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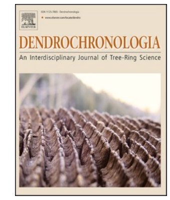


# High resolution radiocarbon spike confirms tree ring dating with low sample depth

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ABSTRACT

Radiocarbon ($^{14}C$) has been used to date carbon-rich objects in Earth science, archeology, and history since the 1940s. New methods, using spikes in $^{14}C$ caused by solar proton events, can be used to annually date wood when crossdating is not possible, such as when sample size is low, samples are floating in time, or external disturbances lead to insecure dates. Here, we use a spike in radiocarbon during a solar energetic particle (SEP) event in 774/775 CE to confirm crossdating of a poorly-replicated King Billy pine (*Athrotaxis selaginoides*) chronology. Low sample depth between 1498 and 1523 CE (two trees) prevented confident dating of the early period of the chronology. Three core samples with strong correlation with the master chronology that likely included the 774/775 CE Miyake SEP event were identified for radiocarbon isotope analysis. We sectioned segments centered on the estimated 774/775 CE date and then isolated the holocellulose in each sample. Samples were sent to an accelerator mass spectrometry (AMS) for radiocarbon measurements. The AMS data confirmed the crossdating accuracy of the tree ring series and reinforces the applicability of this technique to anchor poorly dated tree ring series in time. In addition, we found sample processing with a microtome proved superior for holocellulose extractions and yielded more accurate $^{14}C$ measurements. We recommend sampling with a microtome, processing at least three samples per year, and including sample masses greater than 100 ug C to confirm dating using radiocarbon spikes.

## 1. Introduction

Radiocarbon ($^{14}C$) dating has been used to date carbon-rich objects in the Earth sciences, archeology, and history since the 1940s but until recently, dating estimates included uncertainties ranging from decades to centuries. Spikes in past atmospheric $^{14}C$ concentration, first identified in dated tree ring series (Miyake et al., 2012), have allowed for annually resolved dating of carbon-rich artifacts and geologic deposits that overlap with these events. These spikes in $^{14}C$, likely caused by solar proton events (Güttler et al., 2015), serve as anchors in time and thus have the potential to transform earth sciences by synchronizing geologic events with low-resolution dates, connecting geologic and archeological events to the historical record, and confirming annual dating of tree ring records when crossdating is weak or samples are floating in time (Dee and Pope, 2016; Büntgen et al., 2018; Reinig et al., 2021).

For over 100 years, crossdating has remained the standard for precision dating of tree ring chronologies (Douglass, 1919). However, there are limitations to crossdating even in regions where trees consistently form annual rings (Fritts, 1976). False and locally absent tree rings, as well as disturbances (e.g. defoliation events) can affect the accuracy of crossdating (Büntgen et al., 2018). In addition, limited sample replication may prevent reliable crossdating. Spikes in $^{14}C$ concentration stored in tree rings can be used alongside standard crossdating practices when there are dating uncertainties. Increased concentrations of radiocarbon have now been identified for a series of dates useful for securing multi-millennial length tree ring chronologies, including 660 BCE, 774/775 CE, 993/994 CE, and possibly 3372 BCE, (Miyake et al., 2012; Park et al., 2017; Wang et al., 2017). Combined with crossdating between samples, these anchors have the potential to support development of new tree ring records and increase the length of existing records

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where crossdating is insufficient on its own. This strategy could be used to further develop long tree ring and other proxy records from regions of the world with limited paleoclimate data.

Radiocarbon is produced by highly energetic, charged particles called cosmic rays that come from both our own galaxy and from extra-galactic sources. These cosmic rays bombard the Earth's upper atmosphere creating cascades of particles that interact with atmospheric atoms. Neutrons are emitted during these reactions which are then captured by the nucleus of atmospheric nitrogen ($^{14}N$), resulting in the release of a proton. This loss of said protons directly results in the unstable isotope $^{14}C$ (Hajdas et al., 2021). The radioisotope $^{14}C$ gets oxidized, creating $^{14}CO_2$, which enters the global carbon cycle. The half-oxidation time of $^{14}C$ takes approximately two to seven months (Park et al., 2020) before it can be absorbed by trees through photosynthesis. Once stored as cellulose, the $^{14}C$ remains in the tree decaying according to the half-life of $^{14}C$ (~5370 years). The ratio of absorbed $^{14}C$ concentration relative to the stable carbon isotopes $^{12}C$ and $^{13}C$ can then be used to identify radiocarbon spikes. Once identified in tree rings, these spikes can be used to confirm tree ring records that cannot otherwise be annually dated (Dee and Pope, 2016; Büntgen et al., 2018; Usoskin et al., 2020; Reinig et al., 2021).

The Southern Hemisphere maintains a geographically sparse network of annually resolved climate proxies relative to the Northern Hemisphere. Though several multi-millennial length tree ring records exist for the Southern Hemisphere (Cook et al., 1991; Allen et al., 2017; Boswijk et al., 2006; Lara and Villalba, 1993), the Southern Hemisphere PAGES 2k network maintains only one proxy prior to ~0 CE outside Antarctica (Mt. Read Huon pine tree ring record) (PAGES2k Consortium et al., 2017). In this study, we apply high-resolution $^{14}C$ dating to a chronology with limited sample replication to confirm crossdating. The preliminary chronology has more than > 100 samples from ~42 BCE to 1300 CE and from ~1600–2010 CE. However, the chronology contains 6 measured radii from only two trees covering the period 1498–1523 CE (Fig. 1.). Ring width series from these two trees are highly correlated with the master, ($r = 0.66–0.82$) potentially bridging the two well-replicated parts of the chronology. Further, the early portion of the chronology crossdates well with regional chronologies (Allen et al., 2017). However, in the absence of additional samples to bridge the two sections of the chronology, dating of the early section remains unconfirmed. Here we use the 774/775 CE $^{14}C$ spike to confirm dating of a tree ring chronology with unreliable crossdating using two methods of sample preparation (hand sampling and a microtome). While the

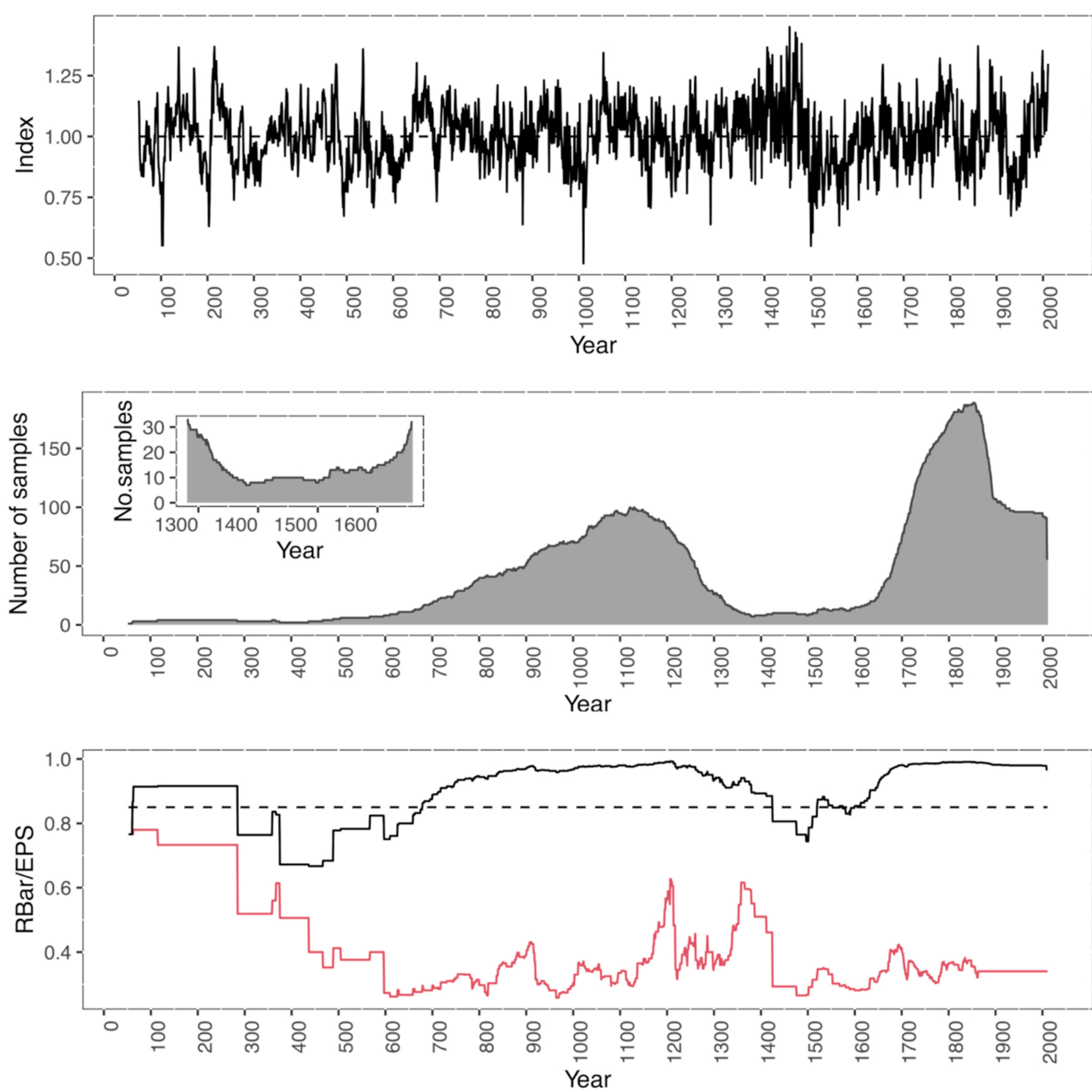


**Fig. 1.** Combined Southern Ranges chronology summary from 42 BCE to 2016 CE. (A) Detrended chronology (black line) from combined Southern Ranges sites (n = 598 series); (B) Sample depth (gray) demonstrating the period of low replication (six radii from two trees) between 1498 and 1523 CE; (C) Running RBar (red line) and expressed population signal (EPS) (black line) with 0.85 threshold (dashed line) (Wigley et al., 1984).

774/775 CE $^{14}$C spike has been used to date completely floating series (Oppenheimer et al., 2017; Reinig et al., 2021), and to identify errors in chronologies previously thought to be well-dated (Büntgen et al., 2018), we believe this is the first application of this method to confirm cross-dating in a poorly replicated chronology.

## 2. Materials and methods

### 2.1. Research sites and species

Tree ring samples from the Southern Ranges King Billy pine (*Athrotaxis selaginoides*) chronology (in development) were collected from live and dead (sub-fossil) trees located on the southwest coast of Tasmania (−43.482366°, 146.757655°). The Southern Ranges King Billy pine collection consists of four living-tree collections and two sub-fossil wood collections from ecologically similar subalpine ridges within a ~100 km$^2$ area in Southwest National Park (Fig. 2.). The sub-fossil wood series include dozens of samples that appear to date to the first millennium (Ooze Lake, Moonlight Ridge; Fig. 1C). Limited overlap between the live and subfossil Southern Ranges sites prevents us from confidently combining records into a single network (Fig. 1.) (Table 1).

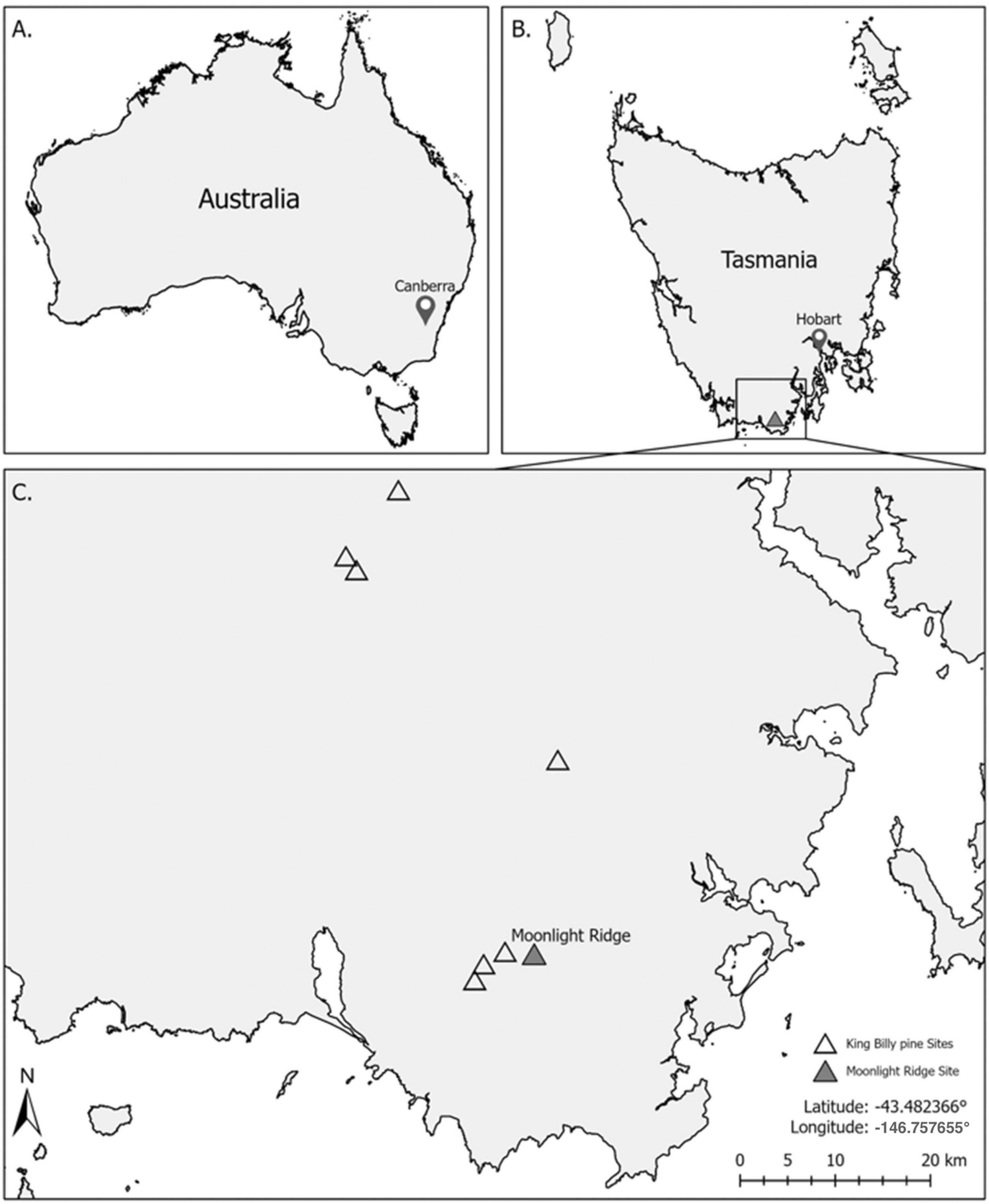


**Fig. 2.** Overview of the Southern Range King Billy Pine site in southern Tasmania, Australia. (A) Map of Australia; (B) map of Tasmania; (C) map of King Billy pine sample site in Tasmania, Australia.

**Table 1**
Core samples used for $^{14}C$ analysis including their crossdated inner ring, outer ring, correlation with the Southern Ranges master chronology, and the sample sectioning technique used.

| Sample ID | Inner Ring | Outer Ring | Correlation with Master | Sample Sectioning Technique |
|---|---|---|---|---|
| MNL09A | 747 CE | 1148 CE | r = 0.58 | Rotary Microtome |
| MNL10A | 718 CE | 1136 CE | r = 0.52 | Hand Sectioned |
| MNL26B | 754 CE | 1278 CE | r = 0.53 | Hand Sectioned |

### 2.2. Sample preparation

Cores were air-dried, mounted, and sanded until individual cells were visible. Samples were visually crossdated, first against samples from the same tree, then against samples from the same site and finally against existing *A. selaginoides* chronologies from elsewhere in Tasmania (Allen et al., 2017). Annual growth rings were measured under magnification to 0.001 mm precision on a sliding stage and crossdated measurement series were checked for accuracy using the quality control program COFECHA (Holmes, 1994).

We selected the 774/775 CE Miyake event to confirm dating as it overlaps with our chronology and has the largest departure from $^{14}C$ values in the years surrounding it. With a pin, we marked 11 years on each sample, centered on the crossdated year 775 CE. We chose 775 CE to adjust for the Austral growing season beginning in October and ending in April of the following year (Schulman, 1956). We used a rotary microtome to section one core and hand sliced two cores from the year 770 CE to 780 CE. From the microtome, we obtained consistent 20-micron-thick shavings from each ring. Hand sectioning produced shavings though we could not control their thickness precisely. To avoid contamination between rings, we focused on collecting the shavings from the center through a thin band of dark latewood (LW) of each ring. The LW fraction is thought to be less likely to contain $^{14}C$ atoms from previous years' growth, avoids boundary contamination, and seasonal differences found in wood as compared to the earlywood (EW) fraction of the ring (Kagawa et al., 2006; Kudsk et al., 2021), however there is some question as to whether LW provides a superior signal of past SEPs given the range of measurement errors. To evaluate sources of error in our sample processing, we created duplicate whole wood samples for the years 773 CE and 779 CE for sample MNL09A. In addition to processing whole wood samples, for samples MNL10A and MNL26B, we processed EW/LW wood fractions for 774, 775, 776, and 777 CE.

Wood formations contain resins, lignins, and soluble compounds that are mobile between annual rings. We removed these by performing an ABA pretreatment (Stuiver and Quay, 1981) and by extracting the holocellulose from each sample to isolate the $^{14}C$ concentration using the method described by Southon and Magana (2010). The ABA pretreatment involves 30-minute treatment washes of 1 M HCl and 1 M NaOH being added to the finely sliced wood shaving samples while being heated at 70 °C. Samples then received several washes of Milli-Q water to remove remaining acid. Samples were then dried for 30 min at 50 °C in an oven. For the holocellulose extractions, the samples were treated with equal parts of 1 M HCl and 1 M $NaClO_2$. This bleaching process allows for removal of lignins. After bleaching, the resulting holocellulose was washed several times with Milli-Q water. Finally, the samples were placed into a warm oven to dry and stored in a vacuum desiccator to reduce the collection of moisture in the tubes. The final product was holocellulose.

### 2.3. Sample analysis

The holocellulose samples were sent to the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) lab, at the Woods Hole Oceanographic Institution in Falmouth, Massachusetts. The $^{14}C$ atoms in our samples were directly counted using the AMS method. The ratio of $^{12}C/^{14}C$ was determined relative to the known ratio in a concurrently measured standard (NBS Oxalic Acid I). Samples were combusted to $CO_2$, and then the $CO_2$ was reduced to graphite with H2 on a Fe catalyst. The resulting graphite was analyzed using AMS, and the measured $^{14}C/^{12}C$ ratios were normalized and blank-corrected before reporting the results.

The sectioned sample from MNL26B for the year 772 CE had less than 100 ug of pure carbon extracted from the sample and thus required small sample handling and analysis. This analysis requires extra process standards and blanks to more accurately characterize the contribution of any contaminating carbon added during processing. For small samples, an estimated added variance has not been determined by NOSAMS. Samples MNL26B years 776, 777, and 779 CE were compromised during analysis, and the sample for the year 778 CE was lost due to a valve failure on one of the vacuum lines. We elected to include the compromised samples here as results were generally in line with the other samples.

Radiocarbon age is calculated by correcting the $\Delta^{13}C$ fraction modern:

$$T_{14C} = -8033 \cdot \ln(Fm)$$

Age-corrected $\Delta^{14}C$ values (Stenström et al., 2011) are calculated using the formula:

$$\Delta^{14}C = \left(e^{-\frac{T_{14C}-T_S}{8033}} \cdot e^{\frac{1950-T_S}{8267}} - 1\right) \bullet 1000‰$$

where $T_S$ is the estimated (crossdated) age. We compared our AMS results against published $\Delta^{14}C$ data for the Southern Hemisphere, including one Huon pine sample from Mount Read, Tasmania (Büntgen et al., 2018, Table 2).

## 3. Results

The AMS results confirm the dates assigned from crossdating the three tree ring series against the Southern Ranges master chronology and regional chronologies (Table 2, Fig. 3). The absolute values of the $\Delta^{14}C$ increased 14.8‰ from the average of 770–773 CE to the peak in 775 CE - 776 CE, though average values were lower than previous studies for the Southern Hemisphere. MNL09A $\Delta^{14}C$ values were within the range previously described, however, samples MNL10A and MNL26B yielded lower values of $\Delta^{14}C$ by ~5.0‰ (Fig. 4). The absolute value of the rapid $\Delta^{14}C$ for sample MNL09A increased by ~9.9‰ between 774 CE and 775 CE and ~13.6‰ between 774 and 776 CE

**Table 2**
$\Delta^{14}C$ results for the three cores centered on the 775 CE $^{14}C$ spike. Two whole-wood (WW) samples were replicated for sample MNL09A for years 773 and 779 CE.

| Year CE | MNL09A | MNL10A | MNL26B |
|---|---|---|---|
| 770 | -29.39‰ | -25.35‰ | -27.20‰ |
| 771 | -23.85‰ | -23.85‰ | -24.55‰ |
| 772 | -21.78‰ | -23.86‰ | -36.89‰ |
| 773 | -25.24‰ (WW)<br>-23.05‰ (WW) | -30.09‰ | -30.32‰ |
| 774 | -18.56‰ | -12.33‰ | -21.21‰ (LW)<br>-29.17‰ (EW) |
| 775 | -8.65‰ | -14.53‰ (LW)<br>-9.23‰ (EW) | -16.37‰ |
| 776 | -4.97‰ | -14.76‰ (LW)<br>-11.18‰ (EW) | -14.83‰* |
| 777 | -11.08‰ | -13.61‰ | -13.21‰ (LW)*<br>-5.32‰ (EW) |
| 778 | -12.47‰ | -10.74‰ | N/A** |
| 779 | -13.74‰ (WW)<br>-18.01‰ (WW) | -14.20‰ | -9.89‰* |
| 780 | -14.55‰ | -14.66‰ | -12.93‰ |

* Compromised Sample
** Lost Sample

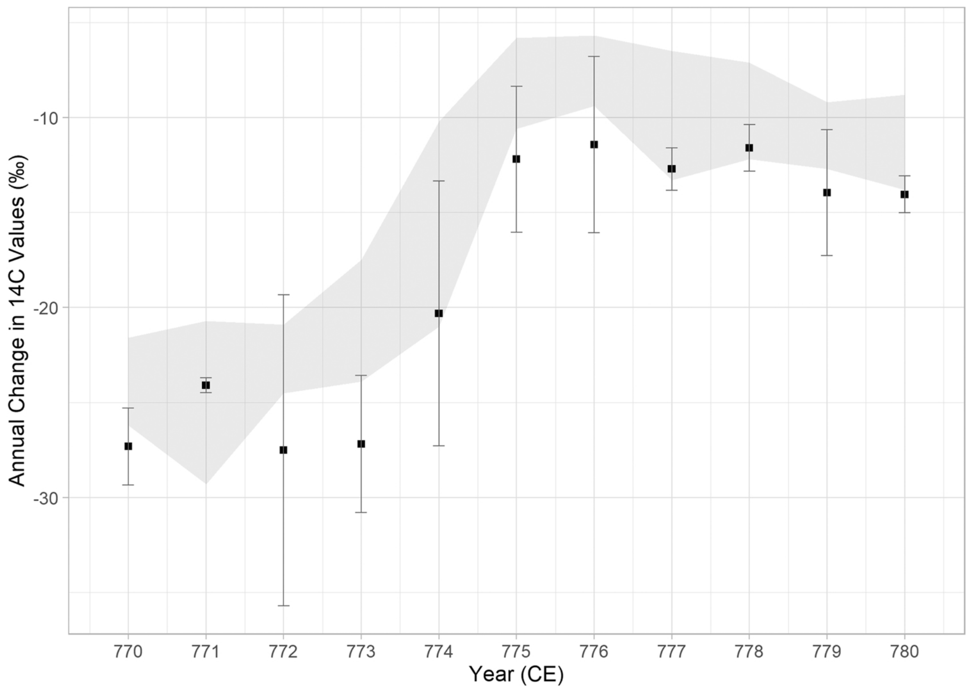


**Fig. 3.** Summary of $\Delta^{14}C$ for three core samples (mean and standard deviation in black error bars) compared to the minimum and maximum values of previously published Southern Hemisphere samples (gray ribbon) (Büntgen et al., 2018).

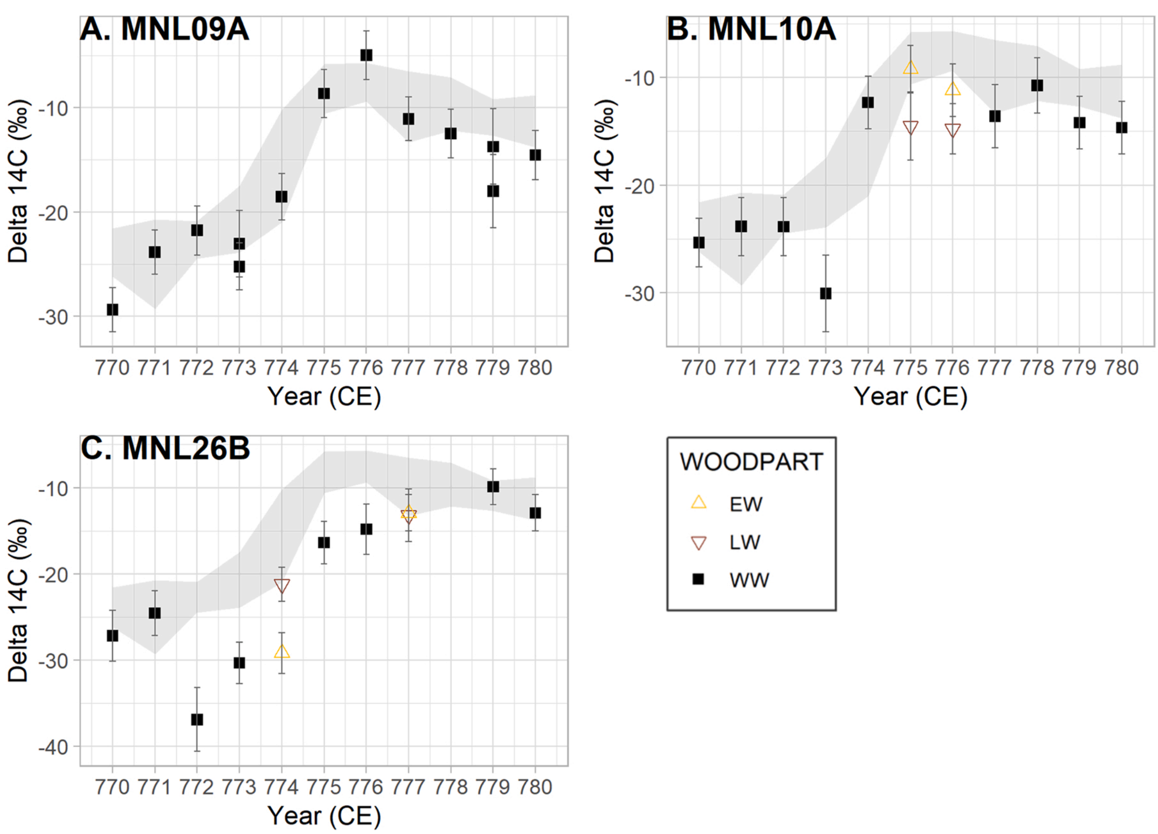


**Fig. 4.** Summary $\Delta^{14}C$ for the three core samples (A) $\Delta^{14}C$ results for MNL09A core sample including samples with duplicate whole wood values for year 773 CE and 779 CE compared to published Southern Hemisphere samples (min and max ranges in gray) (Büntgen et al., 2018). (B) $\Delta^{14}C$ results for MNL10A core sample including samples with earlywood (downward triangle) and latewood values (upward triangle) compared to published Southern Hemisphere samples (min and max ranges in gray) (Büntgen et al., 2018). (C) $\Delta^{14}C$ results for MNL26B core sample including samples with earlywood (upward triangle) and latewood values (downward triangle) compared to published Southern Hemisphere samples (min and max ranges in gray) (Büntgen et al., 2018).

(Fig. 4). Sample MNL10A increased by ~17.8‰ between 773 CE and 774 CE and remained at ~12.0‰ higher from 774 CE through 776 CE. MNL26B maintained a similar pattern to the other samples though values were lower overall. MNL26B $\Delta^{14}C$ values for 772 CE were lower by ~13.0‰ compared to the same ring in MNL09A and MNL10A as well as previously observed data for 772 CE. This sample provided less than 100 ug of pure carbon from extractions and thus required small sample processing. Additionally, results of sample MNL26B showed a ~8.8‰ change between 774 and 775 CE, however, only a total change of ~10.4‰ from 774 CE to 776 CE (Fig. 4).

We used duplicate samples and EW/LW subsampling to assess error in $\Delta^{14}C$ measurements related to sample processing techniques and assess the utility of making seasonal separations to identify radiocarbon spikes. The error in duplicate samples ranged from 2.3‰ to 4.3‰ (n = 2). Earlywood and latewood values also varied across samples. For MNL10A, the LW $\Delta^{14}C$ values were ~5.3‰ lower (775 CE) and ~3.6‰ lower (776 CE) than the respective EW values. For MNL26B, the EW $\Delta^{14}C$ values were ~8.0‰ lower than the LW values for 774 CE. EW and LW values were nearly identical for MNL26B 776 CE (18.8‰ vs 19.7‰).

## 4. Discussion

The mean values of $\Delta^{14}C$ during the years surrounding and including the 774/775 CE Miyake event measured here follow the timing, pattern, and absolute change in $\Delta^{14}C$ previously observed in the Southern Hemisphere over the same period (Büntgen et al., 2018; Güttler et al., 2015). Previous studies observed a Δ14C increase between Southern Hemisphere growing seasons of 773–774 CE. Our results also indicate rising $^{14}C$ first observable in the Southern Hemisphere during the growing season of 774 CE (increase of 6.9% relative to 773 CE), with a slightly larger increase (increase of 8.1%) from 774 to 775 CE. We note that the variability in the measurements of 774 is so large that it cannot be distinguished from measurement in 773 and 775 CE. These results confirm that the original crossdating of the Southern Ranges chronology, reliant on two trees and dating with other regional chronologies, is correct (Fig. 1). This attests to the remarkable reliability of crossdating in this species with limited sample size and demonstrates how Miyake events may be used to confirm poorly replicated tree ring chronologies.

While the mean values of these samples confirm the original crossdating, individual series, repeated measures, and EW/LW comparisons point to several sources of error in the $\Delta^{14}C$ results that should be considered in future studies seeking to assign calendar dates to wood and to calibrate past radiocarbon spikes with the magnitude of SEP events. Possible sources of error include variation in sample processing, contamination from modern $CO_2$ and unwanted resins, lignins, and other mobile wood fractions in the wood sample, seasonal growth differences, instrument error and small sample mass. Here we assess these sources of error and make recommendations for sample processing for future studies.

We observed differences in $\Delta^{14}C$ values related to sample processing by hand sectioning versus using a microtome. Samples that were hand sectioned (MNL10A and MNL26B) had $\Delta^{14}C$ values ~5.0‰ lower than MNL09A, which was sectioned using a rotary microtome (Fig. 4). MNL09A results were largely within the range of previous studies (Büntgen et al., 2018). The rotary microtome was set to slice 20-micron-thick tree ring shavings, resulting in high surface area to volume ratio, allowing for more reliable cellulose extractions. Unwanted substances such as resins, lignins, and other mobile wood fractions not fully extracted from the hand sectioned samples could move in multiple directions, potentially muting the $\Delta^{14}C$ values observed in the hand sectioned samples.

We made EW/LW comparisons to evaluate whether LW samples provided consistently higher values during the SEP event, consistent with seasonal patterns of growth. Some authors argue that latewood is the most robust recorder of past variations in atmospheric $^{14}C$ levels (Park et al., 2021; Kudsk et al., 2018) and could be used to further refine the timing of past SEP events. We observed an average difference between EW and LW samples of 4.5‰ where LW values are higher in only two of four comparisons. Since King Billy pine is an evergreen conifer, we would not expect carbohydrates from previous years to contribute to current ring $\Delta^{14}C$ values from holocellulose (Kozlowski et al., 1967; Leavitt et al., 1993). During an SEP, we would expect $^{14}C$ values to rise over the growing season, however, one would expect only small variation (relative to instrument error), in the years prior to an SEP. In the period before the 774/775 CE SEP, Park et al. (2020) observed five cases where EW/LW $\Delta^{14}C$ were different (>1σ). In three of these cases EW is higher and in two cases LW is higher. Based on our dataset and a review of Park et al. (2020), we do not observe a consistent difference between the EW and LW values and these differences are neither statically significant nor connectable with patterns in the $^{14}C$ record during the growing season or from forerunning years.

Duplicate samples, created by subsampling whole rings (773 and 777 CE), yielded similar differences (3.3‰) to EW versus LW comparisons. This could be due to collecting more EW or LW for the duplicate samples which were not homogenized. However, these errors are only slightly larger than reported instrument error. In 2016, NOSAMS reported the estimated added variance for samples containing more than 100 ug C was 2.6‰ (Radiocarbon Data, 2020). However, the estimated added variance for samples containing less than 100 ug C has not been determined. With the exception of 772 CE MNL26B, our samples were all larger than 100 ug C. This outlying sample was ~13.0‰ lower than other values from this study and previous observations (Büntgen et al., 2018). King Billy pine and other slow growing species with narrow rings may require additional cores and pooling samples by year to ensure adequate sample mass to reduce instrument error.

Previous studies identifying the timing of the 774/775 CE event also relied on small a sample size from 1 to 3 samples per year (Güttler et al., 2015; Park et al., 2020; Uusitalo et al., 2018) and repeat measurements indicate that the variation between samples is less than the departure in $^{14}C$ during the Miyake events. In sum, we observed a range of errors associated with sectioning, subsampling, and sample mass. Errors we attribute to sectioning and subsampling were slightly larger than instrument error. Small sample mass yielded the greatest source of error observed here. Nevertheless, despite the error we can still detect a departure from samples, similar to previous studies that have this sample size (Güttler et al., 2015).

## 5. Conclusions and recommendations

We used high-resolution $^{14}C$ analysis to absolutely date a tree-ring chronology with limited sample depth and unreliable local crossdating. Miyake events, and other $\Delta^{14}C$ excursions can be used in chronology development when crossdating alone is insufficient. However, we note significant variability in the $\Delta^{14}C$ of the three cores we analyzed which may have resulted from our sample processing techniques. In this study, three cores were sufficient to confirm the annual precision of the crossdating. However, if inferences are to be made regarding the magnitude of the SEP events from $\Delta^{14}C$, uncertainties from tree ring measurements must be further constrained. Consistent with previous studies by Usoskin and others (2020), we recommend the following strategies for reducing error in $^{14}C$ measurements from tree ring samples.

Due to limited funding, we were only able to focus on the 774/775 Miyake event that had the greatest magnitude of change in radiocarbon at ~12‰ for 774/775 CE compared to 993/994 CE at ~10‰. An additional tie point would provide a check on the internal uniformity of any questionable crossdating during that time (Dee and Pope, 2016). If only one tie point is possible, we recommend repeat samples ($n \geqq 3$) for each ring due to the possibility of contamination during the sectioning and extraction processes. To eliminate unwanted mobile wood fractions, we recommend using a microtome as opposed to hand sectioning to ensure adequate holocellulose extractions. The microtome provides consistent sample thickness, ensuring adequate surface area to volume during the extraction process and may prevent contamination with compounds like resins or lignins from previous or subsequent years. Since our largest outlier was from a sample with less than 100 ug C, we highly recommend samples larger than 100 ug C, even if that means pooling samples from different cores. Further, although studies have shown that holocellulose and alpha cellulose extractions will yield similar results and are both accurate (Lange et al., 2019), it may be helpful to extract alpha cellulose from samples to ensure that all contaminants are eliminated.

We recommend using this method to confirm weak or floating chronologies and to date important historical events in which the date is disputed. Future discoveries of radiocarbon spikes in addition to 660 BCE, 774/775 CE, 993/994 CE, and 3372 BCE events (Miyake et al., 2012; Park et al., 2017; Wang et al., 2017) will further improve chronologies and transform our understanding of early civilizations and past climate. Additionally, understanding the sources of error in annual $\Delta^{14}C$ values will not only inform the frequency and timing of past SEPs, but may also help resolve their magnitude, critical for improvements in understanding and forecasting solar hazards.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data are contained within the manuscript itself and code has been submitted.

## Acknowledgements

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