Coupled abiotic-biotic cycling of nitrous oxide in tropical peatlands

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37 Abstract

38 Atmospheric nitrous oxide (N₂O) is a potent greenhouse gas thought to be mainly
 39 derived from microbial metabolism as part of the denitrification pathway. Here, we report that
 40 in unexplored peat soils of Central and South America, N₂O production can be driven by abiotic
 41 reactions ($\leq 98\%$) highly competitive to their enzymatic counterparts. Extracted soil iron
 42 positively correlated with *in-situ* abiotic N₂O production determined by isotopic tracers.
 43 Moreover, we found that microbial N₂O reduction accompanied abiotic production, essentially
 44 closing a coupled abiotic-biotic N₂O cycle. Anaerobic N₂O consumption occurred ubiquitously
 45 (pH 6.4-3.7), with proportions of diverse clade II N₂O-reducers increasing with consumption
 46 rates. Our findings show denitrification in tropical peat soils is not a purely biological process,
 47 but rather a “mosaic” of abiotic and biotic reduction reactions. We predict hydrological and
 48 temperature fluctuations differentially affect abiotic and biotic drivers and further contribute to
 49 the high N₂O flux variation in the region.

50 Introduction

51 The atmospheric accumulation of nitrous oxide (N_2O), a potent greenhouse gas, has
 52 continued to increase^{1,2}, calling for a better mechanistic understanding of its sources and sinks.
 53 Tropical soils are a major source of N_2O . The largest contribution to global N_2O flux, along
 54 with the highest uncertainties, have been observed over South America³⁻⁵ with large flux
 55 variations described in ground-based measurements from extensive peatlands of the Amazon
 56 basin^{6,7}.

57 In waterlogged tropical peat soils, anoxic, reducing, humic acid-rich, and Fe-holding
 58 conditions are favorable for the abiotic formation of N_2O ⁸. Nitrous oxide can abiotically form
 59 from the reduction of nitrite (NO_2^-) via intermediary nitric oxide (NO), or hydroxylamine
 60 (NH_2OH)⁹, both of which have typically low steady-state concentrations in soils.
 61 Hydroxylamine conversion into N_2O relies on oxidants such as manganese (IV) minerals that
 62 are unlikely to persist in sufficient levels in the reducing milieu of peat. Thus, peatlands would
 63 generally favor the spontaneous chemical reduction of nitrogenous compounds – also called
 64 chemodenitrification. Some environments appear to sustain abiotic N_2O production rates based
 65 on dissolved Fe and Fe mineral phases¹⁰⁻¹², while others have shown an influence from organic
 66 matter (OM)⁸, presumably by providing complexed Fe^{2+} and/or humic electron shuttles¹³.
 67 Abiotic N_2O formation has been recorded in polar^{10,14} and temperate¹¹ environments, but the
 68 extent and distribution of this process in tropical peatlands have remained unexplored. With a
 69 recently estimated area of 1.7 million km^2 (ref. ¹⁵), tropical peatlands under varying climatic
 70 regimes could play a major role in global N_2O gas cycling.

71 Denitrification, generally occurring at oxygen concentrations below $6 \mu\text{M}$ ¹⁶, is
 72 considered to be driven predominantly by microbial communities using Fe- and Cu-dependent
 73 reductase enzymes¹⁷ through a modular pathway structure with different populations mediating
 74 only one or two reduction steps^{18,19}. Denitrifying microbes are well adapted to the conditions

found in peat soils because they anaerobically respire organic substrates using nitrogen oxides as terminal electron acceptors^{20,21}. Also, the extensive N₂O sink potential previously observed in diverse soils^{22,23} can be better explained with the discovery of the abundant clade II N₂O-reducing bacteria. While clade I N₂O-reducers are affiliated to the *Proteobacteria*, clade II N₂O-reducers are more diverse and scattered across multiple phyla²⁴. Interestingly, the clade II members tend to lack NO₂⁻ reductases more so than clade I members²⁴. From an ecological perspective, this trait might correspond with an intrinsic capability of the soil habitat to reduce NO₂⁻ via chemodenitrification. Cellular resources can be saved and relocated to the expression of NO and N₂O reductases²⁵ to catalyze a thermodynamically more favorable redox reaction (ΔG of N₂O reduction is ~100 kJ mol⁻¹ higher than ΔG of NO₂⁻ reduction).

While interactions between microbial guilds have been proposed as the basis for modularity²³, the interplay of denitrifiers with abiotic reactions has received little attention, even though chemodenitrification can reduce or contribute to different inorganic nitrogen pools, including N₂O and NO. The compatibility of abiotic N₂O production and modular microbial denitrification led us to hypothesize that a coupled abiotic-biotic N₂O cycle could operate in tropical peatlands. To test our hypothesis, we explored the dynamics and underlying factors of abiotic N₂O formation and microbial N₂O reduction in six peatlands located across Central and South America using isotopic tracers. Simultaneously, we quantified and sequenced the *nosZ* gene as a marker for the N₂O-reducing microbial community. Our results provide evidence for concomitant abiotic N₂O production and microbial consumption active under various peat soil conditions.

96 Results

97 *Fe²⁺ drives abiotic formation of N₂O in high-N₂O soils*

98 We assessed soil denitrification in six tropical peatlands, of which four were located
 99 within or near the Amazon basin (San Jorge, SJO; Melendez, MEL; Sítio do Cacau, SCB,
 100 Fazenda Córrego da Areia, FCA) and two in Central America (Medio Queso, MQE and Las
 101 Vueltas, VUL) (Fig. 1). Measured steady-state concentrations of NO₂⁻ in soil pore water were
 102 below detection (< 1 μM) in the majority of sites, indicating rapid cycling^{26,27}. The iron content
 103 and redox balance were highly variable, with higher Fe²⁺ concentrations in mountainous peat
 104 (~5 mM) and lower Fe²⁺ in oligotrophic peat (0.01-0.04 mM). To determine abiotic N₂O
 105 production rates under near-natural conditions, we induced a ten-fold spike with ¹⁵NO₂⁻ *in-situ*
 106 and measured ¹⁴N¹⁵NO + ¹⁵N¹⁴NO + ¹⁵N¹⁵NO evolution. Biotic activity was arrested by
 107 amending the soil with 87.5 mM ZnCl₂. Because the addition of Zn can liberate Fe²⁺ ions
 108 inevitably stimulating N₂O production⁸, we repeated the soil incubations in the laboratory, with
 109 100 μM NO₂⁻, using both gamma-irradiated and Zn-treated peat soil. We then deduced a site-
 110 specific correction factor for estimates of *in-situ* rates. All our reported abiotic N₂O production
 111 rates are therefore corrected for Zn-induced N₂O production.

Fig. 1. Contribution of abiotic and biotic reactions to overall N₂O production in tropical peatlands. Rates were derived *in situ* from the enrichment of ¹⁵N in N₂O after addition of ¹⁵NO₂⁻ to soil in the field (n = 4). Dissolved nitrogen (measured *in-situ*) and Fe species (extracted) concentrations are given in μM. Sites are color-coded based on their NO to N₂O yield (Table 1), showing high-NO yield (red shades) or high-N₂O yield (blue shades).

Abiotic N₂O production was observed in all peatlands. *In-situ* rates ranged from low (0.05-0.3 nmol N₂O g⁻¹ d⁻¹) at FCA and SCB, moderate (2.4-3.3 nmol N₂O g⁻¹ d⁻¹) at MQE and SJO, and high (9.2-39.0 nmol N₂O g⁻¹ d⁻¹) at MEL and VUL. Abiotic N₂O production contributed to the overall N₂O flux to a greater extent than biotic N₂O production at half the field sites (Fig. 1). Soil Fe²⁺ concentrations measured after extraction positively correlated with abiotic N₂O production rates ($R^2 = 0.99$, n = 6, Supplementary Fig. 1). To determine the nitrogen yield of the chemodenitrification reaction, we incubated gamma-irradiated peat soil under anoxic conditions with 100 μM NO₂⁻, and quantified NO₂⁻, NO and N₂O in time (Supplementary Fig. 2). In two peatlands (SJO, SCB), complete denitrification was achieved

almost based purely on abiotic reactions (Table 1). The transformation of NO_2^- into NO and N_2O resulted in dominant yields of either product across sites, suggesting unequal nitrogen diversion directed by local peat chemistry. Our analytical approach could not confirm N_2 as a byproduct²⁸, which was presumably dominant at circum-neutral pH sites (MEL and VUL). We used this observed divergent NO_2^- conversion to group the diverse peat soils into high- N_2O (MQE, VUL, MEL) and high-NO (FCA, SJO, SCB) abiotic-yield sites (Table 1).

To our knowledge, this study represents the first assessment of the relative contribution of abiotic N_2O production to the overall N_2O production at near-natural NO_2^- levels. Based on our results, abiotic reactions outcompete biotic reactions in three peatlands and are highly competitive as a source of N_2O at another two. The measured N_2O production rates were comparable to reported rates from a coniferous forest and grasslands²⁹, although the amount of added NO_2^- was at least one order of magnitude lower in our study. Relative to other evaluated ecosystems¹⁰, peat soils have less oxidized Fe or Mn minerals and are enriched in recalcitrant organic carbon, which would hold additional reducing power, particularly in the structurally disparate OM. For instance, pi-electron bonds are an integral part of the chemical structures found in recalcitrant organic carbon, such as phenolic or humic substances, and they are prone to interact with NO_2^- (refs. ^{30,31}). Besides serving as reactants, humic substances can act as regenerable electron shuttles for redox reactions in soils and sediments³². Iron reduction and dissolution are greatly enhanced in the presence of humic substances^{33,34}, which increases the availability of Fe^{2+} . The distinct production of NO and N_2O across a gradient of Fe^{2+} concentrations suggests divergent reaction mechanisms in high-NO and high- N_2O soils. Previous reports agree with our data that indicate the larger production of NO as the final product of chemodenitrification, which is stimulated by the self-decomposition of nitrous acid in increasingly acidic soil milieu^{35,36}. High- N_2O soils coincided with high soil Fe^{2+} abundances,

and high-NO sites were associated with low Fe^{2+} (Fig. 1, Table 1). Besides Fe^{2+} , the mixture of functional groups in peat OM may also be crucial in determining the NO to N_2O balance. Except for dimethyl glyoxime and quinone oximes, oxime groups preferentially produce N_2O and aromatics tend to produce NO^{37} .

Nitrite incorporation into OM may explain lower nitrogen yields in either NO or N_2O , in addition to the possible production of N_2 . For example, nitrosophenol (a phenol moiety that binds one NO_2^- ion) tautomerizes to quinone monoxime and requires another NO_2^- ion to eject hyponitrous acid which decomposes to $\text{N}_2\text{O}^{30,31}$. Without the consecutive incorporation of 2 NO_2^- ions, nitrosophenol remains stable, and a significant amount of NO_2^- could reside in nitrosated functional groups.

Table 1. Abiotic nitrogen yield fractions based on sterilized batch incubations. Gamma-irradiated peat soil was used for anoxic incubations initiated with the addition of 100 μM NO_2^- . Yield was calculated after all NO_2^- was consumed and based on stable NO and N_2O concentrations in at least two consecutive measurements. Sites with replicated samples ($n = 3$) included: Las Vueltas (VUL), Medio Queso (MQE), Fazenda Córrego da Areia (FCA), Melendez (MEL), Sítio do Cacao (SCB), San Jorge (SJO).

	SCB	SJO	FCA	MEL	VUL	MQE
Yield in NO (%)	97.2	92.8	74.3	0.2	0.2	1.3
Yield in N_2O (%)	4.8	4.8	4.0	12.1	24.6	55.9
Total yield (%)	102	97.6	78.3	12.3	24.8	57.2

Active microbial N_2O reduction in acidic peat soils

Concomitant to N_2O production, we measured N_2O consumption. Only non-sterilized samples exhibited active consumption. In sterilized samples, N_2O was a stable end product after NO_2^- addition, and $^{15}\text{N}_2$ was not produced in $(^{15}\text{N})_2\text{O}$ amendments. Incubations with $(^{15}\text{N})_2\text{O}$ in the field resulted in an accumulation of the ^{15}N label in N_2 (Fig. 2) and were used to derive N_2O reduction rates. Enrichment of $^{15}\text{N}_2$ decreased with soil pH (Fig. 2), while N_2O reduction was

180 surprisingly observed in soils with pH as low as 3.7 (SJO). This finding is significant because
 181 N₂O reductase assembly is post-transcriptionally inhibited by acidic pH³⁸, and exposure to pH <
 182 4 disrupts a histidine amino acid ligand to the Cu cofactor in N₂O reductase, possibly
 183 inactivating the catalytic function (pers. com. W. Nitschke). The measured N₂O reduction rates
 184 were higher than previously observed rates at similar acidic pH values³⁹ and would extend the
 185 known physiological limits for microbial N₂O consumption. Thus, these results demonstrate the
 186 presence and activity of N₂O-reducing communities adapted to a wide range of peat soil pH.

187
 188 **Fig. 2. Isotopic enrichment in molecular nitrogen during *in-situ* incubations of (¹⁵N)₂O**
 189 **with anoxic peat soil.** Replicates per sites (n = 3), as listed, are colored in a gradient according
 190 to their pH. VUL, Las Vueltas; MQE, Medio Queso; FCA, Fazenda Córrego da Areia; MEL,
 191 Melendez; SCB, Sítio do Cacao; SJO, San Jorge.

196 **Diverse Clade II *N₂O*-reducers are associated with higher *N₂O* sink potential**

197 To evaluate the relationship between the abiotic formation and microbial consumption
 198 of *N₂O*, we compared reaction rates against *nosZ* gene abundances. Both processes revealed
 199 similar trends ranging from low (0.1-0.3 nmol *N₂O* g⁻¹ d⁻¹) in SCB and FCA to moderate (0.7-
 200 1.5 nmol *N₂O* g⁻¹ d⁻¹) in MQE and SJO to high (3-9.5 nmol *N₂O* g⁻¹ d⁻¹) in VUL and MEL.
 201 Consumption never outpaced production, except at two sites (FCA and MEL, Fig. 3). While the
 202 variation in *nosZ* gene copies from both clades showed no significant differences among high-
 203 NO sites (FCA, SJO, SCB), they differed (ANOVA, *p* = 0.05) among high-*N₂O* sites (MQE,
 204 MEL). Consumption rates gradually increased with clade II *nosZ* gene abundance at high-*N₂O*
 205 sites. A clear dominance of clade II *nosZ* genes over those from clade I coincided with the
 206 elevated rates of *N₂O* consumption in MEL peatland. Thus, *N₂O* reducers from clade II
 207 establish an increased microbial *N₂O* sink in peatlands with high abiotic *N₂O* fluxes.

208

Fig. 3. Microbial N₂O consumption and abiotic N₂O production (bars) along with *nosZ* gene quantities (open and filled circles). Error bars denote SD (consumption rates, n = 3; production rates, n = 4). Clade I and II *nosZ* was quantified by qPCR assays and are significantly different within sites as denoted by asterisks (ANOVA, **p* = 0.012, ***p* = 0.008, n = 4). Two outliers for *nosZ-II* (SCB, ~6,200 copies g⁻¹; FCA, ~590) are not shown and another two datapoints are missing due to non-amplification. See previous figures for site acronyms.

Next, to evaluate the N₂O-reducing microbial community, we analyzed 183,265 and 80,050 taxonomically assigned *nosZ* gene amplicon sequence variants (ASVs) for clade I and II, respectively. Our analysis focused on the most abundant taxa that made up at least 1% of the total ASVs in at least one site (Fig. 4). The most abundant ASV was affiliated to the alpha-proteobacterium *Nitrospirillum amazonense* (Fig. 4). This phylotype constituted 59-64 % of clade I ASVs in the Amazon bogs SCB and SJO but was least represented in the MEL peatland (~10 %). In MEL, 23 % of ASVs belonged to *Methylocystis* species, which were also abundant in FCA (27 %). The clade II N₂O reducers were more diverse (comprised more phyla) across all soils (Fig. 4), with *Magnetospirillum* (consistently > 10 % in high-NO sites and 30 % in VUL) and unclassified *Myxococcales* (8-50 %) as the most abundant phylotypes. To examine the observed trend of N₂O reduction rates corresponding with *nosZ* clade II gene frequencies in the high-N₂O sites (MEL > VUL > MQE, Fig. 3), we derived diversity indices and performed a principal component analysis (PCA). The Shannon diversity index showed a coinciding order of diversity levels (1.73 > 1.49 > 1.39, Supplementary Tables 1a-b) for high-N₂O sites. This was also supported by a relatively high average Bray-Curtis dissimilarity of clade II *nosZ* gene sequences in MEL (0.71, Supplementary Tables 1c-d), identifying the clade II N₂O reducer community in this peatland as most dissimilar to all others. In addition, the community structure variation among clade II was most parsimoniously explained by N₂O consumption rates in the PCA (Supplementary Figs. 3, 4). Rather than single dominant taxa, it appeared to be the contribution of a diverse group of clade II N₂O reducers to be responsible for the high N₂O sink potential observed.

The intrinsic capacity of the peat to reduce NO_2^- abiotically could provide non-denitrifying microbes that do not possess denitrification enzymes other than N_2O reductase, called “chemodenitrifiers”⁴⁰, an advantage over canonical denitrifiers. Chemodenitrifiers do not have to compete with chemodenitrification and simply harvest the end product N_2O to oxidize organic substrates. Figure 4 illustrates the NO_2^- reductase (either NirS or NirK) and NO reductase (NorB) enzyme repertoires present in available reference proteomes of relatives of the predicted taxa in both clades. At least 2 out of the 11 clade I taxa (including the abundant *Nitrospirillum*) and 10 out of the 14 clade II taxa indicated the absence of Nir enzymes (Fig. 4). Half of the clade II reference proteomes were missing both Nir and Nor proteins. Importantly, the Myxococcales ASVs showed no differences in abundance among high-NO sites but gradually increased, similar to the N_2O yield, in the high- N_2O sites. This order, which also includes *Anaeromyxobacter dehalogenans*, the hallmark organism of clade II N_2O reducers^{22,41}, is frequently represented in acidic, organic-rich soils^{40,42}, presumably with abiotic NO and N_2O production potential. Further, other bacteria such as *Dechloromonas*⁴³, *Ardenticatena*⁴⁴, and *Melioribacter*⁴⁵ also mediate iron reduction, an additional trait that could promote chemodenitrification by recycling Fe^{2+} . Therefore, chemodenitrifiers may outcompete canonical denitrifiers in the studied peatlands (e.g., *Nitrospirillum*) and abundance patterns of the Myxococcales suggest a notable benefit for some chemodenitrifiers in soils associated with high abiotic N_2O yields.

Another abundant taxon of the N_2O -reducing community was *Magnetospirillum* (clade II) that includes several iron-oxidizing species. These alpha-proteobacteria synthesize the mixed-valence iron mineral magnetite, which can accumulate in soils, also under the influence of abiotic crystallization⁴⁶. Secondary iron mineral formation can be widespread in the tropics, driven by dissolved and particulate iron originating from weathering and desilication⁴⁷⁻⁴⁹. Ferrous iron-bearing minerals, such as magnetite, can serve as catalysts for NO_2^- reduction by

providing reaction sites at the mineral surface^{50,51}. The possession of an N₂O reductase makes sense for *Magnetospirillum*, assuming cells are associated with, or at least grow in proximity to, magnetite. Analyses of the iron phases present would be necessary to follow up on this in more detail, which was off the scope of our study. We also acknowledge that our insight into the temporal activity response is limited. Information on the actual *in-situ* transcription levels is needed to better assess how the clades react to fluctuating abiotic pulses of N₂O⁵².

271 **Fig. 4. NosZ phylogeny and taxonomy in tropical peat soils.** Only the most abundant
 272 amplicon sequence variants (ASVs) > 1 % were included in the analysis. Maximum likelihood
 273 phylogenetic trees are based on 1000 iterations. Nodes with 60 % or higher bootstrap support
 274 are labeled. The right panel indicates the presence (filled box) or absence (crossed box) of Nir
 275 or Nor enzyme sequences in reference proteomes. Boxes with question marks indicate an
 276 ambiguous distribution of Nir or Nor within the taxonomic group (Supplementary Table 2).

277 Discussion

278 *Decoupling of abiotic N₂O production and microbial N₂O consumption*

279 Our data show the concomitant occurrence of both abiotic N₂O production and
 280 microbial consumption (Fig. 3 and Supplementary Fig. 2), and their positive correlation points
 281 to the coupling of both processes in several sites (Supplementary Fig. 5). However, the
 282 mountain bog site (VUL) exhibited unusually high abiotic production rates and relatively low
 283 consumption rates (Fig. 3). A lower soil temperature than in the other peatlands and the
 284 differential sensitivity of production and consumption (Supplementary Table 3 and
 285 Supplementary Text) could lead to such kinetic effects⁵³⁻⁵⁵. Along these lines, the decoupling of
 286 production and consumption establishes the potential for vast N₂O emissions when changing
 287 environmental conditions impart selectively negative effects on consumption. For instance,
 288 while peatland drainage occurs naturally between wet and dry seasons⁵⁶, N₂O cycling could
 289 become decoupled by aerobic conditions created by extended peatland drainage with microbial
 290 denitrification persisting only in anoxic microsites. Nitrite, fueled by increased nitrification,
 291 could still be abiotically reduced to N₂O because acidic peat soil stabilizes Fe²⁺ via two
 292 mechanisms. First, Fe²⁺ oxidation by oxygen is kinetically hindered at low pH. Oxidation rates
 293 are significantly slowed at pH ≤ 6.5⁵⁷, a pH regime applying to most peatlands, including all in
 294 our study. Second, Fe²⁺ complexed by OM is resilient to oxidation. Experimental evidence
 295 suggests that tannic acid⁵⁸, phenolics⁵⁹, or natural humic acid⁶⁰ stabilize the Fe²⁺ pool in the
 296 presence of oxygen by the formation of a redox-buffering shell⁶⁰ and re-reduction of Fe³⁺.
 297 However, little is known concerning how Fe²⁺ complexation affects the NO₂⁻ accessibility and
 298 reduction. Nevertheless, these previous findings indicate that the reactants for
 299 chemodenitrification are sufficiently available even at higher oxygen concentrations (> 6 μM),
 300 leading to a potential predominance of abiotic N₂O production over biotic N₂O production.

301

Fig. 5. Schematic representation of denitrification pathways in tropical peatlands. Nitrate (NO_3^-) reduction to NO_2^- occurs at significant rates only with catalysis by NO_3^- reductases. Nitrite is rapidly reduced via abiotic and biotic reactions. At lower pH (≤ 5.4), NO is the dominant product. Nitrosation into OM may be an alternative abiotic process in soils with minor nitrogenous gas production reliant on organic compounds containing pi-electron bonds (π). In Fe^{2+} -rich peat, N_2O is the dominant product, involving Fe redox cycling that can fuel dissimilatory Fe reduction¹¹. The only N_2O consumption pathway in peat soil is N_2O reductase-dependent reduction to N_2 , which is active even in the most acidic soils tested (pH 3.7). All related heterotrophic reactions induce oxidation of OM ($\text{OM}_{\text{red}} \rightarrow \text{OM}_{\text{ox}}$) and eventually peat carbon mineralization.

We present evidence that active abiotic-biotic N_2O cycling is prevalent in tropical peatlands, where denitrification is not a purely biological pathway, but rather a “mosaic” of biotic and abiotic reduction reactions (Fig. 5). Furthermore, our results support the idea that functional modularity complements not only the interrelationship of microbial groups but also concomitant interactions between microbes and spontaneous chemical reactions. Abiotic N_2O formation in tropical peatlands can have important regional consequences in the context of observed N_2O fluxes and higher rates in response to drainage⁶¹, and putatively drought⁶², as well as possible effects on reducing organic carbon release⁶³. For example, compared to the other soils, abiotic N_2O production was moderate in SJO, an acidic oligotrophic site, showing a net production of $1.8 \text{ nmol } \text{N}_2\text{O } \text{g}^{-1} \text{ day}^{-1}$. With the estimation of the global extent of acidic oligotrophic tropical peatlands alike SJO at $1,003,719 \text{ km}^2$ (ref. ¹⁵), this could amount to a total depth-integrated abiotic N_2O flux ranging from 0.1 to $3.9 \text{ Tg } \text{N}_2\text{O } \text{yr}^{-1}$ depending on the depth of NO_2^- diffusion. This contribution is a major uncertainty that could account for two percent to

327 more than half of all combined terrestrial N₂O fluxes from natural sources of South America,
 328 Africa, and Southeast Asia. With a factor of 298 g CO₂ -equivalents per g N₂O over a 100 year
 329 time⁶⁴, abiotic N₂O fluxes could also alter the net radiative effect of tropical peatlands.
 330 Accordingly, by bypassing heterotrophic respiration through chemodenitrification, less organic
 331 carbon is mineralized to CO₂. Considering ~50 mg CO₂-C m⁻² yr⁻¹ emitted from SJO-like
 332 tropical peatlands⁷ and 4 moles of N₂O required to mineralize 1 mole of organic carbon,
 333 chemodenitrification would suppress at least 2.7 % of the organic carbon mineralization,
 334 promoting the retention of 2 Tg C yr⁻¹ across oligotrophic tropical peatlands. These estimates
 335 are conservative because they do not include processes such as diversion of nitrogen oxides into
 336 NO, nitrosation of OM, and the consumption deficit observed in the high-altitude peatland.
 337 Sensitivity to lower temperatures could also impede microbial N₂O reduction in northern
 338 peatlands, which would imply an imbalanced cycling of N₂O and substantial N₂O release from
 339 abiotic origins.

340 **Methods**

341
342 **Study sites.** Six peatlands were chosen to cover a geochemical spectrum, including acidic (pH
343 3.7-5) soils, low (10 μM) and high ($> 5 \text{ mM}$) Fe^{2+} concentrations, varying OM content and soil
344 temperature (Supplementary Table 4). Most sites were under little to no anthropogenic
345 influence (Supplementary Table 5), with two exceptions: Fazenda Córrego da Areia (FCA)
346 located within a catchment experiencing agricultural run-off in Brazil, and Medio Queso
347 (MQE) in a Costa Rican river delta surrounded by agricultural run-off and cattle raising. The
348 San Jorge (SJO) peatland is located in the Pastaza-Marañón foreland basin and Melendez
349 (MEL) is in the Madre de Dios river terraces, both in Peru. Sítio do Cacau (SCB) is located in
350 Central Amazonia (Amanã Reserve) in Brazil. Las Vueltas (VUL), located in Costa Rica's
351 cloud forests of the Cerro Las Vueltas Reserve, differed most drastically from the other sites
352 due to its higher altitude (2,500 m a.s.l.). Field work was conducted in September 2017 (Peru)
353 and between April (Costa Rica) and July (Brazil) in 2018.

354
355 **^{15}N tracer experiment in the field.** Colorimetric assays to determine ambient soil NO_2^- and
356 NO_3^- concentrations were performed in the field using a YSI 9500 portable spectrophotometer
357 (YSI Inc.), including reagent kits, according to the manufacturer's instructions. Dissolved N_2O
358 was sampled by collecting pore water into a pre-evacuated vial and subsequent degassing by
359 shaking for 5 minutes. Thereafter, headspace was transferred into a pre-evacuated vial and
360 stored underwater prior to analysis with a GC-ECD. Soil temperature and pH were measured
361 with a YSI A10 pH probe (Ecosense, YSI).

362 Anaerobic glove bags filled with argon (Ar) were used to provide anaerobic conditions
363 in the field while distributing soil into glass incubation vials (160 mL). Topsoils (10 cm) were
364 sampled with 30 ml-barrel customized plastic corers. Inside the glove bag, the center 5 cm (~ 15
365 g) soil was diluted 1:5 (w/v) into vials with anoxic water extracted directly from the deep peat

soil via a water line connected to the glove bag. Separate sample sets received $^{15}\text{NO}_2^-$ (label fraction = 0.1, Cambridge Isotopes) at 10× the soil ambient NO_2^- concentration and doubly labeled $(^{15}\text{N})_2\text{O}$ (label fraction = 1.0, Cambridge Isotopes) at 5× the soil ambient N_2O concentration. Thus, the amount of ^{15}N tracer applied varied slightly between sites but reflected a similar order of magnitude. $^{15}\text{NO}_2^-$ incubations included untreated and 87.5 mM zinc chloride-poisoned (ZnCl_2 , Fisher Scientific) soils in replicates of four ($n = 4$). Soil slurries were incubated in insulating containers to avoid temperature fluctuations, and gas samples were taken for $(^{15}\text{N})_2\text{O}$ analysis at the beginning of incubation and after 24 h ($n = 4$), and for $^{30}\text{N}_2$ analysis at four time points spread over 36 h ($n = 3$). Gas sampling was destructive (entire headspace used) for $(^{15}\text{N})_2\text{O}$ analysis or by replacement with 5 mL Ar gas for $^{30}\text{N}_2$ analysis. The sample times for the $^{30}\text{N}_2$ analysis were adapted from a previous study⁶⁵. We also prepared zinc-poisoned $(^{15}\text{N})_2\text{O}$ incubations to test for abiotic N_2O consumption. The gas samples were stored underwater in borosilicate glass vials closed with thick butyl rubber stoppers⁶⁶ prior to analysis at Michigan State University. Isotopic compositions of N_2O and N_2 were measured using an Elementar Isoprime isotope ratio mass spectrometer (IR-MS) interfaced with an Elementar TraceGas chromatographic system. Rate calculations closely followed a previously developed and tested protocol¹⁴. Given the constraints of sterilant applications in the field, we repeated the zinc-amended incubations in the lab, complementarily to incubations with gamma-irradiated soils. The rates from both experiments were used to calculate a correction factor accounting for artifacts caused by the zinc addition⁸. The rates derived in the field were then multiplied by the correction factor (Supplementary Fig. 6 and Supplementary Table 6). The Brazilian sites SCB and FCA have associated data from gamma-irradiated soil, but data from zinc-treated soil are missing because of logistic issues concerning the shipment of non-sterilized (not gamma-irradiated) soil. The final rates were combined according to the following equation for net *in-situ* N_2O formation:

$$B = M + C - A$$

where B is the biotic N_2O production rate, M is the mixed rate (from untreated $^{15}\text{NO}_2^-$ incubations), C is the microbial N_2O consumption rate [from $(^{15}\text{N})_2\text{O}$ incubations], and A is the abiotic N_2O production rate (from poisoned $^{15}\text{NO}_2^-$ incubations).

Laboratory incubations. In an anoxic glove box (0.1% H_2 for O_2 reduction in N_2), gamma-sterilized and zinc-treated soil were prepared separately. Gamma sterilization followed a previous method⁸. Zinc chloride was applied, as described for field experiments. Roots and coarse particles (> 5 mm in diameter) were removed from soils, and soils were slurried 1:5 (w/v) in anoxic, sterile 18.2 MΩ·cm water. The slurry was homogenized before equal quantities were distributed into culture vials and sealed with sterile butyl rubber stoppers. An anoxic and filter-sterilized NO_2^- solution was injected (final concentration 100 μM) into microcosms that were previously flushed with pure N_2 . The microcosms were agitated briefly to disperse the added substrate and then kept under dark, static conditions at room temperature for ~100 h. Dissolved NO_2^- was quantified with the Griess reagent (Promega, Kit G2930), and NO and N_2O were analyzed as described below.

Soil Fe measurements. Soils for Fe analysis were kept in anoxic serum bottles and refrigerated during transport. The species Fe^{2+} and Fe^{3+} were extracted and separated as previously described⁸ and quantified in acidified aqueous solution by inductively coupled plasma–optical emission spectrometry (ICP-OES; Thermo iCAP6300 at the Goldwater Environmental Laboratory at Arizona State University). The ICP-OES pump rate for the Ar carrier was set to 50 rpm, and Fe2395 and Fe2599 lines were used for Fe quantification. Iron concentrations were determined from a calibration curve (0.01-10 mg L^{-1}) by diluting a standard solution (100 mg L^{-1} , VHG Labs, product no. SM75B-500) in 0.02 N HNO_3 .

416

417 ***N₂O gas measurements.*** Using a gas-tight syringe (VICI Precision Sampling), 200 μ L of gas
 418 sample was injected into a gas chromatograph (GC, SRI Instruments) equipped with an
 419 electron-capture detector (ECD). Two continuous HayeSep-D columns were kept at 90 °C
 420 (oven temperature), and N₂ (UHP grade 99.999 %, Praxair Inc.) was used as carrier gas. The
 421 ECD current was 250 mV, and the ECD cell was kept at 350 °C. The N₂O measurements were
 422 calibrated over a range of 0.25-100 ppmv using customized standard mixtures (Scott Specialty
 423 Gases, accuracy ± 5 %). Gas concentrations were corrected for solubility effects using Henry's
 424 law and the dimensionless concentration constant $k_H^{cc}(\text{N}_2\text{O}) = 0.6112$ to account for gas
 425 partitioning into the aqueous phase at 25 °C and 1 atm⁶⁷.

426

427 ***NO gas measurements.*** Nitric oxide (NO) was quantified in the microcosm headspace with a
 428 chemiluminescence-based analyzer (LMA-3D NO₂ analyzer, Unisearch Associates Inc.,
 429 Concord, Canada). Headspace gas (50 μ L) was withdrawn with a CO₂-N₂-flushed gas-tight
 430 syringe and injected into the analyzer. The injection port was customized to fit the injection
 431 volume and consisted of a T-junction with an air filter at one end and a septum at the other end.
 432 An internal pump generated consistent airflow. Our method followed a previous protocol⁶⁸,
 433 with minor adjustments. Briefly, NO was oxidized to NO₂ by a CrO₃ catalyst. The NO₂ was
 434 carried across a fabric wick saturated with a Luminol solution (Drummond Technology Inc.,
 435 Canada). Readings were corrected for background NO₂ every 15 min ("zeroing"). Shell airflow
 436 rate was kept at 500 mL min⁻¹, and the span potentiometer was set to 8. Measurements were
 437 calibrated with a 0.1 ppm NO (in N₂) standard (< 0.0005 ppm NO₂, Scott-Marin, Riverside,
 438 CA, USA) over a range of 5-1,000 ppbv. Gas concentrations were corrected using Henry's law
 439 and the dimensionless concentration constant $k_H^{cc}(\text{NO}) = 0.0465$ to account for gas partitioning
 440 into the aqueous phase at 25 °C and 1 atm⁶⁷.

441

442 ***Molecular analyses.*** Peat samples from four randomly distributed locations (coinciding with
443 incubation locations) within a peatland were collected and frozen at -20°C for subsequent
444 DNA extraction. Genomic DNA was extracted using a NucleoSpin Soil DNA extraction kit
445 (Macherey-Nagel GmbH, Düren, Germany).

446 For quantitative polymerase chain reactions (qPCR), we used two primer pairs and a
447 total reaction volume of $15\ \mu\text{L}$ with $1.5\ \mu\text{L}$ DNA template (35-50 ng genomic DNA). The clade
448 I *nosZ* gene was amplified with PowerUp SYBR Green master mix (Applied Biosystems,
449 Foster City, CA), to which $3\ \text{mM}$ MgCl_2 was added. Forward and reverse primer concentrations
450 were $1\ \mu\text{M}$, and previous cycler conditions were used⁶⁹. The clade II *nosZ* gene was amplified
451 using SYBR Fast, ROX low master mix (Kapa Biosystems, Roche Holding AG, Basel,
452 Switzerland), and $1.2\ \mu\text{M}$ primer concentration⁷⁰. Thermal cycling was initiated with 3 min of
453 denaturing at 95°C , followed by 40 cycles of the following stages: 30 s at 95°C , 60 s at 58°C ,
454 30 s at 72°C , 30 s at 80°C , and a final melting-curve. Samples were run in technical duplicates
455 on 96-well plates using a Quantstudio 3 thermocycler (Applied Biosystems, Foster City, CA).
456 Standards were prepared using linearized plasmids. Standard curves indicated efficiencies of 94
457 % ($R^2 = 0.99$, *nosZ* clade I) and 85 % ($R^2 = 0.99$, *nosZ* clade II) and melting curves showed no
458 detectable primer dimers over the duration of 40 amplification cycles.

459 For Illumina amplicon sequence analysis, we developed independent *nosZ* clade I and II
460 libraries. PCR-amplification of both *nosZ* genes used the Promega GoTaq qPCR kit (Promega,
461 Madison, WI) and $1\ \mu\text{L}$ of DNA template (25-50 ng genomic DNA) in a total reaction volume
462 of $20\ \mu\text{L}$. Targeting the clade I *nosZ* gene, we used a novel primer pair⁷¹. The reaction mix
463 included $0.2\ \text{mg mL}^{-1}$ bovine serum albumin (BSA) and $0.8\ \mu\text{M}$ primer concentration. For the
464 clade II *nosZ* gene, we used the same primer as used for qPCR in reactions of $1\ \text{mg mL}^{-1}$ BSA
465 and $0.8\ \mu\text{M}$ primer concentration. Cycling conditions for clade II *nosZ* amplification were used

as described⁷⁰. Thermal cycling conditions for clade I *nosZ* amplification were an initial 2 min denaturing step at 95 °C, followed by 33 cycles of 95 °C for 45 s, annealing by 53 °C for 45 s, and a 72 °C extension for 30 s, and a final extension at 72 °C for 7 min. Amplification was verified by gel electrophoresis using 1 % agarose in 1 Tris-acetate-EDTA buffer. Samples were multiplexed⁷², normalized (SequalPrep kit #1051001, Invitrogen), and submitted for sequencing to the DNASU core facility at Arizona State University, with 2× 300-bp paired-end Illumina MiSeq.

Paired-end sequences were merged and demultiplexed, then we used the USEARCH pipeline⁷³ to 1) correct strand orientations, 2) sort out singletons, and 3) denoise the dataset. We used $\alpha = 2$ for a stringent denoising of sequences⁷⁴ because reads were not clustered with any identity radius to obtain ASVs. The sequences were translated and frameshift-corrected by Framebot⁷⁵ with low sequence loss (< 10 %). The amino acid sequences obtained were classified using Diamond⁷⁶ version 0.9.25. The search was conducted in Diamond's *sensitive* mode, with an e-value cutoff of 10^{-5} , resulting in the top 5 % hits. Sequences were parsed through two databases; the NCBI database RefSeq (release 95) containing 146,381,777 non-redundant protein sequences and manually curated databases built from 2,817 (clade I) and 2,929 (clade II) sequences off the FunGene repository⁷⁷ using the search parameters 80 % HMM coverage and a minimum length of 550 amino acids. The taxonomy achieved with the curated databases was used for downstream analysis because of a higher number of classified sequences. The output was imported into Megan⁷⁸ version 6.18.0, where a weighted lowest common ancestor (LCA) algorithm [default parameters according to Huson et al.⁷⁹] was run to assign a single taxonomic lineage to each read. ASV tables were pasted into Krona⁸⁰ for visual inspection of results. Reads with abundances of > 1 % in at least one site were extracted, and consensus sequences were determined for each taxonomic group. Maximum-likelihood phylogenetic trees were constructed with consensus sequences in Mega X⁸¹. To infer

491 presence/absence of Nir and Nor enzymes in representative proteomes, UniProt reference
 492 (manually curated) proteomes were screened using BlastP with default parameters. NirS
 493 (Q51700, *Paracoccus denitrificans* PD1222), NirK (O31380, *Bradyrhizobium japonicum*),
 494 NorB (Q51663, *Paracoccus denitrificans*) were used as amino acid query sequences.

495 The *nosZ* sequences have been deposited in the GenBank, EMBL, and DDBJ databases
 496 as SRA Bioproject XXX.

497

498 ***Statistical analyses.*** All statistical tests were performed with JMP Pro software (Version 13.1.0,
 499 SAS Institute Inc.). Analysis of variance (ANOVA) was used with $p < 0.05$ to test significantly
 500 different values for gene quantities across soils. Plotting and regression analysis were done with
 501 the Matlab R2018a software package (Version 9.4.0.813654, Mathworks Inc.).

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720 **Data availability**

721 All data to evaluate the conclusions of the study are present in the paper and the Supplementary
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740 **Author contributions**

741 S.B., N.E.O., and H.C.-Q. designed the study. S.B. conducted the field work with essential
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743 M.F.O., A.F.S., and M.C. R. performed laboratory experiments and molecular analyses. S.J.H.
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745 analysis. H.G. analyzed isotopic abundances of gas samples. S.B., I.G.B., B.G., N.E.O., and
746 H.C.-Q. performed the data analysis. S.B. and H.C.-Q. wrote the manuscript, and all co-authors
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749 **Competing interests**

750 The authors have no competing interests to declare.