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Grass cultivation regulates soil hydrogen cyanide formation in continuously cropped karst peach orchards by modulating precursor biosynthesis via nitrogen availability

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Grass cultivation regulates soil hydrogen cyanide formation in continuously cropped karst peach orchards by modulating precursor biosynthesis via nitrogen availability

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Abstract

Aims Orchard grass cultivation is widely used to improve soil conditions and alleviate continuous cropping obstacles, yet its capacity to reduce soil hydrogen cyanide (HCN, an autotoxin in replant orchard soils) and associated mechanisms remain unclear.

Methods A field trial was carried out in a degraded continuously cropped peach orchard in the karst mountains of southwest China. Two non-nitrogen-fixing grasses (Dactylis glomerata, Lolium perenne) and the nitrogen-fixing legume Trifolium repens were planted as monocultures and mixtures. We measured soil physicochemical properties, enzyme activities, bacterial and fungal communities, and quantified soil HCN concentrations.

Results Monocultures of D. glomerata and L. perenne, plus the three-species mixture, markedly lowered HCN: 90.29%, 62.69% and 81.75% reduction in 0–20 cm topsoil, and 47.67%, 52.93% and 82.83% reduction in 20–40 cm subsoil relative to control. By contrast,

white clover monoculture and its two-species mixtures did not reduce topsoil HCN and significantly increased subsoil HCN. PLS-SEM revealed total nitrogen, alkali-hydrolyzable nitrogen, sucrase and alkaline phosphatase as key drivers of HCN accumulation. Effective grass treatments reduced soil nitrogen availability, limiting nitrogen translocation to peach trees and suppressing biosynthesis of HCN precursors in plant tissues, thereby decreasing soil HCN.

Conclusions This study highlights species-specific effects of cover crops on autotoxicity mitigation. We recommend monocultures of non-nitrogen-fixing grasses for managing HCN pollution in karst replant peach orchards.

Keywords: grass cultivation; continuous cropping stress; hydrogen cyanide; mitigation mechanism; karst soil

Introduction

Continuous cropping obstacles severely restrict the growth, yield formation, and fruit quality of perennial fruit trees, threatening the sustainable development of commercial orchard systems worldwide (Laurent et al., 2008; Lei et al., 2022; Mei et al., 2022; Wang, 2024; Xiong et al., 2024; Xiao et al., 2023). As a sustainable soil management practice, cover cropping can effectively improve soil structure, modulate soil nutrient cycling, and reshape rhizosphere microbial community composition and function (Fu, 2020; Haruna, 2020; Li, 2022, 2023; Xiang, 2023; Culumber et al., 2019; Xu, 2013; Wang et al., 2023; Yu et al., 2023; He et al., 2023; Jiang, 2019; Yang, 2024; Zhuo et al., 2025). Due to these ecological benefits, grass cultivation has been widely applied for soil remediation in intensive fruit orchards (Guan et al., 2024; Lee et al., 2009; Xiao et al., 2022). Previous studies have confirmed that crop–forage rotation and cover crop systems can effectively alleviate continuous cropping stress in grain and cash crop fields (Li, 2019; Qin et al., 2017; Sun et al.,

2025; Xu et al., 2024). However, the functional variability and intrinsic mechanisms of orchard grass cultivation in mitigating continuous cropping obstacles remain insufficiently understood.

Hydrogen cyanide (HCN) is a critical autotoxin driving continuous cropping disorders in Rosaceae fruit tree systems (Handriati et al., 2019; Yin, 2018). Excessive soil HCN not only suppresses root growth and nutrient absorption of fruit trees but also disrupts the structural stability and functional balance of soil microbial communities. Cover cropping is generally considered capable of modifying soil nutrient status and microbial assemblages, thereby alleviating continuous cropping stress. Soil organic matter can adsorb cyanide compounds, and specific soil microorganisms are theoretically capable of HCN degradation (Rennert et al., 2002; Ajao et al., 2025). Nonetheless, certain cyanogenic grass species may increase soil HCN accumulation after long-term field coverage (Ballhorn and Elias, 2014; Kuo et al., 2024; Yu et al., 2016; Zagrobelny and Møller, 2011). To date, systematic research regarding the effects of different cover crop species and sowing patterns on soil HCN dynamics in Rosaceae orchards, as well as the underlying regulatory mechanisms, remains limited, constraining the targeted optimization of orchard ground cover management.

Karst peach orchards in southwest China are pivotal ecological and economic systems for rocky desertification control and rural ecological revitalization (Xiang et al., 2025). However, long-term monoculture has induced severe continuous cropping degradation in local peach orchards, severely restricting industrial sustainability. Currently, no studies have systematically reported the responses of soil HCN accumulation and microecological characteristics to grass cultivation in karst peach orchard soils. To fill this research gap, we conducted a two-year field trial using three typical cover crop species with contrasting functional traits (non-nitrogen-fixing cool-season grasses and nitrogen-fixing legume) under monoculture and mixed sowing modes. The primary objectives were to clarify: (1) whether

orchard grass cultivation can mitigate soil HCN accumulation under continuous cropping conditions; (2) how grass species identity and sowing patterns regulate soil HCN reduction efficiency; and (3) the core mechanism by which grass cultivation modulates soil HCN formation in karst continuous cropping peach orchards.

Materials and Methods

Study Area Overview

The field experiment was carried out in Suosai Village, Chenjiazhai Town, Puding County, Anshun City, Guizhou Province, China (26.37°N, 105.81°E). The region has a history of large-scale peach cultivation exceeding 20 years, with widespread severe continuous cropping problems, including declined fruit yield and quality and low survival rate of newly replanted seedlings. The study site belongs to a typical subtropical monsoon climate zone, with an altitude of approximately 1200 m, annual sunshine duration of 1800 h, annual mean temperature of 15.2 °C, a frost-free period of 301 d, and average annual precipitation of 1200 mm. The soil type is calcareous soil characterized by thin soil layers and exposed bedrock, typical of karst fragile ecosystems.

Experimental Design

The field trial was implemented from August 2024 to August 2025 in a 15-year-old continuously cropped peach orchard. Uniform and healthy grass seeds were selected for sowing. A completely randomized block design was adopted for field arrangement. Grass seeds were sown in a 1 m × 1 m area centered on each peach tree trunk. Three typical cover crop species with distinct functional traits were selected: two non-nitrogen-fixing grasses (*Dactylis glomerata* L., perennial cock's-foot; *Lolium perenne* L., perennial ryegrass) and one nitrogen-fixing legume (*Trifolium repens* L., white clover). The experimental treatments

included three monocultures, four mixed sowing combinations, and a no-grass control (CK), with three biological replicates for each treatment, forming 24 experimental plots in total. Detailed sowing treatments are listed in Table 1.

Soil Sample Collection

Soil sampling was conducted in August 2025. For each plot, soil samples were collected from four corners around the peach tree trunk at two soil depths (0–20 cm and 20–40 cm). The four subsamples from the same depth were fully mixed to form one composite sample per depth per treatment, generating 48 soil samples in total. All fresh soil samples were immediately placed in an ice box and transported to the laboratory within 6 h. Each composite sample was divided into three subsamples for subsequent analysis: (1) air-dried, root-removed, and 2-mm sieved subsample for the determination of soil physicochemical properties and enzyme activities; (2) fresh soil stored in PTFE-capped amber bottles at 4 °C for soil HCN content measurement within 48 h; (3) liquid nitrogen-quick-frozen subsamples stored at −80 °C, which were delivered on dry ice to Shanghai Majorbio Bio-pharm Technology Co., Ltd. for high-throughput microbial sequencing within one week.

Soil Sample Determination Methods

Soil organic matter was determined via the potassium dichromate oxidation method (Wan, 2003). Soil total nitrogen was measured using the Kjeldahl digestion method (Feng, 2018). Alkali-hydrolyzable nitrogen was determined by the alkaline diffusion method (Agricultural Press, 2014). Available phosphorus was extracted by sodium bicarbonate and measured using the molybdenum-antimony colorimetric method (Lu, 2000). Available potassium was quantified via flame photometry (Lin, 2015). Soil pH was determined using a potentiometric method at a soil-to-water ratio of 2.5:1 (Gu, 2025).

The activities of soil urease, sucrase, catalase, and alkaline phosphatase were determined

using commercial detection kits following standard protocols (An, 2025). Soil cyanide ion (CN^-) concentration was measured using a spectrophotometric cyanide assay kit according to the modified method described by Zhu (2016).

For soil microbial community analysis, total soil genomic DNA was extracted using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, USA). DNA quality and purity were assessed by 1% agarose gel electrophoresis and NanoDrop 2000 spectrophotometry (Thermo Scientific, USA). The bacterial 16S rRNA gene (V3–V4 region) was amplified using primers 338F and 806R with specific barcodes. PCR products were purified using a DNA gel recovery kit and quantified with a Qubit 4.0 fluorometer. Sequencing libraries were constructed using the NEXTFLEX Rapid DNA-Seq Kit and sequenced on the Illumina PE300 platform at Majorbio Biotechnology Co., Ltd.

Data Statistical Analysis

Basic data sorting and calculation were performed in Microsoft Excel 2021. One-way ANOVA with homogeneity of variance test and LSD multiple comparison was conducted using IBM SPSS 26.0, with $p < 0.05$ defined as statistically significant. Bar graphs of soil physicochemical properties, enzyme activities, and HCN contents were plotted using GraphPad Prism 10.1.2. Microbial community analysis and visualization were completed on the Majorbio BioCloud platform. Environmental factor correlation heatmaps were generated via the CNSknowall analysis platform. Partial least squares structural equation modeling (PLS-SEM) was performed using Smart-PLS 4.1.0.9 to elucidate the causal relationships among soil properties, enzyme activities, microbial communities, and HCN accumulation.

Results

Responses of Soil Physicochemical Properties to Different Grass Cultivation

Patterns

In the 0–20 cm topsoil layer, compared with the control, white clover monoculture (C) increased soil total nitrogen by 14.98%, while perennial cock's-foot monoculture (A) and perennial ryegrass monoculture (B) reduced total nitrogen, with treatment A showing a significant reduction of 38.65%. Among mixed treatments, AB and BC mixtures significantly increased total nitrogen by 50.48% and 12.02%, respectively, whereas the three-species mixture (ABC) significantly decreased total nitrogen by 58.68% (Figure 1-a). For alkali-hydrolyzable nitrogen, monoculture A and B reduced the content by 43.08% and 41.08%, respectively, while AB and BC mixtures increased hydrolyzable nitrogen by 59.80% and 47.96%. The ABC mixture significantly reduced alkali-hydrolyzable nitrogen by 64.05% (Figure 1-c).

For soil available potassium, monoculture A and B decreased the content by 64.07% and 73.74%, while white clover monoculture dramatically increased available potassium by 811.89%. All two-species mixtures increased available potassium, and the three-species mixture reduced available potassium by 66.49% (Figure 1-e). Soil available phosphorus was extremely elevated (2161.66%) in white clover monoculture, while no significant differences were observed in grass monocultures A and B. The BC mixture showed the highest increase in available phosphorus (6265.71%), whereas the ABC mixture remained comparable to the control (Figure 1-g). Soil organic matter was significantly reduced by 21.79% in treatment B, increased in all two-species mixtures (maximum 110.01% in AB), and unchanged in the three-species mixture (Figure 1-i). Soil pH slightly increased in treatment B and ABC mixture and decreased in the BC mixture (Figure 1-k), with no substantial variation in other treatments.

In the 20–40 cm subsoil layer, soil physicochemical properties exhibited similar overall variation trends to the topsoil, with treatment-specific differences in variation magnitude. The

total nitrogen of monoculture A and B showed no significant difference from the control in subsoil, differing from the significant depletion observed in topsoil (Figure 1-b). The alkali-hydrolyzable nitrogen of white clover monoculture increased by 15.05% in subsoil (Figure 1-d). The available phosphorus of the BC mixture shifted from a significant increase in topsoil to a significant decrease (32.97%) in subsoil (Figure 1-h). The organic matter of treatment B recovered to the control level in subsoil (Figure 1-j), and the AC mixture significantly reduced subsoil pH by 6.81% (Figure 1-l).

Variations in Soil Enzyme Activities Under Different Grass Cultivation Treatments

In the 0–20 cm soil layer, soil urease activity decreased significantly by 15.81% in treatment A but increased in treatments B and C and the BC mixture (Figure 2-a). Soil sucrase activity was universally suppressed in effective HCN-reducing treatments, with significant reductions in monoculture B (65.79%), BC mixture (37.52%), and ABC mixture (56.96%) (Figure 2-c). Catalase activity decreased in AC and BC mixtures but increased in the three-species mixture (Figure 2-e). Alkaline phosphatase activity was significantly inhibited in monoculture A, AC, BC, and ABC treatments (Figure 2-g).

In the 20–40 cm subsoil layer, enzyme activity variations were partially distinct from the topsoil. The BC mixture showed no significant subsoil urease activity, while the ABC mixture significantly reduced urease activity by 33.77% (Figure 2-b). Subsoil sucrase activity was significantly decreased in AB and AC mixtures (Figure 2-d). Additionally, monoculture grass treatments and the AB mixture showed no significant catalase variation in topsoil but exhibited significant catalase inhibition in subsoil (Figure 2-f).

Differential Responses of Soil Hydrogen Cyanide Content to Grass Cultivation

In the 0–20 cm topsoil, all grass treatments reduced soil HCN content relative to the control, but only monoculture A, monoculture B, and the three-species mixture achieved

192 significant HCN reduction, with reduction rates of 90.29%, 62.69%, and 81.75%,
193 respectively. The HCN reduction in white clover monoculture and two-species mixtures was
194 statistically insignificant (Figure 3-a).

195 In the 20–40 cm subsoil layer, monoculture A, B, C, and ABC mixture significantly
196 reduced HCN by 47.67%, 52.93%, 24.24%, and 82.83%, respectively. In contrast, AB, AC,
197 and BC two-species mixtures significantly increased subsoil HCN concentrations by 39.64%,
198 4.85%, and 31.51%, respectively (Figure 3-b).

199 Overall, monocultures of two non-nitrogen-fixing grasses and the three-species mixed
200 treatment achieved efficient and consistent HCN mitigation across both soil layers. Other
201 sowing patterns failed to reduce topsoil HCN and even aggravated subsoil HCN
202 accumulation, showing strong species- and pattern-dependent effects.

203 **Shifts in Soil Bacterial and Fungal Community Structure**

204 Grass cultivation significantly altered soil microbial diversity and community assembly
205 (Supplementary Table S1). In topsoil, the AB mixture significantly reduced bacterial richness
206 (Sobs, Chao) and diversity (Shannon) and increased Simpson index, while other treatments
207 slightly improved bacterial diversity. In subsoil, A and B monocultures increased bacterial
208 diversity, whereas the ABC mixture reduced bacterial diversity. Bray–Curtis distance-based
209 β-diversity analysis showed that the bacterial community of the monoculture A treatment was
210 distinct from that of the control (CK) in the topsoil but became similar to it in the subsoil,
211 whereas the two-species mixtures clustered with the control and the three-species mixture
212 formed a distinct bacterial assemblage (Supplementary Figure S1a-b).

213 For soil fungi, topsoil fungal richness showed no significant treatment differences, while
214 white clover-containing treatments reduced fungal Shannon diversity. In subsoil, treatment A
215 and ABC mixture decreased fungal richness, whereas all two-species mixtures increased

fungal richness. Fungal β-diversity further verified that single grass treatments and three-species mixtures significantly reshaped fungal community structure (Supplementary Figure S1c-d).

The dominant bacterial phyla included Pseudomonadota, Actinobacteriota, Acidobacteriota, and Chloroflexota. All grass treatments increased the relative abundance of Chloroflexota, with significant increases in treatments A, B, and ABC. The dominant fungal phyla were Ascomycota, unclassified Fungi, and Mortierellomycota. Correlation heatmaps revealed that topsoil HCN was significantly negatively correlated with Chloroflexota, Latescibacterota, and Gemmatimonadota. Subsoil HCN was negatively correlated with Chloroflexota, GAL15, and Methylomirabilota and positively correlated with Verrucomicrobiota (Figure 4-b). For fungi, topsoil HCN was positively correlated with Rozellomycota and negatively correlated with Glomeromycota and unclassified fungi (Figure 4-c).

PICRUSt2 functional prediction showed abundant nitrogen metabolism functional genes in soil microbial communities, while no typical cyanide-degrading functional genes were detected in either soil layer (Supplementary Figure S2).

Correlations Between Soil Properties, Enzyme Activity, and HCN Content

Spearman correlation analysis demonstrated that soil HCN concentration was significantly positively correlated with total nitrogen, alkali-hydrolyzable nitrogen, organic matter, available potassium, sucrase, and alkaline phosphatase, and significantly negatively correlated with soil pH and catalase activity (Figure 5).

PLS-SEM Analysis of HCN Regulatory Pathways

PLS-SEM was constructed to clarify the direct and indirect driving factors of soil HCN

accumulation (Figure 6). The results showed that soil physicochemical properties (total nitrogen, alkali-hydrolyzable nitrogen, organic matter) and soil enzyme activities (sucrase, alkaline phosphatase) exerted significant positive effects on soil HCN content. Although Chloroflexota and Glomeromycota were closely associated with soil nutrient and enzyme variables, microbial community variation showed no significant direct effect on soil HCN accumulation. These results indicated that soil nitrogen availability and related enzyme activities, rather than microbial direct degradation, were the core drivers regulating soil HCN dynamics in karst peach orchards.

Discussion

This study confirmed that monocultures of perennial cock's-foot and perennial ryegrass, as well as the three-species mixed cultivation, achieved efficient soil HCN mitigation across topsoil and subsoil layers, whereas white clover-dominated mixed treatments failed to reduce HCN and even increased subsoil HCN accumulation. These distinct differences are primarily attributed to the functional traits of different grass species and their differential regulation of soil nitrogen cycling.

Previous studies have confirmed that cyanogenic glycoside synthesis is mainly confined to specific genera such as *Sorghum*, while cool-season grasses including perennial cock's-foot and perennial ryegrass lack complete cyanogenic synthesis pathways (Cheeke, 1995; Foito et al., 2009; Goslee et al., 2017; Shan et al., 2026). White clover exhibits variable cyanogenic characteristics depending on cultivars, but its HCN production capacity is extremely limited under field conditions (Gabriel et al., 2023; Kuo et al., 2023; Olsen et al., 2021). Combined with our finding that grass monoculture significantly reduced soil HCN compared with the blank control, we verified that the soil HCN in continuously cropped peach orchards was not derived from grass exudation or decomposition but originated from

the autotoxic metabolism of peach trees themselves.

Although correlation analysis showed that Chloroflexota and Gemmatimonadota were negatively correlated with soil HCN, PICRUSt2 functional prediction failed to identify canonical cyanide-degrading genes in soil microbial communities. Instead, core nitrogen metabolism genes were highly enriched, indicating that microorganisms regulate soil HCN indirectly by mediating soil nitrogen transformation rather than through direct HCN biodegradation.

Soil HCN accumulation in Rosaceae orchards originates from the hydrolysis of plant-synthesized cyanogenic glycosides (Liu, 2017; Sher et al., 2023; Sun, 2025). Nitrogen availability is a critical positive regulator of cyanogenic glycoside biosynthesis in fruit trees. High soil nitrogen levels upregulate the key enzyme activities of CYP79A1 and CYP71E1 in the cyanogenic synthesis pathway, promoting precursor compound accumulation and ultimately increasing HCN release (Busk and Møller, 2002; Kongsawadworakul et al., 2009; Neilson et al., 2013; Pičmanová et al., 2015). Consistent with previous physiological findings that high nitrogen enhances plant cyanogenic metabolism (Astuti et al., 2019; Gleadow and Møller, 2014; Woldeyohannes et al., 2025), our field results confirmed that HCN content increased significantly in high-nitrogen soil induced by nitrogen-fixing clover mixtures, while HCN decreased significantly in nitrogen-depleted soil under non-fixing grass monocultures and three-species mixtures.

The differential nitrogen regulation capacity of cover crops originates from their inherent functional traits. Non-nitrogen-fixing perennial cock's-foot and ryegrass have strong root nitrogen absorption capacity, effectively depleting soil mineral nitrogen and reducing nitrogen availability (Bloor et al., 2015; Personeni and Loiseau, 2005). In contrast, white clover, as a leguminous plant, promotes soil nitrogen accumulation via biological nitrogen fixation (Fu et al., 2020). Mixed sowing of ryegrass and white clover further increases soil

nitrogen pools through legume–grass synergistic nitrogen input (Song et al., 2016). Notably, the three-species mixture significantly reduced soil nitrogen because interspecific competition suppressed white clover growth and nitrogen fixation, while grass species maintained strong nitrogen uptake capacity (Griffith et al., 2000; Lazzarotto et al., 2009; Qiao et al., 2022). The abnormal nitrogen increase in the two-grass mixture may be attributed to litter decomposition and short-term soil nitrogen priming effects (Van Eekeren et al., 2010), which requires further long-term verification.

In summary, orchard grass cultivation regulates soil nitrogen availability in a species-dependent manner, further modulating the biosynthesis of HCN precursor compounds in peach trees and ultimately controlling soil HCN accumulation. Effective HCN mitigation does not rely on microbial terminal degradation but on source inhibition via nitrogen nutrient regulation.

Conclusion

This study systematically clarified the species-specific effects and nitrogen-mediated mechanism of orchard grass cultivation on soil HCN mitigation in karst continuously cropped peach orchards. Non-nitrogen-fixing grass monocultures (perennial cock's-foot, perennial ryegrass) and three-species mixed cultivation significantly depleted soil nitrogen availability, inhibited the biosynthesis of HCN precursor compounds in peach trees, and achieved efficient soil HCN reduction. In contrast, white clover-containing mixed treatments increased soil nitrogen pools, promoted tree HCN precursor synthesis, and failed to alleviate soil HCN contamination, even exacerbating subsoil HCN accumulation. Soil nitrogen availability and nitrogen-regulated enzyme activities, rather than direct microbial degradation, were the core drivers of soil HCN dynamics. This study reveals a novel source-control mechanism for orchard autotoxicity mitigation: rational grass cultivation regulates soil nitrogen cycling to

suppress plant autotoxin synthesis. We recommend the monoculture of low-protein, non-nitrogen-fixing grass species as a practical and efficient field management strategy to relieve HCN-mediated continuous cropping obstacles in high-nitrogen karst peach orchard soils. Given the regional soil nitrogen characteristics and short-term trial duration, future long-term positioning experiments are required to verify the stability and universality of this regulation mechanism.

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Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement The raw datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Ethics statement This field experiment did not involve endangered or protected species, and no human or animal subjects were included in this study.

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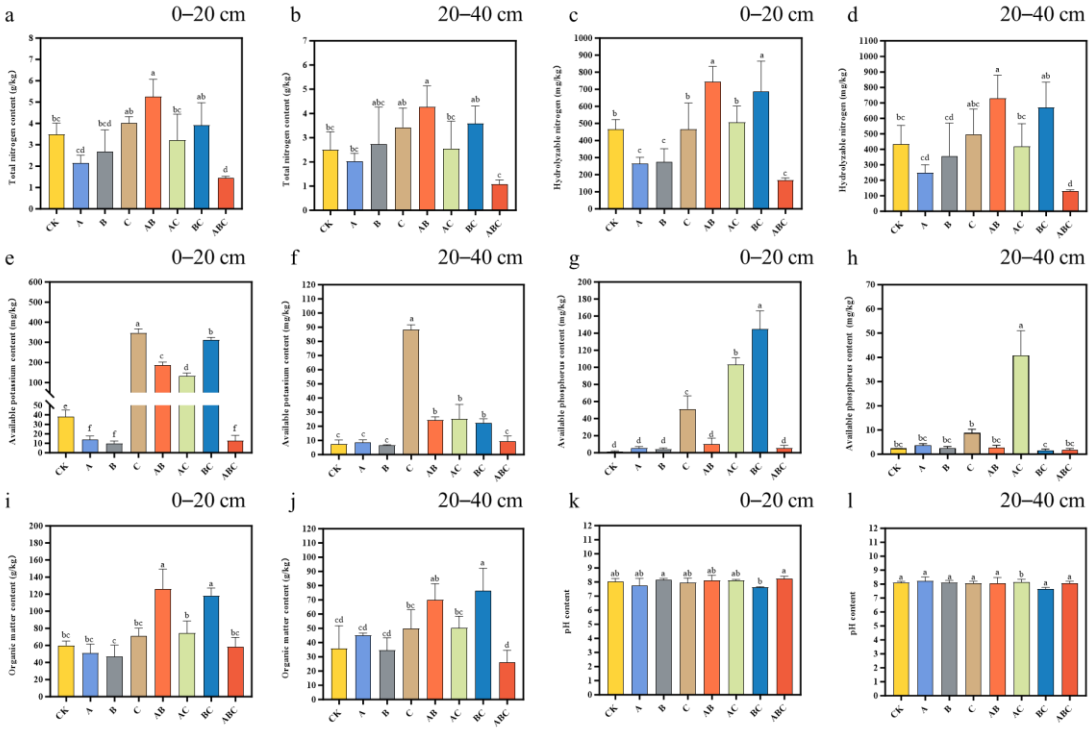
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551 Table 1 Experimental design and grass species

Case number	Sowing method	Grass varieties and seeding rates
CK	No planting; regular weed removal	Compare, remove weeds
A	Live stream	15 g of perennial cock's-foot
B	Live stream	15 g of perennial ryegrass
C	Live stream	15 g white clover
AB	Mixed planting in pairs	15 g perennial cock's-foot + 15 g perennial ryegrass
AC	Mixed planting in pairs	15 g of perennial cock's-foot + 15 g of white clover
BC	Mixed planting in pairs	15 g perennial ryegrass + 15 g white clover
ABC	Mixed planting of three grasses	15 g perennial cock's-foot + 15 g perennial ryegrass + 15 g white clover

552



553

554 Figure 1: Changes in soil physicochemical properties at depths of 0–20 and 20–40 cm

555 Note: ① Data in the figure represent the mean ± standard deviation; ② different lowercase letters indicate
556 statistically significant differences between groups based on one-way analysis of variance (ANOVA) and
557 least significant difference (LSD) tests, $p < 0.05$; ③ CK: control group (no grass planted); A: pure-seeded
558 perennial cock's-foot; B: pure-seeded perennial ryegrass; C: pure-seeded white clover; AB: perennial
559 cock's-foot + perennial ryegrass mixed sowing; AC: perennial cock's-foot + white clover mixed sowing;
560 BC: perennial ryegrass + white clover mixed sowing; ABC: perennial cock's-foot + perennial ryegrass +
561 white clover three-species mixed sowing.

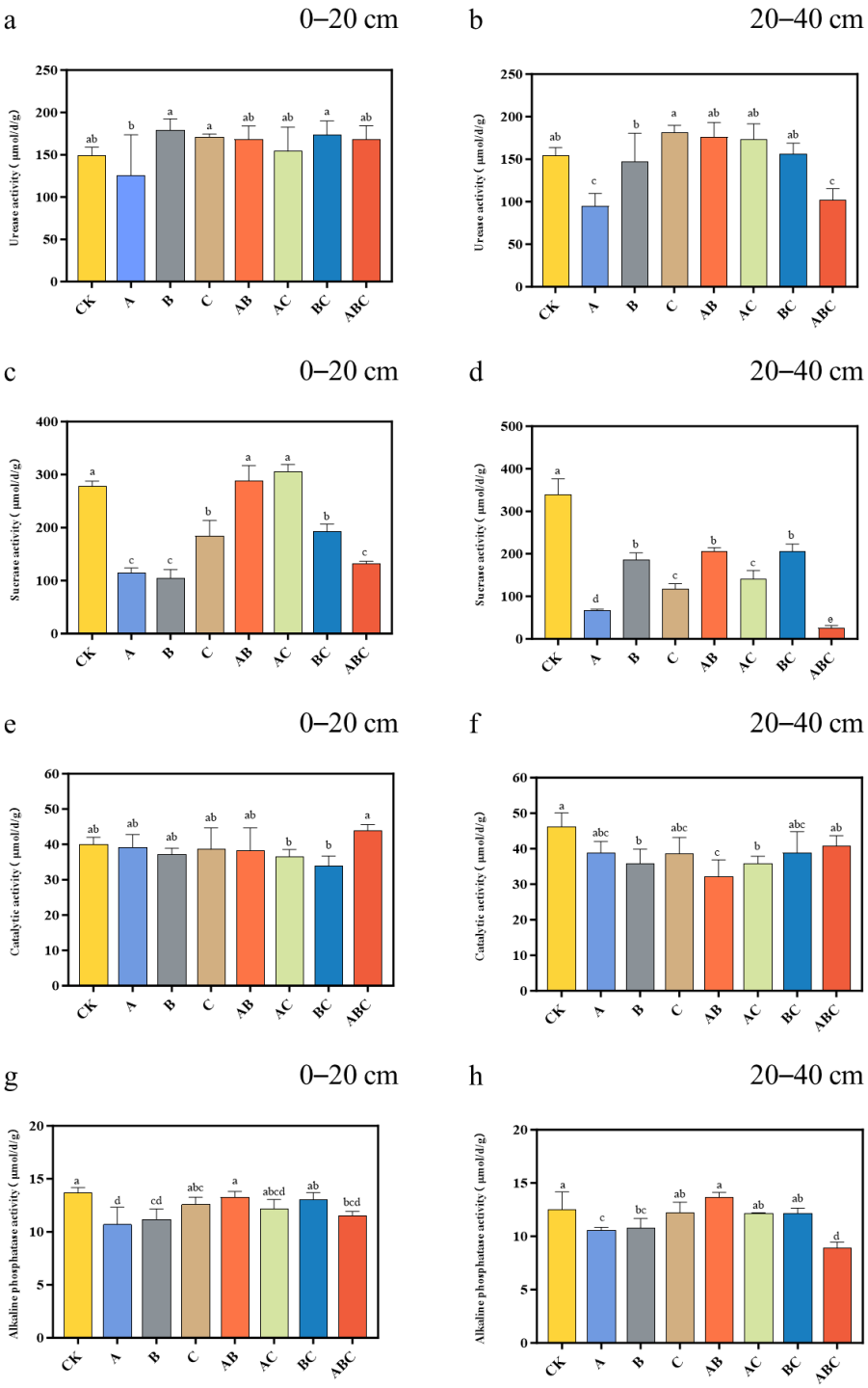


Figure 2: Soil enzyme activity at different depths

Note: ① Data in the figure represent the mean ± standard deviation; ② different lowercase letters indicate statistically significant differences between groups based on one-way analysis of variance (ANOVA) and least significant difference (LSD) tests, $p < 0.05$; ③ CK: control group (no grass planted); A: pure-seeded perennial cock's-foot; B: pure-seeded perennial ryegrass; C: pure-seeded white clover; AB: perennial

cock’s-foot + perennial ryegrass mixed sowing; AC: perennial cock’s-foot + white clover mixed sowing; BC: perennial ryegrass + white clover mixed sowing; ABC: perennial cock’s-foot + perennial ryegrass + white clover three-species mixed sowing.

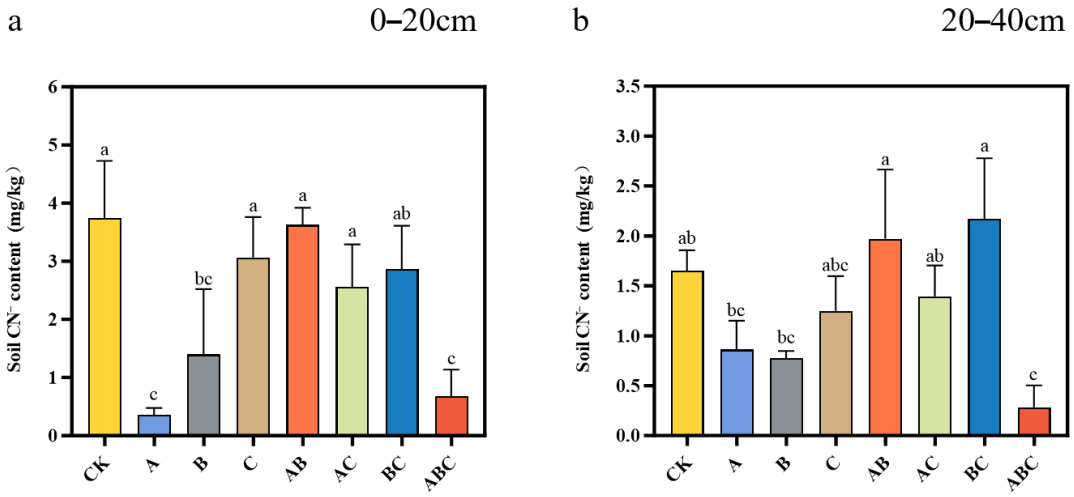


Figure 3: Hydrocyanic acid content in the 0–20 and 20–40 cm soil layers

Note: ① Data in the figure represent the mean ± standard deviation; ② different lowercase letters indicate statistically significant differences between groups based on one-way analysis of variance (ANOVA) and least significant difference (LSD) tests, $p < 0.05$; ③ CK: control group (no grass planted); A: pure-seeded perennial cock's-foot; B: pure-seeded perennial ryegrass; C: pure-seeded white clover; AB: perennial cock's-foot + perennial ryegrass mixed sowing; AC: perennial cock's-foot + white clover mixed sowing; BC: perennial ryegrass + white clover mixed sowing; ABC: perennial cock's-foot + perennial ryegrass + white clover three-species mixed sowing.

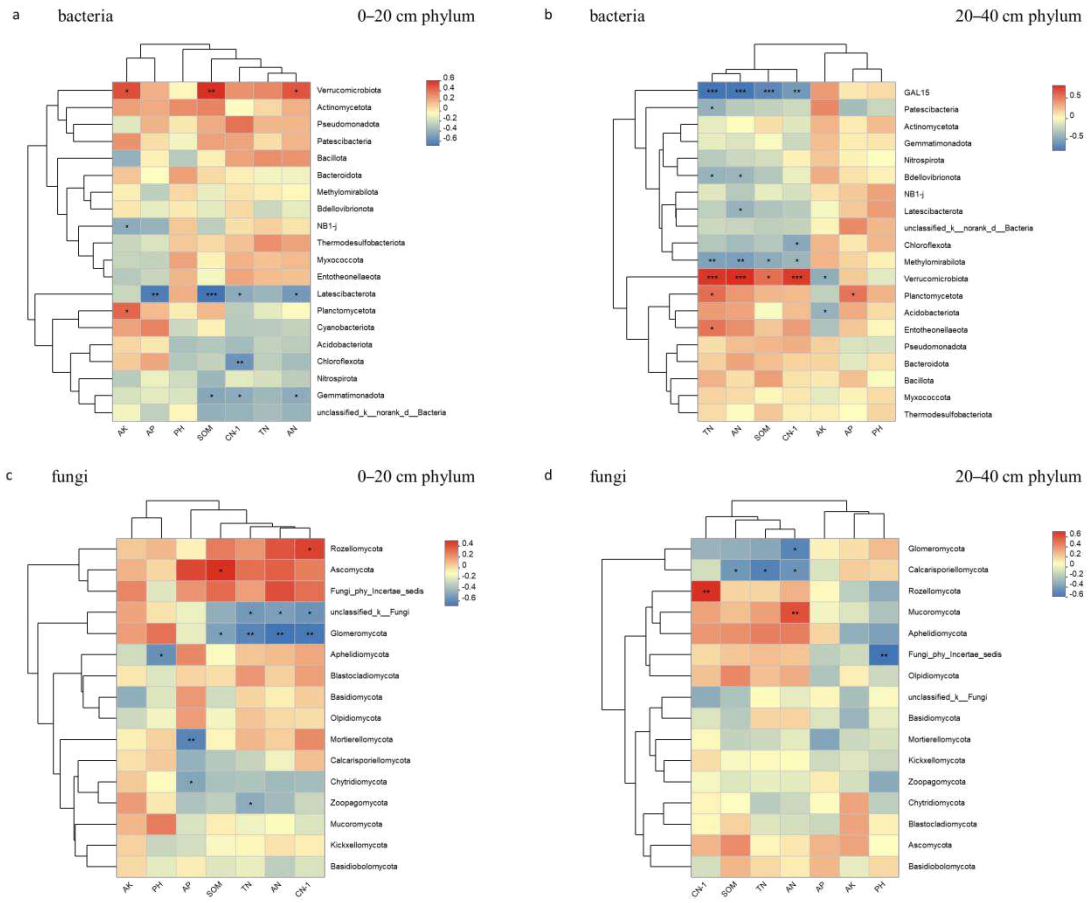


Figure 4: Spearman's correlation analysis of physicochemical properties, hydrogen cyanide, and microbial phyla at different soil depths

Note: ① AP: available phosphorus; SOM: soil organic matter; TN: total nitrogen; AN: alkali-hydrolyzable nitrogen; AK: available potassium; CN: hydrogen cyanide in soil. ② Statistically significant differences are indicated by asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$).

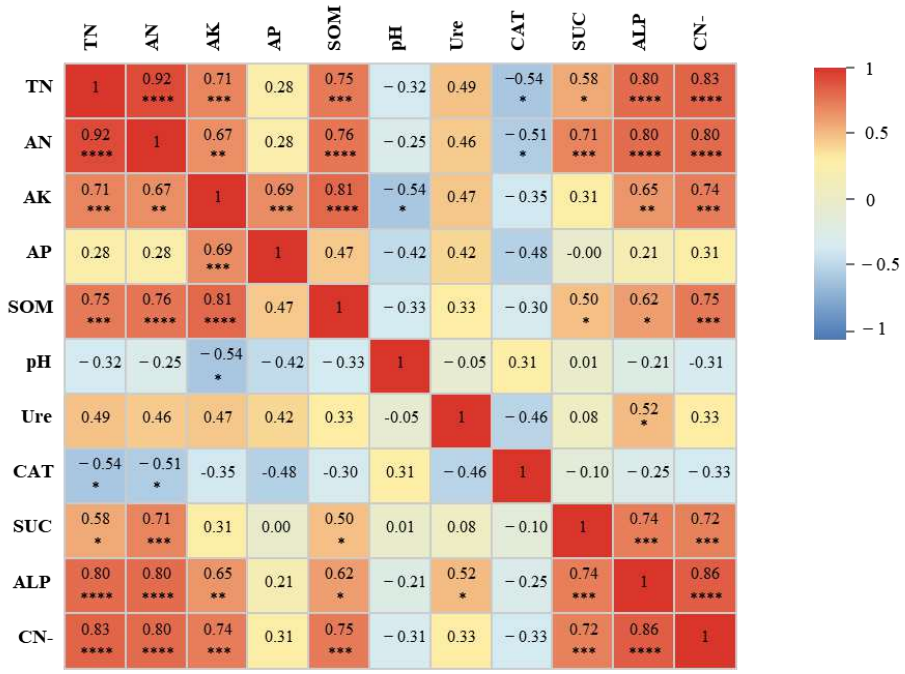
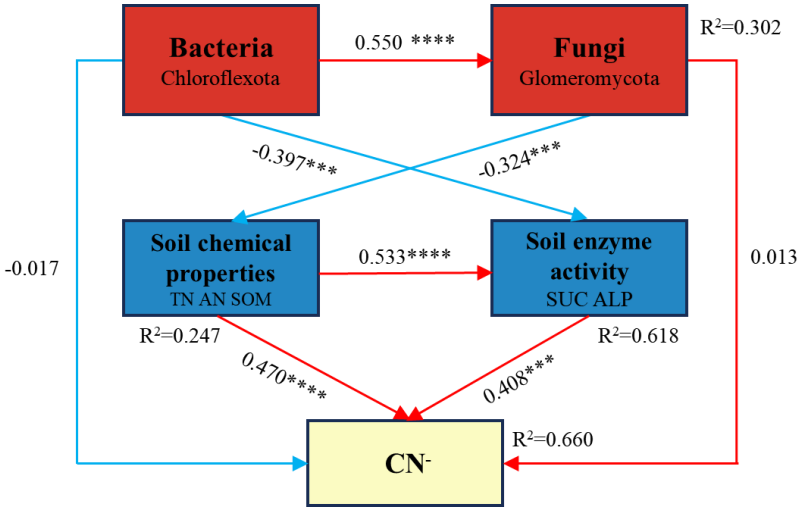


Figure 5: Correlations among physicochemical properties, enzyme activity, and hydrocyanic acid

Note: ① TN: the total nitrogen; AN: the hydrolyzable nitrogen; AK: the available potassium; AP: the phosphorus; SOM: The organic matter; Ure: urease; CAT: catalase; SUC: sucrase; ALP: alkaline phosphatase; CN^-: the hydrogen cyanide. ② Statistically significant differences are indicated by asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.001$)



592

593 Figure 6: Partial least squares path model of factors driving the reduction of hydrocyanic acid in soil

594 Note: ① Red lines indicate positive correlations, while blue lines indicate negative correlations; ② path

595 coefficients with statistically significant differences are marked with asterisks (*$p < 0.05$, **$p < 0.01$, ***p

596 < 0.005, ****$p < 0.001$); the R^2 value indicates the proportion of variance in the dependent variable

597 explained by the model.

598

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