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Spatial and temporal variability of foliar mineral concentration in beech (*Fagus sylvatica*) stands in northeastern France

A. DUQUESNAY,[1] J. L. DUPOUEY,[1,4] A. CLEMENT,[1] E. ULRICH[2] and F. LE TACON[3]

[1] *Ecophysiology Unit, INRA-Nancy, 54280 Champenoux, France*

[2] *O.N.F., Department of Technical Research, Boulevard de Constance, 77300 Fontainebleau, France*

[3] *Microbiology Team, INRA-Nancy, 54280 Champenoux, France*

[4] *Author to whom correspondence should be addressed*

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Summary Foliar mineral concentration may provide a basis for monitoring the consequences of long-term environmental changes, such as eutrophication and acidification of soils, or increase in atmospheric CO_2 concentration. However, analytical drifts and inter-tree and year-to-year variations may confound environmental effects on long-term changes in foliar mineral concentration. We have characterized the relative effects of these potentially confounding factors on foliar carbon, nitrogen, phosphorus, calcium, potassium, magnesium and manganese concentrations in 118 pure beech (*Fagus sylvatica* L.) stands, sampled in 1969–71 and 1996–97. Interannual fluctuations of these elements were quantified in a subset of six beech stands monitored for 5 years.

Intercalibration between the methods used at each sampling period for nitrogen and phosphorus analyses showed significant, but low, relative differences (0.8 and 3.3% for N and P, respectively). Based on inter-tree variability, elements could be arranged in four groups: C (constant), N and P (low variability), K and Ca (medium variability), Mn and Mg (high variability). Inter-tree coefficients of variation were 2, 6, 8, 15, 18, 22 and 27%, respectively. Year-to-year fluctuations increased in the order N, P, Mg, K, Ca, and Mn (coefficients of variation of 4, 4, 7, 9, 11, 15 and 29%, respectively).

Between the two sampling periods, foliar N concentration increased 12%, whereas decreases were observed for P (−23%), Mg (−38%) and Ca (−16%). Ratios of N/P, N/K and N/Mg increased by 42, 19 and 77%, respectively. These changes were larger than the interannual variations for P, Mg, N/P, N/Mg and Mg/Ca. Decreasing concentrations of P and cations were particularly marked for trees growing on acidic soils, whereas the positive N trend did not depend on soil type. Both increasing atmospheric CO_2 concentrations and acidification of forest soils could contribute to decreasing P and cation concentrations in foliage. The increase in foliar N concentration with time suggests a nitrogen deposition effect. Whatever the causes of these changes, the large shift in element ratios indicates an accelerating imbalance between nitrogen and cation status.

Keywords: calcium, cations, foliar analysis, long-term trend, magnesium, manganese, mineral analysis, nitrogen, nutrition, phosphorus, potassium, sampling strategy.

Introduction

Tree leaf mineral analysis could be a useful tool for monitoring the effects of long-term environmental changes on tree nutrition. In controlled CO_2-enrichment conditions, foliar mineral concentrations decrease, suggesting that mineral uptake does not increase at the same rate as dry matter accumulation in response to increased atmospheric CO_2 concentrations (Eamus and Jarvis 1989, Amthor 1995). Ammonium deposition causes increases in foliar nitrogen concentration and decreases in cation uptake, resulting in nutrient imbalances (Pearson and Stewart 1993). Soil acidification (see Rehfuess 1990) also leads to a decrease in leaf cation concentration (Glatzel and Kazda 1985). However, few of these experimental observations have been scaled up to adult trees in the forest, despite the major role of foliar chemistry in tree physiology (Marschner 1995). Moreover, despite acidification-induced decreases in soil fertility, positive growth trends were recently reported for several sites and species in Europe (Spiecker et al. 1996). Relationships between these apparently contradictory trends and tree nutrition are still unknown.

Most studies of long-term foliar mineral changes have been undertaken with coniferous species growing in the control plots of fertilization, spacing or thinning trials. Increases in nitrogen concentration over time have been reported for Scots pine and spruce (Grimm and Rehfuess 1986, Nebe 1991, Sauter 1991, Hippeli and Branse 1992, Prietzel et al. 1997, Uebel and Heinsdorf 1997). For the same species, increase (Sauter 1991), stability (Grimm and Rehfuess 1986, Prietzel et al. 1997, Uebel and Heinsdorf 1997) and decrease (Hippeli and Branse 1992) in phosphorus concentration have been observed. Deterioration of calcium nutrition (Grünhage and Jäger 1988, Sauter 1991, Prietzel et al. 1997) and magnesium nutrition (see review by Landmann et al. 1997) has also been reported for coniferous species. Comparable data for deciduous species are scarce. Flückiger and Braun (1998) reported an increase in nitrogen concentration and a decrease in phosphorus concentration in beech foliage between 1984 and 1995 in 51 plots in northwestern Switzerland. In both coniferous and deciduous species, the foliar potassium concentration has remained more or less stable during the last few decades. However, in individual years, large deviations from the mean of longer periods have frequently been reported for foliar concentrations of several minerals (Sauter 1991, Prietzel et al. 1997, Stefan et al. 1997). Nutrient concentrations, especially nitrogen, usually decrease during dry and cold years (Bonneau 1988, Hippeli and Branse 1992).

Nutrient concentration ratios may reveal nutritional imbalance, even when element concentrations are in the normal range. An increase in the nitrogen to magnesium ratio has been observed for spruce at several sites in the East German subalpine mountains between 1964 and 1988 (Nebe 1991). Increases in the ratios of nitrogen to phosphorus, nitrogen to potassium and nitrogen to magnesium have been reported for beech and spruce in Switzerland between 1984 and 1995 (Flückiger and Braun 1998), and for pine in Brandeburg between 1964 and 1988 (Hippeli and Branse 1992).

We investigated changes in foliar nutrient concentrations (nitrogen, phosphorus, potassium, calcium, magnesium and manganese) in beech stands between 1969–71 and 1996–97 in northeastern France. Changes were assessed diachronically by resampling beech stands previously sampled in the 1970s. Because the assay methods for inorganic elements have changed between the sampling periods, we intercalibrated the new and old methods to counter any methodological bias. Because variability of foliar nutrient concentrations between trees within a plot, between plots, or from year-to-year, could hide long-term changes, we also quantified these sources of variation.

Materials and methods

Sampling strategy to assess inter-tree variability and long-term changes

The initial sampling was conducted in 118 beech stands in northeastern France. Beech represents more than 80% of the total basal area in these stands. Mean stand age was 120 years in 1995 (52% of the stands were between 80 and 120 years old, and 31% were between 120 and 160 years old). Stands extend over a wide range of soils, from calcareous rendzina to acidic podzol (see Thimonier 1994). The climate is intermediate between Atlantic and continental, with a mean annual temperature of 9.6 °C (Nancy), and mean annual rainfall of 760 mm.

In August 1969, leaves from the upper crown of the five beech trees with the largest breast-height diameter were sampled in 65 of the 118 plots by shooting off small branches with a shotgun. Leaves were separated from twigs in the field and pooled to give one composite sample per tree (at least 100 leaves per tree) (cf. Le Tacon and Toutain 1973). In August 1971, all 118 plots were sampled in the same way as in August 1969. Twenty-five years later, in August 1996, 85 of the 118 initial plots remained undisturbed and were resampled. They were resurveyed using the methodology used in 1969–71, except that the six largest trees per plot were sampled, instead of five. Leaf samples were collected in 42 of the 85 plots during August 18–22, 1997 from the same trees that were sampled in 1996. Because of selective cutting, the sampled trees were not the same in 1969–71 and 1996–97.

For long-term comparison of foliar mineral concentrations, we used data from the 25 plots that were sampled on all four dates. Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and manganese (Mn) concentrations were determined in 1969, 1971, 1996 and 1997. Carbon (C) concentration was measured in beech leaves only in 1996 and 1997.

Sampling strategy to assess interannual variability

To assess interannual variability, six beech stands belonging to the French network for long-term monitoring of forest ecosystems RENECOFOR (Office National des Forêts 1996) were selected in northeastern France and sampled annually between 1993 and 1997. Foliage was collected in the same manner as previously described, but the same eight largest trees were sampled in each plot during each of the 5 years. Foliar concentrations of N, P, K, Ca, Mg and Mn were determined.

Foliar mineral analysis

Foliage samples that were oven-dried at 65 °C for 48 h, and subsequently ground in a coffee mill, were analyzed by the methods listed in Table 1. Concentrations of N, P, K, Ca and Mg were expressed as mg g_{dw}^{-1}, Mn concentration was expressed as µg g_{dw}^{-1} and C concentration was expressed as $\%_{dw}$. Accuracy was controlled by cross-checking the samples with a level II laboratory reference (hornbeam foliage), calibrated from a BCR reference level I. Analyses of samples from both sampling periods were carried out in the same laboratory (INRA-Nancy) and supervised by the same technical staff.

Comparisons of previous and current methods for assay of N and P were carried out on a subset of 1996 samples (54 trees for N and 122 trees for P). We did not compare the methods used for analysis of K, Ca, Mg and Mn in 1969–71 and 1996–97, because atomic absorption spectrometry (AAS) and inductively-coupled plasma atomic emission spectrometry (ACP-AES) are standard methods (Maier et al. 1989) that have been compared previously in inter-laboratory workshops.

Elements were analyzed separately for each tree in 1969 (325 trees) and 1971 (590 trees). In 1996 and 1997, as well as for the interannual 1993–1997 study, equal weights of oven-dried leaf powder of each tree were combined to provide one mean sample per plot for chemical analyses. For the assessment of inter-tree variability, trees from a subset of 29 plots (174 trees) of the 1996 sample were analyzed separately.

Data analysis

We determined the mean number of trees necessary to provide a given accuracy of the mean concentration of each element at the plot level:

Table 1. Sample digestion and identification processes used at each sampling date.

Element	Sampling year	Open digestion system	Identification process
N	1969–71	H_2SO_4, H_2O_2, Se	Distillation and titration of NH_3 with H_2SO_4 N/50
N	1996–97	H_2SO_4, H_2O_2, Se	Colorimetry (Analysis of NH_4^+ by indophenol blue method)
P	1969–71	$HClO_4$, H_2O_2	Colorimetry (Vanado-molybdate)
P	1996–97	$HClO_4$, H_2O_2	Inductively-coupled plasma atomic emission spectrometry
Ca, Mg, Mn	1969–71	$HClO_4$, H_2O_2	Atomic absorption spectrometry (AAS)
Ca, Mg, Mn	1996–97	$HClO_4$, H_2O_2	Inductively-coupled plasma atomic emission spectrometry
K	1969–71	$HClO_4$, H_2O_2	Flame emission spectrometry
K	1996–97	$HClO_4$, H_2O_2	Inductively-coupled plasma atomic emission spectrometry
C	1996–97	–	Carmograph

$$n = \frac{t^2_{1-\alpha/2} CV^2}{d_r^2}, \tag{1}$$

where t is the Student t value at the α significance level with $n - 1$ degrees of freedom, CV is the mean coefficient of variation over all plots and d_r is the accepted relative error in percentage.

The number of plots necessary to provide a given accuracy of the mean concentration of each element at the regional level was calculated as:

$$p = \frac{t^2_{1-\alpha/2}\left[\sigma_A^2 + \sigma^2/n\right]}{d_r^2 \bar{X}^2}, \tag{2}$$

where t is the Student t value at the α significance level with $p - 1$ degrees of freedom, σ_A and σ are between-plot and within-plot standard deviations of foliar concentration, respectively (these parameters were estimated with the VARCOMP procedure of SAS (1988)), n is the number of trees per plot (5 in 1971, 6 in 1996), and $\bar{X}$ is the regional mean foliar concentration.

Estimates of the number of trees per plot (n) and the number of plots (p) were calculated based on the 1996 data set. Values for the other sampling years for which individual tree values were available (1969 and 1971) were also calculated and gave similar results.

Because the same plots were sampled on two occasions, the number of plots necessary to provide a given accuracy of the mean variation between the two dates is lower than in the case of non-paired plots:

$$p' = \frac{t^2_{1-\alpha/2}\sigma^2}{d_r^2 \bar{X}^2}, \tag{3}$$

where t is the Student t value at the α significance level with $p' - 1$ degrees of freedom, σ is the standard error of the paired differences between the two sampling periods over all plots (because two years were available for each sampling period (1969 and 1971 for the first period, and 1996 and 1997 for the second period), we took the mean of the two years as the value of each sampling period), and $\bar{X}$ is the initial mean foliar mineral concentration.

All calculations were made with the SAS statistical software package (1988; SAS Institute, Cary, NC). All estimates were calculated for a confidence level $(1 - \alpha)$ of 0.95 and relative deviations from the mean (d_r) of 5, 10 and 20%. Departures from normality were small for all variables, except for Mn at the inter-plot level because of low values on calcareous soils. Consequently, Mn concentrations of trees growing on calcareous soils were not taken into account when calculating p in Equation 2.

Results

Comparison between previous and current analytical methods for N and P

Comparison of foliar N concentrations obtained by distillation (old method) and colorimetry (current method) for the same samples showed significant differences (paired t test: $t = 2.14$, $P < 0.05$, $n = 54$ trees), with lower N concentrations (–0.16 mg g^{-1} and –0.8% in relative value) obtained with the colorimetric method than with the distillation method. Moreover, the difference between the two methods was dependent on sample concentration. Based on the information obtained, we corrected the 1969 and 1971 concentrations as follows: $Y = 1.07X - 0.17$, $r^2 = 0.98$, $P < 0.001$, where Y is the corrected N concentration and X is the N concentration obtained with the distillation method.

Comparison of methods for P analysis showed significant differences between the colorimetric method (old method) and inductively-coupled plasma atomic emission spectrometry (ICP-AES) (current method) (paired t test: $t = 13.57$, $P < 0.001$, $n = 122$ trees), with lower P concentrations (–0.04 mg g^{-1} and –3% in relative value) obtained by spectrometry than by colorimetry, and the difference between the two methods depended on sample concentration. Based on the information obtained, we corrected the 1969 and 1971 concentrations as follows: $Y = 0.958X + 0.00097$, $r^2 = 0.99$, $P < 0.001$, where Y is the corrected P concentration and X is the P concentration obtained with the colorimetric method.

Table 2. Inter-tree variability and inter-plot variability based on six trees per plot averaged over 29 plots. Coefficients of variation between trees within plots and number of trees required to estimate the plot value of beech foliar concentration to within 5, 10 and 20% of the true mean at the 95% confidence level are presented, as well as the number of plots required to estimate the mean regional value of beech foliar concentration to within 5, 10 and 20% of the true mean at the 95% confidence level. The percentage of total variance explained by the variability between plots is also presented.

Element	Inter-tree variability				Inter-plot variability			
	Number of trees per plot			CV %	Number of plots of six trees			% of variance associated with the plot effect
	5%	10%	20%		5%	10%	20%	
N	8	4	3	6.2	11	5	3	52
P	13	5	3	8.4	78	21	7	86
K	36	11	5	14.8	63	15	6	60
Ca	52	15	6	17.9	291	75	20	82
Mg	111	30	9	26.6	242	63	17	54
Mn	79	22	7	22.4	302	77	21	86
C	3	2	2	2.2	3	2	2	35

Inter-tree and inter-plot variability

At the plot level, the relative deviation of the measurements based on 6 trees per plot was < 5% of the true mean for C, 5–10% for N and P, 10–20% for K and Ca, and ≥ 20% for Mg and Mn (Table 2).

At the regional level, the relative deviation of the measurements based on 25 plots was < 5% of the true mean for C and N, 5–10% for P and K, 10–20% for Ca and Mg, and > 20% for Mn (Table 2). The percentage of variance associated with the plot effect was low for N and P, intermediate for Mg and P and high for Ca and Mn.

Based on a resampling of 25 plots, Equation 3 shows that it should be possible to obtain evidence of relative variations between the two sampling periods of < 5% of the initial value for N and P, 5–10% for Ca, Mg and K, and 10–20% for Mn (Table 3).

Interannual variability

Mean, standard deviation and coefficient of variation of foliar mineral concentrations between years were calculated for each of the 6 plots where annual data were available. Data, averaged over the 6 plots, are presented in Table 4. Nitrogen, P and N/P showed the smallest inter-year variability (coefficients of variation of 5, 7 and 6% respectively). The greatest year-to-year variation in elemental concentrations was found for Mn (coefficient of variation of 20%). Sampling year had a significant impact on all elements, except Mg. The ANOVA *F* values of both year and site effects were especially high for Ca. In all cases, plot effect was higher than year effect. Sampling year and plot effects combined accounted for more than 70% of the observed variance.

Table 3. Number of plots required to show evidence of a 5, 10 or 20% relative variation of the initial mean foliar concentrations at the 95% confidence level between two sampling dates. Parameters were estimated from a set of 25 plots.

Element	Number of plots		
	Relative variation between two sampling dates		
	5%	10%	20%
N	13	5	3
P	10	4	3
K	46	13	5
Ca	26	8	4
Mg	54	15	6
Mn	126	33	10

Long-term changes

Variations in foliar nutrient concentrations (N, P, K, Ca, Mg, Mn) and element ratios (N/P, N/K, N/Ca, N/Mg, N/Mn, K/Ca, Mg/Ca) over time for the 25 plots common to the four sampling years are presented in Figures 1 and 2. Foliar nutrient concentrations and nutrient ratios of the six RENECOFOR stands are plotted on the same graphs.

Mean values of foliar nutrient concentrations and nutrient ratios for the 25 plots sampled in both 1969–71 and 1996–97 are presented in Tables 5 and 6, respectively. Foliar nutrient concentrations showed significant changes between 1969–71 and 1996–97. Over this period, N concentration increased 12%, whereas there were decreases in the concentrations of P (−23%), Ca (−16%), Mg (−38%) and K (−6%). The 4% decrease in Mn concentration was not significant. Among the 25 plots, decreases in concentrations of foliar cations and P were higher for the sample subset comprising the nine most acidic plots (upper soil layer pH < 4 .5)—with decreases of 27% for P, 27% for Ca, 44% for Mg, and 14% for K—whereas the 10% increase in foliar N concentration on these acidic plots was comparable with that for the whole set of plots.

Between 1969–71 and 1996–97 there were large changes in some element ratios. Significant increases were registered for the N/P (+42%), N/K (+19%), N/Ca (+30%), N/Mg (+77%) and N/Mn (+18%) ratios, whereas the Mg/Ca ratio decreased by 27%.

Tables 7 and 8 give a classification of the plots for each year, element and ratio according to threshold values for nutrition as defined by Stefan et al. (1997). The N status of the plots did

Table 4. Interannual and inter-plot variability of element concentrations and ratios in the six RENECOFOR plots for the 5 sampling years. Concentrations of N, P, K, Ca and Mg are expressed in mg g^{-1}, and the concentration of Mn is in μg g^{-1}. The ANOVA of plot and sampling year effects on element concentrations are also presented: (*) = $P < 0.10$, * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$.

Element	Element concentration			*F*-value		r^2
	Mean ± SD	Range (min–max)	CV %	Sampling year	Plot	
N	25.90 ± 1.39	24.18–27.72	5.4	3.36*	7.08***	0.71
P	1.06 ± 0.07	0.96–1.14	6.9	2.76(*)	35.14***	0.90
K	8.78 ± 1.00	7.63–10.07	11.1	6.12**	10.99***	0.80
Ca	10.41 ± 1.30	8.60–11.81	14.1	8.99***	112.77***	0.97
Mg	1.09 ± 0.15	0.91–1.28	13.5	1.94ns	23.09***	0.86
Mn	1357 ± 264	1033–1701	19.6	2.77(*)	70.92***	0.95
N/P	24.96 ± 1.54	23.15–27.07	6.3	10.48***	52.5***	0.94
N/K	3.02 ± 0.40	2.55–3.51	13.5	13.52***	8.37***	0.83
N/Ca	3.21 ± 0.60	2.52–4.05	15.6	3.62*	42.79***	0.92
N/Mg	26.72 ± 3.62	22.48–31.45	13.2	1.67ns	41.61***	0.92
N/Mn	0.06 ± 0.01	0.047–0.070	20	4.81**	156.13***	0.98
K/Ca	1.12 ± 0.21	0.90–1.37	15.6	2.03ns	40.32***	0.91
Mg/Ca	0.11 ± 0.02	0.094–0.136	13.9	2.30(*)	18.22***	0.83

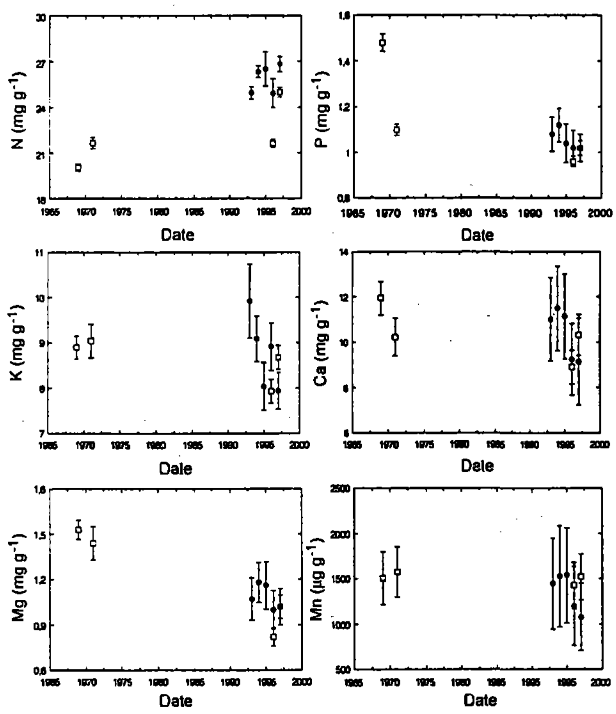

Figure 1. Beech foliar concentrations of N, P, K, Ca, Mg and Mn plotted against year. Symbols: □ = mean of 25 plots sampled in 1969, 1971, 1996 and 1997; ● = mean of six plots sampled annually from 1993 to 1997. Error bars denote ± 1 standard error of the mean. Concentrations of N, P, K, Ca and Mg are expressed in mg g^{-1}, Mn concentration is in μg g^{-1}.

not change significantly between 1969–71 and 1996–97, although a large percentage of plots exceeded the upper limit of the N-sufficiency range by 1997. Conversely, despite some interannual variations, a large proportion of the plots had entered the P and Mg deficiency range by 1996–97. Potassium displayed small interannual variations within the two periods, with a tendency to decrease between 1969–71 and 1996–97. Apart from year-to-year variations, no clear trend was observed for Ca concentration. Although no plots exceeded the adequate N/P range of values in 1969–71, many plots exceeded this range in 1996–97. Values of N/Mg exceeded the adequate range in about 60% of the plots in 1996–97. A majority of plots with adequate values of Mg/Ca in 1969–71 were below the lower boundary of the adequate range in 1996–97, and the reverse was observed for N/Ca.

Discussion

Comparison between previous and current analytical methods for N and P

Relative variations between analytical methods (0.8 and 3% for N and P, respectively) were small enough to allow the detection of long-term signals, despite systematic and significant differences between the methods. A recent test among 39 laboratories in Europe confirmed the reliability of current digestion–extraction methods, including ours, for determining N, P and cation concentrations in tree leaves (Stefan et al. 1997). Among the laboratories, the ranges of variation for N and P (5.5–6.5% and 6–12%, respectively) were much larger than the ranges of variation found in this study.

Inter-tree and inter-plot variability

The elements can be ordered along an increasing gradient of inter-tree variability as follows: C < N < P < K < Ca < Mn < Mg. This order is similar to that reported by Erdmann et al. (1988), who studied the sample-size requirements for foliar analysis of *Acer rubrum* L. in Michigan; however, the number of trees required to obtain a given accuracy is lower in our case. Ouimet and Fortin (1992) also observed low variation in foliar N concentration in *Acer saccharum* Marsh. trees growing in Quebec. Current intensive monitoring projects generally employ a sampling intensity of < 10 trees per plot (De Vries et al.

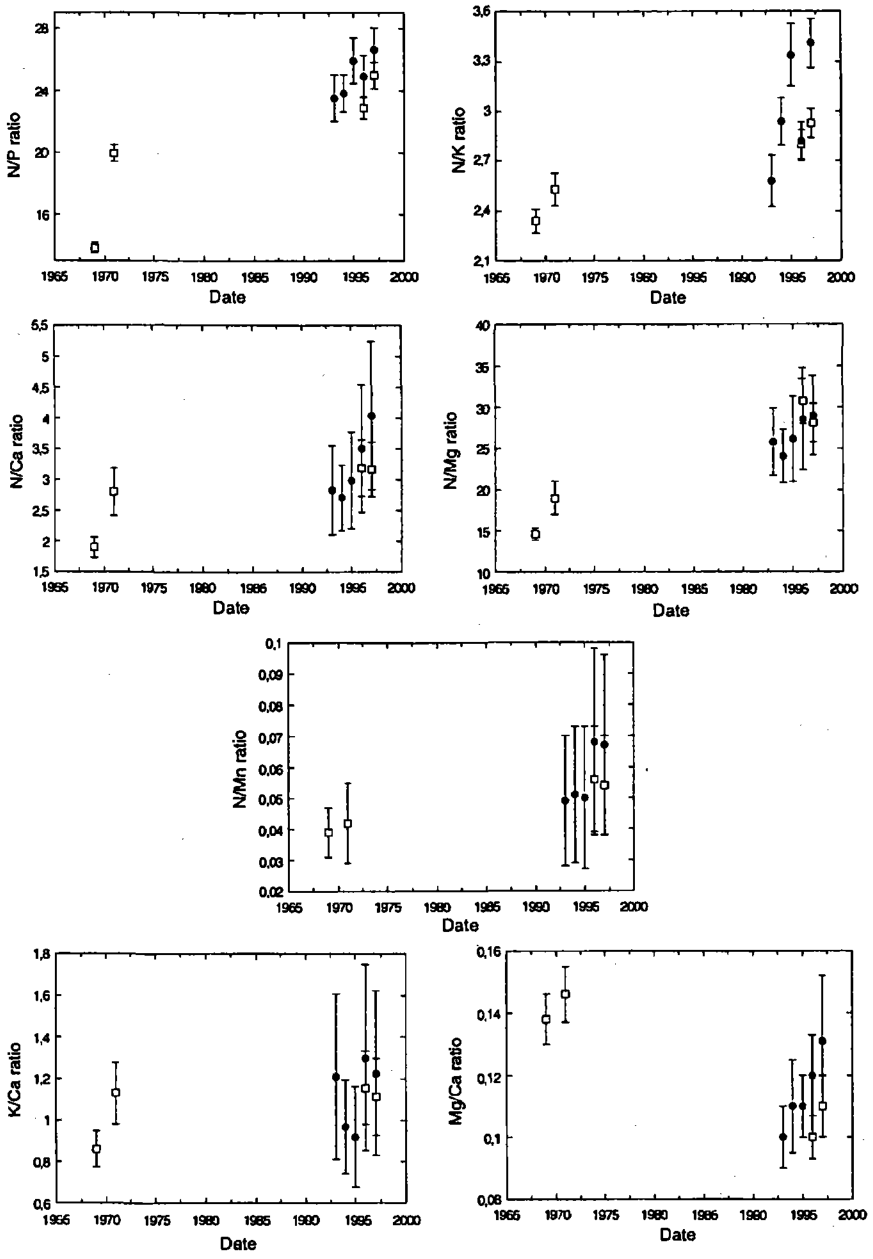

Figure 2. Beech foliar N/P, N/K, N/Ca, N/Mg, N/Mn, K/Ca and Mg/Ca ratios plotted against year. Symbols: □ = mean of 25 plots sampled in 1969, 1971, 1996 and 1997; ● = mean of six plots sampled annually from 1993 to 1997. Error bars denote ± 1 standard error of the mean. Concentrations of N, P, K, Ca and Mg are expressed in mg g^{-1}, Mn concentration is in µg g^{-1}.

Table 5. Mean element concentrations for 1969–71 and 1996–97. Means, standard deviations, mean differences, percentage changes relative to the 1969–71 values and paired *t*-test values are presented: * = $P < 0.05$, *** = $P < 0.001$, and ns = not significant. Concentrations of N, P, K, Ca and Mg are expressed in mg g^{-1}, and the concentration of Mn is in µg g^{-1}. The number of plots was 25, except for Ca ($n = 23$).

Element	Element concentration (mean ± SD)		Difference 1996–1970 (mg g^{-1})	Relative difference 1996–1970 (% of the initial value)	Paired *t*-test
	1969–71	1996–97			
N	20.9 ± 1.2	23.3 ± 1.2	+2.4	+12	7.14***
P	1.29 ± 0.14	0.99 ± 0.13	−0.30	−23	−16.56***
K	8.97 ± 1.37	8.31 ± 1.20	−0.66	−6	−2.20*
Ca	11.08 ± 3.65	9.61 ± 3.92	−1.47	−16	−5.15***
Mg	1.49 ± 0.39	0.92 ± 0.3	−0.56	−38	−10.33***
Mn	1540 ± 1411	1472 ± 1270	−68	−4	−0.73ns

Table 6. Mean element ratios for 1969–71 and 1996–97. Means, standard deviations, mean differences, percentage changes relative to the 1969–71 values and paired *t*-test values are presented: (*) = $P < 0.10$, *** = $P < 0.001$, and ns = not significant. The number of plots was 25, except for N/Ca, Mg/Ca and K/Ca ($n = 23$).

Ratio	Ratio values (mean ± SD) 1969–71	Ratio values (mean ± SD) 1996–97	Difference 1996–1970 ($mg\ g^{-1}$)	Relative difference 1996–1970 (% of the initial value)	Paired *t*-test
N/P	16.88 ± 1.96	23.94 ± 3.75	+7.06	+42	15.68***
N/K	2.44 ± 0.37	2.87 ± 0.41	+0.43	+19	5.3***
N/Ca	2.35 ± 1.30	3.18 ± 2.16	+0.83	+30	3.87***
N/Mg	16.80 ± 6.38	29.44 ± 12.48	+12.64	+77	8.07***
N/Mn	0.043 ± 0.053	0.055 ± 0.081	+0.012	+18	1.87(*)
K/Ca	0.996 ± 0.560	1.135 ± 0.862	+0.139	+11	1.55ns
Mg/Ca	0.142 ± 0.037	0.105 ± 0.042	−0.037	−27	−8.83***

Table 7. Percentage of plots classified in the (1) deficient range, (2) optimum range and (3) supra-optimum range for beech nutrition, by element and sampling year for 25 plots. The limits for each range ($mg\ g^{-1}$) as defined by Stefan et al. (1997) are also presented.

Element	N			P			K			Ca			Mg		
Class	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Limit	< 18	18–25	> 25	< 1	1–1.7	> 1.7	< 5	5–10	> 10	< 4	4–8	> 8	< 1	1–1.5	> 1.5
Date															
1969	4	96	–	–	88	12	–	80	20	–	12	88	4	36	60
1971	4	96	–	12	88	–	–	72	28	4	26	70	20	36	44
1996	–	100	–	68	32	–	–	92	8	8	28	64	76	20	4
1997	–	52	48	44	56	–	–	92	8	8	20	72	56	40	4

1998). Based on our study, the relative error of measurement for a sampling intensity of 10 trees per plot is < 10% for N, P and C and > 10% for K, Ca, Mg and Mn. At a commonly used sampling intensity of 3 trees per plot, relative errors are > 20% for all elements, except C.

At the regional level, the order of increasing inter-plot variability is: $C < N < K < P < Mg < Ca < Mn$. This order corresponds with that for within-plot variability, except for Mg, which is less variable between than within plots, and for P and K, which are transposed. Le Tacon and Toutain (1973) obtained the same order at the between-plot level, except for Mg.

At a given date, the number of plots necessary to obtain the regional mean with a given accuracy is high for all elements (Table 2). But when the same plots are resampled, a much lower sampling intensity is needed (Table 3) to obtain a similar accuracy. This finding illustrates the high statistical power of resampling studies for monitoring long-term changes. Based on the number of plots sampled and the long-term relative variations in foliar concentration observed, we conclude that our sampling intensity was adequate for N, P, Ca and Mg, barely adequate for K and too low for Mn.

Interannual variability

The order of increasing between-year variation in foliar mineral concentrations was: $N < P < K < Mg < Ca < Mn$. A similar order, except for Mg, was found by Ljungström and Nihlgård (1995) for beech seedlings. Hippeli and Branse (1992) showed that increasing rainfall and higher mean temperatures during the growing season increased foliar concentrations of N, P, Ca and Mg in pine. For our study area, variations in cumulated rainfall (January to August) between 1960 and 1997 indicate that 1969 and 1971 were not characterized by high water stresses when compared with the mean rainfall for the period 1961–1990 (Figure 3). Conversely, 1996 was characterized by low precipitation (34% below the mean of the period 1961–1990), which could explain the low foliar concentrations of all elements in 1996 compared with 1997. However, in a comparison of two years, 1969 and 1997, with similar climatic conditions (1969 and 1997 had 550 and 580 mm of precipitation from January to August, respectively), the interannual difference between foliar concentrations remained large and even increased for nitrogen (Table 7).

Changes in nutrient concentrations between 1969–71 and 1996–97 (Table 5) were larger than the between-year variations over the period 1993–1997 (Table 4) for N, P, Mg, N/P, N/Mg and Mg/Ca (1.7, 4.3, 3.7, 4.6, 3.5 and 2.2 times the standard errors, respectively). In contrast, interannual variations for K, Ca and Mn and other ratios were of similar or greater amplitude than changes between 1969–71 and 1996–97.

Long-term changes

Our results are in accordance with previous observations of increasing concentrations of N, and decreasing concentrations of cations and P during the last few decades in leaves of

Table 8. Percentage of plots in each of three classes of beech nutrition, by element ratio and sampling year. The limits of the three classes, defined at the European level (Stefan et al. 1997), are presented alongside the class number. The middle class (2) is defined as the "harmonious" range of nutrition. Ratio values belonging to class (1) or class (3) indicate nutritional imbalance. The total number of plots is 25.

Ratio	Class	Limit	Date			
			1969	1971	1996	1997
N/P	1	< 11	–	–	–	–
	2	11–25	100	100	80	48
	3	> 25	–	–	20	52
N/K	1	< 1.8	–	8	–	–
	2	1.8–5	100	92	100	100
	3	> 5	–	–	–	–
N/Ca	1	< 2	76	57	56	56
	2	2–6	24	39	36	36
	3	> 6	–	4	8	8
N/Mg	1	< 12	24	28	4	4
	2	12–25	76	52	36	40
	3	> 25	–	20	60	56
K/Ca	1	< 0.63	40	13	16	24
	2	0.63–2.50	60	78	76	68
	3	> 2.50	–	9	8	8
K/Mg	1	< 3.33	–	4	–	4
	2	3.33–10	92	76	52	52
	3	> 10	8	20	48	44
Ca/Mg	1	< 3.67	–	–	–	–
	2	3.67–8	52	57	8	36
	3	> 8	48	43	92	64

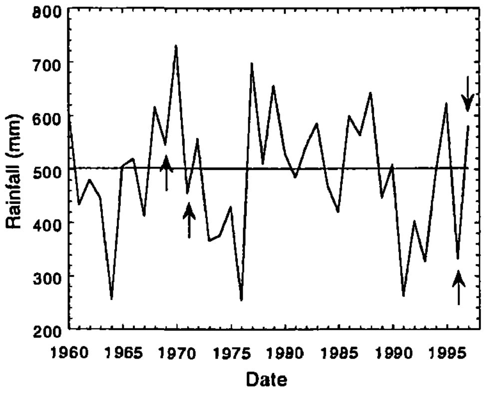

Figure 3. Yearly cumulated rainfall (mm) (January to August) for the 1960–1997 period. Climatic data are from the meteorological station of Nancy-Tomblaine (Météo France). The horizontal line represents mean rainfall (January to August) for the 1961–1990 period. The four sampling dates are indicated by arrows.

Fagus sylvatica (Flückiger and Braun 1998) and coniferous species (Grimm and Rehfuess 1986, Nebe 1991, Sauter 1991, Hippeli and Branse 1992, Prietzel et al. 1997, Uebel and Heinsdorf 1997).

Tree and stand aging during the 27-year study period may partly explain the observed changes in foliar mineral concentrations. Nutrient concentrations in leaves are generally higher in nursery seedlings than in young or mature trees (Bonneau 1988). In West Germany, a comparison of three beech stands, 59, 80 and 122 years old, showed a decrease in foliar N, P and Mg concentrations with age (respective relative variations of −6, −14 and −7% between 80 and 122 years old), but no clear trend for Ca and K (Cole and Rapp 1981).

Environmental changes may also be partly responsible for the observed trends in foliar mineral concentrations. In young beech plants growing under controlled conditions, atmospheric CO_2-enrichment leads to a decrease in all foliar element concentrations by a dilution effect as a result of increased biomass (Overdieck 1993, Epron et al. 1995, Mousseau et al. 1996). Peñuelas and Matamala (1993) also pointed to a CO_2 effect to explain the decrease in all elements in herbarium leaves since 1940–50. However, in our study, N concentration increased, whereas concentrations of the other elements decreased between the sampling periods. This could be explained by the high rate of atmospheric N deposition in the study area (20–30 kg ha^{-1} $year^{-1}$, at the beginning of the period, Aussenac et al. 1972). Increases in foliar N concentration and decreases in P and cation concentrations to deficiency values were also observed in young (Flückiger and Braun 1998) and mature (Balsberg Pahlsson 1992) beech trees when N supply is increased under controlled conditions.

Among the elements that decreased in concentration, Mg and P showed large relative variations. Recent soil acidification and desaturation, which have occurred in the last few decades in beech stands, especially on initially acidic soils (Hallbäcken and Tamm 1986, Falkengren-Grerup and Eriksson 1990, Thimonier et al. 1994), could have resulted in decreased cation and P supply. Glatzel and Kazda (1985) induced decreases in cation concentration by experimental acidification of young beech stands. We observed large decreases in foliar cation and P concentrations in trees growing on the study sites with the most acidic soils, supporting a possible role of long-term soil desaturation.

Mycorrhizae also play a major role in P uptake. Arnolds (1991) and Jaenike (1991) linked a large decrease in mycorrhizal abundance and diversity in European forests during the last decades with atmospheric N deposition. These changes in fungal communities could partly explain the large decrease in beech foliar P concentration.

Reductions in P and Mg concentrations relative to N concentration led to a deterioration of N/P and N/Mg ratios, particularly for trees growing on acidic soils. This imbalance in foliar nutritional status could lead to higher sensitivity to parasite attacks, frost and water stress (Fangmeier et al. 1994, Power et al. 1998). The degree of infestation by beech aphid (*Phyllaphis fagi*) increases significantly with increasing N/P ratio (Flückiger and Braun 1998). Because the degree of cryoprotection depends on carbon allocation between growth and production of cryoprotectants (Sheppard 1994), it could decrease in plants with high N concentrations. Moreover, as much as 75% of foliar nitrogen may be invested in photosynthetic functions, and positive correlations between photosynthetic activity and leaf nitrogen concentration have been repeatedly observed for deciduous species (Field and Mooney

1986). Finally, links between forest decline and low foliar P and high N/P ratio have been reported (Thomas and Büttner 1998). In view of current concerns about forest health and sustainable management in Europe, long-term changes in foliar mineral concentrations deserve further attention.

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