Paludification reduces black spruce growth rate but does not alter ecophysiological mechanisms in Canadian boreal forested peatlands

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Abstract

Background: Paludification is widespread in the boreal biome, inducing tree growth decline in forested peatlands following the development of thick organic layers over the mineral soil. However, the ecophysiological processes involved remain poorly documented and little is known about the interactions between tree growth mechanisms and site conditions in these ecosystems. We investigated changes in stem growth and main ecophysiological processes in a black spruce forested peatland in eastern Canada by combining peat-based and tree-ring stable isotope analyses. These were conducted at three sampling sites located along a paludification gradient with different peat thicknesses.

Results: Organic layer thickening induces black spruce growth decline without altering tree ecophysiological mechanisms. A 40% increase in water use efficiency, or the ratio of carbon assimilated to water losses, was observed at the three sites from 1920 to the 1980s, but did not
translate into enhanced tree growth. A clear shift in the 1980s revealed a decline in black spruce sensitivity to climate and rising atmospheric CO₂ concentration, regardless of the organic layer thickness. Water table reconstructions revealed an important drawdown in the last few decades at the three sites, but we found no evidence of an influence of water table variations on stem growth.

**Conclusions:** This study shows that paludification induces black spruce growth decline without altering tree metabolism in boreal forested peatlands. This underlines that changes in water use efficiency are decoupled from changes in carbon allocation, which are constrained by site, or even tree-specific strategies to access water and nutrients from belowground. Our findings indicate that dynamic changes in edaphic conditions need to be considered in process models. Otherwise, failing to account for the degree of paludification can lead to misleading forest productivity predictions and result in considerable overestimations of aboveground carbon stocks from trees in the boreal regions.

**Keywords:** black spruce growth, boreal biome, climate-growth relationships, ecophysiological response, forested peatland, paludification, stable isotopes, water use efficiency

**Background**

Forested peatlands are widespread ecosystems in the boreal regions of the Northern Hemisphere that result from the paludification process (Korhola 1995; Crawford et al. 2003; Lavoie et al. 2005). Northern forests are particularly prone to paludification due to their cold and humid climates, particularly when combined with flat topography and fine-textured sediments (Payette 2001; Charman 2002). This process is characterised by the development of thick organic layers over the mineral sediment, favored by the establishment and expansion of *Sphagnum* mosses, which leads to humid, acid, cold, and anaerobic soil conditions (Van Cleve et al. 1983; Fenton and Bergeron 2006). This edaphic context causes forest growth decline, primarily by limiting nutrient availability.
eventually results in the establishment of open and/or forested peatlands. Over the past decades, many studies have focused on documenting the effects of paludification on tree biomass productivity (e.g., Lecomte et al. 2006; Simard et al. 2007, 2009) and developing management practices to reduce or even reverse this process in coniferous boreal forests (e.g., Heikurainen 1964; Lavoie et al. 2005; Bergeron et al. 2007; Fenton et al. 2009; Lafleur et al. 2011).

While the impacts of paludification on stem growth are well understood, forest ecophysiological mechanisms associated with growth decline remain unknown at the stand scale. In non-paludified forests, an increase in tree intrinsic water use efficiency (i.e., the ratio of carbon assimilated to water losses through evapotranspiration; Farquhar et al. 1989) associated with rising ambient CO2 concentration has commonly been reported without enhancing carbon use efficiency (Manzoni et al. 2018) or tree radial growth (e.g., Peñuelas et al. 2011; Silva and Horwath 2013; Lévesque et al. 2014). Indeed, while photosynthesis stimulation and stomatal conductance reduction control changes in water use efficiency, stem growth appears to be limited by local edaphic factors, such as nutrient availability (e.g., nitrogen). Paludification is thus expected to alter both water and carbon use efficiency in boreal forested peatlands, given that this process induces significant changes in soil conditions.

Both vegetation and hydrological dynamics in paludified settings have previously been studied over millennial timescales (Ruppel et al. 2013; Le Stum-Boivin et al. 2019; Magnan et al. 2019). Nonetheless, little is known about the implications of changes in ecosystem conditions on tree growth mechanisms in forested peatlands. Paludification is characterized by water table rise (Lavoie et al. 2005; Fenton and Bergeron 2006), and interactions over time may indeed exist between water table fluctuations, tree nutrition, and stem growth responses in boreal ecosystems. These interactions may influence the respective contribution of ecophysiological processes such as
photosynthesis and stomatal conductance, which control tree water use efficiency. Enhanced knowledge of forested peatland ecophysiology may ultimately improve the modelling of the response of these ecosystems to increasing CO\textsubscript{2} concentrations by dynamic global vegetation models (DGVMs) (Pugh et al. 2016).

In this study, we aim to improve the understanding of the mechanisms that support tree radial growth in black spruce \((Picea mariana \text{ (Mill.) BSP})\)-forested peatlands of eastern Canada, using an innovative approach that combines multi-proxy analyses. Peat-based paleoecohydrological analyses were performed in parallel with stable isotope analyses in tree rings to investigate the interactions between black spruce metabolism and site conditions. We hypothesise that peat accumulation triggers changes in tree ecophysiological mechanisms (e.g., reduction in photosynthesis rates) which result in stem growth decline. We expect this effect to be stronger in the most paludified sites characterised by thicker peat deposits.

**Methods**

**Study area**

The study was conducted south of James Bay in eastern Canada, within the Clay Belt region part of the black spruce-feather moss bioclimatic domain (Saucier et al. 2009; Fig. 1). This area is particularly prone to paludification due to the relatively cold and humid climate, the flat topography, and the dominance of poorly-drained clayey sediments left by the proglacial lakes Barlow and Ojibway (Vincent and Hardy 1977; Fenton et al. 2005; Lavoie et al. 2005). Mean annual temperature is 0.3°C (over the 1950-2013 period), ranging from -18.9°C in January to 16.3°C in July, and mean annual precipitation is 818 mm (McKenney et al. 2011). The regional fire cycle is estimated to be ~400 years since 1920 (Bergeron et al. 2004), allowing the accumulation of thick organic layers in forests between fire events.
The Casa forested peatland (49°33'06"N, 78°59'10"O; Fig. S2.1) was selected following the studies of Magnan et al. (2020) and Le Stum-Boivin et al. (2019) due to its regional representativeness in terms of slope, vegetation composition, and canopy openness. The organic layer thickness varies between 40 cm and more than 1 m along the selected transect, and the canopy gradually opens with organic layer thickening, which is typically observed in forested peatlands of the Clay Belt. The aboveground vegetation is largely dominated by black spruce and ericaceous shrubs, such as *Vaccinium angustifolium*, *Rhododendron groenlandicum*, *Kalmia angustifolia*, and *Chamaedaphne calyculata*. The understory is dominated by *Sphagnum* communities, particularly *S. angustifolium/fallax* under the tree canopy, and *S. fuscum* where the tree canopy is more open.

**Figure 1. Location of the studied Casa boreal forested peatland (red dot).**

**Sampling**

Three sampling sites (CAS0, CAS50, CAS100) were established along a 100 m transect following an organic matter thickness gradient within the selected forested peatland (Fig. 2). At each site, one peat monolith was sampled down to the mineral contact using a Box corer (Jeglum et al. 1992). Sampling locations were chosen to be representative of the mean peat thickness of each site.
Relative surface altitude and peat thickness were measured at 5 m intervals along the transect using a high precision altimeter (ZIPLEVEL PRO-2000) and an Oakfield probe. Water table depths were measured at the same intervals a few hours after holes were dug to make sure that the water table level had stabilized. Twenty black spruce trees were also sampled at each site within a 10 m radius of the collected peat core. Only dominant and codominant trees with straight stems and no visible scars were selected. Peat thickness was measured at the bottom of each sampled tree to validate the concordance with the mean peat thickness of the site. The diameter at breast height (DBH) and the height of selected trees were measured and cross-sections were collected at standard height (1.3 m). The root system of one black spruce per site was excavated to verify the depth of the rooting zone, and to identify the growth substrate (i.e., mineral or organic matter). Moreover, tree aboveground biomass of each site was estimated by measuring the diameter at breast height (DBH) of all trees (DBH ≥ 1 cm) within a 10×10 m plot and then using allometric equations adapted to black spruce growth (Lambert et al. 2005; Ung et al. 2008).

Figure 2. Schematic of the three sites along the study transect. Relative altitude of the organic layer (green) and the mineral surface (grey) are shown. Black rectangles represent the locations of the sampled peat cores. The dotted blue line indicates the water table level measured on the field. Trees are not to scale but are representative of variations in canopy openness along the transect.
Peat-based paleoecohydrological reconstructions

Prior to analysis, peat cores were cut into 1 cm-thick slices. Plant macrofossils were analysed at 4 cm intervals along each peat core to reconstruct vegetation dynamics since peat initiation. Samples were prepared following the standard protocol of Mauquoy et al. (2010) and analysed in a gridded Petri dish under a stereoscopic microscope (10-40 × magnification) (SM 1.1). The relative abundances of the main peat components (e.g., Sphagnum, ligneous, Cyperaceae) were estimated visually and expressed as volume percentages, and vascular plant remains (e.g., seeds, needles, leaves) were counted. Macroscopic charcoal particles (>0.5 mm) were analysed at 1 cm intervals along the three peat cores to identify past local fire events.

In order to reconstruct hydrological variations, testate amoeba assemblages were also analysed at 1 cm intervals. Testate amoeba shells were extracted following the standard protocol of Booth et al. (2010) (SM 1.2). Samples were then analysed under an optical microscope (400× magnification). A minimum of 100 tests was counted per sample, except in highly humified peat samples, in which test concentration was very low. In these cases, no water table depth (WTD) was inferred, as the total count (< 20 tests) was insufficient to ensure reliable WTD reconstruction (Payne and Mitchell 2009).

Past WTDs were reconstructed using a weighted average model with tolerance down-weighting and inverse deshrinking (WA.inv.tol). The transfer function was built using the R package rioja (version 0.9-15.1; Juggins 2017), from a modern dataset of 272 surface samples combining non-forested open peatlands (Lamarre et al. 2013) and forested peatlands (Beaulne et al. 2018 and this study) of eastern Canada. High inferred WTD values corresponded to drier surface conditions.
Peat core chronologies

A total of 11 samples were submitted to A. E. Lalonde AMS Laboratory (University of Ottawa, Canada) for accelerator mass spectrometry radiocarbon dating ($^{14}$C). Plant macrofossil remains were carefully selected to date peat initiation, the last fire event, and main transitions in vegetation composition at each sampling site. The $^{14}$C dates were calibrated using the IntCal13 calibration curve (Reimer et al. 2013). Additional $^{210}$Pb dating was achieved for the uppermost 24-26 cm of peat cores at 1 cm intervals by alpha spectrometry (EGG Ortec 476A) at the GEOTOP Research Center (Université du Québec à Montréal, Canada). Ages were inferred by $^{210}$Po activity measurement, using the constant rate of supply model (Appleby and Oldfield 1978) following HNO$_3$-HCl-H$_2$O$_2$ sample digestion. Further details on the $^{210}$Pb dating procedure used in this study can be found in Ali et al. (2008). Age-depth models were generated using the rbacon package in R (version 2.3.9.1; Blaauw and Christen 2019). Ages are expressed in calendar years before present (cal yr BP; 1950 CE) and the age of the peat surface is therefore set to -67 cal yr BP (coring year: 2017 CE).

Black spruce radial growth analysis

Dried cross-sections were finely sanded (from 80 to 600 grit size) prior to ring-width measurements along two radii using CooRecorder software (version 8.1.1; Cybis Elektronik & Data AB 2016). Samples were visually cross-dated using PAST5 software (version 5.0.610; SCIEM 2019), and skeleton plots were generated using the R package dplR (version 1.6.9; Bunn et al. 2018). Ring-width series were converted to annual basal area increment (BAI) to compare tree aboveground productivity between the three sites, as BAI is more representative of three-dimensional stem growth than the linear ring-width measurements (Husch et al. 2003; Biondi and Qeadan 2008). Individual BAI series were produced using the R package dplR (version 1.6.9; Bunn et al. 2018), and yearly averages were then calculated using all trees from the same site.
Ring-width series were standardized using a negative exponential curve to remove cambial age
trends (Fritts 1976). Standardization was performed on all individual series before constructing a
mean standardized chronology for each site. Daily climate data (mean temperature and total
precipitation) from 1950 to 2013 were retrieved from the interpolated gridded climate dataset of
McKenney et al. (2011). Pearson correlation coefficients were calculated between standardized
ring-width series and monthly climate data from March to September of both the current year and
the year preceding ring formation. Because of time series autocorrelation, effective numbers of
degrees of freedom were calculated to generate adjusted $p$-values (Hu et al. 2017).

Isotopic analysis of tree rings

Black spruce ecophysiological response to rising ambient CO$_2$ concentration and climate variability
was evaluated from carbon ($\delta^{13}$C) and oxygen ($\delta^{18}$O) isotopic ratio analyses. These were performed
on five trees per site and from two wood strips per tree (i.e., a total of 30 samples). Sample
preparation was carried out following the protocol described in Giguère-Croteau et al. (2019) (SM
1.3). A five-year resolution over a 100 year period (1919-2018) was considered. Alpha-cellulose
was extracted, as suggested for black spruce samples (Bégin et al. 2015), following the protocol
used by Naulier et al. (2014).

Tree-ring $\delta^{13}$C values vary according to discrimination against $^{13}$C during photosynthesis, defined
as (Farquhar et al. 1982):

$$\Delta^{13}C = \frac{\delta^{13}C_{air} - \delta^{13}C_{tree}}{1 + (\delta^{13}C_{tree}/1000)},$$

where $\delta^{13}C_{air}$ is the carbon isotope ratio of the atmosphere and $\delta^{13}C_{tree}$ is the isotopic value of the
tree ring. $\delta^{13}C_{air}$ values were taken from McCarroll and Loader (2004) for the 1919-2003 period,
and were linearly extrapolated for the 2004-2018 period. Because of the five-year resolution of 
$\delta^{13}C_{\text{tree}}$ values, we averaged the $\delta^{13}C_{\text{air}}$ values over five years. According to Farquhar et al. (1989), 
$\Delta^{13}C$ is related to leaf intercellular CO$_2$ concentration ($c_i$) and ambient CO$_2$ concentration ($c_a$) 
according to the following equation:

\[
\Delta^{13}C = a + (b - a)\left(\frac{c_i}{c_a}\right),
\] (2)

where $a$ (4.4‰) is the fractionation occurring during CO$_2$ diffusion through stomata (O’Leary 1981) and $b$ (27‰) is the fractionation due to carboxylation by the Rubisco enzyme (Farquhar and Richards 1984). Values of $c_a$ were obtained from the Mauna Loa Observatory (esrl.noaa.gov/gmd/ccgg/). Intrinsic water use efficiency (iWUE), defined as the amount of carbon assimilated per unit of water lost, can then be estimated from $c_i$ and $c_a$ as follows (Ehleringer et al. 1993):

\[
iWUE = \frac{A}{g_s} = \left(\frac{c_a - c_i}{1.6}\right),
\] (3)

where $A$ is the rate of CO$_2$ assimilation, $g_s$ is the stomatal conductance, and the constant 1.6 represents the ratio of water vapor and CO$_2$ diffusivity in air. Equation 3 shows that the difference between $c_a$ and $c_i$ is related to the ratio of assimilation ($A$) to stomatal conductance ($g_s$).

Since the $\delta^{18}O$ composition of tree rings is mainly controlled by leaf water composition and enrichment due to transpiration of lighter oxygen isotopes, $\delta^{18}O$ values are assumed to be related to the stomatal conductance and independent of photosynthetic activity (Yakir 1992; Barbour 2007). Therefore, by combining $\delta^{13}C$ and $\delta^{18}O$ analyses it is possible to discriminate the effects of changes in photosynthetic rate ($A$) and stomatal behavior ($g_s$) on iWUE (Scheidegger et al. 2000).
Results

Paleoecohydrological reconstructions

The study sites CAS0, CAS50, and CAS100 have an organic layer thickness of 40, 75, and 100 cm respectively (Table 1). Tree-ring analyses revealed even-aged stands covering the period 1839-2018 CE at each site (see sample depth in Fig. 4 for tree age variability). Radiocarbon dating of the most recent charcoal layer indicates that the last fire event occurred between 0 and 290 cal yr BP (median age: 175-179 cal yr BP; Table S2.1). These results suggest that trees were from the first cohort that grew after the last local fire, which most likely occurred around 200-250 years ago (~1800 CE). The depth of the uppermost charcoal layer in the peat profile indicates that black spruce established in a residual organic layer of 15, 45, and 65 cm at sites CAS0, CAS50, and CAS100 respectively. The root system excavation of the three selected trees suggests that roots reached the mineral soil at CAS0 and CAS50, but were restricted to the organic layer at CAS100.

Table 1. Characteristics of the three study sites.

| Site   | Organic layer thickness (cm) | WTD (cm) | Mean DBH (cm) | Trees (n) | Tree biomass (kg m⁻²) | Tree densityᵃ (trees ha⁻¹) | Mean tree heightᵇ (m) |
|--------|------------------------------|----------|---------------|-----------|-----------------------|---------------------------|----------------------|
| CAS0   | 40                           | 25       | 10.4          | 20        | 8.9                   | 1200                      | 13.8                 |
| CAS50  | 75                           | 19       | 9.4           | 24        | 7.6                   | 1200                      | 11.3                 |
| CAS100 | 100                          | 10       | 5.6           | 34        | 4.6                   | 1000                      | 10.4                 |

ᵃ Include trees with a diameter at breast height (DBH) ≥ 9 cm
ᵇ Calculated from the twenty black spruce trees (dominant and co-dominant) sampled at each site.

Both hydrological conditions and vegetation composition were similar across the three sites throughout the duration of black spruce growth (Fig. S2.2, S2.3). Macrofossil analysis showed that the last fire induced a shift in vegetation composition from high dominance of woody vegetation to a black spruce-*Sphagnum*-dominated stand (Fig. S2.2). The canopy opening allowed rapid
*Sphagnum* moss expansion in the bryophyte layer while the black spruce post-fire cohort established. Testate amoeba records indicate relatively wet conditions (high water tables) shortly after the fire, followed by a gradual lowering of the water table at the three sites (Fig. 3, S2.3). Inferred WTD values show very similar hydrological conditions at CAS50 and CAS100 during the post-fire period (1840-2017). Both sites had stable water table depths between 15 and 20 cm before water tables deepened from the 1990s, and particularly in the very recent horizons (~2010), while the water table lowered more gradually at CAS0.

![Figure 3. WTD reconstructions for the post-fire period based on testate amoeba records. Error bars of both WTD reconstructions and age-depth modelling are shown by pale thin lines.](image)

**Black spruce radial growth**

A decrease in DBH and tree height values was observed in relation with organic layer thickening along the paludification gradient (Table 1). Mean DBHs of 10.4, 9.4, and 5.6 cm were calculated for CAS0, CAS50, and CAS100 respectively. BAIs also indicate a decrease in stem growth with increasing peat thickness (Fig. 4, S2.4). Trees from CAS0 added a greater wood surface with age, especially since 1940, resulting in an increasing BAI trend (mean BAI=70 mm²). At CAS50, tree radial growth was more limited (mean BAI=51 mm²). In contrast, trees from CAS100 maintained relatively constant BAI values, resulting in decreased wood production (mean BAI=40 mm²).
Estimates of tree aboveground biomass showed similar trends with values of 8.9, 7.6, and 4.6 kg/m² for sites CAS0, CAS50, and CAS100 respectively.

![Graph showing basal area increment over time for sites CAS0, CAS50, and CAS100](image)

**Figure 4.** Mean annual basal area increment of black spruce trees since their establishment after the last fire event. The decrease in sample depth at CAS100 since 2000 is explained by some trees for which the latest rings were partly absent. See Fig. S2.4 for BAI distribution.

**Stem growth response to climate**

Pearson correlations between standardized ring-width series (Fig. S2.5) and monthly temperature and precipitation data were performed separately for the 1950-1980 and 1981-2013 periods. These two time periods were chosen because of the shift in black spruce ecophysiology around 1980 (see next section) and the results of moving correlations with climate variables (results not shown).

Temperature’s influence on tree radial growth was stronger than that of precipitation for both time periods. Trees had a similar response to climate at all sites for the period 1950-1980 (Fig. 5). For
all sites, negative correlations were observed between tree stem growth and both temperature of
the previous August and April precipitation of the current year. Stem growth was also positively
correlated with September temperature of the current year. Contrastingly, stem growth barely
responded to climate during the 1981-2013 period, with much less significant and rather
heterogeneous correlations at the three sites (Fig. 5).

Figure 5. Pearson correlations between (a) standardized ring-width and monthly
temperature, and (b) standardized ring-width and monthly precipitation for the periods
1950-1980 and 1981-2013. Correlation coefficients were calculated from March to September of
the current year and the year preceding ring formation. Months from the previous year of stem
growth are marked with an asterisk and significant correlations ($p < 0.05$) are marked with crosses. Results from CAS0, CAS50, and CAS100 are shown in black, red, and blue respectively.

**Trends in $\delta^{13}C$, $\delta^{18}O$, and iWUE**

The $\delta^{13}C$-derived ecophysiological parameters do not differ between the three sites over the 1919-2000 period (Fig. 6a). Over time, black spruce trees used two different strategies in response to rising $c_a$. A substantial increase in iWUE was first observed until the 1980s ($c_a \approx 340$ ppm), along with relatively stable intercellular CO$_2$ concentration ($c_i$). During this period, iWUE increased by 43% at each site. A major shift in tree ecophysiology then occurred in the mid-1980s as $c_i$ began to increase considerably. In parallel, iWUE stabilized until 2018, except for at CAS0, where a new increase seems to have begun around 2000.

Tree-ring cellulose $\delta^{18}O$ analyses show similar trends for the three sites across the whole record (Fig. 6b). However, oxygen stable isotope ratios were systematically lower at the least paludified site (CAS0), suggesting a greater depletion in heavy isotopes. For all series, tree-ring $\delta^{18}O$ values increased until ~1950 and became more constant afterwards.
Discussion

Our study demonstrated that the degree of paludification considerably altered growth conditions and site fertility, but did not influence intrinsic water use efficiency of black spruce trees. Indeed, sites with the thickest organic matter accumulation were characterized by dominant trees that grew
slower, presented smaller heights and diameters (DBH), and had a lower tree density comparatively to the least paludified site (Table 1). Surprisingly, however, $\delta^{13}C$-derived parameters are almost identical in all sites (Fig. 6), both in terms of average iWUE levels or temporal variations, suggesting that the ratio of photosynthesis to stomatal conductance is unaltered by the degree of paludification. We therefore refute our research hypothesis, and cannot ascertain a clear and direct effect of increased peat accumulation on black spruce water use efficiency and growth mechanisms.

Site fertility was recently put forward as an important parameter influencing black spruce iWUE and its evolution with changes in $c_a$ (Marchand et al. 2020). Based on a network of permanent sampling plots in eastern Canada, average iWUE values were shown to be lower in the most fertile sites (i.e., fertility being measured based on tree heights for equivalent age). Fertile sites also experienced the most drastic iWUE increase with rising $c_a$, while this increase remained modest in the least fertile sites. However, in their study, Marchand et al. (2020) examined only sites where organic matter accumulation was inferior to 30 cm. Along our paludification gradient, peat thickness reaches about 100 cm at CAS100 and therefore other processes linking organic matter accumulation, water table depth, and climate need to be invoked in order to shed light on interactions between iWUE and radial growth of black spruce in a paludified context.

Synchronous changes in iWUE over time

1920-1980s: iWUE increases (active response)

From the 1920s and until the 1980s, a ~40% iWUE increase was observed at each site, regardless of the accumulated organic layer thickness. This significant increase, which occurred over a short period of time, is among the highest recorded; most studies report iWUE increases of 20-30% over the last century (e.g., Peñuelas et al. 2011; Silva and Horwath 2013; Saurer et al. 2014; Frank et al. 2015; van der Sleen et al. 2015). The increased iWUE resulted from an active response of trees
characterized by the maintenance of a relatively constant $c_i$ despite rising $c_a$. According to Scheidegger et al. (2000), when both $\delta^{13}C$ and $\delta^{18}O$ values increase, as subtly observed here until the 1950s (Fig. 6), stomatal conductance most likely is the main driver of increased iWUE since it is involved in the fractionation of both stable isotopes. Stomatal closure was possibly induced by the combined effect of rising $c_a$ and severe drought events that took place in the study area at the beginning of the twentieth century (Girardin et al. 2004). This reduction in stomatal conductance could have limited growth during the 1920-1950s period, leading to the formation of smaller and narrower cells (Puchi et al. 2020; Fig. 4, S2.5). After 1950 and until the 1980s, $\delta^{13}C$ values continued to increase, whereas $\delta^{18}O$ values remained relatively constant. This points to a shift in acclimation strategies from a $g_s$-controlled iWUE to an $A$-controlled increase in iWUE, maintaining $c_i$ values constant.

1980s-2010s: iWUE stabilises (passive response)

In the 1980s, tree response to rising $c_a$ suddenly became passive, as shown by the increasing $c_i$ and the relatively constant $c_a$-$c_i$ (iWUE) values at all sites (Fig. 6). Likewise, a shift to a passive response to increasing CO$_2$ concentration has previously been observed for various tree species in the Canadian boreal forest (Giguère-Croteau et al. 2019; Marchand et al. 2020), in China (Wang et al. 2012; Wu et al. 2015), and in Europe (Waterhouse et al. 2004; Gagen et al. 2011; Linares and Camarero 2012). Three reasons might explain this shift in acclimation strategies. Firstly, this finding possibly indicates reduced carbon assimilation rates ($A$). Indeed, in such poor growing environments, the photosynthesis apparatus may saturate and nutrient limitation may downregulate the capacity of trees to assimilate atmospheric carbon (Tognetti et al. 2000; Saurer et al. 2003). Secondly, WTD reconstructions indicate important changes in hydrological conditions over the last 30 years (Fig. 3) that might have altered black spruce iWUE. The recent water table drawdown could have generated stressful growth conditions since black spruce develops adventitious roots
that are generally confined to the upper 20-30 cm of the organic layer (Lieffers and Rothwell 1987; Viereck and Johnson 1990). However, such a drop in WTD would have most certainly been accompanied by a reduction in stomatal conductance, which was not observed here. Moreover, it remains unclear whether the apparent drying trend reflects increasingly drier site conditions or simply an enhanced vertical *Sphagnum* mosses growth that disconnects the peat surface from the water table. Increasingly warmer conditions since the 1990s (Fig. S2.6) could have triggered this rapid peat accumulation (Magnan et al. 2018; van Bellen et al. 2018; Primeau and Garneau, under review; Robitaille et al. submitted). The rapid accumulation of organic matter may have exceeded the capacity of adventitious roots to develop higher in the soil profile, compromising the access to oxygen. Lastly, considering that black spruce trees were approximately 180 years old in the 1980s, we cannot rule out the stand age as another potential cause for the reduction in iWUE (Irvine et al. 2004; Kutsch et al. 2009; Marchand et al. 2020).

Stem growth is decoupled from iWUE variations

Our results indicate that lower radial growth rates are found with increasing peat accumulation (Fig. 4). This effect was also reported in the black spruce feather moss domain of the James Bay and Abitibi lowlands of eastern Canada (Harper et al. 2003; Fenton et al. 2005; Lecomte et al. 2006; Simard et al. 2007). Low stem growth rates have mostly been attributed to the decrease in nutrient availability induced by peat accumulation (Van Cleve and Viereck 1981; Prescott et al. 2000; Simard et al. 2007). Nitrogen concentration is particularly low in peat deposits and consequently, the effect of limited nitrogen availability on boreal forest growth (Macdonald and Lieffers 1990; Tamm 1991; Vitousek and Howarth 1991; Maynard et al. 2014) is certainly exacerbated by paludification. The thickness of the post-fire residual organic layer in which black spruce trees established is thereby critical in determining tree nutrient uptake and aboveground biomass production. It is well established that black spruce roots can penetrate to a depth of about 60 cm,
although they are generally limited to the upper 20-30 cm (Lieffers and Rothwell 1987; Viereck and Johnson 1990). Trees from CAS0 and CAS50 both established in a less than 50 cm-thick organic horizon, and roots could therefore easily reach the mineral soil, resulting in higher radial growth rates at these sites. On the other hand, tree roots were unable reach the mineral soil layer at CAS100 as black spruce trees established in a 67 cm-deep organic layer.

These findings imply that processes controlling carbon use and allocation to radial tree growth are decoupled from those that control iWUE. This is in agreement with studies suggesting that iWUE increases do not directly translate into enhanced radial growth (e.g., Peñuelas et al. 2011; Lévesque et al. 2014; van der Sleen et al. 2015; Giguère-Croteau et al. 2019). Based on a comparison of tree ring widths and eddy-covariance flux towers in boreal Canada, Pappas et al. (2020) showed that aboveground biomass, and most particularly radial stem growth, represents only a minor fraction (~9%) of the total gross ecosystem production (GEP). Rocha et al. (2006) also found that stem growth, as estimated from tree ring widths, was not correlated to eddy-covariance-derived GEP in the boreal forest of central Manitoba. These findings point into the same direction: gas exchanges at the vegetation-atmosphere interface are controlled at the leaf level, but the allocation of newly formed photosynthates to either above- or below-ground compartments may depend on local growing conditions and site-specific growth strategies. Our study must therefore be seen as an extreme case where paludification induced locally-important edaphic changes that resulted in large differences in site fertility. Prioritization of belowground growth was more important in the most paludified (least fertile) sites, neglecting carbon allocation to aboveground compartments. This allocation strategy could reinforce tree anchoring (Nicoll et al. 2006) and enhance nutrient uptake (Vicca et al. 2012; Fernández-Martinez et al. 2014), but further research is needed to shed light on the processes driving allocation changes in black spruce trees.
The proportionality of the $A/g_s$ ratio needs to be preserved between sites in order to maintain comparable iWUE values across the paludification gradient (both in terms of mean and variability). This implies that if $A$ is higher in the least paludified site (e.g., CAS0) and lower in the most paludified site (e.g., CAS100), then $g_s$ will adjust in such a way to maintain nearly identical $A/g_s$ ratio, and consequently, iWUE values. Actually, we suspect that this proportional adjustment in the $A/g_s$ ratio might be an important process driving interactions between iWUE and growth rates in a paludified context. As a supporting evidence for this, we found that black spruce tree ring cellulose from the least paludified site (CAS0) was significantly more depleted in $^{18}$O compared to that of other sites (Fig. 6b). Unsurprisingly, CAS0 is also the site where radial growth rates are the highest. Increased evapotranspiration rates are probably required to sustain enhanced carbon assimilation and growth rates, forcing $g_s$ to level up and proportionally adjust to increases in $A$ (matching the ratio of other sites). Consequently, higher evapotranspiration rates cause black spruce to pump more $^{18}$O-depleted water from soil depths (Evaristo et al. 2017), which in turns decreases average $\delta^{18}$O of tree ring cellulose.

**A shift in tree response to climate in the 1980s**

Difference in peat thickness along the paludification gradient did not have a noticeable influence on tree response to climate. Before the 1980s, tree growth from all sites showed negative correlations with previous summer temperatures (Fig. 5). These climate-growth relationships have previously been reported for the Canadian boreal forest (e.g., Drobyshev et al. 2010; Walker and Johnstone 2014; Girardin et al. 2016; Gennaretti et al. 2017). High summer temperatures in the previous growing season can restrict carbon reserve accumulation, which shapes early wood development the next growing season (Fritts 1976; Skomarkova et al. 2006; Campioli et al. 2011). Black spruce growth also responded positively to September temperature of the current year and negatively to
April precipitation of the current year, which suggests that stem growth benefits from a longer growing season.

After the 1980s, trees however became much less sensitive to temperature and precipitation (Fig. 5). This could possibly represent a collateral effect of paludification. Interestingly, the shift indicating a clear decline in black spruce sensitivity to climate is synchronous with the decline in iWUE. The reduced sensitivity of trees to temperature since the mid-twentieth century has been reported in previous tree ring studies of northern latitudinal forests, and has been referred to as the “divergence problem” (e.g., Briffa et al. 2004; D’Arrigo et al. 2008; Esper and Frank 2009; Schneider et al. 2014). This “divergence” phenomenon could potentially be caused by thresholded responses or stresses induced by changes in growth conditions (D’Arrigo et al. 2008). The important WTD deepening over the last decades (Fig. 3) might have contributed, along with stand age (Szeicz and MacDonald 1994; Konter et al. 2016), to the decline in tree sensitivity to climate (at the stem level) in our sites.

Overall, this study represents a first attempt to unravel the numerous and complex entanglements between paludification dynamics and forest ecophysiology in the boreal forest of eastern Canada. Based on our results, we conclude that taking into account factors reflecting site edaphic conditions is essential to better describe and predict forest response to environmental variability, especially in nutrient-limited ecosystems (Ainsworth and Rogers 2007; Kirschbaum 2011; Lévesque et al. 2016; Guerrieri et al. 2019). Our findings warrant further studies of vegetation/forest dynamics models and their application to forested peatlands, as those models are often biased towards converting increases in iWUE into increases in stem growth. For example, a successful modelling of the c/c_a ratio based on the least-cost optimality principle (Lavergne et al. 2020) would predict comparable iWUE trends, regardless of the degree of paludification and with correct implications for carbon and water cycle interactions at the leaf level. However, failing to account for paludification-related
carbon allocation strategies would result in the overestimation of aboveground biomass production in sites where peat accumulation is rapid. Peatlands are one the largest natural terrestrial ecosystems for carbon sequestration, and forested peatlands represent a major component of these ecosystems in boreal regions (Thompson et al. 2016; Webster et al. 2018). Therefore, additional research on carbon allocation strategies are of utmost importance to understanding the carbon sink capacity of black-spruce-dominated boreal ecosystems.

Conclusions

In this study, we used an innovative approach to evaluate black spruce radial growth in sites undergoing paludification by combining paleoecohydrological, dendrochronological, and geochemical analyses. We provide the first multi-proxy tree-ring chronologies showing how radial tree growth and main metabolic processes change along a paludification gradient.

Contrary to our expectations, results show that the degree of paludification does not alter black spruce metabolism in boreal forested peatlands. The accumulation of thick organic layers does induce stem growth decline, but this tree response to paludification is not reflected in black spruce ecophysiological mechanisms. The increasing iWUE trends observed could suggest increasing carbon assimilation and radial growth rates at the three sites. However, radial growth of black spruce trees clearly declined with organic layer thickness, resulting in different tree aboveground biomass between the study sites. This underlines that changes in iWUE are not necessarily related to changes in carbon use efficiency because of site conditions (Manzoni et al. 2018). Consequently, dynamic changes in edaphic conditions need to be considered in process models (Guiot et al. 2014). Otherwise, based on tree ecophysiological parameters alone, comparable growth between the three study sites would have been assumed. Our results thus suggest that failing to account for degree of paludification in interpreting tree growth mechanisms can lead to misleading forest productivity
predictions in the boreal biome. This could furthermore result in a considerable overestimation of
carbon stocks from trees in the boreal regions where paludified forests and forested peatlands are
widespread.

Declarations

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Authors’ contributions

All authors designed the research and conducted the fieldwork. J.B. performed the research and
É.B., M.G. and G.M. helped analyzing the data. J.B. wrote the first draft of the manuscript and all
authors contributed critically to subsequent drafts and gave final approval for publication.

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Availability of data and materials

Data will be archived on the Tree-Ring network of Qc-Lab database: http://dendro-qc-lab.ca

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

Not applicable.

Consent for publication

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