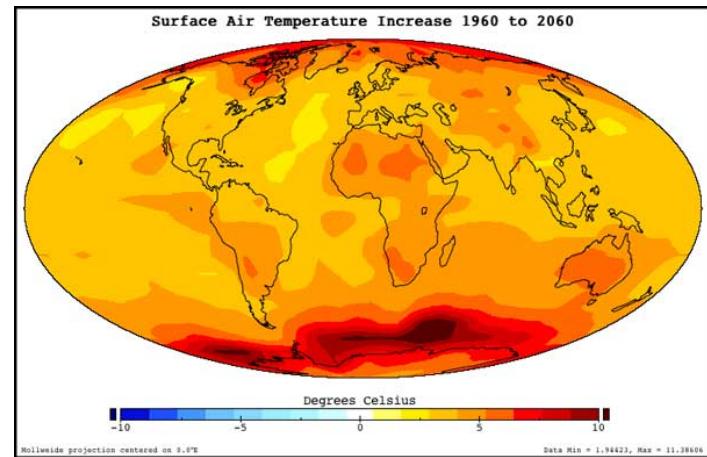
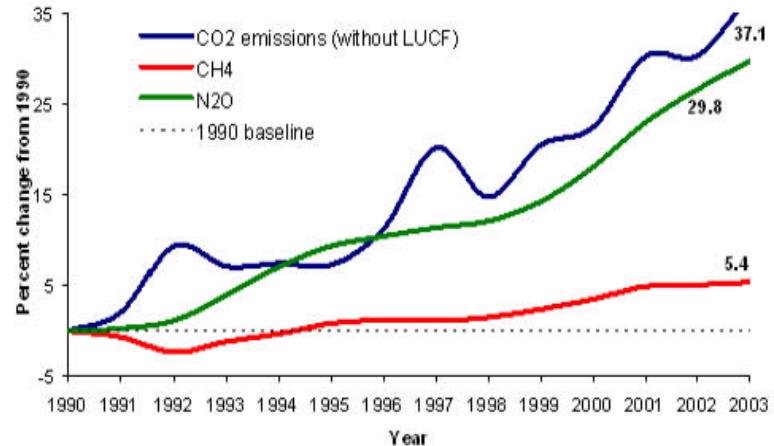
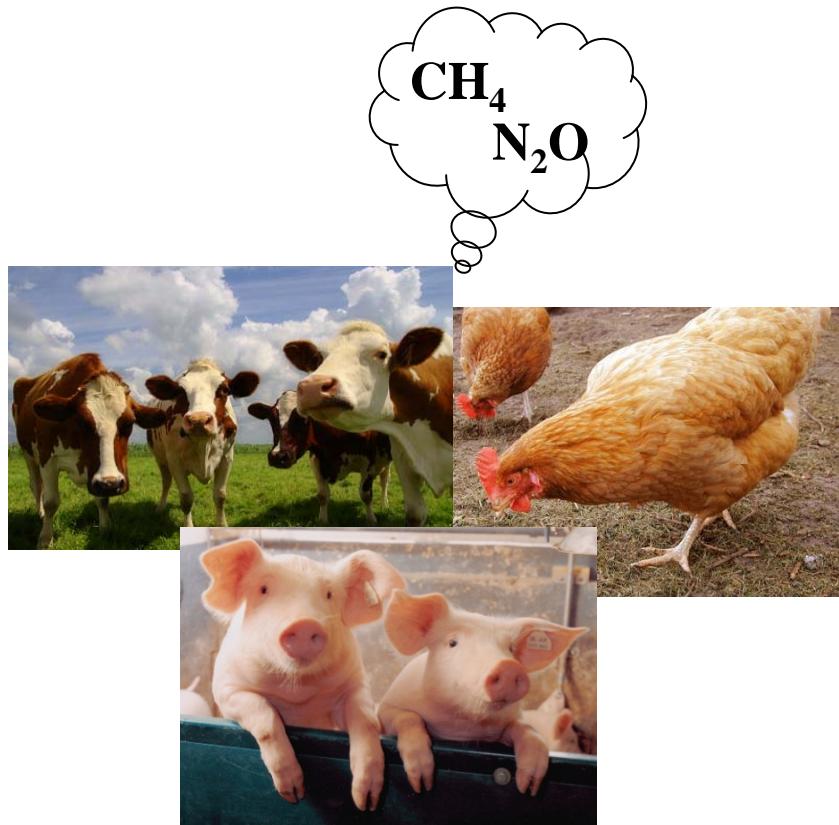




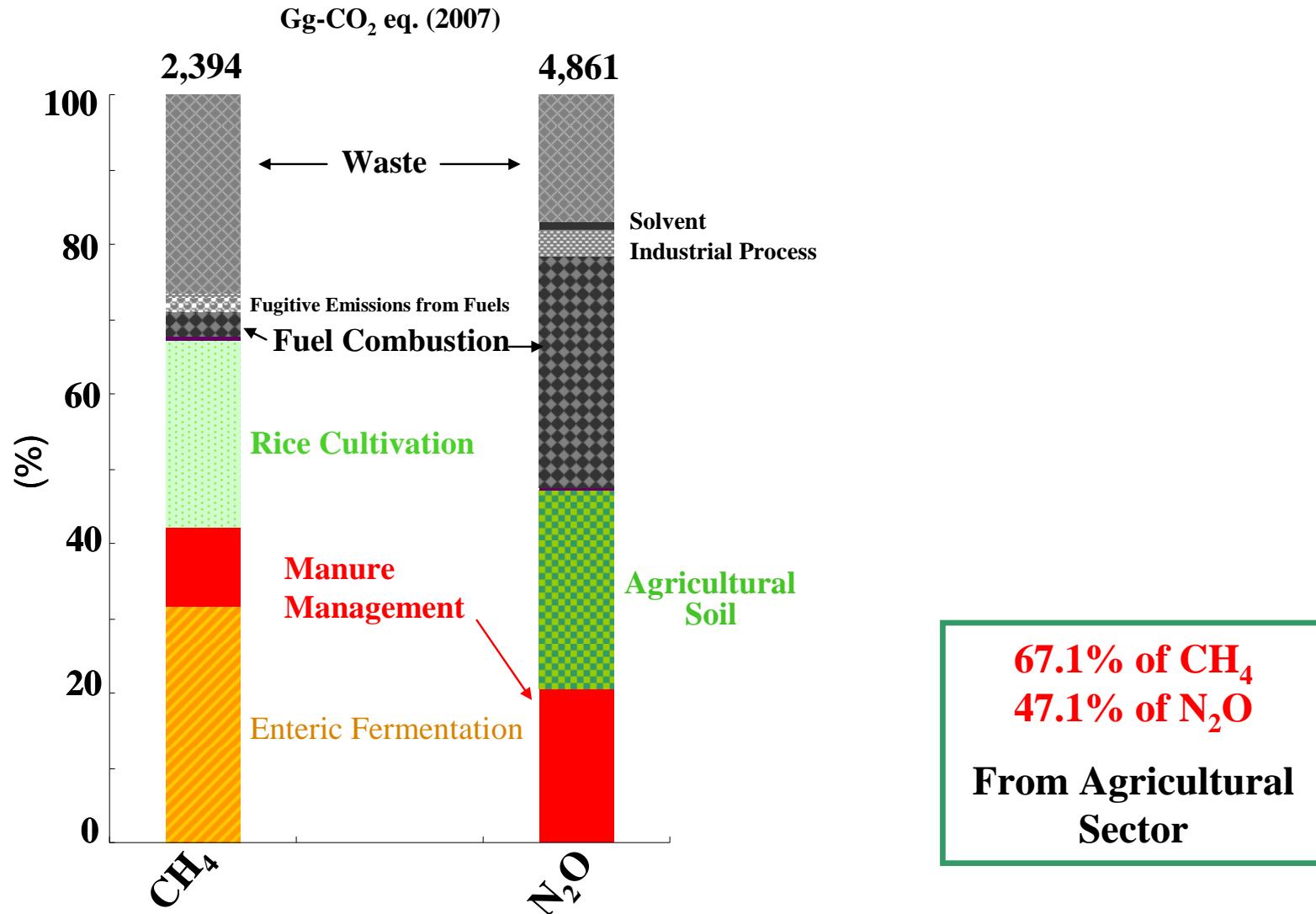
Mitigating strategy of GHG emission from dairy manure composting process



Koki Maeda



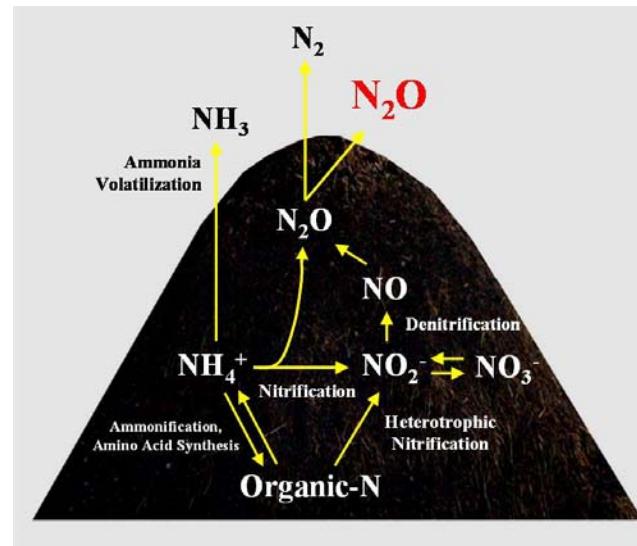
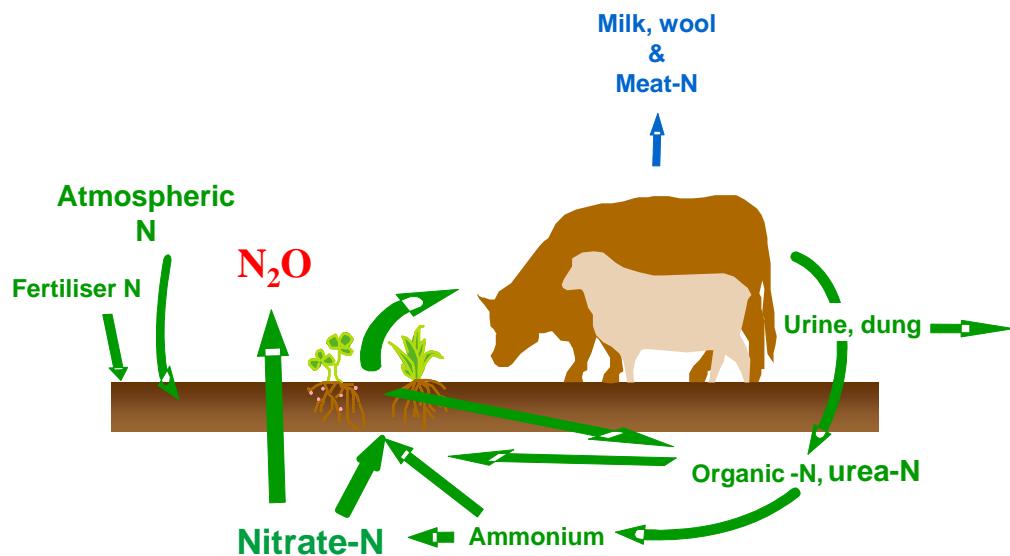
Greenhouse Gas Emission



Nitrogen Cycle Relating Livestock Production



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Complex microbial community
is responsible for N_2O emission



Stable Isotope Analytical Method

Molecular Biological Method

Classical Cultivation Method

For GHG Mitigation Strategy



GHG mitigation option using bulking agent

Measurement of Gas Emission from Composting Process



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Chamber



Moisture 80.8% 4 t

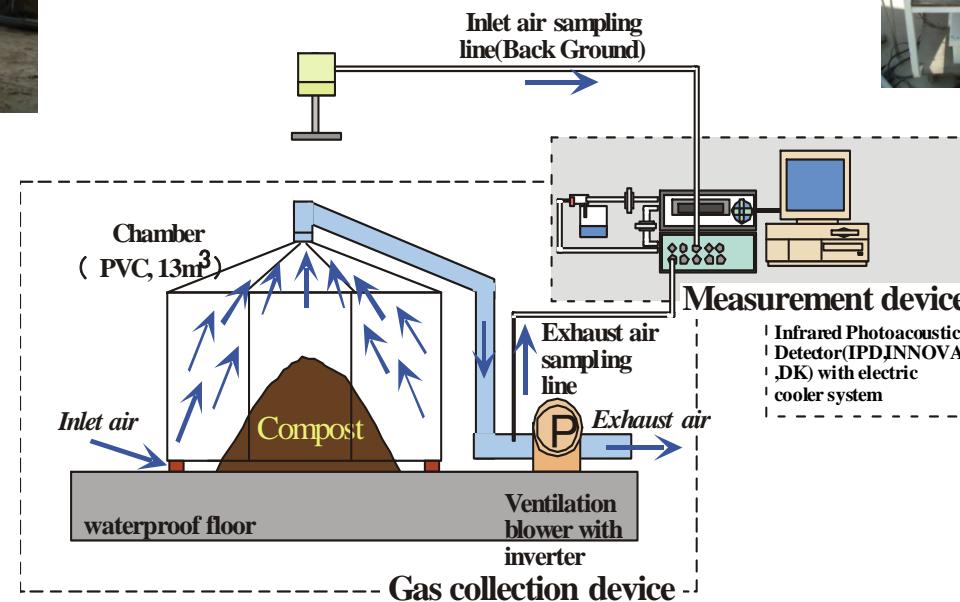
Moisture 73.7% 4 t + 400 kg
Bulking agent

Run 1 7/21-9/17, 2009

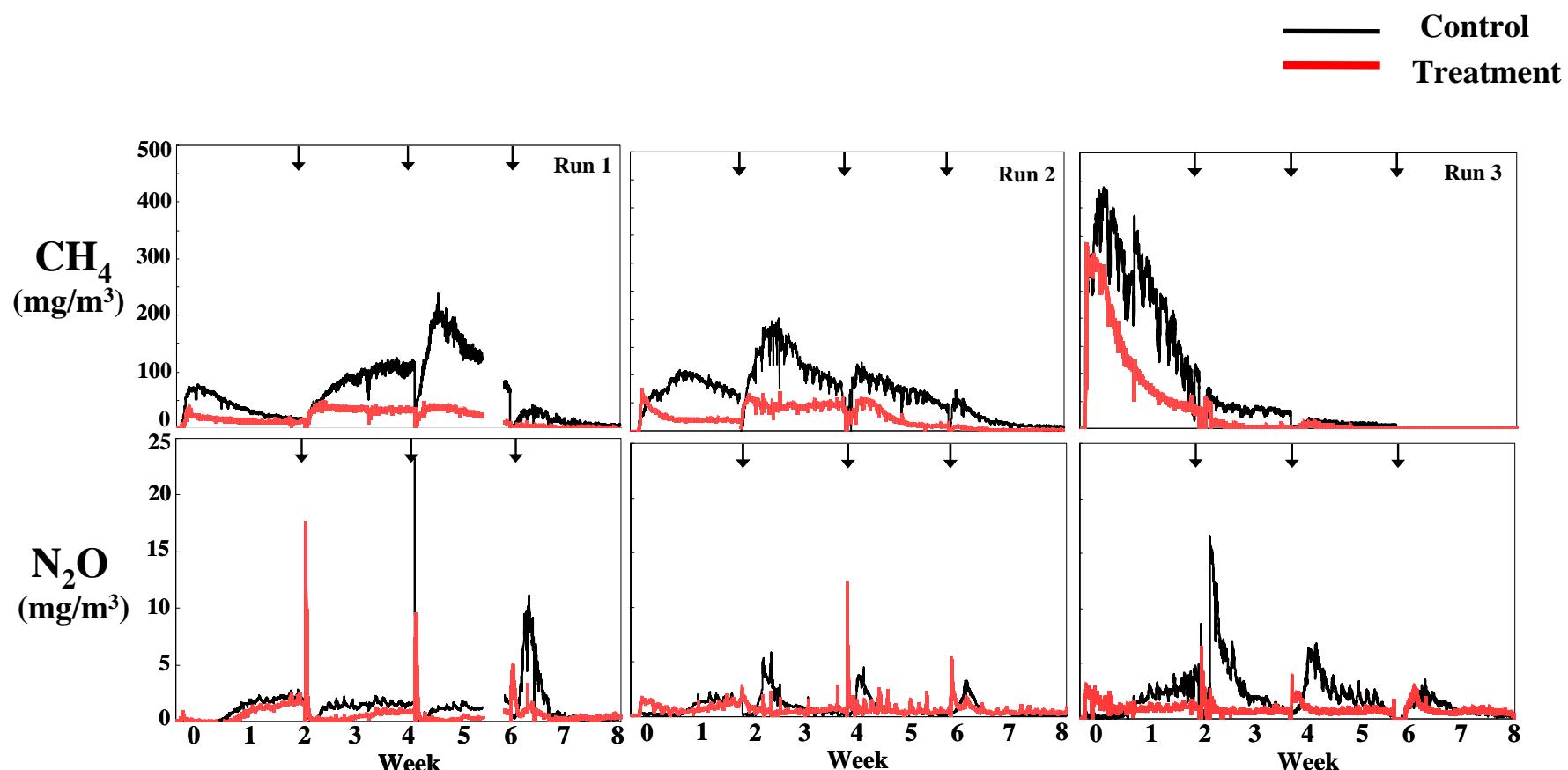
Run 2 5/27-7/21, 2010

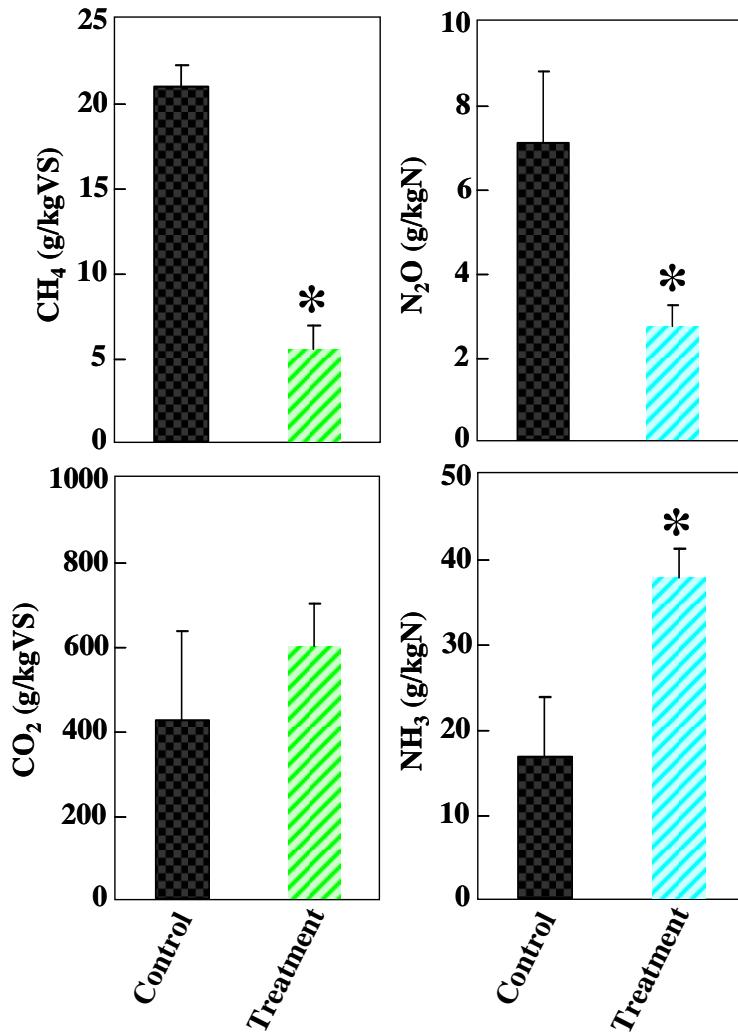
Run 3 9/15-11/10, 2010

Auto Measuring System



Gas Emission





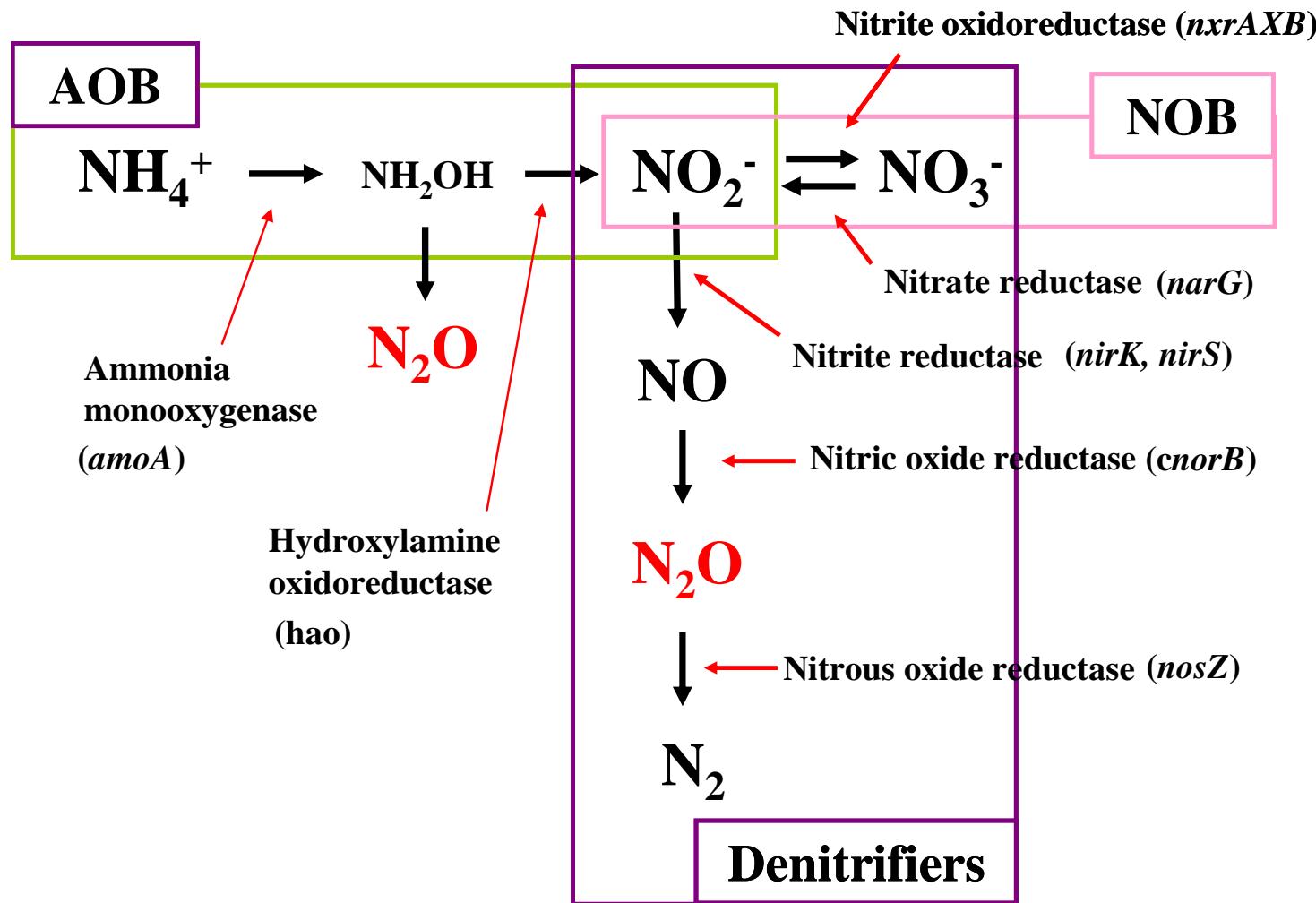
Mitigation

CH_4 74.3%
 N_2O 62.8%

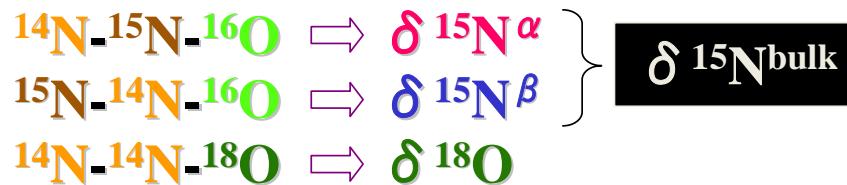
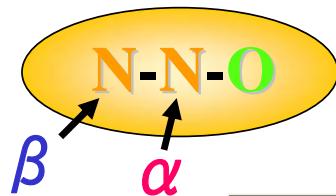
CO_2 -42.2%
 NH_3 -126%



**Source of N_2O ;
Nitrification or Denitrification?**



< N_2O isotopomer>



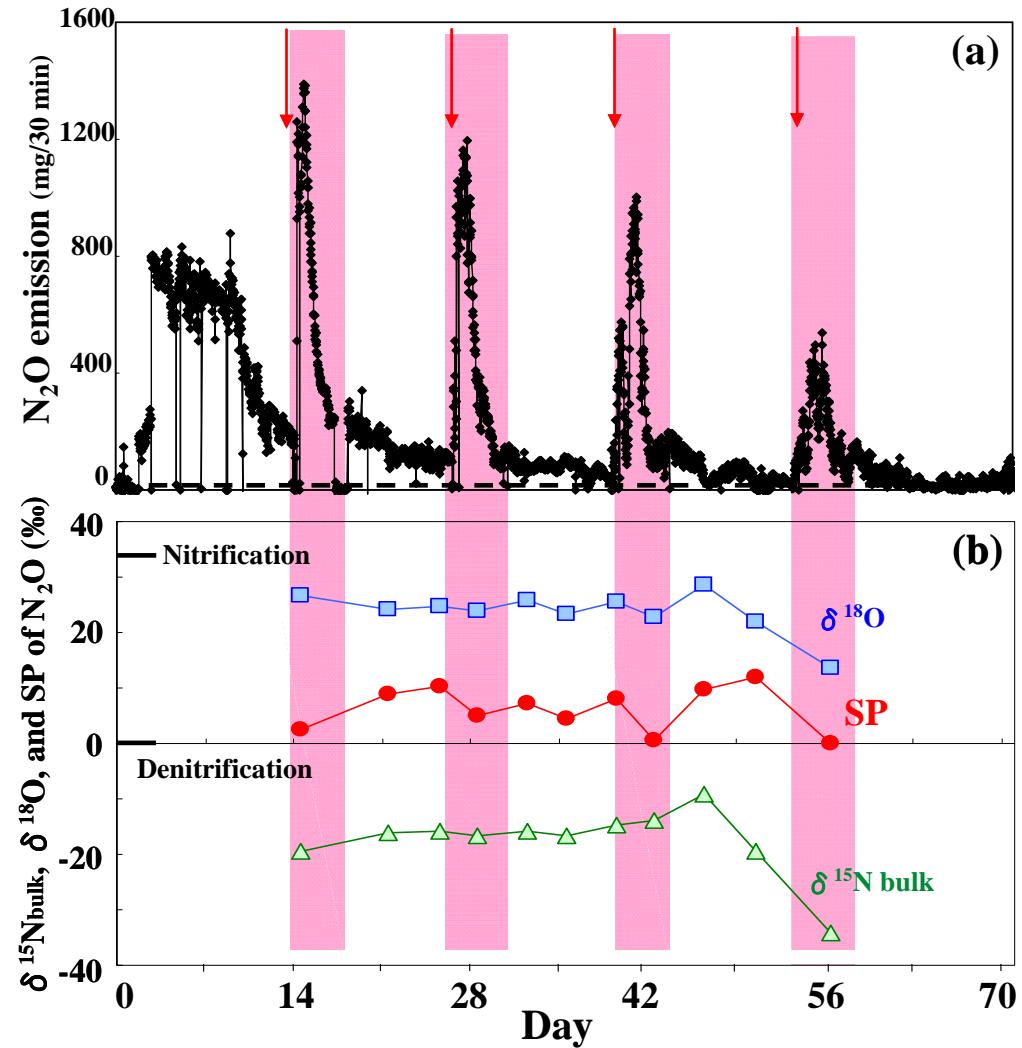
$$\text{Site preference (SP)} = \delta {}^{15}\text{N}^\alpha - \delta {}^{15}\text{N}^\beta$$

	$\delta {}^{15}\text{N-N}_2\text{O} (\text{\textperthousand})$	SP	
<i>Nitrosomonas europaea</i>	-0.3 (4.9)	33.5 (1.2)	
<i>Nitrosospira multiformis</i>	-0.3 (2.9)	32.5 (0.6)	Nitrification
<i>Methylosinus trichosporium</i>	3.4 (1.9)	35.6 (1.4)	
<i>Nitrosospira multiformis</i>	-22.9 (0.6)	0.1 (1.7)	
<i>Pseudomonas chlororaphis</i>	12.7	-0.6 (1.9)	Denitrification
<i>Pseudomonas aureofaciens</i>	36.7	-0.5 (1.9)	

N₂O Emission and Isotopomer Analysis



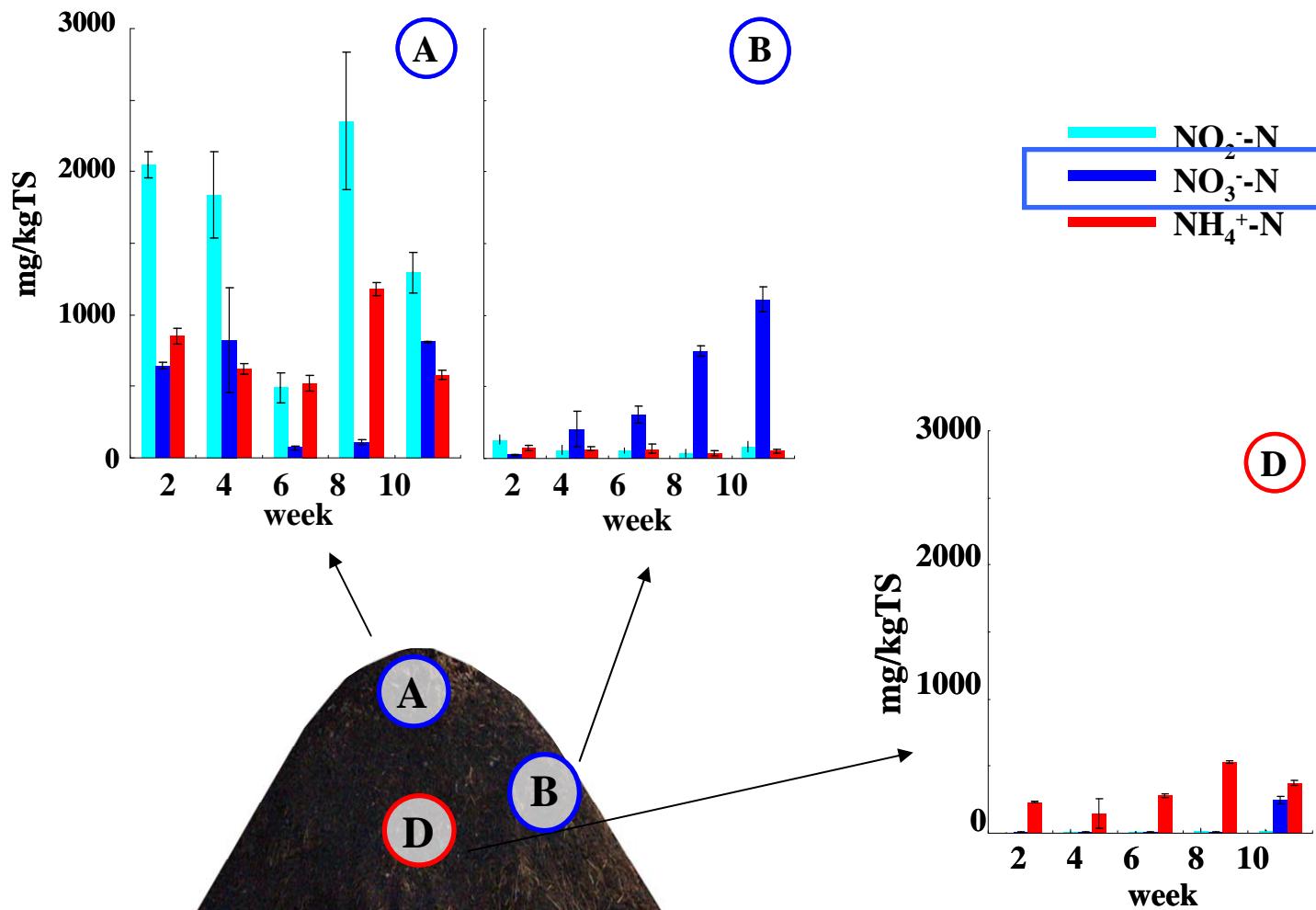
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Accumulation of NO_2^- -N and NO_3^- -N

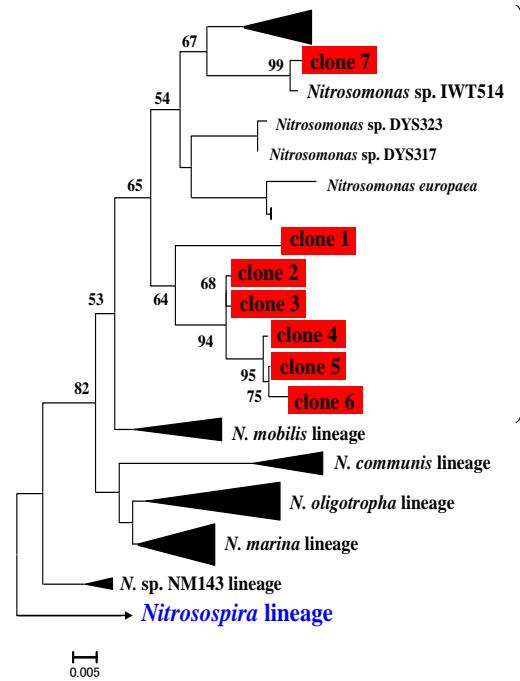


農研機構

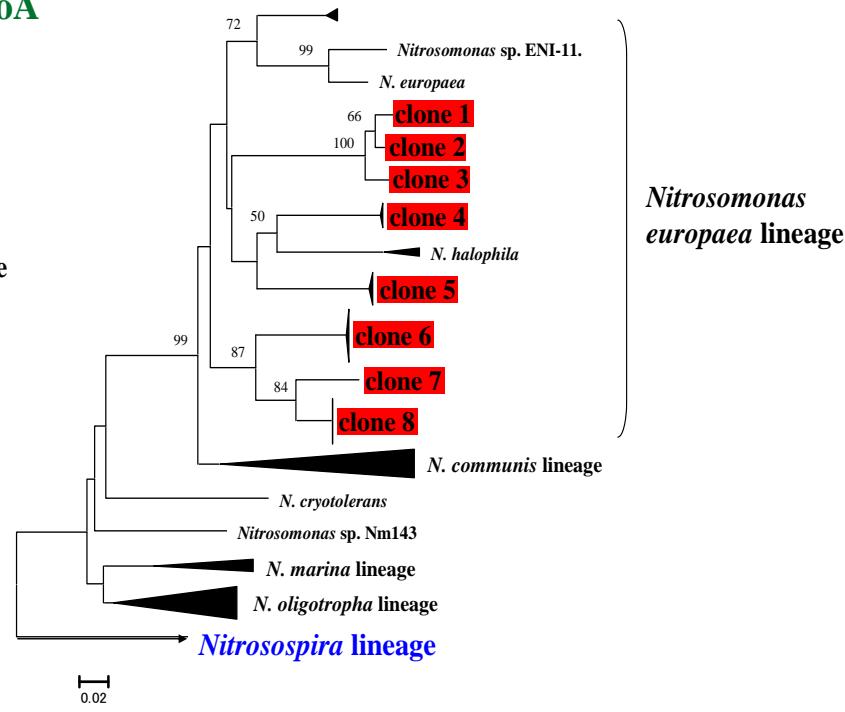


Phylogenetic analysis of 16S rRNA and *amoA* gene

16S rRNA (β -proteobacteria AOB)



amoA



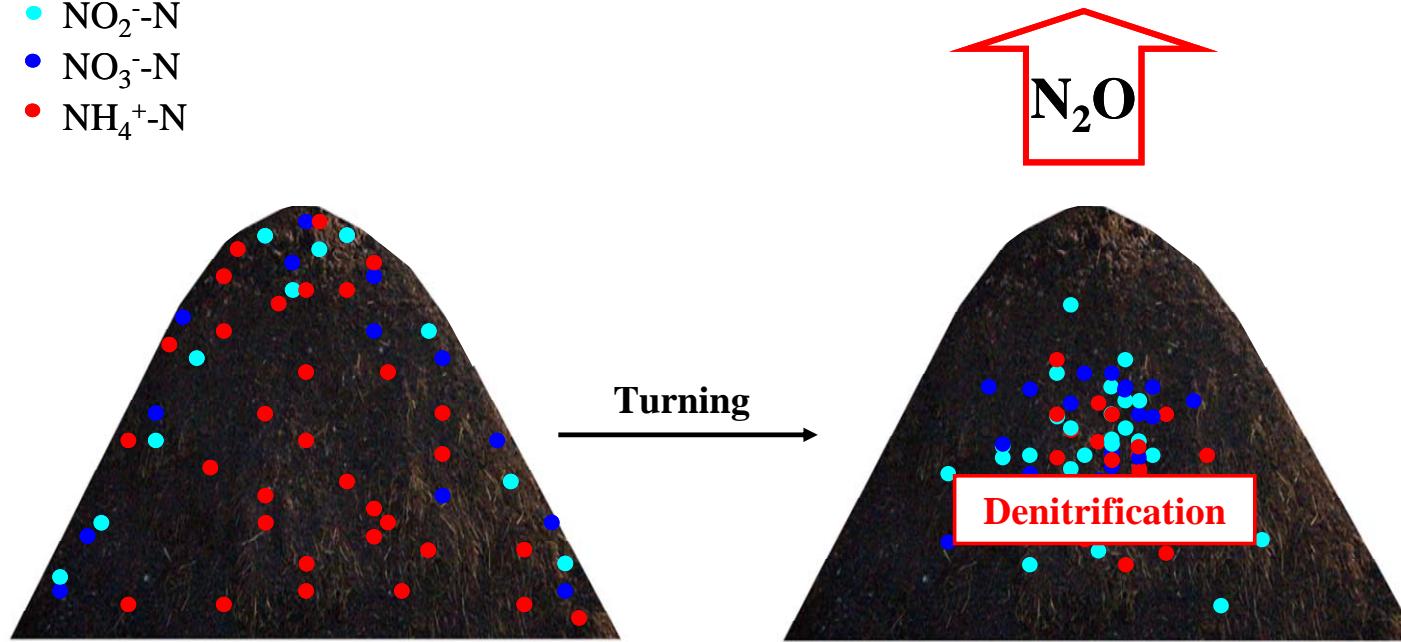
All sequences of both *amoA* and 16S rRNA specific for β -proteobacteria obtained in this study belong to the *Nitrosomonas europaea* cluster. These results suggest that the ammonia oxidizers working in the composting pile are not diverse, but are instead a closely related group contributing to the ammonia oxidation.

The model for N₂O emission



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- NO₂⁻-N
- NO₃⁻-N
- NH₄⁺-N



The reduction of accumulated NO₂⁻-N and NO₃⁻-N (denitrification) occurred just after the turnings.





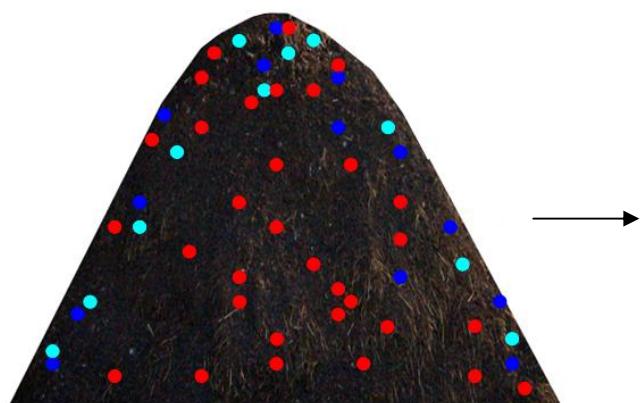
- NO_2 , $\text{NO}_3\text{-N}$ accumulation in the surface samples is responsible for N_2O emission?
- Does NO_2 amendment complement the N_2O emission?

Denitrification Potential Measurement



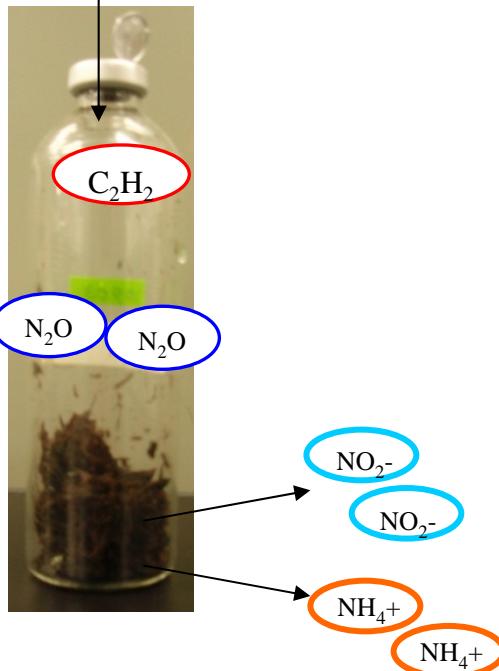
農研機構

- NO_2^- -N
- NO_3^- -N
- NH_4^+ -N



10% C_2H_2

($\text{N}_2\text{O} \rightarrow \text{N}_2$ inhibition)



NH_4^+

NO_2^-

NO_2^- reduction

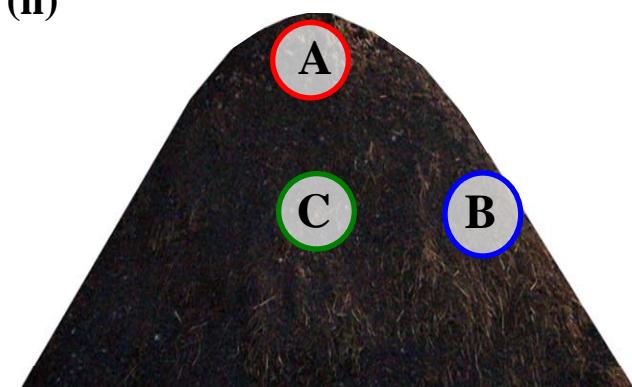
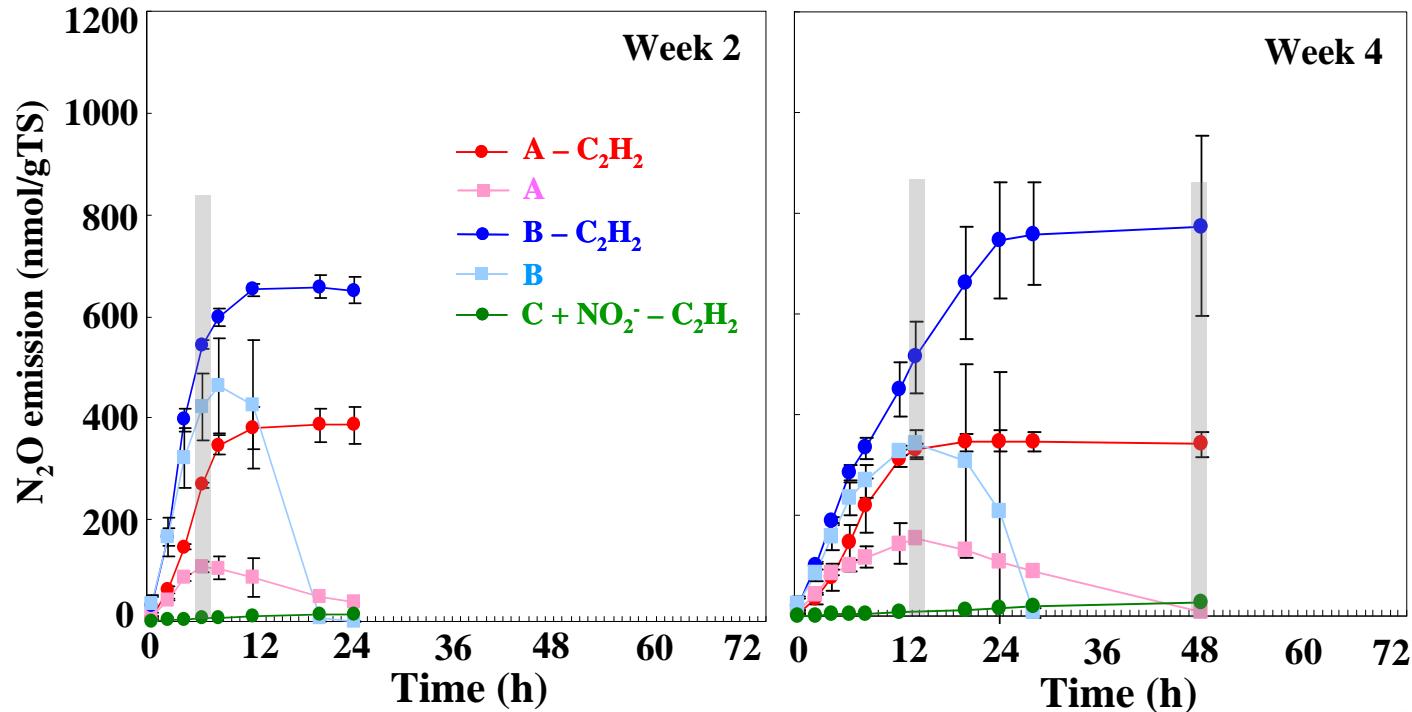
N_2O production

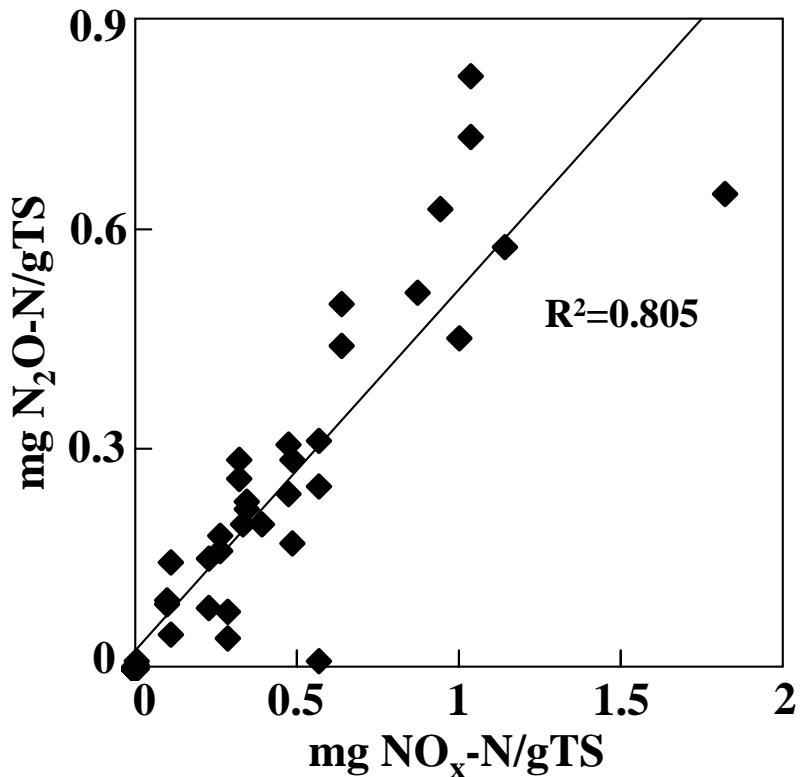
N_2O reduction

Denitrification Potential Measurement



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- N_2O production significantly correlated with NOx- accumulation.

- Surface samples emitted significant N_2O under aerobic condition.
 - N_2O emission correlates NO_x^- accumulation
-
- NO_2^- amended core samples did not produce significant N_2O especially in the initial stage of the process.



?

?

Denitrifiers in the surface zones might be mainly responsible for N_2O production.

?

?

?

?

Denitrifier in the surface zones are responsible for nitrite reduction and subsequent N₂O emission just after the turnings

?

?

① surface samples

N₂O with SP value 0-10 occurs immediately

② Core samples

No N₂O production

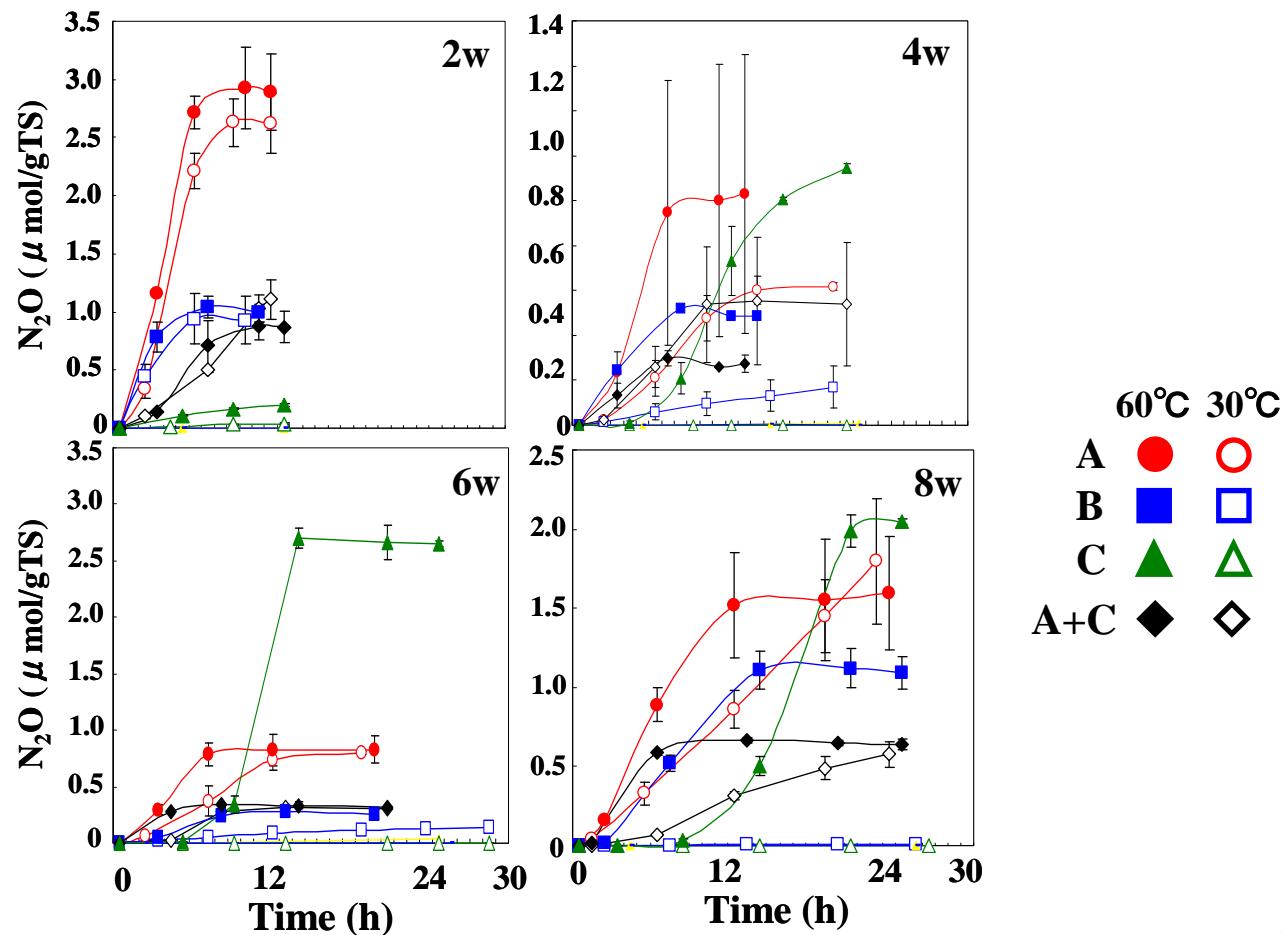
③ Core samples amended with NO₂⁻

Little, slow N₂O production with high SP (>10) value occurs

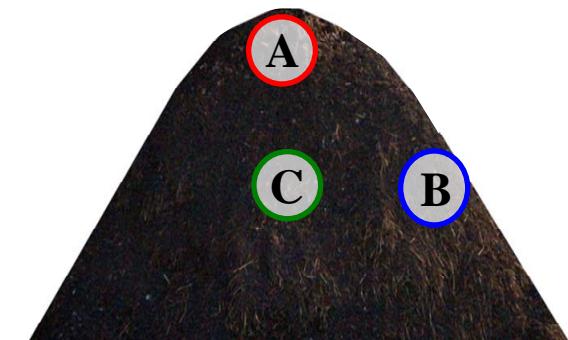
④ Mix of surface and core samples

N₂O production occurs immediately with low SP values?

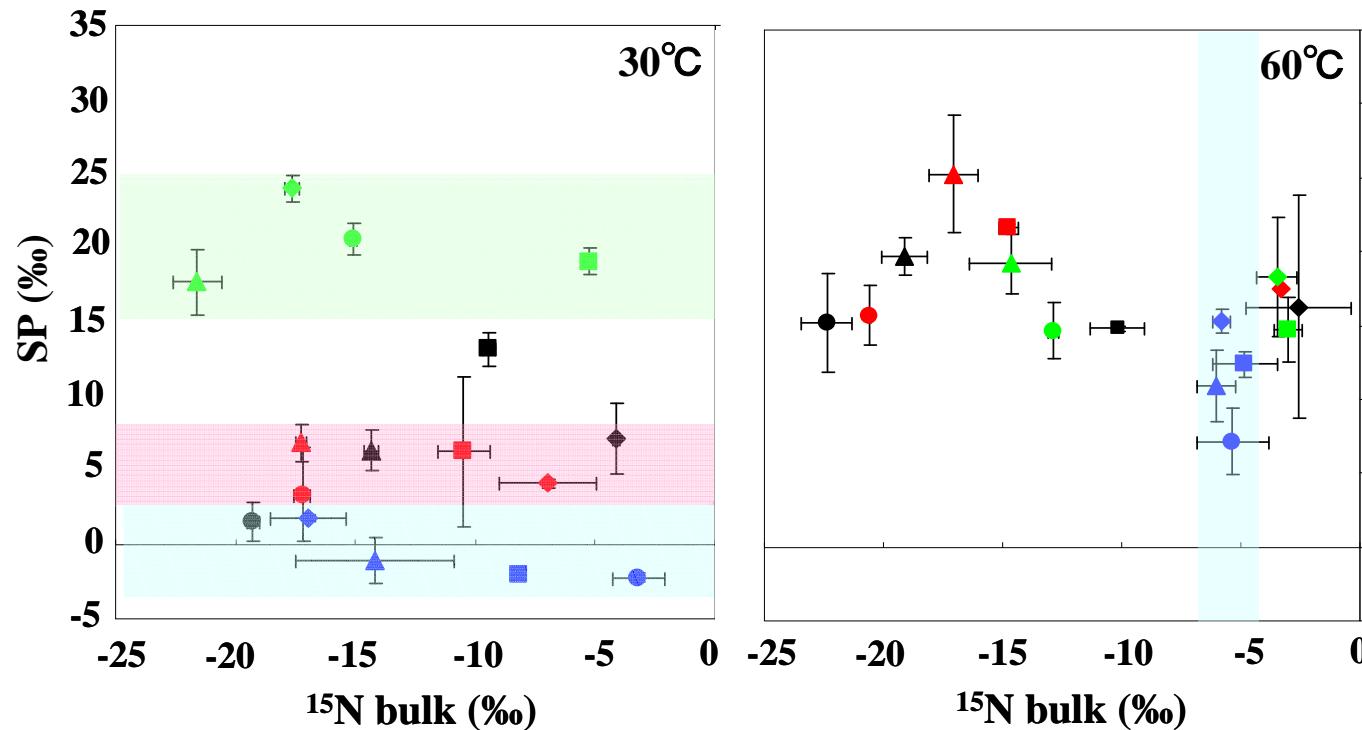
(model samples of the turnings)



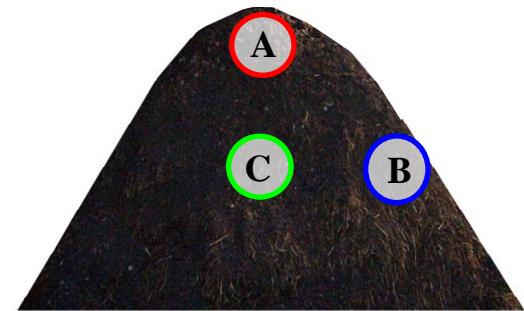
Thermophilic >> Mesophilic
Surface > Core + NO₂

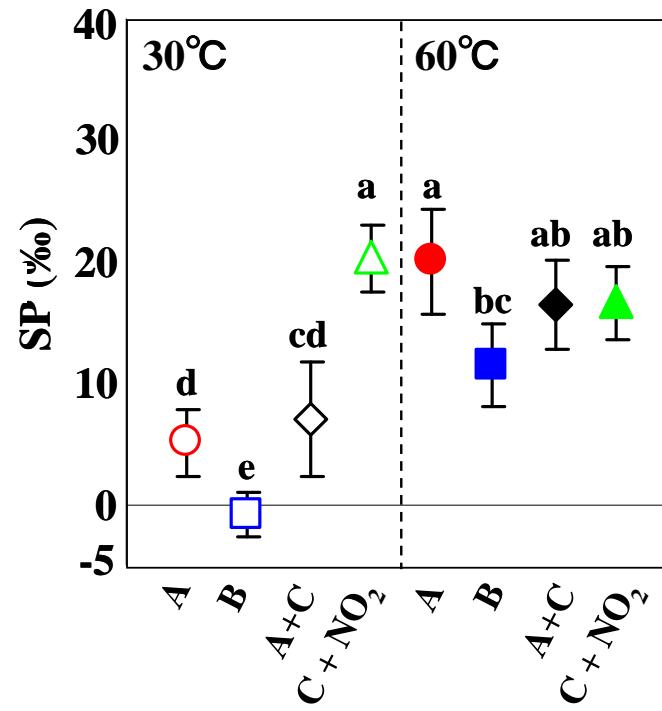
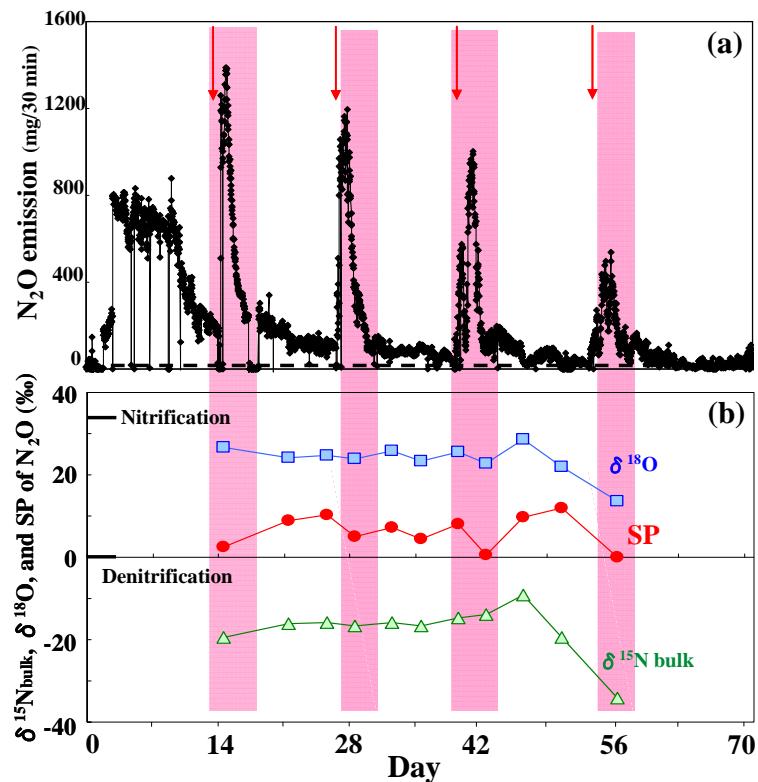


N₂O Isotopomer Analysis

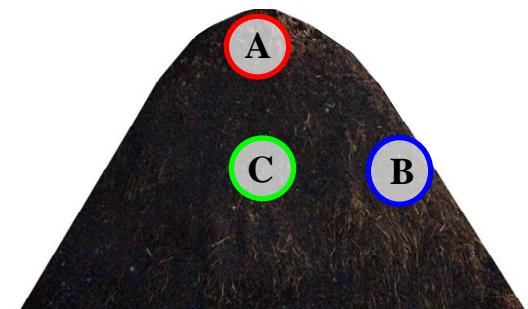


	A	B	C	A+C
2w	●	○	●	●
4w	▲	△	▲	▲
6w	■	□	■	■
8w	◆	◆	◆	◆

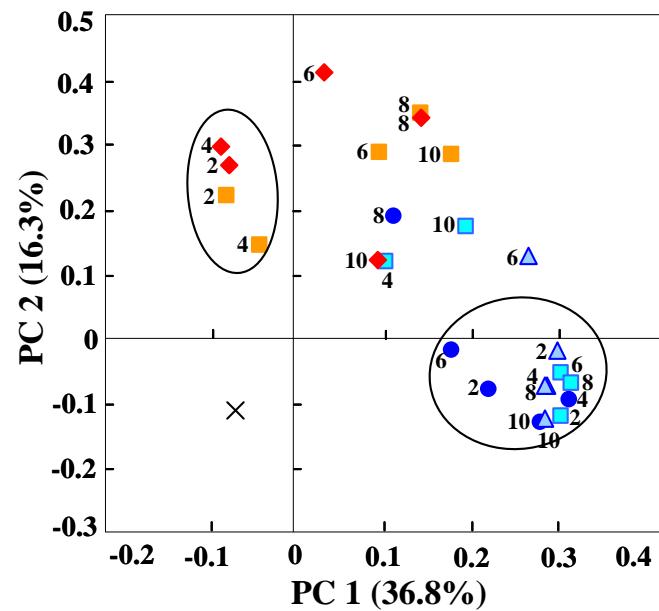
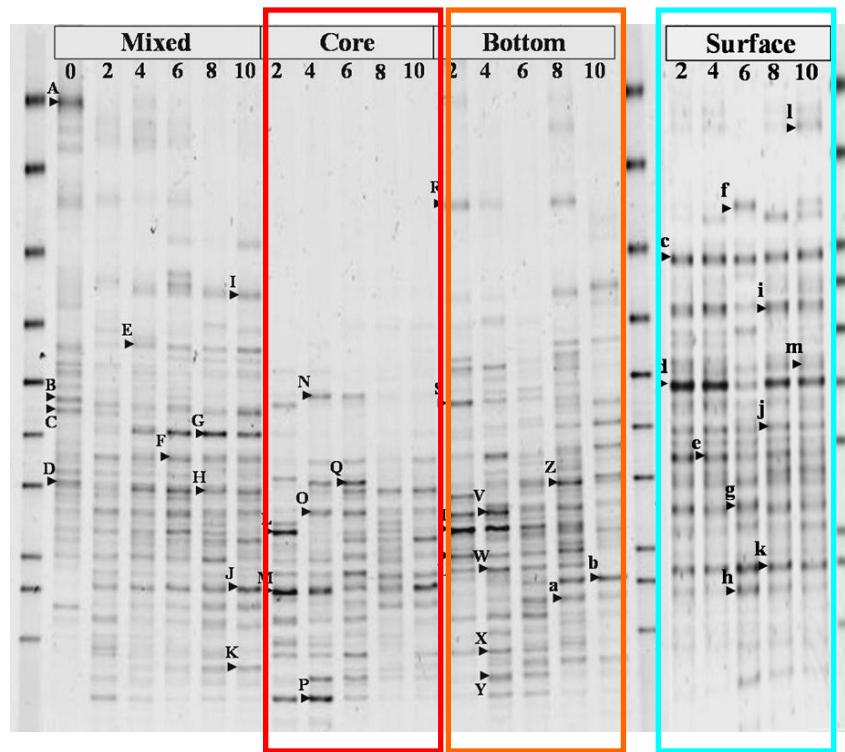




A B C A+C
 30°C ○ □ ▲ ◇
 60°C ● ■ ▲ ◆



What's Going On?- Overall Bacterial Community



Some *Bacillus* species and strictly anaerobic thermophilic *Clostridium* species were dominant only in the core and bottom zones.

In contrast, mesophilic bacteria such as *Bacteroidetes*, *Clostridia*, alpha and gamma-proteobacteria were detected in surface zones, even in the initial thermophilic stage of the process.

Core-Bottom Zone

Thermophiles

Clostridium

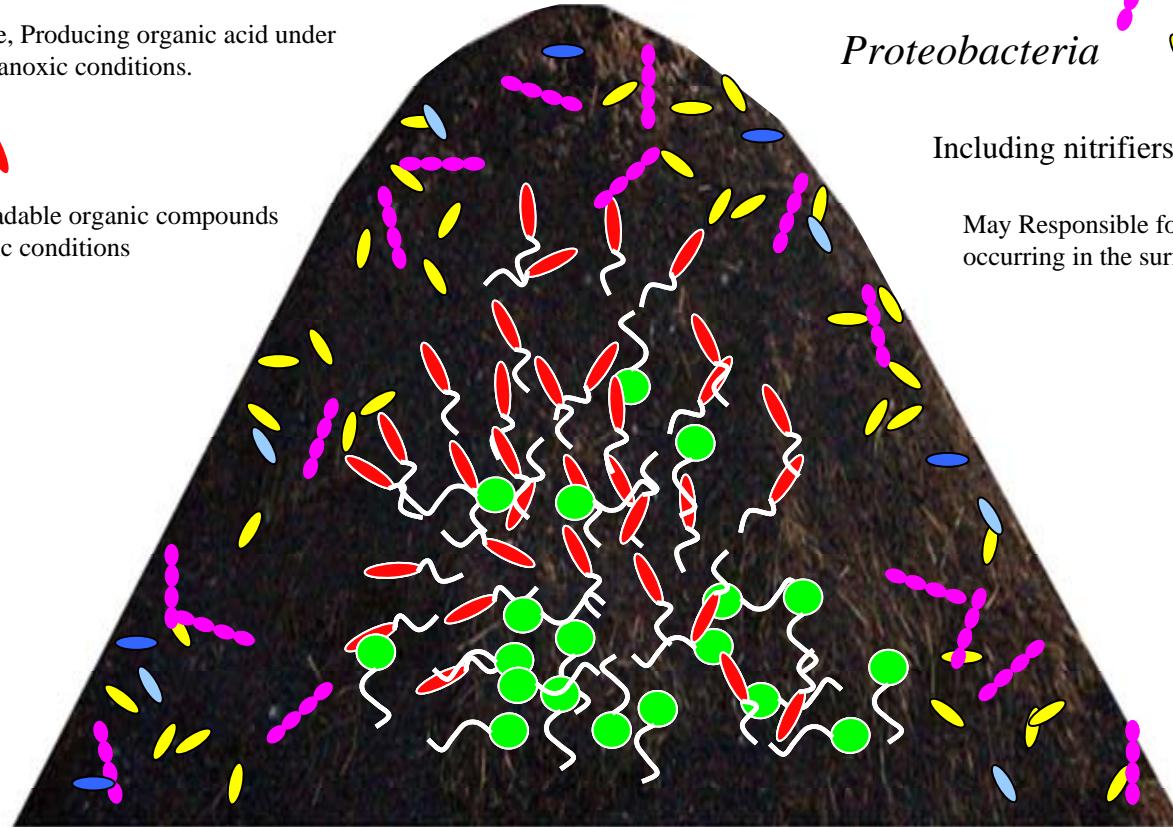


Degrad Cellulose, Producing organic acid under thermophilic and anoxic conditions.

Bacillus



Utilize easy-degradable organic compounds under thermophilic conditions



Surface Zone

Mesophiles

Bacteroidetes



Proteobacteria



Including nitrifiers

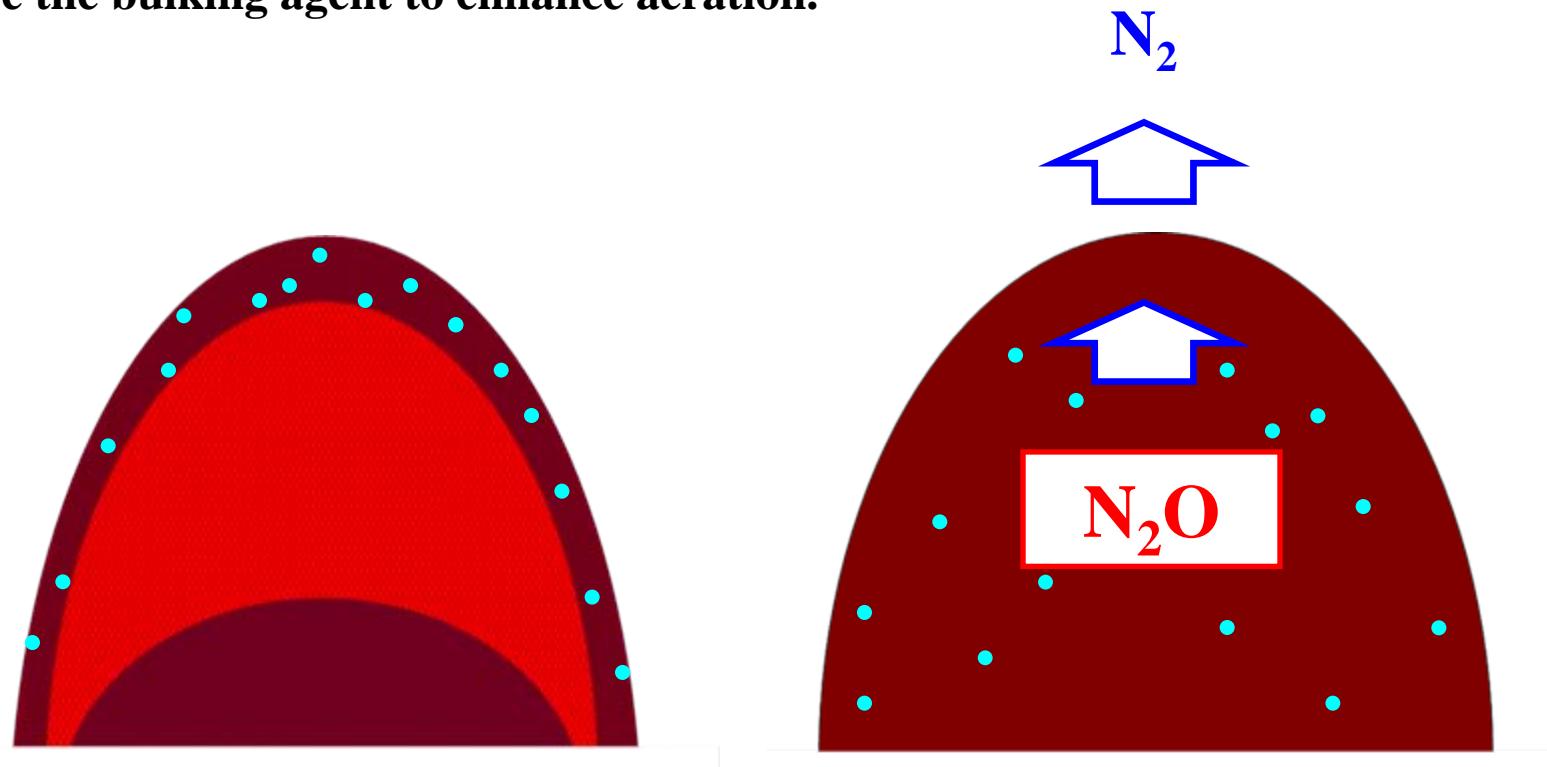


May Responsible for Nitrification mainly occurring in the surface.



- CH_4 and N_2O can be mitigated up to 74 % (CH_4) and 62 % (N_2O) by adding appropriate bulking agent.
- N_2O just after the turnings derives from denitrification of NOx-N accumulated in the pile surface.
- Nitrosomonas-like AOBs are partly responsible for surface nitrification.
- Denitrifiers in the surface zones are primary responsible for N_2O production under mesophilic condition just after the turnings.
- Pile surface are dominated by mesophiles belong to *Bacteroidetes* or *Proteobacteria*,

- Use the bulking agent to enhance aeration.

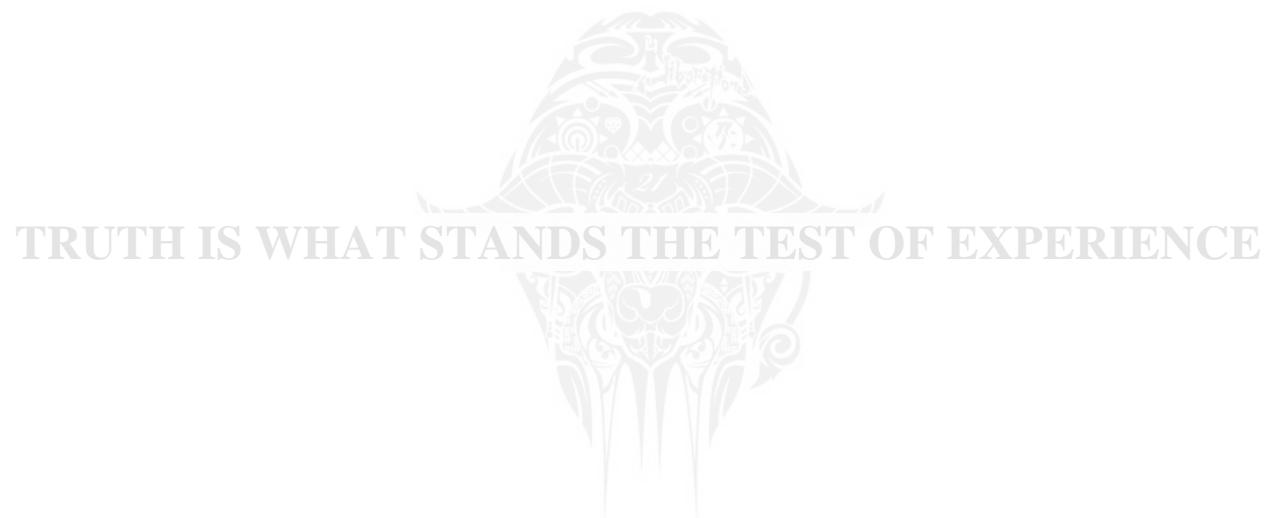


- Control nitrification on the pile surface

- promote N_2O reduction after the turnings



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TRUTH IS WHAT STANDS THE TEST OF EXPERIENCE

...Any Questions?