Impact de la durée d'enneigement sur les cycles biogéochimiques dans les écosystèmes alpins

Florence Baptist

THÈSE

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

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

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Soutenue devant jury le 23 mai 2008

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Michael BAHN, Directeur de recherche, Innsbruck (Autriche)	Examinateur
Richard BLIGNY, Directeur de recherche, CEA, Grenoble	Examinateur
Serge AUBERT, Maître de conférence, UJF – Grenoble I	Directeur de thèse
Philippe CHOLER, Maître de conférence, UJF – Grenoble I	Co-directeur de thèse

Thèse préparée au sein du Laboratoire d'Écologie Alpine (LECA, UMR UJF-CNRS 5553)
et à la Station Alpine Joseph Fourier (UMS 2925)

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« Quel prix, dit encore Pierre Termier, peut se comparer à la joie de la découverte, et quelle récompense ne paraît misérable à coté de celle que la vérité elle-même décerne au chercheur qui l'a dévoilée ». Lorsqu'une parcelle de cette vérité est cachée dans le calice fermé d'une gentiane ou d'un céraiste des glaciers, n'est-elle pas plus attirante encore ? Et ainsi les plantes de Alpes remplissent doublement leur mission, qui est de nous élever au-dessus de nous-mêmes, vers les sommets : ceux que foulent nos pieds et ceux de la réflexion et de la recherche.

Favarger CL et Robert PA, Paris 1956 : Flore et végétation des alpes (Tome I)
Page 12 de l'édition 1995 « Delachaux et Niestlé »

Avant-propos

Ce manuscrit déposé à l'école doctorale Chimie et Science du vivant de l'Université de Grenoble (Université Joseph Fourier) a été rédigé sous la forme d'une thèse sur articles. Il contient une introduction en français suivi de six articles répartis dans deux chapitres. Une synthèse de l'ensemble des travaux réalisés est proposée dans la dernière partie du manuscrit. Les différentes techniques appliquées au cours de ces trois années de travail ne sont décrites que dans les articles par souci de clarté et de concision. Deux des articles ont été acceptés et publiés dans des revues internationales, un est en cours de révision et trois autres sont encore en préparation.

Mon allocation de recherche a été financée par le ministère de la recherche et de l'éducation pour une période de trois ans. L'encadrement scientifique et le support logistique ont été assurés par le Laboratoire d'Ecologie Alpine (UMR CNRS-UJF 5553), la Station Alpine Joseph Fourier (UMS 2925 CNRS-UJF), l'Université de Barcelone (S. Noguès), le Laboratoire Ecologie, Systématique et évolution de l'Université Paris XI (UMR 8079) et le projet MICROALP (ANR 2006-2009) porté par R. Geremia. Ce travail a été réalisé en grande partie au Laboratoire d'Ecologie Alpine de l'Université de Grenoble et à la Station Alpine Joseph Fourier, station d'altitude située au col du Lautaret dans les Hautes-Alpes (05).

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Grenoble, le 08/08/08

Liste des abréviations

Abbreviation	Description	Unité
A_{net}	Net photosynthetic fixation rate	Various unit
AG	*Alopecurus gerardi*	
ANPP	Aboveground Net Primary Production	g m^{-2} d^{-1}
BNPP	Belowground Net Primary Production	g m^{-2} d^{-1}
C	Carbon	-
CF	*Carex foetida*	-
CFP	Community Functional Parameter	-
CWM	Community Weigthed Mean	-
δ	Isotopic signature	-
DO	*Dryas octopetala*	
ENSO	El Niño Southern Oscillation	-
$\gamma^{13}C$ / $\gamma^{15}N$	Labelling-derived ^{13}C or ^{15}N content	µg^{13}C/^{15}N g^{-1} DW
$\gamma^{13}C_R$	Labelling-derived ^{13}C or ^{15}N content in CO_2 respired	µg^{13}C g^{-1} h^{-1}
$\gamma^{13}C_M$ / $\gamma^{15}N_M$	Labelling-derived ^{13}C or ^{15}N mass	µg or mg^{13}C/^{15}N
GPP	Gross Primary Production	g m^{-2} d^{-1}
k	Decay constant	yr^{-1}
KM	*Kobresia myosuroides*	-
LAI	Leaf Area Index	m^2 m^{-2}
LDMC	Lead Dry Matter Content	%
LNC	Leaf Nitrogen Content	%
N	Nitrogen	-
NAO	North Atlantic Oscillation	-
NBP	Net Biome Production	g m^{-2} d^{-1}
NEP	Net Ecosystem Production	g m^{-2} d^{-1}
NPP	Net Primary Production	g m^{-2} d^{-1}
OM	Organic Matter	-
p	Proportion of new carbon inherited from ^{13}C labelling in CO_2 respired	%
PA	*Poa alpina*	
Q_{10}	Factor by which respiration rate increases in response to a 10°C increase	-
R ou ER	Darkness respiration	Various unit
R:S	Root :Shoot ratio	-
RNC	Root Nitrogen Content	%
RTD	Root Tissue Density	g cm^{-3}
SH	*Salix herbacea*	

sANPP	Specific Aboveground Net Primary Production	$g\ g^{-1}\ d^{-1}$
SLA	Specific Leaf Area	$cm^2\ g^{-1}$
SOM	Soil Organic Matter	%
SRL	Specific Root Length	$m\ g^{-1}$

Nota : Les abréviations utilisées dans l'article 1A ne sont pas intégrées dans ce tableau.

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Introduction

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Introduction

A. Contexte et problématique de l'étude

Au sein des écosystèmes froids, la sévérité du climat limite la dégradation de la matière organique entraînant l'accumulation dans le compartiment souterrain de quantités considérables de carbone (Post et al. 1982, Boskheim et al. 1999, Körner 1999). En dépit d'une faible densité de population dans ces régions froides (montagnes, régions arctiques), la menace d'un réchauffement climatique a récemment suscité un regain d'intérêt pour ces systèmes. En effet, l'augmentation de la température, en activant les processus de dégradation de la matière organique, conduit actuellement à la libération de ce carbone dans l'atmosphère sous forme de dioxyde de carbone et ce gaz à effet de serre pourrait fortement accentuer le réchauffement climatique (Oechel et al. 1993). Face à ce constat, les scientifiques tentent depuis une dizaine d'année de préciser les conséquences de cette boucle de rétroaction positive sur la dynamique globale du climat. Différents types de modèle, tels que les modèles de circulation générale ou les modèles atmosphériques inverses ont été appliqués dans ce but, mais les prédictions demeurent largement incertaines (IPCC 2007). En effet, l'enneigement, la présence du permafrost dans les systèmes arctiques (sol gelé en permanence) et la méconnaissance des processus microbiens compliquent fortement l'analyse des flux entre la biosphère des milieux froids et l'atmosphère. De fait, le comportement puits *vs*. sources de ces écosystèmes[1] est difficile à évaluer (McGuire et al. 2006).

Chaque année la neige recouvre plus de la moitié de l'hémisphère nord. Dans les systèmes alpins (voir définition plus loin) ou arctiques où la durée de la saison hivernale atteint 200 à 300 jours par an, cette saisonnalité régule les flux de carbone. Par exemple, la présence de la neige affecte la fixation du carbone en modulant la longueur de la saison de végétation ou la respiration hétérotrophique (respiration des microorganismes du sol) *via* son action sur les conditions édapho-climatiques (climat du sol). **Ainsi, au même titre que la température, l'enneigement conditionne les échanges du carbone entre le sol et l'atmosphère dans les écosystèmes froids. En particulier, un changement du régime des précipitations neigeuses pourrait modifier l'équilibre source-puits de ces systèmes.**

C'est dans ce contexte que les scientifiques se sont récemment intéressés à l'impact de

[1] Bilan annuel positif - accumulation de carbone - *vs.* négatif - libération de carbone

l'enneigement sur les flux biogéochimiques (i.e. flux de matière au sein d'un écosystème, d'un biome ou à l'échelle du globe). Les écosystèmes arctiques ont notamment fait l'objet de nombreuses expérimentations, à des échelles diverses, mettant en évidence le rôle fondamental de la neige ainsi que les forçages exercés par celle-ci sur certains processus, tel que la minéralisation du carbone ou de l'azote (Walker et al. 1999, Welker et al. 2000, Schimel et al. 2004). Etonnamment, très peu d'études ont été menées au sein des systèmes alpins. Or en dépit d'une forte convergence floristique et écologique, les conditions hydriques ainsi que le bilan radiatif diffèrent fortement et limitent l'extrapolation des résultats obtenus en arctique (Körner 1999, Walker et al. 1999). Par ailleurs, les scénarios climatiques prévoient une diminution de l'enneigement hivernal aux latitudes moyennes contrairement aux systèmes arctiques où un renforcement des précipitations est attendu (Serreze et al. 2000, Dye and Tucker 2003, Beniston 2005). Ainsi, au regard des modifications des régimes d'enneigement prédits par les différents modèles climatiques, une étude visant à expliciter les interactions entre neige et processus dans les systèmes alpins s'avère nécessaire afin d'évaluer le devenir des flux et des stocks de carbone contenus dans ces sols.

1) Flux et stocks de carbone

Au sein des systèmes continentaux, les stocks de carbone organique sont principalement localisés dans les sols (1500 Pg[2]), la végétation ne séquestrant que 500 Pg de carbone. Cette accumulation de carbone est régie par les flux entrants *via* la photosynthèse et les flux sortants *via* la respiration autotrophique et hétérotrophique (Fig. 1). Les microorganismes du sol, en dégradant la litière, libèrent rapidement une partie du carbone dans l'atmosphère sous forme de CO_2, mais, selon les écosystèmes, une proportion plus ou moins importante de ce carbone demeure sous forme de matière organique récalcitrante. D'autres flux de carbone liés à l'herbivorie ou au lessivage contribuent à modifier l'équilibre source-puits des écosystèmes, mais leur importance est moindre (Fig. 1). Dans cette étude nous nous sommes donc principalement focalisé sur la production primaire nette et brute du compartiment aérien (GPP et ANPP, respectivement Gross Primary Production et Aboveground Net Primary Productivity) et la minéralisation du carbone par les microorganismes (Fig. 1, R).

Ces flux sont régulés par des facteurs abiotiques et biotiques. La fixation du carbone

[2] $1500\text{Pg} = 1500\times10^{15}\text{gC}$

est dépendante des conditions climatiques, telles que la température de l'air, la lumière, l'eau ou encore la durée de la saison de végétation. Elle dépend également des propriétés des canopées (Leaf Area Index ou LAI, distribution des angles des feuilles dans la canopée), puisque celles-ci déterminent l'efficacité d'interception de la lumière (Anten 2005). De façon similaire, la décomposition des litières et la respiration des sols sont régulées par la température et l'humidité mais aussi par la quantité et la qualité des litières (Joffre and Agren 2001).

Ainsi, les forçages climatiques agissent à court terme en modifiant la réponse physiologique des organismes vivants *via* la modification des conditions édaphoclimatiques (i.e. action sur la cinétique enzymatique, effets directs) et à long terme à travers un changement de composition et de structure des communautés végétales (effets indirects).

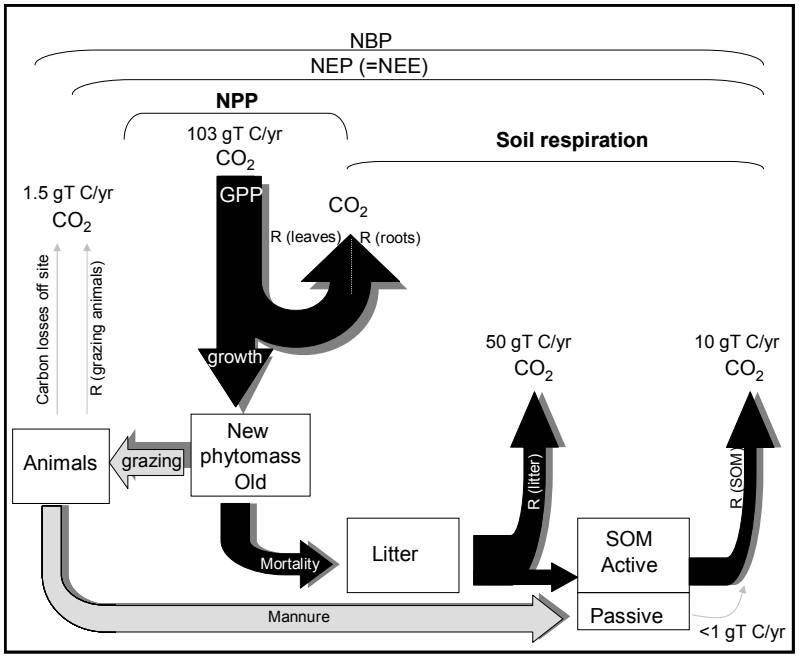


Fig. 1 Schéma conceptuel du cycle du carbone dans les systèmes continentaux. NEP : Net ecosystem production, NBP : Net Biome Production, NPP : Net primary Production, GPP : Gross primary Production, R : Respiration, SOM : Soil Organic Matter. Les chiffres donnés correspondent aux flux estimés moyens pour l'ensemble des biomes continentaux.

Avant de préciser le cadre conceptuel dans lequel s'est inscrite cette étude ainsi que ses objectifs, il est nécessaire de revenir rapidement sur les propriétés physico-chimiques de la neige ainsi que sur les outils permettant d'établir des prédictions quant à l'évolution des précipitations neigeuses dans les décennies à venir.

2) Enneigement et changement climatique

La neige, caractérisée par un albédo élevé (0.85) et une faible conductivité thermique (0.02 – 1 W m^{-1} K^{-1}) modifie fortement le bilan radiatif des systèmes en limitant l'absorption

du rayonnement solaire et donc le réchauffement du sol (Groisman and Davies 2000). Ce pouvoir isolant est à l'origine d'un gradient thermique entre le sol relativement chaud et la surface réfléchissante froide, et permet de maintenir la température des sols aux alentours de 0°C quel que soit le régime thermique atmosphérique (Fig. 2). Il semblerait qu'une épaisseur de 15 à 30 cm suffise à l'établissement de ce gradient et, de ce fait, de nombreux organismes sont protégés des températures extrêmes durant l'hiver. De même, une activité respiratoire peut se maintenir sous le couvert neigeux, sous réserve de l'effet d'autres facteurs limitants (substrat par exemple) (Groisman and Davies 2000).

D'autre part, la productivité des écosystèmes montagnards est fortement reliée à l'enneigement *via* la longueur de la saison de végétation, mais également *via* l'apport en minéraux (azote en particulier) et en eau (Tranter and Jones 2000). Walker et al. (1995) suggèrent que cet apport nutritionnel est critique pour la croissance de la végétation, notamment lorsque la saison de végétation est fortement réduite. D'un point de vue physique, l'eau de fonte permet pour un niveau de nutriments similaire, d'augmenter la diffusion des minéraux dans le sol et de stimuler l'absorption racinaire (Chapin et al. 1988). En modifiant la fertilité et la longueur de la saison de végétation, les durées d'enneigement affectent profondément la distribution des communautés végétales et sélectionnent des espèces à croissance lente dans les zones déneigées et infertiles et des espèces à croissance rapide dans les zones plus enneigées et plus fertiles (Choler 2005).

Ainsi, en modifiant le régime thermique et hydrique des sols, ainsi que la durée de la saison de végétation et la fertilité des sols, la neige affecte les flux biogéochimiques au sein des écosystèmes montagnards (Tranter and Jones 2000).

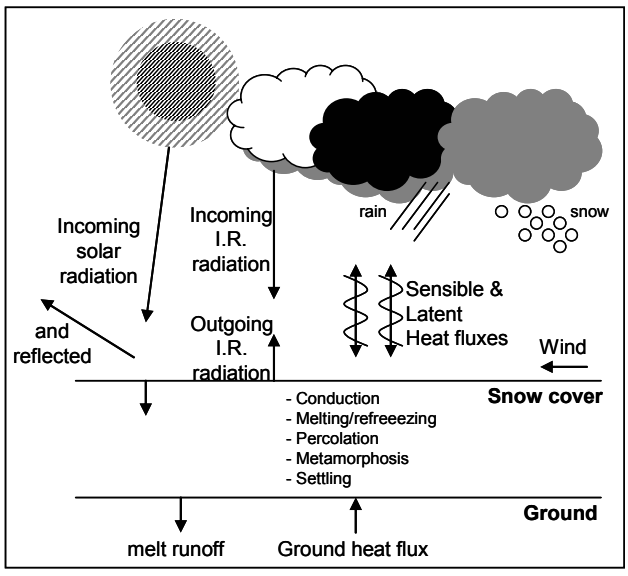


Fig. 2 Schéma conceptuel des flux de masse et d'énergie contrôlant le budget énergétique, la structure et les propriétés de la couverture neigeuse. I.R. : Infra-Rouge. Source : Pomeroy & Brun (2000).

A une échelle globale, d'autres études ont montré que la cryosphère, la biosphère et l'atmosphère sont intimement reliées et qu'une modification des précipitations neigeuses peut affecter l'ensemble des écosystèmes terrestres. Par exemple, des précipitations neigeuses accrues sur le plateau Tibétain ont pour effet de retarder et de limiter l'impact de la mousson indienne (Barnett et al. 1989). De même, une réduction des surfaces enneigées au niveau du pôle nord limiterait l'émission des rayonnements infrarouges vers l'atmosphère, entraînant une hausse des températures à la surface du globe terrestre (Chapin et al. 2005). L'enneigement joue donc un rôle crucial tant à l'échelle locale ou régionale qu'à l'échelle globale.

Actuellement, de nombreuses études tentent d'évaluer quelle sera l'évolution des régimes de précipitations en lien avec le réchauffement climatique. Les scénarios avancés sont beaucoup moins clairs que ceux qui concernent la température et un rapide retour sur les outils permettant d'élaborer ces prédictions s'avère utile. Les indices d'anomalie de pression atmosphérique, tel que le NAO (North Atlantic Oscillation) dans l'hémisphère nord ou le SO (Southern Oscillation) dans l'hémisphère sud, sont de bons indicateurs des régimes de précipitations. Le NAO est une différence de pression normalisée entre les Açores et l'Islande, deux centres d'action primaire de la circulation atmosphérique de l'hémisphère nord. Le SO représente la composante atmosphérique de l' « El Niño Southern Oscillation » (ENSO), c'est-à-dire la différence de pression normalisée entre Tahiti et Darwin (en Australie). Une valeur positive du NAO signifie que les vents de surface tendent à être orientés Nord-Est en direction du Groenland et du Canada et elle est associée à des anomalies de température négatives. Les vents orientés Sud-Ouest apportent, quant à eux, douceur et humidité dans le nord de l'Europe et en Scandinavie. Ces vents sont généralement associés à des faibles précipitations et des températures relativement douces, notamment de la fin de l'automne jusqu'au début du printemps (Serreze et al. 2000). En dépit d'une très forte variabilité interannuelle, le NAO est dans une phase positive depuis les années 1970, et de façon plus marquée depuis les années 80 (Hurrell 1995). A l'échelle régionale, Beniston (2005 pour une revue) détecte les mêmes tendances depuis les années 80, et relie de façon très satisfaisante cet indice à une diminution du régime des précipitations neigeuses dans les Alpes Suisses (Fig. 3-4).

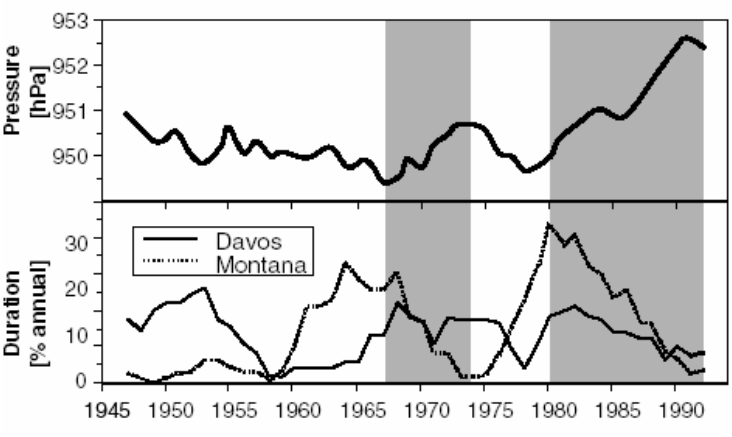


Fig. 3 Série temporelle sur 50 ans de la pression atmosphérique hivernale moyenne et de la durée de la saison hivernale dans deux sites alpins (dans les Alpes suisses à Davos et dans les montagnes du Montana aux USA), pour une profondeur moyenne de neige de 1m. Source : Beniston (2005).

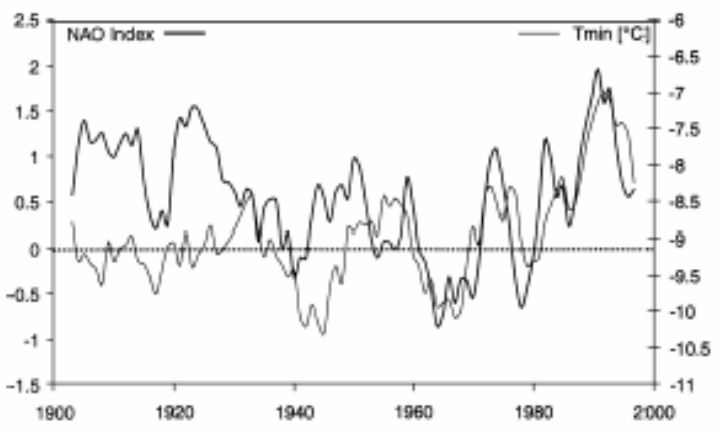


Fig. 4 Série temporelle centennale de l'évolution de l'index NAO et des températures minimales (décembre, janvier, et février) à Säntis (2500m au dessus du niveau de la mer, Suisse). Source : Beniston (2005)

Par ailleurs, les images satellites disponibles depuis 1972 permettent de suivre assez précisément l'évolution des surfaces enneigées à l'échelle continentale. Depuis le début des années 80, le NOAA (National Oceanic and Atmospheric Administration) a observé une diminution de l'enneigement d'environ 10% que ce soit sur le continent Nord-Américain, ou en Eurasie : ceci étant en relation étroite avec une fonte plus précoce au printemps et une modification du ratio pluie : neige. De même, la hausse de température, particulièrement marquée dans les hautes et moyennes latitudes, a déjà été reliée à une modification de la quantité et de la saisonnalité des précipitations neigeuses (Fig. 5, Noguès-Bravo et al. 2007).

Ainsi les systèmes montagnards situés à des latitudes moyennes subissent d'ores et déjà une diminution des précipitations neigeuses qui se caractérise par un retard de l'enneigement en automne et une fonte des neiges de plus en plus précoce (Messerli and Ives 1999). Dans les Alpes, ces effets sont largement visibles à moyenne altitude (1300m) et des tendances sont déjà observables à plus haute altitude (Laternser and Schneebeli 2003). Dans le futur, l'augmentation de la température devrait accélérer ce phénomène.

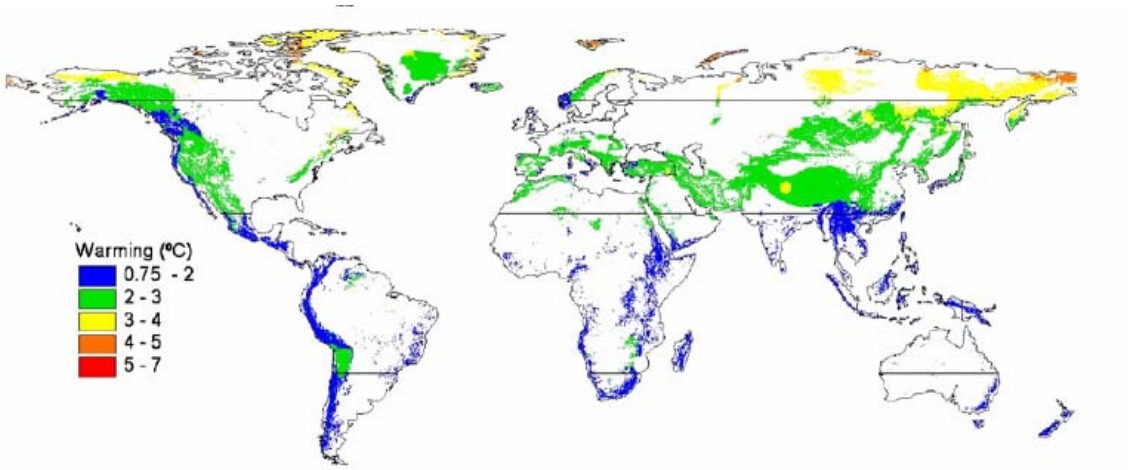


Fig. 5 Réchauffement prédit pour 2055 selon le scénario économique B1 (IPCC 2007). Un gradient latitudinal du réchauffement climatique est observable des systèmes arctiques et boréaux vers les systèmes tropicaux. Source : Noguès-Bravo et al. (2007).

3) Enneigement et cycle du carbone dans les systèmes alpins

Spécificité des systèmes de haute montagne

L'étymologie du mot alpin est « alp », terme Kimri (dialecte Gaulois) qui signifie roches escarpées. Dès l'an -100 av. JC, ce terme était utilisé couramment pour définir la chaîne montagneuse au nord de l'Italie. Par la suite, l'utilisation de ce mot s'est généralisée à l'ensemble des chaînes de montagne du monde initiant de nombreux débats sur la définition même « d'alpin » ou « chaîne alpine ». En effet, les termes tels que "Pyrénéisme", "Andinisme" ou "Himalayisme" voire, en Pologne, "Tatrisme" sont couramment utilisés dans le monde des sports de montagne. Dans le domaine des sciences, les géologues définissent le système alpin « comme toutes les chaînes de montagne formées durant le cycle orogénique alpin et s'étendant sur l'ensemble des ères secondaires et tertiaires, pendant les 225 derniers millions d'années » (Favarger and Robert 1995). En biogéographie, l'adjectif alpin s'applique couramment à l'étage asylvatique (zone située au delà de la limite potentielle des forêts, fig. 6a) de toutes les hautes montagnes du globe. Le déterminisme principal de l'étagement altitudinal de la végétation est la température (Körner 1999). C'est à Alexander Von Humboldt (1769-1859) que l'on attribue la compréhension des mécanismes à l'origine de la répartition des végétaux le long de ce gradient. En observant les flancs du mont Chimborazo en Equateur, il avait remarqué l'étagement de la végétation et s'était le premier interrogé sur la cause de cette répartition (Fig 6b).

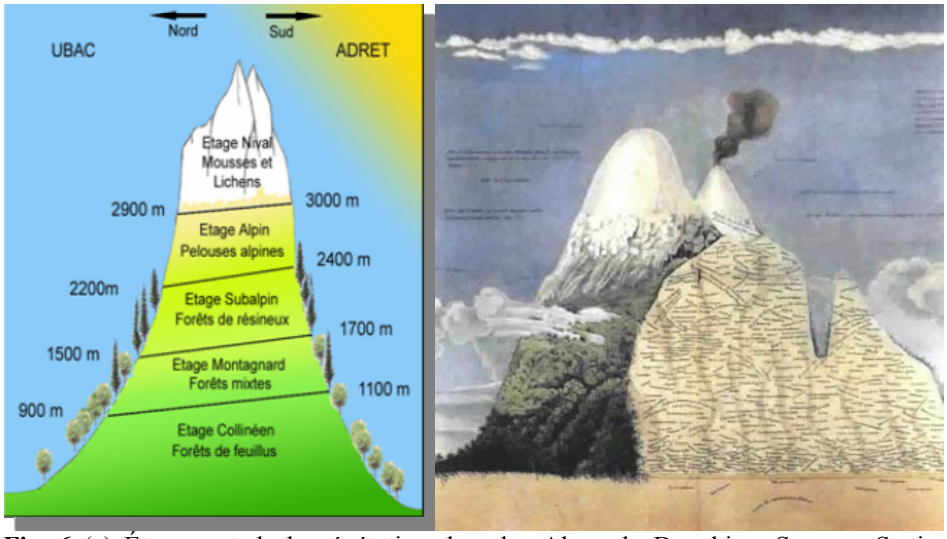


Fig. 6 (a) Étagement de la végétation dans les Alpes du Dauphine. Source : Station Alpine Joseph Fourier. (b) Etagement de la végétation sur les flancs du Chimborazo (Humbold).

À cette époque, l'explication la plus évidente reposait sur les variations climatiques en fonction de l'altitude. Humboldt avait également étudié la question des zones circumterrestres de végétation en fonction des latitudes (toundra, taïga, forêt, etc.), et abouti à la même conclusion : « l'ensemble des facteurs physiques propres aux différents climats semblait être la cause essentielle de la répartition des végétaux à la surface de la Terre » (Humboldt Bonpland 1807[3]). Une forte convergence évolutive des communautés végétales se succédant le long du gradient altitudinal (on parlera d'étagement) et latitudinal (zonalité) est aujourd'hui largement admise. L'étage alpin n'est donc pas uniquement représenté dans les systèmes de haute altitude mais aussi de hautes latitudes (Ozenda 2002). De façon générale on parlera donc de toundras alpines et arctiques.

Les montagnes sont réparties dans le monde entier et représentent plus de 20% de la surface du globe (Beniston and Fox 1996). D'après Körner (1999), les toundras alpines et arctiques couvriraient une surface de 4.5 millions de km^2 entre les parallèles 70°N et 60°S, soit 3% des terres émergées. La durée de la saison hivernale est de façon générale assez

[3] Voyage aux régions équinoxiales du Nouveau Continent, fait en 1799, 1800, 1801, 1803 et 1804 par Alexandre de Humboldt et Aimé Bonpland (30 volumes), rédigé par A. de H., Paris, 1807 et années suivantes.
Les 14 premiers volumes sont consacrés à la botanique, le XV et XVI forment: Vues de cordillères et monuments des peuples anciens de l'Amérique (réédité dans la Collection "Memoria Americana", dirigée par Charles Minguet et Jean-Paul Duviols), le XVIIe, l'Atlas géographique et physique, le XVIIIe, L'Examen critique de l'histoire et de la géographie du Nouveau Monde, le XIXe, L'Atlas géographique et Physique, le XXe Géographie des plantes équinoxiales, le XXI et XXIIeme, Recueil d'observations astronomiques, le XXIII et XXIV, Recueil d'observations de zoologie et d'anatomie comparée, les XXV et XXVI, L'essai politique sur le royaume de la Nouvelle Espagne, le XXVII, Essai sur la géographie des plantes (réédité dans "Memoria Americana"), les volumes XXVIII à XXX, La relation historique du voyage aux régions équinoxiales du Nouveau Continent, dont la dernière partie a été édité à part sous le titre de Essai politique sur l'île de Cuba.

longue (>200 jours) mais varie fortement le long du gradient latitudinal et altitudinal (Beniston and Fox 1996). Malgré leur superficie relativement faible, les montagnes affectent le système climatique dans sa globalité. En tant qu'obstacle physique à la circulation atmosphérique, elles perturbent l'écoulement de l'air et sont considérée comme l'un des facteurs de la cyclogénèse dans les latitudes moyennes (Beniston and Fox 1996). Les montagnes, sources d'un grand nombre de réseaux hydrographiques de la planète et berceaux de nombreux glaciers, constituent également un élément essentiel du cycle de l'eau. Par ailleurs, la sévérité du climat a fortement contraint la productivité de ces systèmes ainsi que la décomposition de la matière organique et le déséquilibre entre ces flux a conduit à l'accumulation de carbone dans les sols. Les quelques études tentant d'évaluer les stocks de carbone séquestrés dans les sols de montagne rapportent des valeurs aussi élevées que dans les systèmes arctiques (Tableau 1) (Becker and Bugmann 2001).

Formation végétale	Localisation	Stock de carbone (kg C m^{-2})	Références
Pelouse alpine		9.3	
Prairie de fauche		12.3	(Dobremez and Eynard-Machet 1997)
Prairie pâturée	Plan de Tueda (Alpes, France)	13.6	
Forêt résineuse		18.2	
Landes		18.2	
Prairies de montagne	Chine centrale	32.4	(Zhou et al. 2003)
Toundra alpine	Plateau Haibei (Chine)	18.2	(Kato et al. 2006)
Toundra mésophile	Moyenne dans le monde	10.9	(Zinke et al. 1998)
Toundra hygrophile		20.7	
Sols de montagne	France	>10.0	(Arrouays et al. 2002)

Tableau 1 Stocks de carbone dans les sols arctiques et de montagne

Au-delà du bilan radiatif, les toundras alpines se distinguent des toundras arctiques par leur orographie. En effet le relief accidenté en montagne est à l'origine d'une forte hétérogénéité spatiale qui gouverne à la fois le bilan radiatif et la distribution de la neige tant à l'échelle régionale que locale (Billings 1973, Körner 1999, Walker et al. 2000). La macrotopographie, c'est-à-dire la variation du relief sur une échelle d'ordre kilométrique, est à l'origine de fortes variations en termes de rayonnement solaire. L'opposition typique entre les faces sud (adret) et les faces nord (ubac) illustre très bien ce phénomène (Fig. 7).

Fig. 7 Opposition entre l'adret et l'ubac. Montagne de Chaillol, Région du Lautaret (2058 m). (Photo : S ; Aubert).

La mésotopographie (variation de la topographie sur des distances inférieures à 100m, Billings 1973) est à l'origine de différences importantes d'enneigement entre des zones convexes et des zones concaves (Fig. 8 et 12). Il s'agit là de l'opposition ancienne entre un alpin dit « thermique » et un alpin dit « nival » (Aubert et al. 1965)[4].

La distribution des communautés végétales est fortement liée à la longueur de la saison de végétation (Walker et al. 1993) et de fait, « gradient de mésotopographie » et « gradient d'enneigement » sont en général utilisés en synonymie. De nombreuses études ont analysé la distribution de la diversité végétale le long de ce gradient (Billings and Bliss 1959, Friedel 1961, Billings 1973, Flock 1978, Bell and Bliss 1979, Walker et al. 1993, Choler 2005). Tous ces auteurs soulignent un important « turn-over » des espèces sur des distances très faibles formant une mosaïque complexe le long du gradient d'enneigement (Fig. 9). Les limites de ces communautés végétales et les dates moyennes de fonte des neiges sont fortement corrélées (Fig. 9).

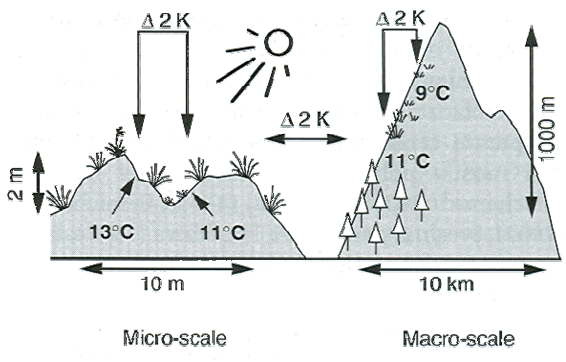
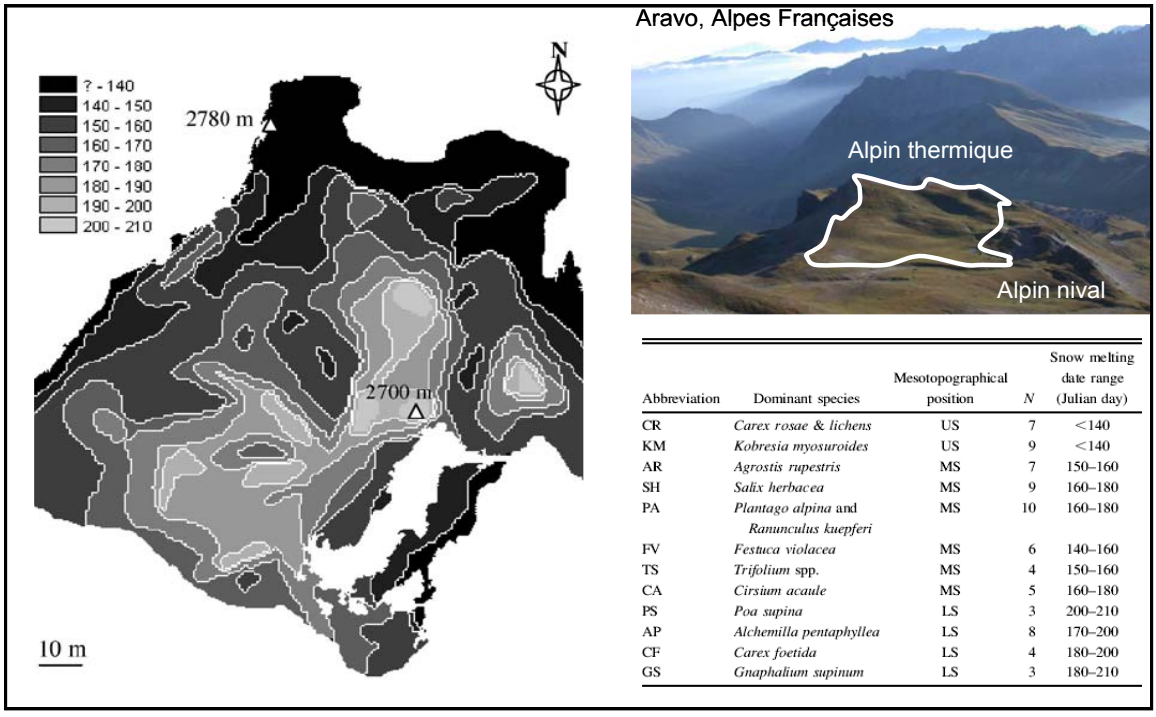


Fig. 8 Les contrastes thermiques liés à la méso- et microtopographie sont aussi marqués que ceux qui sont observés le long du gradient altitudinal. Source : Körner (1999).

[4] Les expressions « Early snowmelt locations » et « late snowmelt locations » sont les appellations correspondantes dans les articles de cette thèse.



Abbreviation	Dominant species	Mesotopographical position	*N*	Snow melting date range (Julian day)
CR	*Carex rosae* & *lichens*	US	7	<140
KM	*Kobresia myosuroides*	US	9	<140
AR	*Agrostis rupestris*	MS	7	150–160
SH	*Salix herbacea*	MS	9	160–180
PA	*Plantago alpina* and *Ranunculus kuepferi*	MS	10	160–180
FV	*Festuca violacea*	MS	6	140–160
TS	*Trifolium* spp.	MS	4	150–160
CA	*Cirsium acaule*	MS	5	160–180
PS	*Poa supina*	LS	3	200–210
AP	*Alchemilla pentaphyllea*	LS	8	170–200
CF	*Carex foetida*	LS	4	180–200
GS	*Gnaphalium supinum*	LS	3	180–210

Fig. 9 Cartographie des dates de déneigement (jour julien) à Aravo prés du col du Galibier dans les Alpes Françaises (panel gauche) et description des espèces dominantes des groupements végétaux associés à ces dates de déneigement (panel droit). Source : Choler (2005).

Cette forte hétérogénéité spatiale a limité la mise en œuvre de certaines techniques d'étude et ce n'est que dernièrement que des travaux, menés dans des systèmes moins accidentés comme le plateau Tibétain, ont tenté d'évaluer l'état source-puits des systèmes alpins (Gu et al. 2005, Kato et al. 2005, Kato et al. 2006, Zhao et al. 2006). En effet, la plupart des modèles de circulation générale possèdent à l'échelle du globe une résolution spatiale limitée et ne peuvent prendre en compte les variations topographiques de l'ordre d'une centaine de mètres ou même du km. De même, jusqu'à récemment, les techniques d'Eddy covariance[5] n'étaient pas adaptées pour mesurer les échanges de carbone dans ces systèmes car une partie trop importante des flux de CO_2 s'écoule le long des pentes et n'était donc pas détectée par les tours à flux (mais voir Hammerle et al. 2007).

Par ailleurs, les études réalisées dans l'arctique visant à évaluer l'impact de la neige sur la minéralisation du carbone et de l'azote se sont basées sur des expérimentations relativement courtes (Brooks and Williams 1999, Groffman et al. 2001a, 2001b, Monson et al. 2006). Dans ce contexte, seuls les effets directs étaient examinés *i.e.* modification des conditions édaphoclimatiques. Or, même si une modification des conditions

[5] Eddy covariance : méthode statistique permettant d'évaluer les flux de carbone sur de larges surfaces. Le système d'Eddy Covariance comprend un anémomètre ultrasonique et un IRGA (Infra Red Gaz Analyzer) fixé en haut d'une tour à flux.

édaphoclimatiques agit de façon transitoire sur le fonctionnement des écosystèmes, les processus biogéochimiques sont largement régulés par les rétrocontrôles et les interactions indirectes exercées par la végétation et les populations microbiennes (Norby and Luo 2004, Rustad 2006). En effet, l'enneigement en contrôlant la longueur de la saison de végétation et la fertilité influence indirectement la composition spécifique des communautés végétales. Un changement de diversité pourrait ainsi affecter le recyclage du carbone indépendamment des conséquences liées aux variations des conditions édaphoclimatiques.

Ainsi dans les systèmes enneigés, les modes de régulation des flux de carbone n'ont pas été identifiés et, de façon générale, la hiérarchisation des effets directs et indirects exercés par la neige et leur contrôle de la productivité des communautés végétales et de la décomposition des litières dans les systèmes froids est encore largement débattue (Hobbie et al. 2000, Grogan and Jonasson 2006). **Quelques travaux ont abordé cette problématique dans les systèmes arctiques (i.e. travaux sur la respiration hétérotrophique, Elberling et al. 2004, Grogan and Jonasson 2006, Elberling 2007) mais, à notre connaissance, aucune étude n'a tenté d'évaluer les contrôles directs et indirects exercés par la neige sur le recyclage du carbone dans les systèmes de montagne et notamment les systèmes alpins (Edwards et al. 2007).**

Récemment, des études ont mis en évidence l'intérêt d'utiliser les caractéristiques fonctionnelles de la végétation afin de prédire le fonctionnement d'un écosystème (Lavorel & Garnier, 2002). La description du cadre dans lequel s'est inscrite cette étude fait l'objet de la partie suivante.

4) Les traits fonctionnels comme outils de changement d'échelle

Des études récentes en écologie des communautés ont mis en évidence la pertinence d'agréger les espèces végétales selon des critères fonctionnels plutôt que selon des critères phytosocioécologiques (Chapin et al. 1996, Woodward and Cramer 1996, Lavorel et al. 1997). Ces groupes fonctionnels se définissent comme des ensembles d'espèces ayant une réponse similaire aux pressions environnementales et/ou ayant un effet similaire sur le fonctionnement des écosystèmes, tel que la productivité primaire ou la décomposition (Lavorel and Garnier 2002). Ils se définissent sur la base de traits d'histoire de vie partagés (i.e. traits fonctionnels). Ces derniers correspondent aux caractéristiques morphologiques, éco-physiologiques, biochimiques ou reproductives d'un organe ou de la plante entière (Chapin et al. 1996, Hodgson et al. 1999, Weiher et al. 1999). Les traits dits « hard » sont des

traits dont la mesure requiert une haute technicité (Tableau 2). Ce sont par exemple des traits en relation directe avec le métabolisme des plantes, comme la conductance stomatique ou le taux d'assimilation d'azote mais leur mesure à grande échelle est difficilement envisageable. Les traits « soft » sont plus faciles à acquérir et sont corrélés avec les traits « hard » (Reich et al. 1999). On définit par ailleurs les traits de réponse comme des adaptations morphologiques ou écophysiologiques des plantes en réponses aux contraintes environnementales (Fig. 10).

La composition fonctionnelle (ou diversité fonctionnelle), c'est-à-dire l'identité, l'abondance et la variation des traits fonctionnels au sein d'une communauté végétale, permet de s'affranchir des distinctions taxonomiques et facilite les études comparatives entre systèmes. Récemment des études de grande ampleur ont mis en évidence un schéma universel de gestion des ressources (Wright et al. 2004). Il est ainsi possible de situer les espèces végétales le long d'un gradient d'exploitation *vs.* conservation des nutriments : les espèces végétales situées dans les systèmes fertiles sont caractérisées par un taux de croissance élevé et un renouvellement rapide des tissus (Specific Leaf Area ou SLA élévé, Leaf Nitrogen Content ou LNC élevé), à la différences de celles situées dans des habitats infertiles.

La théorie proposée par Grime ("the biomass ratio hypothesis", 1998) stipule que le fonctionnement de l'écosystème peut être prédit à partir des valeurs de traits des espèces dominantes pondérées par leur abondance relative dans la communauté végétale. Selon la nouvelle terminologie proposée par Violle et al. (2007), cet index se traduit formellement par la relation suivante :

$$CFP = \sum_{k=1}^{n} A_k \cdot T_k$$

La valeur du trait T dans la communauté (CFP, Community Functional Parameter) est égale à la somme de la valeur du trait pondérée par l'abondance des k espèces de la communauté. Terminologie française : trait agrégé.

Garnier et al. (2004), Garnier et al.(2007) et Quétier et al. (2007) ont montré que les traits foliaires agrégés (SLA, LDMC et LNC) permettaient de prédire de façon satisfaisante l'ANPP (et/ou ANPP spécifique), la production de litière ou encore les taux de décomposition mesurés *in situ*. De façon similaire, la décomposabilité des litières semble être fortement corrélée au taux de matière sèche des feuilles (LDMC agrégé), ainsi qu'au ratio N/[lignine] (Kazakou 2006). Enfin, cette méthodologie a également pu être appliquée dans l'objectif de prédire l'impact d'un changement d'utilisation des terres sur certains services rendus par les écosystèmes tels que la production de biomasse ou encore le maintien de la fertilité des sols

(Diaz et al. 2007).

Fonction	« Hard traits »	« Soft Traits »
Interception de la lumière	Modélisation	Hauteur végétative LAI (Leaf Area Index)
Efficience de la photosynthèse	Mesure de l'assimilation (IRGA)[6]	SLA (Specific Leaf Area) LDMC (Leaf Dry Matter Content) LNC (Leaf Nitrogen Content)
Efficacité d'utilisation de l'eau	Conductance stomatique (IRGA)	Durée de vie des feuilles
Taux de croissance	RGR (Relative growth rate)	Traits foliaires (SLA, LDMC, LNC) Traits racinaires : SRL : Specific Root Length RNC : Root Nitrogen Content
Allocation entre compartiment aérien et souterrain	Marquage $^{13/14}C$ pour suivre le devenir du carbone dans la plante et estimer les flux entre compartiments	Masse racinaire/masse aérienne (Root:Shoot ratio)
Absorption des nutriments	Marquage court ^{15}N	Diamètre des racines, proportion de racine fines, SRL
Résorption des nutriments	Marquage long ^{15}N	Traits foliaires (SLA, LNC de la litière)
Exudation	Marquage $^{13/14}C$	SRL
Respiration foliaire et racinaire	Mesure de la respiration (IRGA)	LNC et RNC
Décomposabilité	Mesure de perte de masse Respiration (IRGA)	LDMC, C/N, N/lignine N/Polyphénol RNC RDT (Root Tissue Density)

Tableau 2 Exemples de traits « softs » et « hards » en lien avec les processus écosystémiques (C,N). Sources : Reich et al. (1999), Weiher et al.(1999), Quested et al (2007). et Kazakou (2006). Voir Roumet et al. (2006) pour une revue sur les traits racinaires.

Ainsi, à la fois révélateurs des pressions environnementales et acteurs potentiels au sein de l'écosystème, les traits fonctionnels sont actuellement considérés comme des outils pertinents pour prédire la performance des espèces dans leur environnement et leur impact sur le fonctionnement des écosystèmes (Fig. 10).

C'est dans ce cadre méthodologique que nous nous proposons d'appréhender les contrôles indirects exercés par l'enneigement (*i.e.* impact liés à la diversité fonctionnelle végétale) sur le cycle du carbone des écosystèmes alpins.

[6] IRGA : Infra Red Gaz Analyser. Appareil permettant la mesure des flux d'eau et de CO_2

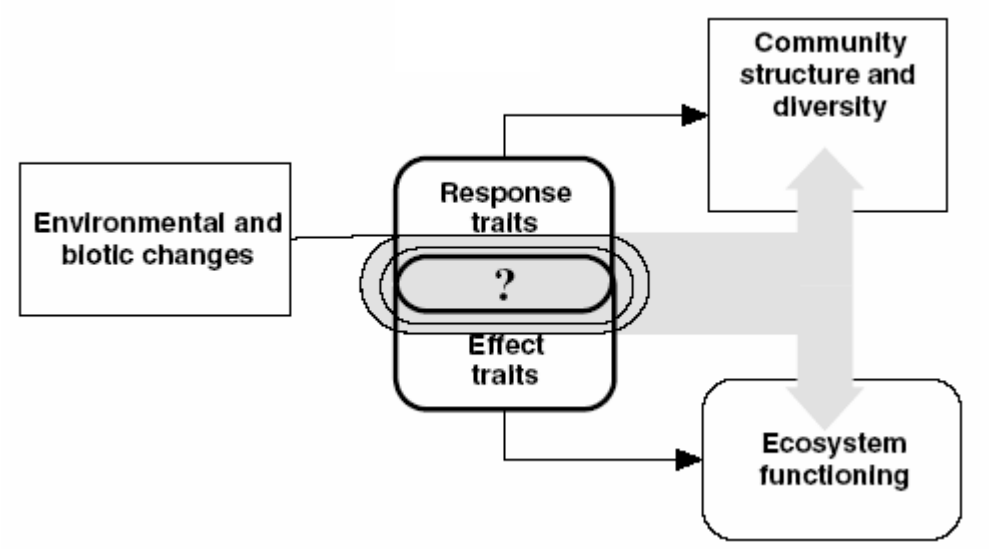


Fig. 10 Diagramme conceptuel permettant l'articulation des effets des conditions environnementales sur les processus écosystémiques via l'impact sur les communautés végétales. Source : Lavorel and Garnier (2002).

5) Interaction entre les cycles de l'azote et du carbone : Influence sur les processus écosystémiques ?

Le recyclage du carbone dans un système ne peut être découplé des processus de minéralisation de l'azote. Les corrélations mises en évidence entre traits fonctionnels et vitesse de recyclage du carbone sont généralement sous-tendues par la disponibilité des ressources (Chapin et al. 1993a). Le compromis fonctionnel associé à la gestion des nutriments et du carbone est en effet directement associé au taux de minéralisation azotée. Une faible disponibilité en azote limite la production de biomasse et contribue à la création de tissus récalcitrants (C/N élevé, [lignine] élevée) affectant en retour le taux de minéralisation de la matière organique (C, N). Cette boucle de rétroaction positive demeure tant que les plantes dépendent des organismes saprophytes dans l'acquisition de l'azote minéral. Elle illustre dans quelle mesure les processus écosystémiques, modulés par la diversité fonctionnelle des communautés végétales, agissent en retour sur la structuration de ces mêmes communautés. Cependant de nombreuses études ont montré que la plupart des plantes alpines sont capables d'assimiler, avec ou sans l'aide des mycorhizes, de l'azote sous forme organique, court-circuitant ainsi la boucle microbienne (Chapin et al. 1993b, Raab et al. 1999, Chapman et al. 2006). Dans ce cas, la production végétale serait en partie découplée de la minéralisation de l'azote par les microorganismes. La mise en évidence de ce nouveau

paradigme a conduit à différentes théories illustrées sur la fig. 11 (Schimel and Bennett 2004, Chapman et al. 2006) : dans les systèmes fertiles, les plantes assimilent majoritairement l'azote sous forme minérale et de fait dépendent des organismes saprophytes pour leur croissance; dans les systèmes infertiles, les espèces végétales assimilent l'azote organique soit par prélèvement direct soit par le biais des mycorhizes et ne dépendent donc plus de l'activité minéralisatrice des microorganismes. Ces hypothèses remettent ainsi en question l'existence, dans certains systèmes, d'un goulot d'étranglement associé à l'activité des microorganismes, pour prédire les processus écosystémiques.

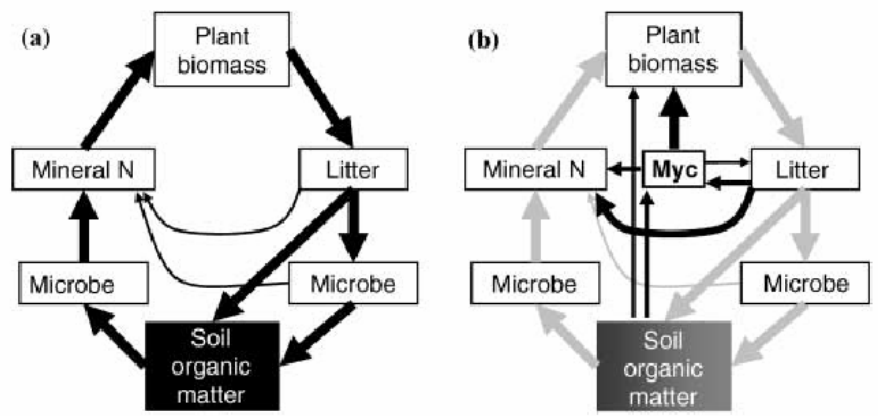


Fig. 11 Deux représentations du cycle de l'azote. Dans chaque cas, la flèche représente l'intensité du flux. (a) Théorie classique selon Knops et al. (2002), (b) Dans le cas d'une l'assimilation directe d'azote organique. Myc, mycorhize. Source : Chapman et al. (2006)

Les contrôles exercés par les plantes et les microorganismes sur la minéralisation de l'azote et de façon générale sur les cycles biogéochimiques sont encore mal connus. Ces résultats montrent la nécessité d'examiner l'intrication des cycles de l'azote et du carbone afin de prédire la réponse des écosystèmes aux changements globaux.

Fig. 12 Illustration de l'impact de la topographie sur les patrons d'enneigement. Le 18/05/07 à Combe Roche Noire près de col du Galibier dans les Alpes Françaises (2550 m). En premier plan, *Kobresia myosuroides*, plante dominante des sites thermiques. Source: F. Baptist.

B. Objectifs et hypothèses de travail

L'objectif de ce travail est d'évaluer les contrôles directs et indirects exercés par la neige sur la fixation et la minéralisation du carbone dans les systèmes alpins.

Dans les systèmes alpins, la longueur de la saison de végétation et l'épaisseur de la couche de neige varient principalement en fonction de la topographie. La succession des communautés végétales et les variations de la diversité fonctionnelle le long du gradient de mésotopographie sont intimement liées aux patrons d'enneigement et résultent d'un long processus de sélection (Choler, 2005). De fait, nous avons considéré ce gradient[7] comme un système modèle pour examiner l'influence respective des effets directs et indirects de la neige. Nous nous sommes principalement focalisé sur les communautés situées aux deux pôles de ce gradient : les communautés nivales ou de combe à neige et les communautés thermiques ou de crête (Tableau 3). Les sites d'étude font l'objet d'une présentation détaillée dans la dernière partie de cette introduction.

Les deux principales étapes du cycle du carbone ont été étudiées, c'est-à-dire :

- La fixation du carbone (*via* la mesure du GPP et de l'ANPP) et allocation du carbone,
- La minéralisation du carbone (décomposition des litières et respiration hétérotrophique).

Afin de dissocier la contribution relative des facteurs directs et indirects, différentes approches et outils ont été privilégiés et appliqués à des échelles variées (Tableau 3). Ces différentes approches nous ont ensuite permis d'évaluer, selon les différents scénarios climatiques envisagés, les conséquences d'une réduction de l'enneigement sur les processus biogéochimiques. Ceci sera développé dans la dernière partie de ce manuscrit (synthèse).

La figure 13 présente de façon schématique les objectifs et les hypothèses de ce travail. De façon générale nous faisons l'hypothèse que les effets indirects de l'enneigement supplantent les effets directs et à court terme sur le cycle du carbone dans les systèmes

[7] Dans la suite du manuscrit, nous utiliserons uniquement le terme « gradient d'enneigement » ou snowmelt gradient.

alpins. Plus précisément, nos hypothèses sont les suivantes :

- Les conditions stationelles dans les systèmes longuement enneigés favorisent les espèces à croissance rapide - réponse adaptive à la limitation de la durée de la saison favorable à la croissance. La productivité instantanée sera donc plus élevée dans ces milieux comparativement aux systèmes thermiques.
- Dans les systèmes nivaux la présence d'espèces dont les tissus sont riches en azote devrait activer la vitesse de décomposition des litières. L'hypothèse inverse est testée au sein des systèmes thermiques.
- Le régime thermique hivernal plus favorable dans les systèmes nivaux devrait cependant contribuer à activer la vitesse de décomposition.

Ces hypothèses, à première vue qualitatives, ont été abordées dans le but de donner des réponses quantitatives soit par le biais de mesures empiriques soit grâce à la modélisation.

Objet d'étude / Echelle géographique	**Outils**	**Type d'expérimentation**	**Articles**
Communautés végétales le long du gradient d'enneigement. Espèces dominantes des communautés nivales et thermiques : *Carex foetida* et *Kobresia myosuroides*	Outils d'écophysiologie Modélisation	*In situ* Conditions semi-controlées	Production primaire Article 1A
Communautés nivales. Espèces dominantes : *Carex foetida*, *Poa alpina*, *Alopecurus gerardi*	Outils d'écophysiologie Analyse chimique	Conditions semi-contrôlées	Production primaire Article 1B
Espèces dominantes des communautés nivales et thermiques : *Carex foetida* et *Kobresia myosuroides*	Outils d'écophysiologie Modélisation	Conditions contrôlées	Allocation Article 1C
Espèces dominantes des communautés nivales (*Carex foetida*, *Salix herbacea*, *Alopecurus gerardi*) et thermiques (*Kobresia myosuroides* et *Dryas octopetala*)	Taux de décomposition Analyse chimique	*In situ*	Décomposition Article 2A
Sols des systèmes nivaux et thermiques	Mesures de flux (C) Respiration potentielle	*In situ* Conditions contrôlées	Respiration Article 2B
Sols des systèmes thermiques	Mesure de flux (C, N) Diversité microbienne Activité enzymatique	Conditions contrôlées	Respiration Article 2C

Tableau 3 Description des expérimentations réalisées dans ce travail : objet et niveaux d'étude, outils et types d'expérimentation.

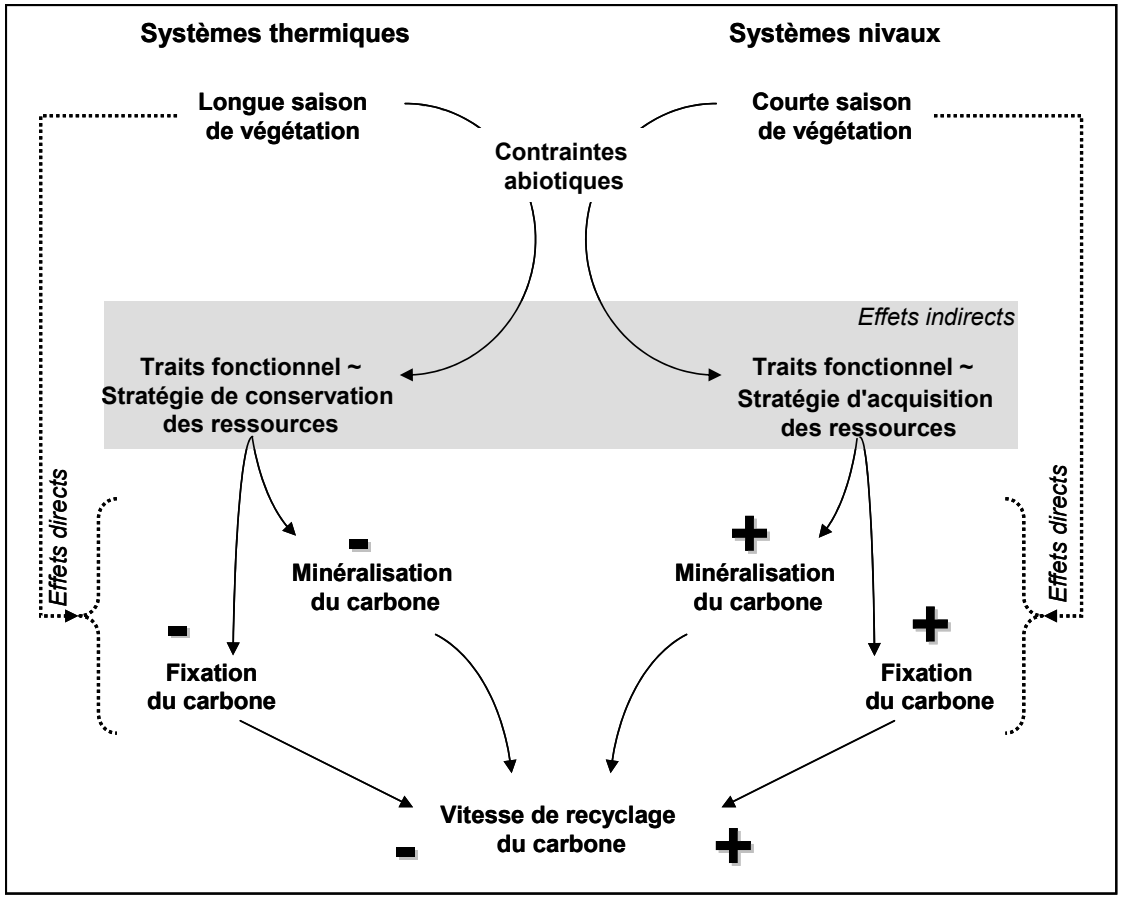


Fig. 13 Hypothèse de travail visant à explorer dans quelles mesures la diversité fonctionnelle végétale (effet indirect) et les conditions édapho-climatiques (effet direct) affectent la productivité, l'allocation du carbone, la décomposition des litières et la respiration hétérotrophique des sols dans les systèmes alpins nivaux et thermiques.

C. Organisation du document

Le premier chapitre de ce manuscrit traite de l'impact de l'enneigement sur les traits fonctionnels foliaires, la production primaire brute et l'allocation du carbone au sein des systèmes nivaux et thermiques.

Notre démarche a tout d'abord consisté à développer un modèle de photosynthèse a l'échelle de la canopée (« bulk canopy photosynthesis model »), à paramétrer ce modèle avec les mesures de traits agrégés puis à examiner l'importance relative des propriétés fonctionnelles des couverts végétaux *vs.* durée de la saison de végétation sur le gain carboné brut des pelouses alpines. Nous avons fait l'hypothèse que les propriétés fonctionnelles des canopées *via* la maximisation de l'interception de la lumière et de la fixation du carbone des systèmes nivaux permettent de compenser la courte durée de la saison de végétation. Une analyse de sensibilité a par ailleurs permis de déterminer l'effet relatif d'un allongement de la durée de la saison de végétation *vs.* modification des traits fonctionnels sur la production primaire de ces communautés végétales (Article 1A).

D'autre part, afin de vérifier d'un point de vue expérimental l'impact de l'allongement de la saison de végétation dans les systèmes nivaux, nous avons mis en place à la Station Alpine Joseph Fourier une expérimentation visant à étudier l'effet d'une réduction de l'enneigement sur la productivité des systèmes de combe à neige. Nous avons fait l'hypothèse qu'une saison de végétation allongée entraînerait une augmentation de la biomasse aérienne produite (Article 1B).

Enfin, la dernière partie de ce chapitre vise à préciser les patrons d'allocation du carbone en relation avec les stratégies d'acquisition de l'azote des deux graminoïdes *Carex foetida* et *Kobresia myosuroides* qui dominent dans les systèmes nivaux et thermiques respectivement. Nous avons fait l'hypothèse que l'espèce caractérisée par une croissance lente (*K. myosuroides*) allouait une quantité importante du carbone nouvellement fixé vers le compartiment souterrain (Article 1C).

Ces trois études font l'objet des articles suivants :

- **Article 1A : Baptist, F.**, and Ph. Choler. 2008. A simulation on the importance of growing season length and canopy functional properties on the seasonal Gross Primary Production of temperate alpine meadows. *Annals of Botany* **101:549-559**.

- **Article 1B : Baptist, F.**, Flahaut C., Streb P., and Ph. Choler. Decreased aboveground primary productivity of alpine tundra in response to earlier snowmelt. En prep. pour *Oecologia*.

- **Article 1C : Baptist, F.,** Tcherkez, G., Aubert, S., Pontailler, J.Y., Choler, Ph. and S. Noguès. ^{13}C and ^{15}N allocations of two alpine species from contrasting habitats reflect their different growth strategies. En prep. pour *New Phytologist.*

Dans le deuxième chapitre, nous nous sommes appliqués à préciser l'impact de l'enneigement sur la décomposition des litières (phase initiale de la dégradation de la SOM) et la respiration hétérotrophique des sols (phase finale de la dégradation de la SOM).

Dans le premier cas, nous avons mis en place un dispositif croisé visant à préciser l'impact respectif des facteurs stationnels et de la qualité des litières sur la décomposition (Article 2A). Cinq espèces dont trois dominantes dans les systèmes nivaux et deux dans les systèmes thermiques ont été choisies afin de mesurer leur taux de décomposition aux deux pôles du gradient d'enneigement sur un pas de temps annuel. Nous faisons l'hypothèse que les processus de minéralisation (décomposition, respiration) sont en premier lieu déterminés par la qualité des litières. Les contrôles indirects exercés par l'enneigement seraient donc plus marqués que les contrôles directs *via* la modification des conditions édapho-climatiques.

Nous avons par ailleurs mesuré la respiration hétérotrophique *in situ* et en conditions contrôlées sur des carottes de sol prélevées en été lors du pic de biomasse et en automne juste avant les premières chutes de neige. Les sols ont été prélevés aux deux pôles du gradient d'enneigement. Nous avons fait l'hypothèse que la qualité de la matière organique, évaluée à partir des valeurs de respiration basale, influence fortement la variabilité spatiale de la respiration hétérotrophique. Par ailleurs, la présence de neige en hiver dans les systèmes nivaux contribuerait à modifier de façon significative le bilan annuel des flux de CO_2 en comparaison avec les systèmes thermiques (Article 2B).

La dernière étude est une ouverture vers l'écologie microbienne. En conditions contrôlées (mésocosmes), nous avons examiné conjointement la dynamique de la diversité microbienne et la minéralisation du carbone et de l'azote suite à un amendement d'extrait tannique (à partir de litière de *Dryas octopetala*). Par ailleurs les sols ont été soumis à différentes contraintes thermiques mimant les conditions hivernales des systèmes nivaux et thermiques. Nous faisons l'hypothèse que les champignons, principaux acteurs de la dégradation l'hiver, sont spécifiquement sélectionnés par les faibles températures hivernales afin de dégrader les composés récalcitrants présents dans les systèmes thermiques (Article 2C).

Ces trois études font l'objet des articles suivants :

- **Article 2A : Baptist, F.**, Yoccoz, G., and Ph. Choler. Snow cover exerts control over decomposition in alpine tundra along a snowmelt gradient. En prep. pour *Plant and soil*..

- **Article 2B : Baptist, F.**, Flahaut, C., and Ph. Choler. Soil respiration in alpine tundra: impacts of seasonal snow cover and soil carbon content. Soumis à *Global Change Biology*.

- **Article 2C : Baptist, F**., Zinger, L., Clement, J.C., Gallet, C., Guillemin, R., Martins, J.M.F., Sage L., Shahnavaz, B. and Ph. Choler. Tannins impacts on microbial diversity and functioning of alpine soils: a multidisciplinary approach. *Environmental microbiology* **10:799-809**

La dernière partie propose une synthèse de l'ensemble des résultats présentés dans les chapitres I et II. Des résultats non publiés à ce jour ont également été intégrés dans cette section.

D. Présentation du site d'étude

Le col du Lautaret (2058 m, 45°20'N, 6°24'E) marque la limite entre la vallée de la Romanche et la vallée de la Guisane. Plus qu'une limite géographique c'est également une limite climatique puisque au-delà de ce col (vers l'Est) les précipitations s'amoindrissent et la région devient beaucoup plus sèche (Fig. 14).

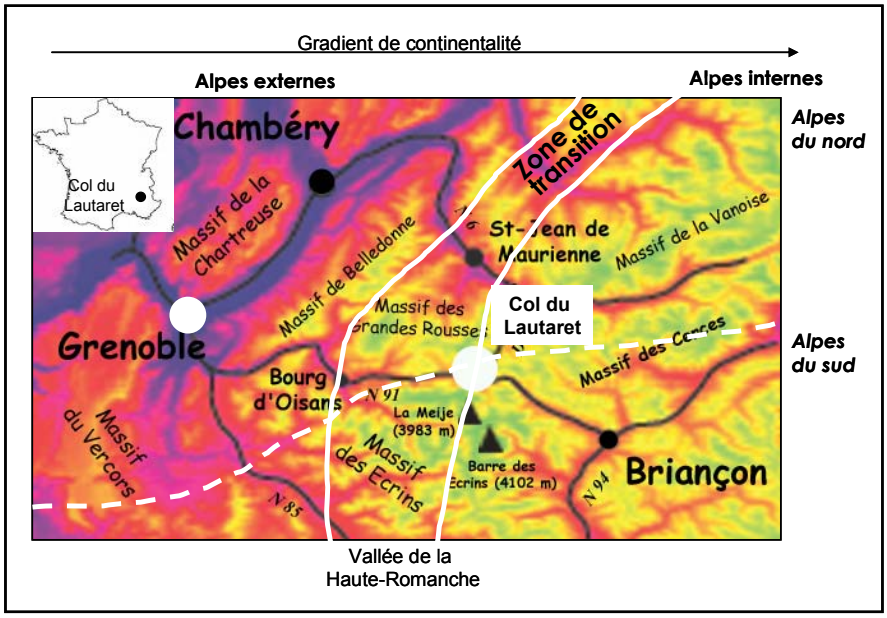


Fig 14 Localisation du site d'étude dans les Alpes Françaises occidentales.

Les sites d'étude se situent au nord du col du Lautaret en direction du col du Galibier (2646 m) (Tableau 4). La limite inférieure de l'alpin se situe aux alentours de 2200-2300 m, et probablement 2400 m sur les adrets francs. Tous les sites d'études sont situés au-delà de 2500 m. L'étage nival lui se situe aux alentours de 2800-3000 m (tableau 4).

Article correspondant	Localisation des sites	Altitude	Cordonnées géographiques	Mesures / prélèvements
Article 1A	Aravo	~ 2750 m	45°04'N, 6°23'E	Mesures
Article 1B	Col Agnel	2744 m	44°41'N, 6°58'E	Prélèvements
Article 1C	Site B, et Col Agnel	2520 m	Site B : 45.05'N, 6.37'E	Prélèvements
		2744 m	Col Agnel : 44°41'N, 6°58'E	
Article 2A	Sites A, B et C	~2550 m	Site A : 45°04N, 6°24E	Mesures
		2520 m	Site B : 45.05'N, 6.37'E	
		~2550 m	Site C : 45.05'N, 6.38'E	
Article 2B	Sites B, C et D	2520 m	Site B : 45.05'N, 6.37'E	Mesures
		~2550 m	Site C :45.05'N, 6.38'E	Prélèvements dans
		~2556 m	Site D : 45.05'N, 6.37'E	le site B
Article 2C	Site B	2520 m	Site B : 45.05'N, 6.37'E	Prélèvements

Tableau 4 Localisation des sites d'étude ou de prélèvement dans les différents articles présentés dans ce manuscrit de thèse.

L'étude développée dans l'article 1A porte sur l'ensemble du gradient d'enneigement et a été réalisé à Aravo (cf carte topographique, Fig. 15, « Aravo »). L'étude 2B a été effectuée à partir de monolithes prélevés dans des systèmes nivaux au col Agnel à la frontière avec l'Italie (2744 m, 44°41'N, 6°58'E). L'étude de décomposition des litières a été menée dans les sites A, B et D (dalle calcaire et vallon de combe roche noire, Article 2A) alors que les mesures de respiration hétérotrophique *in situ* ont été réalisées dans les sites B, C et D. Les carottes de sol ont été prélevées dans le site B, de même en ce qui concerne l'étude développée dans l'article 2C. Au sein de chaque site (A-D) sont représentées les communautés nivales et thermiques (combe à neige / crête).

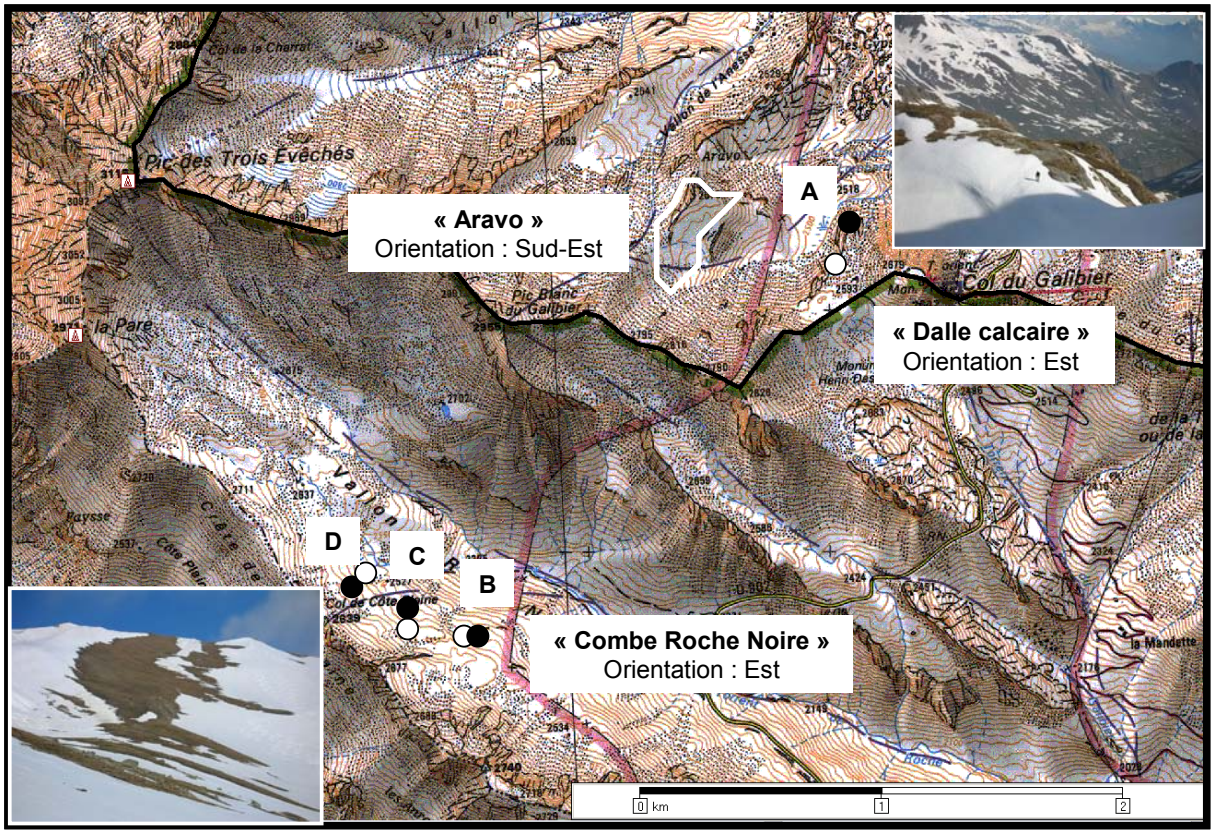


Fig. 15 Localisation des zones et sites d'étude dans la région du col du Galibier (2646m). En blanc, les sites nivaux, en noir, les sites thermiques. Carte IGN Top 25 3435 ET au 25/1000. Photo : F. Baptist.

1) Les conditions climatiques

Une partie de ces sites d'étude a été équipée, dès 1999, de capteurs thermiques enterrés dans le sol (enregistrement horaire en continu). A partir de l'année 2004, des capteurs d'humidité du sol, de température et d'humidité de l'air ont également été ajoutés (enregistrement horaire). Les différentes données climatiques obtenues nous permettent ainsi

d'obtenir une image assez précise des conditions édaphoclimatiques régnant aux deux pôles du gradient d'enneigement.

1.2 La durée de la saison de végétation

La neige, par son pouvoir isolant, limite fortement l'amplitude thermique journalière du sol. L'analyse des données thermiques du sol nous permet de préciser la durée de l'enneigement et donc la durée de la saison de végétation. En général, les systèmes nivaux se déneigent courant juin. La longueur de saison de végétation dure en moyenne 132 ± 4 jours. On observe un différentiel d'environs 40 jours entre les systèmes nivaux et thermiques, ces derniers se déneigeant plutôt fin mai (169 ± 6 jours). Cependant, il n'est pas rare que la neige soit absente une partie de l'hiver. Les températures, dans ce cas largement négatives, empêchent la reprise de la croissance.

Comme le souligne Körner (1999), certaines plantes alpines conservent des tissus chlorophylliens durant toute la saison hivernale. C'est notamment le cas des genres *Geum* et *Potentilla*. Cependant, au-delà de 30 cm de neige, l'activité photosynthétique ne peut se maintenir car la transmittance est quasiment nulle. Cette propriété permet aux plantes d'initier leur croissance très tôt en début de saison de végétation lorsque la couche de neige n'a pas encore totalement disparu optimisant ainsi les chances de compléter leur cycle de vie dans le temps imparti. Néanmoins, dans le cadre de cette étude, nous avons considéré que la date de déneigement marque le début de la croissance végétale dans les systèmes nivaux. Dans le cas des systèmes thermiques, nous avons pris en compte la date à laquelle la température moyenne journalière du sol était supérieure à 0°C.

1.3 Le régime thermique et hydrique

En montagne, la température diminue d'environs 0.6°C lorsque l'on s'élève de 100 m. Il est ainsi possible de prédire la température de l'air moyenne à partir de stations météorologiques située plus bas dans la vallée (Fig. 16). On observe respectivement un maxima d'environs 20°C l'été et un minima proche de -8/-10°C durant l'hiver au col du Lautaret (2058 m). Chaque année, environ 1000 mm d'eau tombent dont la majeure partie sous forme de neige.

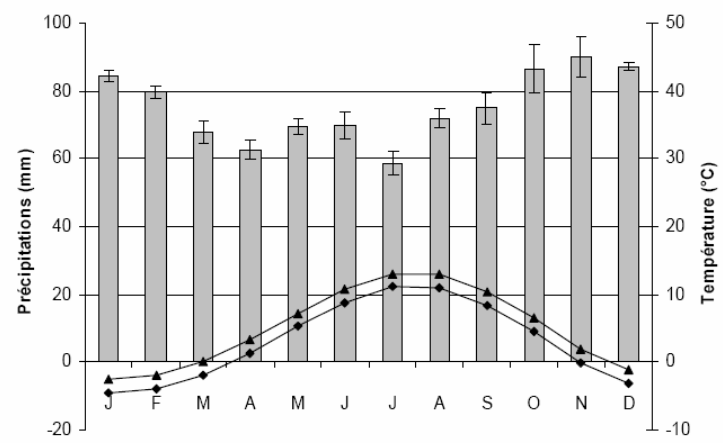


Fig. 16 Diagramme ombro-thermique estimé pour Villar d'Arène (1670 m – courbe du haut) et le col du Lautaret (2058 m courbe du bas). Précipitations moyennes mensuelles (± se) des stations météo France de Besse en Oisans (1525 m), St Christophe en Oisans (1570 m), Valloire (1460 m) et Monêtier les Bains (1490 m). Estimation des températures moyennes mensuelles à partir des enregistrements de la station Météo France de Besse en Oisans. Source : Météo France.

Cependant, en fonction de l'orientation, du vent, des effets de masque, le microclimat diffère grandement des températures moyennées à l'échelle d'un massif. A partir des données climatiques enregistrées depuis 1999 au sein de chaque site, nous avons estimé les températures moyennes des sols dans les systèmes nivaux et thermiques (Fig. 17). Durant l'hiver, le déficit en neige dans les systèmes thermiques est à l'origine d'une forte amplitude des températures, ainsi que d'une fréquence importance de températures extrêmes inférieures. à -10°. Au contraire, le régime thermique de systèmes nivaux est stable et avoisine la valeur moyenne de 0°C.

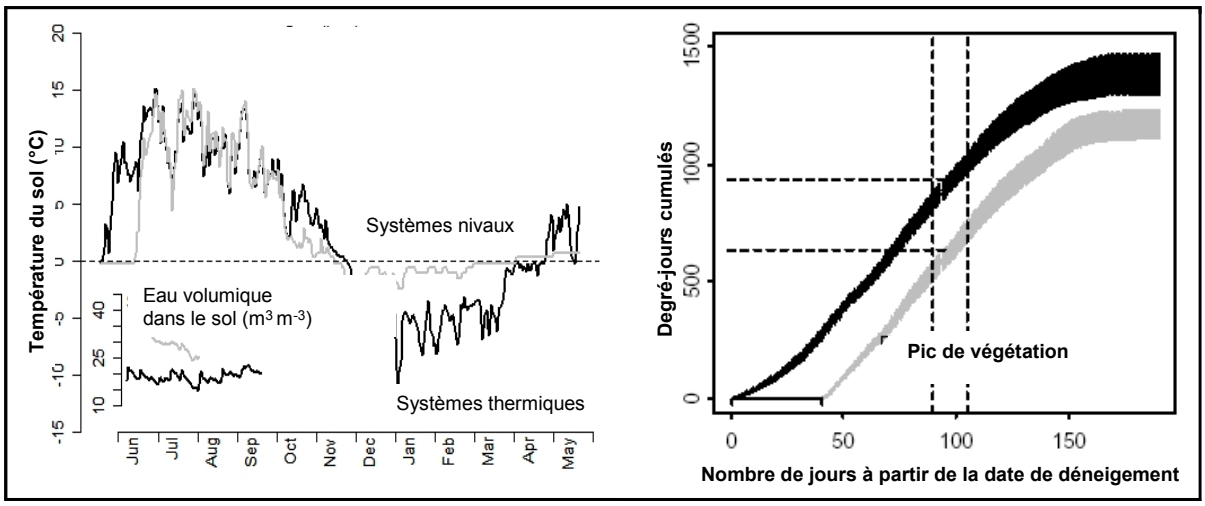


Fig. 17 Température et humidité du sol (encart) dans les systèmes nivaux (gris) et thermiques (noir) au cours de l'année. A droite : somme des degrés jours dans les systèmes nivaux (gris) et thermiques (noir) depuis le déneigement.

Durant l'été, les différences de température du sol entre les systèmes nivaux et thermiques sont très faibles et dépendent du vent et de l'exposition. Cependant, la quantification des degré-jours montre un décalage important d'environs 280 °Cj lié à la fonte précoce de la neige dans les systèmes thermiques (Fig. 17).

En dépit d'une couverture neigeuse très variable le long du gradient, les variations en termes de disponibilité en eau (unité massique) sont peu marquées contrairement aux études de Walker et al.(1993) et de Fisk et al. (1998) (Tableau 4, appendice article 2B). De façon générale, les systèmes thermiques sont légèrement plus secs que les systèmes nivaux, cette différence s'accentuant à la fonte des neiges due à l'engorgement dans les systèmes nivaux.

	Systèmes thermiques	**Systèmes nivaux**
Humidité du sol (% massique)	42.0 (3.0)	37.0 (2.0)
Capacité au champ (%)	57.8 (0.2)	59.6 (3.7)

Tableau 4 Régime hydrique des sols des systèmes thermiques et nivaux moyenné sur les sites A, B et D et sur deux ans. Moyenne ± se.

2) La nature du substrat

La nature du substrat varie entre la zone située près du col Galibier (Dalle calcaire) et les zones correspondantes de Combe Roche Noire (sites B, C et D) et Aravo.

Le panorama du col du Lautaret (2058 m) et du col du Galibier (2646 m) se distingue par la présence de différentes couches sédimentaires autochtones ainsi que de deux nappes de charriages, les nappes Briançonnaise et Sub-briançonnaise. Sur la photo (Fig. 18), on distingue en premier lieu des calcaires marneux jurassiques (Trias inférieur = lias) sur lesquelles reposent directement le tertiaire avec tout d'abord des calcaires nummulitiques puis des grès stratifiés (ou flysh). La Station Alpine Joseph Fourier, que l'on peut apercevoir sur la photo repose sur ce substrat. Par ailleurs, les zones d'étude « Combe Roche Noire » et « Aravo » sont situées sur la nappe sub-briançonnaise formée au crétacé supérieur (calcaire schisteux et grès nummulitiques). Enfin, la nappe Briançonnaise d'age triasique qui se superpose à cette couche tertiaire est constituée notamment de quartzites triasiques et de calcaires dolomitiques qui font les reliefs du massif des Cerces (Grand Galibier). Les sols des sites situés à la « Dalle calcaire » reposent sur ce substrat.

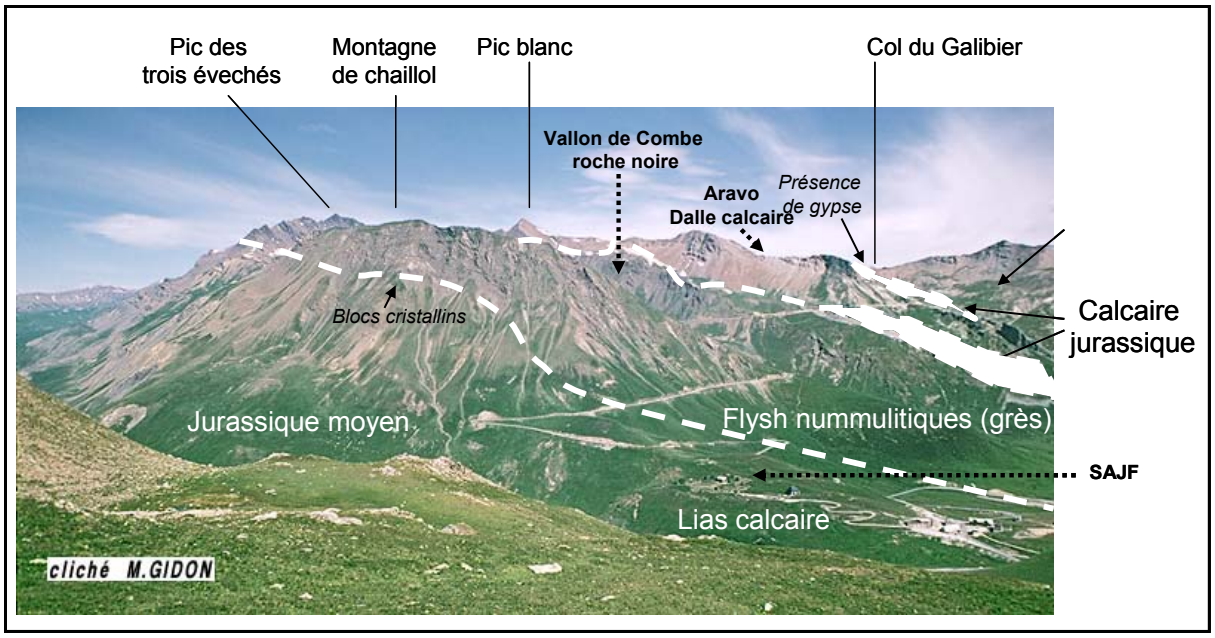


Fig. 18 Panorama des crêtes au nord du col du Lautaret vu du sud, depuis le lac de Laurichard (extrémité nord du massif du Combeynot). SAJF : Station Alpine Joseph Fourier. Source : J. Debelmas.

3) Les conditions édaphiques

Dans la région du Galibier, les sols des systèmes nivaux sont classés comme des stagnogley enrichi en argile (Tableau 5). Les sols des systèmes thermiques correspondent à des ranker alpins (Lamber 1996, Bounemoura et al. 1998). Le pH est faiblement acide et se situe aux alentours de 5. Les sols des systèmes thermiques sont caractérisés par la présence de carbonates à la différence des sols des systèmes nivaux

Caractéristiques des sites d'étude	Systèmes thermiques	Systèmes nivaux
Classification des sols	Ranker alpin	Stagnogley
Densité du sol ($g.cm^{-3}$)	0.31 (0.04)	0.69 (0.02)
Profondeur du sol (cm)	60-80	>120
pH dans l'eau	5.11 (0.0	4.96 (0.06)
Matière organique (%)	14.8 (0.84)	8.70 (0.80)
Carbonates (%)	3.7 (1.1)	<1
Granulometrie (%)[a]		
Argile (<2µm)	9.7 (0.5)	26.4 (2.6)
Limon (2-50µm)	41.4 (1.0)	61.7 (2.0)
Sable (50-2000µm)	48.6 (1.2)	11.9 (4.5)

Tableau 5. Variables édaphiques mesurées sur les sols des systèmes thermiques et nivaux. Moyenne réalisée à partir d'échantillonage dans les sites d'études A-D.
[a] sur la couche 0-10cm (fraction <2mm)

D'autre part, la nature des sols prélevés au col Agnel est décrite dans le tableau ci-dessous :

Caractéristiques du site d'étude	Systèmes nivaux
Classification des sols	Stagnogley
Matière organique (%)	8.10 (0.34)
Carbonates (%)	1.2 (0.1)
Granulometrie (%)[a]	
Argile (<2µm)	27.1 (3.1)
Limon (2-50µm)	47.5 (3.8)
Sable (50-2000µm)	25.4 (4.4)

Tableau 6 Variables édaphiques mesurées sur les sols des systèmes nivaux au col Agnel.

On notera peu de différence entre les systèmes nivaux situés dans la région du col du Galibier, et ceux localisés dans la région du Col Agnel.

L'analyse des concentrations en azote minéral fixé par des résines indique un pic de nitrate dans les combes à neige situées près du col du Galibier en début de saison de végétation (Fig. 19). La présence importance d'eau de fonte s'accumulant dans les zones concaves est en partie responsable de ce phénomène. Durant le reste de la saison de végétation, aucune différence significative n'a pu être observée le long du gradient. Enfin nous ne détenons aucune information sur les concentrations en phosphate de ces sols.

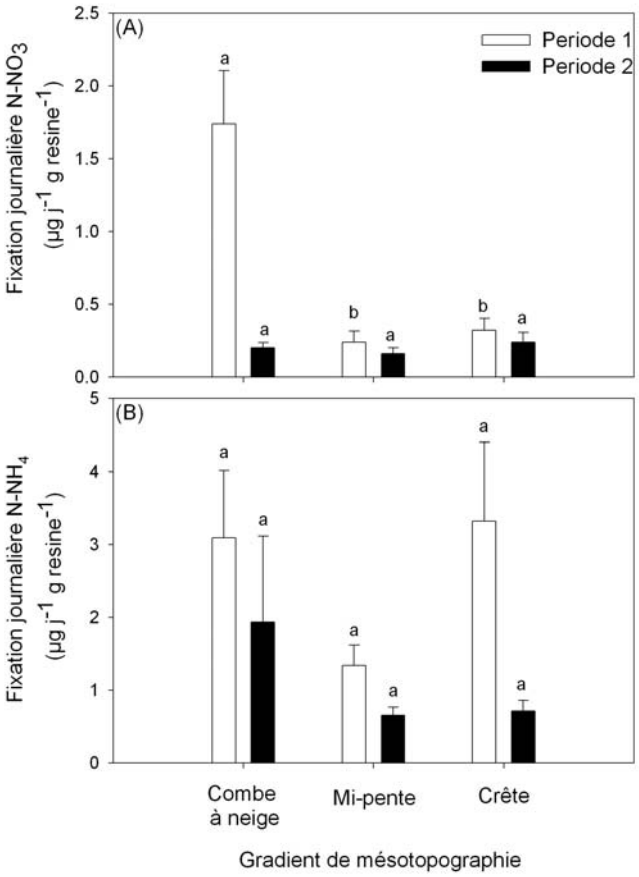


Fig. 19 Fixation journalière des ions nitrate (A) et d'ammonium (B) par des sacs de résines enterrés à 5cm dans les sols situés en combe, mi-gradient et en crête, du déneigement au 21/07 (Période 1) et du 21/07 au 05/10 (période 2). Les différentes lettres indiquent des différences significatives au sein de chaque période (P<0.05). Voir annexe 1 de l'article 1A pour un descriptif détaillé du mode opératoire.

4) Les groupements végétaux du site d'étude

Les systèmes thermiques sont caractérisés par des groupements à Elyne queue de souris (*Kobresia myosuroides = Elyna myosuroides*) et Dryade à huit pétales (*Dryas octopetala*). On distingue plusieurs faciès en fonction du relief et de l'exposition. Le faciès typique, plutôt d'ubac se développe sur un sol superficiel caillouteux, très humifère mais peu fertile. En adret, les sols sont plus profonds, les couverts sont plus denses et on note l'intrusion d'espèces plus xérophiles comme la Seslérie bleue (*Sesleria caerulea)* et l'Avoine des montagnes *(Helictotrichon sedenense)* aux dépens de la Dryade. Les pelouses de milieu de gradient sont des groupements acidicline à sol profond, et caractérisés par une forte richesse spécifique comparativement aux communautés situées aux deux pôles du gradient d'enneigement. Les plantes caractéristiques sont *Festuca violacea*, *Geum montanum*, *Trifolium sp., Alchemilla glaucescens*, *Arnica montana*, *Cirsium acaule*. Les combes à neige se caractérisent par un nombre restreint d'espèces : *Salix herbacea*, *Alchemilla pentaphyllea*, *Sibaldia procumbens, Alopecurus alpinus*. On note l'apparition d'un autre groupement, la pelouse à vulpin des Alpes et Renoncule de Küpfer. Les espèces caractéristiques sont *Plantago alpina*, *Ranunculus kupferi*, *Trifolium alpinum, Geum montanum*, *Potentilla aurea*, etc. (voir annexe 1 pour la liste des espèces dans les sites B,C et D et la richesse spécifique).

Comme résumé dans le tableau 3, les espèces qui ont fait l'objet d'une étude approfondie dans les différents articles de cette thèse sont : *Carex foetida*, *Alopecurus gerardi*, *Salix herbacea*, *Poa alpina* (communautés nivales) et *Kobresia myosuroides*, *Dryas octopetala* (communautés thermiques) (Fig. 20).

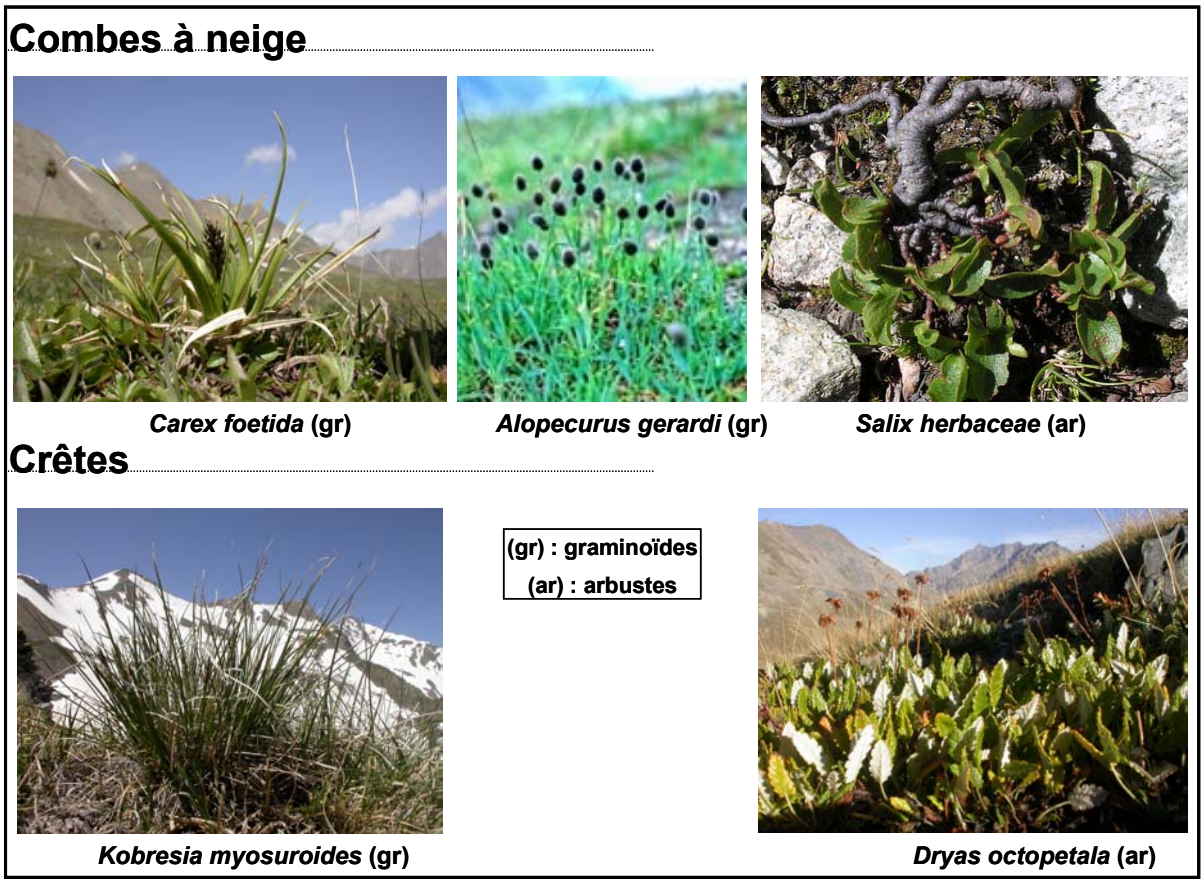


Fig. 20 Illustration des cinq espèces modèles sélectionnées dans cette étude.

Chapitre I

Effet de l'enneigement sur les traits fonctionnels, la fixation et l'allocation du carbone dans les écosystèmes alpins

CHAPITRE I

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Article 1A

A simulation on the importance of growing season length and canopy functional properties on the seasonal Gross Primary Production of temperate alpine meadows.

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Annals of Botany 101:549-559

Mesures des échanges gazeux dans différentes conditions environnementales afin de valider le modèle visant à estimer le gain carboné brut des communautés situées le long du gradient d'enneigement. Espèce présente : *Kobresia myosuroides* dominante dans les communautés thermiques (C140). Photo : Ph. Choler.

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A Simulation of the Importance of Length of Growing Season and Canopy Functional Properties on the Seasonal Gross Primary Production of Temperate Alpine Meadows

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• *Background and Aims* Along snowmelt gradients, the canopies of temperate alpine meadows differ strongly in their structural and biochemical properties. Here, a study is made of the effects of these canopy dissimilarities combined with the snow-induced changes in length of growing season on seasonal gross primary production (GPP).
• *Methods* Leaf area index (LAI) and community-aggregated values of leaf angle and leaf nitrogen content were estimated for seven alpine plant canopies distributed along a marked snowmelt gradient, and these were used as input variables in a sun–shade canopy bulk-photosynthesis model. The model was validated for plant communities of early and late snowmelt sites by measuring the instantaneous CO_2 fluxes with a canopy closed-chamber technique. A sensitivity analysis was conducted to estimate the relative impact of canopy properties and environmental factors on the daily and seasonal GPP.
• *Key Results* Carbon uptake was primarily related to the LAI and total canopy nitrogen content, but not to the leaf angle. For a given level of photosynthetically active radiation, CO_2 assimilation was higher under overcast conditions. Sensitivity analysis revealed that increase of the length of the growing season had a higher effect on the seasonal GPP than a similar increase of any other factor. It was also found that the observed greater nitrogen content and larger LAI of canopies in late-snowmelt sites largely compensated for the negative impact of the reduced growing season.
• *Conclusions* The results emphasize the primary importance of snow-induced changes in length of growing season on carbon uptake in alpine temperate meadows. It was also demonstrated how using leaf-trait values of the dominants is a useful approach for modelling ecosystem carbon-cycle-related processes, particularly when continuous measurements of CO_2 fluxes are technically difficult. The study thus represents an important step in addressing the challenge of using a plant functional-trait approach for biogeochemical modelling.

Key words: Alpine meadows, gross primary production, plant functional traits, snowmelt gradient, sun–shade model.

INTRODUCTION

The carbon budget of cold, snow-covered ecosystems is of particular interest because they are known to sequester a large amount of organic carbon in their soils and to be particularly sensitive to global warming (Hobbie *et al.*, 2000). Much attention has been drawn to the carbon balance of arctic tundra (Vourlitis *et al.*, 2000; Grant *et al.*, 2003; Campbell *et al.*, 2005; Euskirchen *et al.*, 2006), but carbon balance estimations for temperate alpine tundra and meadows are relatively uncommon (Cernusca, 1989; Diemer, 1994). In cold ecosystems, snow determines the length of the season, which is a main driver of carbon exchange between land and atmosphere (Arora and Boer, 2005; Churkina *et al.*, 2005). Recent climatic studies have highlighted the impact of rising temperatures on snow-cover depth and duration at high elevations (Keller *et al.*, 2005), but their consequences for the carbon budget remain poorly understood (Brooks *et al.*, 1997; Monson *et al.*, 2006).

In situ continuous recording of CO_2 fluxes remains difficult in high-elevation terrains (but see Cernusca *et al.*, 1998; Li *et al.*, 2005; Hammerle *et al.*, 2007). The alpine landscape generally exhibits very fine-scale changes in vegetation cover, a feature that would preclude the designation of turbulent fluxes to a particular ecosystem. For the same reason, remote-sensing-based estimates of gross primary production (GPP) in alpine landscapes also suffer from insufficient spatial resolution (Turner *et al.*, 2004). This makes spatial and temporal scaling-up a major challenge. A growing body of literature suggests using plant functional traits in ecosystem modelling in order to scale-up ecosystem processes on a mechanistic basis (Diaz and Cabido, 2001; Lavorel and Garnier, 2002). Although this approach has recently been used in modelling primary productivity and litter decomposition (Quetier *et al.*, 2007), to our knowledge, this promising avenue has seldom been explored to model seasonal variations in GPP of herbaceous ecosystems.

According to the 'biomass ratio hypothesis' (Grime, 1998), ecosystem properties and function (i.e. carbon and nitrogen cycles) should be related to the trait values of the dominant contributors to the plant biomass. Several key ecosystem processes, such as decomposition rate or productivity, may be predicted from the traits of the dominant species (Chapin *et al.*, 1996; Cornelissen *et al.*, 1999; Epstein *et al.*, 2001). A main challenge is to mechanistically link ecosystem processes to a set of key plant

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functional traits that could easily be measured in the field (Diaz and Cabido, 2001). In this respect, it has been proposed that the use of quantitative traits would be more powerful than a broad categorization into discrete plant functional groups (Garnier *et al.*, 2004). The mean of trait values weighted by the relative abundance of each species – i.e. a community-aggregated value (Violle *et al.*, 2007) – provides a route to scale-up from organ to community level and offers a linkage between comparative plant ecology and ecosystem modelling. The goal of this paper is to develop such a modelling approach to estimate the seasonal GPP of temperate alpine meadows. Gross primary production is a key variable of the carbon balance as it quantifies the amount of autotrophic carbon available for growth, reserve and respiratory demands at the ecosystem level.

In alpine ecosystems, the landscape-scale distribution of snow (which is tightly related to the mesotopography) is a main driver of ecosystem structure and functioning. Through its effect on the length of the growing season, snow provides a complex ecological gradient affecting the seasonal course of temperature, light, wind exposure, soil water content and nitrogen availability (Jones *et al.*, 2000). It has long been known that arctic and temperate alpine plant communities exhibit high species turnover along snowmelt gradients (e.g. Komarkova and Webber, 1978; Kudo and Ito, 1992; Onipchenko and Blinnikov, 1994; Theurillat *et al.*, 1994). More recent studies have indicated that consistent shifts in plant functional diversity occur from early to late-snowmelt sites (Kudo *et al.*, 1999; Choler, 2005). A greater leaf nitrogen concentration (N_{mass}), a higher specific leaf area (SLA) and a predominance of horizontal leaves (i.e. trait values generally associated with a high capacity for resource acquisition) are common features of species from snowy sites (Choler, 2005). Conversely, species from early melting sites are characterized by upright and thick leaves and low SLA, i.e. trait values generally associated with nutrient-conservation strategies (Wright *et al.*, 2004).

Many studies have investigated the relationships among several canopy properties – leaf area index (LAI), nitrogen content, canopy architecture – and their effect on carbon uptake in different light conditions (Anten *et al.*, 1995; Hikosaka and Hirose, 1997; Anten, 2005). But, to our knowledge, there has been no attempt to examine these relationships for multispecies assemblages distributed along gradients of length of growing season. In this study, we addressed the following questions. (1) What are the differences in the community-aggregated values of leaf functional traits along the snow-cover gradient? (2) What is the relative effect of these canopy functional properties and environmental factors (light and temperature regime, length of growing season) on the seasonal GPP? (3) To what extent do plant communities from late-snowmelt sites overcome the negative impact of a reduced growing season on GPP?

This study was based on previous work investigating changes in plant functional traits at the species level along snowmelt gradients in the alpine zone of the south-western Alps (Choler, 2005). For this work, a sun–shade canopy bulk-photosynthesis model was implemented to simulate the light interception and the GPP at the ecosystem level. The model was validated with instantaneous measurements of CO_2 fluxes using a closed-chamber technique. Finally, a sensitivity analysis was conducted in order to determine the relative effects of climatic factors and canopy functional properties on the seasonal GPP.

MATERIALS AND METHODS

Study site and plant trait measurements

The research site was located in the south-western Alps (France) between the Lautaret and Galibier Pass (45°7′N, 6°5′E). The 2-ha site is a slightly inclined depression located between 2700 and 2780 m a.s.l. It exhibits a typical mosaic of alpine meadow species, ranging from *Kobresia myosuroides*-dominated plant communities in the early snowmelt sites to *Carex foetida*- and *Alchemilla pentaphyllea*-dominated plant communities in the late-snowmelt sites. For this work, the seven plant communities that are the most abundant in the studied area were selected. The communities are designated here according to the mean date of snowmelt in Julian days (e.g. C130, C140, C150, etc). Leaf angle, specific leaf area and leaf nitrogen concentration [expressed on a mass (N_{mass}) or area (N_{area}) basis] were used in this study because of their known effect on carbon-cycle-related processes. Further details of the site and trait measurements are given in Choler (2005).

For each trait, a community-aggregated value (T_i) was calculated as follows:

$$T_i = \sum_{i=1}^{n} p_i \, t_i \qquad (1)$$

where p_i is the relative cover of species i in the sampling unit (SU), n is the number of species accounting for 80 % of the cover in the SU, and t_i is the trait value of species i. Although the total coverage of the n species could slightly exceed 80 %, we did not correct the community-aggregated value. In practical terms, the change in T_i value because of this discrepancy is of very limited magnitude (Cornelissen *et al.*, 2003).

For each plant community, the peak standing biomass was harvested at the end of July in square plots of 50 × 50 cm. To calculate the leaf area index (LAI), the whole projected surface of green biomass for three harvests per community was measured using a leaf area meter (WinDIAS, Delta-T Devices Ltd). Separation between green and dead phytoelements was hard to achieve in early snowmelt sites, and hence LAI might have been slightly over-estimated for these communities. Material was then dried at 85 °C for 48 h and weighed.

Climate

Hourly soil temperatures were recorded using Hobo probes (Onset Computer Corporation, Bourne, MA)

buried at 5-cm depth over the period 1999–2005. During snow-covered periods, soil temperatures were close to 0 °C (usually between −1 and 1 °C) throughout the day and did not exhibit circadian variations, a recording consistent with a continuous snow cover of at least 1 m depth. The length of the growing season was calculated as the number of snow-free days with a mean soil temperature above 0 °C.

During the summer season, air temperature, relative humidity and photosynthetically active radiation (PAR) were recorded hourly by an automatic weather station [Campbell Scientific (Canada) Corp.] located in the Jardin Alpin du Lautaret (2100 m a.s.l.), 5 km from the study site. The diffuse and direct components of PAR were calculated according to Spitters (1986). Daily integrated values of incoming solar radiation were obtained from a longer time series (1999–2004) at Briançon climatic station (1300 m a.s.l.), located at 30 km from the site and maintained by Météo France.

The Clear Sky Model (CSM) developed in the framework of the European Solar Radiation Atlas (Rigollier *et al.*, 1999) was used to model the incoming global irradiance on a horizontal plane under a cloudless sky. In the CSM, the direct (or beam) radiation (I_{0b}) and the diffuse radiation (I_{0d}) are simulated separately. Details of the CSM can be found in Rigollier *et al.* (1999). Solar geometry was implemented using equations written by L. Wald and O. Bauer (École des mines de Paris, Centre d'Energétique, Groupe de Télédétection, February, 1997). The model was implemented with no mask effect due to the relief. The long-term recordings of daily irradiance in Briançon were used to parameterize the monthly mean atmospheric turbidity in the area.

Canopy bulk-photosynthesis model and simulation of seasonal GPP

We developed a modified version of the sun–shade model of De Pury and Farquhar (1997) to estimate the canopy-intercepted radiation and CO_2 fixation. For light interception, the model splits the canopy into a sunlit and a shaded fraction, which experience distinct light regimes. Shaded leaves receive scattered light and diffuse sky irradiance while sunlit leaves receive direct-beam irradiance in addition to this. CO_2 fixation is based on the von Caemmerer and Farquhar biochemical model of C_3 leaf CO_2 assimilation (Farquhar *et al.*, 1980; von Caemmerer and Farquhar, 1981). Below, we only detail the main changes made to the original sun–shade model. The main constants, parameters and equations of the model are given in the Supplementary Information, available online.

(1) We used the ellipsoidal distribution model (Campbell, 1990) to describe the leaf-inclination distribution (or leaf eccentricity) in the canopy. For simplicity, the canopy is considered as spatially homogeneous, i.e. with no clumped leaves in either the vertical or horizontal dimensions. The model assumes that the leaf-angle distribution is similar to the distribution of area on the surface of an ellipsoid. The model requires only one parameter (ε), which is the ratio of the axes of the ellipsoid. The leaf-azimuth-angle distribution is assumed to be uniform; as such, the leaf-inclination probability density function g will solely depend on z_l, the zenith angle of the leaf normal (see Supplementary Information, Table S2, eqn 1, available online). We estimated the parameter ε from community-aggregated values of leaf angle (Table S2, eqn 2). The use of the ellipsoidal distribution model modifies the calculations of the canopy extinction coefficient for direct radiation (k_b) as well as the canopy reflection coefficient (Table S2, eqns 4–6).

(2) The Rubisco capacity is linearly related to the leaf nitrogen content. The mean slope of the N_{area}–$V_{c,max}$ relationship (the parameter s_N) was derived from AC_i response curves of six alpine species (see Supplementary Information online) and from published data for 13 other species (Wohlfahrt *et al.*, 1998). The minimum leaf nitrogen content, N_{min}, corresponds to the x-intercept of the N_{area}–$V_{c,max}$ linear relationship. Much attention has been devoted to the vertical distribution of nitrogen in models of canopy photosynthesis (Friend, 2001). Under non-uniform distribution of nitrogen within the canopy, $V_{c,max}$ is also a function of canopy depth (Table S2, eqn 12). Following Schieving *et al.* (1992), we used generalized circle equations to model, with a single parameter p_N, different curvatures in the profile of the nitrogen distribution within the canopy while keeping the total amount of nitrogen, N_{tot}, constant (Table S2, eqn 13). If $p_N = 1$, N_{area} decreases linearly with canopy depth, while a uniform distribution is achieved when p_N tends towards infinity. Our preliminary measurements did not support non-uniform nitrogen distribution in early and late-snowmelt alpine meadows. Nevertheless, we maintained this model parameterization in order to compare the potential impact of nitrogen distribution with other factors.

(3) The photosynthesis model was combined with a model of stomatal conductance using the empirical approach developed by Ball *et al.* (1987; Table S2, eqns 24–28, online). Lack of data for accurate parameterization required that several simplifications had to be made. First, we used the relative humidity of ambient air as a surrogate for the leaf surface water vapour pressure. Secondly, boundary layer conductance to H_2O was set constant. Due to the interdependence of leaf net photosynthesis and stomatal conductance, we used an iteration method to calculate C_i (Harley and Tenhunen, 1991). Parameters for the stomatal conductance sub-model were taken from the work of Wohlfahrt *et al.* (1998).

(4) Seasonal GPP was estimated by running the canopy bulk-photosynthesis model from 10 May to 20 August. This 100-d period was divided into ten sub-periods of 10 d. For each sub-period, we calculated on an hourly basis the daily time course of air temperature, light and relative humidity for sunny, cloudy and intermediate days. Each sub-period comprised six sunny days, two cloudy days and two intermediate

days. These relative contributions were derived from an analysis of the long-term recordings of daily irradiance in Briançon (see 'Climate', above). The seasonal evolution of LAI was described as a linear function of cumulative degree days (*DD*) after snowmelt, as follows:

$$LAI(t) = LAI_{max} \times DD(t)/DD(t_{opt})$$

where LAI_{max} is the measured LAI at the time of peak standing biomass (t_{opt}). This parameterization allows the capture of the faster initial development of canopies from late-snowmelt sites.

To assess the relative importance of canopy functional properties and climatic factors on carbon uptake, we conducted sensitivity analyses by systematically varying one factor, i.e. a model parameter or a variable, while keeping the other factors constant and calculating the relative effect on the seasonal GPP.

Model validation by canopy gas-exchange measurements

A closed system was used to measure the CO_2 exchange of alpine meadow monoliths taken from an early and a late-snowmelt site (communities C140 and C180, respectively). Early snowmelt monoliths were sampled in the vicinity of the site of where plant trait measurements were taken. But for practical reasons, late-snowmelt monoliths were sampled near the Agnel Pass at 2760 m a.s.l., located in the Queyras mountain range. For each community, five monoliths of 20-cm depth and 50 × 25 cm area were excavated during autumn 2005, and brought to the Lautaret Alpine field station (2100 m a.s.l.) where they remained until the following summer for measurements. Species' identity and relative cover in the monoliths were assessed before the measurements and no changes were found in the floristic composition compared with the field. This indicated that the monoliths recovered well from excavation.

For CO_2 flux measurements, a 48-L Perspex chamber was placed over each monolith and sealed around the base by seating it in a trough of water. Within the chamber, a fan (Radio Spare) provided air mixing above the canopy. The chamber was connected to a portable IRGA (EGM-4, PPSystems, Hitchin, UK) measuring the CO_2 concentration every minute. The rate of change of CO_2 in the chamber was determined by averaging the measurements under steady-state conditions, which typically began after 1 min. The measurement periods were generally brief, not exceeding 3 min in order to minimize chamber effects. We simultaneously recorded photosynthetically active radiation (PAR), air and soil temperature, and relative humidity (RH) within the chamber. The rate of change in chamber CO_2 concentration was converted into a CO_2 exchange rate per ground area. Net CO_2 fluxes under light conditions (F_n) were collected on both cloudy and sunny days in mid-July, 2006. Dark respiration rates (R_d) were measured before and after the light treatment by placing a dark cover over the chamber. There was no indication that respiration increased after the light treatment. Instantaneous rates of gross CO_2 uptake (GPP) were calculated as $GPP = F_n + R_d$. LAI and N_{tot} were measured on three 5 × 5 cm square plots per monolith. The mean LAI of early snowmelt monoliths was 1·3, and the mean N_{tot} was 208 mmol N m^{-2}. The mean LAI of late-snowmelt monoliths was 2·2, and the mean N_{tot} was 280 mmol N m^{-2}.

Model performance was evaluated quantitatively by calculating the square of Pearson's correlation coefficient (r^2), and qualitatively by the root-mean-square error (RMSE) and the mean absolute error (MAE), summarizing the mean differences between observed and predicted values (Willmott and Matsuura, 2005).

Numerical simulations, statistical analyses and graphics were performed with the R software environment (R Development Core Team, 2006). The source code is available upon request.

RESULTS

Climatic conditions along the snowmelt gradient

The yearly course of soil temperature for the early (C140) and the late (C180) snowmelt sites is depicted on Fig. 1. The delayed onset of growing season, by nearly 40 d, is constant throughout the 7 years of recordings (Fig. 1A). This difference accounts for a loss of around 280 °Cd at the snowy site at the time of peak standing biomass, which is 30 % fewer degree-days than at the early snowmelt site (Fig. 1B). By comparison, inter-annual variability in cumulative degree-days in the snowy sites is about an order of magnitude below that of the early snowmelt sites (Fig. 1B).

The annual cycle of incident solar radiation measured during cloudless days is fitted well by the clear sky model (Fig. 2A). Around 60 % of days during the growing season received more than 70 % clear-sky radiation, hereafter referred to as 'sunny days'. Thus, cloudless weather conditions predominate in this area of the south-western Alps. The effect of cloudiness accounts for a 10 % reduction in total radiation received by the early snowmelt sites compared to the clear sky model (Fig. 2B). Later snowmelt in C180 is responsible for a 40 % reduction in the cumulative incoming solar radiation over the growing season when compared with early snowmelt sites, i.e. from 0·22 MJ cm^{-2} to 0·13 MJ cm^{-2} (Fig. 2B). Inter-annual variations are small compared with those attributable to the timing of snowmelt (Fig. 2B). The daily time course of climatic data for the three types of day is given in the Supplementary Information (Fig. S1, available online).

Plant functional traits along the snowmelt gradient

Graminoids, erect forbs and large leafy rosettes are the main plant life forms accounting for up to 80 % of the vegetation cover in the study area (Table 1). Early snowmelt sites exhibit a discontinuous vegetation cover and a high proportion of bunch graminoids (e.g. *Kobresia myosuroides*), whereas tiny erect forbs and stoloniferous graminoids are predominant at the snowy sites. The snowmelt

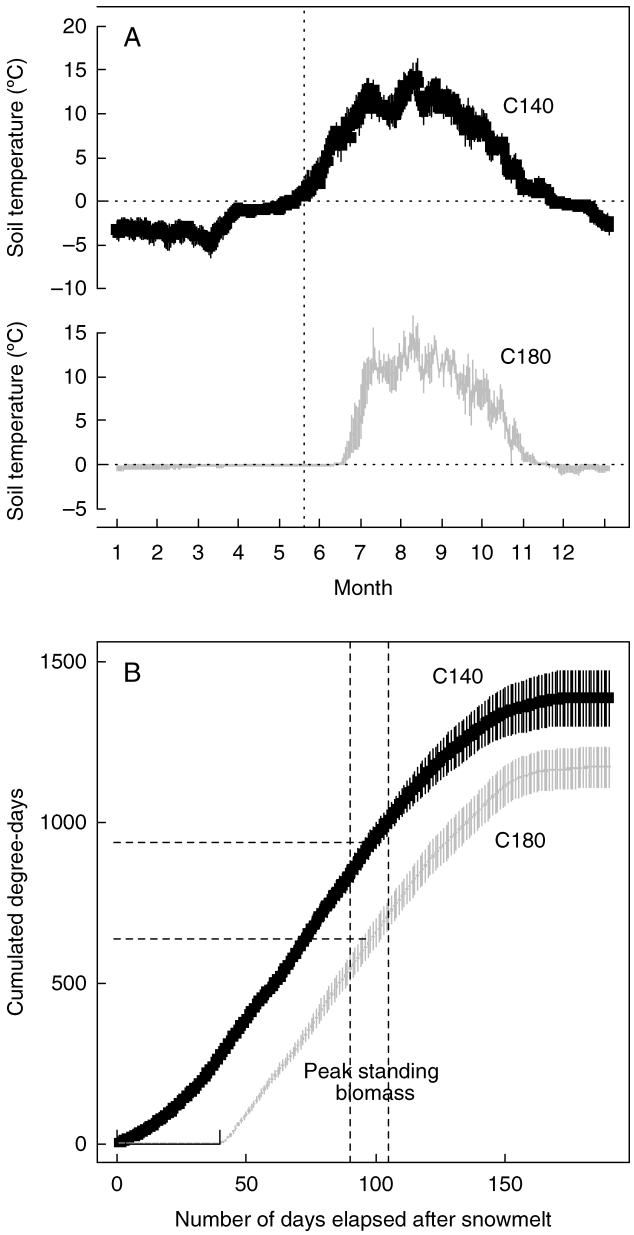

FIG. 1. (A) Time course of daily mean (± s.e.) soil temperature at 5 cm below ground and (B) cumulative degree-days in early and late-snowmelt sites in the C140 and C180 communities (see Table 1). Data are averaged over the period 1999–2005 and were recorded at two or three different sites depending on the year.

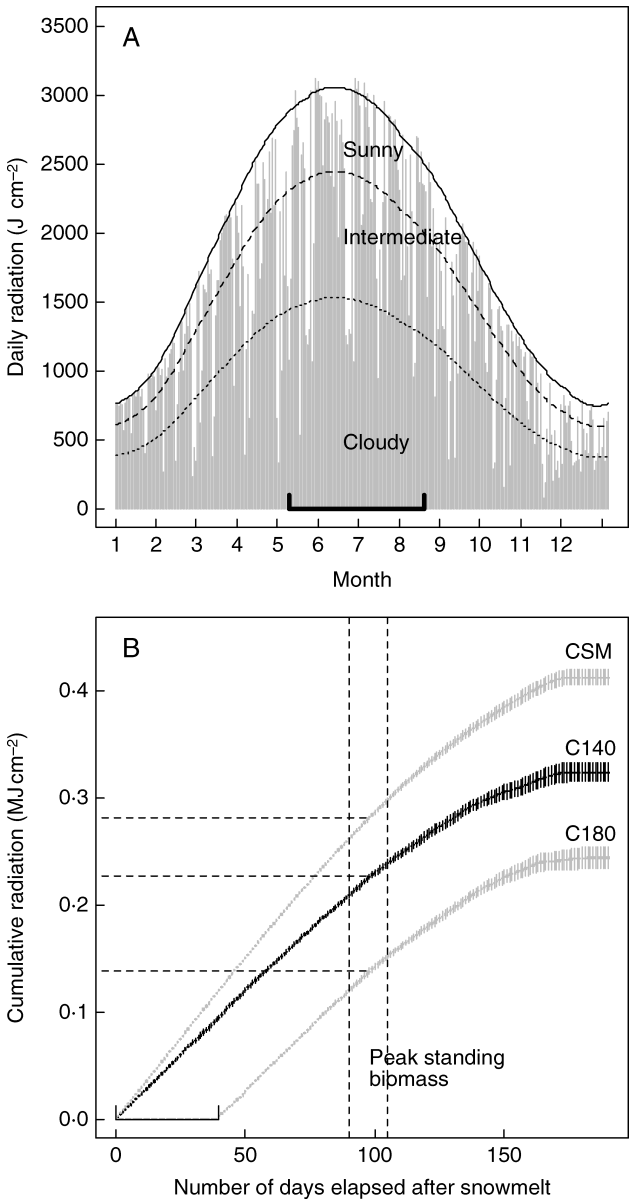

FIG. 2. (A) Time course of daily integrated incoming radiation in 2002 and comparison with a clear sky model (solid line). Broken lines indicate reductions of 20 % and 50 % of the clear sky amount of radiation, i.e. the threshold values chosen for intermediate and cloudy days. The horizontal segment indicates the growing season period. (B) Cumulative radiation during the growing season in the C140 and C180 communities. The clear sky model is shown for comparison. Means (± s.e.) were calculated over the period 1999–2004. Data were recorded at Briançon (1300 m a.s.l.), 30 km from the study site.

gradient was also characterized by a marked shift in community-aggregated values of leaf traits, with planophilous canopies with a high N_{mass} and high SLA at the snowy sites, and erectophilous canopies with low SLA and high N_{area} at the early snowmelt sites (Fig. 3).

Canopies of late-snowmelt sites exhibited larger LAI and greater N_{tot} (Table 1). It should be stressed that the range of variation for LAI (from less than 1·0 at the early snowmelt sites to around 2·5 at the mid-part of the gradient) largely exceeded that of N_{area}. Therefore, the total nitrogen pool in the canopy, $N_{tot} = \text{LAI} \times N_{area}$ is primarily determined by LAI, and to a lesser extent by N_{area}.

Daily and seasonal gross primary production

Modelled values of instantaneous gross CO_2 uptake corresponded well with measurements under cloudless and overcast conditions, although there was a slight overestimation for early snowmelt canopies (Fig. 4). Increased carbon fixation under conditions of high diffuse radiation was particularly noteworthy for canopies with high LAI (Fig. 4A). At a PAR of 1000 μmol m^{-2} s^{-1}, the CO_2 gross uptake of late snowmelt monoliths was 1·5 times

TABLE 1. *Features of the seven alpine meadows distributed along the snowmelt gradient. The measurements were made at the time of peak standing biomass at the end of July 2004. The leaf eccentricity was calculated with an ellipsoidal leaf-distribution model (see Material and Methods)*

	Mean snowmelt (Julian day)						
	130	140	150	160	170	180	190
n	7	9	6	4	9	4	8
Percentage cover of plant life form							
Bunch graminoids	59 ± 1·6	33 ± 1·7	18 ± 0·9	<1	<1	<1	<1
Other graminoids	15 ± 1·4	12 ± 0·8	13 ± 0·5	28 ± 2·5	20 ± 1·1	39 ± 4·3	48 ± 4·8
Erect forbs	14 ± 0·6	30 ± 1·3	33 ± 1·0	36 ± 1·6	43 ± 2·1	52 ± 3·6	52 ± 4·8
Large leafy rosette	3 ± 0·3	17 ± 1·1	17 ± 0·9	22 ± 3·3	17 ± 2·3	4 ± 0·5	<1
Leaf eccentricity	0·5	1·5	1·6	2·7	2·3	2·5	3·1
Leaf area index*	0·8 ± 0·09	1·5 ± 0·06	2·5 ± 0·35	1·2 ± 0·10	1·12 ± 0·01	2·0 ± 0·09	2·4 ± 0·18
N_{tot} (mmol m^{-2})	133 ± 4·0	218 ± 10·3	356 ± 10·2	148 ± 6·1	159 ± 5·9	257 ± 7·4	275 ± 16·7
Above-ground phytomass (g m^{-2})	435 ± 154	486 ± 65	443 ± 89	352 ± 77	402 ± 45	398 ± 67	370 ± 36

Means ± s.e. are shown.
*$n = 3$ for LAI.

higher under overcast conditions compared to sunny conditions (Fig. 4A), even though ambient air temperature was around 5 °C lower (Supplementary Information, Fig. S1).

For sunny and overcast conditions, the daily GPP values for the seven canopies were linearly related to the LAI (Fig. 5). This may be explained by the strong relationship between LAI and N_{tot} (see above and Table 1). For a given amount of N_{tot}, we simulated the daily carbon uptake for a range of LAI (Fig. 5). For low LAI values, the daily GPP strongly increases with LAI, indicating that light capture is the main limiting factor of carbon uptake. Then, the slight decrease of GPP with LAI may be explained by a decrease in N_{mass} due to a 'dilution' effect. The modelled GPP under cloudy skies was lowered by around 10 % compared with cloudless skies, for example 6·5 against 7·3 g C m^{-2} d^{-1} for C180 canopies (Fig. 4). We found a negligible effect of leaf eccentricity (see Table 1) on this trade-off between light capture – driven by LAI – and CO_2 fixation – driven by N_{mass} (data not shown). Finally, it should be noted that for a given $N_{tot,}$ alpine canopies operate at a higher LAI than the optimal LAI (Fig. 4).

A sensitivity analysis of the seasonal GPP model was conducted by estimating the effect of a 10 % increase in a factor while keeping all other characteristics constant (Fig. 6A). The simulations were run for a 100-d period (see Material and Methods). A 10 % increase in the length of growing season (corresponding to a shift of 10 d for the snowmelt) had the greatest impact on the seasonal GPP (Fig. 6A). By comparison, a shift to clear sky conditions throughout the season had a weaker effect. Similarly, increased temperature during the whole season did not compensate for 10 d lost in the growing season. LAI and two nitrogen-related factors, N_{mass} and s_N, had a noticeable impact on GPP, but still lower than that of the length of growing season (Fig. 6A). The shift from a uniform to a linearly decreasing nitrogen distribution ($p_N = 1$) also had a weaker effect on GPP, roughly similar to the effect of a temperature or an atmospheric CO_2 increase. Finally, a 10 % change in relative humidity, leaf angle, or physiological parameters related to stomatal conductance changed the GPP by less than 1 %.

The integrated values of GPP over the growing season were around 200 g C m^{-2}, except for the plant communities C140 and C150 (Fig. 6B). GPP simulations were also performed without the snow-induced shortening of the growing season. For the late-snowmelt sites, the model predicted a severe reduction in the carbon uptake because of the delayed snowmelt. However, the results indicated that the functional properties of these canopies (greater N_{tot} and LAI) largely compensated for this negative impact of a reduced growing season.

DISCUSSION

Our study emphasizes the interplay between short-term and long-term effects of snow-cover duration on the seasonal carbon uptake of alpine canopies. Short-term effects are driven by the direct influence of snow cover on the seasonal light and temperature regimes, whereas long-term effects correspond to the ecological sorting of species and plant traits along snow-cover gradients. A main result is that the snow-induced reduction in the carbon uptake period is counterbalanced by increased efficiency of carbon gain, which is made possible by the particular leaf trait combination of exploitative strategists that occur at the late-snowmelt sites. To our knowledge, this is the first attempt to determine the complex impact of snow on the seasonal GPP of temperate alpine canopies.

Instantaneous CO_2 flux measurements under different light conditions are predicted well by our canopy photosynthesis model. We are thus confident that the use of community-aggregated values of the chosen leaf traits is relevant to simulate the carbon uptake at the community level, as hypothesized in previous conceptual work (Diaz and Cabido, 2001). We found that the assimilation rate of the whole canopy (expressed per unit ground area) was of the same magnitude as the leaf assimilation rate (expressed per unit leaf area), which is consistent with other studies (Grabherr and Cernusca, 1977; Diemer, 1994; Tappeiner

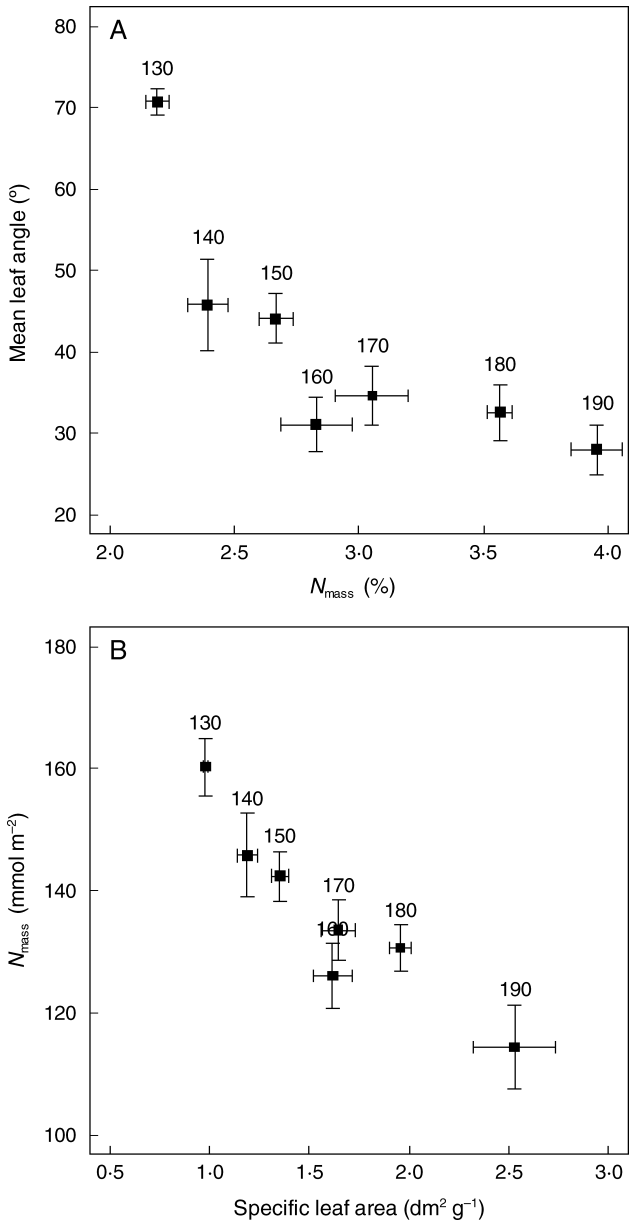

FIG. 3. Relationships between community-aggregated values of (A) leaf nitrogen concentration on a mass basis (N_{mass}) and mean leaf angle, and (B) leaf nitrogen content on an area basis (N_{area}) and specific leaf area (SLA). Means (± s.e.) were calculated from 4–9 different plots (see Table 1). The numbers above the squares indicate the mean snowmelt date for each community in Julian days.

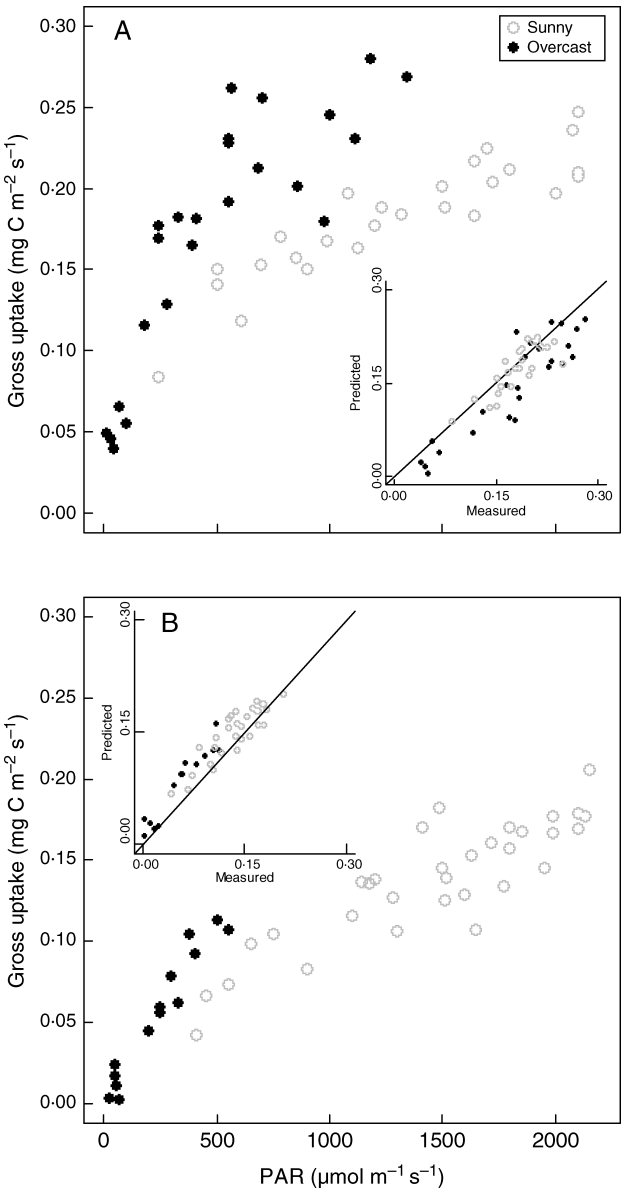

FIG. 4. Canopy gross CO_2 uptake for sunny and overcast conditions in mid-July 2006. Closed chamber measurements were performed (A) on monoliths dominated by *Carex foetida* sampled at a late-snowmelt site (C180), and (B) on monoliths dominated by *Kobresia myosuroides* sampled at an early snowmelt site (C140). The relationships between measured and predicted values are shown in the two insets. Evaluation of the model performance is as follows: (A) $n = 50$, $r^2 = 0{\cdot}89$, RMSE = 0·033, MAE = 0·026; (B) $n = 46$, $r^2 = 0{\cdot}95$, RMSE = 0·025, MAE = 0·021.

and Cernusca, 1998). Compared with the most widely investigated temperate alpine community, i.e. *Carex curvula*-dominated alpine meadow (Diemer and Körner, 1998), the measured and modelled carbon uptake are higher for canopies of late-snowmelt sites and lower for early snowmelt sites.

Scaling-up from detailed plant physiological studies to ecosystem or regional scales requires taking into account variations in vegetation composition and community structure. Approaches based on *a priori* classification of plants into functional types have shown some limitations (Naeem and Wright, 2003; Reich *et al.*, 2004). Our study shows how integrating the trait-based approaches of comparative plant ecology with canopy-functioning models addresses this difficulty. However, recent findings suggest that functional diversity, i.e. the distribution and range of trait values in a given plant community, might be another key driver of ecosystem functioning (Naeem and Wright, 2003; Reich *et al.*, 2004). Clearly, a further challenge in ecosystem modeling would be to explicitly incorporate functional diversity effects into biogeochemical models.

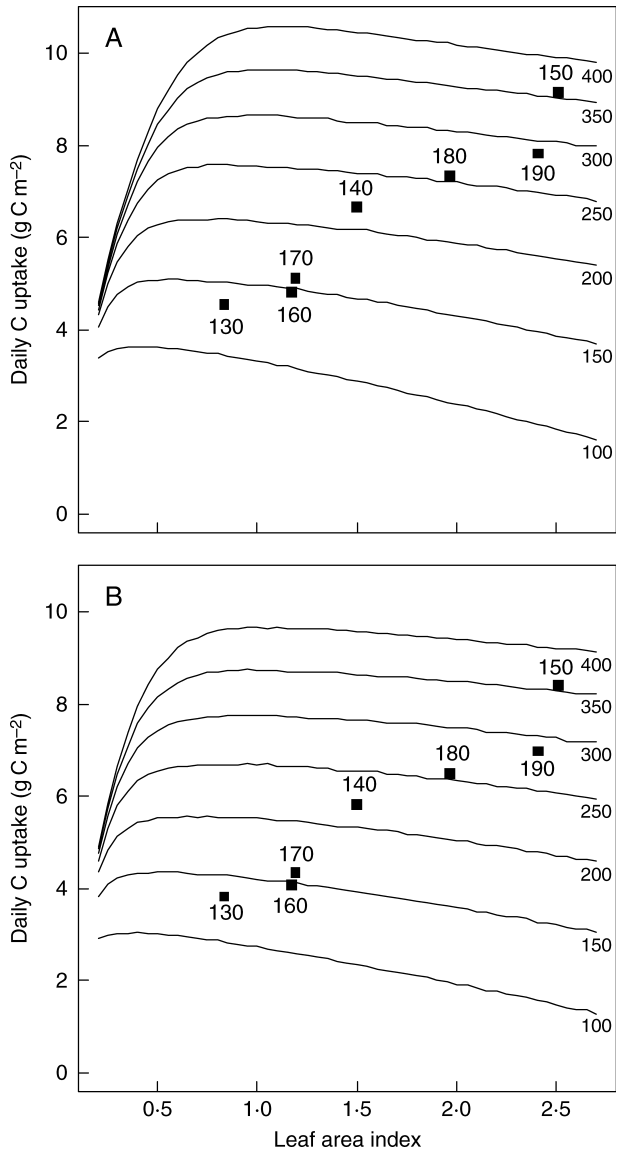

FIG. 5. Daily estimates of GPP (in g C m^{-2} ground d^{-1}) for (A) sunny and (B) overcast conditions in mid-July as a function of LAI. Simulations were run for a range of canopy nitrogen contents, N_{tot} (in mmol N m^{-2} ground), represented by the lines. The black squares correspond to the GPP estimates obtained with the LAI–N_{tot} combinations of the seven alpine plant communities studied. Numbers above the squares as in Fig. 3.

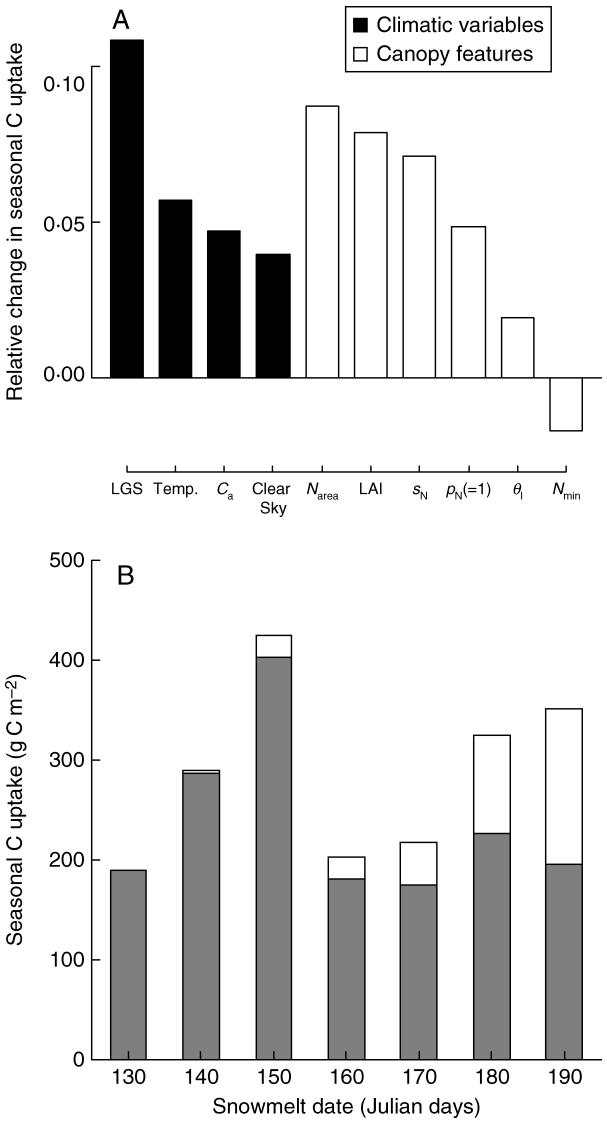

FIG. 6. (A) Sensitivity analysis of the seasonal GPP model. The relative change in GPP following a 10 % increase in a given factor was calculated as (GPP_{new} – GPP)/GPP where GPP_{new} is the GPP obtained after changing the factor. Factors for which the relative effect on GPP did not exceed 1 % are not shown. Climatic variables and canopy features are distinguished. LGS is for a 10-day increase of the length of growing season. C_a, ambient CO_2 partial pressure; p_N, parameter for nitrogen distribution (see Material and Methods); s_N, slope of the linear relation between N_{area} and $V_{c,max}$; N_{min}, minimum leaf nitrogen content; θ_l, curvature of the leaf response of electron transport rate to irradiance. (B) Seasonal estimates of GPP (in g C m^{-2}) for the seven alpine communities studied. For each bar, the grey part accounts for the carbon uptake during the observed length of the growing season, and the white part accounts for the missing carbon uptake due to the delayed snowmelt. Numbers indicate the mean snowmelt date of each community in Julian days.

There are several possible shortcomings when simulating the GPP over the growing season. For example, we considered seasonal variations in incoming radiation and degree-days, excluding the potential impact of changes in soil water content on canopy assimilation. Our continuous measurements of gravimetric soil water content, at 5 cm below ground, did not show significant differences between early and late-snowmelt sites, and only a slight decrease through the growing season (F. Baptist, unpubl. res.). The soils of the study site are deep and the average rainfall between mid-May and mid-August for the three years, 2004–2006, was around 300 mm. These features should ensure enough water is available to plants in the conditions of low evaporative demand normal for high-elevation meadows (Körner, 1999). Moreover, we did not consider all the potential changes in plant functioning over the growing season. For example, the nitrogen content of late-snowmelt canopies is particularly high immediately after their release from snow cover, but then decreases slightly (F. Baptist, unpubl. res.). This may cause an over-estimation of the seasonal GPP at

late-snowmelt sites. These limitations call for further refinements of the model and, as such, absolute values of the seasonal GPP estimates should be considered with caution. However, these limitations should not distort the conclusions about the relative impact of growing season length vs. canopy functional properties on carbon uptake along the snowmelt gradient.

In our simulations, leaf geometrical properties did not exert a strong influence on GPP. Canopy photosynthesis models, based on the Monsi–Saeki theory, also show that the assimilation rate of canopies with a LAI under 2·5 is largely independent of the extinction coefficient, K (Hirose, 2005). However, our model neglects other potential effects of leaf inclination, for example the effect on night-time frost and possible low-temperature photoinhibition (Germino and Smith, 2001). Presently, we do not have enough empirical data to assess the relative importance of such mechanisms at the canopy level and over the growing season.

The results suggest that the alpine canopies do not operate at their optimal LAI (Fig. 5). It is clear that stand-level properties are not exclusively dependent upon maximizing carbon uptake and that individuals are the units under selection, not canopies (Hirose, 2005). Some theoretical approaches have also highlighted that the optimal LAI is not evolutionary stable if one takes into account competition among individuals (Anten and Hirose, 2001). Perhaps more interestingly, the comparison of canopy functioning along the snowmelt gradient might call for a more detailed investigation of the optimal LAI–N relationships for different lengths of growing season.

As reported in other studies (De Pury and Farquhar, 1997; Gu *et al.*, 2002), the sun–shade model allows a more realistic treatment of the difference in the canopy photosynthetic response to direct and diffuse radiation. Increased carbon uptake under diffuse radiation has already been reported (Roderick *et al.*, 2001). The deep-shaded leaf fraction within vegetation canopies is strongly reduced on cloudy days compared to cloudless days as a result of the increased diffuse fraction of incoming radiation. Furthermore, at noon there is a lower probability that canopy photosynthetic saturation will occur under overcast conditions compared to sunny conditions (Gu *et al.*, 2002).

The results support the view that the traits of exploitative strategists (especially the high leaf N_{mass}, the rapid growth of photosynthetic organs) should permit the constraint of a shortened carbon-uptake period to be overcome. However, both conservative and exploitative strategies are known to be adaptive under severe limitation of the carbon uptake period (Kikuzawa and Kudo, 1995). Obviously, the exploitative strategy is strongly dependent upon sufficient soil nutrient availability. The late-snowmelt sites that were investigated benefit from a pulse of inorganic nitrogen at the time of snowmelt, as compared to early snowmelt sites (F. Baptist, unpubl. res.; see Supplementary Information online). These results are also consistent with other comparisons along snowmelt gradients (Bowman *et al.*, 1993; Fisk *et al.*, 1998; Jaeger *et al.*, 1999). It is therefore likely that in the late-snowmelt sites that were studied, this nutrient pulse at the onset of growing season allowed a rapid expansion of photosynthetic tissues, which ensured efficient light capture and carbon fixation.

Conclusions

For cold ecosystems, the carbon-uptake period is primarily determined by the snow-cover duration. Here, we have demonstrated that the snow-induced changes in the length of growing season had the highest impact on the seasonal GPP. Our study is among the first ones to integrate the functional trait approach with instantaneous measurements and integrated estimates of ecosystem functioning. This approach is particularly promising because continuous recordings of CO_2 fluxes have been shown to be technically difficult in alpine environments with strong bioclimatic gradients.

Climatic change in will affect snow regimes in temperate mountains (Keller *et al.*, 2005; Euskirchen *et al.*, 2006). Increased temperature and reduced snow precipitation may be responsible for earlier snowmelt. But the phenological responses of alpine species to a lengthening growing season are still hard to predict (Starr *et al.*, 2000; Wipf *et al.*, 2006; Bjork and Molau, 2007). One might predict a small impact on carbon uptake if plant communities are dominated by periodic species, i.e. species with a fixed, genetically controlled growing period (Sorenson, 1941). Conversely, the short-term consequences for ecosystem productivity would be stronger if aperiodic species, i.e. species able to extend their vegetative growth, are dominatant. Future studies should address these issues for a better estimate of seasonal GPP in alpine meadows in response to global climate change.

SUPPLEMENTARY INFORMATION

Supplementary material is available online at http://aob.oxfordjournals.org/ and consists of the following figures and tables. Figure S1: the daily course of temperature, relative humidity and photosynthetically active radiation for sunny, intermediate and cloudy days in May–August. Figure S2: Relationship between the maximum rate of carboxylation ($V_{c,max}$) and the leaf nitrogen content per unit area (N_{area}). Figure S3: daily uptake of nitrate and ammonium by a resin bag inserted at 5 cm below ground in the C140, C160 and C180 communities. Table S1: list of constants, parameters and lumped variables used in the model. Table S2: list of the main equations of the model.

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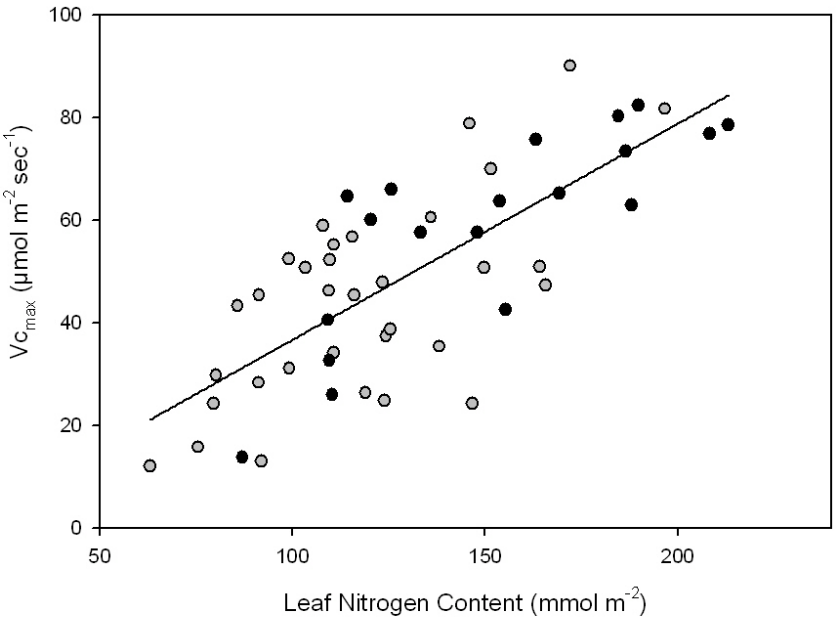
Supplementary material 1.



Relationship between the maximum rate of carboxylation (Vc_{max}) and the leaf nitrogen content per unit area. Black : data from our study. Grey : data from Wohlfahrt et al. (1999). $Vc_{max} = -5.50 + 0.42 \cdot N_a$. R^2=0.57, P<0.0001.

Experimental procedure :

The experimental procedure was similar to the one followed by Wohlfahrt et al. (1999). We used an Infra Red Gaz Analyser (CIRAS-3, PP-System, Hitchin, UK). CO_2 response curve were conducted at saturating light intensity (1500 µmol m^{-2} sec^{-1}). Leaf temperature was equalled to 22°C. Approximately three to four leaves were investigated to estimate the physiological parameters of the alpine species at peak standing biomass (*Carex foetida, Kobresia myosuroides*, *Salix reticulata*, *Dryas octopetala*, *Alchemilla pentaphillea*, et *Alopecurus gerardi).*

Références :

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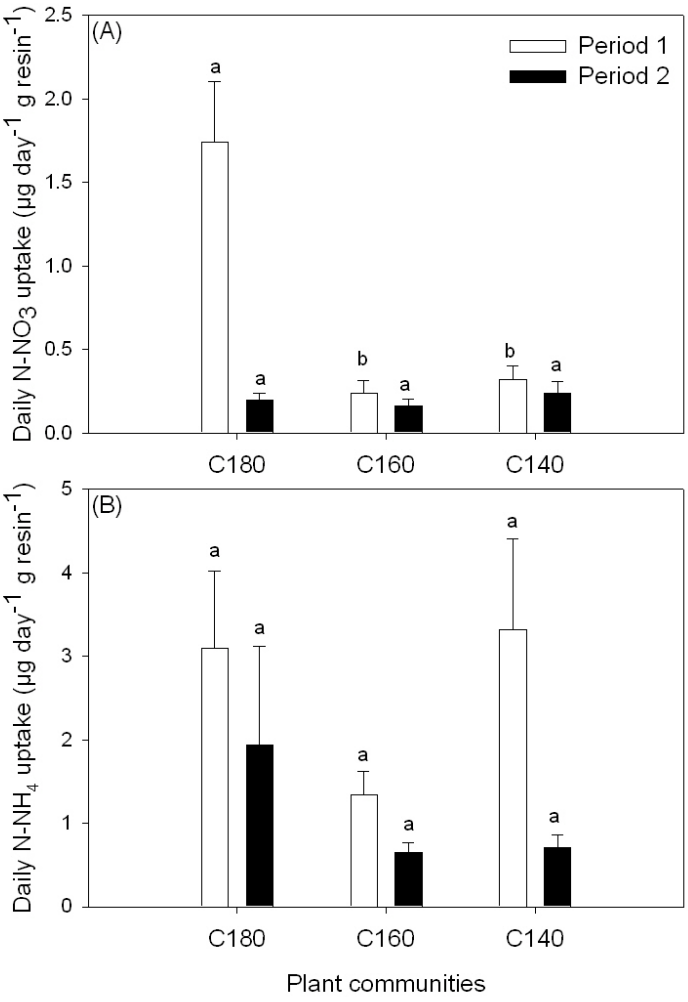
Supplementary material 2.



Daily uptake of nitrate (A) and ammonium (B) by resin bag inserted iat 5 cm depth in soil in C180, C160 and C140 communities from snowmelt until July 2005 (period 1) and from July to September (period 2). Different letters indicate significant differences within each period ($p<0.05$). Values are the mean (se). See below for experimental procedure.

Stastical results :

NO_3^- :

Period 1:

Community effect ($F_{2,27}$ = 15.3, **P<0.0001**)

site effect ($F_{2,27}$ = 1.21, P=0.31)

Community effect × site effect ($F_{4,27}$=1.12, P=0.36)

Period 2 :

Community effect ($F_{2,13}$ = 1.02, P=0.38)

site effect ($F_{2,13}$ = 1.62, P=0.23)

Community effect × site effect ($F_{4,13}$=1.08, P=0.40)

NH_4^+ :

Period 1:

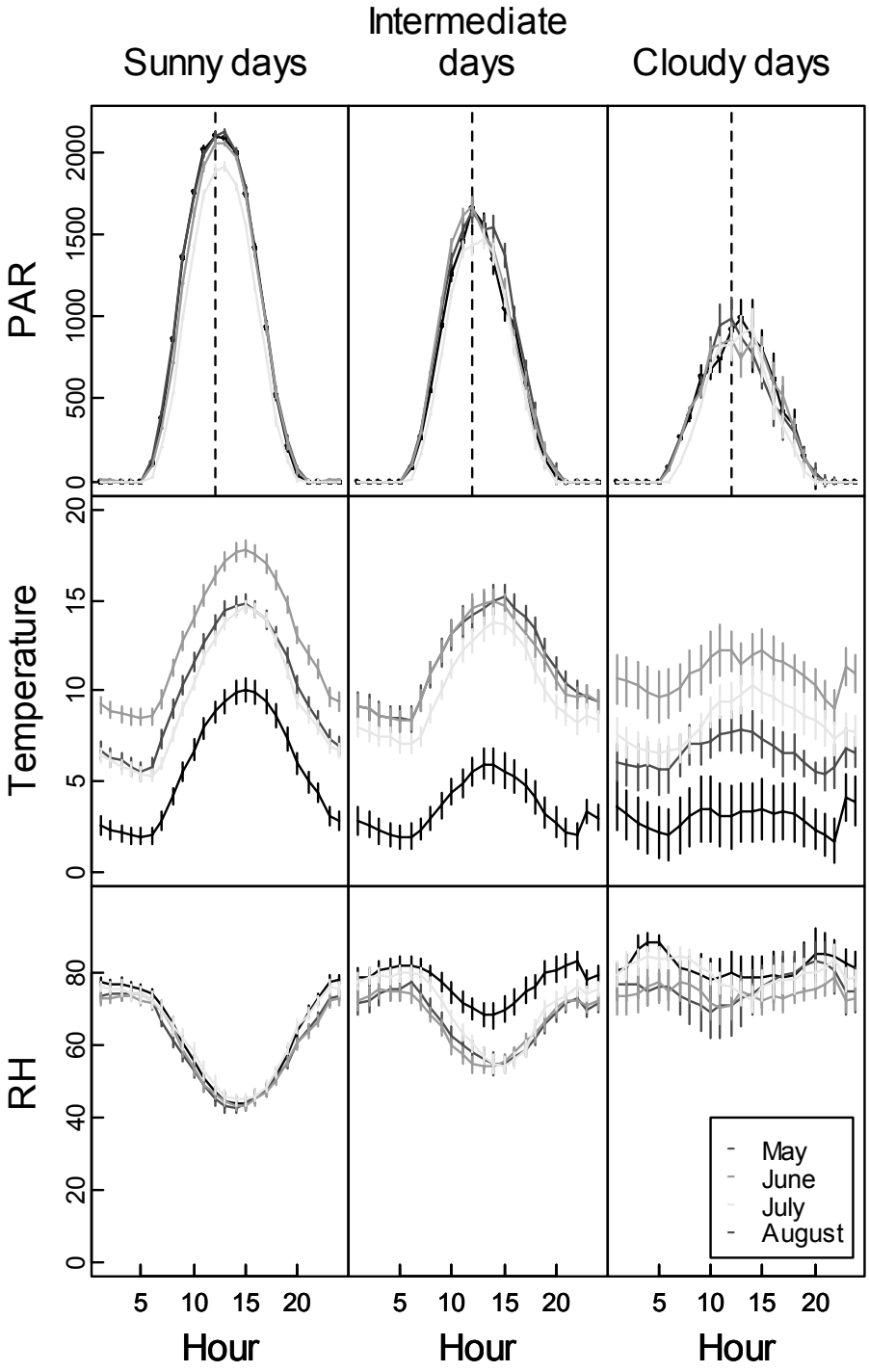
Community effect ($F_{2,27}$ = 1.77, P=0.19)

site effect ($F_{2,27}$ = 2.67, P=0.08)
Community effect × site effect ($F_{4,27}$=1.21, P=0.33)
Period 2 :
Community effect ($F_{2,13}$ = 0.26, P=0.76)
site effect ($F_{2,13}$ = 1.35, P=0.29)
Community effect × site effect ($F_{4,13}$=0.18, P=0.94)

Experimental procedure :
Nylon bags (10 × 5 cm) containing 5 g of mixed anion–cation exchange resin (Amberlite IRN150, VWR InternationalS.A.S., Fontenay-sous-Bois, France) were inserted into the soil at 5cm depth in field site. Bags were installed at four locations in each of the three replicates of the C140 , C160 and C180 communities. Half were incubated (1) from snowmelt until July 2005 (period 1) and the other half (2) for three months from July to September (period 2). Captured nitrate and ammonium were released from the resins by extraction in 100 ml of 2M KCl. The resulting concentrations of nitrate (A) and ammonium (B) were detected using a colorimetric chain (Fiastar 5012 Flow Injection Analyser, Foss Tecator AB, Sweden). Rates of nitrate and ammonium uptake were estimated as μg N uptake day^{-1} g $resin^{-1}$.
A two-way ANOVA was used to test site and community effects on nitrate and ammonium uptake within each period. Statistical analyses were performed with the R software environment (R Development Core Team, 2006).

Supplementary Fig. S1

The daily course of temperature, relative humidity (RH), and photosynthetically active radiation (PAR) for sunny, intermediate and cloudy days. Means and standard errors are given for May, June, July and August. The values are averaged over a 6-year-long period and were collected at the alpine field station at Lautaret (2100 m a.s.l.) located 5 km from the study site.



Supplementary Information: Tables

TABLE S1. Constants, parameters and lumped variables used in the model

Symbol	Value	Units	Description	Reference
A. Solar geometry and irradiance models				
a_S		rad	Solar azimuthal angle	
I_0		μmol m^{-2} s^{-1}	Incident PAR above the canopy	
I_{0b}		μmol m^{-2} s^{-1}	Beam incident PAR above the canopy	
I_{0d}		μmol m^{-2} s^{-1}	Diffuse incident PAR above the canopy	
I_{Sc}	1367	W m^{-2}	Solar constant	
z_s		rad	Solar zenithal elevation	
λ	0.1129	rad	Longitude of the site	
φ	0.7866	rad	Geographic latitude of the site	
B. Canopy architecture and light interception models				
a_l		rad	Leaf azimuthal angle of a normal to the leaf surface	
f	0.15		Spectral Correction Factor	(2)
F_h		m^2 m^{-2}	Cumulated leaf area from the top of the canopy to a given height per unit ground area	
k_d	0.78		Diffuse and scattered PAR extinction coefficients	(2)
LAI		m^2 m^{-2}	Leaf Area Index	
z_l		rad	Zenithal angle of the normal to the leaf surface	
ε			Ratio of the axes of the ellipsoid in the leaf distribution model	
σ	0.15		Leaf scattering coefficient of PAR (sum of leaf reflectance and leaf transmittivity to PAR)	(2)
θ		rad	Incidence angle of the beam light on the leaf foliage element	

C. Photosynthesis and stomatal conductance models				
$E_a\Gamma^*$	37830	kJ mol^{-1}	Activation energy of Γ^*	(4)
E_aK_c	65000	kJ mol^{-1}	Activation energy of K_c	(4)
E_aK_o	36000	kJ mol^{-1}	Activation energy of K_o	(4)
$g_{b,H2O}$	3.4	mol m^{-2} s^{-1}	Boundary layer conductance to water vapour	
$g_{min,H2O}$	80	mmol m^{-2} s^{-1}	Minimum stomatal conductance to water vapour at the light compensation point	(4)
$H_aV_{c,max}$	87624	J mol^{-1}	Activation energy of J_{max}	(5)
H_aJ_{max}	75926	J mol^{-1}	Activation energy of J_{max}	(5)
H_dJ_{max}	194482	J mol^{-1}	De-activation energy of J_{max}	(5)
$H_dV_{c,max}$	201550	J mol^{-1}	De-activation energy of $V_{c,max}$	(5)
$K_c^{T_{ref}}$	404	μbar	Michaelis–Menten constant for Rubisco carboxylation at $T = T_{ref}$	(2)
$K_o^{T_{ref}}$	248	mbar	Michaelis–Menten constant for Rubisco oxygenation at $T = T_{ref}$	(2)
m	14.6		Empirical coefficient for the sensitivity of $g_{s,H2O}$ to A, C_s and RH	(4)
n_{CO2}	0.000335		Molar fraction of carbon dioxide in the atmosphere	
n_{O2}	0.21		Molar fraction of oxygen in the atmosphere	
N_{area}		mmol m^{-2}	Leaf nitrogen content per unit leaf area	
N_{mass}		g g^{-1}	Leaf nitrogen concentration, per unit of dry weighted leaf mass	

N_{min}	13.1	mmol m^{-2}	x-intercept of the linear relationship between N_{area} and $V_{c,max}$	
N_{tot}		mmol m^{-2}	Total canopy leaf nitrogen per unit ground area	
O		mbar	Atmospheric oxygen partial pressure	
P_{atm}		Pa	Mean atmospheric pressure (as a function of the elevation of the site)	
p_N			Curvature coefficient for nitrogen distribution within the canopy	
R	8.31	J mol^{-1} K^{-1}	Universal gas constant	
RH		%	Relative Humidity of the ambient air	
SJ_{max}	643	J K^{-1} mol^{-1}	Entropy term of the J_{max} response parameter	(3)
$SV_{c,max}$	656	J K^{-1} mol^{-1}	Entropy term of the $V_{c,max}$ temperature response	(3)
s_N	0.42	mmol µmol^{-1} s	Slope of the linear relation between N_{area} and $V_{c,max}$ at $T = T_{ref}$	
T		K	Air (ambiant) temperature	
T_{ref}	293	K	Reference temperature	
$\Gamma^{*T_{ref}}$	44	µbar	CO_2 compensation point of photosynthesis in the absence of mitochondrial respiration at $T = T_{ref}$	(1)
θ_l	0.7		Curvature of the leaf response of electron transport (J) to irradiance	(2)
θ_c	0.9		Curvature factor of response of canopy photosynthesis to irradiance	(2)

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TABLE S2. Main equations of the model

Note: The equations of the Clear Sky Model giving I_{0b} and I_{0d} under cloudless conditions are not described.

Equations	Description	No.
Canopy light interception		
$g(z_l) = 2\varepsilon^3 \sin(z_l) / [\Lambda(\cos^2(z_l) + \varepsilon^2 \sin^2(z_l))^2]$ where $\Lambda = \varepsilon + \sin^{-1}(X)/X$, with $X = \sqrt{1-\varepsilon^2}$, if $\varepsilon < 1$ and $\Lambda = \varepsilon + \ln[(1+X)/(1-X)]/2\varepsilon X$, with $X = \sqrt{1-\varepsilon^{-2}}$, if $\varepsilon < 1$	Probability density function describing the fraction of leaf area with an inclination between z_l and $z_l + dz_l$ in the ellipsoidal leaf distribution model	(1)
$\overline{z_l} = \frac{2}{\pi} \int_0^{\pi/2} g(z_l)\, z_l\, d\alpha$	Average leaf inclination angle	(2)
$G(z_S, a_S) = \frac{1}{2\pi} \int_0^{2\pi} \int_0^{\pi/2} \lvert\cos\theta\rvert\, g(z_l) dz_l\, da_l$	Projection of the foliage area on a plane perpendicular to the incident beam direction averaged over leaf elements of all orientations	(3)
$k_b = G(z_S, a_S) / \cos a_S$	Beam radiation extinction coefficient	(4)
$\rho_{cb} = \left\lvert 1 - \exp^{2\rho_{hcb} k_b / (1+k_b)} \right\rvert$ with $\rho_{hcb} = \left[1-(1-s)^{0.5}\right] / \left[1+(1-s)^{0.5}\right]$	Canopy reflection coefficient for beam PAR	(5)
$\rho_{cd} = \int_0^{\pi/2} \cos z_S\, \rho_{cb}\, dz_S$	Canopy reflection coefficient for diffuse PAR	(6)

$f_{sun}(Fh) = \exp^{-k_b F_h}; f_{sha} = 1 - f_{sun}$	Sunlit and shaded fraction at a given height F_h	(7)
$I_{bl}(F_h) = (1-\rho_{cb})\ (1-\sigma)^{0.5}\ k_b\ I_{0b}$	Incident beam light at a given height F_h per unit leaf area	(8)
$I_{dl}(F_h) = (1-\rho_{cd})(1-\sigma)^{0.5}\ k_d\ I_{0d} \exp^{(-(1-\sigma)^{0.5} k_d F_h)}$	Diffuse light at a given height F_h per unit leaf area	(9)
$I_{sl}(F_h) = I_{0b}\left[(1-\rho_{cb})(1-\sigma)^{0.5}\ k_b \exp^{(-F_h (1-\sigma)^{0.5} k_b)} - (1-\sigma)^{0.5}\ k_b \exp^{(-F_h k_b)}\right]$	Scattered light at a given height F_h per unit leaf area	(10)
$I_{csun} = \int_0^{LAI} \left[I_{bl}(F_h) + I_{dl}(F_h) + I_{sl}(F_h)\right] f_{sun}(F_h) dF_h\ ; I_{csha} = \int_0^{LAI} \left[I_{dl}(F_h) + I_{sl}(F_h)\right] f_{sha}(F_h) dF_h$	Incident radiation on the sunlit and shaded fraction	(11)
Nitrogen distribution within the canopy		
$N_{red} = N_{tot} - LAI\ N_{min}$	Nitrogen pool which can be distributed per unit ground area	(12)
$N_{area}(F_h) = N_{min} + N_{red}(1-(F_h / LAI)^{p_N})^{1/p_N} (LAI\Omega)^{-1}$ with $\Omega = \int_0^1 (1-x^{p_N})^{1/p_N} dx$	Nitrogen leaf concentration at a given height F_h per unit leaf area	(13)

Photosynthesis and stomatal conductance models		
$Vc_{max}^{T_{ref}}(F_h) = s_N\left[N_{area}(F_h) - N_{min}\right]$ (in μmol CO_2 m^{-2} leaf s^{-1})	Photosynthetic RUBISCO capacity at $T=T_{ref}$	(14)
$J_{max}^{T_{ref}}(F_h) = 2.1\,Vc_{max}^{T_{ref}}(F_h)$ (in μmol electron m^{-2} leaf s^{-1})	Potential rate of electron transport rate at $T=T_{ref}$	(15)
$X_{max}^{T}(F_h) = X_{max}^{T_{ref}}(F_h)\,\exp^{(H_a\,(T-T_{ref})/(T_{ref}\,T\,R))}\left[1+\exp^{(T_{ref}\,S-H_d)/(T_{ref}\,R)}\right]/\left[1+\exp^{(T\,S-H_d)/(T\,R)}\right]$	Temperature dependence of Vc_{max} and J_{max} (generic form)	(16)
$V_{c(sun/sha)} = \int_0^{LAI} V_{c\,max}(F_h)\,f_{(sun/sha)}(F_h)\,dF_h$ (in μmol CO_2 m^{-2} ground s^{-1})	Photosynthetic capacity of the sunlit / shaded fractions	(17)
$X^{T} = X^{T_{ref}}\ \exp^{(E_a\,(T-T_{ref})/(T_{ref}\,T\,R))}$	Temperature dependence of K_c, K_o and Γ^* (generic form)	(18)
$A_{c(sun/sha)} = Vc_{(sun/sha)}\ \ (C_i - \Gamma^*)/(C_i + K_c\,(1+O/K_o))$	RUBISCO-limited rate of photosynthesis of the sunlit / shaded fractions	(19)
$\theta_l\,J^2 - (I_{le}+J_{max})\,J + I_{le}J_{max} = 0$ with $I_{le} = I_{c(sun/sha)}(1-f)/2$	Irradiance dependence of electron transport rate of the sunlit / shaded fractions	(20)
$A_{j(sun/sha)} = J\,(C_i - \Gamma^*)/4(C_i + 2\Gamma^*)$	Electron-transport limited rate of photosynthesis of the sunlit / shaded fractions	(21)
$A_{gc} = A_{gcsun} + A_{gcsha}$; with A_{gcsun} and A_{gcsha} the smallest roots of $\theta_c\,A_{gc(sun/sha)}^{\ 2} - (A_{j(sun/sha)} + A_{c(sun/sha)})\,A_{gc(sun/sha)} + A_{j(sun/sha)}A_{c(sun/sha)} = 0$	Gross canopy photosynthesis rate	(22)

$R_d = 0.0089\,(V_{csun} + V_{csha})$	Dark respiration rate	(23)
$C_a = n_{CO_2}\,P_{atm}/10^6$	Ambient CO_2 partial pressure	(24)
$C_s = C_a - (A_{gc} + R_d)1.37P_{atm}/g_{sH_20}$	Leaf surface CO_2 partial pressure	(25)
$g_{sH_2O} = g_{min\,H_2O} + m(A_{gc} + R_d)10^{-6}\,(RH/100)/C_s$	Stomatal conductance to water vapour	(26)
$g_{totCO_2} = (1.6/g_{sH_2O} + 1.37/g_{bH_2O})^{-1}$	Total conductance to CO_2	(27)
$C_i = C_a - (A_{gc} + R_d)\,P_{atm}/g_{totCO_2}$	Intercellular CO_2 partial pressure obtained by coupling net assimilation rate conductance using Fick's law	(28)

Article 1B

Decreased aboveground primary productivity of alpine tundra in response to earlier snowmelt.

Baptist, F., Flahaut, C., Streb, P. and Ph. Choler
In prep. for *Oecologia*

Monolithes (traitement E) enterrés au sommet d'une butte afin de limiter l'enneigement durant l'hiver. Lieu : Parcelle expérimentale de la Station Alpine Joseph Fourier avant les premières neiges de l'hiver (2005). Photo : F. Baptist.

Abstract

Snow deposition and thermic regime are rapidly changing in alpine tundra and may lead to longer growing season but also higher risks of frost events in early spring. Alpine tundra from late snowmelt sites may be particularly vulnerable to these climatic changes. Large samples of alpine grassland were grown in monoliths for two consecutive years. Snow cover was manipulated to test for the effects of an early snowmelt (E treatment), an inconsistent winter snow cover (I treatment) and a late snowmelt (L treatment) on plant functional traits, Leaf Area Index (LAI) and Aboveground Net Primary Productivity (ANPP). Besides, we examined the seasonal growth of the locally dominant sedge, *Carex foetida*. To address the potential effect of frost events, we measured freezing temperatures of the dominant graminoid species.
Despite an extended growing season, aboveground biomass, productivity and LAI in the E and I treatments were either reduced or equalled to the values measured in L treatment However, we found no changes in water availability, soil nitrogen content and leaf nitrogen content that could explain these effects. The seasonal growth of *Carex foetida* clearly indicated that this species does not benefit from a prolonged snowfree period. Recorded temperature at the beginning of plant growth in I and E treatments were frequently close to the freezing temperature of the dominant species (≈ -10°C), suggesting that frost events may impair plant productivity. We concluded that (1) the weak plasticity in the phenological response of alpine plants and (2) the detrimental effect of frost events explain why alpine tundras from late snowmelt sites may not benefit from an increased growing season length.

Keywords : ANPP (Aboveground Net Primary Productivity), snowbed species, freezing point, growing season length, *Carex foetida*, *Poa alpina*, *Alopecurus gerardi*, plant functional traits, frost

Introduction

Recent advances in climatic research predict that the increase in greenhouse gaz concentration will lead to an average temperature increase of between 1 and 6°C and a modification of snow precipitation patterns, soil moisture and snow cover (IPCC, 2007). These climatic changes may have a particularly large impact in alpine and arctic tundra ecosystems. In addition to higher temperatures (Noguès-Bravo et al. 2007; Serreze et al. 2000), temperate mountain ranges from the Northern hemisphere may experience decreased snow deposition (Beniston 2005; Dye and Tucker 2003; Serreze et al. 2000). This will have important consequences through changes in the timing of snowmelt and in soil temperature and moisture regime. Indeed, reduced snow cover during winter associated with earlier snowmelt would result in longer growing seasons and higher cumulated Growing Degree Days (GDD) which could benefit vegetation growth (Theurillat and Guisan 2001). In contrast, under these conditions plants may be exposed to higher risks of frost damage, and earlier dehardening or late-summer water stress (Edwards et al. 2007; Inouye 2000; Wipf et al. 2006). Alpine species tightly associated with late snowmelt sites might be particularly vulnerable to such changes.

In snow-covered ecosystems, the growing season is compressed and curtailed at both ends by subzero temperatures. Time available for growth largely determines aboveground biomass and productivity which feedbacks nutrient availability. It also affects community composition by altering the performance of species with different strategies of growth and reproduction (Galen and Stanton 1993). Establishing causal links between growing season length and plant performance is crucial to predict consequences of global climate change on species distribution and carbon balance of snow-covered ecosystems.

In alpine tundra, Choler (2005), Kudo et al. (1992; 1999) and Walker et al. (1993) reported a tight linkage between snowmelt dates and instantaneous productivity along snow cover gradients in alpine habitats. They showed a higher productivity and aboveground biomass in late snowmelt communities dominated by deciduous species. However, these results may reflect not only the effect of growing season length but also others factors such as soil depth, soil fertility or historical contingencies (Thorhallsdottir 1998). The impact of variable snowmelt dates have also been assessed experimentally. A large number of studies adressed the effects of warming (de Valpine and Harte 2001; Dunne et al. 2003; Jones et al. 1997; Molau 1997; Price and Waser 2000; Saavedra et al. 2003; Starr et al. 2000; Stenstrom and

Jonsdottir 1997; Stenstrom et al. 1997; Welker et al. 1997) and earlier or delayed snowmelt (Dorrepaal et al. 2003; Dunne et al. 2003; Saavedra et al. 2003; Scott and Rouse 1995; Wahren et al. 2005) on plant growth, flowering or phenology of particular arctic and alpine species. In general, plants flowered earlier in response to longer growing season and higher cumulated GDD. However, they only considered snowmelt gradients *in natura* or performed experiments with artificial warming leading to an increase in air temperature at snowmelt. (de Valpine and Harte 2001; Starr et al. 2000; Wipf et al. 2006). Besides, only a few studies addressed the effects of decreasing snow deposition on the aboveground biomass or productivity.

Early snowmelt may have different effects on plant growth. Soil nitrogen availability could be modified since snowmelt is associated with an increase in nitrogen availability due to both the penetration of melted water into the soil (Bowman 1992) and to the death of microbial populations (Brooks et al. 1998; Jaeger et al. 1999). Early snowmelt may also lead to a decrease in soil moisture during the growing season. Finally, individual frost events may damage plants and reduce growth (Inouye 2000). It remains unclear to what extent all these parallel and opposite effects will affect plant growth of the dominant species and the aboveground net primary productivity of late snowmelt ecosystems.

The aims of this study were to examine the effects of (a) a late snowmelt (b) an early snowmelt and (c) an inconsistent winter snow, considered as extrems conditions for these communities, on aboveground biomass, productivity, Leaf Area Index (LAI) of late snowmelt communities as well as plant functional trait values and vegetative growth of dominant alpine plant species. The plant functional traits measured were Specific Leaf Area (SLA), Leaf Dry Matter Content (LDMC) and Leaf Nitrogen Content (LNC) as these are related to carbon fixation and productivity (Garnier et al. 2004; Quétier et al. 2007). We also measured soil moisture and air temperature as well as the freezing temperatures of the three dominant species presents in this community.

Materials and methods

Study site and species

On September 27th 2005, 15 monoliths (25 cm deep and 45×35 cm surface) were randomly selected and then excavated from a large late snowmelt site located near the Agnel pass in the South-Western French Alps (2744 m above sea level, abbreviated as m.a.s.l. henceforth). The mean snowmelt time of this site was early june (Choler, personal observations). Monoliths were transferred into plastic pots with drains and transported to the Station Alpine J. Fourier, an alpine field research station located at the Lautaret pass at 2100 m.a.s.l. (45°7'N, 6°5'E). Soils were stagnogley enriched in clay. Organic matter of the soil was 8 ± 0.3 %. *Carex foetida* (Cyperaceae) was the dominant species followed by *Alopecurus gerardi* (Poaceae) and *Poa alpina* (Poaceae). *C. foetida* and *A. gerardi* can be considered as specialists from late snowmelt sites whereas *P. alpina* is a generalist species in these temperate alpine tundras. The relative abundance of these species at harvest time was similar to relative abundance measured in L treatment in 2006. Relative abundance of *C. foetida* accounted for more than 50%, *A. gerardi*, 31%, other graminoïds 3% and forbs 14% (see Table 2).

Experimental design and treatments

At the end of September 2005, in the alpine field station, the 15 monoliths were randomly allocated to the following three treatments: (1) a limited winter snowpack whereby 5 monoliths were disposed on the summit of a hillock (Inconsistent treatment, I), (2) a winter snow-covered but early snowmelting treatment (Early treatment, E) and (3) a delayed snowmelt treatment whereby 1-2 m of snow were added on another 5 monoliths and these were placed in the shade to delay snowmelt (Late treatment, L). This latter treatment resulted in a snowmelt time as close as possible to that of the native site. All sets of monoliths were placed 5 to 10 m away from each others and were buried in the soil. First snow falls occured on the 1st of december 2005. In order to avoid microsite effects during the growing season, all the monoliths were transfered, from snowmelt to the end of October, to a common location in the common garden of the alpine research station and again buried in the soil. The same snow treatments were repeated during the winter 2006-2007.

Edapho-climatic recordings

Hourly soil temperature was recorded during the 2005-2006 and 2006-2007 winters in each treatment using Hobo probes (Onset computer corporation, Bourne, MA, USA) buried at 5 cm

96 belowground. Persistent day temperatures close to 0°C (usually between -1 and 1°C) were
97 considered indicative of a persistent snow cover. Cumulated GDD were calculated as the sum
98 of daily mean temperature above 0°C. Gravimetric soil water content (m^3 m^{-3}) was measured
99 in each monolith at peak standing biomass in July 2006 and 2007. Simultaneously, we
100 measured volumetric soil moisture using Time Domain Reflectometry (TDR) every 3 weeks
101 in each monolith during the 2007 growing season. Finally, we used PAR light sensors located
102 in the common garden to estimate cumulated mean global radiation. To compare climatic
103 conditions in the field and in the garden, we recorded soil temperature at three late snowmelt
104 sites during five years (2000-2005) near the Galibier pass (2650 m.a.s.l.).
105 At peak standing biomass during the 2006 and 2007 growing seasons, 3 soil cores (1 cm
106 diameter) were extracted from each monolith and pooled for analysis of total inorganic
107 nitrogen content. Nitrogen was extracted from fresh soil sample (~ 10 g) with 2M KCl after
108 sieving at 2 mm. Soil extracts were analyzed for ammonia (NH_4^+) and nitrate/nitrite (NO_3^-
109 /NO_2^-) contents using an FS-IV auto-analyzer (OI-Analytical, College Station, TX, USA).
110
111 ***Plant responses***
112 Species abundance, biomass measurements and plant functional traits were realized at
113 peak standing biomass on the 15th of July 2006 and on the 3rd of July 2007. Protocol are given
114 below.
115 The mean cover of plants within each monolith was estimated using line transect
116 method. Linear measurements of plant intercepts were performed along the course of two
117 lines (record each 2 cm along the line, total of 50 points per monolith,). The mean cover of a
118 species corresponded to the area of ground covered by the vertical projection of its green
119 aerial parts. In each monolith, total standing biomass was collected in a randomly located
120 square plot of 5 by 5 cm. Instantaneous productivity was determined by dividing standing
121 biomass by the time between snowmelt and peak biomass. Under these conditions, variations
122 in productivity result both from variations in growing season length and in biomass produced.
123 LAI was determined by measuring total projected area of green leaves with a leaf area meter
124 (WinDIAS, Delta-T Device Ltd, Cambridge, UK).
125 Species selected for trait measurement were *C. foetida*, *A. gerardi* and *P. alpina*
126 (measurements in 2007 only for the latter species). They accounted for more than 70% of the
127 maximum standing live biomass of the community. SLA, LDMC and LNC were measured
128 following standard protocols (Cornelissen et al. 2003) on the cohort 2 in the case of *C. foetida*

(see below) and on the younger leaf in the case of *A. gerardi* and *P. alpina*. Briefly, 3 sets of leaves were collected in each monolith and maintained in moist paper. After rehydratation for 6 hours, they were weighed and total surface was measured using a leaf area meter (Gatehouse scientific instruments LTD, Norfolk, UK) before being dried for 48h at 60°C and weighed for dry mass. Leaves were further grinded and analyzed for carbon and nitrogen using a CHN analyzer (Thermo Electron Corporation, Madison, USA).

Diachronic study of C. foetida growth

We monitored leaf elongation of the dominant sedge *C. foetida,* during the 2006 and 2007 growing seasons, from snowmelt until senescence, on 3 randomly marked tillers per monolith. We considered this measurement as an adequate surrogate of leaf growth as leaf width was strongly correlated to leaf length (F. Baptist, personal observation). Tillers are monocarpic and leaves senesce rapidly if an inflorescence is produced. However, it was impossible to separate vegetative and flowering tillers at snowmelt and reproducing tillers were excluded *a posteriori*. We measured green and total leaf length from the oldest to the youngest leaf every 2 to 4 weeks. The difference due to senescing tips increased over the growing season. Percentage of senescence was estimated from the following formula:

$$\text{Senescent fraction (\%)} = \frac{\text{length}_{\text{total leaves}} - \text{length}_{\text{green leaves}}}{\text{length}_{\text{total leaves}}} \cdot 100$$

The species produced in average 9 to 11 leaves per year, from their basal meristems. We pooled them into three cohorts (three leaves per cohort). Cohort 1 corresponded to leaves which were initiated the previous summer and which displayed weak growth at the beginning of the growing season (senescent fraction > 50%). Cohorts 2 were leaves initiated at snowmelt. Cohorts 3 were initiated later in the growing season. To adequately compare treatments, we expressed growth as a function of cumulated GDD in addition to Julian day following snowmelt.

Freezing point and frost tolerance

In June 2007 just after snowmelt, 3 vegetation cores were collected in late snowmelt sites near the Galibier pass (2650 m.a.s.l.). During the following 2 days, freezing temperatures of *C. foetida*, *A. gerardi* and *P. alpina* leaves were measured (n=3 for *A. gerardi* and 4 for *C. foetida* and *P. alpina*) . We used a metal chamber cooled by an antifreeze liquid. Two thermocouples (0.5mm and 1mm diameter for leaf and air temperature respectively) were connected to a CR800X Campbell data logger (Campbell Scientific Inc. Logan, UT, USA)

and measured every 5 sec. Maximum, minimum and mean temperatures were recorded every 2 min. Freshly cut leaves were placed in contact to the leaf thermocouple and the chamber was closed. The temperature inside the chamber was decreased progressively by a programmed water bath at a rate of 1°C every 5 min to a final leaf temperature of -15°C and then increased up to 25°C. Late treatments were carried out with temperatures declining at 2°C per hour. At their freezing temperature, leaf temperature increased due to heat emission which was detected by the first thermocouple (Ball et al. 2002). Parallel measurements of fluorescence also indicated freezing temperatures through a strong increase in minimal fluorescence F_0 (Neuner and Pramsohler 2006). To confirm freezing-induced damage, intactness of leaf cells was assayed directly before and after determining the freezing temperature as the percentage change in conductivity following incubation of leaves in de-ionized water at room temperature for 24 h, before and after breakage of cells by boiling (10 min) (Webb et al. 1996).

Statistical analysis

Repeated-measures analysis of variance (RMANOVA) was carried out to compare overall differences caused by snow treatment effects (between subject effects) and the interaction between treatments and time (within-subject effects) on aboveground biomass, productivity, LAI and leaf traits. A one-way ANOVA was used to test for the effects of snow treatments on *Poa alpina* leaf functional traits (measured only in 2007). In all cases, individual monoliths were treated as replicates and tiller based-variables were analyzed after averaging per monolith. Similarly, differences in seasonal growth of *C. foetida* leaves between snow treatments were compared only at maximal growth using a RMANOVA. The variable analyzed was the mean green length of each cohort averaged on the 3 tillers selected in each monolith. Freezing temperatures and intactness of cells were analyzed using the non-parametric Kruskall-Wallis test. All analyses were performed with the Jump software (SAS Institute Inc., Cary, North Carolina, USA).

(a) Time	Year	Late	Early	Inconsistent
Snowmelt (date)	2006	08.06.06	15.05.06	14.04.06
	2007	15.05.07	24.04.07	03.04.07
(b) Thermic regime				
Cumulated GDD at :	2006	552/1685	760/1890	974/2067
Biomass peak / End of season	2007	597/1806	746/1955	893/2102
Mean wintertime soil temp. (°C)	2006	-0.8 (1)	-0.9 (1.0)	-3.3 (3.4)
	2007	0.3 (1.5)	0.3 (1.6)	-0.4 (2.3)
Number of frost events	2006	0/ --	6/-5.7	28/-8.1
(<0°C) / Min. air temperature (°C)	2007	6/-4.4	9/-4.4	21/-13.1
(c) Solar radiation				
Cumulated daily mean solar radiation from snowmelt to peak standing biomass (mol photons m^{-2})	2006	1431	2433	3641
	2007	1908	2598	3385
(d) Water status				
Soil moisture measured at mid-season (mass %)	2006	22.0 (1.5)	25.0 (2.4)	29.0 (1.7)
	2007	26.1 (3.5)	27.6 (3.8)	29.4 (2.3)
(e) Soil fertility (mid-season)				
NO_3 (mg/g soil DW)	2006	0.32 (0.25)	0.63 (0.31)	0.45 (0.12)
	2007	3.1 (0.4)	0.92 (0.11)	1.7 (0.4)
NH_4 (mg/g soil DW)	2006	10.2 (1.1)	9.5 (2.1)	11.2 (2.3)
	2007	8.7 (0.8)	5.3 (0.4)	5.1 (0.9)

Table 1 Edapho-climatic conditions experienced by monoliths in response to snow treatments in 2006 and 2007. (a) Time, (b) Thermic regimes, (c) Solar radiation, (d) Water status measured at mid-season (15^{th} and 3^{rd} of July), (e) Soil fertility. Mean ± standard deviation (SD) in the case of mean winter soil temperature, mean ± standard error (SE) for water status and soil fertility. See text for statistical details.

Results

Effects of snow treatment on edapho-climatic conditions

In the common garden, L monoliths experienced a sligthy shorter winter than in the field. In late snowmelt sites (at 2650 m.a.s.l.), snowmelt occurred in early June compare to 08/06/06 and 15/05/07 in the common garden (Table 1). Cumulated GDD reached on average 1200 in the field (average for 5 years) compared to more than 2000 in the common garden. However, at peak standing biomass, cumulated GDD between the L treatment and field did not differ and equalled approximately 600 cumulated GDD (see Baptist and Choler 2008).

Snow treatments were effective in decreasing snow deposition in the I treatment and in delaying snowmelt in L treatment. Cumulated GDD (2006/2007) was offset by 214/147 and by 422/286 in E and L treatments respectively. Winter soil temperature differed strongly between treatments. I monoliths experienced inconsistent snow cover during winter, and minimal air temperatures of -13.1°C in 2006 and -8.1°C in 2007, despite relatively higher mean winter soil temperature (Table 1, Fig. 1 and APPENDIX 1). In the E treatment, winter soil temperatures were not as harsh as in the I treatement. Finally, no frost events occurred in 2006 in the L treatment, whereas, in 2007, six frost events were observed (Table 1, Fig. 1 and APPENDIX 1). In the E and L treatments, mean winter temperature was close to 0°C. During the growing season, all treatments experienced similar temperature and water regimes ($F_{2,12}$=1.0, P=0.39) (Table 1, Fig. 1 and APPENDIX 1). Seasonal records of soil moisture during 2007 did not reveal any differences between snow treatments (data not shown). No effect of snow treatment on [NH_4^+] at peak standing biomass was observed ($F_{2,10}$=1.2, P=0.34) but we did detect a significant year effect ($F_{1,22}$=13.2, P=0.001), indicating higher concentration in 2007 compared to 2006. In contrast, [NO_3^-] was significantly affected by snow treatments ($F_{2,10}$=6.3, P=0.02). A significant year effect was detected with higher concentrations in 2007 than in 2006 ($F_{1,10}$=21.6, P<0.001). The interaction was significant ($F_{2,10}$=6.0, P=0.02) indicating inconsistent patterns in response to snow treatments over the two years : [NO_3^-] was highest in the L treatment in 2007 and lowest in 2006.

Species / Groups		Late	Early	Inconsistent
C. foetida	2006	53 (5)	57 (4)	55 (3)
	2007	39 (7)	50 (4)	46 (5)
A. gerardi	2006	31 (7)	29 (4)	27 (4)
	2007	33 (7)	28 (2)	19 (5)
Others graminoids	2006	3 (1)	7 (2)	4 (3)
	2007	14 (1)	20 (5)	24 (5)
Forbs	2006	14 (5)	6 (5)	11 (4)
	2007	14 (4)	3 (2)	10 (5)

Table 2 Relative abundance (%) of *Carex foetida*, *Alopecurus gerardi* and two functional groups reported as "Others graminoids" and "Forbs" in 2006 and 2007 in each snow treatments. In 2007, the group "Others graminoids" was mainly represented by *P. alpina*. Values are the mean ± standard error (SE) See text for statistical details.

Community composition

Relative abundance of *C. foetida* and *A. gerardi* did not respond to snow treatments (respectively $F_{2,12}$=0.6, P=0.54; $F_{2,12}$=0.6, P=0.55) (Table 2) and was similar to that in the native site (personal observation). Similarly, relative abundance of others graminoïds (mainly *Poa alpina)* and forbs did not respond to snow treatments ($F_{2,12}$=0.98, P=0.40; $F_{2,12}$=1.6, P=0.24). *Poa alpina* showed year effect with a marked increased in 2007 ($F_{1,12}$=23.9, P<0.001) irrespective of treatments. The snow treatment × year interaction was never significant.

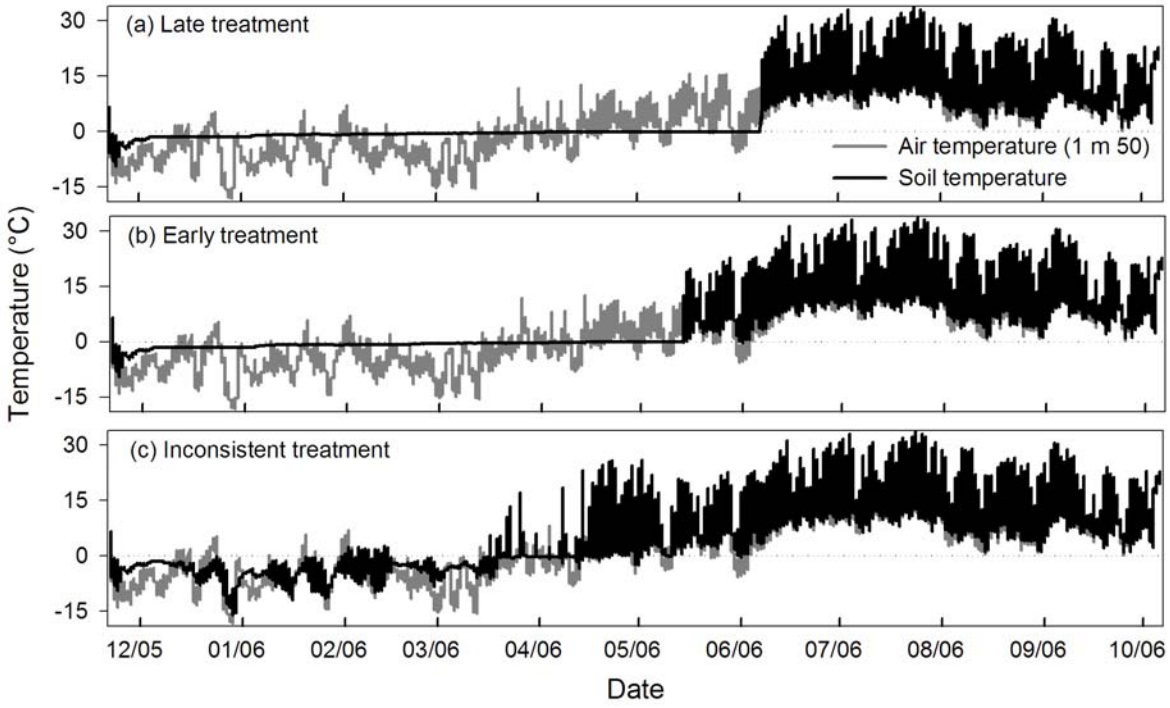

Fig. 1 Daily mean soil temperature (5cm deep) in the Late (a), Early (b) and Inconsistent (c) treatments (black) and air temperature (grey) in 2005-2006 (see APPENDIX 1 for 2006-2007 data).

Plant responses to snow treatments

Aboveground biomass and productivity were significantly affected in the I treatment (respectively $F_{2,12}$=4.2, P=0.04 and $F_{2,12}$=23.9, P<0.0001) and displayed a marked decrease in comparison to the L treatment (Fig. 2A and 2B). Aboveground biomass between the E and I treatments and between the E and L treatments were only marginally different. A year effect was detected ($F_{1,12}$=8.0, P=0.01; $F_{1,\ 12}$=11.5, P=0.005) indicating higher biomass and productivity in 2007 compared to 2006. Interactions were not significant ($F_{2,12}$=0.16, P=0.84; $F_{2,12}$=0.12, P=0.88). LAI was negatively affected in the I treatment ($F_{2,12}$=7.83, P=0.006) over the two seasons (Fig. 2C). Similarly to aboveground biomass and productivity, LAI was lower in the E compared to the L treatments but not significantly. Overall, it was significantly higher in 2007 ($F_{1,12}$=7.4, P=0.02) compared to 2006 as for the previous variables. The treatment × year interaction was not significant ($F_{2,12}$=0.82, P=0.46).

The accumulated leaf length of cohort 2 accounted for more than 46% ± 1 of total accumulated leaf length of the tillers, whereas cohort 1 and 3 accounted for 22% ± 1 and 32% ± 1 respectively. Maximal green leaf length for cohort 1 did not differ between treatments ($F_{2,9}$=0.99, P=0.41) indicating that growth was not enhanced by a longer growing season (Fig. 3). Leaves required similar cumulated GDD to reach their maximal biomass and hence date of peak standing biomass was slightly delayed in L treatment. Leaves from cohorts 2 and 3 displayed lower growth rate (per cumulated GDD) in the E and I treatments. They reached peak standing biomass for a higher cumulated GDD than in the L treatment (Fig. 3). Snow treatments resulted in significant differences in maximal green leaf length of cohort 2 ($F_{2,12}$=4.58, P=0.03). *C. foetida* leaves in the I and E treatments experienced lower maximal green leaf length than in the L treatment. At peak biomass, leaves from cohort 3, initiated later in season, did not show significant patterns in response to snow treatments ($F_{2,12}$=2.4, P=0.13).

SLA, LDMC and LNC of *C. Foetida*, *A. gerardi* and *P. alpina* were not consistently affected by treatments (Fig. 4). We detected a significant year effect in the case of *C. foetida* and *A gerardi* SLA which was nevertheless not consistent : SLA of *C. foetida* was higher in 2007 compared to 2006 ($F_{1,12}$=13.4, P=0.003) whereas the opposite was observed for *A. gerardi* ($F_{1,12}$ = 7.8, P=0.02).

Freezing temperature

Leaf freezing temperature of *C. foetida*, *A. gerardi* and *P. alpina* were -10.0°C, -12.0°C and -

299 9.3°C respectively and did not differ significantly (Kruskal-Wallis $\chi^2(2) = 5.59$, $P = 0.06$)
300 (Fig. 5). Intactness of cells as indicated by electrolyte leakage varied greatly between the three
301 species with less that 40% of cells destroyed in *P. alpina* tissue compared to more than 70%
302 in *C. foetida* and *A. gerardi (*Kruskal-Wallis χ^2 (2) = 7.84, P = 0.02).

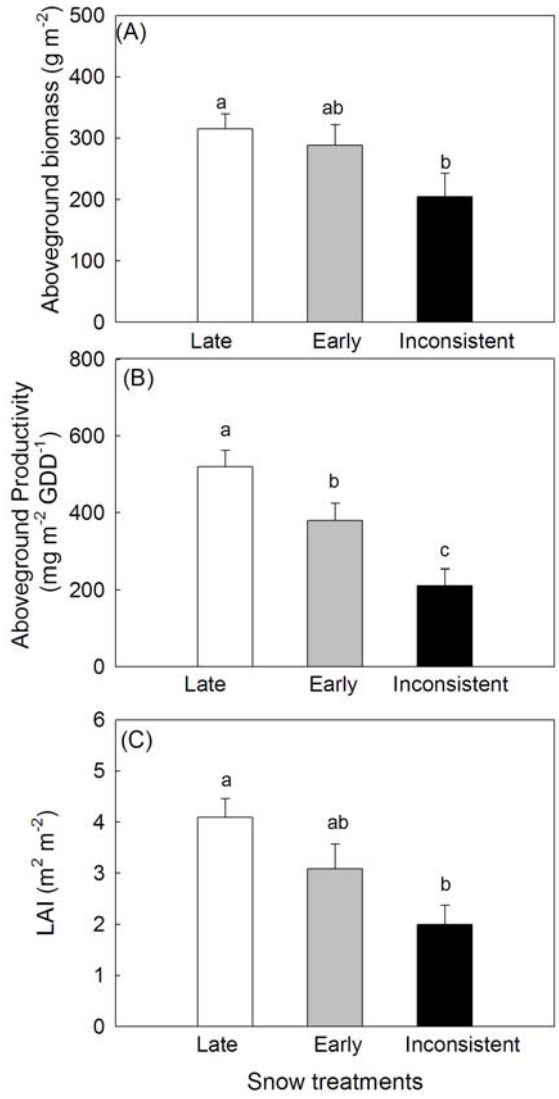

Fig. 2 Aboveground biomass (A), aboveground productivity expressed per cumulated GDD (B) and LAI (D) in response to snow treatments. Mean ± SE for each treatment for both year (n=15). Different letters indicate significant differences between treatments ($P<0.05$). See main text for more details on statistical analysis.

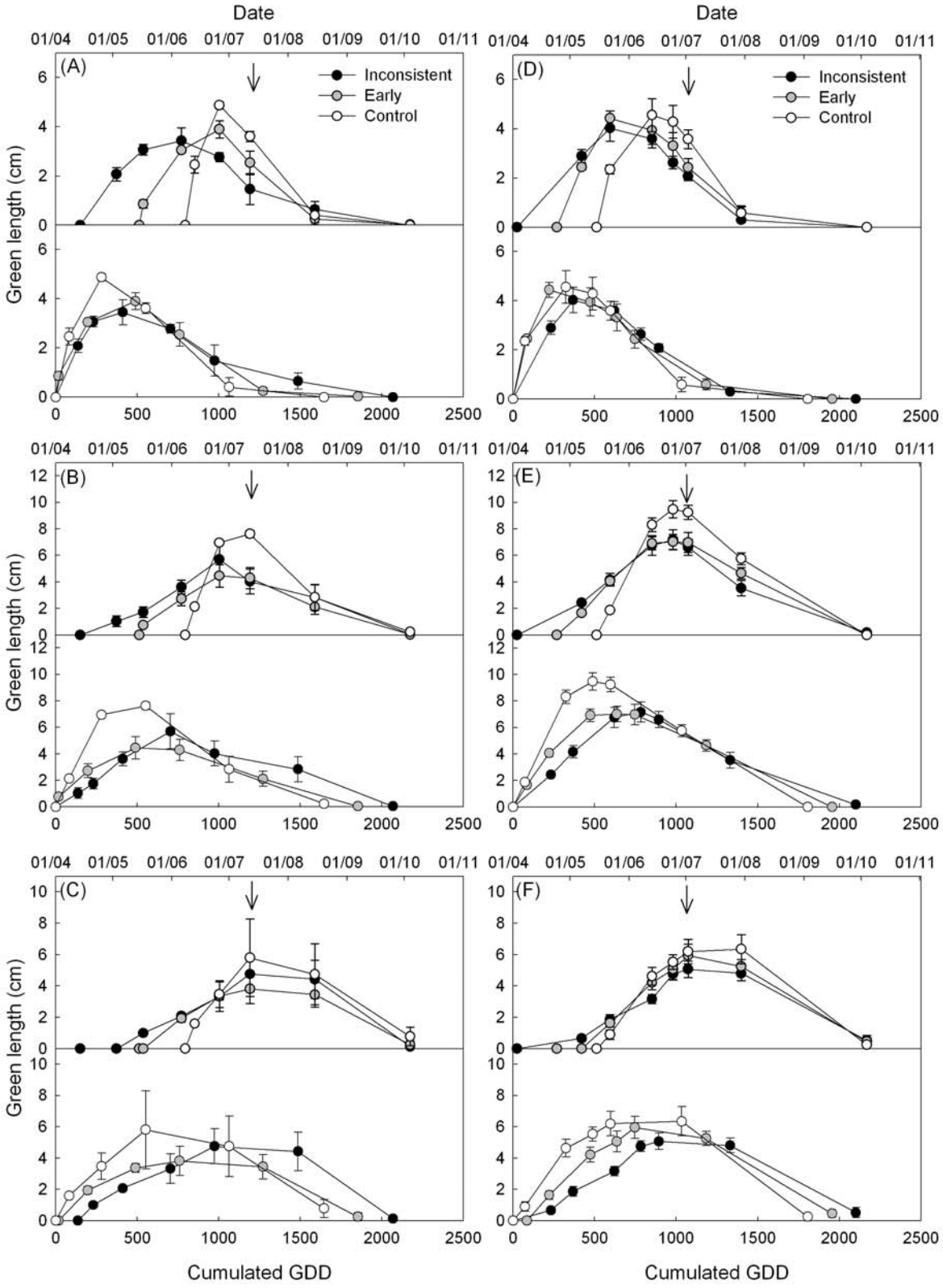

Fig. 3 Seasonal leaf growth for 3 cohorts of *C. foetida* leaves (cohorts 1/2/3, see text for further details) related to Julian days (upper) and to cumulated GDD (lower) in 2006 (A/B/C) and 2007 (D/E/F). Arrows indicate the day when aboveground biomass, LAI and leaf functional trait values were measured. See main text for more details on statistical analysis.

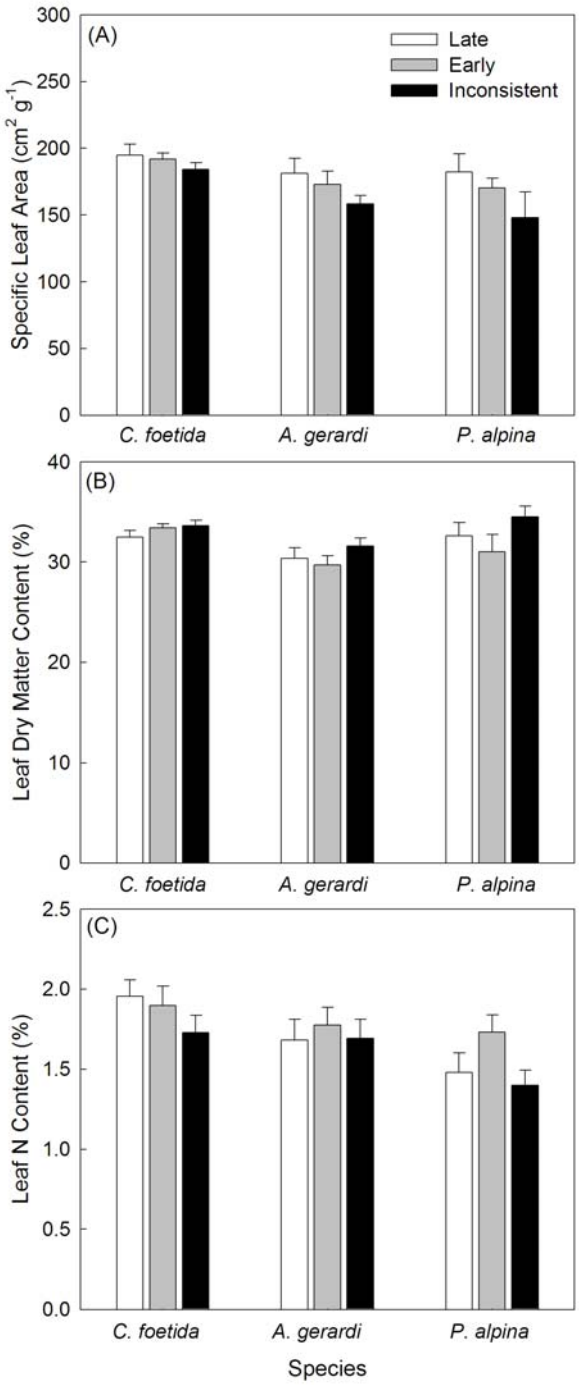

Fig. 4 Specific Leaf Area (A), Leaf Dry Matter Content (B) and Leaf Nitrogen Content (C) of the leaves of the three dominant species of late snowmelt sites (*C. foetida*, *A. gerardi* and *P. alpina*) in response to snow treatments. Leaf functional trait values were measured only in 2007 for *P. alpina*. See main text for more details on statistical analysis.

Discussion

Our result indicate that changes in growing season length and especially snowmelt timing can affect, in the short term, the aboveground biomass of dominant plant species from alpine late snowmelt sites. Indeed, despite an extended growing season, aboveground biomass and LAI in the E and I treatments were either reduced or equalled to the values measured in L treatment. As a result, instantaneous productivity (calculated from snowmelt date until peak standing biomass) was much lower in the I and E treatments compared to the L one. Similarly, the synchronic records of *C. foetida* growth demonstrated clearly that the length of the cohorts were not enhanced by the extending growing season.

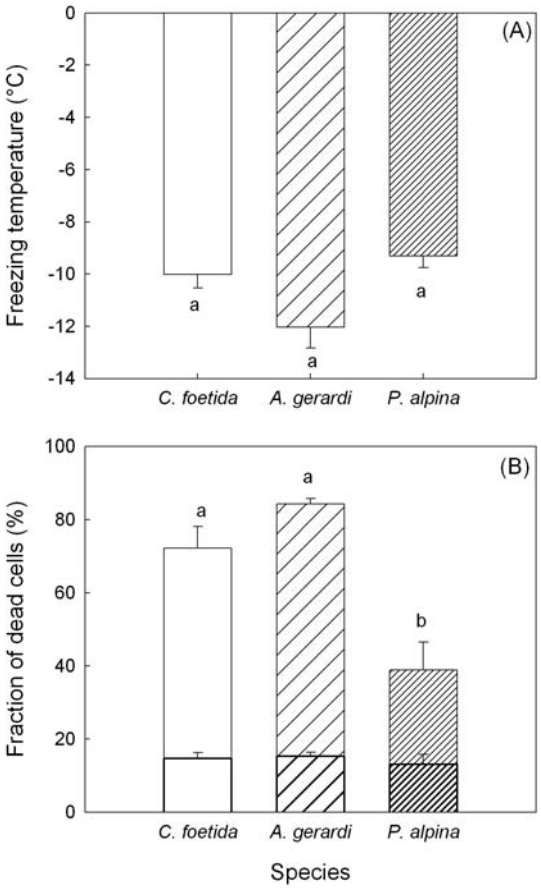

Fig. 5 Freezing temperature of new leaves (A) and electrolyte leakage in % (B) for *C. foetida*, *A. gerardi* and *P. alpina.* Higher and lower values correspond to samples measured after and before freezing treatment. Different letters indicate significant differences between species ($P<0.05$). See main text for more details on statistical analysis.

Galen & Stanton (1993, 1995) postulated that because of lower temperature, earlier snowmelt may limit respiratory costs and so further enhance growth. This is therefore in clear contrast to the results obtained in this study. Neutral and negative effects of early snowmelt on aboveground biomass have already been reported by Starr et al. (2000) and Wipf et al. (2006). Others studies yet suggested a positive effect on plant cover (Galen and Stanton 1993, 1995, de Valpine and Harte 2001) but only considered snowmelt gradients *in natura* or

performed experiments with artificial warming leading to an increase in air temperature at snowmelt.

Various explanations can be proposed to explain the similar aboveground productivity in E treatment compare to L one and can be related to direct environmental constraints or indirect ones through effect on plant functional traits. E Monoliths could be limited by water availability leading to a reduced plant growth. However, the soil moisture measured at peak standing biomass did not differ between treatments. Similarly, measurements through the season did not indicate differences between treatments in 2007 (data not shown). Finally, SLA and LDMC, which are good indicators of water stress (Cunningham et al. 1999, Wright et al. 2001, Gianoli 2004), did not differ between treatments suggesting that the soil moisture did not affect biomass production in this study.

Soil fertility could also lead to lower aboveground productivity. Indeed, during winter, deeper and longer-lasting insulating snowpack lead to higher microbial biomass and litter decomposition (Hobbie and Chapin 1996, Lipson et al. 1999). Furthermore growth is often thought to start at snowmelt as the penetration of melting water into the soil (Bowman 1992) and the death of microbial populations lead to an increase of nitrogen availability (Brooks et al. 1998). We thus expected that a shorter growing season would lead to higher aboveground productivity in the L treatment through increased soil fertility. However, despite a noticeable year effect, soil nitrogen concentrations measured at peak standing biomass could not consistently explain variations in aboveground biomass. Similar results were obtained at snowmelt in 2006 (data not shown). Total soil mineral N content were only instantaneous measurements and might not be representaive of N available to plants during the growing season. However, the values of LNC tend to support these conclusions, as they were not affected by the snow treatments. This corroborates the findings of Jaeger & Monson (1992) and Starr et al. (2000) who showed no variation of LNC in responses to warming or lengthened growing season. Spring growth of alpine plants is mainly sustained by the remobilization of N out of storage structures (Lipson et al. 1996, Monson et al. 2006). These internal constraints buffer the interanual variability of nitrogen availability in the soil and may enable plants to sustain higher tissue production with similar N concentration. Thus, neither fertility and water availability, nor intraspecific changes in leaf trait values explained the observed changes in aboveground biomass in response to earlier snowmelt.

Hence, it appears that only regenerative constraints can explain the lack of response of snowbed community to extend growing season. Sørenson (1941) distinguished two phenological patterns in tundra plants : periodic species, characterized by a fixed growing

period, controlled by genetic constraints and aperiodic species, for which growth and the production of new leaves are prolonged until conditions became unfavorable. Accordingly, the snowbed species studied here and in particularly *C. foetida* are periodic species and do not benefit form lengthened growing season. Hence, these results indicate that the capacity of the plants to increase their productivity will strongly depend on the intrinsic regenerative capacities

The reduced biomass observed in the I treatment compare to L was not expected and the underlying mechanisms are not clear. As stated above, direct environmental constraints, such as water availability or soil fertility and indirect ones through effect on plant functional traits can not explain these patterns. Regenerative constraints may limit enhanced growth in response to extended growing season but can not be responsible for a lower aboveground biomass. An alternative explanation lies in the impact of frost events on early season growth. Indeed, potentially damaging frosts to alpine plants during the growing season were repeatedly described (Körner 1999, Taschler and Neuner 2004). In early spring 2006 and 2007, the higher occurrence of severe frosts (≈ air temperature < -5°C) in the I treatment (Table 1) associated to radiative cooling (exposure to the cold nigth sky, leaf temperature < air temperature) may expose plants to temperature close to their freezing points (about -10°C, Fig. 5, Körner 1999). This hypothesis is comforted by the results of Wipf et al. (2006) who found a strong correlation between the number of frost events (temperature < 0°C) and the aboveground biomass of *Empetrum nigrum* and *Vaccinium vitis-idae*, two subalpine species. During winter, leaf primordial tissues are buried several centimeters belowground and thus are not exposed to damaging frost events (Körner 1999). However, during spring, relatively high diurnal temperatures have the potential to activate growth despite very cold nights (see Fig. 1). The rise of primordia at the soil surface exposes them to overnight freezing temperature and may lead to cell-death, particularly since plants deharden rapidly when temperature and photoperiod increase (Bannister et al. 2005). Despite a capacity to recover very fast, costs for the repair process might be too high. These conclusions are supported by the weak growth of leaves from cohort 2 of *C. foetida* in the I treatments which are initiated at the beginning of the growing season in contrast to cohort 3. Differences between these late and early emerging cohorts may reflect the immediate impact of freezing temperature on growth. However, why leaves from cohort 1 did not show lower growth in the I treatments compared to the E and L treatment remain unclear. Bannister et al. (2005) measured no differences in freezing temperatures between young and old leaves. Leaves from cohort 1

were overwintering leaves and they may display a higher degree of hardening than younger ones, recently grown from primordia. Frost resistance (i.e. the dead cell fraction after a frost event) at the beginning of the season could differ because of higher concentration of carbohydrates in cells (Bell and Bliss 1979).

Conclusion

Our results tend to point out the importance of (1) the alpine plant' capacity to lengthen their growth cycle in response to longer growing season and (2) to support frost events in order to predict the effects of global change on the diversity and the productivity of alpine meadows. During the next decades, the frequency of frosts events may increase in alpine environments due to a reduction of snow cover and earlier snowmelt (Inouye 2000, Inouye 2008). The strong variability in frost resistance observed between the three main species studied here, in addition to the results reported by Bannister et al. (2005) and Taschler & Neuner (2004), suggest that responses to frost events will be species-specific. Aperiodic species might be favored compared to periodic species as the production of new leaves later in season may compensate for losses due to frost events in spring. Larger comparative analysis of frost resistance and leaf phenology along snowmelt gradient in alpine tundras is necessary to assess in greater details how these ecosystems will respond to climatic changes.

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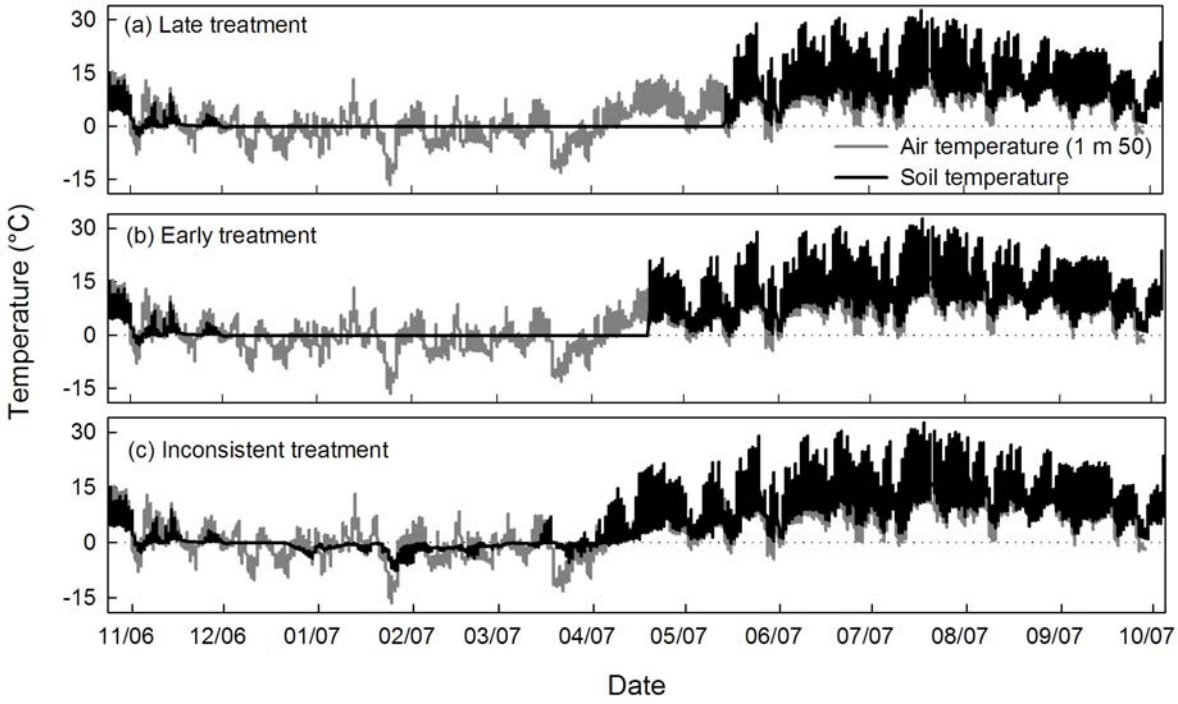
Appendix 1



Appendix 1. Daily mean soil temperature (5cm deep) in the Late (a), Early (b) and Inconsistent (c) treatments (black) and air temperature (grey) in 2006-2007.

Article 1C

^{13}C and ^{15}N allocations of two alpine species from contrasting habitats reflect their different growth strategies

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Système de marquage (^{13}C) mis en place à la Station Alpine Joseph Fourier durant l'été 2005 et 2006. Photo : S. Aubert.

Abstract

- Intense efforts are currently devoted to disentangling the relationships between plant carbon allocation patterns and nitrogen availability.
- Here, we applied $^{12}C/^{13}C$ and $^{14}N/^{15}N$ isotope techniques to elucidate C and N partitioning in two alpine species characterized by constrasting nutrient economies : a slow-growing species, *Kobresia myosuroides* (KM) and a fast-growing species, *Carex foetida* (CF) located at the ends of a snowmelt gradient in the alpine tundra (French Alps).
- CF allocated higher labelling-related ^{13}C content belowground, produced more root biomass. Accordingly, assimilates transfered to roots were preferentially used for growth than respiration and tend to favor N reduction in this compartment. In addition, this species had a higher ^{15}N uptake efficiency than KM and a higher N translocation aboveground.
- The results obtained with this couple of species thus suggest that at the whole-plant level, there is a compromise between N acquisition and C allocation for an optimized growth.

Keywords : carbon and nitrogen isotope labelling, alpine plant, respiration, photosynthesis, root:shoot ratio, *Kobresia myosuroides, Carex foetida*, snowmelt gradient.

Introduction

Distribution, productivity and carbon assimilation of plants are strongly affected by variations in soil fertility. Intense efforts are currently devoted to elucidating the relationships between plant carbon allocation patterns and nitrogen availability in order to improve our understanding of carbon cycles and sequestration pathways in terrestrial ecosystems (Trumbore 2006). This is particularly important in the context of global change, in which growth and carbon fixation tend to be promoted by atmospheric CO_2 increase but depend on N (or other nutrients) availability (e.g. Long et al. 2006). In addition, vegetation shifts to plant species characterized by different nutrient economies may occur in response to natural, climate or human-induced ecosystem perturbations.

Alpine ecosystems are particularly sensitive to such ecological effects because *(i)* alpine habitats are strongly N-limited in many instances (Bowman et al. 1993), *(ii)* snowcover gradients influence the nature of plant communities (Körner 1999, Choler 2005) and *(iii)* the composition of alpine vegetation varies in response to deposition of anthropogenic nitrogen (Bowman et al. 2006).

However, the physiology of carbon allocation patterns of alpine plants in relation to N uptake is poorly understood. It is well known that increased N assimilation promotes carbon fixation and growth at the leaf or biomass level (Wright et al. 2004) and is accompanied by higher leaf and root respiration rates (Reich et al. 1997, Craine et al. 2002, Tjoelker et al. 2005). However, the extent to which carbon allocation patterns are related to N uptake efficiency and allocation remains uncertain (Garnier 1991, Osone et al. 2008). For example, fast-growing species (e.g. *Carex foetida*) have higher leaf N contents and maximum carboxylation rates Vc_{max} than slow-growing species (e.g. *Kobresia myosuroïdes*) found at similar altitude (Baptist and Choler 2008). Therefore, it is currently assumed that fast-growing species allocate less carbon belowground, as suggested by biomass ratio measurements (i.e. the root:shoot ratio, Chapin 1980). Nevertheless, there is no direct evidence of such a relationship (Craine et al. 2002).

As an aid to clarifying these points, we used $^{12}C/^{13}C$ and $^{14}N/^{15}N$ isotope techniques to investigate C and N fixation and allocation patterns of two alpine species along the snowmelt gradient: *Carex foetida* and *Kobresia myosuroides*. *C. foetida* is a fast-growing species found

in late snowmelt habitats, that are characterised by high N availability and a very short growing season (Choler 2005). *K. myosuroides* is a slow-growing species of early snowmelt habitats, that have opposite conditions, that is, low N availability and a longer growing season.

While the respiratory properties are similar in both species, the present labelling experiments show that, in contrast to *K. myosuroides*, *C. foetida* has clear opposite leaf-to-root allocation ratios for C and N. In other words, *C. foetida* allocate more C to roots while N allocation favours the aboveground compartment. We therefore conclude that, in the present couple of species, the adaptation of fast-growing species for an optimal growth involves an increased C and N assimilation efficiencies to feed the belowground biomass production.

Material and methods

Plant material

We collected 37 monoliths of *Kobresia myosuroides* (Cyperaceae, **KM**) and *Carex foetida* (Cyperaceae, **CF**) respectively in the region of the Galibier pass (2646 meters above sea level) and the Agnel pass (2744 m a.s.l.) in the French Alps in October 2005. They were transferred into plastic pots (20×20×30 cm) in a common garden located in the Grand Galibier mountain range in the South-Western Alps (45°7'N, 6°5'E, 2100 m a.s.l.). Snowmelt occurred on the 15 May 2006. The pulse-chase labelling experiments (^{13}C and ^{15}N labelling were independent) started on the 5 July 2006 and finished on the 26 July 2006. All the experiments were conducted in the Station Alpine Joseph Fourier, except the isotope measurements which where carried out at the University of Barcelona. The $\delta^{13}C$ of CO_2 of the air at the Galibier Pass was *ca*. -11.5 ± 0.1 ‰ (Noguès et al. 2006).

^{13}C labelling procedure

In July 2006, at the peak of standing biomass, 12 out of 15 monoliths (here after refered as replicates) were labelled in $^{13}CO_2$ enriched atmosphere. The other three were used as control (for the initial carbon isotope composition before labelling, the corresponding sampling time is denoted as T_{init}). After labelling, a first set of three was immediately harvested (T0). For the others, the chase time lasted 24 h (1 day, T1), 82 h (3.5 days, T3) and 274 h (11.5 days, T11) respectively. The day before the pulse-labelling, the plants were arranged in controlled conditions: 12 h photoperiod, mean air temperature of 18°C and 500 µmol m^{-2} sec^{-1} of light. Labelling was applied following a dark period of 12 h for all plants. After labelling and until the end of the chase period, plants were kept in the same controlled conditions. Every two days, the monoliths were watered with 500 ml of distilled water.

The isotope label was applied during 5 h by enclosing the monoliths, two by two, in a 36 l-Perspex® labelling chamber. Atmospheric air was first CO_2-depleted (decarboxylated) by passing through a soda-lime column and then mixed to $^{13}CO_2$ fluxes from a gas cylinder (enriched at 5 %, Euriso-top, Saint-Aubin, France) in a mixing chamber. The mixing chamber was then connected to the sample air hose of the HCM 1000 Infra Red Gaz Analyser (Heinz Walz GmbH, Effeltrich, Germany) and the CO_2 concentration was estimated. The CO_2 concentration within the chamber was kept between 380 and 420 ppm during all the labelling procedure thanks to mass flow controllers located before the mixing chamber (ROD-4, Aera,

Fort Collins, USA). The air flux passing through the labelling chamber was controlled by the HCM 1000 at a rate of 1.0 l min^{-1}. A second pump was added after the labelling chamber to avoid overpressure. One fan (Radio Spare, Beauvais, France) was fixed into the chamber to ensure air mixing. Aluminium tubes, arranged at the bottom of the chamber and connected to a water bath, maintained the chamber temperature at 22.5 ± 0.4°C during the pulses. Light intensity above the vegetation was kept at 550 µmol m^{-2} s^{-1}.

At the end of each chase time (T0, T1, T3, T11) and also for T_{init}, the three replicates were destructively harvested. We harvested the living aboveground vegetation first (by clipping the leaves at the soil surface). Half of the biomass was immediately lyophilised, while the rest was enclosed in the 1l-Perspex® chamber to measure respiration (5 min) (see below for procedure) and the isotopic signature of dark-respired $^{13}CO_2$. To measure the latter, the 1l-chamber connected to the LICOR 6200 was flushed with CO_2-free air to ensure that only the CO_2 respired in the chamber was measured. We let CO_2 accumulate until it reached approximatively 1000 ppm and air samples were then collected using a special 50 ml syringe (SGE, Ringwood, Australia) and a needle (model microlance 3, BD, Plymouth, UK). The gas samples were passed through a magnesium perchlorate column (water-vapor trap) then immediately injected into a 10 ml vacutainer (BD vacutainer, Plymouth, UK). To avoid contaminations with the air present in the syringe and needle, both were purged with N_2, before taking each sample. The vacutainers were also over-pressurized with N_2 so the pressure inside the vacutainer was above the ambient pressure.

Non woody (new) and woody (old) roots were subjected to a similar procedure after being harvested and washed. Accumulation time was a bit longer for old roots as respiration was much lower than for new roots and leaves. Leaves and roots were subsequently lyophilised for isotopic analysis of Organic Matter (OM). OM analyses were done using an elemental analyzer with a zero-blank autosampler (EA1108, Series 1, Carbo Erba Strumentazione, Milan, Italy) coupled to an isotope ratio mass spectrometer (Delta C, Finnigan Mat, Bremen, Germany) operating in continuous flow.

The $\delta^{13}C$ of respired CO_2 was measured using a Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS). Water vapour and oxygen were removed from the gas samples and carbon dioxide, argon and nitrogen separated by gas chromatography (6890N, Agilent Technologies, Palo Alto, CA, USA) coupled to an isotope ratio mass spectrometer Delta^{plus} through a GC-C Combustion III interphase (ThermoFinnigan, Bremen, Germany). The column used was a 30 m x 0.32 mm i.d. GS-GASPRO (J&W Scientific Inc.,

Folsom, CA, USA). The carrier gas was Helium at a flow rate of 1.2 ml min^{-1}. Injection port temperature was 220°C. The oven temperature was kept at 60°C during the whole run. The injection was done in split mode (injected volume 0.3 ml, split flow 20 ml min^{-1}).

CO_2 gas exchange measurements

In order to evaluate ^{13}C fixation during the pulse-labelling, CO_2 fluxes were measured on each replicate with a LICOR 6200 (LI-COR, Lincoln, Nebraska USA) before and just after labelling. Light intensity, air temperature and relative air humidity were recorded for all the duration of the measurements. The net photosynthetic fixation rate (A_{net}) was calculated as the sum of net CO_2 fluxes in light (NEP) and in darkness (ER, which takes into account the CO_2 evolution rate by both the belowground and aboveground compartments) as follows :

$$A_{net} = NEP + ER \quad \text{Eq. 1}$$

In darkness, respiration by the aboveground compartment was small compared to that of the belowground compartment. In other words, the contribution of photosynthetic organs to ER was small (typically less than 8 %), so the overestimation of the net photosynthetic rate A_{net} was negligible. At the end of the chase period (see section below), leaf, new and old root respiration was estimated every minute during 5 to 10 min by enclosing them in a dark 1l-Perspex® chamber connected to the LICOR 6200.

^{15}N labelling procedure

The $^{15}NO_3$, $^{15}NH_4$ and ^{15}N-glycine uptake by KM and CF was assessed independently from the ^{13}C-labelling, that is to say, on others 18 monoliths (n=3 replicates for each compound and each species). Glycine was chosen among others amino acids, as Lipson *et al.* (1999) indicated that it was the soil amino acid most available to plants. One hundred ml of a 1mM solution of $^{15}NO_3$, $^{15}NH_4$ or ^{15}N-glycine were added with a 5 ml syringe. The syringe was inserted at 5 cm depth in the soil following a 2x2 cm grid layout. We then watered plants with 500 ml of demineralized water to ensure homogeneous labelling in the soil.

^{15}N isotope sampling and processing

After a 24 h chase, leaves, new and old roots were harvested, sorted, washed with demineralized water and lyophilised for isotopic analysis. The same procedure was applied to three others unlabeled monoliths of each species, as a control.

Isotope labelling calculations

To estimate ^{13}C and ^{15}N enrichment in each organ of the plants, %Atom (^{13}C or ^{15}N proportion) for ^{13}C and ^{15}N were calculated using the following the equation:

$$\%\text{Atom} = \frac{\delta + 1000}{\delta + 1000 + \frac{1000}{R_{\text{standard}}}} \quad \text{Eq. 2}$$

where δ is the isotopic signature of CO_2 respired or of OM. $R_{standard}$ is the international standard references (i.e. $^{13}C/^{12}C$, PeeDee Belemnite, and $^{15}N/^{14}N$, atmospheric air).

%Atom excess was then calculated as the %Atom ^{15}N or ^{13}C differences between labelled and unlabelled organs (control, at T_{init}) :

$$\%\text{Atom excess} = \text{Atom}\%_{\text{labelled}} - \text{Atom}\%_{\text{unlabelled}}$$

The labelling-derived ^{13}C content per DW ($\gamma^{13}C$, in µg ^{13}C g^{-1} DW) in each organ of the plant was calculated as follows:

$$\gamma^{13}C = \%\text{Atom excess} \cdot \%C$$

where %C is the percentage of carbon in the organ. The labelling-derived ^{13}C flux associated with root and leaf respiration ($\gamma^{13}C_R$, in µg ^{13}C g^{-1} h^{-1}) was calculated as follows :

$$\gamma^{13}C_R = \frac{\%\text{Atom excess} \cdot R_{\text{organ}}}{\text{mass}_{\text{organ}}} \quad \text{Eq. 3}$$

where $mass_{organ}$ is the mass of the organ (g) considered, R_{organ} is the respiration rate (µg C h^{-1}) and %Atom excess is here the ^{13}C atom excess in CO_2.

As the plants experienced similar conditions during the chase period and the respiration measurements, the cumulated labelling-derived ^{13}C content over time (in mg ^{13}C g^{-1}) was estimated by (1) fitting an exponential decay constant to the labelling derived ^{13}C flux over chase time ($\gamma^{13}C_R$):

$$\gamma^{13}C_R = a \cdot \exp^{(-b \cdot t)} \quad \text{where t is the time in hours, a and b are constants} \quad \text{Eq. 4}$$

and (2) integrating this exponential over time. For CO_2 respired by new and old roots, the maximum concentration sampling point was used as the “time zero” for the exponential curve fit.

Total labelling-derived ^{13}C mass ($\gamma^{13}C_M$, µg or mg ^{13}C) at chase time *T* was calculated by averaging the organ mass over the fifteen replicates (see table 1). For this purpose, we added the labelling-derived ^{13}C mass for each organ and the loss through leaf and new/old root respiration from T0 to *T* as follows :

$$\gamma^{13}C_M(T) = \gamma^{13}C_{leaf}(T)\cdot m_{leaf} + \gamma^{13}C_{new}(T)\cdot m_{new} + \gamma^{13}C_{old}(T)\cdot m_{old} + \int_{T0}^{T}\left(\gamma^{13}C_{Rleaf}(t)\cdot m_{leaf} + \gamma^{13}C_{Rnew}(t)\cdot m_{new} + \gamma^{13}C_{Rold}(t)\cdot m_{old}\right)dt \quad \text{Eq. 5}$$

By doing so, we could see whether the labelling-derived ^{13}C mass was balanced over time for each species, that is, whether the total ^{13}C-amount (remaining + respiratory losses) remained constant (Appendix 2). We also compared $\gamma^{13}C_M$ to the amount of ^{13}C fixed by each replicate during the 5h-labelling based on A_{net} values integrated on the labelling period.

The labelling-derived ^{15}N content per whole plant dry matter ($\gamma^{15}N$, µg ^{15}N g^{-1}) was calculated as following:

$$\gamma^{15}N = \frac{\%\text{Atom excess}\cdot\%N\cdot \text{mass}_{organ}}{\text{mass}_{plant}} \quad \text{Eq. 7}$$

Where %N is the percentage of nitrogen in the organ, $mass_{organ}$, the mass of the organ (g) and $mass_{plant}$, the mass of whole plant (g).

Similarly to ^{13}C data analysis, the labelling-derived ^{15}N mass (µg^{15}N) was calculated for each organ based on the average mass over the nine replicates of each species (see table 1).

Belowground productivity

Below ground productivity was estimated using a root ingrowth core method. The ingrowth cores (5 × 15 cm) were established on the 30/05/06 by removing one soil core in another set of four replicate monoliths for each species and by filling the holes with the same but 2 mm-sieved soil. Roots present within the cores were sampled on the 15/07/06, washed free of soil, dried at 60°C during 48h and weighed. Root production was then calculated as the root biomass divided by time between the 30/05/06 and the 15/07/06 and expressed per unit core surface for each replicate.

Statistical procedure

The statistical analyses referring to isotopic data were performed using the non-parametric Kruskall-Wallis test except for ^{15}N natural abundance which was performed using a one-way ANOVA. The same procedure was used to compare belowground productivity (n=4). The species-induced differences in flux measurements and R:S ratios were analysed using a one-way ANOVA. Finally, an ANCOVA was applied to test the regression between (1) the labelling derived ^{13}C amount ($\gamma^{13}C$) of new roots OM against that of leaf OM and (2) the $\gamma^{13}C$ of root-respired CO_2 against that of leaf-respired CO_2. Species were considered as the

qualitative factor. All analyses were performed with the Jump software (SAS Institute Inc., Cary, North Carolina, USA).

Results

Biomass production

Mean biomass and belowground productivity data are indicated in Table 1 (upper part). Under our experimental conditions, KM has a larger aboveground biomass than CF, that is, has a higher shoot-to-root ratio. In addition, the belowground productivity is larger in CF: the root productivity per unit ground area was higher in CF (χ^2=5.0$_1$, *P*=0.02) compare to KM (Table 1).

Morphological Characteristics	***K. myosuroides***	***C. foetida***
Mean biomass (g)		
- Aboveground	2.6 (0.1)	1.9 (0.1)
- New roots	0.51 (0.07)	0.80 (0.09)
- Old roots	21.6 (1.8)	28.00 (2.0)
Belowground productivity (g m^{-2} d^{-1})	6.9 (1.8)[a]	12.5 (0.6)[b]
Physiological characteristics		
Assimilation rate during labelling (ngC g^{-1} leaf DW sec^{-1})	791.5 (20.3) [a]	622.4 (13.3)[b]
Leaf respiration in darkness (ngC g^{-1} leaf DW sec^{-1})	63.5 (6.7)[a]	100.3 (7.4)[b]
New root respiration (ngC g^{-1} new root DW sec^{-1})	233.6 (21.0)[a]	206.3 (15.0)[a]
Old root respiration (ng C g^{-1} old root DW sec^{-1})	56.4 (4.3)[a]	68.6 (13.0)[a]

Table 1 Morphological and physiological characteristics of *C. foetida* and *K. Myosuroides*. See text for further statistical details. Different letters indicate significant differences between the two species (*P*<0.05).

Photosynthetic rates and total ^{13}C-assimilation

Photosynthesis and respiration were measured during the labelling experiment for the above-ground compartment as well as 'old' and 'new' roots (see Material and Methods). During ^{13}C-pulse time, the net assimilation rate of the above compartment (A_{net}) was significantly larger in KM than in CF ($F_{1,21}$=5.1, *P*=0.03). This does not reflect the general trend: in homogeneous light conditions as CF has higher photosynthetic rates (see APPENDIX 1 and Baptist and Choler 2008). The lower value in CF is here caused by the non-homogeneous light conditions (unidirectional light system, see Material and Methods) and contrast to values of maximal CO_2 assimilation (at saturated light) measured at leaf level (APPENDIX 1). The assimilation values were summed over the pulse time to calculate the total labelling-derived ^{13}C mass ($\gamma^{13}C_M$) fixed by photosynthesis, giving 1.33 ± 0.2 mg for KM and 0.93 ± 0.1 mg for CF (APPENDIX 2).

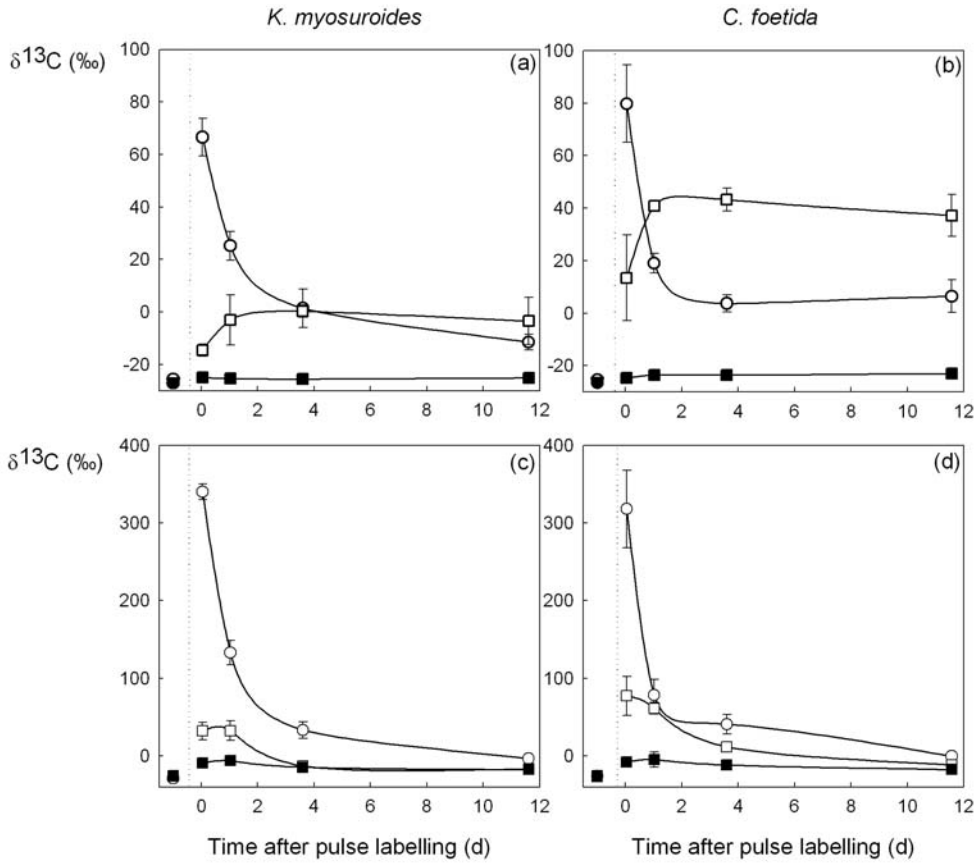

Fig. 1 $\delta^{13}C$ (‰) of leaf, new and old roots of *K. Myosuroides* (a) and *C. Foetida* (b). $\delta^{13}C$ (‰) of CO_2 respired by the leaves, new and old root of *K. Myosuroides* (c) and *C. foetida* (d) following the pulse-labelling. On the left of the dashed vertical line are shown ^{13}C natural abundance of leaves, new and old roots OM and of CO_2 respired by the leaves, the new and the old roots. Leaves: white circle, new roots: white square, old roots: black square. All *X* –axes are time elapsed since the pulse-labelling (in days). Values are the mean ± se (n=3).

Respiratory properties

Leaf respiration in darkness was largely and significantly higher in CF ($F_{1,28}$=13.2, *P*=0.001) (Table 1, bottom part). Thus, in our experimental conditions, the leaf respiration-to-net assimilation ratio was 8.0% in KM and 16.1% in CF. New and old root respiration did not differ significantly between both species.

	K. myosuroides	***C. foetida***
Leaf	-3.40 (0.37)^{a}	0.19 (0.23)^{a}
New roots	0.61 (0.62)^{b}	-0.02 (0.37)^{a}
Old roots	-0.76 (0.14)^{b}	2.73 (1.12)^{ab}
Soil	3.78 (0.12)^{c}	4.49 (0.95)^{b}

Table 2 ^{15}N natural abundance ($\delta^{15}N$, ‰) of unlabelled leaf, new roots, old roots, and soil in *K. Myosuroides* and *C. Foetida* monoliths. Values are the mean ± se. Different letters indicate significant differences between the organs and the soil (*P*<0.05).

^{13}C fixation and partitioning

The $^{12}C/^{13}C$ isotope composition ($\delta^{13}C$) of organic matter (OM) and of respired CO_2 after labelling is shown in Fig. 1. Clearly, at T0, leaves were the most labelled organs, followed by new roots (Fig. 1). Old roots were hardly labelled and always remained nearly not labelled throughout the experiment (corresponding to total chase time). The kinetics were very different for leaves and roots: the $\delta^{13}C$ value of leaves continuously declined during chase time while that of roots increased within 1.5 days and reached a plateau.

The decline of the ^{13}C-amount in leaves may be caused by: *(i)* isotopic dilution (natural $^{12}CO_2$ fixed during day-time of the chase period), *(ii)* respiration (dark-respired CO_2 was strongly ^{13}C-enriched, Fig. 1c and d) and *(iii)* export (^{13}C-increase in roots within a couple of days). Noteworthy, CF showed a more rapid decline of the ^{13}C-abundance in leaf organic matter (the calculated half-time of the exponential decay ($t_{1/2}$) is near 21 h for KM and 13 h only for CF). The isotopic dilution is probably not responsible for such a pattern: plants of both species experienced similar conditions and KM had a slightly higher photosynthetic rate than CF under the conditions of the experiment (see paragraph above, Table 1). By contrast, respiration contributed to such leaf ^{13}C-kinetics, simply because respiration was much larger in the case of CF (Table 1) while having a similar ^{13}C-enrichment (Fig. 1c and d).

The faster decline of leaf ^{13}C in CF also came from the larger export of assimilates to roots: the $\delta^{13}C$ value of new roots organic matter was higher in CF than in KM: in the steady-state, the $\delta^{13}C$ value of new root organic matter is 0 and 40‰ for KM and CF respectively (Fig. 1a and b). That is, the labelling-derived carbon in new roots account for ~0.6 and 1.8% of the total C in KM and CF, respectively.

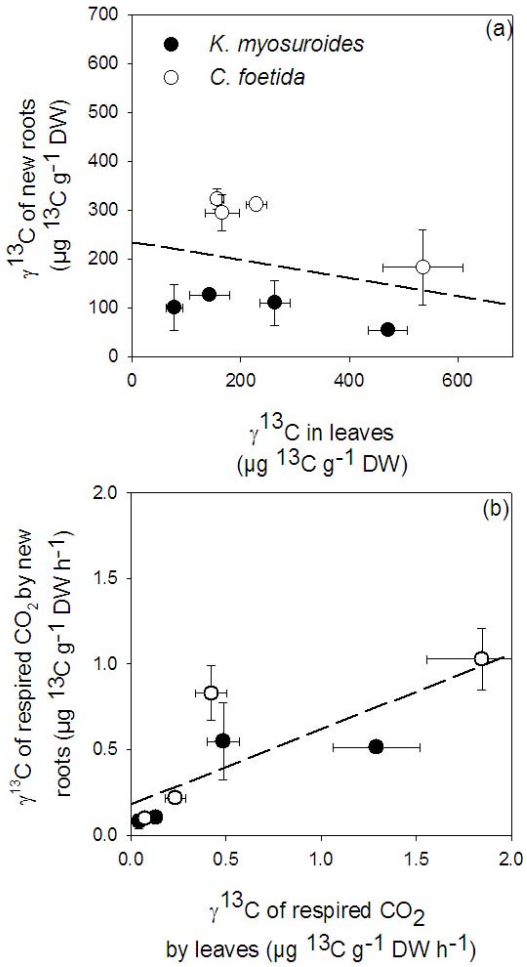

Fig. 2 (a) Labelling-derived ^{13}C content of new roots in relation to labelling-derived ^{13}C content of leaves and (b) labelling-derived ^{13}C content of CO_2 respired by the new roots in relation to the labelling-derived ^{13}C content of CO_2 respired by the leaves for *K. myosuroides* (grey symbol) and *C. foetida* (white symbol) at each chase time. Values are the mean ± se (n=3). See text for further statistical details.

The labelling derived ^{13}C amount ($\gamma^{13}C$) of new roots is plotted against that of leaves in Fig. 2. There was a significant correlation between the $\gamma^{13}C$ value of new roots and that of leaves when species were considered together ($F_{1,22}$=46.1, R^2=0.71, P<0.0001, Fig. 2a). The slope of the regression was slightly (but not significantly) steeper in CF, showing again the more rapid ^{13}C-kinetics in leaves. In addition, the fastest transfer of carbon from leaves to roots was reflected by the shift of data points from the right hand side (T0) to the left hand side (the chase-measurements, at T1 to T3, clustered) in CF while there were intermediate data points in KM. $\gamma^{13}C_R$ of leaf-respired CO_2 and root-respired CO_2 were correlated ($F_{1,23}$=22.2, R^2=0.56, P=0.0001, Fig. 2b) when species were considered together. However, neither the slope of the regression ($F_{1,23}$=0.44, P=0.51) nor the mean value ($F_{1,23}$=2.57, P=0.12) differed between the two species.

Whole-plant carbon partitioning

Eleven days after the pulse-labelling, carbon allocation patterns were calculated, taking into account integrated respiratory losses. The results are shown in Fig. 3 (raw data in APPENDIX

3). In KM, 45% of the labelling-derived ^{13}C remained in leaves, and nearly 5% of the labelling-derived ^{13}C was allocated to new roots. By contrast, 29% of the labelling-derived ^{13}C remained in leaves while new roots accounted for ~20%. Respiratory losses associated with leaves were more pronounced in KM than in CF while that associated with new roots respiration were larger in CF. However, we note that, in the case of new roots CF, the respiration-to-organic matter ratio of ^{13}C allocation was much smaller (Fig. 3, light grey), clearly suggesting that ^{13}C was directed to new root growth to a larger extent than to respiration.

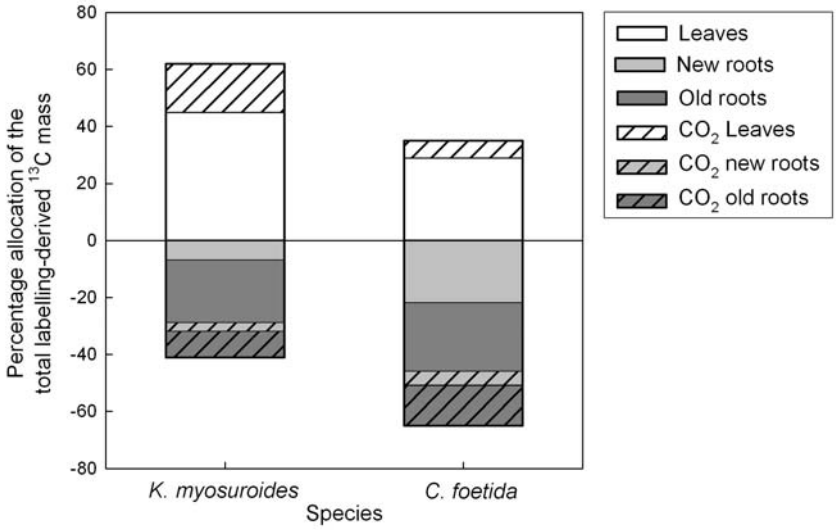

Fig. 3 Percentage allocation of the total labelling-derived ^{13}C mass ($\gamma^{13}C_M$) recovered in the leaves, the new and old roots and lost through leaf, new and old root respiration 11d after the pulse-labelling. Leaves : white, new roots: grey, old roots: dark grey. Hatching patterns correspond to C lost through respiration.

Nitrogen allocation and partitioning

The natural $^{14}N/^{15}N$ isotope composition ($\delta^{15}N$) of organs and soil is indicated in Table 2. Soil N was always ^{15}N-enriched ($P<0.05$) by 1.7 to 7.1‰, so that a $^{14}N/^{15}N$ isotope fractionation during N reduction/assimilation is apparent. KM leaves were clearly and significantly ^{15}N-depleted compared to new and old roots whereas CF leaves nearly had the same $\delta^{15}N$ value as new and old roots and were slighty enriched compared to KM leaves. This indicates that N reduction/assimilation in CF, which discriminates against ^{15}N, occured mainly in the roots, thereby enriching in ^{15}N the remaining N-containing molecules (such as nitrates) transfered to leaves. Inversely, the depleted N compounds in the KM leaves indicate higher proportion of N reduction/assimilation in the aboveground compare to the belowground compartments.

The nitrogen allocation after 24 h ^{15}N-labelling with either ^{15}N-nitrate, ^{15}N-ammonium or ^{15}N-glycine is indicated in Fig. 4. Very clearly, CF was more ^{15}N-labelled than KM at the whole-plant level, whatever the labelling molecule was. In other words, CF had a higher N uptake efficiency (NupE) than KM: the recovery of ^{15}N from the labelling solution was 13.7 ± 2.5% Gly, 19.8 ± 3.1% NO_3^-, 2.9 ± 1.2% NH_4^+ and 4.6 ± 0.5% Gly, 10.4 ± 3.8% NO_3^-, 1.8 ± 0.4% NH_4^+ in CF and KM, respectively. For nitrate, the CF-to-KM ratio of NupE was then as high as 1.9.

In addition, a larger proportion of N (nearly the double) was allocated to leaves in CF than in KM (circle graphs, Fig. 4a) when ^{15}N was supplied as nitrate; such a difference was less pronounced with ^{15}N-ammonium (Fig. 4b) and also less visible with ^{15}N-Gly (Fig. 4c). However, old roots represented a large ^{15}N-sink in Fig. 4, simply because of their high biomass. In other words, the specific ^{15}N-abundance (µg ^{15}N/g DW) was always low in old roots (data not shown). In the case of nitrate, the specific ^{15}N-abundance of leaves is 72.6 ± 26.8 and 20.3 ± 9.7 µg ^{15}N/g DW in CF and KM, respectively, so that the CF-to-KM ratio was 3.5. Such a ratio is larger than the NupE ratio (1.9, see above), clearly demonstrating that preferential N allocation to leaves, rather than whole-plant uptake efficiency, was responsible for the larger ^{15}N mass in CF leaves (Fig. 4a).

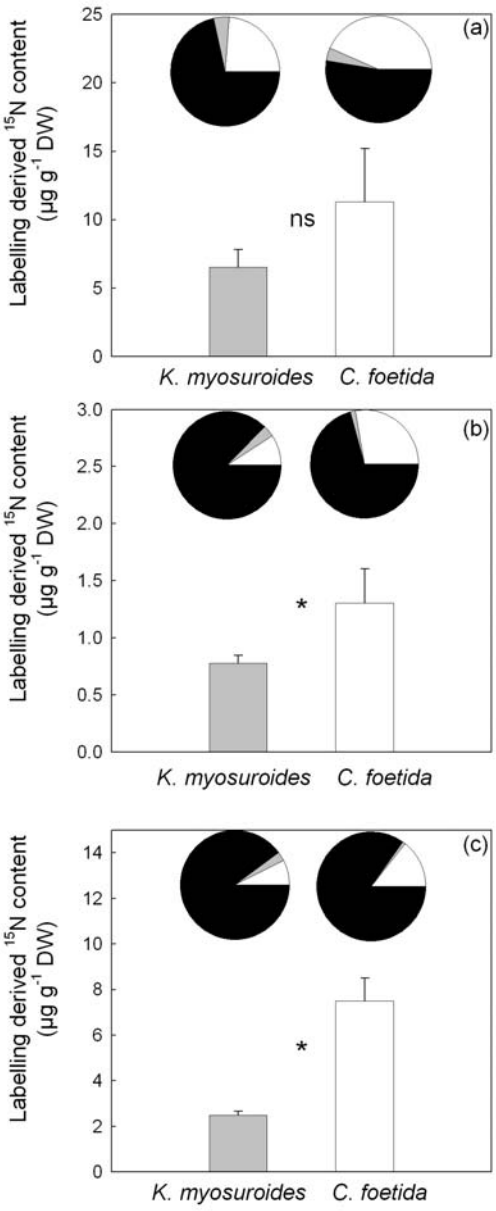

Fig. 4 Labelling-derived ^{15}N content in whole plant dry matter (µg 15N g^{-1} DW) after $^{15}NO_3^-$ (a), $^{15}NH_4^+$ (b), ^{15}N-glycine (c) amendment in *K. myosuroides* (grey bar) and *C. foetida* (white bar). Circle graphs: Percentage allocation of the total labelling-derived ^{15}N mass recovered in the leaves (white), the new (grey) and old roots (black). Values are the mean ± se (n=3). ns : no significant, * P<0.05. See text for further statistical details.

Discussion

Alpine plants are frequently exposed to contrasted micro-environmental conditions (nutrients and water availability, light, etc). Within alpine meadows, a well-documented environmental gradient lies between early and late snowmelt locations. In the latter, the growing season is shorter and accompanied by relatively good nutrient availability (particularly at the beginning of the growing season) (Bowman 1992, Baptist and Choler 2008). Plant growing under such conditions are thus energy-limited rather than nutrient-limited. By contrast, plants growing in early snowmelt habitats are much less energy-limited and soil nutrient is less abundant. Although the adaptation of these plants has been shown to involve growth rapidity (fast and slow-growing species are observed in late and early snowmelt conditions, respectively) and photosynthetic capacity (which is higher in fast-growing species, Wright et al. 2004), the primary carbon metabolism and allocation patterns are poorly known. The aim of this study was thus to clarify C and N partitioning patterns in *Carex foetida* (CF) and *Kobresia myosuroides* (KM), two species found in opposite habitats, that is, growing under late and early snowmelt conditions.

Carbon fixation and partitioning

Adaptation of CF to short growing-season duration involves high relative growth rates (e.g. belowground productivity, Table 1) and photosynthetic capacity (see APPENDIX 1). This agrees with the larger maximal carboxylation rate Vc_{max} and higher leaf N elemental content in CF (Choler 2005, Baptist and Choler 2008), which is an indicator of the specific Rubisco content. Whole-plant carbon allocation favored the root compartment in CF as indicated by the larger ^{13}C-transfer to new roots (Fig. 1, 2 and 3) as compared to KM. Such a transfer to roots plausibly involved a larger flux of assimilates rather than a limited flux of assimilates with high ^{13}C-specific abundance, because the kinetics of the ^{13}C-decline in leaf organic matter are quicker in CF (Fig. 1; $t_{1/2}$ values of 13 h versus 21 h in KM; Fig. 2a) while the initial leaf ^{13}C-abundance is very similar in both species.

The mass-balance after a 11 d chase period indicate that the larger carbon flow from leaves to roots is directed to feeding new root growth rather than respiration in CF plants (Fig. 3). Indeed, the dissimilar ^{13}C-abundance in root-respired CO_2: while the ^{13}C allocation (labelling-derived ^{13}C content) to new root total organic matter is nearly 3 times higher in CF (Fig. 1 and ANNEXE 3), the maximum ^{13}C content in root-respired CO_2 was the double only. In other words, the turn-over (consumption) of the root respiratory pool was lower in CF.

We nevertheless recognize that there were large discrepancies between the two types

of roots, that is, woody (old) and non-woody (new) roots. Old roots were weak ^{13}C-sinks (very low ^{13}C-abundance after labelling, Fig. 1) with small respiratory activity as compared to new roots (nearly 70% less, Table 1). This reflects differences in metabolic activities: new roots are responsible for root growth and nutrients absorption while old roots have a conduction and storage role (Comas et al. 2000, Lipp and Andersen 2003, Volder et al. 2005). However, at the whole plant level, old roots accounted for a substantial ^{13}C content because of their very large biomass (Table 1), while the C allocation pattern to old roots was somewhat similar in both species (Fig.s 1 and 3).

In the present case, carbon allocation closely correlated to belowground productivity rather than to belowground biomass. It should be emphasised that the root-to-shoot biomass ratio is the result of two opposing forces: root turnover (degradation/production balance) and carbon translocation from leaves. That is, as already proposed by Carbone & Trumbore (2007) and Craine *et al.* (2002), the root-to-shoot ratio may be an unreliable trait to predict instantaneous carbon partitioning, so that physiological ^{13}C- or ^{14}C - studies using tracing are necessary to unravel carbon allocation patterns.

Nitrogen uptake and assimilation

Variations in ^{15}N uptake between both species are consistent with previous studies which suggest that fast-growing species display higher specific nitrogen absorption rate than slow-growing species (Garnier 1991, Poorter et al. 1991). Besides, CF and KM exhibited different N allocation patterns : CF experiences higher N allocation to leaves after a 24 h chase compared to KM (Fig. 3). Consequently, one may assume that N translocation toward aboveground compartment was more efficient in CF than in KM plants.

KM exhibited a prevalence of leaf N reduction/assimilation over root reduction in contrast to CF (Table 2). In ordinary conditions where nitrate is reduced by both leaves and roots, the natural ^{14}N/^{15}N isotope composition (δ^{15}N) of leaves is higher (^{15}N-enriched) because nitrate reduction fractionates against ^{15}N thereby enriching the remaining nitrate molecules exported to leaves (for a recent review, see Tcherkez and Hodges 2007). This was typically the case in CF (Table 2). By contrast, KM leaves were not ^{15}N-enriched as compared to new roots, demonstrating that N reduction occured mainly in leaves (Table 2). Under non-limiting nutrients availability, shoots generally appear to be the predominant site of NO_3^- reduction because of the higher content of excess reductants produced by photosynthesis (Scheurwater et al. 2002). As a result, it has been logically stated that fast-growing species may experience higher NO_3^- reduction in the leaves compare to the roots because of larger photosynthetic rate

- which produce important amount of reductants (Gojon et al. 1994). However, the site of NO_3^- reduction is dependant over others factors such as the specific rate of nitrate reductase activity, the biomass allocation (Gojon et al. 1994, Scheurwater et al. 2002) or environmental conditions (Radin 1978, Andrews 1986, Miller and Cramer 2004). In the case of CF, the high C flux allocated to the belowground compartment may furnish enough reductants, through glycolysis and oxidative pentose phosphate pathway, to maintain a significant level of NO_3^- reduction into the roots (Pate 1980). Besides, the ability to reduce efficiently NO_3^- in roots may limit N efflux and contribute to the N uptake efficiency (Mata et al. 2000, Miller and Cramer 2004). In contrast, arctic and alpine slow-growing species, such as KM, exhibit generally lower nitrate reductase activity (Atkin 1996) and as a consequence the important presence of reductants and C skeleton provided by photosynthesis may favor the assimilation/reduction of NO_3^- in the aboveground compartment. Finally, it is also a way for slow-growing species to limit specific respiratory cost associated to N uptake by reducing N losses through efflux (Scheurwater et al. 1999, Mata et al. 2000).

Biomass and energetic root/shoot balance

A larger rate of root respiration was expected in CF compare to KM plants as more respiratory energy was necessary to support higher root productivity and nitrogen uptake (almost two-fold larger). Nevertheless, root respiration did not differ between the two species suggesting that the fast-growing species respired at lower rate than expected from its C and N metabolism. Previous studies already reported similar results and noticed the absence of clear relationships between RGR and root respiration rate (e.g. van der Werf et al. 1988, e.g. Poorter et al. 1991).

Root respiration can be separated into three components, *i.e.* respiration for root growth, for maintenance and for ion uptake. We thus assume a classical relationship between total root respiration (denoted as *R*) and these components as follows (modified version from Van der Werf et al. 1994) :

$$R = R_m + C_u \cdot NupE + C_g \cdot P_r$$

where R is root respiration, R_m is maintenance respiration, NupE is the nitrogen uptake efficiency, *Pr* is the root productivity and C_u and C_g, the specific respiratory growth for ion uptake and growth respectively.

Based on this equation, the reduced respiratory requirements of CF could be explained by a lower energy requirements for nutrients uptake (C_u), a much lower maintenance respiration

(R_R) and/or a reduced growth cost could explained (C_g).

Scheurwater *et al.* (1998) and van der Werf *et al.* (1988) demonstrate that, within a functional group (e.g. Gramineae), maintenance respiration differ only slightly between fast- and slow-growing species. Similarly, numerous studies have proved that construction cost of roots and leaves did not differ between fast and slow-growing species (van der Werf et al. 1988, Navas et al. 2003, Roumet et al. 2006) and it was the case for CF and KM (data not shown). Thus, lower specific respiratory cost associated to growth or maintenance respiration are unlikely. As a result, this suggests that CF has lower costs associated to ion uptake as this has already been demonstrated for various fast-growing graminoids (Scheurwater et al. 1998, Scheurwater et al. 1999). Thus, the low respiratory requirements for N reduction and assimilation in CF might be responsible for the reduced root respiration rate.

Conclusion

Taken as a whole, it is apparent that CF and KM nearly represent two extreme cases of the compromise between shoot and roots involvement in C and N assimilations. In contrast to KM, CF exhibits (1) an improved photosynthetic capacity and N uptake efficiciency (2) a preferential carbon allocation to roots favoring root growth and NO_3^- reduction in this compartment and (3) a higher N aboveground translocation. CF root respiration was much lower than expected, and this was likely caused by a lower cost for ion uptake (lower N efflux, Scheurwater et al. 1999). The results obtained with this couple of species therefore suggest that at the whole-plant level, there is a compromise between N acquisition and C allocation for an optimized growth : the fast growing species, which is energy-limited, exhibits a tighter coupling between C and N metabolisms than the slow-growing's one more nutrient-limited.

Acknowledgements

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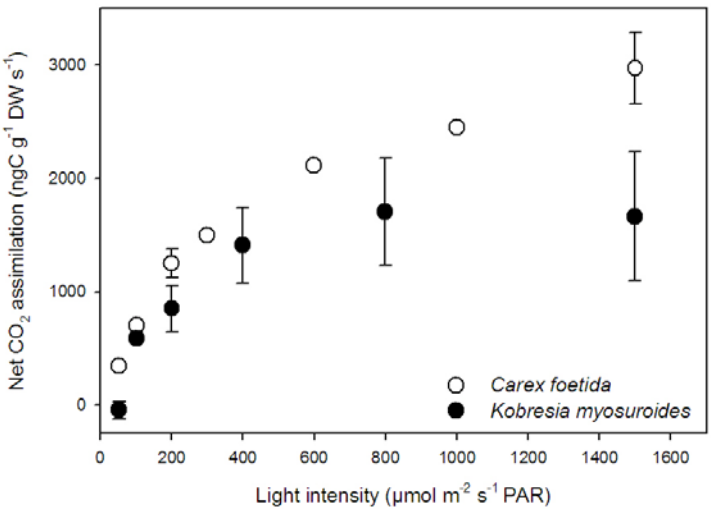
Appendix 1



Appendix 1. Net leaf CO_2 assimilation per g DW of *Kobresia myosuroides* (black circle) and *Carex foetida* (white circle) in relation to PAR (Photosynthetic Active Radiation). Net leaf CO_2 assimilation at saturating light reached 1755.5±1097.7 ng C g^{-1} leaf DW s^{-1} and 2660.2±93.3 ngC g^{-1} leaf DW $s^{-1}$for *Kobresia myosuroides* and *Carex foetida* respectively. Values are the mean ± se (n = 3).

Material and methods :

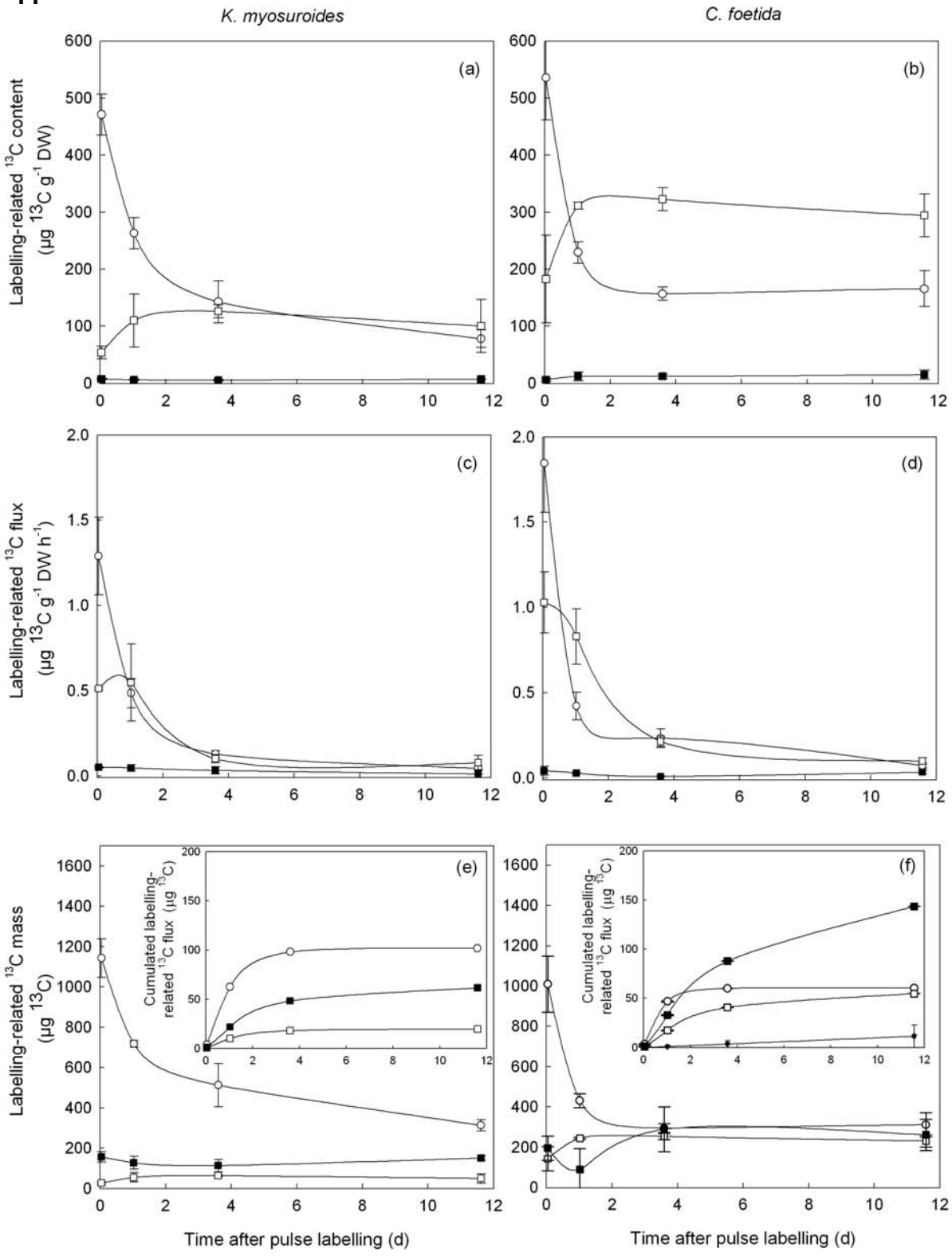
An open-flow photosynthesis system (model 6400, LiCOR, NE, USA) equipped with a CO_2 controller (CO_2 concentration equalled 400 pap) was used to measure light–response curves of *Carex foetida* and *Kobresia myosuroides* under constant temperature and humidity in July 2004. The system was maintained in a closed thermostated chamber and leaf temperature averaged ± 20°C.

Appendix 2

Chase time	*K. myosuroides*	*C. foetida*
T_0	1.28 (0.12)[a]	1.36 (0.22)[a]
T_1	0.95 (0.02)[b]	0.97 (0.09)[a]
T_3	0.80 (0.09)[b]	1.13 (0.14)[a]
T_{11}	0.63 (0.05)[b]	0.94 (0.13)[a]

Appendix 2. Labelling-derived ^{13}C mass ($\gamma^{13}C_M$) at each chase time (mg ^{13}C) for *K. Myosuroides* and *C. Foetida*. See equation 5 for calculations and text for statistical details. Values are the mean ± se. Different letters indicate significant differences between different chase times (P<0.05).

Appendix 3



Appendix 3. Labelling-derived ^{13}C content ($\gamma^{13}C$, µg ^{13}C g^{-1} DW) (a-b), labelling-derived ^{13}C flux ($\gamma^{13}C_R$, µg ^{13}C g^{-1} DW h^{-1}) (c-d) and labelling-derived ^{13}C mass ($\gamma^{13}C_M$, µg ^{13}C) (e-f) in leaves, new and old roots of *K. Myosuroides* and *C. foetida* following the pulse-labelling. Inlet graphs : cumulated labelling-derived ^{13}C mass (µg ^{13}C) respired by the leaves, new and old root of *K. Myosuroides* (e) and *C. foetida* (f) following the pulse-labelling. Leaves: white circle, new roots: white square, old roots: black square. All *X* –axes are time elapsed since the pulse-labelling (in days). Values are the mean ± se (n=3).

Chapitre II

Impact de l'enneigement sur la décomposition des litières et sur la respiration hétérotrophique

CHAPITRE II

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Article 2A

Snow cover exerts control over decomposition in alpine tundra along a snowmelt gradient.

Baptist, F., G. Yoccoz, and Ph. Choler
In prep. for *Plant and soil*

Combe Roche Noire, le 17/10/2006 (~ 2700m). Période durant laquelle une partie des sacs de litière a été prélevée (expérimentation II). En arrière plan, la Meije (massif des Ecrins, Fr). Photo : Ph. Choler.

Abstract

We assessed the interplay between short-term and long-term effects of snowpack on litter decomposition in alpine tundra at early and late snowmelt locations. Short-term effects are driven by the direct influence of snow cover on edaphoclimatic conditions (topographical effect), whereas long-term effects derive from the ecological sorting of species and plant traits (species effect). We examined the relationship of snow cover dynamics to seasonal and annual patterns of litter mass loss and litter nitrogen release. For these purposes, we compared the *in situ* decomposition of four native plant litters (two graminoids and two shrubs) using a two-year reciprocal-transplant litter-bag experiment. Additionally, a seasonal experiment was performed to estimate the relative importance of winter and summer periods for litter decomposition. Our results suggest that decomposition was enhanced in late snowmelt compared to early snowmelt locations irrespective of species identity. Winter decomposition rate was significantly correlated to mean winter soil temperature. Frozen soils at early snowmelt locations reduced winter decomposition. However, species and growth form appeared to determine primarily decomposition rate, with topography exerting only a secondary influence. Mass loss was slower from shrub litter than from graminoid litter regardless of topographical location. No significant effect of topography on N immobilization was detected. The inconsistent snowpack in early snowmelt locations delayed the final stage of N mineralization and may contribute to reduce N availability in the ecosystem. We concluded that reduced snow cover would slow litter decomposition at late snowmelt locations in alpine tundra and hence may favour greater carbon sequestration in these ecosystems. However, changes in litter quality as a result of shifts in growth form would probably be more important in determining litter decomposition rates than the short-term effect of variations in snow cover.

Keywords : snowmelt gradient, alpine tundra, growth form, litter decomposition, litter quality, *Kobresia myosuroides*, *Dryas octopetala*, *Salix herbacea*, *Carex foetida*, *Alopecurus gerardi*.

Introduction

Seasonally snow-covered ecosystems sequester a large pool of organic carbon in the soil, which appears particularly vulnerable in the context of global warming (Hobbie et al. 2000, Monson et al. 2006). Recent climate analyses postulate that, associated with higher temperature, snowfall may decline during the upcoming decades, in the northern hemisphere, especially in American, European and Russian mountain ecosystems (Serreze et al. 2000, Beniston 2005). In these systems, the landscape scale distribution of snow is one of the most important variables controlling mountain ecosystem properties. Variation in the depth and duration of snowpack result in large differences in edaphoclimatic conditions, and plant community composition (Walker et al. 1993). Consequently, changes in snow regime may be of dramatic consequence for nutrient cycling processes and soil carbon sequestration (Robinson 2002). As such, assessing the affect of snow distribution on the rate of decomposition and associated nitrogen release is a crucial element in predicting the impact of global change on carbon stocks in these regions.

Snow can affect decomposition on various levels. It can influence decomposition, in the short term, affecting wintertime soil temperature by insulating soil and/or summertime soil moisture. In the longer term, snow induces consistent and repeated changes in growing-season length, soil fertility, and water availability, thereby driving the ecological sorting of species and plant functional traits (Kudo 1996, Choler 2005). Thus, snow directly and indirectly controls litter decomposition and nitrogen release (Walker et al. 1999, Groffman et al. 2001).

Several recent studies have addressed the impact of variation in snow depth on carbon (C) and nitrogen (N) mineralization (Campbell et al. 2005). They have done this by manipulating snow cover (Walker et al. 1999, Chimner and Welker 2005), or by correlating Net Ecosystem CO_2 Exchange (NEE) to inter-annual variations in snow depth (Monson et al. 2006). These studies focussed on the short-term effect of snow-depth variations on C mineralization, but did not address the effect of long-term snow-induced changes in litter quality or in plant community structure on this process. The latter two factors are interdependent and strongly driven by environmental conditions (Hobbie 1996, Robinson 2002). In order to determine the main controls over litter decomposition and associated N released in snow-covered ecosystems, we need to examine the interplay between (1) snow-induced changes in edaphoclimatic conditions and (2) snow-induced changes in litter quality.

Several approaches have been proposed to undertake the difficult challenge of studying the long-term effects of climate change (Rustad 2006): (1) long-term monitoring (2) modelling or (3) observations of existing environmental gradients treated as space-for-time proxies. In alpine ecosystems, the distribution of snow (and therefore the snowmelt behaviour) is closely related to mesotopographical gradients (i.e. an ecocline along a small to medium-sized hill slope). Vascular plants in early snowmelt locations exhibit a combination of leaf trait attributes, including low LDMC and SLA (Leaf Dry Matter Content, Specific Leaf Area) and high C/N ratio (Kudo 1996, Choler 2005, Baptist and Choler 2008) which are generally associated to low decomposability (e.g. Cornelissen et al. 1999, e.g. Kazakou et al. 2006). Conversely, the leaf traits of species from late snowmelt locations, exhibit high SLA or N_{max}, which give rise to better quality litter.

In this study, we compared litter decomposition in early snowmelt and late snowmelt locations. We addressed the following questions: (1) how important is the influence of species identity compared to that of edaphoclimatic conditions on decomposition in alpine tundra, (2) how do seasonal patterns of decomposition relate to snow cover dynamics in early and late snowmelt locations, and (3) what is the impact of seasonal and annual litter decomposition on N release. To address these questions, we established two litter decomposition experiments: (1) a two-year reciprocal transplant litter experiment to disentangle species effect from topographical effect on decomposition (experiment I), and (2) a seasonal experiment to identify relative influence of summer and winter periods on litter decomposition (experiment II).

Material and methods

Study site

Our research area was located in the mountain ranges of Grand Galibier and Grandes Rousses, South-Western French Alps (45°7'N, 6°5'E) above the potential tree line (2300-2400 m a.s.l.). This study was conducted in three sites. Each site was characterized by both the late and early snowmelt conditions (hereafter resumed as the topographical location) located at a distance of about 30 to 50 m apart (in total three replicates of each topographical location). Sites A and B were orientated East and were situated respectively at 2520m and 2550m (slope : 0° and ~ 30° respectively). The distance between two any sites always exceeded 500m. Site C was orientated North-East at an altitude of 2550m (slope : 0° and 20° in late and early snowmelt locations). In early snowmelt locations, communities were dominated by *Kobresia myosuroides* (Cyperaceae, KM) and *Dryas octopetala* (Rosaceae, DO). In late snowmelt locations, *Carex foetida* (Cyperaceae, CF), *Salix herbacea* (Salicaceae, SH), *Alopecurus gerardi* (Poaceae, AG) and *Alchemilla pentaphyllea* (Rosaceae) were the most abundant species. To estimate standing biomass and aboveground net primary productivity, we randomly harvested aboveground biomass in a 40 × 40 cm quadrat (n=3) on 23/07/03 at peak standing biomass in both topograpical locations of the A, B and C sites (Table 1). In the case of shrubs, only leaves were harvested (see below). Aboveground net primary productivity was determined by dividing standing biomass by the time between the date of snowmelt and peak biomass (Table 1).

The soils are classified as a stagnogley, enriched in clay, in late snowmelt locations, and as an alpine ranker in early snowmelt locations. The bedrock is basic flysh in sites A and B and calcareous in site C.

Climatic recordings

Hourly soil temperature was recorded in each topographical location from 2003 to 2006 with Hobo probes (Onset computer corporation, Bourne, MA, USA) buried at 5 cm belowground (one per topographical location, in total 6 hobo probes). Soil temperatures that remain close to 0 °C (usually between –1 and 1°C) throughout the day indicate a persistent snow cover. The wintertime period was calculated as the number of days exhibiting a mean soil temperature equal or below 0°C (Table 1). Five weeks separated snowmelt in early snowmelt compared to late snowmelt locations (Table 1). Mean wintertime soil temperature was also determined in each topographical location. Finally, volumetric soil water content in each topographical

location at a depth of 5 cm was measured continuously during growing seasons 2005 and 2006 with an Echo Probe (Decagon Devices, Pullman, WA).

Litter decomposition experiments

Experiment I

We chose litter from two dominant species in late snowmelt locations (here after "substrate") which represented more than 70% of aboveground biomass: one graminoid (*Carex foetida*) and one shrub (*Salix herbacea*). In early snowmelt locations, we selected one graminoid (*Kobresia myosuroides*) and one shrub (*Dryas octopetala*) which corresponded to more than 90% of total community biomass. We constructed 10×10-cm polyethylene litter-bags. They were separately filled with 1g of the litter of each species which was removed from the field at the end of the growing season and previously oven dried at 60°C for 48H.

The reciprocal-transplant litter experiment followed a split-plot-design. In total 384 litter-bags were deployed giving 8 replicates for 2 harvests of all substrates (CF, SH, KM and DO) in the three sites A, B and C in both topographical locations. This experiment was conducted in two stages, first with the two graminoid species (2003-2005), and second with the two shrub species (2004-2006). To allow for comparison between the two stages, a "standard" litter (SL), removed from a sweden grassland and used for a similar purpose in a global European survey (Quétier et al. 2007) was monitored in parallel (192 additional litter-bags). The first stage, concerning graminoid decomposition, was set up on 10/10/03 (KM/CF + SLI) and the second stage concerning shrub decomposition on 24/09/04 (DO/SH + SLII). The first harvest took place on 24/09/04 for KM, CF and SLI, the second on 10/10/05 for KM, CF, DO, SH and SLI/II, and the third on 02/10/06 for DO, SH and SLII. Litter remaining was carefully sorted then weighed after drying at 60°C during 48H.

Experiment II

Similarly to experiment I, we harvested at the end of september 2005 the litter from CF, SH, KM, and DO. We added a fifth species, *Alopecurus gerardi* (gramineae, AG) present in late snowmelt locations. This subtrate was harvested still green, because it senesced later under the snow. The same procedure in experiment I was conducted for the litter bag construction and filling. The seasonal litter experiment was set up on 10/10/05. Litterbags filled with substrates from the late snowmelt (CF, SH, AG) and from the early snowmelt locations (KM and DO) were respectively deployed in each topographical location for a total of 225 litter-bags. The design was therefore not orthogonal with respect to topographical effect. Three harvests were performed, at the end of the first winter just after snowmelt, at the end of the

growing season, and after the second winter. In early snowmelt locations, the first harvest (here after reported as "Winter I") took place on the 03/05/06 in all sites. In the late snowmelt locations, harvests were carried out on the 20/06/06 in site A, on the 14/06/06 in site B, and on the 19/06/06 in site C. The second harvest ("summer") took place on the 02/10/06 and the final harvest ("Winter II") was performed after a second wintertime period on the 18/05/07 in early snowmelt locations, and on 14/06/07 and 25/06/07 respectively in sites C, A and B. Treatment of litter-bags followed the same protocol as in Experiment I.

For each experiment I and II, we analysed three sub-samples of each substrate for initial C and N litter content using an elemental analyser (CHS NA1500, Carbo Erba Instrument, Milan, Italy) and for lignin content using the H_2SO_4 digestion method. .

To estimate litter decomposition, we calculate mass loss (%) over the different periods as follows:

$$Mass\ loss_{\Delta t} = (mass_t - mass_{(t-1)}) * 100$$

Statistical procedures

For experiment I, we used Linear Mixed Models (Pinheiro and Bates 2000) to assess effects of topography and species on mass loss. In a split-plot design, sites were considered as random factors, whereas harvest times, topographical location, species and their interactions were fixed factors. We also applied similar models within each harvest, to facilitate the interpretation of results. In the latter case, to estimate the variance explained by each effect compared to the total variance of the response variable, we followed Xu (2003). Partial coefficients of determination R^2 are given by:

$$R^2 = 1 - \frac{RSS}{RSS_0},$$

where RSS and RSS_0 are respectively the residual sums of squares for the model testing the specific effect and the global model. They therefore give an indication on the importance of the effect in addition to its significance. Goodness-of-fit of mixed models was assessed using residuals plotted at the different levels (sites/topographical location). Constant variance of residuals was assessed using Bartlett's test.

In addition, we determined the exponential decay constant, k, assuming a single exponential decay model following Hobbie (2000) and Hobbie and Gough (2004):

$$\mathrm{Mass_t} = \mathrm{Mass_{t0}} \cdot \mathrm{e}^{-\mathrm{kt}}$$

where $Mass_t$ is the remaining mass at time t, $Mass_{t0}$; the initial mass (1 g), and k; the constant

decay (yr^{-1}). Decay constants were determined for each species in each topographical location by fitting a linear-regression of the (LN-transformed) litter remaining against time. An ANCOVA was applied within topographical location to test the significance of the Mass remaining × Species interaction.

Finally, an ANCOVA was applied to test the regression between (1) yearly mass loss of each substrate against mean wintertime soil temperature and (2) litter N content (proportion of initial N content) of each species against mass loss. Analysed data were the mean of each harvest, in each topographical location. Species were considered as the qualitative factor whereas mean wintertime soil temperature (1) or mass loss (2) were the continuous factors.

In Experiment II, we estimated decomposition rate for each period, each species and for each topographical situation by applying the following formula :

$$\text{Decomposition rate}\,(\text{mg}/\text{d}) = \frac{1}{\text{T}} \cdot \left[\text{remaining mass}_{\text{t}} - \overline{\text{remaining mass}_{\text{t-1}}}\right]$$

where T is the period between t-1 and t (in days). We did not estimate the exponential decay constant (as done in experiment I) as the aims of this experiment were to precise for each period the decomposition rate of each substrate rather than the mean decomposition rate over all the period of the experiment. We used a two-way analysis of variance (ANOVA) to compare period and site effects for each species separately and to test differences in litter N content for each species (proportion of initial N content) after the first winter, summer and second winter. Finally, a Student's t-test was used to assess the initial value (=1) litter N content (in proportion to initial) and that after winter I, summer and winter II. Statistical analyses were performed using R software (R Development Core Team 2006).

Characteristics	**Late snowmelt location**	**Early snowmelt location**
Winter duration (d)	240 (12)	206 (15)
Mean winter soil temperature (°C)	-0.15 (0.01)	-3.05 (0.45)
Mean summer soil temperature (°C)	7.7 (1.55)	7.6 (1.62)
Gravimetric summer soil moisture ($gH_2O\ g^{-1}$ soil)	0.35 (0.03)	0.42 (0.03)
Productivity ($g\ m^{-2}\ d^{-1}$)	7.23 (0.45)	2.18 (0.14)
Total biomass ($g\ m^{-2}$)	325.67 (22.04)	185.90 (11.72)

Table 1 Winter duration, soil temperature records and soil moisture are an average of three years records from the three sites A, B and C within each topographical location (from the end of 2003 to the end of 2006). Values are the estimated parameter ± standard error except for winter duration where it is standard deviation.

Species	Location	Growth form	SLA (cm^2/gr)	LDMC (%)	C/N	Lignin content (%)	Experiment I			Experiment II		
							C/N litter	% N	% C	C/N litter	% N	% C
CF	Late snowmelt	Graminoid	194.5 (7.2)	26.1 (0.6)	11.2 (0.5)	6.1 (0.3)	20.6 (0.3)	2.32 (0.04)	47.1 (0.1)	37.7 (2.1)	1.22 (0.07)	45.1 (0.5)
KM	Early snowmelt	Graminoid	123.7 (2.27)	32.2 (0.5)	19.2 (0.7)	7.4 (0.5)	85.6 (5.2)	0.57 (0.03)	48.0 (0.1)	82.5 (5.5)	0.58 (0.05)	47.2 (0.5)
AG	Late snowmelt	Graminoid	191.1 (3.7)	24.5 (0.4)	13.1 (0.6)	3.8 (0.3)	-	-	-	25.6 (0.9)	1.76 (0.09)	44.6 (1.0)
SH	Late snowmelt	Shrub	198.4 (6.1)	31.8 (2.8)	14.7 (0.7)	33.5 (0.8)	23.0 (0.7)	2.20 (0.07)	50.6 (0.3)	21.7 (0.6)	2.27 (0.06)	48.9 (0.8)
DO	Early snowmelt	Shrub	115.9 (4.9)	38.7 (1.7)	20.3 (0.7)	36.1 (0.2)	50.2 (0.7)	1.02 (0.01)	51.0 (1.0)	35.6 (2.4)	1.43 (0.08)	50.4 (0.3)
Standard	-	Graminoid	-	-	-		37.3 (1.9)	1.26 (0.06)	46.2 (0.3)	-	-	-

Table 2 Habitat, growth form, and functional traits of green leaves (SLA, LDMC and C/N) and litter (C/N, lignin content) of *C. foetida*, *K. myosuroides*, *A. gerardi*, *S. herbacea*, *D. octopetala* and standard litter. In case of C/N litter characteristics, n=3, in the case of green leaf traits, n=10. Values are the estimated parameter (standard error).

Results

Standing biomass in late snowmelt communities was twice as large as in early snowmelt communities (Table 1). Late snowmelt communities produced more aboveground biomass in a shorter period, which explains the four-fold difference in productivity between early and late snowmelt locationss when expressed on a daily basis (Table 1). Species had contrasting leaf traits as well as litter chemistry (Table 2). Green tissue from late snowmelt locations species (CF, AG and SH) characteristically had high Specific Leaf Area (SLA), low Leaf Dry Matter Content (LDMC), low green leaf C/N, and litter C/N because of higher %N. Species from early snowmelt locations (KM and DO) had the inverse suite of leaf traits attributes. In contrast, lignin content correlate with growth form : shrubs exhibit logically higher lignin content than graminoids.

Annual litter mass loss: Experiment I

The slope of the relationship between the remaining biomass of SLII and SLI was not significantly different from one (slope = 0.85±0.09, confidence intervals =[0.65,1.04]), whereas the intercept was different from zero (intercept = 10.98±4.75, $t_{1,11}$ = 2.31, P=0.04) (Fig. 1). This indicates that decomposition was higher during the 2004-2006 than during 2003-2005. However, after constraining the slope to a value of 1, the intercept was greatly reduced (intercept: 2.65±0.92, $t_{1,11}$=2.87 P=0.01) and ranged from 0.62 to 4.67 % of mass loss. Compared to the magnitude of the differences observed between graminoids and shrubs (Fig. 2), this effect was negligible and hence was ignored in later analysis. Mass loss from graminoids and shrubs was therefore analysed within the same model.

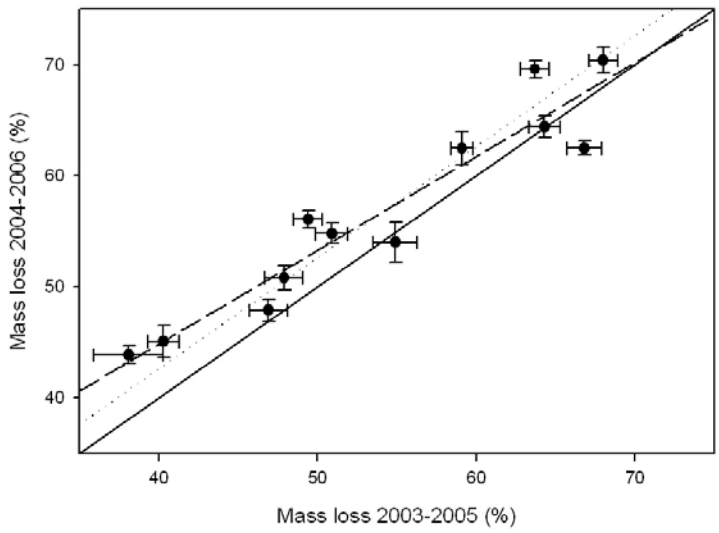

Fig. 1 Mass loss of standard litter set up in 2003 against mass loss of standard litter set up in 2004 (Experiment I). Each point corresponds to the mean of 8 replicates ± se from one site and one year. Short dash line: linear regression to data, dotted line: linear regression when slope is forced to one, solid line 1:1 slope.

There were large species specific differences in total litter mass loss, whereas the effect of topography was only marginally significant (Table 3, Fig. 2A and APPENDIX 1 for raw data within each site and each topographical location). The species × year interaction was highly significant, showing that patterns of mass loss over time depend on species composition. When analysed within the year, the species effect remained highly significant and explained 82 % of the model variability (Table 3). Graminoids exhibited the greatest mass loss (Fig. 2B, Table 4). SH and DO experienced almost two-fold-less mass loss than the graminoids (Fig. 2B). Consistent with the whole-model statistical analysis, the decay constant indicated more rapid decomposition of graminoids compared to shrubs at both early- and late snowmelt locations (Table 4).

Source	F, df	P	Partial R^2
Whole model			
Years	$767.98_{(1,358)}$	**<0.0001**	-
Species	$615.63_{(3,358)}$	**<0.0001**	-
Topography	$8.80_{(1,2)}$	0.10	-
Years × Species	$7.16_{(3,358)}$	**<0.0001**	-
Years × Topography	$0.00_{(1,358)}$	0.95	-
Species × Topography	$2.15_{(3,358)}$	0.09	-
Whole model – year I			
Species	$290.99_{(3,175)}$	**<0.0001**	0.82
Topography	$17.71_{(1,2)}$	0.05	0.00
Species × Topography	$2.58_{(3,175)}$	0.05	0.02
Whole model – year II			
Species	$432.1_{(3,176)}$	**<0.0001**	0.82
Topography	$5.02_{(1,2)}$	0.15	0.00
Species × Topography	$2.00_{(3,176)}$	0.11	0.01

Table 3 Split plot mixed Linear Model results for mass loss, comparing species and topographical effects for the whole data set and for each year (Experiment I).

The estimated uncertainty of the overall effect of topography was large (Table 3). However, the effect size (as measured by the differences between mass loss in the late snowmelt locations and mass loss in early snowmelt locations for each site and each year) always produced the same trend, as illustrated on Fig. 2C (see also Appendix 1). The probability that no negative value is obtained with 12 replicates, as shown on Fig. 3, is equal to 0.0002 (binomial test), providing strong evidence for an effect of topography. Thus, we can conclude that litter degradation was significantly higher in late snowmelt compared to early snowmelt locations.

As no summertime soil temperature and soil moisture differences could be detected between the late snowmelt and the early snowmelt locations (Table 1), we focused on wintertime conditions to explain topographical effect. The inconsistent snow cover in early snowmelt locations lead to low soil temperatures associated with large temperature fluctuations (Table 1). Regression of mass loss of each species after one year in each topographical situation against the respective mean wintertime soil temperatures (Fig. 4), showed a clear effect of both wintertime soil temperature ($F_{1,16}$=76.86, P<0.0001) and species ($F_{1,3}$=38.9, P<0.0001) on mass loss, but the variance explained by soil temperature remained low compared to the importance of the species effect (with temperature effect r^2= 0.90, $F_{7,16}$=31.8, P<0.0001, without temperature effect, r^2 = 0.77, $F_{3,20}$=27.53, P<0.001). The temperature × species interaction was marginally non-significant ($F_{3,16}$=2.60, P=0.08). However, when species were grouped into growth form (shrub *vs.* graminoid), the interaction became significant ($F_{1,20}$=5.48, P=0.03).

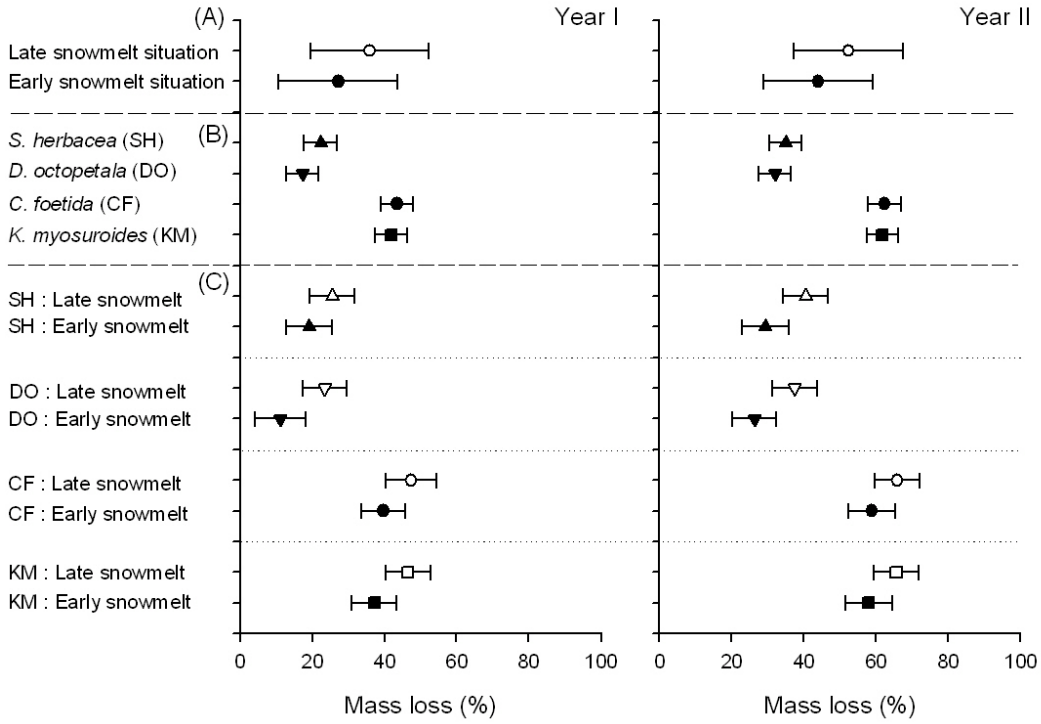

Fig. 2 Mean and 95% confidence intervals of litter mass loss after the first and second year of decomposition (a) in late snowmelt and early snowmelt locations (all species), (b) for each species (both topographical locations) and (c) for each species in each topographical location (Experiment I).

Seasonal litter mass loss: Experiment II

Similarly to Experiment I, there was large and significant effects of species on seasonal mass loss and decomposition rate (Fig. 5). After 1.5 year, the three graminoid species (CF, AG, KM) lost respectively 45.8%, 72.5%, and 49.3%, whereas DO lost 20.60% and SH 23.4% of their initial litter mass. Proportion of mass loss in winter I in comparison to total litter mass loss was high, ranging from 46.2% for SH to 80.7% for AG. In contrast, it ranged from 10 to 40% during summer and 7 to 27 % during Winter II. Only the decomposition of SH followed a different pattern, with similar mass loss observed during Winter I and the summer.

The estimates of decomposition rate were higher during the first winter for most species, except SH (Fig. 5, Table 5). During Winter II, decomposition rates were in general lower than summer and Winter I rates. The periods × sites interaction was significant for all species. This was related to variable patterns of decomposition among sites between summer and winter II. However, the higher decomposition rates recorded during winter I compared to others seasons was consistent throughout the sites.

Finally, the large variation in wintertime decomposition rate between species highlighted the importance of species and litter quality on decomposition, toward lower decomposition rate in the case of shrubs compared to graminoids, as in Experiment I.

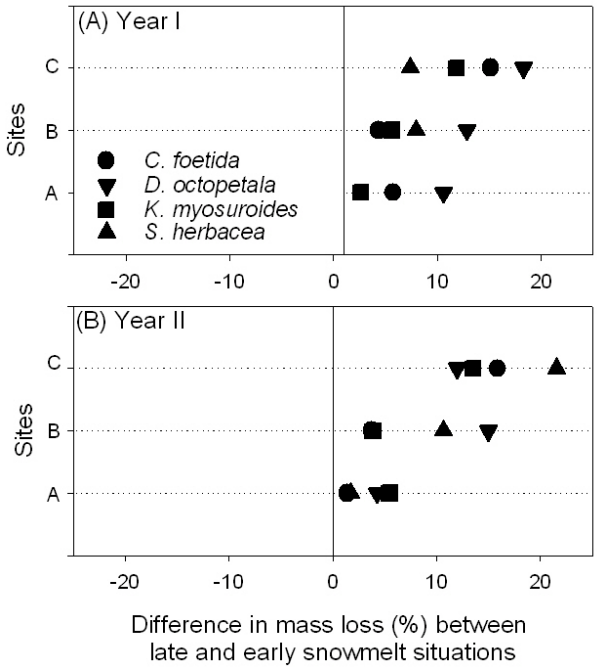

Fig. 3 Mass loss difference (%): late snowmelt locations minus early snowmelt locations mass loss for each species, each site (A, B and C) for (a) year I and (b) year II (experiment I). See text for statistical analysis.

Litter nitrogen dynamics

Litter exhibited very little net N release after two years of decomposition (Fig. 6). Within each species, there were no consistent differences in the patterns of N immobilization between both topographic locations. Significant regression between N immobilization and mass loss suggests that topography controls the timing rather than the pattern of N release (mass loss effect: $F_{1,40}$=294.50, P<0.0001). In other words, at a given percent mass loss, each species retained a similar N concentration (relative to initial) in both topographical conditions. Species differed significantly in net litter N immobilization ($F_{3,40}$=531.3, P<0.0001) but no species-induced changes of N immobilization pattern were detected (Mass loss × Species effect : $F_{3,40}$=1.70, P=0.18). KM, which is characterized by the highest C/N (Table 2), exhibited very strong N immobilization compared to the other species (a more than three-fold increased on the initial N concentration).

On a seasonal basis, all substrates, but DO, exhibited similar patterns of immobilization from the first harvest until the last, similarly to Experiment I (Fig. 7, Table 6). The overall analysis of N immobilization on a yearly (Experiment I) and seasonal basis (Experiment II) was not sufficiently consistent to allow generalisations about the relationship between magnitude of N immobilization and specific species.

Species	Late snowmelt location				Early snowmelt location			
	Decay constant (k, yr^{-1})	F,df	r^2	P	Decay constant (k, yr^{-1})	F,df	r^2	P
C. foetida	0.56 (0.01)	$1462_{1,47}$	0.97	<0.0001	0.47 (0.02)	$538_{1,47}$	0.92	<0.0001
K. myosuroides	0.56 (0.01)	$1672_{1,47}$	0.97	<0.0001	0.45 (0.05)	$805_{1,47}$	0.94	<0.0001
S. herbacea	0.27 (0.01)	$905_{1,45}$	0.95	<0.0001	0.19 (0.01)	$335_{1,43}$	0.88	<0.0001
D. octopetala	0.24 (0.01)	$1086_{1,47}$	0.96	<0.0001	0.15 (0.01)	$340_{1,44}$	0.88	<0.0001

Table 4 Statistics and decay constants (k) from regression of litter mass remaining against time in days. Regressions were done for each species and topographical location separately (Experiment I). Values are the estimated parameter (standard error).

Discussion

In this study, we showed that wintertime soil temperature and litter quality are the two key drivers of dominant-species litter decomposition in early and late snowmelt locations of alpine tundra. Only a few studies have addressed the relative importance of species *vs.* climatic effects on decomposition in alpine or arctic tundra. Hobbie (1996) compared the effects of increased temperature and litter from different Alaskan tundra on carbon and nitrogen mineralization in microcosms. They also tried to disentangle the influence of plant community composition from that of the soil environment at a moist acidic and a moist non-acidic site (Hobbie and Gough 2004). In alpine environments, Bryant et al. (1998) considered that the variation in decomposition rates along a snowmelt gradient was a function of temperature and moisture, however snow was not assessed as a potential determinant of litter decomposition. O'Lear and Seastedt (1994) reported higher decomposition of *Acomastylis rossi* in a late snowmelt compared to an early snowmelt locations, but this study addressed only decomposition of one species. Thus, despite numerous studies in snow-covered ecosystem highlighting the major role played by snow in nutrient cycling (Campbell et al. 2005), ours is to our knowledge the first study which hierarchies the snow-induced change effects on litter decomposition.

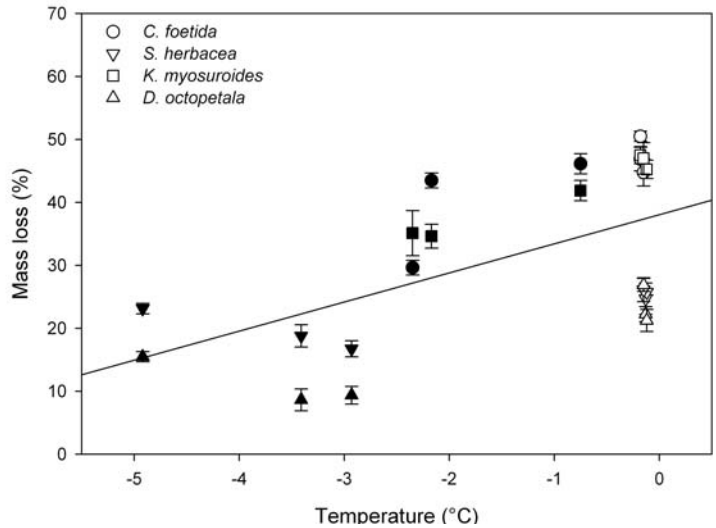

Fig. 4 Regression of mass loss against corresponding wintertime soil temperature for each species (experiment I). Each point corresponds to first year mass loss in each topographical location. White : late snomelt locations and black : early snowmelt locations. See text for statistical analysis.

Seasonal controls on litter decomposition

Our experiment indicated that, on average, 50 to 80% of the two-year mass losses occurred during the winter subsequent to the litter fall. Similarly, previous studies have reported that significant litter mass loss occurred during winter (Bleak 1970, O'Lear and

Seastedt 1994, Hobbie and Chapin 1996, Uchida et al. 2005). Moreover, recent studies have demonstrated that soil respiration persists under the snowpack (Brooks et al. 1997, Oechel et al. 1997, Fahnestock et al. 1999, Zimov et al. 1999, Welker et al. 2000), suggesting that cold-adapted microorganisms may be able to survive even at sub-zero soil temperature (Brooks et al. 2004). Nevertheless, we can not exclude the possibility that physical processes, such as fragmentation, may affect mass loss (Hobbie and Chapin 1996). However, the freeze thaw cycles which can contribute to litter fragmentation are less frequent in late snowmelt locations, so one should reasonably expect a lower litter mass loss in these areas, which is in contradiction to what is observed. Thus, we conclude that biological origin largely predominates over physical fragmentation to explain higher mass loss during winter.

High wintertime decomposition rates compared to summertime rates suggest that specific microbial activity in the surface organic horizon may be at least as important in winter as in summer. This suggestion is supported by evidence that a high level of microbial biomass was also detected during winter in the top soil layer (Baptist, unpublished results), as reported elsewhere by others (Lipson et al. 1999, Schadt et al. 2003, Lipson and Schmidt 2004). Total CO_2 efflux, which is lower during winter, would mainly be the result of fresh litter decomposition, instead of total soil organic matter mineralization. This assumption differs from studies (Clein and Schimel 1995, Hobbie et al. 2000), proposing that much of the wintertime decomposition activity may occur deeper in the soil profile in warmer horizons. But it is supported by Loya et al. (2004), who indicate that C compounds from fresh litter inputs can contribute strongly to winter C respiration in soil, and by Uchida et al. (2005), who show that microorganisms in the litter layer can play an important role in the carbon cycle during the winter. These results illustrate that the growth of wintertime microbial populations may be supported by the large input of litter at the end of the growing season. Thus, seasonal variations in decomposition rate would seem to be closely related to C compound availability during the first stage of decomposition. Brooks et al. (2004) drew similar conclusions as they showed that CO_2 fluxes from snow-covered soil at soil temperatures between 0 and -3°C were carbon-limited.

Seasonal decomposition of SH exhibited very different patterns from the other species. These results may be explained by the high concentration of polyphenols observed generally in *Salix sp.* leaves (Nyman and Julkunen Tiitto 2005). Indeed, the enzymatic degradation of these recalcitrant compounds is hindered by very high activation energy and low temperatures

may restrict their catabolisation (Bosatta and Agren 1999). Warmer conditions during summer would favour their degradation.

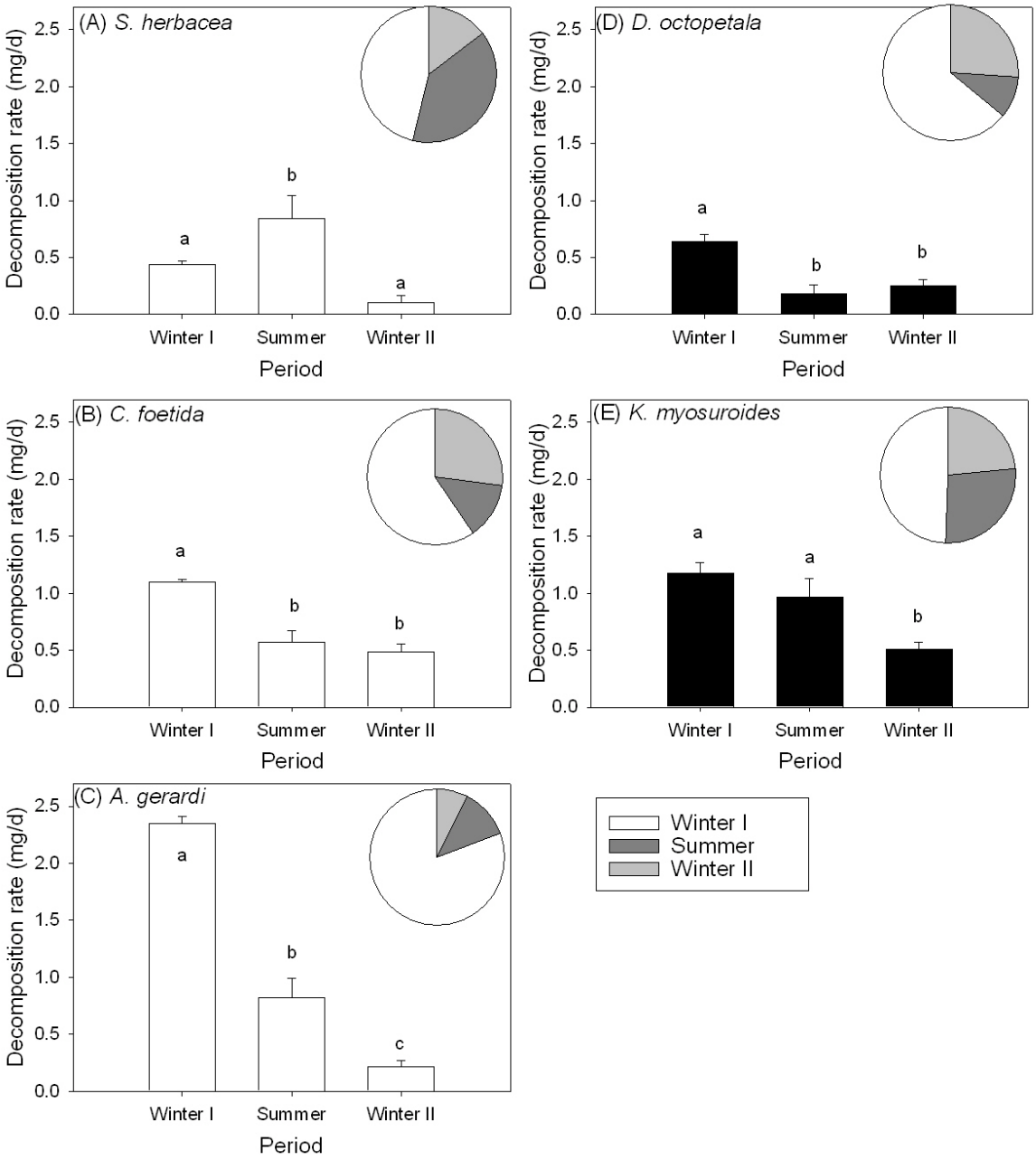

Fig. 5 Decomposition rates by species for each period (mg/d) (experiment II). Circle graphs depict mass loss (in % of total mass loss from fall 2005 to spring 2007) and correspond to each period within species. See Table 5 and text for statistical analysis. Values are the mean ± se.

Topographical controls on decomposition

All the species studied here experienced lower decomposition in early snowmelt compared to late snowmelt locations. During summer, soil temperature and soil moisture were similar in late snowmelt and early snowmelt locations, which is contrary to previous studies considering topographical gradients (O'Lear and Seastedt 1994, Bryant et al. 1998, Fisk et al.

1998). Thus, summertime conditions can not explain the effect of topography on yearly mass loss. In contrast, the positive relationship between mass loss and mean wintertime soil temperature shows clearly that the topographical effect is related to soil temperature and therefore to snow depth during winter. In late snowmelt locations, deep snow cover, which acts as an insulating layer, maintains soil temperature at 0° C, whereas the shallow and variable snow cover on early snowmelt locations leads to very low soil temperatures during winter and tends to limit microbial activity. Snow cover, through soil temperature, may therefore impact decomposition in a significant way. Results from Experiment II support this assumption as they showed that decomposition mainly occurs during winter. Thus, our experiment suggests that deeper snow is creating an abiotic environment which is more favourable for litter decomposition in late snowmelt compared to early snowmelt locations.

Nevertheless, besides a significant effect of topography, the main determinant of litter decomposition remained species composition (Experiment I). Strong interspecific variability in wintertime decomposition rate suggests that the nature of available substrates exerts an important control over microbial activity (Experiment II). Nadelhoffer et al. (1991) reported similar results in arctic ecosystems, as they showed that C mineralization was more strongly related to organic matter quality than to temperature.

Source	F, df	P
C. foetida		
Periods	$39.63_{(2, 36)}$	**<0.001**
Sites	$0.95_{(2, 36)}$	0.40
Periods × Sites	$10.92_{(4, 36)}$	**<0.001**
K. myosuroides		
Periods	$11.18_{(2, 36)}$	**<0.001**
Sites	$0.59_{(2, 36)}$	0.56
Periods × Sites	$3.55_{(4, 36)}$	**0.01**
A. gerardi		
Periods	$154.38_{(2, 36)}$	**<0.001**
Sites	$1.42_{(2, 36)}$	0.25
Periods × Sites	$6.18_{(4, 36)}$	**<0.001**
S. herbacea		
Periods	$13.71_{(2, 36)}$	**<0.001**
Sites	$5.33_{(2, 36)}$	**0.01**
Periods × Sites	$4.83_{(4, 36)}$	**0.003**
D. octopetala		
Periods	$18.27_{(2, 36)}$	**<0.001**
Sites	$0.42_{(2, 36)}$	0.66
Periods × Sites	$4.92_{(4, 36)}$	**0.003**

Table 5 Two-Way ANOVA results for decomposition rates comparing period (seasonal) and site effects (Experiment II).

Source	F, df	P
C. foetida		
Periods	$27.74_{(2,18)}$	**<0.001**
Sites	$1.90_{(2,18)}$	0.18
Periods × Sites	$2.06_{(4, 18)}$	0.12
K. myosuroides		
Periods	$26.93_{(2, 18)}$	**<0.0001**
Sites	$9.80_{(2, 18)}$	**0.001**
Periods × Sites	$0.86_{(4, 18)}$	0.50
A. gerardi		
Periods	$2.49_{(2, 18)}$	0.11
Sites	$0.75_{(2, 18)}$	0.48
Periods × Sites	$0.62_{(4, 18)}$	0.65
S. herbacea		
Periods	$14.57_{(2, 18)}$	**<0.001**
Sites	$0.84_{(2, 18)}$	0.45
Periods × Sites	$0.29_{(4, 18)}$	0.87
D. octopetala		
Periods	$8.36_{(2, 17)}$	**0.003**
Sites	$1.39_{(2, 17)}$	0.27
Periods × Sites	$0.59_{(4, 17)}$	0.67

Table 6 Two-Way ANOVA results for litter N (proportion of initial N) comparing period and site effects (Experiment II).

However, the reciprocal transplant litter experiment underlined that, despite distinct functional trait syndromes along the snowmelt gradient (Choler 2005, Baptist and Choler 2008), species effect was firstly structured by lignin content –growth form - rather than leaf nitrogen content associated to nutrient acquisition strategy (see Table 1). Interspecific variations in litter decomposition largely mirrored growth form diversity in alpine tundra, underlying the importance of lignin in decomposition process. These results are in agreement with previous studies, which showed that shrubs were characterized by a lower decomposition rate compared to sedge species in arctic and sub-arctic systems (e.g. Hobbie 1996, Cornelissen et al. 2007).

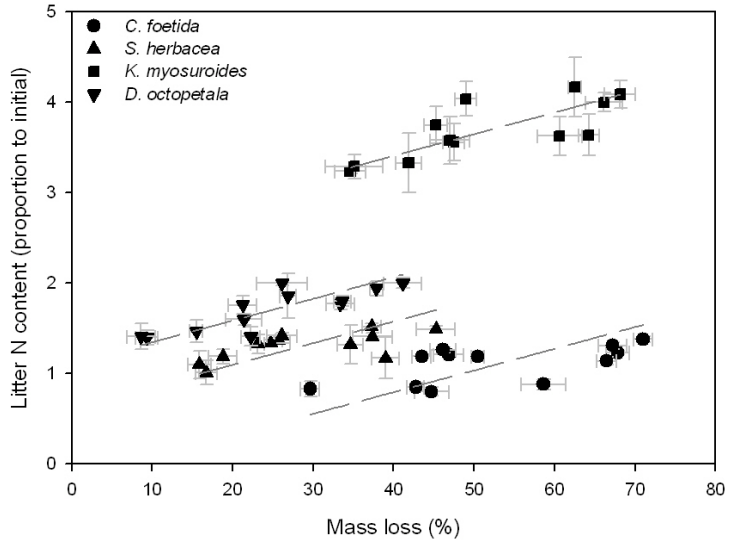

Fig. 6 Litter N content (proportion of initial N) by species in relation to mass loss (Experiment I). Symbols are the mean ± se at each period and each site. See text for statistical analysis.

Implications for N cycling

After two years of decomposition, litter still exhibited N immobilization. We would expect a subsequent period of net N release (mainly N mineralization) (Melillo et al. 1989), however the two-year duration of this experiment was insufficient time to reach this stage. No significant effect of topography on N immobilization was detected. Patterns of N immobilization was primarily the result of faster decomposition. The inconsistent snowpack in early snowmelt locations may delay the final stage of N mineralization, limiting N availability in the ecosystem. However, this phenomenon may also contribute to limit N loss during snowmelt as a result of steep slopes between early and late snowmelt locations and thus promote N retention in the ecosystem, even in an organic form (Steltzer and Bowman 2005). Litter quality affects N immobilization by delaying mass loss and so final N mineralization stage, but also by controlling the magnitude of N immobilization. KM

exhibited the highest immobilization in both experiments, which could be related to high initial C/N whereas CF weakly immobilized nitrogen. However, no significant relationship could be drawn between initial litter C/N and N immobilization after one year of decomposition. The different patterns of N immobilization depend on interspecific differences in nutrient concentration but also resorption and leaching from dead leaves. As a result, they are more likely to be idiosyncratic effects and, as such, difficult to predict.

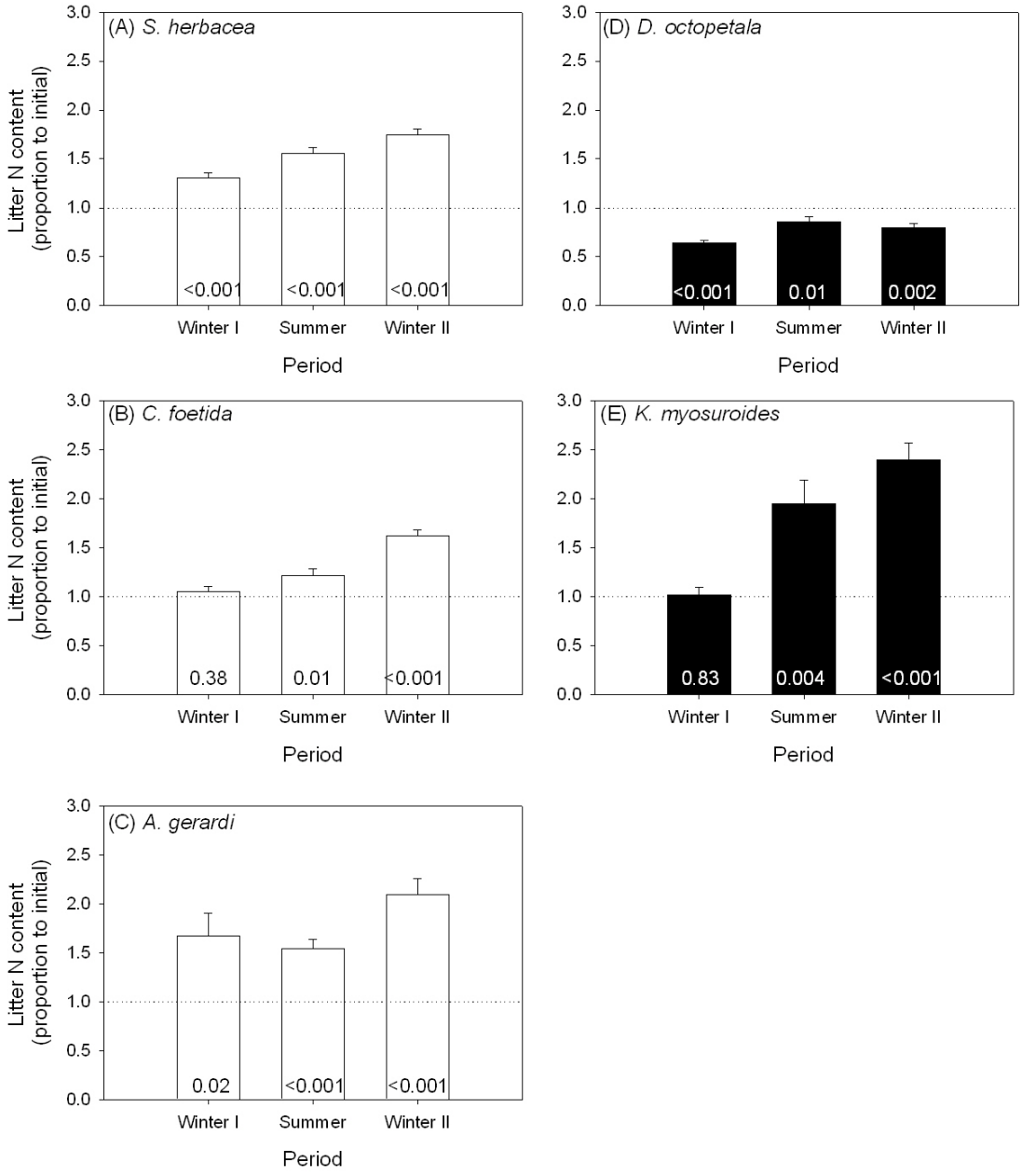

Fig. 7 Litter N content (proportion ofinitial N) by species for each period (Experiment II). Values are the mean ± se of each period for all sites. See Table 6 and text for statistical analysis.

Conclusion

In a context of global change, our results suggest that a decreasing winter snowpack would reduce mass loss during winter and would enhance the potential for C sequestration (Monson et al. 2006). However, the results highlighted growth forms as the primary drivers of decomposition compared to topography. Thus, it is suggested that changes in litter quality, as a result of community-level shifts in growth form will have a stronger impact on litter decomposition in alpine tundra than the direct effect of insulating snowpack.

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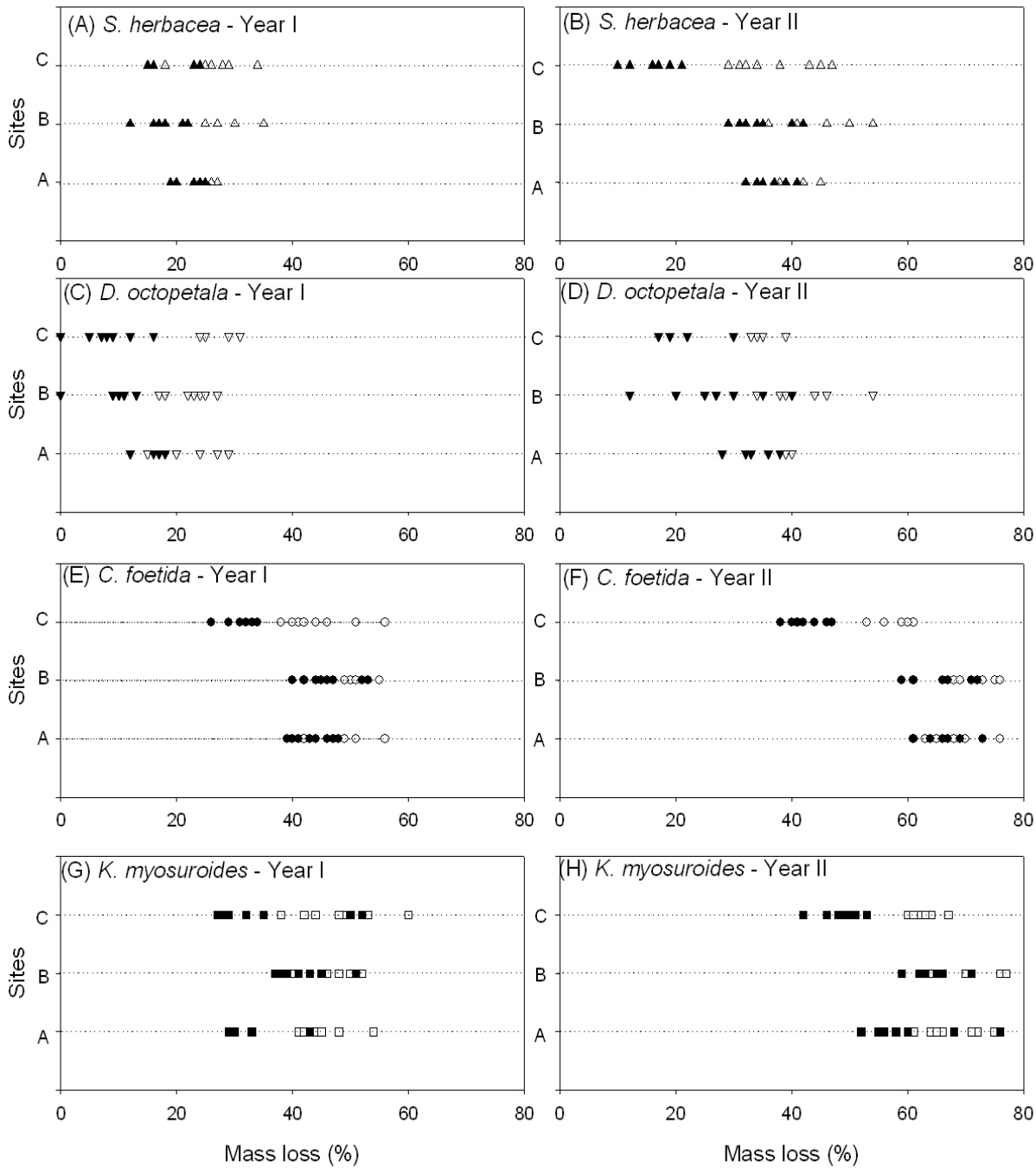
Appendix 1



Appendix 1 Mass loss of each substrate, in each site for early (black) and late (white) snowmelt locations after one (left) and two (right) years of decomposition (Experiment I).

Article 2B

Soil respiration in alpine tundra: impacts of seasonal snow cover and soil carbon content

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In prep. for *Global Change Biology*

Mesure de respiration des sols dans les systèmes thermiques (site B) le 29/05/06. En premier plan, les thermocouples connectés à l'enregistreur de température. Photo : C. Flahaut.

Abstract

Topographical variations and its associated changes in snow cover duration have long been recognized as the primary source of ecosystem structure and functioning in alpine landscapes. Yet, our understanding of the factors controlling soil heterotrophic respiration along snow cover gradients remains incomplete. Here, we examined the temperature sensitivity of alpine soils from early and late snowmelt locations. We conducted laboratory experiments on soil cores to disentangle the relative effect of temperature, soil water content and soil carbon content on heterotrophic respiration. Two set of soil cores, collected in summer and just before snowfall, were submitted to a high (2 - 30 °C) and a low (-8 - 0 °C) temperature range, respectively. A four parameter model explained 90 %, and 80 %, of the variance for the high and the low temperature range, respectively. A noticeable finding was a significant inverse relationship between soil carbon content and basal respiration. The model was adequate in predicting field effluxes measured on a weekly basis during the summer season. We built on these results to simulate summertime and wintertime CO_2 production rates using a 6-years long soil temperature time-series. Despite a much shortened growing season length, summer respiration of late snowmelt locations was 50% above that of soils from early snowmelt locations. This was due to a higher basal respiration rate which over compensated the negative impact of delayed snowmelt. During wintertime, the insulated effect of snowpack along with higher basal respiration explained increased soil CO_2 production rate in late snowmelt locations. The linkage between soil carbon content and substrate quality, and the interplay between carbon quality and soil climate on heterotrophic respiration in alpine tundra are discussed. We emphasized the need to care for topographical variations in any attempt to model soil CO_2 fluxes in cold, snow-covered ecosystems.

Keywords : alpine ecosystems, heterotrophic respiration, snowmelt gradient, soil organic matter, soil climatology

Introduction

In the next decades, snow-covered ecosystems may experience severe and rapid changes in response to global warming and variations in snowfalls (IPCC 2001). For example, high-elevation ecosystems from temperate European Mountains may be specifically exposed to lower snow deposition because of increasing temperature (Diaz and Bradley 1997, Beniston 2005, Noguès-Bravo et al. 2007). Soils of arctic and alpine ecosystem sequester large amount of organic carbon, and the impact of climate forcing on these carbon stocks is a main issue in global carbon cycle studies (Hobbie *et al.* 2000). However, there are still large uncertainties in predicting the impact of global change on the dynamics of this carbon stock (Grogan and Jonasson 2005, Knorr et al. 2005). A main reason is that our knowledge of the relative importance of different drivers controlling soil heterotrophic respiration remains incomplete for seasonally snow-covered ecosystems.

In arctic and alpine landscapes, changes in topography over short distances (i.e. < 100 m), also known as mesotopographical gradients (Billings 1973), are associated with dramatic changes in plant species composition (Chapin *et al.* 1995) and plant functional diversity (Kudo 1996, Choler 2005). Winter snow cover depth and snow cover duration are widely regarded as the main determinants of these mesoscale patterns (Isard 1986, Walker et al. 1999). The length of the favourable period for plant growth, the number of days when soil temperature drops below 0 °C are among the direct consequences of the seasonal dynamics of snow cover. There have been a number of studies comparing ecosystem functioning in early and late snowmelt alpine tundra (e.g. Bowman et al. 1993, Fisk et al. 1998, Soudzilovskaia et al. 2005, Baptist and Choler 2008). But so far, the interplay between soil properties - among which soil carbon content - and soil climate on instantaneous CO_2 effluxes and seasonal production of CO_2 has never been investigated along a snow cover gradient in temperate alpine tundra.

Heterotrophic respiration is the main component of ecosystem respiration in snow-covered ecosystem especially because the favourable period for plant root growth and activity is reduced (Elberling 2007). Temperature and soil moisture are the main soil climatic variables controlling CO_2 effluxes (Reichstein *et al.* 2003). Several authors reported that instantaneous, seasonal and annual CO_2 fluxes in arctic and alpine ecosystems were mainly controlled by temperature while soil moisture conditions had little impact (Kato et al. 2005, Kato et al. 2006, Elberling 2007), though opposite views exist (Illeris *et al.* 2004). The yearly time course of soil temperature is strongly determined by snow cover dynamics, and one may

expect an increased summer CO_2 production rate in early snowmelt locations compared to late snowmelt locations.

Other studies have put the emphasis on substrate quality (Grogan and Jonasson 2005, 2006, Elberling 2007) and substrate availability (Brooks *et al.* 2004) in determining CO_2 effluxes. Basal respiration measured on the upper soil layer is used as a surrogate of either carbon availability when expressed per gram of soil (Fierer *et al.* 2007) or either carbon quality when expressed per gram of carbon (Mikan *et al.* 2002). A much higher soil carbon content in the upper soil layer of early snowmelt locations is a common feature in alpine tundra (Gensac 1990). However, the impact of these contrasting carbon stocks along the snow cover gradient on basal respiration rate remains unknown. It is assumed that slow-growing, stress-tolerant species from early snowmelt locations produce more recalcitrant litter (Choler 2005, Baptist, unpublished results). A working hypothesis is that the negative impact of a lower organic matter quality in early snowmelt locations could counterbalance the positive effect of an extended growing season.

In this study, we combined laboratory and field measurements to examine the sensitivity of heterotrophic respiration of alpine tundra soil to temperature, water and soil carbon content. In a first approach, we favoured laboratory measurements in order (1) to model the relative effect of the main factors controlling soil respiration (Davidson et al. 1998, Reichstein et al. 2005) and (2) to allow a more straightforward measurement of soil respiration per gram of soil or per gram of carbon.

Because laboratory measurements of CO_2 effluxes have sometimes been considered as poorly indicators of field processes (Schimel *et al.* 2006), we also performed field measurements to assess the adequacy of the respiration model derived form laboratory experiments. We focused on a comparison between soils from early and late snowmelt locations, i.e. corresponding to the two extremes of the mesotopographical gradient. The investigated ecosystems corresponded to the dominant temperate alpine tundra in the Alps. We addressed the following questions: (1) are there any difference in the temperature sensitivity of CO_2 effluxes between soil cores from early and late snowmelt locations ?; (2) what is the impact of soil carbon content and soil water content on the basal respiration ?; (3) Is a model based on laboratory experiments adequate to predict summertime field CO_2 efflux measured in early and late snowmelt locations ?; and finally (4) how can we build on this knowledge to assess the relative impact of seasonal snow cover and soil carbon content on the summertime and wintertime CO_2 production in early and late snowmelt locations ?

Materials and methods

Study sites

Our three research sites were located in the mountain ranges of Grand Galibier in South-Western Alps, France (45°7'N, 6°5'E). The bedrock was predominantly made of calcareous shales. The elevation of the three investigated sites were 2520 m (site A), 2550 m (site B), and 2550 m (site C). The lower elevation limit of alpine tundra is thought to occur around 2300 - 2400 m in the area (Ozenda 1985). The distance between two any sites exceeded 500 m. Each site corresponded to a mesotopographical gradient (sensu Billings 1973), with alpine tundra from early and late snowmelt locations at a distance of about 30 to 50 m apart. In early snowmelt locations, the vegetation cover was discontinuous and the two dominant species are a turf graminoid, *Kobresia myosuroides* (Cyperaceae) and a dwarf shrub, *Dryas octopetala* (Rosaceae). In late snowmelt locations, the vegetation cover was higher and the plant community was dominated by tiny species with a marked ability for clonal lateral spread. *Carex foetida* (Cyperaceae), *Salix herbaceae* (Salicaceae), *Alopecurus gerardi* (Poaceae) and *Poa alpina* (Poaceae) were the most common species of late snowmelt locations. Further details on the taxonomy and plant functional diversity along the investigated mesotopographical gradient are given in Choler (2005).

Biogeochemical characterization of the soils

Soils of early snowmelt locations were alpine rankers, those of the late snowmelt locations are stagnogley (Bounemoura *et al.* 1998). At the end of summer 2006, the upper 10 cm of the soils were randomly sampled in early and late snowmelt locations with a coring tube (10 cm diameter) after litter removal. In early snowmelt locations, soil cores were collected between *K. myosuroides* tussocks. Soils were sieved at 2 mm for further biogeochemical analyses. Soil Organic Matter content (SOM) was determined by loss-on-ignition and the C mass was calculated by dividing SOM fraction by 1.72 (Schulte and Hopkins 1996). In order to determine bulk soil density, stone mass was determined and converted to stone volume using an average stone density of 2650 $kg.m^{-3}$ (Hillel 1971). The distribution of grain size (granulometry) was obtained by sieving.

Another set of sampling was designed to quantify the soil carbon stock in early and late snowmelt locations. We excavated a soil cylinder of approximately 5 cm of diameter with a hand auger. Soils of late snowmelt locations are unusually deep for a high-elevation ecosystem and we did not reach the bedrock with a 1 m depth soil column. On the contrary, soils of early snowmelt locations are shallower and the bedrock was located at around 50 to

70 cm depth. Soil carbon content was determined for each 20 cm layers. The total soil carbon on an area basis layer was estimated as in Rodeghiero & Cescatti (2005) on the entire profile.

Soil climatology

Continuous soil temperature recordings were performed at 5 cm belowground using Hobo probes (Onset computer corporation, Bourne, MA, USA). Hourly data were averaged to provide the daily means used in the seasonal simulations of CO_2 effluxes. Soil temperature time series for three to five early and three to five late snowmelt locations (including sites A, B and C) were available from 2000 to 2005. Depending on the site, 10 to 15 % of the presented temperature data are gap filled data. Temperatures closed to 0°C (usually between –0.5 and 0.5°C) all the day long were indicative of a persistent snow cover. Based on soil temperature data, the year was divided in two periods. The summertime period was defined as from the day the mean soil temperature rose above 0°C to the day it kept around 0°C (snow-covered soil) or to the day it dropped below 0°C (frozen soils). The wintertime period corresponded to the snow-covered period for late snowmelt locations, but this did not hold for early snowmelt locations because these locations exhibited inconsistent snow cover during winter (see Fig. 1).

Volumetric soil water content (m^3 m^{-3}) at a depth of 10 cm was measured with the soil dielectric sensor ECH_2O (Decagon Device, Inc. Pullman WA, USA). Data from four different probes per location were recorded at 20 min intervals during part of the summer seasons 2005 and 2006, for a total of around 100 days per location and per year. Data were pooled to have a representative sample of soil moisture at each location.

Laboratory measurements of soil respiration

Our aim was to compare the temperature response of early and late snowmelt soil respiration to summertime and wintertime range of temperature. In the case of summertime soil cores, we measured temperature response of early and late snowmelt soil cores at three levels of soil water content. Water content was not manipulated in the case of wintertime soil cores. The summertime range of temperature was set between 2 °C and 30 °C and the wintertime range between -8 °C and 0 °C. To match field situation as close as possible, we did not apply these two differing temperature treatments to the same soil samples: the summertime temperature range was applied to soils sampled in July 2006 (hereafter referred as unfrozen soil cores), whereas the wintertime temperature range was applied to soils sampled just before the first snowfalls in October 2006 (hereafter referred as frozen soil

cores). Intact soil cores were excavated carefully from in PVC pipes (15 cm depth and 10 cm diameter) in the late and early snowmelt locations of site A. Seven soil cores were randomly sampled in each topographical location. The base of the cores was covered by perforated plastic to contain soil. To limit efflux disturbance due to excavation, the cores were maintained during two weeks in the nearby common garden of Station Alpine Joseph Fourier at an altitude of 2100 m. Mean air temperature was locally 13.3 °C ± 3.4 in July 2006 and 5.7 ± 3.5 in October 2006. We assumed that this period was long enough for the roots to die, and that the measured respiration after two weeks was mainly due to heterotrophic respiration. We determined field capacity for soil cores as follows: an aliquote of soil in three out of seven soil cores was oversaturated with water, the excess water was allowed to drain for 2h and then weighed. Soil water content was assumed to be at field capacity and was determined after sieving at 2mm and oven drying at 105°C during 48h. Field capacity of soil cores corresponded to 59.6 g H_2O / g soil and 57.8 g H_2O / g soil in late and early snowmelt locations respectively (Table 1). During the experiment, soil water content was estimated gravimetrically by sampling small amount of soil in three out of seven soil cores in each snowmelt location. Wet treatment corresponded to 95.8 % (± 2.4) and 95.1 % (± 0.8) of field capacity respectively for late and early snowmelt soil cores. Similarly, moist treatment corresponded to 64.6 % (± 3.9) and 68.3 % (± 3.5) and dry one, 49.0 % (± 2.0) and 55.1 % (± 1.9). We used bulk soil density of each core to convert gravimetric soil water content into volumetric soil water content. Wet treatment corresponded to 29.0 % (± 1.0) and 28.0 % (± 1.0) for late and early snowmelt soil cores respectively. In moist treatment, it equalled 19.0 % (± 1.0) and 20.0 % (± 1.0), and in dry treatment 14.0 % (± 0.1) and 16.0 % (± 0.1). Finally, volumetric soil water content equalled 19.6 % (± 1.6) and 22.6 (± 0.7) for late and early snowmelt wintertime soil cores respectively.

Before starting measurements, the cores were let at least 24 h in a cooled incubator (Fisher Bioblock Scientific, Illkirch, Fr) to let soil CO_2 effluxes stabilize at a given temperature. Soil temperature was measured before each respiration record with thermocouples of 1mm diameter (Thermocoax SAS, Suresnes, Fr) inserted into soil at a depth of 5 cm. In the case of summertime soil cores, we started by measuring CO_2 effluxes for the wet treatment and then let the soil cores dry until adequate soil water content level was reached. To measure CO_2 effluxes, soil cores were enclosed in a respiration chamber connected to a closed system (EGM 4, PP Systems International Ltd., Hertfordshire, UK) staid outside the incubator. Data records lasted 4 to 6 min. Beyond this point, the respiration was decreasing because of an inversion of CO_2 gradient between soil and atmosphere

chamber. Soil respiration was calculated on the basis of a linear increase of CO_2 chamber concentration. At the end of the experiments soil were sieved at 2 mm and SOM fraction was estimated as described above.

Field measurements of soil respiration

In October 2005, we inserted at random three PVC pipes (10×12 cm) (hereafter collars) at about 10 cm in each topographical location (sites A, B and C). We made the assumption that CO_2 efflux measured the following summer was only due to heterotrophic respiration. During summer 2006, we measured soil CO_2 effluxes in each topographical location each week from snowmelt until the end of august with a closed system (EGM 4, PP Systems International Ltd., Hertfordshire, UK). The CO_2 analyser was attached to a dark and closed respiration chamber which was placed on top of the pre-installed collars. Recording lasted from 4 to 6 min depending on signal fluctuation. Soil temperature was simultaneously measured next to each core with thermocouples inserted in the soil (Thermocoax SAS, Suresnes, Fr).

We did not measured soil respiration immediately after rainfall events to avoid CO_2 effluxes due to CO_2 displacement from soil pores (Rodeghiero and Cescatti 2005). Data from the beginning of the vegetation season have been omitted as soil anoxia impacted strongly CO_2 signal and measurements.

Soil respiration modelling

We used non-linear regression models to describe the response of soil respiration (R) to temperature (T) in °C, volumetric soil water content (W) in m^3 m^{-3} and soil carbon content (C) in percentage of soil mass. Unless otherwise stated, respiration rate in this study are expressed in mg C g C^{-1} d^{-1}. The models were fitted separately to the respiration data from frozen and unfrozen soil cores obtained during laboratory temperature experiments. Model coefficients were determined for each soil core at a given soil water content level. The first model we used to fit the experimental data was the widely used Arrhenius-type first-order exponential equation:

(model 1) $R = \alpha_1 e^{(\beta 1 T)}$,

where α_1 is the exponential coefficient of basal respiration, i.e. the respiration rate at a temperature of 0 °C, and β_1 is a temperature scalar.

Empirical log-log linear relationships were found between α_1 and the explanatory

variables W and C. Therefore, we modified structure of model 1 to account for the impacts of C and / or W on soil respiration:

(model 2) $R = \alpha_2 W^{\upsilon 2} e^{(\beta 2 T)}$

(model 3) $R = \alpha_3 C^{\gamma 3} e^{(\beta 3 T)}$

(model 4) $R = \alpha_4 W^{\upsilon 4} C^{\gamma 4} e^{(\beta 4 T)}$

where υ_i and γ_i are empirical coefficients determining the sensitivity of the basal respiration rate to W and C, respectively.

We calculated the respiration coefficient Q_{10} which describes the sensitivity of respiration to a 10 °C change in temperature following the formula $Q_{10} = e^{(10\ \beta i)}$. For each model, we estimated the line that best describes the bivariate scatter between measured and predicted values using a model II regression. This line of best fit corresponds to the standardized major axis (SMA) (Warton *et al.* 2006). Model performance was estimated quantitatively by calculating the square of Pearson's correlation coefficient (r^2), and qualitatively by calculating the root mean square error (RMSE) and the mean absolute error (MAE) (Willmott and Matsuura 2005). Tests of the difference between estimated and hypothesised elevation (or slope) were based on t-statistic. The null hypotheses were slope $\neq 1$ and intercept $\neq 0$. These analyses were performed using the 'smatr' R package (Warton and Ormerod 2007).

We estimated the sensitivity of summertime CO_2 effluxes to C and T by varying C in a range from 5 to 15 % and by varying the summertime period length in a range from 120 to 180 days. The chosen ranges encompassed the observed variations between early and late snowmelt locations. A daily mean temperature of 9 °C was chosen for these simulations because this temperature corresponded to the summertime mean temperature for both early and late snowmelt locations. Therefore, sensitivity to T should be understood as sensitivity to summertime cumulated degree days. These simulations were performed with model 4 using two constant levels of soil water content throughout the simulation period, i.e. 20% and 30%.

A different approach was used for estimating the sensitivity of wintertime CO_2 effluxes to C and T. The main reason was that the daily mean temperature between early and late snowmelt locations strongly differed during winter (see fig. 1). Here we assumed that the key driver was the number of snow-covered days (i.e. days for which the mean soil temperature was around 0 °C). We simulated changes in wintertime respiration by varying the number of snow-covered days during winter. More than 160 days under a deep snowpack as observed in late snowmelt locations corresponded to a full wintertime period under the snow.

In this case, soil temperature of snow-covered days was set to 0 °C. By contrast, a full wintertime period without snow or under a shallow snow pack was typical of early snowmelt locations. In this case, soil temperature was randomly chosen from the distribution of wintertime daily mean soil temperature recorded in snow-free early snowmelt locations. For these simulations, we used model 3, i.e. assuming that the impact of soil water content was negligible during winter. We performed the simulations with the Q_{10} values of frozen and unfrozen soils. All computations and graphs were performed using R software (R Development Core Team 2006).

	Early snowmelt location	**Late snowmelt location**
Summer length (d)	170 ± 3	125 ± 3
Cumulated GDD (°C)	1428 ± 258	1174 ± 198
Summer mean temp. (°C)	8.3 ± 1.4	9.4 ± 1.4
Winter mean temp. (°C)	-1.8 ± 0.6	-0.1 ± 0.2
Bulk soil density ($g.cm^{-3}$)	0.45 (0.03)	0.69 (0.02)
Depth (cm)	60-80	>120
pH in water	5.11 (0.0	4.96 (0.06)
SOC in upper layer (%)	10.6 ± 2.3	6.1 ± 1.0
Soil Carbon stock ($kgC\ m^{-2}$)	26.2	37.8
Granulometry (%)		
Clay (<2µm)	9.7 (0.5)	26.4 (2.6)
Silt (2-50µm)	41.4 (1.0)	61.7 (2.0)
Sand (50-2000µm)	48.6 (1.2)	11.9 (4.5)

Table 1. Climatic and geochemical characteristics of the soils from early and late snowmelt locations. For temperature-related values, data are averaged over the period 2000-2005 and over the three investigated sites (see also Fig. 1). For volumetric soil water content, the mean values of discontinuous measurements performed in the three sites during the growing seasons 2005 and 2006 are given (see Supplementary Material for further details). Geochemical characteristics, including soil pH, bulk density, soil carbon content and granulometry were measured on the soil samples used for respiration (n=5). Soil depth and total soil carbon on an area basis were estimated from 2 soil profiles in each sites.

Results

Soil characteristics and soil climatology

Soils from late snowmelt locations were deeper, slightly more acidic, and strongly enriched in fine particles compared to soils from early snowmelt locations (Table 1). Soil development in early snowmelt locations was weak, and the upper 30 cm layer of the soil profile was characterized by a high content of soil organic carbon (Table 1 and Fig. 1 in Supplementary Material). By contrast, soil profile in late snowmelt locations are more differentiated with a distinguishable anoxic B-horizon and evidence of duplicated soils along the profile. Soil organic matter was more evenly distributed along the profile (Table 1 and Fig.1 in Supplementary Material). Compared to lowland soils, both soil types are sequestering a large amount of organic carbon: from 26 kg C m^{-2} in the early snowmelt locations to 38 kg C m^{-2} in late snowmelt locations.

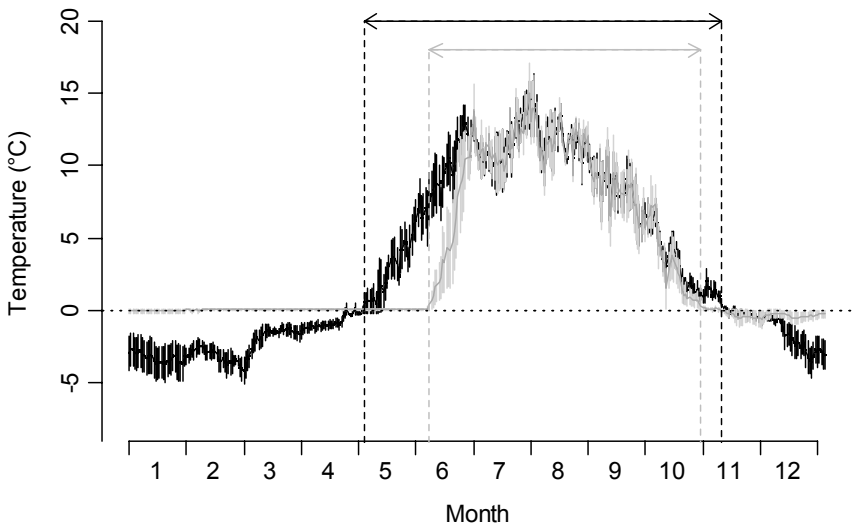

Fig. 1 Annual variations of daily mean soil temperature (± s.e.) observed for early (black) and late (gray) snowmelt locations. Data are averaged over the period 2000-2005 and were recorded in two or three of the investigated sites depending on the year.

The seasonal snow cover was the primary determinant of the yearly course of soil temperature (Fig. 1). In early snowmelt locations, the inconsistent snow cover during winter lead to an extended soil freezing period with a mean wintertime temperature of -2 °C (Table 1). In the late snowmelt locations, a deep and persistent snow pack throughout wintertime maintained daily mean soil temperature around 0 °C (Table 1 and Fig. 1). Soil warming in early snowmelt locations occurred nearly 40 days before what was observed in snowy locations (Fig. 1). This time shift was remarkably constant throughout the 6 years of recordings and across the three sites (Table 1). During the summertime snow free period, both topographical locations exhibited very similar daily mean soil temperatures (Table 1 and Fig. 1). Therefore, the observed difference in summertime cumulated degree days between the

locations was almost entirely due to the delayed onset of the growing period (Table 1).

Volumetric soil water content measured during the growing season showed that, on average, soils of early snowmelt locations were weakly drier that soils from late snowmelt locations (Table 1). This was especially noticeable in site C (Fig. 2 in Supplementary Material).

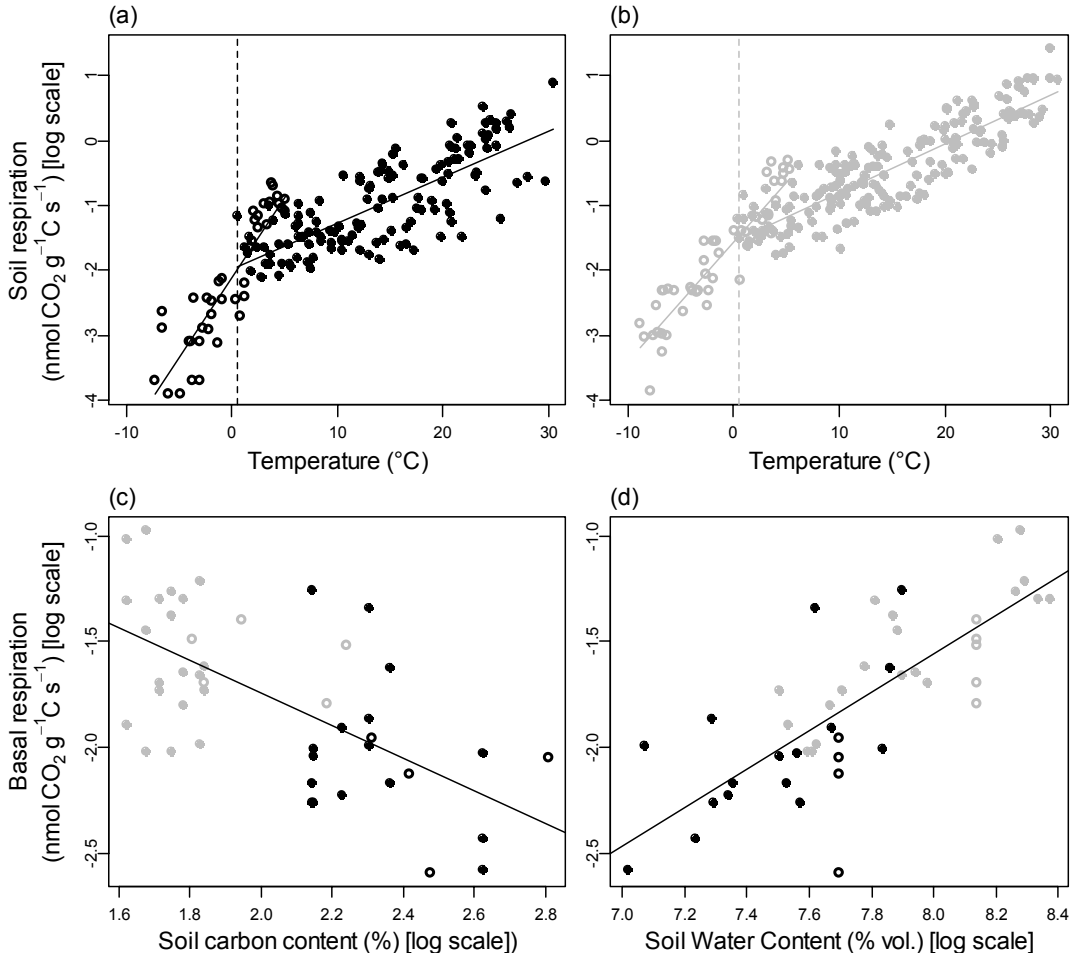

Fig. 2 Laboratory data on CO_2 efflux from -10 to 0 °C (frozen, open symbols) and from 0 to 30 °C (unfrozen, closed symbols). Soils samples represented upper layer of early (a) and late (b) snowmelt locations. The lines of best fit, using a simple first-order exponential model (Respiration = α e $^{(\beta T)}$), are shown separately for the two temperature ranges. The relationships between the exponential constant α and soil carbon content (c), and between α and volumetric relative water content (d). The lines of best fit, using a log-log linear model, were adjusted for a single dataset including unfrozen and frozen samples from early (black) and late (gray) snowmelt locations.

Observed and simulated CO_2 efflux

To investigate the relative effect of temperature, water and carbon content on soil CO_2 effluxes, we conducted laboratory experiments on soil cores collected in early and late snowmelt locations. A simple first-order exponential model (model 1) explained 59 %, and 75 %, of the observed variance with unfrozen soils from early and late snowmelt locations,

respectively (Fig. 2a, b and Table 2). The corresponding values for frozen soils were 86 % and 73 % (Table 2). For each soil type, the results showed an abrupt change of the temperature sensitivity of CO_2 release when comparing frozen and unfrozen soils (Fig. 2). Though the Q_{10} values were very comparable for both soil types incubated at temperatures above 0°C ($F_{1,34}$ = 0.82, P=0.37), there was a difference for frozen soils ($F_{1,7}$ = 6.12, P = 0.04). Soils from early snowmelt locations displayed a higher sensitivity to temperature (Q_{10} around 12) compared to soils from late snowmelt locations (Q_{10} around 7) (Table 2). Finally, we found that the basal respiration, i.e. coefficient α_1, of unfrozen soils was much higher in late snowmelt soils compared to early snowmelt soils ($F_{1,34}$ = 16.54, $P < 10^{-3}$) (Table 2). There were significant log-log linear relationships between α_1 and soil carbon content (r^2 = 0.40, $P < 10^{-3}$), and between α_1 and soil water content (r^2 = 0.61, $P < 10^{-3}$) (Fig. 2c, d). Because part of the observed variance in α_1 was explainable by these two additional variables, models 2, 3 and 4 largely improved on model 1 (Table 2). For example, RMSE and MAE were reduced by nearly a factor two when comparing the model 1 and 4 adjusted for all unfrozen soil cores (Table 2), resulting in a good adequacy between measured and predicted values (Fig. 3a). For all the models, the slope between measured and predicted values was significantly below one (Table 2). By comparison, we did not find significant bias in the elevation coefficient in most of the models except model 1 (Table 2). This led to slight overestimation of CO_2 release in the low range of temperature and to an underestimation at the highest temperature (Fig. 3).

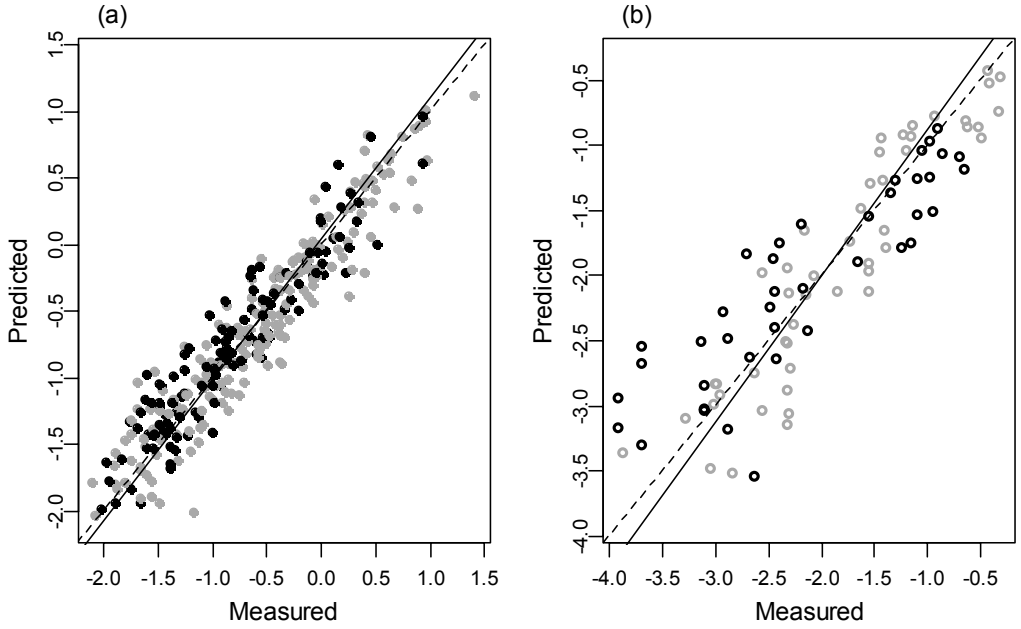

Fig. 3 The relationships between measured (laboratory data) and predicted values of CO_2 efflux for unfrozen (a) and frozen soil samples. Model 4 was used for unfrozen soils and Model 3 for frozen soils (see Material and Methods). Evaluation of the model performances are given in Table 2. Colours and symbols are as shown in Fig. 3.

Plot	Temperature range	Model	n	Model coefficients					Model evaluation						
				α	β	γ	υ	Q_{10}	r^2	Elev.	Elev. = 0	Slope	Slope = 1	RMSE	MAE
Early snowmelt	Unfrozen soils	1	128	-1.98	0.07	-	-	2.03	0.59	-0.22	< 10-3	0.77	< 10-3	0.44	0.37
		2	128	-8.07	0.07	-	1.62	2.11	0.8	-0.1	0.018	0.89	0.006	0.31	0.25
		3	128	-0.27	0.07	-0.74	-	2.04	0.63	-0.2	< 10-3	0.79	< 10-3	0.42	0.36
		4	128	-6.25	0.08	-0.88	1.67	2.12	0.85	-0.08	0.047	0.92	0.018	0.27	0.21
	Frozen soils	1	40	-2.11	0.25	-	-	12.2	0.81	-0.21	**0.167**	0.9	**0.148**	0.43	0.33
		3	40	-2.18	0.25	0.03	-	12.2	0.81	-0.21	**0.167**	0.9	**0.148**	0.43	0.33
Late snowmelt	Unfrozen soils	1	177	-1.57	0.08	-	-	2.13	0.75	-0.06	0.03	0.87	< 10-3	0.34	0.29
		2	177	-5.38	0.07	-	1.04	2.05	0.9	-0.02	**0.216**	0.95	0.029	0.22	0.18
		3	177	1.58	0.07	-1.79	-	2.1	0.79	-0.05	**0.05**	0.89	0.001	0.32	0.25
		4	177	-2.7	0.07	-1.45	1	2.03	0.92	-0.02	**0.284**	0.96	**0.057**	0.19	0.15
	Frozen soils	1	48	-1.57	0.18	-	-	6.26	0.86	-0.14	**0.201**	0.93	**0.172**	0.33	0.27
		3	48	-0.98	0.18	-0.3	-	6.11	0.86	-0.13	**0.208**	0.93	**0.178**	0.33	0.28
Late and early snowmelt	Unfrozen soils	1	305	-1.75	0.07	-	-	2.1	0.62	-0.14	< 10-3	0.78	< 10-3	0.45	0.36
		2	305	-6.07	0.07	-	1.17	2.07	0.75	-0.09	< 10-3	0.87	< 10-3	0.37	0.29
		3	*305*	*0*	*0.07*	*-0.9*	-	*2.09*	*0.75*	*-0.09*	*< 10-3*	*0.86*	*< 10-3*	*0.37*	*0.3*
		4	*305*	*-4.4*	*0.07*	*-0.9*	*1.21*	*2.05*	*0.89*	*-0.04*	*0.038*	*0.94*	*0.003*	*0.24*	*0.19*
	Frozen soils	1	88	-1.81	0.2	-	-	7.16	0.73	-0.29	0.008	0.85	0.006	0.49	0.39
		3	*88*	*-0.1*	*0.2*	*-0.8*	-	*7.29*	*0.79*	*-0.22*	*0.028*	*0.89*	*0.021*	*0.43*	*0.34*

Table 2 Coefficients and evaluation of the performance of the different soil respiration models (see Material and Methods). Results are shown for early and late snowmelt locations as well as for the global data set. Data for frozen and unfrozen soils are presented separately. The fit between measured and predicted values was evaluated quantitatively by the square of Pearson's correlation coefficient (r^2), and qualitatively by the root mean square error (RMSE) and the mean absolute error (MAE). Tests of the difference between estimated and hypothesised elevation (or slope) were based on t-statistic. Bold values indicate when one can not reject the null hypotheses (i.e. slope = 1, elevation = 0) given the data. Rows in italic points to the model used for seasonal estimates (see Fig. 4 and 5).

We examined whether model 4 was adequate in predicting field CO_2 effluxes. Our field measurements were performed in three different sites during the summer 2006, and repeated on a weekly basis. *In situ* heterotrophic respiration rates expressed per surface unit were transformed in mg C g C^{-1} d^{-1}. For this estimate, we made the assumption that only the first 30 cm of the soil profile were really contributing to soil respiration and we used the total amount of carbon in this layer to transform the data. Overall, the model captured most of the variance of the empirical data but had a tendency to underestimate CO_2 fluxes (Fig. 4). It was fairly adequate in predicting actual soil CO_2 effluxes from late snowmelt locations. By contrast, we found much more scattered values when examining data from early snowmelt locations (Fig. 5 and Table 3). We did not find any evidence of a significant effect of the date of soil respiration measurement on this pattern (data not shown).

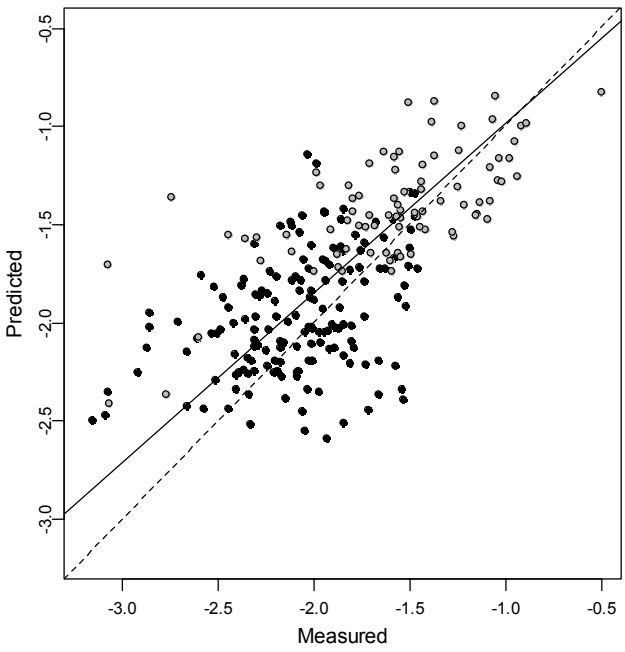

Fig. 4 The relationships between measured (field data) and predicted values of CO_2 efflux. Field measurements were conducted during summer 2006. Measured respiration rates per soil area were transformed to respiration rates per g of soil carbon and were fitted against model 4 (see Material and Methods). Evaluation of the model performance is given in Table 2. Colours are as shown in Fig. 2.

Simulation of seasonal CO_2 production rates

We questioned the relative importance of snow cover dynamics and soil carbon content on total CO_2 production during summer and winter periods. At a given temperature, CO_2 release from early snowmelt unfrozen soils was depressed because of a lower basal respiration rate compared to late snowmelt locations. Simulations revealed that the total CO_2 production during summer is higher in late snowmelt locations than in early snowmelt locations, despite a shortened favourable period (Fig. 5a, b). The same results were found at two levels of soil water content (Fig. 5a, b). The difference between the topographical locations would be even larger if contrasting values of soil water content – i.e. slightly higher in late snowmelt locations - had been considered (data not shown).

Qualitatively similar results were obtained for simulations of wintertime CO_2 production (Fig. 5c, d). For a given soil carbon content, the wintertime CO_2 production rate slightly increased with increasing fraction of snow-covered days (Fig. 5c), and the trend was more pronounced with high Q_{10} values (Fig. 5d). Noticeably, the effect of varying number of snow-covered days on soil respiration remained weak because respiration rates are estimated in a low temperature range (typically from -8 °C to 0 °C). In both simulations, the CO_2 production in late snowmelt locations was approximately twice as much as in early snowmelt locations (around 40 g C g^{-1} against 20 g C g^{-1} C). Clearly, this difference was mainly due to the effect of soil carbon content on basal respiration and to a lesser extent to the difference in the number of snow-covered days (Fig. 5c, d).

Plot	n	Model evaluation						
		r^2	Elev.	Elev. = 0	Slope	Slope = 1	RMSE	MAE
Late and early snowmelt	241	0.44	0.09	**0.342**	0.99	**0.818**	0.39	0.30
Early snowmelt	157	0.15	-0.23	**0.099**	0.88	**0.077**	0.36	0.29
Late snowmelt	84	0.47	-0.39	$< 10^{-3}$	0.59	$< 10^{-3}$	0.45	0.34

Table 3 Performance of the soil respiration models based on laboratory experiments in predicting field CO_2 efflux measurements. See Table 2 and Material and Methods for details on statistical analysis.

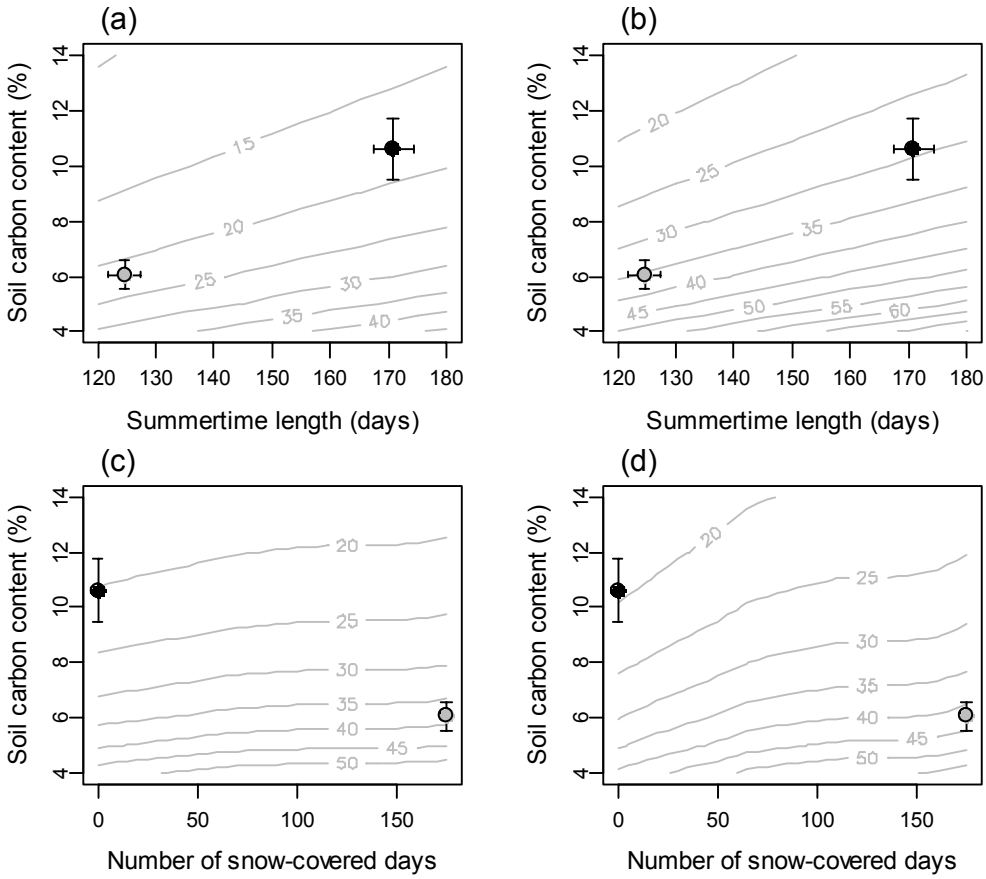

Fig. 5 (a, b) The relative impact of growing season length and soil carbon content on the total summertime CO_2 efflux. Simulations were done with model 4 using two levels of soil water content: 20% (a) and 30% (b). We used a constant daily mean temperature of 9 °C, corresponding to the mean temperature of the growing season period (see Table 1). (c, d) The relative impact of the snow-covered period and soil carbon content on the total wintertime soil respiration. Simulations were done with model 3 using Q_{10} values of unfrozen (c) and frozen (d) soils. For all panels, seasonal CO_2 productions (in g CO_2 g^{-1} C) are represented by the contour gray lines.

Discussion

The goal of the study was to assess the relative effects of temperature, soil water content and soil carbon content on CO_2 effluxes in late and early snowmelt locations in alpine tundra. Combining modelling and empirical approaches, we showed that differences among basal respiration rates accounted for much of the differences on CO_2 effluxes between the two contrasting topographical situations. A higher basal respiration rate in late snowmelt locations explained a higher summertime CO_2 production despite a shortened growing season. This impact of basal respiration rate also overrode temperature effect when simulating CO_2 production during wintertime.

Impact of soil water content and soil carbon content on basal respiration rate

Soil water content and soil carbon content accounted for most of the variation observed in the basal respiration rate of a first-order exponential model of soil respiration temperature dependence. For water, average values of summertime basal respiration were reduced by 34% and 30% from wet to moist conditions in early and late snowmelt cores respectively. This confirmed previous studies (Sjögersten and Wookey 2002, Illeris et al. 2004, Sjogersten et al. 2006) which provided evidence for a significant effect of water on CO_2 effluxes in cold ecosystems. However, the differences of soil water content between early and late snowmelt locations measured in the field were relatively weak. Therefore, it is unlikely that soil water content would represent a key driver of CO_2 effluxes variations along the investigated topographical gradients. These results contrast with those obtained in arctic tundra where soil water table was shown determinant in regulating local and regional and CO_2 effluxes (Ostendorf 1996, Sjogersten et al. 2006). Also they are not in line with other studies performed in temperate alpine tundra suggesting higher hydric limitation in early compare to late snowmelt locations (O'Lear and Seastedt 1994, Bryant et al. 1998, Fisk et al. 1998). In the investigated area, late snowmelt locations are sufficiently well-drained so that the flooding period at the time of snowmelt does not generally exceed one week (Ph. Choler, *pers. obs.*). Later on, low summer rainfall and prolonged clear sky periods rapidly attenuate the initial differences in soil water content along the topographical gradient.

The comparison of early and late snowmelt locations indicated an inverse relationship between soil carbon content and basal respiration (Fig. 2). There are only few examples of such relationships in the literature and they generally report the opposite pattern. Gough & Seiler (2004) reported a weak but significant positive relationship between total soil carbon and soil respiration in a loblolly pine stand (per unit area), while Rodeghiero & Cescatti

(2005) indicated a highly significant relationship between soil respiration at the reference temperature of 10°C and soil carbon content (per unit area). Conversely, no clear trend emerged between soil carbon stock and soil respiration (per unit area) in the study of Reichstein *et al.* (2003). These contrasting reports are firstly explained by the absence of standardized protocols for soil respiration and soil carbon stocks (Rodeghiero and Cescatti 2005). They also result from confounding effect of C quantity and quality as soil respiration is generally expressed per unit area. In this experiment, we expressed CO_2 effluxes per gram of carbon so as to gain insights on the specific activity of microorganisms. Our results showed that this specific activity was strongly reduced in early snowmelt compared to late snowmelt locations, providing evidence for a higher C recalcitrance in early snowmelt locations. This is in agreement with the observation that litter from the dominant *Dryas octopetela* in early snowmelt locations contains high amount of tannin and lignin (Baptist *et al.* 2008, Baptist, unpublished results). By contrast, it has been shown that the presence of a deep snow pack during winter enhanced microbial decomposition, and thus limited the accumulation of recalcitrant compounds in the soil (Hobbie and Chapin 1996).

Seasonal and spatial variations of temperature sensitivity

The range of Q_{10} values observed in this study was in accordance with the values generally reported in arctic and alpine ecosystems. Various studies measured Q_{10} between 2 and 9 above 0°C, and from 8 to 240 below freezing point (Mikan et al. 2002, Elberling and Brandt 2003, Kato et al. 2005, Panikov et al. 2006). The underlying mechanisms responsible for the abrupt change in temperature dependency at freezing point have been widely debated. Mikan et al. (2002) proposed that physical processes such as extracellular substrate diffusion or intracellular dehydratation may affect biological activity under freezing point leading to various temperature sensitivity of saprophytic microorganisms. The occurrence of psychrophylic microbial community exhibiting exponential growth rate and high rates of substrate utilization at below zero temperature tend to support this hypothesis (Monson *et al.* 2006). However, soil water content affects gas diffusion under freezing point and thus modulates CO_2 release around 0°C (Elberling and Brandt 2003, Panikov et al. 2006). These physical processes may also explain the abrupt changes of Q_{10} values around 0°C.

While summertime soil cores from both locations exhibited equivalent temperature sensitivity, soil cores from early snowmelt locations exhibited higher temperature sensitivity below 0°C. Different soil textural properties between the two soil types might impact the dynamics of unfrozen water, microbial access to substrate and therefore modulate CO_2

trapping and release. Another explanation might lay in the contrasting functional properties of microbial communities along the topographical gradient. Molecular studies provided evidence for large shifts in fungal and bacterial communities between early and late snowmelt sites (Zinger, unpublished results). Furthermore, we showed that specific substrate and frozen temperature lead to the development of a psychrophylic fungal community in early snowmelt locations (Baptist et al. 2008). Further studies are now required to test whether these wintertime microbial communities from early snowmelt locations are more sensitive to temperature changes.

The interplay of soil carbon content and soil climate on seasonal CO_2 effluxes

We are aware of only a few number of studies that investigated the impact of carbon quality on CO_2 efflux spatial variation in cold ecosystems (Vance and Chapin III 2001, Smith 2005, Elberling 2007). All of these studies highlighted an important effect of carbon quality on spatial variations of CO_2 effluxes and suggested that, in snow-covered ecosystems, this parameter may exert primary control on heterotrophic soil respiration at least during growing season. Here, we developed a 4-parameter model which predicted reasonably well the field-measured soil CO_2 effluxes during the growing season. We are therefore confident that the relative importance of soil carbon content and soil climate on summertime CO_2 production were well captured by our model.

By contrast, large uncertainties remain for the wintertime estimates of CO_2 production. For example, our simulations showed that in late snowmelt locations, the wintertime estimates of CO_2 production were roughly identical to summertime estimates with low Q_{10} values, but nearly two times higher if Q_{10} values from frozen soils were used. A more accurate estimate of wintertime soil respiration in snow-covered ecosystems remains a challenge because (1) the temperature sensitivity of soil micro-organisms in frozen soils has proven quite difficult to estimate (see above) and (2) there are still considerable technical difficulties in measuring soil CO_2 effluxes under the snowpack (Hubbard *et al.* 2005). Clearly, a better understanding of wintertime soil respiration is required in order to predict impact of climate change on annual net carbon balance in snow-covered ecosystems (Elberling and Brandt 2003).

Conclusion

Our results established that regional models of soil CO_2 fluxes in alpine tundra should properly take into account the interplay of snow-cover dynamics and soil carbon content at the mesotopographical scale. This up-scaling would require further comparative studies of basal respiration rate and temperature sensitivity of soil respiration conducted over a larger set

of dominant subalpine/alpine vegetation types. At a larger scale, it would be worth to examine to what extent soil carbon content could be used as a surrogate for basal respiration.

The effects of that land-use and climate change on species distribution in temperate mountains have received considerable attention over the last decade (Keller et al. 2000, Cannone et al. 2007, Lenoir et al. 2008). Shifts in plant community composition as a result of these changes in elevational ranges could rapidly modify the quantity and quality of soil organic matter, at least in the upper layer. Our results also call for a better understanding of these associated changes in soil organic matter quality to predict CO_2 effluxes from the large carbon stock sequestered in the soils of these ecosystems.

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Appendix 1

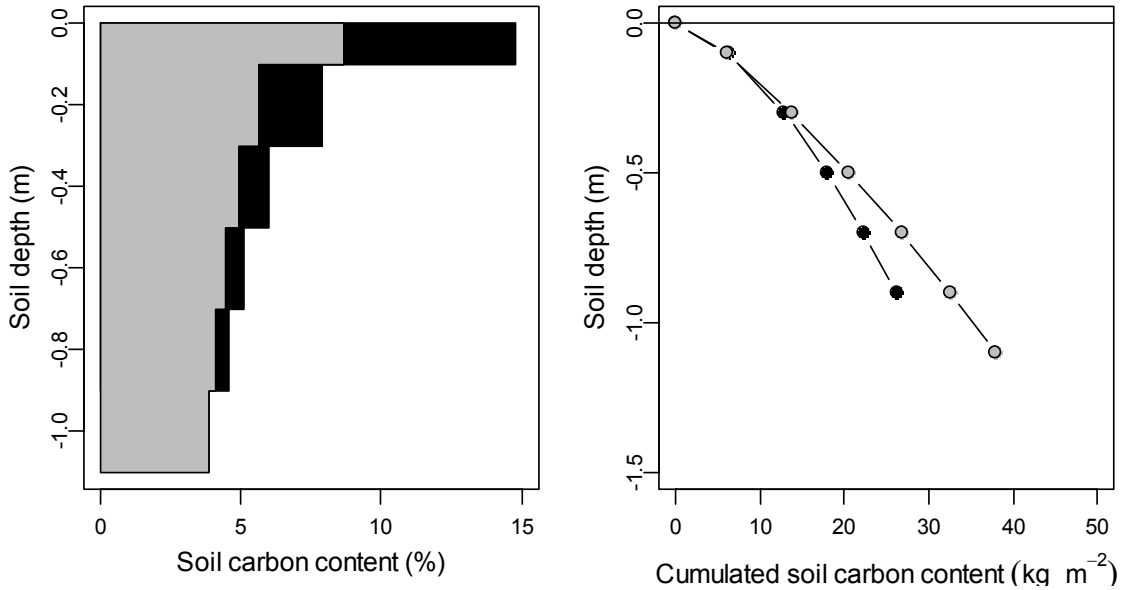

Fig. S1 Soil carbon content along the soil profile for early (black) and late (gray) snowmelt locations.

Appendix 2

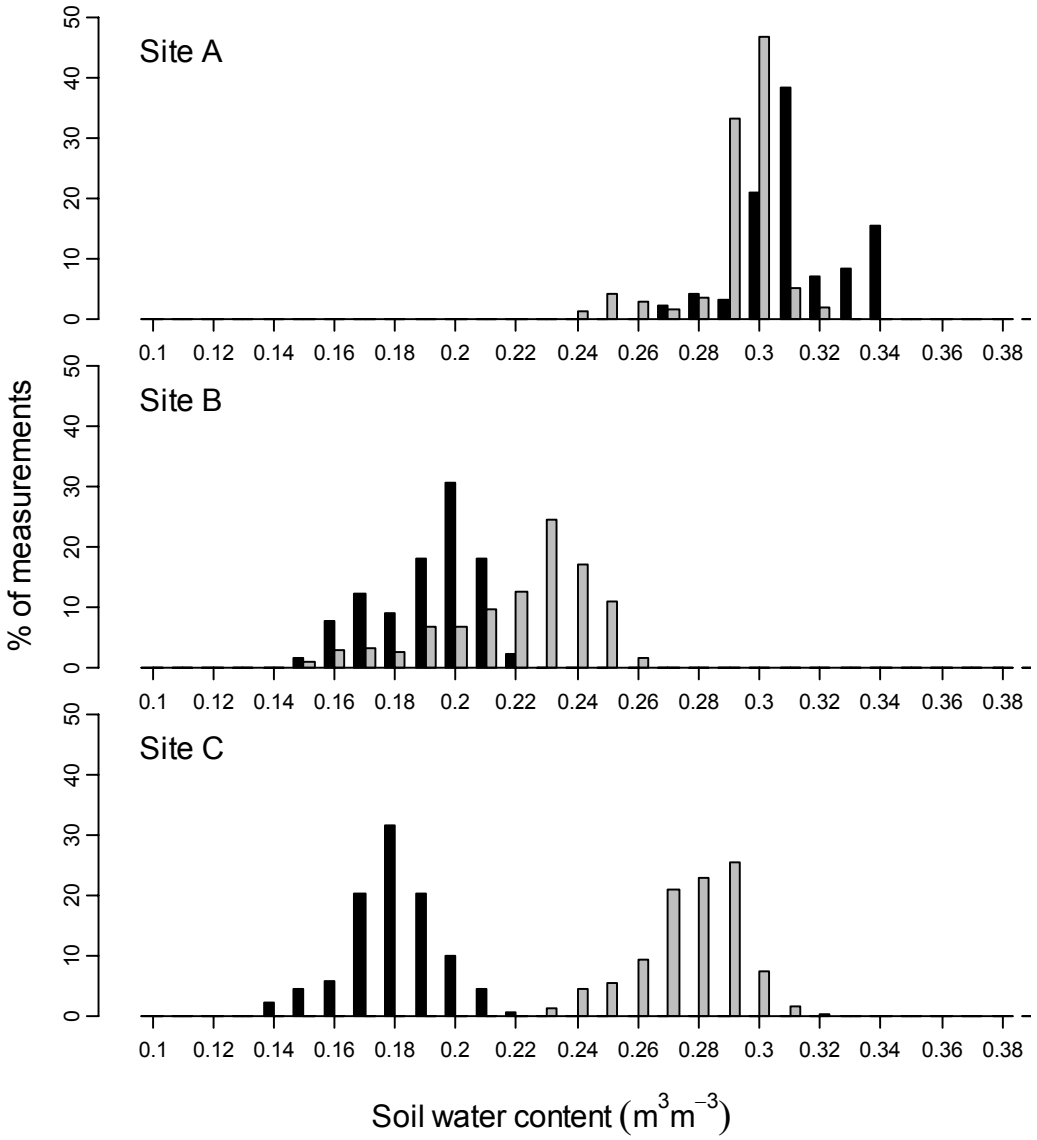

Fig. S2 Volumetric soil water content observed for early (black) and late (gray) snowmelt locations. Measurements were made discontinuously during the growing seasons 2005 and 2006. N is the total number of days for which time-series are available. Histograms show the frequency distribution of values recorded every 20 min.

Article 2C

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

Baptist, F., Zinger, L., Clement, J.C., Gallet, C., Guillemin, R., Martins, J.M.F., Sage, L., Shahnavaz, B. and Choler Ph. 2008. *Environmental Microbiology* 10:799-809

Dryas octopetala dans les systèmes thermiques à Combe Roche Noire durant le mois d'août. Photo : Ph. Choler

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

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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.

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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_2 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

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 proanthocyans as the major tannin compounds. In dry meadows, the low and irregular snow pack leads to frequent periods where soils are frozen (< –5°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°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°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°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°C during 15 days (day 15) and kept at 0°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

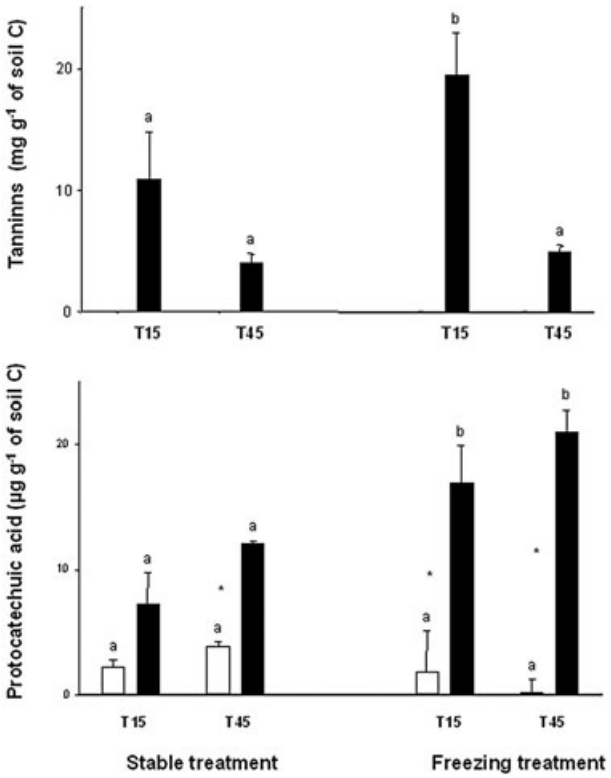

Fig. 1. A. Average tannins concentrations in soil amended with sterilized water (white bars) or a tannin solution (black bars). The concentration of tannins is negligible in the case of sterilized water amendment.
B. Protocatechuic acid concentration in soils amended with sterilized water (white bars) 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_2 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_2 efflux between days 0 and 15 compared with the F/W treatment. However, the total CO_2 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_2 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_2 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^{-1} dry weight (dw), 90.4–99.6% of total dissolved nitrogen (TDN)], while ammonia (~3.2–67.1 µg N g^{-1} dw, 0.3–8.7% of TDN) and nitrate/nitrite (0.1–10.2 µg N g^{-1} 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^{-1} C)	Fungal biomass (µg ergosterol g^{-1} C)	Bacterial count (10^9 cells g^{-1} C)
T15			
S/W	44.9 (11.9)^{aA}	60.9 (12.6)^{aA}	1.13 (0.13)^{aA}
F/W	45.8 (15.5)^{aA}	182.1 (38.1)^{aA}	1.46 (0.28)^{aA}
S/T	35.3 (8.6)^{aA}	53.8 (5.2)^{aA}	1.01 (0.06)^{aA}
F/T	38.0 (17.0)^{aA}	94.1 (37.3)^{aA}	1.12 (0.20)^{aA}
T45			
S/W	16.6 (2.2)^{aA}	114.3 (18.1)^{aA}	1.40 (0.34)^{aA}
F/W	48.1 (5.3)^{aA}	232.0 (42.7)^{aA}	1.59 (0.45)^{aA}
S/T	18.7 (6.3)^{aA}	103.8 (20.3)^{aA}	1.19 (0.50)^{aA}
F/T	19.3 (7.1)^{aA}	117.2 (35.5)^{aA}	0.89 (0.06)^{aA}

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.

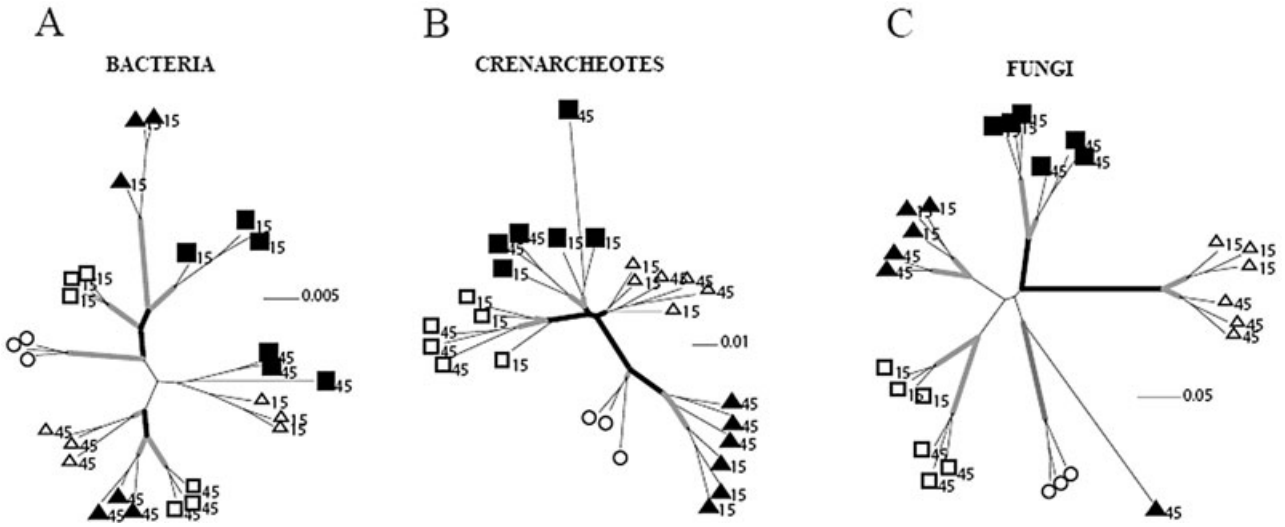

Fig. 2. Neighbour-Joining trees of microbial communities under different treatments: ○= day 0; □= S/W; ■= S/T, △= F/W; ▲= F/T. Lower cases indicate the day of treatments. Supported branches (bootstrap value >50%) are in bold. Grey corresponds to the SSCP replicates after pooling the three soil cores 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_2 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_2 efflux over the days 0–15 and days 15–45.

Treatments	Soil water content (%)	CO_2 efflux (μg C g^{-1} C day^{-1})
T0-T15		
S/W	33.4 (1.0)^{aA}	318.8 (70.4)^{aA}
F/W	34.8 (3.8)^{aA}	76.7 (7.8)^{bA}
S/T	37.6 (4.1)^{aA}	267.9 (71.9)^{abA}
F/T	30.9 (4.5)^{aA}	128.6 (19.2)^{abA}
T15-T45		
S/W	33.2 (1.2)^{aA}	281.3 (131.0)^{aA}
F/W	34.9 (3.1)^{aA}	269.4 (51.5)^{aB}
S/T	39.1 (4.2)^{aA}	210.7 (23.7)^{aA}
F/T	34.0 (4.7)^{aA}	273.8 (94.5)^{aB}

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.

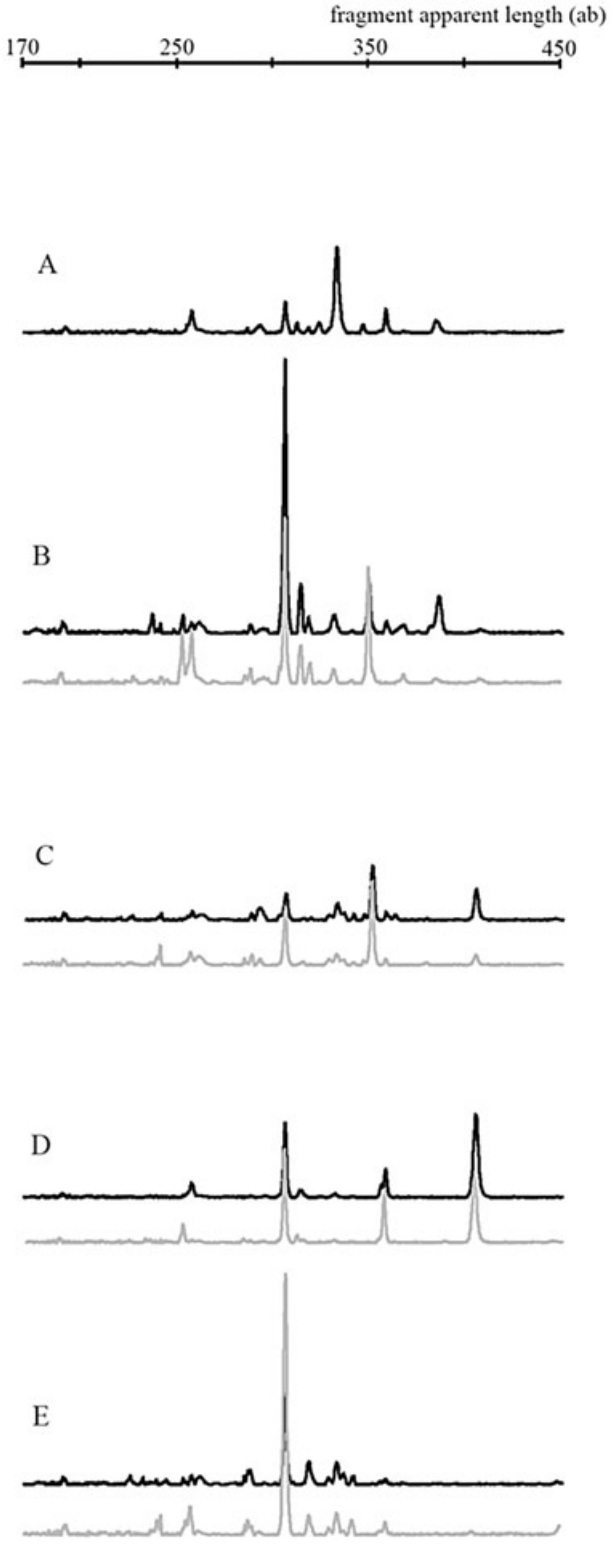

Fig. 3. Capillary electrophoresis-SSCP profiles of fungal communities for each treatment. A = day 0; B = S/W; C = S/T; D = F/W; E = F/T. Black lines: day 15; grey lines: day 45. All profiles are displayed for an arbitrary fluorescence intensity interval of 4000.

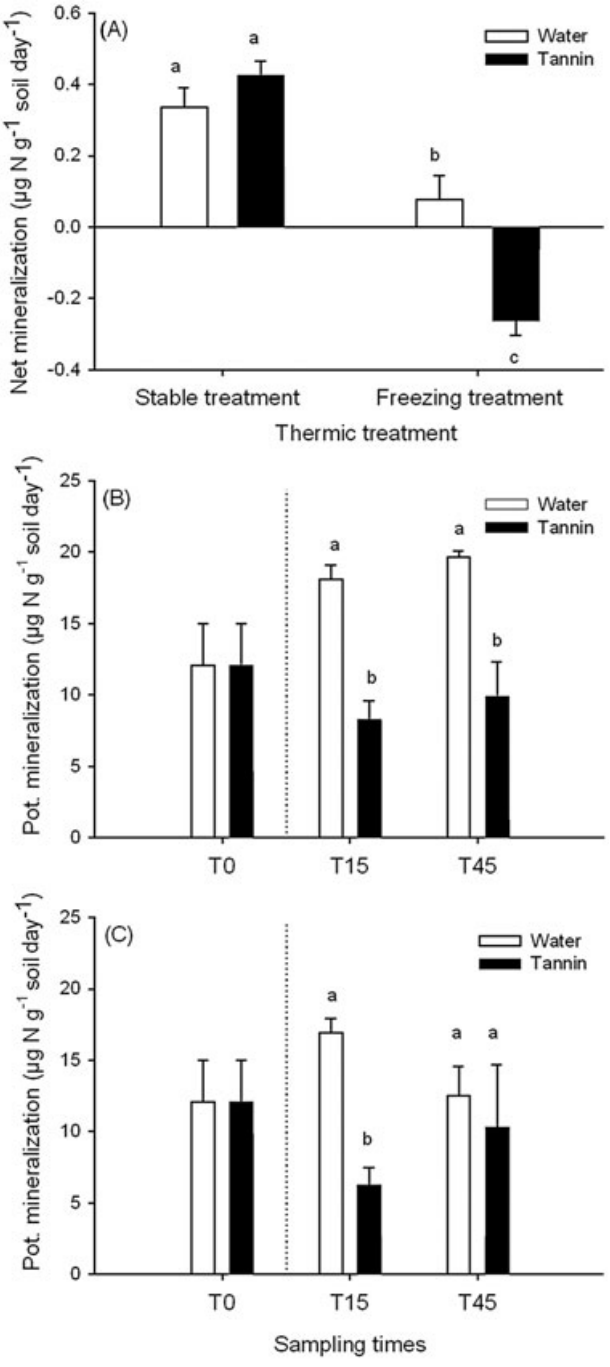

Fig. 4. A. Net N mineralization rates between days 15 and 45 in soils for F and S treatments amended with sterilized water (white bars) or a tannin solution (black bars).
B and C. Net N mineralization potentials in soils after 0, 15 and 45 days of incubation in the F treatment (B) and the S treatment (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°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 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°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°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 N_{org} into NH_4^+ and 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 NH_4^+ and NO_3^- produced).

In F treatment, the reduction of microbial activities reduced the production of NH_4^+ and 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 apparent NH_4^+ and NO_3^- immobilization. This hypothesis is further supported by NMP results (at 30°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 phylotypes (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 phylotypes. 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_2 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'Lear 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 metabolization 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^{-1} 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^{-3})	0.24 (0.04)
Organic matter (%)	16.9 (4.3)
pH (H_2O)	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_0)	
Microbial biomass (mg C g^{-1} C)	170.6 (37.3)
Bacterial count (10^9 cells g^{-1} C)	1.45 (0.21)
Fungal biomass (μg ergosterol g^{-1} C)	130.2 (14.0)
Tannin (mg g^{-1} C)	0.30 (0.10)
NO_3^-(μg N g^{-1} soil)	0.10 (0.02)
NH_4^+ (μg N g^{-1} soil)	3.25 (0.10)
N_{org} (μg N g^{-1} soil)	914.2 (65.7)
Potential mineralization	
NH_4 production (μg N g^{-1} soil day^{-1})	12.1 (2.9)
N_{org} production (μg N g^{-1} soil day^{-1})	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_2 efflux was measured between each harvest.

Soil characterization

Soil water content, pH_{H2O}, pH_{KCl}, 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^{-3} (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. Proanthocyans (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 proanthocyan 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^{-1}. 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_4^+) and nitrate/nitrite (NO_3^-/NO_2^-) contents using an FS-IV autoanalyser (OI-Analytical, College Station, TX). The TDN content in the soil extracts was measured after oxidation with $K_2S_2O_8$ at 120°C. The DON contents in the soil extracts (μg N g^{-1} dw) were calculated as: [DON = TDN – ($N\text{-}NH_4^+$) + ($N\text{-}NO_3^-/NO_2^-$)]. The net nitrogen mineralization (MIN_{net}, μg N g^{-1} dw day^{-1}) between day 15 and day 45 was calculated as: $MIN_{net} = [[(-NH_4^+) + (N\text{-}NO_3^-/NO_2^-)]_{day45} - [(N\text{-}NH_4^+) + (N\text{-}NO_3^-/NO_2^-)]_{day15}]/dw/30$. MIN_{net} 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_4^+. The difference between the NH_4^+ in the fresh soil (t_1) and after the anaerobic incubation (t_2) gave the N mineralization potential: NMP (mg $N\text{-}NH_4$ g^{-1} dw day^{-1}) = [[($N\text{-}NH_4$) t_2 – ($N\text{-}NH_4$) t_1]/dw/7].

Soil CO_2 efflux

Throughout the experiment, CO_2 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_0, T_{10}), 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^{-1} 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; Duthoit *et al*., 2003) and the crenarcheaotal 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 $MgCl_2$, 1× of AmpliTaq Gold™ buffer, 20 g l^{-1} 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 (RDevelopmentCoreTeam, 2006).

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Synthèse et perspectives

« Ceux qui ont commencé à apprendre enchaînent les formules, mais n'en savent pas encore le sens : car il faut qu'elles soient parties intégrantes de notre nature. Or c'est là une chose qui demande du temps »
Aristote. Ethique à Nicomaque,
VI, 1147 a 21-22.

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A. Introduction

Mountains cover more than 20% of the global land surface (Beniston and Fox 1996), and provide goods and services for more than half of humanity. Mountains have a major influence on regional climates and substantially contribute to carbon sequestration (Becker and Bugmann 2001). Because of steep environmental gradients and complex topography, mountains exhibit a greater ecological heterogeneity and are widely viewed as hotspots of biodiversity.

The combination of strong gradients and presence of organisms close to their physiological tolerance explains why alpine ecosystems are particularly vulnerable to global change (Theurillat and Guisan 2001). Remotely-sensed data and climatic recordings indicate that in addition to increasing temperature (Diaz and Bradley 1997, Noguès-Bravo et al. 2007), mountain ecosystems may experience decreased snow precipitation as a result of a higher rain/snow ratio (Serreze et al. 2000, Dye and Tucker 2003, Beniston 2005). In this context, seasonal snow cover has declined by around 10% (surface area) since 1972 (Serreze et al. 2000) and between 1972 and 2000, the duration of the snow-free period in the Northern Hemisphere has increased by five to six days per decade (Dye, 2002).

Predicting the response of alpine ecosystems to global change requires understanding their functioning and sensitivity to key environmental drivers. As formulated by Walker *et al.* (1999) "*The landscape-scale distribution of snow is perhaps the single most important mesoscale variable controlling biological systems in […] alpine ecosystems*"., In alpine landscapes, snow distribution is mainly controlled by mesotopography (Billings, 1973) which determines growing season length, soil temperature and water availability. Therefore, all temperature-dependent processes in alpine ecosystem are under the ultimate control of the snow cover. These effects of snow, mediated by its impact on the physical environment, are hereafter referred to as **direct effects**. Contrasting seasonal snow cover along the mesotopographical gradient has long been associated with strong turn-over in plant species composition, and more recently with important changes in plant functional diversity (Kudo 1996, Kudo et al. 1999, Choler 2005, Baptist and Choler 2008) and soil microbial communities (Schmidt et al. 2007). These changes of plant and microbe patterns along the gradient have important consequences for ecosystem processes. These effects of snow, mediated by plant and microbial functional properties, are hereafter referred as **indirect effects**. Few studies have attempted to disentangle direct and indirect effects of snow cover on ecosystem functioning, and most of them have been conducted in the arctic (e.g. Grogan and

Jonasson 2006, Elberling 2007). Hence, our understanding of the multiple controls exerted by snow cover on biogeochemical cycles, especially C and N cycles (and their coupling) remains largely incomplete in temperate alpine tundra.

Plant functional traits (PFTr) currently form the focus for a broad spectrum of ecological research attempting to quantify the relationship between ecosystem structure and function (Lavorel and Garnier 2002). Response traits are defined as markers selected by environmental conditions whereas effect traits are the traits for which an impact on ecosystem functioning is proven. According to the 'biomass ratio hypothesis' (Grime, 1998), ecosystem properties and functioning are more likely to be related to the trait values of the dominant contributors to the plant biomass. This hypothesis has been successfuly tested in several recent studies (Garnier et al., Garnier et al. 2004, Diaz et al. 2007, Garnier et al. 2007, Quétier et al. 2007) and tends to indicate that focusing on leaf and plant functional properties might be an efficient way to assess the effects of biotic components on ecosystems functioning.

Similarly, this study aimed to dissociate the direct (i.e. climatic variables) and indirect controls (i.e. variations in PFTr) exerted by snow cover along a mesotopographical gradient (hereafter "snowmelt gradient"). It focused on two main steps of the carbon cycle, Primary Production (Gross Primary Production, Aboveground Net Primary Production) and Carbon mineralization (heterotrophic respiration and litter decomposition). Alpine plant communities in the region of Lautaret (French Alps, 2058 m, 45°20'N, 6°24'E were studied along a snowmelt gradient with a particular focus (1) on communities dominated by *Carex foetida* (graminoid, Cyperaceae), *Salix herbacea* (shrub, Salicaceae), *Alopecurus gerardi* and *Poa alpina* (graminoids, Poaceae) corresponding to "late snowmelt location" and (2) on communities dominated by *Kobresia myosuroides* (graminoid, Cyperaceae) and *Dryas octopetata* (shrub, Rosaceae) corresponding to "early snowmelt locations".

The first part of this synthesis emphasizes how climatic drivers and plant functional properties determine carbon cycling in alpine tundra **(section A)**. The second part addresses the linkages between carbon and nitrogen cycling within plants and at the plant-soil interface **(section B)**. This part includes unpublished results on microbial diversity of alpine soils. In the third part **(section Conclusion)**, we will examine the possible effects of climate change in alpine tundra based on our results and the litterature. To conclude, we will suggest new research directions to contribute to a better prediction of the outcomes of climatic change on carbon cycling in alpine tundra **(section Perspectives)**.

B. The interplay of direct and indirect effects on ecosystem processes

1) Features of ecosystem models and methodological considerations

All models in ecosystem physiology make use of variables from the physical environment (climatic forcing) and parameters related to the biological properties of the ecosystem. Examples are the widely-used mechanistic ecophysiological model of leaf carbon gain developped by Farquhar and co-workers (Farquhar et al. 1980, Von Caemmerer and Farquhar 1981) and the stomatal conductance model proposed by Ball et al. (1987). The VC-F model is based on the enzymatic kinetic of RUBISCO, the enzyme which reduces CO_2 (and O_2) in the Calvin cycle. Two key parameters are Vc_{max}, the maximal carboxylation efficiency of RUBISCO and J_{max} the maximal electron flux into the transport chain. These two parameters describe the co-limitation of C fixation by internal CO_2 concentration at the carboxylation sites and by intercepted light. Wohlfahrt et al. (1999) and others demonstrated that these parameters are strongly correlated to leaf N concentration (LNC), and it is therefore possible to infer Vc_{max} and J_{max} from a simple measure of LNC. Leaf geometry within the canopy modulates light interception and leaf temperature and therefore affects the estimated CO_2 fixation. It is therefore necessary to take into account canopy structural properties- the distribution angle of leaves - and the total leaf area per ground area (Leaf Area Index, LAI) to estimate whole-plant C gain.

These models provide opportunities to test for the effects of both physical variables and leaf/canopy features on carbon gain. Along this line, many studies have investigated the relationships among several canopy properties – Leaf Area Index (LAI), nitrogen content, canopy architecture - and their effect on carbon uptake in different light conditions (Anten et al. 1995, Hikosaka and Hirose 1997, Anten 2005). However, these studies mostly focused on single species canopies grown in controlled conditions. Addressing the same questions for multi-specific canopies has rarely been done (see Wohlfahrt et al. 2000).

Recently, Lavorel and Garnier (2002) proposed the use of community-aggregated traits (CFP) to scale up processes from leaf to community level. CFP is defined as follows :

$$CFP = \sum_{k=1}^{n} A_k \cdot T_k$$

Where A_k is the abundance of the k species characterized by the value of trait T_k

A simple idea would be to use these CFP estimates in the framework of ecosystem modeling. Baptist and Choler (2008) provided evidence that this approach was successful for

estimating instantaneous gross carbon gain of alpine plant canopies. Simulations were thus performed to clarify the interplay of growing season length and leaf/canopy properties on the seasonal gross carbon gain of plant communities along the snowmelt gradient.

We tried to develop a similar approach to assess effects of the snow cover on carbon mineralization. This latter process has three steps. Leaching dominates during the first phase of degradation and is associated with litter fragmentation by detritivores. During the second step, the labile compounds are rapidly consumed by microorganisms. A classical litter decomposition experiment which would last from one to two years would only give information about these first two steps in cold ecosystems. The third step is much slower and mainly involves degradation of recalcitrant compounds (phenol-rich compounds). Measurement of heterotrophic respiration essentially provides data on the last two steps. Accordingly, to get a global picture of C mineralization in alpine tundra, we measured (1) an *in situ* litter bag experiment following a reciprocal transplantation design (Article 2A) and (2) heterotrophic respiration in controlled conditions and *in situ* (Article 2C).

Each step of C mineralization is controlled by abiotic and biotic factors. Temperature and soil moisture are the main abiotic drivers. Biochemical compounds such as lignin, polyphenols and nitrogen are major determinants of litter decomposability and the following indexes C:N, N:lignin, N:polyphenols have been widely used in numerous studies (e.g. Hobbie 1996, Perez Harguindeguy et al. 2000, Dorrepaal et al. 2005).

In this study, both statistical models applied to litter bag experiments or semi-empirical models applied to heterotrophic respiration allowed testing of the relative contribution of the climatic variables and biotic factors. However, in contrast to C fixation, we did not use community-aggregated traits to predict litter decomposition (Article 2A, see perspectives). Moreover, because of the low number of species, we did not integrate the index of litter quality (PFTr) within the statistical model. We rather studied the species effect as compared to topographic effect. Relations to litter quality have been assessed *a posteriori*.

Concerning heterotrophic respiration, the simplest predicting model is a first order exponential model (Fang and Moncrieff 2001) :

$$R = \alpha \cdot e^{(\beta \cdot T)}$$

Where α is the exponential coefficient of basal respiration (at a temperature of 0°C) and β a temperature scaler. R : respiration.

If respiration is expressed per gram of soil or C, the parameter A is considered as a surrogate of SOM quantity or quality (e.g. Mikan et al. 2002, Grogan and Jonasson 2005, Fierer et al.

2007). This parameter is therefore related to the quality of organic matter and indirectly to leaf and root-aggregated community traits. As soil moisture is another important factor, model performance is generally enhanced when it is incorporate in the model. Thus, in this study, we applied a more complete model in order to determine the relative importance of the direct and indirect controls exerted by snowcover on heterotrophic respiration (see Article 2B):

$$R = \alpha \cdot W^{\upsilon} \cdot C^{\gamma} \cdot e^{\beta \cdot T}$$

where υ and γ are empirical coefficients determining the sensitivity of the basal respiration rate to volumetric soil water content (W) and soil carbon content (C, i.e. surrogate of carbon quality, see Paper 2B) in percentage of soil mass, respectively.

2) Plant functional diversity and ecosystem processes

2.1 Control of carbon fixation along the snowmelt gradient

2.1.1 *From plant leaf traits to functional properties of canopies*

It is generally accepted that cold, snow-covered ecosystems are strongly limited by N availability (Bowman et al. 1993). In alpine ecosystems, late snowmelt sites exhibit higher soil fertility (soil content in available nitrogen for plants) than early snowmelt sites. This difference is particularly strong shortly after snowmelt (Fig. 19, Bowman 1992). Consistent with these results, strong shifts in plant nutrient economy along this gradient have been identified. A greater LNC, a higher specific leaf area (SLA),- i.e. trait values associated with nutrient acquisition strategies -, are common features of species from late snowmelt locations, (Kudo 1992, Choler 2005, Baptist and Choler 2008). These traits ensure efficient carbon fixation. Conversely, species from early snowmelt locations are characterized by low SLA and high C/N, i.e. trait values generally associated with nutrient conservation strategies (Wright et al., 2004). At the canopy scale, the gradient is characterized by a marked shift from high LAI and planophilous canopies in late snowmelt locations, to low LAI and erectophilous canopies in the early snowmelt locations (Baptist and Choler 2008).

Sensitivity analysis revealed that the most relevant traits in predicting gross carbon uptake were LAI and community-aggregated LNC. In contrast, canopy geometrical properties (parameterized with k, the extinction coefficient of an ellipsoidal leaf distribution model) did not exert a strong influence on the Gross Primary Productivity (GPP), a result which has already been reported for low LAI canopies (Hirose 2005).

Interestingly, empirical models of NPP/GPP resulted in very similar conclusions (Green et al. 2003). Building on remotely-sensed data, these authors developped the following approach:

$$NPP = \varepsilon \cdot PAR_i$$

$$\varepsilon = \frac{N_{canopy} \cdot SLA_{ag}}{f_{PAR}} = \frac{LAI \cdot LNC_{ag}}{f_{PAR}}$$

$$f_{PAR} = 1 - e^{-k \cdot LAI}$$

$$NPP = \frac{LAI \cdot LNC_{ag}}{f_{PAR}} \cdot PAR_i = \frac{LAI \cdot LNC_{ag}}{(1 - e^{-k \cdot LAI})} \cdot PAR_i$$

NPP : Net Primary Productivity
ε: Index of Light Use Efficiency (LUE)
PAR_i : Photosynthetic Active Radiation intercepted
N_{canopy} : canopy N content expressed per unit of ground area
LNC ag / SLA ag : community-aggregated LNC or SLA
f_{Par} : Photosynthetic Active Radiation absorbed par the canopy.
k : extinction coefficient which derived from the Monsi-Saeki model (Monsi and Saeki 1953).

This model highlighted that NPP could be reasonably estimated by using very low-dimensional models with LAI, community-aggregated LNC and light interception/absorption as the main parameters. The empirical model implemented by Green et al. (2003) and the mechanistic one implemented by Baptist and Choler (Article 1A, 2008) confirm that these traits might provide "the best overall integration of the numerous biophysical and biochemical factors" (Green et al. 2003). Their integration in modeling terrestrial NPP may enhance our ability to predict vegetation response in response to global change.

Another interesting result we obtained in this study is that the assimilation rate of the whole canopy (expressed per unit ground area) was of the same magnitude as the leaf assimilation rate (expressed per unit leaf area). This is consistent with previous studies (Grabherr and Cernusca 1977, Diemer 1994, Tappeiner and Cernusca 1998) and tends to show that at the community level the relationship between LNC and photosynthetic capacity is maintained.

2.1.2 Growing season length and plant functional properties as controls of C fixation

In accordance with CFP trends, instantaneous ANPP[8] varies along the snowmelt gradient from high values in late snow melt locations to low values in the early snowmelt locations (Fig. 26, Fisk et al. 1998, Baptist and Choler 2008). We do not have estimates of instantaneous BNPP at the community level but we did measure a higher root production for *C. foetida* (late snowmelt) as compared to *K. myosuroides* (early snowmelt) (Fig. 21). These

[8] Instantaneous ANPP is the biomass produced divided by the number of day elapsed from snowmelt to peak standing biomass. Yearly ANPP is the total biomass produced in a year.

results are in agreement with those obtained by Fisk et al. (1998) at the community level.

The estimation of instantaneous GPP also indicated a marked trend along the snowmelt gradient (Baptist and Choler 2008). But when expressed over season, simulations revealed that growing season length was counterbalanced by the particular CFP combination within each community along the snowmelt gradient (Baptist and Choler 2008). Early snowmelt species benefit from a longer growing season but exhibit lower photosynthetic efficiency. In late snowmelt locations, a set of CFP are associated with a high photosynthetic efficiency compensating for a shorter vegetation season. Hence, along snowmelt gradient, snow affects C fixation through both its influence on the growing season length (direct effect) and the ecological sorting of CFP (indirect effect).

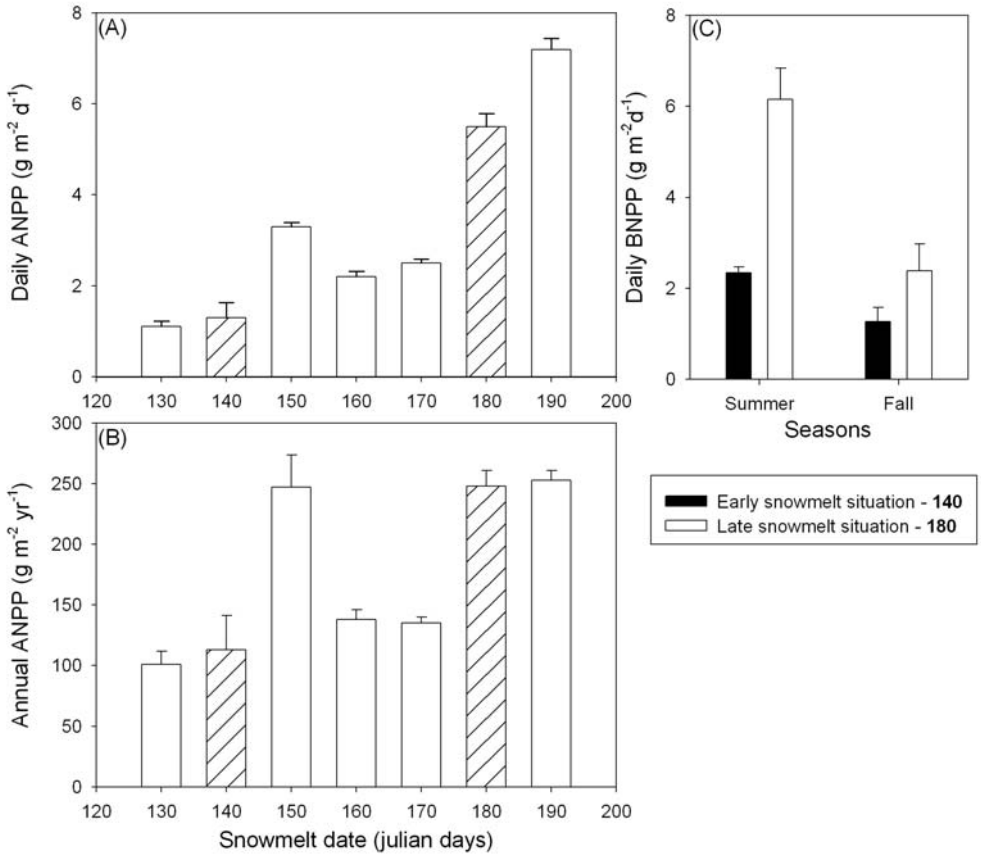

Fig. 21 Instantaneous Aboveground Net Primary Productivity (ANPP expressed on a daily basis) (A) and yearly ANPP (B) for various plant communities located along the snowmelt gradient from the most exposed (120) to the most snowy locations (200) (Aravo, 2750 m). Instantaneous Belowground Net Primary (BNPP) is calculated only for communities dominated by *C. foetida* (180) or *K. myosuroides* (140) (C). BNPP measurements are based on in-growth core methods as defined in the paper 1C. Summer corresponds to the period from the 06/06/2006 to the 16/08/2006 and fall from the 16/08/2006 to the 04/10/2006.

In order to assess the interplay between direct and indirect effects of snow-cover

duration on the seasonal carbon uptake of alpine canopies, a sensitivity analysis was carried out. The results indicated that the GPP was predominantly sensitive to a change in snow-cover duration rather than changes in CFP along the snowmelt gradient (Fig. 6A in the article 1A, Baptist and Choler 2008). This confirms previous studies which proposed that annual carbon gains in arctic ecosystems were limited primarily by the length of the growing season and secondarily by light, temperature and nitrogen (Chapin 1983). Similarly, process-oriented ecosystem models indicate that longer growing seasons may enhance vegetation growth at high latitudes in the Northern Hemisphere (Piao et al. 2007). However, predicting the response of plants to longer growing periods is not easy and it will also depend on the phenological traits of species (Starr et al., 2000; Wipf et al., 2006; Bjork and Molau, 2007). The ability to benefit from a higher number of growing degree days may strongly vary from one species to another. Furthermore, earlier snowmelt may lead to higher frequency of frost events especially at high elevation (Inouye 2000). Short and extreme freezing events have the potential to greatly affect growth of alpine plants especially those located in late snowmelt locations. A semi-controlled experiment carried out at the Joseph Fourier Alpine Station revealed that early frosts significantly affected aboveground biomass, productivity and LAI of late snowmelt communities (Article 1B). Despite a capacity to recover very quickly, frost damage could severely impair leaf growth, even if meristematic tissues are located belowground. *Carex foetida* was the most sensitive species, whereas *Alopecurus gerardi* and *Poa alpina*, two others graminoids were less impacted by these frosts. Hence, it is likely that the detrimental effect of frost might outweigh the benefits of a slight increase in cumulated growing degree day when snowmelt occurs earlier in the year (Wipf et al. 2006, Article 2B).

2.2 Control of decomposition and heterotrophic respiration along the snowmelt gradient

Litter bag experiments indicated that the same substrate decomposes better in late vs. early snowmelt locations (Reciprocal transplant experiment, Article 2A, Bryant et al. 1998). As decomposition occurs mainly in winter (Article 1A), it likely that deep snow pack, which acts as an insulating layer, provides a micro environment that is favourable for litter decomposition. Soil temperature variations associated with changes in snow cover duration affect enzymatic activity and reduce C and N mineralization in early snowmelt locations. Similarly, soil respiration was strongly dependent on soil temperature and moisture during summer and wintertime thus emphasizing the importance of direct effects (Article 2B). However, in late snowmelt location, total summer respiration was 50% above that of soils

from early snowmelt locations and this difference was mainly explainable by the overwhelming impact of higher substrate quality (i.e. high carbon content). During winter, this effect was less marked because of the higher temperature sensitivity of microorganisms (high Q_{10} , Article 2B). The insulating effect of snowpack and the low recalcitrance acted simultaneously to increase soil respiration in late snowmelt locations. Nevertheless, uncertainities remain about the very high Q_{10} values measured on freezing soils. Indeed, Schimel et al. (2006) was not able to predict realistic *in situ* CO_2 efflux based on the high Q_{10} values proposed by Mikan et al. (2002). Similarly, Elberling and Brandt (2003) questionned the impact of physical trapping on CO_2 efflux : it is likely that the important CO_2 efflux observed in response to temperature increment (around 0°C) is primarily related to gas release initially trapped in freezing soil than biological production (Elberling 2007). Consequently, variations in wintertime soil temperatures along the snowmelt gradient may not impact CO_2 efflux to such an extent (i.e. biological activity) as compared to soil organic matter quality (Paper 2B). Hence, these results suggest that within alpine ecosystems, spatial variations in soil respiration is mainly related to SOM quality rather than changes in soil climate, at least during summer. Nadelhoffer et al. (1991) reported similar results in arctic ecosystems in a controlled-condition experiment. Grogan and Jonasson (2005) and Elberling (2007) also indicate that SOM quality was almost as important as soil temperature differences in determining spatial variations in CO_2 efflux in arctic tundra. Their studies principally focused on the winter period.

In addition, we observed that the proportion of shrubs *vs*. graminoids might strongly affect litter decomposition rate (Table 7, Article 2A). Differences in litter mass loss differences between shrubs and graminoids within early and late snowmelt sites largely exceeded the differences observed for one species between these two situations. Similarly, several authors (Hobbie 1996, Dorrepaal et al. 2005, Wahren et al. 2005, Dorrepaal 2007) showed that leaf decomposition rate differed consistently among growth form with low decomposability litters being produced by mosses, followed by evergreen shrubs, deciduous shrubs, graminoids and forbs. These observations emphasized the important role of phenolic compounds (e.g. lignin or tannin) which are a major determinant of litter decomposition, because of their structural stability and biochemical recalcitrance.

Species	Late snowmelt locations			Early snowmelt locations		
	k (yr^{-1})	r^2	P	k (yr^{-1})	r^2	P
	Leaf litter					
C. foetida	**0.56** (0.01)	0.97	<0.0001	**0.47** (0.02)	0.92	<0.0001
K. myosuroides	**0.56** (0.01)	0.97	<0.0001	**0.45** (0.05)	0.94	<0.0001
S. herbacea	**0.27** (0.01)	0.95	<0.0001	**0.19** (0.01)	0.88	<0.0001
D. octopetala	**0.24** (0.01)	0.96	<0.0001	**0.15** (0.01)	0.88	<0.0001

Table 7 Statistics and decay constants (k) from regression of litter mass remaining against time in years. Species underlines are shrubs, the others are graminoids. Data from Baptist et al. (Article 2A).

In summary, the combination of litter bag experiments and CO_2 efflux measurements revealed that C mineralization rate was higher in late compared to early snowmelt locations in both winter and summer. Soil temperature is an important driver of spatial CO_2 efflux variations, especially during winter. However results indicate that the first and the last stages of decomposition are both very vulnerable to changes in SOM quality (*a priori* lignin compounds). Any shift in plant community composition leading to the dominance of one particular growth form may have cascading biogeochemical consequences on ecosystem processes and carbon sequestration (Chapin et al. 2000). A typical example of such possible shifts is the encroachment of deciduous shrubs in arctic tundra (e.g. *Betula nana)*. This shift from graminoids to woody shrubs is predicted to significantly impact ecosystem processes (Walker et al. 2006). Indeed, increased shrub height and cover may also impact albedo and thus the surface energy budget (Sturm et al. 2001). Also, higher shrub abundance will alter biogeochemical processes through changes in litter composition and quantity (Jackson et al. 2002) and thermal insulation (Grogan and Jonasson 2006). Many other examples can be found in the literature (Dorrepaal et al. 2003, Quétier et al. 2007, Wookey et al., accepted manuscript) and emphasize the consequences of such vegetation shifts for ecosystem functioning.

3) The need to integrate a new set of plant traits to predict ecosystem functioning?

Our results indicate that C mineralization will be particularly sensitive to changes in litter quality (i.e. lignin content) which may occur because of snow cover induced shifts in plant communities. They point out the need to assess what are the climatic drivers of the shrub *vs.* graminoid ratio in alpine ecosystems

We identified three sets of response PFTr which may affect community composition in this way. They are briefly described below.

3.1 Regenerative traits and morphological constraints

The preformation of flowers, inflorescences or aerial parts (leaves, branches) is one of the important adaptive features of alpine plants (Körner 1999). As time for growth is limited, this mechanism facilitates the rapid emergence of tissues at the onset of growing season. Conversely, this phenomenon may limit very short-term response to changing environment. Indeed, alpine plant growth is hindered by the number of preformed buds and the number and potential area of associated leaves to be produced by a single meristem (Grelet et al. 2003).

These morphological constraints differ between species (Körner 1999) and also between growth forms. Graminoids are assumed to be generally responsive to perturbation or changes in resource availability because their leaves grow from intercalary meristems (only grasses) and are less limited in the size of the leaves that can be produced. By contrast, most species of deciduous, evergreen shrubs (and many forbs) are limited in their ability to increase their growth in the short term because (1) they must first form new buds, containing new leaves and stems (Shaver *et al.* 1986; Bowman & Conant 1994; Diggle 1997) and (2) they can not increase leaf size as graminoids do because of morphological constraints.

3.2 Phenological traits

Sørenson (1941) distinguished two phenological patterns in tundra plants : periodic species, characterized by a fixed growing period, and aperiodic species, for which both growth and the production of new leaves are maintained as long as conditions are favourable. Periodic species, such as *Polygonum bistorta* in the arctic (Starr, 2000), *Polygonum viviparum* or *C. foetida* in alpine tundra (Article 1B, Diggle 1997) may be disadvantaged compared to aperiodic species such as the shrubs *D. octopetala* (Welker *et al.* 1997), *S. herbacea* (personal observation) or the forb *Ranunculus adoneus* (Galen & Stanton, 1993) in a context of a lengthened growing season.

3.3 Resistance to frost events

During winter, plants are usually protected from low temperature by snow cover, or by specific features (e.g. higher concentration in sugars or supercooling, Bell and Bliss 1979). The situation is different during growing season as plants generally display a rapid dehardenning once snow disapears (Körner 1999). In a context of global change, frost events

are predicted to increase because of reduced snow cover (Inouye 2000), it is therefore relevant to compare the ability of alpine species to support and to recover from freezing events. According to Taschler & Neuner (2004) and Baptist & Streb (unpublished results), graminoids show on average lower freezing points than shrubs, suggesting than they may be favored if the frequency of frosts increases in the future (Table 8). In addition, species from early snowmelt locations displayed similar freezing points to species from late snowmelt locations (Baptist & Streb, unpublished results). However, the fraction of dead cells following the frost was much lower from early snowmelt species (data not shown), suggesting a higher capability to recover.

Species	**Freezing points (°C)**
Dwarf shrubs	
Calluna vulgaris	-8.3 ± 0.1
Juniperus communis sps. nana	-9.0 ± 0.6
Loiseleuria procumbens	-6.6 ± 0.6
Rhododendron ferrugineum	-4.7 ± 0.5
Vaccinium gaultherioides	-5.6 ± 0.5
Vaccinium myrtillus	-4.1 ± 0.4
Vaccinium vitis-idaea	-5.5 ± 0.3
*Dryas octopetala**	-7.6 ± 0.3
*Salix herbacea**	-8.1 ± 0.4
*Salix reticulata**	-7.2 ± 0.6
*Salix retusa**	-8.7 ± 0.4
Mean	**-6.8 ± 0.5^{a}**
Graminoids	
Juncus trifidus	-7.7 ± 0.3
Nardus stricta	-10.3 ± 0.6
Phleum alpinum	-10.8 ± 1.5
Poa alpina	-9.9 ± 1.4
*Poa alpina (2)**	-9.3 ± 0.4
*Kobresia myosuroides**	-10.1 ± 0.5
*Carex foetida**	-10.0 ± 0.5
*Alopecurus gerardi**	-12.0 ± 0.8
Mean	**-10.0 ± 0.4^{b}**

Table 8 Freezing points of alpine species of different growth forms. Data are from Taschler & Neuner (2004) and Baptist et al. (Article 1B and unpublished data, marked with *). Different upperscript letters mean significant differences ($p<0.05$, One-way-ANOVA). See references for further details on methods.

C. Linkages between carbon and nutrient cycling. The role of soil microbial communities

A large body of literature emphasizes the close linkage between the carbon and nitrogen biogeochemical cycles in terrestrial ecosystems (e.g. Hobbie et al. 2000, Long et al. 2006). In plant functional ecology, the continuum between exploitative and conservative strategists is generally related to nutrient availability, especially nitrogen (Chapin et al. 1993a)9. Low N availability limits ANPP, leading to recalcitrant tissue construction and by retroaction affecting N mineralization rates. This positive feedback remains as long as plants rely on saprophytic microorganisms to satisfy their N requirements. However, it has been recently discovered that plants can acquire organic N forms with or without mycorrhiza and therefore subvert the microbial loop (Chapin et al. 1993b, Raab et al. 1999, Chapman et al. 2006). Also, some species can fix atmospheric N limiting their dependance on saprophytic microbes (rhizobial or actinorhizal species). Finally, plants develop special adaptations to reduce dependance on nutrients, such as N storage.

The emergence of this new paradigm questions the existence of a bottleneck associate to SOM mineralization (Schimel and Bennett 2004, Chapman et al. 2006) and calls for further studies to explore the linkage between plant, microbial and biogeochemical cycles in alpine tundras.

1) Links between plants, microbial populations and N availability in alpine tundra

The main constraint in snow-covered ecosystems is the soil temperature which is principally controlled by snow cover throughout the year. Microbial populations experience a marked dynamic throughout the year which impacts on plant production and C and N mineralization.

During summer, bacteria dominate over fungi and feed on both labile carbon exuded by the plants and soil organic matter. During the transition to winter, microbial communities shift to psychrophilous fungal-dominated communities (Schadt et al. 2003, Uchida et al. 2005, Bjork et al. 2008). Wintertime populations display high ability in degrading recalcitrant compounds such as salycilic acid or lignin-rich compounds present in SOM especially in early snowmelt locations (Lipson et al. 2002, Loya et al. 2004, Schmidt and Lipson 2004, Baptist et al. 2008). Additionally, some bacterial populations feed on easy labile substrates which are leached

[9] Water availability can independently initiate the same nutrient acquisition trade-off (Chapin et al. 1993a)

from fresh litter and then contribute to its degradation. These results support our observations concerning high wintertime compared to summertime decomposition rates (Article 2A, Hobbie and Chapin 1996). At snowmelt, microbial populations collapse releasing important quantities of nutrients, especially at snowy sites (Fig. 19, Brooks et al. 1998). The peak of nutrients, coupled to the mineral release from water snowmelt, is crucial as it may support seasonal plant growth. Bilbrough *et al.* (2000) indicated for example that N uptake during snowmelt averaged 7 to 12% of total uptake during the growing season.

In late snowmelt locations, species probably rely on this flush of mineral nitrogen which allows a rapid expansion of photosynthetic tissues and ensures efficient light capture and carbon fixation. Indeed, we demonstrate that a fast growing species from late snowmelt location (*C. Foetida*) displayed higher mineral N uptake compare to a slow growing species present in early snowmelt locations (*K. Myosuroides*) (Article 1C). The fast N incorporation and translocation into the leaves was related to an important belowground C allocation, indicating a tight temporal and spatial C – N coupling in the plant. These features may contribute to optimize mineral N acquisition at snowmelt and plant growth in locations where growing season length is limiting.

By contrast, in early snowmelt locations, Baptist et al. (Article 2A) showed that because of lower soil temperature during winter, litter decomposition and thus N mineralization was slowed down, limiting N availability during the growing season. The presence of species rich in tannin may also contribute to reduce N availability through chemical complexation (Baptist et al. 2008). Finally, as snow cover is limited in early snowmelt locations, species do not benefit from resources contained in melt water at the onset of the vegetation season. Brooks et al. (1998) demonstrate that nitrate exported during snowmelt from the inconsistent snow cover sites was significantly greater than the quantity exported in late snowmelt locations. Thus, it is likely that plant growth in early snowmelt sites does not primarily rely on this pulse of mineral nitrogen at the onset of the growing season.

Analysis of root tissues revealed a strong decrease in nitrogen content in roots of *K. myosuroides* at the beginning of the growing season. This was not observed for *C. foetida* (Fig. 22). These results suggest that, at least for *K. myosuroides*, nitrogen storage from previous years may support growth though allocation into aboveground compartments during the first weeks of the growing season. Regardless of soil N availability, these plants may preserve relatively more N within their biomass than plants from late snowmelt locations. These features are frequent in alpine ecosystems (e.g. Lipson et al. 1996) and confirm that reliance on storage are correlated with the amount of asynchrony between resource supply and

demand (Chapin et al. 1990).

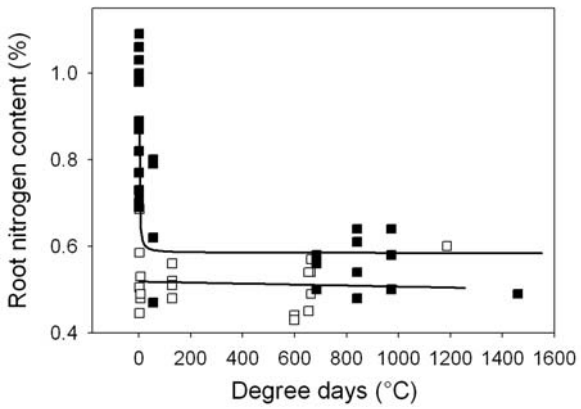

Fig. 22 Root nitrogen content ($g.g^{-1}$) in relation to degrees day since snowmelt (or when sum of degrees days > 0) for *K. myosuroides* (early snowmelt locations, black symbol) and *C. foetida* (late snowmelt locations, white symbol)

Moreover, microbial diversity studies indicated the presence of *Frankia* bacteria in soils from early snowmelt locations (Zinger et al. unpublished results). These bacteria are responsible for biological N_2 fixation and are associated with *D. octopetala* roots (Table 9, Kohls et al. 1994). Thus, an important fraction of N assimilated by this species may result from biological fixation although nitrogenase activity should be investigated to confirm this hypothesis.

	Late snowmelt locations	Early snowmelt locations
May	0	0
June	0	4.7 %
August	0	7.1 %
October	0	20 %

Table 9 Proportion of Frankia sequences within Actinobacteria phylum in late and early snowmelt locations (class: Actinobacteridae, order: Actinomycetales).

In addition, we detected a higher abundance of the genera *Inocyte* and *Russula* and of the species *Cenococcum geophilum* in early compared to late snowmelt locations (Zinger et al., in prep.). These phyla have been previously reported as ectomycorrhiza of *D. octopetala* and *K. myosuroides* respectively (Ascomycota, Gardes and Dahlberg 1996) suggesting these species may display higher efficiency of N incorporation thanks to these symbionts (Lipson et al. 1999). Finally, Chapin et al. (1993b) and Kielland (1994) have recently shown that some plants can assimilate amino acids more rapidly than mineral nitrogen from hydroponic cultures in the absence of mycorrhizae. This phenomenon appears widely developed in alpine and arctic ecosystems as a result of low inorganic N availability (Raab et al. 1999, Jonasson et al. 2001). It is particularly the case for *Kobresia myosuroides* (Raab et al. 1999). Hence, the vegetation has the potential capability to access organic N directly without being dependant on N mineralization. It is however still important to precise that if plants avoid N mineralization, they still depend on soil microbes for soil protease activity (i.e. depolymerization, Schimel and Bennett 2004).

Thus, species from early snowmelt locations appear to subvert the microbial loop of SOM mineralization through different strategies such as the: remobilization of N storage, biological N fixation and organic nitrogen assimilation (Fig. 23). In contrast, species from late snowmelt locations may rely mostly on mineral N at the onset of growing season and developed specific features in order to maximise N assimilation and plant growth. Although these conclusions are mainly indirect and require further experiments, they illustrate how environmental conditions have selected plants with diverse mechanims of obtaining N. They also outline the importance of considering plant and soil communities together over seasonal timescales to identify (1) the controls and bottlenecks and (2) the degree of temporal and spatial coupling between C and nutrient cycles. The incorporation of microbial communities into current conceptual models of biogeochemical dynamics may be a valuable tool in the assessment of plant and microbial mediated controls on carbon and nutrient cycling (Zak et al. 2006).

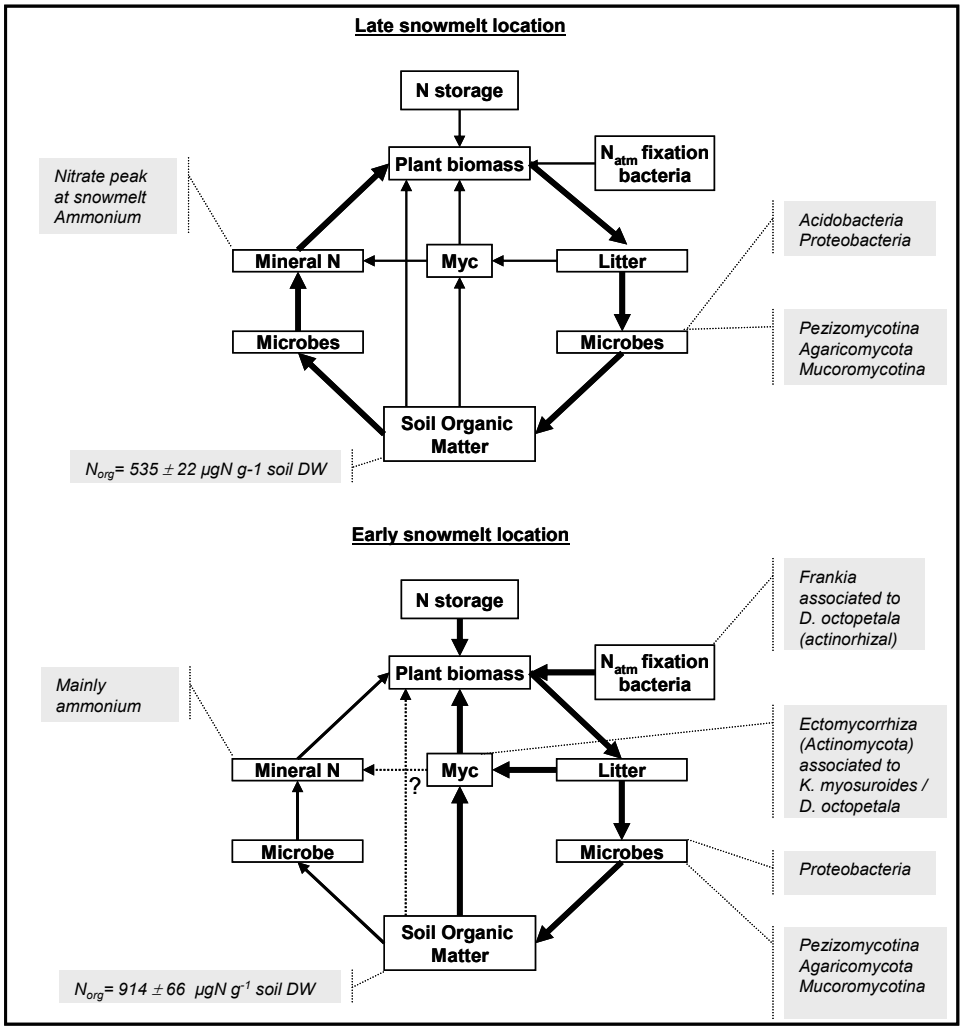

Fig. 23 Hypothesis regarding Carbon and Nutrient interaction in early and late snowmelt locations. Data from Zinger et al. (unpublished results) and Baptist et al. (unpublished results). Modified from Chapman et al. (2006).

2) The need for the integration of microbial community dynamics in biogeochemical models

Microbial populations are absent from biogeochemical models (Zak et al. 2006). Beyond technical difficulties, research is currently limited by (1) the capability to aggregate microbial populations within a functional classification and (2) the absence of a clear conceptual framework which articulates plant and microbial effect traits to predict ecosystem functioning. Accordingly, we propose to modify the conceptual framework proposed by Lavorel & Garnier (2002) (Fig. 24).

In this figure, microbial and plant functional traits interact to determine a potential enzymatic activity which is ultimately regulated by environmental variables. This potential activity refers to RUBISCO and other enzymes involved in plant metabolism when C fixation is analysed and to enzymes associated with SOM degradation when C mineralization is addressed. It it important to note that microbial catalysis depend firstly on (1) the nature of the enzyme selected by climate and substrate and then ultimately on (2) the quality of the substrate and (3) temperature. This situation is different in plants as the enzyme of carboxylation (i.e. RUBISCO) and its substrate (i.e. CO_2 or O_2) are common to all plant species. As a result, the enzymatic efficiency will only depend on the quantity of enzyme present in plants whereas it will depend on both quantity and nature of the enzymes in microbes.

Interdependence of plant and soil microbial communities in alpine tundra can be illustrated by various mechanisms (Fig. 24). Plants impact microbial populations directly through rhyzodeposition, exudation rate, litter quality and through ecosystem processes filters (e.g. litter quantity) (Fig. 24, arrows 4a and 6). Conversely, specific microbial populations enable plants to acquire nutrients in organic or mineral forms (mycorrhizal symbiosis, nitrogen fixing bacteria) (Fig. 24, arrow 4b). They can act as carbon sinks and modulate plant growth and response to disturbances (Grimoldi et al. 2006, Walling and Zabinski 2006). Finally, microorganisms indirectly affect plant growth by controlling mineral N availability within the ecosystem (Fig. 24, arrow 7).

Soil microbes play a key role in ecosystems (e.g. Schmidt et al. 2007). A future challenge will be to determine to which extent changes in microbial diversity may influence plant community dynamics and ecosystem functionning (van der heijden et al. 2008).

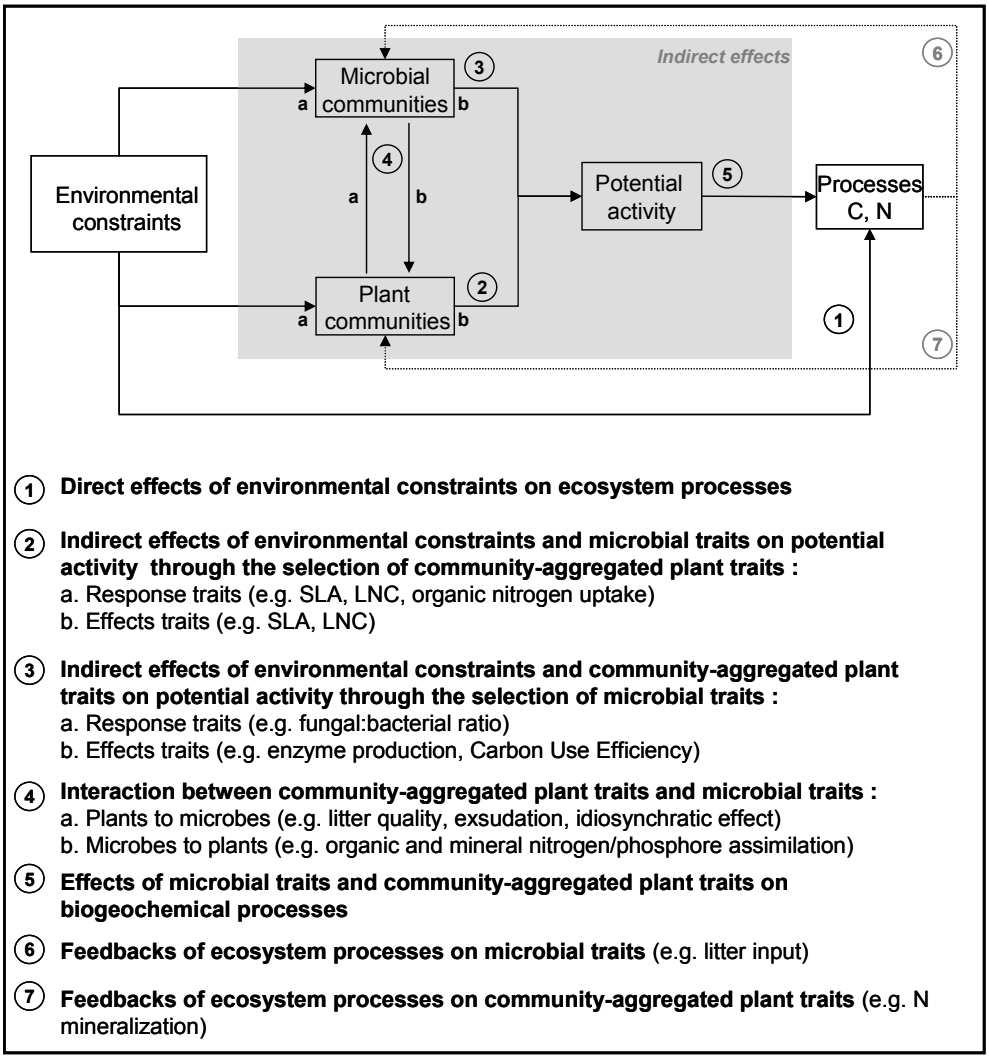

Fig. 24 Conceptual diagram showing the direct and indirect effects of environmental constraints on plant and microbial communities and their impact on biogeochemical processes. These processes are determined by both the potential activity of plants and microbes and by environmental conditions. Modified from Lavorel and Garnier (2002).

D. Conclusions

1) Control of carbon cycling in alpine tundra

- **Along the snowmelt gradient, variations in plant production are determined by both CFP and growing season length. Nevertheless, in a context of reduced snow cover, direct effects of snow through variations in vegetation growth period length should affect primary production to a greater extent than changes in CFP. As vegetation season length is an important limiting factor in alpine ecosystems, the capacity to respond rapidly to longer seasons will be determinant. Freezing events might also affect plant production and their consequences will depend on the ability of species to support these frosts (Fig. 25).**

In order to assess to which extent plant communities will respond to increasing growing degree days, we identified three sets of response trait (set A) :

- Regenerative traits
- Phenological traits
- Freezing points and recovery

Biotic interactions should also be crucial in predicting the evolution of community composition as one of the first effects of lengthened growing season will be to modify competitive relationships between species (Theurillat and Guisan 2001).

- **In early snowmelt locations, carbon mineralization is mainly determined by litter quality. Any changes in litter quality will significantly affect CO_2 efflux. However, variations of wintertime soil temperature (through variations in snow cover) should also slightly alter the turnover of SOM (Fig. 26).**

Three traits are therefore considered crucial (set B):

- N:Lignin
- Tannin content which may contribute to complex proteins in the soil and then decrease N availability
- Allocation coefficients such as the woody:non woody ratio and the root:shoot ratio.

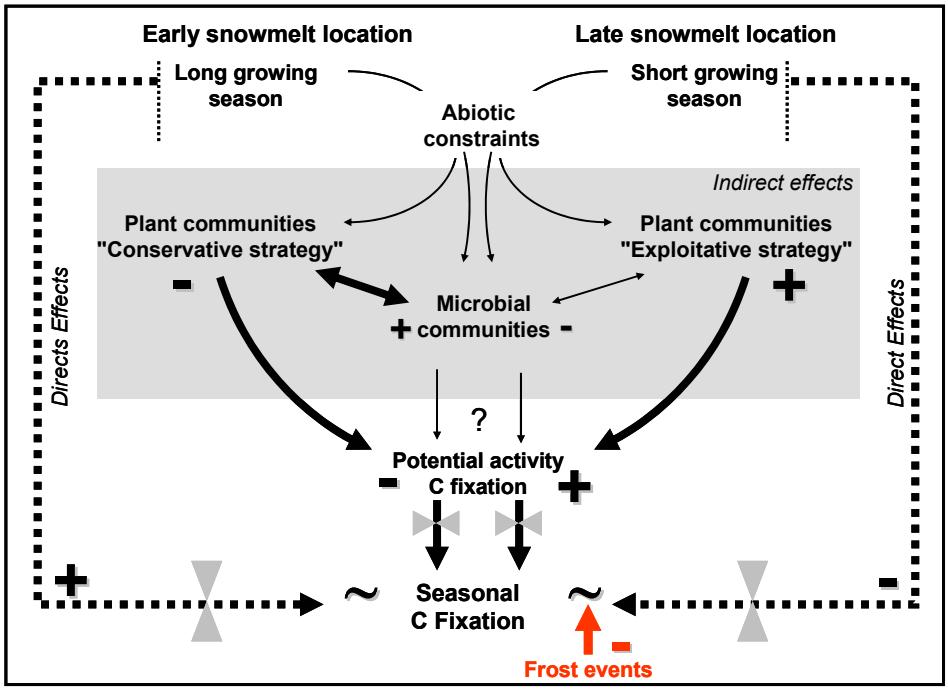

Fig. 25 Conceptual diagram for direct and indirects effects of snow cover on C fixation. In early snowmelt locations, CFP are outweighed by a longer growing season. In late snowmelt locations, we observed the opposite. The presence of mycorrhiza and biological N fixing bacteria may increase the efficiency of N acquisition in early snowmelt locations; however no data is available to confirm this hypothesis. Similarly, uncertainty remains about the impact of mycorrhizal colonization on C fixation. The sensitivity analysis revealed that a longer growing season may affect C fixation more strongly than compared to a change in CFP (represented by the larger regulation button), but this will ultimately depend on extreme events such as the occurence of frosts at snowmelt.

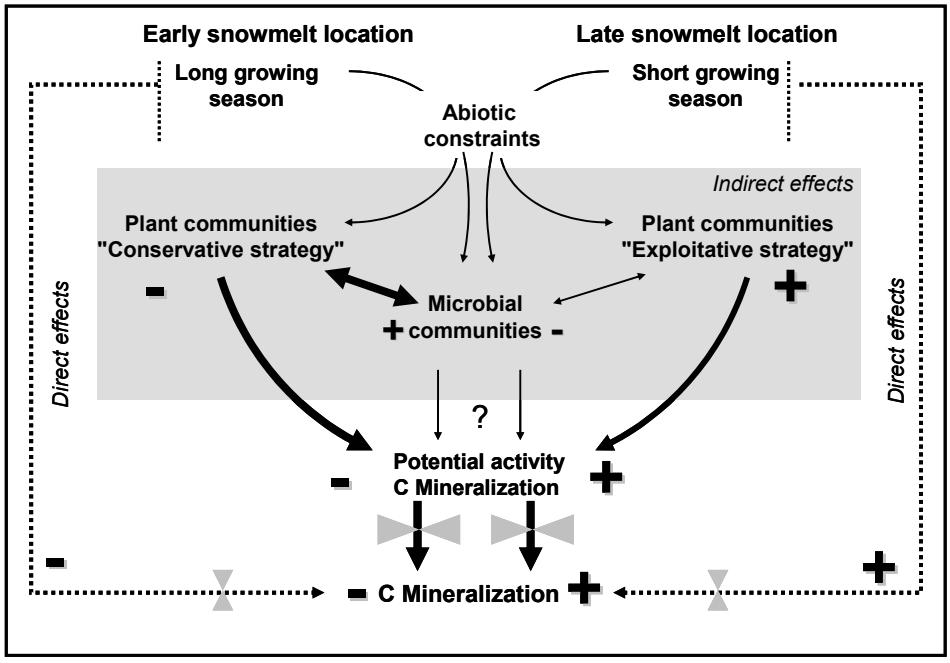

Fig. 26 Conceptual diagram for direct and indirect effects of snow cover on C mineralization. In early snowmelt locations, more efficient microbial populations are selected by both recalcitrant SOM and abiotic conditions (Baptist et al. 2008). However, despite higher efficiency, potential C mineralization remains lower than in late snowmelt locations because of lower C quality. This effect is reinforced by lower soil temperature during winter, but only marginally. Carbon quality is then the main factor determining C mineralization along the snowmelt gradient (represented by larger regulation buttons).

- **Plant species from late snowmelt locations are dependent upon microbial N mineralization whereas species from early snowmelt locations appear to subvert the microbial bottleneck by assimilating organic N and fixing atmospheric N. It is possible to hypothesize contrasting impacts of climate change on these two types of ecosystem functioning. Ecosystems exhibiting faster cycling of C and nutrients and tighter linkages between biogeochemical cycles would probably respond more rapidly and more strongly to climatic forcing. Conversely, a delayed response is likely in early snowmelt locations. This would be reinforced by stressful conditions during wintertime which would be only marginally affected by global warming. (i.e. wintertime (soil) temperatures will remain very low for plant and microbial life in early snowmelt locations).**

Accumulation of SOM, and thus C sequestration, is related to an imbalance between plant production and the decomposition rate. In cold ecosystems, SOM accumulates to high concentrations in relatively narrow horizons, despite low annual productivity (Körner 1999). Carbon mineralization therefore appears to be the limiting step (Körner 1999, De Deyn et al. in press). Thus, we can hypothesize that C mineralization will be the most sensitive step and will eventually determine C cycling and the evolution of C stocks in cold ecosystems. As this process mainly depends on C quality, any shift in plant composition (response traits, set A) either favoring, or not, one of the three traits listed above (effect traits, set B) may impact C mineralization.

The methodological approach based on quantitative CFP was successful in determining the hierarchy of direct and indirect controls exerted by snow cover on C fixation and mineralization. We showed that it is possible to integrate CFP into biogeochemical models, allow the prediction of consequences of changes in biotic and abiotic variables on C processes. This study also indicates that the use of growth-form based groups is not informative when assessing both production and decomposition in an ecosystem (Green et al. 2003, Dorrepaal 2007, Baptist and Choler 2008). This classification has been widely used in arctic ecosystems to predict the indirect impact of global change on ecosystem processes (e.g. Walker et al. 2006). This approach was partly successful because the distinction between growth forms matched differences in lignin and other polyphenol compounds which are determinant traits in decomposition process. However, CFP related to C fixation vary widely within each growth form and thus reduce the pertinence of such classifications to predict production (Dorrepaal 2007). Hence the selective use of a few set of traits at the leaf and plant level seems to be more promising in order to analyze both plant response to environmental

factors and effect on C and nutrient cycles (Dorrepaal 2007).

2) Impact of global changes in alpine ecosystems : a case study of the Lautaret region

Given the lack of any clear consensus concerning possible climate change scenarios, a range of possible changes in attributes of snowpack development are considered. These include potential increases in temperature and decreases in snow precipitation. Decreasing snow precipitation during winter will likely result in:

(1) Longer growing season

(2) Increased frost frequency

(3) Lower N input into the system

(4) Reduced soil moisture during growing season

(5) Lower soil temperature during winter because of higher coupling between air and soil temperature

In addition, IPCC (2007) predict a parallel increase of 1 to 3 °C in mid-latitude, which may reinforce most of these effects, except for freezing events whose frequency might be reduced. Longer growing seasons and a higher frequency of frost events will directly stimulate productivity through community and species specific filters based on the set of response traits defined above (Set A). Shifts in community composition will thus impact C mineralization through variations in effect traits, defined as the set B.

Schöb et al. (in revision) showed that the abundance of the alpine shrub *S. herbacea* decreased with longer growing season, in contrast to graminoids. In parallel, Beerling (1998) indicated that this species was intolerant to dryness and to shade. Its low prostate habit makes it susceptible to competition for light with taller vascular plants. Thus, *S. herbacea* may be rapidly out competed by graminoids because of a slow response time, higher freezing point, and a weak capacity to tolerate shade and competition. In parallel, warming may lead to the rapid expansion of *Poa alpina*, a generalist species (Article 1B, see also Sandvik et al. 2004). This species is relatively tolerant to freezing and is able to maintain photosynthetic tissue during almost of the growing season. Similarly, Körner (1999) indicate that *Poa alpina* may respond rapidly to environmental changes. Hence, late snowmelt locations might evolve toward graminoid-dominated communities. These conclusions are contradictory to the review

of Bjork and Molau (2007) who proposed that more vigorous shrubs from surrounding areas[10] may rapidly invade these communities and out compete graminoids. In the region of Lautaret, the only shrubs which could potentially invade snowbed communities are located at lower altitudes (i.e. subalpine shrubs such as *Vaccinium sp.*). In the very-long term and in a context of a marked temperature increase, this hypothesis cannot be neglected. However, alpine ecosystems are globally dominated by slow-growing species, and the time lag between the initiation of treatment and ecosystem responses may be very long (Chapin et al. 1995, Körner 1999). Thus, it appears essential to predict the evolution of species dominance in nearby communities before predicting the outcomes of invasion by subalpine species. In early snowmelt locations, *D. octopetala* is able to lengthen it life cycle because of its wintergreen habits and therefore its increased seasonal carbon gain (Welker et al. 1997). However, Klanderud and Totland (2005) observed a decreased cover of *D. octopetala* in response to warming and/or nutrient addition. These authors suggested that despite an increase of biomass in the short-term, this species might be outcompeted by graminoids over the longer-term. Similar changes in competition hierarchies may occur in response to a lengthened growing season. Indeed, graminoids are expected to respond faster to longer growing seasons and *D. octopetala* may therefore be excluded by *K. myosuroides* and others sedges such as *Carex rupestris*.

To conclude, we predict that dwarf shrubs may be outcompeted by graminoids along the snowmelt gradient because of lower competitive ability, lower resistance to frost events (case of *S. herbacea*) and slow response times to environmental changes. C fixation will be stimulated by higher growing degree-days, especially in late snowmelt situations where growing season length is the most limiting factor. Additionally, C mineralization may increase because of better C quality in both late and early snowmelt locations. Positive feedbacks between the production of good quality tissue and N availability to plants may take longer in early snowmelt locations because of decoupling between C and N cycles.

Other climatic or anthropogenic drivers may affect ecosystem processes through impacts on plant community composition, and may strengthen these predictions. Numerous studies have showed that graminoids may benefit more from N deposition than forbs or other growth forms. In moist alpine meadows, Bowman et al. (1995) reported an increase in the biomass of the graminoid *Deschampsia caespitosa* as compared to the forb *Acomastylis rossii*. In a recent survey, Korb & Ranker (2001) reported an increase of nitrophilous species

[10] The identity of these shrubs is not given in the paper of Bjork and Molau (2007). We made the hypothesis that these authors refer to lower altitude shrubs compared to dwarf shrubs present in late snowmelt locations.

associated with an increase of a few dominant graminoids, especially *Carex rupestris* (Bowman et al. 2006). Similarly, N deposition in the central Alps and Northern Caucasus resulted in strong changes in dry matter production and community change towards a higher abundance of sedges (especially *Carex sempervirens*) (Soudzilovskaia and Onipchenko 2005, Bassin et al. 2007). Hence, the replacement of forbs and shrubs by graminoids in alpine ecosystems associated with higher production could even accelerate the impact of reduced snow cover on C cycling. Nevertheless, such prediction will have to be made by quantitatively linking these feedbacks to others simultaneously operating identified feedbacks (warming, snow cover).

E. Perspectives

- **The need to up-scale from leaf traits to CFP for predicting C mineralization**

Up-scaling from leaf decomposition to whole-plant decomposition is not straightforward. While leaf litter decomposition has been studied in various ecosystems and for a high number of species, functional groups, etc.., measurements of root and branch decomposition rates are under-investigated because of technical difficulties.

Additionally, predicting community decomposition rate in an ecosystem is hindered by the need to quantify litter fluxes from aboveground *vs.* belowground and from woody *vs.* non woody plants/parts. Differences in C allocation at the whole-plant level affect ecosystem processes through the quality and quantity of litter inputs into the ecosystem (roots/stems/leaves). For example, C allocation to woody or non woody plants/parts affect decomposition rate as woody parts are characterized by higher lignin contents and lower decomposition rates. Similarly, species characterized by higher root/shoot ratios (R:S ratio) may lead to higher mean residence times of C in the ecosystem as root decomposition rates are generally lower than leaf decomposition rates because of higher lignin contents (e.g. Bryant et al. 1998).

In order to predict decomposition rate at the community level, we therefore suggest four relevant steps:

1. Evaluating whole-plant litter flux based on (1) the mean residence time of C within each plant compartment and (2) plant C allocations. Assessing the decay rate of the different compartments of the dominant plants by litter bag experiments.
2. Predicting whole-plant decomposition rate based on the decomposition rate of each organ weighted by its biomass (i.e. additive or not?).

3. Evaluating to which extent whole-plant aggregated Lignin:N ratio can be an accurate indicator of whole-plant decomposition rate.
4. Scaling up to the community level following the same methodology as proposed in section A.

- **Species-specific effects? The case study of tannins**

Tannins are well-known for their capabilities to complex nitrogen *in vitro* or *in situ* and thus reducing its bioavailability (see Kraus et al. 2003 for a review). They therefore contribute to increase SOM recalcitrance within the ecosystem. In cold and snow-covered ecosystems, tannin-rich shrubs are abundant (e.g. *D. octopetala*, *Vaccinium sp.*, *Empetrum sp.*) and synthesis of these compounds is enhanced by environmental factors (UV, low fertility). However, to what extent tannins affect C mineralization remains poorly understood. Baptist et al. (2008) showed that freezing temperatures may stimulate the degradation of tannins extracted from *D. octopetala*. Several hypotheses have been proposed to explain the underlying processes and need to be addressed. In particular, two main questions raised by this study are:

- Is there a relationship between the quantity of litter-rich tannin and SOM recalcitrance? These results suggest the possibility of using tannin quantity as a predictor of C mineralization in snow-covered ecosystems (when weighted by species abundance).
- Do freezing temperatures (<0°C) reduce the strength of the bound of tannins to proteins ? if so, how can these chemical processes shed new light on decomposition rates observed in the field ?

- **The need for a better description of SOM recalcitrance**

Measurement of basal respiration revealed higher SOM recalcitrance in early snowmelt locations (Article 2B). We hypothesized that this recalcitrance was related to lignin and others polyphenols present in the litter of *D. octopetala* and other dominant species from early snowmelt locations. However, this point remains to be confirmed. We are currently assessing the NIRS spectra (Near Infrared Spectroscopy) to obtain a better picture of Soil Organic Matter characteristics along the snowmelt gradient.

- **The need for a better understanding of the interdependence between plants and microbes**

Microbial populations play a crucial role in providing nutrients to vegetation, illustrating the new paradigm proposed by Schimel & Bennett (2004) and further developed by Chapman et al. (2006). However, these results need to be confirmed and many points remain unexplored. The following question should be addressed in future studies:

- How important are mycorrhizae in uncorking the microbial bottleneck?
- Can alpine plants efficiently assimilate organic nitrogen without the presence of these symbionts?
- To what extent are late snowmelt communities dependant on the peak of nutrients at snowmelt?
- Is the uptake of organic nitrogen still efficient in the presence of higher mineral N concentrations? Inversely, are late snowmelt species able to assimilate organic N?, is this property rapidly developed if fertility decreases ?
- To what extent do variations in the temporal dynamics of mineral and organic nitrogen affect plant growth in early and late snowmelt locations respectively?

- **The need for continous measurements of CO_2 fluxes in alpine tundra**

This study remains limited in its capacity to predict carbon balance in alpine tundra. The evaluation of the source-sink dynamic is hindered by the absence of continuous CO_2 measurements. Over the last decade, there has been a burst of new sites where continuous measurements of land-atmosphere exchanges of water and CO_2 were achieved using eddy covariance techniques (Canadell et al. 2000). It remains difficult to interpret eddies sampled over complex terrain, but some mountain ecosystems are now equipped (see Hammerle et al. 2007), and attempts to correct fluxes are under progress (Rana et al. 2007). Hence, this technique appears quite promising for assessing carbon balances in alpine tundra.

Beyond CO_2 efflux measurements, another challenge will be to disentangle the controls over CO_2 efflux during winter in order to quantify adequately yearly CO_2 efflux in snow-covered ecosystems (Elberling and Brandt 2003, Schimel et al. 2006). Finally, experimental manipulations of the environment should be coupled with ecosystem process measurements in order to (1) validate model predictions in regards to the direct effect of snow cover on CO_2 efflux and to (2) evaluate long-term vegetation shifts in response to global change.

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Annexes

ANNEXES

Annexe 1 _____ **249**

Annexe 2 _____ **251**

Annexe 1

Liste et présence des espèces dans les sites B, C et D en situation thermiques et nivales. Relevé réalisé durant le mois de juillet 2007.

	Situation nivale			Situation thermique		
Sites	**B**	**C**	**D**	**B**	**C**	**D**
Alchemilla pentaphyllea L.	4	2	5	0	0	0
Carex foetida All.	2	3	2	0	0	0
Alopecurus alpinus Vill.	1	3	2	0	0	0
Salix herbacea L.	4	0	2	0	0	0
Plantago alpina L.	1	3	1	0	0	0
Sibbaldia procumbens L.	1	1	2	0	0	0
Nardus stricta L.	1	0	2	0	0	0
Omalotheca supina (L.) DC.	2	0	1	0	0	0
Potentilla aurea L.	0	3	0	0	0	0
Geum montanum L.	0	1	1	0	0	0
Ranunculus kuepferi Greuter & Burdet	0	2	0	0	0	0
Ranunculus montanus Willd.	0	2	0	0	0	0
Taraxacum alpinum Weber	1	1	0	0	0	0
Cardamine bellidifolia L. subsp. alpina (Willd.) B.M.G. Jones	0	1	0	0	0	0
Cirsium acaule Scop.	0	0	1	0	0	0
Cirsium spinosissimum (L.) Scop.	0	1	0	0	0	0
Festuca violacea Gaudin	0	1	0	0	0	0
Pedicularis rostratospicata Crantz	0	1	0	0	0	0
Veronica alpina L.	0	1	0	0	0	0
Dryas octopetala L.	0	0	0	4	3	4
Kobresia myosuroides (Vill.) Fiori	0	0	0	2	3	3
Carex curvula All. subsp. rosae Gilomen	0	0	0	2	0	2
Festuca halleri All.	0	0	0	1	1	2
Minuartia verna (L.) Hiern	0	0	0	1	2	1
Androsace vitaliana (L.) Lapeyr.	0	0	0	1	1	1
Avenula versicolor (Vill.) Laínz	0	0	0	1	1	1
Myosotis alpestris F.W. Schmidt	0	0	0	1	1	1
Pachypleurum mutellinoides (Crantz) Vill.	0	0	0	1	1	1
Polygonum viviparum L.	0	0	0	1	1	1
Pulsatilla vernalis (L.) Miller	0	0	0	1	1	1
Sempervivum montanum L.	0	0	0	1	1	1
Alchemilla flabellata Buser	0	0	0	0	2	0
Antennaria carpatica (Wahlenb.) Bluff & Fingerh.	0	0	0	1	0	1
Bartsia alpina L.	0	0	0	1	0	1
Campanula scheuchzeri Vill.	0	0	0	1	0	1
Carex rupestris All.	0	0	0	2	0	0
Draba aizoides L.	0	0	0	1	1	0
Festuca rubra L.	0	0	0	0	1	1
Hieracium glaciale Reyn.	0	0	0	1	0	1
Hieracium piliferum Hoppe gr.	0	0	0	1	1	0
Leucanthemopsis alpina (L.) Heywood	0	0	0	1	1	0
Lotus alpinus (DC.) Schleicher ex Ramond	0	0	0	2	0	0
Minuartia sedoides (L.) Hiern	0	0	0	0	1	1
Oxytropis campestris (L.) DC.	0	0	0	0	1	1
Saxifraga exarata Vill.	0	0	0	1	0	1
Sempervivum arachnoideum L.	0	0	0	1	1	0
Veronica bellidioides L.	0	0	0	0	1	1
Achillea nana L.	0	0	0	1	0	0
Agrostis alpina Scop.	0	0	0	0	1	0
Antennaria dioica (L.) Gaertner	0	0	0	0	1	0
Anthyllis vulneraria L. subsp. alpestris (Kit.) Ascherson & Graebner	0	0	0	0	1	0
Aster alpinus L.	0	0	0	0	0	1
Cerastium arvense L. subsp. strictum (Koch) Gremli	0	0	0	0	1	0
Homogyne alpina (L.) Cass.	0	0	0	0	1	0
Lloydia serotina (L.) Reichenb.	0	0	0	0	0	1
Luzula lutea (All.) DC.	0	0	0	1	0	0
Oxytropis helvetica Scheele	0	0	0	0	0	1
Salix reticulata L.	0	0	0	0	0	1
Saxifraga bryoides L.	0	0	0	0	1	0
Saxifraga paniculata Miller	0	0	0	0	0	1
Trifolium pratense L. subsp. nivale (Koch) Cesati [1844]	0	0	0	0	1	0
Leontodon pyrenaicus Gouan subsp. helveticus (Mérat) Finch & P.D. Sell	0	0	0	0	0	0
Gentiana verna L.	0	1	1	1	0	1
Poa alpina L.	0	1	0	1	1	1
Species richness	**9**	**11**	**17**	**27**	**28**	**28**

Annexe 2

Alpine dandelions originated in the native and introduced range differ in their responses to environmental constraints

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Projet ECOS-Sud avec le Chili 'Facilitation et invasion biologique en zone alpine'. Photo prise au col Agnel (2644 m) le 16/07/05. Au premier plan, coussin de *Silene acaulis*.

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ORIGINAL ARTICLE

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Alpine dandelions originated in the native and introduced range differ in their responses to environmental constraints

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Abstract Few studies have compared the response of native and invasive populations under stressful conditions. Furthermore, there is little consensus as to whether a plastic response is related to invasiveness in stressful environments. Exotic species have recently been reported in the high Andes of central Chile, where individuals have to cope with drought and poor soils, in addition to extreme temperatures. We explored if the exotic species *Taraxacum officinale* (dandelion) has plastic responses to soil moisture and nutrient availability, and whether two sets of alpine populations derived from native and introduced populations can converge to similar plastic responses to environmental constraints. Using a common garden approach, we compared plants grown from seeds collected in alpine populations of its native range (Alps, France) and in alpine populations of its introduced range (Andes, Chile) under a drought experiment, a potassium gradient, and a nitrogen gradient. Plasticity was only found as a response to drought. Moreover, different responses were found between both origins. Andean individuals are drought-resistant, while individuals from the Alps were drought-sensitive. According to the nutrient experiments, Andean dandelions behave as a nitrogen demanding-potassium avoiding species, whereas individuals from the Alps did not show any particular dependency or repulsion tendency to either of these two nutrients. Results suggest that differences in life history traits of both derived sets of populations may have an important role in determining the response of dandelions under the evaluated conditions. However, the relative importance of genetic adaptation in these responses is still unclear. Although *T. officinale* is a cosmopolite weed, this is the first study that compares individuals coming from its native and invaded range under stressful conditions.

Keywords Biological invasions · Common garden · Exotic species · Asteraceae · Environmental stress

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Introduction

The physical environment plays a pivotal role as a filter for successful plant invasions (Chaneton et al. 2002; Dybdahl and Kane 2005; Lake and Leishman 2004; Suding et al. 2004). It has been suggested that stressful abiotic conditions constrain plant invasions (e.g., Richardson et al. 2000; Williamson and Fitter 1996). Hence, the overcome of the barrier imposed by the physical environment is a key step for the invasion success in an extreme environment. Although scarce, there are examples of successful invasions in stressful habitats (e.g., alpine habitats: Dullinger et al. 2003; semiarid habitats: Eggemeyer et al. 2006; salt-marshes: Dethier and Hacker 2005). In these cases, the possession of attributes that enable exotic species to overcome the stressful abiotic conditions of the invaded habitat should be important for explaining the invasion success.

Plasticity has been defined as the ability of an organism to adjust its performance by altering its morphology and/or physiology in response to varying environmental conditions (Sultan 1995). It has been suggested that plasticity could play an important role in explaining successful biological invasions (Sexton et al. 2002; Richards et al. 2006). According to Richards et al.

(2006), pre-existing plasticity in ecologically important traits would promote invasiveness after arrival to a new habitat because it allows the exotic species to cope with the environmental heterogeneity of the invaded habitat. Alternatively, plasticity of these ecologically important traits may evolve rapidly in introduced habitats and thereby contribute to invasion success after a lag time (e.g., Williams et al. 1995; Sakai et al. 2001; Lee 2002). Once naturalized, introduced populations will experience new local selection pressures. Then, recombination of genetic variation among introduced individuals can provide a range of heritable phenotypes to respond to local selection pressures and produce offspring with higher fitness (Ellstrand and Schierenbeck 2000). However, few studies have compared the behavior of exotic and native populations of invasive species under stressful conditions, and whether differences in plasticity could be responsible for the success of the species in the invaded range (Kaufman and Smouse 2001; DeWalt et al. 2004; see review of Bossdorf et al. 2005). The scarce available evidence for differences in plasticity between native and introduced individuals under stressful conditions is still contradictory.

Alpine habitats are well known for their severe environmental conditions (Chambers 1995; Nilsson et al. 2002; Körner 2003). In temperate zones, alpine habitats have strong winds, short growing seasons, high solar radiation, low temperatures and low nutrient availability, especially nitrogen (Billings 1974; Körner 2003). In addition, alpine species could undergo drought stress, which is an important mortality factor for seedlings (Ehleringer and Miller 1975; Bliss 1985). Despite these extreme conditions, some studies have begun to report exotic species establishing in alpine environments (Arévalo et al. 2005; Becker et al. 2005; Cavieres et al. 2005; Daehler 2005; Mc Dougall et al. 2005; Parks et al. 2005; Andersen and Baker 2006). Invasive species of alpine habitats are expected to have high germination and high growth rates to cope with the short growing season, as well as the ability to tolerate drought and very low soil nutrient levels.

Alpine populations are derived from larger source populations occurring at lower altitudes. Therefore, attributes of alpine individuals (such as plasticity) depend on the attributes that are already present in individuals from lower altitudes. Considering that introduced species usually undergo major genetic bottlenecks following their introduction (Sakai et al. 2001), it is expected that such genetic changes will be reflected in derived alpine individuals. Hence, it is expected that alpine individuals from the introduced range show different responses in front of diverse abiotic conditions than alpine individuals from the native range.

Taraxacum officinale Weber (dandelion) is an invasive weed that was introduced to Chile from Europe ca. 150 years ago (Matthei 1995). In its native range, *T. officinale* is present in alpine environments, although it is mostly restricted to disturbed sites (*data not shown*) that are expected to be more fertile than adjacent undisturbed alpine soils (Vitousek et al. 1979; Chambers et al. 1990). However, in central Chile, this exotic species has been found growing abundantly in alpine zones, either in disturbed sites or in undisturbed natural communities (Cavieres et al. 2005).

Due to the influence of the Mediterranean-type climate, drought conditions in the Andes of central Chile are more accentuated than in the majority of the mountains in the Alps (Cavieres et al. 2006). In addition, due to its intrusive volcanic origin, soils in the high-alpine zone of central Chile have very high amounts of phosphorous and potassium (Table 1). Therefore, it can be expected that *Taraxacum officinale* individuals invading the Andes should be able to cope with low amounts of nitrogen (as occur in most of the alpine environments), and high amounts of potassium and phosphorous, as well as drought.

The aim of this study was to explore if *Taraxacum officinale* has plastic responses to soil moisture and soil nutrient availability (Nitrogen and Potassium), and whether two sets of derived alpine populations from native and introduced populations show similar responses to environmental constraints. Using a common garden approach, we compared the performance of plants grown from seeds collected in an alpine environment from its native range, the Alps (France), and an alpine environment from its introduced range, the Andes (Chile).

Methods

Target species

Taraxacum officinale (Asteraceae) (dandelion) is native to Europe, but is now found in most countries of the world (Holm et al. 1997), where it is considered as a noxious weed in several countries (Holm et al. 1997). It is a stemless, deeply rooted perennial herb having a thick taproot and leaves in rosettes at the soil level. Each plant has one or more 2–5 cm diameter capitula or flower heads terminally positioned on 5–45 cm long, hollow,

Table 1 Matric potential (Ψ_m), nitrogen (N), phosphorous (P) and potassium (K) found in Andean soils, matric potential obtained in the drought treatment and nutrient amounts used in gradients of N and K used in greenhouse experiments

	Andean soils	Drought treatment	Nitrogen gradient	Potassium gradient
Ψ_m (KPa)	−31 + 1.98	−30 + 1.01		
N-NO_3 (mg/kg)	12.90 ± 1.63		4 – 17– 30	13
P (mg/kg)	13.63 ± 1.29		14	14
K (mg/kg)	321.37 ± 32.59		320	130 – 415 – 700

cylindrical peduncles. Each capitulum has a composite of 50–250 small bright yellow ligulate or ray florets (Holm et al. 1997). Propagules are mainly dispersed by wind. *T. officinale* is generally apomictic, although sexually reproducing biotypes have been described. Asexual *T. officinale* plants are mostly triploids (Richards 1973). Genetic variation existing among asexually reproducing dandelions is likely to have come exclusively from either mutations or multiple origins of the clones detected (Ellstrand and Roose 1987). However, some traces of sexual recombination have been found in some triploid asexual populations, contributing to the genetic variation of those populations (Van der Hulst et al. 2000).

Although this species is widespread in Europe, the first collection of this species in Chile corresponds to 1870, in the city of Santiago. From that date, multiple introductions have probably toke place (Matthei 1995). Since our study area is about 50 km away from this city, it seems unlikely that this species has been present for more than 100 years in our sampling area or the Andes.

Seed collection

Bulk seed collections of >50 *Taraxacum officinale* individuals were made in the Queyras Mountains at $>2{,}000$ m elevation, in the South Western French Alps (native range), and in the Molina River valley at $>2{,}500$ m elevation in the central Chilean Andes (introduced range). We sampled more than two high-altitude populations in both localities. Seeds from the Alps were collected from individuals found in anthropically disturbed areas (Alps), whereas seeds from the Andes corresponded to individuals located in undisturbed habitats or anthropically disturbed areas.

Although we cannot demonstrate that individuals generated in disturbed and undisturbed sites are similar, Rogstad et al. (2001) showed in clonal populations of *T. officinale* present in North America that seed dispersal is able to maintain the same "clonal families" at short distances and at distances as high as hundreds of km. Based on this evidence, we assume that similar clonal lineages are present in disturbed and undisturbed sites in the sampling area located in the Andes. Since Rogstad et al. (2001) also showed that genetic diversity is similar both at small scales (few meters) and at large scales (hundreds of km), we are confident that possible differences in the area from which seeds were collected will not influence the genetic diversity that was included in all treatments.

Taraxacum officinale individuals found in the study area of the Andes have been found to be triploid (L. San Martin, unpublished data). Hence, we can expect that they reproduce asexually. On the other hand, it has been described that *T. officinale* individuals occurring in the study area of the Alps can be sexual or asexual (Verduijn et al. 2004). Unfortunately, there is no information about the ploidy levels of the high-altitude populations sampled for this study. Nevertheless, in areas where the ranges of sexual and asexual individuals overlap, the range of asexually reproducing individuals usually extends to higher altitudes where abiotic conditions are more severe (Bierzychudek 1985). Thus, it seems likely that for both origins we are in presence of asexually originated individuals.

Seedling preparation

Seeds were carried to the laboratory in Chile where random samples of seeds were germinated in Petri dishes at 20°C and a photoperiod of 12 h light. Emerged seedlings from the two origins of *T. officinale* were planted into one-liter plastic pots and randomly assigned to the different experiments explained below. These experiments were carried out in the greenhouse located in Universidad de Concepción, Concepción, Chile (36°S, 73°W), where the mean maximum and minimum temperatures during the experiments were 24 and 12°C, respectively.

Drought tolerance

To compare the response of *Taraxacum officinale* from both origins to drought, individuals from both origins were exposed to two soil moisture levels, drought and control. The drought treatment mimicked the soil matric potential that is found in the Andes of central Chile during the driest period of the growing season (Table 1). Forty-one-month-old seedlings of each origin were planted in one-liter pots filled with a mixture of commercial soil and sand (50:50). Twenty seedlings were assigned to one of the two following irrigation treatments: (a) addition of 100 ml of water every 2 days (hereafter control) and (b) addition of 100 ml of water every 5 days (hereafter drought). This design resulted in 20 replicates $\times$ 2 moisture levels $\times$ 2 origins $=$ 80 pots in total. Pots were placed in the greenhouse, where their final position was randomly assigned. The experiment was maintained during 2 months, and at the end of this period we recorded survival and flower production. After 2 months, surviving individuals were collected and their final biomass was measured. The root:shoot ratio of surviving plants was also calculated.

Nutrient gradient

To compare the response of *Taraxacum officinale* from both origins to varying levels of soil nutrients, we used levels of N, P and K that are commonly found in alpine soils of the Andes of central Chile (Table 1). Individuals from both origins were grown at three levels of potassium and three levels of nitrogen, whereas P level was maintained constant in both gradients. Ninety-six 1-month-old seedlings of each origin were planted in one-liter pots filled with commercial vermiculite. We randomly selected groups of 16 seedlings that were assigned to each of the following treatments. The three

levels of potassium treatment consisted of a weekly addition of (a) 170 ml of a mineral solution with a low level of potassium (0.004602 mol K_2SO_4/l, hereafter K1, dissolved in 0.00095 mol $Ca(NO_3)_2$/l, 0.002 mol $MgSO_4$/l, 0.0006 mol $NaNO_3$/l, 0.00128 mol NaH_2PO_4/l and Fe-EDTA chelated trace metals), (b) 170 ml of a mineral solution with an intermediate level of potassium (0.01472 mol K_2SO_4/l, hereafter K2, dissolved in the same solution mentioned above), or (c) 170 ml of a mineral solution with a high level of potassium (0.02502 mol K_2SO_4/l, hereafter K3, dissolved in the same solution mentioned above). The three levels of nitrogen treatment consisted of a weekly addition of (a) 170 ml of a mineral solution with a low level of nitrogen (0.0004 mol urea, hereafter N1, dissolved in 0.002 mol $MgSO_4$/l, 0.00128 mol NaH_2PO_4/l, 0.005 mol $CaCl_2$/l, 0.023 mol KCl/l, Fe-EDTA chelated trace metals, and), (b) 170 ml of a mineral solution with an intermediate level of nitrogen (0.0017 mol urea, hereafter N2, dissolved in the same solution mentioned above), or (c) 170 ml of a mineral solution with a high level of nitrogen (0.003 mol urea, hereafter N3, dissolved in the same solution mentioned above). Pots were watered two times per week with 170 ml of distilled water, and once a week with the nutrient solutions.

This design resulted in 16 replicates × 3 nutrient levels × 2 nutrient gradient × 2 origins = 192 pots in total. Pots were placed in the greenhouse, where their positions were randomized. Treatments were applied during 2 months, and at the end of this period we recorded survival, final biomass and root:shoot ratio of surviving plants.

Statistical analyses

In both experiments, survival was analyzed with a two-tailed proportions test. In the drought experiment, morphological and reproduction data did not fit the assumptions for parametric statistics. Thus, final biomass, root:shoot ratio and the number of capitula produced per plant were analyzed with Mann-Whitney non-parametric paired tests. Comparisons were made among every origin and treatment. Since non-parametric tests do not allow to explore interactions between two predictor variables, we used the following procedure for comparing the change in some attributes (final biomass, root:shoot ratio, number of capitula) between the two origins along each gradient. First, for each attribute we calculated for each individual the quotient between the value obtained in the drought treatment and the mean value obtained in the control. After that, individuals of different origin (Andes vs. Alps) were compared with one-way ANOVAs (final biomass and root:shoot ratio), or a Mann-Whitney U test (number of capitula) when assumptions of normality were not met. Root:shoot values were log transformed to fit assumption of homogeneity of variance. In the nutrient gradient experiments, final biomass and root:shoot ratio were analyzed with two-way ANOVAs. Post-hoc comparisons were made by Tukey HSD for unequal sampling sizes, including both origins and the three levels of the gradient. All statistical analyses were performed with Statistica 6.0.

Results

Drought tolerance

Individuals from the two origins differed in their responses to drought. Survival of individuals from the Andes was similar in both irrigation treatments (Fig. 1a, Table 2). In contrast, individuals from the Alps showed lower survival under drought compared to the control individuals (Fig. 1a, Table 2). Individuals from the Alps showed lower final biomass with drought ($U = 9.5$; $P < 0.01$), whereas Andean individuals did not differ in final biomass between the two irrigation treatments ($U = 33.5$; $P = 0.21$) (Fig. 1b, Table 3). Although individuals from both origins increased their root:shoot ratio in drought ($U = 16$; $P < 0.05$ and $U = 4$; $P < 0.001$ for the Andes and the Alps, respectively), this increment was higher in individuals from the Alps ($F_{1,18} = 19.40$; $P < 0.01$) (Fig. 1c, Table 3). With drought, both origins produced lower number of capitula compared to control (Fig. 1d), and the decrease in the number of capitula produced per plant did not differ between origins ($U = 40.0$; $P = 0.450$). Notice that plants from the Alps produced no capitula in the harshest conditions.

Nutrient gradient

Potassium gradient

Alpine individuals showed higher survival along the two higher gradient potassium levels, compared to Andean individuals (Fig. 2a, Table 2). Individuals from the Alps showed the same survival along the three potassium levels, whereas individuals from the Andes decreased survival at the highest level of potassium (Fig. 2a, Table 2). Individuals from the Andes reached lower biomass than individuals from the Alps across the potassium gradient ($F_{1,64} = 5.70$; $P < 0.05$) (Table 4), with no changes among the different potassium levels ($F_{2,64} = 0.71$; $P = 0.49$) (Fig. 2b, Table 4). The root:shoot ratio of both genotypes did not change along this gradient (Fig. 2c, Table 4).

Nitrogen gradient

Although total survival was higher in Alpine individuals along the entire gradient compared to Andean individuals, this difference was only significant at the intermediate level (Fig. 3a, Table 2). The highest survival was found in the intermediate level of nitrogen for individuals from the Alps, while for individuals from the Andes it was found at

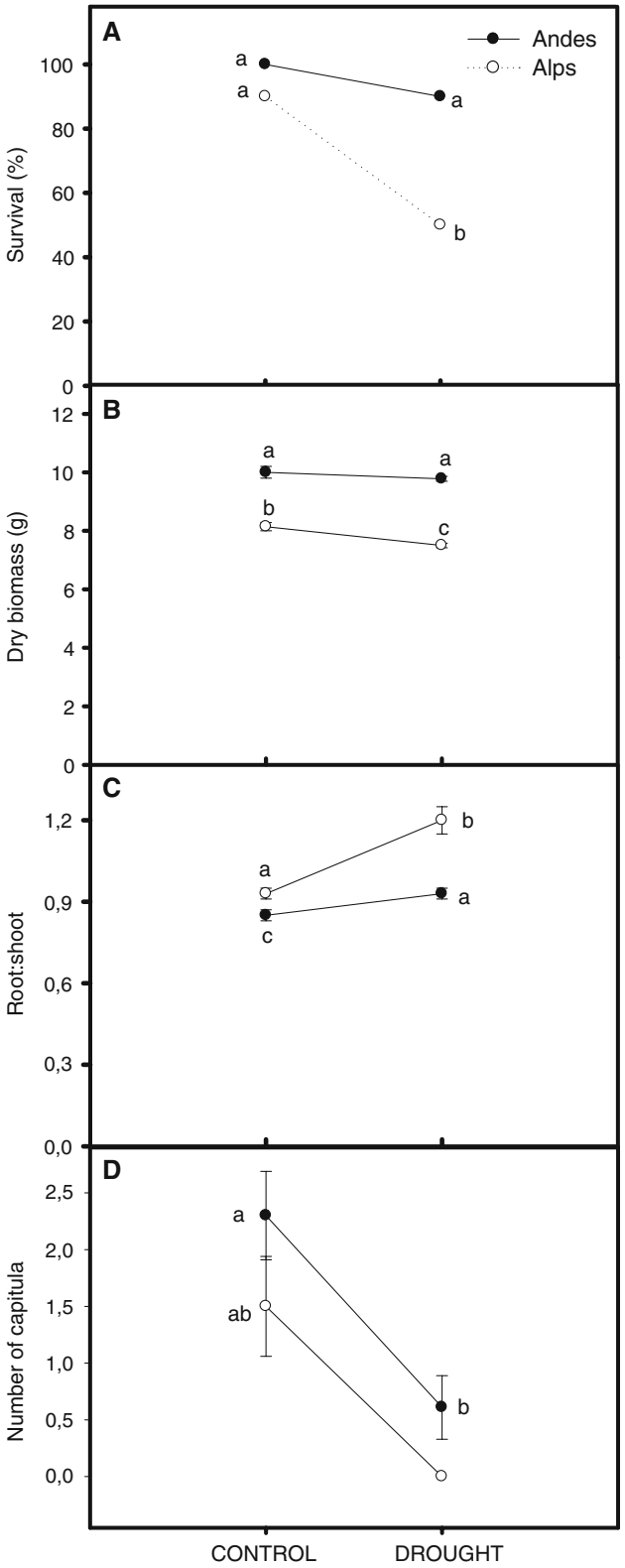

Fig. 1 Survival (%), total dry biomass, root:shoot and number of capitula produced by *Taraxacum officinale* individuals grown from seeds collected in the introduced range (Andes) and in the native range (Alpes), under watered (CONTROL) and drought conditions. *Error bars* indicate S.E. Different *superscript letters* indicate significant differences (Mann-Whitney *U* test)

the two higher levels (Fig. 3a, Table 2). Individuals from the Andes reached lower biomass than individuals from the Alps ($F_{1,40} = 9.88$; $P < 0.01$) across the entire nitrogen gradient ($F_{2,40} = 0.56$; $P = 0.58$) (Fig. 3b, Table 5). Regarding the root:shoot ratio, Andean individuals allocated more resources to the roots than Alpine individuals ($F_{1,40} = 11.69$; $P < 0.01$) across the entire gradient ($F_{2,40} = 0.31$; $P = 0.73$) (Fig. 3b, Table 5).

Discussion

The individuals of *T. officinale* used in this study come from two high-altitude origins, the Andes and the Alps. Although the Alpine dandelions belong to the native range and the Andean dandelions, to the introduced range, both can be considered as derived populations of lower-altitude populations, that had acted as a source of propagules dispersed by wind, cattle, and humans to higher elevations. Therefore, individuals originating in these habitats will not only reflect the genetic pool of the lower-altitude populations of origin, but also the results of new selective pressures that are present in high-altitude environments.

Although there was no indication of plasticity in the two gradients of soil nutrients examined, individuals from both origins differed in their plasticity under drought. With drought, individuals from both origins showed plasticity in the root:shoot ratio, increasing allocation to below ground biomass. However, this change in biomass allocation was bigger in individuals coming from the Alps. This change in biomass allocation is a common strategy to drought that allows plants to increase their water absorption surface (Larcher 2003). Nevertheless, despite the higher allocation to belowground biomass in individuals from the Alps, this was not translated into a higher fitness compared to individuals from the Andes. While individuals from the Alps showed lower survival and reached lower biomass with drought, individuals from the Andes maintained the same survival compared to the control. Indeed, they produced flowers under drought. Therefore, Andean individuals can be considered as drought-resistant, while Alpine individuals were drought-sensitive.

In the context of an exotic species growing in a stressful environment, plasticity in a certain trait is unlikely to have any effect on invasiveness unless that plasticity contributes to fitness in that particular habitat (Richards et al. 2006). Our results suggest that the Andean population (introduced origin) does not show great plasticity under drought. Nevertheless, this lack of plasticity is not related to negative consequences in individual fitness, in terms of survival and reproduction. In the dry growing season occurring in central Chile, the ability to tolerate drought can make the difference between a successful establishment or not (Cavieres et al. 2006). Therefore, differences observed in drought resistance between the two origins of *T. officinale* might be

Table 2 *P*-values of the comparison of the final survival of *Taraxacum officinale* individuals grown in a drought experiment, in a gradient of nitrogen (N), and in a gradient of potassium (K)

	And-Control	And-Drought	Alp-Control	Alp-Drought
And-Control	–	0.155	0.155	< 0.001
And-Drought		–	1	< 0.01
Alp-Control			–	< 0.01
Alp-Drought				

	And-N1	And-N2	And-N3	Alp-N1	Alp-N2	Alp-N3
And-N1	–	0.273	< 0.05	0.054	< 0.001	< 0.05
And-N2		–	0.164	0.344	< 0.01	0.106
And-N3			–	0.599	0.094	0.805
Alp-N1				–	< 0.05	0.43
Alp-N2					–	0.143
Alp-N3						–

	And-K1	And-K2	And-K3	Alp-K1	Alp-K2	Alp-K3
And-K1	–	0.394	< 0.05	1	0.146	1
And-K2		–	0.155	0.394	< 0.05	0.394
And-K3			–	< 0.05	< 0.01	< 0.05
Alp-K1				–	0.131	1
Alp-K2					–	0.131
Alp-K3						–

Survival was compared among groups with a two-tailed proportion test
And = Andean individuals; Alp = Alpine individuals

influenced by differences in specific abiotic conditions between both environments. The ability to cope with drought found in the Andean dandelions may be part of the reason for their success in this part of the Andes. Nevertheless, this ability was accompanied by a high nutrient sensitivity in terms of survival. Survival of the Andes individuals was negatively affected towards increasing levels of potassium and lower levels of nitrogen, whereas survival in dandelions originated in the Alps was not negatively affected by the nutrients availability. Hence, Andean individuals behave as a nitrogen demanding-potassium avoiding species, whereas Alpine individuals did not show any particular dependency or repulsion tendency to either of these two nutrients. In the central Chilean Andes, *T. officinale* is very abundant in anthropically-disturbed sites (Cavieres et al. 2005), which are characterized by higher levels of nitrogen but lower levels of potassium compared to undisturbed soils (Quiroz CL, unpublished data). This last might explain the responses observed for the Andes origin under nutrient availability. Despite the fact that potassium is an essential nutrient for plant growth (Taiz and Zeiger 1998), high concentrations of this cation in the soil have been reported to have negative effects in plants (Russel and Russel 1959). According to this, we presume that *T. officinale* from the Andes is more damaged by higher levels of potassium than their counterparts from the Alps.

Although *T. officinale* is distributed worldwide among a great diversity of environments, this is the first study that compares plastic responses of individuals occurring in the native and invaded ranges under stressful conditions. Actually, the ability of this species to tolerate stressful conditions has only been examined in presence of native co-occurring species in the introduced range and in the native range separately (Brock et al. 2005; Tsialtas et al. 2004). Despite the general assumption that *T. officinale* presents a plastic strategy, the few studies that have actually evaluated phenotypic plasticity for this species (Tsialtas et al. 2004; Brock et al. 2005, this study) have not found evidence to support this common assumption.

Table 3 One-way analysis of variance of the change in the attributes measured for *Taraxacum officinale* individuals grown in the drought experiment

	Final biomass (gr)	Root:Shoot
Origin (O)	19.25***	19.40***

F-values are shown ($df = 1.18$)
***$P < 0.001$

The scarce available evidence for differences in plasticity between native and introduced individuals of other invasive species occurring under stressful conditions is still contradictory. For instance, Kaufman and Smouse (2001) compared plasticity of *Melaleuca quinquenervia* using two soil moisture levels (moist unsaturated soil and flooded soil) and found more phenotypic plasticity in individuals from the introduced range than from the native range. In contrast, DeWalt et al. (2004) found little evidence of differences in plasticity between introduced and native genotypes of the tropical shrub *Clidemia hirta*.

If constant fitness is the key to success, then we expect a Jack-of-all trades situation, where plasticity allows the fitness of the invader to remain relatively constant across environments (Richards et al. 2006). If the success of an invader is due to its ability to rapidly take advantage of available resources, we expect a Master-of-some situation, where the invader shows a greater fitness response to

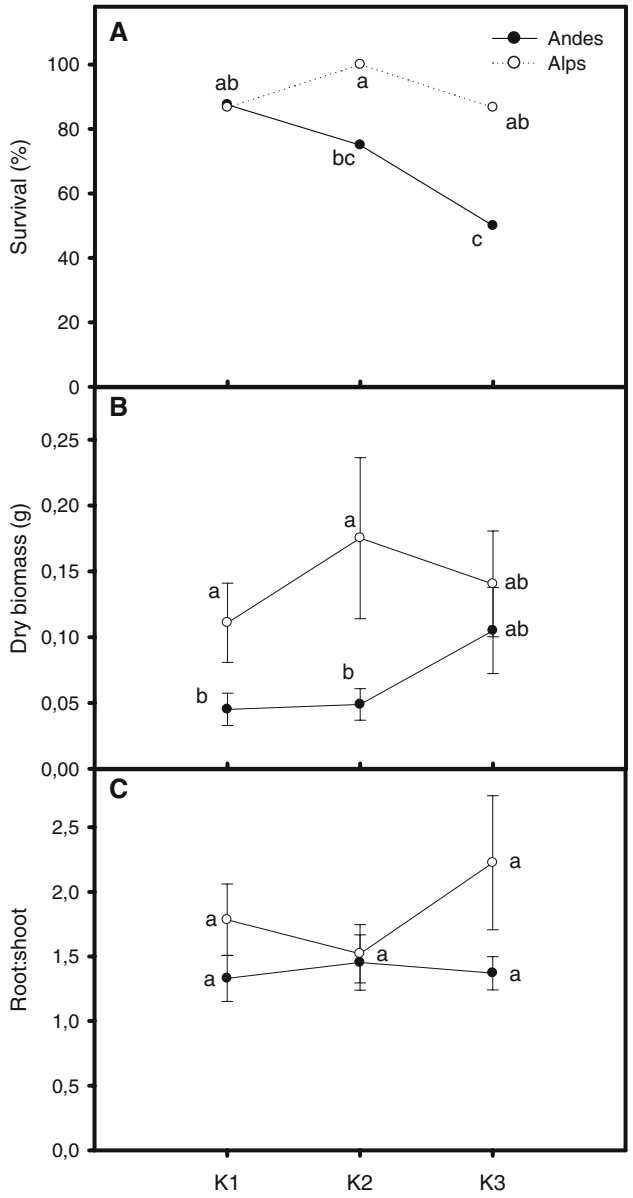

Fig. 2 Survival (%), total dry biomass and root:shoot of *Taraxacum officinale* individuals grown from seeds collected in the introduced range (Andes) (*solid bars*) and in the native range (Alps) (*empty bars*), under and increasing gradient of potassium. *Error bars* indicate S.E. Different *superscript letters* indicate significant differences (Tukey HSD for unequal sample sizes)

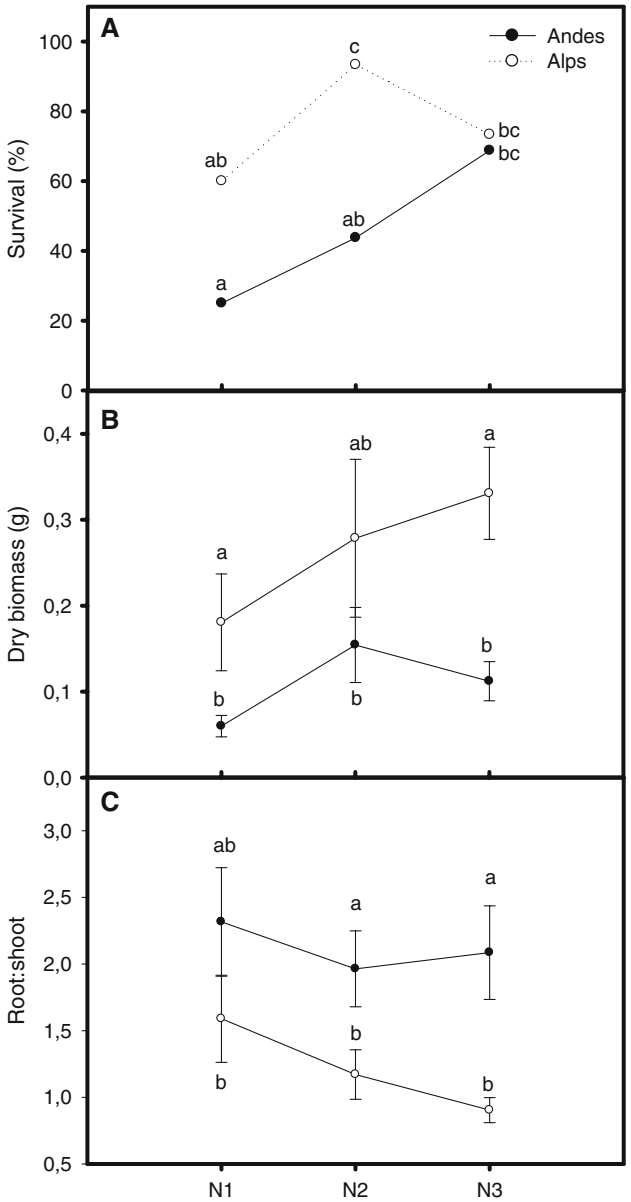

Fig. 3 Survival (%), total dry biomass and root:shoot of *Taraxacum officinale* individuals grown from seeds collected in the introduced range (Andes) (*solid bars*) and in the native range (Alps) (*empty bars*), under and increasing gradient of nitrogen. *Error bars* indicate S.E. Different *superscript letters* indicate significant differences (Tukey HSD for unequal sample sizes)

Table 4 Two-way analysis of variance of final biomass and root-shoot ratio for *Taraxacum officinale* individuals grown in the potassium gradient experiment

	Final biomass (gr)	Root: Shoot
Origin (O) $_{F\ 2.64}$	5.70*	2.56 ns
Treatment (T) $_{F\ 1.64}$	0.73 ns	0.39 ns
O × T $_{F\ 2.64}$	0.71 ns	0.43 ns

F-values are shown
ns $P > 0.05$, *$P < 0.05$

favorable conditions (Richards et al. 2006). Morrison and Molofsky (1998, 1999), and Lavergne et al. (2007) studied the performance of invasive *Phalaris arundinacea* genotypes under different biotic and abiotic conditions. They have found consistent evidence of low phenotypic plasticity among introduced genotypes, and dependence on multiple introductions to increase its geographical spread in the introduced region (Morrison and Molofsky 1998, 1999; Lavergne et al. 2007). According to our results, native individuals of *T. officinale* originated in the Alps behaves like a ''Jack-of-all-trades'' under stressful conditions, whereas introduced *T. officinale* originated in the

Table 5 Two-way analysis of variance of final biomass and root-shoot ratio for *Taraxacum officinale* individuals grown in the nitrogen gradient experiment

	Final biomass (gr)	Root:Shoot
Origin (O) $_{F\ 2.40}$	9.88**	11.69**
Treatment (T) $_{F\ 1.40}$	1.49 ns	1.05 ns
O × T $_{F\ 2.40}$	0.56 ns	0.31 ns

F-values are shown
ns $P > 0.05$, $^{**}P < 0.01$

Andes resembles a ''Master-of-some conditions'' under the same environmental constraints. These conclusions open a window of opportunities for the control of the spread of *T. officinale* in these habitats, by creating the conditions that these individuals are not able to manage.

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Résumé

Les écosystèmes alpins, au même titre que les écosystèmes arctiques, séquestrent des quantités importantes de carbone dans leurs sols. Dans ces écosystèmes, la topographie locale détermine la répartition de la neige; un facteur qui, sur le court terme, affecte les paramètres physiques de l'environnement (effets directs) et qui, sur le long terme, a sélectionné des communautés végétales et microbiennes très différentes aux deux extrêmes du gradient de mésotopographie (effets indirects). Au regard des modifications futures des régimes d'enneigement prédits par les différents modèles climatiques, cette étude vise à explorer les contrôles directs et indirects exercés par l'enneigement sur la fixation du CO_2 et la minéralisation du carbone organique dans les écosystèmes alpins.

Les paramètres physiques des sols (eau et température) ont été mesurés pendant plusieurs années révélant les effets directs. Afin de quantifier les effets indirects de l'enneigement sur les flux biogéochimiques, nous avons utilisé les caractéristiques fonctionnelles des végétaux (leurs traits). Différentes approches (mesures *in situ*, manipulations expérimentales et modélisation) ont été employées.

Cette étude démontre que la fixation du carbone le long des gradients de mésotopographie est à la fois déterminée par les traits fonctionnels végétaux, les propriétés des canopées et la longueur de la saison de végétation. Un allongement de la saison de végétation devrait entraîner une augmentation marquée de la production primaire si les événements de gel en début de saison de végétation demeurent limités. La minéralisation du carbone est au contraire largement dépendante de la qualité de la matière organique contenue dans les sols. Des changements de composition en traits fonctionnels de la végétation, notamment ceux affectant les concentrations en lignine des litières, devraient avoir un impact déterminant sur les vitesses de minéralisation de la matière organique. Enfin, l'étude des flux de carbone et d'azote dans les plantes dominantes et à l'interface plante – sol révèle un couplage temporel et spatial essentiel chez les espèces dont la croissance est limitée par la longueur de la saison de végétation. Ce couplage apparaît plus limité dans les communautés végétales bénéficiant d'une plus longue saison de végétation. L'évolution des flux et stocks de carbone au sein des écosystèmes alpins dans un contexte de changement climatique est discutée.

Abstract

Alpine tundra store large carbon stocks in their soils. In these ecosystems, the local mesotopography determines snow cover distribution, a key variable, which affect the edapho-climatic conditions on the short term (direct effects) and, in the longer-term, select for contrasting plant and microbial communities at both ends of the topographical gradient (indirect effects). In the context of global change, where large changes in snow precipitations are projected, this study explores the controls exerted by snow cover on carbon fixation and carbon mineralization in alpine tundra.

Edapho-climatic variables (water and temperature) were measured during several years and we used vegetation functional characteristics (using plant functional traits) to quantify the indirect effects of snow cover on biogeochemical cycles. Various approaches (*in situ* measurements, experimental manipulations and modeling) were used.

This study demonstrates that carbon fixation along mesotopographical gradients is determined by plant functional traits, canopy properties and growing season length. A longer growing season may lead to a marked increase in primary production, if freezing events at snowmelt remain infrequent. In contrast, carbon mineralization is mainly dependant over soil organic matter quality. Shifts in plant functional traits, in particular those related to litter lignin content, will strongly impact the degradation process. Finally, the quantification of carbon and nitrogen fluxes in plants and at the plant-soil interface reveals a tight spatial and temporal coupling which is essential for species whose growth is limited by growing vegetation length. This coupling is reduced in plant communities which benefit from a longer growing season. The evolution of carbon fluxes and stocks in alpine ecosystems is discussed in the context of climatic changes.