

EFFECTS OF *ARTEMISIA ARGYI* RESIDUE ON COTTON GROWTH AND RHIZOSPHERE SOIL ECO-ENVIRONMENT

HE, L. M.^{1,2} – ZHANG, M. L.^{1,2} – WU, Q. F.^{1,2} – CAI, X.^{1,2,3} – WANG, X. Y.^{1,2} – TIAN, L.^{1,2} –
WANG, J. S.^{1,2*} – ZHANG, K. P.^{1,2*}

¹*College of Biology and Food Engineering, Anyang Institute of Technology, Anyang, China*

²*Henan Province Taihang Mountain Forest Pest Field Scientific Observation and Research Station, Linzhou, China*

³*College of Veterinary Medicine, Shanxi Agricultural University, Taigu, China*

**Corresponding authors*

e-mail: aygallwasp@163.com, zhangkunpengag@163.com;

phone: +86-139-3721-8864, +86-135-9810-3561;

fax: +86-372-290-9876

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Abstract. Cotton production, a vital agricultural sector, faces significant challenges due to soil degradation issues such as compaction and salinization. This study explored the impact of *Artemisia argyi* residue on cotton growth and rhizosphere soil eco-environment. The results demonstrated that *A. argyi* residue application significantly enhanced cotton growth, increasing plant height (38.23%-60.94%), stem diameter (43.59%-62.96%), and biomass (29.35%-48.26%) across developmental stages. *A. argyi* residue also improved soil microbial diversity, with notable increases in metabolic activity (Average Well Color Development, abbreviated as AWCD) and diversity indices (Shannon index increased by 43.2%). Soil fertility parameters, including organic matter (increased by 5.94%), total nitrogen (by 22.18%), available phosphorus (by 292.85%), and available potassium (by 172.34%), were markedly elevated. These findings highlight *A. argyi* residue as a sustainable soil amendment that promotes cotton productivity while enhancing soil health, offering a viable solution for agricultural waste recycling.

Keywords: *Artemisia argyi* residue, cotton cultivation, soil improvement, microbial diversity, sustainable agriculture, resource utilization

Introduction

The cotton industry occupies a central position in agricultural economies worldwide, serving as the principal raw material source for textile production and related manufacturing processes (Feng et al., 2022). Its supply chain encompasses multiple stages, from cultivation to garment production, making it a key driver of international trade and an essential strategic resource for national economic security. However, despite its close ties to agricultural modernization, cotton cultivation faces significant environmental challenges, including excessive water usage and pesticide contamination (Shahzadi et al., 2024). Furthermore, soil degradation issues such as compaction, salinization, and declining organic matter severely restrict cotton growth (Zhao and Chen, 2023). Soil compaction impedes root penetration, limiting water and nutrient absorption. Salinization hinders water uptake, disrupts the absorption of essential nutrients, and damages cell membrane stability (Abbas et al., 2025). Meanwhile, reduced organic matter weakens soil water retention, decreases microbial activity, and disrupts nutrient cycling (Liang et al., 2025). Addressing these challenges is crucial for

advancing sustainable cotton production and ensuring the long-term stability of the cotton industry.

Agricultural waste recycling plays a pivotal role in advancing sustainable agricultural development. In China, approximately 4 billion tons of agricultural waste—including crop straw and livestock manure—are generated each year. Improper disposal of these materials can result in substantial resource loss and environmental pollution (Wang et al., 2016). Although considerable progress has been made in waste valorization through energy conversion, fertilizer production, feed processing, and material applications, numerous challenges remain (Wei et al., 2020). The impact of agricultural waste on soil ecosystems exhibits complex dynamics. When properly returned to fields, it can effectively enhance soil structure and fertility by increasing humus content, promoting aggregate formation, and improving water/nutrient retention capacity (Li et al., 2025). Furthermore, it provides essential nutrients while reducing reliance on chemical fertilizers and associated pollution risks. Soil improvement is intrinsically linked to crop growth performance, where chemo-biological amendments demonstrate remarkable potential in optimizing soil structure, regulating pH balance, and improving nutrient availability, thereby creating favorable conditions for crop development (Dilly et al., 2011; Wang et al., 2020).

Artemisia argyi residue (AR) is a byproduct generated during the processing of *A. argyi* (mugwort), primarily derived from the extraction or crushing of its leaves. Its chemical composition varies significantly and may include organic compounds, inorganic salts, or trace metal elements (Wang et al., 2016). However, the inadequate utilization of AR has created dual problems of resource waste and environmental concerns (Huang et al., 2018). In traditional *A. argyi* processing, about 30% of the raw leaves ultimately become AR. This fiber-rich material containing volatile oils and flavonoids is frequently disposed of through simple incineration or landfilling, which not only leads to the loss of bioactive components but also releases pollutants including PM_{2.5} (Xia et al., 2014). Some farmers have attempted composting AR, but its high cellulose content results in an extended decomposition period. While traditional Chinese medicine processors have explored essential oil extraction from AR, industrial applications remain challenging due to issues such as pesticide residue contamination and high extraction costs. Current research on AR is predominantly limited to laboratory-scale antibacterial testing, failing to develop scalable application solutions. Due to this suboptimal utilization model, *A. argyi* cultivation suffers an economic loss of around 7500 yuan per hectare, which poses a significant barrier to the industry's long-term sustainability.

By integrating waste valorization and agroecological perspectives, this study endeavors to establish a scientific basis for AR utilization by characterizing its growth-promoting mechanisms in cotton and its regulatory effects on soil ecosystem functioning. Through pot experiments, we quantitatively analyze the regulatory effects of AR application on cotton growth and development, including morphological parameters (plant height, stem diameter, aboveground and underground biomass) and dynamic patterns of yield components. Furthermore, we elucidate AR-induced modulations in rhizosphere microbial functional diversity, revealing its potential mechanism for improving soil ecology through microbial mediation. Additionally, the study will evaluate AR's efficacy in enhancing key soil fertility indicators, exploring its potential as an organic amendment to improve soil quality and promote crop growth. This research provides theoretical support for AR resource utilization, contributes to solving soil ecological challenges in cotton cultivation, reduces resource waste and pollution, and promotes waste recycling and sustainable cotton field management. The findings hold

significant value for agricultural green development and are particularly meaningful for achieving ecological balance and yield enhancement under the “dual carbon” goals.

Materials and methods

Experimental materials

Cotton material: Zhongmiansuo 49, an early-maturing upland cotton (*Gossypium hirsutum* L.) variety sourced from the Cotton Research Institute, Chinese Academy of Agricultural Sciences. This variety is widely cultivated in China’s major cotton-producing regions, exhibiting strong adaptability and representativeness. It effectively reflects typical cotton growth responses, thereby enhancing the extensibility and practical value of the research findings.

A. argyi residue (AR): Processing byproduct from moxibustion product manufacturing, supplied by Anyang Jiutouxian Moxa Industry Co., Ltd. After processing, the yield of moxa typically ranges from 5% to 30%, meaning approximately 70% to 95% of the raw material is converted into AR. For example, in Anyang, the annual production of dried *A. argyi* ranges from 15,000 to 20,000 tons, corresponding to AR output of 13,000 to 17,000 tons. Nationwide, the annual production of AR can reach millions of tons.

Experimental design

On December 28, 2024, healthy cotton seeds were sterilized in 1% HgCl₂ for 15 min, rinsed 5 times with sterile water (1 min each), and soaked for 4-6 h. The seeds were sown in nutrient soil under controlled conditions (25°C, 16 h/8 h light/dark cycle) with adequate watering. The experiment included a control group (CK) without AR application and a treatment group (T) with AR incorporated into the nutrient soil (Pindstrup, Denmark) at a 1:3 volume ratio. Each group consisted of 40 pots with one plant per pot. The pots used had an inner diameter of 23 cm and a height of 18 cm. The 1:3 volume ratio of AR to nutrient soil was determined as a safe concentration through preliminary experiments, and is consistent with conventional application rates for organic waste in agriculture. This ratio ensures favorable substrate aeration and water retention capacity, while preventing inhibitory effects on plant growth caused by high salinity or an imbalanced carbon-to-nitrogen ratio.

Growth parameters (plant height, stem diameter, biomass) were measured at bud, flowering-boll, and boll-opening stages. Biolog-ECO microplates were employed to characterize rhizosphere microbial functional diversity according to standard protocols. Soil fertility parameters including organic matter, total nitrogen, available phosphorus and available potassium were assessed through standard assays. All groups received identical cultivation management.

Determination of cotton growth parameters

The measurement of cotton growth parameters is a critical step in evaluating the effects of AR application. Stem diameter enlargement enhances the plant’s structural support for its biomass, particularly during the flowering-boll and boll-opening stages when the plant bears more fruits and seeds. Meanwhile, the dry weights of aboveground and underground parts reflect the biomass accumulation of cotton plants, serving as key indicators of growth status (Tang et al., 2025).

In this study, systematic random sampling was conducted during the bud stage, flowering-boll stage, and boll-opening stage to periodically monitor major growth parameters, with five pots randomly selected for each group. Plant height was measured using a calibrated ruler, while stem diameter was determined with a vernier caliper. Additionally, aboveground and underground dry weights were analyzed to assess plant growth and nutrient allocation. The cotton plants were carefully uprooted to preserve root integrity. After removing large soil clumps, rhizosphere soil was collected (for Experiment *Measurement of rhizosphere soil microbial diversity*), and the roots were gently rinsed with clean water. Residual moisture on the root surface was blotted dry using filter paper. The plants were then cut at the cotyledon node, and both the aboveground parts and roots were subjected to enzyme deactivation at 105°C for 40 min, followed by drying at 80°C to a constant weight before measuring their dry mass.

All growth parameter measurements were conducted under consistent conditions, avoiding rainy days and midday strong sunlight to minimize environmental variability. Data collection was performed by fixed researchers to ensure standardized procedures.

Measurement of rhizosphere soil microbial diversity

The Biolog-ECO microplate method is a technique used to characterize microbial functional diversity by assessing the metabolic capacity of soil microbial communities toward 31 different carbon sources (Molnár, 2021). This method is relatively simple, efficient, accounting for its widespread adoption in soil microbial functional diversity studies. The principle involves the reduction of tetrazolium violet dye when microorganisms metabolize the carbon sources in the microplate wells, resulting in a color change. The metabolic potential of soil microbial communities was quantified using AWCD, computed as the mean absorbance change rate across all 31 carbon substrate wells during the 168-h incubation period. A higher AWCD value indicates stronger carbon source utilization and greater microbial abundance, while a lower value suggests weaker metabolic activity (Su et al., 2025).

Rhizosphere soil samples were collected from potted cotton plants at the bud stage, flowering-boll stage, and boll-opening stage. For each treatment, five replicates were randomly selected and thoroughly mixed. The soil was passed through a 2-mm sieve, and 10 g of fresh sample was transferred into a 250-mL Erlenmeyer flask containing 100 mL of sterile deionized water to obtain the initial 10^{-1} dilution. After 20 min of orbital shaking (200 rpm, 25°C), the suspension was gradiently diluted to 10^{-3} . Using a multichannel pipette, 150 μ L of the 10^{-3} soil suspension was inoculated into every well of a Biolog-ECO microplate.

Plates were incubated at 25°C for 7 d, and optical density (OD₅₉₀) was recorded at 24-h intervals with a BIOLOG reader. Functional diversity indices (McIntosh, Shannon i, and Simpson) were calculated using the 72-h OD data. Detailed descriptions and calculation formulas are given in *Table 1*.

Soil nutrient analysis

Soil samples were collected at the bud stage, flowering-boll stage, and boll-opening stage, with five replicates randomly selected and thoroughly mixed for each treatment. The determination of soil nutrient properties followed the procedures outlined in “Soil

Agrochemical Analysis” (Bao, 2000). Organic matter (OM) was measured using the potassium dichromate volumetric method with external heating; total nitrogen (TN) was determined using a K1100 fully automated Kjeldahl nitrogen analyzer; available phosphorus (AP) was extracted with 0.5 mol·L⁻¹ NaHCO₃ and measured by the molybdenum-antimony anti-spectrophotometric method; and available potassium (AK) was measured using a flame photometer.

Table 1. Description of functional diversity indices for soil microbial communities

Functional diversity indices	Calculation formulas	Description
AWCD	$AWCD = \sum (C - R)/n$	Assess the overall activity of soil microorganisms
McIntosh Index	$U = \sqrt{\sum n_i^2}$	Diversity index based on Euclidean distance in the multidimensional space of community species
Shannon Index	$H' = -\sum p_i \ln p_i$	Evaluate the species richness and evenness of the microbial community
Simpson Index	$D = 1 - \sum p_i^2$	Evaluate the dominance index of some of the most common species

C, the OD value of each micro-well; R, the OD value of the blank control well; n, 31 (types of carbon sources); n_i , the relative OD value of the ith well (C-R); $p_i = n_i / \sum (C - R)$

Data processing and statistical analysis

All raw data were first organized and visualized in Microsoft Excel 2010. Statistical analyses were then conducted in SPSS 20.0: an independent-samples *t*-test was employed to analyze the significance of differences between the control and treatment groups, with the significance level set at $\alpha = 0.05$.

Results

Effects of AR on cotton growth parameters

The analysis results of cotton growth parameters under different treatments are presented in Table 2. The findings demonstrate that *Artemisia* residue application significantly promoted cotton growth throughout all developmental stages. At the bud stage (T-A group), plants exhibited markedly greater height, stem diameter, and both above- and below-ground biomass relative to the control (CK-A). This growth-promoting effect persisted during the flowering-boll (T-B) and boll-opening stages (T-C), with all measured parameters showing significant improvements compared to their respective controls (CK-B and CK-C).

Table 2. Growth parameters and comparative analysis of cotton under different treatments

Growth stage	Treatment	Plant height (cm)	Stem diameter (cm)	Aboveground dry weight (g)	Underground dry weight (g)
Bud stage	CK-A	18.49 ± 0.80	0.27 ± 0.02	19.26 ± 0.45	2.42 ± 0.22
	T-A	25.57 ± 0.89*	0.44 ± 0.02*	25.32 ± 0.66*	3.25 ± 0.25*
Flowering-boll stage	CK-B	26.27 ± 1.16	0.32 ± 0.01	39.56 ± 0.54	6.23 ± 0.31
	T-B	42.28 ± 1.42*	0.50 ± 0.03*	58.64 ± 0.91*	9.16 ± 0.30*
Boll-opening	CK-C	36.50 ± 1.82	0.39 ± 0.03	51.89 ± 0.88	8.83 ± 0.42

stage	T-C	55.33 ± 3.82*	0.56 ± 0.02*	70.38 ± 1.56*	11.42 ± 0.38*
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Data in the table are the mean ± standard deviation of five replicates. * Indicates a significant difference between the control and treatment groups at each growth stage at the 0.05 level

Compared with the control, AR treatment elevated plant height by 38.23% at the bud stage, 60.94% at flowering-boll stage, and 51.59% at boll-opening stage. Corresponding increases in stem diameter were 62.96%, 56.25%, and 43.59%, indicating remarkable enhancement in plant architecture. Dry matter accumulation showed increases of 31.43% (aboveground) and 34.30% (underground) at the bud stage, 48.26% and 47.08% at the flowering-boll stage, and 35.68% and 29.35% at the boll-opening stage. The most pronounced effect was observed at the flowering-boll stage, suggesting that AR might enhance photosynthetic efficiency and nutrient uptake, thereby promoting biomass accumulation. These results provide theoretical support for the agricultural application of AR, with its growth-promoting effects potentially contributing to improved cotton yield and quality.

Effects of AR on rhizosphere soil microbial diversity

The AWCD values of cotton rhizosphere soil microbes in different treatment groups exhibited dynamic changes (Fig. 1). Across all growth stages, the AR-treated groups consistently outperformed their respective controls in AWCD values, demonstrating both faster metabolic activity increases and higher final metabolic potentials. At the bud stage, while both the control (CK-A) and AR-treated group (T-A) exhibited rising AWCD values over time, T-A displayed a markedly steeper growth trajectory, culminating in a significantly elevated endpoint. This trend became more pronounced at the flowering-boll stage, where T-B not only surged ahead of CK-B in metabolic rate but also achieved a substantially greater final AWCD value. By the boll-opening stage, the divergence further intensified: T-C not only sustained a sharper upward trend but also reached a significantly higher AWCD value than CK-C, underscoring the progressive and cumulative benefits of AR treatment on microbial metabolic activity. These results indicate that AR treatment consistently enhanced rhizosphere soil microbial activity at different growth stages, with the promotive effect strengthening over time. This improvement may be attributed to the rich organic matter and nutrients in AR, which provide energy and essential elements for microbial growth and reproduction.

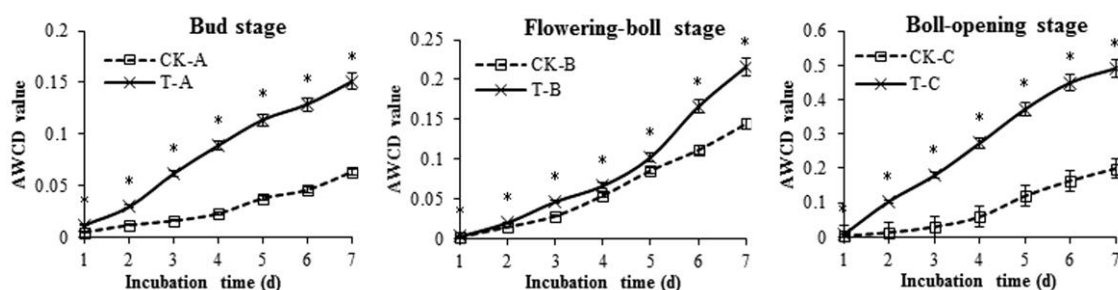


Figure 1. Changes in AWCD values of cotton rhizosphere soil microorganisms. CK-A: Control group at bud stage, T-A: AR-treated group at bud stage; CK-B: Control group at flowering-boll stage, T-B: AR-treated group at flowering-boll stage; CK-C: Control group at boll-opening stage, T-C: AR-treated group at boll-opening stage. The same abbreviations apply hereinafter. * Indicates a significant difference between the control and treatment groups at each incubation time at the 0.05 level

As shown in Figure 2, AR application (T groups) significantly enhanced soil microbial diversity indices compared to the control groups (CK) across all growth stages (A: bud, B: flowering-boll, C: boll-opening). For the McIntosh index, the T groups exhibited consistently higher values (4.39-4.77) than the CK groups (3.23-3.96), with the most pronounced increase (35.91%) observed in T-A (4.39) relative to CK-A (3.23). Similarly, the Shannon index demonstrated marked improvements in the T groups (2.85-3.25) compared to the CK groups (2.03-2.28). The highest value was recorded in T-C (3.25), reflecting a 43.17% increase over its corresponding control (CK-C: 2.27). Most notably, the Simpson index in the T groups (0.92-1.00) substantially exceeded that of the CK groups (0.66-0.88), with T-C approaching near-maximum diversity (0.998). These results demonstrate that AR treatment consistently improved microbial diversity across all three indices, with the most pronounced effects observed during the boll-opening stage (T-C). The Simpson index showed the greatest response to AR application, nearing the theoretical maximum (1.0) in T-C, suggesting a highly balanced microbial community structure. The Shannon index exhibited the largest relative increase (43.17%), indicating AR not only increased species richness (McIntosh) but also improved evenness in microbial distribution. Growth-stage dependency was evident, as the magnitude of improvement generally increased from bud stage (A) to boll-opening stage (C), implying prolonged AR exposure may further enhance microbial diversity. These findings collectively suggest that AR application effectively promotes soil microbial diversity and community stability in cotton cultivation, with progressive benefits observed throughout the growing season.

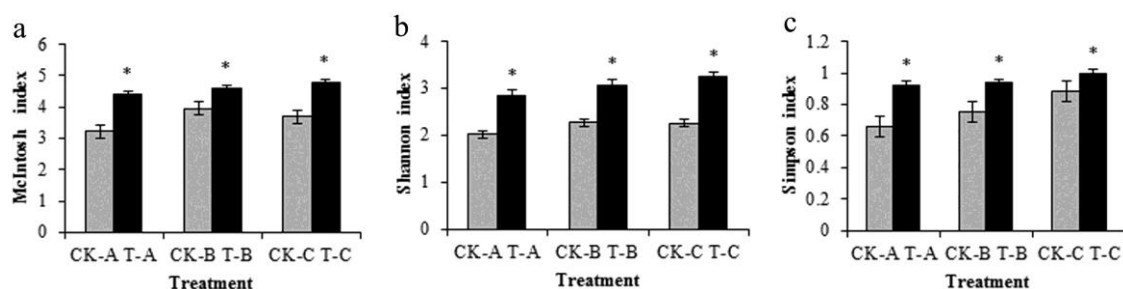


Figure 2. Changes in microbial diversity indices of cotton rhizosphere soil. * Indicates a significant difference between the control and treatment groups at each growth stage at the 0.05 level, and the same notation applies hereinafter

Effects of AR on soil fertility

Comparative analysis of soil fertility indicators (TN, OM, AP, and AK) revealed significant enhancements in AR-treated potted cotton (Group T) relative to controls (Group CK) (Fig. 3). The treatment consistently elevated all measured parameters across growth stages (A: bud; B: flowering-boll; C: boll-opening), with particularly dramatic improvements in AP and AK. TN increased by 14.29-22.17% in T groups (6.16-6.70 g/kg) versus CK (5.23-5.61 g/kg), peaking at T-A (6.39 g/kg; + 22.18%). OM showed moderate but consistent gains (752.94-764.68 g/kg in T vs 720.04-721.69 g/kg in CK), with maximal improvement (5.94%) at flowering-boll stage (T-B: 764.68 g/kg). Most strikingly, AP increased 2.9-fold in T groups (236.02-310.82 mg/kg) compared to CK (79.12-196.61 mg/kg), with T-C demonstrating exceptional 292.9%

elevation (310.82 vs 79.12 mg/kg). Similarly, AK levels surged by 117.33-172.34% in T groups (6.52-8.50 g/kg) relative to CK (2.82-3.55 g/kg), peaking at T-C (7.68 g/kg; + 172.34%).

These findings demonstrate AR's sustained efficacy as a soil amendment throughout cotton development, with particularly profound effects on AP and AK. The treatment's stage-independent performance and exceptional nutrient enhancement capacity position it as a promising sustainable agricultural practice for cotton cultivation systems.

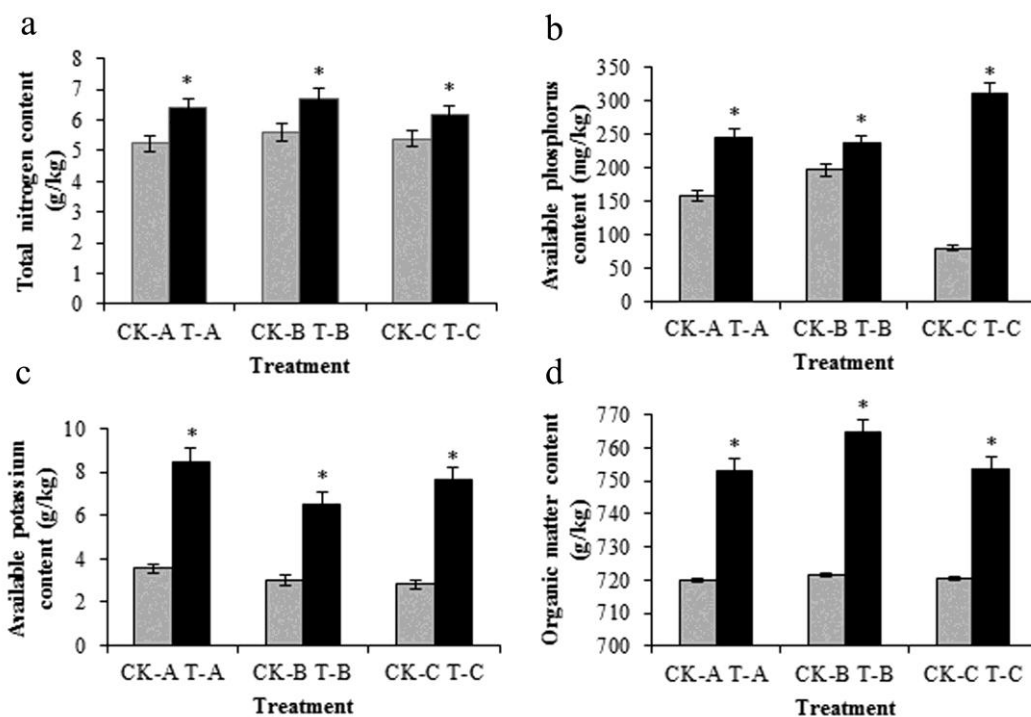


Figure 3. Comparative analysis of soil nutrient contents in cotton across treatment groups

Discussion

This study systematically examined the effects of AR application on cotton growth, rhizosphere soil microbial diversity, and soil fertility. The results demonstrated that AR significantly enhanced cotton growth parameters, improved soil microbial activity and diversity, and increased key soil fertility indicators, providing valuable insights for sustainable cotton cultivation and agricultural waste utilization.

Effects of AR on cotton growth parameters

The application of AR markedly improved cotton growth across all developmental stages, with significant increases in plant height (38.3%-60.9%), stem diameter (43.6%-62.9%), and biomass (29.3%-48.2%). These findings align with previous studies highlighting the role of organic amendments in promoting plant growth by enhancing soil structure and nutrient availability (Das et al., 2006). The pronounced effects during the flowering-boll stage suggest that AR may optimize photosynthetic efficiency and nutrient uptake, critical for biomass accumulation and yield formation. These results demonstrate that organic amendments exert significant physiological regulatory effects on cotton during the flowering-boll stage, which is consistent with previous research (Das et al.,

2006). The progressive enhancement in growth parameters from the bud stage to the boll-opening stage further indicates that AR provides sustained benefits throughout the cotton growth cycle, likely due to the gradual release of nutrients and bioactive compounds.

Effects of AR on rhizosphere soil microbial diversity

AR application significantly enhanced both microbial metabolic activity (AWCD values) and diversity indices (McIntosh, Shannon, and Simpson), indicating comprehensive improvement in soil microbiome functionality. These findings align with the research on organic amendments (Bonanomi et al., 2020) while demonstrating AR's superior efficacy in promoting microbial richness and evenness, as evidenced by the near-maximum Simpson index (0.998) at boll-opening stage. The higher microbial activity in AR-treated soil suggests enhanced carbon utilization capacity, which is crucial for nutrient cycling and soil health (Yazdanpanah, 2016). The stage-dependent effects show particular promise, with a 43.17% Shannon index increase at boll-opening stage suggesting AR helps sustain microbial functions during critical reproductive phases. The mechanism likely involves multiple pathways: substrate diversity theory through heterogeneous organic compounds, nutrient bridge effects via improved TN and AK stoichiometry, and potential bioactive stimulation (Nelson, 2018; Sinsabaugh and Follstad Shah, 2012; Spohn, 2016; Wang, 2025). Notably, the synchronous improvement of microbial parameters and fertility indices (including the 292.85% AP increase) positions AR as a multi-functional amendment addressing both immediate nutrient needs and long-term biological soil health. These results demonstrate AR's unique capacity to restore degraded soils through fostering robust microbial ecosystems while enhancing nutrient availability, making it particularly valuable for intensive cropping systems where microbial diversity depletion often limits sustainability. The cumulative benefits observed across growth stages further suggest AR's potential for creating self-reinforcing soil health improvement cycles in continuous cotton cultivation systems.

Effects of AR on soil fertility

AR application significantly improved soil fertility parameters, with OM increasing by 4.57%-5.94%, TN by 14.29%-22.18%, AP by 20.04%-292.85%, and AK by 117.33-172.34%, demonstrating its effectiveness as a multifunctional soil amendment. The remarkable 292.85% AP increase at boll-opening stage is particularly noteworthy, as it addresses phosphorus limitation in cotton production through mechanisms including reduced fixation and enhanced microbial mineralization (Iqbal et al., 2023). The sustained nutrient improvements across growth stages indicate AR's slow-release properties, supporting long-term soil productivity while reducing reliance on chemical fertilizers (Aasmi et al., 2022). The substantial AK enhancement (up to 172.34%) further highlights AR's ability to correct common nutrient deficiencies in intensive cropping systems (Nouri et al., 2019). These findings position AR as a sustainable solution for improving soil fertility and nutrient cycling efficiency in cotton cultivation, with potential benefits for environmental sustainability and long-term soil health maintenance.

Conclusions

This study establishes *Artemisia* residue (AR) as a highly effective organic soil amendment with multifaceted benefits for sustainable cotton production. The pronounced improvements in plant growth, soil microbial activity, and nutrient

availability underscore AR's potential to serve as an eco-friendly alternative to conventional inputs, thereby supporting agricultural waste valorization and reducing dependency on synthetic fertilizers.

The consistent enhancement observed across cotton developmental stages—especially during critical periods such as the flowering-boll stage—suggests that AR functions through sustained-release mechanisms, progressively enriching rhizosphere ecology and soil fertility. These findings highlight the practical relevance of AR in promoting synergistic plant-microbe interactions and enhancing nutrient use efficiency, which are essential for long-term soil health and system resilience.

From a broader perspective, this work contributes to advancing circular agricultural practices by converting waste biomass into a valuable resource. Future efforts should prioritize field-scale validation to refine application protocols, assess economic feasibility, and evaluate long-term impacts under diverse agroecological conditions. Ultimately, the integration of AR into cotton cultivation systems could play a significant role in developing more sustainable and productive agricultural systems.

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