

Novel Mechanism of Abiotic Ammonium Oxidation Triggers Nitrous Oxide Bursts Upon Soil Rewetting

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27 **Abstract**

28 Drought-rewetting cycles (DWC) in soil are accompanied by a rapid burst of nitrous oxide (N_2O)
29 emission within minutes without reactivation of dormant microbes. Here we present a manifold of
30 evidence for a previously unrecognized abiotic mechanism connecting soil ammonium (NH_4^+) to
31 N_2O with hydroxylamine (NH_2OH) as a key intermediate. DWC experiments in the Negev Desert
32 soil revealed comparable short N_2O pulses in both live and radiation-sterilised soils. N_2O
33 emission increased in a dose-dependent manner following NH_2OH addition, underscoring NH_2OH
34 availability as a key factor of the observed N_2O spikes. Finally, isotope labelling experiments
35 showed that NH_4^+ directly contributes to N_2O emission even in sterilised soil. This pathway is
36 likely facilitated by the extracellular ammonia monooxygenase (AMO) – the only known catalyser
37 of NH_4^+ - NH_2OH conversion in soil. Our finding of an additional source of atmospheric N_2O extend
38 beyond drylands since abiotic processes and DWC are projected to intensify globally including
39 temperate ecosystems.

Introduction

Rewetting of dry soils in deserts (1) and elsewhere (2) results in abrupt bursts of trace gases emissions initially described by Birch (3). Upon rewetting, a cascade of events (driven by the change in water potential) trigger peaks of carbon dioxide (CO_2) and nitrous oxide (N_2O) (4, 5). Both gases are important for atmospheric chemistry; CO_2 concentration has steadily increased in the atmosphere since the start of the industrial revolution (6, 7) and is the main driver of climate change (8). While N_2O is a potent greenhouse gas (GHG) and the main driver of the ozone (O_3) destruction in the stratosphere (9–11). The Birch effect, or post-wetting burst of soil CO_2 emissions, is attributed to lysis of microbial cells and fast increase in microbial and fungal activity in soils (12–14). The mechanism of soil N_2O emissions is less well understood (15, 16).

There are three major sources for N_2O emission from soil. Firstly, microbes contribute to N_2O emission through nitrification and denitrification processes. Nitrification depends on the aeration, and it is facilitated by ammonia oxidizing bacteria (AOB) and archaea (AOA) (17). Supporting this the accumulation of nitrite (NO_2^-) proportionally to ammonium (NH_4^+) level has been shown (18, 19). During droughts soil are well oxygenated (20) and the dominance of nitrification is consequential. However, the exact molecular mechanism of N_2O production by AOB or AOA includes unknown enzymes, whose role is proved but not fully revealed (21, 22). Otherwise, recently the role of the denitrification process has been reconsidered. Analysis of isotope signatures of N_2O gas emitted after drought and rewetting cycle revealed the dominance of denitrifying pathways in N_2O emission (23). Contradiction between oxygen levels and the nitrification abundance was explained by a strong retention of water by hydrophobic microsites, which led to hypoxic conditions (24). Moreover, application of ^{15}N -labeled nitrates (NO_3^-) to the dry soil during the rewetting demonstrated a rapid NO_3^- consumption within minutes with the ^{15}N emitted as N_2O (25).

Secondly, Zhu-Barker et al. (26) collected evidence on the importance of abiotic N_2O production pathways. Chemodenitrification has been known for a long time to produce N_2O via reduction of NO_2^- with soil organic matter or Fe^{2+} ions (27, 28). Another abiotic process includes hydroxylamine (NH_2OH) as a key-intermediate, which can be chemically oxidized to N_2O in the presence of Fe^{3+} or Mn^{4+} (29). It was shown that a turnover of exogenous NH_2OH is mostly abiotic and its addition to γ -irradiated soil results in a pronounced N_2O production under oxic conditions (30). However, this reaction wasn't considered in ecological studies due to questionable plausibility of NH_2OH extracellular release (31). Finally, biochemically mediated abiotic processes may lead to soil N_2O emission during rewetting. This phenomenon is driven by enzymatic production of substrates (i.e., NH_2OH), which is later released into the soil matrix and chemically oxidized (32). Recently, unambiguous evidence was obtained for abiotic conversion of extracellular hydroxylamine produced by AOB and AOA in pure cultures (33). Enzymatically produced hydroxylamine may undergo a set of chemical reactions: disproportionation, reaction with NO_2^- , oxidation by oxygen (O_2) and oxidation by $\text{Fe}^{3+}/\text{Mn}^{4+}$ (34). These cascade of abiotic and enzymatic processes are particularly important for archaea-dominant soils, because archaea lack NO producing nitrification and nitrifier denitrification pathways (35).

Recent introduction of the laser-based instruments capable to measuring air N_2O concentrations online and with high accuracy (36) enabled great reduction of time needed to measure soil N_2O emissions relative to classic static chamber techniques (37, 38). This allowed to measure post-rewetting pulses of soil trace gases emissions with temporal resolution of minutes (39), down from hours or days (38, 40). Indeed, recent reports showed a timeline of minutes from addition of water till the emissions peak of soil N_2O emissions in field (32). Arguably, such a short timescale seems insufficient for “resurrection” and reactivation of dehydrated microbes in soil (41). Changes in microbial communities usually lag the first emission peak (42). Therefore, the initial N_2O pulse upon rewetting can be hypothesized to be completely abiotic. Part of it can be mediated by the availability of extracellular intermediates, which was reported for soil solution (43) facilitating bio-abiotic type of N_2O emission. Considering oxic conditions of dryland soils we

hypothesize that a driving force of the mechanism leading to post-wetting soil N₂O emissions is the extracellular oxidation of NH₄⁺ by ammonia monooxygenase (AMO). This enzyme is released from the periplasm of soil microbes after lysis initiated by abrupt osmotic changes due to rewetting.

In this study we aimed to test the existence of the suggested mechanism at three levels. Firstly, we performed a field rewetting experiment during summer drought and measured instantaneous soil CO₂ and N₂O emissions. Secondly, we conducted a series of laboratory rewetting experiments with minimally disturbed soils cores. In order to distinguish between abiotic and biotic nature of N₂O emissions and assess the ability of AMO reactive site to catalyse NH₄⁺ oxidation in soils, all laboratory experiments were performed with soil cores that were either live or sterilized by long γ -irradiation with the total dose of 5 MRd (5×10⁴ Gray). Finally, we performed batch experiments with sieved and homogenized γ -irradiated soils. A key step in concluding the bio-abiotic mechanism was the usage of ¹⁵N enriched NH₄Cl to assess the trend of isotopic signature dynamics during post-rewetting fluxes from γ -irradiated soil.

Results

Nitrous oxide flux after rewetting of dry soil in field

In order to draw a timeline for N₂O emission from the Negev desert soil, we measured soil trace gases fluxes after rewetting of a mineral soil and soil with plant residues (*Supplementary Information*, Table S2) that had two different C and N availability levels (44). Fifteen minutes after the rewetting, ~ twofold, increase of soil N₂O fluxes was observed in both soils, from a baseline of ~76 and 99 ng N m⁻²min⁻¹ to 168 and 206 ng N m⁻²min⁻¹ for soils with lower and higher inorganic N content, respectively (Fig. 1, *Supplementary Information*, Table S2). After one hour, however, the N₂O emissions dropped in both cases to the pre-wetting levels. Before the rewetting, soil NO₂⁻ concentrations were the same in both soils, while NH₄⁺ and NO₃⁻ concentrations were ~1.5 times higher within the site with higher N-availability (*Supplementary Information*, Table S1). Notably, after the rewetting, a sharp increase of NO₂⁻ in soil with higher N availability was observed (*Supplementary Information*, Table S1). At the same time NO₂⁻ concentration remained unchanged in the soil with lower N availability. One-hour post-wetting the NO₂⁻ levels had returned to pre-wetting concentrations, however, after two hours the accumulation of NO₂⁻ was detected again. Consequently, nitrite fluctuation seemed to have no influence on N₂O fluxes. Yet, considering also the differences in CO₂ emission, NO₂⁻ fluctuation likely indicates that observed fluxes correspond to the genuine N₂O producing rather than physical displacement of soil air upon rewetting. Initial N availability in the forms of NH₄⁺ and NO₃⁻ seems, at least partially to affect the outburst of N₂O emission but the overall post wetting emissions trends remained similar. It should be mentioned that despite the presumable occurrence of nitrification, we observed an increase in NH₄⁺ concentrations which could be driven by post-wetting release of mineral-bounded N and, considering high CO₂ fluxes (*Supplementary Information*, Table S2), by mineralization of organic matter (44). Transient accumulation of NO₂⁻ in soil during the field experiment unlikely affected N₂O emission due to both very low soil concentrations and undetectable NO₂⁻ in low N soils which exhibited similar N₂O emissions compared to high N soils. This contradicts studies which identified denitrification leading to N₂O pulses (39). Arguably, the initial pulse occurring within the first 15 minutes should be forced by abiotic factors (45) but only partially by substrate availability. Thus, differences in N₂O pulses can be attributed to the differences in substrate transforming sites in these two soils. Collectively, field experiments enable us to hypothesize that initial post-wetting availability of AMO affects N₂O emission.

Nitrous oxide flux after rewetting of undisturbed soil cores in laboratory

To obtain second-level evidence of bio-abiotic nature of N₂O pulses we performed laboratory rewetting experiments using irradiated and live minimally disturbed soil cores. Immediately after addition of water CO₂ pulses up to 700 ng C m⁻²min⁻¹ were detected in live soil cores, a phenomenon described previously as the “Birch effect” (Fig. 2). Yet, in case of irradiated soil the primary CO₂

flux was initially trivial and nil after ~two hours, whereas live soil continued to emit CO₂ throughout the entire experiment reaching higher rates of up to 1500 ng C m⁻²min⁻¹ (Fig 2). Upon rewetting heavy N₂O pulses were also detected in both irradiated and live soils (Fig 2). With a peak flux of 300 ng N m⁻²min⁻¹, the maximal emission rate of N₂O in sterilized soil exceeded that of live soil by about 100%. Trends in N₂O emission were rather similar at the beginning and different after two hours. In case of irradiated soil, the fluxes rapidly diminished and after 4 hours N₂O flux was nil. In the live soil the primary pulse also dropped to lower values as compared to irradiated soil. Still, after one hour the descending trend was substituted by constant but persistence flux of ~ 100 ng N₂O-N m⁻²min⁻¹. Likely this flux originated from the source process biological nitrification, given that it did not occur in irradiated soil.

The divergence between live and sterilized soils emerged only after the initial emission pulses, suggesting that the detected CO₂ and N₂O emissions from the sterilized soil were of abiotic origin and not due to microbial contamination, which was prevented using an aluminium seal. Indeed, after rewetting reactive oxygen species (ROS) may appear and alter soil organic matter (46), causing in some cases > 50% of the total CO₂ emission (47). Despite in live soil CO₂ fluxes were of both biotic and abiotic origin, similar pulse patterns were observed for both live and sterilized soils. This can be explained by high quantities of dissolved organic matter, which releases from inorganic matrix during different sterilization methods, including irradiation (48). At the same time, it was shown that unlike alive, sterilized soils exhibited lack of nitrification (49). This indicated the successful damage of microbial cells in soil during irradiation, which alloyed sterilization of soils without disturbance of soil structure (50). Therefore, observed trends support that in both live and sterilized soils initial N₂O pulses are facilitated by non-biological mechanisms involving turnover of reactive intermediates. Under oxic conditions it is most likely coming from NH₂OH (30) and its role was thus further evaluated.

Role of hydroxylamine availability in N₂O emission in undisturbed soil cores

According to ICP OES measurements, iron content in soil exceeded 30×10³ ppm (or 3%). Drought and rapid rewetting (51, 52) most likely lead to cell lysis followed by organic matter and inorganic N released into the soil solution. Consequently, NH₂OH accumulated in cells can reach the soil interface, where it can react with Fe³⁺-bearing minerals yielding N₂O (53). Since γ-irradiation is ionizing by its nature and results in oxidative cell membrane damage (54), higher flux from irradiated soil could be expected and corroborates with Fe³⁺-induced NH₂OH oxidation. In order to assess this reaction two types of experiments were conducted: addition of synthetic iron oxides (*Supplementary Information*, Table S3) and hydroxylamine (*Supplementary Information*, Table S4) directly to undisturbed soil core. Addition of iron oxide didn't change the temporal trend and magnitude in soil N₂O emission (Fig. 3B, *Supplementary Information*, Table S3). In additional experiments with synthetic ferrihydrite and maghemite we observed a first order reaction with liner dependency on the NH₂OH concentration in solution (*Supplementary Information*, Table S5, Fig. S1). This explains the lack of impact of addition of iron oxides to soil: with 3% of Fe in soils, the hydroxylamine concentration seems to be the limiting factor of the reaction.

Addition of NH₂OH to soil resulted in pronounced positive feedback of N₂O fluxes (Fig. 3A, *Supplementary Information*, Table S4). It was found that the addition of hydroxylamine yielded ~700 times higher N₂O fluxes as compared to water control indicating a qualitative oxidation of NH₂OH in soils. Despite the addition of NH₂OH hardly allowing quantitative comparison of gaseous emissions pulses, pronounced changes in the timeline of fluxes between water and hydroxylamine were observed in case of the sterilised soil.

In control experiments with water N₂O emission were quenched after three hours. Addition of hydroxylamine extended N₂O emission to 24 hours post wetting. Such dynamics can result from several factors: first, rapid addition of high concentrations of NH₂OH results in the formation of Fe²⁺ ions, which are oxidized by oxygen to form iron oxides with slower reaction kinetics (55, 56). Additionally, high concentrations of hydroxylamine may change the reaction stoichiometry leading to formation of side products (57), thereby also affecting the kinetics. Another possible explanation involves redox reactions of Fe²⁺ with NO₂⁻, which results in chemodenitrification. Independently of these reactions, collected results support our major hypothesis on the leading role of NH₂OH

availability in N₂O emission during the rewetting. Consequently, N₂O pulses in soils without spikes of hydroxylamine can be explained by cell damage caused by water addition, which resulted in a release of AMO catalysing the oxidation of NH₄⁺.

Mechanism of abiotic N₂O production includes AMO activity as the driving force

In order to acquire direct third level evidence of extracellular AMO pathway of N₂O production, we conducted an incubation experiment with ¹⁵N-labeled NH₄⁺ substrate and sieved γ-irradiated soil slurry. N₂O was collected at 10 time points between 0 to 420 minutes and analysed by isotope-ratio mass-spectrometry. Nitrite content was also determined for each time point. The increase of N₂O concentration in the headspace started immediately and kept increasing up to 2 hours with 45 minutes as an inflection point (*Supplementary Information*, Fig. S2A). Similarly, CO₂ concentration increased steeply starting with 45 minutes up to two hours (*Supplementary Information*, Fig. S2B) however, its accumulation was almost quenched after 2 hours. At the same time NO₂⁻ content slightly increased after 15 minutes as compared to the baseline obtained with KCl extraction (*Supplementary Information*, Fig. S2C). After two hours the NO₂⁻ content decreased but it didn't affect N₂O or CO₂ emission and there was no correlation between NO₂⁻ content and N₂O emission (*Supplementary Information*, Fig. S2D). Still, at the beginning the N₂O and NO₂⁻ contents increased together, which implies the occurrence of the similar abiotic oxidation process, likely, oxidation of NH₂OH.

The first portion of emitted N₂O has been enriched with ¹⁵N (*Supplementary Information*, Fig. S3) with ¹⁵NH₄Cl as its only source. The δ¹⁵N signature of emitted N₂O increased progressively over time, reaching values near 180‰ after 6–7 hours. A positive correlation was observed between δ¹⁵N and emitted N₂O concentrations (*Supplementary Information*, Fig. S3 insert; $r^2 = 0.59$), indicating that higher N₂O production was accompanied by a stronger ¹⁵N enrichment. Given that NH₄⁺ was the only source of label this pattern suggests that emitted N₂O increasingly incorporated N derived from NH₄⁺. It should be noted that even considering the dilution by background air, only modest enrichment in δ¹⁵N-N₂O was detected. That means that in overall abiotic N₂O emission the contribution of NH₄⁺ oxidation is relatively small and overtaken by a multiple of other reactions reported for soils, e.g. chemodenitrification or Fenton oxidation of organic matter. But reliable incorporation of ¹⁵N reflects the conceptual discovery of our work: the abiotic oxidation of NH₄⁺ is a driving force of N₂O formation during rewetting of dry soil.

Ammonia cannot be transformed into N₂O directly implying that the biochemical oxidation of NH₄⁺ to NH₂OH triggers a cascade of redox reactions and N₂O emission. Formed NH₂OH may be oxidized by Fe³⁺ or by reaction with NO₂⁻. To explain the formation of NH₂OH we propose the following mechanism (Fig. 4): At the first stage rapid addition of water leads to an extensive cell lysis, which facilitates the release of membrane-bound AMO from periplasm or periplasm-like space (in case of AOA) into the soil. Under aerobic conditions, extracellular AMO catalyses oxidation of soil NH₄⁺, producing NH₂OH which becomes available for oxidation/reduction reactions. The reaction between extracellular NH₂OH and Fe³⁺ occurs immediately. The abiotic reaction continues until the uptake of NH₄⁺ is complete or until catalytic ability of AMO persists. Reducing iron oxides to soluble Fe²⁺ launch Fenton-type reactions, with yields depending on the quantities and quality of soil organic matter and contributing to N₂O (and CO₂) fluxes.

Discussion

Three lines of evidence collected in the current study point towards a novel abiotic mechanism of N₂O emission during rewetting of dry soil. While the role of Fe chemistry in N₂O production has been previously highlighted in literature, the ability of extracellular AMO to convert NH₄⁺ to NH₂OH *in situ* hasn't been explicitly demonstrated. In this study we observed N₂O pulses in the timeframe of minutes after rewetting of the Negev Desert alkaline soil. These pulses were accompanied by ephemeral accumulation of NO₂⁻. At acidic and neutral soils chemodenitrification plays an important role in N₂O emission (58). This process is strongly pH dependent and may include reaction of HNO₂ with NH₂OH, organic matter, or Fe²⁺ (34). Under acidic pH nitrous acid is highly reactive (59), whereas at higher pH NO₂⁻ ions are stabilized (60, 61). Moreover, the

oxidation rate of Fe^{2+} -species by oxygen also increases with pH (62). Therefore, it was concluded that NH_2OH turnover was a driving force for the observed N_2O pulses upon rewetting.

The distinction between biological and chemical processes was achieved through soil sterilization using γ -irradiation. It should be highlighted that sterilization was conducted for undisturbed soil cores as well as sieved soil. In case of undisturbed cores, the structure and physical-chemical properties of soil remain intact facilitating comparison of post-wetting N_2O emission between live and sterilized soil. At the beginning the trend in N_2O emission between live and sterilized soils was fully correlated (Fig. 1B). This implies the abiotic nature of N_2O emission pulse. Notably, although the high C and N soil contained ~ 1.5 times more NH_4^+ than the low C and N soil, N_2O emissions were comparable between the two. This implies that the rate-limiting factor should be the availability of catalytic centres. The long-lasting abiotic emission of N_2O with the increasing enrichment from $^{15}\text{NH}_4^+$ oxidation supports that catalyst availability drives the reaction - this might explain the comparable or even higher N_2O pulse from sterilized soil where extracellular AMO is easily accessible. Crucially, the detection of ^{15}N -labeled N_2O , even in sterilized soil, confirms that NH_4^+ -derived N was incorporated into N_2O via non-biological pathways. This observation supports our hypothesis. In turn, NH_2OH can drive downstream redox reactions, such as Fe^{3+} reduction to Fe^{2+} , and chemodenitrification via Fe^{2+} and NO_2^- , ultimately leading to abiotic N_2O formation with NH_4^+ oxidation as a biochemical trigger acting upon soil rewetting.

Methods

Study site.

The study site was located in the Negev Desert, in Sde Boker campus, Midreshet Ben-Gurion (475 a.s.l., $30^\circ 52' 27.3''\text{N}$; $34^\circ 47' 33''\text{E}$). In this area, the average annual precipitation was 92.2 mm (2010-2019, IMS Sde Boquer station) falling between November and May only. In 2019, the total precipitation was 94.8 mm. The mean annual temperature was 18.8°C , with a monthly mean temperature of 9.7°C in January and 26.6°C in August (1). Soils are loessal sandy-loam with 55% sand, 32.5% silt, and 12.5% clay (2). The soil pH was 8.24 ± 0.03 (1:5 soil: water). The upper 15 cm of soil had bulk density (BD) of $1.24 \pm 0.04 \text{ g cm}^{-3}$, water holding capacity (WHC) of $44.5 \pm 0.9\%$ and soils contain 0.38 and 0.05% of carbon (C) and nitrogen (N), respectively.

Set up for field and laboratory soil core re-wetting experiments.

Cylindrical opaque PVC collars (15.0 cm height, 19.5 cm diameter, 0.5cm wall thickness) were placed in the field site area within *Salsola* sp., plant patches and at least 80 cm out of the patch as described previously (44). These soils were designated as soils with higher and lower C and N contents, respectively. Collars were left undisturbed in the field for ~ 10 months between wet, rainy season and end of ~ 7 month long dry season without rain. Part of the cores were used for the rewetting experiments in the field, and another part was removed intact for laboratory experiments. The intact, undisturbed cores were removed from the field together with surrounding soils. The cores were carefully placed into plastic boxes, one core per box and were stored in the lab for another ~ 7 months under ambient air-dried conditions prior laboratory experiments.

Re-wetting experiments of soil cores.

In all rewetting experiments with soil cores (field, live, and γ -irradiated soil cores) a rewetting was achieved by slow application of an equivalent of 6.6 mm of water or aquatic solution homogeneously on the surface of the soil, reaching $\sim 35\%$ of the soil water-holding capacity (WHC). Each experiment was performed in five replicates. Field experiments were conducted with distilled water. For laboratory experiments distilled water, Fe-oxides, or hydroxylamine

solutions were used. The pH values of all solutions were adjusted to 7.4 ($\pm$ 0.1) using either NaOH or HCl.

Gas measurements.

Soil N₂O and CO₂ emissions from the intact cores were measured using accumulation chambers in the field and in laboratory modified for our needs (44, 63). An opaque gas-tight lid made of PVC; interior dimensions: diameter 19.5 cm, height 3 cm, wall thickness 0.5 cm was placed on the top of cylindrical PVC collars (15.0 cm height, 19.5 cm diameter, 0.5 cm wall thickness). The lid was equipped with a vent (made of 3.0 mm Swagelok bulkhead union and about 12.0 cm long coiled copper tube, 1.5 mm i.d.) for pressure equilibration. In the chamber, the total headspace volume was 2.69 L and the chamber covered an area of 0.028 m². A foam tape (1.0 x 1.0 cm) inside the chamber lid and a ~one kg weight attached to the top of the lid were placed to ensure a good seal upon closure of the chamber. Gaseous emissions rates were determined by measuring increasing concentration of the gas of interest in the chamber headspace for 5 minutes to assure linearity of the flux (44). The gases were measured by infra-red gas analyzers; the CO₂ was measured by LiCor-820 (LICOR Biogeosciences, Lincoln, Nebraska, USA) and N₂O by quantum-cascade laser analyzer (QCL; GLA151-N2OM1; ABB, Quebec City, QC, Canada). Fluxes of gases of interest were calculated from linear increase of concentration in the chamber headspace and corrected for ambient pressure and temperature.

Soil incubation with ¹⁵N enriched substrate

To assess the role of NH₄⁺ oxidation by extracellular AMO, incubation experiments with soil sterilized by γ -irradiation were conducted. In 20 jars, 50 g of sterilized soil were placed followed by the addition of 50 mL of 0.1 M ¹⁵NH₄Cl (78 atom-% enrichment). Gas samples were taken from the two replicating jars after 0, 15 and 45 minutes followed by sampling each hour from 1 to 7 hours. For the isotope-ratio mass spectrometry (IRMS) analysis, gas samples (15.0 mL) were transferred by the syringe to the 12.0 mL exetainers (Labco, Lampeter, Wales, UK), which were preliminary flashed and filled with N₂. Gas samples were analyzed for ¹⁵N-N₂O using an Isoprime PreciSOL isotope ratio mass spectrometer (Elementar UK Ltd. Stockport, UK), coupled to an isoFLOW GasBench (Elementar UK Ltd. Stockport, UK).

Data availability

All data supporting the findings of this study are presented in the Supplementary materials accompanying this Article.

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Author Contributions

AZ, ED, MP, and IG designed the research, AZ, ED, IY, NK, LF performed the research, MP, MD, RM, FCH, CS contributed analytic tools, AZ, VIG, IG analyzed data, AZ, and IG wrote the paper with contribution from all coauthors.

Competing Interest Statement

The authors declare no competing interests.

Supplementary information

The Supplementary Information contains additional details on experimental procedures and all data used in this study.

Figures and Tables

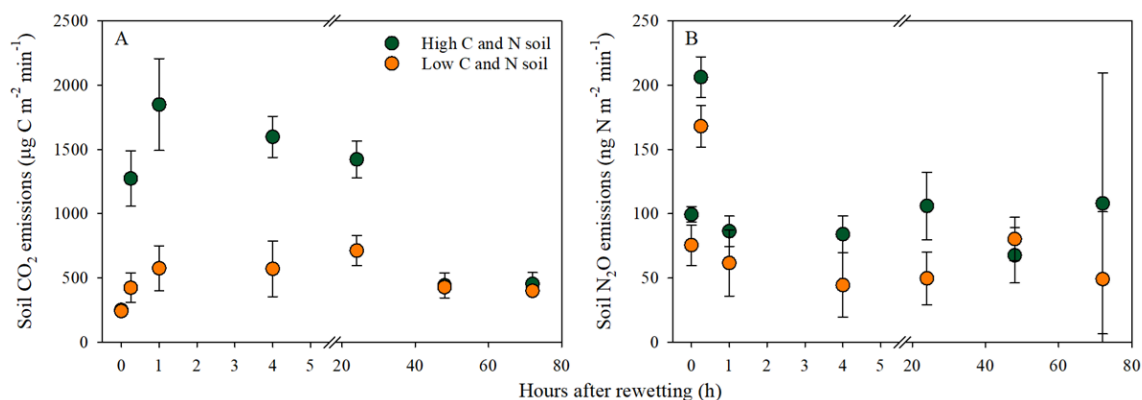


Figure 1. Nitrogen (N) and carbon (C) fluxes from rewetted soil under contrasting C and N availability in field.

(A) CO₂ and (B) N₂O fluxes measured from intact, minimally disturbed soil cores with different C and N content during field rewetting experiments. Error bars indicate the standard error of the mean (n = 6 independent soil cores per treatment). Attention to the Y-scale change between the panels.

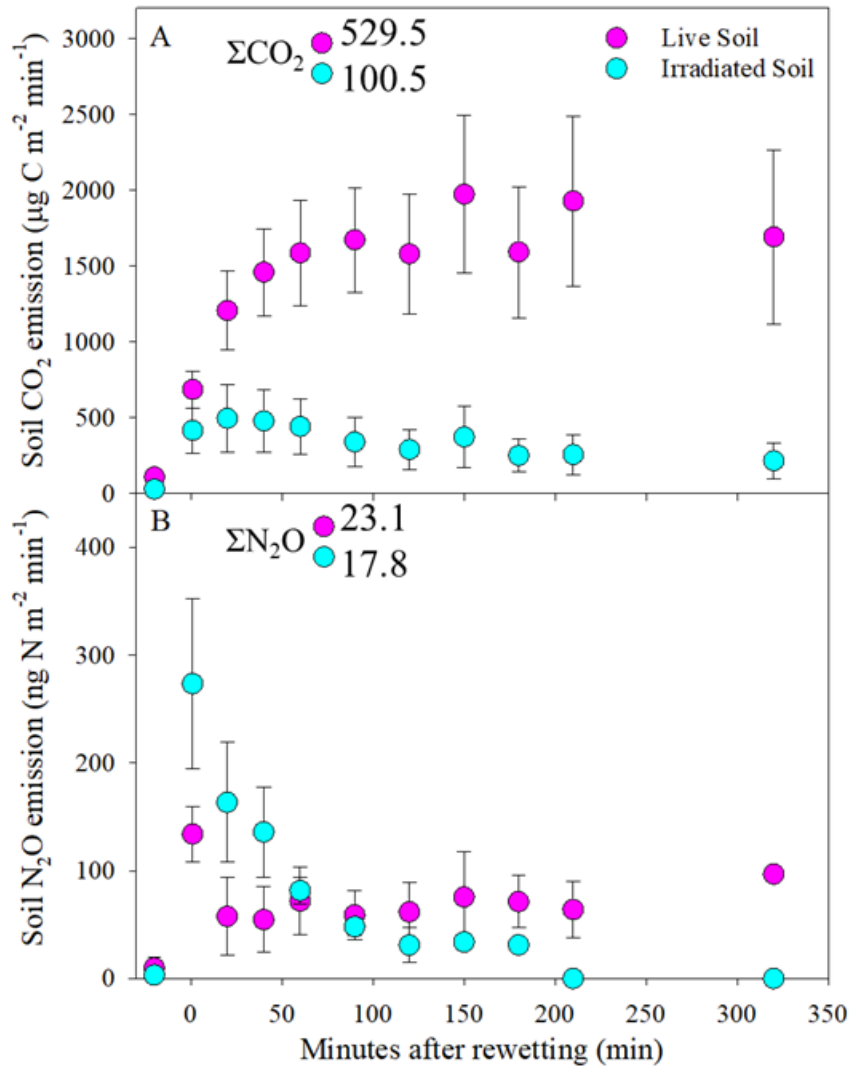


Figure 2. CO₂ and N₂O fluxes following rewetting of live and sterilized soil cores under laboratory conditions.

(A) CO₂ and (B) N₂O net fluxes dynamics measured from intact soil cores following rewetting, comparing live soils and soils sterilised by gamma irradiation. Points represent means $\pm$ standard error (n = 6 independent soil cores per treatment). Cumulative fluxes (Σ CO₂ and Σ N₂O; mg C or µg N) are indicated within each panel. Attention to the Y-scale change between the panels.

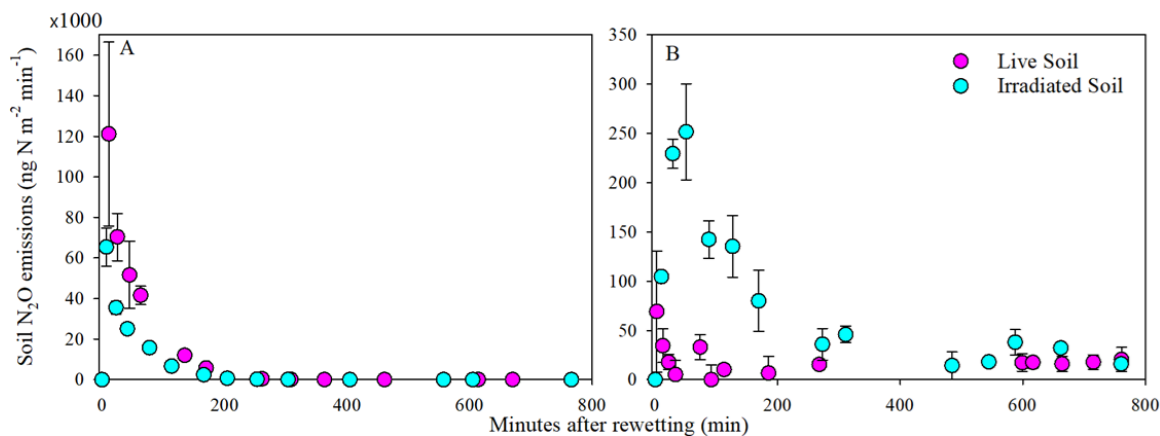


Figure 3. Effects of hydroxylamine (NH₂OH) and iron oxide (γ-Fe₂O₃) addition on soil N₂O emissions following rewetting.

Soil N₂O fluxes measured from intact soil cores following rewetting with the addition of (A) NH₂OH and (B) synthesised γ-Fe₂O₃ (maghemite) under laboratory conditions, comparing live soils and soils sterilised by gamma irradiation. Points represent means ± standard error (n = 6 independent soil cores per treatment).

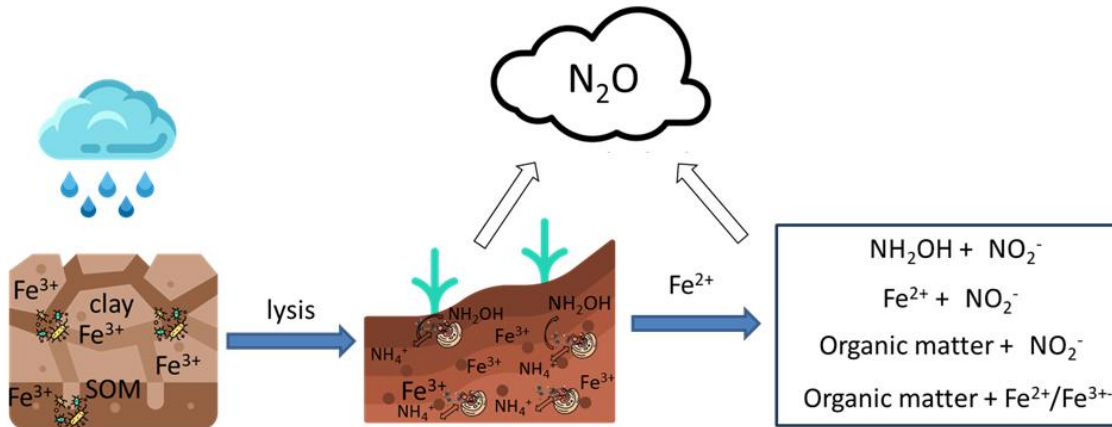


Figure 4. Conceptual scheme for triggering abiotic N₂O production during soil rewetting.

Rewetting events induce microbial cell lysis, releasing extracellular enzymes, including ammonia monooxygenase (AMO), into the soil matrix. Extracellular catalysis promotes the oxidation of ammonium (NH₄⁺) to hydroxylamine (NH₂OH), which subsequently reacts with iron-bearing minerals leading to rapid N₂O formation. These reactions initiate a cascade of Fenton-like and other redox processes involving Fe²⁺/Fe³⁺ and organic matter that further contribute to N₂O emissions.

Supplementary Information for

Novel Mechanism of Abiotic Ammonium Oxidation Triggers Nitrous Oxide Bursts Upon Soil Rewetting.

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This PDF file includes:

Supporting text
Figures S1 to S3
Tables S1 to S7
SI References

Supporting Information Text

1. Materials and methods

1.1. Sterilization of undisturbed cores and soil samples by γ -irradiation

Sterilization of soils was performed at Sorvan (www.sorvan.co.il/) company in Israel with dosage between 50 - 80 kGy. Intact cores were sterilized in their boxes without disturbance and kept air dry under aluminum foil and plastic cover for up to 8 weeks.

1.2. Chemicals and solutions for laboratory experiments

1.2.1. Chemicals

All chemicals were of analytical purity and used as received from Sigma-Aldrich without further purification. Double distilled water (DDW) was used for all measurements, experiments, and solutions preparation. The total iron content was measured by ICP OES (Spectro Arcos) after microwave digestion with HNO_3 .

1.2.2. Preparation of hydroxylamine stock solution

Hydroxylamine stock (100 mM) solution was first prepared by dissolving 347.45 mg of hydroxylamine hydrochloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$; Sigma-Aldrich) in DDW. The solution was then diluted to obtain a NH_2OH concentration of 0.034 mM. The pH was subsequently adjusted to $\text{pH} = 7.4 (\pm 0.1)$ and the solution was used (within the 3 days following its preparation) for the rewetting experiments.

1.2.3. Synthesis of iron oxide

The synthesis of Fe-oxides was performed according to described procedure (1). Briefly, $\text{FeCl}_2\cdot 4\text{H}_2\text{O}$ (4.3 g) were dissolved in 10 mL HCl (2 M) and $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$ (11.7 g) dissolved in 30 mL of DDW (30 mL). The two iron salt solutions were mixed together, and the homogeneous solution obtained was added dropwise to an alkaline solution (NaOH 0.5 M). The pH was maintained during the synthesis at 8-8.5 by adding drops of NaOH (1 M). The obtained black precipitate suspension was left to settle for several hours. Supernatant was removed, the precipitate redispersed and left maturation for a second time. To the washed precipitate 6 mL of H_2O_2 (30%) was added leading to the oxidation of magnetite (Fe_3O_4) to maghemite ($\gamma\text{-Fe}_2\text{O}_3$) followed by the dilution with distilled water until the volume was 1L. The pH was checked again and adjusted to $\text{pH} = 7.4 (\pm 0.1)$.

1.3. Laboratory measurements and experiments

1.3.1. Aqueous inorganic N-species

Ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) concentrations in soil were determined by extractions and standard spectroscopic assays on a microplate spectrophotometer (Infinite F50, TECAN) (2). Extractions were conducted by adding 25 mL of KCl (1 M) to 5 g of dry soil. During the field experiments, soils were samples from the surrounding of chambers before re-wetting, 15-minutes after the rewetting and additionally after 1, 4, 24, 48, and 72 hours, respectively. In all cases the obtained soil slurries were shaken for a minute and left to settle overnight (24 hours). The samples were centrifuged, and supernatants were collected, stored in plastic vials and frozen (-25°C) until NH_4^+ , NO_3^- and NO_2^- analyses were performed. Soil pools of NH_4^+ , NO_2^- or NO_3^- ($\mu\text{g N g}^{-1}$ soil) were calculated using the following equation:

$$C_s(\mu\text{gN} \times g_{\text{soil}}^{-1}) = \frac{C_e \times V}{W}$$

where C_e , V and W stand for concentrations of N (mgL^{-1}) in the extraction solution, solution volumes (mL) and dry mass of soil (g), respectively.

1.3.2. Oxidation of hydroxylamine by iron oxides

Experiments presented here are focused on the reaction between iron and hydroxylamine, particularly the effects of reactants concentrations and Fe-speciation on N₂O production. Hydroxylamine stock solutions were prepared at three different concentrations (0.0068; 0.034; 0.068 mM). Solutions were mixed with iron-oxide suspension (1:2 by volume). Iron oxides were added in three concentrations (0.2; 1.0; 2.0 g L⁻¹) in the form of maghemite or ferrihydrite. Further, the combined mixtures were sealed in 220 mL gas-tight glass jars and 5 mL of gas were sampled from the jar headspace using a needled-syringe and injected in the N₂ flow for N₂O measurements in the QCL analyzer (after (3, 4)). The first sampling was made immediately, followed by sampling every 5 minutes during the first hour, every 20 minutes during the two following hours and then every 45 minutes during the last four hours.

1.3.3. Nitrification potential

Irradiation impact on soil nitrifier communities were assessed by measuring nitrification potential. The assay relies on the aerobic incubation of soil slurries.(5) In brief: 15 grams of live or irradiated sieved (2 µm) soil samples (in 5 replicates) were placed in 220 mL gas-tight glass jars. For the assay, ammonia substrate in phosphate buffer was prepared using the following: 15 mL of (NH₄)₂SO₄ (50 mM), 1.5 mL of KH₂PO₄ (0.2M), 3.5 mL of K₂HPO₄ (0.2 M), and 980 mL of DDW. In order to inhibit NO₂⁻ oxidation potassium chlorate was added to the solution to a final concentration of 10 mM. 100 mL of substrate solution was added to each jar with soil, and the jars were loosely capped and shaken on an orbital shaker. Aliquots of 1 mL (duplicates) were sampled regularly for 48 hours. Each sample was immediately centrifuged (13000 rpm) and kept frozen (-25°C) until NO₂⁻ analysis (1.3.1. Aqueous inorganic N-species).

1.3.4. Gases emission measurement in sterilized soil with chemodenitrification mitigation

To assess the impact of chemodenitrification on N₂O emission, incubation experiments with KNO₂ spikes were performed. Each experiment was conducted with 6 replicates. Three sets of soil slurry experiments (50.0 mL of solution) were performed: DDW (control), 1.5 mM NH₄Cl and 10 mM KNO₂. Each slurry was intensively stirred on an orbital shaker for 8 hours. The gas was sampled from jar headspace using a syringe followed by the injection in the N₂ stream for N₂O measurements as described in the main text.

1.3.5. Soil incubation with ¹⁵N enriched substrate

To assess the role of NH₄⁺ oxidation by extracellular AMO, incubation experiments with soil sterilized by γ-irradiation were conducted. In 20 jars, 50 g of sterilized soil were placed followed by the addition of 50 mL of 0.1 M ¹⁵NH₄Cl (78 atom-% enrichment). Gas samples were taken from the two replicating jars after 0, 15 and 45 minutes followed by sampling each hour from 1 to 7 hours. For the isotope-ratio mass spectrometry (IRMS) analysis, gas samples (15.0 mL) were transferred by the syringe to the 12.0 mL exetainers (Labco, Lampeter, Wales, UK), which were preliminary filled with N₂. Gas samples were analyzed for ¹⁵N-N₂O using an Isoprime PreciSILON isotope ratio mass spectrometer (Elementar UK Ltd. Stockport, UK), coupled to an isoFLOW GasBench (Elementar UK Ltd. Stockport, UK).

Additionally, 5.0 mL were used for measurements of N₂O concentration increase in the jar headspace, see above (Gases emission measurement in sterilized soil with chemodenitrification mitigation). After gas sampling, jars were unsealed, and medium was sampled for inorganic N-species estimation (see 1.3.1. Aqueous inorganic N-species).

δ¹⁵N signatures were calculated following the equation:

$$\delta^{15}N, \text{‰} = \left(\frac{R_{45}^{exp}}{R_{45}^{std}} - 1 \right) * 1000,$$

in which R45 is the ratio of GC IRMS-derived peak areas of parent and labeled N₂O with the subtraction of coalescence with ¹⁷O and ¹⁸O isotopologues.

2. Additional Experiments

2.1. Reactivity of hydroxylamine and iron oxides

Nitrous oxide production systematically increased at higher NH_2OH concentrations. The production of N_2O from NH_2OH and ferrihydrite also responded to increased Fe concentration, while occurred in a lesser extent using maghemite. Overall, although NH_2OH has a leading effect on the induced N_2O emissions, it is also noticeable that iron oxides properties have larger effects on N_2O emissions than the bulk iron concentration does. This outcome could have important implications for soils studies, since the reaction of $\text{Fe}/\text{NH}_2\text{OH}$ is expected to occur in soils made of different Fe content and various Fe minerals.

2.2. Role of NO_2^- in abiotic N_2O production

Besides oxidation of NH_2OH , N_2O can be produced by redox reactions of Fe^{2+} with NO_2^- , which results in chemodenitrification or by reaction with soil organic matter (6), and it may dominate the N_2O emissions (7). In order to examine the role of NO_2^- in abiotic N_2O production we conducted a laboratory incubation of γ -irradiated soil with water, NH_4^+ substrate and/or NO_2^- spike during 8 hours followed by gases and inorganic N species measurements (Table S6, S7). During the experiment no accumulation of NO_2^- was observed as expected from the nitrification potential measurements (Figure S1). Laboratory incubation with a spike of KNO_2^- showed an increase in N_2O compared to initial γ -irradiated soil. Moreover, a significant consumption of NO_2^- during incubation was detected (Table S6). Likely, at high NO_2^- content it may react with organic matter and compete with oxygen for Fe^{2+} ions. The absence of NO_2^- consumption without its excess implies that AMO transformation of NH_4^+ contributes mostly to N_2O formation.

Figures

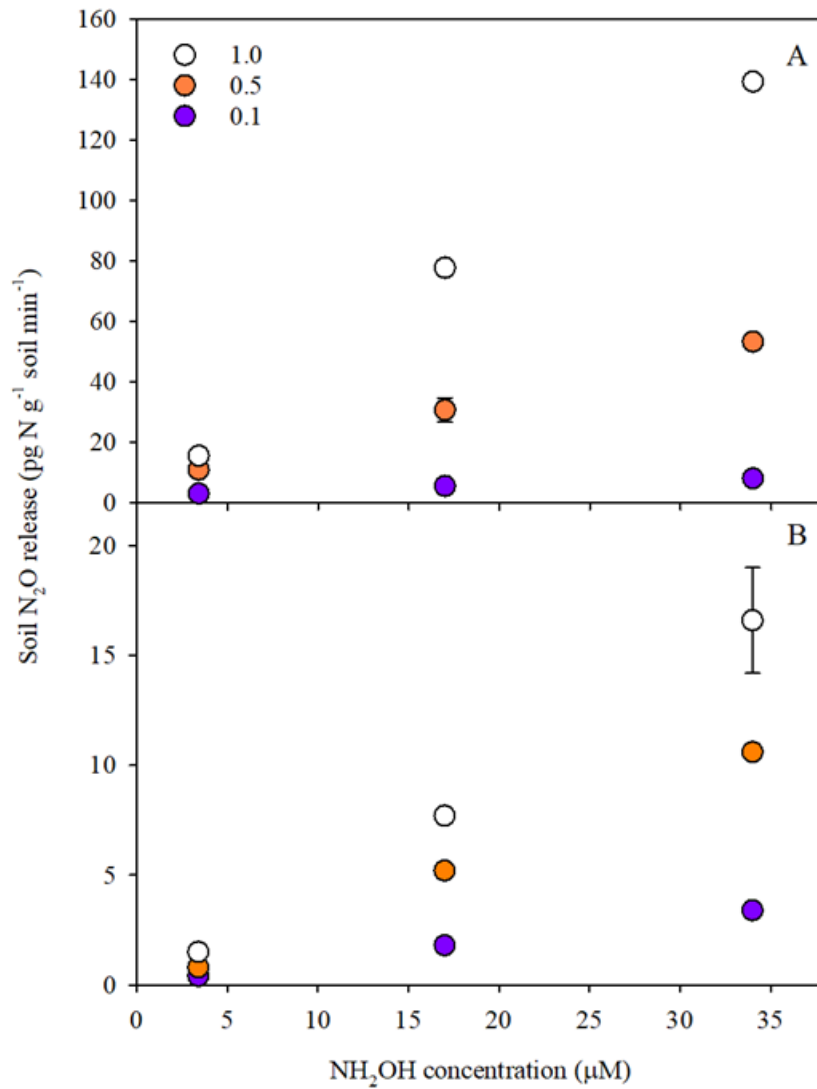


Fig. S1. Nitrous oxide fluxes (N₂O-N, pg g⁻¹ min⁻¹) after oxidation of NH₂OH with increasing concentrations of Fe-oxides of 0.1, 0.5, and 1 (g L⁻¹) in forms of A) Ferrihydrite and B) Maghemite. For each experiment an equation of linear regression is provided. Attention to scale changes across panels.

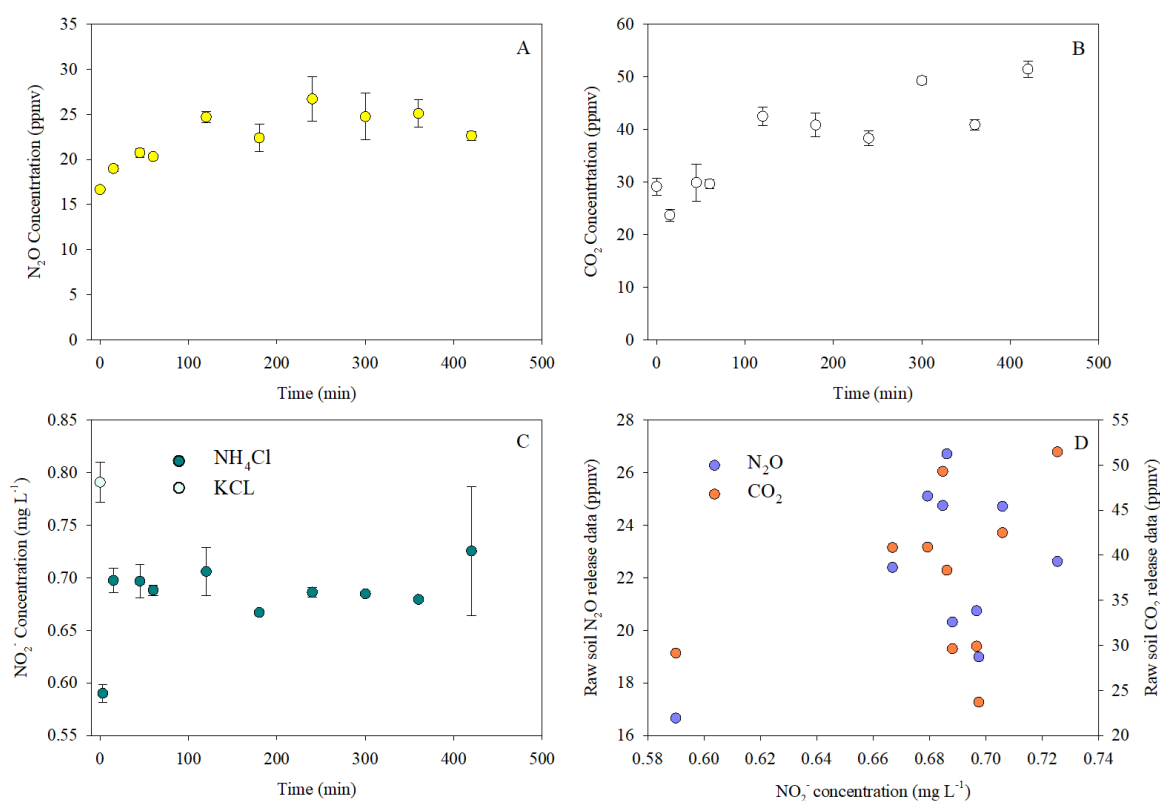


Fig. S2. Time dependence of measured headspace A) N₂O, B) CO₂ with instrumental calibration in γ -irradiated soil slurry experiments after addition of ¹⁵NH₄Cl solution; C) dissolved NO₂⁻ concentrations in filtered soil slurry; D) Dependence of N₂O and CO₂ concentrations on NO₂⁻ concentration.

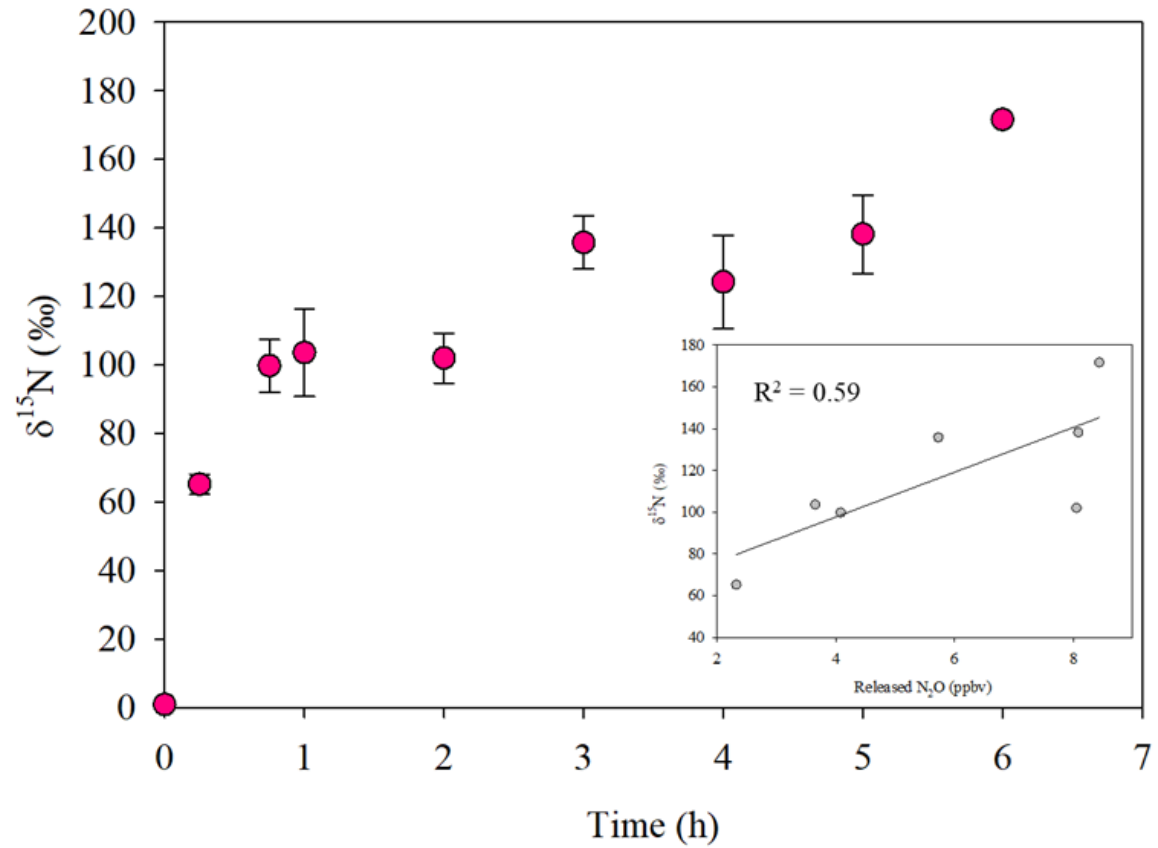


Fig. S3. $\delta^{15}\text{N}$ signature of N_2O emitted from γ -irradiated soil slurry after rewetting with $^{15}\text{NH}_4\text{Cl}$ solution. Inset shows the dependence of ^{15}N enrichment from measured N_2O emission calculated as a difference between measured concentrations at each time points and a starting point

Tables**Table S1.** Results of inorganic N-species quantification ($\mu\text{g N g}^{-1}$ soil DW) in soil during rewetting experiment; (mean $\pm$ standard error, n = 6).

Time	NH_4^+		NO_3^-		NO_2^-	
	Lower C, N	Higher C, N	Lower C, N	Higher C,N	Lower C,N	Higher C,N
Pre-wetting	2.02 (0.08)	3.09 (0.14)	2.93 (1.00)	5.28 (1.31)	0.13 (0.01)	0.17 (0.01)
15 minutes	2.90 (0.45)	4.69 (0.69)	1.25 (0.21)	5.50 (1.27)	0.11 (0.01)	1.23 (0.69)
1 hour	2.91 (0.29)	4.28 (0.50)	1.27 (0.21)	4.85 (1.27)	0.11 (0.01)	0.19 (0.03)
4 hours	3.36 (0.13)	6.12 (0.46)	1.78 (0.46)	6.76 (1.05)	0.17 (0.02)	1.11 (0.31)
24 hours	2.77 (0.14)	5.86 (0.32)	1.35 (0.07)	7.41 (1.5)	0.15 (0.01)	0.90 (0.32)
48 hours	1.79 (0.14)	3.30 (0.34)	1.10 (0.20)	6.26 (1.02)	0.10 (0.01)	0.14 (0.02)
72 hours	1.58 (0.07)	2.61 (0.19)	1.23 (0.25)	4.54 (0.76)	0.09 (0.01)	0.18 (0.03)

Table S2. Soil N₂O (ng m⁻²min⁻¹) and CO₂ (μg m⁻²min⁻¹) efflux during rewetting of minimally disturbed soil cores with different C and N-contents; (mean ± standard error, n = 6).

Time	N ₂ O-N		CO ₂ -C	
	Lower C, N	Higher C, N	Lower C, N	Higher C, N
Pre-wetting	75.64 (21.57)	99.41 (16.97)	242.6 (29.3)	251.4 (35.5)
15 minutes	167.96 (101.37)	206.23 (52.38)	423.7 (114.4)	1274.0 (216.3)
1 hour	61.71 (5.87)	86.41 (15.69)	575.6 (176.1)	1849.2 (357.3)
4 hours	44.46 (11.76)	84.14 (25.81)	571.0 (217.3)	1598.1 (160.3)
24 hours	49.75 (15.83)	106.02 (16.23)	713.7 (115.4)	1422.8 (142.9)
48 hours	80.33 (26.14)	67.74 (20.62)	428.2 (16.1)	442.6 (97.2)
72 hours	49.10 (14.45)	107.99 (24.98)	400.1 (13.8)	453.0 (88.8)

Table S3. Nitrous oxide fluxes ($\text{N}_2\text{O-N}$, $\text{ng m}^{-2}\text{min}^{-1}$) in response to Fe-oxides addition to the undisturbed soil cores; (mean $\pm$ standard error, $n = 6$).

Time	live soil	γ -irradiated soil
10 minutes	34.5 (17.0)	-
30 minutes	-	229.5 (14.9)
60 minutes	33.0 (12.6)	251.7 (48.8)
270 minutes	15.5 (2.3)	35.9 (16.1)
600 minutes	16.1 (7.6)	37.9 (12.9)
24 hours	18.1 (9.4)	12.6 (0.8)

Table S4. Response of soil N₂O fluxes (ng N m⁻²min⁻¹ N₂O-N) to NH₂OH addition in soil cores; (mean ± standard error, n = 6).

Time	live soil	γ-irradiated soil
10 minutes	121169.0 (45328.0)	65388.3 (9461.9)
45 minutes	51671.0 (16526.6)	25064.5 (647.0)
60 minutes	41613.5 (4519.9)	15784.8 (2542.9)
135 minutes	12014.1 (1733.4)	-
170 minutes	5749.1 (1311.1)	2381.9 (145.9)
4 hours	448.2 (35.0)	220.6 (25.2)
24 hours	484.3 (48.0)	91.1 (26.7)

Table S5. Nitrous oxide fluxes ($\text{N}_2\text{O-N}$, $\text{pg g}^{-1} \text{min}^{-1}$) in response to Fe-oxides addition to NH_2OH solution at different concentrations; (mean $\pm$ standard error, $n = 6$).

Concentration		$\text{N}_2\text{O-N}$	
Fe (g L^{-1})	NH_2OH (μM)	Ferrihydrite (Fh)	Maghemite (Mag)
0.1	3.4	3.0 (0.4)	0.4 (0.0)
	17.0	5.5 (0.6)	1.8 (0.0)
	34.0	8.0 (0.7)	3.4 (0.1)
0.5	3.4	10.9 (1.2)	0.8 (0.1)
	17.0	30.7 (3.9)	5.2 (0.0)
	34.0	53.3 (1.2)	10.6 (0.3)
1.0	3.4	15.5 (0.3)	1.5 (0.1)
	17.0	77.8 (1.6)	7.7 (0.3)
	34.0	139.4 (0.9)	16.6 (2.4)

Table S6. Inorganic N-species ($\mu\text{g N g}^{-1}$ soil) in γ -irradiated soil after rewetting with KCl solution (Control), water, NO_2^- and/or NH_4^+ solutions; (mean and standard error, $n = 6$).

Sample	NH_4^+	NO_2^-	Consumed NO_2^-
Control	1.66 (0.02)	0.86 (0.01)	-
Water	-	0.79 (0.01)	~0.07
NH_4Cl (1.5 mM)	-	0.83 (0.01)	~0.03
KNO_2 (0.8 ppm)	-	1.04 (0.04)	~0.62
KNO_2 (10 mM)+ NH_4Cl (1.5 mM)	-	0.97 (0.02)	~0.69

Table S7. Inorganic N-species ($\mu\text{g N g}^{-1}$ soil) in γ -irradiated soil after rewetting with KCl solution (Control), water, NO_2^- and/or NH_4^+ solutions; (mean and standard error, $n = 6$).

Sample	N_2O	CO_2
NH_4Cl (1.5 mM)	21.4 (2.9)	13.2 (0.2)
KNO_2 (0.8 ppm)	74.5 (8.4)	13.0 (0.5)
KNO_2 (10 mM)+ NH_4Cl (1.5 mM)	103.1 (17.6)	13.2 (0.2)

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