Highlights

- Soil nitrification was significantly impeded in the presence of H_2O_2 at 20-50 μM
- Soil texture and organic matter markedly affected the process
- Addition of magnetite enhances inhibition of soil nitrification.

Short Communication

Effects of Micro-molar H_2O_2 on Inhibiting Soil Nitrification

Junhao Qin[1,2] and Chuxia Lin[2*]

[1]College of Natural Resources and Environment, South China Agricultural University, Guangzhou, China

[2]School of Environment and Life Science, University of Salford, Greater Manchester M5 4WT United Kingdom

*Corresponding authors: C.Lin@salford.ac.uk

ABSTRACT

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

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

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

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

It is well established that when H_2O_2 reacts with ferrous iron (Fe^{2+}), Fenton reaction takes place to produce hydroxyl radical ($^{\bullet}OH$), which is a powerful oxidant that can effectively inhibit microbial activities (Ma et al., 2013). Tolar et al. (2016) found that H_2O_2 at nanomolar levels could inhibit ammonia oxidation in ocean environments. This mechanism can be utilized to depress the activities of ammonia-oxidizing microbes. The ingredients for triggering Fenton reaction are naturally available under certain circumstances. H_2O_2 is a common constituent in rainwater (Gonçalves et al., 2010). Under water saturated conditions during rainfall events, reductive dissolution of iron compounds in the soils with sufficient amounts of microbially degradable organic matter could take place to produce Fe^{2+} (Abgottspon et al., 2015). It is also possible to introduce Fe^{2+} into soils to enhance Fenton reaction where the supply of Fe^{2+} becomes a limiting factor.

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

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

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

Briefly, the soil was a sandy loam with a slightly alkaline pH (7.84), an electrical conductivity of 0.154 dS/m and an organic matter content of 1.74%. Two experiments were performed. The first 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. One control and two treatments were set (either without or with added grass clippings). The second experiment was to simulate the process when the soils with different application rate of magnetite are exposed to rainwater containing H_2O_2. Two factors were considered in the experiment (a) dosage level of Fe_3O_4, and (b) dosage level of H_2O_2. Water-extractable and NH_4Cl-extractable cations were measured using an ICP-OES (Varian 720-ES). Water-extractable and NH_4Cl-extractable NH_4^+, NO_2^-, NO_3^-, SO_4^{2-} and PO_4^{3-} were determined by ion chromatography (DIONEX ICS-1000). All experiments were performed in triplicate.

For the first experiment, the pH and dissolved oxygen (DO) in the overlying water layer ranged 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 mg/L and 0.03-0.08 mg/L, respectively. The DO in the treatments with added grass clippings was lower, as compared to their counterparts (see Table S3 in Supplementary Material).

For the system without added grass clippings, there was no significant ($p<0.05$) difference in NH_4^+-N between the control and any of the treatments though NH_4^+-N tended to be lower in the control, as compared to the treatments. This corresponds with the trend that NO_3^--N tended to be higher in the control than in the treatments. At the 10th min, there was a significant ($p<0.05$) difference between C and T2 but no significant ($p>0.05$) difference between C and T1; at the 1st h, there was a significant difference between the control and either T1 or T2; at the 24th h, NO_3^--N was in the following decreasing order (significant at p<0.05): C >T1 >T2 (Table S4). In the system with added grass clippings, there was a clear trend (significant at p<0.05) that NH_4^+-N decreased in the following order: TG2 >TG >CG. Except for CG vs TG1, there was a significant (p<0.05) difference in NO_3^--N between the control and any of the treatments on any sampling occasions (Table S4).

The KCl-extractable nitrogen fraction is used as the estimated amount of retained nitrogen species. Fig. 1 shows that the retained NH_4^+-N tended to increase significantly (p<0.05) with increasing dose of H_2O_2 except for between C and T1. There was a consistent trend that the retained NO_3^--N significantly decreased with increasing H_2O_2 dose for both the soils without and with added grass clippings.

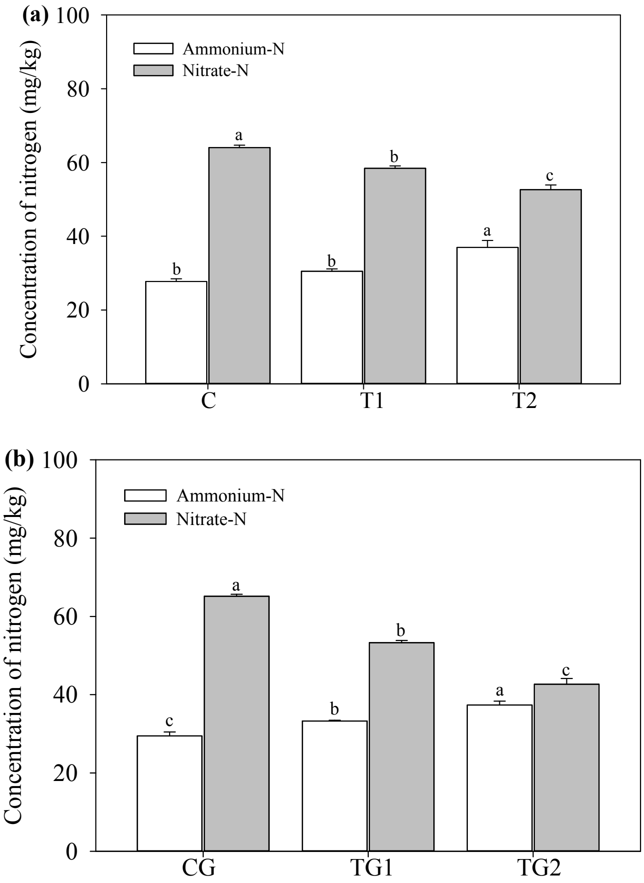

Fig. 1 Comparison of the retained nitrogen among the control and the treatments for (a) soil without added grass clippings, and (b) soil with added grass clippings. Different letters above the bars for the same parameter indicate significant difference (p<0.05) between the treatments.

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

In the presence of grass clippings, nitrate in the overlying water layer tended to decrease to a very low level at the 24th h, corresponding to the low DO level. This indicates the occurrence of denitrification under reducing conditions (Tiedje et al., 1988). Despite that nitrate at the 24th h was much lower in the presence of grass clippings than in the absence of grass clippings, the concentration of retained nitrate in the controls was higher in the former than in the latter, suggesting that much of the nitrate was absorbed before the soil conditions became sufficiently anoxic to initiate denitrification. In contrast with the controls, the concentration of retained nitrate in the treatments tended to be lower in the presence of grass clippings than in the absence of grass clippings. In the former situation, the microbially mediated decomposition of organic matter led to enhanced reductive iron dissolution (Mukwaturi and Lin, 2015), which could optimize production of hydroxyl radical and cause more severe damage to nitrifying microbes. This may be responsible for the observed lower level of nitrate in the combined H_2O_2 and grass clippings-affected system, as compared to the H_2O_2 only-affected system.

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

In the second experiment, the concentration of water-soluble ammonium tended to be lower in the control than in the H_2O_2 treatments for all the three Fe_3O_4 dosage levels though there was no significant difference ($p>0.05$) between the control and some treatments (Fig. 2). At the highest Fe_3O_4 dose, all H_2O_2 treatments with a dose at 10 μM (H10c) and above (H20c and H50c) had significantly higher soluble ammonium, as compared to the control (Ckc). For the retained ammonium, this trend was even clearer with all the H_2O_2 treatments at the lower Fe_3O_4 dosage levels (H5a, H10a, H20a and H50a) showed significantly ($p<0.05$) higher concentrations than that of the control (Cka).

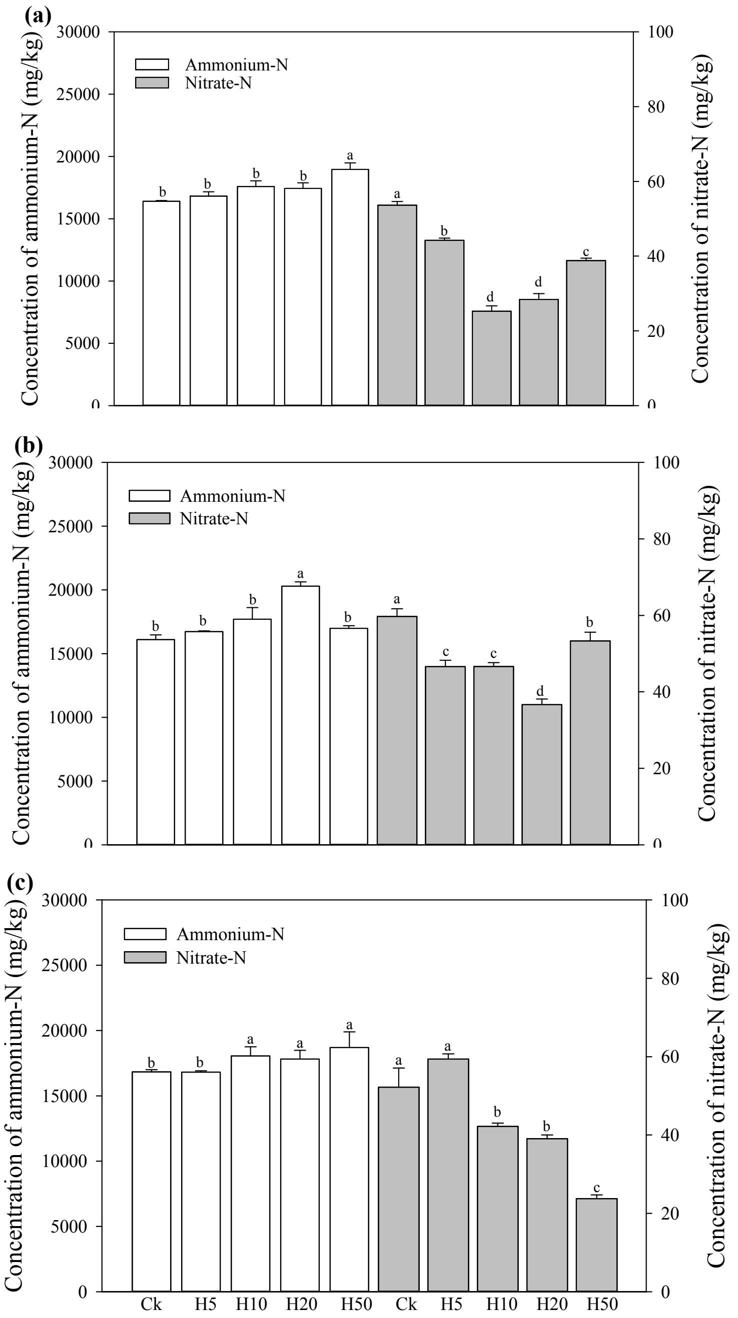

Fig. 2 Comparison of water-soluble ammonium-N and nitrate-N between the control and the treatments in the presence of Fe_3O_4 at an application rate of (a) 2.31 mg/kg, (b) 4.63 mg/kg, and (c) 9.26 mg/kg. Different letters above the bars for the same parameter indicate significant difference (p<0.05).

In contrast, an opposite trend was observed for both water-soluble and retained nitrate (Fig. 2 and Fig. 3). There was a clear trend showing that soluble nitrate ($R^2 = 0.85$) and retained nitrate ($R^2 = 0.79$) decreased with increasing dose of H_2O_2 in the presence of the highest Fe_3O_4 amount. This was in contrast with that for the soluble ammonium (R^2 = 0.79) and retained ammonium (0.93), which showed a clear trend to increase with increasing dose of H_2O_2 in the presence of the highest Fe_3O_4 amount (Fig. 2c and Fig. 3c).

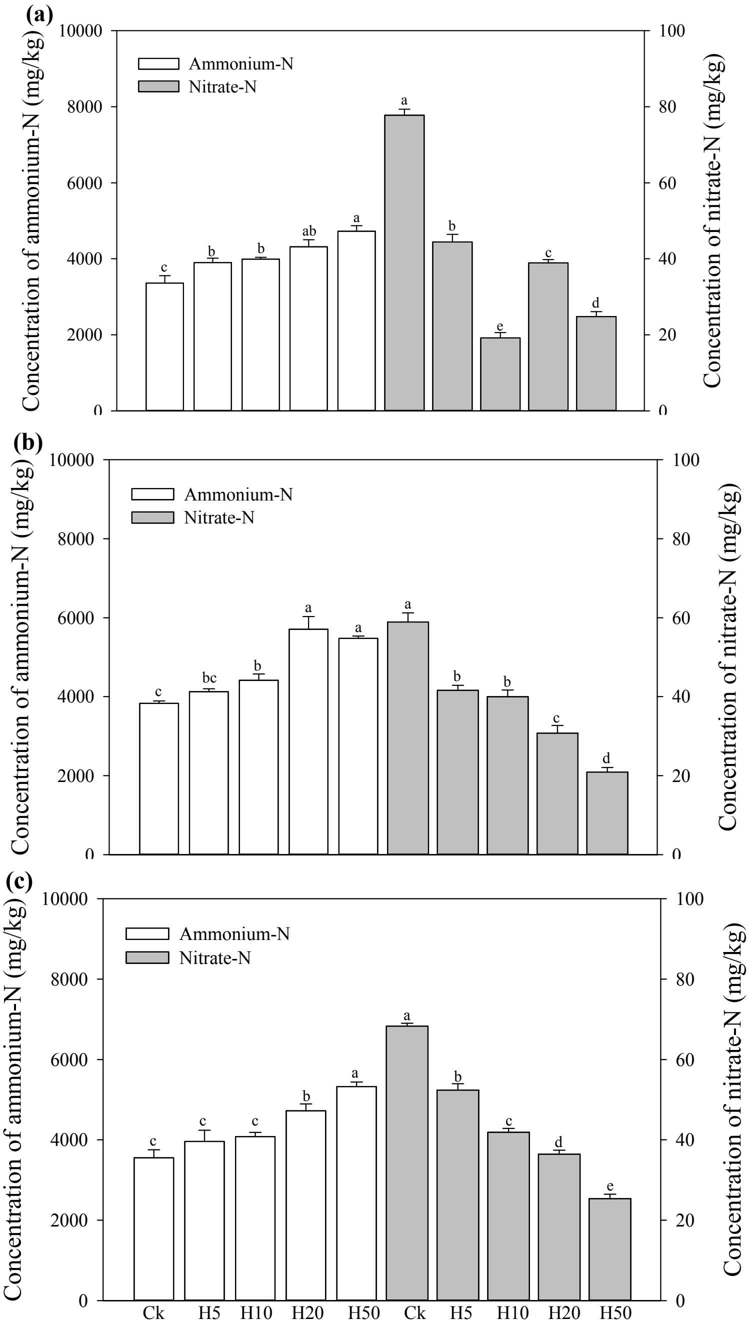

Fig 3 Comparison of exchangeable-soluble ammonium-N and nitrate-N among the control and the treatments for 20 g N/kg soil added 2.31 mg/kg (a), 4.63 mg/kg (b) and 9.26 mg/kg (c) Fe_3O_4. Different letters above the bars for the same parameter indicate significant difference (*p*<0.05) between the treatments.

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

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

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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 triplicate. Plastic cups with a capacity of 125 mL were used as reactors for the batch experiment. One control and two treatments were set (either without or with added grass clippings): (a) the control: soil inundated by ultrapure water (18.2 MX cm); (b) Treatment 1 soil inundated by ultrapure water containing H_2O_2 at a concentration of 20 µM; and (c) Treatment 2: soil inundated by ultrapure water containing H_2O_2 at a concentration of 50 µM. The use of grass clippings in the first experiment was to enhance generation of ferrous iron (Fe^{2+}) by microbially mediated iron reduction and the reason for setting 20 and 50 µM of H_2O_2 for the experiment was that rainwater frequently has a concentration of H_2O_2 ranging from 20 µM to 50 µM. The amount of added NH_4Cl was set at 9 mg to allow the soil to have a concentration of nitrogen at 50 mg N per kg soil. Details on experimental design are given in Table S1.

Table S1 Details of experimental set-up for Experiment 1

Code	Soil (g)	Grass clippings (g)	H_2O_2 (µM)	NH_4Cl (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

In each cup, 50 g of the soil (plus 1.25 g fresh grass clippings for each of CG, TG1 and TG2) was mixed with 50 mL of the relevant synthetic rainwater. An appropriate amount of ammonium chloride was added to the soil with the synthetic rainwater to allow a theoretical application rate of 50 mg N/kg (mg of ammonium-N per kg of soil) at the onset of the experiment. The cup was then capped with the lid and hand-shaken for 30 seconds. After this, the cup was placed on the bench with the lid being removed. At 10 min, 1 h and 24 h after the commencement of the experiment, pH and dissolved oxygen (DO) in the overlying water layer were measured and 10 mL of the water sample was also collected for analysis. At the 24th h (the end of the incubation experiment), the supernatant was decanted and the soil residue was washed with ultrapure water for three times before being extracted by 1 M KCl at a soil/liquid ratio of 2.5. After shaking for 1 h, an aliquot of KCl extract was taken and stored in a clean container at -25 °C prior to analysis.

1.3 Experiment 2: Soil with Added Magnetite

This experiment was to simulate the process when the soils with different application rate of magnetite are exposed to rainwater containing H_2O_2. The experiments were performed in triplicate. Plastic cups with a capacity of 125 mL were used as reactors for the batch experiment. Two factors were considered in the experiment (a) dosage level of Fe_3O_4, and (b) dosage level of H_2O_2. Details on experimental design are given in Table S2. 0.7634 g of NH_4Cl was added to the reaction system in order to formulate a soil with 2% of ammonium-N, which is expected to encounter in the surface soils with heavy application of chemical fertilizer. The incubation experiment lasted for 24 h after the addition of synthetic H_2O_2-containing rainwater. Water-soluble nitrogen species were then extracted, followed by extraction of retained nitrogen species by 1 M KCl. An aliquot of water and KCl extracts was stored in a clean container at -25 °C prior to analysis.

1.4 Analytical Methods

For soil characterization, soil particle size analysis was conducted using a HORIBA Laser Particle Size Analyzer (LA-952V2), coupled with sieving for the separation of coarse fractions. The total concentrations of Na, K, Ca, Mg, Fe and Mn were determined by X-ray fluorescence (XRF). Water-extractable and NH_4Cl-extractable Na, K, Ca, Mg, Fe and Mn were measured using an ICP-OES (Varian 720-ES). Water-extractable and NH_4Cl-extractable NH_4^+, NO_2^-, NO_3^-, SO_4^{2-} and PO_4^{3-} were determined by ion chromatography (DIONEX ICS-1000). Electrical conductivity (EC) and pH were measured in 1:5 (soil: water) extract using a calibrated EC meter (Mettler Toledo) and pH meter (JENWAY-3510), respectively. Organic matter content was determined by a loss-on-ignition method (Walkley-Black method).

In situ pH and DO measurements for the overlying water layers were made using a calibrated pH meter (JENWAY-3510) and DO meter (Handy Polaris), respectively. The concentration of Fe, Na, K, Ca and Mg in the overlying water layers were determined by an ICP-OES. The concentrations of NH_4^+, NO_2^-, NO_3^-, SO_4^{2-} and PO_4^{3-} in various aqueous samples were determined by ion chromatography (DIONEX ICS-1000). For the determination of NH_4^+, IonPac® CS12A analytical column (4 mm×250 mm), IonPac® CG12A guard column (4 mm×50 mm), and RFIC cation *self-regenerating suppressor 300* (4 mm) were used. 20 mM methanesulphonic acid was used as mobile phase. Flow rate was set at 1.0 mL/min with 20 μL injection volume. For the measurements of NO_2^- and NO_3^-, IonPac® AS14 anion analytical column (4 mm×250 mm), IonPac® AG14 guard column (4 mm×50 mm), and ULTRA Ⅱ *anion self-regenerating suppressor* (4 mm) were used. A mixed 0.8 mM sodium carbonate (Na_2CO_3)

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

1.5 Quality Control/Quality Assurance and Statistical Methods

Repeatability analysis shows that the mean RSD for pH, DO, ammonium and nitrate was <0.82 %, <3.03 %, <8.97 %, and <7.78 %, respectively. Statistical difference analysis was performed using one-way analysis of variance (ANOVA) with the Duncan's multiple range test being used for mean separation. The linear regression analysis for the concentration of ammonium/nitrate vs the dosage level of H_2O_2 was performed in excel (Microsoft Excel 2016).

2. Supplementary Tables

Table S1 Details of experimental set-up for Experiment 1

Code	Soil (g)	Grass clippings (g)	H_2O_2 (μM)	NH_4Cl (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

Table S2 Details of experimental set-up for Experiment 2

Code	Soil (g)	Fe_3O_4 (mg)	H_2O_2 (µM)	NH_4Cl (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

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

	Time		pH	DO (mg/L)	Fe (mg/L)	Mn (mg/L)
Soil without added grass clippings	10th 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
	1st 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
	24th 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	10th 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
	1st 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
	24th 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

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

Table S4 Various chemical parameters in the water layer overlying the soils during the period 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	10th 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
	1st 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
	24th 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	10th 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
	1st 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
	24th 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

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

Table S5 Sum of water-soluble and exchangeable nitrogen (mg/kg) in the 20 g N/kg soils with added different concentration of Fe_3O_4 and H_2O_2

Fe_3O_4 (μM)	H_2O_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

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

3. Supplementary Figures

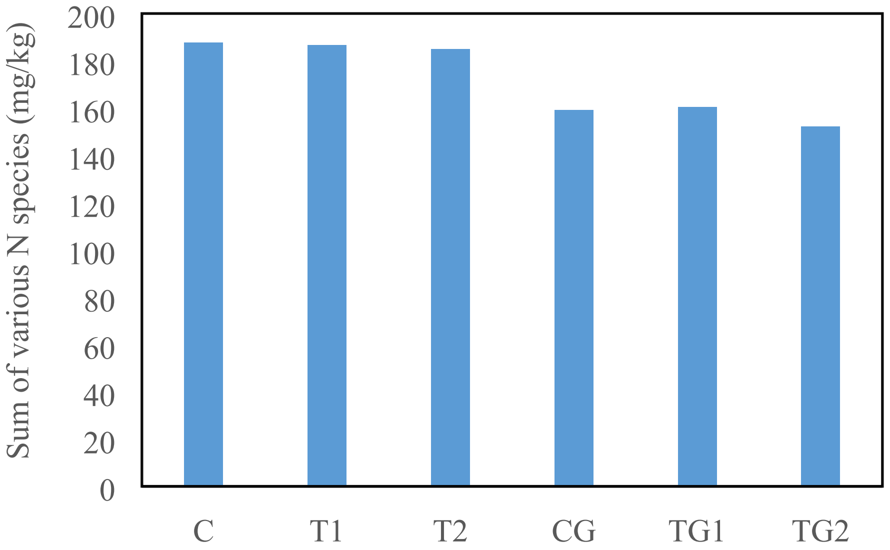

Figure S1 Comparison of the sum of soluble and retained nitrogen species in the soil after the 1-day incubation experiment