Atmospheric Environment 43 (2009) 410–418

Contents lists available at ScienceDirect

Atmospheric Environment

journal homepage: www.elsevier.com/locate/atmosenv

Impact of different nitrogen emission sources on tree physiology as assessed by a triple stable isotope approach

M.R. Guerrieri [a,b,*], R.T.W. Siegwolf [b], M. Saurer [b], M. Jäggi [b], P. Cherubini [c], F. Ripullone [a], M. Borghetti [a]

[a] *Department of Crop Systems, Forestry and Environmental Sciences, University of Basilicata, Viale dell'Ateneo Lucano 10, I-85100 Potenza, Italy*
[b] *Laboratory of Atmospheric Chemistry, Paul Scherrer Institute, CH-5232 Villigen PSI, Switzerland*
[c] *Swiss Federal Institute for Forest, Snow and Landscape Research, Zürcherstrasse 111, CH-8903 Birmensdorf, Switzerland*

ARTICLE INFO

Article history:
Received 29 February 2008
Received in revised form
7 August 2008
Accepted 12 August 2008

Keywords:
Dendrochronology
N deposition
NO_x emissions
Picea abies
Quercus cerris
Stable isotopes

ABSTRACT

The importance that nitrogen (N) deposition has in driving the carbon (C) sequestration of forests has recently been investigated using both experimental and modeling approaches. Whether increased N deposition has positive or negative effects on such ecosystems depends on the status of the N and the duration of the deposition. By combining $\delta^{13}C$, $\delta^{18}O$, $\delta^{15}N$ and dendrochronological approaches, we analyzed the impact of two different sources of NO_x emissions on two tree species, namely: a broad-leaved species (*Quercus cerris*) that was located close to an oil refinery in Southern Italy, and a coniferous species (*Picea abies*) located close to a freeway in Switzerland. Variations in the c_i/c_a ratio and the distinction between stomatal and photosynthetic responses to NO_x emissions in trees were assessed using a conceptual model, which combines $\delta^{13}C$ and $\delta^{18}O$. $\delta^{15}N$ in leaves, needles and tree rings was found to be a bioindicator of N input from anthropogenic emissions, especially at the oil refinery site. We observed that N fertilization had a stimulatory effect on tree growth near the oil refinery, while the opposite effect was found for trees at the freeway site. Changes in the c_i/c_a ratio were mostly related to variations in $\delta^{13}C$ at the freeway site and, thus, were driven by photosynthesis. At the oil refinery site they were mainly related to stomatal conductance, as assessed using $\delta^{18}O$. This study demonstrates that a single method approach does not always provide a complete picture of which physiological traits are more affected by N emissions. The triple isotope approach combined with dendrochronological analyses proved to be a very promising tool for monitoring the ecophysiological responses of trees to long-term N deposition.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

The increase of reactive nitrogen (N) in the atmosphere in both oxidized (NO_x) and reduced (NH_x) forms and its deposition in terrestrial ecosystems raise relevant questions about its effects on tree growth and forest health. The productivity of many natural ecosystems, e.g. temperate and boreal forests, is often limited by low N availability (Vitousek et al., 2002). In this context, the contribution that N deposition makes toward driving productivity, and thus, carbon (C) sequestration by forests, can be important, as shown in recent investigations (Högberg, 2007; Magnani et al., 2007). Fertilization by N deposition only has a positive effect initially; however, over time, the accumulation and saturation of inorganic N in forests can perturb the biogeochemical cycles, causing, for example, a nutrient imbalance in the soil, reduced root/shoot ratio of trees, changes in the composition of vegetation (Kirchner et al., 2005; Bernhardt-Römermann et al., 2006) and a decline in biodiversity (Phoenix et al., 2006).

The impact of N deposition on forest ecosystems can be investigated near the pollution sources, where the effects are expected to be easily detectable. The analysis of stable N isotope composition ($\delta^{15}N$) has proven to be a very useful tool for detecting changes in N deposition and the incorporation of atmospheric N into leaves (e.g. Ammann et al., 1999; Siegwolf et al., 2001; Pardo et al., 2006) and tree rings (e.g. Poulson et al., 1995; Saurer et al., 2004; Bukata and Kyser, 2007). The contribution of different sources of N could potentially be distinguished using $\delta^{15}N$, since each source has a distinct $^{15}N/^{14}N$ ratio (Heaton, 1986). While the $\delta^{15}N$ in fossil fuels is typically close to 0‰, corresponding to the $\delta^{15}N$ of the atmospheric N_2, widely different values have been found for NO_x from combustion processes. Heaton (1986) reported that $\delta^{15}N$ values measured directly at the exhaust pipes of vehicles without

* Corresponding author. Department of Crop Systems, Forestry and Environmental Sciences, University of Basilicata, Viale dell'Ateneo Lucano, 10, 85100 Potenza, Italy. Tel.: +39 0971 205277; fax +39 0971 205278.
E-mail address: maria.guerrieri@unibas.it (M.R. Guerrieri).

1352-2310/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.atmosenv.2008.08.042

catalyzers ranged from −13‰ to −2‰. In the atmosphere, N isotopic exchange between NO and NO_2 may occur, which causes an enrichment of ^{15}N in the more oxidized form (Freyer et al., 1993). Ammann et al. (1999) measured positive $\delta^{15}N$ values (up to +7.7‰) for NO_2 emitted from vehicles, which was also reflected in needles and tree rings (Saurer et al., 2004) of *Picea abies* trees growing close to the freeway in the Swiss Middle Land. In contrast, negative $\delta^{15}N$ values were found in the tree rings of *Tsuga canadensis* (Poulson et al., 1995), *Pinus densiflora* (Choi et al., 2005), *Quercus rubra* and *Quercus alba* (Bukata and Kyser, 2007) that were located near areas of industrial and urban pollution.

Using the Scheidegger et al. (2000) model, the stable carbon ($\delta^{13}C$) vs. oxygen ($\delta^{18}O$) isotope composition results in a typical NO_x-induced isotopic pattern: $\delta^{13}C$ in leaf material increased with increasing NO_x exposure, while $\delta^{18}O$ decreased (Siegwolf et al., 2001; Saurer and Siegwolf, 2007). This pattern of $\delta^{13}C$ suggests the stimulation of photosynthesis (A) due to N fertilization, while $\delta^{18}O$ indicates an increase in stomatal conductance (g_s), resulting in enhanced transpiration. Despite the axiomatic and strong positive relationship between A and N availability (Warren and Adams, 2006) due to N investment in the photosynthetic apparatus, the mechanistic link between N and g_s is still not well understood (Grassi et al., 2002). Rather than NO_x having a direct effect, variations in g_s could be more closely related to stimulation of A by N, altering the c_i/c_a ratio (the ratio of intercellular and ambient CO_2 concentrations, respectively), and changing the C and O isotope fractionation (Farquhar et al., 1989; Farquhar and Lloyd, 1993). These C and O isotope ratios in leaves are then transferred to tree-ring organic materials.

Additionally, the dendrochronological approach is an efficient way to retrieve information about past pollution events and their influences on tree-growth. By examining ring widths, several studies showed that pollution plays an important role in reducing tree growth (e.g. Tolunay, 2003; Muzika et al., 2004; Wilczyński, 2006).

To our knowledge, there are no investigations that combine $\delta^{15}N$, $\delta^{13}C$ and $\delta^{18}O$ with the dendrochronological approach in order to gain valuable information on the physiological responses of trees to NO_x emissions near point sources.

The main purpose of this study was the retrospective assessment of the impact of N emissions on trees that are heavily exposed to gaseous N-compounds (especially NO_x). Two different sources of N emissions (traffic and oil refinery) and two tree species (*P. abies* and *Quercus cerris*) were considered in this investigation. In particular, we addressed the following questions: how clear is the fingerprint of anthropogenic disturbance in tree rings detected by $\delta^{15}N$; is growth rate enhanced through higher atmospheric N availability; how are emissions of N-compounds linked to tree physiological traits assessed by $\delta^{13}C$ and $\delta^{18}O$, as investigated by Siegwolf et al. (2001); and do trees exposed to increased pollution show similar responses in terms of $\delta^{13}C$ and $\delta^{18}O$, irrespective of N emission sources?

2. Materials and methods

2.1. Study sites and sampling

This study was conducted in two locations: (i) two *Q. cerris* L. stands close to an oil refinery in the Agri Valley (40°18′53″N, 15°53′59″E, 600 m a.s.l.), in the South of Italy; and (ii) a *P. abies* L. Karst forest, along the A2 freeway, near Faido (46°29′N, 8°48′E, 715 m a.s.l.) in Switzerland (Fig. 1).

2.1.1. Oil refinery site

Since April 1996, all the crude oil extracted from the deep soil layers in the Agri Valley area has been collected and processed in an oil refinery, which mainly produces SO_2, NO, NO_2 and CO as exhaust gases. The predominant wind direction in the area is N–NW; the annual mean temperature and the total precipitation are approximately 12 °C and 602 mm, respectively (measured for the period from 1980 to 2005). Climatic data were recorded at the Grumento Nova meteorological station (40°01′20″N, 15°45′53″E, 771 m a.s.l. – 14 km away from the oil refinery). Two plots, located in contrasting positions with respect to the oil refinery and wind direction, were chosen for the investigation. The first plot, labeled OR1, was situated in a *Q. cerris* stand, relatively close to the oil

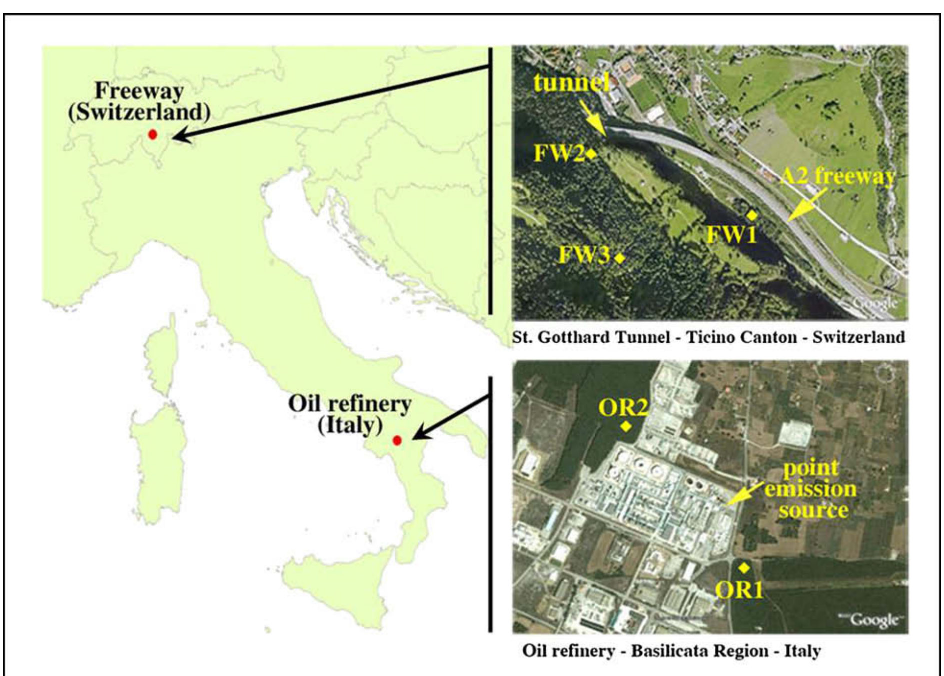

Fig. 1. Map showing the two experimental sites (oil refinery in Italy and freeway in Switzerland) and the location of the plots. OR1 and OR2 indicate the plots at 300 and 1000 m from the oil refinery, respectively. Plots FW1, FW2 and FW3 are 50 m, 175 m and 400 m distant from the freeway, respectively.

refinery (300 m away). This plot was constantly exposed to flue gases since it was located downwind from the prevailing winds. The second plot, OR2, was situated in a *Q. cerris* stand that was upwind from the prevailing winds and was farther from the emission source (1000 m away). Samples of wood cores and leaves were taken from six trees in each plot. Leaves were sampled at two different times: November 2005 (old leaves that had already been shed and exposed to the NO_x emissions throughout their entire life span) and April 2006 (young leaves at the beginning of their differentiation). Three wood cores, two for isotopic analyses and one for dendrochronological analyses, were sampled from each tree stem at breast height using a 0.5 cm diameter increment borer. In addition, six soil samples were collected from each plot in the vicinity of the monitored trees. Two layers (0–5 and 5–10 cm in depth) were separated and examined individually for $\delta^{15}N$.

2.1.2. Freeway site

The A2 freeway is one of the busiest roads in Switzerland, since it represents the main North–South axis from Basel to Chiasso, and carries international traffic between Italy and Germany. The A2 track close to Faido and the St. Gotthard tunnel opened in 1980. The period before this event is also of scientific interest because a significant increase of vehicular traffic was observed on the old cantonal road during that time. Climatic data were taken from the meteorological station based at Lugano (46°00′N; 8°57′E, 273 m a.s.l. – 68 km away from Faido). The mean annual temperature and annual precipitation amount for the investigated time span (1945–2004) were 12 °C and 1634 mm, respectively. A transect perpendicular to the A2 freeway was identified in a *P. abies* forest near Faido. Within this transect, three plots were selected at different distances from the freeway (FW1 = 50 m, FW2 = 175 m, FW3 = 400 m), with FW1 and FW3 differing by approximately 200 m in altitude. The effects of emissions were investigated on 2-year-old needles. For this purpose, we used samples collected in October 2002 to measure heavy metal content (Peter Waldner, WSL, Birmensdorf, Switzerland, data unpublished). Three wood cores (two for isotopic measurements and one for dendrochronolgy) were sampled from each tree stem at breast height using a 0.5 cm diameter increment borer. Three soil samples were collected at each plot in the vicinity of the sampled trees. As in the case of the oil refinery samples, two layers (0–5 and 5–10 cm in depth) were separated and measured individually for $\delta^{15}N$.

2.2. Dendrochronological measurements

For each wood core, each annual tree ring was identified, dated and measured from bark to pith with a Leica MS5 stereoscope (Leica Microsystems, Germany). Ring-width (RW) measurements were made with a resolution of 0.01 mm using the Time Series Analysis and Presentation (TSAP) software package (Frank Rinn, Heidelberg, Germany). The ring-width series were plotted and visually synchronized to identify measurement errors and to locate any missing or double rings (Fritts, 1976; Schweingruber, 1996). The agreement of each curve with the main standard chronology curve was examined using the following statistical parameters (data not shown): (1) "Gleichläufigkeit", which is a measure of year-to-year agreement between the interval trends of two chronologies, based on the sign of agreement; and (2) Student's *t*-test, which determines the degree of correlation between the curves. The age of trees was 30 years for *Q. cerris* and 100 years for *P. abies*. The time periods investigated were 1980–2005 for the oil refinery and 1945–2004 for the freeway.

2.3. Sample preparation and isotopic measurements

Before the isotopic measurements, the N-mobile compounds were extracted from the wood cores using a Soxhlet apparatus, according to the procedure of Sheppard and Thompson (2000), as modified by Saurer et al. (2004). For trees at the freeway site, we considered sections of three consecutive annual rings instead of a single annual ring. Leaves, soil and tree rings were dried, ground with a centrifugal mill and weighed in tin capsules. To determine the $\delta^{13}C$ and $\delta^{15}N$ in leaf and needle samples, an amount (3.8–4.2 mg) of organic material was combusted in the elemental analyzer (EA-1100, Carlo Erba, Milano, Italy), which was connected to the isotope ratio mass spectrometer (Delta S, Finnigan MAT, Bremen, Germany) through a variable open split interface (ConFlo II, Finnigan MAT, Bremen, Germany). For $\delta^{18}O$, in a separate measurement, an aliquot (1.1–1.3 mg) of bulk organic material was decomposed to CO by thermal pyrolysis at 1080 °C (according to Saurer et al., 1998) in a different elemental analyzer (EA-1108, Carlo Erba, Milano, Italy), which was connected to a continuous flow mass spectrometer (Delta S, Finnigan MAT, Bremen, Germany). For tree rings, $\delta^{18}O$ was measured as described above, while $\delta^{13}C$ and $\delta^{15}N$ were each determined in a separate analysis by taking an amount of 0.6–0.8 mg and 20 mg of dry matter, respectively. Samples were combusted under an excess of oxygen in an elemental analyzer (EA-1100, Carlo Erba, Milano, Italy). For $\delta^{13}C$, the CO_2 and N_2 gas were carried in a helium stream to the mass spectrometer (Delta S, Finnigan MAT, Bremen, Germany) via the ConFlo II interface. For $\delta^{15}N$ analysis, the standard measuring procedure for organic materials was modified due to the low N and high C content in wood, to minimize the interference of the CO_2 signal and to optimize the detection of $^{15}N/^{14}N$. A CO_2 (carbosorb) and a water trap (magnesium perchlorate) were mounted between the GC column and the ConFlo interface. The chemicals were replaced after every 50 samples. To eliminate the carry-over effect of the large amount of CO_2, a blank (an empty tin capsule) was inserted after every wood sample. The isotope values were expressed in the δ-notation (in per mil; ‰) as a relative deviation from the international standard (atmospheric N_2 for $\delta^{15}N$, V-PDB for $\delta^{13}C$ and V-SMOW for $\delta^{18}O$). The relative precision of the repeated analysis was ±0.1‰ for $\delta^{13}C$ and ±0.3‰ for $\delta^{15}N$ and $\delta^{18}O$. $\delta^{13}C$ values measured in the tree rings were corrected for the SUESS effect (the decline of the $^{13}C/^{12}C$ ratio of atmospheric CO_2).

2.4. Statistical analysis

Using paired sample *t*-tests, we explored the differences between the soil samples collected at two different depths (0–5 and 5–10 cm) in plots OR1 and OR2 near the oil refinery and plots FW1, FW2 and FW3 near the freeway. Independent sample *t*-tests were used to explore the differences between OR1 and OR2 in terms of ring width, %N, $\delta^{15}N$, $\delta^{13}C$ and $\delta^{18}O$ measured in leaf and tree-ring samples. For the same parameters, differences among FW1, FW2 and FW3 plots at the freeway site were tested by multiple ANOVA comparisons. Furthermore, all parameter differences between the periods before and after the onset of pollution were tested using an independent sample *t*-test. We employed bivariate Pearson correlations to quantify the relationships between climatic factors and isotope signals. All statistical analyses were carried out with the SPSS 10.0 statistical package (SPSS, Chicago, IL). The significance level for all the statistical tests was $\alpha = 0.05$.

3. Results

3.1. $\delta^{15}N$ in soil, leaves and needles

3.1.1. Oil refinery site

The *t*-test showed significant differences for $\delta^{15}N$ between the top (0–5 cm; OR1 = 0.24 ± 0.61‰, OR2 = 0.52 ± 0.32‰) and the deep (5–10 cm; OR1 = 3.17 ± 0.47‰, OR2 = 3.97 ± 0.64‰) soil layers in each plot. Although not statistically significant, the

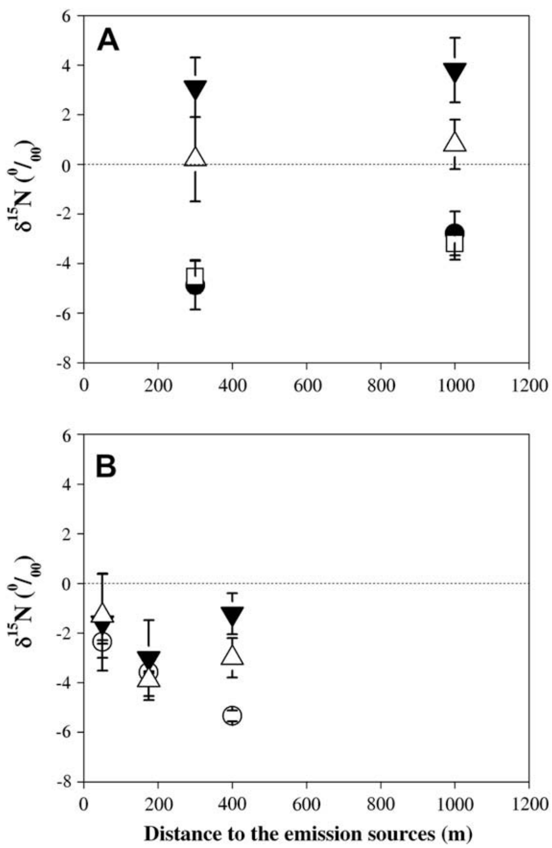

Fig. 2. Trend of δ^{15}N values measured: (A) in soil (▼ deep soil; △ top soil; mean ± SD) and leaves (□ young leaves harvested in April 2006; ● senescent leaves harvested in November 2005) for the oil refinery; and (B) in soil (▼ deep soil; △ top soil; mean ± SD) and 2-year-old needles (○) for the freeway. The dotted line represents the δ^{15}N of atmospheric N_2.

measured δ^{15}N values were lower at OR1 than OR2 for both soil layers (Fig. 2A). The %N was higher in the top soil (OR1 = 0.44 ± 0.05%, OR2 = 0.53 ± 0.08%) than in the deeper layers (OR1 = 0.19 ± 0.02%, OR2 = 0.22 ± 0.09%). δ^{15}N measured in the leaves showed the same trend as observed in the soil (Fig. 2A). For both old (OR1 = −4.53 ± 0.27‰, OR2 = −3.20 ± 0.26‰) and young (OR1 = −4.86 ± 0.39‰, OR2 = −2.78 ± 0.36‰) leaves, those that were more exposed to pollution had δ^{15}N values significantly ($P < 0.01$) lower than leaves further away from the pollution source. At each plot, the δ^{15}N in old leaves was not statistically different from values measured in young leaves. As for the %N, the difference between plots was significant only for old leaves, with higher values in OR2 (1.07 ± 0.09%) than OR1 (0.83 ± 0.02%). In general, δ^{15}N in the topmost soil samples was enriched in ^{15}N compared to that measured in old and new leaves, with enrichment factors of 4.7‰ and 5.1‰, respectively.

3.1.2. Freeway site

δ^{15}N values measured in the two soil layers did not show any significant differences, except for FW3, where higher values were measured in the top soil layers than the deeper layers. The δ^{15}N in the soil decreased with the distance to the freeway (Fig. 2B), with significantly less negative values for the topmost soil in the plot FW1 (−1.26 ± 0.99‰) compared to FW2 (−3.93 ± 0.40‰), while differences between FW1 and FW3 (−2.67 ± 0.45‰) were not significant. Soil samples were not different with respect to %N. The δ^{15}N measured in the needles of *P. abies* trees showed a decreasing trend with increasing distance to the freeway (Fig. 2B). In particular, δ^{15}N was higher in needles that were more exposed to vehicle emissions (FW1) (−2.63 ± 0.81‰) than needles that were less exposed such as those at FW2 (−3.58 ± 0.27‰) and FW3 (−5.34 ± 0.32‰). Significant differences were only found between FW1 and FW3. No significant changes were observed for %N among the plots (FW1 = 1.23 ± 0.04%, FW2 = 1.23 ± 0.02%, FW3 = 1.40 ± 0.06%). The δ^{15}N in needle samples, except those in FW2, was more depleted in ^{15}N compared to the soil samples.

3.2. δ^{13}C and δ^{18}O in leaves and needles

Near the oil refinery, δ^{18}O values (Table 1) showed no significant variations in either old or young leaves between the two sites. The δ^{13}C values in the young leaves were more negative at OR1 than at the less polluted site (OR2), and this trend was reversed for old leaves, with significantly higher δ^{13}C values found in leaves from OR1 than OR2. In general, δ^{13}C values were more negative in old leaves than in young ones, with observed differences of 2‰ and 5‰ for OR1 and OR2, respectively. Along the freeway in Switzerland, the variations were not significant between plots for either δ^{13}C or δ^{18}O values.

Fig. 3 shows the shifts of mean δ^{13}C and δ^{18}O values between the trees that were highly exposed to NO_x and the non-exposed trees at the two study sites. As indicated by the arrows, shifts in δ^{13}C vs. δ^{18}O values were in the opposite directions for the two sites, with larger variations of δ^{13}C and δ^{18}O occurring in leaves next to the oil refinery.

3.3. Tree-ring widths

At the oil refinery site, tree growth did not differ significantly between the plots until 1995 (Fig. 4A). After the establishment of the oil refinery (1996), the growth rates measured at OR1 were significantly ($P < 0.001$) higher than at OR2. In particular, the differences between OR1 and OR2 increased from 1994 to 1996, with the highest values in ring width (RW) occurring in 1998.

In general, a high variability in RW is apparent among trees at the freeway site (Fig. 4B). For trees at FW1, we observed a relevant increase of growth rates between 1960 and 1980. Particularly after the tunnel opening (1980), the differences between FW1 and FW2 became smaller, while growth rates decreased in FW3 compared to FW1.

Table 1
δ^{13}C and δ^{18}O values (mean ± SD) measured in young (harvested in April 2006) and old (harvested in November 2005) leaves of *Quercus cerris* at the oil refinery and 2-year-old needles of *Picea abies* at the freeway.

Site	Plot	Old leaves		Young leaves		Needles	
		δ^{13}C (‰) (SD)	δ^{18}O (‰) (SD)	δ^{13}C (‰) (SD)	δ^{18}O (‰) (SD)	δ^{13}C (‰) (SD)	δ^{18}O (‰) (SD)
Refinery	OR1	−29.05 (0.63)	27.15 (1.05)	−26.29 (1.02)	27.93 (0.43)		
	OR2	−30.42 (0.65)	27.93 (1.15)	−25.16 (1.29)	27.21 (0.36)		
Freeway	FW1					−26.49 (0.16)	29.63 (0.34)
	FW2					−26.98 (0.38)	29.59 (0.1)
	FW3					−26.28 (0.36)	30.13 (0.05)

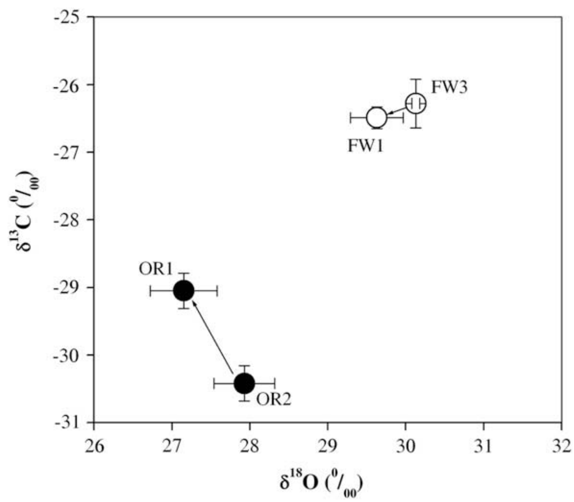

Fig. 3. Combination of δ^{13}C and δ^{18}O (mean ± SD) in leaves of *Quercus cerris* (●) at the oil refinery (OR1 = 300 m vs. OR2 = 1000 m) and in needles of *Picea abies* (○) at the freeway (FW1 = 50 m vs. and FW3 = 400 m).

3.4. δ^{15}N in tree rings

At the oil refinery site, δ^{15}N has diverged since 1992 for the two investigated plots, with a decreasing trend for trees more exposed to NO_x emissions (Fig. 5A). The *t*-test showed a significant ($P < 0.001$) difference between the two plots for δ^{15}N and %N in tree rings. In particular, the average value of δ^{15}N was significantly lower (−1.08‰) for OR1 compared to OR2 (0.05‰), while we observed a higher %N at OR2 (0.28%) than at OR1 (0.23%). The comparison of δ^{15}N between the time periods (1980–1995 and 1996–2005) only showed a significant difference ($P < 0.001$) for trees in the plot more exposed to pollution. In this case, after the establishment of the oil refinery, further depletion of the heavier isotope was observed for δ^{15}N. However, at both plots, we observed a significant ($P < 0.01$) increase of %N in tree rings soon after the industrial activity began, with average values of 0.26% and 0.34% for OR1 and OR2, respectively. We did not observe significant correlations between δ^{15}N and %N for trees more exposed to pollution, either before or after the establishment of the oil refinery. For trees in OR2, correlations between %N and δ^{15}N were significant only for the years before the establishment of the oil refinery ($r = 0.44$; $P < 0.001$).

At the freeway site (Fig. 5B), trees more exposed to traffic (FW1) showed significantly more positive δ^{15}N values than those at FW2 and FW3. This was found to be true even when the time periods before (1945–1980) and after (1981–2004) the opening of the tunnel were considered separately. However, differences between δ^{15}N values measured before and after the tunnel opening were significant only for trees in FW3. Significantly ($P < 0.01$) higher %N values were measured for FW1 (0.11%) than FW2 (0.09%) and FW3 (0.08%) only after the tunnel opening. At each plot, the %N was significantly correlated with δ^{15}N for the years before the tunnel opening, while no significant relationship was observed between %N and δ^{15}N after the tunnel opening.

3.5. δ^{13}C and δ^{18}O in tree rings

The time series for δ^{13}C and δ^{18}O did not show a clear signal of perturbations due to the industrial activity of the oil refinery (Fig. 6A and C). When the entire monitoring period was analyzed, no significant differences were observed between plots for δ^{13}C or δ^{18}O. By splitting the time periods into 1980–1995 and 1996–2005, δ^{13}C became slightly, but significantly, higher after the establishment of the oil refinery, but only for trees in OR1 (from −26.1‰ to −25.9‰). Moreover, δ^{18}O showed significant ($P < 0.01$) differences between the two time periods at each plot. We only observed significant correlations between δ^{13}C–δ^{18}O and climate (in particular precipitation) before the establishment of the oil refinery (Table 2).

In the case of the freeway, trees in FW1 showed significantly higher (more positive) δ^{13}C values (Fig. 6B and D), which began to deviate from the values of the non-exposed sites after 1950. Values of δ^{18}O were significantly higher for tree rings at FW1 and FW3 than at FW2. Between the 1980s and the 1990s, we observed an increase in δ^{18}O, along with a decrease in δ^{13}C. No significant differences were observed for δ^{18}O between the two time intervals (before and after 1980) at any of the plots, while δ^{13}C was significantly ($P < 0.001$) more negative after the tunnel opening at all plots. The multiple ANOVA comparison only showed significant differences for δ^{13}C ($P < 0.01$) among plots for both the time intervals, where the difference was significant only before 1980 for δ^{18}O. With respect to the climate relationship, δ^{13}C and δ^{18}O generally reflected the precipitation more than the temperature signal, particularly in the years before the tunnel was opened (Table 3).

3.6. Tree physiological traits vs. N emission sources

Significant correlations were observed only for trees directly exposed to N emission between δ^{15}N and δ^{18}O ($r = -0.66$; $b = -0.54$; $P < 0.001$) and δ^{15}N and ring widths ($r = -0.64$; $b = -71.58$, $P < 0.001$) at the oil refinery site. In contrast, near the

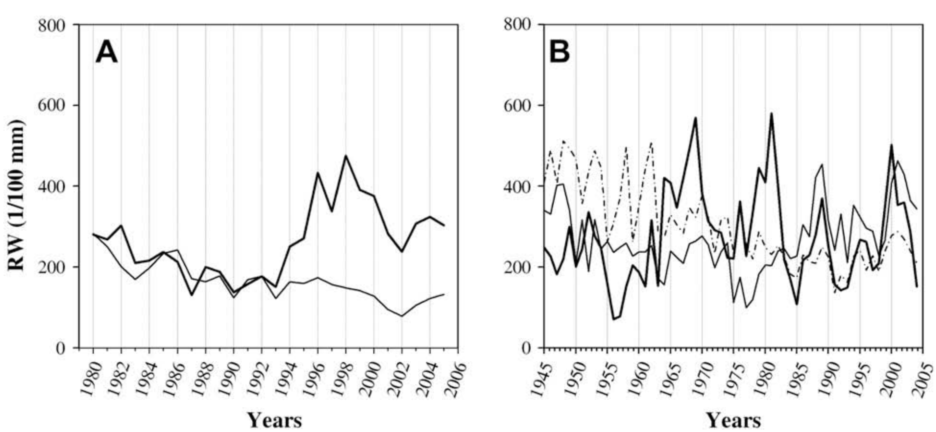

Fig. 4. Mean ring width (RW) curves for: (A) *Quercus cerris* at the oil refinery (OR1 (—) and OR2 (-)); (B) *Picea abies* at the freeway (FW1 (—), FW2 (-) and FW3 (- - -)). The oil refinery was established in 1996, and the St. Gotthard tunnel was opened in 1980.

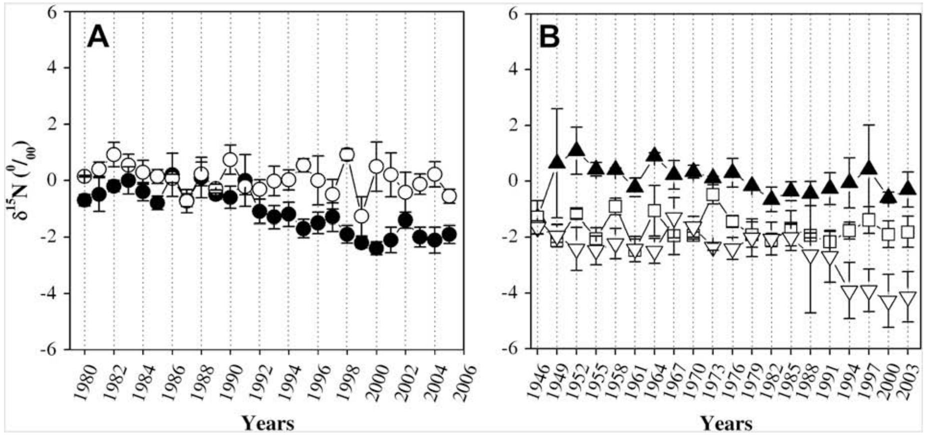

Fig. 5. Trend of $\delta^{15}N$ values (mean ± SE) measured in: (A) annual tree rings (from 1980 to 2005) for *Quercus cerris* at the oil refinery ((●) OR1 = 300 m and (○) OR2 = 1000 m); and (B) in groups of three consecutive annual rings (from 1945 to 2004) for *Picea abies* at the freeway (FW1 = 50 m (▲), FW2 = 175 m (□), FW3 = 400 m (▽)).

freeway, $\delta^{13}C$ was significantly correlated with $\delta^{15}N$ for trees at FW1 ($r = -0.49$; $b = -0.71$) and FW3 ($r = -0.7$; $b = -0.36$; $P < 0.001$). We observed a significant ($r = -0.7$; $b = -75.39$; $P < 0.001$) and negative relationship between $\delta^{15}N$ and ring widths only for trees at FW3.

Fig. 7 showed the shifts in $\delta^{13}C$ vs. $\delta^{18}O$ values for trees more exposed to pollution at the two investigated sites. At the oil refinery site, NO_x exposure caused an increase in $\delta^{13}C$ and $\delta^{18}O$, while at the freeway site, an increase of $\delta^{13}C$ was accompanied by a decrease in $\delta^{18}O$.

4. Discussion

4.1. δ^{15} N as a primary indicator

Our results reflected the appreciable input of N from anthropogenic activities on trees at the investigated sites, even though the signal was not as strong as expected in all the organic pools examined.

It is not clear if the isotopic fingerprint of NO_x emissions, which was detected in the leaves, needles and tree rings, was also contributing to the isotopic signature in the soil, particularly at the oil refinery. The isotopic variability of $\delta^{15}N$ at the soil level depends on the amount of N and the isotopic fractionations that occur during the processes of mineralization, nitrification and denitrification (Högberg, 1997). At the undisturbed site, the $\delta^{15}N$ found in leaf, needle and tree ring material should mostly reflect the $\delta^{15}N$ of the soil (Gebauer et al., 1994). Therefore, the significant variations in $\delta^{15}N$ observed in tree pools showed clear disturbances due to anthropogenic emissions.

In our study, the %N in tree pools was not helpful in detecting the changes in tree N cycling due to pollution, particularly when tree rings were considered. We observed a significant increase in the %N after the oil refinery establishment and the tunnel opening,

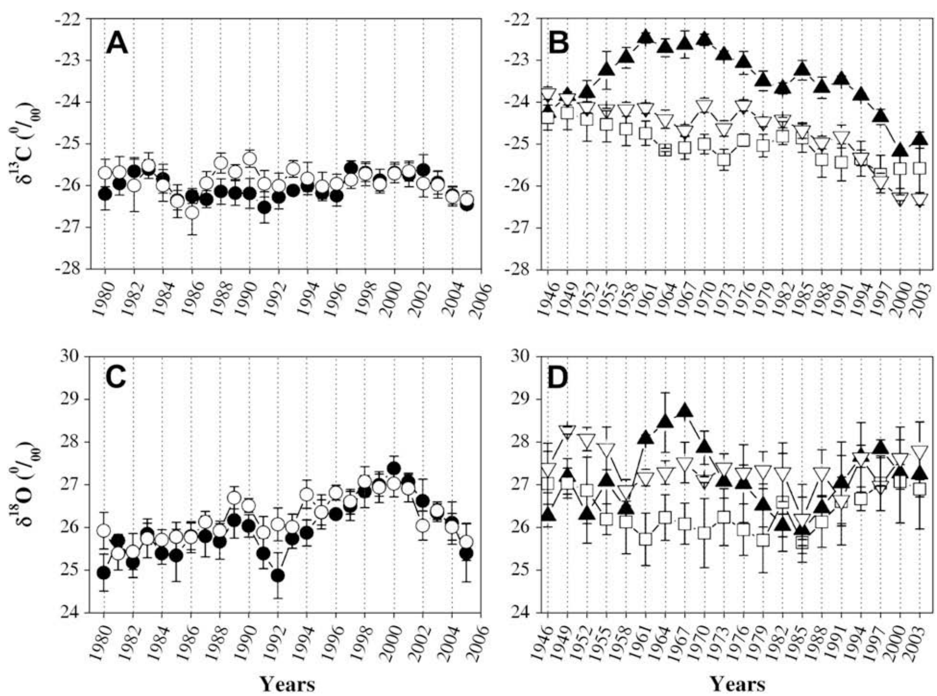

Fig. 6. Trends of $d^{13}C$ and $d^{18}O$ values (mean ± SE) measured in: (A),(C) annual tree rings (from 1980 to 2005) for *Quercus cerris* at the oil refinery ((·) OR1 at 300 m and (○) OR2 at 1000 m); and (B),(D) in groups of three consecutive annual rings (from 1945 to 2004) for *Picea abies* at the freeway (FW1 = 50 m (▲), FW2 = 175 m (□), FW3 = 400 m (Ñ)).

Table 2
Pearson correlations between $\delta^{13}C$ and $\delta^{18}O$ in tree rings and temperature (T), precipitation (Prec) for *Quercus cerris* at the oil refinery (OR1 and OR2).

Parameters (1980–1995)	Correlation coefficients		Parameters (1996–2005)	Correlation coefficients	
	OR1	OR2		OR1	OR2
$\delta^{13}C \times T_{min}$	−0.64*	−0.34	$\delta^{18}O \times T_{average}$	−0.59	−0.72*
$\delta^{13}C$ × annual Prec	0.68**	−0.17	$\delta^{18}O \times T_{min}$	−0.57	−0.78**
$\delta^{13}C$ × Prec Feb	0.81**	−0.02			
$\delta^{13}C$ × Prec Apr	0.55*	−0.08			
$\delta^{13}C$ × Prec Oct	0.54*	0.09			
$\delta^{13}C$ × Prec Dec	0.61*	0.41			
$\delta^{18}O$ × annual Prec	−0.40	−0.70**			
$\delta^{18}O$ × Prec Jun	−0.11	−0.58*			
$\delta^{18}O$ × Prec Aug	−0.09	−0.58*			
$\delta^{18}O$ × Prec Oct	−0.58*	−0.66**			
$\delta^{18}O$ × Prec Nov	−0.76**	−0.31			
$\delta^{18}O$ × Prec Dec	−0.24	−0.53*			

The time periods before (1980–1995) and after (1996–2005) the establishment of the oil refinery were considered separately. Correlation coefficient values (r) were significant at: $^{*}P < 0.05$ and $^{**}P < 0.01$.

not only for trees more exposed to pollution, but also for those growing at the more distant plot. $\delta^{15}N$ and %N were not significantly correlated after the anthropogenic disturbances, suggesting that variations in $\delta^{15}N$ in tree rings are not exclusively related to the natural N background. Similar results for leaves have been found by Pardo et al. (2006), suggesting that $\delta^{15}N$ is a better measure of internal N cycling in response to N deposition than is %N alone.

Inverse patterns between the two sites were found for $\delta^{15}N$ in the leaves, needles and tree rings. At the oil refinery site, the $\delta^{15}N$ was more negative in the plot close to the emission source than in the distant plot, while we found the opposite pattern along the freeway. Although these results might seem contradictory, these values reflect the difference in $\delta^{15}N$ between the two pollution sources, which are likely due to the different combustion processes and treatments of the exhaust fumes (e.g. Heaton, 1986; Ammann et al., 1999).

The decreasing strength of pollution effects relative to the source is reflected in the $\delta^{15}N$ of leaves and needles, irrespective of age (Ammann et al., 1999). Similarly, $\delta^{15}N$ values in tree rings were more positive at the freeway site than the oil refinery site. We observed clear changes in $\delta^{15}N$ in tree rings for directly exposed trees near the refinery for the time period since 1992, although the oil refinery began operation in 1996. We can exclude changes in the isotopic composition of soil due to livestock or agricultural activity, since it is an industrial area. Rather, these changes are related to the disturbances during the construction of the oil refinery (i.e. exhaust fumes of trucks and construction machines). Moreover, the slight difference between the year in which $\delta^{15}N$ decreased and the

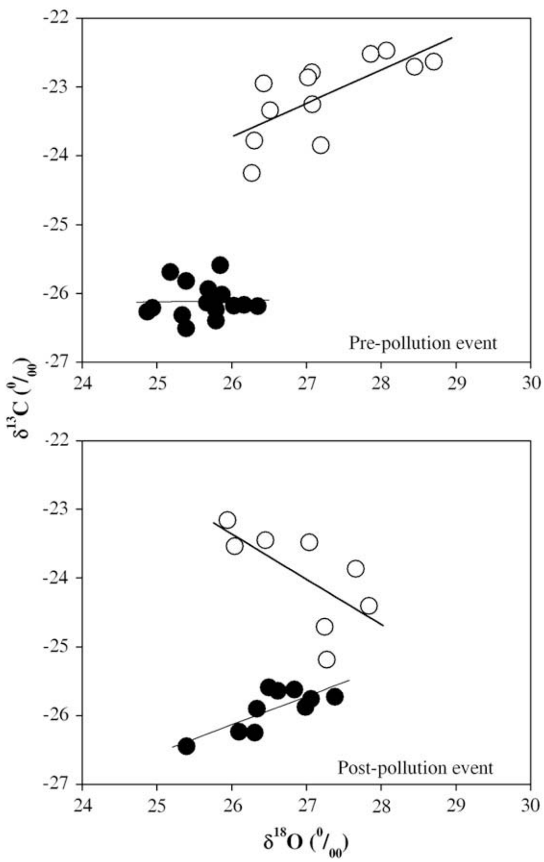

Fig. 7. Combinations of $\delta^{18}O$ and $\delta^{13}C$ (mean values) in tree rings for *Quercus cerris* at the oil refinery ((●), OR1) and for *Picea abies* at the freeway ((○), FW1) directly exposed to pollution. Pre- (1980–1995 for the oil refinery and 1945–1980 for the freeway) and post- (1996–2005 for the oil refinery and 1981–2004 for the freeway) pollution events are shown separately.

beginning of the operation of the oil refinery could be due to lateral transport of mobile N-compounds in the stem, where an exchange between the individual tree rings occurs (Elhani et al., 2003). This possibility cannot be completely excluded even after the extraction of the soluble N-compounds. At the more exposed site along the Swiss freeway, the $\delta^{15}N$ values increased only moderately after the tunnel opening, indicating possible exposure of trees to NO_x even before the 1980s. In fact, before the freeway and tunnel construction, the main cantonal road, which was close to the monitored trees in FW1, experienced high traffic. Therefore, our results could indicate either a moderate NO_x impact for the trees or a small increase in NO_x deposition after the opening of the tunnel. Changes in the $^{15}NO_x$ signal of emitted traffic pollution over time are also possible due to engine and catalyzer improvements, or to local factors like traffic density or wind direction (Freyer et al., 1993).

4.2. Tree-ring growth

Ring-width curves suggest an opposite tendency for growth rates at the two investigated sites. In fact, near the oil refinery, trees that were more exposed to pollution showed the highest growth rate, particularly during the period 1996–2000, soon after the oil refinery began operating. Variations in ring widths were significantly correlated with changes in NO_x emissions, as detected by $\delta^{15}N$. Thus, in this case, we observed a fertilizer effect of the enhanced N availability on tree growth rate. Conversely, trees at OR2 did not seem to be affected by industrial activity, as they showed slower growth. Since trees at OR1 and OR2 were homogeneous in terms of age and environmental conditions, the

Table 3
Pearson correlations between $\delta^{13}C$ and $\delta^{18}O$ in tree ring and temperature (T), precipitation (Prec) for *Picea abies* at the freeway (FW1, FW2 and FW3).

Parameters (1945–1979)	Correlation coefficients			Parameters (1980–2004)	Correlation coefficients		
	FW1	FW2	FW3		FW1	FW2	FW3
$\delta^{13}C$ × Prec Feb	0.21	−0.65*	−0.42	$\delta^{13}C$ × Prec May	0.29	0.30	0.74*
$\delta^{13}C$ × Prec Aug	−0.59*	−0.65*	0.52	$\delta^{13}C$ × Prec Nov	−0.74*	−0.805**	−0.47
$\delta^{18}O$ × Prec Aug	−0.41	0.58*	0.29	$\delta^{13}C \times T_{average}$	−0.81*	−0.88**	−0.82*
$\delta^{18}O$ × Prec Sep	0.64*	0.06	0.10	$\delta^{18}O$ × Prec Apr	−0.47	−0.77*	−0.44
$\delta^{18}O$ × Prec Nov	0.64*	0.10	0.06	$\delta^{18}O \times T_{average}$	0.77*	0.75*	0.68
$\delta^{18}O \times T_{max}$	−0.35	0.74**	0.05	$\delta^{18}O \times T_{min}$	0.73*	0.70	0.70
$\delta^{18}O \times T_{average}$	−0.23	0.66*	0.28				

The time periods before (1945–1980) and after (1981–2004) the construction of St. Gotthard tunnel were considered separately. Correlation coefficient values (r) were significant at: $^{*}P < 0.05$ and $^{**}P < 0.01$.

differences in growth rates could be related to the differences in exposure to N emissions.

In the case of the freeway, the beginning of the anthropogenic N-input mostly occurred after the tunnel opened in 1980. The effect of fertilization on tree growth is not as clearly visible for these trees, and there was even a decreasing tendency for growth after 1980. Corroborating this, Saurer et al. (2004) did not observe any changes in secondary growth for *P. abies* trees after the construction of the freeway in Switzerland between Zurich and Berne.

4.3. Physiological traits vs. N emission sources

4.3.1. $\delta^{13}C$ and $\delta^{18}O$ signals as secondary indicators

Our results only partly agree with the findings of Siegwolf et al. (2001) (lab experiments) and Saurer and Siegwolf (2007) (results from the field). As described above, the known C and O isotope pattern caused by NO_2 was observed in old leaves for trees close to the oil refinery (Fig. 3) and in tree rings at the freeway site (Fig. 7). In this case, NO_2 exposure causes an increase in $\delta^{13}C$ and, at the same time, a decrease in $\delta^{18}O$. Both isotope responses are a result of N fertilization (Siegwolf et al., 2001); the increase in $\delta^{13}C$ reflects the stimulation of photosynthesis, while the decrease in $\delta^{18}O$ indicates an increase in stomatal conductance. This pattern was not observed for needles near the freeway or for tree rings at the oil refinery. In this latter case, exposure to NO_x led to an increase in both $\delta^{13}C$ and $\delta^{18}O$. The increase of $\delta^{13}C$, which indicates a reduction in c_i/c_a, could be the result of either (1) reduced g_s (at constant A), or (2) increased A (at constant g_s). We are aware that the trees were also assimilating ^{13}C-depleted CO_2 originating from fossil fuel combustion. Therefore it is well possible that the $\delta^{13}C$ values indicated in Figs. 6 and 7 are somewhat underestimated. A correction of the absolute values is hardly possible since we could not quantify the $\delta^{13}C$ values at the sites, because the air masses are too turbulent and the $\delta^{13}C$ signal in the organic matter represents a long-term average value. Since $\delta^{18}O$ also increased, indicating an enhanced ^{18}O enrichment of leaf water due to lower transpiration (Farquhar and Lloyd, 1993), we can assume a reduction in g_s, which is likely a result of a drought condition masking the stomatal opening N-effect. As for needles along the freeway, an appreciable signal of N emissions from $\delta^{15}N$ did not correspond with strong variations in C and O isotopic signatures, suggesting a physiological adjustment of 2-year-old needles to NO_x exposure.

These diverging results suggest a competing effect between drought and NO_x exposure for trees at the oil refinery site, located in a semi-arid region. In dry conditions, trees have to minimize water loss by reducing the g_s, hence $\delta^{18}O$ value increased. We found a diminished correlation between precipitation and $\delta^{18}O$ values after the oil refinery began operating, which could be an indicator for the interfering impact of NO_x. Close to the freeway site, however, we found the expected NO_2 induced isotope pattern. This site is well water supplied; therefore NO_x exposure facilitated an enhanced carbon gain and led to an increased g_s (Fig 7).

4.3.2. Contrasting expression of the NO_x impact in trees

The temporal trends of $\delta^{13}C$ and $\delta^{18}O$ were not similarly related to NO_x emissions at the two investigated sites. We observed significant correlations between $\delta^{13}C$ and $\delta^{15}N$ at the freeway site for trees in plots FW1 and FW3. However, the extent of the relationship was different for the two plots, as indicated by the slope coefficient (b), which was higher for FW1 ($b = -0.71$) than FW3 ($b = -0.36$). This indicates a reduced NO_x load at the FW3 site. In contrast, $\delta^{18}O$ was not significantly affected by changes in $\delta^{15}N$, and variations in the ratio of c_i/c_a, as assessed by stable isotopes, were mainly related to changes in A and, to a smaller degree, to g_s. Our results also suggest that trees farther away from the freeway can be influenced by pollution in the long term, though to a lesser extent than the trees closest to the freeway. At the oil refinery site, $\delta^{15}N$ was not significantly related to $\delta^{13}C$, while we observed a significant correlation with $\delta^{18}O$ throughout the entire study period. A decrease in $\delta^{15}N$ corresponded with an increase in $\delta^{18}O$, which indicated an enrichment of ^{18}O in the leaf water due to a reduction in leaf transpiration. This suggests that a decrease in g_s (which affects the c_i/c_a ratio) is a result of more frequent drought events at this site, rather than a direct effect of NO_x exposure.

5. Conclusions

- Irrespective of the source of NO_x emissions, the $\delta^{15}N$ in leaves, needles and tree rings were found to be a more specific bio-indicator for anthropogenic N-compound emissions than %N. The strongest fingerprint of N emissions was detected for *Q. cerris* at the oil refinery site.
- A stimulatory effect on tree growth caused by N fertilization was only found at the oil refinery site.
- Long-term exposure to NO_x emissions had a different impact on c_i/c_a in the two experimental sites: at the oil refinery (*Q. cerris*), g_s influenced c_i/c_a more, as assessed by $\delta^{18}O$, while at the freeway site (*P. abies)* the c_i/c_a ratio was mainly altered by variations in A, as assessed by $\delta^{13}C$. These long-term findings are the result of different water regimes at the two locations. The oil refinery is located in a semi-arid site and the trees must preserve their water reserves by closing their stomates, thus diminishing the effect of NO_x. At the freeway site, the trees are not water limited and respond in accordance with the expected C and O isotope NO_2 induced pattern.
- This study highlights that the triple isotope approach can give a differentiated insight into the A–g_s relationship, representing a promising tool to investigate the effect of N emissions on trees.

Acknowledgements

We acknowledge Anne Kress and Christina Reynolds from PSI for their technical support in the laboratory, Peter Waldner from the WSL and Antonio Lapolla from the University of Basilicata for their help in the field. Climatic data were provided by the "Agenzia Lucana di Sviluppo ed Innovazione in Agricoltura" and the "Agenzia Regionale per la Protezione dell'Ambiente della Basilicata" for Grumento Nova and by the Swiss Meteorological Institute for Lugano. Research was funded by the European Science Foundation, Scientific Program 'Stable Isotope in Biospheric–Atmospheric Exchange' – grant Nr. 800, the Swiss Federal Office for the Environment-Grant 003 MFM-U/Ökonomie und Umweltbeobachtung Nr. 810.3129.008, "Ministero Italiano dell'Università e della Ricerca" – PRIN-project prot. 2005077185_002. We would like to thank the two anonymous referees for helpful discussions and comments on the manuscript.

References

Ammann, M., Siegwolf, R.T.W., Pichlmayer, F., Suter, M., Saurer, M., Brunold, C., 1999. Estimating the uptake of traffic-derived NO_2 from ^{15}N abundance in Norway spruce needles. Oecologia 118 (2), 124–131.

Bernhardt-Römermann, M., Kirchner, M., Kudernatsch, T., Jakobi, G., Fischer, A., 2006. Changed vegetation composition in coniferous forests near to motorways in Southern Germany: the effects of traffic-born pollution. Environmental Pollution 143 (3), 572–581.

Bukata, A.R., Kyser, T.K., 2007. Carbon and nitrogen isotope variations in tree-rings as records of perturbations in regional carbon and nitrogen cycles. Environmental Science & Technology 41, 1331–1338.

Choi, W.J., Lee, S.M., Chang, S.X., Ro, H.M., 2005. Variations of $\delta^{13}C$ and $\delta^{15}N$ in *Pinus densiflora* tree-rings and their relationship to environmental changes in Eastern Korea. Water, Air and Soil Pollution 164 (1–4), 173–187.

Elhani, S., Lema, B.F., Zeller, B., Brechet, C., Guehl, J.M., Dupouey, J.L., 2003. Inter-annual mobility of nitrogen between beech rings: a labelling experiment. Annals of Forest Science 60 (6), 503–508.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 40, 503–537.

Farquhar, G.D., Lloyd, L., 1993. Carbon and oxygen isotope effects in the exchange of carbon dioxide between plants and the atmosphere. In: Ehleringer, J.R., Hall, A.E., Farquhar, G.D. (Eds.), Stable Isotope and Plant Carbon–Water Relations. Academic Press, New York, USA, pp. 47–70.

Freyer, H.D., Kley, D., Volz-Thomas, A., Kobel, K., 1993. On the interaction of isotopic exchange processes with photochemical reactions in atmospheric oxides of nitrogen. Journal of Geophysical Research 98, 14,791–14,796.

Fritts, H.C., 1976. Tree Rings and Climate. Academic Press, New York.

Gebauer, G., Giesemann, A., Schulze, E.D., Jäger, H.J., 1994. Isotope ratios and concentrations of sulfur and nitrogen in needles and soils of *Picea abies* stands as influenced by atmospheric deposition of sulfur and nitrogen compounds. Plant and Soil 164, 267–281.

Grassi, G., Meir, P., Cromer, R., Tompkins, D., Jarvis, P.G., 2002. Photosynthetic parameters in seedlings of *Eucalyptus grandis* as affected by rate of nitrogen supply. Plant, Cell and Environment 25, 1677–1688.

Heaton, T.H.E., 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. Chemical Geology (Isotope Geoscience Section) 59, 87–102.

Högberg, P., 1997. ^{15}N natural abundance in soil–plant systems. New Phytologist 137, 179–203.

Högberg, P., 2007. Nitrogen impacts on forest carbon. Nature 447, 781–782.

Kirchner, M., Jakoby, G., Feicht, E., Bernhardt, M., Fischer, A., 2005. Elevated NH_3 and NO_2 air concentrations and nitrogen deposition rates in the vicinity of a highway in Southern Bavaria. Atmospheric Environment 39 (25), 4531–4542.

Magnani, F., Mencuccini, M., Borghetti, M., Berbigier, P., Berninger, F., Delzon, S., Grelle, A., Hari, P., Jarvis, P.G., Kolari, P., Kowalski, A.S., Lankreijer, H., Law, B.E., Lindroth, A., Loustau, D., Manca, G., Moncrieff, J.B., Rayment, M., Tedeschi, V., Valentini, R., Grace, J., 2007. The human footprint in the carbon cycle of temperate and boreal forests. Nature 447 (7146), 848–850.

Muzika, R.M., Guyette, R.P., Zielonka, T., Liebhold, A.M., 2004. The influence of O_3, NO_2 and SO_2 on growth of *Picea abies* and *Fagus sylvatica* in the Carpathian Mountains. Environmental Pollution 130 (1), 65–71.

Pardo, L.H., Templer, P.H., Goodale, C.L., Duke, S., Groffman, P.M., Adams, M.B., Boeckx, P., Boggs, J., Campbell, J., Colman, B., Compton, J., Emmett, B., Gundersen, P., Kjønaas, J., Lovett, G., Mack, M., Magill, A., Mbila, M., Mitchell, M.J., McGee, G., McNulty, S., Nadelhoffer, K., Ollinger, S., Ross, D., Rueth, H., Rustad, L., Schaberg, P., Schiff, S., Schleppi, P., Spoelstra, J., Wessel, W., 2006. Regional assessment of N saturation using foliar and root $\delta^{15}N$. Biogeochemistry 80, 143–171.

Phoenix, G.K., Hicks, W.K., Cinderby, S., Kuylenstierna, J.C.I., Stock, W.D., Dentener, F.J., Giller, K.E., Austin, A.T., Lefroy, R.D.B., Gimeno, B.S., Ashmore, M.R., Ineson, P., 2006. Atmospheric nitrogen deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N deposition impacts. Global Change Biology 12 (3), 470–476.

Poulson, S.R., Chamberlain, C.P., Friendland, A., 1995. Nitrogen isotope variation of tree rings as a potential indicator of environmental change. Chemical Geology (Isotope Geoscience Section) 125, 307–315.

Saurer, M., Robertson, I., Siegwolf, R.T.W., Leuenberger, M., 1998. Oxygen isotope analysis of cellulose: an interlaboratory comparison. Analytical Chemistry 70, 2074–2080.

Saurer, M., Cherubini, P., Ammann, M., De Cinti, B., Siegwolf, R.T.W., 2004. First detection of nitrogen from NO_x in tree rings: a $^{15}N/^{14}N$ study near a motorway. Atmospheric Environment 38, 2779–2787.

Saurer, M., Siegwolf, R.T.W., 2007. Human impacts on tree-ring growth reconstructed from stable isotopes. In: Dawson, T.E., Siegwolf, R.T.W. (Eds.), Stable Isotopes as Indicators of Ecological Change Terrestrial Ecology Series. Elsevier, Amsterdam, Boston, pp. 49–62.

Scheidegger, Y., Saurer, M., Bahn, M., Siegwolf, R.T.W., 2000. Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. Oecologia 125, 350–357.

Schweingruber, F.H., 1996. Tree Rings and Environment: Dendroecology. Swiss Federal Institute for Forest, Snow and Landscape Research, Paul Haupt Verlag, Berne, pp. 609.

Sheppard, P.R., Thompson, T.L., 2000. Effect of extraction pre-treatment on radial variation of nitrogen concentration in tree rings. Journal of Environmental Quality 29 (6), 2037–2042.

Siegwolf, R.T.W., Matyssek, R., Saurer, M., Maurer, S., Günthardt-Georg, M.S., Schmutz, P., Burcher, B.J., 2001. Stable isotope analysis reveals differential effects of soil nitrogen and nitrogen dioxide on the water use efficiency in hybrid poplar leaves. New Phytologist 149, 233–246.

Tolunay, T., 2003. Air pollution effects on annual ring widths of forest trees in mountainous land of Izmir (Turkey). Water, Air and Soil Pollution: Focus 3, 227–242.

Vitousek, P.M., Hättenschwiler, S., Olander, L., Allison, S., 2002. Nitrogen and nature. Ambio 31 (2), 97–101.

Warren, C.R., Adams, R.A., 2006. Internal conductance does not scale with photosynthetic capacity: implications for carbon isotope discrimination and the economics of water and nitrogen use in photosynthesis. Plant, Cell and Environment 29, 192–201.

Wilczyński, S., 2006. The variation of tree-ring widths of Scots pine (*Pinus sylvestris* L.) affected by air pollution. European Journal of Forest Research 125, 213–219.