Carbon assimilation, nitrogen, and photochemical efficiency of different Himalayan tree species along an altitudinal gradient

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Abstract

In the area of Jumla region in Western Nepal, measurements of saturated leaf net photosynthetic rate (P_{sat}), nitrogen content, leaf fluorescence, carbon isotopic composition, and water status were performed on woody coniferous (*Pinus wallichiana, Picea smithiana, Abies spectabilis, Juniperus wallichiana, Taxus baccata*), evergreen (*Quercus semecarpifolia, Rhododendron campanulatum*), and deciduous broadleaved species (*Betula utilis, Populus ciliata, Sorbus cuspidata*) spreading from 2 400 m up to the treeline at 4 200 m a.s.l. With the exception of *J. wallichiana*, P_{sat} values were lower in coniferous than broadleaved species. *Q. semecarpifolia*, that in this area grows above the coniferous belt between 3 000 and 4 000 m, showed the highest P_{sat} at saturating irradiance and the highest leaf N content. This N content was higher and P_{sat} lower than those of evergreen oak species of temperate forests at middle and low altitudes. For all species, P_{sat} and N content were linearly correlated, but instantaneous nitrogen use efficiency was lower than values measured in lowland and temperate plant communities. The values of carbon isotopic composition, estimated by $\delta^{13}C$, showed the same range reported for temperate tree species. The ranking of $\delta^{13}C$ values for the different tree types was conifers< evergreen broadleaved<deciduous, suggesting tighter stomatal closure and higher water use efficiency for the evergreen types, confirming trends found elsewhere. No relevant differences of $\delta^{13}C$ were found along the altitudinal gradient. Quantum yield of photochemistry at saturating irradiance, measured by leaf fluorescence ($\Delta F/F_m'$), was highest in *J. wallichiana* and lowest in *T. baccata*. Overall, photochemical efficiency was more strongly related to species than to altitude. Interestingly, changes of $\Delta F/F_m'$ along the altitudinal gradient correlated well with the reported altitudinal distribution of the species.

Additional key words: *Abies*; *Betula*; carbon isotopic composition; nitrogen; photosynthesis; *Picea*; *Pinus*; *Populus;* PS2 efficiency; *Quercus semecarpifolia*; *Rhododendron*; *Sorbus*.

Introduction

Vegetation at high altitude experiences extreme environmental conditions that become unaffordable or disadvantageous, particularly for tree species. High irradiance, low temperature, large diurnal temperature fluctuations, low O_2 and CO_2 partial pressure, together with the limited nutrient availability as a consequence of slow mineralization and low atmospheric deposition are all influencing net photosynthetic rate (P_N) (Tranquillini 1964, Billings and Mooney 1968, Larcher 1983, Smith and Young 1987, Friend and Woodward 1990). All these factors lead to slow growth (Sakai and Malla 1981) and to the development of high resistance to photoinhibitory damage at low temperature. Nevertheless, the photosynthetic response of high altitude plants appears to be well adapted and the photosynthetic rates are sometimes not limited and even similar to lowland plants (Körner 1989, Terashima *et al.* 1993). Higher P_N per unit leaf area is associated with greater carboxylation efficiency at high altitude as shown by the trend of carbon isotopic discrimination that decreases with increasing elevation (Körner and Mayr 1981, Körner *et al.* 1986, Körner 1988, Morecroft and Woodward 1990). Also mineral nutrients are important and among them leaf N particularly exhibits an increasing altitudinal trend (Körner 1989).

Forest ecosystems in the Himalayan region reach particularly high altitudes, occurring up to 4 500 m a.s.l. In comparison, in the northern hemisphere, at around 40°N, the treeline generally occurs between 2 100 and 3 700 m a.s.l. Isolated tree or forest patches are present above the treeline in various parts of the world, but they never occur at altitudes comparable with the Himalayan region. For example, in the rocky outcrops of Swiss Alps, *Pinus cembra* L. reaches 2 500 m a.s.l., 200–300 m above treeline, while trees of *Juniperus recurva* Buch-Ham. in the Everest region occur at 4 220 m a.s.l., several hundred meters above the continuous treeline of central Himalaya

(Troll 1973).

In Western Nepal, the distribution of vegetation along altitude shows high peculiarity with different forest types, ranging from temperate, cool-temperate to subalpine (Dobremez 1976, Shrestha 1982, 1989, Ohsawa *et al.* 1986). A broad generalisation of the altitudinal vegetation belts can be sketched as follows (Fig. 1): *Pinus wallichiana* A.B. Jacks and *Picea smithiana* Wall. forests appear from 2 000 to 3 000 m a.s.l., a mixed forest dominated by *Quercus semecarpifolia* Smi. and *Abies spectabilis* (D. Don) Spach from 3 000 to 3 800 m a.s.l.; on southern slopes pure stands of *Q. semecarpifolia* up to 4 000 m
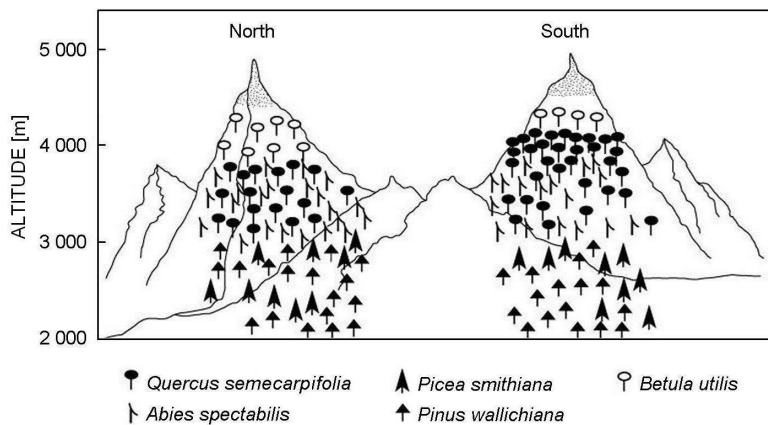

Fig. 1. Scheme of the altitudinal distribution of vegetation for Western Nepal on North- (*left*) and South-facing (*right*) aspects.

a.s.l., while on northern slopes *Betula utilis* D. Don accompanied by *Rhododendron campanulatum* D. Don. grows from 4 000 to 4 200 m a.s.l.

Ecophysiology of the major tree species of high altitude Himalayan vegetation has seldom been studied and most of the findings on ecophysiological traits of high altitude vegetation came from studies performed on herbaceous species. Data on tree and shrub species are limited,

particularly in the Himalayan region (Zobel and Singh 1995). Hence, the main objectives of this study were to investigate high altitude Himalayan woody species in their natural habitat in order to characterise some of their ecophysiological responses, to investigate possible differences along the altitudinal gradients and to compare the traits of these high mountain species with those of the temperate zone.

Materials and methods

Study area: The study was conducted in Western Nepal, in the Jumla region in the Karnali valley during the scientific expedition "Jumla Forest 1994" organised by the University of Tuscia (Viterbo, Italy). The expedition was carried out between end of September and beginning of October 1994, when a trekking of 180 km was performed. The altitude reached during the trek ranged from the 2 300 m a.s.l. of the Jumla village to the 4 200 m of the treeline and, along this gradient, different forest types of conifers and broadleaved species were crossed. Most of the measurements and plant samples were collected at Rara Lake National Park, at an altitude around 3 000 m a.s.l., during a seven days long camp. Geographical coordinates and altitudes of the main sampling sites are given in Table 1.

Nepal has a monsoon climate with relatively moderate winters and humid summers. The area of Western Nepal is drier than other parts of Nepal falling into the 500–1 000 mm annual rainfall class, with a period of aridity in autumn and, sometimes, in spring (Dobremez and Shrestha 1980). On the average, almost 80 % of annual precipitation falls from June to August. The study area lies in the temperate and subalpine bioclimatic zone, where very deep snow cover is rare (Shresta 1989).

The long term climate of Jumla and of the main sampling site of the expedition (Rara Lake) is presented in Table 2. Mean annual temperature at Jumla is 12.0 °C, while the annual precipitation reaches 842 mm. Rara Lake, being at higher altitude, has a cooler mean temperature (9.3 °C) and a slightly higher precipitation

Table 1. Geographical coordinates and altitudes of the main sampling sites visited during the scientific expedition "Jumla Forest 1994". Geographical coordinates were measured by mean of a Trimble Navigation Global Positioning System (GPS).

Date, place	Altitude	Latitude (°N)	Longitude (°E)
23–24 Sep 1994 Jumla	2 300–2 400	29°16'00"	82°10'00"
24 Sep 1994	2 600–2 800	29°18'31"	82°14'02"
25 Sep 1994	3 200–3 250	29°18'22"	82°17'20"
26 Sep 1994 Path to Rara Lake	3 800	29°22'50"	82°15'48"
29 Sep – 2 Oct 1994 Rara Lake	3 000	29°32'30"	82°04'10"
5 Oct 1994 Danphe Lagna	3 650	29°22'15"	82°08'59.9"

(929 mm). At both sites, 60–70 % of annual precipitation occurs during the summer monsoon months.

Table 2. Mean monthly temperature and precipitation for the main study site (Rara Lake) and for the village of Jumla, the starting point for the expedition. Rara Lake: temperature: 12 data – years between 1979 and 1996; precipitation: 23 data – years between 1971 and 1994. Jumla: temperature: 23 data – years between 1972 and 1996; precipitation: 24 data – years between 1970 and 1994. Meteorological data gathered from the Regional Hydrological data Centre of the Hindu Kush Himalayan – Flow Regimes from International Experimental and Network Data (HKH-FRIEND), at http://www.hkh-friend.net.np/

	Temperature [°C]		Precipitation [mm]	
	Jumla	Rara Lake	Jumla	Rara Lake
Jan	3.5	1.2	32	28
Feb	5.0	1.9	38	42
Mar	8.3	5.0	65	51
Apr	11.9	8.8	44	42
May	15.2	11.9	62	76
Jun	18.8	14.6	79	95
Jul	19.5	15.9	183	215
Aug	19.2	15.8	183	215
Sep	17.4	14.5	98	107
Oct	12.2	11.0	37	24
Nov	7.8	7.3	6	6
Dec	4.9	4.0	15	30
Annual	12.0	9.3	842	929

In 1994, mean annual temperature was very close to the long-term average at both sites (Jumla 12.0 °C, Rara Lake 9.6 °C). For the same year, precipitation was within the mean at Jumla (814 mm), while at Rara Lake, due to very limited autumn and winter precipitation, the year was significantly drier (600 mm). At both sites, summer monsoon rains were similar to the long-term average and represented 70–80 % of the annual total precipitation.

Leaf sampling and ecophysiological measurements were carried out on eight woody species (Table 3): the evergreen conifers *Pinus wallichiana*, *Picea smithiana*, *Abies spectabilis*, *Juniperus wallichiana*, *Taxus baccata*, the evergreen broadleaved *Quercus semecarpifolia* and *Rhododendron campanulatum,* and the broadleaved deci-

duous *Betula utilis*, *Populus ciliata* Wall. *ex* Royle and *Sorbus cuspidata* (Spach) Hedl. For the last two species only leaf nutrient and stable isotope analyses were performed.

Measurements and sampling were taken along an altitudinal gradient 2 500–3 800 m a.s.l. Generally, measurements were performed at one plot per altitude (three in the case of Rara Lake area), where two to four trees per species and three to six leaves per tree were sampled. Sample trees and shrubs were selected according to representativity within the stands. Generally, sun-exposed specimens (South, South West) with well-developed crowns were chosen and sampling was performed on attached foliage in the first three meters of crown.

Leaf gas exchange was measured with two portable infrared gas analysers (IRGA), *LCA-2* and *LCA-3* (*ADC*, Hoddesdon, UK). The analysers were equipped with two cuvettes, one for coniferous (cylindrical, 200 cm^3) and the other for broadleaved species (flat, 6 cm^2). The analysers are open systems, measuring the CO_2 difference between the reference and the cuvette gas flows and have an auto-zeroing procedure. They were intercalibrated in the laboratory before the expedition with three gas bottles of different CO_2 concentrations (300, 420, and 900 μmol mol^{-1} in nitrogen; *Rivoira*, Florence, Italy). The calibration of the analysers was checked again at the end of the expedition and the CO_2 span was found to be within ±1 % against the calibration gas (300 μmol mol^{-1}). The cuvette temperature sensors were calibrated in a climatised chamber in 6 temperature steps (from 5 to 30 °C). Gas exchange measurements were performed under natural conditions on attached leaves or shoots (in the case of conifers), from the sun-exposed portion of the crown. Generally, current year's mature leaves and shoots were selected for measurements. For conifers, only one needle age class (the current one) was included in the cuvette. Photosynthesis at saturating irradiance (P_{sat}) was measured at photosynthetically active radiation (PAR) above 1 300–1 500 μmol m^{-2} s^{-1}, generally on three to six samples. During the measurements, care was taken to avoid excessive heating of the attached samples and the difference between ambient air and cuvette temperature was limited to a maximum of 2 °C. All measurements were performed *in vivo* and under natural environmental conditions occurring at the time of sampling. The sampling period

Table 3. Structural characteristics of the studied species. Basal area, altitude, and soil water content (SWC) of the surveyed forest plots (sample areas 100–625 m^2) are also given.

	Species	Growth form	Maximum diameter [cm]	Height [m]	Basal area [m^2 ha^{-1}]	Altitude [m a.s.l.]	SWC [m^3 m^{-3}]
Conifers	*Abies spectabilis*	tree	73	21	17.0	3 060	0.265±0.016
	Juniperus wallichiana	shrub	15	3	n.d.	3 000	0.119±0.012
	Picea smithiana	tree	73	24	62.4	3 000	0.259±0.016
	Pinus wallichiana	tree	43	15	22.4	3 000	0.119±0.012
	Taxus baccata	tree	18	8	n.d.	3 050	0.265±0.016
Broadleaved	*Betula utilis*	tree	42	18	16.5	3 800	0.241±0.012
	Populus ciliata	tree	35	18	n.d.	2 600	n.d.
	Sorbus cuspidata	shrub	12	4	n.d.	3 800	0.241±0.012
	Quercus semecarpifolia	tree	40	15	22.4	3 000	0.119±0.012
		tree	105	26	41.7	3 600	0.241±0.012
	Rhodendron spp.	shrub	12	3	n.d.	3 800	0.241±0.012

was restricted to one week around the beginning of October 1994 that was characterised by clear weather; the measurements were generally performed during the first part of the morning. The average temperature of P_{sat} measurements ranged between 25.8 °C (*P. smithiana*) and 27.8 °C (*Q. semecarpifolia*), while for the species typically growing on the North facing slopes it was around 22–22.6 °C (*A. spectabilis, B. utilis, R. campanulatum*). Only for *T. baccata* P_{sat} was measured at a slightly higher temperature (29.7 °C). Hence, the limited differences in temperature during measurements make possible the comparison of P_{sat} between the species. P_{sat} values were expressed at standard conditions (*i.e.* sea level pressure) and, to be consistent with the stomata distribution of the species, they were referred to the projected leaf area for broadleaved species, *A. spectabilis* and *T. baccata*, and to actual total surface area for *P. wallichiana* and *P. smithiana*. Because of airline safety regulation and for portability, it was not possible to use a Scholander pressure chamber for measuring water potential. Hence, foliage water potential was measured on detached samples with a *J-14* clamp-on system (*Decagon*, Tacoma, WA, USA). The *J-14* system was tested against a Scholander chamber (*PMS*, Corvallis, Oregon, USA) on samples of temperate deciduous and coniferous species in the laboratory, and a regression was performed to derive water potential (WP) [MPa] from the *J-14* measurements, that are in absolute units from a manometer [WP$_{Scholander}$ = 0.0071 WP$_{J-14}$, $n = 25$, $r^2 = 0.92$].

Volumetric soil water content (SWC) was determined by the time domain reflectometry (TDR) technique, using a portable system equipped with a tri-furcated 18 cm probe (*Trime, IMKO*, Germany). The technique determines SWC assessing the changes in electrical properties of soils with increasing water content.

The quantum yield of photosystem 2, PS2 ($\Delta F/F_m'$), which is linearly correlated to electron transport (Genty *et al.* 1989) and may be referred to the photon use efficiency in photochemistry, was measured by chlorophyll *a* fluorescence on attached leaves utilising a *PAM-2000* portable fluorometer (*H. Walz*, Effeltrich, Germany). Measurements were taken at saturating PAR (1 400–1 500 µmol m^{-2} s^{-1}). To verify if the sampled trees were experiencing photoinhibitory damage, the photochemical efficiency of dark-adapted samples (F_V/F_M) was measured, both at predawn and at midday. Dark adaptation of detached foliage samples was obtained by means of a black cloth in the shade and fluorescence measurements were performed after 15 min, a period considered sufficient to relax the fast- and medium-energetical quenching components (Krause and Weis 1991). The batteries of all the electronic equipments were charged by means of 3 solar panels.

Leaf N content was determined on the same samples as those used for photosynthesis measurements; the samples were collected and dried during the trekking, put in paper bags, and kept in the dark. N content was determined by the Kjeldahl method (Raveh and Avnimelech 1979) in the laboratory of the Department of Plant Biology (University of Rome, Italy).

The determination of the carbon isotopic composition (^{13}C) was carried out in the laboratory of the National Research Council, Institute for Agroenvironmental and Forest Biology (CNR-IBAF, Porano, Italy) on three to five leaves sampled from the crown of the same trees on which the other ecophysiological measurements were performed; care was taken to ensure that the sampled leaves had the same prevalent exposure. Leaf material was dried and finely ground before the combustion process. Carbon isotope composition (δ^{13}C), relative to the PeeDee belemnite (PDB) standard, was determined by combusting samples in an elemental analyser coupled to a stable isotope mass spectrometer (IRMS model *SIRAII, VG Isotech*, Middlewich, UK). Overall precision of the ^{13}C analysis was 0.1 ‰, as tested against a standard with known isotopic composition.

Results

Two different groups can be distinguished based on P_{sat} (Table 4) of broadleaved evergreen and deciduous species. They ranged from 3.6 (*B. utilis*) to 5.2 μmol(CO_2) m^{-2} s^{-1} (*Q. semecarpifolia*) being greater than P_{sat} of conifer species that ranged from 2.3 (*T. baccata*) to 3.2 μmol(CO_2) m^{-2} s^{-1} (*P. smithiana*). According to *t*-test, the two groups were statistically different at $p < 0.01$. Due to particular leaf morphology, it was not possible to calcuate area-based P_{sat} for *J. wallichiana*: its mass-based P_{sat} was the highest among all the studied species.

Predawn water potential (ψ, Table 4) indicated general good hydric conditions for all tested species. The lowest ψ was measured in *Q. semecarpifolia* (-1.1 MPa). Compared to the general trend, *Q. semecarpifolia*,

T. baccata, and *R. campanulatum* exhibited lower midday water potentials, reaching -2.7, -2.4, and -2 MPa, respectively. The good hydric status of the plants is confirmed by the fact that predawn photochemical efficiency (F_V/F_M), an indicator of long lasting stress effects, is close to the theoretical maximum (0.81–0.83) for almost all species (Table 4). Furthermore, soil water content in the first 20 cm of soil ranged between 0.12 and 0.27 m^3 m^{-3} (Table 3) which indicated good hydration of the soil. The lowest values were registered in re-colonised open woodland, with S-W exposure, dominated by *Q. semecarpifolia*, *J. wallichiana*, and *P. wallichiana*.

Nitrogen content ranged from 14 (*T. baccata*) to 21.6 g kg^{-1} (*Q. semecarpifolia*, Table 4) and, overall,

Table 4. Ecophysiological parameters measured on Himalayan deciduous and evergreen tree species at different altitudes. P_{sat}: net photosynthetic rate at saturating irradiance; N: leaf nitrogen content; PWP and MWP: predawn and minimum leaf water potential; LMA: leaf mass per area; F_V/F_M: photochemical efficiency of photosystem 2 measured before dawn. *The value of *J. wallichiana* P_{sat} is calculated on a dry mass basis.

	Species	Altitude [m a.s.l.]	P_{sat} [μmol m^{-2} s^{-1}]	N [g kg^{-1}]	Leaf Water Potential [MPa] PWP	MWP	LMA [g m^{-2}]	F_V/F_M
Conifers	*Abies spectabilis*	3 000	3.03±0.16	17.1±2.4	−0.5	n.d.	120.0±0.0	0.805±0.008
	Juniperus wallichiana	3 000	(0.08±0.00)*	15.5±0.5	−0.79	−1.02	n.d.	0.807±0.009
		3 800		20.9±0.4	n.d.	n.d.	n.d.	n.d.
	Picea smithiana	3 000	3.20±0.22	17.2±0.5	−0.68	−0.91	123.8±0.1	0.812±0.007
	Pinus wallichiana	3 000	2.57±0.21	14.9±0.6	n.d.	−0.74	51.0±2.2	0.772±0.004
	Taxus baccata	3 000	2.26±0.11	14.0±0.5	−0.64	−2.43	188.3±9.7	0.824±0.005
Broadleaved	*Betula utilis*	3 800	3.56±0.26	21.1±0.5	n.d.	−0.88	126.5±0.0	n.d.
	Quercus semecarpifolia	3 200	4.78±0.23	21.5±0.6	−1.12	−2.69	213.3±4.0	0.811±0.011
		3 800	5.21±0.51	21.6±0.4	n.d.	n.d.	n.d.	n.d.
	Rhodendron spp.	3 800	4.40±0.19	17.8±0.2	−0.61	−2.02	253.8±3.2	n.d.

broadleaved species were characterised by higher values than conifers (grouped analysis, *t*-test, $p < 0.05$) confirming what was already found for P_N. Along the altitudinal
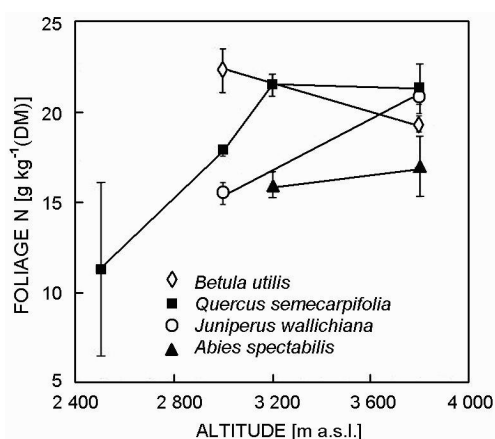

Fig. 2. Foliage nitrogen content in dry mass of Himalayan tree species sampled at different altitudes. *Error bars* represent ± 1 standard error calculated on three to five leaves.

gradient (Fig. 2), *A. spectabilis* and *B. utilis* had constant leaf N content, while in *J. wallichiana* foliage N content increased ($p < 0.05$). Leaf N content of *Q. semecarpifolia* trees growing at 2 500 and 3 000 m a.s.l. was lower than that of trees living at higher altitudes ($p < 0.05$). Above 3 000 m a.s.l., also this species had constant N contents (Fig. 2).

The values of leaf mass per unit leaf area (LMA) found in our survey (Table 4) ranged, on average, from 51 g m^{-2} (all sided, *P. wallichiana*) to 254 g m^{-2} (projected, *R. campanulatum*). LMA and N content allowed calculate area-based foliage N content, which can be related to area-based P_{sat} (Field and Mooney 1986). The relationships between area-based foliage N and P_{sat} is presented in Fig. 3. For almost all the species, higher leaf N resulted in higher P_{sat}, with a significant linear relationship ($r^2 = 0.75$, $p < 0.05$). The relationship even improved when *T. baccata* was omitted ($r^2 = 0.95$). The low P_{sat} of this species could be linked to the less efficient photon use at saturating PAR, as confirmed by fluorescence measurements (see below).
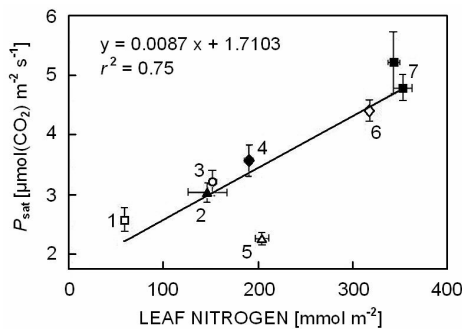
Fig. 3. Relationship between foliage nitrogen content on an area basis and net photosynthetic rate at photon saturation, P_{sat}. N and P_{sat} were measured on the same samples. *Error bars* represent ± 1 standard error calculated on three to five samples. 1: *Pinus wallichiana*; 2: *Abies spectabilis*; 3: *Picea smithiana*; 4: *Betula utilis*; 5: *Taxus baccata*; 6: *Rhododendron campanulatum*; 7: *Quercus semecarpifolia*.

At 3 000 m a.s.l., $\delta^{13}C$ of the nine sampled species ranged from –24.08 ‰ (*J. wallichiana*) to –28.9 ‰ (*P. ciliata*, Fig. 4). At higher altitude (3 800 m a.s.l.), on a lower number of species, the range of $\delta^{13}C$ values was larger, from –22.8 ‰ (*J. wallichiana*) to –29 ‰ *(S. cuspidata)*. Between 3 000 and 3 800 m a.s.l., carbon isotopic composition increased significantly for *R. campanulatum* (+9 %, *t*-test, $p<0.01$), but was relatively constant for *Q. semecarpifolia*, *A. spectabilis*, and *B. utilis*, while the slight increase for *J. wallichiana* (+5 %) was not statistically significant.
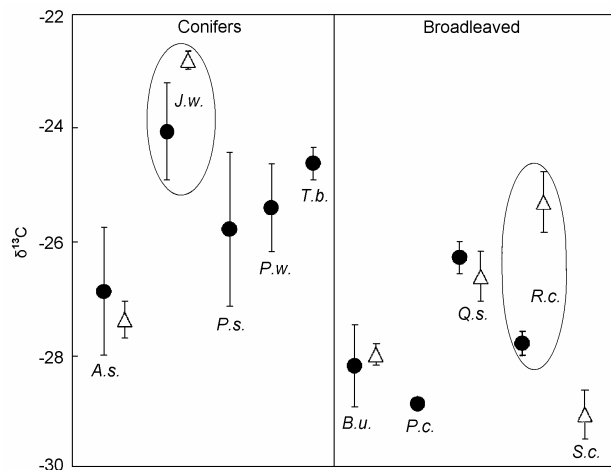

Fig. 4. Foliage carbon isotopic composition, $\delta^{13}C$ [‰] of deciduous and evergreen tree species sampled at 3 000 m (*closed circles*) and 3 800 m a.s.l. (*open triangles*). *Error bars* represent ±1 standard error calculated on three to six samples. *The ovals* connect values of the species that have shown the largest difference between two altitudes. Abbreviations: *A.s.*, *Abies spectabilis*; *J.w.*, *Juniperus wallichiana*; *P.s.*, *Picea smithiana*; *P.w.*, *Pinus wallichiana*; *T.b.*, *Taxus baccata*; *B.u.*, *Betula utilis*; *P.c.*, *Populus ciliata*; *Q.s.*, *Quercus semecarpifolia*; *R.c.*, *Rhododendron campanulatum*; *S.c.*, *Sorbus cuspidata*.

When the data of isotopic composition were analysed at the same altitude, it was possible to identify some interesting features. At 3 000 m a.s.l., around the Rara Lake, *P. ciliata, B. utilis, R. campanulatum,* and *A. spectabilis* showed lower $\delta^{13}C$ than *Q. semecarpifolia, P. smithiana, P. wallichiana, T. baccata,* and *J. wallichiana*. Isotopic composition of different tree types ranked as follows: conifers (–25.38±0.34 ‰, $n = 28$)< evergreen broadleaved (–26.65±0.22 ‰, $n = 23$)< deciduous (–28.47±0.25 ‰, $n = 10$). The differences were significant and a single factor analysis of variance resulted in a stronger role of tree type in determining $\delta^{13}C$ values ($p<0.001$).
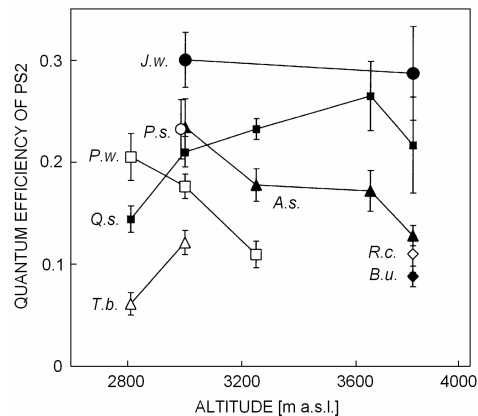

Fig. 5. Quantum efficiency of photosystem 2, PS2 ($\Delta F/F_m'$) measured at saturating irradiance (PAR 1 400–1 500 µmol m^{-2} s^{-1}) in Himalayan tree species along an altitudinal trend. *Error bars* represent ±1 standard error calculated on five to ten samples. Abbreviations: *B.u.*, *Betula utilis* (*closed diamonds*); *R.c.*, *Rhododendron campanulatum* (*open diamonds*); *A.s.*, *Abies spectabilis* (*closed triangles*); *Q.s.*, *Quercus semecarpifolia* (*closed squares*); *P.s.*, *Picea smithiana* (*open circles*); *P.w.*, *Pinus wallichiana* (*open squares*); *T.b.*, *Taxus baccata* (*open triangles*); *J.w.*, *Juniperus wallichiana* (*closed circles*). *B.u.*, *R.c.*, and *P.s.* were measured at only one altitude.

The efficiency of PS2 ($\Delta F/F_m'$) at saturating PAR ranged from 0.05 to 0.30 (Fig. 5). Overall, this parameter was correlated to the tree species (single factor ANOVA, $p<0.05$), while altitude was not a significant factor. *J. wallichiana* was the most photochemically efficient species, in accordance with its tolerance of high irradiance in open habitats. By contrast, *B. utilis* and *T. baccata* were less efficient species for photon use.

At 3 000 m, *Q. semecarpifolia, P. smithiana*, and *A. spectabilis* were clustered together (no statistical differences), while *P. wallichiana* showed a slightly lower $\Delta F/F_m'$ ($p<0.05$). Close to the upper limit of forest vegetation, at 3 800 m, *Q. semecarpifolia* and *J. wallichiana* were more efficient in energy use that the other three species ($p<0.05$, Fig. 5).

Among the species sampled within this large altitudinal range, oak and juniper were the only ones that increased or maintained constant the photon use efficiency with increasing altitude.

Discussion

Despite environmental constraints, photosynthesis of European and Himalayan alpine plants is similar to those of lowland plants (Gale 1972, Körner and Diemer 1987, Körner 1989, Terashima *et al.* 1993). These results have been attributed to the high photosynthetic capacity, associated with high leaf N contents (Friend *et al.* 1989) and to an increased mesophyll diffusion rate of carbon dioxide or an increased stomatal conductance (Gale 1972, Terashima *et al.* 1993). However, these findings concern mainly herbaceous species.

Our results on Himalayan woody species documented lower P_{sat} [2.26–5.21 µmol(CO_2) m^{-2} s^{-1}, Table 4] if compared to those of the same growth form at lower altitudes (Ceulemans and Saugier 1991). Although the amount of precipitations in the Karnali valley falls into the annual range of 500–1 000 mm, our findings on leaf predawn water potential and F_V/F_M suggest that during the study period most of the tested western Himalayan species did not undergo water stress (Table 4) and their low P_{sat} was not caused by drought effects. In this respect, Zobel *et al.* (2001) analysing seasonal trends of water potential in several tree species in Central Himalaya found that, apart from the rainy season, fall (October) was the period of the year with optimal water status of trees.

Unlike other mineral nutrients, foliage N content consistently increases with altitude (Körner 1989). In European mountains, some differences have been reported between growth forms, with mean N content increasing with altitude in herbaceous plants and remaining stable and lower in evergreen woody plants such as *Rhododendron ferrugineum* (Körner 1989). On the other hand, Friend *et al.* (1989) found that N content of *Vaccinium myrtillus* increased with altitude. In our study, leaf N content in various species remained constant or showed a tendency to increase with altitude (Fig. 2).

The species tested in this paper exhibited relatively high leaf N content, similar to woody species at high altitude in eastern Nepal (Terashima *et al.* 1993) or central Himalaya (Rawat and Singh 1988) and slightly higher that reported for *Larix* and other evergreens along altitudinal gradients in North America (Kloeppel *et al.* 1998). Our findings agree with other studies, which reported leaf N contents up to 25 % higher for Himalayan forests than for other forest types, reaching 3.47 g m^{-2} (Turner 1994).

The instantaneous nitrogen use efficiency (NUE) values found in our study [0.014–0.044 mmol(CO_2) mol^{-1}(N)] are in the same range as those reported by Terashima *et al.* (1993) for two woody species (*Salix, Rhododendron*; 0.020–0.044). The linearity of the relationship between leaf N and photosynthesis found for different vegetation types at lower altitude (Field and Mooney 1986) holds even at high altitudes in these Himalayan forest communities (Fig. 3). However, the slope of the relationship found in our study was less steep than that reported by Field and Mooney (1986), ind-

cating that for Himalayan tree species a higher proportion of leaf N is invested in processes other than photosynthesis.

Due to their relatively high leaf N content, limited nutrient retranslocation and large leaf mass, Himalayan evergreen-broadleaved forests have a lower integrated nutrient use efficiency (calculated as net primary production per unit of net nutrient uptake) than other central Himalayan and temperate forests (Zobel and Singh 1997). The consequences of these differences in leaf properties may be important: a lower specific leaf area has higher costs and longer photosynthetic periods are needed to compensate for that (Griffin 1994, Aerts 1995).

P. wallichiana and *Q. semecarpifolia* presented, respectively, high and low instantaneous NUE. These differences could be possibly linked to the ability of the two species in N re-use from senescing leaves. In this respect, Singh *et al.* (1984) reported, for different pine and oak species in the same region at lower altitude, the greater ability of pine in N use and the creation of N shortage in the soil that makes it difficult for the oak to re-invade areas occupied by pine, hence influencing vegetation dynamics.

Gas exchange characteristics of high altitude plants can also be investigated by measuring their carbon isotopic composition, which integrates plant gas exchange activity over medium-long periods. Availability of data on $\delta^{13}C$ of tree species over 3 000 m a.s.l. is generally very scanty and is almost completely lacking from Himalayan mountains. Data on trees of the temperate region between 3 000 and 4 000 m a.s.l. were reported in a global survey, with $\delta^{13}C$ values ranging between –24.5 and –28.0 ‰ (Körner *et al.* 1988). $\delta^{13}C$ for conifers from several area of the world (none from the Himalaya), growing between sea level and 2 200 m a.s.l., ranged between –22 to –29 ‰ (Warren *et al.* 2001). The range of isotopic composition we found (–22.1 to –29.8 ‰) is comparable to other surveys. Furthermore, our data are valuable in completing the available datasets in a region, which, up to now, was not covered. The values we measured for *Rhododendron* are similar to those already reported for the same species (Körner *et al.* 1988, –26.63 ‰).

In C_3 plants the carbon isotope composition increases with altitude and this is paralleled by greater carboxylation efficiency related to changing partial pressure of CO_2 and O_2, temperature, and leaf structure (Körner *et al.* 1991, Marshall and Zhang 1994, Kloeppel *et al.* 1998, Warren *et al.* 2001). In our study, the variation of $\delta^{13}C$ with altitude was smaller than previously reported. This can be probably linked to the limited altitudinal difference sampled (800 m). Nevertheless, For a comparable altitudinal difference, also another data set showed more limited changes (Körner *et al.* 1988). For some species, there is a tendency to increase carbon isotopic composition with altitude. This increase was evident for

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J. wallichiana and partially for *R. campanulatum* between 3 000 and 3 800 m a.s.l.

Carbon isotopic composition has been used to calculate plant discrimination for ^{13}C that has been related to stomatal control and water use efficiency, WUE (Farquhar *et al.* 1982, Marshall and Zhang 1994, Kloeppel *et al.* 1998, Warren *et al.* 2001). Lower δ^{13}C values generally indicate lower WUE; in North Western Nepal we found that tree types were clearly ranked for δ^{13}C with conifers exhibiting higher values than evergreen broadleaved and deciduous broadleaved trees. These findings are in agreement with what has been reported for native trees of the Central Rockies (Marshall and Zhang 1994), for larch and other sympatric evergreens (Kloeppel *et al.* 1998), and for tree species of the Italian Alps (Valentini *et al.* 1994).

A greater stomatal control and WUE seem to be ecophysiological traits pertaining to evergreen species more than to deciduous ones, also in a different vegetation type such as the maquis of the Mediterranean environment (Valentini *et al.* 1992). The highest carbon isotopic value was found in *Juniperus* spp. both in Mediterranean maquis (*J. communis* L., Valentini *et al.* 1992), in the present (*J. wallichiana*) and other studies (Warren *et al.* 2001), signalling its adaptability to harsh environments.

Alpine plants have to face high irradiance, involving several functional responses. High irradiance may photo-inactivate PS2; nevertheless, leaves of high-mountain plants are highly resistant, applying different adaptation strategies (Streb *et al.* 1988).

The photochemical response of *J. wallichiana* reported here indicates its characteristics of high photon use efficiency, emphasising its adaptability to high irradiance and open habitats already at lower altitudes (Fig. 5). By contrast, *B. utilis* and *T. baccata* were the less efficient species in photon use. The former is indeed a species that occurs on northern slopes in open habitats, indicating a better adaptation to cooler and less irradiated environments, while *T. baccata* is a typical shade tolerant species generally occurring in the understory.

The altitudinal variation of the efficiency of photon use in photochemistry may also contribute to the understanding the species altitudinal distribution. The photon-use efficiency of *P. wallichiana* progressively decreased from 2 800 to 3 200 m a.s.l. (Fig. 5). Nevertheless, this species is generally found between 2 500 and 3 000 m and sporadically occurs at higher altitudes (Shrestha 1982). *A. spectabilis* showed a maximum value at about 3 000 m a.s.l., which was maintained up to 3 650 m a.s.l. and then decreased. In the region, this species occurs preferentially in mixed forests up to 3 800 m, at higher altitudes being gradually replaced by *Q. semecarpifolia*. The evergreen oak showed an increasing trend in quantum efficiency of PS2 from 2 800 m up to a maximum value at 3 650 m, with a slight decrease at 3 800 m a.s.l. Indeed, this species is found in closed and open formations in the vegetation belt occurring from 3 000 to 4 000 m a.s.l.

The distribution and dynamics of forest vegetation are linked to the efficiency of using the available resources. In the present study, irradiance and N use were found to be important for adaptation of Himalayan tree species to altitude. For nitrogen, the results indicate that, with respect to lowland vegetation, a higher proportion of leaf N is invested in processes other than photosynthesis. Concerning photon energy, photochemical efficiency of PS2 reaches, species by species, higher values where each species becomes dominant along the altitudinal gradient. Hence, the quantum efficiency of PS2 linked to photochemistry is a useful tool to infer some ecophysiological explanations of altitudinal distribution of plant species.

The analysis of carbon isotopes confirmed, also in this Himalayan region, that conifers, evergreen broadleaved, and deciduous species differ in terms of WUE, a feature that is relevant for adaptation to environmental conditions of high altitude. Furthermore, the data set reported here will contribute to the data sets on δ^{13}C available so far.

Among the species considered, *Q. semecarpifolia* and *J. wallichiana* reached the highest P_{sat}, the highest leaf N content, concurrently with a high photochemical efficiency. All these features can help to explain the peculiarity of the distribution of these two species, with *Q. semecarpifolia* able to form extensive woodlands at high altitude, above the coniferous belt, and *J. wallichiana* able to colonise open, harsh habitats.

Hence the ecophysiological traits can be an important tool for understanding resource use, climate responses, and the distribution of tree species. Moreover, our findings further document the relevant ecological value of Nepal Himalayan forests.

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