Supplementary information

Article title: The interaction of CO_2 concentrations and water availability on organic acids in root exudates of native Australian species

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Table S1. Summary ANOVA table for analyses of plant and soil measurements. The number of cluster roots per unit root biomass was only tested for *Hakea sericea*. Statistical significance is shown alongside the corresponding *F*-values: *P* <0.1 (†), <0.05 (*), <0.01 (**) and <0.001 (***). First, a linear mixed-effects model (LMM) was fitted to each variable with block as a random factor. Where block did not explain a significant amount of the variation in response variables and did not improve the models, it was removed, and a linear model (LM) was fitted instead. Degrees of freedom for *F* tests are shown in parentheses.

Species	CO_2	Water	CxW	Model
Biomass				
E. tereticornis	F(1, 8)=0.23	F(1, 8)=31.60***	F(1, 8)=0.23	LMM
H. sericea	F(1, 28)=6.76*	F(1, 28)=7.78**	F(1, 28)=0.56	LM
M. stipoides	F(1, 28)=3.77†	F(1, 28)=4.66*	F(1, 28)=0.01	LM
RSR				
E. tereticornis	F(1, 8)=0.19	F(1, 8)=5.80*	F(1, 8)=0.47	LMM
H. sericea	F(1, 14)=1.79	F(1, 14)=18.43***	F(1, 14)=0.04	LMM
M. stipoides	F(1, 14)=1.71	F(1, 14)=0.59	F(1, 14)=0.90	LMM
Soil nitrate				
E. tereticornis	F(1, 8)=0.09	F(1, 8)=0.48	F(1, 8)=0.90	LMM
H. sericea	F(1, 14)=0.53	F(1, 14)=63.68***	F(1, 14)=0.11	LMM
M. stipoides	F(1, 14)=1.47	F(1, 14)=26.98***	F(1, 14)=1.21	LMM
Soil ammonium				
E. tereticornis	F(1, 16)=41.57***	F(1, 16)=35.52***	F(1, 16)=26.35***	LM
H. sericea	F(1, 14)=0.18	F(1, 14)=6.78*	F(1, 14)=1.40	LMM
M. stipoides	F(1, 14)=19.33***	F(1, 14)=29.69***	F(1, 14)=13.41**	LMM
Soil phosphate				
E. tereticornis	F(1, 8)=0.03	F(1, 8)=46.00***	F(1, 8)=0.05	LMM
H. sericea	F(1, 14)=0.45	F(1, 14)=64.58***	F(1, 14)=3.07	LMM
M. stipoides	F(1, 14)=13.38**	F(1, 14)=19.33***	F(1, 14)=6.53*	LMM
No. of cluster root per root				
H. sericea	F(1, 28)=1.07	F(1, 28)=9.62**	F(1, 28)=0.23	LM

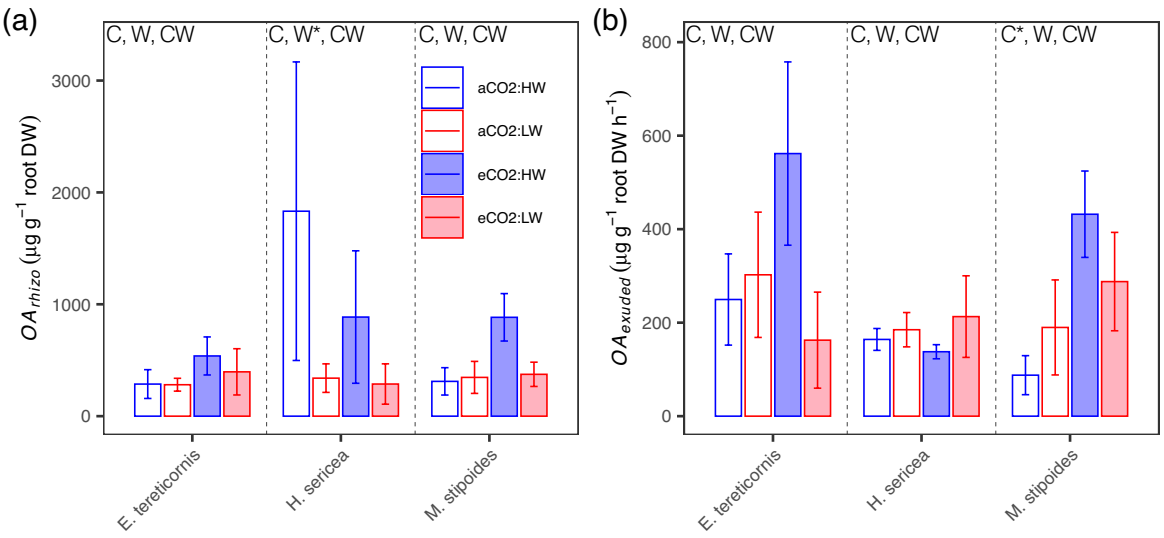

Figure S1. The amounts of organic acids a) in the rhizosphere soil (OA_{rhizo}) and b) exuded from roots (OA_{exuded}), expressed on a root dry weight (DW) basis, for *Eucalyptus tereticornis*, *Hakea sericea* and *Microlaena stipoides* (mean ± 1 standard error, n = 5 for *E. tereticornis* and n = 8 for the others). Ambient (aCO_2) and elevated (eCO_2) CO_2 treatments are depicted with open and filled boxes, respectively. High-watered (HW) and low-watered (LW) plants are in blue and red, respectively. The statistical analysis results are shown for CO_2 (C), water (W) and their interaction (CW) at the top of corresponding figures for each species, with significance codes: P <0.1 (†), <0.05 (*), <0.01 (**) and <0.001 (***).

Table S2 Summary of ANOVA with CO_2, water and CO_2×water interaction (C×W) effects on the amounts of organic acids (OAs) in the rhizosphere soil (OA_{rhizo}) and exuded from roots (OA_{exuded}) for *Eucalyptus tereticornis*, *Hakea sericea* and *Microlaena stipoides*. Analyses are presented for amounts per plant and per unit root biomass and OA-derived carbon (C)-mass per plant and per unit root mass. Statistical significance is shown alongside the corresponding *F*-values: P <0.1 (†), <0.05 (*), <0.01 (**) and <0.001 (***). First, a linear mixed-effects model (LMM) was fitted to each of the variables, with block as a random factor. Where block did not explain any of the variation in response variables and did not improve the model, it was removed, and a linear model (LM) was fitted instead. Degrees of freedom for *F* tests are shown in parentheses.

Variable	CO_2	Water	CxW	Model
OA_{rhizo} per plant				
E. tereticornis	F(1, 8)=0.33	F(1, 8)=0.48	F(1, 8)=0.02	LMM
H. sericea	F(1, 28)=0.35	F(1, 28)=2.08	F(1, 28)=0.40	LM
M. stipoides	F(1, 14)=1.27	F(1, 14)=0.01	F(1, 14)=0.61	LMM
OA_{rhizo} per root				
E. tereticornis	F(1, 16)=0.63	F(1, 16)=0.13	F(1, 16)=0.63	LM
H. sericea	F(1, 14)=0.97	F(1, 14)=8.71*	F(1, 14)=0.26	LMM
M. stipoides	F(1, 14)=1.24	F(1, 14)=0.91	F(1, 14)=0.58	LMM
OA_{rhizo} C per plant				
E. tereticornis	F(1, 8)=0.50	F(1, 8)=0.29	F(1, 8)=0.01	LMM
H. sericea	F(1, 28)=0.28	F(1, 28)=2.30	F(1, 28)=0.40	LM
M. stipoides	F(1, 14)=1.24	F(1, 14)=0.03	F(1, 14)=0.65	LMM
OA_{rhizo} C per root				
E. tereticornis	F(1, 16)=1.03	F(1, 16)=0.33	F(1, 16)=0.55	LM
H. sericea	F(1, 14)=0.93	F(1, 14)=9.80**	F(1, 14)=0.24	LMM
M. stipoides	F(1, 14)=1.19	F(1, 14)=1.09	F(1, 14)=0.64	LMM
OA_{exuded} per plant				
E. tereticornis	F(1, 16)=0.07	F(1, 16)=0.52	F(1, 16)=0.53	LM
H. sericea	F(1, 28)=0.09	F(1, 28)=4.51*	F(1, 28)=0.12	LM
M. stipoides	F(1, 14)=5.00*	F(1, 14)=0.00	F(1, 14)=0.65	LMM
OA_{exuded} per root				
E. tereticornis	F(1, 16)=0.04	F(1, 16)=1.82	F(1, 16)=1.40	LM
H. sericea	F(1, 28)=0.36	F(1, 28)=0.18	F(1, 28)=0.00	LM
M. stipoides	F(1, 14)=5.24*	F(1, 14)=0.51	F(1, 14)=0.35	LMM
OA_{exuded} C per plant				
E. tereticornis	F(1, 16)=0.11	F(1, 16)=0.43	F(1, 16)=0.54	LM
H. sericea	F(1, 28)=0.25	F(1, 28)=4.68*	F(1, 28)=0.01	LM
M. stipoides	F(1, 14)=5.39*	F(1, 14)=0.09	F(1, 14)=0.47	LMM
OA_{exuded} C per root				
E. tereticornis	F(1, 16)=0.05	F(1, 16)=2.18	F(1, 16)=1.56	LM
H. sericea	F(1, 28)=0.34	F(1, 28)=0.07	F(1, 28)=0.02	LM
M. stipoides	F(1, 14)=6.10*	F(1, 14)=0.36	F(1, 14)=0.68	LMM

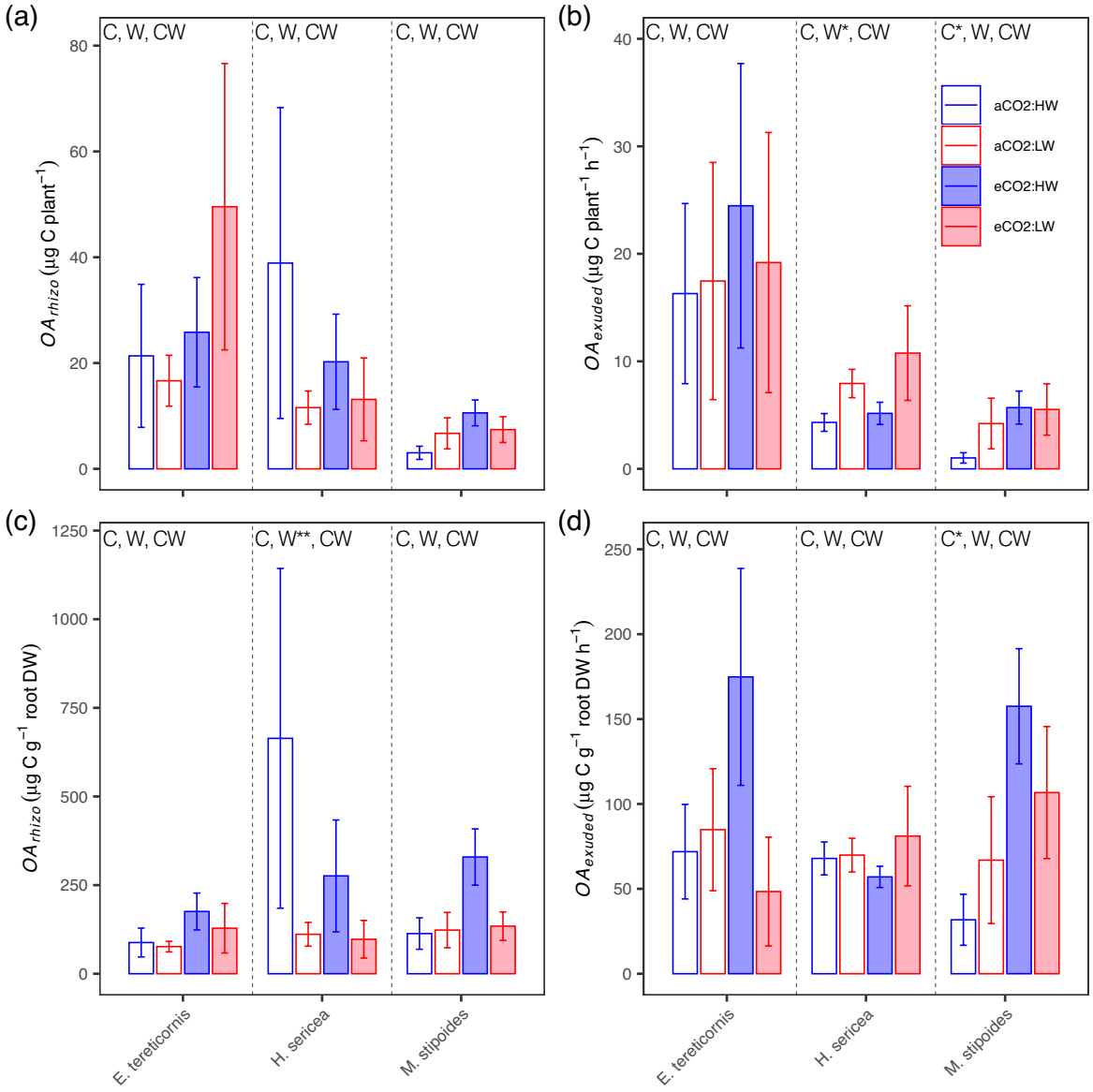

Figure S2. Total amounts of carbon (C) derived from organic acids (OAs) in the rhizosphere soil (OA_{rhizo}) and exuded from roots (OA_{exuded}) (a, b) per plant and (c, d) per unit root dry weight (DW) for *Eucalyptus tereticornis*, *Hakea sericea* and *Microlaena stipoides* (Mean ± 1 standard error, n = 5 for *E. tereticornis* and n = 8 for the others). Ambient (aCO_2) and elevated (eCO_2) CO_2 treatments are depicted with open and filled boxes, respectively. High-watered (HW) and low-watered (LW) treatments are in blue and red, respectively. The statistical analysis results are shown for CO_2 (C), water (W) and their interaction (CW) at the top of corresponding figures for each species, with significance codes: P <0.1 (†), <0.05 (*), <0.01 (**) and <0.001 (***).

Table S3. Summary of multiple regression analyses on the total amounts of organic acids per plant in the rhizosphere soil (OA_{rhizo}) and exuded from roots (OA_{exuded}) for *Eucalyptus tereticornis*, *Hakea sericea* and *Microlaena stipoides*. The following plant/soil measurements were fitted: total biomass, root:shoot ratio (RSR) and soil nutrients. The number of cluster roots per unit root biomass was also fitted for *H. sericea.* Response variables were transformed ($\log_e$ or Box-Cox transformation) as required to ensure homogeneity of variances and normality of errors prior to analysis. Explanatory variables were Z-standardised so that values had mean=0 and standard deviation=1. Ninety-five percent confidence intervals (CIs) on the coefficients of the multiple regression model were estimated by parametric bootstrap with 999 simulations, shown within square parentheses alongside variable estimates. Coefficients whose 95% CIs do not overlap zero are shown in bold.

	E. tereticornis	***H. sericea***	***M. stipoides***
OA_{rhizo}			
Transformation	*y^(1/3)*	*y^(–0.4)*	*y^(1/3)*
Terms			
Intercept	**3.74 [3.16, 4.32]**	**0.30 [0.26, 0.34]**	**2.86 [2.11, 3.61]**
Biomass	**1.19 [0.31, 2.06]**	–0.03 [–0.09, 0.03]	**1.13 [0.50, 1.87]**
log(RSR)	–0.35 [–1.03, 0.33]	0.00 [–0.04, 0.05]	–0.10 [–0.89, 0.65]
Soil nitrate	–0.74 [–1.57, 0.09]	0.01 [–0.06, 0.07]	0.57 [–0.18, 1.23]
log(soil ammonium)	–0.33 [–1.02, 0.37]	–0.01 [–0.05, 0.03]	0.56 [–0.15, 1.27]
Soil phosphate	0.13 [–0.53, 0.78]	–0.05 [–0.11, 0.01]	**0.83 [0.14, 1.51]**
CR per root	–	–0.01 [–0.07, 0.04]	–
OA_{exuded}			
Transformation	*log(y+0.1)*	*log(y)*	*y^(1/3)*
Terms			
Intercept	**2.53 [1.32, 3.73]**	**2.58 [2.37, 2.79]**	**2.23 [1.70, 2.75]**
Biomass	0.30 [–1.52, 2.12]	**0.47 [0.12, 0.81]**	**1.39 [0.87, 1.97]**
log(RSR)	0.24 [–1.18, 1.66]	**0.27 [0.01, 0.53]**	0.32 [–0.24, 0.90]
Soil nitrate	–1.45 [–3.17, 0.27]	0.13 [–0.23, 0.48]	–0.17 [–0.74, 0.43]
log(soil ammonium)	–0.09 [–1.54, 1.37]	0.08 [–0.16, 0.32]	0.19 [–0.39, 0.77]
Soil phosphate	0.45 [–0.91, 1.82]	0.15 [–0.18, 0.47]	0.42 [–0.12, 0.96]
CR per root	–	0.06 [–0.30, 0.41]	–

Table S4 Amounts of each organic acid in the rhizosphere soil (OA_{rhizo}) and exuded from roots (OA_{exuded}) (mean±1 standard error). Statistical significance is shown alongside the corresponding Chi-squared test values with significance codes: P <0.1 (†), <0.05 (*), <0.01 (**).

	Mean±SE (µg plant^{-1})				Chi-squared test (χ^2)		
Compound	**aCO_2:HW**	**aCO_2:LW**	**eCO_2:HW**	**eCO_2:LW**	**CO_2**	**Water**	**CxW**
OA_{rhizo}							
E. tereticornis							
Citric	ND	ND	ND	23.49±23.49	–	–	–
Fumaric	1.34±0.45	1.70±0.68	1.77±0.53	1.32±0.63	0.00	0.00	0.37
Malic	26.26±26.26	ND	48.39±20.73	62.50±34.19	5.17*	0.89	0.88
Oxalic	40.10±17.81	57.00±15.64	26.75±12.09	63.43±24.41	0.03	5.46*	0.24
Shikimic	1.45±0.63	1.54±0.86	1.27±0.26	1.82±0.80	0.60	0.32	0.09
H. sericea							
cis-aconitic	0.68±0.25	0.28±0.19	0.77±0.35	0.41±0.28	0.00	7.65**	0.39
Citric	20.75±15.90	ND	ND	ND	–	–	–
Fumaric	0.12±0.12	ND	ND	ND	–	–	–
Malic	55.08±55.08	ND	6.50±6.50	ND	–	–	–
Oxalic	11.75±7.75	19.04±12.48	33.95±33.95	21.35±21.35	–	–	–
Shikimic	0.28±0.24	ND	0.28±0.28	ND	–	–	–
trans-aconitic	18.80±5.47	15.41±2.80	20.28±4.05	17.56±5.29	0.17	0.47	0.01
M. stipoides							
Acetic	ND	ND	11.58±5.68	ND	–	–	–
Citric	4.89±4.89	32.32±12.65	29.43±7.23	36.35±11.98	1.47	5.47*	4.86*
Fumaric	0.04±0.04	0.04±0.04	1.61±1.41	0.21±0.08	–	–	–
Malic	19.33±7.37	13.80±9.62	41.14±9.61	19.70±8.42	0.95	10.77**	1.16
Oxalic	ND	10.52±10.52	ND	4.19±2.89	–	–	–
Shikimic	0.65±0.25	0.47±0.16	1.36±0.37	0.73±0.23	0.19	2.83†	0.22
OA_{exuded}							
E. tereticornis							
Fumaric	0.64±0.35	0.94±0.41	1.23±0.20	0.93±0.65	0.29	0.24	2.46
Malic	12.89±12.89	20.57±20.57	36.40±29.27	17.88±17.88	–	–	–
Oxalic	42.38±17.59	34.19±16.18	37.63±11.86	45.10±21.70	0.03	0.00	0.20
Shikimic	0.23±0.17	1.22±0.85	1.82±0.64	0.76±0.48	2.73†	0.03	5.74*
H. sericea							
cis-aconitic	0.70±0.28	0.39±0.26	0.95±0.26	1.09±0.48	2.07	0.21	0.63
Oxalic	ND	5.00±5.00	ND	6.95±6.95	–	–	–
trans-aconitic	9.72±1.77	15.56±2.98	11.52±2.26	20.43±6.02	0.80	6.34*	0.00
M. stipoides							
Citric	ND	18.06±11.84	14.26±9.34	26.82±11.74	1.50	4.95*	3.55†
Fumaric	0.33±0.10	0.33±0.14	0.63±0.21	0.51±0.13	2.33	0.24	0.00
Malic	7.94±3.90	11.66±8.04	31.35±6.41	17.10±9.92	2.52	1.89	2.21
Oxalic	ND	5.80±5.80	ND	ND	–	–	–
Shikimic	0.12±0.09	ND	0.50±0.13	0.36±0.17	3.89*	2.34	5.76*

ND, undetected OAs. When OA was detected in only one pot, means and standard errors are the same.

Table S5 Summary of redundancy analysis on the composition of organic acids in rhizosphere soil (OA_{rhizo}) and exuded from roots (OA_{exuded}), with CO_2 and water treatments as explanatory variables, for *Eucalyptus tereticornis*, *Hakea sericea* and *Microlaena stipoides*. The results of statistical analyses are shown alongside the corresponding *F*-values with significance codes: *P* <0.1 (†), <0.05 (*), <0.01 (**). Degrees of freedom for *F* tests used for permutation tests are shown in parentheses.

Variable	CO_2	Water	Model
OA_{rhizo}			
E. tereticornis	F(1, 17)=3.79**	F(1, 17)=2.98*	F(2, 17)=3.38**
H. sericea	–	–	F(2, 29)=0.72
M. stipoides	F(1, 29)=2.69*	F(1, 29)=2.63*	F(2, 29)=2.66*
OA_{exuded}			
E. tereticornis	–	–	F(2, 17)=0.27
H. sericea	–	–	F(2, 29)=1.48
M. stipoides	F(1, 29)=2.42†	F(1, 29)=3.21*	F(2, 29)=2.82*