes involved in the wintertime heterotrophic respiration in relation to snow cover duration in cold ecosystems (McGuire *et al.*, 2000; Monson *et al.*, 2006). The variations of soil microbial activity in relation with the dynamic of the snow cover and litter inputs are poorly documented, though some seasonal changes in the structure and function of microbial communities in alpine soils have been described (Lipson *et al.*, 2002; Schadt *et al.*, 2003; Lipson and Schmidt, 2004). It has been shown that the cooler temperature at the end of the growing season triggers a marked shift to a psychrophilic microflora dominated by fungi (Lipson *et al.*, 2002). Additionally, the microbial biomass increased sharply during wintertime. These microbial communities are able to decompose efficiently recalcitrant carbon sources, such as polyphenols, which are likely to be abundant in alpine plants tissues (Steltzer and Bowman, 2005). It is widely recognized that phenolics play a major role in nutrient cycling and litter decomposition through their multilevel interactions with mineralization processes (Cornelissen *et al.*, 1999; Hattenschwiler and Vitousek, 2000). Aside from their toxicity towards some microorganisms, polyphenols, especially the tannin fraction, are expected to affect the availability of nitrogen to plants during their growing season, mainly through complexation

Received 27 June, 2007; accepted 23 October, 2007. \*For correspondence. E-mail [christiane.gallet@univ-savoie.fr](mailto:christiane.gallet@univ-savoie.fr); Tel. 33 (0) 4 79 75 86 01; Fax 33 (0) (0) 4 79 88 80.

of the organic nitrogen in soils (Kraus *et al.*, 2003; Kaal *et al.*, 2005; Nierop and Verstraten, 2006).

Fungus-dominated microbial communities are particularly abundant in the most constraining habitats of the alpine landscape such as exposed dry meadows (Nemergut *et al.*, 2005). These ecosystems are dominated by slow-growing plant species – mainly *Kobresia myosuroides*, *Dryas octopetala* – and are characterized by a low net primary productivity, a high soil organic matter (SOM) content, and a limited supply of soil nutrients (Choler, 2005). Furthermore, *D. octopetala* produces high amounts of polyphenols, with proanthocyanins as the major tannin compounds. In dry meadows, the low and irregular snow pack leads to frequent periods where soils are frozen ( $< -5^{\circ}\text{C}$ ) between plant senescence and renewal. However, the impact of repeated low temperature events on both recalcitrant litter decomposition and soil functioning remains unknown. Additionally, these abiotic constraints are likely to be modified by climatic change. Recent climate scenarios for the Alps show changes in the seasonality and quantity of snow at high altitude, i.e. above 2000 m (Beniston, 2003; Keller *et al.*, 2005). The predicted decrease in precipitation between autumn and early spring will most likely reduce the winter snow-covered period of alpine dry meadows, consequently, increasing the length of the soil freezing period. It is not known to what extent these changes will affect the wintertime decomposition of organic matter, recalcitrant compounds in particular.

In this study, we focused on the combined effect of low temperature ( $< 0^{\circ}\text{C}$ ) and the input of recalcitrant compounds on alpine soil functioning. We expected a shift in microbial communities as a consequence of changes in these two ecological factors during the late-fall critical period. We set up an incubation experiment with soil cores under laboratory conditions to disentangle the effects of temperature and tannin addition on the diversity of microbial communities and the carbon and nitrogen cycles. More specifically, we addressed the following questions: how does tannin input affect (i) carbon and nitrogen mineralization and (ii) overall soil bacterial and fungal phylogenetic structures? (iii) how are these functional and phylogenetic responses are modulated by a prior treatment at freezing temperature ( $-6^{\circ}\text{C}$ )?

We simulated a late-fall litter flux by adding tannins extracted from *D. octopetala* leaves to soil cores collected in dry meadows during the fall, and we mimicked the snow-pack reduction by a freezing treatment. We monitored the C and N soil dynamics (including tannin evolution) and assessed the microbial soil diversity through rRNA genes (16S rRNA gene for prokaryotes, ITS for fungi) using molecular profiling [single-strand conformation polymorphism (SSCP)] in addition to classical microbial techniques.

## Results

Impact of tannin on structure and metabolism of microbial populations were addressed in an incubation experiment with soil cores under laboratory conditions. Four treatments were applied ( $n = 3$ ). In W/S and T/S treatments, soil cores were amended, respectively, with water and tannins extracted from *D. octopetala* leaves, and they were all maintained at  $0^{\circ}\text{C}$  during 45 days. In W/F and T/F, soil cores were also amended with water and tannin solution respectively, but then were stored at  $-6^{\circ}\text{C}$  during 15 days (day 15) and kept at  $0^{\circ}\text{C}$  for four more weeks.

### Phenolic metabolization

At day 15, more than 10% of the added tannins were recovered from the soil samples, 12% for S/T (Stable/Tannin treatment) and 17% for F/T (Freezing/Tannin treatment) (non-significant difference,  $U = 2.5$ ,  $P = 0.376$ ). After 45 days, both temperature treatments had a recovery fraction of around 5%. In treatment W, no tannins were detected in the soils at days 15 and 45, while they were present in low but detectable amounts at day 0. When comparing the phenolic acids, significantly higher levels of protocatechuic acid (last aromatic degradation metabolite before ring fission) were observed in the treatment T, than in the treatment W, irrespective of temperature and sampling times (Fig. 1). The accumulation of this degradation product was higher in the F/T treatment than in the S/T treatment for both dates, indicating a better metabolizing of the tannins in soils submitted to the freezing treatment. Similar patterns were observed for other phenolic acids: vanillic and p-hydroxybenzoic acids (data not shown).

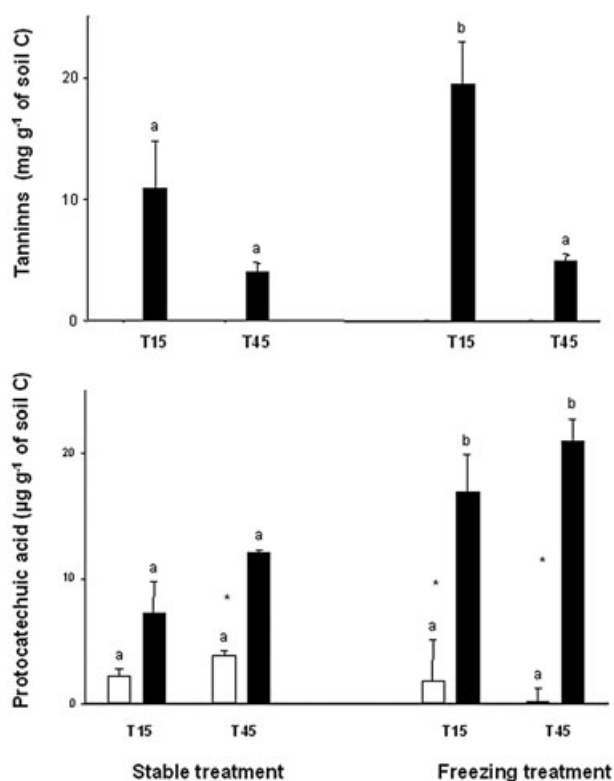
### Changes in microbial biomass and diversity

Microbial and fungal biomasses and bacterial counts were not significantly affected by temperature or tannin amendment (Table 1). However, these treatments affected microbial diversity differently. The F/T and S/W treatments had the strongest impact on the bacterial communities (Fig. 2A). Moreover marked differences between day 15 and day 45 are supported for all the treatments except for F/W.

For crenarcheotes, we observed the formation of new SSCP peaks for all the treatments (data not shown). The F/T treatment had a contrasted effect on the structure (peak distribution) of the crenarcheote communities compared with other conditions (data not shown) as there were less peaks, which suggests the dominance of few phylotypes.

Diversity of fungal communities was significantly affected for all conditions and especially for S/T and F/W. The SSCP profile of day 45 (F/T) was an outlier because





**Fig. 1.** A. Average tannins concentrations in soil amended with sterilized water (white bars) or a tannin solution (black bars). The concentration of tannins is negligible in the case of sterilized water amendment. B. Protocatechuic acid concentration in soils amended with sterilized water (white bars) or a tannin solution (black bars). Differences between water and tannin treatments (\* $P < 0.05$ ) and between temperature treatments (a, b;  $P < 0.05$ ) were tested with Mann–Whitney tests.  $n = 3 \pm$  standard error of the mean.

the data file containing the migration value for statistical analysis was corrupted. The longest distance corresponded to the F/W cores, which showed fewer peaks than the other treatments (Fig. 3D). The day 0 and S/W profiles (Fig. 3A and B) had more than 10 peaks. The S/T profiles (Fig. 3C) presented a low signal, but as many peaks as S/W and day 0. A broad analysis of the raw data for S/T revealed that the baseline increased, which may indicate the co-migration of numerous fungal phylotypes (Loisel *et al.*, 2006). For all profiles, the predominant phylotypes became relatively more abundant between days 15 and 45. Moreover, the F/T profiles presented more peaks than the S/T ones (Fig. 3E). These results suggest that tannins prevented the loss of fungal phylotypes due to freezing.

#### Impact on carbon mineralization

Between days 0 and 15, the total CO<sub>2</sub> efflux measured with the F treatment was significantly lower (approximately two- to fourfold) than with the S treatment

(Table 2). Although marginally significant, the tannin treatment (F/T) led to an increase (approximately twofold) in CO<sub>2</sub> efflux between days 0 and 15 compared with the F/W treatment. However, the total CO<sub>2</sub> efflux at 0°C was not affected by the presence of tannins.

On day 15, when the temperature shifted from –6°C to 0°C, the CO<sub>2</sub> efflux doubled from soils of F/T treatment and increased fourfold in the F/W treatment. Between days 15 and 45, no additional differences were detected between the treatments with and without tannins (Table 2). These results indicated that tannins enhanced the CO<sub>2</sub> efflux only with the freezing treatment between days 0 and 15.

#### Impact on nitrogen cycling

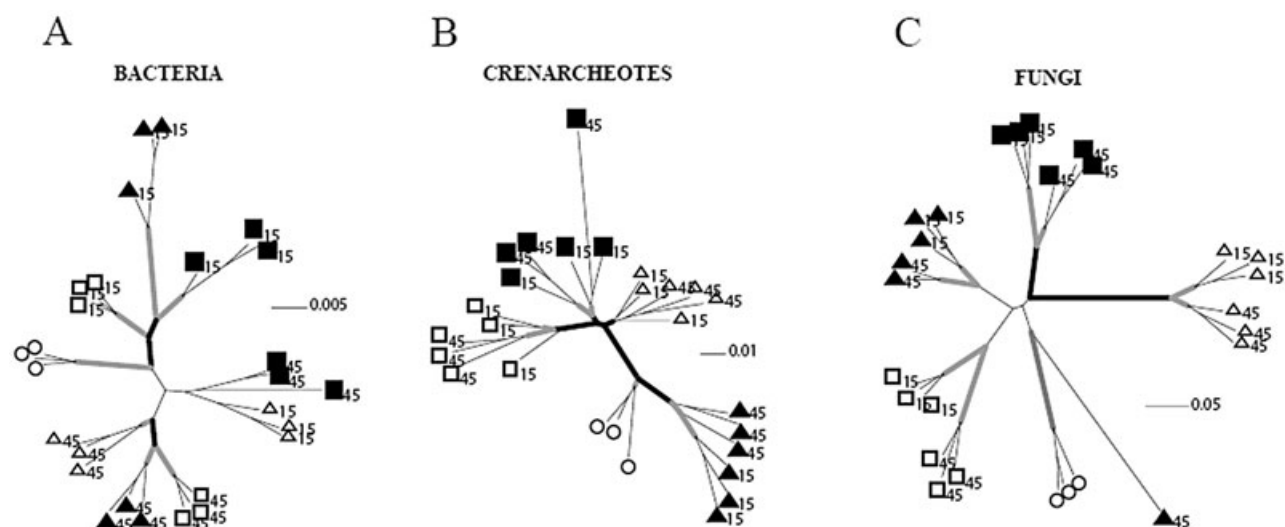
The nitrogen dissolved in the soil extracts was mainly in organic forms [–529.6–1416.7 µg N g<sup>-1</sup> dry weight (dw), 90.4–99.6% of total dissolved nitrogen (TDN)], while ammonia (–3.2–67.1 µg N g<sup>-1</sup> dw, 0.3–8.7% of TDN) and nitrate/nitrite (0.1–10.2 µg N g<sup>-1</sup> dw, 0.0–1.3% of TDN) made up smaller proportions of the TDN. Total dissolved nitrogen and dissolved organic nitrogen (DON) soil contents were changed neither by the temperature (F versus S treatments, data not shown) nor by the addition of tannins (T versus W treatments). Within S treatment, net N mineralization rates between days 15 and 45 were not influenced by the presence of tannins, whereas they were significantly reduced in soils previously stored at –6°C (F treatment, Fig. 4A). This effect was even stronger in soils in the F/T treatment, for which we measured net N immobilization values suggesting that the production of inorganic N was not sufficient to compensate for its disappearance.

Net mineralization potentials (NMP) measured on soil subsamples at days 0, 15 and 45 were 10–400 times

**Table 1.** Microbial and fungal biomasses and bacterial count estimated from soil cores on days 15 and 45 ( $n = 3$ ).

Treatments	Microbial biomass (mg C g <sup>-1</sup> C)	Fungal biomass (µg ergosterol g <sup>-1</sup> C)	Bacterial count (10 <sup>9</sup> cells g <sup>-1</sup> C)
T15			
S/W	44.9 (11.9) <sup>aA</sup>	60.9 (12.6) <sup>aA</sup>	1.13 (0.13) <sup>aA</sup>
F/W	45.8 (15.5) <sup>aA</sup>	182.1 (38.1) <sup>aA</sup>	1.46 (0.28) <sup>aA</sup>
S/T	35.3 (8.6) <sup>aA</sup>	53.8 (5.2) <sup>aA</sup>	1.01 (0.06) <sup>aA</sup>
F/T	38.0 (17.0) <sup>aA</sup>	94.1 (37.3) <sup>aA</sup>	1.12 (0.20) <sup>aA</sup>
T45			
S/W	16.6 (2.2) <sup>aA</sup>	114.3 (18.1) <sup>aA</sup>	1.40 (0.34) <sup>aA</sup>
F/W	48.1 (5.3) <sup>aA</sup>	232.0 (42.7) <sup>aA</sup>	1.59 (0.45) <sup>aA</sup>
S/T	18.7 (6.3) <sup>aA</sup>	103.8 (20.3) <sup>aA</sup>	1.19 (0.50) <sup>aA</sup>
F/T	19.3 (7.1) <sup>aA</sup>	117.2 (35.5) <sup>aA</sup>	0.89 (0.06) <sup>aA</sup>

Differences between day 15 and day 45 were tested by Wilcoxon signed rank test (upper case,  $P < 0.05$ ) and differences between treatment (temperature or tannin amendment) by Mann–Whitney rank sum test (lower case,  $P < 0.05$ ).  $n = 3 \pm$  standard error of the mean.



**Fig. 2.** Neighbour-Joining trees of microbial communities under different treatments: ○= day 0; □= S/W; ■= S/T, △= F/W; ▲= F/T. Lower cases indicate the day of treatments. Supported branches (bootstrap value >50%) are in bold. Grey corresponds to the SSCP replicates after pooling the three soil cores from the same treatment. Black corresponds to supported clustering of treatments. Edwards's distances are shown by a scale bar.

higher than the net N mineralization measured between days 15 and 45 (Fig. 4B and C). Yet, NMP increased during the incubation in the case of the F/W treatment but not in the F/T treatment. On days 15 and 45, NMP from soils amended with tannins were significantly lower than for the unamended ones (Fig. 4B). In treatment S, soils incubated with tannins (S/T) had also lower NMP than on soils amended with water (S/W) but only on day 15 (Fig. 4C). Net mineralization potentials were therefore strongly reduced in F/T treatment compared with the others.

## Discussion

In our study, the role of tannins was evaluated through the combination of biogeochemical analyses with molecular profiling approach. The few other studies which examined the impact of polyphenols through purified tannin addition focused on forested ecosystems, characterized by faster nutrient cycling and higher productivity (Bradley *et al.*, 2000; Fierer *et al.*, 2001). Furthermore, unlike those studies, we used undisturbed soil cores instead of composite and homogenized samples to maintain the vertical stratification and its associated physical and microbiological properties.

### Impact on carbon and nitrogen cycles

The organic and inorganic nitrogen soil concentrations measured in the alpine soils, as well as the dominance of organic N forms, were in accordance with the literature (Tosca and Labroue, 1986; Lipson *et al.*, 1999; Zeller

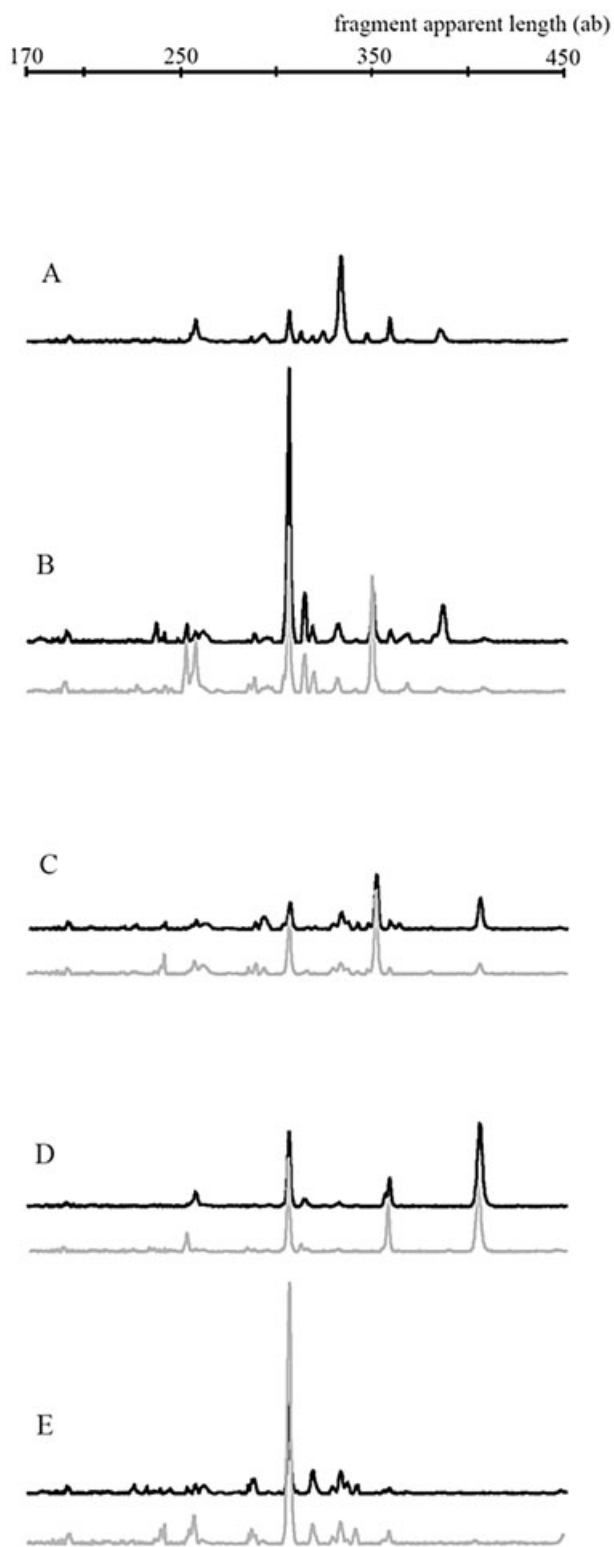
*et al.*, 2000). Similarly, CO<sub>2</sub> efflux measurements were within the range of previous observations (Leifeld and Fuhrer, 2005; Schimel and Mikan, 2005). After 1 month, only a minor fraction of the added tannins was recovered from the amended soils. The disappearance of tannins could be explained either by degradation or by insolubilization, due to complexation with proteins or adsorption on organo-mineral soil fractions (Kaal *et al.*, 2005; Nierop and Verstraten, 2006).

In stable treatment, tannin addition had limited effects on C and N mineralization as well as on microbial biomass indicating (i) that tannins were not used as a significant extra C source and (ii) that they did not inhibit microbial communities. The slight increase in protocatechuic acid

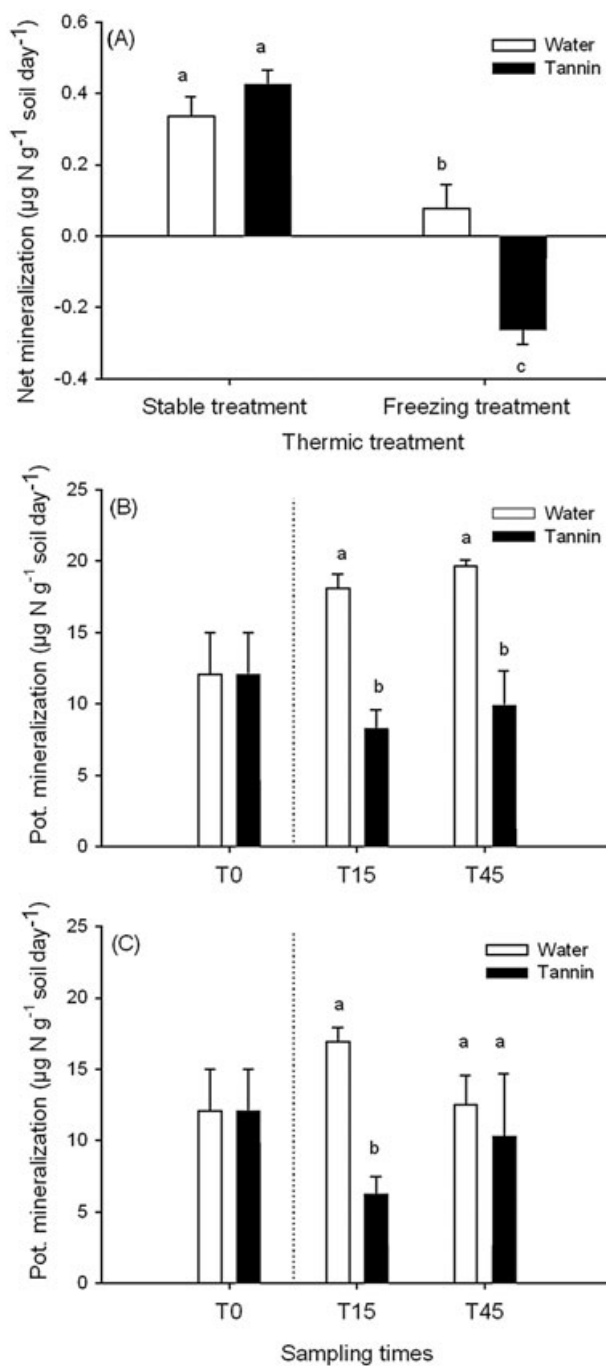
**Table 2.** Soil water content, daily mean CO<sub>2</sub> efflux over the days 0–15 and days 15–45.

Treatments	Soil water content (%)	CO <sub>2</sub> efflux (µg C g <sup>-1</sup> C day <sup>-1</sup> )
T0-T15		
S/W	33.4 (1.0) <sup>aA</sup>	318.8 (70.4) <sup>aA</sup>
F/W	34.8 (3.8) <sup>aA</sup>	76.7 (7.8) <sup>bA</sup>
S/T	37.6 (4.1) <sup>aA</sup>	267.9 (71.9) <sup>abA</sup>
F/T	30.9 (4.5) <sup>aA</sup>	128.6 (19.2) <sup>abA</sup>
T15-T45		
S/W	33.2 (1.2) <sup>aA</sup>	281.3 (131.0) <sup>aA</sup>
F/W	34.9 (3.1) <sup>aA</sup>	269.4 (51.5) <sup>ab</sup>
S/T	39.1 (4.2) <sup>aA</sup>	210.7 (23.7) <sup>aA</sup>
F/T	34.0 (4.7) <sup>aA</sup>	273.8 (94.5) <sup>ab</sup>

The differences between days 15 and 45 within each treatment (upper case,  $P < 0.05$ ) were tested by Wilcoxon signed rank test and the differences between treatment (temperature or tannin addition) within each period (lower case,  $P < 0.05$ ) were tested by Mann-Whitney rank sum test.  $n = 3 \pm$  standard error of the mean.



**Fig. 3.** Capillary electrophoresis-SSCP profiles of fungal communities for each treatment. A = day 0; B = S/W; C = S/T; D = F/W; E = F/T. Black lines: day 15; grey lines: day 45. All profiles are displayed for an arbitrary fluorescence intensity interval of 4000.



**Fig. 4.** A. Net N mineralization rates between days 15 and 45 in soils for F and S treatments amended with sterilized water (white bars) or a tannin solution (black bars). B and C. Net N mineralization potentials in soils after 0, 15 and 45 days of incubation in the F treatment (B) and the S treatment (C), amended with sterilized water (white bars) or a tannin solution (black bars). Mann-Whitney rank sum tests were performed to test for differences between treatment (temperature or tannin addition) within each period (lower case,  $P < 0.05$ ).  $n = 3 \pm$  standard error of the mean.

indicated only weak tannin degradation. This suggests that tannins were preferentially adsorbed on organo-mineral soils or complexed with decomposing organic compounds (Fierer *et al.*, 2001). But this hypothesis, largely mentioned in the literature, is hypothetical as efficient methods to quantify such insoluble compounds are missing (Lorenz *et al.*, 2000; Kanerva *et al.*, 2006). Also, we did not assess the impact of a prolonged exposure to tannins as it occurs in natural habitats, but rather try to mimic a pulse of tannins corresponding to the litter fall period. Thus, it should not be considered as a long-term effect.

The incubation of alpine soils at  $-6^{\circ}\text{C}$  led to a decrease in C mineralization between day 0 and day 15. Lower temperatures strongly affect soil processes by lowering microbial enzymatic activities (Schimel *et al.*, 2004) but do not affect microbial biomass as already shown by others authors (Lipson *et al.*, 2000; Groffman *et al.*, 2001; Grogan *et al.*, 2004). Surprisingly, higher  $\text{CO}_2$  effluxes were detected in F/T than in F/W treatments and higher concentrations of protocatechuic acid in F/T than in S/T treatments in the first step of the experiment. These results indicate a higher tannin degradation at  $-6^{\circ}\text{C}$ . Added to limited microbial growth, this suggests that some microorganisms may be able to use this C source, unlike the populations present at  $0^{\circ}\text{C}$  (S/T). However, the lack of data on N mineralization between day 0 and day 15 prevents us from drawing further conclusions.

From day 15 to day 45, the only significantly affected process was net N mineralization, which decreased in F/W-treated alpine soils. This confirms that a prolonged pre-period of frost slowed down N cycling in alpine soils (Schimel *et al.*, 2004). The reduction of metabolic activities in the soil microbial community may be responsible for this effect. The addition of tannins in our alpine soils amplified the freezing effect on net N mineralization described previously and even led to apparent N immobilization (F/T, Fig. 4A). Possibly, the complexing capacity of tannins may have been detected only when microorganisms were less active due to freezing, suggesting that the kinetics of N mineralization by living microorganisms was faster than that of abiotic N immobilization by tannins. Both processes occurred in the stable treatment, but N microbial mineralization was dominant and microorganisms transformed  $\text{N}_{\text{org}}$  into  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in much larger quantities than could be complexed by tannins. As a result, we measured no tannin effect on net N mineralization in soils for the S treatment (measured as the amount of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  produced).

In F treatment, the reduction of microbial activities reduced the production of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , which did not compensate for the complexation by tannins (Fierer *et al.*, 2001; Castells *et al.*, 2003). In our experiment, this was perceived as a negative net N mineralization or an appar-

ent  $\text{NH}_4^+$  and  $\text{NO}_3^-$  immobilization. This hypothesis is further supported by NMP results (at  $30^{\circ}\text{C}$ ) which were significantly lower in soils amended with tannins, most probably due to complexation of organic compounds (Fierer *et al.*, 2001).

The high concentration of protocatechuic acid and C mineralization during the first step of freezing treatment suggests that the added tannins were metabolized despite very low temperatures. C mineralization was strongly affected by the temperature shift and no long-term effect of tannin addition was detected, possibly due to the shortage of easily decomposable tannin (Kraus *et al.*, 2004). The absence of relationship between N immobilization and C mineralization between days 15 and 45 suggests a decoupling between both processes, as reported by Mutabaruka and colleagues (2007). This is probably because N immobilization is driven by both abiotic and biotic factors, whereas C mineralization depends on biotic controls.

#### *Impact on microbial diversity*

There have been several studies of microbial diversity fingerprints for bacterial communities in mesocosm experiments (Hewson *et al.*, 2003; Hendrickx *et al.*, 2005; Lejon *et al.*, 2007), but none, in alpine soils, were carried out on the three main groups of microorganisms as we did here. We determined three distinct patterns, one for each microbial community. Previous studies showed that the bacterial SSCP patterns are specific for a given bacterial community (Godon *et al.*, 1997; Mohr and Tebbe, 2006; Zinger *et al.*, 2007a). Here, the bacteria profiles showed a high baseline suggesting a large number of rare phylogenotypes (Loisel *et al.*, 2006), preventing the detection of minor changes. We found effects supported by bootstrapping for most treatments. However, because of the high baseline masking the community shifts (Fig. 3), the branch length remained very short between treatments (Fig. 2). Therefore, minor relevant changes in bacterial diversity cannot be detected and a more detailed study is needed to assess the tannin impact on bacterial communities.

For the crenarcheotes, freezing and tannin amendment resulted in a reduction in the number of peaks. Possibly, the convergence of both factors led to the disappearance of some crenarcheote phylogenotypes. However, the effects on nutrient cycling were likely to be negligible, as no decrease in population biomass or in C mineralization was detected.

Fungal communities, whose biomasses were found to increase during winter (Schadt *et al.*, 2003), showed strong responses to all treatments. Tannin amendment associated with low temperature maintained a relatively high diversity whereas freezing temperatures alone led to a decrease in fungal richness. This result suggests that



some wintertime fungal strains may be able to benefit from the addition of tannins, as it has been already shown for bacterial communities (Chowdhury *et al.*, 2004) or fungal populations (Mutabaruka *et al.*, 2007). Previous studies on alpine meadows also suggested that a strong supply of allelochemical-rich litter in the fall may select wintertime populations able to grow on phenolic compounds (Lipson *et al.*, 2002; Schmidt and Lipson, 2004). Unexpectedly, the phylotypes reacted differently in response to the addition of these compounds, depending on the thermic regime. This interaction may be related to the presence of psychrophilic fungi which were excluded at relatively high temperature. Another explanation is that the available labile C, which decreased at lower temperatures, created a selective pressure in favour of fungal strains which metabolize more resistant C substrates (Bradley *et al.*, 2000).

#### Ecological implications

CO<sub>2</sub> efflux measurements showed that there were significant levels of microbial activities even well below 0°C (Brooks *et al.*, 1998). In snow-covered ecosystems, litter decomposition occurs principally during the winter (Hobbie and Chapin, 1996) and recent studies indicate that winter microbial communities degrade phenolic compounds (vanillic and salicylic acids) better than summer microbial communities (Schmidt *et al.*, 2000; Lipson *et al.*, 2002). However, because of inconsistent snow cover during winter, dry meadows frequently experience very low temperatures (< -5°C) reducing soil microbial activity and litter decomposition rates (F. Baptist, unpubl. results, O'Leary and Seastedt, 1994). Furthermore, high concentrations of tannins in the fresh litter of *D. octopetala*, which is a dominant species in this ecosystem, potentially contribute to a decrease in N mineralization by complexing soil organic compounds (Northup *et al.*, 1995; Hattenschwiler and Vitousek, 2000). Severe soil edapho-climatic conditions probably act by inhibiting microbial activity. However, we detected no toxic effects of compounds extracted from *D. octopetala* on microbial activity which indicates that plants producing phenolic compounds may select microbial populations able to use these compounds, or at least able to withstand them (Schmidt *et al.*, 2000). Changes in phylogenetic composition coupled with higher C mineralization and protocatechuic acid contents showed that freezing temperatures selected psychrophilic fungi. These may be able to degrade *D. octopetala*'s tannins, and their activities are potentially initiated by a decrease in temperature. However, this particular effect of temperature remains unclear and could also be related to a decrease in labile C availability.

This study illustrates how soil and climatic conditions interact with soil microorganisms to enhance the metabo-

lization of the tannins released by the plants which dominate alpine ecosystems. The degradation of recalcitrant compounds, during winter, produces a less recalcitrant litter which becomes available by the time plant growth starts. This limits nutrient immobilization thanks to a reduced litter C/N ratio (Schmidt and Lipson, 2004). Consequently, the microbial catabolism of these compounds during winter is of functional importance. A variation in snowfall might affect microbial functional diversity with cascading biogeochemical consequences on ecosystem processes and carbon sequestration. Nevertheless, further investigations remain necessary to identify the exact role of microorganisms in tannin catabolism and their vulnerability to climate change.

## Experimental procedures

### Field site

The study site was located in the Grand Galibier massif (French south-western Alps, 45°0.05'N, 06°0.38'E) on an east facing slope at 2520 m. The growing season lasts around 169 ± 6 days and the mean soil temperature is 7.7 ± 1.5°C in summer and -2.2 ± 1.7°C in winter. The mean soil temperature reaches very low values (< -5°C) during relatively long periods because of inconsistent snow cover. Dry meadow soils are classified as typical alpine rankers. The bedrock is calcareous shales. The dominant plant community in the field site consist mainly of *K. myosuroides* (Cyperaceae) and *D. octopetala* (Rosaceae). Fifteen soil cores were sampled in October 2005 using sterilized (ethyl alcohol 90°) PVC pipes (h = 10 cm, Ø = 10 cm) and tools, to avoid contamination. In the laboratory, the plants were separated from the soil cores which were covered with perforated plastic bags and stored at 0°C until the beginning of the experiment.

### Experimental design

On day 0, three soil cores were destructively harvested and used as controls (Table 3), six soil cores were amended with 19 ml of a tannin solution (with a mean of 749 mg of C/core or 32.4 mg C g<sup>-1</sup> soil C, tannin treatment, T) and six cores with sterilized water (water treatment, W) to reach similar gravimetric soil moisture contents (34.2 ± 1.7% and 34.1 ± 1.6% for cores amended with tannins and water respectively). Three W cores and three T cores were incubated at -6°C for 2 weeks (freeze treatment, F) and then at 0°C for four more weeks. The six remaining cores were kept at 0°C (stable temperature treatment, S) during the whole period. To limit temperature gradients inside the incubators, the soil cores were regularly rotated. At the end of the first period (day 15), half of each soil core (3 S/T, 3 S/W, 3 F/T, 3 F/W) was harvested for a first analysis (longitudinal section). To limit disturbance, the harvested soil was replaced by a sterile and closed bag full of sand. The remaining soil cores were placed back in the incubator for four more weeks at 0°C and then were harvested for final analysis.

**Table 3.** A. Soil characteristics of the cores. B. Initial parameters estimated on the three control soil cores.

(A) Soil characteristics	
Soil water content (%)	32.9 (3.7)
Bulk soil density on < 2 mm fraction (g cm <sup>-3</sup> )	0.24 (0.04)
Organic matter (%)	16.9 (4.3)
pH (H <sub>2</sub> O)	5.1 (0.1)
pH (KCl)	4.1 (0.1)
Grain size analysis	
Clay (< 2 µm)	9.7 (0.5)
Silt (2–50 µm)	41.4 (1.0)
Sand (50–2000 µm)	48.6 (1.2)
(B) Initial parameters (T <sub>0</sub> )	
Microbial biomass (mg C g <sup>-1</sup> C)	170.6 (37.3)
Bacterial count (10 <sup>9</sup> cells g <sup>-1</sup> C)	1.45 (0.21)
Fungal biomass (µg ergosterol g <sup>-1</sup> C)	130.2 (14.0)
Tannin (mg g <sup>-1</sup> C)	0.30 (0.10)
NO <sub>3</sub> <sup>-</sup> (µg N g <sup>-1</sup> soil)	0.10 (0.02)
NH <sub>4</sub> <sup>+</sup> (µg N g <sup>-1</sup> soil)	3.25 (0.10)
N <sub>org</sub> (µg N g <sup>-1</sup> soil)	914.2 (65.7)
Potential mineralization	
NH <sub>4</sub> production (µg N g <sup>-1</sup> soil day <sup>-1</sup> )	12.1 (2.9)
N <sub>org</sub> production (µg N g <sup>-1</sup> soil day <sup>-1</sup> )	145.9 (40.9)

$n = 3 \pm$  standard error of the mean.

At each sampling time (day 0, day 15 and day 45), the soils were sieved (2 mm) and further analysed to determine the tannins and phenolic acid contents, microbial and fungal biomasses, bacterial counts, microbial diversity and nitrogen mineralization rates. The CO<sub>2</sub> efflux was measured between each harvest.

#### Soil characterization

Soil water content, pH<sub>H<sub>2</sub>O</sub>, pH<sub>KCl</sub>, bulk soil density and texture were determined following standard methods (Robertson *et al.*, 1999). The SOM content was determined by loss-on-ignition and the C mass was calculated by dividing SOM fraction by 1.72. In order to determine bulk soil density, the stones mass was determined and converted to stone volume using mean stone density of 2650 kg m<sup>-3</sup> (Hillel, 1971).

#### Tannin extraction and phenolic analysis

*Dryas octopetala* leaves were collected at the end of July, and air-dried. Tannins were extracted from about 300 g of ground leaves, using liquid sequential extractions and a final purification on Sephadex LH-20 (Preston, 1999). The elemental composition of the dried final fraction was obtained by CHN analysis (C %: 62.4; N %: 0). The addition of tannins was performed with a solution of 15.85 g of purified tannins dissolved in 250 ml of distilled water. Proanthocyanins (here after referred to tannins) were quantified in the soil extracts by spectrophotometry, after hydrolysis with butanol/HCl using the proanthocyanidin assay (Preston, 1999). The calibration curves were prepared with a previously purified proanthocyanin fraction from *D. octopetala*. Phenolic acids were obtained (5 g FW) by a double ethanolic extraction (ethanol

70%) under reflux. Aliquots (20 µl) of the ethanolic solution filtered at 0.5 µm, were used for high-performance liquid chromatography (HPLC) analysis on a RP C18 µBondapak column (4.6 mm × 250 mm) monitored by a Waters 600 Controller with a UV detection at 260 nm (Waters 996 PDA). Phenolic acids were separated using a linear gradient from 0 to 20% of solvent B (acetonitrile) in solvent A (acetic acid 0.5% in distilled water) in 45 min, at 1.5 ml min<sup>-1</sup>. Standards of common phenolic acids (including protocatechuic) were obtained from Sigma-Aldrich (L'Isle d'Abeau, France).

#### Nitrogen mineralization

Nitrogen was extracted from fresh soil samples with 2 M KCl. The soil extracts were analysed for ammonia (NH<sub>4</sub><sup>+</sup>) and nitrate/nitrite (NO<sub>3</sub><sup>-</sup>/NO<sub>2</sub><sup>-</sup>) contents using an FS-IV autoanalyser (OI-Analytical, College Station, TX). The TDN content in the soil extracts was measured after oxidation with K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> at 120°C. The DON contents in the soil extracts (µg N g<sup>-1</sup> dw) were calculated as: [DON = TDN - (N-NH<sub>4</sub><sup>+</sup>) + (N-NO<sub>3</sub><sup>-</sup>/NO<sub>2</sub><sup>-</sup>)]. The net nitrogen mineralization (MIN<sub>net</sub>, µg N g<sup>-1</sup> dw day<sup>-1</sup>) between day 15 and day 45 was calculated as: MIN<sub>net</sub> = [((-NH<sub>4</sub><sup>+</sup>) + (N-NO<sub>3</sub><sup>-</sup>/NO<sub>2</sub><sup>-</sup>))<sub>day45</sub> - ((-NH<sub>4</sub><sup>+</sup>) + (N-NO<sub>3</sub><sup>-</sup>/NO<sub>2</sub><sup>-</sup>))<sub>day15</sub>]/dw/30. MIN<sub>net</sub> was not calculated between day 0 and day 15, because the day 15 did not originate from the same soil cores as those for day 0. The NMP was determined from subsamples, using anaerobic incubations (Waring and Bremner, 1964). This protocol allows comparisons of relative organic matter degradability in different soils. Under optimized conditions (dark, 7 day, 30°C, anaerobic) organic N in fresh soils was mineralized and accumulated as NH<sub>4</sub><sup>+</sup>. The difference between the NH<sub>4</sub><sup>+</sup> in the fresh soil (t<sub>1</sub>) and after the anaerobic incubation (t<sub>2</sub>) gave the N mineralization potential: NMP (mg N-NH<sub>4</sub> g<sup>-1</sup> dw day<sup>-1</sup>) = [(N-NH<sub>4</sub>)<sub>t<sub>2</sub></sub> - (N-NH<sub>4</sub>)<sub>t<sub>1</sub></sub>]/dw/7].

#### Soil CO<sub>2</sub> efflux

Throughout the experiment, CO<sub>2</sub> efflux measurements were conducted just after tannin amendment (day 0), one before temperature shift (day 15) and three times between days 15 and 45 on all soil cores. The cores were enclosed in a hermetic Plexiglas™ chamber equilibrated to 400 p.p.m. prior to measurements. The chamber was connected to a LiCor 6200 gas exchange systems (LiCor, Lincoln, NE, USA). Data recording lasted 3–5 min, depending on the signal fluctuations, and the soil temperature was monitored.

#### Microbial community analyses

**Microbial biomass and ergosterol determination.** Microbial carbon biomass was determined by the fumigation-extraction method (Jocteur Monrozier *et al.*, 1993; Martins *et al.*, 1997) adapted from Amato and Ladd (1988). Duplicated soil samples (10 g) were fumigated for 10 days with chloroform. Total organic nitrogen was extracted with 20 ml of 2 M KCl from both the non-fumigated and fumigated soil samples (T<sub>0</sub>, T<sub>10</sub>), microbial nitrogen biomass being determined from the difference between the two treatments. After reaction with ninhydrin, the absorbance (570 nm) of all samples was deter-

mined by spectrophotometry using leucin as standard. The microbial carbon biomass calculated using a conversion factor of 21 (Amato and Ladd, 1988; Martins *et al.*, 1997). The soil ergosterol content was evaluated as an indirect estimate of the soil fungal biomass (Nylund and Wallander, 1992; Gors *et al.*, 2007). Ergosterol was extracted from 5 g of soil (FW) with 30 ml of 99.6% ethanol by shaking for 30 min at 250 r.p.m. The soil solution was filtered and immediately submitted to HPLC under isocratic flow of 1.5 ml min<sup>-1</sup> of MeOH, on a Lichrosorb RP18 column (250 × 4.6 mm, 5 µm). Calibration curves at 282 nm were recorded with standard ergosterol solution from Sigma-Aldrich (L'Isle d'Abeau, France).

**Bacterial counts.** The soil bacterial counting was conducted using the method described by Martins and colleagues (1997). Briefly, 10 g of soil (duplicated) was blended in 50 ml of sterile NaCl 0.9%. After flocculation of the soil particles, an aliquot of the soil suspension (1 ml) was collected and used to enumerate the bacteria after successive dilutions. One millilitre of the diluted suspension was filtered on 0.2 µm polycarbonate membrane filters (Millipore). Bacteria were then stained using a sterile solution (filtered at 0.2 µm) of 4',6-diamidino-2-phenylindole (DAPI) and enumerated by direct counting with a motorized epifluorescent microscope (Axioscope, Zeiss) under UV excitation (Hg lamp) with a filter set for DAPI (365 nm) at 1000-fold magnification.

**SSCP analysis of microbial diversity.** DNA extraction and PCR: the protocols for fungal and prokaryotic signatures have already been described in Zinger and colleagues (2007a,b). Briefly, the soil DNA was amplified using microbial community-specific primers and submitted to capillary electrophoresis-SSCP (CE-SSCP). The 16S rRNA gene was used as the prokaryotic specific marker. The bacterial primers were W49 and W104-FAM (Zumstein *et al.*, 2000; Dutoit *et al.*, 2003) and the crenarchaeotal primers were 133FN6F-NED and 248R5P (Sliwinski and Goodman, 2004). Fungal ITS1 was amplified with the primers ITS5 and ITS2-HEX (White *et al.*, 1990). The soil DNA extraction was performed with the Power Soil™ Extraction Kit (MO BIO Laboratories, Ozyme, St Quentin en Yvelines, France) using 250 mg (fresh weight) of soil per core sample, according to manufacturer's instructions. The DNA extracts from the same-condition cores were then pooled to limit the effects of soil spatial heterogeneity. The PCR reactions (25 µl) were set up as follows: 2.5 mM of MgCl<sub>2</sub>, 1× of AmpliTaq Gold™ buffer, 20 g l<sup>-1</sup> of bovine serum albumin, 0.1 mM of each dNTP, 0.26 µM of each primer, 2 U of DNA polymerase (Applied Biosystems, Courtaboeuf, France) and 1 µl of DNA (1–10 ng DNA). The PCR reaction was performed as follows: an initial step at 95°C (10 min), followed by 30 cycles at 95°C (30 s), 56°C (15 s) and 72°C (15 s), and final step at 72°C (7 min). The PCR products were visualized on a 1.5% agarose gel. Then, amplicons of each microbial community were then pooled for each sample to perform multiplex CE-SSCP. Capillary electrophoresis-SSCP: 1 µl of the PCR product was mixed with 10 µl formamide Hi-Di (Applied Biosystems, Courtaboeuf, France), 0.2 µl standard internal DNA molecular weight marker Genescan-400 HD ROX (Applied Biosystems, Courtaboeuf, France), and 0.5 µl NaOH (0.3 M). The sample mixtures were denatured at 95°C for 5 min and immediately cooled on ice before loading on the instrument. The non-

denaturing polymer consisted of 5% CAP polymer, 10% glycerol and 3100 buffer. Capillary electrophoresis-SSCP was performed on an ABI PRISM 3130 XL Genetic Analyzer (Applied Biosystems, Courtaboeuf, France) using a 36-cm-long capillary. The injection time and voltage were set to 22 s and 6 kV. Electrophoresis was performed for 35 min. The CE-SSCP profiles were normalized in order to control for differences in the total fluorescence intensity between profiles.

**Statistical analysis.** We tested for differences between the temperature and tannin amendment treatments using Mann-Whitney rank sum test ( $P < 0.05$ ) (Statistica 5.0, Statsoft. (1995) Statistica 5.0 Software. Statsoft, Tulsa, USA). Paired differences between days 15 and 45 sampling were tested using the Wilcoxon signed rank test ( $P < 0.05$ ). The normalized profiles of SSCP were analysed by Neighbour-Joining analysis based on a matrix of Edwards distances (Edwards, 1971). The robustness of the resulting tree was assessed using 1000 bootstraps. The data analysis was performed using the Ape package of the R software (RDevelopment-CoreTeam, 2006).

### Acknowledgements

We gratefully acknowledge Olivier Alibeu, Mathieu Buffet, Alice Durand, Niu Kechang, Claire Molitor and Tarafa Mustafa for their help in the field and in the laboratory. Logistical supports were provided by the 'Laboratoire d'Ecologie Alpine' (UMR 5553 CNRS/UJF, University Joseph Fourier) and the 'Station Alpine Joseph Fourier', the alpine field station of the University Joseph Fourier. This study was supported by CNRS and MicroAlpes project funding (ANR). We thank Sophie Rickebusch, Stéphane Reynaud and Michel Tissut for their helpful comments on an earlier version of this manuscript. We also thank the technical platform MOME of the Envirhonalp Pole, Rhône-Alpes Region.

### References

- Amato, M., and Ladd, J.N. (1988) Essay for microbial biomass based on ninhydrin – reactive nitrogen in extracts of fumigated soils. *Soil Biol Biochem* **20**: 107–114.
- Beniston, M. (2003) Climatic change in mountain regions: a review of possible impacts. *Clim Change* **59**: 5–31.
- Bradley, R.L., Titus, B.D., and Preston, C.P. (2000) Changes to mineral N cycling and microbial communities in black spruce humus after additions of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and condensed tannins extracted from *Kalmia angustifolia* and balsam fir. *Soil Biol Biochem* **32**: 1227–1240.
- Brooks, P.D., Williams, M.W., and Schmidt, S.K. (1998) Inorganic N and microbial biomass dynamics before and during spring snowmelt. *Biogeochemistry* **43**: 1–15.
- Castells, E., Penuelas, J., and Valentine, D.W. (2003) Influence of the phenolic compound bearing species *Ledum palustre* on soil N cycling in a boreal hardwood forest. *Plant Soil* **251**: 155–166.
- Choler, P. (2005) Consistent shifts in alpine plants traits along a mesotopographical gradient. *Arct Antarct Alp Res* **37**: 444–453.
- Chowdhury, S.P., Khanna, S., Verma, S.C., and Tripathi, A.K.



- (2004) Molecular diversity of tannic acid degrading bacteria isolated from tannery soil. *J Appl Microbiol* **97**: 1210–1219.
- Cornelissen, J.H.C., Perez Harguindeguy, N., Diaz, S., Grime, J.P., Marzano, B., Cabido, M., *et al.* (1999) Leaf structure and defence control litter decomposition rate across species and life forms in regional floras on two continents. *New Phytol* **143**: 191–200.
- Duthoit, F., Godon, J.J., and Montel, M.C. (2003) Bacterial community dynamics during production of registered designation of origin Salers cheese as evaluated by 16S rRNA gene single-strand conformation polymorphism analysis. *Appl Environ Microbiol* **69**: 3840–3848.
- Edwards, A.W.F. (1971) Distance between populations on the basis of gene frequencies. *Biometrics* **27**: 873–881.
- Fierer, N., Schimel, J.P., Cates, R.G., and Zou, J. (2001) Influence of balsam poplar tannin fractions on carbon and nitrogen dynamics in Alaskan taiga floodplain soils. *Soil Biol Biochem* **33**: 1827.
- Godon, J.-J., Zumstein, E., Dabert, P., Habouzit, F., and Moletta, R. (1997) Molecular microbial diversity of an anaerobic digester as determined by small-subunit rDNA sequence analysis. *Appl Environ Microbiol* **63**: 2802–2813.
- Gors, S., Schumann, R., Haubner, N., and Karstena, U. (2007) Fungal and algal biomass in biofilms on artificial surfaces quantified by ergosterol and chlorophyll a as biomarkers. *Int Biodeterior Biodegradation* **60**: 50–59.
- Groffman, P.M., Driscoll, C.T., Fahey, T.J., Hardy, J.P., Fitzhugh, R.D., and Tierney, G.L. (2001) Effects of mild winter freezing on soil nitrogen and carbon dynamics in a northern hardwood forest. *Biogeochemistry* **56**: 191–213.
- Grogan, P., Michelsen, A., Ambus, P., and Jonasson, S. (2004) Freeze-thaw regime effects on carbon and nitrogen dynamics in sub-arctic heath tundra mesocosms. *Soil Biol Biochem* **36**: 641–654.
- Hattenschwiler, S., and Vitousek, P.M. (2000) The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends Ecol Evol* **15**: 238.
- Hendrickx, B., Dejonghe, W., Boenne, W., Brennerova, M., Cernik, M., Lederer, T., *et al.* (2005) Dynamics of an oligotrophic bacterial aquifer community during contact with a groundwater plume contaminated with benzene, toluene, ethylbenzene, and xylenes: an *in situ* mesocosm study. *Appl Environ Microbiol* **71**: 3815–3825.
- Hewson, I., Vargo, G.A., and Fuhrman, J.A. (2003) Bacterial diversity in shallow oligotrophic marine benthos and overlying waters: effects of virus infection, containment, and nutrient enrichment. *Microb Ecol* **46**: 322.
- Hillel, D. (1971) *Soil and Water; Physical Principles and Processes*. New York, USA: Academic Press New York.
- Hobbie, S.E., and Chapin, F.S. (1996) Winter regulation of tundra litter carbon and nitrogen dynamics. *Biogeochemistry* **35**: 327–338.
- Hobbie, S.E., Schimel, J.P., Trumbore, S.E., and Randerson, J.R. (2000) Controls over carbon storage and turnover in high-latitude soils. *Glob Change Biol* **6** (Suppl. 1): 196–210.
- Jocteur Monrozier, L., Guez, P., Chalamet, A., Bardin, R., Martins, J.M.F., and Gaudet, J.P. (1993) Distribution of micro-organisms and fate of xenobiotic molecules in unsaturated soil environments. *Sci Total Environ* **136**: 121–133.
- Kaal, J., Nierop, K.G.J., and Verstraten, J.M. (2005) Retention of tannic acid and condensed tannin by Fe-oxide-coated quartz sand. *J Colloid Interface Sci* **287**: 72–79.
- Kanerva, S., Kitunen, V., Kiikkila, O., Loponen, J., and Smolander, A. (2006) Response of soil C and N transformations to tannin fractions originating from Scots pine and Norway spruce needles. *Soil Biol Biochem* **38**: 1364–1374.
- Keller, F., Goyette, S., and Beniston, M. (2005) Sensitivity analysis of snow cover to climate change scenarios and their impact on plant habitats in alpine terrain. *Clim Change* **72**: 299–319.
- Knorr, W., Prentice, I.C., House, J.I., and Holland, E.A. (2005) Long-term sensitivity of soil carbon turnover to warming. *Nature* **433**: 298–301.
- Kraus, T.E.C., Dahlgren, R.A., and Zasoski, R.J. (2003) Tannins in nutrient dynamics of forest ecosystems – a review. *Plant Soil* **256**: 41–66.
- Kraus, T.E.C., Zasoski, R.J., Dahlgren, R.A., Horwath, W.R., and Preston, C.M. (2004) Carbon and nitrogen dynamics in a forest soil amended with purified tannins from different plant species. *Soil Biol Biochem* **36**: 309.
- Leifeld, J., and Fuhrer, J. (2005) The temperature response of CO<sub>2</sub> production from bulk soils and soil fractions is related to soil organic matter quality. *Biogeochemistry* **75**: 433–453.
- Lejon, D.P.H., Nowak, V., Bouko, S., Pascault, N., Mougel, C., Martins, J.M.F., and Ranjard, L. (2007) Fingerprinting and diversity of bacterial *copA* genes in response to soil types, soil organic status and copper contamination. *FEMS Microbiol Ecol* **61**: 424–437.
- Lipson, D.A., and Schmidt, S.K. (2004) Seasonal changes in an alpine soil bacterial community in the Colorado Rocky Mountains. *Appl Environ Microbiol* **70**: 2867–2879.
- Lipson, D.A., Schmidt, S.K., and Monson, R.K. (1999) Links between microbial population dynamics and nitrogen availability in an alpine ecosystem. *Ecology* **80**: 1623–1631.
- Lipson, D.A., Schmidt, S.K., and Monson, R.K. (2000) Carbon availability and temperature control the post-snowmelt decline in alpine soil microbial biomass. *Soil Biol Biochem* **32**: 441–448.
- Lipson, D.A., Schadt, C.W., and Schmidt, S.K. (2002) Changes in soil microbial community structure and function in an alpine dry meadow following spring snow melt. *Microb Ecol* **43**: 307–314.
- Loisel, P., Harmand, J., Zemb, O., Latrille, E., Lobry, C., Delgenes, J.-P., and Godon, J.-J. (2006) Denaturing gradient electrophoresis (DGE) and single-strand conformation polymorphism (SSCP) molecular fingerprintings revisited by simulation and used as a tool to measure microbial diversity. *Environ Microbiol* **8**: 720–731.
- Lorenz, K., Preston, C.M., Raspe, S., Morrison, I.K., and Feger, K.H. (2000) Litter decomposition and humus characteristics in Canadian and German spruce ecosystems: information from tannin analysis and C-13 CPMAS NMR. *Soil Biol Biochem* **32**: 779–792.
- McGuire, A.D., Melillo, J.M., Randerson, J.T., Parton, W.J., Heimann, M., Meier, R.A., *et al.* (2000) Modeling the effects of snowpack on heterotrophic respiration across northern temperate and high latitude regions: comparison



- with measurements of atmospheric carbon dioxide in high latitudes. *Biogeochemistry* **48**: 91–114.
- Mack, M.C., Schuur, E.A.G., Bret Harte, M.S., Shaver, G.R., and Chapin, F.S. (2004) Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. *Nature* **431**: 440–443.
- Martins, J.M.F., Jocteur Monrozier, L., Chalamet, A., and Bardin, R. (1997) Microbial response to repeated applications of low concentrations of Pentachlorophenol in an Alfisol under pasture. *Chemosphere* **35**: 1637–1650.
- Mohr, K.I., and Tebbe, C.C. (2006) Diversity and phylotype consistency of bacteria in the guts of three bee species (Apoidea) at an oilseed rape field. *Environ Microbiol* **8**: 258–272.
- Monson, R.K., Lipson, D.L., Burns, S.P., Turnipseed, A.A., Delany, A.C., Williams, M.W., and Schmidt, S.K. (2006) Winter forest soil respiration controlled by climate and microbial community composition. *Nature* **439**: 711–714.
- Mutabaruka, R., Hairiah, K., and Cadisch, G. (2007) Microbial degradation of hydrolysable and condensed tannin polyphenol-protein complexes in soils from different land-use histories. *Soil Biol Biochem* **39**: 1479–1492.
- Nemergut, D.R., Costello, E.K., Meyer, A.F., Pescador, M.Y., Weintraub, M.N., and Schmidt, S.K. (2005) Structure and function of alpine and arctic soil microbial communities. *Res Microbiol* **156**: 775–784.
- Nierop, K.G.J., and Verstraten, J.M. (2006) Fate of tannins in corsican pine litter. *J Chem Ecol* **32**: 2709–2719.
- Northup, R.R., Yu, Z., Dahlgren, R.A., and Vogt, K.A. (1995) Polyphenol control of nitrogen release from pine litter. *Nature* **377**: 227–229.
- Nylund, J.E., and Wallander, H. (1992) Ergosterol analysis as a means of quantifying mycorrhizal biomass. In *Methods in Microbiology*. Norris, J.R., Read, D.J., and Varna, A.K. (eds). London, UK: Academic press, pp. 77–88.
- O’Lear, H.A., and Seastedt, T.R. (1994) Landscape patterns of litter decomposition in alpine tundra. *Oecologia* **99**: 95–101.
- Preston, C.A. (1999) Condensed tannins of salal (*Gaultheria shallon* Pursh): a contributing factor to seedling, ‘growth-check’ on northern Vancouver island? In *Plant Polyphenols 2: Chemistry, Biology, Pharmacology, Ecology*. Gross, G.G., Hemingway, R.W., and Yoshida, T. (eds). New York, USA: Kluwer Academic/Plenum Publishers, pp. 825–841.
- RDevelopmentCoreTeam (2006) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Robertson, G.P., Coleman, D.C., Bledsoe, C.S., and Sollins, P. (1999) *Standard Soil Methods for Long-Term Ecological Research*. New York, USA: Oxford University Press.
- Schadt, C.W., Martin, A.P., Lipson, D.A., and Schmidt, S.K. (2003) Seasonal dynamics of previously unknown fungal lineages in tundra soils. *Science* **301**: 1359–1361.
- Schimel, J.P., and Mikan, C. (2005) Changing microbial substrate use in Arctic tundra soils through a freeze-thaw cycle. *Soil Biol Biochem* **37**: 1411.
- Schimel, J.P., Bilbrough, C., and Welker, J.M. (2004) Increased snow depth affects microbial activity and nitrogen mineralization in two Arctic tundra communities. *Soil Biol Biochem* **36**: 217.
- Schmidt, S.K., and Lipson, D.A. (2004) Microbial growth under the snow: implications for nutrient and allelochemical availability in temperate soils. *Plant Soil* **259**: 1–7.
- Schmidt, S.K., Lipson, D.A., and Raab, T.K. (2000) Effects of willows (*Salix brachycarpa*) on populations of salicylate-mineralizing microorganisms in alpine soils. *J Chem Ecol* **26**: 2049–2057.
- Sliwinski, M.K., and Goodman, R.M. (2004) Spatial heterogeneity of crenarchaeal assemblages within mesophilic soil ecosystems as revealed by PCR-single-stranded conformation polymorphism profiling. *Appl Environ Microbiol* **70**: 1811–1820.
- Steltzer, H., and Bowman, W.D. (2005) Litter N retention over winter for a low and a high phenolic species in the alpine tundra. *Plant Soil* **275**: 361–370.
- Tosca, C., and Labroue, L. (1986) Nitrogen-cycle in sub-alpine meadows of the central pyrenees – nitrogen mineralization in soil of sub-alpine meadows. *Acta Oecol-Oec Plant* **7**: 57–73.
- Waring, S.A., and Bremner, J.M. (1964) Ammonium production in soil under waterlogged conditions as an index of nitrogen availability. *Nature* **201**: 951–952.
- White, T.J., Bruns, T., Lee, S., and Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*. Innis, M.A., Gelfand, D.H., Shinsky, J.J., and White, T.J. (eds). San Diego, California, USA: Academic press, pp. 315–322.
- Zeller, V., Bahn, M., Aichner, M., and Tappeiner, U. (2000) Impact of land-use change on nitrogen mineralization in subalpine grasslands in the Southern Alps. *Biol Fertil Soils* **31**: 441–448.
- Zinger, L., Gury, J., Giraud, F., Krivobok, S., Gielly, L., Taberlet, P., and Geremia, R.A. (2007a) Improvements of polymerase chain reaction and capillary electrophoresis single-strand conformation polymorphism methods in microbial ecology: toward a high-throughput method for microbial diversity studies in soil. *Microb Ecol* **54**: 203–216.
- Zinger, L., Gury, J., Alibeu, O., Rioux, D., Gielly, L., Sage, L., et al. (2007b) CE-SSCP and CE-FLA, simple and high throughput alternatives for fungal diversity studies. *J Microbiol Methods* (in press).
- Zumstein, E., Moletta, R., and Godon, J.J. (2000) Examination of two years of community dynamics in an anaerobic bioreactor using fluorescence polymerase chain reaction (PCR) single-strand conformation polymorphism analysis. *Environ Microbiol* **2**: 69–78.



## Résumé

---

Les bactéries jouent un rôle clé dans les cycles biogéochimiques. Bien que l'effet du manteau neigeux en hiver dans la fonction et la composition des communautés bactériennes du sol ait été signalé, l'effet de la variation spatio-temporelle du manteau neigeux reste à étudier. Dans cette étude, nous avons caractérisé la dynamique spatio-temporelle des communautés bactériennes à partir de deux sites extrêmes selon un gradient de couvert neigeux. Pour cela, nous avons utilisé des approches moléculaires (SSCP et clonage / séquençage) et traditionnel (isolation par culture bactérienne).

Les résultats présentés montrent que l'ensemble de la diversité bactérienne, sa composition et sa structure phylogénétique sont fortement liés à la durée de la couverture de neige. En outre, ces effets sont détectables au cours de la saison de végétation des plantes. Les facteurs biotiques et abiotiques (i.e. la sénescence des plantes et le pH du sol) jouent un rôle essentiel conduisant au regroupement de certaines bactéries en clades spécifiques (*Acidobactéries*, *Actinobactéries*,  $\alpha$ -et  $\beta$ -*Proteobactéries*). Au cours de la saison de végétation des plantes, les clades de bactéries sont plus dispersés. La présente étude montre que, à un niveau taxonomique fin, la variation temporelle est un facteur plus important que la variation spatiale sur la diversité bactérienne. A un niveau taxonomique supérieur (i.e. sousphylum), la conclusion est inverse. Seule une petite fraction du total de la diversité bactérienne est cultivable et il se peut que certains groupes bactériens soient surreprésentés dans les plaques de culture. Cette étude apporte un nouvel éclairage sur le rôle de l'hiver et de la couverture neigeuse dans les distributions des communautés bactériennes. Cette étude peut-être utile pour prédire le comportement des bactéries dans les cycles des éléments nutritifs dans un contexte de réchauffement de la planète.

## Abstract

---

Bacteria play a key role in biogeochemical cycles. While the effect of winter snow cover in function and composition of soil bacterial communities has been reported, the effect of spatio-temporal variation of snow cover remains to be studied. In this study, we characterised the spatio-temporal dynamics of bacterial communities from two sites at the extremes of a snow cover gradient. We used molecular (SSCP and cloning/sequencing) and traditional (bacterial isolation by culture) approaches.

The presented results show that the overall bacterial diversity, composition and phylogenetic structure are strongly related to snow cover duration. Moreover, these effects are also detectable during the plant productive season. The biotic and abiotic factors (i.e. plant senescence and soil pH) play an essential role leading to the clustering of certain bacterial clades (*Acidobacteria*, *Actinobacteria*,  $\alpha$ - and  $\beta$ -*Proteobacteria*). During the plant productive season, the bacterial clades are overdispersed. The present study shows that, at a fine taxonomic level, the temporal variation is more important than the change over space. At higher taxonomic levels (i.e. sub-phylum), the space are more important than temporal variations. Only a minor fraction of the total bacterial diversity is cultivable, and may bacterial groups be overrepresented in culture plates. This study provides new insights in role of snow cover in bacterial communities' distribution and role of winter. This study may be useful in predicting of bacterial behaviour in nutrient cycle in a context of global warming.