Dicyandiamide shifts the production pathway from denitrification to incomplete nitrification dominated by the *amo*A gene during composting, delaying rather than continuously inhibiting the nitrous oxide emission

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Keywords: Nitrous oxide; Composting; Dicyandiamide; Ammonia-oxidizing bacteria; Denitrification

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Introduction: Nitrous oxide (N_2O) is a powerful greenhouse gas (GHG) with a warming potential of 265–298 times of CO_2 on a 100 year scale (Chen et al., 2023). And about 10% of non-CO_2 greenhouse gases (calculated based on CO_2-eq) worldwide come from the management process of manure generated (Møller et al., 2022). For example, during the composting process, N_2O contributes over 74% to the non-CO_2 greenhouse effect, which is much higher than other gases such as CH_4.

The continuous nitrification process during the maturation period leads to the accumulation of NO_3^- and NO_2^-, while denitrification of them results in a large amount of N_2O emissions (Chang et al., 2024). So, nitrification plays an important role in the production of N_2O. Therefore, nitrification inhibitors are used. The overall situation is that the mechanism by which DCD affects N_2O during composting has not yet been revealed. Previous studies have only simplified assumptions about the reaction pathway, or focused only on the response of dominant microorganisms to DCD, rather than functional microorganisms.

Therefore, the purpose of this study is to: (1) investigate the effect of adding DCD during the emission duration on the overall N_2O emission pattern; (2) By combining functional gene analysis and structural modeling, reveal the main pathways of N_2O production in the presence and absence of DCD; (3) By combining functional microbial analysis, reveal the types of key functional microorganisms and their mechanisms for producing N_2O.

Materials and methods: Composting materials included pig manure and corn straw, they were mixed in a ratio of 85:15 (w/w). Dicyandiamide (DCD) was used as an external additive during the composting, various amounts of DCD (based on TN) were added to the mixture on day 28. The labeling method for each treatment were as follows: CK: no addition; L: 5%; H: 10%. Mixed the materials evenly and placed them in three closed reactor for composting. The composting experiment was conducted for 53 days.

The N_2O was analyzed using a gas chromatograph (3420A, Beifen, China) equipped with both electron capture and flame ionization detectors. We collected and measured the gas sample each day with three replicates per sample. Turning and sampling of compost piles on days 0, 7, 14, 21, 28, 35, 42, and 53. To determine the content of NH_4^+-N, NO_3^--N and NO_2^-, we weighed 10 g sample in a conical flask and added 100 mL of 2 $mol \cdot L^{-1}$ KCl solution. Then shook the conical flask for 30 minutes and let it stand before filtration. The supernatant was analyzed using a flow analyzer (Technicon Auto Analyzer 3; Seal Analytical GmbH, Norderstedt, Germany).

On the days of turning and sampling, separate samples were collected for microbial community analysis, and the samples were promptly stored in an environment with a temperature of –80°C. DNA extraction and high-throughput sequencing analysis of bacterial 16S rRNA were performed on the samples, as well as High throughput sequencing analysis of nitrification and denitrification functional genes.

Results and discussion: N_2O was mainly produced after the 28th day, and groups L and H slowed the

growth of N_2O emission rate, delayed and reduced its emission peaks (**Fig. 1a**). Over the full N_2O emission during the composting (days 28–53), DCD did not reduce cumulative N_2O emissions (**Fig. 1b**). But compost usually does not require such a long time to mature, so the effect of DCD is closely related to the composting duration.

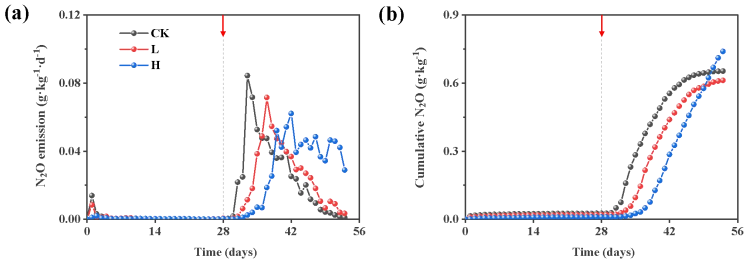

Fig. 1. Changes in nitrogen content index: N_2O emission rate (a) and cumulative N_2O emission (b).

In summary, under the action of DCD, the source of N_2O during the composting process gradually shifts from nitrite denitrification pathway dominated by the *nir*K and *nor*B genes to incomplete nitrification pathway dominated by the *amo*A gene (**Fig. 2**).

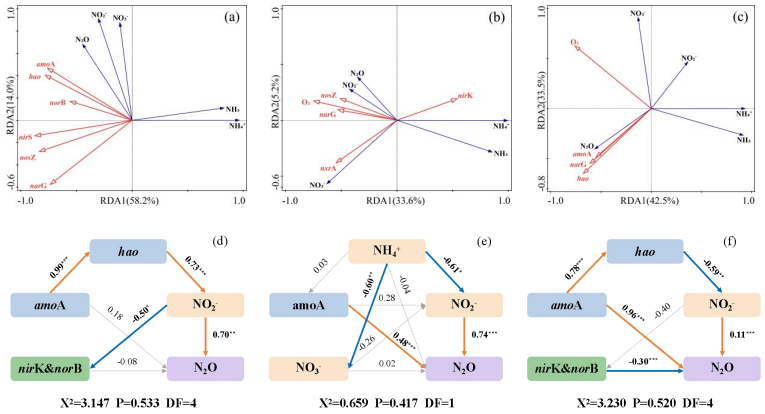

Fig. 2. Redundancy analysis (RDA) between N_2O emission rate, inorganic nitrogen, functional genes, and physicochemical properties (CK, a; L, b; H, c). The structural equation model (SEM) between N_2O emission rate, inorganic nitrogen, and functional genes (CK, d; L, e; H, f).

Conclusion: The N_2O generated during composting mainly comes from denitrification, and ammonia oxidizing bacteria (AOB, mainly *Nitrosomonas*) not only contribute to this through nitrification but also through nitrifier denitrification. The addition of DCD delayed the enrichment of AOB carrying *amo*A gene, delaying N_2O emissions by 4–9 days. DCD also gradually reduces the contribution of denitrification to N_2O by continuously inhibiting the enrichment of *hao* genes and reducing the abundance of *Nitrosomonas europaea nir*K genes encoding AOB. When the addition of DCD reaches 10%, the main pathway for N_2O production is transformed into an incomplete nitrification process dominated by *Nitrosomonas* carrying the *amo*A gene.

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