Restoring effect of soil acidity and Cu on N₂O emissions from an acidic soil

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Dear Editor,

Journal of Environmental Management

Thank you for email regarding providing opportunity to revise our manuscript. We have addressed all comments of the reviewer and improved the manuscript accordingly. Response to point by point comments of the reviewers is given in the attached file.

Best regards

Dr. Muhammad Shaaban on the behalf of

Professor Ronggui Hu

Reviewer #2:

Comment: The authors address to my comments is satisfactory.

There are two typos in line 133 (the last line) of the updated manuscript that need to be addressed:

"..in filed enironment, should be:"...in the field environment."

Response: Thank you for kind comment. We have corrected it.

Highlights

- We examined the effect of soil restoration and Cu on N₂O emissions
- Soil pH adjustment highly influenced the bio-available Cu
- N₂O emissions were higher in +Cu soil than –Cu added soil
- Increasing soil pH suppressed N₂O emissions in both +Cu and –Cu soils

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Abstract

Heavy metals are believed to impact soil processes by influencing microbial communities, nutrient cycling or exchanging for essential plant nutrients. Soil pH adjustment highly influences the bio-availability of nutrients and microbial processes. We examined the effect of soil pH manipulation and copper (Cu as $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$) application on nitrogen (N) cycling and nitrous oxide (N_2O) emissions from an acid soil. Increasing amounts of Cu (0, 250, 500 and 1000 mg kg^{-1}) were added to an acidic soil ($\text{pH}=5.44$) that was further amended with increasing amounts of dolomite [$\text{CaMg}(\text{CO}_3)_2$] to increase soil pH. Dolomite increased soil pH values, which reached a maximum without Cu application (-Cu) at day 42 of the experiment. The soil pH values decreased with increasing dose of Cu, and remained low as compared with both control and dolomite amended soil. Ammonium ($\text{NH}_4^+\text{-N}$) concentrations were higher in Cu contaminated soil as compared with the control and dolomite treated soil. Nitrate ($\text{NO}_3^-\text{-N}$) concentrations increased in dolomite treated soil when compared with the +Cu alone treatments and control. Microbial biomass carbon (MBC) and nitrogen (MBN) contents were higher in dolomite treated soil as compared with the +Cu treatments and control. The application of increasing amounts of Cu progressively decreased soil MBC and MBN. Nitrous oxide emissions were higher ($p \leq 0.01$) in +Cu soil as compared with the control, and increased with increasing Cu concentration in soil. Application of dolomite highly suppressed soil N_2O emissions in both +Cu and -Cu soils. The results indicate that the effects of heavy metal contamination (specifically Cu contamination) can increase N_2O emissions, but this can be effectively mitigated through increasing soil pH, also decreasing potential toxic effects on soil microorganisms.

Keywords: soil restoration; low soil pH; greenhouse gas; copper stress; mineral nitrogen

1. Introduction

Atmospheric greenhouse gases concentrations are increasing, which contribute to climate change and negatively affect sustainable development (IPCC, 2014). Nitrous oxide (N₂O) is one of the main greenhouse gases (GHG) with highest global warming potential of 265 over 100 years (IPCC, 2014). Accumulation of N₂O in the atmosphere also favors the destruction of stratospheric ozone (Ravishankara and Portmann, 2009). Soils are the largest source of N₂O emissions to the atmosphere (Li et al., 2016). Various factors regulate N₂O emissions from soils including temperature (Luo et al., 2013), soil moisture (Chen et al., 2017), fertilizer N (Siqueira Neto et al., 2016), organic matter content (Wu et al., 2017) and soil pH (Shaaban et al., 2016).

Adjusting the pH to counteract soil acidity is essential for sustainable crop production (Dai et al., 2017). Changes in soil pH and its adjustment can have profound effects on activities of microbial communities and C and N cycling, with both potentially influencing N₂O emissions through affecting nitrifier's and denitrifier's activities (Shaaban et al., 2015a). The use of N fertilizers not only increases N₂O emissions, they often decrease soil pH affecting both nitrification and denitrification (Guo et al., 2010), which aggregate its emission risk.

Nitrous oxide production is widely considered to be influenced by changing soil pH (Cheng et al., 2013; Dai et al., 2017). N₂O is more likely to be produced as the terminal denitrification product in acidic soils since N₂O-reductase is generally not functional at low pH for the reduction of N₂O to N₂ (Bakken et al., 2012; Shaaban et al., 2018). Acidic soils may therefore produce higher N₂O via denitrification compared with

neutral and alkaline soils. Although earlier studies have revealed that N₂O emissions from agricultural lands are highly sensitive to soil pH (Bakken et al., 2012; Samad et al., 2016), contradictory viewpoints have also been reported for increases (Rn et al., 2011; Qu et al., 2013) or decreases (Shaaban et al., 2018) in soil N₂O emissions in response to pH manipulation. Baggs et al. (2010) revealed that changes in soil pH with application of lime to an acid soil increased mineral nitrogen (NH₄⁺-N and NO₃⁻-N), which subsequently increased N₂O emissions. In contrast, recent studies showed that liming (with dolomitic lime) of acidic soils from Xianning (China) resulted in a significant reduction in soil N₂O emissions (Shaaban et al., 2014; Shaaban et al., 2018; Shaaban et al., 2016). The discrepancy between N₂O emissions from limed acid soils might be due to differences in soil properties, mineral N and C contents and change in microbial communities (Page et al., 2010).

In addition to soil pH, metals can also influence N cycling, thus influencing N₂O emissions (Holtan-Hartwig et al., 2002). The availability of metals in soil is pH dependent. It has been recognized that heavy metals affect the structure and activity of soil microbial communities, soil respiration, nitrification, denitrification and N-mineralization (Giller et al., 1998; Holtan-Hartwig et al., 2002). Mining, smelting activities and agrochemicals are main sources of soil contamination with heavy metals throughout the world (Mao et al., 2015; Morse et al., 2016). Geochemical weathering processes acting upon metallurgical wastes and by-products initiate the process of transporting heavy metals from contaminated areas and redistributing them to surrounding soils, streams, and groundwater. Thus, heavy metals can adversely affect soil and water resources and endanger the health of surrounding ecosystems and human

populations (Morse et al., 2016). Copper (Cu) is an essential element for crops (Rajput et al., 2018) and compulsory component of many microbial enzymes (Hansda et al., 2017), but excess availability of Cu makes it heavy metal which is toxic to microbes and crops (Theriault and Nkongolo, 2016). Generally, the concentration of Cu in unpolluted soils varies from 3 to 100 mg kg⁻¹ (Adriano, 2001). In agricultural soils, normal Cu concentration ranges between 5 and 30 mg kg⁻¹ based on type of soil, but vineyards soils contain 200 to 500 Cu mg kg⁻¹ (Brun et al., 1998). The effects of Cu on N₂O emissions from soils are less reported so far. The present study was conducted with the following aims: (1) to evaluate soil pH manipulation effects on N₂O emissions, (2) to assess Cu stress effects on soil N₂O emissions, and (3) to investigate linkage of soil pH and Cu stress on N₂O emissions. In addition to N₂O emissions, soil microbial biomass and respiration were also measured as a proxy to determine Cu stress effects on soil microbial processes.

2. Materials and methods

2.1. Soil characteristics

Soil was collected from arable land (under rapeseed-rice rotation over 50 years) after rice harvest, located in Xianing, China. The soil is classified as a Ultisol according to USDA (Soil Survey Staff, 2010). Soil samples (0-20 cm) were collected from 7 multiple-points within the selected field and composited into one bulk soil sample. Plant roots and visible organic residues were picked from the soil. Air dried soil was passed through a 2 mm sieve and analyzed as per standard methods (Tan, 1996) for its physical and chemical properties: cation exchange capacity: 13.0 cmol_c kg⁻¹, pH_(H₂O, 1:2.5): 5.44, total nitrogen: 1.33 g kg⁻¹, total organic C: 11.79 g kg⁻¹, bulk density: 1.45 g cm⁻³, sand: 11%, clay: 36%,

silt: 53%, and texture: silt loam.

2.2. Experimental design

Air dried soil was preincubated with moisture of 40% water filled pore space (WFPS, equivalent to the soil sampling field) and $25\pm 2^{\circ}\text{C}$ for 6 days. Following the preincubation, Cu as $\text{CuCl}_2\cdot 2\text{H}_2\text{O}$ was dissolved in distilled water, applied to soil and thoroughly mixed using a spatula. The Cu application rates were 0 (Cu0), 250 (Cu250), 500 (Cu500) and 1000 (Cu1000) mg Cu kg^{-1} soil. These treated soil samples were further amended with dolomite $\text{CaMg}(\text{CO}_3)_2$ (<0.3 mm particle size; Xinjing Lingshou Co., Ltd. Hebei, China) at a rate of 5 g kg^{-1} soil to obtain: Cu0+dolomite, Cu250+dolomite, Cu500+dolomite, and Cu1000+dolomite. Each of the 8 treatments had 3 replicates. Moisture content of treated soils was increased to 60% WFPS (equivalent to soil sampling field), calculated using soil bulk density of sampling field (Wu et al., 2017). Treated soils (100 g) were incubated in 1000 ml glass jars under controlled temperature of $25\pm 0.5^{\circ}\text{C}$ in an automated dark chamber (HP-400S Ruihua, Wuhan, China), for 42 days. A thin plastic sheet containing small pin holes was placed on the jar tops to minimize moisture loss and provide gas exchange. Soil water content was kept constant by weighing jars and replenishing water every 24 h.

2.3. Collection and analysis of headspace gas

Gas sampling from the jars (headspace volume: 1 L, sample volume: 30 ml) was performed at days 1, 2, 3, 4, 5, 6, 7, 9, 11, 13, 15, 21, 24, 27, 30, 34, 38 and 42 from treatment jars using an air-tight syringe (HA23-Nonill, Nonill International Co., Ltd. Fujian, China). Prior to gas sampling, the plastic sheet cover was removed from tops of jars, allowing to equilibrate with ambient air for 20 min. The sampling jars were then

sealed air-tight using lids containing a pipe (3mm diameter) for collection of gas. Two gas headspace samples were taken over a period of 60 min (T_0 and T_{60}). The collected gas from each treatment jar was analyzed for N_2O and CO_2 concentration using a gas chromatograph equipped with electron capture and thermal conductivity detectors (Agilent 7890-A, USA). The N_2O and CO_2 emissions were calculated using following equation as described by Shaaban et al. (2015b).

$$F = p \times V / W \times \Delta c / \Delta t \times 273 / (T + 273)$$

where F is emission rate for N_2O ($\mu g\ kg^{-1}\ h^{-1}$) and CO_2 ($mg\ kg^{-1}\ h^{-1}$), p is density ($kg\ m^{-3}$) of corresponding gas (N_2O or CO_2) at standard conditions, V is glass jar volume (m^3), W is the weight of the dry soil (kg), Δc is the gas production (μg or $mg\ kg^{-1}\ h^{-1}$) during the closure time, Δt is closure time (h) of jars, and T is temperature of experiment ($25\ ^\circ C$).

Cumulative gas fluxes were calculated using the following equation as described by Shaaban et al. (2015b).

$$\text{Cumulative gas flux} = \sum_{i=1}^n (R_i \times 24 \times D_i)$$

where R_i is the gas emission rate ($\mu g\ kg^{-1}\ h^{-1}$ for N_2O and $mg\ kg^{-1}\ h^{-1}$ for CO_2), of the sampling dates, D_i is the number of days in the sampling interval, and n is the number of sampling times.

2.4. Analysis of treated soil samples

A duplicate set of the treatments, each with three replicates, as described for gas collection was also established for soil analysis. Soil (275 g, oven dry basis equivalent) was incubated in a 1-L polyvinyl beaker. The same conditions of moisture and temperature as for gas treatments were adopted for these soils analyzing treatments. Soil

subsamples were collected and analyzed (as described below) at days 1, 7, 14, 21, 28, 35 and 42 of the experiment.

The pH of soil was tested by making a soil slurry (soil:distilled water ratio of 1: 2.5). The slurry was shaken for 1 h under the controlled temperature (25°C) with 300 revolutions per min (rpm) on an electrical orbital shaker (Ts-2000A, Wincom Co., Ltd, Shanghai, China). The mixture was then allowed to wait for 30 min and pH was measured using a pH-meter (PHS-2F, Wincom Co., Ltd, Shanghai, China) (Shaaban et al., 2013). Nitrate and ammonium concentrations in soil were determined using a flow injection system (Auto-analyzer, Seal, Germany) (Wu et al., 2017). Soil was extracted with 1 M potassium chloride (KCl) solution (1: 5, soil: KCl) by shaking for 1 h. After shaking, the slurry was passed through a filter paper Whatman no. 40, and the extract was tested for ammonium and nitrate concentrations. Soil microbial biomass C and N were measured using chloroform fumigation method (Vance et al., 1987). Bio-available Cu contents were determined with EDTA extraction method (Lakanen and Erviö, 1971).

2.5. Data analysis

Data (both gases and soil properties) were analyzed using a two-way analysis of variance (ANOVA) employing “application of Cu and dolomite” as separate factors. Significant differences among treatments were identified by employing Tukey tests. The normality distribution of variables was tested using Kolmogorov–Smirnov test (Razali and Wah, 2011). All the data were analyzed using the IBM SPSS Statistics 23 software package.

3. Results

3.1. Soil pH

The application of Cu and dolomite significantly ($p \leq 0.01$) influenced soil pH values. The

dolomite amendment raised ($p \leq 0.01$) the soil pH from 5.44 to 7.24 without Cu application at day 7, and reached its maximum value of 7.94 at day 42 of experiment (Fig. 1). The soil pH was lower in Cu contaminated soil as compared to the control and dolomite amended soil, and decreased with increasing Cu concentration.

3.2. Concentrations of soil ammonium and nitrate

Ammonium ($\text{NH}_4^+\text{-N}$) concentrations were significantly ($p \leq 0.01$) affected by Cu and dolomite application. Higher concentrations of $\text{NH}_4^+\text{-N}$ were observed in Cu contaminated soil as compared with the control and dolomite treated soil, and the concentrations increased with increasing Cu concentration (Fig. 2). In Cu contaminated soils, the $\text{NH}_4^+\text{-N}$ concentrations continuously increased until day 21, and then decreased until the end of the incubation. Dolomite favored a faster decrease in ammonium concentration as compared with treatments without dolomite.

Nitrate concentration increased significantly ($p \leq 0.01$) following dolomite additions, and this increase was higher than in the control (Fig. 3). Cu addition generally decreased $\text{NO}_3^-\text{-N}$ concentrations and dolomite addition increased $\text{NO}_3^-\text{-N}$ compared to no dolomite regardless of Cu addition rate. The maximum nitrate concentration detected was 96.08 mg kg^{-1} in the dolomite amended soil without Cu contamination.

3.3. Microbial biomass C and N

Dolomite application and Cu significantly ($p \leq 0.01$) influenced microbial biomass C (MBC) and microbial biomass N (MBN). The application of Cu decreased MBC and MBN contents and further decreased with increasing Cu application rate regardless of dolomite treatment (Fig. 4). Microbial biomass C and N were higher in dolomite treated soil as compared with the control. Interestingly, dolomite application began to increase

MBC and MBN contents in Cu contaminated soil after three weeks of its application to soil (Fig. 5).

3.4. Bio-available Cu

Bio-available copper contents were significantly ($p \leq 0.01$) influenced by dolomite application. Bio-available Cu contents were higher in soil without dolomite addition and dolomite addition decreased bio-available Cu contents (Fig. 6). Furthermore, application of dolomite decreased Cu contents over time and the lowest Cu contents were observed in Cu250 treatment at day 42.

3.5. Soil respiration (CO₂ emissions)

Soil CO₂ emissions indicates microbial respiration. Therefore, CO₂ emissions were monitored following Cu and dolomite additions to determine the extent of Cu stress and the effect of increasing pH on the soil microbial activity. Soil respiration (CO₂) was significantly ($p \leq 0.01$) reduced by Cu and increased by dolomite application. Soil CO₂ emissions were significantly ($p \leq 0.01$) lower in Cu treated soil as compared with the control, and decreased with increasing Cu application dose regardless of treatment (Fig. 7). The highest cumulative CO₂ emissions were observed in Cu0 treatment with dolomite application.

3.6. N₂O emissions

The emissions of N₂O were significantly ($p \leq 0.01$) influenced by Cu and dolomite application. N₂O emissions were significantly ($p \leq 0.01$) higher in Cu treated soil and increased with increasing Cu concentration compared with the control. Dolomite application significantly ($p \leq 0.01$) decreased soil N₂O emissions (Fig. 8). The highest N₂O emissions were 0.37 $\mu\text{g N}_2\text{O-N kg}^{-1} \text{ h}^{-1}$ at day 4 in the Cu1000 treatment without

application of dolomite, while the lowest N₂O emission was observed in dolomite application without Cu contamination. After 7 days of incubation, the pH in Cu contaminated soils started to recover slightly. The magnitude of soil N₂O emission was reduced with increasing pH indicating a negative relation.

4. Discussion

The effect of micronutrients, especially those that can be toxic (if in excess) and essential for microorganisms, are less understood in regard to N₂O emissions. Soil pH management can substantially alter soil properties, particularly the availability of nutrients and thus influences N cycling (Goulding and Annis, 1998; Kunhikrishnan et al., 2016). In the present study, dolomite application increased soil pH and reduced N₂O emissions from the acidic soil. The earlier laboratory and field experiments have indicated decreases, increases or no effects of increasing soil pH on N₂O emissions (Baggs et al., 2010; Kunhikrishnan et al., 2016; Qu et al., 2013).

Khan et al. (2011) reported that application of lime and increased soil pH raised cumulative soil N₂O emissions. A laboratory and field study in New Zealand revealed that soil pH manipulation by lime application reduced N₂O emissions (Zaman and Nguyen, 2010; Zaman et al., 2008). Incubation experiments carried out for 80 days found that lime application decreased N₂O emissions with low soil water contents but not with high soil water contents (Clough et al., 2004). Lime application to an acidic soil (pH 4.71) revealed that the total N₂O emissions were 547 g N₂O-N ha⁻¹ in un-limed and 134 g N₂O-N ha⁻¹ in lime amended soil (Mkhabela et al., 2006). A two year study of soil pH manipulation with lime addition displayed that average daily emission was 0.96 mg N m⁻¹ d⁻¹ in un-limed and 0.88 mg N m⁻¹ d⁻¹ in lime treated soils (Galbally et al., 2010). A study

conducted by Shaaban et al. (2014) reported that addition of dolomitic lime to two acid paddy soils markedly reduced N₂O emission because of pH manipulation. They suggested that elevated pH increased N₂O-reductase activity and thus reduced N₂O emissions. Later on, they analyzed *nosZ* gene in acidic soils following dolomite application and reported that increment in soil pH highly reduced soil N₂O emissions by increasing *nosZ* gene abundance (Shaaban et al., 2018). The decreased soil N₂O emissions following liming shows that soil pH plays an important role in controlling N₂O emissions (Page et al., 2010).

The mechanism involved in reduced N₂O emission in soil where pH is increased are likely directly affecting soil microorganisms that reduce N₂O to N₂. Application of dolomite supplies Mg²⁺, Ca²⁺ and HCO₃⁻ in soil solution, which subsequently decrease sources of soil acidity. The reduction of N₂O to N₂ at high pH levels has been previously observed (Bakken et al., 2012). N₂O-reductase is a key enzyme for the reduction of nitrous oxide and very sensitive to acidic soil pH suggesting increased N₂O production in acidic soils (Samad et al., 2016). Altering microbial community or its functional traits as a result of short-term changes in soil pH has been observed (Lalande et al., 2009; Peng et al., 2016). We conjecture that, in the present study, dolomite addition changed the microbial population or associated function to create suitable conditions for N₂O reduction.

The dynamics of mineral N also highly influenced N₂O emissions in the present study. The NH₄⁺-N concentrations increased with dolomite application at the beginning of the incubation. The early accumulation of NH₄⁺-N points out that dolomite addition likely accelerated mineralization more than nitrification. At later stages, decreased NH₄⁺-

N concentrations indicated that the increase in soil pH increased nitrification. The oxidation of $\text{NH}_4^+\text{-N}$ and subsequent increase of $\text{NO}_3^-\text{-N}$ was seen in the dolomite amended soil. Higher $\text{NO}_3^-\text{-N}$ concentration at higher soil pH level suggests the reduction of N_2O to N_2 , instead of $\text{NO}_3^-\text{-N}$ used as an electron acceptor (Feng et al., 2003). However, in this study, the soil moisture was controlled as 60% of WFPS. It is difficult to infer that the difference in the N_2O reduction rates determined the difference in $\text{NO}_3^-\text{-N}$ concentrations in this aerobic condition because N_2O reduction becomes dominant in more anaerobic conditions. In addition, nitrification process as main pathway of N_2O emissions is not clear. Looking at the time-series changes in $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations, the effect of Cu and lime application on nitrification seems to be great. Therefore, we do not think that the contribution of nitrification to N_2O emission is negligible.

The presence of heavy metals has been observed to interfere with the reduction of N_2O to N_2 (Holtan-Hartwig et al., 2000). The repression of N_2O -reductase would enhance N_2O emissions from soils. The present study showed that N_2O emissions were higher in +Cu soil treatments demonstrating a pronounced inhibitory effect on specific step or microorganisms involved in N_2O reduction. Bollag and Barabasz (1979) also observed an increase in soil N_2O emissions with increasing Cu concentration indicating greater inhibition of N_2O -reductase during denitrification due to toxicity of Cu. Higher N_2O emissions from +Cu treatments can also be explained by the decrease in soil microbial biomass C (MBC) and N (MBN) and associated activity. This likely affected the activity and growth rate of N_2O reducing microbial communities. Soil CO_2 emissions also decreased with Cu application corroborating the microbial biomass C and N results. The

application of dolomite counteracted the reduction in microbial respiration. Further, microbial respiration increased by relieving and eliminating Cu stress at high pH values. Soil CO₂ emissions started to increase at the later stage of the study, which implies that relief of soil microbial growth by pH manipulation could substantially decrease soil N₂O emissions in dolomite treated soil. However, emissions of N₂O decreased with time indicating the recovery of soil N₂O-reductase enzyme activity. The recovery of N₂O-reductase enzyme activity and decreased N₂O emissions with time could be due to: (i) decreased availability of Cu, (ii) a shift in community towards a tolerance of Cu or augmented tolerance genes within the community (Holtan-Hartwig et al., 2002), (iii) increased soil pH, and (iv) decreased in NO₃⁻-N concentration.

Bio-available amount of heavy metals is affected by soil pH (Angle et al., 1993). Dolomite treated soils with Cu additions showed lower overall N₂O emissions as compared with Cu only treatments. Dolomite application substantially decreased bio-available Cu. The decrease in available Cu under higher pH has been previously reported in several studies (Kirchmann and Eskilsson, 2010; Moore et al., 2018; Senkondo, 2016). The application of dolomite likely decreased the toxic effects and stress caused by Cu on denitrification and also stimulated activity of N₂O-reductase activity. Lower soil N₂O emissions at later stage showed that higher pH in dolomite treated soil alleviated Cu stress on microorganisms which were involved in denitrification. The results suggest that complete denitrification occurred i.e. stimulation of N₂O to N₂ reduction, the terminal step of denitrification (Holtan-Hartwig et al., 2002). The results indicate that liming of acid soils even with high Cu levels decreases N₂O emissions (Holtan-Hartwig et al., 2002).

5. Conclusion

This study showed that N₂O emissions were highly affected by Cu content and soil pH. High Cu levels likely interfered with the reduction of N₂O to N₂. Dolomite markedly decreased soil N₂O emissions through increasing soil pH and alleviating stress of Cu on soil microorganisms. Higher soil respiration and microbial biomass C and N in dolomite treated soil also inferred the relief from Cu stress that reduced N₂O production at higher pH values. Considering the reference to pH and heavy metal contamination, further research is suggested how consistent these results of N₂O emissions are under high soil water regimes in the filed environment.

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Figures

Fig. 1. Soil pH dynamics in dolomite and Cu amended soil. (A): Cu treated soil without dolomite application. (B): Cu treated soil with dolomite application. Cu0: 0 Cu mg kg⁻¹ soil, Cu250: 250 Cu mg kg⁻¹ soil, Cu500: 500 Cu mg kg⁻¹ soil, Cu1000: 1000 Cu mg kg⁻¹ soil. -d: no dolomite, +d: with dolomite. Error bars represent standard errors of means (n=3).

Fig. 2. Soil ammonium dynamics in dolomite and Cu amended soil. (A): Cu treated soil without dolomite application. (B): Cu treated soil with dolomite application. Cu0: 0 Cu mg kg^{-1} soil, Cu250: 250 Cu mg kg^{-1} soil, Cu500: 500 Cu mg kg^{-1} soil, Cu1000: 1000 Cu mg kg^{-1} soil. -d: no dolomite, +d: with dolomite. Error bars represent standard errors of replicates (n=3).

Fig. 3. Soil nitrate dynamics in dolomite and Cu amended soil. (A): Cu treated soil without dolomite application. (B): Cu treated soil with dolomite application. Cu0: 0 Cu mg kg^{-1} soil, Cu250: 250 Cu mg kg^{-1} soil, Cu500: 500 Cu mg kg^{-1} soil, Cu1000: 1000 Cu mg kg^{-1} soil. -d: no dolomite, +d: with dolomite. Error bars represent standard errors of replicates (n=3).

Fig. 4. Microbial biomass carbon in dolomite and Cu amended soil. (A): Cu treated soil without dolomite application. (B): Cu treated soil with dolomite application. Cu0: 0 Cu mg kg⁻¹ soil, Cu250: 250 Cu mg kg⁻¹ soil, Cu500: 500 Cu mg kg⁻¹ soil, Cu1000: 1000 Cu mg kg⁻¹ soil. -d: no dolomite, +d: with dolomite. Error bars represent standard errors of replicates (n=3).

Fig. 5. Microbial biomass nitrogen in dolomite and Cu amended soil. (A): Cu treated soil without dolomite application. (B): Cu treated soil with dolomite application. Cu0: 0 Cu mg kg⁻¹ soil, Cu250: 250 Cu mg kg⁻¹ soil, Cu500: 500 Cu mg kg⁻¹ soil, Cu1000: 1000 Cu mg kg⁻¹ soil. -d: no dolomite, +d: with dolomite. Error bars represent standard errors of replicates (n=3).

Fig. 6. Bio-available copper in dolomite and Cu amended soil. Cu250: 250 Cu mg kg⁻¹ soil, Cu500: 500 Cu mg kg⁻¹ soil, Cu1000: 1000 Cu mg kg⁻¹ soil. -d: no dolomite, +d: with dolomite. Error bars represent standard errors of replicates (n=3). Different alphabet letters denote significant differences at p<0.05. Data were analyzed at each time course for -d and +d separately using one-way ANOVA.

Fig. 7. (A and B): Soil respiration rate. (C and D): Cumulative soil CO₂-C emissions. Cu0: 0 Cu mg kg⁻¹ soil, Cu250: 250 Cu mg kg⁻¹ soil, Cu500: 500 Cu mg kg⁻¹ soil, Cu1000: 1000 Cu mg kg⁻¹ soil. -d: no dolomite, +d: with dolomite. Error bars represent standard errors of replicates (n=3).

Fig. 8. (A and B): Soil N_2O-N emission rate. (C and D): Cumulative soil N_2O-N emissions. Cu0: 0 Cu $mg\ kg^{-1}$ soil, Cu250: 250 Cu $mg\ kg^{-1}$ soil, Cu500: 500 Cu $mg\ kg^{-1}$ soil, Cu1000: 1000 Cu $mg\ kg^{-1}$ soil. -d: no dolomite, +d: with dolomite. Error bars represent standard errors of replicates ($n=3$).