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## Science of the Total Environment

journal homepage: [www.elsevier.com/locate/scitotenv](http://www.elsevier.com/locate/scitotenv)Traffic pollution affects tree-ring width and isotopic composition of *Pinus pinea*Giovanna Battipaglia<sup>a,b,\*</sup>, Fabio Marzaioli<sup>b</sup>, Carmine Lubritto<sup>b</sup>, Simona Altieri<sup>b</sup>, Sandro Strumia<sup>b</sup>, Paolo Cherubini<sup>a</sup>, M. Francesca Cotrufo<sup>b,c</sup><sup>a</sup> WSL Swiss Federal Institute for Forest, Snow and Landscape Research, CH-8903 Birmensdorf, Switzerland<sup>b</sup> Department of Environmental Sciences, Second University of Naples, Via Vivaldi 43, I-81100 Caserta, Italy<sup>c</sup> Department of Soil and Crop Sciences, Colorado State University, CO, USA

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## ABSTRACT

This study presents new evidence that radiocarbon, combined with dendrochronological and stable isotopes analysis in tree rings and needles, can help to better understand the influence of pollution on trees. *Pinus pinea* individuals, adjacent to main roads in the urban area of Caserta (South Italy) and exposed to large amounts of traffic exhaust since 1980, were sampled and the time-related trend in the growth residuals was estimated. We found a consistent decrease in the ring width starting from 1980, with a slight increase in  $\delta^{13}\text{C}$  value, which was considered to be a consequence of environmental stress. No clear pattern was identified in  $\delta^{15}\text{N}$ , while an increasing effect of the fossil fuel dilution on the atmospheric bomb-enriched  $^{14}\text{C}$  background was detected in tree rings, possibly as a consequence of the increase in traffic exhausts. Our findings suggested that radiocarbon is a very sensitive tool to investigate small-scale (i.e. traffic exhaust at the level crossing) and large-scale (urban area pollution) induced disturbances.

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## 1. Introduction

Urbanization and industrialization are rapidly growing, and as a consequence roads and their associated vehicular traffic exert major and increasing impacts on adjacent ecosystems (Angold 1997). The effects of a road on adjacent ecosystems are complex and include disturbances during road construction and deterioration of air quality caused by the traffic of the established road. Various studies have shown the impact of vehicle exhausts on road side vegetation through their visible and non-visible effects (Farmer and Lyon 1977, Angold 1997, Nuhoglu, 2005) but, presently little known is about the long-term effects of air pollution on vegetation and on trees in particular. Developing proxies for atmospheric pollution that would be used to identify the physiological responses of trees to roadside car exhaust pollution stress are needed.

In this context, tree rings offer a useful tool to decipher historical changes in the atmospheric environment. Trees in temperate and boreal forests form a new wood-growth layer every year (annual ring). The physical and chemical characteristics of the wood cell formed in each particular year reflect the environmental conditions in which the tree grew in that year, and can be used to reconstruct past environmental conditions (including climatic conditions and air quality). In addition, stable isotope signals in the annual rings of trees are useful proxies for

reconstructing past climates and tree ecophysiology (McCarroll and Loader, 2004; Switsur and Waterhouse 1998). Stable carbon isotopes in tree rings have often been used as long-term and sensitive indicators of tree carbon uptake limitations (Panek and Waring 1997) and water use efficiency (Leffler and Evans 1999). Some environmental factors, such as atmospheric pollutants (e.g. increase of  $\text{SO}_2$  or  $\text{NO}_x$ ) can alter  $\delta^{13}\text{C}$  signals by influencing carbon discrimination (Niemelä et al., 1997; Rennenberg and Gessler, 1999). Thus, the analysis of  $^{13}\text{C}$  has the potential to reveal the effects of pollutants exposure, showing a possible stimulation of photosynthesis (N-fertilization) and/or an inhibition of stomatal conductance (Siegwolf et al., 2001).

The  $\delta^{15}\text{N}$  ratio can provide a historical record of N deposition, since the nitrogen isotope ratio in compounds produced from anthropogenic activities may be significantly different from the natural background N in the soil (Freyer, 1991).

To date, a limited number of studies attempted to assess the relationships between car exhausts, tree growth and isotopic composition of tree rings in order to reconstruct past atmospheric pollution history (Poulson et al., 1995; Savard et al., 2008; Saurer et al., 2004; Wagner and Wagner, 2006; Guerrieri et al., 2009; Kwar et al., 2009). Those studies rarely considered other substrates (such as soil and foliage) and selectively measured certain isotopes, rather than combining all the potential indicators.

We propose a novel method to determine the effects of car exhaust pollution on tree growth, coupling classical dendrochronological analyses and analyses of  $^{15}\text{N}$  and  $^{13}\text{C}$  in tree rings, soils and leaves with tree-ring radiocarbon ( $^{14}\text{C}$ ) data. The  $^{14}\text{CO}_2$  atmospheric content

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is characterized by a peak around the early 1960s when  $^{14}\text{C}$  activity doubled in the northern hemisphere, due to the tropospheric tests of nuclear weapons in the late 1950s. This was followed by an approximately exponential decrease (bomb spike signal; Burchuladze et al., 1989), caused by natural  $\text{CO}_2$  atmospheric exchange fluxes with terrestrial ecosystems and oceans. Additionally, the combustion of fossil fuel contributes  $^{14}\text{C}$ -dead  $\text{CO}_2$  to the atmosphere, thus further diluting the atmospheric  $^{14}\text{C}$  background. The  $^{14}\text{C}$  content of  $\text{CO}_2$  arising from petroleum use is equal to zero because of the age ( $>>50\text{ka}$ ) of the reservoir where the fossil fuel originates. This effect, known as the Suess effect is clearly evident during the years prior to the bomb peak, when the atmospheric  $^{14}\text{CO}_2/^{12}\text{CO}_2$  signal decreased below the standard value (1890 atmospheric 14/12 ratio) (Suess, 1955). Local emissions (i.e. on regional to local scales) can be estimated by comparing the measured local  $^{14}\text{CO}_2/^{12}\text{CO}_2$  ratio with regional background data (Levin et al., 1989). Trees preserve the local radiocarbon signature over their life spans in their annual rings. This makes it possible to use tree rings to monitor changes in the rate of  $\text{CO}_2$  emissions from fossil fuel combustion.

The scope of this research was to evaluate the novel method proposed above. To achieve this, we analyzed width, stable isotopes and radiocarbon content of annual rings from *Pinus pinea* L. trees growing along a heavily polluted road built in 1980. Since then, these trees have been exposed to a large amount of pollutants emitted by cars and trucks. This road presents a railway crossing, which makes an ideal research site because here vehicles are forced to stop close to the trees for a relatively long time when trains cross. Based on the potential relationships between traffic pollution, trees growth and isotopic compositions of tree samples, it was hypothesized that trees, collected from a polluted area, may exhibit a decrease in tree-ring width and a change in the isotopic composition, after the construction of a road.

The results of this research will provide further insights into the interplay between environmental condition and physiological processes, and thus, will improve our understanding of pollution effects on trees.

## 2. Materials and methods

### 2.1. Study site

The study was conducted along a heavily polluted road in the city of Caserta, Southern Italy ( $41^\circ 4' \text{N}$ ,  $14^\circ 20' \text{E}$ , 68 m.a.s.l.). The study site, the level crossing and the position of the seven sampled trees are shown in Fig. 1. All the trees were about 70 to 80 years old, while the street and the level crossing were completed in 1980. Since the 1980s there has been a progressive increase (64%) in the level of traffic in the area (ACI, 2006), especially around 1990–1995 due to the construction of new buildings. A comparison of three topographic maps of the area in 1981, 1987 and 1998 (see supplemental Fig. S1) shows a marked spread in human settlement and a consequent increase in traffic exhausts during this period. In the recent past the urban traffic management of Caserta city has been strongly regulated by a local government with a decrease of the total numbers of vehicles into the urban area (ACI, 2006) and, consequently, along the sampling site. Air quality measurements of the urban area, monitored by ARPAC (Regional Agency for Environmental Protection of Campania), are scarce, limited to the period 1995–2004 and related to the  $\text{NO}_x$  emissions. The five different monitoring stations located close to the sampling site (15 km away) revealed a consistent increase of  $\text{NO}_x$  emissions with time, from  $29.8 \pm 4.4 \text{ mg/m}^3 \text{ N}$  recorded in 1995 to  $58 \pm 6.2 \text{ mg/m}^3 \text{ N}$  in 2004.

### 2.2. Meteorological data

Monthly mean precipitation data for the study period (1915–2005) were obtained from the meteorological station at Caserta, 5 km from the sampling site.

Missing monthly data were obtained from other meteorological stations situated nearby (within 30 km). When meteorological data were not available, they were estimated (only 2% of missing dataset) using a gap-filling program where the missing value was set equal to the average of the previous and the following month.

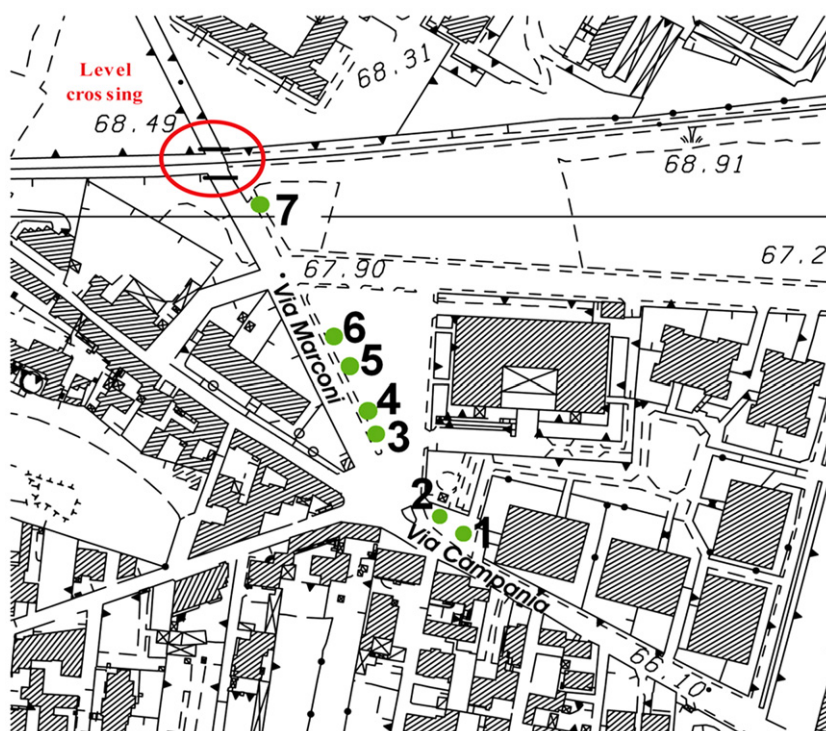


Fig. 1. Map of the site (1:25,000-scale) showing the seven sampled trees and the location of the level crossing.

Meteorological data (not shown) were used for the regression analyses as described below.

### 2.3. Wood samples

Three cores were taken from each individual with a 5 mm diameter borer (Suunto, Finland). The cores were carefully dated by counting the rings from bark to pith and the ring widths were measured using LINTAB measurement equipment (Frank Rinn, Heidelberg, Germany). The early and latewood were separated in all the cores and the latewood of different radii was pooled for each year, resulting in a single wood sample per year. Dried samples were ground (<0.1 mm) in a centrifugal mill and then each sample was split into two parts. From the first part we removed extractable mobile N-compounds according to the method described in Sheppard and Thompson (2000). Cores were first washed in a Soxhlett-apparatus for 18 h in a 50:50 solution of toluene and ethanol, then for 18 h in ethanol, and finally for 18 h in distilled water. From the second part, cellulose was extracted according to the method described in Loader et al. (1997), based on a double-step digestion and modified according to Battipaglia et al. (2008). We performed an extraction of resin, fatty acids, ethereal oils and hemicellulose with a solution of 5% NaOH for 2 h at 60 °C, repeating the operation twice. In the second step, lignin was extracted with a 7% NaClO<sub>2</sub> solution for a minimum of 36 h at 60 °C. Because the solution only reacts for 10 h, it was changed daily and refilled as necessary. This step was repeated until the sample was “white”, which is a characteristic that can be determined by experience or by comparison with commercial cellulose. Samples were washed three to four times with boiling distilled water (until pH = 7 ± 1) and dried overnight at 50 °C. Finally, the samples were weighed in tin cups to perform isotopic analyses.

### 2.4. Soil and needle samples

Samples of needles were taken from each tree at the end of vegetation season in 2005 to analyze needle material that had been exposed to NO<sub>2</sub> emissions for a long time. In the planters where the trees are growing, soil samples were collected at a depth of 10 cm using a soil corer. Five samples per planter were taken and pooled together before drying in the lab. The pH was measured and the carbonates were removed with acid fumigation (Harris et al., 2001) prior to δ<sup>13</sup>C and δ<sup>15</sup>N analysis.

### 2.5. Isotope measurements

Carbon and nitrogen concentrations and their stable isotopes were measured by combustion in an elemental-analyser (Carlo Erba 1112 Flash EA) connected in continuous-flow mode to an isotope ratio mass-spectrometer (MS-Delta-Plus, Finnigan, Bremen, Germany).

The δ<sup>13</sup>C values were recorded in units (‰) relative to V-PDB and calibrated using a laboratory graphite standard (δ<sup>13</sup>C = −26.65 ± 0.33‰) and a laboratory α-cellulose standard (δ<sup>13</sup>C = −24.65 ± 0.20‰). The δ<sup>15</sup>N values, which were recorded in units (‰) relative to air (δ<sup>15</sup>N = 0‰), were calibrated using IAEA N1 (δ<sup>15</sup>N = 0.40 ± 0.20‰) and a laboratory vegetal standard (VR2, δ<sup>15</sup>N = −0.06 ± 0.06‰). The relative precision of the repeated analysis was ± 0.1‰ for δ<sup>13</sup>C and ± 0.5‰ for δ<sup>15</sup>N.

Due to the low N-concentration of the wood samples (0.06% to 0.08%), the usual measurement procedure for organic materials had to be modified. After testing the linearity of mass spectrometry in the range below 1 V, it was necessary to prevent incomplete sample combustion. Thus, the oxygen injection was enhanced, the carrier flow rate was reduced, the acquisition time was increased, a blank was processed after each sample and the column was replaced after 30 samples.

The wood samples to be measured for radiocarbon (<sup>14</sup>C) abundance were further treated for graphite target production. In detail, cellulose was combusted (6 h at 920 °C) in a sealed quartz tube, and the resulting CO<sub>2</sub> was cryogenically purified from other combustion gases (mainly water) and graphitized according to Passariello et al. (2007). Graphite samples were pounded into holed Al cathodes and mounted on a wheel with 40 samples, together with the normalising and control standards and the radiocarbon-processed blanks. <sup>14</sup>C was measured using a 3 MV National Electrostatic Corporation pelletron accelerator system (Terrasi, 2001). The results are reported in Δ<sup>14</sup>C notation, corrected for isotopic fractionation based on the measured or estimated δ<sup>13</sup>C values, and radioactive decay since the year of growth (Stuiver and Polach 1977). The following equation was used:

$$\Delta^{14}\text{C} = \left( \frac{A_{\text{SN}} e^{-\lambda(1950-x)}}{A_{\text{ON}}} - 1 \right) * 1000$$

The <sup>14</sup>C/<sup>12</sup>C activities (A) of the sample (A<sub>S</sub>) that were measured and the standard (A<sub>O</sub>) were normalised (A<sub>SN</sub>, A<sub>ON</sub>) for the observed isotopic fractionation to conventional values of −25‰ and −19‰ respectively. A<sub>SN</sub>, A<sub>ON</sub> were, finally, scaled to the sampling date (x; the date when the tree ring was measured) and to 1950 respectively.

### 2.6. C isotope data analysis

With respect to <sup>13</sup>C, we used the data provided in Francey et al. (1999) and McCarroll and Loader (2004) to remove the atmospheric δ<sup>13</sup>C trend from the carbon isotope data series. The corrected series were used for all statistical analyses.

It was assumed that tree rings preserve unvaried the mean annual atmospheric radiocarbon signature of the CO<sub>2</sub> used for their net primary production. The approach used by Saurer et al. (2003), which combined tree ring and isotopic methodologies, could then be adopted to reconstruct the isotopic content of past atmospheric composition. With annually resolved tree rings, it is possible to quantify the fraction of CO<sub>2</sub> derived from a fossil source (in this case traffic exhausts, see Marzaioli et al. 2005), applying a double source mass balance approach to the <sup>14</sup>C tree-ring (Δ<sup>14</sup>C<sub>meas</sub>) values measured:

$$\begin{cases} \Delta^{14}\text{C}_{\text{meas}} = \Delta^{14}\text{C}_{\text{atm}} * \chi_{\text{atm}} + \Delta^{14}\text{C}_{\text{fos}} * \chi_{\text{fos}} \\ \chi_{\text{atm}} + \chi_{\text{fos}} = 1 \end{cases} \quad (1)$$

Briefly this approach assumes that the atmospheric CO<sub>2</sub> reservoir “sampled” by the tree comes from mixing gases from two sources, the natural atmosphere (Δ<sup>14</sup>C<sub>atm</sub>) and the fossil CO<sub>2</sub> (Δ<sup>14</sup>C<sub>fos</sub>) released in the traffic exhausts with their characteristic Δ<sup>14</sup>C and relative contributions (χ<sub>atm</sub> = molar fraction from atmosphere; χ<sub>fos</sub> = molar fraction from fossil source).

This approach leads to:

$$\chi_{\text{fos}} = \frac{\Delta^{14}\text{C}_{\text{meas}} - \Delta^{14}\text{C}_{\text{atm}}}{\Delta^{14}\text{C}_{\text{fos}} - \Delta^{14}\text{C}_{\text{atm}}} \quad (2)$$

According to Eq. (2), the mean annual fossil fuel dilution can then be measured by determining the <sup>14</sup>C abundance of the tree ring, estimating the natural atmospheric background (Δ<sup>14</sup>C<sub>atm</sub>) from measurements (Nydal and Lövseth, 1983) and assuming Δ<sup>14</sup>C<sub>fos</sub> is equal to −1000.

### 2.7. Data analysis

The single-core ring width chronologies were cross-dated using the *Gleichläufigkeit*, a statistical measure of the year-to-year agreement between the interval trends of the chronologies based upon the sign of

agreement (Kaennel and Schweingruber, 1995). A Student *t*-test was also applied to determine the degree of correlation between curves. Series were detrended to remove long-term growth trends embedded in the raw tree-ring series, which were thought to be induced by non-climatic influences, such as aging and competition between trees (Fritts, 1976). Tree-ring indices were calculated as residuals from the estimated age-trend. Data were checked for normality and homogeneous variance before applying inferential statistics. The new dataset was used for all the statistical analyses. Correlation and regression analysis were carried out with the program STATVIEW (SAS Institute Inc., Cary, NC, USA) and SIGMASTAT (Systat Software, CA, USA), and the correlation between the climate and the raw data was assessed using the Pearson product moment correlation coefficient. In order to identify patterns in data, and express the data in such a way as to highlight their similarities and differences, a Principal Component Analysis (PCA; Cook and Kairiukstis, 1990) was applied using the package SYN-TAX 2000 (Podani, 2001). For each tree we pooled together data collected in 5 periods of 10 years in order to have 5 analyzed periods (*a* = 1955–1964; *b* = 1965–1974; *c* = 1975–1984; *d* = 1985–1994; *e* = 1995–2005). Finally a data matrix of 5 period per each tree (35 periods in total, indicated with progressive numbers, 5 for each tree) and three variables (ring width,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measured for each tree) was created and the data of tree nos. 1–3–4–6–7 were processed with PCA as the ordination method for indirect gradient analysis (Podani, 2000). Table 1 shows, for each individual tree and for each pooled period, the values of width,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  used as raw matrix for the statistical analysis.

**Table 1**

Tree-ring data from the study, which are pooled into five 10 year-periods (*a* = 1955–1964; *b* = 1965–1974; *c* = 1975–1984; *d* = 1985–1994; *e* = 1995–2005).

Trees	Periods	Ring widths (1/100 mm)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
1	1a	694.75	-25.47	8.36
1	2b	630.61	-25.28	9.10
1	3c	712.89	-25.20	9.77
1	4d	495.00	-23.35	10.53
1	5e	416.73	-24.94	9.18
2	6a	503.90	-24.06	35.87
2	7b	637.44	-25.09	29.13
2	8c	912.00	-25.52	29.54
2	9d	707.00	-25.94	29.77
2	10e	554.83	-24.97	27.39
3	11a	368.15	-26.13	5.35
3	12b	536.78	-25.81	7.65
3	13c	751.39	-25.24	14.19
3	14d	295.44	-24.91	13.74
3	15e	299.83	-23.94	12.96
4	16a	487.20	-23.55	5.73
4	17b	594.94	-24.97	4.51
4	18c	626.28	-26.62	4.85
4	19d	383.11	-25.91	4.82
4	20e	178.35	-23.22	6.07
5	21a	349.55	-25.15	27.65
5	22b	485.78	-25.25	26.26
5	23c	711.44	-25.13	28.73
5	24d	414.67	-24.10	25.43
5	25e	253.41	-22.73	32.85
6	26a	750.20	-24.02	7.80
6	27b	541.28	-24.06	6.52
6	28c	691.22	-24.67	5.26
6	29d	401.33	-24.76	4.36
6	30e	166.99	-24.75	3.17
7	31a	977.10	-23.48	4.79
7	32b	735.94	-22.79	5.53
7	33c	628.65	-23.82	5.22
7	34d	129.67	-23.80	5.31
7	35e	348.53	-23.37	4.18

A number from 1 to 7 indicates each tree, while the 10 year-periods are in total 35, 5 per tree. The periods are indicated with progressive numbers and the correspondent years with a letter from a to e. For each period, the average values of the ring widths,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are given.

### 3. Results

Annual raw tree-ring width values for the seven trees located along the road studied were plotted versus time in Fig. 2a. Due to the high variability in tree-ring widths between trees, there was not a significant ( $P > 0.05$ ) difference between trees in tree growth during the period studied. Tree number (no.) 7 showed an abrupt growth decline that started in 1980, after the construction of the road. We believe this was due to external physical factors or root damage, which were independent of the traffic and which may have had a long-term effect. In Fig. 2b the mean chronology of raw data and the mean standard chronology with standard errors (SE) are shown. Tree no. 7 was excluded. Despite being non-significant, a trend towards lower mean growth rates was observed during the 1980s.

Tree-ring carbon isotope values were not significantly different (analysis of variance  $F = 2.70$ ,  $P = 0.80$ ) among trees and ranged between  $-22\%$  and  $-28\%$ , showing a considerable variation between years (with a coefficient of variance of 3%, Fig. 3, grey line). The years were reported accordingly with the periods described before.

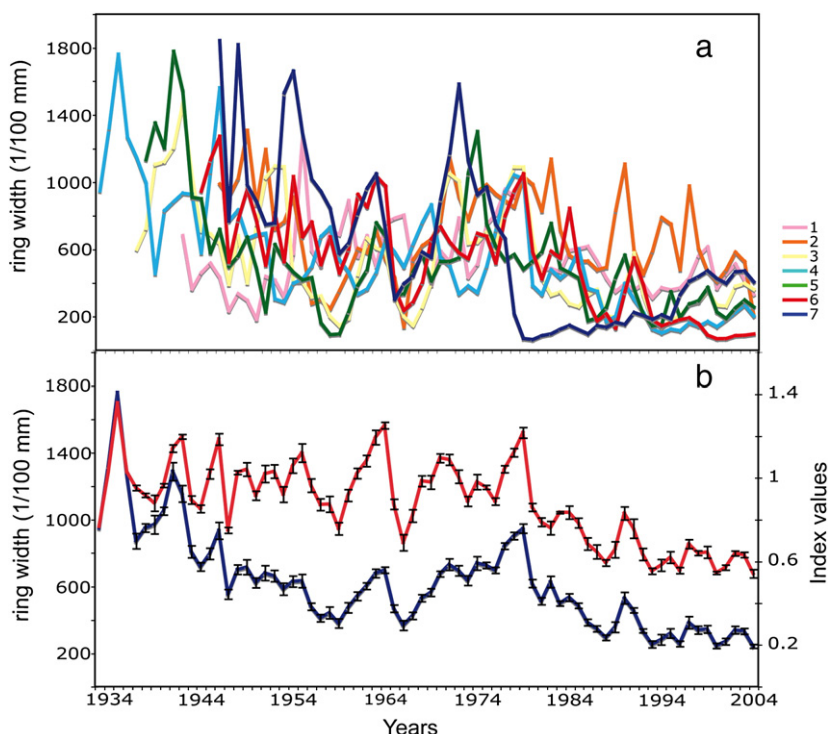
To identify the effects of the traffic on tree growth, we divided the dataset into two periods, prior to building the road (1955–1979) and after building the road (1980–2005). We also excluded the individual no. 7 from the analysis because of its anomalous growth behaviour. The mean  $\delta^{13}\text{C}$  values of the six trees are significantly correlated with the mean standard tree-ring growth during 1955–1979 ( $r = 0.62$ ,  $P \leq 0.01$ ) and with the mean annual precipitation and temperature ( $r = 0.78$ ,  $P < 0.001$ ;  $r = 0.52$ ,  $P \leq 0.01$ , respectively). After 1980,  $\delta^{13}\text{C}$  values increased while standard tree-ring width slightly declined. These two variables correlated only slightly during this period ( $r = 0.32$ ,  $P \leq 0.05$ ). After 1980,  $\delta^{13}\text{C}$  values correlated with precipitation ( $r = 0.43$ ,  $P \leq 0.05$ ) but no other climatic parameters.

The values of  $\delta^{15}\text{N}$  in tree nos. 1, 3, 4, 6 ranged from 2.6 to 15‰, with an increase in variability in the recent decades. Surprisingly, tree nos. 2 and 5 had  $\delta^{15}\text{N}$  values outside the natural abundance range for  $^{15}\text{N}$ , ranging between 25‰ and 37.2‰. They, as discussed below, were considered as outliers and excluded from further statistical analysis. The  $\delta^{15}\text{N}$  values of the mean chronology (except tree nos. 2, and 5) were plotted versus time (Fig. 3, black line).

The  $\delta^{15}\text{N}$  of each tree was compared with the *P. pinea* needle and soil values measured at the same site in 2005. The  $\delta^{15}\text{N}$  tree-ring values were consistent with the  $\delta^{15}\text{N}$  values measured in the soil ( $10.5 \pm 1.9\%$ ,  $n = 7$ ), but not with those in the needles ( $0.7 \pm 0.2\%$ ,  $n = 7$ ).

Fig. 4 shows the ordination diagram (I and II components) resulting from PCA applied to  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and ring widths values for the selected trees, grouped in five periods (*a* = 1955–1964; *b* = 1965–1974; *c* = 1975–1984; *d* = 1985–1994; *e* = 1995–2005). The biplot was used so both objects (grouped tree rings) and variables (vectors) were represented in the same best plane, defined by the first two components, therefore explaining the maximum variance accounted for in the data. The first and second components accounted for 42.55% and 31.26% of the total variance, respectively. From the graph it was possible to observe a clear temporal pattern with a difference between the period 1955–1979 and the period 1980–2005 mostly due to differences in the  $\delta^{13}\text{C}$  and ring-width vectors (the closer the vector is to a year, the larger is its influence in that year). The fact that  $\delta^{15}\text{N}$  variable vector was perpendicular to the other vectors showed a lack of correlation with the other variables.

Fig. 5a shows the mean time trend in  $\delta^{14}\text{C}$  recorded in the tree rings of tree no. 6, compared to an atmospheric background curve for the observed period. This latter curve was obtained from the best fit (double exponential) annual curve for the atmospheric  $^{14}\text{C}$  database of Hua and Barbetti (2004) and Levin et al. (2008) for the period of the bomb spike (1955 to 2003) and the INTCAL04 (Reimer et al., 2004) for the 1941–1954 period. Our results indicated that the measured points



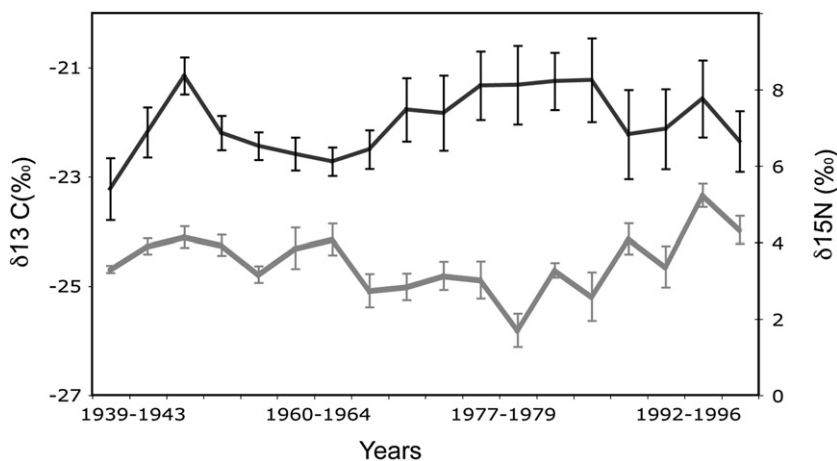
**Fig. 2.** (a) Individual ring-width chronologies for the seven *Pinus pinea* L. trees, plotted against calendar. Different colours indicate different individuals: pink: tree no.1; orange: tree no. 2; yellow: tree no. 3; light blue: tree no. 4; green: tree no. 5; red: tree no. 6; blue: tree no. 7. (b) Average ring width chronology for the trees using either the raw data (blue) or data after detrending (red). Tree no 7 was excluded.

generally corresponded well with the mean annual radiocarbon values of the background atmosphere prior to the 1980s. The effect of the fossil source  $^{14}\text{C}$  dilution on the atmospheric bomb-enrichment background was clearly evidenced by the significant depletion in the tree rings'  $^{14}\text{C}$  values with respect to the atmosphere after 1985. The mean annual  $\text{CO}_2$  fraction derived from the fossil source ( $\chi_{\text{fos}}$ ) was determined and showed clearly how the traffic fossil effect peaked in the 1990s and declined in the following years back to previously observed values (Fig. 5b).

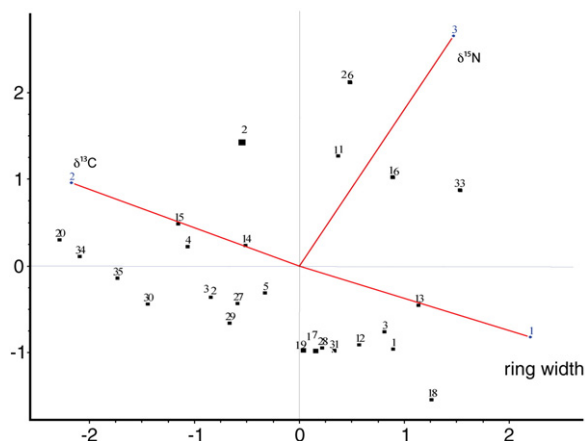
#### 4. Discussion

In this paper we investigated the influence of traffic emission on roadside vegetation, using a novel combination of different methods of tree-ring analysis. The stable isotopes and ring-width results showed

how the trees responded in terms of physiology and growth, while the  $\delta^{14}\text{C}$  was used to derive incorporation of fossil carbon and therefore gave a measure of the amount of anthropogenic influence. The relationships between air pollution, tree growth and isotopic composition of different trees species have been previously investigated: e.g. between steel plants vs  $\delta^{13}\text{C}$  of subfossil oak wood (Jedrysek et al., 2003),  $\text{SO}_2$  emissions vs  $\delta^{13}\text{C}$  of *Abies firma* and *Picea* species (Sakata and Suzuki, 2000; Savard et al., 2008), local environment vs  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of *Quercus* species and *Pinus densiflora* (Bukata and Kyser, 2007; Kwar et al., 2009), traffic exhausts vs  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  of *Pinus sylvestris* (Wagner and Wagner, 2006),  $\text{NO}_x$  emissions and  $\delta^{15}\text{N}$  of *Tsuga canadensis* (Poulson et al., 1995), of *Pinus ponderosa* (Hart and Classen, 2003), of *Fagus sylvatica* (Ehlani et al., 2003), of *Picea abies* (Saurer et al., 2004) of *Quercus cerris* and *P. abies* (Guerrieri et al., 2009), of *Fagus grandifolia* and *Pinus strobus* (Savard et al., 2009) and between variations of  $^{14}\text{C}$

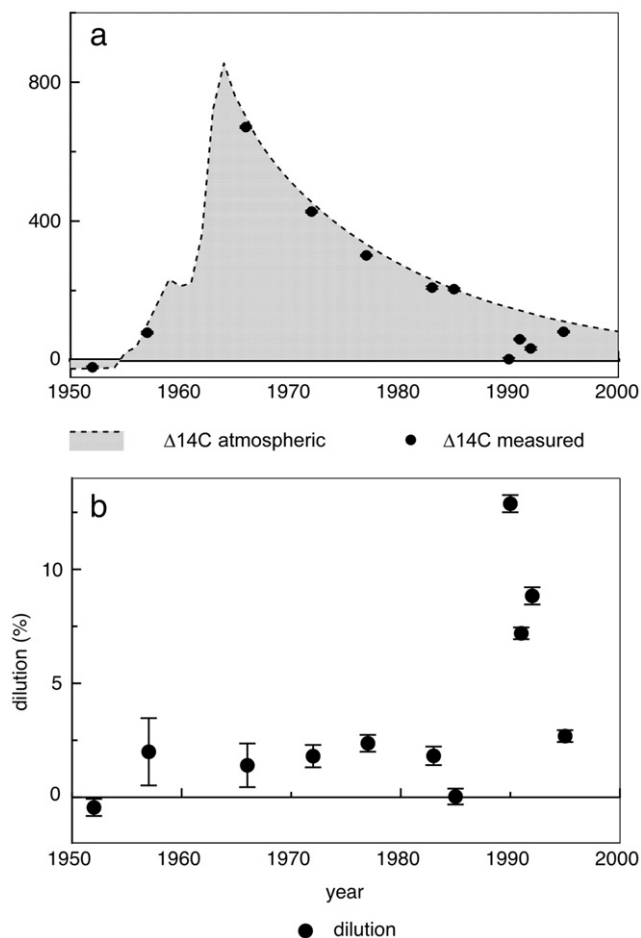


**Fig. 3.** Average  $\delta^{13}\text{C}$  values  $\pm$  standard error (SE) of the tree rings (grey line) and average  $\delta^{15}\text{N}$  values  $\pm$  standard error (SE) of the tree rings (black line).



**Fig. 4.** Biplot diagram resulting from Principal Component Analysis of a sample plot in the *Pinus pinea* L. community. The biplot in the first and second component planes, obtained with PCA applied to the five calculated periods (see Table 1) for each tree, is reported with either the periods (squares) or the parameters (vectors) in which they have been measured. The first and second components accounted for 42.55% and 31.26% of the total amount of variation, respectively. Vector 1 = ring width. Vector 2 =  $\delta^{13}\text{C}$ . Vector 3 =  $\delta^{15}\text{N}$ .

concentration of atmospheric  $\text{CO}_2$  and radiocarbon concentration in *P. densiflora* tree rings (Rakowski et al., 2008). However, those proxies were seldom combined together and tested to reconstruct the effect of traffic exhausts on tree growth.



**Fig. 5.** a. Atmospheric  $\Delta^{14}\text{C}$  time series from  $^{14}\text{C}$  in tree rings, compared with the annual average atmospheric  $\Delta^{14}\text{C}$  (Nydal and Lövseth); b. Dilution in percent of  $\Delta^{14}\text{C}$  due to the local effect of fossil fuel  $\text{CO}_2$  source.

In our study, tree rings  $\delta^{13}\text{C}$  values were measured along with  $\delta^{15}\text{N}$  primarily to determine whether the isotopic signals of these two elements covary over time, indicating a possible coupling between tree physiological condition and N availability. In previous studies (Poulson et al., 1995; Hart and Classen, 2003), the two isotopes have been found to correlate sporadically among trees at a given site, while in our data the correlations were very low and not significant throughout the whole period considered. There was only a slight increase in  $\delta^{13}\text{C}$  after 1980, which could be due to external variation and which would be consistent with the theory proposed by Farquhar et al. (1982) and Ehleringer and Cooper (1988). Not only  $\text{NO}_x$ , but also  $\text{SO}_x$ , both emitted with  $\text{CO}_2$  during fossil fuel combustion, were demonstrated to reduce carbon isotope discrimination via either an increase of carboxylation or a decrease of stomatal activity (Rennenberg and Gessler, 1999). In addition, Siegwolf et al. (2001) observed an increase in  $\delta^{13}\text{C}$  under  $\text{NO}_2$ -exposure, possibly due to an increase in PEP carboxylase activity, triggered by  $\text{NO}_2$ . Our data seem to verify this hypothesis; the general trends we found suggest that changes in  $\delta^{13}\text{C}$  values were representative of the inherent physiological tree responses to external factors even if it is not easy to distinguish between climatic or pollution factors. On the other hand, the reduction of correlation between growth and climate post 1980 and the results from the PCA supported the idea that external factors linked to the road affect carbon isotope discrimination, leading to a less negative  $\delta^{13}\text{C}$ , in trees at the study site after 1980.

The results from the  $\delta^{15}\text{N}$  analysis were extremely problematic and difficult to interpret. In fact, tree-ring  $\delta^{15}\text{N}$  values varied significantly in different trees and over time, with five of the seven analyzed trees, showing less variability before 1980, with values around  $6.8 \pm 2.5\%$ , and an increase in the variability after 1980. Two trees presented very high value of  $\delta^{15}\text{N}$ , outside the range of reported values in the literature for organic material and far higher than the measured soils and needle values, which would imply an enormous isotope fractionation. The limited amount of wood material prevented the re-measurement of samples and being able to distinguish between methodological or operative problems and ecophysiological explanations. Our best explanation for the differences found with the other trees growing in the same area could be internal N cycling in the trees and/or an external N source. The sampled trees grow along the same road but they are separated in different planter areas by a fence. Trees can uptake from N different sources, from internal reserves or external output, depending on local N availability and climatic conditions. The sampled trees have been influenced by various anthropogenic disturbances, over past decades, as well as increasing human impacts, e.g. scars caused by car accidents, deposition of domestic animals and different tree management practices, e.g. pruning. Moreover the anthropogenic fractionation of N isotopes varies depending on heat of combustion and the  $\delta^{15}\text{N}$  values could be either negative or positive depending on whether a car was idling or at high speed during the exposed period (Heaton, 1990). Therefore, caution is required when  $\delta^{15}\text{N}$  is correlated with atmospheric pollution and further analyses are clearly warranted.

This is also confirmed by the lack of any relationship between  $\delta^{15}\text{N}$  in tree rings and  $\delta^{15}\text{N}$  in the soil and needles that may, especially in some of the trees, be related to the time at which the parameters were measured. The wood  $\delta^{15}\text{N}$  values were determined by analyzing the annual tree-rings formed throughout an entire growing season, whereas the soil and needle  $^{15}\text{N}$  analyses were based on individual needles and soil cores sampled in 2005 and thus could not capture the annual and seasonal N availability to trees. In addition, precipitation events or stochastic factors may influence soil N values, and not all photosynthates are translocated from the needle to the rings (Jäggi et al., 2002).

We found a clear signal of the increase in the trees' uptake of fossil fuel, because of the increase in traffic. This is indicated by the  $^{14}\text{C}$  dilution in tree rings, which was around 10% less than the  $^{14}\text{C}$  bomb-enriched

background of the atmosphere. This was when the road was built at the beginning of the construction of the residential area, in the so-called "167 residential plan" (Fig. S1). After the 1980s  $^{14}\text{C}$  activity slightly decreased under atmospheric background level with the lowest values around 1990s. This decline indicated a decrease in the traffic in the recent past, probably as a consequence of the urban traffic management of the city, and suggested that the  $^{14}\text{C}$  is extremely sensitive to local changes in the emission of carbon dioxide from fossil fuel combustion.

## 5. Conclusion

This study showed that  $^{14}\text{C}$  analysis can be a strong and innovative tool to map the impact of traffic exhaust on roadside trees, while ring width and  $\delta^{13}\text{C}$  add information about the plants' physiological responses to long-term air pollution. Analyses of the nitrogen isotopic composition of tree rings seem to be very problematic, which makes it difficult to draw clear conclusions. It is, nevertheless, important to assess the history of the impact of pollutants on plant communities, and tree rings are one of the few sources of information that go back in time with annual resolution. Our paper gives a contribution to improving the methodology to determine the effect of the roads and road traffic on tree growth. The next research step in this area should be to process different species and different plant organs. This would help to refine this combined approach to be able to monitor the ecophysiological responses of plants to high traffic levels.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.scitotenv.2009.09.036.

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