Paludification reduces black spruce growth rate but does not alter tree water use efficiency in Canadian boreal forested peatlands

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Supplementary material
1. Supplementary methods

1.1 Tree rings and climate analysis

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).

1.2 Isotopic analysis of tree rings

Black spruce ecophysiological mechanisms were investigated through $\delta^{13}$C and $\delta^{18}$O analyses. These analyses were performed on cross-sections from five trees per site. For each selected cross-section, two wood strips of $3 \times 10$ mm were cut and finely sanded on all sides. A five-year resolution covering a 100-year period (1919-2018) was considered. After having carefully cross-dated the wood strips, tree rings were cut with a razor blade under a binocular microscope. Rings of the same years were pooled together in equal amounts, resulting in 20 subsamples of five years per site, which were then grinded using a mixer mill (Retsch MM400) to ensure homogeneity (Borella et al. 1998). Alpha-cellulose was then extracted as suggested for black spruce samples (Bégin et al. 2015) following the protocol used by Naulier et al. (2014).

All isotopic analyses were carried out at the Light stable isotope geochemistry laboratory of the GEOTOP Research Center (Université du Québec à Montréal, Canada). Stable isotope ratios of
carbon and oxygen were analyzed with an isotope ratio mass spectrometer (Isoprime 100 for $\delta^{13}$C and Isoprime VisIon for $\delta^{18}$O) coupled to an elemental analyser (Elementar Vario MicroCube for $\delta^{13}$C and Elementar Vario PyroCube for $\delta^{18}$O) in continuous flow mode. Results were normalized with three internal standards on NBS19-LSVEC and VSMOW-SLAP scales for $\delta^{13}$C and $\delta^{18}$O respectively. Results are reported in ‰ ($\pm 0.1‰$ for $\delta^{13}$C and $\pm 0.3‰$ for $\delta^{18}$O) relative to VPDB for carbon isotopic ratios and to VSMOW for oxygen isotopic ratios. Because of the high combustion of $^{13}$C-depleted fossil fuels since the industrial period (~1850 CE), atmospheric $^{13}$CO$_2$ concentration is significantly decreasing, which causes a declining trend in tree ring $\delta^{13}$C values. This Suess effect was therefore corrected as proposed by McCarroll and Loader (2004).

1.3 Testate amoeba analysis

Testate amoeba shells were extracted from 1 cm$^3$ peat subsamples following the standard protocol of Booth et al. (2010). Subsamples were gently boiled in distilled water and washed through 300 and 15 µm sieves. The material remaining in the 15 µm mesh was stained and mounted on glass slides before being analysed under an optical microscope (400 × magnification). Testate amoebae were identified following the taxonomy of Mitchell (2002), Siemensma (2018), and Charman et al. (2000), with the modifications of Booth and Sullivan (2007).

The dataset of the transfer function used to reconstruct WTD variations (Lamarre et al. 2013) was improved by adding 40 surface samples collected along the study transect, and 40 others sampled in another forested peatland in the study area. Peat surface samples of approximately 10 cm$^3$ were cut with a serrated knife, following the method described in Lamarre et al. (2013). Samples were collected in lawns and hummocks, as these are the only microforms found in our sites.
1.4 Macrofossil analysis

Subsamples of 4 cm$^3$ were gently boiled in a 5% KOH solution before being washed through a 125 µm mesh sieve, following the protocol of Mauquoy et al. (2010). Macrofossils were analysed in a gridded Petri dish under a stereomicroscope (10-40 × magnification), using Lévesque et al. (1988) and Mauquoy and van Geel (2007) for plant identification. 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. The degree of plant material decomposition was also determined visually using an index ranging from 1 (poorly decomposed) to 5 (highly decomposed). Macroscopic charcoal particles (>0.5 mm) were analysed at 1 cm intervals along the three peat cores to identify past local fire events (Beaulne et al. 2021).
2. Supplementary figures and tables

Figure S2.1. Photographs of the Casa forested peatland showing sites CAS100 (top) and CAS0 (bottom).
Figure S2.2. Comparison of the three study sites in terms of peat accumulation, water table depth, tree height and diameter at breast height (DBH). Results of Tukey’s test indicate that CAS0 and CAS100 are significantly different for all of these parameters ($P<0.01$). Different letters above the boxes indicate significant differences between the sites based on Tukey’s test.
Figure S2.3. Basal area increment distribution between the 25\textsuperscript{th} and the 75\textsuperscript{th} quantiles. Sites CAS0, CAS50, and CAS100 are shown in black, red, and blue respectively. The solid line represents the mean annual values of each site.
Figure S2.4. Plant macrofossil diagrams of the three peat cores analysed. Data are presented in percentages (silhouettes) and in counts (bars, except for charcoals). The main peat components (%) are shown in the left column: *Sphagnum* (green), non-*Sphagnum* mosses (black), ligneous material (brown), Pteridophyte (orange), and Cyperaceae (white). The visual decay index, the dry density of peat, and the water table depth (WTD) reconstructions from testate amoeba assemblages (see figure S2.4) are also presented in the right columns.
Figure S2.5. Testate amoeba diagrams. Diagrams show the abundance (%) of the dominant taxa in the three peat cores analysed. The water table depth (WTD) values inferred from testate amoeba records are presented in the right column (high values indicate dry conditions). Blanks in WTD reconstructions are due to exceptionally low test concentrations in some horizons, where the minimum count (20 tests) was not reached. The main peat components (%) are shown in the left column: *Sphagnum* (green), non-*Sphagnum* mosses (black), ligneous material (brown), Pteridophyte (orange), and Cyperaceae (white).
Figure S2.6. March to September climate trends in the study area for the period 1950-2013. (a) precipitation, (b) temperature, and (c) growing degree days (>0°C). Linear trends are shown by dotted lines. Data were extracted from McKenney et al. (2011).
Figure S2.7. Raw tree-ring-width series and standardized ring-width series. Sites CAS0, CAS50, and CAS100 are shown in black, red, and blue respectively.
Figure S2.8. 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.
| Core    | Depth (cm) | Laboratory number | Material dated           | Radiocarbon age ± error (14C yr BP) | Calibrated age range (cal yr BP) | Median age (cal yr BP) |
|---------|------------|-------------------|--------------------------|-------------------------------------|---------------------------------|-----------------------|
| CAS0    | 26-27      | UOC-9614          | Charcoal, charred needles| 158±29                              | 0-285                           | 175                   |
|         | 38-39      | UOC-8508          | Bulk peat                | 1314±32                             | 1182-1296                      | 1255                  |
| CAS50   | 26-27      | UOC-9615          | Charcoal, charred needles| 171±29                              | 0-290                           | 179                   |
|         | 33-34      | UOC-8509          | Charcoal, charred needles| 1264±24                             | 1175-1281                      | 1224                  |
|         | 56-57      | UOC-9617          | Sphagnum stems           | 6610±29                             | 7440-7565                      | 7502                  |
|         | 69-70      | UOC-9616          | Bulk peat                | 6838±29                             | 7610-7724                      | 7667                  |
| CAS100  | 28-29      | UOC-8512          | Charcoal, charred needles| 154±24                              | 35-284                         | 178                   |
|         | 44-45      | UOC-8513          | Picea needles, Sphagnum stems| 4691±31                             | 5320-5577                      | 5397                  |
|         | 52-53      | UOC-8514          | Picea needles, Sphagnum stems| 5469±38                             | 6190-6386                      | 6274                  |
|         | 72-73      | UOC-8515          | Picea and Larix needles  | 6466±34                             | 7311-7436                      | 7374                  |
|         | 95-96      | UOC-6053          | Bulk peat                | 6635±41                             | 7441-7578                      | 7522                  |

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