Managing Nitrogen for Greenhouses Gases Mitigation in Tropical Agricultural

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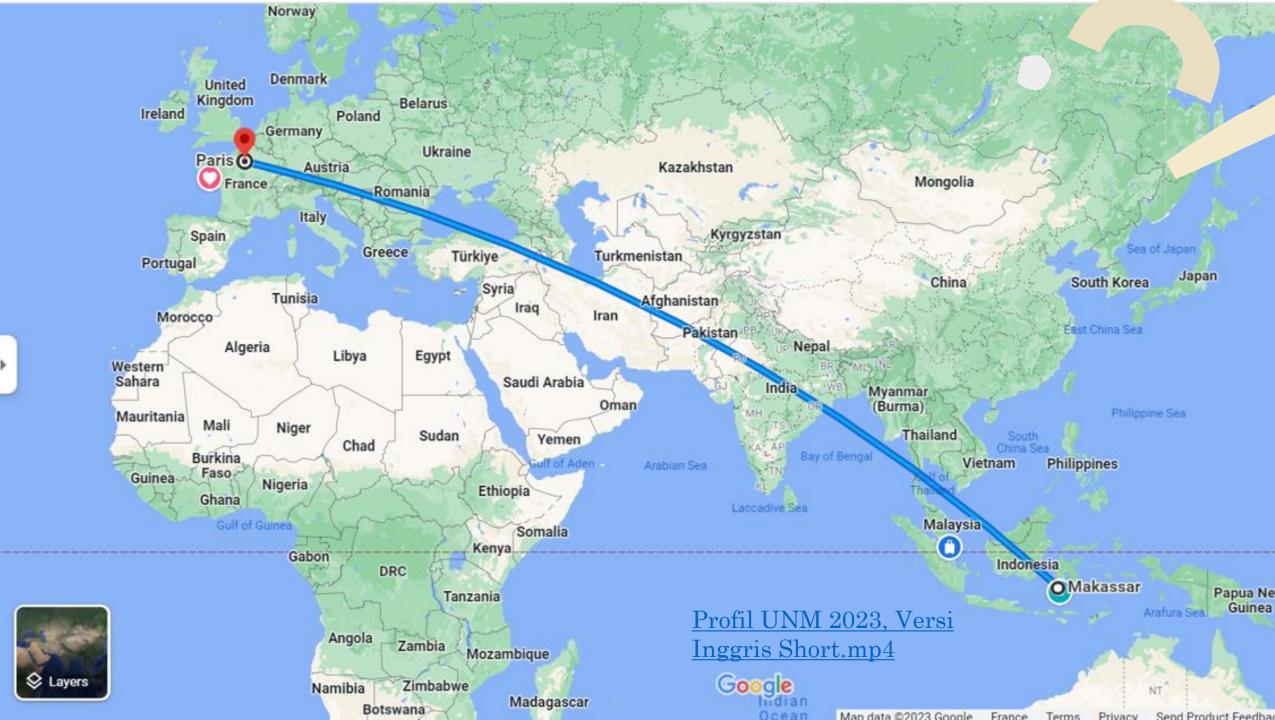
Acknowledgments

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Research Group Oslan Jumadi Department of Biology, Universitas Negeri Makassar Sokolia Field.mp4







RESEARCH TEAM The Nitrogen and Carbon Cycling in Soil - from cells to ecosystem processes-

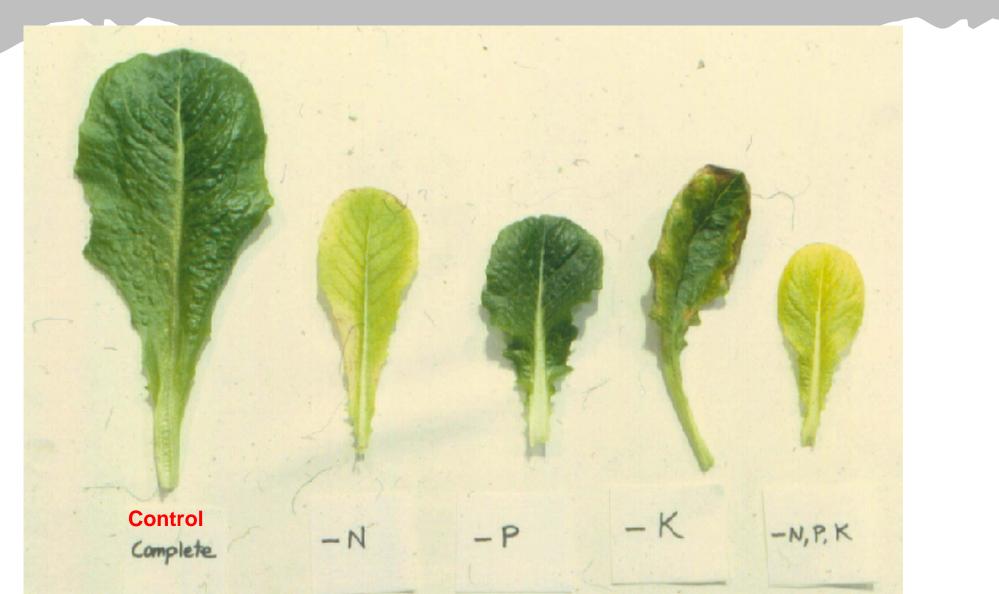
Why nitrogen fertilizer should be managed,

• <u>Without</u> fertilizers, the soil would be depleted and therefore plants would be particularly difficult to grow.

Fig.: Fertilizer adds nutritional value: <u>To the right, tomato plant with</u> <u>nitrogen deficiency</u>, to the left, tomato plant with optimum nutritional balance.



Macronutrients N, P, K Deficiencies Leaf Lettuce



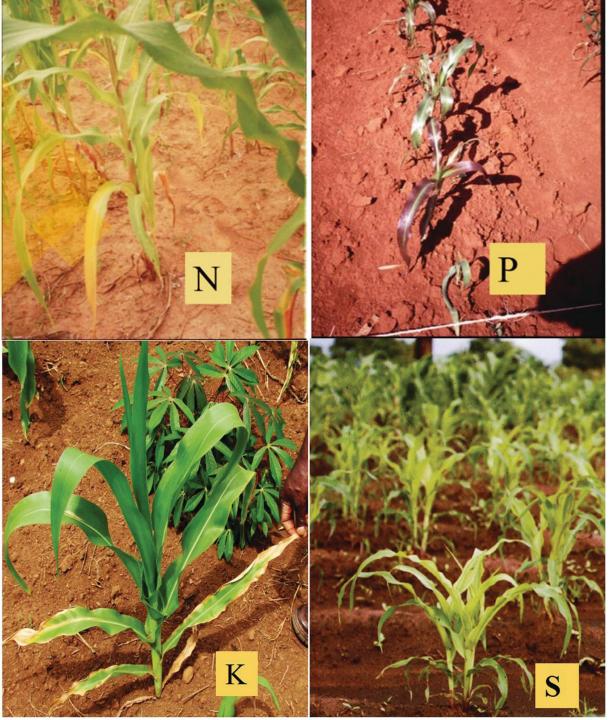


Fig.: Maize deficiency symptoms of the four main limiting macronutrients in the tropics. Nitrogen (N) phosphorus (P) potassium (K) sulfur (S) deficiency. (Sanchez, P. A. (2019). Properties and Management of Soils

in the Tropics. Cambridge University Press)

world population and fertilizer use

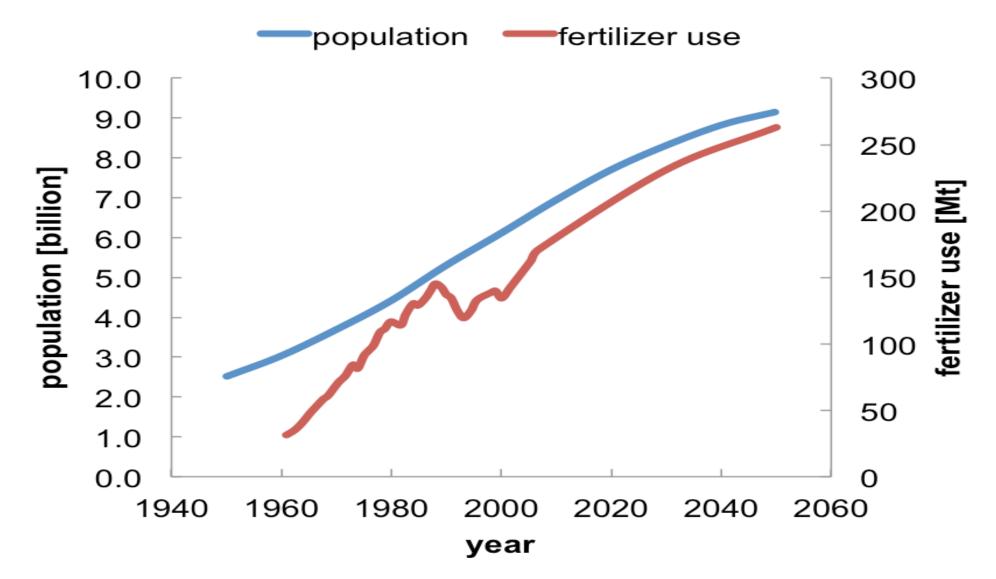


Fig.: World population and fertilizer consumption, with projections to 2050 (Alexandratos, N. and J. Bruinsma. 2012. World agriculture towards 2030/2050: the 2012 revision. ESA Working paper No. 12-03. Rome, FAO.)

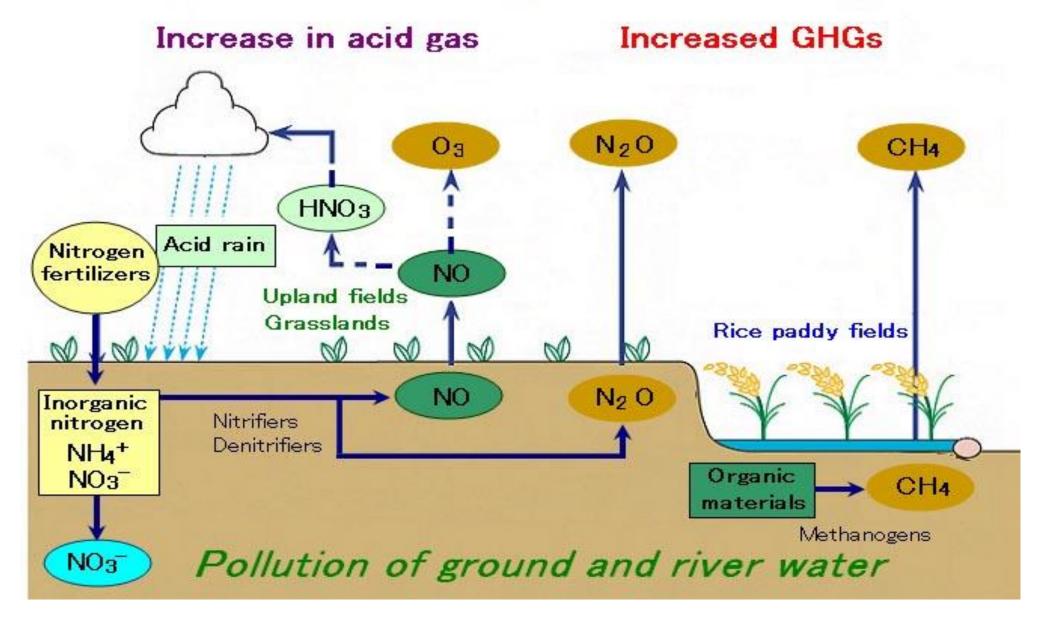


Fig. Greenhouse gases production from agriculture system. (*Research Project for Mitigation of Greenhouse Gas Emissions, National Institute for Agro-Environmental Sciences Japan*).

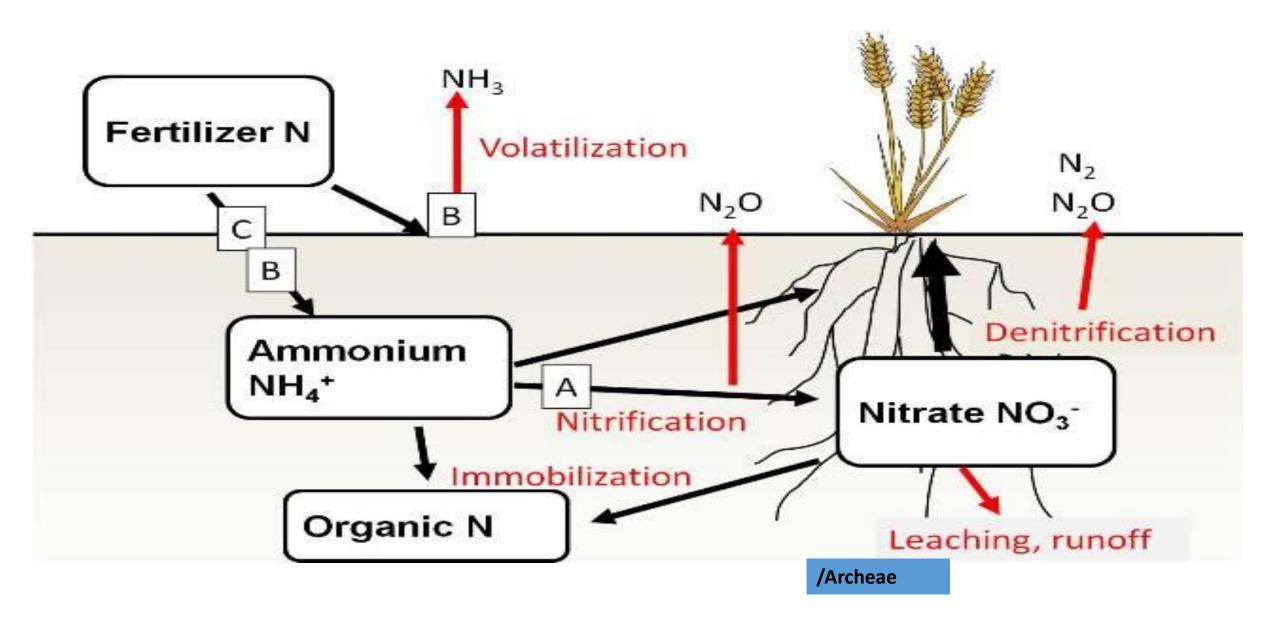


Figure. Main sources of Nitrous Oxide (N₂O) Gas Production

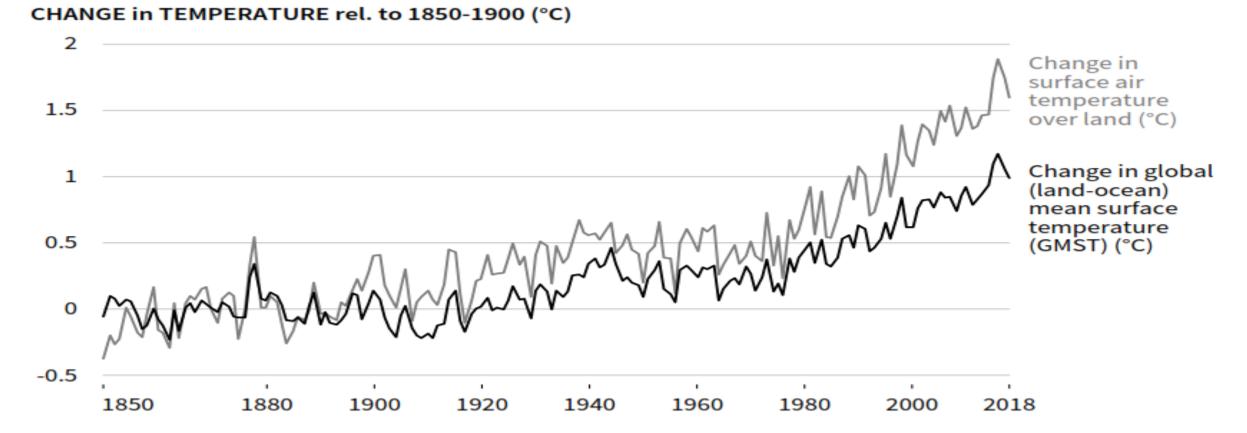
Nitrogen Buffering Mechanisms



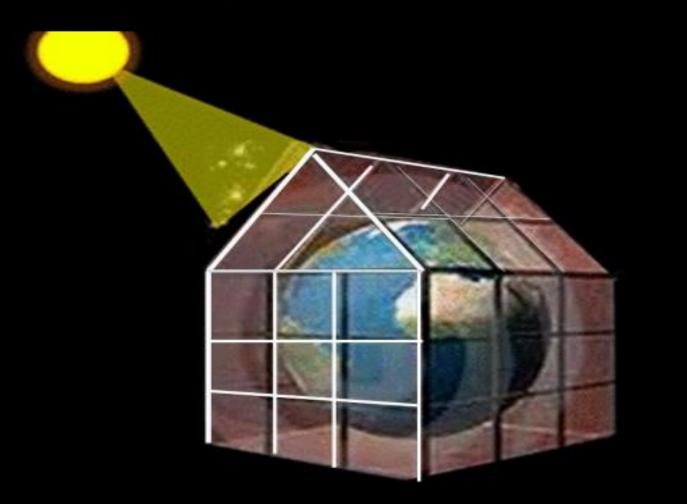
- 1. Increased Applied N results in increased = plant N loss (NH₃)
- 2. Higher rates of applied N increased = volatilization losses
- 3. Higher rates of applied N increased = denitrification
- Higher rates of applied N increased organic C, = increased organic N
- 5. Increased applied N increased = grain protein
- 6. Increased applied N increased = forage N
- 7. Increased applied N increased = straw N

Trends of greenhouse gases emission and global warming

Observed temperature change relative to 1850-1900



IPCC, 2019: Summary for Policymakers. In: Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems [P.R. Shukla, J. Skea, E. Calvo Buendia, V. Masson-Delmotte, H.- O. Pörtner, D. C. Roberts, P. Zhai, R. Slade, S. Connors, R. van Diemen, M. Ferrat, E. Haughey, S. Luz, S. Neogi, M. Pathak, J. Petzold, J. Portugal Pereira, P. Vyas, E. Huntley, K. Kissick, M. Belkacemi, J. Malley, (eds.)]. Greenhouse gases GHGs) in the atmosphere have a heat trapping effect, like the glass of a greenhouse traps heat from the sun, heating up the greenhouse. Light energy passes easily through glass into the greenhouse but the heat energy cannot pass through the glass well.



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"The Paris Agreement confirm the irreversible transition to a low carbon, safer and healthier world."

Christiana Figueres
UNFCCC Executive Secretary

#ParisAgreement #COP81



Whilst the Glasgow Climate Pact agreed at the UN Climate Change Conference of the Parties (COP26) firms up the global commitment to accelerate action on climate this decade, it left many wondering if this deal is enough to limit global warming to 1.5°C over pre-industrial levels.



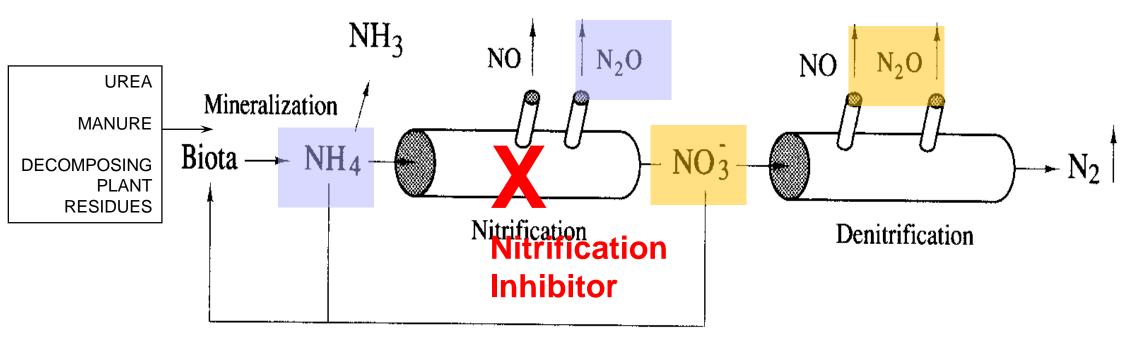
The possible option of mitigation strategies for N_2O and CH_4

- Fertilizer managements
 - Slow Release, *Nitrification Inhibitor (Neem or DCD*, Switching Fertilizer (synthetic to organic)
- Drainage Managements
 - Intermitted Drainage
 - Midseason Drainage
- Crop management (short duration varieties, aerial Parenchyma)

Mitigation Options: Nitrification Inhibitor

Nitrification Conversion of Nitrogen Containing Materials to a Plant Useable Form

"Leaky pipe" analogy for N₂O production



Immobilization and Plant Uptake

Oxidation step

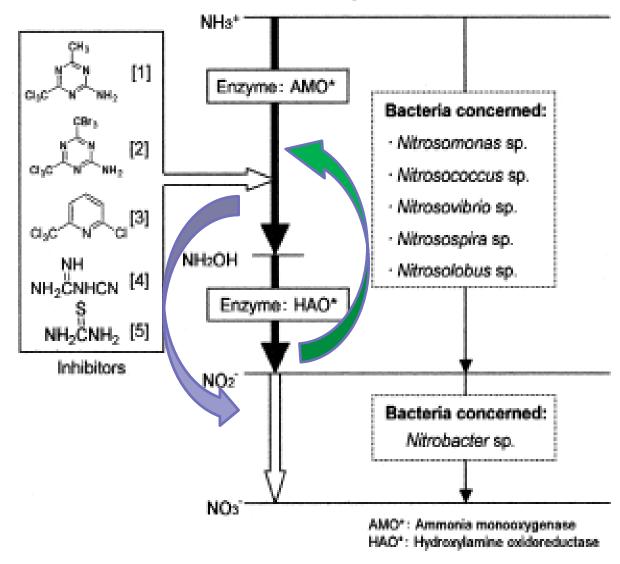
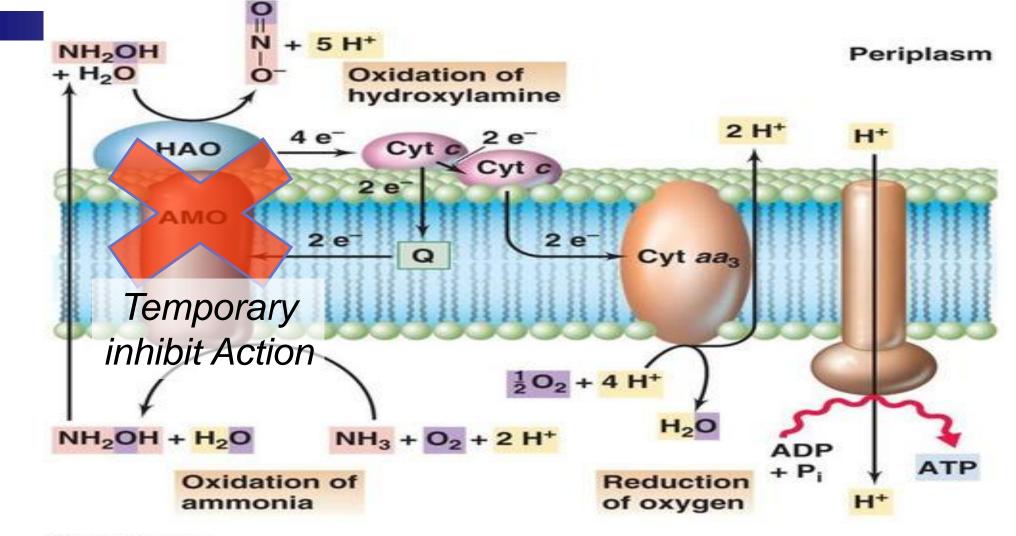


Fig. 2. Effect of inhibitors on nitrification of ammonia.



Cytoplasm

Fig. Hypothesis inhibition two enzymes, ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO) are involved in the oxidation of ammonia to nitrite.

Several chemical/synthetics nitrification inhibitors (NIs);

- 1. AM (2-amino 4-chloro 6-methyl pyrimidine)
- 2. ST (2-sulfanilamide thiazone), DCS (N-2,5-dichlorophenyl succeinamic acid)
- 3. ASU (1-amino 2-thiourea),
- 4. DCD (dicyandiamide),
- 5. Nitrapiryn
- 6. CCC (wax-coated calcium carbide)
- 7. DMPP (3,4-dimethylpyrazol-phosphate).

(Mosier. 1996; Zerulla et al. 2001; Weiske et al. 2001; Liu et al. 2013).

The synthetics NIs are mostly expensive and limited available in market particularly in Indonesia.

In addition, several studies showed that NI like dicyandiamide (DCD) has dissatisfactory impact to environment due to the coumpund of DCD belong to the group of organic chlorine. (Zerulla et al. 2001).

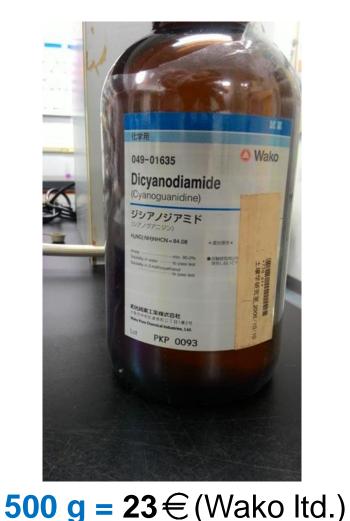
The Alternative:

Several plant materials/extracts can also inhibit the nitrification with the potency to compete with the synthetic NI (Subbarao et al., 2012).

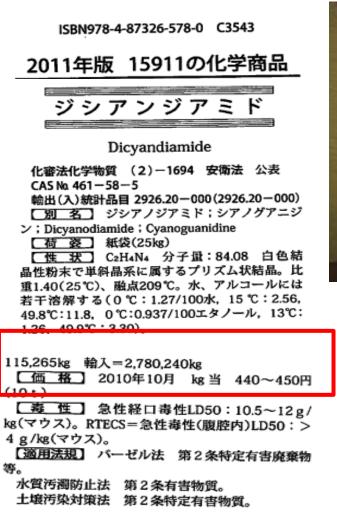
Some of these plant products are easily available, less expensive and easily degradable in soil than the synthetic NI.

Organic nitrification inhibition;

- 1. Karanja (Pongamia glabra)(Singh, 1966), and
- 2. Neem (*Azadiracta indica*) and Nimin (plant extract of A. indica) possess wide spectrum antimicrobial action and the properties to be a potent NI) (Sharma and Prasad 1995); (Biswas et al. 2002); (Kumar et al. 2007); (Abbasi et al. 2011): (Jumadi et al. 2019:2020).



Comparative price of NIs Synthetics Vs Organic





Neem Cake (Waste	Neem Oil (pure
product)	extract)
0.19€	4.4€

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RESEARCH ARTICLE

https://doi.org/10.48048/tis.2023.6395

Efficiency of Nitrification Inhibitor on Designing Nitrogen Fertilizer by Neem Compounds Based on Molecular Docking

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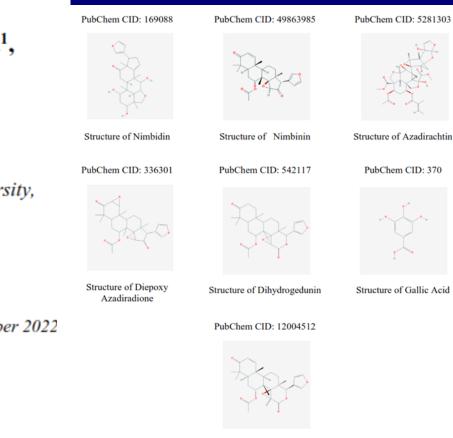


Figure 1 Ten chemicals structure of Neem compounds with highest energy binding score. Source: https://pubchem.ncbi.nlm.nih.gov/.https://pubchem.ncbi.nlm.nih.gov/.

Structure of Gedunir

https://www.youtube.com/watch?v=b_RJUdi4tD8

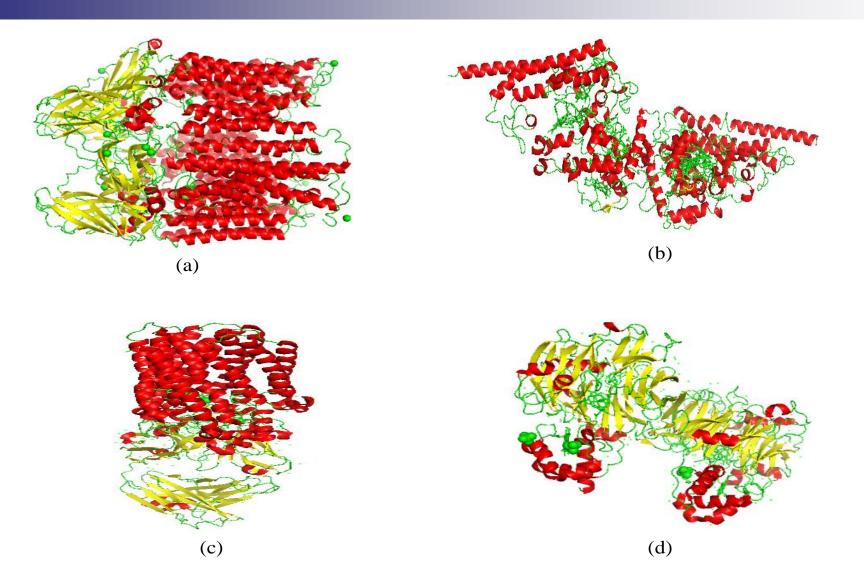
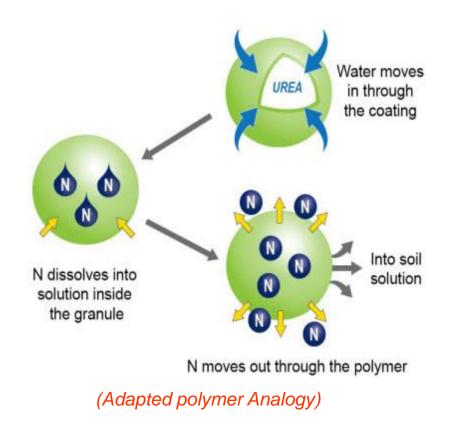


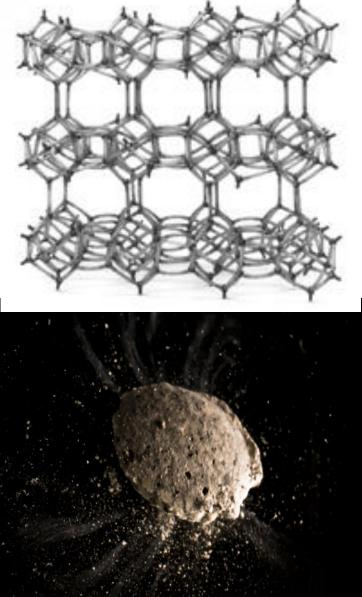
Figure 2 The tertiary structure of enzymes (a) Methane Monooxygenase, (b) Hydroxylamine Oxidoreductase, (c) Nitric Oxide Reductase and (d) Nitrite Reductase. The structures of alpha-helix and beta-sheet are presented by red and green colors in cartoon models, respectively.

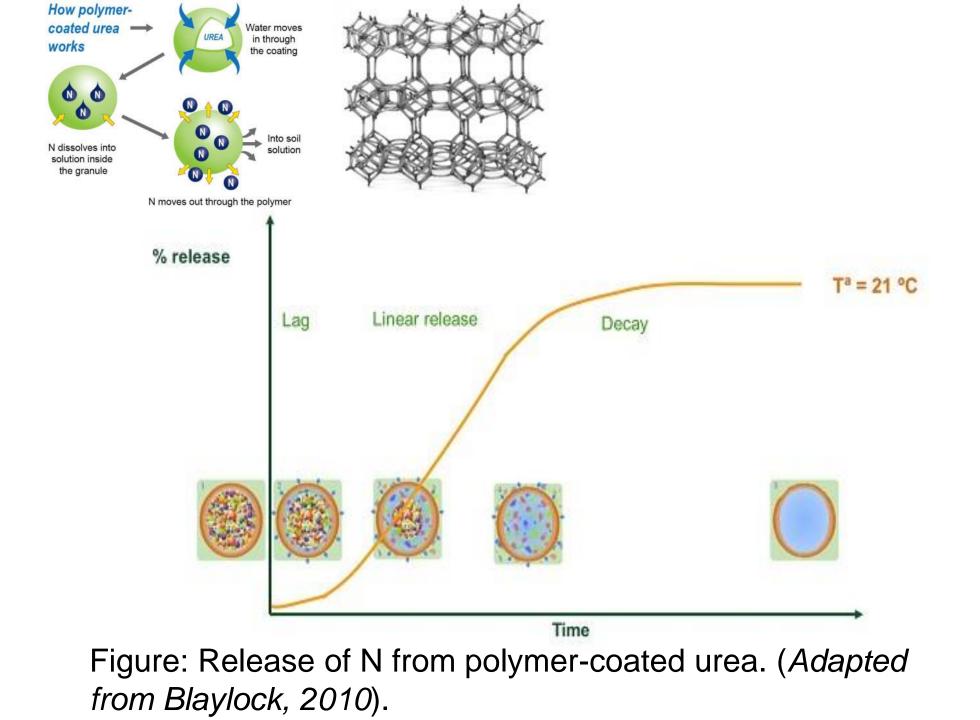
Table 6. The binding energy of ligands in complex with Nitric Oxide Reductase enzyme obtained by molecular docking.

No.	Compound	Binding energy (Kcal/mol)
1	Nimbin	-7.5
2	Nimbidin	-8.4
3	Nimbic Acid	-7.8
4	Nimbidinin	-8.3
5	Nimbinin	-7.8
6	Azadirachitin	-7.4
7	Diepoxy Azadiradione	-8.7
8	Dyhidrogedunin	-8.1
9	Gallic	-6.3
10	Gedunin	-8.2

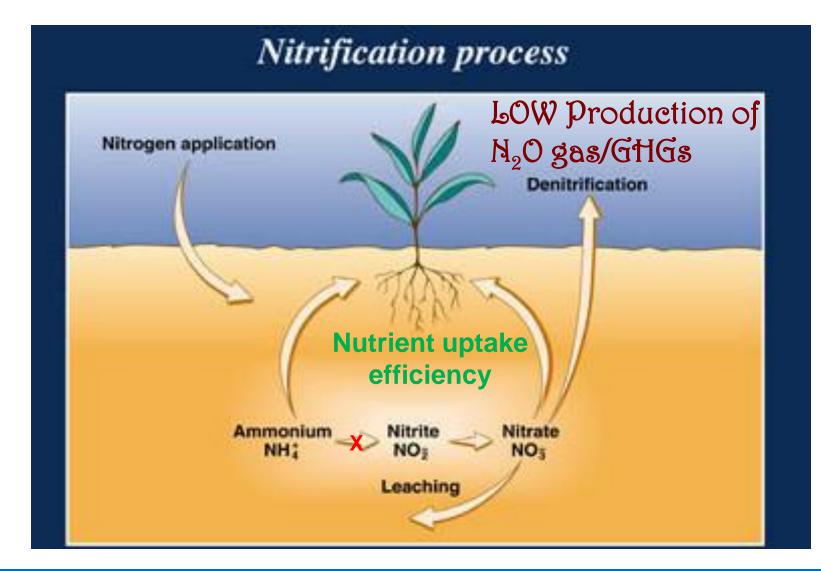
While zeolite act as slow release releases nutrients gradually into the soil (nitrogen or phosphor)



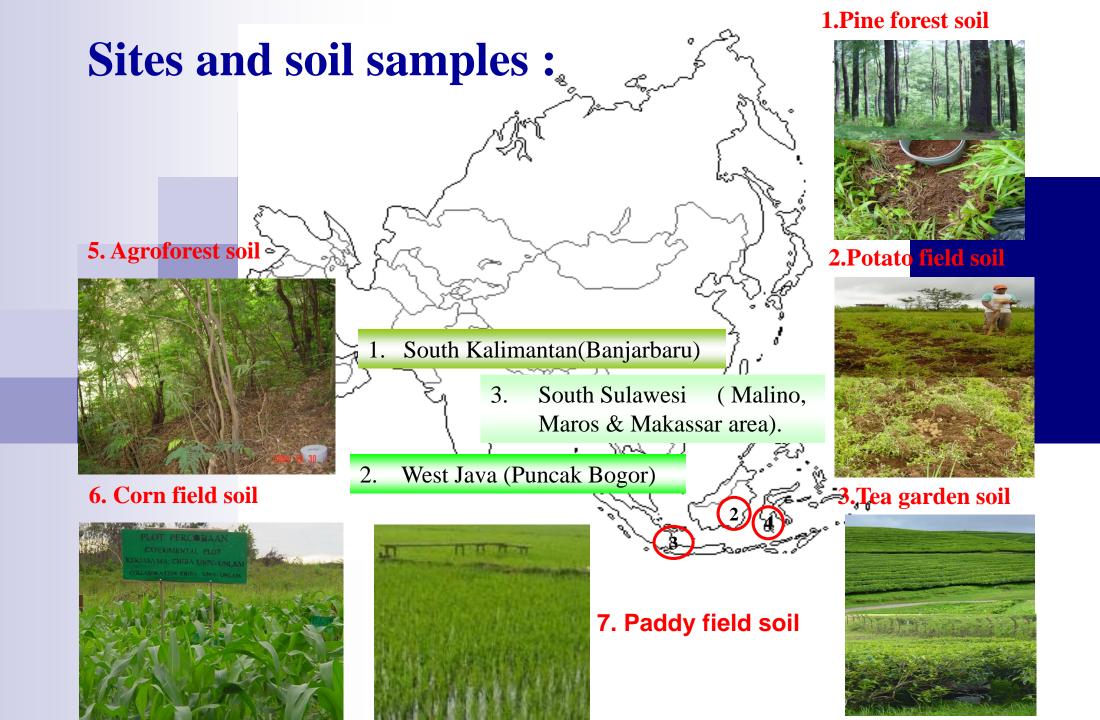




Mitigation Options: Nitrification Inhibitor and Slow-Release Fertilizer



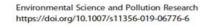




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ARTICLE

Emissions of nitrous of



RESEARCH ARTICLE

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Brown algae (Sa microbe ferment

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e Department of Agricultural Chemist

TRUESOIL Project: Understanding Trade-offs and Dynamic Interactions between SOC Stocks and GHG Emissions for Climate Smart Agrisoil Management

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Field preparations



Rice field experiments

Nitrogen Fertilizer application (130 kg-N ha⁻¹) split time (60 and 70 kg-N ha⁻¹)

- Urea Granule
- Urea Granule Zeolit
- Urea Granule Zeolit and Neem
- Urea Granule Zeolit and Dicyandinamide

Water Managements

- 1. Continuous flooded
- 2. Non continuous flooded









Results and discussions

Continuous flooded

Non Continuous flooded

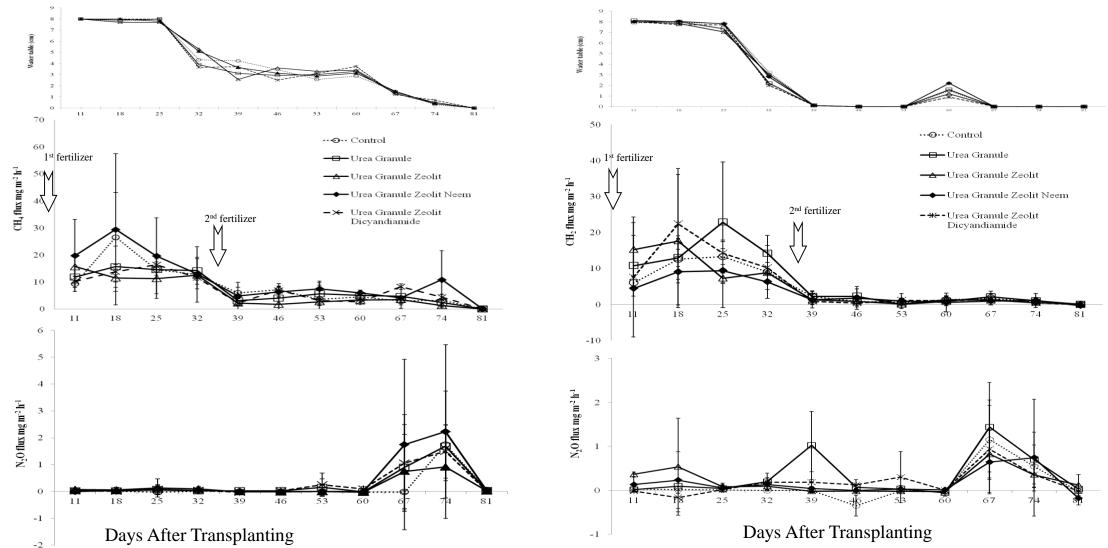


Figure. A. Change of water table height, N₂O and CH₄ fluxes in continuous flooded rice field; B. Change of water table height, N₂O and CH₄ fluxes in non continuous flooded rice field, during rice cropping seasons March 13rd, 2015 to August 5th, 2015). Vertical bars indicate <u>+</u> standard deviations.

Table. Total and reduction of N₂O emission (kg h⁻¹ season⁻¹)

Treatment	Continuous Flooded	Non Flooded	Reduction	%
Control	2.9 ^a	2.0 ^a	0.9	<mark>31.0</mark>
Urea Granule	7.2 ^a	5.9 ^a	1.3	<mark>18.0</mark>
Urea Granule Zeolit	3.4 ^a	3.3 ^a	0.1	2.9
Urea Zeolite Neem	7.0 ^a	3.0 ^a	4.0	57.0
Urea Zeolite Dyciandiamide	4.7 ^a	3.2 ^a	1.5	<mark>31.9</mark>

Description: Means followed by the same letter are not significantly different at 5% Tukey HSD.

Table. Total and reduction of CH₄ emission (kg h⁻¹ season⁻¹)

Treatment	Total Emission Continuous Flooded	Total Emission Non Flooded	Reduction	%
Control	141.7 ^a	74.1 ^a	67.6	47.7
Urea Granule	137.7 ^a	102.8 ^a	34.9	25.3
Urea Granule Zeolit	229.6 ^a	92.3ª	137.3	59.7
Urea Zeolite Neem	245.2 ^a	60.4 ^a	184.8	75.3
Urea Zeolite Dicyandiamide	147.7 ^a	101.1ª	46.6	31.0

Description: Means followed by the same letter are not significantly different at 5% Tukey HSD.

Table. The average of rice grain yield

Trootmonto	Weight kg	J/8m ⁻²
Treatments	Continuous Flooded	Non Flooded
С	4.30 ^a	3.72 ^a
UG	5.99 ^b	5.06 ^b
UZN	5.90 ^b	4.85 ^b
UZD	6.03 ^b	4.70 ^b
UZ	6.11 ^b	5.36 ^b

Description: Means followed by the same letter are not significantly different at 5% Tukey HSD.

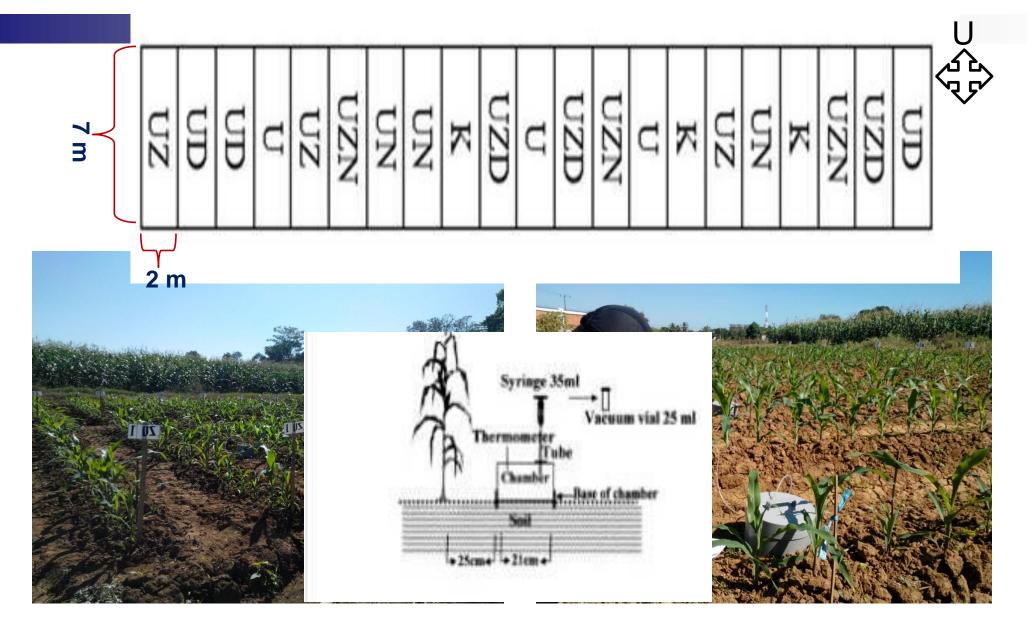


Fig. Gas sampling activities and arrangement of experimental plots and position of the chamber in each plot. K= Control, U= Urea, UZ= Urea Zeolite, UD+Urea+DCD, UZN = Urea+Zeolite+Neem, UZD = Urea+Zeolite+DCD, UN=Urea+Neem







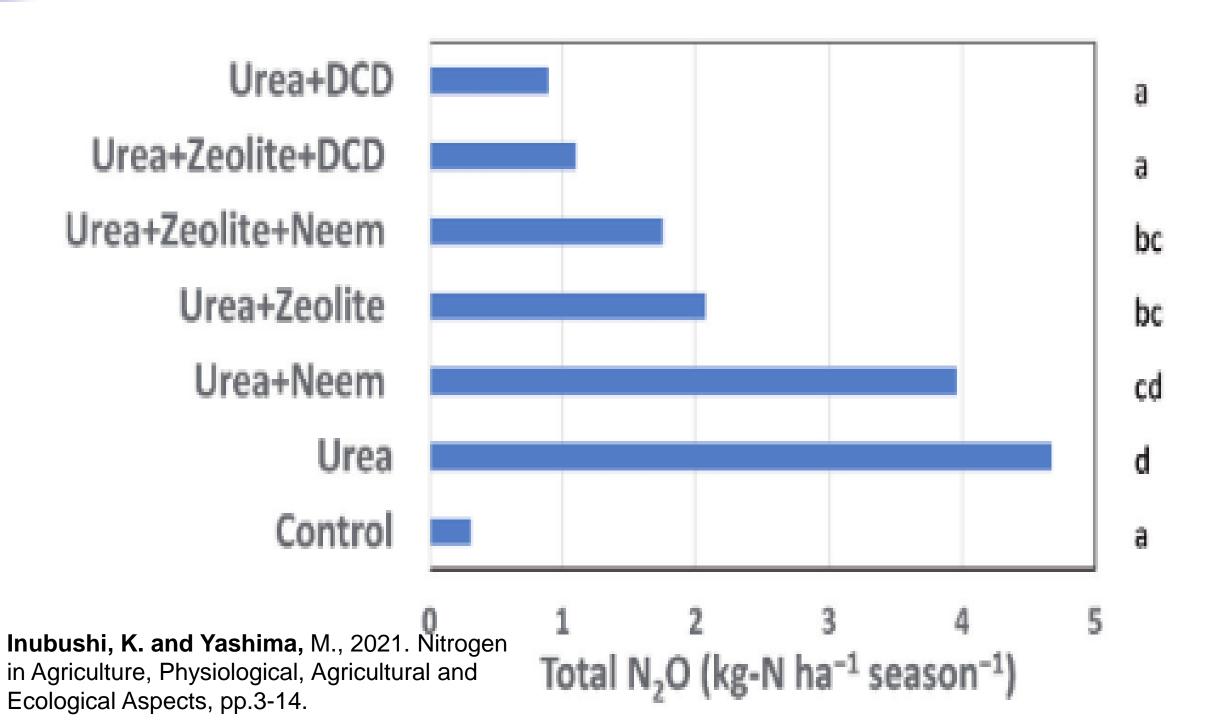




Table. Total gas, Emission Factor (EF) and N₂O Reduction in a season corn plantation

Treatment	Total gas (kg N ₂ O-N ha ⁻¹ season ⁻¹)	EF %	Reduction %
С	0,31 ^a		
U	4,6 ^d	<u>2,1</u>	
UZ	2,0 ^{bc}	0,8	59,6
UN	3,9 ^{cd}	1,8	16,3
UD	<u>0,8</u> ª	0,2	<u>86,7</u>
UZN	<u>1,7</u> ^a	0,7	66,8
UZD	<u>1,1</u> ^a	0,3	81,9

Description: Means followed by the same letter are not significantly different at 5% LSD.

Table. Dry weight of five corn's cobs and chlorophyll content after harvest.

Treatments	Five Weight of Cobs (gram)	Chlorophyll (%)
С	754 ^a	38.2 ^a
U	1421 ^c	52.5 ^{bc}
UZ	1271 ^b	53.5 ^c
UZN	1303 ^{bc}	47.1 ^{bc}
UZD	1394 ^{bc}	50.1 ^{bc}
UD	1407c	49.2 ^{bc}
UN	1374 ^{bc}	45.7 ^b

Description: Means followed by the same letter are not significantly different at 5% Tukey.





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Laboratory test dan Field experiments

- 1. Plant responds
 - a. Vegetative b. Generative
- 2. Change of NH_4^+ dan NO_3^-
- 3. Emission of N_2O , CH_4 , dan O_2
- 4. Soil Microbial -> MPN and

Soil Bacteria Metagenomic

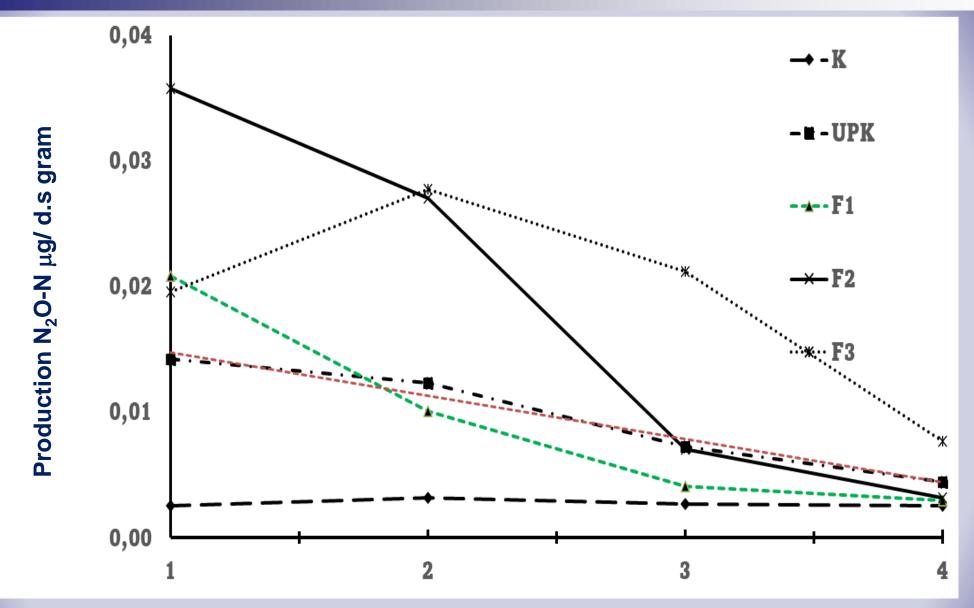












Incubation time (week) Fig.: Nitrous oxide production affected by additional frass

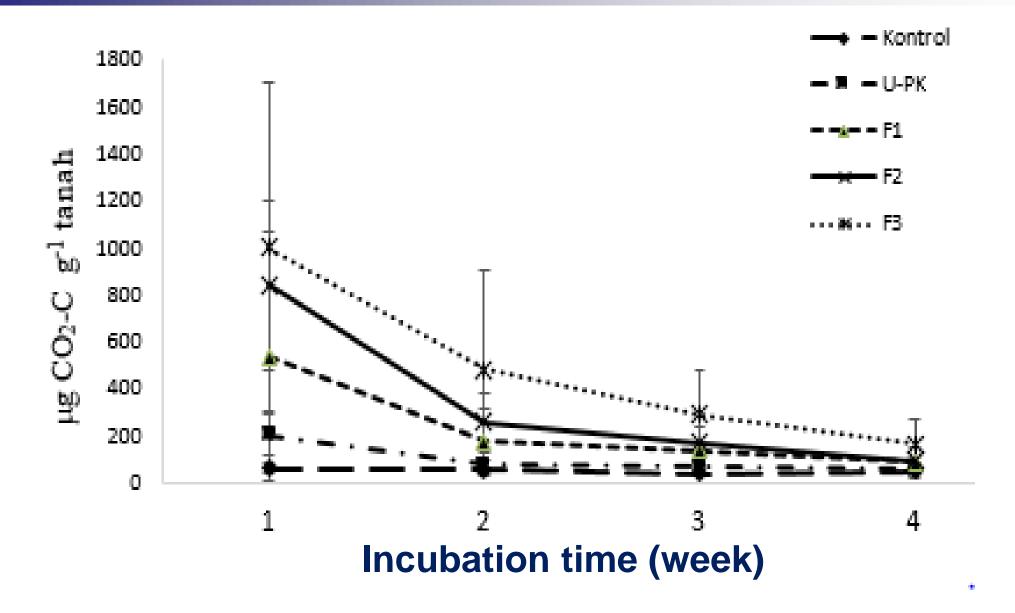
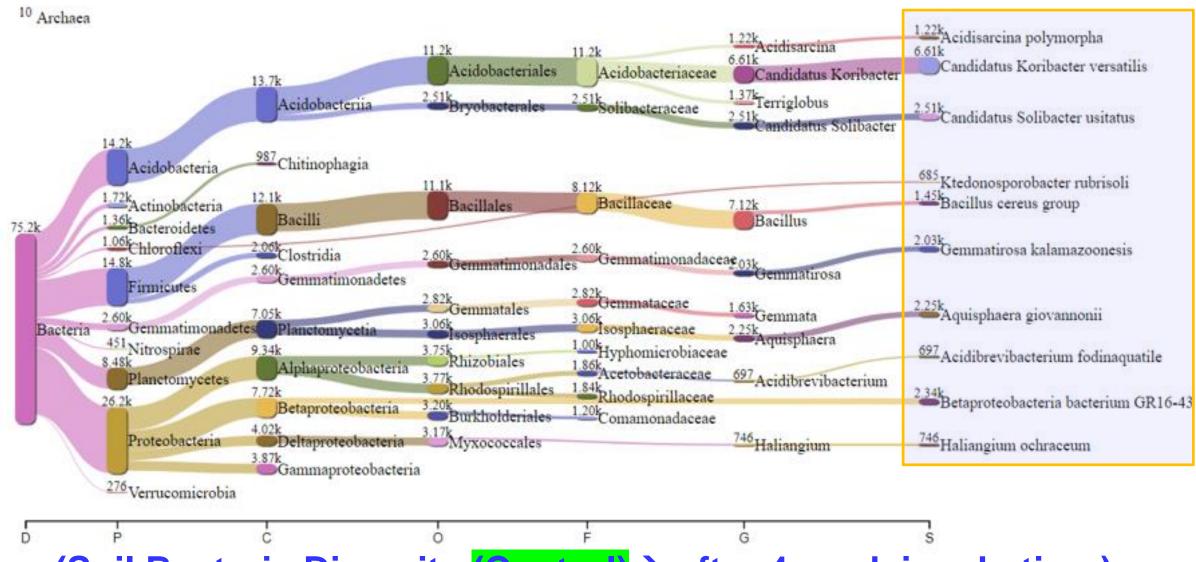


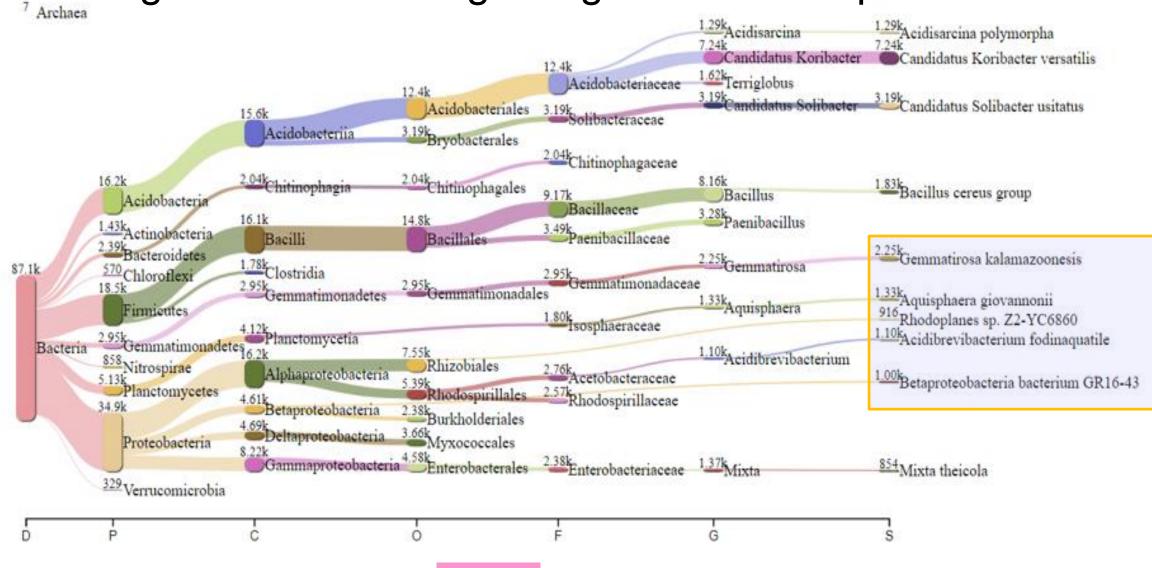
Fig.: Carbon dioxide production affected by additional frass

Full Length 16S Barcoding using Oxford Nanopore Platform



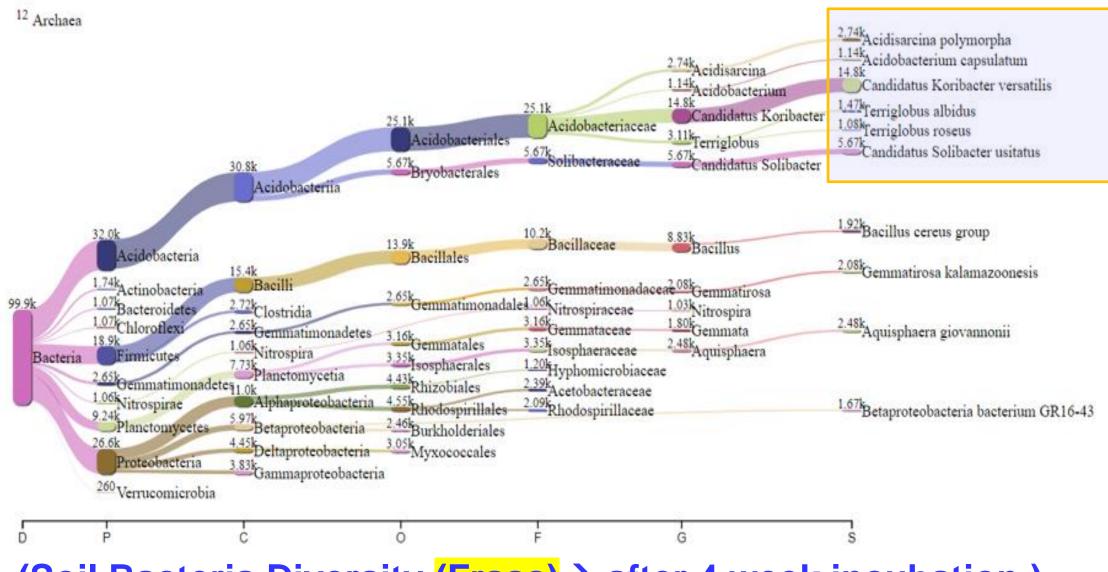
(Soil Bacteria Diversity (Control) \rightarrow after 4 week incubation)

Full Length 16S Barcoding using Oxford Nanopore Platform



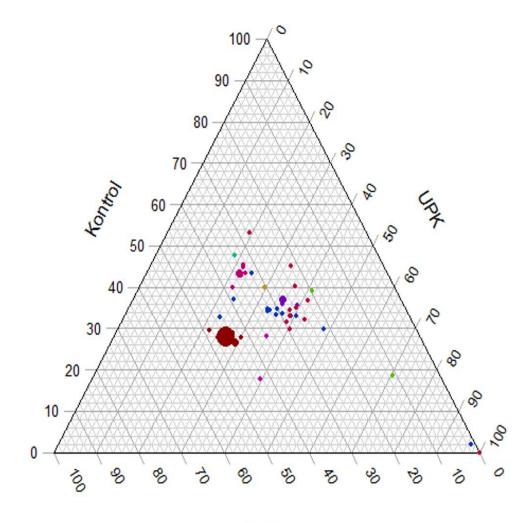
(Soil Bacteria Diversity (UPK) → after 4 week incubation)

Full Length 16S Barcoding using Oxford Nanopore Platform



(Soil Bacteria Diversity (Frass) → after 4 week incubation)

Metagenomic Soil Microbe affected by frass Ternary Plot

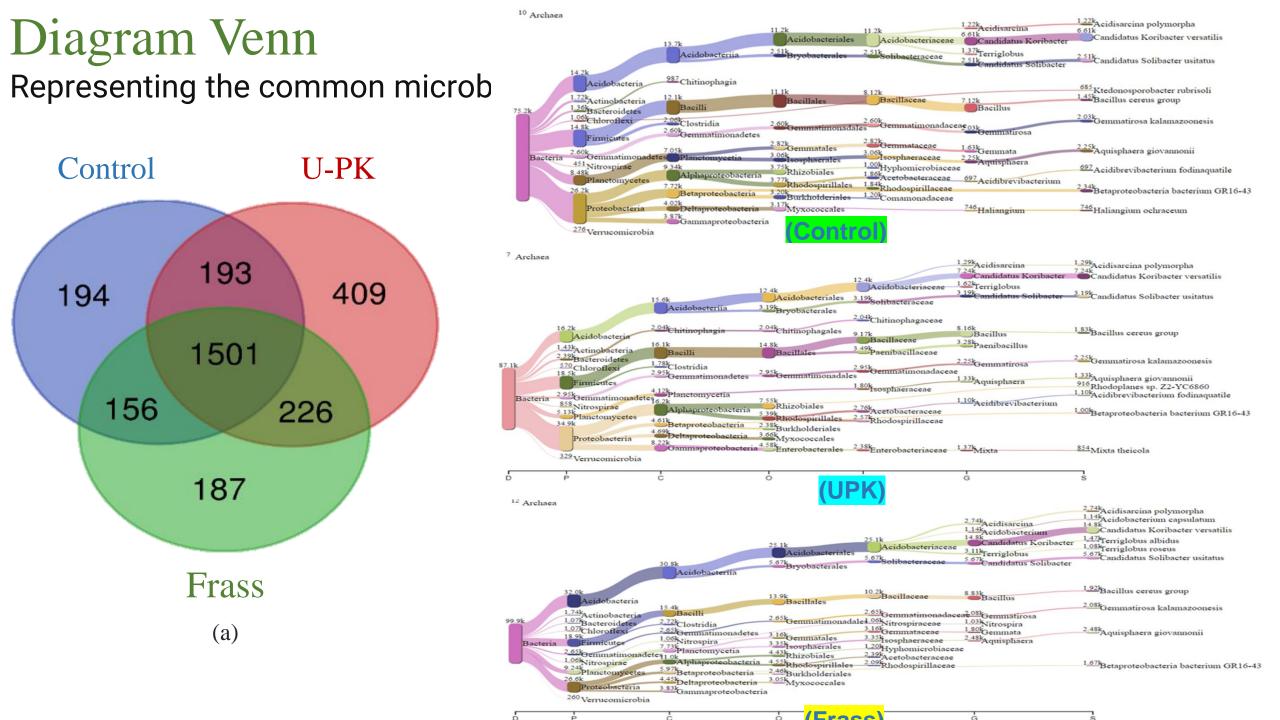


Phylum Acidobacteria Actinobacteria Bacteroidetes Chloroflexi Firmicutes Gemmatimonadetes Nitrospirae Planctomycetes Proteobacteria

The ternary plot depicts the ratio of the three variables in an equilateral triangle position. The axis reflects the percentage of isolates detected at each location.

Visually, Phylum Acidobacteria with the highest quantity in the Frass sample reached 70%, UPK 50%, and control 30%.

Frass





Development of sustainable liquid *biostimulant extracted* from Indonesia seaweeds to improve soil quality, production food and the crop yield in the corn field

The aims of this work are to develop a marine sustainable liquid biostimulants extracted from Indonesian seaweed by enzymeassisted extraction and fermentation methods with selecting three species (*Kappaphycus alvarezii, kappaphycus stratum* and *Sargassum polycystum*). The soluble extracts were characterized by their respective biochemical composition, antiradical activities, pigments and sugar fractions and as biostimulan.











The Biochemical composition analyses were conducted

Protein by BCA – KIT MICRO BC ASSAY (Smith et al., (1985); Wiechelman et al.(1988)) Neutral Sucrose by DUBOIS (Dubois et al. 1956) Group Sulfates by AZURE A (Jacques et al. (1968); Pierre (2010)) Polyphenols by FOLIN CIOCALTEAU (Chandler et al. (1983); Heo et al. (2004); Wang et al. (2010)) Uronic acid by META-HYDROXY-DIPHENYL (MHDP) (Blumenkrantz et al 1973); Montreuilet al. (1963)

Antiradical activities of extracts were measured with the 2,2- diphenyl-1-picrylhydrazyl (DPPH) method.

Pigment analysis Chlorophyll a, Chlorophyll b and ß-carotene performed by high performance liquid chromatography (HPLC) (Wright and Jeffrey (1997) and Pinto et al. 2002).

The composition of unitary sugars present in the polysaccharide chains 'was' determined by High Pressure Anion Exchange Chromatography (Dionex).

Seed Coating Using Brown Algae (Sargassum sp.) Extract Fermented with Bacteria from Digestive Organs of Abalone (Haliotis sp.) on Germination of Corn (Zea mays) and Tomato

Table 1. Biochemical co	mposition of raw material (%).
-------------------------	------------------------------	----

	Neutral Sugars	Uronic Acids	Sulfate	Total Phenol	Proteins
K. alvarezii	27.62±0.20	4.67±0.04	3.42±0.95	0.74±0.00	11.59±1.16
K. striatum	27.38±0.15	4.06±0.07	3.89±1.00	0.71±0.00	12.70±1.26
Sargassum 125 µm	3.62±0.13	2.24±0.59	4.75±0.97	0.32±0.00	11.66±0.50
Sargassum 250 µm	5.01+0.99	1.06±0.04	4.92±0.46	0.27±0.00	10.08±1.05
Sargassum 500 µm		2.72±0.91	4.55±0.41	0.05±0.00	8.32±1.43

Biochemical composition of raw material and enzymatic extracts (% dw and g).

		Ash	Organic matter	Neutral sugars	Uronic acids	Sulfate	Proteins	Total phenols
Raw algae	% dw	15.9 ± 1.2	84.1 ± 1.2	23.2 ± 1.4	21.1 ± 0.1	20.1 ± 0.9	24.4 ± 0.1	0.6 ± 0.1

Table 3-1 Biochemical composition of Indonesian Sargassum species.

Algeo		Content (% dry algal material)											
Algae	Uronic acid	Protein	Sugar	Sulfates groups	Polynhenol		Nitrogen	Total	Other				
S. aquifolium	4.5 ± 2.0	20.9 ± 1.1	8.4 ± 0.1	10.7 ± 0.6	1.7 ± 0.3	32.5 ± 0.3	1.6 ± 0.1	69.2	30.8				
S. ilicifolium	7.8 ± 3.3	30.5 ± 1.2	10.8 ± 0.2	11.4 ± 0.5	2.1 ± 0.4	29.0 ± 2.6	1.6 ± 0.1	77.1	22.9				
S. polycystum	6.1 ± 2.6	19.6 ± 0.6	10.3 ± 0.1	13.7 ± 0.7	1.7 ± 0.3	$\textbf{33.6} \pm \textbf{1.9}$	1.0 ± 0.2	72.0	28.0				

Hardouin et al., 2016

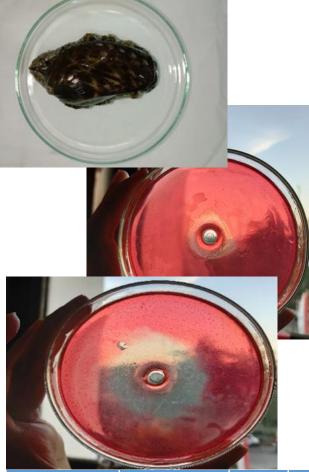
Table 2. Antioxidant Activities of raw material

C1

			IC50 (μg m						
BHA*			0.00192:	±0.00					
BHT*			0.00245:	±0.00	Table 3-18 Antira and enzyme-assist		g activity of Indo	onesian Sargassur	n in solid-liquid
K. alvai	rozii		0.975:	+0 00	Samples	Methods	DPPH IC ₅₀ (mg/mL)	Sig. (p<0.05) of Species	Sig. (p<0.05) of Solvents
n. aivai	6211		0.975.	10.03	BHA BHT		7.5±0.4 11.7±4.5		
K. striat	tum		0.685:	±0.23		Water MeOH	8.9 ± 2.1 n.a		
S. polyc			0.649:	±0.24	S. aquifolium	MeOH 50% EtOH 75% Viscozyme Protamex	3.2 ± 1.7 n.a n.a 1.4 ± 0.7		
	ted Hydroxy Anisole. ted HydroxyToluene.	Crude extracts		_	S. ilicifolium	Water MeOH MeOH 50% EtOH 75% Viscozyme	n.a 3.4 ± 0.8 9.0 ± 0.7 9.9 ± 1.8 9.9 ± 6.1	.014 (df = 2 ; F= 4.802)	.008 (df = 5; F = 3.691)
	Cytotoxicity	Antiviral	Antioxidant			Protamex	9.9 ± 0.1 4.4 ± 2.4		
	CC ₅₀ (µg/ml)	EC ₅₀ (µg/ml)	IC ₅₀ (μg/ml)			Water MeOH	n.a		
Acyclovir	> 500.0	0.3 ± 0.1	-		S. polycystum	MeOH 50%	n.a 20.3 ± 2.5		
BHA* BHT**	-	-	0.0048 0.0069			EtOH 75% Viscozyme	5.5 ± 0.3 n.a		
Blk	> 500.0	> 500.0	> 25.0			Protamex	9.5 ± 2.4		

Hardouin et al., 2016

Puspita, M. 2017



Isolation of Abalone Digestive Organ Bacteria (Gomare et al., 2011). The hydrolysis ability of polysaccharides was carried out using the CMC test medium and evaluated for each isolate using the plate test according to the procedure described by Teather and Wood (1982).

Molecular and Phylogenetic Identification

DNA extraction was carried out using the ZR Bacterial DNA Kit[™]. PCR amplification was performed using MyTaq Red Mix (Bioline). The primers used were Sequence 27F AGAGTTTGATCMTGGCTCAG and Sequence 1492R TACGGYTACCTTGTTACGACTT. The phylogeny tree schematic was created using the Molecular Evolutionary Genetics Analysis (MEGA-X) application (Kumar et al., 2018). The nucleotide sequences in this study have been deposited in the NCBI under Accession number OP218021 and OP218022.

Seed Extraction, Coating and Germination (Suo et al., 2017).

Isolate Code/ Access Number	CMC hydrolysis zone diameter (cm)	Indo Test	MR Uji test	VP test	Citrate Test	OF test	Test Catalase	Gram stain results	Cell shape	Colony form (form)	Elevation (elevation)	Shape of the edge of the colony (margin)	Colony form (form)
OP218021	2.56	-	+	-	-	-	+	-	Cocci	circular	Raised	Entire	circular
OP218022	4.50	-	+	-	+	-	+	+	Basil	circular	Flat	Entire	circular



0.10





Figure. 1 Phylogenetic tree based on 16s rRNA sequences of selected isolates and sequences from Genbank NCBI with 1000 bootstrap Neighbor Joining method using MEGA-X The treatment was Sargassum sp. using several types of starter microbes that have the ability to excrete polysaccharide-degrading enzymes namely a) Sargassum sp. fermented abalone consortia (pasteurized), b) Sargassum sp. fermented abalone consortia (non-pasteurized) c) Metalaxyl (an acylalanine fungicide with systemic function as a positive control), and 4) without treatment (distillated water as negative control).

Treatment	Average Corn	Corn	Average Corr
	Sprout Height (cm)	Germination	Seed Vigor Ind
		(%)	(%)
Pasteurized Abalone	8.26 ^{ab}	57.99 ^c	481.65 ^c
Algae			
Non Pasteurized	4.96 ^{ab}	41.67^{ab}	264.42^{b}
Abalone Algae			
Metalaxyl	1.94 ^a	11.11 ^a	46.30 ^a
Control	11.67 ^c	50.69 ^c	710.29 ^d







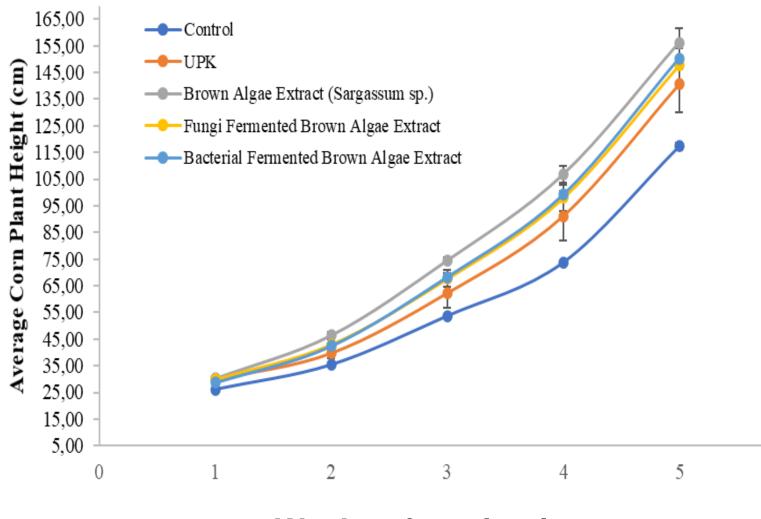






The growth of vegetative corn

Fig. The average height of corn



Weeks after planting

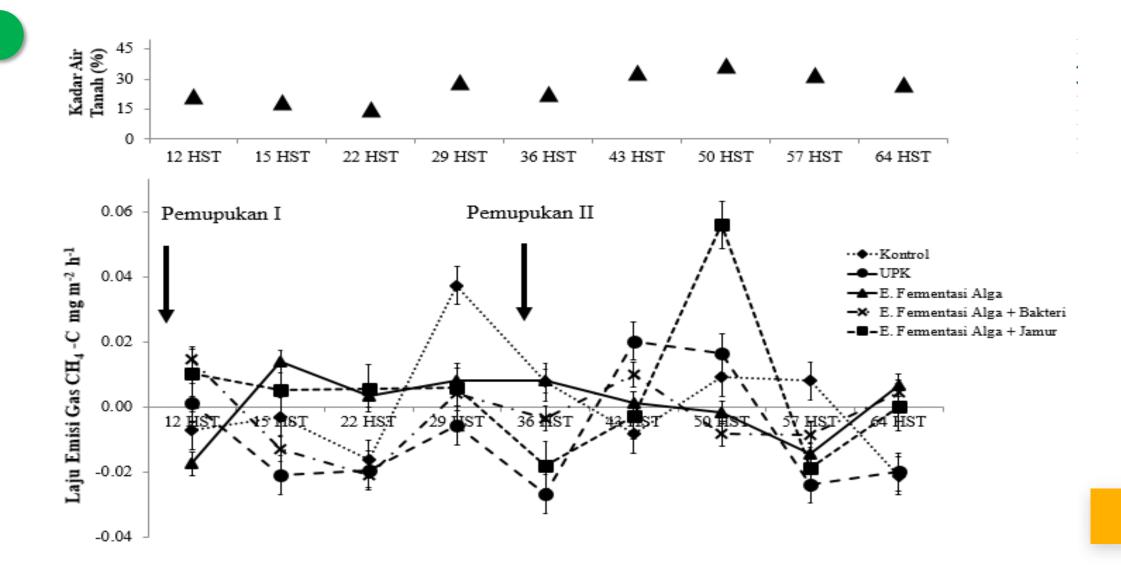
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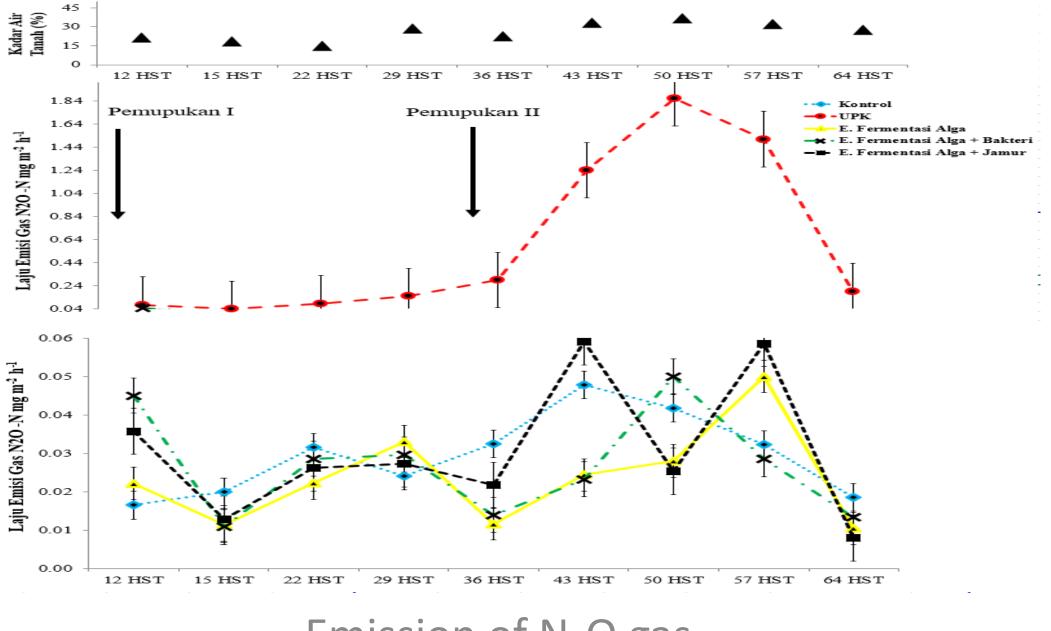
CORN CROP YIELDS

The harvest of corn crops with Five treatments is Control, UPK, Brown Algae Extract, Fungi Fermented Brown Algae Extract and Bacterial Fermented Brown Algae.



Emission of CH₄ gas





Emission of N₂O gas

Conclusion

Mitigation or efforts to reduce greenhouse gases (N_2O) can be done by nitrogen management such as application of organic nitrification inhibition (neem), organic fertilizer shifting, and *a combination of water management in paddy fields (CH₄).*

Fertilizer management can affect soil microbial communities that carry out the nitrogen and carbon cycling processes in soil.



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