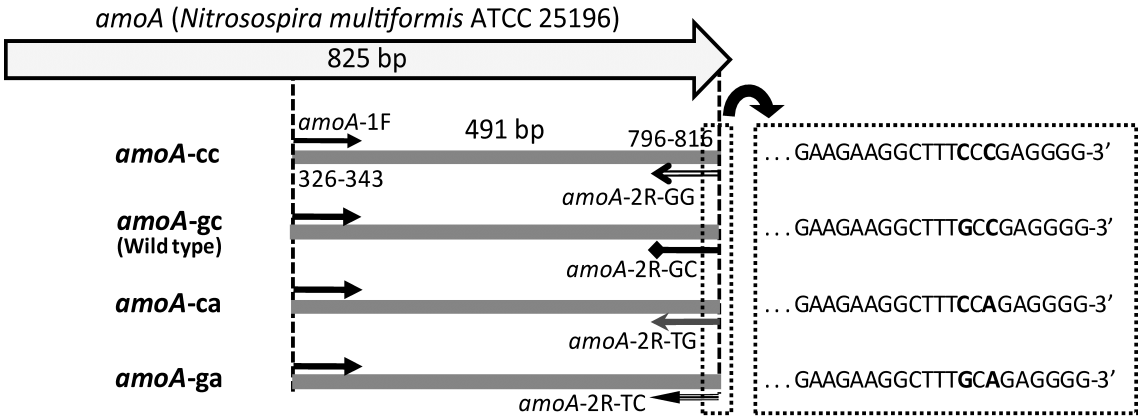
Legends of Figures

Fig. S1: Amplification positions of *amoA* from *Nitrosospira multiformis* ATCC 25196 (accession number CP000103) for construction of *amoA* clones.

Fig. S2: DGGE band patterns of *amoA* fragments retrieved from gray lowland soil and andosol using the three primers at four different annealing temperatures.



Shimomura et al. Figure S1

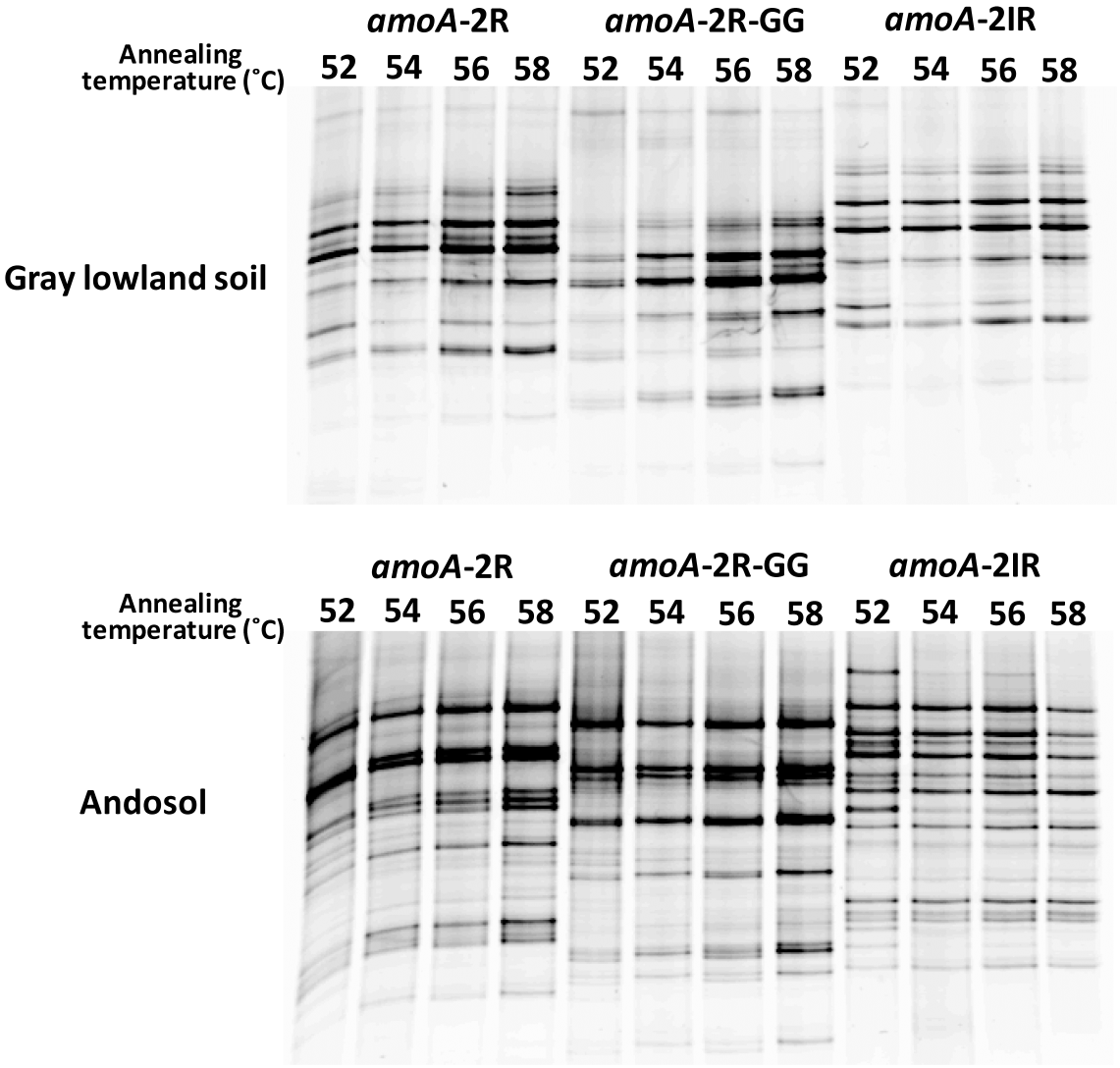


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Table S1. PCR primers for the amplification of *amoA* gene fragments having complementary sequences with *amoA*-2R.

Primer		Sequence (5'-3')	Tm (ºC)	Referrence
Forward	*amoA*-1F	GGGGTTTCTACTGGTGGT	46.5	(26)
Reverse	*amoA*-2R-GG	CCCCTC**GGG**AAAGCCTTCTTC	54.4	(20)
	amoA-2R-GC	CCCCTC**GGC**AAAGCCTTCTTC	54.4	(20)
	amoA-2R-TG	CCCCTC**T**G**G**AAAGCCTTCTTC	52.5	(20)
	amoA-2R-TC	CCCCTC**T**G**C**AAAGCCTTCTTC	52.5	(20)

Table S2. PCR conditions used in this study for the amplification of *amoA* gene fragments.

Experiment	Template	Composition of reaction mixture	Thermocycling conditions
Construction of standard clones	*Nitrosospira multiformis* ATCC 25196 *amoA*	25 μL of Premix Ex Taq™ Hot Start Version (Takara, Kyoto, Japan), 2 μL of 10 μM each primer (shown in Table S1), 1 μL of template (including 10^5 *amoA* copies), and 20 μL of SDW.	2 min at 94ºC, followed by 30 cycles at 94ºC for 30 s, at 54ºC for 30 s, and at 72ºC for 30 s.
Real-time PCR	DNA of standard clones or extracted soil DNA*	10 μL of SYBR® Premix Ex Taq™ (Takara, Kyoto, Japan), 0.8 μl of 10 μM each primer (shown in Table 1), 0.2 μL of 20 mg/ml BSA, 0.4 μL of ROX Dye, 1.0 μL of template, and 6.8 μL of SDW.	2 min at 94ºC, followed by 40 cycles at 94ºC for 30 s, at 54ºC for 30 s, and at 72ºC for 30 s.
PCR-DGGE	Extracted soil DNA	25 μL of Premix Ex Taq™ Hot Start Version, 2 μL of 10 μM each primer (shown in Table 1), 1 μL of 20 mg/mL BSA, 1 μL of template (around 50 ng of soil DNA), and 19 μL of SDW.	2 min at 94ºC, followed by 30 cycles at 94ºC for 60 s, at 52 to 58ºC by 2 ºC for 60 s, and at 72ºC for 60 s.

Ten-fold dilution was used to measure *amoA* copy numbers in soil samples.

Table S3. Real-time PCR standard curves and calculated amplification efficiency of the three primer sets.

Forward primer	Reverse primer	Template	Standard curve	Coefficient of determination	Amplification efficiency (%)
amoA-1F	*amoA*-2R	*amoA*-cc	y=−3.391+35.277	R^2=0.9999	97.2
		amoA-gc	y=−3.551+35.49	R^2=0.9999	91.3
		amoA-ca	y=−3.471+34.777	R^2=1.0000	94.1
		amoA-ga	y=−3.382+31.804	R^2=1.0000	97.6
	amoA-2R-GG	*amoA*-cc	y=−3.4421x+36.115	R^2=0.9999	95.2
		amoA-gc	y=−3.4031x+36.039	R^2=0.9996	96.7
		amoA-ca	y=−3.5012x+36.433	R^2=0.9999	93.0
		amoA-ga	y=−3.4455x+36.217	R^2=0.9999	95.1
	amoA-2IR	*amoA*-cc	y=−3.5105x+35.796	R^2=0.9995	92.7
		amoA-gc	y=−3.4571x+35.663	R^2=0.9991	94.7
		amoA-ca	y=−3.5559x+36.379	R^2=0.9999	91.1
		amoA-ga	y=−3.4916x+36.097	R^2=0.9998	93.4