

BEHAVIORAL VARIABILITY IN ABA CHEMICAL PRETREATMENT CLOSE TO THE ¹⁴C AGE LIMIT

Guaciara M Santos¹ • Kaelyn Ormsby

Earth System Science, University of California, Irvine, B321 Croul Hall, Irvine, California 92697-3100, USA.

ABSTRACT. The acid-base-acid (ABA) procedure is a common chemical pretreatment used on most organic matter samples. Although this pretreatment is straightforward, there is no consensus among labs about the optimum strength of the chemicals, or even the temperature, to be used for digestions. Comparisons between ¹⁴C results obtained by samples undergoing ABA against other wet oxidations, such as acid-base oxidation followed by stepped-combustion (ABOX-SC) or α -cellulose on wood, have sometimes suggested that ABA does not always remove all contaminating carbon. In addition, if not all ABA protocols are the same, could it be said that one procedure is better than others or comparable to wet-oxidation pretreatments, if rigorously applied? To determine whether ¹⁴C-free samples showed any fraction modern carbon (FmC) deviations due to pretreatment strategies, 3 experiments were carried out. The first compared ¹⁴C results for wood samples at or near the limit of ¹⁴C dating, pretreated with a standard ABA protocol used at UC Irvine's Keck Carbon Cycle Accelerator Mass Spectrometry (KCCAMS) facility, with those obtained from ABA- and ABOX-SC-prepared aliquots of the same samples performed at Australian National University (ANU) in 2001. The second experiment subjected wood samples, ranging from ~12 ka BP to ¹⁴C-free, to 5 selected published ABA pretreatments. Third, we evaluated whether the ABA protocol needs a higher-strength final acid step (particularly important for removing CO₂ absorption when samples are submerged in alkaline solutions). We are able to show that rigorous ABA treatments exist that can provide robust, reproducible results for many wood samples that are known to be >>50 ka BP, implying that a lack of control over the procedure's outcome might be sample-related, rather than due simply to inadequacies in the chemical pretreatment.

INTRODUCTION

For radiocarbon accelerator mass spectrometry (AMS) dating to obtain accurate ages, samples must undergo chemical pretreatments capable of removing exogenous carbon (C) incorporated on or within the material, during *in situ* burial or laboratory handling. A common pretreatment for many organic matter samples that most laboratories worldwide have used for decades is the well-known ABA (acid-base-acid) procedure. During this pretreatment, samples are initially washed using weak acids and bases in order to remove secondary carbon-containing species, such as carbonates and humic or fulvic acids that might have accumulated on the sample over time. These washes are usually followed by a final acid bath, designed to remove any CO₂ absorption that may have occurred when the samples were submerged in the base solution. Although this pretreatment is relatively straightforward, there is no consensus among labs about the optimal strength of the chemical solutions, or even the temperature, used for such chemical digestions. Apart from the obvious exceptions when the method must be adapted or diluted (e.g. to treat components that are soluble in an alkali solution, such as organic fractions of soils and sediments, and protein building materials such as feather, hair, horns, nails and claws, or chitin), this method has been far from standardized. Literature searches show that chemical solution strengths can vary from 0.1 to 6M, with temperature digestions spanning from room temperature to 90 °C. The duration of each step is also highly variable and can run from 20 min to 21 hr (Bird et al. 1999; Hatté et al. 2001; Kim et al. 2001; Santos et al. 2001; Hajdas et al. 2004; Sakamoto et al. 2004; Catanzariti et al. 2007; Mayer et al. 2008; Brock and Higham 2009). Moreover, several publications do not directly describe the ABA chemical pretreatment used; rather, they refer to it by its epithets (e.g. "standard," "conventional," "classical," and "well-known" or "widely used"), without further explanation.

¹Corresponding author. Email: gdossant@uci.edu.

Comparisons between ^{14}C age results obtained by organic samples (mostly charcoal and wood) undergoing ABA versus other wet oxidations, such as the acid-base-oxidation (ABOX) followed by stepped-combustion procedure (SC) or alpha-cellulose, have sometimes suggested that ABA does not always remove all contaminating C (Santos et al. 2001; Higham et al. 2009 and references therein). Others have shown that to some extent the ABA pretreatment's accuracy may be sample-specific (Turney et al. 2001a,b; Southon and Magana 2010), instead of reflecting a simple inadequacy in the chemical pretreatment. If all ABA protocols are not the same, then how should the effects of chemical procedural differences be evaluated? Moreover, can a given ABA pretreatment protocol be better than others or even comparable to other wet-oxidation pretreatments, if rigorously applied? To determine whether pretreatment strategies caused any fraction modern carbon (FmC) deviations in ^{14}C -free samples, 3 experiments were carried out.

SAMPLE SELECTION

Due to the difficulty of obtaining large amounts of charcoal, all samples tested were well-preserved wood samples. USGS coal (Argonne Premium Coal POC#3) was used as an independent reference blank material. The second experiment used a subfossil wood sample (SR7269 Two Creeks) as secondary standard. To determine levels of postdepositional impurities, we also measured "raw" wood materials (i.e. those that did not undergo any chemical pretreatment), except for Takanini (described below) and SR7269 Two Creeks wood samples. The sample list and its rationale are given below.

1. Queets-A: This wood is from a conifer specimen collected near the Queets River mouth, Olympic Peninsula, Washington, USA, from a stratigraphic unit corresponding to oxygen isotope stage (OIS) 5a or 5c, and has been routinely used as a procedural blank in our prep laboratory since 2005 (Southon and Magana 2010).
2. Takanini: This wood was sampled at South Auckland, New Zealand, immediately beneath a hyperconcentrated silica flood deposit dated 0.49 ± 0.05 Ma using the isothermal plateau fission-track technique (Alloway et al. 2004).
3. AR1, AR2, and AR3: These are subfossil wood samples collected from Airedale Reef, a coastal section of North Taranaki in New Zealand, from under a 4-m-thick debris avalanche deposit at OIS 5c (Newnham and Alloway 2004). They have been routinely dated by liquid scintillation spectrometry at Waikato University since 2001 (Hogg 2004).
4. Wk5385: Well-preserved kauri wood previously dated to $>>50$ kyr BP (on α -cellulose extraction fraction; Turney et al. 2007). It was chosen for this study because several chemical pretreatments have significant difficulty cleaning it completely (Southon and Magana 2010).
5. MT04-109-2005B: Highly humified twigs from a stratigraphy deposit at the Prophet River, northeastern British Columbia, Canada, previously dated to >54 kyr BP, after background correction (Trommelen and Levson 2008). This sample was chosen for this study because its humic acids have the same age as the wood fibers' cellulosic elements.

SAMPLE PRETREATMENT STRATEGIES, COMBUSTION, AND GRAPHITIZATION

Each experiment's pretreatment strategies will be described and discussed in detail within that experiment's section. At UCI, combustions are performed off-line at $900\text{ }^\circ\text{C}$ by loading clean material in evacuated sealed quartz tubes with CuO (~ 60 mg) and silver wire. Following cryogenic purification, CO_2 is reduced to filamentous graphite on ~ 5 mg of Fe powder catalyst through a H_2 reaction (Santos et al. 2007) at $550\text{ }^\circ\text{C}$, using $\text{Mg}(\text{ClO}_4)_2$ to remove reaction water (Santos et al. 2004).

The KCCAMS graphitization vacuum lines each have 12 H_2/Fe reactors, which are made of glass and stainless steel. Those vacuum lines reduce a broad array of $^{14}\text{CO}_2$ samples, from modern to ^{14}C -

Behavioral Variability in ABA Chemical Pretreatment

free, to graphite on a daily basis. Therefore, our reactors show some memory effect, which, although it is very small (Southon 2007), can be totally eliminated after the reactor vessels are loaded with empty tubes and baked using ^{14}C -free CO_2 at 550 °C for 1 hr. Once this procedure is complete, Fe catalyst can be loaded into tubes and preconditioned (H_2 reduced) as usual. To avoid reactor selection bias due to memory effect and to closely reproduce the conditions found in most low- ^{14}C -level dedicated vacuum lines (i.e. vacuum lines that are used only with low-background samples; Bird et al. 1999), graphitization reactors were treated as described above, before CO_2 from blank samples was transferred to reactors for graphite reduction.

DESCRIPTION OF EXPERIMENTS

First Experiment (Conventional ABA Versus ABA-SC and ABOX-SC)

The first experiment compared ^{14}C results from wood samples known to be beyond the limit of ^{14}C dating (see Sample Selection section), pretreated with a standard ABA protocol used at our laboratory, with those obtained from ABA-SC and ABOX-SC prepared from aliquots of the same samples performed at ANU in 2001. Physical and chemical procedures are briefly described below, and summarized in Table 1.

Table 1 Chemical procedures used for the first experiment.

Protocol	Carbonate removal			Humics removal			Alkaline contamination removal and/or wet-oxidation step		
	Chemical	Temp.	Time	Chemical	Temp.	Time	Chemical	Temp.	Time
ABA-SC ^a	6M HCl	RT ^b	1 hr	1M NaOH	RT ^b	10 hr	6M HCl	RT ^b	1 hr
ABOX-SC ^a	6M HCl	RT ^b	1 hr	1M NaOH	RT ^b	10 hr	0.1M $\text{K}_2\text{Cr}_2\text{O}_7$ in 2M H_2SO_4	60.5 °C	1–21 hr
ABA/UCI	1N HCl	~90 °C	30 min	1N NaOH ^c	~90 °C	1 hr	1N HCl	~90 °C	30 min

^aSC = stepped-combustion (330 °C, 650 °C, and 910 °C) and reduction to graphite were performed using the Bird et al. (1999) vacuum line at Australian National University (ANU) in 2001 (see Santos et al. 2001 for details).

^bRT = room temperature.

^cRepeat until clear.

The wood samples analyzed at ANU used both ABA and ABOX chemical pretreatments. The steps of those chemical procedures have been previously described in Santos et al. (2001), and were carried out mostly at room temperature (RT), except for the acid-dichromate oxidant solution (0.1M $\text{K}_2\text{Cr}_2\text{O}_7$ in a 2M solution of H_2SO_4) step, which was performed at 60.5 °C (in a hot box). Approximately 800 mg of wood were shaved and subdivided into 2 aliquots, as the acid-dichromate oxidant solution digestion and SC portion of the full procedure were expected to produce great losses, which occasionally happens to some wood samples (Santos et al. 2001). Upon completing the chemical pretreatments (ABA and ABOX), the samples were rinsed with ultrapure Milli-Q™ lukewarm water to pH neutral, and dried at ~80 °C before SC (Bird et al. 1999). The SC method is based on online combustion of chemically clean material at 330 °C for 2 hr, 650 °C for 1 hr, and 910 °C for 12 hr, while loaded in prebaked quartz tubes with ~100 mg of CuO and Ag wire. A minimum of 8–10 mg of pretreated wood was needed for a single SC combustion run. The combined combustion and graphitization steps of the ABA- and ABOX-SC methods performed at ANU were carried out on a low- ^{14}C -level dedicated vacuum line (Bird et al. 1999). After ~24 hr (from combustion to graphitization completion), only 2 graphite targets (i.e. the 650 and 910 °C fractions) were produced, since the CO_2 evolved at 330 °C was normally discarded. Graphite targets were measured by AMS using ANU's 14UD accelerator (~1.6% precision). The ^{14}C results obtained were corrected for a presumed $\delta^{13}\text{C}$ value of –25%.

Our tests at UCI used just 5–10 mg of shaved wood samples, as we anticipated that a single combustion would need just 2.5 mg or less of clean material. The ABA/UCI pretreatment applied to organic samples consisted of heated baths at 70–90 °C with 1N HCl for 30 min and 1N NaOH for 1 hr, with the base washes repeated until humic acids were fully removed (Table 1). Chemically clean material was rinsed with ultrapure Milli-Q water close to pH neutral, and dried at 70–90 °C prior to undergoing combustion and graphitization (see above).

Each sample undergoing the ABA/UCI pretreatment was assayed in triplicate or quadruplicate to ensure that our experimental techniques were accurately quantitated. The harsh digestions of the ABA- or ABOX-SC protocols allowed us to obtain just 1 ¹⁴C result from each ancient wood studied.

Second Experiment (Evaluation of 5 Published ABA Procedures)

The second experiment subjected wood samples ranging from 12 ka BP to ¹⁴C-free to selected ABA pretreatment protocols, including the ABA/UCI, that were built from information found in the literature. Our choices were mostly based on major differences, such as weak versus strong base solutions, 65–90 °C versus room temperature digestions, and pretreatment step durations. To avoid an unwieldy number of combinations, just 5 protocols were tested. These protocols were designated as groups and are summarized in Table 2. To retain fidelity to the information in the literature, after each acid and base bath, samples were rinsed with Milli-Q water to pH neutral rather than moving to the next chemical step (as called for the ABA/UCI protocol; see above). Once the samples were chemically pretreated, they were dried and combusted offline, and the CO₂ produced was reduced to graphite (see section Sample Pretreatment Strategies, Combustion, and Graphitization).

Table 2 These ABA procedures were built from information found in the literature as cited.

Group#	Carbonate removal			Humics removal			Alkaline contamination removal			References
	Chemical	Temp. (°C)	Time	Chemical	Temp. (°C)	Time	Chemical	Temp. (°C)	Time	
A - base at RT ^a	6M HCl	RT*	1 hr	1M NaOH	RT ^a	30 min	6M HCl	RT ^a	1 hr	Kim et al. 2001; Bird et al. 1999
B - long duration	0.5M HCl	80	12 hr	0.1M NaOH	80	12 hr	0.5M HCl	80	12 hr	Catanzariti et al. 2007
C - weak base	1M HCl	60	2 hr	0.1M NaOH	60	>8 hr	1M HCl	60	2 hr	Hajdas et al. 2004; Mayer et al. 2008
D - strong base	1N HCl	~90	30 min	1N NaOH ^b	~90	1 hr	1N HCl	~90	30 min	Sakamoto et al. 2004; ABA/UCI
E - short duration	1M HCl	80	20 min	0.2M NaOH ^b	80	20 min	1M HCl	80	20 min	Brock and Higham 2009; Higham et al. 2009

^aRT = room temperature.

^bRepeat until clear.

To evaluate precision and accuracy, ¹⁴C analyses of the wood standard (SR7269 Two Creeks) were performed side by side with the set of blank wood samples subjected to the 5 selected pretreatments. Aliquots of Queets-A wood and coal (subjected to group D pretreatment) were also analyzed to provide independent blank data.

Third Experiment (Need for an Aggressive Acid in the Last Step of ABA Procedure)

Hatté et al. (2001) proposed the use of a stronger acid (2M H₂SO₄ with an ionic affinity for carbonates, following the steps of Bird et al. 1999) as an alternative chemical solution to neutralize modern carbonates, which can be formed during the alkaline step washes of the standard ABA pretreatment. Their tests subjected old samples with confirmed presence of contaminant C younger than the ¹⁴C age of the sample matrix to 2 chemical procedures, summarized in Table 3.

Table 3 Chemical procedures used for the third experiment.

Procedure ^a	Carbonate removal			Humics removal			Alkaline contamination removal		
	Chemical	Temp.	Time	Chemical	Temp.	Time	Chemical	Temp.	Time
WB/WA	1M HCl	RT ^b	unknown ^c	2 × 10 ⁻⁴ M NH ₄ OH	25–30 °C	3 hr	0.4M H ₂ SO ₄	RT ^b	1 hr
WB/SA	1M HCl	RT ^b	unknown ^c	2 × 10 ⁻⁴ M NH ₄ OH	25–30 °C	3 hr	2M H ₂ SO ₄	RT ^b	2 hr

^aWB/WA = weak base/weak acid; WB/SA = weak base/strong acid.

^bRT = room temperature.

^cUnknown = until total decarbonation, as reported by authors.

To directly evaluate Hatté et al.'s (2001) hypothesis and findings, we reproduced their chemical strategies on 2 ¹⁴C-free wood samples (samples 4 and 5). Those samples were chosen for this study because a) Wk5385 has labile and recalcitrant C younger than cellulose fibers, and therefore mimics the C contamination levels of Hatté et al.'s (2001) previously tested samples, and b) MT04-109-2005B contains ¹⁴C-free humic acids. Because this ancient wood lacks younger C contaminants, it should show the effects Hatté et al. (2001) proposed: the increased uptake of modern CO₂ during alkaline washes, and its later removal by a higher-strength final acid step.

In the first stage of our tests, we subjected both shaved wood sample aliquots to Hatté et al.'s (2001) protocols (Table 3), our regular combustion and graphitization procedures (as described above), and AMS ¹⁴C measurements. Later, we re-subjected the leftovers of previous chemically treated samples to our regular ABA/UCI protocol (Table 1). Each wood sample was surveyed in triplicate to ensure accurate quantitation of our experimental analyses. To obtain independent blank data, coal aliquots were processed and measured together in the same batch of pretreated wood samples.

DATA ANALYSIS

At UCI, all ¹⁴C results from the 3 experiments were normalized to an independent set of OX-I samples, which were measured by a NEC compact (1.5 SDH) AMS system. Isotopic fractionated corrections were performed using the on-line δ¹³C AMS values of the respective graphite targets that were measured, following the instrumental analysis described in Santos et al. (2007). The ¹⁴C results were not background-corrected by either sample processing or AMS machine blanks (see below, Figures 1, 2, and 3), except in the case of SR7269 Two Creeks, used during the second experiment as a secondary standard. The background subtraction applied to this set of samples corresponded to ¹⁴C values from their respective chemically pretreated blanks (see above). The uncertainties plotted on the figures represent the 1σ scatter in the results from each sample.

RESULTS AND DISCUSSION

First Experiment (Conventional ABA Versus ABA-SC and ABOX-SC)

Santos et al. (2001) have shown that ancient wood samples treated by ABA-SC or ABOX-SC cluster near ~ 55 ka BP. These results suggest that the chemical pretreatment did not have a significant bearing on the overall procedure for those woods studied. However, the combination of SC and a dedicated signature featured vacuum extraction system (with backing line through which all atmospheric CO_2 can be removed, and that is used only with low-background ^{14}C samples; Bird et al. 1999) might be of more significance.

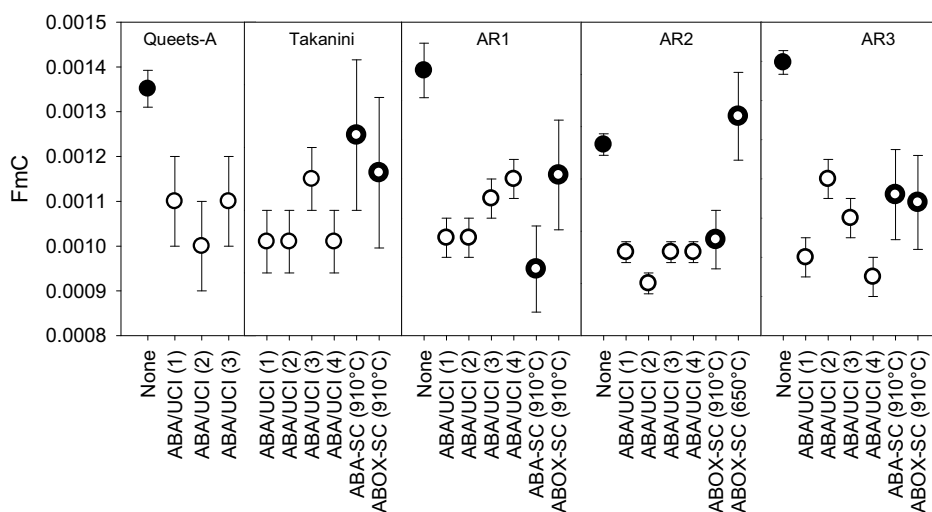


Figure 1 Fraction modern carbon (FmC) results of ^{14}C -free wood samples untreated and chemically pretreated according to the procedures summarized in Table 1, and briefly described in text. Queets-A wood subjected to 1 chemical pretreatment (ABA/UCI) is shown for reference. Symbols are defined on the lower scale. They were chosen to match pretreatments applied as described in the text for easy visualization.

Here, we compared ^{14}C results from another set of ancient woods that were pretreated by ABA-SC and/or ABOX-SC protocols and further graphitized using the dedicated vacuum extraction system of Bird et al. (1999), versus an ABA procedure used at UCI coupled with a standard graphitization line (Santos et al. 2004). Except for the chemically untreated and ABOX-SC (650 °C) residues, all ^{14}C results are indistinguishable within uncertainties (i.e. $\text{FmC} = 0.0011 \pm 0.0002$, ~ 55 ka BP, $n = 19$; see Figure 1). Despite the fact that we removed the memory effect from our multi-use ^{14}C vacuum lines, previous analyses had shown that the effect itself is too small to explain large shifts in age results (Southon 2007). Thus, our ^{14}C results suggest that in most cases it may be unnecessary to use both the SC procedure and the dedicated vacuum extraction and graphitization systems.

However, these results do not imply that for selected samples that are difficult to clean, it is unnecessary to use ABA-SC and/or ABOX-SC procedures, or any other high-strength wet oxidations and surface cleaning pyrolysis (Bird et al. 1999; Santos et al. 2001, 2004; Turney et al. 2001a,b; Ascough et al. 2009; Brock and Higham 2009). Southon and Magana (2010) have shown that a subset of well-preserved ancient wood does exist for which even rigorous ABA treatment is somewhat ineffective. Our ^{14}C results confirm that the ABA treatment appears to provide reliable results for many ancient samples, implying that the lack of control over this procedure's outcome may be sam-

ple-related (see below), rather than that the pretreatment is inadequate. Nevertheless, because more elaborate/harsh treatments critically reduce the sample yield needed for further processing (Santos et al. 2001, 2004; Ascough et al. 2009; Higham et al. 2009), those complex treatments should only be used when they are clearly essential.

Second Experiment (Evaluation of 5 Published ABA Procedures)

Examining the MT04-109-2005B ^{14}C results (Figure 2a), we noticed that the ABA procedures that do not refresh the solutions used (Group B) and/or digest most of the humics (Group E, and possibly D) kept and/or introduced exogenous C. The MT04-109-2005B sample is highly humified, with humic acids matching in age with the wood fiber's cellulosic elements. Unfortunately, it is difficult to confirm the extent to which a medium- to high-strength chemical protocol can actually introduce undesirable C to this type of sample (as responses to the already "clean" material's excessive exposure to chemical baths and/or surfaces).

The Wk5385 kauri wood (Figure 2b), which is significantly challenging to clean, yielded better results from the ABA treatments that used stronger oxidizing solutions (Group D) and/or were coupled with long heat intervals (Group C). It seems that those combined strategies are better at removing young labile and recalcitrant carbon. Still, the final ^{14}C values obtained here do not match those from holocellulose, as reported by others (Southon and Magana 2010).

The ^{14}C results from all aliquots of SR7269 Two Creeks subjected to the 5 selected ABA pretreatments yielded an average $\text{FmC} = 0.2236 \pm 0.0003$ ($\pm 1\sigma$; $n = 5$) and are basically indistinguishable, indicating that those pretreatments are suitable for samples within this age range (Figure 2c).

All samples in this trial were subjected to similar steps, except for the chemical pretreatment that was applied, so the presence or absence of high ^{14}C levels could be related solely to the chemical digestions. However, given the potential for unique bias, and the limited number of duplicates in this experiment, these results should be interpreted and used with caution.

Third Experiment (Need for an Aggressive Acid in the Last Step of ABA Procedure)

Triplicates of Wk5385 wood that were initially subjected to Hatté et al.'s (2001) procedures (Table 3) yielded very high FmC values. Figure 3 shows the ^{14}C values from the following combinations: a) weak base with a weak acid (WB/WA) yielded an average $\text{FmC} = 0.0045 \pm 0.0002$ ($\pm 1\sigma$; $n = 3$); and b) weak base followed by a strong acid (WB/SA) gave an average $\text{FmC} = 0.0044 \pm 0.0003$ ($\pm 1\sigma$; $n = 3$). Although the WB/WA was expected to be high, the WB/SA yielded ^{14}C results that were indistinguishable of the first combination.

Hatté et al.'s (2001) paper stated that the standard ABA method's shortcoming (i.e. the use of an extremely weak base for 3 hr) was needed to purposely increase modern CO_2 uptake during the alkaline baths. However, we suspected that the alkaline treatment's actual weakness was that it failed to remove postdepositional young labile and recalcitrant C from the sample matrix. To investigate our hypothesis, we subjected the pretreated leftover material to a full-strength alkaline solution from our ABA/UCI procedure. The ^{14}C values of the chemically re-pretreated material from both combinations yielded $\text{FmC} = 0.0026 \pm 0.0002$ ($\pm 1\sigma$; $n = 6$). Although this average ^{14}C value did not come closer to the age expected for this difficult-to-clean wood sample, at the very least this experiment demonstrates that subjecting samples that contain postdepositional young exogenous carbon to a very weak base bath ($2 \times 10^{-4}\text{M NH}_4\text{OH}$) can prevent accurate assessment of chemical protocols.

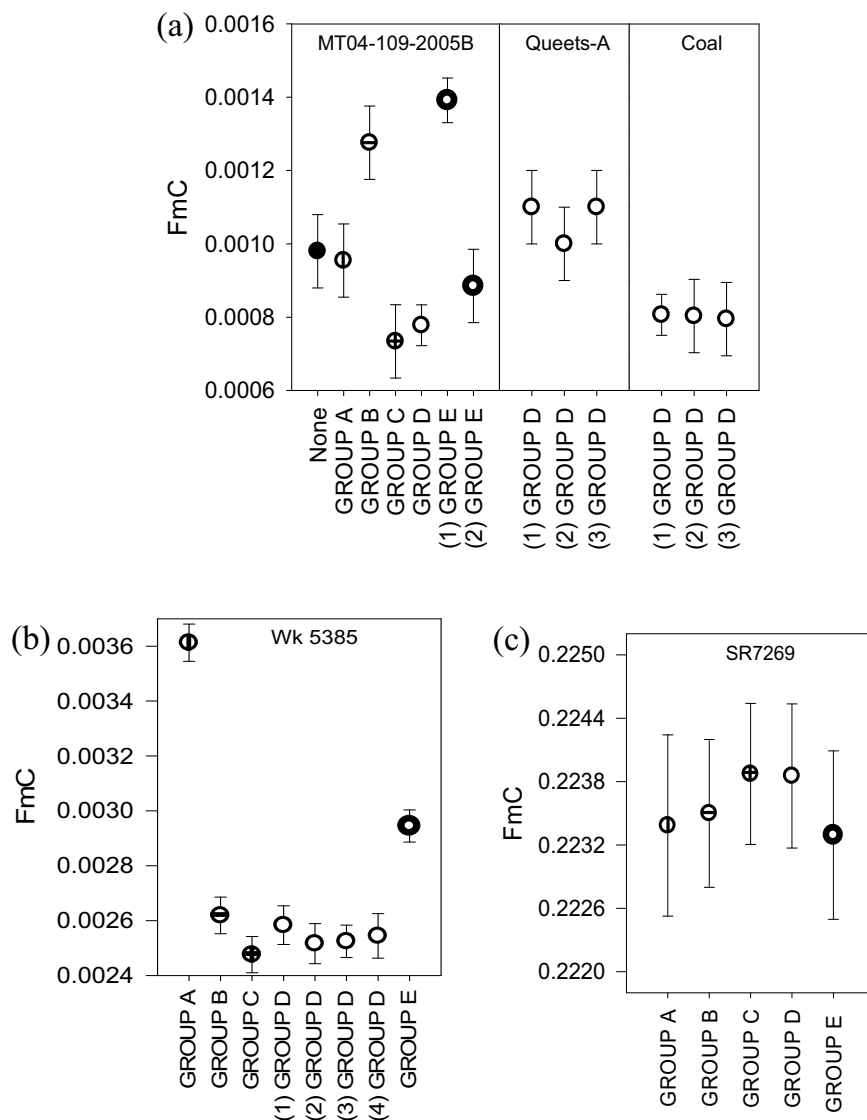


Figure 2 Fraction modern carbon (FmC) results of ^{14}C -free and SR7269 Two Creeks wood chemically pretreated by 5 selected published ABA pretreatments (Table 2). Group D represents the ABA procedure used at UCI. SR7269 Two Creeks wood was used as secondary standard for each of the chemical pretreatments in this trial. Queets-A wood and coals were subjected to just 1 chemical pretreatment (group D) and are shown for reference. Symbols are defined on the lower scale. They were chosen to match pretreatment groups applied as described in the text for easy visualization.

To conclusively elucidate whether the high-strength acid step (after NaOH baths) is somewhat consistent in neutralizing modern carbonates formed during the ABA pretreatment's alkaline step washes, triplicates of another sample (MT04-109-2005B wood) were also subjected to the procedures in Table 3. The WB/WA ^{14}C values yielded an average FmC of 0.0010 ± 0.0001 ($\pm 1\sigma$; $n = 3$), when the WB/SA yielded an average Fm = 0.0007 ± 0.0001 ($\pm 1\sigma$; $n = 3$). These results indicate that even when applied under the most unlikely conditions, the alkaline pretreatment did not introduce a

Behavioral Variability in ABA Chemical Pretreatment

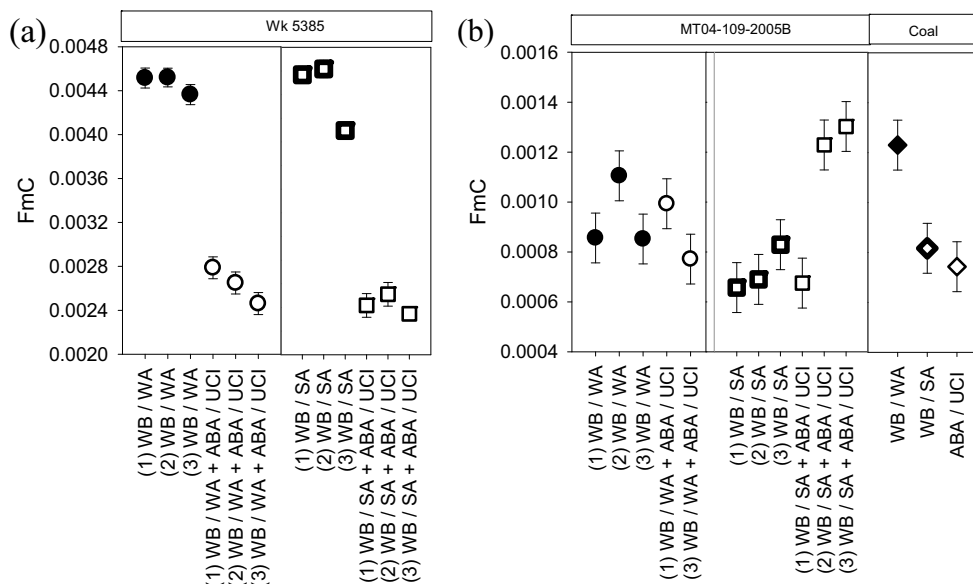


Figure 3 Fraction modern carbon (FmC) results of ^{14}C -free Wk5385 (which contains young recalcitrant carbon) and MT04-109-2005B (which contains ^{14}C -free humics). The x axis outlines the main features of the procedures applied (as shown in Table 3). Coal aliquots were also pretreated, and ^{14}C results are shown as reference. Symbols are defined on the lower scale. They were chosen to match sample types and pretreatments applied as described in the text for easy visualization.

significant amount of contamination. Figure 3b shows that the high-strength acid step resulted in little to indistinguishable improvement in ^{14}C values of MT04-109-2005B aliquots.

Similarly, the leftovers of the pretreated wood fibers were further pretreated by the ABA/UCI procedure. The ^{14}C results indicate that the repeated digestions introduced some carbon to 2 of the aliquots processed (Figure 3b), which were previously subjected to the procedures described in Table 3. The ^{14}C values of the chemically re-pretreated material from both combinations yielded $\text{FmC} = 0.0010 \pm 0.0002$ ($\pm 1\sigma$; $n = 6$), which is inappreciably higher than the $\text{FmC} = 0.0008 \pm 0.0001$ ($\pm 1\sigma$; $n = 1$) from the ABA/UCI (group D) pretreatment alone (Figure 2).

Unfortunately, it is very difficult to ascertain what triggered the large shifts in age observed by Hatté et al. (2001) after they applied the high-strength acid step pretreatment to their alkaline-treated samples. We suspect that the combination of a weak base and the strong acid decontamination procedure played a part; however, the phenomenon was not observed here. Our observations make it apparent that replacing the ABA's conventional last acid treatment with a high-strength 2M H_2SO_4 (2 hr at 70 °C) is unlikely to have a significant impact on the final ^{14}C values. Therefore, it may be necessary to perform additional tests on multiple types of samples to identify when a high-strength acid decontamination procedure may be essential.

CONCLUSIONS

Recently, several concerns have been raised about the ABA protocol's efficiency close to the ^{14}C age limit. To investigate its effectiveness, we tested our rigorous ABA/UCI protocol against ABA-SC and ABOX-SC procedures on a set of ancient wood samples. The ABA/UCI treatment applied to 5 woods (Queets-A, Takani, AR1, AR2, and AR3) yielded an average ^{14}C age of ~55 ka BP

($n = 19$; $FmC = 0.0011 \pm 0.0002$). This result is indistinguishable from the values ANU obtained previously (Santos et al. 2001), using more complex protocols to test 4 of those woods. Therefore, when the standard ABA protocol is rigorously applied, it seems appropriate to use it with confidence for a large array of ancient samples.

Second, we subjected wood samples (12 ka BP and ^{14}C -free) to 5 selected published ABA pretreatments. For the ^{14}C -free set, we selected wood samples containing younger recalcitrant C that cannot be easily removed (Wk5383 kauri) and ^{14}C -free humic acids (MT04-109-2005B twigs). Previously published ABA protocols appear to be reasonably suitable for most of the blank wood samples in this trial. Most of the ambiguities in ^{14}C results are attributable to the sample type (e.g. samples with or without younger recalcitrant C), instead of to differences in chemical sample processing. However, the ^{14}C results appear to be more consistent in procedures using stronger oxidizing solutions that are refreshed frequently, heated, and applied for a longer duration.

Third, we evaluated whether the ABA protocol needs a higher-strength acid final step (important for removing CO_2 absorption that occurs when samples are submerged in alkaline solutions), as raised by Hatté et al. (2001). As yet, we cannot explain exactly what mechanism causes the ^{14}C shifts the authors previously observed. Our experiments suggest that the young labile and recalcitrant carbon left behind by the very weak-base bath was somewhat important. We suspect that the combination of it and the strong acid decontamination procedure (2M H_2SO_4 per 2 hr at 70 °C) play a part in their results, rather than just removing modern CO_2 adsorbed during the final neutralization step after alkaline solutions. More tests are needed to clarify this issue.

Our tests on wood aliquots that are originally ^{14}C -free (such as MT04-109-2005B twigs) failed to show the C contamination effect expected from the sequential alkaline solution treatments. In addition, when applied to this type of sample, the strong acid pretreatment (2M H_2SO_4 per 2 hr at 70 °C) failed to improve the final ^{14}C results enough to justify its use as a replacement for the conventional last acid treatment of the standard ABA. Therefore, it may be necessary to perform additional tests on multiple sample types to identify when a stronger acid decontamination procedure might be useful.

ACKNOWLEDGMENTS

The authors would like to acknowledge Dr Alan Hogg for kindly providing aliquots of the samples Takanini, AR1, AR2, AR3, and Wk5385; Dr Jack McGeehin, of the US Geological Survey, for the Argonne Premium Coal POC#3; and Dr John Southon for valuable discussions. We also thank the anonymous reviewers for feedback and suggestions, and *Radiocarbon* editors Prof Tim Jull and Mark McClure for their support and work.

REFERENCES

- Alloway BV, Westgate JA, Pillans B, Pearce N, Newnham R, Byrami M, Aarburg S. 2004. Stratigraphy, age and correlation of middle Pleistocene silicic tephra in the Auckland region, New Zealand: a prolific distal record of Taupo Volcanic Zone volcanism. *New Zealand Journal of Geology and Geophysics* 47(3): 447–79.
- Ascough PL, Bird MI, Meredith W, Snape CE, Vane C, Brock F, Higham T. 2009. Hydrolysis as a tool for enhanced ^{14}C age measurement and quantification of black carbon in depositional environments. *Quaternary Geochronology* 4(2):140–7.
- Bird MI, Ayliffe LK, Fifield LK, Turney CSM, Cresswell RG, Barrows TT, David B. 1999. Radiocarbon dating of “old” charcoal using a wet oxidation, stepped-combustion procedure. *Radiocarbon* 41(2):127–40.
- Brock F, Higham TFG. 2009. AMS radiocarbon dating of Paleolithic-aged charcoal from Europe and the Mediterranean Rim using ABOX-SC. *Radiocarbon* 51(2): 839–46.
- Catanzariti G, McIntosh G, Osete ML, Nakamura T, Rakowski AZ, Ramirez González I, Lanos P. 2007. A comparison of radiocarbon and archaeomagnetic dating from an archaeological site in Spain. *Radiocarbon* 49(2):543–50.
- Hajdas I, Bonani G, Thut J, Leone G, Pfenninger R,

Behavioral Variability in ABA Chemical Pretreatment

- Maden C. 2004. A report on sample preparation at the ETH/PSI AMS facility in Zurich. *Nuclear Instruments and Methods in Physics Research B* 223–224:267–71.
- Hatté C, Morvan J, Noury C, Paterne M. 2001. Is classical acid-alkali-acid treatment responsible for contamination? An alternative proposition. *Radiocarbon* 43(2):177–82.
- Higham T, Brock F, Peresani M, Broglio A, Wood R, Douka K. 2009. Problems with radiocarbon dating the Middle to Upper Palaeolithic transition in Italy. *Quaternary Science Reviews* 28(13–14):1257–67.
- Hogg AG. 2004. Towards achieving low background levels in routine dating by liquid scintillation spectrometry. *Radiocarbon* 46(1):123–31.
- Kim JC, Park JH, Kim IC, Lee C, Cheoun MK, Kang J, Song YM. 2001. Progress at the Seoul National University AMS Facility. *Radiocarbon* 43(2A):163–7.
- Mayer JH, Burr GS, Holliday VT. 2008. Comparisons and interpretations of charcoal and organic matter radiocarbon ages from buried soils in north-central Colorado, USA. *Radiocarbon* 50(3):331–46.
- Newnham R, Alloway B. 2004. A terrestrial record of Last Interglacial climate preserved by voluminous debris avalanche inundation in Taranaki, New Zealand. *Journal of Quaternary Science* 19(3):299–314.
- Sakamoto M, Kodaira A, Imamura M. 2004. An automated AAA preparation system for AMS radiocarbon dating. *Nuclear Instruments and Methods in Physics Research B* 223–224:298–301.
- Santos GM, Bird MI, Pillans B, Fifield LK, Alloway BV, Chappell J, Hausladen PA. 2001. Radiocarbon dating of wood using different pretreatment procedures: application to the chronology of Rotoehu Ash, New Zealand. *Radiocarbon* 43(2A):239–48.
- Santos GM, Southon JR, Druffel-Rodriguez KC, Griffin S, Mazon M. 2004. Magnesium perchlorate as an alternative water trap in AMS graphite sample preparation: a report on sample preparation at KCCAMS at the University of California, Irvine. *Radiocarbon* 46(1):165–73.
- Santos GM, Moore RB, Southon JR, Griffin S, Hinger E, Zhang D. 2007. AMS ¹⁴C sample preparation at the KCCAMS/UCI Facility: status report and performance of small samples. *Radiocarbon* 49(2):255–69.
- Southon JR. 2007. Graphite reactor memory—Where is it from and how to minimize it? *Nuclear Instruments and Methods in Physics Research B* 259(1):288–92.
- Southon JR, Magana AL. 2010. A comparison of cellulose extraction and ABA pretreatment methods for AMS ¹⁴C dating of ancient wood. *Radiocarbon* 52(3):1371–9.
- Trommelen M, Levson V. 2008. Quaternary stratigraphy of the Prophet River, northeastern British Columbia. *Canadian Journal of Earth Science* 45:565–75.
- Turney CSM, Bird MI, Fifield LK, Kershaw AP, Cresswell RG, Santos GM, di Tada ML, Hausladen PA, Youping Z. 2001a. Development of a robust ¹⁴C chronology for Lynch's Crater (North Queensland, Australia) using different pretreatment strategies. *Radiocarbon* 43(1):45–54.
- Turney CSM, Kershaw AP, Moss P, Bird MI, Fifield LK, Cresswell RG, Santos GM, di Tada ML, Hausladen PA, Zhou Y. 2001b. Redating the onset of burning at Lynch's Crater (North Queensland): implications for human settlement in Australia. *Journal of Quaternary Science* 16(8):767–71.
- Turney CSM, Fifield LK, Palmer JG, Hogg AG, Baillie MGL, Galbraith R, Ogden J, Lorrey A, Tims SG. 2007. Towards a radiocarbon calibration for Oxygen Isotope Stage 3 using New Zealand kauri (*Agathis australis*). *Radiocarbon* 49(2):447–57.