

COMBINED SHEEP MANURE AND VINEGAR RESIDUE IMPROVES SOIL PROPERTIES, YIELD AND NUTRITIONAL QUALITY OF *Morchella sextelata*

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ABSTRACT

Morchella sextelata is a high-value edible fungus with a growing cultivation area across China. Shanxi produces abundant vinegar residue and sheep manure, while studies on their combined application for morel cultivation remain insufficient. This field greenhouse experiment was carried out in Shanxi with a randomized complete block design and three biological replicates. Four treatments were arranged: topsoil as the control (CK), sole application of decomposed sheep manure (YF), sole application of vinegar residue (CZ), and combined application of sheep manure and vinegar residue (YF+CZ). The YF+CZ treatment increased soil alkali-hydrolyzable nitrogen by 47.59% and yielded the highest morel output of 332.56 g m⁻². It also raised the contents of essential, umami and functional amino acids, calcium, magnesium, crude protein, and crude polysaccharides, and reduced crude fat content. Soil alkali-hydrolyzable nitrogen was positively correlated with morel yield, crude protein and total amino acids. This study provides data and technical support for the resource recycling of both sheep manure and vinegar residue as well as eco-friendly morel cultivation in Shanxi. Further research is suggested to explore soil microbial mechanisms and optimize substrate formulas.

Keywords: *Morchella*; vinegar residue; yield; quality

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INTRODUCTION

Morchella sextelata has attracted considerable attention due to its distinctive honeycomb-like pileus morphology, rich nutritional profile, smooth texture, notable medicinal properties, and high market value (Sunil and Xu, 2022; Li *et al.*, 2023; Qiu *et al.*, 2024). It contains a variety of bioactive compounds, including amino acids, polysaccharides, minerals, sterols, vitamins, and dietary fiber (Lan *et al.*, 1999). Its recognized physiological functions, including hypolipidemic, antimicrobial, anti-fatigue, antioxidant, and immunomodulatory activities (Xu *et al.*, 2018, 2021; Peng *et al.*, 2021), have contributed to a sustained increase in market demand. Notably, as early as the 1950s, China had already established semi-artificial cultivation techniques and corresponding theoretical foundations for *Morchella* (Yu *et al.*, 2018). At present, experimental cultivation of *Morchella* is practiced across most regions of China (Liu, 2020; Zhao *et al.*, 2020) and the total cultivation area has remained stable at over 7000 hectares (Luo *et al.*, 2022).

At present, the research on *Morchella* in China mainly focuses on artificial cultivation, nutrient composition analysis, industrialization strategy, and optimization of field cultivation mode (Liu *et al.*, 2022). Clarifying how the cultivation substrate regulates the quality and yield of *Morchella* is the key research direction and core breakthrough point of current industrial upgrading. Conventional substrates currently used for morel cultivation—including sheep manure, wood chips, and mushroom residue—have been extensively optimized and are widely used in production; however, they still suffer from significant limitations, such as unstable nutrient supply, soil nutrient imbalances, and the risk of carrying or introducing pathogens. Shen *et al.* (2021) demonstrated that a composite substrate consisting of raw soil, sheep manure, and mushroom residue effectively improved the morphological characteristics of *Morchella*, including larger stipe diameter, stipe length, and pileus size, as well as higher individual fruiting body weight. In addition, this treatment increases the content of moisture, essential amino acids, and flavor-contributing amino acids in the fruiting bodies, while reducing the content of crude polysaccharides and crude fat. Song *et al.* (2022) reported that the application of a mixed substrate comprising peat, *Lentinula edodes* residue, and organic fertilizer significantly improved soil physicochemical properties. It is worth noting that the combination of *L. edodes* residue and organic fertilizer specifically enhanced the accumulation of calcium, potassium, and magnesium in morel fruiting bodies. These

findings are further supported by Shen *et al.* (2009), who noted that additives such as sawdust have a positive effect on morel yield.

China's vinegar industry produces more than 3 million tons of vinegar residue annually. (Feng *et al.*, 2025). The production of one ton of vinegar generates 60–70% vinegar residue (Li *et al.*, 2015). Currently, landfilling and incineration are the primary methods for disposing of vinegar still residue. These practices lead to secondary pollution of the atmosphere and soil, as well as the waste of biological resources. (Li *et al.*, 2015). There is an urgent need to develop alternative disposal methods for vinegar stills. Vinegar residue is currently reused only to a limited extent as animal feed, growing media, and raw materials for organic fertilizer production. (Tian *et al.*, 2017). Its overall utilization rate remains low, and more efficient utilization approaches are urgently required. As vinegar residue is derived from rice bran, wheat bran and sorghum husk, it is rich in crude protein, cellulose, hemicellulose and other valuable nutrients (Chen *et al.*, 2022). Its nutritional profile is suitable for the growth of edible fungi (Cui *et al.*, 2015). Additionally, the application of vinegar residue exerts remarkable effects on soil properties and microbial communities (Zhou *et al.*, 2021). To explore high-value utilization ways of vinegar residue, researchers have paid increasing attention to its application in edible fungi cultivation in recent years. When mixed with other substrates, vinegar residue enhances the crude protein content of *Agaricus bisporus* fruiting bodies by up to 40.2% (Jing *et al.*, 2008), shortens the growth cycle of *Pleurotus nebrodensis* by 25 days and raises its biological efficiency by 15% (Hou *et al.*, 2010). The biological efficiency of *Pleurotus ostreatus* can reach 110.8% with the addition of vinegar residue (Li *et al.*, 2018). However, relevant research on the application of vinegar residue in *Morchella* cultivation is still insufficient. Existing studies rarely examine the combined effects of sheep manure and vinegar residue on alkaline soil properties and *Morchella* quality when applied together, which remains an underexplored research area. Therefore, this study proposes the core hypothesis that compound substrate formulated with appropriate proportion of vinegar residue and sheep manure can improve soil physicochemical properties, optimize nutrient supply status, and further promote the growth and nutritional quality accumulation of *Morchella*, realizing efficient resource utilization of vinegar waste and green production of *Morchella*. Considering the local conditions of Shanxi Province, this study proposes a formula of *Morchella* cultivation substrate composed of vinegar residue and sheep manure. This research mainly explores efficient cultivation techniques, aiming to promote the industrial development of *Morchella* in the local area, and provide theoretical reference and practical guidance for the construction of a green ecological circular agriculture model.

MATERIALS AND METHODS

Experimental Materials: The fungal strain used in this study was *Morchella sextelata*, which was provided by Mianyang Jingke Edible Fungi Co., Ltd. (Sichuan, China). The basal medium for strain activation comprised 200 g potatoes, 10 g glucose, 4 g peptone, 1 g magnesium sulfate, 2 g potassium dihydrogen phosphate, and 20 g agar powder dissolved in 1000 mL deionized water, with an unadjusted natural pH of approximately 5.6. The medium for mother spawn and cultivated spawn consisted of 60% wheat (*Triticum aestivum* L.), 10% millet (*Setaria italica* L. Beauv.), and 30% sorghum (*Sorghum bicolor* L. Moench) grains; the moisture content was adjusted to 55%–60%, and its natural pH was 5.7–6.1 without pH adjustment. Each 500 g exogenous nutrient bag consisted of 46% wheat grains, 17% sawdust, 18% rice husk (*Oryza sativa* L.), 18% topsoil, and 1% light calcium carbonate, with a standardized moisture content of 55%–60%.

Experimental Design: The field experiment was carried out in a solar greenhouse at the Shanxi Academy of Agricultural Sciences (37.54° N, 112.67° E) from October 10, 2023, to April 25, 2024. A randomized complete block design with three biological replications was adopted. Each experimental plot measured 1.5 m × 1.0 m = 1.5 m². The plots were separated by 0.5-meter-wide isolation trenches to prevent cross-contamination between different treatments. The experiment included four treatments: unamended (CK, control); topsoil amended with 600 kg ha⁻¹ of sheep manure (YF); topsoil amended with 1,800 kg ha⁻¹ of vinegar lees (CZ); and topsoil amended with 300 kg ha⁻¹ of sheep manure and 900 kg ha⁻¹ of vinegar lees (YF + CZ). The substrate application rates adopted in this study were set with reference to conventional organic amendment rates for *Morchella* cultivation in Shanxi Province, as well as the initial nutrient status of the local calcareous cinnamon soil, and were further verified through pre-experiments. The experimental soil type was cinnamon soil. The initial physicochemical properties of the 0–20 cm plow layer prior to the experiment were as follows: soil organic carbon 9.97 g kg⁻¹, total nitrogen 0.91 g kg⁻¹, available phosphorus 7.99 mg kg⁻¹, available potassium 157.33 mg kg⁻¹, electrical conductivity 0.26 mS cm⁻¹, pH 8.51. Vinegar residue was provided by a traditional aged vinegar factory in Qingxu County, Shanxi Province, China, while sheep manure was obtained from local livestock farms. Sheep manure was fully decomposed via high-temperature composting prior to field application. The physicochemical properties of the tested substrates are shown in Table 1.

Table 1 Chemical properties of different substrates

Treatment	Organic matter (g kg ⁻¹)	Total nitrogen (g kg ⁻¹)	EC mS cm ⁻¹	C/N	pH
CZ	845.33	30.11	0.41	16.28	5.57
YF	683.11	17.92	3.21	22.11	7.85
YF+CZ	801.13	26.33	1.15	17.27	6.23

YF: Sheep manure; CZ: Vinegar residue; YF+CZ: Sheep manure + Vinegar residue; EC, electrical conductivity; C/N, carbon-to-nitrogen ratio.

Land Preparation and Sowing: Field preparation took place on October 10, 2023. Topsoil was first air-dried to a semi-moist condition to reduce soil-borne pathogen loads, then sheep manure and vinegar residue were spread uniformly across the plot surface and incorporated into the 0–20 cm layer with a rotary tiller. Raised seedbeds were formed to 20 cm height, 1.0 m width, and 1.5 m length. Sowing was carried out on October 15, 2023, using uniform broadcasting at 0.4 kg m⁻², and seeds were immediately covered with 5 cm of sieved topsoil. Black plastic mulch was then laid over both seedbeds and walkways to buffer soil temperature and moisture and suppress weeds.

Exogenous Nutrient Bag Application: Ten days after inoculation, place exogenous nutrient bags (500 g each) on the soil surface, spacing them evenly at 5 bags per plot. Before placing them, use a sterile blade to make 10 to 15 uniform slits on one side of each bag, and press the slit side firmly into the topsoil to promote nutrient exchange and release.

Fruiting Period Management: Keep the greenhouse clean and well-ventilated throughout the entire fruiting period, and use micro-sprinkler irrigation to maintain soil moisture at 70% to 80% of field capacity to support the stable development of fruiting bodies.

Harvesting: From March 20 to April 25, 2024, fruiting bodies were harvested daily at 08:00, 12:00, and 18:00. Fruiting bodies were considered commercially mature when they had fully expanded but had not yet opened their honeycomb-like caps, their surface color had darkened, and the mature fruiting bodies were slightly tilted. Each fruiting body was cut at the base using a sharp, sterile blade to avoid damaging residual mycelium and nearby immature primordia. The fresh yield for each plot was weighed and recorded immediately after collection.

Sample collection: After harvest, representative fruiting body samples of uniform size were selected from each treatment (3 replicates per treatment), rinsed with distilled water, and dried in an oven at 60°C to constant weight for subsequent nutritional analysis. Using the five-point sampling method described by Zhang *et al.* (2020), soil samples were collected from the 0–20 cm layer. After removing debris, the samples were air-dried, ground, and sieved, then stored in light-proof, sealed bags for subsequent chemical analysis.

Determination of soil properties: The soil pH value was measured by shaking a pH metre in a suspension of soil/water (1:2.5) for 30 min (Wen *et al.*, 2024); alkali-hydrolyzable nitrogen content was determined using the alkali diffusion method after NaOH hydrolysis (Wen *et al.*, 2024); available phosphorus content was determined using the molybdenum-antimony colorimetric method after extraction with ammonium fluoride-hydrochloric acid and sodium bicarbonate solutions (Guo *et al.*, 2025); and available potassium content was measured using a flame photometer following ammonium acetate extraction (Wen *et al.*, 2024).

Determination of mineral elements in morel: Calcium (Ca) and magnesium (Mg) contents were determined using inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7850, Agilent Technologies, Inc., Santa Clara, CA, USA). (Ma *et al.*, 2017).

Determination of nutritional substances in morel: The crude protein content was quantified following the National Food Safety Standards of China (GB 5009.5-2016 and GB/T 15673–2009), both based on the Kjeldahl digestion principle (Page *et al.*, 1982). Crude polysaccharides were extracted from morel ascocarps in boiling ultrapure water and measured spectrophotometrically via the phenol-sulfuric acid method, as outlined by Tan *et al.* (2019). Crude fat content was analyzed by Soxhlet extraction according to Zhang *et al.* (2017). The free amino acids in the pileus and stipe were analyzed using a previously established method (Bakır *et al.*, 2018). Seventeen amino acids were detected, including aspartic acid (Asp), threonine (Thr), serine (Ser), glutamic acid (Glu), glycine (Gly), alanine (Ala), valine (Val), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), histidine (His), lysine (Lys), arginine (Arg), proline (Pro), cysteine (Cys), and methionine (Met). Quantification was performed on an ultra-high-speed automatic amino acid analyzer (LA8080, HITACHI, Tokyo, Japan).

Statistical analysis: Two-way analysis of variance (ANOVA) was performed using SPSS 23.0 (IBM Corp., Armonk, NY, USA) for the randomized complete block design, with cultivation substrate as the fixed factor and block as the random factor, to evaluate the overall effects of cultivation substrates on soil properties, morel yield and nutritional quality. Results of ANOVA F-tests are denoted in tables as ns (not significant) and * ($p \leq 0.01$). For post-hoc multiple comparisons among treatment means, Duncan's multiple range test was applied at $P \leq 0.01$ (Duncan, 1955). Graphs were generated using Origin Pro 2019 (OriginLab Corp., Northampton, MA, USA). Pearson correlation analysis was performed to quantify the linear relationships between soil properties (pH, alkali-hydrolyzable N, available P, available K) and *Morchella* yield, mineral elements, crude protein, crude fat, total amino acids. Correlation analysis was conducted and visualized using the GGally package in R software (version 4.3.2), with significance levels denoted as * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.

RESULTS

Effect of Cultivation Substrates on Soil Properties: Table 2 shows that the cultivation substrates had no significant effect on soil pH, but significantly influenced soil alkali-hydrolyzable nitrogen, available phosphorus, and available potassium. The soil pH across the four treatments ranged from 8.28 to 8.49, with no significant differences among treatments (Fig. 1). Compared to CK, the alkali-hydrolyzable nitrogen content in sheep manure, vinegar residue, and sheep manure + vinegar residue treatments increased by 27.44%, 41.55%, and 47.59%, respectively, with the YF+CZ treatment being significantly higher than other treatments. Available phosphorus content increased by 50.71%, 6.10%, and 19.48%, respectively, and available potassium increased by 53.90%, 10.01%, and 20.59%, respectively, with the YF treatment being significantly higher than other treatments.

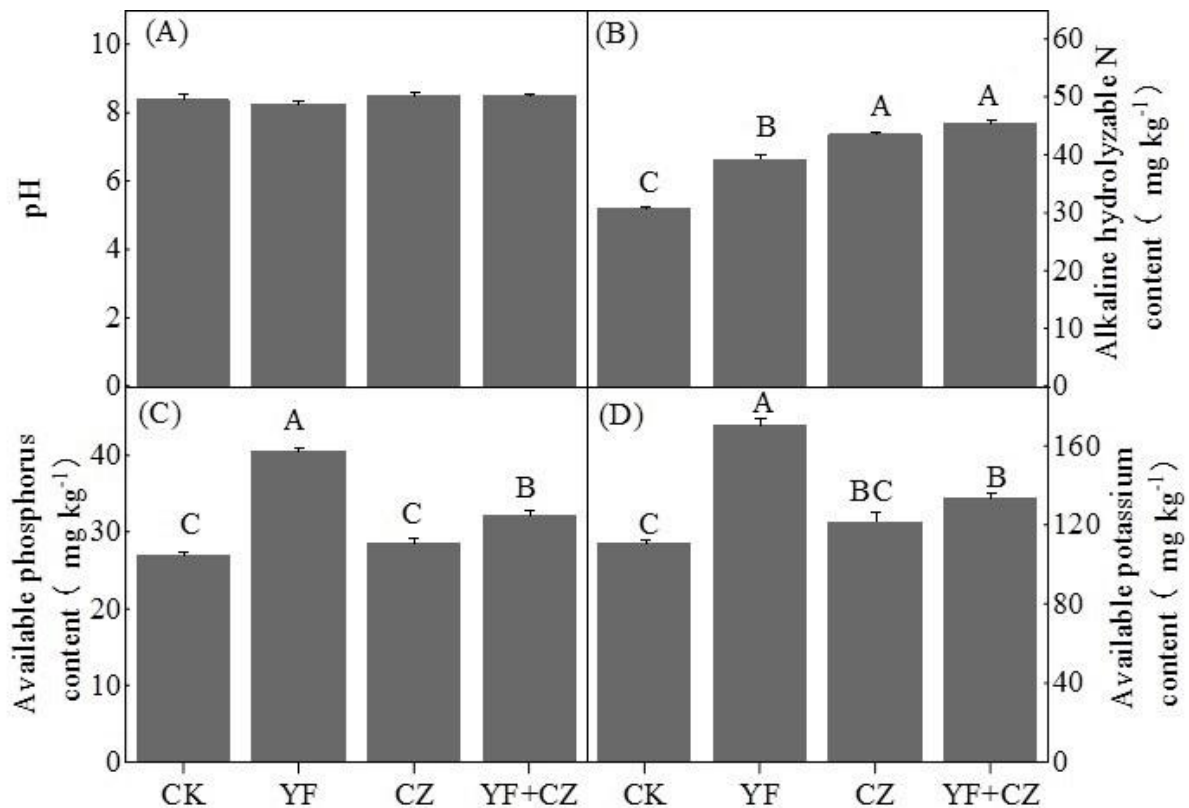


Fig.1 Effects of different cultivation substrates on soil properties. CK: Topsoil; YF: Sheep manure; CZ: Vinegar residue; YF+CZ: Sheep manure +Vinegar residue. Means $\pm$ SE with capital letters indicate a significant difference at $p \leq 0.01$.

Table 2. ANOVA of effects of cultivation substrates on soil properties, yield and nutritional quality of *Morchella*

	pH	AN	AP	AK	Yield	Ca	Mg	CPS	CPR	CF	TAA
F-value	4.32	413.27	523.14	222.38	4002.70	1398.60	448.56	23.55	197.79	158.22	25.83
P	ns	*	*	*	*	*	*	*	*	*	*

AN, alkali-hydrolyzable; AP, available phosphorus; AK, available potassium; CPS, crude polysaccharide; CPR, crude protein; CF, crude fat; TAA, total amino acids. ns, not significant; * $p \leq 0.01$.

Effect of Cultivation Substrates on Essential and Non-essential Amino Acid Contents in *Morchella*: Table 2 shows that cultivation substrates had significant effect on the total amino acid content of *Morchella*. As shown in Table 3, among the seven essential amino acids for humans, leucine was the most abundant (2.10%–2.21%), while glutamic acid dominated the ten non-essential amino acids (6.13%–6.46%). The contents of total essential amino acids (TEAA), total non-essential amino acids (TNEAA), and total amino acids (TAA) all followed the consistent order YF+CZ > CZ > YF > CK. Compared with CK, the YF+CZ treatment increased TEAA, TNEAA, and TAA by 9.79%, 8.45%, and 8.89%, respectively. Notably, the TEAA content in YF+CZ reached 9.98%, accounting for 32.8% of TAA.

Table 3 Effects of different cultivation substrates on the contents of essential and non-essential amino acids for humans in morel (%)

Amino Acid Category		CK	YF	CZ	YF+CZ
Essential amino acids	Valine	1.32 ^C	1.42 ^B	1.52 ^A	1.46 ^{AB}
	Lysine	1.64 ^C	1.67 ^C	1.78 ^B	1.91 ^A
	Threonine	1.28 ^{AB}	1.25 ^B	1.27 ^{AB}	1.31 ^A
	Isoleucine	1.21 ^B	1.31 ^B	1.24 ^B	1.42 ^A
	Phenylalanine	1.12 ^B	1.22 ^A	1.27 ^A	1.23 ^A
	Methionine	0.42 ^A	0.52 ^A	0.51 ^A	0.46 ^A
	Leucine	2.1 ^A	2.21 ^A	2.18 ^A	2.19 ^A
	Total	9.09 ^B	9.6 ^{AB}	9.77 ^A	9.98 ^A
Non-essential amino acids	Arginine	2.51 ^A	2.34 ^B	2.41 ^{AB}	2.51 ^A
	Serine	1.42 ^B	1.45 ^B	1.44 ^B	1.57 ^A
	Proline	1.01 ^A	0.98 ^{AB}	0.95 ^B	0.95 ^B
	Glutamic Acid	6.13 ^A	6.26 ^A	6.45 ^A	6.46 ^A
	Aspartic Acid	2.78 ^C	3.01 ^{AB}	2.98 ^{BC}	3.21 ^A
	Tyrosine	0.94 ^A	0.84 ^B	0.87 ^B	0.86 ^B
	Alanine	1.74 ^C	2.18 ^B	2.22 ^B	2.41 ^A
	Cysteine	0.20 ^A	0.22 ^A	0.24 ^A	0.24 ^A
	Glycine	1.27 ^B	1.29 ^{AB}	1.31 ^{AB}	1.33 ^A
	Histidine	0.81 ^A	0.83 ^A	0.84 ^A	0.86 ^A
Total Amino Acids		27.9 ^A	29.00 ^A	29.48 ^A	30.38 ^A

CK: Topsoil; YF: Sheep manure; CZ: Vinegar residue; YF+CZ: Sheep manure + Vinegar residue. Means with capital letters indicate a significant difference at $p \leq 0.01$.

Effect of Cultivation Substrates on Flavor and Functional Amino Acid Contents in *Morchella*: Flavor amino acids in *Morchella* mainly consist of umami and sweet types. The umami components are primarily aspartic acid and glutamic acid, accounting for 31.93% of total amino acids. Sweet components include four amino acids: serine, glycine, alanine, and proline, comprising 20.14% of total amino acids (Table 4). Both umami and sweet amino acids followed the order YF+CZ > CZ > YF > CK. Compared to CK, the YF+CZ, CZ, and YF treatments increased total umami amino acid content by 8.53%, 5.84%, and 4.04%, respectively, and total sweet amino acid content by 15.07%, 8.82%, and 8.46%, respectively. Table 2 also shows that among functional amino acids, glutamic acid and aspartic acid were the main components, their average content accounting for 47.67% of total functional amino acids. Total functional amino acid content followed the order YF+CZ > CZ > YF > CK. Compared to CK, the YF+CZ, CZ, and YF treatments increased functional amino acid content by 6.61%, 4.49%, and 2.38%, respectively.

Table 4 Effects of different cultivation substrates on the contents of flavor and functional amino acids in morel (%)

Amino acid category	Amino acid kind	CK	YF	CZ	YF+CZ
Umami amino acids	Aspartic acid	2.78 ^C	3.01 ^{AB}	2.98 ^{BC}	3.21 ^A
	Glutamic acid	6.13 ^A	6.26 ^A	6.45 ^A	6.46 ^A
	Total	8.91 ^C	9.27 ^B	9.44 ^{AB}	9.67 ^A
Sweet amino acids	Serine	1.42 ^B	1.45 ^B	1.44 ^B	1.57 ^A
	Glycine	1.27 ^B	1.29 ^{AB}	1.31 ^{AB}	1.33 ^A
	Alanine	1.74 ^C	2.18 ^B	2.22 ^B	2.41 ^A
	Proline	1.01 ^A	0.98 ^{AB}	0.95 ^B	0.95 ^B
	Total	5.44 ^B	5.9 ^A	5.92 ^A	6.26 ^A
Functional amino acids	Glutamic acid	6.13 ^A	6.26 ^A	6.45 ^A	6.46 ^A
	Aspartic acid	2.78 ^C	3.01 ^{AB}	2.98 ^{BC}	3.21 ^A
	Arginine	2.51 ^A	2.34 ^B	2.41 ^{AB}	2.51 ^A
	Glycine	1.27 ^B	1.29 ^{AB}	1.31 ^{AB}	1.33 ^A
	Phenylalanine	1.12 ^B	1.22 ^A	1.27 ^A	1.23 ^A
	Tyrosine	0.94 ^A	0.84 ^B	0.87 ^B	0.86 ^B
	Methionine	0.42 ^A	0.52 ^A	0.51 ^A	0.46 ^A
	Leucine	2.1 ^A	2.21 ^A	2.18 ^A	2.19 ^A
	Lysine	1.64 ^C	1.67 ^C	1.78 ^B	1.91 ^A
	Total	18.91 ^B	19.36 ^{AB}	19.76 ^{AB}	20.16 ^A

CK: Topsoil; YF: Sheep manure; CZ: Vinegar residue; YF+CZ: Sheep manure + Vinegar residue. Means with capital letters indicate a significant difference at $p \leq 0.01$.

Effect of Cultivation Substrates on *Morchella* Yield and Nutritional Components: As indicated in Table 2, different cultivation substrates significantly affected the yield and nutritional quality of *Morchella*, with the YF+CZ composite treatment showing the best overall performance. As the core economic indicator, the fresh yield of *Morchella* was significantly increased by all substrate amendments; the YF+CZ treatment achieved the highest yield of 332.56 g m⁻², which was 65.94% higher than CK, 26.37% higher than YF, and 16.11% higher than CZ. Substrate amendments also promoted accumulation of mineral element in fruiting bodies. The Ca content in YF+CZ treatment increased by 26.66% compared with CK, exceeding that of both sole treatments, and Mg content in YF+CZ treatment increased by 34.89%, while YF treatment slightly decreased Mg content by 3.88%. Crude protein and crude polysaccharides are key indicators of the nutritional value of morels. Compared with CK, the YF+CZ treatment increased crude protein content by 21.80% and crude polysaccharide content by 9.64%, both reaching the highest levels among all treatments. Conversely, all substrate amendments reduced crude fat content, with YF+CZ treatment showing the largest reduction of 19.49%.

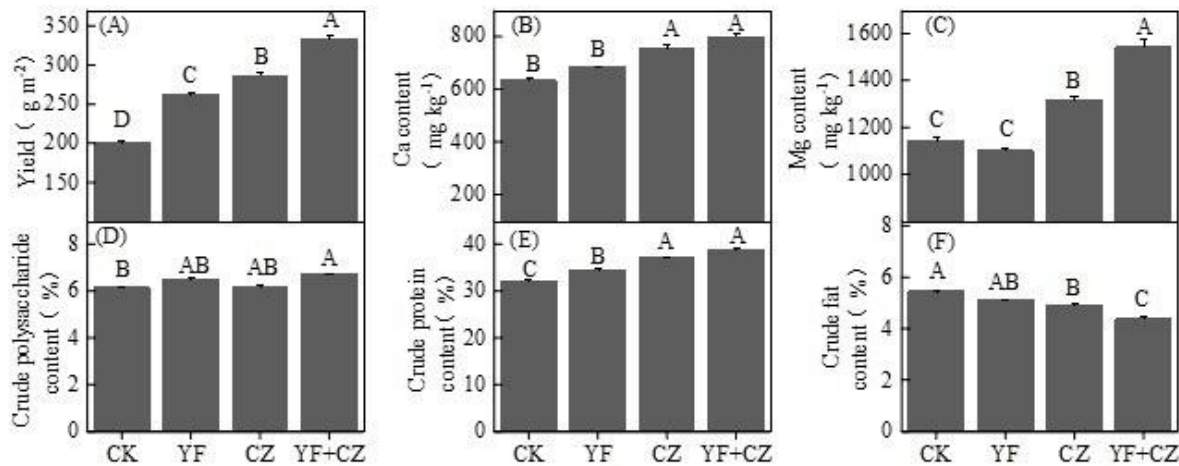


Fig.2 Effects of different cultivation substrates on fruiting body yield and nutritional components of morel. CK: Topsoil; YF: Sheep manure; CZ: Vinegar residue; YF+CZ: Sheep manure + Vinegar residue. Means±SE with capital letters indicate a significant difference at $p \leq 0.01$.

Correlation Analysis: To clarify the relationship between soil nutrients and *Morchella* yield and quality, a correlation analysis of relevant indicators was conducted. The results indicated that soil alkali-hydrolyzable nitrogen content was significantly positively correlated with Mg content, crude protein content, total amino acid content, and yield of *Morchella*, while it was significantly negatively correlated with crude fat content (Fig. 3).

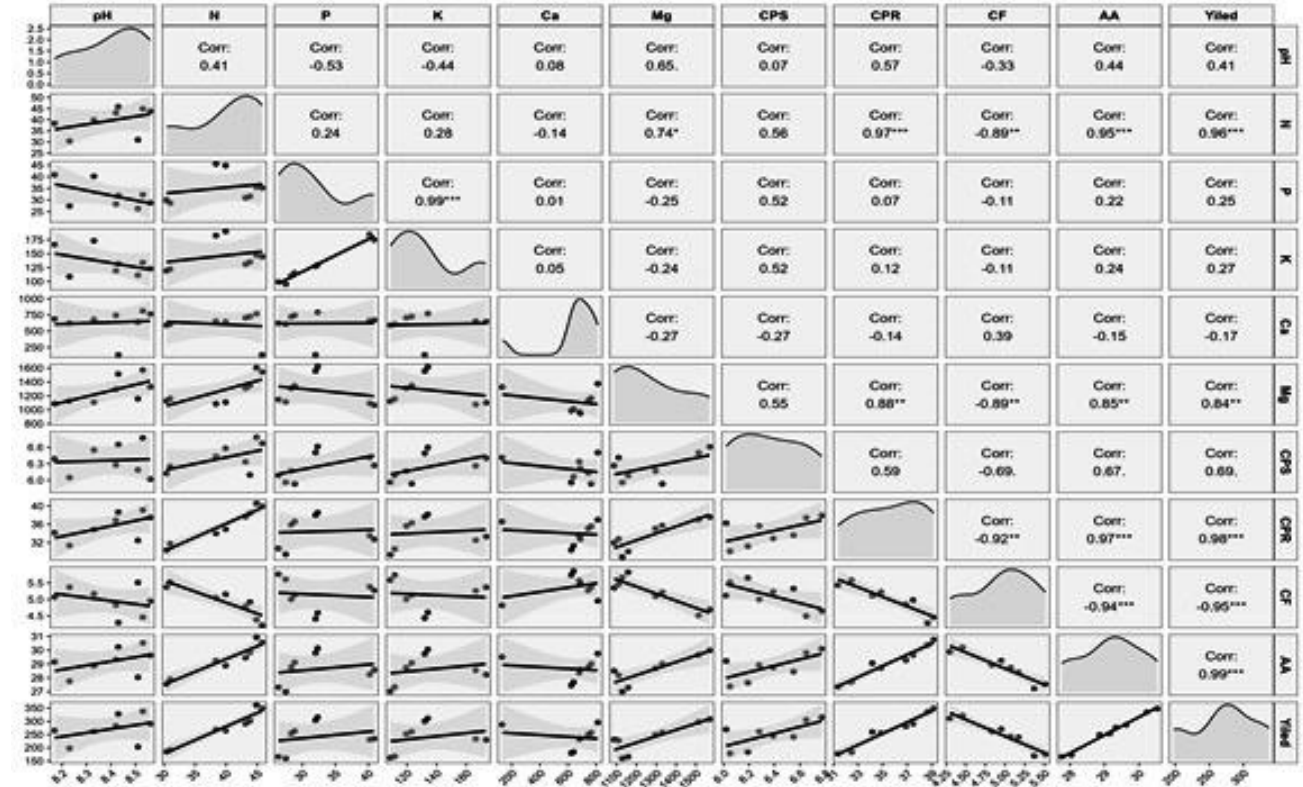


Fig. 3 Correlation analysis. N: alkali-hydrolyzable nitrogen content; P: Available phosphorus content; K: available potassium content; CPS: crude polysaccharide content; CPR: crude protein content; CF: crude fat content; AA: total amino acid content. * $P \leq 0.05$; ** $p \leq 0.01$, and *** $p \leq 0.001$.

DISCUSSION

Soil nutrient levels and ecological environmental conditions are key limiting factors determining the growth status of *Morchella* (Sambyal and Singh, 2021). Current cultivation techniques rely on a dual nutrient supply mode involving both the native soil and exogenous nutrient bags, further confirming the crucial role of soil nutrients in the *Morchella* cultivation system (Li *et al.*, 2018a; Li *et al.*, 2018b). Contrary to the widely accepted optimal pH range of 7.0–7.6 for *Morchella* in Shanxi (Han *et al.*, 2017), all treatments in this study maintained stable soil pH values of 8.28–8.49 throughout the 6-month cultivation cycle, with no significant acidification effect from acidic vinegar residue (pH 5.57). This stability can be attributed to: (1) the strong buffering capacity of calcareous cinnamon soil, which rapidly neutralizes the organic acids released by decomposition residues (Zhang *et al.*, 2016); (2) the complementary pH effects of the two substrates, where near-neutral decomposed sheep manure (pH 7.85) counteracts the acidity of vinegar residue, resulting in a composite substrate with a moderate pH of 6.23. The successful cultivation of *M. sextelata* under these alkaline conditions challenges previous assumptions about its pH requirements, consistent with the findings of Li *et al.* (2026), who reported normal growth and fruiting of *M. sextelata* at pH values up to 8.7 in northern China. This wider pH tolerance extends the range of regions where *Morchella* can be cultivated in alkaline soils.

This study found that the combination of sheep manure and vinegar residue achieved the highest *Morchella* yield (332.56 g m⁻²) and nutritional quality. Nitrogen availability is a critical determinant of morel fruiting (Tan *et al.*, 2019; Zhang *et al.*, 2020), and enhancing soil N supply has been shown to improve *Morchella* yield (Kang *et al.*, 2026). The significant positive correlations between soil alkali-hydrolyzable nitrogen and morel yield, crude protein, and total amino acids support this interpretation and are consistent with the findings of Li *et al.* (2024). Although sole sheep manure significantly increased soil available phosphorus and potassium contents, it did not achieve the same yield and

quality improvements as the composite substrate. This may be because nitrogen has a much stronger regulatory effect on fungal community structure and diversity than phosphorus and potassium (Hu *et al.*, 2022). The superior yield and quality performance of the composite substrate relative to single amendments is likely attributable to its elevated alkali-hydrolyzable nitrogen concentration measured at harvest, which may sustain adequate nitrogen supply throughout mycelial colonization and fruiting development. The improved nutrient availability under the combined treatment likely stems from more than just direct nutrient input. Vinegar residue contributes carbon-rich lignocellulose, whereas sheep manure supplies readily available nitrogen, and together these two substrates may help shape soil microbial conditions that favor *Morchella* growth (Wen *et al.*, 2020; Cai *et al.*, 2024). However, since the present study did not conduct microbial community detection, such microbial-mediated processes remain unconfirmed. Future research relying on high-throughput sequencing is required to verify this potential pathway.

Amino acids, as fundamental nutritional elements for life activities, are crucial for food quality and flavor formation, and the nutritional value of food protein is primarily determined by the types and contents of essential amino acids (Wiedemair *et al.*, 2020; Li *et al.*, 2021). This study showed that the sheep manure and vinegar residue composite substrate increased the total essential amino acid content in *Morchella* fruiting bodies to 9.98%, effectively enhancing the nutritional quality of *Morchella* protein, and also elevated the contents of flavor amino acids including umami and sweet amino acids to improve the edible taste of *Morchella*. Functional amino acids, as essential components for maintaining human nitrogen balance (Feng *et al.*, 2016), are predominantly composed of glutamic acid and aspartic acid that exert vital functions in promoting children's growth and development, alleviating fatigue and enhancing liver function, and the highest functional amino acid content detected in the sheep manure and vinegar residue treatment verified that this substrate formulation can effectively strengthen the health benefits of *Morchella*. Correlation analysis further confirmed that soil alkali-hydrolyzable nitrogen content was significantly positively correlated with the total amino acid content of *Morchella*, indicating that the composite substrate may promote nitrogen uptake and assimilation in *Morchella* by increasing soil available nitrogen levels, thereby facilitating the accumulation of total amino acids in fruiting bodies. Substrate modification significantly regulates the nutritional component content of *Morchella* (Shen *et al.*, 2021), and mineral elements, as important nutritional substances in *Morchella*, are critical to the nutritional value and comprehensive quality of its ascocarps. The YF+CZ treatment produced the highest Ca and Mg contents in *Morchella* fruiting bodies, and the calcium content was significantly positively correlated with soil alkali-hydrolyzable nitrogen content. In addition, the effects of cultivation substrates on crude protein content were consistent with the yield variation trend, while crude fat content showed an opposite change trend. The synchronous increase in crude polysaccharide and crude protein contents and the reduction in crude fat content jointly optimized the edible taste of *Morchella*, fully demonstrating that the YF+CZ composite substrate can effectively improve the nutritional components and overall quality of *Morchella* fruiting bodies.

Some limitations of this work should be acknowledged, and point to directions for future research. For one, soil nutrients were only quantified at the end of the cultivation cycle, and tracking nutrient dynamics across the full growth period would help clarify how supply and demand align over time. Second, while our results strongly suggest a microbial-mediated mechanism, direct measurement of soil microbial community composition and function using high-throughput sequencing is needed to confirm this hypothesis. Third, further studies using nitrogen isotope tracers are required to directly quantify nitrogen uptake and allocation in *Morchella* fruiting bodies. Finally, the optimal substrate ratio may vary depending on soil type, *Morchella* species, and climatic conditions, and future research should investigate these factors to develop region-specific recommendations.

Conclusions: Combined application of sheep manure and vinegar residue effectively increased soil alkali-hydrolyzable nitrogen and achieved the maximum yield of *Morchella* at 332.56 g m⁻². This composite substrate elevated the contents of amino acids, calcium, magnesium, crude protein and crude polysaccharides in fruiting bodies, and decreased crude fat content. Significant positive correlations existed between soil alkali-hydrolyzable nitrogen and morel yield, crude protein and total amino acids. The mixture of the two agricultural wastes shows good application potential for morel cultivation.

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