



# HONO Emissions from Soil Bacteria as a Major Source of Atmospheric Reactive Nitrogen

R. Oswald, T. Behrendt, M. Ermel, D. Wu, H. Su, Y. Cheng, C. Breuninger, A. Moravek, Éric Mougin, Claire Delon, et al.

## ► To cite this version:

R. Oswald, T. Behrendt, M. Ermel, D. Wu, H. Su, et al.. HONO Emissions from Soil Bacteria as a Major Source of Atmospheric Reactive Nitrogen. *Science*, 2013, 341 (6151), pp.1233-1235. 10.1126/science.1242266 . hal-00910702

**HAL Id: hal-00910702**

**<https://hal.science/hal-00910702>**

Submitted on 8 Jul 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

# HONO Emissions from Soil Bacteria as a Major Source of Atmospheric Reactive Nitrogen

R. Oswald,<sup>1,2\*</sup>† T. Behrendt,<sup>1,3†</sup> M. Ermel,<sup>1,2†</sup> D. Wu,<sup>1,4</sup> H. Su,<sup>5</sup> Y. Cheng,<sup>5</sup> C. Breuninger,<sup>1</sup> A. Moravek,<sup>1,6</sup> E. Mougín,<sup>7</sup> C. Delon,<sup>8</sup> B. Loubet,<sup>9</sup> A. Pommerening-Röser,<sup>10</sup> M. Sörgel,<sup>1</sup> U. Pöschl,<sup>5</sup> T. Hoffmann,<sup>2</sup> M.O. Andreae,<sup>1</sup> F.X. Meixner,<sup>1</sup> I. Trebs<sup>1\*</sup>

Abiotic release of nitrous acid (HONO) in equilibrium with soil nitrite ( $\text{NO}_2^-$ ) was suggested as an important contributor to the missing source of atmospheric HONO and hydroxyl radicals (OH). The role of total soil-derived HONO in the biogeochemical and atmospheric nitrogen cycles, however, has remained unknown. In laboratory experiments, we found that for nonacidic soils from arid and arable areas, reactive nitrogen emitted as HONO is comparable with emissions of nitric oxide (NO). We show that ammonia-oxidizing bacteria can directly release HONO in quantities larger than expected from the acid-base and Henry's law equilibria of the aqueous phase in soil. This component of the nitrogen cycle constitutes an additional loss term for fixed nitrogen in soils and a source for reactive nitrogen in the atmosphere.

Soil biogenic NO emissions account for ~20% of the total NO sources to the atmosphere (1) and vary as a function of microbial activity and physicochemical soil properties. NO is produced during nitrification, in which soil microbes convert ammonium ( $\text{NH}_4^+$ ) via  $\text{NO}_2^-$  to nitrate ( $\text{NO}_3^-$ ), both of which can accumulate in soil (2, 3). In addition, the reduction of  $\text{NO}_3^-$ , which is known as denitrification, can cause a release of NO. The two microbial processes are mainly influenced by temperature, soil water content, pH value, and mineral nitrogen availability in the soil (4–6). Previous studies have shown that HONO may also be emitted from soil; this release may originate from the transformation of soil  $\text{NH}_4^+$  to  $\text{NO}_2^-$  (7) or from soil  $\text{NO}_2^-$  because of a chemical acid-base equilibrium (8).

To estimate the contribution of soil HONO emissions to the total reactive nitrogen flux (HONO + NO) from the soil to the atmosphere and to elucidate the major processes influencing

HONO release from soil, we studied the relation of soil HONO emissions to biogenic soil NO emissions under controlled laboratory conditions using the dynamic chamber method (9, 10). Earlier studies have shown that results from using this technique are consistent with those from field measurements (9, 11, 12). We investigated soils from various ecosystems around the world, covering a wide range of soil pH, organic matter, and soil nutrient contents (table S1). The soil samples were wetted in order to reach water holding capacity (WHC) (10) and placed into the chamber, which was then continuously flushed with purified air (free of HONO,  $\text{NO}_x$ ,  $\text{O}_3$ , hydrocarbons, and water vapor), leading to a slow drying of the soil sample during the course of the experiment. The gas-phase mixing ratio of HONO released by the soil sample was measured at the chamber exit with a long path absorption photometer

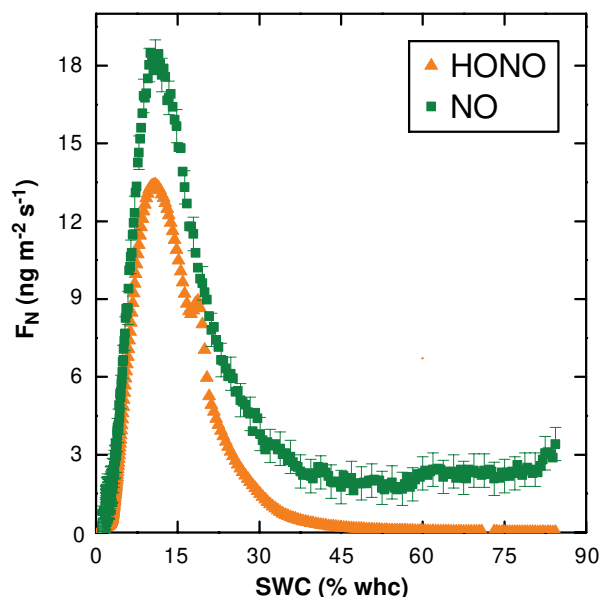
(LOPAP) (13). Mixing ratios of NO and water vapor were also measured (14).

The characteristic moisture dependency of HONO and NO fluxes that is known from previous studies of soil biogenic NO emissions is shown in Fig. 1 (4, 9, 15, 16). We found that the maximal emission fluxes of HONO and NO [henceforth denoted as optimum fluxes;  $F_{\text{N,opt}}(\text{HONO})$  and  $F_{\text{N,opt}}(\text{NO})$ ] are of comparable magnitude and occur at similar optimum soil water content (SWC) (10)—within 10% WHC of one another for all investigated samples.

Chemical acid-base equilibrium calculations predict that abiotic HONO emissions from soil nitrite should be largest for soils with low pH and high  $\text{NO}_2^-$  content (8). The soil pH reflects a sum parameter, which depends on the amount of acidic and basic species in soil, and regulates the solubility of soil constituents and the protonation equilibria. These variables, however, also influence nitrifier and denitrifier activity in soil. In general, abundance and diversity of bacteria are positively correlated with pH (17), and individuals mostly possess a maximum activity at a certain pH (18). In contrast to expectations based on the acid-base equilibrium, the results from different soil samples presented in Fig. 2 do not show a decrease of HONO fluxes with increasing pH. In fact, the neutral soil sample S12, taken from a wheat field in Germany, features extremely high values for HONO and NO emissions ( $F_{\text{N,opt}}$ :  $257.5 \pm 0.1 \text{ ng m}^{-2} \text{ s}^{-1}$  HONO,  $134.8 \pm 0.6 \text{ ng m}^{-2} \text{ s}^{-1}$  NO). The second highest emission of HONO and NO was found for the alkaline, sodic soil represented by sample S17. Comparison with soil  $\text{NO}_2^-$  and  $\text{NH}_4^+$  concentrations (Fig. 2) clearly demonstrates that high HONO and NO emissions are favored for soils with high nutrient content.

The ratio of  $F_{\text{N,opt}}(\text{HONO})$  to  $F_{\text{N,opt}}(\text{NO})$  was found to be higher for arid and arable soils (on

**Fig. 1. Soil emissions of HONO and NO feature similar optimum curves.** Characteristic HONO (triangles) and NO (squares) emission fluxes ( $F_{\text{N}}$  in terms of nitrogen mass) from soil sample S15 (jujube field, semi-arid, fertilized, and irrigated; Mingfeng, Xinjiang, PR China) as a function of SWC expressed in percent of WHC. Experimental error bars (10) are shown for every fourth data point for NO. Error bars of HONO fluxes have the size of the symbols and were omitted.



<sup>1</sup>Biogeochemistry Department, Max Planck Institute for Chemistry, Post Office Box 3060, 55020 Mainz, Germany. <sup>2</sup>Institute for Inorganic and Analytical Chemistry, Johannes Gutenberg University Mainz, 55128 Mainz, Germany. <sup>3</sup>Institute for Geography, Johannes Gutenberg University Mainz, 55128 Mainz, Germany. <sup>4</sup>Key Laboratory of Agricultural Water Research, Center for Agricultural Resources Research, Institute of Genetic and Developmental Biology, Chinese Academy of Sciences, 050021 Shijiazhuang, China. <sup>5</sup>Multiphase Chemistry Department, Max Planck Institute for Chemistry, Post Office Box 3060, 55020 Mainz, Germany. <sup>6</sup>Department of Micrometeorology, University of Bayreuth, 95447 Bayreuth, Germany. <sup>7</sup>Géosciences Environnement Toulouse (GET), Observatoire Midi-Pyrénées, Université de Toulouse, CNRS, Institut pour la Recherche et le Développement, 14 Avenue Edouard Belin, 31400 Toulouse, France. <sup>8</sup>Laboratoire d'Aérodologie, Université de Toulouse, Toulouse, France. <sup>9</sup>Institut National de la Recherche Agronomique (INRA), Unité mixte de Recherche INRA-AgroParistech, Environnement et Grandes Cultures, 78850 Thiverval-Grignon, France. <sup>10</sup>Department of Microbiology and Biotechnology, University of Hamburg, 22609 Hamburg, Germany.

\*Corresponding author. E-mail: robert.oswald@mpic.de (R.O.); i.trebs@mpic.de (I.T.)

†These authors contributed equally to this work.

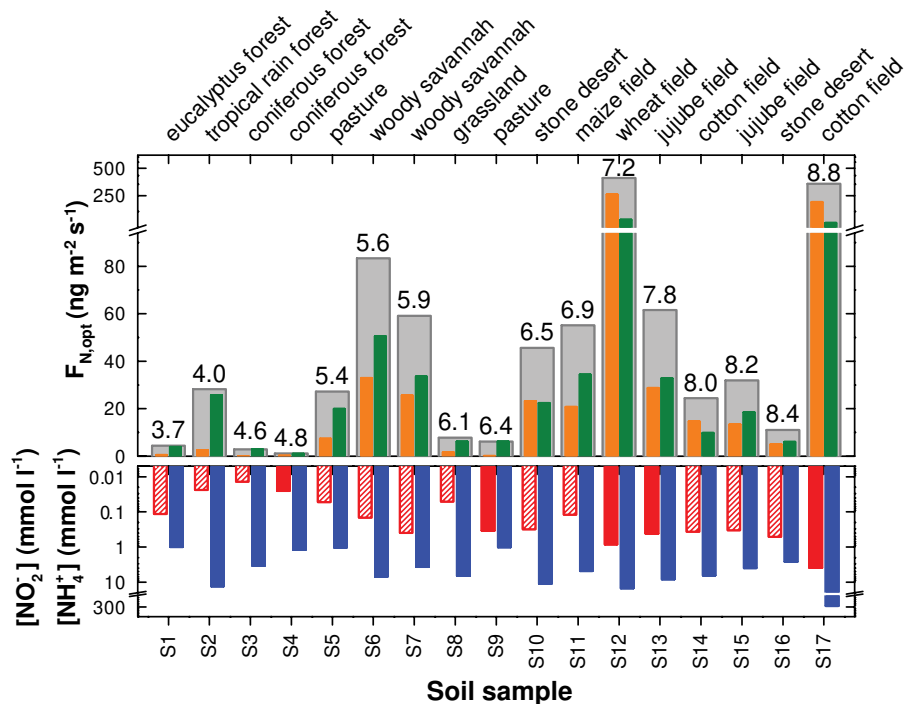
average  $1.06 \pm 0.44$ ) than for nonarable soils of humid and temperate regions (on average  $0.16 \pm 0.12$ ) (fig. S1). For soil pH values higher than 7, the optimum HONO emission fluxes always exceeded  $5 \text{ ng m}^{-2} \text{ s}^{-1}$  (in terms of N) and even reached  $\sim 258 \text{ ng m}^{-2} \text{ s}^{-1}$  (at  $25^\circ\text{C}$ ). We anticipate that HONO emissions are particularly relevant for arid and arable areas with neutral or alkaline soil pH, where they may substantially influence tropospheric chemistry. Potential HONO soil emission hot spots comprise, for instance, large areas of northern Africa, central/southwestern

Asia, and North America as well as some regions around the Mediterranean Sea (fig. S2), covering in total  $\sim 20\%$  of the terrestrial surface (excluding Antarctica). Given the high spatial variability of soil properties (such as pH and nutrients) and our limited amount of soil samples, these hot spot areas may be even larger. This previously neglected ground source of reactive nitrogen may explain the unexpectedly high daytime HONO mixing ratios observed in many studies (19). In addition, NO is produced on a time scale of  $\sim 30 \text{ min}$  from the photolysis of HONO during

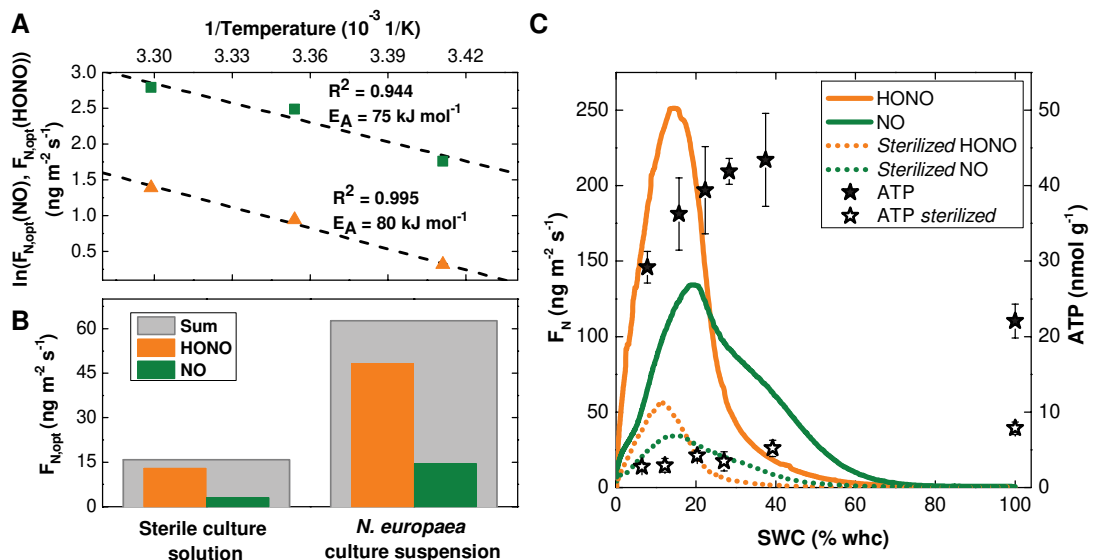
daytime. Hence, soil HONO emissions in the identified hot spot areas (fig. S2) may account for the observed discrepancies between soil emissions of reactive nitrogen estimated with global models by using the Yienger and Levy algorithm and those derived from “top-down” approaches by using nitrogen dioxide ( $\text{NO}_2$ ) columns measured by satellites over arid ecosystems (20, 21).

Biogenic NO emissions are known to depend strongly on soil temperature (22). We measured the temperature dependency of  $F_{\text{N}}(\text{HONO})$  and

**Fig. 2. Optimum emission fluxes of HONO are comparable with those of NO and are largest for  $\text{NO}_2^-$  rich, neutral-to-basic soils in arid and arable regions. (Top)** Optimum emission fluxes of HONO (orange bars), NO (green bars), and their sum (gray bars) in terms of nitrogen for each soil sample (at  $25^\circ\text{C}$ ), arranged by increasing pH. Numbers on top of gray bars represent the soil pH. Land use of soil samples is shown on the top axis. **(Bottom)** Calculated concentrations of  $\text{NH}_4^+$  (blue bars) and  $\text{NO}_2^-$  (red bars) in the soil solution at  $F_{\text{N,opt}}(\text{HONO})$  [striped bars refer to theoretical  $\text{NO}_2^-$  values at the limit of detection (10)].



**Fig. 3. Ammonia-oxidizing bacteria directly release HONO and cause high emissions from soil. (A)** Arrhenius plot of the optimum fluxes of HONO (triangles) and NO (squares) for soil sample S8. **(B)** Optimum fluxes of HONO, NO, and their sum for a sterile AOB nutrient solution and a *N. europaea* culture suspension (activity equivalent to  $1.1 \cdot 10^{-3} \text{ nmol ATP l}^{-1}$ ) applied to glass beads as a soil proxy. **(C)** Influence of bacterial activity on HONO and NO emissions for soil sample S12. Fluxes of HONO and NO for the untreated soil (solid orange and green line, respectively) and for a sterilized subsample (dotted orange and green line, respectively). ATP concentrations serve as indicators for bacterial activity during the dry-out of the untreated sample (black filled stars) and the sterilized sample (black open stars). Error bars denote SDs of three replicates.



$F_N(\text{NO})$  from soil sample S8 (an example for HONO is provided in fig. S3). A temperature increase from 20 to 30°C yielded  $Q_{10}$  values (averaged over the whole SWC range) of 3.7 ( $\pm 1.4$ ) for HONO and 2.1 ( $\pm 0.2$ ) for NO, which is typical for soil respiratory systems (16, 23, 24). From an Arrhenius plot (Fig. 3A), we obtained similar activation energies for HONO (80 kJ mol<sup>-1</sup>) and for NO (75 kJ mol<sup>-1</sup>). These values are much lower than the activation energies reported for denitrification (202 to 250 kJ mol<sup>-1</sup>) (25) but are within the range reported for nitrification by ammonia-oxidizing bacteria (AOB) (25 to 149 kJ mol<sup>-1</sup>) (3, 25), suggesting that the latter process governs the observed co-emission of HONO and NO.

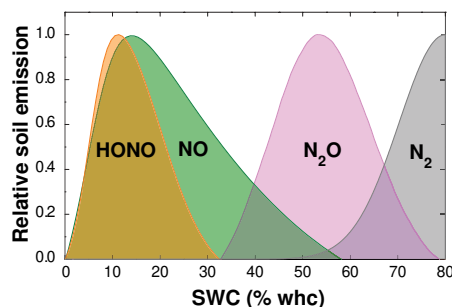
To test this hypothesis, we investigated a pure culture of *Nitrosomonas europaea*, a common and well-studied AOB (26). A suspension of the pure culture (buffered at pH = 8.2) was applied to glass beads serving as an inert soil-like matrix (16), and the model system was treated like a soil sample (10).  $F_{N,\text{opt}}(\text{HONO})$  and  $F_{N,\text{opt}}(\text{NO})$  of the *N. europaea* culture suspension are compared in Fig. 3B with the emissions by using a sterile AOB nutrient solution additionally containing 0.14 mmol l<sup>-1</sup> NO<sub>2</sub><sup>-</sup>, which equals 0.5 mg kg<sup>-1</sup> of NO<sub>2</sub><sup>-</sup> (in terms of N) in soil. The NO<sub>2</sub><sup>-</sup> added to the sterile solution equals the NO<sub>2</sub><sup>-</sup> that would have been produced by the bacteria during the experiment and reflects the chemical contribution to the HONO emission from the model system, whereas the observed difference in  $F_{N,\text{opt}}(\text{HONO})$  between the sterile solution and the culture suspension can be attributed to the direct emissions by the AOB. The *N. europaea* culture emits four times more HONO than does the sterile reference, demonstrating that AOB can indeed act as a strong direct source of HONO.

Measured adenosine 5'-triphosphate (ATP) concentrations during the dry-out of S12 (Fig. 3C) show that soil microbes are active also under relatively dry conditions (% WHC < 20%), where

$F_{N,\text{opt}}(\text{HONO})$  is observed. Because ATP is an indicator for microbial activity in general, the maximum activity of AOB might not coincide with the maximal ATP concentration. We applied methyl iodide—a strong sterilization agent for soil (27) also targeting nitrification (28)—to a subsample of S12 (Fig. 3C). Both HONO and NO emission fluxes were reduced by ~75%, revealing a strong microbial source. This demonstrates that the findings from the model system shown in Fig. 3B are transferable to a real soil sample. The residual emissions can largely be attributed to the chemical source because the ATP content and, hence, the microbial activity was reduced by ~92% at the HONO emission optimum. These results explain the high HONO emissions from nonacidic soil samples.

The conceptual model in Fig. 4 shows that  $F_{N,\text{opt}}(\text{HONO})$  and  $F_{N,\text{opt}}(\text{NO})$  occur in the lower SWC range (~0 to 40% WHC) (16, 29), whereas at high SWC (~40 to 80% WHC), nitrogen is released from soil mainly as the greenhouse gas N<sub>2</sub>O. In general, substrate diffusion is limited at low SWC, and gas diffusion is limited at higher SWC (30). HONO is produced and emitted during nitrification, which predominates at low SWC (5). Samples from different soil and land-use types show their maximal release of the respective nitrogen compound at different optimum SWC (15). The magnitude of the maximal emission of each compound varies depending on, for example, nutrient availability and abundance of soil bacteria.

HONO emissions by AOB and possibly other types of bacteria represent an additional component for gaseous losses from the soil nitrogen pool to the atmosphere. Our survey of soils from different ecosystems indicates that HONO emissions may account for up to 50% of the reactive nitrogen release from soil. This contribution of soil HONO emissions is currently not considered in model estimates of global soil reactive nitrogen emissions (1) and may constitute one of the major uncertainties in this budget. Furthermore, these HONO emissions contribute to atmospheric chemistry by enhancing the oxidation capacity of the lower atmosphere.



**Fig. 4. HONO is a major component of nitrogen emissions from soil.** The conceptual model of soil nitrogen emissions as a function of SWC was adopted from Firestone and Davidson (31). The curves are based on measurements of HONO, NO, and N<sub>2</sub>O emissions from soil sample S12 [N<sub>2</sub> emissions were fitted from (31)].

## References and Notes

- IPCC, *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (Cambridge, UK, 2007).
- J. H. Quastel, *Annu. Rev. Plant Physiol.* **16**, 217–240 (1965).
- Q. Q. Jiang, L. R. Bakken, *FEMS Microbiol. Ecol.* **30**, 171–186 (1999).
- J. Ludwig, F. X. Meixner, B. Vogel, J. Forstner, *Biogeochemistry* **52**, 225–257 (2001).
- M. Gödde, R. Conrad, *Soil Biol. Biochem.* **30**, 433–442 (1998).
- W. Naegle, R. Conrad, *FEMS Microbiol. Ecol.* **74**, 49 (1990).
- M. Kubota, T. Asami, *Soil Sci. Plant Nutr.* **31**, 35–42 (1985).
- H. Su *et al.*, *Science* **333**, 1616–1618 (2011).
- S. M. van Dijk *et al.*, *J. Geophys. Res. D Atmos.* **107**, 8058 (2002).

- Materials and methods are available as supplementary materials on Science Online.
- U. Rummel, C. Ammann, A. Gut, F. X. Meixner, M. O. Andreae, *J. Geophys. Res.* **107**, 8050 (2002).
- A. Remde, J. Ludwig, F. X. Meixner, R. Conrad, *J. Atmos. Chem.* **17**, 249–275 (1993).
- J. Kleffmann, J. Heland, R. Kurtenbach, J. Lörzer, P. Wiesen, *Environ. Sci. Pollut. Res. Int.* **9**, 48 (2002).
- C. Breuninger, R. Oswald, J. Kesselmeier, F. X. Meixner, *Atmos. Meas. Tech.* **5**, 955–989 (2012).
- K. Pilegaard, *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **368**, 20130126 (2013).
- G. T. Feig, B. Mantimin, F. X. Meixner, *Biogeosciences* **5**, 1723–1738 (2008).
- J. Rousk *et al.*, *ISME J.* **4**, 1340–1351 (2010).
- S. Park, W. Bae, J. Chung, S.-C. Baek, *Process Biochem.* **42**, 1671–1676 (2007).
- J. Kleffmann, *ChemPhysChem* **8**, 1137–1144 (2007).
- J. J. Yienger, H. Levy II, *J. Geophys. Res. D Atmos.* **100**, 11447 (1995).
- J. Steinkamp, M. G. Lawrence, *Atmos. Chem. Phys.* **11**, 6063–6082 (2011).
- M. Gödde, R. Conrad, *Biol. Fertil. Soils* **30**, 33–40 (1999).
- E. A. Davidson, I. A. Janssens, Y. Q. Luo, *Glob. Change Biol.* **12**, 154–164 (2006).
- L. Breuer, R. Kiese, K. Butterbach-Bahl, *Soil Sci. Soc. Am. J.* **66**, 834 (2002).
- O. Saad, R. Conrad, *Biol. Fertil. Soils* **15**, 21–27 (1993).
- H. P. Koops, A. Pommerening-Röser, *FEMS Microbiol. Ecol.* **37**, 1 (2001).
- M. E. Stromberger, S. Klose, H. Ajwa, T. Trout, S. Fennimore, *Soil Sci. Soc. Am. J.* **69**, 1987 (2005).
- D. Yan *et al.*, *Soil Sci. Plant Nutr.* **59**, 142–148 (2013).
- J. S. Levine *et al.*, *J. Geophys. Res. D Atmos.* **101**, 23689 (1996).
- J. Skopp, M. D. Jawson, J. W. Doran, *Soil Sci. Soc. Am. J.* **54**, 1619 (1990).
- M. K. Firestone, E. A. Davidson, in *Exchange of Trace Gases Between Terrestrial Ecosystems and the Atmosphere*, M. O. Andreae, D. S. Schimel, Eds. (Wiley, Chichester, UK, 1989), pp. 7–21.

**Acknowledgments:** This project was funded by the Max Planck Society. The work was supported by the Max Planck Graduate Center with the Johannes Gutenberg University Mainz (MPGC). We are grateful to S. Hohlmann, S. Müller, M. Welling, P. Stella, A. Held, B. L. Simmons, N. Mascher, C. Becker, A. C. Hertel, F. Hertel, and Z. Wu for supporting the experiments and helping to collect the soil samples and to S. Böhnke for assistance in creating the global map. We thank K. Emde and team as well as the company Envityx for analyzing chemical and physical soil properties. Soil sample S7 was collected in Mali at the Agoufou site belonging to the African Multidisciplinary Monsoon Analysis–Couplage de l’Atmosphère Tropicale et du Cycle Hydrologique (AMMA-CATCH) observatory ([www.amma-catch.org](http://www.amma-catch.org)). The soil sample S11 from Grignon was collected within the framework of the European Commission project NitroEurope-IP (017841). The work of H.S. was supported by the European Commission within the framework of PEGASOS (265148). Supporting soil data are available in the supplementary materials.