Supplemental material

Abiotic-denitrification in tropical highland rainforest soils

Tropical rainforest soils are often characterized by high concentrations of crystalline reactive Iron (Fe) and fluctuating redox conditions, which are likely to support Fe reduction coupled to anaerobic ammonium oxidation (Feammox) (Yangh et al., 2012) or chemo-denitrification (Van Cleemput and Baert, 1984). Luther et al. (1997) pointed out that high Fe concentrations and pH values below 6.8 provide conditions under which Feammox based produce N_2 production could take place. Furthermore, chemo-denitrification can occur in Fe-rich and moderately acidic soils, reducing NO_2^- (nitrite) to NO (nitric oxide), N_2O (nitrous oxide), or N_2 (di-nitrogen) through self-decomposition of nitrous acid or in reaction with metallic cations such as Fe (II) (Van Cleemput and Baert, 1984).

We performed a preliminary experiment to assess if abiotic denitrification could occur in tropical mountain forest soils of the Nyungwe national park. We focused on the acidic soils of the Nyungwe forest to test if chemo-denitrification plays a role for NO and N_2O production in addition to microbial processes.

Laboratory experiment

We selected seven soil samples from a dataset of 31 soil samples from the Nyungwe forest for which NO and N_2O production potentials have been determined previously (Gharahi Ghehi et al., 2012). These 7 samples cover different organic carbon content, pH and Fe levels (Table S1).

Mercuric chloride ($HgCl_2$) was used as the sterilizing agent because among sterilization techniques (i.e., autoclaving, fumigation), $HgCl_2$ results in minimal changes in chemical and physical soil properties, with no significant effects on nutrient concentrations (Trevors, 1996; Wolf et al., 1989). The rate of $HgCl_2$ addition of 3000 mg $HgCl_2$ kg^{-1} soil was in the range recommended by Wolf and Skipper (1994) (500-20000 mg kg^{-1} of dry soil) to achieve effective inhibition of microbial metabolism. In addition, Trevors (1996) pointed out that generally, a concentration of 500 mg $HgCl_2$ kg^{-1} dry soil is satisfactory.

Table S1 Soil chemical characteristics, organic carbon (OC), Iron (Fe II& III), Nitrite-N (NO_2^--N), ammonium-N (NH_4^+-N) and Nitrate-N (NO_3^--N), for seven soil samples selected from a dataset of 31 soil samples from the Nyungwe forest

Soil sample	OC	Soil-pH	Fe (II)	Fe (III)	NO_2^--N	NH_4^+-N	NO_3^--N
	%		%	%	(µg g^{-1} soil)	(µg g^{-1} soil)	(µg g^{-1} soil)
1	3.9	4.47	0.13	0.44	0.0258	266.26	1.02
2	6.5	4.14	0.41	2.70	0.0338	216.76	14.93
3	1.0	3.98	0.31	4.55	0.0422	47.09	9.87
4	4.2	3.74	0.47	6.29	0.0546	160.51	16.10
5	6.6	3.85	0.33	2.04	0.0444	17.55	1.26
6	3.2	4.15	0.45	5.17	0.0336	25.05	5.23
7	4.2	3.40	0.46	6.05	0.0376	72.42	14.50

One day before the start of the experiment we first tested microbial activity with sterilization treatment ($HgCl_2$ sterilized) and four incubation times (1, 2, 5 and 24 h). Therefore, 25 g dry soil was added to two 1200 ml airtight sealed glass containers. Five milliliters of 15 mg $HgCl_2$ L^{-1} was added to one the soil sample in a solution of deionized water and mixes thoroughly into the soil. After 1, 2, 5 and 24 h, the headspace of the glass jar was sampled for dioxide carbon (CO_2) analyses both from live soil and Hg-treated soil. Addition of 3000 mg $HgCl_2$ kg^{-1} dry soil clearly eliminated CO_2 productions (Fig. S1a, b). This indicates that the Hg-treatment was effective in eliminating the activity of soil microorganisms.

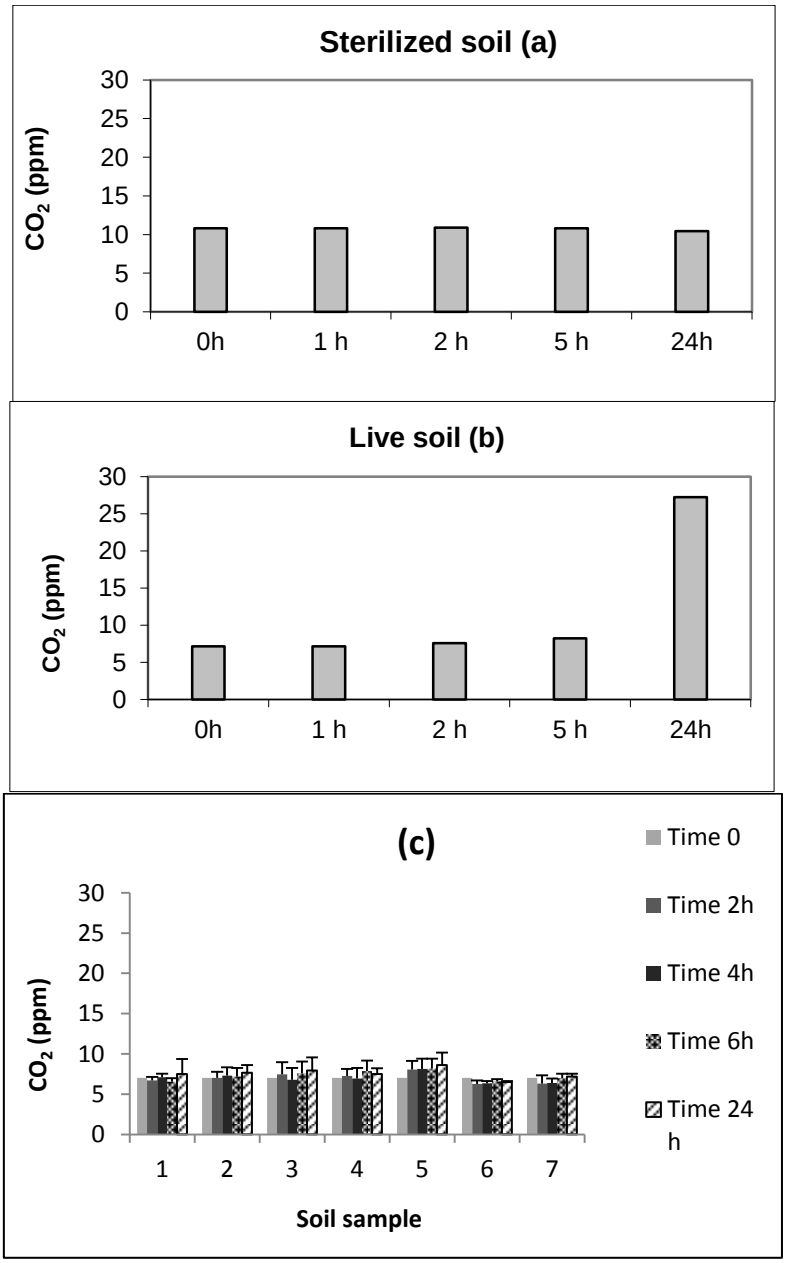

Fig. S1 Comparison of headspace carbon dioxide (CO_2) concentrations between $HgCl_2$-treated (a) and live soil (b) for 1, 2, 5 and 24 h after $HgCl_2$ addition; headspace CO_2 concentrations for $HgCl_2$-treated soils for the seven Nyungwe soil samples for each nitric oxide (NO) and nitrous oxide (N_2O) sampling time during the incubation (c), error bars are plus one standard deviation

All seven soil samples in three replicates were incubated at 70% water filled pore space (WFPS). We applied the same laboratory techniques that were used by Gharahi Ghehi et al. (2012) to measure soil N_2O and NO production. For each treatment 25 g dry soil was added to an incubation tube that was placed into an airtight sealed glass container with a volume of 1200 ml. Soils received 10 atom% $Na^{15}NO_2$ and 5 milliliters of 15 mg $HgCl_2$ L^{-1} . The rate of ^{15}N addition was equivalent to ~1 fold of the soil NO_2^--N concentration in each soil sample (range 0.025-0.054 μg $^{15}NO_2^-$-N g^{-1} soil). Immediately after addition of $^{15}NO_2^-$-N -, the glass containers were closed. After 0, 2, 4, 6 and 24 h, NO concentration of the headspace was determined using an NO analyzer (CLD 77AM, Eco Physics, Switzerland) and immediately after the NO measurements, a 12 ml gas sample was withdrawn from the headspace for CO_2 and N_2O analyses. The stored gas vials was analyzed on a gas chromatograph (14B, Shimadzu, Japan) equipped with an electron capture detector, ECD) for CO_2 and N_2O detection. Following the sampling at 24 h, all glass continents were opened and 0.2 g of wet soil was sampled from each tube for analyzes of Fe (II) and Fe (III). The remaining soil was extracted in 50ml of 1M KCl for analysis of NO_2^- concentration and ^{15}N as NO_2^-.

Soil Fe (II) was measured colorimetrically by dissolving 10 mg of soil sample in a solution of H_2SO_4 and HF in the presence of powdered orthophenantroline on a steam bath (Shapiro, 1960). Total Fe (Fe (II) + Fe (III)) was measured upon sodium dithionite extraction (Mehra and Jackson, 1960) and measured via optical emission spectrometer (Varian ICP-OES) (720 ES, Mulgrave VIC 3170, Australia). Soil ^{15}N as NO_2^- content were measured by trace gas (TG II) coupled to an isotope ratio mass spectrometer (TG-IRMS) (20–20, SerCon, Crewe, UK). Extractable NO_2^- concentrations was determined using copper-cadmium reduction on a Brann+Luebbe autoanalyser AA3.

Results

The negligible rates of CO_2 production in all seven Hg-treated soils over 24 h (Fig. S1c) showed that the Hg-treatment was effective in eliminating the activity of soil microorganisms. Twenty four hours after addition of ^{15}N-NO_2^-, no ^{15}N was observed in NO_2^- for all soils that received $^{15}NO_2^-$. Furthermore, Figure S2a shows that the large proportion of soil NO_2^- (added $^{15}NO_2^-$+ Soil NO_2^-) disappeared after 24 h of incubation.

Except two soil samples, N_2O concentrations showed no significant increase between the 0 and 2 h incubation time. Figure S2b also illustrates that N_2O concentrations from few sterilized soil samples have a significant increase after 24 h of incubation.

Multiple comparisons of all NO concentrations from all sterilized soil samples showed a significant increase between 0 and 2 h ($p<0.05$), and small increase observed in concentration thereafter (no significant differences between 2, 4, 6 and 24h) (Fig. S2b, c). Therefore, we used N_2O and NO emissions during a 2 h incubation time to calculate N-gas fluxes in μg m^{-2} h^{-1} (Table S2). Follow up measurement of Fe (II) and Fe (III) showed no significant differences between 0 and 24 h of incubation time (Fig. S3). The results also showed that most of the Fe is in the oxidized form Fe (III).

Table S2 Nitrous oxide (N_2O) and nitric oxide (NO) flux rates calculated in μg m^{-2} h^{-1}

Soil sample	μg N_2O-N m^{-2} h	μg NO-N m^{-2} h
1	8.75 ± 3.54	67.62 ± 29.05
2	10.77 ± 15.75	95.11 ± 36.66
3	24.71 ± 10.66	110.24 ± 20.69
4	20.63 ± 3.37	149.64 ± 26.28
5	13.97 ± 4.08	104.04 ± 13.90
6	17.88 ± 14.97	98.86 ± 2.39
7	23.14 ± 5.02	235.97 ± 14.62

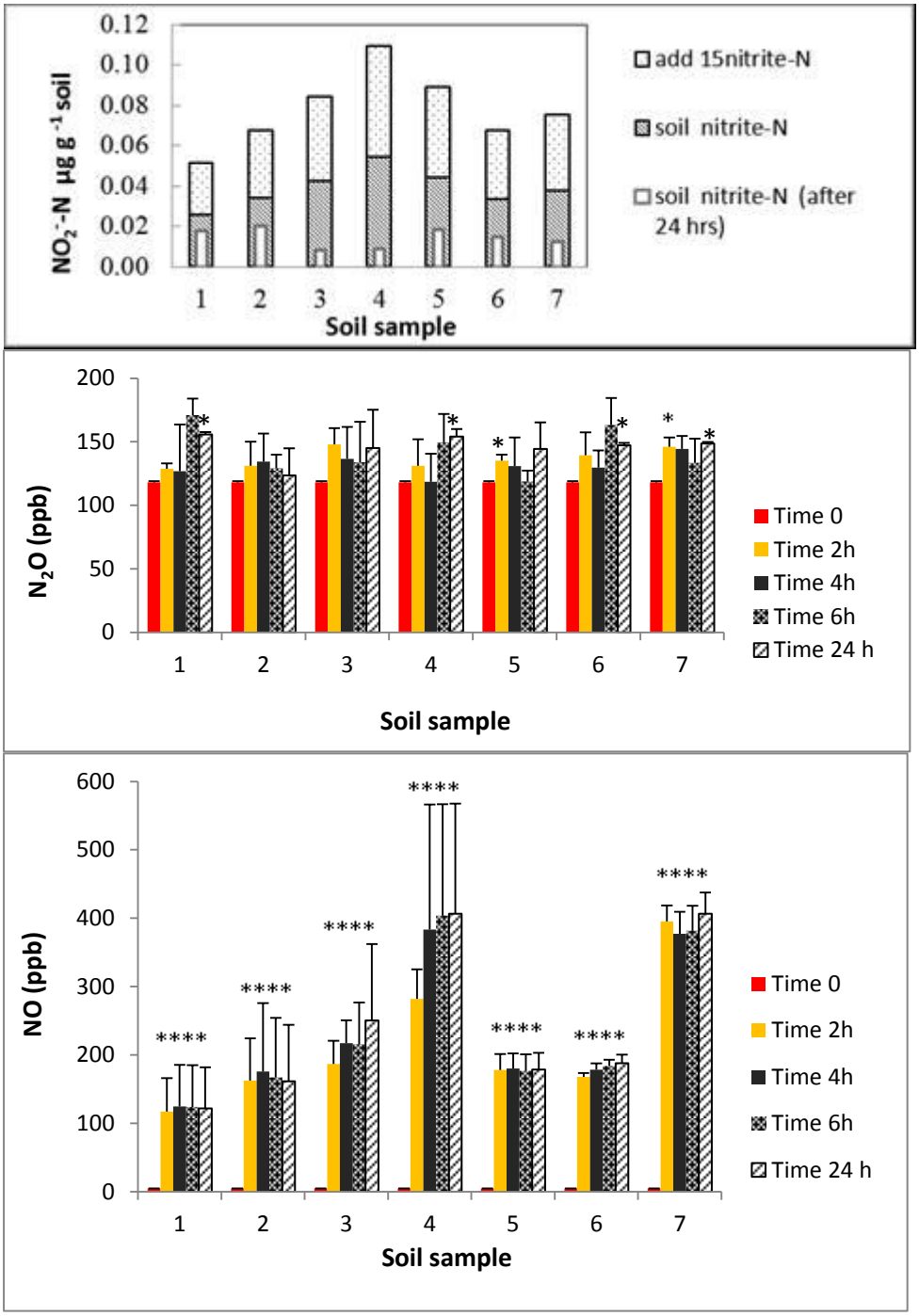

Fig. S2 Nitrite (NO_2^-) concentration in soil before incubation and after 24 hours of incubation among seven soil samples (a); nitrous oxide (N_2O) and nitric oxide (NO) concentration in the headspace of the glass jar for each sampling time for the seven Nyungwe soil samples (a,b); error bars are plus mines one standard deviation, significant deference from 0 h time have been indicated by a star

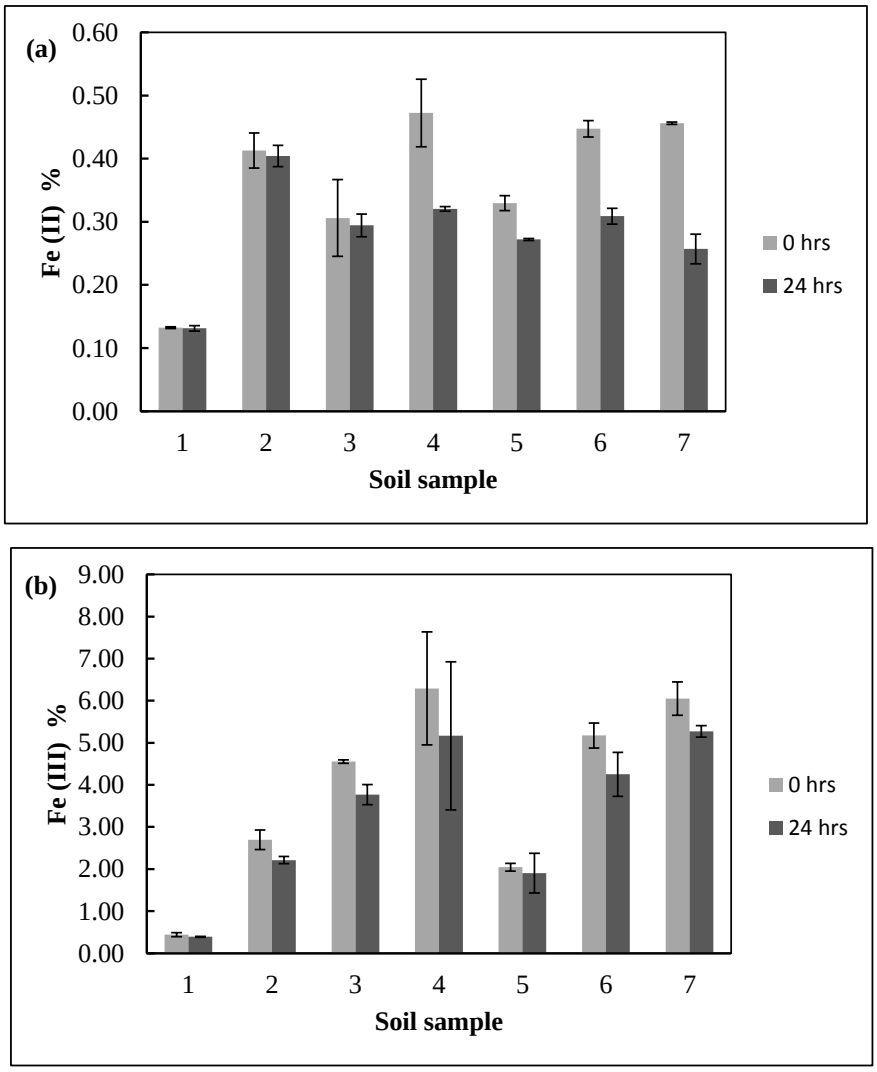

Fig. S3 Extractable Fe (II) and Fe (III) concentrations before and after 24 hours of incubation. error bars are plus mines one standard deviation

As a result, we acknowledge that consumption of all added $^{15}N\text{-}NO_2^-$ and concurrent N_2O and NO emissions from the Nyungwe forest soils are possibly due to abiotic processes, such as chemo-denitrification.

Nitrite is known to react readily and abiotically with Fe (II). Ammonium (NH_4^+) can follow a similar fate if it is first oxidised to NO_2^-. So, abiotic NO and N_2O production is thought to occur in all our soil samples with pH around 4 or <4 as follows:

First, oxidation of soil NH_4^+ coupled to reduction of Fe (III) (Feammox, Yang et al., 2012), may provide a source of Fe (II) and NO_2^-. Second, by reaction of Fe (II) with NO_2^-, Fe (III) and NO, N_2O or N_2 can be formed (Fig. S4) (Van Cleemput and Baert, 1984; Van Cleemput

and Samater 1996). However, we note that NH_4^+ concentration in all seven soil samples were high due to long storage (~2 years) for dry soil samples (Table S1).

Moreover, Davidson et al. (2003) pointed out that Nitrate (NO_3^-) can abiotically reduced to NO_2^- via reduced inorganic components (e.g. Fe (II)). So we speculate that this reaction pathway wherby Fe (II) reduces NO_3^- to NO_2^- most likely play a role in producing NO/N_2O through abiotic processes (see NO_3^- concentration for seven soil samples in Table S1).

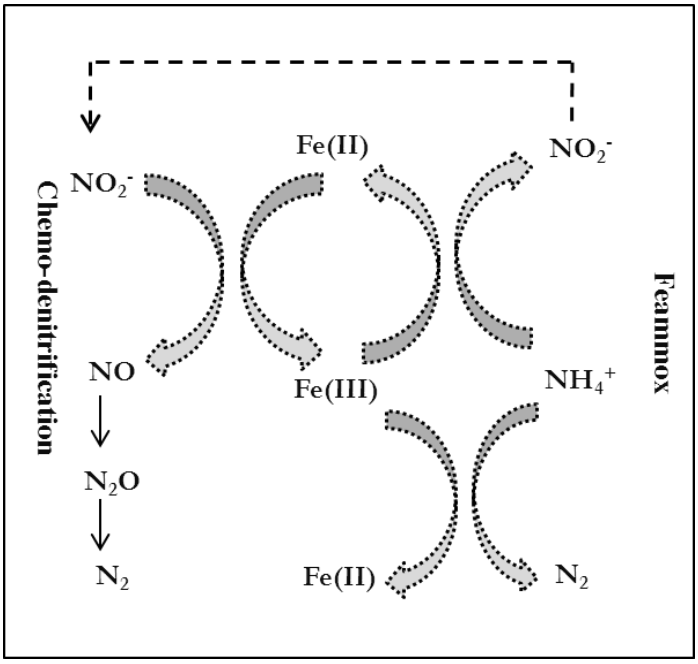

Fig. S4 Chemo-denitrification and Feammox pathway for nitric oxide (NO), nitrous oxide (N_2O) and di-nitrogen (N_2) production

Furthermore, the variation in abiotic N_2O emissions was significantly positively correlated with Fe (III) ($r = 0.85$, $p < 0.05$). N_2O emissions also correlated negatively with pH and positively with Fe (II) but not significant. NO emissions was negatively, significantly correlated with pH ($r = 0.92$, $p < 0.05$). The correlation was also positive with Fe (II) and Fe (III) but not significant. These results are supportive to other studies which were showing abiotically produce soil NO (and N_2O) is thermodynamically favorable at acidic soil with reduced metals.

To summarize, based on our results the abiotic NO (and N_2O) production is a very fast (< 2 h) process, which can be a major pathway responsible for production NO (and N_2O) in the Nyungwe forest soils. Furthermore, we point out that a mixed abiotic-biotic pathway of N_2O production may be also accrued in soils. An abiotic pathway in which, NO_2^- is reduced to NO

followed by the biotic reduction of NO to N_2O. However, we do not have evidence for the contribution of the abiotically produced NO to microbially produced N_2O from our experiment.

In addition, since no ^{15}N-NO and $^{15}N_2O$ could be measured (concentrations < detection limit) in our experiment, there was no evidence to determine if NO_2^- is source of NO or N_2O emissions.

References

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