Plant Photosynthesis-Irradiance Curve Responses to Pollution Show Non-Competitive Inhibited Michaelis Kinetics

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Abstract

Photosynthesis-irradiance (PI) curves are extensively used in field and laboratory research to evaluate the photon-use efficiency of plants. However, most existing models for PI curves focus on the relationship between the photosynthetic rate (Pn) and photosynthetically active radiation (PAR), and do not take account of the influence of environmental factors on the curve. In the present study, we used a new non-competitive inhibited Michaelis-Menten model (NIMM) to predict the co-variation of Pn, PAR, and the relative pollution index (I). We then evaluated the model with published data and our own experimental data. The results indicate that the Pn of plants decreased with increasing I in the environment and, as predicted, were all fitted well by the NIMM model. Therefore, our model provides a robust basis to evaluate and understand the influence of environmental pollution on plant photosynthesis.

Introduction

Photosynthesis-irradiance (PI) curves, which show the efficiency and capacity of plant photosynthesis with respect to light intensity, have widely been used in both field and laboratory research to evaluate the influences of abiotic and biotic factors (e.g., nutrient limitation, photoacclimation) on plant performance, e.g., phytoplankton [1–9], Alnusrubra [10], winter wheat [11, 12], Oriza sativa [13, 14], Atriplex hastate [15], Alocasia macrorrhiza [15], Tidestromia oblongifolia [15], Trillium grandiflorum [16], alga [17], and carrots [18]. Accurate assessment of such relationships is of fundamental importance for understanding the photochemical yield of the process and for studying the responses of plants to environmental changes, such as pollution, temperature, water, and light stresses.

Many models have been used to assess the relationship between the photosynthetic rate (Pn) and photosynthetically active radiation (PAR), including the exponential function (EF, [8, 10]), hyperbolic tangent function (HTF, [1]), non-rectangular hyperbola model (NHM, [11],...
13), rectangular hyperbolic model (RHM, [18]), binomial regression function (BRF, [9, 13]), and the modified model based on the rectangular hyperbolic model (MM, [14]). All of these models, except for the three functions (EF, HTF, and BRF), are derived from the Michaelis-Menten equation [11, 14, 17–19, 20]. Biochemically, photosynthesis is essentially a process of reversible enzymatic reaction kinetics, because the primary process in photosynthesis is an oxidation-reduction reaction [17] and photosynthetic efficiency relies on photon use efficiency by antenna pigments and the catalytic reaction efficiency of CO2 by ribulose diphosphate carboxylase. Thus, photons play the role of a resource in photosynthesis, and the relationship between individual gross photosynthesis and PAR can be described by the Michaelis-Menten model [21]. Therefore, the Michaelis-Menten model is optimal to assess the relationship between Pn and PAR. Namely, the RHM, NHM, and MM are all suitable for mathematical fitting of the relationship between Pn and PAR.

However, the PI curve varies significantly with abiotic factors [7], especially environmental pollution [22–25]. Soil pollution, which results from elevated concentrations of pollutants in soil or water, has become a widespread environmental problem because of increased industrialization [26], the land application of sewage sludge [27], and the use of feed additives and/or premixes containing heavy metals in animal husbandry [28]. Thus, it is necessary to build a further model that takes into account the effect of pollution on the relationship between Pn and PAR.

The objectives of the present study were to: 1) build a model for predicting the relationship of Pn, PAR, and I (the relative pollution index) in a contaminated environment; and 2) determine why and whether the non-competitive inhibited Michaelis-Menten model (NIMM) is suitable for predicting the PI curve of plant responses to pollution. However, because there are three kinds of pollutant-induced inhibited enzymatic reactions, including competitive, non-competitive, and un-competitive, it is also important to determine which is the most suitable to show the inhibiting effect of pollutants on the PI curve.

Materials and Methods
2.1 The non-competitive inhibited Michaelis-Menten model

Michaelis and Menten [29] proposed the Michaelis-Menten equation (Eq 1) to describe the relationship between v and [S] in enzymatic reactions,

\[ v = \frac{V_m [S]}{K_m + [S]} \]  

(1)

where v is the velocity of the enzymatic reaction, \( V_m \) is the maximum velocity of the enzymatic reaction, [S] is the content of the substrate in the enzymatic reaction, and \( K_m \) is the Michaelis constant. Further, in an inhibitor-induced enzymatic reaction, three general types of inhibition kinetics equations (i.e., competitive, Eq 2; non-competitive, Eq 3; and uncompetitive, Eq 4) can be derived from the Michaelis-Menten equation [29, 30],

\[ v = \frac{V_m [S]}{K_m \cdot \left(1 + \frac{I}{K_i}\right) + [S]} \]  

(2)

\[ v = \frac{V_m [S]}{(K_m + [S]) \cdot \left(1 + \frac{I}{K_i}\right)} \]  

(3)
\[
v = \frac{V_m \cdot [S]}{K_m + [S] \cdot \left(1 + \frac{I}{K_i}\right)}
\]  

(4)

in these equations, \(v\), \(V_m\), \([S]\), and \(K_m\) are the same as mentioned above; \(I\) is the content of the inhibitor; and \(K_i\) is the inhibition constant. As mentioned above, photosynthesis is a process of enzymatic reactions, and photons play the role of a resource [21], the PAR in photosynthesis is similar to the \([S]\) in an enzymatic reaction.

The RHM (Eq 5) was derived from the Michaelis-Menten equation [11, 14, 18, 19],

\[
P_n = \frac{\alpha \cdot P_m \cdot \text{PAR}}{\alpha \cdot \text{PAR} + P_m} - \text{Rd}
\]

(5)

where \(\alpha\) is the photochemical efficiency of photosynthesis at low light, \(P_m\) is the maximum photosynthetic rate, PAR is the photosynthetically active radiation, and Rd is the dark respiration rate.

Ye [14] presented a new model (Eq 6) modified from the RHM (Eq 5) for predicting the relationship between \(P_n\) and PAR,

\[
P_n = \frac{\alpha \cdot (1 - \beta \cdot \text{PAR}) \cdot \text{PAR}}{1 + \gamma \cdot \text{PAR}} - \text{Rd}
\]

(6)

Where \(\alpha\) is the photochemical efficiency of photosynthesis at low light, i.e., the initial slope of the PI curve; \(\beta\) is a correction factor for the decreasing trend of \(P_n\) when PAR exceed light saturation point due to photoinhibition, and the \(\beta\) is similar to the convexity [9, 11] or the sharpness of the knee [20] of the PI curve; \(\gamma\) is a correction factor for the \(\alpha\) (i.e., the initial slope of the PI curve) and the \(P_m\) (i.e., the maximum photosynthetic rate), and the \(\gamma\) is proportional to the ratio of \(\alpha\) and \(P_m\) (i.e., \(\gamma \propto \frac{\alpha}{P_m}\)); \(\alpha\), \(\beta\), and \(\gamma\) are coefficients that are independent of irradiance [14]; PAR is the photosynthetically active radiation, and Rd is the dark respiration rate. Here, we assumed that, 1) the \(P_n\) of plants decreased with increasing concentrations of a pollutant; and 2) the effect of the pollutant on the PI curve is non-competitive inhibited, and we presented our new non-competitive inhibited Michaelis-Menten model (NIMM) as:

\[
P_n = \frac{\alpha \cdot (1 - \beta \cdot \text{PAR}) \cdot \text{PAR}}{(1 + \gamma \cdot \text{PAR}) \cdot \left(1 + \frac{I}{K_i}\right)} - \text{Rd}
\]

(7)

Where \(\alpha\), \(\beta\) and \(\gamma\) are the same as mentioned above; \(P_n\) denotes the net photosynthetic rate; \(K_i\) denotes an inhibition constant; \(I\) is the relative pollution index and

\[
I = \frac{C_i}{C_{\text{max}}}
\]

(8)

Where \(C_i\) is the actual concentration of pollutant i in water or soil; and \(C_{\text{max}}\) is the maximum concentration of pollutant i in water or soil.

2.2 Experimental design

Establishing a single pollutant model is the first step in the research of effects of pollution on plants. Here, we chose one pollutant to a plant research model. We tested effects of a variety of common pollutants to corresponding representative plants as shown in Table 1. Phenolic pollution is often the chemical hazards and accidents that take place in the chemical industry. And
the soil heavy metal pollution result from rapid industrialization and urbanization during industrial and agricultural development and population growth. So, we tested the pollutants including phenol and some common metal pollutants, e.g., Cu^{2+}, Pb^{2+}, Cd^{2+}, and Al^{3+}. The Bordeaux mixture (a mixture of coppersulfate and lime) or animal manure use in agriculture results in the potential risk of soil copper pollution. The lead and cadmium pollution also result from automobile exhaust. The soil acidity increasing leads to aluminum pollution. The plants we considered including monocotyledonous or dicotyledonous plant, C_{3} or C_{4} plant, herbaceous or woody plant, or crop. We collected and analyzed the data of effects of phenol and Cu^{2+} on plants from pot-culture experiments. For additional information, we also extracted and analyzed the data about the effects of other pollutants such as Pb^{2+}, Cd^{2+} and Al^{3+} on plants from published literatures [22–25].

2.3 Pot-culture experiment and PI curves measurement

The pot-culture experiments were carried out in a greenhouse at the Fuqing Branch of Fujian Normal University from June to September in 2013. *T. pratense* L. and *W. trilobata*, two types of ornamental groundcover that often appear on roadsides and plantations, were planted in flowerpots filled with \( 1.8 \) kg soil. Each treatment had \( 15 \) replicates. The properties of the soil were pH: \( 6.4 \), total nitrogen: \( 24.2 \) mg kg\(^{-1} \), total phosphorus: \( 1.15 \) g kg\(^{-1} \), available phosphorus: \( 9.03 \) mg kg\(^{-1} \), total potassium: \( 68 \) mg kg\(^{-1} \), and clay particles: \( 21.7\% \).

*T. pratense* seeds were germinated for \( 48 \) h in the dark (on wet filter paper at \( 25^\circ\)C) and sown into a flowerpot (diameter: \( 200 \) mm, height: \( 200 \) mm) filled with phenol treated soil. Before being filled into pot, air-dried soil was treated with \( 0 \) (as control), \( 100, 200, \) or \( 300 \) mg kg\(^{-1} \) of phenol. *W. trilobata* were collected from the roadsides and cut, and the apex meristem with two leaves (\( \approx 100\)-mm length, two internodes) were planted in a flowerpot (diameter: \( 200 \) mm, height: \( 200 \) mm). Three apex meristems were planted in every flowerpot with CuSO\(_{4}\)-5H\(_{2}\)O added soil. Air-dried soil was added with \( 0 \) (as control), \( 500, 1000, \) or \( 2000 \) mg kg\(^{-1} \) of CuSO\(_{4}\)-5H\(_{2}\)O, and then was filled into the flower port.

We selected a sunny day (three months after planting) to measure the PI curves using a CIRAS-2 Portable Photosynthesis System (PP Systems, USA) with an LED radiation source.

2.4 Data collection and detailed data descriptions

PI data for plants under different concentrations of pollutants from four studies were gathered from published literatures (Table 1) to further evaluate our NIMM. All data were collected from pot-culture experiments.

| Species                  | Species types                                      | pollutant | Data source                        |
|--------------------------|---------------------------------------------------|-----------|------------------------------------|
| *Trifolium pratense*     | monocotyledonous, herbaceous, C\(_3\) plant       | phenol    | Measured in this study             |
| *Wedelia trilobata*      | dicotyledonous, herbaceous, C\(_3\) plant         | Cu\(_{2+}\) | Measured in this study             |
| *Zea mays*               | monocotyledonous, crop, C\(_2\) plant             | Pb\(_{2+}\) | Data collected from literature [22]|
| *Citrus sinensis Osbeck* | dicotyledonous, woody, C\(_3\) plant              | Cu\(_{2+}\) | Data collected from literature [23]|
| *Zea mays*               | monocotyledonous, crop, C\(_2\) plant             | Cd\(_{2+}\) | Data collected from literature [24]|
| *Plantago asiatica*      | dicotyledonous, herbaceous, C\(_3\) plant         | Al\(_{3+}\) | Data collected from literature [25]|

doi:10.1371/journal.pone.0142712.t001

The pot-culture experiments of *Z. mays* seedling [22] were conducted in silica culture. And the seedlings consisting of one bud and two leaves were treated with three Hoagland solution (including equal amount of Pb\(_{2+}\) and EDTA at different concentrations: \( 0, 0.25 \) or \( 0.5 \) mg kg\(^{-1} \).
mmol L\(^{-1}\)). After 15 days of treatment, the PI curves were measured with a Ciras-2 portable photosynthesis system (PP systems, UK). For more detailed information, please see S1 Table.

The one-year old C. sinensis Osbeck [23] was grafted onto Citrus aurantium L. before Cu stress treatment. The pot-culture experiments of C. sinensis Osbeck were conducted in a 10-L pot filled with 8 L of Alva nutrient solution (pH 6.5). The Alva nutrient solution was aerated 3 times with each time for 2 h in every day, and it was renewed every 10 days; And the C. sinensis Osbeck were treated with five Alva nutrient solution (containing Cu\(^{2+}\) concentration at 0, 0.1, 5, 20 or 40 μmol L\(^{-1}\)). After 60 days of treatment, the PI curves were measured with a CID-301 PS (CID Bio-Science, Inc., USA). For more detailed information, please see S2 Table.

The other pot-culture experiments of Z. mays [24] were conducted in paddy soil. The properties of the paddy soil were pH: 6.42, organic matter: 1.63%, total Cd: 0.32 mg kg\(^{-1}\), total nitrogen: 0.09%, available phosphorus: 0.05%, available potassium: 0.04%. The paddy soil was air-dried and sieved through a 2-mm sieve, mixed with different amount of CdCl\(_2\)-2H\(_2\)O, and then the post-treated paddy soil was added into each pot up to three kg with one gram of compound fertilizer (including N 15%, P 15%, K 15%). Finally, the germinated Z. mays were planted; So far, the germinated Z. mays were treated with six paddy soil (including Cd concentration at: 0.32, 1, 5, 15, 50 or 100 mg kg\(^{-1}\)). After 20 days of treatment, the PI curves were measured with Li-6400 (Li-Cor Inc., USA). For more detailed information, please see S3 Table.

The P. asiatica [25] seed was sterilized with 0.1% HgCl\(_2\) for 10 min, following by washing and soaking in distilled water for 8 h, and then the seed was sowed in sterilized silica culture. The two-leaves old plants were transplanted into a 20 cm × 23 cm flowerpot with three kg medium (peat soil: sand = 3:1). On the six-leaves old plant, the Al stress was performed. 10 mL of AlCl\(_3\) solution (pH 4.0) with different concentration at 0, 100, 500, 800 or 2000 mg L\(^{-1}\) were respectively poured into the flowerpot to simulate different leaching of Al\(^{3+}\) in soil every day. After 20 days of treatment, the PI curves were respectively measured with a Ciras-2 portable photosynthesis system (PP systems, UK). For more detailed information, please see S4 Table.

### 2.5 Mathematical fitting and model testing

To obtain the equation parameters (i.e., \(\alpha, \beta, \gamma, K_0\), and Rd), mathematical fitting of NIMM was performed using 1stOpt software (7D-Soft High Technology Inc. Beijing, China) with the Levenberg-Marquardt method. In addition, mathematical fitting of the relationship of \(P_n\) and \(I\) and that of \(P_n\) and PAR were performed to obtain the equation parameters using the same software and method as in the previous case. The relationship between the \(P_n\) and PAR of T. pratense response to different concentrations of phenol in our pot-culture experiment was calculated according to the mathematical fitting results to test the NIMM. The relationship between the \(P_n\) and PAR of W. trilobata response to different concentrations of Cu\(^{2+}\) was calculated using the same method.

### Results

#### 3.1 Experimental results

The \(P_n\) in our pot-culture experiments was measured with a Ciras-2 under conditions of natural ambient CO\(_2\) at different PAR. Our results were similar to the references [22–25]. Clearly, the PI curves of the plants were saturation curves. The results also showed that, either in W. trilobata or in T. pratense, the \(P_n\) increased with PAR increasing below the PAR\(_{\text{sat}}\) (i.e., light saturation point, \(\approx 1000 \mu\text{mol photon m}^{-2} \text{s}^{-1}\) in T. pratense, \(\approx 1400 \mu\text{mol photon m}^{-2} \text{s}^{-1}\) in W. trilobata), while decreased as PAR increasing above PAR\(_{\text{sat}}\). The results also showed that the pollutant obviously negatively affected the PI curves. For more detailed information, please see S5 and S6 Tables.
### 3.2 Effect of a pollutant on the normalized Pn of plants

The normalized Pn of plants decreased with increasing concentrations of the pollutant under 1000 μmol photon m⁻² s⁻¹ PAR (Fig 1). Akaike’s information criterion (AIC) was proposed by Akaike [31, 32] and defined as Eq 9,

\[
AIC = N \cdot \ln R_e + 2 \cdot p
\]  

Where N is the number of experimental data points, p is the number of parameters in an estimated model, and R_e is the residual sum of squares. In addition, the model with the lowest AIC is regarded as the best representation of a curve [32]. The Pn values for all five species were normalized to the pollutant-free control value of Pn, and the normalized Pn were regressed with respect to I using linear (Eq 10), power (Eq 11), exponential (Eq 12), and hyperbolic (Eq 13) functions,

\[
Pn' = a + b \times I
\]  

\[
Pn' = \frac{a}{I^b}
\]  

\[
Pn' = \frac{a}{b^I}
\]  

\[
Pn' = \frac{a}{b + I}
\]  
in these equations (Eqs 10 ~ 13), Pn' is the normalized net photosynthetic rate, a and b are coefficients, I is the relative pollution index.

And the results showed that all functions (Eqs 10, 11, 12 and 13) were significant (P < 0.01), and the hyperbolic function (Eq 13) was the optimal function based on having the greatest goodness-of-fit (R²) of 0.5983 and the lowest AIC of -9.0 (Fig 1a). The normalized Pn of each species was regressed with respect to I using a hyperbolic (Eq 13) function, and the results were all significant (P < 0.01) (Fig 1b).

### 3.3 Mathematical fitting of PI curves using different models

The Pn of *T. pratense*, *Z. mays* seedling, *C. sinensis* Osbeck, *Z. mays*, *P. asiatica*, and *W. trilobata* were respectively regressed with respect to PAR using an EF [8, 10], HTF [1], NHM [11, 13], RHM [17, 18], BRF [9,13], and MM [14]. The R² was significant for all models (P < 0.001). In *T. pratense* or *P. asiatica*, the three largest R² values (associated with the lowest AIC) of models were for HTF, NHM, and MM (Fig 2a and 2b). In *C. sinensis* Osbeck or *Z. mays* seedling, the three largest R² values (associated with the lowest AIC) of models were for HTF, BRF, and MM (Fig 2c and 2d). In *Z. mays*, the three largest R² values (associated with the lowest AIC) of models were for RHM, NHM, and MM (Fig 2e). In *W. trilobata*, the three largest R² values (associated with the lowest AIC) of models were for EF, HTF, and MM (Fig 2f). The MM and BRF were both better than other models at describing the photoinhibition phenomenon at high PAR (Fig 2).

### 3.4 Evaluation of NIMM

The Pn of each species was regressed on PAR and I using NIMM, and the results are shown in Table 2. The R² values were greater than 0.95 except for *Z. mays*. For Cu pollution, the K_i of *W.
Trilobata was greater than that of Citrus sinensis Osbeck. The Ki of Cu to W. trilobata was greater than that of the phenol to T. pratense. For Z. mays, the Ki of Cd was greater than that of Pb.

The NIMM was tested using our pot-culture experimental data. Either in T. pratense, or in W. trilobata, the R² values were all significant (P < 0.001) under different pollution levels (Fig 3, Table 3). Either in T. pratense, or in W. trilobata, the light saturation point (PARsat) and the light compensation point (PARcom) both increased with worsening pollution, while the maximum photosynthetic rate (Pm), quantum efficiency at PARcom (φc), and intrinsic quantum efficiency (φ0) all decreased (Table 3). The φc represents the light energy use efficiency at PARcom, the φ0 represents the intrinsic light energy use efficiency at darkness, i.e., the optimal light use potential of plant. The results suggested that the pollutant inhibited the light use potential of plant. In order to analyze the credibility of the assessment results, we performed paired sample test analysis, and the results showed that in T. pratense, the calculated Pm was no significant difference to the measured Pm (t = -1.975, df = 3, P2-tailed = 0.143), in W. trilobata, the calculated Pm was also no significant difference to the measured Pm (t = -1.777, df = 3, P2-tailed = 0.174).

Discussion

All of the above mentioned existing models (i.e., EF; HTF; NHM; RHM; BRF; and MM) provide useful protocols for PI curve assessment. Jassby and Platt reported that, from zero light up to the onset of photoinhibition, the PI curve for natural populations of coastal phytoplankton is best described by HTF, and they recommended its use as an operational model for the elucidation of physiological parameters in photosynthesis-light experiments and for the theoretical investigation [1]. The shape of PI curve described by EF suggests that a linear relation holds only for low light intensities, then the photosynthetic rate tends towards a maximum value when the light intensity is increasing [8, 10]. The NHM was found to be objective to calculate the photosynthetic parameters of the PI curve [9, 11, 13, 20], the PI curve could also be described by BRF [13, 33], but the BRF could not be used to calculate the quantum...
Fig 2. Mathematical fitting of the PI curve using different models. AIC is Akaike's information criterion.

doi:10.1371/journal.pone.0142712.g002
efficiency and explain that the predicted Pn declines quickly when PAR exceeds the light saturation point [13]. In addition, the BRF has the shortcoming of sometimes inferring a positive dark respiration rate, which has no biological significance. The RHM can be obtained from the NHM by putting $\theta = 0$, it is a special case of the NHM [20]. And the RHM is preferred to the NHM by some workers on the grounds of simplicity [18, 20], though it is rather tedious to take the limit as $\theta \to 0$ in the NHM equation [20]. Our experimental results showed that the shapes of PI curves were similar to that of the literatures. Our experimental results also showed that the PI curves have photoinhibition phenomenon at high irradiance, i.e., the Pn decreased when the PAR exceeded light saturation point. These results were fully consistent with that of the literature [8, 11, 13, 14, 23, 34]. Although the HTM, EF, NHM and RHM have been extensively applied [11, 14, 17, 18, 20, 34–38], they do not consider the photoinhibition of plants. The MM, which is based on the RHM, is useful to study photoinhibition and photosynthetic behavior at high irradiance and, especially, is the best model to describe the PI curve because its fitted values were close to the measured data [14]. Therefore, the MM (Eq 6) was the optimal

Table 2. Mathematical fitting results of the NIMM for plant responses to pollution.

| Species (Pollutant)   | Data source          | Model parameters |
|----------------------|----------------------|------------------|
|                      |                      | $K_i$  | $\alpha$ | $\beta$ | $\gamma$ | $R_d$ | $R^2$ |
| Trifolium pratense (Phenol) | Measured in this study | 1.17   | 0.086   | 0.0002 | 0.0022 | 1.03  | 0.9886 |
| Wedelia trilobata (Cu)   | Measured in this study | 4.48   | 0.044   | 0.0001 | 0.0042 | 1.00  | 0.9629 |
| Zea mays (Pb)           | Reference [22]       | 0.395  | 0.044   | 0.0003 | 0.0002 | 1.78  | 0.9841 |
| Citrus sinensis Osbeck (Cu) | Reference [23]     | 0.321  | 0.013   | 0.0003 | 0.0002 | 0.42  | 0.9862 |
| Zea mays (Cd)           | Reference [24]       | 0.923  | 0.061   | 0.0001 | 0.0015 | 1.65  | 0.8984 |
| Plantago asiatica (Al)  | Reference [25]       | 0.501  | 0.058   | 0.0003 | 0.0005 | 1.59  | 0.9576 |

K. denotes the inhibition constant; $\alpha$ denotes the photochemical efficiency of photosynthesis at low light, i.e., the initial slope of the PI curve; $\beta$ and $\gamma$ are the coefficients that are independent of irradiance; $R_d$ denotes the dark respiration rate.

doi:10.1371/journal.pone.0142712.t002

Fig 3. The test results for the NIMM. a, in T. pratense; b, in W. trilobata; *** means significant at $P < 0.001$.

doi:10.1371/journal.pone.0142712.g003
Plant Photosynthesis-Irradiance Curve Responses to Pollution

**Table 3. Model testing results of the NIMM.**

| Species | Pollutant in soil (mg kg⁻¹) | Calculated equation | Measured $P_n$ (μmol CO₂ m⁻² s⁻¹) | Calculated $P_m$ (μmol CO₂ m⁻² s⁻¹) | $\text{PAR}_{\text{com}}$ (μmol photon m⁻² s⁻¹) | $\text{PAR}_{\text{sat}}$ (μmol photon m⁻² s⁻¹) | $\phi_e$ | $\phi_0$ | $R^2$ |
|---------|-----------------------------|---------------------|----------------------------------|----------------------------------|------------------------------------|------------------------------------|-------|-------|-------|
| T. pratense | Phenol (0) | $P_n = \frac{0.04 (1 - 0.0002 \text{PAR}) \text{PAR}}{1 - 0.0002 \text{PAR}} - 1.03$ | 19.5 | 20.5 | 12.0 | 1140.7 | 0.083 | 0.089 | 0.9835*** |
| | Phenol (100) | $P_n = \frac{0.04 (1 - 0.0002 \text{PAR}) \text{PAR}}{1 - 0.0002 \text{PAR}} - 1.03$ | 15.0 | 15.8 | 15.4 | 1146.2 | 0.065 | 0.070 | 0.9850*** |
| | Phenol (200) | $P_n = \frac{0.04 (1 - 0.0002 \text{PAR}) \text{PAR}}{1 - 0.0002 \text{PAR}} - 1.03$ | 11.3 | 12.8 | 18.7 | 1152.2 | 0.053 | 0.057 | 0.8708*** |
| | Phenol (300) | $P_n = \frac{0.04 (1 - 0.0002 \text{PAR}) \text{PAR}}{1 - 0.0002 \text{PAR}} - 1.03$ | 10.9 | 10.6 | 22.2 | 1158.1 | 0.044 | 0.049 | 0.9924*** |
| W. trilobata | CuSO₄·5H₂O (500) | $P_n = \frac{0.04 (1 - 0.0002 \text{PAR}) \text{PAR}}{1 - 0.0002 \text{PAR}} - 1.00$ | 6.7 | 6.7 | 23.0 | 1397.0 | 0.040 | 0.048 | 0.9848*** |
| | CuSO₄·5H₂O (1000) | $P_n = \frac{0.04 (1 - 0.0002 \text{PAR}) \text{PAR}}{1 - 0.0002 \text{PAR}} - 1.00$ | 5.6 | 6.3 | 24.0 | 1400.0 | 0.038 | 0.046 | 0.7705*** |
| | CuSO₄·5H₂O (2000) | $P_n = \frac{0.04 (1 - 0.0002 \text{PAR}) \text{PAR}}{1 - 0.0002 \text{PAR}} - 1.00$ | 5.6 | 5.9 | 25.3 | 1404.0 | 0.036 | 0.044 | 0.8408*** |
| | CuSO₄·5H₂O (3000) | $P_n = \frac{0.04 (1 - 0.0002 \text{PAR}) \text{PAR}}{1 - 0.0002 \text{PAR}} - 1.00$ | 5.2 | 5.3 | 27.8 | 1411.8 | 0.032 | 0.040 | 0.9593*** |

$\phi_e$ is light saturation point; $\text{PAR}_{\text{com}}$ is light compensation point; $P_m$ is maximum photosynthetic rate; $\phi_0$ is the quantum efficiency at $\text{PAR}_{\text{com}}$. *** means significant at $P \leq 0.001$.

doi:10.1371/journal.pone.0142712.t003

Moreover, based on the lowest AIC values [31, 32], the HTF, NHM, and MM are more suitable for characterizing the PI curve (Fig 2).

Temperature, intensity of irradiation, and concentration of carbon dioxide in the surrounding medium are the three important controlling factors could influence the rate of photosynthesis in plant, and of the three controlling factors, the most important is the temperature [11, 17]. However, the concentration of carbon dioxide in the atmosphere remains relatively constant, and it is unlikely to be a major factor effecting variations in the rate of photosynthesis, simultaneously, the temperature could not influence the shape of the PI curve of plant, therefore, temperature and concentration of carbon dioxide need not appear explicitly in a PI curve model [11]. On the other hand, the shapes of PI curves in our pot-culture experiments (Fig 3) were fully consistent with that of the literatures [8, 11, 13, 14, 22–25, 34], and showed that $\alpha$ and $P_m$ both decreased along with the increasing concentrations of pollutant, but the convexity [11, 13, 37], or the sharpness of the knee [20] of the PI curve described by the NHM increased along with increasing pollutant concentrations. It indicated that the pollutants negatively affected on the photosynthesis of plants, and the impact degree increased with rising pollutant concentrations. This conclusion was similar to that of the literature [17]. The literature [17] reported that a poison may materially to reduce the rate of photosynthesis, because the poison may either decrease the velocity of the Blackman reaction, or decrease the velocity of the primary photosynthetic reaction by being preferentially adsorbed by the chlorophyll a and thus preventing the latter from adsorbing or combining with hydrated carbon dioxide. So, pollutant was significant and necessary appear explicitly in a PI curve model. And even though some metals, such as zinc and copper, are essential trace elements for plants as the natural active sites of an enzyme, plant growth and development only need low concentrations of these metals of around 10 μg g⁻¹ dry plant tissue [39, 40]. Some studies [41, 42] have also shown that pollutants...
(heavy metals) significantly affect the Pn of plants. Hence, in the present study, an attempt was made to build a new model, which was integrated I (i.e., pollution index) into the MM, for predicting the relationship of Pn, PAR and I.

Then, how to integrate the I into the MM? The relationship of normalized Pn and I were respectively regressed using linear (Eq 10), power (Eq 11), exponential (Eq 12), and hyperbolic (Eq 13) functions. And, the effect of pollutants on the Pn of plants (Fig 1) indicated that the hyperbolic function (Eq 13) was optimal for predicting the relationship of Pn and I. Thus, we integrated the I into the MM as:

$$Pn = \frac{\alpha \cdot (1 - \beta \cdot PAR) \cdot PAR}{1 + \gamma \cdot PAR} \frac{a}{b + I} - Rd$$

(Eq 14)

Eq 14 can be converted into:

$$Pn = \frac{\alpha \cdot (1 - \beta \cdot PAR) \cdot PAR}{(1 + \gamma \cdot PAR) \cdot b} - Rd$$

(Eq 15)

Further, Eq 15 can be converted into:

$$Pn = \frac{\alpha \cdot (1 - \beta \cdot PAR) \cdot PAR}{(1 + \gamma \cdot PAR) \cdot \delta \cdot (1 + \frac{a}{b})} - Rd$$

(Eq 16)

If $b = K_i$ and $\delta = \frac{b}{a}$, Eq 16 can be expressed as:

$$Pn = \frac{\alpha \cdot (1 - \beta \cdot PAR) \cdot PAR}{(1 + \gamma \cdot PAR) \cdot \delta \cdot (1 + \frac{1}{K_i})} - Rd$$

(Eq 17)

Where $\delta$ is a non-zero coefficient, Eq 17 is equivalent to the NIMM, i.e., Eq 7.

Further, our mathematical fitting results showed that the NIMM was suitable for predicting the relationship of Pn, PAR, and I because of their high $R^2$ (Table 2) and their significance at the $P < 0.001$ level (Table 3), that is, the NIMM was suitable for fitting the PI curve of plant responses to pollution (Fig 3, Table 3). The NIMM showed that the Pn is a function of PAR and I, thus, the Eq 18 denotes the influence rate of I on Pn, and the Eq 19 denotes the influence rate of PAR on Pn,

$$\frac{\partial Pn}{\partial I} = -\frac{\alpha \cdot (1 - \beta \cdot PAR) \cdot PAR}{K_i \cdot (1 + \gamma \cdot PAR) \cdot \left(1 + \frac{1}{K_i}\right)^2}$$

(Eq 18)

$$\frac{\partial Pn}{\partial PAR} = \frac{\alpha \cdot (1 - \beta \cdot PAR) \cdot PAR - \alpha \cdot \beta \cdot \gamma \cdot PAR^2}{K_i \cdot (1 + \gamma \cdot PAR)^2 \cdot \left(1 + \frac{1}{K_i}\right)}$$

(Eq 19)

Where $\frac{\partial Pn}{\partial I}$ and $\frac{\partial Pn}{\partial PAR}$ are partial derivative, denotes the influence rate of I on Pn, and the influence rate of PAR on Pn respectively; $\alpha$, $\beta$, $\gamma$, $K_i$, PAR, and I are the same as above mentioned.

In all the published models (i.e., HTF [1], EF [8], EF [10], NHM [11, 13], BRF [9, 13], RHM [17, 18]), the researchers focused more on the relationship between the Pn and PAR, however, they didn’t take account of the influence of I on the PI curve. In the present study, we have integrated the I into the MM [14] as the NIMM to predict the co-variation of Pn, PAR, and the I. Here, we also integrated the I into the published models (i.e., HTF [1], EF [8], EF [10], NHM [11, 13], BRF [9, 13], RHM [17, 18], respectively) to predict the co-variation of Pn, PAR, and the I. Then, we compared the NIMM with the modified models based on our pot-culture experimental data (Table 4). In T. pratense, the AIC of the NIMM (i.e., 242.5) was lower than...
that of the models which were modified from the EF [8, 10], RHM [17, 18], and BRF [9, 13]
(i.e., 277.0, 249.6, 308.2, and 357.3 respectively), while, the AIC of the NIMM was higher than
that of the models modified from the HTF [1] or NHM [11, 13] (i.e., 229.0 or 235.3 respectively). In W. trilobata, the AIC of the NIMM (i.e., 131.2) was lower than that of the models
which were modified from the EF [8], RHM [17, 18], and BRF [9, 13] (i.e., 164.8, 151.5, and
209.2 respectively), while, the AIC of the NIMM was higher than that of the models modified
from the EF [10], HTF [1] or NHM [11, 13] (i.e., 126.4, 124.1, and 128.4 respectively).

Although the model with the lowest AIC is regarded as the best representation of a curve [32],
the models of the EF [10], HTF [1], NHM [11, 13], and RHM [17, 18] cannot fit the data that
shows the photoinhibition phenomenon at high irradiance. The NIMM modified from the
MM [14], is more reliable at unveiling the photoinhibition phenomenon. Therefore, the
NIMM provides a robust tool to evaluate and understand the influence of environmental pollu-
tion on plant photosynthesis, and it is relative improved model comparing to the previous
models published [1, 8–11, 13, 17, 18, 20].

Pollutants (metals) are harmful to plants because they inhibit various metabolic processes
[41–43]. Some metal pollutants directly affect enzymes of the chlorophyll biosynthesis pathway
[44–46], and some affect the proper assembly of the photosynthetic pigment–protein com-
plexes [47, 48]. Some metals replace the central Mg ion in chlorophyll molecules, destroying
the chlorophyll [49]. Conversely, some studies have not found that metal pollutants directly affect
the biosynthesis of pigments or influence the photosynthetic machinery, and have claimed that
the metal pollutants interfere with cell division and chloroplast replication, thus decreasing
the number of chloroplasts and ultimately lowering the photosynthetic efficiency [50]. Thus,
regardless of whether elevated concentrations of pollutants in contaminated environments
bind equally well to enzymes, they will already have negatively affected plant growth and develop-
ment through the inhibition of photosynthetically related enzyme activity. Our mathematical
fitting results indicate that the elevated concentrations of pollutants not only inhibited $\alpha$
(i.e., photosynthetic potential, light use efficiency, or the slope of the PI curve), but also lowered
$P_n$ (Tables 2 and 3, Figs 1 and 3). The former (i.e., decreased $\alpha$ associated with increasing pol-
lutant concentrations) suggested that the pollution decreased the activity of the photosyntheti-
cally related enzyme. Our pot-culture experimental results showed that in W. trilobata, the
pollutant (Cu$^{2+}$) did not significantly affect the pigment content, above-ground biomass, or
belowground biomass, but did significantly affect the $P_n$ (Please see S7 Table). Our pot-culture
experimental results also showed that the pollutant (phenol) significantly affected the biomass
and $P_n$ of T. pratense, but did not affect its pigment contents (Please see S8 Table). The results
indicate that the pollutants acted as a non-competitive inhibitor because they varied the $P_n$
(whch is equivalent to the maximum enzymatic reaction rate in the Michaelis-Menten model). Combining with the above-mentioned relationship between individual gross photo-
synthesis and PAR following the Michaelis-Menten model [21], that is, our results were similar
to the literature [21]. And, the NIMM was suitable for reasonably predicting the relationships
of $P_n$, PAR, and $I$.

To compare the three Michaelis kinetics (i.e., non-competitive, competitive, and un-com-
petitive inhibition), we integrated the pollution factor into the MM in different ways, and per-
formed mathematical fitting using our pot-culture experimental data for T. pratense. The result
for un-competitive inhibited Michaelis-Menten (UIMM) kinetics was

$$P_n = \frac{0.0811 - 0.0003 \text{PAR}/\text{PAR}}{1 + 0.002 \text{PAR} / (1 + 0.002 \text{PAR})} - 1.56, \ R^2 = 0.9777, \text{and an AIC of 283.2.}$$

The result for competitive inhibited Michaelis-Menten (CIMM) kinetics was

$$P_n = \frac{0.0783 - 0.0003 \text{PAR}/\text{PAR}}{1 + 0.002 \text{PAR}/(1 + 0.003 \text{PAR})} + 0.006, \ R^2 = 0.9723, \text{and an AIC of 306.2.}$$

Both AIC values were greater than 242.5 (i.e., the AIC of the
Table 4. The comparation of model application results in *T. pratense* or *W. trilobata*.

| Species        | Published model | The model equation modified from the published model                                      | Parameters |          |          | R²  | AIC    |
|----------------|-----------------|------------------------------------------------------------------------------------------|------------|---------|------------|-----|--------|
| *T. pratense*  | EF, [8]         | \( P_n = \frac{a \times \text{PAR} \times \exp(b \times \text{PAR})}{1 + a \times \text{PAR}} - \text{Rd} \) | 1.06       | 0.05    | a = 0.05, \( P_m = 20.3 \) | 0.9811     | 277.0 |
|                | EF, [10]        | \( P_n = \frac{\text{P}_n (1 - \exp(b \times \text{PAR} \times \text{PAR}))}{1 + a \times \text{PAR}} - \text{Rd} \) | 1.19       | 1.18    | a = 0.24, \( P_m = 20.6 \) | 0.9870     | 249.6 |
|                | HTF, [1]        | \( P_n = \frac{\text{P}_n \times \text{tan}(c \times \text{PAR})}{1 + a \times \text{PAR}} - \text{Rd} \) | 1.13       | 0.70    | \( P_m = 19.9, a = 0.06 \) | 0.9903     | 229.0 |
|                | NHM, [11, 13]   | \( P_n = \frac{a \times \text{PAR} \times \text{P}_n - \exp(b \times \text{PAR} \times \text{PAR})}{1 + a \times \text{PAR}} - \text{Rd} \) | 1.11       | 0.46    | \( P_m = 20.1, a = 0.05, \theta = 0.9463 \) | 0.9897     | 235.3 |
|                | RHM, [17, 18]   | \( P_n = \frac{\text{P}_n \times \exp(c \times \text{PAR})}{a \times \text{PAR} + \text{P}_n} - \text{Rd} \) | 1.23       | 1.52    | \( \alpha = 0.13, P_m = 24.3 \) | 0.9708     | 308.2 |
|                | BRF, [8, 13]    | \( P_n = \frac{a \times \text{PAR} + b \times \text{PAR}}{1 + \text{PAR}} - \text{Rd} \) | 0.92       | -1.39   | \( a = -1.73, b = 0.0371 \) | 0.9422     | 357.3 |
|                | NIMM, modified based on MM [14] | \( P_n = \frac{a \times \text{PAR} \times \exp(b \times \text{PAR})}{(1 + \text{PAR})} - \text{Rd} \) | 1.17       | 1.03    | \( \alpha = 0.086, \beta = 0.0002, \gamma = 0.0022 \) | 0.9886     | 242.5 |

| Species        | Published model | The model equation modified from the published model                                      | Parameters |          |          | R²  | AIC    |
|----------------|-----------------|------------------------------------------------------------------------------------------|------------|---------|------------|-----|--------|
| *W. trilobata* | EF, [8]         | \( P_n = \frac{a \times \text{PAR} \times \exp(b \times \text{PAR})}{1 + \text{PAR}} - \text{Rd} \) | 3.17       | -0.46   | \( a = 0.02, P_m = 6.1 \) | 0.9372     | 164.8 |
|                | EF, [10]        | \( P_n = \frac{\text{P}_n (1 - \exp(b \times \text{PAR} \times \text{PAR}))}{1 + a \times \text{PAR}} - \text{Rd} \) | 4.25       | 0.73    | \( a = 0.09, P_m = 7.0 \) | 0.9643     | 126.4 |
|                | HTF, [1]        | \( P_n = \frac{\text{P}_n \times \text{tan}(c \times \text{PAR})}{1 + a \times \text{PAR}} - \text{Rd} \) | 3.76       | 0.17    | \( \alpha = 6.4, P_m = 0.02 \) | 0.9655     | 124.1 |
|                | NHM, [11, 13]   | \( P_n = \frac{a \times \text{PAR} \times \text{P}_n - \exp(b \times \text{PAR} \times \text{PAR})}{1 + a \times \text{PAR}} - \text{Rd} \) | 3.67       | 0.06    | \( \alpha = 6.5, \gamma = 0.02, \beta = 0.9200 \) | 0.9644     | 128.4 |
|                | RHM, [17, 18]   | \( P_n = \frac{\text{P}_n \times \exp(c \times \text{PAR})}{a \times \text{PAR} + \text{P}_n} - \text{Rd} \) | 5.71       | 2.36    | \( \alpha = 0.09, P_m = 9.4 \) | 0.9484     | 151.5 |
|                | BRF, [8, 13]    | \( P_n = \frac{a \times \text{PAR} + b \times \text{PAR}}{1 + \text{PAR}} - \text{Rd} \) | 2.47       | -1.25   | \( a = -4.68, b = 0.01 \) | 0.8795     | 209.2 |
|                | NIMM, modified based on MM [14] | \( P_n = \frac{a \times \text{PAR} \times \exp(b \times \text{PAR})}{(1 + \text{PAR})} - \text{Rd} \) | 4.48       | 1.00    | \( \alpha = 0.044, \beta = 0.0001, \gamma = 0.0042 \) | 0.9629     | 131.2 |

EF, exponential function; HTF, hyperbolic tangent function; NHM, nonrectangular hyperbola model; RHM, rectangular hyperbolic model; BRF, binomial regression function; MM, modified model based on the rectangular hyperbolic model; NIMM, non-competitive inhibited Michaelis-Menten model; \( K_i \) denotes the inhibition constant; \( P_m \), maximum net photosynthetic rate; e is natural logarithm, 2.71828; a and b is constant; \( \theta \) is convexity of the PI curve; \( \alpha \) denotes the photochemical efficiency of photosynthesis at low light, i.e., the initial slope of the PI curve; \( \beta \) and \( \gamma \) are the coefficients that are independent of irradiance; Rd denotes the dark respiration rate; AIC, Akaike's information criterion.

doi:10.1371/journal.pone.0142712.t004

We also tested the two models (UIMM and CIMM) using our pot-culture experimental data for *T. pratense*; the results are shown in Table 5. Based on the UIMM, it’s unreasonable that the \( q_0 \) increased but the calculated \( P_m \) decreased with the increasing phenol pollution. Based on the CIMM, we performed paired samples t test analysis, and the results showed that the calculated \( P_m \) was significant higher than the measured \( P_m \) \( (t = -5.184, df = 3, P_{2-tailed} = 0.014) \), i.e., the calculated \( P_m \) deviated greatly from the measured \( P_m \). So, the UIMM and CIMM were both unsuitable for predicting the relationship of \( P_n, \text{PAR}, \) and \( I \). The NIMM, however, was suitable for predicting the relationship of \( P_n, \text{PAR}, \) and \( I \) because the calculated
### Table 5. Model testing results of the un-competitive inhibited and the competitive inhibited model.

| Model type | Concentration of phenol (mg kg⁻¹) | Calculated equation | Measured \( P_m (\mu mol \ CO_2 \ m^{-2} \ s^{-1}) \) | Calculated \( P_m (\mu mol \ CO_2 \ m^{-2} \ s^{-1}) \) | \( PAR_{com} (\mu mol \ photon \ m^{-2} \ s^{-1}) \) | \( PAR_{sat} (\mu mol \ photon \ m^{-2} \ s^{-1}) \) | \( \Phi_c \) | \( \Phi_o \) | \( R^2 \) |
|------------|----------------------------------|---------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|------|------|------|
| **UIMM**   |                                  |                     |                                             |                                             |                                            |                                            |      |      |      |
| 0          | \( P_n = \frac{(0.001 \times PAR)}{(1 + 0.0002 \times PAR)} - 1.56 \) | 19.5                | 19.4                                        | 19.3                                        | 1171.0                                      | 0.078                                       | 0.085 | 0.9877*** |
| 100        | \( P_n = \frac{(0.001 \times PAR)}{(1 + 0.0002 \times PAR)} - 1.56 \) | 15.0                | 14.6                                        | 19.3                                        | 1038.0                                      | 0.076                                       | 0.086 | 0.9851*** |
| 200        | \( P_n = \frac{(0.001 \times PAR)}{(1 + 0.0002 \times PAR)} - 1.56 \) | 11.3                | 12.0                                        | 19.3                                        | 956.0                                       | 0.075                                       | 0.087 | 0.9520*** |
| 300        | \( P_n = \frac{(0.001 \times PAR)}{(1 + 0.0002 \times PAR)} - 1.56 \) | 10.9                | 9.9                                         | 19.3                                        | 887.0                                       | 0.074                                       | 0.089 | 0.9079*** |
| **CIMM**   |                                  |                     |                                             |                                             |                                            |                                            |      |      |      |
| 0          | \( P_n = \frac{0.073 \times PAR}{1 + 0.0005 \times PAR} + 0.006 \) | 19.5                | 20.4                                        | 0.008                                       | 966.3                                       | 0.073                                       | 0.073 | 0.9650*** |
| 100        | \( P_n = \frac{0.073 \times PAR}{1 + 0.0005 \times PAR} + 0.006 \) | 15.0                | 16.8                                        | 0.014                                       | 1143.6                                      | 0.043                                       | 0.043 | 0.8973*** |
| 200        | \( P_n = \frac{0.073 \times PAR}{1 + 0.0005 \times PAR} + 0.006 \) | 11.3                | 13.6                                        | 0.020                                       | 1220.1                                      | 0.030                                       | 0.030 | 0.8155*** |
| 300        | \( P_n = \frac{0.073 \times PAR}{1 + 0.0005 \times PAR} + 0.006 \) | 10.9                | 12.2                                        | 0.026                                       | 1318.8                                      | 0.023                                       | 0.023 | 0.7567*** |

UIMM is the un-competitive inhibited Michaelis-Menten; CIMM is the competitive inhibited Michaelis-Menten; \( PAR_{sat} \) is the light saturation point; \( PAR_{com} \) is the light compensation point; \( P_m \) is the maximum photosynthetic rate; \( \Phi_c \) is the quantum efficiency at \( PAR_{com} \); \( \Phi_o \) is the intrinsic quantum efficiency; \( P_{n0} \) values were close to the measured \( P_m \) (Table 3), and the fitted results were close to measured data (Fig 3).

Interestingly, pollutants play a role in the inhibition of photosynthetically related enzyme activity; the \( K_i \) decreased with the combination of the pollutant with the photosynthetically related enzyme. The mathematical fitting results (Table 2) indicate that \( W. \) *trilobata* is tolerant of Cu pollution [51].

Finally, we put forward a perspective that the field investigation still needs to be further done for model validation. The published results [22–25] and the present study showed that the pollution factor could affect the PI curve in controlled experiment. In natural environment, many other uncontrolled variables such as temperature, humidity, \( CO_2 \) concentrations and so on, can also affect photosynthetic parameters. Therefore, it is important to justify and reveal the accuracy of the NIMM in practice.

### Supporting Information

**S1 Table.** Effect of \( Pb^{2+} \) on the \( P_n \) of *Zea mays*.

(DOCX)

**S2 Table.** Effect of \( Cu^{2+} \) on the \( P_{n0} \) of *Citrus sinensis* Osbeck.

(DOCX)

**S3 Table.** Effect of \( Ca^{2+} \) on the \( P_n \) of *Zea mays*.

(DOCX)

**S4 Table.** Effect of \( Al^{3+} \) on the \( P_{n0} \) of *Plantago asiatica*.

(DOCX)
S5 Table. Effect of phenol on the Pn of *Trifolium pratense* L.

S6 Table. Effect of CuSO₄·5H₂O on the Pn of *Wedelia trilobata*.

S7 Table. Effect of Cu²⁺ on *W. trilobata*.

S8 Table. Effect of phenol on *T. pratense* L.

Acknowledgments

We would like to thank Meifang Jin, Qiaoli Zeng and Jiahui Kang, Fuqing Branch of Fujian Normal University, for their help in experiment. We are grateful to the three anonymous reviewers and the academic editor for their comments that helped us improve the submitted manuscript.

Author Contributions

Conceived and designed the experiments: ML DC GW. Performed the experiments: ML DC. Analyzed the data: ML ZW LH KX DC GW. Contributed reagents/materials/analysis tools: ML DC GW. Wrote the paper: ML ZW LH KX DC GW. Designed the software language used in analysis: ML.

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