Global changes and the balsam fir ecosystem: Effects of increased soil temperature, nitrogen deposition and water stress on soil chemistry and xylogenesis

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Abstract

The nature and intensity of environmental changes projected by climate models for Eastern Canada could significantly affect the functioning of boreal forest ecosystems. In this thesis, I tested experimentally the effects of some of these changes (increased soil temperature and atmospheric N deposition, and summer droughts) in a mature boreal balsam fir stand (*Abies balsamea* (L.) Mill.) in the Réserve faunique des Laurentides (Quebec). The general objective of the thesis was to evaluate the adaptation of the soil/plant system to these changes.

In the first experiment, using heat-resistance cables buried in the ground and water nozzles set up above the trees, soil temperatures were increased (+4°C), spring snowmelt hastened (2-3 weeks), and higher levels of atmospheric N were deposited on the canopy (3x the natural NH₄NO₃ concentration in the rain) for three years. In the second experiment, a summer drought (starting in July) was simulated in two consecutive years by means of throughfall exclusion using polyethylene sheets maintained 1.3-2 m aboveground and redirecting the water outside the plots. In the soil warming – N deposition experiment, volumetric soil water content was assessed weekly, soil chemistry was monitored with ion-exchange membranes (PRS-probesTM) and soil extractions, while soil respiration was measured in a laboratory incubation experiment. Tree nutrition was assessed through leaf sampling and nutrient analyses, radial growth was monitored using dendrometers and seasonal xylogenesis was assessed using microscopic analyses of wood microcores extracted weekly from April to October each year. In the second experiment, the analysis of wood microcores was also used to assess the effects of throughfall exclusion on balsam fir xylogenesis, while the volumetric soil water content was monitored weekly.

Three years of warmer soils decreased the soil mineralizable C pool by 11-15% with limited effects on the availability of inorganic N (NH₄ and NO₃). The availability of K, Mg and SO₄ increased by 43, 44 and 79% in the forest floor of heated plots, respectively, while Mg, SO₄ and Al increased by 29, 66 and 23% in the mineral horizon, respectively. Increased nitrogen deposition did not affect soil nutrient availability. The treatments had no effects on the amount of wood produced or the foliar nitrogen concentration of balsam fir. The

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experimentally induced earlier snowmelt did not hasten resumption of xylogenesis, although an eight-day earlier peak in diameter growth was observed in heated plots in the second year. Finally, two consecutive years of summer drought reduced by 16.1% the diameter of tracheids and increased the thickness of their cell wall by 14.1% during both years. In addition, the treatment delayed by more than a week the tracheid differentiation process in the second year, with a concomitant decrease of 26% in the number of tracheids produced.

If the short-term responses of balsam fir observed in our studies persist over time, it should be considered relatively resilient to warmer soils and higher N deposition but responsive to drought episodes. Thus, a higher frequency and/or intensity of droughts could have major consequences on the productivity of the balsam fir ecosystem. However, other environmental factors that are expected to change in the future were not included in this thesis (e.g. increasing air temperature and atmospheric CO₂) and could affect the response of the ecosystem to increased soil temperature, N deposition and water stress.

Résumé

La nature et l'intensité des changements environnementaux projetés par les modèles climatiques pour l'est du Canada pourraient affecter de manière significative l'écosystème de la forêt boréale. Dans cette thèse, j'ai testé expérimentalement l'effet de certains de ces changements (accroissement de la température du sol et des dépôts atmosphériques de N, sécheresse estivale) dans une sapinière boréale mature de la Réserve faunique des Laurentides, au Québec. L'objectif général de cette thèse consistait à évaluer l'adaptation du système sol/plante à ces changements.

Dans une première expérience, des câbles chauffants ont été enfouis dans le sol et des gicleurs installés au-dessus de sapins baumiers (Abies balsamea (L.) Mill.) afin d'accroître la température du sol (+4°C), hâter la fonte de neige au printemps (2-3 semaines), et augmenter les dépôts atmosphériques d'azote (3x la concentration naturelle de NH₄NO₃ dans la pluie) pendant trois ans. Dans une seconde expérience, un épisode de sécheresse estivale (débutant en juillet) a été simulé deux années de suite à l'aide de membranes de polyéthylène maintenues 1.3-2 m au-dessus du sol et redirigeant l'eau de pluie hors des parcelles. Dans l'expérience de chauffage du sol et d'accroissement des dépôts de N, la chimie du sol était mesurée à l'aide de résines échangeuses d'ions (PRSprobesTM) et d'extractions de sol, tandis que la respiration du sol a été mesurée dans une expérience d'incubation en laboratoire. La nutrition des arbres était évaluée à l'aide de l'analyse du contenu nutritif d'échantillons foliaires. La croissance radiale était suivie à l'aide de dendromètres, et la xylogénèse saisonnière était mesurée grâce à l'analyse microscopique de micro-carottes de bois récoltées chaque semaine d'avril à octobre. Dans le cadre de la deuxième expérience, cette même approche d'analyse de micro-carottes de bois a permis de mesurer l'impact de la sécheresse sur la xylogénèse du sapin baumier, en plus de mesures hebdomadaires de contenu en eau du sol.

Trois années de chauffage du sol ont réduit la quantité de carbone minéralisable dans le sol de 11-15%, avec des effets toutefois limités sur la quantité d'azote inorganique disponible (NH₄ et NO₃). La disponibilité du K, Mg et SO₄ s'est respectivement accrue de 43,

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44 et 79% dans l'horizon organique des sols chauffés, tandis que dans l'horizon minéral, la disponibilité du Mg, SO₄ et Al était plus élevée de 29, 66 et 23%, respectivement. Les apports supplémentaires d'azote n'ont pas modifié le contenu du sol en éléments nutritifs disponibles. Ces traitements n'ont pas affecté la quantité de bois produite ou les concentrations foliaires en azote du sapin baumier. La fonte de neige hâtive provoquée par le réchauffement du sol n'a pas avancé l'initiation de la xylogénèse, bien que le pic de croissance radiale ait été atteint en moyenne huit jours plus tôt chez les arbres chauffés la deuxième année. Finalement, deux années consécutives de sécheresse estivale ont significativement réduit de 16.1% le diamètre des trachéides et accru l'épaisseur de leurs parois de 14.1% durant les deux années. De plus, le traitement a retardé de plus d'une semaine le processus de différentiation cellulaire la deuxième année, conjointement avec une baisse de 26% du nombre de trachéides produits.

Si les réactions du sapin baumier observées ici se maintiennent à long-terme, l'espèce devrait être considérée relativement résiliente au réchauffement du sol et à la hausse des apports atmosphériques en N, mais sensible aux épisodes de sécheresse. Ainsi, une hausse de la fréquence et/ou de l'intensité des sécheresses pourrait avoir des conséquences majeures sur la productivité de la sapinière boréale. Cependant, d'autres variables environnementales majeures qui devraient être modifiées par les changements globaux n'ont pu être prises en compte dans le cadre de cette thèse (hausse de la température de l'air et de la concentration en CO₂, etc.) et pourraient modifier la réponse de l'écosystème à l'accroissement de la température du sol, des dépôts de N et des sécheresses.

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Preface and Contribution of Authors

This manuscript-based thesis consists of a general introduction written in French and presented in Chapter 1, five chapters (Chapters 2 to 6) submitted for publication in peerreviewed journals (three of which are accepted, the other two under review), a summary and a general conclusion. Four of the five chapters/papers are based on the same experiment, thus some duplication is inevitable in the introductions and Material and methods sections. The cited literature from all chapters was compiled in a single section.

For all manuscripts, I am listed as the first author. My contributions consisted in the complete analysis of the data and preparation of the manuscripts. I was directly involved in some, if not all of the data collection for increment core analysis, soil and plant nutrient analysis and soil water content. Finally, I was solely responsible for the design, setup and maintenance of the experiments for chapter four and five. In all papers, my thesis advisors B. Côté and D. Houle are listed as second and third authors due to their assistance in most phases of my research from designing the experiments to the writing of manuscripts. L. Duchesne is co-author of chapters 2, 4 and 5. As the person in charge of the experimental station at Montmorency Forest, L. Duchesne played a significant role in the compilation of some of the data, the maintenance of the soil warming experimental site and the revision of the dendrological analysis of wood samples, maintenance of some materials at the soil warming experiment as well as some data collection. Finally, J. Whalen is co-author of chapters 3. In addition to helping with the improvement of the manuscript, Dr. Whalen provided some laboratory equipment and was responsible for the analysis of gas samples.

My thesis provides several original contributions to knowledge in the area of global climate change impacts on plant-soil nutrient relationships for the Eastern Canada balsam fir ecosystem. In my thesis, I report on the first experimental study of the effects of increased soil temperatures and N deposition levels on the Eastern Canada balsam fir ecosystem, specifically soil chemistry and respiration, and xylogenesis (chapters 2, 3, 4 and 5). This is one of very few studies in which realistic amounts of N were applied to simulate

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predicted levels of atmospheric deposition. In addition, the N was applied as artificial precipitation directly over the canopy of a mature stand. This unique study design provides the necessary data to test hypotheses about the adaptation of the balsam fir ecosystem to some of the projected global changes. More specifically,

- I show in Chapters 4 and 5, using two different approaches, that in the short-term (three years), balsam fir radial growth responds weakly to an increase in soil temperature and atmospheric N deposition as projected by climate scenarios.
- I provide evidence that, contrary to common hypotheses reported in the literature, these environmental changes have limited effects on plant available nitrogen in the soil (Chapter 4).
- Chapter 2 describes for the first time the effects of increased soil temperature on plant-available nutrients in the Eastern Canada boreal forest. I show significant increases in available potassium, magnesium, sulfate and aluminum in warmer soils. Due to the small size of the exchangeable cation pool in the balsam fir ecosystem as well as the phytotoxicity of aluminum, these results could have significant implications for the long-term productivity of the ecosystem.
- I provide the first experimental validation that the spring resumption of radial growth in balsam fir is not driven by the timing of snowmelt (Chapter 5).
- Using standard laboratory soil incubations, I show that chronically higher soil temperatures (for three years) reduce the amounts of readily mineralizable soil carbon (Chapter 3). This result could help us predict future trends in the long-term carbon cycle of the balsam fir boreal ecosystem.

In addition, I present in Chapter 6 results from the first experimental study of the effects of a summer drought on wood formation in balsam fir. Results from this study will help us predict how well the balsam fir boreal ecosystem will adapt to the projected increased frequency and intensity of droughts. I show that:

• Balsam fir trees respond to droughts by reducing their radial growth and the size of tracheids produced.

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• A drought can delay the start of cambial activity the following year. This is the first report of a delayed effect of water stress on the phenology of xylogenesis.

This thesis provides a rather comprehensive description of how balsam fir, one of the dominant species of the Canadian boreal forest, could react to global climate changes.

Chapter 1. Introduction générale

1.1 Mise en contexte

Située dans les régions nordiques d'Amérique du Nord, d'Asie et d'Europe, la forêt boréale représente 32% des superficies forestières mondiales. Elle est caractérisée par un climat hautement saisonnier d'hivers froids et secs et de courtes saisons de croissance (Burton *et al.* 2003). La forêt boréale recouvre la majorité du territoire canadien. En fait, un tiers (~4 millions de km²) des forêts boréales mondiales se trouvent au Canada (Canadian Forest Service 2012). La forêt boréale canadienne appartient presque exclusivement au domaine public (97.2%), et le secteur forestier contribue à hauteur de 23.5 milliards \$ au produit intérieur brut annuel (Canadian Forest Service 2012). La forêt boréale canadien comprend environ 700 000 km² de milieux humides à haute valeur écologique (Tarnocai 2006), et abrite plus de 23 000 espèces (Burton *et al.* 2003), dont les plus grandes populations de caribou du monde et un tiers des populations d'oiseaux migrateurs terrestres d'Amérique du Nord (Krawchuk *et al.* 2012).

Le sapin baumier (*Abies balsamea* (L.) Mill.) représente une des principales essences forestières de l'est du Canada. Sa présence s'étend de Terre-Neuve et Labrador au centre de l'Alberta ainsi que dans le nord-est des États-Unis, de la Virginie au sud jusqu'au Minnesota à l'ouest (Burns & Honkala 1990). Au Québec, le sapin baumier domine 139 000 km² de territoire, soit le quart le plus au sud de la forêt boréale québécoise. Le sapin est une des principales essences exploitées par l'industrie forestière, principalement pour la production de pulpe et de bois de charpente ainsi que de sapins de Noël.

Il est maintenant bien établi que l'accumulation des gaz à effets de serre dans l'atmosphère, d'origine anthropique, fait désormais dévier le climat global hors de sa course naturelle, et cette tendance devrait s'accélérer au XXI^e siècle (IPCC 2007). Parmi les effets des changements climatiques, les modèles prédisent pour la sapinière boréale un air plus chaud de 3°C en 2041-2060 relativement à 1990 (Plummer *et al.* 2006), un printemps

avancé d'un mois et une diminution de l'humidité du sol de 20-40% durant la saison de croissance pour 2070-2099 (relativement à 1971-2000; Houle *et al.* 2012).

Étant donné l'importance à la fois écologique et économique de la sapinière boréale canadienne, il est impératif de pouvoir prédire adéquatement son évolution en réponse aux changements globaux. Nous devons pouvoir estimer l'impact sur plusieurs biens et services, dont les futurs volumes de bois produit, importants économiquement, et les futurs niveaux de séquestration de carbone, qui ont une incidence majeure sur la trajectoire future du climat global. Les modèles d'aménagement forestier ne prennent présentement pas en compte les changements globaux. De telles connaissances permettraient d'alimenter ces modèles avec des données qui intègrent ces changements et ainsi tendre vers une utilisation adéquate de la ressource.

Pour cela, nos niveaux de connaissance sont encore largement insuffisants. Par exemple, nous ne connaissons pas l'impact qu'aura la diminution projetée des niveaux d'eau du sol (Houle et al. 2012) sur la croissance et la survie du sapin baumier, alors que ce nouveau régime est susceptible de bouleverser la dynamique forestière (Choat et al. 2012). De plus, l'hypothèse communément admise qu'une hausse de la température du sol devrait stimuler la décomposition de la matière organique du sol, accroître la disponibilité des éléments nutritifs et augmenter la croissance des arbres (Mäkipää et al. 1999; Jarvis & Linder 2000) serait actuellement remise en question. Entre autres, certaines études ont mis en évidence que les éléments nutritifs ajoutés dans un écosystème forestier peuvent être presqu'entièrement immobilisés dans le sol, sans que les arbres en bénéficient (Berg & Matzner 1997; Nadelhoffer et al. 1999b; Friedrich et al. 2011). Également, des études de chauffage du sol en prairie (Luo et al. 2001) et en forêt tempérée (Melillo et al. 2011) ont récemment démontré que l'activité microbienne du sol, initialement stimulée par le traitement, retournait à son niveau initial après quelques années de chauffage. Enfin, certains auteurs soulignent le rôle inhibiteur d'un assèchement du sol sur ces mêmes taux de décomposition (Allison & Treseder 2008).

En plus des changements prévus au climat, l'accroissement à venir des activités industrielles et agricoles mondiales devrait avoir pour effet d'accroître les dépôts

atmosphériques de N à l'échelle de la planète et sur la forêt boréale (Galloway *et al.* 2004). Bien que la forêt boréale est reconnue pour être limitée en N (Tamm 1991), l'effet de tels ajouts sur sa croissance est difficile à prévoir. En effet, nos connaissances sont basées sur des expériences de fertilisation en N dont les quantités ne reproduisent pas les niveaux actuels ou prédits, généralement au-dessus de 50 kg N ha⁻¹ an⁻¹ tandis que les taux moyens de déposition en N dans l'ensemble des écosystèmes boréaux sont estimés à 2.34 kg N ha⁻¹ an⁻¹ (Holland *et al.* 1999). De plus, ces expériences appliquent le N au sol, contournant les multiples interactions avec la canopée et le sous-bois (Hobbie *et al.* 2002; Gundale *et al.* 2011).

Ainsi, les changements globaux devraient affecter de nombreux paramètres déterminants de la dynamique forestière, mais le nombre limité d'études expérimentales ne nous permet pas d'évaluer la capacité d'adaptation de la sapinière boréale à ces changements.

1.2 Revue de littérature

Située principalement entre les 45^e et 70^e parallèles nord, au-dessus des forêts tempérées et des prairies mais au sud de la toundra circumpolaire, la forêt boréale est principalement dominée par l'épinette (*Picea*), le pin (*Pinus*), le mélèze (*Larix*) et le sapin (*Abies*), accompagnés de feuillus comme le peuplier (*Populus*) et le bouleau (*Betula*). La présence de bryophytes est également caractéristique de cet écosystème, notamment les Hypnaceae comme *Hylocomium splendens* (Hedw.) Schimp. et *Pleurozium shrebeirii* (Brid.) Mitt. ainsi que les sphaignes (*Sphagnum*).

À cause de son climat hostile, peu d'êtres humains y habitent. Les feux, épidémies d'insectes et chablis demeurent les principaux facteurs de renouvellement des peuplements, assurant la stabilité de la structure d'âge, de la composition floristique et de la biodiversité de l'écosystème boréal (Bonan & Shugart 1989). En moyenne, 1.6 millions d'hectares de forêt boréale sont brûlés annuellement en Amérique du Nord (Soja *et al.* 2007). L'intérêt récent des industries pour les ressources minières, énergétiques (pétrole et

gaz naturel) et hydriques soulève d'importants défis pour la conservation de cet écosystème (Krawchuk *et al.* 2012). La proportion actuelle de forêts boréales intactes est estimée à 20% (Hannah *et al.* 1995).

1.2.1 La sapinière boréale de l'est du Canada

Caractérisé par une tolérance à l'ombre plus élevée que la plupart des essences qui lui sont associées, comme l'épinette noire (*Picea mariana* (Mill.) Britton, Sterns & Poggenb) ou blanche (*Picea glauca* (Moench) Voss), le sapin baumier maintient une importante banque de semis dans le sous-bois forestier qui lui permet de dominer les peuplements en l'absence de perturbations (Morin 1994). L'espèce se retrouve le plus souvent sur des sols loameux, associée à des climats froids et humides (Burns & Honkala 1990). Au Québec, la sapinière boréale est moins affectée par les feux que les pessières plus au nord. Ce sont plutôt les épidémies d'insectes, notamment la tordeuse des bourgeons de l'épinette (*Choristoneura fumiferana* (Clem.)), qui représentent le facteur dominant de remplacement des peuplements (Morin 1994).

Les podzols (spodosols aux États-Unis) constituent les principaux sols associés à la sapinière boréale (Jones *et al.* 2009). Ce sont des sols bien développés dominants sous les forêts conifériennes de l'est du Canada et de la Fennoscandie mais également retrouvés en Eurasie (Jones *et al.* 2009). Étant donné leurs basses températures, les sols boréaux tendent à accumuler les débris partiellement décomposés de végétation en surface, une strate de sol décrite comme l'horizon LFH (O dans la classification américaine) qui constitue le plus grand réservoir de carbone terrestre (McGuire *et al.* 1995). Dans un podzol, la grande quantité d'acides organiques relâchés dans l'horizon LFH forme des complexes avec les ions métalliques, entre autres l'aluminium (Al) et le fer (Fe), ensuite lessivés par l'eau de pluie le long du profil de sol (Lundström *et al.* 2000a). Cette migration ionique entraîne la formation d'un horizon éluvié, décrit comme l'horizon Ae, caractérisé par sa couleur grise et son faible contenu en Al, Fe et en cations basiques. Logiquement, l'horizon Ae repose sur un horizon illuvié rougeâtre, l'horizon B, où s'accumulent les ions d'Al et Fe selon des processus

d'adsorption, de précipitation ou de biodégradation qui ne sont pas parfaitement connus encore aujourd'hui (Lundström *et al.* 2000b).

1.2.2 Le rôle de l'azote

Les basses températures des forêts boréales ne restreignent pas seulement la durée de la saison de croissance (Bonan & Shugart 1989), elles limitent également les taux de décomposition de la matière organique (Van Cleve et al. 1983). Ces faibles taux, combinés à des pH bas, maintiennent de faibles concentrations d'éléments nutritifs disponibles pour la croissance de la végétation (Bonan & Shugart 1989). La croissance optimale d'une plante est dépendante d'apports suffisants en énergie, en eau et en minéraux, et la Loi de Liebig sur le minimum énonce que la ressource qui est la plus rare détermine la productivité d'un système. Les forêts boréales étant pauvres en éléments nutritifs, le N est le principal facteur limitant des forêts boréales car il s'agit de l'élément que les plantes requièrent en plus grande quantité (Magnani *et al.* 2007; Melillo *et al.* 2011). Bien que les plantes aient la capacité d'assimiler le N organique (Nashölm et al. 1998), elles assimilent surtout le N inorganique rendu disponible par la minéralisation microbienne du N organique (Friedland et al. 1991). Le paradigme de la limitation en N s'est cristallisé autour des nombreuses expériences de fertilisation en N qui montraient des réponses de croissance drastiques (Tamm 1991; Jarvis & Linder 2000). De plus, les analyses stœchiométriques comme les ratios C/N ont confirmé indirectement cette limitation (Gutschick 1981; Magnani et al. 2007). Il en résulte dans les podzols un cycle du N très conservateur maintenu par des compartiments édaphiques avec un fort potentiel de prélèvement du N inorganique disponible et de faibles pertes par lessivage (Nadelhoffer et al. 1999b; Berg & Dise 2004; Friedrich et al. 2011).

Les activités humaines ont, dans le dernier siècle, considérablement accru les quantités de N déposées sur les écosystèmes forestiers sous forme de pluie, de brouillard ou de dépôts secs, particulièrement dans l'est de l'Amérique du Nord (Driscoll *et al.* 2001; Galloway *et al.* 2004). Ces dépôts atmosphériques de N peuvent représenter une source significative de N inorganique. La majorité des forêts boréales sont soumises à des taux

annuels de dépôt de N égales ou moindres à 3 kg ha⁻¹, bien que les dépôts puissent s'élever à 12 kg ha⁻¹ à proximité de sites industriels (Gundale *et al.* 2011). L'impact de cet apport sur la croissance des forêts boréales et tempérées d'Europe et d'Amérique du Nord est généralement positif (Magnani *et al.* 2007), bien que des dépôts chroniquement excessifs par rapport aux besoins de l'écosystème peuvent perturber l'équilibre nutritif des plantes et réduire la productivité (Aber *et al.* 1989). De tels cas sont principalement causés par l'acidification du sol, le lessivage des cations basiques et une augmentation toxique de la disponibilité de certains éléments comme l'aluminium (Cronan & Schofield 1990).

Étant donné cette forte compétition pour le N, la plupart des dépôts atmosphériques de N sont interceptés par la strate de bryophytes au sol (Gundale *et al.* 2011). Les arbres, dont le sapin baumier (Houle *et al.* 1999), peuvent également intercepter une fraction du N atmosphérique dans leur canopée (Lovett 1992). L'influence de cette voie nutritionnelle sur la croissance des arbres n'est pas encore bien comprise, mais l'application de traceurs de ¹⁵N dans un peuplement d'épinettes et de pruches du nord-est des États-Unis a confirmé que le N intercepté n'était pas seulement retenu à la surface des branches mais également incorporé dans le feuillage et le bois des arbres (Bryan Dail *et al.* 2009).

1.2.3 Les changements globaux

La population humaine s'est multipliée par 10 depuis les trois derniers siècles, et devrait s'accroître d'encore trois milliards de personnes d'ici 2100 (United Nations 2011). Afin de soutenir l'expansion du nombre d'humains sur la planète, l'exploitation des ressources terrestres s'est accrue à un rythme semblable. Le couvert forestier global ne représenterait plus que 30 à 50% de sa taille d'origine, et 13 millions d'hectares de superficies forestières disparaissent chaque année (FAO 2010). La consommation croissante de combustibles fossiles et l'intensification de l'agriculture – la population de bétail, un important émetteur de méthane, atteint 1.4 milliards d'individus (FAO 2011) – ont favorisé l'accumulation dans l'atmosphère de CO₂, de CH₄ et de N₂O à des niveaux inégalés depuis 650 000 ans (IPCC 2007). Cette concentration de gaz à effets de serre est en train de créer des distorsions climatiques : les températures globales ont augmenté de 0.74°C au XX^e siècle, les niveaux

des océans augmentent de 3.1 mm an⁻¹, alors que les superficies arctiques couvertes de neige ou de glace rapetissent au rythme de 2.7% par décennie (IPCC 2007). L'importance de ce phénomène a mené la communauté scientifique à donner le nom d'Anthropocène à notre époque géologique (Crutzen 2002).

L'impact des changements globaux sur la forêt boréale est déjà perceptible. Alors que les températures plus chaudes entraînent un déplacement vers le nord des isothermes climatiques, les habitats des espèces suivent le mouvement, avec de potentielles conséquences écologiques importantes (Root et al. 2003; Aerts et al. 2006). Par exemple, les populations de dendroctone du pin ponderosa (Dendroctonus ponderosae Hopkins), à l'origine d'une mortalité élevée dans les pinèdes de l'ouest du Canada, s'étendent actuellement à l'est et au nord de leur aire de répartition habituelle étant donné les températures hivernales favorables des dernières décennies (Robertson et al. 2009). De plus, les changements climatiques auraient également favorisé l'accroissement observé des superficies brûlées au Canada depuis les années 1970 (Gillett et al. 2004), bien que la fréquence des feux de forêt ait diminué durant le XX^e siècle (Flannigan *et al.* 1998). Les changements globaux affectent également la productivité forestière. Selon des données de terrain et satellitaires, ces changements auraient eu en général un impact positif sur la productivité forestière dans les 55 dernières années (Boisvenue & Running 2006). Au Canada, des peuplements d'épinette noire de la toundra forestière subarctique du nord du Québec ont accru leur croissance terminale dans les dernières décennies (Gamache & Payette 2004). De plus, la période d'activité photosynthétique des plantes se serait allongée de 12 jours en Amérique du Nord entre 1981 et 1999 (Zhou et al. 2001). Des valeurs similaires ont été obtenues dans une étude limitée aux sites nordiques de toundra (Myneni et al. 1997).

La productivité de la forêt boréale de l'est du Canada pourrait être affectée par l'accroissement additionnel de 25 à 90% des émissions de gaz à effets de serre projeté pour 2030 relativement à 2000 (IPCC 2007). Entre autres, les dépôts atmosphériques globaux de N devraient s'accroître de 70% en 2050 par rapport à 2000 (Galloway *et al.* 2004). Également, les niveaux d'ozone près de la surface terrestre devraient atteindre 40 ppm en

moyenne d'ici 2100, voire 70 ppm pour l'est de l'Amérique du Nord, des niveaux potentiellement toxiques puisque l'ozone cause des dommages cellulaires à l'appareil foliaire des plantes (Sitch et al. 2007). De plus, le Modèle régional climatique canadien projette un accroissement de 3°C de la température annuelle de l'air pour 2041-2060 relativement à 1971-1990 (Plummer et al. 2006). Les simulations de température du sol prévoient pour cette même période que la fonte printanière du couvert de neige arrivera un mois plus tôt (Houle et al. 2012). Les précipitations devraient s'accroître de 7 à 14%, surtout en hiver, bien que ces projections soient caractérisées par d'importantes incertitudes (Plummer et al. 2006). Ces précipitations accrues pourraient cependant être contrebalancées par des taux plus élevés d'évapotranspiration causés par les températures plus chaudes. De plus, étant donné qu'une part importante de l'eau disponible dans les écosystèmes boréaux provient de la fonte de neige printanière, une fonte hâtive pourrait faire sécher les sols trop tôt durant la saison de croissance. Ainsi, des réductions de 20 à 40% de l'humidité du sol sont projetées durant la saison de croissance pour la période 2070-2099 relativement à 1971-2000 pour plusieurs sites forestiers de l'est du Canada (Houle et al. 2012). Une telle baisse du contenu en eau du sol devrait probablement entraîner un accroissement de la fréquence et de l'intensité des sécheresses (Schar et al. 2004). Ainsi, les faibles précipitations combinées à la canicule de 2003 en Europe ont causé un réduction globale de 30% de la productivité primaire, annulant en une seule année l'équivalent de quatre années de séquestration nette de carbone (Ciais et al. 2005). En parallèle, les superficies forestières canadiennes touchées par les feux de forêt devraient s'étendre à la faveur de conditions climatiques plus favorables (Flannigan et al. 2005).

L'adaptation des systèmes naturels à ces changements globaux est probablement un des plus grands problèmes auxquels est confrontée la communauté scientifique, qui tente surtout de répondre à deux questions : quels seront les impacts, et quels degrés de changements climatiques les écosystèmes peuvent-ils soutenir (Hiramatsu *et al.* 2010) ? D'importantes incertitudes persistent quant aux prévisions à long-terme, notamment à cause des nombreuses interactions potentielles (IPCC 2007). Afin de diminuer ces incertitudes, les études expérimentales de changements climatiques se sont multipliées

depuis le début des années 1990. Les forêts boréales ne sont cependant que peu représentées : des 32 études de réchauffement expérimental d'écosystème incluses dans une méta-analyse, seulement trois portaient sur la forêt boréale (Rustad *et al.* 2001). La communauté scientifique est également en train de reconnaître les évènements climatiques extrêmes (sécheresses, inondations, etc.) comme une composante importante des changements globaux, alors que les études expérimentales de ce type représentaient en 2006 qu'un cinquième de l'ensemble des études expérimentales sur les changements climatiques (Jentsch *et al.* 2007).

1.2.4 Impacts potentiels sur la diversité et la superficie de la sapinière boréale

La hausse des températures moyennes devrait étendre vers le nord l'habitat potentiel de nombreuses espèces d'arbres à une vitesse bien supérieure à leur capacité migratoire. En effet, il est estimé que ces espèces devraient migrer à la vitesse de 3000-5000 m an⁻¹ pour suivre l'évolution du climat, alors que la vitesse des migrations mesurées lors de la dernière fonte du glacier laurentien au début de l'Holocène est estimée à environ 100 m an⁻¹ (Mohan *et al.* 2009). Ainsi, le sapin baumier pourrait s'installer plus au nord, un phénomène actuellement observé chez l'épinette blanche à sa limite nordique (Payette 2007), tout en subissant une compétition accrue à sa limite sud de distribution.

De plus, la diminution projetée du contenu en eau du sol ainsi que l'augmentation prévue des sécheresses risquent de modifier la compétitivité du sapin baumier. Bien que le sapin baumier soit reconnu comme intolérant à la sécheresse (USDA 2013), il n'y a pas, à ma connaissance, de données scientifiques permettant de comparer la vulnérabilité du sapin baumier au stress hydrique avec celle d'autres espèces.

Des effets des changements globaux sur la tordeuse des bourgeons de l'épinette pourraient avoir une incidence majeure sur les niveaux de mortalité et de croissance du sapin baumier. L'insecte étant déjà présent sur une grande majorité de l'aire de répartition du sapin baumier, son expansion ne devrait pas avoir d'effets importants (Régnière *et al.* 2012). Par contre, un hiver et un printemps plus doux devraient favoriser l'incidence d'épidémies (Volney & Fleming 2000). De plus, les interactions trophiques, la sélection

naturelle et l'impact des évènements extrêmes vont certainement modifier les épidémies en termes d'intensité et de durée, mais la complexité des interactions limite notre capacité à prédire les changements à venir (Fleming & Volney 1995).

Le sapin baumier est considéré comme le conifère le moins résistant au feu du nordest de l'Amérique du Nord, entre autres à cause de son système racinaire superficiel et de la faible épaisseur de son écorce (Starker 1934). Cela pourrait expliquer en partie que la sapinière boréale du Québec domine des secteurs où le feu ne joue pas un rôle déterminant, mais ce n'a pas toujours été le cas. Jusqu'à il y 4 500 ans, des conditions climatiques plus sèches favorisaient une plus grande fréquence de feux dans les sapinières du centre-sud du Québec (Couillard *et al.* 2013). Outre la disparition du pin gris (*Pinus banksiana* Lamb.), l'assemblage d'espèces est le même qu'à cette période mais sous différentes proportions. Le retour de conditions plus sèches pourrait accroître la fréquence de feux, mais ces résultats suggèrent que le sapin baumier se maintiendrait dans son écosystème sous de telles conditions.

1.2.5 Impacts potentiels sur la productivité du sol de la sapinière boréale

Comme la croissance végétale dans les écosystèmes boréaux est souvent limitée par le N disponible (Tamm 1991), la réponse des plantes aux changements globaux pourrait être déterminée par l'impact de ces changements sur la disponibilité du N (Magnani *et al.* 2007; Melillo *et al.* 2011). En plus des dépôts de N d'origine atmosphérique, le sol fournit une fraction significative des apports en N des plantes. Ainsi, il est indispensable de pouvoir anticiper les impacts potentiels des changements globaux sur la mise en circulation du N dans le sol. La température et l'humidité du sol contrôlent grandement les processus de décomposition et de minéralisation responsables de la mise en disponibilité du N dans le sol (Campbell *et al.* 2009). Ainsi, en plus des hausses attendues des dépôts atmosphériques de N, les changements les plus significatifs au niveau de la disponibilité du N dans la sapinière boréale pourraient provenir des hausses projetées de températures et de stress hydrique.

Disponibilité des éléments nutritifs

La décomposition de la litière permet de remettre en circulation les éléments nutritifs immobilisés dans la matière organique du sol (MOS) tout en limitant l'accumulation de la MOS. Les champignons sont les principaux décomposeurs de la forêt boréale (Högberg et al. 2007), et des études portant sur leur réaction à des dépôts de N atmosphérique accrus ou des températures plus élevées ont montré jusqu'ici des réponses très variables en termes de diversité, d'activité ou de biomasse (Allison et al. 2009). Il reste que les taux de décomposition de la MOS sont généralement favorisés par une hausse de la température (Davidson & Janssens 2006), car celle-ci réduit le degré d'agrégation physique ou la force des liaisons chimiques des composés organiques, accélérant d'autant la vitesse des réactions enzymatiques (Knorr et al. 2005b). Cependant, plusieurs études expérimentales de réchauffement du sol ont mesuré une hausse initiale du taux de décomposition due au réchauffement qui revenait à la normale après seulement quelques années (Luo et al. 2001; Melillo et al. 2002). Pour l'instant, certains avancent que l'épuisement du carbone facilement décomposable pourrait expliquer ces résultats (Luo et al. 2001; Knorr et al. 2005b), alors que d'autres suggèrent que la communauté microbienne du sol pourrait s'être adaptée aux températures plus chaudes (Davidson & Janssens 2006; Bradford et al. 2008). Une réduction de la population microbienne pourrait constituer une forme d'adaptation, réduisant ainsi la production enzymatique et contrebalançant l'accroissement initial du taux de respiration (Davidson & Janssens 2006). Des changements aux propriétés enzymatiques pourraient représenter une autre forme d'adaptation, i.e conformation enzymatique, production d'isoenzymes (enzymes différentes mais catalysant la même réaction chimique), ainsi que des changements à la structure de la communauté microbienne (Davidson & Janssens 2006). Enfin, des effets négatifs du réchauffement expérimental du sol sur les taux de respiration du sol ou de décomposition ont été rapportés (Peterjohn et al. 1994; McHale *et al.* 1998; Rustad & Fernandez 1998b; Verburg *et al.* 2009). De tels cas, attribués à la diminution de l'eau disponible causée par le réchauffement du sol et l'accroissement de

l'évapotranspiration, ont été observés dans des écosystèmes vulnérables au stress hydrique qui pourraient ainsi pâtir de conditions plus sèches.

Quoi qu'il en soit, les études de chauffage d'écosystèmes montrent que des hausses de températures allant de 0.3 à 6°C peuvent accroître la minéralisation du N de 30 à 64% (Rustad et al. 2001). Ce processus microbien, influencé par la température et l'humidité, permet la libération de NH₄ à partir de composés organiques complexes (Schimel & Bennett 2004). Ainsi, l'hypothèse que le NH₄ disponible devrait augmenter à l'avenir est relativement bien corroborée par les expériences de chauffage (Rustad et al. 2001). La nitrification du NH₄ étant également influencée par la température, une hausse du NO₃ disponible est également attendue (Sabey et al. 1956; Malhi & McGill 1982). Cependant, l'utilisation de traceurs isotopiques de ¹⁵N appliqués sous forme de NH₄NO₃ (de 4 à 75 kg N ha⁻¹ an⁻¹) pendant un à trois ans dans neuf forêts tempérées nordiques d'Europe et d'Amérique du Nord limitées en N a montré que seulement 3% du N inorganique ajouté étaient incorporés dans la biomasse ligneuse (Nadelhoffer *et al.* 1999b). La majorité du N ajouté était immobilisé dans la litière du sol ainsi que dans les horizons organique et minéral. De tels résultats révèlent la présence d'une compétition intense pour le N dans le sol, causée par une importante immobilisation du N par la communauté microbienne au détriment de la communauté végétale. Des résultats similaires ont été obtenus dans une sapinière boréale de la réserve faunique des Laurentides, au centre-sud du Québec. En effet, des expériences de dilution isotopique avec ¹⁵N de courte durée indiguent que la presque totalité du NH₄NO₃ nouvellement ajouté étaient immobilisée en moins d'une journée (Ste-Marie & Houle 2006). Au même site, 95% des 17 et 57 kg N ha⁻¹ an⁻¹ ajoutés au sol pendant trois ans étaient immobilisés dans les strates supérieures du sol où se trouvent les racines des arbres, et ces ajouts n'ont pas modifié les concentrations de N inorganique, exception faite de hausses éphémères durant quelques jours suivant chaque ajout (Houle & Moore 2008). D'autres expériences similaires menées dans divers écosystèmes boréaux présentant des niveaux de N inorganique disponible également faibles ont confirmé la forte compétition pour le N (Berg & Matzner 1997; Nadelhoffer et al. 1999a; Friedrich et al. 2011).

Les sols acides de la sapinière boréale reposent sur une roche-mère d'origine granitique et sont caractérisés par un faible bassin de cations basiques échangeables – dont le K, le Ca et le Mg, des éléments nutritifs essentiels à la croissance végétale – et un faible pouvoir tampon (Watmough et al. 2005; Duchesne & Houle 2006). De telles conditions permettent de supposer que ces sols pourraient être susceptibles aux apports d'acidité accrus associés aux changements globaux. Entre autres, des dépôts atmosphériques de N supplémentaires vont ajouter des ions hydrogène. Les principales formes de N déposées au sol sont le NO₃ (issu d'interactions atmosphériques du NO et NO₂) et le NH₄ (Driscoll et al. 2001). En solution, les NO_x produisent de l'acide nitrique (HNO₃), un acide fort qui libère des ions H tandis que le NH₄ peut être nitrifié (transformé en NO₃), ce qui libère deux protons par ion NO₃ produit. Il est important de mentionner que parallèlement aux formes de N retrouvées dans les dépôts acides, le principal agent acidifiant est le soufre et que les dépôts acides de soufre ont diminué dans les deux dernières décennies (Houle et al. 1997; Driscoll et al. 2001). Ainsi, malgré un pouvoir tampon limité dans la sapinière boréale, un accroissement des apports atmosphériques en N n'aurait probablement qu'un effet marginal sur le pH du sol. Une expérience de réchauffement du sol a montré que des taux de décomposition plus élevés pouvaient également acidifier le sol (Van Cleve et al. 1990), car ce processus entraîne une augmentation des acides organiques en solution (Lundström et al. 2000a). De plus, la ré-humidification de sols secs suite à un épisode de sécheresse peut engendrer d'importants pics de nitrification acidifiants (Lamersdorf et al. 1998).

L'acidification d'un sol entraîne principalement la libération des cations basiques K, Ca, Mg et Na ainsi que des cations acides Al et H, afin de maintenir un équilibre des charges ioniques en solution. À court terme, lorsque des protons sont ajoutés dans le sol, la dissociation des cations basiques adsorbés sur les sites d'échange permet d'éliminer le H de la solution, mais accroît la mobilité de ces ions et le risque qu'ils soient lessivés hors de la zone racinaire. Le K est le plus mobile des cations basiques, entre autres à cause de sa plus faible charge ionique. Suivant une perturbation telle qu'un feu, une coupe forestière ou une épidémie d'insecte, cet élément subit généralement les pertes par lessivage les plus importantes (Lamontagne *et al.* 2000; Houle *et al.* 2009). C'est aussi le cation basique le plus

facilement libéré lors du processus de décomposition, étant donné qu'à la différence du Ca ou du Mg, il n'est pas incorporé dans des composés organiques complexes mais se retrouve plutôt sous forme d'électrolyte soluble (Likens *et al.* 1994). Ainsi, une expérience de chauffage de litière d'épinette rouge (*Picea rubens* Sarg.) a montré que le K était le seul cation basique montrant une réduction significative dans la litière après six mois de traitement (Rustad & Fernandez 1998a). Ainsi, si le réchauffement du sol entraîne un accroissement de la décomposition de la matière organique, tel que suggéré par les expériences de chauffage jusqu'ici (Rustad *et al.* 2001), cela aurait pour effet d'accélérer la remise en circulation des cations basiques immobilisés dans la matière organique.

En plus des précipitations qui peuvent constituer une source non-négligeable de cations, l'altération des minéraux ajoute des cations dans le sol et permet d'accroître son pouvoir tampon (Cronan & Schofield 1990). Les taux annuels d'altération estimés pour les cations basiques d'une pessière du Québec étaient de 1 kg ha⁻¹ pour le K, 2.7 kg ha⁻¹ pour le Mg et de 8.5 kg ha⁻¹ pour le Ca (Duchesne & Houle 2006). Ce processus étant fortement influencé par la température, des sols plus chauds pourraient libérer davantage d'éléments nutritifs, dans la mesure où le contenu en eau du sol ne devient pas limitant (White et al. 1999). Néanmoins, l'analyse de 31 parcelles forestières tempérées et boréales du Québec a montré que 61% des parcelles boréales ne présentaient pas un pouvoir tampon suffisant pour neutraliser les intrants atmosphériques d'acidité (Ouimet et al. 2001). Ainsi, la faible disponibilité de cations basiques des sols de la sapinière boréale pourrait ne pas être suffisante pour neutraliser les intrants acides. De tels cas entraînent alors la mise en solution d'ions Al, un cation acide très abondant sur les sites d'échange. En solution, l'ion Al tend à s'hydrolyser, produisant des complexes $AI(OH)_AI(OH)_2$ ou encore $AI(OH)_3$ libérateurs de protons. En général, l'abondance du Al en solution est associée aux sols acides présentant une faible saturation en bases ainsi qu'un ratio Al/bases échangeable élevé (Cronan & Schofield 1990). La dissolution d'ions Al peut ensuite déplacer des cations basiques des sites d'échange et faciliter leur lessivage hors d'accès des racines des arbres, diminuant d'autant le pouvoir tampon des sols (Galloway et al. 1983; Zysset et al. 1999). Le contrôle de la solubilité du Al est un processus complexe qui fait encore l'objet de débats.

Sa solubilité peut être contrôlée par des réactions d'adsorption sur des composés organiques ou des colloïdes argileux, ou encore par des réactions de précipitationdissolution de composés minéraux plus stables comme la gibbsite (Al(OH)₃) ou la kaolinite (un silicate cristallin de formule Al₂Si₂O₅(OH)₄) ainsi que les phosphates (AlPO₄), sulfates (Al₂(SO₄)₃) et divers silicates d'Al amorphes (Cronan *et al.* 1986). L'importance relative de ces processus est l'objet de nombreuses études puisque une abondance excessive de cet élément en solution peut interférer avec l'assimilation racinaire d'autres éléments nutritifs – particulièrement Mg et Ca – et inhiber la croissance racinaire (Driscoll *et al.* 1985; Cronan & Grigal 1995). Par exemple, plusieurs sapinières et pessières du Nord-Est des États-Unis et d'Allemagne aux sols acides (pH≤4.5) ont montré des problèmes de croissance attribués aux effets antagonistes du Al sur l'assimilation racinaire du Ca (Shortle & Smith 1988). Des expériences portant sur les seuils de tolérance de semis de sapin baumier ont montré que l'Al pouvait inhiber l'élongation racinaire et l'assimilation des éléments nutritifs (Schier 1985).

Racines et mycorhizes

La réponse des radicelles du sapin baumier pourrait moduler la réponse de l'écosystème aux changements globaux, étant donné leur forte influence sur la dynamique souterraine de l'eau, du carbone et des éléments nutritifs (Yuan & Chen 2010). Anticiper les effets de sols plus chauds et plus secs sur les radicelles est compliqué par des réponses antagonistes à ces changements. D'un côté, l'élongation racinaire, l'absorption d'éléments nutritifs et la mortalité augmentent dans des sols plus chauds (Gessler *et al.* 1998; BassiriRad 2000; Pregitzer *et al.* 2000), et la production totale de radicelles est corrélée avec la durée de la saison de croissance (Majdi & Öhrvik 2004). De telles relations supposent que la production racinaire devrait bénéficier d'un climat plus chaud. Cependant, les arbres réduisent généralement l'allocation en carbone aux racines lorsque le N devient plus abondant (Nadelhoffer 2000). Ainsi, une réduction de la biomasse racinaire a été observée suite à un réchauffement expérimental du sol (Bergner *et al.* 2004; Majdi & Öhrvik 2004; Bronson *et*

al. 2008). De plus, les arbres répondent à des conditions plus sèches le plus souvent en accroissant leur ratio racine/tige et en orientant la croissance racinaire vers des zones profondes du sol où l'eau est davantage disponible (Kozlowski *et al.* 1991; Joslin *et al.* 2000). Cependant, des sécheresses sévères et de courte durée peuvent entraîner une réduction de la biomasse des radicelles causée par une mortalité accrue, de la cavitation et une diminution de la croissance (Joslin *et al.* 2000; Palatova 2002).

Les champignons mycorhiziens sont associés aux racines des arbres de façon symbiotique, fournissant eau et éléments nutritifs en échange de carbone. Il est généralement établi que la disponibilité des éléments nutritifs est inversement corrélée avec le degré de colonisation mycorhizienne (Lilleskov *et al.* 2002; Nilsson & Wallander 2003), bien que des exceptions soient rapportées (Treseder *et al.* 2007). Ainsi, l'augmentation attendue du N disponible pourrait réduire l'importance des mycorhizes dans la sapinière boréale. Une réduction du degré d'infection mycorhizienne chez le pin sylvestre (*Pinus sylvestris* L.) a également été observée en réponse à une sécheresse expérimentale (Palatova 2002). Quant à l'effet de la température du sol sur cette communauté, le manque d'études en forêt coniférienne ainsi que le manque de connaissances sur la composition de ces communautés rend toute anticipation pour l'instant incertaine (Staddon *et al.* 2002).

1.2.6 Impacts potentiels sur la croissance de la sapinière boréale

Les forêts représentent un réservoir de 2 477 Gt de carbone et captent chaque année environ 3.3 Gt de CO₂ (IPCC 2007). La formation du bois, ou xylogénèse, constitue ainsi un des mécanismes majeurs permettant de limiter la concentration de gaz à effets de serre dans l'atmosphère. Cependant, notre capacité à prédire la croissance dans les prochaines décennies est limitée par notre faible compréhension des processus et facteurs qui régissent la xylogénèse (Savidge 2000b). La formation du bois est initiée par les divisions cellulaires dans le cambium qui produisent des cellules cambiales dérivées. Ces nouvelles cellules, encore indifférenciées, entrent ensuite dans une phase d'élargissement suivie d'une phase d'épaississement et de lignification des parois, avant d'entrer en apoptose, ou mort cellulaire programmée, et de devenir des cellules fonctionnelles du xylème (Plomion

et al. 2001). Plusieurs des changements globaux projetés pourraient affecter chacune des phases de la xylogénèse, et ainsi altérer la quantité annuelle de bois produit et de carbone séquestré.

Réactivation du cambium

La température de l'air est le facteur le plus corrélé avec la réactivation du cambium au printemps (Begum et al. 2008; Begum et al. 2010; Dufour & Morin 2010; Gruber et al. 2010; Begum et al. 2013). La mitose cellulaire étant physiologiquement impossible à des températures inférieures à 1-2° (Körner 2003), des seuils de température minimale de 4-5°C ont été associés à l'activité cambiale de divers conifères en milieu froid (Rossi et al. 2008). De plus, de nombreuses expériences de chauffage du tronc d'arbres en dormance ont confirmé le rôle prépondérant de la température dans la réactivation cambiale (Savidge & Wareing 1981; Barnett & Miller 1994; Oribe & Kubo 1997; Oribe et al. 2001; Oribe et al. 2003; Gricar et al. 2006; Gričar et al. 2007). Ainsi, avec des températures plus chaudes, le début de la mitose du cambium au printemps pourrait être devancé. En même temps, plusieurs de ces expériences de chauffage ont produit des réactivations partielles (réactivation temporaire, absence de différentiation cellulaire, etc.) indiquant que d'autres facteurs pourraient influencer la réactivation (Oribe et al. 2003; Gricar et al. 2006). Entre autres, certains ont observé un épuisement des réserves de carbohydrates peu après le début de la xylogénèse (Krabel 2000; Oribe et al. 2003), ce qui pourrait révéler l'importance que la réactivation cambiale soit synchrone avec l'activité photosynthétique afin d'assurer un apport en sucres constant pour maintenir la croissance. Également, certaines hormones, elles-mêmes sensibles à des signaux environnementaux, pourraient moduler la réponse du cambium. Par exemple, l'acide β indolacétique (AIA), une forme d'auxine, est reconnu comme le déclencheur chimique de la réactivation du cambium (Little & Bonga 1974; Little & Savidge 1987; Rensing & Owens 1994; Uggla et al. 1998; Savidge 2000a; Frankenstein et al. 2005). Cependant, l'acide abscissique (ABA), une phytohormone stockée dans les racines et relâchée lors de stress hydrique afin d'initier la fermeture des stomates (Davies & Zhang

1991), peut inhiber les récepteurs d'auxine du cambium et ainsi affecter la réactivation (Hou *et al.* 2006). Plusieurs auteurs ont justement rapporté que la réactivation du cambium était corrélée avec la fonte de la neige (Vaganov *et al.* 1999; Jarvis & Linder 2000; Kirdyanov *et al.* 2003; Körner 2003; Carrer *et al.* 2007; Jyske *et al.* 2012). Ainsi, le faible contenu en eau du sol au printemps, très faible jusqu'à la fonte de la neige, pourrait limiter l'activité cambiale (Jarvis & Linder 2000). Cependant, l'analyse de l'effet d'un réchauffement hâtif du sol sur la réactivation du cambium de l'épinette noire et de l'épinette de Norvège (*Picea abies* L. Karst.) n'a pu établir de lien de causalité entre les deux (Strömgren & Linder 2002; Lupi *et al.* 2012a). De telles études n'ont pas encore été menées sur d'autres espèces de conifères comme le sapin baumier.

Croissance radiale

Un allongement de la saison de croissance – période qui débute lorsque la température moyenne est ≥5°C pour cinq jours consécutifs et se termine quand elle passe sous la barre des -2°C – devrait allonger l'activité photosynthétique et ainsi accroître les quantités de carbohydrates disponibles pour le maintien et la croissance des arbres. Des expériences de fertilisation ont montré que des quantités plus importantes de N disponible, causées par des sols plus chauds et/ou des dépôts atmosphériques accrus, pourraient également augmenter le nombre de cellules produites (Puech *et al.* 2000; Plavcovà *et al.* 2013). Également, une corrélation positive a été établie chez le sapin baumier entre les dépôts de N et le contenu foliaire en N (McNeil *et al.* 2007) ainsi que la croissance radiale (Quinn Thomas *et al.* 2010). De plus, l'accroissement des concentrations atmosphériques en CO₂ a en général des effets positifs sur les taux de photosynthèse et la croissance, bien que l'intensité de la réponse varie selon l'espèce (Hyvönen *et al.* 2007; Ellsworth *et al.* 2012).

Cependant, ces effets positifs sur la production de xylème pourraient être contrebalancés par d'autres facteurs. Par exemple, l'augmentation prévue des niveaux atmosphériques d'ozone pourrait réduire la photosynthèse, demander plus de ressources pour réparer les dommages foliaires (chlorose légère, pigmentation foliaire anormale,
sénescence foliaire prématurée) et réduire d'autant la croissance (Ollinger et al. 2002; Sitch et al. 2007). De plus, une diminution de l'eau disponible, dans des sites déjà limités en eau, est souvent liée à une plus faible production de xylème liée à un ralentissement de l'activité photosynthétique (Hsiao 1973; Eilmann et al. 2009; Jyske et al. 2010). Également, des températures plus chaudes accroissent les pertes d'eau via les stomates et réduisent l'efficacité des plantes à utiliser l'eau disponible (Campbell *et al.* 2009). Pour des cas extrêmes comme les sécheresses, tel que mentionné précédemment, nous manquons de données permettant d'évaluer la résistance du sapin baumier. Néanmoins, la vulnérabilité des conduits de xylème aux embolismes induits par une sécheresse n'est pas limitée aux environnements secs (Choat et al. 2012), et les forêts humides de sapin baumier du Québec pourraient être tout autant affectées. Une sécheresse expérimentale n'a révélé aucune réduction de croissance radiale chez l'épinette noire adulte au Québec (Belien et al. 2012). Cependant, dans le sud de la Finlande, des arbres matures d'épinette de Norvège sujets à des sécheresses printanières de 60 à 75 jours pendant 4 à 5 années de suite ont réduit leur cerne de croissance annuel de 11 à 21% chaque année (Jyske et al. 2010). Des coups de chaleur pourraient également affecter la croissance du sapin baumier. En 2006, la croissance radiale de sapins baumiers dans la réserve faunique des Laurentides s'est terminée quatre semaines plus tôt que les autres années suite à une combinaison de températures chaudes, d'humidité relative élevée et de faibles précipitations (Duchesne & Houle 2011). De plus, les impacts de tels évènements peuvent persister plusieurs années et parfois se révéler mortels, notamment à cause des dommages hydrauliques persistants (Anderegg et al. 2013) ainsi que de l'épuisement des réserves en carbone (McDowell et al. 2008). Enfin, la forte compétition pour le N dans les sols boréaux pourrait également limiter l'accroissement attendu du nombre de trachéides produits. Un tel mécanisme a été avancé pour justifier l'absence d'effets d'un accroissement durant quatre ans de la température de l'air et du sol ainsi que de la concentration atmosphérique en CO₂ sur le xylème produit par une forêt mixte de Norvège, dans le cadre du projet CLIMEX où plusieurs parcelles de forêt matures étaient isolées dans des serres à climat contrôlé (Rasmussen et al. 2002).

Caractéristiques des trachéides

Outre le nombre de cellules du xylème, leur diamètre ainsi que l'épaisseur de leurs parois déterminent les quantités de carbone séquestrées annuellement dans un arbre. En réaction à une réduction de la disponibilité en eau, les conifères, incluant le sapin baumier, peuvent réduire la taille de leurs trachéides et/ou accroître l'épaisseur de leurs parois (Abe & Nakai 1999; Abe *et al.* 2003; Eilmann *et al.* 2009; Rossi *et al.* 2009; Gruber *et al.* 2010; Jyske *et al.* 2010). Par exemple, la simulation en serre d'une sécheresse estivale de 20 jours a produit chez des semis de sapin baumier une réduction de 50% du diamètre des trachéides et de la superficie de leur lumen (Rossi *et al.* 2009). La diminution du diamètre cellulaire est une adaptation à la réduction de la pression hydrostatique, puisqu'elle diminue les risques de cavitation (Sperry *et al.* 2006). De plus, des parois plus épaisses permettent d'éviter l'implosion des conduits du xylème que pourrait causer une trop forte pression négative (Hacke *et al.* 2001). Dans les sites non limités en eau, l'accroissement attendu de la photosynthèse (causée par les hausses de CO₂, de température ou d'éléments nutritifs) devrait engendrer plus de transpiration, et ainsi nécessiter un accroissement du diamètre des cellules de xylème (Beets *et al.* 2001; Roderick & Berry 2001; Mäkinen *et al.* 2002).

La résilience du sapin baumier aux épisodes de sécheresses pourrait également être influencée par le stade de développement de l'individu. Par exemple, les semis de sapin présentent un enracinement réduit et davantage superficiel que les arbres adultes, ce qui peut limiter leur accès à l'eau disponible dans strates plus profondes du sol (McDowell *et al.* 2008). De plus, bien que leurs besoins soient réduits, les semis présentent également des réserves en carbone limitées qui pourraient entraîner un dépérissement suite à un arrêt prolongé de la photosynthèse (Hartmann 2011). Ainsi, bien que les effets d'une sécheresse soient de nature semblable chez les semis et les arbres matures (Abe *et al.* 2003; Rossi *et al.* 2009; Galle *et al.* 2010), une plus grande vulnérabilité des semis pourrait altérer la structure d'âge des peuplements (Beier *et al.* 2012).

1.2.7 Défis à venir

Plusieurs difficultés freinent notre capacité à prédire l'impact des changements globaux à venir sur la sapinière boréale de l'est du Canada. Le handicap le plus évident pourrait être la diversité des échelles de temps de réponse, allant de processus microbiens modifiés en l'espace de quelques secondes à la composition floristique qui peut prendre des décennies, voire des siècles, pour s'adapter. Shaver et al. (2000) donnent l'exemple de la productivité écosystémique nette: un réchauffement aurait comme effet immédiat un accroissement de la productivité causé par une hausse de la photosynthèse, mais à plus long terme, l'assimilation et l'accumulation plus élevée de N dans la biomasse pourrait réduire la productivité en diminuant le N disponible, sans compter que d'éventuels changements dans la composition floristique viendraient probablement également altérer la productivité. Anticiper cette variabilité temporelle est davantage compliqué par le grand nombre d'interactions qui risquent d'affecter la réponse globale à chaque instant. Notre capacité d'anticipation et d'adaptation à ces changements viendra ultimement de modèles forestiers à grande échelle intégrant l'ensemble des interactions, mais d'ici là, les expériences de changements climatiques en milieu forestier demeurent une source essentielle de données pour le développement de tels modèles.

1.3 Objectifs et hypothèses de recherche

L'objectif général de cette thèse consiste à déterminer expérimentalement l'impact de certains changements globaux projetés d'ici la fin du XXI^e siècle sur la disponibilité des éléments nutritifs du sol et la croissance du sapin baumier dans la sapinière boréale québécoise. L'ensemble des recherches présentées ici ont été menées dans la forêt Montmorency, une sapinière boréale typique située dans la réserve faunique des Laurentides, poussant sur un podzol et présentant un bassin échangeable réduit de cations basiques (Duchesne & Houle 2008). Le monitoring du bassin versant a permis une excellente caractérisation des principaux cycles biogéochimiques, et des placettes permanentes y sont suivies depuis de nombreuses années. De plus, la haute résolution du

Modèle régional climatique canadien, développé par le consortium Ouranos, a permis de projeter avec précision les conditions de température et d'humidité du sol pour ce site pour les années 2070-2100 (Houle *et al.* 2012).

Les chapitres 2, 3, 4 et 5 portent sur les impacts d'un accroissement expérimental de la température du sol de 4°C, incluant une fonte de neige hâtive de 2-3 semaines au printemps, et des dépôts atmosphériques de N (3x la concentration naturelle de NH₄NO₃ dans la pluie) dans une sapinière boréale mature. Le chapitre 2 aborde les effets de ces traitements sur les éléments chimiques disponibles du sol, en posant comme hypothèses que les deux traitements devraient accroître la disponibilité du N et que le chauffage devrait également accroître la disponibilité des cations basiques. Le chapitre 3 permet de vérifier si trois années de chauffage ont réduit la quantité de carbone minéralisable dans le sol, en mesurant la respiration microbienne à l'aide d'incubations en laboratoire. Le chapitre 4 fait le pont entre la chimie du sol et la croissance du sapin baumier, en abordant l'impact des traitements sur le N inorganique disponible du sol ainsi que sur le contenu en N foliaire des arbres et leur croissance radiale. Nous nous attendions à ce que les deux traitements accroissent le N disponible et que les arbres répondent en accroissant leur contenu en N foliaire et leur croissance. Enfin, le chapitre 5 détaille l'impact des traitements sur la formation saisonnière du xylème chez le sapin baumier. Deux hypothèses étaient vérifiées, soit 1) que les traitements allaient accroître le nombre et le diamètre des cellules de bois produites et allonger la saison de croissance, et 2) que le moment de fonte de neige ne déterminait pas le début de la xylogénèse.

Le chapitre 6 aborde une autre composante majeure des changements climatiques, soit le risque accru de sécheresse. L'objectif consiste à établir la vulnérabilité du processus de xylogénèse du sapin baumier soumis à deux années consécutives de sécheresse estivale simulées à l'aide d'un dispositif d'exclusion des précipitations. Plusieurs hypothèses sont avancées : 1) la sécheresse entraînerait une réduction de la taille des cellules et un épaississement de leurs parois, 2) la sécheresse avancerait la fin de xylogénèse, 3) le nombre de cellules produites serait surtout diminué la deuxième année puisque la majorité des cellules auraient déjà été formées lors de la première année de traitement, et 4) la

réduction de croissance serait plus sévère pour les semis de sapin que pour les arbres matures.

Connecting paragraph

This chapter constitutes a fine scale study of the impact of three years of experimentally increased soil temperature and N deposition on the soil chemistry of a boreal balsam fir stand. We used ion exchange membranes to determine the availability of soil nutrients for tree growth. This chapter is being reviewed for publication by the 'Soil Science Society of America Journal' with co-authors Daniel Houle, Benoît Côté, and Louis Duchesne.

Chapter 2 - Effects of increased soil temperature and atmospheric nitrogen deposition on soil element fluxes in a mature balsam fir forest

2.1 Abstract

The projected increase in atmospheric N deposition and air/soil temperature will likely affect soil nutrient dynamics in boreal ecosystems. The potential effects of these changes on soil ion fluxes were studied in a mature balsam fir stand (Abies balsamea [L.] Mill) in Québec, Canada, that was subjected to three years of experimentally increased soil temperature (+4°C) and increased inorganic N concentration in artificial precipitation (3x the current N concentrations using NH_4NO_3). Soil element fluxes (NO_3 , NH_4 , PO_4 , K, Ca, Mg, SO₄, Al and Fe) in the organic and upper mineral horizons were monitored using buried ion exchange membranes (PRSTM-probes). There were no effects of the treatments on soil inorganic N availability. While N additions did not affect soil element fluxes, three years of soil warming increased the cumulative fluxes of K, Mg and SO_4 in the forest floor by 43, 44 and 79%, respectively, and Mg, SO₄ and Al in the mineral horizon by 29, 66 and 23%, respectively. We attribute these changes to increased rates of soil organic matter decomposition. Significant interactions of the heating treatment with time were observed for most elements although no clear seasonal patterns emerged. The increase in soil K and Mg in heated plots resulted in a significant but small K increase in balsam fir foliage while no change was observed for Mg. A 6-15% decrease in foliar Ca content with soil warming could be related to the increase in soil available Al in heated plots, as Al can interfere with the root uptake of Ca.

2.2 Introduction

Plant growth in boreal ecosystems is often constrained by nutrient availability (Hobbie *et al.* 2002). In the last decades, the human-induced increase in atmospheric N deposition has increased soil N availability (Driscoll *et al.* 2001) and improved the growth of

many tree species in some regions of North America (Quinn Thomas et al. 2010) and Europe (Kenk & Fischer 1988). The global levels of atmospheric N deposition are projected to be 70% higher in 2050 relative to 2000 (Galloway et al. 2004). In addition, boreal soils are also expected to undergo significant warming in the coming decades, with projected increases in mean annual temperature of 2.0-3.3°C in 2070-2099 relative to the current 30-yr average (1971-2000) for some sites in Eastern Canada (Houle *et al.* 2012). Many soil processes such as litter decomposition and mineralization of soil organic matter (SOM) are expected to proceed faster with such warming (Campbell et al. 2009). Because of the temperature dependence of enzymatic reactions in the soil, decay rates for the labile fraction of the SOM respond well to temperature increases (Davidson & Janssens 2006). A priori, these changes could be beneficial for the availability of N as well as for base cations (K, Ca and Mg). As boreal soils of the Canadian Shield generally have small reservoirs of available N (Tamm 1991) and base cations (Duchesne & Houle 2006, Watmough *et al.* 2005), the combination of increased N deposition and warmer soils is expected to have beneficial effects on tree nutrition and growth in the Canadian boreal forest. Additive effects of these factors on plant productivity have been reported in temperate ecosystems (Hutchison & Henry 2010, Ma et al. 2011).

The soil nutrient response to increased temperature and N deposition may however be more difficult to predict than initially thought. For instance, the decomposition of more recalcitrant SOM fractions is relatively limited by other environmental factors such as physical aggregation or strong chemical bonds that can attenuate the positive effect of temperature on decomposition over time (Davidson & Janssens 2006, Luo *et al.* 2001, Melillo *et al.* 2002). Also, soil water content is projected to decrease in boreal forests during the growing season (Houle *et al.* 2012). Such changes could affect soil nutrient dynamics, as organic matter decomposition rates are affected by soil moisture (Swift *et al.* 1979). On the other hand, soil water content and temperature are projected to increase earlier in the season due to an earlier snowmelt (Houle *et al.* 2012). Changes in redox potentials associated with wetter and warmer soils early in the growing season could increase the

amounts of Fe, Mn, PO₄ and SO₄ in soil solution (Darke & Walbridge 2000, Ponnamperuma 1972).

Increased soil temperature and atmospheric N deposition may also affect the acidbase status of soils. The higher SOM decomposition commonly observed in warming experiments (Rustad *et al.* 2001) is usually associated with increased releases of organic acids and can result in soil acidification (Lundström *et al.* 2000; Van Cleve *et al.* 1990). Additional inputs from the atmosphere, namely NO_x, NH_y, and SO₄, also have the potential to increase soil acidity, a process that may have contributed to the decline of some fir and spruce forests of Germany and Eastern North America (Shortle & Smith 1988). In acidic soils with a low buffering capacity, acidic inputs can result in the dissolution of aluminium, a phytotoxic ion that can inhibit root elongation and nutrient uptake (Cronan & Grigal 1995). Both Al and H ions can displace base cations from exchange sites resulting in increased leaching of these nutrients out of the soil rooting zone (Galloway *et al.* 1983).

Very few studies have been conducted to assess the combined effects of soil warming and increased N deposition in boreal forests and in those that did, the focus was generally on N and C cycling (*e.g.* the study by Mäkipää *et al.* (1999) with Scots pine in Finland). Other nutrients (K, Ca and Mg) but also elements potentially toxic to growth, Al for example, were not considered. Our objective is to characterize the potential effect of global changes on soil nutrient availability of a typical balsam fir (*Abies balsamea* [L.] Mill.) boreal forest of Quebec. For this purpose, we experimentally combined an increase in soil temperature (+4°C) and length of growing season (earlier snowmelt, +2-3 weeks) with an increase in rain N concentration during the growing season (3x the current N concentration in the rain at the site), to study their effects on ion availability (NO₃, NH₄, PO₄, K, Ca, Mg, S, Fe and Al). We hypothesized that i) NO₃ and NH₄ availability would increase in the N deposition and the warming treatments ii) NO₃ and NH₄ availability would be higher in the combined treatment (N deposition + warming soils) and iii) that higher soil temperatures would increase base cations availability.

2.3 Materials and methods

Study area

The study site is located in the Lake Laflamme watershed north of Quebec City (47°17′ N; 71°14′ O; 800 m above sea level). The experimental plots were established in a 60-year-old even-aged balsam fir stand. The soil is an Orthic Ferro-Humic Podzol (Spodosol in the U.S. soil taxonomic system), laying on a bedrock of Precambrian charnockitic gneiss and characterized by a sandy loam texture. The C and the N content of the forest floor are 416 g C kg⁻¹ and 16.6 g N kg⁻¹, respectively, for a C:N ratio of 25 (unpublished data). The pH of the forest floor (FH horizon) and mineral horizons (first 30 cm) is 3.0 and 4.1 (NH₄Cl-based), respectively. The climate is continental with cold winters and warm summers. Between 1981 and 2006, annual precipitation was 1535 mm and mean temperature was -0.4°C. Annual N deposition (NH₄ + NO₃) averages 5.7 kg N ha⁻¹ yr⁻¹ (Houle & Moore 2008).

Experimental design

The experiment was set up in autumn 2008 and laid out according to randomized split-plot design with two levels of warming (ambient and 4°C above ambient) nested within two levels of artificial precipitations (no added NH₄NO₃ and 3 times the ambient NH₄NO₃ concentration in incoming precipitations, details below). Because of the intensive manipulations and sampling effort, we limited the experiment to twelve plots split up into three blocks. The blocks were 12m x 60m and laid out perpendicular to the slope with a 12m buffer strip in between. Each block was divided in two 24mx12m sub-blocks separated by a 12m x 12m buffer zone. Each sub-block was divided into two 12m x 12m plots. Within each plot, a balsam fir tree was selected based on a visual estimation of good health and dominance or co-dominance in the canopy. The N deposition treatment was randomly

distributed within the two sub-blocks of each block, and the soil warming treatment was randomly distributed within the two plots of each sub-block.

Soil warming

In each plot, 70 m of heating cables (non-heating cables for the control) were buried in a spiral pattern 5-10 cm below the ground at the interface between the organic and mineral soil with an average distance between cables of approximately 30 cm. Great care was taken to minimize root damage and roots >5mm in diameter were bypassed by running the wire below or above the root. Soil temperature in each plot was measured continuously with thermistors (precision: $\pm 0.2^{\circ}$ C; model 107-L from Campbell Scientific Inc., Utah, USA) buried between cables. The warming cables were activated and disabled when the average difference of soil temperature between heated and non-heated trees reached 3°C and 5°C, respectively, in order to maintain an average difference of 4°C. This value was chosen based on climate simulations for the site (Houle *et al.* 2012). Storage of temperature data and control of warming were done with a CR1000 datalogger (Campbell Scientific, Inc., Utah, USA). Soil heating was started in the spring of 2009. From the beginning of spring snow melt until the first autumn frost, the average soil temperature in treated plots was higher by $4.0\pm0.4^{\circ}$ C, $4.1\pm0.3^{\circ}$ C and $3.7\pm0.9^{\circ}$ C in 2009, 2010 and 2011, respectively (Fig. 2.1).

In addition to the maintenance of a higher soil temperature during the growing season, the heating treatment was also designed to simulate an earlier snowmelt and an earlier snow-pack disappearance. In 2009, soil warming was started on April 27 with a remaining snowpack of 0.75 m, with snowmelt in control plots ending 19 days later. A system malfunction caused an early interruption of the soil warming in mid-August that year. In 2010, soil heating was started on March 30 because of a warm spring. The snowpack was 0.85 m and the snowmelt was completed 28 days later. The treatment was stopped before the first freeze-thaw events, on October 19. The cold spring of 2011 delayed the start of soil heating to May 5. The snowpack was then 0.60 m and snowmelt was completed 16 days after beginning of treatment. Heating was terminated on October 18.

Application of artificial rain

The protocol used to apply artificial rain was designed to simulate how natural atmospheric N is deposited on forest ecosystems. The rain solution (control and N-enriched) was applied on the tree canopy using nozzles set up above each individual tree, within each plot, using a system of pumps and reservoirs. The composition of the artificial rain solution was based on the average concentrations of Na (0.046 mg L⁻¹), Ca (0.089 mg L⁻¹), Mg (0.018 mg L⁻¹), K (0.026 mg L⁻¹), H (0.014 mg L⁻¹), Cl (0.07 mg L⁻¹) and SO₄ (1.01 mg L⁻¹) in incoming precipitation measured at the site. For the N treatment, NH₄NO₃ was added to reach a final rain concentration of 1.0 and 3.4 mg L⁻¹ of NH₄⁺ and NO₃⁻ respectively which corresponds to approximately three times the concentrations measured in the local precipitation. Each year, from mid-June to mid-September, each plot was simultaneously subjected to a weekly artificial precipitation of 70 litres. The applications resulted in an increased N deposition of 1.544 g N tree⁻¹ year⁻¹, which was dispersed over an area estimated to 20-50 m², resulting in a rain event of 1.3-3.4 mm, or an additional 1.1-2.7 kg N ha⁻¹ year⁻¹. Watering was rescheduled to the next day on rainy days.

Soil sampling and analysis

Soil ion fluxes were determined during the three years of the study using ion-exchange membranes (PRSTM-probes, Western Ag Innovations Inc.; Saskatoon, Canada). Ion exchange membranes allow the assessment of nutrient supply rates by continuously adsorbing free ions on charged surfaces. Contrary to classical soil extractions or lysimeters, ion exchange membranes simulate the action of plant roots that accumulate nutrients from soils through exchange reactions (Qian & Schoenau 1997). At first, the probes typically adsorb the labile pool of ions until depletion, followed by measurements of ion supply rates through mineralization, leaching or dissolution. Being in contact with plant roots, the measured values reflect the difference between nutrient release and root immobilization. The probe

data will be referred to as 'available nutrients' hereafter. Avoiding direct contact with heating cables, six pairs of probes – a pair consisting of one cation and one anion adsorbing membrane – were evenly distributed around the tree at a distance of 1.5 m from the stem, three pairs in the organic soil layer (approx. 5-10 cm deep) and three pairs in the mineral soil layer (approx. 15-20 cm deep) inserted vertically in the soil. In 2009, the probes were replaced every four weeks to avoid potential saturation of the membranes, from May 21 to November 2. Based on these results, it was decided to replace the ion exchange membranes every six weeks for the following years, each burial period corresponding to spring, summer and autumn, and keep one set of probes buried between the growing seasons to measure the elemental flux during winter and the snowmelt period.

At the end of each burial period, the probes were removed and rinsed with deionized water to remove soil particles and then sent to the laboratory (Western Ag Innovations) for analysis. The probes were pooled per soil horizon for each tree. Membranes were eluted with 17.5 ml of 0.5 M HCl for 1h. Concentrations of NH₄, NO₃ and PO₄ in the extract were determined colorimetrically using a Technicon Autoanalyzer (Technicon AA2, Seal Analytical Inc., Mequon, USA), while K, SO₄, Ca, Mg, Al and Fe concentrations were measured using inductively-coupled plasma-atomic emission spectrometry (IPC-AES; Perkin Elmer Optima 3000-DV ICP; Perkin-Elmer Inc., Shelton, USA). Although sulfur levels measured with ICP are composed of SO₄ as well as dissolved organic S, they will be referred to as SO₄ as it is the most abundant fraction (Vendrell *et al.* 1990). The values were reported as µg 10 cm⁻² of resin surface. To compare seasonal or annual fluxes, values were divided by the duration of burial in days to account for the time differences between burial periods. However, such comparison should be interpreted with caution as nutrient fluxes measured with PRS-probes are not linear over time.

Soil water content

Volumetric soil water content (SWC) in the plots was measured weekly during the snow free period from 2009 to 2011. SWC was measured with a ThetaProbe ML1 (Delta-T Devices Ltd,

Cambridge, England) in 2009 and with a FieldScout TDR-300 device with 20-cm rods and standard calibration (accuracy: ±3% vol; Spectrum technologies Inc., Plainfield, USA) in 2010 and 2011. A broken rod delayed the start of the 2011 data collection. For each plot, nine measurements were randomly taken at a distance of 1.5m from each tree and averaged.

Leaf chemistry

Current-year foliage was collected from the upper third of the canopy on three distinct branches of each tree. Sampling was done at the end of each growing season. Needles were oven-dried at 65°C, ground at 250 µm and digested with H₂SO₄. Nitrogen was determined with a Kjeldahl autoanalyser (Kjeltec Auto model 1030 Analyser, Tecator, UK), and P, K, Ca and Mg were determined by ICP-AES (Perkin Elmer Optima 3000-DV ICP; Perkin-Elmer Inc., Shelton, CO, USA).

Statistical Methods

A linear mixed-model analysis of variance (ANOVA) with repeated measures was used to test for the effect of the warming treatment on SWC, considering warming, year and sampling date as fixed factors and plot within sub-block within block as random factors. A compoundsymmetry variance-covariance structure was assumed for repeated measures. Soil (per horizon) and leaf nutrient data were analyzed using a similar ANOVA model. When *treatment* and *treatment*time* interactions were significant, each time period was analyzed separately. Data were tested *a priori* to meet the assumptions of ANOVA, and Tuckey posthoc tests were used when needed to identify which means were significantly different from one another. Finally, principal component analyzes (PCA) were applied on the matrices of nutrients, with the soil warming treatment transformed to binary factor, for each soil horizon. The N deposition treatment was excluded because of the absence of significant effects measured with ANOVA. All statistical analysis and plots were done using the R software (R Development Core Team 2012).

2.4 Results

Soil water content

The average SWC during the growing season was significantly lower in 2010 (20.7 \pm 0.7%) relative to 2009 (28.1 \pm 0.7%) and 2011 (26.6 \pm 0.9%) (*P*<0.01; Fig.2.2). This was mainly caused by low precipitations in August (38mm compared to 122 mm in 2009 and 226 mm in 2011) and early September. Heated plots had higher SWC than control plots at times but the overall difference was not significant (*P*=0.08).

Soil available nutrients – Temporal variations

Except for Fe in the organic horizon, all ions measured with ion-exchange membranes displayed significant temporal fluctuations in both horizons (P<0.01; Fig. 2.3). In 2009, forest floor available NH₄ and NO₃ were 9.5 and 3.2 times higher than the average of the following years, respectively. With the exception of Fe and SO₄, a similar pattern was observed for other ions in that horizon, with levels of Ca, Mg, K, PO₄ and Al being 1.6, 1.4, 3.1, 2.0 and 1.5 times higher, respectively, in 2009 relative to 2010-2011. In the mineral horizon, only NH_4 , NO₃ and Al were highest in 2009, with levels 4.5, 7.4 and 1.4 times higher relative to 2010-2011. The high concentrations observed in 2009 in the forest floor for all elements were characterized by an early peak in the growing season followed by a marked decrease in autumn, particularly for NH₄ and K with a 10- and 6-fold decrease, respectively (Fig. 2.3). In the mineral horizon, decreasing trends were also observed for NO₃, NH₄, PO₄, SO₄ and Al with levels 7.1, 4.8, 1.6, 3.8 and 1.7 times lower in autumn, respectively, while Mg, K and Ca remained stable over the growing season. This decreasing seasonal trend was similar in 2010 for Mg, K, SO₄, Fe and Al for the forest floor, with levels 1.5, 1.4, 2.8, 6.1 and 1.8 times lower in autumn (Fig. 2.3), while NO₃ and NH₄ values remained steadily low (Fig. 2.3). No such seasonal pattern was observed in 2011 for both soil horizons.

Soil available nutrients – Effects of treatments

Soil warming significantly increased available K in the forest floor, Mg and SO₄ in both soil horizons, and Al in the mineral horizon (P<0.05; Fig. 2.4). The cumulative amounts of forest floor available K, Mg and SO₄ were 43, 44 and 79% higher in heated plots, respectively, relative to control plots. In the mineral horizon, available Mg, SO₄ and Al were 29, 66 and 23% higher in heated plots, respectively. The corresponding Ca:Al molar ratios in that horizon were 3.1 ± 0.3 and 3.8 ± 0.2 in heated and control plots, respectively. Exclusion of the first year data from the calculations has negligible effects ($\pm3\%$) on the cumulative values for all elements except for K, whose increase in the warming treatment went from 43 to 20%. Peaks of available Fe were measured in the organic and mineral horizons of heated plots over the course of the experiment (Fig. 2.3), but differences in cumulative fluxes between treatments were not significant (P>0.15; Fig. 2.4). The peaks in available Fe were exclusively observed in three of the six heated plots, with concentrations as high as 46- and 91-fold the average of other plots in the forest floor and mineral horizon, respectively (Fig. 2.5). These extreme values were correlated with elevated SWC (>30%) during spring or fall (Fig. 2.5).

Significant interactions of the heating treatment with time were observed for K, Mg and SO₄ in the forest floor as well as for Mg in the mineral horizon (P<0.05; Fig. 2.3). When tested per burial period, available K in the forest floor was significantly higher in heated plots in the summer of 2009, spring 2010 and fall 2011 (P<0.05). For available Mg, significant effects were limited to spring and fall of 2009 in the forest floor as well as spring 2010, winter-snowmelt in 2010-2011 and summer 2011 in both soil horizons (P<0.05). For SO₄ in the forest floor, the treatment effect was observed at the end of the 2009 summer as well as during the summer of 2010 and the winter-snowmelt period between 2010 and 2011 (P<0.05).

Finally, no significant effects (or interactions with warming) were detected for the N treatment on soil nutrients (*P*>0.05).

The two first principal components (PC1 and PC2) for available ions measured explain 59.2% and 49.5% of the total variability of the dataset in the organic and mineral horizons, respectively (Fig. 2.6). In both soil horizons, the elements are clustered in two groups relative to PC1 and PC2 (Fig. 2.6). In the forest floor, Ca, Mg, SO₄, Al and Fe are correlated with PC1 (r = 0.45, 0.46, 0.48, 0.44, and 0.26, respectively), while NO₃, NH₄, K and PO₄ are correlated with PC2 (r = 0.24, 0.50, 0.43, and 0.37, respectively). The soil heating vector is weakly correlated with PC2 (r=0.17) and PC1 (r=0.09). The only notable difference between horizons comes from Fe, whose correlation with PC2 increases from 0.05 in the forest floor to 0.19 in the mineral soil (Fig. 2.6).

Leaf nutrients

From 2009 to 2011, no statistical differences in leaf nutrient concentrations were detected between treatments for N (12.5±0.26 g kg⁻¹), P (1.98±0.05 g kg⁻¹) and Mg (0.90±0.04 g kg⁻¹) (Table 2.1). Significantly higher K (3%, P=0.02) and lower Ca concentrations (6-15%, P<0.01) were measured in balsam fir needles from heated plots compared to controls. Trees subjected to combined heating and increased N deposition displayed 2% lower needle K levels (P<0.01) relative to controls.

2.5 Discussion

Initial soil disturbance effects

High levels of available soil nutrients were observed in the first year of treatment, especially N and K. This is probably the result of the soil disturbance caused by the burial of the heating and control cables during the preceding fall. In boreal ecosystems, about fifty

percent of the fine root biomass (<2mm in diameter) is located in the upper 10 cm of the soil profile, and up to 90% can be found in the top 30 cm (Yuan & Chen 2010). The impact of the trenching on roots was minimized by running the cable below or above large roots. The effect of cable burial should therefore be mostly restricted to fine-roots. In general, N mineralization rates are stimulated by the input of fresh organic matter or the mixing of the soil (Booth et al. 2006, Weintraub & Schimel 2003). The higher NH₄ concentrations measured in the forest floor early in the season were synchronous with increased NO₃ concentrations in the mineral horizon, which suggests that some of the NH₄ was leached into the mineral soil where the higher pH could have allowed its transformation into NO₃ (Ste-Marie & Paré 1999). As for K, it is found in living organisms as a soluble electrolyte and its concentration is relatively high in soil microbes and woody plants in comparison to other ions (Bowden et al. 1989). Therefore, K cycles rapidly following ecosystem disturbance (Likens et al. 1994). Other elements like Ca or Mg are partly incorporated in cell organelles and plant tissue, for example Ca in cell wall. They also have a higher affinity for soil exchange sites because of their higher valence, and are therefore more easily retained following soil disturbance. Similar nutrient pulses were observed in hardwood forests of northeastern U.S. in the first year following cable burial (McHale & Mitchell 1996, Peterjohn et al. 1994) and soil lysimeter installation (Johnson et al. 1991). During a spruce budworm outbreak in 1981-1984 at the site, N and K also displayed the highest leaching losses amongst soil nutrients in reaction to increased organic matter decomposition rates and reduced nutrient uptake (Houle et al. 2009). Similar patterns were observed after fire and forest harvesting in boreal watersheds of Québec (Lamontagne et al. 2000), providing further evidence of the responsiveness of soil N and K to perturbations.

Effects of treatments on N availability

Given that N is the most common growth-limiting nutrient in boreal ecosystems (Hobbie *et al.* 2002), increased N availability due to soil warming or additional N deposition has the potential to increase ecosystem productivity. However, contrary to our first two

hypotheses, the treatments, alone or in combination, had no effect on N availability. This result is consistent with the lack of effects of both treatments observed on needle N concentration and radial growth (D'Orangeville *et al.* in press). A similar lack of response of soil N was also observed in a N fertilization experiment (18 and 60 kg N ha⁻¹ yr⁻¹ added during three years) in a neighbouring stand and was associated with high N retention in the upper soil profile with nearly no inorganic N in the soil solution at 30 or 60 cm depth (Houle & Moore 2008). This result suggests the presence of strong N sinks in the soil that may quickly immobilize the N input from the N deposition treatment (Berg & Matzner 1997, Friedrich *et al.* 2011, Nadelhoffer *et al.* 1999). Because of the temperature-dependence of N mineralization (Jackson *et al.* 2008, Rustad *et al.* 2001), the soil warming treatment was also expected to increase N availability. If SOM decomposition rates were indeed stimulated by the treatment, the resulting N compounds – including organic N, which can be taken up by ectomycorrhizal fungi (Chalot & Brun 1998) – could have been quickly immobilized due to the high competition mentioned earlier.

Response patterns to increased soil temperature

A soil warming effect was measured during the three years of treatment on available Mg and K, in agreement with our third hypothesis. Calcium behave similarly to Mg in the organic horizon (r=0.91, df=154, *P*<0.05), but the differences between treatments were not significant (*P*=0.11), perhaps because of the lower mobility of Ca, relative to Mg. The K response to the warming treatment was 2-fold stronger than Mg and Ca during the first year (see PCA results in Fig. 2.5). As discussed above, the high mobility of K, as compared to Ca and Mg, probably increased its release following soil disturbance.

In addition to the predicted increases in base cations with warming, SO₄ and Al availability also increased in heated plots. With the notable exception of K, the intensity of the response of Mg, SO₄ and Al (relative to control plots) remained similar between years. Therefore, we can assume for these elements that the influence of the initial disturbance on the soil warming response was limited. However, the soil warming effect for these elements

was inconsistent and no common seasonal pattern emerged after three years of treatment. We can only speculate on the numerous environmental factors (e.g. precipitations, tree and microbial nutrient uptake, temperature) which could have interacted with the soil response. Such lack of seasonal pattern was also observed in a study of N cycling under various warming treatments in an eastern North American old-field (Auyeung *et al.* 2013).

Higher rates of litter decomposition and mineralization of organic compounds were probably responsible for the additional K, Mg and SO₄ made available in the forest floor of heated plots. The temperature-dependence of these processes for base cations (Campbell et al. 2009, Rustad et al. 2001), and S (MacDonald et al. 1995) are well known. Such positive temperature effects suppose that the warming treatment did not induce a water stress. SWC was not affected by heating in our plots which should have allowed the soil heating to produce the expected effects of increased decomposition and mineralization. The high correlation between Mg and SO₄ availability ($r \ge 0.72$, df=154, P<0.05 in both horizons, see Fig. 2.6) as well as the increase observed in the mineral horizon of heated plots for both elements support the possibility that the SO_4 liberated in soil solution was accompanied by Mg as a counter ion and that both were leached together into the mineral horizon. Although we observed no effect of soil warming on Ca availability, the latter was also correlated with Mg and SO₄ in both horizons ($r \ge 0.91$ and $r \ge 0.68$ respectively, with df=154 and P<0.05) which suggest that Ca behave like Mg. As for Al, its proximity with SO₄ and Mg vectors in PCAs of both horizons (Fig. 2.6) suggests that the additional release of AI in the mineral soil could also be linked with the increased decomposition in the forest floor of heated plots. To the contrary, the lack of a warming effect on K availability in the mineral soil could suggest that K was not leached from the humus to the mineral soil horizons in greater amounts in heated plots.

Effects of soil warming and soil water content on Fe availability

A significant interaction between time and soil heating was observed for Fe. In addition, the peaks in Fe coincided with SWC > 30%. In the study of a floodplain forest soil in the U.S., it

was observed that Fe oxides were very sensitive to flooding-induced declines in soil redox potential (Darke & Walbridge 2000). Reducing soil conditions associated with prolonged periods of water saturation can lead to the dissolution of large amounts of Fe oxides through a temperature-sensitive reaction (Schwertmann 1991, Szilas *et al.* 1998). Based on these observations, we suggest that the peaks of Fe were caused by the increased soil temperature at times of high soil moisture, in spring or fall. The soil temperature gradient between heating cables of heated plots could explain the variability of the response to warming at high soil moisture. As SO₄ is commonly adsorbed on Fe oxides in mineral podzolic horizons at the lake Laflamme site (Houle & Carignan 1992), the dissolution of Fe oxides may have contributed to the higher SO₄ availability in the mineral horizon. In the future, the projected increases in soil temperature and water content in spring could favor similar changes in redox potential.

Consequences for tree nutrition and ecosystem health

Like a majority of eastern Canada boreal forests, our study site rests upon shallow soils derived from granitic rocks with small exchangeable base cation reservoirs (Duchesne & Houle 2006). In the longer term, the higher base cation availability (K and Mg) measured in the forest floor of heated soils could have a positive effect on tree nutrition. However, this possibility is not well supported by the measured foliar nutrient levels, as heated balsam fir trees only displayed a 3% increase in K while Mg levels remained unaffected by the treatment. A more significant change in heated plots was the 6-15% reduction in foliar Ca, an effect that could be related to the 23% higher available Al measured in the mineral horizon of heated plots. Aluminium is known to produce antagonistic interference with Mg and Ca uptake as well as root damages from Al interactions with plant cell components (Cronan & Grigal 1995). The decline of spruce and fir in acid soils of the northeastern United States and in Germany (pH 3.0-4.5) has been attributed to Al-mediated impairment of Ca uptake by tree roots (Shortle & Smith 1988). In balsam fir, inhibition of root elongation is a primary effect of Al toxicity, but root deterioration and reduced uptake of essential nutrients

have also been observed (Schier 1985). A Ca to Al molar ratio ≤ 1 in the soil solution is commonly used as an indicator for potential toxicity (Cronan & Grigal 1995). Based on this threshold, the mean Ca:Al molar ratios of 3.1 ± 0.3 and 3.8 ± 0.2 measured in the mineral horizon of heated and control plots, respectively, with seasonal lows of 1.7 ± 0.3 in heated plots, should not affect the tree health, but critical threshold values are difficult to establish as they show great variability between sites and species (Andersson 1988).

It is also possible that root injuries reduced nutrient uptake. Most of the soil at the interface of the organic and the mineral soil maintained a temperature approximately 4°C higher than in the control plots, but soils in close contact with heating cables could occasionally have reached temperatures lethal to roots. Living organisms in contact with these ranges of temperature (i.e. bacteria, fungi or roots) are also likely to have suffered injuries or mortality (Hare 1961). Based on the small growth and nutrient response of trees to the heating treatment, we believe that these localized "hot spots" did not have significant effects on soil nutrient cycling but could nevertheless have contributed to the high variability of the results.

2.6 Conclusion

Climate manipulations of mature stands are an effective way of studying forest adaptation to climate change. In this study, a mature balsam fir stand was subjected to a 3-year manipulation of soil temperature and atmospheric N deposition, and the soil response was measured with buried ion exchange membranes. The N deposition and soil warming treatments alone or together had no effect on inorganic N availability. Soil warming did increase forest floor Mg and K availability, which was probably induced by increased rates of SOM decomposition, a temperature-dependant process. Three years of treatment had limited effects on leaf nutrition, with a 3% increase in K and no changes in Mg in the needles of heated trees. Owing to the potential phytotoxicity of Al, the increased Al availability in the mineral soil caused by higher soil temperature could have significant consequences for ecosystem health. The higher soil Al could have interfered with the trees Ca uptake capacity, which would explain the 6-15% lower Ca content observed in needles from heated balsam fir trees. Although we observed significant and substantial effects on ion availability after three years of experimental manipulation, more time is necessary to verify if the observed effects will persist on the long-term.

2.7 Acknowledgements

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	Ν	Р	К	Ca	Mg
	(g kg ⁻¹)	(g kg⁻¹)	(g kg ⁻¹⁾	(g kg⁻¹)	(g kg⁻¹)
С	12.7 ± 0.74	2.05 ± 0.16	5.1 ± 0.19	4.1 ± 0.42	0.94 ± 0.07
Ν	13.1 ± 0.39	2.11 ± 0.07	6.1 ± 0.27	4.4 ± 0.54	0.95 ± 0.08
Н	12.4 ± 0.44	1.96 ± 0.05	5.3 ± 0.12	3.9 ± 0.48	0.90 ± 0.09
HN	11.8 ± 0.48	1.79 ± 0.07	5.0 ± 0.28	3.5 ± 0.27	0.79 ± 0.06
Statistical analyses					
Warming (H)	0.15	0.16	0.02*	<0.01**	0.14
Fertilization (N)	0.92	0.73	0.07	0.74	0.53
Year	0.02*	0.02*	0.18	<0.01**	<0.01**
H x N	0.36	0.38	<0.01**	0.07	0.33



Figure 2.1. Average soil temperature and the differential temperature between heated and control plots in 2009, 2010 and 2011. Coloured ribbons are standard error. The periods of soil warming are represented by the shaded areas.



Figure 2.2. Average soil water content in control and heated plots in 2009, 2010 and 2011. Vertical lines are standard error.



Figure 2.3. Soil element availability per burial period in the organic and mineral horizons, as measured with ion exchange membranes (PRS-probes). Vertical bars are standard error. Asterisks indicate significant differences (P<0.05) between heated and non-heated plots per burial period, as indicated by post-hoc tests when significant warming-time interactions (P<0.05) were observed.



Figure 2.4. Soil element availability over the three growing in the organic and mineral horizons, as measured with ion exchange membranes (PRS-probes). Vertical bars are standard error. Asterisks indicate significant differences (P<0.05) between heated and non-heated plots over the three growing, as indicated by post-hoc tests when significant warming-time interactions (P<0.05) were observed.



Figure 2.5. Effect of soil water content on daily available Fe in the organic and mineral horizon measured with ion exchange membranes (PRS-probes).



Figure 2.6. Principal component analysis biplots of the soil warming treatment and available nutrients measured with ion-exchange membranes in the organic and mineral horizons. Values next to the axis captions show the fractions of variance accounted for by that axis.

Connecting paragraph

Chapter two discussed the influence of increased soil temperature and N deposition on the soil chemistry of a balsam fir boreal stand. In the following chapter, the effects of the treatments on the amounts of mineralizable carbon in the soil are measured using standard laboratory soil incubations of samples taken at the end of the third year of treatment. This manuscript was accepted for publication in the journal 'Canadian Journal of Soil Science' as a short communication, with co-authors Benoît Côté, Daniel Houle, and Joann Whalen.

Chapter 3. Reduced mineralizable carbon in a boreal forest soil after three years of artificial warming

3.1 Abstract

Warmer soils are expected to display reduced organic carbon pools. We incubated soils from a balsam fir stand previously subjected to three years of *in situ* experimental warming (+4°C). Mineralizable carbon was significantly reduced (16-25%) in heated soils, corresponding to a 0.4-0.8% decline in the organic carbon pool.

3.2 Introduction

Building soil organic C reserves will help to offset greenhouse gas emissions, but the response of the soil organic C pool (SOC) to future climate scenarios is still highly uncertain (Friedlingstein et al. 2006). Under non-limiting availability of water and nutrients, the SOC decomposition rates and C release to the atmosphere are expected to increase with temperature (Davidson & Janssens 2006). However, long-term in situ warming experiments in temperate forests (Melillo et al. 2011) and prairie ecosystems (Luo et al. 2001) suggest that the effects of temperature on soil respiration may be temporary with increased rates not persisting more than a few years. The rapid depletion of the most labile pools of SOC in the first years of soil warming could explain why soil respiration rates decline with time. Decreasing soil respiration rates could also be explained by the thermal adaptation of soil microbes to warmer soil temperatures (Davidson & Janssens 2006). Both mechanisms were observed in a long-term (>15 years) soil warming experiment in a hardwood forest of Eastern North America (Bradford *et al.* 2008). In boreal ecosystems, decomposition of labile C is generally limited by the low levels of available N as well as soil temperature (Hobbie et al. 2002). However, the interactive effect of soil warming and soil N levels on C mineralization and SOC dynamics remains to be verified for boreal forest soils.

A climate change simulation experiment was set up in a boreal balsam fir forest of Eastern Canada, combining two levels of *in situ* soil warming and two levels of N deposition. After three years of treatment, soils were collected and incubated under standard laboratory conditions for 72 days at 5, 10, 15 and 20°C and soil respiration was monitored. We hypothesized that previously heated soils would have a lower rate of respiration due to labile C depletion and that increased N deposition would exacerbate the depletion of labile C.

3.3 Materials and methods

Study area

The soil warming – N deposition experiment was established in a 60-year-old even-aged boreal balsam fir (*Abies balsamea* (L.) Mill.) stand (47°17′ N; 71°14′ O; 800 m above sea level), about 100 km north of Québec City, in the province of Québec, Canada. The soil is an Orthic Ferro-Humic Podzol laying on a bedrock of Precambrian charnockitic gneiss. The SOC of the organic and upper mineral horizon in 2011 averaged 399±11 and 39.9±3.2 g kg⁻¹, respectively, with a C:N ratio of 25 and 23, respectively. The pH of the forest floor and upper mineral horizon (NH₄Cl-based) was 3.03 ± 0.03 and 4.05 ± 0.03 , respectively. The climate is continental with cold winters and warm summers. From 1981 to 2006, mean annual air temperature and total precipitation, measured in a clearing located ~300m from the plots, were -0.3°C and 1535 mm, respectively, with maximum daily temperature averaging 21°C in July.

Experimental design and treatments

The experiment was established in autumn 2008 and laid out according to a randomized split-plot design with two levels of soil temperature (ambient and 4°C above ambient) nested within two levels of artificial precipitation (without added N and three times the

natural NH_4NO_3 concentration in ambient precipitation; details below) for a total of four treatments. The treatments were distributed amongst twelve plots covering a 60m x 60m area, each plot centered on a single balsam fir tree, evenly distributed amongst three blocks.

Based on climate model projections, a +4°C difference was maintained in the organic and upper mineral soil layers of heated plots during the 2009, 2010 and 2011 growing seasons with heat-resistance cables buried 5-10 cm belowground (see D'Orangeville *et al.* in press for details). As for the N treatment, the protocol was designed to simulate the predicted increase in atmospheric N deposition over forest ecosystems (Galloway *et al.* 2004). Each year and every week from mid-June to mid-September, all plots received identical amounts of artificial rainwater (70 L) applied on the canopy using nozzles set up above each individual tree using a system of pumps and reservoirs. The rain solution in Nenriched plots contained 1.0 and 3.4 mg·L⁻¹ of NH₄ and NO₃ respectively, approximately three times the concentrations measured in the precipitation at the site.

Soil sampling and analysis

In September 2011, three soil cores were collected within each plot from the organic (FH horizon) and mineral horizon (first 20 cm of the B horizon) and pooled by soil layer to form one bulk sample per horizon per plot. Moist samples were gently sieved (6 mm) in order to remove coarse fragments and woody debris while preserving as much as possible the soil structure and aggregations. The water content of each soil sample was determined by the difference of weight after drying a subsample (10 g) to a constant weight at 105°C for 24 hours. Assuming a similar soil structure and texture between samples, the soil water content (SWC) for all samples from each horizon was adjusted to that of the sample with the highest moisture in the organic (74.2%) and mineral (31.8%) horizons with the addition of demineralized water, in order to even out moisture conditions between samples.

For each soil sample of each horizon, four subsamples (15 g for organic soil, 50 g for mineral soil; fresh weight) were put in 120 cm³ acid-washed graduated plastic vials and

placed inside open 1-L Mason jars along with 10 mL of distilled water to maintain soil humidity, for a total of 96 jars. The exact weight and volume of soil were noted. All jars were sealed with an air-tight lid and incubated at 4°C for 10 days in the dark to stabilize the samples microbial community (Bowden *et al.* 2004). Air-tight rubber septa were fitted into the lids to allow CO₂ sampling.

The four subsamples were then incubated in the dark for 72 days at four temperatures covering the natural range of temperatures experienced at the site during the growing season: 5, 10, 15 and 20°C. Gas samples (20 mL) were taken at the beginning of incubation and after 1, 3, 6, 10, 17, 31, 45 and 72 days with a gas-tight syringe and injected into pre-evacuated 12 mL Exetainers (Labco, Wycombe, UK) with an extra 60 mil thick Teflon-silicone septa (National Scientific, Rockwood, TN) containing a small amount of magnesium perchlorate to absorb moisture. After sampling, jar lids were removed 30 minutes to allow air circulation into the jar. Within one week of gas sampling, the CO₂ concentration in every sample was measured using a gas chromatograph (6890 Series II, Hewlett-Packard Company, Avondale, PA) equipped with a Porapak Q column (ethylvinylbenzene and divinylbenzene copolymer beads; 80-100 mesh; length 25 m; internal diameter, 0.2 mm; Supelco 20331). The carrier gas was He (50 mL min⁻¹). Oven and detector temperatures were 120 and 250°C, respectively, and CO₂ detection was achieved with a thermal conductivity detector. Fluxes of CO₂ (F_{CO2}, mg CO₂-C kg⁻¹ soil hr⁻¹) were calculated using the following equation (Rochette & Bertrand 2007):

$$F_{CO_2} = \frac{\mathrm{d}C}{\mathrm{d}t} \frac{V}{M} \frac{\mathrm{Mm}}{\mathrm{Mv}} \left(1 - \frac{e_a}{P}\right)$$

where dC/dt (mol CO₂ mol⁻¹ h⁻¹) is the rate of change of headspace gas concentration in dry air samples, V (m³) is the volume of air inside the jar, M (kg) is the soil mass inside the jar, Mm (mg mol⁻¹) is the molecular weight of CO₂-C (12 000 mg C mol⁻¹ CO₂⁻¹), Mv (m³ mol⁻¹) is the molecular volume at 25°C (0.024 m³ mol⁻¹), e_a (kPa) is the partial pressure of water vapor in the jar headspace and P (kPa) is the barometric pressure. The volume of air inside the jar (V) was calculated according to the following equation:

$$V = V_H + V_A + \alpha V_W$$

where V_{H} (m³) is the headspace volume, V_{A} (m³) is the soil air porosity volume, α is the dissolution constant of CO₂ in water (in m³ CO₂ m⁻³ of water), and V_{W} (m³) is the volume of water in the soil at -38 kPa. The term αV_{W} accounts for the CO₂ dissolved in soil water (Tiedje 1994). The dissolution constant α used was 0.834, as calculated at standard barometric pressure and at a 25°C temperature (Li & Tsui 1971). We calculated temperature response coefficients Q₁₀ according to the following equation:

$$Q_{10} = \left(\frac{F_{CO_2(t_1)}}{F_{CO_2(t_2)}}\right)^{10/(t_1 - t_2)}$$

where t_1 and t_2 are incubation temperatures and F_{CO2} values are the corresponding mineralized C value measured at each sampling. We averaged the Q_{10} values across multiple sampling dates in order to obtain one Q_{10} value per sample. An exponential function was fitted to cumulative CO_2 concentrations in order to describe the process of C respiration for each soil sample at each incubation temperature (Stanford & Smith 1972):

$$C_{min} = C_o(1 - e^{-kt})$$

where C_o is the upper asymptote corresponding to potential mineralizable C, C_{min} is C respiration at time t (in days) and k is the respiration constant (day⁻¹). Parameter estimation was achieved through an iterative approach using nonlinear least-squares estimates, giving a mean coefficient of determination (R^2) of 0.979 and normal distribution of the residuals.

Statistical analysis

A linear mixed-effect analysis of variance (ANOVA) was used to analyse the effects of the treatments on C respired and temperature sensitivity coefficients Q₁₀, assuming a compound symmetry variance-covariance structure for repeated measures. Parameters of the C respiration function (C₀ and *k*) were tested against soil warming, N deposition and incubation temperature as fixed factors and plot within sub-block within block as random factors. A similar ANOVA structure was used to test Q₁₀ values, this time with soil warming, N deposition and temperature range as fixed factors. Data were tested *a priori* to meet the assumptions of ANOVA, log-transformed when necessary and Tukey post-hoc tests were
used for multiple comparisons of means. Statistical analysis, modelling and plots were done using the R software (R Development Core Team 2012).

3.4 Results

In both soil horizons and for all incubation temperatures, the general pattern of CO₂ production was similar, with high rates in the first three days followed by an exponential decrease until day 17 and a steady CO₂ production rate for the remainder of the incubation (Figure 3.1). Three years of artificial soil warming and/or increased N deposition had no significant effect on the decomposition constant *k* or temperature sensitivity coefficient Q₁₀ (Table 3.1). Relative to control soils, soils exposed to higher temperatures *in situ* had significantly less C₀, with a 15-32% (average 25%) and 2-23% (average 16%) reduction in C₀ in the organic and mineral horizons, respectively ($P \le 0.04$; Table 3.1). Nitrogen additions alone had no significant effect on potential mineralizable C (C₀). However, the decrease in C₀ for the organic horizon relative to controls was significantly less in heated-fertilized plots than in heated-only plots (P=0.01; Table 3.1).

In the organic and mineral horizons, C_o ranged from 3.8 to 18.7 g C kg soil⁻¹ and between 0.5 and 1.5 g C kg soil⁻¹, respectively, and was found to differ significantly among incubation temperatures (P<0.01; Table 3.1). In both soil horizons, C_o was statistically similar at 5 and 10°C, but increased significantly from 10 to 15°C and from 15 to 20°C (P<0.05). The corresponding temperature response coefficient Q₁₀ was 2.9±0.2 and 2.5±0.1 in the organic and mineral horizons, respectively (Table 3.1). The incubation temperature also had a significant effect on the respiration constant k in both soil horizons (P<0.01; Table 3.1). In the organic horizon, k was higher at 10°C (k=0.13) than at 5, 15 or 20°C (k=0.07; P<0.05). According to post-hoc tests, there were no significant differences between incubation temperatures in the mineral horizon (P≥0.06).

3.5 Discussion

The measured absolute rates and patterns of CO₂ production are consistent with studies of various soil types, including boreal soils, using similar incubation protocols (Cook & Allan 1992; Dalias *et al.* 2001; Rey *et al.* 2008; Gillabel *et al.* 2010). The differences in the initial total C content of the organic (399 g kg⁻¹) and the mineral (40 g kg⁻¹) horizons could account for the different respiration rates between soil horizons since C₀ values, relative to total C, varied little in both horizons (on average, 2.5 and 2.3% of the total C was mineralizable in the organic and mineral horizons, respectively). Being mineralized in a matter of weeks and months in this experiment, C₀ can be considered as a readily decomposable substrate, or labile C. Labile C is generally composed of carbohydrates and some lignin-containing compounds (Berg 2000). Identifying the chemical characteristics of the SOC before and after incubation, using for instance pyrolysis-gas chromatography/mass spectrometry (e.g. Wickland & Neff 2008), would have enabled us to confirm the composition of this labile C pool.

The temperature dependence of C_0 has been previously reported for similar incubation temperature ranges (Dalias *et al.* 2001; Gillabel *et al.* 2010). Higher incubation temperatures increase enzymatic depolymerisation rates, giving microbes better access to nutrients, and also reduce the physical occlusion of SOC within soil aggregates as well as the strength of SOC-mineral bonds (Conant *et al.* 2011). Thus, more C is mineralized at higher temperatures, especially during the first three days. The calculated temperature sensitivity, or Q_{10} values, fell within the 1.6-3.2 range observed for various soil types at temperatures from 10 to 40°C (Schlesinger 1977). As for *k*, the higher *k* observed at 10°C, relative to lower and higher incubation temperatures, is close to the average soil temperature of 11.0°C measured at the site during the growing season (June to September of 2009 to 2011). Because microbial communities adapt to their environment, their efficiency is often characterized by a temperature optimum corresponding to their natural climate (Dalias *et al.* 2001). Therefore, we interpret the *k* value to represent the optimal respiration conditions for the naturally-occurring microbial community, which did not increase at

temperatures above the average soil temperature of the site (15 and 20°C). Our results suggest that the optimal temperature for the soil microbial community was not changed by the three-year warming treatment.

Based on the calculated Q₁₀ values, the 4°C difference maintained in heated plots for three years corresponded to a 53.1% increase in respiration. Such an increase without a concomittant increase in litter inputs should eventually reduce the mineralizable C pool. In our study, three years of soil heating significantly reduced that C pool by 16-25%, on average, a result consistent with previous observations in a hardwood forest (Bradford *et al.* 2008) as well as with recent models (Knorr *et al.* 2005). This reduction represents an average of 0.4-0.8% of the total SOC found in the organic and mineral horizons, and suggests a negative effect of increased soil temperature on SOC sequestration.

The quantities of N added in this experiment were small and deposited on top of the canopy, and had no detectable effect on the availability of soil inorganic N (D'Orangeville *et al.* in press). Therefore, the effects of N fertilization on soil respiration were small and non-significant. Nitrogen additions in N-limited environments are generally expected to increase the amount of labile C, but the microbial response to N additions in boreal ecosystems has been shown to be highly variable (see Allison *et al.* 2009). For instance, higher NH₄ levels can inhibit the production of certain ligninolytic enzymes by fungi (Berg 2000). More time could be needed in order to detect an effect of increased N deposition on the soil C dynamics and composition.

3.6 Conclusion

As we hypothesized, three years of *in situ* artificial soil warming in a boreal forest were sufficient to reduce the amount of labile organic C. However, the additional N deposited on top of the tree canopy did not exacerbate the depletion of the SOC. Whether the treatments could also have affected the microbial community structure, activity or composition remains to be determined.

3.7 Acknowledgements

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Table 3.1. Potential mineralizable C (C_0), decomposition rate constant (k) and temperature response coefficient (Q_{10}) in the organic
and mineral horizons of control (C), fertilized (N), heated (H) and heated-fertilized (HN) soils incubated for 72 days at four
temperatures, and mixed-model ANOVA treatment effects (N=12). Values are the mean ± standard error. ANOVA interactions were
removed when non-significant.

	O	rganic horizon		Mineral horizon						
	Co	k	Q ₁₀	Co	k	Q ₁₀				
	(g C-CO ₂ kg ⁻¹ soil)	(day⁻¹)		(g C-CO ₂ kg ⁻¹ soil)	(day ⁻¹)					
С	11.5 ± 2.3	0.09±0.01	2.9±0.3	1.0±0.2	0.12±0.02	2.3±0.3				
Ν	10.0±1.9	0.08±0.01	2.9±0.3	0.9±0.2	0.11±0.03	2.3±0.3				
Н	8.2±1.5	0.08±0.01	2.8±0.3	0.8±0.1	0.11±0.01	2.9±0.4				
HN	9.8±1.8	0.09±0.01	2.8±0.3	0.9±0.1	0.13±0.03	2.4±0.3				
5°C	3.8±0.2 <i>a</i>	0.07±0 <i>a</i>	-	0.5±0 <i>a</i>	0.07±0	-				
10°C	5.9±0.3 <i>a</i>	0.13±0.01 <i>b</i>	-	0.6±0.1 <i>a</i>	0.19±0.03	-				
15°C	11.2±0.8 <i>b</i>	0.07±0 <i>a</i>	-	1.0±0.1 <i>b</i>	0.13±0.02	-				
20°C	18.7±1.4 <i>c</i>	0.08±0 <i>a</i>	-	1.5±0.1 <i>c</i>	0.07±0	-				
Treatment effects										
Warming (H)	<i>P</i> = 0.0042**	NS	NS	$P = 0.0490^*$	NS	NS				
Fertilization (N)	NS	NS	NS	NS	NS	NS				
Temperature	<i>P</i> < 0.0001**	$P < 0.0001^{**}$	NS	<i>P</i> < 0.0001**	<i>P</i> < 0.0001**	NS				
H x N	<i>P</i> = 0.0114*	NS	NS	NS	NS	NS				

a, *b*, *c* Within a column, values with the same letter within a treatment did not differ significantly at *P* < 0.05 (Tukey test).

*, ** Treatment effects significant at *P* < 0.05 and *P* < 0.01, respectively; NS, non-significant.



Figure 3.1. Average hourly C mineralization rates in organic (black) and mineral soils (grey) for control, heated, fertilized and heated-fertilized soils at 5, 10, 15 and 20°C.

Connecting paragraph

Chapters two and three discussed the influence of increased soil temperature and N deposition on the soil chemistry and carbon mineralization in a balsam fir boreal stand. In the following chapter, an attempt is made to link the soil response to the treatments with tree nutrition and radial growth. The focus is put on nitrogen (N) as this element is the most common growth-limiting factor in boreal ecosystems. This manuscript is in press in the journal 'Biogeosciences', with co-authors Daniel Houle, Benoît Côté, Louis Duchesne and Hubert Morin.

Chapter 4 - Increased soil temperature and atmospheric N deposition have no effect on the N status and growth of a mature balsam fir forest

4.1 Abstract

Nitrogen (N) is a major growth-limiting factor in boreal forest ecosystems. Increases of temperature and atmospheric N deposition are expected to affect forest growth directly and indirectly, by increasing N availability due to higher rates of N mineralization. In order to understand the potential impacts of these changes, a mature balsam fir stand in Québec, Canada, was subjected during three consecutive growing seasons (2009-2011) to i) experimentally increased soil temperature (4°C) and earlier snowmelt (2-3 weeks) as well as ii) increased inorganic N concentration in artificial precipitation (3x current N concentrations using $^{15}NH_{4^{-}}$ NO₃). Soil inorganic N was measured using buried ion exchange membranes (PRSTM-probes) and standard soil extractions. Dendrometers were used to monitor the variations in diameter growth and needles were analyzed annually for N to assess the nutritional response of trees. Results from the second (2010) and third (2011) year of treatment are reported.

After three years of treatment, there was no significant increase in soil nitrate (NO₃) or ammonium (NH₄) availability either in the organic or in the mineral soil as measured with standard soil extractions. Similar results were obtained with ion exchange membranes, except for NH₄ in the forest floor which increased by an average of 54% over the two years. No effect of treatments were observed on needle N or diameter growth, but an eight-day earlier peak in diameter growth was measured in heated plots in 2010.

We attributed the limited effects of our treatments to the acute soil competition for available N at the site. As a result, the projected modifications of the forest N cycle and concomitant increased forest growth due to an earlier snowmelt, increased soil temperature and N deposition should be considered with caution in similar cold N-poor ecosystems.

4.2 Introduction

The boreal forest of Canada accounts for one tenth of the world forests (Burton *et al.* 2003). Recent simulations of the future climate from the Canadian Regional Climate Model (CRCM) for the Eastern boreal forest of Canada suggest an average annual temperature increase of 3°C by 2041-2060 relative to 1971-1990 (Plummer *et al.* 2006) and a one-month earlier snowmelt by 2050 relative to 1971-2000 (Houle *et al.* 2012). Understanding the impact of climate change on boreal forest soils is of primary importance, as recent studies show that the response of plants to climate in such biomes is limited by inorganic nitrogen (N) availability (Magnani *et al.* 2007; Melillo *et al.* 2011) which is in turn affected by temperature and water availability. A meta-analysis of 32 ecosystem warming studies, of which three were in boreal forests, suggests a positive effect of increased soil temperature on soil inorganic N and plant productivity (Rustad *et al.* 2001). In addition, the start of snowmelt was found to coincide with the start of nutrient uptake and radial growth by conifer trees (Jarvis & Linder 2000). Based on these relationships, many researchers expect the growth rates in boreal forests to increase in the future (Jarvis & Linder 2000; Strömgren & Linder 2002; Bronson *et al.* 2009).

Aside from soil N mineralization, atmospheric deposition of N represents an alternative and significant source of inorganic N in boreal forests. The amounts deposited in forest ecosystems through rain, fog or dry deposits strongly increased in the last century, especially in Eastern North America (Driscoll *et al.* 2001), and an increase of 70% is projected by 2050 (Galloway *et al.* 2004). The current impacts of N deposition on forests are well documented, with a generally positive effect on growth (Magnani *et al.* 2007) although its acidifying potential can also impair tree nutrition (Shortle & Smith 1988).

The combination and likely interaction of increasing temperatures and N deposition on plants has garnered the attention of many researchers (Chapin *et al.* 1995; Mäkipää *et al.* 1999; Strömgren & Linder 2002; Majdi & Öhrvik 2004; Sager & Hutchinson 2005; Zhao & Liu 2009; Hutchison & Henry 2010). Carbon sequestration models predict a positive impact on plant growth based primarily on increased organic matter decomposition and nutrient

availability (Mäkipää *et al.* 1999), but our current understanding of the combined effect of climate change and increased N deposition on mature forest stands remains limited. In northern Sweden, Norway spruce stands subjected to soil warming and N fertilization increased stemwood production (Strömgren & Linder 2002), but detrimental effects were measured on root longevity and elongation (Majdi & Öhrvik 2004). In China, planted *Picea asperata* Mast. and *Pinus tabulaeformis* Carr. seedlings increased photosynthetic rates and grew faster when exposed to infrared warming or N additions (Zhao & Liu 2009). When combined, the treatments further increased the performance of *P. tabulaeformis* but reduced that of *P. asperata*, suggesting that the interactive effects of increased soil temperature and N addition are complex and potentially species specific.

The amounts of N added in the experimental studies of soil warming and N deposition interactions cited earlier vary between 50 to 250 kg N ha⁻¹ yr⁻¹, although the average atmospheric N deposition in boreal ecosystems is estimated to be 2.34 kg N ha⁻¹ year⁻¹ (Holland *et al.* 1999). Thus, these quantities do not accurately mimic the magnitude of the additional inputs of N to boreal forest ecosystems that can be expected in the future (Galloway *et al.* 2004). Furthermore, nutrients are generally added in one or two massive doses on the forest floor, avoiding the canopy and understory interactions that are likely to take place in forested ecosystems as well as the buffering role of natural N sinks (Hobbie *et al.* 2002; Gundale *et al.* 2011). These aboveground interactions can be taken into account by adding N over the forest canopy, and the fate of the added N can be followed using traceable isotopic ¹⁵N.

In this paper, we report on the effect of experimentally increased soil temperature $(+4^{\circ}C)$ by means of heat-resistance cables, increased inorganic N concentration in artificial precipitation (3x current N concentrations using $^{15}NH_{4}$ - $^{15}NO_{3}$) and earlier onset of snowmelt (2-3 weeks) in a typical mature balsam fir stand growing in the boreal ecosystem of the Lake Laflamme watershed (Québec, Canada). We hypothesized that these treatments would increase soil N availability, needle N concentration and radial growth. The monitoring of tree growth and soil parameters was conducted over three growing seasons following the setup of the experiment. Significant changes in soil N availability associated with the burial

of cables were detected until the end of the first year of treatment (data not shown). Therefore, the results presented in the present paper are limited to years two and three (2010 and 2011).

4.3 Materials and methods

Study area

The study site is located near the Lake Laflamme watershed, north of Quebec City (47°17' N; 71°14' O; 800 m above sea level). The experimental plots were established in a 60-year-old even-aged balsam fir (*Abies balsamea* (L.) Mill.) stand. The soil is an Orthic Ferro-Humic Podzol (Spodosol in the American classification system), laying on a bedrock of Precambrian charnockitic gneiss. The average thicknesses for LFH, Ae, Bhf and Bf horizons (based on the Canadian soil classification system) are 7.4, 3.6, 7.6 and 28.7 cm, respectively. The C and the N content of the forest floor are 416.1 and 16.6 g kg⁻¹, respectively, for a C:N ratio of 25. The forest floor and upper mineral horizon display a pH of 3.03 and 4.04, respectively. According to reference foliar nutrient levels for balsam fir (Michel 2010), the study site displays no major nutrient deficit. A weather tower, located 200 meters from the study area, records precipitation and air temperature. The climate is continental with cold winters and warm summers. From 1981 to 2006, mean annual air temperature and total precipitation were -0.3°C and 1535 mm, respectively. Annual N deposition (NH₄ + NO₃) averages 5.7 kg N ha⁻¹ year⁻¹ (Houle & Moore 2008).

Contrasting weather conditions were observed in the two years of monitoring (2010-2011). In 2010, the summer was particularly dry with 58% less precipitations and an average temperature 0.7°C warmer in July-August relative to 2011. In 2011, precipitation was higher for the May to September growing season (747 mm compared to 596 mm). Relative to the average precipitation of 654±47 mm received at the site from May to September 1975-2008, both monitoring years were nonetheless representative of the normal precipitation regime during the growing season.

Experimental design

The experiment was set up in autumn 2008 and laid out according to a randomized splitplot design with two levels of soil warming (ambient and +4°C) nested within two levels of artificial precipitation (no added NH₄NO₃ and three times the natural NH₄NO₃ concentration in ambient precipitation; details below). Because of the extensive manipulations and intensive sampling effort, we limited the experiment to twelve plots evenly distributed amongst three blocks. The blocks were 12m x 60m and laid out parallel to the slope with a 12m buffer strip in between. Each block was divided in two 24m x 12m sub-blocks separated by a 12m x 12m buffer zone. Each sub-block was divided into two 12m x 12m plots. Within each plot, a balsam fir tree was selected based on a visual estimation of good health and dominance or co-dominance in the canopy. The height and diameter of every selected tree was noted, ranging from 12.9 to 18.1 m and from 14.0 to 22.8 cm, respectively. The fertilization treatment was randomly distributed within the two sub-blocks of each block, and the soil warming treatment was randomly distributed within the two plots of each sub-block (Fig. 4.1).

Soil warming

Heat-resistance cables were used to warm the soil as this method, relative to infrared warming, provides the most homogeneous warming throughout the soil strata (Aronson & McNulty 2009). Around each experimental tree, 70 m of heating cables were buried in a spiral pattern 5-10 cm belowground at the interface of the organic and mineral soil. To avoid potential bias between treatments due to cable burying, non-heating cables were also installed around control trees. The beginning and end of the cable were at 0.9 m and 2.5 m, respectively from the base of the tree with an average distance between cables of approximately 30 cm. Great care was taken to avoid root damage and roots >5 mm were bypassed. Soil temperature under the canopy of each tree was measured continuously with

thermistors (precision: ±0.2°C; model 107-L from Campbell Scientific Inc., Utah, USA) buried between cables. The warming cables were activated and deactivated when the average difference of soil temperature between heated and non-heated trees reached 3°C and 5°C, respectively, in order to maintain an average difference of 4°C on the basis of projections for the site for the 2070-2100 period (Houle *et al.* 2012). No warming was applied during winter as the soil, being insulated by a thick snowpack, is expected to experience only small temperature changes (Houle *et al.* 2012).

Soil heating was started in the spring of 2009. From the beginning of spring snow melt until the first autumn frost, the average soil temperature in treated plots was higher by 4.0±0.4°C, 4.1±0.3°C and 3.7±0.9°C in 2009, 2010 and 2011, respectively (Fig. 4.2). Storage of temperature data and control of warming were done with a CR1000 datalogger (Campbell Scientific, Inc., Utah, USA). At a depth of 31 cm (approximately 20 cm deep in the mineral soil), the soil temperature in heated plots was maintained approximately 2°C above controls, as measured in July 2009 with 107-L thermistors linked to a CR1000 datalogger (Campbell Scientific, Inc., Utah, USA). In addition to the maintenance of a higher soil temperature during the growing season, the heating treatment was also designed to simulate an earlier snowmelt and an earlier snow-pack disappearance. In 2009, soil warming was started on April 27 with a remaining snowpack of 0.75 m, with snowmelt in control plots ending 19 days later. A system malfunction caused an early interruption of the soil warming in mid-August that year. In 2010, soil heating was started on March 30 because of a warm spring. The snowpack was 0.85 m and the snowmelt in control plots was completed 28 days later. The treatment was stopped before the first freeze-thaw events, on October 19. The cold spring of 2011 delayed the start of soil heating to May 5. The snowpack was then 0.60 m and the snowmelt in control plots was completed 16 days after beginning of treatment. Heating was terminated on October 18.

Increasing atmospheric N deposition

The protocol used to increase atmospheric N deposition was designed to simulate how atmospheric N is deposited on forest ecosystems. The rain solution (control and N-enriched) was applied on the canopy using nozzles set up above each individual tree using a system of pumps and reservoirs. The composition of the artificial rain solution was based on the average ion concentration in incoming precipitation measured at the site in previous years. The concentration of the different elements added was as follows: Na (0.046 mg L⁻¹), Ca (0.089 mg L⁻¹), Mg (0.018 mg L⁻¹), K (0.026 mg L⁻¹), H (0.014 mg L⁻¹), Cl (0.07 mg L⁻¹) and SO₄ (1.01 mg L⁻¹). For the N treatment, NH₄NO₃ labelled with a 10% fraction of ¹⁵N was added to reach a final rain concentration of 1.0 and 3.4 mg·L⁻¹ of NH₄⁺ and NO₃⁻ respectively, which corresponds to approximately three times the concentrations measured in the precipitation at the site. Each year and every week from mid-June to mid-September, 70 liters of artificial precipitation was applied on every tree. The applications resulted in an increased N deposition of 1.544 g N tree⁻¹ year⁻¹, which was dispersed over an area estimated to 20-50 m², resulting in a rain event of 1.3-3.4 mm (approximately 16-48 mm added annually), or an additional 1.1-2.7 kg N ha⁻¹ year⁻¹. Watering was rescheduled to the next day on rainy days.

Soil sampling and analysis

Soil N availability was monitored during the snow free period using ion-exchange membranes (PRSTM-probes, Western Ag Innovations Inc.; Saskatoon, Canada). Exchange membranes allow the assessment of nutrients supply rates by continuously adsorbing free ions on charged surfaces similarly to plant roots. At first, the probes typically adsorb the labile pool of ions until depletion, followed by measurements of ion supply rates through mineralization, leaching or dissolution. Being in contact with plant roots, the measured values reflect the difference between nutrient release and root immobilization. The probe data will be referred to as "available" N hereafter. Avoiding direct contact with heating cables, six probes were evenly distributed around each tree at a distance of 1.5 m from the

stem and inserted vertically, three in the organic soil layer (approx. 5-10 cm deep) and three in the mineral layer (approx. 15-20 cm deep). The probes were buried on May 12 and May 30 in 2010 and 2011 respectively, following snowmelt, and replaced every six weeks. In autumn, the last set of probes was removed on September 30 and October 7 in 2010 and 2011, respectively.

At the end of each burial period, the probes were sent to Western Ag Innovations for extraction after rinsing off the remaining soil from the membranes with deionized water. The probes were pooled per soil horizon for each tree. Membranes were eluted with 17.5 ml of 0.5 M HCl for 1h. Concentrations of NH_4 and NO_3 in the extract were determined colorimetrically using a Technicon Autoanalyzer (Technicon AA2, Seal Analytical Inc., Mequon, USA). The values were reported as μ g 10 cm⁻² of resin surface and cumulated for each year.

Soils were sampled at the end of the growing season (September) for extractable N. Three evenly spaced cores around trees at a distance of 2 m from the stem were taken in the organic (FH horizon) and mineral horizons (first 20 cm of the B horizon) and pooled by soil horizon for each tree to form one bulk sample per horizon per plot. Being thin and discontinuous, the eluvial horizon (Ae) was not sampled. Samples were air-dried for a week and sieved to 2 mm. Soils were extracted with KCl for 12 hours. Levels of NO₃ were measured by ion chromatography (Dionex 2120i, Thermo Fisher Scientific Inc., Sunnyvale, USA) and NH₄ colorimetrically with a Technicon Autoanalyzer (Technicon AA2), and will be referred to as "extractable" hereafter. Levels of soil nitrates were close to the detection limit of the analytical devices used for the analyses (limit of <1 mg kg⁻¹ for extractable NO₃; 31% of samples and of <2µg 10 cm⁻² for available NO₃; 51% of samples). Therefore, caution should be used when interpreting these results.

Volumetric soil water content (SWC) was measured weekly using a FieldScout TDR-300 device with 20-cm rods and standard calibration (accuracy: ±3% vol; Spectrum technologies Inc., Plainfield, USA). For each plot, nine measurements were taken at a distance of 1.5m from each tree and averaged.

Foliar sampling and analysis

The current-year foliage of experimental and reference trees was sampled from the upper third of the canopy on three branches. Sampling was done in September of both years at the end of each growing season. In 2010, a sub-sample of thirty needles for each tree was weighted to account for any change in the leaf specific mass of each tree. Needles were oven-dried at 65°C and then ground to 250 µm-sized fragments. The total N content was determined with a Kjeldahl Autoanalyzer (Kjeltec Auto model 1030 Analyser, Tecator, UK) after digestion with H₂SO₄. The degree of isotopic N enrichment of the samples (¹⁵N) was measured on sub-samples (5.8–6.1 mg) using isotope ratio mass spectrometry with a Isoprime100 IRMS continuous flow spectrometer (Isoprime Ltd, Cheadle, UK) at GEOTOP Research Center (Université du Québec à Montréal). The analytical error was ±0.2‰.

Calculation of N pools

Two labile N pools (foliage N and soil extractable N) were determined to assess the relative contribution of fertilization to N cycling in the plots. Foliage N of each tree was determined by multiplying the average foliar N concentrations measured in 2010 and 2011 with the total foliage mass estimated with the allometric equation of Lambert *et al.* (2005) for balsam fir using tree height and stem diameter as independent variables. The inorganic N pool of the forest floor (FH-horizon and first 20 cm of B-horizon) was obtained by multiplying i) the average level of extractable N (NH₄ + NO₃) of the organic and mineral horizons of plots in 2010 and 2011 by ii) the horizon thickness, recorded using a soil corer at twelve sampling points next to the experimental site, and by iii) the average bulk densities (mass of oven dry soil divided by its volume) measured on the same twelve soil samples cited earlier. The Ae horizon was not included in the calculation because it was not analyzed for extractable N.

Calculation of ¹⁵N abundance and recovery

The ¹⁵N abundance was converted into the common per mill deviation from atmospheric standard of 0.3663 atom% ¹⁵N and noted in δ^{15} N:

$$\delta^{15} N = \left(\frac{R_{sample}}{R_{standard}} - 1\right) * 1000$$

where R is the ratio of ${}^{15}N/{}^{14}N$. The ${}^{15}N$ tracer recovery in the foliage was calculated as:

$${}^{15}N_{rec} = m_{foliage} * \left(\frac{at.\%^{15}N_{foliage} - at.\%^{15}N_{ref}}{at.\%^{15}N_{tracer} - at.\%^{15}N_{ref}} \right)$$

where $m_{foliage}$ is the estimated N pool in the foliage, at.%¹⁵N_{tracer} is the at.%¹⁵N in the fertilizer solution, at.%¹⁵N_{ref} is the at.%¹⁵N in the foliage of non-fertilized trees, and at.%¹⁵N_{foliage} is the at.%¹⁵N in the foliage of fertilized trees.

Seasonal course of basal area increment

Dendrometers (Ecomatik; Munich, Germany) were installed on every tree 2 m above ground to monitor radial variations with a precision of $<5 \mu$ m. Data was recorded every 15 minutes on the CR1000 datalogger and averaged to obtain hourly values. To account for the difference in stem size between trees, the recorded radial variations were converted to basal area increments (BAI):

$$BAI_t = \pi[(X + R_t)^2 - (X + R_i)^2]$$

Where R_t is the tree radius increment at time t of measurement, R_i is the initial radius read by the dendrometers upon their installation, and X is the radius of the tree measured upon the installation of the dendrometers.

The Gompertz function provides a good description of seasonal tree growth patterns (Rossi *et al.* 2003). The seasonal BAI was modelled using data spanning from May 1st, a time when tree rehydration is expected to be completed (Turcotte *et al.* 2009), to the first frosts, characterized by large negative BAI values (Fig. 3a). Even during cambium dormancy, the changes in tree water potential produce significant day-to-day stem diameter variations confusing the identification of the start of the seasonal growth, *i.e.* the moment when the

BAI values must be set to 0 (Duchesne *et al.* 2012). To avoid arbitrary choices, a formulation of the Gompertz model that includes a fourth parameter indicative of the initial stem size at the beginning of the growing season (Duchesne et al., 2012) was fitted to hourly BAI data for each tree:

 $Y = Y_0 + A * \exp\left[-\exp(\beta - \kappa * t)\right]$

where Y is the maximal daily measured BAI, Y₀ is the lower asymptote, *A* is the upper asymptote, β is the abscissa of inflection point, κ is the rate of change of curve shape and t is the time in day of year (DOY). Therefore, (A-Y₀) corresponds to the total annual BAI while (Y_t-Y_{t-1}) corresponds to the daily BAI. All regressions had R² > 0.92 and normal distribution of the residuals (Motulsky & Ransnas 1987). The 'nlm' procedure from the R software was used with ordinary least square method for parameter estimation (R Development Core Team 2012). Gompertz growth curves were fitted for each tree (12) and each year (2), for a total of 24 models (Fig. 3b). The beginning and end of radial growth were determined as the day of year when modeled daily basal increment values went above and below 0.1 mm² day⁻¹, respectively.

Pre-treatment growth

Using a Trephor tool (Rossi *et al.* 2006a), a wood microcore 15 mm in length and 1.9 mm in diameter was collected on each tree next to the dendrometer to check for potential pre-treatment differences in BAI (Linares *et al.* 2009). The microcores were stored in a 10% ethanol solution and embedded in paraffin (Rossi *et al.* 2006a). Transverse sections were cut using a rotary microtome (6-10 μ m in thickness), stained with safranin (1:100 with water) and fixed on slides. A camera linked to an optical microscope recorded numerical images at 400x magnification. The ring widths of 2007 and 2008 were measured along three radial rows using Wincell software (Regents Instruments Inc., Québec, Canada) and transformed into BAI with:

$$BAI_{2008} = \pi [(X + R_i)^2 - (X + R_i - R_{2008})^2]$$

and

$$BAI_{2007} = \pi [(X + R_i - R_{2008})^2 - (X + R_i - R_{2008} - R_{2007})^2]$$

where R_i is the initial radius read by the dendrometers upon their installation while R_{2008} and R_{2007} are the mean ring widths for the years 2008 and 2007.

Statistical methods

A linear mixed-effects analysis of variance (anova) with repeated measures was used to analyse the effects of the treatments, assuming a compound symmetry variance-covariance structure for repeated-measures. The effect of soil warming on soil water content (SWC) was tested with temperature treatment and year as fixed factors and plot within sub-block within block as random factors. Soil, foliar and growth parameters were analyzed with temperature treatment, fertilization and year as fixed factors and plot within sub-block within block as random factors. Because SWC and pre-treatment BAI (average of BAI₂₀₀₇ and BAI₂₀₀₈) tended to differ between treatments (Fig. 4.4 and Table 4.1), they were included as covariates in the anovas for soil and growth analysis, respectively, when significantly correlated with the response variable at P<0.05. The relations between the covariates and the dependent variables were linear. KCl-extracted NO₃ values in the mineral horizon were too low for detection in 2010. Therefore, only the 2011 data was analyzed and the factor 'year' was removed. Due to the important variability of the growth-related response variables, we assessed the statistical power of the experimental design to estimate the minimum detectable difference between treatments. This minimum detectable difference was calculated for the commonly used type I (α =0.05 and α =0.10) and type II (β =0.20) error probabilities (Peterman 1990; Stroup 1999). Mixed-effects anovas were computed using the 'Ime' procedure in R (R Development Core Team 2012), and statistical power was assessed using SAS statistical package (SAS version 9.2, SAS Institute, Cary, NC, USA).

4.4 Results

Soil water content

The average soil water content during the growing season was significantly higher in 2011 than in 2010, ranging from 18.4 to 42.1% in 2011 compared to 12.8 to 34.9% in 2010 (P<0.01; Fig. 4.4). There was no significant difference between heated and non-heated plots (P=0.14).

Soil available and extractable N

Significant between-year differences ($P \le 0.05$) were detected for available N (NH₄ and NO₃) in both horizons and for extractable NH₄ in the mineral horizon (Fig. 4.5). The forest floor was higher in extractable NH₄ than the mineral horizon in both years (60.6 and 8.3 mg kg⁻¹, respectively). Extractable NO₃ showed a different pattern with concentrations below the detection limit in the mineral horizon in 2010 but higher concentrations in the mineral horizon than in the organic horizon in 2011 (1.7 and 3.8 mg kg⁻¹ in 2011, respectively; Fig. 4.5).

Available NH₄ levels were higher in the organic than the mineral horizon in 2010 (22.4 and 14.6 μ g 10 cm⁻², respectively), but were similar in 2011 (Fig. 4.5). Available NH₄ decreased by 70% and 54% in the organic and mineral horizons in 2011, respectively (P<0.05; Fig. 4.5). No differences between horizons were observed for available NO₃ levels in both years. Levels of available NO₃ increased in 2011 relative to 2010 in both soil horizons (P<0.01; Fig. 4.5).

In 2010 and 2011, the soil warming treatment increased available NH_4 in the organic soil horizon by 53.8% in average (*P*=0.02; Fig. 4.5). No such effect was observed for available NO_3 , and extractable NH_4 and NO_3 in either soil horizon. Except for a likely increase in forest floor extractable NH_4 (*P*=0.09; Fig. 4.5), the N deposition treatment did not have a significant effect on soil inorganic N. The annual input of N from the fertilization treatment

represented 2.1% of the inorganic N pool contained in the organic soil horizon and the first 20 cm of mineral soil (3.7 g extractable-N m⁻²).

Average leaf weight and foliar N

Needle N concentrations and needle mass did not respond significantly to any treatment nor did they vary significantly over time. Needle N concentration averaged 12.3±0.4 g N kg⁻¹ in 2010 and 12.1±0.4 g N kg⁻¹ in 2011 (Fig. 4.6) whereas needle mass averaged 3.6 mg needle⁻¹ over the two years of sampling (data not shown).

Needle δ^{15} N concentrations measured in fertilized trees averaged 4.29±0.71‰ and were significantly higher than those of control trees (-2.04±0.17‰, *P*<0.01; Fig. 4.6). With a calculated foliage N pool of 101 g tree⁻¹, an average of 1.1% of the added N (1.54 g tree⁻¹ yr⁻¹) was recovered in the foliage of fertilized trees, representing 0.02% of the total foliage N pool.

Seasonal course of basal area increment

The BAI was highly variable between trees, ranging from 191 to 1329 mm² yr⁻¹ (Fig. 4.3). No effect of treatments on BAI (A-Y_o) was detected in either year with BAI averaging 740±103 mm² and 700±104 mm² in 2010 and 2011, respectively (Table 4.1). BAI started to increase May 1st 2010 and May 9 2011 and reached a maximum on September 28 2010 and October 8 2011. The rate of growth increased steadily until mid-June (June 8 in 2010 and June 17 in 2011) before decreasing until the end of the growing season (Table 4.1 & Fig.4.3). The onset of growth and maximum growth rate were reached on average one week earlier in 2010 compared to 2011 (*P*<0.01; Table 4.1). Heated trees reached their maximal growth rate significantly earlier than control trees (*P*<0.01), although the significant warming-year interaction revealed that this effect was stronger in 2010 (*P*<0.01), when the mean inflection point was reached on June 4, 8 days earlier than for non-heated trees.

The power analysis for growth variables revealed that for type I error probabilities of 0.05 and 0.1, respectively, the smallest detectable differences between treatments was nine and seven days for growth onset, 50 and 40 days for growth ending, 700 and 500 mm² for total growth, 0.02 and 0.018 for the slope of growth curve, eight and six mm² day⁻¹ for maximum daily growth and five and four days for the timing of maximum daily growth (Table 4.1). Relative to the natural variance observed in the population of balsam fir trees, the power of our experimental design to detect true treatment effects was highest for the timing of maximum daily growth.

4.5 Discussion

Treatment effect on soil and foliar N

The hypothesis of increased soil N availability due to the treatments was rejected for N fertilization and verified in part for soil heating. Increased soil temperature did not raise NH₄ or NO₃ availability in the forest floor or mineral soil as measured by the two methods except for an increase (54%) in NH₄ availability as measured with the ion exchange membranes in the forest floor. The significant differences in available N observed in 2010 and 2011 as well as the three-fold increase in available NH₄ measured in 2010 in heated plots compared to the control plots suggest a strong influence of the prevailing climatic conditions of 2010 on the availability of soil N. Indeed, the warmer temperatures observed in 2010 prior to the August drought could have promoted N mineralization rates (Van Cleve *et al.* 1990; Rustad *et al.* 2001; Allison & Treseder 2008; Brzostek *et al.* 2012) and increased the NH₄ levels that year.

Given the influence of temperature on nitrification rates (Sabey *et al.* 1956; Malhi & McGill 1982), an increase in NO₃ in the heated plots was expected. The lack of detectable effect of soil warming on NO₃ in the forest floor, despite an increase in NH₄, could be explained by its low pH (3.03), a well-known nitrification inhibitor (Ste-Marie & Paré 1999). In addition, the forest floor of balsam fir stands typically displays a high polyphenolic

content, which can also inhibit nitrification (Olson & Reiners 1983). However, conditions favorable to nitrification are encountered in the mineral soil (i.e. higher pH, lower polyphenolic content), as shown by a strong increase in NO₃ measured during a spruce budworm outbreak at the site in 1981-1984 (Houle *et al.* 2009). The absence of increased NO₃ in the mineral horizon of heated plots thus suggests that the increased NH₄ measured in the forest floor, although found on the buried ion exchange membranes, did not reach the underlying mineral soil where it could have been transformed to NO₃.

In contrast with available NH₄, extractable NH₄ in the forest floor did not respond to the soil warming treatment. Adsorbing membranes interact with the inorganic ions present in the soil solution and are generally more sensitive to changes in the environment (Johnson *et al.* 2005), while soil extractions measure a larger pool of elements that includes inorganic N bound to exchange sites. The fact that the soil warming treatment increased the pool of NH₄ in the soil solution but not the larger pool of extractable NH₄ suggests that the effect of that treatment was relatively modest. Since the air drying of the samples prior to extraction may have resulted in some loss of labile N, data from ion-exchange membranes should be considered a more robust assessment of the potential changes in nutrient availability.

The limited effect of the soil heating on inorganic N availability could be due to the site condition. The levels of available N at the site were compared with published data from boreal forest soils of Canada sampled with identical ion-exchange membranes (PRS-probes) including studies spanning over one to 11 growing seasons, monitoring unmanaged plots in a black spruce forest of Alberta (Jerabkova & Prescott 2007), a wet spruce-fir forest of British-Columbia (Hope 2009), and another wet mixed-conifer forest in British-Columbia (Bengtson *et al.* 2007). On a daily basis, the organic soil horizon sampled in the current study displayed 46% less available NH₄ (range 15-67%) and 62% less NO₃ (range 30-77%) than the other three sites. A previous study conducted in the same watershed using ¹⁵N isotopic pool dilutions showed that almost all of the NH₄ and NO₃ made available in the forest floor was immobilized in less than a day (Ste-Marie & Houle 2006). In another experiment at the site, the addition of 3- and 10- fold the current atmospheric N deposition (17 and 57 kg N ha⁻¹year⁻¹ respectively) for three years did not have prolonged effects on

inorganic N concentrations in the soil solution except for transitory increases that lasted less than a week with the result that 95% of the added N was immobilized above the rooting zone (Houle & Moore 2008). All together, these results suggest that the low levels of extractable inorganic N at our site, 0.8% of the total N contained in the forest floor, and the relatively high C:N ratio (25) maintain strong N sinks in the soil, including microbes and humus (Berg & Matzner 1997; Nadelhoffer *et al.* 1999b; Friedrich *et al.* 2011) which could be responsible for the relatively weak response of inorganic N after three years of soil warming.

There were no effects of the treatments on leaf N concentration although fertilized trees had significantly higher leaf ¹⁵N levels, showing that a fraction of the inorganic ¹⁵N in the artificial precipitation was immobilized in the foliage. In the long term, increased N deposition has been shown to have positive impacts on balsam fir N nutrition (McNeil *et al.* 2007) and carbon sequestration (Quinn Thomas *et al.* 2010). In the short term however, the calculated 1.1% recovery of added N in the foliage of trees in the present study represents 0.02% of the foliage N pool and probably a minor fraction of the new foliage N pool as well. While in good agreement with previous results (Bowden *et al.* 1989; Boyce *et al.* 1996; Bryan Dail *et al.* 2009), this suggests a limited assimilation of deposited N through the leaf epidermis and limited impacts on the trees N nutrition.

Treatment effect on growth

The phenology of radial growth was hastened by soil warming in 2010, when an earlier peak of growth was observed for heated trees. To our knowledge, this is the first study to detect an effect of soil warming on the phenology of basal area increment in conifers. The course of xylem production in black spruce trees growing in Québec (Canada) and subjected to an identical experimental design was not affected by three years of soil warming (Lupi *et al.* 2012b). In a boreal Norway spruce stand of northern Sweden, six years of soil warming did not affect the phenology of basal area increment, although the maximum growth rate and seasonal production of wood were repeatedly higher for heated trees (Strömgren & Linder

2002).

With a limited response in soil N availability and no changes in foliar N, there is little evidence to support that differences in N availability could account for the change in BAI phenology. An alternative explanation for this change in phenology could be an earlier uptake of water and nutrients induced by the early snowmelt and increase in soil temperature associated with the soil warming The effect would have been more obvious in 2010 as the heated trees benefited from a longer additional growing season compared to 2011 (+12 days). Indeed, soil temperatures below 6°C have been shown to significantly inhibit root activity in conifers (Alvarez-Uria & Körner 2007). It is thus probable that trees growing in heated soils were able to start water and nutrient uptake earlier, as well as photosynthesis. Soil thaw is a prerequisite for the resumption of photosynthesis in balsam fir trees (Goodine *et al.* 2008), and higher rates of light-saturated photosynthesis were noted in boreal Norway spruce stands subject to soil warming (Bergh & Linder 1999).

Although strong positive relationships have previously been established between rates of carbon sequestration in plants and increased levels of soil available N through increased natural atmospheric deposition (Magnani *et al.* 2007; Quinn Thomas *et al.* 2010), and microbial mineralization (Rustad *et al.* 2001), fertilization (Tamm 1991) or soil warming (Rustad *et al.* 2001), the beneficial effects of a higher rate of growth early in the season, in terms of carbon allocation and wood production, did not translate into higher annual BAI. This absence of effects is logical with the lack of changes in foliar N between treatments. However, it should be interpreted cautiously. Due to the high natural BAI variability between trees, treatment effects on annual BAI were less susceptible to be detected than for other growth variables (Table 4.1). Cumulating additional years of data will increase our confidence in the absence of effects. Nonetheless, it was previously observed that higher growth rates at a certain point in a growing season do not necessarily enhance the annual stem growth (Deslauriers *et al.* 2003; Schmitt *et al.* 2004; Gruber *et al.* 2009). Four years of experimental warming of an entire pine-birch forest in Norway also resulted in similar radial growth between treatments (Rasmussen *et al.* 2002).

Most reported cases of null or negative effects of experimental soil warming on forest ecosystem productivity are related to water stress (Rustad et al. 2001). In the current study, soil water content and supply was not decreased by soil warming which suggests that trees in the heated plots were not more water stressed than control trees. This is also supported by the lack of relationship measured between soil temperature and soil water content (P=0.69; data not shown). Alternatively, there is little evidence that the soil disturbance associated with the burial of heating and control cables could have confounded our results. The impact of soil trenching on roots was minimized by running the cable below or above large roots. Disturbance was therefore restricted to fine-roots, which have a relatively rapid turnover of ca. one year (Yuan & Chen 2010). Given that cables were buried in fall 2008 and the data presented here span from April 2010 to October 2011, fine roots are highly likely to have recovered by the presented time period. The comparison of radial growth in treated trees with non-disturbed trees monitored during the entire experiment suggests no significant impairment of nutrient and water uptake capacity (D'Orangeville et al. in press). As for the soil nutrient availability, disturbed nutrient levels in the soil following cable burial have been shown to return to normal within a year (Peterjohn et al. 1994; McHale & Mitchell 1996).

4.6 Conclusion

Overall, and despite the changes in radial growth phenology, soil warming and/or additional N deposition did not significantly increase tree growth. This result, contrary to our hypothesis, suggests that some N-poor boreal forests could not respond as expected to increases in soil temperature and N availability when N is added in experimental conditions that mimic natural deposition and inorganic N concentrations. As a result, the modifications of the forest N cycle expected with climatic warming and increased N deposition (Rustad *et al.* 2001; Galloway *et al.* 2004) as well as the expected increases in forest growth and C sequestration (Jarvis & Linder 2000; Magnani *et al.* 2007) may simply not happen in the short term for these sites. Given the crucial importance of the boreal forest in the global C

cycle, more studies are necessary to better understand how these forest types will respond to climate change in order to improve the predictions of forest productivity and carbon sequestration models.

4.7 Acknowledgements

We would like to thank Mathieu Gélinas-Pouliot for the help with field maintenance and sampling, and Josianne De Blois for statistical advices. Funding for this research was provided by Ouranos and the Fonds Québécois de la recherche sur la nature et les technologies for the Réal-Décoste doctoral research scholarship to L. D'Orangeville, the Ministère des Ressources naturelles du Québec and Le Plan Vert du Ministère du Développement Durable, Environnement, Parc et Faune du Québec within the framework of the Action Plan 2006-2012 on Climate Change. Table 4.1. Mean basal area increment (BAI) parameters (and standard error) for balsam fir trees growing in control, heated, fertilized and heated-fertilized plots, and results from power analysis and mixed-model anovas applied to BAI parameters. Pre-BAI was included as covariable when significant. Significant p-values are in bold (P<0.05). Maximum daily growth corresponds to the highest ($Y_t - Y_{t-1}$) value.

		Pre- BAI	onset	ending	Annual BAI (mm ²)	Slope of growth	Maximum daily	Timing of maximum
		(mm²)	(DOY)	(DOY)		curve	growth	daily growth
							(mm² day ⁻¹)	(DOY)
2010	control	818 (196)	119 (1)	297 (6)	894 (109)	0.046 (0.001)	15.4 (1.5)	165 (1)
	fertilized (f)	1006 (102)	124 (5)	275 (28)	947 (297)	0.062 (0.015)	19.5 (2.4)	162 (3)
	heated (h)	615 (121)	121 (4)	261 (15)	674 (169)	0.058 (0.006)	14.3 (2.8)	156 (1)
	h-f	473 (237)	121 (4)	250 (13)	444 (165)	0.059 (0.006)	9.4 (2.6)	155 (1)
2011	control	818 (196)	124 (5)	294 (11)	785 (74)	0.046 (0.004)	15.3 (0.6)	167 (1)
	fertilized (f)	1006 (102)	132 (4)	286 (16)	936 (325)	0.051 (0.005)	20.6 (5.0)	170 (1)
	heated (h)	615 (121)	131 (3)	275 (7)	655 (98)	0.055 (0.003)	16.0 (1.5)	168 (2)
	h-f	473 (237)	128 (2)	270 (5)	430 (223)	0.050 (0.005)	10.3 (4.7)	165 (1)
ANOVA								
	Pre-BAI	-	0.68	0.03	<0.01	0.22	<0.01	<0.01
	Heating (H)	<0.01	0.81	0.45	0.85	0.39	0.89	0.03
	Fertilization (N)	0.82	0.75	0.32	0.60	0.41	0.75	0.47
	Year (Y)	-	<0.01	0.08	0.35	0.20	0.45	<0.01

HxN	0.12	0.30	0.42	0.84	0.24	0.16	0.56
HxY	-	0.87	0.23	0.59	0.91	0.79	0.01
NxY	-	0.38	0.37	0.52	0.34	0.83	0.13
HxNxY	-	1.0	0.72	0.57	0.77	0.73	0.07
Power analysis (for β =0.2)							
standard deviation (σ)	-	6	26	353	0.012	5.7	6
detectable delta (δ) at α =0.05	-	9	50	700	0.020	8.0	5
detectable delta (δ) at α =0.10	-	7	40	500	0.018	6.0	4



Figure 4.1. Schematic description of the experimental design. Each circle corresponds to an experimental tree.



Figure 4.2. Average soil temperature in control, fertilized, heated and heated-fertilized plots in 2010 and 2011. Coloured ribbons are standard error. The periods of soil warming are represented by the shaded areas.



Figure 4.3. A. Average daily basal area increment of the 12 monitored balsam fir trees in 2010 and 2011 at the Lake Laflamme watershed (Québec, Canada). The non-shaded areas identify the periods used for growth modelling each year and each monitored tree is represented by a different color. B. Modeled seasonal basal area increment of each monitored balsam fir tree (12) in 2010 and 2011 using the Gompertz equation.



Figure 4.4. Box plot of soil water content in 2010 and 2011 (N=12). Soil warming was not significant (P=0.14) and SWC was different between years (P<0.01).



Figure 4.5. Extractable and available NH₄ and NO₃ in the forest floor and mineral horizon in control (C), fertilized (+N), heated (+H) and heated-fertilized (+NH) plots in 2010 and 2011 (N=12). Extractable N corresponds to inorganic N species measured on soil samples taken in September of each year and extracted with KCl 1M. Available N corresponds to the cumulative amount of inorganic N species measured with ion exchange membranes (PRS-probes) from May 12 to September 30 in 2010 and from May 30 to October 7 in 2011. Values of extractable NO₃ in the mineral horizon in 2010 were all below the detection limit of the analytic device (<1 mg kg⁻¹). Error bars are standard error. Significant between-year differences ($P \le 0.05$) were detected for available N (NH₄ and NO₃) in both horizons and for extractable NH₄ in the mineral horizon. The only significant treatment effect was soil warming for available NH₄ in the organic horizon (P=0.02) with no effect of years (P=0.11).



Figure 4.6. Total N and ¹⁵N concentration in the needles of balsam fir trees in 2010 and 2011. C: control trees (no heating, no fertilization); +N: fertilized trees; +H: heated trees; +NH: fertilized and heated trees. Error bars are standard error. Foliar N was not significantly affected by the treatments ($P \ge 0.47$). Levels of ¹⁵N were significantly affected by fertilization (P < 0.01) but not by soil warming (P = 0.13). Foliar N and ¹⁵N were not different between years ($P \ge 0.45$).

Connecting paragraph

While the previous two chapters addressed the influence of soil warming and increased N deposition on the soil chemistry and radial growth of a boreal balsam fir stand, the following chapter presents a fine-scale analysis of the influence of these treatments on the xylogenesis of balsam fir. We measured the characteristics of the xylem cells produced, the number of cells produced as well as the general phenology of the process. This manuscript is in press in the journal 'Trees: Structure and Function', with co-authors Benoît Côté, Daniel Houle, Hubert Morin and Louis Duchesne.

This is a pre-copy-editing, author-produced version of an article accepted for publication in Trees: Structure and Function following peer review. The final publication is available online at http://link.springer.com (DOI: 10.1007/s00468-013-0899-4).
Chapter 5 - A three-year increase in soil temperature and atmospheric N deposition has minor effects on the xylogenesis of mature balsam fir

5.1 Abstract

Tree growth in most boreal forests is strongly regulated by temperature and nitrogen (N) availability. The expected increases in soil temperature and N deposition over the next decades have the potential to affect the phenology of tree growth and xylogenesis. In order to test for these changes on xylogenesis of balsam fir (Abies balsamea (L.) Mill), 12 mature trees were subjected to a combination of experimentally increased soil temperature (+4°C) with an earlier snowmelt (2-3 weeks) and N deposition (3x ambient rain N concentrations using NH₄-NO₃ in artificial precipitation) over a 3-year period. Increased soil temperature and atmospheric N deposition had no significant effect on the number of tracheids produced (38-51), tracheid diameter (27.2-29.0 μ m) and cell wall thickness (2.5-3.1 μ m). For the three years of treatment, xylogenesis was initiated at minimum and average daily air temperatures of 0.6±0.5 and 6.5±0.6°C, respectively, with inter-annual differences of 17 days in the onset of xylogenesis. The earlier snowmelt induced by soil warming did not hasten resumption of xylogenesis, and the time dynamics of xylogenesis was not affected by higher N deposition. Our results suggest that soil temperature and the timing of snowmelt have no direct influence on the breaking of cambium dormancy in balsam fir. The shortterm effects of increased soil temperature and N deposition on xylogenesis of mature balsam fir appear to be small compared with the effects of air temperature and are likely to be associated with a persistent N limitation.

5.2 Introduction

Recent climate model simulations for the Eastern boreal forest of Canada predict a 3°C temperature increase by 2041-2060 relative to 1971-1990 (Plummer *et al.* 2006) and a one-month earlier snowmelt by 2050 relative to 1971-2000 (Houle *et al.* 2012). In N-poor

ecosystems like the boreal forest, the plant response to climate change could be regulated by N availability (Magnani *et al.* 2007; Melillo *et al.* 2011). By increasing N mineralization rates and available N, warmer soils could promote growth and C sequestration in plants (Bronson *et al.* 2009; Jarvis & Linder 2000; Rustad *et al.* 2001; Strömgren & Linder 2002). Atmospheric N deposition is also increasing at the global scale and this trend is expected to continue in the next decades (+70% by 2050; Galloway *et al.* 2004). Hence, atmospheric N deposition could contribute an increasing proportion of the N required for tree growth (Magnani *et al.* 2007) and affect the tree response to climate change.

Understanding the interaction of additional N inputs from increased soil temperatures and increased N deposition is therefore of great importance to better predict forest growth. Results from most recent studies on increased N availability suggest a positive effect on plant growth (Chapin et al. 1995; Hutchison & Henry 2010; Ma et al. 2011; Majdi & Öhrvik 2004; Mäkipää et al. 1999; Sager & Hutchinson 2005; Strömgren & Linder 2002; Zhao & Liu 2009). However, the mechanisms by which environmental factors affect wood production are not well understood (Savidge 2000). The volume of wood produced by a tree in a year is function of the length of growing season, the rate of cell division and the amount of cell expansion (Creber & Chaloner 1984). How much each of these components is affected by increasing soil temperature and N deposition is unknown, but clues can be found in studies on the separate effects of N availability and soil temperature on tree growth. The positive effect of N fertilization on the number of xylem cells produced annually is well established (Plavcovà et al. 2013; Puech et al. 2000). Improving the N status of trees can also enhance the size of xylem cells due to increased photosynthetic rates and the associated increase in transpiration requirements (Beets et al. 2001; Mäkinen et al. 2002; Roderick & Berry 2001). Thus, the future increase in available N could lead boreal trees to produce larger xylem cells with thinner walls, which implies a reduction in the density and possibly the quality of the wood. Nitrogen fertilization can also affect the phenology of tree growth. Mitotic activity in the stem (Hawkins et al. 1995) and in the foliage (Rikala & Repo 1997) can be prolonged, and bud formation can be delayed following N fertilization (Bigras et al. 1996). Warmer soils, on the other hand, could have a negative

effect on plant growth by inducing water stress through increased evapotranspiration (Rustad et al. 2001). Although plants could compensate with increased aquaporins activity in the roots (Maurel & Chrispeels 2001), this could lead conifers to produce smaller tracheids with thicker cell walls (Jyske et al. 2010). In addition to the above-mentioned potential effects of higher available N and warmer soils on various aspects of xylogenesis, warmer air temperatures could affect plant growth by inducing an earlier cambium reactivation in spring. Studies aimed at identifying the most important environmental factors affecting the onset of spring growth often found that air temperature was the most important factor (Begum et al. 2008; Begum et al. 2010; Dufour & Morin 2010; Gruber et al. 2010). Minimal temperature thresholds for cambial activity of many conifers were found to be ranging from 4 to 5°C (Rossi et al. 2008). However, how temperature triggers cambium reactivation remains uncertain. Cell division proceeds extremely slowly at temperatures below 1-2°C (Körner, 2003). Air temperature could control the supply of some hormones (i.e. auxin, cytokinin) to the cambium (Fonti et al. 2007). Alternatively, some have hypothesized that snowmelt could be the starting signal. In some cold ecosystems, soil temperature is typically maintained at or below 0°C during winter which inhibits water and nutrient uptake (Jarvis & Linder 2000). Experimentally delayed soil thawing has also been shown to affect the physiology of Norway spruce (Picea abies L. Karst.) needles (Repo et al. 2008). Several studies report cambial reactivation following snowmelt (Hoch and Körner 2003; Turcotte et al. 2009) and annual growth has been repeatedly correlated with the timing of snowmelt (Carrer et al. 2007; Jarvis & Linder 2000; Kirdyanov et al. 2003; Körner 2003; Vaganov et al. 1999). Delayed budburst has also been observed in trees subject to colder soils (Lopushinsky & Max 1990; Repo et al. 2007). However, recent results on the effects of experimental warming of forest soils suggests that soil temperature is not related to cambial reactivation in black spruce (Picea mariana (Mill.) Britton, Sterns & Poggenb.; Lupi et al. 2012a) and Norway spruce (Strömgren & Linder 2002). Whether other species of conifers e.g. Abies spp and Pinus spp respond similarly to soil warming remains unknown.

In this study, we experimentally increased soil temperature (+4 $^{\circ}$ C), hastened spring snowmelt (2-3 weeks) and increased atmospheric N deposition (+50% of annual NH₄NO₃ in

incoming precipitation during the growing season) during three years in a typical mature balsam fir stand of Eastern Canada. We hypothesized that i) higher soil temperature and N deposition would increase xylem cell production, extend the duration of xylogenesis and increase the diameter of tracheids, and ii) an earlier snowmelt would not affect the onset of xylogenesis.

5.3 Materials and methods

Study area

The study site is located in the Laurentian Mountains of eastern Québec, Canada (47°17' N; 71°14' O; 800 m above sea level). The 60-year-old even-aged stand is dominated by balsam fir with some paper birch (*Betula papyrifera* Marsh.) and white spruce (*Picea glauca* (Moench) Voss) as companion species. Stem density in 2008 was 2024 trees ha⁻¹ (diameter at breast height \ge 9 cm) and the site index is 14 m at 50 years. The soil is an Orthic Humo-ferric podzol (Spodosol) with a sandy loam texture. Annual N deposition (NH₄ + NO₃) averages 5.7 kg N ha⁻¹ year⁻¹ (Houle & Moore 2008), while mean annual air temperature and precipitation between 1981 and 2006 averaged -0.3°C and 1535 mm.

Experimental design

In autumn 2008, three 12m x 60m blocks were laid out with the long axis perpendicular to the slope with a 12 m buffer strip in between. Each block was divided into two 24 m x 12 m sub-blocks separated by a 12 m x 12 m buffer zone. Each sub-block was then divided into two 12 m x 12 m plots. The fertilization treatment was randomly distributed within each block, and the soil warming treatment was randomly nested within each sub-block. Thus, the buffer zone within each block was set up to avoid contamination between fertilization treatments. Within each plot, a balsam fir tree was selected based on a visual estimation of good health and dominance or co-dominance in the canopy. The height and DBH of every

selected tree was noted and ranged from 12.9 to 18.1 m and from 14.0 to 22.8 cm, respectively.

Around each experimental tree, heating cables (non-heating cables for the heating control) were buried in a spiral pattern 5-10 cm belowground (at the interface between the organic and the mineral layers) at 0.9-2.5 m from the base of the tree, with an average distance between cables of 30 cm. Great care was taken to avoid root damages and roots >5mm in diameter were bypassed.

Soil temperature under the canopy of each tree was measured continuously with thermistors buried 5cm belowground (precision: ±0.2°C; model 107-L from Campbell Scientific Inc., Utah, USA) buried between cables. Dataloggers (CR1000, Campbell Scientific, Inc., Utah, USA) were used to control the activity of the warming cables and maintain an average difference of 4°C on the basis of projections for the site for the 2070-2100 period (Houle *et al.* 2012). In order to evaluate potential effects of the burial of cables on the trees water and nutrient uptake, three nearby non-disturbed trees (control) were also monitored during the entire course of the experiment.

The rain solution used for the N fertilization treatment (control and N-enriched) was applied on the canopy using nozzles set up above each individual tree using a system of pumps and reservoirs, in order to simulate how atmospheric N is deposited on forest ecosystems. The composition of the artificial rain solution was based on the average ion concentration in incoming precipitation measured at the site in previous years. Deionized water in which were added Na (0.046 mg L⁻¹), Ca (0.089 mg L⁻¹), Mg (0.018 mg L⁻¹), K (0.026 mg L⁻¹), H (0.014 mg L⁻¹), Cl (0.07 mg L⁻¹) and SO₄ (1.01 mg L⁻¹) was used as rain solution. For the N treatment, 1.0 and 3.4 mg·L⁻¹ of NH₄ and NO₃ were respectively added to the solution, which corresponds to approximately three times the concentrations measured in the local precipitation. Each year, from mid-June to mid-September, every tree was simultaneously subjected to a weekly artificial precipitation of 70 liters, summing to 1.544 g N tree⁻¹ year⁻¹. On rainy days, watering was rescheduled to the next day.

Weather data

In addition to the soil temperature measured at the site, air temperature and snow depth were measured in a clearing located approximately 200 m from the plots. Temperature sensors (HMP35CF, Campbell Scientific Inc., USA) recorded air temperature at a height of 3.3 m, and a sonic ranging sensor (SR-50, Campbell Scientific Inc., USA) was used to monitor the thickness of the snowpack. Measurements were made every 15 min and hourly averages recorded by the data logger (CR-1000, Campbell Scientific Inc., USA).

From the beginning of spring snowmelt until the first autumn frost, the average soil temperature in treated plots was maintained $4.0\pm0.4^{\circ}$ C, $4.1\pm0.3^{\circ}$ C and $3.7\pm0.9^{\circ}$ C higher than controls in 2009, 2010 and 2011, respectively (Fig. 5.1). In 2009, soil warming was started on April 27 with a remaining snowpack of 0.75 m. A system malfunction caused an early interruption of the soil warming in mid-August that year. In 2010, soil heating was started earlier (March 30) because of a warm spring, with a snowpack of 0.85 m. The treatment was stopped before the first freeze-thaw, on October 19. In 2011, the soil heating was started on May 5 (Fig. 5.1). The snowpack was then 0.60 m. Two consecutive system malfunctions reduced the temperature difference to +2.8°C from May 17 to June 4 and to +0.7°C from August 9 to August 23 that year. Heating was terminated on October 18 before the first freeze-thaw events.

Soil water content

Volumetric soil water content (SWC) in the plots was measured weekly during the snow free period from 2009 to 2011. Soil water content was measured with a ThetaProbe ML1 (Delta-T Devices Ltd, Cambridge, England) in 2009 and with a FieldScout TDR-300 device with 20-cm rods and standard calibration (accuracy: ±3% vol; Spectrum technologies Inc., Plainfield, USA) in 2010 and 2011. For each plot, nine measurements were randomly taken at a distance of 1.5m from each tree and averaged.

Sample collection

The effect of treatments on xylogenesis was studied over three years (2009-2011) by extracting wood microcores (2.5 mm in diameter and 20 mm long) weekly from April to October from each tree using a Trephor tool (Rossi *et al.* 2006a). The sampling followed an upward spiral starting at 1.3 m aboveground. All samples were taken at least 10 cm apart to minimize the formation of resin ducts (Forster *et al.* 2000). The samples were stored at 4°C in Eppendorf microtubes filled with a 10% ethanol solution for tissue preservation.

Histological analyses

At the laboratory, the water from the microcores was removed through successive immersions in ethanol and HistosolTM and embedded in paraffin according to (Rossi *et al.* 2006b). Transverse sections were cut with a rotary microtome (6-10 μ m in thickness), stained with cresyl violet acetate (0.16% in water) after removal of the paraffin, and observed under visible and polarized light at a magnification of x400-500 to differentiate cells in the cambium and the differentiating xylem.

The number of cambial cells, enlarging cells, wall thickening cells and mature cells were counted along three radial files and averaged for every sample of each tree. Cambial cells were characterized by thin cell walls and less than half the diameter of enlarging cells. Both cambial and enlarging cells could be differentiated from other cell phases by the lack of secondary cell wall, a structure that shines under polarized light (Kutscha *et al.* 1975). Cells in the process of lignification and wall thickening showed violet and blue walls, the colour blue revealing the deposition of lignin on the cell walls (Thibeault-Martel *et al.* 2008). Mature tracheids were characterized by completely blue cell walls.

An additional series of microcores was collected upon termination of xylogenesis, in mid-October of each year, and used for additional anatomical measurements. Following the standard preparation procedure described above, the sections were stained with safranin (1%) and numerically photographed with a camera fixed on an optical microscope at a

magnification of x400. Cell and lumen diameter as well as cell wall thickness were measured on three radial files per section, including pre-treatment year 2008, using Wincell software (Regent Instruments Inc.). Tracheids were classified as earlywood or latewood according to Mork's formula, where latewood cells are characterized by a lumen smaller than twice a double cell wall (Denne 1988).

Model fitting

The cumulative amount of cells produced throughout the growing season, calculated as the sum of enlarging, wall thickening and mature cells, was modelled with a Gompertz function (Rossi *et al.* 2003) for each tree (12) and each year (3), for a total of 36 models :

$$Y = A \exp\left[-e^{\left(\beta(\chi-t)\right)^2}\right]$$

where Y is the cumulative amount of cells produced, *A* is the upper asymptote, β sets the rate of change, κ sets the x displacement, which is the date when maximal growth rate is reached, and *t* is the time computed in day of year (DOY). All regressions had R² > 0.85 and showed a normal distribution of the residuals (Motulsky & Ransnas 1987). The 'nlm' procedure from the R software was used with ordinary least square method for parameters estimation (R Development Core Team 2012). The beginning and ending of xylogenesis were determined as the day when the daily cell production (Y_t -Y_{t-1}) went above and below 0.1 cell day⁻¹, respectively. The duration of xylogenesis was calculated as the number of days between the onset and termination of cell production. Maximum growth rate was calculated as the maximum daily growth. Finally, we averaged the daily minimal and mean air temperature of the seven days preceding the onset of xylogenesis for each tree and each year in order to estimate the threshold temperature for the onset of xylogenesis at the site.

Statistical Methods

A linear mixed-model analysis of variance (ANOVA) with repeated measures was used to test for the effect of the warming treatment on soil water content, considering warming,

year and sampling date as fixed factors and plot within sub-block within block as random factors. A compound symmetry variance-covariance structure was assumed for repeated measures. A similar approach was used to detect any significant effect of treatments on the phenology of growth and cell characteristics, considering soil warming, fertilization and year as fixed factors and plot within sub-block within block as random factors. Strong correlations of pre-treatment growth ring width (year 2008) were found with the onset of growth (P=0.06) as well as with the total number of cells produced (P<0.01). Because pretreatment growth was significantly smaller in heated-fertilized trees (P=0.02; Table 5.2), it was used as covariate for the analysis of phenological and productivity parameters when significant at P<0.05. As for the analysis of cellular morphology, no significant pre-treatment differences were detected, thus no covariates were included in the models. Similar ANOVA models were used to compare control trees with experimental trees, this time with cable burial and year as fixed factors. Data were tested *a priori* to meet the assumptions of ANOVA, and Tukey post-hoc tests were used to identify which means differed from one another. Statistical analysis, modelling and plots were done using the R software (R Development Core Team 2012).

5.4 Results

The spring snowmelt was completed on May 16 in 2009 and on May 21 in 2011, but ended three weeks earlier in 2010 (April 27) because of warmer temperatures (Fig. 5.1). For the period of sampling (May to September), air temperature averaged 11.1°C in 2009 compared with 12.5°C in 2010 and 12.2°C in 2011 (Fig. 5.1). In addition to its extended snow-free period, 2010 was characterized by a growing season with very low precipitation in August (38 mm compared to 122 mm in 2009 and 226 mm in 2011, data not shown). For the May to September period, 2011 was the wettest year with 747 mm in precipitation, compared to 585 mm in 2009, and 596 mm in 2010. Soil water content was significantly lower (P<0.05) during the 2010 growing season (20.7±0.2%) relative to 2009 (28.1±0.3%) and 2011

(26.6±0.2%; Fig. 5.2). Soil water content tended to be higher in heated plots but the difference was not statistically significant (P>0.05).

Growth dynamics

For all treatment, the cambium averaged five to six cells prior to the onset of xylogenesis (Fig. 5.4). The number of cambial cells averaged 11±1 at the peak of mitosis in all treatments except fertilized-only trees with 14±1 cambial cells. On average, up to 8±1 and 11±1 enlarging cells could be counted on a single sample in heated and non-heated trees, respectively (Fig. 5.4). The mean number of cells in the phase of wall thickening was 14±2, 19±2, 17±2 and 16±2 in control, fertilized, heated and heated-fertilized trees, respectively. Overall, fertilized trees produced the largest number of tracheids with an average of 68±7 tracheids produced each year, followed by control (56±6), heated (52±5) and heated-fertilized (46±5) trees.

The onset, ending and duration of xylogenesis were not significantly affected by the treatments (Table 5.1). The onset of cambial activity occurred significantly later in 2009 (May 29; *P*<0.01) relative to 2010 (May 12) and 2011 (May 21; Table 5.1). Xylogenesis was initiated at a minimum daily air temperature of 0.8 ± 0.7 °*C*, -0.5 ± 0.8 °*C* and 1.9 ± 0.8 °C in 2009, 2010 and 2011, respectively, and at average daily air temperatures of 7.4 ± 0.6 °C, 5.3 ± 1.2 °*C* and 7.0 ± 0.8 °C in 2009, 2010 and 2011, respectively. In 2010, the maximum rate of growth was reached around June 14, ten days earlier than in 2009 or 2011 (*P*<0.01; Table 5.1). Cambial activity was maintained until September 4 in 2011, significantly later than in 2009 (August 10; *p*<0.01) but similar to 2010 (August 29; Table 5.1). The overall period of cambial activity was significantly shorter in 2009 (73 days) relative to 2011 (106 days; *P*<0.01) but similar to 2010 (108 days) due to the high variability measured that year.

Amount of tracheids produced and anatomical characteristics

There were no statistical differences in the number of tracheids produced between control trees and experimental trees (*P*=0.98; Fig. 5.3). Furthermore, the average lumen diameter and wall thickness did not differ between the two groups (*P*≥0.24; Fig. 5.3). The annual production of xylem cells was not significantly different between years or treatments, with a mean annual production ranging from 38 to 51 tracheids (Table 5.2 & Fig. 5.5). The average tracheid lumen diameter and wall thickness differed significantly between years (*P*<0.01; Table 5.2 & Fig.5.5) with the average tracheid diameter displaying a similar trend (*P*=0.08). When considering all treatments together, the tracheid lumen was significantly reduced in 2010 (21.1±0.6 µm) relative to 2009 (23.5±0.6 µm; *P*<0.05) while the year 2011 did not differ from both previous years (22.9±0.7 µm; Fig. 5.5). The average cell diameter in 2010 (27.2±0.7 µm) also tended to be smaller than that of 2009 (28.6±0.8 µm) and 2011 (29.0±0.8 µm). The wall thickness of tracheids tended to be higher in 2011 (3.17±0.03 µm) relative to 2009 (2.70±0.02 µm; *P*=0.08), while that of 2010 was intermediate (3.12±0.03 µm; Fig. 5.5).

The treatments had no significant effect on the average cell diameter, lumen diameter and cell wall thickness of tracheids (Table 5.2) or the ratio of earlywood to latewood (data not shown). The separate analysis of earlywood and latewood anatomical characteristics yielded similar results (data not shown).

5.5 Discussion

Initial root disturbance

Soil trenching was necessary to bury the heating and control cables in each experimental plot. The impact of the trenching on roots was minimized by running the cable below or above large roots. The effect of cable burial should therefore be mostly restricted to fine-roots. The average fine-root turnover rate in boreal forests is about 1.08 yr⁻¹ (Yuan & Chen

2010). Therefore, tree nutrient and water uptake could have been impaired during the first year of treatment. Since nutrient availability and xylem cell production are usually correlated (Puech *et al.* 2000; Plavcovà *et al.* 2013), the similar growth observed for our negative control trees and soil-disturbed trees suggests no significant impairment of nutrient and water uptake capacity. Cell lumen diameter and wall thickness of many conifers (Abe & Nakai 1999; Abe *et al.* 2003; Eilmann *et al.* 2009; Gruber *et al.* 2010; Jyske *et al.* 2010), including balsam fir (Rossi *et al.* 2009), are also known to respond to changes in water uptake. The fact that these two variables were not significantly different for our negative-control trees and treated trees provides further evidence that the initial root disturbance did not interfere significantly with the response of trees to the experimental treatments.

Earlier snowmelt and the onset of xylogenesis

The lack of soil heating effect on the spring resumption of radial growth supports the hypothesis that soil temperature and the timing of snowmelt have no direct influence on the breaking of balsam fir cambium dormancy. Similar results and conclusions were obtained for black spruce (Lupi *et al.* 2012a) and Norway spruce (Strömgren & Linder 2002) which suggests that the lack of response to earlier soil warming and rewetting on the onset of xylogenesis is likely generalized for conifers of the boreal forest. In our study, the thick snowpack generally maintained the soil slightly above the freezing point during the winter (Fig. 5.1). Other sites may however experience soil frost with concomitant effects on water availability (Jarvis & Linder 2000). Such conditions may require that snowmelt and soil thawing occur for xylogenesis to resume in the spring. If soil temperature and snowmelt are to be rejected as signals for cambium reactivation, air temperature remains the most likely triggering factor.

A strong argument for air temperature to be the main trigger of xylogenesis comes from experiments using artificial warming applied to parts of the main trunk of a tree. This treatment induced cell division and xylogenesis at various degrees for various evergreen

conifer species even when a snow cover was present (Barnett & Miller 1994; Oribe et al. 2001; Oribe et al. 2003; Gricar et al. 2006; Gričar et al. 2007). The preponderant effect of air temperature on spring growth onset over other environmental signals has been demonstrated in many studies (Begum et al. 2008; Deslauriers et al. 2008; Begum et al. 2010; Dufour & Morin 2010; Gruber et al. 2010). The recent analysis of cambial activity in conifers located at their latitudinal or altitudinal distribution limits have revealed the existence of threshold air temperatures for radial growth of 5.6 and 8.5°C for Swiss stone pine (Pinus cembra L.) and Norway spruce, respectively (Rossi et al. 2007), of 8.2°C for Bosnian pine (Pinus leucodermis Antoine; Deslauriers et al. 2008), and minimal and average daily temperatures of 4-5°C and 8-9°C, respectively, for seven conifer species (balsam fir, European larch, Swiss stone pine, Scots pine, Bosnian pine, mountain pine and Norway spruce) at ten sites in Italy, Slovenia, Canada and Finland (Rossi et al. 2008). Relative to these last threshold values, xylogenesis at our site started at lower minimum and average daily temperatures of approximately 1°C and 6°C, respectively. These lower threshold temperatures could be explained in part by the different methodological approaches used to calculate the threshold values, as no logistic regressions were used in the current study to produce these numbers.

The comparison of naturally contrasting years in terms of temperature can give clues as to the growth phenology of a given tree species in a warmer world. In the current study, May 2010 was 1.1°C warmer than the average for 1970-2008, while May 2009 was 2.2°C colder. In 2010, cambial derivatives resumed differentiation 17 days earlier than in 2009. This result highlights the importance of air temperature for the onset of xylogenesis at our study site. Similar results have been obtained in Europe, where a warmer spring (+2.6°C) induced a 20-day advanced onset of radial growth in Bosnian pine (Deslauriers *et al.* 2008).

The effect of increased soil temperature and N deposition

The average five to six cells measured in the dormant cambium in our study is consistent with the range of values observed in other balsam fir trees of Eastern Canada (Riding &

Little 1986; Thibeault-Martel *et al.* 2008). Upon cambial reactivation, trees from the current study produced 1.6-2 times the number of differentiating xylem cells than balsam fir trees growing in a colder and dryer boreal stand of Québec, Canada (Deslauriers *et al.* 2003) although the overall number of tracheids produced (38-51) was not higher than the mean number of tracheids observed in balsam fir trees from the colder site (Rossi *et al.* 2003; Deslauriers & Morin 2005). The cell division rates in the cambium being mainly driven by temperature (Körner 2003), the warmer temperature at the study site could explain that a higher number of cells were differentiating simultaneously although the total number of cells produced was the same. The phenology of growth was also similar to previous studies, with xylogenesis starting in early to late May and ending in late August (Deslauriers *et al.* 2003).

Contrary to our hypothesis, neither treatment significantly affected the course of xylogenesis, the number of tracheids produced or their anatomy. The application of similar treatments to black spruce trees in Québec also yielded no effects on the number of tracheids produced although increases in cell wall thickness were noted (Lupi et al. 2012b). The combination of increased CO_2 and air temperature in a pine-birch forest of Norway also produced no significant effect on the number of cells (Rasmussen et al. 2002). It was suggested that the availability of N limited the response to increased CO₂ and air temperature. In our study, we hypothesized that the heating treatment would affect xylogenesis through its effects on N mineralization/availability and water availability. However, the effects of the treatments on soil inorganic N at our site were negligible (D'Orangeville et al. in press). Although current forest carbon models predict positive effects from similar treatments on tree growth (Mäkipää et al. 1999), boreal forest soils are known to immobilize a large fraction of N inputs at the expense of the aboveground vegetation (Nadelhoffer et al. 1999a; Berg & Dise 2004; Houle & Moore 2008; Friedrich et al. 2011). The existence of such N sinks at the study site was confirmed with a short term ¹⁵N isotopic pool dilutions approach, as almost all of the NH₄ and NO₃ made available in the forest floor was immobilized in less than a day (Ste-Marie & Houle 2006). Alternatively, balsam fir trees have been shown to intercept with their canopy a fraction of the nutrients deposited from

the atmosphere, thereby avoiding soil competition (Houle *et al.* 1999). However, the lack of effects from three years of increased N deposition on xylogenesis suggests that this nutritional pathway has a limited effect on radial growth of balsam fir.

As for water, soil warming did not decrease its availability in the soil. The size of tracheids and the thickness of their wall usually respond to water stress (Beth Kirkham *et al.* 1972; Deri Tomos *et al.* 1989). Balsam fir is no exception, as the smaller lumen diameter and thicker walls of tracheids produced in 2010 are probably linked to the reduced water availability measured that year. However, no anatomical changes were detected in the tracheids wall thickness or lumen diameter of trees in heated plots, which confirms the absence of a water stress caused by the treatment. Although negative control trees did not display any signs of water deficiency in 2010 based on their xylogenesis, we cannot exclude that the additional irrigation applied evenly in all plots limited the negative impact of the 2010 water shortage as well as the higher soil temperature forcing on evapotranspiration rates.

Considering the realistic scenarios of increased soil temperature and N deposition used in our study and the lack of effect they had on the number of tracheids produced and their anatomical characteristics, the potential changes in future xylogenesis – and hence on radial growth – due to higher N uptake could be smaller than expected for similar boreal forests. The absence of effects from the combination of increased soil temperature and N deposition in the current study does not exclude that effects could arise on the longer term in presence of co-occurring changes in other climatic variables such as increased air temperature, reduced water availability and increased CO₂ concentrations (Bonan 2008). In addition, it should be noted that due to the high variability of the stand under study, our capacity to detect significant treatment effects would have benefited from a larger sample size.

5.6 Conclusions

The earlier warming and rewetting of soil in the spring did not hasten the differentiation of xylem cells of balsam fir. This study supports the hypothesis that air temperature is the main trigger of cambial cell differentiation in conifers. The limited response of xylogenesis of balsam fir to the realistic scenario of increased soil temperature and N deposition used in our study suggests that the impact of these two components of global change could be small for this species in the short-term. The influence of soil temperature on radial growth could therefore be limited to indirect effects on available soil nutrients which are more likely to respond significantly on more productive sites.

5.7 Acknowledgements

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Figure 5.1. Average air temperature, soil temperature and differential between heated and heating control plots, and snowpack in 2009, 2010 and 2011. Coloured ribbons are standard error. The periods of soil warming are represented by the shaded areas.



Figure 5.2. Average soil water content in control and heated plots in 2009, 2010 and 2011. Vertical lines are standard error.



Figure 5.3. Average number of tracheids, lumen diameter and wall thickness for control trees (grey line; no cable burial) and experimental trees (black line; cable burial) from 2008 to 2011. Vertical bars are standard error. The shaded area represents the period of treatment.



Figure 5.4. Average number of cambial cells, enlarging cells, wall thickening-lignification cells and total number of cells in control, heated, fertilized and heated-fertilized balsam fir trees in 2009, 2010 and 2011.



Figure 5.5. Average number of tracheids, cell diameter, lumen diameter and wall thickness for control (dotted grey line), heated (dotted black line), fertilized (full grey line) and heated-fertilized balsam fir trees (full black line) from 2008 to 2011. Vertical bars are standard error. The shaded area represents the period of treatment.

Connecting paragraph

The four previous chapters concerned the response of a balsam fir stand to an artificial increase in soil temperature and N deposition. Such experiment aims at reproducing the trends associated with climate change, expressed as shifts in the mean value of various climatic parameters (temperature, CO₂, etc.). However, the future climate is also projected to display more extreme events, in numbers and in intensity. Here we present the results of a throughfall exclusion experiment that simulates natural summer droughts. The impacts on balsam fir xylogenesis are described. This manuscript was published in the journal 'Tree Physiology' with co-authors Benoît Côté, Daniel Houle and Hubert Morin.

This is a pre-copy-editing, author-produced version of an article accepted for publication in Tree Physiology following peer review. The definitive publisher-authenticated version (D'Orangeville et al. (2013). The effects of throughfall exclusion on xylogenesis of balsam fir. Tree physiology, 33: 516-526) is available online at www.treephys.oxfordjournals.org/content/33/5/516

Chapter 6. The effects of throughfall exclusion on xylogenesis of balsam fir

6.1 Abstract

A 20-40% reduction in soil moisture is projected for the boreal forest of Eastern Canada for the period 2070-2099 relative to 1971-2000. In order to better predict the effects of a reduced water supply on the growth of balsam fir (*Abies balsamea* (L.) Mill.), a dominant tree species of the boreal forest, we simulated two consecutive years of summer droughts (starting in July) by means of throughfall exclusion. Four 100-m² plots were established in 2010 with polyethylene sheets maintained 1.3-2m aboveground and redirecting the water outside the plots. Wood microcores were extracted weekly from mature trees from April to October 2011 to analyse the time dynamics of wood formation in that year. The number of tracheids formed during and before treatment and their anatomical characteristics was determined through microscopic analyses. The growth of lateral and terminal branches and the water potential of balsam fir seedlings were also monitored.

Throughfall exclusion significantly reduced soil water content by 5.8% in 2010 and 10.5% in 2011. Xylogenesis was affected significantly by the treatment. Tracheids were 16.1% smaller in diameter and their cell wall was 14.1% thicker during both years. The treatment delayed by more than a week the start of the tracheid differentiation process in the second year with a concomitant decrease (26%) in the number of tracheids produced. The seedlings displayed a 32% reduction in growth and a 40% reduction in leaf water potential. Our results suggest that a future regime of increased frequency and intensity of droughts could have negative effects on the duration of xylogenesis and the production of xylogenesis and the production of xylogenesis in balsam fir.

6.2 Introduction

Global aridity and drought frequency have increased significantly in the last 60 years in Africa, southern Europe, East and South Asia, eastern Australia, Alaska and northern Canada

(IPCC 2012). For the boreal forest of Québec, Canada, a 20-40% reduction in soil moisture is projected during the growing season for the period 2070-2099 compared to 1971-2000 (Houle *et al.* 2012). Such a decrease in soil moisture is likely to be associated with an increase in the frequency and intensity of droughts (Schar *et al.* 2004). In comparison with other forest growth drivers expected to undergo significant changes in the coming decades (e.g. temperature, CO₂), reduced water availability has received little attention (Hartmann 2011; Beier *et al.* 2012).

Water deficits affect a variety of physiological processes in plants. A reduction in plant turgor pressure triggers trees to close stomata to reduce transpiration (Hsiao 1973). As a result, both photosynthesis and growth can be negatively affected (Hsiao *et al.* 1976). Our understanding of drought-related impacts on tree growth is, however, complicated by delayed responses resulting in non-linear effect of drought over time (Allen *et al.* 2010). For instance, the growth of beech (*Fagus sylvatica* L.) was lowest in the year following a severe drought in Europe (Granier *et al.* 2007). In the study of growth patterns from dead oak trees (*Quercus* spp. L.), this response lag was shown to persist much longer: a majority of trees exhibited pre-mortality growth declines often correlated with drought events that occurred two to 52 years prior to mortality (Pedersen 1998). The post-drought decline of trembling aspen trees (*Populus tremuloides* Michx.) in western North America was related to the persistence of hydraulic damages that increase the susceptibility to cavitation (Anderegg *et al.* 2013). Alternatively, the exhaustion of carbon reserves is also put forward as a possible explanation for post-drought decline (McDowell *et al.* 2008).

In this context, the anticipated increase in carbon sequestration rates of boreal forest ecosystems due to global warming (Hyvönen *et al.* 2007) could be partially offset by growth reductions caused by an increased frequency of drought events. For example, the severe rainfall deficit and summer heat of 2003 in Europe caused a massive reduction of 30% in gross primary productivity, cancelling in a single year the equivalent of four years of net ecosystem carbon sequestration (Ciais *et al.* 2005). Of 95 recent experiments involving changes to precipitation regimes, only two looked at tree growth in response to drought in boreal ecosystems, highlighting the need for additional studies on that matter (see Beier *et*

al. 2012). The formation of wood in conifers, or xylogenesis, involves cambium mitosis, enlargement of the cambial derivatives, wall thickening and lignification, with cell apoptosis marking the completion of the differentiation process (Catesson 1994). The successive phases of cell division and differentiation, or phenophases, can display contrasting temporal patterns as they occur at different times in the growing season (Deslauriers *et al.* 2003). Therefore, the consequences of a drought event on tree growth will depend on the timing of the event and the affected phenophases. A better understanding of the effects of drought on each phenophase could improve our capacity to predict how trees will be affected by future changes in water availability.

Studies of xylem formation in trees subjected to natural episodes of drought have shown a reduction in the rate of xylem differentiation and the annual amount of xylem produced (Hsiao 1973; Abe *et al.* 2003; Eilmann *et al.* 2009; Jyske *et al.* 2010). Xylem cells produced under drought stress can have different properties, including smaller diameters and thicker cell walls (Abe & Nakai 1999; Abe *et al.* 2003; Eilmann *et al.* 2009; Rossi *et al.* 2009; Gruber *et al.* 2010; Jyske *et al.* 2010). This reduction in size is the direct consequence of a reduced turgor pressure during cell enlargement and is thought to limit the risk of cavitation (Sperry *et al.* 2006). With increasing water stress and negative pressure in the xylem, a thicker cell wall is needed to increase the resistance of the xylem conduits against the risk of implosion (Hacke *et al.* 2001).

The phenology of xylem formation could also be affected by droughts. In Québec, Canada, balsam fir (*Abies balsamea* (L.) Mill.) radial growth ended four weeks earlier and this was attributed to warm temperatures combined with high relative humidity and low precipitation which caused stomata to close, impairing transpiration during the two weeks preceding the end of growth (Duchesne & Houle 2011). There are no reported effects of drought on the beginning of growth, although stem rehydration is a prerequisite for cambium mitosis (Turcotte *et al.* 2009) perhaps because of the preponderant role of temperature on cambium reactivation (see (Begum *et al.* 2013). To the best of our knowledge, the effects of drought on each of the phenophases of xylogenesis have yet to be demonstrated experimentally. Throughfall exclusion for approximately 90 days on mature

black spruce trees (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.) was limited to changes in the tracheids morphology (Belien *et al.* 2012). In another study, balsam fir seedlings subjected to 20-day water exclusion did not, however, alter their xylogenesis phenology (Rossi *et al.* 2009).

The susceptibility of trees to drought depends on their life stage. For instance, seedlings in the understory could be more susceptible to drought because of their small and superficial rooting that prevent water uptake from the deeper soil layers (McDowell *et al.* 2008). Their limited carbon reserves could also make them more vulnerable to carbon starvation following an extended period of reduced photosynthesis (Hartmann 2011). Nevertheless, the effects of experimental drought on the xylogenesis of younger trees appear to be quite similar to those observed in mature trees with effects on xylem morphology or cell division rates (Abe *et al.* 2003; Rossi *et al.* 2009; Galle *et al.* 2010). A good understanding of the differential response of young and mature trees to drought is necessary to better predict the consequences of a drought stress on the age structure of uneven-aged forest stands (Beier *et al.* 2012).

In this study, a throughfall exclusion experimental design was used to study the effect of two consecutive years of summer drought on the xylogenesis of balsam fir, a dominant species of the boreal forest of Eastern Canada. Our hypotheses were that the drought would i) reduce the size of the differentiating tracheids and ii) increase tracheid wall thickness during both treatment years, iii) hasten the ending of xylogenesis and iv) reduce the number of tracheids produced particularly in the second year of throughfall exclusion as 65-80% of the annual ring will be formed when the treatment will be started in July (Deslauriers *et al.* 2003). We hypothesized that the growth reduction of seedlings would be more severe than for mature trees.

6.3 Materials and methods

Study area

The study area is located in the Laurentian Mountains of Québec, Canada (47°17′ N; 71°14′ O; 800 m above sea level). The 60-year-old even-aged balsam fir stand is dominated by balsam fir mixed with paper birch (*Betula papyrifera* Marsh.) and white spruce (*Picea glauca* (Moench) Voss). The stand density is 2024 stems ha⁻¹ (diameter at breast height \geq 9cm) with a basal area of 18.6 m² ha⁻¹. The climate is continental with cold winters and warm summers. Between 1981 and 2006, mean temperature and annual precipitation were -0.3°C and 1535 mm, respectively. The soil is a sandy till classified as an Orthic Humo-Ferric Podzol. The mean slope is 8% with a western aspect.

Throughfall exclusion

Four plots were established in the spring of 2010 at least 50 m apart from each other. Each plot was centered on a group of three healthy dominant or co-dominant balsam fir trees in order to have at least three meters from each stem to the edges of the plot. For each plot, a throughfall exclusion shelter was established with a transparent 10m x 10m polyethylene film (Harnois Industries Inc., Saint-Thomas de Joliette, Canada) laid down over a tent-like structure made out of ropes and wooden posts with the apex aligned with the slope (Fig. 6.1). By gravity, the intercepted water was redirected outside the plots and perpendicularly to the slope. Openings were made in the polyethylene sheets to surround every tree stem, and the sheet was sealed around each stem with sheathing tape. The polyethylene sheets were maintained 1.3m to 2m aboveground to allow proper air flow beneath them. The built-up of heat was limited to 0.3°C on average, as measured with temperature data loggers (HOBO H8 Pro Series, Onset Computer Corp., USA). In 2010, the experiment was started in the first week of July and ended on August 5. In 2011, the experiment was also started in the first week of July, but ended one month later, on September 8.

In addition to the three trees located in the throughfall exclusion shelters, two balsam fir trees were used as a control (Fig. 6.1). They were also healthy dominant or codominant trees and were located within a 5m x 10m area contiguous to the upper side of each shelter. Control (N=8) and treated trees (N=12) had on average a diameter at breast height (DBH) of 21±3.5 cm and 22±5.7 cm, respectively, and a height of 16.1±2.2 m and 16.7±2.7 m, respectively. No significant differences in DBH or height were detected between treatments.

Weather data

Weather parameters were measured in a clearing located approximately 500 m from the plots. Temperature sensors (HMP35CF, Campbell Scientific Inc., USA) recorded air temperature at a height of 3.3 m and rainfall was measured by averaging readings from two different pluviometers: a tipping bucket rain gauge (TE-525, Texas Electronics, Dallas, USA) and a precipitation gauge (35-1558, Fisher and Porter, Albany, NY). Daily soil volumetric water content of the mineral soil (22 cm depth) was measured in a nearby stand using time-domain reflectometry (CS615, Campbell Scientific Inc., USA). Measurements were made every 15 min and hourly averages were recorded with a datalogger (CR-1000, Campbell Scientific Inc., USA).

Volumetric soil water content (SWC) in the plots was measured weekly during treatment in both years using a FieldScout TDR-300 device with 20-cm rods and standard calibration (accuracy: ±3% vol; Spectrum technologies Inc., Plainfield, USA). Rods were inserted vertically and measurements were made at 1-m intervals along five 15m transects parallel to the slope and 2m apart crossing both control and treatment areas (Fig. 6.1).

Sample collection

Using a Trephor tool (Rossi *et al.* 2006a), wood microcores (2.5 mm in diameter and 20mm long) were extracted weekly from each tree stem during the second treatment year (2011),

from April to October. No wood samples were taken during the first year of treatment (2010) because the experiment was setup in June of that year and the analysis of wood cores (see 'Model fitting' section below) required a sampling period covering the entire snow-free season. The sampling followed a spiralling upward transect centered at breast height and all samples were taken at least 10cm apart to minimize the formation of resin ducts (Forster *et al.* 2000). The samples were stored at 4°C in Eppendorf microtubes filled with a 10% ethanol solution. An additional series of microcores was collected upon termination of xylogenesis, in mid-October 2011, and used for anatomical measurements of the tracheids formed in the years 2008 to 2011.

Histological analyses

In the laboratory, the water from the microcores was removed through successive immersions in ethanol and HistosolTM and embedded in paraffin according to Rossi *et al.* (2006a). Transverse sections were cut with a rotary microtome (6-10 μ m in thickness), stained with cresyl violet acetate (0.16% in water) after removal of the paraffin and observed under visible and polarized light at a magnification of x400-500 to differentiate cells in the cambium and the differentiating xylem.

The number of cambial cells, enlarging cells, wall thickening cells and mature cells were counted along three radial files and averaged for every sample of each tree. Cambial cells were characterized by thin cell walls and less than half the radial diameter of enlarging cells. Both cambial and enlarging cells could be differentiated from other cell phases by their absence of secondary cell wall, a structure that shines under polarized light (Kutscha *et al.* 1975). Cells in the process of lignification and wall thickening showed violet and blue walls, the colour blue revealing the deposition of lignin on the cell walls (Thibeault-Martel *et al.* 2008). Mature tracheids were characterized by blue cell walls.

For anatomical measurements, parallel sections were stained with safranin (1%) following the standard preparation procedure described above, and numerically photographed with a camera fixed on an optical microscope at a magnification of x400. Cell

and lumen diameter (μ m) as well as cell wall thickness (μ m) were measured on three radial files per section using Wincell software (Regent Instruments Inc., QC, Canada).

Model fitting

The cumulative amount of cells produced was modelled with a modified Gompertz function (Rossi *et al.* 2003) for each tree:

$$Y = A \exp\left[-e^{\left(\kappa(\beta-t)\right)}\right]$$

where Y is the cumulative amount of cells produced, A is the annual amount of cells produced, κ is the rate of change, t is the time computed in days of year (DOY) and β is the time at inflection point, i.e. when maximal growth rate is reached. The cumulative amount of cells produced was calculated as the sum of enlarging, wall thickening and mature cells. Three phenological signals were extracted from the models: i) beginning of cell enlargement, determined as the moment when the daily cell production (Yt-Yt-1) reached 0.1 cell day⁻¹, ii) maximal growth rate and its corresponding date, determined as the highest value of daily cell production (Yt-Yt-1) and iii) end of wall thickening, determined as the moment when daily cell production $(Y_t - Y_{t-1})$ declined below 0.1 cell day⁻¹. In addition, the starting date for the wall thickening phase was obtained by applying the Gompertz function to the sum of wall thickening and mature cells (enlarging cells were excluded) and determining the moment when the daily cell production $(Y_t - Y_{t-1})$ reached 0.1 cell day⁻¹. All regression showed a normal distribution of the residuals (Motulsky & Ransnas 1987). The 'nlm' procedure from the R software was used with ordinary least square method for parameters estimation (R Development Core Team 2012). In addition to the abovementioned phenological signals, the duration of xylogenesis was calculated as the difference in days between the beginning of cell enlargement and the ending of wall thickening.

Separating the tracheids produced before and during treatment

With xylogenesis starting in May, the differentiation of many cells was already in progress or even completed prior to the initiation of the treatment in early July (Fig. 6.5). These cells were probably not affected in their development (i.e. diameter and wall thickness) contrary to differentiating cells produced after the start of the treatment. In order to better detect treatment effects on the differentiation of tracheids, it was thus decided to narrow the study to morphological impacts of the tracheids produced after the beginning of the treatment for the measured years. To do so, the individual Gompertz models previously fitted on the cumulative amount of cells produced after July 1st relative to the total number produced that year, on average 44±3%. Assuming similar growth patterns between years and based on the total number of tracheids produced in 2008-2010, we identified for each tree and each previous year the tracheids corresponding to the fraction produced after July 1st in 2011. The morphological parameters corresponding to each tracheid were then averaged per tree for each year (2008-2011).

Seedling growth and water potential

Due their shade tolerance, balsam fir stands generate important banks of seedlings in the understory (Morin 1994), and this study site was no exception. In each plot, three balsam fir seedlings located under the throughfall exclusion shelters (>2m from the edges), and two others located outside the treatment (>2m from the edges) were sampled in late August 2011. Based on the homogeneity of the canopy structure in this even-aged stand, we assumed a similar light regime between treatments. Sampled seedlings height ranged between 70 and 110 cm, and their age was visually estimated between 10 to 20 years. Because of their size, seedlings are not suited for weekly extractions of wood cores. In addition, the terminal growth of seedlings is perhaps more ecologically significant than radial growth in order to assess their capacity to survive and grow in the understory. For

each seedling, the 2011 terminal growth (leader) was measured to the nearest 5mm, and the 2011 lateral growth of three distinct upper branches was also measured to the nearest 5mm and averaged. To determine the water status of each seedling, the midday water potential of three distinct lateral branches was measured using a pressure chamber instrument (model 610, PMS Instrument Co., Albany USA) and their water potential was averaged.

Statistical Methods

To test our hypotheses of the effects of drought on tracheid characteristics, the two years of treatment were combined in a mixed-model linear analysis of covariance (ANCOVA) with repeated measures, assuming a compound symmetry variance-covariance structure between years. The pre-treatment value of each morphological parameter (average of 2008 and 2009), limited to tracheids produced since July 1st as for treatments years, was used as covariate when significant at P<0.05, considering throughfall exclusion treatment and year as fixed factors and subject and block as random factors. In order to verify the hypothesis that xylem production would not be affected in the first year but perhaps in the second year, each year was tested separately in an ANCOVA with previous growth (average of 2008 and 2009) as a covariate. A similar approach was used to detect a treatment effect on the phenology of xylem formation in 2011. Significant correlations with pre-treatment growth ring width (average of 2008 and 2009) were often observed (P<0.05). For such cases, pretreatment growth ring width was used as a covariate. Finally, 'treatment' was used in a mixed-model ANOVA as fixed factor, with random factors 'subject' and 'block', to detect an effect of the treatment on the seedling growth and water potential. Data were tested a priori to meet the assumptions of ANOVA (e.g. normality and homoscedasticity). Statistical analysis, modelling and plots were done using the R software (R Development Core Team 2012).

6.4 Results

Climate

From May to September, average temperatures were 12.6 and 12.2°C in 2010 and 2011, respectively (Fig. 6.2), more than one degree higher than the normal of 11.2°C (1971-2000). The precipitations recorded for the period of May to September 2010 (596 mm) were slightly lower than the average for 1975-2000 (654±47 mm), mostly because of small amounts of precipitation in August (38 mm compared to an average of 130±46 mm for 1975-2000). Precipitations were higher in 2011 totalling 747 mm for the period of May to September, with 226 mm falling in August (Fig. 6.2). The average volumetric soil water content (SWC) measured in a nearby stand for the period of May to September was also lower in 2010 (30±8.5%) than in 2011 (40±4.5%). SWC was highest following snowmelt in May (42±2.7% in 2010 and 46±1.3% in 2011) and decreased somewhat steadily in the following months with large between-years differences in August and September probably due to contrasting levels of precipitation (Fig. 6.2). SWC was 23±1.6% and 41±2.5% in August and September 2010, respectively, compared to 34±4.6% and 41±2.5% in August and September 2011, respectively.

Treatment effect on soil water content

The SWC measured inside the treated plots was significantly higher in 2011 than in 2010 (P<0.01). During both years, the throughfall exclusion shelter significantly reduced SWC relative to the control (P<0.01; Fig. 6.3). The average SWC in treated and control plots, respectively, was 7.7±0.2% and 13.5±0.3% in 2010, and 11.7±0.3% and 22.2±0.5% in 2011. The contrasting precipitation regime between years affected the efficiency of the treatment, with reductions in SWC of 5.8% and 10.5% being recorded under the throughfall exclusion shelter in 2010 and 2011, respectively (Fig. 6.3). Indeed, lower precipitations caused a reduction in throughfall exclusion in 2010, decreasing the treatment effect over

SWC that year. Probably due to the lateral movement of incoming rain as well as the water coming from the shelter itself, SWC was higher near the edges of the throughfall exclusion shelters (10.2±0.5%) than in the center (7.5±1.0%; Fig. 6.3).

Characteristics of the new tracheids

No differences in tracheid characteristics (wall thickness, cell and lumen diameter) were detected between the two years of treatment (*P*>0.34; Table 6.1). No initial differences were observed between treatments in 2008-2009 (*P*>0.55). All three characteristics were however significantly different between treatments (*P*≤0.02; Table 6.1). The diameter of tracheids was 16.1% smaller in treated trees with a mean cell diameter of 20.1±1.3 µm compared to 23.9±0.9 µm for the control (Fig. 6.4). A reduction in lumen size was also observed, with a diameter that was 30.8% smaller in treated trees (16.5±0.9 µm and 11.4±1.4 µm in control and treated trees, respectively). The cell walls of treated trees were 14.1% thicker than those of control trees (4.3±0.1 and 3.7±0.1 µm, respectively; Fig. 6.4).

Phenology of wood formation in 2011

In 2011, the cambium was in general composed of five cells prior to the onset of xylogenesis but reached eight to nine cells at the peak of its mitotic activity (Fig. 6.5). The first enlarging cells were observed on average on May 19 for control trees, but eight days later in trees under the throughfall exclusion shelters (May 27; *P*<0.01; Table 6.2). The number of enlarging cells during the growing season followed a bell-shaped curve, reaching seven cells at its peak. The first cells in the final stage of wall thickening were observed on May 31 in control trees and nine days later in treated trees (June 9; *P*<0.01; Table 6.2). The seasonal amount of cells in that phenophase also followed a bell-shaped curve, with an average maximum of ten cells (Fig. 6.5). At its peak, the daily growth rate was 0.61 ± 0.05 cells day⁻¹, with no significant differences between treatments (Table 6.2). The growing season averaged 99 days and ended on August 30 for all trees (Table 6.2). Xylogenesis was already

in progress when the treatment was initiated in early July, with eight cambial cells, five enlarging cells, seven wall thickening cells and approximately five mature tracheids already formed (Fig. 6.5). When the treatment ended in September, there were no more enlarging cells although the process of wall thickening was not completed.

Number of tracheids

In the two years preceding the experiment, an average of 32±2 tracheids were produced annually (Fig. 6.6), with no difference in annual xylem production being detected between treatments (*P*=0.64). An average of 33±3 tracheids were produced in the first year of treatment (2010), also without a significant difference between treatments (*P*=0.80). In 2011 however, annual xylem production in control trees increased to 39±3 tracheids while treated trees production was reduced by 26% (29±4 tracheids, *P*=0.03; Fig. 6.6). On average, 56±3% of the annual amount of tracheids was already differentiating or mature when the treatment was started in 2011.

Seedlings growth and water potential

The growth of the leader and lateral branches of treated seedlings were reduced by an average of 31 % (P=0.10) and 32 % (P=0.01), respectively, in 2011 (Fig. 6.7), and no seedling mortality was observed. Seedlings subjected to the throughfall exclusion treatment had a 40% reduction in midday water potential averaging -1.38±0.05 relative to -0.98±0.04 MPa in control seedlings (P<0.01; Fig. 6.7).

6.5 Discussion

Effects on tracheids characteristics

In agreement with our first two hypotheses, throughfall exclusion affected the morphology of the tracheids. The reduction in cell and lumen diameter and the increase in cell wall thickness show that the enlargement and wall thickening stages of xylogenesis were affected by the treatment. The formation of tracheids starts with cell divisions in the cambium producing cambial derivatives. Cell expansion depends exclusively on hydrostatic pressure: as water potential lowers, cell turgor decreases, limiting the expansion of new cells (Beth Kirkham *et al.* 1972; Deri Tomos *et al.* 1989). Experimental water limitation has been previously observed to reduce tracheid diameter in balsam fir seedlings (Rossi *et al.* 2009) as well as in mature trees of *Pinus sylvestris* L. (Sterck *et al.* 2008; Gruber *et al.* 2010) and *Cryptomeria japonica* (L.f.) D. Don (Abe & Nakai 1999; Abe *et al.* 2003). This phenomenon could be considered as an adaptation to ensure sufficient water transport while minimizing the negative pressure in order to avoid xylem injuries due to cavitation (Sperry *et al.* 2006).

Consistent with the reduction in tracheid diameter, an increase in cell wall thickness with reduced water availability has also been documented (Eilmann *et al.* 2009; Jyske *et al.* 2010). A higher negative pressure in the xylem enhances the risk of implosion, and the vessel walls thicken to prevent the cells from collapsing (Hacke & Sperry 2001). Comparing 47 woody species, Hacke *et al.* (2001) determined that the thickness of the xylem cell wall is proportional to the negative pressure it has to withstand. In our study, we observed a 40% decrease in xylem water potential in seedlings growing in the throughfall exclusion plots. Although the water status of mature trees was not measured *per se* in this experiment, the changes observed in the tracheids morphology suggest that the prolonged throughfall exclusion also decreased the trees water potential. Balsam fir could be relatively sensitive to extended drought periods as it is a shallow rooted species (Schultz 1969 cited by Hix *et al.* 1987).
Effects on the phenology of wood formation

The hypothesis stating that xylogenesis would end prematurely in treated plots was not verified. In fact, the 26% reduction in the number of tracheids produced in 2011 by treated trees was not associated with an earlier growth ending. This hypothesis, based on the comparison of trees growing under naturally contrasting water regimes (see (Gruber et al. 2010), has not been supported yet by data from experimental drought studies (Arend & Fromm 2007; Rossi et al. 2009; Belien et al. 2012). Our result is consistent with previous findings that the timing of growth cessation is a highly variable parameter poorly correlated with climate variables (Rossi et al. 2006c; Duchesne et al. 2012). While the hypothesis of a premature ending of xylogenesis was rejected, the phenology was nonetheless affected by the treatment. We observed a delay of more than a week in the beginning of cell differentiation in the second year (2011) of treatment. Cell enlargement and wall thickening phases started in late May and early June, respectively, at least one month before the treatment was started in July. Therefore, the observed delay in cell differentiation was caused by a reaction to the preceding-year treatment. While drought-induced mortality in trees can be observed many years following a given event (Pedersen 1998; Bigler et al. 2007; Hartmann 2011), only a few examples of non-lethal lagged effects have been documented. Following the exceptional European drought in the summer of 2003, a pronounced growth reduction was observed in the following year (Granier *et al.* 2007). The same observation was made in a mature boreal aspen stand of Canada following a 3-year long drought (2001-2003) (Krishnan et al. 2006). However, to our knowledge, our study is the first to report a delayed onset of cell differentiation caused by a drought stress in the preceding year. During the xylogenesis process, the enlargement of a cambial derivative is prior to its wall thickening. Thus, as both phases were delayed by the drought, the delay observed for wall thickening can be accounted for by the delay in cell enlargement. In turn, the start of cell enlargement relies on the start of mitosis in the cambium and the production of cambial derivatives. It is therefore possible that the delayed cell enlargement

was due to delayed mitosis in the cambium. The reduced number of tracheids produced that year proves that the cambium activity was affected, although we cannot determine if this effect was specific to the start of cambium mitosis. Both cambium mitosis and cell enlargement rely heavily on available water (Beth Kirkham *et al.* 1972; Hacke & Sperry 2001), and cambium reactivation was preceded by four to six weeks of stem rehydration in black spruce (Turcotte *et al.* 2009). Droughts can cause hydraulic damages in trees that can affect various water-dependent processes for several years following the end of the event (Anderegg *et al.* 2013). In the current study, a persistent deterioration of the trees hydraulic capacities could have slowed down the spring rehydration process of the cambium. The cambium water potential serves as a metabolic regulator of mitotic activity and has the capacity to influence later stages of differentiation (Abe & Nakai 1999; Arend & Fromm 2007). Perhaps a delay in reaching sufficient water potential in the cambium could have provoked a later initiation of cambium mitosis and cell differentiation.

Effects on the production of tracheids

As we hypothesized, the effect of drought on xylem production was more pronounced in the second year of treatment. Indeed, the treatment had no effect on the number of tracheids produced in 2010, while a 26% decline was observed in 2011. The lack of effects on xylem production in the first year of treatment was expected given that a majority of the new tracheids forming the growth ring (56±3%) were already in the differentiation process and/or completed when the treatment was started. Delayed effects from the first year drought cannot be separated from current aggravating effects due to the second-year drought, and several mechanisms related to carbon and water availability can explain this result. Among them, impaired hydraulic conductivity could have reduced the rates of waterdependent processes of wood formation such as cambium mitosis and tracheid differentiation, as cited earlier to explain the delayed onset of xylogenesis. Other waterdependent processes occurring during the growing season could have been impacted, such as photosynthesis or carbon distribution within the tree, increasing belowground carbon

allocation for root growth (Krishnan *et al.* 2006), reducing the amounts of carbon allocated to xylogenesis (e.g. Hartmann 2011). For instance, the reduced growth of oak saplings (*Quercus pubescens* Willd.) subjected to two consecutive years of summer drought (22-38% reduction in ring width) was attributed to impaired photosynthesis but also to enhanced protection and repair processes which consumed significant amounts of carbon (Galle *et al.* 2010). The comparison of balsam fir growth vulnerability to drought relative to other species is difficult to establish due to the lack of published data. Mature black and Norway spruce trees growing in Canada and Denmark, respectively, displayed no reduction in radial growth in response to a single experimental drought (Beier *et al.* 1995; Belien *et al.* 2012). However, mature Norway spruce trees subjected to spring droughts of 60 to 75 days for 4-5 consecutive years in southern Finland reduced their annual ring width by 11-21% every year (Jyske *et al.* 2010). The rooting plasticity as well as the water reserves of a given species is often cited, but the vulnerability of the water transport system to drought-induced embolism could also play a critical role in the drought tolerance of forest species (Choat *et al.* 2012).

Effects on seedlings growth

A 32% reduction in the terminal growth of balsam fir seedlings growing under the exclusion shelter as compared to control seedlings was observed. In this experiment, seedlings and trees were not subjected to an identical treatment, as the seedlings canopy was not in contact with rainwater while trees could benefit from the foliar absorption of a fraction of the intercepted rainfall (Breshears *et al.* 2008). In a greenhouse study with 7-year-old balsam fir seedlings subjected to a 20-day drought in June-July, (Rossi *et al.* 2009) observed a significant reduction in shoot growth during the treatment and in the following weeks that was compensated for before the end of the growing season after the cessation of the treatment. The shorter drought duration in that study could explain the different results. The water potentials observed in the current study were similar to those of two-year-old Scots pine and Sitka spruce trees (approx. -1.5 MPa) after 30 days of water stress under

controlled conditions (Jackson *et al.* 1995). Initial differences in available light between treatments could have affected the growth of seedlings, but the even-aged structure of the stand supposes a homogenous understory light regime. In addition, the likelihood of contrasting light environments between treatments was reduced by the random selection of seedlings as well as the proximity of control and treated seedlings within each plot.

6.6 Conclusions

In order to study the impact of droughts on balsam fir growth, an in situ experiment consisting in throughfall exclusion during summer was conducted for two consecutive years in a typical balsam fir boreal forest of Québec, Canada. The treatment reduced the soil water content and had significant impacts on several aspects of xylogenesis. The new tracheids formed were smaller, with thicker cell walls, suggesting a significant response to decreasing tree water potential. The treatment also delayed by more than a week the start of the differentiation process of cambial derivatives in reaction to the preceding-year treatment. Although previous observations have shown that drought may have delayed effect on tree growth, this is the first experimental observation of a drought stress affecting the phenology of xylogenesis. The drought also reduced by 26% the number of tracheids produced in the second year of treatment. Balsam fir seedlings were also affected, with a 32% reduction in terminal growth. Our results suggest that a future regime of increased frequency and intensity of droughts will have negative effects on the duration of xylogenesis, the production of xylem cells and the regeneration of balsam fir. Balsam fir could be particularly sensitive to droughts as compared to black spruce trees since a similar protocol applied to this species did not affect its xylem production or phenology (Belien et al. 2012). The delayed effect of the throughfall exclusion treatment observed here underlines the importance of conducting longer-term studies on the effect of drought on tree growth.

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Table 6.1. Probability values from mixed-model ANOVAs applied to the tracheid morphology data from the treated (12 trees) and control (8 trees) plots, before and during the treatment. Pre-treatment tracheid characteristics (*covariate*: average of 2008-2009) were used as covariate when significant at *P*<0.05. Significant p-values are in bold (*P*<0.05).

		Tracheid	Lumen diameter	Wall thickness
		diameter		
Before treatment	Treatment (T)	0.97	0.86	0.55
During treatment	covariate	0.04	<0.01	0.58
	т	0.02	<0.01	<0.01
	Year (Y)	0.34	0.35	0.19
	Τ*Υ	0.99	0.80	0.34

Table 6.2. Average values for phenology and productivity parameters (and standard error) of balsam fir in control and treated plots in 2011 (N=20 trees). Probability values from mixed-model ANOVAs with pre-treatment ring width (covariate: average of 2008-2009) as covariate when significant at P<0.05. Significant p-values are in bold (P<0.05).

	2008-2009 ring width (µm)	Onset of enlargement	Onset of wall thickening	End of wall thickening	Duration ^a (days)	Maximum growth rate ^b (cells day ^{_1})	Timing of maximum growth	
Control	1242 (91)	May 19	May 31	Sept. 5 (7	109 (8)	0.61 (0.05)	June 26	
		(2 days)	(2 days)	days)			(2 days)	
Treated	1033 (159)	May 27	June 9	Aug. 24	89 (9)	0.60 (0.06)	June 27	
		(2 days)	(2 days)	(8 days)			(2 days)	
Statistical analysis								
Covariate	-	<0.01	<0.01	0.07	0.03	0.08	0.97	
Treatment	0.42	<0.01	<0.01	0.46	0.24	0.28	0.64	

^a Duration was calculated as the difference in days between the onset of enlargement and the ending of wall thickening.

^b Maximum growth rate was calculated as the maximum daily growth.



Figure 6.1. Schematic description of the throughfall exclusion shelter, including the location of the three treated balsam fir trees as well as the two control balsam fir trees. The dashed lines identify the transects used to monitor soil water content.



Figure 6.2. Daily air temperature (black curve), soil water content (grey curve) and rainfall (vertical bars) from May to October 2010 and 2011 measured 500m from the experimental site. The periods of throughfall exclusion are in the shaded areas.



Figure 6.3. Average soil water content in control and throughfall exclusion plots during treatment in 2010 and 2011.



Figure 6.4. Anatomical characteristics of the tracheids produced after July 1st before treatment (Pre-Tx; average of 2008 and 2009) and during treatment in 2010 and 2011. Control trees are in grey, treated trees in black and vertical bars are the standard error.



Figure 6.5. Average number of cambial cells, enlarging cells, wall thickening cells and differentiated tracheids in control and treated balsam fir in 2011. Control trees are in grey and treated trees in black, and the period of throughfall exclusion is in the shaded area.



Figure 6.6. Annual number of tracheids produced before (Pre-Tx; average of years 2008 and 2009) and during throughfall exclusion years. Control trees are in grey, treated trees in back, and vertical bars are the standard error. P-values refer to the effect of the treatment as measured with mixed-model ANCOVAs.



Figure 6.7. Average growth and water potential in control and treated balsam fir seedlings. P-values refer to the effect of the treatment as measured with mixed-model ANOVAs.

Chapter 7. Summary and Conclusions

7.1 Summary

The balsam fir boreal ecosystem plays an important role at both ecological and economical levels. It is therefore crucial to predict accurately its response to global changes. Although climate manipulations of mature stands are an effective way of studying forest adaptation to global change, few have been established in the Canadian boreal ecosystem. Two climate change experiments were set up in a balsam fir forest of Québec, Canada. One combined two treatments: i) increased soil temperature (+4°C) and earlier spring snowmelt and ii) increased nitrogen (N) deposition, using buried heat-resistance cables and water nozzles spraying N-added rain above the trees canopy. In the other experiment, a summer drought was simulated for two consecutive years by excluding throughfall with transparent plastic sheets.

The global objective of chapters 2 to 5 was to study the effects of three years of experimentally increased soil temperature and N deposition on a balsam fir boreal stand. In chapter 2, ion exchange membranes were used to determine changes in the availability of soil nutrients for tree growth. I hypothesized that i) both treatments would increase soil N availability and ii) increased soil temperature would increase base cations availability. I report no effects of the treatments on soil inorganic N, although three years of soil warming increased the soils fluxes of potassium (K), magnesium (Mg), sulfate and aluminum (Al). The higher K and Mg availability observed in warmer soils could have positive effects on ecosystem productivity although this gain could be offset by the concomitant increase in available Al.

In chapter 3, soil respiration – after three years of warming – was monitored in order to verify if previously heated soils would display reduced mineralizable carbon due to the maintenance of higher respiration rates. To do so, experimental plots were sampled at the end of the third year and soil samples were incubated under standard laboratory conditions at 5, 10, 15 and 20 °C for 72 days. We found 14.8 and 10.5% less mineralizable

carbon in heated organic and mineral soils, respectively. Such result could help us predict the stand long-term effect of climate change on the ecosystem C cycle.

In chapter 4, the focus was put on N, by linking soil N availability with balsam fir foliar N content and radial growth. I hypothesized that both treatments would increase soil N availability as measured with ion-exchange membranes and soil extractions, needle N content and radial growth as measured with dendrometers. After three years of treatments, there were little changes to the soil N content. No effects of the treatment were observed on needle N or diameter growth, except for an earlier peak in diameter growth observed in heated plots in 2010. The high competition for soil N at the site is suspected to be responsible for this limited response.

In chapter 5, wood microcores were extracted weekly from every tree stem and analyzed under a microscope to study the effects of the treatments on seasonal wood formation. I expected both treatments to increase the production of tracheids as well as their diameter and extend the duration of xylogenesis. In addition, I hypothesized that an earlier snowmelt would not affect the onset of xylogenesis. Results were consistent with those from chapter 4, as both treatments had no significant effect on the production of tracheids as well as their diameter and the time dynamics of wood production. Finally, as predicted, the earlier snowmelt induced by soil warming did not hasten resumption of xylogenesis.

Climate models predict more frequent and intense droughts in the coming decades. The objective of chapter 6 was to study the effect of two consecutive years of summer drought on the xylogenesis of balsam fir. I hypothesized smaller and thicker tracheids, a precocious ending of xylogenesis and a reduced production of tracheids in response to drought. In addition, I expected the growth reduction of seedlings to be more severe than for mature trees. Analyzing wood microcores as described in chapter 5, I found smaller and thicker tracheids in drought-subjected trees. The ending of growth was not affected, but the treatment delayed by more than a week the start of the tracheid differentiation process in the second year, concomitant with a decrease in the number of tracheids produced. The seedlings displayed a 32% reduction in growth. Our results suggest that a future regime of

increased frequency and intensity of droughts could have negative effects on the duration of xylogenesis and the production of xylem cells in balsam fir.

7.2 Conclusions

Relative to the short duration of the experiments presented in this thesis, the response of boreal ecosystems to global changes will develop over several decades, and various climate feedbacks may change the ecosystem trajectory at different points in time. For instance, the eventual depletion of more labile carbon in warmer soils, discussed in Chapter 3, will probably affect the sensitivity of the soil carbon cycle to increased temperatures. Therefore, caution should be exercised when extrapolating the results from this thesis over longer time scales.

In light of my results, the balsam fir ecosystem should not be rapidly affected by the projected increase in atmospheric N deposition. Canopy interactions were limited, and three years of increased deposition did not affect the trees N nutrition or the soil N availability. As for the effect of warmer soil temperature, nutrient cycling should be affected, with notable effects on base cations as well as sulfates and aluminum. However, the increased carbon mineralization measured in heated plots had only marginal effects on available N, balsam fir N nutrition and growth. Strong competitive N sinks in the soil could be responsible for this poor sensitivity of the balsam fir ecosystem to both increased soil temperature and N deposition. Nonetheless, the chronic addition of N to the system and chronically warmer soil temperatures should eventually reduce the competition for N and benefit balsam fir growth. However, this benefit could be offset by the strong vulnerability of balsam fir to droughts as water levels are projected to decrease in the future. Thus, a higher frequency and/or intensity of droughts could drive a global reduction in productivity. However, many more environmental factors are expected to change in the future which will certainly alter the ecosystem patterns and processes. For instance, the effects on increased air temperature, atmospheric CO₂ and near-surface O₃ were not addressed in this thesis although we can assume they will affect major processes like photosynthesis, plant carbon

allocation and available water.

7.3 Future research

Exploratory studies commonly end up with more questions than answers, and this thesis is no exception. In general, the experiments described here should be extended over several more years in order to validate if the short-term trends observed so far persist in time. In addition, these experiments were limited to a single site, making our results hard to generalize to the whole balsam fir ecosystem. Therefore, the response of one or more balsam fir stands to similar treatments would provide an estimate of the variability of the response across the ecosystem. Aside from these broad issues, results from my thesis have exposed several unsolved questions:

- Because of the small size of the exchangeable cation pool found in most boreal forests in Eastern Canada, the higher flux of base cations found in warmer soils should be studied further in order to estimate the proportion that is leached out of the system.
- We are lacking Al toxicity thresholds for balsam fir to understand the potential threat that warmer soil temperatures pose on balsam fir health.
- Our understanding of the processes by which increased soil temperatures drive nutrient cycling would be greatly improved by gathering data on the composition, biomass and mineralization dynamics of microbial fungi in warmer soils. Such knowledge could help us understand the process of N immobilization and eventually identify response thresholds.
- Nitrogen tracer studies would help improve our understanding of the faith of atmospheric N deposition when deposited on the canopy of trees.
- The response of seedlings to global changes could reveal to be capital for the successional dynamic of balsam fir forests. Especially, the response to low available water could become an increasingly important adaptive trait that should be studied

further by comparing responses of coexisting species and eventually identify threshold values where water becomes limiting.

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Appendix A. Initial tree data for the soil warming experiment

I measured the initial diameter at breast height (DBH), height and live crown ratio of the experimental trees described in chapters 4 and 5 before the start of the treatments. Here I present the average values per treatment, and the standard deviation of the mean.

	DBH (mm)	Height (m)	Live Crown ratio ^a (%)
Control	180 ± 51	14.9 ± 4.4	47.9 ± 8.4
Fertilized	202 ± 8	17.6 ± 4.5	51.6 ± 4.5
Heated	173 ± 17	14.9 ± 4.2	50.9 ± 10.9
Heated-fertilized	187 ± 3	15.9 ± 4.7	43.2 ± 13.2

^a Calculated as crown height divided by total tree height

Appendix B. Soil extractions

In addition to KCI-extractable NO₃ and NH4 values presented in the fourth chapter of my thesis in regard with the effects of increased soil temperature and N deposition, several more elements were extracted from the soil samples and measured. Here I provide for each year, soil horizon and treatment (C: control, N: fertilized, H: heated, HN: heated-fertilized) the average values for soil C, K, Ca, Mg, Mn, S, Na, Al and Fe. The detailed methodology is described in Chapter 4.

	C ^a g∙kg⁻¹	pH⁵	K mg∙kg⁻¹	Ca mg∙kg ⁻¹	Mg mg∙kg⁻¹	Mn mg∙kg⁻¹	Al mg∙kg⁻¹	Fe mg∙kg ⁻¹	Na mg∙kg ⁻¹	S mg∙kg⁻¹	
2009 Forest floor											
С	425	3.1	475	1883	201	190	466	162	20	122	
Ν	434	3.0	462	1883	197	195	357	108	18	130	
Н	433	3.1	502	1703	198	151	614	184	20	117	
HN	407	3.0	469	1715	201	159	377	142	19	105	
2009 Mineral horizon											
С	36	4.0	17	34	6	2	316	101	4	16	
Ν	41	4.0	22	31	7	1	397	113	4	17	
Н	39	4.2	16	27	5	1	272	66	3	14	
HN	40	4.1	18	31	6	1	315	75	3	13	
2010 Fo	rest floo	r									
С	447	3.1	481	1907	177	177	606	340	31	144	
Ν	429	3.1	390	1413	157	131	668	372	29	158	
н	413	3.2	423	1527	150	128	602	464	36	137	
HN	411	3.1	416	2303	211	184	291	310	32	111	
2010 M	ineral ho	rizon									
С	44	4.0	27	37	7	2	374	175	5	18	
Ν	33	4.2	16	20	4	1	291	103	4	17	
Н	39	4.1	24	30	6	2	360	155	4	21	
HN	32	4.1	17	26	5	1	309	111	5	17	
2011 Fo	orest floo	r									
С	400	2.8	381	1490	146	79	598	132	45	106	
Ν	403	2.8	312	1271	124	60	798	162	34	91	
Н	424	3.1	318	987	114	55	1275	215	43	91	
HN	369	3.0	310	1588	171	70	814	174	35	93	
2011 M	ineral ho	rizon									
С	48	3.9	25	40	9	1	463	145	6	15	
Ν	44	4.0	23	41	8	1	481	124	7	16	
Н	26	4.1	18	31	5	1	291	71	5	9	
HN	42	4.0	25	50	9	1	419	111	6	11	

^a Total carbon (C) concentrations were measured with an automated dry combustion instrument (LECO CR-412, LECO Corp., St.Joseph, Michigan, USA).

^b All remaining elements were extracted with NH₄Cl 1M. Hydrogen, K, Ca, Mg, Na, S, Mn, Al and Fe concentrations were measured by inductively-coupled plasma-atomic emission spectrometry (ICP Jarrell Ash, Waltham, USA)