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Highlights

- Soil nitrification was significantly impeded in the presence of H₂O₂ at 20-50 μM
- Soil texture and organic matter markedly affected the process
- Addition of magnetite enhances inhibition of soil nitrification.

1 *Short Communication*

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3 **Effects of Micro-molar H₂O₂ on Inhibiting Soil Nitrification**

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17 **ABSTRACT**

18 Microcosm experiments were conducted to examine the effects of micro-molar hydrogen
19 peroxide (H_2O_2) on soil nitrification in three scenarios: (a) without added fresh biomass and
20 magnetite, (b) with added fresh biomass, and (c) with added magnetite. The results show that
21 nitrification of the added ammonium was impeded in the presence of H_2O_2 at a concentration range
22 of 20-50 μM probably as a result of weakened activities of ammonia-oxidizing microbes due to
23 oxidative stress caused by H_2O_2 and possibly hydroxyl radical ($\bullet OH$). Application of grass clippings
24 or magnetite enhanced the effects of H_2O_2 on inhibiting soil nitrification. The research findings
25 obtained from this study have implications for understanding the complication of soil nitrification by
26 rainwater-borne H_2O_2 during flood events and developing cost-effective and environmentally
27 friendly techniques for nitrification inhibition in fertilized soils.

28 **Key words:** Nitrogen, Hydrogen peroxide, Magnetite, Chemical fertilizer, Rainwater

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35 Heavy use of chemical fertilizers is common in intensive agricultural systems. Upon application
36 to soils, the ammonium contained in chemical fertilizers tends to undergo nitrification to form more
37 leachable nitrate and gaseous nitrogen species (Ju et al., 2009; Zhang et al., 2013), which not only
38 reduces fertilizer use efficiency but also bring about a series of environmental problems such as
39 eutrophication, contamination of groundwater etc. (Davidson et al., 2012; Xu et al., 2015). Where
40 surface broadcast is used, large amounts of fertilizer are spread over the soil surface and can only be
41 incorporated into soil layers by downward movement mediated by rainwater or irrigation water.

42 One of the strategies to minimize nitrogen loss after application of chemical fertilizers is the use
43 of nitrification inhibitors (Lam et al., 2017). Currently, most of the nitrification inhibitors are
44 chemical substances that depress the activities of ammonia-oxidizing microbes (Nardi et al., 2013).
45 Nitrification inhibiting chemicals have been detected in open water environments receiving
46 agricultural runoff (Smith et al., 2013), and their effects on aquatic ecosystems and human health are
47 still unclear. To minimize the environmental risk from potentially toxic nitrification inhibitors, the
48 use of more environmentally-friendly nitrification inhibitors is desirable.

49 It is well established that when H_2O_2 reacts with ferrous iron (Fe^{2+}), Fenton reaction takes place
50 to produce hydroxyl radical ($\bullet OH$), which is a powerful oxidant that can effectively inhibit microbial
51 activities (Ma et al., 2013). Tolar et al. (2016) found that H_2O_2 at nanomolar levels could inhibit
52 ammonia oxidation in ocean environments. This mechanism can be utilized to depress the activities
53 of ammonia-oxidizing microbes. The ingredients for triggering Fenton reaction are naturally
54 available under certain circumstances. H_2O_2 is a common constituent in rainwater (Gonçalves et al.,

55 2010). Under water saturated conditions during rainfall events, reductive dissolution of iron
56 compounds in the soils with sufficient amounts of microbially degradable organic matter could take
57 place to produce Fe^{2+} (Abgottspon et al., 2015). It is also possible to introduce Fe^{2+} into soils to
58 enhance Fenton reaction where the supply of Fe^{2+} becomes a limiting factor.

59 Magnetite (Fe_3O_4) is a mineral that contains Fe^{2+} . Unlike ferrous sulfate (FeSO_4) that are subject
60 to rapid oxidation by molecular oxygen, magnetite is relatively inert when exposed to molecular
61 oxygen but highly reactive when in contact with H_2O_2 (Horst et al., 2006). Magnetite is a readily
62 available commodity with no known harmful effects on biota. Therefore, this material can be an ideal
63 candidate of Fe^{2+} source for triggering Fenton reaction in fertilized surface soils.

64 Microcosm experiments were performed to test the above hypothesis. The objectives were to
65 evaluate the possible effects of H_2O_2 at a concentration range frequently encountered in rainwater on
66 impeding nitrification in (a) soils that are subject to reductive iron dissolution under water inundated
67 conditions (with and without the presence of grass clippings), and (b) soils with added magnetite.

68 The soil used for the experiments was a brown calcareous alluvial soil (Avery, 1973) collected
69 from the floodplain of River Irwell in England. The first experiment was to simulate the process
70 when the soil is saturated by rainwater containing H_2O_2 in the presence or absence of plant debris
71 and the second experiment was to simulate the process when the soils with different application rate
72 of magnetite are exposed to rainwater containing H_2O_2 . The major characteristics of the soil,
73 experimental design, analytical methods, QA/QC and statistical methods are provided in the
74 Description of Materials and Methods (including Tables S1 and S2) in Supplementary Material.

75 Briefly, the soil was a sandy loam with a slightly alkaline pH (7.84), an electrical conductivity of
76 0.154 dS/m and an organic matter content of 1.74%. Two experiments were performed. The first
77 experiment was to simulate the process when the soil is saturated or inundated by rainwater
78 containing H₂O₂ in the presence or absence of plant debris. One control and two treatments were set
79 (either without or with added grass clippings). The second experiment was to simulate the process
80 when the soils with different application rate of magnetite are exposed to rainwater containing H₂O₂.
81 Two factors were considered in the experiment (a) dosage level of Fe₃O₄, and (b) dosage level of
82 H₂O₂. Water-extractable and NH₄Cl-extractable cations were measured using an ICP-OES (Varian
83 720-ES). Water-extractable and NH₄Cl-extractable NH₄⁺, NO₂⁻, NO₃⁻, SO₄²⁻ and PO₄³⁻ were
84 determined by ion chromatography (DIONEX ICS-1000). All experiments were performed in
85 triplicate.

86 For the first experiment, the pH and dissolved oxygen (DO) in the overlying water layer ranged
87 from 7.30 to 7.72 and from 6.70 to 7.77 mg/L, respectively. Fe and Mn had the range of 0.09-0.19
88 mg/L and 0.03-0.08 mg/L, respectively. The DO in the treatments with added grass clippings was
89 lower, as compared to their counterparts (see [Table S3 in Supplementary Material](#)).

90 For the system without added grass clippings, there was no significant ($p < 0.05$) difference in
91 NH₄⁺-N between the control and any of the treatments though NH₄⁺-N tended to be lower in the
92 control, as compared to the treatments. This corresponds with the trend that NO₃⁻-N tended to be
93 higher in the control than in the treatments. At the 10th min, there was a significant ($p < 0.05$)
94 difference between C and T2 but no significant ($p > 0.05$) difference between C and T1; at the 1st h,

95 there was a significant difference between the control and either T1 or T2; at the 24th h, NO₃⁻-N was
96 in the following decreasing order (significant at $p<0.05$): C >T1 >T2 (Table S4). In the system with
97 added grass clippings, there was a clear trend (significant at $p<0.05$) that NH₄⁺-N decreased in the
98 following order: TG2 >TG >CG. Except for CG vs TG1, there was a significant ($p<0.05$) difference
99 in NO₃⁻-N between the control and any of the treatments on any sampling occasions (Table S4).

100 The KCl-extractable nitrogen fraction is used as the estimated amount of retained nitrogen
101 species. Fig. 1 shows that the retained NH₄⁺-N tended to increase significantly ($p<0.05$) with
102 increasing dose of H₂O₂ except for between C and T1. There was a consistent trend that the retained
103 NO₃⁻-N significantly decreased with increasing H₂O₂ dose for both the soils without and with added
104 grass clippings.

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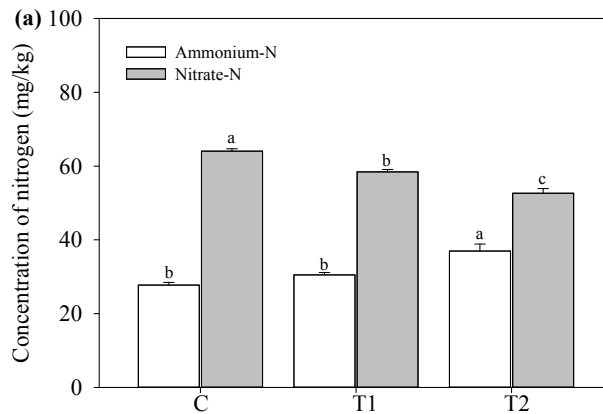
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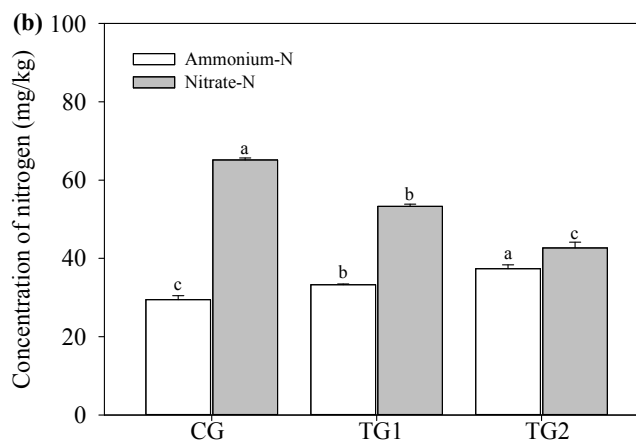
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114 **Fig. 1 Comparison of the retained nitrogen among the control and the treatments for (a) soil**
115 **without added grass clippings, and (b) soil with added grass clippings. Different letters above**
116 **the bars for the same parameter indicate significant difference ($p < 0.05$) between the**
117 **treatments.**

118

119 The consistently lower level of ammonium in the control than in the treatments indicates that
120 ammonia oxidation was impeded in the presence of H_2O_2 at a concentration range of 20-50 μM . This
121 can be attributed to the weakened activities of ammonia-oxidizing microbes due to oxidative stress
122 caused by H_2O_2 and possibly $\bullet OH$ because soluble Fe was present in the systems. Since the pH of the
123 soil solutions was above 7 at which ferric iron (Fe^{3+}) is not stable (Willey et al., 2015), it is therefore
124 likely that the iron was mainly in Fe^{2+} . Consequently, Fenton reaction could take place (Oturán et al.,
125 2014).

126 In the presence of grass clippings, nitrate in the overlying water layer tended to decrease to a
127 very low level at the 24th h, corresponding to the low DO level. This indicates the occurrence of
128 denitrification under reducing conditions (Tiedje et al., 1988). Despite that nitrate at the 24th h was
129 much lower in the presence of grass clippings than in the absence of grass clippings, the
130 concentration of retained nitrate in the controls was higher in the former than in the latter, suggesting
131 that much of the nitrate was absorbed before the soil conditions became sufficiently anoxic to initiate
132 denitrification. In contrast with the controls, the concentration of retained nitrate in the treatments
133 tended to be lower in the presence of grass clippings than in the absence of grass clippings. In the
134 former situation, the microbially mediated decomposition of organic matter led to enhanced
135 reductive iron dissolution (Mukwaturi and Lin, 2015), which could optimize production of hydroxyl
136 radical and cause more severe damage to nitrifying microbes. This may be responsible for the
137 observed lower level of nitrate in the combined H₂O₂ and grass clippings-affected system, as
138 compared to the H₂O₂ only-affected system.

139 Mass balance analysis indicates that the sum of soluble and retained nitrogen was smaller than
140 the amount of nitrogen added into the system (Fig. S1 in Supplementary Material). This suggests that
141 part of the added nitrogen was removed from the soil-water system after ammonia oxidation took
142 place, possibly through the formation of gaseous nitrogen species. Emission of gaseous nitrogen
143 species from soils is frequently associated with nitrification, denitrification and/or nitrifying
144 denitrification (Dobbie and Smith, 2003; Heil et al., 2016). The relatively higher sum of soluble and
145 retained nitrogen in a no-added grass clippings treatment than its counterpart in the added grass
146 clippings treatment (Fig. S1 in Supplementary Materials) suggests that more nitrogen was lost

147 through gas evolution, possibly due to intensified denitrification in the presence of the grass
148 clippings.

149 In the second experiment, the concentration of water-soluble ammonium tended to be lower in
150 the control than in the H₂O₂ treatments for all the three Fe₃O₄ dosage levels though there was no
151 significant difference ($p>0.05$) between the control and some treatments (Fig. 2). At the highest
152 Fe₃O₄ dose, all H₂O₂ treatments with a dose at 10 μM (H10c) and above (H20c and H50c) had
153 significantly higher soluble ammonium, as compared to the control (Ckc). For the retained
154 ammonium, this trend was even clearer with all the H₂O₂ treatments at the lower Fe₃O₄ dosage levels
155 (H5a, H10a, H20a and H50a) showed significantly ($p<0.05$) higher concentrations than that of the
156 control (Cka).

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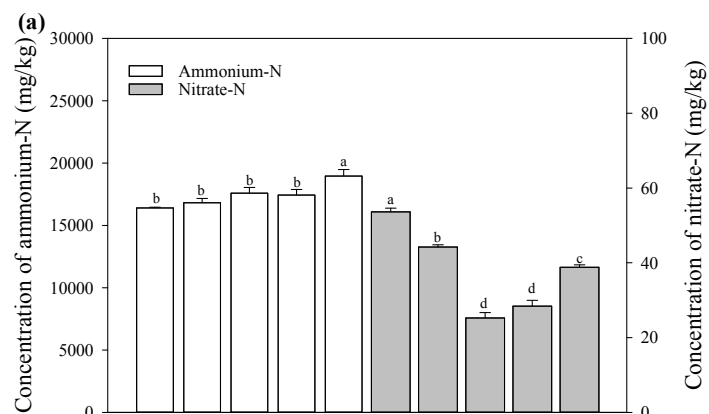
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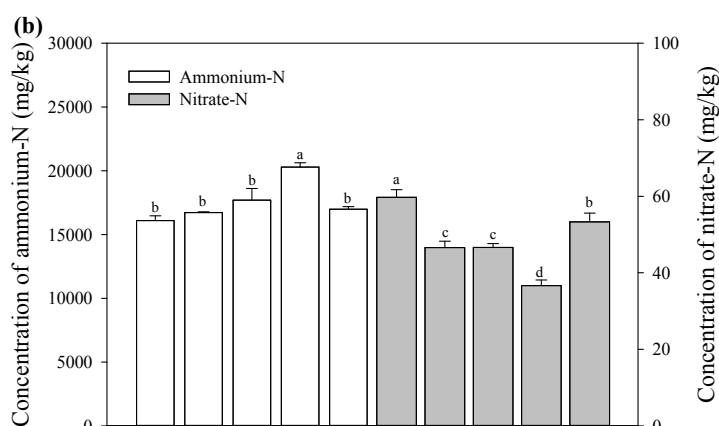
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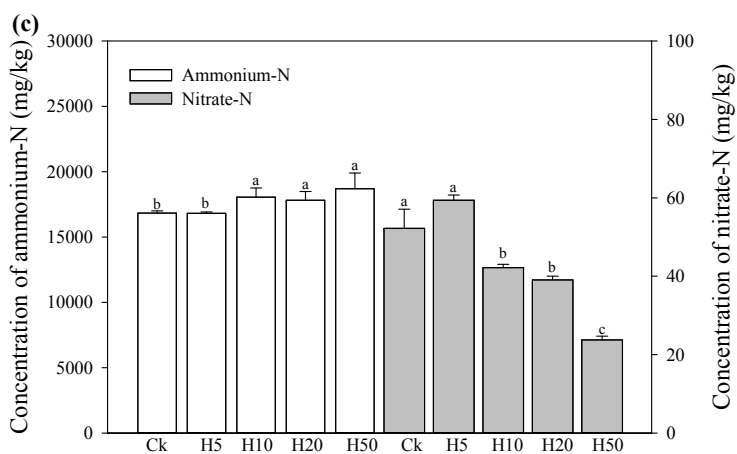
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165 **Fig. 2 Comparison of water-soluble ammonium-N and nitrate-N between the control and the**
166 **treatments in the presence of Fe₃O₄ at an application rate of (a) 2.31 mg/kg, (b) 4.63 mg/kg,**
167 **and (c) 9.26 mg/kg. Different letters above the bars for the same parameter indicate significant**
168 **difference ($p < 0.05$).**

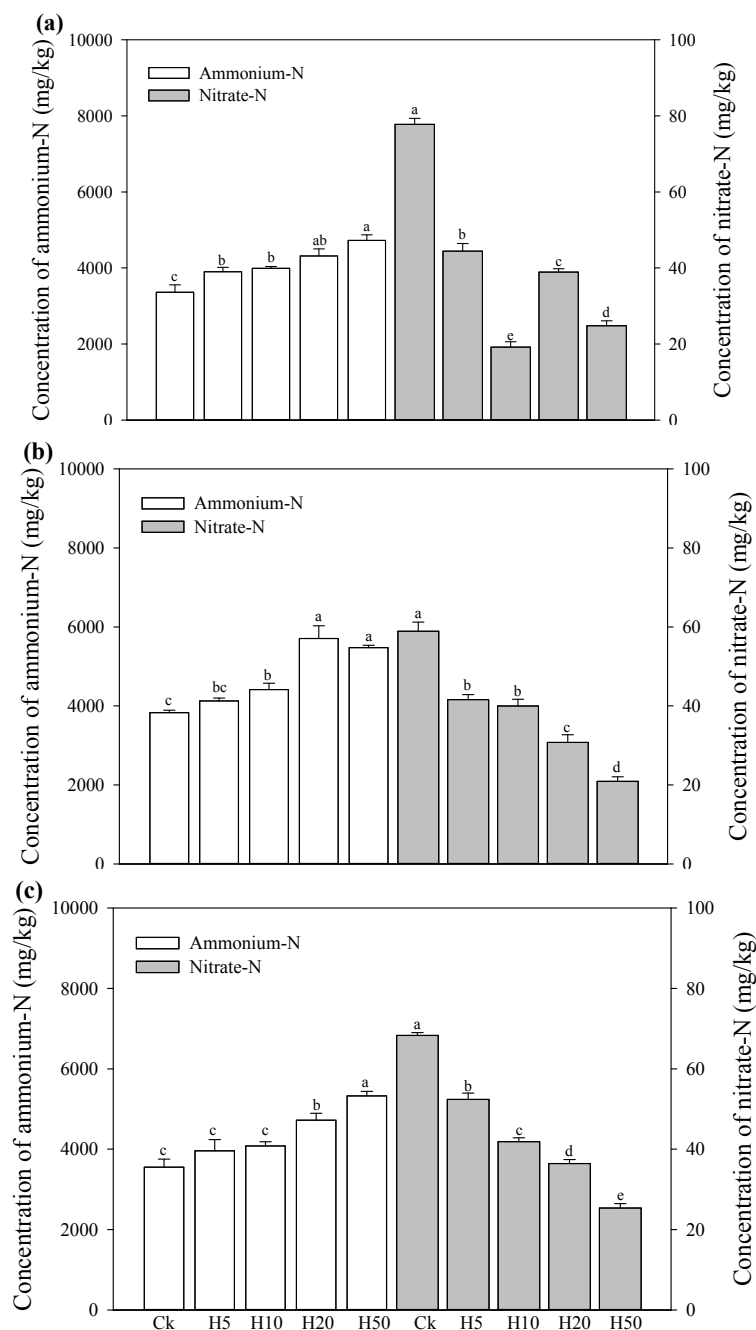
169 In contrast, an opposite trend was observed for both water-soluble and retained nitrate (Fig. 2
170 and Fig. 3). There was a clear trend showing that soluble nitrate ($R^2 = 0.85$) and retained nitrate ($R^2 =$
171 0.79) decreased with increasing dose of H₂O₂ in the presence of the highest Fe₃O₄ amount. This was

172 in contrast with that for the soluble ammonium ($R^2 = 0.79$) and retained ammonium (0.93), which
 173 showed a clear trend to increase with increasing dose of H_2O_2 in the presence of the highest Fe_3O_4
 174 amount (Fig. 2c and Fig. 3c).

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178 **Fig 3 Comparison of exchangeable-soluble ammonium-N and nitrate-N among the control and**
 179 **the treatments for 20 g N/kg soil added 2.31 mg/kg (a), 4.63 mg/kg (b) and 9.26 mg/kg (c)**
 180 **Fe_3O_4 . Different letters above the bars for the same parameter indicate significant difference**
 181 **($p < 0.05$) between the treatments.**

182 The effective inhibition of nitrification in the presence of magnetite suggests that magnetite is
183 an ideal Fe^{2+} source for triggering Fenton reaction when it is in contact with micro-molar H_2O_2 , and
184 the increase in the application rate of Fe_3O_4 tended to enhance the inhibitory effects of soil
185 nitrification. The sum of soluble and retained nitrogen in the soil increased with increasing H_2O_2
186 dose (Table S5). Emission of gaseous nitrogen species, including greenhouse gas N_2O is frequently
187 observed following application of nitrogen-based chemical fertilizers (Sistani et al., 2011; Volpi et
188 al., 2017). The results obtained from this experiment suggest that the application of Fe_3O_4 , in
189 combination with rainwater-borne H_2O_2 could potentially reduce N gaseous losses from fertilized
190 soils.

191 The research findings obtained from this study have implications for understanding the possible
192 complication of soil nitrification by rainwater-borne H_2O_2 during flood events. To provide further
193 evidence to support the hypothesis that rainwater-borne H_2O_2 could impede soil nitrification,
194 mesocosm experiment using actual rainwater as a source of H_2O_2 is currently underway to validate
195 the observed phenomenon under outdoor conditions. The preliminary findings obtained from this
196 work have implications for developing a cost-effective and environmentally friendly technique for
197 controlling environmental issues caused by application of ammonium-containing chemical fertilizers

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201

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259

Supplementary Material

1. Description of Materials and Methods

1.1 The Soil Sample Used in the Experiment

The soil sample used for the incubation experiments was collected from the floodplain of River Irwell in the Greater Manchester, England. The soil had a pH and electrical conductivity of 7.84 and 0.154 dS/m, respectively. It was a sandy loam with 1.74% of organic matter. The water-extractable ammonium-N and nitrate-N was 1.89 mg/kg and 6.60 mg/kg, respectively, and the KCl-extractable ammonium-N and nitrate-N (after water extraction) was 10.1 mg/kg and 36.5 mg/kg, respectively. No nitrite was detected. The water-extractable Na, Mg, Ca, Fe, Mn, SO_4^{2-} and PO_4^{3-} in the soil sample were 24.7 mg/kg, 3.15 mg/kg, 35.3 mg/kg, 0.40 mg/kg, 0.12 mg/kg, 46.5 mg/kg and 6.35 mg/kg, respectively. The KCl-extractable Na, Mg, Ca, Fe, Mn, SO_4^{2-} and PO_4^{3-} were 144.2 mg/kg, 23.8 mg/kg, 1404 mg/kg, 0.45 mg/kg, 0.17 mg/kg, 73.9 mg/kg and 54.2 mg/kg, respectively. Total Na, K, Mg, Ca, Fe, Al and Mn were 1.05 g/kg, 13.2 g/kg, 7.81 g/kg, 17.8 g/kg, 47.1 g/kg, 18.2 g/kg and 0.67 g/kg, respectively. After collection, the soil sample was air-dried, crushed and passed through a 2 mm sieve before being thoroughly homogenized for use in the incubation experiment.

1.2 Experiment 1: Inundated Soil with and without Added Grass Clippings

This experiment was to simulate the process when the soil is saturated or inundated by rainwater containing H_2O_2 in the presence or absence of plant debris. The experiments were performed in

20 triplicate. Plastic cups with a capacity of 125 mL were used as reactors for the batch experiment. One
 21 control and two treatments were set (either without or with added grass clippings): (a) the control: soil
 22 inundated by ultrapure water (18.2 MX cm); (b) Treatment 1 soil inundated by ultrapure water
 23 containing H₂O₂ at a concentration of 20 μM; and (c) Treatment 2: soil inundated by ultrapure water
 24 containing H₂O₂ at a concentration of 50 μM. The use of grass clippings in the first experiment was to
 25 enhance generation of ferrous iron (Fe²⁺) by microbially mediated iron reduction and the reason for
 26 setting 20 and 50 μM of H₂O₂ for the experiment was that rainwater frequently has a concentration of
 27 H₂O₂ ranging from 20 μM to 50 μM. The amount of added NH₄Cl was set at 9 mg to allow the soil to
 28 have a concentration of nitrogen at 50 mg N per kg soil. Details on experimental design are given in
 29 [Table S1](#).

30

31 **Table S1 Details of experimental set-up for Experiment 1**

Code	Soil (g)	Grass clippings (g)	H ₂ O ₂ (μM)	NH ₄ Cl (mg)	Water (mL)
C	50	0	0	9	50
T1	50	0	20	9	50
T2	50	0	50	9	50
CG	50	1.25	0	9	50
TG1	50	1.25	20	9	50
TG2	50	1.25	50	9	50

32

33 In each cup, 50 g of the soil (plus 1.25 g fresh grass clippings for each of CG, TG1 and TG2) was
 34 mixed with 50 mL of the relevant synthetic rainwater. An appropriate amount of ammonium chloride

35 was added to the soil with the synthetic rainwater to allow a theoretical application rate of 50 mg N/kg
36 (mg of ammonium-N per kg of soil) at the onset of the experiment. The cup was then capped with the
37 lid and hand-shaken for 30 seconds. After this, the cup was placed on the bench with the lid being
38 removed. At 10 min, 1 h and 24 h after the commencement of the experiment, pH and dissolved oxygen
39 (DO) in the overlying water layer were measured and 10 mL of the water sample was also collected
40 for analysis. At the 24th h (the end of the incubation experiment), the supernatant was decanted and the
41 soil residue was washed with ultrapure water for three times before being extracted by 1 M KCl at a
42 soil/liquid ratio of 2.5. After shaking for 1 h, an aliquot of KCl extract was taken and stored in a clean
43 container at -25 °C prior to analysis.

44 **1.3 Experiment 2: Soil with Added Magnetite**

45 This experiment was to simulate the process when the soils with different application rate of
46 magnetite are exposed to rainwater containing H₂O₂. The experiments were performed in triplicate.
47 Plastic cups with a capacity of 125 mL were used as reactors for the batch experiment. Two factors
48 were considered in the experiment (a) dosage level of Fe₃O₄, and (b) dosage level of H₂O₂. Details on
49 experimental design are given in [Table S2](#). 0.7634 g of NH₄Cl was added to the reaction system in
50 order to formulate a soil with 2% of ammonium-N, which is expected to encounter in the surface soils
51 with heavy application of chemical fertilizer. The incubation experiment lasted for 24 h after the
52 addition of synthetic H₂O₂-containing rainwater. Water-soluble nitrogen species were then extracted,
53 followed by extraction of retained nitrogen species by 1 M KCl. An aliquot of water and KCl extracts
54 was stored in a clean container at -25 °C prior to analysis.

55 1.4 Analytical Methods

56 For soil characterization, soil particle size analysis was conducted using a HORIBA Laser Particle
57 Size Analyzer (LA-952V2), coupled with sieving for the separation of coarse fractions. The total
58 concentrations of Na, K, Ca, Mg, Fe and Mn were determined by X-ray fluorescence (XRF). Water-
59 extractable and NH₄Cl-extractable Na, K, Ca, Mg, Fe and Mn were measured using an ICP-OES
60 (Varian 720-ES). Water-extractable and NH₄Cl-extractable NH₄⁺, NO₂⁻, NO₃⁻, SO₄²⁻ and PO₄³⁻ were
61 determined by ion chromatography (DIONEX ICS-1000). Electrical conductivity (EC) and pH were
62 measured in 1:5 (soil: water) extract using a calibrated EC meter (Mettler Toledo) and pH meter
63 (JENWAY-3510), respectively. Organic matter content was determined by a loss-on-ignition method
64 (Walkley-Black method).

65 In situ pH and DO measurements for the overlying water layers were made using a calibrated pH
66 meter (JENWAY-3510) and DO meter (Handy Polaris), respectively. The concentration of Fe, Na, K,
67 Ca and Mg in the overlying water layers were determined by an ICP-OES. The concentrations of NH₄⁺,
68 NO₂⁻, NO₃⁻, SO₄²⁻ and PO₄³⁻ in various aqueous samples were determined by ion chromatography
69 (DIONEX ICS-1000). For the determination of NH₄⁺, IonPac® CS12A analytical column (4 mm×250
70 mm), IonPac® CG12A guard column (4 mm×50 mm), and RFIC cation *self-regenerating suppressor*
71 *300* (4 mm) were used. 20 mM methanesulphonic acid was used as mobile phase. Flow rate was set at
72 1.0 mL/min with 20 µL injection volume. For the measurements of NO₂⁻ and NO₃⁻, IonPac® AS14
73 anion analytical column (4 mm×250 mm), IonPac® AG14 guard column (4 mm×50 mm), and ULTRA
74 II *anion self-regenerating suppressor* (4 mm) were used. A mixed 0.8 mM sodium carbonate (Na₂CO₃)

75 and 1 mM sodium bicarbonate solution was used as mobile phase. Flow rate was set at 1.0 mL/min
76 with 20 µL injection volume.

77 1.5 Quality Control/Quality Assurance and Statistical Methods

78 Repeatability analysis shows that the mean RSD for pH, DO, ammonium and nitrate was <0.82 %,
79 <3.03 %, <8.97 %, and <7.78 %, respectively. Statistical difference analysis was performed using one-
80 way analysis of variance (ANOVA) with the Duncan's multiple range test being used for mean
81 separation. The linear regression analysis for the concentration of ammonium/nitrate vs the dosage
82 level of H₂O₂ was performed in excel (Microsoft Excel 2016).

83 2. Supplementary Tables

84 Table S1 Details of experimental set-up for Experiment 1

Code	Soil (g)	Grass clippings (g)	H ₂ O ₂ (µM)	NH ₄ Cl (mg)	Water (mL)
C	50	0	0	9	50
T1	50	0	20	9	50
T2	50	0	50	9	50
CG	50	1.25	0	9	50
TG1	50	1.25	20	9	50
TG2	50	1.25	50	9	50

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89 **Table S2 Details of experimental set-up for Experiment 2**

Code	Soil (g)	Fe ₃ O ₄ (mg)	H ₂ O ₂ (μM)	NH ₄ Cl (g)	Water (mL)
Ca	10	0.02315	0	0.7634	10
H5a	10	0.02315	5	0.7634	10
H10a	10	0.02315	10	0.7634	10
H20a	10	0.02315	20	0.7634	10
H50a	10	0.02315	50	0.7634	10
Cb	10	0.0463	0	0.7634	10
H5b	10	0.0463	5	0.7634	10
H10b	10	0.0463	10	0.7634	10
H20b	10	0.0463	20	0.7634	10
H50b	10	0.0463	50	0.7634	10
Cc	10	0.0926	0	0.7634	10
H5c	10	0.0926	5	0.7634	10
H10c	10	0.0926	10	0.7634	10
H20c	10	0.0926	20	0.7634	10
H50c	10	0.0926	50	0.7634	10

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91 **Table S3 pH, dissolved oxygen, Fe and Mn in the water layer overlying the soils during the period**
92 **of the incubation experiment**

	Time		pH	DO (mg/L)	Fe (mg/L)	Mn (mg/L)
Soil without added grass clippings	10 th min	C	7.30±0.01b	7.07±0.07b	0.19±0.02a	0.08±0.01a
		T1	7.37±0.03ab	6.70±0.10c	0.17±0.01a	0.05±0.00a
		T2	7.45±0.02a	7.60±0.06a	0.16±0.01a	0.05±0.00a
	1 st h	C	7.48±0.01a	6.87±0.12c	0.11±0.00a	0.03±0.01a
		T1	7.45±0.01a	7.20±0.06b	0.11±0.00a	0.03±0.00a
		T2	7.41±0.01b	7.77±0.03a	0.11±0.01a	0.03±0.01a
	24 th h	C	7.67±0.03a	7.60±0.10a	0.11±0.01a	0.03±0.00a
		T1	7.72±0.03a	7.43±0.09a	0.10±0.01a	0.03±0.00a
		T2	7.70±0.02a	7.30±0.06a	0.09±0.01a	0.03±0.00a
Soil with added grass clippings	10 th min	CG	7.21±0.02a	4.63±0.09a	0.19±0.01a	0.09±0.00a
		TG1	7.33±0.10a	3.87±0.19b	0.19±0.01a	0.08±0.01a
		TG2	7.32±0.04a	3.70±0.06b	0.17±0.01a	0.08±0.00a
	1 st h	CG	6.75±0.06a	3.10±0.21a	0.15±0.02a	0.16±0.02a
		TG1	6.77±0.04a	2.90±0.21a	0.13±0.01a	0.17±0.00a
		TG2	6.69±0.11a	2.27±0.09b	0.13±0.01a	0.16±0.01a
	24 th h	CG	7.03±0.03a	3.47±0.23a	0.10±0.01a	0.19±0.02a
		TG1	7.06±0.03a	2.73±0.07b	0.10±0.02a	0.20±0.01a
		TG2	7.02±0.02a	2.30±0.06b	0.10±0.01a	0.19±0.03a

93 Means of each water chemical parameter with different letters for the control and the treatments at the same sampling
94 occasion are significantly different at $p<0.05$.

95 **Table S4 Various chemical parameters in the water layer overlying the soils during the period**
 96 **of the incubation experiment**

	Time		NH ₄ ⁺ -N (mg/L)	NO ₃ ⁻ -N (mg/L)	NO ₂ ⁻ -N (mg/L)
Soil without added grass clippings	10 th min	C	8.76±0.25a	0.54±0.03a	nd
		T1	9.45±0.61a	0.43±0.04ab	nd
		T2	9.68±0.12a	0.34±0.03b	nd
	1 st h	C	8.99±0.22a	0.56±0.06a	nd
		T1	9.71±0.41a	0.42±0.02ab	nd
		T2	9.49±0.25a	0.35±0.02b	nd
	24 th h	C	8.71±0.20a	0.97±0.05a	nd
		T1	9.58±0.49a	0.67±0.02b	nd
		T2	9.58±0.10a	0.28±0.04c	nd
Soil with added grass clippings	10 th min	CG	9.21±0.11c	0.29±0.02a	nd
		TG1	10.05±0.34b	0.22±0.03ab	nd
		TG2	10.93±0.08a	0.19±0.02b	nd
	1 st h	CG	8.28±0.23c	0.58±0.04a	nd
		TG1	9.11±0.19b	0.34±0.07b	nd
		TG2	9.83±0.06a	0.42±0.05ab	nd
	24 th h	CG	8.82±0.18b	0.21±0.01a	nd
		TG1	9.36±0.25b	0.14±0.00b	nd
		TG2	10.27±0.25a	0.13±0.01b	nd

97 Means of each water chemical parameter with different letters for the control and the treatments at the same sampling
 98 occasion are significantly different at $p<0.05$.

99

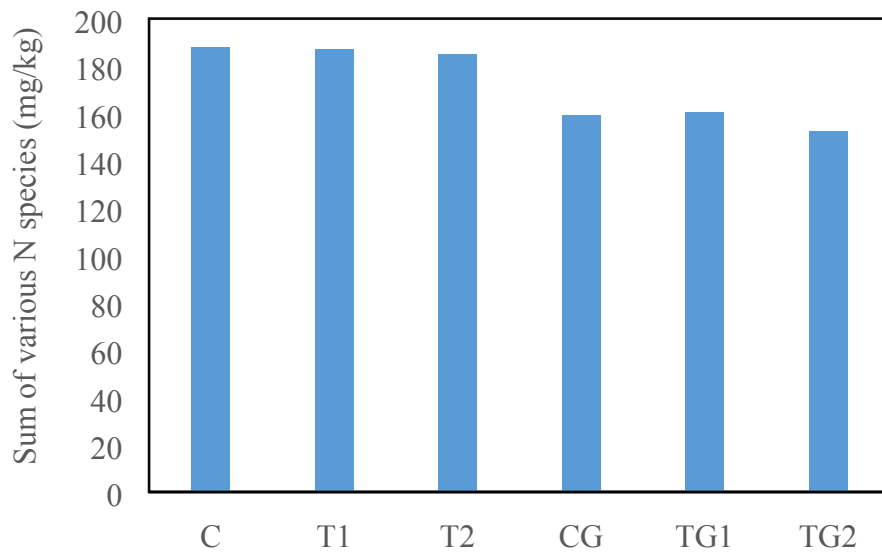
100

101 **Table S5 Sum of water-soluble and exchangeable nitrogen (mg/kg) in the 20 g N/kg soils with**
 102 **added different concentration of Fe₃O₄ and H₂O₂**

Fe ₃ O ₄ (μM)	H ₂ O ₂ (μM)	Water-soluble	Retained	Sum
10	0	16459±56.1b	3438±195c	19897±247c
	5	16865±346b	3943±114b	20809±308bc
	10	17615±457b	4008±50.8b	21623±504b
	20	17470±446b	4352±188ab	21823±628b
	50	18999±524a	4750±149a	23749±658a
20	0	16158±375b	3886±65.5c	20044±327d
	5	16776±61.7b	4165±75.6bc	20942±136cd
	10	17743±918b	4454±164b	22198±776bc
	20	20330±338a	5740±318a	26071±319a
	50	17042±202b	5498±58.6a	22540±150b
40	0	16891±161a	3620±199c	20511±358b
	5	16882±101a	4011±278c	20893±250b
	10	18096±704a	4120±105c	22217±785ab
	20	17859±667a	4760±171b	22620±654ab
	50	18727±1196a	5352±112a	24080±1243a

103 All values are presented as mean ± standard error (n=3). Means with different letters for the control
 104 and the treatments in the same row are significantly different at $p<0.05$.

105 **3. Supplementary Figures**



106

107 **Figure S1 Comparison of the sum of soluble and retained nitrogen species in the soil after the 1-**
108 **day incubation experiment**

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