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Variation of the photosynthetic capacity across a chronosequence of maritime pine correlates with needle phosphorus concentration

Sylvain DELZON^{a,b*}, Alexandre BOSCA^a, Lisa CANTET^a, Denis LOUSTAU^a

^a Unité de Recherche EPHYSE, INRA-Bordeaux, 69 route d'Arcachon, 33610 Gazinet, France

^b Present address: UMR INRA 1202 BIOGECO, Équipe Écologie des Communautés, Université Bordeaux 1, Bât. B8, avenue des Facultés, 33405 Talence, France

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Abstract – Changes in needle photosynthetic capacity has been studied across a chronosequence of four maritime pine stands aged 10-, 32-, 54- and 91-yr. We determined photosynthetic parameters from response curves of assimilation rate to air CO₂ concentration ($A-C_i$) and radiation ($A-Q$) using gas exchange measurements on branches in the laboratory. Our data showed no shift in photosynthetic parameters (V_{cmax} , J_{max} , α and R_d) with increasing stand age. This result means that the decline in productivity observed throughout our maritime pine chronosequence cannot be explained by a decrease in photosynthetic capacity but by a decline in stomatal conductance evidenced in a previous paper [7]. However, V_{cmax} was higher in the 32-yr-old stand compared to the other stands and these between-stand differences were explained by leaf phosphorus concentration. Moreover, additional data of V_{cmax} suggest that the photosynthetic capacity may be higher at younger stages due to initial fertilisation. Therefore the P nutrition may contribute to productivity decline over the duration of the management cycle.

Pinus pinaster Ait. / forest aging / NPP decline / maximum carboxylation rate / nutrient limitation

Résumé – Les variations de la capacité de la photosynthèse des aiguilles de pin maritime dans une chronoséquence sont corrélées à celles des teneurs en phosphore. Nous avons étudié l'évolution des capacités photosynthétiques foliaires dans une chronoséquence constituée de quatre peuplements équiennes de Pin maritime âgés de 10, 32, 54 et 91 ans. Les paramètres photosynthétiques ont été estimés sur des courbes de réponse du taux d'assimilation à la concentration en CO₂ ($A-C_i$) et à la lumière ($A-Q$) obtenues à partir de mesures d'échange gazeux foliaire. Dans la chronoséquence étudiée, aucune tendance n'a été mise en évidence entre les paramètres photosynthétiques (V_{cmax} , J_{max} , α et R_d) et l'âge du peuplement. Ce résultat démontre que le déclin de productivité foliaire observée dans la chronoséquence ne peut être expliqué par les capacités photosynthétiques, mais bien par une diminution de la conductance stomatique mise en évidence dans un précédent article [7]. Toutefois, des valeurs de V_{cmax} plus élevées ont été observées dans le peuplement de 32 ans. Ces variations de capacités photosynthétiques entre peuplements sont bien expliquées par la teneur foliaire en phosphore. L'ajout de données antérieures suggère cependant des taux supérieurs de capacités photosynthétiques chez les jeunes peuplements sans doute en lien avec une fertilisation lors de la plantation. La nutrition en phosphore pourrait ainsi contribuer au déclin de productivité dans le contexte sylvicole des Landes de Gascogne.

Pinus pinaster Ait. / vieillissement / déclin de productivité / vitesse maximale de carboxylation

1. INTRODUCTION

In even-aged forests, growth and biomass accumulation decline after reaching a peak relatively early in a stand's life [10, 28, 29]. The primary reason for a decrease in forest net primary production with increasing stand age is the decline in photosynthesis [3, 30, 37]. This decline could be due to both reduced leaf area and reduced leaf photosynthesis. Reduced leaf assimilation may be caused by changes in (i) diffusive limitation via a decrease in stomatal conductance and internal CO₂ concentration, (ii) an increased in mesophyll resistance, and (iii) a biochemical and photochemical limitation via the RubisCO

activity and photochemistry respectively. The former has been increasingly demonstrated to be linked to the decline in hydraulic transfer capacity accompanying the increase in height and architectural complexity with tree development [4, 11, 30]. Both photosynthesis and stomatal conductance are reduced with tree age [11, 12, 37]. Indeed, when trees grow taller, height may reduce the ability of tall trees to transport water to the top of the canopy due to combination of factors including gravity and a longer and more ramified water path-length. Stomatal adjustment must occur therefore to maintain homeostasis of minimum needle water potential [31, 33] and keep the water transport away from cavitation threshold. On the other hand,

* Corresponding author: s.delzon@ecologie.u-bordeaux1.fr

Table I. Characteristics of the four stands of the chronosequence. Values are mean \pm standard error.

Stand age	10 yr	32 yr	54 yr	91 yr
Latitude	44° 44' N	44° 44' N	44° 44' N	44° 37' N
Longitude	0° 46' W	0° 46' W	0° 46' W	0° 34' W
Mean height (m)	8.46 \pm 0.08	20.21 \pm 0.11	26.65 \pm 0.11	28.36 \pm 0.26
Diameter at 1.3 m (mm)	142.5 \pm 0.1	298.8 \pm 0.1	436.7 \pm 0.3	513.2 \pm 0.4
Basal area (m ² ha ⁻¹)	19.23 \pm 0.21	36.00 \pm 0.26	38.22 \pm 0.50	32.96 \pm 0.52
Tree number (trees ha ⁻¹)	1180	500	250	155
Biometric measurements (trees)	637	1921	485	463
Plant area index (PAI, m ² m ⁻²)	3.41	3.04	2.51	1.85
Leaf area index (LAI, m ² m ⁻²)	2.86	2.26	1.78	1.76
Growth efficiency (gC m ⁻² leaf yr ⁻¹)	121.3 (12.1)	76.2 (3.9)	51.7 (7.0)	37.8 (4.2)

changes in mesophyll resistance with tree age have never been studied in our knowledge and the rational behind an age-related decrease of photosynthetic capacities are not fully understood. In the literature, few studies of woody plants have investigated rigorously the variations in photosynthetic capacity with age. Few, if any studies were designed to isolate variation caused by age from all other sources of variation, e.g. size and environment. Thus, the present study was focused on quantifying the possible change in photosynthetic capacity with increasing tree age.

Declining nutrient availability during stand development adversely affects tree leaf area and leaf photosynthesis [10]. Thus, foliar nutrient concentration might be lower in older and taller trees [21, 34] and might limit the activity of photosynthetic enzymes but see e.g. Mencuccini and Grace [19]. This hypothesis has been rarely investigated in detail in literature and most often, only nitrogen was considered as a potential limitation of tree photosynthesis (especially in the temperate zone [26]) whereas other nutrients such as phosphorus may limit tree growth and forest productivity depending on the type of soil.

To examine the possible changes in photosynthetic capacity independently of diffusive limitations, we characterized the parameters controlling the photosynthetic capacity of maritime pine needles across a chronosequence composed of four stands aged of 10-, 32-, 54- and 91-yr respectively. Maximal carboxylation capacity, maximal electron transport rate and apparent quantum use efficiency were determined from gas exchange measurements in the laboratory and this was complemented by foliar nutrient concentration analyses. This study was part of the French contribution to the European CARBO-AGE project where the hydraulic and stomatal conductance limitations on tree growth were investigated in details as reported by Delzon et al. [7].

2. MATERIALS AND METHODS

2.1. Chronosequence description

Studies were carried out in four pure, even-aged maritime pine stands located 20 km southwest of Bordeaux in the "Landes de Gascogne" forest in south-western France. Trees were grown as even aged

stands aged 10, 32, 54 and 91 year-old in 2002, from seeds originating from the same geographical provenance (Tab. I). Stands were located in a 20 km wide area and exhibited similar environmental conditions (altitude, climate and soil characteristics) and management practices. The climate is temperate maritime with cool wet winters and warm dry summers. Mean annual temperature (1950–2000) was 13 °C, and mean annual precipitations (1970–2000) were 977 mm. The soil was a sandy hydromorphic humic podzol with a cemented B_h horizon limiting the root zone depth to -0.8 m, low soil phosphorus and nitrogen levels and mean pH of 4.0. Soil texture analysis showed the soil is 90% sand, 5% silt and 5% clay. In each stand, aboveground biomass increment per unit of leaf area (i.e. growth efficiency) was estimated from an allometric relationship between tree biomass, diameter at 1.3 m and tree age [7]. Mean values of growth efficiency (1996–2001) dramatically declined with stand age from 121 gC m⁻²leaf yr⁻¹ for the 10-yr-old stand to 38 gC m⁻²leaf yr⁻¹ for the 91 yr-old stand (Tab. I).

2.2. Gas exchange measurements

Measurements were carried out during May–June 2003 on a total of 24 branches, i.e. two branches per tree and three trees per stand. Characteristics of the sampled trees as measured in December 2002 are presented in Table II. Six series of measurements were carried out where each series, a randomised block, included one branch taken from each stand. Each branch was cut in the early morning wrapped within a wet cloth and brought back to the laboratory, then re-cut under water. Branches were chosen within the 3 year-old whorl among branches exposed South. This corresponded to the upper third of the tree canopy which was made accessible by a scaffolding. Measurements were carried out using three one-year-old fascicles (six needles) positioned across the minicuvette and kept hydraulically connected to the branch during gas exchange measurements. The branch was kept covered with a humid cloth during the measurements.

Gas exchange measurements were made in the laboratory inside an air-conditioned room, using an open gas exchange system, with a controlled environment minicuvette (Compact Minicuvette System CMS 400, Walz, Effeltrich, Germany). The protocol used was similar to Porté and Loustau [25] and Medlyn et al. [18] except for the following points. Air temperature (T_a) was set at 25 °C as controlled with a Peltier element, dewpoint (T_{dp}) fixed at 19 °C with a dew-point generator and air composition (O₂, CO₂, N₂) was controlled by mass flow meters (Gas Mixing Unit GMA-2, Walz). The upper and lower sides of the cuvette were illuminated each by a bundle of 200 optic fibres arranged uniformly and connected to a metal halide lamp (Fiber Illuminator FL-440, Special Fiberoptics 400-F, Walz, Effeltrich, Germany). The

Table II. Characteristics of the sampled trees in each maritime pine stand. The tree leaf area was calculated using an allometric relationship from diameter under the live crown (Delzon et al. [7]).

Stand age	Diameter (mm)	Height (m)	Leaf area (m ²)
10 yr	149	10.2	66
	145	10.4	63
	131	9.3	54
32 yr	300	20.4	124
	259	19.9	94
	341	19.9	158
54 yr	455	28.1	172
	491	27.0	200
	442	27.2	162
91 yr	523	27.7	159
	508	27.7	226
	556	26.9	214

required range of irradiances was obtained by an electronic regulator and neutral filters controlling light intensity sent to the two upper and lower sides of the cuvette through a bundle of 200 optic fibres. Incident PAR onto the needles surface was mapped in the cuvette with a PAR sensor (LI-190, LI-Cor, Inc., Lincoln, NE) and needles were positioned so that the illumination received by the upper and lower surfaces did not show spatial variation exceeding $\pm 5\%$ of the average illumination received. Differential (CO₂) and (H₂O) concentrations between the measuring and reference circuits were measured by a Bios 100 IRGA differential analyser calibrated with gas standards and cross-checked against a Licor 6262. Environmental parameters that were continuously measured in the chamber included air temperature (T_a), relative humidity (RH), and absolute CO₂ concentration (C_a) (Analyser IRGA Li-800, Li-Cor, Lincoln Nebraska, U.S.A.). The needle temperature was not measured with the constructor thermocouple because of problems with direct heating of the thermocouple by incident light and the spherical shape of the thermocouple which forbids a close contact between needle surface and the thermocouple. Instead, the needle temperature was estimated from light intensity and cuvette temperature using an energy balance calculation parameterised using a heated needle replicas, aluminium 2 mm diameter half-cylinder of known emissivity whose temperature was measured with an internal Cu-Cn thermocouple embedded in resin. The average aerodynamic conductance of the needle replica over a range of locations in the cuvette was estimated to 3000 mmol H₂O m⁻² s⁻¹. It is worth noting that the difference between needle and air temperature during subsequent measurements attained +0.8 °C on average. Assimilation (A , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration (E , mmol H₂O m⁻² s⁻¹), stomatal conductance (g_s , mmol CO₂ m⁻² s⁻¹) and the internal CO₂ concentration (C_i , $\mu\text{mol CO}_2 \text{ mol}^{-1}$) were calculated according to Farquhar and von Caemmerer [9].

To determine the photosynthetic parameters, the response curves of assimilation rate to air CO₂ concentration ($A-C_i$) and radiation ($A-Q$) were operated as follows. Before measurements, needles were acclimated in the chamber for 90 min at a CO₂ concentration of 360 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ and incident photosynthetic flux density (PPFD) of 900 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. The branch xylem water potential was measured using needles outside of the chamber of which transpiration were prevented by a wet cloth each 30 min all along the measurements. The branch was eventually recut to keep the water potential above -0.3 MPa. First measurement was made at CO₂ concentration of 350 $\mu\text{mol mol}^{-1}$ and PPFD of 1500 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ respectively fol-

lowed by a full $A-C_i$ response curve and a light response curve. The air CO₂ concentrations used to generate $A-C_i$ curves were decreased from 1500 to 800, 350, 200, 100, 50 and 0 $\mu\text{mol mol}^{-1}$ while O₂ concentration was switched between 2% and 21% at each CO₂ value except for the first four series where the 2% concentration was applied only from 0 to 350 $\mu\text{mol CO}_2 \text{ mol}^{-1}$. For the $A-Q$ curves, the air CO₂ concentration was kept constant at 1100 $\mu\text{mol mol}^{-1}$ and Q was sequentially lowered from 1500 to 900, 490, 270, 150, 100, 50 and 30 $\mu\text{mol m}^{-2} \text{ s}^{-1}$. To make respiration measurements, needles were kept in the dark with a T_{dp} of 5 °C and values were recorded at the end of the night.

Photosynthetic capacities, V_{cmax} the maximum rate of carboxylation ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), J_{max} the maximum rate of electron transport ($\mu\text{mol e}^- \text{ m}^{-2} \text{ s}^{-1}$), the quantum use efficiency ($\mu\text{mol e}^- \text{ mol}^{-1} \text{ photons}$) and TPU, the rate of triose phosphate utilisation were estimated altogether from the data observed by minimizing the sum of squares between the predicted values and observed values according to the Farquhar model of leaf photosynthesis [8], including the phosphate utilisation rate as proposed by von Caemmerer [35]. Needle temperature fluctuations observed during measurements were accounted for using the equations of activation energy, values published by Medlyn et al. [18] so that the photosynthetic parameters fitted were given at a reference leaf temperature of 25 °C.

2.3. Nutrient content analysis

Immediately after the gas exchange measurements, needle length (l), diameter (d) and thickness (t) were measured with an electronic calliper on the six needles sampled in order to estimate the total photosynthetic surface area, calculated as $((2t + d)/4 \times \pi + d) \times l$. Needles were dried subsequently at 65 °C for 72 h, weighted and specific leaf area (SLA, m² kg⁻¹) was calculated as the ratio of needle area to dry weight. Needles were re-dried at 70 °C, mineralised with hot sulphuric acid and assayed colorimetrically for concentrations of nitrogen and phosphorus using the Technicon auto-analyser [23]. Nitrogen and phosphorus concentrations are expressed either on a mass basis (%; N_m , P_m) or on a leaf area basis (g m⁻²; N_a , P_a).

2.4. Statistical analysis

To determine whether the variation in photosynthetic parameters (V_{cmax} , J_{max} , α , R_d), nutrient concentrations (N_m , P_m , N_a , P_a) and specific leaf area (SLA) were related to stand age, data were analysed by simple linear regression. Regressions were performed using SAS software package (SAS 8.01, SAS Institute Inc., Cary, NC) with the REG procedure. The effect of the series of measurements was analysed using ANOVA and Student-Newman-Keuls's test for eventual mean comparison.

3. RESULTS

3.1. Photosynthetic parameters

No significant relationship was found between needle photosynthetic capacities and stand age (Tab. III). However, V_{cmax} (J_{max}) showed differences between stands, reaching its maximum value of 50.4 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (147.2 $\mu\text{mol e}^- \text{ m}^{-2} \text{ s}^{-1}$) in the 32-yr-old stand. No significant interaction with date of measurements (series) was found and the series effect itself was significant only for R_d , the respiration rate at 25 °C which the overall mean decreased from 1.2 to 0.7 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ throughout the experiment. No large difference appeared between stands either for the quantum use efficiency (α) or dark

Table III. Linear regression coefficients for photosynthetic parameters (V_{cmax} , J_{max} , α and R_d) versus stand age for data pooled by age ($n = 4$). P represents the significance of the slope and non-zero intercept.

	Intercept	Slope	R^2	P
V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	44.690	-0.032	0.050	0.777
J_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	119.692	-0.116	0.032	0.820
α	0.215	-0.0004	0.778	0.118
R_d ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.849	-0.0005	0.0507	0.775

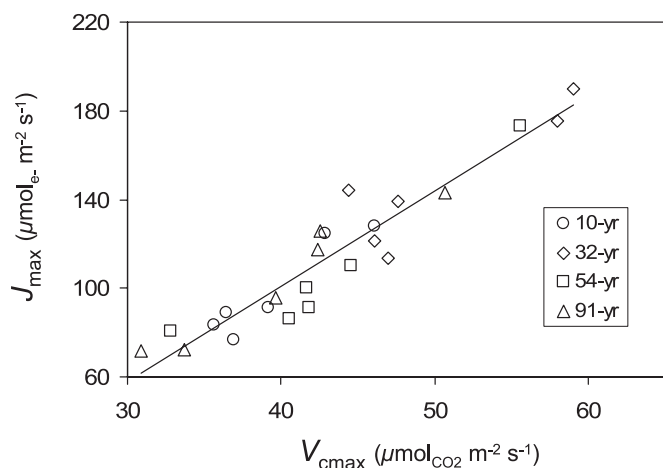


Figure 1. Relationship between maximum electron transport rate, J_{max} , and maximum carboxylation rate, V_{cmax} . The linear regression is $J_{\text{max}} = 4.2871 V_{\text{cmax}} - 70.811$ ($R^2 = 0.90$). Values were compiled for the four stands of different age.

respiration (R_d). The rate of triose phosphate utilisation, TPU, could be estimated only for 5 shoots collected in the 10-, 54- or 91-yr-old stands. Values were closed from $5.5 \mu\text{mol TP m}^{-2} \text{s}^{-1}$ while no major difference emerged among stands. TPU was never limiting under ambient CO_2 and O_2 concentrations. We observed a close linear relationship ($r^2 = 0.90$) between J_{max} and V_{cmax} (Fig. 1) and the ratio $J_{\text{max}}/V_{\text{cmax}}$ ratio were about $2.5 \text{ mol e}^- \text{ mol}^{-1} \text{ CO}_2$ at the 10-, 54- and 91-yr-old stands and reached its highest value of 2.9 at the 32-yr-old stand.

3.2. Specific leaf area and mineral concentrations

Specific leaf area decreased significantly with increasing age from 6.2 to $5.2 \text{ m}^2 \text{ kg}^{-1}$ (Tab. IV). In addition, leaf nitrogen concentration on an area basis (N_a) significantly increased with stand age from 1.70 to 2.51 g m^{-2} . This results mainly from the change in the specific leaf area. By contrast, all other parameters such as leaf nitrogen and phosphorus concentration on a mass basis (N_m and P_m) did not vary across the chronosequence (Tab. IV). However, the leaf phosphorus concentration on mass basis (P_m) was highest in the 32-yr-old stand, following the same pattern than photosynthetic parameters. This pattern held

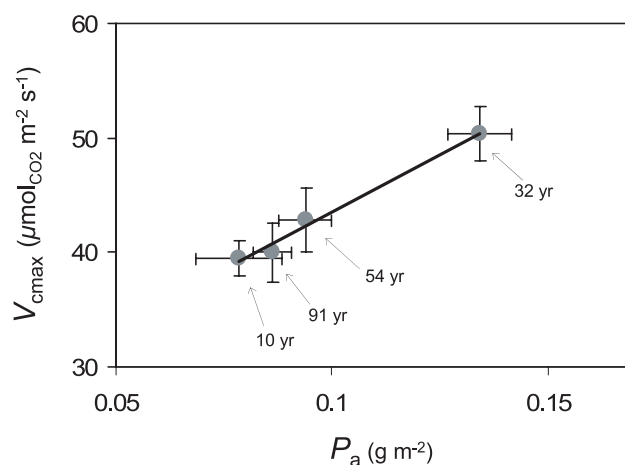


Figure 2. Relationship between maximum carboxylation rate, V_{cmax} , and phosphorus concentration on a leaf area basis, P_a , across the chronosequence. For each stand, value represents the mean and standard error of 6 measurements. Correlation between V_{cmax} and P_a : intercept 23.43 , slope 200.93 , $n = 4$, $R^2 = 0.988$, $P < 0.0064$.

true for the phosphorus expressed on an area basis (P_a). This trend was confirmed by other independent measurements carried out across the same stands both in January 2002 and 2003 [6]. Figure 2 shows the relationship between V_{cmax} and P_a for all measurement series pooled by stand. The between-stand variation in V_{cmax} was mostly explained by the phosphorus concentration expressed on area basis whereas N concentrations showed non relationship with photosynthetic parameters (data not shown).

4. DISCUSSION

The growth efficiency measured across the four stands composing the chronosequence declined asymptotically from 121 to $38 \text{ gC m}^{-2} \text{ leaf yr}^{-1}$ between 10 and 91 years. No variable studied in the present study follows a similar pattern except the specific leaf area. However, the carbon isotope discrimination as studied in a companion paper declined continuously with age [7].

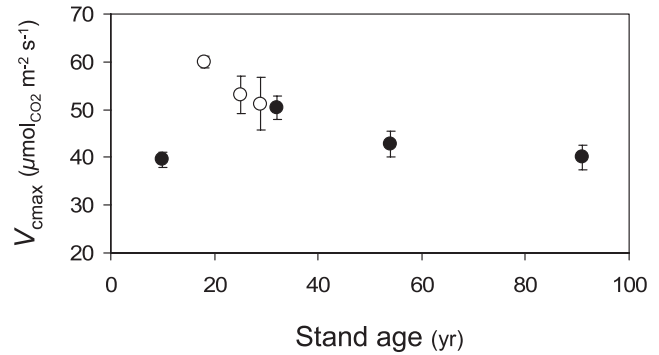
Photosynthetic values reported here were close to those found in previous studies made on the same species for a 25-yr-old stand, where V_{cmax} value was $49.3 \mu\text{mol m}^{-2} \text{s}^{-1}$ for one

Table IV. Regression coefficients for leaf structural parameters (N_m , P_m , SLA, N_a and P_a) versus stand age for data pooled by age $n = 4$ (linear regressions for all parameters except specific leaf area (SLA); log-linear regression for SLA).

	Intercept	Slope	R^2	P
N_m (%)	1.03	0.003	0.836	0.086
P_m (%)	0.061	-0.0001	0.109	0.671
SLA ($\text{m}^2 \text{kg}^{-1}$)	1.807	-0.002	0.908*	0.047
N_a (g m^{-2})	1.689	0.009	0.948*	0.026
P_a (g m^{-2})	0.103	-0.0001	0.021	0.856

year old needles sampled in the top of the canopy [25]. Medlyn et al. [18] reported a range of V_{cmax} between 35 and 60 for a 18-yr-old stand throughout the year. In our study, photosynthetic parameters were only measured at the canopy top and we assumed that they were representative of the whole crown. Indeed, Porté and Loustau [25] demonstrated that crown height did not influence V_{cmax} and J_{max} in maritime pine trees. The lack of variation in photosynthetic parameters was due to the weak attenuation of light with canopy depth. The range of LAI observed in this chronosequence makes therefore unlikely that the photosynthetic parameters may vary strongly in tree crowns and the parameters as measured may be considered as spatially representative of entire crowns. Although Medlyn et al. [18] showed that the V_{cmax} and J_{max} values may change by $16 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ and $32 \mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$ respectively on a seasonal basis, we did not detect any time effect over the course of our experiment.

We did not find any relationship between photosynthetic parameters (V_{cmax} , J_{max} , α and R_d) and stand ages across our chronosequence (Tab. III). Therefore, the lack of difference in photosynthetic parameters means that the decline in growth efficiency (Tab. I) cannot be explained by a decline in photosynthetic capacity. This result supports the hypothesis that the drop in stomatal conductance observed in this stage by Delzon et al. [7] and confirmed by isotope discrimination could alone explain the change in growth efficiency throughout our maritime pine chronosequence. Maintenance of the photosynthetic parameters observed in this study has also been observed in other studies, even though photosynthetic capacity along tree life cycle have been poorly quantified and just tackled in few studies. Indeed, the results reported so far support apparently the idea that V_{cmax} and J_{max} do not correlate with the age decline in forest productivity. For instance, Barnard and Ryan [1] found that *Eucalyptus saligna* trees of 1- (7 m) and 5-years (26 m) had V_{cmax} values of 76 and $85 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. Phillips et al. [24] did not detect any difference in either V_{cmax} or J_{max} between 10- and 25-m height oak trees. Likewise, no significant change was found for Ponderosa pine [11, 37]. However, Law et al. [14] found different results for 10- and 50-yr-old Ponderosa pine stands, where V_{cmax} decreased by 35% from the young to the older stand. On the other hand, for Douglas-fir, McDowell et al. [17] reported V_{cmax} values reaching a maximal value at intermediate age, i.e. 27.5, 47.9 and $38.9 \mu\text{mol m}^{-2} \text{s}^{-1}$ for the 15-, 32- and 60-m trees, respectively. However, it must be mentioned that at large with present and past results obtained on maritime pine, none of these V_{cmax} determinations were made under constant temperature and humidity conditions and

**Figure 3.** Mean values of maximum carboxylation rate, V_{cmax} , versus stand age. Full circles, mean values measured across our chronosequence in this study. Open circles, mean values from previous studies (Porté and Loustau [28] and Medlyn et al. [20]) measured using the same gas exchange system in three stands among which two do not belong to our chronosequence; bars are standard errors.

satürating light. Discrepancies in the measurement protocol might cause large bias when comparing data from different authors.

Our data show no trend in photosynthetic capacity (V_{cmax} , J_{max} , α and R_d) with stand age despite the fact that maximum rate of carboxylation was higher in the 32-yr-old stand. This higher value at age 32-yr is confirmed by data measured previously in three stands among which two do not belong to the chronosequence [18, 25] (Fig. 3). In agreement with the observation that V_{cmax} is affected at this level of P concentrations in maritime pine [2, 15], the between-stand difference in V_{cmax} is well correlated to the needle P concentration measured across sites ($R^2 = 0.99$, $n = 4$). This conclusion holds true for the additional data issued from Porté and Loustau [25] and Medlyn et al. [18] where needle P_a is in the range $0.11 - 0.13 \text{ g P m}^{-2}$ (data not shown). We may suspect an impact of the fertilisation provided shortly after planting to the trees of the 32-yr-old stand; indeed, the other stands of our chronosequence never received any fertilisation.

The Δ of annual ring cellulose decreased significantly with increasing stand ages (intercept 18.466 slope -0.008 $R^2 = 0.769$, $P < 0.0096$) independently of the year from 18.5 to 17.68‰ [7]. There was no relationship between Δ and maximum carboxylation rates or electron transport rates. On the other hand, the photosynthetic parameters results conformed to the concurrent decline in stomatal conductance and carbon discrimination

observed across the chronosequence studied. Indeed, our data suggested that at a given photosynthesis performance lower stomatal conductance occurred, inducing lower C_i , and decreasing Δ . Moreover, the lower value of Δ in the 32-yr-old stand can be explained by the higher values of photosynthetic capacity and intermediate level of stomatal conductance measured in the trees of this stand.

Leaf nitrogen concentration on an area basis appeared to increase slightly throughout the chronosequence and did not play a role in the photosynthesis decline in maritime pine trees. We found that most of the variations in N_a across the chronosequence were a result of thicker needles (SLA) rather than difference in nitrogen concentration (N_m). Because leaf nitrogen concentration of leaves is usually correlated with photosynthetic capacity and its measurement was less time-consuming than $A-C_i$ curves, a lot of studies have investigated age-related change in N_a . Leaf N on a mass basis did not present a general trend in response to tree height or age [22]; in some studies, it was lower in older trees [10, 21, 34] while it remained constant with increasing tree age in others [11, 19, 37]. The leaf mass to area ratio is known to increase as trees become older and taller [22] which increases the nitrogen concentration on an area basis [27, 34], as observed in our study.

The data presented in Figure 3 suggest that the photosynthetic capacity may decrease with increasing stand age after canopy closure (LAI max observed between 15 and 25 years). Since the 18- and 32-yr-old stands had received a larger initial fertilisation in P than the other stands, we cannot disentangle unambiguously the effects of P nutrition from the eventual age effect. The change in phosphorus concentration in needle correlates well with the variation in V_{cmax} observed between stands consistently with previous studies on the impact of phosphorus starvation on the photosynthesis in this species [15]. Therefore, P nutrition is likely the main cause of the changes in photosynthetic capacity observed among maritime pine stands. Having acknowledged that the differential fertilisation of the stands composing the chronosequence studied may explain the pattern observed, we cannot exclude that the sequestration of P under unavailable forms in soil, soil organic matter and biomass may play a role in photosynthesis, growth and productivity decline [10, 20, 36]. Moreover, deficiency in P can also affect total leaf area [5] and not only nutrient concentration per unit of leaf area or photosynthesis. Results from fertilisation experiments in this area have shown that a 42-yr-old stand responds positively to a late fertilisation in phosphorus, which demonstrates that nutrient is still limiting at this age even for stands having received as much as 250 kg P ha⁻¹ during site preparation (Trichet, unpublished results). A positive response of tree growth to thinning in old growth stands of Douglas-fir and Ponderosa pine provides an additional support to the hypothesis that the availability of resources, not an inherent decadence with age, limits tree growth in old stands [13, 16, 32].

5. CONCLUSION

In our chronosequence, we observed no trend in photosynthetic capacity (V_{cmax} , J_{max} , α and R_d) with increasing stand age. Elsewhere, in a previous paper [7], we related a marked

decrease in both stomatal conductance and wood Δ with increasing tree height across stand development, reducing CO_2 diffusion into the leaf. Together with the results presented in this study, our results demonstrated that the decrease in foliar assimilation, inducing the growth efficiency decline observed in the studied chronosequence, could be explained only by stomatal closure in response to greater hydraulic constraints as trees grow taller. However, additional data from previous studies showed that V_{cmax} might be higher in young stands due to initial fertiliser application with respect to forest management in south-western France. So, we cannot exclude the idea that P nutrition as a limiting factor of tree growth might play a role in productivity decline throughout the rotation cycle of maritime pine stands.

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