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Soil δ^{13}C and δ^{15}N as a good indicator for predicting the site index of Galician pine forests *(Pinus pinaster* Ait. and *Pinus sylvestris* L.)

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Abstract

Stable isotopes natural abundance (^{13}C, ^{15}N) was studied in soils from *P. pinaster* Ait. and *P. sylvestris* L. plantations located in Galicia, NW Spain, to evaluate the use of isotopic techniques for stand quality estimation. Combinations of old/young forest plantations of high/low site index, growing on acidic soils over two different parent materials (granite/schists) were studied. Significantly lower δ^{13}C in soils under *P. pinaster* (–27.03‰) as compared with soils under *P. sylvestris* (–26.13‰) were found ($P < 0.001$). However, soil δ^{15}N of both species did not differ significantly. Significant linear regressions between soil δ^{13}C and site index for *P. pinaster* were found, pointing at the δ^{13}C signature of the soil as a good stand quality indicator, especially over schists. For *P. sylvestris* over schists, linear regression between soil δ^{15}N and site index suggests that δ^{15}N could be used as a complementary tool in quality determinations. As a general trend for both tree species, stands with high site index showed more depleted soils in ^{13}C and ^{15}N as compared with low site index stands.

Key words: Soil organic matter, Stable isotopes, ^{13}C/^{12}C, ^{15}N/^{14}N, Forest quality, NW Spain.

Resumen

δ^{13}C y δ^{15}N del suelo como un buen indicador para predecir el índice de sitio en pinares gallegos *(Pinus pinaster* Ait. and *Pinus sylvestris* L.)

Se determinó la abundancia natural de los isótopos estables ^{13}C y ^{15}N en suelos ácidos bajo *P. pinaster* Ait. y *P. sylvestris* L. de Galicia (NO España) para evaluar su uso en estimaciones de calidad de estación. Se estudiaron combinaciones de las siguientes variables: roca (granito/esquistos), edad (joven/madura) y calidad (alto/bajo índice de sitio) de la masa arbórea. Los suelos bajo *P. pinaster* presentaron δ^{13}C (–27,03‰) menores ($P < 0{,}001$) que los suelos bajo *P. sylvestris* (–26,13‰). El δ^{15}N del suelo no difirió significativamente entre ambas especies. Las regresiones lineales significativas entre el índice de sitio y la composición isotópica del suelo, especialmente sobre esquistos, señalan al δ^{13}C del suelo como un buen indicador de calidad para *P. pinaster,* mientras que para suelos bajo *P. sylvestris* es el δ^{15}N el isótopo más útil en las determinaciones de calidad. En general para ambas especies, parcelas con alto índice de sitio presentaron suelos más pobres en ^{13}C y ^{15}N que parcelas con bajo índice de sitio.

Palabras clave: Materia orgánica del suelo, Isótopos estables, ^{13}C/^{12}C, ^{15}N/^{14}N, Calidad forestal, NO España.

Introduction

The study of the processes involved in organic matter transformations of forest soils (Carballas *et al.,* 1979, 1980, 1983; Fernández *et al.,* 1999) and soil organic matter composition (Fernández *et al.,* 2001; Ussiri and Johnson, 2003) has a great importance, affecting to multitude of disciplines related to silviculture and environmental sciences. Forest soil organic matter has a great influence on the sustainability of ecosystems as well as on their soil fertility and forest productivity. The site index, the most widely accepted index of the quality of standing tree mass (Carmean, 1975) based in the determination of the height of dominant trees at a specific age, is a required variable for the modelling of the present and future growth and yield and it is also used for purposes of forest exploitation on a sustainable yield bases

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(García, 1983). However, site index determinations have some limitations, since it can not be used before a minimum time of stand growth. For this reason, when early soil fertility estimation is requited, several authors use changes on soil organic matter content and dynamics to quantify the success of a sustainable silviculture (Morris *et al.,* 1997). Therefore, the study of soil organic matter characteristics should be a priority research line for the knowledge of the present and future status of nutrient cycles in forest ecosystems (Nilsson *et al.,* 1995; Sánchez *et al.,* 1997; Fernández *et al.,* 1999).

The two biogeochemical cycles that provides more information about soil organic matter composition and dynamics are those of carbon and nitrogen. Both radioactive and stable heavy isotopes have been frequently utilized to follow these cycles in order to monitor edaphic processes where organic matter is involved (Cabaneiro *et al.,* 1987; González-Prieto and Villar, 2003; Van Dam *et al.,* 1997).

Stable isotopic techniques can provide an integrated and quantitative view of chemical, biological and ecological transformations in diverse research fields (Boutton *et al.,* 1998; Griffiths *et al.,* 1999). Due to the precision and efficiency of these techniques, at present many organic matter studies use stable isotopes (^{13}C, ^{15}N) to trace the different processes and transformations that take place in forests ecosystems (Fernández *et al.,* 2004), such as atmospheric CO_2 and N_2 fixation, decomposition of complex plant debris (Fernández *et al.,* 2003; Schleser *et al.,* 1999), soil formation, etc.

As regards to the carbon biogeochemical cycle, it is well known that during each chemical transformation some variation in the ^{13}C concentration occurs (Connin *et al.,* 2001; Fernández and Cadisch, 2003; Fernández *et al.,* 2003; Fleck *et al.,* 1996) and, due to this fact, analyses of this stable isotope at natural abundance levels can be used in organic matter research to quantify carbon fluxes from a great diversity of ecosystems (Bernoux *et al.,* 1998; Bird and Pousai, 1997; Ehleringer *et al.,* 2000; Mary *et al.,* 1992). Nevertheless, only recently this kind of techniques has been used to study Galician forest ecosystems. Previous studies on soil organic matter from Galician coniferous forest showed the existence of a clear relationship between the characteristics of this soil organic matter and the tree mass quality (González-Prieto and Villar, 2003). Therefore, the isotopic composition of these ecosystems and the factors influencing the ^{13}C distribution in the soil organic matter could be a good index to successfully evaluate tree growth and photosynthetic conditions of a forest plantation. However, few studies aim to relate this edaphic characteristic with different silvicultural qualities.

In the case of the nitrogen biogeochemical cycle, the high N concentration of living organisms, as compared with their environment, points to this element as the more common limiting factor of the biosphere (Haynes, 1986; Jenkinson, 1981; Margalef, 1980) and particularly of forest ecosystems (Casals *et al.,* 1995; Nilsson *et al.,* 1995; Raison *et al.,* 1990), so much so that it is considered as the main growth limiting element since vascular plants evolution (Ericsson, 1995). In terrestrial ecosystems, nitrogen fertility is so closely related with soil organic N dynamics that organic matter and its properties are considered the key factor for long term soil sustainability and productivity (Peoples *et al.,* 1995), as well as promising indicators to evaluate soil quality in forest management (Schoenholtz *et al.,* 2000; Wander and Drinkwater, 2000). Thus, several variables related to N status and dynamics in the soil, such as total N content, $\delta^{15}N$, potentially mineralizable N and nitrification, have been proposed as basic soil quality indicators (Karlen *et al.,* 1994; Knoepp *et al.,* 2000; Lal, 1997; McCarty and Meisinger, 1997) and as predicting variables for N balance and hydric stress in forests (Nilsson *et al.,* 1995) and for the site index and potential productivity in *Pinus radiata* plantations (González-Prieto and Villar, 2003). Soil richness in N as well as soil $\delta^{15}N$ values related with small N losses and, therefore, with «closed» N cycles (Abadín *et al.,* 2002; Chang and Handley, 2000; Eshetu and Högberg, 2000; González-Prieto and Villar, 2003; Högber *et al.,* 1995).

Therefore, the main objective of this study was to evaluate the usefulness of isotopic techniques as a tool for quality estimation and management of *P. pinaster* Ait. and *P. silvestris* L. forests, tree species very abundant in the north of Spain.

Material and Methods

Experimental design

A total number of 48 pine forest plantations of both *P. pinaster* Ait. and *P. sylvestris* L. located in Galicia,

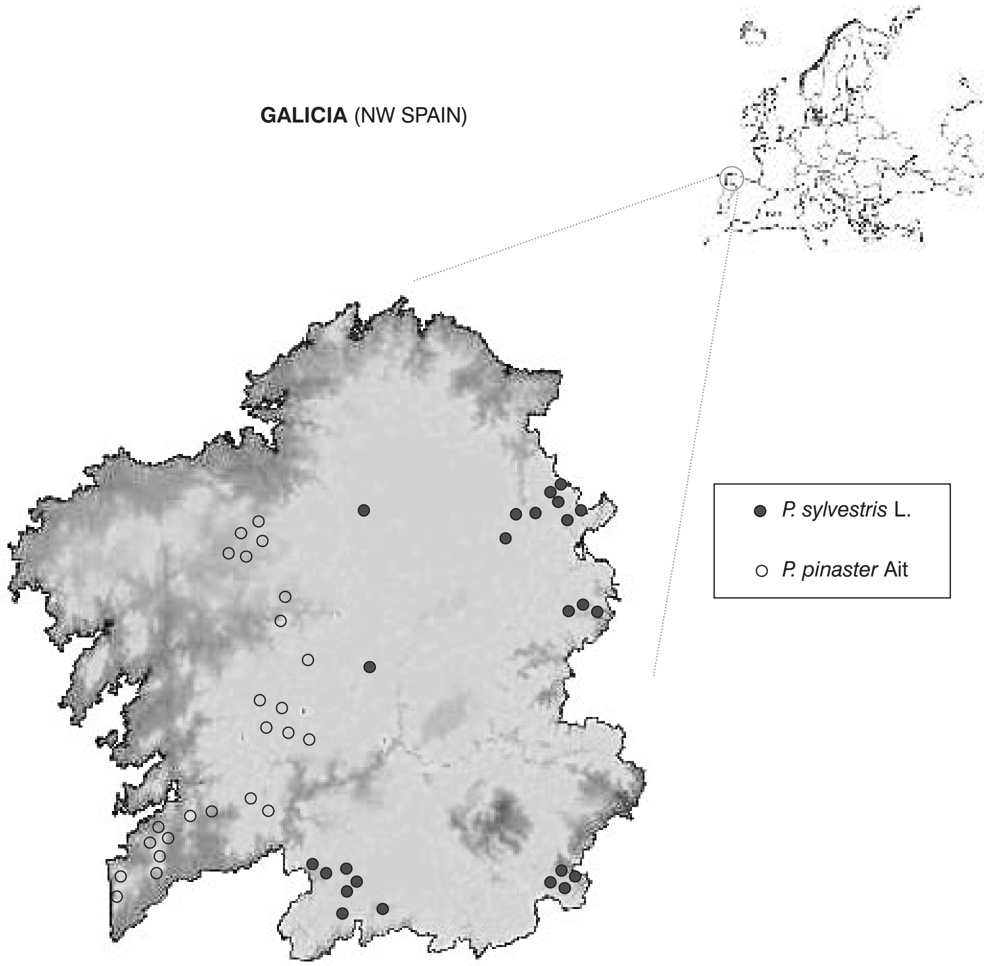

Figure 1. Location of *P. pinaster* and *P. sylvestris* forest plots studied in Galicia (NW Spain).

NW Spain (Figure 1), were selected to study the C and N isotopic natural abundance of samples from the upper 15 cm of the soil to evaluate the use of isotopic techniques to estimate the quality of the stand. The plots were located throughout Galicia and were subjectively selected to represent the whole range of stand densities, undergrowth types and growing conditions in Galician pine forests but restricted to two different ranges of age and site index. More often than not, none or scarce (only in some cases low pruning) silvicultural treatments were applied to the forests selected for this study. For *P. pinaster,* forest stands from natural regeneration were selected in the coastal area of Galicia (Pontevedra and La Coruña provinces) due to the high genetic homogeneity of their seeds. These forests are often considered as an autochthonous type perfectly adapted to this environment. For *P. sylvestris,* in Galicia only found in the highest areas [>800 m above sea level (a.s.l.)], forest plantations originally established by making soil holes for tree placement (or higher mechanization in some young forest plantations) were selected. The genetic origin of the trees used in these plantations corresponds to Scots pines from the Spanish Sistema Central mountains.

Three replicates for each possible combination of forests stands with high (17 m - 23 m for *P. pinaster* and 12 m - 17 m for *P. sylvestris*) or low site index (9 m - 14 m for *P. pinaster* and 5 m - 10 m for *P. sylvestris*) and with old (25-35 years for *P. pinaster* and 45-55 years for *P. sylvestris*) or young trees (10-20 years for *P. pinaster* and 20-35 years for *P. sylvestris)* growing on acidic soils developed over two different parent materials (granite/schists) have been chosen for each species (see following diagram).

Tree species (n = 24)	Granite (n = 12)	Young (n = 6)	Low quality (n = 3)
			High quality (n = 3)
		Old (n = 6)	Low quality (n = 3)
			High quality (n = 3)
	Schists (n = 12)	Young (n = 6)	Low quality (n = 3)
			High quality (n = 3)
		Old (n = 6)	Low quality (n = 3)
			High quality (n = 3)

Values of the site index of the plots studied were obtained using the equations proposed by Álvarez González *et al.* (1999) and Martínez Chamorro *et al.* (1997). The site index is defined as the dominant height of the stand, in meters, at the age of 20 years for *P. pinaster* (Álvarez González *et al.,* 1999) and at the age of 40 years for *P. sylvestris* (Dieguez Aranda, 2004).

Soil sampling

According to forest tree density, forest plots of 550 m^2 to 1200 m^2 with at least 30 tree specimens were established for site index determination and from each plot 30 subsamples from the upper 15 cm of the soil were collected with a stainless steel probe (3.5 cm in diameter) and mixed to constitute a representative soil sample.

Main soil characteristics

The methods described by Guitián Ojea and Carballas (1976) were used to determine the following soil properties: texture, water holding capacity, pH in H_2O (1:2.5) and extractable Fe and Al oxides (by extraction with a mixture of hydrosulfite and Tamm's reagent). Total organic C was determined by dry combustion and measurement of the CO_2 evolved in a Carmhograph 12 (Whösthoff, Germany).

Isotopic analysis (^{13}C and ^{15}N)

Approximately 4 kg of soil were collected from each forest plot. The soil samples were air dried, sieved (4 mm), thoroughly homogenized and a representative subsample of each soil (80 g approximately) was finely ground (<0.100 μm) for isotopic analysis.

The ^{13}C and ^{15}N isotopic composition of soil samples were measured using an automated CN analyzer coupled on line to a Finnigan MAT Delta-C isotope ratio mass spectrometer. Carbon and nitrogen isotopic composition were calculated relative to the Pee Dee Belemnite standard (Craig, 1957) and relative to air-N_2, respectively. The stable isotope reference materials IAEA-CH-6 and IAEA-CH-7 calibrated against the international PDB standard were run between different C sample groups. The same was made for N analyses with the reference materials IAEA-N-1 and IAEA-N-2. Sample size was adjusted to give a similar amount of C or N in the sample and in the standard.

The results of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios were expressed in the relative δ scale (‰) according to the following equation:

$$\delta\ (‰) = (R_{sample}/R_{standard} - 1) \times 10^3$$

where $R = {}^{13}C/{}^{12}C$ or $R = {}^{15}N/{}^{14}N$.

Statistical analysis

The results are the average of the replicate determinations in three subsamples of the same material and they were expressed as mean ± standard deviation (SD).

Statistical analysis were performed using the computer software SPSS 11.5.1 (2002). After checking the assumptions of homocedasticity and normality, ANOVA test was applied to analyse the variations between different groups of soils. The least significant difference (LSD) test was applied to the results. Multilinear regression data were used to ascertain the relative importance of the variables included in the best models. To prevent problems of multicollinearity among the soil properties used as independent variables in the multiple regression analyses, the models selected included only variables with a tolerance higher than 0.6.

Results and Discussion

Forest soils selected to carry out this study were acidic (pH ranged from 3.4 to 4.8) and sandy (coarse sand from 10% to 73%; fine sand from 10% to 62%), with contents of non-crystalline Al_2O_3 ranging from 6.5 to 47.0 g $kg^{-1}_{dry\ soil}$ and contents of non-crystalline Fe_2O_3 ranging from 6.7 to 86.4 g $kg^{-1}_{dry\ soil}$. The soil organic matter of these soils exhibited C-to-N ratios between 12 and 22, with soil C and N contents highly scattered. The total soil C contents ranged from 39 to 162 g C $kg^{-1}_{dry\ soil}$ and total soil N contents ranged from 1.7 to 13.0 g N $kg^{-1}_{dry\ soil}$.

The ^{13}C natural abundance values of all the soils studied are included within the range reported for soils developed under a C_3 vegetation (Arrouays *et al.,* 1995; Boutton *et al.,* 1998; González-Prieto *et al.,* 1999; Spaccini *et al.,* 2000). However, the soil ^{13}C isotopic composition of these soils was found to be substantially different under the two tree species considered. The distribution of samples from the upper 15 cm of all forest soils studied on the plane defined by ^{13}C and ^{15}N isotopic composition (Figure 2) shows two groups

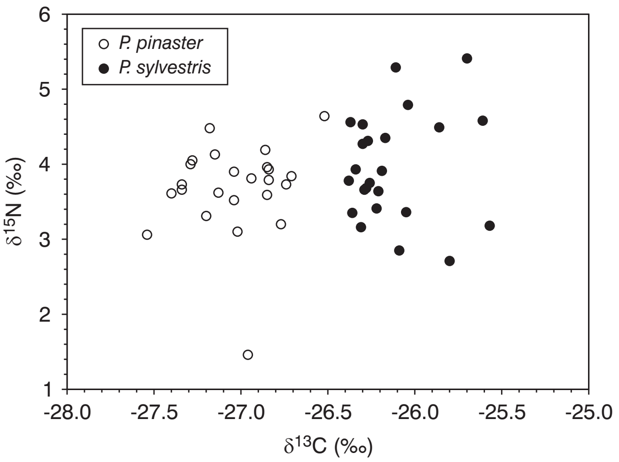

Figure 2. Isotopic distribution $\delta^{13}C$ (‰) vs. $\delta^{15}N$ (‰) of soils under *P. pinaster* and *P. sylvestris*.

clearly-defined: soils colleted under *P. pinaster* (white circles on the left) and soils collected from *P. sylvestris* plots (black circles on the right). The $\delta^{13}C$ values of soils under *P. pinaster* ranged from –27.54‰ to –26.52‰, whereas values of soils under *P. sylvestris* ranged from –26.38‰ to –25.57‰. Therefore, as compared with *P. pinaster* soils (mean value $\delta^{13}C$ = –27.03‰ ± 0.25), significantly (ANOVA, $P < 0.001$, n = 24) higher $\delta^{13}C$ values from *P. sylvestris* soils (mean value $\delta^{13}C$ = –26.13‰ ± 0.24) were found. When a general correlation matrix was obtained considering all samples from both type of forests together in order to analyse the possible relationships of the soil isotopic composition with other main soil characteristics, soil $\delta^{13}C$ values were negatively correlated with the soil pH in water ($P < 0.01$) and sand content of the soil ($P < 0.001$) and, accordingly with the latter, positively correlated with the water holding capacity ($P < 0.001$).

As compared with $\delta^{13}C$ values, a wider dispersion for $\delta^{15}N$ values was observed for soils under both *P. pinaster* as well as under *P. sylvestris.* The ranges of $\delta^{15}N$ values of soils under both tree species slightly differed. The $\delta^{15}N$ values of soils under *P. pinaster* varied from 1.46‰ to 4.64‰ with a mean value of 3.68‰ ± 0.61. Values of $\delta^{15}N$ for soils under *P. sylvestris* ranged from 2.71‰ to 5.41‰, with a mean value of 3.96‰ ± 0.71. The $\delta^{15}N$ isotopic signature of these soils fell within the range of –1‰ to + 8‰ usually found in forest or culture soils (Cheng *et al.,* 1964; Binkley *et al.,* 1985; Mordelet *et al.,* 1996; Hadley and Scrimgeour, 1997; Koba *et al.,* 1998; González-Prieto and Villar, 2003; Eshetu, 2004). Results from ANOVA test showed that soil isotopic ^{15}N composition for *P. pinaster* and *P. sylvestris* were not significantly different. However, the isotopic ^{15}N composition of all soils studied positively correlated with the non-crystalline Fe oxides content and statistically significant correlations between $\delta^{15}N$ values and soil texture data were also found, negative ($P < 0.05$) with sand and positive ($P < 0.005$) with silt and clay contents. Similarly to the $\delta^{13}C$, this resulted again in a significant positive correlation between $\delta^{15}N$ values and the soil water holding capacity ($P < 0.05$). Mean values of the $\delta^{13}C$ and $\delta^{15}N$ isotopic composition of the diverse groups of 6 soils, with the same age and similar site index but without taking into account the soil parent material, are shown in Table 1.

Table 1. Mean values ± standard deviation (n = 6) of $\delta^{13}C$ and $\delta^{15}N$ isotopic composition for soils under young and old trees of *P. pinaster* and *P. sylvestris* from plots of high or low site index

Site index	Age	*P. pinaster*		*P. sylvestris*	
		$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Low	Young	–26.86 ± 0.12	3.65 ± 0.28	–26.12 ± 0.23	4.38 ± 0.89
	Old	–26.86 ± 0.23	4.08 ± 0.38	–26.13 ± 0.25	3.60 ± 0.74
High	Young	–27.26 ± 0.18	3.56 ± 0.47	–26.15 ± 0.27	4.02 ± 0.44
	Old	–27.16 ± 0.22	3.42 ± 0.99	–26.12 ± 0.29	3.78 ± 0.67

Although some differences were observed on soil $\delta^{15}N$ isotopic composition according to the age of the trees for *P.* sylvestris, these differences between young and old trees were only significant for *P. sylvestris* plots of low quality ($P < 0.05$). Soils under *P. pinaster* trees do not show any significant isotopic difference between young and old plots but significant ^{13}C isotopic differences between low and high tree quality ($P < 0.02$) were observed.

When only forests with the same tree species were considered, some changes on the relationships between the stable isotopic composition of soils and other soil characteristics were found. The previously mentioned correlation between $\delta^{13}C$ and water holding capacity and most of the relationship of $\delta^{15}N$ with other soil characteristics were not found when *P. pinaster* forests are studied independently, however in these forests $\delta^{13}C$ significantly correlated with non-crystalline Al oxides ($P < 0.05$). Moreover, when only *P. pinaster* soils developed over granite were considered separately, the $\delta^{13}C$ significantly correlated with variables related to soil total carbon content ($P < 0.05$, positive correlation) and soil organic matter quality ($P < 0.05$, negative correlation). On the other hand, the $\delta^{13}C$ of *P. pinaster* soils developed over schists exhibited significant correlation with soil texture variables and with the soil content in non-crystalline Al oxides ($P < 0.05$).

Figure 3 shows not only the isotopic composition (^{13}C and ^{15}N) of soils under *P. pinaster* stands but also their site index values, the size of the bubble in the figure being proportional to this index. The position on the plane defined by $\delta^{13}C$ vs. $\delta^{15}N$ points to the $\delta^{13}C$ value as a good indicator for stand quality estimation, since the size of the bubble decreased as the $\delta^{13}C$ values increased, particularly for stands developed over schist (grey bubbles). This hypothesis was confirmed by the high significance of the linear regressions between soil $\delta^{13}C$ and site index values found for this tree species (Figure 4). The $\delta^{13}C$ decrease with increasing values of the site index of *P. pinaster* forests was clear ($P < 0.05$) for stands developed over granite (Figure 4b), but especially obvious ($P < 0.001$) for stands developed over schists (Figure 4a).

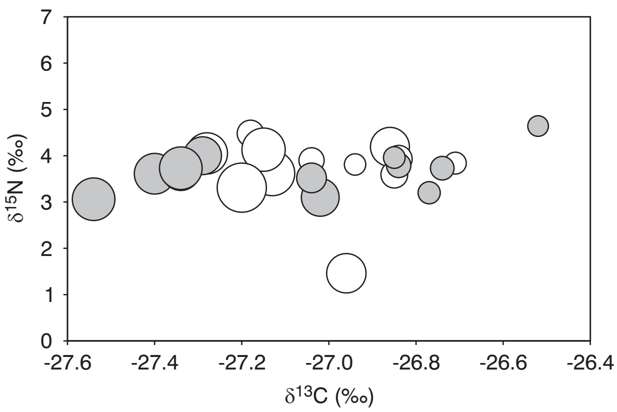

Figure 3. Isotopic $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) composition of *P. pinaster* soils developed over schists (grey bubbles) and granite (white bubbles), the size of the bubbles being proportional to the value of the site index of the corresponding plot.

When only *P. sylvestris* forests were considered, all relationships between $\delta^{13}C$ values and other soil characteristics disappeared. However, the correlations with $\delta^{15}N$ found for all soils studied remained for *P. sylvestris* independently and in this case a new significant negative correlation with soil pH was also found ($P < 0.005$). On the other hand, when we consider *P. sylvestris* soils developed over different parent materials separately, soil $\delta^{15}N$ appeared significantly correlated with soil $\delta^{13}C$ and with some

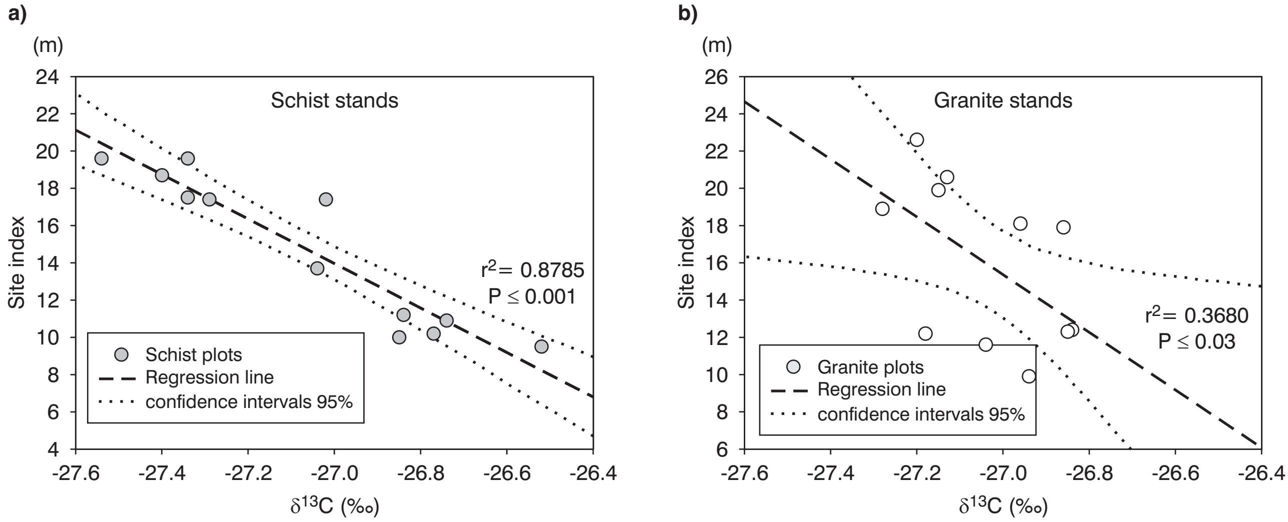

Figure 4. Relationships between the site index (m) and the δ^{13}C (‰) value of soils under *P. pinaster* stands developed over schists (a) and granite (b).

soil organic matter characteristics: i) for *P. sylvestris* soils developed over granite, soil δ^{15}N correlated negatively ($P < 0.05$) with soil δ^{13}C and soil organic matter quality, ii) for *P. sylvestris* soils developed over schists, soil δ^{15}N correlated positively ($P < 0.05$) with soil δ^{13}C and total soil organic matter content.

Figure 5 shows the isotopic composition (^{13}C and ^{15}N) of soils under *P. sylvestris* stands with the size of the bubble being again proportional to the site index values. The position on the plane defined by δ^{13}C vs. δ^{15}N suggests that the soil δ^{15}N signature could be used as a complementary tool in stand quality determination. For this tree species, a quite good linear regression between δ^{15}N and site index values was also found for soils developed over schists, where stands with higher site index showed lower δ^{15}N values ($P < 0.05$). However, in *P. sylvestris* forests developed over granite this relation was not confirmed.

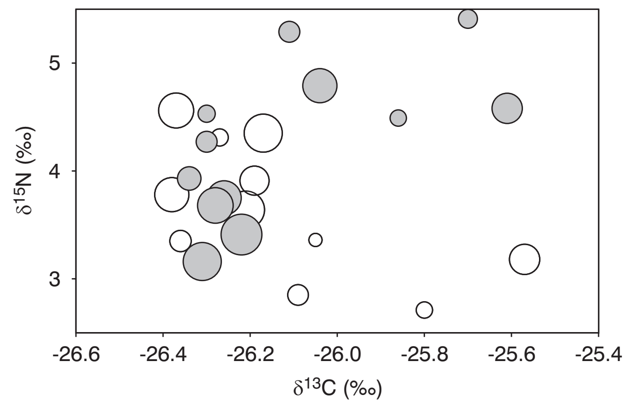

Figure 5. Isotopic δ^{13}C (‰) and δ^{15}N (‰) composition of *P. sylvestris* soils developed over schists (grey bubbles) and granite (white bubbles), the size of the bubbles being proportional to the value of the site index of the corresponding plot.

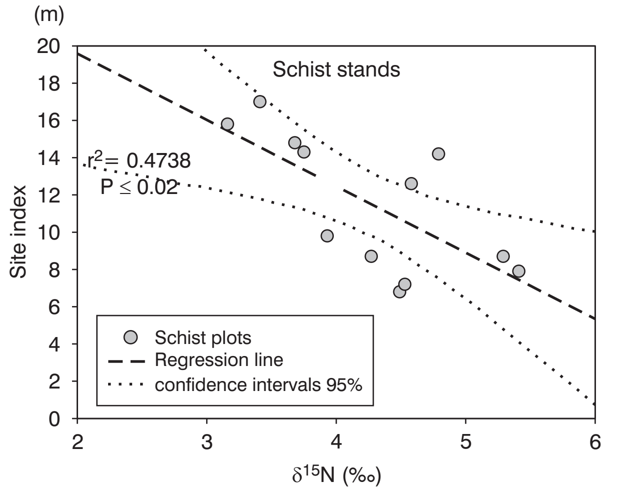

Figure 6. Relationships between the site index (m) and the δ^{15}N (‰) value of soils under *P. sylvestris* stands developed over schists.

Table 2. Best regression models obtained for the site index of *P. pinaster* or *P. sylvestris* plots

Adjusted R^2	Independent variables	Beta standardised	Significance	Tolerance
Best models for ***P. pinaster*** stands				
GRANITE + SCHIST (n = 24)				
0.542	^{13}C	–0.749	0.001	1
0.562	^{13}C ^{15}N	–0.720 –0.198	0.001 0.170	>0.979
GRANITE (n = 12)				
0.305	^{13}C	–0.607	0.036	1
0.361	^{13}C ^{15}N	–0.646 –0.333	0.026 0.203	>0.986
SCHIST (n = 12)				
0.867	^{13}C	–0.938	0.001	1
Best models for ***P. sylvestris*** stands				
SCHIST (n = 12)				
0.421	^{15}N	–0.688	0.013	1
0.417	^{13}C ^{15}N	+0.285 –0.866	0.359 0.016	>0.612

Dependent variable: SITE INDEX.

Finally, from an applied point of view it is important to highlight that multiple regression models (Table 2) indicate that more than half of the site index variations found in the *P. pinaster* stands studied could be predicted with $\delta^{13}C$ alone and the percentage of variance explained slightly improved when $\delta^{15}N$ was included, with small changes in the tolerance. These models explained even more site index variance (surpassing 80%) when applied only to *P. pinaster* plots developed over schists. Earlier quoted results were also confirmed for *P. sylvestris* forests, the soil ^{15}N isotopic composition appearing as the best indicator of stand quality, since soil $\delta^{15}N$ values explained more than 40% of the site index variations for this species.

Conclusions

The isotopic composition of soils under *P. pinaster* differed from soils under *P. sylvestris* forests, values of $\delta^{13}C$ from *P. pinaster* forests soils being significantly lower than those from *P. sylvestris* plots. Differences in $\delta^{15}N$ between soils from forest plantations with different tree age were only found for *P. sylvestris* plots of low quality, soils under young plantations being ^{15}N enriched as compared with old ones. In both tree species, the relationships between stand quality and soil isotopic composition (^{13}C and ^{15}N) were more evident for forest stands developed over schists.

Soil ^{13}C content was found to be a good indicator of stand quality for *P. pinaster* forests, whereas stand quality of *P. sylvestris* plantations was more related with their soil ^{15}N content.

Therefore, the use of C and N stable isotopes natural abundances has been proved to be a useful tool for quality tree and quality site estimations. For both pine species, stands with high site index showed more depleted soils in ^{13}C and ^{15}N as compared with low site index stands, suggesting the possible use of soil isotopic fingerprint as a source of useful information for a suitable forest establishment. Nevertheless, further investigation would be necessary in order to confirm if the data obtained in this research can be applied to other areas and/or to different tree species.

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