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Calcium dynamics during the growth of *Agaricus bisporus*: implications for mushroom development and nutrition

Juan Wu[1*], Rui Wang[1], Xue Liu[1], Ying Ni[1], Hui Sun[1], Xiaonan Deng[1], Lingzhong Wan[1], Fangzhi Liu[1], Jun Tang[2], Junjie Yu[2] and Xiaoming Yan[1*]

Abstract

Background *Agaricus bisporus* (*A. bisporus*) is highly valued for its nutritional benefits and delicious taste, making it one of the most widely cultivated, highest yielding, and most consumed edible mushrooms worldwide. The yield and quality of *A. bisporus* were affected by its culture medium and environment. Among the culture base, the precise impact of calcium on *A. bisporus* cultivation and the dynamic changes in calcium concentration and chemical environment during the cultivation process remain unclear. This study aims to investigate the changes in calcium content and forms during the growth of *A. bisporus* and their implications for mushroom growth and nutrition.

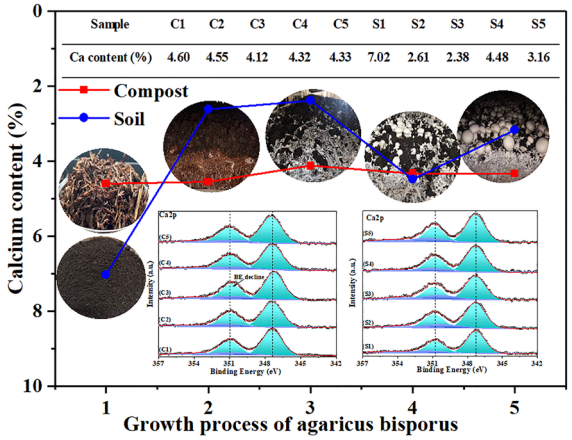
Results Through the analysis of samples collected during the composting phase, mycelial development phase, and *A. bisporus* harvesting phase, the role of calcium in the growth process of button mushrooms is revealed. During the composting phase, the calcium content remains relatively stable, suggesting a consistent calcium source in the compost. The fermentation process shows a significant decrease in carbon content and an increase in oxygen content, indicating the degradation and oxidation of organic matter. In the mycelial development phase, both the cover soil and compost experience a decrease in calcium content, with a more pronounced reduction observed in the covering soil, indicating its primary role as an energy source for enzymatic activity and metabolic processes of the mycelium. During the *A. bisporus* harvesting phase, the changes in calcium, carbon, and oxygen content become less prominent, indicating a stable state of fruiting bodies growth that no longer requires a significant supply of organic matter.

Conclusions Analysis of calcium forms reveals the presence of different calcium compounds, likely influenced by soil calcium sources, microbial activities, and mushroom metabolic byproducts. Calcium plays a crucial regulatory role in the growth and quality of *A. bisporus*. This study provides valuable insights into the significance of calcium in *A. bisporus* growth and offers theoretical guidance for optimizing mushroom production and quality improvement.

Keywords *Agaricus bisporus*, Growth process, Calcium content and forms

*Correspondence:
Juan Wu
ecjtutmtm@163.com
Xiaoming Yan
19909699660@163.com
Full list of author information is available at the end of the article

Graphical Abstract



Background

Agaricus bisporus, commonly known as the white button mushroom, is highly valued for its nutritional benefits and delicious taste, making it one of the most widely cultivated, highest yielding, and most consumed edible mushrooms worldwide [1–3]. The commercial cultivation of *A. bisporus* typically involves three distinct stages [4, 5]: composting Phase (Stage 1): during this phase, compost (substrate) undergoes a process of fermentation and conversion, resulting in the formation of a selective growth medium that promotes the development of *A. bisporus* mycelium [6]. Mycelial Development Phase (Stage 2): during this stage, a covering layer of soil is applied to stimulate the growth and maturation of the mycelium, ultimately leading to the formation of mature fruiting bodies. Mature Harvesting Phase (Stage 3): when the fruiting bodies reach full maturity, they are harvested. Subsequently, the spent compost and covering soil are typically disposed of as waste materials. The successful growth of *A. bisporus* depends on the careful selection and management of materials to provide optimal growth conditions throughout these stages [7]. For instance, in Stage 1, the compost (substrate) serves as the foundation for the growth of *A. bisporus* mycelial and the development fruiting bodies. Typical growth substrates consist of a mixture of wheat straw, horse manure, and poultry manure [8]. In Stage 2, the covering soil is composed of peat moss, vermiculite, and/or a mixture of these materials with calcined clay or other aggregates. The covering soil helps maintain appropriate moisture and humidity levels while providing support for the developing fruiting bodies [9]. In addition, spawning materials are substances used to introduce mushroom mycelium into the compost and provide additional nutrients for the developing mushrooms. Common examples include soybean meal, cottonseed meal, and gypsum. Water is also a crucial component in maintaining the optimal moisture levels for the substrate and covering soil. Careful selection and management of these materials is essential to provide the best growth conditions for mushrooms and ensure successful harvests [4, 10].

Calcium (Ca) is essential for the structural integrity of cell walls and membranes and regulates various physiological processes, including cell division, differentiation, enzyme activity, and nutrient absorption. In the context of mushroom cultivation, Ca also influences the physicochemical properties of both the compost and the covering soil [11, 12]. Moreover, Ca is an essential macronutrient for plants and fungi, and its effectiveness within the growth substrate can significantly impact the yield and quality of harvested products [13, 14]. Prior research has demonstrated that the availability of Ca can affect the growth and development of diverse plant species, including fruit trees, vegetables, and cereals [15]. For instance, Reitz et al. reported that the moderate application of Ca to tomato plants effectively reduced the incidence and severity of blossom-end rot while enhancing the Ca content in fruits, thereby increasing both tomato yield and quality [16]. Furthermore, Ca plays a pivotal role in plant responses to abiotic and biotic stresses, such as drought, salinity, and diseases. Similarly, there have been reports indicating that Ca also influences the growth and development of certain edible mushrooms, such as button mushrooms and shiitake mushrooms [17, 18].

Within the context of *A. bisporus* cultivation, several studies have investigated the impact of Ca on mushroom growth and development [19, 20]. For instance, Philippoussis et al. found that irrigating the growth substrate with a 0.35% $CaCl_2$ concentration enhances the yield and quality attributes of *A. bisporus* [21]. These studies demonstrate the potential benefits of optimizing Ca levels in the cultivation of *A. bisporus* and emphasize the necessity for further research to elucidate the specific effects of Ca on this widely cultivated edible mushroom [22]. However, the precise impact of Ca on *A. bisporus* cultivation and the dynamic changes in Ca concentration and the chemical environment during the cultivation process remain unclear.

Therefore, this study aims to address this knowledge gap by investigating changes in Ca concentration and the chemical environment in the substrate and covering soil during *A. bisporus* cultivation. We hypothesize that the Ca concentration and the chemical environment in the substrate and covering soil may vary over time during the cultivation process, and these variations may impact the growth and development of *A. bisporus*. To test our hypothesis, we conducted a series of experiments using standard commercial substrates and covering soil with a balanced nutrient composition for the cultivation of *A. bisporus*. The Ca content and chemical environment in the compost and covering soil at different stages of mushroom growth were monitored using ICP, FTIR, and XPS techniques. The findings of this study will offer valuable insights for mushroom growers and researchers to enhance cultivation methods and nutrient management strategies in edible mushroom production. This research holds significance in optimizing the cultivation processes of *A. bisporus* and other edible mushrooms, thereby contributing to the sustainable production of nutritionally rich and high-quality edible mushrooms.

Materials and methods

Sampling

The compost, mushroom cultivation samples, and their initial ingredients were obtained from commercial farms (Funan Lian Mei Agricultural Products Co., LTD). Sixteen samples were collected from compost batches during both the composting and mushroom cropping stages (Fig. 1). In Stage 1 (samples B1–3 and C0–1): the compost undergoes fermentation and is converted into mature compost, which is then incubated with mushroom mycelium. Commercial compost production typically consists of three phases [23]. Phase I: at the start of the composting process, the basic mixture of chicken manure (B1) and prewetted straw (B2) is watered and mixed. Thermophilic micro-organisms, including fungi, begin to decompose the basic mixture material, causing the temperature to rise to 80 ℃, resulting in Phase I (C0)

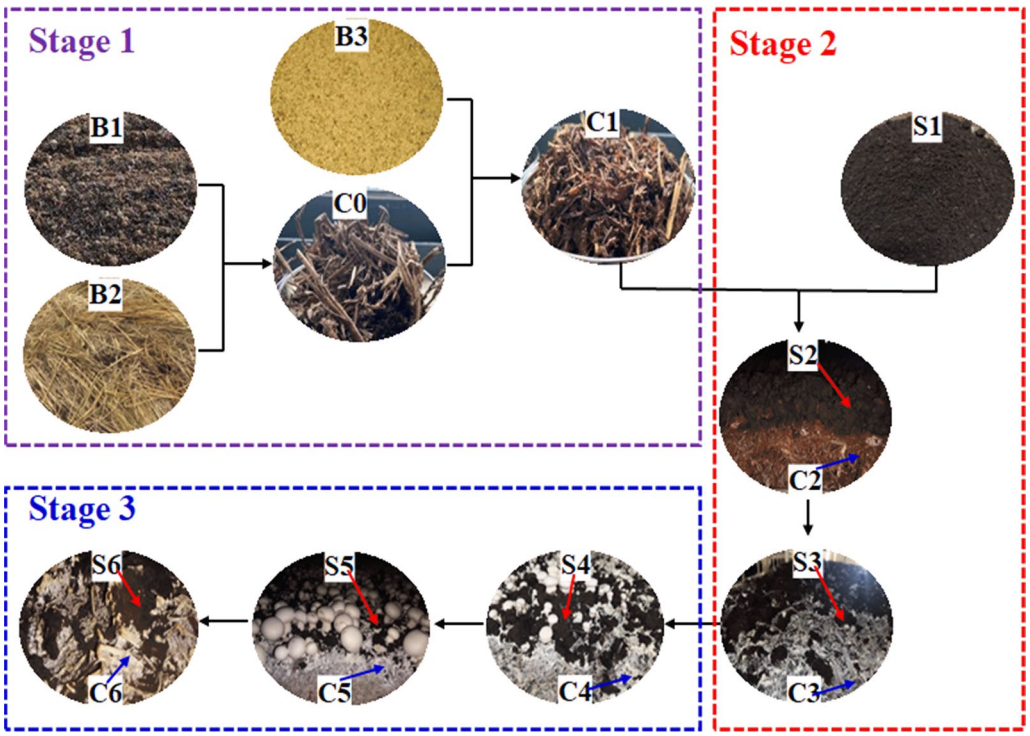


Fig. 1 Schematic representation of the composting, mycelium, and mushroom growth in *A. bisporus* cultivation

compost. Phase II: the compost is conditioned at 45–50℃ until it is free of ammonia, after which the temperature is lowered to about 25 ℃ [8]. Phase III: *A. bisporus* mycelium is inoculated through the spawning material (B3: soybean meal). Temperatures are maintained at around 25 ℃, and mycelium grows, within 16 days, mature compost (C1) is obtained. Stage 2 (samples C2–3 and S1–3): mulching induced mycelium growth into fruiting bodies. A layer of soil (S1) about 5 cm thick is spread over the mature compost, which is about 25 cm thick. The temperature inside the mushroom house is maintained at 16–20 ℃, the pile temperature at 24–29 ℃, and the humidity is kept at 98–99% to promote mycelial growth. Samples S2 and C2 represent covering soil and compost samples covered with soil for about 7 days, respectively. When the mycelium grew to the surface of the covering soil (about 17 days), the upper soil (S3) and the underlying compost (C3) were sampled. Stage 3 (samples C4–6 and S4–6): this stage represents the maturation of fruiting bodies, and waste compost and soil are generated after harvesting *A. bisporus* were harvested for approximately three flushes. Samples S4 and S5 represent the covering soil from the second and third flushes, respectively, during the harvesting period. Samples C4 and C5 correspond to the base compost materials from the same periods. After harvesting, waste compost (C6) and soil (S6) were produced. To minimize the effects of experimental variability, all samples were collected and mixed at three different points, followed by freeze-drying. The resulting dried samples were then ground into a powder state.

Chemical analysis

The Ca content in composts and covering soil during *A. bisporus* cultivation was measured using inductively coupled plasma spectrometry (ICP-MS: Thermo Fisher iCAP PRO, USA). Due to the complex composition of covering soil and chicken manure, the samples were pre-treated by microwave digestion. The Ca content is calculated by Eqs. 1 and 2 [24, 25]:

$$C_X(\mathrm{mg/kg}) = \frac{C_0\,(\mathrm{mg/L}) * f * V_0\,(\mathrm{mL}) * 10^{-3}}{m_0(\mathrm{g}) * 10^{-3}} \quad (1)$$

$$W(\%) = \frac{C_x\,(\mathrm{mg/kg})}{10^6} * 100\% \quad (2)$$

where m_0 (g) represents the mass of the sample used for analysis; V_0 (mL) denotes the constant volume of the sample after digestion; f stands for the dilution ratio; C_0 (mg/L) signifies the concentration of elements in the test solution, as determined by the instrumental analysis; C_x (mg/kg) represents the final test result, calculated using Eq. (1); and W (%) is the final test result expressed as a percentage, calculated using Eq. (2).

To analyze changes in the surrounding environment of Ca during composting and the growth of *A. bisporus*, all samples underwent analysis using Fourier transform infrared (FTIR) and X-ray photoelectron spectroscopy (XPS) spectra. Sample B1 was not subjected to XPS analysis due to the strong odor of chicken manure, which could potentially damage the instrument. FTIR spectra of all samples were recorded using a Thermo Scientific Nicolet iS50 FT-IR spectrometer (USA) within the range of 4000–450 cm^{-1} [26]. XPS measurements of all samples were conducted using a Thermo Scientific K-Alpha spectrometer (UK) equipped with a monochromatic Al K radiation source (1486.6 eV). The binding energy values were calibrated using the C 1 s signal at 284.8 eV as a reference [27].

Results

Stage 1: compost fermentation phase

The Ca content in freeze-dried chicken manure (B1) was determined to be 7.13% through ICP analysis (Table 1). Ca in chicken manure primarily originates from feed ingredients, skeletal tissues, eggshells, and forage materials [28]. Ca exists in both inorganic forms, such as calcium carbonate ($CaCO_3$), hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), and calcium phosphate ($Ca_3(PO_4)_2$), as well as organic forms, such as organic Ca salts. Straw (B2), primarily composed of cellulose, hemicellulose, and lignin [29], exhibits a Ca content of 0.91% as determined by ICP analysis. The presence of Ca in straw is predominantly derived from plant bio-cycling and the surrounding soil. Plants absorb inorganic Ca ions from the soil through their root systems, which are subsequently transported to various plant tissues and organs, including stems, leaves, and straw. In straw, Ca primarily exists in an inorganic form, mainly as Ca salts [30]. Common Ca salts found in straw include $CaCO_3$, $Ca_3(PO_4)_2$, and $Ca_{10}(PO_4)_6(OH)_2$. These Ca salts can interact with the cell wall structures and organic compounds present in plant tissues, forming stable compounds. The actual Ca content of the Phase I compost (C0), composed of chicken

Table 1 Calcium content of Stage 1 measured by ICP

Sample	Calcium content
B1: chicken manure	7.13
B2: straw	0.91
C0: Phase I compost	4.90
B3: soybean meal	3.10
C1: mature compost	4.60

manure and straw, as measured by ICP, is 4.90%, which may deviate from the expected values. To maintain a C/N ratio of approximately 30:1 in the compost, the ratio of chicken manure to straw can be adjusted as needed [31]. During the fermentation process, Ca present in chicken manure and straw may undergo release and transfer into the mixture. Factors such as microbial metabolic activities and changes in acidity or alkalinity during fermentation could facilitate the release of Ca from organic matter or inorganic salts, redistributing it within the mixture. For instance, certain micro-organisms may possess the ability to utilize or fix Ca, potentially resulting in an increase in the Ca content of the mixture [32].

The main components of soybean meal (B3) include protein, cellulose, oil, and carbohydrates [33]. In soybean meal, Ca primarily exists in an inorganic form, mainly in the form of Ca salts. Common Ca salts found in soybean meal include $CaCO_3$ and $Ca_3(PO_4)_2$. During the growth process of soybean plants, they absorb inorganic Ca ions from the soil through their root systems and transport them to various tissues and organs, including soybean meal [34]. Therefore, the Ca in B3 mainly originates from soybean plants themselves and the soil in which they are grown, with the actual Ca content measured at 3.1% by ICP. The Ca content in mature compost (C1), obtained by further fermenting C0 and then mixing it with B3, is 4.6%. Consequently, we can conclude that the mature compost obtained after a series of substrate fermentations has a Ca content proportion of 4.6%.

The FTIR spectra are examined to identify the types of chemical bonds and functional groups associated with Ca [26]. Changes in the absorption bands are observed to reveal interactions between Ca and functional groups, such as carbonate or phosphate groups. Specific functional groups, including hydroxyl, carboxyl, or amine groups, influence the coordination and bonding of Ca. By correlating the FTIR data with the Ca content, insights into the chemical environment surrounding Ca and its interactions with other compounds or functional groups in the samples are obtained. This analysis provides valuable information about the coordination and bonding of Ca in the studied samples and sheds light on its interactions with different chemical species.

The FTIR spectrum (Fig. 2) of chicken manure may vary due to feeding conditions, feed composition, and treatment methods. The FTIR analysis results for B1 are as follows: the peaks at 3269 cm^{-1}, 2920 cm^{-1}/2849 cm^{-1}, 1621 cm^{-1}, 1548/1416 cm^{-1}, and 1031 cm^{-1}, correspond to the O–H stretch vibrations [35], C–H stretch vibrations [36], C=C stretch vibrations, asymmetric and symmetric C–O bonds stretching vibrations (CO_3^{2-}) [37], and phosphate (PO_4^{3-}) group [38], respectively. The presence of –OH, CO_3^{2-}, and

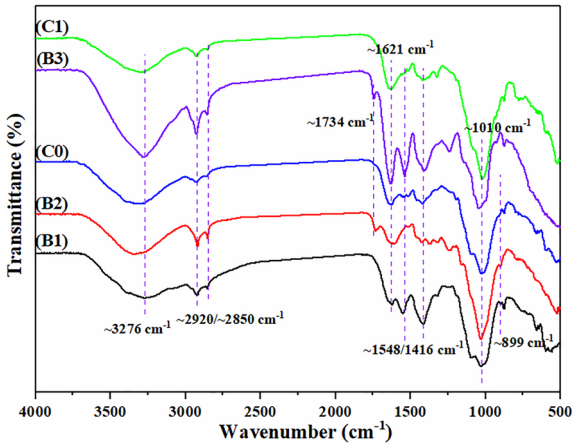


Fig. 2 FTIR spectra of Stage 1

PO_4^{3-} groups indicates a suitable environment for the presence of Ca. Furthermore, the presence of multiple functional groups suggests a complex and diverse chemical environment in chicken manure samples. In addition to the characteristic functional group peaks observed in B1, the FTIR spectrum of B2 also exhibits a peak at 1733 cm^{-1} corresponding to the C=O bonds [39]. Based on these observations, we speculate that the presence of these functional groups creates an environment conducive to the formation of $CaCO_3$. The strong vibrational peaks of –OH (~ 3276 cm^{-1}), C=O (~ 1733 cm^{-1}), C–O (~ 1548/1416 cm^{-1}), and PO_4^{3-} (~ 1010 cm^{-1}) in sample B3 indicate the presence of corresponding Ca salts [40]. Sample C0 is a mixture of B1 and B2 obtained through thermophilic microbial degradation. Therefore, the FTIR spectrum of sample C0 exhibits vibrational peaks corresponding to the respective functional groups present in samples B1 and B2. From the spectrum, it can be observed that the intensity of vibration peaks located around 2924/2855 cm^{-1} and 1548 cm^{-1} is significantly reduced. This reduction suggests the involvement of micro-organisms in the decomposition of organic matter (proteins) during the fermentation process [6], leading to changes in the chemical environment surrounding Ca. A similar phenomenon was also observed in sample C1. Based on these observations, it can be inferred that the fermentation process, involving microbial activity and organic matter degradation, leads to changes in the chemical environment of compost, potentially resulting in the dynamic transformation of Ca compounds. This suggests that microbial activity and chemical reactions during the fermentation process may influence the stability of Ca compounds in compost.

In XPS analysis, it is customary to conduct the analysis under vacuum conditions. However, the strong odor

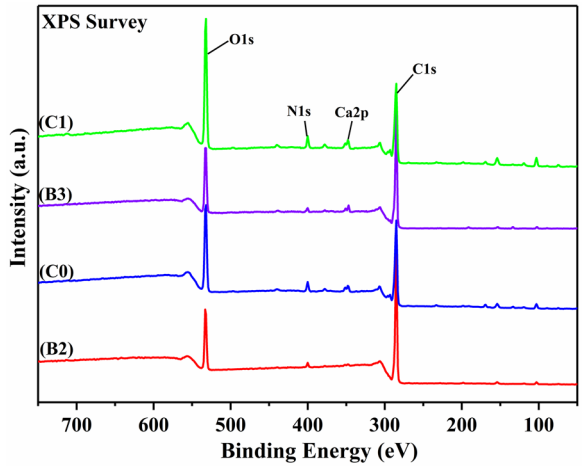


Fig. 3 XPS survey spectra of Stage 1

emitted by B1 could potentially interfere with the instrument and compromise the accuracy of the data [27]. To ensure the safety and accuracy of the laboratory and analysis environment, the decision was made not to conduct XPS analysis on B1. Analysis of the XPS survey spectrum revealed the presence of peaks for C1s, Ca2p3/2, N1s, and O1s (Fig. 3). The relative content of each element (Additional file 1: Table.S1) can be determined based on the peak areas or peak heights in the survey spectrum [40]. It is important to note that the relative content obtained from XPS analysis should be interpreted as relative proportions rather than absolute quantification. Sample C0, derived from the fermentation of B1 and B2, exhibited an increase in the relative content of oxygen (O) and nitrogen (N) elements, while showing a decrease in the relative content of carbon (C) was observed. The increased in the relative content of O and N in sample C0 can be attributed to the degradation and transformation of organic compounds during the fermentation process [32]. Micro-organisms involved in the fermentation process utilize the organic matter in the samples as sources of energy and nutrients, leading to the generation of more oxidative products and N-containing compounds. Microbial activities during fermentation result in the production of CO_2 and H_2O, which could contribute to the increased relative content of O. Furthermore, the fermentation process may facilitate the conversion of N in the organic matter into amino compounds or other N-containing compounds, leading to an increased relative content of N [41]. On the other hand, the decrease in the relative content of C in sample C0 can be attributed to the metabolic utilization and degradation of organic matter by micro-organisms during fermentation. Some of the organic C may be released as CO_2 and expelled from the system, thus reducing the C content in the sample [23]. In addition, certain organic compounds may undergo structural transformations during the fermentation process, resulting in a decrease in the C content in the sample. Similar phenomena were also observed in sample C1. Moreover, in comparison with C0, C1 exhibited an increased relative content of N and O elements, while a decrease in the relative content of C. These findings suggest that the changes in the relative content of O, N, and C observed in samples C0 and C1 are consistent with the ongoing microbial processes and metabolic activities taking place during the fermentation of the organic mixture.

After deconvolution analysis, we observed peaks associated with the C element at approximately 284.80 eV, 286.4 eV, and 287.8 eV, respectively (Fig. 4A). The positions of these peaks provide information about the chemical state and environment of the C element. Specifically, the peak at around 284.80 eV may correspond to the presence of C–C or C–H bonds, indicating the presence of hydrocarbon compounds or alkyl groups in the sample. Similarly, the peaks at 286.4 eV and 287.8 eV, associated with C–O or C=O bonds, align with the FTIR bands attributed to carbonyl compounds or functional groups involving O [42]. Following the fermentation process, there was a significant decrease in the relative content of C–C bonds in samples C0 (65.89–45.54%) and C1 (60.27–34.91%), while the relative content of C–O and C=O bonds in sample C1 increased significantly. This outcome further supports the notion that micro-organisms, during the fermentation process, utilize the organic compounds (C–C) in the samples as a source of energy and nutrients, engaging in metabolic and degradation activities, and generating chemical substances with C–O/C=O structures or releasing CO_2.

The peak observed at approximately 399.8 eV in the N 1 s spectrum may correspond to amino groups (–NH_2) or amine compounds (Fig. 4B), while the peak observed around 402.2 eV may correspond to imine groups (–NH–) or amino groups (–NH_2) [43]. The fermentation process involves protein degradation and amino acid metabolism, which could result in a slight increase in the relative content of -NH_2 groups in samples C0 and C1 [44]. The peak observed at approximately 532.2 eV in the O 1 s spectrum can be attributed to carbonyl groups (C=O) or carboxylic acid groups (–COOH), while the peak observed at around 532.8 eV is likely associated with hydroxyl groups (–OH) or alcohol groups (–ROH) (Fig. 4C) [40]. It is worth noting that in comparison with sample C0, which underwent only one round of fermentation, the mature compost sample C1 exhibited a blue shift in the O 1 s binding energy (from 532.13 eV to 532.00 eV, and from 532.94 eV to 532.66 eV), accompanied by an increase in the relative O content (from 27.75% to 32.23%, and from 1.75% to 4.49%). These

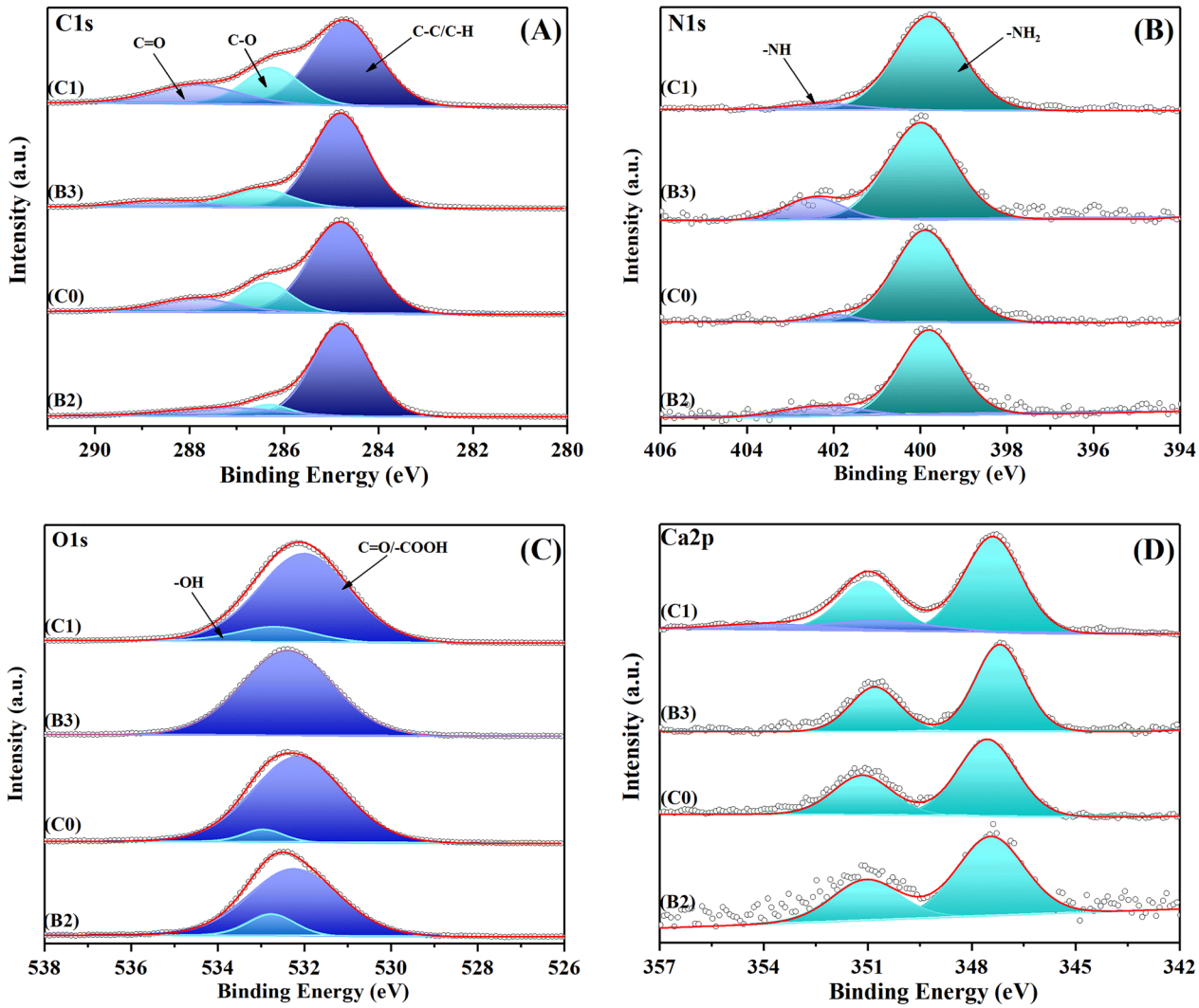


Fig. 4 XPS spectra of C1s (**A**), N1s (**B**), O 1 s (**C**), and Ca2p (**D**) of Stage 1

observations further support the degradation and transformation of organic matter during the fermentation process [45].

Due to the low Ca content in the straw, the Ca2p peak in B2 exhibits higher noise levels (Fig. 4D). The Ca2p3/2 binding energy is 347.55 eV in sample C1, slightly higher than in B1 (347.45 eV), suggesting a slight change in the chemical environment of Ca compounds during the fermentation process. The shift of the binding energy to higher values suggests an increase in the relative abundance of electronegative species in the environment surrounding Ca [46]. Because N and O have higher electronegativity than C, during the fermentation process, there is an increase in the relative content of N and O, accompanied by a decrease in the relative content of C. This provides a reasonable explanation for the observed increase in Ca2p binding energy. Similarly, an upward shift in binding energy was observed in sample C1. Furthermore, the Ca2p3/2 binding energy in C1 appeared at both 347.38 eV and 350.65 eV, indicating the presence of multiple coexisting Ca compounds. This split peak pattern suggests that the composition of C1 has become more complex after undergoing three rounds of fermentation processes.

The complex composition of chicken manure, straw, and soil contributes to the diverse and intricate chemical environment of Ca in the fermented mixture. The fermentation process involves microbial activities, decomposition of organic matter, and transformation of nutrients, all of which can result in dynamic changes in Ca compounds. The FTIR and XPS results provide further evidence that the consumption of organic C sources occurs during fermentation, leading to an increase in the relative content of N and O, which are more

Table 2 Calcium content of Stage 2 measured by ICP

Sample	Calcium content
S1: raw soil	7.02
S2: covering soil about 7 days	2.61
S3: covering soil about 17 days	2.38
C2: compost about 7 days	4.55
C3: compost about 17 days	4.12

electronegative. Consequently, Ca loses more electrons and undergoes a shift towards higher oxidation states. Overall (based on ICP analysis), the total Ca content remains relatively stable, indicating a consistent source of Ca in the compost.

Stage 2 mycelial growth phase

The frozen and dried raw soil (S1) was analyzed by ICP, and the Ca content was found to be 7.02% (Table 2). $CaCO_3$ is a common mineral in soil and serves as one of the primary sources of Ca [47]. After approximately 7 days of incubation in the mushroom cultivation room, the Ca content in the soil of S2 decreased to 2.61%. Subsequently, after 17 days, the Ca content in the soil (S3) further declined to 2.38%. This decrease in Ca content is likely attributed to the migration and utilization of Ca that occurs during the mycelial growth process. It is hypothesized that the growth and metabolic activities of the mycelium efficiently utilize the nutrients present in the soil, leading to a reduction in the Ca content within the soil. In the compost, the Ca content exhibits a minor declining trend (from 4.60% in C1 to 4.55% in C2, and further to 4.12% in C3). Accordingly, we hypothesize that the mycelium primarily acquires Ca nutrients from the soil during its growth and development.

In the FTIR spectra of the raw soil (S1), peak positions were observed at approximately 3292 cm^{-1}, 2918 cm^{-1}/2849 cm^{-1}, 1610 cm^{-1}, 1416 cm^{-1}, 1007 cm^{-1}, and 874 cm^{-1}, which can be attributed to the O–H bond, C–H bond, C=C/C=O bond, C–H bond, PO_4^{3-} group, and C–H bond, respectively (Fig. 5) [37, 38]. The blue shift observed in the C=C/C=O band at around 1620 cm^{-1} in samples S2 (after 7 days) and S3 (after 17 days) may be indicative of an increased electron density of ligands binding to Ca ions, thereby strengthening their interaction with Ca ions. Conversely, a noticeable decrease in the intensity of infrared peaks in S2 and S3 compared to S1 suggests the degradation or consumption of organic compounds in the samples during mycelial development. The mycelium absorbs and utilizes nutrients, including Ca and organic compounds, from the soil, leading to changes in the chemical composition of the samples. In addition, metabolic byproducts of the mycelium, such as enzymes or secondary metabolites, can interact with functional groups in the samples and influence the infrared spectrum. The decrease in infrared peak intensities may indicate the dynamic transformation and redistribution of Ca during the fermentation process to accommodate microbial activity and organic matter transformation.

The presence of a prominent vibration peak around 1320 cm^{-1} in the compost samples (C2 and C3), which is not observed in the soil samples (Fig. 5), suggests the presence of amino compounds or other N-containing compounds in the compost [48]. In addition, with increased time spent in the mushroom cultivation facility, as observed in compost sample C3, the intensity of the vibration peak at 1016 cm^{-1} shows a slight increases. This can be attributed to the decomposition and polymerization reactions of organic matter, resulting in the accumulation of new substances in the compost pile. Consequently, their concentration increases, subsequently enhancing the intensity of the infrared peak. The decrease in intensity of the infrared peaks in the soil samples, accompanied by an increase in the compost samples, suggests a dynamic transformation and redistribution of Ca during the fermentation process. The mycelium in its developmental stage primarily consumes nutrients, including Ca, from the soil. Meanwhile, the byproducts of organic matter reactions may accumulate in the compost, leading to significant consumption and utilization of Ca in the soil, with a less noticeable change in the compost samples. These results align with the data obtained from ICP analysis.

The XPS survey spectrum analysis revealed the presence of C1s, Ca2p3/2, N1s, and O1s peaks in the sample (Fig. 6). The relative content of each element was

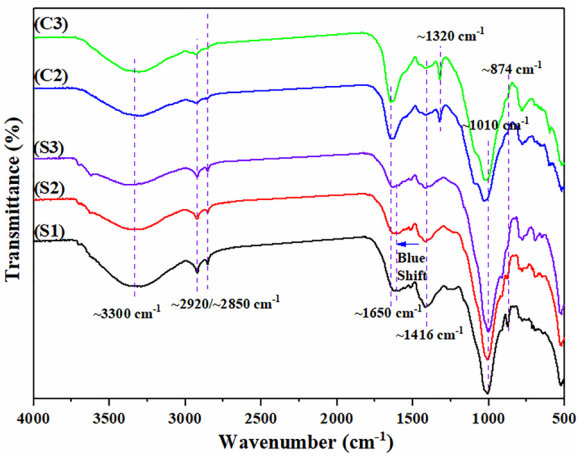


Fig. 5 FTIR spectra of Stage 2

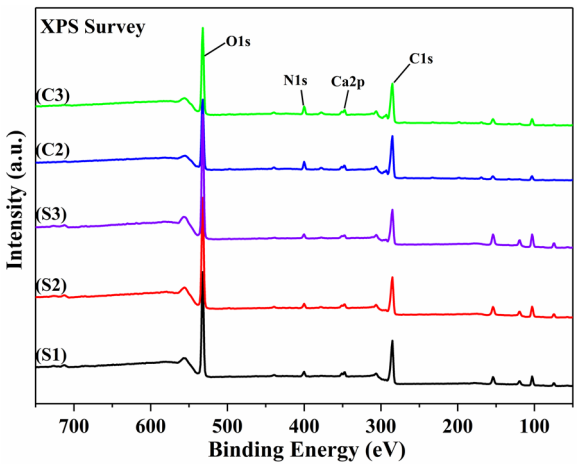


Fig. 6 XPS survey spectra of Stage 2

determined by analyzing the peak areas or peak heights, as summarized in Additional file 1: Table S2. The relative content of Ca in the covering soil decreased continuously with an increasing number of days spent inside the mushroom cultivation room, following a similar trend to that observed in the ICP results. As the number of days in the mushroom cultivation process increased, the relative content of C and N in the covering soil gradually decreased, while the relative content of O increased. This observation suggests that C and N elements are being utilized and metabolized by mycelium or other microorganisms, leading to their decreased levels. Simultaneously, the increase in O content may be attributed to the production of oxidative by-products or an enhanced occurrence of oxidation reactions resulting from the metabolic activities of mushrooms or micro-organisms. These changes are likely associated with the decomposition of organic matter, microbial activities, and the growth and development of mycelium in the soil. Similar trends of decreasing relative content in Ca, C, and N, accompanied by an increase in relative O content, were observed in the composting process. This suggests that the decomposition and transformation of organic matter during composting result in a reduction of Ca, C, and N levels, while the increase in O content may be attributed to microbial respiration and oxidative processes. These changes indicate the dynamic nature of nutrient transformations and microbial activities occurring within the composting system.

Subsequent deconvolution analysis of the C1s in the covering soil of Stage 2 revealed binding energy peaks similar to those observed in Stage 1. The peak positions were determined to be approximately at 284.60 eV, 286.2 eV, and 287.7 eV, corresponding to the presence of C–C/C–H, C–O, and C=O functionalities, respectively (Fig. 7A). The analysis of C–C, C–O, and C=O bonds in the covering soil (S1, S2, and S3) reveals interesting trends. The decreasing binding energy and relative content of the C–C bond suggest a potential reduction in the presence of C–C bonds, indicating the degradation or transformation of organic compounds containing C–C bonds during mushroom cultivation. In contrast, the decreasing binding energy but increasing relative content of the C–O bond imply an accumulation or enhanced formation of O-containing functional groups, possibly due to the breakdown of organic matter and increased incorporation of O-rich compounds. The increasing binding energy and decreasing relative content of the C=O bond suggest a potential decrease in the presence or formation of carbonyl groups, indicating a transformation or reduction of organic compounds containing C=O functional groups. These observations reflect the complex nature of organic transformations occurring in the covering soil during mushroom cultivation. The compost (C2 and C3) exhibited a slight decrease in both the binding energy and relative content of C–C, C–O, and C=O bonds (Fig. 7B), indicating significant changes in the compost matrix. These observations suggest the decomposition, degradation, or transformation of organic compounds. The declining C–C and C–O bonds signify the breakdown or conversion of C–C and O-containing groups, respectively, while the diminishing C=O bonds indicate a reduction in carbonyl compounds. These findings highlight the dynamic nature of the composting process and the intricate biochemical reactions involved.

After entering the mushroom cultivation facility, the N 1 s spectrum of the covering soil samples exhibited changes in peak positions. In S1, two peaks were observed at approximately 399.81 eV and 400.43 eV, which could be associated with amino groups ($-NH_2$) or amine compounds. In S2, only one peak was observed at around 399.93 eV, indicating the presence of a different type of N-containing compound. Sample S3 exhibited two peaks at approximately 399.76 eV and 401.82 eV (Fig. 7C). These variations suggest that the composition and properties of N-containing species in the samples have undergone changes, possibly related to the development of mycelium. The observed similarities in the compost (C2 and C3) suggest that the presence of N-containing substances (Fig.7D), such as proteins and amines, played a pivotal role in the mycelium's development upon entering the mushroom cultivation facility. These findings underscore the substantial impact of N-containing compounds on mycelium growth and development throughout the process of mushroom cultivation. Upon entering the mushroom cultivation facility, both the soil and compost samples exhibited a consistent

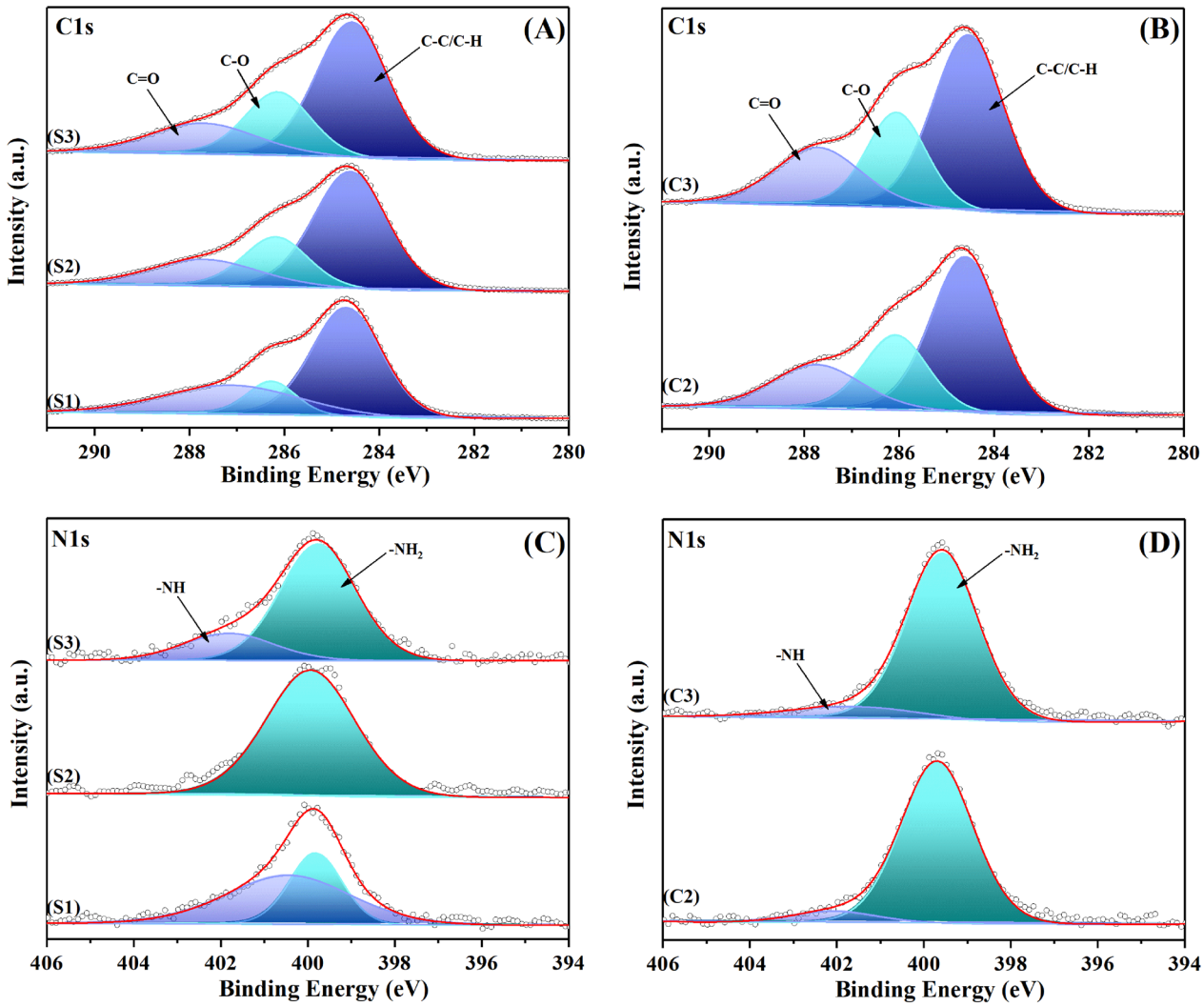


Fig. 7 XPS spectra of C1s (**A**, **B**) and N1s (**C**, **D**) of Stage 2

trend of decreasing binding energy and increasing relative content of O1s (Fig. 8A and B). The presence of mycelium and its associated microbial activity likely contribute to the degradation of organic matter, leading to the release of O-containing functional groups or compounds. These discernible changes in the O1s spectrum emphasize the dynamic nature of the samples and underscore the impact of mushroom mycelium growth on the O-related composition of the soil and compost.

The variations in C, N, and O during the growth and development of mushroom mycelium indicate a significant impact on the surrounding environment, particularly regarding Ca. Analysis of the Ca2p deconvolution results reveals the presence of multiple Ca environments in all samples (Fig. 8C and D), but their overall relative content exhibits a consistent downward trend, consistent with the findings from the ICP analysis. Specific analysis reveals that in the covering soil samples, the relative content of Ca at the binding energy position around 347.3 eV continuously decreases, while the relative content of Ca at the binding energy position around 350.5 eV shows a slight increasing trend. This could be attributed to the influence of an increased O content, which has a higher electronegativity. The phenomenon of decreased relative content also occurs in the compost samples. However, it is worth noting that the binding energy of Ca2p3/2 in sample C2 and C3 also show a decreasing trend. The declining Ca2p binding energies may be attributed to the presence of different chemical species or interactions with other elements or compounds present in the compost matrix. These findings emphasize the dynamic nature of Ca interactions and its sensitivity to the composting process.

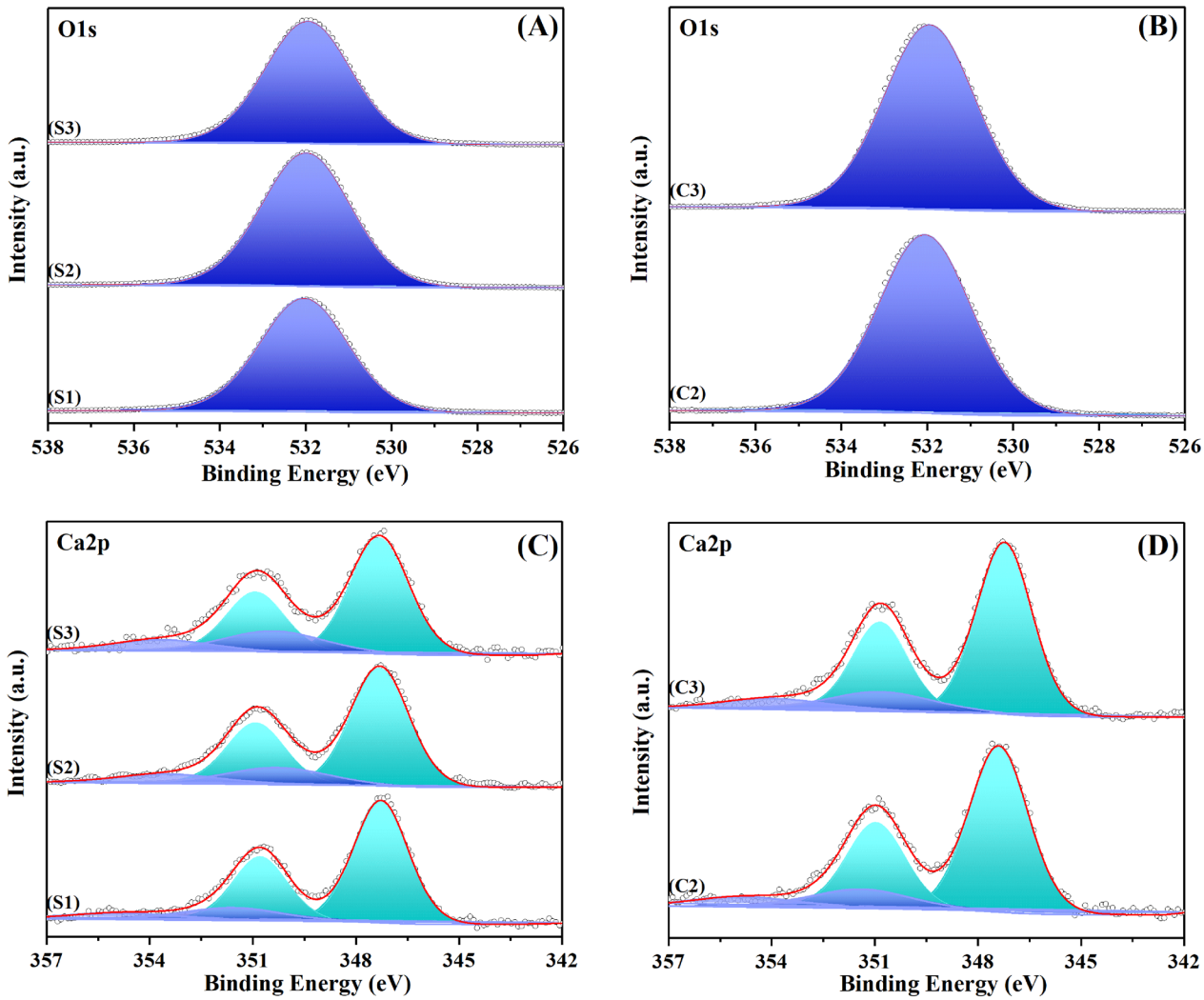


Fig. 8 XPS spectra of O1s (**A**, **B**) and Ca2p (**C**, **D**) of Stage 2

The changes in C, N, O, and Ca binding energies and relative contents indicate dynamic interactions and transformations of organic compounds in the compost and covering soil during the growth and development of *A. bisporus*. Enzymatic activity and metabolic processes of mycelium play a crucial role, actively participating in the decomposition, mineralization, and synthesis of organic matter. The Ca nutrition for mycelial growth is largely derived from the transformation of Ca in the covering soil. These findings highlight the dynamic nature of nutrient cycling and organic matter transformation, emphasizing the significance of cover soil and compost in the mycelial growth environment of *A. bisporus*.

Table 3 Calcium content of Stage 3 measured by ICP

Sample	Calcium content/%
S4: covering soil of 2 stubble mushrooms	4.48
S5: covering soil of 3 stubble mushrooms	3.16
S6: waste soil	3.75
C4: compost of 2 stubble mushrooms	4.32
C5: compost of 3 stubble mushrooms	4.33
C6: waste compost	5.79

Stage 3 *A. bisporus* harvesting phase

The results of the ICP analysis revealed that during the harvesting phase of *A. bisporus*, a decreasing trend in Ca content was observed in the covering soil (from 4.48% to 3.16%), whereas the Ca content in the compost

remained relatively stable (Table 3). Intriguingly, an increase in Ca content was observed in the discarded *A. bisporus* residues, both in the covering soil (3.75%) and the compost (5.79%). These findings suggest that during the growth process of *A. bisporus*, the mycelium might have absorbed Ca from the covering soil, resulting in a reduction in Ca content. The increase in Ca content in the discarded mushroom residues could be attributed to the decomposition of fungal biomass and the release of Ca compounds from the waste materials. These observations highlight the dynamic interplay and transformation of organic compounds in the growth environment of button mushrooms, emphasizing the significance of topsoil and compost in nutrient cycling and organic matter transformation.

Based on FTIR characterization, it was observed that during the harvesting phase of *A. bisporus,* there were no significant changes in the wavenumbers of characteristic functional groups in the covering soil samples (Fig. 9). However, compared to the second flush (S4), the intensity of the infrared peaks corresponding to functional groups in the third flush (S5) and mushroom residue (S6) samples exhibited a noticeable decrease. This phenomenon can be attributed to the combined effects of organic matter consumption, metabolic activities of mycelium and mushrooms, and associated chemical reactions. In the compost samples (C4, C5, and C6), the prominent vibration peak near 1320 cm^{-1} is likely associated with amino compounds or other N-containing compounds [48]. However, compared to the mycelial development stage (Stage 2), the changes in the intensity of the infrared vibration peak in the Stage 3 samples can be considered negligible. This situation may indicate that in Stage 3, the organic matter in the compost has been effectively decomposed and transformed, or the supply of organic

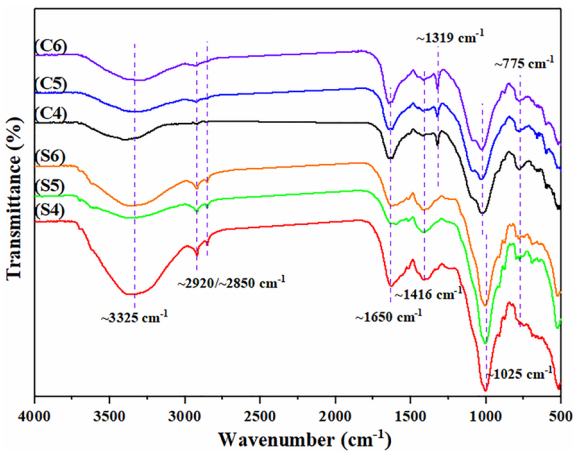


Fig. 9 FTIR spectra of Stage 3

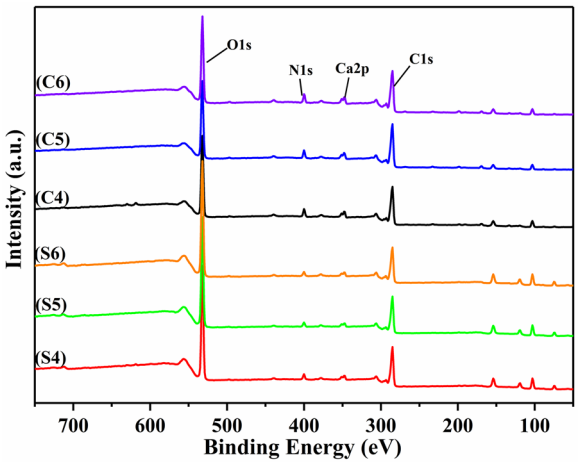


Fig. 10 XPS survey spectra of Stage 3

matter in the compost has reached a relative equilibrium with the mushroom's demand. This could be attributed to the metabolic coordination between the mycelium and fruiting bodies, as well as the interactions with other micro-organisms in the environment. These interactions may lead to changes in the content, forms, and availability of Ca in soil and compost.

XPS survey spectrum analysis was conducted on the samples obtained during the harvesting phase of *A. bisporus*, revealing the presence of peaks corresponding to C1s, Ca2p3/2, N1s, and O1s (Fig. 10). The relative content of each element was determined by analyzing the peak areas or peak heights, and the results are summarized in Additional file 1: Table S3. During the harvesting phase of *A. bisporus*, XPS analysis revealed a very slight decrease in the relative content of Ca in the covering soil samples (S4 and S5), whereas ICP characterization showed a more pronounced decrease. This difference can be attributed to the surface-sensitive nature of XPS analysis, which primarily detects the surface within a few nanometers, while ICP analysis provides a comprehensive measurement of the entire sample, including surface and internal components. Both characterization methods indicate a decrease in Ca content, suggesting that the soil provides Ca nutrients during the growth of *A. bisporus*. The relatively minor changes in the relative content of Ca in the compost samples (C4 and C5), which are consistent with the findings from ICP analysis, suggest that the compost has a relatively stable Ca composition during the harvesting phase. During the harvesting phase of *A. bisporus*, the relative content of C and O in the soil cover showed no significant changes. However, in the compost, there was an increase in C content and a decrease in O content, changes that were not observed during the fermentation and mycelial development phases. This

phenomenon may indicate that during the harvesting phase of *A. bisporus*, the mycelium has fully utilized the C and O elements present in the soil cover, converted them into biomass. The growth of the fruiting bodies has reached a stable state, and a substantial amount of C and O elements is no longer required for their growth and development. The increase in C content in the compost may be attributed to organic matter decomposition and microbial metabolic activities, while the decrease in O content may be related to organic matter respiration and oxidation processes. These findings highlight the differences in nutrient utilization and requirements during different growth stages and the interplay between mycelial growth and compost microbial activities.

During the deconvolution analysis of the C1s peak in the covering soil, it was observed that the relative content of the C–C bond at 284.6 eV remained relatively unchanged for S4 and S5 (Fig. 11A). However, the relative content of the C–O bond at 286.2 eV decreased, while the relative content of the C=O bond at 287.8 eV increased. The variations in the relative content of C–O and C=O bonds may be associated with the dynamic transformation and interaction of organic compounds during the growth and development of *A. bisporus*. In the mushroom residue stage (S6), the relative contents of C–O and C=O bonds recovered to 11.85% and 7.02%, respectively. When performing deconvolution analysis of the C1s peak in the compost samples (C4 and C5), a different trend was observed (Fig. 11B). The relative content of the C–C bond at 284.6 eV showed an increase, while the relative contents of the C–O and C=O bonds remained relatively unchanged. The increase in the relative content of the C–C bond could be attributed to the degradation and transformation of organic compounds, complex C-containing compounds are broken down into simpler compounds, leading to the formation of new C–C bonds. The relatively stable relative content of C–O and C=O bonds in the compost samples indicates that the abundance of C–O functional groups did not undergo significant changes. This suggests that during the mature harvesting phase of *A. bisporus*, there is no substantial formation or consumption of O-containing compounds in the compost. Alternatively, it could imply that there is a dynamic equilibrium in the transformation of C–O groups. Overall, these findings suggest that during the harvesting phase of *A. bisporus*, microbial degradation and transformation of organic compounds, as well as enzymatic activity and mycelium metabolism, significantly impact the chemical environment of C elements.

The deconvolution analysis of the N1s spectrum demonstrated the presence of two distinct N forms across all samples during the harvesting phase (Fig. 11C and D). These forms were identified at approximately 399.8 eV, corresponding to -NH_2, and approximately 402.0 eV, corresponding to –NH–. This suggests that N in the samples is primarily associated with organic compounds containing amino groups, such as proteins or amino acids. The significantly higher relative content of -NH_2 compared to –NH observed in all samples during the harvesting phase indicates a greater number of amino groups, possibly attributed to the degradation of proteins or other N-containing compounds during the mushroom growth and development process. According to the deconvolution analysis of O1s, only one form of O was observed, with a binding energy of 532.0 eV (Fig.12A and B). This suggests that the O atoms in the sample may share a similar chemical environment. Another possibility is that the sample contains a higher proportion of this particular form of O, which could result in the contributions of other O species or O-containing functional groups being averaged or masked, leading to the observation of a single peak. During the harvesting phase of *A. bisporus*, it is evident that the relative N content in the covering soil is lower compared to that in the compost. On the other hand, the O content in the covering soil is higher than in the compost. These findings underscore the distinct contributions of covering soil and compost to the growth of *A. bisporus*.

The deconvolution analysis of the Ca2p spectra revealed the presence of two distinct forms of Ca in all samples collected during the harvesting phase of *A. bisporus*. These forms were characterized by binding energies of approximately 347.3 eV and 351.0 eV, respectively. The existence of these two forms indicates the presence of different chemical environments or binding states for Ca in the samples (Fig. 12C and D). The occurrence of multiple Ca compounds with varying binding energies can be attributed to factors such as the origin of Ca in the soil, microbial activities, and the metabolites produced by the mushrooms themselves. Throughout the growth process of *A. bisporus*, changes in the soil's chemical environment, including fluctuations in soil pH or the introduction of additional substances, may contribute to the transformation or formation of different Ca compounds. Moreover, the metabolic processes of mushrooms can release specific compounds that may interact with Ca or form Ca compounds, resulting in the observed diversity of Ca forms. The presence of these distinct Ca forms underscores the complexity of Ca chemistry in the context of *A. bisporus* growth.

Based on the comprehensive results, the investigation conducted during the third stage reveals that the changes in Ca content in both soil and compost samples are no longer significant, especially in the soil samples. This suggests that during the maturation phase of the fruiting bodies, there is reduced dependence on the soil or compost for nutrient supply. It is possible that the metabolic

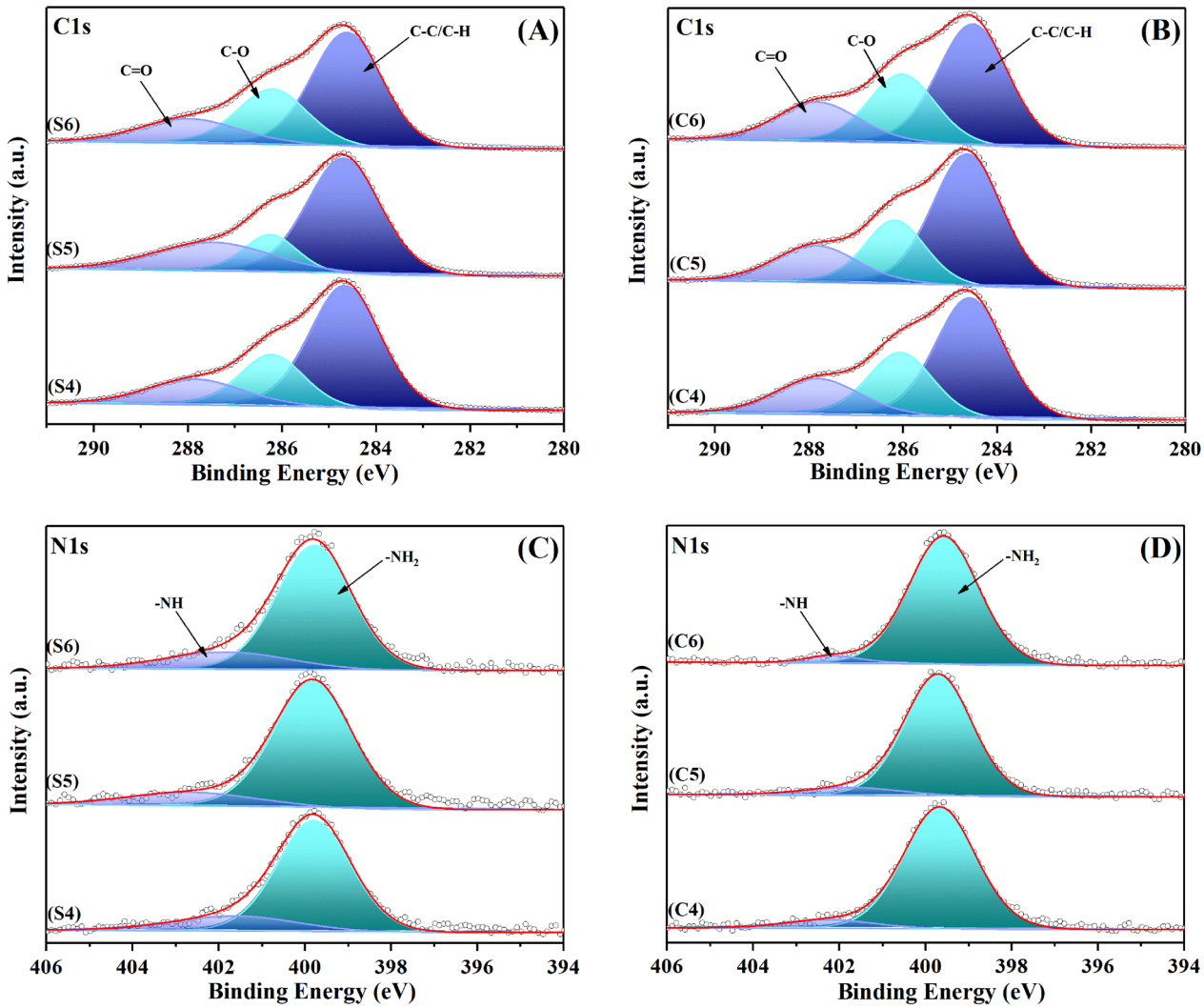


Fig. 11 XPS spectra of C1s (**A**, **B**) and N1s (**C**, **D**) of Stage 3

coordination between the mycelium and the fruiting bodies, as well as interactions with other micro-organisms in the environment, have led to the depletion of nutrient resources in the soil or compost, resulting in less noticeable variations in Ca, C, N, and O.

Discussion

Impact of elemental changes on the growth of *A. bisporus*

Fluctuations in elemental composition, including C, N, O, and Ca, during different stages of *A. bisporus* growth provide intriguing insights into the potential processes governing mushroom development and nutrient dynamics within the soil–compost–mushroom ecosystem. In the compost fermentation phase, characterized by peak organic matter decomposition and microbial activity, substantial changes in elemental composition serve as the foundation for *A. bisporus* growth. Microbial respiration and the breakdown of complex organic compounds lead to a significant decrease in C levels and an increase in O levels. Simultaneously, the mineralization of organic N sources by microbes results in a slight increase in N content. Notably, Ca content remains relatively stable during compost fermentation, indicating a consistent source of calcium within the compost materials.

During the mycelial growth phase, a noteworthy feature is the dynamic variation in elemental composition as the mycelium establishes itself within the substrate. The mycelium consumes available organic carbon sources for energy and growth, leading to a continuous decrease in C content during this stage, whether in the covering soil or compost. Similarly, owing to the mycelium's efficient utilization of N for protein synthesis and mycelial expansion, N content decreases in both the covering soil and compost. Furthermore, the substantial increase in O

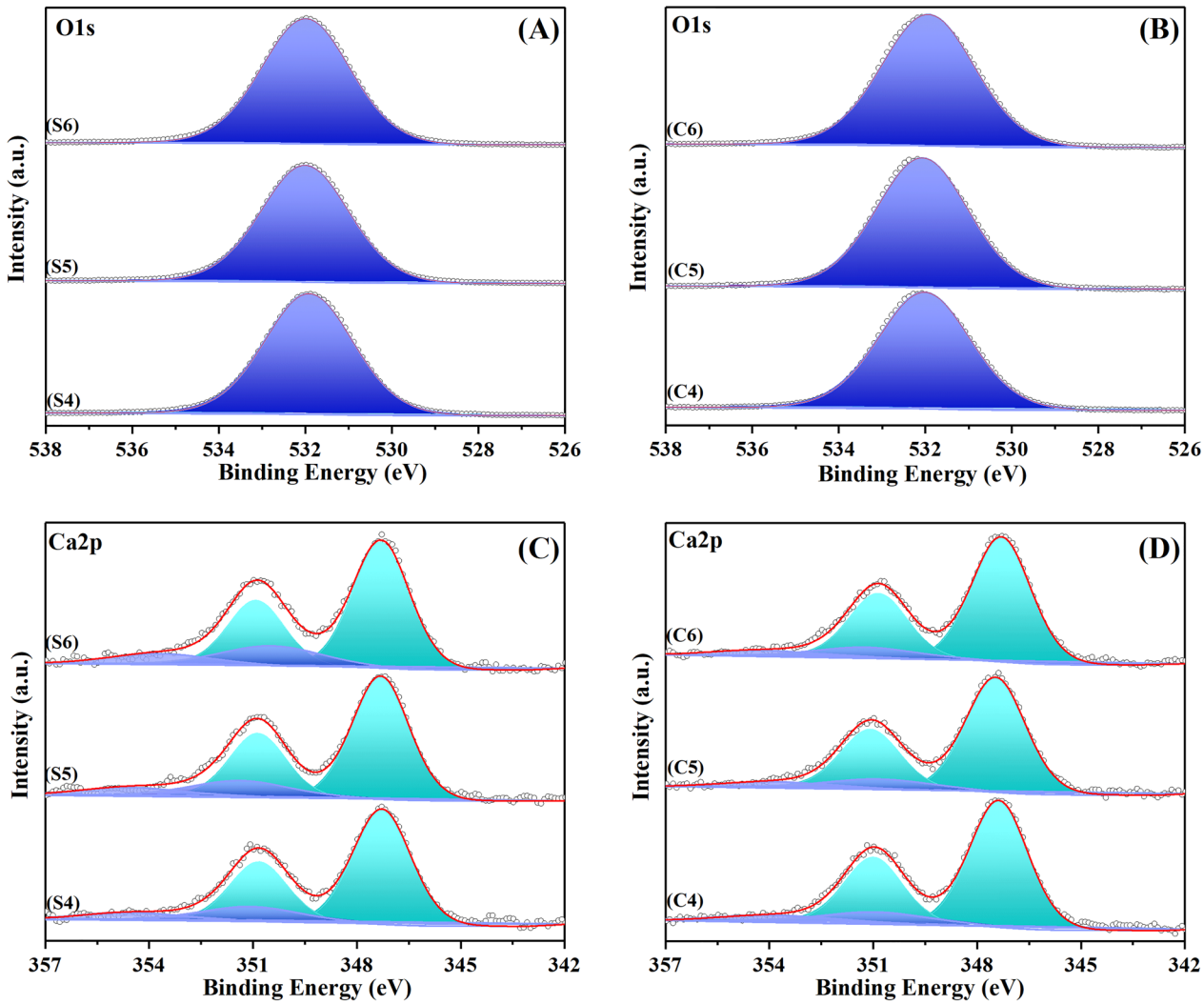


Fig. 12 XPS spectra of O1s (**A**, **B**) and Ca2p (**C**, **D**) of Stage 3

content may be attributed to the release of O-rich organic compounds as metabolic byproducts. A notable decline in Ca content occurs during this stage, particularly in the covering layer, underscoring the crucial role of Ca in primordia and subsequent fruiting body development.

As *A. bisporus* approaches maturity, they may rely more on internal nutrient reserves, resulting in less pronounced changes in elemental content. The increase in Ca content in the discarded mushroom residues is attributed to the breakdown of fungal biomass, releasing Ca compounds from the waste material. In summary, each growth stage of *A. bisporus* exhibits specific elemental changes, reflecting the organism's nutritional demands, metabolic variations, and ecological interactions.

Nutrient cycling and microbial contributions

Nutrient cycling is a fundamental process in the growth of *A. bisporus*. Microbial communities play a crucial role in nutrient transformations throughout the entire growth cycle, particularly influencing the availability of Ca. One of their primary tasks is the decomposition of organic matter, producing essential metabolites and releasing nutrients from organic reservoirs. The relentless pursuit of nutrients by mycelia and the transformative capacity of microbial communities collectively supports the growth of *A. bisporus*.

Throughout the entire growth stages, the dynamics of C reflect the metabolic activities of mycelia. Initially, mycelia absorb C from the soil–compost matrix, breaking down organic matter and converting it into biomass. As the *A. bisporus* mature, a shift occurs, reducing the mycelium's demand for C, while the compost microbial

community becomes active. This subtle transition underscores the complex balance between mycelia and microbes in utilizing available C resources. In the early stages, fluctuations in N content occur as mycelia break down N-rich compounds. Microbes enhance N mineralization, converting organic N into forms accessible to mycelia. This microbial contribution helps meet the mycelium's N requirements during growth. The concentration of O reveals its own narrative in nutrient cycling. Its levels are closely linked to microbial respiration and oxidation processes. Microbes actively decompose organic matter, consuming O through respiration, potentially leading to a decrease in O content. Conversely, the presence of oxidative byproducts suggests the involvement of mycelia and microbes in redox reactions, likely facilitating nutrient transformations.

Calcium demonstrates intriguing dynamics within the nutrient cycling process. The microbial community plays a significant role by enzymatically breaking down organic compounds containing Ca, releasing Ca ions into the soil–compost matrix, which significantly promotes Ca mobilization. Mycelia, acting as nutrient foragers and mobilizers, can directly access these released Ca ions, thus facilitating growth and development. In addition, microbial metabolic activities generate various byproducts, including organic acids and enzymes, further influencing nutrient dynamics. Organic acids can chelate Ca, affecting its solubility and effectiveness, while enzymes catalyze reactions that either release or sequester Ca within the growth environment. During the mycelial growth phase, there is a sharp decline in soil Ca content, whereas compost maintains relatively stable Ca levels. This indicates that microbial communities coordinate complex interactions, including organic matter decomposition, nutrient mineralization, Ca mobilization, and the production of metabolic byproducts. These multifaceted contributions serve as key factors driving nutrient transformations, especially Ca, within the *A. bisporus* growth environment. Nutrient cycling represents a multifaceted and dynamic pathway in the growth of *A. bisporus*. The continually changing nutrient demands of mycelia, microbial regulation of nutrient effectiveness, and the complex interactions among C, N, O, and Ca elements underscore the intricacy of this process.

Some cultivation guidelines

Here are some guiding points for further research and practical applications based on the understanding of elemental changes during *A. bisporus* growth:

Nutrient Management Strategies: During the early growth phases, when mycelium dominates, providing a nutrient-rich substrate can promote robust mycelial development. Adjusting C/N ratios in compost formulations can ensure a steady supply of these essential elements during mycelial expansion. As the *A. bisporus* matures, a shift towards microbial nutrient cycling becomes evident. Therefore, managing compost composition to support microbial communities during later growth stages is essential. Consider using microbial supplements or organic matter amendments to enhance microbial activities, thus contributing to nutrient availability.

Ca Supplementation: The study underscores the importance of soil as a primary source of Ca for *A. bisporus*. Growers can consider soil treatments or Ca-rich supplements to maintain adequate Ca levels, especially in the early growth phases when mycelial demands are high. Ca plays a crucial role in fruiting body development and can significantly impact yield and quality.

Monitoring and Adaptation: Regular monitoring elemental changes during cultivation can guide real-time decision-making. Nutrient management should be adaptable, with adjustments made based on the observed nutrient dynamics. Monitoring microbial communities and their activities, along with elemental content, can provide growers with insights into the health and progress of the cultivation system. Understanding the nutrient dynamics and microbial contributions can aid in reducing resource inputs, optimizing nutrient use efficiency, and minimizing environmental impacts associated with *A. bisporus* production.

Conclusion

In summary, the comprehensive analysis of various parameters and spectroscopic techniques during the growth and harvesting phase of *A. bisporus* provides valuable insights into the dynamics of organic compounds, elemental composition, and chemical environments in the soil and compost. The results indicate that the microbial degradation and transformation of organic compounds, as well as the enzymatic activity and metabolic processes of the mycelium, significantly influence the dynamics of C and N. The observed changes in the relative content of C and N elements suggest the involvement of microbial activity and nutrient availability during the growth stages of *A. bisporus*. Furthermore, the distinctive patterns in the relative content of Ca and O elements in the soil and compost highlight the different roles played by the soil cover and compost in the mushroom growth process. The variations in Ca forms and the presence of multiple binding energies emphasize the complexity of Ca chemistry and its interaction with soil components, microbial activities, and mushroom metabolites. These findings contribute to our understanding of the chemical environment and nutrient dynamics during the growth

of *A. bisporus* and have implications for optimizing cultivation practices and enhancing mushroom productivity. Further investigations are warranted to explore the underlying mechanisms and interactions among organic compounds, elemental dynamics, microbial communities, and mushroom development, ultimately advancing our knowledge of sustainable mushroom cultivation and nutrient management strategies.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-023-00471-y.

Additional file 1: Table S1. Relative content of C1s, Ca2p3/2, N1s, and O1s on Stage 1 measured by XPS. **Table S2.** Relative content of C1s, Ca2p3/2, N1s, and O1s on Stage 2 measured by XPS. **Table S3.** Relative content of C1s, Ca2p3/2, N1s, and O1s on Stage 3 measured by XPS.

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Author contributions
JW, JT, and XY designed the experiment. HS, XD, and NW prepared the samples, JW, RW, XL, and YN performed the experiments, analyzed data and wrote the paper. JW, FL, XD, JT, JY, and XY reviewed and checked all the details. All authors read and approved the final manuscript.

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Availability of data and materials
The data sets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
This research has been confirmed for publication in the journal.

Competing interests
The authors have no competing interests.

Author details
[1]Institute of Cotton, Anhui Academy of Agricultural Sciences, Hefei 230001, Anhui, China. [2]College of Biology and Food Engineering, Fuyang Normal University, Fuyang 236037, Anhui, China.

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