

# Manganese distribution in the Mn-hyperaccumulator *Grevillea meisneri* from New Caledonia

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## SUPPLEMENTARY METHODS

### *Nursery growth conditions*

The germination of seeds was made in a mixture of river sand and coir. Round particles of river sand avoid abrasion on seedlings during removal. The sprouts were then transplanted into small pots with a mixture of topsoil (1/3), compost (1/3) and coir (1/3). No manganese was added during the plants' growth in the nursery.

Plants were watered twice a day during 5 minutes in the wet season and twice a day during 10 minutes during the dry season. One month before plantation, watering was gradually decreased: seedlings were watered only once each day during the first two weeks, then once every two days for the last two weeks. The temperature during nursery growth was not regulated, but was the ambient temperature, which ranged from about 30 °C (day) to 20 °C (night) in the wet season and 35 °C to 25 °C in the dry season.

### *Monitoring of the plants' growth*

Survival and size of each transplanted plant, and of plants naturally developing *in situ* near the transplanted plants, was monitored once a year in the studied mining site. Two parameters, height of the plant and root collar diameter, were chosen as measures of plant size. Both can be quickly and easily measured in a reproducible manner and provide useful information on the response of plants to environment.

Two other parameters were monitored to study the efficiency of the phytoremediation program: the biomass production and the foliar mineral composition. The biomass production was estimated by counting the number of leaves and measuring the minimal and maximal dimensions of each plant. In order to minimize any impact of sampling on the plant's growth, only a few leaves were sampled for analyses of mineral composition.

## SUPPLEMENTARY FIGURES AND TABLES

**Table S1.** Composition and chemical characteristics of soils of the 16 parcels of ‘Creek à Paul’ under rehabilitation, established by ICP-MS analyses.

Site	pH		Available N (mg/kg)		Available P (mg/kg)	Extrait DTPA (mg/kg)		Elemental composition (mg/kg)											
	H <sub>2</sub> O	KCl	N(NO <sub>3</sub> )	N(NH <sub>4</sub> )		Mn	Ni	Ca	Mg	Na	K	Fe	Co	Cr	Mn	Ni	Al	P	Zn
CAP A	7.07	5.51	0.1	8.9	1	604	170	7 196	56 506	2 389	710	127 476	1 204	12 119	8 479	6 104	36 082	13	124
CAP B	7.00	5.19	0.2	4.8	1	424	135	187	27 526	458	118	120 489	699	7 113	1 240	15 316	13 692	1	93
CAP C	7.08	5.47	0.0	7.2	0	492	131	1 148	32 094	859	106	123 277	724	14 177	4 178	7 053	24 080	14	133
CAP D	6.98	5.46	0.1	7.2	0	343	144	5 458	72 842	2 506	303	126 155	1 447	13 019	9 333	5 600	41 703	4	120
CAP E	6.33	4.69	0.2	5.9	0	416	216	9 699	57 289	2 934	331	120 178	1 182	12 902	8 667	4 723	44 143	12	119
CAF F	6.44	4.82	0.3	7.4	0	531	138	446	28 481	628	104	142 882	1 264	20 883	6 915	5 638	31 025	22	176
CAP G	6.31	4.95	1.0	7.1	0	331	200	701	34 510	657	151	158 153	1 135	17 846	8 167	9 455	29 295	24	147
CAP H	6.74	4.56	0.2	4.3	0	227	202	1 305	59 585	742	113	122 635	381	11 871	2 649	12 152	21 866	16	111
CAP I	6.89	4.42	0.6	3.7	2	231	232	2 401	68 530	660	164	119 412	475	12 267	3 384	12 132	21 213	1	128
CAP J	6.84	5.27	0.2	4.8	2	255	201	1446	46987	859	96	101863	526	8946	3052	9142	19419	5	162
CAP K	6.58	5.02	0.4	4.1	1	167	233	1504	49610	891	95	110301	449	11182	2015	10325	22204	14	180
CAP L	6.51	4.89	0.7	5.7	2	342	189	885	35757	592	56	109096	560	11867	2877	7856	22647	21	160
CAP M	5.95	4.48	0.3	9.4	2	163	185	713	28522	460	125	101200	395	7397	1749	7576	13700	4	140
CAP N	6.59	4.90	0.5	5.9	1	509	144	744	26779	634	88	99035	555	16125	2794	4740	24822	14	166
CAP O	6.05	4.48	0.6	5.6	0	370	189	829	26714	761	70	99570	572	11839	2885	6948	22860	9	162
CAP P	6.29	4.76	0.5	7.8	1	501	192	1154	29977	1267	103	115313	1225	14077	7480	6886	24119	5	174

**Table S2.** Composition and quantity of fertilizers applied to transplanted *G. meisneri* seedlings at 'Creek à Paul'.<sup>a</sup>

	<b>Quantity (g/plant)</b>	<b>N (kg/ha)</b>	<b>P (kg/ha)</b>	<b>K (kg/ha)</b>
<b>Yates nutricote</b>	13.85	200	88	166
<b>Superphosphate triple</b>	8.8	0	200	0
<b>Total</b>	22.65	200	288	166

<sup>a</sup> The quantity of fertilizer added was adjusted in order to have the same amount of nitrogen provided for the different treatments on the various mining sites under rehabilitation by our group.

**Table S3.** Analysis of Yates NUTRICOTE fertilizer provided by the supplier.

<b>Yates NUTRICOTE</b>	<b>Composition (%w/w)</b>
Nitrogen (N) as nitrate	7.0
Nitrogen (N) as ammonium	7.0
<b>Total Nitrogen</b>	<b>14.0</b>
Phosphorus (P) as water soluble	5.2
Phosphorus (P) as citrate soluble	0.9
<b>Total Phosphorus</b>	<b>6.1</b>
<b>Total Potassium (K) as nitrate</b>	<b>11.6</b>
Calcium (Ca) as phosphates	4.0
Inert coating	5.7

**Table S4.** Analysis of Superphosphate fertilizer provided by the supplier.

<b>Superphosphate</b>	<b>Composition (%w/w)</b>
<b>Total Nitrogen</b>	<b>0</b>
P <sub>2</sub> O <sub>5</sub>	46
<b>Total phosphorus</b>	<b>20</b>
K <sub>2</sub> O	0
CaO	15
S	1,3

**Table S5.** Foliar elemental composition of *G. rosa* ssp. *jenkinsii*, determined by MP-AES analyses.

	<b>Composition (ppm (%RSD))</b>				
Leaves of ' <i>G. rosa</i> <i>Jenkinsii</i> '	<b>Mn</b>	<b>Ca</b>	<b>K</b>	<b>Mg</b>	<b>Na</b>
	920 (1,32)	17640 (1,21)	4051 (0,82)	2390 (2,60)	122 (2,77)

**Table S6.** Foliar P and S composition of various species of *Grevillea* genus, determined by ICP-MS.

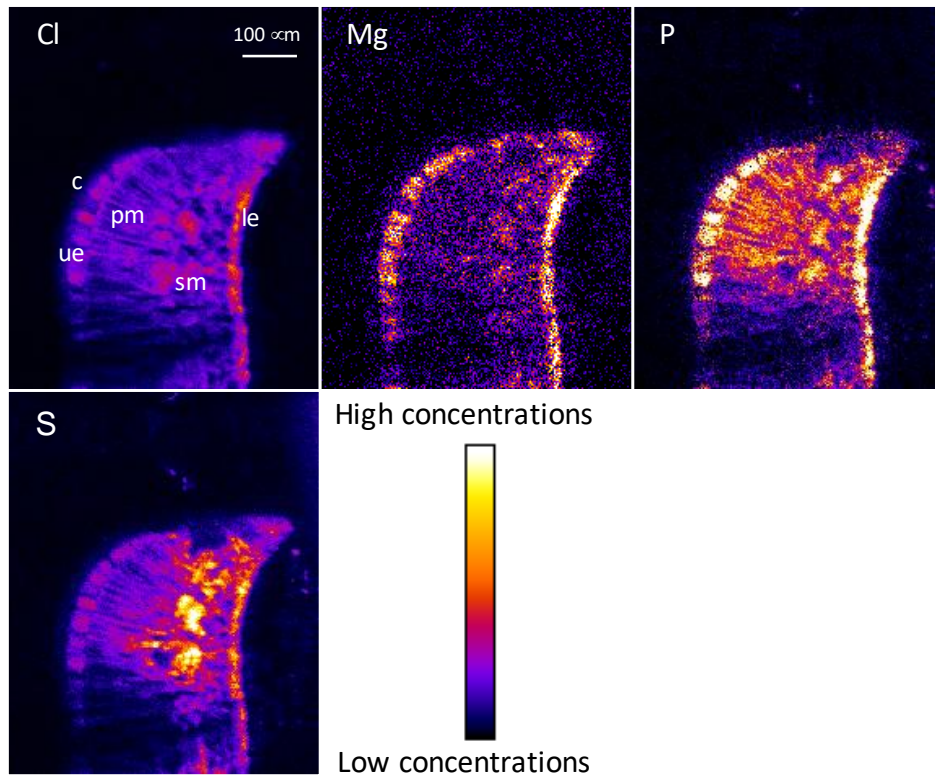
	Composition (ppm (%RSD))	
	P	S
<i>Grevillea meisneri</i> leaves	228.79 (2.15)	1294.50 (6.48)
<i>Grevillea gillivrayi</i> leaves	240.56 (2.35)	1816.94 (11.23)
<i>Grevillea exul</i> ssp. <i>exul</i> leaves	282.99 (1.26)	2561.52 (15.03)
<i>Grevillea 'rosa Jenkinsii'</i> leaves	1183.58 (0.83)	2232.81 (8.00)



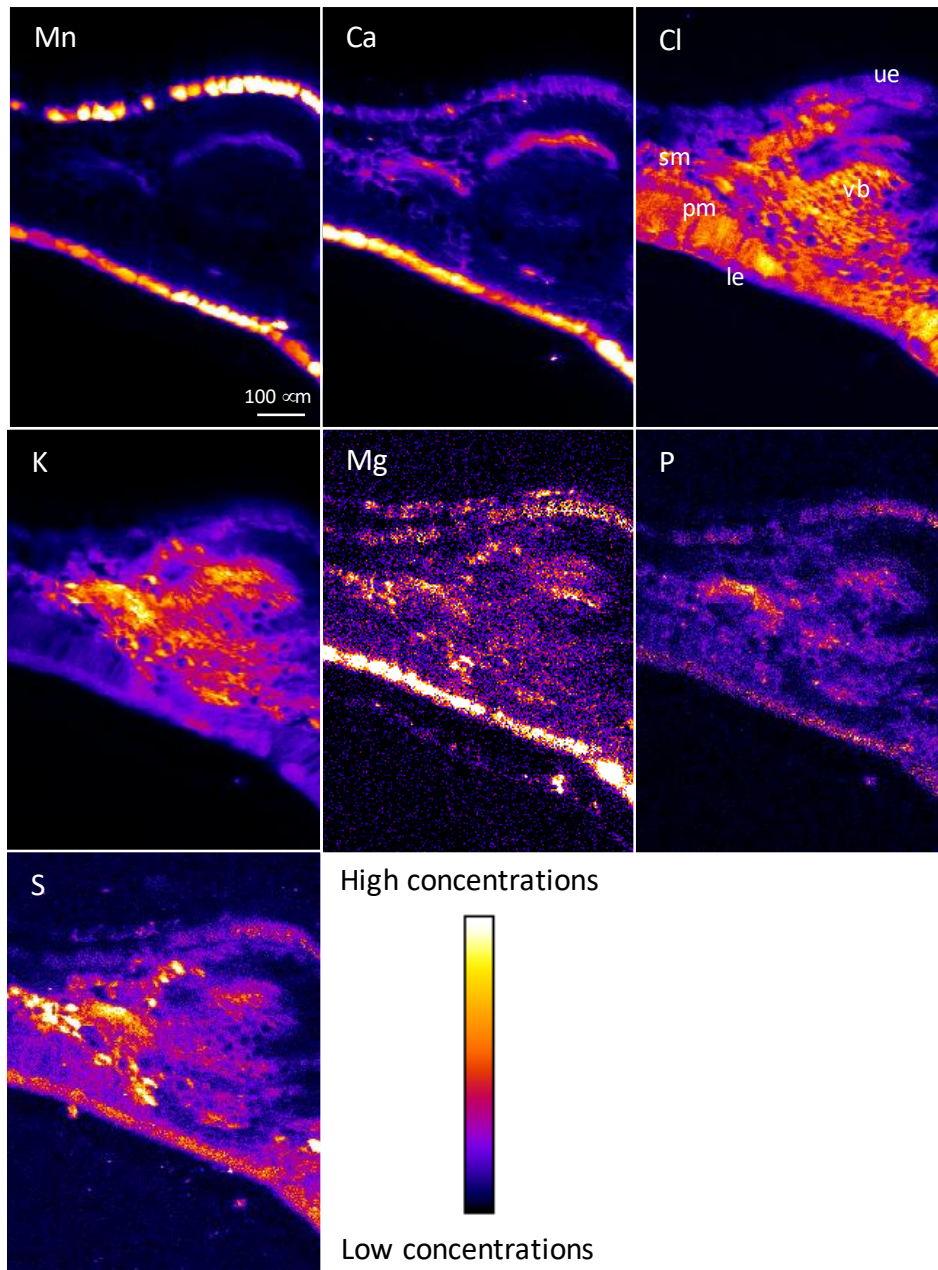
**Table S7.** Concentrations of anions in leaves of *Grevillea meisneri*, determined by ion chromatography analyses.

<b>Anions</b>	<b>Malate</b>	<b>Oxalate</b>	<b>Citrate</b>	<b>SO<sub>4</sub><sup>2-</sup></b>	<b>PO<sub>4</sub><sup>3-</sup></b>
<b>Concentration (mg.L<sup>-1</sup>)</b>	14.6	7.7	nd	10.9	39.2

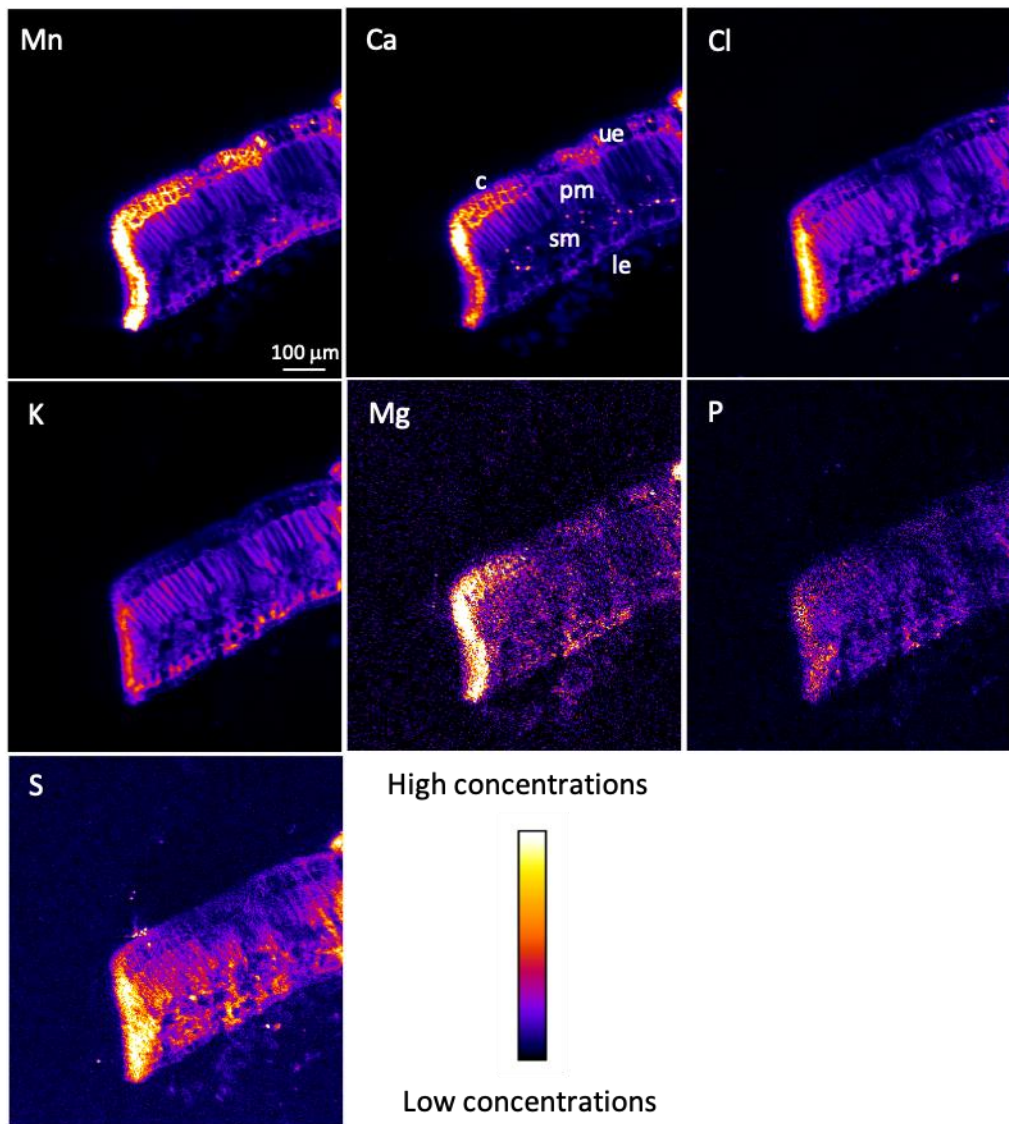
*nd: not detected.*



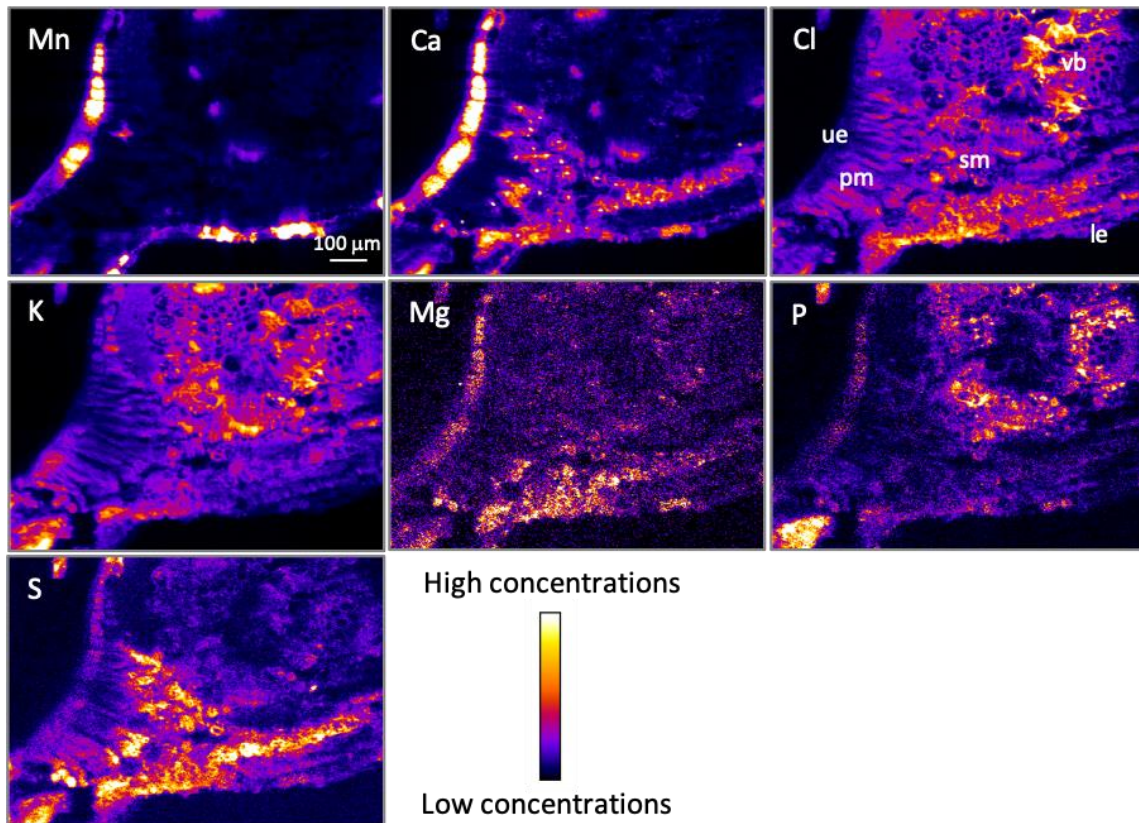
**Figure S1.** Cl, Mg, P and S  $\mu$ XRF maps of a frozen hydrated leaf margin cross-section of transplanted *G. meisneri*. The pixel size is 3  $\mu$ m. The intensity scales are different between elements. c, cuticle; le, lower epidermis; pm, palisade mesophyll; sm, spongy mesophyll, ue, upper mesophyll.



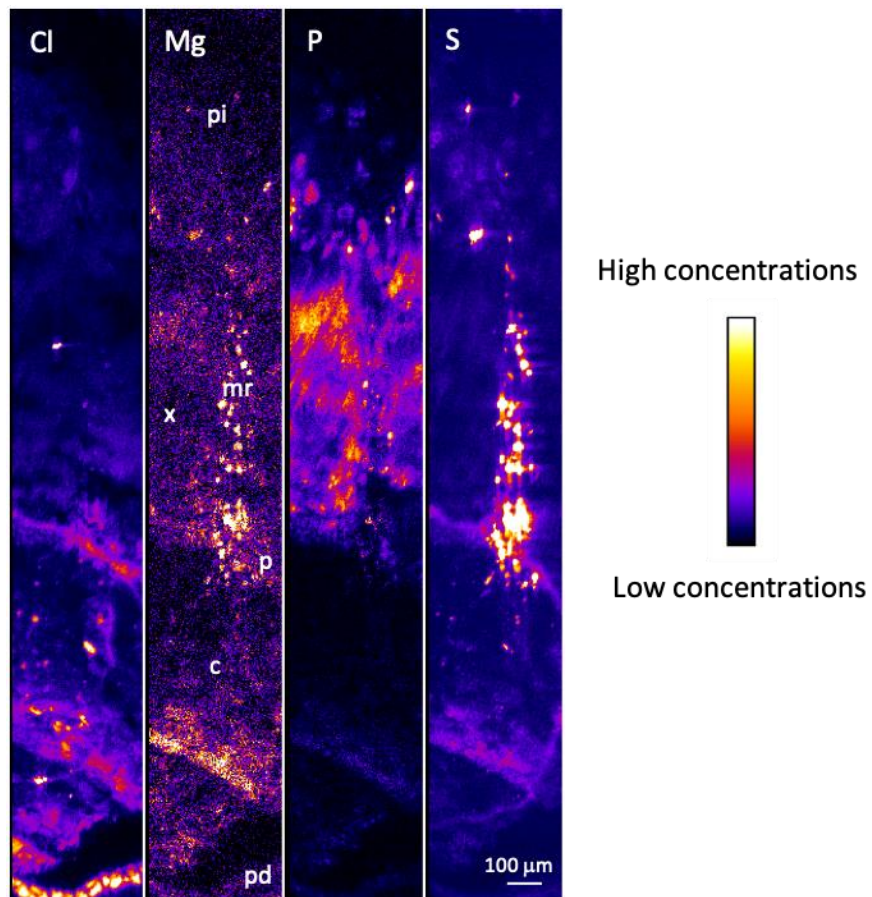
**Figure S2.** Mn, Ca, Cl, K, Mg, P and S  $\mu$ XRF maps of a frozen hydrated leaf mid-rib cross-section of transplanted *G. meisenri*. The pixel size is 3  $\mu$ m. The intensity scales are different between elements. le, lower epidermis; pm, palisade mesophyll; sm, spongy mesophyll; ue, upper epidermis; vb, vascular bundles.



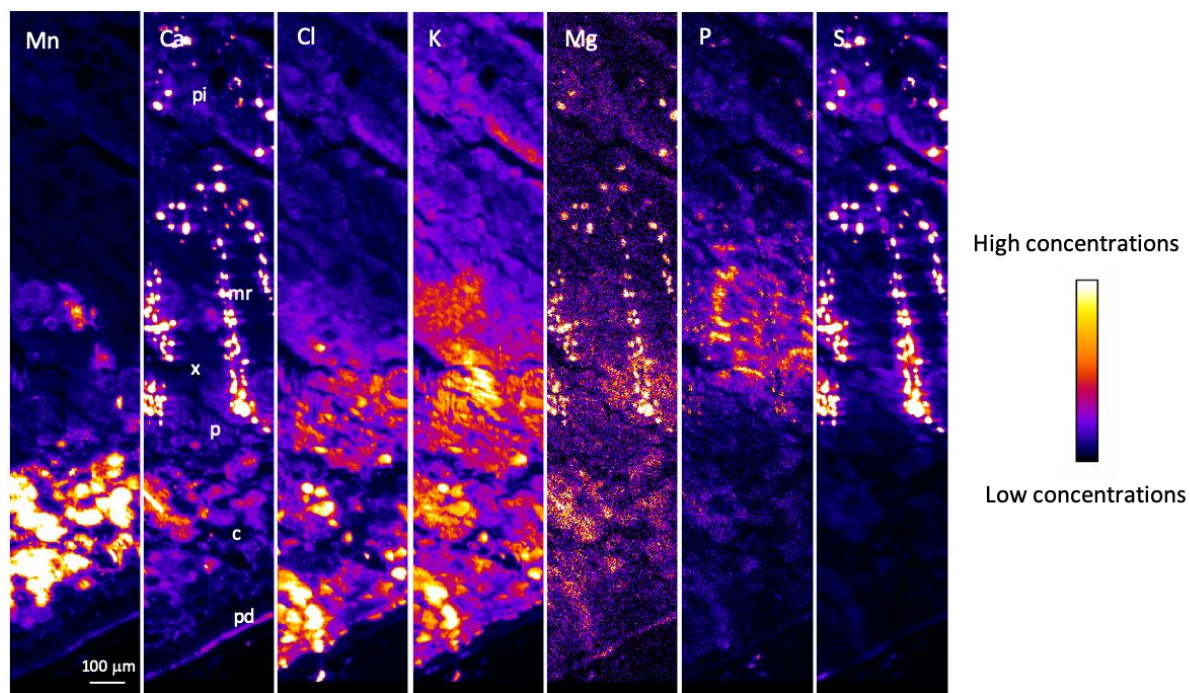
**Figure S3.** Mn, Ca, Cl, K, Mg, P and S  $\mu$ XRF maps of a frozen hydrated leaf margin cross-section of *G. meisneri* spontaneously growing in the site. The pixel size is 3  $\mu$ m. The intensity scales are different between elements. c, cuticle; le, lower epidermis; pm, palisade mesophyll; sm, spongy mesophyll, ue, upper mesophyll.



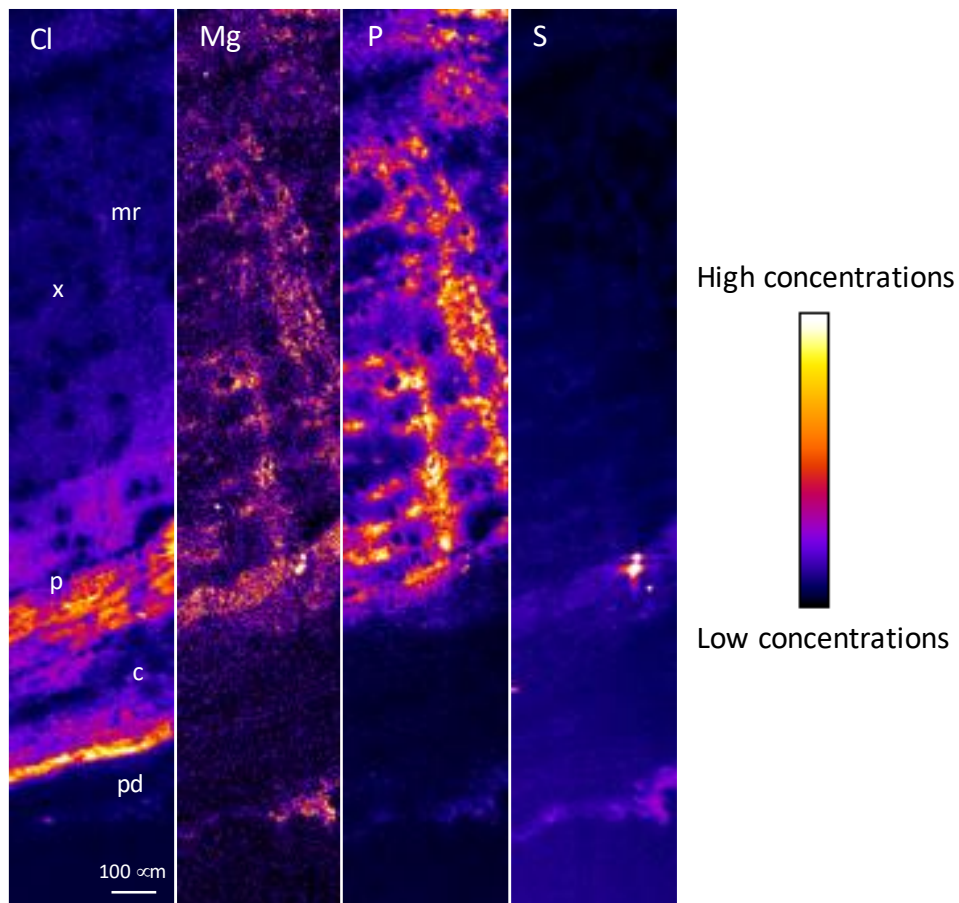
**Figure S4.** Mn, Ca, Cl, K, Mg, P and S  $\mu$ XRF maps of a frozen hydrated leaf mid-rib cross-section of *G. meisneri* spontaneously growing in the site. The pixel size is 3  $\mu$ m. The intensity scales are different between elements. le, lower epidermis; pm, palisade mesophyll; sm, spongy mesophyll; ue, upper epidermis; vb, vascular bundles.



**Figure S5.** Cl, Mg, P and S  $\mu$ XRF maps of a frozen hydrated stem cross-section of transplanted *G. meisneri*. The pixel size is 3  $\mu$ m. The intensity scales are different between elements. c, cortex; mr, medullary ray; p, phloem; pi, pith; pd, periderm; x, xylem.

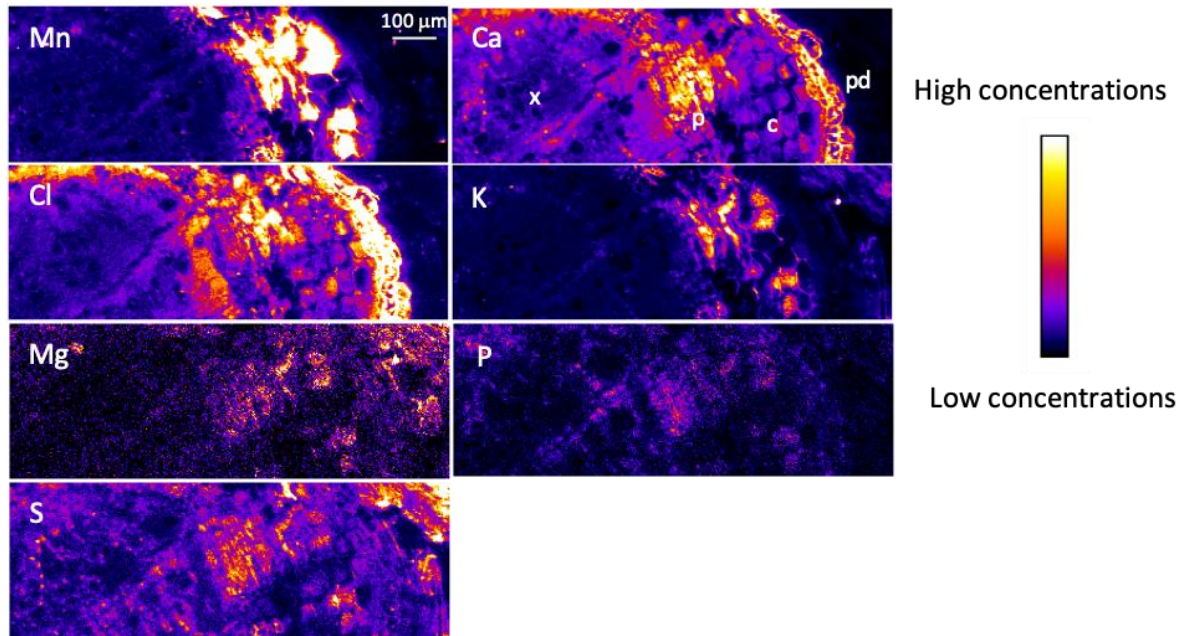


**Figure S6.** Mn, Ca, Cl, K, Mg, P and S  $\mu$ XRF maps of a frozen hydrated stem cross-section of *G. meisneri* spontaneously growing in the site. The pixel size is 3  $\mu$ m. The intensity scales are different between elements. le, lower epidermis; pm, palisade mesophyll; sm, spongy mesophyll; ue, upper epidermis; vb, vascular bundles.

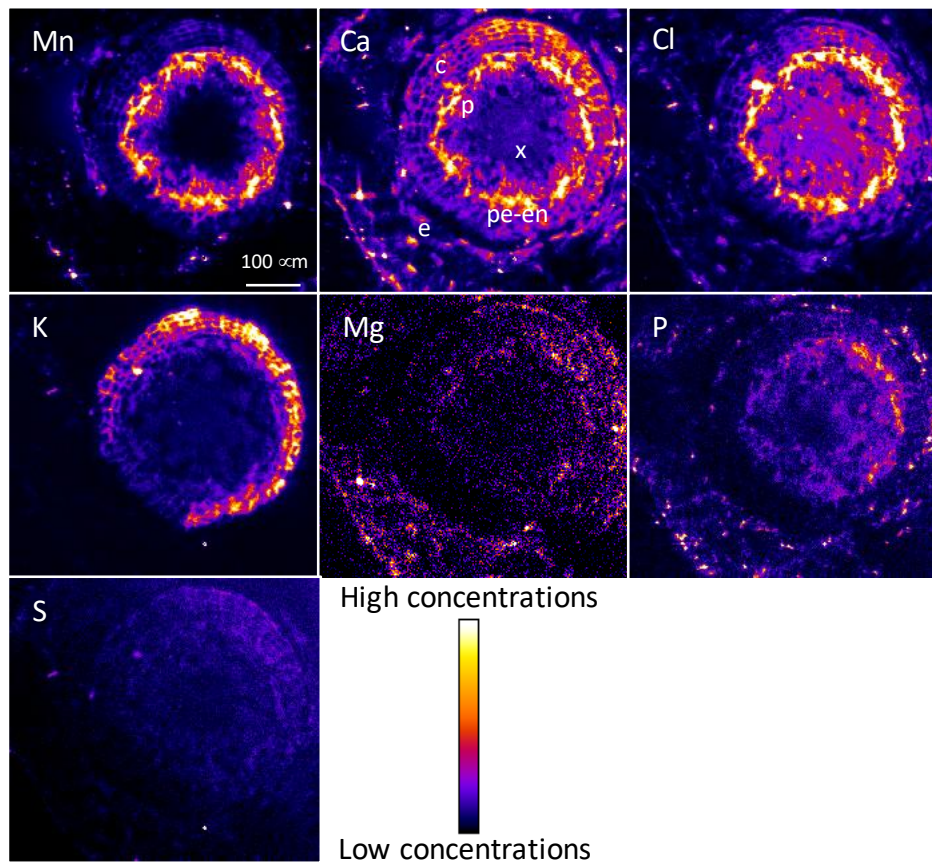


**Figure S7.** Cl, Mg, P and S  $\mu$ XRF maps of a frozen hydrated primary root cross-section of transplanted *G. meisneri*. The pixel size is 3  $\mu$ m. The intensity scales are different between elements. c, cortex; mr, medullary ray; p, phloem; pd, periderm; x, xylem.

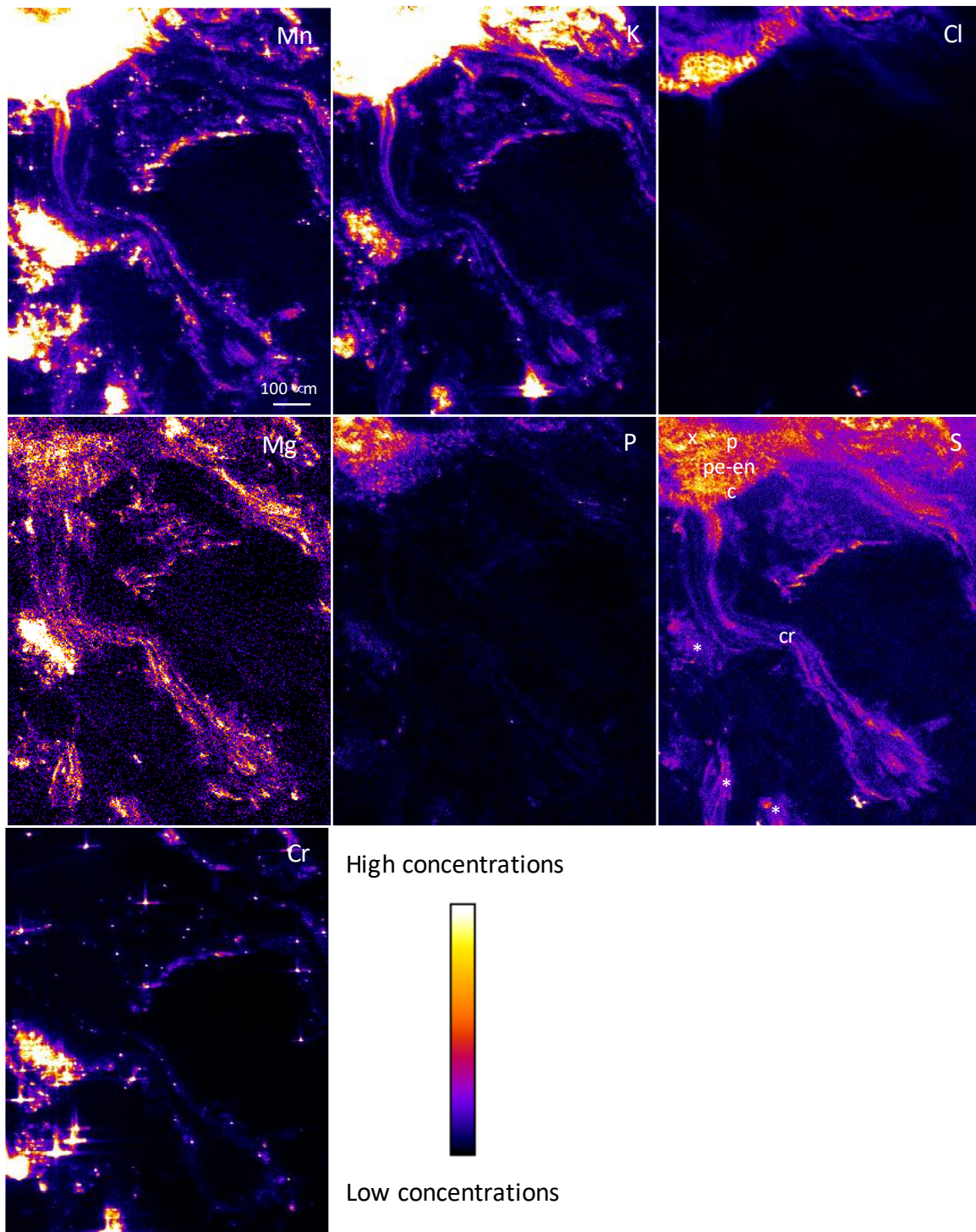




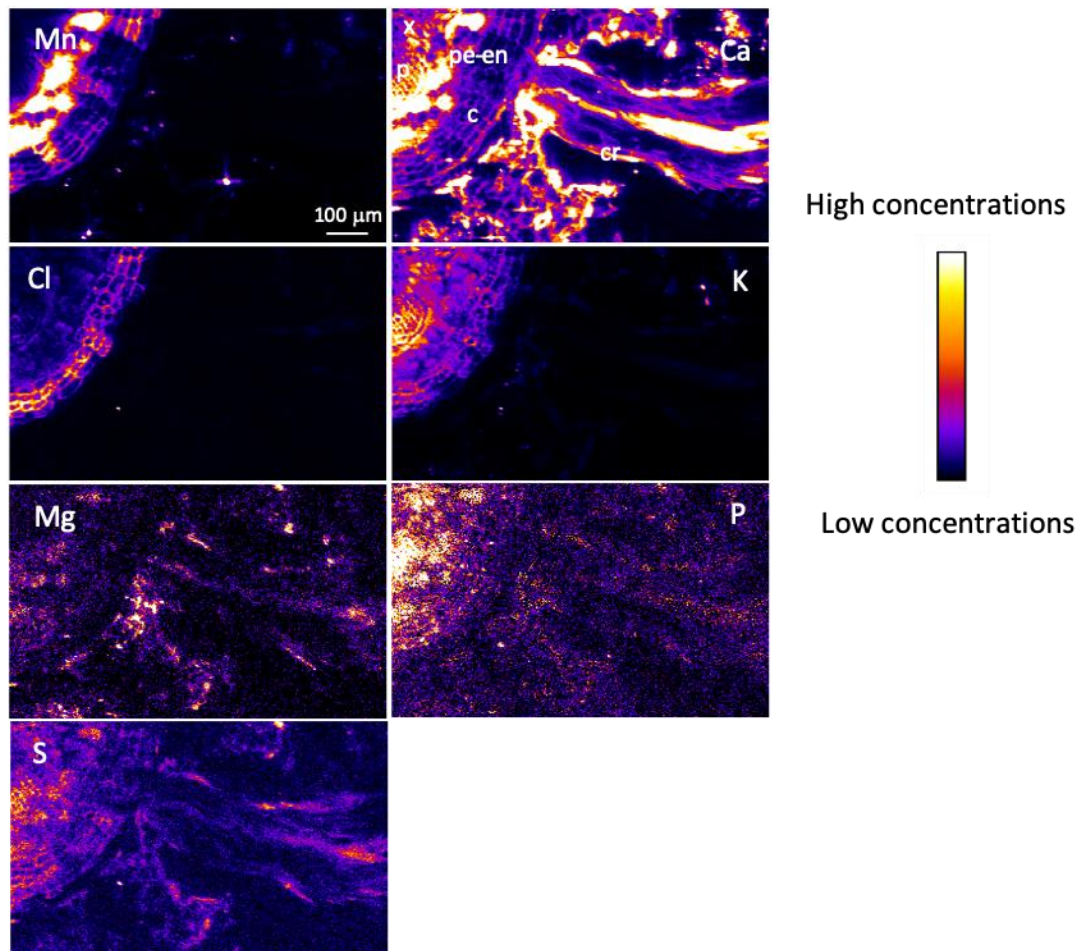
**Figure S8.** Mn, Ca, Cl, K, Mg, P and S μXRF maps of a frozen hydrated primary root cross-section of *G. meisneri* spontaneously growing in the site. The pixel size is 3 μm. The intensity scales are different between elements. c, cortex; p, phloem; pd, periderm; x, xylem.



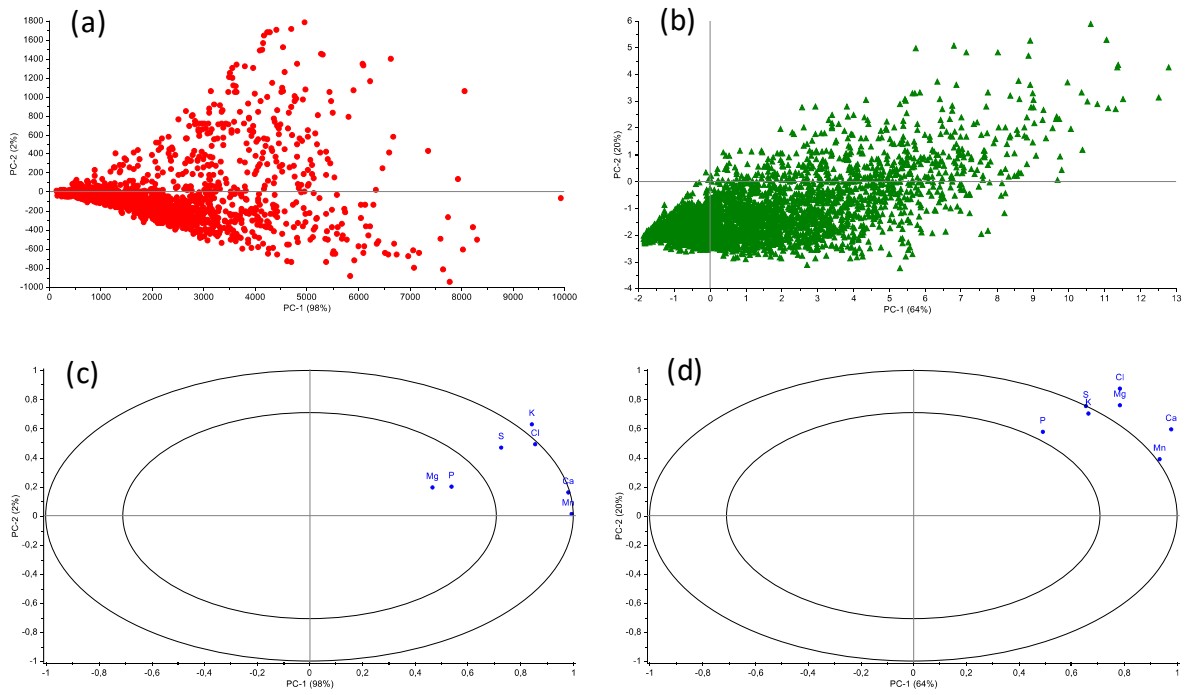
**Figure S9.** Mn, Ca, Cl, K, Mg, P and S  $\mu$ XRF maps of a frozen hydrated young root cross-section of transplanted *G. meisneri*. The pixel size is 3  $\mu$ m. The intensity scales are different between elements. c, cortex; e, epidermis; p, phloem; pe-en, pericycle-endodermis; x, xylem.



**Figure S10.** Mn, K, Cl, Mg, P, S and Cr  $\mu$ XRF maps of a frozen hydrated cluster root cross-section of transplanted *G. meisneri*. The pixel size is 3  $\mu$ m. The intensity scales are different between elements. Pixel intensities on Mn and K maps were over-increased to observed cluster rootlets. c, cortex; cr, cluster rootlet; p, phloem; pe-en, pericycle-endodermis; x, xylem. Asterisks indicate soil particles.



**Figure S11.** Mn, Ca, Cl, K, Mg, P and S  $\mu$ XRF maps of a frozen hydrated cluster root cross-section of *G. meisneri* spontaneously growing in the site. The pixel size is 3  $\mu$ m. The intensity scales are different between elements. c, cortex; cr, cluster rootlet; p, phloem; pe-en, pericycle-endodermis; x, xylem.

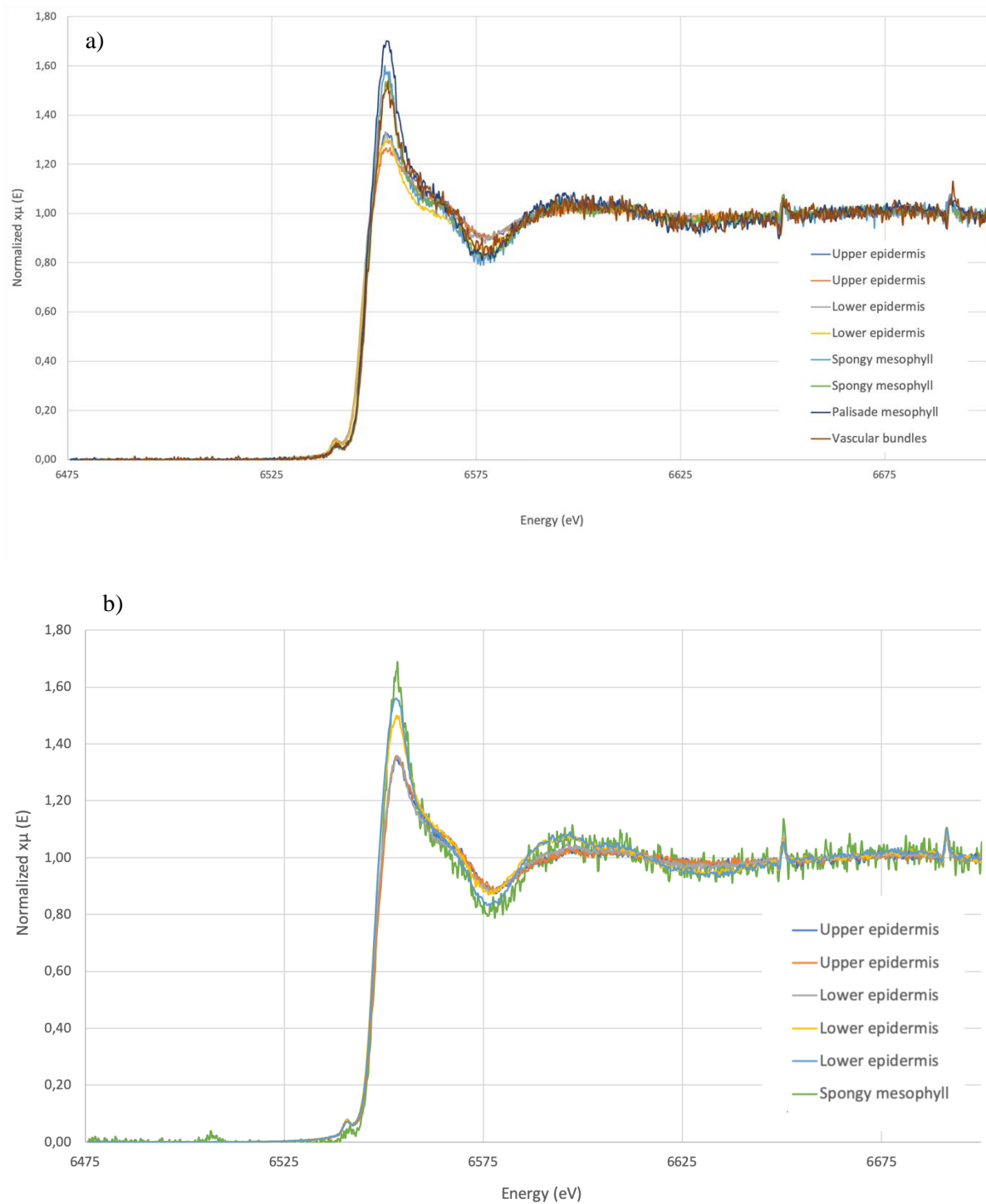


**Figure S12.** Graph depicting the position of all the pixels of fluorescence intensity, for all the elements from the a) lower epidermis and b) upper epidermis, as well as the correlation circle between the first two principal components of the PCA of the c) lower epidermis and d) upper epidermis of the leaf margin cross section of *G. meisneri*.

**Table S8.** Correlation coefficients between physiological elements among the main Mn sinks of the different organs of *G. meisneri* (ue: upper epidermis; le: lower epidermis; c: cortex; pe-en: pericycle and endodermis layers; p: phloem). Correlation *P*-values of correlation coefficients are all  $\leq 0.0001$  except where noted otherwise. The most significant correlations are put in bold type ( $< 0.5$ ).

	Leaf margin		Leaf mid-rib		Stem	Primary root	Cluster parent root	
	ue	le	ue	le	c	c	pe-en	p
Ca/Cl	<b>0.72</b>	<b>0.59</b>	0.36	0.49	0.29	0.45	0.44	<b>0.83</b>
Ca/K	0.48	<b>0.63</b>	<b>0.51</b>	<b>0.53</b>	0.42	0.41	<b>0.59</b>	<b>0.83</b>
Ca/Mg	<b>0.68</b>	0.36	<b>0.76</b>	0.45	<b>0.53</b>	0.35	<b>0.61</b>	0.36
Ca/P	<b>0.69</b>	0.48	0.40	0.42	0.42	0.32	<b>0.62</b>	0.06#
Ca/S	<b>0.69</b>	<b>0.54</b>	0.49	<b>0.53</b>	<b>0.65</b>	0.47	<b>0.57</b>	0.22
Cl/K	<b>0.68</b>	<b>0.69</b>	<b>0.90</b>	<b>0.90</b>	<b>0.77</b>	<b>0.81</b>	<b>0.70</b>	<b>0.90</b>
Cl/Mg	<b>0.56</b>	0.46	0.38	0.41	0.24	0.34	0.47	<b>0.55</b>
Cl/P	<b>0.70</b>	<b>0.70</b>	0.47	<b>0.59</b>	0.28	0.42	0.44	0.38
Cl/S	<b>0.85</b>	<b>0.79</b>	<b>0.68</b>	<b>0.75</b>	0.33	0.20	<b>0.55</b>	<b>0.52</b>
K/Mg	0.42	0.29	0.49	0.43	0.39	0.34	0.49	<b>0.54</b>
K/P	0.28	0.44	<b>0.54</b>	<b>0.62</b>	0.41	<b>0.50</b>	<b>0.68</b>	0.45
K/S	<b>0.64</b>	<b>0.60</b>	<b>0.58</b>	<b>0.78</b>	0.46	0.20	<b>0.78</b>	<b>0.55</b>
Mg/P	<b>0.50</b>	<b>0.52</b>	0.37	0.34	0.40	0.26	<b>0.64</b>	0.47
Mg/S	<b>0.55</b>	<b>0.52</b>	0.46	0.45	0.44	0.21	<b>0.63</b>	0.48
P/S	<b>0.67</b>	<b>0.78</b>	0.40	<b>0.59</b>	0.39	0.20	<b>0.75</b>	<b>0.80</b>

# *P*-value: 0.04



**Figure S13.**  $\mu$ XANES spectra at Mn K-edge obtained in different points of interest spotted among the upper epidermis, lower epidermis, spongy mesophyll, palisade mesophyll and vascular bundles of a) a frozen hydrated leaf mid-rib cross-section and b) a frozen hydrated leaf margin cross-section of transplanted *G. meisneri*