Supplemental

Messant *et al.*

Manganese excess and deficiency affect chloroplast structure and the photosynthetic apparatus in *Marchantia polymorpha*

Table S1. Chlorophyll and Mn content in thalli grown in Mn excess

sample	mg Chl/g DW	mg Mn/g DW	µg Mn/mg Chl	µg Mn in Chloroplast/ g DW
Crtl (agar)	14.6 ± 1.2	0.23	2.1	30.3 ± 0.10
30x	10.6 ± 1.7*	2.71	23.6	250.8 ± 0.09***
200x	8.8 ± 1.3*	23.12	178.7	1566.2 ± 0.06***

Chlorophyll content and dry weight was determined by three consecutive acetone extractions (N=6). Values of Mn content are the same as in Figs 1. 2 of the main manuscript. Mean value and SE is given. Stars indicate significant differences based on a Mann and Whitney test (* $p<0.05$. *** $p<0.001$). Chlorophyll content was determined according to Porra and Scheer (2019).

Table S2. List of 96 metabolites identified by GC-MS in *Marchantia polymorpha*

	Control		30X		200X		Starch+Mn		Starch-Mn	
Metabolites	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1.3-propanediol	0.011	0.000	0.012	0.000	0.011	0.000	0.011	0.001	0.011	0.001
1.6-anhydro-glucose	0.005	0.001	0.004	0.000	0.002	0.000	0.002	0.001	0.002	0.001
2.3-butanediol	0.005	0.001	0.005	0.000	0.004	0.000	0.005	0.000	0.004	0.000
2-hydroxypyridine	0.021	0.003	0.017	0.002	0.014	0.001	0.015	0.001	0.012	0.002
3.4-dihydroxymandelic acid	0.003	0.001	0.002	0.000	0.008	0.002	0.002	0.000	0.001	0.000
3-hydroxy-3-methylglutaric acid	0.005	0.000	0.004	0.000	0.003	0.000	0.003	0.001	0.002	0.001
4-hydroxypyridine	0.003	0.000	0.002	0.000	0.002	0.000	0.002	0.000	0.002	0.000
acetohydroxamic acid	0.030	0.004	0.028	0.003	0.034	0.006	0.030	0.005	0.034	0.006
allantoin	0.007	0.004	0.012	0.008	0.008	0.003	0.001	0.001	0.000	0.000
alpha ketoglutaric acid	0.029	0.004	0.006	0.006	0.000	0.000	0.011	0.010	0.010	0.009
arabinose	0.003	0.000	0.003	0.000	0.003	0.000	0.001	0.000	0.001	0.000
arbutin	0.002	0.000	0.002	0.000	0.002	0.000	0.001	0.000	0.000	0.000
aspartic acid	0.750	0.207	0.585	0.114	0.385	0.053	0.086	0.027	0.155	0.130
benzoic acid	0.003	0.000	0.003	0.000	0.003	0.000	0.003	0.000	0.003	0.000
Beta- alanine	0.004	0.001	0.003	0.001	0.004	0.001	0.001	0.000	0.001	0.001
beta-cyano-L-alanine	0.002	0.000	0.004	0.002	0.020	0.007	0.001	0.000	0.001	0.000
beta-sitosterol	0.004	0.001	0.004	0.001	0.004	0.001	0.002	0.001	0.002	0.001
citramalic acid	0.005	0.000	0.004	0.000	0.002	0.000	0.004	0.001	0.004	0.003
citric acid	0.285	0.010	0.295	0.017	0.037	0.005	0.289	0.063	0.247	0.091
citrulline	0.001	0.000	0.001	0.000	0.003	0.001	0.000	0.000	0.000	0.000
D-(+) trehalose	0.053	0.003	0.049	0.008	0.083	0.012	0.036	0.011	0.021	0.008
dehydroascorbic acid	0.007	0.001	0.004	0.000	0.005	0.001	0.001	0.000	0.001	0.000
D-glucose	0.239	0.035	0.252	0.068	0.218	0.058	0.411	0.233	0.234	0.081
D-glucose-6-phosphate	0.015	0.002	0.010	0.003	0.005	0.001	0.010	0.005	0.006	0.003
DL-isoleucine	0.060	0.015	0.061	0.010	0.046	0.014	0.028	0.006	0.023	0.009
D-malic acid	0.453	0.067	0.288	0.051	0.048	0.009	0.654	0.299	0.618	0.257
D-mannitol	0.013	0.010	0.009	0.007	0.009	0.006	0.023	0.022	0.024	0.019
D-mannose	0.005	0.000	0.004	0.000	0.004	0.000	0.045	0.042	0.002	0.000
dopamine (hydroxytyramine)	1.589	0.178	1.144	0.201	1.810	0.252	0.612	0.074	0.260	0.019
D-sorbitol	0.008	0.001	0.007	0.000	0.006	0.000	0.007	0.001	0.006	0.000
D-threitol	0.010	0.003	0.008	0.002	0.008	0.003	0.017	0.013	0.023	0.013
fructose	0.182	0.027	0.203	0.060	0.173	0.045	0.261	0.134	0.166	0.065
fructose 6-phosphate	0.008	0.001	0.006	0.001	0.003	0.001	0.005	0.002	0.003	0.001
fumaric acid	0.180	0.031	0.126	0.030	0.022	0.002	0.070	0.008	0.067	0.028

galactonic acid	0.001	0.000	0.001	0.000	0.000	0.000	0.092	0.088	0.009	0.005
galacturonic acid	0.004	0.000	0.004	0.001	0.008	0.001	0.013	0.008	0.005	0.003
gluconic acid	0.003	0.000	0.003	0.000	0.002	0.000	0.002	0.001	0.002	0.000
glyceric acid	0.016	0.001	0.014	0.001	0.006	0.001	0.016	0.005	0.013	0.006
glycerol	0.014	0.001	0.013	0.001	0.011	0.001	0.011	0.002	0.010	0.002
glycerol 1-phosphate	0.021	0.002	0.016	0.002	0.011	0.002	0.010	0.003	0.007	0.001
glycine	0.007	0.001	0.006	0.001	0.006	0.001	0.002	0.000	0.003	0.001
glycolic acid	0.013	0.001	0.015	0.001	0.014	0.001	0.009	0.000	0.009	0.002
L-(+) lactic acid	0.012	0.001	0.016	0.004	0.012	0.001	0.014	0.003	0.012	0.002
L-alanine	0.010	0.002	0.010	0.002	0.004	0.001	0.003	0.001	0.003	0.002
L-asparagine	0.372	0.021	0.461	0.074	0.478	0.085	0.059	0.036	0.108	0.095
leucrose	0.003	0.000	0.004	0.001	0.005	0.001	0.001	0.000	0.001	0.000
L-glutamic acid	2.642	0.178	2.568	0.065	2.024	0.148	1.143	0.298	1.078	0.449
L-glutamine	0.107	0.013	0.080	0.022	0.202	0.078	0.023	0.019	0.023	0.022
L-homoserine	0.002	0.000	0.002	0.000	0.002	0.000	0.001	0.000	0.000	0.000
L-leucine	0.004	0.001	0.007	0.001	0.007	0.001	0.007	0.002	0.006	0.001
L-lysine	0.003	0.001	0.002	0.001	0.004	0.002	0.001	0.000	0.000	0.000
L-methionine sulfoxide	0.001	0.000	0.001	0.000	0.001	0.000	0.000	0.000	0.000	0.000
L-norleucine	0.059	0.013	0.066	0.015	0.043	0.015	0.025	0.007	0.018	0.005
L-ornithine	0.003	0.001	0.003	0.000	0.007	0.001	0.001	0.000	0.001	0.001
L-proline	0.010	0.002	0.011	0.001	0.009	0.001	0.004	0.000	0.005	0.002
L-serine	0.625	0.028	0.617	0.037	0.616	0.113	0.157	0.040	0.138	0.069
L-threonine	0.080	0.008	0.070	0.010	0.042	0.006	0.028	0.007	0.024	0.010
L-threonine	0.025	0.005	0.036	0.018	0.070	0.019	0.005	0.001	0.004	0.000
L-tryptophan	0.043	0.019	0.036	0.017	0.134	0.035	0.005	0.002	0.003	0.002
L-valine	0.046	0.007	0.054	0.014	0.060	0.013	0.026	0.005	0.022	0.006
maltose	0.002	0.001	0.003	0.000	0.002	0.001	0.084	0.067	0.022	0.011
myo-inositol	0.042	0.003	0.041	0.001	0.031	0.003	0.022	0.005	0.018	0.004
myristic acid	0.005	0.000	0.004	0.001	0.004	0.000	0.005	0.001	0.004	0.000
N-ethylglycine	0.849	0.030	0.888	0.093	1.101	0.039	0.755	0.089	0.743	0.053
N-methylalanine	0.021	0.002	0.670	0.237	1.101	0.038	0.607	0.210	0.743	0.053
norepinephrine (noradrenalin)	0.008	0.002	0.006	0.002	0.020	0.003	0.007	0.003	0.003	0.001
norvaline	0.086	0.015	0.092	0.015	0.069	0.020	0.047	0.008	0.044	0.019
oleic acid	0.005	0.000	0.004	0.001	0.004	0.000	0.004	0.001	0.005	0.001
O-phosphocolamine	0.005	0.001	0.003	0.001	0.002	0.000	0.002	0.001	0.001	0.001
oxalic acid	0.024	0.004	0.023	0.002	0.018	0.003	0.013	0.002	0.013	0.005
palmitic acid	0.146	0.019	0.134	0.025	0.140	0.025	0.164	0.034	0.146	0.021
phenanthrene	0.007	0.001	0.006	0.001	0.007	0.001	0.006	0.000	0.007	0.001
Phenylalanine	0.018	0.002	0.019	0.002	0.009	0.002	0.006	0.003	0.005	0.001
phosphoric acid	0.615	0.138	0.318	0.124	0.093	0.019	0.589	0.176	0.510	0.280
phytol	0.050	0.002	0.046	0.006	0.089	0.024	0.016	0.007	0.011	0.005
porphine	0.032	0.003	0.030	0.002	0.039	0.002	0.029	0.001	0.024	0.001
putrescine	0.003	0.001	0.002	0.000	0.008	0.001	0.000	0.000	0.001	0.000
pyruvic acid	0.006	0.001	0.005	0.000	0.003	0.000	0.001	0.001	0.002	0.001
rhamnose	0.004	0.000	0.004	0.001	0.008	0.001	0.002	0.000	0.001	0.000
shikimic acid	0.209	0.043	0.168	0.031	0.068	0.010	0.265	0.106	0.127	0.074
stearic acid	0.076	0.017	0.073	0.021	0.087	0.019	0.098	0.022	0.098	0.023

stigmasterol	0.022	0.003	0.023	0.003	0.021	0.002	0.009	0.002	0.007	0.002
succinic acid	0.074	0.006	0.080	0.008	0.040	0.007	0.093	0.019	0.096	0.041
Sucrose	2.261	0.326	2.004	0.232	1.473	0.293	1.571	0.421	1.042	0.277
tagatose	0.005	0.000	0.004	0.000	0.074	0.071	0.079	0.046	0.085	0.084
talose	0.005	0.000	0.004	0.000	0.004	0.000	0.004	0.001	0.002	0.000
threonic acid	0.102	0.010	0.120	0.016	0.098	0.008	0.082	0.008	0.062	0.010
Threonic acid-1.4-lactone	0.007	0.001	0.008	0.001	0.016	0.003	0.004	0.000	0.003	0.001
trans-aconitic acid	0.475	0.054	0.362	0.049	0.031	0.006	0.407	0.151	0.324	0.151
tyramine	0.073	0.006	0.074	0.015	0.106	0.020	0.076	0.036	0.021	0.012
tyrosine	0.044	0.007	0.043	0.007	0.052	0.013	0.018	0.009	0.011	0.008
uracil	0.003	0.001	0.003	0.000	0.002	0.000	0.002	0.000	0.002	0.000
urea	0.011	0.002	0.010	0.002	0.003	0.001	0.005	0.002	0.002	0.001
xylitol	0.005	0.000	0.003	0.001	0.001	0.000	0.001	0.000	0.001	0.000
xylose	0.004	0.000	0.004	0.000	0.002	0.000	0.002	0.000	0.001	0.000
xylulose	0.007	0.000	0.005	0.000	0.003	0.000	0.004	0.001	0.003	0.001

List of 96 metabolites identified and quantified in plants grown in Agar Control. 30X. 200X. Starch Control and Starch-Mn condition. N=4 biological replicates. $P<0.05$ for all values according to two-way ANOVA test.

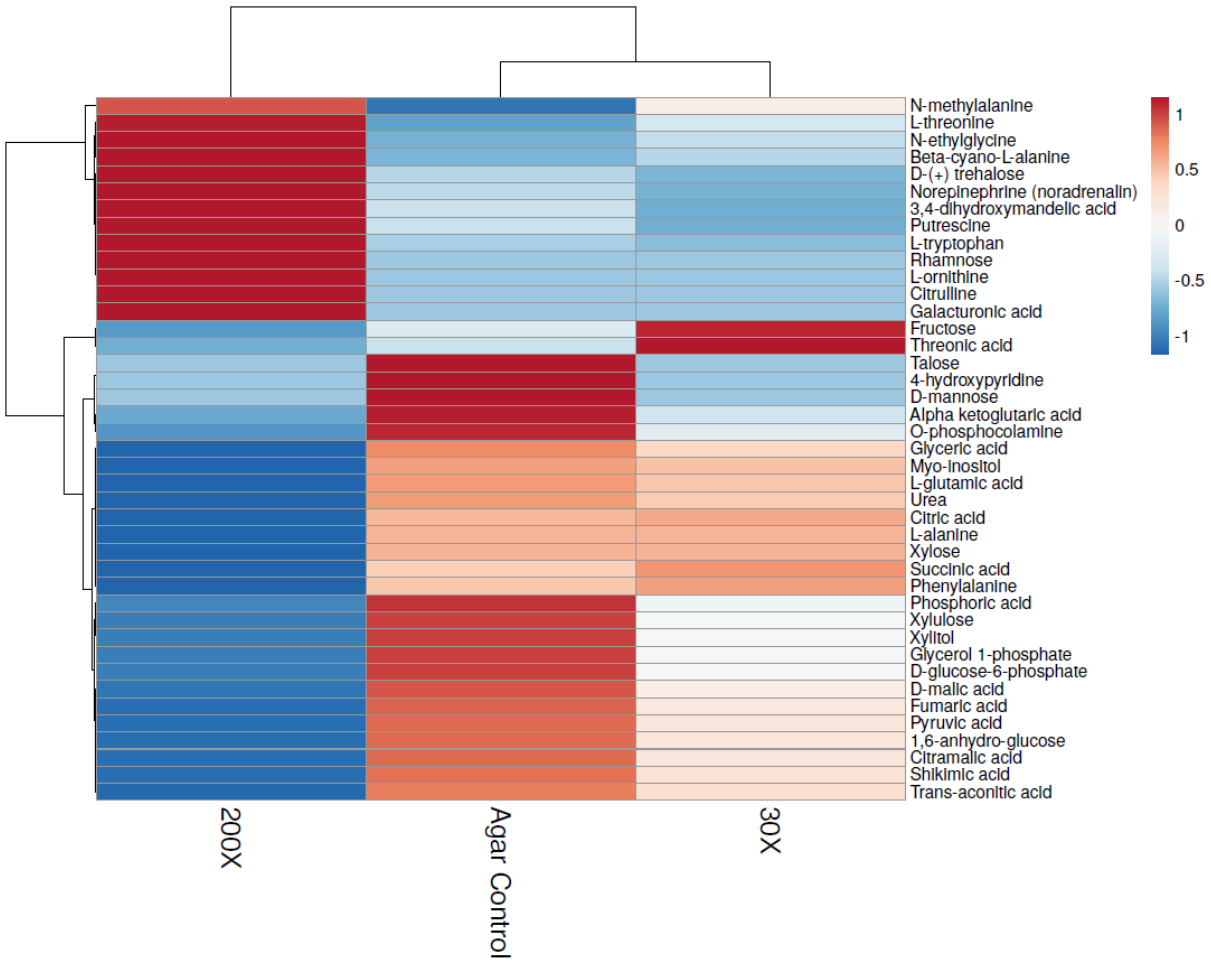
Table S3. Metabolites significantly decreased in Starch condition compared to Agar Control in *Marchantia polymorpha*

	Agar Control		Starch (+/-)	
Metabolites	Mean	SD	Mean	SD
1.6-anhydro-glucose	0.005	0.001	0.002	0.001
2-hydroxypyridine	0.021	0.003	0.013	0.003
3.4-dihydroxymandelic acid	0.003	0.001	0.001	0.001
arabinose	0.003	0.000	0.001	0.001
arbutin	0.002	0.000	0.001	0.000
aspartic acid 2	0.750	0.207	0.120	0.177
beta-cyano-L-alanine	0.002	0.000	0.001	0.000
citrulline 2	0.001	0.000	0.000	0.000
D-(+) trehalose	0.053	0.003	0.029	0.020
dopamine (hydroxytyramine)	1.589	0.178	0.436	0.213
fumaric acid	0.180	0.031	0.068	0.038
glycerol 1-phosphate	0.021	0.002	0.008	0.005
L-alanine 1	0.010	0.002	0.003	0.003
L-asparagine 2	0.372	0.021	0.083	0.136
leucrose	0.003	0.000	0.001	0.001
L-glutamic acid 3 (dehydrated)	2.642	0.178	1.110	0.707
L-glutamine 1	0.107	0.013	0.023	0.038
L-homoserine 2	0.002	0.000	0.000	0.000
L-lysine 2	0.003	0.001	0.000	0.001
L-methionine sulfoxide 3	0.001	0.000	0.000	0.000
L-norleucine 1	0.059	0.013	0.022	0.012
L-serine 1	0.625	0.028	0.148	0.105
L-threonine 1	0.080	0.008	0.026	0.016
L-valine 2	0.046	0.007	0.024	0.010
myo-inositol	0.042	0.003	0.020	0.009
N-methylalanine	0.021	0.002	0.675	0.293
O-phosphocolamine	0.005	0.001	0.002	0.001
Phenylalanine 1	0.018	0.002	0.005	0.004
phytol 1	0.050	0.002	0.013	0.012
putrescine	0.003	0.001	0.000	0.001
pyruvic acid	0.006	0.001	0.001	0.002
rhamnose 1	0.004	0.000	0.002	0.001

stigmasterol	0.022	0.003	0.008	0.005
talose 1	0.005	0.000	0.003	0.002
threonic acid	0.102	0.010	0.072	0.019
Threonic acid-1.4-lactone	0.007	0.001	0.003	0.002
tyrosine 2	0.044	0.007	0.015	0.016
urea	0.011	0.002	0.004	0.003
xylitol	0.005	0.000	0.001	0.001
xylose 2	0.004	0.000	0.002	0.001
xylulose	0.007	0.000	0.003	0.001

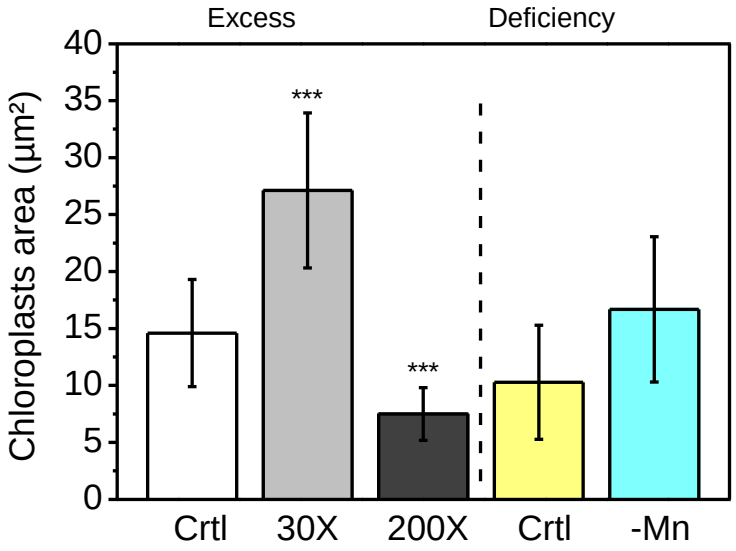
List of 41 metabolites significantly different between Agar Control and both Starch Control and Starch-Mn conditions combined ($p<0.05$). The column Starch (+/-) represents the mean of all starch samples (Starch Control and Starch-Mn). A statistical test ANOVA 2 was conducted. Absolute values represent the mean of 4 biological replicates for each condition.

Figure S1. Metabolite analysis in *M. polymorpha* grown in Mn excess condition.



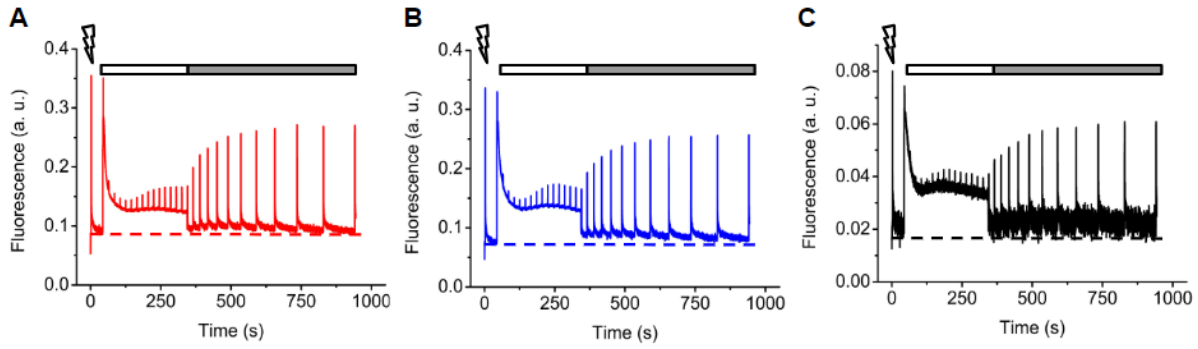
Heatmap showing significantly different metabolites in whole thalli grown under Control. 30X and 200X conditions. This figure is a result of an ANOVA 2 statistical test. The color scale represents the changes between the three conditions taking into account the maximum and the minimum value for a given metabolite in each condition.

Figure S2. Estimation of the chloroplast size under the different growth conditions



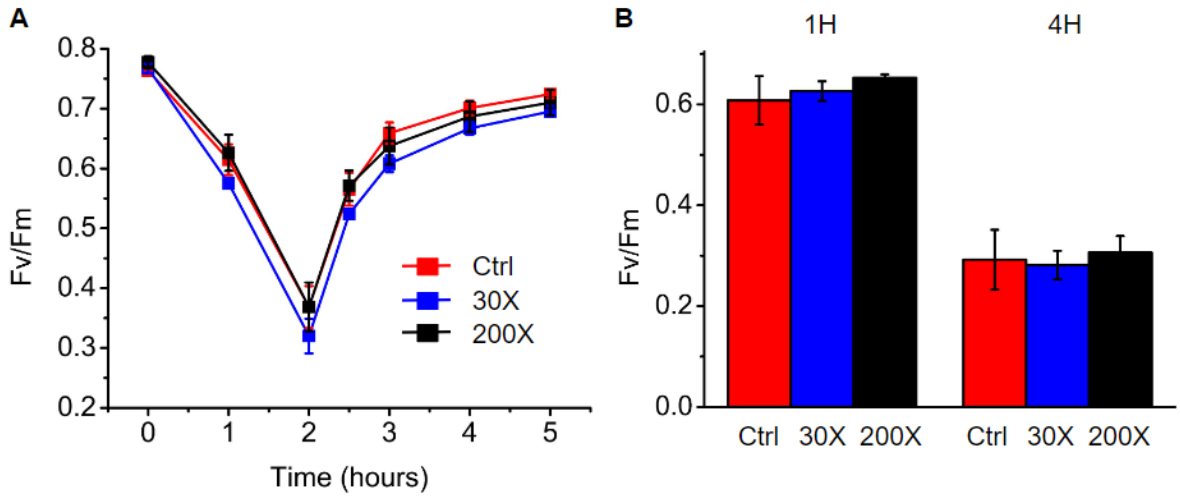
Area of the chloroplasts was determined from super-resolution fluorescence microscopy images using ImageJ. Number of images analyzed: Crtl agar: N=12, 30X: N=12; 200X: N=23; Crtl starch: N=25, starch –Mn : N=7. Mean value and SD are given. Stars indicate significant differences, compared to the control, based on a Mann and Whitney test (*** $p<0.001$).

Figure S3. Chlorophyll fluorescence at room temperature in *M. polymorpha* grown in control and Mn excess conditions



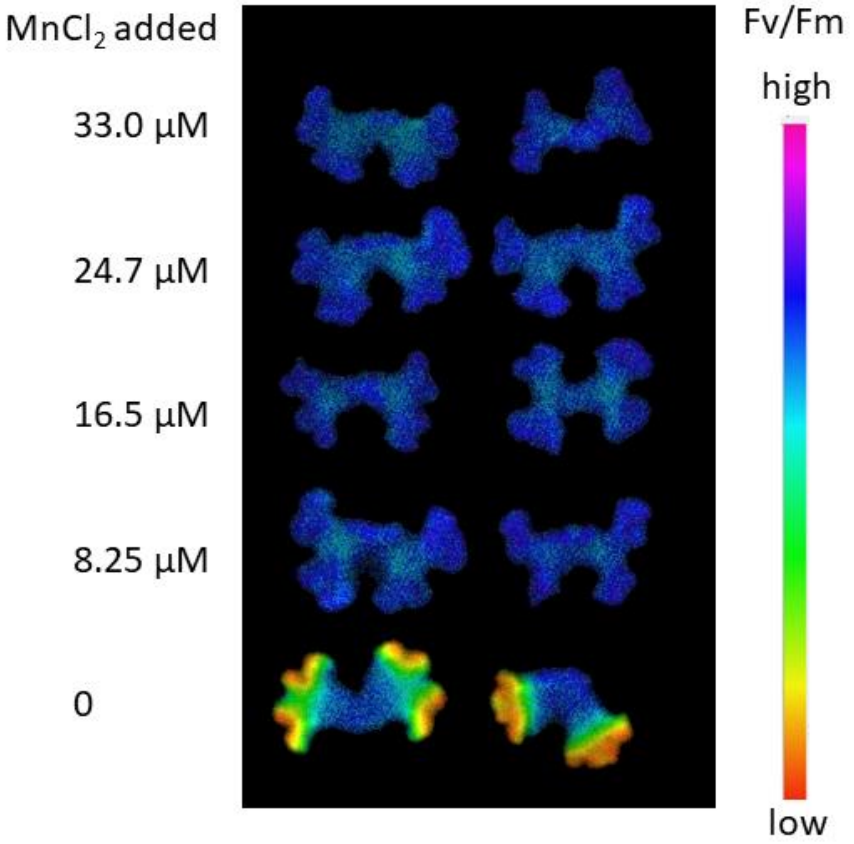
Induction and recovery of chlorophyll measured on thalli grown in Control (red), 30X (blue) and 200X (black) conditions. After 5 min dark adaptation, thalli were exposed to a measuring light to determine the minimum fluorescence level (dashed lines) and to a saturating flash to obtain the maximum fluorescence level. Actinic light (50 µmol photons m^{-2} s^{-1}) was applied for 5 min (white bar). Recovery in the dark was followed (grey bar). Representative curves are shown (N=3).

Figure S4. Photoinhibition of photosystem II in *M. polymorpha* grown in control and Mn excess condition in the presence and absence of lincomycin



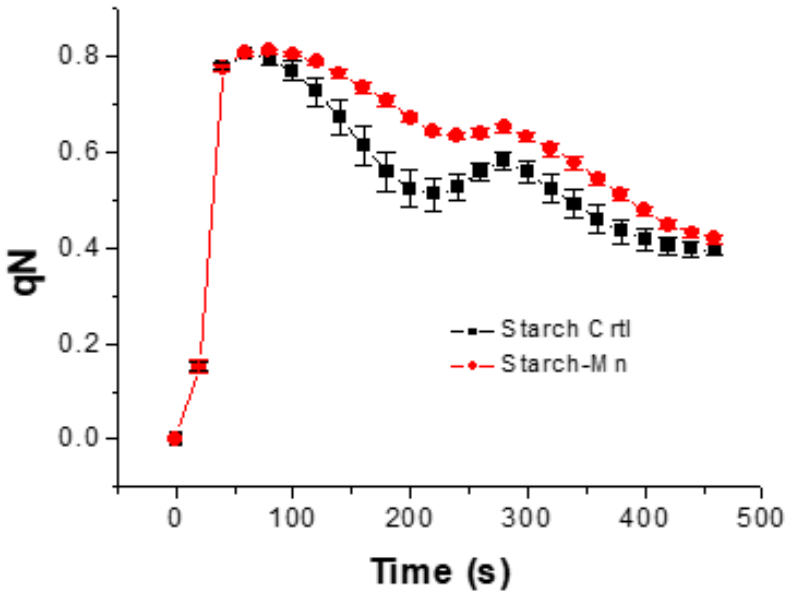
(A) Photoinhibition without lincomycin was measured in thalli cultivated in Control (red), 30X (blue) and 200X (black) conditions. Fv/Fm was followed on plants exposed to a strong light (800 µmol photons $m^{-2}s^{-1}$) for 2h and during the recovery at room light. **(B)** Fv/Fm was recorded during photoinhibition of thalli preincubated with lincomycin for 4h. Mean and SD are shown (N=3, biological replicates).

Figure S5. Chlorophyll fluorescence (Fv/Fm) in *M. polymorpha* grown on starch plates in low Mn concentrations



Thalli were grown for 2 weeks on standard medium before transferred for 1 week to starch media with the addition of the indicated Mn concentration. After 1 week growth on starch medium, thalli were dark-adapted for 5 min before Fv/Fm values were determined using an Imaging-PAM (Walz, Effeltrich, Germany). Right: Colour scale corresponds to Fv/Fm value between 1 (pink/high) and 0 (red/low).

Figure S6. qN determination in the presence of lincomycin in *Marchantia polymorpha* grown on starch in control and Mn-deficient conditions



Thalli were incubated for 4 h in lincomycin solution (1g L^{-1}) prior to the measurements. After 5 min dark adaptation. thalli were exposed to measuring light and to saturating flashes. Actinic light (55 µmol quanta $m^{-2}s^{-1}$) was applied during 5 min. Mean and SD are shown (N=4).