Mitigating strategy of GHG emission from dairy manure composting process
Livestock Production and Global Warming

Greenhouse Gas Emission

CH₄
N₂O

Surface Air Temperature Increase 1960 to 2060
GHG Emission from Agricultural Sector

67.1% of CH$_4$
47.1% of N$_2$O From Agricultural Sector
Nitrogen Cycle Relating Livestock Production

Complex microbial community is responsible for N$_2$O 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

Chamber

Auto Measuring System

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
Gas Emission

**Mitigation**

- CH$_4$: 74.3%
- N$_2$O: 62.8%
- CO$_2$: -42.2%
- NH$_3$: -126%
Source of $\text{N}_2\text{O}$;
Nitrification or Denitrification?
Nitrification, Denitrification and N$_2$O

AOB

$\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NO}_2^- \leftrightarrow \text{NO}_3^-$

$\downarrow$ $\text{N}_2\text{O}$

Ammonia monooxygenase ($amoa$)

Hydroxylamine oxidoreductase ($hao$)

NOB

Nitrite oxidoreductase ($nxrAXB$)

Nitrate reductase ($narG$)

Nitrite reductase ($nirK, nirS$)

Nitric oxide reductase ($cnorB$)

Nitrous oxide reductase ($nosZ$)

Denitrifiers

N$_2$
\[ \delta^{15}N_{\text{N}_2\text{O}} \] 

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

<table>
<thead>
<tr>
<th></th>
<th>( \delta^{15}N_{\text{N}_2\text{O}} ) (%)</th>
<th>SP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrosomonas europaea</td>
<td>-0.3 (4.9)</td>
<td>33.5 (1.2)</td>
<td></td>
</tr>
<tr>
<td>Nitrosospora multiformis</td>
<td>-0.3 (2.9)</td>
<td>32.5 (0.6)</td>
<td>Nitrification</td>
</tr>
<tr>
<td>Methylosinus trichosporium</td>
<td>3.4 (1.9)</td>
<td>35.6 (1.4)</td>
<td></td>
</tr>
<tr>
<td>Nitrosospora multiformis</td>
<td>-22.9 (0.6)</td>
<td>0.1 (1.7)</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas chlororaphis</td>
<td>12.7</td>
<td>-0.6 (1.9)</td>
<td>Denitrification</td>
</tr>
<tr>
<td>Pseudomonas aureofaciens</td>
<td>36.7</td>
<td>-0.5 (1.9)</td>
<td></td>
</tr>
</tbody>
</table>

N$_2$O Emission and Isotopomer Analysis

- **N$_2$O emission (mg/30 min)**
  - 1600
  - 1200
  - 800
  - 400

- **Day**
  - 70

- **Nitrification**
  - SP

- **Denitrification**
  - δ$^{15}$N bulk

- **δ$^{18}$O, δ$^{15}$N bulk, δ$^{18}$O, and SP of N$_2$O (‰)**
Accumulation of NO$_2^-$-N and NO$_3^-$-N

![Graph showing accumulation of NO$_2^-$-N and NO$_3^-$-N over 10 weeks.](image)

- **NO$_2^-$-N:** Cyan bars
- **NO$_3^-$-N:** Blue bars
- **NH$_4^+$-N:** Red bars

Weeks: 2, 4, 6, 8, 10

Nitrogen content in mg/kg TS:
- 0, 1000, 2000, 3000

Graph indicates a significant increase in NO$_2^-$-N and NO$_3^-$-N over the 10-week period.
Phylogenetic analysis of 16S rRNA and amoA gene

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 N$_2$O emission

The reduction of accumulated NO$_2$-N and NO$_3$-N (denitrification) occurred just after the turnings.

Maeda et al. AEM (2010)
• NO$_2$, NO$_3$-N accumulation in the surface samples is responsible for N$_2$O emission?

• Does NO$_2$ amendment complement the N$_2$O emission?
Denitrification Potential Measurement

10% C$_2$H$_2$

(N$_2$O $\rightarrow$ N$_2$ inhibition)

- NO$_2^-$-N
- NO$_3^-$-N
- NH$_4^+$-N

C$_2$H$_2$

- NH$_4^+$
- NO$_2^-$
- NO$_2^-$ reduction
- N$_2$O production
- N$_2$O reduction
Denitrification Potential Measurement

**Week 2**
- A – C\(_2\)H\(_2\)
- B – C\(_2\)H\(_2\)
- C + NO\(_2\) – C\(_2\)H\(_2\)

**Week 4**

**N\(_2\)O emission (nmol/gTS)**

- 1200
- 1000
- 800
- 600
- 400
- 200
- 0

**Time (h)**
- 720 12 24 36 48 60 72

Denitrification Potential Measurement
Denitrification Potential Measurement

- $N_2O$ production significantly correlated with NOx- accumulation.

$R^2 = 0.805$

$Y = 0.3 + 0.7X$

$mg \text{N}_2O\text{-N/gTS}$

$mg \text{NO}_x\text{-N/gTS}$
• Surface samples emitted significant N<sub>2</sub>O under aerobic condition.
• N<sub>2</sub>O emission correlates NO<sub>x</sub>-<sup>-</sup> accumulation

• NO<sub>2</sub>- amended core samples did not produced significant N<sub>2</sub>O especially in the initial stage of the process.

Denitrifiers in the surface zones might be mainly responsible for N<sub>2</sub>O production.
Hypothesis

Denitrifier in the surface zones are responsible for nitrite reduction and subsequent $\text{N}_2\text{O}$ emission just after the turnings.

① surface samples  
N$_2$O with SP value 0-10 occurs immediately

② Core samples  
No N$_2$O production

③ Core samples amended with NO$_2^-$  
Little, slow N$_2$O production with high SP (>10) value occurs

④ Mix of surface and core samples  
(model samples of the turnings)  
N$_2$O production occurs immediately with low SP values?
Thermophilic >>>> Mesophilic
Surface > Core + NO₂
N\textsubscript{2}O Isotopomer Analysis

![Graph showing N\textsubscript{2}O Isotopomer Analysis results at 30\textdegree C and 60\textdegree C. The graph displays the relationship between SP (‰) and 15N bulk (‰). The data points are color-coded for different time periods: 2w, 4w, 6w, and 8w, with each time period represented by different shapes and colors. The graph also includes shaded areas to indicate the range of 15N bulk values for each SP level.]}
(a) N$_2$O emission (mg/30 min)

(b) Nitrification and Denitrification

$\delta^{15}$N$_{bulk}$, $\delta^{18}$O, and SP of N$_2$O (‰)

30°C vs 60°C

A B C A+C

30°C A+A+C

60°C B B B
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, Clostoridia, alpha* and *gamma-proteobacteria* were detected in surface zones, even in the initial thermophilic stage of the process.

Maeda et al., BITE (2010).
**What’s Going On?- Overall Bacterial Community**

**Core-Bottom Zone**

**Thermophiles**

*Clostridium*

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

*Bacillus*

Utilize easy-degradable organic compounds under thermophilic conditions.

**Surface Zone**

**Mesophiles**

*Bacteroidetes*

Function unknown

*Proteobacteria*

Including nitrifiers

May Responsible for Nitrification mainly occurring in the surface.

Maeda et al., BITE (2010).
Conclusion

• CH₄ and N₂O can be mitigated up to 74 % (CH₄) and 62 % (N₂O) by adding appropriate bulking agent.

• N₂O 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 N₂O production under mesophilic condition just after the turnings.

• Pile surface are dominated by mesophiles belong to Bacteroidetes or Proteobacteria,
GHG mitigation strategy

- Use the bulking agent to enhance aeration.
- Control nitrification on the pile surface
- Promote N\textsubscript{2}O reduction after the turnings
TRUTH IS WHAT STANDS THE TEST OF EXPERIENCE

…Any Questions?