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► **To cite this version:**

Marine Messant, Umama Hani, Thaïs Hennebelle, Florence Guérard, Bertrand Gakière, et al.. Manganese concentration affects chloroplast structure and the photosynthetic apparatus in *Marchantia polymorpha*. *Plant Physiology*, 2023, 192 (1), pp.356-369. 10.1093/plphys/kiad052 . hal-04176185

HAL Id: hal-04176185

<https://hal.science/hal-04176185>

Submitted on 2 Aug 2023

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

3
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14
15 Total word count: 5351

16 Number of Figures: 7; colour figures: 6

17 Tables: 3

18 Supporting Material: 3 tables and 6 figures

19
20 **Running title:** Manganese excess and starvation in *M. polymorpha*

21
22
23
24 **Key words:** manganese homeostasis, *Marchantia polymorpha*, metabolomic analysis,
25 photosynthetic electron transport, super-resolution microscopy

29 **Abstract**

30 Manganese is an essential metal for plant growth. The most important Mn-containing enzyme
31 is the Mn_4CaO_5 cluster that catalyses water oxidation in Photosystem II. Mn deficiency
32 primarily affects photosynthesis, while Mn excess is generally toxic. Mn excess and
33 deficiency were studied in the liverwort *Marchantia polymorpha*, an emerging model ideally
34 suited for analysis of metal stress since it accumulates rapidly toxic substances due to the
35 absence of well-developed vascular and radicular systems and a reduced cuticle. We
36 established growth conditions for Mn excess and deficiency, performed analysis of metal
37 content in thalli and in isolated chloroplasts. *In vivo* super-resolution fluorescence
38 microscopy and transmission electron microscopy revealed changes in the organization of the
39 thylakoid membrane under Mn excess and deficiency. Both, Mn excess and Mn deficiency,
40 increased the stacking of the thylakoid membrane. We investigated photosynthetic
41 performance by chlorophyll fluorescence at room temperature and at 77K, P700 absorption
42 and by studying the susceptibility of thalli to photoinhibition. Non-optimal Mn concentrations
43 changed the ratio of photosystem I to photosystem II. Upon Mn deficiency higher non-
44 photochemical quenching was observed, electron donation to photosystem I was favoured,
45 and photosystem II was less susceptible to photoinhibition. Mn deficiency seems to favour
46 cyclic electron flow around photosystem I protecting thereby photosystem II in high light.
47 The results presented here suggest an important role of Mn for the organisation of the
48 thylakoid membrane and photosynthetic electron transport.

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53 **Introduction**

54 Manganese (Mn) is an essential element for plant growth. Mn homeostasis is
55 disturbed under suboptimal or excessive Mn availability (Schmidt et al., 2016; Alejandro et
56 al., 2020). The most important Mn-containing enzyme is the Mn_4CaO_5 cluster at Photosystem
57 II (PSII) that catalyses water oxidation. Although Mn is involved as cofactor in a range of
58 biochemical pathways, the primary effect of Mn deficiency in photosynthetic organisms is a
59 decrease in photosynthetic activity (Marschner, 1995). Furthermore, Mn is involved in
60 Reactive Oxygen Species (ROS) metabolism and in the antioxidant response. Manganese is
61 the cofactor of the Manganese Superoxide Dismutase (MnSOD) found in mitochondria and
62 peroxisomes (Bowler et al., 1994; Corpas et al., 2017). Oxalate oxidase (OxOx) present in the
63 apoplast also requires Mn. This enzyme catalyses the oxidation of oxalate to carbon dioxide
64 coupled with the reduction of oxygen to hydrogen peroxide (Requena and Bornemann, 1999),
65 the latter having an essential role in the defence against pathogens (Lane et al., 2002). When
66 accumulated in excess, Mn can be toxic causing oxidative stress (Marschner, 1995; Pittman,
67 2005; Delhaize et al., 2007; Peiter et al., 2007; Eroglu et al., 2016).

68 In the presence of excess Mn in the soil, there is a competition between the uptake of
69 Mn and other metals that are also essential for the plant (Alam et al., 2005; St. Clair and
70 Lynch, 2005; Blamey et al., 2015; Leskova et al., 2017). Indeed, at the level of the roots,
71 plants do not have transporters that are completely selective for a single metal. Thus, a high
72 abundance of Mn can lead to a decrease in the absorption of other essential metals such as
73 calcium, magnesium, iron or even phosphorus, negatively affecting photosynthesis (Nable et
74 al., 1988; Amao and Ohashi, 2008) and inhibiting chlorophyll synthesis (Clairmont et al.,
75 1986; Subrahmanyam and Rathore, 2001). Manganese excess causes chlorosis followed by
76 necrosis leading to plant death, but these symptoms are very variable and species-dependent
77 (Millaleo et al., 2010). To overcome Mn toxicity, plants have developed different ways of Mn
78 storage in vacuoles (Ducic and Polle, 2007), cell walls (Führs et al., 2010) and even Golgi
79 vesicles (Marschner, 1995; Pittman, 2005). In the literature, plants species have been divided
80 into tolerant and non-tolerant to Mn excess. Some species can hyperaccumulate Mn at levels
81 above $10\ 000\ mg.kg^{-1}\ DW$ (van der Ent et al., 2013).

82 The effect of Mn deficiency on photosynthetic electron transport and chloroplast
83 structure has been studied for decades in a number of different organisms ranging from
84 cyanobacteria to higher plants (e.g., Homann, 1967; Salomon and Keren, 2011). In general, a
85 decline of PSII activity is observed although symptoms of Mn deficiency are species-

86 dependent (Homann, 1967). Depending on the severity of Mn deficiency the ultrastructure of
87 chloroplasts may be perturbed. In slightly Mn-deficient spinach plants, the stroma lamellae
88 are affected while the grana stacks are normal. Under more severe Mn deficiency, grana
89 stacks are also disorganized (Mercer et al., 1962). More recently, similar observations were
90 reported in the Arabidopsis knockout mutant Chloroplast Mn Transporter1 (CMT1), a
91 transporter localized in the inner envelope membrane, where chloroplast development was
92 abnormal and thylakoids appeared disorganized, with either hypo- or hyper-stacked grana
93 lamellae (Eisenhut et al., 2018; Zhang et al., 2018). In this mutant, a large heterogeneity
94 between the chloroplasts was observed with chloroplasts containing a well-structured, normal
95 organization of the thylakoid membranes next to chloroplasts with completely disorganized
96 architectures (Eisenhut et al., 2018). The consequences of the loss of PSII activity and the
97 disorganization of the thylakoid membrane on the ratio of linear and cyclic photosynthetic
98 electron transport have not been investigated in the previous studies.

99 In this present study, we investigated for the first time the consequences of both
100 excess and deficiency of Mn on photosynthesis in the liverwort *Marchantia polymorpha*, an
101 emerging model system. *Marchantia* has been described in the 1990s as being able to hyper-
102 accumulate certain metals such as for example lead (Samecka-Cymerman et al., 1997). Due
103 to the morphology of the thalli, the ventral part and hyaline parenchyma of the plant is in
104 direct contact with the substrate allowing uptake of the metals from the medium without
105 having to pass through the root system as occurs in higher plants. Compared to higher plants,
106 the leaf anatomy of *Marchantia* is simpler and the tissues are thinner. Chloroplasts are less-
107 structured than in higher plants (Tanaka et al., 2017) with smaller grana stacks, making these
108 chloroplasts an ideal system for super-resolution fluorescence microscopy.

109 We have established conditions for cultivating *Marchantia* on plates under Mn
110 deficiency and excess. The metal content, metabolome and antioxidant activities as well as
111 photosynthetic activity and chloroplast structure were determined to investigate the response
112 of *Marchantia* to non-optimal Mn supply. Manganese excess and deficiency affect these
113 processes in different ways. Manganese excess led to a strong response of the metabolome
114 but subtle defects in photosynthesis, while in contrast Mn deficiency affected the activity of
115 PSII and promoted cyclic electron transport around PSI. Under Mn deficiency, an increase in
116 non-photochemical fluorescence quenching was observed, protecting PSII against
117 photoinhibition.

118

119

120 **Results**

121 ***Part I: Mn excess induces a stress response and affects the photosynthetic apparatus***

122 In order to determine the effect of Mn excess on *M. polymorpha*, plants were
123 cultivated on media containing different MnCl₂ concentrations, ranging from 33 μM (Agar
124 Control) to 6.5 mM (200X) manganese. Gemmae were cultured on a standard Gamborg's B5
125 medium (33 μM MnCl₂) for two weeks before being transferred for one week to the different
126 Mn concentrations before analysis. Fig. 1A shows that thalli were able to grow normally up
127 to 1 mM Mn. However, thalli showed reduced growth from 2 mM up to 6.6 mM Mn, with
128 signs of stress visible as brown spots. Chlorophyll content of the thalli was significantly
129 decreased in excess Mn conditions (SI Table 1). Determination of Mn concentration in thalli
130 (Fig. 1B) showed that there is a positive correlation between Mn accumulation and Mn
131 concentration in the medium. In addition to Mn, we determined Fe and Mg content since high
132 Mn may impact negatively Fe import into the chloroplasts and thereby the assembly of FeS
133 clusters in PSI (Millaleo et al., 2013), and Mg is important for stacking of the thylakoid
134 membranes. Neither Fe nor Mg content were affected by the high Mn concentrations.

135 In the following, we focus on the conditions of Mn excess 30X and 200X. The first
136 being the highest concentration at which Marchantia showed no obvious signs of stress and
137 the second being the most stressful condition. We conducted a metabolomic analysis by GC-
138 MS on thalli. In total, 94 metabolites have been identified and quantified (Supplemental Fig.
139 S1; Tables S2, S3). Regarding the 200X condition, certain metabolites involved in the
140 response to heavy metal or oxidative stresses were strongly increased while all other
141 metabolites decreased compared to the control. A very high concentration of Mn was
142 associated with a strong induction of polyamine synthesis with an accumulation of ornithine,
143 citrulline and putrescine (Table 1). Among sugars and their derivatives, strong increases in
144 trehalose, rhamnose and galacturonic acid were also observed. All these compounds are
145 known to accumulate in plants and other organisms upon exposure to heavy metals (Sharma
146 and Dietz, 2006 ; Hasanuzzaman et al., 2019, Liu et al., 2017; Singh et al., 2016; Alejandro et
147 al., 2020). Most striking, N-methylalanine content was 30 and 50 times higher in 30X and
148 200X conditions, respectively. To further investigate the response to oxidative stress, the
149 activity of a few antioxidant enzymes was measured (Table 2). A twofold increase in SOD
150 activity was observed at the highest Mn concentration. The activity of class III peroxidases
151 was also slightly increased at 200X, while catalase activity gradually decreased.

152 Next, we studied the impact of Mn excess on chloroplast ultrastructure and
153 photosynthetic activity. The concentration of Mn, Fe and Mg was measured in isolated intact

154 chloroplasts (Fig. 2A). The results show a gradual and significant increase of Mn comparable
155 with the observation made in whole thalli. We observed an increase from 2 to 175 $\mu\text{g Mn mg}$
156 Chl^{-1} in the 200X condition compared to the Control. We determined the total chlorophyll
157 content of the thalli to determine the proportion of Mn allocated to chloroplasts (Fig. 1B; Fig.
158 2A; Supplemental table S1). 7-13% of the total Mn content of the thalli was detected inside
159 the isolated chloroplasts with 13% in Control and 30X and 7% in 200X. There were no
160 significant changes in the chloroplast Fe and Mg concentrations under Mn excess.

161 We used first bright-field and epifluorescence microscopy to see whether Mn excess
162 changes the morphology of the cells as well as the number of chloroplasts (Fig. 2B). In
163 Control Agar, most chloroplasts show typical plant-type spherical morphology, while in 30X,
164 there was a divergence from this typical architecture. Some chloroplasts had a deformed
165 shape. In 200X, this progression of deformation of the morphology was further enhanced and
166 the chloroplasts were smaller on average (Fig. 2C, Supplemental Fig. S2). Next, we used
167 super-resolution fluorescence nanoscopy and transmission electron microscopy to determine
168 better the change in the organization of the thylakoid membranes triggered by excess Mn
169 (Fig. 3). At physiological temperatures, chlorophyll fluorescence is mainly due to PSII
170 emission, which are essentially concentrated in the grana lamellae (for reviews see Dekker
171 and Boekema 2005; Kirchhoff, 2019). Hence, the fluorescence images partly represent the
172 spatial organisation and relative abundance of grana stacks in *in vivo* chloroplasts. The
173 organization of the chloroplast in the Control (Fig. 3A top panel) is similar to that of
174 *Arabidopsis thaliana* (Iwai et al., 2018; Streckaite, 2021) with a 'round' shape and the
175 presence of numerous grana stacks visualised as highly fluorescent yellow/orange patches in
176 the false-colour images, albeit with less clear separation between stroma and grana lamellae.
177 Under the 30X condition, the chloroplast is smaller, indicating a more compact spatial
178 organization of the inter-connected granae. Furthermore, the presence of a non-fluorescent
179 region was observed in the centre corresponding to a void in bright field images. In 200X, the
180 smaller chloroplasts still exhibited strong chlorophyll fluorescence emissions with a well-
181 developed distribution of patches indicative of thylakoid grana stacks. Electron microscopy
182 images confirm clear grana stacking under control and 200X conditions, while the
183 organisation at 30X was different with three thylakoid membranes always attached to each
184 other concomitant with a lack of major grana stack formation (Fig. 3B, TEM insert). This
185 uniform distribution of attached membranes under 30X Mn is in agreement with the quasi-
186 uniform false-colour chlorophyll emission maps observed using *in vivo* fluorescence
187 nanoscopy, attributed to mainly PSII emission.

188 Since the size of the chloroplasts and the distribution of the chlorophyll fluorescence
189 within the chloroplasts was affected by the growth conditions, photosynthesis was studied in
190 more detail. Table 3 shows that the Chl *a/b* ratio remained unchanged at 30X but decreased at
191 200X Mn, indicating that the antenna size relative to reaction centre increased since Chl *b* is a
192 major constituent of the Light Harvesting Complex II (LHCII). 77K fluorescence emission
193 spectra (Fig. 4A) showed three characteristic peaks at 685 nm, 695 nm and 725 nm, the
194 former two peaks are attributed to LHCII and PSII, and the latter to PSI with its antenna
195 (Sato and Butler, 1978). For chloroplasts isolated from thalli grown at 200X, the emission
196 attributed to PSI decreased relative to that of PSII, while there was no change when grown at
197 30X (Fig. 4A). In line with the lower PSI fluorescence relative to PSII fluorescence in 200X,
198 the capacity of PSI electron transport was slightly lower although the effect was not
199 statistically significant (Table 3). However, the capacity of PSII electron transfer was
200 significantly decreased. A damaging effect of high Mn concentration on PSII activity was
201 confirmed by fluorescence induction curves (Fig. 4B). The appearance of the K-phase under
202 200X condition can be attributed to a damage of the Mn₄CaO₅ cluster at the PSII donor side
203 according to Strasser (1997). In 200X, Fv/Fm and chlorophyll quenching fluorescence was
204 altered during illumination with actinic light, but there was no increase in the susceptibility of
205 PSII to photoinhibition (Table 3, Supplemental Figs S3, S4).

206

207 ***Part II: Mn deficiency affects the photosynthetic apparatus and may favour cyclic electron*** 208 ***flow***

209 In the following, we investigated the effect of Mn starvation on *Marchantia*
210 *polymorpha*. Agar plates were not suitable to induce Mn deficiency since agar contains too
211 much Mn as an impurity ($12.8 \pm 0.4 \mu\text{M}$). Therefore, we substituted agar by starch as a
212 gelling agent. Gemmae were cultured during 2 weeks on Gamborg's B5 Agar medium (33
213 μM MnCl₂) before young thalli were transferred to Mn-free Gamborg's B5 Starch medium
214 with or without addition of Mn (33 μM Mn). It was not possible to culture gemmae directly
215 on starch because they were not able to develop. Fig. 1C shows that plants exhibited no
216 visible growth defect after one week culturing on Starch Control or without Mn. However,
217 chlorophyll fluorescence showed a clear decrease in the quantum yield of PSII, Fv/Fm) in the
218 developing part of the thallus when no MnCl₂ had been added to the medium (Supplemental,
219 Fig. S5). The amount of metals in thalli was measured (Fig. 1D). The use of starch allowed a
220 reduction of Mn content from 75 μg Mn g DW⁻¹ (Starch Control) and to 20 μg Mn g DW⁻¹
221 (Starch-Mn). Fe and Mg contents were not affected.

222 As for Mn excess, a metabolic analysis was carried out. No significant difference in
223 the metabolomics profiles was observed between the two starch conditions (Supplemental
224 Table 1) while there were clear differences between Agar Control and Starch Control
225 (Supplemental Table 2). The activity measurements of the antioxidant enzymes showed a
226 significant decrease in the activity of type III peroxidases (Table 3). Using a spin-trapping
227 assay, the generation of $\cdot\text{OH}$ derived from $\text{H}_2\text{O}_2/\text{O}_2\cdot^-$ was detected in starch-grown plants.
228 ROS levels were two times higher in the Starch-Mn condition.

229 Next, the impact of Mn deficiency on chloroplasts was investigated. The
230 measurements (Fig. 2D) revealed a decrease in the Mn content by about 50% in the Starch-
231 Mn condition compared with the Starch Control. There was a tendency that the Starch-Mn
232 growth condition lowers slightly the Fe and Mg content, however the differences were not
233 statistically significant. Overall, the metal content (Mn, Fe, Mg) of the chloroplasts under
234 starch conditions was almost double compared to chloroplasts from thalli grown under Agar
235 Control conditions. This may be due to effects on the metal uptake and transport system or
236 also be connected to a higher intactness of the isolated chloroplasts. The epifluorescence
237 microscopy images (Fig. 2F) showed more “horseshoe-shaped” chloroplasts under the Starch
238 control condition. In Starch-Mn chloroplasts appeared rounder. These observations were
239 confirmed by super-resolution nanoscopy (Fig. 3B), however the area of the chloroplasts was
240 unaltered (Supplemental Fig. S2). In the Starch Control, the thylakoid membranes were
241 organized in grana/lamellae-like structures. In the Starch-Mn condition, the chloroplasts had
242 fewer voids in the 3-D volume. Compared with a standard chloroplast as represented in the
243 Agar Control (Fig. 3A, top panel), or *Arabidopsis* (Iwai et al., 2018; Streckaite, 2021), the
244 strongly fluorescent regions were localized more distinctly, indicating a change in the
245 mesoscopic organization of the thylakoid membrane. This indicates smaller grana stacks with
246 a more pronounced segregation between them and the stroma lamellae as confirmed by TEM.
247 The TEM image shows a much higher degree of stacking of the thylakoid membranes (Fig.
248 3B).

249 77K fluorescence (Fig. 4B) showed a decrease in the emission at 680 nm in
250 chloroplasts isolated from Starch-Mn thalli, indicating a loss of PSII content compared with
251 PSI. PSII activity was lower (Table 3). The quantum yield of PSII, Fv/Fm, was significantly
252 lower upon Mn deficiency. PSI activity increased significantly, confirming the change in the
253 PSI/PSII ratio under Mn deficiency seen in 77K fluorescence spectra. In the Starch-Mn
254 condition, the fluorescence induction curves showed a dip phase at about 2 ms, indicative of
255 damage to the Mn_4CaO_5 cluster at the PSII donor side (Fig. 4D).

256 Figure 5 shows changes in room temperature chlorophyll fluorescence upon
257 illumination with actinic light. Upon Mn deficiency, energy dissipation as heat was enhanced
258 (qN; Fig. 5C). However, photochemical quenching (qP; Fig. 5D) was not affected.
259 Furthermore, the slightly higher minimum fluorescence level during the recovery phase
260 (Post-Illumination Fluorescence Transient, PIFT) upon Mn deficiency indicates an increase
261 in chlororespiratory and/or cyclic electron flow. To show whether the stability of PSII or the
262 repair of damaged PSII was affected under Mn deficiency, photoinhibition experiments were
263 carried out with or without the protein synthesis inhibitor lincomycin (Fig. 6). Plants
264 cultivated on Starch-Mn were more susceptible to high light in the absence of lincomycin. In
265 the presence of lincomycin, the opposite was observed with PSII in plants cultivated on
266 Starch-Mn being more resistant to the photoinhibitory treatment. This may be explained by
267 the higher NPQ protecting the photosynthetic apparatus. A higher NPQ was observed also in
268 the presence of lincomycin (Supplemental Fig. S6).

269 The higher PSI fluorescence at 77K, the higher NPQ and the protection of PSII
270 against photoinhibition in plants cultivated on Starch-Mn may indicate that cyclic electron
271 transport is favoured under these conditions. Absorption changes of P700 show that P700 is
272 more slowly oxidized, both in red actinic and in far-red light, in plants cultivated on Starch-
273 Mn indicating that more electrons are available in the electron transport chain (Fig. 7A). To
274 show that indeed more electrons are available for P700⁺ reduction at the luminal side, we
275 determined the yield of donor side limitation, Y(ND) as a function of the intensity of the
276 actinic light. Figure 7B shows less donor-side limitation of PSI over a wide range of light
277 intensities in plants cultivated on Starch-Mn compared to those cultivated on Starch Control.
278 These data further indicate a higher activity of cyclic electron flow under Mn-deficiency.
279 Under both conditions, acceptor side limitation at the stromal side of PSI was not observed
280 because *Marchantia* possesses flavodiiron proteins that use ferredoxin as electron donor to
281 reduce oxygen to water (Shimakawa et al., 2021).

282

283 Discussion

284 In this study, we established growth conditions for manganese excess and deficiency in
285 *Marchantia polymorpha*. *Marchantia* is able to absorb up to 25 mg Mn g DW⁻¹ (Fig. 1B)
286 which is higher than the hyperaccumulator *Polygonum lapathifolium* which contains
287 approximately 18 mg Mn g DW⁻¹ in its aerial parts (Liu et al., 2016). However, *Marchantia*
288 shows symptoms of toxicity under the 200X conditions, and thalli only survive for a few
289 weeks. Therefore, *Marchantia* does not qualify as hyperaccumulator despite its high Mn

290 uptake capacity. Mn excess conditions induced an increase in SOD and PRX activity while
291 catalase activity decreased (Table 2). Increase in SOD activity could reflect a higher amount
292 of MnSOD present under Mn excess conditions. The metabolomics analysis showed that
293 known stress-induced metabolites accumulate upon excess Mn (Table 1; Supplemental Fig.
294 S2, tables S2, S3), however, the metabolomics response of *Marchantia* is distinct from that of
295 angiosperms and requires in depth functional analysis of metabolites and metabolic pathways.
296 An excess of Mn affected the ratio between it and other metals (e.g. Fe and Mg) in both thalli
297 and chloroplasts. Indeed, cation transporters are known not to be completely selective under
298 stressed conditions (Barberon et al., 2011; Ducic et al., 2005; Lei et al., 2007; Millaleo et al.,
299 2010; Vert et al., 2002); well-documented examples of excess of Mn in the soil are known to
300 perturb the absorption and translocation of other elements including iron, magnesium and
301 phosphorus (Millaleo et al., 2010; Ducic et al., 2005; Lei et al., 2007), causing Fe deficiency
302 in plants. However, *Marchantia* grown at 200X does not show alterations in the Fe and Mg
303 content inside the thalli (Figs 1B, 2A). Nevertheless, an alteration in the Mn/Fe ratio may
304 induce Fe deficiency through competitive binding. Fe deficiency is known to affect primarily
305 PSI that is rich in 4Fe4S clusters. As shown in Fig. 4B, PSI content is lowered in comparison
306 to PSII in 200X. A similar reduction of PSI upon exposure to Mn excess has been reported
307 for *Arabidopsis* (Millaleo et al., 2013). Aside PSI, the water-splitting activity of PSII seems
308 also to be affected in the 200X condition as seen by the appearance of the K phase in
309 fluorescence induction curves (Fig. 4C) and the decrease in PSII activity (Table 2). A toxic
310 effect of Mn excess on PSII activity is in accordance with data on higher plants (Liang et al.,
311 2019). 200X Mn negatively affected not only the photosynthetic apparatus but also led to a
312 reduction in chloroplast size and an alteration of the well-separated grana stack and stroma
313 lamellae distribution (Figs. 2 and 3).

314 Transfer of thalli from agar to starch plates allowed to establish Mn deficiency
315 conditions. Growth on starch allowed lowering drastically the Mn content in the thalli to 21
316 $\mu\text{g Mn g DW}^{-1}$. According to Mengel and Kirkby (1987), the minimum Mn content required
317 for growth of higher plants is about 20 to 40 mg Mn kg DW^{-1} , and most higher plants usually
318 contain 30 to 500 mg Mn kg DW^{-1} . Manganese deficiency led to a decrease in Fv/Fm, a
319 change in the ratio of the activity PSI/PSII (Table 3) and less PSII content relative to PSI
320 according to 77K fluorescence (Fig. 4B). In higher plants, Mn deficiency lowers PSII activity
321 while PSI activity remains unaffected (Homann, 1967; for review see Schmidt et al., 2016).
322 Furthermore, the structural organization of the thylakoids was affected (Fig. 3). It is known
323 that Mn deficiency leads to disorganization of the thylakoid membrane in higher plants

324 (Mercer et al., 1962; Homann, 1967). In *M. polymorpha*, the smaller compartmentation of the
325 grana stacks could be partly responsible for the changes in electron transport. In addition to a
326 change in the stoichiometry between active PSI and active PSII, a different distribution of
327 photosynthetic complexes may favour cyclic electron flow around PSI by allowing the
328 formation of supercomplexes likely to be required for cyclic electron flow (Iwai et al., 2010).
329 As shown in Fig. 7, thalli grown on Starch-Mn show less limitation of electron donation to
330 P700⁺ than those of the Starch Control indicating a stimulation of cyclic electron flow under
331 Mn deprivation. Cyclic electron flow is known to be induced under specific physiological
332 conditions like anaerobiosis in *Chlamydomonas reinhardtii* (Joliot et al., 2022). Changes in
333 the organization of PSI complexes was also observed under Mn limitation in cyanobacteria
334 (Salomon and Keren, 2011). Increased cyclic electron flow generates a higher proton gradient
335 across the thylakoid membrane and thereby an increase in NPQ (Fig. 5C). A higher NPQ
336 protects photosystem II against photoinhibition as seen when thalli grown on Starch-Mn were
337 exposed to high light in the presence of lincomycin (Fig. 6) while they were more susceptible
338 to photoinhibition in the absence of lincomycin (Fig. 6) likely due to a slowdown of PSII
339 repair. A slowdown of PSII repair under Mn deficiency has been observed previously in the
340 Mn transporter mutant *nramp3nramp4* in Arabidopsis (Lanquar et al., 2010). The repair of
341 PSII may be slowed down in the light because of the higher ROS levels in Starch-Mn (Table
342 2) that affects the synthesis of the D1 protein (Nishiyama *et al.*, 2011).

343 In conclusion, we show here that unfavourable Mn concentrations during growth of
344 *Marchantia* affect not only the photosynthetic apparatus but also the organisation of the
345 thylakoid membrane. Future work is needed to explore the link between changes in the
346 organization and stacking of the thylakoid membrane and stimulation of cyclic electron flow
347 under Mn deficiency.

348

349 **Material and Methods**

350 **Plant growth conditions**

351 Gemmae from *Marchantia polymorpha*, Takaragaike (Tak-1) accession (male) were
352 asexually cultured on ½ Gamborg's GB5 1% Agar medium (Gamborg et al., 1968) for two
353 weeks under a light-dark cycle of 16 h-light, 22°C, 120 μmol quanta m⁻²s⁻¹, white fluorescent
354 lamp / 8 h-dark, 20°C. For Mn excess condition, young thalli were transferred to ½
355 Gamborg's GB5 1% Agar medium containing 33 μM (control), 0.5 mM (15X), 1 mM (30X),
356 2 mM (60X), 5 mM (150X) or 6.5 mM (200X) MnCl₂ for one week before measurements.
357 According to the metal quantification, the control medium contained 2.52 ± 0.27 μg Mn g⁻¹

358 fresh weight of the solid medium. For Mn deficiency, young thalli were transferred on 1/2
359 Gamborg's GB5 6% starch medium with or without addition of 33 μM MnCl_2 for one week
360 before performing measurements. According to the metal quantification, the starch medium
361 with MnCl_2 contained $2.03 \pm 0.13 \mu\text{g Mn g}^{-1}$ and the medium without addition of MnCl_2
362 contained $0.26 \pm 0.08 \mu\text{g Mn g}^{-1}$ fresh weight of the solid medium.

363

364 **Chloroplast and thylakoid isolation**

365 10 g thalli were collected, the media carefully mechanically removed, and ground in 100 ml
366 of GR buffer (50 mM Hepes-KOH pH 7.5, 0.33 M sorbitol, 1 mM MgCl_2 , 2 mM EDTA, 5
367 mM Na-ascorbate) with a spatula of BSA. The slurry was filtered through two layers of
368 Miracloth and centrifuged at 1200g (4°C) for 7 min. Pellets were resuspended in 2 mL of GR
369 buffer then layered on top of a percoll gradient (15 ml of 30% percoll/GR buffer and 10 ml of
370 70% Percoll/GR buffer), centrifuged at 7000g (4°C) for 17 min (no brake). Intact chloroplasts
371 were collected and washed in 25 ml of GR buffer; centrifugation 1500g (4°C) for 5 min. The
372 intactness was checked by light microscopy. For thylakoids, the same procedure was used
373 without the percoll gradient.

374

375 **Metal quantification**

376 Thalli were collected and dried at 70°C for 2 days before being weighted. 1 mL of nitric acid
377 (65%) was added to 1 mg plant material. For intact chloroplasts, the chlorophyll
378 concentration was adjusted to $100 \mu\text{g chl mL}^{-1}$ before the addition of 2.5 volume of nitric
379 acid. After 10-fold dilution in trace-metal-free water, the metal content of the samples was
380 determined by atomic emission spectroscopy using a MP AES 1200 spectrometer (Agilent,
381 USA).

382

383 **Super-resolution microscopy**

384 For each sample, a small quantity of thallus was placed in a sandwich of two glass coverslips
385 (22 mm diameter, Paul Marienfeld GmbH & Co. KG), sealed and placed inside an in-house
386 built sample holder. The sample holder was fixed to a nano-positioning system (P-733.2CD,
387 P-725.4CD, E-725.3CD, Physikinstrumente) coupled to an inverted microscope (Ti-U,
388 Nikon) equipped with a CFI Apochromat Lambda 100x oil immersion objective. Room-
389 temperature fluorescence emission was recorded by an iXon ULTRA 897 camera (Andor
390 Technology Ltd., Belfast) coupled to the microscope with a custom-made Optomask (Cairn
391 research Ltd, Faversham). Total chlorophyll fluorescence emission was isolated using a

392 dichroic mirror/emission filter doublet (59022BS/ET655LP, Chroma Technology
393 Corporation). Epifluorescence images were recorded using an excitation provided by a
394 plasma light source (HPLS245 Thorlabs Inc.) and an excitation filter (MF469-35 Thorlabs
395 Inc.). The excitation source for the laser-scanning measurements was a 445 nm emitting laser
396 (OBIS LX, Coherent Inc.). Laser-scanning and final image reconstruction (resolution (d)
397 $d_{xy}=126$ nm, $d_z=320$ nm), was as previously described (Streckaite, 2021) with a dwell time of
398 10-30 ms, 60 nm X/Y-scan steps and 300 nm Z-scan steps.

399

400 **Transmission electron microscopy (TEM)**

401 1 mm strips of thalli were cut under glutaraldehyde/paraformaldehyde fixative and
402 impregnated by the 1% osmium and 1.5% potassium hexacyanoferrate *en bloc* staining
403 protocol as described (Hawes et al., 1981; Juniper et al., 1982). 70 nm thick sections were cut
404 with an EM UC6 ultramicrotome (Leica Microsystems) and deposited on to copper grids.
405 Ultrathin sections were stained with 2% uranyl acetate (Merck) and Reynolds lead citrate
406 according to standard procedures. Grids were examined under a JEOL 1400 TEM operating
407 at 80 kV (JEOL, <http://www.jeol.com>). Images were acquired using a nine megapixel high-
408 speed camera (RIO9; Gatan, <http://www.gatan.com>) and processed using Digital Micrograph
409 (Gatan).

410

411 **Pigment analysis**

412 Thalli were weighed and incubated in 100% acetone for 16 h in the dark. For complete
413 pigment extraction, acetone incubation was repeated three times. Pigment extract was diluted
414 to 80% acetone before measurement. Chlorophyll *a* and chlorophyll *b* contents were
415 calculated according to Porra and Scheer (2019).

416

417 **77K Chlorophyll fluorescence measurements**

418 Fluorescence spectra of intact chloroplasts diluted in GR buffer were measured with a Carry
419 Eclipse fluorimeter (Agilent, USA); excitation wavelength: 430 nm. The spectra were
420 normalized to the intensity of the PSI emission.

421

422 **Chlorophyll fluorescence analysis at room temperature**

423 Chlorophyll fluorescence analysis was performed on 5 min dark-adapted thalli using a Dual-
424 PAM-100 fluorimeter (Walz, Effeltrich, Germany). F_m and F_m' were determined using
425 saturating flashes ($10,000 \mu\text{mol quanta m}^{-2}\text{s}^{-1}$, duration 300 ms). F_o , minimum and F_m ,

426 maximum fluorescence in a dark-adapted sample; F_m' , maximum fluorescence and F' ,
427 fluorescence emission from a light-adapted sample. Induction and recovery curves were
428 measured using actinic light of $50 \mu\text{mol quanta m}^{-2}\text{s}^{-1}$. $F_v/F_m = (F_m - F_0)/F_m$, $qN = (F_m -$
429 $F_m')/(F_m - F_0')$ and $qP = (F_m' - F)/(F_m' - F_0')$. For induction curves (OJIP) one saturating flash
430 was given.

431

432 **Photoinhibition**

433 Thalli were placed on wet filter paper and illuminated with white light ($800 \mu\text{mol quanta m}^{-2}$
434 s^{-1}); LED panel SL3500 (Photon Systems Instrument, Drasov, Czech Republic). For
435 recovery, samples were placed in low light ($10 \mu\text{mol quanta m}^{-2}\text{s}^{-1}$). When indicated, thalli
436 were incubated in lincomycin (1 g L^{-1}) for 4 h prior to the photoinhibition treatment. F_v/F_m
437 was measured using an Imaging-PAM (Walz, Effeltrich, Germany).

438

439 **P700 measurements**

440 P700 absorbance was measured using a Dual-PAM-100 fluorometer (Walz, Effeltrich,
441 Germany). Near-infrared measuring lights (830 and 870 nm) were applied to measure the
442 transmittance of oxidized P700. Prior to the measurements, the plants were kept in the light in
443 the growth chamber so that the Calvin-Benson cycle enzymes were active. Five minutes dark-
444 adapted thalli were exposed to actinic light followed by far-red light. Then, a saturating flash
445 was given at the end of the far-red light period. To determine quantum yields of PSI donor,
446 $Y(\text{ND})$, and acceptor side-limitations, $Y(\text{NA})$, saturating pulse analysis was used
447 (Klughammer and Schreiber, 1994). Each actinic light intensity was applied for 180 s before
448 determining $Y(\text{ND})$ and $Y(\text{NA})$.

449

450 **Antioxidant enzymes activity**

451 Thalli were harvested and ground in 50 mM HEPES buffer pH 6.5. The slurry was filtered
452 through one layer of miracloth, centrifuged for 5 min at $10.000g$ (4°C). Protein concentration
453 in the crude extract was determined by amido black since the supernatant contained pigments
454 (Schaffner and Weissmann, 1973). $10 \mu\text{g mL}^{-1}$ protein was used for the enzymatic tests.
455 Guaiacol peroxidase activity was determined spectrophotometrically by measuring the
456 oxidation of guaiacol to tetraguaiacol at 470 nm ($\epsilon: 26.6 \text{ mM}^{-1}\text{cm}^{-1}$) (Chance and Maehly,
457 1955). The reaction mixture contained 50 mM $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ pH 7.5, 3 mM H_2O_2 ,
458 0.01% (v/v) guaiacol. Catalase activity was measured polarographically at 20°C with a
459 Clark-type electrode in 50 mM Tris buffer pH 8.5 and 1 mM H_2O_2 as substrate. Superoxide

460 dismutase (SOD) activity was measured using a solution of 500 μM Xanthine, 20 mM
461 HEPES buffer pH 7; 0.2 U mL^{-1} xanthine oxidase and 100 μM XTT (Na,3'-(1-
462 (phenylaminocarbonyl)-3,4-tetrazolium)-bis-(4-methoxy-6-nitro) benzene sulfonic acid
463 hydrate) as substrate. The kinetics of superoxide production were measured as increase in
464 absorbance at 470 nm, and the SOD activity was determined by following the inhibition of
465 the superoxide production after addition of the crude extract (Molins et al. 2013). For $\cdot\text{OH}$
466 detection, 15 mg thalli were incubated for 1 h in 3 ml 10 mM phosphate buffer, pH 6.0, 50
467 mM N-tert-butyl- α -(4-pyridyl)nitron N'-oxide (4-POBN) and 4% ethanol (Heyno et al.,
468 2008).

469

470 **Metabolite Analysis**

471 GC-MS profiling of metabolites was performed on thalli cultured on Agar Control, 30X and
472 200X conditions and thalli grown on Starch Control and Starch-Mn. Dried and grounded
473 samples (5 mg of dry weight) were incubated in 1 mL of H₂O/ACN/isopropanol (2/3/3) with
474 4 mg l⁻¹ of ribitol for 10 min at 4°C with shaking at 250g in an Eppendorf Thermomixer.
475 Insoluble material was removed by centrifugation at 20 400g for 10 min. 700 μl of
476 supernatant were recovered, 70 μl of a mix of H₂O/MeOH/isopropanol (2/5/2) with 0.3 g L⁻¹
477 of myristic acid d₂₇ was added as an internal standard for retention time locking. Aliquots of
478 each extract (100 μL) extracts were dried for 4 h at 30°C in a Speed-Vac and stored at -80°C.
479 After thawing, samples were dried again in a Speed-Vac for 1 h at 30°C before adding 10 μL
480 of 20 mg mL⁻¹ methoxyamine in pyridine to the samples. The reaction was performed for 90
481 min at 30°C under continuous shaking in an Eppendorf thermomixer. A volume of 90 μL of
482 N-methyl-N-trimethylsilyl-trifluoroacetamide was then added and the reaction continued for
483 30 min at 37°C. After cooling, 80 μL were transferred to an Agilent vial for injection. Four
484 hour after derivatization, 1 μL sample was injected in splitless mode on an Agilent 7890B gas
485 chromatograph coupled to an Agilent 5977A mass spectrometer (column: RESTEK RXI
486 5SIL MS 30MX0.25MMX0.25UM). An injection in split mode with a ratio of 1:30 was
487 systematically performed for quantification of saturated compounds. Raw Agilent data files
488 were analysed with AMDIS <http://chemdata.nist.gov/mass-spc/amdis/>. The Agilent Fiehn
489 GC-MS Metabolomics RTL Library was employed for metabolite identifications. Peak areas
490 were determined with the Masshunter Quantitative Analysis (Agilent) in splitless and split 30
491 modes. Peak areas were normalized to ribitol and dry weight. Metabolite contents are
492 expressed in arbitrary units (semi-quantitative determination).

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Acknowledgements

We thank Claire Boulogne (I2BC) for performing electron microscopy. This work was supported by the Labex Saclay Plant Sciences-SPS (ANR-17-EUR-0007), the platform of Biophysics of the I2BC supported by the French Infrastructure for Integrated Structural Biology (FRISBI; grant number ANR-10-INSB-05) and IBiSA. M.M. is supported by a CEA PhD fellowship.

Author Contribution

M.M, and A.K-L. designed the project. M.M, U.H., T.H., F.G., B.G., A.G., S.T. and A.K-L. performed the experiments and analysed the data. M.M. and A.K-L. wrote the initial version of the manuscript that was read and revised by all authors.

Data availability

Data will be made available on demand.

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724 **Tables**

725

726 **Table 1. Increase in metabolites involved in oxidative stress response upon growth on**
727 **high Mn concentration**

728

	Ctrl	30X	200X
Citrulline	0.001 ± 0	0.001 ± 0	0.003 ± 0.001
Galacturonic acid	0.004 ± 0	0.004 ± 0.001	0.008 ± 0.001
L-ornithine	0.003 ± 0.001	0.003 ± 0	0.007 ± 0.001
N-Methylalanine	0.021 ± 0.002	0.67 ± 0.237	1.101 ± 0.038
Putrescine	0.003 ± 0.001	0.002 ± 0	0.008 ± 0.001
Rhamnose	0.004 ± 0	0.004 ± 0.001	0.008 ± 0.001
Trehalose	0.053 ± 0.003	0.049 ± 0.008	0.083 ± 0.012

729

730

731 List of selected metabolites showing an increase in plants grown in 200X condition. Mean
732 value and SD are given (N=4). P<0.05 for all values, according to two-way ANOVA test. See
733 Supplemental Table S2 for the full list.

734

735

736

737 **Table 2. Antioxidant enzymes activities in thalli from control and manganese excess and**
738 **deficiency conditions**

	Ctrl	30X	200X	Starch+Mn	Starch-Mn
SOD activity	3.01 ± 0.63	4.19 ^{**} ± 0.86	6.00 ^{***} ± 1.48	5.63 ± 1.43	5.08 ± 0.63
CAT activity	1.18 ± 0.16	0.87 ^{**} ± 0.26	0.54 ^{***} ± 0.12	0.72 ± 0.16	0.70 ± 0.13
PRX activity	1.27 ± 0.38	1.17 ± 0.25	1.59 [*] ± 0.42	1.52 ± 0.20	1.04 ^{**} ± 0.40
ROS	-	-	-	44.4 ± 4.2	100 ± 1.4

739

740 Superoxide dismutase, Catalase and peroxidase activities ($\mu\text{mol substrat. mg proteins}^{-1} \text{ min}^{-1}$)
741 were measured in crude extracts. $\cdot\text{OH}$ derived from $\text{H}_2\text{O}_2/\text{O}_2^{\cdot-}$ was measured using electron
742 paramagnetic resonance (EPR) as a hydroxyethyl-N-tert-butyl- α -(4-pyridyl)nitron N' -oxide
743 (4-POBN) adduct. Stars indicate significant difference compared to the control condition,
744 based on a Mann and Whitney test (* p<0.01, ** p<0.01, *** p<0.001, N=3 biological
745 replicates).

746 **Table 3. Chl *a/b* ratio and electron transport capacities of thylakoids from thalli**
 747 **cultured on manganese excess and deficiency.**

	Ctrl	30X	200X	Starch+Mn	Starch-Mn
Chl <i>a/b</i>	1.23 ± 0.09	1.30 ± 0.10	0.97 ± 0.06	0.95 ± 0.22	0.91 ± 0.20
Fv/Fm	0.775±0.005	0.773±0.003	0.765±0.004	0.772±0.012	0.751*±0.004
PSI/PSII	1.47 ± 0.02	1.42 ± 0.01	1.19* ± 0.02	1.11 ± 0.02	1.51* ± 0.01
PSII activity	48 ± 13	43 ± 17	15.0*** ± 5.1	30 ± 9	22 ± 8
PSI activity	116 ± 20	114 ± 27	100 ± 40	59 ± 8	75* ± 16

748 Chlorophyll content was determined after extraction in acetone. PSI to PSII ratio was
 749 determined by integrating the area of the fluorescence at 77K ($\lambda_{\max 725}/\lambda_{\max 685}$). PSII and PSI
 750 activities ($\mu\text{mol O}_2 \mu\text{g chl}^{-1} \text{h}^{-1}$) were measured using an O₂ electrode. PSII activity was
 751 measured as oxygen evolution in the presence of 1 mM DCBQ, 10 mM NH₄Cl. PSI activity
 752 was measured as oxygen uptake in the presence of 10 μM DCMU, 5 mM ascorbate, 30 μM
 753 DCPIP, 500 μM methylviologen, 10 mM NH₄Cl. Stars indicate significant difference
 754 compared to the control condition, based on a Mann and Whitney test (* p<0.01, ***
 755 p<0.001, N=6).
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 757

758 **Figures legends**

759

760 **Figure 1: Effect of non-optimal Mn supply on growth and metal content of *Marchantia***
761 ***polymorpha*.**

762 **Mn excess: A:** Two-week old thalli were transferred for one week to agar plates containing
763 33 μ M (control, Ctrl), 0.5 mM (15X), 1 mM (30X), 2 mM (60X), 5 mM (150X) and 6.5 mM
764 (200X) MnCl_2 , respectively. White scale bars represent 1 cm. **B:** Manganese, iron and
765 magnesium concentrations were determined after one-week growth with the given Mn
766 concentrations. **Mn deficiency: C:** Two week old thalli were transferred and grown for one
767 week on starch plates with or without addition of 33 μ M MnCl_2 . Black scale bars represent 1
768 cm. **D:** Manganese, iron and magnesium concentrations in thalli were measured after one
769 week of growth on starch plates with (Ctrl, hatched bars) or without MnCl_2 (-Mn, white bars)
770 in the medium. Error bars represent standard errors. Stars indicate significant differences,
771 compared to the control condition, based on a Mann and Whitney test (* $p < 0.05$, ** $p < 0.01$,
772 *** $p < 0.001$; $N \geq 3$ biological replicates).

773

774 **Figure 2: Chloroplast metal content and structure in *Marchantia polymorpha* grown on**
775 **non-optimal Mn supply.**

776 **Mn excess: A:** Metal content of intact chloroplasts isolated from thalli cultured on agar
777 (control, 33 μ M; 30X, 1 mM; 200X, 6.5 mM MnCl_2). **B:** Microscopy images of thalli
778 cultured on agar (a, Control; b, 30X; c, 200X). Scale bar represents 20 μ m. **C:**
779 Epifluorescence microscopy images of chloroplast surfaces of thalli (d, Control; e, 30X; f,
780 200X). **Mn deficiency: D:** Metal content of intact chloroplasts isolated from thalli cultured
781 on starch (Starch+Mn, 33 μ M Mn; Starch-Mn, without Mn). **E:** Microscopy images of
782 chloroplasts in thalli (g, Starch+Mn; h, Starch-Mn). **F:** Epifluorescence microscopy images of
783 chloroplast surfaces of thalli (i, Starch+Mn; j, Starch-Mn). Stars indicate significant
784 differences, compared to the control condition, based on a Mann and Whitney test (***
785 $p < 0.001$; $N \geq 3$ biological replicates).

786

787 **Figure 3: Organisation of chloroplast membranes as evidenced by room-temperature *in***
788 ***vivo* super-resolution chlorophyll fluorescence emission nanoscopy and by TEM.**

789 **A:** Super-resolution fluorescence microscopy. **1:** Agar Control, 30X and 200X conditions. **2:**
790 Starch Control and Starch-Mn. For each culture condition, reading from left to right, the three
791 reconstructed fluorescence maps are separated by 300 nm in the axial (z) direction. The false-

792 colour scale is represent by horizontal colour bar (top). Scale bar is 1 μ m. One chloroplast is
793 represented of a total of ten chloroplasts analysed for each condition. **B:** Transmission
794 electron microscopy images. 10-20 chloroplasts were analysed, exhibiting the same features
795 for each culture condition. Scale bar is 1 μ m; 2.5x amplification for inset in 30X.

796

797 **Figure 4: Chlorophyll fluorescence at 77K and room temperature.**

798 **A:** 77K fluorescence emission of chloroplasts extracted from thalli grown on agar: Ctrl
799 (black), 30X (red) and 200X (blue) (N=3, biological replicates). **B:** Same as (a) carried out on
800 starch cultivated thalli with (Ctrl, black) or without MnCl₂ (-Mn, red). Spectra in **A** and **B**
801 were normalized to the maximum emission at 725 nm. **C:** Room temperature fluorescence
802 induction curves (OJIP) were measured on 5 min dark-adapted thalli by the application of a
803 saturating flash with 300 ms duration. Culture conditions as in **A**. **D:** Fluorescence induction
804 curves on thalli grown as in **B**. One representative curves is shown for each condition.

805

806 **Figure 5: Analysis of variable chlorophyll fluorescence in Mn deficiency.**

807 **A, B:** Induction and recovery fluorescence curves were measured on thalli transferred for 1
808 week to starch medium with (black) or without added MnCl₂ (red). After 5 min dark
809 adaptation, thalli were exposed to a measuring light to determine the minimum fluorescence
810 level (dashed lines) and to a saturating flash to obtain the maximum fluorescence level.
811 Actinic light (50 μ mol photons m⁻² s⁻¹) was applied for 5 min (white bar). Recovery in the
812 dark was followed (grey bar). **C:** qN and **D:** qP parameters were calculated thanks to
813 saturating flashes applied during the light and dark period (N=12, 3 biological replicates).

814

815 **Figure 6: Photoinhibition in Mn deficiency in the absence and presence of lincomycin.**

816 Photoinhibition was measured on starch-cultivated thalli with (black symbols) or without
817 MnCl₂ (red symbols). Fv/Fm was followed during exposure to high light (800 μ mol photons
818 m⁻² s⁻¹) for 90 min (yellow bar) and during the recovery at room light (black bar). Squares:
819 without lincomycin, triangles with lincomycin. As a control, lincomycin samples incubated in
820 the dark are shown (stars).

821

822 **Figure 7: Activity of photosystem I in Mn deficiency.**

823 **A:** Normalized P700⁺ signal was obtained on thalli cultivated on Starch (+Mn, black; -Mn,
824 red). The redox state of the PSI primary donor P700 was monitored through the changes in

825 absorbance at 830 versus 875 nm. Leaves were kept in the dark for 5 min prior to the
826 measurements. Thalli were exposed to actinic light for 15s (white bar) and to far-red light
827 until reaching a plateau (red bar). Then, a saturating flash was applied (yellow bar) and the
828 decay was observed in the dark (grey bar). **B:** PSI donor-side limitation Y(ND) based on
829 saturating pulse analyses. Following the initial determination of maximal oxidation of P700,
830 actinic light of the indicated intensities was given for 180 s.

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836 **Supplemental**

837 **Table S1** Chlorophyll and Mn content in thalli grown in Mn excess

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

839 **Table S3** Metabolites significantly decreased in Starch condition compared to Agar Control
840 in *M. polymorpha*

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

842 **Figure S2** Estimation of the chloroplast size under the different growth conditions

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

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

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

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

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