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Effects of micro-molar H_2O_2 on inhibiting soil nitrification

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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 ($\cdot$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.

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 and Schallenberg, 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 ($\cdot$OH), which is a powerful oxidant that can effectively inhibit microbial activities (Ma and Lin, 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

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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.

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 $\cdot OH$ 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 and Aaron, 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, 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.

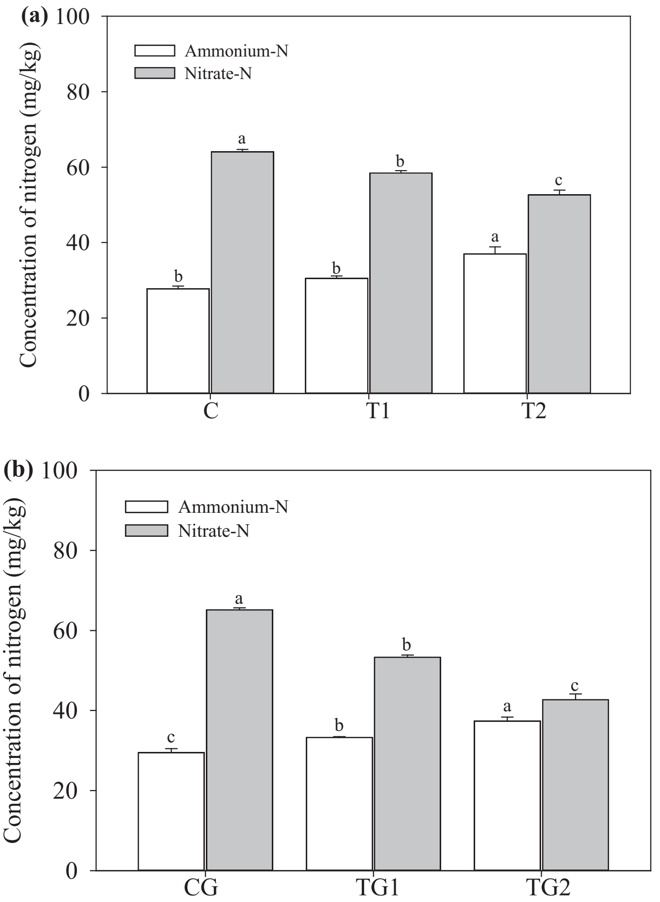

Fig. 1. Comparison of the retained nitrogen among the control and the treatments for (a) soil without added grass clippings, and (b) soil with added grass clippings. Different letters above the bars for the same parameter indicate significant difference ($p < 0.05$) between the treatments.

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