Supporting Information for

High-Throughput Screening for Engineered Nanoparticles That Enhance Photosynthesis Using Mesophyll Protoplasts

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Chloroplast Isolation Assay
Light-Absorption and Photoelectrochemical Measurements of NPs
ROS generated by MoS_2

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Methods

Chloroplast Isolation Assay. The chloroplasts were isolated from hydroponic cultivated 3-week-old spinach leaves by a mechanical method according to Weise et al.[1] First, 4-5 pieces of spinach leaves were cut into 5 mm strips along the main vein, followed by transfer to a pre-ice-cold mortar. The buffer used in the bowl consisted of 20 mL of ice-cold 30 mM HEPES buffer (pH 7.6) with 350 mM mannitol, 3 mM EDTA, and 0.05 g of acetone-washed fraction-V BSA. When grinding, the grinding pestle should not be direct contact with the wall of the grinding bowl. After grinding for two minutes to fully release chloroplasts, the solution should then be filtered through four layers of cheesecloth. The suspension was then centrifuged using refrigerated centrifuge for 2 min at 100 g. After removal of the supernatant, the tube (containing chloroplasts) was placed on ice. The isolated chloroplasts were re-suspended in solution consisting of 30 mM Hepes buffer (pH 7.6) with 350 mM mannitol, 2.5 mM $MgCl_2$, 5 mM KH_2PO_4 and 2.5 mg per ml BSA. The isolated chloroplasts were then counted by Hemocytometer.

Light-Absorption and Photoelectrochemical Measurements of NPs. NM light-adsorption was investigated by monitoring the absorbance spectra with a UV-vis spectrophotometer (UV-3010, Shimazu, Japan). Ten mg of the sample was dissolved in 100 mL of absolute ethanol and was subjected to ultrasonic oscillation for 30 minutes. Afterwards, 3 mL mixed suspension was transferred to a cuvette for measurement of the absorbance spectra by UV-visible spectrophotometry.

The photoelectrochemical performance of NPs was tested using a standard three-electrode system with Pt filament as a counter electrode, Ag/AgCl as a reference electrode, and KCl (0.1 mol/L) as the electrolyte solution. The photoelectrochemical properties of the NPs was tested by the bulk electrolysis with coulometry (BE) under visible light irradiation. Ten mg of sample powder was mixed with 30 μL of water and 30μL of PEDOT/PSS conductive ink so that a well dispersed solution was obtained. The solution was uniformly painted onto ITO conductive glass substrate ($\leqslant$ 7 Ω/cm^2) having an area of 0.49 cm^2 with a glass rod. After the electrode was nearly dry, it was transferred to an oven and dried at 80 °C for 30 min. A Pt filament was selected as the counter electrode, an Ag/AgCl was used as the reference electrode, and KCl (0.1 mol/L) was used as the electrolyte solution. A 150 W xenon lamp (*Beijing Newbit Co., Ltd.*) was used as the light source, and a 420 nm filter was used to obtain visible light with a wavelength greater than 420 nm. The photoelectrode was irradiated from the back side (light intensity was 300 W/m^2). The difference between the photocurrent and the dark current was defined as the net current.

ROS generated by MoS_2. The photodegradation of Rhodamine B was used to investigate if MoS_2 can generate hydroxyl radicals under visible light (λ> 420 nm, 150 W Xe lamp). Five milligrams of MoS_2 was dispersed in the Rhodamine B solution (80 mL, 1.625 mg/mL). Before the photocatalytic reaction, the suspensions were stirred under darkness for 120 min so that the adsorption of Rhodamine B reached saturation. For each test, 0.8 mL of the reaction solution was centrifuged and analyzed by UV-vis spectrophotometry (UV-3010, Shimazu, Japan). To determine the major active species in

the photocatalytic process, an active species trapping experiment was conducted under the same conditions as the photodegradation experiment, except that an additional capture agent was added. Ethylene diamine tetraacetic acid (EDTA) was used as the hydrogen ion (h^+) trap and ethylene glycol (EG) was used as the hydroxyl radical trap.

Reference

1. Weise, S. E.; Weber, A. P. M.; Sharkey, T. D., Maltose is the major form of carbon exported from the chloroplast at night. *Planta* **2004,** *218*, (3), 474-482.

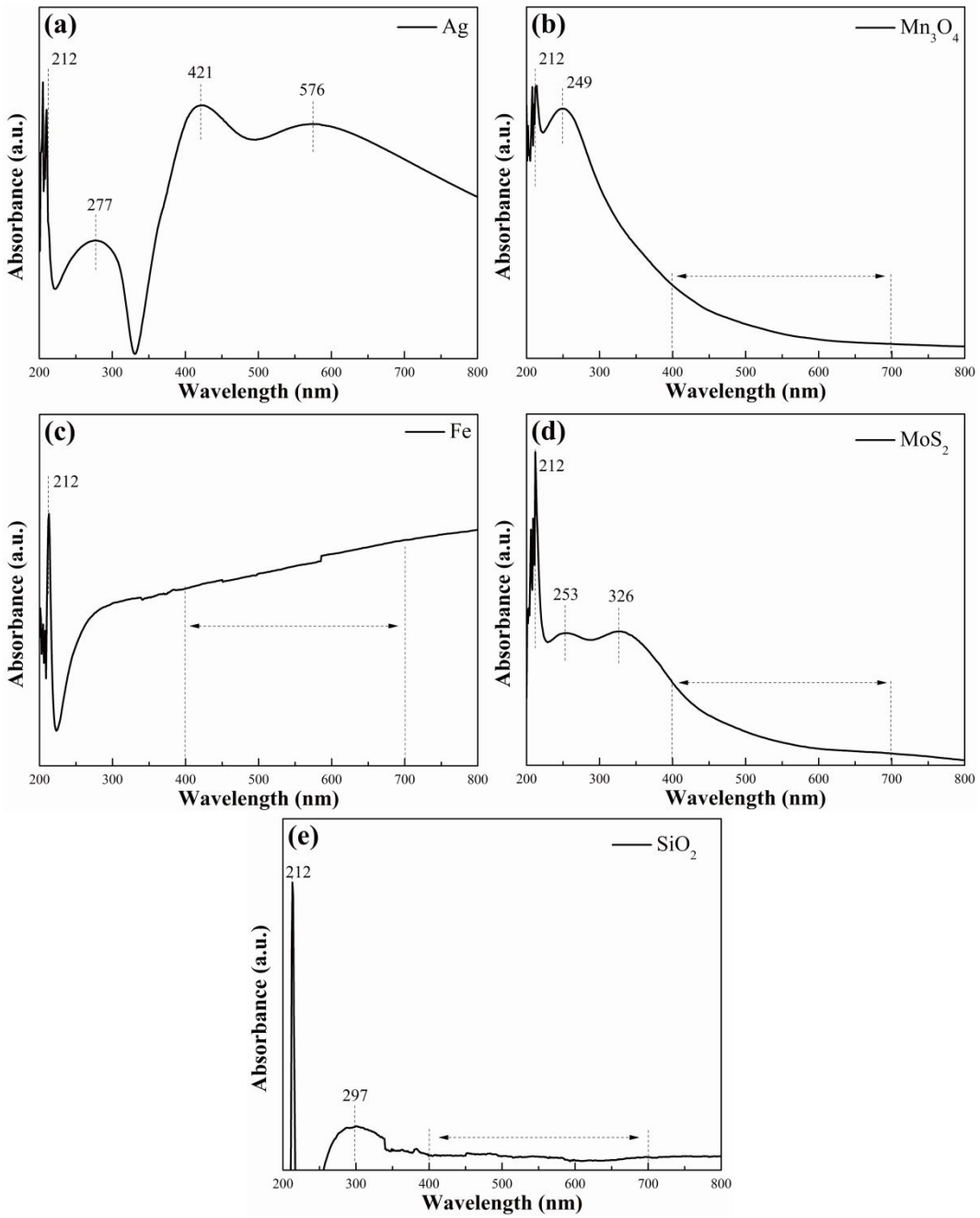
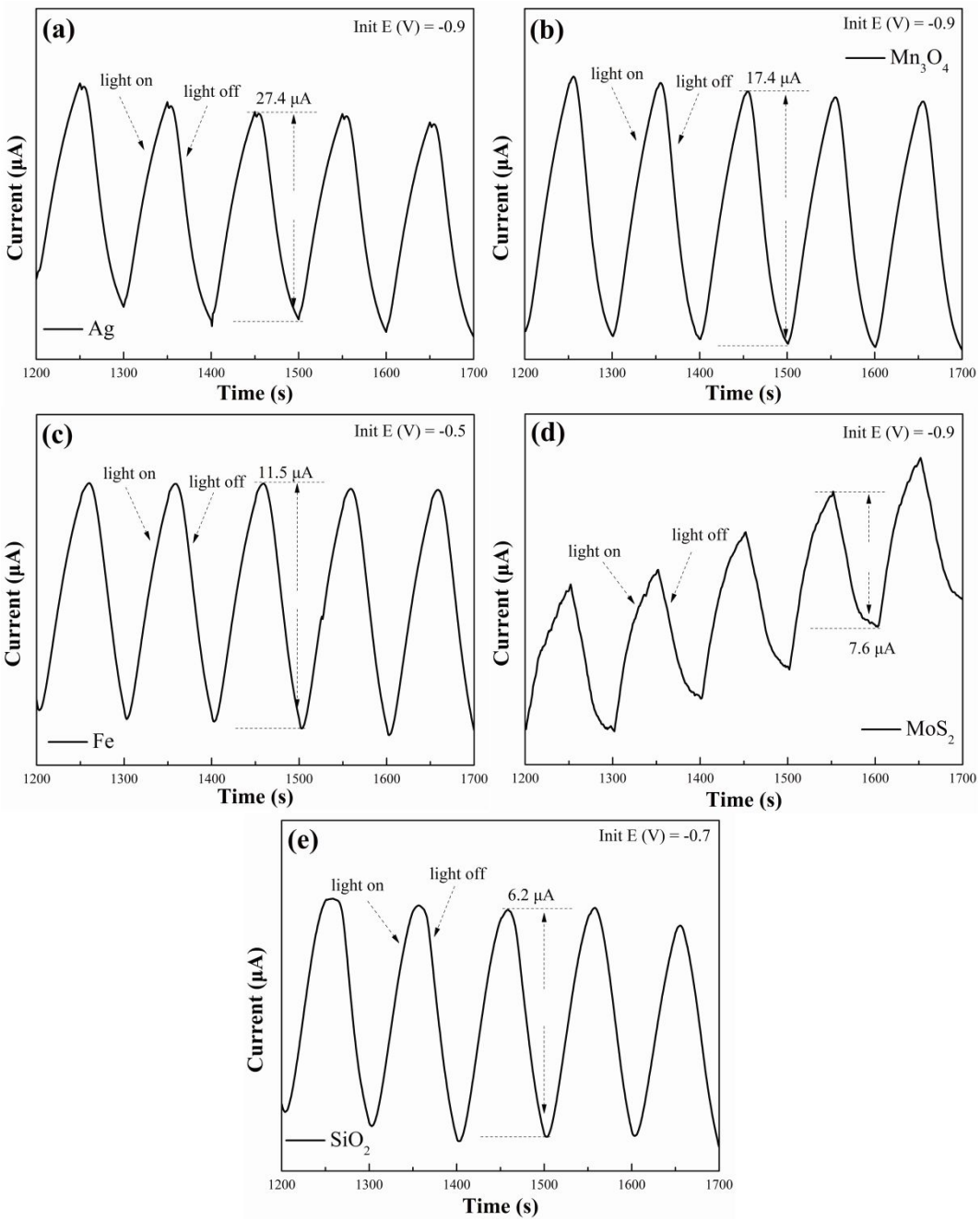


Figure S1. Light absorption: UV-vis light absorption spectra of (**a**) AgNPs, (**b**) Mn_3O_4 NPs, (**c**) FeNPs, (**d**) MoS_2 NPs and (**e**) SiO_2 NPs.



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106 **Figure S2**. Light-induced electron: photocurrent profiles of (**a**) AgNPs, (**b**) Mn_3O_4 NPs,

107 (**c**) FeNPs, (**d**) MoS_2 NPs and (**e**) SiO_2 NPs.

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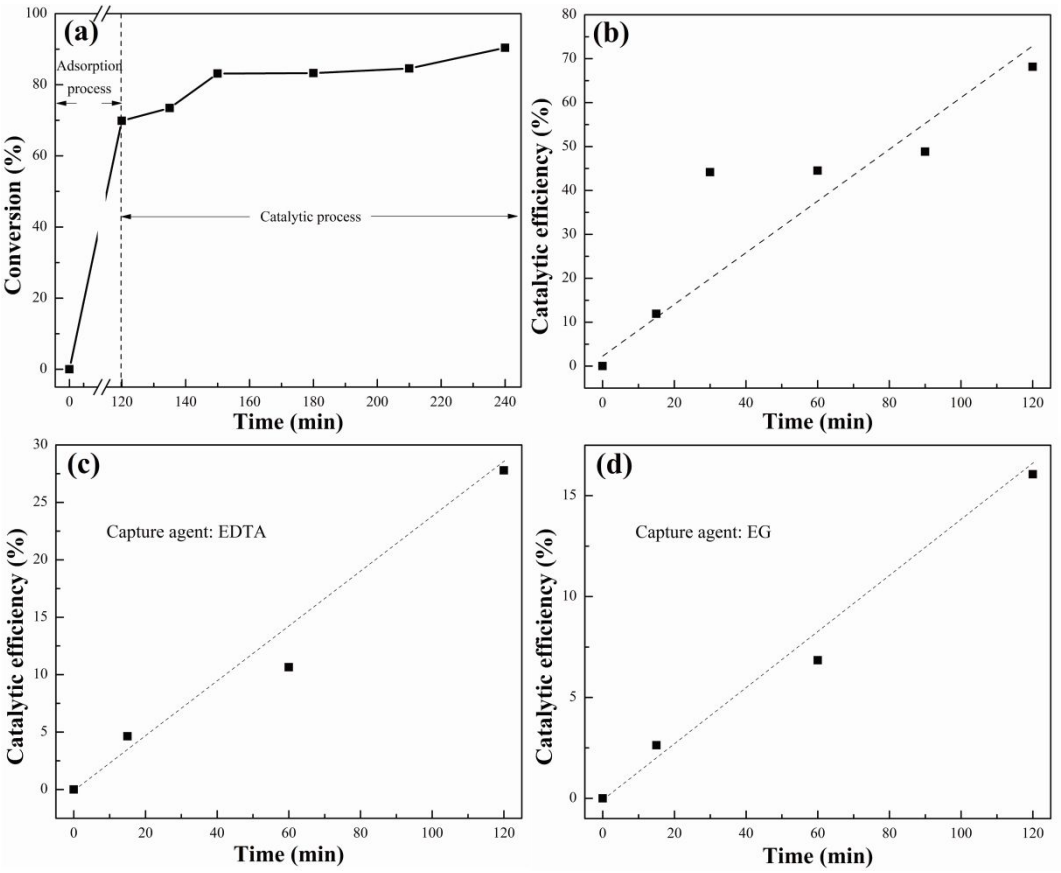

Figure S3. ROS generated by MoS_2: (a) the conversion of Rhodamine B in the adsorption and catalytic processes, (b) the catalytic efficiency of Rhodamine B without the capture agent, (c) the catalytic efficiency of Rhodamine B with the capture agent of EDTA and (d) the catalytic efficiency of Rhodamine B with the capture agent of EG.

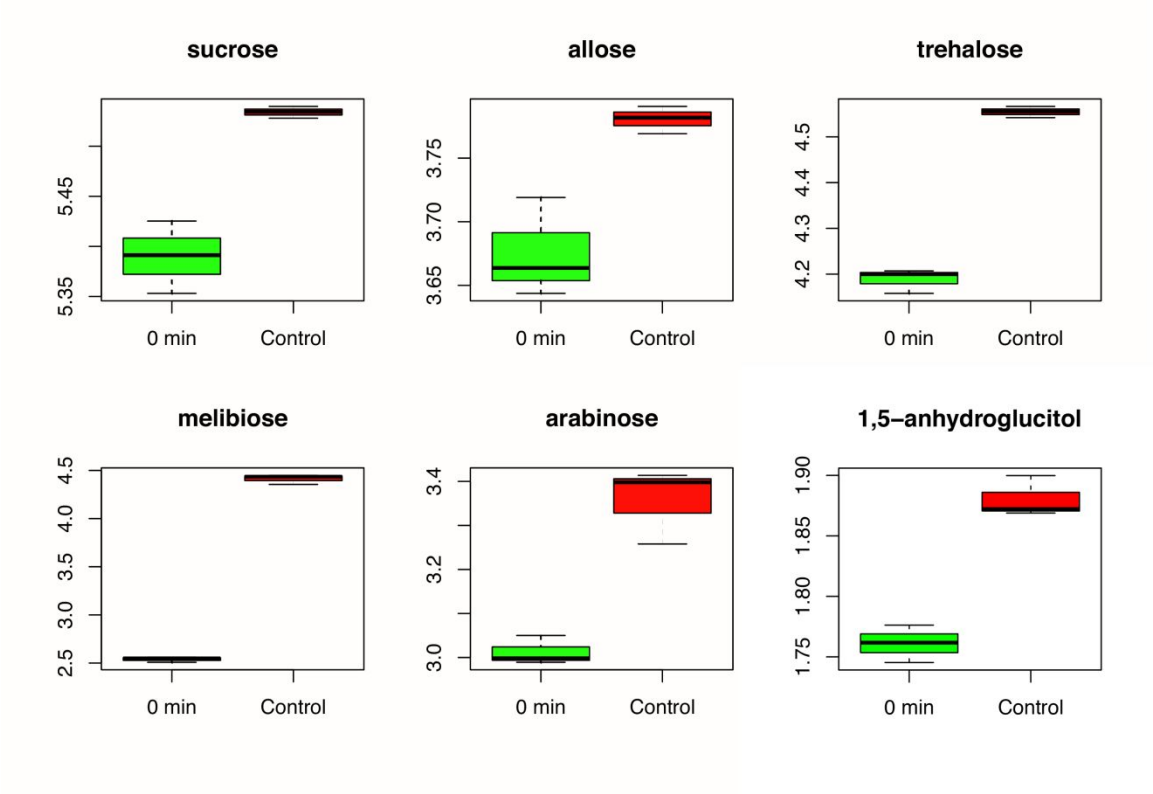

Figure S4 A. Box plots of GC-MS data showing the relative abundance of significantly changed sugars in mesophyll protoplasts under dark (0 min) and light illumination (control). The *y*-axis indicates the absolute signal from GC-MS.

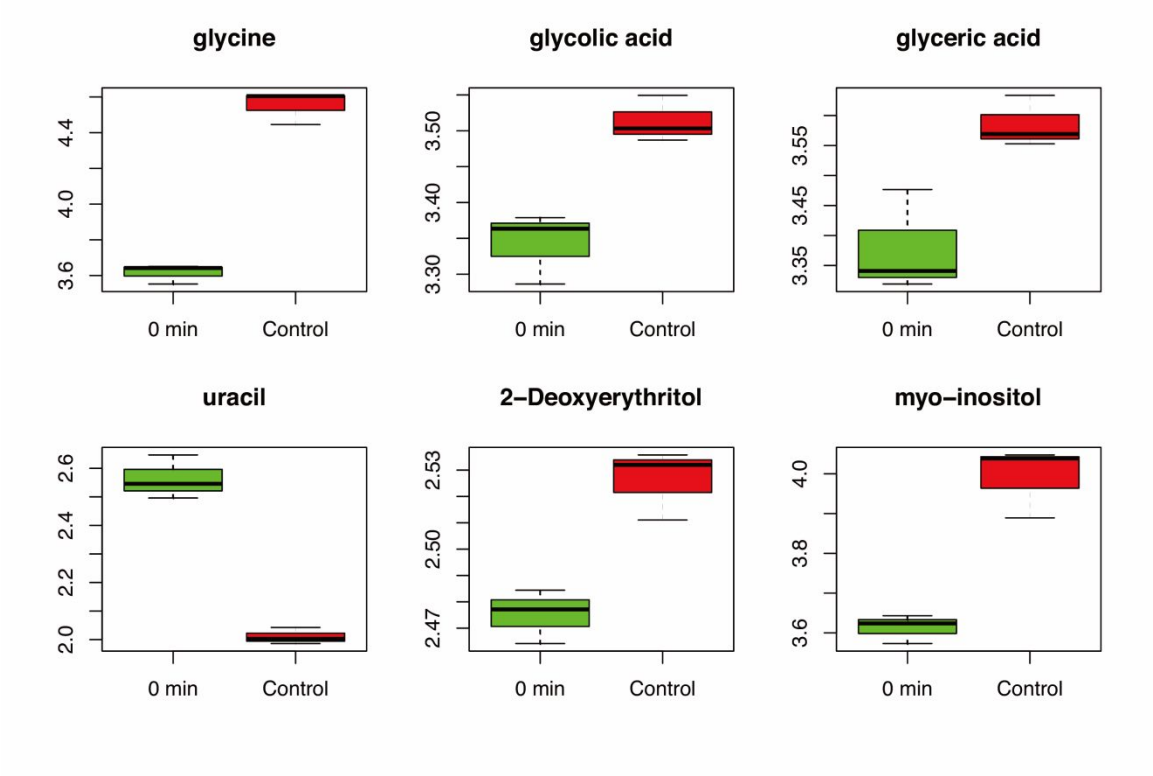

Figure S4 B. Box plots of GC-MS data showing the relative abundance of significantly changed metabolites in mesophyll protoplasts under dark (0 min) and light illumination (control). The *y*-axis indicates the absolute signal from GC-MS.

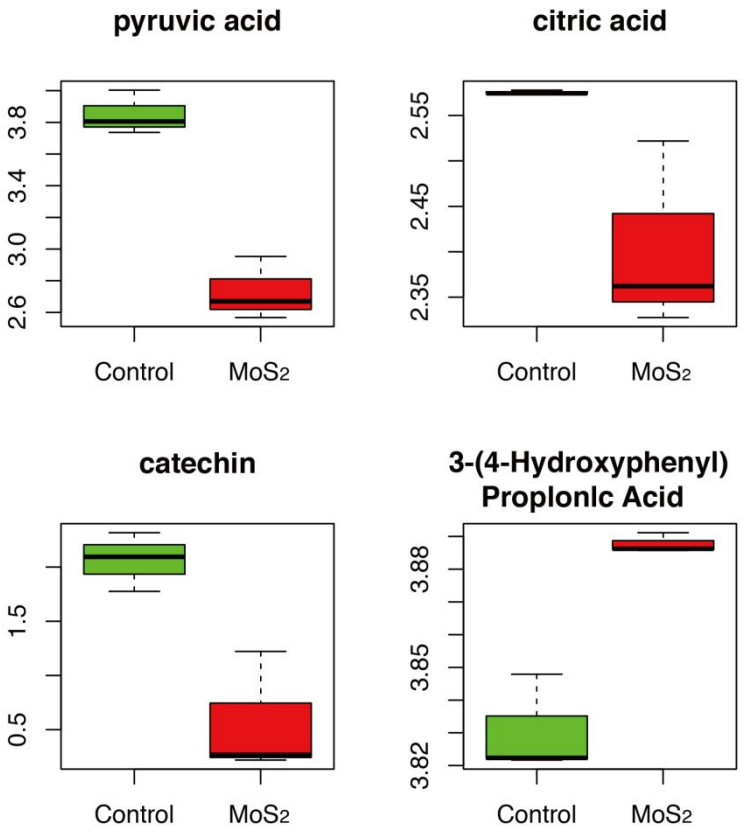

Figure S5. Box plots of GC-MS data showing the relative abundance of significantly changed metabolites in mesophyll protoplasts exposed to MoS_2 (50 mg/L) for 2 hours under illumination. The *y*-axis indicates the absolute signal from GC-MS.

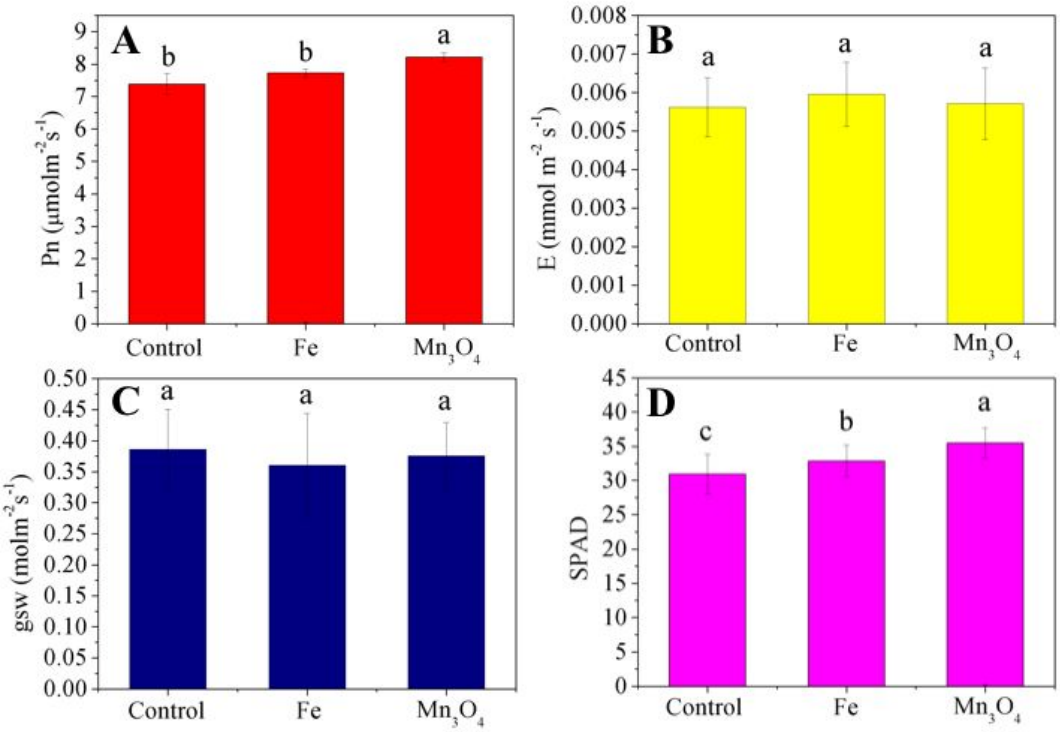

Figure S6. Phenotypic changes of spinach plants exposed to NPs; **A**) net photosynthetic rate; **B**) respiration rate; **C**) stomatal conductance; **D**) chlorophyll content of spinach leaves. Three-week-old spinach plants were foliar applied by NPs for 10 days. Data are means ± SD (n=4).

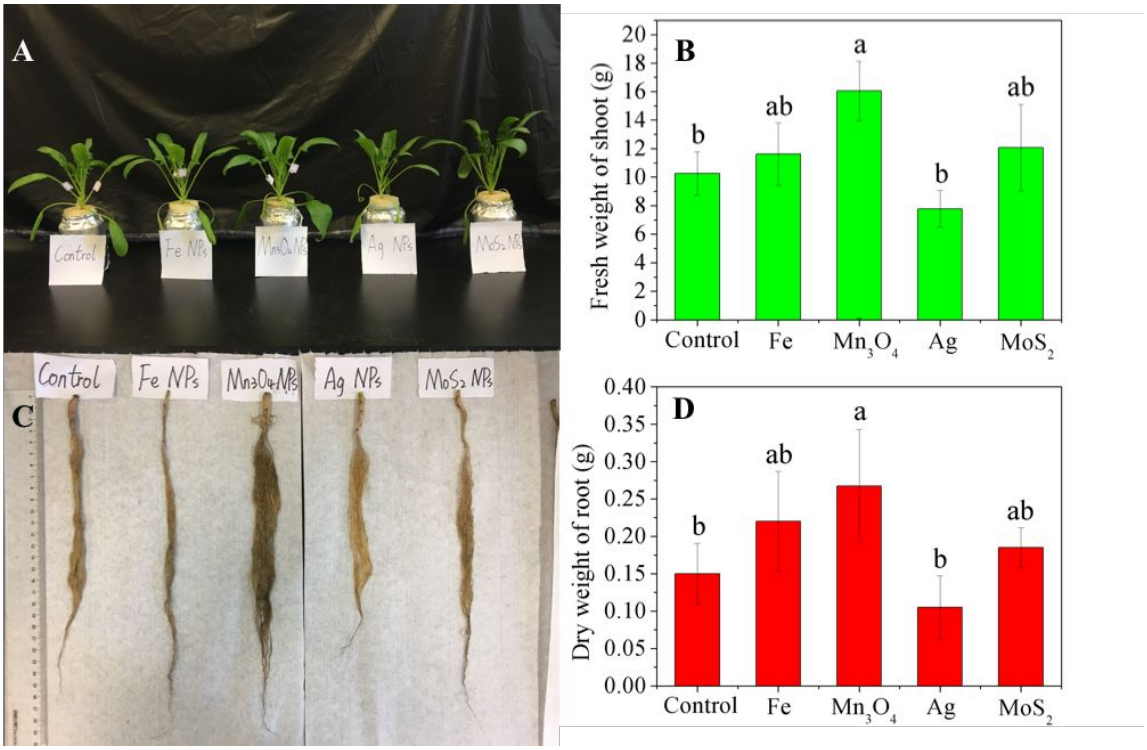

Figure S7. Phenotypic images of spinach leaves and roots (**A** and **C**); fresh biomass of leaf and dry weight of root (**B** and **D**). Data are means ± SD (n=4).

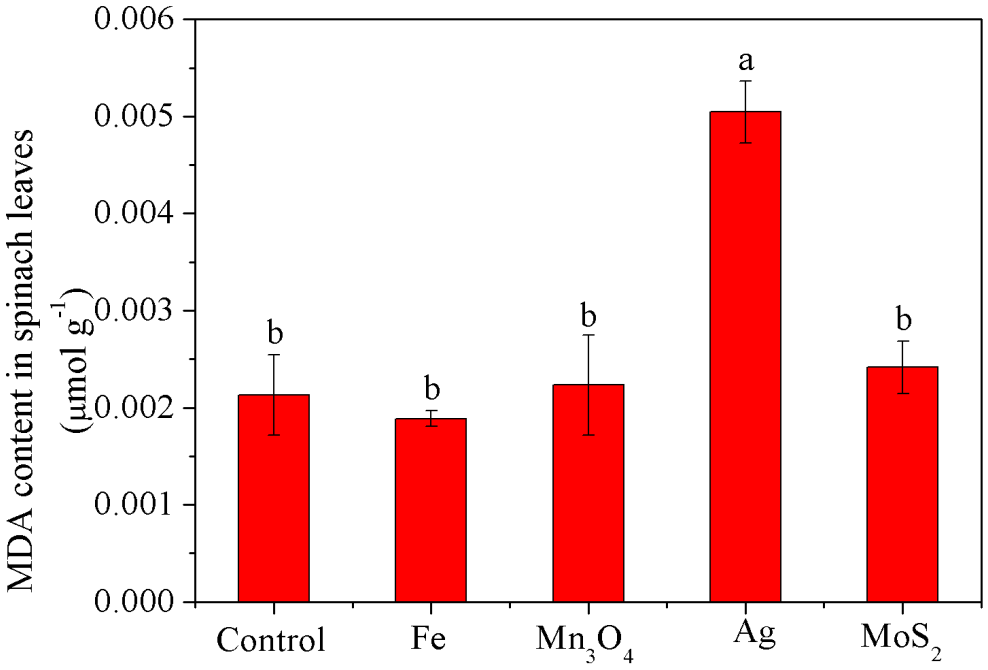

Figure S8. MDA content in spinach leaves exposed to different NPs.