

Supplementary Information

Effects of different fertilization practices on anammox activity, abundance and community compositions in a paddy soil

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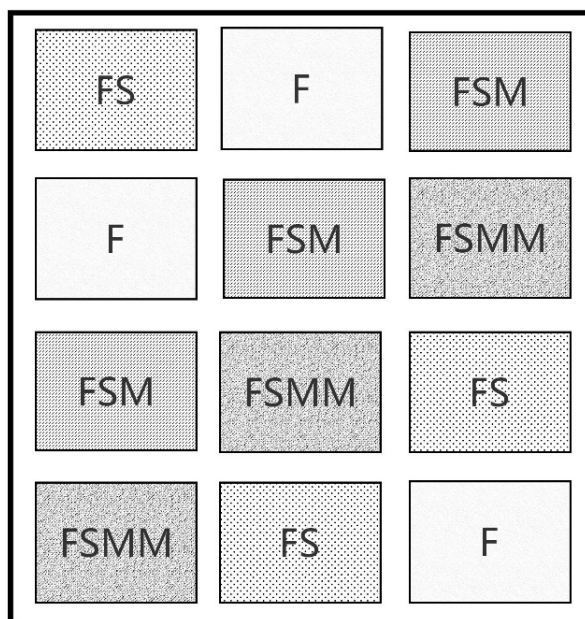


Figure S1 Plot design with different fertilizing practices (F, FS, FSM, FSMM) in this study.

The calculation equations and explanations used for the activity and contribution of anammox and denitrification with ^{15}N -tracer technique.

Calculation: The fractions of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ in soil treated with $\text{Na}^{15}\text{NO}_3$ were calculated using equations (1) and (2). The mass of headspace N_2 (M_{total}) was calculated using the equation (3). The production rates of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ in the vials could be calculated based on the molecular fractions of F^{29} and F^{30} using equations (4) and (5).

Explanations of the equations: F^{29} and F^{30} denote the fraction of $^{29}\text{N}_2$ and $^{30}\text{N}_2$, R^{29} and R^{30} represent the ratio of $^{29}\text{N}_2$ to $^{28}\text{N}_2$ and $^{30}\text{N}_2$ to $^{28}\text{N}_2$, M_{total} is the mass of headspace N_2 , ρ_{N_2} is the density of N_2 , $V_{headspace}$ is the volume of headspace, t is the incubation time and M_{soil} represents the dry soil weight in vials, P_{29} and P_{30} are the measured production of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ from each vial in the $^{15}\text{NO}_3^-$ treatment,

respectively. Denitrification and anammox rates were calculated by equation (6) and (7), respectively. The contribution of anammox to total N₂ production was assessed by equation (8). In slurries treated with negative control, no significant accumulation of ²⁹N₂ or ³⁰N₂ could be detected, suggesting that initial NO₃⁻ and NO₂⁻ had been depleted during the pre-incubations. In positive control treatment, ²⁹N₂ could be detected while ³⁰N₂ was undetectable. The slurries treated with Na¹⁵NO₃ indicated that anammox and denitrification process were detectable in the examined soils. Based on different time point, a liner regression of different N₂ concentration was performed to estimate the potential rates of anammox and denitrification.

Table S1 Isotope pairing technique equations used for the calculation of anammox and denitrification activity, and their contribution to N₂ production.

Equations	Equation No.
$F^{29} = \frac{{}^{29}\text{N}_2}{\text{N}_2^{\text{total}}} = \frac{\frac{{}^{29}\text{N}_2}{{}^{28}\text{N}_2}}{1 + \frac{{}^{29}\text{N}_2}{{}^{28}\text{N}_2} + \frac{{}^{30}\text{N}_2}{{}^{28}\text{N}_2}} = \frac{R_{29}}{1 + R_{29} + R_{30}}$	(1)
$F^{30} = \frac{{}^{30}\text{N}_2}{\text{N}_2^{\text{total}}} = \frac{\frac{{}^{30}\text{N}_2}{{}^{28}\text{N}_2}}{1 + \frac{{}^{29}\text{N}_2}{{}^{28}\text{N}_2} + \frac{{}^{30}\text{N}_2}{{}^{28}\text{N}_2}} = \frac{R_{30}}{1 + R_{29} + R_{30}}$	(2)
$M_{\text{total}} = \rho_{\text{N}_2} \times V_{\text{headspace}}$	(3)
$P_{29} = (M_{\text{total}} \times F^{29}) / (t \times M_{\text{soil}})$	(4)
$P_{30} = (M_{\text{total}} \times F^{30}) / (t \times M_{\text{soil}})$	(5)
$D_{\text{total}} = P_{30} \times F_N^{-2}$	(6)
$A_{\text{total}} = F_N^{-1} \times [P_{29} + 2 \times (1 - F_N^{-1}) \times P_{30}]$	(7)
$R_a \% = \frac{D_{\text{total}}}{D_{\text{total}} + A_{\text{total}}} \times 100\%$	(8)

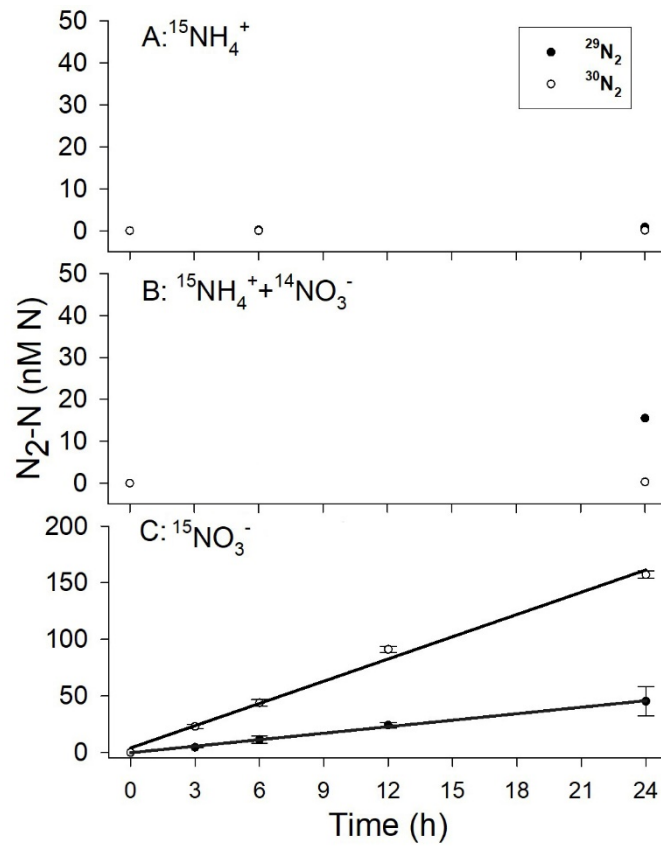


Figure S2 Examples of concentrations of $^{29}N_2$ and $^{30}N_2$ in samples amended with ^{15}N labeled on ammonium and nitrate separately. (A) negative control; (B) positive control; (C) $Na^{15}NO_3$.

Table S2 Pearson correlations between anammox activity, *hzsB* gene copies, relative abundance of community compositions and soil physiochemical properties in this study.

	Anammox activity	<i>hzsB</i> gene copies	Relative abundance of community compositions		
			<i>Brocadia</i>	<i>Scalindua</i>	<i>Kuenenia</i>
pH	-0.763	0.775	-0.792	0.869	-0.486
Bulk density	-0.090	0.079	-0.050	-0.625	-0.887
TOC	-0.210	0.222	-0.184	0.755	-0.969*
TN	-0.371	0.383	-0.359	0.836	-0.903
AOC	-0.378	0.386	-0.166	0.672	-0.936
AN	-0.358	0.363	-0.032	0.545	-0.930
Ammonium	0.053	-0.044	0.120	0.555	-0.797
Combined nitrate-nitrite	0.121	-0.113	0.169	0.521	-0.789
CEC	-0.451	0.457	-0.146	0.609	-0.900
Salinity	-0.564	0.572	-0.309	0.695	-0.840
Fe ²⁺	-0.519	0.527	-0.283	0.701	-0.868

* Correlation is significant at 0.05 level (two tailed).

TOC, total organic carbon; TN, total nitrogen; AOC, active organic carbon; AN, Alkali-hydrolysable N; CEC, cation exchange capacity.