Photosystem II of *Ligustrum lucidum* in response to different levels of manganese exposure

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The toxic effect of excessive manganese (Mn) on photosystem II (PSII) of woody species remains largely unexplored. In this study, five Mn concentrations (0, 12, 24, 36, and 48 mM) were used, and the toxicity of Mn on PSII behavior in leaves of *Ligustrum lucidum* was investigated using in vivo chlorophyll fluorescence transients. Results showed that excessive Mn levels induced positive L- and K-bands. Variable fluorescence at 2 ms (V₁) and 30 ms (V₃), absorption flux (ABS/RC), trapped energy flux (TRₒ/RC), and dissipated energy flux (DIₒ/RC) increased in Mn-treated leaves, whereas the performance index (PIABS), electron transport flux (ETₒ/RC), maximum quantum yield (ϕpₒ), quantum yield of electron transport (ϕeo), and probability that an electron moves further than QA⁻ (ψₒ) decreased. Also, excessive Mn significantly decreased the net photosynthesis rate and increased intercellular CO₂ concentration. The results indicated that Mn blocked the electron transfer from the donor side to the acceptor side in PSII, which might be associated with the accumulation of QA⁻, hence limiting the net photosynthetic rate.

It is well-known that manganese (Mn) is an essential micronutrient element required for the growth and development of plants. Especially, Mn is involved in metabolic pathways of chlorophyll (Chl) synthesis and breakdown in the chloroplasts. The oxygen-evolving complex (OEC) of photosystem II (PSII) contains a Mn-containing metalloenzyme core. This inorganic core, binding to the reaction center (RC) protein D1 in PSII, has the empirical formula Mn₄CaO₅ and is known as the tetra-nuclear Mn cluster. However, Mn, in excess, is also considered as one of the most toxic trace metals to plants. Mn pollution often originates from industrial disposal, manufacturing sewage, as well as mineral exploitation.

Toxic effects of Mn on plants is well documented. Excessive Mn level impedes plant growth and development by interfering with metabolic processes. Moreover, a number of studies have shown that Mn mainly exerts its toxicity to plant leaves by inhibiting photosynthesis and chloroplast activity leading to reduced chloroplast content and suppressed CO₂ assimilation. PSII is highly sensitive to Mn level. Feng *et al.* have reported that excessive Mn inhibits the maximum photochemical efficiency (Fᵥ/Fₘₐₓ) and effective quantum yield of PSII (ϕPSII) in cucumber. Doncheva *et al.* have shown that excessive Mn significantly affects the quantum efficiency of PSII in Mn-sensitive maize (*Zea mays L.*), 'Kneja 605', but not in Mn-tolerant maize 'Kneja 434'. Interestingly, some other studies have revealed that Fᵥ/Fₘₐₓ is not substantially affected by Mn accumulation in tobacco and rice bean seedlings. In addition, Kitao *et al.* have suggested that excessive Mn affects the activity of CO₂ reduction cycle rather than Fᵥ/Fₘₐₓ in white birch, while increased Qₑ reduction and thermal energy dissipation, as well as decreased quantum yield of PSII, have been observed. Similar results have been found in *Alnus hirsuta* Turcz., *Betula ermanii* Cham., *Ulmus davidiana* Planch., and *Acer mono* Maxim. Li *et al.* have reported that excessive Mn impairs the whole photosynthetic electron transport chain from the donor side of PSII up to the reduction of end acceptors of PSI in *Citrus grandis* seedlings, followed by the increase in ABS/RC, TRₒ/RC, and DIₒ/RC, as well as decrease in ETₒ/RC, ϕpₒ, and ψₒ. However, the mechanism of toxicity of Mn to PSII remains largely unexplored, and most previous studies have only focused on herbaceous plants. Chl a fluorescence, a non-invasive spectroscopic technique, has been widely used to detect and measure the in vivo behavior of PSII under different environmental stresses. Analysis of the polyphasic fluorescence transient under physiological conditions shows that the fluorescence increases in the typical shape of OJIP kinetics.

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The “JIP-test” analysis of the OJIP transients allows the calculation of structural, conformational, and functional parameters quantifying the PSII behavior under environmental stresses, including absorption flux, trapped energy flux, electron transport flux, and dissipated energy flux. 

Hunan Province in the southern area of China has a high density of Mn mines. Severe pollution in agricultural lands, stream water, sediments, and soils have been reported in this area, threatening human health. As an important evergreen broad-leaved tree species in the southern regions of China, *Ligustrum lucidum* is highly tolerant to heavy metals. In this study, we aimed to investigate changes in the OJIP transient and related parameters in the leaves of *L. lucidum* in the presence of Mn. In addition, we evaluated the toxicity of Mn to PSII behavior when *L. lucidum* was cultured under Mn stress for up to 40 days.

### Materials and Methods

#### Plant culture and Mn treatments.

Two-year-old *L. lucidum* seedlings (average diameter, ~9 mm; height, ~133 cm) were purchased from a local nursery. All plants were individually transplanted into plastic pots (diameter, 25.4 cm; height, 17.8 cm) filled with 7 kg of air-dried soil. The plants were grown under natural illumination (~133 cm) and a day/night temperature of 30–25 °C day/night temperature, 12/12 h day/night cycle and a maximum photosynthetically active radiation of 1,000 μmol photons m⁻² s⁻¹) for 4 months to acclimatize them to the soil microclimate before initiating Mn treatment. Each pot was supplied with 400 mL of pure water every 2 to 3 days.

Samples of soil free from heavy metal pollution were collected from the CSUFT campus soil at a depth of 5–20 cm. The soil samples were taken back to the laboratory, and were sieved through 5 × 5 mm sieves to remove rocks, and were then air-dried at room temperature. The chemical properties of soil samples were as measured: pH 4.9, 0.227 g N/kg, 0.129 g P/kg, and 355.978 mg Mn/kg.

For the Mn-treated soil, distilled water containing 1.2 mM, 2.4 mM, 3.6 mM and 4.8 mM Mn from MnCl₂·5H₂O was added to the pots every other day at a rate of 400 mL per day for 20 days. The four Mn treatments were designated as L1, L2, L3, and L4, respectively. For the control (CK), about 400 mL of distilled water without Mn was added into the pots. In total we have five treatments: CK, L1, L2, L3 and L4, and each treatment was replicated five times. Measurements were carried out on three fully expanded leaves of *L. lucidum* with similar size and shape on days 10, 25, and 40 after the Mn treatment.

#### Fast Chl a fluorescence kinetics and JIP-test.

Fast Chl a fluorescence was measured by M-PEA (Multifunctional Plant Efficiency Analyzer, Hansatech Instrument, UK). Leaves were exposed to a pulse of saturating red light (5,000 μmol m⁻² s⁻¹, peak 625 nm, duration 50 μs–2 s, records of 128 points) and measured daily between 8:30–11:00 am after 1 h of dark adaptation using dark adaptation clips. The fluorescence transients (OJIP curves) were analyzed to determine energy distribution through PSII per RC (ABS/RC, TR o/RC, ETo/RC, DIo/RC, see Table 1), flux ratios (ϕo, ψo, and ψl), and performance index (PIABS) according to the JIP-test. Relative variable fluorescence at time *t*, at the J-step, and at the I-step (i.e., VJ, VI, and VO, respectively) was calculated using the following equations:

\[ V_J = (F_J - F_0)/(F_M - F_0) \]  
\[ V_I = (F_I - F_0)/(F_M - F_0) \]  
\[ V_o = (F_o - F_0)/(F_M - F_0) \]  
\[ \Delta V_J = V_J - V_J^{(control)} \]  
\[ \Delta V_I = V_I - V_I^{(control)} \]

where \( F_J \) is fluorescence intensity at 2 ms and \( F_I \) is the fluorescence intensity at 30 ms.

To further characterize the effect of Mn on *L. lucidum* PSII, some functional parameters were calculated from the JIP-test. The OJIP transients were double normalized between O (50 μs) and P steps to estimate relative variable fluorescence \( W_{OP} = (F_P - F_0)/(F_P - F_o) \). Normalization between O and K (300 μs) steps revealed L-band (150 μs), resulting in the variable fluorescence:

\[ W_{OK} = (F_K - F_0)/(F_K - F_o) \]  
\[ W_{OK}^{(control)} = (F_K - F_0)/(F_K - F_o) \]  
\[ \Delta W_{OK} = W_{OK} - W_{OK}^{(control)} \]

Normalization between O and J (2 ms) steps revealed K-band (300 μs), resulting in the variable fluorescence:

\[ W_{OJ} = (F_J - F_0)/(F_J - F_o) \]  
\[ \Delta W_{OJ} = W_{OJ} - W_{OJ}^{(control)} \]

The \( F_o, F_P, F_K, F_{O0}, \) and \( F_J \) represent fluorescence at I-step, J-step, and K-step, dark-adapted maximum fluorescence, and dark-adapted minimum fluorescence, respectively. \( \Delta V_J, \Delta V_I \), \( \Delta W_{OK} \), and \( \Delta W_{OJ} \) represent the J-band, I-band, L-band, and K-band, respectively, and are associated with the accumulation of QA. The proportion of
40 was higher than that of the controls. The changes of K-band were similar to L-band except that the K-band in the L1-treated group on day 40. In the L2-treated group, the maximum value of L-band appeared on day 25 and was maintained in the L4-treated group. In the L1-treated group, the L-band achieved the maximum value on day 10 and then decreased continuously to reach the control group, while there was no significant difference between the L3- and L4-treated groups and the control group. Significant differences were also observed between the L4-treated group and the L1- or L2-treated groups. Variable fluorescence at 2 ms ($V_t$) increased with the Mn levels (Table 2). There was no significant difference between the L1- or L2-treated groups and control group except for the L2-treated group on day 40. A significant difference was observed between the L3- and L4-treated groups and the control group. Significant differences were also observed between the L4-treated group and the L1- or L2-treated groups. $V_t$ was significantly higher on day 40 compared with day 10 in all Mn treatment levels. The variable fluorescence at 30 ms ($V_t$) increased with Mn levels (Table 2). $V_t$ was significantly increased in L2-, L3- and L4-treated groups compared with the control group, while there was no significant difference between the L1-treated group and the control group. $V_t$ was significantly increased in the L4-treated group compared with the L2-treated group on days 25 and day 40. There were no significant differences among the different stress time points.

**Effects of Mn on the performance index, energy distribution, and the quantum yield of excitation energy trapping of PSII in *L. lucidum* leaves.** We analyzed several functional parameters from the JIP-test to further characterize the effect of Mn on PSII of *L. lucidum*. Performance index ($P_{ABS}$) showed a declining trend during the test period (Fig. 3a). $P_{ABS}$ in the L4-treated group was significantly decreased compared with the control group, while there was no significant difference between the L3- and L4-treated groups and the control group.

### Table 1. Definition of terms and formulae of the selected JIP-test parameters.

| **Data extracted from the recorded fluorescence transient OJIP** |
|---------------------------------------------------------------|
| $F_o$ ≅ $F_{mm}$ | Minimal fluorescence, when all RCs are open |
| $F_{300}$, $F_{2ms}$, $F_{30ms}$ | Fluorescence intensity at 300 μs, 2 ms, 30 ms, respectively |

| **FPs derived from the extracted data** |
|----------------------------------------|
| $V_t$ = $(F_t - F_o)/(F_{mm} - F_o)$ | Relative variable fluorescence at time $t$ |
| $V_{J}$ = $(F_{30ms} - F_o)/(F_{mm} - F_o)$ | Relative variable fluorescence at the J-step (2 ms), reflects the activity of acceptor side of PSII |
| $V_{I}$ = $(F_{3ms} - F_o)/(F_{mm} - F_o)$ | Relative variable fluorescence at the I-step (30 ms), reflects the activity of acceptor side of PSII |

| **Specific energy fluxes (per Q$_A$ reducing PSII RC)** |
|------------------------------------------------------|
| $ABS/RC$ | Absorption flux (of antenna Chls) per RC (also a measure of PSII apparent antenna size) |
| $TRo/RC$ | Trapped energy flux (leading to $Q_A$ reduction) per RC at $t = 0$ |
| $ETo/RC$ | Electron transport flux (further than $Q_A$) per RC at $t = 0$ |
| $DIo/RC$ | Dissipated energy flux per RC at $t = 0$ |

| **Quantum yields and efficiencies/probabilities** |
|--------------------------------------------------|
| $\phi_{ABS}$ | Performance index (potential) for energy conservation from photons absorbed by PSII to the reduction of interystem electron acceptors |
| $\phi_{ET}$ | Quantum yield for electron transport |
| $\phi_{TR}$ | Efficiency/probability that an electron moves further than $Q_A$ |
| $\psi_{o}$ | Maximum quantum yield for primary photochemistry |

*Q$_A$*: non-reducing PSII RCs; energetic connectivity of antennae to PSII RC units; and the activity of OEC of PSII donor side, respectively.

**Gas exchange.** Net photosynthetic rate ($P_n$) and intercellular CO$_2$ concentration ($C_I$) were measured by LI-COR 6400 portable photosynthesis system (LI-COR Bioscience, Lincoln, NE, USA). Measurements were carried out on three leaves per plant on day 40 with 1,000 μmol photon m$^{-2}$ s$^{-1}$, and a 2 min measurement duration per sample.

**Determination of total Mn content.** The dried biomass of different organs (roots, stems, and leaves) was powdered and used to digest with 15 mL acid mixture (HClO$_4$/HNO$_3$ = 1/4). The concentration of Mn was determined by ICP-AES (Optima 8300, American platinum Elmer, USA).

**Data analysis.** Data were reported as means of each group based on at least six independent replicates. Results are presented as means ± standard error (SE). Statistical differences between measurements were analyzed using one-way ANOVA, followed by a least significant difference (LSD) test at $P < 0.05$. Chl a fluorescence parameters (FPs) associated with Mn levels and stress time were assessed using two-way ANOVA with $\alpha = 0.05$. All graphs were made using Sigmaplot 12.0.
with the L1-treated group and the control group in a time-dependent manner. There was no significant difference between the L1- or L2-treated groups and the control group at any time point, except between the L2-treated group and the control group on day 40. In L3- and L4-treated groups, PI_ABS was significantly decreased on day 40 compared with day 10.

Absorption ABS/RC (Fig. 3b), trapping TRo/RC (Fig. 3c), and dissipation DIo/RC (Fig. 3e) were increased in a Mn-concentration dependent manner during the test period, and these parameters were significantly increased in the L4-treated group compared with the control group during the test period. No significant difference at the same levels of Mn was observed among various time points (day 10, 25 and 40). Electron transport ETo/RC

Figure 1. Changes in O-P phase relative variable fluorescence intensity ($\Delta V_t$) in control group and four Mn-treated groups on day 10 (a), day 25 (b) and day 40 (c).
(Fig. 3d) first increased and then decreased along with the increase of Mn levels, and the maximum value of ETₒ/RC was observed in the L2-treated group. There was no significant difference between the Mn-treated groups and control group at various time points, except between the L4-treated group and the control group on day 40.

Maximum quantum yield ϕₚₒ (Fig. 3f), the probability that an absorbed photon moves an electron further than QA−(ϕₑₒψₒ) (Fig. 3g), and the probability that a trapped exciton moves an electron further than QA−(ψₒ) (Fig. 3h) at various time points showed a declining trend with increasing Mn levels. ϕₚₒ was significantly decreased in the L4-treated group compared with the control on days 10, 25, and 40, and ϕₑₒ was significantly decreased in the L2-, L3-, and L4-treated groups compared with the control on day 40. ϕₑₒ and ψₒ were significantly decreased in the L2-, L3- and L4-treated groups compared with the control on day 40.

**Interaction of stress time and Mn levels for Chl a fluorescence parameters (FPs) in L. lucidum leaves.** All JIP-test parameters significantly varied with the Mn levels, and all parameters, except Vₜ, ABS/RC, TRₒ/RC, DIₒ/RC, and ϕₑₒ, were significantly affected by the stress time. However, none of the parameters significantly responded to the interaction between Mn levels and stress time.

**Figure 2.** Changes in O-K phase relative variable fluorescence intensity ($\Delta W_{OK}$, a–c), and in O-J phase relative variable fluorescence intensity ($\Delta W_{OJ}$, d–f) in control group and four Mn-treated groups on day 10 (a,d), day 25 (b,e) and day 40 (c,f).
Effects of Mn on net photosynthesis rate (Pn) and intercellular CO₂ concentration (Cᵢ) in *L. lucidum* leaves. As reported in Table 4, Pn was significantly decreased in L2-, L3-, and L4-treated groups compared with the control. Further, Pn in the L3- and L4-treated groups was significantly decreased compared with the L1-treated group. Cᵢ increased with the Mn levels (Table 4), but no significant differences were observed between the control and the Mn-treated groups.

Total contents of Mn in *L. lucidum* leaves, stems, and roots. The total Mn contents of plant organs were significantly higher in the Mn-treated *L. lucidum* than in the control except for the Mn levels in the leaves of the L1-treated group (Table 5). The Mn concentrations were highest in the roots, followed by the leaves, and the stems. The Mn content was significantly different among roots, stems, and leaves, except for between the stems and leaves in the L1-treated group.

Discussion
In this study, the OJIP curve was observed to be O-L-K-J-I-P when the Mn levels increased (Figs 1 and 2). The OJIP curve is very sensitive to environmental stress\(^{16,21,24}\). In leaves that have been exposed to a disturbed environment for a short period of time, Chl a fluorescence shows a polyphasic rise before J step, and the O-J-I-P becomes O-L-K-J-I-P\(^{16,21,24}\).

The L-band (~150 μs) is an indicator of energetic connectivity of the antennae to PSII units\(^{24,27}\), implying better excitation energy utilization and system stability of PSII units\(^{21,27}\). In our study, the presence of positive L-band in the Mn-treated leaves indicated an inferior performance of antennae connectivity compared to the control leaves and might be a sign of disturbed energy transfer\(^{28}\). According to the Grouping Concept and JIP-test\(^{21,26}\), the

Table 2. Fluorescence parameters (V₁ and V₂) in the control and Mn-treated groups on day 10, 25 and day 40. Values represent mean ± SE. Different capital letters represent significant difference between the different Mn-treatments in the same stress time points, and different lowercase letters represent significant difference between the same Mn- treatments in the different stress time points (\(P < 0.05\)). Mn-treatment = Manganese-treatment; CK = control; L₁ = 12 mM manganese -treatment; L₂ = 24 mM manganese -treatment; L₃ = 36 mM manganese -treatment; L₄ = 48 mM manganese -treatment; \(V₁ = \) values of relative variable fluorescence at 2 ms; \(V₂ = \) values of relative variable fluorescence at 30 ms.

| Mn-treatment | Day 10 | Day 25 | Day 40 |
|--------------|--------|--------|--------|
| CK           | 0.530 ± 0.009 Aa | 0.537 ± 0.010 Aa | 0.542 ± 0.022 Aa |
| L₁           | 0.539 ± 0.005 ABa | 0.538 ± 0.008 Aa | 0.557 ± 0.004 ABB |
| L₂           | 0.534 ± 0.010 ABa | 0.557 ± 0.014 Aab | 0.588 ± 0.015 BCB |
| L₃           | 0.572 ± 0.009 Ba | 0.576 ± 0.004 Bab | 0.606 ± 0.005 Cb |
| L₄           | 0.614 ± 0.029 Ca | 0.638 ± 0.045 Bab | 0.720 ± 0.017 Db |

Table 3. Two-way ANOVA results for JIP-test parameters. *\(P < 0.05\), **\(P < 0.01\). \(V₁ = \) values of relative variable fluorescence at 2 ms; \(V₂ = \) values of relative variable fluorescence at 30 ms; PIABS = Performance index (potential) for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors; ABS/RC = Absorption flux (of antenna Chls) per RC (also a measure of PSII apparent antenna size); TRo/RC = Trapped energy flux (leading to QA reduction) per RC at \(t = 0\); ETo/RC = Electron transport flux (further than \(Q_A^-\)) per RC at \(t = 0\); DIo/RC = Dissipated energy flux per RC at \(t = 0\); \(\psi_o\) = Maximum quantum yield for primary photochemistry; \(\psi_o^{*}\) = Quantum yield for electron transport; \(\psi_o^\prime\) = Efficiency/probability that an electron moves further than \(Q_A^-\).

| PIABS | V₁  | V₂  | Mn level × Stress time |
|-------|-----|-----|------------------------|
| 24.389** | 23.176** | 10.695** | 1.519 |
| 27.454** | 22.823** | 10.573** | 0.571 |
| 14.637** | 12.174** | 2.039 | 0.367 |
| 16.874** | 14.893** | 1.802 | 0.279 |
| 3.879** | 3.379** | 7.108** | 0.065 |
| 5.753** | 5.253** | 2.257 | 0.625 |
| 0.065 | 0.065 | 0.065 | 0.204 |
| 0.065 | 0.065 | 0.065 | 0.204 |
| 27.176** | 27.176** | 10.695** | 1.519 |
positive L-band implies that the PSII units were less tightly grouped, or that less energy was exchanged between the independent PSII units. Therefore, PSII units of Mn-treated leaves had lower stability and became more fragile. However, an amplitude change in the L-band (from positive to negative) of the L1-treated group was observed.

Figure 3. Mn induced changes in performance index (PI_{ABS}, a), absorption (ABS/RC, b), trapping (TR_{o}/RC, c), electron transport (ET_{o}/RC, d), dissipation (DL_{o}/RC, e), the maximum quantum yield of primary photochemistry ($\phi_{Po}$, f), the quantum yield of electron transport ($\phi_{Eo}$, g) and the efficiency ($\psi_{o}$, h). Values represent mean ± SE. Different capital letters represent significant difference between the different Mn-treatments in the same stress time points, and different lowercase letters represent significant difference between the same Mn-treatments in the different stress time points ($P < 0.05$).
from day 10 to day 40 (Fig. 2a,c,e) suggesting that the PSI units had better excitation energy utilization and system stability on day 40 without any irreversible damage. This may be associated with a lack of significant Mn accumulation in the leaves of the L1-treated groups (compared to controls, Table 5).

The K-band can be explained by the imbalance of electron flow from the donor side to the acceptor side in the PSII RCs25. When the electron transfer from the OEC to tyrosine Z (Yz) is slower than the electron transfer from P680 to QA and beyond, there is a high accumulation of Yz–25. Thus, this accumulation of Yz– causes the appearance of K-step, which is directly associated with an inactivation of the OEC25. In this study, the appearance of K step suggested that Mn inhibits the electron flow from the donor to the acceptor side of PSII even at low levels (L1) (Fig. 1a–c). Meanwhile, the presence of positive K-band in the Mn-treated leaves indicates an inactivation of the OEC24,25 (Fig. 2d–f). Therefore, it may be inferred that the competition between Ca2+ and Mn2+ in the OEC led to more sites held by Mn2+ in the OEC, and this may depend on the similar ion radius and charge properties of Mn2+ and Ca2+30.

OJIP transients can be used to examine the electron transport flux from PSI RCs to PSII through QA and QB. In this study, leaves in the L3- and L4-treated groups had significantly increased Vj compared with the control leaves (Table 2), indicating that high levels of Mn induced the accumulation of QA–. This result is consistent with the previous findings26,29,35. The increased value of Vj could be related to the blockade of electron transport downstream of QA by Mn stress31. This finding is also supported by the decrease of φo25 and Ψo (Fig. 3g,h), as QA was unable to be reduced by QA–-non-reducing PSII RCs25,35. Correspondingly, the higher levels of QA–-non-reducing centers blocked electron transport towards PSII. Lower redox state of QA, in turn, implies altered reduction potential of PSII at the acceptor side in Mn-stressed plants17. Since QA is in quasi-equilibrium with QB and the PQ pool, the lower redox potential of QA will decrease the probability of forward electron transfer between the two quinone acceptors by shifting the redox equilibrium between QA/QB and QA/QB33,34.

The significant reduction of PIK25, which is a very sensitive indicator of plant functionality33, suggests that Mn may down-regulate PSIII function, resulting in prolonged negative effect with irreversible damage. An increase in both ABS/RC and TRo/RC, and a decrease in φo25 indicates inactivation of a certain part of RCs, which was most likely due to inactivation of OEC as well as the transformation of active RCs to silent ones, because the functional antenna that supplies excitation energy to active RCs was increased in size34,37. However, an increase in ETo/RC under low levels of Mn (L1 and L2) implies that these inactive RCs35 could prevent further damage to themselves and protect neighboring active RCs in response to the absorbed light energy in the active RCs36. Significantly increased DIo/RC and decreased ETo/RC in the highest Mn treatment group (L4) shows that the excess excitation energy was mostly dissipated41,24.

ANOVA results revealed that all JIP-test parameters used in this study were significantly affected by Mn stress (P < 0.05), but the interactive influences of Mn stress and stress time on the examined parameters were not significant (P > 0.05) (Table 3). We also found that L. lucidum leaves were more sensitive to the Mn levels compared with the stress time. Additionally, ETo/RC, φo25, and Ψo were significantly influenced by Mn stress time, indicating that the blockage of PSII electron flow beyond QA was more severe in response to the increasing stress time. The blockage of PSI electron flow was also supported by the phenomena of the accumulation of QA, and the increase in Vj.

The Mn-induced changes in the shape of OJIP transient curves and other related parameters of L. lucidum as observed in this study were also found in the studies of Mn-treated Citrus grandis seedlings44, Al-treated
Citrus grandis, and Cd-treated Solanum lycopersicum. But different from our results here, Cr-treated Spirodela polyrhiza was found to have a decreasing trend of TR/RC, indicating that the Cr damages LHCs. Therefore, the sensitivity of different parts of the PSII units vary, and this response is different for different heavy metals and is species-dependent.

This study found that Pn of the plants in L2, L3 and L4 treatments was significantly lower than that in the control (Table 4), and Pn and Ci were negatively correlated. Therefore the reduced Pn observed in our study was not caused by Ci limitation. A negative correlation between Ci and Pn was suggested as an indicator to describe the decrease in carboxylation efficiency by Rouhi et al. A positive relationship between maximum quantum yield of PSII (Fv/Fm) and Pn was also found by Tezara et al. These results suggested that the reduction of Pn could be explained by the limitation in photochemical activity of PSII, which impeded the utilization of CO2 in the assimilation process. The current study found that excessive Mn impaired the functional PSII, as supported by the observed positive L-band and the observed decrease in PIABS. Thus, Mn toxicity contributed to the observed significant reduction of Pn through its effects on photosynthetic apparatus.

Conclusions

We conclude that an excess level of Mn affected the net photosynthesis rate, the OJIP transient, and other related parameters of L. lucidum seedlings. The imaging of JIP-Test parameters revealed Mn-induced photo-damage on the PSII RCs, including a decrease in energy absorption and excitation energy trapping, and an increase in energy dissipation. The disturbance of the PSII electron transport from the donor side to the acceptor side might be associated with inactivation of OEC. This, in turn, resulted in a decrease in the rate of electron transport beyond QA and an accumulation of QA−.

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Author Contributions
H.Z.L. and F.Z. designed the research and wrote the main manuscript, H.Z.L., R.J.W. and X.H.H. conducted the experiment, R.J.W., X.H.H. and J.J.C. analyzed data. All authors reviewed the manuscript.

Additional Information
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