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ORIGINAL PAPER

Tree-ring growth and stable isotopes (^{13}C and ^{15}N) detect effects of wildfires on tree physiological processes in *Pinus sylvestris* L.

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Abstract Forest fires may alter the physiological and growth processes of trees by causing stress in trees and modifying the availability of soil nutrient. We investigated if, after a high-severity fire, changes in tree-ring growth can be observed, as well as changes in the nitrogen and carbon isotope composition of tree rings of surviving trees. Two wildfires that occurred in *Pinus sylvestris* L. stands in Northern Italy, one at the beginning and one at the end of the vegetative season, were chosen as the focus of this study. After the fires, the surviving trees showed growth suppression with very narrow tree rings or locally absent rings. The carbon isotope ratio was more negative in tree rings formed in the 5 years following fire, indicating better water supply in a situation of less competition. The nitrogen isotope ratio followed opposite trends in the two wildfire stands. In trees cored in the stand where the fire happened at the beginning of the vegetative season, there was no change in the nitrogen isotope ratio, whereas in samples collected in the other fire site, higher nitrogen isotope ratios were observed in the tree rings formed after the fire, reflecting changes in the soil nitrogen supply. Modifications in the growth and isotope composition of the fire-stressed trees disappeared from 6 to 10 years after the fire. By studying trees before and after fire, we were able to show that fire affects not only the growth of surviving trees, but also their physiological processes.

Keywords Wildfires · Tree rings · δ^{13}C · δ^{15}N · *Pinus sylvestris* L.

Introduction

Fire is one of the most important disturbances that can affect an ecosystem's dynamics (Neary et al. 1999), as it can modify nutrient cycles, species composition and plant growth. Fires differ in their intensity, frequency, season of occurrence, and spatial extent according to vegetation and physical factors such as topography, soil type and local climatic conditions (Whelan 1995). Fire can kill or injure a tree directly depending on the magnitude of the crown scorch or bole injury (Bond and van Wilgen 1996; DeBano et al. 1998; McHugh and Kolb 2003) and how much the vegetation physiology is affected (Rieske 2002). Indirectly, it can modify the soil environment and the availability of resources (Giovannini and Lucchesi 1997; Certini 2005). Although, such effects of wildfires on forest soil and on trees are closely related and critical for understanding post-fire dynamics better, they are still poorly understood.

In studies of the ecological impact of wildfires, tree-ring analysis has often been applied, mainly in North America (Weaver 1951; Arno and Sneck 1977; Dieterich 1980; Bergeron 1991; Swetnam 1993; Grissino-Mayer et al. 2004), but also in Northern Patagonia (Veblen et al. 1999), and Scandinavia (Niklasson and Granström 2000).

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The most common application involves dating the fire frequency by analysing the fire scars that usually occur in the cambium zone, when wood and cambial cells are killed by fire-induced high temperatures (Brown and Smith 2000). However, scars are not always induced, and where no fire scars are evident, it would be useful to develop proxies of fire disturbances to be used as tracers of historical fires when visible signs are not present. In this context, we tested tree-ring width, carbon isotope ratio ($^{13}C/^{12}C$ expressed as $\delta^{13}C$) and nitrogen isotope ratio ($^{15}N/^{14}N$ expressed as $\delta^{15}N$) in wood, along with wood N concentrations as potentially useful proxies for detecting past occurrence of fire and their impact.

The stable isotope composition of wood material has often been used in ecophysiological studies to supply information about past environmental conditions and plant physiological processes (McCarroll and Loader 2004). The $\delta^{13}C$ of plant tissue is an indicator of long-term intercellular carbon dioxide concentrations, which are determined by the balance between stomatal conductance and photosynthesis, and thus an indicator of water-use efficiency in C_3 plants. It also provides an integrated measure of plant responses to changes in the environment such as water availability (Ehleringer and Cooper 1988), or irradiance (Zimmerman and Ehleringer 1990). Fires can alter the water and nutrient availability in a forest ecosystem. Trees' responses to these environmental constraints have an impact on leaf gas-exchange and possibly also on plant growth.

The nitrogen concentrations and $\delta^{15}N$ in plant samples are not only indicators of plant and soil nitrogen metabolism, but can also function as indicators of fire effects. Several previous studies have tried to assess the effects of fire on stable isotopes in plants after fire. Högberg (1997) noted that fire consumes the relatively ^{15}N-depleted organic soil layers, causing the plants' roots to penetrate deeper into the soil after the fire to seek deeper, more ^{15}N-enriched sources, which results in a net increase in foliar $\delta^{15}N$ after a fire. Similarly, Grogan et al. (2000) found that all plant and soil samples were enriched in ^{15}N after fire in a Californian bishop pine (*Pinus muricata* D. Don) forest. On the other hand, Cook (2001) found greater foliar $\delta^{15}N$ in rainforests and fire-excluded mesic savannahs than in fire-prone savannahs in Australia. He speculated that this pattern was attributable to post-fire nitrification and nitrate uptake by plants. Post-fire nitrification has been previously found to cause a strong increase in soil NO_3^-. Because nitrification discriminates against ^{15}N, this may in turn lead over time to a ^{15}N-enriched residual total N pool over (Herman and Rundel 1989; Högberg 1997). While the processes influencing $\delta^{13}C$ are well understood, the relationship between $\delta^{15}N$, and soil and wood is still poorly understood mainly because little is known about the fractionating processes that occur at root level.

To explore the effects of fire on the stable isotopic ratio in the tree rings of surviving trees, we established two different hypotheses:

1. A high-severity fire, can badly damage a tree's cambium, which is insulated from fire by bark (Bond and van Wilgen 1996), if it reaches a lethal temperature (*T* of 60°C for longer than 1 min). The crown may be scorched and the leaf surface reduced, thus altering the plant's photosynthetic rates and efficiency (DeBano et al. 1998). A fire makes the soil lose nutrients accumulated in above-ground biomass to the atmosphere (Grogan et al. 2000). In particular, it may result in less nitrogen in the soil due to volatilization and erosion (Duran et al. 2008). If these processes occur, we expect lower tree-ring growth rates after fire, a decrease in photosynthesis rates and, consequently, less negative $\delta^{13}C$ values. Probably, after fire, $\delta^{15}N$ will change due to the different quantity and type of nitrogen available for trees.
2. Trees are damaged by fire, but among those that survive, there is less competition for both water and nutrients. As a result more water and more photosynthates are available, accompanied by more negative $\delta^{13}C$, inducing higher tree-growth rates and stomata-conductance activities. Much more nitrogen is available in the soil, resulting in very high nitrogen concentrations.

In this study, we explore these two hypotheses to find out more about the effects of fire on plant ecophysiological processes in the Mediterranean region, where fire plays an important ecological role. The long-term aim is to improve silvicultural strategies and techniques for managing forest ecosystems after wildfires.

Materials and methods

Study sites

Our study was performed in two pure *Pinus sylvestris* L. which stands in the north-western Italian Alps.

The two areas were chosen according to two criteria: (a) the trees growing there should be old enough to have sufficient tree rings formed after the fire occurred, (b) detailed information about fire behaviour, size and timing should be available from written records of local foresters.

The first study area (Lat. 45°46′, Long. 7°33′) is located in the Valle d'Aosta Region near Verrayes (Aosta Province). Here, on 4th September 1995 a high-severity fire burned an area of 51 ha including 36 ha of *Pinus sylvestris* forest, and only a few trees survived. We analysed one pine stand (site AOB) in the burned area, and a control site (AOC) about 100 m from the edge of the burned area in the same Scots pine forest.

The second study area (Lat. 44°30′, Long. 7°11′), where the third site (MCB) is located, is situated in Piedmont Region near Macra (Cuneo Province). Here, a high-severity fire occurred on 1st March 1990 burning an area of 3,190 ha, of which 2,066 ha were covered by *Pinus sylvestris* forest. Here it was impossible to select a control site, because the wildfire burned a much larger area and no similar site could be found outside the burned area.

At AOB and AOC, the mean annual temperature is 7.4°C, and mean annual precipitation is 718 mm. These data (from 2002 to 2006) were recorded at the Saint Denis (Aosta) weather station, 840 m a.s.l., about 8 km from the study site. The soils are classified as entisols (Soil Taxonomy USDA). The herbaceous layer is characterized by *Arctostaphylos uva-ursi* L., *Epilobium angustifolium* L., *Bromus erectus* Huds., *Ononis* sp..

At MCB the mean annual temperature is 9.2°C and the mean annual precipitation 771 mm. These data (from 2001 to 2008) were recorded at the San Damiano Macra (Cuneo) weather station, 1095 m a.s.l., about 10 km from the study site. The soils are classified as entisols (Soil Taxonomy USDA) (IPLA 2007). In the understory, we can find *Cytisus sessilifolius* L., *Teucrium* sp., *Bromus erectus* Huds., *Lavandula angustifolia* Mill., *Ononis* sp..

Sampling and sample preparation

We sampled 20 trees which survived at each "burned" sites (AOB and MCB) and 20 trees at the control site (AOC). Trees were sampled at the end of 2008. From 15 trees (out of the 20 trees), we collected two cores to build a mean ring-width chronology, and from five trees, we collected four cores to provide enough material for isotopic analyses. All cores were collected at 130 cm above ground using an increment borer 5 mm in diameter. The selected trees did not have any evident fire scars.

In the laboratory, the cores selected for the ring-width analyses were glued on channelled wood, dried at room temperature, and sanded with progressively finer grade abrasive paper until optimal surface resolution allowed the annual rings to be recognized and detected under magnification.

The cores for isotopic analyses were prepared for further dating analysis using only a scalpel. Glue and sandpaper were not used, as they would have contaminated the samples. Glue has an own isotopic ratio and sandpaper might have transported wood dust from one ring to others.

Dendrochronological and dendroclimatic analyses

The tree rings of each core were dated, and each tree ring assigned to its exact year of formation. Tree-ring chronologies were developed using standard dendrochronological procedures (Stokes and Smiley 1968). Tree rings were identified and dated by counting them from bark to pith with the help of a stereomicroscope (magnification 6.4–40×, Wild M3Z, Leica, Germany). Ring-width measurements were made to the nearest 0.01 mm using Time Series Analysis and Presentation (TSAP) software package and LINTAB measuring table (Rinntech, Heidelberg, Germany).

The tree-ring series were visually synchronized to identify local absent rings (LARs) and to check for errors (Fritts 1976). To facilitate comparisons between cores, we also used digital photographs of the samples. The raw tree-ring widths of each dated core were visually checked using TSAP, and then synchronized according to the *Gleichläufigkeit*, a measure of the year-to-year agreement between the interval trends of two chronologies based on the sign of agreement (Schweingruber 1988; Kaennel and Schweingruber 1995), and Student's *t* test, which determines the degree of correlation between the curves. The COFECHA software was used to perform a data quality control and to evaluate the cross-dating (Grissino-Mayer 2001). LARs were given ring widths equal to zero (Fritts and Swetnam 1986).

The first differences method ($DY_t = Y_t - Y_{t-1}$), i.e., one-year ring width (Y_t) minus the previous one (Y_{t-1}), was applied to all series to remove the age trend without altering the fire effects (Saurer et al. 1995; Loader and Switsur 1996; Robertson et al. 1997; Anderson et al. 1998; Schleser et al. 1999; Battipaglia et al. 2007). The resulting curves are called index curves.

For all the raw ring-width series, we calculated the mean sensitivity, i.e., the mean percentage change between each annual ring value measured and the next (Fritts 1976), and the autocorrelation coefficient, which is a measure of the correlations between rings lagged in time. Between each series and the average series, we calculated a correlation coefficient which measures the interdependence of association between two data sets and Student's *t* test to verify significance.

Dendroclimatic analysis was used to evaluate the effects of monthly precipitation and temperature on tree-ring growth. We considered the grid (Coarse Resolution Subregional LOW 6) from the HISTALP dataset (Auer et al. 2007) and data from 1902 to 2005 for the AOB and AOC sites, and data from 1923 to 2005 for the MCB site. To correlate monthly precipitation (P) and temperature (T) with the tree-ring index, we applied the first differences method both to temperature and precipitation. To exclude the effect of climate, we analyzed the correlations between *T* and *P* and the tree-ring index for the periods before and after the fire.

Isotopic analysis and statistics

Cores selected for isotopic analyses were dated in order to define the exact year of formation of each tree ring. No pencil was used during any part of the analysis because of contamination risks. From each core, we analyzed only 15 tree rings split into blocks of 5 years each: five before the year of occurrence of the wildfire and ten (divided into two pools of 5 years) after the wildfire. At AOB, the fire occurred after the vegetation period 1995, so that the first tree ring after the fire was formed in 1996. At MCB, the first tree ring after the fire was formed in 1990 because the fire occurred in March, when the vegetative season had started. At AOB and AOC, we considered the tree rings dated between 1991 and 1995 as those formed before the fire, and these between 1996 and 2000 and between 2001 and 2005 as those formed after the fire. At MCB, the years before the fire were 1985–1989, and after the fire 1990–1994 and 1995–1999.

Before starting with nitrogen extraction, all samples were divided into groups (of 5 years each one) from which we removed extractable mobile N-compounds according to the methods described in Sheppard and Thompson (2000). A Soxhlet apparatus was used to remove extractable N-compounds from the samples. The samples were left 18 h in a mixture 50:50 toluene and ethanol, then for 18 h in ethanol, and finally for 18 h in distilled water (Sheppard and Thompson 2000; Saurer et al. 2004). After each treatment, the samples were washed with distilled water and finally dried in an oven for 20 h at 60°C. The weight lost from the beginning to the end was 8%. The samples were then milled using a centrifugal mill and each sample prepared according to the required weight (20 mg for δ^{15}N‰ analysis and 0.7–0.8 mg for δ^{13}C‰) and enclosed in tin capsules.

The δ^{13}C‰ and δ^{15}N‰ of the tree rings were determined using an elemental analyzer connected to an isotope-ratio mass spectrometer (Delta S, Finningan MAT, Bremen, Germany) via a variable open-split interface (ConFlo II, Finningan MAT, Bremen, Germany). Wood samples have a low N-concentration respect to the C-content and a blank (empty tin capsule) was measured after every sample to eliminate the effect of the large amount of CO_2. We used the data provided in Francey et al. (1999) and McCarroll and Loader (2004) to remove the atmospheric δ^{13}C trend from the carbon isotope data series. The corrected series were used for all statistical analyses. The results of carbon and nitrogen isotope ratio are presented in per mil (‰) as: $\delta = [(R_{sample}/R_{standard}) - 1] \times 1{,}000$, where R_{sample} is the ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$ in the sample and $R_{standard}$ in the standard. The international standard is atmospheric N_2 for δ^{15}N and VPDB for δ^{13}C.

Differences between the isotopic data were tested using the Mann–Whitney *U* test, a non-parametric test that can be used with a small number of observations or, alternatively, when observations in one sample tend to be larger than observations in the other. We tested for differences between data from the same area during different periods and, for the same periods, for differences between samples collected at the sites AOB and AOC. Differences in the tree-ring index width during the periods before and after the fire were tested using Student's *t* test. All statistical analyses were carried out with the program SPSS (Version 16, Chicago, IL, USA).

Results

Site chronology

Mean ring-width chronologies were built at all the sites, and both raw ring-width and index chronologies are shown in Figs. 1a, b and 2. The dendrochronological statistical characteristics of the raw ring-width data are summarized in Table 1.

LARs were individuated in several of the trees that survived in the burned areas. At AOB, 60% of the cores had LARs and at MCB 69%, all occurring after fire. At

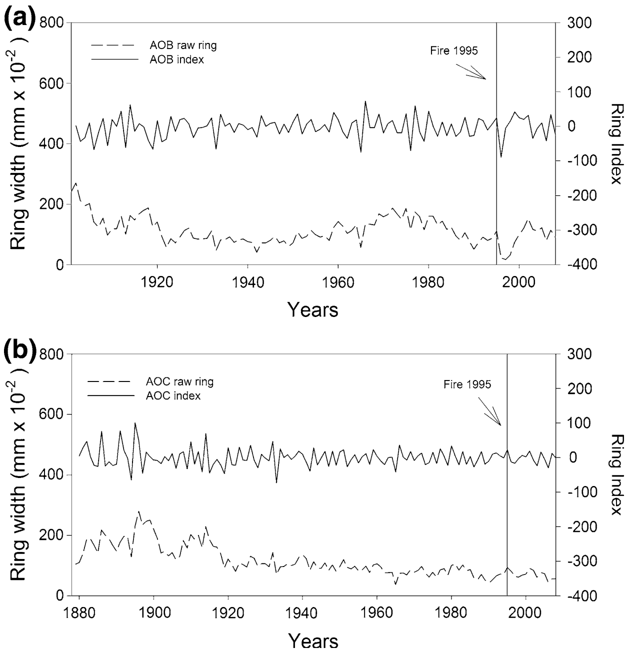

Fig. 1 Raw ring-width chronologies (*dashed lines*) and index ring values (*continuous lines*) built for site AOC (**a**) and AOB (**b**). Year of fire is 1995

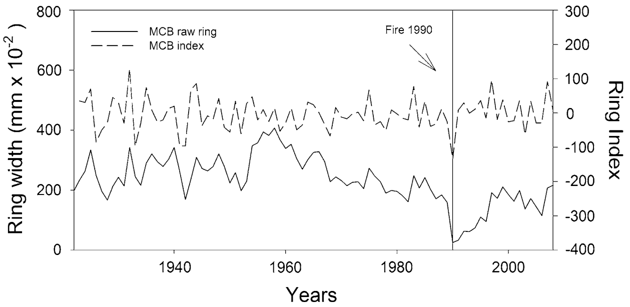

Fig. 2 Raw ring-width chronologies (*dashed lines*) and index ring values built for site MCB. Year of fire is 1990

AOC, only one core had LARs but in the time period before the fire occurred (years 1917 and 1934).

According to Student's *t* test, significant differences in the indexed ring-widths ($P < 0.05$) were found between the period before the fire (1991–1995) and the two periods after the fire (1996–2000 and 2001–2005) at AOC, but the standard deviations were similar for all three periods (17.80, 19.55, 23.22). At AOB significant differences were found between the period before the fire (1991–1995) and the two periods after the fire (1996–2000 and 2001–2005), but none between the two periods after the fire. The standard deviations were different between the period before the fire (23.44) and the two periods after the fire (51.51 and 48.38). At MCB, there were significant differences ($P < 0.05$) between the period before the fire (1985–1989) and the second period after the fire (1995–1999), and between the two periods after the fire (1990–1994 and 1995–1999). The standard deviations were 43.38 for 1985–1989, 83.90 for 1990–1994 and 71.01 for 1995–1999.

At AOB the mean sensitivity (MS) of the raw ring-width series was 0.345, the lowest was 0.269 and the highest 0.463. Furthermore, for a more detailed analysis, we considered the MS for each of the three periods separately. The MS before the fire was 0.320 (0.099–0.569), for 1996–2000 it was 1.062 (0.417–1.828), and for 2001–2005 it was 0.398 (0.061–1.311). Since there were many LARs, we calculated the MS considering only cores without LARs. The MS was 0.313 (0.167–0.470) before the fire, 0.763 (0.417–1.223) in the 5 years after the fire and 0.295 (0.061–0.701) for the period 2001–2005.

At AOC, the MS was 0.245 (0.178–0.331) for all the period, for the period before the fire it was 0.307 (0.100–0.492), for 1996–2000 it was 0.251 (0.102–0.530), and for 2001–2005 it was 0.287 (0.092–0.511).

At MCB, MS was 0.292 (0.172–0.407). The MS for the period before the fire was 0.232 (0.068–0.403), for 1996–2000 it was 0.973 (0.198–1.466), and for 2001–2005 it was 0.506 (0.240–0.758). As with AOB, we also considered samples without LARs. Then the MS for all periods was 0.245 (0.172–0.328), for the 5 years before the fire it was 0.185 (0.068–0.354), for the 5 years after the fire it was 0.640 (0.198–0.987), and from 6 to 10 years after the fire it was 0.380 (0.240–0.639).

Overall, we observed a difference in both the growth and the mean sensitivity during the 5 years following the fire. From 6 to 10 years after the fire, however, all the parameters considered had values similar to those for the period before fire. We correlated the temperature, precipitation and tree ring-width index time series (obtained from applying the first differences method) to clarify the effect of climate on the tree growth. Table 2 shows the correlation coefficients between the tree-ring index and *T* and *P* for each site.

At AOB, the tree-ring index correlated significantly with both temperature (February, $P < 0.005$) and precipitation (May, August, September, $0.005 > P < 0.001$) during the period before the fire, but there was no significant correlation after the fire. At AOC, the tree-ring index correlated significantly with precipitation (May, June, July, September, $0.005 > P < 0.001$) during the whole period and also during the periods before (May, July, September, $0.005 > P < 0.001$), and after the fire (March, $P < 0.001$). At the same site, there was no significant correlation with temperature during the whole period or that before the fire, but there was a significant correlation after the fire (June,

Table 1 Dendrochronological characteristics of the raw ring-width data

	AOC	AOB	MCB
Number of dated cores	48	46	45
Master series length (years)	130 (1879–2008)	108 (1901–2008)	87 (1922–2008)
Mean correlation with the master*	0.718	0.694	0.620
Mean ring width (1/100 mm)	96	109	214
Mean standard deviation	0.502	0.615	1.262
Mean sensitivity	0.245	0.345 (0.331)	0.294 (0.245)
Mean sensitivity before fire	0.280	0.283 (0.281)	0.219 (0.203)
Mean sensitivity after fire	0.262	0.596 (0.465)	0.474 (0.361)

* Critical coefficient at $P < 0.01 = 0.4226$; (values of only cores without LAR)

Table 2 Correlation coefficient (r) between tree ring index and monthly temperature (T) and precipitation (P)

Sites	Monthly T all period	Monthly T before fire	Monthly T after fire	Monthly P all period	Monthly P before fire	Monthly P after fire
AOB	February (0.237*) March (0.197*)	February (0.212*)	–	March (0.212*) May (0.285**) September (−0.254**)	May (0.276**) August (0.217*) September (−0.319**)	–
AOC	–	–	June (−0.727*) October (0.812**)	May (0.365**) June (0.195*) July (0.258**) September (−0.201*)	May (0.359**) July (0.271**) September (−0.247*)	March (0.762*)
MCB	June (−0.232*) September (0.247*)	January (0.250*)	December (0.517*)	July (0.345**)	July (0.294*)	July (0.606*)

Correlation was calculated considering all period for which data were available (AOC, 1880–2005, AOB, 1902–2005, MCB, 1923–2005) and for the period before and after fire

* $P < 0.005$

** $P < 0.001$

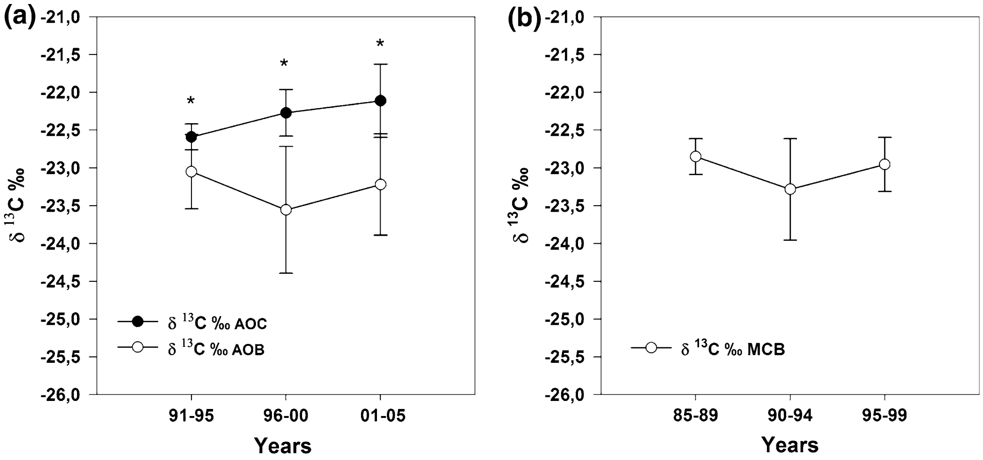

Fig. 3 The mean δ^{13}C-values of the tree rings (5-year intervals) measured for sites AOB, AOC (**a**) and MCB (**b**). Groups of years 1991–1995 and 1985–1989 are before fire, intervals 1996–2000, 2001–2005, 1990–1994, 1995–1999 are after fire. *Bars* are standard deviation

October, $0.005 > P < 0.001$). At MCB, the correlations with precipitation were significant in July ($0.005 > P < 0.001$), in the periods before and after the fire, and in the whole period. Temperatures were significantly correlated in June and September ($P < 0.001$) in the whole period, in January ($P < 0.001$) for the period before the fire, and in December ($P < 0.001$) for the period after the fire.

Carbon isotopic analyses

The tree-ring δ^{13}C ‰ values at the three sites are shown in Fig. 3. In both study areas, the pines that survived the fire had slightly, but not significantly, more negative values in the 5 years after the fire than in the 5 years before the fire, and in the 6 to 10 years after the fire. Tree-ring δ^{13}C ‰ values at AOC became less negative with time. Mann–Whitney's U test showed significant differences between AOC and AOB for the same periods (1991–1995: AOC vs. AOB, $P = 0.028$; 1996–2000: AOC vs. AOB, $P = 0.016$; 2001–2005: AOC vs. AOB, $P = 0.028$), whereas there were no significant differences between the periods at the same sites.

Considering the single trees (data not shown), at AOC all δ^{13}C ‰ values were less negative than at AOB and MCB. At AOB, all the surviving trees presented more negative δ^{13}C ‰ values in the 5 years after the fire (1996–2000) than before it. In the period 2001–2005, values rose again to levels of those in the period before the fire (1991–1995). In contrast, at MCB no such homogeneous trend was found among the trees that survived.

Nitrogen isotopic and concentration analyses

At AOB and AOC, the δ^{15}N‰ signals in the tree rings were not significantly different before and after fire. There were, however, significant differences during the periods 1991–1995 ($P = 0.009$) and 1996–2000 ($P = 0.009$) between AOB and AOC (Fig. 4a). At MCB, the mean tree-

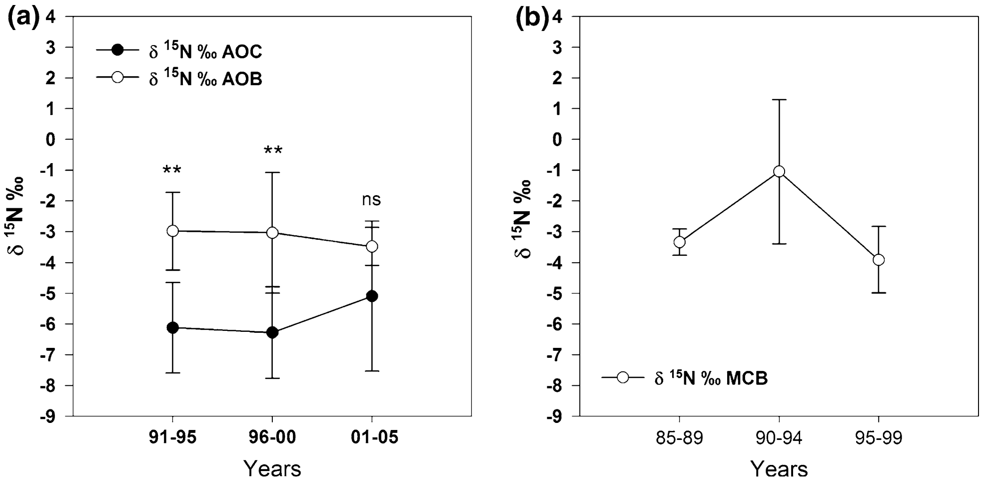

Fig. 4 The mean δ^{15}N-values of the tree rings (5-year intervals) measured for sites AOB, AOC (**a**) and MCB (**b**). Groups of years 1991–1995 and 1985–1989 are before fire, intervals 1996–2000, 2001–2005, 1990–1994, 1995–1999 are after fire. *Bars* are standard deviation

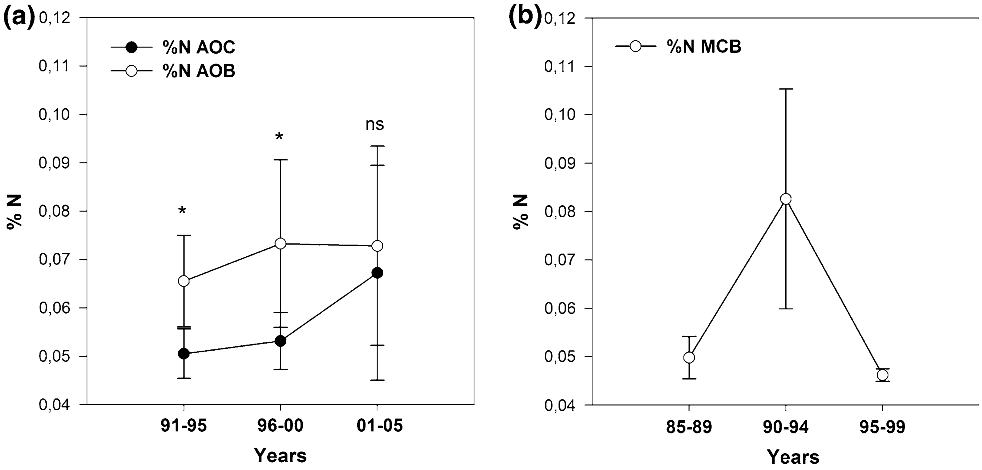

Fig. 5 The mean N concentration-values of the tree rings (5-year intervals) measured for sites AOB, AOC (**a**) and MCB (**b**). Groups of years 1991–1995 and 1985–1989 are before fire, intervals 1996–2000, 2001–2005, 1990–1994, 1995–1999 are after fire. *Bars* are standard deviation

ring δ^{15}N‰ values of the surviving trees were significantly different in the years 1990–1994 and 1995–1999 ($P = 0.047$) (Fig. 4b). The δ^{15}N‰ values of single trees at MCB present a homogenous trend in comparison with those at AOB. All δ^{15}N‰ measured in the tree rings of the surviving trees, at AOB were less negative than those at AOC.

The nitrogen concentrations (%N) in tree rings also differed at the two sites AOB and AOC (Fig. 5), but not during the different time periods. As with the δ^{15}N‰ values, the nitrogen concentrations in the AOB and AOC areas during 1991–1995 differed significantly from those in 1996–2000 (1991–1995: AOB vs. AOC, $P = 0.016$; 1996–2000: AOB vs. AOC, $P = 0.047$). At MCB, the nitrogen concentrations in the years 1985–1989 differed significantly from those in 1990–1994 ($P = 0.027$) and in the years 1990–1994 differed significantly from those in 1995–1999 ($P = 0.009$).

At AOB the surviving trees had varying trends in their nitrogen concentrations, whereas the trees sampled at MCB all had the same trend, i.e., the values of nitrogen were higher in the 5 years following the fire than in those before the fire.

Accordingly, at MCB both δ^{15}N and %N tended to be higher after the fire, while from 6 to 10 years after the fire, the values were more similar to the period before fire. At AOB, δ^{15}N had the same values in all three periods, whereas %N was higher after the fire than before. At AOC, both δ^{15}N and %N were higher after the fire than before.

Discussion

Analyses of ring width

We had difficulties cross-dating the ring-width series because of LARs. However, the synchronous occurrence of pointer years, i.e., years in which particularly narrow (1965, 1933, 1922) or wide (1932) rings formed, in the mean ring-width chronologies for all the trees in both areas indicate that the tree rings were correctly dated.

Although sites AOC and AOB are close together, they present differences in tree-ring growth, and in relation with precipitation and temperature. The reduction in ring width during the period after 1995, i.e., year of fire, are probably due to the effect of fire on the trees' cambium, on soil properties and on nutrient availability. The differences between the periods before and after the fire at AOC site may have been caused by the local topography, the effects of which depend on soil and air temperatures, evapotranspiration and other complex aspects (Oberhuber and Kofler 2000). At the MCB site, in contrast, we found significant differences in tree-ring growth before and after the fire which probably only have to do with the fire, since tree-ring growth correlated significantly with precipitation in July in all years, and in the years before and after the fire considered separately.

The differences in tree-ring growth at AOB and AOC even before the fire show how the micro-topography and local conditions may affect growth (Cherubini et al. 2003). This is one reason why we could not have a control stand in Macra, where the fire affected a very large area. The closest area that had not been burned by the fire was at least at 5 km far away from the MCB site, and had very different topographic properties.

Carbon isotope variations

The isotopic composition of carbon stored in the tree rings represents a record of the trees' physiological responses to environmental changes (Francey and Farquhar 1982) and to natural disturbances (Simard et al. 2008). In particular, it also reflects the water-use efficiency (Elhani et al. 2005). Plants can discriminate against ^{13}C (O'Leary 1988), because biological processes tend to use ^{12}C in preference to ^{13}C (McCarroll and Loader 2004). An increase in δ^{13}C (less negative values) reflects faster tree photosynthesis rates or stomata closure and consequently less discrimination against ^{13}C by Rubisco. More negative values mean that stomata are relatively open and Rubisco can discriminate against ^{13}C. Stomatal activity can be influenced by water availability and is expressed in the δ^{13}C of the photosynthetic products, and ultimately in the tree rings (Dawson and Siegwolf 2007).

We found more negative δ^{13}C values during the 5 years after the fire at both the AOB and MCB sites, whereas in the control area, during the same period, the δ^{13}C values were less negative. This difference can be explained by the weaker competition for water among the trees that survived in the burned areas than prior to the fire, when there were more competitors. It also shows that the damage at the crown level can be compensated for, if there is more water available. Ring widths are smaller after fire while reduced δ^{13}C values indicate an improvement in water use. This seemingly contradictory result has to do with the fact that, although the canopy is smaller after a fire, the wood structure was damaged (Bigio et al. 2010), and trees'-ring widths are therefore narrower and the remaining leaves have optimal conditions.

Variation in nitrogen concentration

Nitrogen concentrations in tree rings increase with age and are normally highest in the outermost ring which mainly consists of living cells (Merrill and Cowling 1966; Helmisaari and Siltala 1989). This trend is clear at AOC, where no disturbance occurred. The stems concentrations of nutrients in tree change under different conditions for nutrient uptake and phases in the annual cycle. In the inner bark of *Pinus sylvestris*, Helmisaari and Siltala (1989) found the highest concentrations of nutrients were in February and May, which shows that nutrients can be stored in the inner bark during winter because there are no active nutrient sinks. These stored nutrients can then be used for growth during the next growing season.

During maturation, trees are able to recycle the nitrogen present in their cambial cells (Pallardy and Kozlowski 2008) and re-allocate it from the older tissues (Helmisaari and Siltala 1989). At MCB, during the 5 years after the fire, the nitrogen concentration was higher than the period before the fire, but then decreased. This suggests the nitrogen is re-used to counter stress caused by fire. Five years after a fire, however, a tree seems no longer to need to reallocate nitrogen. Alternatively, the lower nitrogen concentration may reflect a lack of nitrogen. At AOB, the nitrogen concentration was also higher during the 5 years after the fire than before, but after that (from 6 to 10 years after the fire) it remained constant. These differences between AOB and MCB may have arisen because the fires occurred during different seasons at the two sites, i.e., in spring at MCB and in autumn at AOB.

Nitrogen isotope variation

Nitrogen is the chemical element that limits plant growth in the most forest ecosystems (Evans 2001).

During fire, organic nitrogen in soil is removed through volatilisation caused by heat, but fires may also convert soil organic nitrogen into inorganic forms (ammonium NH_4^+ and nitrate NO_3^-) that then become available for plant absorption (Certini 2005). Covington and Sackett (1992) found that ammonium also increases immediately after fire, and that 1 year later, nitrate concentrations also increases. Five years after the fire, however, both ammonium and nitrate disappear. Duran et al. (2008) measured higher concentrations of ammonium and nitrate 1 year after a fire than at control plots, but 5 years after the fire differences between the control and burned samples were no longer found.

We obtained similar results. At MCB, we found higher values of δ^{15}N in the period of 5 years after the fire, but later (6–10 years after fire) δ^{15}N‰ values returned to a similar level to those before the fire. We found no such effect at AOB, probably because the fire occurred in the autumn, when there was more erosion and nitrogen leaching. Tree-physiological enzyme-mediated reactions, which mostly take place at the root level, discriminate against ^{15}N. Consequently there are differences in δ^{15}N composition between the N source in the soil and the tree (Dawson et al. 2002). Each species has different strategies for using ammonium and nitrate. For example, *Pinus* sp. prefers NH_4 to NO_3 (Certini 2005).

Conclusions

Our work is the first study on tree-ring isotopic carbon and nitrogen concentrations in trees that had survived a high-severity wildfire. In this paper, we have described some of our more important findings namely:

- After a wildfire, significantly more locally absent rings seem to form in surviving trees during the 5 years following fire, than at an unburned control site and during the period prior to fire in the burned areas.
- δ^{13}C‰ seems to dramatically decrease in burned areas during the 5 years following a fire, probably due to the trees being in less competition for water and nutrients.
- Finally, the ecophysiological response of trees that survived fire is different depending on the season, in which, the fire occurred. Both δ^{15}N‰ and N% tend to reach a maximum during the 5 years following a wildfire, if it occurs in spring.

We observed a variation in the isotopic signal of tree rings after fire. However, only two stands were studied, and therefore, it is difficult to attribute different responses between the two stands to spring versus autumn fires. Further studies at more sites are needed to understand the different impact of early versus late growing season fires on tree physiological processes.

Some authors assert that δ^{15}N cannot be used as a tracer, but others claim that δ^{15}N can serve as method to analyse past changes in the N regime in forests (Elhani et al. 2005). We believe the use of isotopic analyses to detect past fires is promising.

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