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Assessing the Effects of Nitrification Inhibitor DMPP on Acidification and Inorganic N Leaching Loss from Tea (*Camellia sinensis* L.) Cultivated Soils with Increasing Urea–N Rates

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Abstract: The effects of nitrification inhibitor in tea gardens with different urea–N rates have rarely been assessed. For eight months, a glasshouse experiment was conducted to investigate the effects of a nitrification inhibitor (3, 4–dimethylpyrazole phosphate, DMPP) on the changes of soil pH and inorganic N loss. Urea (0, 300, 500, and 800 kg N ha^{-1}) with or without DMPP (1% of urea–N applied) were added to pots that hosted six plants that were three years old. Next, three leaching events were conducted with 600 mL of water after 7, 35, and 71 days of intervention while soil samples were collected to determine pH and inorganic N. Averaged across sampling dates, urea–N application at an increasing rate reduced soil pH with the lowest values at 800 kg urea–N ha^{-1}. Adding DMPP increased soil pH up to a rate of 500 kg ha^{-1}. Irrespective of the addition of DMPP, gradient urea–N application increased the leaching loss of inorganic N. On overage, DMPP increased soil pH and decreased leaching losses of total inorganic N, suggesting a higher soil N retention. Therefore, we believe that this increase in soil pH is associated with a relatively lower proton release from the reduced nitrification in the DMPP–receiving pots. This nitrification reduction also contributed to the N loss reduction (NO_3^-–N). Altogether, our results suggest that DMPP can reduce N leaching loss while maintaining the pH of tea–cultivated soils. Therefore, DMPP application has a significant potential for the sustainable N management of tea gardens.

Keywords: DMPP; soil acidification; nitrification inhibitor; inorganic N leaching; soil N

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1. Introduction

China is the largest tea–producing country in the world, comprising of 45% of the total global production, consumption, and exportations [1]. Tea production requires high nitrogen (N) input since new shoots are recurrently harvested. Synthetic N fertilization is an essential way to replenish soil N supply in tea gardens. However, extensive use of synthetic N could also alter soil properties and cause environmental pollution, which could threaten the sustainable development of tea gardens.

Globally, croplands annually receive a large amount of N fertilizer (~109 Tg N (85% of the total anthropogenic N production)), while China uses > 25% (30 Tg N) [2,3]. The average N supplementation rate in tea–producing areas in China is about 553 kg ha^{-1} year^{-1} (281~745 kg ha^{-1} year^{-1}), which is much higher than the basic N demand of tea gardens (300~450 kg ha^{-1} year^{-1}) [4–6]. Extensive external N inputs have been demonstrated to cause soil acidification and reactive N pollution of soil and water in the environment [7–11].

Soil acidification is a common problem in tea gardens [12–15], as it intensifies after extensive usage of synthetic NH_4^+–N fertilizers [16,17]. For example, a recent meta–analysis suggested that soil pH in tea–growing areas reduced by 0.41 units [18]. Soil acidification in tea cultivation may occur in different ways, including N uptake and leaching [19,20]. When NH_4^+–N fertilizers such as urea are applied to soil, it mineralized to NH_4^+ while releasing hydroxyl ions (OH^-) that counterbalance soil pH. However, these NH_4^+ are taken up by plants and protons (H^+) are released from plant roots causing acidification. Soil acidification also occurs at a double rate when NH_4^+–N is converted to NO_3^-–N and leached from soil. Moreover, 2 mol protons can be released when 1 mol NH_4^+–N is oxidized to NO_3^-–N by microbial nitrification.

Nitrate leaching with water is the primary pathway of soil N loss from the tea gardens since tea is grown in mountainous areas and thus NO_3^-–N is easily leached with rainfall or irrigation water. Moreover, tea garden soil is mostly sandy in nature, facilitating leaching loss. The leaching losses of N could significantly be reduced if the supplied N is efficiently utilized by plants [21,22]. Therefore, different measures, including the use of nitrification inhibitors (NIs), are suggested as potential ways to increase N efficiency while maintaining soil pH in the tea garden.

In recent years, NIs have received extensive attention as a potential way to improve N fertilizer use [23–25]. The combination of NIs with synthetic N fertilizers could suppress microbial nitrification by inhibiting *Nitrosomonas* spp. mediate oxidation of NH_4^+–N, and, thus, slow down the conversion of ammonium to nitrate [26,27]. Moreover, NIs have been shown to increase N retention and uptake since NH_4^+–N is less prone to leach than NO_3^-–N [27]. However, it is still unclear how the combined application of NIs and synthetic N at an increasing rate affect soil acidification and leaching loss of inorganic N from tea cultivated soils with light in texture. Here, we examined the effects of DMPP (3, 4–dimethylpyrazole phosphate, DMPP) on soil acidification risk and available N losses from tea cultivated soil along a urea–N gradient. We hypothesized the following: (a) the urea–N application at an increasing rate may decrease soil pH and increase leaching loss of inorganic N from soils; (b) the combined application of DMPP and urea–N at an increasing rate may increase soil pH by reducing nitrification; (c) the combined application of DMPP and urea–N at an increasing rate may increase soil inorganic N retention, since more stable NH_4^+–N and less mobile NO_3^-–N is produced.

2. Materials and Methods

2.1. Soil and Sapling Preparation

On 4 November 2017, surface soil (0~30 cm) was collected from the Biological Garden at Xinyang Normal University (32.14° N, 114.04° E). The soil was classified as sandy loam (FAO classification) with an initial pH of 6.11 (0~20 cm depth). Roots were removed from soils by passing through a 2 mm sieve. Three–year–old tea plants of equal canopy sizes (height 30~45 cm, with more than three branches) were collected from a tea garden in the Cheyun Mountain area (32.20° N, 113.79° E). Six tea plants were replanted in pots (24 cm in diameter and 35 cm in height) filled with 5 kg of fresh soil. All pots were kept in a glasshouse with 28~30 °C day temperature and 25~27 °C night temperature. Every two days, 100 mL deionized water was added to maintain soil moisture at 65% of water holding capacity.

2.2. Pot Experiment

After five months of replanting, a pot study was started including two factors: (a) urea–N application rates (0, 300, 500, and 800 kg ha^{-1}) and (b) NI addition (with or without, 1% *w*/*w* of urea–N). Twenty–four pots were chosen from the prepared 30 pots and divided into two sets. One set of pots were only fertilized by urea (0, 1.35, 2.26 and 3.62 g N pot^{-1}), 4 N treatments × 3 replicates = 12 pots; and the other set of pots received combined urea (0, 1.35, 2.26 and 3.62 g N pot^{-1}) and corresponding DMPP addition (1, 13.6, 22.6, 36.2 mg pot^{-1}), 4 N+DMPP treatments × 3 replicates = 12 pots. On 31 March 2018 (day 0), urea with or without DMPP was applied to surface soil of each pot with 500 mL of deionized water. All pots were then equally watered with 200 mL water every two days for the complete ammonification and nitrification of the applied urea. The pots were placed on a tray to collect leachate. On day 7 (7 April), day 35 (5 May), and day 71 (11 June) after treatment intervention, we collected soil samples and added 600 mL deionized water to collect leachate. Since each pot received the same amount of water, the inorganic N concentration in the leachate would represent the N loss. Soil pH was determined in water (1:2.5 w/w). One 2 cm–diameter soil core was collected through the pot on day 7 after fertilization. Next, 10 g of soil samples (equivalent dry weight) were extracted with 50 mL 2 M KCl to determine inorganic N (NH_4^+–N and NO_3^-–N) concentration. The inorganic N concentration of the leachate and soil extracts was determined using a colorimetric method with a continuous flow analyzer (Futura II, Alliance Instruments, Frépillon, France).

2.3. Statistical Analysis

Three–way ANOVA analysis was applied to soil pH, leachate pH, and NH_4^+–N and NO_3^-–N leachate concentrations to test the main and interactive effects of urea–N application, DMPP addition, and sample collection dates. Two–way ANOVA was then used to determine the main and interactive effects of DMPP and urea–N application on soil and leachate variables at each sampling date. The means were separated with Tukey's Honestly Significant Difference (HSD) test ($p < 0.05$). Data were statistically analyzed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). All figures were prepared with SigmaPlot 14.0 (Systat Software, Inc., San Jose, CA, USA).

3. Results

3.1. Soil and Leachate pH

After seven days of treatment, urea–N application significantly reduced soil pH, but DMPP addition did not change soil pH (Figure 1a). Similar to day 7, urea–N at increasing rates reduced soil pH when compared to the control on days 35 and 71. On average, DMPP increased soil pH by 0.12 and 0.06 units, respectively (Figure 1b,c). Averaged across all sampling dates, urea–N at increasing rates gradually decreased soil pH with a considerable reduction at 500 kg N ha^{-1} and above, while DMPP increased soil pH compared to the control treatment ($p < 0.05$, Table S1). However, there was a significant urea–N×DMPP interaction after the increase of soil pH with DMPP because it received higher N (Figure S1a,b).

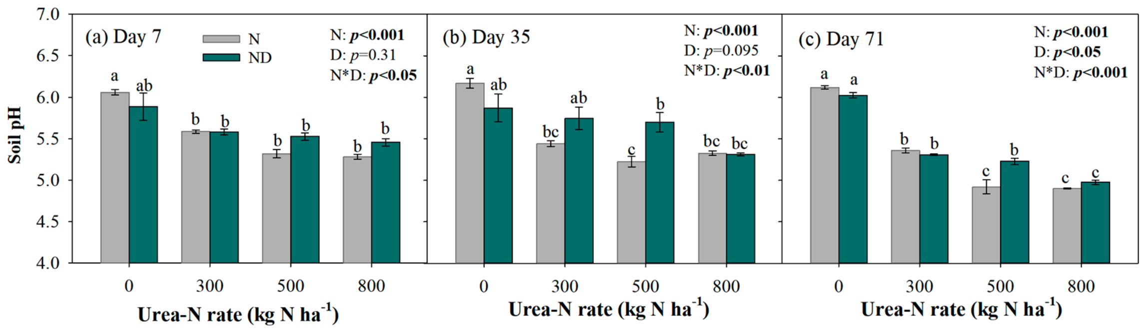

Figure 1. Soil pH on day 7 (**a**), day 35 (**b**), and day 71 (**c**). Grey bars: soil pH with N application; green bars: soil pH with N+DMPP; N: N fertilizer; ND: N+DMPP; D: DMPP; N*D: interaction of N and DMPP. Means with same lowercase letters are not significantly different among treatments by Tukey's HSD at the $p = 0.05$ level. DMPP: 3, 4–dimethylpyrazole phosphate.

The use of DMPP increased leachate pH for all the three sampling dates ($p < 0.001$, Table S1), while the increment was specific to urea–N rates, suggesting a significant N×DMPP interaction (Figure 2a–c). Specifically, there was no difference between control and DMPP treatments on day 7, but DMPP increased leachate pH at a rate of 800 kg urea–N ha^{-1} on days 35 and 71. Both soil pH and leachate pH showed nonlinear decreases with urea–N levels across DMPP treatment (Figure S1a,c). The differences of soil/leachate pH with or without DMPP were both nonlinearly increased when urea–N rates increased (Figure S1b,d). Across all sampling dates, leachate pH showed a nonlinear increase with soil pH in only urea–N received pots (r^2 = 0.59), but no relationship was detected between leachate and soil pH in DMPP added pots (Figure S2).

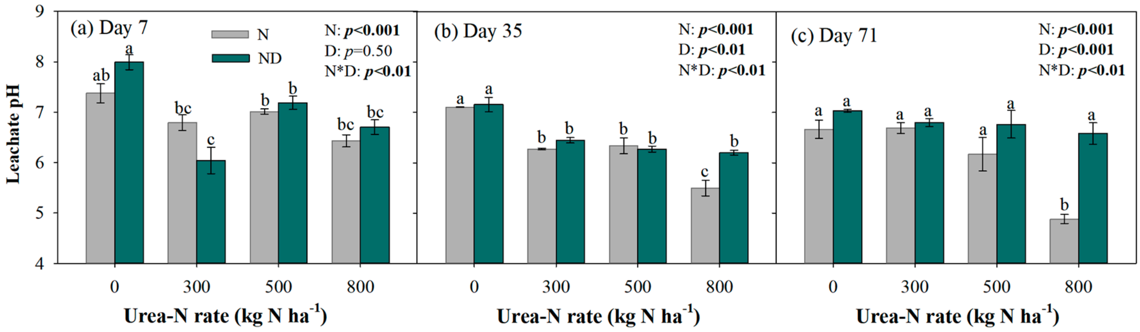

Figure 2. Leachate pH on day 7 (**a**), day 35 (**b**), and day 71 (**c**). Grey bars: leachate pH with N application; green bars: leachate pH with N+DMPP; N: N fertilizer; ND: N+DMPP; D: DMPP; N*D: interaction of N and DMPP. Means with same lowercase letters are not significantly different among treatments by Tukey's HSD at the p = 0.05 level.

3.2. Inorganic Nitrogen Concentration of Soil and Leachate

Soil NH_4^+–N concentration measured on day 7 increased after urea–N application, reaching its maximum at the highest application rate (800 kg N ha^{-1}, Figure 3a). Similar to NH_4^+–N concentration, soil NO_3^-–N concentration was also elevated when urea–N rates increased. Altogether, the inorganic N status of soil was higher with larger N supply. However, DMPP addition did not change the concentration of soil NH_4^+–N and NO_3^-–N (Figure 3b).

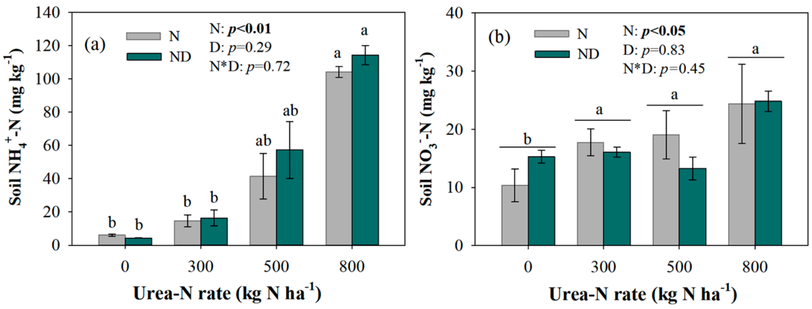

Figure 3. Concentration of soil NH_4^+–N (**a**) and NO_3^-–N (**b**) on day 7. Grey bars: with urea–N application; green bars: with urea–N+DMPP; N: urea–N; ND: urea–N+DMPP; D: DMPP; N*D: interaction of urea–N and DMPP; means with same lowercase letters are not significantly different among treatments by Tukey's HSD at the p = 0.05 level.

Urea–N at increasing rates increased leachate NH_4^+–N and NO_3^-–N concentration (also their combined loss) on all three sampling dates (Figures 4a–c and 5a–c; Table S1). Averaged across all sampling dates, the leachate NH_4^+–N concentration was stimulated by DMPP application (p < 0.001, Table S1). In contrast, DMPP application led to less NO_3^-–N leaching from soils than the control treatment. Both NH_4^+–N and NO_3^-–N leaching losses (and their combined losses) showed nonlinear increases along the urea–N gradient after DMPP addition (Figure S3a,c). The differences of NH_4^+–N leaching loss induced by DMPP were nonlinearly increased along the urea–N gradient, but NO_3^-–N loss showed no relationship with urea–N application rates (Figure S3b,d).

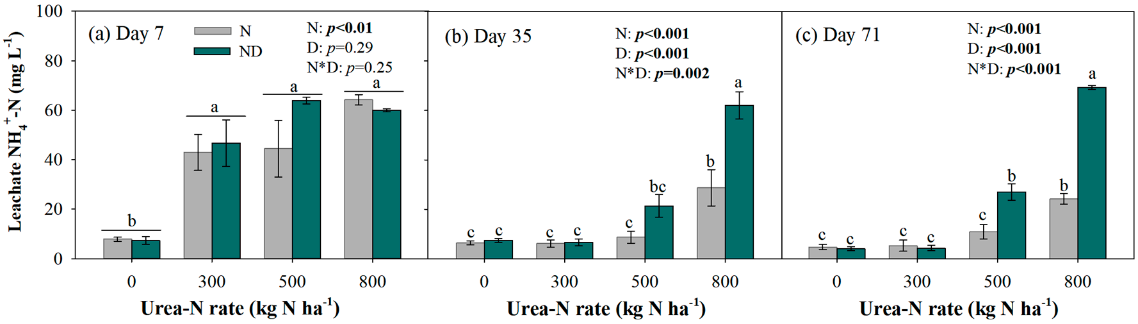

Figure 4. Leachate NH_4^+–N concentration on day 7 (**a**), day 35 (**b**), and day 71 (**c**). Grey bars: leachate NH_4^+–N with urea–N application; green bars: leachate NH_4^+–N with urea–N+DMPP combination; N: urea–N; ND: urea–N+DMPP; D: DMPP; N*D: interaction of urea–N and DMPP; means with same lowercase letters are not significantly different among treatments by Tukey's HSD at the $p = 0.05$ level.

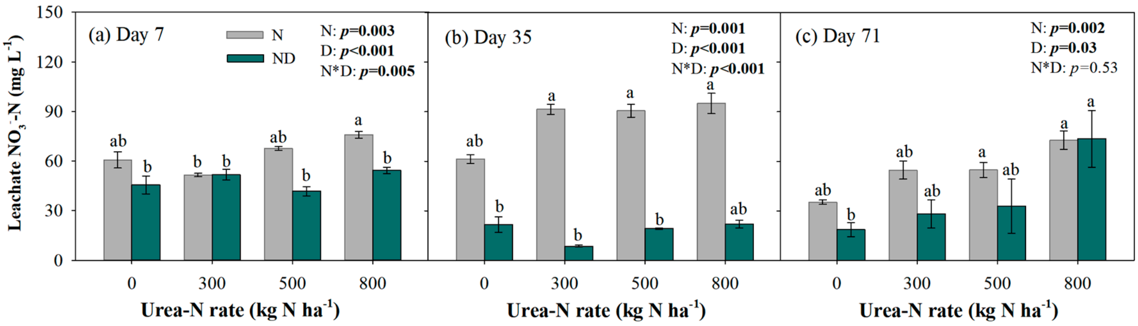

Figure 5. Leachate NO_3^-–N concentration on day 7 (**a**), day 35 (**b**) and day 71 (**c**). Grey bars: leachate NO_3^-–N with urea–N application; green bars: leachate NO_3^-–N with urea–N+DMPP; N: urea–N; ND: urea–N+DMPP; D: DMPP; N*D: interaction of urea–N and DMPP; means with same lowercase letters are not significantly different among treatments by Tukey's HSD at the $p = 0.05$ level.

4. Discussion

4.1. Soil Acidification with Increasing Rates of Urea Application

Synthetic N fertilization could boost soil N supply and the yield of tea shoots [28] while simultaneously causing tea garden soil acidification [29,30]. In our experiment, urea–N application at increasing rates reduced soil and leachate pH with the largest reduction in the highest application rate, demonstrating that high N supply (e.g., 500 kg ha^{-1} and above) causes acidification in tea–cultivated soils. Similar to previous reports, this acidification was caused by protons releasing in exchange of NH_4^+ uptake and nitrification of NH_4^+ and its leaching [19,20]. Soil acidification was reported to decrease the growth and N uptake of tea trees (Ruan et al., 2007). With intensified soil acidification, the losses of base cations (e.g., Ca^{2+}, Mg^{2+}, and K^+) and the accumulation of toxic aluminum (Al^{3+}) may reduce the buffering capacity and modify microbial activity and function, leading to imbalanced nutrient supply [15,31]. Acidic leachate resulted from large amounts of urea–N application may simultaneously induce acidification of the adjacent surface and underground water with consequences for environmental security and biodiversity of aquatic organisms.

4.2. Effects of DMPP on Soil Acidification

Ammonium–based N application can accelerate nitrification–induced soil acidification while NI can slow the rate of acidification [15,29,32]. Our results indicated that DMPP elevated soil and leachate pH along the urea–N gradient. The effects of DMPP on soil/leachate pH were higher when DMPP was applied with higher N rates (500 kg ha^{-1} and above). We believe that these increases in soil and leachate pH were due to the inhibition of microbial nitrification and retention of N as NH_4^+. Soil can be acidified when N uptakes as NH_4^+ via plants since proton exchanges for NH_4^+–N occur between soil and roots. However, soil acidification can be twice the NH_4^+–N uptake if NH_4^+–N is converted to NO_3^-–N and leached, since 2 mol protons are released when 1 mol NH_4^+ is oxidized to NO_3^- [33]. We found that DMPP had higher efficacy in maintaining soil pH when N was applied at 500 kg ha^{-1} (close to the average N rate in tea gardens). This was possibly due to less nitrification of NH_4^+–N and uptake of this NO_3^-–N by plants. The current results demonstrated that the simultaneous addition of urea–N and DMPP is a potential way to alleviate environmental acidification risks during the tea cultivated process.

4.3. Inorganic N Leaching Loss with Increasing Rates of Urea–N Application

Nitrogen leaching losses in tea–planted areas is relatively high since tea garden soils (light in texture) receive high frequency and large amounts of rainfall during the growing season. Our results indicated that urea–N application at increasing rates increased soil NH_4^+–N and NO_3^-–N concentrations. These increases of soil inorganic N led to higher N losses across DMPP addition. As expected, higher concentrations of NH_4^+–N and easily mobile NO_3^-–N in soils with high urea–N rates led to more reactive N leaching with 600 mL of water (equivalent to 13 mm rainfall), since these inorganic N were either not fixed or loosely bonded with soil reactive surfaces. These results indicate that N supply beyond the nutrient holding capacity of soils results in N leaching losses in tea garden soils, irrespective of NI addition.

4.4. Effects of DMPP on Inorganic N Leaching Loss

Use of NIs with synthetic N fertilizers can change NH_4^+–N and NO_3^-–N dynamics in soil, including their retention and losses [34]. In our study, we did not find increased NH_4^+–N in soils on day 7, although other studies reported an increment of soil NH_4^+–N concentration with the addition of DMPP [35]. Moreover, the concentration of soil NO_3^-–N was similar between control and DMPP treatments. These results suggest that the inhibition of nitrification after DMPP addition may take a longer time (more than a week) to have a significant reduction. Considering the combined loss of NH_4^+–N and NO_3^-–N, DMPP significantly reduced the soil's total inorganic N loss ($p < 0.001$, Table S1), even though the NH_4^+–N leaching loss was stimulated with DMPP. This occurred due to the less NO_3^-–N production and reduced leaching loss with nitrification inhibition after adding DMPP [35,36]. These results suggest that DMPP could increase soil N retention, improve plant N use efficiency, and potentially stimulate the shoot yield of tea trees.

5. Conclusions

As the most commonly used synthetic fertilizer in artificial ecosystems, urea–N at high rates can intensify soil acidification while causing environmental pollution. By conducting this pot trail, we observed that urea–N application at increasing rates (up to 800 kg N ha^{-1}) reduced soil pH, while DMPP, a nitrification inhibitor, increased soil pH with a higher efficacy combined with an urea–N application rate of 500 kg N ha^{-1} and above. Gradient urea–N applications generally increased inorganic N leaching losses, with the largest lost amid the highest rates, irrespective of DMPP. On average, the use of DMPP decreased leaching losses of nitrate (and total inorganic N), indicating it may be a significant way to abate eutrophication in an adjacent environment. Adding DMPP may potentially elevate the yield of tea trees with concerns about a higher soil N retention with DMPP. Altogether, our results highlight that adding DMPP can alleviate soil acidification and enhance inorganic N retention by reducing nitrate leaching losses with positive impacts for tea garden cultivation systems. Nevertheless, the wide use of DMPP in tea gardens should still be cautionary, as the yield and quality responses of tea shoots still need to be better assessed.

Supplementary Materials: The following are available online at https://www.mdpi.com/2071-1050/13/2/994/s1. Figure S1: Relationships of DMPP with mean soil/leachate pH and with the differences of mean soil/leachate pH; Figure S2: Relationship of soil pH and leachate pH with increasing urea–N rates across DMPP; Figure S3: Relationships of DMPP with leachate NH_4^+–N/

NO_3^-–N and the differences of mean leachate NH_4^+–N/leachate NO_3^-–N between treatment and control; Table S1: Three–way ANOVA analysis of different treatments on soil pH, leachate pH, and leachate N loss.

Author Contributions: Conceptualization, C.Q. and B.X.; software, J.H.; formal analysis, C.Q. and S.M.; investigation, Y.W.; writing—original draft preparation, C.Q. and B.X.; writing—review and editing, C.Q. and S.M.; supervision, B.X.; All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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