Supporting Information

Shifts in the composition and activities of denitrifiers dominate CO_2-stimulation of N_2O emissions

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Number of pages: 11

Number of figures: 8

Number of tables: 1

Figure S1. The experiment was conducted in the USDA-ARS Plant Science Research CO_2 facility at North Carolina State University. The facility consisted of 8 continuously stirred tank reactor (CSTR) chambers designed for the exposure of plants to CO_2 and other gases. Each CSTR is a cylindrical chamber covered with Teflon and measured 1.2 m in diameter by 1.4 m tall. Compressed CO_2 was mixed with air and dispensed to the CSTR chambers using a rotometer to control flow so that CO_2 concentration was maintained at a target level. The air continuously moved out the CSTR and thus alleviated the heating effect of chambers. To monitor CO_2 concentrations, an infrared analyzer (model 6252, LiCor Inc., Lincoln, NE, USA) was used to measure CSTR chamber air CO_2 concentrations every two minutes. Two experimental microcosms (Figure S2, see below) were placed into each CSTR chamber.

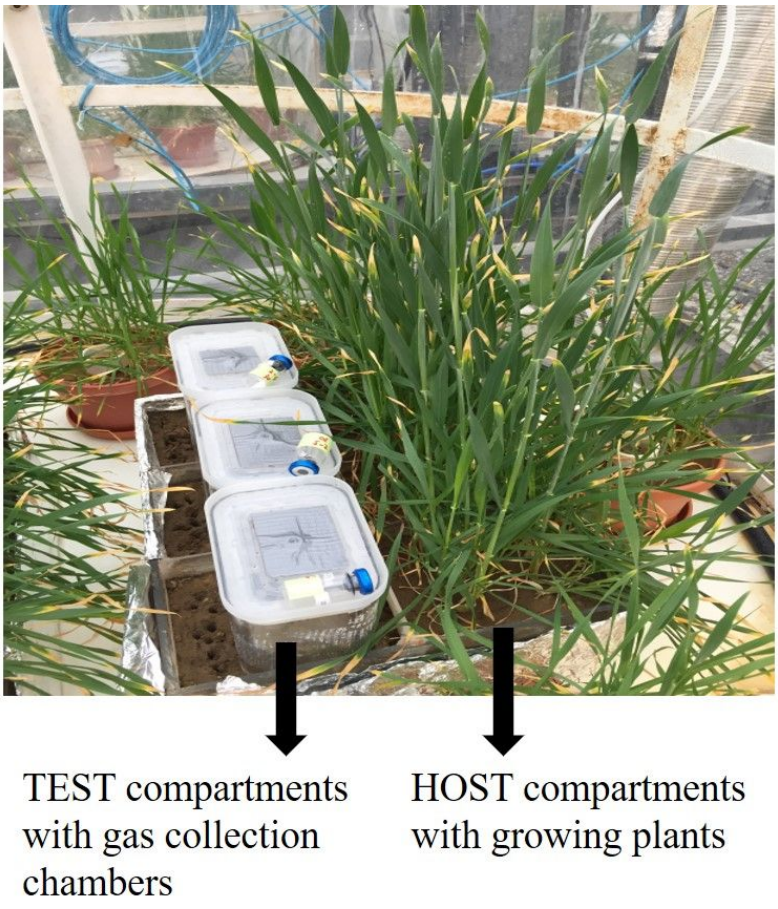

Figure S2. A microcosm unit composed of six equal-size compartments for N_2O sampling in the presence of growing plants. Three compartments in one row were assigned as the HOST compartments where wheat was planted, and the other three were the TEST compartments where gas samples were collected. When a mesh of 0.45 μm was placed between the HOST and TEST compartments, neither arbuscular mycorrhizal hyphae nor plant roots grow into the TEST compartments. When a 20 μm mesh screen was placed between the two compartments, only AM hyphae grow into the TEST compartments. When a 1.6 mm mesh was placed between the two compartments, both AM hyphae and plant roots grow into the TEST compartments. Two experimental microcosm units were placed into each CSTR chamber during the experiment.

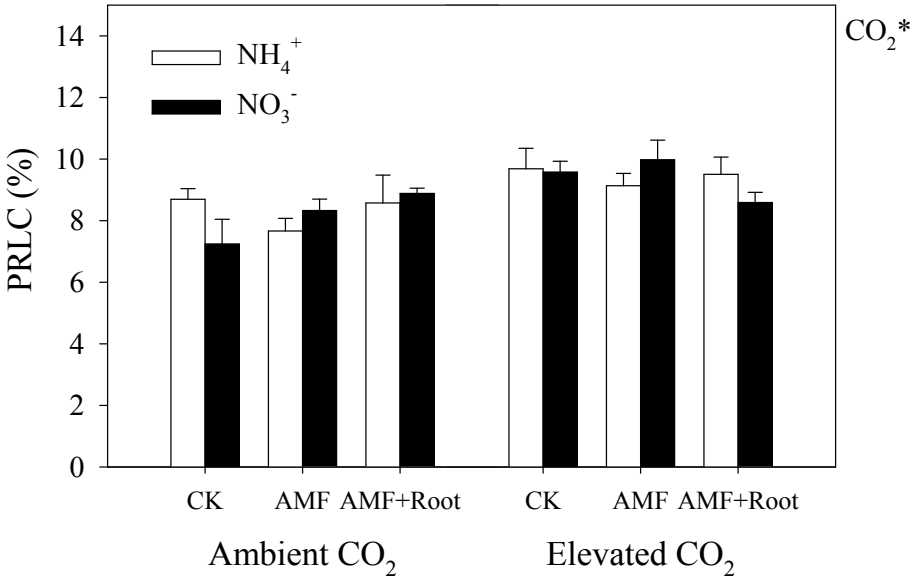

Figure S3. Effects of CO_2 enrichment, N forms and AMF or plant roots on wheat root colonization. Values are means ± 1 SE (n=4). The significance levels are labeled with: $^{*}0.01 < P \leq 0.05$.

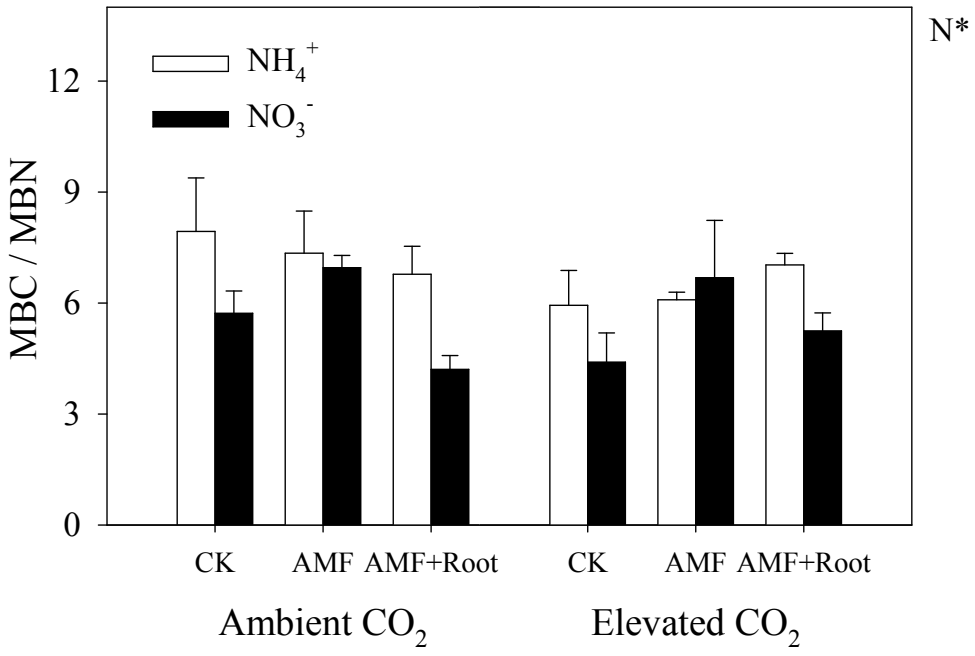

Figure S4. Effects of CO_2 enrichment, N forms and AMF or plant roots on TEST soil microbial biomass C to N ratio (MBC/MBN). Values are means ± 1 SE (n=4). The significance levels are labeled with: $^{*}0.01 < P \leq 0.05$.

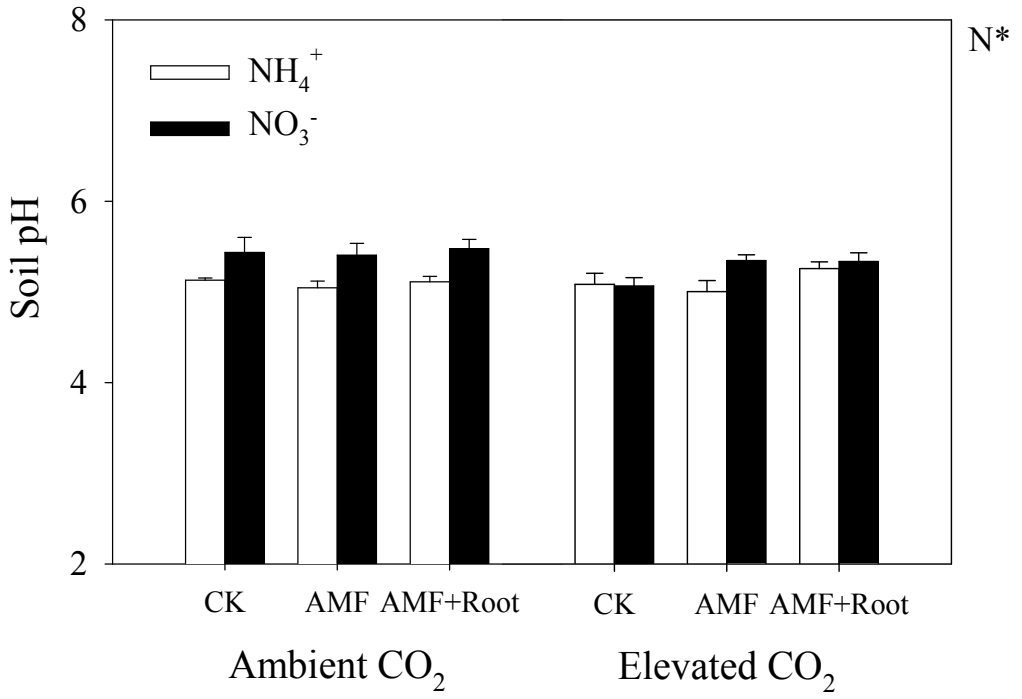

Figure S5. Effects of CO_2 enrichment, N forms and AMF or plant roots on TEST soil pH. Values are means ± 1 SE (n=4). The significance levels are labeled with: $^{*}0.01 < P \leq 0.05$.

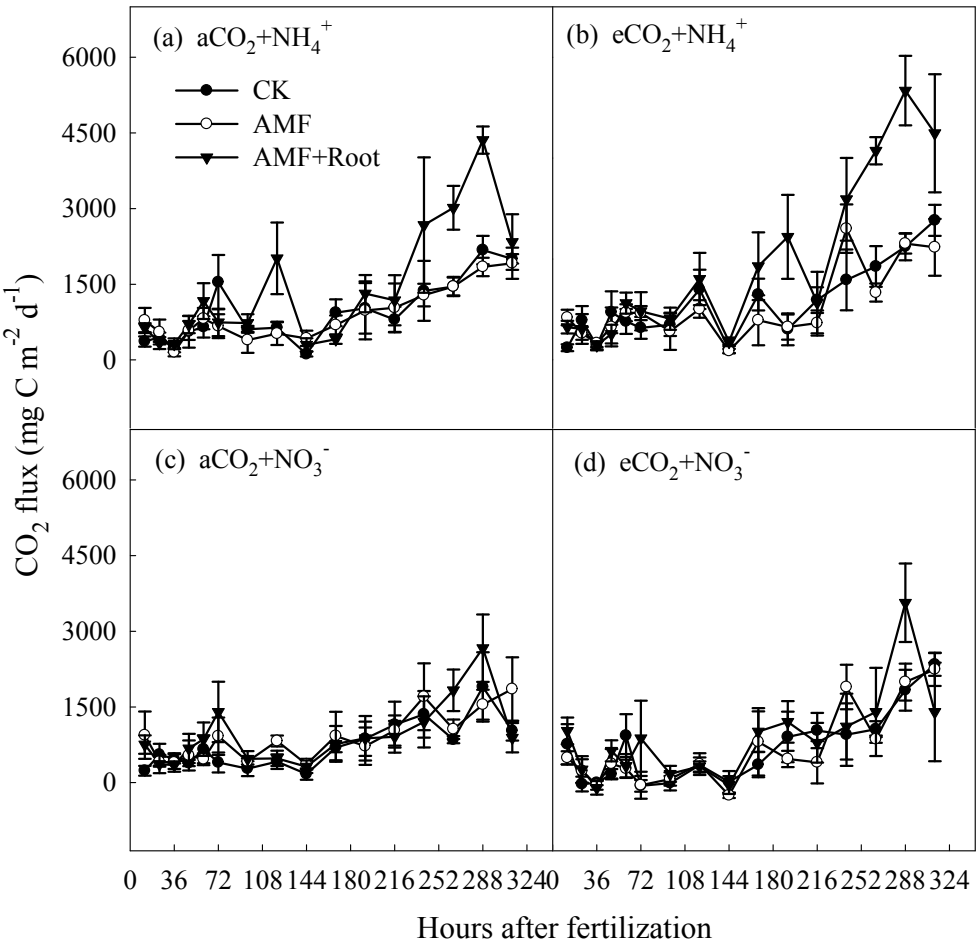

Figure S6. Effects of CO_2 enrichment, N forms and AMF or plant roots on soil CO_2 fluxes after a water and fertilization pulse corresponding to 40 kg N ha^{-1} at 12th week of plant growth. (a) $aCO_2+NH_4^+$: ambient CO_2 and NH_4^+ fertilization; (b) $eCO_2+NH_4^+$: elevated CO_2 and NH_4^+ fertilization; (c) $aCO_2+NO_3^-$: ambient CO_2 and NO_3^- fertilization; (d) $eCO_2+NO_3^-$: elevated CO_2 and NO_3^- fertilization. Values are means ± 1 SE (n=4) at any given time point.

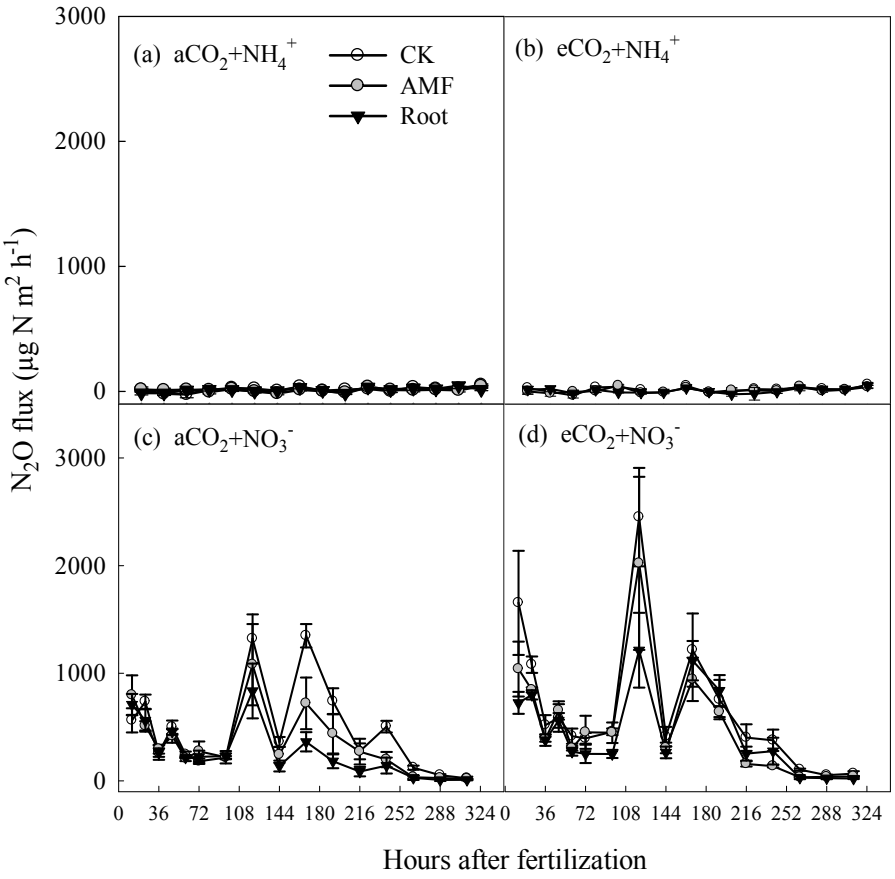

Figure S7. Effects of CO_2 enrichment, N forms and AMF or plant roots on soil N_2O fluxes after a water and fertilization pulse corresponding to 40 kg N ha^{-1} at 12th week of plant growth. (a) aCO_2+NH_4^+: ambient CO_2 and NH_4^+ fertilization; (b) eCO_2+NH_4^+: elevated CO_2 and NH_4^+ fertilization; (c) aCO_2+NO_3^-: ambient CO_2 and NO_3^- fertilization; (d) eCO_2+NO_3^-: elevated CO_2 and NO_3^- fertilization. Values are means ± 1 SE (n=4) at any given time point.

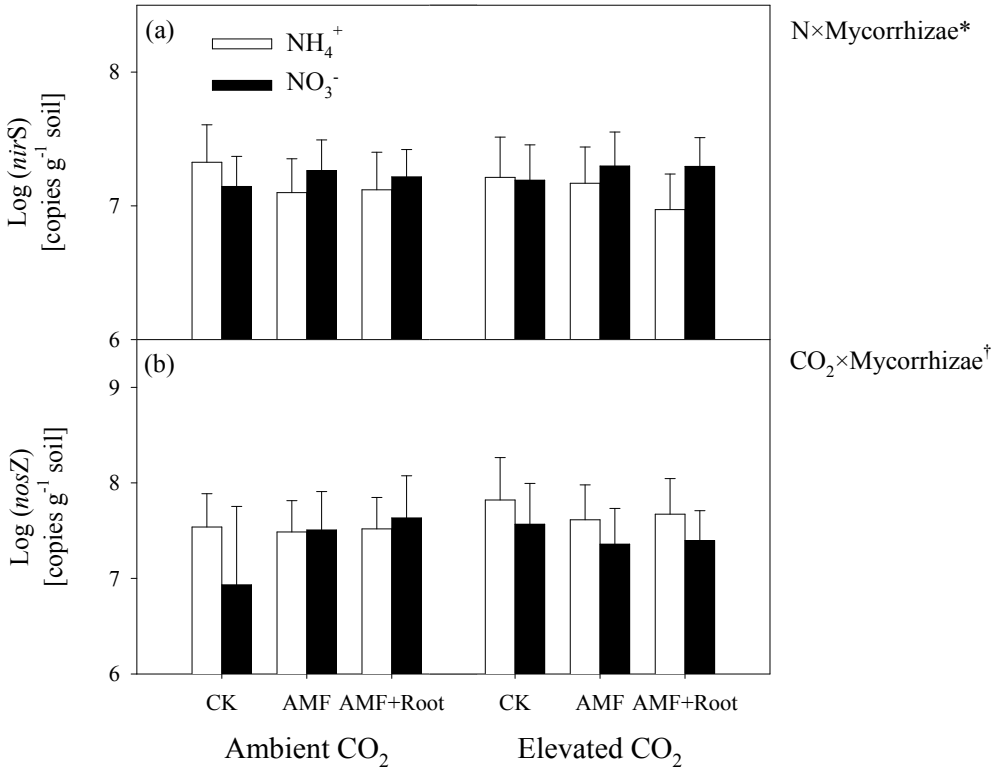

Figure S8. Effects of CO_2 enrichment, N forms and AMF or plant roots on abundances of denitrification genes (*nir*S and *nos*Z). (a) gene copy numbers of *nir*S (log-transformed) (b) gene copy numbers of *nos*Z (log-transformed). Values are means ± 1 SE (n=4). The significance levels are labeled with: $^{\dagger}0.05 \leq P < 0.10$; $^{*}0.01 < P \leq 0.05$.

Table S1 Primers and qPCR conditions for the real-time PCR quantifications of *nir*S, *nir*K, and *nosZ* genes extracted from soils

Target genes	Primer	Sequence	qPCR conditions	References
*nir*S	nirSCd3aF	AACGYSAAGGARACSGG	Six TD CL: 98 °C for 10 s, 63 °C for 30 s, and 72 °C for 30 s with AT dropped by 1 °C to 58 °C; 40 CL: 98 °C for 10 s, 58 °C for 30 s, and 72 °C for 30 s	1
	nirSR3cd	GASTTCGGRTGSGTCTTSAYGAA		
*nir*K	nirK876	ATYGGCGGVAYGGCGA	Same as *nir*S	2
	nirK1040	GCCTCGATCAGRTTRTGGTT		
nosZ	nosZ1F	WCSYTGTTCMTCGAGCCAG	Six TD CL: 98 °C for 10 s, 67 °C for 30 s, and 72 °C for 30 s with AT dropped by 1 °C to 62 °C; 40 CL: 98 °C for 10 s, 62 °C for 30 s, and 72 °C for 30 s	3
	nosZ1R	ATGTCGATCARCTGVKCRTTYTC		

CL, TD, AT are short for cycles, touchdown, and annealing temperature, respectively

REFERENCES

(1) Kandeler, E.; Deiglmayr, K.; Tscherko, D.; Bru, D.; Philippot, L. Abundance of *nar*G, *nir*S, *nir*K, and *nos*Z genes of denitrifying bacteria during primary successions of a glacier foreland. *Appl. Environ. Microbiol.* **2006,** *72* (9), 5957–5962.

(2) Henry, S.; Baudoin, E.; López-Gutiérrez, J. C.; Martin-Laurent, F.; Brauman, A.; Philippot, L. Quantification of denitrifying bacteria in soils by *nir*K gene targeted real-time PCR. *J. Microbiol. Methods,* **2004,** *59* (3), 327–335.

(3) Henry, S.; Bru, D.; Stres, B.; Hallet, S.; Philippot, L. Quantitative detection of the *nos*Z gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, *nar*G, *nir*K, and *nos*Z genes in soils. *Appl. Environ. Microbiol.* **2006,** *72* (8), 5181-5189.