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Supplementary Material

Delayed greening in phosphorus-efficient *Hakea prostrata* (Proteaceae) is a photoprotective and nutrient-saving strategy

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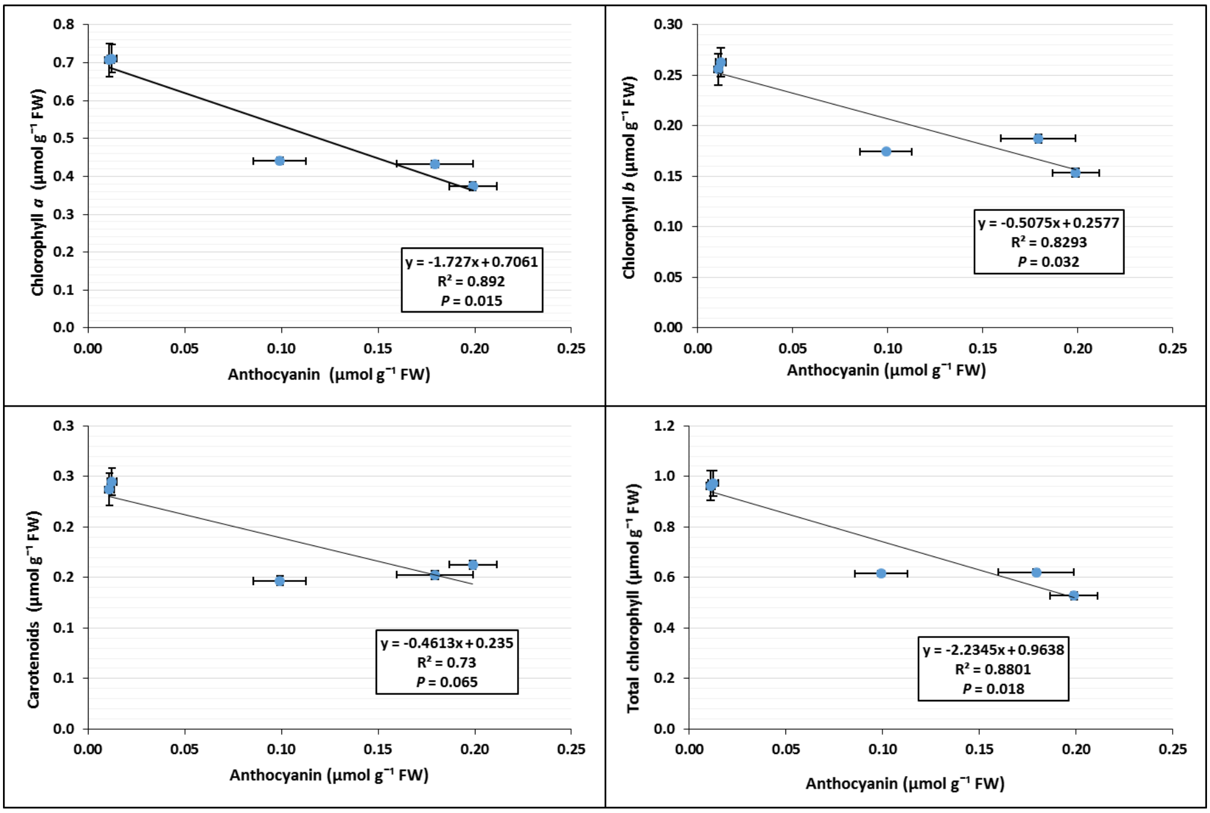


Fig. S1. Correlation between concentrations of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, carotenoids, and total chlorophyll) and anthocyanins at five developmental stages of *Hakea prostrata* leaves.

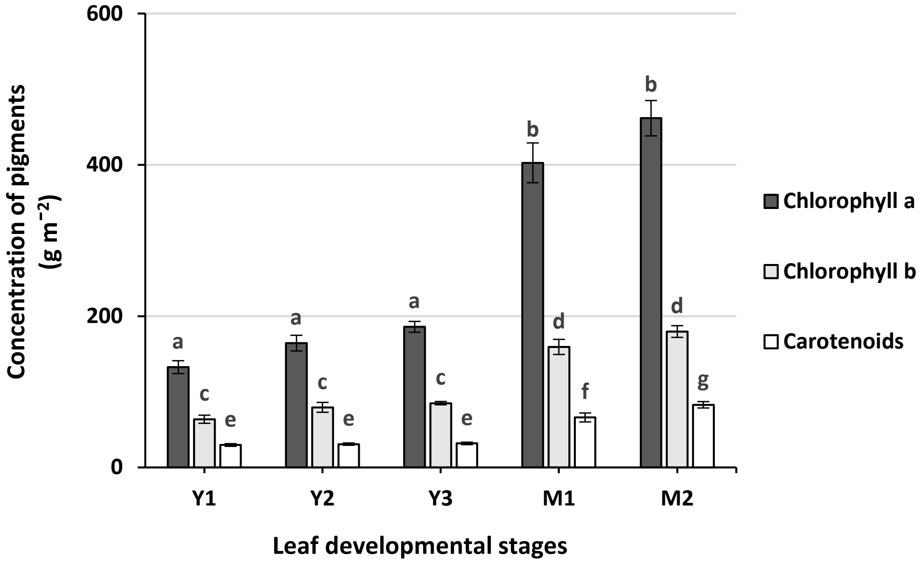


Fig. S2. Photosynthetic pigment concentrations per unit area in the leaves of *Hakea prostrata* during development. Values are means ± s.e.; $n = 9$ (nine sprigs of leaves from seven plants). Variation across the developmental stages was analysed separately for each pigment using one-way ANOVA. Different letters above the bars indicate significant differences among leaf stages at $P < 0.05$ level. Y1: Young 1, Y2: Young 2, Y3: Young 3, M1: Mature 1 and M2: Mature 2.

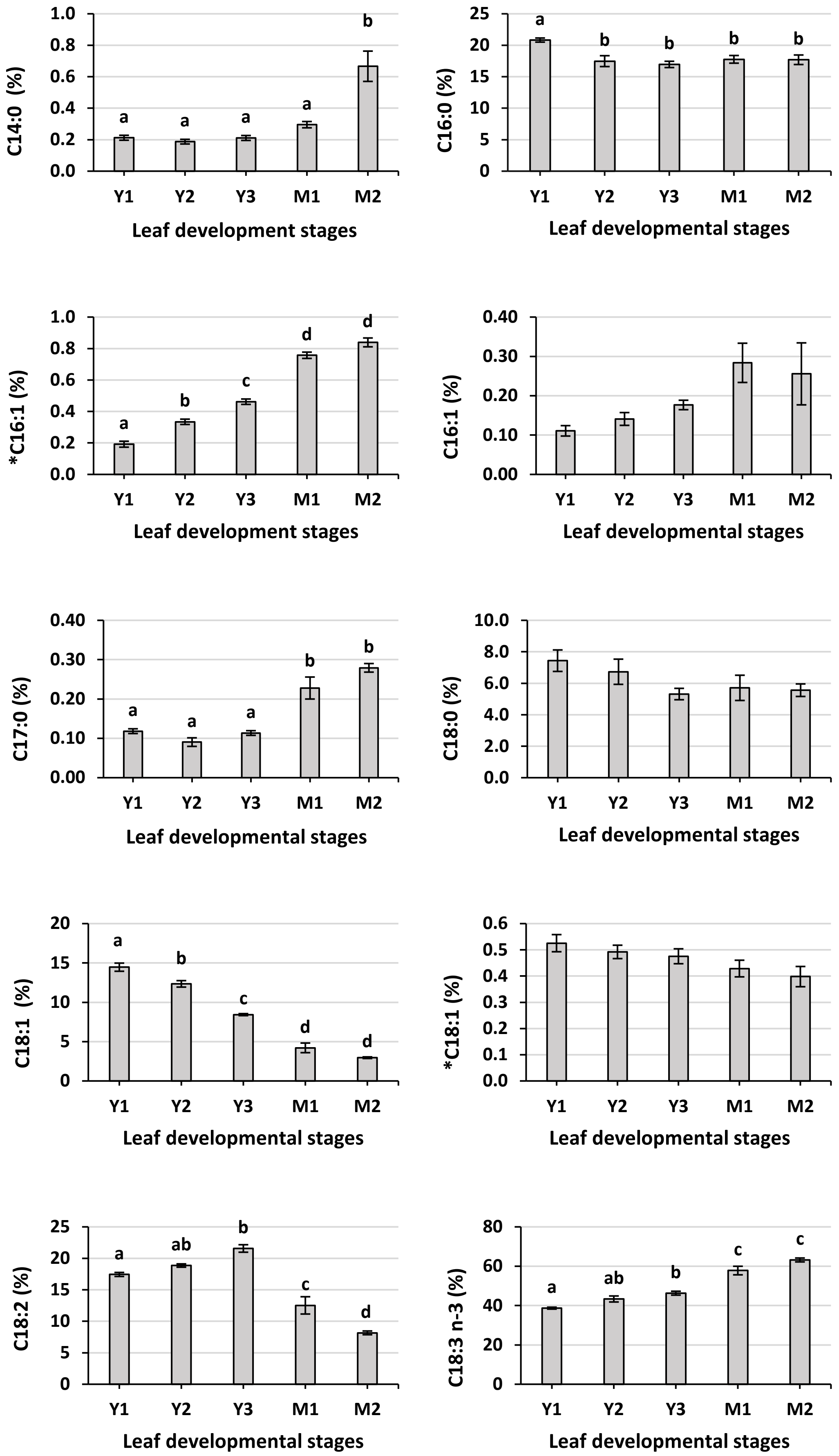

C14:0 (%)
a a a a b
Y1 Y2 Y3 M1 M2
Leaf development stages
C16:0 (%)
a b b b b
Y1 Y2 Y3 M1 M2
Leaf developmental stages
*C16:1 (%)
a b c d d
Y1 Y2 Y3 M1 M2
Leaf development stages
C16:1 (%)
Y1 Y2 Y3 M1 M2
Leaf developmental stages
C17:0 (%)
a a a b b
Y1 Y2 Y3 M1 M2
Leaf developmental stages
C18:0 (%)
Y1 Y2 Y3 M1 M2
Leaf developmental stages
C18:1 (%)
a b c d d
Y1 Y2 Y3 M1 M2
Leaf developmental stages
*C18:1 (%)
Y1 Y2 Y3 M1 M2
Leaf developmental stages
C18:2 (%)
a ab b c d
Y1 Y2 Y3 M1 M2
Leaf developmental stages
C18:3 n-3 (%)
a ab b c c
Y1 Y2 Y3 M1 M2
Leaf developmental stages

Fig. S3. Differences in the proportion of 10 fatty acids at five developmental stages of *Hakea prostrata* leaves. Values from FAME analysis are means ± s.e.; *n* = 5 (one sprig of leaves from each of five plants). Different letters represent significant differences between leaf stages at $P < 0.05$ based on ANOVA followed by Tukey's HSD post-hoc test. Y1: Young 1, Y2: Young 2, Y3: Young 3, M1: Mature 1 and M2: Mature 2. C14:0 = myristic acid, C16:0= palmitic acid, C16:1 = palmitoleic acid, *C16:1: unconfirmed isomer 1, C17:0 = margaric acid, C18:0 = stearic acid, C18:1 = oleic acid, *C18:1 = unconfirmed isomer 2, C18:2 = linoleic acid and C18:3 n-3 = α-linolenic acid.

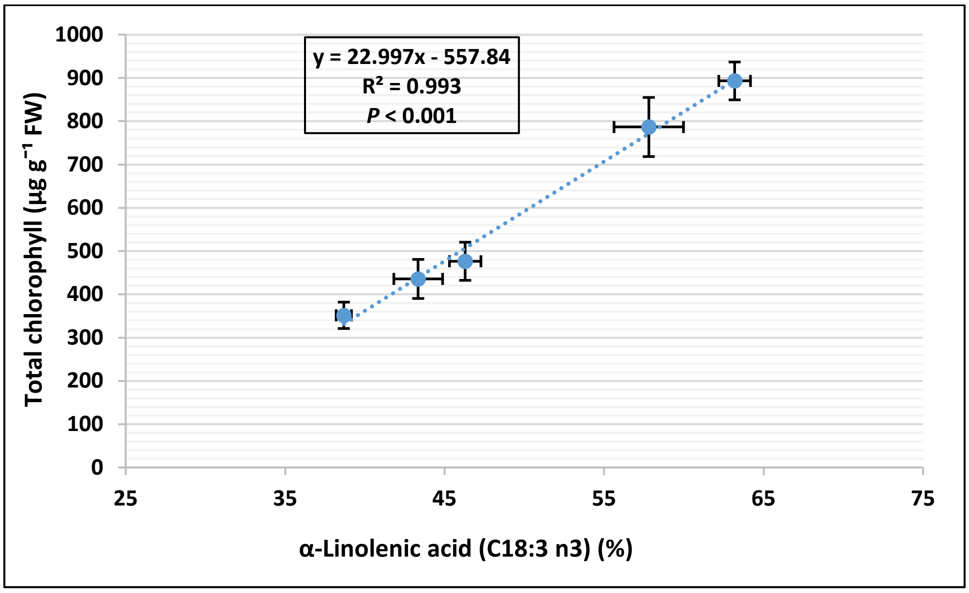


Fig. S4. Correlation between total chlorophyll and α-linolenic (C18:3 n3) concentrations at five developmental stages of *Hakea prostrata* leaves. Values are means ± s.e., *n* =7 (one sprig of leaves from each of seven plants) for total chlorophyll and *n* = 5 (one sprig of leaves from each of five plants) for α-linolenic acid.

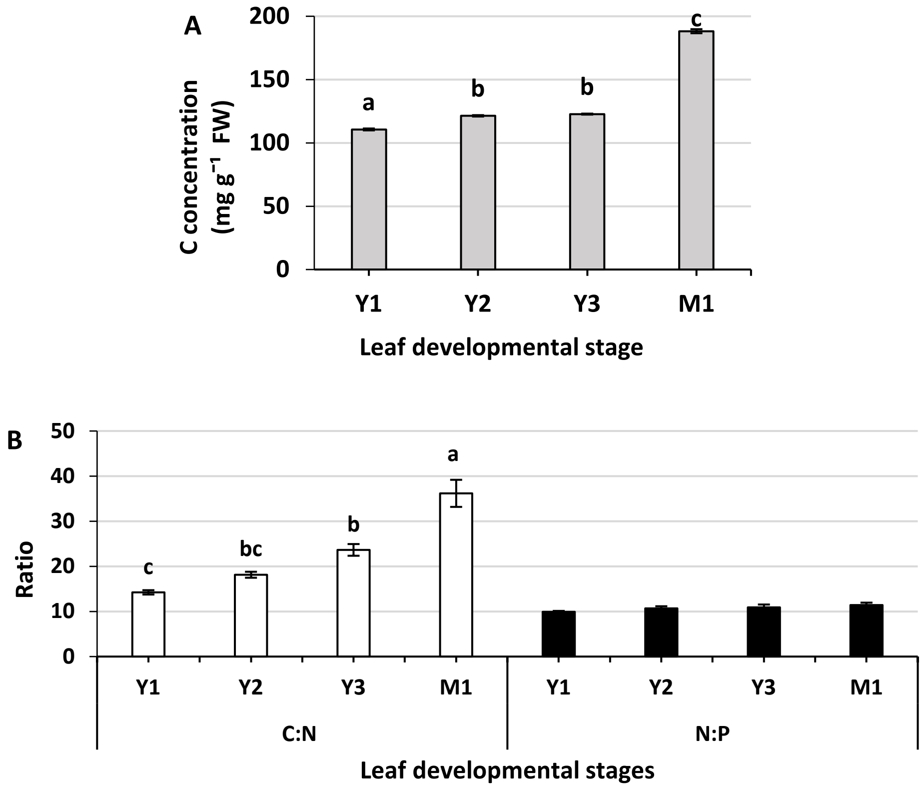
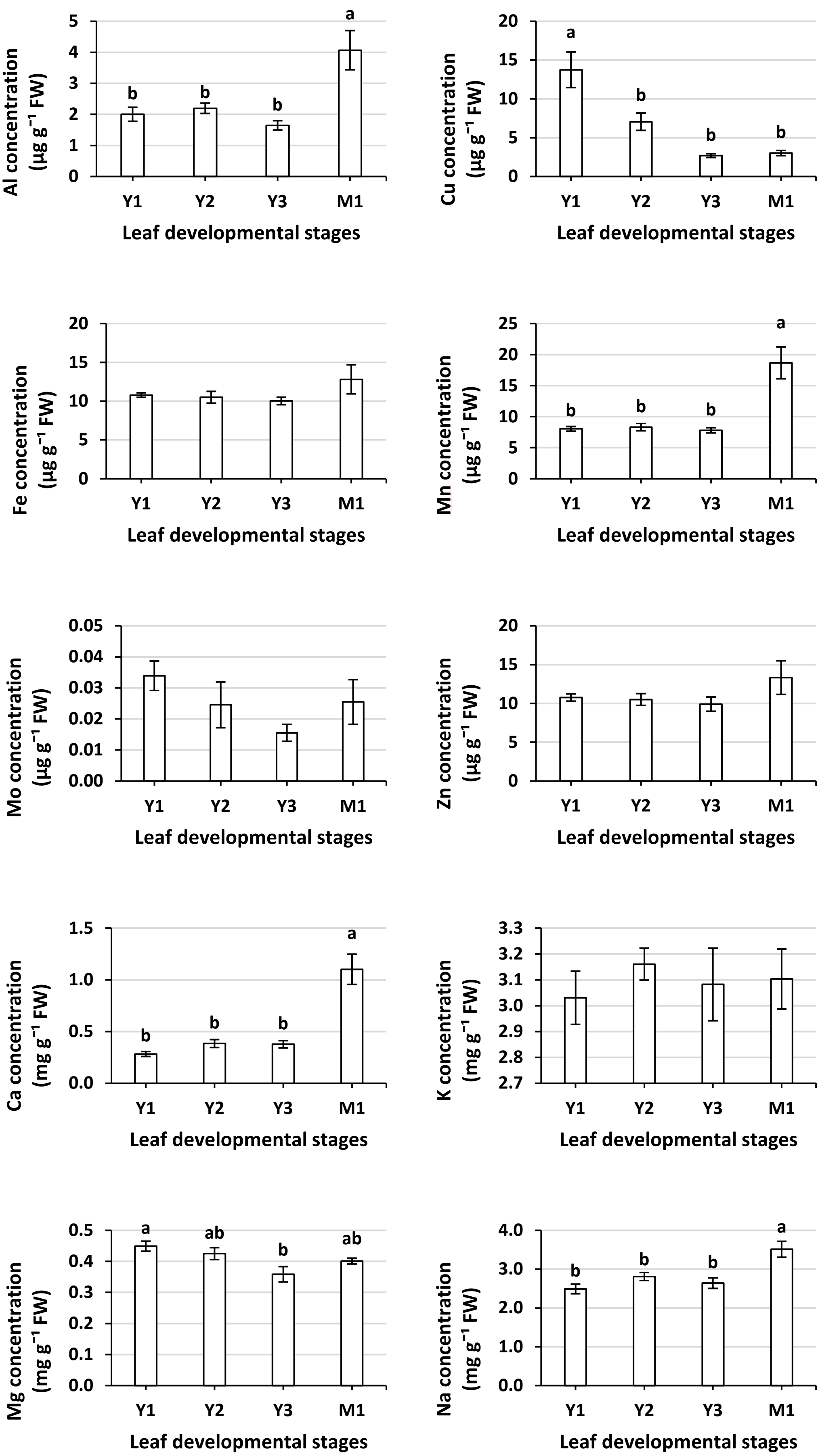


Fig. S5. Differences in (**A**) carbon (C) concentrations and (**B**) carbon : nitrogen (C : N) and nitrogen – phosphorus (N : P) ratios in developing leaves of *Hakea prostrata* grown in fertilised soil. Values are means ± s.e.; *n* = 10 (10 sprigs of leaves from five plants) in **A**, and means ± s.e. in **B**. Different letters represent significant differences among leaf stages at $P < 0.05$ based on ANOVA followed by Tukey's HSD post-hoc test. Y1: Young 1, Y2: Young 2, Y3: Young 3, M1: Mature 1 and M2: Mature 2.



Al concentration (µg g⁻¹ FW)
0
1
2
3
4
5
a
b
b
b
Y1
Y2
Y3
M1
Leaf developmental stages
Cu concentration (µg g⁻¹ FW)
0
5
10
15
20
a
b
b
b
Y1
Y2
Y3
M1
Leaf developmental stages
Fe concentration (µg g⁻¹ FW)
0
5
10
15
20
Y1
Y2
Y3
M1
Leaf developmental stages
Mn concentration (µg g⁻¹ FW)
0
5
10
15
20
25
a
b
b
b
Y1
Y2
Y3
M1
Leaf developmental stages
Mo concentration (µg g⁻¹ FW)
0.00
0.01
0.02
0.03
0.04
0.05
Y1
Y2
Y3
M1
Leaf developmental stages
Zn concentration (µg g⁻¹ FW)
0
5
10
15
20
Y1
Y2
Y3
M1
Leaf developmental stages
Ca concentration (mg g⁻¹ FW)
0.0
0.5
1.0
1.5
a
b
b
b
Y1
Y2
Y3
M1
Leaf developmental stages
K concentration (mg g⁻¹ FW)
2.7
2.8
2.9
3.0
3.1
3.2
3.3
Y1
Y2
Y3
M1
Leaf developmental stages
Mg concentration (mg g⁻¹ FW)
0.0
0.1
0.2
0.3
0.4
0.5
a
ab
b
ab
Y1
Y2
Y3
M1
Leaf developmental stages
Na concentration (mg g⁻¹ FW)
0.0
1.0
2.0
3.0
4.0
a
b
b
b
Y1
Y2
Y3
M1
Leaf developmental stages

Fig. S6. Concentration profiles of all mineral elements analysed (aluminium (Al), calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na) and zinc (Zn)) in developing leaves of *Hakea prostrata* grown in fertilised soil. Values are means ± s.e.; $n = 5$ (one sprig of leaves from five plants). Different letters represent significant differences between leaf stages at $P < 0.05$ based on ANOVA followed by Tukey HSD post-hoc test. Y1: Young 1, Y2: Young 2, Y3: Young 3 and M1: Mature 1.

Table S1. Fresh to dry weight ratio and percentage moisture content at five developmental stages of *Hakea prostrata* leaves

Values are means ± s.e.; *n* = 7 (one sprig of leaves from seven plants).

Leaf developmental stage	Fresh weight : dry weight ratio	Moisture content (%)
Young 1 (Y1)	4.4 ± 0.08	77 ± 0.4
Young 2 (Y2)	4.0 ± 0.23	74 ± 1.5
Young 3 (Y3)	3.9 ± 0.18	74 ± 1.2
Mature 1 (M1)	2.5 ± 0.08	60 ± 1.2
Mature 2 (M2)	2.2 ± 0.06	54 ± 1.2

Biomass analysis

Leaves of *H. prostrata* (n = 3 to 10, depending on stage) were harvested and their fresh weight (FW) was recorded. The leaf material was dried at 60°C for four days to determine leaf dry weight (DW). Leaf FW:DW ratios were used to calculate leaf moisture percentage (%) according to the formula:

$$\left(\frac{FW - DW}{FW}\right) \times 100$$

Table S2. List of 10 fatty acids identified through fatty acid methyl ester (FAME) analysis in *Hakea prostrata* leaves from developmental stages Y1 to M2

Fatty acid	Common name	Lipid numbers	Retention time (Minutes)
Tetradecanoic acid	Myristic acid	C14:0	9.34
Hexadecanoic acid	Palmitic acid	C16:0	11.17
Hexadecenoic acid Iso. 1	Palmitoleic acid	C16:1	11.49
Hexadecenoic acid Iso. 2	Unconfirmed isomer 1	*C16:1	11.74
Heptadecanoic acid	Margaric acid	C17:0	12.32
Octadecanoic acid	Stearic acid	C18:0	13.68
cis-9-Octadecenoic acid	Oleic acid	C18:1	14.02
Octadecenoic acid Iso. 2	Unconfirmed isomer 2	*C18:1	14.12
cis-9, 12-Octadecadienoic acid	Linoleic acid	C18:2	14.76
cis-9, 12, 15-Octadecatrienoic acid	α-Linolenic acid	C18:3 n-3	15.84

Table S3. Concentrations of mineral elements in mature leaves of *Hakea prostrata* compared with adequate concentrations in crop plants (Epstein and Bloom 2005; White and Brown 2010)

NA= not applicable

Element	**Concentrations in mature leaves of *Hakea prostrata***	**Adequate concentrations in leaves of crop plants**
Aluminium	10 μg g^{-1}	NA
Calcium	3 mg g^{-1}	5 mg g^{-1}
Copper	8 μg g^{-1}	6 μg g^{-1}
Iron	33 μg g^{-1}	100 μg g^{-1}
Magnesium	1 mg g^{-1}	2 mg g^{-1}
Manganese	47 μg g^{-1}	50 μg g^{-1}
Molybdenum	0.06 μg g^{-1}	0.1 μg g^{-1}
Nitrogen	14 mg g^{-1}	15 mg g^{-1}
Phosphorus	0.3 mg g^{-1}	2 mg g^{-1}
Potassium	8 mg g^{-1}	10 mg g^{-1}
Sodium	9 mg g^{-1}	10 μg g^{-1}
Sulfur	2 mg g^{-1}	1 mg g^{-1}
Zinc	34 μg g^{-1}	20 μg g^{-1}

References

Epstein E, Bloom AJ (2005) Mineral nutrition of plants: principles and perspectives. Sinauer Associates, Inc., Sunderland, MA, USA.

White PJ, Brown PH (2010) Plant nutrition for sustainable development and global health. *Annals of Botany* **105**, 1073–1080.