

# Coupled abiotic-biotic cycling of nitrous oxide in tropical peatlands

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37 **Abstract**

38 Atmospheric nitrous oxide (N<sub>2</sub>O) 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, N<sub>2</sub>O 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 N<sub>2</sub>O production determined by isotopic tracers.  
43 Moreover, we found that microbial N<sub>2</sub>O reduction accompanied abiotic production, essentially  
44 closing a coupled abiotic-biotic N<sub>2</sub>O cycle. Anaerobic N<sub>2</sub>O consumption occurred ubiquitously  
45 (pH 6.4-3.7), with proportions of diverse clade II N<sub>2</sub>O-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 N<sub>2</sub>O flux variation in the region.

## 50 **Introduction**

51           The atmospheric accumulation of nitrous oxide (N<sub>2</sub>O), a potent greenhouse gas, has  
52 continued to increase<sup>1,2</sup>, calling for a better mechanistic understanding of its sources and sinks.  
53 Tropical soils are a major source of N<sub>2</sub>O. The largest contribution to global N<sub>2</sub>O flux, along  
54 with the highest uncertainties, have been observed over South America<sup>3-5</sup> with large flux  
55 variations described in ground-based measurements from extensive peatlands of the Amazon  
56 basin<sup>6,7</sup>.

57           In waterlogged tropical peat soils, anoxic, reducing, humic acid-rich, and Fe-holding  
58 conditions are favorable for the abiotic formation of N<sub>2</sub>O<sup>8</sup>. Nitrous oxide can abiotically form  
59 from the reduction of nitrite (NO<sub>2</sub><sup>-</sup>) via intermediary nitric oxide (NO), or hydroxylamine  
60 (NH<sub>2</sub>OH)<sup>9</sup>, both of which have typically low steady-state concentrations in soils.  
61 Hydroxylamine conversion into N<sub>2</sub>O 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<sub>2</sub>O production rates based  
65 on dissolved Fe and Fe mineral phases<sup>10-12</sup>, while others have shown an influence from organic  
66 matter (OM)<sup>8</sup>, presumably by providing complexed Fe<sup>2+</sup> and/or humic electron shuttles<sup>13</sup>.  
67 Abiotic N<sub>2</sub>O formation has been recorded in polar<sup>10,14</sup> and temperate<sup>11</sup> 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<sup>2</sup> (ref. <sup>15</sup>), tropical peatlands under varying climatic  
70 regimes could play a major role in global N<sub>2</sub>O gas cycling.

71           Denitrification, generally occurring at oxygen concentrations below 6 μM<sup>16</sup>, is  
72 considered to be driven predominantly by microbial communities using Fe- and Cu-dependent  
73 reductase enzymes<sup>17</sup> through a modular pathway structure with different populations mediating  
74 only one or two reduction steps<sup>18,19</sup>. Denitrifying microbes are well adapted to the conditions

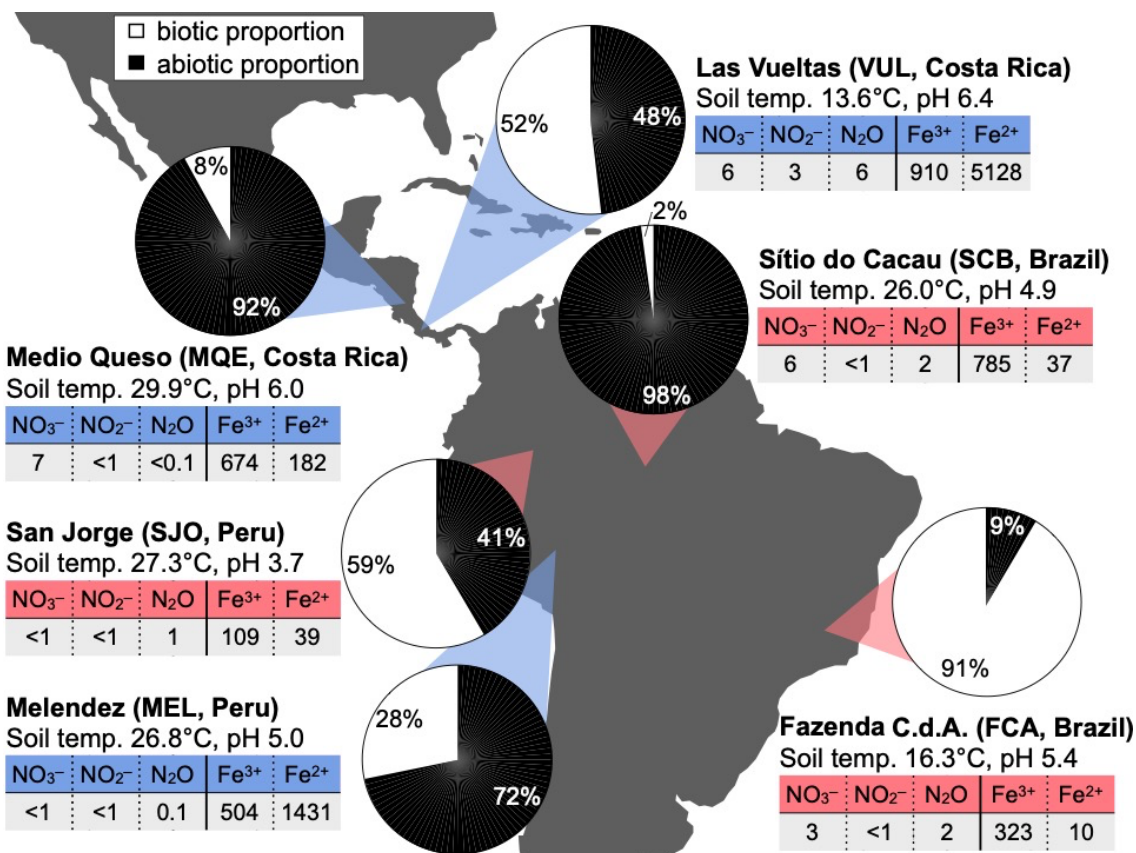
75 found in peat soils because they anaerobically respire organic substrates using nitrogen oxides  
76 as terminal electron acceptors<sup>20,21</sup>. Also, the extensive N<sub>2</sub>O sink potential previously observed  
77 in diverse soils<sup>22,23</sup> can be better explained with the discovery of the abundant clade II N<sub>2</sub>O-  
78 reducing bacteria. While clade I N<sub>2</sub>O-reducers are affiliated to the *Proteobacteria*, clade II N<sub>2</sub>O  
79 reducers are more diverse and scattered across multiple phyla<sup>24</sup>. Interestingly, the clade II  
80 members tend to lack NO<sub>2</sub><sup>-</sup> reductases more so than clade I members<sup>24</sup>. From an ecological  
81 perspective, this trait might correspond with an intrinsic capability of the soil habitat to reduce  
82 NO<sub>2</sub><sup>-</sup> via chemodenitrification. Cellular resources can be saved and relocated to the expression  
83 of NO and N<sub>2</sub>O reductases<sup>25</sup> to catalyze a thermodynamically more favorable redox reaction  
84 ( $\Delta G$  of N<sub>2</sub>O reduction is  $\sim 100$  kJ mol<sup>-1</sup> higher than  $\Delta G$  of NO<sub>2</sub><sup>-</sup> reduction).

85         While interactions between microbial guilds have been proposed as the basis for  
86 modularity<sup>23</sup>, the interplay of denitrifiers with abiotic reactions has received little attention,  
87 even though chemodenitrification can reduce or contribute to different inorganic nitrogen pools,  
88 including N<sub>2</sub>O and NO. The compatibility of abiotic N<sub>2</sub>O production and modular microbial  
89 denitrification led us to hypothesize that a coupled abiotic-biotic N<sub>2</sub>O cycle could operate in  
90 tropical peatlands. To test our hypothesis, we explored the dynamics and underlying factors of  
91 abiotic N<sub>2</sub>O formation and microbial N<sub>2</sub>O reduction in six peatlands located across Central and  
92 South America using isotopic tracers. Simultaneously, we quantified and sequenced the *nosZ*  
93 gene as a marker for the N<sub>2</sub>O-reducing microbial community. Our results provide evidence for  
94 concomitant abiotic N<sub>2</sub>O production and microbial consumption active under various peat soil  
95 conditions.

96 **Results**

97 ***Fe<sup>2+</sup> drives abiotic formation of N<sub>2</sub>O in high-N<sub>2</sub>O 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 NO<sub>2</sub><sup>-</sup> in soil pore water were  
102 below detection (< 1 μM) in the majority of sites, indicating rapid cycling<sup>26,27</sup>. The iron content  
103 and redox balance were highly variable, with higher Fe<sup>2+</sup> concentrations in mountainous peat  
104 (~5 mM) and lower Fe<sup>2+</sup> in oligotrophic peat (0.01-0.04 mM). To determine abiotic N<sub>2</sub>O  
105 production rates under near-natural conditions, we induced a ten-fold spike with <sup>15</sup>NO<sub>2</sub><sup>-</sup> *in-situ*  
106 and measured <sup>14</sup>N<sup>15</sup>NO + <sup>15</sup>N<sup>14</sup>NO + <sup>15</sup>N<sup>15</sup>NO evolution. Biotic activity was arrested by  
107 amending the soil with 87.5 mM ZnCl<sub>2</sub>. Because the addition of Zn can liberate Fe<sup>2+</sup> ions  
108 inevitably stimulating N<sub>2</sub>O production<sup>8</sup>, we repeated the soil incubations in the laboratory, with  
109 100 μM NO<sub>2</sub><sup>-</sup>, 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 N<sub>2</sub>O production  
111 rates are therefore corrected for Zn-induced N<sub>2</sub>O production.



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**Fig. 1. Contribution of abiotic and biotic reactions to overall N<sub>2</sub>O production in tropical peatlands.** Rates were derived *in situ* from the enrichment of <sup>15</sup>N in N<sub>2</sub>O after addition of <sup>15</sup>NO<sub>2</sub><sup>-</sup> 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 N<sub>2</sub>O yield (Table 1), showing high-NO yield (red shades) or high-N<sub>2</sub>O yield (blue shades).

121 Abiotic N<sub>2</sub>O production was observed in all peatlands. *In-situ* rates ranged from low  
122 (0.05-0.3 nmol N<sub>2</sub>O g<sup>-1</sup> d<sup>-1</sup>) at FCA and SCB, moderate (2.4-3.3 nmol N<sub>2</sub>O g<sup>-1</sup> d<sup>-1</sup>) at MQE and  
123 SJO, and high (9.2-39.0 nmol N<sub>2</sub>O g<sup>-1</sup> d<sup>-1</sup>) at MEL and VUL. Abiotic N<sub>2</sub>O production  
124 contributed to the overall N<sub>2</sub>O flux to a greater extent than biotic N<sub>2</sub>O production at half the  
125 field sites (Fig. 1). Soil Fe<sup>2+</sup> concentrations measured after extraction positively correlated with  
126 abiotic N<sub>2</sub>O production rates ( $R^2 = 0.99$ , n = 6, Supplementary Fig. 1). To determine the  
127 nitrogen yield of the chemodenitrification reaction, we incubated gamma-irradiated peat soil  
128 under anoxic conditions with 100 μM NO<sub>2</sub><sup>-</sup>, and quantified NO<sub>2</sub><sup>-</sup>, NO and N<sub>2</sub>O in time  
129 (Supplementary Fig. 2). In two peatlands (SJO, SCB), complete denitrification was achieved

130 almost based purely on abiotic reactions (Table 1). The transformation of  $\text{NO}_2^-$  into NO and  
131  $\text{N}_2\text{O}$  resulted in dominant yields of either product across sites, suggesting unequal nitrogen  
132 diversion directed by local peat chemistry. Our analytical approach could not confirm  $\text{N}_2$  as a  
133 byproduct<sup>28</sup>, which was presumably dominant at circum-neutral pH sites (MEL and VUL). We  
134 used this observed divergent  $\text{NO}_2^-$  conversion to group the diverse peat soils into high- $\text{N}_2\text{O}$   
135 (MQE, VUL, MEL) and high-NO (FCA, SJO, SCB) abiotic-yield sites (Table 1).

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137 To our knowledge, this study represents the first assessment of the relative contribution  
138 of abiotic  $\text{N}_2\text{O}$  production to the overall  $\text{N}_2\text{O}$  production at near-natural  $\text{NO}_2^-$  levels. Based on  
139 our results, abiotic reactions outcompete biotic reactions in three peatlands and are highly  
140 competitive as a source of  $\text{N}_2\text{O}$  at another two. The measured  $\text{N}_2\text{O}$  production rates were  
141 comparable to reported rates from a coniferous forest and grasslands<sup>29</sup>, although the amount of  
142 added  $\text{NO}_2^-$  was at least one order of magnitude lower in our study. Relative to other evaluated  
143 ecosystems<sup>10</sup>, peat soils have less oxidized Fe or Mn minerals and are enriched in recalcitrant  
144 organic carbon, which would hold additional reducing power, particularly in the structurally  
145 disparate OM. For instance, pi-electron bonds are an integral part of the chemical structures  
146 found in recalcitrant organic carbon, such as phenolic or humic substances, and they are prone  
147 to interact with  $\text{NO}_2^-$  (refs. <sup>30,31</sup>). Besides serving as reactants, humic substances can act as  
148 regenerable electron shuttles for redox reactions in soils and sediments<sup>32</sup>. Iron reduction and  
149 dissolution are greatly enhanced in the presence of humic substances<sup>33,34</sup>, which increases the  
150 availability of  $\text{Fe}^{2+}$ . The distinct production of NO and  $\text{N}_2\text{O}$  across a gradient of  $\text{Fe}^{2+}$   
151 concentrations suggests divergent reaction mechanisms in high-NO and high- $\text{N}_2\text{O}$  soils.  
152 Previous reports agree with our data that indicate the larger production of NO as the final  
153 product of chemodenitrification, which is stimulated by the self-decomposition of nitrous acid  
154 in increasingly acidic soil milieu<sup>35,36</sup>. High- $\text{N}_2\text{O}$  soils coincided with high soil  $\text{Fe}^{2+}$  abundances,



155 and high-NO sites were associated with low Fe<sup>2+</sup> (Fig. 1, Table 1). Besides Fe<sup>2+</sup>, the mixture of  
156 functional groups in peat OM may also be crucial in determining the NO to N<sub>2</sub>O balance.  
157 Except for dimethyl glyoxime and quinone oximes, oxime groups preferentially produce N<sub>2</sub>O  
158 and aromatics tend to produce NO<sup>37</sup>.

159 Nitrite incorporation into OM may explain lower nitrogen yields in either NO or N<sub>2</sub>O, in  
160 addition to the possible production of N<sub>2</sub>. For example, nitrosophenol (a phenol moiety that  
161 binds one NO<sub>2</sub><sup>-</sup> ion) tautomerizes to quinone monoxime and requires another NO<sub>2</sub><sup>-</sup> ion to eject  
162 hyponitrous acid which decomposes to N<sub>2</sub>O<sup>30,31</sup>. Without the consecutive incorporation of 2  
163 NO<sub>2</sub><sup>-</sup> ions, nitrosophenol remains stable, and a significant amount of NO<sub>2</sub><sup>-</sup> could reside in  
164 nitrosated functional groups.

165

166 **Table 1. Abiotic nitrogen yield fractions based on sterilized batch incubations.** Gamma-  
167 irradiated peat soil was used for anoxic incubations initiated with the addition of 100 μM NO<sub>2</sub><sup>-</sup>.  
168 Yield was calculated after all NO<sub>2</sub><sup>-</sup> was consumed and based on stable NO and N<sub>2</sub>O  
169 concentrations in at least two consecutive measurements. Sites with replicated samples (n = 3)  
170 included: Las Vueltas (VUL), Medio Queso (MQE), Fazenda Córrego da Areia (FCA),  
171 Melendez (MEL), Sítio do Cacau (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 <sub>2</sub> O (%)	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

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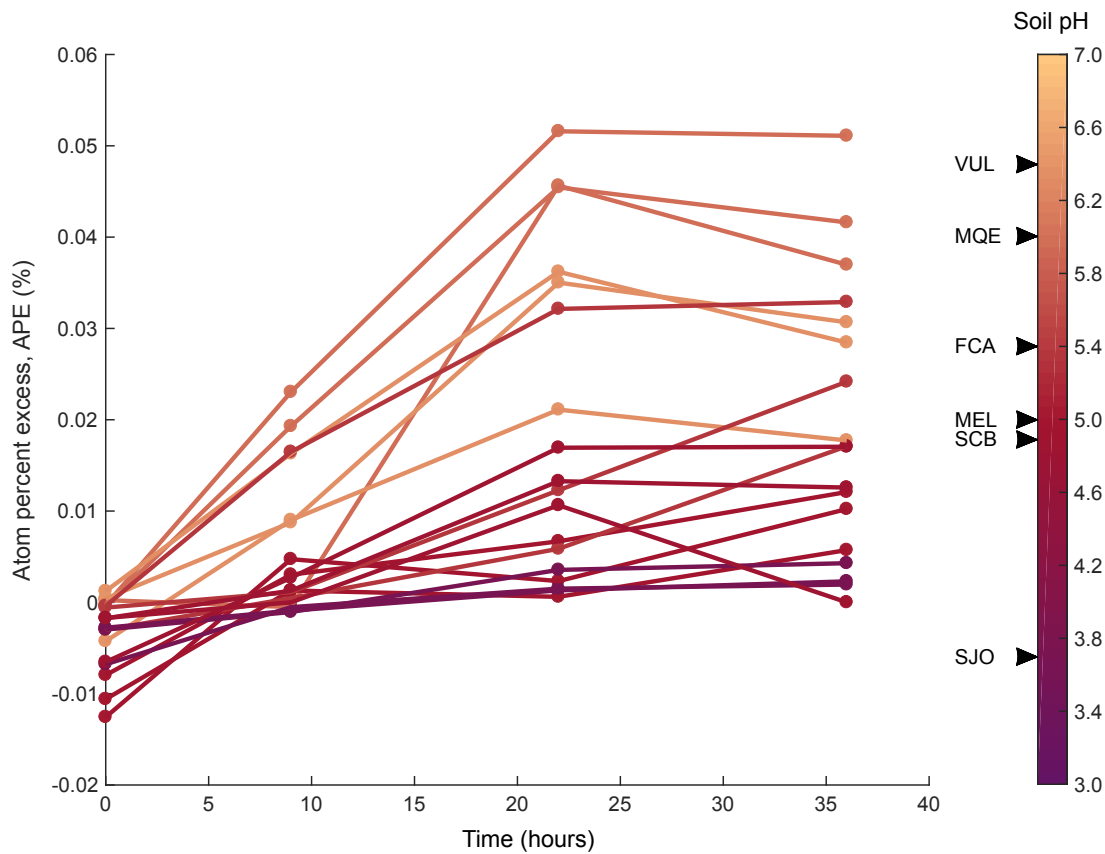
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#### 174 *Active microbial N<sub>2</sub>O reduction in acidic peat soils*

175 Concomitant to N<sub>2</sub>O production, we measured N<sub>2</sub>O consumption. Only non-sterilized  
176 samples exhibited active consumption. In sterilized samples, N<sub>2</sub>O was a stable end product after  
177 NO<sub>2</sub><sup>-</sup> addition, and <sup>15</sup>N<sub>2</sub> was not produced in (<sup>15</sup>N)<sub>2</sub>O amendments. Incubations with (<sup>15</sup>N)<sub>2</sub>O in  
178 the field resulted in an accumulation of the <sup>15</sup>N label in N<sub>2</sub> (Fig. 2) and were used to derive N<sub>2</sub>O  
179 reduction rates. Enrichment of <sup>15</sup>N<sub>2</sub> decreased with soil pH (Fig. 2), while N<sub>2</sub>O reduction was



180 surprisingly observed in soils with pH as low as 3.7 (SJO). This finding is significant because  
181 N<sub>2</sub>O reductase assembly is post-transcriptionally inhibited by acidic pH<sup>38</sup>, and exposure to pH <  
182 4 disrupts a histidine amino acid ligand to the Cu cofactor in N<sub>2</sub>O reductase, possibly  
183 inactivating the catalytic function (pers. com. W. Nitschke). The measured N<sub>2</sub>O reduction rates  
184 were higher than previously observed rates at similar acidic pH values<sup>39</sup> and would extend the  
185 known physiological limits for microbial N<sub>2</sub>O consumption. Thus, these results demonstrate the  
186 presence and activity of N<sub>2</sub>O-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 (<sup>15</sup>N)<sub>2</sub>O**  
189 **with anoxic peat soil.** Replicates per sites (n = 3), as listed, are colored in a gradient according  
190 to their pH. VUL, Las Vuelas; MQE, Medio Queso; FCA, Fazenda Córrego da Areia; MEL,  
191 Melendez; SCB, Sítio do Cacau; SJO, San Jorge.

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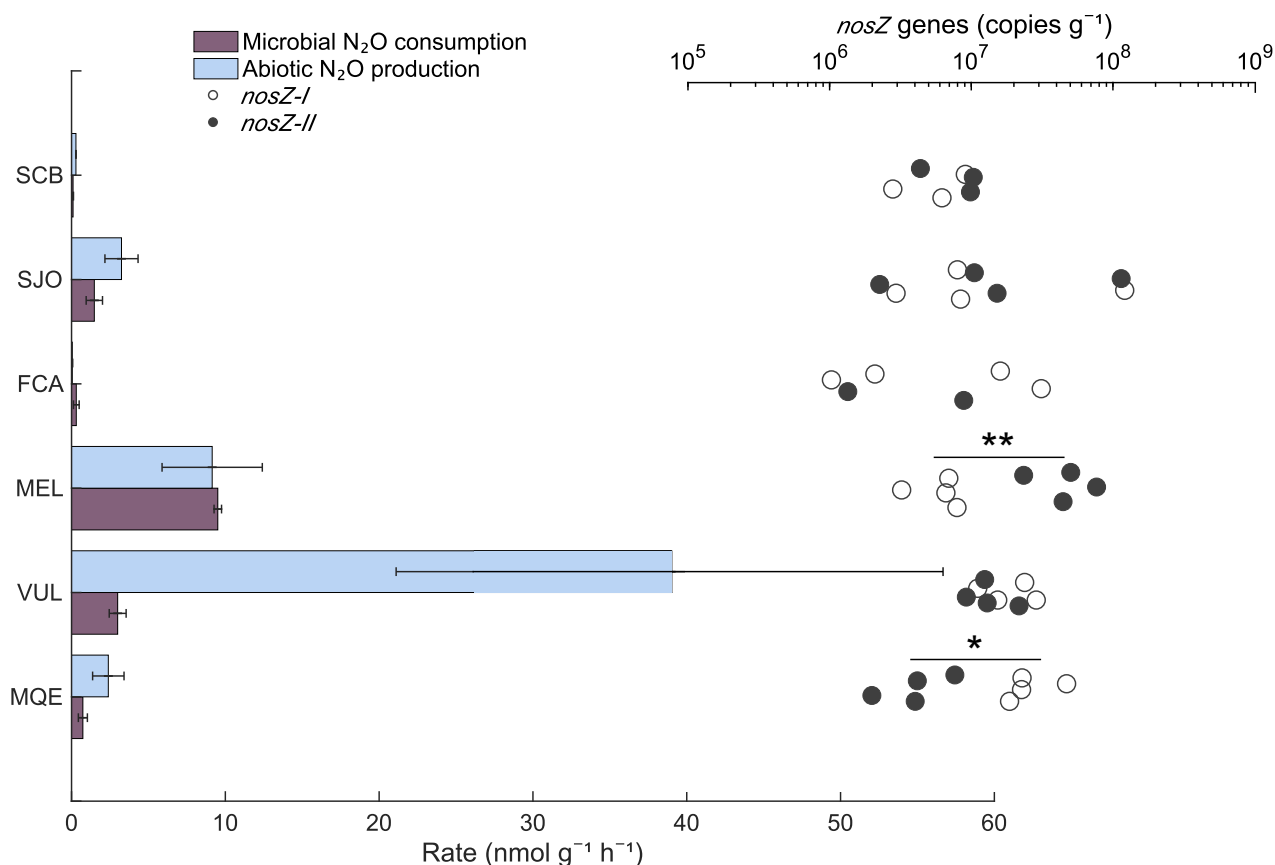
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## 196 ***Diverse Clade II N<sub>2</sub>O-reducers are associated with higher N<sub>2</sub>O sink potential***

197 To evaluate the relationship between the abiotic formation and microbial consumption  
198 of N<sub>2</sub>O, we compared reaction rates against *nosZ* gene abundances. Both processes revealed  
199 similar trends ranging from low (0.1-0.3 nmol N<sub>2</sub>O g<sup>-1</sup> d<sup>-1</sup>) in SCB and FCA to moderate (0.7-  
200 1.5 nmol N<sub>2</sub>O g<sup>-1</sup> d<sup>-1</sup>) in MQE and SJO to high (3-9.5 nmol N<sub>2</sub>O g<sup>-1</sup> d<sup>-1</sup>) 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-N<sub>2</sub>O sites (MQE,  
204 MEL). Consumption rates gradually increased with clade II *nosZ* gene abundance at high-N<sub>2</sub>O  
205 sites. A clear dominance of clade II *nosZ* genes over those from clade I coincided with the  
206 elevated rates of N<sub>2</sub>O consumption in MEL peatland. Thus, N<sub>2</sub>O reducers from clade II  
207 establish an increased microbial N<sub>2</sub>O sink in peatlands with high abiotic N<sub>2</sub>O fluxes.



208

209 **Fig. 3. Microbial N<sub>2</sub>O consumption and abiotic N<sub>2</sub>O production (bars) along with *nosZ***  
210 **gene quantities (open and filled circles).** Error bars denote SD (consumption rates, n = 3;  
211 production rates, n = 4). Clade I and II *nosZ* was quantified by qPCR assays and are  
212 significantly different within sites as denoted by asterisks (ANOVA, \**p* = 0.012, \*\**p* = 0.008, n  
213 = 4). Two outliers for *nosZ-II* (SCB, ~6,200 copies g<sup>-1</sup>; FCA, ~590) are not shown and another  
214 two datapoints are missing due to non-amplification. See previous figures for site acronyms.  
215

216 Next, to evaluate the N<sub>2</sub>O-reducing microbial community, we analyzed 183,265 and  
217 80,050 taxonomically assigned *nosZ* gene amplicon sequence variants (ASVs) for clade I and  
218 II, respectively. Our analysis focused on the most abundant taxa that made up at least 1% of the  
219 total ASVs in at least one site (Fig. 4). The most abundant ASV was affiliated to the alpha-  
220 proteobacterium *Nitrospirillum amazonense* (Fig. 4). This phylotype constituted 59-64 % of  
221 clade I ASVs in the Amazon bogs SCB and SJO but was least represented in the MEL peatland  
222 (~10 %). In MEL, 23 % of ASVs belonged to *Methylocystis* species, which were also abundant  
223 in FCA (27 %). The clade II N<sub>2</sub>O reducers were more diverse (comprised more phyla) across all  
224 soils (Fig. 4), with *Magnetospirillum* (consistently > 10 % in high-NO sites and 30 % in VUL)  
225 and unclassified *Myxococcales* (8-50 %) as the most abundant phylotypes. To examine the  
226 observed trend of N<sub>2</sub>O reduction rates corresponding with *nosZ* clade II gene frequencies in the  
227 high-N<sub>2</sub>O sites (MEL > VUL > MQE, Fig. 3), we derived diversity indices and performed a  
228 principal component analysis (PCA). The Shannon diversity index showed a coinciding order of  
229 diversity levels (1.73 > 1.49 > 1.39, Supplementary Tables 1a-b) for high-N<sub>2</sub>O sites. This was  
230 also supported by a relatively high average Bray-Curtis dissimilarity of clade II *nosZ* gene  
231 sequences in MEL (0.71, Supplementary Tables 1c-d), identifying the clade II N<sub>2</sub>O reducer  
232 community in this peatland as most dissimilar to all others. In addition, the community structure  
233 variation among clade II was most parsimoniously explained by N<sub>2</sub>O consumption rates in the  
234 PCA (Supplementary Figs. 3, 4). Rather than single dominant taxa, it appeared to be the  
235 contribution of a diverse group of clade II N<sub>2</sub>O reducers to be responsible for the high N<sub>2</sub>O sink  
236 potential observed.

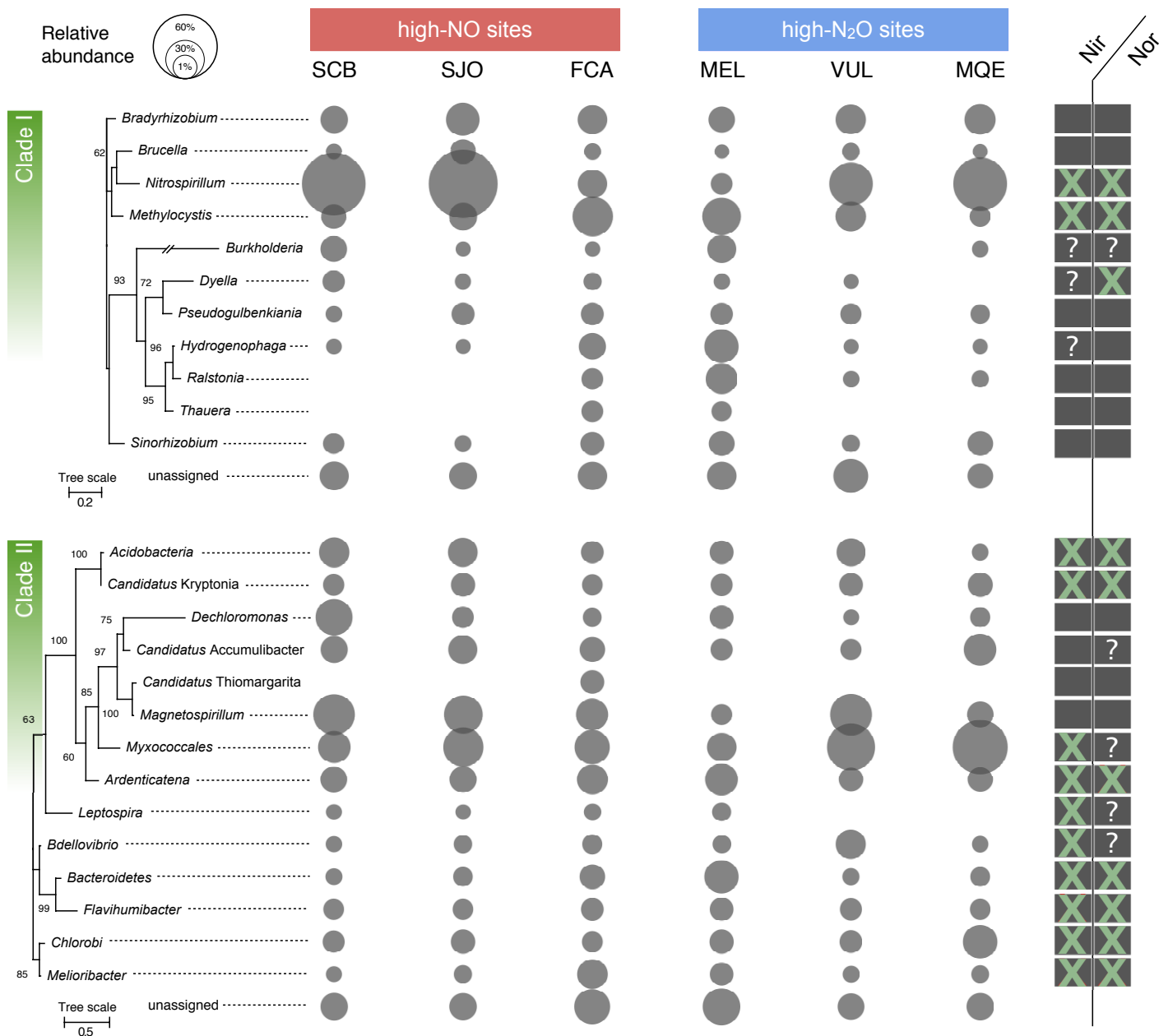
237 The intrinsic capacity of the peat to reduce  $\text{NO}_2^-$  abiotically could provide non-  
238 denitrifying microbes that do not possess denitrification enzymes other than  $\text{N}_2\text{O}$  reductase,  
239 called “chemodenitrifiers”<sup>40</sup>, an advantage over canonical denitrifiers. Chemodenitrifiers do not  
240 have to compete with chemodenitrification and simply harvest the end product  $\text{N}_2\text{O}$  to oxidize  
241 organic substrates. Figure 4 illustrates the  $\text{NO}_2^-$  reductase (either NirS or NirK) and NO  
242 reductase (NorB) enzyme repertoires present in available reference proteomes of relatives of the  
243 predicted taxa in both clades. At least 2 out of the 11 clade I taxa (including the abundant  
244 *Nitrospirillum*) and 10 out of the 14 clade II taxa indicated the absence of Nir enzymes (Fig. 4).  
245 Half of the clade II reference proteomes were missing both Nir and Nor proteins. Importantly,  
246 the Myxococcales ASVs showed no differences in abundance among high-NO sites but  
247 gradually increased, similar to the  $\text{N}_2\text{O}$  yield, in the high- $\text{N}_2\text{O}$  sites. This order, which also  
248 includes *Anaeromyxobacter dehalogenans*, the hallmark organism of clade II  $\text{N}_2\text{O}$  reducers<sup>22,41</sup>,  
249 is frequently represented in acidic, organic-rich soils<sup>40,42</sup>, presumably with abiotic NO and  $\text{N}_2\text{O}$   
250 production potential. Further, other bacteria such as *Dechloromonas*<sup>43</sup>, *Ardenticatena*<sup>44</sup>, and  
251 *Melioribacter*<sup>45</sup> also mediate iron reduction, an additional trait that could promote  
252 chemodenitrification by recycling  $\text{Fe}^{2+}$ . Therefore, chemodenitrifiers may outcompete canonical  
253 denitrifiers in the studied peatlands (e.g., *Nitrospirillum*) and abundance patterns of the  
254 Myxococcales suggest a notable benefit for some chemodenitrifiers in soils associated with  
255 high abiotic  $\text{N}_2\text{O}$  yields.

256 Another abundant taxon of the  $\text{N}_2\text{O}$ -reducing community was *Magnetospirillum* (clade  
257 II) that includes several iron-oxidizing species. These alpha-proteobacteria synthesize the  
258 mixed-valence iron mineral magnetite, which can accumulate in soils, also under the influence  
259 of abiotic crystallization<sup>46</sup>. Secondary iron mineral formation can be widespread in the tropics,  
260 driven by dissolved and particulate iron originating from weathering and desilication<sup>47-49</sup>.  
261 Ferrous iron-bearing minerals, such as magnetite, can serve as catalysts for  $\text{NO}_2^-$  reduction by

262 providing reaction sites at the mineral surface<sup>50,51</sup>. The possession of an N<sub>2</sub>O reductase makes  
 263 sense for *Magnetospirillum*, assuming cells are associated with, or at least grow in proximity to,  
 264 magnetite. Analyses of the iron phases present would be necessary to follow up on this in more  
 265 detail, which was off the scope of our study. We also acknowledge that our insight into the  
 266 temporal activity response is limited. Information on the actual *in-situ* transcription levels is  
 267 needed to better assess how the clades react to fluctuating abiotic pulses of N<sub>2</sub>O<sup>52</sup>.

268

269



270

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**

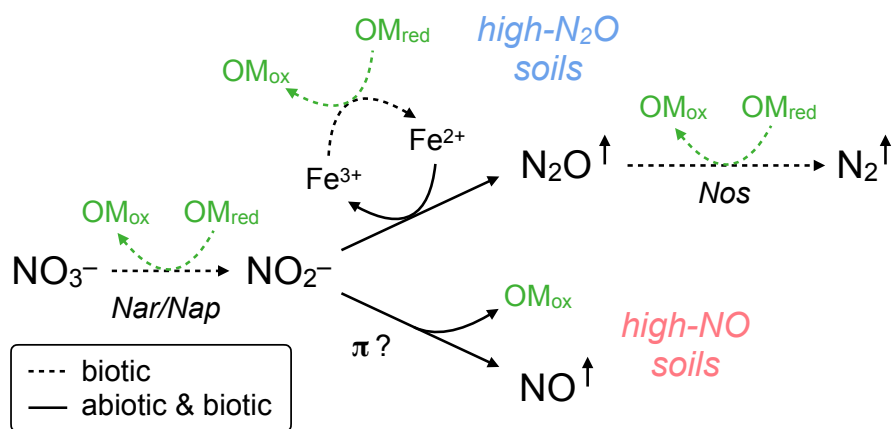
278 ***Decoupling of abiotic N<sub>2</sub>O production and microbial N<sub>2</sub>O consumption***

279 Our data show the concomitant occurrence of both abiotic N<sub>2</sub>O 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 effects<sup>53-55</sup>. Along these lines, the decoupling of  
286 production and consumption establishes the potential for vast N<sub>2</sub>O emissions when changing  
287 environmental conditions impart selectively negative effects on consumption. For instance,  
288 while peatland drainage occurs naturally between wet and dry seasons<sup>56</sup>, N<sub>2</sub>O 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 N<sub>2</sub>O because acidic peat soil stabilizes Fe<sup>2+</sup> via two  
292 mechanisms. First, Fe<sup>2+</sup> oxidation by oxygen is kinetically hindered at low pH. Oxidation rates  
293 are significantly slowed at pH ≤ 6.5<sup>57</sup>, a pH regime applying to most peatlands, including all in  
294 our study. Second, Fe<sup>2+</sup> complexed by OM is resilient to oxidation. Experimental evidence  
295 suggests that tannic acid<sup>58</sup>, phenolics<sup>59</sup>, or natural humic acid<sup>60</sup> stabilize the Fe<sup>2+</sup> pool in the  
296 presence of oxygen by the formation of a redox-buffering shell<sup>60</sup> and re-reduction of Fe<sup>3+</sup>.  
297 However, little is known concerning how Fe<sup>2+</sup> complexation affects the NO<sub>2</sub><sup>-</sup> 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 N<sub>2</sub>O production over biotic N<sub>2</sub>O production.

301



302



303 **Fig. 5. Schematic representation of denitrification pathways in tropical peatlands.** Nitrate  
 304 ( $\text{NO}_3^-$ ) reduction to  $\text{NO}_2^-$  occurs at significant rates only with catalysis by  $\text{NO}_3^-$  reductases.  
 305 Nitrite is rapidly reduced via abiotic and biotic reactions. At lower pH ( $\leq 5.4$ ),  $\text{NO}$  is the  
 306 dominant product. Nitrosation into OM may be an alternative abiotic process in soils with  
 307 minor nitrogenous gas production reliant on organic compounds containing pi-electron bonds  
 308 ( $\pi$ ). In  $\text{Fe}^{2+}$ -rich peat,  $\text{N}_2\text{O}$  is the dominant product, involving Fe redox cycling that can fuel  
 309 dissimilatory Fe reduction<sup>11</sup>. The only  $\text{N}_2\text{O}$  consumption pathway in peat soil is  $\text{N}_2\text{O}$  reductase-  
 310 dependent reduction to  $\text{N}_2$ , which is active even in the most acidic soils tested (pH 3.7). All  
 311 related heterotrophic reactions induce oxidation of OM ( $\text{OM}_{\text{red}} \rightarrow \text{OM}_{\text{ox}}$ ) and eventually peat  
 312 carbon mineralization.

313

314 We present evidence that active abiotic-biotic  $\text{N}_2\text{O}$  cycling is prevalent in tropical  
 315 peatlands, where denitrification is not a purely biological pathway, but rather a “mosaic” of  
 316 biotic and abiotic reduction reactions (Fig. 5). Furthermore, our results support the idea that  
 317 functional modularity complements not only the interrelationship of microbial groups but also  
 318 concomitant interactions between microbes and spontaneous chemical reactions. Abiotic  $\text{N}_2\text{O}$   
 319 formation in tropical peatlands can have important regional consequences in the context of  
 320 observed  $\text{N}_2\text{O}$  fluxes and higher rates in response to drainage<sup>61</sup>, and putatively drought<sup>62</sup>, as  
 321 well as possible effects on reducing organic carbon release<sup>63</sup>. For example, compared to the  
 322 other soils, abiotic  $\text{N}_2\text{O}$  production was moderate in SJO, an acidic oligotrophic site, showing a  
 323 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  
 324 oligotrophic tropical peatlands alike SJO at  $1,003,719 \text{ km}^2$  (ref. 15), this could amount to a total  
 325 depth-integrated abiotic  $\text{N}_2\text{O}$  flux ranging from 0.1 to  $3.9 \text{ Tg } \text{N}_2\text{O } \text{yr}^{-1}$  depending on the depth  
 326 of  $\text{NO}_2^-$  diffusion. This contribution is a major uncertainty that could account for two percent to

327 more than half of all combined terrestrial N<sub>2</sub>O fluxes from natural sources of South America,  
328 Africa, and Southeast Asia. With a factor of 298 g CO<sub>2</sub>-equivalents per g N<sub>2</sub>O over a 100 year  
329 time<sup>64</sup>, abiotic N<sub>2</sub>O 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 CO<sub>2</sub>. Considering ~50 mg CO<sub>2</sub>-C m<sup>-2</sup> yr<sup>-1</sup> emitted from SJO-like  
332 tropical peatlands<sup>7</sup> and 4 moles of N<sub>2</sub>O 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<sup>-1</sup> 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 N<sub>2</sub>O reduction in northern  
338 peatlands, which would imply an imbalanced cycling of N<sub>2</sub>O and substantial N<sub>2</sub>O 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  $\mu\text{M}$ ) and high ( $> 5 \text{ mM}$ )  $\text{Fe}^{2+}$  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 ***<sup>15</sup>N tracer experiment in the field.*** Colorimetric assays to determine ambient soil  $\text{NO}_2^-$  and  
356  $\text{NO}_3^-$  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  $\text{N}_2\text{O}$   
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

366 soil via a water line connected to the glove bag. Separate sample sets received  $^{15}\text{NO}_2^-$  (label  
367 fraction = 0.1, Cambridge Isotopes) at  $10\times$  the soil ambient  $\text{NO}_2^-$  concentration and doubly  
368 labeled  $(^{15}\text{N})_2\text{O}$  (label fraction = 1.0, Cambridge Isotopes) at  $5\times$  the soil ambient  $\text{N}_2\text{O}$   
369 concentration. Thus, the amount of  $^{15}\text{N}$  tracer applied varied slightly between sites but reflected  
370 a similar order of magnitude.  $^{15}\text{NO}_2^-$  incubations included untreated and 87.5 mM zinc chloride-  
371 poisoned ( $\text{ZnCl}_2$ , Fisher Scientific) soils in replicates of four ( $n = 4$ ). Soil slurries were  
372 incubated in insulating containers to avoid temperature fluctuations, and gas samples were  
373 taken for  $(^{15}\text{N})_2\text{O}$  analysis at the beginning of incubation and after 24 h ( $n = 4$ ), and for  $^{30}\text{N}_2$   
374 analysis at four time points spread over 36 h ( $n = 3$ ). Gas sampling was destructive (entire  
375 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  
376 sample times for the  $^{30}\text{N}_2$  analysis were adapted from a previous study<sup>65</sup>. We also prepared zinc-  
377 poisoned  $(^{15}\text{N})_2\text{O}$  incubations to test for abiotic  $\text{N}_2\text{O}$  consumption. The gas samples were stored  
378 underwater in borosilicate glass vials closed with thick butyl rubber stoppers<sup>66</sup> prior to analysis  
379 at Michigan State University. Isotopic compositions of  $\text{N}_2\text{O}$  and  $\text{N}_2$  were measured using an  
380 Elementar Isoprime isotope ratio mass spectrometer (IR-MS) interfaced with an Elementar  
381 TraceGas chromatographic system. Rate calculations closely followed a previously developed  
382 and tested protocol<sup>14</sup>. Given the constraints of sterilant applications in the field, we repeated the  
383 zinc-amended incubations in the lab, complementarily to incubations with gamma-irradiated  
384 soils. The rates from both experiments were used to calculate a correction factor accounting for  
385 artifacts caused by the zinc addition<sup>8</sup>. The rates derived in the field were then multiplied by the  
386 correction factor (Supplementary Fig. 6 and Supplementary Table 6). The Brazilian sites SCB  
387 and FCA have associated data from gamma-irradiated soil, but data from zinc-treated soil are  
388 missing because of logistic issues concerning the shipment of non-sterilized (not gamma-  
389 irradiated) soil. The final rates were combined according to the following equation for net *in-*  
390 *situ*  $\text{N}_2\text{O}$  formation:

391 
$$B = M + C - A$$

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

395  
396 **Laboratory incubations.** In an anoxic glove box (0.1%  $\text{H}_2$  for  $\text{O}_2$  reduction in  $\text{N}_2$ ), gamma-  
397 sterilized and zinc-treated soil were prepared separately. Gamma sterilization followed a  
398 previous method<sup>8</sup>. Zinc chloride was applied, as described for field experiments. Roots and  
399 coarse particles (> 5 mm in diameter) were removed from soils, and soils were slurried 1:5  
400 (w/v) in anoxic, sterile 18.2  $\text{M}\Omega\cdot\text{cm}$  water. The slurry was homogenized before equal quantities  
401 were distributed into culture vials and sealed with sterile butyl rubber stoppers. An anoxic and  
402 filter-sterilized  $\text{NO}_2^-$  solution was injected (final concentration 100  $\mu\text{M}$ ) into microcosms that  
403 were previously flushed with pure  $\text{N}_2$ . The microcosms were agitated briefly to disperse the  
404 added substrate and then kept under dark, static conditions at room temperature for ~100 h.  
405 Dissolved  $\text{NO}_2^-$  was quantified with the Griess reagent (Promega, Kit G2930), and  $\text{NO}$  and  
406  $\text{N}_2\text{O}$  were analyzed as described below.

407  
408 **Soil Fe measurements.** Soils for Fe analysis were kept in anoxic serum bottles and refrigerated  
409 during transport. The species  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  were extracted and separated as previously  
410 described<sup>8</sup> and quantified in acidified aqueous solution by inductively coupled plasma–optical  
411 emission spectrometry (ICP-OES; Thermo iCAP6300 at the Goldwater Environmental  
412 Laboratory at Arizona State University). The ICP-OES pump rate for the Ar carrier was set to  
413 50 rpm, and Fe2395 and Fe2599 lines were used for Fe quantification. Iron concentrations were  
414 determined from a calibration curve (0.01-10  $\text{mg L}^{-1}$ ) by diluting a standard solution (100 mg  
415  $\text{L}^{-1}$ , VHG Labs, product no. SM75B-500) in 0.02 N  $\text{HNO}_3$ .

416

417 ***N<sub>2</sub>O gas measurements.*** Using a gas-tight syringe (VICI Precision Sampling), 200  $\mu$ 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 N<sub>2</sub> (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 N<sub>2</sub>O measurements were  
422 calibrated over a range of 0.25-100 ppmv using customized standard mixtures (Scott Specialty  
423 Gases, accuracy  $\pm$ 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 atm<sup>67</sup>.

426

427 ***NO gas measurements.*** Nitric oxide (NO) was quantified in the microcosm headspace with a  
428 chemiluminescence-based analyzer (LMA-3D NO<sub>2</sub> analyzer, Unisearch Associates Inc.,  
429 Concord, Canada). Headspace gas (50  $\mu$ L) was withdrawn with a CO<sub>2</sub>-N<sub>2</sub>-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 protocol<sup>68</sup>,  
433 with minor adjustments. Briefly, NO was oxidized to NO<sub>2</sub> by a CrO<sub>3</sub> catalyst. The NO<sub>2</sub> was  
434 carried across a fabric wick saturated with a Luminol solution (Drummond Technology Inc.,  
435 Canada). Readings were corrected for background NO<sub>2</sub> every 15 min ("zeroing"). Shell airflow  
436 rate was kept at 500 mL min<sup>-1</sup>, and the span potentiometer was set to 8. Measurements were  
437 calibrated with a 0.1 ppm NO (in N<sub>2</sub>) standard (< 0.0005 ppm NO<sub>2</sub>, Scott-Marlin, 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 atm<sup>67</sup>.

441

442 ***Molecular analyses.*** Peat samples from four randomly distributed locations (coinciding with  
443 incubation locations) within a peatland were collected and frozen at  $-20\text{ }^{\circ}\text{C}$  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  $15\text{ }\mu\text{L}$  with  $1.5\text{ }\mu\text{L}$  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  $3\text{ mM}$   $\text{MgCl}_2$  was added. Forward and reverse primer concentrations  
450 were  $1\text{ }\mu\text{M}$ , and previous cycler conditions were used<sup>69</sup>. The clade II *nosZ* gene was amplified  
451 using SYBR Fast, ROX low master mix (Kapa Biosystems, Roche Holding AG, Basel,  
452 Switzerland), and  $1.2\text{ }\mu\text{M}$  primer concentration<sup>70</sup>. Thermal cycling was initiated with 3 min of  
453 denaturing at  $95\text{ }^{\circ}\text{C}$ , followed by 40 cycles of the following stages: 30 s at  $95\text{ }^{\circ}\text{C}$ , 60 s at  $58\text{ }^{\circ}\text{C}$ ,  
454 30 s at  $72\text{ }^{\circ}\text{C}$ , 30 s at  $80\text{ }^{\circ}\text{C}$ , 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  $1\text{ }\mu\text{L}$  of DNA template (25-50 ng genomic DNA) in a total reaction volume  
462 of  $20\text{ }\mu\text{L}$ . Targeting the clade I *nosZ* gene, we used a novel primer pair<sup>71</sup>. The reaction mix  
463 included  $0.2\text{ mg mL}^{-1}$  bovine serum albumin (BSA) and  $0.8\text{ }\mu\text{M}$  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  $0.8\text{ }\mu\text{M}$  primer concentration. Cycling conditions for clade II *nosZ* amplification were used



466 as described<sup>70</sup>. Thermal cycling conditions for clade I *nosZ* amplification were an initial 2 min  
467 denaturing step at 95 °C, followed by 33 cycles of 95 °C for 45 s, annealing by 53 °C for 45 s,  
468 and a 72 °C extension for 30 s, and a final extension at 72 °C for 7 min. Amplification was  
469 verified by gel electrophoresis using 1 % agarose in 1 Tris-acetate-EDTA buffer. Samples were  
470 multiplexed<sup>72</sup>, normalized (SequalPrep kit #1051001, Invitrogen), and submitted for sequencing  
471 to the DNASU core facility at Arizona State University, with 2× 300-bp paired-end Illumina  
472 MiSeq.

473 Paired-end sequences were merged and demultiplexed, then we used the USEARCH  
474 pipeline<sup>73</sup> to 1) correct strand orientations, 2) sort out singletons, and 3) denoise the dataset. We  
475 used  $\alpha = 2$  for a stringent denoising of sequences<sup>74</sup> because reads were not clustered with  
476 any identity radius to obtain ASVs. The sequences were translated and frameshift-corrected by  
477 Framebot<sup>75</sup> with low sequence loss (< 10 %). The amino acid sequences obtained were  
478 classified using Diamond<sup>76</sup> version 0.9.25. The search was conducted in Diamond's *sensitive*  
479 mode, with an e-value cutoff of  $10^{-5}$ , resulting in the top 5 % hits. Sequences were parsed  
480 through two databases; the NCBI database RefSeq (release 95) containing 146,381,777 non-  
481 redundant protein sequences and manually curated databases built from 2,817 (clade I) and  
482 2,929 (clade II) sequences off the FunGene repository<sup>77</sup> using the search parameters 80 %  
483 HMM coverage and a minimum length of 550 amino acids. The taxonomy achieved with the  
484 curated databases was used for downstream analysis because of a higher number of classified  
485 sequences. The output was imported into Megan<sup>78</sup> version 6.18.0, where a weighted lowest  
486 common ancestor (LCA) algorithm [default parameters according to Huson et al.<sup>79</sup>] was run to  
487 assign a single taxonomic lineage to each read. ASV tables were pasted into Krona<sup>80</sup> for visual  
488 inspection of results. Reads with abundances of > 1 % in at least one site were extracted, and  
489 consensus sequences were determined for each taxonomic group. Maximum-likelihood  
490 phylogenetic trees were constructed with consensus sequences in Mega X<sup>81</sup>. 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.).

502 **References**

503

- 504 1. Thompson, R. L. *et al.* Acceleration of global N<sub>2</sub>O emissions seen from two decades of  
505 atmospheric inversion. *Nat. Clim. Change* **9**, 993–998 (2019).
- 506 2. Tian, H. *et al.* A comprehensive quantification of global nitrous oxide sources and sinks.  
507 *Nature* **586**, 248–256 (2020).
- 508 3. Zhuang, Q., Lu, Y. & Chen, M. An inventory of global N<sub>2</sub>O emissions from the soils of  
509 natural terrestrial ecosystems. *Atm. Environ.* **47**, 66–75 (2012).
- 510 4. Huang, J. *et al.* Estimation of regional emissions of nitrous oxide from 1997 to 2005  
511 using multinetwork measurements, a chemical transport model, and an inverse method. *J.*  
512 *Geophys. Res.* **113**, 197–19 (2008).
- 513 5. D'Amelio, M. T. S., Gatti, L. V., Miller, J. B. & Tans, P. Regional N<sub>2</sub>O fluxes in  
514 Amazonia derived from aircraft vertical profiles. *Atmos. Chem. Phys.* **9**, 8785–8797  
515 (2009).
- 516 6. Teh, Y. A., Murphy, W. A., Berrío, J.-C., Boom, A. & Page, S. E. Seasonal variability in  
517 methane and nitrous oxide fluxes from tropical peatlands in the western Amazon basin.  
518 *Biogeosciences* **14**, 3669–3683 (2017).
- 519 7. Finn, D. R. *et al.* Methanogens and methanotrophs show nutrient-dependent community  
520 assemblage patterns across tropical peatlands of the Pastaza-Marañón basin, Peruvian  
521 Amazonia. *Front. Microbiol.* **11**, (2020).
- 522 8. Buessecker, S. *et al.* Effects of sterilization techniques on chemodenitrification and N<sub>2</sub>O  
523 production in tropical peat soil microcosms. *Biogeosciences* **16**, 4601–4612 (2019).
- 524 9. Heil, J., Liu, S., Vereecken, H. & Brüggemann, N. Abiotic nitrous oxide production from  
525 hydroxylamine in soils and their dependence on soil properties. *Soil Biol. Biochem.* **84**,  
526 107–115 (2015).
- 527 10. Samarkin, V. A. *et al.* Abiotic nitrous oxide emission from the hypersaline Don Juan  
528 Pond in Antarctica. *Nat. Geosci.* **3**, 341–344 (2010).
- 529 11. Otte, J. M. *et al.* N<sub>2</sub>O formation by nitrite-induced (chemo)denitrification in coastal  
530 marine sediment. *Sci. Rep.* **9**, 1–12 (2019).
- 531 12. Jones, L. C., Peters, B., Pacheco, J. S. L., Casciotti, K. L. & Fendorf, S. Stable isotopes  
532 and iron oxide mineral products as markers of chemodenitrification. *Environ. Sci.*  
533 *Technol.* **49**, 3444–3452 (2015).
- 534 13. Stevenson, F. J. & Swaby, R. J. Nitrosation of soil organic matter: I. Nature of gases  
535 evolved during nitrous acid treatment of lignins and humic substances. *Soil Sci. Soc. Am.*  
536 *J.* **28**, 773–778 (1964).
- 537 14. Ostrom, N. E., Gandhi, H., Trubl, G. & Murray, A. E. Chemodenitrification in the  
538 cryoecosystem of Lake Vida, Victoria Valley, Antarctica. *Geobiology* **14**, 575–587  
539 (2016).
- 540 15. Gumbrecht, T. *et al.* An expert system model for mapping tropical wetlands and  
541 peatlands reveals South America as the largest contributor. *Glob. Change Biol.* **23**, 3581–  
542 3599 (2017).
- 543 16. Seitzinger, S. *et al.* Denitrification across landscapes and waterscapes: A synthesis. *Ecol.*  
544 *Appl.* **16**, 2064–2090 (2006).
- 545 17. Glass, J. B. & Orphan, V. J. Trace metal requirements for microbial enzymes involved in  
546 the production and consumption of methane and nitrous oxide. *Front. Microbiol.* **3**, 1–20  
547 (2012).
- 548 18. Graf, D. R. H., Jones, C. M. & Hallin, S. Intergenomic comparisons highlight modularity  
549 of the denitrification pathway and underpin the importance of community structure for  
550 N<sub>2</sub>O emissions. *PLoS ONE* **9**, e114118 (2014).

- 551 19. Roco, C. A., Bergaust, L. L., Bakken, L. R., Yavitt, J. B. & Shapleigh, J. P. Modularity  
552 of nitrogen-oxide reducing soil bacteria: Linking phenotype to genotype. *Environ.*  
553 *Microbiol.* **19**, 2507–2519 (2017).
- 554 20. Pihlatie, M., Syväsalo, E., Simojoki, A., Esala, M. & Regina, K. Contribution of  
555 nitrification and denitrification to N<sub>2</sub>O production in peat, clay and loamy sand soils  
556 under different soil moisture conditions. *Nutr. Cycl. Agroecosystems* **70**, 135–141  
557 (2004).
- 558 21. Palmer, K. & Horn, M. A. Actinobacterial nitrate reducers and Proteobacterial  
559 denitrifiers are abundant in N<sub>2</sub>O-metabolizing peat. *Appl. Environ. Microbiol.* **78**,  
560 5584–5596 (2012).
- 561 22. Sanford, R. A. *et al.* Unexpected nondenitrifier nitrous oxide reductase gene diversity  
562 and abundance in soils. *Proc. Natl. Acad. Sci. USA* **109**, 19709–19714 (2012).
- 563 23. Jones, C. M. *et al.* Recently identified microbial guild mediates soil N<sub>2</sub>O sink capacity.  
564 *Nat. Clim. Change* **4**, 801–805 (2014).
- 565 24. Hallin, S., Philippot, L., Löffler, F. E., Sanford, R. A. & Jones, C. M. Genomics and  
566 Ecology of Novel N<sub>2</sub>O-Reducing Microorganisms. *Trends Microbiol.* **26**, 43–55 (2018).
- 567 25. Lycus, P. *et al.* A bet-hedging strategy for denitrifying bacteria curtails their release of  
568 N<sub>2</sub>O. *Proc. Natl. Acad. Sci. USA* **115**, 11820–11825 (2018).
- 569 26. Burns, L. C., Stevens, R. J. & Laughlin, R. J. Determination of the simultaneous  
570 production and consumption of soil nitrite using <sup>15</sup>N. *Soil Biol. Biochem.* **27**, 839–844  
571 (1995).
- 572 27. Burns, L. C., Stevens, R. J. & Laughlin, R. J. Production of nitrite in soil by  
573 simultaneous nitrification and denitrification. *Soil Biol. Biochem.* **28**, 609–616 (1996).
- 574 28. Wullstein, L. H. & Gilmour, C. M. Non-enzymatic formation of nitrogen gas. **210**, 1150–  
575 1151 (1966).
- 576 29. Liu, S., Schloter, M., Hu, R., Vereecken, H. & Brüggemann, N. Hydroxylamine  
577 contributes more to abiotic N<sub>2</sub>O production in soils than nitrite. *Front. Environ. Sci.* **7**, 1–  
578 10 (2019).
- 579 30. Thorn, K. A. & Mikita, M. A. Nitrite fixation by humic substances nitrogen-15 nuclear  
580 magnetic resonance evidence for potential intermediates in chemodenitrification. *Soil*  
581 *Sci. Soc. Am. J.* **64**, 568–582 (2000).
- 582 31. Thorn, K. A., Younger, S. J. & Cox, L. G. Order of Functionality Loss during  
583 Photodegradation of Aquatic Humic Substances. *J. Environ. Qual.* **39**, 1416–1428  
584 (2010).
- 585 32. Klüpfel, L., Piepenbrock, A., Kappler, A. & Sander, M. Humic substances as fully  
586 regenerable electron acceptors in recurrently anoxic environments. *Nat. Geosci.* **7**, 195–  
587 200 (2014).
- 588 33. Lovley, D. R. & Blunt-Harris, E. L. Role of humic-bound iron as an electron transfer  
589 agent in dissimilatory Fe(III) reduction. *Appl. Environ. Microbiol.* **65**, 4252–4254  
590 (1999).
- 591 34. Kappler, A., Benz, M., Schink, B. & Brune, A. Electron shuttling via humic acids in  
592 microbial iron(III) reduction in a freshwater sediment. *FEMS Microbiol. Ecol.* **47**, 85–92  
593 (2004).
- 594 35. Van Cleemput, O., Patrick, W. H. & McIlhenny, R. C. Nitrite decomposition in flooded  
595 soil under different pH and redox potential conditions. *Soil Sci. Soc. Am. J.* **40**, 55–60  
596 (1976).
- 597 36. Van Cleemput, O. & Baert, L. Nitrite: A key compound in N loss processes under acid  
598 conditions? *Plant Soil* **76**, 233–241 (1984).

- 599 37. Porter, L. K. Gaseous products produced by anaerobic reaction of sodium nitrite with  
600 oxime compounds and oximes synthesized from organic matter. *Soil Sci. Soc. Am. J.* **33**,  
601 696–702 (1969).
- 602 38. Liu, B., Mørkved, P. T., Frostegård, Å. & Bakken, L. R. Denitrification gene pools,  
603 transcription and kinetics of NO, N<sub>2</sub>O and N<sub>2</sub> production as affected by soil pH. *FEMS*  
604 *Microbiol. Ecol.* **72**, 407–417 (2010).
- 605 39. Palmer, K., Biasi, C. & Horn, M. A. Contrasting denitrifier communities relate to  
606 contrasting N<sub>2</sub>O emission patterns from acidic peat soils in arctic tundra. *ISME J.* **6**,  
607 1058–1077 (2012).
- 608 40. Onley, J. R., Ahsan, S., Sanford, R. A. & Löffler, F. E. Denitrification by  
609 *Anaeromyxobacter dehalogenans*, a common soil bacterium lacking the nitrite reductase  
610 genes *nirS* and *nirK*. *Appl. Environ. Microbiol.* **84**, 1–14 (2018).
- 611 41. Sanford, R. A., Cole, J. R. & Tiedje, J. M. Characterization and Description of  
612 *Anaeromyxobacter dehalogenans* gen. nov., sp. nov., an Aryl-Halo-respiring Facultative  
613 Anaerobic Myxobacterium. *Appl. Environ. Microbiol.* **68**, 893–900 (2002).
- 614 42. Mohr, K. I., Zindler, T., Wink, J., Wilharm, E. & Stadler, M. Myxobacteria in high moor  
615 and fen: An astonishing diversity in a neglected extreme habitat. *MicrobiologyOpen* **6**,  
616 e00464 (2017).
- 617 43. Hori, T., Müller, A., Igarashi, Y., Conrad, R. & Friedrich, M. W. Identification of iron-  
618 reducing microorganisms in anoxic rice paddy soil by <sup>13</sup>C-acetate probing. *ISME J.* **4**,  
619 267–278 (2010).
- 620 44. Kawaichi, S. *et al.* *Ardenticatena maritima* gen. nov., sp. nov., a ferric iron- and nitrate-  
621 reducing bacterium of the phylum ‘*Chloroflexi*’ isolated from an iron-rich coastal  
622 hydrothermal field, and description of *Ardenticatena* classis nov. *Int. J. Sys. Evol.*  
623 *Microbiol.* **63**, 2992–3002 (2013).
- 624 45. Podosokorskaya, O. A. *et al.* Characterization of *Melioribacter roseus* gen. nov., sp.  
625 nov., a novel facultatively anaerobic thermophilic cellulolytic bacterium from the class  
626 *Ignavibacteria*, and a proposal of a novel bacterial phylum *Ignavibacteriae*. *Environ.*  
627 *Microbiol.* **15**, 1759–1771 (2013).
- 628 46. Maher, B. A. & Taylor, R. M. Formation of ultrafine-grained magnetite in soils. *Nature*  
629 **336**, 368–370 (1988).
- 630 47. Sanchez, P. A. *Properties and management of soils in the tropics*. (Wiley, 1976).
- 631 48. White, A. F. *et al.* Chemical weathering in a tropical watershed, Luquillo Mountains,  
632 Puerto Rico: I. Long-term versus short-term weathering fluxes. *Geochim. Cosmochim.*  
633 *Acta* **62**, 209–226 (1998).
- 634 49. Hall, S. J., Liptzin, D., Buss, H. L., DeAngelis, K. & Silver, W. L. Drivers and patterns  
635 of iron redox cycling from surface to bedrock in a deep tropical forest soil: a new  
636 conceptual model. *Biogeochemistry* **130**, 177–190 (2016).
- 637 50. Buchwald, C., Grabb, K., Hansel, C. M. & Wankel, S. D. Constraining the role of iron in  
638 environmental nitrogen transformations: Dual stable isotope systematics of abiotic NO<sub>2</sub><sup>-</sup>  
639 reduction by Fe(II) and its production of N<sub>2</sub>O. *Geochim. Cosmochim. Acta* **186**, 1–12  
640 (2016).
- 641 51. Grabb, K. C., Buchwald, C., Hansel, C. M. & Wankel, S. D. A dual nitrite isotopic  
642 investigation of chemodenitrification by mineral-associated Fe(II) and its production of  
643 nitrous oxide. *Geochim. Cosmochim. Acta* **196**, 388–402 (2017).
- 644 52. Drewer, J. *et al.* Linking nitrous oxide and nitric oxide fluxes to microbial communities  
645 in tropical forest soils and oil palm plantations in Malaysia in laboratory incubations.  
646 *Front. For. Glob. Change* **3**, 1–14 (2020).



- 647 53. Yvon-Durocher, G., Jones, J. I., Trimmer, M., Woodward, G. & Montoya, J. M.  
648 Warming alters the metabolic balance of ecosystems. *Philos. Trans. R. Soc. B: Biol. Sci.*  
649 (2010). doi:10.1098/rstb.2010.0038
- 650 54. Yvon-Durocher, G. *et al.* Reconciling the temperature dependence of respiration across  
651 timescales and ecosystem types. *Nature* **487**, 472–476 (2012).
- 652 55. Jauhiainen, J., Keröjoki, O., Silvennoinen, H., Limin, S. & Vasander, H. Heterotrophic  
653 respiration in drained tropical peat is greatly affected by temperature – a passive  
654 ecosystem cooling experiment. *Environmental Research Letters* **9**, 105013 (2014).
- 655 56. Wang, S., Zhuang, Q., Lähteenoja, O., Draper, F. C. & Cadillo-Quiroz, H. Potential shift  
656 from a carbon sink to a source in Amazonian peatlands under a changing climate. *Proc.*  
657 *Natl. Acad. Sci. USA* **115**, 12407–12412 (2018).
- 658 57. Stumm, W. & Lee, G. F. Oxygenation of ferrous iron. *Ind. Eng. Chem.* **53**, 143–146  
659 (1961).
- 660 58. Theis, T. L. & Singer, P. C. Complexation of iron(II) by organic matter and its effect on  
661 iron(II) oxygenation. *Environ. Sci. Technol.* **8**, 569–573 (1974).
- 662 59. Wan, X. *et al.* Complexation and reduction of iron by phenolic substances: Implications  
663 for transport of dissolved Fe from peatlands to aquatic ecosystems and global iron  
664 cycling. *Chem. Geol.* **498**, 128–138 (2018).
- 665 60. Daugherty, E. E., Gilbert, B., Nico, P. S. & Borch, T. Complexation and redox buffering  
666 of iron(II) by dissolved organic matter. *Environ. Sci. Technol.* **51**, 11096–11104 (2017).
- 667 61. Prananto, J. A., Minasny, B., Comeau, L.-P., Rudiyanto, R. & Grace, P. Drainage  
668 increases CO<sub>2</sub> and N<sub>2</sub>O emissions from tropical peat soils. *Glob. Change Biol.* **26**, 4583–  
669 4600 (2020).
- 670 62. Stirling, E., Fitzpatrick, R. W. & Mosley, L. Drought effects on wet soils in inland  
671 wetlands and peatlands. *Earth-Sci. Rev.* **210**, 1–15 (2020).
- 672 63. Hodgkins, S. B. *et al.* Tropical peatland carbon storage linked to global latitudinal trends  
673 in peat recalcitrance. *Nat. Commun.* **9**, 1–13 (2018).
- 674 64. Arias, P. *et al.* Climate change 2021: The physical science basis. Contribution of  
675 Working Group I to the sixth assessment report of the intergovernmental panel on  
676 climate change. (2021).
- 677 65. Babbin, A. R., Bianchi, D., Jayakumar, A. & Ward, B. B. Rapid nitrous oxide cycling in  
678 the suboxic ocean. *Science* **348**, 1127–1129 (2015).
- 679 66. Hamilton, S. K. & Ostrom, N. E. Measurement of the stable isotope ratio of dissolved N<sub>2</sub>  
680 in <sup>15</sup>N tracer experiments. *Limnol. Oceanogr.-Meth.* **5**, 233–240 (2007).
- 681 67. Stumm, W. & Morgan, J. J. *Aquatic chemistry., 3rd edn (John Wiley & Sons: New York).*  
682 (1996).
- 683 68. Homyak, P. M., Kamiyama, M., Sickman, J. O. & Schimel, J. P. Acidity and organic  
684 matter promote abiotic nitric oxide production in drying soils. *Glob. Change Biol.* **23**,  
685 1735–1747 (2017).
- 686 69. Henry, S., Bru, D., Stres, B., Hallet, S. & Philippot, L. Quantitative detection of the *nosZ*  
687 gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA,  
688 *narG*, *nirK*, and *nosZ* genes in soils. *Appl. Environ. Microbiol.* (2006).  
689 doi:10.1128/AEM.00231-06
- 690 70. Jones, C. M., Graf, D. R., Bru, D., Philippot, L. & Hallin, S. The unaccounted yet  
691 abundant nitrous oxide-reducing microbial community: a potential nitrous oxide sink.  
692 *ISME J.* **7**, 417–426 (2013).
- 693 71. Zhang, B. *et al.* A new primer set for clade I *nosZ* that recovers genes from a broader  
694 range of taxa. *Biol. Fertil. Soils* **57**, 523–531 (2021).

- 695 72. Herbold, C. W. *et al.* A flexible and economical barcoding approach for highly  
696 multiplexed amplicon sequencing of diverse target genes. *Front. Microbiol.* **6**, 8966  
697 (2015).
- 698 73. Edgar, R. C. Search and clustering orders of magnitude faster than BLAST.  
699 *Bioinformatics* **26**, 2460–2461 (2010).
- 700 74. Edgar, R. C. UNOISE2: Improved error-correction for Illumina 16S and ITS amplicon  
701 sequencing. *bioRxiv* 081257 (2016). doi:10.1101/081257
- 702 75. Wang, Q. *et al.* Ecological patterns of *nifH* genes in four terrestrial climatic zones  
703 explored with targeted metagenomics using Framebot, a new informatics tool. *mBio* **4**,  
704 e00592–13 (2013).
- 705 76. Buchfink, B., Xie, C. & Huson, D. H. Fast and sensitive protein alignment using  
706 DIAMOND. *Nat. Methods* **12**, 59–60 (2015).
- 707 77. Fish, J. A. *et al.* FunGene: the functional gene pipeline and repository. *Front. Microbiol.*  
708 **4**, 1–14 (2013).
- 709 78. Huson, D. H. *et al.* MEGAN Community Edition - Interactive exploration and analysis  
710 of large-scale microbiome sequencing data. *PLoS Comput. Biol.* **12**, e1004957 (2016).
- 711 79. Huson, D. H. *et al.* MEGAN-LR: New algorithms allow accurate binning and easy  
712 interactive exploration of metagenomic long reads and contigs. *Biol. Direct* **13**, 1–17  
713 (2018).
- 714 80. Ondov, B. D., Bergman, N. H. & Phillippy, A. M. Interactive metagenomic visualization  
715 in a Web browser. *BMC Bioinform.* **12**, 1–10 (2011).
- 716 81. Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. MEGA X: Molecular  
717 evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **35**, 1547–  
718 1549 (2018).
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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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723

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739

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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748

749 **Competing interests**

750 The authors have no competing interests to declare.