Couto-Vázquez, A. and González-Prieto, S.J. (2010). Effects of climate, tree age, dominance and growth on $\delta^{15}\text{N}$ in young pinewoods. *Trees* 24, 507-510.

Abstract

Needles, annual rings from basal stem disks and bark of 3 dominant and 3 suppressed *Pinus pinaster* from a 12-year-old pine stand (naturally regenerated after a wildfire) were analysed to study the effects of climate, tree age, dominance and growth on tree $\delta^{15}\text{N}$. Foliar-N concentration in dominant pines (0.780-1.474 % N) suggested that soil N availability was sufficient, a circumstance that allowed isotopic discrimination by plants and (greater) differences in $\delta^{15}\text{N}$ among trees. The $\delta^{15}\text{N}$ decreases in the order wood (-0.20 to +6.12 ‰), bark (-1.84 to +1.85 ‰) and needles (-2.13 to +0.77 ‰). In all trees, before dominance establishment (years 1-8), the N stored in each ring displayed a decreasing $\delta^{15}\text{N}$ tendency as the tree grows, which is mainly due to a more "closed" N cycle or an increasing importance of N sources with lower $\delta^{15}\text{N}$. After dominance establishment (years 9-12), wood $\delta^{15}\text{N}$ values were higher in suppressed than in dominant trees (2.62‰ and 1.46‰, respectively; $P < 0.01$) while the reverse was true for needles and bark; simultaneously, the absolute amount of N stored by suppressed pines in successive rings decreased, suggesting a lower soil N assimilation. These results could be explained by lignification acting as major N source for needles in suppressed pines because products released and reallocated during lignification are ^{15}N -depleted compared to the source. According to principal component analysis, wood $\delta^{15}\text{N}$ appears associated with wood N concentration and precipitation during the growing season, but clearly opposed to age, basal area increment and mean temperature in spring and summer.

Key words: bark, needles, tree rings, *Pinus pinaster*.

Introduction

Forests currently cover 30 % of Europe and, after a very long human influence, most of them are 'semi-natural' planted forests of native (70 %) and non-native species (30 %). As a result, European forests are dominated by commercial plantations of even-aged stands of few tree species, intensively managed and characterised by highly simplified forest structure and composition (European Environment Agency 2006). *Pinus pinaster* Ait. has a broad natural distribution in the western

Mediterranean Basin and the Atlantic coast of Portugal, Spain and France; it is also widely used in afforestation programmes even outside the natural range, either for ecological or economical reasons, because of its ability to grow in very poor soils and under prolonged drought.

Plant and soil $\delta^{15}\text{N}$ is a basic and powerful tool in environmental sciences and N-cycling studies (Handley and Scrimgeour 1997; Robinson 2001). Soil $\delta^{15}\text{N}$ values reflect the net effect of biotic and abiotic environment on N-cycling processes (Dawson et al. 2002). At the ecosystem level, $\delta^{15}\text{N}$ is influenced by the quantity and quality of organic matter inputs, N sources and isotopic fractionation during N transformations (Nadelhoffer and Fry 1988). At the plant level, $\delta^{15}\text{N}$ is affected by N source, physiological transformations in the plant, rooting depth and mycorrhizal association (Handley and Scrimgeour 1997; Hobbie and Hobbie 2006). Following the first results of Poulson et al. (1995) proving the potential utility of N isotope analysis of tree rings as a method to investigate the long-term biogeochemical behaviour of N, in recent years there is an increasing use of $\delta^{15}\text{N}$ in tree rings to study temporal changes on N cycle. Most of the published studies deal with the use of tree-ring $\delta^{15}\text{N}$ as a registry of the long-term effects of fertilization and N deposition on forests (Elhani et al. 2003; Hart and Classen 2003; Saurer 2004; Choi et al. 2005a,b; Elhani et al. 2005; Bukata and Kyser 2007; McLauchlan et al. 2007; Savard et al. 2009). A few papers have used tree-ring $\delta^{15}\text{N}$ to monitor the effects of tree-clearing, land-use changes and elevating levels of atmospheric CO_2 on N availability (Peñuelas and Estiarte 1997; Bukata and Kyser 2005; McLauchlan et al. 2007). No published studies were found that deal with the effects of intrinsic tree factors, for example dominance or growth, on tree-ring $\delta^{15}\text{N}$.

It is well known that N recycling by trees during maturation leads to radial translocation of N across rings boundaries (see Poulson et al. 1995 and references therein). Aiming to avoid the interference of mobile N compounds in dendrochemical studies, the removal of extractives (i.e. the more mobile N fractions) has been proposed by several authors. There is strong evidence that the removal of extractives is necessary in ^{15}N -labelling studies (Elhani et al. 2003), but removal of extractives may not be necessary at natural $\delta^{15}\text{N}$. Hart and Classen (2003) found that the removal of extractives from the wood reduced their $\delta^{15}\text{N}$ value, but the change was fairly small and consistent among unlabelled trees (about 2 to 3 ‰). In spite of wood N content being reduced by 36% after removal of mobile compounds, Elhani et al. (2003) reported a $\delta^{15}\text{N}$ decrease less than 0.4 ‰ for most annual rings, a figure actually close to analytical precision of $\delta^{15}\text{N}$ in wood (st.dev. typically around 0.3 ‰); moreover, they only found significant differences between extracted and non-extracted wood in 4 out 14 rings. Bukata and Kyser (2005) did not find significant changes in heartwood $\delta^{15}\text{N}$ after de-ionized water extractions, while sapwood $\delta^{15}\text{N}$ decreased significantly by 0.59 ‰, although it was still

within the range of the analytical errors (0.30 ‰). The decrease in $\delta^{15}\text{N}$ of tree rings by removal of extractive N fractions suggests that mobile N fractions were enriched in ^{15}N as compared with non-extractable N fractions; however, this result contrasts with the fact that most intra-plant enzymatic reactions discriminate against ^{15}N , and reallocation of N during growth should result in products with lower $\delta^{15}\text{N}$ than the original source (Evans, 2001). As lignification releases a high amount of N recycled in other tree tissues (Suárez et al. 2002), we might expect that, compared with the tissues of origin, the more physiologically active tissues (needles, bark and new wood) that are the destinations of the recycled N should have higher N concentrations and: a) higher $\delta^{15}\text{N}$ if the mobile N is ^{15}N -enriched, as the chemically extractable compounds, or b) lower $\delta^{15}\text{N}$ if the mobile N is ^{15}N -depleted due to isotopic fractionation during the breakdown of N compound into mobile forms.

The aim of present paper was to study the effects of biotic (tree growth, dominance and age) and abiotic factors (seasonal temperature and precipitation) on wood, bark and needle $\delta^{15}\text{N}$ in young pinewoods. This information can provide useful insight into: a) changes on N cycle during the critical step of early stand development, and b) $\delta^{15}\text{N}$ variation due to N translocation between wood, bark and needles.

Material and methods

Study area

The study was conducted at Barbantes site (Galicia, NW of Spain; 370 m a.s.l.), a 2.7 ha stand originated by natural regeneration after a wildfire that in August 1991 completely destroyed the original plantation of *Pinus pinaster* Aiton. In these circumstances, seedling recruitment takes place almost exclusively during the first year after the fire and depends upon the germination of seeds in a transient soil bank that is produced by the postfire dispersal of pine seeds stored in the canopy seed bank (see Daskalakou and Thanos, 1996, and Vega et al., 2008). Therefore, although originated from natural post-fire regeneration, the result is an even-aged stand.

Mean annual temperature is 14 °C, and total annual precipitation and evapotranspiration are 900-1100 mm and 650-700 mm, respectively (<http://www2.meteogalicia.es/galego/observacion/estacions/>). The soil, a Distric cambisol over adamellitic granite, has sandy loam texture and an N content of 0.273 ± 0.040 % in the 0-30 cm layer, with a $\delta^{15}\text{N}$ value of 5.07 ± 0.48 ‰.

In winter 2003-04, three dominant and three suppressed 12-year-old pines were randomly selected to collect: a) 1-, 2- and 3-year-old needles; b) stem disks of wood (6.4-13.5 cm diameter) cut

at basal height to sample all annual rings of growth; and c) bark.

Sample processing and analysis

Needle samples were oven-dried at 60 °C to a constant mass and crushed by hand (protected with gloves to prevent N contamination) to < 4 mm size. Wood samples were air dried in the laboratory, cut into disks 1-1.5 cm thick and planed to reveal all rings that might be present: rings are visible as thin brown lines representing the latewood. The annual rings were measured with a Vernier caliper (0.05-mm precision), separated with a set of stainless steel cutting tools and crushed with a carpenter pincer to < 4 mm. Between successive samples, tools were cleaned with denatured ethanol. The narrower tree rings (years 1-2 always, and years 11-12 in two suppressed trees) were jointly processed because of the extreme difficulty to separate them. In total, 88 samples were processed (64 of wood, 6 of bark and 18 of needles).

All plant material was finely ground (< 100 µm) in a Planetary Ball Mill [Retsch PM 100]. Between consecutive samples, vessel and balls (both made of zirconium oxide) were thoroughly cleaned with water, DI-water and ethanol.

Because of mixed results regarding the need for the extraction of labile organic compounds before total N and $\delta^{15}\text{N}$ analyses in unlabelled wood samples (see Introduction), we did not apply this pretreatment.

Subsamples of all vegetal material were weighed into tin capsules (approx. 5-6 mg for needles and bark; approx. 9-10 mg for wood) and analysed for total N and $\delta^{15}\text{N}$ in an elemental analyser (EA) coupled on-line with an isotopic ratio mass spectrometer (Finnigan Mat, delta C, Bremen, Germany). The isotopic values are given as relative deviation from the international standard (atmospheric N_2) in the δ -notation: $\delta^{15}\text{N} = [({}^{15}\text{N}/{}^{14}\text{N}_{\text{sample}})/({}^{15}\text{N}/{}^{14}\text{N}_{\text{air}}) - 1] \cdot 1000$. Due to the extremely high C/N ratio (from 250 to 530) of the wood samples, a CO_2 trap was installed in the EA between the ovens and the GC column to prevent CO^+ -derived interferences in the $\delta^{15}\text{N}$ measurements.

The following rules, some of them also recommended by Jardine and Cunjak (2005), were taken into account in isotopic analysis. We constraint the weights of samples and standards such that their peaks' amplitudes were within a small range, and we adjusted to this range the peak of the internal reference injected in each analysis (N_2 from a pressure bottle calibrated against IAEA standards). All samples were analysed on duplicate and when the differences between replicates were greater than 0.5 ‰ (around 9 %, 30 % and 40 % of needles, bark and wood samples, respectively), a third analysis was done. The analytical precision (± 1 st. dev.) was 0.10 ‰, 0.25 ‰ and 0.32 ‰ for

needles, bark and wood, respectively. Analytical accuracy and precision for isotopic standards IAEA-N1 and IAEA-N2 (included, alternately, after every tenth sample) were always within the certified values ($0.40 \pm 0.20 \text{ ‰}$ and $20.3 \pm 0.20 \text{ ‰}$, respectively) with new combustion and reduction ovens in the elemental analyser; when a drift in accuracy of 0.5-0.6 ‰ showed that ovens became exhausted, they were replaced. When necessary, the isotopic values of samples were corrected by the difference between the measured and the certified values of the IAEA standards that bracketed the samples in the analytical sequence.

Data analysis

Initially, an exploratory data analysis of N content and $\delta^{15}\text{N}$ values was made to detect outliers and anomalies that could affect the results. Data on N content and $\delta^{15}\text{N}$ were analysed by two-way ANOVA (with age and dominance as factors). Significant differences among the mean groups were established at $P < 0.05$ using the Bonferroni test for multiple comparisons, after applying Levene's test to verify homocedasticity. Relationships among $\delta^{15}\text{N}$ in tree rings, as dependent variable, and the biotic (tree age and basal area increment) and abiotic (seasonal temperature and precipitation) factors, as independent variables, were established by linear and curvilinear regression analyses; "best" models were selected based on the criteria of maximizing the adjusted R^2 and minimizing the mean square error. Relationships among all variables were also studied by principal component analysis (PCA; using Varimax normalization with Kaiser as the rotation method) after checking sampling adequacy with Kaiser-Meyer-Olkin measure and sphericity with Barlett's test. Before running the PCA with wood variables, the matrix of anti-image correlations (the negative of the partial correlation coefficients) was studied to find and discard inadequate variables for the factor analysis. All statistical analyses were made using SPSS 15.0. statistical package.

Results

Pinus pinaster development of dominant and suppressed trees was similar until the seventh year of growth. Thereafter, suppressed tree development slowed, especially during the last two years (with a nearly suspended growth), so the basal area increment was fitted with quadratic equations ($r^2 = 0.422$ to $r^2 = 0.546$; Fig. 1). Conversely, dominant trees' growth remained constant and was fitted with linear models ($r^2 = 0.633$ to $r^2 = 0.708$; Fig. 1). Consequently, significant differences in basal area increments were established after the seventh year ($P < 0.05$ to $P < 0.005$) and, when trees were 12 years old, the basal area in dominants became, on average, 72% higher than in suppressed.

Nitrogen concentration decreased in the order needles (0.780-1.474 % N, d.w.) > bark (0.250-

0.329 % N) > wood (0.109-0.176 % N) (Fig. 2). While N concentration fell steadily with needle age, there were no significant differences among tree rings. No dominance effects on N concentration were found, except for 2-year-old needles (% N of dominants < % N of suppressed).

Fig. 1. Annual basal area increment (cm²) in: A) dominant (● n=3), and B) suppressed (○ n=3) *P. pinaster* trees. Continuous line: best regression fitting with all values. Dashed line: best regression fitting excluding the highest value (identified with an arrow), which is a statistically extreme value in the case of suppressed trees.

Wood samples, all but one with positive $\delta^{15}\text{N}$ values, were significantly different (Fig. 3) from bark and needles, whose $\delta^{15}\text{N}$ values ranged from -2.13 to -0.11 ‰, except in 4 samples from dominant trees. Although differences were not significant, a general decrease was observed with needle age; no relationship between tree dominance and needle $\delta^{15}\text{N}$ was found.

Wood $\delta^{15}\text{N}$ ranged from -0.20 to +6.12 ‰ and tended to decrease with successively formed rings, although differences among age groups rarely became significant (Fig. 3). Curvilinear regression analyses showed that the year of ring formation explained nearly half the wood $\delta^{15}\text{N}$ variance when dominant and suppressed trees were separately considered: the former fit to a linear model ($r^2 = 0.485$; Fig. 4A) and the latter to a quadratic model ($r^2 = 0.444$; Fig. 4B). No simple relationship was found between wood $\delta^{15}\text{N}$ and any other of the studied variables, except a weaker one with basal area increment: wood $\delta^{15}\text{N}$ decrease with BAI was fitted with a linear ($r^2 = 0.285$) or a quadratic model ($r^2 = 0.344$) in the dominant trees and with a linear model ($r^2 = 0.394$) in the suppressed pines.

Fig. 2 Variation with age and organs of the nitrogen content (% N d.w.) in the plant material of dominant (● n=3) and suppressed (○ n=3) *P. pinaster* trees. Different letters (a, b,...) indicate significant differences ($P < 0.05$). Note: bark includes a small quantity formed at age= 9 years.

Fig. 3 Variation with age and organs of $\delta^{15}\text{N}$ in the plant material of dominant (● n=3) and suppressed (○ n=3) *P. pinaster* trees. Different letters (a, b,...) indicate significant differences ($P < 0.05$). Note: bark includes a small quantity formed at age= 9 years.

Fig. 4 Best curvilinear regressions between wood $\delta^{15}\text{N}$ and age of tree rings in: A) dominant ($\bullet$ $n=3$), and B) suppressed ($\circ$ $n=3$) *P. pinaster* trees.

After discarding the inadequate variables for the factor analysis (autumn and winter mean temperatures, and autumn, winter and annual precipitation), the Kaiser-Meyer-Olkin measure of sampling adequacy increased from an unacceptable value of 0.348 to a good figure of 0.719, and the Barlett's test of sphericity was significant at $P < 0.001$. On the plane defined by factors 1 and 2, which explained respectively 45.2% and 15.8% of the total variance, both nitrogen variables (% N and $\delta^{15}\text{N}$) appeared loosely grouped with precipitation during the growing season and clearly opposed (especially $\delta^{15}\text{N}$) to age, basal area increment and mean temperature in spring and summer (Fig. 5).

Discussion

Basal area increment showed that dominance relationships in *P. pinaster* were not established until the seventh year, after which suppressed trees growth declined due to the intraspecific competition among the dominants for light, water and nutrients (Blumfield et al. 2004; Turner et al. 2001). Therefore, dominance effects on $\delta^{15}\text{N}$ must not be expected before the eighth year. The declining growth of suppressed pines after dominance establishment leads to nearly suspended growth during the last two years, the corresponding rings being hardly discernable. Several authors have also reported the existence of partial or missing rings in trees under environmental stress (Kramer and

Kozłowski 1979; Lorimer et al. 1999), being most evident during prolonged periods of suppression.

Fig. 5 Principal Component Analysis showing the relationships between annual tree ring characteristics ($\delta^{15}\text{N}$, %N d.w. and basal area increment), age and climatic conditions (precipitation, temperature) in 12-year old *P. pinaster*.

Nitrogen concentration increased in the order wood < bark < needles, as usually reported for conifer and deciduous forests (Liu 1995; Nadelhoffer et al. 1999), which is probably due to the increasing ratio of metabolic vs structural tissues, the latter with a higher N percentage and the former relatively poor in this nutrient (Kramer and Kozłowski 1979; Lemaire and Gastal 1997). In our study, N concentration in bark and wood was higher than those reported for mature conifers (0.04-0.12 % N in wood; 0.08-0.20 %N in bark; see Kauppi et al. 1995; Poulson et al. 1995; Montero et al. 1999), but it was within the range found by Montero et al. (1999) in branches of mature *P. pinaster* (0.22-0.29 % N in bark; 0.09-0.20 % N in wood), which suggests that the youth of our stand was associated with those high N concentrations. On the other hand, needle N concentration: a) decreased with needle age, always within the range (0.7-1.6 %) usually found for pine needles (Choi et al. 2005a; Montero et al. 1999; Tausz et al. 2004; Warren 2006; Zhang and Allen 1996); and b) was higher in the suppressed trees than in the dominants as Van den Driessche (1974) reported for foliar nutrient concentrations. Considering that needle N concentration in the dominant pines (see Brockley 2001) is within the adequate range reported for *P. pinaster* (see Bergmann 1993 and Fürst 1997, in Tausz et al. 2004), it seems that soil N availability was sufficient for this species in the studied site. As isotopic discrimination by the plants increases as a function of N concentration (see Pritchard and Guy 2005,

and references therein), this circumstance allowed (greater) differences in isotopic signatures among trees.

Except for one wood sample from a 1-2 year ring, all plant material was ^{15}N depleted compared with the $\delta^{15}\text{N}$ of the bulk soil in the 0-30 cm layer (Fig. 3), as also reported in most ecosystems studied (see Högberg 1997). In *P. pinaster* the $\delta^{15}\text{N}$ was significantly higher in wood than in needles and bark (Fig. 3), disagreeing with the results of Emmett et al. (1998), who did not observe any difference between wood and needles, and with those of Nadelhoffer et al. (1999), who found the opposite tendency in *Pinus resinosa*. These contrasting results support the lack of a common trend for different coniferous species and/or sites.

Considering the high contribution of N remobilised from other tree parts in early leaf formation stages (Cantón et al. 2005), the higher $\delta^{15}\text{N}$ in young needles than in the old ones might be due to the high proportion of N in the former that came from wood and bark, which were relatively ^{15}N enriched compared with needles. The general decrease of needles $\delta^{15}\text{N}$ with age agrees with the results of Gebauer and Schulze (1991) and Emmett et al. (1998), although Choi et al. (2005a) did not find any consistent tendency. As also indicated Martinelli et al. (1999), there was a strong positive correlation ($r=0.713$; $p<0.0005$) between $\delta^{15}\text{N}$ and N content in all leaf material samples jointly studied. No significant relationship between tree dominance and needle $\delta^{15}\text{N}$ was found.

Concerning the $\delta^{15}\text{N}$ values in *P. pinaster* wood, it is noteworthy that only 1 out of 64 samples analysed has a negative value. Short and medium-term effects of fire on soil N could partly account for this result in the studied *P. pinaster* stand, which come from a natural post-fire regeneration. As Högberg et al. (1995) reported, fire influences soil N balance by promoting N losses that discriminate against the heavy isotope. Couto-Vázquez and González-Prieto (2006) also highlighted a significant increase of $\delta^{15}\text{N}$ (+0.83 ‰) on the surface of burnt soils.

Plant $\delta^{15}\text{N}$ is affected by the amount and form of inorganic N taken up, rooting depth and mycorrhizal associations (Handley and Scrimgeour 1997; Emmerton et al. 2001; Kolb and Evans 2003). The $\delta^{15}\text{N}$ decreasing tendency with the year of wood formation before dominance establishment (years 1-8) showed that, as a whole, the N stored in successive rings became ^{15}N depleted owing to one or more of the following factors: a) deposition of NO_x , which are usually ^{15}N depleted relative to soil N (Poulson et al. 1995; Choi et al. 2005b; Bukata and Kyser 2007; Savard et al. 2009); b) higher N availability because more negative whole plant $\delta^{15}\text{N}$ values have been found when N supply was increased in hydroponic experiments (Högberg et al. 1999; Yoneyama et al. 2001; Kolb and Evans 2003); c) change from open to closed N cycle thanks to decreasing nutrient losses with increasing stand

age (see Jussy et al. 2000) and to the decrease with time of N losses triggered by the fire (Thomas et al. 2000); d) increasing importance of N sources with lower $\delta^{15}\text{N}$ values, as NO_3^- or N transferred by ectomycorrhizal fungi (Högberg 1997; Hobbie and Hobbie 2006, 2008); and e) increasing supply of N released during lignification of preceding ring(s), which are progressively wider, because most intra-plant enzymatic reactions discriminate against ^{15}N and reallocation of N during growth should result in products with lower $\delta^{15}\text{N}$ than the original source (Evans 2001).

Although there are not available data, pollutant deposition in the study area must be low considering that it is neither industrialized nor densely populated (50-60 people km^{-2} in a 10-km radius), Ourense (100,000 inhabitants, 13 km East) being the only noticeable city in a radius of 50 km. Moreover, most of the year dominant winds have a westerly direction and come from a clean area: the Atlantic Ocean 70 km away. A relatively close motorway (450 m away) was opened when the stand was 6 years old but, as reported Saurer et al. (2004), $\delta^{15}\text{N}$ pollutant effects were only evident in direct proximity to a motorway and already disappeared in 150 m distance. On the other hand, as mineral N availability decreased with time (1 to 5 years) after the fire (Durán et al. 2008), it seems that the wood $\delta^{15}\text{N}$ decrease we found was not related to increasing N availability. Therefore, the progressive decrease of wood $\delta^{15}\text{N}$ must mainly be a consequence of a more "closed" N cycle or increasing importance of N sources with lower $\delta^{15}\text{N}$ (NO_3^- , N transferred by ectomycorrhizal fungi or N released during lignification of preceding rings).

After dominance establishment (years 9-12), wood $\delta^{15}\text{N}$ continued to decrease linearly with age in dominant trees, likely due to the same factors as in the previous years, while it tended to increase in suppressed pines (see Fig. 4). Consequently, after dominance establishment, wood formed in dominant trees became significantly ^{15}N depleted compared with that of suppressed (1.46 ‰ and 2.62 ‰, respectively; $P < 0.01$) when rings from age 9-12 years were jointly considered, suggesting that there was a dominance effect on wood ^{15}N values. Several explanations for the tendency of increasing wood $\delta^{15}\text{N}$ with age in the suppressed pines are possible. The first one is that N sources for suppressed pines in years 9-12 had globally higher $\delta^{15}\text{N}$ values than previous years in all trees (years 6-8) and than those of dominant pines in years 9-12, but the lower $\delta^{15}\text{N}$ values in needles and bark of suppressed pines than those of the dominants (see Fig. 3) invalidates this account. A second possible explanation is growth-related isotope fractionation because BAI and wood $\delta^{15}\text{N}$ have an inverse linear relationship ($r^2=0.285$ and $r^2=0.394$ in dominant and suppressed trees, respectively). Finally, if we take into account BAI decline and stabilization of wood N concentration from year 9 to 12 in suppressed pines (Figs. 1 and 2), we should conclude that suppression resulted into a reduction in the absolute amount of soil N assimilated; this lead us to think that wood is acting as major N source for needles in suppressed

trees via lignification (and the associated isotopic discrimination) causing higher $\delta^{15}\text{N}$ values in wood and lower $\delta^{15}\text{N}$ in needles compared with dominant trees.

Considering the lower ^{15}N values in dominant trees and the clear opposition between $\delta^{15}\text{N}$ and basal area increment showed by the PCA, we could conclude that dominant trees had better access to soil N reserves thanks to a more developed root system and/or (stronger) symbiosis with ectomycorrhizal fungi. However, as fire effects on soil are depth-dependent and rooting depth may be different in dominant and suppressed trees, our results may also be influenced by dominance-related differences of post-fire effects on tree growth. Therefore, more research is needed on this topic before accepting as a general trend the relationship we found between dominance and wood $\delta^{15}\text{N}$.

As lignification releases a high amount of N recycled in other tree tissues (Suárez et al. 2002), we might expect that, compared with “old” wood, tissues acting as a sink of the N recycled (needles, bark and recently formed wood) should have higher N concentration and: a) higher $\delta^{15}\text{N}$ values if mobile N is ^{15}N -enriched, as compounds chemically extracted by Elhani et al. (2003), Hart and Classen (2003) and Bukata and Kyser (2005); or b) lower $\delta^{15}\text{N}$ values if products released and reallocated during lignification were ^{15}N -depleted compared with the source (Evans 2001). Results of the present study support the latter expectation; consequently, without more experimental data, it seems that we should be cautious in considering that proposed chemical extractions are removing the more physiologically mobile N compounds from wood.

Conclusion

Among the abiotic variables considered, only the climatic conditions during the growing season were related with wood $\delta^{15}\text{N}$, which appeared loosely grouped with precipitation and clearly opposed to mean temperature.

All the biotic factors studied (tree growth, dominance and age) exerted influence upon $\delta^{15}\text{N}$ of some compartment (wood, bark and needles) and, combined with measurements of N concentration and basal area increment, provide useful insight into the changes on N cycle during the critical step of early stand development and dominance establishment:

- a) Before dominance establishment, the progressive decrease of wood $\delta^{15}\text{N}$ with age must be mainly a consequence of a more “closed” N cycle or an increasing importance of N sources with lower $\delta^{15}\text{N}$ (NO_3^- , N transferred by ectomycorrhizal fungi or N released during lignification of preceding rings).
- b) After dominance establishment, wood $\delta^{15}\text{N}$ values were higher in suppressed than in dominant

trees while the reverse was true for needles and bark; simultaneously, the absolute amount of N stored by suppressed pines in successive rings decreased. These results could be explained by suppression resulting in a reduction in the absolute amount of soil N assimilated and lignification becoming a major N source for needles.

More experimental data are needed to identify the nature and ^{15}N isotopic signature of the nitrogenated compounds released and reallocated during lignification and to check if they coincide with those removed from wood by chemical extractants.

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References

- Blumfield, TJ, Xu, ZH, Saffigna, PG (2004) Carbon and nitrogen dynamics under windrowed residues during the establishment phase of a second-rotation hoop pine plantation in subtropical Australia. *Forest Ecol Manag* 200: 279-291
- Brockley, RP (2001) Foliar analysis as a planning tool for operational fertilization. In: Bamsey C (ed) *Proceedings of Enhanced Forest Management: Fertilization & Economics Conference*. Clear Lake Ltd., Edmonton, pp 63-68
- Bukata, AR, Kyser, TK (2005) Response of the nitrogen isotopic composition of tree-rings following tree-clearing and land-use change. *Environ Sci Technol* 39: 7777-7783
- Bukata, AR, Kyser, TK (2007) Carbon and nitrogen isotope variations in tree-rings as records of perturbations in regional carbon and nitrogen cycles. *Environ Sci Technol* 41: 1331-1338
- Cantón, FR, Suárez, MF, Cánovas, FM (2005) Molecular aspects of nitrogen mobilization and recycling in trees. *Photosynth Res* 83: 265-278
- Choi, WJ, Chang, SX, Allen, HL, Kelting, DL, Ro, HM (2005a) Irrigation and fertilization effects on foliar and soil carbon and nitrogen isotope ratios in a loblolly pine stand. *Forest Ecol Manag* 213: 90-101
- Choi, WJ, Lee, SM, Chang, SX, Ro, HM (2005b). Variations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in *Pinus densiflora* tree-rings and their relationship to environmental changes in eastern Korea. *Water Air Soil Pollut* 164: 173-187
- Couto-Vázquez, A, González-Prieto, SJ (2006) Short- and medium- term effects of three fire fighting chemicals on the properties of a burnt soil. *Sci Total Environ* 371: 353-361

- Daskalakou, EN, Thanos, CA (1996) Aleppo Pine (*Pinus halepensis*) postfire regeneration: the role of canopy and soil seed banks. *Int J Wildland Fire* 6: 59-66
- Dawson, TE, Mambelli, S, Plamboeck, AH, Templer, PH, Tu, KP (2002) Stable isotopes in plant ecology. *Ann Rev Ecol Syst* 33: 507-559
- Durán, J, Rodríguez, A, Fernández-Palacios, JM, Gallardo, A (2008) Changes in soil N and P availability in a *Pinus canariensis* fire chronosequence. *Forest Ecol Manag* 256: 384-387
- Elhani, S, Lema, BF, Zeller, B, Brechet, C, Guehl, JM, Dupouey, JL (2003) Inter-annual mobility of nitrogen between beech rings: a labelling experiment. *Ann For Sci* 60: 503-508
- Elhani, S, Guehl, JM, Nys, C, Picard, JF, Dupouey, JL (2005) Impact of fertilization on tree-ring $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ and in beech stands: a retrospective analysis. *Tree Physiol* 25: 1437-1446.
- Emmerton, KS, Callaghan, TV, Jones, HE, Leake, JR, Michelsen, A, Read, DJ (2001) Assimilation and isotopic fractionation of nitrogen by mycorrhizal and nonmycorrhizal subarctic plants. *New Phytol* 151: 513-524
- Emmett, BA, Kjenaas, OJ, Gundersen, P, Koopmans, C, Tietema, A, Sleep, D (1998) Natural abundance of ^{15}N in forests across a nitrogen deposition gradient. *Forest Ecol Manag* 101: 9-18
- European Environmental Agency (2006) European forest types: Categories and types for sustainable forest management reporting and policy. Tech. Rep. 9, Brussels
- Evans, RD (2001) Physiological mechanisms influencing plant nitrogen isotope composition. *Trends Plant Sci* 6: 121-126
- Gebauer, G, Schulze, ED (1991) Carbon and nitrogen isotope ratios in different compartments of a healthy and declining *Picea abies* forest in the Fichtelgebirge NE Bavaria. *Oecologia* 87: 198-207
- Handley, LL, Scrimgeour, CM (1997) Terrestrial plant ecology and ^{15}N natural abundance: The present limits to interpretation for uncultivated systems with original data from a Scottish old field. *Adv Ecol Res* 27: 133-212
- Hart, SC, Classen, AT (2003) Potential for assessing long-term dynamics in soil nitrogen availability from variations in $\delta^{15}\text{N}$ of tree rings. *Isot Environ Health Stud* 39: 15-28
- Hobbie, EA, Hobbie, JE (2008) Natural abundance of ^{15}N in nitrogen-limited forests and tundra can estimate nitrogen cycling through mycorrhizal fungi: a review. *Ecosystems* 11: 815-830
- Hobbie, JE, Hobbie, EA (2006) ^{15}N in symbiotic fungi and plants estimates nitrogen and carbon flux rates in arctic tundra. *Ecology* 87: 816-822
- Högberg, P (1997) N-15 natural abundance in soil-plantsystems. *New Phytol* 137: 179-203
- Högberg, P, Johannisson, C, Högberg, M, Högbom, L, Näsholm, T, Hällgren, JE (1995) Measurements of abundances of ^{15}N and ^{13}C as tools in retrospective studies of N balances and water stress in forests: A discussion of preliminary results. *Plant Soil* 168-169: 125-133
- Högberg P, Högberg MN, Quist ME, Ekblad A, Nasholm T (1999) Nitrogen isotope fractionation during nitrogen uptake by ectomycorrhizal and non-mycorrhizal *Pinus sylvestris*. *New Phytol* 142: 569-576
- Jardine, TD, Cunjak, RA (2005) Analytical error in stable isotope ecology. *Oecologia* 144: 528-533
- Jussy, JH, Colin-Belgrand, M, Ranger, J (2000) Production and root uptake of mineral nitrogen in a chronosequence of Douglas-fir (*Pseudotsuga menziesii*) in the Beaujolais Mounts. *Forest Ecol Manag* 128: 197-

- Kauppi, PE, Tomppo, E, Ferm, A (1995) C and N storage in living trees within Finland since 1950s. *Plant Soil* 168-169: 633-638
- Kolb KJ, Evans RD (2003) Influence of nitrogen source and concentration on nitrogen isotopic discrimination in two barley genotypes (*Hordeum vulgare* L.). *Plant Cell Environ* 26: 1431-1440
- Kramer, PJ, Kozlowski, TT (1979) *Physiology of Woody Plants*. Academic Press, New York
- Lemaire, G, Gastal, F (1997) N uptake and distribution in plant canopies. In: Lemaire G (ed) *Diagnosis of the nitrogen status in crops*. Springer Verlag, Berlin, pp 3-43
- Liu, S (1995) Nitrogen cycling and dynamic analysis of man made larch forest ecosystems. *Plant Soil* 168-169: 391-397
- Lorimer, CG, Dahir, SE, Singer, MT (1999) Frequency of partial and missing rings in *Acer saccharum* in relation to canopy position and growth rate. *Plant Ecol* 143: 189-202
- McLauchlan, KK, Craine, JM, Oswald, WW, Leavitt, PR, Likens, GE (2007) Changes in nitrogen cycling during the past century in a northern hardwood forest. *Proc Nat Acad Sci USA* 104: 7466-7470
- Martinelli, LA, Piccolo, MC, Townsend, AR, Vitousek, PM, Cuevas, E, McDowell, W, Robertson, GP, Santos, OC, Treseder, K (1999) Nitrogen stable isotopic composition of leaves and soil: Tropical versus temperate forests. *Biogeochemistry* 46: 45-65
- Montero, G, Ortega, C, Cañellas, I, Bachiller, A (1999) Productividad aérea y dinámica de nutrientes en una repoblación de *Pinus pinaster* Ait. sometida a distintos regímenes de claras. *Invest. Agr. Sist. Recur. For. Fuera de Serie* nº 1: 175-206
- Nadelhoffer, K, Fry, B (1988) Controls on natural nitrogen-15 and carbon-13 abundances in forest soil organic matter. *Soil Sci Soc Am J* 52: 1633-1640
- Nadelhoffer, KJ, Downs, MR, Fry, B (1999) Sinks for ¹⁵N-enriched additions to an oak forest and a red pine plantation. *Ecol Appl* 9: 72-86
- Peñuelas, J, Estiarte, M (1997) Trends in plant carbon concentration and plant demand for N throughout this century. *Oecologia* 109: 69-73
- Poulson, SR, Chamberlain, CP, Friedland, AJ (1995) Nitrogen isotope variation of tree rings as a potential indicator of environmental change. *Chem Geol* 125: 307-315
- Pritchard, ES, Guy, RD (2005) Nitrogen isotope discrimination in white spruce fed with low concentrations of ammonium and nitrate. *Trees Struct Funct* 19: 89-98
- Robinson, D (2001) Delta N-15 as an integrator of the nitrogen cycle. *Trends Ecol Evol* 16: 153-162
- Saurer, M, Cherubini, P, Ammann, M, De Cinti, B, Siegwolf, R (2004) First detection of nitrogen from NO_x in tree rings: a ¹⁵N/¹⁴N study near a motorway. *Atmos Environ* 38: 2779-2787
- Savard, MM, Begin, C, Smirnoff, A, Marion, J, Rioux-Paquette, E (2009) Tree-ring nitrogen isotopes reflect anthropogenic NO_x emissions and climatic effects. *Environ Sci Technol* 43: 604-609
- Suárez, MF, Ávila, C, Gallardo, F, Cantón, FR, García-Gutiérrez, A, Claros, MG, Cánovas, FM (2002) Molecular and enzymatic analysis of ammonium assimilation in woody plants. *J Exp Bot* 53: 891-904

- Tausz, M, Trummera, W, Wonischa, A, Goesslerb, W, Grilla, D, Jiménez, MS, Morales, D (2004) A survey of foliar mineral nutrient concentrations of *Pinus canariensis* at field plots in Tenerife. *Forest Ecol Manag* 189: 49–55
- Thomas, AD, Walsh, RPD, Shakesby, RA (2000) Solutes in overland flow following fire in eucalyptus and pine forests, northern Portugal. *Hydrol Process* 14: 971–985
- Turner, J, Lambert, MJ, Hopmans, P, McGrath, J (2001) Site variation in *Pinus radiata* plantations and implications for site specific management. *New Forests* 21: 249–282
- Van den Driessche, R (1974) Prediction of mineral nutrient status of trees by foliar analysis. *Bot Rev* 40: 337–394
- Vega, JA, Fernández, C, Pérez-Gorostiaga, P, Fonturbel, T (2008) The influence of fire severity, serotiny, and post-fire management on *Pinus pinaster* Ait. recruitment in three burnt areas in Galicia (NW Spain). *Forest Ecol Manage* 256: 1596–1603
- Warren, CR (2006) Why does photosynthesis decrease with needle age in *Pinus pinaster*? *Trees Struct Funct* 20: 157–164
- Yoneyama T, Matsumaru T, Usui K, Engelaar WMHG (2001) Discrimination of nitrogen isotopes during absorption of ammonium and nitrate at different nitrogen concentrations by rice (*Oryza sativa* L.) plants. *Plant Cell Environ* 24:133–139
- Zhang, SS, Allen, HL (1996) Foliar nutrient dynamics of 11-year-old loblolly pine (*Pinus taeda*) following nitrogen fertilization. *Can J For Res* 26: 1426–1439