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Supplementary: Carbon Availability and Nitrogen Mineralization Control Denitrification Rates and Product Stoichiometry During Initial Maize Litter Decomposition

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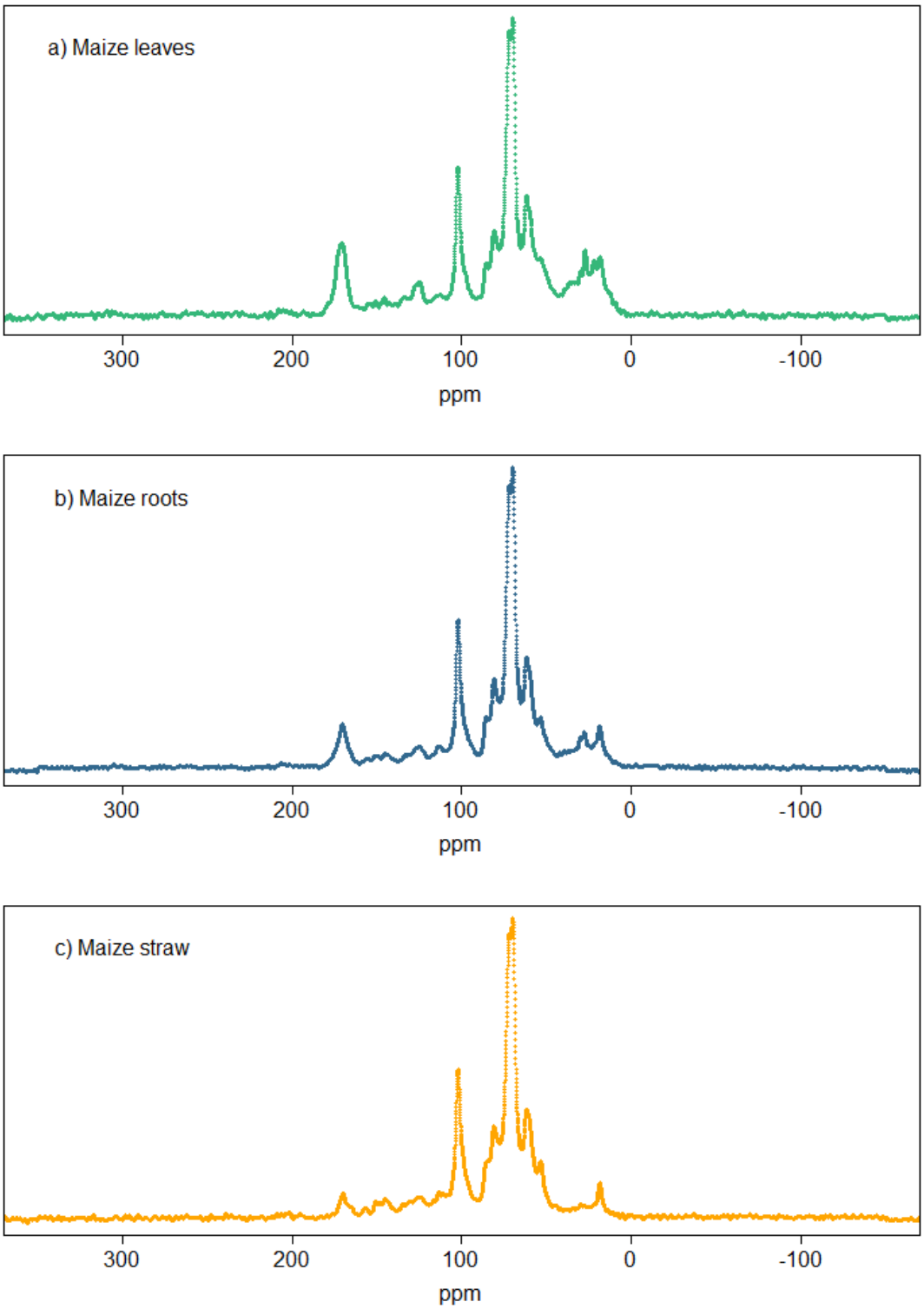

Figure S1. a–c: Solid state ^{13}C-CPMAS NMR spectra of maize litter used in the incubation experiment.

Determination of $\delta^{13}C$ of CO_2 respired from maize litter

To determine the $\delta^{13}C$ of CO_2 released from maize litter decomposition, dried maize litter was incubated without soil. Then, 20 g of washed quartz sand were filled into 100 ml glass bottles. Dried and ground maize litter was mixed with sand in amounts equaling 2 mg C g^{-1}. To provide incubation conditions similar to the main experiment, 70 g of pre-incubated soil was homogenized with 140 ml of H_2O_{bidest} and stirred for 45 min. The soil suspension was filtered through paper filters and centrifuged at 5000 rpm for 30 min. The supernatant was discarded, and the pellet was resuspended in KNO_3 solution. Two milliliters of the soil-microorganisms-KNO_3 solution were added to the sand-litter-mixture providing 50 µg N g^{-1}.

Gas samples were taken from the gas bottles 1, 5, 9, 14, 19, 26, and 33 days after mixing. Before sampling, bottles were flushed for 30 min with HeO_2 (80:20) at a flowrate of 50ml min^{-1} to remove any CO_2 from the bottles. Then, soil gases were accumulated in the bottles for 60 min and 25 ml of gas samples were filled in evacuated 12 ml Exetainer® septum-capped vials (Labco, High Wycombe, UK). Samples were introduced by a Combi-Pal autosampler (CTC-Analytics, Zwingen, Switzerland) to a GC (GC-Box, Thermo Fisher Scientific, Bremen, Germany) coupled to an isotope mass spectrometer (Delta plus XP, Thermo Fisher Scientific, Bremen, Germany) via a Conflo III Interface (Thermo Fisher Scientific, Bremen, Germany).

$\delta^{13}C$ values of CO_2 derived from litter are shown in Figure S2. On the first sampling day, values were similar to the Control without litter (−13.75δ‰). Thus, the mean of 5 to 33 DAO was taken as the average $\delta^{13}C$ of CO_2 derived from maize litter (Leaf = −7.910δ‰, Root = −7.497δ‰, Straw = −9.327δ‰).

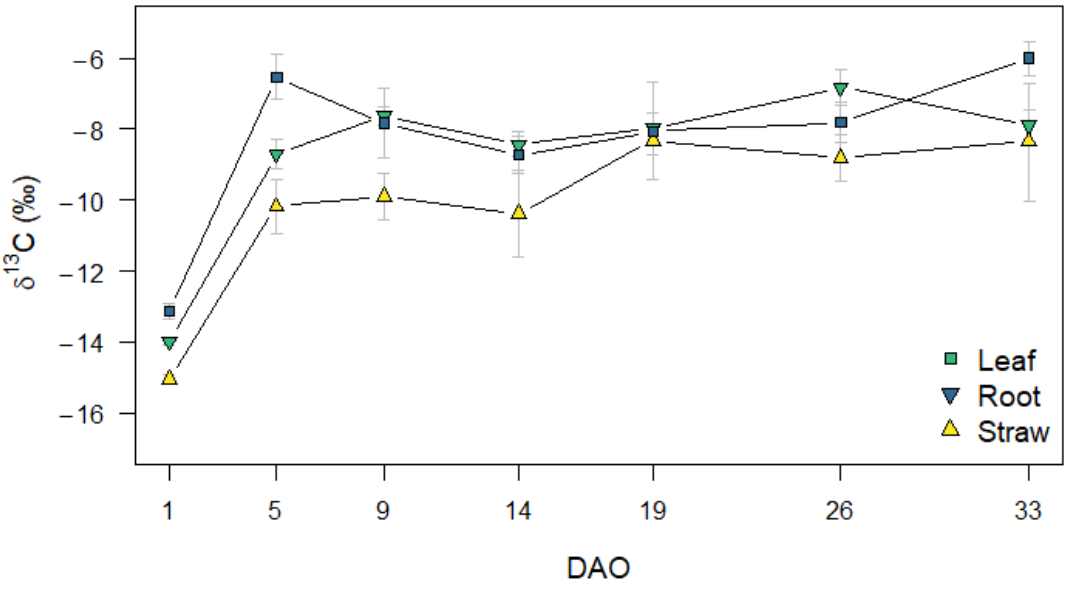

Figure S2. $\delta^{13}C$ of CO_2 derived from maize litter.

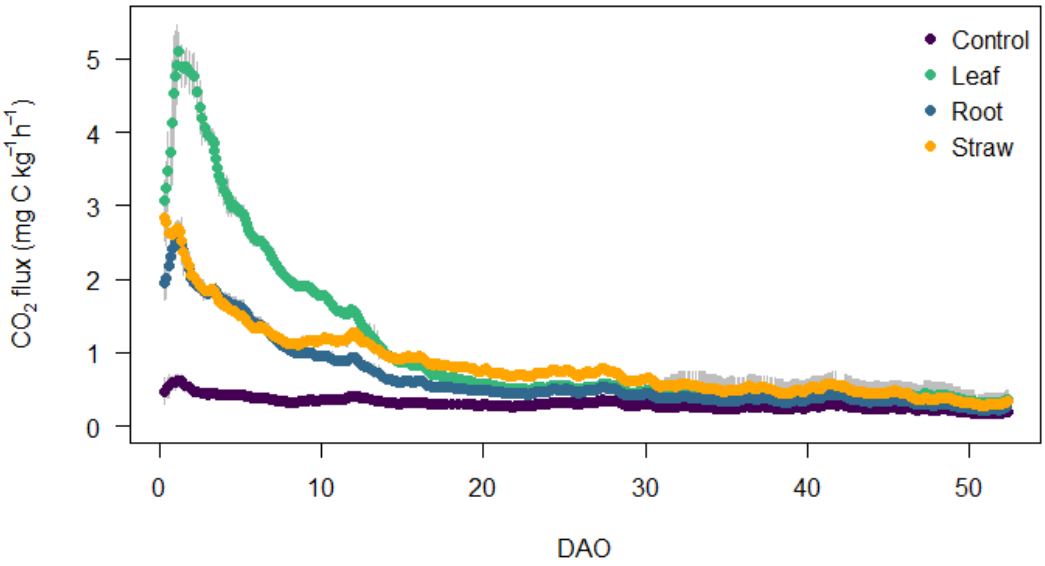

Figure S3. Total CO_2 efflux from soil during oxic incubation from Day 0 to Day 46 and during anoxic incubation from Day 47 to Day 55 (means and standard deviation for n = 5, n = 4 for Control, when not visible, error bars are smaller than the symbols).

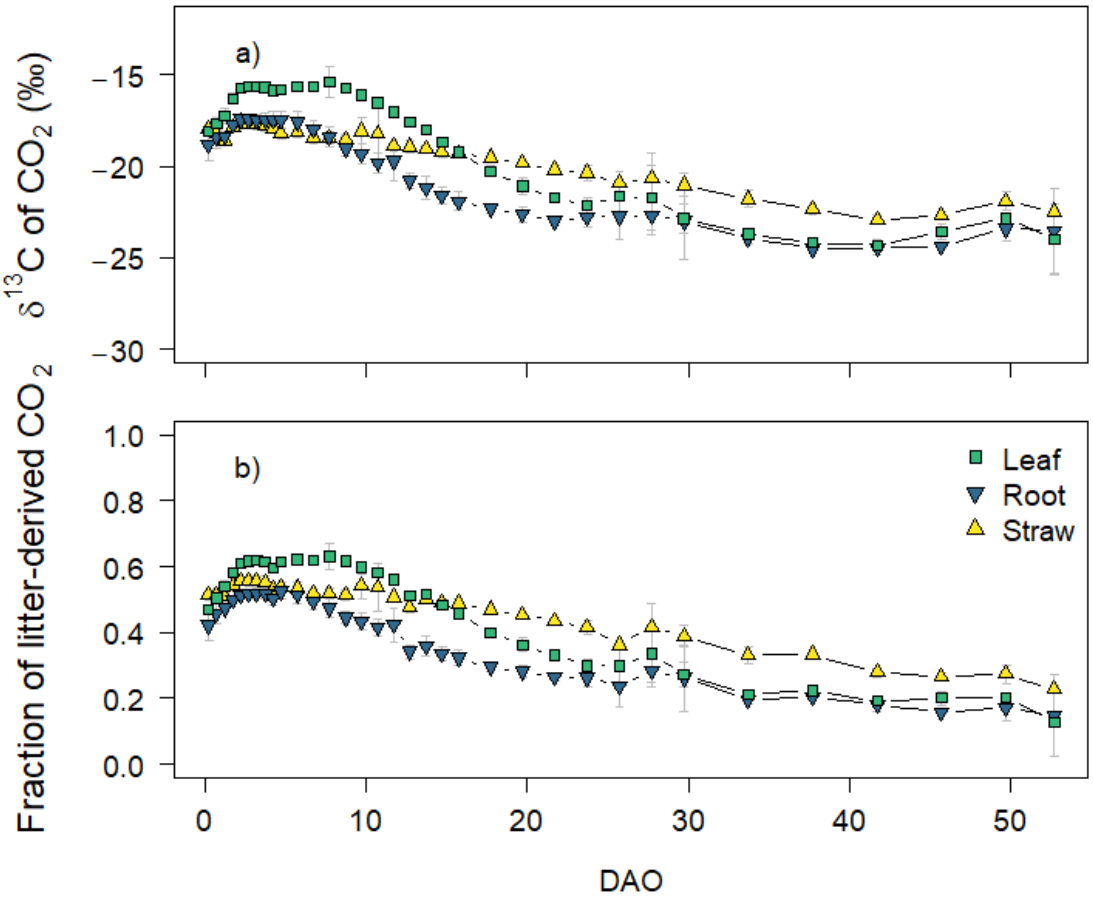

Figure S4. a: $\delta^{13}C$ of CO_2 evolving from soil and b: fraction of litter-derived CO_2 (means and standard deviation for n = 5, n = 4 for Control, when not visible, error bars are smaller than the symbols).

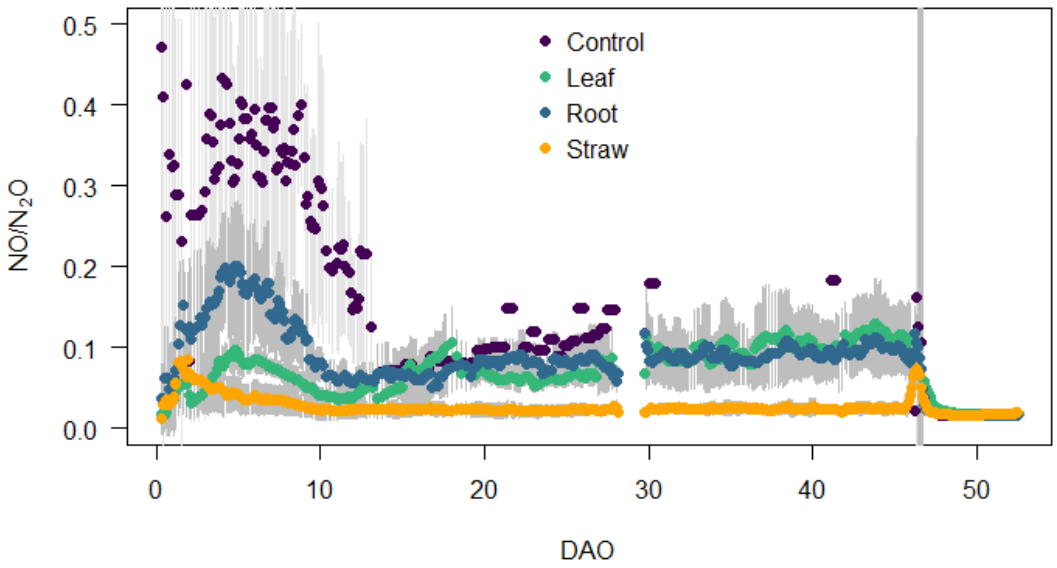

Figure S5. NO/N_2O ratio during oxic incubation from Day 0 to Day 46 and during anoxic incubation from Day 47 to Day 55 (means and standard deviation for n = 5, n =4 for Control, when not visible, error bars are smaller than the symbols).

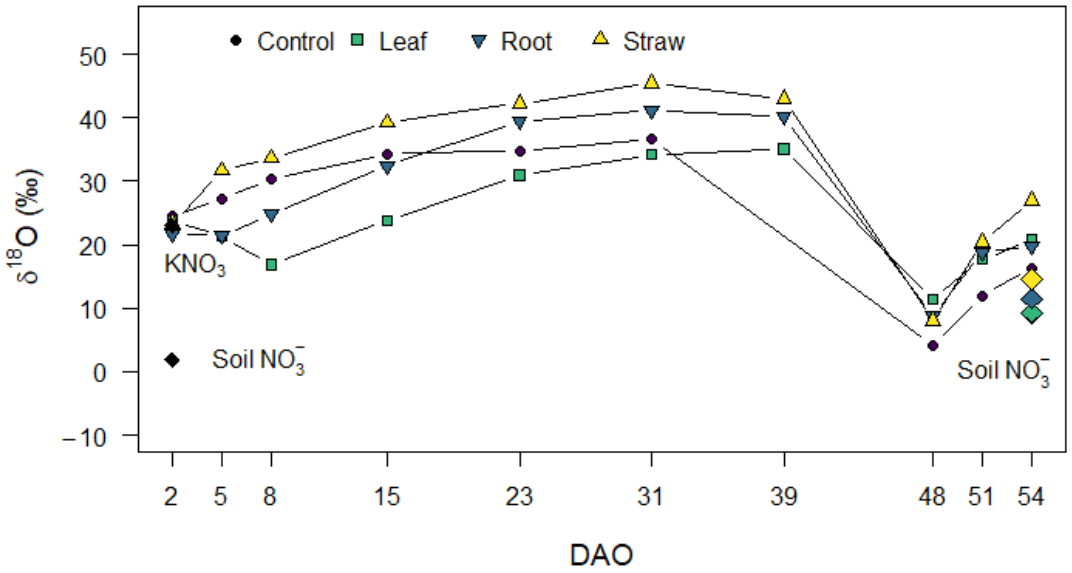

Figure S6. $\delta^{18}O$ of N_2O (colored symbols and lines), added KNO_3, and soil NO_3^- at first and last day of incubation. $\delta^{18}O$ of N_2O was corrected for $\delta^{18}O$ of soil water (−6.7 δ‰) (means for n = 5, n = 4 for Control).

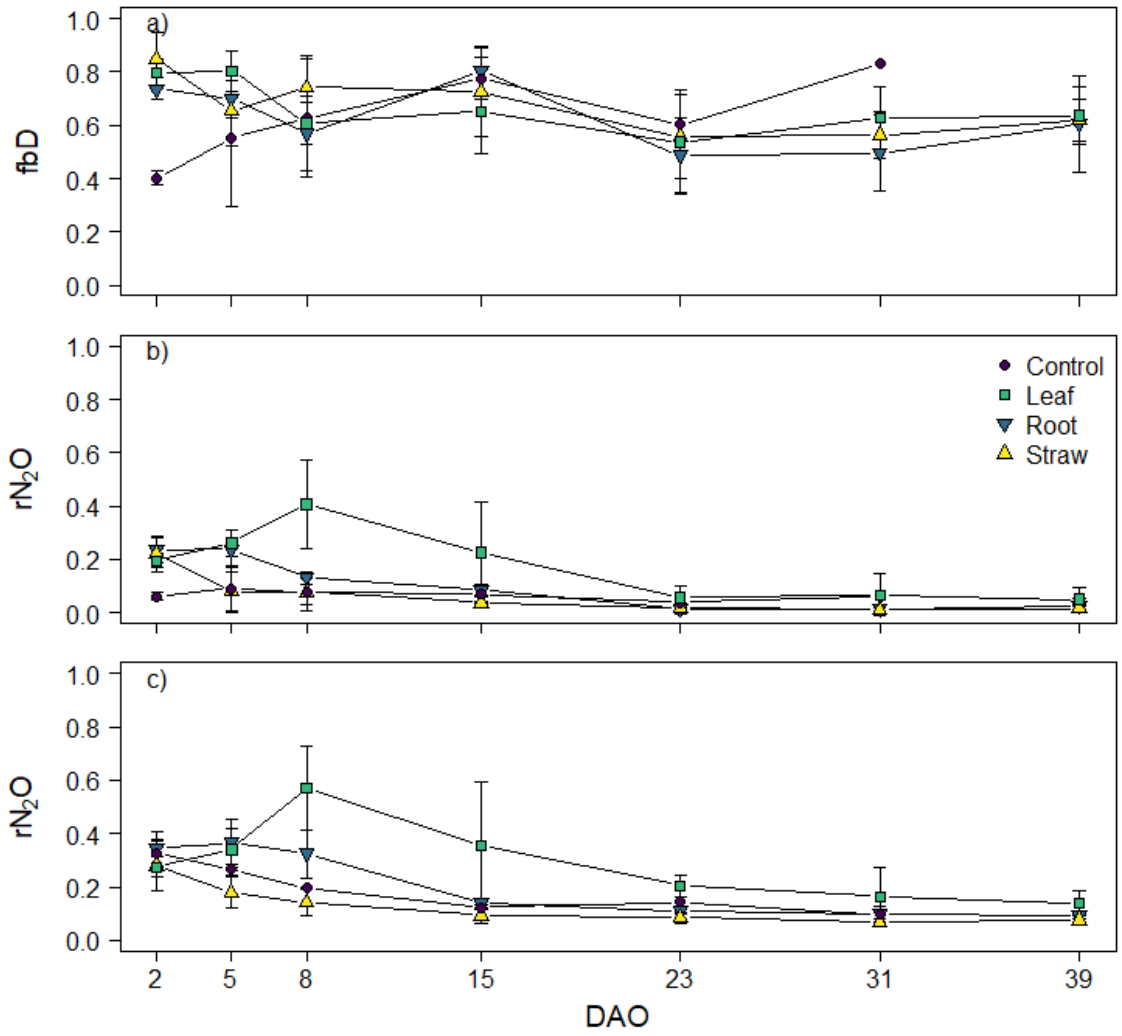

Figure S7. a: Fraction of N_2O originating from heterotrophic bacterial denitrification/nitrifier denitrification (fbD) and b+c: fraction of residual unreduced N_2O (rN_2O). Values were calculated based on the isotopocule mapping approach by Lewicka-Szczebak et al. (2017) [1] and represent results for scenario 1 (b, reduction-mixing) and scenario 2 (c, mixing-reduction) of bacterial denitrification with nitrification (mean and standard deviation for n = 5, data points missing for samples with an isotopic signature outside the reduction-mixing area, no N_2O emitted from Control on 39 DAO).

Table S1. $\delta^{15}N^{SP}_{N2O}$, $\delta^{18}O_{N2O/H2O}$, and $\delta^{15}N^{bulk}_{N2O}$ endmember values from literature used for isotopocule mapping.

Process	$\delta^{15}N^{SP}_{N2O}$				$\delta^{18}O_{N2O/H2O}$				$\delta^{15}N^{bulk}_{N2O}$			References
	Min	**Max**	Mean		Min	Max	Mean		Min	Max	Mean	
Heterotrophic bacterial denitrification	−7.5	3.7	−1.9	−3.9	16.7	23.3	19.2	17.55	−52.8	2.3	−25.9	[2–5]
Nitrifier denitrification	−13.6	1.9	−5.9		12.4	19.4	15.9		−60.7	−53.1	−56.9	[3,6]
Fungal denitrification	27.2	39.9	33.5		42.0	55.1	47.2		−46.0	−31.0	−38.0	[7–10]
Nitrification	32.0	38.7	35.0		20.5	26.5	23.5		−64.0	−47.0	−57.0	[3,6]

Table S2: Cumulative CO_2, NO, N_2O, and N_2 emissions and denitrification product ratio standardized against litter C input.

	Total CO_2 (mg C g^{-1} C input)		**Total N_2O (mg N g^{-1} C input)**		**Total N_2 (mg N g^{-1} C input)**		**Total NO (mg N g^{-1} C input)**		**$N_2O/(N_2O+N_2)$**	
Maize Leaves	240.2 ± 22.5	n.s.	5.73 ± 1.72	n.s.	1.30 ± 0.84	n.s.	0.13 ± 0.02	n.s.	0.83 ± 0.04	a
Maize Roots	227.9 ± 20.7	n.s.	6.62 ± 0.46	n.s.	1.15 ± 0.48	n.s.	0.13 ± 0.02	n.s.	0.85 ± 0.05	a
Maize Straw	220.1 ± 7.8	n.s.	5.74 ± 0.20	n.s.	1.90 ± 0.48	n.s.	0.11 ± 0.01	n.s.	0.75 ± 0.05	b

Values represent means (n = 5) ± standard deviation. Different letters in the same column indicate a significant difference according to the LSD post hoc tests at $p \leq 0.05$. n.s. indicates no significant difference.

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