Electronic Supplementary Information (ESI) for Metallomics.

Evaluating effects of iron on manganese toxicity in soybean and sunflower using synchrotron-based X-ray fluorescence microscopy and X-ray absorption spectroscopy

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Supplementary Information
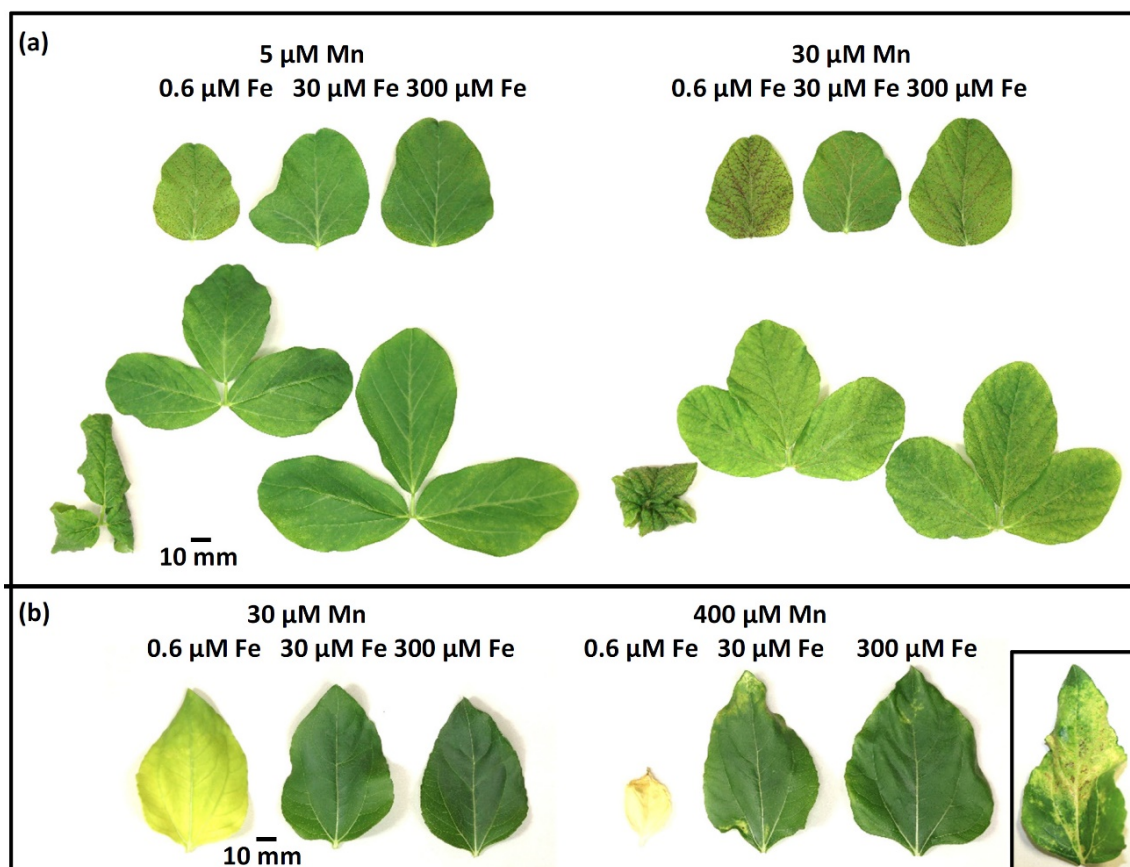
Supplementary Fig. S1 Visible effects of Mn and Fe in solution on soybean and sunflower leaves. (a) Unifoliate and trifoliate leaves of soybean grown for 1 week at 5 and 30 µM Mn with 0.6, 30, and 300 µM Fe (Experiment 2). (b) Lower alternate leaves of sunflower grown at 30 and 400 µM Mn with 0.6, 30, and 300 µM Fe. Insert: Unpublished image from the study of Blamey et al. (2018a) showing the chlorosis and distortion of a lower alternate leaf of sunflower grown at 400 µM Mn and 6 µM Fe.
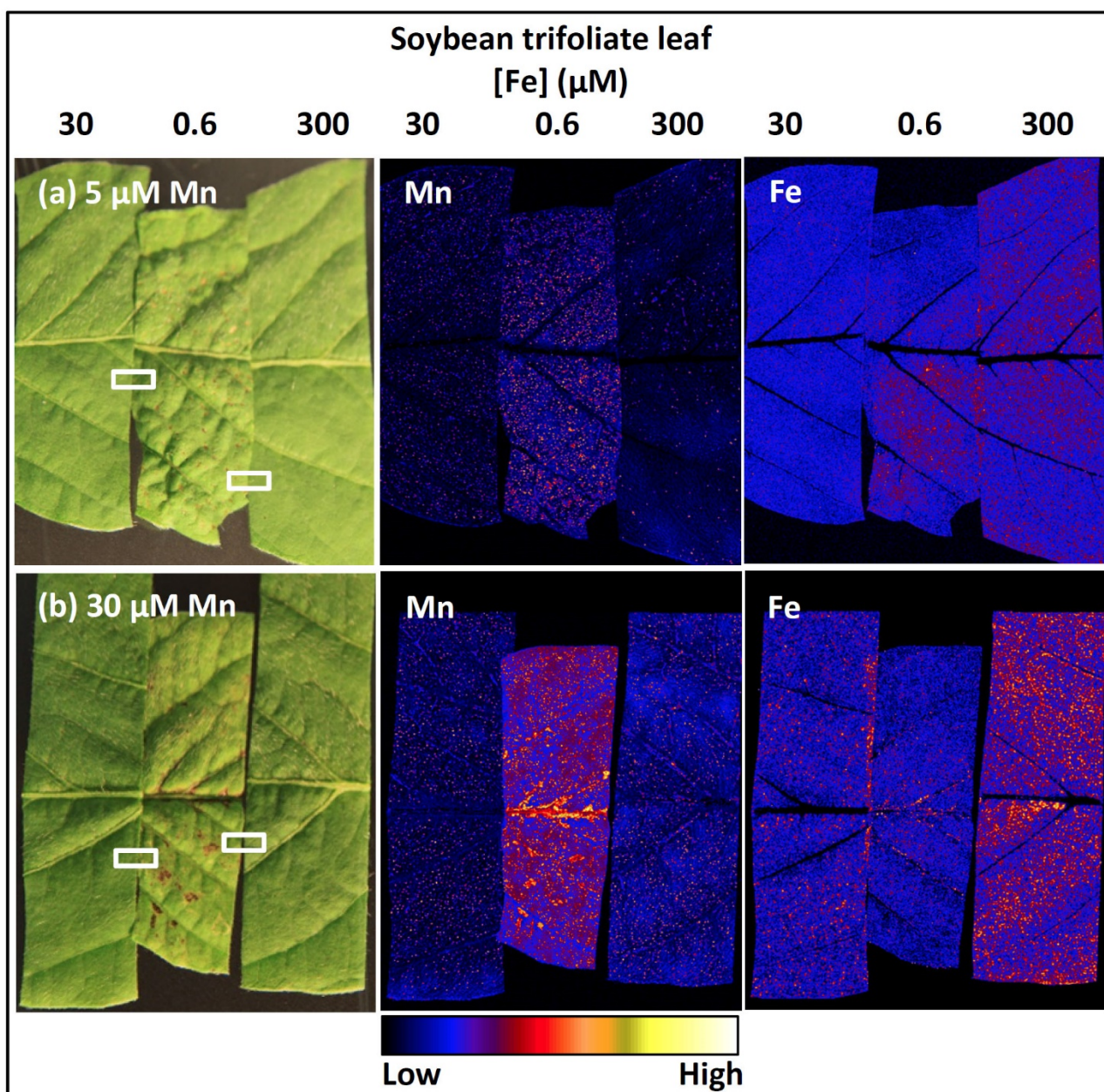
Supplementary Fig. S2 Optical images and μ-XRF survey scans of Mn and Fe distributions in trifoliate leaf sections of soybean grown for 1 week in solutions with (a) 5 and (b) 30 μM Mn and with 30, 0.6, and 300 μM Fe in solution (Experiment 2). The white boxes in the optical images indicate the locations of the 3.05 mm × 1.00 mm μ-XRF detailed scans and the color scale in (b) applies to all Mn and Fe distributions.
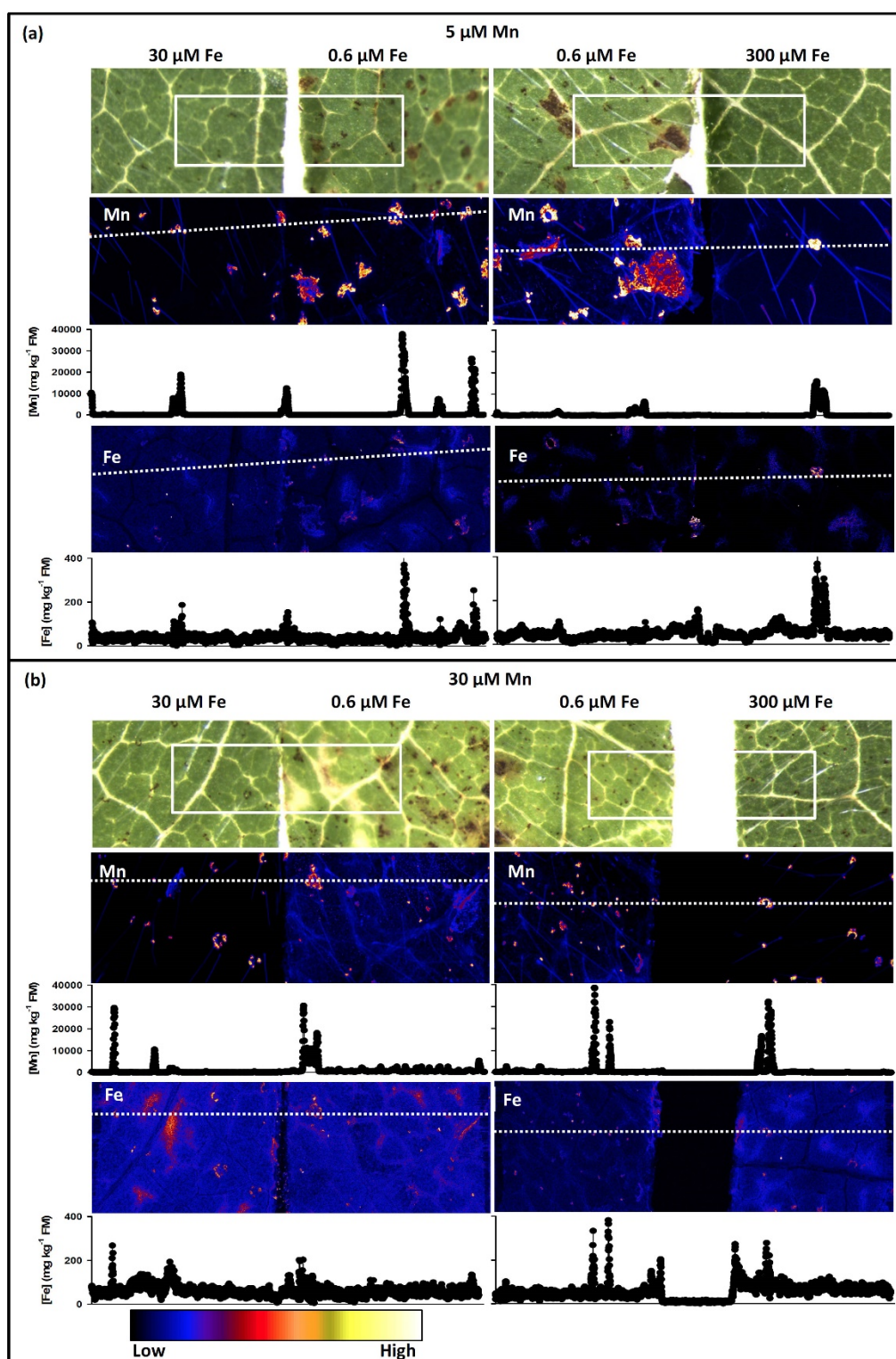
Supplementary Fig. S3 Optical images and μ-XRF detailed scans (3.05 mm × 1.00 mm) of Mn and Fe distributions in trifoliate leaf sections of soybean grown at (a) 5 and (b) 30 μM Mn with 30, 0.6, and 300 μM Fe in solution (Experiment 2). The dotted white lines indicate transects along which the Mn and Fe concentrations were determined. Valid comparisons may be made between Fe treatments within each image. The color scale in (b) applies to all Mn and Fe μ-XRF scans.
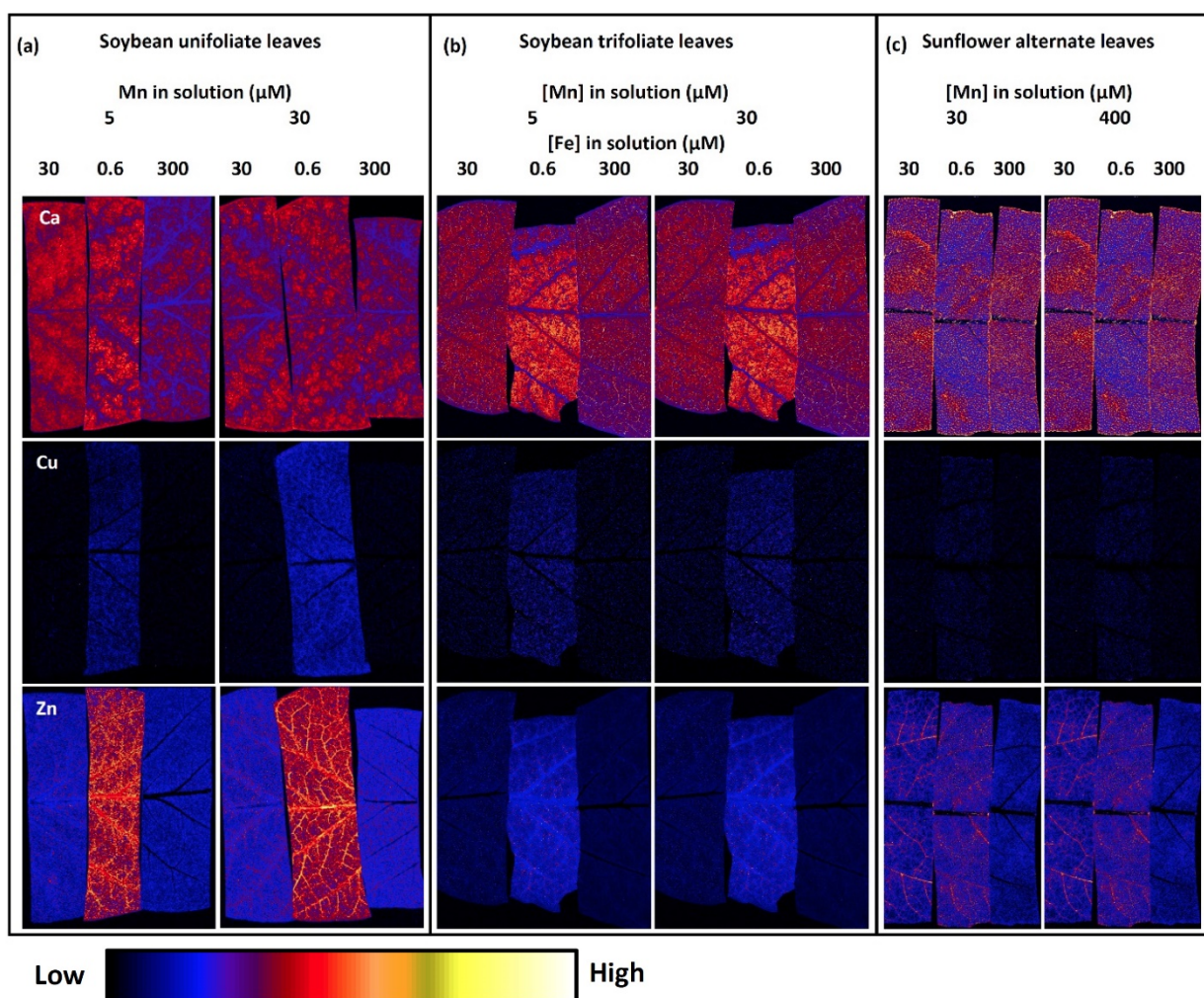
Supplementary Fig. S4 μ-XRF survey scans of Ca, Cu, and Zn distributions and concentrations in sections of (a) soybean unifoliate leaves, (b) soybean trifoliate leaves, and (c) sunflower lower alternate leaves after 1 week's growth in complete nutrient solutions with two Mn treatments and three Fe treatments (Experiment 2). The color scale applies to all images.
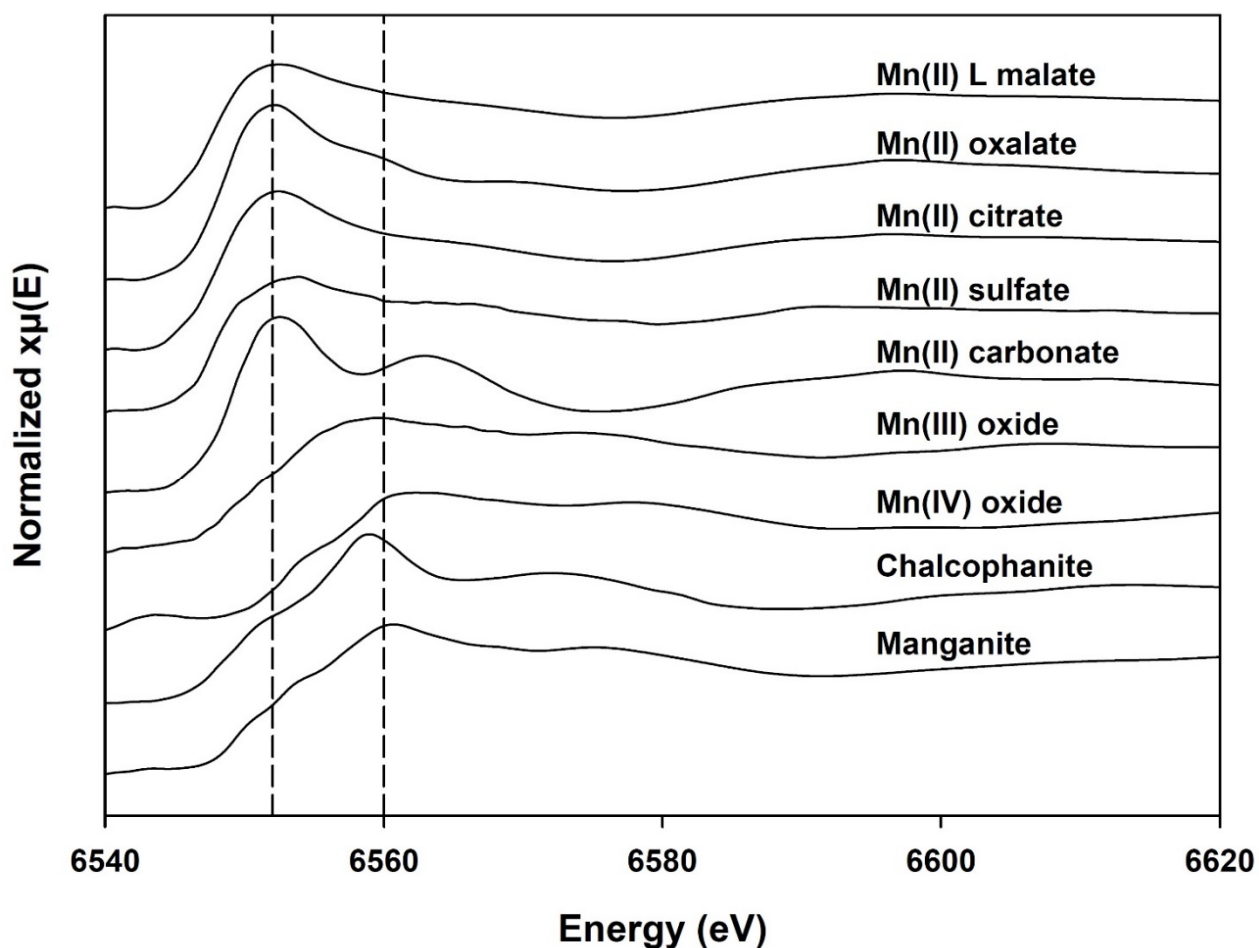
Supplementary Fig. S5 Normalized K-edge XANES spectra of nine Mn compounds showing differences over the range from 6540 to 6620 eV. The vertical dashed lines correspond approximately to the white-line peak of Mn(II) at 6,552 eV and that of Mn(III) and Mn(IV) at 6,560 eV.

Supplementary Table S1 Main effects and interactions of Mn and Fe in solution on concentrations of nine selected nutrients in soybean unifoliate leaves on a dry mass (DM) basis. A value of 19.5 % DM may be used to convert concentrations to a fresh mass basis. (Experiment 1)

[Mn] in unifoliate leaves (mg kg⁻¹) Mn** Fe** Mn×Fe**		[Mn] in solution (μM)			Fe means
		0.5	5	30	
[Fe] in solution (μM)	0.6	220 ± 30	850 ± 140	3480 ± 240	2010 ± 330
	30	240 ± 40	220 ± 10	820 ± 40	520 ± 70
	300	280 ± 40	220 ± 10	800 ± 40	530 ± 60
	Mn means	250 ± 20	430 ± 90	1700 ± 230	1020 ± 140
[Fe] in unifoliate leaves (mg kg⁻¹) Mn** Fe** Mn×Fe**		**[Mn] in solution (μM)**			**Fe means**
		0.5	5	30	
[Fe] in solution (μM)	0.6	90 ± 10	100 ± 10	50 ± 0	70 ± 10
	30	1260 ± 200	150 ± 20	360 ± 70	530 ± 10
	300	1340 ± 170	250 ± 60	510 ± 100	650 ± 10
	Mn means	900 ± 160	170 ± 30	310 ± 50	420 ± 60
[Cu] in unifoliate leaves (mg kg⁻¹) Mn** Fe** Mn×Fe NS		**[Mn] in solution (μM)**			**Fe means**
		0.5	5	30	
[Fe] in solution (μM)	0.6	25.0 ± 3.9	20.5 ± 2.8	29.2 ± 2.2	26.0 ± 1.7
	30	11.7 ± 1.3	6.2 ± 0.2	10.9 ± 1.1	9.9 ± 0.8
	300	14.0 ± 0.9	7.3 ± 0.7	9.2 ± 0.6	9.9 ± 0.7
	Mn means	16.9 ± 1.9	11.3 ± 1.8	16.4 ± 1.7	15.3 ± 1.1
[Zn] in unifoliate leaves (mg kg⁻¹) Mn NS Fe** Mn×Fe NS		**[Mn] in solution (μM)**			**Fe means**
		0.5	5	30	
[Fe] in solution (μM)	0.6	350 ± 40	400 ± 70	420 ± 20	390 ± 20
	30	170 ± 20	90 ± 10	160 ± 20	150 ± 10
	300	200 ± 20	80 ± 10	140 ± 20	140 ± 10
	Mn means	240 ± 20	190 ± 40	240 ± 20	230 ± 20
[Ca] in unifoliate leaves (%) Mn NS Fe NS Mn×Fe NS		**[Mn] in solution (μM)**			**Fe means**
		0.5	5	30	
[Fe] in solution (μM)	0.6	3.14 ± 0.21	3.13 ± 0.11	3.07 ± 0.17	3.10 ± 0.10
	30	2.82 ± 0.11	3.02 ± 0.11	3.09 ± 0.10	3.01 ± 0.07
	300	2.82 ± 0.07	3.02 ± 0.22	2.70 ± 0.06	2.81 ± 0.07
	Mn means	2.93 ± 0.09	3.06 ± 0.08	2.95 ± 0.07	2.97 ± 0.05
[Mg] in unifoliate leaves (%) Mn** Fe** Mn×Fe**		**[Mn] in solution (μM)**			**Fe means**
		0.5	5	30	
[Fe] in solution (μM)	0.6	0.38 ± 0.04	0.42 ± 0.03	0.48 ± 0.03	0.44 ± 0.02
	30	0.40 ± 0.04	0.27 ± 0.02	0.34 ± 0.02	0.33 ± 0.02
	300	0.43 ± 0.03	0.31 ± 0.01	0.34 ± 0.02	0.36 ± 0.01
	Mn means	0.40 ± 0.02	0.33 ± 0.02	0.39 ± 0.02	0.38 ± 0.01
[K] in unifoliate leaves (%) Mn* Fe NS Mn×Fe*		**[Mn] in solution (μM)**			**Fe means**
		0.5	5	30	
[Fe] in solution (μM)	0.6	1.98 ± 0.17	1.69 ± 0.08	1.64 ± 0.09	1.74 ± 0.07
	30	1.99 ± 0.12	1.43 ± 0.06	1.99 ± 0.07	1.85 ± 0.07
	300	1.85 ± 0.13	1.92 ± 0.14	1.89 ± 0.10	1.89 ± 0.07
	Mn means	1.94 ± 0.08	1.68 ± 0.07	1.84 ± 0.05	1.83 ± 0.04
[P] in unifoliate leaves (%) Mn** Fe** Mn×Fe*		**[Mn] in solution (μM)**			**Fe means**
		0.5	5	30	
[Fe] in solution (μM)	0.6	0.12 ± 0.01	0.22 ± 0.02	0.25 ± 0.02	0.21 ± 0.02
	30	0.10 ± 0.01	0.14 ± 0.00	0.14 ± 0.02	0.13 ± 0.01
	300	0.11 ± 0.00	0.16 ± 0.01	0.13 ± 0.01	0.13 ± 0.01
	Mn means	0.11 ± 0.00	0.18 ± 0.01	0.17 ± 0.01	0.16 ± 0.01
[S] in unifoliate leaves (%) Mn** Fe* Mn×Fe NS		**[Mn] in solution (μM)**			**Fe means**
		0.5	5	30	
[Fe] in solution (μM)	0.6	0.20 ± 0.01	0.25 ± 0.01	0.23 ± 0.01	0.23 ± 0.01
	30	0.16 ± 0.01	0.23 ± 0.00	0.22 ± 0.01	0.21 ± 0.01
	300	0.17 ± 0.01	0.24 ± 0.01	0.20 ± 0.01	0.20 ± 0.01
	Mn means	0.18 ± 0.01	0.24 ± 0.01	0.22 ± 0.01	0.21 ± 0.00

Values are means ± standard error

**, *, NS = significant at P ≤ 0.01, significant at P ≤ 0.05, and not significant, respectively

Supplementary Table S2 Main effects and interactions of Mn and Fe in solution on concentrations of nine selected nutrients in soybean trifoliate leaves on a dry mass (DM) basis. A value of 20.3 % DM may be used to convert concentrations to a fresh mass basis. (Experiment 1)

[Mn] in trifoliate leaves (mg kg^{-1})		[Mn] in solution (μM)			Fe means
Mn** Fe** Mn×Fe**		0.5	5	30	
[Fe] in solution (μM)	0.6	70 ± 10	580 ± 60	3080 ± 170	1700 ± 300
	30	80 ± 10	230 ± 10	770 ± 50	460 ± 70
	300	80 ± 20	180 ± 10	810 ± 50	470 ± 80
	Mn means	80 ± 10	330 ± 50	1550 ± 190	880 ± 130
[Fe] in trifoliate leaves (mg kg^{-1})		[Mn] in solution (μM)			Fe means
Mn** Fe** Mn×Fe**		0.5	5	30	
[Fe] in solution (μM)	0.6	30 ± 0	70 ± 10	50 ± 10	50 ± 0
	30	200 ± 30	110 ± 10	90 ± 10	120 ± 10
	300	230 ± 70	110 ± 10	170 ± 20	170 ± 20
	Mn means	150 ± 30	90 ± 10	100 ± 10	110 ± 10
[Cu] in trifoliate leaves (mg kg^{-1})		[Mn] in solution (μM)			Fe means
Mn** Fe** Mn×Fe**		0.5	5	30	
[Fe] in solution (μM)	0.6	13.9 ± 0.9	16.1 ± 1.1	23.1 ± 0.9	19.1 ± 1.0
	30	8.4 ± 0.2	6.2 ± 0.2	8.5 ± 0.4	7.9 ± 0.3
	300	8.4 ± 0.6	6.4 ± 0.5	9.0 ± 0.3	8.2 ± 0.3
	Mn means	10.2 ± 0.7	9.6 ± 1.2	13.5 ± 1.2	11.7 ± 0.7
[Zn] in trifoliate leaves (mg kg^{-1})		[Mn] in solution (μM)			Fe means
Mn NS Fe** Mn×Fe**		0.5	5	30	
[Fe] in solution (μM)	0.6	120 ± 10	220 ± 20	240 ± 30	210 ± 20
	30	60 ± 0	70 ± 0	70 ± 0	70 ± 0
	300	60 ± 10	60 ± 0	60 ± 0	60 ± 0
	Mn means	80 ± 10	110 ± 20	130 ± 20	110 ± 10
[Ca] in trifoliate leaves (%)		[Mn] in solution (μM)			Fe means
Mn NS Fe NS Mn×Fe NS		0.5	5	30	
[Fe] in solution (μM)	0.6	1.76 ± 0.08	2.09 ± 0.07	1.85 ± 0.09	1.89 ± 0.06
	30	1.94 ± 0.05	1.81 ± 0.05	1.74 ± 0.08	1.81 ± 0.05
	300	2.01 ± 0.16	1.81 ± 0.06	1.72 ± 0.10	1.82 ± 0.07
	Mn means	1.90 ± 0.06	1.91 ± 0.05	1.77 ± 0.05	1.84 ± 0.03
[Mg] in trifoliate leaves (%)		[Mn] in solution (μM)			Fe means
Mn** Fe NS Mn×Fe**		0.5	5	30	
[Fe] in solution (μM)	0.6	0.24 ± 0.01	0.27 ± 0.00	0.32 ± 0.01	0.29 ± 0.01
	30	0.32 ± 0.01	0.24 ± 0.00	0.27 ± 0.01	0.27 ± 0.01
	300	0.34 ± 0.03	0.24 ± 0.01	0.29 ± 0.01	0.29 ± 0.01
	Mn means	0.30 ± 0.01	0.25 ± 0.01	0.29 ± 0.01	0.28 ± 0.01
[K] in trifoliate leaves (%)		[Mn] in solution (μM)			Fe means
Mn* Fe** Mn×Fe**		0.5	5	30	
[Fe] in solution (μM)	0.6	1.89 ± 0.16	2.46 ± 0.13	2.18 ± 0.08	2.18 ± 0.07
	30	1.55 ± 0.10	1.58 ± 0.06	1.77 ± 0.05	1.67 ± 0.04
	300	1.77 ± 0.06	1.63 ± 0.12	1.94 ± 0.09	1.82 ± 0.06
	Mn means	1.74 ± 0.07	1.89 ± 0.11	1.97 ± 0.05	1.89 ± 0.04
[P] in trifoliate leaves (%)		[Mn] in solution (μM)			Fe means
Mn** Fe** Mn×Fe*		0.5	5	30	
[Fe] in solution (μM)	0.6	0.13 ± 0.01	0.33 ± 0.03	0.44 ± 0.03	0.34 ± 0.03
	30	0.11 ± 0.01	0.19 ± 0.01	0.17 ± 0.01	0.16 ± 0.01
	300	0.10 ± 0.00	0.20 ± 0.02	0.18 ± 0.02	0.16 ± 0.01
	Mn means	0.11 ± 0.01	0.24 ± 0.02	0.26 ± 0.02	0.22 ± 0.01
[S] in trifoliate leaves (%)		[Mn] in solution (μM)			Fe means
Mn** Fe** Mn×Fe**		0.5	5	30	
[Fe] in solution (μM)	0.6	0.18 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.25 ± 0.01
	30	0.19 ± 0.01	0.23 ± 0.01	0.21 ± 0.01	0.21 ± 0.00
	300	0.19 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.22 ± 0.01
	Mn means	0.19 ± 0.00	0.24 ± 0.01	0.23 ± 0.01	0.22 ± 0.00

Values are means ± standard error
**, *, NS = significant at P ≤ 0.01, significant at P ≤ 0.05, and not significant, respectively

Supplementary Table S3 Main effects and interactions of Mn and Fe in solution on concentrations of nine selected nutrients in sunflower lower alternate leaves on a dry mass (DM) basis. A value of 16.9 % DM may be used to convert concentrations to a fresh mass basis. (Experiment 1)

[Mn] in unifoliate leaf tissues (mg kg⁻¹)		[Mn] (μM)		Fe means
Mn**, Fe**, Mn×Fe*		30	400	
	0.6	3450 ± 300	8650 ± 500	6050 ± 610
[Fe] (μM)	30	1110 ± 120	5130 ± 340	3120 ± 460
	300	810 ± 70	4450 ± 260	2630 ± 400
	Mn means	1790 ± 230	6080 ± 380	3950 ± 340
[Fe] in unifoliate leaf tissues (mg kg⁻¹)		**[Mn] (μM)**		**Fe means**
Mn**, Fe**, Mn×Fe *		30	400	
	0.6	28 ± 2	26 ± 7	27 ± 4
[Fe] (μM)	30	158 ± 28	58 ± 8	108 ± 18
	300	127 ± 22	84 ± 14	105 ± 13
	Mn means	104 ± 15	56 ± 7	80 ± 9
[Cu] in unifoliate leaf tissues (mg kg⁻¹)		**[Mn] (μM)**		**Fe means**
Mn NS, Fe**, Mn×Fe**		30	400	
	0.6	14.8 ± 0.6	25.5 ± 3.7	20.1 ± 2.1
[Fe] (μM)	30	11.6 ± 1.7	8.9 ± 0.4	10.2 ± 0.9
	300	8.9 ± 0.7	8.0 ± 0.5	8.4 ± 0.4
	Mn means	11.8 ± 0.7	14.1 ± 1.8	12.9 ± 1.0
[Zn] in unifoliate leaf tissues (mg kg⁻¹)		**[Mn] (μM)**		**Fe means**
Mn*, Fe**, Mn×Fe NS		30	400	
	0.6	113 ± 6	98 ± 10	105 ± 6
[Fe] (μM)	30	71 ± 6	53 ± 6	62 ± 5
	300	39 ± 3	36 ± 3	38 ± 2
	Mn means	74 ± 6	62 ± 6	68 ± 4
[Ca] in leaf tissues (%)		**[Mn] (μM)**		**Fe means**
Mn**, Fe **, Mn×Fe NS		30	400	
	0.6	2.70 ± 0.14	1.97 ± 0.17	2.33 ± 0.13
[Fe] (μM)	30	1.75 ± 0.12	1.04 ± 0.08	1.40 ± 0.10
	300	1.50 ± 0.09	0.89 ± 0.07	1.20 ± 0.08
	Mn means	1.99 ± 0.11	1.30 ± 0.10	1.64 ± 0.09
[Mg] in leaf tissues (%)		**[Mn] (μM)**		**Fe means**
Mn**, Fe**, Mn×Fe NS		30	400	
	0.6	0.23 ± 0.02	0.17 ± 0.02	0.20 ± 0.02
[Fe] (μM)	30	0.17 ± 0.02	0.07 ± 0.00	0.12 ± 0.01
	300	0.15 ± 0.01	0.06 ± 0.01	0.11 ± 0.01
	Mn means	0.19 ± 0.01	0.10 ± 0.01	0.14 ± 0.01
[K] in leaf tissues (%)		**[Mn] (μM)**		**Fe means**
Mn NS, Fe**, Mn×Fe NS		30	400	
	0.06	4.21 ± 0.46	4.78 ± 0.56	4.49 ± 0.36
[Fe] (μM)	30	2.65 ± 0.19	2.08 ± 0.16	2.36 ± 0.14
	300	2.41 ± 0.25	2.30 ± 0.16	2.36 ± 0.15
	Mn means	3.09 ± 0.23	3.05 ± 0.29	3.07 ± 0.18
[P] in leaf tissues (%)		**[Mn] (μM)**		**Fe means**
M**, Fe**, Mn×Fe**		30	400	
	0.6	0.15 ± 0.02	0.89 ± 0.23	0.52 ± 0.14
[Fe] (μM)	30	0.14 ± 0.02	0.11 ± 0.01	0.12 ± 0.01
	300	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
	Mn means	0.13 ± 0.01	0.37 ± 0.10	0.25 ± 0.05
[S] in leaf tissues (%)		**[Mn] (μM)**		**Fe means**
Mn NS, Fe**, Mn×Fe NS		30	400	
	0.6	0.51 ± 0.03	0.52 ± 0.07	0.51 ± 0.03
[Fe] (μM)	30	0.38 ± 0.04	0.29 ± 0.03	0.33 ± 0.03
	300	0.32 ± 0.03	0.29 ± 0.03	0.30 ± 0.02
	Mn means	0.40 ± 0.02	0.36 ± 0.03	0.38 ± 0.02

Values are means ± standard error

**, *, NS = significant at P ≤ 0.01, significant at P ≤ 0.05, and not significant, respectively

Supplementary Table S4 Effects of Mn and Fe in solution on the concentrations of micronutrients and macronutrients in soybean and sunflower leaf tissues on a dry mass basis (Experiment 3).

Tissue	Solution composition (μM)		Micronutrient concentration in leaf tissues (mg kg^{-1})			
	[Mn]	[Fe]	[Mn]	[Fe]	[Cu]	[Zn]
Soybean unifoliate leaf	5	0.6	380 ± 50	67 ± 6	20.3 ± 2.5	170 ± 11
	5	30	160 ± 10	146 ± 13	7.8 ± 0.6	78 ± 5
Soybean trifoliate leaf	5	0.6	360 ± 30	39 ± 2	13.2 ± 1.2	66 ± 4
	5	30	190 ± 20	110 ± 10	7.2 ± 0.2	45 ± 3
Sunflower alternate leaf	30	0.6	3100 ± 260	24 ± 2	15.0 ± 0.2	79 ± 5
	30	30	1020 ± 160	62 ± 6	9.4 ± 0.6	39 ± 2
Tissue	Solution composition (μM)		Macronutrient concentration in leaf tissues (%)				
	[Mn]	[Fe]	[Ca]	[Mg]	[K]	[P]	[S]
Soybean unifoliate leaf	5	0.6	2.24 ± 0.12	0.28 ± 0.03	2.07 ± 0.04	0.12 ± 0.01	0.19 ± 0.01
	5	30	2.12 ± 0.07	0.17 ± 0.01	1.97 ± 0.11	0.09 ± 0.01	0.16 ± 0.01
Soybean trifoliate leaf	5	0.6	1.29 ± 0.09	0.23 ± 0.03	1.78 ± 0.07	0.18 ± 0.02	0.22 ± 0.02
	5	30	1.14 ± 0.07	0.19 ± 0.01	1.54 ± 0.14	0.15 ± 0.02	0.21 ± 0.01
Sunflower alternate leaf	30	0.6	3.03 ± 0.27	0.29 ± 0.02	3.06 ± 0.04	0.15 ± 0.01	0.62 ± 0.05
	30	30	1.96 ± 0.63	0.22 ± 0.01	1.71 ± 0.13	0.09 ± 0.01	0.28 ± 0.02

Values are means ± standard error