

## Microbial Characteristics of Tobacco-Cultivated Soil and Isolation, Identification, and Activity Study of Ammonifying and Nitrosifying Bacteria (Postprint)

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### Abstract

To address the issue of difficult regulation of organic nitrogen decomposition rate in tobacco-planting soil after organic fertilizer application in flue-cured tobacco production, and to provide superior strains for preparing microbial inoculants for organic nitrogen decomposition, this study utilized rhizosphere soil of flue-cured tobacco under treatments of chemical fertilizer, cattle manure, corn straw, and oil cake as experimental materials to isolate and screen highly active indigenous ammonifying and nitrosating bacteria, and evaluated their effects on organic nitrogen degradation and ammonia nitrogen decomposition, respectively. The results showed that: during the vigorous growth stage of flue-cured tobacco, the quantities of culturable bacteria, fungi, actinomycetes, and ammonifying bacteria in soils treated with organic fertilizers were all higher than those in the chemical fertilizer alone treatment; during the maturity stage of flue-cured tobacco, the quantities of ammonifying bacteria, nitrosating bacteria, fungi, and actinomycetes in the rhizosphere soil under organic fertilizer treatments were all higher than under chemical fertilizer alone. Through analysis of the soil sample sources of various strains, the dominant strains with higher ammonification intensity all originated from soil samples treated with organic fertilizers, indicating that applying organic fertilizers to tobacco-planting soil could obtain ammonifying bacteria with higher activity compared with chemical fertilizer alone. Studies on the activities of various bacteria showed that the culture medium of *Bacillus pumilus*, *Geobacillus stearothermophilus*, and *Bacillus megaterium* showed the greatest reduction in organic nitrogen content, decreasing by 84.74%, 92.74%, and 79.52% compared with the initial organic nitrogen content, respectively; *Stenotrophomonas* sp. possessed both nitrosating and nitrifying functions, with nitrate nitrogen content in the culture medium

increasing by  $0.617 \text{ mg} \cdot \text{L}^{-1}$  after 7 days of cultivation; *Stenotrophomonas maltophilia* had the strongest nitrosating activity, with nitrite nitrogen content in the culture medium reaching  $0.518 \text{ mg} \cdot \text{L}^{-1}$  after 7 days of cultivation. The organic nitrogen decomposition rate of ammonifying bacteria decreased after 48 h of cultivation, while strains with nitrosating and nitrifying functions still maintained relatively high activity levels when cultivated up to day 7. Therefore, when preparing nitrogen-releasing compound microbial inoculants using the functionally different bacteria isolated in this experiment, strains need to be added at different time points for fermentation to obtain highly active microbial inoculants. Based on the organic nitrogen decomposition and ammonia decomposition experiments, along with functional analysis of each strain from relevant literature, eight highly efficient nitrogen-functional strains were selected for the preparation of organic nitrogen-decomposing microbial inoculants: *Naxibacter* sp., *Stenotrophomonas* sp., *Bacillus pumilus*, *Geobacillus stearothermophilus*, *Bacillus stratosphericus*, *Cellulosimicrobium cellulans*, *Bacillus altitudinis*, and *Bacillus megaterium*.

## Full Text

### Isolation, Identification and Activity of Ammonifiers, Nitrosifying Bacteria and Microbial Characteristics in Tobacco-Planted Soils

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**Abstract:** The decomposition rate of organic nitrogen in tobacco-planted soils is difficult to regulate after organic fertilizer application, which affects normal tobacco growth and leaf quality. This study isolated and screened highly active indigenous ammonifying and nitrosifying bacteria from tobacco root-zone soils treated with chemical fertilizer, cow manure, maize straw, and rapeseed cake, and evaluated their effects on organic nitrogen degradation and ammonia decomposition. Results showed that during the vigorous growth period of flue-cured tobacco, the numbers of culturable bacteria, fungi, actinomycetes, and ammonifiers in organic fertilizer treatments were higher than those in chemical fertilizer treatment. At maturity, soils receiving organic amendments also exhibited higher populations of ammonifiers, nitrosifying bacteria, fungi, and actinomycetes compared to chemical fertilizer alone. Analysis of strain origins revealed that dominant strains with high ammonification activity were all

derived from organic fertilizer treatments, indicating that organic amendment promotes more active ammonifying bacteria in tobacco soils. Activity studies demonstrated that *Bacillus pumilus*, *Geobacillus stearothermophilus*, and *B. megaterium* achieved the greatest reductions in organic nitrogen content, decreasing by 84.74%, 92.74%, and 79.52% respectively compared to initial levels. *Stenotrophomonas* sp. exhibited both nitrosifying and nitrifying activities, increasing nitrate nitrogen by  $0.617 \text{ mg} \cdot \text{L}^{-1}$  after 7 days of culture. *S. maltophilia* showed the strongest nitrosifying activity, producing  $0.518 \text{ mg} \cdot \text{L}^{-1}$  nitrite nitrogen after 7 days. The organic nitrogen decomposition rate of ammonifying bacteria decreased after 48 hours of culture, whereas strains with nitrosifying and nitrifying activities maintained high activity levels through day 7. Therefore, when preparing composite nitrogen-decomposing microbial agents, different functional strains should be added at different fermentation stages to obtain highly active microbial preparations. Based on organic nitrogen decomposition, ammonia degradation tests, and literature analysis of strain functions, eight highly efficient nitrogen-functional strains were selected for microbial agent formulation: *Nazibacter* sp., *Stenotrophomonas* sp., *B. pumilus*, *G. stearothermophilus*, *B. stratosphericus*, *Cellulosimicrobium cellulans*, *B. altitudinis*, and *B. megaterium*.

**Keywords:** Tobacco-growing soil; Microbial agents; Ammonifier; Nitrosifying bacteria; Isolation and identification; Nitrogen decomposition

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## Introduction

Long-term heavy application of chemical fertilizers while neglecting organic amendments in tobacco production leads to decreased soil humus content, poor biological activity, and increasingly severe soil degradation. Relying solely on high-quality chemical fertilizers to regulate soil nutrition cannot compensate for nutritional imbalances caused by soil impoverishment, resulting in low industrial usability of tobacco leaves with high nicotine and starch content, dense structure, insufficient aroma quantity, poor aroma quality, and even loss of characteristic style. This severely constrains the sustainable development of tobacco (*Nicotiana tabacum*) production. Modern tobacco agriculture is gradually moving toward green and ecological production, with higher safety requirements for tobacco and tobacco products. Reducing chemical fertilizer application and increasing organic fertilizer use are crucial for improving soil physicochemical properties, enhancing soil fertility, improving tobacco leaf quality and safety, and promoting sustainable tobacco production. However, organic fertilizer nutrient decomposition and release are constrained by multiple environmental factors, making their effectiveness difficult to predict and control. When nitrogen application timing does not match tobacco's nitrogen demand patterns, excessive application results in slow early growth, delayed vigorous growth and maturity, and ultimately affects both yield and quality. Guizhou's tobacco-planted soils are generally heavy and clayey, with low temperatures and high

rainfall during early growth stages. After organic fertilizer application, soil nitrogen bioavailability is low, creating a supply shortage that fails to meet early growth requirements. Soil microbial activity plays a decisive role in nitrogen transformation, supply, and bioavailability in tobacco fields.

Previous research has focused primarily on changes in soil microbial activity and physicochemical indicators after organic fertilizer application in tobacco fields, as well as the production effects of microbial fertilizers on flue-cured tobacco. Few studies have addressed the development of nitrogen-decomposing microbial agents adapted to local ecological conditions to regulate organic nitrogen decomposition in tobacco-planted soils, thereby solving the problem of delayed available nitrogen release during the tobacco growth period. Ammonifying bacteria, nitrifying bacteria, and nitrifying bacteria play decisive roles in regulating organic nitrogen decomposition rates. While organic nitrogen-decomposing bacteria are typically isolated from wastewater and applied in wastewater treatment, few studies have reported their isolation and application from soils. Different plant rhizospheres harbor different bacterial strains, and different strains exhibit varying environmental adaptability. Moreover, indigenous strains do not cause secondary environmental pollution. Therefore, this study comprehensively investigated microbial activity changes during the tobacco growth period, isolated and screened highly active indigenous ammonifying and nitrifying bacteria from tobacco root-zone soils under different organic fertilizer treatments, and evaluated their effects on organic nitrogen degradation and ammonium decomposition. The goal was to establish a foundation for producing composite microbial agents that can regulate organic fertilizer nitrogen decomposition in local environments.

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## Materials and Methods

### 1.1 Microbial Characteristics and Screening of Ammonifying and Nitrifying Bacteria in Tobacco-Planted Yellow Soil with Organic Amendments

#### 1.1.1 Experimental Materials

The experiment was conducted at the Guizhou University Tobacco Base in Longshan Village, Pingba County, Anshun City, Guizhou Province (26°37'N, 106°54' E), located in a subtropical monsoon climate zone at 1,898 m average elevation with an annual mean temperature of 17.2°C. The experimental soil was yellow loam with the following properties: organic matter 46.79 g · kg<sup>-1</sup>, total nitrogen 1.79 g · kg<sup>-1</sup>, ammonium nitrogen 9.42 mg · kg<sup>-1</sup>, nitrate nitrogen 5.71 mg · kg<sup>-1</sup>, bulk density 1.20 g · cm<sup>-3</sup>, pH 5.37, total phosphorus 1.08 g · kg<sup>-1</sup>, available phosphorus 17.78 mg · kg<sup>-1</sup>, total potassium 9.63 g · kg<sup>-1</sup>, and available potassium 207.60 mg · kg<sup>-1</sup>.

The flue-cured tobacco variety 'K326' was used. Test fertilizers included tobacco-specific compound fertilizer (N:P O :K O = 10:11:23), cow manure, straw, and rapeseed cake. All organic fertilizers were fully decomposed with relatively sta-

ble nutritional structures. Nutrient characteristics of the organic fertilizers are shown in .

### 1.1.2 Experimental Design

A completely randomized design was employed with four treatments: (1) compound fertilizer  $1,500 \text{ kg} \cdot \text{hm}^{-2}$  (control); (2) compound fertilizer  $1,200 \text{ kg} \cdot \text{hm}^{-2}$  + cow manure  $2,326 \text{ kg} \cdot \text{hm}^{-2}$ ; (3) compound fertilizer  $1,200 \text{ kg} \cdot \text{hm}^{-2}$  + straw  $2,703 \text{ kg} \cdot \text{hm}^{-2}$ ; and (4) compound fertilizer  $1,200 \text{ kg} \cdot \text{hm}^{-2}$  + rapeseed cake  $993 \text{ kg} \cdot \text{hm}^{-2}$ . Each treatment had three replications, totaling 12 plots of  $33.6 \text{ m}^2$  each, with planting density of  $0.65 \text{ m}$  (within-row)  $\times$   $0.90 \text{ m}$  (between-row). Plots were separated by 1 m intervals, with 2 m protective borders around the entire experimental area.

Fertilization was based on a pure nitrogen rate of  $150 \text{ kg} \cdot \text{hm}^{-2}$ , ensuring that the nitrogen content from organic fertilizer accounted for 20% of total nitrogen in all organic treatments. All organic fertilizer was applied as base fertilizer. At 30 days after transplanting, a topdressing of tobacco-specific compound fertilizer (N:P O :K O = 13:0:26) was applied at  $1,000 \text{ kg} \cdot \text{hm}^{-2}$ .

### 1.1.3 Soil Sampling

Based on soil nutrient dynamics and the tobacco growth cycle, soil samples were collected at 60 days and 90 days after transplanting (corresponding to the vigorous growth and maturity stages, respectively). Sampling avoided fertilizer application zones, collecting 4-6 points radially 10 cm from the plant base at 5-20 cm depth. In each plot, root-zone soils from three healthy, disease-free tobacco plants were mixed to form composite samples, placed in sterile sampling bags, sealed, transported at low temperature, and processed immediately for strain isolation.

### 1.1.4 Culture Media

Peptone agar medium was used for ammonifier counting and isolation. Modified Stephenson medium was used for nitrosifying bacteria counting and isolation. Beef extract-peptone liquid medium was used for bacterial counting, ammonifier characterization, and ammonification intensity determination. LB liquid medium was used for strain activation and enrichment. Martin medium was used for fungal counting. Potato-sucrose agar (PDA) medium was used for actinomycete counting. Sporulation medium was used to identify spore formation.

### 1.1.5 Microbial Population Determination

After serial dilution, ammonifiers were enumerated using  $10^{-1}$  and  $10^{-2}$  dilutions on peptone agar medium via the MPN method. Nitrosifying bacteria were enumerated using  $10^{-3}$  and  $10^{-4}$  dilutions on modified Stephenson medium via the MPN method. Total bacteria were counted using  $10^{-1}$  and  $10^{-2}$  dilutions on beef extract-peptone medium via spread plate method. Fungi were counted using  $10^{-2}$  and  $10^{-3}$  dilutions on Martin medium via spread plate method. Actinomycetes

were counted using  $10^{-2}$  and  $10^{-3}$  dilutions on PDA medium via spread plate method.

### 1.1.6 Isolation, Purification, and Identification of Ammonifying and Nitrosifying Bacteria

Inoculated plates were inverted in an incubator. Based on colony quantity, morphology, color, surface characteristics, and staining features, representative colonies were selected and streaked on appropriate solid media until pure cultures were obtained. Preliminary verification was conducted for target isolates. For ammonifying bacteria, single colonies were inoculated into beef extract-peptone broth (three replicates per strain). After 2 days of culture, a small aliquot was placed on a white porcelain colorimetric plate, and Nessler's reagent was added. Red-brown or yellow precipitate indicated  $\text{NH}_4^+$  production from peptone decomposition, confirming ammonifying activity. For heterotrophic nitrosifying bacteria, purified colonies were inoculated into Stephenson broth (three replicates). After 14 days, two drops of culture were placed on a white porcelain plate, and Griess reagents I and II were added. A red reaction indicated  $\text{NO}_2^-$  production, confirming nitrosifying bacteria. Additionally, two drops of culture were treated with diphenylamine reagent; a blue reaction indicated  $\text{NO}_3^-$  production, confirming nitrifying bacteria. Uninoculated medium served as control.

Strain morphology was observed through plate culture, Gram staining, and spore formation tests. Physiological and biochemical tests included methyl red test, starch hydrolysis, V-P test, D-glucose acid production, glucose gas production, nitrate reduction, catalase test, citrate utilization, D-mannitol utilization, gelatin hydrolysis, urea hydrolysis, indole production, oxidase test, and phenylalanine deaminase test.

For 16S rDNA molecular identification, PCR amplification and sequencing were performed using primers 1492r/27f. Bacterial DNA was extracted using a commercial kit. The 16S rRNA universal primers 27f (5'-AGAGTTTGGATCCTGGCTCAG-3') and 1492r (5'-GGTTACCTTGTTACGACTT-3') were used for PCR amplification of the rDNA region. The 25  $\mu\text{L}$  reaction mixture contained: ddH<sub>2</sub>O 9.5  $\mu\text{L}$ , 2 $\times$ Taq Master Mix 12.5  $\mu\text{L}$ , forward primer (10 pmol  $\cdot$  L<sup>-1</sup>) 1  $\mu\text{L}$ , reverse primer (10 pmol  $\cdot$  L<sup>-1</sup>) 1  $\mu\text{L}$ , and DNA template 1  $\mu\text{L}$ . PCR conditions were: 95°C pre-denaturation for 5 min; 35 cycles of 94°C denaturation for 1 min, 50–52°C annealing for 1 min, and 72°C extension for 2 min; final extension at 72°C for 5 min; storage at 4°C. PCR products were detected by 1% agarose gel electrophoresis and sequenced by Shanghai Hengyin Genome Research Center. Sequences were analyzed via BLAST in the GenBank database ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)).

## 1.2 Nitrogen Decomposition Activity of Isolated Strains

### 1.2.1 Experimental Materials

Fifteen ammonifying strains and five nitrosifying strains obtained from preliminary screening were used. Beef extract-peptone liquid medium, LB medium,

and modified Stephenson liquid medium were employed.

### 1.2.2 Experimental Design

- 1) Isolated ammonifying and nitrosifying strains were activated in LB liquid medium at appropriate temperatures for 12 h.
- 2) Activated cultures were inoculated at 4% (v/v) into 150 mL beef extract-peptone liquid medium (three replicates per strain) and incubated in a shaking incubator ( $130 \text{ r} \cdot \text{min}^{-1}$ ,  $30^{\circ}\text{C}$ ). Samples were collected at 0, 12, 24, 48, and 72 h to determine Kjeldahl nitrogen, organic nitrogen, and ammonia nitrogen contents.
- 3) For nitrosifying bacteria, activated cultures were inoculated at 4% into 150 mL modified Stephenson liquid medium (three replicates per strain) and incubated under the same conditions. Samples were collected at 0, 1, 3, 5, and 7 days to determine nitrate and nitrite nitrogen contents.

Ammonia nitrogen was measured by titration. Organic nitrogen content was calculated as Kjeldahl nitrogen minus ammonia nitrogen. Nitrate nitrogen was determined by phenoldisulfonic acid photometry, and nitrite nitrogen by N-(1-naphthyl)-ethylenediamine photometry.

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## Results

### 2.1 Microbial Population Dynamics and Strain Characteristics 2.1.1 Microbial Population Changes in Tobacco Root-Zone Soil

As shown in , except for higher fungal numbers in cow manure treatment and actinomycete numbers in chemical fertilizer treatment at 90 days, all microbial populations were higher during the vigorous growth stage (60 days) than at maturity (90 days), likely due to abundant soil nutrients promoting microbial growth during the vigorous period. Organic fertilizer application, alone or in combination with chemical fertilizer, can improve soil physicochemical properties and enhance microbial populations, with increased microbial activity facilitating the release of available nutrients from organic fertilizers.

At 60 days after transplanting, all microbial groups in organic fertilizer treatments were generally more abundant than in chemical fertilizer treatment, with bacterial, fungal, and actinomycete populations in straw and rapeseed cake treatments reaching significant levels. At 90 days, nitrosifying bacteria and fungal populations in organic treatments were significantly higher than in chemical fertilizer treatment, while bacterial and actinomycete populations were also elevated. Soil nitrosifying bacteria populations (representing the first stage of nitrification) were measured to reflect overall nitrifying activity. Since tobacco primarily absorbs nitrate nitrogen, nitrosifying bacteria promote the conversion of ammonium to nitrite and subsequently to nitrate. At maturity, nitrosifying

bacteria populations in cow manure, straw, and rapeseed cake treatments were significantly higher than chemical fertilizer by 101.75%, 104.39%, and 55.26%, respectively, indicating high nitrosifying activity in organic treatments during this stage. However, elevated nitrosifying activity can accelerate organic nitrogen mineralization, maintaining high ammonium and nitrate levels during the curing period, which is detrimental to proper leaf yellowing and may cause high nicotine content and reduced usability in upper leaves.

Fungi are important components of soil ecosystems and key decomposers involved in carbon and nitrogen cycling. Arbuscular mycorrhizal fungi form symbiotic associations that enhance nutrient uptake. The high fungal activity observed in organic treatments at maturity may promote rapid nutrient absorption, potentially making it difficult to maintain the low nitrogen status desirable for tobacco.

### 2.1.2 Characteristics of Strains Isolated from Tobacco-Planted Yellow Soil

Based on colorimetric reactions with Nessler's, Griess, and diphenylamine reagents, 11 deep yellow to orange ammonifying strains and 5 pink, red, or blue nitrosifying/nitrifying strains (A-4, A-6, A-8, A-17, X-4) were selected. All 15 strains underwent colony characterization, Gram staining, and physiological-biochemical testing. Based on surface features, spore formation, Gram staining, and reference to *Bergey's Manual of Determinative Bacteriology* and *Handbook of Common Bacterial System Identification*, preliminary genus-level identification was achieved. Strains A-9, A-11, A-12, A-19, and A-36 produced spores, were Gram-positive and catalase-positive, preliminarily identified as *Bacillus* spp. Strains A-4 and A-8 were short to medium-sized Gram-negative rods with active motility, oxidase-negative, slow glucose oxidation, gelatin liquefaction, and negative nitrate gas production, preliminarily identified as *Stenotrophomonas* spp. Other strains could not be definitively identified at this stage. shows that only three strains were isolated from chemical fertilizer-treated soils, while all others originated from organic fertilizer treatments.

### 2.1.3 16S rDNA Identification of Ammonifying and Nitrosifying Bacteria

Combined biochemical test results ( ) and molecular identification ( ) showed excellent correspondence between phenotypic and genotypic identification, confirming result reliability. Identification revealed that strains A-9, A-11, A-12, A-19, and A-36 represented different *Bacillus* species, all derived from various organic fertilizer treatments. Strains A-4 and A-8 belonged to *Stenotrophomonas* spp. Strains A-2 and A-10 were phenotypically similar but genotypically distinct *Burkholderia* spp. Strain A-5 was identified as *Flavobacterium* spp.

## 2.2 Dynamics of Nitrogen Content in Culture Solutions and Ammonia Decomposition by Nitrosifying Bacteria

### 2.2.1 Dynamics of Nitrogen Content in Culture Solutions

Kjeldahl nitrogen includes ammonia nitrogen and organic nitrogen compounds convertible to ammonium salts under test conditions, primarily proteins, amino acids, nucleic acids, urea, and synthetic organic nitrogen compounds with nitrogen in the -3 oxidation state. It excludes azo compounds, nitrates, nitrites, nitriles, and related nitrogenous compounds.

As shown in , Kjeldahl nitrogen content decreased to varying degrees in all strains after 72 h of culture, due to both microbial nitrogen assimilation and conversion of nitrogen to forms outside the Kjeldahl nitrogen pool through metabolic processes. Among the 15 strains, strains A-9, A-11, and A-36 showed significantly greater Kjeldahl nitrogen reduction than others, with decreases of 56.84%, 64.66%, and 55.46%, respectively. These high-performing strains were predominantly *Bacillus* spp. Notably, *B. stratosphericus* (A-12) and *B. megaterium* (A-36) showed substantial Kjeldahl nitrogen reduction within 12 h, while *G. stearothermophilus* (A-11) showed the maximum reduction, and *Stenotrophomonas* sp. (A-8) the minimum.

Organic nitrogen content (calculated as Kjeldahl nitrogen minus ammonia nitrogen) followed similar trends. *B. pumilus* (A-9), *G. stearothermophilus* (A-11), and *B. megaterium* (A-36) achieved the largest reductions, decreasing organic nitrogen by 84.74%, 92.74%, and 79.52% from initial levels. *B. megaterium* showed particularly rapid degradation, with 20.86% and 44.17% reductions at 12 h and 48 h, respectively, but minimal further reduction after 48 h, likely due to culture aging.

Ammonia nitrogen content increased linearly in most strains, except A-4 (*S. maltophilia*), A-6 (*Naxibacter* sp.), A-8 (*Stenotrophomonas* sp.), A-17 (*C. cellulans*), and X-4 (*S. fradiae*). *B. pumilus* (A-9) and *G. stearothermophilus* (A-11) showed the highest ammonia increases, significantly greater than other strains, followed by *B. altitudinis* (A-19) and *B. megaterium* (A-36). The low ammonia accumulation in the five exceptional strains may reflect weak ammonification activity or concurrent ammonia decomposition by nitrosifying bacteria. The decreasing ammonia trend from 48–72 h in these strains, consistent with reduced Kjeldahl and organic nitrogen degradation rates, suggests declining activity. Strains A-4, A-6, A-8, A-17, and X-4 showed positive Griess and diphenylamine reactions, indicating potential nitrosifying or nitrifying activities requiring further verification.

### 2.2.2 Dynamics of Nitrite and Nitrate in Nitrosifying and Nitrifying Bacteria Cultures

As shown in , strain A-8 (*Stenotrophomonas* sp.) exhibited both nitrosifying activity (converting ammonium to nitrite) and nitrifying activity (converting nitrite to nitrate). Strains A-17 (*S. maltophilia*), A-6 (*Naxibacter* sp.), and X-4 (*S. fradiae*) showed only nitrosifying activity, converting  $\text{NH}_4\text{-N}$  to  $\text{NO}_2\text{-N}$  in medium with  $\text{NH}_4$  as the sole nitrogen source. After one week of culture, nitrosifying activity ranked as  $\text{A-4} > \text{A-17} > \text{A-6} > \text{X-4}$ . Strains with both nitrosifying and nitrifying activities grew more slowly but maintained high activity through

day 7.

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## Discussion and Conclusion

Analysis of microbial populations across tobacco growth stages revealed three key findings: First, microbial populations were higher during the vigorous growth stage than at maturity under organic fertilizer treatments, consistent with previous reports that humic acid increases bacterial numbers during mid-growth but declines rapidly thereafter. Second, organic fertilizer treatments supported higher microbial populations than chemical fertilizer alone. Third, during the vigorous growth stage, organic fertilizer treatments significantly increased culturable bacteria, fungi, actinomycetes, and ammonifiers compared to chemical fertilizer. At maturity, organic treatments enhanced ammonifiers, nitrifying bacteria, fungi, and actinomycetes. Soil nutrient transformation occurs primarily through biological activity, with ammonifiers and nitrifying bacteria playing decisive roles in organic nitrogen decomposition. High microbial activity in organic-amended rhizosphere soils accelerates organic nitrogen mineralization, maintaining high available nitrogen levels at maturity. Therefore, developing nitrogen-decomposing microbial agents to regulate nitrogen release timing has significant practical value for tobacco production.

Isolation and identification results demonstrated that dominant strains with high ammonification activity originated exclusively from organic fertilizer treatments, confirming that organic amendment promotes active ammonifying bacteria in tobacco soils. Literature analysis of identified species confirmed the suitability of strains A-6, A-8, A-9, A-11, A-12, A-17, A-19, A-36, X-1, X-3, and X-4 as functional components for nitrogen-transformation biofertilizers without causing plant or soil diseases. Key literature findings include: *Burkholderia* spp. are used as biocontrol agents against fungal pathogens but can be opportunistic pathogens in cystic fibrosis; *Stenotrophomonas* spp. degrade organic pollutants and exhibit denitrifying and phosphorus-solubilizing activities valuable for bioremediation, though *S. maltophilia* is an emerging opportunistic pathogen; *Flavobacterium* spp. can cause infections; *Naxibacter* sp. may have cellulolytic activity; *Bacillus* spp. are robust biocontrol agents with broad antimicrobial spectra; *B. megaterium* is widely applied in phosphorus solubilization, nitrogen fixation, bioremediation, and enzyme production; *G. stearothermophilus* produces thermostable proteases; *B. pumilus* degrades organic phosphorus and controls pests; *Streptomyces* spp. promote growth of beneficial bacteria involved in nutrient cycling and disease suppression, with *S. fradiae* effectively degrading feather keratin.

Researchers typically assess ammonification indirectly by calculating organic nitrogen as total nitrogen minus ammonia, nitrate, and nitrite. This study improved the method by using beef extract-peptone medium containing only organic nitrogen, theoretically preventing nitrifying and nitrifying activities. Di-

rect measurement of Kjeldahl nitrogen and ammonia nitrogen effectively demonstrated ammonification capacity. *G. stearothermophilus* showed the greatest Kjeldahl nitrogen reduction, while *B. pumilus*, *G. stearothermophilus*, and *B. stratosphericus* achieved the highest organic nitrogen degradation rates, consistent with literature reports of 86.91–90.6% conversion efficiency under optimal conditions. Ammonifying activity was strongest in *B. pumilus*, *G. stearothermophilus*, *B. stratosphericus*, *B. altitudinis*, and *Stenotrophomonas* sp. Nitrosifying/nitrifying tests revealed that *Stenotrophomonas* sp. possessed both activities, while *S. maltophilia* is pathogenic. Therefore, suitable nitrosifying/nitrifying strains for application are *Nazibacter* sp. and *Cellulosimicrobium* spp.

Based on organic nitrogen decomposition, ammonia degradation, and literature analysis, eight highly efficient nitrogen-functional strains were selected for microbial agent formulation: *Nazibacter* sp., *Stenotrophomonas* sp., *B. pumilus*, *G. stearothermophilus*, *B. stratosphericus*, *C. cellulans*, *B. altitudinis*, and *B. megaterium*. Activity monitoring revealed that bacilli ammonifiers showed reduced organic nitrogen degradation after 48 h, likely due to culture maturation and aging, as bacilli can sporulate within 2 days under suitable conditions. In contrast, nitrosifying and nitrifying bacteria maintained strong activity after one week. Therefore, when preparing composite nitrogen-decomposing agents, nitrosifying and nitrifying bacteria should be added first, with ammonifying bacteria introduced during the final two days of fermentation to ensure high activity of all components.

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