

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

Mn excess: **A:** Two-week old thalli were transferred for one week to agar plates containing 33 μ M (control, Ctrl), 0.5 mM (15X), 1 mM (30X), 2 mM (60X), 5 mM (150X) and 6.5 mM (200X) MnCl_2 , respectively. White scale bars represent 1 cm. **B:** Manganese, iron and magnesium concentrations were determined after one-week growth with the given Mn concentrations.

Mn deficiency: **C:** Two week old thalli were transferred and grown for one week on starch plates with or without addition of 33 μ M MnCl_2 . Black scale bars represent 1 cm. **D:** Manganese, iron and magnesium concentrations in thalli were measured after one week of growth on starch plates with (Ctrl, hatched bars) or without MnCl_2 (-Mn, white bars) in the medium. Error bars represent standard errors. Stars indicate significant differences, compared to the control condition, based on a Mann and Whitney test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; $N \geq 3$ biological replicates).

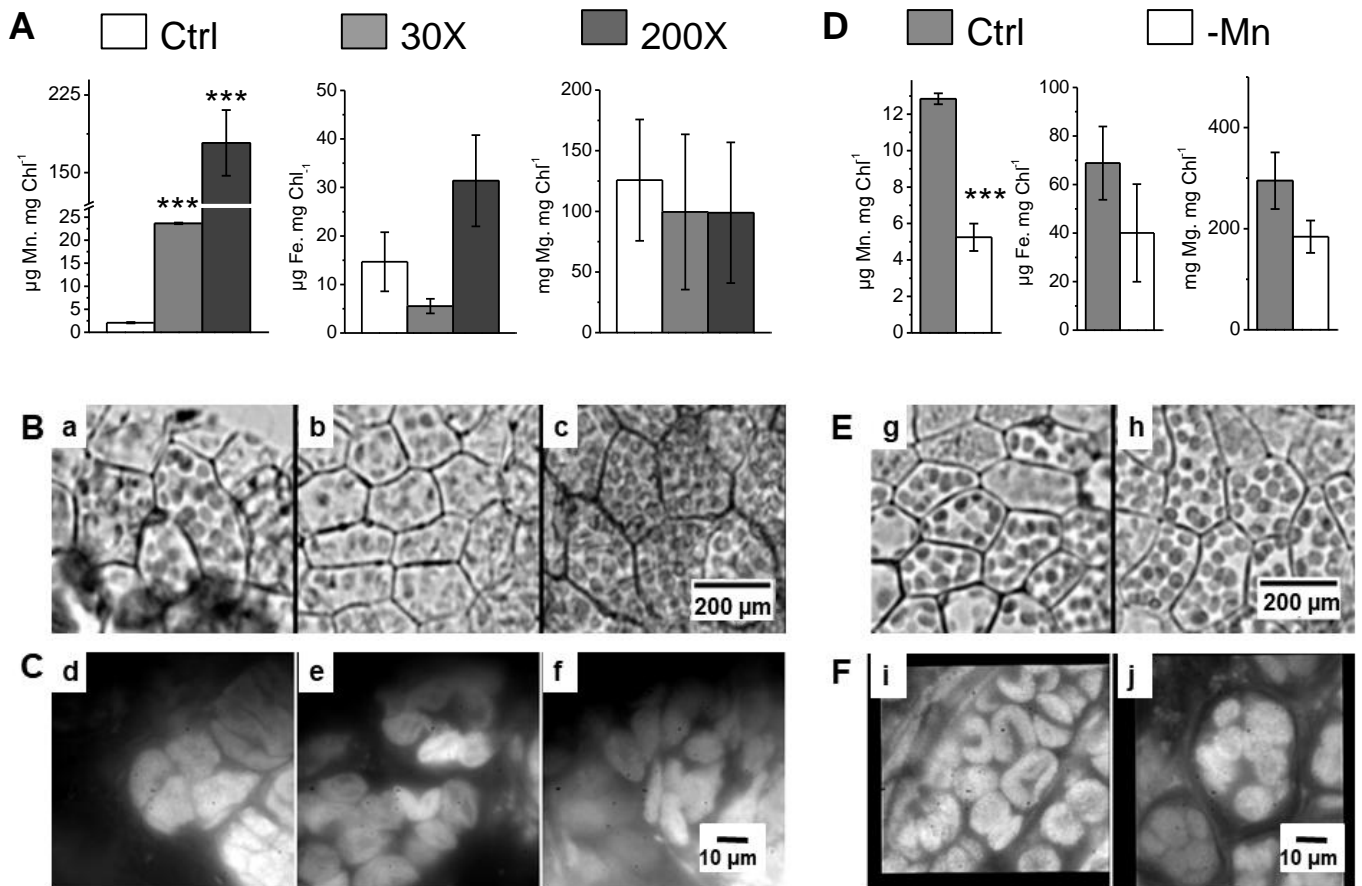


Figure 2: Chloroplast metal content and structure in *Marchantia polymorpha* grown on non-optimal Mn supply.

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

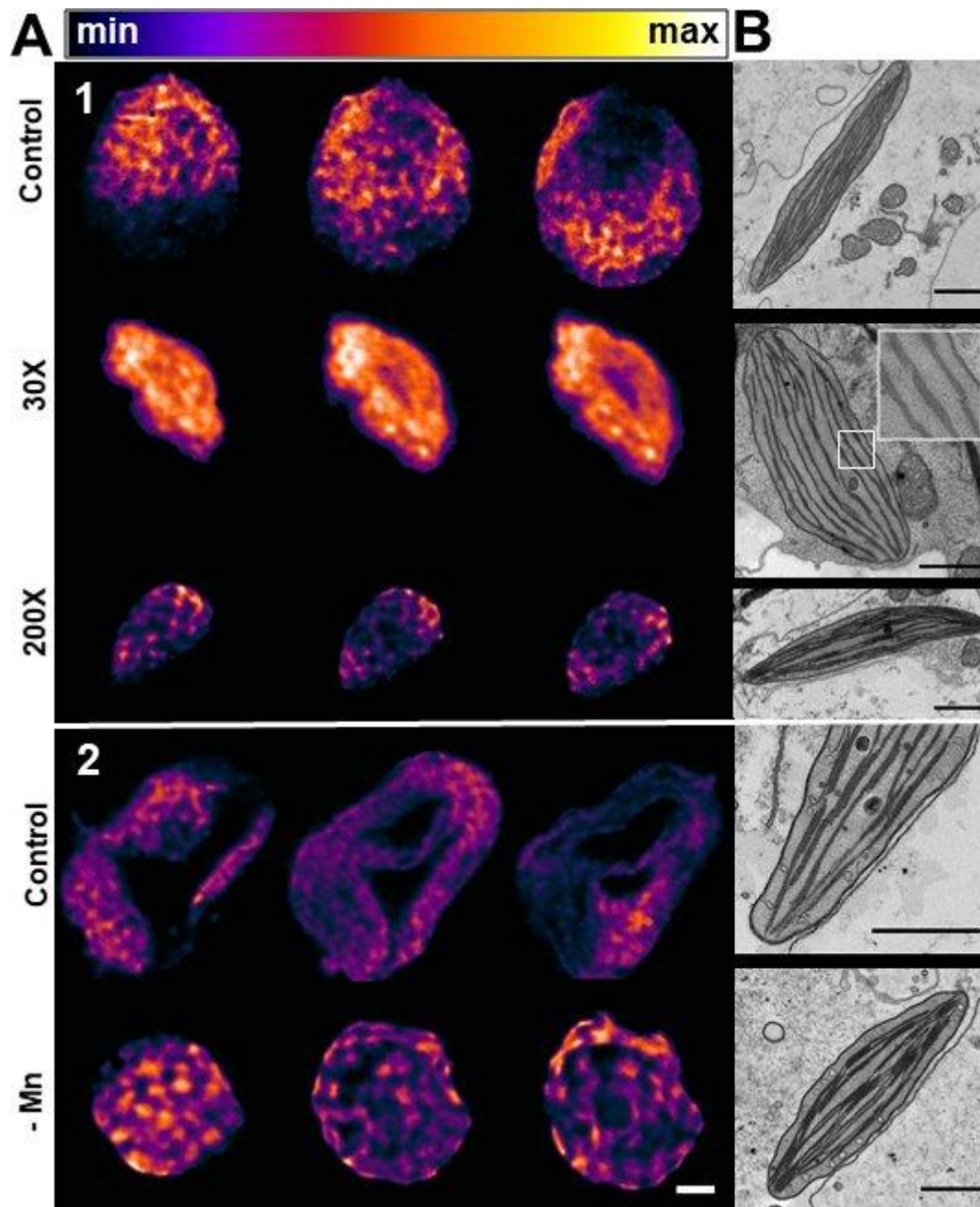


Figure 3: Organisation of chloroplast membranes *in vivo* as evidenced by room-temperature super-resolution chlorophyll fluorescence emission nanoscopy and by TEM images.

A: Super-resolution microscopy. **1:** Agar Control, 30X and 200X conditions. **2:** Starch Control and Starch-Mn. For each culture condition, reading from left to right, the three reconstructed fluorescence maps are separated by 300 nm in the axial (z) direction. Light yellow represents highest fluorescence intensity and dark blue, lowest fluorescence intensity. Scale bar is 1 μ m. One chloroplast is represented of a total of ten chloroplasts analysed for each condition. **B:** Transmission electron microscopy images. 10-20 chloroplasts were analysed and showed the same features for each culture condition. Scale bar is 1 μ m.; 2.5x amplification for inset in 30X.

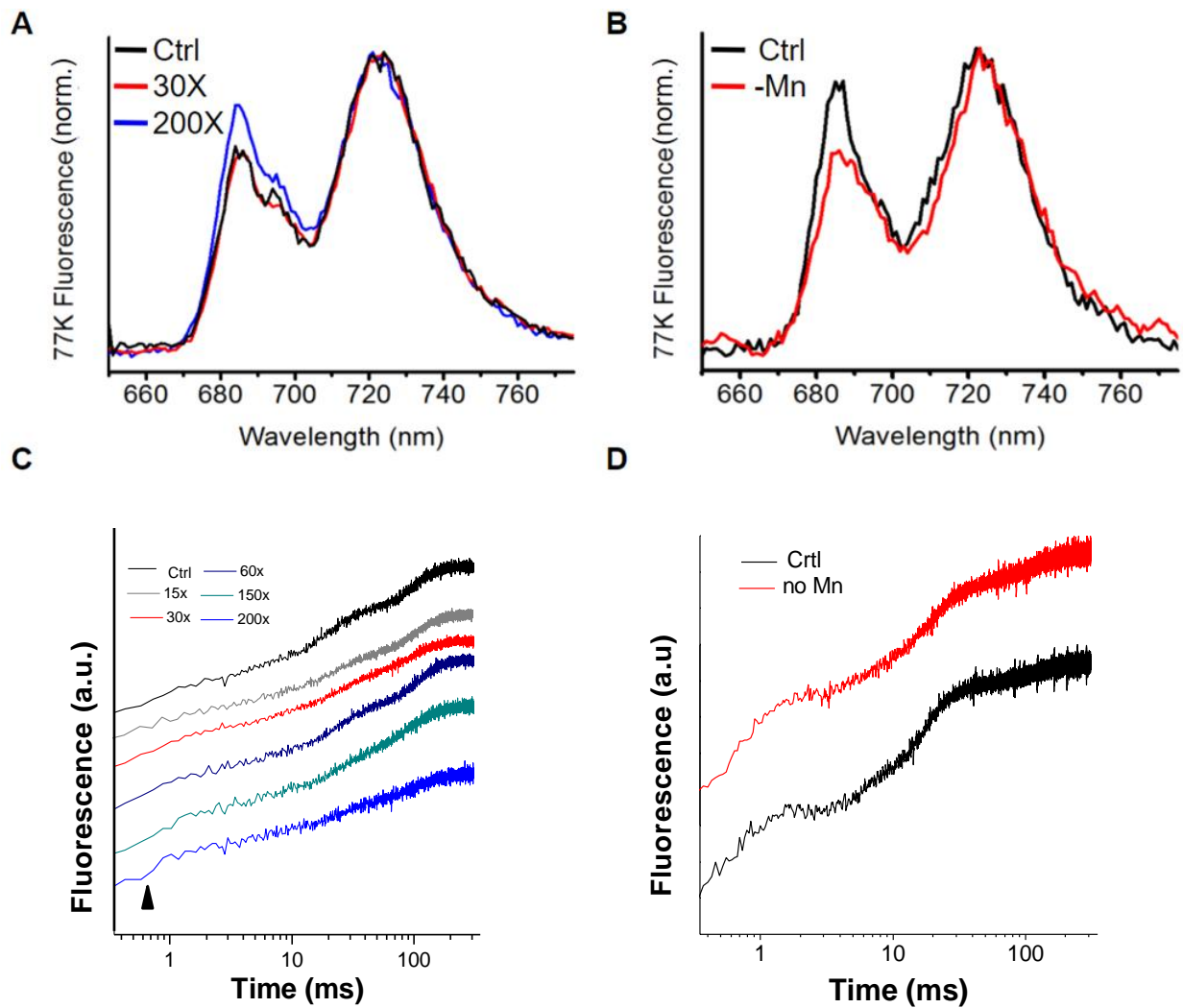


Figure 4: Chlorophyll fluorescence at 77K and room temperature.

A: 77K fluorescence emission of chloroplasts extracted from thalli grown on agar: Ctrl (black), 30X (red) and 200X (blue) (N=3, biological replicates). **B:** Same as (a) carried out on starch cultivated thalli with (Ctrl, black) or without MnCl_2 (-Mn, red). Spectra in **A** and **B** were normalized to the maximum emission at 725 nm. **C:** Room temperature fluorescence induction curves (OJIP) were measured on 5 min dark-adapted thalli by the application of a saturating flash with 300 ms duration. Culture conditions as in **A**. **D:** Fluorescence induction curves on thalli grown as in **B**. One representative curves is shown for each condition.

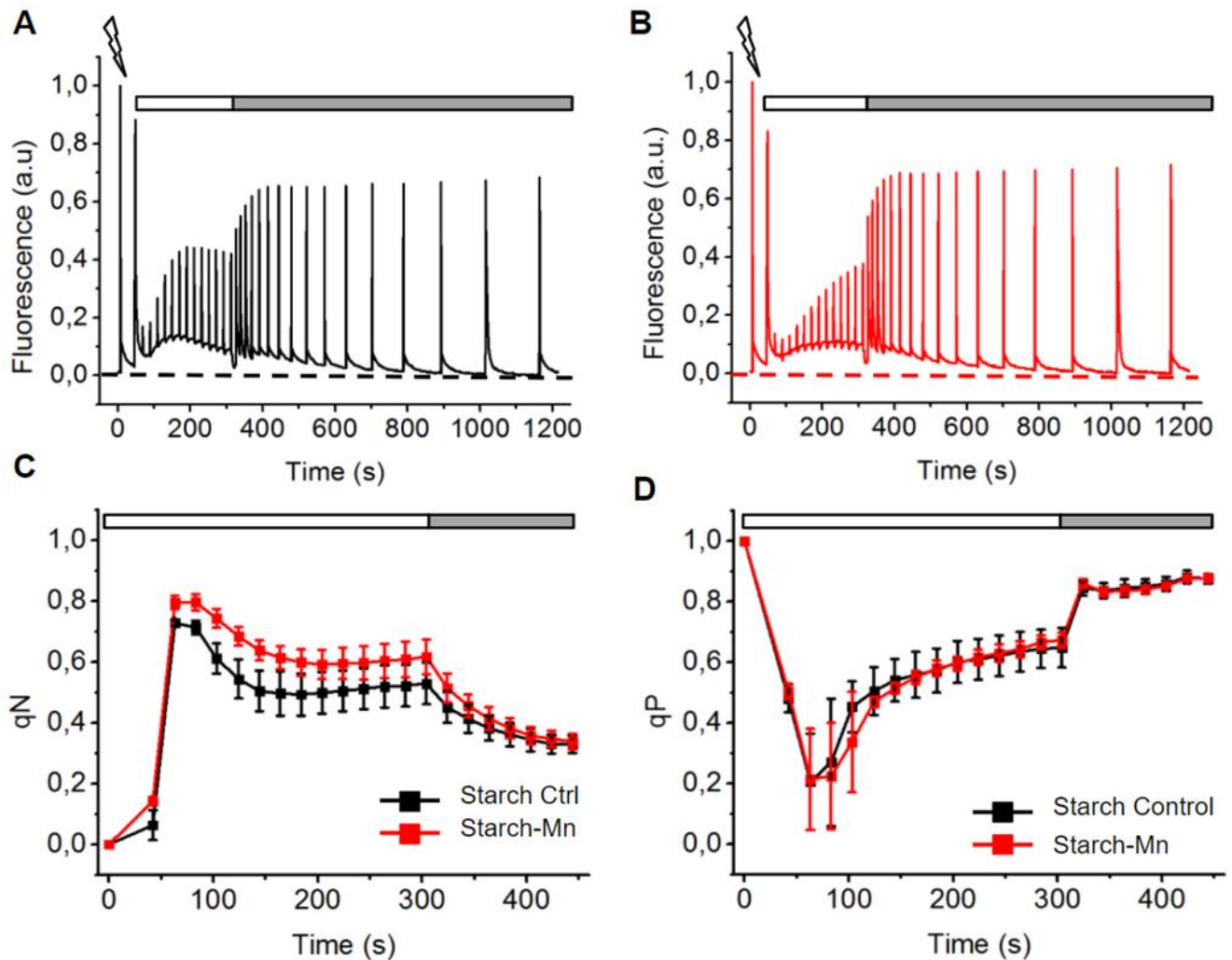


Figure 5: Analysis of variable chlorophyll fluorescence in Mn deficiency.

A, B: Induction and recovery fluorescence curves were measured on thalli transferred for 1 week to starch medium with (black) or without added MnCl_2 (red). 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 ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) was applied for 5 min (white bar). Recovery in the dark was followed (grey bar). **C:** qN and **D:** qP parameters were calculated thanks to saturating flashes applied during the light and dark period ($N=12$, 3 biological replicates).

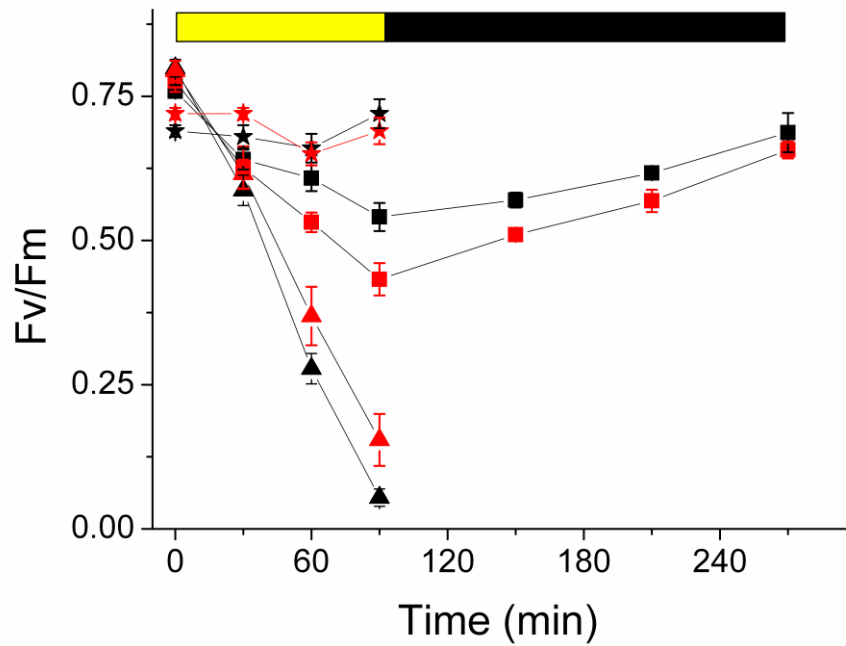


Figure 6: Photoinhibition in Mn deficiency in the absence and presence of lincomycin.

Photoinhibition was measured on starch-cultivated thalli with (black symbols) or without MnCl_2 (red symbols). Fv/Fm was followed during exposure to high light ($800 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) for 90 min (yellow bar) and during the recovery at room light (black bar). Squares: without lincomycin, triangles with lincomycin. As a control, lincomycin samples incubated in the dark are shown (stars).

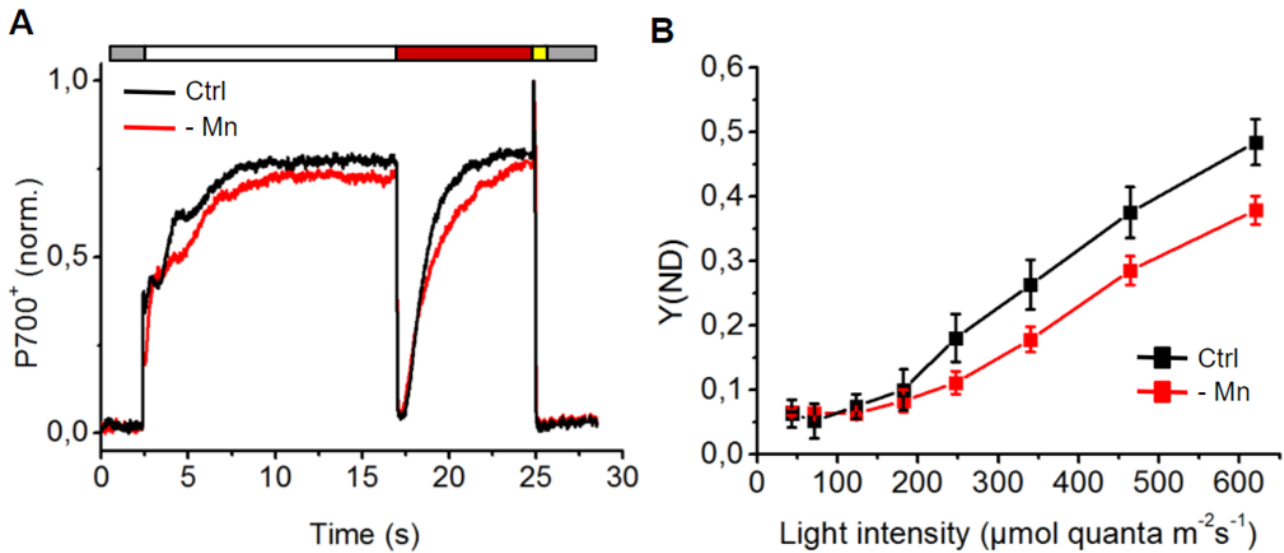


Figure 7: Activity of photosystem I in Mn deficiency.

A: Normalized P700⁺ signal was obtained on thalli cultivated on Starch (+Mn, black; -Mn, red). The redox state of the PSI primary donor P700 was monitored through the changes in absorbance at 830 versus 875 nm. Leaves were kept in the dark for 5 min prior to the measurements. Thalli were exposed to actinic light for 15s (white bar) and to far-red light until reaching a plateau (red bar). Then, a saturating flash was applied (yellow bar) and the decay was observed in the dark (grey bar). **B:** PSI donor-side limitation Y(ND) based on saturating pulse analyses. Following the initial determination of maximal oxidation of P700, actinic light of the indicated intensities was given for 180 s.