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Abstract

Through the variations of their dimension, density, anatomy or isotopes compositions, tree rings have provided invaluable proxies to evaluate past changes of the environment. Whereas long-term records of changes in soil fertility are particularly desired for forest ecosystem studies, the use of the chemical composition of tree rings as potential marker is still controversial. Dendrochemistry has sometimes been considered as a promising approach to study past changes in soil chemistry, whereas some authors stated that element translocations in the wood preclude any possibility of reliable retrospective monitoring. Here, we aimed at testing whether the wood elemental content of fertilized oaks (*Quercus petraea*) differed from control trees more than 30 years after a NPKCaMg fertilization and, if so, if the date of fertilization might be retrieved from the ring analysis. The contents in N, Mg, P, K, Ca and Mn were measured for each of the 43 sampled trees and in every ring of the 58-year long chronology with a non-destructive method coupling a Wavelength Dispersive Spectroscope (WDS) with a Scanning Electron Microscope (SEM). The results showed significantly higher contents in Ca and lower contents in Mn in fertilized compared to control trees. However, there was no difference of elemental content between the rings of the fertilized trees build in the 20 years before and those build after fertilization. Thus, whereas the effect of fertilization on increasing ring width was dramatic, immediate and relatively short-lasting, the elemental composition of the entire ring sequence was impacted, precluding the dating of the event. These results question the possibility to reconstruct long-term changes in soil fertility based on dendrochemistry.

1. Introduction

Despite the recent decrease in acidifying atmospheric deposition in Europe (Waldner et al., 2014), changes towards a degradation in soil forest fertility is still topical (Jonard et al., 2015). Nitrogen deposition still exceeds 20 kg $N.ha^{-1}.yr^{-1}$ in Western Europe, Southern Asia and Eastern China (Smith et al., 2016) and became the main driver of soil acidification. As a consequence, the percentage of ecosystems area considered at risk for acidification was higher than 50% in Europe and China in 2000 (Posch et al., 2015). Moreover, a decreasing trend in atmospheric deposition of Ca and Mg has been identified concomitantly with the decline in SO_4 deposition (Hedin et al., 1994; Lajtha and Jones, 2013). In addition to these long-term changes in atmospheric deposition, new practices in forest harvest are emerging, pushed by the need to increase the use of biomass for renewable energy. By removing logging residues (*i.e.* branches and tree tops) for the energy industry a significant part of the mineral nutrients is exported from the forest ecosystems, in addition to the traditional logs and firewood, potentially leading to a soil fertility decrease (Moldan et al., 2017; Thiffault et al., 2011).

It is also known that the increase in forest productivity in response to elevated CO_2 will depend on soil fertility and might be limited by growing nutrient imbalance (Fernandez-Martinez et al., 2014). Evaluating the dynamics of soil fertility recovery, or deterioration, and understanding its limiting effect on tree growth require long-term monitoring. Most studies currently rely on sites providing soil data since the 1980s, for the earliest (Akselsson et al., 2013; Pannatier et al., 2011). Alternatively, dendrochemistry, *i.e.* the study of the chemical content of tree rings, might constitute a powerful way to obtain long-time records of tree nutrition variations, providing that the chemical composition of the wood does reflect the soil conditions prevailing when the wood was formed.

Nutrients in the tree rings are mainly originating from root uptake but foliar and bark pathways have also been identified (Odabasi et al., 2016; Wright et al., 2014). The involvement of K in the regulation of xylem cell expansion and Ca in wood structure and chemistry have been shown (Fromm, 2010; Lautner and Fromm, 2010). However, the physiological processes involved in nutrient incorporation

in the wood and possible subsequent translocations are still mostly unknown. The relationship between soil chemistry and xylem sap content is unclear (Dambrine et al., 1995) but metabolic controls are thought to occur on the mineral composition of the xylem sap (Smith and Shortle, 2001). Some studies suggested that tree rings do not passively record the variations in soil nutrient availability but that their chemical content is instead the result of complex physiological responses to environmental changes (Bukata and Kyser, 2008; Hevia et al., 2019; Kirchner et al., 2008; Sanchez-Salguero et al., 2019). However, the elemental concentration of the wood displayed more concordance with those of the soil than foliar elemental concentrations (Cote and Camire, 1995). The latter are yet commonly used for nutrition diagnosis. Inter-ring translocations apparently depend on the elements (Martin et al., 2003), the tree species (Cutter and Guyette, 1993; Burnham et al. 2019) and, at some point, the studies (*i.e.* the chosen experimental design and analytical techniques) so that a synthesis does not seem currently possible (Martin et al., 2006).

Several authors claimed that the dendrochemical approach can be a valid way to study past changes in soil chemistry (Cui et al., 2013; Lévy et al., 1996; Marten et al., 2015; Watmough, 1997) while other ones stated that element translocations preclude any attempt to do so (Bilodeau-Gauthier et al., 2008; Hagemeger, 1995; McClenahen et al., 1989; Nabais et al., 1999). This debate remained without consensus for over 40 years and is regularly rekindled with instrumental advances. However, dendrochemical studies conducted in experimental design where the changes in soil conditions could be controlled are still lacking (but see DeWalle et al., 2003; Kohler et al., 2019). Indeed, most studies attempted to evaluate the changes in wood element content of trees subjected to suspected soil chemistry modifications without real control modality (Augustin et al., 2005; Cui et al., 2013; Lévy et al., 1996; but see Witte et al., 2004), thus strongly weakening the demonstration. Fertilization trials constitute valuable experimental designs to test the tree-ring potential to monitor tree nutrition but have been rarely used (Kashuba-Hockenberry and DeWalle, 1994; McClenahen et al., 1989; Smith et al., 2009).

White oaks (subgenus *Quercus*, section *Quercus*) are important resources of European forests that have been extensively used in classical dendrochronological studies because of their longevity and large geographic distribution. Temperate white oaks have a ring-porous wood where the majority of the vessels definitely loses their ability to conduct water (and the dissolved elements that it contains) at the end of the growing season so that the conducting wood is almost limited to the current-year ring (Cochard and Tyree, 1990). Whereas elements transported in the xylem sap gradually accumulate in the vessel cell walls over many years in diffuse-porous wood species, the accumulation time in a ring is limited to a single growing season in white oaks (Watmough and Hutchinson, 2003). This, in addition to the presence of a clear and large heartwood area (composed by definition of dead tissues only), make white oak species a good theoretical candidate for dendrochemical approaches. So far, most studies concluded that, despite a strong influence of the sapwood/heartwood conversion on elemental redistribution (Chun and Hui-yi, 1992; De Visser, 1992; Wardell and Hart, 1973), deciduous oaks were rather valuable species for dendrochemical analysis (Chun and Hui-yi, 1992; Cutter and Guyette, 1993; DeWalle et al., 2003; Lévy et al., 1996), even if their ring-porous wood structure is more complex than that of conifers (Legge et al., 1984).

Most of the techniques used in the past to measure the content of trace elements in the wood required extensive sample preparation procedure and large sample size (*e.g.* Atomic Absorption Spectroscopy, Neutron Activation Analysis, Proton-Induced X-ray fluorescence, Inductively Coupled Plasma spectroscopy) and practically led to the analysis of only a few trees (Monticelli et al., 2009). These demanding techniques appear rather incompatible with tree-ring ecological studies that typically involve a large number of analyses (long chronologies, many trees) performed on small size samples (*ca.* 5-50 mg). On the opposite, X-ray fluorescence (XRF) spectroscopy encompasses different techniques that can be performed directly on the sample with only limited preparation and thus appear well-suited for the analysis of large sampling sets of small-size samples. Several studies displayed measurements performed on wood or other plant material with Energy Dispersive X-Ray fluorescence (Hevia et al., 2018; Maclauchlan et al., 1987; Scharnweber et al., 2016; Smith et al.,

2014) or Wavelength Dispersive X-Ray fluorescence (Andersen et al., 2013; Margui et al., 2009), known to exhibit higher peak resolution. In the current study, we coupled a Wavelength Dispersive Spectroscope (WDS) with a Scanning Electron Microscope (SEM), using the electron beam as an excitation source. In such a configuration, the microscopy imaging system offers an efficient way to precisely localize the analyzed area and to associate image and elemental analyses. To our knowledge, such a technique has never been used for tree-ring elemental analysis before.

Here, we developed an automated routine in a SEM-WDS environment to analyze the variations in N, Mg, P, K, Ca and Mn in tree rings of *Quercus petraea* trees subjected to a fertilization experiment and compare them to control trees. The objectives were:

- i) to test if a difference in wood elemental content still existed between fertilized and control trees more than 30 years after the fertilization event.

- ii) to determine the temporal accuracy of the retrospective analysis of the elemental content.

- iii) to test if the fertilization chemical signal differed between early- and latewood, and between sapwood and heartwood.

- iv) to validate a new methodology to measure elemental composition in tree rings, by comparing SEM-WDS analyses with the more classical inductively coupled plasma and atomic emission spectroscopy (ICP-AES) analyses.

2. Materials & Methods

2.1 Site characteristics and experimental design

The study site was located in a fertilization trial set up in 1969 in the national forest of Bercé, France, in a 40-year old stand of sessile oak (*Quercus petraea* (Matt.) Liebl.). The experimental design was described in Garbaye *et al.* (1974) as the "Route des Trembles" trial (47° 48' 23'' North; 0° 23' 18''

East). The goal of the fertilization was to increase the tree growth during the young stages of the stand development in order to shorten the forest rotation length. The trial included nine square plots of 0.33 ha each, distributed in three blocks. Each block was composed of (1) a control plot, (2) a plot supplied with nitrogen (220 kg N per ha) and calcium (1500 kg per ha), referred to as *NCa* hereafter, (3) a plot supplied with nitrogen (same amount as *NCa* treatment), phosphorus (200 kg of P_2O_5 per ha), potassium (120 kg of K_2O per ha), calcium (same amount as *NCa* treatment) and magnesium (120 kg of MgO per ha), referred to as *NPKCaMg* hereafter. The fertilizers were spread by hand as Patentkali®, scories and slaked lime in August 1969 and as ammonitrate in May 1970 and 1971.

The soil is a stagnic luvisol (FAO classification), acidic (pH=4.2 in the 5-40 cm soil layer), rather poor in calcium ($[Ca]_{5-40}$=0.13 cmol+.kg^{-1}), with a low C/N (9.3 in the 5-40 cm soil layer) and a maximum 172 mm extractable water content estimated for the 0-120 cm soil layer (Ponton et al., 2019). The climate is mainly oceanic with a continental influence. Climate data came from the weather station of Météo-France Le Mans Airport (47° 56' 27'' North; 0° 11' 23'' East), 22 km from the study site. For the 1950-2009 period, the mean annual temperature was 11.9°C and the mean annual rainfall was 687 mm.year^{-1}.

2.2 Sampling and measurements

In each of the nine plots, five dominant trees were cored in the central zone of the plot (excluding a 5 m peripheral band) in April 2008. The 5mm-diameter cores, extracted from the trunk at 1.3m above ground, were stored in the laboratory at -18°C and rapidly lyophilized to avoid water movement in the wood during drying. Tree-ring widths were measured to the nearest 1/100 mm from the bark to the pith and the obtained chronologies were cross-dated according to the procedure of Becker *et al.* (1994). The heartwood/sapwood boundary was determined according to color changes and occurrence of tylose-filled earlywood vessels. Two cores with poor quality (*i.e.* highly fragmented with missing pieces) had to be discarded from one of the control plot. Thus, the sampling design was

finally composed of 43 cores coming from 15 *NPKCaMg*-fertilized trees, 15 *NCa*-fertilized trees and 13 control trees. The analyses were performed over the 1950–2007 period.

The microscope stage was 8x4 cm and thus imposed the cores, whose length was between 7 and 18 cm, to be fragmented. To ensure the best layout of the cores on the stage, they were fractionated in 2-5cm length fragments (including 10-20 rings). The microanalysis technique used in this study requires flat surfaces. To avoid working on a surface riddled with large vessels, as typically found in ring-porous species such as sessile oak, each fragment was split in two halves with a razor blade along the radial-longitudinal plane. The microdensitometry measurements were made on one half and microanalyses on the other, with extreme care to avoid chemical contamination throughout the preparation steps.

2.2.1 Microdensitometry

The half-cores dedicated to microdensitometric analyses were sawn in the radial-longitudinal plane to a thickness of 2 mm. The radiography of the samples was performed by a combination of an X-ray source (Hamamatsu L9181-02 130 kV, Hamamatsu Photonics KK, Hamamatsu City, Japan) and an X-ray detector (PaxScan 4030R, Varian X-Ray Products, Salt Lake City, UT, USA). The grey levels obtained by radiography were converted in density levels using a wedge machined in a homogeneous material of near wood atomic composition (kemetal) in fixed thickness steps and a ramp of variable thickness . Then, mean ring density was computed (for more information on the technique, see: Mothe et al., 1998).

2.2.2 Microanalysis

The microanalysis was performed by a wavelength dispersive spectroscope (WDS; INCA Wave 700, Oxford Instrument, Oxfordshire, UK) combined with a scanning electron microscope (SEM; Leo 1450 VP, Zeiss, Oberkochen, Germany). The principle of the technique is that, by scanning the sample surface, the SEM electron beam excites the electron of the constitutive atoms, which in turn immediately emit X-ray photons to revert to a stable state. The wavelength of the X-ray photons

8

being specific of the emitting element, the WDS is set-up to count photons of a chosen element between Z=4 (Be) and Z=92 (U). The conversion of measured fluorescence intensity to accurate element concentration is affected by a matrix effect that requires calibration with matrix-matched reference materials (Andersen et al., 2013).

In regular XRF spectrometry and microanalysis, organic samples are usually ground to a fine powder to obtain a constant and homogeneous matrix of organic constituents including a low concentration in inorganic elements (Andersen et al., 2013). The powder is then compressed into a flat-surface pellet in order to assure a penetration of the electron beam at a constant angle (Margui et al., 2009). On top of being time-consuming, this preparation requires a relatively large quantity of biological material (> 8 g) that would prevent the analysis of the mineral content of a single ring. In this study, for instance, the average ring size was 2.3 mm (corresponding to ca. 32 mg of wood) and the 10 % smallest rings were less than 1.4 mm (corresponding to ca. 19 mg of wood).

In this study, the microanalysis was achieved directly on the raw unground material. A strict control over the WDS parameterization (*i.e.* accelerating voltage = 10kV for N and Mg and 20kV for P, K, Ca and Mn; beam current adjusted to maintain a sample current = 50 nA; measuring time = 20 s; optimization of the analytical position of the spectrometer) was imposed to maintain stable and repeatable measures along the entire measurement session. Since no standard was available, the count rates of fluorescent photons of each element (expressed in $counts.s^{-1}.nA^{-1}$) did not lead to a measure of the absolute content but provided instead semi-quantitative (or relative) estimations.

Prior to analysis, the cores were scanned on their radial-tangential plane and the relative coordinates of the tree-ring boundaries were recorded using CooRecorder 7.8 (Larsson and Larsson, 2014). These coordinates were then used to automatically retrieve the ring boundaries on the radial-longitudinal plane under the SEM. For each ring of the 1950-2007 period, the microanalysis was performed over two randomly selected areas, one within the earlywood and the other in the latewood.

The analysis was made on carbon coated cores (Desk IV, Denton Vacuum LLC., Moorestown, USA), to avoid charging effects, placed under a high vacuum ($<10^{-4}$ mTorr). The working distance was set at 15mm and the magnification at x1000, leading to a constant scanning surface (270.1 μm x 203.2 μm). Every ten measurements, a measure of the sample current was made (and corrected if needed) to assure its stability during the measurement period. Since the measurement area is small compared to the whole ring, we performed preliminary tests by measuring the element content (N = 10) of different wood tissues (*i.e.* latewood, empty vessels, and tylose-filled vessels) to assess the within-ring variation. Overall, the normalized root-mean-square deviation was 19% with no significant difference between elements or between wood tissues (P > 0.07).

2.2.3 Wood and leaf chemical analysis

In addition to microanalysis measurements, the wood elemental content was also analyzed by ICP-AES (Agilent 700, Agilent, Santa Clara, CA, USA) after sample digestion with 50% nitric acid on a subset of 60 samples coming from 20 trees (10 control and 10 *NCa*). The protocols for the sample digestion and the analysis itself were adapted for very small sample sizes. Our preliminary tests established that the lowest limit for quantification by ICP-AES was 15mg. Since this quantity of ground wood coming from half-cores could not be obtained from a single ring, six consecutive rings were pooled, for each of the 20 trees, corresponding to the years 1950-55, 1970-75 and 2001-06. Measurements were eventually done on samples whose size was between 15 and 30mg. Three out of 60 samples were too small to be measured. Finally, Mg, P, K, Ca and Mn contents were quantified on 57 samples and these values were compared to the microanalysis measurements described in section 2.2.2.

Leaf chemical analyses were conducted, as a well-established method of nutritional diagnosis, and used to gain insights on the intensity and duration of the effect of fertilization on trees. Green leaves were sampled with a rifle in late August 1993 and 2014 from three branches in the top of the canopy collected from three to five dominant trees. A composite sample was generated for each plot. The

nutrient content in green leaves was measured after oven-drying (65 °C) and sample-milling: N content was measured in 1993 by calorimetry (Technicon Autoanalyser II, Technicon Industrial System, Tarrytown, NY, USA) after Kjeldahl mineralisation and with an elemental analyser in 2014 (NCS 2500, ThermoQuest, Milano, Italy). P, K, Ca, Mg, and Mn contents were determined by ICP-AES after sample digestion with perchloric acid in 1993 (JY38 plus, Jobin Yvon, Paris, France) and 50% nitric acid in 2014 (Agilent 700, Agilent, Santa Clara, CA, USA). Limits of quantification were estimated to be: 0.5 mg.l^{-1} for P and K, 0.25 mg.l^{-1} for Ca, 0.05 mg.l^{-1} for Mg and 0.025 mg.l^{-1} for Mn.

2.3 Data analysis

The effect of fertilization on ring width and ring density data was tested using a mixed model where the fertilization treatment and the year were considered as fixed effects, the tree and the block as random effects and measurements from the same tree were considered as repeated measurements linked together with a first-order autoregressive structure. Tukey-Kramer post-hoc tests were applied to assess differences between the three fertilization modalities. Fragmented cores generated missing values in the microdensitometric series. In order to avoid any bias, the within-treatment averages were considered only when at least 10 trees contributed to the mean. The observations corresponding to the ring density of the years 1959-1962, 1982-1984, 1994-1996 and 2007 were therefore discarded because of missing data (therefore N=47 years instead of 58; Table S1).

The relationship between wood density and ring width was tested with the same mixed model where ring width was introduced as a regressor to explain the variations in wood density. A test on slope estimates was computed to compare this relationship between treatments.

Differences in element contents between earlywood and latewood, and between heartwood and sapwood were tested by using a mixed model where the tree and the block were considered as random effects and measurements from the same tree were considered as repeated measurements linked together with a first-order autoregressive structure. Radial trends within the heartwood were tested with linear regression model. For statistical comparison of the elemental content between (as

well as for description within) heartwood and sapwood, a transition zone corresponding to ± 2 rings (*i.e.* inwards and outwards) from the heartwood/sapwood boundary (identified visually, as described above) was excluded from the computation.

The autocorrelations for each element, ring width and mean wood density were analysed in the control trees, for earlywood and latewood separately, after removal of the effects of the tree and of the heartwood/sapwood factor. The relationships between elements were assessed independently within sapwood and heartwood of control trees by means of Pearson correlation coefficient calculation and Principal Component Analysis (PCA). These analyses were performed both on raw values (including between-trees variations) and on values centered per tree (to remove the tree effect), for earlywood and latewood separately. Ring width and mean density were also included in the PCA to examine their link with element contents.

Differences in wood elemental contents between trees from the three different fertilization treatments were first tested year after year from 1950 to 2007 (*i.e.* including heartwood and sapwood) with a One-Way ANOVA. Then, a mixed model was applied to test the effect of the treatment on the element content of the heartwood, including all the observations of the 1950-1988 period (thus excluding any year corresponding to sapwood, see 3.1.1). The treatment and the year were defined as fixed effects and the tree and block as random effects. Measurements made on rings from a same tree were considered as repeated measurements linked together with a first-order autoregressive structure. Tukey-Kramer post-hoc tests were subsequently performed to assess differences between the three fertilization modalities.

The elemental contents of the rings formed after and before the fertilization event (*i.e.* 1970) were compared for each fertilization modality independently. In order to limit the influence of long-term differences independent from fertilization, the comparisons were made on contents averaged over the rings 1960 to 1969, and 1970 to 1979, for the pre- and post-fertilization periods, respectively. The statistical significance of these differences were tested by paired t-tests.

Average values are accompanied in the text with standard deviations. For all statistical tests, the code of significance used in tables and figures throughout the article is: p < 0.001: ***; p < 0.01: **; p < 0.05: *. All tests were performed using SAS 9.4 (Statistical Analysis Systems, SAS Institute Inc., Cary, NC, USA).

3. Results

3.1 Effect of fertilization on ring width, wood density and leaf elemental content.

3.1.1 Ring width & wood density

Over the 1950-2007 period, the ring width averaged 2.2 ± 0.5 mm for the control trees (N=13) and was characterized by a slightly decreasing trend (R^2 = 0.11, P = 0.01, N = 58; Figure 1) and an expressed population signal (EPS) of 0.96 (Wigley et al. 1984). Before fertilization (*i.e.* from 1950 to 1969), radial growth was similar in the three treatments (2.3 ± 0.7 mm, 2.2 ± 0.7 mm, 2.3 ± 0.5 mm, for *NCa*, *NPKCaMg* and control trees, respectively). Between 1969 and 1971, the average ring width increased by 40% and 65% for *NCa* and *NPKCaMg* trees, respectively, whereas no significant changes were observed for the control trees (ring width was 3.6 ± 0.7mm, 3.8 ± 0.9mm and 2.5 ± 0.9 mm in 1971 for *NCa*, *NPKCaMg* and control trees, respectively). After 1971, the differences in ring width between fertilized and control trees grew smaller but stayed significant until 1978 (but appeared non-significant in 1976 and 1977 when the overall growth dramatically decreased because of a severe drought in 1976). It is noticeable that the increase in ring width in the fertilized trees was not accompanied with significant changes in the earlywood / latewood proportion nor in wood microdensity (Figure S1).

In the control treatment, the mean wood density significantly decreased from ring 1950 to ring 2007 (R^2=0.60, P < 0.0001, N=47; Figure 1). There was no significant difference in wood density between the control and fertilized trees neither before fertilization (1950-1969) nor after (1970-2007), and the decreasing trends were similar for the three treatments. The relationship between ring width and

ring mean density, calculated for the 1950-2007 tree rings, was significant and positive for the control trees (R=0.65, P<0.0001, N=47) and differed from the fertilized trees (t=-4.30, P<0.0001 and t=-2.21, P=0.03 for *NCa* and *NPKCaMg* trees, respectively).

For two trees (from the control treatment) among the 43 studied trees, the boundary between sapwood and heartwood could not be identified. The sapwood included between 10 and 22 rings at the time of sampling (*i.e.* the oldest sapwood rings corresponded to the years 1989 to 2001), with an average of 15 rings (1993). No significant difference regarding the number of rings included in the sapwood was observed between treatments.

3.1.2 Leaf elemental content

Only values of foliar element content averaged over each treatment were available from the 1971 sampling and, as a result, no statistical test could confirm the highest observed leaf content in N and Ca in the fertilized trees, nor the slight decrease in N, Mg, P and K and increase in Ca leaf content between 1971 and 1993 samples (Table 1). In 2014, 44 years after fertilization, the leaves appeared significantly more concentrated in Ca, P and K in the *NPKCaMg* treatment than in the other ones. In the 1993 sampling, although the same tendency was observed for Ca, not any significant differences were evidenced between treatments.

3.2 Elemental variation in the wood of control trees

3.2.1 Heartwood versus sapwood composition.

The abundance in Mg, P, K, Ca and Mn (but not N, Figure 2) appeared higher in the rings that constituted the sapwood than those that formed the heartwood, for both earlywood and latewood measurements (for comparison between earlywood and latewood data, see Figure S2). Since the position of the heartwood/sapwood boundary varied between trees, the heartwood/sapwood effect on elemental composition was smoothed when annual values from the 13 control trees were averaged. So, beneath these apparent gradual increases of the average contents lied more abrupt

individual changes, with a transition period of only 2-3 rings (Figure S3). Compared to heartwood, the average sapwood content was significantly higher by 87% for K, 62% for P, 30% for Ca, 29% for Mg, and 26% for Mn, and lower by 12% for N (Figure S2). Similar results were obtained for both earlywood and latewood measurements.

3.2.2 Autocorrelation and co-variation among elements.

The autocorrelations and correlations between elements were assessed separately in the heartwood and in the sapwood of the control trees (N=13). Significant first-order autocorrelations were found regarding the wood element content, except for the Ca in the earlywood (Table S2). On the whole, they were larger in the latewood than in the earlywood (except for Mn) and the correlations between the earlywood content of a year and the latewood content of the previous year displayed intermediate values. The first-order autocorrelations of ring width and mean density (measured at the entire ring level) were larger than those of the element content.

When considering the 13 trees together, nearly all elemental contents appeared positively correlated with each other, both in the earlywood and the latewood (see Table S3 for correlations in the heartwood, data for the sapwood not shown). The highest correlations were observed between earlywood Mg, P and N contents, both in the sapwood and in the heartwood. In the latewood, Mg, P and N contents were slightly less strongly correlated, both in the sapwood and in the heartwood. Relatively high correlations were also calculated between Ca and Mn, especially in the earlywood. These relationships were also depicted in the PCA where measurements made in the earlywood and latewood were considered together (Figure 3). The first four components accounted for were 30, 18, 13 and 9% of the variance in the heartwood, respectively, and clearly separated the elements N, Mg and P to Ca, Mn and K measured in the earlywood and in the latewood. The relationships were mostly unchanged when the effect of the tree was removed (by performing the PCA on the residuals of the ANOVA testing the effect of the tree). The explained variance became 24, 18, 15 and 11%. Comparable values were obtained in the sapwood.

The earlywood content was significantly correlated with the latewood content of the same ring (Table S3) but the largest part of the relationship was due to large between-tree variations in element contents. Once the effect of the tree was removed (Table S3b, right side of Figure 3), covariations of elemental contents between earlywood and latewood were very low (r<0.22).

3.2.3 Relationships between physical properties and elemental content of the ring.

Overall, only weak correlations were found between the elemental content and ring width or mean density mainly in the heartwood (Table 2). The N, Mg and P contents were positively but slightly correlated to the ring width, both in the latewood and in the earlywood. But, in the sapwood, this relationship became negative for Mg and P in the latewood, and not significant for N. All significant correlations between element contents and mean ring densities were negative. Both Mn and Ca contents in the heartwood, and P, K and Mn contents in the sapwood appeared slightly negatively related to the mean ring densities.

3.3 Effect of fertilization on tree-ring elemental composition

3.3.1 Comparison between fertilized and control trees

When tested year per year, only a few isolated significant differences in N, Mg, P and K were observed between treatments over the whole studied period (*i.e.* 1950-2007). Most of them corresponded to measurements made in the latewood (Figure 4). Significant differences in Mn content were observed between treatments for six of the 58 analyzed years when measurements were performed in the earlywood (with invariably higher content in the control trees than in the fertilized trees). The number of years with significant differences increased to nine when the measurements were compared in the latewood (note that significance in Figure 4 was only tested and displayed between the treatments and control, not between both treatments). Calcium clearly appeared as the most discriminating element between treatments. The measurements made in the earlywood showed significant differences for three years only (in the period 1962 to 1985). When the

measurements were compared in the latewood, the Ca content appeared significantly higher in the *NCa*-fertilized trees for 20 years (spread over the period 1952 to 2006). Intermediate values were obtained in *NPKCaMg*-fertilized trees that could not be significantly differentiated from the control trees (except in 1968 and 1969).

The effect of the treatment observed on the Ca content was emphasized when expressed as a Ca/Mn ratio, especially in the latewood (Figure 5). Overall, 21 and 29 out of 58 years showed a clear increase in the Ca/Mn ratio measured in the earlywood and latewood, respectively, of the fertilized trees compared to the control trees. When measured in the earlywood, Ca/Mn appeared significantly higher, compared to control trees, for 19 years in *NPKCaMg*-fertilized trees and seven years in *NCa*-fertilized trees, five of these years only being shared. One year only (*i.e.* 2007) corresponded to a ring located in the sapwood. *NCa* trees were intermediate between controls and *NPKCaMg* trees. Measurements performed in the latewood evidenced 22 years with a higher ratio in *NCa*-fertilized trees and 14 years with a higher ratio in *NPKCaMg*-fertilized trees (seven being shared between the two fertilized modalities), compared to control trees.

When all the analyses performed in the heartwood (*i.e.* 1950-1988) were included together in a mixed model, no effect of the treatment were identified on the N, Mg, P, K and Mn contents of the trees, neither for measurements made in the earlywood, nor in the latewood. The Ca content was significantly higher in the fertilized trees than in the control trees both in earlywood ($F=3.30$, $P=0.037$, $N=1668$) and latewood ($F=5.27$, $P=0.0052$, $N=1668$). Based on the Tukey-Kramer post-hoc tests, the differences between fertilization modalities were as follow: *NPKCaMg>(NCa*=control) in the earlywood, and (*NCa= NPKCaMg*)>control in the latewood. The effect of the year was barely significant on Mg ($F=1.43$, $P=0.045$, $N=1668$) and Ca contents ($F=1.41$, $P=0.0496$, $N=1668$) measured in earlywood but higher for K (earlywood: $F=2.79$, $P<0.0001$, $N=1668$; latewood: $F=1.65$, $P=0.0079$, $N=1668$) and Mn (earlywood: $F=1.92$, $P=0.0007$, $N=1668$; latewood: $F=2.39$, $P<0.0001$, $N=1668$). Whatever the element, there was no interaction between the treatment and the year effects. Regarding the Ca/Mn ratio, both earlywood ($F=15.24$, $P<0.0001$, $N=1668$) and latewood ($F=12.1$,

P<0.0001, N=1668) measurements showed a clear effect of the fertilization with values sorted as follows (Tukey-Kramer post-hoc test): *NPKCaMg>NCa*>control in the earlywood, and (*NPKCaMg=NCa)*>control in the latewood. The effect of the year was only significant for measurements performed in the earlywood (F=1.76, P=0.0031, N=1668) and there was no interaction with the treatment effect.

3.3.2 Comparison between pre- and post-fertilization rings

The elemental composition did not differ between the tree rings corresponding to the period 1960-1969 (*i.e.* before fertilization) and those corresponding to the period 1970-1979 (*i.e.* after fertilization) for the control trees. The trees that received a *NPKCaMg* treatment did not display any differences in elemental contents between the rings of the two periods either. In the *NCa* treatment, a higher Mg and N contents were observed in the rings formed before the fertilization occurred (t=3.54, P=0.0033, N=15; t=3.63, P=0.0027, N=15; t=2.68, P=0.018, N=15; for Mg in the earlywood, Mg in the latewood and N in the latewood, respectively).

3.4 Methods comparison

The relationships between ICP-AES and WDS measurements (Figure 6) were rather good for Mn (R=0.83, P<0.0001, N=56) and K (R=0.71, P<0.0001, N=56). For P, the correlation coefficient was also quite high (R=0.75, P<0.0001, N=56) but the numerous ICP measurements below the detection level likely biased the results. Relationships were weaker although very significant for both Mg (R=0.57, P<0.0001, N=56) and Ca (R=0.54, P<0.0001, N=56).

4. Discussion

The experimental design used in this study was initially only set up to evaluate the effect of a fertilization on tree growth. No soil analysis were performed in the years following the fertilization event. However, the sudden and dramatic radial growth increase observed on fertilized trees, right after 1970, attested the impact of fertilization on soil chemistry. This increased tree-ring width in

fertilized trees was in agreement with the increased circumference, mainly in *NCa* but also *NPKCaMg* trees, following the fertilization event (Garbaye et al., 1974). The width data was extensively discussed in Ponton et al. (2019). The effectiveness of the fertilization was also confirmed through foliar analyses that repeatedly (*i.e.* in 1971, 1993 and 2014) showed higher Ca contents in *NPKCaMg* (and *NCa*, to a lesser extent, but only significant for *NPKCaMg* in 2014) trees than in the control ones.

4.1 Radial variations in element contents

Whatever the fertilization treatment, expected differences in elemental contents were observed between heartwood and sapwood. Indeed, as in many studies (De Visser, 1992; Lévy et al., 1996; Penninckx et al., 2001), much higher contents were measured in the sapwood than in the heartwood, except for nitrogen. N, Mg, P and K are considered as mobile elements that are transferred from parenchyma cells during the conversion to heartwood and accumulate in the sapwood (Arp and Manasc, 1988; Colin-Belgrand et al., 1996; Cutter and Guyette, 1993; Frelich et al., 1989; Meerts, 2002; Wardell and Hart, 1973). Although Ca and Mn cations are considered less mobile, the observed trend with increasing contents in the more recent rings is common, especially in the *Quercus* genus ((Chun and Hui-yi, 1992; De Visser, 1992; DeWalle et al., 1991b; Herbauts et al., 2002). This shows that a fraction of these elements, at least, can be withdrawn during sapwood senescence. Additionally, Herbauts et al. (2002) reported that the cation exchange capacity (CEC) was decreasing from the pith outwards in the heartwood and increasing in the sapwood of *Quercus robur* (L.). They claimed that radial changes in Ca and Mg contents primarily reflect these intrinsic variations in CEC. These decreasing trends in the radial variations of the heartwood contents of Mg, Ca and Mn were also visible in our samples. Contrary to what is usually observed, the general radial pattern of N did not mimic that of P (but N and P still appeared closely related, Table S3 and Figure 3). The slight decreasing trend of N content in xylem from the pith to the bark was shared with the wood mean density. However, wood density and element contents, including N, seemed to have only marginal relationships.

In this study, the heartwood/sapwood boundary was located within the years 1989 to 2001, in average 23 years after the fertilization event. Considering that the fertilized-induced increase in radial growth rapidly abated after 1974, the heartwood/sapwood transition, and its associated abrupt changes in chemical composition, should not hamper the monitoring of the main fertilization effects.

4.2 Fertilization effect

The fertilized trees were characterized by a higher content in Ca and a slightly lower content in Mn than the control trees (Figure 4). A higher Ca content and lower Mn content in the wood of trees exposed to higher soil pH (and base saturation) has been observed on many tree species (DeWalle et al., 1999; Guyette et al., 1992; Jensen et al., 2014; Kuang et al., 2008; McClenahen et al., 1989) including oaks (Bukata and Kyser, 2008; DeWalle et al., 1991a; DeWalle et al., 2003; Kashuba-Hockenberry and DeWalle, 1994). Tree-ring content in Mn was found to be related to soil pH, rather than Mn concentration in the soil (Bukata and Kyser, 2008). Although expected from the literature (Ouimet and Moore, 2015; Prietzel et al., 2008), it is not possible to confirm in the current study whether the soil pH was increased or not by the fertilization since no soil analysis was performed in the following years. The fertilization effect was magnified when considering the Ca/Mn ratio. Element ratios are often preferred as indicators of wood composition changes and seem to amplify the external environmental signal (Berger et al., 2004; Bondietti et al., 1989; Chen et al. 2010; Scharnweber et al., 2016). Over the years that followed fertilization, the excess in wood Ca (as compared to the control treatment) could have mirrored differences in the soil or, alternatively, could have been translocated towards newly-formed rings. Whatever the cause, the observed highest Ca content in the leaves of *NPKCaMg* trees sampled in 2014 testified that nutrition differences between treatments remained long after 1970.

The rings formed before 1970 also showed differences in elemental contents between fertilized and control trees. Moreover, except a slightly higher content in N and Mg before fertilization in *NCa*

trees, the element content in fertilized trees did not appear different between rings built before and after fertilization. These results are similar to those observed by McClenahen et al. (1989) in fertilized *Liriodendron tulipifera* trees or Houle et al. (2002) in *Acer saccharum* trees subjected to liming. In *Picea abies* (L.) and *Pinus sylvestris* (L.), while translocation of phosphorus outwards to the sapwood was exhibited in the two species, the authors did not found any evidence for translocation inwards (*i.e.* towards older rings) (Kohler et al., 2019). Two processes likely contributed to these element distribution over the rings. First, when the fertilization occurred, the many rings included in the sapwood were able to conduct xylem sap and could thus transport the elements made available by the fertilization. Although irreversible embolism of the large vessels lead to a 90% loss of hydraulic conductivity after the first hard frost (Cochard and Tyree, 1990), a significant residual sap flow has been observed in the deeper layers of *Quercus petraea* sapwood (Granier et al., 1994). Thus, even if most of the sap flow was supplied through the earlywood vessels of the current-year ring, tracheids and latewood vessels of previous-years rings certainly kept the capacity to conduct sap and dissolved elements. In addition, even if most vessels were non-functional in the 1970 sapwood, living tissues like ray parenchyma were likely able to make elements movement possible in the inward direction (Okada et al., 2012). In wood tissue, N and P are mainly involved in mobile non-structural molecules accumulating at their highest level in the most recent ring (Cutter and Guyette, 1993; Gerhart and McLauchlan, 2014; Hevia et al., 2019). Even if N and P appeared clearly associated in their variations in wood (Figure 3), our data suggests that N did not accumulate in the sapwood (Figure 2), contrary to previous results on the closely-related species *Quercus robur* (Penninckx et al., 2001). K is known to be actively accumulated and translocated through the wood symplasm whereas Ca is preferentially translocated via the apoplastic pathway (Smith et al., 2014). Interestingly, Kashuba-Hockenberry and DeWalle (1994) did not find any evidence of significant radial translocation of Mn on *Q. coccinea* (Muenchh.) trees subjected to liming.

Some differences in tree-ring content were observed between the *NPKCaMg* and *NCa* treatments. Our dataset does not allow explaining precisely the involved processes. However, we may

hypothesize that changes in the stoichiometry of elements in soil occurred after the products application, inducing changes in tree nutrition strategy. In addition, while fertilization and liming operations may help enhancing the chemical, biological and physical properties of forest soil fertility, they may also cause long-term nutrient imbalances in forest stands and soils. For instance, Court et al. (2018) suggested that adding compounds without Mg (which is the case for the *NCa* plots) may induce a Mg deficiency to the soil-plant system when its availability in the soil is low. Indeed, the enhanced tree growth following the product application (*NCa* in our study) caused an increase of Mg uptake to produce biomass. A decrease of soil bioavailable Mg pools was actually observed in 1995 in the *NCa* plots compared to the control plots (data not shown). However, no difference was reported in foliar Mg concentrations between the *NCa* plots and the control plots over the study period, suggesting that tree internal remobilization could have occurred and compensate for this Mg deficiency in the *NCa* plots. This could partly explain the lowest Mg content, observed in the *NCa* treatment only, in tree rings formed after the fertilization occurred.

Since the oldest rings encountered in our chronology were 20-year old at the time of fertilization, in 1970, they could potentially have been included in the sapwood (since up to 22 rings were counted in the current sapwood). It is then not possible to know whether the chemical content of rings already converted to heartwood at the time of fertilization could be changed (*i.e.* get a higher Ca content) or not. Although the absence of living cells and sap flow are unfavourable for element mobility, significant diffusion in heartwood has been proven for alkaline metals (Okada et al., 2012), yet lower in oaks than in other species (Ohashi et al., 2017).

Even though they showed the same general tendencies, some differences were observed between the earlywood and the latewood elemental contents. A lower content in K, Ca and Mn was observed in the latewood as already mentioned for conifers (Hevia et al., 2018; Silkin and Ekimova, 2012) and *Castanea sativa* (for Ca only, though; Scharnweber et al., 2016). This was likely the results of the higher proportion of primary cell walls (known to offer higher concentrations of pectins on which cations can bind) in the thin cells of the earlywood (Hietz et al., 2015). In comparison, the thicker

cells of the latewood have a larger proportion of secondary walls and therefore contains relatively less cation exchange sites. Alternatively, this difference could result from a dilution effect, as suggested by the negative correlation between element content and mean density (Table 2). The higher autocorrelations in latewood (and higher correlations between contents of earlywood and latewood of the previous year) than the ones in earlywood suggested a higher rate of transport from the latewood and/or a higher effect of the year on the earlywood elemental content. When analyzed year by year, Ca and Mn measurements made in the latewood discriminated more efficiently the fertilized trees from the control ones compared to measurements made in the earlywood. This result might be related to the fact that a part of the earlywood is necessarily built from remobilized material (mainly carbohydrates but probably also inorganic elements incorporated in the chemical structure). Indeed, in white oaks the cambium activity resumes its activity in spring a few days before leaf unfolding (Michelot et al., 2012), thusbefore leaves can export photosynthesis products. Furthermore, winter embolism leads the vessel lumen to be progressively filled with tylose (Cochard and Tyree, 1990). Since tylose walls are built from new material (De Micco et al., 2016), differences in elemental composition are also likely to occur. Besides, measurement errors may have been higher in earlywood than in latewood. WDS analysis should be realized on flat polished surface to perform optimally, which is not the case of the large vessel structures even when cut along the radial-longitudinal plane.

The WDS technique has been traditionally used for analysis of metal or glasses. Here, we extended its use beyond these classical conditions with convincing results. This extension proved to be efficient in *i)* observing the expected heartwood/sapwood differences, and *ii)* identifying significant differences in the wood of fertilized and control trees. Admittedly, this technique is far from being the most sensitive and accurate one. On glass material, the detection limit in WDS varied from 15 ppm (for K and Ca) to 500 ppm (for N) with intermediate values for Mg (20 ppm), Mn (35 ppm) and P (120 ppm) (Kuisma-Kursula, 2000). For an element concentration of 500 ppm, WDS yielded a precision of ± 25-50% and an accuracy of ± 40-80%, except for Mn where the accuracy was ± 11% (Kuisma-Kursula,

2000). In the current study, we can expect even lower accuracy and precision, with measurements performed on uneven wood and element concentration <200 ppm for Mg and Mn, and <100 ppm for P (according to ICP analyses). However, the observed relationship between ICP and WDS measurements (Figure 6), *i.e.* rather poor for Mg but good for Mn (and intermediate for P, K and Ca), are well in agreement with the results obtained on glass. To our knowledge, there is no existing study using SEM-WDS analysis on wood or other biological tissue. However, the advantages of this method are that measurements are possible with a high spatial resolution on small size samples, with only minor preparation, and do not affect the sample integrity. Our data suggests that this standardless method may be less precise. However, it could be compensated for by a higher measurement number, which allows the screening of numerous samples. It then appears particularly suitable to dendrochemical analysis.

5. Conclusion

The tree ring chronologies revealed that the sudden change in soil fertility in 1970 induced a dramatic and immediate increase in ring width but no change in wood density and no apparent change in element content. However, in oak trees sampled in 2008, the Ca content in the wood appeared clearly higher for those that were fertilized in 1969-1970 than for the control ones. Thus, the wood did record and store this environmental information for almost 40 years.

As previously observed from dendrochemical studies, the Ca/Mn ratio appeared to be the most discriminating variable between the fertilized and control trees. The use of ratios is particularly sound when using semi-quantitative method as done here by improving the comparability of the between- and within-tree (*i.e.* periods of distinct ages) results (Scharnweber et al., 2016).

We showed that for oaks, even after a clear and sudden change in tree nutrition imposed in experimental conditions, the time spent in the sapwood by a ring and the subsequent elements radial transfers may preclude, or at least complicate the posterior dating of the event by tree ring chemical analyses. The change in element composition was registered by the trees, but spread over

the entire radial profile, at least 20 years before and 38 years after the date of fertilization. It could be interesting to extent the width of this observation window in order to check if some signal could appear on a longer term. However, compared with most fertilization trials used to document the stability of the dendrochemical record in tree rings (McClenahen *et al.* 1989, Smith *et al.* 2009, Ortega Rodriguez *et al.* 2018), the current experiment offered a long time sequence of monitoring and older fertilization experiments are rare. Alternatively, species characterized by thinner sapwood, as *Castanea sativa* (Mill.) or *Robinia pseudoaccacia* (L.) for instance, might be better suited to record environmental changes with a fine time resolution.

Even if the size of their sapwood complicates the dendrochemical use of white oak species to monitor high-frequency environmental variations, these long-lived species might still offer valuable chemical chronologies to study long-term changes (Vaganov et al., 2013).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Tables

Table 1: Mean leaf chemical content (in %) per treatment in 1971, 1993 and 2014. Statistical tests were not applicable for the 1971 sampling where only averages values per treatment were available (from Garbaye *et al.* 1974). Tukey's HSD tests were performed in 1993 and 2014 where three and six repetitions (trees) per treatment were available, respectively. Letters denote statistical differences among fertilization treatments at P<0.05. Bold values are used for significant treatment effects.

Year	Number of years after fertilization beginning	Treatment	N	Mg	P	K	Ca	Mn
1971	2	*Control*	1.93	0.16	0.10	0.98	0.58	*na*
		NCa	2.21	0.17	0.09	0.79	0.69	*na*
		NPKCaMg	2.02	0.20	0.15	0.90	0.88	*na*
1993	24	*Control*	1.95 [a]	0.16 [a]	0.12 [a]	0.79 [a]	0.79 [a]	0.28 [a]
		NCa	1.99 [a]	0.17 [a]	0.10 [a]	0.71 [a]	0.79 [a]	0.30 [a]
		NPKCaMg	1.96 [a]	0.17 [a]	0.12 [a]	0.75 [a]	0.88 [a]	0.32 [a]
2014	45	*Control*	*na*	0.14 [a]	**0.09 [a]**	**0.72 [a]**	**0.56 [a]**	0.33 [a]
		NCa	*na*	0.13 [a]	**0.09 [a]**	**0.70 [a]**	**0.58 [a]**	0.30 [a]
		NPKCaMg	*na*	0.16 [a]	**0.13 [b]**	**0.88 [b]**	**0.70 [b]**	0.29 [a]

Table 2: Correlations between the elemental content, ring width and mean density for the control trees. EW: earlywood; LW: latewood. When significant, Pearson correlation coefficient is displayed with its associated p-value (p<0.05: *; p<0.01: **; p<0.001: ***), n.s. otherwise. For the sapwood, N=117 (relationships with mean density) or N=159 (relationships with ring width). For the heartwood, N=420 (relationships with mean density) or N=530 (relationships with ring width).

		Heartwood		Sapwood	
		Ring width	Mean density	Ring width	Mean density
N	EW	**0.26** ***	ns	ns	ns
	LW	0.11 **	ns	ns	ns
Mg	EW	**0.30** ***	ns	ns.	ns
	LW	**0.22** ***	ns	**-0.16** *	ns
P	EW	**0.26** ***	ns	ns	ns
	LW	0.10 *	ns	**-0.17** *	-0.24 **
K	EW	0.11 *	ns	ns	ns
	LW	**N.S.**	ns	ns	**-0.19** *
Ca	EW	-0.09 *	**-0.13** *	-0.16 *	ns
	LW	**-0.13** **	**-0.15** **	ns	ns
Mn	EW	ns	**-0.17** ***	ns	ns
	LW	ns	**-0.23** ***	ns	**-0.22** *

Figures caption

Figure 1: Mean ring width (▲) and mean ring density (●) of the control (N=13), NCa (N=15) and NPKCaMg trees (N=15) from 1950 to 2007. Error bars are standard errors of the mean. The grey area indicates the average position of the sapwood. The date of the fertilization event is specified by the vertical dashed line. The observations corresponding to the ring density of the years 1959-1962, 1982-1984, 1994-1996 and 2007 were discarded due to missing data.

Figure 2: Mean elemental content (in counts.s^{-1}.nA^{-1}) in the earlywood and latewood of the tree rings of the control trees, from 1950 to 2007. Error bars are standard errors of the mean. The grey area indicates the average position of the sapwood.

Figure 3: Principal component analyses calculated on the elemental analysis (N=530) performed in the heartwood (earlywood: green dots, latewood: brown plus) of the control trees (N=13). The top and bottom rows display the variable position in the plane composed by the components 1 (x axis) and 2 (y axis) and in the plane composed by the components 2 (x axis) and 3 (y axis), respectively. The left column shows the results of the analysis performed on raw values whereas the right column shows the results after the removal of the tree effect (*i.e.* PCA performed on element contents standardized by tree to a zero mean). Note that PC2 is almost unchanged in the two calculations whereas PC1 of the first one corresponds to PC3 of the second one, and vice versa.

Figure 4: Mean elemental composition (in counts.s^{-1}.nA^{-1}) in the latewood of the control and fertilized trees. Error bars are standard errors of the mean (N=15 for *NPKCaMg* and *NCa* and N=13 for control). The date of the fertilization event is represented by the vertical dashed line. The grey area indicates the position of the average sapwood. The horizontal arrow shows the 1950-1988 period, which excludes any ring belonging to the sapwood. Differences in elemental content between treatments were tested for each year with Tukey-Kramer post-hoc tests and are indicated by color bars as follows: <*NPKCaMg* ≠ control> in yellow, <*NCa* ≠ control> in red, both <*NCa* & *NPKCaMg* ≠

control> in orange. In order to keep the figure legible, significant differences between *NCa* and *NPKCaMg* are not shown.

Figure 5: Mean Ca/Mn ratio calculated in the earlywood and latewood of the control, NCa-fertilized and NPKCaMg-fertilized trees. Legend as in figure 3.

Figure 6: Comparison between microanalysis (WDS, in counts.s^{-1}.nA^{-1}) and ICP-AES (in ppm) methods. Samples for dual analysis were selected from control (N=30) and *NCa* (N=30) modalities.
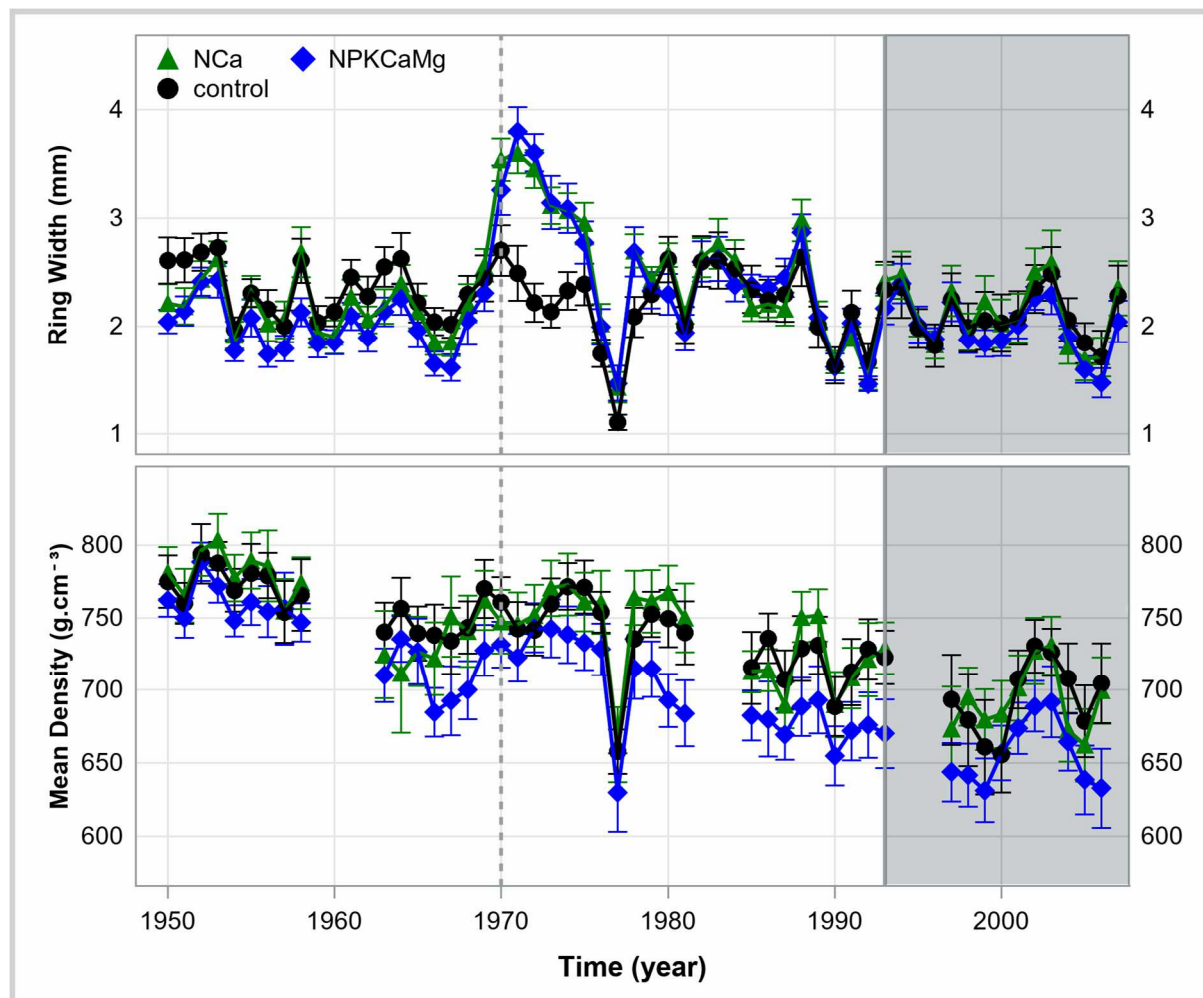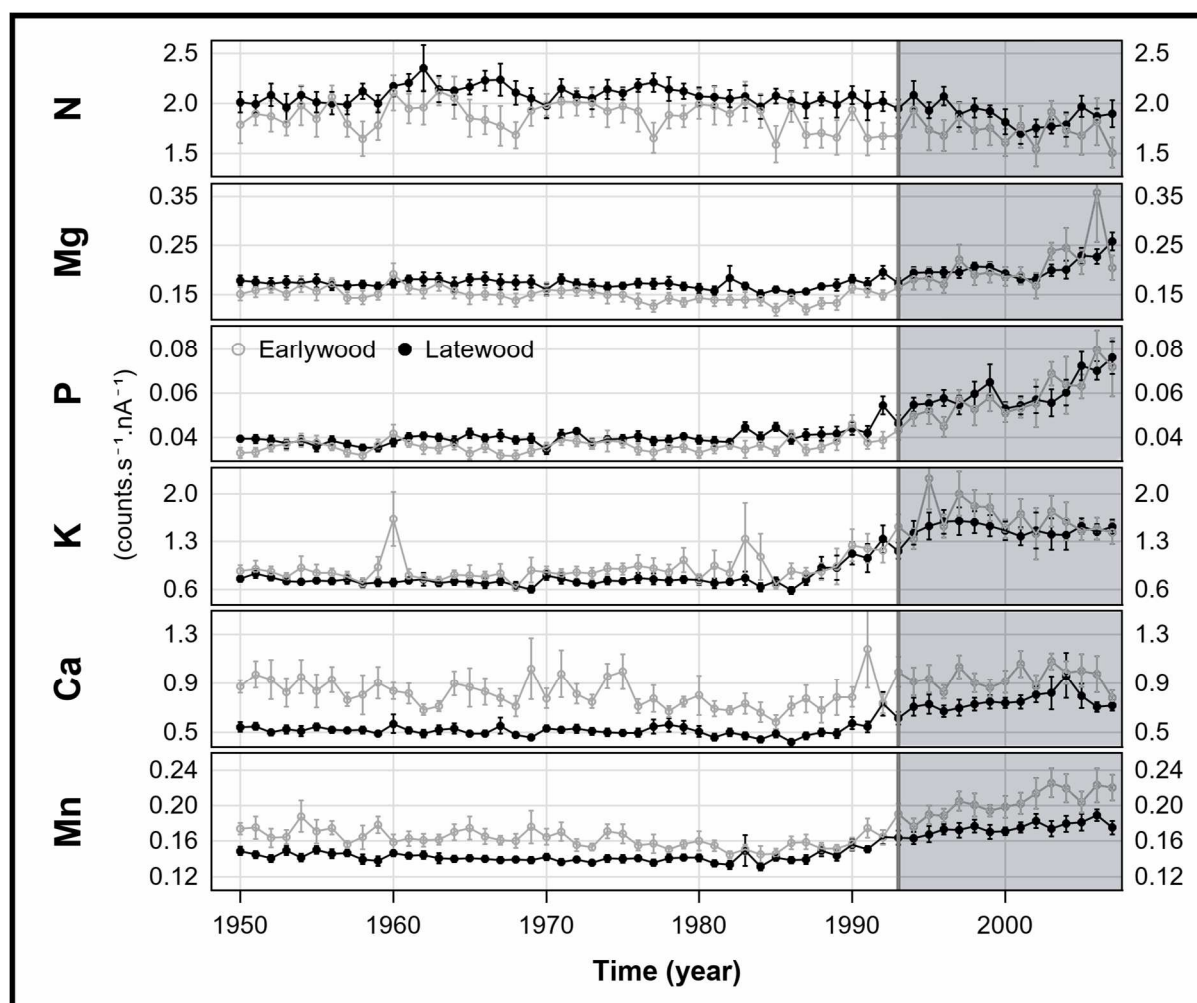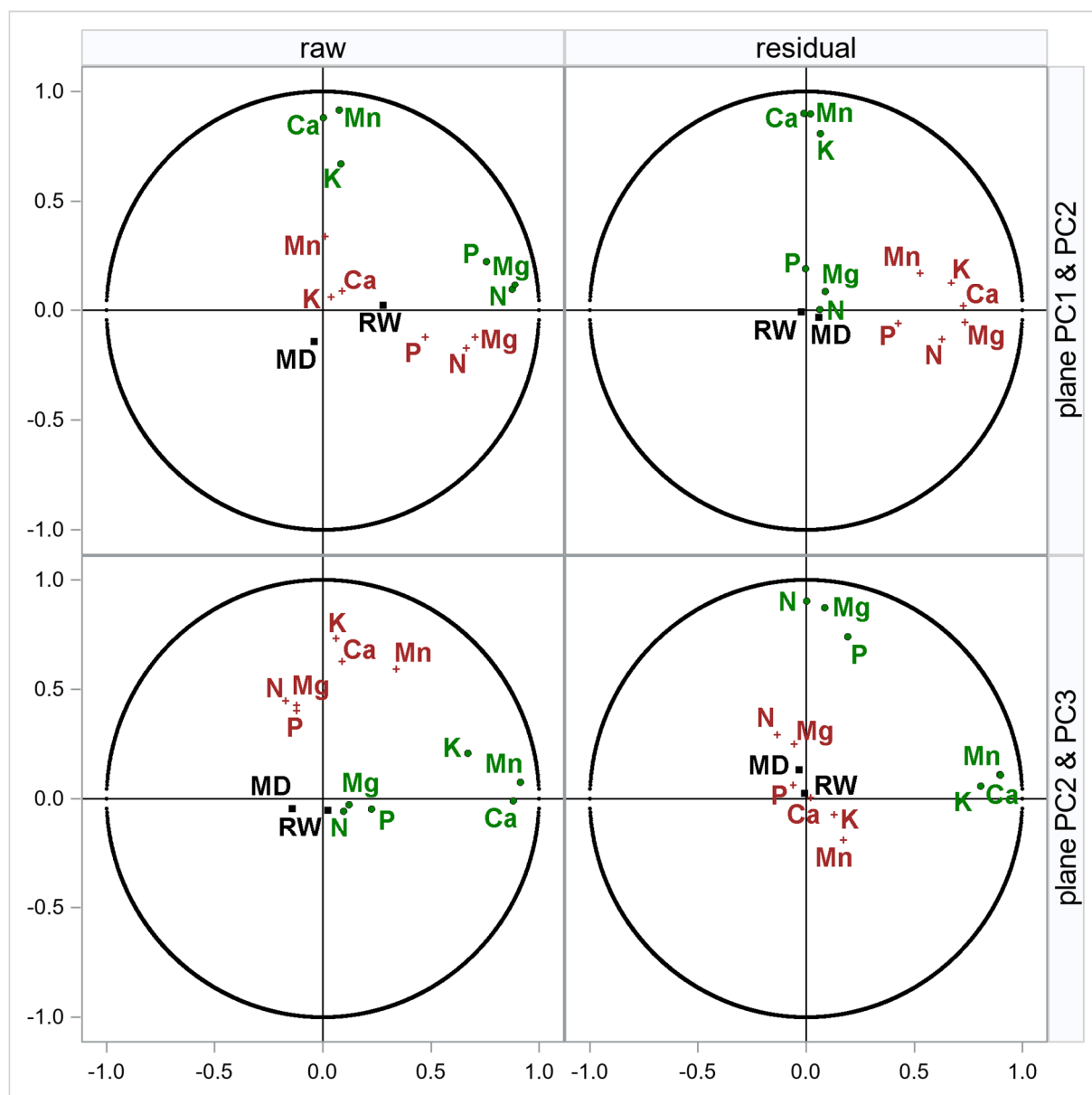
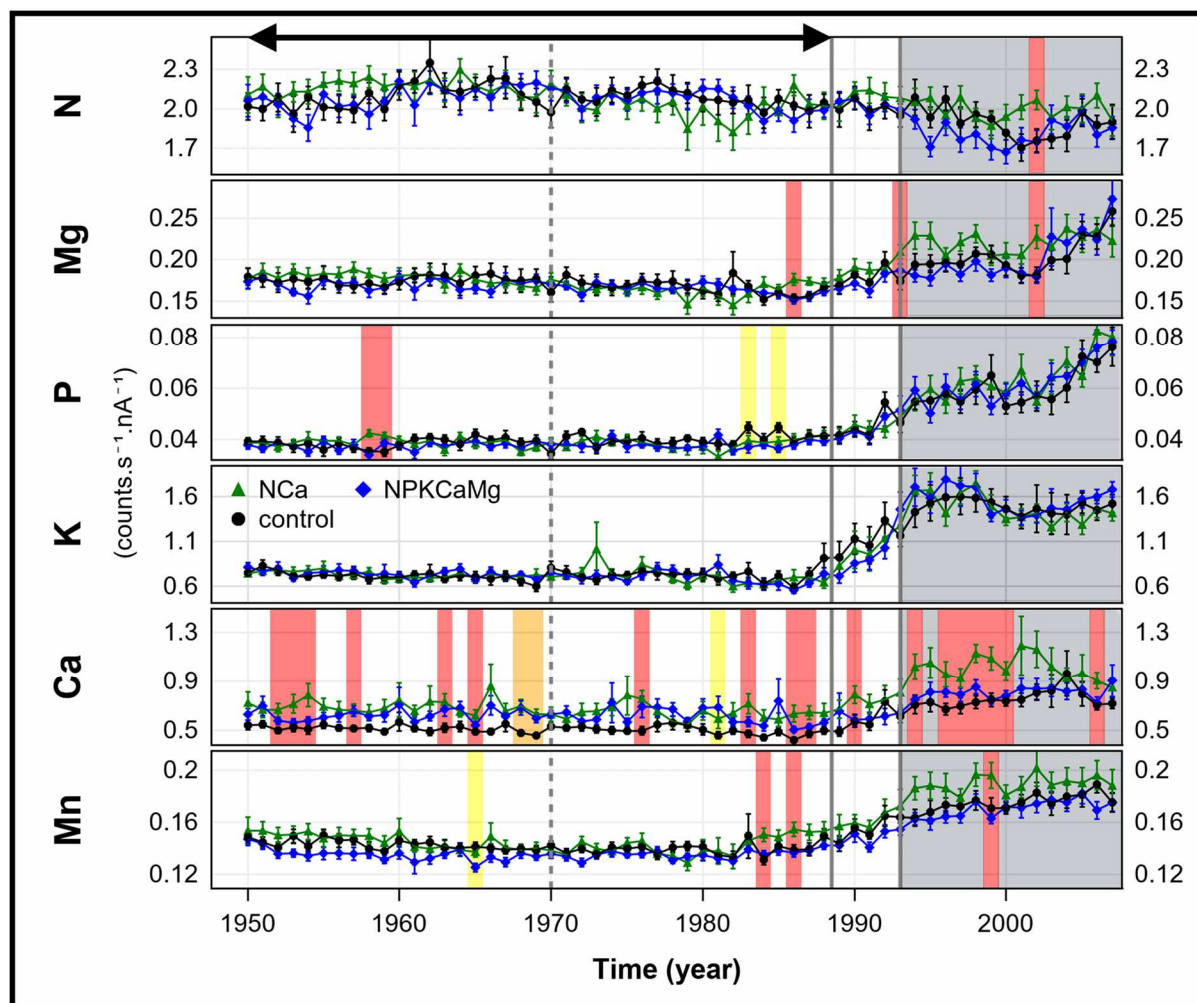# Figures

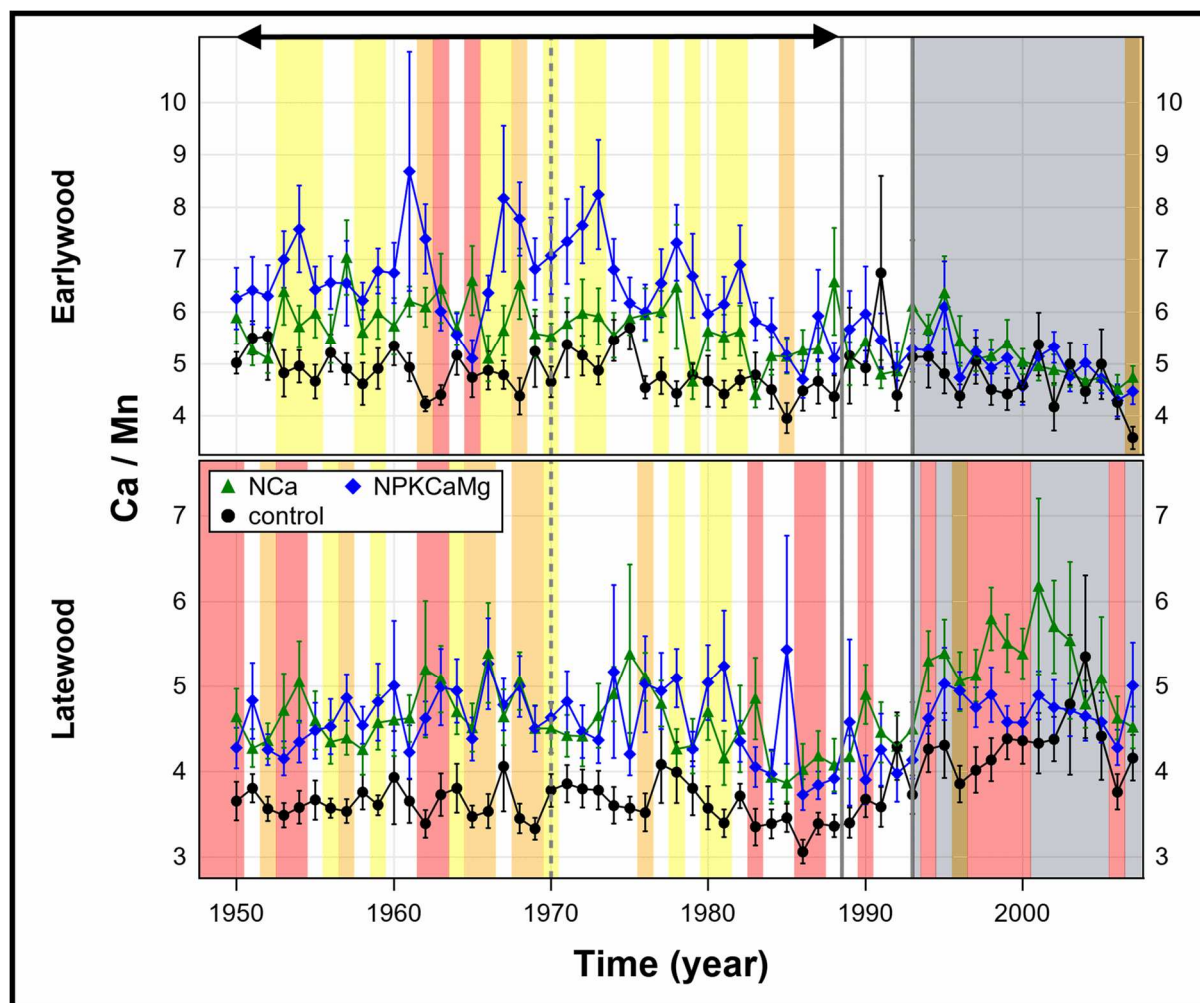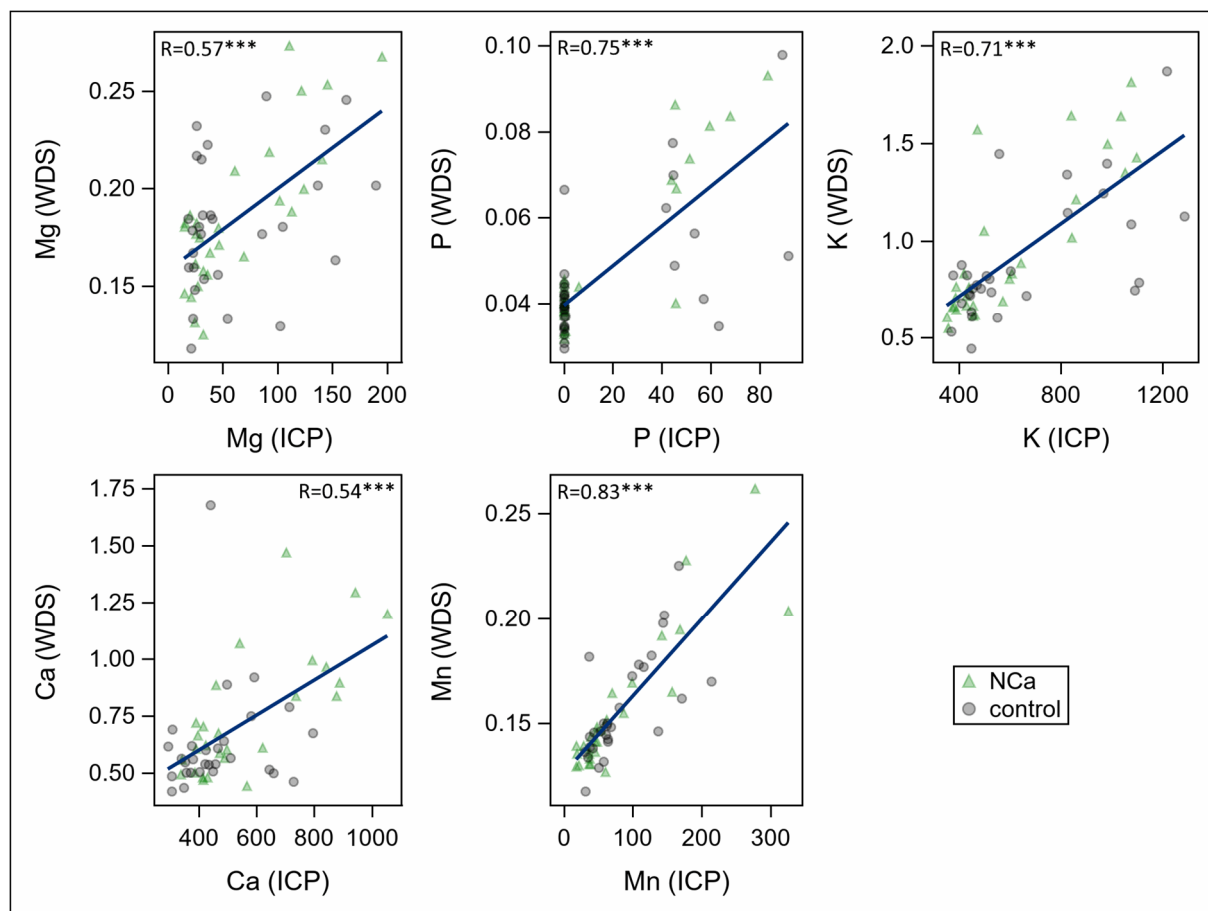Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Supplementary material

Table S1: Sample size (in number of trees) and time period (in calendar years) considered for the analysis of ring width (RW), mean density (MD), element contents in the wood with WDS and ICP-AES and leaf chemical analysis with ICP-AES.

	RW	MD	Element content (WDS)	ICP (wood)	ICP (leaf)	
Time period	1950-2007	1950-1958 1963-1981 1985-1993 1997-2006	1950-2007	1950-1955 1970-1975 2001-2006	1993	2014
Control (3 plots)	13	13	13	10	3	6
NCa (3 plots)	15	15	15	10	3	6
NPKCaMg (3 plots)	15	15	15	-	3	6

Table S2: First-order autocorrelations within the earlywood (EW) and the latewood (LW) and correlations between earlywood of the current year (EW_n) and the latewood of the previous year (LW_{n-1}). The calculations were performed on ring width (RW), mean density (MD) and N, Mg, P, K, Ca and Mn contents after removal of the effects of the tree and of the heartwood/sapwood factor. RW and MD were measured for the entire ring. The value of the Pearson's correlation coefficient is given (N=741 except for MD where N=521). $p < 0.05$: *; $p < 0.01$: **; $p < 0.001$: *** *ns*: non-significant.

		EW	LW	LW_{n-1} / EW_n
	RW	0.54 ***		
	MD	0.55 ***		
Element content	N	0.15 ***	0.38 ***	0.24 ***
	Mg	0.13 ***	0.33 ***	0.31 ***
	P	0.10 **	0.29 ***	0.25 ***
	K	0.08 *	0.38 ***	0.30 ***
	Ca	*ns*	0.37 ***	0.14 ***
	Mn	0.21***	0.15 ***	0.26 ***

Table S3: Correlations between heartwood elemental contents for the control trees. The analyses were performed on (a) raw values and (b) on data after removal of the tree effect (*i.e.* correlations calculated on series standardized by tree to a zero mean). The bottom left side shows correlations in the earlywood whereas the top right side shows correlations in the latewood. The grey diagonal line shows correlation between earlywood and latewood values. The value of the Pearson's correlation coefficient is given (N=530). $p < 0.05$: *; $p < 0.01$: **; $p < 0.001$: *** *ns*: non-significant.

a)

	N	Mg	P	K	Ca	Mn
N	0.43 ***	0.69 ***	0.51 ***	0.26 ***	0.15 ***	0.13 **
Mg	0.85 ***	0.53 ***	0.42 ***	0.23 ***	0.18 ***	0.13 **
P	0.70 ***	0.66 ***	0.23 ***	0.14 **	0.10 *	0.13 **
K	0.23 ***	0.27 ***	0.33 ***	0.25 ***	0.30 ***	0.27 ***
Ca	ns	ns	0.11 **	0.31 ***	0.19 ***	0.34 ***
Mn	ns	0.10 *	0.11 *	0.28 ***	0.83 ***	0.30 ***

b)

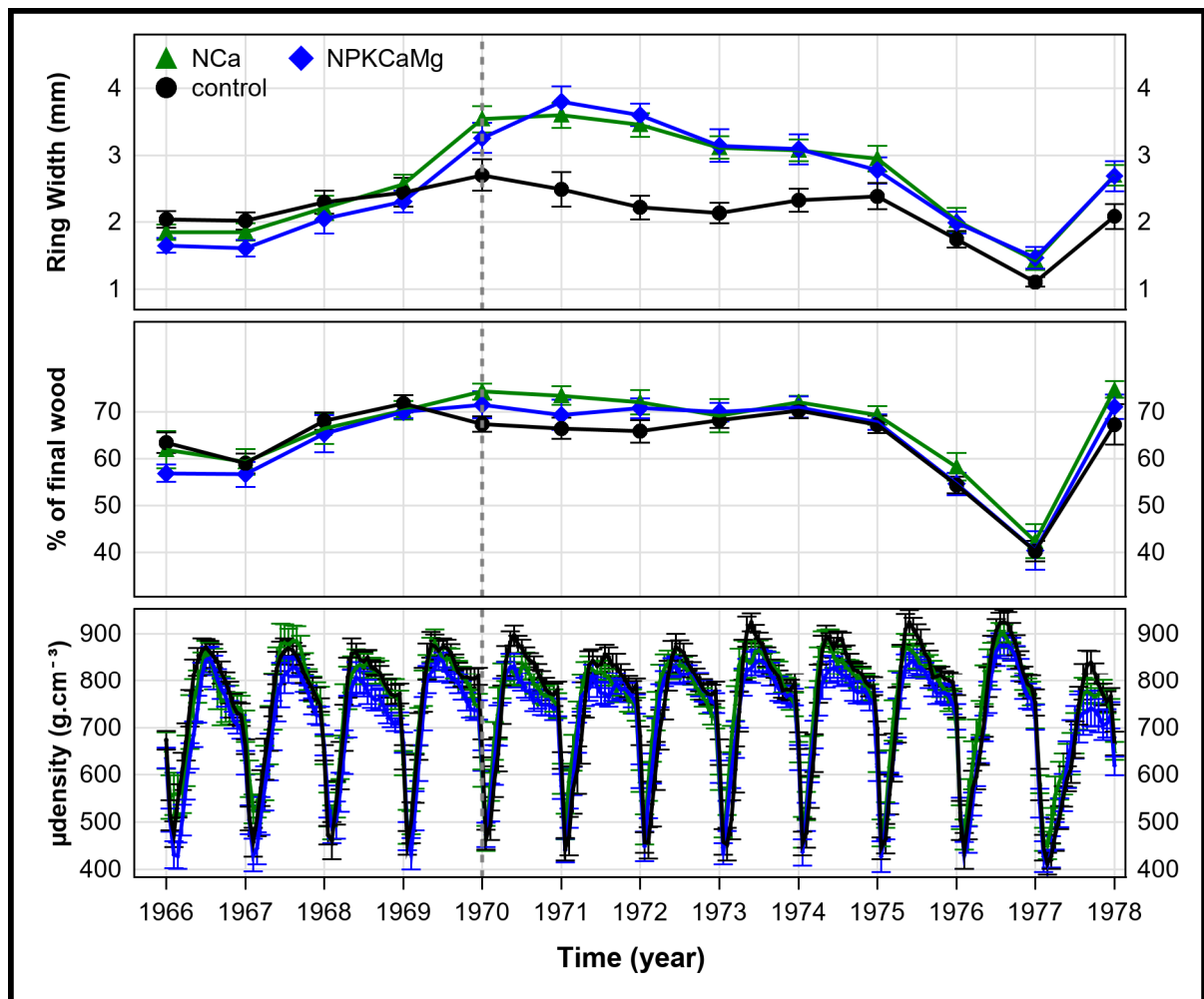|	N	Mg	P	K	Ca	Mn
N	0.22 ***	0.63 ***	0.33 ***	0.22 ***	0.20 ***	ns
Mg	0.81 ***	0.16 ***	0.30 ***	0.25 ***	0.28 ***	0.18 ***
P	0.58 ***	0.54 ***	n.s.	ns	0.10 *	ns
K	0.18 ***	0.25 ***	0.28 ***	0.09 *	0.51 ***	0.34 ***
Ca	0.10 *	0.16 ***	0.20 ***	0.40 ***	NS	0.31 ***
Mn	ns	0.13 *	0.12 **	0.29 ***	0.81 ***	0.09 *

Figure S1: Ring width, percentage of latewood and ring density (upper, middle and lower panel, respectively) for the years 1966 to 1978. Percentage of latewood was assessed by microdensitometry measurements. Error bars are standard errors of the mean. The vertical dashed gray line show the fertilization event.
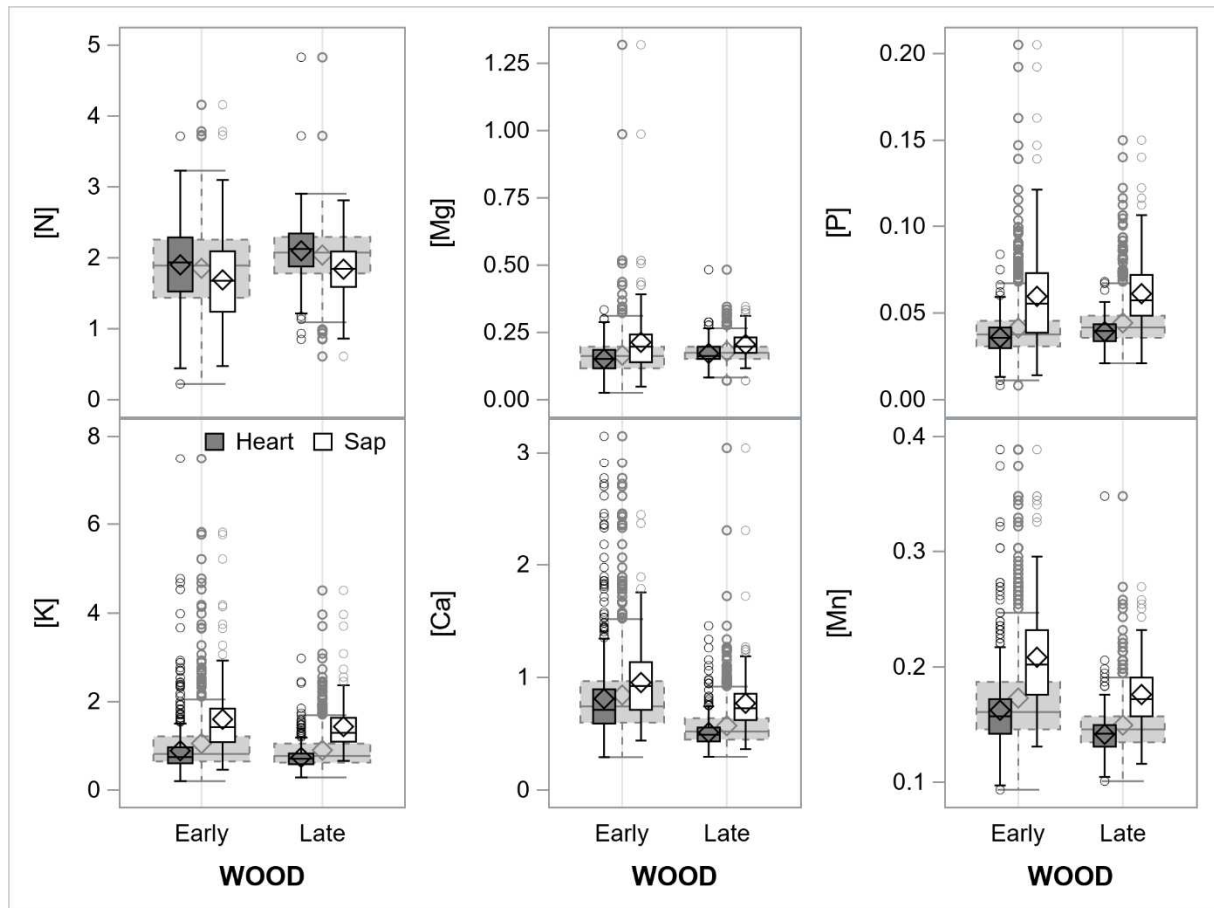
Figure S2: Distribution of the elements in the earlywood and latewood of the heartwood (dark grey) and sapwood (white) of control trees. Units are counts.s^{-1}.nA^{-1}. The boxplots display the mean (diamond), median, first and third quartiles, lower and upper fences (± 1.5 interquartile range) and extremes values (dots). The dashed boxplots (light grey) show the distribution when measurements performed in the heartwood and sapwood are pooled together. The K, Ca and Mn contents appeared significantly higher in the earlywood than in the latewood by 14, 32 and 12%, respectively (P<0.0001, N= 1508). On the opposite, the N, Mg and P were significantly more abundant in the latewood than in the earlywood by 11, 13 and 7%, respectively (P<0.0001, N= 1508).
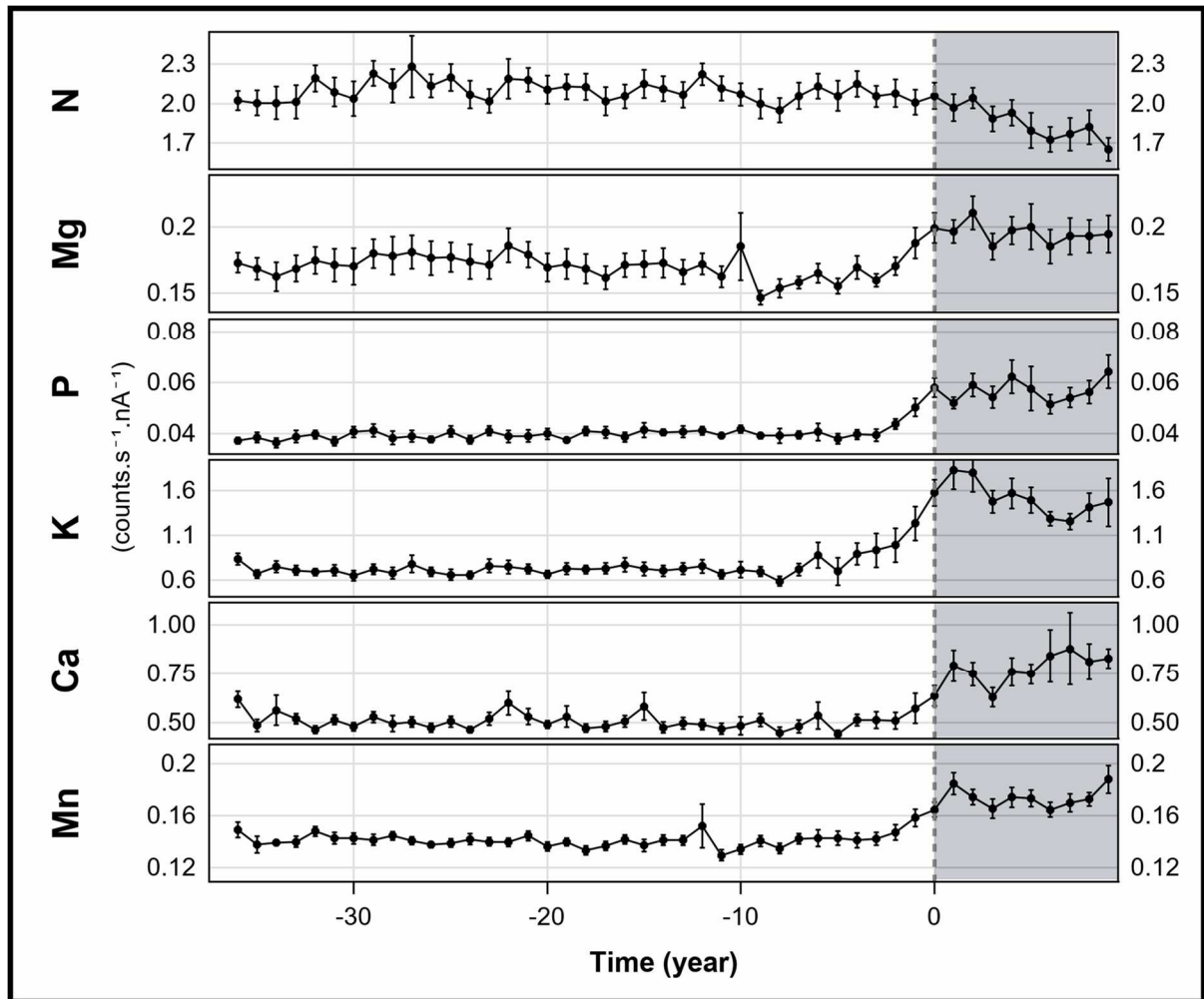
Figure S3: Mean elemental composition (in counts.s^{-1}.nA^{-1}) in the latewood of the tree rings of the control trees according to the position of the heartwood/sapwood transition (vertical dashed line). Error bars are standard errors of the mean. Within the heartwood, significant decreasing contents outwards (*i.e.* from 1950 to 1988) were observed for Mg, Ca and Mn on the average values of the control trees (Figure 2). When tested for each tree separately, it appeared that these trends were significant for only four or five of the 13 control trees. These trends were more pronounced in the earlywood (F=30.92, P<0.0001; F=16.73, P=0.0002; F=32.45, P<0.0001; for Mg, Ca and Mn contents, respectively; N=39) than in the latewood (F=16.55, P=0.0002; F=10.14, P=0.0029; F=7.92, P=0.0078; for Mg, Ca and Mn contents, respectively; N=39).