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ARTICLE



Measuring mercury in wood: challenging but important

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ABSTRACT

Mercury (Hg) in tree wood has been overlooked, in part because concentrations are so low as to be below detection limits of some analytical methods, but it is potentially important to forest ecosystem processes and budgets. We tested methods for the preparation and determination of Hg in tree wood by analysing samples of four tree species at the Hubbard Brook Experimental Forest, New Hampshire, USA, using thermal decomposition, catalytic conversion, amalgamation and atomic absorption spectrophotometry (USEPA Method 7473). Samples that were freeze-dried or oven-dried at 65°C were suitable for determination of Hg, whereas oven-drying at 103°C resulted in Hg losses, and air-drying resulted in Hg gains, presumably due to sorption from indoor air. Mean (\pm SE) concentrations of Hg tree bole wood were 1.75 ± 0.14 ng g⁻¹ for American beech, 1.48 ± 0.23 ng g⁻¹ for sugar maple, 3.96 ± 0.19 ng g⁻¹ for red spruce and 4.59 ± 0.06 ng g⁻¹ for balsam fir. Based on these concentrations and estimates of wood biomass by species based on stand inventory, we estimated the Hg content of wood in the reference watershed at Hubbard Brook to be 0.32 g ha⁻¹, twice the size of the foliar Hg pool (0.15 g ha⁻¹). Mercury in wood deserves more attention and is feasible to measure using appropriate techniques.

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Fagus grandifolia Ehrh; *Acer saccharum* Marsh; *Picea rubens* Sarg; *Abies balsamea* (L.) Mill; wood mercury; sample preparation

1. Introduction

Mercury (Hg), a neurotoxic pollutant, has increased greatly in the environment due to emissions from anthropogenic activities such as coal combustion and gold mining [1]. A potentially important but poorly characterised source of Hg emissions is biomass burning [2]. Mercury has been studied extensively in aquatic ecosystems but is less well described in forests, although forests are important receptors and biological Hg hotspots largely occur in forested regions [3,4].

Studies have been conducted in forest ecosystems to determine Hg concentrations in tree foliage [5–8], leaf litter [9–11], and bark [12,13]. Mercury concentrations in wood were lower than values in the foliage or branches in a study of 14 forest sites across the United States [14]. Because wood is the largest component of forest biomass, it can represent a larger Hg pool than foliage. Thus, quantifying concentrations in wood is

important to Hg budgets in forests. Current studies of concentrations of Hg in wood are often reported for conifer species [15–17], whereas the reported values in hardwood species are often below the analytical detection limits [14,18]. As Hg concentrations in wood are low relative to other tree tissues, it is important to understand the consequences of choices of instrumental techniques, size of the sample and sample preparation.

The method of cold vapour atomic fluorescence spectrometry (CV AFS) [19] used by Siwik et al. [18], like inductively coupled plasma-atomic emission spectrometry [20], requires a liquid sample, which might be 1% of the concentration of the tissue sample prior to digestion and dilution. Solid samples can be analysed directly by thermal decomposition, catalytic conversion, amalgamation and atomic absorption spectrophotometry through a direct Hg analyser, giving much lower detection limits [21]. However, if too small a mass of sample is analysed, values will still be below detection [14]. Also, if too large a mass of sample is analysed, the sample itself can produce interference due to high carbon content. Soot can form due to incomplete combustion of organic matter, decreasing the precision of the analysis and shortening the lifetime of the instrument.

Samples are commonly oven-dried before analysis to allow the results to be reported on a dry-weight basis. One study of soil preparation found greater Hg losses upon oven-drying (24%) than air-drying (3–8%) [22], whereas peat samples lost more Hg upon oven-drying (8–10%) than air-drying, which increased Hg by 2% [23]. Contamination of samples during air-drying was attributed to sorption from the atmosphere.

The effects of sample preparation on bole wood have not previously been reported. Freeze-drying is a standard procedure of pretreatment for measuring Hg in tree tissues, but many wood samples that were previously air-dried or oven-dried could be appropriate for Hg determination if these approaches could be validated.

The purpose of this study was to determine the methods necessary to accurately quantify Hg in bole wood, using four North American tree species. We analysed wood tissue samples by thermal decomposition, catalytic conversion, amalgamation and atomic absorption spectrophotometry, using a direct Hg analyser. We determined the relationship between aliquot size and detection limits using dosing techniques. We evaluated the effect of air-drying and oven-drying samples on Hg recovery, compared to freeze-drying samples prior to analysis, which is the standard procedure. Because we observed Hg contamination in air-dried samples, air-drying was tested in multiple labs and locations to determine whether this procedure should generally be avoided.

2. Experimental

2.1. Sample sources

Tissue samples were collected from the dominant species, American beech (*Fagus grandifolia* Ehrh.), sugar maple (*Acer saccharum* Marshall.), red spruce (*Picea rubens* Sarg.) and balsam fir (*Abies balsamea* (L.) Mill.), at the Hubbard Brook Experimental Forest in the White Mountain National Forest in central New Hampshire. Soils are well-drained Spodosols developed in glacial drift. Average temperature is -9°C in January and 18°C in July; annual average precipitation is 1400 mm [24].

2.2. Sample collection and preparation

2.2.1. Samples for method comparison

One tree >10 cm in diameter at breast height (1.3 m) of each species (beech, maple, spruce and fir) was felled, and cross-sectional discs about 5 cm in thickness were cut near breast height in July 2014. Samples were stored in Ziploc bags and transported on ice in a cooler to the laboratory. The sawn surfaces were shaved with a plane and rinsed with methanol. Samples were stored frozen before further processing. From each disc, dedicated stainless- steel drill bits were used to obtain a homogenised subsample of wood particles. We did not sample the dead heartwood or dark wood at the centre of the disc, which might differ in Hg concentration [17,18]. To prevent cross contamination, drill bits were rinsed with methanol before processing each disc.

From each of the four sampled trees, five replicates of ~0.8 g were prepared for each of five different processing methods: fresh, air-drying, freeze-drying and oven-drying at 65 and 103°C. Fresh samples were analysed immediately. Freeze-dried samples were dried at -80°C and 7 Pa for 5 days, using FreeZone Plus 6 Freeze Dry System (Labconco, Kansas City, MO). Air-dry samples were dried in covered foil trays in a lab drawer for a week. Oven-dry samples were dried at 65 or 103°C in an oven for 2 days. Samples were weighed before drying and after drying.

2.2.2. Samples for air-dry contamination test

To test for Hg contaminations during air-drying, we used an additional disc cut near breast height from one sugar maple tree in August 2015. Saw-dust size samples of ~0.8 g were prepared by the same methods described above. Triplicate samples were dried in each of seven locations: a clean room at Syracuse University in New York; a drying room at the State University of New York College of Environmental Science and Forestry (ESF); a soil room at the Coweeta Hydrological Laboratory in North Carolina; a garage containing sample drying racks at the Bartlett Experimental Forest in New Hampshire and the barn, the archive building and soil sample processing room at the Hubbard Brook Experimental Forest in New Hampshire. All the locations were heated (~20°C) except the garage at Bartlett and barn at Hubbard Brook, which were unheated (~5°C). Samples were stored in folded foil trays to protect them from dust deposition during the drying period. Two sets of triplicate samples were used as controls. For minimal contamination, one set was kept frozen. For extreme contamination, one set was dried in a closed chamber with air exposed to liquid Hg. The concentration of mercury in the atmosphere of the closed chamber was measured every week (four times in total) on a 10- μ L air sample using a mercury vapour analyser (Tekran 2537A, Canada). The average concentration was 6480 ng m⁻³.

After air drying for 30 days (33 days for samples at Hubbard Brook), all the samples were freeze dried before analysis to eliminate differences in moisture content.

2.3. Hg determination and detection limits

We conducted the analyses by thermal decomposition, catalytic conversion, amalgamation and atomic absorption spectrophotometry [21], using a Milestone DMA 80 direct Hg analyser (Shelton, CT). This method requires a smaller sample size and less preparation

Table 1. Methods used for measuring mercury.

Method	Method detection limit (ng g ⁻¹)	Sample size (mg)	Preparation procedure	Method number	References
CV AFS	0.0005	>500	Digestion	1631	US EPA [19]
ICP-MS	0.1	>500	Acid digestion	6020A	US EPA [25]
Manual cold vapour atomic absorption spectrometry	0.2	500–600	Heating or digestion	7471B	US EPA [26]
Thermal decomposition, amalgamation and atomic absorption spectrophotometry	1.0	2–1000	None	7473	US EPA [21]
Microwave digestion	1.75	>500	Acid digestion	3051A	US EPA [27]
ICP-AES	17	>500	Acid digestion	6010B	US EPA [20]

CV AFS: Cold vapour atomic fluorescence spectrometry; ICP-MS: inductively coupled plasma mass spectrometry; ICP-AES: inductively coupled plasma atomic emission spectroscopy.

than other methods (Table 1). Parameter settings were drying temperature at 300°C, drying time at 60 s, decomposition temperature at 925°C, decomposition time at 270 s, waiting time at 70 s and amalgam time at 18 s. For each sample, two replicate samples of ~100 mg of tissue were weighed into tared nickel boats and autoloading into the instrument. About 5–10 mg of aluminium oxide (Al₂O₃) was added to each tissue sample to ensure that the samples were fully combusted. The usage of aluminium oxide is recommended by the manufacturer to slow the combustion process, which facilitates complete burning of samples high in organic matter and increases the lifetime of the analytical tube of the instrument. The Hg concentration reported is the average of the two replicate samples. A standard reference material (NIST 1515 apple leaves) was used to test the dosing technique. Five aliquots were accumulated as one burn, to be compared with the burn with one aliquot.

2.4. Quality control

The certified reference material we used for quality control was NIST 1515, apple leaves. This CRM was validated for Hg analysis by CV AAS (cold-vapour atomic absorption spectrometry) and radiochemical neutron activation analysis. Before running tissue samples, we analysed two blanks, two primers (NIST DORM-2, dogfish muscle, ~50 mg, 410 ± 41 ng g⁻¹), two continuing calibration verification samples (NIST 2976 mussel tissue, ~15 mg, 61 ± 6 ng g⁻¹, Gaithersburg, MD, USA), two quality control samples (NIST 1515, ~5 mg, 44 ± 4 ng g⁻¹) and one method blank sample (with Al₂O₃), to verify the calibration curve; we did not proceed with sample analysis unless the difference between measured and certified values of our quality control samples was <10%. After every 10 wood samples, we ran continuing calibration verifications (NIST 2976) and continuing calibration blanks. A sample batch consisted of a method blank, a quality control sample (NIST 1515), a duplicate, a matrix spike and a matrix spike duplicate. The matrix spike was a wood sample matrix spiked with a standard reference material (NIST 2976). The average recovery for Hg was 99% (*n* = 32, *rsd* = 8%) of NIST 2976, 100% (*n* = 16, *rsd* = 5%) of DORM-2, 100% (*n* = 8, *rsd* = 5.7%) of NIST 1515 and 107% (*n* = 8, *rsd* = 14%) of the matrix spike, which were all within the accepted range of values. This

information indicated that there was no interference during the analysis. The blanks and the method blank had a Hg concentration of $0.01 \pm 0.02 \text{ pg g}^{-1}$. Thus, we did not perform standard additions.

2.5. Data analysis

The moisture content of wood samples was calculated as the weight loss on drying divided by the fresh weight. The moisture contents of the five replicates determined under each drying condition were compared with values determined by freeze-drying samples, which is the method that removes the most moisture (Table 2). To calculate Hg concentrations on a freeze-dried mass basis, we corrected the measured concentrations using the remaining moisture content under other drying conditions.

To test the effect of the five drying treatments on moisture loss of wood samples, we used one-way ANOVA separately for each species, using the five replicates for each treatment. We used the same model to examine the effect of drying treatment on wood Hg concentrations. We tested the normality of the residuals in this and other analyses using the Shapiro–Wilk test.

To test for differences in Hg concentration in sugar maple wood samples dried in different locations, we used one-way ANOVA, using three replicates dried at each location. Tukey's honestly significant difference was used to compare means.

Statistical tests were performed using SAS 9.4.

3. Results

3.1. Detection limits and dosing technique

The instrument-detection limit (IDL) is the smallest quantity of Hg that can be detected by the analytical instrument. The IDL for Method 7473 [21] was calculated using the US Environmental Protection Agency Method Detection Limit procedure found in Title 40 Code of Federal Regulations Part 136 [28], where the Student *t* value was multiplied by the standard deviation of concentrations of seven replicate samples. We analysed seven blanks, which had a mean Hg content of 0.009 ng and a standard deviation of 0.002 ng. Thus, the IDL was 0.01 ng.

The method detection limit (MDL) is the smallest quantity of Hg that can be quantified with the method. To calculate the MDL, we analysed seven replicates of 5 mg of a standard reference material (NIST1515-apple leaves) with a multiplication factor of 3.14 derived from a *T* table. The mean Hg content was 0.22 ng (44 ng g^{-1} in units of concentration) with a standard deviation of 0.01 ng (0.41 ng g^{-1}). Thus, the MDL was

Table 2. Moisture loss (Ave \pm SE) of five replicate samples prepared by different methods as a percentage of the fresh weight.

	Moisture loss (%)			
	American beech	Sugar maple	Red spruce	Balsam fir
Freeze dry	13.8 \pm 0.1	11.2 \pm 0.1	27.5 \pm 0.04	28.8 \pm 0.09
Oven 65°C	11.3 \pm 0.4	8.7 \pm 0.3	23.4 \pm 0.4	25.8 \pm 0.1
Oven 103°C	11.6 \pm 0.5	9.1 \pm 0.3	25.0 \pm 0.2	26.1 \pm 0.1
Air dry	3.2 \pm 0.4	6.3 \pm 0.1	14.2 \pm 0.5	15.1 \pm 0.4

0.05 ng in units of mass (1.27 ng g^{-1}). The IDL and MDL should be reported in units of mass because the calibration curve is based on mass. The MDL can be calculated in units of concentration but the result is specific to the concentration of the material analysed.

Dosing is a technique that accumulates multiple aliquots as one burn to increase the absorbance for detection in the direct Hg analyser. Five aliquots were dosed as one burn, which increased of absorbance by a factor of five (Table 3). This approach shows that wood samples with Hg concentrations that are below the detection limits we report using one aliquot could be analysed successfully by increasing the effective mass of the sample.

3.2. Sample preparation and Hg concentrations

Freeze-drying resulted in greater moisture loss than air-drying or oven-drying ($p < 0.001$) (Table 2). The freeze-dried samples lost 11–28% of their fresh weight, depending on the species, averaged over the five replicates of each species. Oven-drying removed almost as much moisture as freeze-drying, with moisture losses of 9–26% for both drying temperatures. Drying at 103°C removed only 0.7% more of the fresh weight, on average, than drying at 65°C . Air-drying resulted in moisture losses of only 3–15% of the fresh weight. We used the mass loss from the freeze-dried samples as the total moisture content and corrected the measured Hg concentration of the samples prepared by other drying treatments using the difference in average moisture contents of the five replicate samples in that drying treatment compared to the freeze-drying treatment.

Concentrations of Hg in samples that were analysed fresh, freeze-dried or oven-dried at 65°C were in close agreement after correcting for moisture content for all four species

Table 3. A standard reference material (NIST 1515 Apple leaves) was analysed using a single sample and a dosing technique, which allows multiple aliquots to be burned before desorption.

Procedure	Sample	Mass (g)	Signal (absorbance)	Content (ng)	Concentration (ng g^{-1})	Recovery (%)	Relative standard deviation of each condition (%)
Single sample	1	0.0051	0.004	0.24	46.5	105.6	0.48
	2	0.0049	0.0039	0.23	46.9	106.6	
	3	0.0050	0.004	0.24	46.6	105.9	
Dosing technique	4	0.0051					
		0.0056					
		0.0053					
		0.0056					
		0.0050					
	Total	0.0266	0.0172	1.21	45.6	103.6	
	5	0.0047					
		0.0054					
		0.0056					
		0.0058					
Total		0.0046	0.017	1.20	46.1	104.7	
6	0.0261						
	0.0050						
	0.0055						
	0.0054						
	0.0056						
	0.0048						
Total	0.0263	0.0171	1.20	45.9	104.3	0.53	

We used 5 mg of the standard reference material, consistent with the Hg content of the wood samples. In this test, we used five samples, which increase the absorbance by a factor of five.

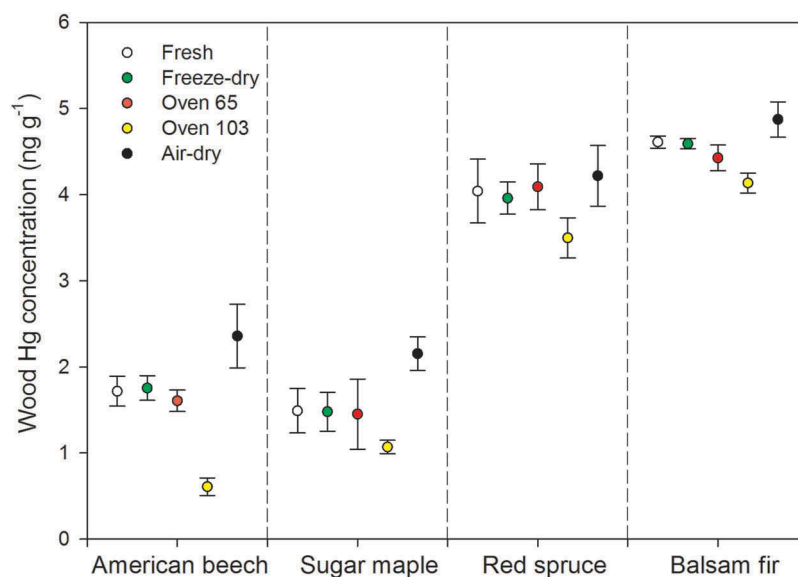


Figure 1. Corrected Hg concentrations measured in samples from four trees of different species prepared by five different methods. Oven-drying at 103°C results in Hg loss, while air-drying results in Hg gain.

(Figure 1). Thus, it appears that samples could be analysed fresh, freeze-dried or oven-dried at low temperature without altering Hg concentrations by more than 4%. The difference in Hg concentrations for the different preparation treatments was significant for American beech ($p < 0.001$) and balsam fir ($p = 0.01$), but not for sugar maple ($p = 0.10$) or red spruce ($p = 0.20$). For American beech and sugar maple, concentrations of Hg were overestimated by 34–45% in the air-dry samples and were underestimated by 44–66% in the samples oven-dried at 103°C, compared to Hg concentrations in the freeze-dried samples. For red spruce and balsam fir, concentrations of Hg were higher in the air-dry samples, but by only 6.1–6.5%, and they were only 9.9–12% low in the samples oven-dried at 103°C.

Although Hg losses due to oven-drying at 103°C were greater in the two hardwood trees than the two conifers when reported as a percentage of the concentration, they were more similar across samples when reported in units of concentration: 1.1 ng g⁻¹ for American beech and 0.4–0.5 ng g⁻¹ for the other three trees. Similarly, gains in Hg due to air-drying ranged from 0.3 ng g⁻¹ for the conifers to 0.6–0.7 ng g⁻¹ for the hardwoods, which was a much smaller percentage of the total Hg concentration in the conifers than the hardwoods (Figure 1).

3.3. Contamination of air-dried samples

Mercury concentration of air-dried samples differed by location ($p < 0.001$, Figure 2). Samples dried in the clean room at Syracuse University, the barn at Hubbard Brook and the soil sample processing room at Hubbard Brook all had Hg concentrations indistinguishable from the standard (freeze-dried samples), based on Tukey's honestly

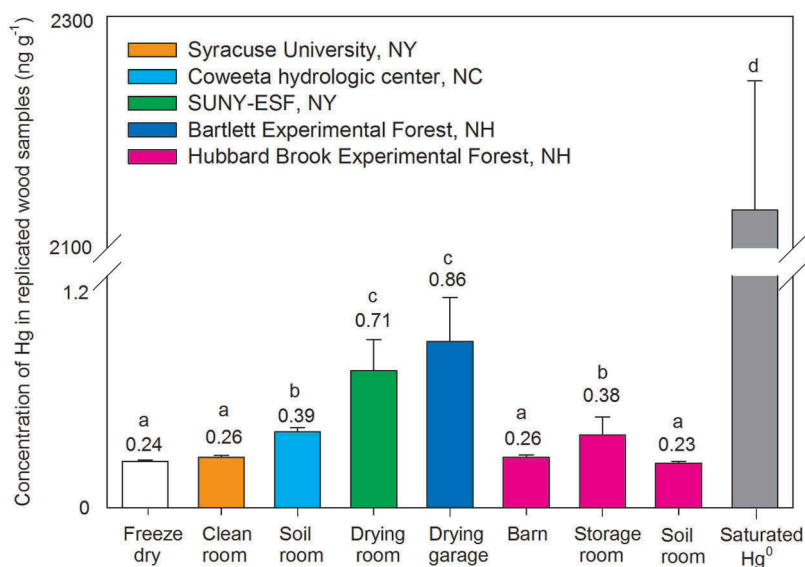


Figure 2. Mercury in sugar maple wood air-dried in different locations. For comparison, samples were prepared without any contamination (freeze-dried) and fully contaminated (in air exposed to liquid Hg). Different letters indicate significant differences (Tukey's honestly significant difference test).

significant difference. Not surprisingly, samples in air exposed to liquid Hg had the highest Hg concentration ($2133 \pm 111 \text{ ng g}^{-1}$). Samples dried in the drying room at ESF and the garage at Bartlett had quite elevated concentrations, 196–258% higher than the freeze-dried samples. Samples dried in the soil room at Coweeta and the archive building at Hubbard Brook had 63% and 57% higher concentrations compared to the freeze-dried samples.

4. Discussion

4.1. Techniques for measuring Hg in wood

We demonstrated that thermal decomposition, amalgamation and atomic absorption spectrophotometry can be used to measure concentrations of Hg in wood with an MDL of 0.05 ng (1.27 in unit of ng g^{-1}). The MDL should be reported in units of ng per sample because the calibration curve in used for this method is based on a mass of Hg, not a concentration.

Concentrations of Hg in wood samples have been measured using other techniques, such as CV AFS [15,18] and manual CV AAS [16]. These methods provide a lower detection limit than the method used in this paper (Table 1). However, both require sample preparation that involves a digestion process, whereas the Milestone DMA 80 direct Hg analyser allows the processing of solid samples. The digestion process is especially complex and challenging for CV AFS, for which all the organic Hg must be converted into inorganic Hg to ensure the transformation of inorganic Hg to elemental Hg using SnCl_2 [29].

Our method could be modified to further improve the detection of Hg at low concentration. We analysed samples of 100 mg, analysing a larger sample would increase the amount of Hg in the sample and thus reduce the detection limit in units of concentration. However, there is a limit to the mass of sample that can be placed into a tared nickel boat, which depends on the sample density and the boat volume. Ten times more sample can be analysed by using the instrument dosing feature, which allows multiple aliquots to be burned before desorption. We demonstrated this approach using five aliquots (Table 3). Using 10 aliquots would bring the detection limit down by an order of magnitude compared to only 1 aliquot.

4.2. Sample preparation

The loss of Hg from wood samples by oven-drying at 103°C was presumably due to volatilisation at this high drying temperature. The loss of Hg by oven-drying at 65°C was negligible, compared to freeze-drying, and the moisture content was similar between samples dried at 65 and 103°C. Thus, there is little advantage to drying at temperatures above 65°C.

Air-drying in some locations resulted in significantly elevated Hg concentrations. Indoor air is generally elevated in Hg [30] and varies by location due to history of Hg exposure. A dentist's office was reported to have high indoor airborne Hg due to the use of dental amalgam containing Hg [31,32]. Broken Hg thermometers or Hg manometers [33], gas pressure regulators containing Hg [34] and Hg in paint [35] are possible sources of elevated concentrations of Hg in indoor air. The highest Hg contamination we observed was in a garage at the Bartlett Experimental Forest, which houses snowmobiles. Vehicle exhaust and brake wear are both likely sources of Hg contamination [36].

We dried wood samples in air exposed to liquid Hg, which showed that wood absorbs Hg in gaseous form (Figure 2). The samples that were air dried in various laboratories were covered to prevent contamination by dust particles, which showed that the contamination we observed was due to gaseous Hg. Studies that reported Hg losses during air-drying of soils [22,37] attributed these losses to microbiological process that would reduce Hg²⁺ to gaseous Hg⁰. Wood samples have very little Hg to begin with and less microbial activity than soils, so it is not surprising that we did not observe Hg losses with air-drying.

We recommend that samples be freeze-dried or oven-dried at low temperatures for determination of Hg. Oven-drying is easier than freeze-drying, and the difference in water content was small as a fraction of sample fresh mass (5% for spruce, 3% for fir, 2% for sugar maple and 3% for beech; Table 2). Thus, although oven-dried tissues contain slightly more moisture than freeze-dried tissues, most tissue concentrations are reported on an oven-dry basis, and this should be acceptable for Hg concentrations.

Archived samples that have been oven-dried at high temperatures or stored exposed to the air may not be suitable for measurement of Hg. It is possible that large wood samples, such as logs or solid wood products, would contain interior tissue that is not contaminated. Researchers interested in using such materials, for example using Hg in tree rings to evaluate historical changes in Hg exposure, would need to characterise the rate of Hg loss through the wood in the case of oven drying or the rate of transport of air-derived Hg through wood in the case of atmospheric exposure.

4.2.1. Importance of wood to Hg budgets

To evaluate the possible importance of Hg in wood to ecosystem budgets, we compared the pool size of Hg in wood to that in leaves at the Hubbard Brook Experimental Forest, New Hampshire. The four tree species we studied account for 76% of the wood biomass and 78% of the leaf biomass at Hubbard Brook [38]. Using our data for these four species and the average of our values for the remaining softwood and hardwood species, we estimated the Hg content of bole wood at the ecosystem scale to be 0.32 g ha^{-1} . We sampled leaves from the same four species in 2015 (unpublished data) and found Hg concentrations of $22.7 \pm 1.6 \text{ ng g}^{-1}$ for American beech, $18.0 \pm 1.5 \text{ ng g}^{-1}$ for sugar maple, $19.5 \pm 2.2 \text{ ng g}^{-1}$ for red spruce and $33.2 \pm 3.5 \text{ ng g}^{-1}$ for balsam fir. Although these concentrations are 8 times those of wood, the mass of wood is 30 times the mass of leaves. Thus, the Hg content of foliage was calculated to be 0.15 g ha^{-1} , only half of the Hg content of wood. Similarly, in a Douglas-fir stand in Washington State, the wood contained more Hg (0.5 g ha^{-1}) than the foliage (0.3 g ha^{-1}), because of its greater biomass [39].

Including wood in estimates of Hg contained in forest vegetation is important, in spite of the low concentrations, because of the large mass of wood in forests. The magnitude of this pool suggests that biomass burning is potentially an important source of Hg to the atmosphere [2]. Our study shows that it is feasible to detect and report concentrations of Hg in wood if the right methods are selected.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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