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Significant Links Between Photosynthetic Capacity, Atmospheric CO_2 and the Diversification of C_3 Plants During the Last 80 Million Years

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ABSTRACT

Changing CO_2 concentrations will continue to affect plant growth with consequences for ecosystem functioning. The adaptive capacity of C_3 photosynthesis to changing CO_2 concentrations is, however, insufficiently investigated so far. Here, we focused on the phylogenetic dynamics of maximum carboxylation rate (V_{cmax}) and maximum electron transport rate (J_{max})—two key determinants of photosynthetic capacity in C_3 plants—and their relation to deep-time dynamics in species diversification, speciation and atmospheric CO_2 concentrations during the last 80 million years. We observed positive relationships between photosynthetic capacity and species diversification as well as speciation rates. We furthermore observed a shift in the relationships between photosynthetic capacity, evolutionary dynamics and prehistoric CO_2 fluctuations about 30 million years ago. From this, we deduce strong links between photosynthetic capacity and evolutionary dynamics in C_3 plants. We furthermore conclude that low CO_2 environments in prehistory might have changed adaptive processes within the C_3 photosynthetic pathway.

1 | Introduction

Increasing CO_2 concentrations have profound effects on global climate and ecosystems (Körner 2006; Li et al. 2017; Liu et al. 2019). Rising CO_2 concentrations are assumed to strongly affect plant growth with significant consequences for ecosystem functioning and agricultural production (Böhnisch et al. 2021; Kaur, Singh, and Behl 2016). Knowledge about the adaptive capacity of plants to changing CO_2 concentrations is therefore imperative to understand and predict ecosystem responses that happened in prehistory and will happen in the future (Sniderhan, McNickle, and Baltzer 2018).

C_3 photosynthesis predominates carbon fixation in terrestrial plants with more than 90% of terrestrial plants exhibiting this pathway—accounting for approximately 77% of terrestrial primary productivity (Sage, Li, and Monson 1999; Still et al. 2003). Understanding the adaptive capacity of the C_3 photosynthetic pathway to changing CO_2 concentrations is therefore imperative to assess past and future ecosystem responses. However, most studies on CO_2 adaptation focus on single species in short-term experiments (e.g., Li et al. 2017) or contemporary dynamics, for example, via remote sensing studies (Higgins, Conradi, and Muhoko 2023; Wang et al. 2020). Evolutionary perspectives on physiological adaptations of C_3 plants to changing CO_2 concentrations are underrepresented (see e.g., Li et al. 2014; Neto-Bradley et al. 2021).

Carbon concentrating mechanisms (CCMs) are a well-known adaptation of plants to variations in atmospheric CO_2 that

evolved to maximise photosynthetic capacity under insufficient CO_2 concentrations (Raven, Cockell, and De La Rocha 2008; Sage 2004; Smith et al. 1999). Close links have been shown to exist between prehistoric changes in atmospheric CO_2 concentrations and the evolutionary history of CCMs in plants. C_4 photosynthesis is one of the most famous CCMs which spread among dicots in the late Pleistocene during periods of exceptionally low atmospheric CO_2 concentrations (Lundgren et al. 2019; Sage 2004; Sage, Sage, and Kocacinar 2012). Existing studies on the macroevolutionary history of plant physiology almost exclusively focus on this C_3 to C_4 photosynthesis transition but neglect evolutionary dynamics in the globally predominant C_3 photosynthesis mode (Neto-Bradley et al. 2021).

Maximum rubisco carboxylation rate (V_{cmax}) and maximum electron transport rate (J_{max}) describe key limitations of C_3 photosynthesis with high adaptive capacity in terms of changing CO_2 concentrations (Farquhar, von Caemmerer, and Berry 1980). The carboxylation rate is strongly coupled to substrate specificity (CO_2 vs. O_2) and reaction kinetics of RuBisCO (Ribulose 1,5-Bisphosphate Carboxylase/Oxygenase)—the key enzyme of C_3 photosynthetic carbon fixation (Nisbet et al. 2007). The electron transport rate is controlled by the energy demand of the carboxylation process in the Calvin–Benson cycle and the amount of energy provided by the light-harvesting photosystems. V_{cmax} quantifies the capacity of RuBisCO carboxylation whereas J_{max} indicates ribulose-1,5-biphosphat (RuBP) regeneration via energy transferred through the electron transport chain to ADP/ATP and NADP+/NADPH. V_{cmax} therefore quantifies limitations of carboxylation caused by the enzymatic characteristics of RuBisCO under given CO_2 concentrations whereas J_{max} quantifies limitations in the regeneration of RuBP caused, for example, by insufficient or excessive light energy.

Rates of C_3 carbon fixation are determined by the activity and substrate specificity of RuBisCO (Raven, Cockell, and De La Rocha 2008; Tabita et al. 2008). RuBisCO is generally characterised by a low affinity for CO_2 and a relatively slow carboxylation turnover rate—features that characterise this enzyme to be quite inefficient in carbon fixation under present-day CO_2 concentrations (Iñiguez et al. 2020). RuBisCO and C_3 photosynthesis evolved early in the history of life under high CO_2 concentrations—conditions under which its capacity was considerably higher (Hayes 1994; Sage 2004). However, C_3 photosynthesis persisted during extremely low CO_2 periods in Earth history and is nowadays still the most widespread form of photosynthetic carbon acquisition (Busch et al. 2013). This raises the question of alternative forms of adaptation to low CO_2 conditions acting on C_3 plant evolution.

C_3 photosynthesis requires minimum levels of CO_2 of 100–150 $mmol\,mol^{-1}$ to maintain growth and reproduction in warm environments (Campbell et al. 2005; Lovelock and Whitfield 1982). With CO_2 concentrations being close to this limit, C_3 plants must have experienced strong limitations with effects on their competitive ability during periods of low CO_2 in Earth history (Li et al. 2014). Such low CO_2 environments are well known to have initiated the evolution of alternative, more efficient forms of carbon concentration mechanisms such as C_4 photosynthesis (Busch et al. 2013; Christin et al. 2008; Galmés et al. 2014; Iñiguez et al. 2020; Lundgren et al. 2019; Nisbet et al. 2007). Low CO_2 environments that happened in prehistory are therefore likely to have not only affected plant physiology but also shaped evolutionary dynamics in C_3 plant lineages by affecting photosynthetic capacity (cf. Busch et al. 2013). More specifically, we hypothesise that periods of low CO_2 concentrations in prehistory strongly limited C_3 photosynthetic carbon fixation and were selected for higher photosynthetic capacity, namely more efficient rubisco carboxylation and electron transport. This should be reflected in higher maximum rates of rubisco carboxylation (V_{cmax}) and higher maximum electron transport rates (J_{max}) for species that emerged in periods of low CO_2 concentrations in contrast to species that originated in higher CO_2 periods. We furthermore hypothesise that low CO_2 periods in prehistory amplified evolutionary dynamics in C_3 plants by facilitating speciation and diversification in C_3 plant lineages similar to C_4 plant speciation and diversification in these low CO_2 periods. To test these hypotheses, we focussed on the phylogenetic variation of V_{cmax} and J_{max} during the last 80 million years (Myr) of C_3 evolution and its relation to deep-time dynamics in speciation and diversification rates of C_3 plants as well as atmospheric CO_2 concentrations.

2 | Methods

For our study, we gathered information on the photosynthetic capacity (i.e., J_{max} and V_{cmax} at standard temperature) and other related traits (i.e., Specific Leaf Area as well as leaf N and P contents) for 232 contemporary C_3 plant species— that is, 196 woody angiosperms, 15 woody gymnosperms, 2 woody monocots (palms), 7 Grasses (*Poaceae* and *Cypercaea*), 8 herb and 4 crop species (see Table S1). Information was extracted from trait databases with global coverage, that is, TRY and the Botanical Information and Ecology Network (BIEN). V_{cmax} and J_{max} values were extracted from TRY (Kattge et al. 2020, request 29,688) whereas information on specific leaf area as well as leaf nitrogen and leaf phosphorus contents were derived for the respective species from the Botanical Information and Ecology Network (BIEN, Enquist et al. 2016) using the BIEN R package (v.1.2.4, Maitner et al. 2018). We assume most of the V_{cmax} and J_{max} estimates in the TRY database to be deduced from A-Ci curves rather than net assimilation-chloroplastic CO_2 concentration (A-Cc) curves—a fact that can result in considerable differences in parameter estimation for V_{cmax} and J_{max} (Miao et al. 2009). We therefore define the parameter estimates as apparent V_{cmax} and J_{max} but stay with the terms 'V_{cmax}' and 'J_{max}' for readability. Both, J_{max} as well as V_{cmax} estimations are sensitive to temperature differences (Medlyn et al. 2002). To account for this temperature dependency, we used V_{cmax} and J_{max} values which were estimated at a standard temperature of 25°C with calculating the mean out of multiple available values per species. If species estimates did not include 25°C measurements, we interpolated to this standard temperature using multiple species measurements at different temperatures. Furthermore, all plant-related traits used in this study can show high intraspecific variation. To account for this uncertainty in trait estimates, we estimated species-specific trait variation by calculating the quartile-based coefficient of variation (qcv) of the species-specific trait measurements and tested its effect on the median trait measurements we were using in our analyses.

Information on the phylogenetic relationships among the investigated plant species was extracted from an updated version of Zanne et al.'s (2014) mega-phylogeny using the S.PHYLOMAKER R package (Qian and Jin 2016). Uncertainty about the phylogenetic relationships and the phylogenetic age was implemented in the analyses. For this, we used two scenarios of adding missing species or genera to the backbone phylogeny (i.e., scenarios 2 and 3, cf. Jin and Qian 2022; Qian and Jin 2016). In the first case (i.e., scenario 3), missing species or genera are added to their corresponding families or genera by using the same approach as implemented in Phylomatic and BLADJ phylogenetic tools, that is, by binding them to the 1/2 point of the family branch, that is, the branch between the family root node and basal node. If the family branch length was longer than 2/3 of the whole family branch (i.e., from the family root node to the tip) length, the new genus tip was added to the upper 1/3 point of the whole family branch length. In any other case, the new species is added to the basal node of the corresponding genus. In scenario 2 case, missing species were added at a randomly selected node, thus, phylogenetic age within their families or genera lineages. Both scenarios are commonly used in current literature (Jin and Qian 2022; Qian and Jin 2016). To account for uncertainty in the phylogenetic relations and ages, we reran scenario 2 reconstructions 100 times and replicated our analyses for each of these 100 scenario outputs (cf. Jin and Qian 2022).

Prehistoric CO_2 concentrations were extracted from a dataset provided by Foster, Royer, and Lunt (2017) consisting of 1241 independent estimates of atmospheric CO_2 concentration based on five different proxy methods and 112 published studies covering the past 420 Myr with a temporal resolution of 0.5 Myr. In addition to the phylogenetic uncertainty, we accounted for uncertainty in the CO_2 concentration reconstructions. For this, we constructed normal distributions of CO_2 concentrations for each point in time based on the maximum probability of CO_2 as well as the value for the 5% and 95% percentile provided by Foster et al. for the respective times. From these constructed normal distributions, we repeatedly, randomly sampled 100 times for each considered age. The estimates from each of these 100 sampling runs are depicted as light blue lines in Figure 1. Each of these 100 sampling runs was combined with the 100 scenario 2 phylogenetic reconstruction runs and served as the bases of our statistical analyses.

We quantified temporal changes of V_{cmax} and J_{max}, 8 of leaf P and N contents as well as of specific leaf area (SLA) over the last 80 Myr by subdividing the total time span in intervals of 5 Myr for which we calculated median trait values from all species that originated in the respective time period. The width of the time window was chosen to have a sufficiently high sample size of species for each of the respective time intervals. All time intervals with less than 5 species were excluded from the analyses. Times of species origin were quantified as the time when a respective species branched of the phylogenetic tree. Species in the respective time intervals were identified using the cutPhylo()- command of the RRPHYLO package 2.7.0 (v.2.7.0, Castiglione et al. 2018). The total time span of the investigation was restricted to 80 Myr as the number of investigated species originating earlier than 80 Myr was too low in our dataset to be still representative (i.e., more than 5 species, see Table S1 for species details). The CO_2 concentrations for the respective time intervals were calculated as the median concentrations in the respective time intervals based on the CO_2 timeseries of Foster, Royer, and Lunt (2017). Speciation and diversification rates were calculated by using the bd.ms() and bd.mk()- command of the GEIGER package (v.2.0.7, Pennell et al. 2014). Rates were calculated for each 5-million-year interval based on the subset of the respective species which were identified to originate in the respective time interval.

Relationships between the different variables were tested by using simple linear regression models. We used untransformed or transformed predictors (i.e., cubic transformation for the J_{max}—Diversification relationship) based on the model diagnostics of the respective model (i.e., the amount of explained variation and Q-Q-plot). We identified breakpoints in the relationship between the different, plant-related trait medians and prehistoric CO_2 concentrations by using the STRUCCHANGE-R-package

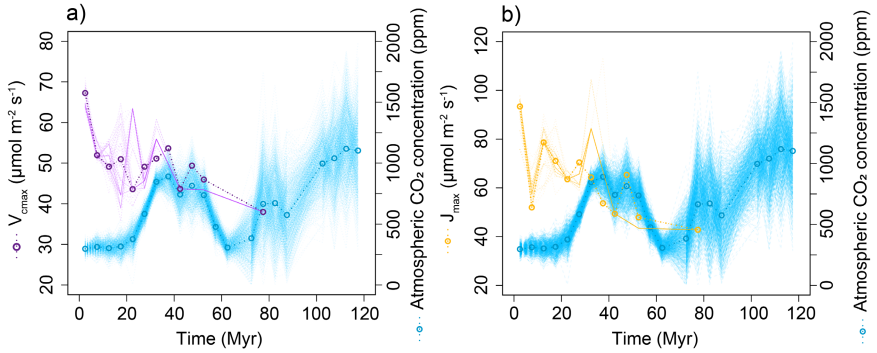

FIGURE 1 | Variation in atmospheric CO_2 concentration (blue), maximum carboxylation rate (V_{cmax}, purple, a) and maximum electron transport rate (J_{max}, yellow, b) of C_3 plant species during the last 80 Myr before presence. V_{cmax} and J_{max} per time were quantified as median values for plant species which originated in the respective time intervals of 5 Myr. Variation is depicted along the time axis with points being positioned at the mean of each time interval (i.e., 2.5 Myr for the interval 0–5 Myr). Bold, dark yellow and purple lines indicate the results from scenario 3, phylogenetic reconstruction and the respective, maximum probability CO_2 concentrations. Dark blue lines indicate median values of CO_2 concentrations for the respective time intervals. Additionally, results from each of the 10,000 sample runs for the phylogenetic reconstruction (scenario 2) are depicted as light yellow and purple lines. Uncertainty in the CO_2 reconstructions is depicted as light blue lines (see methods for details).

(v.1.5–3, Zeileis et al. 2002, 2003). We tested for phylogenetic signals in V_{cmax} and J_{max} using Pagel's lambda as implemented in the phylosig()-command of the PHYTOOLS package (v.0.7–70, Revell 2012). Measures such as Pagel's lambda quantify the degree to which observable species characteristics such as functional traits contain information about phylogenetic relationships among the tested species and are commonly used as a simple measure to estimate the degree to which functional traits are phylogenetically conserved (Münkemüller et al. 2015). Significance testing of the phylogenetic signals was based on a randomisation test with 1000 simulations implemented in the phylosig()-command. For each analysis, we repeated 100 sample runs of phylogenetic reconstruction and CO_2 concentration estimation.

In addition to the analyses on the full dataset, we ran all analyses only for the woody angiosperms, which comprised 197 species the biggest subset of the considered plants. We performed this additional analysis to reduce the bias which might be based on different life forms of C_3 such as angiosperms and gymnosperms with potential differences in photosynthesis limitation (see the results of this additional analysis in Appendix S1 and details on the lifeforms in Table S1). To evaluate the generality of our hypothesis, we furthermore performed an analysis on 35 species of ferns and fern allies (Lycopodiopsida and Polypodiopsida) as other types of land plants which show significant differences in photosynthesis limitation (i.e., stomatal limitation, biochemical limitation and especially mesophyll conductance limitation, Flexas and Carriquí 2020; Gago et al. 2019). The results of these analyses are summarised in Appendix S2. In this study, we primarily focus on the effect of pre-historic CO_2 fluctuations. However, the effect of prehistoric temperature fluctuations cannot be ignored due to a strong coupling of prehistoric CO_2 and temperature fluctuations (CENCO2PIP Consortium 2023).

All analyses were performed in R (v.4.2.3, R Core Team 2023) with a level of significance of alpha = 0.05.

3 | Results

Species medians of maximum carboxylation rate (V_{cmax}) and maximum electron transport rate (J_{max}) visibly fluctuated through the 80 Myr but generally increased towards the more recent past (Figure 1). Median V_{cmax} of the species that originated in the respective time periods was positively correlated with the corresponding, prehistoric CO_2 concentrations until 32.5 million years ago ($R^2 = 0.95$, $p = 5.6 \times 10^{-4}$) but showed no significant correlation with CO_2 fluctuations in the more recent past ($R^2 = 0.11$, $p = 0.28$, Figure 2a). This shift in the

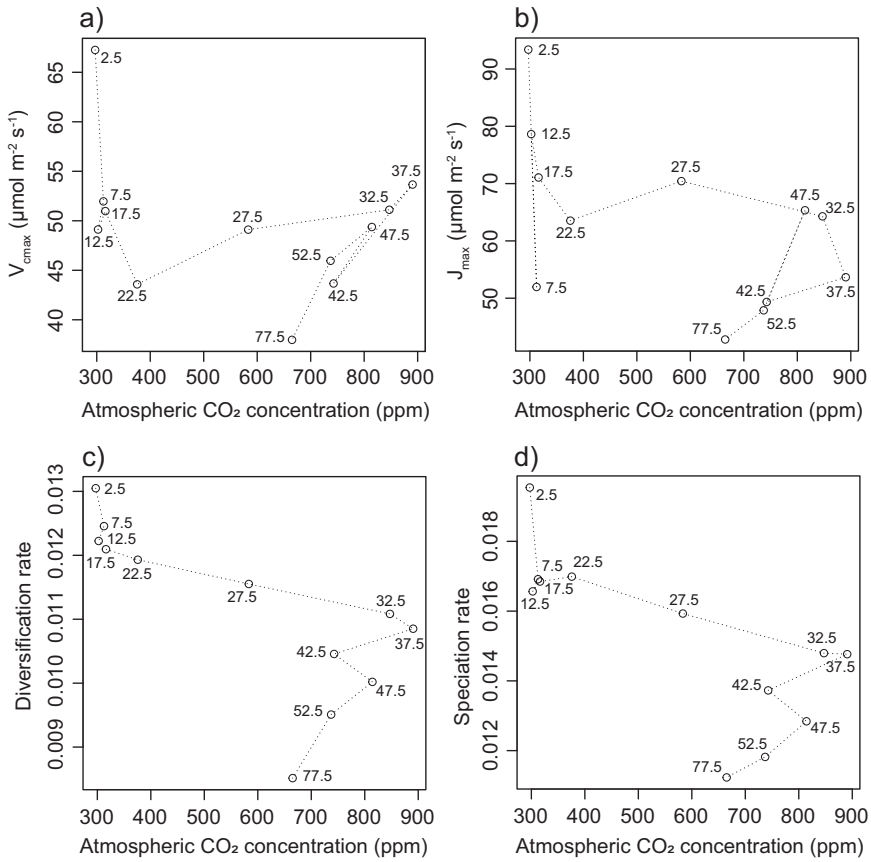

FIGURE 2 | Relationship between maximum carboxylation rate (V_{cmax}, a), maximum electron transport rate ($J_{max,}$ b), species diversification rate (c) as well as species speciation rate (d) and atmospheric CO_2 concentration during the last 80 Myr of evolutionary history. J_{max} and V_{cmax} were quantified as median values for plant species which originated in the respective time intervals of 5 Myr (see Figure 1). CO_2 concentrations reflect the median of the maximum probability CO_2 concentrations of the respective time interval. Diversification and speciation rates were calculated for the same set of species of the corresponding time intervals. Points of subsequent times are connected by dashed lines with the respective time (i.e., mean of each time interval) being indicated as a number beside each point.

relationship at 32.5 Myr was also detected as the most probable breakpoint for the relationships of J_{max}, species diversification and species speciation rate with prehistoric CO_2 concentrations (Figure S1). J_{max} and prehistoric CO_2 concentrations were positively correlated until 32.5 Myr ($R^2 = 0.68$, $p = 0.03$) but showed no correlation after passing the threshold ($R^2 = 0.11$, $p = 0.28$, Figure 2b). Species diversification rate decreased with decreasing CO_2 until 32.5 Myr ($R^2 = 0.69$, $p = 0.025$) but increased with decreasing CO_2 concentrations in the more recent time interval ($R^2 = 0.46$, $p = 0.084$, Figure 2c). Species speciation rate and prehistoric CO_2 concentrations were positively correlated until 32.5 Myr ($R^2 = 0.68$, $p = 0.03$) but showed no significant correlation after passing the threshold ($R^2 = 0.11$, $p = 0.28$, Figure 2d).

Prehistoric fluctuations in species diversification rate were positively correlated with the species median J_{max} with a non-linear increase of J_{max} with increasing diversification rate ($R^2 = 0.52$, $p = 0.005$ for scenario 3 and $R^2 = 0.58 \pm 0.04$ with $p = 0.01 \pm 0.004$ for the uncertainty scenarios, Figure 3a; Figure S2a). Similar strong, positive relationships were observed for species speciation rate and J_{max} ($R^2 = 0.57$, $p = 0.003$ for scenario 3 and $R^2 = 0.60 \pm 0.05$ with $p = 0.008 \pm 0.004$ for the uncertainty scenarios, Figure 3b; Figure S2b).

V_{cmax} increased with increasing species diversification rate ($R^2 = 0.62$, $p = 3.1 \times 10^{-4}$ for scenario 3 and $R^2 = 0.52 \pm 0.1$ with $p = 0.02 \pm 0.015$ for the uncertainty scenarios, Figure S3a,b). This relationship, however, weakened when excluding points with high leverage, that is, the lowest and highest species median V_{cmax}, which was calculated for 77.5 and 2.5 Myr ($R^2 = 0.08$, $p = 0.44$ for scenario 3 and $R^2 = 0.38 \pm 0.11$ with $p = 0.08 \pm 0.06$ for the uncertainty scenarios). V_{cmax} showed a linear increase with species speciation rate ($R^2 = 0.46$, $p = 0.009$ for scenario 3 and $R^2 = 0.59 \pm 0.1$ with $p = 0.009 \pm 0.009$ for the uncertainty scenarios, Figure S3c). This relationship also weakened when excluding the point with high leverage, that is, V_{cmax} for 2.5 Myr ($R^2 = 0.20$, $p = 0.09$ for scenario 3 and $R^2 = 0.34 \pm 0.1$ with $p = 0.10 \pm 0.07$ for the uncertainty scenarios).

Species medians of leaf P content were negatively related to prehistoric CO_2 concentrations ($R^2 = 0.32$, $p = 0.03$ for scenario 3 and $R^2 = 0.23 \pm 0.14$ with $p = 0.08 \pm 0.09$ for the uncertainty scenarios, Figure 4a). When considering the most probable breakpoint in this relationship at 27.7 Myr (Figure S4), this negative relationship became very strong in the more recent past (i.e., younger 27.7 Myr; $R^2 = 0.9$, $p = 0.003$) but non-existent in the earlier time period ($R^2 = 0$, $p = 0.97$).

Leaf P was positively correlated with species diversification rates ($R^2 = 0.26$ with $p = 0.05$ in scenario 3 and $R^2 = 0.42 \pm 0.06$ with $p = 0.04 \pm 0.02$ in the uncertainty scenarios, Figure 4b; Figure S5a). A positive relationship was also detectible between leaf P and the respective species speciation rates ($R^2 = 0.19$ with $p = 0.09$ in scenario 3 and $R^2 = 0.37 \pm 0.06$ with $p = 0.06 \pm 0.03$ in the uncertainty scenarios, Figure 4c; Figure S5b).

No correlations were detected between SLA or leaf N and the respective species diversification or speciation rates ($p > 0.35$ in all cases). SLA and leaf N were not correlated with prehistoric CO_2 concentrations ($p > 0.6$ in all cases). Furthermore, no relationship was detectable between the time-specific species medians of leaf chemical and morphological traits (leaf N, leaf P and SLA) and the corresponding species medians of the physiological traits J_{max} and V_{cmax} ($p > 0.3$ in all cases).

Variation in the time-specific species trait medians showed no relation to intraspecific trait variation (correlations between the trait median and the species-specific quartile-based variation coefficient per time interval, V_{cmax}: $p = 0.16$, J_{max}: $p = 0.35$, leaf P: $p = 0.60$, leaf N: $p = 0.23$, SLA: $p = 0.50$).

We detected a strong phylogenetic signal for J_{max} (Pagel's lambda = 0.88, $p = 6.7\ 10^{-13}$ for scenario 3 and lambda = 0.88 ± 0.0042 for 100 runs of scenario 2 phylogenies). The phylogenetic signal for V_{cmax} was significantly weaker (i.e., no overlap of the 95% confidence intervals in Figure 5; lambda = 0.74 with $p = 1.5 \times 10^{-12}$ for scenario 3 and lambda = 0.72 ± 0.011 for 100 runs of scenario 2 phylogenies).

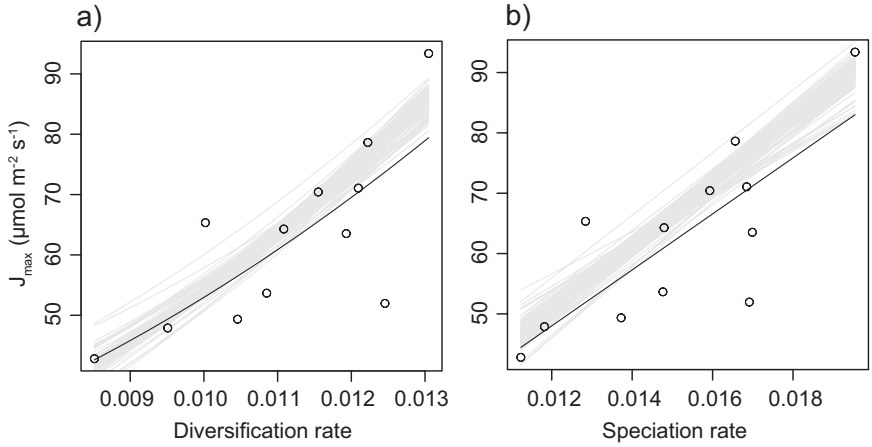

FIGURE 3 | Relationship between maximum electron transport rate (J_{max}) and species diversification rate (a) as well as species speciation rate (b) of C_3 plant species during the last 80 Myr of evolutionary history. J_{max} was quantified as median values for plant species which originated in the respective time intervals of 5 Myr (see Figure 1). Diversification and speciation rates were calculated for the same set of species of the corresponding time intervals. Relationships are visualised as solid, black lines (i.e., scenario 3 phylogeny) as well as grey lines (100 runs of scenario 2 phylogenetic reconstruction, see methods).

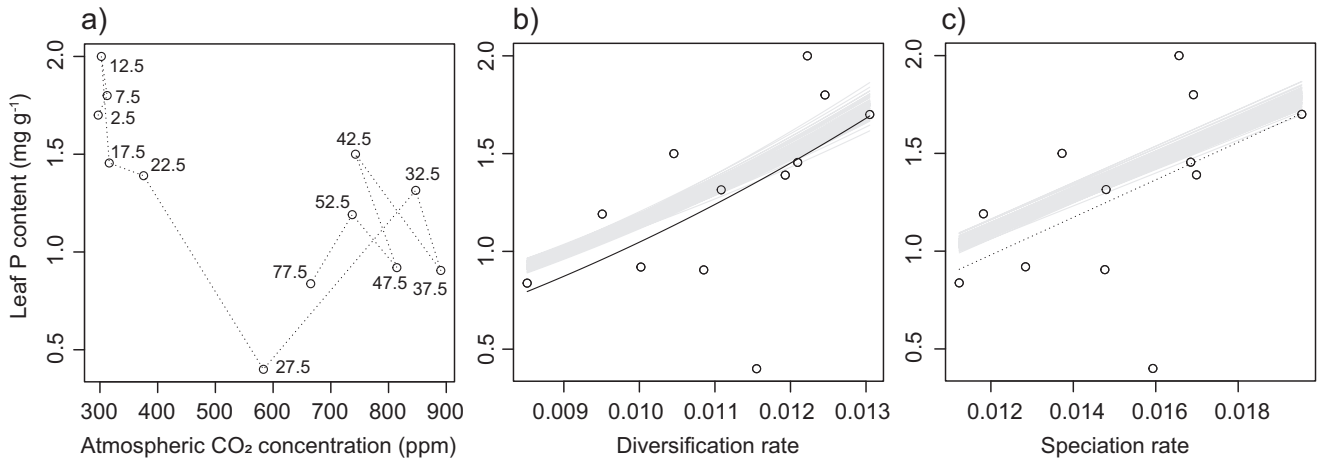

FIGURE 4 | Relationship between leaf phosphorous content of C_3 plant species and atmospheric CO_2 concentration (a) as well as species diversification and speciation rates (b, c) during the last 80 Myr of evolutionary history. Leaf P content was quantified as median values for plant species which originated in the respective time intervals of 5 Myr. CO_2 concentrations reflect the median of the maximum probability CO_2 concentrations of the respective time interval. Diversification and speciation rates were calculated for the same set of species of the corresponding time intervals. Points of subsequent times are connected in (a) by dashed lines with the respective time (i.e., mean of each time interval) being indicated as a number beside each point. Relationships in (b) and (c) are visualised as solid ($p < 0.05$) or dashed ($p > 0.05$), black lines (i.e., scenario 3 phylogeny) as well as grey lines (100 runs of scenario 2 phylogenetic reconstruction, see methods).

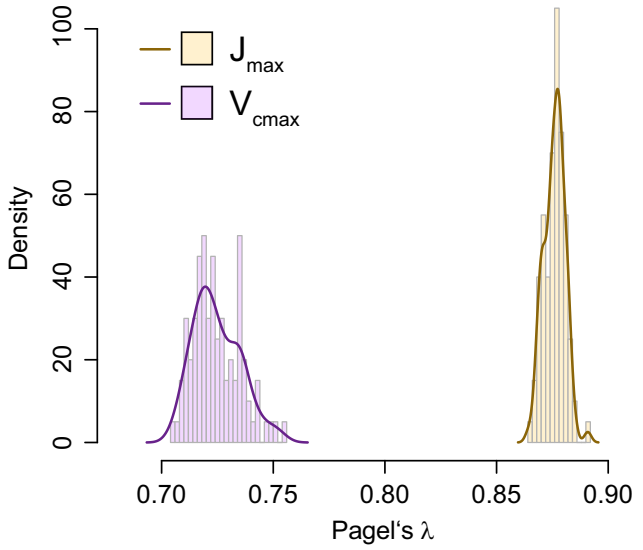

FIGURE 5 | Phylogenetic signal (Pagel's lambda) in maximum carboxylation rate (V_{cmax}, purple) and maximum electron transport rate (J_{max}, yellow) in C_3 plants for the last 80 Myr. Histograms and solid lines (i.e., corresponding kernel density estimates) visualise the outcome of 100 model runs based on scenario 2 phylogenies (see methods).

Species median J_{max} and V_{cmax} of the respective time intervals were positively correlated ($R^2 = 0.50$, $p = 0.006$ for scenario 3 and $R^2 = 0.64 \pm 0.16$ with $p = 0.006 \pm 0.007$ for the uncertainty scenarios, Figure S6). This relationship, however, weakened when data points with high leverage were excluded, that is, 2.5 Myr ($R^2 = 0.13$, $p = 0.15$ for scenario 3 and $R^2 = 0.48 \pm 0.20$ with $p = 0.04 \pm 0.05$ for the uncertainty scenarios).

The results on the woody angiosperms were consistent with the observations we made for the full dataset although relationships between become less clear between J_{max}, V_{cmax}, species diversification as well as speciation rates and prehistoric CO_2 concentrations. The positive relationships between J_{max}, V_{cmax} and species diversification as well as species speciation rates became more pronounced in the subset analysis. Details on the results of these additional analyses are given in Appendix S1. The additional analyses on ferns and fern allies generally confirm our observations on C_3 angiosperms with regard to the positive relationship between V_{cmax} and species diversification and speciation rate and the negative correlations between diversification rates and prehistoric CO_2 concentrations (Appendix S2 for details).

4 | Discussion

Increasing atmospheric CO_2 concentrations generally increase photosynthetic carbon acquisition by increasing intercellular CO_2 concentrations and, thus, carboxylation capacity as well as by reducing photorespiration (Ainsworth and Long 2005; Bowes 1993; Stiling et al. 2013). All this facilitates plant growth under elevated CO_2 but reduces it under limited CO_2 availability with variation between different species and photosynthetic pathways (e.g., C_3 vs. C_4) (Luo and Reynolds 1999; Nowak, Ellsworth, and Smith 2004; Prior et al. 2011). C_3 photosynthesis is generally more limited under decreasing CO_2 concentrations than the alternative C_4 photosynthetic pathway.

In our analyses, both components of photosynthetic capacity, carboxylation and electron transport rates were linked to fluctuations in prehistoric CO_2 concentrations. However, the direction of these relationships changed over the course of the last 80 Myr of C_3 plant evolution. Our analyses revealed the most probable time for this change was about 30 million years ago. At this point in time, the relationship between photosynthetic capacity and CO_2 concentration changed from a positive with increasing V_{cmax} and J_{max} with increasing CO_2 concentrations to a period with weaker relations into a period with a negative relationship between photosynthetic capacity and CO_2 concentration in recent prehistory (i.e., from about 20 million years ago). These

negative relationships in recent prehistory are clearly visible in Figure 2 though statistically not significant due to few data points. We detected this breakpoint about 30 million years ago also for the relationships of species diversification and speciation with prehistoric CO_2 concentrations. Whereas both, diversification and speciation increased with increasing CO_2 before this threshold, diversification rates increased with decreasing CO_2 after passing this threshold. All these observations hint towards a shift in the evolutionary trajectory of C_3 photosynthesis which happened approximately 30 million years ago.

Around 30 million years ago, during the transition from the Eocene to the Oligocene epoch, Earth climate experienced significant cooling, that is, a shift from the warmer, more tropical climate of the Eocene to the cooler, more temperate climate of the Oligocene. This cooling was driven by a combination of factors, including changes in ocean circulation, the isolation of Antarctica and a strong decrease in atmospheric CO_2 concentrations. These environmental changes, especially the decrease in temperatures and CO_2 concentrations, spurred significant evolutionary changes in plants. The most prominent example is the evolution of C_4 photosynthesis as an alternative, photosynthetic pathway that has a significantly higher carboxylation efficiency than the C_3 photosynthetic pathway. C_4 photosynthesis first emerged in grasses, most probably during the Oligocene epoch, that is, 24–35 Myr (Sage 2004). Atmospheric conditions favouring significant levels of photorespiration during these periods, that is, low CO_2 concentrations and/or high O_2 concentrations are named as key drivers of C_4 emergence (Sage 2004; Sage, Sage, and Kocacinar 2012). RuBP oxygenation was negligible throughout most of Earth history (Sage 1999). However, decreasing CO_2 concentrations during, for example, the Eocene–Oligocene-Transition very likely decreased the carboxylation capacity of C_3 photosynthesis and increased the likelihood of photorespiration to have happened via RuBP oxygenation (Busch et al. 2013). Competition between oxygenase and carboxylase activity under low CO_2 concentrations strongly increases energetic costs of carbon assimilation and, thus, very likely created extreme environments for C_3 plants. This might have put intense selection pressure on C_3 photosynthesis. Based on our observations, we argue that these low CO_2 environments might have favoured adaptations in carboxylation capacity not only via the evolution of C_4 photosynthesis but also within the C_3 evolutionary trajectory and might have facilitated species speciation and diversification in C_3 plants similar to the diversification of C_4 plants (cf. Busch et al. 2013). Despite the apparent shift in the trajectory of C_3 plant evolution about 30 million years ago, we observed a fundamental relationship between photosynthetic capacity and rates of species diversification and speciation covering the entire 80 Myr of C_3 plant evolution. Based on these observations, we argue for a close link between the capacity of C_3 photosynthesis and the evolutionary dynamics in C_3 plants with carboxylation capacity as well as evolutionary dynamics seem to have been facilitated in during periods of low atmospheric CO_2 in recent prehistory, that is, in the last 30 Myr.

In our analyses, J_{max} turned out to be phylogenetically more conserved, that is, showed a significantly higher phylogenetic signal than V_{cmax}. This higher degree of phylogenetic conservatism might be related to the fact, that carboxylation directly depends on energy supply via the electron transport chain. This might make J_{max} more important to be phylogenetically conserved. Based on these observations, we hypothesise a higher adaptive capacity of V_{cmax} compared to J_{max}. Evolutionary adaptation of C_3 plants to changing CO_2 concentration in prehistory might therefore not have happened via adjustments of the electron transport chain but by enzymatic down- or upregulation of the carboxylation process. However, both V_{cmax} and J_{max} showed high and significant phylogenetic signals in our analyses—a fact that might indicate overall high degrees of phylogenetic conservatism for these important regulatory traits of C_3 photosynthesis. This high degree of phylogenetic conservatism might be related to the limited adaptive capacity of RuBisCO. The evolution of RuBisCO has been shown to be strongly constrained by trade-offs between the enzyme's activity and its stability. Studer et al. (2014) were able to show that mutations favouring the activity of RuBisCO are destabilising its chemical structure. These destabilising mutations were most often followed by evolutionary periods in which stabilising mutations were predominant, preserving enzyme stability but hampering the adaptive capacity to increase activity, thus, carboxylation capacity. Based on this lack of adaptive capacity of RuBisCO, other mechanisms and/or components in the process of carboxylation must have been more adaptive to explain the close links between the phylogenetic dynamics in photosynthetic capacity and species diversification/speciation, which we observed in our study. Such mechanisms are detrimental to maintain the fitness of C_3 photosynthesis in low CO_2 environments but are also of high importance under increasing CO_2 concentrations.

C_3 plants have long been thought to lack mechanisms to compensate productivity losses caused by, for example, photorespiration in low CO_2 environments. However, several recent phylogenetic studies provide evidence for an evolutionary fine tuning of RuBisCO as well as for other forms of carbon concentration mechanisms acting in C_3 plants (Kapralov and Filatov 2007; Wang, Kapralov, and Anisimova 2011). Recent investigations demonstrate that C_3 plants such as rice and wheat are capable to trap and reassimilate photorespired CO_2 (Busch et al. 2013). This reassimilation is estimated to account for up to 50% of the net carbon gain in wheat (Pärnik and Keerberg 1995). Such carbon concentration mechanisms (CCMs) are assumed to explain the persistence of C_3 plants under low CO_2 conditions in Earth history even under high competition with plants being equipped with the more effective C_4 pathway (Busch et al. 2013). Li et al. (2014) demonstrated that experimental exposure to low CO_2 concentrations (i.e., 100 ppm for 42 days) initiated the upregulation of PEPC and PEPC-K gene expression, altered expression of transcription factors related to photosynthesis development and downregulated the light-harvesting and thylakoid protein complexes in the C_3 plant *Arabidopsis thaliana*. This again highlights the high adaptive capacity of C_3 plants to low CO_2 environments. This adaptive capacity might have emerged in C_3 plants in Earth history during periods with exceptionally low CO_2 concentrations. However, in-depth investigations on the evolutionary history of CCMs in C_3 plants are necessary to thoroughly assess these assumptions.

Generally, we observed strong links between photosynthetic capacity and the evolutionary dynamics in C_3 plants during the last 80 Myr. We see visible evidence for low CO_2 environments during prehistory having been a strong force of adaptations within the C_3 evolutionary trajectory that might have facilitated species speciation and diversification in C_3 plants similar to the diversification

of C_4 plants. Selective forcing of temperature during low CO_2 periods seems less likely as low temperatures coupled with low CO_2 concentration might have favoured rather than limited carboxylation. However, the role of temperature cannot be entirely ruled out due to a strong coupling of prehistoric CO_2 and temperature fluctuations (CENCO2PIP Consortium 2023). Our analyses are based on reconstructions of prehistoric CO_2 concentrations and of the phylogenetic history (i.e., speciation events) as well as on contemporary, species-specific, empirical data on functional traits such as V_{cmax}, J_{max}, leaf P and N contents and specific leaf area. All these data sources are subject to biases which might have affected the results of our study. Empirical estimations of V_{cmax} and J_{max} are well known to differ between genotypes of single species or even within single plant individuals depending on their acclimation to the growth environment including the nutritional and water status of the measured plants and the environmental factors set for the gas exchange measurements. We tried to account for these biases as good as possible by including uncertainty of the phylogenetic and CO_2 reconstruction and intraspecific trait variation in the analyses and by accounting for temperature differences during measurements in the V_{cmax} and J_{max} estimations. Our analyses are furthermore based on median values of multiple species per time interval and thus should be not affected by single species outliers which might originate, for example, from unreliable estimates of V_{cmax} and J_{max} values. All this in combination with the strong relationships, we observed for the key aspects of our study make us confident about the significant links between photosynthetic capacity and the diversification of C_3 plants. Generally, we conclude that current and future increases in atmospheric CO_2 concentrations might rather hinder than facilitate evolutionary dynamics in C_3 plants and, by this diminish the adaptive capacity of a major share of terrestrial primary productivity to ongoing CO_2 emissions. Nevertheless, parts of our discussion on the evolutionary drivers and trajectories of C_3 photosynthesis are speculative and need to be rigorously tested in follow-up studies.

Author Contributions

A.H.S. and J.M.-I.S. jointly developed the idea for this study; A.H.S. performed the analyses and led the writing with major input from J.M.-I.S.

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Data Availability Statement

All data used in this study is freely available. References of data sources are provided in the text. All scripts and data environments are available on Zenodo via https://zenodo.org/records/11547361.

Peer Review

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.