



# Canopy Nitrogen Addition and Soil Warming Affect Conifer Seedlings' Phenology but Have Limited Impact on Growth and Soil N Mineralization in Boreal Forests of Eastern Canada

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The increase in soil organic matter mineralization rate in boreal forests that may result from global warming is a major concern as it could release large amounts of C to the atmosphere. On the other hand, this may also release N to the soil and stimulate tree growth, which could partly offset the C losses from the soil. The long-term interaction between increased N deposition and soil warming on soil organic N mineralization and tree growth is still uncertain in the boreal zone. In this study, soil temperature was increased by +2–4°C from April to July with heating cables for a period of 9 years and N was applied above the canopy of mature stands from June to September for 7–9 years in two eastern Canada boreal forests [Bernatchez (BER) and Simoncouche (SIM)]. We assessed the effects of these treatments on organic N mineralization rate and on the growth, phenology and foliar N of *Abies balsamea* (L.) Mill. (balsam fir; BF) and *Picea mariana* (Mill.) BSP (black spruce; BS) seedlings. The soil warming (SW) treatment had no significant effect on N mineralization rates, whereas canopy N addition (CNA) decreased gross N mineralization rate by 23% and forest floor's alkyl/O-alkyl C ratio by ~15% relative to unfertilized plots. Foliar  $\delta^{15}\text{N}$  in the control plots was markedly lower in BS than in BF and at BER than at SIM (−4.8 and −2.9‰ in BS at BER and SIM, respectively; 1.6 and 3.8‰ in BF at BER and SIM, respectively) likely due to a higher contribution of  $^{15}\text{N}$ -depleted N derived from mycorrhizal fungi in BS and at BER, the colder and the more N-depleted site. The treatments had non-significant effects on seedling growth and foliar chemistry but SW caused a premature bud outbreak and faster bud development for both species at both sites. Overall, our results show that

increased soil temperature and N deposition in boreal forests may not impact soil fertility and vegetation growth as much as previously thought, but climate warming, by initiating earlier and faster bud development, may however expose seedlings to late spring frosts in the future.

**Keywords: soil warming experiment, nitrogen addition experiment, boreal forest, nitrogen mineralization, conifer regeneration, foliar  $\delta^{15}\text{N}$ , NMR**

## INTRODUCTION

Nitrogen mineralization is crucial in determining soil fertility and forest productivity because plant N nutrition largely relies upon  $\text{NH}_4^+$ , especially in cold ecosystems such as taiga and boreal forests (Schimel and Chapin, 1996; Aerts and Chapin, 2000; McFarland et al., 2002; Persson et al., 2003; Schimel and Bennett, 2004; Shenoy et al., 2013), although several plants of these habitats can absorb small organic-N compounds such as amino acids (Persson and Näsholm, 2001; McFarland et al., 2002; Persson et al., 2003). Since the mineralization of organic N is an enzymatic process and that N can be a limiting factor for soil microbial activity, it is thought that increased N deposition and soil temperature will affect soil OM decomposition and N mineralization (D'Orangeville et al., 2014). A recent meta-analysis compiling 528 observations from 51 articles reports that, on average, experimental warming treatments increased N mineralization rate by 52% although the direction and the magnitude of the effect strongly vary among ecosystems and warming methods and duration (Bai et al., 2013). Similarly, several studies have shown that experimental N addition impacts soil microorganisms, enzymatic activity, and reduces litter or soil OM decomposition (Allison et al., 2008; Frey et al., 2014; Pisani et al., 2015), but the direction and the magnitude of the effect depend on the level of experimental N inputs relative to natural rates of N deposition (Knorr et al., 2005). It is expected that increased soil temperature and changes in N cycling will particularly impact boreal forests because these habitats are characterized by low temperatures, low N availability and high C:N ratio litter (Marty et al., 2017). These changes may increase N availability with subsequent effects on forest regeneration and growth (Hobbie et al., 2002; D'Orangeville et al., 2013a; Blaško et al., 2015). It is important to anticipate the impact of these perturbations on boreal forests because these ecosystems are a major C store at the global scale and provide many services to human societies (e.g., timber and food production, wildlife refuge) (Dixon et al., 1994; Batjes, 1996; Chapin et al., 2004; Lal, 2005). Significant shifts in tree species performances (e.g., survival, regeneration, photosynthetic and growth rates) under a warmer climate may, for instance, impact forest productivity and require adapting silvicultural and management practices (e.g., assisted migration) (Benomar et al., 2018; Otis Prud'Homme et al., 2018).

In Quebec, Canada, the mean annual air temperature (MAAT) has increased by 1–3°C between 1950 and 2011 and is expected to rise by another 2.5–10°C in its northern ecosystems by 2080, depending on the representative concentration pathway (RCP) scenarios (Ouranos, 2015). These increases in air temperature

will likely increase soil temperature by up to 2°C by the middle of the century and result in earlier snowmelt (Houle et al., 2012). Large decreasing trends in nitrate deposition have been observed throughout North America (Ackerman et al., 2018; Zhang et al., 2018) and Europe (Vuorenmaa et al., 2018) in the last decades as a response of emission regulations, particularly in remote areas such as boreal forests (Gilliam et al., 2019). The deposition of ammonium has however remained stable and now represents about 50% of total N atmospheric inputs in eastern Canadian boreal forests (Houle et al., 2015). Although the long-term effects of soil warming and N addition on soil processes and OM composition have been studied (Pisani et al., 2015; Melillo et al., 2017; Marty et al., 2019a), how these treatments interactively and simultaneously impact both soil N mineralization and tree regeneration is poorly documented for boreal forests. In addition, whether these treatments affect soil processes differently among sites with contrasting temperature and soil OM composition, and how different conifer species respond to these potential changes is still unclear in these habitats.

In this study, two boreal forests of eastern Canada with contrasting mean annual temperature (MAT) and soil characteristics, were subjected to soil warming (SW) for a period of 9 years and canopy N addition (CNA) for 7–9 years in order to assess the effects of projected environmental changes on N mineralization and on the growth and phenology of *Abies balsamea* (L.) Mill. (balsam fir; BF) and *Picea mariana* (Mill.) (black spruce; BS) seedlings, two commercially important species in boreal Canada that have contrasting growth dynamics and resource use strategies with respect to environmental conditions (Chen et al., 2019). Large areas of the eastern Canadian boreal forests are managed for timber and wood fibers, which plays a significant economic role both locally and nationally (Natural Resources Canada, 2019). It is therefore insightful to study how planted seedlings react to changes in soil temperature and N addition in order to adapt silvicultural practices and species or seed sources selection. Both the applied SW treatment (+ 2–4°C during the growing season) and the CNA rate (+0.30–0.35 kg N ha<sup>-1</sup> yr<sup>-1</sup>) were in agreement with regional projections for 2050 (Houle et al., 2012). Seedling growth, phenology, foliar chemistry and  $\delta^{15}\text{N}$  as well as N mineralization rates were measured for each experimental treatment over the eighth and ninth years of the experiment. The goal of this study was to investigate the impact of increased soil temperature and N deposition on N cycling and on seedling growth, phenology and N sources in the two species. We hypothesized that (i) the SW treatment would increase net and gross N mineralization rates; (ii) the CNA treatment would decrease both N mineralization rates and the relative degree of degradation of forest floor OM due to a

decrease in soil microorganisms mining activity; and (iii) both soil warming and CNA would stimulate seedling growth and trigger bud break earlier at both sites.

## MATERIALS AND METHODS

### Study Sites

The study was carried out in two boreal forest sites located within the balsam fir–paper birch (*Betula papyrifera* Marsh.) bioclimatic domain of Québec, Canada. The first site (abbreviated SIM) is located within the Simoncouche research station (48° 13'N, 71° 15'W; 350 m asl.) in the Laurentides Wildlife Reserve, Québec, Canada. The second site (abbreviated BER) is located at higher altitude in the Monts-Valin near Lake Bernatchez (48°51'N, 70° 20'W; 611 m asl.). At both sites, the forest is mostly composed of even-aged mature black spruce. Both sites were affected by major wildfires, in 1922 for SIM (De Barba et al., 2016) and around 1865–1870 for BER. The two sites are characterized by thick undifferentiated glacial tills as parental material and slopes ranging between 8 and 17% (Lupi et al., 2012). The soils are Podzols with a mor-humus. The thickness of the organic soil (LFH) averages 10 and 18 cm at SIM and BER, respectively (Rossi et al., 2013). In the LFH, total N concentration and C:N ratio respectively averaged 8.5 g kg<sup>-1</sup> and 50.2 at BER, and 9.7 g kg<sup>-1</sup> and 39.1 at SIM. Similar low pH values of 2.5–3.6 were observed at both sites (Lupi et al., 2012).

The climate at both sites is continental, with long cold winters and short cool summers. Mean annual air temperature averages 0.2 and 1.9°C at BER and SIM, respectively (Rossi et al., 2011a), whereas the mean air temperature during the growing season (from May to September) is 11.4°C at BER and 13.3°C at SIM (Rossi et al., 2011a). Snow cover lasts from November to May and complete snowmelt occurs 20 days earlier at SIM [day of year (DOY): 120] than at BER (DOY: 140) (De Barba et al., 2016). Maximum snow cover reaches 132 and 108 cm at BER and SIM, respectively (Rossi et al., 2011b). The growing season at BER is ~15 days shorter than at SIM (Boulouf Lugo et al., 2012).

### Experimental Design

The field experiment combined soil warming (SW) and canopy N addition (CNA) and was conducted for a period of 9 years between 2008 and 2016. Twelve 7.5 m × 7.5 m square plots were delimited within a square area of 60 m × 60 m and distributed across three blocks at each site. Four treatments were randomly assigned to these plots in a split-plot design: warming with N addition (WN), warming with no N addition (W), no warming with N addition (N), and no warming and no N addition (C).

Sprinklers were installed in each experimental plot types (N, W, WN, and C) above the canopy of one selected tree located within each plot in order to simulate N deposition. Once a week, during the frost-safe period (June to September), the equivalent of 2 mm of rainfall was applied over a circular area of 3 m radius centered on the stem of each selected tree. We used a <sup>15</sup>N-enriched ammonium-nitrate solution (NO<sub>3</sub>NH<sub>4</sub>; 10% <sup>15</sup>N) to irrigate the trees. Nitrogen addition-free plots (W and C) received a solution reproducing the chemical composition of

natural rainfall (14.93 μmol L<sup>-1</sup> of both NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>), while N-enriched plots (N and WN) received a solution with 44.78 μmol L<sup>-1</sup> of both NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> in order to simulate a threefold increase in inorganic N concentration. This is equivalent to a ~50% increase in N deposition in forests that naturally receive 0.7–1 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Rossi et al., 2013). Artificial rainfall was applied from 2008 to 2016 at SIM and from 2008 to 2014 at BER.

The W and WN plots were heated using heating cables at an approximate depth of 15 cm beneath the forest floor (FF) surface. The heating system aimed to simulate a 2–4°C increase in soil temperature. The cables were installed in the fall of 2007, in circles (following a spiral pattern) around the same selected tree's stem collar, leaving 30 cm between cables' coils. To account for potential soil disturbance and root damage during cable laying, non-heating cables were installed in the same way in non-heated plots (C and N). Soil warming was conducted from April to July between 2008 and 2016, simulating an early snowmelt and soil thawing. Soil warming started 2 weeks later at BER (higher altitude) than at SIM to reflect the natural difference in temperature between the two sites (Lupi et al., 2012). This treatment resulted in an increased FF temperature by up to 4°C in summer months in warmed plots (W and WN) relative to C plots at SIM (Marty et al., 2019a).

At the beginning of the summer 2015, i.e., after 7 years of treatments, each experimental plot was split in two subplots. Five three-year-old black spruce (BS; *Picea mariana*) seedlings were planted in monoculture in one of these subplots and five three-year-old balsam firs (BF; *Abies balsamea*) seedlings in the other (**Supplementary Material 1**). Seedlings were planted with their 110 cm<sup>3</sup> soil carrot from the tree nursery, where they were grown from local seed sources. The phenology and the growth of the seedlings were monitored during the second year only (the first year was an acclimatization period) before we harvested them at the end of September 2016, i.e., 2 year after they were planted.

### Forest Floor Sampling

After 9 years of treatment (October 2016), we collected four cores (depth of 5–10 cm and diameter of 8 cm) from the F horizon (Fibric) of the organic layer (beneath the litter layer) in each of the four experimental plots (C, W, N, and WN) where the seedlings were planted (two soil cores in the portion with BS seedlings and two soil cores in the portion with the BF seedlings) with a hammer drill. We then mixed and homogenized the samples (4 treatments × 3 blocks × 2 sites = 24 samples) in polyethylene bags that were taken to the lab and kept at 4°C in the dark for 5 months until preparation for analyses. Forest floor samples were then dried at 55°C until reaching a constant weight, ground to fine powder and sent to the lab for analyses.

### Forest Floor Chemical Analyses

Subsamples of the air-dry forest floor were analyzed for remaining humidity, organic matter (OM) by loss on ignition, pH (soil:water 1:2.5 ratio wt/vol), and total C and N determination by dry combustion (LECO CR-412, LECO Corporation, St-Joseph, MI, United States). Total P, K, Ca, Mg, Mn, Cu, Zn, Al, and



Fe concentrations were analyzed by digestion in concentrated  $\text{H}_2\text{SO}_4$  (Parkinson and Allen, 1975) and total S by digestion in hot concentrated  $\text{HNO}_3$ ; measurements were made by inductively coupled plasma emission spectrophotometry (ICP-AES).

## Solid-State $^{13}\text{C}$ Nuclear Magnetic Resonance (NMR) Analysis

Solid-state  $^{13}\text{C}$  NMR analyses were performed as described in Marty et al. (2019a). We packed  $\sim 250$  mg of dried and ground soil subsamples into 4 mm zirconium rotors and sealed with a Kel-F cap. Solid-state  $^{13}\text{C}$  cross polarization magic angle spinning (CP-MAS) spectra were measured using a 500 MHz Bruker BioSpin Avance III spectrometer having a 4 mm H-X MAS probe. A MAS rate of 11 kHz was used with a 1 ms ramp-CP contact time and a 1 s recycle delay (Conte et al., 2004). NMR spectra were processed using a zero-filling factor of 2 and line broadening of 50 Hz. Spectra were baseline-corrected manually and phased using TopSpin (v3.5). NMR spectra were integrated into four main regions using TopSpin (v3.5), which included: alkyl C (0–50 ppm); O-alkyl C (50–110 ppm), aromatic and phenolic C (110–165 ppm) and carboxylic and carbonyl C (165–210 ppm) (Preston et al., 1997). Alkyl C to O-alkyl C ratios were calculated to compare the relative stage of degradation between samples (Baldock et al., 1992).

## Net N Mineralization

Net N mineralization rates were assessed during the 8th and 9th years of treatments using the buried bag incubations technique (Hart et al., 1994). In each plot, a sample was taken from the F horizon (fibric), manually sorted (i.e., rocks, cones, branches, fresh leaves, and coarse roots were removed) and homogenized. A subsample ( $\sim 5$  g of dry weight) was taken for  $\text{NH}_4^+$  extraction (see below). The remaining sample was put in a closed polyethylene bag ( $\sim 0.030$  mm thick) and buried under the surface (L) horizon. The bag was left *in situ* and collected about 30 days later to extract  $\text{NH}_4^+$  (time  $t$ ). The same procedure was repeated for each plot six times from May to late October in 2015 and five times from May to late September in 2016. In addition, one incubation of about 210 days (from late October 2015 to late May 2016) was carried out to cover the winter period.

Soil  $\text{NH}_4^+$  extractions were conducted as follows: soil subsamples ( $\sim 5$  g of dry weight) were mixed with 100 ml of 2 M KCl in glass bottles and shaken ( $\sim 130$  rpm) for 60 min. All soil extracts were then filtered (No. 1 filter paper; Whatman Inc., Little Chalfont, United Kingdom) and filtered extracts were stored at  $-19^\circ\text{C}$  until analysis. Ammonium concentrations from the filtered extracts were determined by colorimetry following the nitroprusside-hypochlorite-salicylate method (Mulvaney, 1996) using a Technicon AutoAnalyzer II (Pulse Instrumentation Ltd., Saskatoon, CAN) in 2015 and a QuikChem 8500 (Lachat Instruments, Milwaukee, United States) in 2016. The net N mineralization rate was then calculated for each incubation period as follows (Hart et al., 1994):

$$m_N = \frac{[\text{N-NH}_4^+]_t - [\text{N-NH}_4^+]_{t_0}}{t} \quad (1)$$

where  $m_N$  is the net N mineralization rate (in  $\text{mg N-NH}_4^+ \text{ kg}^{-1}$  soil  $\text{day}^{-1}$ ),  $t$  is the incubation time (in days),  $[\text{N-NH}_4^+]_{t_0}$  is the total  $\text{N-NH}_4^+$  concentration (in  $\text{mg kg}^{-1}$  soil) at time 0, and  $[\text{N-NH}_4^+]_t$  is the total  $\text{N-NH}_4^+$  concentration (in  $\text{mg kg}^{-1}$  soil) at time  $t$ .

## Gross N Mineralization

Gross N mineralization rates were estimated during the 8th and 9th years of treatments using the N isotope dilution technique (Davidson et al., 1991; Hart et al., 1994). Soil samples (150–200 g) were taken from the F horizon, sorted and homogenized as described above, then put in polyethylene bags ( $\sim 0.044$  mm thick). Using a syringe and a needle 30G1/2 (Becton Dickinson & Co., Franklin Lakes, United States), 4 ml of  $(^{15}\text{NH}_4)_2\text{SO}_4$  solution (15 mg N  $\text{L}^{-1}$  at 98%  $^{15}\text{N}$ ) was spread uniformly onto the soil sample. The sample was then homogenized and 15 min later, a subsample ( $\sim 5$  g of dry weight) was transferred into a bottle for  $\text{NH}_4^+$  extraction (time  $t = 0$ ). The soil bag was closed, buried beneath the L-horizon and the moss layer and left to incubate *in situ* for about 24 h before another soil subsample was taken for  $\text{NH}_4^+$  extraction (time  $t$ ). All soil extracts were kept at  $\sim 2^\circ\text{C}$  until filtration. Gravimetric water content was determined like previously and volumetric water content of the F horizon was measured using a GS3 probe (Decagon Devices, Inc., Pullman, United States). The same steps ( $^{15}\text{N}$  dilution) were repeated twice a year both in 2015 and in 2016 during the soil warming period, i.e., one incubation in June and one in July. These procedures were done 1–2 weeks later at BER than at SIM due to delayed growing season.

Soil extracts were filtered as described previously and stored at  $-19^\circ\text{C}$  until analysis. A diffusion method was used to measure the *atom%*  $^{15}\text{N}$  as previously described (Davidson et al., 1991; Paré and Bedard-Haughn, 2012). Approximately 0.3 g of magnesium oxide (MgO) was added to a 20 ml subsample of the filtered extract to convert  $\text{N-NH}_4^+$  into  $\text{N-NH}_3$ . Ammonia was then trapped on a 7 mm in diameter paper disk (No. 3; Whatman Inc., Little Chalfont, United Kingdom) previously acidified with 10  $\mu\text{l}$  of 2.5 M  $\text{KHSO}_4$ . The acidified disk was sealed between two Teflon strips (Rona X-Pert; Rona Inc., Boucherville, CAN). The 20 ml subsample, MgO and sealed paper disk were gently shaken ( $\sim 45$  rpm) in an airtight 60 ml Nalgene bottle (High-Density polyethylene; VWR International, Mississauga, CAN) for 7 days. Thereafter, the paper disk was removed from its Teflon casing, dried ( $50^\circ\text{C}$  for 1 h) and inserted into a Tin capsule (D1008; Elemental Microanalysis Ltd., Okehampton, United Kingdom). The *atom%*  $^{15}\text{N}$  and  $\text{N-NH}_4^+$  concentration was determined using an elemental analyzer Costech ECS 4010 (Costech Analytical Technologies, Inc., Valencia, United States) coupled to an isotope ratio mass spectrometer Delta V with a ConFlo IV interface (Thermo Fisher Scientific, Bremen, GER). For 2015 samples,  $\text{N-NH}_4^+$  concentration was assessed by colorimetry (Mulvaney, 1996) using a Technicon AutoAnalyzer II (Pulse Instrumentation Ltd., Saskatoon, CAN). Gross N

mineralization rate calculated according to Eq. 2 (Hart et al., 1994), respectively:

$$m_B = \frac{[N-NH_4^+]_{t0} - [N-NH_4^+]_t}{t} \times \frac{\log \frac{APE_0}{APE_t}}{\log \frac{[N-NH_4^+]_{t0}}{[N-NH_4^+]_t}} \quad (2)$$

where  $m_B$  is the gross N mineralization rates (in mg N-NH<sub>4</sub><sup>+</sup> kg<sup>-1</sup> soil day<sup>-1</sup>), and  $APE$  is the *atom%* <sup>15</sup>N enrichment of a N pool enriched with <sup>15</sup>N minus the *atom%* <sup>15</sup>N enrichment of that pool prior to <sup>15</sup>N addition.

## Seedlings Phenology and Growth

Seedling buds were surveyed throughout the second growing season following planting (2016) between May and late June, twice a week at SIM and once a week at BER. The phenological stages of the buds (0–7; **Supplementary Material 2**) were determined based on the Numainville and Despons (2004) procedure. The survey was ended when all trees had reached the last stage of phenological development (stage 7).

Seedling stem diameter at the base and height (from the base to the top of the apical shoot) were measured before planting and at the end of the first and second growing seasons (August 2015 and 2016, respectively) to calculate radial and apical growth rates. The apical shoots of BF seedlings were grazed by herbivores during the first growing season. As a consequence, we show apical shoot growth for BS only. At the end of the second growing season, all seedlings were harvested (4 treatments × 3 blocks × 2 sites × 2 species × 5 seedlings = 240 seedlings). The roots, stems and needles were separated, dried at 65°C for 48 h and weighted.

## Foliar Chemistry and δ<sup>15</sup>N

The foliar biomass of the five BS and BF seedlings per plot ( $n = 240$ ) was dried at 65°C for 48 h, ground to a fine powder and sent to the lab for analyses. Total N concentrations (g N g<sup>-1</sup>) were determined through the Kjeldhal digestion method (Kjeltec Tecator 1030). Total P, K, Ca, Mg, Mn, Cu, Zn, Al, Fe, and Mo were determined by ICP-AES after sample digestion with concentrated H<sub>2</sub>SO<sub>4</sub> (Parkinson and Allen, 1975).

Foliar biomass subsamples were pooled in order to obtain one sample for each species in each experimental plot at each site (4 treatments × 3 blocks × 2 sites × 2 species = 48 samples) for δ<sup>15</sup>N measurements. Analyses were performed at the Centre de recherche en géochimie et géodynamique (GEOTOP; UQÀM, Québec, Montréal). N isotopic ratios were measured using an elemental analyzer in continuous flow mode, coupled to an isotope ratio mass spectrometer (IRMS; Micromass Isoprime Isoprime 100, Cheadle, United Kingdom). Values of δ<sup>15</sup>N are expressed in ‰ vs. air (± 0.2‰ at 1σ). Raw values were corrected with a calibration line obtained from two reference materials: urea and dogfish tissue (δ<sup>15</sup>N = −0.22 and +14.36‰, respectively). Internal reference materials were normalized to IAEA-N1, N-2, and N-3 scales for δ<sup>15</sup>N. A third internal reference material was used to verify the exactitude of the calibration (leucine δ<sup>15</sup>N = −0.06‰).

## Statistical Analyses

Gross mineralization data were log-transformed to meet the assumptions of normality and homogeneity of variance prior to statistical analyses. Net N mineralization data could not be log-transformed because of the presence of several negative values (34 values out of 264). We assessed the impact of soil warming (two levels: “W+” and “W-”), CNA (two levels: “N-” and “N+”), site (two levels: “BER” and “SIM”) and species (two levels: “BF” and “BS”) with blocks as random effect on inorganic N concentrations, N mineralization rates, seedling growth and foliar chemistry by using mixed-model analyses. We used the lmerTest package in R, which uses the Satterthwaite’s degrees of freedom method (Kuznetsova et al., 2017) as in Marty et al. (2019a). A paired *t*-test was used to assess the impact of the soil warming treatment on foliar δ<sup>15</sup>N in both N- and N+ plots by comparing the foliar δ<sup>15</sup>N values in W and C plots, and WN and N plots, respectively. Wilcoxon’s rank-sum tests were performed on the mean phenological stage of the buds at each survey date to assess the differences between treatments and species within each site. We performed principal component analyses (PCA) on both forest floor and foliar chemistry by using the ade4 package in R (R Core Team, 2019). For all analyses, *P*-values ≤ 0.05 were considered significant.

## RESULTS

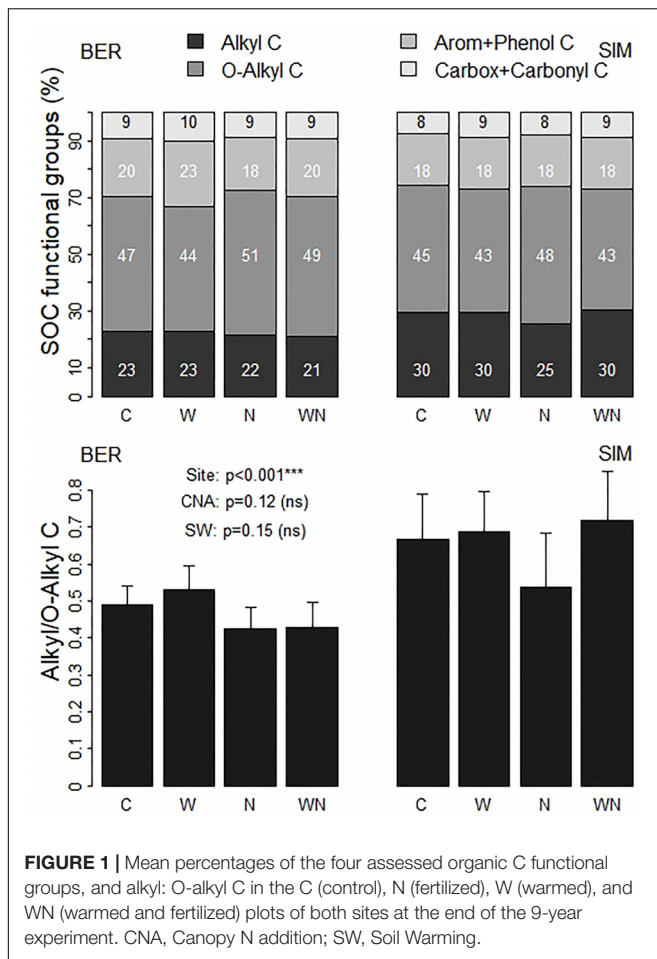
### Soil Chemistry at Initial Conditions

A previous study conducted at the SIM site showed that the presence of warming cables did not affect the soil conditions (Marty et al., 2019a). The control (C) plots can thus be used to characterize the chemical composition of the forest floor at initial conditions. In these C plots, the O-alkyl functional group represented about half of the organic C in the soil samples at both sites (**Figure 1**). The second most represented functional group was the alkyl C group, which accounted for 23–30% of forest floor organic C. Aromatic and phenolic C together accounted for 18–20%. Finally, carboxylic and carbonyl C represented the smaller fraction of soil C (8–9%).

The forest floor at BER had a lower proportion of alkyl C as well as a lower alkyl/O-alkyl C ratio, and a slightly higher proportion of aromatic + phenolic C functional groups than at SIM (**Figure 1**). Forest floor OM content and C:N ratio were higher at BER than at SIM, whereas Cu, Zn, Al, Fe, and N concentrations were much higher at SIM than at BER (**Table 1**).

### Effects of the Treatments on Soil Chemistry

The PCA graphical representation (**Figure 2A**) shows an obvious separation of the two sites on the PC1 axis, all BER samples having negative PC1 values and all but one SIM samples having positive PC1 values. This separation on the PC1 axis reflected the higher OM, Ca, Mn concentrations, as well as higher C:N ratio and contents of carboxyl + carbonyl and aromatic + phenolic functional groups at BER than at SIM (**Table 1** and **Figure 1**).



Conversely, SIM samples were more associated with high P, N and Fe concentrations and a higher alkyl/O-alkyl C ratio.

In contrast, the PCA did not reveal clear differences in forest floor's chemical characteristics between warmed (W+) and unwarmed (W-) samples (Figure 2B), or between fertilized (N+) and unfertilized (N-) samples (Figure 2C). Although not statistically significant, the alkyl/O-alkyl C ratio was slightly reduced by the N+ treatment relative to control at both sites. At SIM, this ratio was on average 25% lower in the N plots ( $0.54 \pm 0.15$ ) than in the WN plots ( $0.72 \pm 0.13$ ), whereas it was similarly lower in both N and WN plots (0.43) than in the other plots (0.49–0.53) at BER (Figure 1).

## Differences in Soil Inorganic N Concentrations Among Sites and Treatments

On May 19 2015, before the first incubation of the soil (buried bag method),  $\text{N-NH}_4^+$  represented about 100% of total soil inorganic N ( $\text{N-NO}_3^- + \text{N-NH}_4^+$ ) as  $\text{N-NO}_3^-$  concentrations were below the detection limit at both sites, which is common in boreal soils. In C plots (i.e., initial conditions), soil  $\text{N-NH}_4^+$  concentration was on average more than twice higher at SIM ( $16.9 \text{ mg kg}^{-1}$ ) than at BER ( $7.8 \text{ mg kg}^{-1}$ ; Table 1). At SIM,

$\text{N-NH}_4^+$  concentrations were significantly lower in N+ (N and WN plots) than in N- plots (C and W plots; Table 1).

## Differences in Net and Gross N Mineralization Rates Among Sites and Treatments

Net N mineralization rate was on average about three times higher at SIM than at BER across experimental treatments (Figure 3A). The CNA and soil warming treatments had no significant effect on net N mineralization rate (Figures 3B,C).

The rate of gross N mineralization was similar at both sites (Figure 3D) but 23% lower in N+ than in N- plots (Figure 3E). In contrast, the soil warming treatment had no significant effect on gross nitrogen mineralization rates (Figure 3F).

## Seedling Growth

Soil warming and CNA had no effect on BS annual apical shoot growth, which significantly varied only among sites (Table 2). Stem radial growth (mm) was higher at BER than at SIM (Figure 4A) and for BS than for BF (Figure 4B). There was a significant interaction between species and CNA for stem radial growth (Table 2). Stem radial growth was 20% higher in N- than in N+ plots for BS but not for BF (Figure 4C). Annual apical shoot growth was significantly higher at BER than at SIM in both 2015 and 2016 (Supplementary Material 3).

## Effects of Site and Treatments on Seedling Growth and Phenology

Seedling budbreak occurred earlier and was more rapid i) at SIM than at BER; and ii) for BF than for BS (Supplementary Material 4). The SW treatment significantly accelerated BF bud development at both sites (Figures 5A,B). The impact was not as pronounced for BS. The effect of CNA was non-significant for both species at BER, although the mean phenological stage was lower in N+ than in N- plots at the beginning of the growing season (Figure 5C). In contrast, CNA decreased BS bud development rate at SIM, particularly at the very beginning and at the end of the growing season (Figure 5D).

## Seedling Foliar Chemistry and $\delta^{15}\text{N}$

The PCA shows a contrast in foliar chemical composition between the two sites (Figure 6A) and seedling species (Figure 6D), reflecting the significant effects of these variables on most elements (Table 3). BF seedlings were associated with P and N concentrations on the PC1 axis (Figure 6D), reflecting higher foliar N and P concentrations in BF than in BS (Table 4). All other nutrients were more concentrated in BS than in BF (Table 4). Foliar concentrations of N, P, K, and Fe were significantly higher at SIM than at BER. The N+ and N- plots (Figure 6B) as well as the SW- and SW+ plots were scattered through the graphical representation of the PCA (Figures 6B,C), reflecting the absence of CNA and SW effects on foliar chemistry (Table 4).

BS and BF seedlings from the C plots had distinct foliar N isotopic signatures.  $\delta^{15}\text{N}$  values were markedly higher in BF (range from 0.2 to 4.9‰) than in BS (range from -5.3 to -2.6‰) at both sites (Figure 7). Foliar  $\delta^{15}\text{N}$  was also higher at SIM than at

**TABLE 1 |** Forest floor chemical composition in the C (control), N (fertilized), W (warmed), and WN (warmed and fertilized) plots of both sites at the end of the 9-year experiment.

Site		Treatments			
		C	N	W	WN
BER	N-NH <sub>4</sub> <sup>+</sup> (mg kg <sup>-1</sup> soil DW)	7.8 ± 0.9	12.3 ± 6.0	8.7 ± 0.9	13.1 ± 3.0
SIM	–	16.9 ± 3.2	7.2 ± 4.9	14.2 ± 3.4	7.1 ± 2.9
BER	OM (g kg <sup>-1</sup> soil DW)	947 ± 7.2	953.3 ± 9.3	953.7 ± 4.2	950.7 ± 21.2
SIM	–	826.3 ± 112.1	934 ± 12.1	911.3 ± 57.5	879.3 ± 50.9
BER	pH	3.5 ± 0.2	3.5 ± 0.2	3.6 ± 0.2	3.5 ± 0.1
SIM	–	4.0 ± 0.2	3.8 ± 0.1	3.8 ± 0.1	3.8 ± 0.1
BER	C (g kg <sup>-1</sup> soil DW)	476.7 ± 11	476.0 ± 6.0	481.3 ± 1.5	475.3 ± 7.5
SIM	–	430 ± 54.5	490 ± 25.2	475.7 ± 27.5	467 ± 29.8
BER	N (g kg <sup>-1</sup> soil DW)	9.7 ± 1.5	9.8 ± 0.6	9.3 ± 1.0	9.4 ± 0.7
SIM	–	12.2 ± 1.8	11.9 ± 0.9	12.6 ± 1.1	12.0 ± 0.2
BER	C: N	50.2 ± 8.4	48.6 ± 3.6	52.1 ± 5.7	50.9 ± 3.1
SIM	–	35.4 ± 3.7	41.4 ± 3.3	38 ± 2.4	38.8 ± 2.9
BER	P (mg kg <sup>-1</sup> soil DW)	140.0 ± 35.0	119.7 ± 36.5	121.3 ± 30.7	137.7 ± 8.3
SIM	–	145.3 ± 45.1	150.7 ± 17	151.0 ± 21.8	140.7 ± 38.7
BER	K (mg kg <sup>-1</sup> soil DW)	548 ± 67.5	540.7 ± 103.4	542 ± 65.5	547.7 ± 75.5
SIM	–	492.3 ± 149.1	527.3 ± 126.4	520.3 ± 80.6	474.0 ± 68.5
BER	Ca (mg kg <sup>-1</sup> soil DW)	1609 ± 722	1530 ± 650	1893 ± 514	1670 ± 475
SIM	–	1478 ± 633	1593 ± 510	1670 ± 92	1273 ± 363
BER	Mg (mg kg <sup>-1</sup> soil DW)	269.3 ± 64.5	247.7 ± 23.2	299.7 ± 58.5	274 ± 55.5
SIM	–	435 ± 216.8	334.3 ± 40.1	384.7 ± 114	301 ± 45.7
BER	Mn (mg kg <sup>-1</sup> soil DW)	42.3 ± 27.2	36.7 ± 3.2	83.7 ± 36.7	49.3 ± 19.2
SIM	–	48.0 ± 20.3	60.0 ± 33.0	59.3 ± 9.3	36.3 ± 13.1
BER	Cu (mg kg <sup>-1</sup> soil DW)	2.3 ± 0.6	2.0 ± 0.0	2.0 ± 0.0	2.3 ± 0.6
SIM	–	40.7 ± 33.3	19.7 ± 15.9	35.7 ± 22.6	27.3 ± 12
BER	Zn (mg kg <sup>-1</sup> soil DW)	32 ± 9.5	31.7 ± 8.7	35 ± 3	35.3 ± 4
SIM	–	144.7 ± 39.7	117.7 ± 46.3	132.7 ± 49.7	113.7 ± 45.6
BER	Al (mg kg <sup>-1</sup> soil DW)	502.3 ± 73.9	453.7 ± 96.2	468 ± 151.5	502.3 ± 74.9
SIM	–	940 ± 198	789.3 ± 797.3	770.7 ± 493.4	1181.7 ± 798.6
BER	Fe (mg kg <sup>-1</sup> soil DW)	194.7 ± 27.1	393.7 ± 393.9	174 ± 2.6	192.3 ± 31.3
SIM	–	434.3 ± 300.7	342.3 ± 330.8	293.3 ± 193.5	398.7 ± 357.2
BER	S (mg kg <sup>-1</sup> soil DW)	60.7 ± 5.5	64.0 ± 6.9	54.7 ± 6.7	61 ± 2.6
SIM	–	62.7 ± 6.7	65.0 ± 13.5	64.0 ± 5.2	63.7 ± 13.5

BER for both species, reflecting the higher  $\delta^{15}\text{N}$  in the forest floor at SIM (7.6‰) than at BER (0.8‰). The foliar  $\delta^{15}\text{N}$  average values were similar to those of the seedlings prior to planting for both species (+3.3 and -2.8‰ for BF and BS, respectively) at SIM, and markedly lower at BER (decrease of 1.7 and 2.0‰ for BF and BS, respectively). Foliar  $\delta^{15}\text{N}$  appeared slightly higher in warmed than in unwarmed plots both in N- and N+ treatments (except for BF at SIM in N-) but these differences were not statistically significant ( $P > 0.05$ ; Table 5).

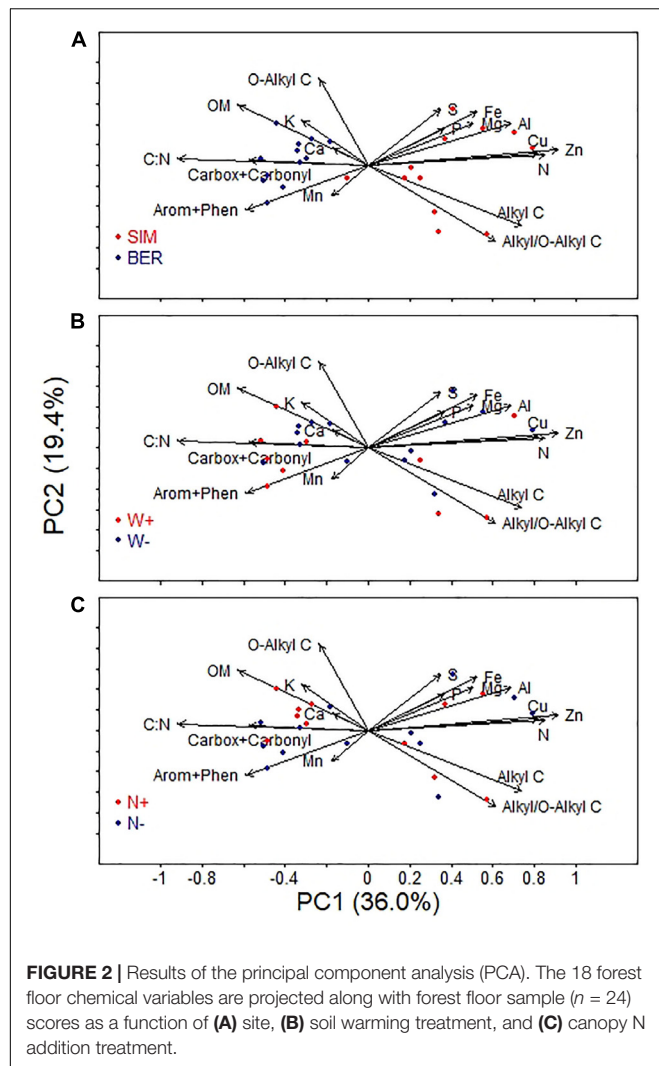
## DISCUSSION

### Soil Inorganic N at the Two Boreal Sites

Although soil organic matter of Canadian temperate and boreal forest soils store large amounts of N (up to 1.2 kg N m<sup>-2</sup>), particularly in the mineral horizons of the soil (Marty et al., 2017), N is one of the main factors limiting the growth of trees

in boreal forests (Schulte-Uebbing and de Vries, 2018), because most of this N is in its organic forms due to low mineralization rates, and thus not directly and readily available to support plant development and growth. The very low NO<sub>3</sub><sup>-</sup> concentrations we measured in the soil of both study sites agree with what is generally observed in boreal forests of the region, where N-NH<sub>4</sub><sup>+</sup> is the main inorganic N form in the soil solution (Ste-Marie and Houle, 2006). At the beginning of the growing season, NH<sub>4</sub><sup>+</sup> concentration in the forest floor was more than twice higher at SIM than at BER (Table 1), which is consistent with the higher net N mineralization rate observed at SIM (Figure 3A). These differences were likely due to a higher immobilization rate at BER, as suggested by the similar gross mineralization rates at both sites (Figure 3D) and the much higher soil C:N ratio at BER than at SIM (50.2 vs. 35 at SIM and BER, respectively; Table 1), a condition known to stimulate inorganic N immobilization. The forest floor organic matter at SIM also had a higher alkyl/O-alkyl C ratio (Figure 1C), which is consistent with the

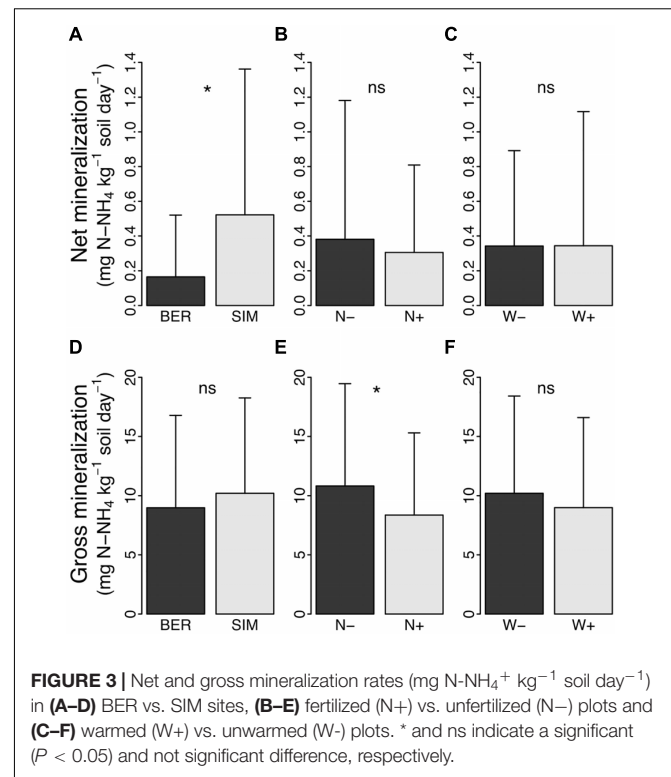




generally observed negative relationship between OM C:N ratio and alkyl/O-alkyl C ratio (Simpson and Simpson, 2012). As O-alkyl C corresponds to more labile and easily degraded OM constituents, a higher alkyl/O-alkyl C ratio generally indicates a higher degree of soil OM degradation (Simpson and Simpson, 2012; Marty et al., 2019a). The sometimes large variability in  $\text{NH}_4^+$  concentrations (large standard deviation values; Table 1) sometimes observed within experimental treatments at each site was certainly caused by a variation over time and space in factors controlling OM mineralization, such as soil temperature, soil moisture, resources availability (e.g., labile organic C) as well as microbial activity (Moorhead and Sinsabaugh, 2006).

## Effect of N Addition and Soil Warming on Soil N

In contrast with our hypothesis, the CNA treatment did not impact soil  $\text{NH}_4^+$  concentrations and net N mineralization rates. This absence of effect agrees with a meta-analysis reporting an overall negligible effect of N addition on litter decomposition (Knorr et al., 2005) but contrasts with other studies reporting



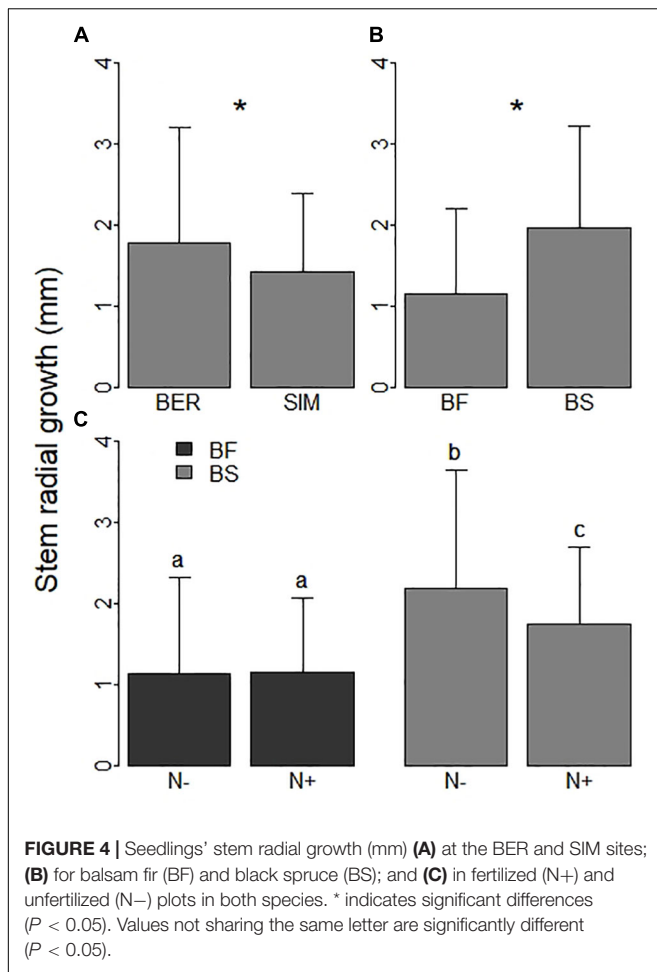
**TABLE 2 |** Result of the mixed model analysis conducted on black spruce annual shoot growth (BS ASG) and stem radial growth of black spruce and balsam fir (SRG) between June 2015 and August 2016 (two growing seasons) with site, species, canopy N addition (CNA), and soil warming (SW) as fixed effects and blocks as random effect.

	BS ASG	SRG
	P-value	P-value
Site	<b>&lt;0.001</b>	<b>0.05</b>
Species	–	<b>&lt;0.001</b>
CNA	0.23	0.18
SW	0.40	0.54
Site × Species	–	0.24
Site × CNA	0.21	0.80
Species × CNA	–	<b>0.04</b>
Site × SW	0.65	0.15
Species × SW	–	0.31
CNA × SW	0.58	0.61
Site × Species × CNA	–	0.92
Site × Species × SW	–	0.89
Site × CNA × SW	0.83	0.65
Species × CNA × SW	–	0.46
Site × Species × CNA × SW	–	0.75

The table shows the results of type III analyses of variance computed via Satterthwaite's degree of freedom method. Values in bold characters indicate significant effects.

either a decrease (Berg and Matzner, 1997; Magill and Aber, 1998; Craine et al., 2007; Frey et al., 2014) or an increase (Aber et al., 1998; Knorr et al., 2005; Contosta et al., 2011; Ma et al., 2011;





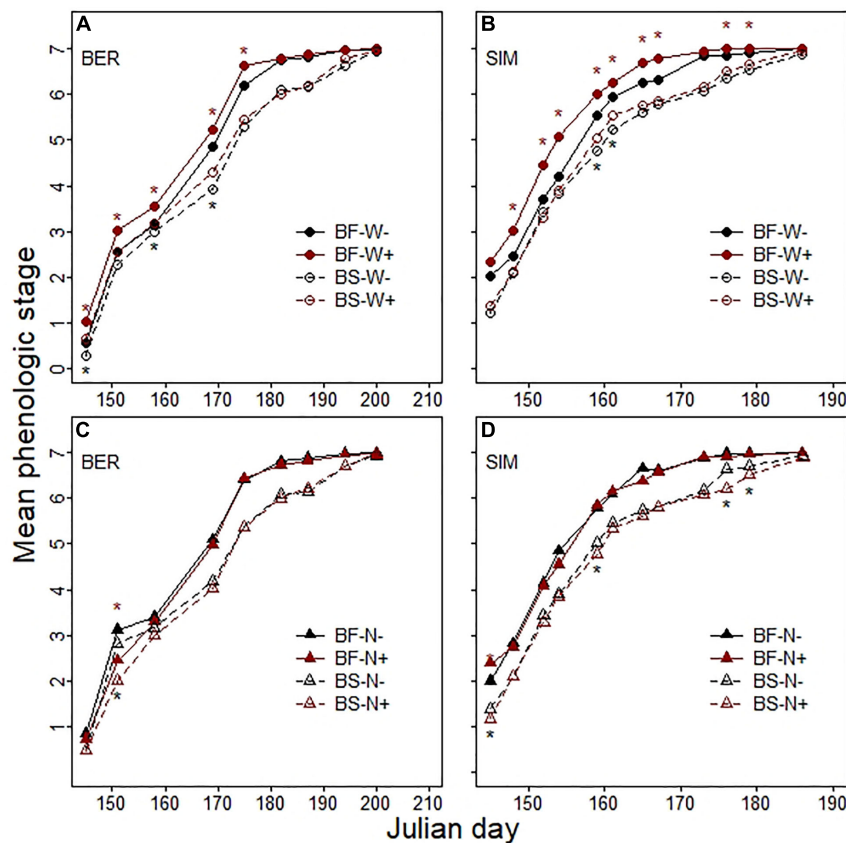
Zhang et al., 2012) in soil OM decomposition in response to increased N addition, either directly onto the forest floor or on top of the forest canopy. The absence of effect of CNA in our experimental setting may result from the low absolute amount of N actually added to the soil-plant system. Although we doubled the N concentration in precipitation relative to natural rainfall, our treatment corresponded to a total input of only 0.3–0.5 kg N ha<sup>-1</sup> yr<sup>-1</sup>, which is much lower than in other studies which added up to 50–100 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Frey et al., 2014; Maaroufi et al., 2015). Such N inputs are nevertheless unrealistic in eastern Canadian boreal forests which receive low and decreasing N deposition rates (Houle et al., 2014). Moreover, a significant fraction of CNA may have been immobilized in the canopy (Dail et al., 2009; Adriaenssens et al., 2012; Fenn et al., 2013; Houle et al., 2014) or in the upper moss layers (Gundale et al., 2011; Houle et al., 2014), and may thus have not reached the sampled forest floor.

Nevertheless, the 23% lower gross N mineralization in N+ plots as compared to plots that received no additional N as well as the higher foliar  $\delta^{15}\text{N}$  in N+ plots (Figure 7) show that at least a fraction of the added N reached the forest floor. A decrease in the N mineralization rate following inorganic N addition is generally ascribed to a decrease in soil microorganism mining

effort (Moorhead and Sinsabaugh, 2006; Craine et al., 2007), which preferentially use inorganic N rather than alternative N sources such as organic compounds (Geisseler et al., 2010). This phenomenon is illustrated by an increase in soil OM decomposition with N limitation across a wide range of soil types (Craine et al., 2007). Several studies have also shown that chronic N fertilization reduces soil microbial and fungal biomass, activity and diversity (Lilleskov et al., 2001; Frey et al., 2004, 2014; Maaroufi et al., 2015), but that the effect depends on the amount of N added and on the context. Litter decomposition is for instance inhibited when N inputs are 2–20 times the N deposition rate and when litter quality is low, i.e., high lignin litter (Knorr et al., 2005). Also, inorganic N addition to fresh litter generally stimulates the decomposition of cellulose and soluble compounds, whereas inorganic N addition to stabilized soil OM may reduce microbial decomposition activity (Berg and Matzner, 1997). In early successional boreal forests, N addition has been shown to increase litter mass loss as well as the activity of cellulose- and chitin-degrading enzymes (Allison et al., 2010). In our study, we observed slightly lower alkyl/O-alkyl C ratios with N at both sites relative to the control. The alkyl/O-alkyl C ratio typically increases with soil OM degradation and as such, N addition appears to have suppressed biodegradation at both BER and SIM (Figure 1), which is consistent with the decreased gross mineralization rate associated with the CNA treatment (Figure 3E).

The observed decrease in gross mineralization rate caused by N addition was not accompanied by a significant decrease in net mineralization rate, suggesting that N immobilization by soil microorganisms was also decreased with the same magnitude by the CNA treatment. However, we cannot exclude a methodological bias. Gross mineralization was indeed measured over a short time scale (24 h-long incubation), whereas net mineralization was measured over several incubation periods of 30 days. This may explain a part of the discrepancy between the observed effect of N addition on net and gross mineralization rates.

Contrary to our hypothesis, soil warming did not stimulate net and gross N mineralization rates. This also contrasts with a recent meta-analysis showing that soil warming increases the rate of several variables related to N fluxes, such as net N mineralization, net nitrification, denitrification and N fixation, especially in experiments where the soil was warmed with heating cables (Bai et al., 2013). A recent study also reports a stimulation of microbial activity and cutin degradation by *in situ* soil warming (Pisani et al., 2015). This also contrasts with a previous study conducted at the SIM site which showed that soil warming had a significant impact on the temperature sensitivity and the basal respiration rate of the forest floor organic C (Marty et al., 2019a). Our results are however in agreement with other studies reporting no significant effect of soil warming on N mineralization rate and inorganic N fluxes in boreal forests. For instance, 3 years of soil warming (+4°C) in a boreal forest of Quebec did not increase soil inorganic N fluxes (Houle et al., 2014) nor tree growth and N status in a mature balsam fir boreal forest (D'Orangeville et al., 2013b). A recent study also reported that soil N mineralization and inorganic N pools in the soil



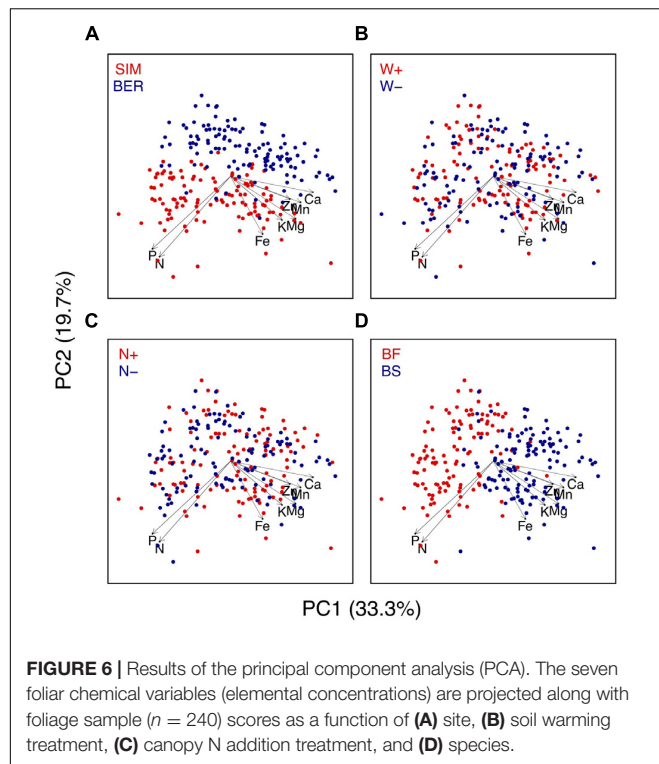
**FIGURE 5 |** Change in the mean phenologic stage for balsam fir (BF) and black spruce (BS) in **(A,B)** warmed (W+) and unwarmed (W-) plots and **(C,D)** in fertilized (N+) and unfertilized (N-) plots across the 2016 growing season at the BER and SIM sites. \* indicate significant differences between treatments for BF (red symbols) and BS (black symbols) (Wilcoxon's rank-sum test;  $P < 0.05$ ).

solution were not affected by experimental warming in northern agro-ecosystems of eastern China (Fu et al., 2019). This treatment also did not significantly affect the alkyl/*O*-alkyl C ratio relative to the control, indicating an absence of significant impact on the degree of soil OM degradation. Several factors may explain the absence of effect of soil warming on N mineralization in the long term. First, a reduction in microbial biomass and/or changes at the individual and community levels, i.e., replacement by a microbial community with lower temperature sensitivity, have been observed following *in situ* long-term soil warming (Zogg et al., 1997; Luo et al., 2001; Bradford et al., 2008; Craine et al., 2013). At the BER site, Rossi et al. (2013) have for instance observed a reduction in the proportion of EcM fungi in response to increased soil temperature. Second, increased soil temperature may have reduced soil moisture resulting in decreased microbial activity and nutrient diffusion throughout the soil matrix, or enhanced the magnitude and the frequency of freeze/thaw cycles by removing the snow cover earlier in spring. This phenomenon (freeze/thaw cycle) is known to have large detrimental effects on tree N uptake (Templer et al., 2007), nutritional status (Comerford et al., 2013) and growth (Reinmann et al., 2019), as well as on soil organic N mineralization rates (Durán et al., 2016). Interestingly, the combination of soil warming and N addition

had a different impact on the alkyl/*O*-alkyl C ratio depending on the site. The alkyl/*O*-alkyl C ratio of WN plots was similar to that of the N plots at BER, whereas it was similar to that of the W plots at SIM (Figure 1). This discrepancy is difficult to interpret but shows that specific ecosystem properties (e.g., temperature, soil OM properties, soil microbial communities) influence soil OM stability and may control the net output of N addition and soil warming on soil OM degradation (Schmidt et al., 2011). The greater similarity between WN and N plots at BER corroborates observations from the Harvard Forest, MA, United States after 4 years of similar treatments (Pisani et al., 2015), suggesting a predominant influence of the N treatment at this site, at least over the duration of the study. Given the dynamic nature of soil processes and microbial communities (Melillo et al., 2017), the relative influence of these treatments may change over time, which could explain the difference between the two sites.

## Seedling Phenology and Growth

Our data showed an earlier budbreak and faster bud development at SIM than at BER, which is consistent with latitudinal and climate differences between the two sites. We also observed differences between the two seedling species. The phenological development was faster for BF than for BS seedlings at both



sites (Supplementary Material 4). The phenology seemed to be decoupled with the growth of seedlings, as our data point toward higher growth rates for BS than for BF and at BER than at SIM.

Despite the higher  $\text{NH}_4^+$  pool and net mineralization rate at SIM, the growth of both BF and BS seedlings was higher at BER, suggesting that the seedlings could not access this N pool at SIM,

or that soil net mineralization and  $\text{NH}_4^+$  concentration at the beginning of the growing season did not represent the actual N availability for seedlings in boreal forests where immobilization rates are high. In these systems, N availability may rather be more precisely estimated by the gross mineralization rate as suggested by the positive (but not significant) correlation between the growth mineralization rate and BS radial growth at BER ( $R^2 = 0.30$ ;  $P = 0.06$ ; Supplementary Material 5) and the coupled decreases in both stem radial growth of BS seedlings (Figure 4C) and gross mineralization consecutive to canopy N addition (Figure 3E).

Our data also suggest that the contribution of ectomycorrhizae (EcM) to seedling N nutrition contributed to the observed growth differences among sites and seedling species. Foliar  $\delta^{15}\text{N}$  was much lower for BS than for BF at both sites, which likely resulted from a different degree of EcM association or different N fertilization treatments in the tree nursery, as their isotopic signatures were already much different prior to planting in the field ( $-2.7$  and  $3.3\text{‰}$  for BS and BF, respectively; Figure 7). The particularly low foliar  $\delta^{15}\text{N}$  of BS as compared to other tree species has been reported in other studies (Marty et al., 2011; Houle et al., 2014) and may result from its high reliance on N derived from EcM fungi (Mayor et al., 2012), which provide trees with  $^{15}\text{N}$ -depleted N (Hobbie and Hobbie, 2008; Hobbie et al., 2009; Mayor et al., 2015). The lower foliar  $\delta^{15}\text{N}$  at BER than at SIM for both seedling species is consistent with the much higher forest floor  $\delta^{15}\text{N}$  at SIM (Figure 7). Interestingly, foliar  $\delta^{15}\text{N}$  of both species decreased at BER, whereas it remained unchanged after 2 years in the field at SIM. This indicates the use of a more  $^{15}\text{N}$ -depleted N source, such as EcM-derived N, or in larger amount at BER than at SIM. The first hypothesis is consistent with the lower inorganic N availability and organic N mineralization rate as well as the higher C:N ratio at BER, which

**TABLE 3 |** Result of the mixed model analysis conducted on seedlings' foliar chemistry with site, species, canopy N addition (CNA) and soil warming (SW) as fixed effects and blocks as random effect.

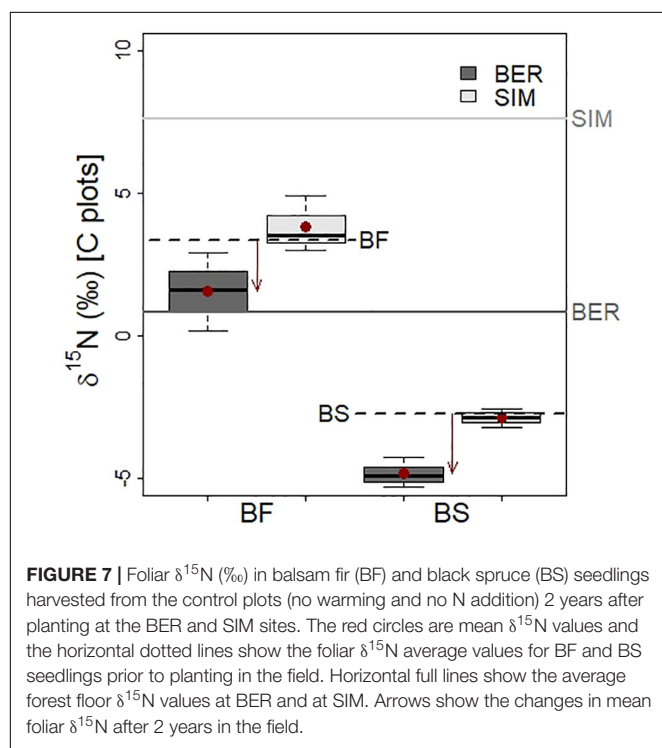
	P-values							
	N	P	K	Ca	Mg	Fe	Mn	Zn
Site	<0.01	<0.01	<0.01	0.07	0.07	0.05	0.35	0.98
Species	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
CNA	0.70	0.55	0.86	0.99	0.35	0.74	0.80	0.82
SW	0.61	0.22	0.49	0.57	0.41	0.15	0.94	0.28
Site × Species	<0.01	<0.01	0.09	0.19	0.64	0.04	0.40	0.98
Site × CNA	0.68	0.52	1.0	0.21	0.14	0.53	0.58	0.38
Species × CNA	0.14	0.23	0.43	0.05	0.75	0.75	0.05	0.35
Site × SW	0.52	0.55	0.46	0.55	0.57	0.27	0.99	0.57
Species × SW	0.14	0.05	<0.01	0.05	0.05	0.08	0.47	0.65
CNA × SW	0.59	0.77	0.35	0.14	0.83	0.03	0.50	0.59
Site × Species × CNA	0.46	0.61	0.43	0.39	0.30	0.30	0.39	0.78
Site × Species × SW	0.37	0.88	0.10	0.04	0.57	0.53	0.41	0.30
Site × CNA × SW	0.99	0.72	0.59	0.57	0.63	0.17	0.53	0.45
Species × CNA × SW	0.78	0.37	0.01	<0.01	0.36	0.04	0.25	0.56
Site × Species × CNA × SW	0.15	0.65	0.51	0.24	0.59	0.13	0.14	0.18

The table shows the results of type III analyses of variance computed via Satterthwaite's degree of freedom method. Values in bold characters indicate significant effects.

**TABLE 4** | Seedlings' foliar chemistry for balsam fir (BF) and black spruce (BS) at the BER and SIM sites.

Concentrations (mg g <sup>-1</sup> )	BF	BS	BER	SIM
N	14.49 ± 4.09 (a)	11.9 ± 2.77 (b)	10.29 ± 2.25 (a)	15.99 ± 2.43 (b)
P	1.93 ± 0.50 (a)	1.43 ± 0.39 (b)	1.35 ± 0.31 (a)	1.99 ± 0.46 (b)
K	5.6 ± 1.15 (a)	6.78 ± 0.95 (b)	5.99 ± 1.20 (a)	6.44 ± 1.17 (b)
Ca	4.69 ± 1.04 (a)	6.35 ± 1.47 (b)	5.75 ± 1.72 (a)	5.35 ± 1.29 (a)
Mg	1.14 ± 0.23 (a)	1.53 ± 0.35 (b)	1.32 ± 0.35 (a)	1.37 ± 0.36 (a)
Fe	0.09 ± 0.03 (a)	0.18 ± 0.22 (b)	0.12 ± 0.07 (a)	0.16 ± 0.22 (b)
Mn	0.36 ± 0.18 (a)	0.53 ± 0.22 (b)	0.46 ± 0.25 (a)	0.43 ± 0.18 (a)
Zn	0.06 ± 0.04 (a)	0.10 ± 0.06 (b)	0.08 ± 0.07 (a)	0.08 ± 0.05 (a)

Values are means ± SD (n = 55–65). Values not sharing the same letter are significantly different (P < 0.05).



generally promotes mycorrhizal associations and increases the relative contribution of EcM fungi to trees' N demand (Hobbie and Ouimette, 2009; Marty et al., 2019b). Thus, the decline in

foliar  $\delta^{15}\text{N}$  at BER may have resulted from a rapid infection by EcM fungi and from a greater dependence of seedlings upon N derived from EcM fungi at this site. This N source may explain the higher growth rate of seedlings at BER than at SIM and for BS than for BF. By contrast, the absence of a significant change in foliar  $\delta^{15}\text{N}$  and the lower growth rate at SIM suggests that the seedlings' root system did not develop enough to access soil N pools (yet larger at SIM than at BER) and that the seedlings mainly have relied on internal recycling of N or N resources contained in the soil carrot to support annual growth. This hypothesis is supported by the lower root biomass at SIM than at BER (**Supplementary Material 6**). The lower root development at SIM may have been caused by various local factors such as soil texture, moisture or temperature, and likely resulted in the lower growth rate at this site.

### Effect of N Addition and Soil Warming on Seedling Growth, Foliar $\delta^{15}\text{N}$ and Phenology

Our hypothesis that N addition and soil warming would result in higher seedling growth was not supported by the data. Our results also indicate that N addition may not have the same effect on all tree species. Whereas the CNA treatment had no effect on BF seedling stem radial growth, it decreased it by about 20% for BS (**Figure 4D**). This may be due to the observed decrease in gross mineralization rate consecutive to the CNA treatment. Seedling growth, particularly for BS, may be related more closely to the gross mineralization rate and EcM association than to

**TABLE 5** | Foliar  $\delta^{15}\text{N}$  (‰) (mean ± SD; n = 3) in balsam fir (BF) and black spruce (BS) seedlings in the four experimental plots at the BER and SIM sites.

Site	Species	$\delta^{15}\text{N}$ (‰)					
		C			Treatments		
		C	W	$\Delta_{[W-C]}$	N	WN	$\Delta_{[WN-N]}$
BER	BF	1.56 ± 1.37	1.69 ± 0.02	0.13 ± 1.39 (ns)	2.46 ± 0.56	2.75 ± 1.51	0.29 ± 1.82 (ns)
	BS	-4.83 ± 0.53	-4.26 ± 0.52	0.57 ± 0.96 (ns)	-2.43 ± 0.59	-1.80 ± 0.83	0.62 ± 0.28 (ns)
SIM	BF	3.81 ± 0.99	2.52 ± 0.74	-1.29 ± 0.60 (ns)	5.88 ± 2.20	6.78 ± 0.60	0.90 ± 2.62 (ns)
	BS	-2.89 ± 0.33	-2.02 ± 0.70	0.87 ± 0.80 (ns)	-0.27 ± 0.63	2.32 ± 2.35	2.59 ± 1.79 (ns)

The differences in foliar  $\delta^{15}\text{N}$  between the warmed (W) and the control (C) plots ( $\Delta_{[W-C]}$ ) and between the warmed and fertilized (WN) and fertilized-only (N) plots ( $\Delta_{[WN-N]}$ ) are displayed to show the effect of soil warming on foliar  $\delta^{15}\text{N}$  in the unfertilized and fertilized plots, respectively. ns, not significant (P > 0.05).



soil  $\text{NH}_4^+$  concentration in the long term. Although not statistically significant, the soil warming treatment slightly increased BS foliar  $\delta^{15}\text{N}$  in both N+ and N- treatments (Table 5), which may be the result of a decrease in the contribution of N derived from EcM to the seedling N nutrition. This hypothesis is consistent with the reported decrease in the proportion of EcM fungi consecutive to soil warming at the BER site (Rossi et al., 2013).

The most significant effect of soil warming was on seedling phenology. We clearly observed an earlier and more rapid budbreak in W+ than in W- plots at both sites and for both seedling species. This likely resulted from a ~2-week earlier snow melting in warmed plots, which mimicked an earlier initiation of the growing season. This also reflects the commonly reported influence of soil temperature on the triggering of budbreak in trees and confirms the potential of climate warming to impact the phenology of trees (Hugues, 2000; Hannah, 2015). The induction of budbreak earlier in the spring due to climate warming is not without risks for the trees as it exposes them to late frosts (Silvestro et al., 2019).

## CONCLUSION

Our data show the strong stability of the soil in these boreal forests in which N net mineralization rate, soil chemistry and soil OM quality in the forest floor were barely impacted by 9 years of soil warming and canopy N addition. This absence of impact on the soil may explain that the growth and the foliar chemistry of the seedlings were not affected during their 2 first years in the field. The higher growth rate of seedlings at BER—where net N mineralization rate and soil  $\text{NH}_4^+$  pool is lower—suggests that seedlings may not be limited only by inorganic N availability at our sites. Seedling growth may be more tied to the level of EcM association and to gross mineralization rate, particularly for BS. The observed decreased foliar  $\delta^{15}\text{N}$  after 2 years in the field at BER actually points toward a larger use of N derived from EcM fungi at this site. Although not statistically significant, the slightly higher BS foliar  $\delta^{15}\text{N}$  in warmed than in unwarmed plots suggests that this treatment reduced EcM contribution to seedling N nutrition, which corroborates the previously reported decrease in EcM biomass resulting from soil warming at BER. The main effect of soil warming was on seedling phenology. The treatment caused a more precocious and rapid budbreak for both BS and BF at both sites, which confirms the influence of soil temperature and the impact of climate warming

on the phenology of trees. Overall, our data showed only a small effect of N addition and soil warming on soil N availability and seedling growth. Climate warming, by initiating earlier and faster bud development, may however expose seedlings to late spring frosts in the future and eventually impact tree growth and forest community establishment and structure.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

## AUTHOR CONTRIBUTIONS

HM, RB, DH, NT, and MP designed the study. JP and ÉD-C performed field and lab work. MS performed the NMR analyses. CM analyzed the data and drafted the manuscript. MP, HM, RB, DH, NT, RO, and MS reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/ffgc.2020.581363/full#supplementary-material>

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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