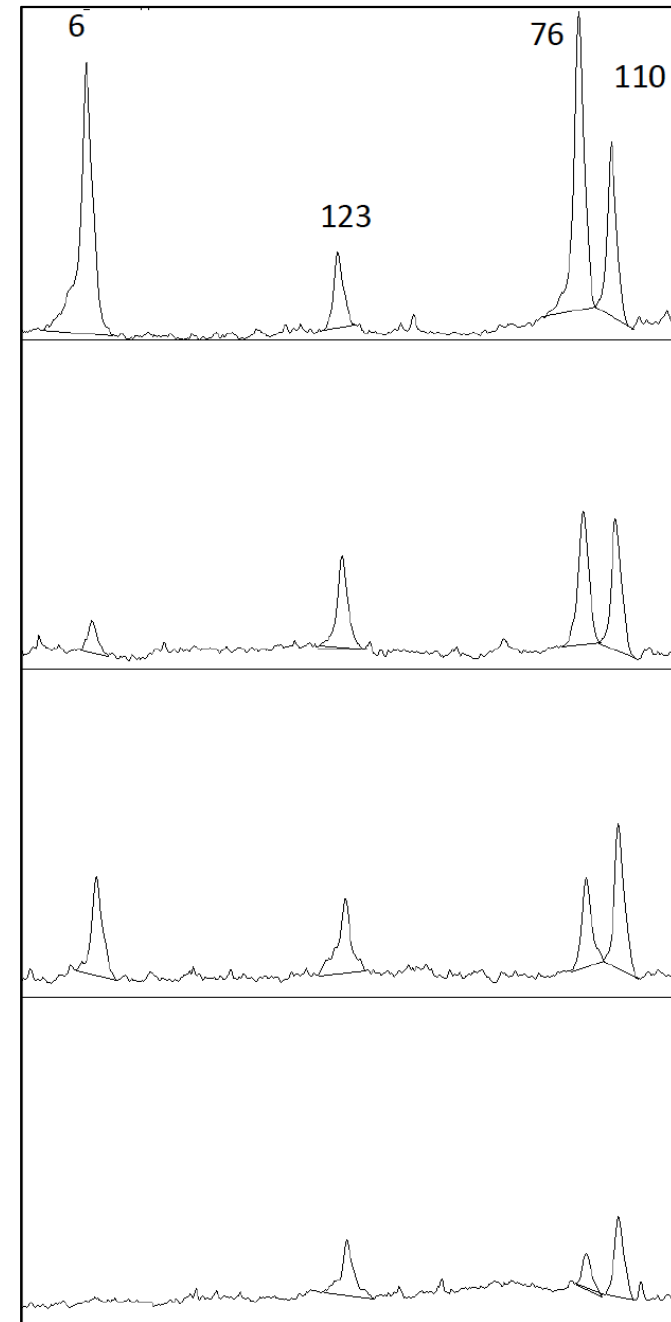
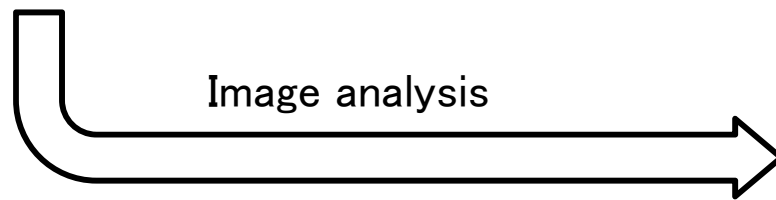
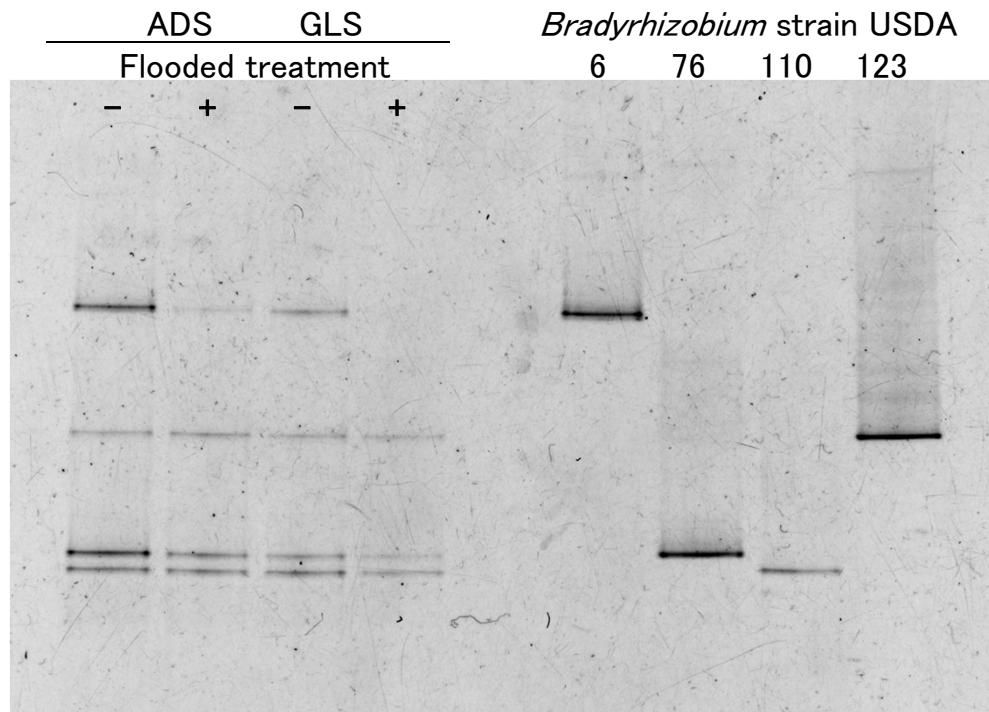


# Denaturing gradient gel electrophoresis

Denaturing gradient: 41–44%  
Electrophoresis: 60°C, 100V, 14h



Measurement of peak area

Fig. S1. Schematic representation of a DGGE analysis and the image analysis procedure for the estimation of community structures.

Table S1 pH, EC values, and population densities of bradyrhizobia in soil microcosms.

	pH(H <sub>2</sub> O) 1:5 extract*			EC (dS m <sup>-1</sup> ) 1:5 extract*			Population density (CFU g <sup>-1</sup> dry soil)		
	0 day	30 day	60 day	0 day	30 day	60 day	0 day	30 day	60 day
ANW_H	5.93 ± 0.02 a	6.09 ± 0.05 ab	6.05 ± 0.00 b	0.38 ± 0.00 a	0.28 ± 0.02 b	0.33 ± 0.01 b	(1.26 ± 0.45) × 10 <sup>6</sup>	(3.17 ± 0.28) × 10 <sup>7</sup>	(3.64 ± 1.30) × 10 <sup>7</sup>
ANW_M		6.06 ± 0.01 b	6.05 ± 0.02 b		0.31 ± 0.01 b	0.35 ± 0.01 a		(3.09 ± 1.25) × 10 <sup>7</sup>	(3.81 ± 2.09) × 10 <sup>7</sup>
ANW_L		6.11 ± 0.07 ab	6.05 ± 0.00 b		0.31 ± 0.04 a	0.34 ± 0.02 a		(3.17 ± 1.56) × 10 <sup>7</sup>	(8.38 ± 1.22) × 10 <sup>6</sup>
AFW_H	5.93 ± 0.02 a	6.80 ± 0.13 b	6.78 ± 0.05 b	0.38 ± 0.00 a	0.21 ± 0.05 ab	0.19 ± 0.00 b	(1.26 ± 0.45) × 10 <sup>6</sup>	(1.04 ± 0.56) × 10 <sup>8</sup>	(1.09 ± 0.39) × 10 <sup>7</sup>
AFW_M		6.65 ± 0.02 b	6.74 ± 0.08 b		0.18 ± 0.00 b	0.18 ± 0.00 b		(1.27 ± 0.30) × 10 <sup>8</sup>	(3.85 ± 0.80) × 10 <sup>7</sup>
AFW_L		6.81 ± 0.18 b	6.63 ± 0.02 b		0.17 ± 0.01 b	0.16 ± 0.01 b		(9.83 ± 1.84) × 10 <sup>7</sup>	(1.18 ± 1.62) × 10 <sup>7</sup>
AND_H	6.49 ± 0.02 a	6.21 ± 0.19 ab	6.33 ± 0.04 b	0.41 ± 0.02 a	0.37 ± 0.01 b	0.32 ± 0.02 b	(8.82 ± 3.29) × 10 <sup>5</sup>	(1.04 ± 0.33) × 10 <sup>8</sup>	(4.72 ± 1.09) × 10 <sup>7</sup>
AND_M		6.13 ± 0.04 b	6.19 ± 0.03 b		0.40 ± 0.01 a	0.31 ± 0.03 a		(1.04 ± 0.11) × 10 <sup>8</sup>	(5.48 ± 0.76) × 10 <sup>7</sup>
AND_L		6.18 ± 0.05 b	6.17 ± 0.01 b		0.42 ± 0.07 ab	0.31 ± 0.01 b		(1.27 ± 0.75) × 10 <sup>8</sup>	(8.20 ± 1.16) × 10 <sup>7</sup>
AFD_H	6.49 ± 0.02 a	6.92 ± 0.05 b	6.98 ± 0.04 b	0.41 ± 0.02 a	0.25 ± 0.01 b	0.23 ± 0.01 b	(8.82 ± 3.29) × 10 <sup>5</sup>	(2.76 ± 0.95) × 10 <sup>8</sup>	(7.77 ± 4.44) × 10 <sup>7</sup>
AFD_M		6.94 ± 0.03 b	6.92 ± 0.16 a		0.25 ± 0.01 b	0.22 ± 0.01 b		(1.37 ± 0.25) × 10 <sup>8</sup>	(8.76 ± 1.68) × 10 <sup>7</sup>
AFD_L		6.95 ± 0.04 b	7.20 ± 0.05 c		0.24 ± 0.01 b	0.26 ± 0.01 b		(1.14 ± 0.27) × 10 <sup>8</sup>	(1.01 ± 0.82) × 10 <sup>8</sup>
GNW_H	6.09 ± 0.02 a	5.88 ± 0.03 b	5.95 ± 0.02 c	0.28 ± 0.01 a	0.32 ± 0.04 a	0.33 ± 0.03 a	(1.33 ± 1.00) × 10 <sup>6</sup>	(3.81 ± 1.28) × 10 <sup>7</sup>	(4.78 ± 1.76) × 10 <sup>7</sup>
GNW_M		6.00 ± 0.07 a	5.89 ± 0.12 a		0.33 ± 0.01 a	0.28 ± 0.04 a		(6.76 ± 2.36) × 10 <sup>7</sup>	(3.26 ± 1.44) × 10 <sup>7</sup>
GNW_L		5.99 ± 0.02 b	5.96 ± 0.03 b		0.26 ± 0.03 a	0.31 ± 0.08 a		(5.59 ± 0.43) × 10 <sup>7</sup>	(3.25 ± 1.65) × 10 <sup>7</sup>
GFW_H	6.09 ± 0.02 a	6.51 ± 0.21 ab	6.42 ± 0.06 b	0.28 ± 0.01 a	0.13 ± 0.01 b	0.16 ± 0.03 b	(1.33 ± 1.00) × 10 <sup>6</sup>	(1.43 ± 0.25) × 10 <sup>8</sup>	(1.06 ± 1.21) × 10 <sup>8</sup>
GFW_M		6.68 ± 0.10 b	6.20 ± 0.08 a		0.14 ± 0.02 b	0.12 ± 0.00 b		(9.24 ± 1.74) × 10 <sup>7</sup>	(9.81 ± 5.87) × 10 <sup>7</sup>
GFW_L		6.53 ± 0.11 ab	6.28 ± 0.05 b		0.12 ± 0.01 b	0.12 ± 0.08 b		(1.64 ± 0.51) × 10 <sup>8</sup>	(1.83 ± 1.41) × 10 <sup>7</sup>
GND_H	6.09 ± 0.02 a	6.01 ± 0.07 a	5.99 ± 0.06 a	0.28 ± 0.01 a	0.35 ± 0.04 a	0.34 ± 0.05 a	(1.13 ± 0.95) × 10 <sup>6</sup>	(4.81 ± 1.78) × 10 <sup>7</sup>	(3.65 ± 0.78) × 10 <sup>7</sup>
GND_M		5.96 ± 0.09 a	6.01 ± 0.03 a		0.34 ± 0.02 a	0.33 ± 0.03 a		(6.26 ± 3.66) × 10 <sup>7</sup>	(3.61 ± 0.55) × 10 <sup>7</sup>
GND_L		5.85 ± 0.02 b	6.10 ± 0.10 ab		0.36 ± 0.05 a	0.28 ± 0.02 a		(5.60 ± 0.34) × 10 <sup>7</sup>	(3.16 ± 0.74) × 10 <sup>7</sup>
GFD_H	6.09 ± 0.02 a	6.69 ± 0.05 c	6.28 ± 0.04 b	0.28 ± 0.01 a	0.18 ± 0.01 b	0.16 ± 0.00 b	(1.13 ± 0.95) × 10 <sup>6</sup>	(1.03 ± 0.61) × 10 <sup>8</sup>	(7.22 ± 0.50) × 10 <sup>7</sup>
GFD_M		6.53 ± 0.13 a	6.42 ± 0.21 a		0.17 ± 0.01 b	0.16 ± 0.01 b		(8.43 ± 0.49) × 10 <sup>7</sup>	(6.73 ± 0.60) × 10 <sup>7</sup>
GFD_L		6.57 ± 0.09 b	6.33 ± 0.10 ab		0.16 ± 0.00 b	0.16 ± 0.01 b		(1.87 ± 0.62) × 10 <sup>8</sup>	(7.50 ± 2.57) × 10 <sup>7</sup>

Each value is the mean ± SD ( $n=3$ ). Community nomenclature is indicated as follows: A: andosol (ADS) or G: gray lowland soil (GLS), and N: non-flooded or F: flooded conditions, and W: microcosm with the USDA110<sup>T</sup> wild-type or D: microcosm with the USDA110Δ*nosZ* variant. Additionally, High: high incubation temperature (H: 30°C), Medium: medium incubation temperature (M: 25°C), Low: low incubation temperature (L: 20°C). Although these incubations were conducted using sterile soil, and sterile water was supplied where necessary, the water supply was conducted under semi-sterile conditions. These cfu values are indicated as references.

a,b,c: different characters indicate significant differences among incubation periods (0, 30, 60 d) in the treatment (Bonferroni,  $p<0.05$ ).

Table S2 Abundance (%) of bradyrhizobia in soil microcosms under different temperature and flooding conditions

		Oday	IP <sup>1</sup>	30day	IP <sup>1</sup> T <sup>2</sup> F <sup>3</sup>	60day	IP <sup>1</sup> T <sup>2</sup> F <sup>3</sup>			Oday	IP <sup>1</sup>	30day	IP <sup>1</sup> T <sup>2</sup> F <sup>3</sup>	60day	IP <sup>1</sup> T <sup>2</sup> F <sup>3</sup>
ANW_H	U6	44.76 ± 9.84		32.07 ± 1.49	x	31.85 ± 3.61		AND_H	U6	51.81 ± 15.99	59.06 ± 3.81	*	53.61 ± 3.51	*	
	U76	27.11 ± 12.02		42.28 ± 4.70	*	48.02 ± 6.72			U76	16.32 ± 7.74	32.14 ± 0.52	x *	34.08 ± 5.08	*	
	U110	12.71 ± 7.34		23.13 ± 2.79	*	16.97 ± 7.31	*		U110	7.86 ± 6.05	1.02 ± 0.50		1.43 ± 0.97		
	U123	15.42 ± 9.35		2.52 ± 0.94	x	3.16 ± 1.08			U123	24.01 ± 3.60 a	7.78 ± 4.39 b		10.87 ± 3.38 b		
ANW_M	U6	44.76 ± 9.84		48.37 ± 2.52	y *	41.09 ± 7.33		AND_M	U6	51.81 ± 15.99	58.95 ± 3.35		56.54 ± 2.04	*	
	U76	27.11 ± 12.02		30.29 ± 1.18	*	37.24 ± 1.75			U76	16.32 ± 7.74	23.15 ± 1.73	y	29.97 ± 0.52	*	
	U110	12.71 ± 7.34		15.08 ± 1.21	*	17.58 ± 6.38	*		U110	7.86 ± 6.05	0.54 ± 0.23	*	1.07 ± 0.75		
	U123	15.42 ± 9.35		6.25 ± 0.47	y	4.08 ± 0.74			U123	24.01 ± 3.60	17.37 ± 2.13		12.42 ± 1.60		
ANW_L	U6	44.76 ± 9.84		37.21 ± 2.73	xy	35.41 ± 2.44		AND_L	U6	51.81 ± 15.99	53.74 ± 4.11	*	53.34 ± 3.92		
	U76	27.11 ± 12.02		37.64 ± 2.37	*	43.11 ± 3.08	*		U76	16.32 ± 7.74	26.04 ± 1.01	y *	29.95 ± 3.99		
	U110	12.71 ± 7.34		16.44 ± 3.27	*	16.49 ± 5.91	*		U110	7.86 ± 6.05	0.46 ± 0.19		0.96 ± 0.34		
	U123	15.42 ± 9.35		8.72 ± 1.71	y *	4.98 ± 0.64	*		U123	24.01 ± 3.60	19.76 ± 2.99		15.76 ± 2.28	*	
AFW_H	U6	44.76 ± 9.84		16.93 ± 7.00		14.07 ± 12.21		AFD_H	U6	51.81 ± 15.99 a	38.18 ± 5.58 ab	x *	18.60 ± 10.70 b	*	
	U76	27.11 ± 12.02		24.30 ± 3.13	*	47.61 ± 17.66			U76	16.32 ± 7.74 a	44.98 ± 4.17 b	x *	63.72 ± 9.27 b	x *	
	U110	12.71 ± 7.34 a		54.44 ± 6.14 b	*	36.25 ± 6.22 b	*		U110	7.86 ± 6.05	11.71 ± 4.98		12.14 ± 6.19		
	U123	15.42 ± 9.35		4.33 ± 1.54		2.06 ± 0.24			U123	24.01 ± 3.60 a	5.12 ± 0.94 b		5.54 ± 2.97 b		
AFW_M	U6	44.76 ± 9.84		35.35 ± 3.05	*	31.04 ± 0.59		AFD_M	U6	51.81 ± 15.99	48.80 ± 5.50	x	29.22 ± 8.37	*	
	U76	27.11 ± 12.02		14.03 ± 1.32	*	28.62 ± 7.59			U76	16.32 ± 7.74 a	26.59 ± 8.72 ab	x	48.56 ± 1.37 b	x *	
	U110	12.71 ± 7.34 a		45.87 ± 3.58 b	*	36.97 ± 8.87 ab	*		U110	7.86 ± 6.05	7.64 ± 2.64	*	13.02 ± 7.81		
	U123	15.42 ± 9.35		4.75 ± 1.61		3.37 ± 1.17			U123	24.01 ± 3.60 a	16.98 ± 3.64 ab		9.20 ± 0.79 b		
AFW_L	U6	44.76 ± 9.84		40.54 ± 2.71		40.42 ± 5.00		AFD_L	U6	51.81 ± 15.99	70.55 ± 1.46	y *	49.97 ± 7.13	ns	
	U76	27.11 ± 12.02		16.88 ± 2.28	*	20.34 ± 3.80	*		U76	16.32 ± 7.74	15.34 ± 3.01	y *	33.20 ± 9.56	y	
	U110	12.71 ± 7.34 a		37.70 ± 3.57 b	*	36.52 ± 6.16 b	*		U110	7.86 ± 6.05	1.17 ± 0.55		7.66 ± 3.16		
	U123	15.42 ± 9.35		4.88 ± 0.79	*	2.72 ± 0.96	*		U123	24.01 ± 3.60 a	12.95 ± 4.16 ab		9.17 ± 1.08 b	*	
GNW_H	U6	7.89 ± 5.49		19.82 ± 13.55		25.18 ± 7.02	*	GND_H	U6	8.77 ± 5.49 a	36.39 ± 11.75 ab	xy	36.78 ± 4.65 b		
	U76	7.57 ± 0.27 a		37.69 ± 7.17 ab		39.08 ± 4.63 b			U76	16.09 ± 0.27	32.09 ± 7.77		40.46 ± 6.89		
	U110	57.84 ± 5.70 a		31.57 ± 9.19 ab	*	31.27 ± 5.81 b	*		U110	49.39 ± 5.70 a	20.82 ± 9.29 b		16.73 ± 2.99 b	x	
	U123	26.70 ± 0.39 a		10.92 ± 3.32 b	x *	4.47 ± 2.67 b	x		U123	25.74 ± 0.39 a	10.70 ± 4.05 ab	x	6.02 ± 3.35 b	x	
GNW_M	U6	7.89 ± 5.49 a		38.17 ± 3.75 b	*	35.41 ± 1.13 b	*	GND_M	U6	8.77 ± 5.49 a	51.08 ± 4.10 b	x	29.90 ± 14.82 ab		
	U76	7.57 ± 0.27 a		16.15 ± 2.10 a		23.49 ± 1.66 b			U76	16.09 ± 0.27	15.42 ± 0.67		34.60 ± 7.73		
	U110	57.84 ± 5.70 a		18.44 ± 1.98 b	*	17.89 ± 3.13 b	*		U110	49.39 ± 5.70 a	1.53 ± 0.42 b		6.65 ± 6.92 b	xy	
	U123	26.70 ± 0.39		27.24 ± 0.37	y *	23.22 ± 1.85	y *		U123	25.74 ± 0.39	31.97 ± 3.60	y	28.85 ± 1.24	y	
GNW_L	U6	7.89 ± 5.49 a		29.82 ± 2.66 b		23.37 ± 6.25 ab		GND_L	U6	8.77 ± 5.49 a	37.74 ± 2.48 b	y	32.67 ± 4.25 b		
	U76	7.57 ± 0.27 a		16.49 ± 5.64 ab		30.89 ± 2.64 b			U76	16.09 ± 0.27	19.19 ± 7.02		28.60 ± 4.28		
	U110	57.84 ± 5.70 a		17.85 ± 3.66 b	*	19.09 ± 3.31 b	*		U110	49.39 ± 5.70 a	1.30 ± 0.37 b	*	1.43 ± 0.39 b	y *	
	U123	26.70 ± 0.39		35.84 ± 9.44	xy *	26.64 ± 0.66	y *		U123	25.74 ± 0.39	41.77 ± 6.27	y	37.29 ± 4.59	y	
GFW_H	U6	7.89 ± 5.49		10.02 ± 1.29	x	4.70 ± 4.00	x *	GFD_H	U6	8.77 ± 5.49	25.25 ± 3.21		16.49 ± 12.12		
	U76	7.57 ± 0.27		28.64 ± 9.68		24.78 ± 11.35			U76	16.09 ± 0.27 a	40.74 ± 5.59 b	x	43.34 ± 8.63 ab		
	U110	57.84 ± 5.70		58.63 ± 9.68	*	69.00 ± 15.60	*		U110	49.39 ± 5.70	31.39 ± 3.48	x	33.40 ± 12.81		
	U123	26.70 ± 0.39 a		2.71 ± 0.19 b	x *	1.52 ± 0.27 b	x		U123	25.74 ± 0.39 a	2.62 ± 1.00 b		6.77 ± 5.51 ab		
GFW_M	U6	7.89 ± 5.49		15.63 ± 8.23	xy *	14.65 ± 2.37	xy *	GFD_M	U6	8.77 ± 5.49	50.98 ± 29.27		20.48 ± 11.54		
	U76	7.57 ± 0.27 a		12.18 ± 0.96 b		23.53 ± 8.79 ab			U76	16.09 ± 0.27	12.89 ± 7.57	y	38.36 ± 16.56		
	U110	57.84 ± 5.70		64.28 ± 8.36	*	57.40 ± 10.10	*		U110	49.39 ± 5.70 a	13.43 ± 4.94 b	y	22.66 ± 14.89 ab		
	U123	26.70 ± 0.39 a		7.92 ± 3.22 b	xy *	4.42 ± 0.53 b	y *		U123	25.74 ± 0.39	22.69 ± 17.52		18.50 ± 14.14		
GFW_L	U6	7.89 ± 5.49		26.45 ± 2.12	y	21.81 ± 5.21	y	GFD_L	U6	8.77 ± 5.49	52.98 ± 18.61		29.25 ± 18.14		
	U76	7.57 ± 0.27		12.47 ± 2.95		26.32 ± 4.97			U76	16.09 ± 0.27 ab	10.78 ± 3.72 a	y	24.79 ± 3.49 b		
	U110	57.84 ± 5.70		53.57 ± 2.44	*	45.74 ± 6.80	*		U110	49.39 ± 5.70 a	7.09 ± 1.91 b	y *	7.55 ± 2.01 b	*	
	U123	26.70 ± 0.39 a		7.51 ± 0.67 b	y *	6.13 ± 2.44 b	y *		U123	25.74 ± 0.39	29.15 ± 15.30		38.42 ± 20.81		

Each value is the mean ± SD ( $n=3$ ). Community nomenclature is indicated as follows: A: andosol (ADS) or G: gray lowland soil (GLS), and N: non-flooded or F: flooded conditions, and W: microcosm with the USDA110<sup>T</sup> wild-type or D: microcosm with the USDA110ΔnosZ variant. Additionally, High: high incubation temperature (H: 30°C), Medium: medium incubation temperature (M: 25°C), Low: low incubation temperature (L: 20°C).

<sup>1</sup>IP(a,b): different characters indicate significant differences among incubation periods (0, 30, 60 d) in the treatment (Bonferroni,  $p<0.05$ ).

<sup>2</sup>T(x,y): different characters indicate significant differences among incubation temperatures (H, M, L) in the treatment (Bonferroni,  $p<0.05$ ).

<sup>3</sup>F(\*): asterisk indicates a significant difference between flooded and non-flooded conditions in the treatment (Welch's t-test,  $p<0.05$ ).