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ARTICLE

Sources of variability in tissue chemistry in northern hardwood species[1]

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Abstract: Measurements of tree tissue chemistry are influenced by the precision and accuracy of laboratory analyses, sampling position within the tree, variation among replicate trees of the same species, and variation from year to year. We characterized these sources of uncertainty for six northern hardwood species and compared them with observed rates of long-term change. Uncertainty associated with laboratory quality control was small (1%–5%) and differed among elements, with K concentrations exhibiting the lowest accuracy and precision. Sampling position within the tree was more important for branches (the coefficient of variation was 23%) and wood (37%) than for foliage or bark (12% for both) ($p < 0.001$). Foliar N and P concentrations in leaves were less variable than other elements or tissue types both from tree to tree ($p = 0.02$) and from year to year ($p = 0.03$), which means that more samples would be needed to detect differences over space or time for Ca, Mg, or K in branches or wood. Concentrations of foliar N increased over 25 years at the Huntington Forest ($p \leq 0.03$) by > 16%. Uncertainty analysis can be used to guide the allocation of sampling effort, depending on the elements and tissue types of interest and the objectives of the study.

Key words: Fagus grandifolia Ehrh., *Acer saccharum* Marsh., *Acer rubrum* L., *Betula papyrifera* Marsh., *Betula alleghaniensis* Britt., *Prunus pensylvanica* L.f.

Résumé : Les mesures chimiques dans les tissus des arbres sont influencées par la précision et l'exactitude des analyses de laboratoire, la position de l'échantillonnage dans l'arbre, la variation parmi les arbres de la même espèce faisant partie d'un échantillonnage répété et la variation d'une année à l'autre. Nous avons caractérisé ces sources d'incertitude chez six espèces de feuillus nordiques et nous les avons comparées aux taux de changement observés à long terme. L'incertitude associée au contrôle de la qualité en laboratoire était faible (1–5 %) et différente selon les éléments; l'exactitude et la précision des concentrations de K étaient les plus faibles. La position de l'échantillonnage dans l'arbre était plus importante dans le cas des branches (coefficient de variation de 23 %) et du bois (37 %) que du feuillage et de l'écorce (12 % dans les deux cas) ($p < 0{,}001$). La concentration foliaire de N et P était moins variable que celle des autres éléments ou des types de tissus tant d'un arbre à l'autre ($p = 0{,}02$) que d'une année à l'autre ($p = 0{,}03$), ce qui signifie que davantage d'échantillons seraient nécessaires pour détecter des différences dans l'espace et le temps dans le cas de Ca, Mg ou K dans les branches et le bois. La concentration foliaire de N a augmenté de plus de 16 % ($p \leq 0{,}03$) sur 25 ans à la forêt de Huntington. L'analyse de l'incertitude peut être utilisée pour orienter l'allocation de l'effort d'échantillonnage selon les éléments et les types de tissus visés et les objectifs de l'étude. [Traduit par la Rédaction]

Mots-clés : Fagus grandifolia Ehrh., *Acer saccharum* Marsh., *Acer rubrum* L., *Betula papyrifera* Marsh., *Betula alleghaniensis* Britt., *Prunus pensylvanica* L.f.

Introduction

Changes in the nutritional status of forests and thus tree tissue chemistry can result from many different factors, including natural disturbances, forest management (Purahong et al. 2014), and pollutant loading (Aber et al. 2003; Elvir et al. 2006). Examples of reported changes in foliar chemistry include increases in nitrogen concentration and decreases in phosphorus, calcium, magnesium, and potassium concentrations in Europe from 1969 to 1997 (Duquesnay et al. 2000), from 1984 to 1995 (Flückiger and Braun 1998), and from 1993 to 2005 (Jonard et al. 2009). However, there are many sources of uncertainty in estimating long-term changes in tree nutrients, some of which are not commonly accounted for.

Laboratory analyses contribute uncertainty in measurements of nutrient concentrations; this uncertainty is usually characterized with replicate analyses and standard reference materials. Sampling

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Table 1. Site descriptions for the Huntington Wildlife Forest (HWF), Hubbard Brook Experimental Forest (HBEF), and Bartlett Experimental Forest (BEF).

Studies	Stands	Year cut	Latitude (N)	Longitude (W)	Elevation (m asl)	Aspect	Slope (%)	Dominant overstory vegetation	Basal area (m²·ha⁻¹)
HWF	IFS hardwood site	~1915	43°59′	74°14′	530	NE	5–15	American beech, sugar maple, red maple	26
HBEF	Base of W7	~1915	43°56′	74°45′	689	NE	12–14	American beech, sugar maple, yellow birch	26
BEF	C1	1990	44°02′	71°19′	570	SE	5–20	Pin cherry, white birch, American beech	12
	C2	1988	44°04′	71°16′	340	NE	15–30	Red maple, American beech, white birch	15
	C4	1979	44°03′	71°16′	410	NE	20–25	White birch, pin cherry, red maple	26
	C6	1975	44°02′	71°16′	460	NNW	13–20	White birch, pin cherry, red maple	27
	C9	1890	44°03′	71°17′	440	NE	10–35	American beech, sugar maple, yellow birch	30
	C8	1883	44°03′	71°18′	330	NE	5–35	American beech, sugar maple, yellow birch	32

error due to variation among trees in the population sampled is also commonly reported. Sampling position within the tree may contribute uncertainty to estimates of changes in nutrient concentrations, as samples collected from different parts of a tree may differ in concentration. For example, observed differences in nutrient concentrations of branches sampled at different places or times may be due to differences in the diameter of the branches sampled (Whittaker et al. 1979). This source of uncertainty is more difficult to characterize than laboratory and sampling error. Interannual variation in nutrient concentrations is another source of uncertainty that could be mistaken for change over time if a limited number of sampling dates are compared.

Foliage has been well studied for variation in nutrient concentrations within the tree (Le Tacon and Toutain 1973; Van den Driessche 1974; Ellis 1975; Morrison 1985; Erdmann et al. 1988), from tree to tree (Ellis 1975; Morrison 1985; Erdmann et al. 1988), and from year to year within a 5-year period (Alban 1985; Duquesnay et al. 2000; Bussotti et al. 2000). Other tissues such as boles and branches are less often studied and are more difficult to sample repeatedly but are more important to forest nutrient budgets due to their greater biomass (Pardo et al. 2004; Paré et al. 2013).

The sampling intensity required to detect a change over time depends on the magnitude of uncertainty sources and is an important consideration when budgeting for a monitoring program (Levine et al. 2014). A comparison of the relative magnitude of all sources of uncertainty could be used to improve allocation of sampling effort to best detect change over time in tree tissue nutrient concentrations. In this paper, we report the coefficient of variation (CV), which is the standard deviation as a percentage of the mean, to facilitate comparisons across tissue types and elements that differ widely in concentration.

This study reports uncertainty in concentrations of N, P, Ca, Mg, and K of bark, branches, foliage, and wood in six northern hardwood species: American beech (*Fagus grandifolia* Ehrh.), sugar maple (*Acer saccharum* Marsh.), red maple (*Acer rubrum* L.), white birch (*Betula papyrifera* Marsh.), yellow birch (*Betula alleghaniensis* Britt.), and pin cherry (*Prunus pensylvanica* L.f.). We report the accuracy of analysis of standard reference material, the precision of replicate laboratory analyses, and the magnitude of interannual variation using samples collected from the Huntington Wildlife Forest (HWF) in the Adirondacks of New York State. We characterize the effect of sampling position within trees from the Hubbard Brook Experimental Forest (HBEF) and sampling uncertainty due to tree-to-tree variability at the Bartlett Experimental Forest (BEF), both in the White Mountains of New Hampshire. Long-term changes in tree tissue nutrient concentrations are reported with associated uncertainty sources using samples collected from HWF over a 28-year interval. Quantifying the magnitude of these various sources of uncertainty provides a basis for optimizing sampling efforts and makes it possible to predict the sampling intensity necessary to detect a possible change in nutrient concentrations.

Materials and methods

We sampled trees at three sites (HWF, HBEF, and BEF) to provide a comprehensive assessment of sources of variation in tissue nutrient concentrations. At HWF, bark, branch, foliage, and wood were collected in 1985, 1986, 1987, 2012, and 2013, which allows an analysis of interannual variability and long-term change. At HBEF, samples of the same tissue types were collected at different positions within the trees. At BEF, samples of the same tissue types were collected from replicate trees in multiple stands, which allows for an analysis of within- and between-species variability.

Study sites

The HWF is located in the Adirondack Mountains of northern New York. The HBEF and the BEF are located in the White Mountain National Forest in central New Hampshire. The annual mean temperature and precipitation were 5.0 °C and 105 cm at HWF (1940–2007; Mitchell et al. 2009), 5.7 °C and 140 cm at HBEF (1955–2005; Campbell et al. 2007), and 4.4 °C and 130 cm at BEF (1932–2000; Smith and Martin 2001). Soils at all three sites are dominantly well drained, loamy, Haplorthods developed in glacial drift (Somers 1986; Huntington et al. 1988; Vadeboncoeur et al. 2014). Stands differ in age, slope, aspect, elevation, and species composition (Table 1).

We sampled trees in mature stands (>100 years after harvest) at HBEF and HWF. At BEF, trees were sampled in two stands in each of three age classes (15, 30, and >100 years after harvest). Young and middle-aged stands were dominated by American beech, yellow birch, red maple, white birch, and pin cherry, while mature stands at all three sites were dominated by American beech, sugar maple, and yellow birch.

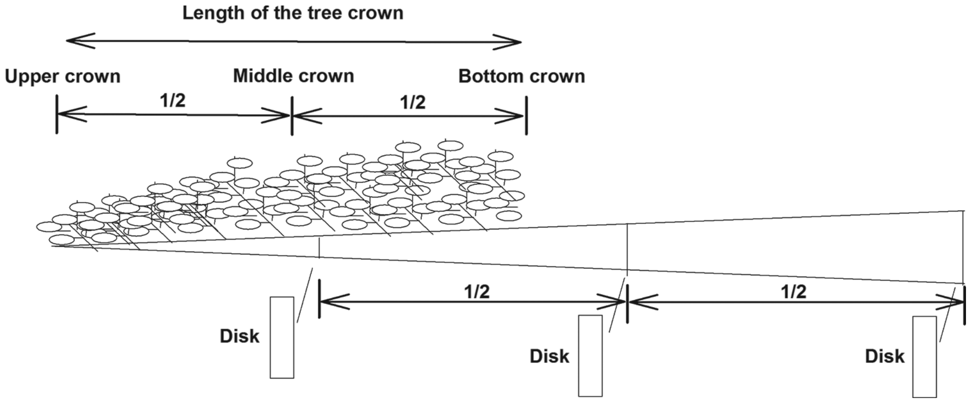
Field sampling

Sampling at HWF

In 1985, a survey line was established consisting of 39 points encompassing 4.7 ha (at the Integrated Forest Study site; Johnson and Lindberg 1992). The same survey line was resampled every sampling period. In August of 1985, 1986, 1987, 2012, and 2013, at least five trees of each of four species (American beech, sugar maple, red maple, and yellow birch) with diameter at breast height (DBH) > 10 cm were selected for sampling along the survey line. Trees nearest each sample point were selected in the 1980s. Because of destructive sampling for allometric analysis (Briggs et al. 1989), trees were not tagged or repeatedly sampled in 1985–1987. The trees sampled in 2013 were the same as the trees sampled in 2012.

Bark was collected from the stem 1.3 m above the ground with a chisel and hammer. Two branches from each tree were cut from the base of the crown, at least 1 m from the trunk, using a ladder and pruner. Twenty to 30 healthy leaves, without signs of herbivory or pathogens, were collected from the cut branches of each tree. Three cores were collected from each tree at breast height using a Pressler's increment borer 5 mm in diameter.

Fig. 1. Sampling design for characterizing variability in tissue nutrient concentrations due to sampling position within the tree. Disks for wood and bark were collected at the base of the tree, the top of the merchantable bole (10 cm in diameter), and the midpoint. Branches were collected by diameter class. Leaves were collected from the top, midpoint, and bottom of the crown.



Sampling at HBEF

To address the variability of nutrient concentrations within a tree, one tree of each of three species (American beech, sugar maple, and yellow birch) with DBH of ~30 cm was felled in July 2013. Branch samples were collected with diameters of 0.5, 1, 2, and 3 cm. Thirty leaves without petioles were collected at three canopy positions (bottom, middle, and upper). Disks were collected from the bole of each tree at three heights (Fig. 1) and separated into bark and wood in the laboratory.

Sampling at BEF

A total of 101 trees of six species were sampled in 2005 and 2006 (American beech, red maple, sugar maple, white birch, yellow birch, and pin cherry; Fatemi et al. 2011). In young and middle age stands, 71 trees ranging from 2 to 12 cm DBH were felled. We collected leaves from the entire canopy, sampled the branches by size class, and cut disks every 2 m along the stem (every 1 m if the tree height was less than 6 m). Disks were separated into bark and wood in the laboratory. In mature stands, 30 trees with DBH > 12 cm were selected for three species (American beech, sugar maple, and yellow birch). Leaves were sampled using a 12-gauge shotgun. Bark was collected from the stem at 1.5 m above the ground with a chisel and hammer. Two tree cores were taken to the pith from each tree at approximately 1.0 m above the ground using a Haglof increment borer 4 mm in diameter.

Sample processing and analysis

Samples from HWF and HBEF

Wood samples were separated into lightwood and darkwood based on color using a chisel. Tissue samples were dried at 60 °C and ground in a Wiley mill to pass a 20 mesh screen. Total N was analyzed using a Kjeldahl digestion method in the 1980s and a carbon–nitrogen elemental analyzer (Thermo Electron Corporation, EA1112 elemental analyzer, SUNY-ESF) in 2012 and 2013. Subsamples were ground to pass 40 mesh screen, ashed at 470 °C, and dissolved in 5 mL of 6 mol·L^{-1} HNO_3 on a hot plate (Siccama et al. 1994). Concentrations of P, Ca, Mg, and K were determined by Perkin-Elmer Optima 3300DV inductively coupled plasma optical emission spectroscopy (ICP-OES) for all samples. National Institute of Standards and Technology (NIST) solid standard reference material (NIST 1515, apple leaves) was run after every 10 samples. Samples were reprocessed and the analyzer was recalibrated when the error in recovery of the SRM was larger than 5%.

Samples from BEF

Samples were oven-dried at 60 °C and ground in a Wiley mill to pass a 20 mesh screen. Subsamples were ground to a fine powder; total N was determined using a carbon–nitrogen combustion analyzer. Plant tissue was digested either in a microwave oven (9 mL of 6 mol·L^{-1} HNO_3) or by dry ashing in a muffle furnace at 470 °C and acid digestion on a hot plate (Bickelhaupt and White 1982). These two digestion methods gave comparable results for tissue samples and standard reference materials (Rechcigl and Payne 1990). Concentrations of P, Ca, Mg, and K were then determined by ICP-OES. Standard reference material (NIST 1515, apple leaves) was used for quality control as described above.

Data analysis

To describe the precision of laboratory analyses, the SD and CV of nutrient concentrations were calculated for 12 samples (four tissue types of each of three species: American beech, yellow birch, and red maple) collected from HWF in the 2010s and run in duplicate. These statistics were used as the dependent variable in a general linear model to test the effects of element and tissue type on the precision of laboratory analyses. For this and all other models, Tukey's honestly significant difference was used to compare means where the null hypothesis of no effect was rejected ($\alpha = 0.10$). The independent variables in this and all other models were treated as fixed factors because we were interested in their effects. The SD and CV were log-transformed in all of the analyses to meet the assumption of normality of the residuals.

To describe the accuracy of laboratory analyses, we used the bias in concentrations of a certified standard reference material (NIST 1515, apple leaves) run 20 times for N and 12 times for P, Ca, Mg, and K. The recovery (the difference between the measured value and the certified value) was calculated, and a one-sample *t* test was used to determine whether the recovery was different from 100%.

To analyze the variability in nutrients sampled from different positions in the tree, we used data from three trees at HBEF. A general linear model was used to test the effects of element and tissue type on the SD and CV of nutrient concentrations, with the three trees as replicates. The total number of observations (SDs or CVs) included in the model was 75 (5 elements × 5 tissue types × 3 species).

To describe variability among individuals of a species, we used data from BEF in 2005. The SD and CV of nutrient concentrations were calculated by element, tissue type and species based on the 3–5 replicate trees sampled in each stand (Fatemi 2007). A general linear model was used to test the effects of element, tissue type, species, stand age, and their interactions on SDs and CVs of replicate trees, with stand treated as a nested variable within stand age (Table 2). The total number of observations (SDs or CVs) included in the model was 570 (110 for American beech, sugar maple, and yellow birch; 80 for pin cherry, red maple, and white birch).

Table 2. ANOVA table for the general linear model testing the effects of age, species, tissue type, and element on the CV among individuals of a species using data from BEF in 2005.

Source	Degrees of freedom	Sum of squares	Mean square	*F* value	*p* value
Model	17	9.81	0.58	5.34	<0.0001
Error	552	59.71	0.11		
Corrected total	569	69.52			
Stand (age)	2	1.39	0.69	6.40	0.002
Species	5	1.17	0.23	2.16	0.06
Tissue	3	5.35	1.78	16.50	<0.0001
Element	4	1.20	0.30	2.78	0.03
Stand	3	0.70	0.23	2.16	0.09

Note: Stand was nested within stand age.

Table 3. Analysis of reference material (NIST 1515, apple leaves) for evaluating accuracy of laboratory analyses.

	N	P	Ca	Mg	K
Observed value (%)	2.33±0.02	0.16±0.004	1.58±0.04	0.27±0.08	1.54±0.32
Certified value (%)	2.25±0.20	0.16±0.02	1.53±0.20	0.27±0.03	1.61±0.20
Recovery (%)	103.6±1.0	101.9±1.4	103.4±1.4	100.7±1.8	95.7±1.0
CV (%)	2.47±0.20	1.31±0.04	2.41±0.10	0.52±0.05	3.14±0.20

Note: For N, $n = 20$; for P, Ca, Mg, and K, $n = 12$. Means and standard error are shown.

To describe interannual variation in nutrient concentrations, we used data collected in 1985, 1986, and 1987 at HWF. The SD and CV of nutrient concentrations across the 3 years was calculated by element, tissue type, and species using the median nutrient concentration of the 5–16 trees sampled of each species in each sampling year. A general linear model was used to test the effects of element, tissue type, and species and their interactions on these SDs and CVs. The total number of observations (SDs or CVs) included in the model was 80 (5 elements × 4 tissue types × 4 species).

Long-term changes in nutrient concentrations were calculated between the two sampling periods at HWF. The 3 years in the 1980s and 2 years in the 2010s were compared using a two-sample *t* test, using the median nutrient concentration of replicate individuals by element, tissue type, and species for each sampling year. The change was calculated as the difference in average across years of the median nutrient concentrations between the two sampling periods (1980s and 2010s), expressed as a percentage of the 1980s value.

We estimated the number of replicate trees required to detect a 20% difference in nutrient concentrations between our observations and those collected at a future date or in another stand using eq. 1, with power $(1 - \beta) = 0.8$ and $\alpha = 0.05$:

$$n = 2 \times (Z_{1-\alpha/2} + Z_{1-\beta})^2 \times \mathrm{CV}^2/\mathrm{PC}^2 \qquad (1)$$

where n = sample size, PC = percentage change, the Z statistic describes the probability that two populations differ, based on a normal distribution, α = level of significance, and $\beta = 1 -$ statistical power (Van Belle and Millard 1998). Note that for power = 0.8 and $\alpha = 0.05$, $(Z_{1-\alpha/2} + Z_{1-\beta})^2 = 8$, which makes this calculation easy to implement.

We used data from BEF to estimate the number of trees needed to detect a change for each element, tissue type, species, and stand age. A general linear model was used to test the effects of element, tissue type, species, stand age, and their interactions on the sample sizes required to detect a difference.

Fig. 2. Variability of nutrient concentrations among replicates in the laboratory using data from HWF in 2010s.

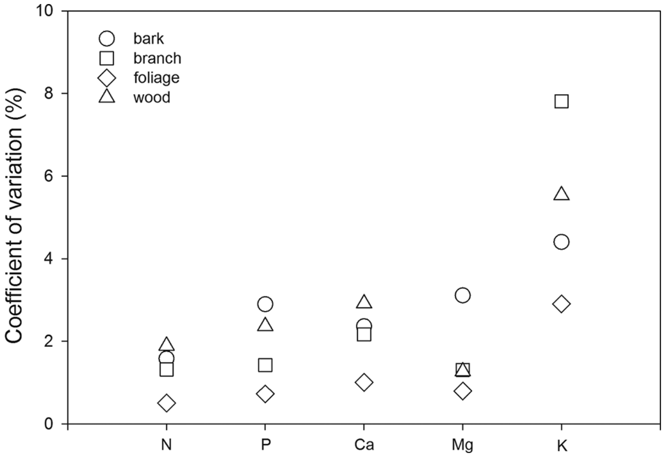
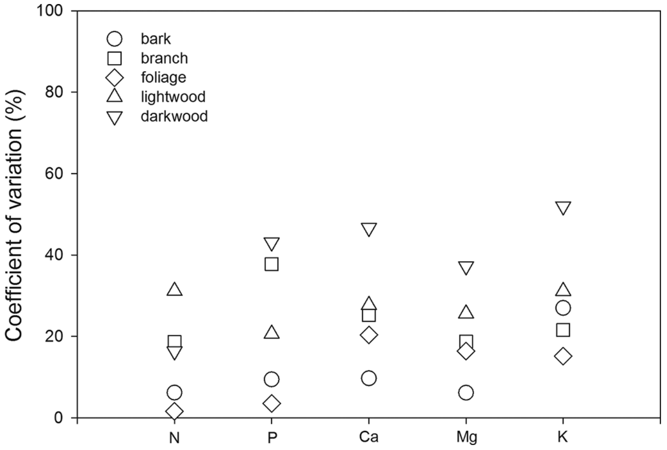


Fig. 3. Variability of nutrient concentrations across three sampling positions for each tissue type (Fig. 1) using datasets from HBEF in 2013. Each symbol represents the average CV for three individuals, one each of American beech, sugar maple, and yellow birch.



Statistical analyses were conducted with SAS 9.4 (SAS Institute Inc. 2013).

Results

Accuracy and precision in laboratory analyses

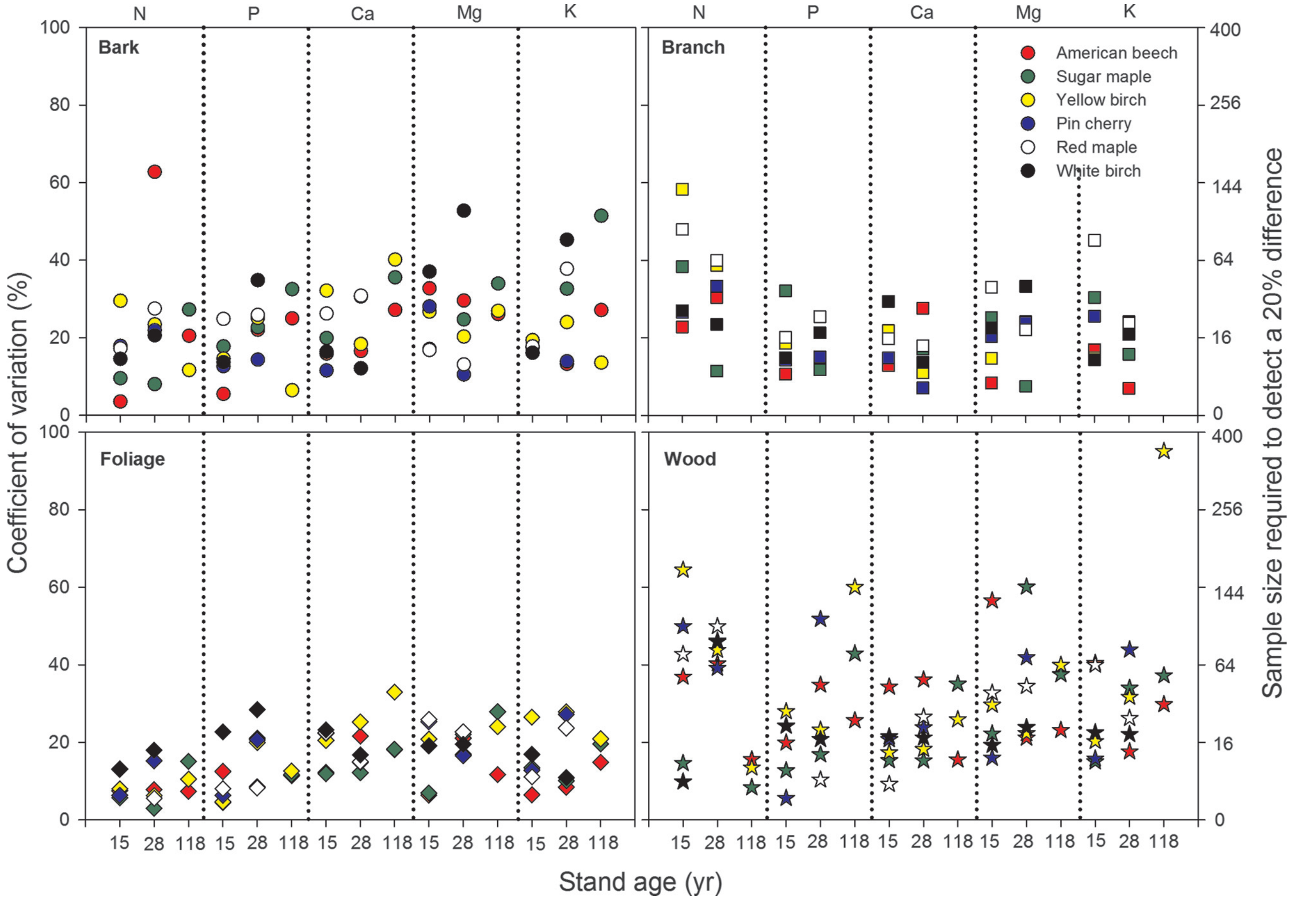
The precision of laboratory analysis was calculated from duplicate analyses of three samples of each tissue type collected from HWF (2012–2013). Coefficients of variation of these replicates ranged from 1% to 8%, depending on the tissue and element (Fig. 2). Precision differed among tissue types ($p = 0.05$); bark had the largest CV (3.2%) and foliage had the smallest (1.6%). Elements also differed significantly in precision ($p = 0.01$), with K exhibiting the largest CV (5.2%) and N the smallest (1.3%).

Accuracy was assessed via analysis of standard reference material (NIST1515, apple leaves). Recovery averaged from 95% to 104%, depending on the element (Table 3). Reported values averaged 3% higher than the reference values for N and Ca and 4% lower for K; elements differed significantly in accuracy ($p = 0.01$).

Variability within and among trees

Nutrient concentrations differed depending on sampling position within trees of three species sampled at HBEF in 2013 (Fig. 6; Appendix Fig. A1). Tissue types differed in the amount of variation due to sampling position, represented by the CV ($p < 0.001$): dark-

Fig. 4. Tree-to-tree coefficient of variation (CV) (left axis) and sample size required to detect a 20% difference in nutrient concentrations (right axis) of stands of different ages using data from BEF. (This figure is available in colour on the Web.)



wood exhibited the largest CV (44% across three heights, averaged for the three trees), while foliage and bark exhibited the smallest (12%) (Fig. 3). Elements also differed in variability within trees ($p = 0.08$), with K having the largest CV (29%) and N having the smallest (18%).

Variability in nutrient concentrations among trees was reported for trees sampled at BEF in 2005. Species had similar tree-to-tree CVs within stands (21%–25%, averaged across tissues and elements) at BEF ($p = 0.19$; Fig. 4). Tree-to-tree variability depended on the tissue type ($p < 0.001$), with wood having the largest CV (30%) and foliage having the smallest (16%) (Fig. 4). Elements also differed ($p = 0.03$), with K having the largest CV (24%) and P having the smallest (19%) (Fig. 4). Stand age had a significant effect on tree-to-tree variability ($p = 0.002$) in that mature stands had a high CV (26%), while young stands showed the least variation (21%). Wood N was especially variable in units of CV (35%), and foliage N and P were the least variable (11%), resulting in a significant interaction of tissue and element ($p = 0.02$). Sugar maple in mature stands varied most from tree to tree (CV = 29%), and American beech in young stands varied the least (19%), resulting in a significant interaction of stand age and species ($p = 0.05$).

Interannual variability and long-term nutrient dynamics

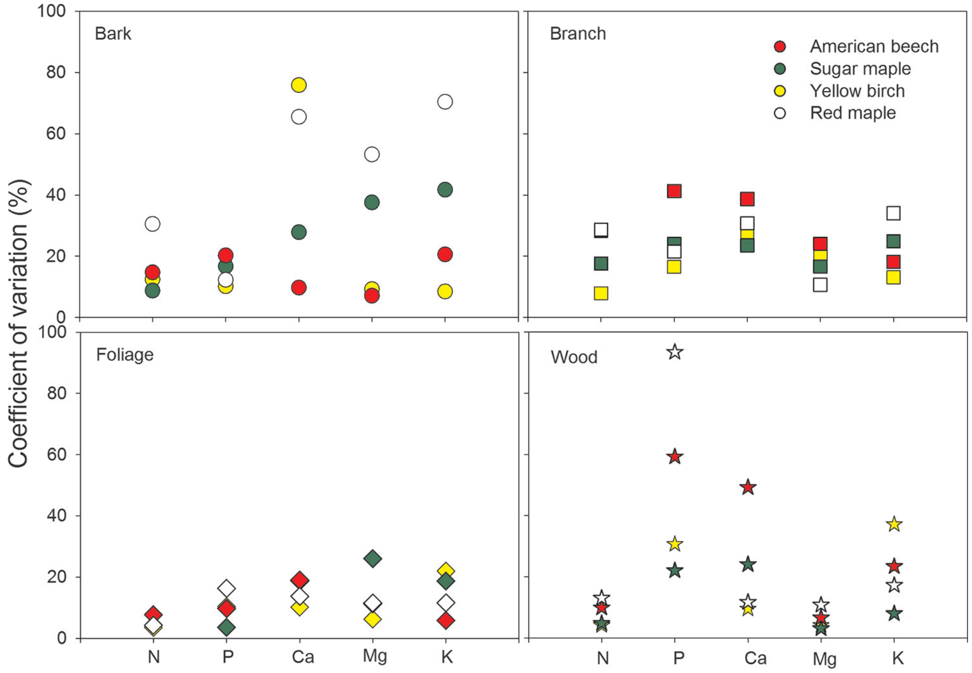
Species differed in interannual variability based on trees sampled at HWF in 1985, 1986, and 1987 ($p = 0.06$), with red maple exhibiting the largest CV (28%) and yellow birch showing the smallest (17%) (Fig. 5). Elements also differed in interannual variability ($p = 0.001$), with Ca exhibiting the largest CV (28%) and N again having the smallest (13%) (Fig. 5). Tissue types also differed ($p = 0.001$), with bark having the largest interannual variability (28%) and foliage having the smallest (12%). Wood P was especially variable in units of CV (51%), and foliar N was the least variable (6%), resulting in a significant interaction of tissue and element ($p = 0.03$).

There were significant differences between tissue nutrient concentrations of trees in the 1980s and 2010s (Table 4). Concentrations of foliar N reported in red maple, sugar maple, and yellow birch increased ($p \leq 0.03$), and concentrations of foliar K reported in American beech decreased ($p = 0.02$). For non-leaf tissues, concentrations of bark N reported in American beech ($p = 0.02$) and concentrations of branch Ca reported in red maple and sugar maple increased from the 1980s to 2010s ($p \leq 0.02$). Concentrations of wood Ca and Mg reported in red maple ($p \leq 0.02$) and concentrations of branch K reported in yellow birch ($p = 0.04$) decreased from the 1980s to 2010s.

Variability in units of concentrations

Wood and bark exhibited greater variability than foliage in units of CV, but in some cases, such as the calculation of nutrient pools, units of concentration are more relevant. We tested whether the greater variability of wood and bark persisted in comparisons using concentration (SD) as the dependent variable. Recall that wood had the highest variability within the tree and from tree to tree in units of CV, and foliage exhibited the smallest variability in laboratory precision, within the tree, and from tree to tree and year to year. In contrast, using SD as the dependent variable instead of CV, foliage had the largest variability in laboratory precision ($p < 0.001$), within the tree ($p < 0.001$), from tree to tree ($p < 0.001$), and from year to year ($p < 0.001$), whereas wood exhibited the smallest.

Fig. 5. Interannual variability of nutrient concentrations in trees sampled at HWF in 1985, 1986, and 1987. (This figure is available in colour on the Web.)



Sample size required to detect a 20% difference in nutrient concentrations

We compared the sampling intensity required to detect changes over time of a given magnitude depending on the element, species, tissue type, and stand age, using the data from BEF. The sample size required is proportional to the variance (CV^2) and inversely proportional to the square of the difference to be detected. We selected 20% as a magnitude of change that might reasonably be expected to be detectable, and we assumed that the variability of concentrations would be the same at a future sampling date. To detect a 20% change in nutrient concentration, wood required the largest number of replicates (47 trees, on average, depending on the element, species, and stand age) and foliage required the fewest (14 trees on average) ($p < 0.001$) (Fig. 4). Stand age also affected the sample size required to detect a concentration difference; mature stands required more replication (38 trees on average, depending on the element, tissue type, and species) than middle-aged (31) or young (26) stands ($p = 0.01$). The sampling intensity required to detect a difference did not vary by species ($p = 0.36$) or elements ($p = 0.11$). The interaction of tissue type and element was significant ($p = 0.002$). Foliar N and P required the fewest replicates (8 trees on average, depending on species and stand age), and N in branches or wood required the most (59 trees on average). The interaction of stand age and species was significant ($p = 0.03$); sugar maple in mature stands required the largest number of replicates (43 trees on average, depending on element and tissue type) and American beech in young stands required the fewest (24 trees on average).

Discussion

Precision of laboratory analyses by element

Laboratory analyses introduced ≤ 5% uncertainty in tree tissue concentrations, which was small compared with other sources in this study (Table 5). For the five elements that we studied here, variation in laboratory analysis of replicate samples was mostly < 10% for standard tree leaf samples analyzed by 21 laboratories in Holland (La Bastide and Van Goor 1978), for foliar tissues of agricultural crops analyzed by 8 laboratories in Ohio and Illinois (Watson 1981), and for tree foliage analyzed by 54 laboratories in 25 countries through the Needle/Leaf Interlaboratory Comparison Test (Furst 2015). For trace elements, variation can be much higher (Furst 2015). Among the elements that we studied, K had the poorest precision in laboratory analyses; the other elements were not statistically distinguishable from one another in precision (Fig. 2). Because K suffers from ionization effects in the presence of other alkali metals, it is necessary to quantify K in a radial mode (torch positioned vertically in relation to the optical system) when using ICP-OES (Method 200.7, U.S. Environmental Protection Agency (USEPA) 2004). Concentrations of P, Ca, and Mg are quantified in an axial mode, which is about 10 times more sensitive than the radial mode. Thus K suffers from low signal magnitudes, and detection limits are high (700 ppb) compared with P (76 ppb), Ca (30 ppb), and Mg (30 ppb) in ICP-OES (Method 200.7, USEPA 1994). These detection limits are not a problem for nutrient analysis as they are low relative to tree tissue concentrations (Table 4).

Tree-to-tree and interannual variability by element

Elements differed in variability from tree to tree and from year to year. Potassium was found to be the most variable element from tree to tree, with a CV 2% higher, on average, than the other elements (24% CV compared with 22% on average for other elements; Table 5). Potassium is highly mobile in plant tissues, and this characteristic has been invoked to explain the higher variability of K in foliage than other elements, such as for sugar maple and white ash (*Fraxinus americana* L.) in Ontario (Ellis 1975). In our study, however, the greater observed variability in K could be due to the poorer laboratory precision for this element, because K was 3% more variable than the other elements (5% CV compared with 2% on average for the other elements; Table 5). Calcium varied the most from year to year and N varied the least, especially in foliage (Fig. 5), perhaps reflecting the greater degree of biological control of N cycling (Chapman et al. 2006). Where this difference applies, fewer trees could be sampled in studies devoted to N than those monitoring other elements.

Foliar N and P exhibited less variability from tree to tree and from year to year compared with Ca, Mg, and K in bark, branches,

Table 4. Median nutrient concentrations in bark, branch, foliage, and wood at HWF. The average of these values was computed for the two sampling periods (1980s vs. 2010s) and the change is the difference divided by the average for the 1980s.

			Median nutrient concentration at each sampled year ($mg \cdot g^{-1}$)					
Tissue type	Nutrient element	Species	1985	1986	1987	2012	2013	Change (%)
Bark	N	AB	6.9	8.0	6.1	12.6	10.6	66*
		SM	5.5	5.3	6.5	10.8	7.5	59
		YB	5.7	5.2	6.9	7.6	6.6	20
		RM	6.0	5.4	9.3	8.3	6.0	4
	P	AB	0.3	0.4	0.3	0.4	0.4	20
		SM	0.3	0.3	0.3	0.4	0.4	33
		YB	0.3	0.3	0.3	0.3	0.3	0
		RM	0.3	0.3	0.5	0.4	0.4	9
	Ca	AB	37.3	33.7	29.5	31.2	27.8	–12
		SM	21.9	26.6	37.8	24.2	29.0	–8
		YB	9.8	10.1	38.7	6.6	13.4	–49
		RM	15.2	12.2	36.7	20.7	18.2	–9
		AB	0.5	0.4	0.5	0.7	0.5	29
		SM	0.8	0.8	0.4	1.3	1.2	88
		YB	0.4	0.5	0.6	0.5	0.5	0
		RM	0.3	0.4	0.8	0.5	0.5	0
	K	AB	1.5	1.7	1.2	1.8	2.1	33
		SM	2.6	3.3	1.2	2.0	2.2	–11
		YB	0.9	1.1	1.0	0.6	0.8	–30
		RM	0.9	1.2	3.2	0.8	1.6	–32
Branch	N	AB	2.4	4.6	4.2	6.6	6.0	69
		SM	3.2	4.6	4.0	6.8	6.4	68
		YB	4.6	5.0	5.2	6.7	5.6	25
		RM	6.0	5.4	9.3	8.3	6.0	4
	P	AB	0.2	0.4	0.4	0.3	0.3	–10
		SM	0.3	0.4	0.3	0.5	0.5	50
		YB	0.4	0.4	0.5	0.4	0.4	–8
		RM	0.3	0.2	0.4	0.4	0.4	33
	Ca	AB	3.8	10.3	9.6	10.3	8.4	18
		SM	6.9	8.8	6.0	7.5	7.2	2
		YB	6.3	5.4	8.5	9.4	8.4	32
		RM	7.1	4.9	2.7	11.9	13.2	156*
	Mg	AB	0.4	0.6	0.5	0.6	0.4	0
		SM	0.3	0.5	0.4	0.6	0.4	25
		YB	0.5	0.6	0.7	0.6	0.6	0
		RM	0.4	0.4	0.4	0.5	0.5	0
	K	AB	1.2	1.2	1.7	1.2	1.1	–16
		SM	1.4	2.2	2.0	2.4	1.9	15
		YB	1.1	1.2	1.3	0.9	0.9	–25*
		RM	1.4	1.1	2.1	1.5	1.7	4
Foliage	N	AB	24.1	26.5	22.0	26.2	25.1	6
		SM	19.7	19.0	16.5	24.9	24.5	34*
		YB	25.2	26.0	25.2	27.0	26.8	6*
		RM	19.2	20.3	18.5	22.5	22.5	16*
	P	AB	1.3	1.5	1.2	1.2	1.2	–10
		SM	1.1	1.1	0.9	1.5	1.2	31
		YB	1.4	1.7	1.5	1.3	1.3	–15
		RM	1.2	1.4	1.1	1.2	1.2	–3
	Ca	AB	6.7	8.6	N/A	9.6	8.6	61
		SM	8.6	6.7	N/A	8.2	6.9	38
		YB	12.0	11.5	N/A	12.8	11.2	37
		RM	8.3	6.9	N/A	8.5	8.5	52
	Mg	AB	1.3	1.8	1.7	2.0	1.8	19
		SM	1.7	1.2	1.0	1.1	1.2	–12
		YB	3.1	2.9	3.2	2.7	2.5	–15
		RM	1.9	1.7	1.5	1.9	1.9	12
	K	AB	7.8	7.7	7.1	5.6	4.7	–32*
		SM	7.7	6.1	6.1	5.2	4.5	–27
		YB	14.4	8.6	8.8	8.0	5.9	–34
		RM	6.4	7.7	6.0	4.8	4.8	–28

Table 4. (*concluded*).

Tissue type	Nutrient element	Species	Median nutrient concentration at each sampled year (mg·g^{-1}) 1985	1986	1987	2012	2013	Change (%)
Wood	N	AB	1.3	1.3	1.1	1.3	2.0	34
		SM	1.0	0.9	1.0	1.1	1.7	45
		YB	0.8	0.9	0.9	1.1	1.8	67
		RM	0.9	0.9	0.7	1.0	1.5	50
	P	AB	0.07	0.06	0.02	0.04	0.05	–10
		SM	0.1	0.1	0.1	0.1	0.1	0
		YB	0.04	0.06	N/A	0.03	0.04	–30
		RM	0.04	0.09	0.08	0.05	0.05	–29
	Ca	AB	0.8	1.0	2.2	1.0	1.5	–6
		SM	1.7	1.5	2.3	1.4	1.2	–29
		YB	0.8	0.9	1.1	1.0	1.0	7
		RM	1.9	1.5	2.1	0.8	0.8	–56*
	Mg	AB	0.2	0.2	0.2	0.2	0.2	0
		SM	0.2	0.2	0.2	0.2	0.2	0
		YB	0.2	0.2	0.2	0.2	0.2	0
		RM	0.3	0.2	0.3	0.1	0.2	–44*
		AB	0.8	0.6	0.9	0.3	0.4	–54
		SM	0.5	0.6	0.7	0.4	0.5	–25
		YB	0.4	0.2	0.3	0.1	0.3	–33
		RM	0.8	1.1	1.7	0.7	0.6	–46

Note: Asterisks indicate significance at $\alpha = 0.05$.

Table 5. Magnitude of different sources of uncertainty in tissue nutrient concentrations.

		Coefficient of variation (%)								
		Nutrient element					Tissue type			
Dataset used for analysis	Source of uncertainty	N	P	Ca	Mg	K	Bark	Branch	Foliage	Wood
HWF	Laboratory	1	3	2	2	5	3	3	2	3
HBEF	Within tree	18	27	25	20	29	12	23	12	35
BEF	Among tree	23	19	21	23	24	23	22	16	30
HWF	Among years	13	25	28	16	23	28	23	12	22

Note: The CVs for nutrient element are based on tissue types as replicates. The CVs for tissue type are based on nutrient elements as replicates.

Table 6. Variability in foliar nutrient concentrations within trees, among trees, and among years calculated from other studies.

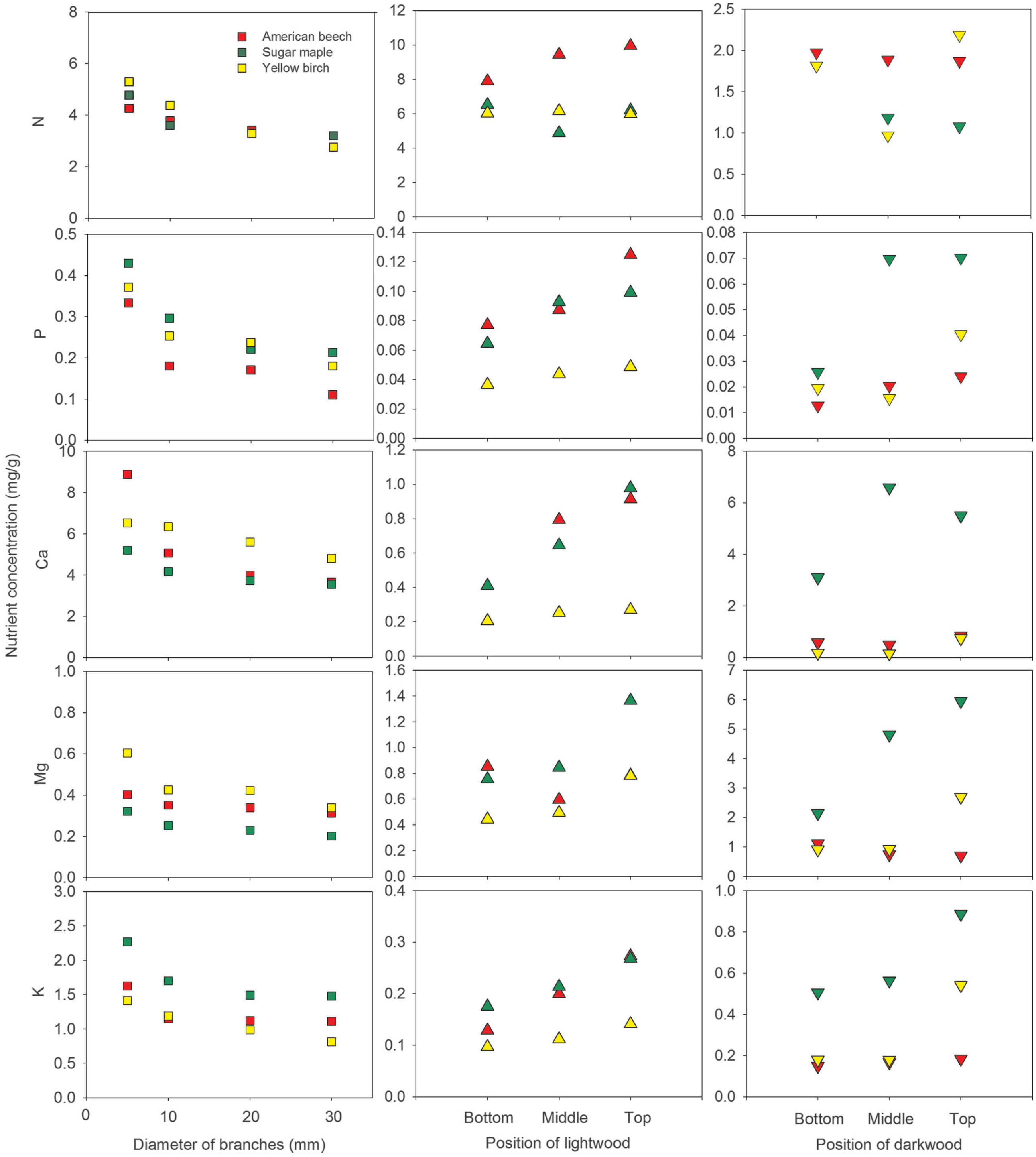
			Variability of nutrient element in foliage (CV)					
Type of variability	Location	Species	N	P	Ca	Mg	K	Sources
Within the tree	Ontario	Maple and birch	3	3	13	10	7	Morrison 1985
	Michigan	Maple	2	4	12	11	14	Erdmann et al. 1988
	Southern Ontario	Maple and ash	2	2	12	13	11	Ellis 1975
	New Hampshire	Maple, birch, and beech	2	3	20	16	15	This study
Tree-to-tree	Southern Ontario	Maple and ash	11	12	12	14	17	Ellis 1975
	New Hampshire	Maple, birch, beech, and pin cherry	9	13	19	19	18	This study
Interannual	France	European beech	6	8	18	27	15	Duquesnay et al. 2000
	New York	Maple, birch, and beech	8	10	18	20	19	This study

or wood. In this study, we found foliar N and P varied 11% (CV) from tree to tree, which was similar to the 8%–15% CVs reported in other studies of sugar maple, yellow birch, and white ash (Table 6). Foliar N and P varied only 6% from year to year, which was similar to the variation of 5%–7% in studies of European beech (*Fagus sylvatica* L.) (Ljungström and Nihlgård 1995; Duquesnay et al. 2000). In study systems such as these, fewer trees could be sampled to monitor N or P in foliage than would be needed to characterize Ca, Mg, or K in bark, branches, or wood. We found that nine trees would be adequate to detect a 20% change in foliar N or P, which is similar to sample size requirements reported for red maple in Michigan (Erdmann et al. 1988), sugar maple in Quebec (Ouimet and Fortin 1992), and European beech in France (Duquesnay et al. 2000).

Importance of sampling position

Bark and wood are usually sampled at a standard height (~1.3 m) that is convenient to measure from the ground. The variation in wood concentration along the bole (35% CV) was the highest source of error that we observed in this study (Table 5). Sampling at a consistent height is important for accurate detection of change over time or comparisons among stands. Because concentrations tend to increase with height (Fig. 6), samples collected at breast height will likely underestimate the average concentration and thus the content of nutrients in tree boles, which is the largest pool of all plant tissues. Sampling sugar maple is especially sensitive because of the variability of nutrient concentrations in darkwood with height (averaging 38% CV across all elements).

Fig. 6. Nutrient concentrations in branches, lightwood, and darkwood at different sampling positions within the tree using datasets from HBEF in 2013. (This figure is available in colour on the Web.)



Branches exhibited the second largest uncertainty (23%) of all of the tissue types that we examined. Other studies have also reported high variability in branch nutrient concentrations, with the highest concentrations in the finest branches. Branches of sugar maple, yellow birch, and American beech at HBEF were sampled from 0–30 mm in diameter, and N and K concentrations were found to vary by as much as 38% (Whittaker et al. 1979). Quaking aspen (*Populus tremuloides* Michx.) and white birch in Canada had a branch wood CV of 46% and branch bark CV of 26%, using branches from 0–75 mm in diameter (Hendrickson 1987). Inconsistency in the diameter of branches sampled could introduce a large uncertainty in comparisons of tissue chemistry over space or time.

Foliage had the smallest variation due to sampling position (Table 5). The magnitude of variation that we found (12% CV) was similar to other studies that sampled foliage in different canopy positions (Table 6). Because of the height of tree canopies, representative foliar samples are difficult to collect, but the effect of sampling position is less important than in other tissue types. Note that the canopies in the young and middle-aged stands at BEF were sampled by felling the trees and homogenizing all of the leaves from each tree. Such destructive approaches to representative sampling are not always compatible with the goals of long-term studies.

Interpreting long-term change in the context of sampling uncertainty

Knowing the magnitude of different sources of uncertainty is important to interpreting differences in reported concentrations. For example, to interpret the differences that we observed in foliage and wood at HWF over a 25-year interval, we need to know that these exceed the uncertainty due to sampling different trees, because the same trees were not sampled over time. For foliage, we found statistically significant increases in concentrations of N in sugar maple (34% of the initial concentration), red maple (16%), and yellow birch (6%), but because foliar N varied by 8%–9% (CV) from tree to tree for these species, the difference in birch could be due to sampling uncertainty. We found decreases in foliar concentrations of K in American beech (32%) that exceed the 15% tree-to-tree variability for K. Previous studies have also reported increases in foliar N for European beech in Switzerland (Flückiger and Braun 1998; Duquesnay et al. 2000) and decreases in foliar K for European beech in France (Duquesnay et al. 2000) and have attributed this change to N deposition.

Wood is rarely sampled repeatedly in long-term studies, although it is easier to sample than foliage or branches. We found decreases in concentrations of Ca (56%) and Mg (46%) in red maple wood (Table 4), which exceed the 30% tree-to-tree variability that we found for both Ca and Mg. Analysis of tree rings has been used to test for change over time in nutrient concentrations in wood (Lévy et al. 1996; Ferretti et al. 2002; Read 2008). For example, Ca, Mg, and K decreased in xylem wood of sugar maple in Wisconsin in wood formed from 1886 to 1986 (Frelich et al. 1989). Soil cation depeletion might be expected to result from N deposition at HWF (Pardo and Driscoll 1996). Cation depletion in forest soils and tree foliage over time has been widely observed in North America (Fenn et al. 2006).

To interpret the reported change in Ca in branches at HWF, we need to account for uncertainty within the tree, as well as from tree to tree, because we are not sure if the branches were sampled at the same diameter over time. The observed increase in branch Ca concentrations in red maple (156%) is higher than expected from the 25% CV within trees and 18% between trees (Table 4). An increase in tissue Ca is unexpected given the depletion of base cations at HWF due to acid rain (Jenkins et al. 2005, pp. 129–142).

Recommendations for sampling

Decisions about sampling intensity should be made with knowledge of which measurements are most variable (Levine et al. 2014). Rarely is sampling intensity adjusted to reflect differences in variability of tissue types and nutrient elements. For example, accurately estimating nutrient concentrations of foliage would require fewer replicate samples than for wood, according to our dataset. Among elements, N and P exhibited the smallest variation across trees and years, and thus fewer samples would be needed to detect differences across sites or over time for these than for other elements.

The selection of a sampling scheme depends on the objectives of the study. To sample nutrient concentrations repeatedly requires careful attention to sampling position within the tree. Foliage should be sampled at the same canopy position, bark and wood should be sampled at a consistent height, and branches should be sampled at a consistent branch diameter. To estimate change over time or to compare stands or species, it may not be necessary to collect samples that are representative of the entire tree.

To estimate the nutrient contents of trees, representative samples of tissues are needed, especially for wood and branches, which vary depending on the position sampled. For example, samples taken at breast height would underestimate the mean nutrient concentration for wood in our dataset (Fig. 6). The variability of nutrient concentrations of wood is small in units of concentration, because concentrations are low in wood, but wood is the most massive component of trees. Taking into account the mass of the tissues, nutrient contents of wood have the greatest uncertainty of all the tissue types. Whether sampling effort should be allocated to minimize the uncertainty in nutrient concentrations or contents depends on the objectives of the study.

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Appendix A

Appendix Fig. A1 appears on the following page.

Fig. A1. Nutrient concentrations in bark and foliage at different sampling positions within the tree using datasets from HBEF in 2013. (This figure is available in colour on the Web.)