

# Evidence of altitudinal increase in photosynthetic capacity: gas exchange measurements at ambient and constant CO<sub>2</sub> partial pressures

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## Abstract

- Because all microclimatic variables change with elevation, it is difficult to compare plant performance and especially photosynthetic capacity at different elevations. Indeed, most previous studies investigated photosynthetic capacity of low- and high-elevation plants using constant temperature, humidity and light but varying CO<sub>2</sub> partial pressures ( $P_{CO_2}$ ).
- Using gas exchange measurements, we compared here maximum assimilation rates ( $A_{max}$ ) at ambient and constant-low-elevation  $P_{CO_2}$  for two temperate tree species along an altitudinal gradient (100 to 1600 m) in the Pyrénées mountains.
- Significant differences in  $A_{max}$  were observed between the CO<sub>2</sub> partial pressure treatments for elevations above 600 m, the between-treatment differences increasing with elevation up to 4  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . We found an increase in  $A_{max}$  with increasing elevation at constant-low-elevation  $P_{CO_2}$  but not at ambient  $P_{CO_2}$  for both species. Given a 10% change in  $P_{CO_2}$ , a proportionally higher shift in maximum assimilation rate was found for both species.
- Our results showed that high elevation populations had higher photosynthetic capacity and therefore demonstrated that trees coped with extreme environmental conditions by a combination of adaptation (genetic evolution) and of acclimation. Our study also highlighted the importance of using constant CO<sub>2</sub> partial pressure to assess plant adaptation at different elevations.

## Résumé – Augmentation de la capacité photosynthétique avec l'altitude : mesures d'échanges gazeux à pressions partielles de CO<sub>2</sub> ambiante et constante.

- Les conditions microclimatiques étant très variables avec l'altitude, il est difficile de comparer les performances d'une espèce végétale à différentes altitudes, particulièrement la capacité photosynthétique. En effet, la plupart des études antérieures ont estimé le taux maximal d'assimilation à basses et hautes altitudes en maintenant la température, l'humidité de l'air et la lumière constantes mais en laissant varier la pression partielle de CO<sub>2</sub> ( $P_{CO_2}$ ).
- Afin de comparer le taux maximum d'assimilation ( $A_{max}$ ) à pressions partielles de CO<sub>2</sub> constantes de basse altitude et variables, nous avons effectué des mesures d'échanges gazeux sur deux espèces d'arbres tempérés le long d'un gradient altitudinal de 1600 m de dénivelé dans les Pyrénées françaises.
- La différence entre les deux traitements de  $P_{CO_2}$  est significative au-dessus de 600 m d'altitude et atteint un maximum de 4  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Pour les deux espèces, nous avons mis en évidence une augmentation de  $A_{max}$  avec l'altitude à  $P_{CO_2}$  constantes mais pas à  $P_{CO_2}$  ambiantes. Pour une modification de  $P_{CO_2}$  de 10 %, le changement du taux maximum d'assimilation est proportionnellement supérieur chez les deux espèces.
- Nos résultats montrent que les populations de hautes altitudes possèdent une capacité photosynthétique supérieure, démontrant que les arbres font face aux conditions environnementales extrêmes grâce à des adaptations génétiques ou des acclimations. Notre étude souligne ainsi l'importance de fixer la  $P_{CO_2}$  pour comparer l'adaptation des plantes à différentes altitudes.

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## 1. INTRODUCTION

Trees are commonly well adapted to local conditions, having evolved ecophysiological characteristics (Chabot and Hicks, 1982; Kikuzawa, 1989; Reich et al., 1992), especially in mountain areas where selection pressure is very important. The main geophysical drivers along altitudinal gradients from an ecological point of view are temperature, air pressure, precipitation and radiation (Körner, 2008). Indeed, morphological, phenological and physiological changes allow trees to maintain a relatively high level of growth-related activity despite increasingly constraining environmental conditions towards high elevation (Cordell et al., 1999). The main physiological traits that control the carbon uptake and water loss in plants and generally vary according to elevation are photosynthesis and stomatal conductance.

To our knowledge, about twenty studies have dealt with photosynthetic capacity along altitudinal gradients (Körner, 2003). However, variations in plant performance with altitude are difficult to predict, partly because of the complexity of the geophysical effects of altitude and partly because of biological responses to these changes. Indeed, maximum rates of  $\text{CO}_2$  assimilation in plants from different elevations, measured at ambient  $\text{CO}_2$  partial pressure, have been found to be equal (Benecke et al., 1981; Cordell et al., 1999; Körner and Diemer, 1987), lower (Kao and Chang, 2001; Slatyer and Morrow, 1977; Zhang et al., 2005), or higher at high elevation (Premoli and Brewer, 2007).

When comparing along an altitudinal gradient, the distinction between pressure and concentration of gases becomes crucial. Unfortunately, many studies have used  $\text{CO}_2$  molar fractions instead of  $\text{CO}_2$  partial pressure to express  $\text{CO}_2$  availability, neglecting the fact that partial pressure decreases dramatically with elevation while molar fraction remains stable. As is the case for respiration in animals (Zhang et al., 2007), reduced partial pressure has a significant impact on gas exchange by plants;  $\text{CO}_2$  assimilation closely depends on  $\text{CO}_2$  partial pressure ( $P_{\text{CO}_2}$ ) and not  $\text{CO}_2$  molar fraction (Farquhar et al., 1980). The decline in  $P_{\text{CO}_2}$  with elevation has led various authors to contend that the lack of  $\text{CO}_2$  may be an important factor in the reduction of photosynthesis at high elevations (Decker, 1947; Tranquillini, 1964). However, more recent modelling studies suggested that air pressure effects on photosynthesis are smaller than predicted based on the decline in ambient  $P_{\text{CO}_2}$  alone (Gale, 1972; Terashima et al., 1995). They concluded (i) that low pressure has only a small effect on the availability of  $\text{CO}_2$  for plants photosynthesis, and (ii) that when suppression of photosynthesis in alpine plants occurs it can mostly be attributed to limited  $\text{CO}_2$  diffusion or low temperatures rather than to lowered  $P_{\text{CO}_2}$  itself.

In order to compare photosynthetic capacity of populations at various elevations, all microclimatic determinants must be kept constant (temperature, humidity, light and  $\text{CO}_2$  partial pressure). While many experiments have been carried out at constant temperature and saturating light, few studies have yet compared photosynthetic capacity of low- and high-elevation plants at uniform  $\text{CO}_2$  partial pressure. Rather, for gas exchange measurements, researchers tended to impose a constant

molar fraction of  $\text{CO}_2$  in the cuvette (Rada et al., 1998; Zhang et al., 2005), and therefore compared  $\text{CO}_2$  assimilation at different  $\text{CO}_2$  partial pressures. For example, if the molar fraction of  $\text{CO}_2$  in air is 375 ppm (equal to  $375 \mu\text{mol mol}^{-1}$ ) and the temperature  $20^\circ\text{C}$ , then the partial pressure ( $P_{\text{CO}_2}$ ) is 38 Pa ( $380 \mu\text{bar}$ ) at sea level, but only 32 Pa at 600 m and 27 Pa at 3000 m of elevation. Consequently, these studies have measured photosynthetic capacity under local conditions but did not compare populations in the same air pressure conditions across different elevations. Although such results are useful for studying leaf performance in ambient conditions (instantaneous assimilation) and for quantifying the carbon balance, they do not allow assessment of plant adaptation or acclimation (photosynthetic capacity).

In the present study, we focus on the importance of  $\text{CO}_2$  partial pressure when studying plant adaptation/acclimation at different elevations. We compare maximum assimilation rates of two temperate tree species growing naturally along an altitudinal gradient (100 to 1600 m) in the Pyrénées Mountains. Gas exchange measurements were performed at each elevation and at two different  $\text{CO}_2$  partial pressure treatments: (i) at ambient  $\text{CO}_2$  partial pressure ( $P_{\text{CO}_2\text{-A}}$ ), as is generally employed and (ii) at constant-low-elevation  $P_{\text{CO}_2}$  ( $P_{\text{CO}_2\text{-C}}$ ). Therefore, we were able to quantify the effect of decreasing  $\text{CO}_2$  partial pressure on photosynthetic capacity ( $A_{\text{max}}$ ) for two common European tree species (*Fagus sylvatica* L. and *Quercus petraea* (Matt.) Liebl.). Three main questions are addressed here: (i) how does maximum assimilation rate vary with increasing elevation? (ii) do maximum assimilation rate and partial pressure vary to the same extent with increasing elevation? and (iii) do measurements made at ambient and constant  $P_{\text{CO}_2}$  lead to similar results when examining plant adaptation or acclimation?

## 2. MATERIALS AND METHODS

### 2.1. Altitudinal gradient and microclimate

The altitudinal study was conducted in the Gavarnie's valley on the west side of the Pyrénées Mountains in France (from  $42^\circ 53' \text{N}$ ,  $0^\circ 25' \text{W}$  to  $43^\circ 45' \text{N}$ ,  $0^\circ 14' \text{W}$ ). This region is characterized by an oceanic mountain climate, with a mean annual temperature of  $12^\circ\text{C}$  and precipitation of 1079 mm (1946–2001) at low elevation (Tarbes,  $43^\circ 11' \text{N}$   $0^\circ 00' \text{W}$ , 360 m ASL, Météo France). We selected two common European tree species (*Fagus sylvatica* L. and *Quercus petraea* (Matt.) Liebl.) along a 1500 m altitudinal gradient. For each species, natural established populations were sampled at six elevations: 100 m, 400 m, 600 m, 800 m, 1200 m and 1600 m ASL ( $\pm 50$  m).

For each population, we used a GPS receiver (GPS Pathfinder ProXR, Trimble Navigation, Sunnyvale, USA) to determine the exact elevation of each site (Tab. I). Air temperature was measured using data loggers (HOBO Pro RH/Temp, Onset Computer Corporation, Bourne, USA) located in each population along the altitudinal gradients. Sensors were mounted 1.5 m above the ground on poles located in clearings near the studied populations, and protected by white plastic shelters to prevent exposure to rain and direct sunlight. All sensors were inter-calibrated in the laboratory before installation. Data

**Table I.** Altitudinal variations in summer air temperature ( $T_{as}$ ), air pressure ( $P_{atm}$ ), ambient CO<sub>2</sub> partial pressure ( $P_{CO_2-A}$  at 375 ppm of molar fraction), and molar fraction ( $x_{CO_2-C}$ ) imposed to obtain  $P_{CO_2-C}$  for each species.

	Elevation (m)	$T_{as}$ (°C)	$P_{atm}$ (hPa)	$P_{CO_2-A}$ (Pa)	$x_{CO_2-C}$ (ppm)
Beech-sites	131	19.6	997.9	37.4	376
	495	17.7	956.0	35.9	392
	773	16.9	925.1	34.7	405
	1190	13.1	879.1	33.0	427
	1604	13.4	836.9	31.4	448
Oak-sites	131	19.6	997.9	37.4	376
	425	18.9	964.1	36.2	386
	627	18.0	941.4	35.3	389
	803	17.5	922.0	34.6	398
	1235	16.4	875.9	32.8	407
	1608	14.0	836.9	31.4	428

were recorded hourly from January 1<sup>st</sup> 2006 to December 31<sup>st</sup> 2007 and mean summer air temperatures ( $T_{as}$ ) were calculated as the mean daily temperature between May 1<sup>st</sup> and August 31<sup>st</sup> 2006 (Tab. I). The altitudinal gradient used here provided mean annual temperature ranges of 6.5 and 5.6 °C for beech and oak, respectively (Tab. I). The average rate of decrease in temperature with increasing elevation (lapse rate) was 0.42 °C per 100 m (Vitasse et al. 2008). Analysis of monthly precipitation data in 2005–2007 indicated no water stress along the altitudinal gradient. Indeed, average values (2005–2007) ranged from 957 to 1376 mm of water per year between low and high elevations. Variations in air pressure ( $P_{atm}$  in Pa) according to elevation were estimated according to Jones (1983):

$$P_{atm} = \frac{101325}{e^{\left(\frac{z}{29.3 \times T_{as}}\right)}} \quad (1)$$

where  $z$  is elevation above sea level (m) and  $T_{as}$  is summer air temperature (K). Values of  $P_{atm}$  decreased with increasing elevation by about 10.9 hPa 100 m<sup>-1</sup>, ranging from 997.9 to 836.9 hPa (16.1% lower at the highest elevation sites; Tab. I). These values were used to calculate the CO<sub>2</sub> partial pressure based on the molar fraction reported in situ by the gas analyser. According to the ideal gas law, the fractional abundance of gas expressed in dimensionless percentage or ppm reflects equivalently a molar fraction ( $\mu\text{mol mol}^{-1}$ ), a volumetric fraction ( $\mu\text{L L}^{-1}$ ) or a fractional pressure ( $\mu\text{bar bar}^{-1}$ ). Such a measure of CO<sub>2</sub> has no physical dependence on elevation, and infrared gas analyzers (IRGAs) generally allow researchers to fix this value within cuvettes. By contrast, partial gas pressure depends directly on other state variables (temperature and density), with strong altitudinal variation in the atmosphere.

## 2.2. Gas exchange measurements

Measurements were carried out using a portable steady-state, flow-through chamber (PLC6) connected to an IRGA (CIRAS-2, PP Systems, Hitchin, UK) equipped with temperature, humidity, light and CO<sub>2</sub> control modules. The IRGA measures CO<sub>2</sub> density ( $\rho_{CO_2}$ ) as a function of infrared absorption via the Beer-Bouguer-Lambert law, in an optical chamber where data on temperature and pressure allow calculation of the molar fraction ( $X_{CO_2}$ ). Since  $X_{CO_2}$  is unaffected during the passage from the cuvette to the optical chamber

(i.e., is conserved when humidity is maintained constant as in the cuvette; Kowalski and Serrano-Ortiz, 2007), the system effectively determines the cuvette's  $X_{CO_2}$ , whose changes are due exclusively to leaf CO<sub>2</sub> exchange. In addition, this analyser (CIRAS-2 PP System) automatically corrects measurements for water vapour and changes in air pressure. Before measurement campaigns, the analyser was calibrated in the laboratory using 400 ppm standard gas. Full CO<sub>2</sub> and H<sub>2</sub>O zero and differential calibration have been performed in the field at every setting change, or after a set of four measurements.

We sampled adult individuals of comparable height on a North-facing slope for beech and South-facing slope for sessile oak. Five to nineteen mature individuals were randomly selected per population to monitor leaf gas exchange. Measurements were always done in the field between 8:00 and 11:00 solar time on fully expanded leaves during two consecutive weeks in August 2007. Measurements were carried out on two leaves per tree immediately after cutting the branch using a pole tree pruner between 5 and 7 m height in the crown. As defined by Larcher (1969), maximum assimilation rate and stomatal conductance ( $A_{max}$  and  $g_{smax}$ , respectively) were measured under optimum temperature and relative humidity, saturated light and non-saturated CO<sub>2</sub> partial pressure. To compare maximum rates of assimilation at light saturation ( $A_{max}$ ) between populations, all gas exchange measurements were made at equal temperature (20.0 °C ± 1.5), VPD (1200 Pa ± 250) and saturating light (1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of light in the leaf chamber, determined by a light response curve made on five individual per species). At each elevation, measurements were performed at two CO<sub>2</sub> partial pressures (treatments A and C), by imposing different CO<sub>2</sub> molar fractions in the chamber: (A) measurements at ambient CO<sub>2</sub> partial pressure ( $P_{CO_2-A}$ ; Tab. I) were done using a constant CO<sub>2</sub> molar fraction ( $x_{CO_2} = 375$  ppm) in the leaf chamber along the gradient; (C) measurements at constant-low-elevation CO<sub>2</sub> partial pressure ( $P_{CO_2-C} = 37.5$  Pa) along the altitudinal gradient were done by adjusting CO<sub>2</sub> molar fractions ( $x_{CO_2-C}$ ; Tab. I) at each elevation. We calculated CO<sub>2</sub> partial pressure as:  $P_i = X_i \times P_{atm} \times 10^6$  where  $P_i$  is the partial pressure of gas  $i$ ,  $X_i$  the molar fraction (in ppm) and  $P_{atm}$  the air pressure. Maximum assimilation rates were performed for both treatments (ambient and constant-low-elevation  $P_{CO_2}$ ) on the same leaf and data were recorded when steady state of assimilation was reached (within ten minutes). For each tree, measurements were randomly conducted between treatments. In the laboratory, leaf samples were dried at 70 °C, mineralised with hot sulphuric acid and assayed

**Table II.** Comparison of mean values of maximum assimilation rate ( $A_{max}$ ) and maximum stomatal conductance ( $g_{smax}$ ) between CO<sub>2</sub> partial pressure treatments for each elevation and species. Na is the leaf nitrogen content. Values in parenthesis correspond to the standard error and  $n$  is the number of replicates per population. Differences between treatments were performed using a paired  $t$ -test: ns, non-significant differences between  $P_{CO_2}$  treatments.

Species	Elevation (m)	$n$	$A_{max}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )			$g_{smax}$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ )			Na ( $\text{mg m}^{-2}$ )
			At ambient		ns	At constant		ns	
			$P_{CO_2}$	$P_{CO_2}$		$P_{CO_2}$	$P_{CO_2}$		
Beech	131	9	8.9 (0.72)	8.8 (0.74)	ns	131.6 (9.9)	133.0 (12.6)	ns	1.22 (0.1)
	495	11	8.4 (0.24)	8.8 (0.44)	ns	116.4 (6.7)	115.0 (9.4)	ns	1.01 (0.07)
	773	16	8.7 (0.75)	9.9 (0.77)	*	130.7 (14.4)	128.5 (15.6)	ns	1.41 (0.13)
	1190	10	8.8 (0.66)	11.3 (0.64)	**	149.3 (17.4)	153.0 (16.7)	ns	1.62 (0.11)
	1604	15	9.5 (0.42)	12.7 (0.50)	***	183.7 (11.1)	177.7 (8.0)	ns	2.26 (0.14)
Oak	131	19	10.0 (0.80)	9.9 (0.81)	ns	148.2 (15.2)	151.5 (14.7)	ns	1.55 (0.07)
	425	12	11.7 (0.66)	11.9 (0.74)	ns	173.3 (13.3)	177.8 (15.3)	ns	1.87 (0.07)
	627	11	9.6 (0.85)	10.5 (0.87)	**	152.7 (19.8)	141.3 (15.1)	ns	–
	803	12	12.2 (0.69)	13.7 (0.74)	*	91.1 (13.9)	87.8 (13.0)	ns	1.82 (0.13)
	1235	5	9.1 (1.30)	12.8 (0.88)	*	153.2 (32.2)	203.8 (21.1)	ns	1.85 (0.12)
	1608	10	11.9 (0.74)	14.2 (0.84)	***	366.4 (17.0)	331.5 (17.1)	*	2.41 (0.07)

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

colorimetrically for concentrations of nitrogen and phosphorus using the Technicon auto-analyser.

### 2.3. Data analysis

In order to compare the two treatments (ambient and constant-low-elevation  $P_{CO_2}$ ), the relative differences in  $A_{max}$  ( $D_{Amax}$ , %) and  $P_{CO_2}$  ( $D_{PCO_2}$ , %) were calculated as follows:

$$D_{Amax} = \left| \frac{A_{max-C} - A_{max-A}}{A_{max-C}} \right| \times 100 \quad (2)$$

$$D_{PCO_2} = \left| \frac{P_{CO_2-C} - P_{CO_2-A}}{P_{CO_2-C}} \right| \times 100 \quad (3)$$

where  $A_{max-C}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) is the maximal rate of assimilation measured at constant-low-elevation  $P_{CO_2}$ ,  $A_{max-A}$  is the maximal rate of assimilation measured at ambient  $P_{CO_2}$ ,  $P_{CO_2-C}$  is the constant-low-elevation partial pressure of CO<sub>2</sub> (37.5 Pa) and  $P_{CO_2-A}$  (Pa) is the ambient partial pressure of CO<sub>2</sub> along the elevational gradient.

Within each population (at each elevation), we used a paired  $t$ -test to characterize the effects of  $P_{CO_2}$  treatments on ecophysiological variables ( $A_{max}$  and  $g_{smax}$ ). Then, to determine whether variations in photosynthetic capacity, stomatal conductance and foliar nitrogen content were related to elevation, the data were analysed by linear regression (for each  $P_{CO_2}$  treatment and species). In addition, to compare  $A_{max}$  and  $g_{smax}$  between elevations, analysis of variances (ANOVA) with the Tukey test at  $P < 0.05$  was used for each species. All analyses were performed using the SAS software package (SAS 9.1, SAS Institute Inc., Cary, NC).

## 3. RESULTS

Overall, values of  $A_{max}$  ranged from 8.8 to 12.7 and from 9.1 to 14.2  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for beech and oak, respectively

(Tab. II). Oak exhibited higher values of  $A_{max}$  than beech independent of elevation; mean values for the whole experiment were about 11.5 and 9.6  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for oak and beech, respectively. Values of maximum stomatal conductance ( $g_{smax}$ ) were less variable for beech (115 to 184  $\text{mmol m}^{-2} \text{s}^{-1}$ ) than for oak (88 to 366  $\text{mmol m}^{-2} \text{s}^{-1}$ ) (Tab. II).

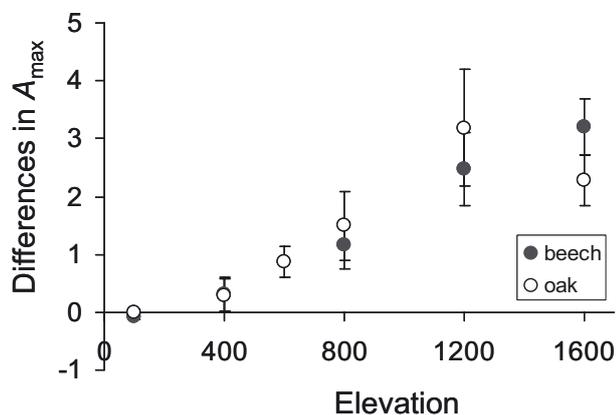
At ambient CO<sub>2</sub> partial pressure,  $A_{max}$  values did not vary with elevation for either species (Tab. III), whereas  $A_{max}$  values measured at constant-low-elevation  $P_{CO_2}$  significantly increased with increasing elevation for both species. Indeed, for both species,  $A_{max}$  values at the highest elevation were about 4  $\mu\text{mol m}^{-2} \text{s}^{-1}$  higher than those at the lowest elevation (Tab. II). The increase in  $A_{max}$  with elevation was gradual for beech and more variable for oak (values at 1200 m were lower than those at 800 m). Whatever the treatment, we observed a slight but non-significant increase in  $g_{smax}$  for beech whereas no altitudinal trend was found for oak (Tab. III). However, values of  $g_{smax}$  were significantly higher at high elevation sites (1600 m) compared to lower elevations (ANOVA,  $P < 0.0001$ ) for both species. Leaf nitrogen content significantly increased with increasing elevation for beech but this trend was not significant for oak, despite the higher values observed at high elevations (Tabs. II and III).

We found no difference in  $A_{max}$  values between  $P_{CO_2}$  treatments at low elevation whereas significant differences were observed at high elevations (paired  $t$ -test, Tab. II). Values of  $\Delta A_{max}$  were significantly different of 0 only above 600 m and 800 m for oak and beech, respectively. These differences became more marked with increasing elevation (Fig. 1), reaching high values at the highest elevation, about 2.3 and 3.2  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for oak and beech, respectively. Table II shows that there was no difference between  $g_{smax}$  values measured at ambient versus constant  $P_{CO_2}$  for either species, except for the oak population at 1600 m where  $g_{smax}$  values at ambient  $P_{CO_2}$  were higher than those at constant-low-elevation

**Table III.** Statistical coefficients of linear regression for  $A_{max}$ ,  $g_{smax}$  and Na versus elevation for each partial pressure treatments and for both species.

Species	$A_{max}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )				Na ( $\text{mg m}^{-2}$ )	
	At ambient		At constant			
	$P_{\text{CO}_2}$	$P_{\text{CO}_2}$	$P_{\text{CO}_2}$	$P_{\text{CO}_2}$		
Beech	$R^2$	0.5114	0.9493	0.7434	0.7273	0.8230
	$P$ -values	0.1745	0.0049 **	0.0601	0.0663	
Oak	$R^2$	0.0403	0.6863	0.4241	0.4982	0.7326
	$P$ -values	0.7028	0.0416 *	0.1612	0.1171	

\*  $P < 0.05$ , \*\*  $P < 0.01$ .



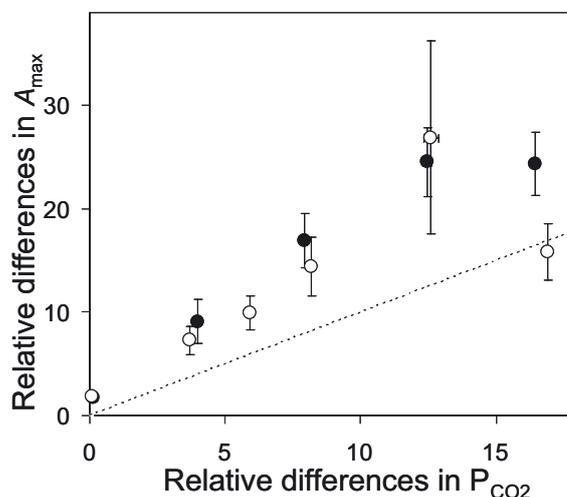
**Figure 1.** Differences in maximum assimilation rates ( $\Delta A_{max}$   $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) between  $\text{CO}_2$  partial pressure treatments ( $\Delta A_{max} = A_{max-C} - A_{max-A}$ ) in relation to elevation (m) for both species.  $A_{max-C}$ : maximal rate of assimilation measured at constant- low-elevation  $P_{\text{CO}_2}$  (37.5 Pa);  $A_{max-A}$ : maximal rate of assimilation measured at ambient  $P_{\text{CO}_2}$ . Values correspond of the mean of 5 to 19 individuals per site  $\pm$  standard errors

$P_{\text{CO}_2}$ . The  $\text{CO}_2$  partial pressure has no effect on stomatal conductance; therefore the observed differences result from non stomatal effects.

Relative differences in  $A_{max}$  ( $D_{Amax}$ ) reached 25% for both species (Fig. 2). However, for oak,  $D_{Amax}$  was lower at 1600 m than 1200 m, due to the very high  $A_{max}$  values measured at the highest elevation. Meanwhile, the highest relative differences in  $P_{\text{CO}_2}$  ( $D_{P_{\text{CO}_2}}$ ) were less than 16% at the highest elevation. Figure 2 shows the steeper increase in  $\text{CO}_2$  assimilation rate with elevation than that of  $\text{CO}_2$  partial pressure, since a given increase in  $D_{P_{\text{CO}_2}}$  resulted in a larger increase in  $D_{Amax}$ .

#### 4. DISCUSSION

This study shows that the maximum assimilation rate ( $A_{max}$ ) increases significantly with increasing elevation, about 2.8 and 2.6  $\mu\text{mol m}^{-2} \text{s}^{-1}$  per 1000 m of elevation for beech and oak, respectively when measurements were done at constant-low-elevation  $P_{\text{CO}_2}$ . By contrast, at ambient  $P_{\text{CO}_2}$  no significant trend was found in  $A_{max}$  according to elevation. Along the altitudinal transect, we found that values of  $A_{max}$  measured at



**Figure 2.** Relative differences in  $A_{max}$  ( $D_{Amax}$ ) versus relative differences in  $P_{\text{CO}_2}$  ( $D_{P_{\text{CO}_2}}$ ) in percentage (%). Dashed line represents the 1:1 relationship.

ambient  $P_{\text{CO}_2}$  were significantly lower than those measured at constant-low-elevation  $P_{\text{CO}_2}$  above 600 m of elevation: these between-treatment differences increase as elevation increases. We found that gas exchange measurements realised along altitudinal gradient at ambient or constant  $P_{\text{CO}_2}$  lead to different results in terms of photosynthetic capacity and therefore to different conclusions regarding plant adaptation or acclimation.

In the literature, most studies that have examined photosynthetic capacity ( $A_{max}$ ) over altitudinal gradients have kept temperature, humidity and light level constant in order to compare populations, but either (i) did not mention how they managed the  $\text{CO}_2$  variable (Cordell et al., 1998; Rundel et al., 2003), or (ii) used constant  $\text{CO}_2$  molar fraction (ppm or  $\mu\text{mol mol}^{-1}$  or  $\mu\text{bar bar}^{-1}$ ) instead of constant partial pressure (Gonzalez-Real and Baille, 2000; Yin et al., 2004). These studies found results similar to those of Treatment A in our study: no significant variations of  $A_{max}$  along the gradient (Cordell et al., 1999; Körner and Diemer, 1987; Kumar et al., 2006). However, a few studies have showed either slight increases in  $A_{max}$  with increasing elevation (Friend et al., 1989; Premoli and Brewer 2007) or a decrease (Kao and Chang, 2001; Zhang et al., 2005). Only a few studies have used  $\text{CO}_2$  partial pressure when comparing population adaptation for photosynthesis at

different elevations (Friend et al., 1989), but they carried out measurements at ambient  $P_{\text{CO}_2}$ . To our knowledge, only one study estimated photosynthetic capacity of low- and high-elevation plants applying constant cuvette  $\text{CO}_2$  partial pressure (Körner and Diemer, 1987). They also found an increase in  $A_{\text{max}}$  over a gradient of 2000 m of elevation; values were 20% higher for high elevation versus low elevation plants, using a constant  $\text{CO}_2$  partial pressure of 25.1 Pa. In other words, they found that photosynthesis varied to the same extent as the variation in  $P_{\text{CO}_2}$ : a 22.5% shift in  $A_{\text{max}}$  for 20.8% variations in  $P_{\text{CO}_2}$  along their gradient. In our study, we found an even higher shift in  $A_{\text{max}}$  (25%) compared to that of  $P_{\text{CO}_2}$  (16%) for both species.

For beech, the higher values of leaf nitrogen content observed for high-elevation populations partially explained their greater photosynthetic capacity. For oak, we only found a slight but non-significant increase in leaf nitrogen content with increasing elevation. Altitudinal increase in leaf nitrogen content has already been observed for other species (Cordell et al., 1998; Premoli and Brewer, 2007). Photosynthetic capacity tends to be positively related to higher values of foliar nitrogen and phosphorus due to increased levels of Rubisco and other N-containing constituents of the photosynthetic apparatus (Delzon et al., 2005; Field and Mooney, 1986; Marron et al., 2008). Moreover, only for beech, our results also suggest that at least part of the increase in maximum assimilation rate at high elevation may be attributed to a slight increase in stomatal conductance, implying an increase in  $\text{CO}_2$  diffusion into the leaf. Stomatal density of beech was greater in high elevation populations (data not shown), as were  $g_s$  and  $A_{\text{max}}$ . The relationship between stomatal conductance (or stomatal density) and elevation is not as clear cut, with various authors reporting increases (Körner and Cochrane, 1986; Körner et al., 1986; Premoli and Brewer, 2007), decreases (Körner et al., 1989) or no clear trend with elevation (Cordell et al., 1998), depending of species.

The increase in photosynthetic capacity with increasing elevation suggested that trees accomplish a relatively high level of photosynthesis to compensate for extreme environmental conditions and short growing seasons at high elevation. This ability to cope with varying environment is likely be achieved by genetic adaptation or by acclimation. In order to discern adaptation and acclimation in the future, we have set up a lowland common garden with the same populations for both species. For other species, previous studies have showed that in a common environment, photosynthetic capacity of plants from high elevation remained higher than those of lowland plants (Hovenden and Brodribb, 2000; Oleksyn et al., 1998). Premoli and Brewer (2007) showed that assimilation rates were 40% higher for high-elevation plants in the field (measurements done at ambient  $P_{\text{CO}_2}$ ), and 18% higher in the common garden, than those for low-elevation plants. Therefore, photosynthetic capacity seems to be under strong selective pressures typically observed at high elevation. Genetic adaptation to high elevation conditions could be a result of maintaining of higher leaf nitrogen, chlorophyll content (Oleksyn et al., 1998), stomatal density (Kouwenberg et al., 2007) and car-

boxylation efficiency (Körner and Diemer, 1987), and would allow trees to adapt to their local conditions.

The low partial pressure of  $\text{CO}_2$  prevailing at high elevations has been hypothesized to be responsible for the reduced photosynthesis (Decker, 1947; Tranquillini, 1964). However, this is in contradiction to some theoretical studies (Gale, 1972; Terashima et al., 1995). Indeed, Gale (1972) demonstrated using a simple diffusion model that low pressure has a small effect on photosynthesis for plants with efficient  $\text{CO}_2$ -uptake mechanisms. The effect of a reduction in  $P_{\text{CO}_2}$  could indeed be counterbalanced by an increase in the diffusion coefficient with decreasing air pressure (Terashima et al., 1995). Nevertheless, this compensating effect is only observed in plants with low values of mesophyll resistance (Gale, 1972), which correspond to  $\text{C}_4$  and not to  $\text{C}_3$  type plants as in our study. In addition, Terashima et al. (1995) only found no effect of partial pressure on photosynthesis at low temperature; this effect reached 23% at higher leaf temperatures (35 °C) for an elevation of 3000 m. Whereas this last study suggested that changes in the photosynthetic capacity of alpine plants can be attributed mostly to low temperature, we suggest that low  $\text{CO}_2$  partial pressure at high elevation could also play a role in plant adaptation. Indeed, the decrease in  $\text{CO}_2$  partial pressure is expected to increase stomatal density and stomatal conductance (Kouwenberg et al., 2007; Woodward and Bazzaz, 1988) and therefore maximum assimilation rate.

In our study, we were not able to take the decrease in partial pressure of oxygen ( $\text{O}_2$ ) into account with a decrease in air pressure. Rubisco catalyzes both the carboxylation of RuBP and its oxygenation. The ratio of carboxylation to the oxygenation reaction (initiating photorespiration) strongly depends on the relative concentrations of  $\text{CO}_2$  and  $\text{O}_2$ , and on leaf temperature (Lambers et al., 1998). Because of competition between  $\text{CO}_2$  and  $\text{O}_2$  for Rubisco, the decreased partial pressure of  $\text{O}_2$  at high elevations combined with the constant  $\text{CO}_2$  partial pressure treatment likely reduces photorespiration, and could partly explain the large increase in  $A_{\text{max}}$  observed with elevation here (Cornic, 1980; Sun, 1999; Ramonell, 2001).

Our study demonstrates the importance of using constant  $\text{CO}_2$  partial pressure to assess plant adaptation/acclimation at different elevations; i.e. to accurately quantify photosynthetic capacity. Measurements done at ambient and constant  $P_{\text{CO}_2}$  lead to different results, with different patterns according to elevation. Had we conducted experiments only at ambient  $\text{CO}_2$  partial pressure, we might have concluded that high-elevation populations did not evolve to be more efficient, and therefore that adaptation/acclimation does not occur along altitudinal gradient. In this case (ambient  $\text{CO}_2$  partial pressure), a finding that photosynthetic capacity stays constant with increasing elevation suggests the conclusion that high elevation populations did adapt to constraining environmental conditions. However, it is worth knowing that a decrease in photosynthetic capacity at ambient  $P_{\text{CO}_2}$  did not allow conclusions to be drawn regarding plant adaptation as the effect of decreasing  $\text{CO}_2$  partial pressure cannot be removed. Therefore, to assess plant adaptation or acclimation to elevation, we recommend carrying out gas exchange measurements at constant partial pressure.

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