

# Greenhouse gas emissions from cassava production influenced by 47 years of diverse fertilizer application practices in Thailand: insights from two years of measurements

Patikorn SRIPHIROM<sup>1,2</sup> (✉), Amnat CHIDTHAISONG<sup>3,4,5</sup> (✉), Wanlee AMORNAPON<sup>6</sup>, Kazuyuki YAGI<sup>7</sup>, Wanida NOBUNTOU<sup>8</sup>, Nimaradee BOONAPATCHAROEN<sup>9</sup>, Wantanasak SUKSONG<sup>9</sup>

1 Department of Environmental Science, Faculty of Science, Silpakorn University, Nakhon Pathom 73000, Thailand.

2 Greenhouse Gas Unit for Sustainability, Department of Environmental Science, Faculty of Science, Silpakorn University, Nakhon Pathom 73000, Thailand.

3 The Joint Graduate School of Energy and Environment, King Mongkut's University of Technology Thonburi, Bangkok 10140, Thailand.

4 Center of Excellence on Energy Technology and Environment, Postgraduate Education and Research Development Office, Ministry of Higher Education, Science, Research and Innovation, Bangkok 10400, Thailand.

5 Earth System Science Research Cluster, King Mongkut's University of Technology Thonburi, Bangkok 10140, Thailand.

6 Field and Renewable Energy Crops Research Institute, Rayong Field Crop Research Center, Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok 10900, Thailand.

7 Faculty of International Communication, Aichi University, Nagoya 4538777, Japan

8 Soil Science Research Group, Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok 10900, Thailand.

9 Excellent Center of Waste Utilization and Management, Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi, Bangkok 10150, Thailand.

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Correspondence: [sriphirom\\_p@su.ac.th](mailto:sriphirom_p@su.ac.th), [amnat.chi@kmutt.ac.th](mailto:amnat.chi@kmutt.ac.th)

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## SUPPLEMENTARY MATERIALS

**Table S1** Crop management practices implemented for cassava cultivation in this study

Activity	Date (DAP)	Conducted treatment
2021 season		
Soil tillage	Jul. 06, 2021 (-29 DAP)	All treatments
Crop residue incorporation into the soil	Jul. 06, 2021 (-29 DAP)	CR <sub>I</sub> and NPKCR <sub>I</sub>
Planting	Aug. 04, 2021 (0 DAP)	All treatments
Weeding	Sep. 27, 2021 (54 DAP)	All treatments
Municipal compost application	Oct. 14, 2021 (71 DAP)	NPK <sub>F</sub> CP <sub>A</sub>
Chemical fertilizer application	Oct. 18, 2021 (75 DAP)	N <sub>F</sub> , NP <sub>F</sub> , NK <sub>F</sub> , NPK <sub>F</sub> , NPK <sub>F</sub> CR <sub>I</sub> , and NPK <sub>F</sub> CP <sub>A</sub>
1 <sup>st</sup> spraying weeding chemical	Oct. 27, 2021 (84 DAP)	All treatments
2 <sup>nd</sup> spraying weeding chemical	May 16, 2021 (285 DAP)	All treatments
Harvest	Aug. 23, 2022 (384 DAP)	All treatments
2022 season		
Soil tillage	Aug. 31, 2022 (-21 DAP)	All treatments
Crop residue incorporation into the soil	Aug. 31, 2022 (-21 DAP)	CR <sub>I</sub> and NPKCR <sub>I</sub>
Planting	Sept. 21, 2022 (0 DAP)	All treatments
1 <sup>st</sup> weeding	Oct. 30, 2022 (49 DAP)	All treatments
Municipal compost application	Nov. 07, 2022 (57 DAP)	NPK <sub>F</sub> CP <sub>A</sub>
Chemical fertilizer application	Nov. 23, 2022 (73 DAP)	N <sub>F</sub> , NP <sub>F</sub> , NK <sub>F</sub> , NPK <sub>F</sub> , NPK <sub>F</sub> CR <sub>I</sub> , and NPK <sub>F</sub> CP <sub>A</sub>
1 <sup>st</sup> spraying weeding chemical	Dec. 07, 2022 (87 DAP)	All treatments
2 <sup>nd</sup> weeding	Dec. 14, 2022 (94 DAP)	All treatments
2 <sup>nd</sup> spraying weeding chemical	Jun. 28, 2022 (290 DAP)	All treatments
Harvest	Oct. 03, 2023 (387 DAP)	All treatments

Note: DAP refers to days after planting. Control, no amendments; CR<sub>I</sub>, crop residue incorporation; N<sub>F</sub>, NP<sub>F</sub>, NK<sub>F</sub> and NPK<sub>F</sub> refer to N, NP and NK, NPK fertilizer applications, respectively; NPK<sub>F</sub>CR<sub>I</sub>, combination of NPK<sub>F</sub> and CR<sub>I</sub> treatments; and NPK<sub>F</sub>CP<sub>A</sub>, combination of NPK<sub>F</sub> and CP addition.

**Table S2** Primer pairs and thermocycler conditions employed for qRT-PCR analysis

Target gene	Primer	Primer sequence (5'-3')	Size (bp)	Thermal condition	Source
16S RNA gene of total bacteria	EUB338F	ACTCCTACGGGAGGCAGCAG	200	95 °C for 3 min; 40 cycles of 95 °C for 10 s, 58 °C for 10 s, and 72 °C for 10s	[1]
	EUB518R	ATTACCGCGGCTGCTGG			
16S RNA gene of total archaea	Arch915F	AGGAATTGGCGGGGAGCAC	112	95 °C for 3 min; 40 cycles of 95 °C for 10 s, 58 °C for 10 s, and 72 °C for 10s	[2]
	Arch1017R	GGCCATGCACCWCCTCT			
<i>amoA</i> -AOB	<i>amoA</i> -1F	GGGGTTTCTACTGGTGGT	491	95 °C for 3 min; 40 cycles of 95 °C for 10 s, 58 °C for 10 s, and 72 °C for 25 s	[3]
	<i>amoA</i> -2R	CCCCTCKGSAAAGCCTTCTTC			
<i>amoA</i> -AOA	Arch- <i>amoA</i> F	STAATGGTCTGGCTTAGACG	635	95 °C for 3 min; 40 cycles of 95 °C for 10 s, 58 °C for 10 s, and 72 °C for 30 s	[4]
	Arch- <i>amoA</i> R	GCGGCCATCCATCTGTATGT			
<i>nirK</i>	<i>nirK</i> F1aCu	ATCATGGTSCTGCCGCG	455	95 °C for 3 min; 40 cycles of 95 °C for 10 s, 55 °C for 10 s, and 72 °C for 25 s	[5]
	<i>nirK</i> R3Cu	GCCTCGATCAGRTTGTGGTT			
<i>nirS</i>	<i>nirS</i> cd3aF	G TSAACG TSAAGGARACSGG	425	95 °C for 3 min; 40 cycles of 95 °C for 10 s, 55 °C for 10 s, and 72 °C for 25 s	[6]
	<i>nirS</i> R3cd	GASTTCGGRTGSGTCTTGA			
<i>nosZ</i>	<i>nosZ</i> 2F	CGCRACGGCAASAAGGTSMSST	267	95 °C for 3 min; 40 cycles of 95 °C for 10 s, 55 °C for 10 s, and 72 °C for 10 s	[7]
	<i>nosZ</i> 2R	CAKRTGCAKSGCRTGGCAGAA			

**Table S3** Pearson's correlation coefficients between CO<sub>2</sub> and N<sub>2</sub>O emissions, cassava yield, soil pH, % water-filled pore space (WFPS), soil organic carbon (SOC) sequestration, and various inputs (C, N, P, and K) during the study period

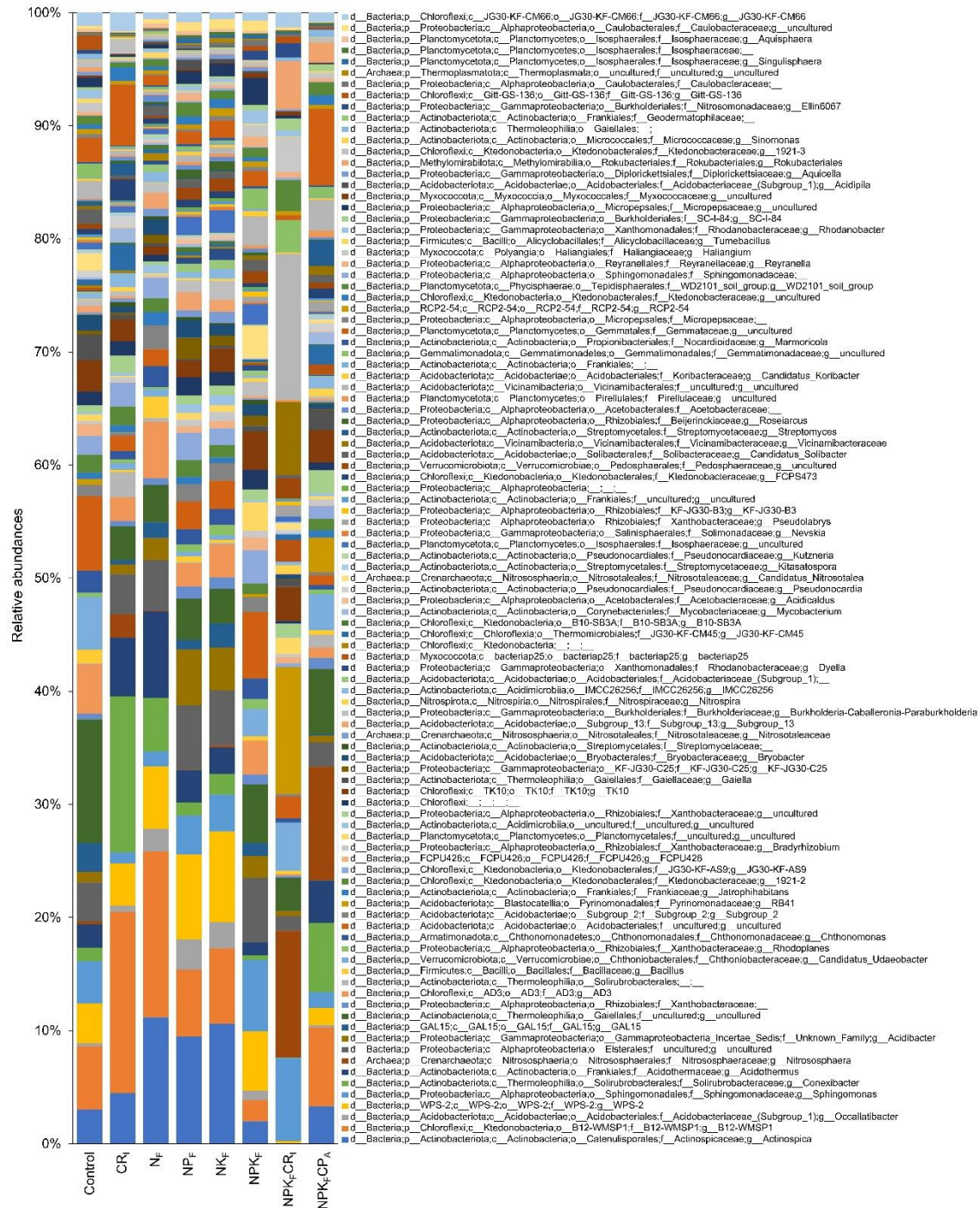
Property	CO <sub>2</sub>	N <sub>2</sub> O	Cassava yield	pH	%WFPS	SOC	C input	N input	P input	K input
CO <sub>2</sub>	1	0.289*	0.648**	0.414**	0.423**	0.670**	0.693**	0.944**	0.868**	0.871**
N <sub>2</sub> O	0.289*	1	0.113	0.097	0.062	0.259*	0.585**	0.947**	0.796**	0.874**
Cassava yield	0.648**	0.113	1	0.272*	0.380**	0.738**	0.600**	0.854**	0.802**	0.893**
pH	0.414**	0.097	0.272*	1	0.285*	0.637**	0.260	0.511**	0.429*	0.689**
%WFPS	0.423**	0.062	0.380**	0.285*	1	0.507**	0.526**	0.596**	0.645**	0.490**
SOC	0.670**	0.258*	0.738**	0.637**	0.507**	1	0.436*	0.855**	0.810**	0.884**
C input	0.693**	0.585**	0.600**	0.260	0.526**	0.436*	1	0.786**	0.779**	0.703**
N input	0.944**	0.947**	0.854**	0.511**	0.596**	0.855**	0.786**	1	0.891**	0.923**
P input	0.868**	0.796**	0.802**	0.429*	0.645**	0.810**	0.779**	0.891**	1	0.820**
K input	0.871**	0.874**	0.893**	0.689**	0.490**	0.884**	0.703**	0.923**	0.820**	1

Note: \*\* and \* indicate that the correlation is significant at the 0.01 and 0.05 levels, respectively.

**Table S4** Pearson's correlation coefficients between CO<sub>2</sub> and N<sub>2</sub>O emissions, various inputs (C, N, P, and K), and microbial groups (total bacteria (EUB), total archaea (ARC), ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA), and denitrifying bacteria associated with *nirK*, *nirS*, and *nosZ* genes) during the study period

Property	CO <sub>2</sub>	N <sub>2</sub> O	C input	N input	P input	K input	EUB	ARC	AOB	AOA	<i>nirK</i>	<i>nirS</i>	<i>nosZ</i>
CO <sub>2</sub>	1	0.289*	0.693**	0.944**	0.868**	0.871**	0.513**	0.228**	0.363**	0.257**	0.343**	0.587**	0.533**
N <sub>2</sub> O	0.289*	1	0.585**	0.947**	0.796**	0.874**	0.070	-0.095	0.117	0.121	-0.031	0.289**	0.108
C input	0.693**	0.585**	1	0.786**	0.779**	0.703**	0.667**	0.119	0.795**	0.261	0.435*	0.078	0.689**
N input	0.944**	0.947**	0.786**	1	0.891**	0.923**	0.903**	0.549**	0.910**	0.604**	0.785**	0.514**	0.919**
P input	0.868**	0.796**	0.779**	0.891**	1	0.820**	0.852**	0.501**	0.853**	0.628**	0.737**	0.371*	0.889**
K input	0.871**	0.874**	0.703**	0.923**	0.820**	1	0.954**	0.705**	0.900**	0.751**	0.891**	0.629**	0.959**
EUB	0.513**	0.070	0.667**	0.903**	0.852**	0.954**	1	0.791**	0.916**	0.830**	0.952**	0.715**	0.985**
ARC	0.228**	-0.095	0.119	0.549**	0.501**	0.705**	0.791**	1	0.542**	0.939**	0.934**	0.896**	0.757**
AOB	0.363**	0.117	0.795**	0.910**	0.853**	0.900**	0.916**	0.542**	1	0.598**	0.793**	0.560**	0.916**
AOA	0.257**	0.121	0.261	0.604**	0.628**	0.751**	0.830**	0.939**	0.598**	1	0.929**	0.723**	0.820**
<i>nirK</i>	0.343**	-0.031	0.435*	0.785**	0.737**	0.891**	0.952**	0.934**	0.793**	0.929**	1	0.837**	0.937**
<i>nirS</i>	0.587**	0.289**	0.078	0.514**	0.371*	0.629**	0.715**	0.896**	0.560**	0.723**	0.837**	1	0.645**
<i>nosZ</i>	0.533**	0.108	0.689**	0.919**	0.889**	0.959**	0.985**	0.757**	0.916**	0.820**	0.937**	0.645**	1

Note: \*\* and \* indicate that the correlation is significant at the 0.01 and 0.05 levels, respectively.



**Fig. S1.** Genus-level taxonomic profiles of dominant bacteria identified during cassava cultivation in 2021. Control, no amendments; CR<sub>1</sub>, crop residue incorporation; N<sub>F</sub>, N<sub>Pf</sub>, N<sub>Kf</sub> and N<sub>PKf</sub> refer to N, NP and NK, NPK fertilizer applications, respectively; N<sub>PKf</sub>-CR<sub>1</sub>, combination of N<sub>PKf</sub> and CR<sub>1</sub> treatments; and N<sub>PKf</sub>-CP<sub>A</sub>, combination of N<sub>PKf</sub> and CP addition.

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