UNIVERSITÉ DU QUÉBEC À CHICOUTIMI

# WOOD FORMATION IN BLACK SPRUCE: INFLUENCE OF GROWING SEASON LENGTH, SOIL TEMPERATURE AND NITROGEN

THÈSE
PRESENTÉE
COMME EXIGENCE PARTIELLE
DU DOCTORAT EN SCIENCES DE L'ENVIRONNEMENT

PAR
CARLO LUPI

9 JUILLET 2012

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# FORMATION DU BOIS CHEZ L'ÉPINETTE NOIRE: INFLUENCE DE LA SAISON DE CROISSANCE, DE LA TEMPÉRATURE DU SOL ET DE L'AZOTE

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# Remerciements

"The thing the ecologically illiterate don't realize about an ecosystem is that it's a system. A system! A system maintains a certain fluid stability that can be destroyed by a misstep in just one niche. A system has order, a flowing from point to point. If something dams the flow, order collapses. The untrained might miss that collapse until it was too late. That's why the highest function of ecology is the understanding of consequences."

Pardot Kynes in "Appendix I: The Ecology of Dune", in the science fiction novel "Dune", written by Frank Herbert (1920-1986) in 1965

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# Résumé

La croissance des arbres dans la forêt boréale est souvent considérée limitée par les basses températures et par la disponibilité en azote (N). Avec le réchauffement climatique, la disponibilité en azote pourrait augmenter grâce à l'effet positif d'une augmentation de la température du sol sur la minéralisation et la décomposition de la matière organique. De plus, les dépôts azotés d'origine anthropique ont grandement altéré le cycle de l'azote et la nutrition des plantes dans les deux derniers siècles et ils devraient augmenter dans le futur. Il est donc primordial de comprendre si, et dans quelle mesure, les perturbations d'origine anthropique (réchauffement climatique et augmentation de dépôts azotés) peuvent influencer la croissance de la forêt boréale. Le but de cette thèse a été de comprendre de quelle manière la formation du cerne ligneux et la productivité en termes de bois chez l'épinette noire (*Picea mariana* (Mill.) BSP) pourraient varier dans le futur sous l'influence des perturbations d'origine anthropique évoquées ci-dessus. La recherche a eu lieu en forêt boréale dans deux peuplements équiennes constitués d'épinettes noires adultes, dans la région du Saguenay-Lac-St-Jean, au Québec (Canada). Premièrement, nous avons déterminé les dynamiques actuelles de formation du cerne de croissance chez l'épinette noire, afin de pouvoir décrire d'éventuelles différences entre les deux sites et connaître l'impact de la variabilité climatique interannuelle (Chapitre II). Ces résultats nous ont permis de développer un modèle qui analyse la relation entre la phénologie et la productivité du cerne de croissance dans les conditions actuelles. Ce modèle, en plus de fournir une nouvelle clé d'interprétation de la xylogenèse dans les conditions actuelles, a permis d'interpréter de façon plus approfondie les résultats d'une expérience de manipulation de la température du sol et des dépôts azotés (Chapitres III et IV) simulant les conditions attendues pour le futur.

Dans les deux sites étudiés la dynamique de formation du cerne de croissance était similaire. Toutefois, la différence entre les sites, d'environ deux à trois semaines, dans la date de fonte de la neige, se reflétait par un délai d'une à deux semaines dans le début de l'activité cambiale observé dans la tige des arbres sans traitement. La date de fin de la xylogenèse a été assez variable d'un arbre à l'autre, mais aucune différence significative n'était mise en évidence entre sites et années (entre 2006 et 2009) (Chapitre II). Dans les conditions actuelles, la xylogenèse dans les sites étudiés variait entre 77 et 126 jours, en accord avec des études précédentes sur des espèces des milieux froids. La durée a été significativement différente entre les années, principalement à cause d'une différence dans le début de la formation du cerne (Chapitre II et III). Un nombre plus élevé de cellules est lié à un début plus hâtif et une fin plus tardive de la différentiation cellulaire. La relation est plus forte avec le début (r=0.74) qu'avec la fin (r=0.61) de la xylogenèse. L'absence d'une relation directe entre début et fin de la xylogenèse, indique que c'est bien le nombre de

cellules qui modifie les rapports entre ces deux phases de la xylogenèse. La relation causale entre la production cellulaire et la date de fin de différentiation cellulaire mise en évidence dans cette thèse (Chapitre II) a ainsi fourni une nouvelle clé d'interprétation de certains mécanismes de formation du cerne de croissance et des observations que les arbres plus jeunes, vigoureux ou fertilisés produisent plus de cellules et terminent plus tard leur croissance.

Dans les chapitres III et IV, des hypothèses stipulant que la phénologie de formation du xylème et l'anatomie du bois sont affectées par des augmentations de la température du sol et les dépôts azotés ont été testées. Pendant trois ans, la température du sol a été augmentée de 4 K pendant la première partie de la saison de croissance (pour simuler une fonte hâtive de la neige de 1 à 2 semaines) et des précipitations contenant 3 fois la concentration actuelle en N ont été ajoutées sur le terrain à travers des applications fréquentes au niveau de la canopée. Le réchauffement du sol a eu comme effet des débuts plus hâtifs de la xylogenèse et a interagi avec l'ajout de N produisant des durées de la xylogenèse plus longues dans les arbres traités. L'effet du chauffage a été particulièrement marqué au niveau de la phénologie des racines, tandis que la production de bois, en termes de nombre de cellules et de largeur de cerne, n'a pas été influencée par les traitements. Nos résultats démontrent donc que le chauffage du sol a été capable de modifier la phénologie de formation du xylème, pas seulement dans les racines (effet localisé) mais aussi dans la tige, probablement à travers une influence indirecte due à une absorption et un transport améliorés des nutriments et de l'eau. De plus, le contrôle de l'activité cambiale et la formation du bois dans la tige et les racines pourraient dépendre en partie de différentes variables, c.à.d. que la température de l'air pourrait être plus importante pour la tige tandis que la température du sol le serait pour les racines. À court terme, la variation de la production des cellules semble être principalement liée au début de la réactivation cambiale et aux conditions favorables dans la première partie de la saison de croissance (Chapitre II), tandis qu'à long terme d'autres facteurs peuvent affecter la croissance radiale, comme par exemple la disponibilité des nutriments. D'ailleurs, l'interaction significative du traitement d'irrigation azotée et de réchauffement du sol sur la durée de la xylogenèse a confirmé le rôle important à la fois de l'azote et de la température du sol sur la formation du cerne de croissance. L'anatomie du xylème et la composition chimique du sol et des aiguilles n'ont pas été affectées par les traitements, excepté pour l'épaisseur de la paroi cellulaire dans le bois initial du site plus au Nord. Les effets cumulés du réchauffement du sol et de l'ajout de N sur la disponibilité des nutriments lors d'expériences à plus long terme restent quand même à évaluer.

En analysant ensemble les résultats de notre étude nous pouvons mettre en évidence comment le modèle causal, liant phénologie de formation du xylème et production du bois, élaboré dans le Chapitre II en se basant sur les conditions actuelles, semble fonctionner même dans les conditions futures simulées (Chapitre III et IV). Par

exemple, dans le chapitre III, nous avons observé qu'à partir de la deuxième année de chauffage, les plantes chauffées ont montré une durée de la xylogenèse plus longue. Même si la différence entre les traitements n'a pas toujours été statistiquement significative, nous avons observé un début plus hâtif et une fin plus tardive dans les arbres chauffés par rapport aux arbres non-chauffés. À partir de la troisième année, l'écart en nombre de cellules produites entre plantes chauffées et plantes non-chauffées a augmenté. Ces observations et tendances suggèrent que le modèle arrive à bien représenter comment des variations dans la phénologie de formation du bois sont associées avec des variations au niveau de la production, même dans les conditions environnementales prévues pour le futur.

Mots clés: activité cambiale; anatomie du bois; dépôts azotés; épinette noire formation du bois; phénologie cambiale; *Picea mariana*; température du sol; xylème; xylogenèse;

# Chapitre I

# Introduction générale

# 1. Introduction générale

## 1.1. Problématique

La zone boréale circumpolaire est l'un des plus vastes biomes terrestres, constitué en bonne partie par des forêts, et en Amérique du Nord, elle couvre plus de 600 millions d'hectares ou environ 30% du continent nord-américain (Brandt 2009). Selon Burton *et al.* (2003), au Canada, 60% des revenus de l'exploitation de la forêt proviennent des forêts boréales. Plus d'un demi-million de canadiens ont des emplois liés à la ressource forestière et plus de trois millions de canadiens habitent dans la zone climatique occupée par la forêt boréale (Burton *et al.* 2003). Malgré son importance environnementale, économique et sociale, la sensibilité de cet écosystème aux changements climatiques et à la pollution d'origine anthropique est encore peu connue.

Le climat de la Terre s'est considérablement réchauffé pendant les derniers cent ans et, en particulier, dans la dernière décennie (IPCC, 2007). Parmi les écosystèmes forestiers du globe, la forêt boréale est celui pour lequel on s'attend aux plus importantes augmentations de température (Strömgren and Linder 2002). Les plus récentes simulations du modèle canadien du climat régional pour la forêt boréale de l'Est du Canada estiment qu'il y aura une hausse des températures annuelles moyennes de 3°C et une augmentation des précipitations annuelles entre 5% et 20% d'ici l'an 2050 (Plummer *et al.* 2006). Les températures devraient augmenter surtout en hiver, avec des augmentations saisonnières pour l'an 2050 qui varient en moyenne entre 3 et 5°C au Québec (Logan et al. 2011). Dans cet écosystème, la croissance des arbres est limitée par les basses températures et par la disponibilité de certains nutriments, en particulier l'azote (Vitousek *et al.* 1997, Boisvenue and Running 2006, Hyvonen *et al.* 2008). Ainsi, certaines expériences conduites en forêt boréale ont observé que la température du sol peut affecter la croissance radiale et en biomasse aérienne des plantes (Van Cleve *et al.* 1990, Strömgren and Linder 2002). De plus, la disponibilité en azote devrait augmenter avec le réchauffement climatique, et ce, grâce à l'effet positif sur la minéralisation de la substance organique dû à une hausse de la température du sol (Rustad *et al.* 2001, Sushama *et al.* 2006). Toutefois, le rôle de la température du sol et de la disponibilité des éléments nutritifs sur la croissance de la forêt boréale est encore peu compris (Vaganov *et al.* 1999, Jarvis and Linder 2000).

Les activités anthropiques ont grandement modifié le cycle de l'azote et la nutrition des arbres dans les deux cents dernières années (Galloway *et al.* 2004). Dans les forêts boréales et tempérées, traditionnellement considérées comme des écosystèmes limités en azote, l'ajout d'azote à travers des dépôts atmosphériques accrus par la pollution est très probablement une des causes de l'augmentation de la croissance en biomasse des arbres observée au XX$^{e}$ siècle (Boisvenue and Running 2006, Magnani *et al.* 2007). Certains auteurs ont également observé que l'accroissement en volume des peuplements de la forêt boréale dans le sud de la Suède était plus important par rapport aux peuplements au nord, en lien avec l'augmentation des dépôts azotés d'origine anthropique décroissante du sud au nord (moins peuplé et industrialisé) (Akselsson *et al.* 2008, Hyvonen *et al.* 2008).

Même si les sols de la forêt boréale sont riches en matière organique et, donc, en azote, seulement une petite partie de cet azote est sous forme disponible pour les plantes et la compétition pour cet élément nutritif est alors très élevée (Jackson *et al.* 2008). De plus, dans les pessières noires, le temps de séjour de l'azote inorganique dans la solution du sol est très court (Ste-Marie and Houle 2006). Néanmoins, les arbres de la forêt boréale semblent capables d'absorber une partie de l'azote qui arrive avec les dépôts azotés directement à travers leur canopée, et cela, en contournant la compétition dans les sols (Houle *et al.* 1999, Ignatova and Dambrine 2000). À la différence des sites plus productifs, où l'absorption de l'azote par la canopée représente visiblement une petite fraction de l'accroissement foliaire et des besoins du peuplement, dans des sites peu productifs, comme certains sites dominés par l'épinette et le sapin, l'absorption par la canopée pourrait contribuer presque entièrement à la formation de biomasse foliaire (Lovett and Lindberg 1993). En plus, une augmentation des dépôts azotés d'origine anthropique est prévue dans le futur et couvrira de plus vastes étendues de la forêt boréale (Galloway *et al.* 2004). L'importance d'évaluer son impact sur la production de bois chez les conifères est donc fondamentale.

L'épinette noire est l'une des espèces les plus communes de la forêt boréale de l'est du Canada et est économiquement importante étant donné son utilisation pour la production de papier et de bois d'œuvre (Gagnon and Morin 2001, Krause and Morin 2005). La compréhension du rôle des facteurs comme la température du sol et la disponibilité en azote sur la croissance de cette espèce s'avère fort importante pour connaître de quelle façon sa productivité, en termes de bois, pourrait varier avec les changements attendus pour le futur. Par exemple, des facteurs comme la température et la disponibilité en éléments nutritifs peuvent avoir une influence sur le «timing» et la durée de la croissance des racines (Gill and Jackson 2000) et ainsi influencer, par ricochet, la croissance dans tout le restant de l'arbre. De même, les caractéristiques du bois pourraient être modifiées indirectement par l'azote, car cet élément influence la photosynthèse (donc la disponibilité en C), le taux de croissance de l'arbre et la synthèse de la lignine (Alcubilla *et al.* 1976, Reich *et al.* 1997, Canovas *et al.* 2007, Kaakinen *et al.* 2007).

Le bois ou xylème est produit grâce à l'activité d'un méristème secondaire, le cambium vasculaire (Plomion *et al.* 2001). Dans les écosystèmes tempérés et boréaux, la croissance radiale chez les conifères a lieu à travers la formation annuelle des cellules du xylème qui sont produites par le cambium et qui entourent le cerne de croissance de l'année précédente (Rossi *et al.* 2006). À la suite des divisions dans la zone cambiale, pour terminer leur développement, les cellules produites doivent passer à travers trois phases de différentiation cellulaire, c.à.d. l'élargissement, la formation de la paroi secondaire et la lignification. Par rapport à la formation du bois, la saison de croissance peut ainsi être définie comme la période comprise entre le début de la formation des nouvelles cellules du xylème et la fin de leur développement. Pendant la saison de croissance, dès que des cellules commencent leur différentiation, des nouvelles cellules sont produites par le cambium, de sorte que des cellules localisées dans la même file radiale peuvent se trouver à différentes phases de leur développement (Plomion *et al.* 2001). Le moment du développement des différentes cellules détermine les caractéristiques anatomiques propres à chaque cellule du xylème (Wodzicki 1971) et, en retour, les propriétés du bois (Butterfield 2003). L'analyse des processus de croissance radiale doit distinguer les phases de production et de différentiation cellulaire pour caractériser correctement les facteurs qui influencent la formation du bois (Antonova and Stasova 1993, Deslauriers and Morin 2005, Rossi *et al.* 2006). La compréhension des dynamiques de croissance actuelles et futures des arbres de la forêt boréale requiert une évaluation de l'impact des facteurs environnementaux qui sont en train de changer (réchauffement climatique et augmentation des dépôts azotés) sur la croissance radiale, incluant l'activité cambiale et la phénologie de formation du bois.

En résumé, les effets des changements climatiques et des dépôts azotés sur la formation du bois sont peu connus en forêt boréale. Étant donné les implications écologiques et socio-économiques, une évaluation plus détaillée du rôle de ces facteurs environnementaux est nécessaire afin de comprendre de quelle manière la formation du cerne de croissance et donc la productivité de l'épinette noire pourraient varier dans le futur.

## 1.2. Approche méthodologique

Une des méthodes les plus précises et intéressantes pour étudier la croissance radiale de l'arbre et l'impact des facteurs environnementaux sur la formation du bois est l'utilisation des analyses cellulaires intra-annuelles du cerne en formation (Wodzicki *et al.* 1971, Antonova and Stasova 1993). La prise hebdomadaire de petits échantillons de bois (micro-carottes) pendant la saison de croissance permet de reconstruire la façon dont le cerne ligneux est formé et d'analyser en détail comment les facteurs extérieurs influencent les différentes phases de développement et de différentiation des cellules du bois (Antonova and Stasova 1993, Deslauriers *et al.* 2003, Deslauriers and Morin 2005). Qui plus est, les échantillons pris après la fin du développement du cerne annuel peuvent être utilisés pour des analyses de l'anatomie du bois et le calcul de variables liées, entre autres, aux propriétés mécaniques et, par conséquent, à la qualité du bois (Butterfield 2003). L'étude de la phénologie des tissus de la zone cambiale et du développement du cerne de croissance dans différentes parties de l'arbre (ex. tige et racines) offre le moyen de mieux comprendre la dynamique temporelle de la formation du bois et la manière dont la production de bois peut varier dans l'arbre (Thibeault-Martel *et al.* 2008, Steinaker and Wilson 2008).

Le projet a pour but d'étudier les effets du réchauffement du sol et de l'augmentation des dépôts azotés atmosphériques sur la formation et la structure du bois de l'épinette noire mature dans la tige et les racines. La formation du cerne de croissance sera suivie à l'aide des analyses histologiques pendant la saison de croissance (phénologie) et après la fin de la différentiation des cellules du bois (anatomie). Les arbres expérimentaux seront soumis à deux traitements de manipulation de conditions environnementales afin de simuler les conditions attendues pour le futur : (1) un traitement de hausse de la température du sol, et ce, à l'aide de câbles chauffants placés autour des racines et (2) un traitement de précipitations artificielles enrichies d'azote appliquées sur la canopée des arbres expérimentaux à l'aide de gicleurs.

### 1.3. Hypothèses et objectifs spécifiques

Plusieurs études se sont penchées sur la question du début de l'activité cambiale et de la xylogenèse et les résultats indiquent que, dans les milieux froids, la température est le facteur le plus important qui agit sur cette phase (Deslauriers *et al.*

2003, Deslauriers and Morin 2005). Beaucoup moins d'études ont toutefois analysé les facteurs affectant la fin de la xylogenèse (Rossi et al. 2012). Or, pour comprendre l'impact des changements environnementaux attendus pour le futur (réchauffement climatique et hausse de dépôts azotés), il est fondamental d'interpréter correctement les mécanismes qui régissent la xylogenèse actuellement (en la décrivant en détail et en développant, si possible, des modèles interprétatifs) et les liens entre la phénologie de la formation du xylème et la production du bois. Par exemple, des délais dans la réactivation du cambium et le début de la formation du xylème peuvent réduire la période disponible pour la croissance radiale et la quantité du bois produit (Vaganov *et al.* 1999, Deslauriers *et al.* 2003, Gričar *et al.* 2005). Cependant, un nombre plus élevé de trachéides en différentiation pourrait retarder la fin de la différentiation cellulaire et ainsi prolonger la saison de croissance (Gričar *et al.* 2005, Thibeault-Martel *et al.* 2008). Nous posons donc l'hypothèse de l'existence d'une relation complexe de cause à effet entre la phénologie de la formation et la production du bois (voir Chapitre II).

Après avoir décrit la xylogenèse dans les conditions actuelles et mis en évidence d'éventuelles relations entre les variables étudiées, il sera possible d'analyser l'impact de changements attendus pour le futur, en modifiant artificiellement les facteurs environnementaux d'intérêt de cette étude. Étant donné le rôle fondamental de la température pour la réactivation cambiale, on se concentrera d'abord sur la température au début de la saison de croissance et, en conséquence, sur la fonte de la neige. Il faut d'abord rappeler que la neige, à travers son effet isolant, maintient la température du sol près de 0°C pendant l'hiver (Decker *et al.* 2003) et des fontes tardives de la neige au XX$^{e}$ siècle semblent être responsables de réactivations cambiales plus tardives et d'accroissements radiaux réduits (Vaganov *et al.* 1999). En effet, une température du sol inferieure à 6°C peut fortement inhiber l'activité racinaire et l'absorption de l'eau dans les conifères (Alvarez-Uria and Korner 2007) et ainsi affecter la croissance dans tout l'arbre. Étant donné que le cambium semble se réactiver de façon synchrone dans la tige et les racines (Thibeault-Martel *et al.* 2008) et que l'activité cambiale est souvent positivement influencée par la température (Oribe *et al.* 2001, Deslauriers *et al.* 2003, Begum *et al.* 2007), nous avançons l'hypothèse qu'un réchauffement du sol modifiera la phénologie de formation du xylème et stimulera la production du bois dans la tige (voir Chapitre III).

Pour terminer, on intégrera dans notre analyse le « facteur azote », ceci, car la croissance des arbres de la forêt boréale est souvent considérée limitée par les basses températures et la disponibilité en azote (Stromgren and Linder 2002, Reich *et al.* 2006). L'augmentation de la température du sol peut augmenter la disponibilité en azote en stimulant la minéralisation de la matière organique (Rustad *et al.* 2001). Nous posons donc l'hypothèse que le réchauffement du sol et l'augmentation de la disponibilité en azote attendus stimuleront la formation de bois chez l'épinette noire en forêt boréale davantage que chaque facteur pris individuellement et que les effets seront plus évidents au niveau des racines, étant donné que celles-ci pourront profiter directement soit de l'augmentation de la température du sol, soit de la disponibilité accrue en azote (voir Chapitre IV).

Les objectifs spécifiques de la thèse sont: (1) de caractériser la formation du bois dans l'épinette noire mature soumise aux conditions environnementales actuelles et étudier la relation entre phénologie et production du bois en forêt boréale; (2) d'étudier l'effet d'une hausse de la température du sol et d'une fonte hâtive de la neige sur l'activité cambiale et la formation du bois et d'analyser d'éventuelles tendances; (3) d'analyser l'effet de la température du sol et de la disponibilité en azote et leur interaction sur la production du bois dans différentes parties de l'arbre (tiges et racines). Ces objectifs seront traités dans trois articles scientifiques indépendants qui constitueront les trois chapitres de cette thèse de doctorat.

### 1.4. Sites d'étude

Deux sites d'étude avec des caractéristiques abiotiques différentes (altitude, latitude, température moyenne annuelle, date de fonte de la neige), mais une végétation et une structure du peuplement similaire ont été utilisés pour évaluer si oui ou non et comment la réponse des arbres aux traitements est affectée par des conditions environnementales différentes. Les sites d'étude utilisés dans cette thèse sont localisés dans deux peuplements matures et équiennes d'épinette noire dans la forêt boréale du Québec (Canada). Plus précisément, les sites se situent dans la région du Saguenay-Lac-Saint-Jean. Le site plus au sud et à plus basse altitude (SIM, 350 m, 48°13.78' N, 71°15.18' W), se situe près du lac Simoncouche, dans la réserve faunique des Laurentides, tandis que le site plus au nord et plus en altitude (BER, 611 m, 48°51.55' N, 70°20.34' W) est localisé dans les monts Valins, près du lac Bernachez (Figure 1.1).

Les sites sont caractérisés par un climat continental avec des hivers froids et longs et des étés courts et chauds (Tableau 1.1). Entre la fin de l'automne et le début du printemps, la majorité des précipitations tombe sous forme de neige qui s'accumule au sol jusqu'au début du mois de mai, quand la fonte commence, avec environ deux ou trois semaines de délai entre les deux sites (plus hâtive dans SIM, le site plus au sud; Tableau 1.1). Dans chaque site d'étude, une station météorologique est présente et mesure, entre autres, la température de l'air, du sol organique et minéral, les précipitations sous forme de pluie, la hauteur de la neige, l'humidité relative et le contenu en eau du sol.

### 1.5. Structure de la thèse

La thèse comprend trois chapitres principaux (II, III et IV) rédigés en anglais sous forme d'article scientifique et une conclusion générale (chapitre V) rédigée en français. Le chapitre II porte sur la formation du cerne de croissance dans des épinettes noires matures en proposant un modèle qui explique la relation causale entre la phénologie et la production du bois. La méthodologie de ce chapitre explique en détail l'utilisation des analyses cellulaires pour l'étude du cerne de croissance en formation et introduit la statistique derrière les modèles causaux. Les relations de cause à effet entre la phénologie (début et fin) de formation du bois et la productivité ligneuse (en nombre de cellules) sont analysées et un modèle explicatif est développé. *Note : Ce chapitre est le fruit du travail du candidat. Hubert Morin, Annie Deslauriers et Sergio Rossi ont participé à l'élaboration des idées de base de ce chapitre.*

Le chapitre III étudie l'effet d'une augmentation de la température du sol sur la phénologie de la formation du bois et la productivité en utilisant une approche statistique à mesures répétées. Les dates de début et de fin des différentes phases de la différentiation des cellules et la durée de la formation du bois ainsi que la production de cellules ont été comparées entre les arbres soumis aux traitements de chauffage du sol (sols non-chauffés vs chauffés) dans les trois années de l'expérience (2008-2010). Ceci a permis de mettre en évidence les effets du réchauffement du sol et d'isoler l'influence de la température du sol par rapport à la température de l'air sur l'activité cambiale et la production de bois dans la tige. L'approche à mesures répétées permet de mettre en évidence des tendances dans le temps et d'avancer des hypothèses quant aux effets à plus long terme. *Note : Ce chapitre est le fruit du travail du candidat. Hubert Morin, Annie Deslauriers et Sergio Rossi ont participé à l'élaboration des idées de base de ce chapitre.*

Le chapitre IV aborde aussi les conséquences du réchauffement du sol pour la formation du bois dans l'épinette noire, mais, en plus, il inclut l'étude des effets des dépôts azotés et leur interaction avec le réchauffement du sol. La phénologie de formation du bois, ainsi que la productivité en termes de nombre de cellules et d'accroissement radial, sont analysées et comparées entre les traitements de réchauffement du sol et d'enrichissement en azote dans la tige et les racines. Le chapitre inclut également l'analyse des effets des traitements sur l'anatomie du bois dans la tige et les racines, et cela, à la fin des trois années de l'expérience. Ce chapitre se concentre davantage sur les effets cumulés des traitements en analysant plusieurs variables après trois ans d'application des traitements de chauffage et des pluies artificielles. *Note : Ce chapitre est le fruit du travail du candidat. Hubert Morin, Annie Deslauriers, Sergio Rossi et Daniel Houle ont participé à l'élaboration des idées de base de ce chapitre.*

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Tableau 1.1 Paramètres météorologiques des sites d'étude pour la période 2002-2007, qui précède l'expérience (débutée en 2008).

| | BER | SIM |
|---|---|---|
| Température moyenne annuelle (°C) | 0.3 | 1.9 |
| Température maximale absolue (°C) | 32 | 34 |
| Température minimale absolue (°C) | -36 | -35 |
| Température moyenne entre Mai et Septembre (°C) | 11.4 | 13.3 |
| Précipitations entre Mai et Août (mm) | 323 | 321 |
| Hauteur maximale de la neige (m) | 1.3 | 1.1 |
| Date moyenne de la fonte complète de la neige | 20 Mai | 1 Mai |
| Date moyenne du début d'accumulation de la neige | 3 Nov | 10 Nov |

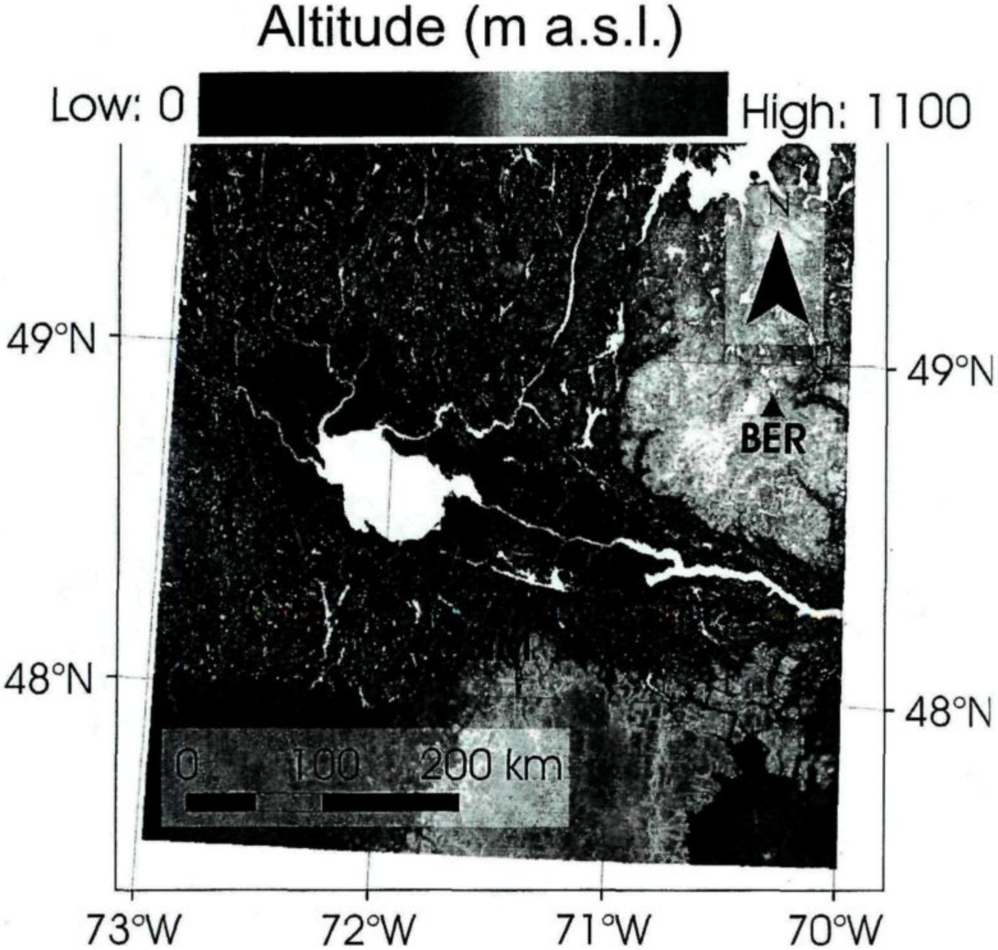

Figure 1.1 Localisation des sites d'étude (BER et SIM)

# Chapitre II

# Xylem phenology and wood production: resolving the chicken-or-egg dilemma

**Title** Xylem phenology and wood production: resolving the chicken-or-egg dilemma

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## 2.1 Abstract

Delays in the start of the growing season reduce the period available for growth and the amount of xylem production. However, a higher number of developing tracheids could prolong cell differentiation and, consequently, lengthen the growing season. The relationship between the amount and duration of cell production in the xylem remains an unresolved issue. The aim of this study was to resolve the chicken-or-egg causality dilemma about duration of growth and cell production through simple- and double-cause models. This was achieved by (i) analyzing the intra-annual growth dynamics of the xylem in *Picea mariana* (Mill.) BSP during 2006-2009 in two contrasting sites of the boreal forest of Quebec, Canada, and (ii) extracting the dates of onset and ending of xylem formation and the number of radial cells along the tree ring.

A higher number of cells was linked to an earlier onset ($r=0.74$) and later ending ($r=0.61$) of cell differentiation. The absence of a relationship between the residuals of the onset and ending of xylogenesis ($r_p=-0.06$) indicated that cell production influenced the correlation between the two phenophases of the xylem.

These results demonstrated that a higher number of cells produced delays the ending of xylem maturation, so extending the duration of wood formation.

**Keywords:** cambial activity, causal modeling, cell production, cold environments, duration of cell differentiation, growth cessation, *Picea mariana*, xylogenesis, wood formation

## 2.2 Résumé

Des retards dans le début de la saison de croissance réduisent la période disponible pour la croissance et la quantité de xylème produit. Cependant, un nombre plus élevé de trachéides en développement pourrait prolonger la différentiation cellulaire et, par conséquent, allonger la saison de croissance. La relation entre la quantité et la durée de la production des cellules dans le xylème demeure une question non résolue. Le but de cette étude était de résoudre le dilemme de causalité de la poule et de l'œuf sur la durée de la croissance et la production des cellules à travers des modèles simples et avec des doubles causes. Ceci a été réalisé par (i) l'analyse de la dynamique de croissance intra-annuelle du xylème dans *Picea mariana* (Mill.) BSP pendant la période 2006-2009 dans deux sites différents de la forêt boréale du Québec, Canada, et (ii) l'extraction des dates de début et de fin de formation du xylème et le nombre des cellules le long du cerne de croissance.

Un nombre plus élevé de cellules était lié à un début plus hâtif ($r=0.74$) et une fin plus tardive ($r=0.61$) de la différentiation cellulaire. L'absence d'une relation entre les résidus du début et de la fin de la xylogenèse ($r_p=0.06$) a indiqué que la production de cellules influence la corrélation entre les deux phénophases du xylème.

Ces résultats ont démontré qu'un nombre plus élevé de cellules produites retarde la fin de la maturation du xylème, et ainsi prolonge la durée de la formation du bois.

**Mots-clés:** activité cambiale, modélisation causale, production de cellules, environnements froids, durée de la différentiation cellulaire, fin de la croissance, *Picea mariana*, xylogenèse, formation du bois

## 2.3 Introduction

The length of the growing season is one of the main determinants of tree production in all forest ecosystems of the world (Nemani *et al.* 2003; Boisvenue and Running 2006). Although the role of cambial reactivation in controlling the quantity and quality of wood is well known (Begum *et al.* 2007; Rossi *et al.* 2008a), the relationship between the amount and duration of cell production in the xylem remains an intriguing but unresolved issue. Delays in the start of the growing season reduce the period available for growth, so significantly affecting tree ring width and, consequently, radial growth (Vaganov *et al.* 1999; Deslauriers *et al.* 2003). Recent studies suggested that a higher number of developing tracheids could prolong cell differentiation and lengthen the growing season (Gričar *et al.* 2005; Thibeault-Martel *et al.* 2008). Given the ecological and economic importance of wood production, determining the causal relationships between timing, duration and formation of the xylem is crucial to understanding the complex dynamics of tree growth.

Recently, several studies underlined the importance of temperature in both cambial reactivation and cell production and pointed out the existence of a temperature threshold above which growth occurred (Oribe *et al.* 2001; Begum *et al.* 2007, 2010; Deslauriers *et al.* 2008; Rossi *et al.* 2008b). According to Deslauriers and Morin (2005), daily minimum temperatures affected the rate of tracheid production in *Abies balsamea* (L.) Mill., while Rossi *et al.* (2006a) observed that, in conifers of cold climates, maximum growth rate was reached at the summer solstice, with cambium concluding its activity in mid-summer, when temperatures are still favorable for growth. Different physiological processes may act at the beginning and ending of growth (Thibeault-Martel *et al.* 2008), with the different phases of cell division and differentiation being independent of one another (Deslauriers *et al.* 2003). The closing stages of xylem growth are related to the ending of cell division in cambium and can last up to 40 days for a latewood cell, involving several interconnected metabolic activities to accomplish maturation of the secondary wall (Rossi *et al.*

2006b). However, neither the physiological nor environmental factors affecting the cessation of growth are well understood.

Some studies have proposed that external factors, like photoperiod or water availability, or internal factors, like hormonal signals and vigour, may be more important than temperature for growth cessation (Schmitt *et al.* 2003; Schmitt *et al.* 2004; Gričar and Čufar 2008). In an experiment with *Pinus sylvestris* L. saplings from different regions, cessation of shoot and needle elongation and diameter growth was related to cold hardening and tended to follow the latitude of origin (Repo *et al.* 2000). Repo *et al.* (2000) also attributed the delayed hardening of stems, compared with needles, to the longer period of growth in stems. At the alpine timberline, the shorter periods of cell production, due to later cambial reactivations and cell enlargement, corresponded to narrow tree rings observed in the older trees (Rossi *et al.* 2008a). Similarly, less vigorous *Abies alba* Mill. trees produced fewer cells along the tree rings and had reduced duration of wood formation with delayed onset and earlier cessation of cambial activity (Schmitt *et al.* 2003). Gričar *et al.* (2005) observed that lignification ended earlier in trees with narrower rings. Instead, other authors observed delays in the onset of cambial activity associated with similar endings of cell differentiation along altitudinal gradients, despite decreases in wood production with altitude (Tranquillini 1979; Moser *et al.* 2010). Thus, the way in which the timings and duration of xylem growth are connected with wood production is still a debated subject because the available knowledge does not allow the causality relationship between these growth variables to be clarified.

The aim of this study was to resolve the chicken-or-egg causality dilemma about duration of growth and cell production by testing two different hypotheses through simple- and double-cause models. It was achieved by (i) analyzing the intra-annual growth dynamics of the xylem in *Picea mariana* (Mill.) BSP (Figure 2.1a-b) in two contrasting sites of the boreal forest of Quebec and (ii) extracting the dates of onset and ending of xylem formation and the number of radial cells along the tree ring.

## 2.4 Material and methods

### 2.4.1 Study site

The study took place in the boreal forest of Quebec, Canada, in two mature even-aged *P. mariana* (black spruce) stands at different altitudes. The first site (SIM) is located in the Laurentides Wildlife Reserve, within the Simoncouche research station (48°13' N, 71°15' W, 350 m a.s.l.). The second site (BER) is located at a higher altitude, near lac Bernatchez, in the Monts-Valin (48°51' N, 70°20' W, 611 m a.s.l.). The region is included in the balsam fir-white birch ecological domain (Saucier *et al.* 1998), with an understory vegetation mainly composed of *Kalmia angustifolia* L., *Ledum groenlandicum* Oeder, *Cornus Canadensis* L., *Vaccinium myrtilloides* Michx., and soil vegetation of *Sphagnum* spp. and mosses (*Hylocomium splendens* (Hedw.), *Pleurozium schreberi (*Brid.), *Ptilium crista-castrensis* (Hedw.) De Not.). SIM derived from a forest fire in 1922, while in BER, the forest fire at the origin of the stand has been estimated to have occurred between 1865 and 1870. The stands are growing on gentle slopes (8-17%) and drain glacial tills.

### 2.4.2 Meteorological data

In each site, a standard weather station was installed in a forest gap to measure air and soil temperature. The soil temperature was measured both on the mineral and organic layers, at 20-30 and 5-10 cm depth, respectively. Measurements were taken every 15 minutes and data were stored as hourly averages in CR10X dataloggers (Campbell Scientific Corporation, Utah, USA). The rainfall sum and snow cover height were measured hourly and daily values were calculated.

### 2.4.3 Sample collection and preparation

In each site, three dominant trees with upright stem and similar growth patterns were selected in 2006 and 2008. The homogeneity in growth rates was assessed during a preliminary investigation by extracting wood cores and counting the number of tracheids in three previous tree rings (Rossi *et al.* 2007).

During 2006-2009, wood microcores (2.5 mm in diameter and 25 mm long) were collected weekly from the stem from April to October with a Trephor (Rossi *et al.* 2006c) following a counter-clockwise elevating spiral centered at breast height. In 2006-2007 in BER, samples were collected weekly in early summer (May-June) and every 2 weeks during July-October. Microcores usually contained the previous 5-10 tree rings and the developing annual layer with the cambial zone and adjacent phloem tissues. Wood samples were always taken at 5-10 cm intervals to avoid the formation of resin ducts as reaction to disturbance (Deslauriers *et al.* 2003). The microcores were placed in Eppendorf microtubes containing a water:ethanol solution (1:1). Microcores were oriented by marking the transversal side with a pencil, dehydrated through successive immersions in ethanol and Histosol™ and embedded in paraffin (Rossi *et al.* 2006c). Transverse sections 6-10 μm in thickness were cut with a rotary microtome, stained with cresyl violet acetate (0.16% in water) and observed within 20-30 minutes under visible and polarized light at a magnification of 400-500× to differentiate cambium and developing xylem cells. The cambial zone and cells in radial enlargement showed only a primary wall, which, unlike the secondary wall, did not shine under polarized light (Gričar *et al.* 2006). Cambial cells were characterized by thin cell walls and small radial diameters, while enlarging cells had a radial diameter at least twice that of a cambial cell (Figure 2.1c). Cells in wall thickening shone under polarized light and, during the maturation process, showed a coloration varying from light to deep violet (Figure 2.1d-e). As lignification advanced, a blue coloration starting from the cell corners spread into the secondary walls. Since lignin deposition may persist after the end of cell wall thickening (Gindl *et al.* 2000), cells were considered mature when the violet coloration was completely replaced by the blue coloration (Rossi *et al.* 2006c). The number of cells in each phase was counted along three radial rows and the total number of xylem cells was calculated as the sum of cells in radial enlargement and wall thickening and lignification and mature cells. In spring, xylem formation was considered to have begun when the average number of cells in enlarging phase between the three radial rows was higher than one. In late summer, when no further cells were observed undergoing wall thickening and lignification, xylem formation was considered complete.

The phenology of xylem development was assessed for each tree. Four phenophases, computed in days of the year (DOY), were considered, including onset and ending of cell enlargement, wall thickening and lignification. Duration of xylem formation was calculated as the difference between the onset of cell enlargement and the ending of cell wall thickening and lignification.

### 2.4.4 Statistical Analyses

To calculate cell production, for each tree the total number of cells counted on each sampling date was fitted with a Gompertz function using the nonlinear regression (NLIN) procedure with the Marquardt iterative method in the SAS statistical package (SAS Institute, Cary, NC, USA). The Gompertz function was defined as:

$$y = A\exp(-e^{\beta-\kappa t})$$

where $y$ is the number of cells, $t$ is time computed in days of the year (DOY), $A$ is the upper asymptote of the total number of xylem cells, $\beta$ is the $x$-axis placement parameter, and $\kappa$ is the rate of change parameter (Deslauriers *et al.* 2008). The asymptote represented the number of radial cells produced by a tree during the growing season.

Onset, duration and ending of xylogenesis and cell production were compared between sites and years using analysis of variances (ANOVA ) and Welch's test after having examined the normality of distributions and uniformity of variances (Quinn and Keough 2002; Ruxton 2006).

### 2.4.5 Causal models

Based on two initial hypotheses defining the causality links between variables, two causal models were applied to assess the relationships between onset and ending of xylogenesis and cell production. Hypothesis 1 was that onset and end of xylogenesis (i.e. the duration) affect cell production. Hypothesis 2 was that the number of cells produced during growth affect the duration of xylogenesis. The general forms of the two models of causal relationship were defined as:

Model 1

$$\begin{tikzcd} V_1 \arrow[r] & V_2 \arrow[r] & V_3 \end{tikzcd}$$

Model 2

$$\begin{tikzcd} V_1 \arrow[r] \arrow[dr] & V_2 \arrow[d] \\ & V_3 \end{tikzcd}$$

where $v$ were the independent measured variables given in Table 2.1, and the arrows described the interactions between $v_1$ and $v_3$ in the presence of possible interactions caused by $v_2$ (Legendre and Legendre 1998). In Model 1, $v_1$ influenced $v_2$ which, in turn, influenced $v_3$, but no causal relationship existed between $v_1$ and $v_3$ (single cause). In Model 2, $v_1$ affected the variable $v_2$ and both influenced $v_3$ (double cause).

Simple and partial correlations were performed between the three variables of the two models to verify each hypothesis. A partial correlation coefficient (e.g. $r_{v1v3.v2}$) measured the connection between two variables (e.g. $v_1$ and $v_3$), controlling for the effect of a third (e.g. $v_2$) being constant at its mean. According to Legendre and Legendre (1998), both the assumptions for model applicability and the expectations, described in Table 2.2, had to be respected for accepting a model. If the assumptions for model applicability were not met, neither model could be formulated because of missing causal links. Before performing the analyses, linearity of the correlations was verified visually and by analyzing the distribution of residuals.

## 2.5 Results

### 2.5.1 Meteorological patterns at the two sites

The climate is continental with long cold winters and short warm summers. During 2006-2009 mean annual temperature was 0.5 °C at BER and 2.2 °C at SIM, with absolute maximum temperatures reaching 30 °C and 33 °C, respectively. During winter, the absolute minimum temperatures were between -33 and -40 °C, with the lowest values in 2009 (Table 2.3). During the four years of study, the May-September temperature in BER was, on average, almost two degrees lower than in SIM (11.4 vs. 13.1 °C) (Table 2.3). In May, the monthly mean temperature was 5.2 °C in BER and 7.7 °C in SIM. Above-zero temperatures were reached at the end of May in BER, two weeks later than in SIM, with the larger temperature differences between sites observed in late spring and late summer (Figure 2.2). From June to August, mean daily temperatures were always above 5 °C at both sites, even if, in 2008 and 2009, early June was colder than in 2006 and 2007 (Figure 2.2). The temperatures of the organic and mineral layers were lower at BER, with the daily differences between sites varying during June-August between 0.2-6.3 and 1.3-3.7 degrees, respectively, with a different pattern between years. In general, winter temperatures of the organic soil were around 0 °C and, after snow melted, increased within one week in 2006 and 2008, while remaining stable for about two weeks in 2007 and 2009, because of the low air temperatures in early May (Figure 2.2). The colder site received higher precipitations during summer (Table 2.3). Although rainfall in May was relatively low (on average 55.4 mm in BER and 58.1 mm in SIM), there was an important water input from snowmelt. Snow depth varied between 0.9 and 1.6 m, with 2007 and 2009 being the years with less (Table 2.3). The date of snowmelt varied between years, but on average it disappeared 17 days later in BER (Table 2.3).

### 2.5.2 Cambial activity

The number of cambial cells varied between 3 and 5 in spring and autumn, reaching a maximum of 7-12 between June and July (Figure 2.3). On average, cambium was active between mid-May and the end of August. In BER, the number of cells in the cambial zone began to increase 1-2 weeks later than in SIM, while, in summer, the decrease was synchronous and the cambial cells attained their initial values during the same periods (Figure 2.3).

### 2.5.3 Cell differentiation

The dynamics of the cells in enlargement and in wall thickening and lignification showed similar patterns with evident bell-shaped curves (Figure 2.3). Both increased in late spring, after cambial reactivation, reaching a maximum and then decreasing to zero in late summer. The mature cells showed an S-shaped curve, with major increment rates observed in July (Figure 2.3). The onset of cell enlargement occurred between DOY 143 and 171 (Table 2.4) and was significantly different between sites (ANOVA, $F=13.71$, $p<0.01$) and years (ANOVA, $F=8.06$, $p<0.01$). The onset of cell enlargement occurred 7.3 days earlier in SIM on average, while 2009 was the year with the latest resumption of cell differentiation. Different onsets of cell wall thickening and lignification and mature cells were observed between sites, except in 2007 when these phases began during the same periods. Lignification was complete between DOY 243 and 272 and no significant difference was observed between sites (ANOVA $F=0.07$, $p>0.05$) and years (ANOVA $F=2.19$, $p>0.05$). Overall, xylogenesis lasted 77-126 days, and was significantly different between years (ANOVA $F=3.76$, $p<0.05$) but not between sites (ANOVA $F=2.58$, $p>0.05$). The shortest and longest duration of cell differentiation occurred in 2009 and 2007 (on average 94.3 and 117.7 days), respectively (Table 2.4). The time required for the first cell in enlargement to begin wall thickening significantly differed between years (ANOVA $F=4.50$, $p<0.05$) and varied on average between 11.5 and 17.0 days, in 2009 and 2008, respectively.

The time spent in wall thickening and lignification increased through the growing season, reaching 24 and 70 days, for the first and last cell of the xylem, respectively.

Curve fitting of the Gompertz was good, with 0.68 to 0.97 variance explained. In general, a better fitting was observed in BER than in SIM, with average $R^2$ of 0.89 and 0.84, respectively, because of the higher variability between the measured trees in SIM. The Gompertz function usually fitted the data well at the start of the growing period, and was always able to reach an asymptote by the end.

On average, trees in SIM produced more cells along the tree ring, with the highest and lowest values of 34 and 25 cells estimated in 2007 and in 2009, respectively. In SIM, a higher variability was observed between trees in the number of cells produced, especially in 2008-2009, as one tree produced fewer cells than those in BER. This affected the results of Welch's test that showed no significant difference in the number of cells between the sites (Welch's test, F=4.14, $p>0.05$) and years (Welch's test, F=1.20, $p>0.05$). However, the causal modeling analyses, which were performed by considering all individuals pooled together, were not affected.

### 2.5.4 Causal modeling

A negative correlation between the onset of xylogenesis and the total number of xylem cells in the ring was found, while the total number of xylem cells–was positively correlated with the ending of xylogenesis (Figure 2.4). Thus, a higher number of cells was linked to an earlier onset and a later ending of cell differentiation. In accordance with the absolute value of the coefficient of correlation, the number of cells was more correlated with the onset ($r=0.74$) than the ending ($r=0.61$) of xylogenesis. The non-significant relationship between the residuals of the onset and ending of xylogenesis ($r_p=-0.06$, $p>0.05$) indicated that cell production influenced the correlation between the two phenophases of the xylem (Figure 2.4). Nevertheless, a significant partial correlation between the onset of xylogenesis and number of cells was observed ($r_p=-0.64$, $p<0.01$). So, even holding the ending of xylogenesis constant, the influence of the timing of its onset on cell production emerged (Figure 2.4).

The assumptions for model applicability were met for both hypotheses and the model could be evaluated by means of the expectations, by analyzing the results of the simple and partial correlations, reported in Figure 2.4. For Hypothesis 1, one of the expectations for both models was not respected, because the partial correlation between the onset and ending of xylogenesis was not significantly different from zero. Both models were therefore rejected for Hypothesis1. Although the expectations for both models were respected for Hypothesis 2, only Model 1 was accepted, according to the specific expectations of each model (Table 2.5). Model 2 was rejected because expectations were not met, since the partial correlation between the onset and ending of xylogenesis was not significantly different from zero ($r_p$=-0.06, p>0.05). Therefore, the onset of cell differentiation influenced the number of cells, which in its turn, influenced the end of cell differentiation, but there was a lack of causal relationship between onset and ending of xylogenesis.

## 2.6 Discussion

In this study, two different hypotheses linking the duration of growth with cell production were tested on *P. mariana* by simple and double causal models. Results clearly showed that only the simple cause for one hypothesis could be accepted, with the onset of xylogenesis influencing the number of cells produced by the cambium which, in turn, influenced the ending of cell differentiation. In addition, no causal relationship was observed between the onset and ending of xylogenesis. These findings allow the chicken-or-egg dilemma to be resolved on which is the cause and the effect between duration of growth and cell production in trees. The higher xylem production observed in younger trees (Rossi *et al.* 2008a) or at lower altitudes and latitudes (Moser *et al.* 2010; Rossi *et al.* 2011) delays the period required to complete the maturation of xylem, so extending the wood formation period. This longer time window for xylogenesis is considered to lead to a dilution of the climatic signal during tree-ring formation and to the reduced climate-growth relationships as observed in the younger trees having a longer growing period (Carrer and Urbinati 2004).

Cambial reactivation, and consequently the onset of cell differentiation, is strongly influenced by the temperatures in late spring, as also demonstrated by Gričar *et al.* (2007). Although the reaching of a certain threshold is necessary for cell formation to occur (Rossi *et al.* 2007, 2008b; Deslauriers *et al.* 2008), other factors may contribute to define the onset of xylem growth. In our study, the delay in the timing of snowmelt between the two sites was reflected in the difference in the onset of cell differentiation, confirming the deductions of Kirdyanov *et al.* (2003), who stated that snow melting and soil thawing must begin before growth can start. Moreover, Turcotte *et al.* (2009) observed that stems of *P. mariana* were completely rehydrated before the onset of growth. In addition, with its insulating property, snow plays an important role by maintaining the soil at a constant temperature around 0 °C, which is below the minimum threshold for growth (Decker *et al.* 2003; Körner and Paulsen 2004; Alvarez-Uria and Körner 2007; Shi *et al.* 2008). Even if needles in evergreen conifers can be temporarily active during the warmer days of early spring and produce photoassimilates, there is evidence that low temperatures preclude the production of new cells by meristematic tissues (e.g. the cambium, Alvarez-Uria and Körner 2007, Shi *et al.* 2008).

In some ecosystems, precipitation is also an important factor in controlling the length of the growing season. In drought-subjected areas, dry periods were associated to narrow tree rings and earlier conclusions of growth (Giovannelli *et al.* 2007; Gričar and Čufar 2008). By affecting wood production, water stress can thus indirectly determine growth cessation (Antonova and Stasova 1993). However, in the boreal forest of Quebec, there is no evidence that precipitation could be a limiting factor for growth of *P. mariana* (Krause *et al.* 2010). The spring snowmelt and the regularly distributed precipitations during summer (on average, 414.9 mm of rain and 95 rainy days during May-September for the four study years) seem to guarantee the water requirements of trees regardless of the reduced water retention capacity of the soils.

The causal relationship between cell production and the timings of growth cessation found in this work allowed clarification of some mechanisms of tree-ring formation and the observations that younger, vigorous, or fertilized trees produced more cells and showed later cessation of growth (Peltola *et al.* 2002; Deslauriers *et al.* 2003; Schmitt *et al.* 2003). In boreal and temperate forests, temperature mainly affects cell production in spring and early summer, during the first part of the growing season. Moreover, according to our findings, cell production may affect the ending of xylogenesis, thus potentially establishing an indirect relationship with temperature. In fact, cooling treatment of the stems affected xylem development by reducing the width of tree rings and shortening the period of cambial activity (Gričar *et al.* 2007). In addition, Thibeault-Martel *et al.* (2008) observed larger tree rings in roots than in the stem, and, consequently, later endings of cell wall lignification. So, a higher number of cells produced leads to larger accumulations of cells in differentiation in the developing xylem. In latewood of conifers, although cell enlargement, when occurring, is a very rapid phase that is completed within a few days, wall thickening and lignification is an expensive and long-lasting deposition of cellulose and lignin within the cell wall, requiring up to 40 days (Deslauriers *et al.* 2003). In spite of the continuous requirement of energy to accomplish radial growth, cambium is one of the sinks with the lowest priority, being preceded by respiration and root growth and storage (Oribe *et al.* 2003; Polák *et al.* 2006; Deslauriers *et al.* 2009). The delays in completing cell maturation caused by a higher number of cells produced clearly demonstrated the presence of constraints to sink activity for secondary growth, namely cell differentiation, in xylem.

Our findings may also supply new insight on the development of the hydraulic architecture of trees. Indeed, the additional cells produced during an earlier beginning of the growing period belong to earlywood, which is characterized by larger diameters and higher hydraulic efficiency than latewood (Domec and Gartner 2002, Deslauriers *et al.* 2008). A warmer spring may induce an earlier onset of xylogenesis and result in larger earlywood cells (Deslauriers *et al.* 2008). In fact, spring conditions determine cell size of earlywood elements influencing the rate of cell division and differentiation (Fonti *et al.* 2010). Consequently, a hypothesis is proposed that, during the more thermally-favourable years, trees growing in cold climates could benefit of proportionally more xylem conduits, which could allow a more efficient water transport within the plant and a higher height growth.

In the cold forest ecosystems, climate warming will undoubtedly widen the thermally favourable period available to the trees for growth, with the more pronounced effects being observed in the boreal zones of the planet (Menzel 2000; Chmielewski and Rötzer 2001). For eastern Canada, predictions estimate a raised mean annual temperature of 3 °C for the year 2050 with a 5 - 20% increase in precipitation (Plummer *et al.* 2006). Kilpeläinen *et al.* (2007) concluded that, since air temperature influences tree phenology and diameter growth, climate warming is likely to affect both development and growth of the boreal forest. Based on a temperature-driven phenological model, Rossi *et al.* (2011) calculated increases of 8-11 days/°C in the period of xylogenesis, but no consequence on wood production could be inferred by the study. According to our results, it is possible to infer that a warmer spring will lead to earlier cambial reactivation, thus increasing cell production and delaying cell maturation in autumn. Consequently, our findings suggest that a future climate warming will indirectly affect xylem phenology acting on the production of cells.

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Table 2.1 Variables used in the models of causal relationship for testing the two hypotheses

| | $v_1$ | $v_2$ | $v_3$ |
|---|---|---|---|
| Hypothesis 1 | Onset of xylogenesis | Ending of xylogenesis | Number of cells |
| Hypothesis 2 | Onset of xylogenesis | Number of cells | Ending of xylogenesis |

Table 2.2 Assumptions and expectations in terms of simple and partial correlation coefficients for causal models involving the variables $v_1$, $v_2$ and $v_3$

| Assumptions for model applicability | Expectations | | |
|---|---|---|---|
| | Both Models | Model 1 | Model 2 |
| $r_{v1v2}$ signif. $\neq 0$ | $r_{v1v2.v3}$ signif. $\neq 0$ | $\lvert r_{v1v2} \rvert \geq \lvert r_{v1v3} \rvert$ | $r_{v1v3}$ signif.$\neq 0$* |
| $r_{v2v3}$ signif. $\neq 0$* | $r_{v2v3.v1}$ signif. $\neq 0$ | $\lvert r_{v2v3} \rvert \geq \lvert r_{v1v3} \rvert$ | $r_{v1v3.v2}$ signif.$\neq 0$ |
| | | $r_{v1v3.v2}$ not signif.** | |
| | | $\lvert r_{v1v2.v3} \rvert \leq \lvert r_{v1v2} \rvert$ | |
| | | $\lvert r_{v2v3.v1} \rvert \leq \lvert r_{v2v3} \rvert$ | |
| | | $r_{v1v2} \times r_{v2v3} \approx r_{v1v3}$ | |

* For model 2, model holds even if *only one* of these two simple correlations is not significant.

**' The correlation is not necessarily significantly different from zero.

Table 2.3 Meteorological parameters at the two sites (BER and SIM) during 2006-2009

| Year | Air temperatures (°C) | | | | | | May-September air temperature (°C) | | May-September precipitation (mm) | | Maximum snow cover (m) | | Day of complete snowmelt (DOY) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Annual mean | | Absolute maximum | | Absolute minimum | | | | | | | | | |
| | BER | SIM | BER | SIM | BER | SIM | BER | SIM | BER | SIM | BER | SIM | BER | SIM |
| 2006 | 1.21 | 3.27 | 29.8 | 32.7 | -35.3 | -32.9 | 11.5 | 13.4 | 522.0 | 372.5 | 1.6 | 1.2 | 137 | 119 |
| 2007 | 0.38 | 1.75 | 30.4 | 31.4 | -34.3 | -32.6 | 11.5 | 13.3 | 373.2 | 371.8 | 1.1 | 1.0 | 131 | 116 |
| 2008 | 0.23 | 1.99 | 27.9 | 29.3 | -37.6 | -34.1 | 11.3 | 13.0 | 440.6 | 369.0 | 1.5 | 1.4 | 140 | 124 |
| 2009 | 0.07 | 1.69 | 27.7 | 30.7 | -39.8 | -39.7 | 11.3 | 12.8 | 478.3 | 391.8 | 1.1 | 0.9 | 135 | 114 |

Table 2.4 Timings of xylogenesis and total number of cells produced during 2006-2009 in the two study sites. Values are reported as mean ± standard deviation between three trees

| | Xylogenesis | | | | | | Number of cells | |
|---|---|---|---|---|---|---|---|---|
| year | Onset (DOY) | | Ending (DOY) | | Duration (days) | | | |
| | BER | SIM | BER | SIM | BER | SIM | BER | SIM |
| 2006 | 152.0±2.6 | 145.7±3.1 | 254.7±8.5 | 256.3±9.3 | 102.7±9.3 | 110.7±8.0 | 22.3±3.3 | 25.9±7.9 |
| 2007 | 149.3±4.0 | 147.3±1.5 | 246.3±12.4 | 267.7±3.1 | 115.0±15.7 | 120.3±2.1 | 26.2±5.6 | 34.0±7.4 |
| 2008 | 159.7±3.1 | 149.3±6.5 | 255.0±8.7 | 255.0±10.1 | 95.3±11.4 | 105.7±16.6 | 18.6±5.8 | 28.9±17.2 |
| 2009 | 165.3±4.9 | 155.0±8.5 | 255.0±8.2 | 254.0±9.8 | 89.7±12.5 | 99.0±17.3 | 18.1±4.5 | 24.8±12.6 |

Table 2.5 Outcomes for the two models and hypotheses

| | Assumptions for model applicability | Expectations | | |
|---|---|---|---|---|
| | | Both Models | Model 1 | Model 2 |
| Hypothesis 1 | yes | no | - | - |
| Hypothesis 2 | yes | yes | accepted | rejected |

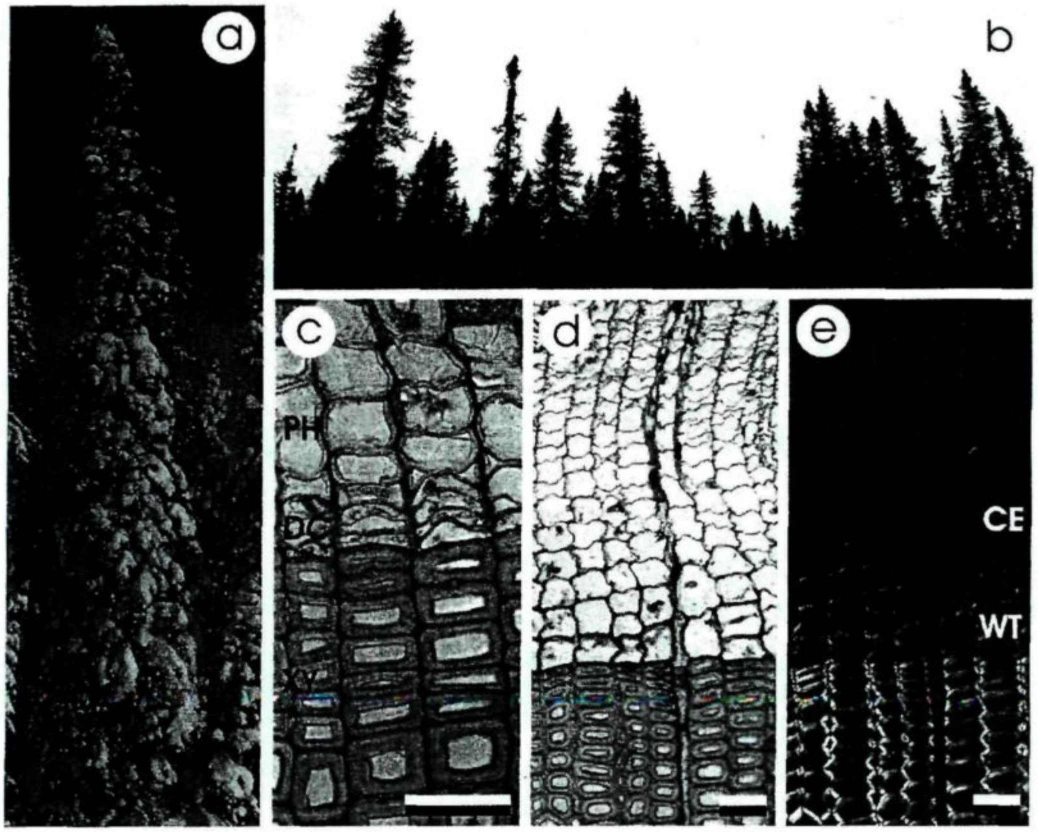

Figure 2.1 Winter and summer appearance of *Picea mariana* in the balsam fir-white birch ecological domain of Quebec, Canada (a, b). Cross section of the outermost xylem (XY) showing dormant cambium (DC) and phloem (PH) collected at the beginning of April (c). Developing xylem collected in June, representing the maximum growth period, with cells in enlargement (CE) and in wall thickening (WT) observed under visible (d) and polarized (e) light. Scale bars = 20 μm

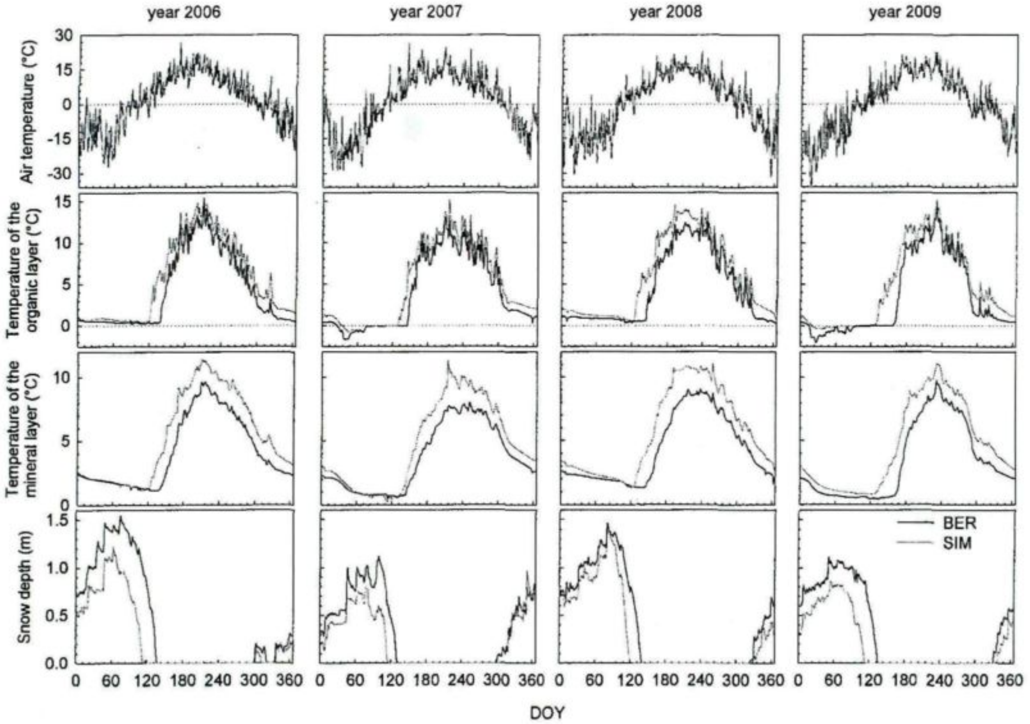

Figure 2.2 Daily patterns of air and soil temperature and snow depth in the two study sites during 2006-2009. X-axis unit is day of the year (DOY).

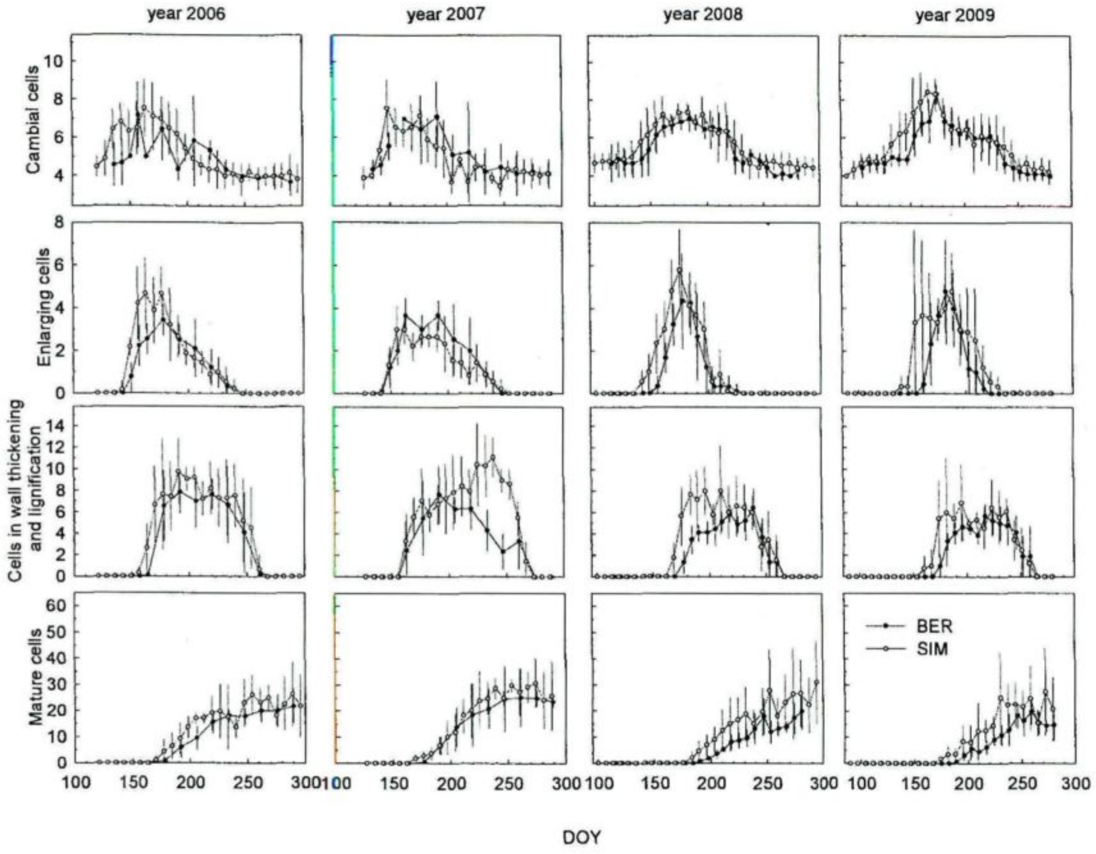

Figure 2.3 Cambial, enlarging, wall thickening and lignification and mature cells in the two study sites during 2006-2009. Vertical bars correspond to the standard deviation between three trees

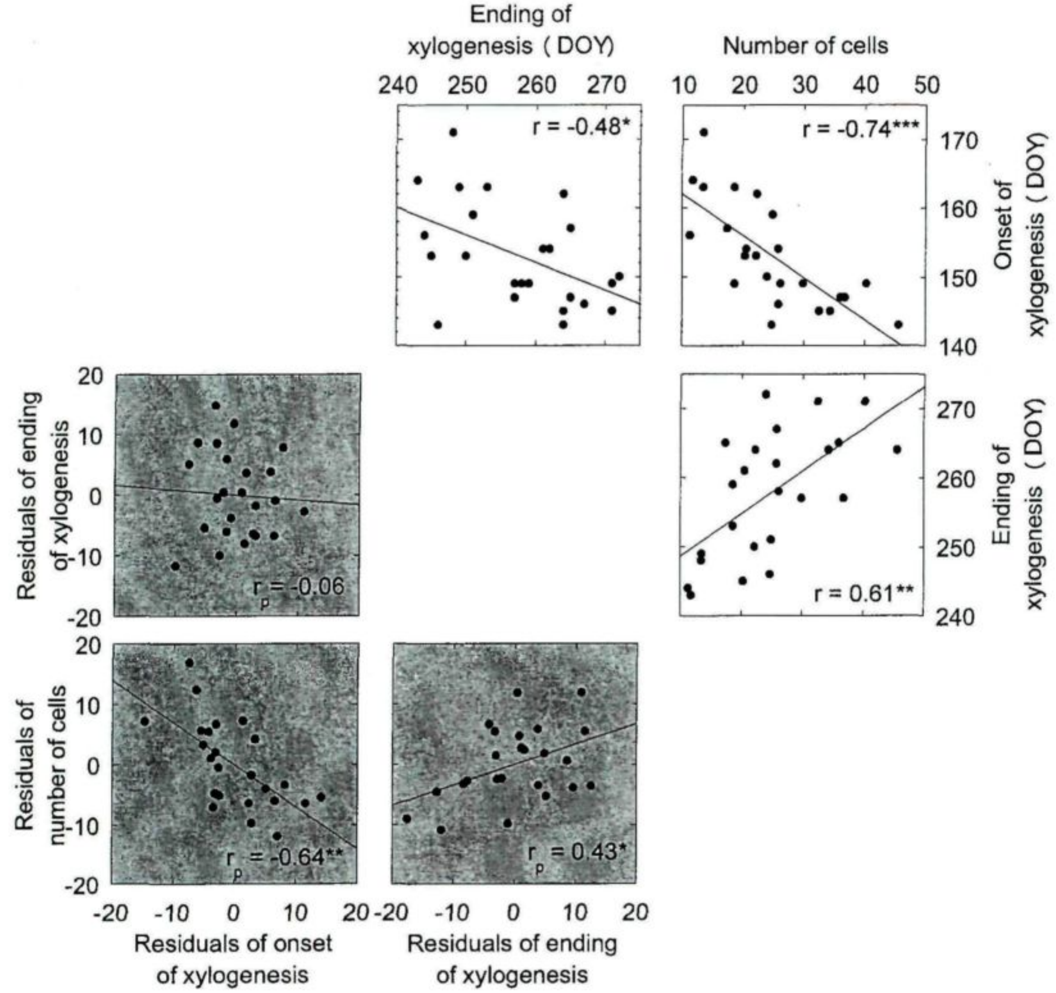

Figure 2.4 Simple (r, white background) and partial ($r_p$, gray background) correlations between onset and ending of xylogenesis and the total number of cells produced. One, two and three asterisks correspond to a probability lower than 0.05, 0.01 and 0.001, respectively

# Chapitre III

## Xylogenesis in black spruce: does soil temperature matter?

**Title:** Xylogenesis in black spruce: does soil temperature matter?

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**Keywords**: boreal forest, cambium, climate change, intra-annual growth, microcores, soil warming, soil temperature, wood production, xylem phenology

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### 3.1 Summary

In boreal ecosystems, an increase in soil temperature can stimulate radial growth and cambial phenology. However, cambium phenology in trees was better explained by air than soil temperature, which suggested that soil temperature is not the main limiting factor affecting xylogenesis. Since soil temperature and snowmelt are correlated to air temperature, the question whether soil temperature directly limits xylogenesis in the stem will remain unresolved without experiments disentangling air and soil temperatures. This study investigated the effects of an increase of 4 °C in soil temperature and a consequent one-week earlier snowmelt on growth of black spruce [*Picea mariana* (Mill.) BSP] in the boreal forest of Quebec, Canada. The soil of two natural stands at different altitudes was warmed up with heating cables during 2008-2010 and cambial phenology and xylem production were monitored weekly from April to October. The results showed no significant effect of the treatment on the phenological phases of cell enlargement and wall thickening and lignification. The number of cells produced in the xylem also did not differ between control and heated trees. These findings allowed the hypothesis of a direct influence of soil temperature on stem growth to be rejected and supported the evidence that, in the short term, air temperature is the main limiting factor for xylogenesis in trees of these environments.

**Keywords**: boreal forest, cambium, climate change, intra-annual growth, microcores, soil warming, soil temperature, wood production, xylem phenology

## 3.2 Résumé

Dans les écosystèmes boréaux, une augmentation de la température du sol peut stimuler la croissance radiale et la phénologie du cambium. Toutefois, la phénologie du cambium dans les arbres est mieux expliquée par la température de l'air plutôt que du sol, ce qui suggère que la température du sol n'est pas le principal facteur limitant qui influence la xylogenèse. Étant donné que la température du sol et la fonte de la neige sont corrélées à la température de l'air, la question de savoir si la température du sol limite directement la xylogenèse dans le tronc restera non résolue sans expériences qui démêlent la température de l'air et du sol. Cette étude examine les effets d'une augmentation de la température du sol de 4°C et d'une conséquente fonte de la neige d'une semaine plus hâtive sur la croissance de l'épinette noire [*Picea mariana* (Mill. BSP] dans la forêt boréale du Québec, Canada. Le sol de deux peuplements naturels situés à différente altitude a été réchauffé avec des câbles chauffants pendant la période 2008-2010 et la phénologie cambiale et la production du xylème ont été surveillées de façon hebdomadaire d'avril à octobre. Les résultats n'ont montré aucun effet significatif du traitement sur les phases phénologiques de l'élargissement cellulaire et de l'épaississement des parois secondaires et de la lignification. Le nombre des cellules produites dans le xylème ne différait pas entre les arbres témoins et les arbres chauffés. Ces constats ont permis de rejeter l'hypothèse d'une influence directe de la température du sol sur la croissance dans la tige et de fournir des preuves indirectes que, à court terme, la température de l'air est le principal facteur limitant la xylogenèse dans les arbres de ces environnements.

**Mots-clés:** forêt boréale, cambium, changement climatique, croissance intra-annuelle, microcarottes, réchauffement du sol, température du sol, production du bois, phénologie du xylème

## 3.3 Introduction

The boreal forest is the biome with a greater expected increase in surface temperature than that of any other forest ecosystem in the world (Strömgren and Linder 2002, Bronson and Gower 2010). Recent forecasts for eastern Canada estimate increases of 3 °C in mean annual temperature and of 5-20% in annual rainfall for the year 2050 (Plummer *et al.* 2006). In this biome, plant growth is limited by low temperatures and, indirectly, by availability of nutrients, especially nitrogen, which is expected to increase with climate warming according to the change in the rate of mineralization of the soil (Rustad *et al.* 2001, Strömgren and Linder 2002, Campbell *et al.* 2009). Some experiments conducted in boreal ecosystems have observed that soil temperature can affect plant growth (Van Cleve *et al.* 1990, Strömgren and Linder 2002, Jyske *et al.* 2011). Needles of black spruce growing on heated soils contained greater amounts of N, P and K and showed 20% higher photosynthetic rates (Van Cleve *et al.* 1990). Strömgren and Linder (2002) reported increases in stem production attaining 115% in irrigated and heated plots of Norway spruce. The understanding of current and future growth dynamics of forest ecosystems requires a clear assessment of the mechanism of action of climate on growth, including cambial activity and phenology. However, the direct and indirect role of temperature in growth still remains uncertain (Vaganov *et al.* 1999, Jarvis and Linder 2000, Rossi *et al.* 2011).

The key role of temperature in cambial reactivation and cell production has recently been demonstrated (Oribe *et al.* 2001, Begum *et al.* 2007, Deslauriers *et al.* 2008, Rossi *et al.* 2008) and may be explained by the evidence that a soil temperature below 6 °C strongly inhibits root activity and water uptake in various conifers (Alvarez-Uria and Körner 2007). Also, observations carried out at the northern treeline showed no cambial activity when soil temperature was below 3-5 °C (Körner 2003). Since cambium seems to have synchronous activity in stem and roots (Thibeault-Martel *et al.* 2008), and root growth is limited by low temperatures (Alvarez-Uria and Körner 2007), xylogenesis in the stem could be assumed to begin after soil starts to warm up (Rossi *et al.* 2007, Turcotte *et al.* 2009, Lupi *et al.* 2010). Nevertheless, Rossi *et al.* (2007) and Swidrak *et al.* (2011) found that the onset of wood formation could be better explained by air than soil temperature and concluded that soil temperature was not the main limiting factor affecting xylogenesis in the stem. When air temperature increases, soil temperature increases accordingly. Thus, only manipulative experiments addressing separately the effect of air and soil temperatures can resolve the question whether soil temperature directly limits xylogenesis in the stem (Vogel *et al.* 2008).

Because of the well-known insulating effect of snow, soil maintains a temperature close to 0 °C until complete snowmelt, which is below the minimum threshold for growth (Decker *et al.* 2003, Körner 2003). Vaganov *et al.* (1999) suggested that the later snowmelts occurring as a result of the increased snowfalls of the last century in subarctic Eurasia delayed the onset of xylogenesis and reduced tree growth. Indeed, it has been observed that the resumption of radial growth follows, not precedes, soil thaw and snowmelt (Graumlich and Brubacker 1986, Cairns and Malanson 1998, Turcotte *et al.* 2009, Rossi *et al.* 2011). According to Jarvis and Linder (2000), neither cambial division nor uptake of nutrients and $CO_2$ can occur while the soil is frozen, thus the length of the snow-free period should influence tree growth and C-assimilation by affecting photosynthesis in spring and nutrient cycling and availability in summer.

Even if the effect of soil temperatures and timings of snowmelt on tree growth is considered obvious, there is a need to ultimately rule out whether the relationship is direct or derives from the fact that soil temperatures and snowmelt are correlated to air temperature. This study investigated the effects of an increase in soil temperature and the consequent earlier snowmelt on cambial phenology by warming the soil of natural stands with heating cables during 2008-2010 in two sites of the boreal forest of Quebec, Canada. The treatment produced earlier snowmelts and longer snow free periods in accordance with the increase in winter temperature predicted for the eastern Canada (Plummer *et al.* 2006). Many studies underline the importance of soil temperature for cambial reactivation and wood production (Vaganov *et al.* 1999, Körner 2003, Alvarez-Uria and Körner 2007), thus the hypothesis was tested that increased soil temperatures and earlier snowmelts produce earlier onsets of growth and greater amount of xylem.

## 3.4 Materials and Methods

### 3.4.1 Study sites and tree selection

The study took place in the boreal forest of Quebec, Canada, in two mature and even-aged black spruce [*Picea mariana* (Mill.) BSP] stands at different altitudes. The first site (BER) was located near Lake Bernatchez, in the Monts-Valin (48°51' N, 70°20' W, 611 m a.s.l.). The second site (SIM) was at a lower altitude, in the Laurentides Wildlife Reserve, within the Simoncouche research station (48°13' N, 71°15' W, 350 m a.s.l.). The density of the two stands was similar, c.a 3000 trees ha$^{-1}$, even if slightly smaller trees were present at BER. The region is included in the balsam fir-white birch ecological domain (Saucier *et al.* 1998), with an understory vegetation mainly composed of *Kalmia angustifolia*, *Ledum groenlandicum*, *Cornus canadensis*, *Vaccinium myrtilloides*, and soil vegetation of *Sphagnum* spp. and mosses (*Hylocomium splendens*, *Pleurozium schreberi*, *Ptilium crista-castrensis*) (Marie-Victorin 1995). The average May-September rainfall is 401.8 and 425.4 mm, at SIM and BER respectively. SIM derived from a forest fire in 1922, while in BER, the forest fire at the origin of the stand has been estimated to have occurred between 1865 and 1870. The stands are growing on gentle slopes (8-17%) and drain glacial tills.

In each site, six co-dominant trees were chosen with upright stem, healthy overall appearance and similar growth patterns. The homogeneity in growth rates was assessed during a preliminary investigation by extracting wood cores and counting the number of tracheids along three previous tree rings (Rossi *et al.*, 2007). The average diameter at breast height and the average height of sampled trees were 17±2 cm and 21±4 cm and 15±2 m and 14±2 m, at BER and SIM respectively.

### 3.4.2 Experimental design

During autumn 2007, heating cables were installed in the soil at 20-30 cm depth, where the majority of the root system of black spruce is localized (Ruess *et al.* 2003), between the organic and mineral layers, following a spiral pattern at a distance of 90-200 cm from the stem collar, leaving 30 cm between each coils of the cables. The experimental design included a control and a heated treatment, each applied to three trees per site. Non-heating cables were similarly installed around the control trees to account for root damage at the moment of cable laying. In the treated trees, soil temperature was increased by 4 °C according to the forecasts for 2050 proposed by the FORSTEM climatic model developed for the boreal forest of eastern Canada (Houle *et al.* 2002). Heating started on different dates according to year and site, usually with two weeks delay between SIM and BER, to reflect the difference in temperature between the two altitudes (Lupi *et al.* 2010), and in order to obtain a 1-2 week earlier snowmelt. In SIM, heating usually started at the end of March except in 2008 when it started in mid-April. Soil temperature was measured, at about 1-2 m from the stem, between the coils of the cables in three heated and three non-heated points per site. A diesel generator was used to maintain the temperature differential between control and treated trees during April-July, the period in which most cambial division takes place (Rossi *et al.* 2006a, Thibeault-Martel *et al.* 2008). Measurements were taken every 15 minutes and data were stored as hourly averages in CR1000 dataloggers (Campbell Scientific Corporation, Canada). Soil volumetric water content of heated and non-heated plots was measured during June-July 2009 but no difference was found between treatments. No particular side effects, like shrub or moss dieback was observed, even if during the heating period moss seemed drier near the heating cables.

Standard weather stations were installed in a forest gap close to the experimental plots to measure air temperature. Measurements were taken at the same time interval as that of soil temperature and stored in CR10X dataloggers.

### 3.4.3 Sample collection and preparation

During 2008-2010, wood microcores (2.5 mm in diameter and 25 mm long) were collected from the stem weekly from April to October with a Trephor (Rossi *et al.* 2006b) following a counter-clockwise rising spiral centered at breast height. Microcores usually contained the previous five tree rings and the developing annual layer with the cambial zone and adjacent phloem tissues. Wood samples were always taken at 5-10 cm intervals to avoid the formation of traumatic resin ducts (Deslauriers *et al.* 2003). The microcores were stored in Eppendorf microtubes containing a water:ethanol solution (1:1). In the lab, the microcores were dehydrated through successive immersions in ethanol and Histosol™ and embedded in paraffin (Thibeault-Martel *et al.* 2008). Transverse sections 6-10 µm in thickness were cut with a rotary microtome, stained with cresyl violet acetate (0.16% in water) after the paraffin was removed and observed within 20-30 minutes under visible and polarized light at a magnification of 400-500× to differentiate cambium and developing xylem cells.

The cambial zone and cells in radial enlargement showed only a primary wall, which, unlike the secondary wall, did not shine under polarized light (Gričar *et al.* 2006). Cambial cells were characterized by thin cell walls and small radial diameters, while enlarging cells had a radial diameter at least twice that of a cambial cell. Cells in wall thickening shone under polarized light and, during the maturation process, showed a coloration varying from light to deep violet. As lignification advanced, a blue coloration starting from the cell corners spread into the secondary walls. Since lignin deposition may persist after the end of cell wall thickening (Gindl *et al.* 2000), cells were considered lignified and mature when the violet was completely replaced by the blue coloration (Thibeault-Martel *et al.* 2008). The number of cells in enlargement and in wall thickening and lignification and mature was counted along three radial rows. The number of total cells was calculated as the sum of cells in enlargement, wall thickening and lignification and mature cells. In spring, xylem formation was considered to have begun when the average of number of cells in the enlarging phase was greater than one. In late summer, when the average number of cells undergoing wall thickening and lignification was less than one, xylem formation was considered complete. The phenology of xylem development was assessed for each tree. Four phenophases, computed in days of the year (DOY), were considered, including onset and ending of (i) cell enlargement and (ii) wall thickening and lignification. Duration of xylem formation was calculated as the difference between the onset of cell enlargement and the ending of cell wall thickening and lignification.

Four microcores were also taken around the stem of each tree after the end of the experiment, in October 2010. The number of xylem cells produced during 2005-2010 was counted on each sample along two radial rows per tree ring.

### 3.4.4 Statistical analyses

Linear fixed effect models with repeated measures and different variance-covariance structures were used to analyze the statistical differences in onset and ending of each differentiation phase and in duration of xylogenesis. Analyses were conducted using PROC MIXED of the SAS statistical package (SAS version 9.2, SAS Institute, Cary, NC, USA) considering site, temperature treatment, year and all interactions as fixed factors. The denominator degrees of freedom for testing the fixed effects were calculated using the Satterthwaite method of approximation and different variance-covariance structures were tested to select the best model (SAS version 9.2, SAS Institute, Cary, NC, USA). The following variance-covariance structures were tested using the TYPE option in the REPEATED statement of the MIXED procedure in SAS, for selecting the best model for each variable: ante-dependence (ANTE), autoregressive (AR(1)), heterogeneous autoregressive (ARH(1)), Huyn-Feldt (HF), Toeplitz (TOEP), heterogeneous Toeplitz (TOEPH), unstructured (UN), variance components (VC). All of variance-covariance structures were tested with and without the option GROUP=site to test for heterogeneous variance models grouping or not by site. Akaike's information criterion (AIC) was used to find a suitable covariance structure and to select the best model for each variable (Quinn and Keough 2002, Kilpeläinen *et al.* 2007). When significant effects of a factor or interactions between factors were found, multiple comparisons of least squares means were performed using the method of Scheffe for adjusting the *p*-values.

Specifically for cell production, the tests were conducted on the treatment period (2008-2010) by removing the initial differences between trees and treatments using as covariate the average number of cells of the three tree rings formed prior to the treatment (i.e. 2005-2007) (Jyske *et al.* 2009). The relation between the covariate and dependent variable was linear. Results are reported as least squares means calculated by the mixed models.

## 3.5 Results

### 3.5.1 Air and soil temperatures

The climate of the sites is continental with long, cold winters and warm summers. During 2008-2010, the mean annual temperature was 0.2 °C in BER and 2.2 °C in SIM (Figure 3.1). May-September average temperature varied between 11.2 °C and 14.2 °C, with the highest values recorded in 2010, 12.5 °C and 14.2 °C in BER and SIM, respectively. Mean monthly temperatures during winter were close to or below zero for a period ranging between the beginning of November and the end of March and dropping in January 2009 to -21.2 °C and -19.1 °C in BER and SIM, respectively (Figure 3.1). The coldest months showed absolute minimum temperatures reaching -39.8 °C in BER in 2009 and -32.0 °C in SIM in 2010. Summers were generally short with absolute maximum temperatures attaining 31 °C in 2010 (Figure 3.1).

Soil temperatures were close to 0 °C from December to April. The 4 °C differential between treatments was achieved in about three days after heating started and was maintained up to the end of the heating period (Figure 3.1). Heating took between two and three weeks before the snow completely melted, about one week before that around the control trees (Figure 3.2). Complete snowmelt was generally two weeks later in BER, the site at the higher altitude. Treatment stopped at the beginning of July in SIM and two weeks later in BER (Figure 3.1). The warmest soil temperatures were measured around the heated trees, with 19.1 °C in BER and 21.6 °C in SIM, both occurring in 2010.

### 3.5.2 Cambial activity and cell differentiation

In both treatments, during the inactive period, the cambium was constituted by 4-6 cells (Figure 3.3). In May, the number of cells in the cambial region rapidly increased, reaching its maximum in June when up to 14 cells were counted, with higher values observed in 2009 and 2010. The number of cells in the cambial zone decreased to minimum values at the end of August (Figure 3.3). The number of cells in enlargement increased rapidly at the beginning of xylogenesis, reached its maximum between the end of June and mid-July, and then decreased to zero, following the decrease in cambial divisions. No clear differences between treatments, except a higher maximum number of cells in enlargement in the heated trees at BER, were observed (Figure 3.3). The number of cells in wall thickening and lignification followed a similar pattern, but usually decreased to zero 2-4 weeks later than the number of cells in enlargement (Figure 3.3). At both sites, 1-2 more cells in wall thickening and lignification were often observed in heated trees than in control trees (Figure 3.3).

The maximum number of cambial cells was similar between the two sites and varied between 9 and 14, depending on year. More differences were observed for cells in enlargement and in wall thickening and lignification, especially during the period of maximum cell production. Up to 8 and 15 enlarging cells were counted in BER and SIM, respectively. Similarly, up to 12 and 24 cells in wall thickening and lignification were observed in BER and SIM, respectively. However, in SIM the maximum number of cells in wall thickening and lignification was more frequently 10-12, while 7-9 was more common in BER (Figure 3.3). Higher total number of cells was generally observed in SIM than BER (Figure 3.3).

The number of cambial cells was similar between the two treatments both before and during the period of cambial activity (Figure 3.3). Heated trees occasionally showed more enlarging cells in BER, and heated trees had more cells in wall thickening and lignification than control trees in both sites (Figure 3.3). The total number of cells was usually higher in the heated trees (usually 5-7 more cells), especially in BER.

### 3.5.3 Xylem phenology and cell production

The onset of xylogenesis was statistically different between sites and years (Table 3.1). On average, xylogenesis started on DOY 156 in BER, 9 days later than in SIM (Scheffe's test, t=2.77, $p<0.05$). The year with the earliest onset was 2010 (DOY 145), while that with the latest was 2009 (DOY 156). The onset of wall thickening and lignification differed between years, with the earliest and latest onset observed in 2010 (DOY 150) and 2009 (DOY 171), respectively (Table 3.1, Figure 3.4). No effect of treatment was observed on the phenological phases of xylem (Table 3.1, Figure 3.4).

The ending of cell enlargement differed between years (Table 3.1), with 2008 being that with the earliest ending (DOY 204). The ending of xylogenesis was not affected by treatment, site or year (Table 3.1). The duration of xylogenesis varied between years and showed a significant interaction between treatment and year (Table 3.1). Overall, during 2009 and 2010 the duration of xylogenesis was longer in the heated trees of both sites, lasting on average 18 days more than in control trees. However, no significant difference was detected by the multiple comparisons performed for each year.

The overall number of cells decreased up to 2009, while a higher amount of cells was produced in 2010 (Figure 3.5). Between the three study years, cell production differed significantly, but no significant results were detected between treatments and sites (Table 3.1). Before the treatment began, more cells were counted in heated than control trees. This difference was maintained during 2008-2009, the first two years of the experiment. In 2010, the gap between treatments increased substantially in both sites, but no statistically significant difference was observed between heated and control trees (Scheffe's test, t=0.95, p>0.05).

### 3.6 Discussion

In this study the hypothesis that cambial phenology and cell production is directly affected by soil temperature and snowmelt was explicitly and experimentally tested in the field through a manipulative approach that disentangled air and soil temperature. The treatment consisted of an increase of 4 °C in soil temperature and a consequent one-week earlier snowmelt. After three years of experiment, no difference in xylem phenology or cell production was observed. Nonetheless during the last two years of the treatment the duration of xylogenesis was longer in the heated trees and an increasing gap was observed between heated and control trees for the number of cells. As a result, the initial hypothesis was partly rejected: the relation between soil temperature and xylem phenology and production in the short term seems absent or weak, however longer term indirect effects may exist.

During late winter, localized heating of the stem could induce localized reactivation of the cambium, which however ceased its activity soon after a few cells had been produced, suggesting that meristems require additional conditions to maintain cell division (Oribe *et al.* 2001, 2003, Gričar *et al.* 2006). Depending on the species, cambial reactivation may be limited by factors other than temperature (Oribe and Kubo 1997). For example, in the deciduous Japanese larch cambium was not responsive to heating before bud break (Oribe and Kubo 1997). Moreover certain studies suggest that, after cambial reactivation, a continuous downward flow of auxin through the phloem may be needed for the maintenance of cambial divisions and cell differentiation (Oribe and Kubo 1997, Oribe *et al.* 2003). According to some authors, air temperature should affect the transport of auxin inside the stem and thus an insufficient supply of auxin may inhibit the reactivation of cambial tissues in early spring (Oribe *et al.* 2003, Fonti *et al.* 2007). A hypothesis is that the responsiveness of cambium to auxin may indeed be modulated by temperature and the presence of cytokinins supplied by the root apices (Aloni *et al.* 2006, Fonti *et al.* 2007). The production of cytokinins followed by the synthesis of auxin in the apical region of active shoots seems to be promoted by warm temperatures (Aloni *et al.* 2003, Friml 2003). Thus we could assume that although the raised soil temperature in our experiment may have increased the production of cytokinins, the absence of a favourable air temperature prevented the production and transport of auxin, denying one of the conditions required for cambial activity along the stem.

Although no significant increase in soil $CO_2$ efflux was observed in an experiment of soil heating (Vogel *et al.* 2008), extreme soil heating of 8 °C increased the aboveground productivity and canopy N content (Van Cleve *et al.* 1990). Also, greater allocations to the aboveground growth than to fine roots of Norway spruce were observed in a heated and irrigated treatment than in a treatment with just irrigation (Strömgren and Linder 2002, Majdi and Ohrvik 2004). Similarly, in a study along a gradient of soil temperature in the black spruce forest, Kane and Vogel (2009) observed a shift in C distribution from below- to above-ground pools as temperature increased. However the production was insensitive to the increasing heat sums of soil in the warmer sites (>600 soil summed degree-days, above 0 °C). Since both sites of the present study can be considered in this category, this could explain the absence of a response in xylem production following the soil warming.

Rossi *et al.* (2007) found different soil temperatures at the onset of growth between treeline and timberline sites, deducing that soil temperature was not the main limiting factor for growth. Soil temperature and timings of snowmelt co-vary with air temperatures and this logically explains the lack of a direct relationship between these variables and growth, in terms of xylem phenology and production. In the short term, the variation in cell production is mainly related to the timings of cambial resumption (Lupi *et al.* 2010), while in the long term other factors are considered to affect growth, such as nutrient availability (Jarvis and Linder 2000, Rossi *et al.* 2011). Nutrition, especially the availability of nitrogen, is one of the most limiting factors for the boreal forest. The decomposition of soil organic matter and nutrient cycles are affected by soil temperatures and by the duration of snow cover. Thus, in the long term, increases in soil temperature and earlier snowmelts may affect wood production, although no effect could be observed in the short term (Vaganov *et al.* 1999, Jarvis and Linder 2000, Rustad *et al.* 2001). Indeed, even if the mixed models applied in the present work were unable to detect differences between treatments in terms of productivity during the first three years of monitoring, the average difference between heated and control trees increased exponentially across the three years of the experiment and significant differences could reasonably be expected during the future prolongation of the experiment.

In experiments with localized heating of the stem, cambial activity and cell differentiation were influenced only within the treated area, which pointed out the non-transference of the effect of temperature from the site of its application to other parts of the tree (Gričar *et al.* 2006, 2007). This may explain the absence of any effect on wood production and phenology of xylogenesis in our experiment. Several studies have found changes in C-allocation between aboveground and belowground pools (Strömgren and Linder 2002, Majdi and Ohrvik 2004, Vogel *et al.* 2008, Kane and Vogel 2009). Nevertheless, although synchronous xylogenesis in stem and roots has been observed in natural stands (Thibeault-Martel *et al.* 2008), the question whether the manipulative treatment of soil heating affects xylem phenology and production in the roots of black spruce still remains unanswered.

## 3.7 Conclusions

This paper described a heating treatment of 4 °C applied to the root system of mature black spruce in the field over three years to simulate the warmer soil and earlier snowmelt predicted for 2050 by the climatic models for eastern Canada. The results showed no significant effect of the treatment on cambial phenology and xylem production, which allowed the hypothesis of a direct influence of soil temperature on tree growth in the stem to be rejected. However, the observed high variability among trees and the limited size of the sample could have affected the significance of the statistical analyses. Moreover, different results could be expected with a soil warming performed over larger areas or with heating cables penetrating to greater soil depths. Nonetheless our findings support the evidence that, in the short term, air temperature is the main factor determining annual increment variation in the boreal forest. However, the observed trends in the amount of cells produced by cambium during 2008-2010 suggests that the future prolongation of the experiment might reveal long-term effects of soil temperature on nutrient cycles, especially nitrogen, and, indirectly, on tree growth. This may have important implications for the growth of boreal forest trees given the increase in soil and air temperature and the consequently increased availability of nutrients expected with climate change.

## 3.8 Acknowledgments

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Table 3.1 F-values of the mixed models for the type III test of fixed effects applied to the phenological phases and cell production during the three years of treatment (2008-2010). The average number of cells of the three years preceding the treatment (2005-2007) was used for cell production as covariate to remove the initial differences in the number of cells between trees, treatments and sites. Significant values are reported with one or two asterisks for $p<0.05$ and $p<0.01$, respectively.

| | Cell enlargement | | Wall thickening and lignification | | Duration of xylogenesis | Number of cells |
|---|---|---|---|---|---|---|
| Effect | Onset | Ending | Onset | Ending | | |
| Covariate | - | - | - | - | - | 598.20** |
| Treatment (Tt) | 3.37 | 1.32 | 1.70 | 2.24 | 2.09 | 0.09 |
| site | 7.68* | 2.15 | 4.88 | 1.35 | 2.69 | 0.03 |
| year | 16.43** | 14.82** | 159.79** | 0.76 | 7.64** | 27.78** |
| site*year | 0.54 | 0.10 | 1.17 | 0.36 | 0.82 | 1.09 |
| site*Tt | 0.69 | 0.01 | 0.01 | 1.16 | 0.05 | 0.81 |
| Tt*year | 2.05 | 1.87 | 0.62 | 1.10 | 4.73* | 1.23 |
| site*Tt*year | 1.05 | 0.38 | 1.50 | 0.66 | 1.95 | 0.75 |

* $p<0.05$, ** $p<0.01$

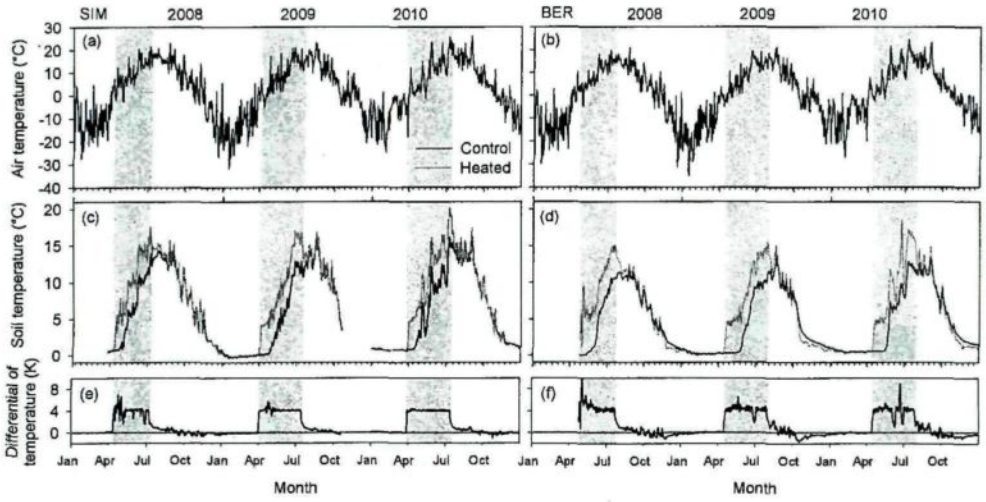

Figure 3.1 Temperature of air (a,b) and soil (c,d) around control (black line) and heated (gray line) trees, and differential (e,f) between treatments in BER and SIM during 2008-2010. The gray background indicates the period of heating.

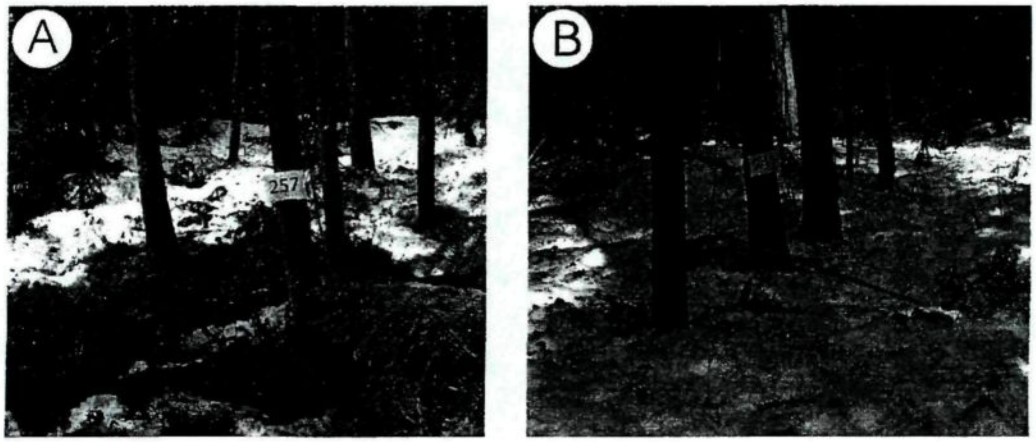

Figure 3.2 Snow conditions around a heated (A) and control (B) tree on DOY 117 (2010, April 24th), one week before the complete snowmelt around control trees.

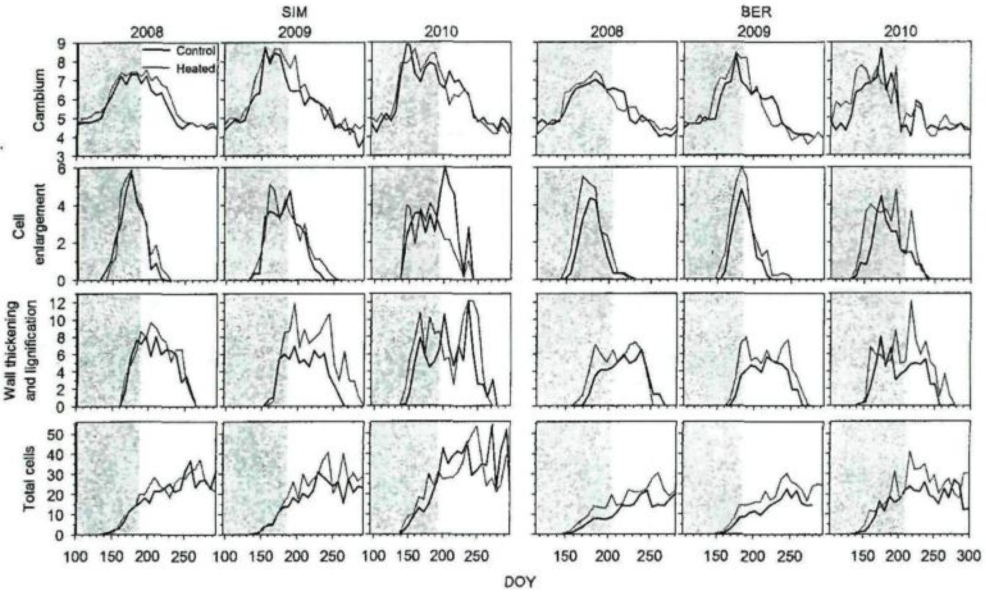

Figure 3.3 Average number of cambial and differentiating xylem cells in control and heated trees in BER and SIM during 2008-2010. The gray background indicates the period of heating.

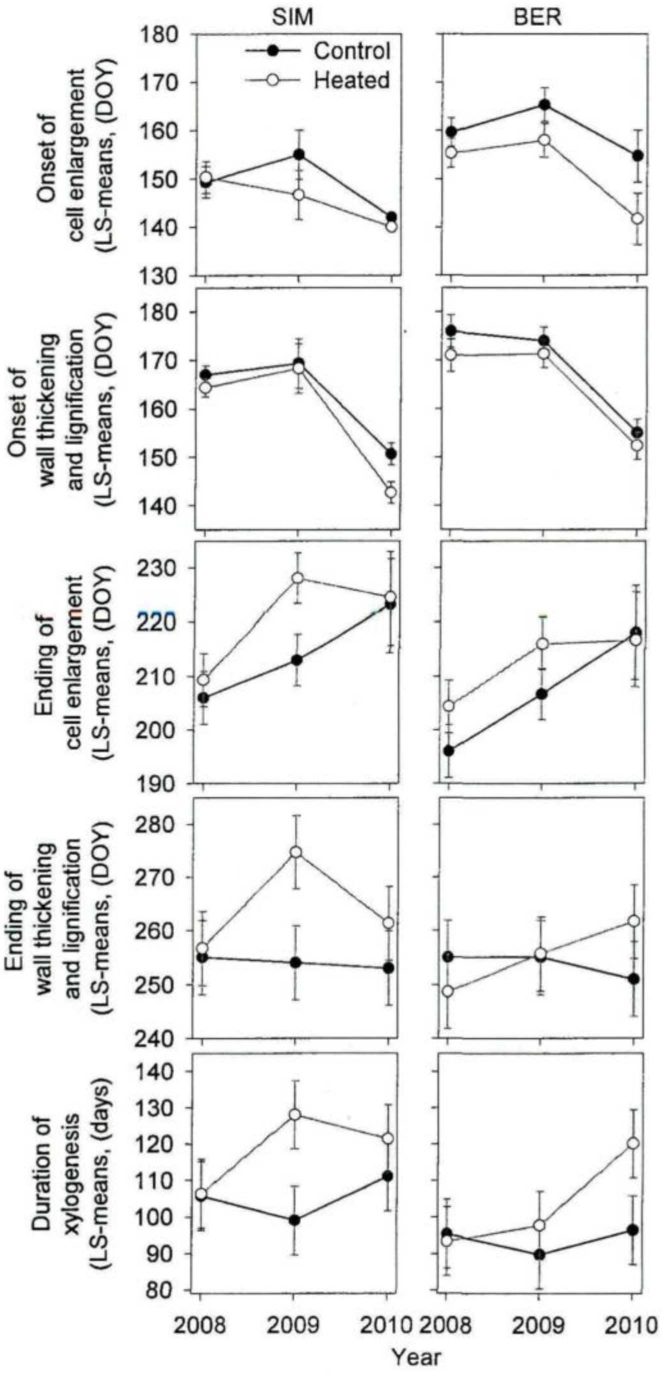

Figure 3.4 Phenological phases of xylem and duration of xylogenesis in control and heated trees in BER and SIM during 2008-2010. Values are reported as LS-means ± standard error.

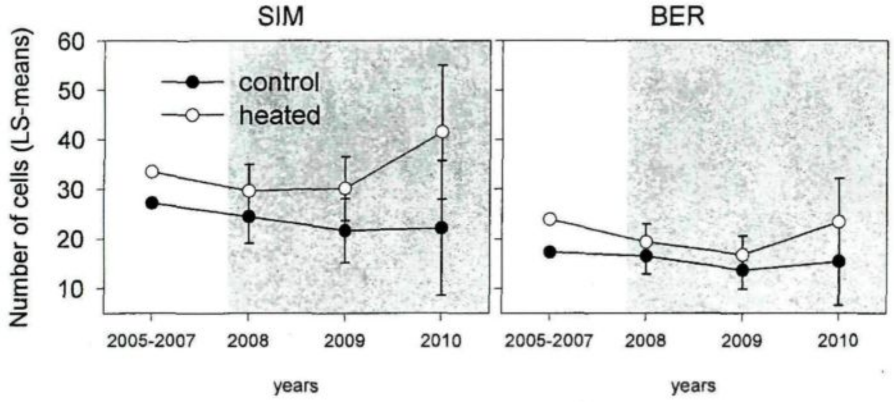

Figure 3.5 Number of cells produced before (white background) and during (gray background) the heating treatment in control (black line) and heated (gray line) trees in BER and SIM. The average of the three years preceding the treatment (2005-2007) was used as a covariate to account for initial differences. Values are reported as LS-means ± standard error.

# Chapitre IV

# Increasing nitrogen availability and soil temperature: effects on xylem phenology and anatomy of mature black spruce.

**Title**: Increasing nitrogen availability and soil temperature: effects on xylem phenology and anatomy of mature black spruce.

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## 4.1 Abstract

Since plant growth in the boreal forest is often considered to be limited by low temperatures and low nitrogen (N) availability and these variables are projected to increase due to climate warming and increased anthropogenic activities, it is important to understand whether and to what extent these disturbances may affect the growth of boreal trees. In this study, the hypotheses that wood phenology and anatomy were affected by increased soil temperatures and N-depositions have been tested in two mature black spruce stands at different altitude in Quebec, Canada. For three years, soil temperature was increased by 4 °C during the first part of the growing season and precipitations containing three times the current N concentration were added in the field by frequent canopy applications. Soil warming resulted in earlier onsets of xylogenesis and interacted with N addition producing longer durations of xylogenesis for the treated trees. The effect of warming was especially marked in the phenology of roots, while wood production, in terms of number of tracheids, was not affected by the treatment. Xylem anatomy and soil and needle chemistry showed no effect of the treatments, except for an increase of cell wall thickness in earlywood of treated trees. This short-term experiment with black spruce suggested that previous fertilization studies that used large and unrealistic rates of nitrogen addition may have overestimated the impact of N-depositions on boreal forest productivity.

**Keywords** cambial activity, N-deposition, needle chemistry, phenology, *Picea mariana*, snow melting, soil chemistry, soil warming, wood anatomy, xylogenesis

## 4.2 Résumé

Comme on considère souvent que les basses températures et la faible disponibilité du N limitent la croissance des plantes dans la forêt boréale, et qu'une augmentation de ces variables est prévue à cause du réchauffement du climat et de l'augmentation des activités anthropiques, il est important de comprendre si, et dans quelle mesure, ces perturbations pourraient influencer la croissance des arbres boréaux. Dans cette étude, les hypothèses que les augmentations de la température du sol et des dépôts de N ont un effet sur l'anatomie et la phénologie du bois ont été testées dans deux peuplements matures d'épinette noire (*Picea mariana* (Mill.) BSP) situés à différentes altitudes au Québec, Canada. Pendant 3 ans, la température du sol a été élevée de 4°C durant la première partie de la saison de croissance et des précipitations contenant trois fois la concentration actuelle de N ont été ajoutées au champ par de fréquentes applications aériennes. Le réchauffement du sol a entraîné le déclenchement plus hâtif de la xylogénèse et a interagi avec l'addition de N pour allonger la durée de la xylogénèse chez les arbres traités. L'effet du réchauffement s'est fait particulièrement sentir sur la phénologie des racines tandis que la production de bois, en termes de nombre de trachéides, n'a pas été influencée par le traitement. Les traitements n'ont eu aucun effet sur l'anatomie du xylème ni sur les caractéristiques chimiques du sol et des aiguilles, à l'exception d'une augmentation de l'épaisseur de la paroi cellulaire dans le bois initial des arbres traités. Cette expérience à court terme avec l'épinette noire indique que les études précédentes de fertilisation, dans lesquels des taux élevés et irréalistes d'apport en N ont été utilisés, ont pu surestimer l'impact des dépôts de N sur la productivité de la forêt boréale.

**Mots-clés:** activité cambiale, dépôts azotés, chimie des aiguilles, phénologie, *Picea mariana,* fonte de la neige, chimie du sol, réchauffement du sol, anatomie du bois, xylogenèse

## 4.3 Introduction

The boreal forest is expected to experience a greater increase in surface temperature than any other forest ecosystem in the world (Strömgren and Linder 2002). For eastern Canada, recent simulations for the year 2050 project increases of up to 3 °C in annual temperature and 20% in rainfall (Plummer et al. 2006). Plant growth in the boreal forest is often considered to be limited by low temperatures and the availability of nitrogen (N) (Reich et al. 2006). The soil warming associated with the increase in surface air temperature expected for eastern Canada (Sushama et al. 2006) may favor mineralization and enhance the availability of N (Campbell et al. 2009). Moreover, anthropogenic N-depositions have greatly altered the N-cycle and plant nutrition in the last two centuries and are projected to increase in the future (Galloway et al. 2004, Thomas et al. 2010). Thus it is important to understand whether and to what extent the combination of soil warming and increased N depositions may affect the growth of boreal trees.

Investigations on N-deposition effects have used very high amounts of N, often in a few concentrated applications, supplied directly to the forest floor, which poorly represent a natural process (Gaige et al. 2007, Gundale et al. 2011). More than 80% of the boreal region receives 4 kg ha$^{-1}$ year$^{-1}$ or less of N deposition, while past studies have more often added quantities 1-3 orders of magnitude higher (de Vries et al. 2009, Gundale et al. 2011). Low doses of N and frequent applications directly to the canopy would better represent the natural depositions and account for the difference between foliar and soil N uptake (Sparks 2009, Guerrieri et al. 2011). Indeed, canopy uptake has been proposed as an important sink of N where N availability in the soil is constrained by low mineralization (Ignatova and Dambrine 2000, Gaige et al. 2007, Sparks 2009). Tomaszewski et al. (2003) estimated that N uptake of the canopy in a spruce-fir forest was 2-3 kg N ha$^{-1}$, contributing 10-15% of the foliar N requirement for canopy growth. According to Lovett and Lindberg (1993), canopy uptake can be nearly equal to foliar biomass requirements in low productivity sites. No measurable canopy uptake is usually observed during winter months (Houle et al. 1999, Klopatek et al. 2006), suggesting the biological nature of this phenomenon.

The importance of temperature in cambial reactivation and wood production has been clearly proven for cold environments like boreal and montane forests (Gričar et al. 2007, Rossi et al. 2008, Swidrak et al. 2011). In spring, snow maintains soil temperature close to freezing point and is assumed to delay the onset of xylogenesis (Vaganov et al. 1999, Rossi et al. 2011). According to Lupi et al. (2010), delayed growth resumption results in fewer xylem cells produced by cambium and reduced duration of wood formation. Moreover, root activity is strongly inhibited by a soil temperature below 6 °C (Alvarez-Uria and Körner 2007), which is may influence root uptake and growth of the whole tree (Jarvis and Linder 2000). A study by Thibeault-Martel et al. (2008) reported similar dates of onset of xylogenesis between lower stem and roots in black spruce [*Picea mariana* (Mill.) BSP]. However, delays between root and shoot phenology in woody plants, with root activity occurring later, were also reported (Steinaker and Wilson 2008, Steinaker et al. 2010). A difference of 3 °C between air and soil temperature resulted in a difference of 15 days between the phenology of leaves and roots (Steinaker et al. 2008). According to Alvarez-Uria and Körner (2007), shoot growth was unaffected by soil temperature, contrary to roots that were limited by cold soils. Changes in root phenology may affect stem growth via modifications in water and nutrient uptake and *vice versa* through the transport of photosynthates and hormonal signaling driving cambial reactivation and xylem production (Fonti et al. 2007, Nord and Lynch 2009). For example, soil warming and forest floor fertilization enhanced stem growth of Norway spruce in long-term fertilization in northern Sweden, with significant increases in volume growth from the second year of heating (Strömgren and Linder 2002). In addition, a combined study of N fertilization and soil warming demonstrated the existence of interactions between N-availability and soil temperature on root activity (Majdi and Öhrvik 2004).

The effect of warming and fertilization can also be observed in xylem anatomy (Kostiainen et al. 2004, Kilpeläinen et al. 2007). Kilpeläinen et al. (2007) reported that elevated temperature resulted in thicker tracheid walls and greater wood density in Scots pine. Fertilization usually increased radial increment, but decreased wood density and cell wall thickness in conifers (Hättenschwiler et al. 1996, Kostiainen et al. 2004). However, the majority of previous studies analyzed juvenile wood and/or used high rates of N-addition, so it is not possible to extend these results to natural conditions of a mature boreal forest. Moreover, differences in xylem characteristics are observed between roots and stem and their responses to the environment may also be expected to differ (Krause et al. 2010). The growth response to environmental disturbances should consequently be investigated in both stem and root tissues of a mature tree.

The aim of this study was to test the effects of soil warming and increased N-deposition on wood formation in roots and stem of mature black spruces. Based on previous findings, we hypothesized that soil warming would induce an earlier onset of xylogenesis, especially in roots, and, together with canopy N-addition would increase tracheid production. The potential increase in tracheid production may, in turn, affect the duration of xylogenesis (Lupi et al. 2010). Concerning wood anatomy, we hypothesized that soil warming and N fertilization treatments would act in different directions at the level of cell wall thickness (elevated temperature translating into thicker cell walls, with N-deposition corresponding to decreased cell wall thickness), but together would further increase radial increment. The study took place in two sites at different altitude, corresponding to a 2 °C difference in mean annual temperature, to check for any potential effect of different starting conditions on the response to the treatments.

## 4.4 Material and Methods

### 4.4.1 Study sites and tree selection

The study was conducted in two mature, even-aged and monospecific black spruce [*Picea mariana* (Mill.) BSP] stands of the boreal forest of Quebec, Canada. The first site (abbreviated as BER) was located near Lake Bernatchez, in the Monts-Valin (48°51' N, 70°20' W, 611 m a.s.l.). The second site (SIM) was at a lower altitude, in the Laurentides Wildlife Reserve, within the Simoncouche research station (48°13' N, 71°15' W, 350 m a.s.l.). The stands are growing on gentle slopes (8-17%) and drain glacial tills. At BER, the water table is within 10-30 cm of the ground surface in the low-lying areas of the site, especially at the time of snowmelt and in autumn. SIM is better drained throughout the year. The climate is continental, with short summers and long, cold winters with a mean annual temperature of 1.9 °C and 0.2 °C, in SIM and BER, respectively (Rossi et al. 2011). The average May-September temperature is 13.3 °C and 11.4 °C and rainfall is 401.8 mm and 425.4 mm, in SIM and BER, respectively. Snow cover lasts from November to May, reaching a depth of up to 150 cm in both sites.

### 4.4.2 Experimental design

In each site, a square area of 60×60 m was delimited and divided in 25 12×12 m square plots, of which 12 acted as experimental plots with the others being buffer zones (Fig. 4.1). In each experimental plot, a tree was chosen with upright stem and healthy overall appearance. The homogeneity in growth rates among the selected trees had been assessed during a preliminary investigation by extracting wood cores from five preselected trees per plot and counting the number of tracheids along three previous tree rings. Tree height and diameter at breast height of experimental trees were measured during the autumn of 2007, before the beginning of treatments (Table 4.1). On average, trees were 16 m and 17 m tall with a diameter at breast height of 18 cm and 21 cm at BER and SIM, respectively. The stands at both sites were even-aged and originated from forest fires, which were estimated to have occurred around 1865-1870 and in 1920 at BER and SIM, respectively. Thus, the age of the trees was estimated to be about 140 and 85 years at BER and SIM, respectively.

Two treatments were combined: an increase in soil temperature (H-treatment) and a canopy application of artificial rain enriched with nitrogen (N-treatment). The combination of the treatments resulted in four experimental groups: heated only trees (H), N-enriched only trees (N), heated and N-enriched trees (NH) and control trees, for which the soil was not heated and that received no N-enrichment (C). The two treatments were attributed randomly to experimental trees resulting in a random split plot design with three replications (Fig. 4.1).

For the H-treatment, heating cables were installed during the autumn of 2007 between the organic and mineral soil layers, at about 20 cm depth, where the majority of the root system of black spruce is localized (Strong and La Roi 1983), following a spiral pattern at a distance of 90-200 cm from the stem collar. Cables were laid by cutting the soil vertically with a shovel or a knife and manually inserting the cable in the resulting narrow "trench", which was then rapidly reclosed. To account for potential root damage and soil disturbance during cable laying, non-heating cables were also installed around non-heated trees (C and N). Power was supplied by a diesel generator located at 200 m from the site. H-treatment consisted of increasing the soil temperature by 4 °C during the first part of the growing season. This led to an earlier snowmelt and an increase in annual soil temperature in agreement with the estimates for 2050 of the FORSTEM climatic model developed for the boreal forest of eastern Canada (Houle et al. 2002, Houle et al. *in press*). Heating started on different dates according to year and site (Table 4.2), usually with a two weeks delay between SIM and BER to reflect the difference in temperature between the two sites (Lupi et al. 2010) and achieve a 1-2 week earlier snowmelt in heated plots. Soil temperature was measured between the coils of the cables in three heated and three control trees. The differential in temperature between control and treated trees was maintained during April-July (Table 4.2), the period in which most cambial division takes place (Thibeault-Martel et al. 2008), to reproduce an earlier snow melt and a longer snow free period. Soil temperature was measured every 15 minutes and data were stored as hourly averages in CR1000 dataloggers (Campbell Scientific

Corporation, Canada). Volumetric water content of heated and non-heated plots was measured in July 2009 using a portable device mounted with a CS-616 probe (Campbell Scientific Corporation, Edmonton, Canada) to check for differences in soil moisture content. No significant difference was found between heated (H and NH) and non-heated trees (C and N, data not shown).

The artificial rain was produced by sprinklers installed above the canopy of each tree. Each week, the equivalent of 2 mm rainfall was applied to the canopy, during the frost-safe period (June to September), for a number of weeks varying between 12 and 16 (Table 4.2). Rain was applied over a circular area of three-meter radius centered on the stem of each experimental tree, which allowed the canopy area to be covered. Non-N-enriched trees (C and H) were irrigated with a water solution reproducing the chemical composition of natural rainfall over the studied sites (Duchesne and Houle 2006, 2008), while for N-enriched trees (N and NH), a 3-fold increase in ammonium nitrate ($NH_4NO_3$) concentration was used (Table 4.3). It is expected that frequent artificial rain additions directly to the canopy, with relatively low inorganic N concentration, imitate the way anthropogenic derived N depositions are reaching boreal forest ecosystems better than massive soil applications do.

### 4.4.3 Phenology and wood anatomy

During April-October of 2010, the third year of experimentation, wood microcores were collected weekly from stem and roots with a Trephor (Rossi et al. 2006). Microcores usually contained the previous 5-10 tree rings and the developing annual layer including the cambial zone and adjacent phloem tissues. In the stem, sample collection followed a counter-clockwise elevating spiral centered at breast height. For roots, samples were collected on the upper part of one main horizontal adventitious root for each tree, starting the sampling near the stem and going outward at about 3-4 cm intervals following a sinusoidal path (Thibeault-Martel et al. 2008).

The microcores were placed in Eppendorf microtubes containing a water:ethanol solution (1:1), dehydrated through successive immersions in ethanol and Histosol™, embedded in paraffin and cut with a rotary microtome to obtain transverse sections 6-10 μm in thickness (Rossi et al. 2006). The sections were stained with cresyl violet acetate (0.16% in water) and observed within 20-30 minutes under visible and polarized light at a magnification of 400-500× to differentiate cambium and developing xylem cells (Rossi et al. 2006). In spring, xylem formation was considered to have begun when at least one row of differentiating cells was observed. In late summer, when no further cells were observed undergoing cell wall differentiation, xylem formation was considered complete. Duration of xylem formation was calculated as the difference between the onset and ending of cell differentiation.

Two additional microcores were collected in October, at the end of the growing season for analyses of cell size in the 2010 tree ring. Wood sections were stained with safranine (1% in water) and fixed with a mounting medium. Digital images of the tree ring at a magnification of 200× were taken using a camera mounted on the microscope. Measures were taken along three paths using Wincell™ (Regent Instrument). For each cell along the paths, the software gave diameter of cell lumen, tracheid width and single cell wall thickness (μm). Single cell wall thickness was calculated by dividing the double cell wall thicknesses measured left and right of the cell lumen by two and then averaging the measurements for each cell. Tracheid width was calculated summing two single cell wall thicknesses and cell lumen. Tracheids were assigned to earlywood or latewood according to Mork's formula, which classified as latewood the cells with lumen smaller than twice a double cell wall (Denne 1989). Percentage and number of tracheids of earlywood and latewood were also calculated. Since diameter of cell lumen and tracheid width data are very similar and can be derived from one another given cell wall thickness, only data for tracheid width are presented.

### 4.4.4 Chemical analyses of soil and needles

During October 2010, samples of soil and needles were collected for chemical analyses. Cylindrical soil cores were collected in three points around each experimental tree between 1 and 2 m from the stem, representing the area in which the treatments were applied. The moss layer, organic and mineral horizons were visually distinguished, separated in the field and sealed in plastic bags. One and two-years-old needles were collected from three branches cut from the top third of the canopy. All samples were stored at -10 °C in the dark and air-dried at 65 °C for 48 hours. Soil samples were mixed by hand and sieved through a 2 mm mesh to remove roots and coarse woody debris. For soil, the amount of mineral nitrogen ($N_{min}$) representing the sum of ammonium, nitrate and nitrite was determined after extraction with KCl using a combustion-element analyzer. The amount of nitrate and nitrite was often below the detection threshold (1 mg $kg^{-1}$). Total soil N (mainly organic) was determined following Kjeldahl digestion (Kjeltec Tecator 1030). Exchangeable cations (K, Ca, Mg) were measured through an inductively coupled plasma emission spectrometer, after extraction in an unbuffered 1 mol $l^{-1}$ $NH_4Cl$ solution for 12 hours (Duchesne and Houle 2006). For needles, N content was determined following Kjeldahl digestion (Kjeltec Tecator 1030) while P and K were measured after digestion with $H_2SO_4$ by inductively coupled plasma-atomic emission spectroscopy (Houle and Moore 2008).

### 4.4.5 Statistics

Sites, treatments and locations on the tree (roots and stem) were compared using general linear models [GLM procedure in SAS 9.2 (SAS Institute Inc., Cary, NC)]. Overall tests for the significance of the fixed effects were conducted using Type III sum of squares. When the effects were significant ($p < 0.05$), Tukey-Kramer tests were used for multiple comparisons. Statistics for anatomical characteristics were conducted separately for earlywood and latewood.

## 4.5 Results

### 4.5.1 Air and Soil temperature

The mean annual air temperature in 2010 was 2.4 and 3.7 °C at BER and SIM, respectively, thus higher than the annual mean for the period 2002-2008 (see Rossi et al. 2011). Mean monthly air temperatures from January to March and from November to December were below zero at both sites. From April to June, minimum air temperature varied between -11.4 °C at BER and -8.2 °C at SIM, to 16.1 °C and 19.0 °C at BER and SIM, respectively (Fig. 4.2*a*). At both sites, the monthly average of minimum air temperatures was higher than 4 °C in September and dropped to around zero in October.

Soil temperature, at 20 cm depth, was always above zero in 2010 in both non-heated and heated plots. The 4 °C difference between heated (H and NH) and non-heated (C and N) plots was maintained throughout the heating period (Fig. 4.2*b*, Table 4.2). This led to a difference in annual soil temperature in heated vs. non-heated plots of about 1.2 °C at both sites, in agreement with forecasts for 2050. Soil heating affected snow cover (data not shown), leading to one week earlier snowmelt around heated trees at both sites. At BER, soil temperature in non-heated plots began to steeply increase above zero almost a month after heated plots, while at SIM the delay was about two weeks (Fig. 4.2*b*). Soil temperatures in all plots dropped again to zero in December 2010, at both sites.

### 4.5.2 Soil and Needle Chemistry

Results for the organic layer are reported to illustrate the characteristics of the topsoil, the part where the majority of roots are localized. Data for the moss layer and mineral horizon are not reported since no significant differences were found. Overall, the organic horizon showed total N varying between 6.8 and 12.8 g kg$^{-1}$, with significantly greater amounts detected at SIM (Table 4.4, ANOVA, $F=3.55$, $p<0.05$). The C:N ratio varied between 30-65 and was significantly lower at SIM (Table 4.4, ANOVA, $F=4.24$, $p<0.01$). $N_{min}$ varied between 30 and 65 mg $kg^{-1}$ at both sites. Similar low pH values of 2.5-3.6 were observed at both sites. At BER, N content in one and two year-old needles was similar and varied between 5.0 and 8.5 g $kg^{-1}$. Higher needle N contents were detected at SIM, ranging between 6.8 and 9.4 g $kg^{-1}$. No significant difference among treatments or age classes of needles was observed for any of the chemical variables analyzed, even if average N content in needles of N-enriched trees was generally higher than in control trees (Table 4.4).

In the organic horizon, K was higher at SIM (504 vs. 471 mg $kg^{-1}$ at SIM and BER, respectively), while needles of BER showed higher K contents (Table 4.4). In the organic horizon, Ca was higher and with a greater variation at BER (2462 vs. 1643 mg $kg^{-1}$ at BER and SIM, respectively). Mg varied between 203 and 553 mg $kg^{-1}$ and was on average higher at BER. In needles, P was similar between age classes and sites, averaging 1.4 g $kg^{-1}$.

### 4.5.3 Phenology

#### 4.5.3.1 Control trees: stems

In 2010, onset of xylogenesis in the stem of control trees occurred between the end of May and beginning of June (DOY 155 and 142 at BER and SIM, respectively, Fig. 4.3*a*). Even if xylogenesis at BER began 13 days later than at SIM, no statistically significant difference was found between sites ($p>0.05$). In the stem of control trees, xylogenesis was completed between DOY 237 and DOY 275, but no significant difference was observed between sites (Fig. 4.3*a*). Xylogenesis in the stem of control trees lasted on average 96 and 111 days at BER and SIM, respectively, without significant difference between sites.

### 4.5.3.2 Control trees: roots

Seven out of 24 roots did not produce a tree ring in 2010, thus the analysis was conducted with an unbalanced design. Xylogenesis in roots of control trees began within a few days between DOY 157 and 159 and at the same time at BER and SIM (Fig. 4.3*b*). In the roots of these trees, the ending of xylogenesis was observed on DOY 239 and 223 at BER and SIM, respectively (Fig. 4.3*b*). In roots of control trees, the duration of xylogenesis lasted 82 and 65 days at BER and SIM, respectively.

### 4.5.3.3 Treatment effects

An overall significant difference was observed in the onset and duration of xylogenesis between stem and roots. By analyzing sites and treatments together, xylogenesis started 14 days earlier in the stem (ANOVA, $F=50.11$, $p<0.001$). The model also detected a significant effect of H-treatment (ANOVA, $F=9.01$, $p<0.01$), with trees in the H-treatments (H and NH) starting xylogenesis 6 days before control trees. The model was not significant for the ending of xylogenesis (ANOVA, $F=1.40$, $p>0.05$), probably because a high variability among treatments and individual trees was observed for both stem and roots (Figs. 4.3*a* and 4.3*b*). Xylogenesis lasted longer in the stems (112 days) compared to the roots (91 days) (ANOVA, $F=18.56$, $p<0.01$, Figs. 4.3*a* and 4.3*b*). Moreover, significant effects of the H-treatment (ANOVA, $F=6.74$, $p<0.05$) and of the interaction H×N (ANOVA, $F=6.26$, $p<0.05$) were found. By analyzing the interaction H×N more in detail with a Tukey-Kramer test, it was possible to show that the control trees significantly differed from H-trees, with xylogenesis lasting on average 25 days longer in H-trees while the N and NH trees were intermediate (102 days).

#### 4.5.4 Wood Anatomy

#### 4.5.4.1 Control trees: stems

In 2010, the number of tracheids counted in the stem of control trees was 16 and 22 at BER and SIM, respectively, with latewood percentage being around 23% at both sites (Table 4.5). Average tracheid width in the earlywood varied between 19.7 and 31.3 µm, with higher variability between trees at BER (Table 4.5). Earlywood single cell wall thickness was also more variable at BER and ranged between 1.8 and 2.8 µm.

#### 4.5.4.2 Control trees: roots

Based on phenological observations, roots that did not show tracheids in differentiation in 2010 were also deleted from the dataset for anatomical characteristics, thus leading to an unbalanced design. The roots of control trees produced 12 and 4 tracheids at BER and SIM, respectively, while the latewood percentage was 29 and 35%, respectively (Table 4.5). Earlywood tracheid width and cell wall thickness were 25.2 and 23.4 µm and 2.1 and 2.5 µm at BER and SIM, respectively.

#### 4.5.4.3 Treatment effects

Except for single cell wall thickness of earlywood, the statistical models for the anatomical variables in earlywood and latewood (tracheid width, single cell wall thickness and number of tracheids) and for the whole 2010 tree ring (number of tracheids, percentage of latewood) were not significant. Concerning cell wall thickness in earlywood (ANOVA, $F=3.12$, $p<0.01$), significant effects were found of the location on the tree (ANOVA, $F=8.99$, $p<0.01$) and of the interactions site × N-treatment (ANOVA, $F=7.00$, $p<0.05$) and N-treatment × H-treatment (ANOVA, $F=4.34$, $p<0.05$). Roots exhibited earlywood tracheids with thicker walls than cells in the stem. At BER, earlywood tracheid walls of the N-enriched trees (N and NH) were thicker than tracheids of non-N-enriched trees (C and H) and thicker than earlywood tracheid walls of both N-enriched and non-N-enriched trees at SIM. Overall, pooling parts of the tree and sites together, all treated trees (H, N and NH) showed earlywood tracheids with thicker cell walls than control trees (C). Average single cell wall of earlywood tracheids in treated trees was 2.7 μm, while it was 2.3 μm in control trees.

## 4.6 Discussion

In this study, the hypotheses that wood phenology and anatomy were affected by increased soil temperatures and N-depositions were tested in two mature black spruce stands. For three years, soil temperature was increased by 4 °C during the first part of the growing season and precipitations containing three times the current N concentration were applied. The experiment consisted of frequent canopy applications of inorganic N at low concentrations, in order to reproduce future rain composition, rather than analyzing the effects of N-fertilization per se. Soil warming resulted in earlier onsets of xylogenesis and interacted with N-treatment producing longer durations of xylogenesis in the treated trees at both sites. The effects of warming were especially marked in the phenology of roots, while wood production, in terms of number of tracheids, was not affected by the treatments. Xylem anatomy was slightly affected by the treatments, but only at the level of cell wall thickness in earlywood (thicker in treated trees). There was no clear effect of the treatments on soil and needle chemistry.

The experiment confirmed the role of temperature on cambial reactivation and xylem phenology, as observed by other authors (Gričar et al. 2006, 2007, Begum et al. 2010). The differences between control and treated trees were especially marked for the roots, which are more likely to be affected by increased soil temperature. Indeed, soil temperature below 6 °C strongly limited root activity and water uptake in various conifers (Alvarez-Uria and Körner 2007). However, previous studies of localized warming of the stem often reported reactivations of cambial tissues and sometimes increased cell productions, but only in the zone of application (Oribe et al. 2003, Gričar et al. 2006, 2007). On the contrary, our results showed that soil warming is able to alter xylem phenology not only in the root (localized effect) but also along the stem. Different mechanisms may be involved, including improved nutrient and water uptake and transport and interactions with hormonal signaling. For example, Fonti et al. (2007) advanced the hypothesis that the responsiveness of cambial tissues in sweet chestnut was modulated by temperature and the presence of cytokinines supplied by the root apices. Even if no site effect was detected on xylem phenology, the effect of the H-treatment on the onset of xylogenesis was stronger in roots at SIM. The onset of xylogenesis in roots of heated trees (H and NH) happened on average 5 and 11 days before that in non-heated trees (C and N), while in the stem it occurred 5 and 3 days before control trees at BER and SIM, respectively. According to Jyske et al. (2011), the soil type and, in particular, the water holding capacity may affect the response of xylem formation to soil temperature. Thus, the differences observed between the soils at SIM and BER might explain the different delays at these sites.

The monitoring of xylem phenology allowed us to assess that seven out of 24 roots did not produce a tree ring in 2010. It is known that missing rings are found much more frequently in roots than in the stem (Schweingruber 1996). A root can stop secondary growth for a certain number of years and then reactivate again, even if primary growth of thin roots may continue throughout those years (Coutts 1987). The mechanisms controlling this phenomenon are not well understood. In this and other studies (e.g. Thibeault-Martel et al. 2008), roots were observed to start xylogenesis at the same time or later than the stem. This may depend on the different patterns of warming between air and soil. The air temperature increases faster than that of the soil and root production is positively related to soil temperature (Alvarez-Uria and Körner 2007, Steinaker and Wilson 2008), while growth in the aboveground parts (stem and shoots) is better explained by air temperature (Rossi et al. 2008, Swidrak et al. 2011). The number of tracheids produced may vary between roots and stem, although no clear pattern has been observed (Thibeault-Martel et al. 2008).

Other soil-warming experiments have been conducted in the boreal forest, but, to our knowledge, none of them has observed xylem phenology or tracheid production, so it is difficult to make direct comparisons. Indeed, the majority of studies analyzing phenology regarded primary meristems (e.g. bud burst and shoot elongation), while observations of growth responses concentrated on tree volume or photosynthesis, rather than tracheid production. For example, in a soil warming experiment in a 12-year-old black spruce plantation in central Canada, soil-only warming did not result in changes in bud burst, while the air and soil warming treatment resulted in a bud burst 9-11 days earlier but without a significant effect on shoot elongation (Bronson et al. 2009). In this study, no significant difference was observed in tracheid production among treatments. However, the variation in cell production in the short term may be mainly related to climatic variability affecting cambial phenology (i.e. earlier onset of wood formation in warmer years, Deslauriers et al. 2008), while in the medium and long term, the cumulative effect of other factors, such as nutrient availability (influenced by soil temperature and N-depositions), could affect wood formation and growth (Jarvis and Linder 2000, Yarie and Van Cleve 2010, Rossi et al. 2011). Indeed growth limitation may vary at different time scales and between species (Bonan 1993, Yarie and Van Cleve 2010, Rossi et al. 2011). For example, in 40-year-old Norway spruces in Northern Sweden, six years of soil warming resulted in a significant increase of stem volume in heated plots (115% higher than in control plots), even if no difference in bud break or timing of basal area growth was observed (Strömgren and Linder 2002). However, the effect reported in the latter study on Norway spruce was much higher than that found in a three-year extreme soil-warming (up to 10 °C) experiment with 140-year-old black spruce trees in Alaska (estimated around 33%, Van Cleve et al. 1990, Bonan 1993). This may be due to age, habitat and species differences. Indeed, black spruce is known to be a slow-growing species, often found on poor soils (Bonan 1993, Krause et al. 2010). Moreover, Bonan (1993) suggested that black spruce has a low capacity to respond to increased nitrogen mineralization via increased photosynthesis and estimated that forest floor decomposition and N mineralization rates were the lowest in black spruce stands compared to other boreal coniferous and mixed forest stands he studied in Alaska. This may also due to the competition by soil microbes rapidly immobilizing mineralized N and to the presence of Kalmia sp. in this type of ecosystems, a species which is known to produce a tannin-rich litter which may reduce black spruce growth (Wallstedt et al. 2002, Joanisse et al. 2007, 2009). Moreover other sources of N, like organic N, seem to be available to boreal forest trees (Kielland et al. 2006, 2007, Näsholm et al. 2009) and may limit the impact of increased mineralization on N availability. As Houle and Moore (2008) observed, tree growth may be affected by N availability in the longer term, but in the short term, no significant growth enhancement was evident. These observations agree with the hypothesis advanced by Rossi et al. (2011) who demonstrated that timing of snowmelt, a proxy of air and soil temperature in spring, was strongly related to both xylem phenology and production, but just in the longer term, probably through changes in the rate of nutrient cycling. Indeed, changes in nutrient cycling and its potential effect on tree growth may become significant only in the medium and long term, since trees seem less receptive than other plants and microorganisms to the uptake of inorganic and organic N in the short term (Näsholm et al. 2009).

In this study, anatomical analyses did not show a significant effect of treatments, except for cell wall thickness of earlywood tracheids. Altered cell characteristics have already been observed in trees subject to N additions and warming. Elevated temperatures were reported to increase wood density and result in smaller lumina and an earlier switch to latewood in young Scots pine trees (Kilpeläinen et al. 2007). Many studies on the effect of N on wood anatomy pointed out that N usually increased annual ring width and radial lumen diameter, but reduced cell wall thickness and density (Hättenschwiler et al. 1996, Kostiainen et al. 2004). Our results, on the contrary, showed thicker cell walls in earlywood tracheids of treated trees, including N-enriched trees. However, past experiments have usually been conducted on young trees and with much higher amounts of N than in the present study. Besides, Kostiainen et al. (2004) warned that the effects of elevated nutrients can greatly depend on developmental stage of trees and that there are difficulties in scaling up the responses of young seedlings to mature trees or of growth chamber to natural stands. Moreover the changes commonly observed in wood properties of fertilized trees are suspected to be related to the higher growth rates observed (Mäkinen et al. 2002). In our experiment, no significant difference was found between treatments regarding the number of tracheids produced. Thus, the effect of our treatments on xylem anatomy seems to be independent of growth rate. Wodzicki (1971) proposed that changes in wall thickness were caused by changes in the duration of cell wall material deposition rather than by the rate of deposition. So it is worth underlining that in our experiment treated trees also showed longer duration of xylem differentiation. Whatever the reason is for the difference between previous studies and the present one, our results may have important implications for wood utilization. Cell wall thickness, together with cell width and the relative proportion of latewood and earlywood, is an important determinant of wood density (Lundgren 2004). If, in a future warmer and N-richer environment, cell wall thickness of earlywood becomes thicker, this may affect the pulp and paper industry as well as the wood manufacturing industry.

In many studies, soil warming often resulted in increased decomposition of the forest floor and mineralization of N, in both permafrost and non-permafrost environments (Van Cleve et al. 1990, Rustad et al. 2001). In our study, even if no significant difference in nutrient concentrations in the soil or needles was observed between treatments, the small amount of N added or the potential increase in N availability resulting from soil warming may have been diluted in larger pools of the soil and possibly of canopy components, without significantly changing the concentration at the end of the growing season (David et al. 1998, Houle et al. 1999). Houle and Moore (2008) added 17 kg ha$^{-1}$ year$^{-1}$ (which is two orders of magnitude greater than that used in our experiment) directly to the forest floor in a black spruce stand and after three years of treatment no change in foliar N concentration or tree basal area increment was observed in their low N-treatment. Indeed, despite occasional increases of inorganic N concentrations in the soil solution, the changes were only transitory (less than one week) and no effect in the pool of N in the soil was detectable on a yearly basis (Houle and Moore 2008). Houle and Moore (2008) hypothesized that N was removed from the soil solution mainly as a result of root and/or microbial absorption and, to a lesser extent, as leaching. In short-term studies with $^{15}$N tracers, low recoveries (1-6% of added N) in woody tissues and live foliage have been reported, probably pointing out the existence of other important sinks for N (e.g. soil and epiphytes) than tree uptake (Nadelhoffer et al. 2004, Dail et al. 2009). Frequent and small additions of N, correctly simulating the future N-deposition, could maximize tree canopy uptake as well as the uptake by epiphytes in general, which seem to be very effective in capturing low atmospheric N inputs (Reiners and Olson 1984, Turetsky 2003, Gundale et al. 2011). The absence of significant differences in nutrient concentrations in our study may also be due to the small sample size, the spatial variability of the soils surrounding the experimental trees and the competition by nearby trees.

The novelty of our research was the use of soil warming and the frequent addition of low N doses directly to the canopy of mature trees in two natural stands in order to simulate future conditions expected for the boreal forest. It was demonstrated that soil warming can affect xylem phenology, in particular cambial resumption, especially in roots. Although the sites differed by almost 2 °C in mean annual temperature, no significant effect of the site on xylem phenology was detected by the statistical model, probably due to the high variability observed. Also, the absence of changes in growth increments and in the concentration of nutrients in soil and needles following treatments may be linked to the short-term nature of our experiment and the high variability of natural stands. Moreover, changes in the chemical variables, if any, may have been masked by the large pool of N present in the soil or by the retention by other sinks (e.g. epiphytic lichens). Nonetheless, our investigation demonstrated that, even in the short term (3 years of experimentation), increased soil temperature interacted with simulated N-depositions determining changes in xylem phenology and anatomy of mature black spruce. Thicker earlywood cell walls were observed in treated trees. Cumulative effects of soil warming and N addition on the availability of nutrients by longer-term experiments may produce significant increases in tree growth (e.g. number of tracheids). However, previous fertilization studies using larger and unrealistic rates of nitrogen addition may have overestimated the impact of N-depositions on boreal forest productivity.

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Table 4.1 Diameter at breast height (DBH) and height of treated trees at BER and SIM at the beginning of the experiment (spring 2008). C, control; H, heated only; N, N-enriched only; NH, N-enriched and heated. Data are presented as mean ± standard deviation.

| | BER | | SIM | |
|---|---|---|---|---|
| | DBH (cm) | Height (m) | DBH (cm) | Height (m) |
| C | 17.3±3.0 | 17.1±2.0 | 19.4±3.2 | 15.6±2.0 |
| H | 17.0±2.3 | 15.0±2.5 | 23.5±3.2 | 17.8±1.5 |
| N | 18.7±2.5 | 16.9±2.5 | 18.8±0.8 | 18.5±1.6 |
| NH | 17.0±2.7 | 14.6±3.4 | 20.2±1.3 | 16.8±2.5 |

Table 4.2 Date of onset and ending (DOY) and number of days (in parentheses) of the N-enrichment and heating treatments in 2008-2010 for the two study sites.

| Year | N-enrichment | | Heating | |
|---|---|---|---|---|
| | BER | SIM | BER | SIM |
| 2008 | 179-253 (13) | 171-252 (12) | 115-204 (89) | 102-188 (86) |
| 2009 | 168-259 (14) | 153-258 (16) | 105-210 (105) | 90-195 (105) |
| 2010 | 169-257 (13*) | 151-258 (15) | 103-208 (105) | 88-194 (106) |

* One week irrigation was cancelled due to strong wind and heavy rain

Table 4.3 Ion concentrations (μeq $l^{-1}$) in the artificial rain applied for the N-treatment. Ammonium ($NH_4^+$) and nitrate ($NO_3^-$) concentrations for the N-enriched treatment are reported in parentheses.

| Artificial rain (μeq $l^{-1}$) | | |
|---|---|---|
| $Na^+$ | 2.24 | |
| $Ca^{+2}$ | 5.00 | |
| $Mg^{+2}$ | 1.66 | |
| $K^+$ | 0.76 | |
| $H^+$ | 16.18 | |
| $Cl^-$ | 2.24 | |
| $SO_4^{-2}$ | 23.69 | |
| $NH_4^+$ | 14.93 | (44.78) |
| $NO_3^-$ | 14.93 | (44.78) |

Table 4.4 Chemistry of organic soil and one- and two-year-old needles at BER and SIM measured at the end of the treatment. C, control; H, heated only; N, N-enriched only; NH, N-enriched and heated. Data are presented as mean ± standard deviation.

| | | | | BER | | | | SIM | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | C | H | N | NH | C | H | N | NH |
| Soil | Organic layer | C:N | | 50.2±8.7 | 58.4±10.0 | 54.2±2.8 | 53.5±5.1 | 39.1±9.6 | 38.6±5.9 | 39.6±7.5 | 37.9±3.4 |
| | | pH | | 2.9±0.6 | 2.8±0.3 | 2.7±0.3 | 2.7±0.1 | 2.8±0.2 | 3.1±0.1 | 3.1±0.4 | 3.1±0.4 |
| | | N | (g $kg^{-1}$) | 8.5±0.1 | 8.0±1.5 | 8.9±0.7 | 8.7±1.1 | 9.7±1.1 | 11.1±0.9 | 10.9±1.7 | 10.3±0.6 |
| | | $N_{min}$ | (mg $kg^{-1}$) | 42.3±8.7 | 46.0±7.0 | 40.0±11.3 | 60.0 | 43.0±9.8 | 52.7±13.7 | 40.0±9.2 | 40.0±8.2 |
| | | K | (mg $kg^{-1}$) | 471.0±213.0 | 504.3±177.2 | 401.7±147.3 | 505.7±94.6 | 552.0±112.6 | 523.7±194.2 | 410.3±177.4 | 529.7±139.1 |
| | | Ca | (mg $kg^{-1}$) | 2636.7±2025.7 | 2260.0±980.6 | 2920.0±1437.1 | 2030.0±737.3 | 1893.3±602.5 | 1830.0±862.6 | 1503.3±502.4 | 1346.7±263.5 |
| | | Mg | (mg $kg^{-1}$) | 444.0±96.6 | 366.3±92.4 | 356.7±128.9 | 402.3±119.3 | 332.0±106.2 | 282.3±39.2 | 276.7±75.0 | 328.0±48.7 |
| Needles | 1-year | N | (g $kg^{-1}$) | 6.3±0.4 | 7.0±0.8 | 8.0±0.5 | 6.7±0.5 | 7.5±0.6 | 7.9±0.6 | 8.2±1.1 | 7.8±0.4 |
| | | P | (g $kg^{-1}$) | 1.5±0.2 | 1.7±0.2 | 1.4±0.3 | 1.5±0.2 | 1.3±0.2 | 1.4±0.2 | 1.5±0.2 | 1.4±0.0 |
| | | K | (g $kg^{-1}$) | 7.3±1.7 | 7.2±1.0 | 6.7±1.4 | 7.1±0.9 | 5.4±1.6 | 6.5±1.2 | 6.7±1.8 | 7.3±0.3 |
| | 2-years | N | (g $kg^{-1}$) | 7.0±0.9 | 7.2±0.5 | 7.8±0.3 | 6.9±1.8 | 7.7±0.9 | 8.5±0.6 | 8.0±0.6 | 8.2±1.0 |
| | | P | (g $kg^{-1}$) | 1.3±0.3 | 1.6±0.4 | 1.3±0.4 | 1.4±0.4 | 1.1±0.1 | 1.3±0.2 | 1.1±0.2 | 1.3±0.1 |
| | | K | (g $kg^{-1}$) | 5.7±1.0 | 6.3±1.5 | 6.0±1.1 | 5.9±0.9 | 4.7±1.4 | 6.0±0.8 | 5.4±1.8 | 6.2±0.7 |

Table 4.5 Anatomical characteristics of the 2010 tree ring in stem and root at BER and SIM, presented by treatment, for earlywood, latewood and the whole ring. The variables presented in the table are the number of observations used for the analysis (No. obs.), single cell wall thickness (WT), tracheid width (TW), percentage of earlywood and latewood, number of tracheids (NT) in earlywood, latewood and the whole ring. C, control; H, heated only; N, N-enriched only; NH, N-enriched and heated. Values are presented as mean ± standard deviation.

| | | | BER | | | | SIM | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | C | H | N | NH | C | H | N | NH |
| Stem | No. obs. | | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | Early-wood | WT (μm) | 2.4±0.5 | 2.6±0.6 | 2.7±0.4 | 2.6±0.4 | 2.1±0.1 | 2.6±0.3 | 2.3±0.5 | 2.1±0.1 |
| | | TW (μm) | 24.9±5.9 | 26.6±4.0 | 29.6±1.4 | 24.6±2.4 | 26.4±2.6 | 29.9±4.4 | 26.3±6.2 | 26.7±3.9 |
| | | % EW | 76.6±2.2 | 76.7±5.5 | 77.4±3.7 | 71.7±13.2 | 77.1±4.7 | 76.4±5.8 | 73.0±6.9 | 73.2±4.4 |
| | | NT | 10.2±2.2 | 13.0±7.9 | 10.8±0.4 | 14.2±11.0 | 13.7±7.7 | 13.9±2.9 | 17.8±11.8 | 12.4±4.5 |
| | Late-wood | WT (μm) | 3.8±0.7 | 3.8±0.9 | 4.3±0.5 | 4.1±1.1 | 3.8±0.3 | 4.5±0.8 | 4.6±1.1 | 4.2±0.8 |
| | | TW (μm) | 12.8±1.3 | 13.7±2.3 | 13.7±1.3 | 13.4±1.4 | 13.4±2.6 | 15.7±3.5 | 15.7±2.8 | 13.4±3.1 |
| | | % LW | 23.4±2.2 | 23.4±5.5 | 22.6±3.7 | 28.3±13.2 | 22.9±4.7 | 23.6±5.8 | 27.0±6.9 | 26.8±4.4 |
| | | NT | 6.1±1.3 | 7.4±4.8 | 6.7±0.4 | 7.4±2.7 | 7.9±4.7 | 7.9±5.1 | 9.6±3.7 | 9.4±4.7 |
| | Ring | NT | 16.2±3.4 | 20.4±12.5 | 17.5±0.7 | 21.6±13.7 | 21.6±12.3 | 21.8±7.1 | 27.4±15.3 | 21.8±9.1 |
| Root | No. obs. | | 2 | 1 | 3 | 2 | 2 | 3 | 2 | 2 |
| | Early-wood | WT (μm) | 2.1±0.1 | 2.8 | 2.9±0.2 | 3.6±0.8 | 2.5±0.1 | 2.8±0.3 | 2.9±0.1 | 2.4±0.1 |
| | | TW (μm) | 25.2±2.2 | 28.9 | 28.2±3.8 | 33.7±2.5 | 23.4±8.6 | 29.3±2.5 | 26.1±4.2 | 26.4±1.8 |
| | | % EW | 71.4±8.7 | 78.7 | 67.0±4.9 | 64.7±27.7 | 65.5±16.5 | 59.7±3.2 | 55.3±9.0 | 75.3±6.8 |
| | | NT | 7.6±3.7 | 6.8 | 5.3±4.8 | 9.8±10.8 | 2.0±1.4 | 7.1±3.7 | 4.0±2.8 | 2.2±1.2 |
| | Late-wood | WT (μm) | 3.4±1.0 | 4.1 | 3.9±0.7 | 4.5±0.9 | 2.8±0.4 | 4.0±0.4 | 3.7±0.7 | 2.8±0.4 |
| | | TW (μm) | 13.3±2.7 | 14.3 | 17.0±4.4 | 16.6±0.9 | 11.3±1.1 | 14.9±2.3 | 15.5±5.3 | 11.0±1.5 |
| | | % LW | 28.6±8.7 | 21.3 | 33.0±4.9 | 35.3±27.7 | 34.5±16.5 | 40.3±3.2 | 44.7±9.0 | 24.7±6.8 |
| | | NT | 4.7±1.2 | 3.7 | 3.7±2.3 | 6.0±0.7 | 1.8±0.5 | 7.7±3.7 | 5.8±5.2 | 1.4±0.1 |
| | Ring | NT | 12.3±2.5 | 10.5 | 9.0±7.1 | 15.8±11.5 | 3.8±1.9 | 14.7±7.4 | 9.8±8.0 | 3.6±1.3 |

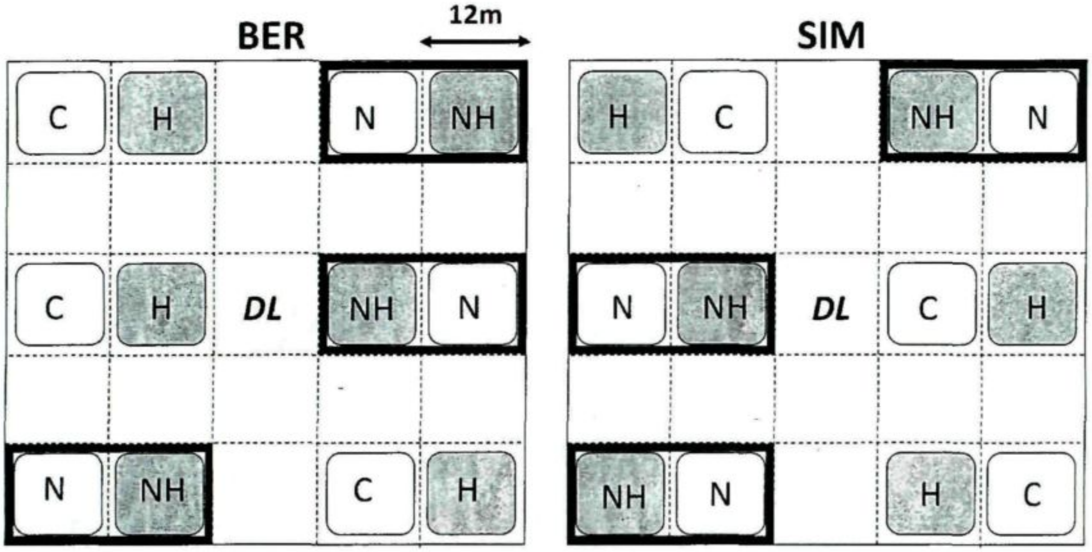

Figure 4.1 Experimental design at BER and SIM. C, control; H, heated only; N, N-enriched only; NH, N-enriched and heated; DL, datalogger. The N-enriched plots (N and NH) are surrounded by a black rectangle. The heated plots have a gray background (H and NH).

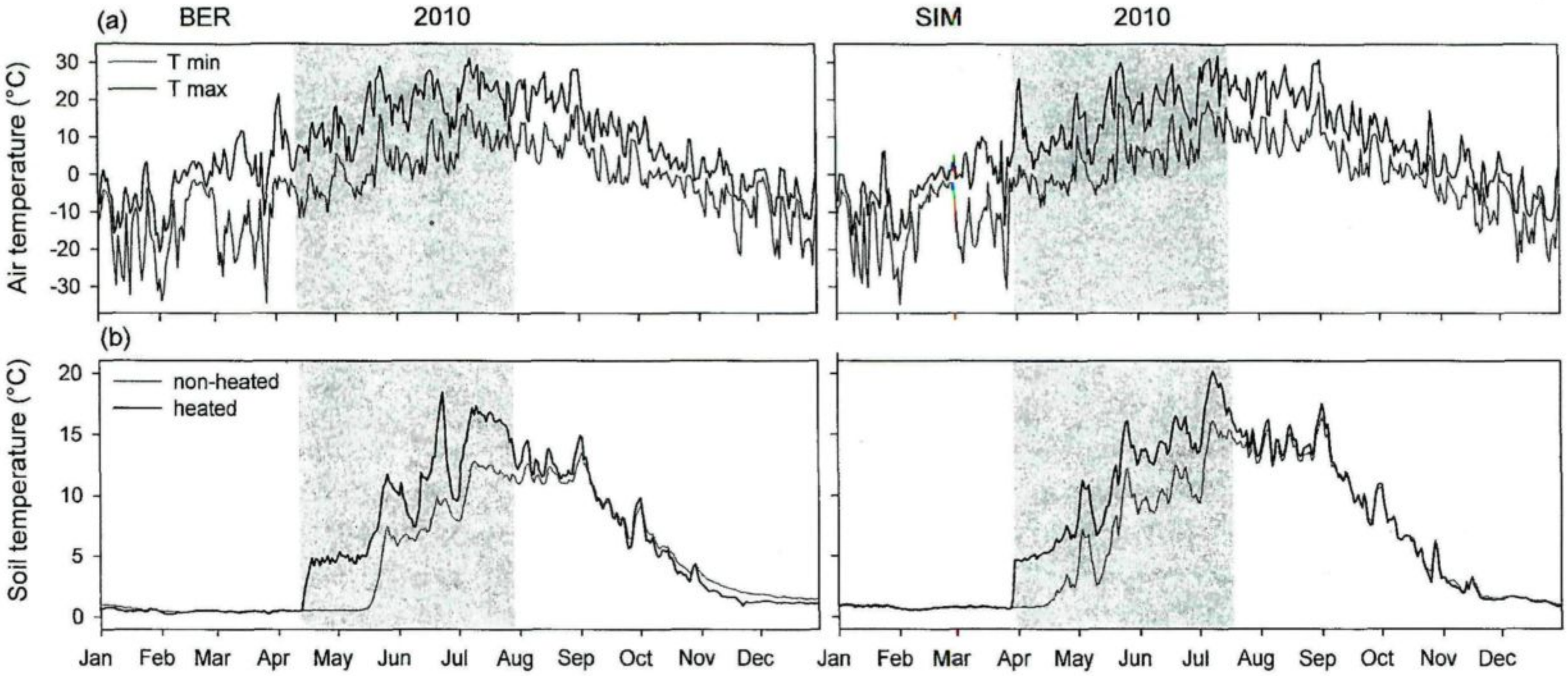

Figure 4.2 Daily air (minimum and maximum) and soil temperatures (in non-heated and heated plots) at BER and SIM in 2010. Gray background indicates the period during which the H-treatment was applied.

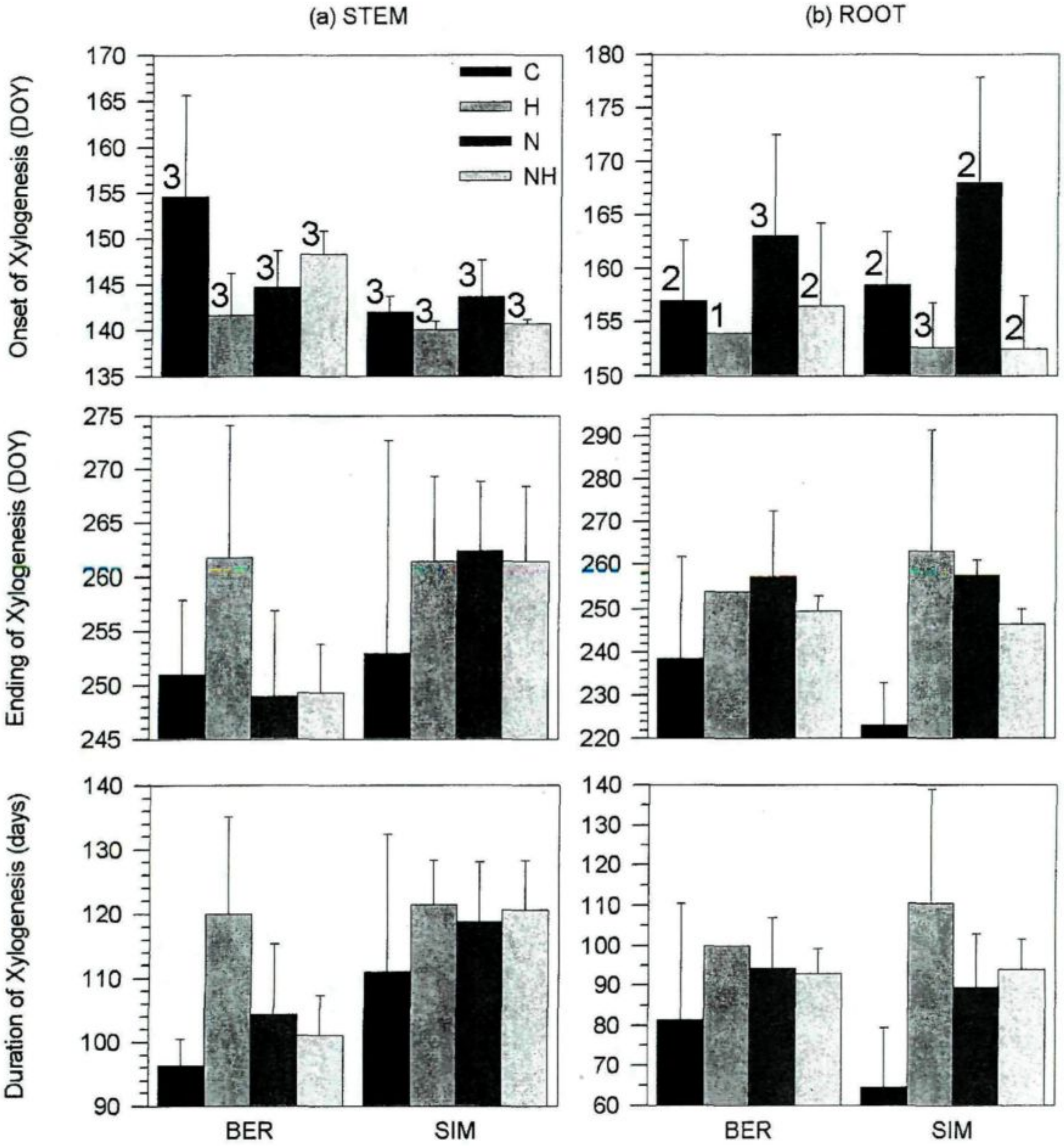

Figure 4.3 Onset, ending and duration of xylogenesis in (a) stem and (b) roots at BER and SIM in 2010. C, control; H, heated only; N, N-enriched only; NH, N-enriched and heated. Error bars represent the standard deviation. The number of observations used for the analyses is reported over the bar in the upper part of the figure.

# Chapitre V

# Conclusion générale

## 5. Conclusion générale

Le but de cette thèse a été de comprendre de quelle manière la formation du cerne ligneux et la productivité en termes de bois de l'épinette noire pourront varier dans le futur sous l'influence du réchauffement climatique et de l'augmentation de dépôts azotés. La recherche a eu lieu dans deux peuplements équiennes issus de feu constitués d'épinettes noires adultes, dans la région du Saguenay-Lac-St-Jean, au Québec (Canada).

### 5.1. Principaux mérites et limites de l'étude

Notre étude a eu le mérite d'essayer de représenter de manière réaliste les mécanismes (p.ex. absorption foliaire) qui agissent avec les dépôts azotés atmosphériques (Sparks 2009). Par rapport à d'autres méthodes de chauffage, l'utilisation des câbles chauffants permet un contrôle précis sur la température du sol ciblée et le chauffage d'une surface suffisante, nécessaire dans le cas des arbres adultes (Strömgren & Linder 2002). En plus, nous avons étudié pour la première fois, à notre connaissance, la xylogenèse des arbres soumis à de tels traitements non seulement dans les tiges, mais aussi dans les racines. À ce jour, il y a très peu d'études qui ont décrit la phénologie racinaire (Steinaker & Wilson 2008, Thibeault-Martel et al. 2008) et aucune avec des arbres sujets à des traitements qui simulent des perturbations liées aux prévisions futures de réchauffement climatique et d'augmentation des dépôts azotés (Houle et al. 2002, Galloway et al. 2004, Sushama et al. 2006). De plus, l'analyse chimique des sols et du feuillage a permis de mettre en évidence la nécessité de prévoir dans le futur des analyses plus fines et précises, comme l'étude de la dynamique saisonnière de l'azote dans différents compartiments pendant toute la saison de croissance (sols, lichens et épiphytes, feuillage mais aussi le xylème et le phloème) et l'utilisation des isotopes ($^{15}$N) pour reconstruire le destin de l'azote dans l'arbre et en général dans l'écosystème. Notre étude a aussi le mérite d'avoir essayé de simuler les perturbations futures avec des arbres adultes en forêts non-aménagées. En effet, beaucoup des recherches dans le passé ont été conduites sur des semis ou des jeunes arbres en conditions contrôlées (p.ex. serre et chambre de croissance), avec des difficultés à extrapoler les résultats à des conditions naturelles et, en particulier, aux forêts matures. Enfin, nous avons choisi les niveaux des traitements en se basant sur des scénarios vraisemblables de dépôts azotés et de température du sol prévus pour le futur pour la zone étudiée (et pour une grande partie du nord-est de l'Amérique du Nord).

Notre recherche a aussi certaines limites qui suggèrent une interprétation prudente des résultats. D'abord nous n'avons pas augmenté la température de l'air ni la quantité de dioxyde de carbone, qui pourrait stimuler directement la croissance de l'arbre (effet de fertilisation carbonique) ou influencer sa réponse aux facteurs environnementaux (comme l'azote et l'eau). Cependant, il faut souligner que les coûts prohibitifs et les difficultés techniques empêchent actuellement d'augmenter la température de l'air en travaillant en forêt naturelle avec des arbres adultes. En ce qui concerne le $CO_2$, une revue de littérature récente a mis en évidence que pour les environnements où la croissance des arbres est limitée par la température, il n'y avait pas de preuves qui supportaient l'hypothèse de la fertilisation carbonique (Huang et al. 2007). De plus Körner (2006) suggère que dans des forêts fermés et matures, comme la notre, la stimulation de la croissance suite à une hausse de 160 à 200 ppm de $CO_2$ par rapport aux conditions actuelles semble être nulle ou beaucoup moins importante que celle attendue selon des études en conditions contrôlées et sur des jeunes plantes. Ainsi, après 9 ans d'enrichissement de l'air avec de la $CO_2$ (expérience FACE à la limite supérieure de la forêt en Suisse), aucun stimulation de la croissance radiale ou de la formation de nouveau feuillage n'était observée chez des arbres matures de *Pinus mugo* ssp. *uncinata* et la réponse initiale chez une espèce décidue comme le mélèze ne semblait pas perdurer dans le temps (Dawes et al. 2011).

Notre expérience est de courte durée rendant difficile l'interprétation des processus qui agissent à plus long terme (Chapitre III). De plus des espèces à croissance lente, comme l'épinette noire, pourraient demander davantage de temps pour réagir aux traitements par rapport à des espèces à croissance plus rapide (Aerts 1995, Patterson et al. 1997, Reich et al. 1998). La variabilité entre les plantes au niveau de la croissance (surtout dans le site plus chaud) et la petite dimension de l'échantillon pourraient avoir réduit notre capacité à mettre en évidence des différences significatives pendant l'étude ou des tendances futures à court terme (ex. effet du chauffage dans la tige, Chapitre III). Ceci pourrait être amélioré dans des expériences futures en augmentant le nombre d'arbres par site. Toutefois il reste que la grande variabilité génétique intra-peuplement de l'épinette noire ne peut pas être contrôlée efficacement dans des peuplements naturels comme ceux que nous intéressait d'étudier (Isabel et al. 1995, Perry & Bousquet 2001, Rajora & Pluhar 2003, Bouillé et al 2011, Prunier et al. 2011).

Enfin, la technique d'échantillonnage nous permet d'utiliser seulement des racines très proches de la surface et d'une certaine dimension. Le fait que plusieurs racines (presque un tiers de toutes les racines échantillonnées) n'aient pas formé un cerne de croissance pendant la dernière année de l'expérience (Chapitre IV), nous a forcés a utiliser un design expérimental déséquilibré qui nous oblige à interpréter ces résultats avec prudence. Dans des expériences futures d'étude sur la xylogenèse des racines, nous conseillons, si possible, d'échantillonner au moins deux racines par arbre par année tout en évaluant par un échantillonnage préliminaire si les racines sont actives dans la croissance secondaire (Coutts & Lewis 1983).

### 5.2. La xylogenèse de l'épinette noire dans les conditions actuelles

Dans les deux sites étudiés (BER et SIM) la dynamique de formation du cerne de croissance était similaire. Toutefois, en général, le début de l'activité cambiale et de la formation du cerne était significativement différent entre les sites et les années

(Chapitre II). La différence entre les sites, d'environ deux à trois semaines, dans la date de fonte de la neige, se reflétait par un délai d'une à deux semaines dans le début de l'activité cambiale observé dans la tige des arbres témoins. Ceci suggérait une possible influence de la date de fonte de la neige sur la réactivation cambiale et potentiellement sur la production de bois (Vaganov et al. 2011, Kirdyanov et al. 2003, Rossi et al. 2011a). La date de fin de la xylogenèse était assez variable d'un arbre à l'autre et aucune différence significative n'était mise en évidence entre sites et années (Chapitre II), suggérant que la phase de formation de la paroi secondaire et de lignification est moins sujette à l'influence de la variabilité climatique interannuelle. La variabilité entre les arbres pourrait être en partie expliquée par la variabilité génétique (Bouillé et al. 2011). Ainsi en utilisant de plantations de différents taxa de mélèze génétiquement sélectionnées, Gauchat (2011) a observé que au niveau de la xylogenèse les différences plus importantes entre familles étaient au niveau de la date de fin de la formation du bois. Dans les conditions actuelles, la xylogenèse dans les sites étudiés variait entre 77 et 126 jours, en accord avec des études précédentes sur des espèces des milieux froids (Deslauriers et al. 2003, Rossi et al. 2007, Rossi et al. 2011a). La durée était significativement différente entre les années, principalement à cause d'une différence dans le début de la saison de croissance (Chapitre II et III), en accord avec les observations de Deslauriers et al. (2008) sur le *Pinus leuçodermis* Ant. dans des environnements limités par les basses températures (limite altitudinale).

Dans certains écosystèmes, les précipitations sont aussi un facteur important dans le contrôle de la durée de la saison de croissance (Pichler & Oberhuber 2007, Gruber et al. 2010a). Dans des endroits sujets à la sécheresse, des périodes sèches sont associées à des cernes étroits et à des fins hâtives de la croissance (Giovannelli et al. 2007, Gričar & Čufar 2008, Gruber et al. 2010a). En affectant la production de bois, le stress hydrique peut ainsi indirectement déterminer la fin de la croissance (Antonova & Stasova 1993). Toutefois, dans les sites étudiés, la fonte printanière de la neige et les précipitations régulièrement distribuées pendant l'été (en moyenne, 415 mm et 95 jours de pluie de mai à septembre pour les années étudiés) semblent garantir les besoins en eau des arbres malgré la capacité réduite de rétention hydrique des sols (Chapitre II).

À cause de la variabilité plus élevée dans les accroissements des arbres du site plus chaud (plus au Sud et moins en altitude), aucune différence significative entre les deux sites n'a été mise en évidence concernant l'accroissement radial (nombre de cellules des cernes de croissance), même si en général moins de cellules étaient présentes dans les cernes du site plus froid (Chapitre II). Le site plus chaud était aussi celui dans lequel on observait en général des concentrations plus élevées en azote total dans les sols et dans les aiguilles des arbres, même si la différence n'était pas statistiquement significative (Chapitre IV). Le site plus chaud était aussi celui dans lequel le peuplement était le plus jeune (86 vs 138-143 ans). Même si Rossi et al. (2008a) ont mis en évidence que la xylogenèse pourrait être influencée par l'âge et/ou la dimension des arbres, dans leur étude la différence était beaucoup plus marquée (50-80 vs 200-350 ans) et, ainsi, dans notre design expérimental on ne s'attend pas à un effet significatif de l'âge.

### 5.3. La relation entre phénologie de la xylogenèse et production du bois

Notre étude (Chapitre II) a mis en évidence comment un début plus hâtif de la xylogenèse correspondait à un nombre total de cellules du xylème plus élevé dans le cerne de croissance, tandis que le nombre total de cellules était positivement corrélé avec la fin de la xylogenèse. Ainsi, un nombre plus élevé de cellules était lié à un début plus hâtif et une fin plus tardive de la différentiation cellulaire. La relation était plus forte avec le début (r=0.74) qu'avec la fin (r=0.61) de la xylogenèse. L'absence d'une relation directe entre début et fin de la xylogenèse, indiquait que c'était bien le nombre de cellules qui modifiait les rapports entre ces deux phases de la xylogenèse.

La relation causale entre la production cellulaire et la date de fin de la croissance radiale mise en évidence dans cette thèse (Chapitre II) a fourni une nouvelle clé d'interprétation de certains mécanismes de formation du cerne de croissance et des observations que les arbres plus jeunes, vigoureux ou fertilisés produisent plus de cellules et terminent plus tard leur croissance (Peltola et al. 2002, Deslauriers et al. 2003, Schmitt et al. 2003). La production plus élevée en terme de bois observée dans les arbres plus jeunes (Rossi et al. 2008a) ou à des plus basses altitudes et latitudes (Moser et al. 2010, Rossi et al. 2011b) devrait donc allonger la période requise pour compléter la maturation du xylème, prolongeant ainsi la période de formation du bois. On peut avancer l'hypothèse que cette fenêtre temporelle plus longue pour la xylogenèse soit une des causes de la dilution du signal climatique, qui se base sur la formation du cerne de croissance en entier, et de la réduction des relations climat-croissance observée dans les arbres plus jeunes, qui ont une plus longue saison de croissance (Carrer & Urbinati 2004), et dans les arbres plus vigoureux ou dans les sites plus favorables.

Dans les conifères des forêts boréales et tempérées, des températures plus élevées au printemps et, en général, dans la première partie de la saison de croissance, alors que le bois initial est formé, stimulent une réactivation cambiale hâtive et la production cellulaire (Chapitre II, Rossi et al. 2012). De plus, selon nos résultats, la production cellulaire peut influencer la fin de la xylogenèse, et, ainsi, potentiellement établir un lien indirect avec la température dans la première partie de la saison de croissance (Chapitre II, Rossi et al. 2012). D'ailleurs, certains auteurs ont observé qu'un traitement de refroidissement de la tige influençait le développement du xylème en réduisant la largeur des cernes de croissance et raccourcissant la période de l'activité cambiale (Gričar et al. 2007). De plus, Thibeault-Martel et al. (2008) observaient des cernes plus larges dans les racines que dans la tige, et, en accord avec notre modèle, des fins plus tardives de la lignification des parois cellulaires. Donc, un nombre plus élevé de cellules produites entraîne de plus importantes accumulations de cellules en différentiation dans le xylème en formation. Dans le bois final des conifères, bien que l'élargissement des cellules soit très rapide et complété en peu de jours, l'épaississement des parois et la lignification sont le résultat d'une longue et coûteuse (en terme de sucres et d'éléments nutritifs) déposition de cellulose et de lignine dans la paroi secondaire, qui peut durer jusqu'à 40 jours (Deslauriers et al. 2003, Oberhuber et al. 2011).

En dépit du besoin continu d'énergie pour accomplir la croissance radiale, l'activité cambiale est un des puits pour les glucides avec la plus basse priorité dans l'arbre, étant précédée par la croissance primaire, la respiration, la croissance des racines et l'accumulation des réserves (Oribe et al. 2003, Polák et al. 2006, Deslauriers et al. 2009). Les délais dans la terminaison de la maturation des cellules correspondant à un nombre plus élevé de cellules confirment que la croissance secondaire, en particulier la différentiation cellulaire dans le xylème, est donc limitée par la disponibilité d'énergie et de ressources nécessaires pour compléter la xylogenèse (Chapitre II). Pour appuyer notre interprétation, Oberhuber et al. (2011) ont récemment mis en évidence que l'utilisation des sucres liée à la xylogenèse montrait deux pics, un au début de la saison de croissance, au printemps (quand beaucoup de cellules sont produites), et l'autre pendant la formation du bois final, en été (quand beaucoup d'énergie est demandée pour terminer l'épaississement des parois secondaires et la lignification).

Dans les écosystèmes forestiers froids, le réchauffement climatique étendra sans doute la période thermiquement favorable à la disposition des arbres pour leur croissance et les effets les plus prononcés sont attendus pour les zones boréales de la planète (Menzel 2000, Chmielewski & Rötzer 2001). En se basant sur un modèle phénologique « piloté » par la température, Rossi et al. (2011) ont calculé des augmentations de 8-11 jours par degré Celsius (°C) dans la période de la xylogenèse. Grâce à notre modèle, nous pouvons affirmer que dans le futur, des printemps plus chauds devraient amener à une réactivation cambiale plus hâtive, et, ainsi, augmenter la production cellulaire et prolonger la maturation des cellules en automne. Par conséquent, nos résultats suggèrent que le réchauffement climatique attendu pour le futur pourrait entre autres influencer la phénologie de formation du xylème en agissant sur le début de la xylogenèse et sur la production de cellules (Chapitre II).

### 5.4. L'impact de la fonte de la neige et de la température du sol sur la xylogenèse

Notre recherche a confirmé en partie le rôle de la température du sol sur l'activité cambiale et la phénologie du xylème. Même si dans le Chapitre III aucune différence significative (soit au niveau de la durée de la xylogenèse soit du nombre de cellules produites) n'était mise en évidence entre arbres chauffés et arbres témoins, on observait une durée en moyenne plus longue dans les arbres chauffés et la différence en nombre de cellules entre arbres chauffés et non augmentait au fil des années de l'expérience (Figure 5.1), soulignant la probable existence d'un effet à long terme. Les résultats du Chapitre III diffèrent un peu des résultats du Chapitre IV, dans lequel on trouve une différence significative au niveau soit du début que de la durée de la xylogenèse, même si encore une fois il n'y a pas de différences significatives dans le nombre de cellules entre arbres chauffés et arbres témoins. Il faut toutefois souligner que dans les deux chapitres différents analyses statistiques sur des jeux de données différents (les trois années de mesures sur les tiges des arbres seulement chauffés et des arbres témoins dans le Chapitre III vs les mesures de l'année 2010 sur les tiges et les racines des tous les arbres du design dans le Chapitre IV) ont été conduites, ce qui peut en partie expliquer les différents résultats. La différence entre les arbres témoins et les arbres traités était particulièrement marquée dans les racines (augmentation de la durée de 18 jours dans les arbres chauffés), directement influencées par le chauffage du sol. La durée de la croissance des tiges (+ 8 jours dans les arbres chauffés) était moins influencée par le traitement, ce qui, ensemble avec le fait que en 2008 on avait à peu près la même durée dans les tiges chauffées et les tiges témoins, peut en partie expliquer pourquoi l'analyse à mesure répétées faite dans le Chapitre

III seulement sur les tiges n'ait pas mis en évidence des différences significatives même si, sauf pour le 2008, la xylogenèse a été plus longue de 18 jours en moyenne dans les arbres chauffés. Nos résultats démontrent donc que le chauffage du sol peut provoquer une modification de la phénologie de formation du xylème, pas seulement dans les racines (effet localisé) mais aussi dans la tige, probablement à travers une absorption et un transport améliorés des nutriments et de l'eau (Alvarez-Uria et al. 2007). Qui plus est, plusieurs études observent une relation positive entre la température du sol et la photosynthèse: une plus grande quantité de sucres, utilisés par le cambium pendant la saison de croissance, pourrait être disponible dans les arbres qui poussent sur des sols plus chauds et, par ricochet, stimuler la croissance dans la tige. (Gruber et al. 2010b, Wieser et al. 2010, Oberhuber et al. 2011).

Nos observations nous suggèrent que le contrôle de l'activité cambiale et la formation du bois dans la tige et les racines pourrait dépendre en partie des différentes variables, c.à.d. que la température de l'air pourrait être plus importante pour la tige tandis que la température du sol le serait pour les racines. Ainsi, selon Steinaker et al. (2008) une température de l'air de 3°C plus haute que celle du sol a eu comme effet une reprise de la croissance dans les parties aériennes de 15 jours plus hâtive par rapport aux racines. La température de la zone racinaire semble avoir un impact sur beaucoup de processus physiologiques (comme la photosynthèse et la respiration; Van Cleve et al. 1990, Wieser et al. 2010) et pourrait ainsi influencer indirectement la xylogenèse dans la tige (Chapitres III et IV). Par exemple, le chauffage du sol et la fertilisation augmentaient la croissance dans la tige de l'épinette de Norvège dans une expérience de fertilisation de longue durée dans le Nord de la Suède, avec des augmentations significatives de la croissance en volume à partir de la deuxième année de chauffage (Strömgren & Linder 2002). Qui plus est, plusieurs études ont observé une différente allocation du C entre les parties aériennes et les racines des plantes en lien avec la température du sol (Strömgren & Linder 2002, Majdi & Ohrvik 2004, Vogel et al. 2008, Kane & Vogel 2009). Enfin, une étude combinant fertilisation avec de l'azote et réchauffement du sol démontrait, pour l'activité racinaire, l'existence d'interactions entre la disponibilité d'azote et la température du sol (Majdi &Öhrvik 2004).

À court terme, la variation de la production des cellules semble être principalement liée au début de la réactivation cambiale et aux conditions favorables dans la première partie de la saison de croissance (Lupi et al. 2010), tandis qu'à long terme d'autres facteurs peuvent affecter la croissance, comme par exemple la disponibilité des nutriments (Jarvis & Linder 2000, Rossi et al. 2011a). Ainsi, à long terme, des augmentations dans la température du sol et des fontes de la neige plus hâtives pourraient augmenter la production de bois dans la tige, même si parfois aucun effet significatif ne peut être observé à court terme (Vaganov et al. 1999, Jarvis & Linder 2000, Rustad et al. 2001, Jyske et al. 2011, Rossi et al. 2011a). À l'appui de cette interprétation, nous avons observé une certaine tendance, c.à.d. que la différence, entre les arbres chauffés et les arbres témoins, en nombre de cellules produites dans les tiges, a augmenté au cours des trois années de l'expérience de façon exponentielle, même si aucune différence statistiquement significative n'a pu encore être mise en évidence à l'aide de l'analyse à mesures répétées (Figure 5.1, Chapitre III). Étant donné que les effets du chauffage pourraient être liés en partie à des modifications des cycles de nutriments par une augmentation graduelle de la disponibilité en azote (Rustad et al. 2001), on s'attend à ce que des différences significatives dans la production de bois soient mises en évidence avec la prolongation future de l'expérience.

### 5.5. Les dépôts azotés et la disponibilité en azote : effets sur la croissance

Dans notre expérience, aucune différence significative dans les concentrations des nutriments dans le sol et les aiguilles n'a été observée entre les traitements y compris l'apport de N (Chapitre IV). Toutefois, la petite quantité d'azote ajoutée au niveau de la canopée ou la potentielle augmentation de la disponibilité en azote dans le sol (liée à la stimulation de la minéralisation par le traitement de chauffage au niveau du système racinaire) pourraient avoir été diluées dans l'azote du sol et de la canopée sans changer significativement la concentration des éléments nutritifs à la fin de la saison de croissance (David et al. 1998, Houle et al. 1999). Ainsi, après trois ans d'ajout d'azote (au sol) sous-forme de $NH_4NO_3$, simulant une forte augmentation dans les dépôts azotés (3× et 10× plus), Houle & Moore (2008) n'observaient aucune différence persistante dans les concentrations d'azote inorganique entre les parcelles fertilisées et les parcelles témoins dans des sites dominés par le sapin baumier et l'épinette noire dans l'est du Canada. Ils supposaient que l'azote était soustrait de la solution du sol principalement à cause de l'absorption des racines et/ou des microorganismes et, dans une moindre mesure, à cause du lessivage. Ceci dit, ce n'est peut-être pas surprenant que les petits et fréquents ajouts d'azote appliqués dans nos sites n'aient pas influencé les concentrations d'azote dans le sol et les aiguilles. Dans des études avec des traceurs de $^{15}N$ appliqués par hélicoptère, des faibles quantités (1-6% de l'azote ajouté) se retrouvaient dans les tissus ligneux et dans le feuillage vivant, soulignant l'existence à court terme d'autres puits pour l'azote (p.ex. les épiphytes) plus forts que l'absorption des arbres (Nadelhoffer et al. 2004, Dail et al. 2009). De plus ces petites quantités d'azote captées ne stimulaient pas beaucoup l'assimilation de C des arbres et se retrouvaient en très faible quantités dans les tissus ligneux, amenant les auteurs à suggérer que la rétention de l'azote au niveau de la canopée pouvait être le résultat d'un processus physico-chimique (adsorption) plutôt que l'effet d'une vraie absorption et assimilation par l'arbre (Dail et al. 2009).

Dans une grande majorité d'études, l'azote est ajouté directement au sol et très rarement au niveau de la canopée, à des quantités très élevées et avec peu d'applications (trois ou quatre fois par année ou même moins fréquemment), ce qui représente mal les processus naturels (Gaige et al. 2007, Dail et al. 2009). Plus de 80% de la zone boréale reçoit 4 kg $ha^{-1}$ $an^{-1}$ ou moins sous forme de dépôts azotés, tandis que les études du passé ont souvent ajouté des quantités de 1 à 3 ordres de grandeur plus élevées (de Vries et al. 2009, Gundale et al. 2011). Des doses plus basses d'azote et des applications plus fréquentes directement au niveau de la canopée (comme dans notre expérience) simuleraient mieux les dépôts atmosphériques naturels et prendraient en compte les mécanismes potentiels d'absorption foliaire. Toutefois des ajouts fréquents et modérés, qui simulent correctement les dépôts azotés actuels et ceux attendus pour le futur, maximisent non seulement l'absorption à travers la canopée mais aussi l'absorption des épiphytes, qui semblent être très efficaces dans la captation des inputs atmosphériques d'azote à faible dose (Reiners & Olson 1984, Turetsky 2003, Gundale et al. 2011). Ainsi, l'absence de différences significatives dans les concentrations de nutriments de notre étude (Chapitre IV) peut être aussi liée à une augmentation de la rétention de l'azote par des composants de l'écosystème autres que les arbres (ex. épiphytes). Également, l'absence d'effets des traitements sur la composition chimique des sols et des aiguilles pourrait être en partie aussi liée à la petite dimension et à la variabilité de l'échantillon (Chapitre IV). Enfin, des changements dans le cycle de nutriments et ses effets potentiels sur la croissance des arbres pourraient être visibles seulement à moyen et long terme, étant donné que les arbres semblent être moins efficaces que les autres plantes et les microorganismes dans l'absorption de l'azote à court terme, mais qu'ils sont capables d'en accumuler de plus en plus au fil du temps (Nasholm et al. 2009).

D'ailleurs, l'interaction significative du traitement d'irrigation azotée et de réchauffement du sol sur la durée de la xylogenèse confirme le rôle important à la fois de l'azote et de la température du sol sur la croissance. Il reste toutefois à comprendre de quelle façon cet azote a pu agir. Une hypothèse pourrait être avancée: l'azote pourrait avoir stimulé la production de nouvelle biomasse foliaire, augmentant ainsi légèrement la photosynthèse et la disponibilité des sucres, stimulant l'activité cambiale, assez pour influencer la durée de la xylogenèse sans modifier la concentration moyenne d'azote dans les aiguilles. Pour tester cette hypothèse, il faudrait être en mesure de reconstruire comment les traitements pourraient avoir modifié la biomasse foliaire, par rapport à la période avant l'expérimentation. Luszczynski (2009) a mis en évidence que, au cours d'une défoliation causée par la tordeuse des bourgeons de l'épinette (TBE), les branches présentaient une réduction de croissance qui survenait 1 à 2 ans plus tôt que celle de l'accroissement radial. En plus, au niveau de la tige, il y avait un délai de réduction de l'accroissement radial entre la cime et la base de l'arbre. Donc, en raisonnant à l'inverse, on peut avancer l'hypothèse qu'une augmentation de la biomasse foliaire devrait se refléter en premier dans les branches. Une façon indirecte de procéder pourrait consister à comparer les cernes de croissance des branches produites après le début de l'expérience avec les cernes de croissance avant l'expérience, entre les différents traitements, étant donné qu'une augmentation de la surface photosynthétique devrait se refléter dans une augmentation de la croissance des branches.

Enfin, dans notre expérience, avec des épinettes matures en forêt boréale non-aménagée, les traitements de chauffage et de précipitations azotées n'ont pas eu d'effet sur l'anatomie du bois, sauf dans le site plus froid au niveau de la largeur de la paroi cellulaire du bois initial, qui était légèrement plus épaisse dans les arbres traités. Cette différence significative est difficile à expliquer et en partie en contraste avec la majorité des études précédentes. En effet, plusieurs études sur l'effet de l'azote sur la formation du bois ont mis en évidence que l'azote pouvait réduire l'épaisseur de la paroi cellulaire (Hättenschwiler et al. 1996, Kostiainen et al. 2004, Kaakinen et al. 2007, Wielinga et al. 2008). La fertilisation azotée à des niveaux élevés peut amener à la production de cernes de croissance plus larges, surtout à cause d'une augmentation du bois initial et à une augmentation de la largeur du lumen (Kostiainen et al. 2004). Toutefois, la majorité des études ont étés faits sur des jeunes semis et/ou avec des concentrations élevées d'azote. De plus, Kostiainen et al. (2004) mettent en garde que les effets d'une disponibilité accrue de nutriments dépendent en grande partie du stade de développement de l'arbre et qu'il y a des dangers à extrapoler les réponses des jeunes semis à des arbres adultes

### 5.6. Leçons tirées de l'ensemble des trois chapitres

À travers les chapitres II, III et IV nous avons pu confirmer le rôle fondamental de la température pour la réactivation cambiale en forêt boréale (Tableau 5.1). L'impact d'un début hâtif sur la production de bois en termes de nombre de cellules a été démontré dans le Chapitre II. Analysant ensemble les résultats des trois chapitres, on peut tirer quelques conclusions. La température de l'air semble être le facteur qui agit plus à court terme et directement sur la xylogenèse dans la tige. Ainsi, par exemple, en 2010 la différence de température moyenne annuelle de l'air de 2°C entre les deux sites d'études se traduisait par un délai de 13 jours dans le début de la xylogenèse de la tige des arbres témoins entre le site plus chaud et le site plus froid (Chapitre II et IV). La température du sol a montré une influence sur la xylogenèse dans la tige, bien que probablement de façon indirecte (Chapitre III et IV). Ainsi, les 4°C de différence dans la température du sol entre les parcelles chauffées et les parcelles non-chauffées se traduisaient par un début de la xylogenèse dans la tige de 4 jours plus hâtif dans les arbres chauffés, tandis qu'au niveau des racines, le début était plus hâtif d'environ 8 jours (Chapitre IV) (Tableau 5.1). L'azote, par contre, n'a pas démontré d'influence sur le début de la xylogenèse et semble agir à plus long terme, probablement à travers des effets cumulés et indirects, liés à une augmentation de la croissance dans tout l'arbre, y compris la tige (Chapitre IV). Toutefois, le traitement d'ajout d'azote montrait son influence en interaction avec le traitement de chauffage du sol seulement au niveau de la durée de la xylogenèse et de l'épaisseur de la paroi cellulaire dans le bois initial, sans influencer de façon évidente la production du bois après trois ans (Chapitre IV) (Tableau 5.1).

En analysant ensemble les résultats des trois chapitres on peut aussi mettre en évidence comment le modèle causal, liant phénologie de formation du xylème et production du bois et élaboré dans le Chapitre II en se basant sur les conditions actuelles, semble fonctionner même dans les conditions futures simulées (Chapitre III et IV). Par exemple, dans le chapitre III, on a observé qu'à partir de la deuxième année de chauffage les plantes chauffées montraient une durée de la xylogenèse plus longue. Même si la différence entre les traitements n'était pas toujours statistiquement significative, on observait un début plus hâtif et une fin plus tardive dans les arbres chauffés par rapport aux arbres non-chauffés. À partir de la troisième année l'écart en nombre de cellules produites entre plantes chauffées et plantes non-chauffées augmentait. Ces observations et tendances suggèrent que le modèle arrive à bien représenter comment des variations dans la phénologie de formation du bois sont associées avec des variations au niveau de la production (Nord & Lynch 2009, Rossi et al. 2011a, 2012). Toutefois les processus concernés pourraient être plus complexes et agir à différentes échelles temporelles. Dans le chapitre IV les plantes chauffées, dans lesquelles un début plus hâtif de la xylogenèse était observé avaient aussi une durée plus longue de la xylogenèse et produisaient en général plus de cellules, même si la différence entre les traitements n'était pas encore significative (Chapitre III et IV). En outre, dans les chapitre IV, les racines, qui produisaient moins de cellules que les tiges, avaient aussi un début plus tardif et une fin plus hâtive de la xylogenèse, encore en accord avec le modèle du chapitre II, qui a été développé initialement pour les tiges. Notre modèle a été le point de départ qui a amené Rossi et al. (2012) à développer un nouveau modèle causal, beaucoup plus complexe, qui prend en considération plusieurs phases de différentiation des cellules du xylème. Il serait intéressant de vérifier si ce nouveau modèle s'applique aussi aux conditions futures simulées dans notre expérience.

### 5.7. Perspectives de recherche

De plus en plus d'études soulignent l'importance de considérer les interactions potentielles entre le cycle de l'azote et le cycle du carbone et les implications au niveau des modèles bioclimatiques (Luo et al. 2004, Reich et al. 2006, Jain et al. 2009, Yang et al. 2009, Zaehle et al. 2010). Nos résultats seront donc fort utiles pour l'amélioration de ce genre de modèles et les prévisions de productivité ligneuse dans des scénarios de changements climatiques. En outre, nos données et résultats pourront être utilisés pour calibrer ou valider des modèles de prévision comme TreeRing, CaBala et CAMBIUM, qui permettent de tester la compréhension actuelle des processus qui contrôlent la formation et la production du bois (en termes d'accroissements radiaux) dans les arbres (Downes & Drew 2008, Vaganov et al. 2011). Par exemple, TreeRing, développé par Fritts et al. (1999), est un modèle qui met ensemble différents processus écophysiologiques qui règlent la croissance, comme la photosynthèse et la xylogenèse, pour reconstruire les caractéristiques anatomiques du bois.

Dans le futur, des chercheurs pourraient profiter de notre expérience pour essayer d'améliorer la compréhension de l'impact des perturbations étudiées. L'inclusion de plantes de différents âges dans un nouveau design expérimental permettrait de vérifier si la réponse aux traitements (ex. chauffage du sol et de l'air) reste la même à plusieurs stades de développement (Xu et al. 2012). De plus, à l'intérieur du même peuplement, des arbres qui appartiennent à différentes classes sociales (dominant, supprimé ou intermédiaire) présenteraient aussi des différences dans la phénologie de la xylogenèse (Rathgeber et al. 2011). Compte tenu de ces nouveaux résultats et des implications qui découlent de notre modèle liant phénologie et production (Lupi et al. 2010, dans le Chapitre II), on suggère que les chercheurs intègrent ces facteurs (âge et classe sociale) dans des projets futurs. La « classe sociale » (définie à partir de la dimension de la canopée) pourrait être en partie à la base de la grande variabilité observée, en termes de croissance, entre nos arbres, surtout dans le site plus chaud (Chapitre II). En plus d'augmenter les critères de sélection des arbres, on pourrait augmenter le nombre d'arbres échantillonnés dans chaque traitement pour inclure la grande variabilité qui se retrouve dans les milieux naturels.

Le dispositif expérimental qui a été mis en place pourrait être utilisé non seulement pour continuer l'expérience en cours et ainsi examiner l'impact à moyen terme des perturbations étudiées, mais aussi pour des nouvelles recherches intéressantes avec différents objectifs. Par exemple, on pourrait se poser comme question si le réchauffement du sol entraîne des changements de la phénologie des bourgeons. Et si oui, s'ils peuvent avoir un impact sur la nutrition et donc la vitalité de la tordeuse des bourgeons de l'épinette qui elle est dépendante de cette phénologie. Un changement dans la phénologie des bourgeons pourrait influencer la dynamique future des épidémies de la tordeuse, ce qui aurait un impact majeur dans la pessière. Ce genre d'étude pourrait être très important à court terme, car les données sur la tordeuse nous indiquent qu'on est au début d'une nouvelle période épidémique (MRNF 2011) et que la prochaine épidémie (qui devrait avoir lieu dans plus de 35-40 ans) pourrait se produire dans des conditions climatiques différentes à cause du réchauffement climatique.

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Tableau 5. 1 Tableau de synthèse des principaux résultats de recherche

| | *Saison de croissance* | ↑ *Température du sol* | ↑ *Dépôts azotés* |
|---|---|---|---|
| *Phénologie* | • Début plus hâtif => nombre de cellules plus élevé | • Début plus hâtif (surtout racines, 8 jours);<br>• Durée plus longue;<br>• Fin ? | • Début pas d'effet;<br>• Durée intermédiaire entre témoins et arbres seulement chauffés;<br>• Fin ? |
| *Nombre de cellules* | • Nombre de cellules plus élevé => fin plus tardive | • Encore pas clair;<br>• Tendance à la hausse dans les plantes seulement chauffées | • Encore pas clair |
| *Anatomie* | | • Paroi cellulaire plus épaisse dans le bois initial | • Paroi cellulaire plus épaisse dans le bois initial |

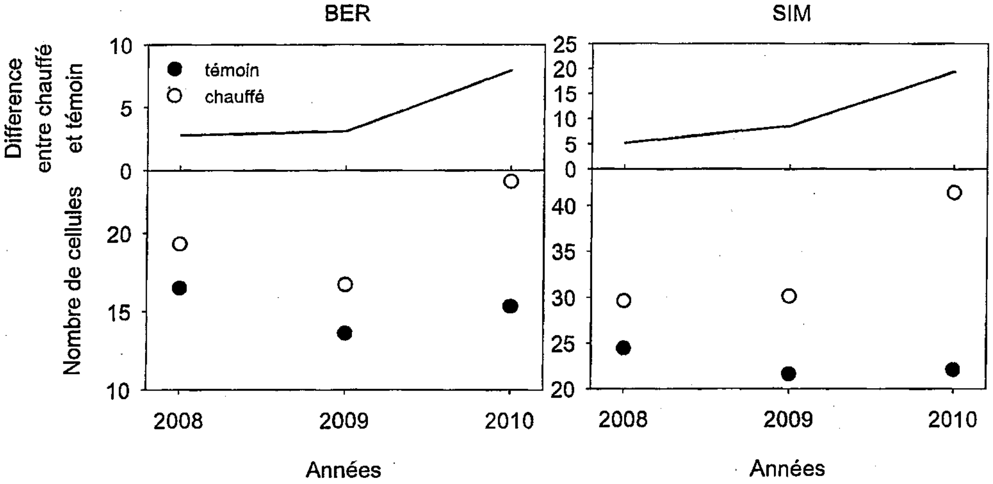

Figure 5.1 Nombre moyen de cellules dans les tiges des arbres témoins et chauffés (chauffage seulement) et leur différence dans les deux sites d'étude pendant les trois années de l'expérience