The role of microbial diversity in controlling greenhouse gas emissions from conserved forage

Seongmin Yang, Maheen mahmood **Jeongdae Im (jeongdaei@ksu.edu)**
Department of Civil Engineering, Kansas State University, Manhattan, KS

KANSAS STATE UNIVERSITY

Background

- Forages can be conserved to feed livestock during periods of shortage
- Historically and throughout the world, conserved forages are an integral part of raising livestock
- Two main conservation methods in forage preservation are hay and silage.

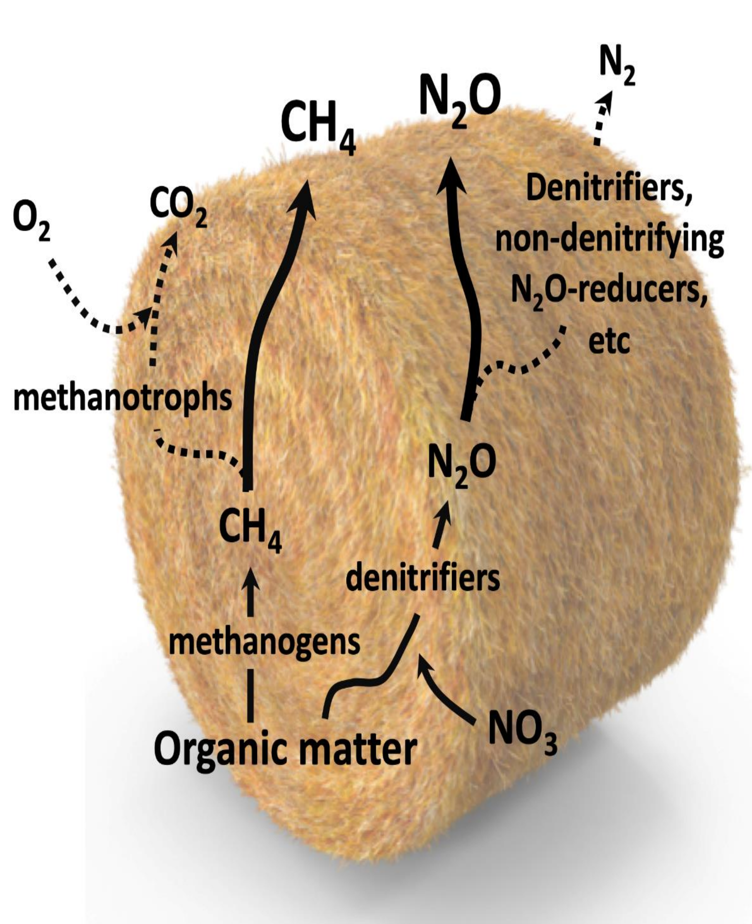
Forage conservation methods

Hay | **Silage**

< 20% moisture
Aerobic storage

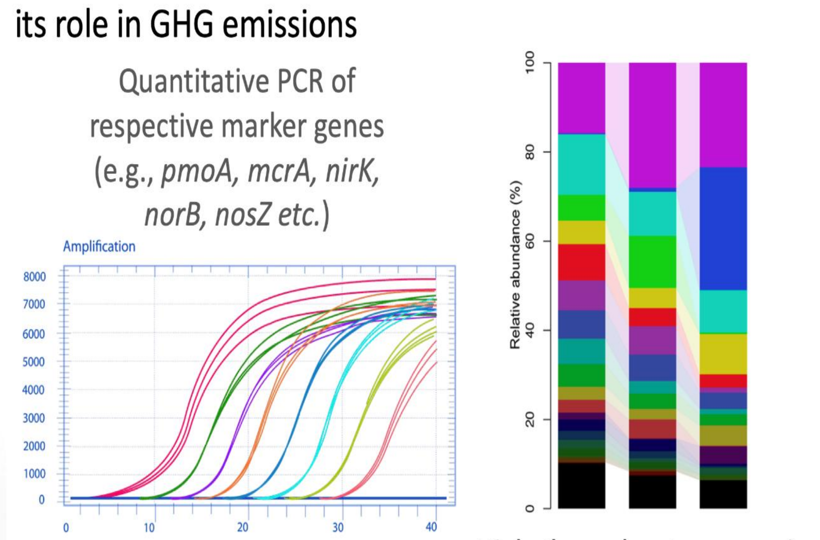
40~70% moisture
Anaerobic storage

- In other words, conserved forages are *nutrient-rich ecosystems that are maintained partly or completely under anoxic conditions*.
- Therefore, they can serve as ideal habitats for diverse microorganisms especially those involved in the production and reduction of greenhouse gases (GHGs), e.g., methanogens, methanotrophs, denitrifiers, and non-denitrifying N_2O reducers
- Despite the high production volume and widespread commercial use, little is know about these ecosystems



Objectives
- Quantify GHG emission potentials from forage conservation
- Characterize the conserved forage microbiome and analyze its role in GHG emissions

Quantitative PCR of respective marker genes (e.g., *pmoA*, *mcrA*, *nirK*, *norB*, *nosZ* etc.)



High-throughput sequencing

Impact
- Reduce environmental footprint of agriculture
- Promote sustainable agricultural practices

Figure 1. Objectives, hypothetical results, and impact of the proposal

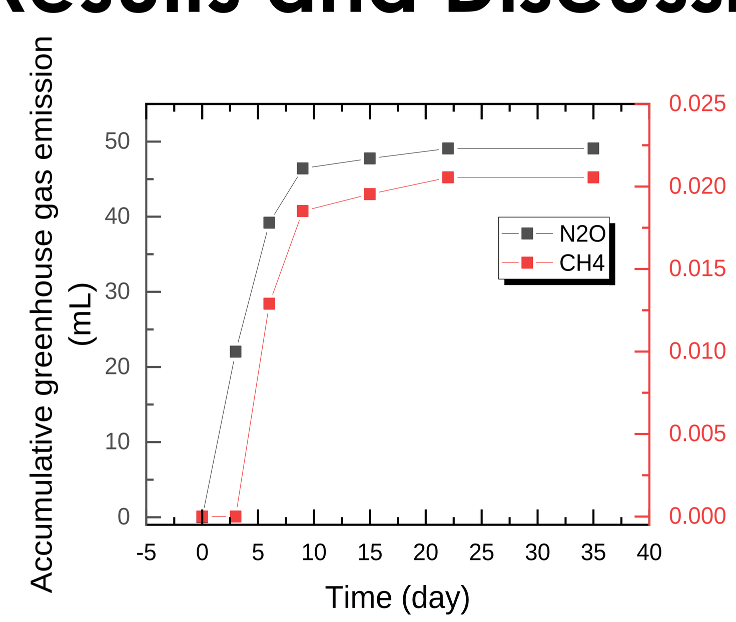
Materials and Methods

- Three bales (**Figure 3**) were placed outside at the Kansas Beef Stocker Unit and monitored for GHG emissions.
- For the laboratory experiment simulating silage, 648 g of forage samples were added in 1L bottles to achieve the packing density of 45 lb/ft^3, and the moisture contents were adjusted to 70% (w/w) by adding sterilized deionized distilled water.
- Four round bales (4 ft width x 4 ft diameter) from second cut alfalfa (*Medicago sativa L.*) for laboratory and field experiment.
- For the field experiment, three round bales were monitored for surface GHG emissions once a month for 4 months. During each field sampling event, three forage core samples were collected using a hay coring probe at two different depths (5-25, 35-55 cm), and DNA was extracted.
- Geochip 5.0S a functional gene microarray was used to examine relative abundance of functional genes.

Figure 2. Laboratory experiment simulating silage

Figure 3. Surface GHG emissions measurement from round hay bales

Results and Discussion



0.66 μmol N_2O/day/m^2
No detectable CH_4

Figure 5. CH_4 and N_2O production from forages under anaerobic conditions.

Table1. Greenhouse gas emissions from agriculture (MMT[a] CO_2 Eq.)

Gas/Source	1990	2005	2012	2013	2014	2015	2016
CO_2	**7.1**	**7.9**	**10.3**	**8.4**	**8.1**	**8.7**	**9.0**
Urea Fertilization	2.4	3.5	4.3	4.4	4.5	4.9	5.1
Liming	4.7	4.3	6.0	3.9	3.6	3.8	3.9
CH_4	**217.6**	**242.1**	**244.0**	**240.6**	**240.1**	**245.4**	**251.8**
Enteric Fermentation	164.2	168.9	166.7	165.5	164.2	166.5	170.1
Manure Management	37.2	56.3	65.6	63.3	62.9	66.3	67.7
Rice Cultivation	16.0	16.7	11.3	11.5	12.7	12.3	13.7
Field Burning of Agricultural Residues	0.2	0.2	0.3	0.3	0.3	0.3	0.3
N_2O	**264.5**	**270.1**	**265.5**	**294.2**	**291.6**	**312.8**	**301.8**
Agricultural Soil Management	250.5	253.5	247.9	276.6	274.0	295.0	283.6
Manure Management	14	16.5	17.5	17.5	17.5	17.7	18.1
Field Burning of Agricultural Residues	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Total	**489.2**	**520.0**	**519.8**	**543.1**	**539.8**	**566.9**	**562.6**

Note: Totals may not sum due to independent rounding. [a]MMT: million metric tons.

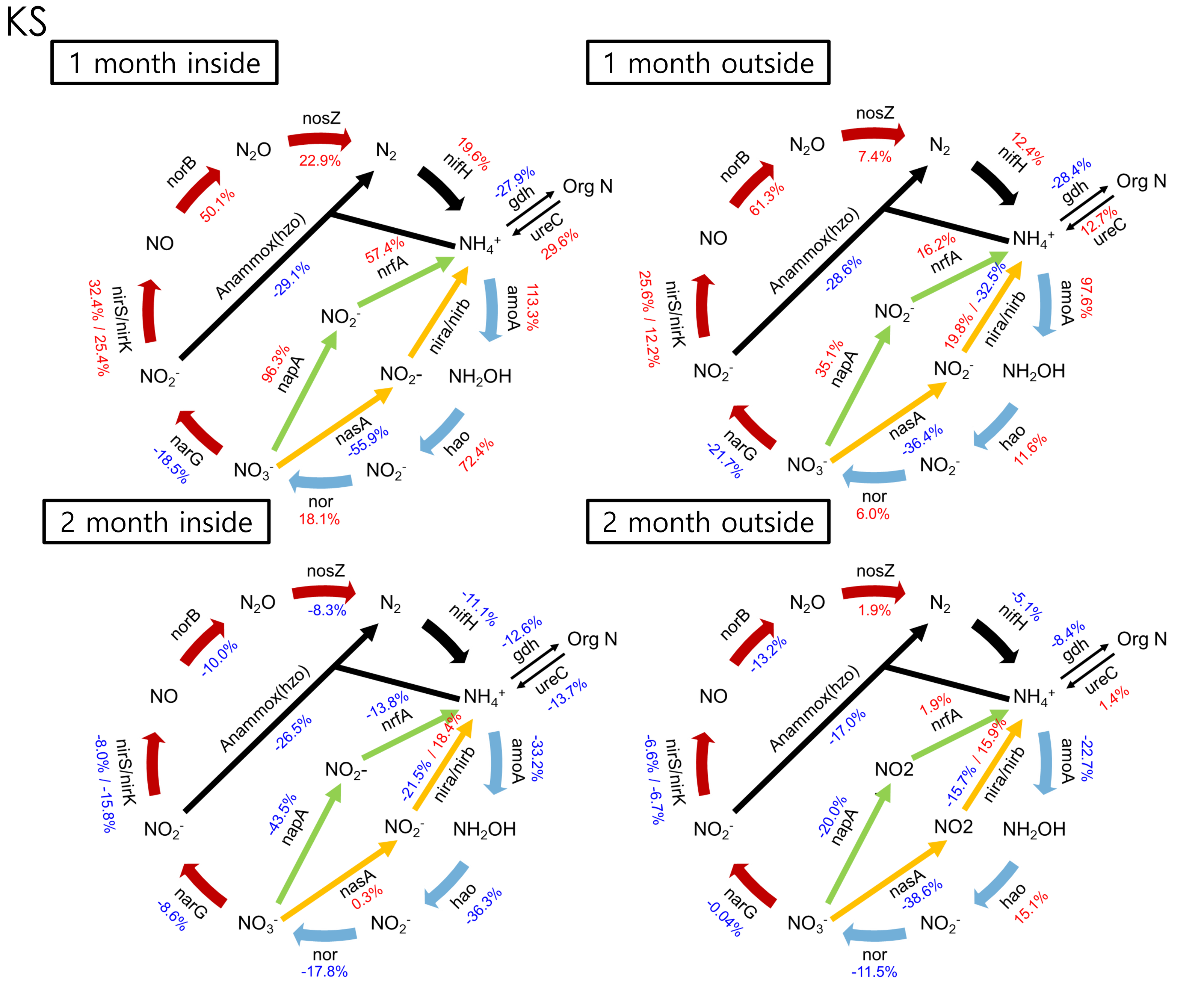


Figure 4. Percentage changes of functional genes (N-cycle)

- Functional genes related with N-cycle is increased in oxic and anoxic condition at 1st month
- After 2 month of incubation, N-related genes abundances plateaued or decreased by nutrient depletion

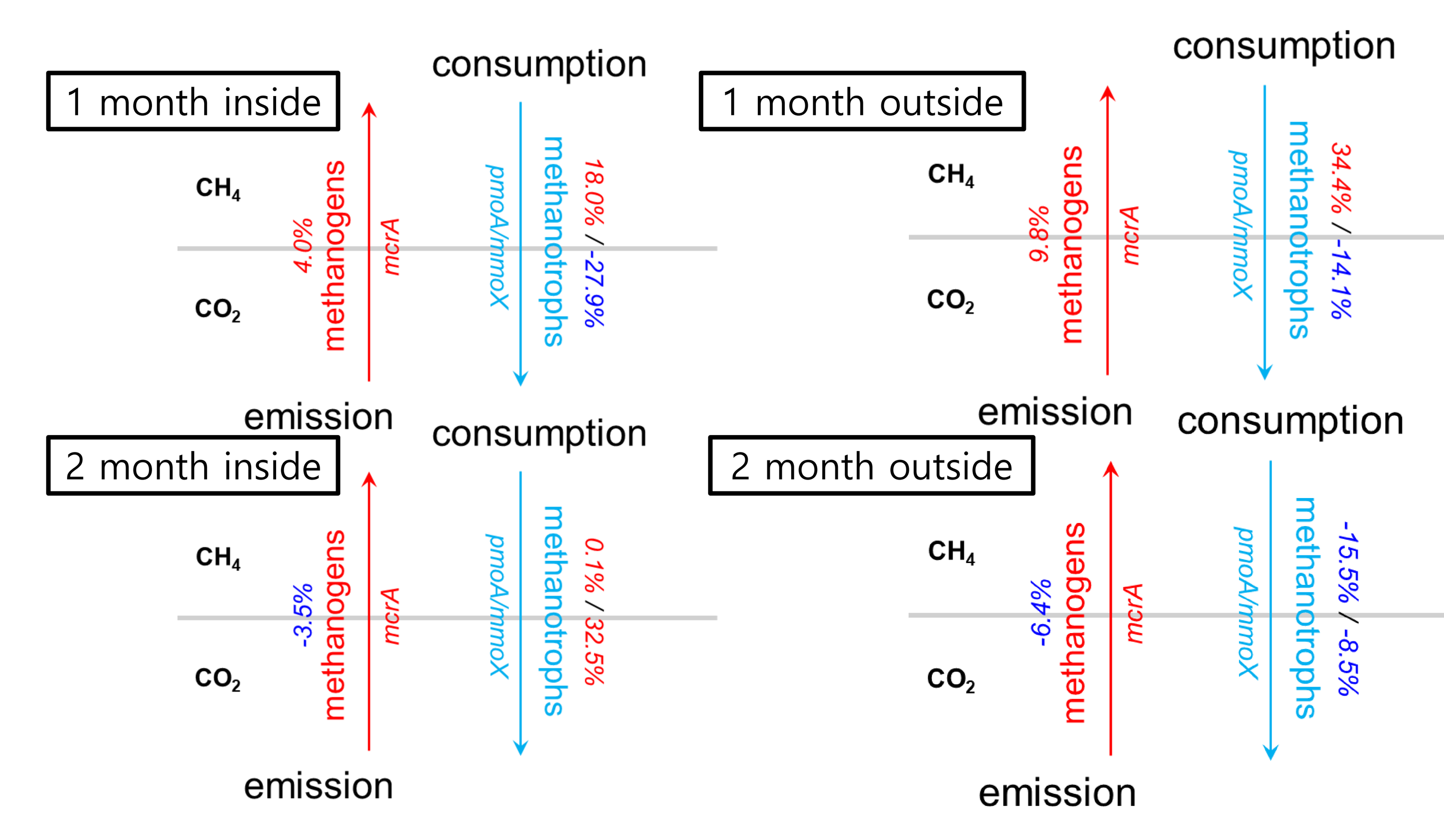


Figure 5. Percentage changes of functional genes (CH_4 cycle)

- Methane oxidation functional gene (pmoA) increase their abundance in oxic region of forage at 1 month.
- pmoA genes abundance is decreased at 2 months.

Conclusions

- <u>Forage fermentation process produce the greenhouse gas (CH_4, N_2O) in oxic and anoxic region and the majority of genes involved in the transformation of nitrogenous compounds correlated positively at 1st month</u>
- Due to the depletion of the nutrient and pH drops, methane and nitrous oxide production stops and functional gene abundance is plateaued and decreased.