

## Water sources and carbon isotope composition ( $\delta^{13}\text{C}$ ) of selected tree species of the Italian Alps

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Selected species of the Italian Alpine region (*Picea abies* (L.) Karst., *Pinus sylvestris* L., *Pinus cembra* L., and *Larix decidua* L.) have been investigated in terms of water-source utilization and carbon fixation estimated by the analysis of hydrogen and carbon stable isotopes composition at two sites of differing altitude (1000 and 1500 m above sea level). *Larix decidua* is the species most dependent on groundwater in both sites, while *Pinus sylvestris* utilizes rainwater to a greater extent. Concurrently, *Pinus sylvestris* displayed the highest value of the carbon isotopic ratio ( $\delta^{13}\text{C} = -25.9 \pm 0.6\text{‰}$ ), while *Larix decidua* had the most negative one ( $\delta^{13}\text{C} = -29.0 \pm 0.4\text{‰}$ ). A relationship was found between water-source utilization and carbon-isotope discrimination.

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Nous avons étudié l'utilisation de l'eau et la fixation du carbone telles qu'estimées par l'analyse de la composition en isotopes stables d'hydrogène et de carbone chez une sélection d'espèces (*Picea abies* (L.) Karst., *Pinus sylvestris* L., *Pinus cembra* L., et *Larix decidua* L.) sur deux sites de la région alpine d'Italie localisés à 1 000 et 1 500 m d'altitude. Les résultats indiquent que *L. decidua* est l'espèce qui dépend le plus de l'eau souterraine alors que *P. sylvestris* dépend le plus de l'eau de pluie. En même temps, *P. sylvestris* démontre la plus haute valeur de rapport isotopique pour le carbone ( $\delta^{13}\text{C} = -25.9 \pm 0.6 \text{‰}$ ) alors que *L. decidua* affiche la valeur la plus négative ( $\delta^{13}\text{C} = -29.0 \pm 0.4 \text{‰}$ ). Nous avons établi une relation entre l'utilisation de l'eau et la discrimination isotopique pour le carbone.

[Traduit par la rédaction]

### Introduction

The use of stable isotopes at natural abundance levels as tracers of important biological processes in plant ecology and physiology has gained considerable interest (Rundel et al. 1988).

Measurement of the carbon isotope composition of plant tissue has become an important technique for assessing water-use efficiency of crops and natural vegetation (Farquhar et al. 1988).

The  $^{13}\text{C}/^{12}\text{C}$  composition of plant materials is lower than that of atmospheric carbon dioxide, owing to the discrimination during carboxylation and slower diffusion of  $^{13}\text{CO}_2$  relative to  $^{12}\text{CO}_2$ . The amount of fractionation with respect to the air-source composition can be related to intercellular  $\text{CO}_2$  concentration by a model that takes into account discrimination at carboxylation site and fractionation during diffusion (Farquhar et al. 1982; O'Leary et al. 1992). Because intercellular  $\text{CO}_2$  concentration is greatly controlled by stomata and is related itself to the water-use efficiency, the use of carbon stable isotopes allowed to understand better the simultaneous regulation of carbon and water exchanges by plants (Farquhar et al. 1989).

At the same time, considerable progress has been achieved in understanding water-source utilization by means of the hydrogen stable isotope analysis.

Because there is no fractionation of isotopes by plant roots during water uptake (White et al. 1985), hydrogen isotope analysis of plant xylem water reflects the isotopic composition of the actual water source in use by the plant. This makes it possible to distinguish the type of water source (i.e., rain- or ground-water) that the plant is using at the time of analysis (Sternberg and Swart 1987; Flanagan and Ehleringer 1991; Dawson and Ehleringer 1991; Ehleringer and Dawson 1992).

Recently, the comparative analysis of water-use efficiency and water-source utilization for a Mediterranean macchia community (Valentini et al. 1992) showed that species depending mostly on rainwater are more water-use efficient than species whose predominant water source is ground-water. Similar studies by DeLucia and Schlesinger (1991) and Flanagan et al. (1992), on different species, reported an analogous trend.

The aim of the present work was to investigate a similar relationship among plant species occurring in the Italian Alpine region.

Alpine trees are exposed to severe constraints on carbon and water-resource acquisition and utilization. Previous

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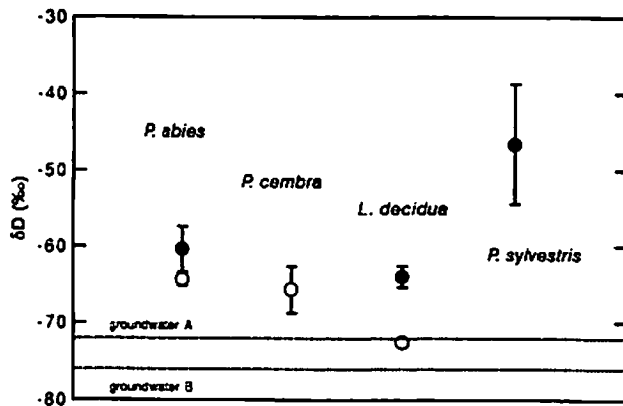


FIG. 1. Distribution of the hydrogen isotope ratio ( $\delta D$ ; ‰) for the study species in the two sites (site A; 1000 m asl, solid circles; site B, 1500 m asl, open circles). Vertical bars are  $\pm 1$  S.E.

works (Körner et al. 1988, 1991) have focussed on a global survey of the carbon stable isotope composition of plant tissues along altitudinal and latitudinal gradients. Nevertheless, a parallel analysis between water-use efficiency and water-source utilization has never been carried out for the alpine regions.

For this reason we have selected both evergreen (*Picea abies* (L.) Karst., *Pinus sylvestris* L., and *Pinus cembra* L.) and deciduous (*Larix decidua* L.) tree species at two different altitudinal sites, representative of the Italian Alpine region.

#### Materials and methods

Two sites were selected in the eastern Italian Alps (Dolomites) near Cortina D'Ampezzo. The first site is located at an altitude of 1000 m above sea level (asl) (Villanova di Cadore; Site A), on a northeast alluvial detritus slope characterized by a brown forest soil. The study species are *Larix decidua*, *Picea abies*, and *Pinus sylvestris*, which form a later successional stage of an old grassland.

The second site is located at an altitude of 1500 m asl (Ospitale di Cardore; Site B) on a north-northwest slope characterized by a rendzina soil with a thick organic layer. The study species are *Pinus cembra*, *Picea abies*, and *Larix decidua*.

The two sites are 20 km apart and experience different climatic conditions. Site A is warmer than site B (about 3°C difference in mean annual temperature, and more xeric due to a decreased soil field capacity).

The study species were sampled in the two sites taking care to maximize homogeneity among individuals with respect to age and social position in the stand.

#### Hydrogen stable isotope analysis

Plant source water was obtained by sampling bole cores directly to prevent possible enrichment fractionation due to transpiration, which occurs in young stems or close to the leaf site. Cores were immediately inserted into bags, sealed, and then taken to the laboratory. Samples were kept frozen until the water was extracted. Two bole cores were sampled per tree, with three trees per species, in the two sites.

To determine the water sources used by the these plants, well water, taken in close proximity to the sampled trees, was sampled as an indicator of groundwater isotopic composition. These samples were stored in glass vials until isotopic analysis was conducted.

Water from stems was cryogenically extracted under vacuum (Dawson and Ehleringer 1991) and hydrogen in water was reduced to its diatomic form using zinc (Coleman et al. 1982). The procedure was modified slightly in that combustion occurred at

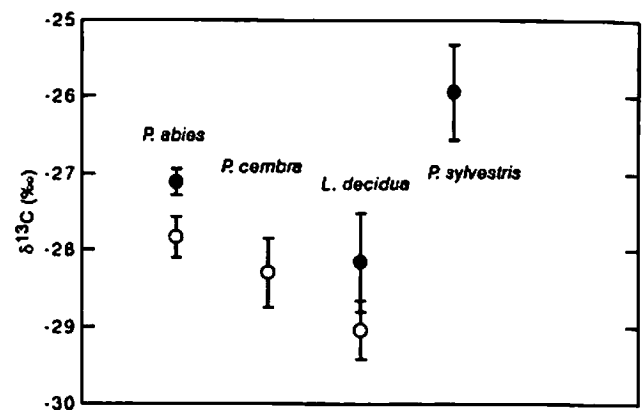


FIG. 2. Distribution of  $^{13}C$  discrimination ( $\delta^{13}C$ ) among the study species (site A; 1000 m asl, solid circles; site B; 1500 m asl, open circles). Vertical bars are  $\pm 1$  S.E.

500°C. This modification to a higher temperature of combustion resulted in a greater precision than observed in the original study of Coleman et al. (1982).

The deuterium content was measured on a Finnigan MAT, model  $\delta S$ , isotope ratio mass spectrometer.

Deuterium content is expressed in  $\delta$ -notation as  $\delta D$  (parts per thousand) relative to standard mean oceanic water (SMOW):

$$\delta D = (R_{\text{sample}}/R_{\text{standard}} - 1)1000$$

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  represent the molar deuterium/hydrogen ratios of samples and the SMOW standard, respectively. The overall precision of preparation and analysis was  $\pm 1.0\text{‰}$ .

#### Carbon-isotope analysis

For carbon-isotope determination, five small twigs of the study species were sampled randomly in the upper part of the crown (on the same trees as for xylem sap hydrogen-isotope ratio). The leaves were dried and finely ground before combustion. Carbon isotope ratio ( $\delta^{13}C$ ), relative to the Pee Dee belemnite (PDB) standard (using an equivalent equation as above), was determined by combusting samples in an elemental analyzer coupled to the same isotope ratio mass spectrometer. Overall precision of the  $^{13}C$  analysis was 0.11‰.

Data on isotopic composition are expressed as means  $\pm 1$  SE.

#### Results

Figure 1 presents the  $\delta D$ -values for the study species in the two sites. The study species show  $\delta D$ -values ranging from -72.5 to -46.5. The groundwater isotopic composition is -72.5 and -76.5 for site A and site B, respectively. Among the four study species of site A (lower altitude site), *Pinus sylvestris* xylem water seems to show the highest deuterium composition ( $\delta D = -46.5 \pm 7.8\text{‰}$ ), followed by *Picea abies* and *Larix decidua* ( $-60.4 \pm 3.0\text{‰}$  and  $-64.0 \pm 1.4\text{‰}$ , respectively). On the other hand *Larix decidua* of site B (higher altitude site) shows the minimum  $\delta D$ -value ( $\delta D = -72.5 \pm 0.5\text{‰}$ ). At site B, *Picea abies* and *Pinus cembra* show intermediate  $\delta D$ -values ( $-64.4 \pm 0.8\text{‰}$  and  $-65.7 \pm 3.0\text{‰}$ , respectively).

Discrimination of carbon isotopes for the study species in both sites range from -25.9 to -29.0‰ (Fig. 2), confirming the expected values for  $C_3$  species.

The maximum  $\delta^{13}C$  value is achieved by *Pinus sylvestris* ( $\delta^{13}C = -25.9 \pm 0.6\text{‰}$ ) while *Larix decidua*, belonging to the higher altitude site, shows the lowest value ( $\delta^{13}C = -29.0 \pm 0.4\text{‰}$ ). At site A, *Picea abies* and *Larix decidua* show intermediate values,  $-27.1 \pm 0.2\text{‰}$  and  $-28.3 \pm 0.5\text{‰}$ , respec-

tively. At site B, *Picea abies* shows a  $\delta^{13}\text{C}$  value of  $-27.8 \pm 0.3\text{‰}$  and *Pinus cembra* a value of  $-28.3 \pm 0.5\text{‰}$ .

### Discussion and conclusions

The isotopic composition of rainwater varies seasonally (Dansgaard 1964). Precipitation falling as rain is enriched in deuterium, compared with snow, owing to differences in the vapour pressures of heavier and lighter water (i.e., DHO versus  $\text{H}_2\text{O}$ ) (Ehleringer and Dawson 1992).

Groundwater isotopic composition reflects a weighted average of annual precipitation inputs and is fairly constant throughout the year. Both precipitation and groundwater, however, are more depleted in deuterium than ocean water, which is taken as the standard. This explains the usually negative isotopic ratios of water in plant tissues.

We were not able to analyze isotopic composition of rainwater during the period of sampling, but summer rain is far more enriched in deuterium than the groundwater collected in the same period (Dawson and Ehleringer 1991). For this reason, deviations from the groundwater base line towards positive values are to be considered as indications of a certain amount of rainwater use. We cannot exclude other sources of groundwater with different isotopic composition, but the ones more enriched in deuterium are the result of the most recent rainfall events.

The stability of the groundwater hydrogen isotopic signature is confirmed also by the small variation among the two sites (4.0‰) despite the fact that they are located far from each other and characterized by different soils.

*Pinus sylvestris*, at site A, shows the higher  $\delta\text{D}$  isotopic ratio, indicating the greater rainwater use among the study species.

This species grows well on poor and rocky soils, where it is rather competitive as a result of its shallow rooting system (Gellini 1985; Jacamon 1987). In contrast, *Larix decidua* shows, in both sites, the most negative  $\delta\text{D}$ -value, indicating an almost exclusive use of groundwater, particularly at site B.

This species, which is the only deciduous conifer of the Alpine region, is capable of developing a deep root system.

At site A (lower altitude site),  $\delta\text{D}$ -values of both *Picea abies* and *Larix decidua* are more positive than the corresponding ones at site B. The difference of  $\delta\text{D}$ -value between the two groundwaters (4.0), can explain the shift of *Picea abies*  $\delta\text{D}$ -value between altitudes, but not the *Larix decidua* one, which is of greater magnitude (8.5). It is also evident that the difference in water-source utilization, between *Picea abies* and *Pinus cembra* versus *Larix decidua* (the latter using essentially groundwater), that was significant at the higher altitude site, becomes less important at the lower altitude site. This trend can be explained by the thinner organic layer and greater compactness of site A, which restricts the development of deep roots for *Larix decidua* and determines its rainwater use to a greater extent. Actually, *Larix decidua* in this environment is a pioneer species, which is gradually replaced by *Picea abies*, *Abies alba* L., and *Fagus sylvatica* L. Root conformation and development probably do not follow the usual trend for this species in its favourable environment.

Discrimination of carbon isotopes ranged from  $-25.9$  to  $-29.0\text{‰}$  confirming the expected values for  $\text{C}_3$  species.

Taking into account both discrimination at carboxylation sites and fractionation during diffusion (Farquhar et al. 1982;

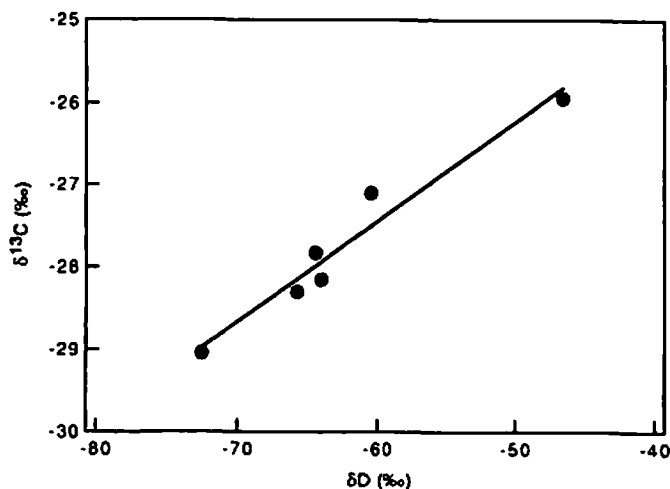


FIG. 3. Relationship between carbon discrimination ( $\delta^{13}\text{C}$ ) and hydrogen isotopic ratio ( $\delta\text{D}$ ) for the study species.

Ehleringer et al. 1986), it is possible to relate  $\delta^{13}\text{C}$  leaf values with intercellular  $\text{CO}_2$  concentration ( $c_i$ ):

$$\delta^{13}\text{C}_{\text{leaf}} = \delta^{13}\text{C}_{\text{air}} - \frac{a(c_a - c_i)}{c_a} - \frac{bc_i}{c_a}$$

where  $c_a$  is the air  $\text{CO}_2$  concentration;  $\delta^{13}\text{C}_{\text{air}}$  and  $\delta^{13}\text{C}_{\text{leaf}}$  are the carbon isotope ratio of air (typically  $-7.8\text{‰}$ ) and leaves, respectively;  $a$  is the discrimination associated with the slower diffusion rate of  $^{13}\text{CO}_2$  ( $a = 4.4\text{‰}$ ); and  $b$  is the net discrimination against  $^{13}\text{CO}_2$ , associated with RuBP carboxylase ( $b = 29\text{‰}$ ).

Altitudinal effects could be responsible for the differences observed in the  $\delta^{13}\text{C}$  values of *Larix decidua* and *Picea abies*, going from 1000 to 1500 m asl. Both species show higher  $\delta^{13}\text{C}$  values, hence, higher intercellular concentrations than the lower elevation site. *Larix decidua* and *Picea abies* change from  $c_i/c_a$  equal to 0.65 and 0.61 at site A to 0.68 and 0.64 at site B, respectively. *Pinus sylvestris* at site A shows the lowest intercellular  $\text{CO}_2$  concentration among the study species ( $c_i/c_a = 0.56$ ), while *Pinus cembra* at site B shows intermediate values ( $c_i/c_a = 0.66$ ). The estimate of intercellular concentrations is to be considered indicative, because  $\delta^{13}\text{C}$  values do not consider isotopic discrimination associated with source or respiratory effects.

As air pressure decreases with altitude, the partial pressures of its constituents (including  $\text{CO}_2$ ) decrease in proportion. In principle,  $\text{CO}_2$  concentration should be constant, besides possible stratification effects, which are negligible for this altitude variation.

This was also confirmed by Levin (1984), who showed that the carbon isotope composition of the atmosphere did not change significantly along an altitudinal gradient. Nevertheless, changes in the intercellular  $\text{CO}_2$  concentration have been observed.

Korner et al. (1988) carried out a survey of carbon isotope composition in plants from high altitudes. They reported that plants occurring at high-altitude sites showed a decrease in intercellular  $\text{CO}_2$  concentration, in contrast with our results. However, our altitudinal gradient was smaller than the range taken into consideration by their survey, and site-specific characteristics, like soil depth and moisture conditions, probably had a greater effect on the intercellular  $\text{CO}_2$  concentration.

Actually water relations of *Picea abies* during July show that trees growing in the lower altitude site have a greater water potential at turgor loss (-1.4 MPa) than those growing at higher altitude (-2.0 MPa) (T. Anfodillo, personal communication). This difference can partly explain the observed decrease of intercellular CO<sub>2</sub> concentration at lower altitude, where trees are more sensitive to stomatal closure in response to drought.

Combining hydrogen and carbon isotopes observations on the study species, irrespectively of altitudes, a general linear trend of water-source use and carbon discrimination is obtained (Fig. 3).

Species that are using groundwater as the principal source of water show the highest intercellular CO<sub>2</sub> concentration, while species using both groundwater and rainwater show lower intercellular concentration.

A similar result was obtained by Valentini et al. (1992) on a Mediterranean macchia ecosystem, where for all the study species carbon and hydrogen isotope ratios were closely correlated in the same direction, i.e., species only utilizing groundwater were the least water-use efficient.

DeLucia and Schlesinger (1991) and Flanagan et al. (1992) have observed a negative relationship between plant water-use efficiency and water potential in a variety of perennial species.

Although indirectly, water potential reflects water-source utilization, because more negative water potentials are observed for shallow-rooting species, which are likely to utilize mostly summer rains. In particular, the data of Flanagan et al. (1992) clearly show a close correlation between water use and carbon fixation reflected in the isotope values, in the same direction as the present study.

Also Ehleringer et al. (1991) observed a similar trend between water-use efficiency and water potential, but the correlation between water potential and water-use efficiency was present also in spring when all the plants were utilizing the same source of water.

Although we cannot show a direct relationship between water-use efficiency and water sources, per se, it is rather clear that, considered on a long-term basis, stomatal control plays a fundamental role in linking water sources with intercellular CO<sub>2</sub> concentration.

Species that depend more on rainwater seem to need to develop strong stomatal control to regulate transpiration losses, hence, photosynthesis according to the frequency and intensity of rain events. On the other hand, those species depending more on groundwater sources are likely to show stomatal control to a lesser extent, being capable of utilizing a rather constant water source.

In the cited study of Valentini et al. (1992), it was noted also a clear differentiation among deciduous and evergreen species for both water-source utilization and water-use efficiency. The former were using almost exclusively groundwater with the least water-use efficiency, while the latter were using rainwater to a greater extent, with the higher water-use efficiency. Also in the present data set *Larix decidua* shows a similar trend, being the only deciduous

species of the investigated group, which presents, particularly in the higher altitude site the most negative  $\delta D$ - and  $\delta^{13}C$ -values.

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