Research paper

Potassium supply modulates *Eucalyptus* leaf water-status under PEG-induced osmotic stress: integrating leaf gas exchange, carbon and nitrogen isotopic composition and plant growth

Nikolas de Souza Mateus[1,6], Eric Victor Oliveira Ferreira[2], Antonio Leite Florentino[3], Alexandre Vicente Ferraz[4], Jean-Christophe Domec[5], Lionel Jordan-Meille[5], José Albertino Bendassolli[1], José Leonardo Moraes Gonçalves[3] and José Lavres[1]

[1]Center for Nuclear Energy in Agriculture, University of São Paulo, São Paulo 13400-970, Brazil; [2]Amazon Rural Federal University, Pará 68650-000, Brazil; [3]University of São Paulo, Luiz de Queiroz College of Agriculture, São Paulo 13418-900, Brazil; [4]Forest Science and Research Institute, IPEF, São Paulo 13415-000, Brazil; [5]ISPA Bordeaux Sciences Agro UMR 1391, INRA, 33140 Villenave d'Ornon, France; [6]Corresponding author (nikolas.mateus@usp.br)

The objective of this study was to quantify the effect of potassium (K) supply on osmotic adjustment and drought avoidance mechanisms of *Eucalyptus* seedlings growing under short-term water stress. The effects of K supply on plant growth, nutritional status, leaf gas exchange parameters, leaf water potential (Ψ_w), leaf area (LA), stomatal density (SD), leaf carbon (C) and nitrogen (N) isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$ ‰) and leaf C/N ratio under polyethylene glycol (PEG)-induced water deficit were measured. Under both control (non-PEG) and osmotic stress (+PEG) conditions, K supply increased plant growth, boosting dry matter yield with decreased C/N leaf ratio and $\delta^{15}N$ ‰ values. The +PEG significantly reduced LA, plant growth, dry matter yield, Ψ_w, number of stomata per plant and leaf gas exchange, relative to non-PEG condition. Potassium supply alleviated osmotic-induced alterations in *Eucalyptus* seedlings by better regulating leaf development as well as SD, thus improving the rate of leaf gas exchange parameters, mesophyll conductance to CO_2 (lower $\delta^{13}C$ ‰ values) and water use efficiency (WUE). Consequently, K-supplied plants under drought better acclimated to osmotic stress than K-deficient plants, which in turn induced lower CO_2 assimilation and dry matter yield, as well as higher leaf $\delta^{13}C$ ‰ and $\delta^{15}N$ ‰ values. In conclusion, management practices should seek to optimize K-nutrition to improve WUE, photosynthesis-related parameters and plant growth under water deficit conditions.

Keywords: carbon and nitrogen leaf isotopes, K-nutrition, leaf gas exchange parameters, water deficit.

Introduction

In Brazil, *Eucalyptus* is the most planted tree species, and predominantly on non-arable and unfertile dry lands (Stape et al. 2004). In those planted forests, water supply and potassium (K) are the co-limiting growing factors, in particular on highly degraded tropical soils (Battie Laclau et al. 2016, Cakmak 2010, Sardans and Peñuelas 2015, Zörb et al. 2014). As a consequence, fertilization is one of the most important management practices, enabling yield gains of up to 50% (Gonçalves 2011).

As climate is changing (IPCC 2013), future biomass production of those *Eucalyptus* plantations will even be more negatively affected in areas already limited by water and nutrient availability (Booth 2013, Silva et al. 2020). Drought is an increasingly common climatic event that can dramatically disturb ecosystems as well as surrounding agricultural and forestry sites (Law 2019). Plants respond to water stress by osmotic regulation to avoid turgor loss and by reducing stomatal conductance (g_s) to prevent excessive water loss and too negative leaf water potential (Ψ_w). Those physiological adjustments then

lower internal CO_2 concentration and net carbon assimilation (photosynthesis), thus impairing *Eucalyptus* growth by up to 40% (Santos et al. 2020).

Potassium is an essential element playing a key role in regulating tree metabolism and growth in forest plantations (Asensio et al. 2020, Christina et al. 2015). This nutrient is involved in cell osmotic control, sucrose phloem transport, enzymatic activation and protein synthesis, cytosolic pH control and CO_2 assimilation (Anschütz et al. 2014, Drosdoff et al. 1947, Marschner 2012, Mateus et al. 2019). Specifically, K starvation partially inhibits water stress-induced stomatal closure, reduces biomass accumulation and carbon partitioning (Gerardeaux et al. 2009, Jordan-Meille et al. 2018, Makhdum et al. 2007, Martineau et al. 2017) and alters numerous metabolic processes involving carbon (C) and nitrogen (N) metabolism (Dong et al. 2004, Hu et al. 2016). Changes in energy levels raised by photosynthetic electron transport have also been observed (Fait et al. 2011), since C and N metabolism share organic carbon, CO_2 fixation or respiration (Huppe and Turpin 1994). Adequate K supply provides the necessary driving force for water influx into guard cell vacuoles and cell turgor maintenance under osmotic stress, thus regulating stomata opening and limiting leaf transpiration (Oddo et al. 2020, Peiter 2011). Numerous studies have shown that K application mitigates the adverse effects of long-term drought on plant growth (Asensio et al. 2020, Gonçalves et al. 2017). However, the importance in photosynthetic processes and the multiple physiological mechanisms modulated by K are subject of debate (Battie Laclau et al. 2014, Egilla et al. 2005), highlighting the importance of further investigation of K in water deficit stress alleviation in *Eucalyptus*.

The stable isotope composition of C and N ($\delta^{13}C$ and $\delta^{15}N$) can be used as an indicator of environmental (Kohzu et al. 1999) and drought stress (Lauteri et al. 1993, Robinson et al. 2000). Specifically, $\delta^{13}C$ is a good predictor of water use efficiency (WUE; Mateus et al. 2019, 2021). As a consequence of water shortage, the increase in leaf ^{13}C abundance is usually associated with a decrease in g_s and an increase in WUE (Farquhar et al. 1989). Similarly, leaf $\delta^{15}N$ can act as a potential indicator of N metabolism and adequate growing conditions (Serret et al. 2018, Spangenberg et al. 2021, Yousfi et al. 2012), where a better ability for plants to accumulate N is indicated by more negative $\delta^{15}N$ values (Robinson 2001). Even though the mechanisms underlying environmental relationships between $\delta^{15}N$ and biomass are not fully elucidated (Yousfi et al. 2010), $\delta^{15}N$ can be used as plant performance screening tool (Yousfi et al. 2009). In addition, the combination of $\delta^{13}C$ and $\delta^{15}N$ assessment can provide a comprehensive understanding of the impact of K application on C-N interaction and plant response to water deficit.

Even though the role of K on tree growth and metabolism is well-known, less is known on the effect of K supply on drought adjustment and drought-avoidance mechanisms on nutrient-demanding and fast-growing tree species. This work aimed to evaluate the role of K nutrition in limiting the effect of osmotic stress on leaf gas exchange, carbon assimilation and WUE in *Eucalyptus* seedlings. In addition, the study also aimed at providing new insights on the use of $\delta^{13}C$, $\delta^{15}N$ and leaf C/N ratio as metabolic screening tools between plants growing in well-watered conditions and under polyethylene glycol (PEG)-induced osmotic stress. We hypothesized that (i) osmotic-induced stress inhibits plant development independently of K supply, but that (ii) the optimized K-nutrition can potentially modulate leaf-structural features, physiological mechanisms and C and N metabolism by upregulating photosynthesis-related parameters and WUE, thus alleviating stress-induced impacts on plant growth.

Material and methods

Experimental design and growth conditions

The experiment was carried out in greenhouse conditions at the Center for Nuclear Energy in Agriculture at University of São Paulo (Piracicaba, Brazil), using *Eucalyptus* clones (*Eucalyptus urophylla* × *Eucalyptus grandis* hybrid) that were 90 days old and 30 cm in height. The mean air temperature and relative humidity in the greenhouse were 28.0 °C and 65%, respectively. The experiment setup represented a complete randomized block design, with four replicates in a 2 × 5 factorial combination: two levels of osmotic conditions and five K levels (totaling 40 experimental units). Prior to the experiment, the substrate was removed from the roots with tap water, and the plants were repotted to a collective tray (10 L) containing Clark's nutrient solution (Clark 1975). Plants were grown for 30 days, which represented the acclimatization stage of the aerated nutrient solution whose concentration gradually increased weekly from 25, 50, 75 and to 100% to avoid osmotic shock. At the end of the acclimatization stage, homogeneous plants were selected according to height and collar diameter and transferred to individual plastic pots (3 L); treatments were applied over a period of 60 days.

The fertilization treatments consisted of five K application levels (0, 0.90, 1.80, 2.70 and 3.60 mmol L^{-1} of K) using potassium chloride (KCl). Since 1.80 mmol L^{-1} of K represents the original level of K in Clark's nutrient solution, the lower applied K level (0 mmol L^{-1}) indicated the highest K deficiency stress treatment, while the levels from 1.80 to 3.60 mmol L^{-1} represented K-sufficient treatments (Mateus et al. 2019, 2021). Two osmotic conditions were used: well-watered (non-PEG) treatment (water potential of −0.05 MPa in nutrient solution) and osmotic stress (+PEG) treatment (water potential of −1 MPa in nutrient solution), which was induced by polyethylene glycol 6000 (PEG 6000) to simulate drought induced-stress. Approximately 28 days after the onset of treatments, PEG 6000 was added every 7 days and for 49 days to gradually reduce the water potential of the solutions to −0.15 MPa (100 g L^{-1}), −0.65 MPa (200 g L^{-1})

Table 1. Mean values (± standard errors; $n = 4$) of LA, SLA, SD and the total number of stomata per plant of *Eucalyptus* seedlings submitted to five potassium (K) levels and grown under controlled (non-PEG condition; −PEG) or under PEG-induced osmotic stress (+PEG)

K Rates (mmol L⁻¹)	LA (m² plant⁻¹)[*,**,***]		SLA (m² kg⁻¹)[*,**,***]		SD (stomates mm⁻²)		Stomates (10⁷ plant⁻¹)[*]	
	non-PEG[w.a]	+PEG[n.s]	non-PEG[w.a]	+PEG[n.s]	non-PEG[n.s]	+PEG[n.s]	WW[w.a]	WS[n.s]
0.0	0.12 ± 0.01b	0.06 ± 0.01b	15.31 ± 0.66b	9.25 ± 1.03a	438.47 ± 43.9a	400 ± 25.7a	54.59 ± 8.7a	23.29 ± 8.3a
0.9	0.57 ± 0.03a	0.09 ± 0.02ab	19.93 ± 0.78a	9.06 ± 0.90a	413.55 ± 42.6a	442.15 ± 9.5a	193.28 ± 28.6a	42.29 ± 13.3a
1.8	0.42 ± 0.08a	0.06 ± 0.01b	20.55 ± 1.34a	7.92 ± 1.11a	313.96 ± 35.3a	333.62 ± 32.7a	150.61 ± 32.7a	13.08 ± 4.1a
2.7	0.40 ± 0.04a	0.05 ± 0.00b	19.39 ± 0.65ab	8.28 ± 1.03a	379.14 ± 40.5a	340.42 ± 26.5a	157.62 ± 62.6a	43.07 ± 1.5a
3.6	0.48 ± 0.05a	0.13 ± 0.02a	21.68 ± 0.46a	8.91 ± 1.25a	438.99 ± 18.7a	340.45 ± 15.1a	172.53 ± 58.2a	48.53 ± 5.5a
Models/Means	0.38	0.09	19.37	8.84	396.82	371.32	145.72	34.05
R^2	0.86	0.86	0.91	0.91	0.43	0.43	0.68	0.68

For each parameter *, ** and *** indicate the statistical influence (F test with a significance threshold of $P < 0.05$) of +PEG, K levels and +PEG × K interaction, respectively. The adjustment model is indicated by not significant (n.s) and without suitable adjustment (w.a). Within a column, different letters indicate significant differences between K rates according to Tukey test ($P < 0.05$).

and −1.0 MPa (250 g L⁻¹) (Michel and Kaufmann 1973, Tavakol et al. 2018).

Leaf gas exchange parameters and leaf water potential

Before harvesting seedlings for assessing plant biomass, the youngest and fully expanded leaf of each plant was used to measure gas exchange (between 7 a.m. and 11 a.m.) with a Li-Cor 6400XT (Li-Cor, Inc., Lincoln, NE, USA). Cuvette temperature was set to 25 °C and relative humidity to 65% maintaining vapor pressure deficit in the cuvette at around 1.1 kPa. The CO_2 concentration was kept at 380 ppm throughout the measurements. A LED array provided a photosynthetic photon flux density of 1200 μmol m⁻² s⁻¹. Leaf photosynthesis (A), stomatal conductance (g_s) and transpiration (E) were measured and averaged over 5–10 minutes after values had stabilized (Mateus et al. 2019). Instantaneous leaf WUE was then calculated by dividing values of A by E. Leaf water potential (Ψ_w) was measured weekly (12 p.m.) on 2 leaves per plant using a Scholander pressure chamber (PMS Instrument Company, Albany, OR, USA) (Turner 1981).

Stomatal density and leaf area

Four leaves per plant were collected in the middle section of the leafy stem (one on each side) for stomatal density (SD) evaluation (number of stomates per mm⁻²) by printing the abaxial leaf surface on a glass microslide. The sampled foliar region was pressed for 10 s against a drop of superglue on the glass microslide. Then, using an optical microscope mounted with a digital camera, the leaves were imaged at 100× magnification using four random fields of 0.116 mm² each. The ImageJ® program with 150× zoom was used to count the stomata and determine SD (Mateus et al. 2019). Ninety days after the treatments were applied, the plants were harvested and their leaves, stems, branches and roots were separated. Surface area of newly expanded and mature leaves (leaf area, LA) was measured using a digital area meter (LICOR® LI-3100, Li-Cor Inc., Lincoln, NE, United States).

Elementary and isotopic analyses

Roots and leaves were ground using a Wiley mill to quantify K concentrations (Malavolta et al. 1997), isotope composition ($\delta^{13}C$ and $\delta^{15}N$) and leaf C/N ratio. The isotope composition and total leaf C and N of the samples were determined using a mass spectrometer (ANCA-GSL Hydra 20–20 model, SERCON Co., Crewe, GBR) coupled to a C-automatic analyzer (Barrie and Prosser 1996). The isotope values (‰) were calculated by Eq. 1 described by Farquhar et al. (1982)

$$\delta\,(\text{‰}) = \left(R_{(sample)} / R_{(standard)} - 1\right) \times 1000, \qquad (1)$$

where R is the isotope ratio of ¹³C/¹²C or ¹⁵N/¹⁴N. The $\delta^{13}C$ results were reported relative to the VPDB standard ($\delta a = -8$‰), and $\delta^{15}N$ is relative to the standard atmospheric

Table 2. Mean values ($\pm$ standard errors; $n = 4$) of height and collar diameter of *Eucalyptus* seedlings submitted to five potassium (K) levels and grown under controlled (non-PEG condition; −PEG) or under PEG-induced osmotic stress (+PEG)

Growth				
K rates (mmol L^{-1})	Height (cm plant^{-1})[*],[**]		Diameter (mm plant^{-1})[*]	
	non-PEG[w.a]	+PEG[n.s]	non-PEG[w.a]	+PEG[n.s]
0	49 $\pm$ 0.58a	40.0 $\pm$ 1.00b	7.5 $\pm$ 0.87a	6.65 $\pm$ 0.29a
0.9	51.9 $\pm$ 3.06a	41.0 $\pm$ 2.29ab	9.4 $\pm$ 1.57a	7.40 $\pm$ 0.70a
1.8	53 $\pm$ 3.14a	49.5 $\pm$ 0.96a	12.5 $\pm$ 1.18a	6.95 $\pm$ 1.17a
2.7	55.6 $\pm$ 3.89a	47.5 $\pm$ 1.06ab	9.1 $\pm$ 1.10a	5.90 $\pm$ 0.90a
3.6	56.1 $\pm$ 3.62a	48 $\pm$ 1.50ab	8.6 $\pm$ 1.30a	6.53 $\pm$ 0.24a
Models/Means	52.87	45.33	9.16	6.69
R^2	0.69	0.69	0.67	0.67

For each parameter *, ** and *** indicate the statistical influence (F test with a significance threshold of $P < 0.05$) of +PEG, K levels and +PEG $\times$ K interaction, respectively. The adjustment model is indicated by not significant (n.s) and without suitable adjustment (w.a). Within a column, different letters indicate significant differences between K rates according to Tukey test ($P < 0.05$).

N$_2$ (δ^{15}N = 0‰). The leaf C/N ratio was then calculated by dividing values of C by N.

Plant growth and dry matter production

A graduated ruler (in cm) and a digital pachymeter (in mm) were used to measure the weekly growth in height and root collar diameter, respectively. At the end of the experiments, seedlings were separated into leaves, stems, branches and roots. Dry matter of each plant organ was determined after oven-drying at 65 °C until constant weight.

Statistical analysis

The data were analyzed by the F test ($P < 0.05$), and the significant differences among means were submitted to post hoc multiple comparisons using Tukey's test ($P < 0.05$) to compare WW and WS treatments. The effects of K supply, when significant, were described by linear, quadratic and square root regression models, and the highest determination coefficient (R^2) was selected. The original data were standardized to achieve zero mean and one variance to be analyzed in Pearson's Correlation ($P < 0.05$) and multivariate statistics, with Heatmap-Clustering and principal components analysis (PCA). A heat map was produced calculating the Euclidian distance of similarity among treatments and dependent variables. PCA was used to characterize the effect of K fertilization on the structural and physiological parameters, and confidence ellipses with 95% of confidence were used to visualize the multivariate trends of treatments with WW and WS. Statistical tests were performed using SAS version 9.3 (SAS Institute Inc. 2004) and R version 3.5.1 (R Development Core Team 2008).

Results

LA, specific leaf area and SD

Total LA of *Eucalyptus* seedlings was strongly affected by K supply and osmotic treatments (Table 1). The LA values of K-supplied plants (from 0.9 to 3.6 mmol L^{-1} of K) were ~277

and 50% higher than those of K-deficient plants (0 mmol L^{-1} of K) in both non-PEG and +PEG treatments, respectively (Table 1). The PEG-induced osmotic stress decreased the LA of K-supplied and K-deficient plants by up to 82 and 50%, respectively. Potassium application increased specific leaf area (SLA) by 42% in non-PEG and reduced it by 15% in +PEG conditions (Table 1), relative to K-deficient plants.

Irrespective of K supply, SD was not affected by osmotic stress, but the total number of stomata per plant was 7% higher in treatments with no osmotic stress (−PEG) relative to +PEG treatments (Table 1).

Plant growth and dry matter production

Compared with K-deficient plants, K fertilization increased plant height by 15 and 20% in non-PEG and +PEG treatments, respectively. Moreover, the induced osmotic stress reduced the height of K-sufficient and K-deficient plants by 15 and 20%, respectively. Osmotic stress reduced overall root collar diameter by 27%. Despite being non-significant, higher K concentrations increased collar diameter by up to 60% in the non-PEG treatment (Table 2).

Plant dry matter was influenced by K supply and by osmotic stress (Table 3). Root and shoot dry matter production increased with K supply, notably under non-PEG condition. Besides, osmotic-induced stress reduced root and shoot dry matter production by 30% compared with the non-PEG treatment. The induced osmotic stress increased the root/shoot ratio by 50%, compared with non-PEG treatment. Compared with K-deficient plants, K supply increased the total plant dry matter by 140 and 40% under non-PEG and +PEG treatments, respectively.

Leaf gas exchange and leaf water potential

Under non-PEG condition, K application increased A, E, g_s and WUE by 55, 40, 100 and 43%, respectively (Figure 1). Moreover, under +PEG condition, K-supplied plants also showed an increase in A (85%), E (50%), g_s (400%) and WUE (20%)

Table 3. Root and shoot dry matter yield, root/shoot ratio and total dry matter production of *Eucalyptus* seedlings submitted to five potassium (K) levels and grown under controlled (non-PEG condition; −PEG) or under PEG-induced osmotic stress (+PEG)

Dry matter production (g plant^{-1}) *, **, ***							
K rates (mmol L^{-1})	Root		Shoot		Root/Shoot Ratio		Total	
	non-PEG$^{w.a}$	+PEG	non-PEG$^{w.a}$	+PEG$^{n.s}$	non-PEG	+PEG$^{w.a}$	non-PEG$^{w.a}$	+PEG$^{n.s}$
0	4.38 ± 0.49b	4.43 ± 0.38a	12.83 ± 1.3b	10.18 ± 0.7a	0.33 ± 0.02a	0.43 ± 0.01ab	17.22 ± 1.8b	14.62 ± 1.1a
0.9	9.78 ± 0.17a	5.38 ± 0.84a	36.85 ± 1.6a	15.20 ± 2.9a	0.26 ± 0.01a	0.36 ± 0.04ab	46.64 ± 1.5a	20.59 ± 3.9a
1.8	8.57 ± 0.51ab	4.82 ± 0.41a	32.60 ± 4.7a	14.76 ± 0.2a	0.25 ± 0.01a	0.37 ± 0.06ab	41.17 ± 0.5a	19.58 ± 2.5a
2.7	6.84 ± 0.85ab	5.33 ± 0.69a	28.94 ± 3.5a	10.38 ± 1.0a	0.23 ± 0.01a	0.52 ± 0.02a	35.79 ± 4.2a	15.71 ± 1.7a
3.6	9.44 ± 2.04ab	7.64 ± 0.65a	32.78 ± 3.6a	19.24 ± 1.2a	0.28 ± 0.03a	0.35 ± 0.03b	42.22 ± 5.3a	26.88 ± 1.1a
Models/Means	7.8	$y = 3.51 + 0.71x$	28.8	19.95	$y = 0.33 - 0.08x + 0.018x^2$	0.41	36.6	19.47
R^2	0.83	0.78	0.93	0.93	0.94	0.77	0.93	0.93

For each parameter *, ** and *** indicate the statistical influence (*F* test with a significance threshold of $P < 0.05$) of +PEG, K levels and +PEG × K interaction, respectively. The adjustment model is indicated by not significant (n.s) and without suitable adjustment (w.a). Within a column, different letters indicate significant differences between K rates according to Tukey test ($P < 0.05$).

relative to K-deficient plants. Overall, the induced osmotic stress was more responsive in K-deficient plants, reducing *A* (66%), *E* (70%) and g_s (85%), and increasing WUE (30%), while osmotic stress decreased *A* (55%), *E* (65%), g_s (65%) and increased WUE (10%) in K-supplied plants.

Leaf water potential (Ψ_w) of K-deficient plants growing under non-PEG condition (low osmotic stress of −0.05 MPa) varied from −0.8 to −1.8 MPa (Figure 2a), with similar values observed for K supplied plants. Overall, Ψ_w of K-deficient plants under osmotic stress showed higher Ψ_w values (close to zero) relative to plants supplied with K (Figure 2b). Moreover, under osmotic stress, the highest K level (3.6 mmol L^{-1} of K) induced the lowest Ψ_w values, which were kept constant up to the 35th day after the start of the experiment, before declining progressively (Figure 2b).

Potassium concentrations in plant tissue and isotopic analyses

Root K-concentrations were influenced by K supply and by the osmotic condition (Figure 3a). Plants grown in non-PEG conditions showed increased root K content as a result of higher K application rates. Conversely, K supply had no effect on plant root concentrations in +PEG treatment, with values 54% lower than for plants grown in non-PEG treatment. On the other hand, leaf K concentration was affected by the interactive effects of K rates and osmotic condition treatments (Figure 3b). Plants supplied with K showed a leaf K concentration of 27 g kg^{-1} in either non-PEG or +PEG conditions. Moreover, K rates and osmotic condition treatments had significant effects on the δ^{13}C, δ^{15}N and the leaf C/N ratio values. Leaf carbon isotopic values (δ^{13}C) were less negative in K-supplied plants subjected to osmotic stress when compared with healthy K plants without PEG in the solution (Figure 3c). Leaf nitrogen isotopic values (δ^{15}N ‰) decreased with increasing K levels in the nutrient solution, and these values were more negative for plants exposed to osmotic stress. The leaf C/N ratio decreased with higher K rates in the +PEG treatment (Figure 3e). On the other hand, plants under non-PEG conditions showed an average C/N value of 15.4, which decreased by 14% at a K concentration of 1.8 mmol L^{-1}. In addition, K supply led to substantial variation in isotopic abundances. Leaf K concentration was positively correlated to δ^{13} ($P < 0.01$) and negatively to δ^{15}N ($P < 0.01$) (Figure 3f).

Pearson correlations and multivariate analyses: morpho-physiological variables, isotopic and nutritional responses

There was a positive correlation among the morpho-physiological variables (*A*, g_s, e *E*, Std, LA and TDM) and a negative correlation between the isotopic values (δ^{13}C and δ^{15}N) (Figure 4a). Tissue K concentration was negatively correlated with δ^{15}N. The [C/N] ratio presented a negative correlation with the morpho-physiological variables, except for Ψ_w, which did not correlate
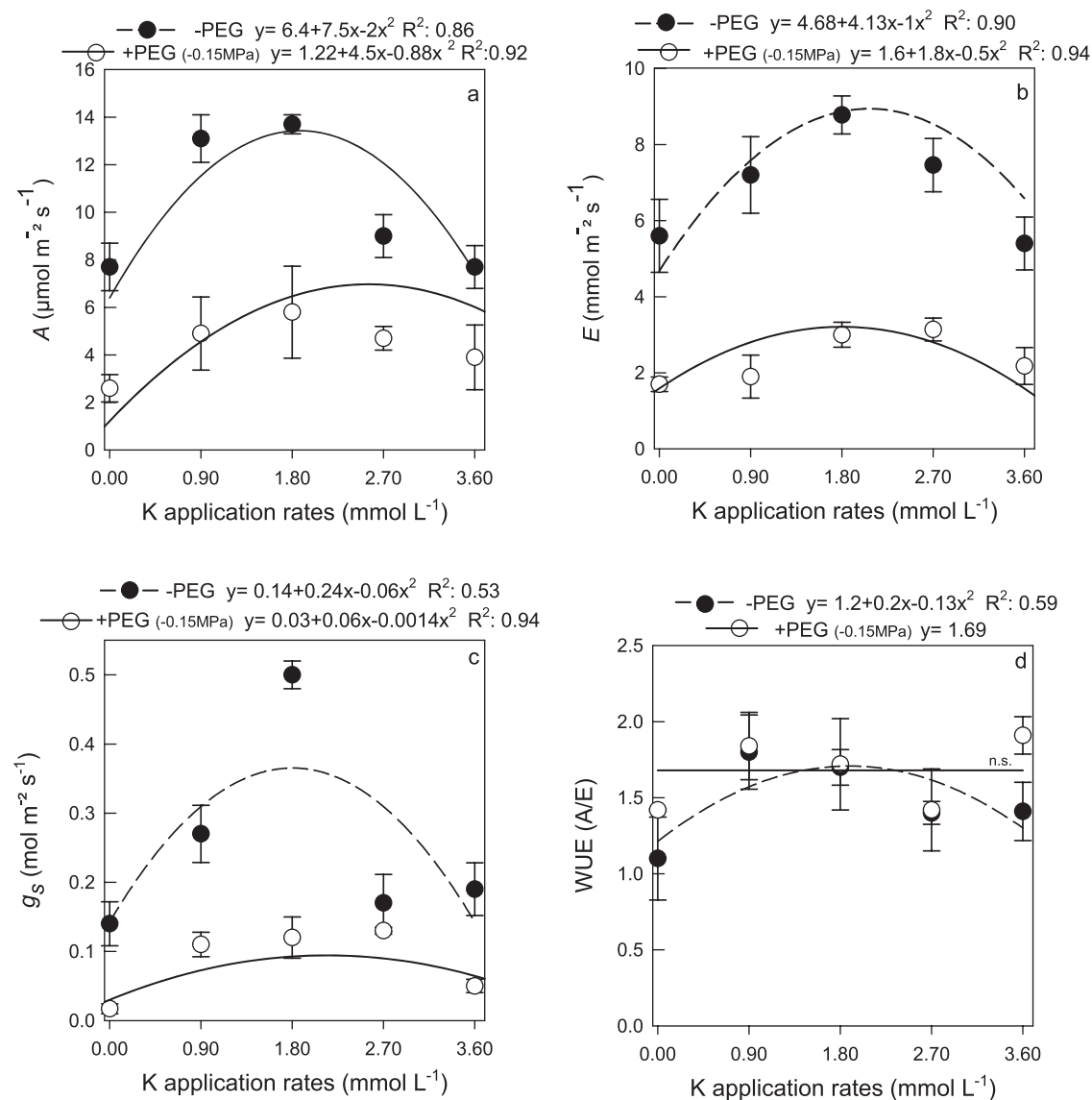
Figure 1. Leaf gas exchange parameters of *Eucalyptus* seedlings grown under five potassium (K) fertilization rates, and under non-PEG (−PEG) and osmotic induced-stress (+PEG) conditions: (a) CO_2 assimilation rate, (b) stomatal conductance−g_s, (c) leaf transpiration (E) and (d) WUE. Vertical bars indicate standard errors between blocks ($n = 4$). The adjustment model is indicated by not significant (n.s) and without suitable adjustment (w.a). *F* test, respectively, (a) +PEG ($P < 0.05$), K rate ($P < 0.05$), K* + PEG ($P > 0.05$); (b) +PEG ($P < 0.05$), K rate ($P > 0.05$), K* + PEG ($P > 0.05$); (c) +PEG ($P < 0.05$), K rate ($P > 0.05$), K* + PEG ($P > 0.05$); (d) +PEG ($P > 0.05$), K rate ($P < 0.05$), K* + PEG ($P > 0.05$).

with any other variables. Taking 95% of the confidence ellipse as indicative of significant correlation among variables, we observed a negative correlation among the mean vectors of variables under non-PEG and +PEG treatments, with a low correlation between the two osmotic conditions and a higher variance in the non-PEG treatment (Figure 4b).

Heatmap and hierarchical cluster analysis showed the formation of three main groups among the treatments (columns) and variables (lines). For treatments, Group 1 was represented by plants supplied with K and grown under non-PEG condition, while Group 2 consisted of plants without K supply, regardless of the osmotic condition. Group 3 was represented by

K-supplied plants subjected to osmotic stress (Figure 4c). For variables, Group 1 was represented by the [C/N] ratio and $\delta^{15}N$, Group 2 by $\delta^{13}C$ and [K] and Group 3 by morpho-physiological variables (Figure 4c).

More than 80% of the total variance was explained by the main principal components (PC1 + PC2) (Figure 4b and d), with PC1 being driven by the morpho-physiological variables (A, g_s, E, Std, LA and TDM) and PC2 by the isotopic parameters ($\delta^{13}C$ and $\delta^{15}N$) and K-tissue concentrations (Figure 4d). The variables Ψ_w, WUE and [C/N] ratio contributed with average weights to explain the data variance in PC1 and PC2. Under non-PEG conditions, increasing K rates from 0.9 to 3.6 mmol L⁻¹
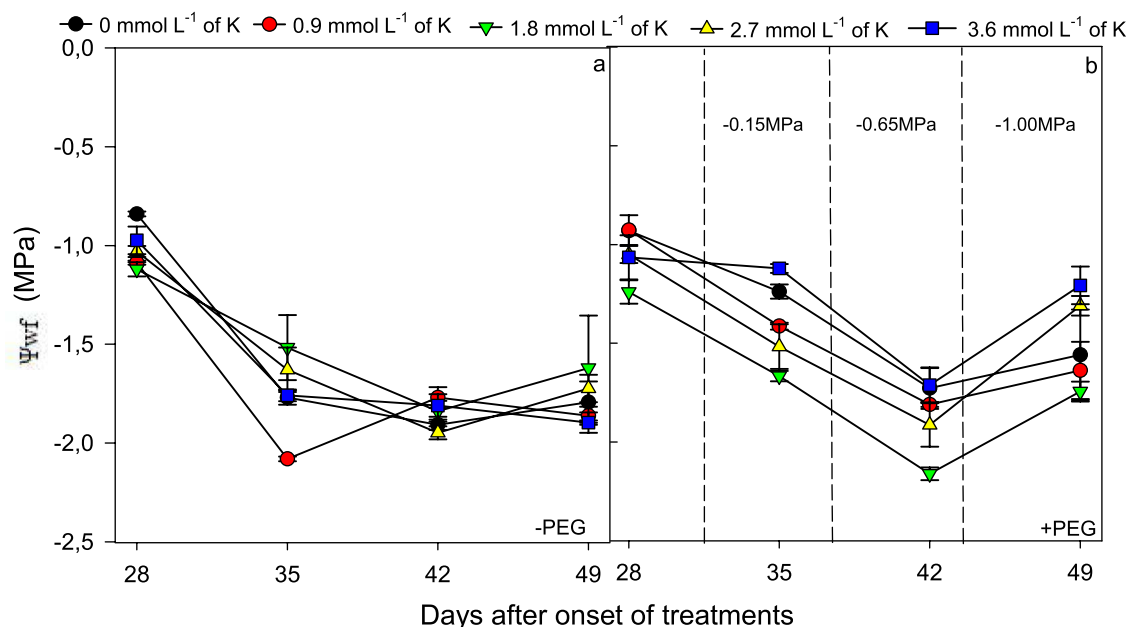
Figure 2. Leaf water potential (Ψw) of *Eucalyptus* seedlings submitted to five levels of potassium (K) and grown under non-PEG (−PEG) condition at an osmotic potential of −0.05 MPa (a) and under osmotic induced-stress (+PEG) at osmotic potentials of −0.15, −0.65 and −1 MPa (b). Vertical bars indicate standard errors between blocks ($n = 4$). F test on each day after onset of treatments: 28 days +PEG ($P > 0.05$), K rate ($P < 0.05$), K* + PEG ($P > 0.05$); 35 days + PEG ($P < 0.05$), K rate ($P < 0.05$), K* + PEG ($P < 0.05$); 42 days +PEG ($P > 0.05$), K rate ($P < 0.05$), K* + PEG ($P > 0.05$); 49 days +PEG ($P < 0.05$), K rate ($P < 0.05$), K* + PEG ($P < 0.05$).

resulted in higher values of morpho-physiological variables and a lower [C/N] ratio. Under +PEG condition, K levels from 0.9 to 3.6 mmol L^{-1} also showed higher δ^{13}C and K-tissue concentrations and lower Ψ_w and δ^{15}N values (Figure 4d). Plants fertilized with low K concentrations and grown in non-PEG and +PEG conditions were characterized by high δ^{15}N levels and a high [C/N] ratio, as well as by low K tissue concentrations and low values of morpho-physiological variables (Figure 4d).

Discussion

Osmotic stress-induced reduction in *Eucalyptus* growth has also been observed in other studies (Christina et al. 2015, Silva et al. 2016), even in K-fertilized plants (Epron et al. 2015, Tavakol et al. 2018). As observed in our study, K supply altered structural and physiological responses of *Eucalyptus* seedlings. Overall, plants growth was higher under control conditions (non-PEG) relative to osmotic-induced water stress (+PEG). These assumptions can be confirmed by the greater dry matter production (non-PEG: 140%; +PEG: 40%) and LA (non-PEG: 270%; +PEG: 50%) observed in K-supplied, relative to K-deficient plants.

The K supply also alleviated the negative impacts of osmotic stress by partially closing stomata to minimize water loss, as also observed by Battie Laclau et al. (2016). Stomatal aperture and closure are modulated by CO_2 assimilation and water loss in guard cells, altering turgor pressure consequently affecting leaf development, SLA and *A* (Battie Laclau et al.

2013, Schulze et al. 2006). Coordinated mechanisms between stomatal closure (Teixeira et al. 2008) and biomass allocation generally act in concert to prevent water loss during water shortage and alleviate the effects of drought stress (Merchant et al. 2007). Regardless of K supply, plants under +PEG also showed higher root/shoot ratio, indicative of a greater root carbon allocation for more efficient water and nutrient uptake. This increase was accompanied by a decrease in shoot and LA thus representing a compensated mechanism minimizing transpiration and mitigating drought effect (Chaves et al. 2002, Christina et al. 2015, Gonçalves et al. 2017, Muller et al. 2017).

Plants use a myriad strategies involving complex molecular mechanisms (Fox et al. 2018) to cope with water shortage. Under drought stress, plants can alter their metabolism accordingly via changes in gene expression and cell structure (Gugger et al. 2017), as well as decreases in Ψ_w and cambial activity (Chołuj et al. 2008, Foyer and Shigeoka 2010, Shi et al. 2017). Lower Ψ_w induces stomatal closure, an essential drought tolerance mechanism (Mateus et al. 2021) that prevents Ψ_w from further declining below critical levels that could induce loss of water transport capacity via xylem embolism (Li et al. 2019, Li et al. 2020, Marenco et al. 2006). These mechanisms allow continuous plant growth and development even under lower water supply (Hubbard et al. 2010, Silva et al. 2004, Wildhagen et al. 2018).

The significant differences in leaf K concentration were translated to substantial variation in δ^{13}C and in δ^{15}N (Figure 3f), which were reliable integrative indicators of whole plant-scale
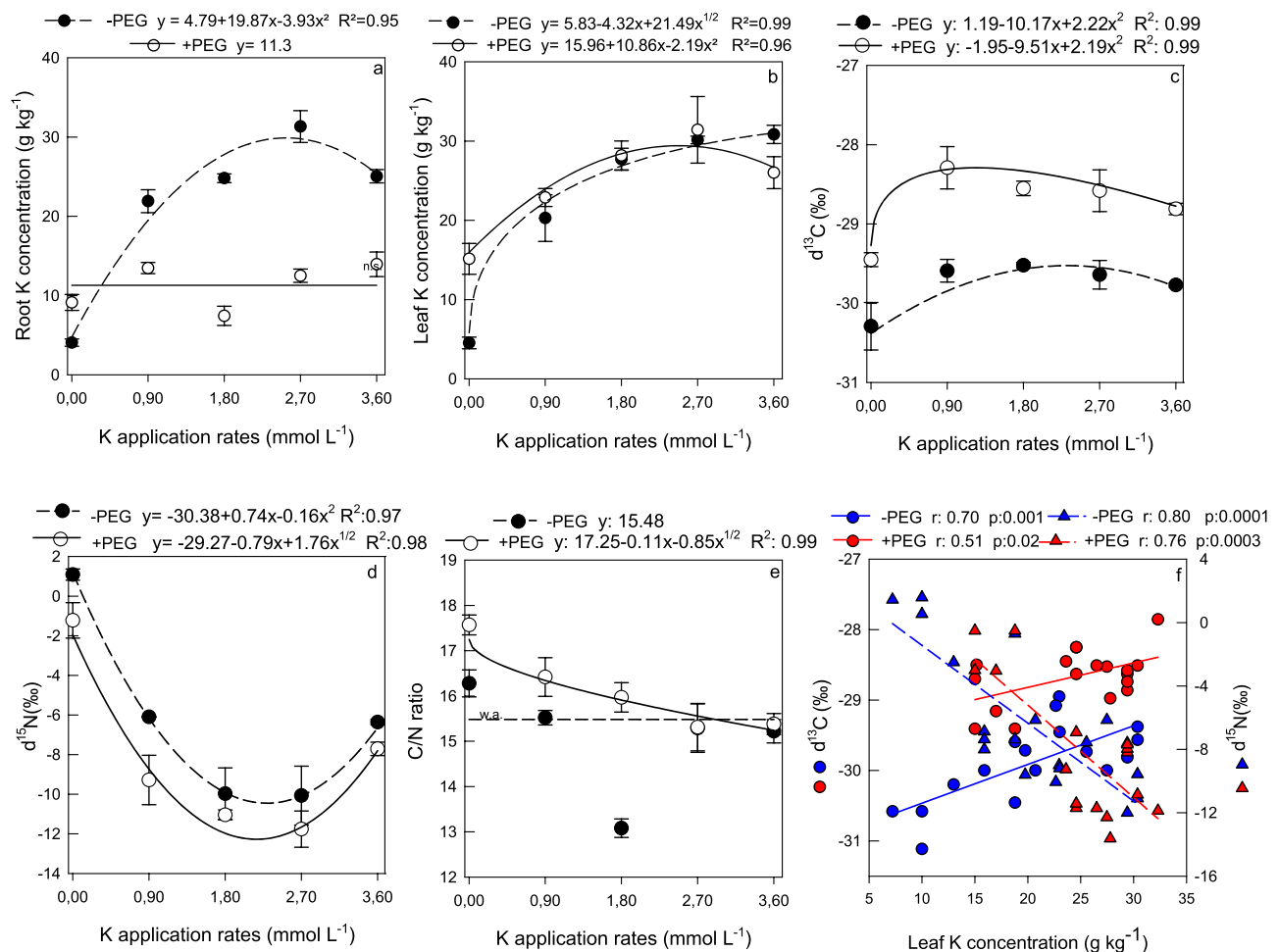
Figure 3. (a) Root potassium (K) concentration, (b) leaf K concentration, (c) leaf carbon isotope composition ($\delta^{13}C$ ‰), (d) leaf nitrogen isotope composition ($\delta^{15}N$ ‰), (e) C/N ratio and (f) the correlation among $\delta^{13}C$, $\delta^{15}N$ and leaf K concentration of *Eucalyptus* seedlings submitted to five K concentrations grown under non-PEG condition (−PEG) and under osmotic induced-stress (+PEG). Vertical bars around means represent standard errors ($n = 4$). The adjustment model is indicated by not significant (n.s) and without suitable adjustment (w.a). *F* test, respectively, (a) WS ($P < 0.05$), K rate ($P < 0.05$), K*WS ($P < 0.05$); (b) WS ($P > 0.05$), K rate ($P < 0.05$), K*WS ($P < 0.05$); (c), (d) and (e) WS ($P < 0.05$), K rate ($P < 0.05$), K*WS ($P > 0.05$).

responses to changes in environmental condition (Mateus et al. 2021), and reflected the effects of K supply throughout the experimental period. The decrease (more negative values) in tissue carbon isotopic composition ($\delta^{13}C$) values observed in K-deficient plants was a consequence of larger g_s (Farquhar et al. 1989), leading to lower WUE. Moreover, the reduction in g_s under high osmotic and water stress leads plants to fix any available CO_2 molecule, therefore increasing (more positive) their $\delta^{13}C$ values (Robinson et al. 2000). The higher values of $\delta^{13}C$ observed in K-supplied plants also confirmed the crucial role of K in affecting photosynthesis and transpiration, through probably a stronger effect on mesophyll diffusion conductance than on g_s (Jákli et al. 2017, Mateus et al. 2019). Consequently, $\delta^{13}C$ correlated significantly to dry matter and WUE, justifying the use of $\delta^{13}C$ as a tool in breeding programs to select the genotypes with genetic improvement of WUE and to provide additional insights into photosynthetic metabolism and

responses to abiotic stress. Long-term WUE and instantaneous WUE (*A/E*) measurements express different time scales of CO_2 assimilation (Fullana-Pericas et al. 2017, Medrano et al. 2015). Therefore, instantaneous WUE represents a short window of time, whereas $\delta^{13}C$ indicates the plant's fitness and its relation with the environment from the onset of photosynthesis until sampling (Araus et al. 2002, Araus et al. 2003).

Photosynthesis process demands a large amount of N for CO_2 assimilation and sugar synthesis, since Rubisco, stromal enzymes and thylakoid proteins are highly dependent on N metabolism in leaves (Champigny 1995, Wang et al. 2015). Our results showed that K application positively influenced leaf N concentration, notably under non-PEG conditions, by enhancing N demand and assimilation. Thus, under K deficiency, a pronounced increase in the C/N ratio was observed due to the significant reduction in total leaf N (Figure 3e). We can hypothesize that K supply enhanced drought stress tolerance
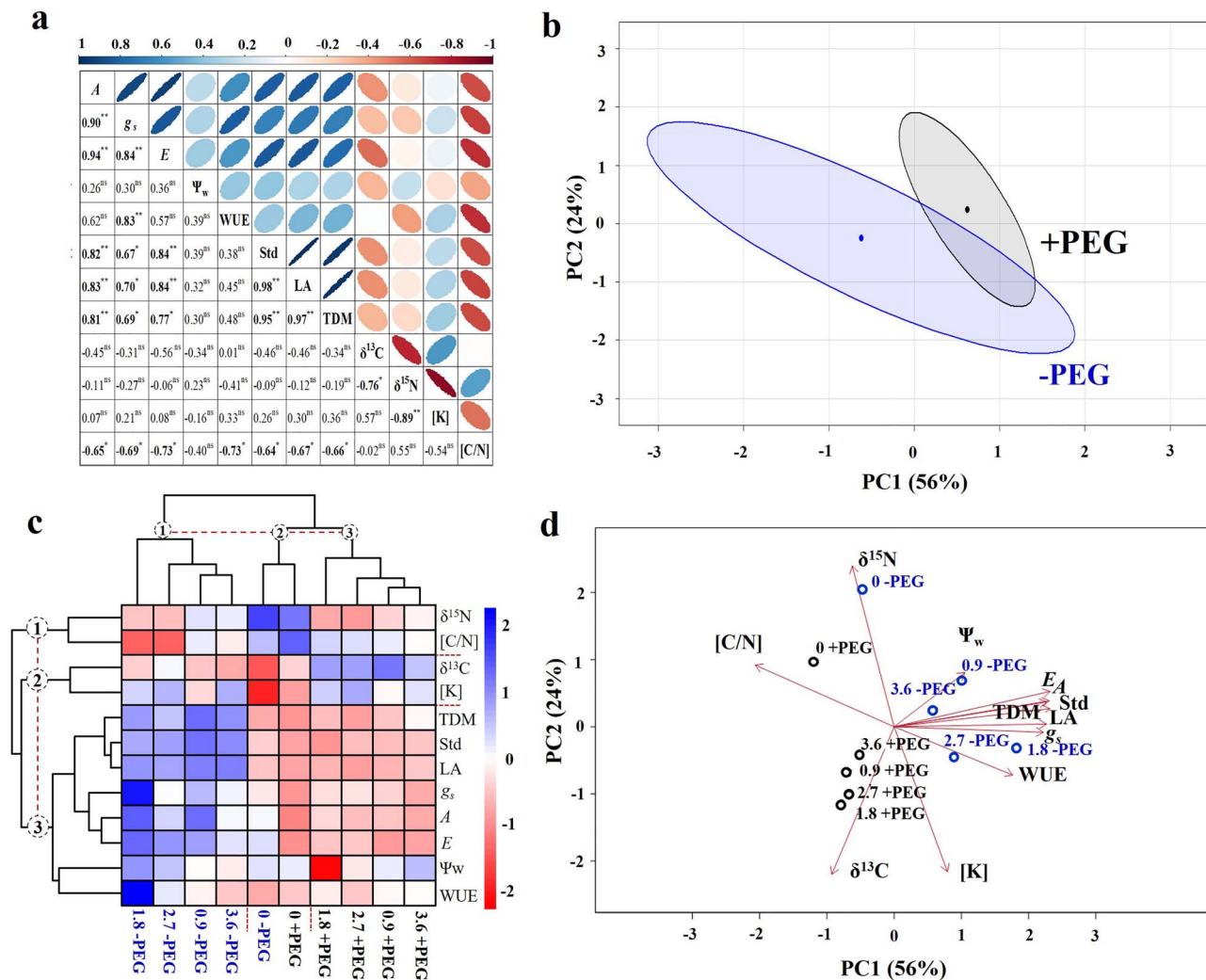
Figure 4. Multivariate analyses showing (a) Pearson correlations, (b) confidence ellipse, with 95% of confidence, (c) heatmap and hierarchical cluster and (d) PCA in *Eucalyptus* seedlings submitted to five potassium (K) levels under water-stress (PEG addition; +PEG) and under controlled (well-watered; −-PEG) conditions. The heatmap indicates values close (blue tones) and distant (red tones) from the samples' average ($n = 4$). Abbreviation: CO_2 assimilation (*a*), stomatal conductance (g_s), transpiration (*E*), leaf water potential (Ψw), stomatal density (std), total dry matter (TDM), $\delta^{13}C$ (isotope composition of C), $\delta^{15}N$ (isotope composition of N), leaf K concentration ([K]) and leaf C/N ratio (leaf [C/N]).

of *Eucalyptus* seedlings by upregulating the concentrations of osmolytes (Hu et al. 2017), e.g., soluble sugar and proline, and by upregulating antioxidant enzyme activities (Song et al. 2015), modulating plant growth-related responses to different extents.

K-deficient plants under both osmotic conditions were mainly characterized by higher $\delta^{15}N$ and C/N ratio levels (Figure 4a and d), suggesting both stable isotope natural abundance as a feasible screening tool and a physiological indicator of *Eucalyptus* response to K deficiency. The osmotic stress decreased $\delta^{15}N$ whereas increased $\delta^{13}C$ (Gouveia et al. 2019, Handley et al. 1999, Robinson et al. 2000), also confirming both as an effective drought stress mechanism response of *Eucalyptus*. The negative relationship between $\delta^{13}C$ and $\delta^{15}N$ (Figure 4a and d) relates the effect of water stress

on gas exchange to leaf N uptake and accumulation. Under drought stress, $N-NO_3^-$ assimilation and translocation from root to shoot are restricted, resulting in decreased $\delta^{15}N$ levels (Sahoo et al. 2010). Thus, the negative relationship observed between $\delta^{15}N$ and leaf K concentration (Figures 3f and 4a and d) was associated with N assimilation capacity and demand (Coque et al. 2006, Pritchard and Guy 2005, Robinson et al. 2000, Yousfi et al. 2009) as well as the plant's transpiration (Medina et al. 2016).

Due to many non-controllable factors occurring under field conditions, the environmentally controlled study presented here allowed the investigation of the direct K-nutrition effects combined with several levels of plant water stress. The PEG addition to nutrient solution has been receiving considerable attention, allowing homogeneous drought-induced conditions, while

field-based drought events cannot be carefully manipulated (Kaufmann and Eckard 1971). However, the short duration of studies in controlled environment precludes the drought-induced effects in the whole plant morphology and anatomy, which are often major components of plant response to field conditions. In hydroponic system production, the nutrients supply is not hampered by factors that limit nutrient availability in the soil and accessibility by plant roots. Thus, environmentally controlled studies provide useful insights into the mechanisms that underpin more mature tree response in the field (Barker and Stratton 2019).

Root K concentration under non-PEG conditions kept increasing up to a K fertilization level of 2.7 mmol L^{-1} and then decreased, indicating an optimum root K absorption. Plants exposed to osmotic stress showed an average of 11.3 g/kg^{-1} of K, with a maximum root concentration irrespective of the K rates (Figure 3a). Conversely, the increased leaf K concentration due to enhanced K rates was important in osmotic-stressed plants, highlighting the strategy of upregulating leaf K concentration and optimizing WUE to maintain shoot dry matter production in drought conditions (Santos et al. 2020). Even though the complete withdrawal of K (0 mmol L^{-1}) mimicked an extreme field-growing condition, the aim of this treatment was to simulate a scarcity of K during a phase of high nutritional demand, or when seasonal demand for growth exceeds external nutrient supply.

Conclusion

Osmotic-induced stress decreased plant growth, regardless of K supply; however, these effects were more accentuated for K-deficient plants. *Eucalyptus* seedling plants showed a positive response to K supply in non-PEG and +PEG conditions, since K positively modulated the plants' physiology, upregulating LA, SLA and leading to higher leaf gas exchange parameters, e.g., A, E, g_s and WUE, and resulting in lower values of leaf carbon and nitrogen isotopes ($\delta^{13}C$ and $\delta^{15}N$). In addition, K supply led to changes in leaf structural features such as SD, resulting in better plant growth and dry matter production. Altogether, our results show that better nutritional management strategy should seek to optimize K-nutrition to improve WUE, photosynthesis-related parameters and plant growth under water deficit conditions.

Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

N.S.M., E.V.O.F., A.V.F., J.L.M.G. and J.L. conceived and designed the study. N.S.M. performed most experiments, A.L.F. and E.V.O.F. assisted with the management of pot culture, plant material,

analysis and interpretation of data. J.C.D., L.J.M. and J.A.B. revised this draft by commenting and rewriting. All authors checked and approved the submitted version.

References

Anschütz U, Becker D, Shabala S (2014) Going beyond nutrition: regulation of potassium homoeostasis as a common denominator of plant adaptive responses to environment. J Plant Physiol 171:670–687.

Araus JL, Slafer GA, Reynolds MP, Royo C (2002) Plant breeding and drought in C3 cereals: what should we breed for? Ann Bot 89:925–940.

Araus JL, Villegas D, Aparicio N, Garcı LF, Hani S, El Rharrabti Y, Ferrio JP, Royo C (2003) Environmental factors determining carbon isotope discrimination and yield in durum wheat under Mediterranean conditions. Crop Sci 43:170–180.

Asensio V, Domec JC, Nouvellon Y, Laclau JP, Bouillet, Li JP, Jordan-Meille L, Lavres J, Delgado-Rojas J, Guillemot J, Abreu-Junior CH (2020) Potassium fertilization increases hydraulic redistribution and water use efficiency for stemwood production in *Eucalyptus grandis* plantations. Environ Exp Bot 176:104085.

Barker AV, Stratton ML (2019) Nutrient density of fruit crops as a function of soil fertility. In: Fruit crops: diagnosis and management of nutrient constraints. Elsevier, Amsterdam, pp. 13–31.

Barrie A, Prosser SJ (1996) Automated analysis of light-element stable isotopes by isotope ratio mass spectrometry. In: Boutton TW, Yamasaki S (eds) Mass spectrometry of soils. Marcel Dekker, New York, pp. 1–46.

Battie Laclau P, Delgado-Rojas JS, Christina M, Nouvellon Y, Bouillet JP, Piccolo MC, Moreira MZ, Gonçalves JLM, Roupsard O, Laclau JP (2016) Potassium fertilization increases water-use efficiency for stem biomass production without affecting intrinsic water-use efficiency in *Eucalyptus grandis* plantations. For Ecol Manage 364:77–89.

Battie Laclau P, Laclau JP, Domec JC et al. (2014) Effects of potassium and sodium supply on drought-adaptive mechanisms in *Eucalyptus grandis* plantations. New Phytol 203:401–413.

Battie Laclau P, Laclau JP, Piccolo MC et al. (2013) Influence of potassium and sodium nutrition on leaf area components in *Eucalyptus grandis* trees. Plant and Soil 371:19–35.

Booth TH (2013) Eucalypt plantations and climate change. For Ecol Manage 301:28–34.

Cakmak I (2010) Potassium for better crop production and quality. Plant Soil 335:1–2.

Champigny ML (1995) Integration of photosynthetic carbon and nitrogen metabolism in higher plants. Photosynth Res 46:117–127.

Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CPP, Osório ML, Pinheiro C (2002) How plants cope with water stress in the field? Photosynthesis and growth. Ann Bot 89:907–916.

Chołuj D, Karwowska R, Ciszewska A, Jasińska M (2008) Influence of long-term drought stress on osmolyte accumulation in sugar beet (*Beta vulgaris* L.) plants. Acta Physiol Plant 30:679–687.

Christina M, Le Maire G, Battie Laclau P, Nouvellon Y, Bouillet JP, Jourdan C, Gonçalves JLM, Laclau JP (2015) Measured and modeled interactive effects of potassium deficiency and water deficit on gross primary productivity and light-use efficiency in *Eucalyptus grandis* plantations. Glob Chang Biol 21:2022–2039.

Clark RB (1975) Characterization of phosphatase of intact maize roots. J Agric Food Chem 23:458–460.

Coque M, Bertin P, Hirel B, Gallais A (2006) Genetic variation and QTLs for 15N natural abundance in a set of maize recombinant inbred lines. Field Crop Res 97:310–331.

Dong H, Tang W, Li Z, Zhang D (2004) On potassium deficiency in cotton-disorder, cause and tissue diagnosis. Agric Conspec Sci 69:77–85.

Drosdoff M, Sell HM, Gilbert SG (1947) Some effects of potassium deficiency on the nitrogen metabolism and oil synthesis in the tung tree (*Aleurites fordii*). Plant Physiol 22:538.

Egilla JN, Davies FT, Boutton TW (2005) Drought stress influences leaf water content, photosynthesis, and water-use efficiency of Hibiscus rosa-sinensis at three potassium concentrations. Photosynthetica 43:135–140.

Epron D, Cabral OMRC, Laclau JP et al. (2015) In situ $^{13}CO_2$ pulse labelling of field-grown eucalypt trees revealed the effects of potassium nutrition and throughfall exclusion on phloem transport of photosynthetic carbon. Tree Physiol 36:6–21.

Fait A, Nesi AN, Angelovici R, Lehmann M, Pham PA, Song LH, Haslam RP, Napier JA, Galili G, Fernie AR (2011) Targeted enhancement of glutamate-to-gamma-aminobutyrate conversion in *Arabidopsis* seeds affects carbon-nitrogen balance and storage reserves in a development-dependent manner. Plant Physiol 157:1026–1042.

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Ann Rev Plant Physiol Plant Mol Biol 40:503–537.

Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust J Plant Physiol 9:121–137.

Fox H, Doron-Faigenboim A, Kelly G, Bourstein R, Attia Z, Zhou J, Moshe Y, Oshelion M, David-Schwartz R (2018) Transcriptome analysis of *Pinus halepensis* under drought stress and during recovery. Tree Physiol 38:423–441.

Foyer CH, Shigeoka S (2010) Understanding oxidative stress and antioxidant functions to enhance photosynthesis. Plant Physiol 155:93–100.

Fullana-Pericas M, Conesa MA, Soler S, Ribas-Carbo M, Granell A, Galmes J (2017) Variations of leaf morphology, photosynthetic traits and water-use efficiency in western-Mediterranean tomato landraces. Photosynthetica 55:121–133.

Gerardeaux E, Saur E, Constantin J, Porté A, Jordan-Meille L (2009) Effect of carbon assimilation on dry weight production and partitioning during vegetative growth. Plant and Soil 324:329–343.

Gonçalves JLM (2011) Fertilização de plantação de eucalipto. In: Encontro Brasileiro de Silvicultura Campinas, Anais.

Gonçalves JLM, Alvares CA, Rocha JHT, Brandani CB, Hakamada R (2017) Eucalypt plantation management in regions with water stress. South For 79:169–183.

Gouveia CSS, Ganança JFT, Slaski J, Lebot V, Carvalho MAAP (2019) Stable isotope natural abundances ($\delta^{13}C$ and $\delta^{15}N$) and carbon-water relations as drought stress mechanism response of taro (*Colocasia esculenta* L. Schott). J Plant Physiol 232:100–106.

Gugger PF, Peñaloza-Ramírez JM, Wright JW, Sork VL (2017) Whole-transcriptome response to water stress in a California endemic oak, *Quercus lobata*. Tree Physiol 37:632–644.

Handley LL, Austin AT, Robinson D, Scrimgeour CM, Raven JA, Heaton TH, Schmidt S, Stewart GR (1999) The ^{15}N natural abundance (d^{15}N) of ecosystem samples reflects measures of water availability. Aust J Plant Physiol 26:185–199.

Hu W, Coomer TD, Loka DA, Oosterhuis DM, Zhou Z (2017) Potassium deficiency affects the carbon-nitrogen balance in cotton leaves. Plant Physiol Bioch 115:408–417.

Hu W, Zhao W, Yang J, Oosterhuis DM, Loka DA, Zhou Z (2016) Relationship between potassium fertilization and nitrogen metabolism in the leaf subtending the cotton (*Gossypium hirsutum* L.) boll during the boll development stage. Plant Physiol Bioch 101:113–123.

Hubbard RM, Stape J, Ryan MG, Almeida AC, Delgado-Rojas J (2010) Effects of irrigation on water use and water use efficiency in two fast growing *Eucalyptus* plantations. For Ecol Manage 259:1714–1721.

Huppe HC, Turpin DH (1994) Integration of carbon and nitrogen metabolism in plant and algal cells. Annu Rev Plant Physiol 45:577–607.

IPCC (2013) In: Stocker TF, Qin D, Plattner GK, Tignor MMB, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) Climate change: the physical science basis contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge & New York, NY.

Jákli B, Tavakol E, Trankner M, Senbayram M, Dittert K (2017) Quantitative limitations to photosynthesis in K deficient sunflower and their implications on water-use efficiency. J Plant Physiol 209:20–30.

Jordan-Meille L, Martineau E, Bornot Y, Lavres J, Abreu Junior CH, Domec JC (2018) How does water-stressed corn respond to potassium nutrition? A shoot-root scale approach study under controlled conditions. Agri 8:180.

Kaufmann MR, Eckard AN (1971) Evaluation of water stress control with polyethylene glycols by analysis of guttation. Plant Physiol 47:453–456.

Kohzu A, Nishizawa N, Boontanon N, Wada E (1999) The use of natural ^{13}C and ^{15}N abundances for monitoring of environmental stress. Aust J Biol Sci 2:1–10.

Lauteri M, Brugnoli E, Spaccino L (1993) Carbon isotope discrimination in leaf soluble sugars and in whole-plant dry matter in *Helianthus annum* L. grown under different water conditions. In: Ehleringer JR, Hall AE, Farquhar GD (eds) Stable isotopes and plant carbon—water relations. Academic Press, Inc., San Diego, CA, pp. 93–106.

Law SR (2019) Eucalypt seedlings are aided by phosphorus in the face of drought. Physiol Plantarum 166:892–893.

Li X, Blackman CJ, Peters JMR, Choat B, Rymer PD, Medlyn BE, Tissue DT (2019) More than iso/anisohydry: hydroscapes integrate plant water use and drought tolerance traits in 10 eucalypt species from contrasting climates. Funct Ecol 33:1035–1049.

Li X, Smith R, Choat B, Tissue DT (2020) Drought resistance of cotton (*Gossypium hirsutum*) is promoted by early stomatal closure and leaf shedding. Funct Plant Biol 47:91.

Makhdum M, Pervez H, Ashraf M (2007) Dry matter accumulation and partitioning in cotton (*Gossypium hirsutum* L.) as influenced by potassium fertilization. Biol Fert Soils 43:295–301.

Malavolta E, Vitti GC, Oliveira AS (1997) Avaliação do Estado Nutricional das Plantas: princípios e aplicações. Associação Brasileira para Pesquisa da Potassa e do Fosfato, Piracicaba.

Marenco RA, Siebke K, Farquhar GD, Ball MC (2006) Hydraulically based stomatal oscillations and stomatal patchiness in *Gossypium hirsutum*. Funct Plant Biol 33:1103–1113.

Marschner H (2012) Mineral nutrition of higher plants, 3rd edn. Academic Press, London.

Martineau E, Domec JC, Bosc A, Denoroy P, Fandino VA, Lavres J, Jordan-Meille L (2017) The effects of potassium nutrition on water use in field-grown maize (*Zea mays* L.). Environ Exp Bot 134:62–71.

Mateus NS, Ferreira EVO, Arthur Junior JC, Domec JC, Jordan-Meille L, Gonçalves JLM, Lavres J (2019) The ideal percentage of K substitution by Na in *Eucalyptus* seedlings: evidences from leaf carbon isotopic composition, leaf gas exchanges and plant growth. Plant Physiol Bioch 137:102–112.

Mateus NS, Leite AF, Santos EF, Ferraz AV, Goncalves JLM, Lavres J (2021) Partial substitution of K by Na alleviates drought stress and increases water use efficiency in *Eucalyptus* species seedlings. Front Plant Sci 12:632342.

Medina S, Vicente R, Amador A, Araus JL (2016) Interactive effects of elevated [CO_2] and water stress on physiological traits and gene expression during vegetative growth in four durum wheat genotypes. Front Plant Sci 7:1738.

Medrano H, Tomas M, Martorell S, Flexas J, Hernandez E, Rossello J, Pou A, Escalona JM, Bota J (2015) From leaf to whole-plant water use efficiency (WUE) in complex canopies: limitations of leaf WUE as a selection target. Crop J 3:220–228.

Merchant A, Callister A, Arndt S, Tausz M, Adams M (2007) Contrasting physiological responses of six *Eucalyptus* species to water deficit. Ann Bot 100:1507–1515.

Michel BE, Kaufmann MR (1973) The osmotic potential of polyethylene glycol 6000. Plant Physiol 51:914–916.

Muller C, Hodecker BER, Merchant A, Barros NF (2017) Nutritional efficiency of *Eucalyptus* clones under water stress. Rev Bras Cienc Solo 41:e0160528.

Oddo E, Abbate L, Inzerillo S, Carimi F, Motisi A, Sajeva M, Nardini A (2020) Water relations of two Sicilian grapevine cultivars in response to potassium availability and drought stress. Plant Physiol Bioch 148:282–290.

Peiter E (2011) The plant vacuole: emitter and receiver of calcium signals. Cell Calcium 50:120–128.

Pritchard ES, Guy RD (2005) Nitrogen isotope discrimination in white spruce fed with low concentrations of ammonium and nitrate. Trees 19:89–98.

R Development Core Team, (2008). R: a language and environment for statistical computing. Austria: R foundation for statistical computing. Vienna. ISBN 3-900051-07-0.

Robinson D (2001) $\delta^{15}N$ as an integrator of the nitrogen cycle. Trends Ecol Evol 16:153–162.

Robinson D, Handley LL, Scrimgeour CM, Gordon C, Forster BP, Ellis RP (2000) Using stable isotope natural abundances ($\delta^{15}N$ and $\delta^{13}C$) to integrate the stress responses of wild barley (*Hordeum spontaneum* C. Koch.) genotypes. J Exp Bot 51:41–50.

Sahoo MR, Dasgupta M, Kole PC, Mukherjee A (2010) Biochemical changes in leaf tissues of taro [*Colocasia esculenta* L. (Schott)] infected with *Phytophthora colocasiae*. J Phytopathol 158:154–159.

Santos EF, Mateus NS, Rosário MO, Garcez TB, Mazzafera P, Lavres J (2020) Enhancing potassium content in leaves and stems improves drought tolerance of eucalyptus clones. Physiol Plantarum 172:552–563.

Sardans J, Peñuelas J (2015) Potassium: a neglected nutrient in global change. Glob Ecol Biogeogr 24:261–275.

SAS Institute Inc (2004) SAS/STAT 9.1®. User's Guide. SAS Institute Inc., Cary, NC.

Schulze ED, Turner NC, Nicolle D, Schumacher J (2006) Leaf and wood carbon isotope ratios, specific leaf areas and wood growth of *Eucalyptus* species across a rainfall gradient in Australia. Tree Physiol 26:479–492.

Serret MD, Yousfi S, Vicente R, Piñero MC, Otálora-Alcón G, del Amor FM, Araus JL (2018) Interactive effects of CO_2 concentration and water regime on stable isotope signatures, nitrogen assimilation and growth in sweet pepper. Front Plant Sci 8:2180.

Shi H, Ma W, Song J et al. (2017) Physiological and transcriptional responses of *Catalpa bungei* to drought stress under sufficient and deficient-nitrogen conditions. Tree Physiol 37:1457–1468.

Silva FC, Shvaleva A, Maroco JP, Almeida MH, Chaves MM, Pereira JS (2004) Responses to water stress in two *Eucalyptus globulus* clones differing in drought tolerance. Tree Physiol 24:1165–1172.

Silva PHM, Campoe OC, De Paula RC, Lee DJ (2016) Seedling growth and physiological responses of sixteen eucalypt taxa under controlled water regime. Forests 7:1–13.

Silva VE, Nogueira TAR, Abreu-Junior CH, He Z, Buzetti S, Laclau JP, Teixeira Filho MCM, Grilli E, Murgia I, Capra GF (2020) Influences of edaphoclimatic conditions on deep rooting and soil water availability in Brazilian *Eucalyptus* plantations. Forest Ecol Manag 445:117673.

Song ZZ, Duan CL, Guo SL, Yang Y, Feng YF, Ma RJ, Yu ML (2015) Potassium contributes to zinc stress tolerance in peach (*Prunus persica*) seedlings by enhancing photosynthesis and the antioxidant defense system. Genet Mol Res 14: 8338–8351.

Spangenberg JE, Schweizer M, Zufferey V (2021) Carbon and nitrogen stable isotope variations in leaves of two grapevine cultivars (Chasselas and pinot noir): implications for ecophysiological studies. Plant Physiol Bioch 163:45–54.

Stape JL, Binkley D, Ryan MG (2004) *Eucalyptus* production and the supply, use and efficiency of use of water, light and nitrogen across a geographic gradient in Brazil. For Ecol Manage 193: 17–31.

Tavakol E, Jákli B, Cakmak I, Dittert K, Karlovsky P, Pfohl K, Senbayram M (2018) Optimized potassium nutrition improves plant-water-relations of barley under PEG-induced osmotic stress. Plant and Soil 430:23–35.

Teixeira PC, Gonçalves JLM, Arthur JC Jr, Dezordi C (2008) *Eucalyptus sp*. seedling response to potassium fertilization and soil water. Ciênc Florestal 18:47–63.

Turner NC (1981) Techniques and experimental approaches for the measurement of plant water status. Plant and Soil 58:339–366.

Wang ZN, Lu JY, Yang M, Yang HM, Zhang QP (2015) Stoichiometric characteristics of carbon, nitrogen, and phosphorus in leaves of differently aged lucerne (*Medicago sativa*) stands. Front Plant Sci 6:1062.

Wildhagen H, Paul S, Allwright M, et al. (2018) Genes and gene clusters related to genotype and drought-induced variation in saccharification potential, lignin content and wood anatomical traits in *Populus nigra*. Tree Physiol 38:320–339.

Yousfi S, Serret MD, Araus JL (2009) Shoot $\delta^{15}N$ gives a better indication than ion concentration or $\Delta^{13}C$ of genotypic differences in the response of durum wheat to salinity. Func Plant Biol 36: 144–155.

Yousfi S, Serret MD, Márquez AJ, Voltas J, Araus JL (2012) Combined use of $\Delta^{13}C$, $\Delta^{18}O$ and $\Delta^{15}N$ tracks nitrogen metabolism and genotypic adaptation of durum wheat to salinity and water deficit. New Phytol 194:230–244.

Yousfi S, Serret MD, Voltas J, Araus JL (2010) Effect of salinity and water stress during the reproductive stage on growth, ion concentrations, $\Delta^{13}C$, and $\delta^{15}N$ of durum wheat and related amphiploids. J Exp Bot 61:3529–3542.

Zörb C, Senbayram M, Peiter E (2014) Potassium in agriculture - status and perspectives. J Plant Physiol 171:656–669.