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Opinion

Overcoming the homogenization dilemma of forest-derived food and medicine homology resources: A multidimensional authentication strategy system based on ecological process memory

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1 Introduction

Forest understory ecosystems nurture a rich variety of food and medicine homology resources (FMHR), including forest fungi, fruits, and flowers. FMHR are understood here as materials used in food and traditional medicinal contexts, their permitted uses depend on the relevant jurisdiction. The term forest-derived includes both wild-harvested products and products cultivated beneath a forest canopy. These two production categories should be distinguished when the declared origin requires it. The fundamental difference between forest-derived and conventionally cultivated products may arise from growth-process-mediated metabolic adaptation rather than from simple differences in component concentrations. Light, nutrient availability, and biological interactions can affect plant metabolism and environmental responses. For forest fungi, interactions with trees, substrates, and associated soil microorganisms also provide a reason to investigate growing conditions. However, forest growth does not necessarily involve continuous stress, nor does conventional cultivation always provide a uniform environment. The effects of these conditions on product quality must be assessed for the species and properties concerned.

In distribution and consumption channels, products of the same species and grade are often treated as interchangeable, regardless of their actual growing history. This is the homogeneous competition discussed in this paper. The resulting homogenization dilemma is the difficulty of establishing a verifiable distinction between production categories. It does not mean that all forest-derived products have higher quality or should command a price premium. How widespread this problem is can be assessed by two practical measures: How often unverifiable production claims appear on the market, and whether production categories remain distinguishable after grade and processing are accounted for. Commercial mushroom studies illustrate the need to verify product descriptions. DNA barcoding has identified mislabelling in commercial porcini products[1]. This example concerns species identity and does not establish the extent of same-species forest-origin fraud or price competition. It nevertheless shows the limits of relying on labels alone. Once species identity is confirmed, a further question remains: Whether the material was produced under the growing conditions claimed.

Accordingly, this paper proposes a multidimensional authentication strategy centered on the concept of ecological process memory, extending the authentication target from the product's end state to its growth process. Ecological process memory refers here to endogenous and surface-associated biological signals that may retain information about previous growing conditions. It includes epigenetic states, associated microorganisms, and deposited environmental particles. The term therefore describes a group of possible sources of growth-history evidence, rather than a single biological memory mechanism. The aim is to provide additional evidence for forest-production claims and thereby support product differentiation.

2 Limitations of existing strategies for the homogenization dilemma

Chemical composition profiling is useful for quality assessment and adulteration detection, but end-state compositional measurements do not directly record the growing conditions

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under which the product was produced. Metabolic responses can vary with environmental conditions. Environmental responses can alter metabolic profiles, which makes a single measurement difficult to interpret as a record of the entire growing process. This does not mean that a short-term treatment can reproduce the full chemical profile of a forest-derived product. Chemical profiling remains useful for quality assessment and adulteration detection, while a claim about understory growth requires evidence appropriate to that claim. Stable isotope analysis has been used to investigate geographic origin and production conditions in different foods. Its performance depends on the isotope measured, the reference samples and the production contrast[2]. For understory and conventional cultivation within the same region, water sources, soil, fertilizers, and management must therefore be considered before drawing conclusions about discrimination. Isotope analysis can be informative for origin authentication, but distinguishing understory from open-field production within a single region remains to be tested. Certification and traceability systems record production requirements and product movements and may include inspection and analytical testing. Their effectiveness depends on maintaining the connection between the physical product and its records. Product substitution or misidentification can break the link between the actual sample and its documented production history. This gap is why additional biological tests on the material itself are needed. Such testing, however, also depends on controlled sampling and a secure chain of custody. The methods therefore address different questions. Chemical analysis describes composition and detects adulteration; isotope analysis can reflect environmental inputs; certification documents declared production practices and product movements. Ecological process memory would add candidate product-associated evidence of growing conditions, provided that its persistence, specificity and added value are demonstrated. Metabolites and isotope ratios can themselves reflect earlier exposure; the proposed strategy extends the sources of evidence rather than claiming that only the new modules can provide information about the past.

3 Strategy for reading ecological process memory

The core of the strategy proposed in this paper is that organisms growing under forest understory conditions may retain growth-associated signals within their bodies and on their surfaces. As shown in Fig. 1, the strategy consists of three complementary modules. DNA methylation provides a possible source of endogenous molecular evidence, while surface microbiome fingerprinting and spore-pollen imprint analysis provide evidence of ecological association and environmental exposure. Together, these modules connect endogenous molecular states with surface-associated microbiota and deposited environmental traces.

Their roles do not establish a fixed ranking of authentication performance. The choice of modules should depend on the species, tissue, processing state, and available validation results. In addition, the signals may share environmental influences and cannot be treated as statistically independent. Reading ecological process memory therefore means evaluating whether the available evidence supports a declared production category. It does not imply that the entire growth history can be reconstructed. Reference collections should distinguish wild harvesting, understory cultivation, greenhouse cultivation, and open-field cultivation where relevant. Material transferred between environments requires separate consideration or an inconclusive result.

3.1 DNA methylation: Endogenous molecular evidence

This module is based on the theoretical foundations of epigenetics. DNA methylation can affect genome regulation without altering the underlying sequence. Plant studies have associated methylation and other epigenetic processes with environmental responses and stress memory[3]. Work on wild strawberry clones has also provided evidence that some epigenetic differences may persist beyond the original exposure[4]. These findings provide a basis for investigating whether methylation patterns retain information relevant to the growing conditions of harvested products. Related applications also indicate the factors that require attention. These precedents justify testing methylation markers, but do not demonstrate an understory-specific signature in forest fruits or fungi. Inherited variation and population history may also influence methylation patterns. Genotype, tissue, and developmental stage must therefore be considered when a pattern is attributed to the environment.

For macrofungi, the method should be selected according to the

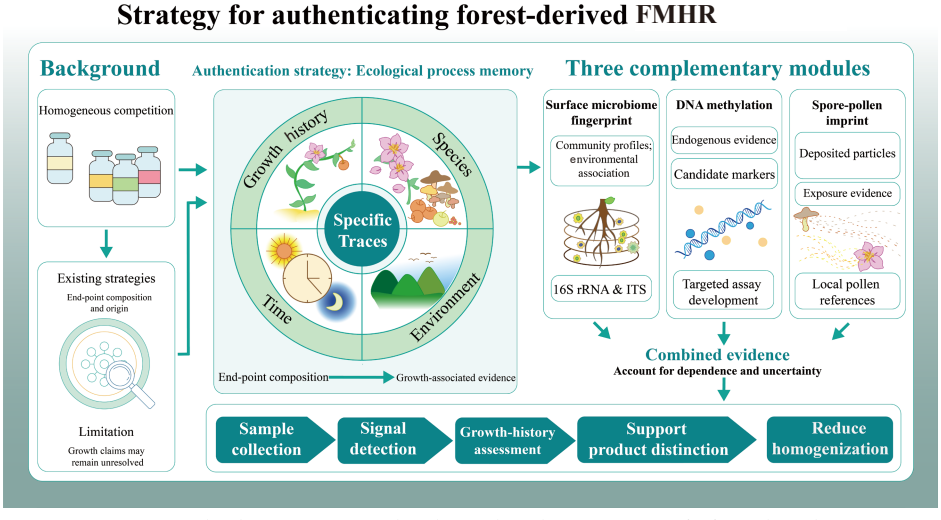

Figure 1 Proposed authentication strategy based on ecological process memory for forest-derived FMHR.

methylation characteristics of the target species. Comparative studies show considerable variation in cytosine methylation among fungal lineages[5]. Consequently, the presence of 5-methylcytosine (5mC), or a proposed role for N^6-methyladenine (6mA), cannot be taken as evidence that either modification records understory growth in *Ganoderma* or *Poria*. The relevant modification and its reversibility must first be established for the species concerned. The number, persistence, and specificity of candidate markers must be determined rather than inferred from other fungi.

The technical approach of this module consists of two steps. First, standardized samples of target species with strictly traceable habitats are collected, and their genome-wide methylation profiles are mapped separately for each target taxon. Samples from different production categories should be compared within regions, using the same cultivar or strain where possible and matching tissue and developmental stage. Replicated sites, seasons, and harvests are needed to account for biological variation. Genotyping, common-garden comparisons, and controlled stress or transfer experiments can help distinguish understory-associated changes from inherited differences and general stress responses. Whole-genome bisulfite sequencing (WGBS), enzymatic methylation sequencing, or validated long-read methods can be used for discovery, depending on the target modification and genome. WGBS does not measure 6 mA. Long-read modification calls require appropriate controls and calibration, and reference-genome completeness, repetitive regions, and strain-specific mapping bias may limit analysis in less-characterized macrofungi. These conditions should be examined before candidate markers are selected. Second, reproducible differentially methylated regions (DMRs) are selected for targeted assays. A DMR is a genomic interval showing a group difference under a specified statistical model. Coverage, informative sites, cytosine context, effect size, and multiple-testing criteria should be defined in advance. The required number of markers depends on independent classification performance; it cannot be prescribed for all species. Methylation-sensitive or methylation-specific PCR, multiplex assays, custom arrays, and targeted sequencing may reduce routine workload after validation. Ordinary PCR requires an appropriate conversion or methylation-sensitive step to provide information about methylation. These methods still require species- and tissue-specific development. The main limitation is that methylation states are not permanently fixed. Their reversibility and persistence vary among loci and biological conditions. Storage may involve further biological change, while processing can affect DNA integrity and the measurement of modifications. Paired samples taken at harvest and after storage or processing are needed to establish the period over which a marker remains useful. Detection of DNA alone does not demonstrate retention of an informative methylation pattern, and resistance to short-term stress manipulation requires direct testing.

3.2 Surface microbiome fingerprint analysis

This module is suitable for forest fungi and forest fruits with intact surface structures. The scientific rationale is that surface microbial communities develop through interactions with the host and the surrounding environment. Interactions between hosts and their environment provide a basis for considering this approach. An adjacent food-authentication study combined microbiome data and machine learning to classify the geographical origin of mozzarella[6]. These findings support further investigation in forest products, rather than demonstrating that an understory-specific fingerprint has already been established.

Operationally, standardized sampling of the sample surface can be performed using sterile cotton swabs. After total DNA extraction, amplicon sequencing of bacterial 16S rRNA and fungal internal transcribed spacer (ITS) regions can be conducted to obtain a microbiome fingerprint profile. The sampled area, tissue position, and interval before preservation should be standardized. Field, transport, and extraction blanks, technical replicates, and mock-community controls should accompany sampling and analysis. Packaging, washing, temperature, and storage duration should also be recorded.

Bioinformatics analysis can further be used to construct a random forest classification model for evaluation against the declared production categories. Training samples should cover regions, forest types, seasons, years, and handling conditions in a balanced manner. Feature selection and preprocessing should be conducted within the training data, and samples from the same individual, lot, or site should not be distributed between training and test groups. Independent locations and harvests are needed to assess performance beyond the collection used to develop the model[7]. One limitation is that surface microbial communities may be affected by post-harvest handling and storage conditions. Washing, waxing, and cold storage have been shown to change apple-associated microbial communities[8]. Therefore, standardized sample pretreatment procedures should be established for practical application, and modeling should incorporate samples from different batches and under various storage conditions to enhance the model's robustness. Matched harvest and post-handling samples can help separate production signals from subsequent changes. Sealed retained samples and blinded tests involving soil contact, microbial transfer, washing, and mixed batches should be used to examine susceptibility to manipulation. Surface sampling may be useful for preliminary screening, but low microbial biomass, contamination, differences between production sites and changes in handling can all undermine generalization. Its cost, speed and reliability must be established for the product concerned.

3.3 Spore-pollen imprint analysis

Spore-pollen imprint analysis is applicable in principle to products with rough surfaces, complex structures, or secretions that retain external particles. The scientific basis is that deposited pollen and spores can provide information about the surrounding vegetation and period of exposure. Modern pollen-rain studies demonstrate relationships between deposited assemblages and local ecological conditions[9]. Honey-origin research provides an example of combining pollen analysis with other measurements[10]. Pollen DNA barcoding may assist identification, but its reference coverage and taxonomic resolution need to be assessed separately. Operationally, particles are recovered from a defined surface by a validated rinse or extraction and prepared for microscopic examination. The number and relative abundance of different pollen types are counted under an optical microscope. Collection blanks and recovery controls are needed, together with reference samples from forest interiors, forest edges, neighboring fields, and handling environments. Seasonal sampling, voucher material, and checks of agreement among observers are important for building a local pollen-rain database. For flower products and harvested fungi, the product's own pollen or spores should be distinguished from externally deposited particles.

The advantages of this method lie in its simple equipment requirements and the ability to examine environmental traces

directly. Its limitations include the need for specialized spore-pollen identification skills; the accuracy of authentication depends on the completeness of the local pollen reference database, and the resolution may decrease in forest edges and areas where forests and farmland intermingle. Wind, insects, people, and packaging can transfer particles, while washing and polishing can remove them. The chemical stability of pollen walls does not make the surface assemblage resistant to artificial addition or physical removal. Blinded addition, transfer, and washing experiments are therefore required. The resulting evidence should support an environmental interpretation, rather than be treated as direct proof of the complete growth history.

4 Practical application and limitations

In practice, the three modules can be applied in a tiered workflow. Before any testing, species, tissue, lot identity, and reference domain availability should be confirmed. The first tier uses validated microbiome or pollen assays for preliminary screening, with throughput determined by the assay and product type. A prespecified subset of screen-negative samples should also enter follow-up testing, so that missed misclassification can be estimated rather than assumed. The second tier applies a validated targeted methylation panel (or another corroborative method) to samples selected by screening and audit. The third tier reserves genome-wide analysis and specialist review for unresolved cases or reference development, rather than routine testing of every sample. Per-sample cost and throughput should be measured in a pilot study, including controls, repeat tests, staff time, and interpretation effort. Estimated time and capacity must refer to a defined assay rather than to an assumed rapid method.

Module selection requires different reference materials and should not assume that one test is always confirmatory. For DNA methylation, the relevant domain is a specified species, tissue and developmental stage, with genotype-aware methylomes, appropriate genome resources and matched production and handling groups. Genome-wide discovery is resource-intensive, whereas a validated targeted panel may allow batch testing; plasticity, mapping bias and processing effects remain limitations. For surface microbiomes, intact fungi or fruits are the main candidates. Reference data should cover host, region, season, lot and handling conditions, with curated microbial sequences. Amplicon sequencing and analysis require batch resources; faster targeted assays would need separate validation, especially when biomass is low or the test site differs from the reference sites. Spore–pollen analysis is relevant only when external particles can be recovered. It requires local pollen-rain samples, vouchers, seasonal and neighboring-field data and handling controls. Microscopy uses relatively simple equipment but requires trained observers and counting time; molecular identification introduces further costs. Particle transfer or removal and incomplete reference coverage make this supporting exposure evidence, not proof of origin.

When combining results, the correlations among signals must be taken into account. A calibrated joint classifier or hierarchical Bayesian model can account for shared environmental influences and site or batch effects. Agreement among the three modules does not automatically count as three independent confirmations. If results conflict, identity, contamination and handling records should be checked; samples that remain unresolved should be reported as inconclusive. If one module is unavailable, the combined approach must have been validated for that specific missing-data pattern. Validation must disentangle production category from confounding factors such as geography, genotype, soil type and management practices, and should draw on independent sites and multiple harvests[7]. Sampling design should account for clustered data structure, and learning curves can guide how many reference samples are needed. Decision thresholds must be set before final testing. Reported outcomes should include sensitivity, specificity, false-positive and false-negative rates, confidence intervals, calibration, and the proportion of samples reported as inconclusive. Here, a false positive means incorrectly accepting a declared forest-production category. Predictive values also depend on how commonly each production category appears in practice. Finally, the combined strategy should be benchmarked against chemical, isotope and documentary evidence to demonstrate what additional information it provides.

Post-harvest treatment determines which signals remain useful. Fresh, intact material offers the broadest range of candidate signals, but tissue, collection time and sampling position must still match the reference data. During cold transport or storage, living tissues may change, microbial communities may drift or acquire contaminants, and retained particles cannot be assumed to remain unchanged, time, temperature and packaging should therefore be recorded and validated. Drying does not necessarily remove microbial DNA or external particles, but a model trained on fresh material should not be transferred to dried samples without validation. Slicing changes the exposed surface and may affect which tissue is represented, cut and intact materials need matched sampling rules. Milling does not necessarily destroy DNA, but it removes the original surface location of particles and can mix material from different lots. Ingredient identification in powder is not equivalent to reconstructing a production history. Heating or extraction may leave no interpretable biological evidence at all. For such products, the approach is outside scope unless a usable signal has been independently validated. These distinctions require processing-matched controls, when the appropriate reference domain is unavailable, the result should be reported as inconclusive.

The strategy should operate alongside existing traceability and inspection systems. Organic-production and geographical-indication regulations provide examples of schemes that require more than an analytical origin result. Understory production is not equivalent to organic status, and origin testing cannot replace food-safety testing or demonstrate medicinal efficacy. Reports should identify the production claim, reference population, sample handling, and uncertainty. Wild harvesting and understory cultivation should have separate references where needed, with attention to lawful collection, sustainable access, and protection of sensitive locality data.

5 Conclusions and outlook

This authentication strategy based on ecological process memory extends the assessment of forest-derived FMHR beyond end-point composition to include biological signals associated with growing conditions. DNA methylation, surface microbiome fingerprinting, and spore–pollen analysis capture different layers of biological information, and their combined value depends on the species, product state, and quality of the reference collection. The integrated framework has not yet been tested on these resources, and no single module should be expected to reconstruct a complete growth history. If validated, the strategy could support product differentiation by providing evidence of growing

conditions alongside compositional measurements. Processing methods could also be designed to preserve the biological signals that carry this information. Downstream brands would then have measured evidence to back production claims, instead of relying on broad origin labels. Such evidence can inform pricing, but it does not by itself prove superior quality or justify a premium.

Further work should start with a small set of well-defined species and product states. Artificial intelligence may help with feature selection[6], combining the three evidence types, and flagging samples that fall outside the reference population, as food-microbiome applications already suggest. Model outputs, however, do not establish causality, and any change to the reference database or model requires revalidation. With these caveats, ecological process memory provides a testable framework for growth-related authentication and a practical route for forest-derived FMHR to move beyond commodity competition.

Data availability

All data needed to support the conclusions in the paper are presented in the manuscript. Additional data related to this paper may be requested from the corresponding author upon request.

Conflicts of interest

The authors declare that there are no conflicts of interest in this work.

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Author contribution statement

Sheng-Ling He: Data curation, project administration, validation, writing manuscript, experimental design. Yu-Ting He: Project administration, writing manuscript, validation, experimental design. MD SHAON: Data curation. Dan Xiang: Data curation. Yong-Xiang Liu: Project administration, validation. Yu-Mei Tan: Conceptualization. Chao Zhao: Conceptualization. Zhan-Nan Yang: Data curation. Zhu Li: Conceptualization, project administration, validation, funding acquisition. All the authors have approved the final manuscript.

Informed consent

Not applicable.

Ethics statement

Not applicable.

Use of AI statement

None.

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