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Insecticidal effects of nitrogen-based hypoxia–anoxia and pressure regulation on Lasioderma serricorne in paper artefacts

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Paper-based artifacts are particularly susceptible to pest infestations. To overcome the limitations associated with conventional treatments, this study introduces a nitrogen-based anoxia/hypoxia and pressure regulation (NPR) system as a safer alternative for artifacts. In tests against *Lasioderma serricorne* (*L. serricorne*), a prevalent museum pest, the NPR-99 treatment achieved complete mortality of adult beetles and larvae within 4 and 12 h, respectively, without causing significant damage to the mechanical strength or color stability of the paper. Mechanistic insights indicate that the treatment disrupts physiological functions by interfering with genes related to stress response and cuticle development. This method provides an effective, non-invasive strategy for safeguarding paper-based cultural heritage.

Paper-based artifacts constitute fundamental collections, archives, and libraries, serving as vital documentation of the evolution of human civilization[1]. However, these invaluable heritage materials face multiple threats, among which pest infestation represents a significant concern. Pests can cause substantial damage on organic materials, including paper, binding, pulp, starch glue, and cellulose compounds, and can serve as vectors for pathogens such as bacteria and viruses, facilitating disease transmission[2]. Furthermore, within the confined environments of archives and libraries, pests can contribute to allergic reactions, thereby jeopardizing the health of personnel[1].

In the domain of paper-based artifact preservation, numerous studies have established a correlation between pest occurrence and environmental conditions. Elevated temperatures and humidity levels can markedly expedite the life cycle activities of pests and facilitate the proliferation of microorganisms, thereby indirectly supplying food sources for pests. Additionally, inadequate ventilation may result in the creation of localized micro-environments, maintaining temperature and humidity within the optimal range for pest survival, consequently leading to pest infestations[2]. Historically, the management of pests affecting traditional paper cultural relics predominantly relied on chemical methods, encompassing the use of inorganic compounds (such as arsenic, mercury, and lead-based substances) and organic pesticides (including ethylene oxide, methyl bromide, and formaldehyde)[3,4]. However, the prolonged application of these chemicals has resulted in the development of pest resistance, a reduction in the efficacy of pest control measures, and potential damage to the paper artifacts themselves[5,6]. With the growing awareness of environmental issues, it has been recognized that these chemicals pose significant risks to both the environment and human health[7–9]. Consequently, there has been a gradual shift towards the adoption and implementation of physical methods, such as freezing, heating, gamma radiation, and anoxia treatment[10–15]. Nonetheless, Various physical treatment methods require processing times ranging from 3 to 21 days and exhibit specific limitations. While the freezing method is recognized for its efficacy, it may inadvertently increase the cold resistance of insects[16,17]. Additionally, low-temperature treatments can significantly compromise the flexural strength and tear resistance of paper due to ice crystal formation[18]. Excessive doses of gamma irradiation not only inflict damage on paper but also incur substantial costs[13,19,20]. Furthermore, heat treatment is constrained by the thermal stability of paper; excessively high temperatures can lead to cellulose denaturation and discoloration of the paper[14,21]. The long-term maintenance of extremely low oxygen concentrations through anoxic treatment, utilizing nitrogen or carbon dioxide, results in a marked increase in expenses[22,23]. Compounding these challenges, prolonged processing times may not only damage the paper but also induce genetic

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mutations in insect heat shock proteins and unfolded proteins, thereby enhancing their tolerance to hypoxia[24,25].

Insects exhibit behavioral adaptations in response to variations in air pressure. Research indicates that several critical behaviors, including flight, oviposition, parasitism, and mating, across diverse insect taxa such as Diptera, Orthoptera, Coleoptera, Lepidoptera, Hemiptera, and Hymenoptera, are markedly influenced by fluctuations in air pressure[26,27]. Specifically, under low-pressure conditions, distinct insect species display unique behavioral responses: for instance, the motor activity of *Diabrotica speciosa* is significantly diminished, whereas the calling behavior of *Pseudaletia unipuncta* and *Macrosiphum euphorbiae* is notably reduced[28]. This environmental sensitivity offers novel insights for the development of advanced pest management strategies. Integrating air pressure modulation with anoxia could therefore offer significant advantages. By optimizing air pressure parameters, it is feasible to reduce the duration of insecticidal treatment and lower the oxygen concentration threshold necessary for effective pest control, thereby improving insecticidal efficacy. This combined approach has not yet been systematically evaluated for insecticidal efficacy or material safety in heritage paper. This integrated approach could inform the development of safer, more efficient pest control strategies for paper-based artifacts.

This study focuses on *Lasioderma serricorne* (*L. serricorne*), which was isolated and identified from pest- and disease-affected documents of the Tang Dynasty, specifically the *Dongji of Liuhe*, and the Qing Dynasty *Qianlong Buddhist Sutra*, housed in the Liuzhou Museum in Guangxi. The research investigates the efficacy of a combined treatment involving nitrogen anoxia/hypoxia and pressure regulation technology (NPR) on the larvae and adults of *L. serricorne* in paper-based artifacts, accompanied by a transcriptomic analysis to assess the impact of NPR treatment on gene expression in *L. serricorne*, thereby elucidating its potential insecticidal mechanisms. Additionally, the safety of the NPR treatment for paper materials is evaluated from multiple perspectives, including the mechanical properties of the paper and colorimetric changes. This study aims to evaluate the efficacy and mechanism of a novel NPR system for insect pest eradication in paper-based artefacts, while assessing its safety for cultural materials.

Methods

Isolation and identification of pests

The Tang Dynasty document *Dongji of Liuhe* and the Qing Dynasty *Qianlong Buddhist Sutra* in the collection of Liuzhou Museum in Guangxi were sampled and cultured for 48 h. The adult insects were then taken out until the detection of larvae, pupae and adults was completed. Observation and identification were conducted by stereomicroscopy and Tungsten filament scanning electron microscopy (TFSEM, SU3500, Hitachi, Japan).

Insect cultivation

In this study, *L. serricorne* specimens were cultivated in a controlled environment consisting of a mixture of 5% yeast and whole wheat flour, maintained at 28 °C and 70% relative humidity. New colonies were established by transferring newly emerged adult *L. serricorne* into rearing containers. Specimens were collected at two distinct intervals: 21–24 days post-colony establishment for third instar larvae, and 31–35 days post-establishment for first to fifth instar adults. The experimental procedure involved the extraction of larvae and adults from the flour medium using a sieving technique.

Table 1 | Experimental conditions of each group

Group	pressure regulation	N_2 concentration
Control	−	80%
PR	+	80%
N_2	−	99.9%
NPR-85	+	85%
NPR-99	+	99.9%

The insecticidal effect of NPR treatment

The collected larvae and adults of L. serricorne were placed in non-woven bags containing Xuan paper and subsequently sealed within a 51 L programmable environmental test chamber (40 × 40 cm, FZG1, Jiangsu, China). These specimens were categorized into six groups: the control group, PR group, N_2 group, NPR-85 group, and NPR-99 group. A single cycle of pressure regulation is defined as follows: Initially, the air pressure is sustained at 0.4 MPa for a duration of one hour using a vacuum pump. Subsequently, pre-dried nitrogen is introduced from a high-pressure gas cylinder into a sealed test chamber, reducing the air pressure to 0.01 MPa for a period of five minutes. Finally, the pressure is restored to 0.4 MPa. (Fig. 3A). Throughout this entire cycle, the concentration of N_2 is continuously monitored and recorded by an integrated nitrogen detector (PGD4-C-N2, Shenzhen, China). The environmental test chamber was situated within a temperature-controlled laboratory maintained at 23 ± 2 °C. Throughout these particular pressure cycles, the relative humidity was not actively regulated, remaining at 50 ± 10%. The specific experimental conditions for each group are outlined in Table 1. The control group was concurrently subjected to treatment in the identical programmable environmental test chamber as the experimental group, maintaining consistent environmental pressure and standard atmospheric composition, with an oxygen concentration of 20.9%. Following exposure to the various conditions, the mortality rates of the larvae and adults were assessed immediately. In this study, "hypoxia" refers to an oxygen concentration of ~15% (as in the NPR-85 group), while "anoxic" denotes an oxygen concentration below 1% (as in the NPR-99 group). These definitions are based on established standards for therapeutic environments and prior research by ref.[29]. Direct application of these pressure parameters to sensitive historical paper-based cultural artifacts is not advisable without first conducting thorough preliminary verification on simulated samples. An absence of movement upon gentle prodding with a brush was used as the criterion for determining death in both larvae and adults. Each group consisted of 20 replicate samples, and the entire experiment was conducted in triplicate.

Observe the microstructure of *L. serricorne*

To investigate the alterations in stomatal structures at the microscopic level, the treated larvae of *L. serricorne* were fixed in glutaraldehyde overnight. Following a gradient dehydration process using 30–100% ethanol, the specimens were subjected to gold sputter coating for 120 s. The modifications in the larval stomata were subsequently examined using TFSEM (SU3500, Hitachi, Japan).

Transcriptomic analysis

Twenty adult and twenty larval of *L. serricorne* specimens were subjected to a NPR-99 treatment for 2 h. Subsequently, the specimens were rapidly frozen in liquid nitrogen for 30 min, after which they were stored at −80 °C. Following a quality assessment of the samples, transcriptomic sequencing was carried out by the Shanghai Meggie Bioassay Center (Meiji Biological Medicine Co., Ltd., Shanghai, China), with three replicates for each group. Differentially expressed genes were identified and compared against the UniProt database using Diamond software. Data analysis was performed on the Majorbio cloud platform.

Paper fiber composition

The Xuan paper, originating from Gutangge, Jing County, Anhui Province, China, is characterized by dimensions of 10 × 10 cm, a thickness of 116 ± 5 μm, and a basis weight of 50 ± 10 g/m^2. It is composed of approximately 80% blue sandalwood fiber and 20% rice straw fiber. In contrast, the printing paper, supplied by Shenzhen Qixin Group Co. Ltd., exhibits a thickness of approximately 100 ± 10 μm and a basis weight of 80 ± 5 g/m^2. This substrate primarily consists of about 80% cork fiber and 20% hardwood fiber.

Micromorphology characterization

The paper treated with NPR-99 was cut to 0.5*0.5 cm. The microscopic morphology of the paper was observed by TFSEM after gold spraying 90 s using a magnetron sputtering vacuum coating instrument (Q150R + , QUORUM, UK).

Mechanical characterization

The mechanical properties were assessed by determining the tensile strength and folding endurance of paper samples (15 × 1.5 cm) following a one-day NPR-99 treatment, in accordance with ISO standard 1942–2:1994 and ISO standard 5256-1993.0. The tensile strength was measured by applying force parallel to the length of the paper strip and recording the maximum force per unit width that the strip could withstand before failure. Tensile strength, with a stretching speed of 10 mm/min, was evaluated in both the transverse and longitudinal directions using a universal testing machine (QT-1136PC, Guangzhou, China), with ten repetitions conducted for each group. This strain rate was selected due to the difficulty of testing brittle samples at higher strain rates.

The folding endurance was assessed by repeatedly folding the paper in the transverse and longitudinal directions at the same position using a paper folding endurance tester (LB-MIT135, Bluepard Instruments Co., Ltd. Shanghai, China) until the paper cracked or broke, applying a weight of 2.5 N. The number of times the paper is folded without breaking is recorded as the folding endurance value, which is expressed as the number of double folds. Each group consists of 6 repetitions, and all tests are conducted in a room with controlled temperature and humidity.

Colorimetric measurements

To assess the impact of NPR-99 treatment on the colorimetric variation of paper, paper samples were subjected to NPR-99 treatment for durations of 4, 12, and 24 h. The resulting color changes were quantified using the CIB LAB system, with the color difference represented as ΔE, and each treatment group comprised three replicates. A ΔE value of less than 3 indicates that there is no statistically significant difference in color[30]. ΔE is determined by formula (1).

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (1)$$

The symbol ΔE represents the color deviation, while ΔL, Δa, and Δb denote changes in brightness and shifts along the red-green and yellow-blue axes, respectively.

Analysis of X-ray diffraction (XRD)

The crystallinity index (Xc) of fibers in various treated paper samples was analyzed using X-ray diffraction. The samples were positioned on the sample stage of the X-ray diffractometer for step scanning detection. The operating parameters were configured as follows: a tube current of 100 milliamperes, a tube voltage of 40 kilovolts, a step angle of 0.02° per step, and a scanning angle range of 5°to 50°. The crystallinity index of the paper fibers was calculated using the Segal empirical formula. The Xc of paper fibers was calculated by the Segal empirical formula (2):

$$X_c = (I_{002} - I_{am})/I_{002} \times 100\% \quad (2)$$

I_{002} represents the diffraction peak intensity in the crystalline region (2θ = 22.5 °), and *I*am represents the diffraction peak intensity in the amorphous region (2θ = 18.0°).

Statistical analysis

Statistical analyses were conducted utilizing GraphPad Prism version 8.0. The data are presented as mean ± standard deviation (SD). The significance of the results was assessed through one-way analysis of variance (ANOVA). A *P*-value of less than 0.05 was deemed statistically significant, whereas a *P*-value greater than 0.05 was considered not statistically significant (ns). The complete results of the ANOVA have been provided in the Supplementary Materials (as shown in Supplementary Data 1).

Results

Isolation and identification of pests

We systematically separated and morphologically identified pest samples from the Tang Dynasty document *Dongji of Liuhe* and the Qing Dynasty *Qianlong Buddhist Sutra*, housed in the Liuzhou Museum in Guangxi (Fig. 1). Utilizing stereomicroscopy and TFSEM, we observed that the adult insect possesses a slender, oval body with a moderately elevated dorsal surface, covered in medium-density short soft hairs, and exhibits a semi-reclined posture. The body color is reddish-brown (Fig. 2A, B). The larval stage is characterized by a distinct scale-like morphology, with a generally pale yellow coloration (Fig. 2C, D). The oocytes are predominantly regular in shape, appearing mostly oval or oblong (Fig. 2E, F).

Insecticidal effect of NPR treatment

In this study, various treatment methodologies were employed to assess their lethality on both adult and larval stages of *L. serricorne*. The findings revealed that 24 h of pressure regulation treatment resulted in mortality rates of 48.33% for adults and 41.45% for larvae, demonstrating a moderate lethal effect. Although N_2 anoxia treatment alone achieved a 100% mortality rate after 22 h for adults and 24 h for larvae, this approach is associated with longer treatment durations, higher costs, and the potential induction of insect tolerance. Consequently, we integrated N_2 hypoxia with air pressure regulation to significantly reduce treatment duration. At an 85% N_2 concentration, the NPR-85 hypoxia treatment achieved 100% mortality after 6 h for adults and 18 h for larvae. Furthermore, increasing the N_2 concentration to 99.9% enabled complete lethality within just 4 h for adults and 12 h for larvae using NPR-99 anoxia treatment (Fig. 3B–E). The findings indicate that the integrated application of nitrogen-induced anoxia and pressure regulation markedly enhances insecticidal efficacy. Furthermore, the insecticidal effect is progressively augmented with increasing concentrations of N_2.

Effect of NPR treatment on the stomata of *L. serricorne* larvae

Insect larvae predominantly depend on stomata located on their body surface for gas exchange, with additional respiration facilitated by osmosis through the body wall. As illustrated in Fig. 4, exposure to NPR-99 resulted in a significant dilation of the stomata across each segment of the *L. serricorne* larva's body ($P < 0.01$), indicating a disruption in its normal gas exchange mechanism. This alteration in stomatal structure is typically interpreted as a compensatory physiological response by insects to respiratory resistance. It suggests that the high nitrogen concentration and hypoxic conditions associated with NPR-99 treatment may impair the larvae's stomatal regulation function, thereby reducing their air intake efficiency and hindering the respiratory metabolic process.

Transcriptomic analysis

We performed a transcriptomic analysis on both adult and larval stages of *L. serricorne* following exposure to sublethal doses of NPR. Differential expression analysis was conducted using DESeq2, with genes deemed differentially expressed if they satisfied the criteria of Padjust < 0.05 and log2 fold change (log2FC) ≥ 1. The findings were illustrated using differential expression volcano plots, as depicted in Fig. 5A, B. In adult *L. serricorne*, 18 differentially expressed genes were identified between the control and NPR-treated groups, comprising 9 up-regulated and 9 down-regulated genes. In contrast, the larval stage exhibited 87 differentially expressed genes, with 54 genes up-regulated and 33 down-regulated. A heat map clustering analysis was performed on all differentially expressed genes, revealing a distinct separation between the four samples of *L. serricorne* adults and larvae in the control group and the four samples subjected to NPR treatment. This separation indicates a high degree of repeatability within the experimental groups and substantial differences between the groups. The experimental

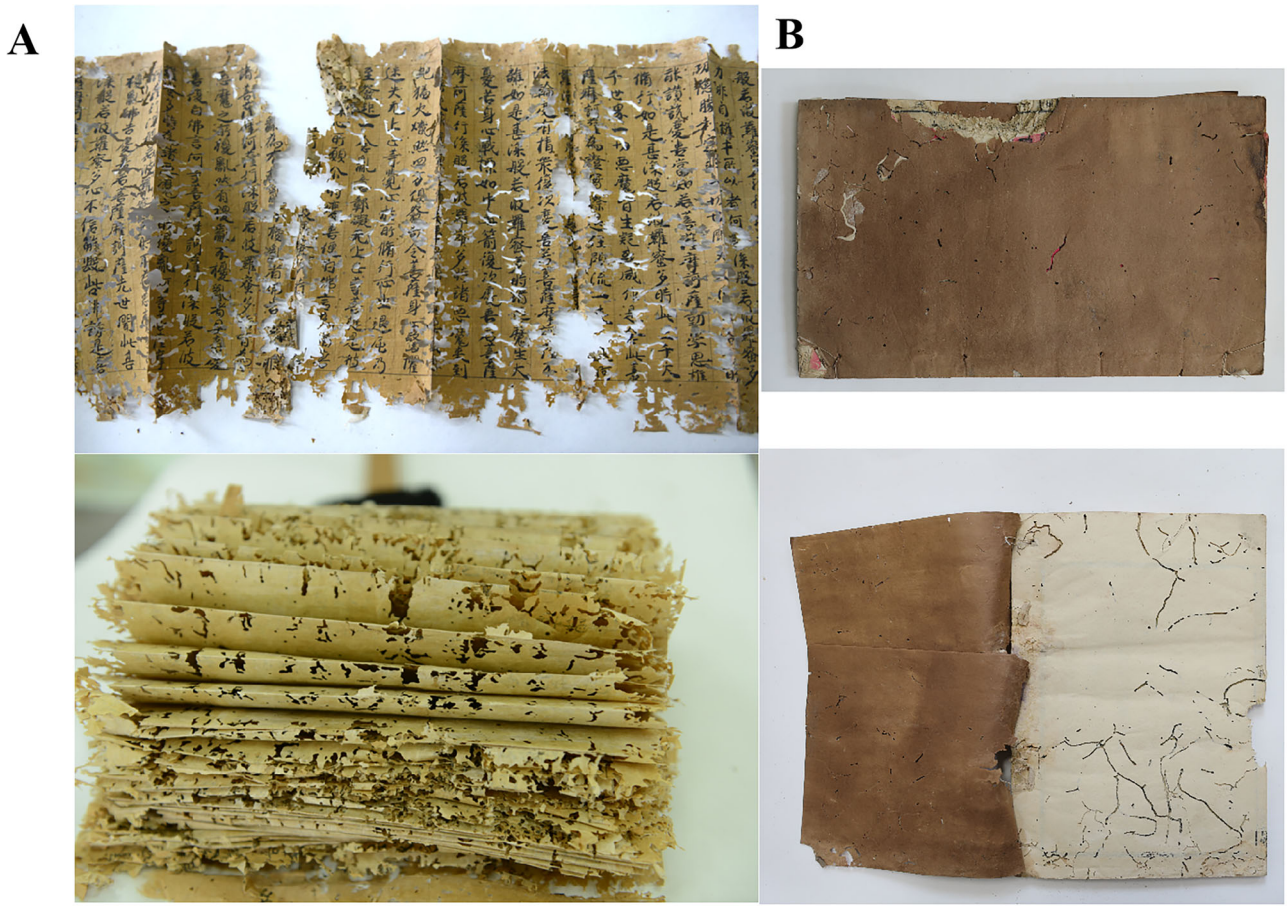

Fig. 1 | Books and archives affected by pests. A The *Qianlong Buddhist Sutra* of the Qing Dynasty. **B** The *Liuhe Dongji* of the Tang Dynasty.

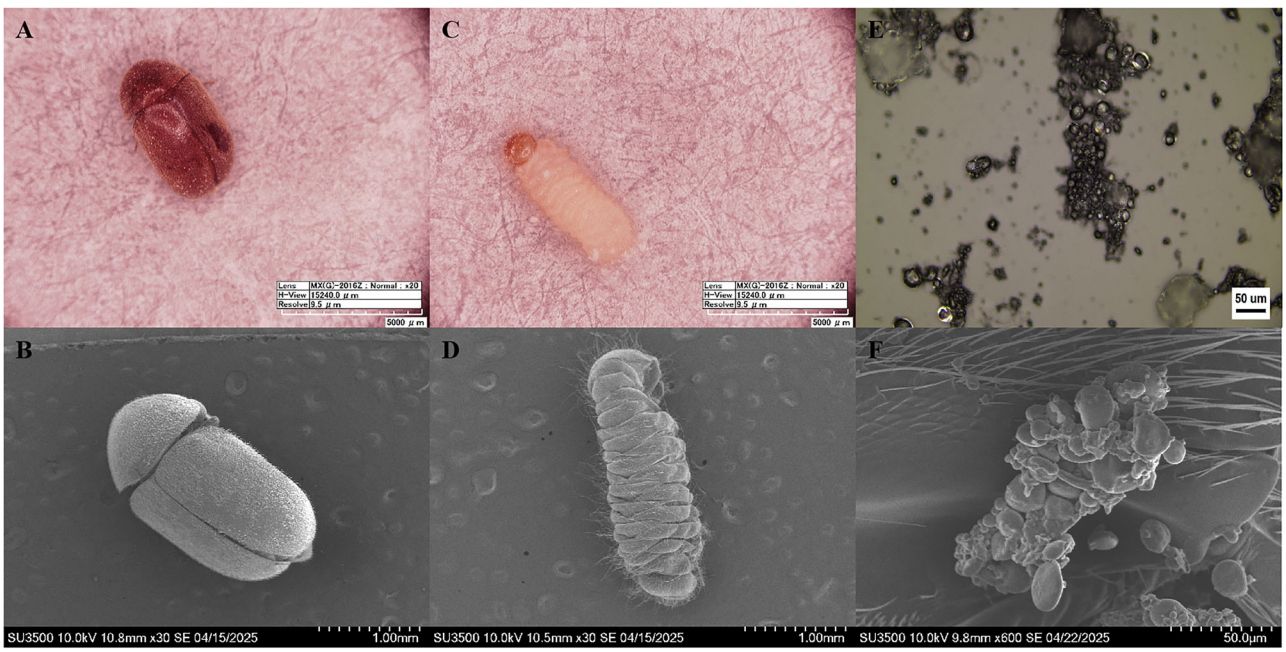

Fig. 2 | Isolation and identification of pests. A Stereomicroscopic image and (**B**) TFSEM image of the adult insect. **C** Stereomicroscopic image and (**D**) TFSEM image of the larva. **E** Stereomicroscopic image and (**F**) TFSEM image of the eggs.

data demonstrated stability and reliability, making it suitable for subsequent analyses (Fig. 5C, D).

Functional annotation and enrichment analysis of differentially expressed genes in *L. serricorne* adults and larvae, subjected to NPR-99 treatment compared to a control group, were conducted utilizing the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. The GO functional annotation analysis indicated that the differentially expressed genes in both adult and larval groups were significantly

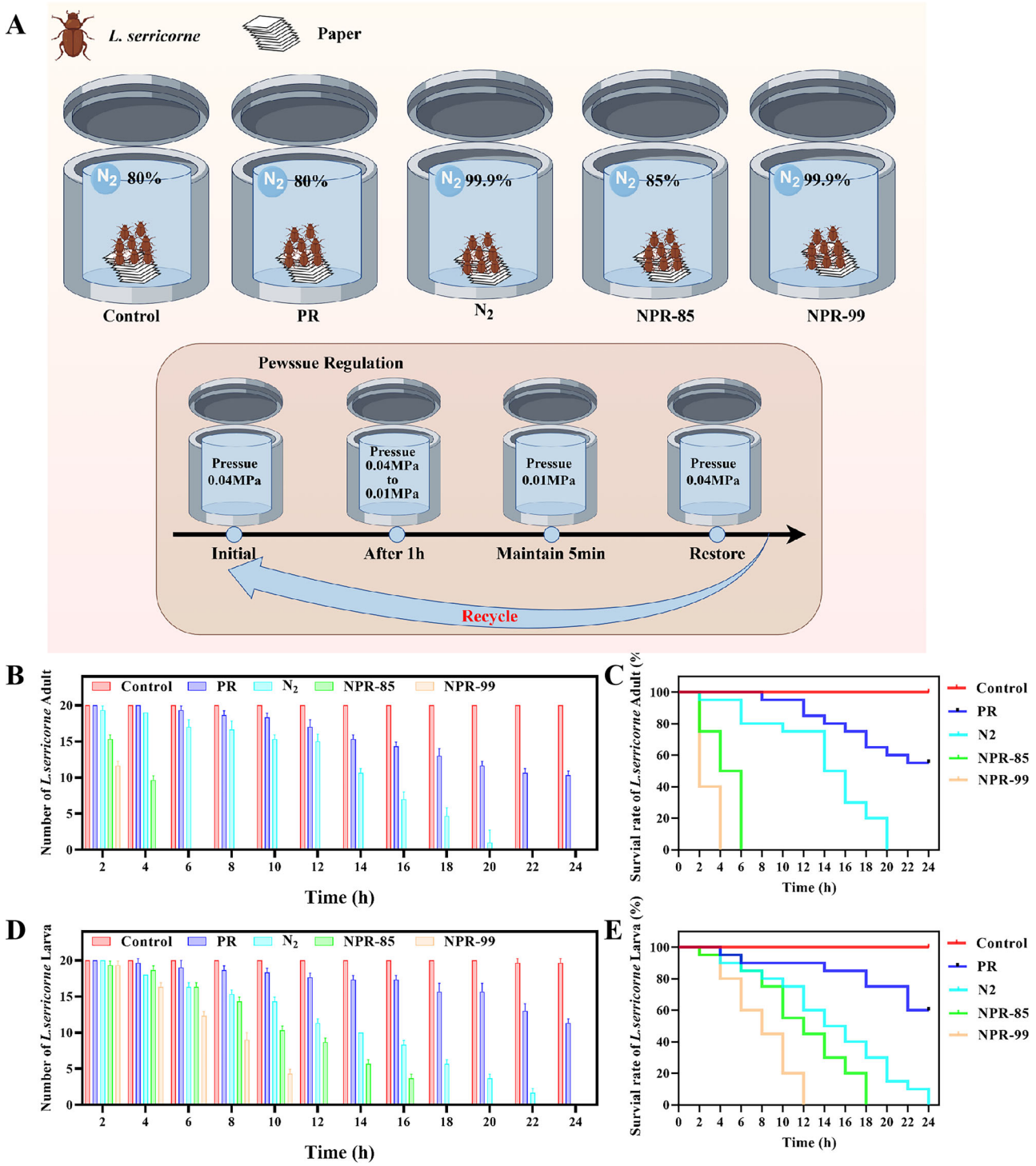

Fig. 3 | Insecticidal effect of NPR treatment. A Different pest control treatment schemes. **B** The number of deaths and (**C**) the survival rate of adult *L. sericorne* after treatment at different times. **D** The number of deaths and **E** The survival rate of larvae *L. sericorne* after treatment at different times. Values are mean ± SD from three independent experiments ($n = 20$ per group per experiment).

associated with the three principal categories: biological processes, cellular components, and molecular functions. Within the more detailed secondary classification, the differentially expressed genes in adult insects were predominantly concentrated in the plasma membrane category (3). In contrast, the differentially expressed genes in larvae were more prominently associated with categories such as membrane (13), plasma membrane (10), cytoplasm (8), and cytosol (7) (Fig. 6A, B). KEGG functional annotation analysis identified that the differentially expressed genes in adult insects subjected to NPR-99 treatment were significantly associated with three primary categories: Cellular Processes (1), Genetic Information Processing (3), and Organismal Systems (1). Within the Genetic Information Processing category, the differentially expressed genes in adult insects were predominantly enriched in Folding, Sorting, and Degradation (2). Conversely, in larvae, the differentially expressed genes were primarily concentrated in Cellular Processes (3), Environmental Information Processing (5), Genetic Information Processing (3), Metabolism (9), and Organismal Systems (2).

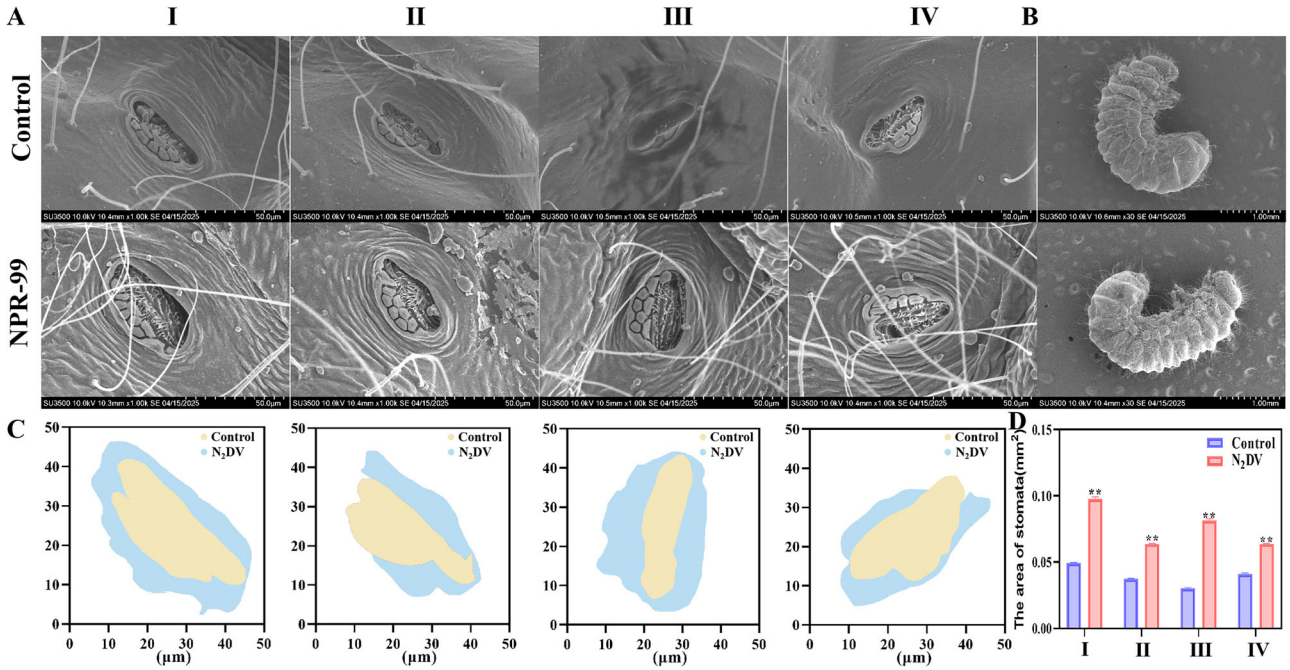

Fig. 4 | TFSEM observation of the stomata of *L. serricorne* larvae. TFSEM of (**A**) Stomata of *L. serricorne* larvae and (**B**) *L. serricorne* larvae. **C** Stomatal simulation diagram. **D** Stomatal area statistical diagram. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with control. Values are mean ± SD from three independent experiments ($n = 20$ per group per experiment).

Specifically, in the Metabolism category, the differentially expressed genes in adults were mainly enriched in Carbohydrate Metabolism (2) and Amino Acid Metabolism (2) (Fig. 6C, D). The comprehensive analysis of the two databases suggests that there are certain similarities in the differentially expressed genes between NP-99-treated adult and larval insects compared to the control group. However, larvae demonstrate more complex and specific gene expression regulation characteristics, particularly in Metabolism and Environmental Information Processing.

Functional enrichment analysis was performed on the differentially expressed genes of *L. serricorn* adults and larvae, revealing significant differences in functional enrichment between the two developmental stages. GO enrichment analysis demonstrated that the differentially expressed genes in adults were predominantly associated with molecular functions such as unfolded protein binding, response to heat, and protein folding, and were localized within cellular components including the plasma membrane and cytosol. These genes were also implicated in biological processes such as ATP binding and cellular response to heat. Furthermore, KEGG pathway analysis indicated that the differentially expressed genes in adults were significantly enriched in pathways related to Longevity regulation across multiple species and Protein processing in the endoplasmic reticulum (Fig. 7A, C). In contrast, the differentially expressed genes in larvae were predominantly enriched in the structural constituents of cuticle and chitin-based larval cuticle, as well as in chitin-based processes, according to GO analysis. Functional categories identified included extracellular matrix, chitin-based cuticle development, and wound healing. Furthermore, KEGG analysis revealed that the larval differential genes were significantly enriched in pathways such as ECM-receptor interaction, thiamine metabolism, ABC transporters, glycine, serine and threonine metabolism, apoptosis, the MAPK signaling pathway, and protein processing in the endoplasmic reticulum (Fig. 7B, D).

Characterization of paper properties

This study conducted a systematic evaluation of the safety implications of various treatments on Xuan paper and printing paper, examining multiple parameters including microstructural observation, mechanical property assessment, color difference analysis, pH measurement, and fiber crystallinity. The results from TFSEM indicated that post-treatment with NR-85 and NR-99, the fibers in both printing and Xuan papers remained intact and coherent, exhibiting a random arrangement and interweaving into a complex network structure. No significant differences were noted in comparison to the control group (refer to Fig. 8A). The color difference of paper significantly influences its visual appearance and functional applications. Samples subjected to treatment durations of 4, 12, and 24 h were assessed using the CIELAB color space system. The findings revealed that the ΔE values for both printing and Xuan papers were below 3, suggesting that the color change was not significant (refer to Fig. 8B). Mechanical strength is a critical parameter for assessing the applicability and durability of paper. As illustrated in Fig. 8C, the deviation in the number of double folds in both the horizontal and vertical directions for the printing paper and Xuan paper in the NPR-85 and NPR-99 groups was minimal when compared to the control group, with no statistically significant difference observed ($P > 0.05$). Furthermore, the tensile strengths of the processed Xuan paper in the transverse and longitudinal directions were measured at 0.4548 MPa and 0.1633 MPa, respectively, whereas the tensile strength of the printing paper was 1.7303 MPa. These tensile strength values did not significantly differ from those of the control group ($P > 0.05$), as depicted in Fig. 8D. Additionally, post-NPR processing, the pH values of both Xuan paper and printing paper remained largely unchanged (Fig. 8E). As shown in Fig. 8F, G, the crystallinity index (Xc) of Xuan paper and printing paper exhibited only slight variations following NPR treatment, suggesting that NPR treatment exerted a relatively minor effect on the hydrogen bond interactions within the paper fibers. The aforementioned findings suggest that the NPR insecticidal technique does not alter the fiber structural properties of the paper, does not result in significant color variations, and exerts a relatively minimal effect on the paper's performance

Discussion

This study successfully isolated and identified pest samples from the Tang Dynasty's *Dongji of Liuhe* and the Qing Dynasty's *Qianlong Buddhist Sutra*, both housed in the Liuzhou Museum in Guangxi, using stereomicroscopy and TFSEM. The morphological characteristics observed in each pest sample were consistent with the description of *L. serricorne*, suggesting that

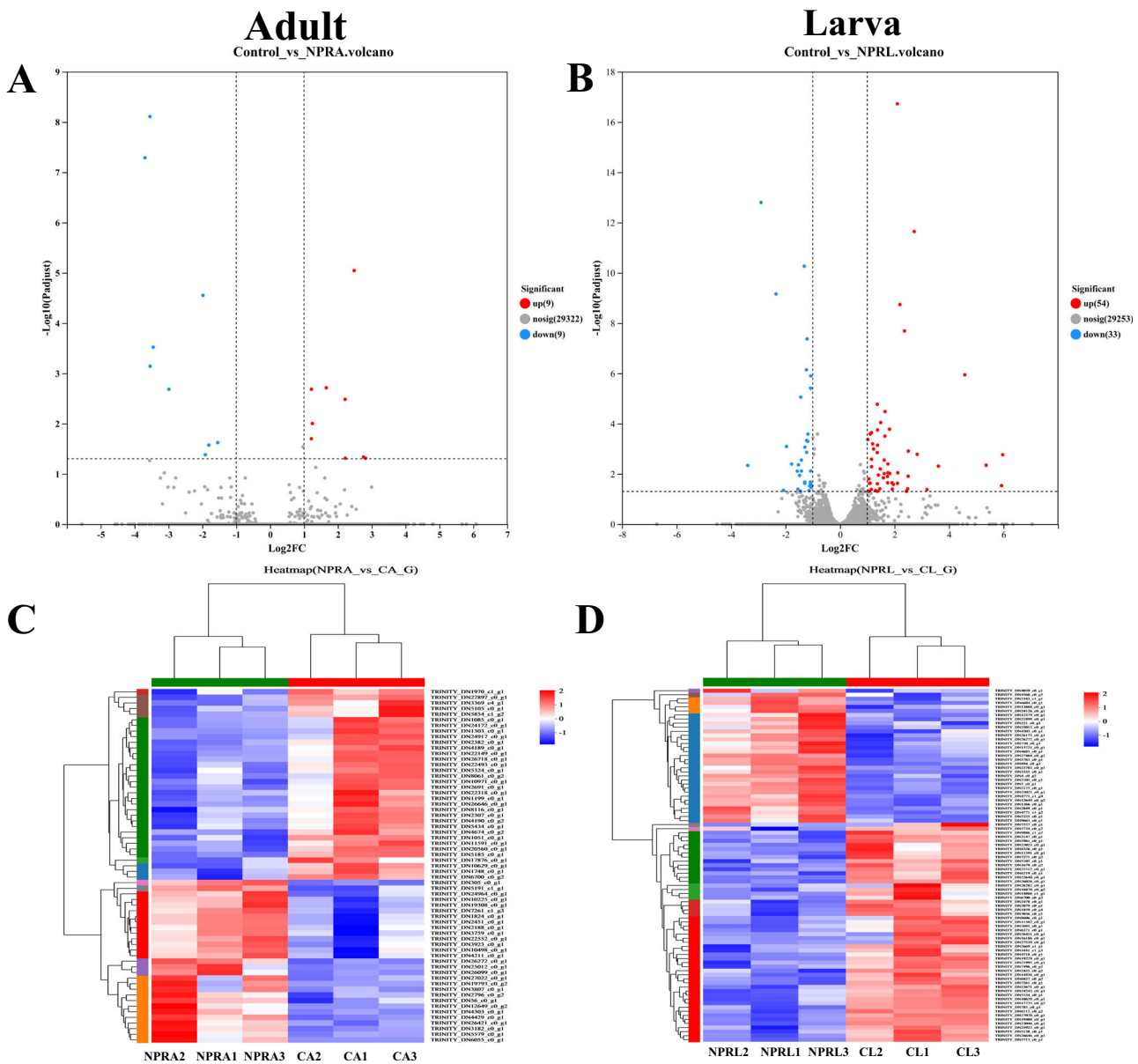

Fig. 5 | Analysis of differentially expressed genes treated by Control and NRR-99. Differentially metabolized volcanic diagrams of (**A**) *L. sericorne* adults and (**B**) *L. sericorne* larvae. Red dots represent significantly upregulated differentially expressed genes, blue dots represent significantly downregulated differentially expressed genes, and gray dots represent non-significantly differentially expressed genes. Cluster analysis of (**C**) *L. sericorne* adults and (**D**) *L. sericorne* larvae. Red indicates a higher expression level in the sample, while blue indicates a lower expression level. Values are mean ± SD from three independent experiments ($n = 20$ per group per experiment).

the infestation was attributable to *L. sericorne*[31,32] (Fig. 2). *L. sericorne* is recognized as one of the primary pests affecting paper-based artifacts in museums, archives, and libraries. This insect is characterized by its rapid reproduction and strong dispersal capabilities, which can lead to significant damage to paper-based cultural heritage items[2,33]. The larvae of *L. sericorne* exhibit a particular preference for feeding on bookbinding materials containing starch glue, often resulting in extensive damage to historical documents and books[2]. Consequently, *L. sericorne* was selected as a representative species for further investigation.

The insecticidal efficacy of anoxic treatments, such as N_2 and CO_2, on pests has been extensively documented[22]. However, further research has revealed that short-term anoxia may induce hypoxic excitation effects in insects, which paradoxically enhance their survival rate, lifespan, and mating success under starvation conditions[34]. Additionally, prolonged exposure to anoxia can facilitate the development of tolerance in insects[24]. The findings of this study also suggest that achieving a complete lethality effect through N_2-induced anoxia alone requires a relatively extended duration. Therefore, there is substantial practical importance in developing methods that can reduce processing time while maintaining high levels of pest control. Pellegrino et al. found that alterations in air pressure can significantly suppress the motor activity of *Diabrotica speciosa* and diminish the calling behavior of *Pseudaletia unipuncta* and *Macrosiphum euphorbiae*[28]. In this study, the regulation of air pressure independently caused a measurable degree of physiological harm to *L. sericorne*. Building on this finding, we integrated nitrogen-induced anoxia with pressure regulation to develop a combined treatment approach, which effectively reduced the complete lethality time to 4 h for adults and 12 h for larvae. These results suggest that NPR-99 treatment holds significant potential for the efficient and rapid management of archival pests, including *L. sericorne* (Fig. 3). It should be noted that the test results obtained from small-sized

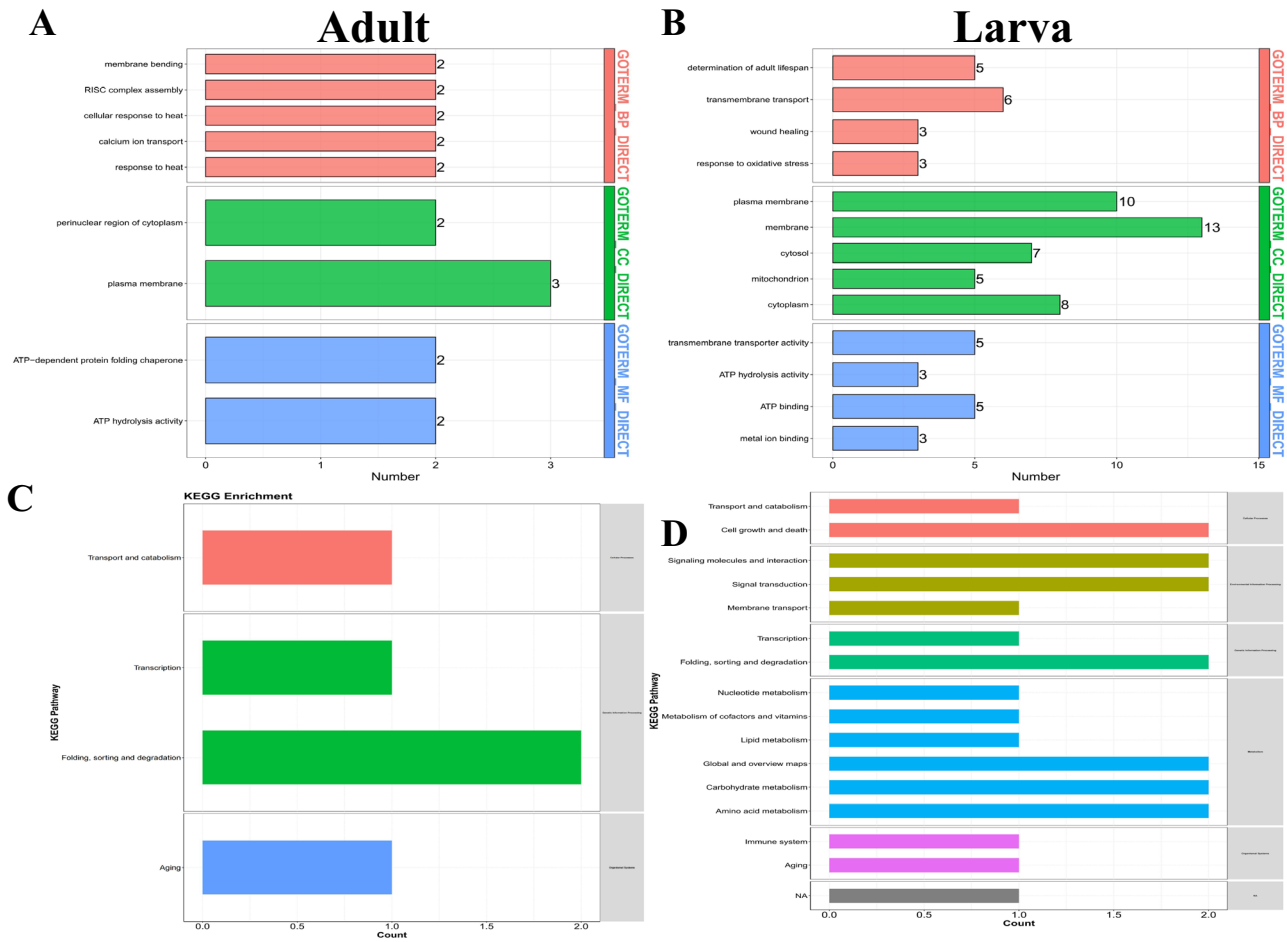

Fig. 6 | Functional annotation analysis of differentially expressed genes treated by Control and NPR-99. GO database functional annotation analysis of (**A**) *L. serricorne* adults and (**B**) *L. serricorne* larvae. KEGG database Functional annotation analysis of (**C**) *L. serricorne* adults and (**D**) *L. serricorne* larvae. Values are mean ± SD from three independent experiments ($n = 20$ per group per experiment).

paper samples under laboratory conditions in this study necessitate further validation at a larger scale, as well as considerations of humidity control and safety assessment, within the context of actual cultural heritage conservation environments.

To further investigate the mechanisms underlying the mortality of *L. serricorne* after NPR-99 treatment, changes in the spiracular structures of the larvae were examined using TFSEM. The findings revealed a significant increase in stomatal size after NPR-99 treatment (Fig. 4). Insect gas exchange capacity is modulated by both internal physiological state and external environmental conditions. Previous research by Frank Hanson et al. has demonstrated that insects can maintain essential physiological functions by regulating stomatal opening and closing in response to environmental stress[35]. During NPR-99 treatment, pressure regulation significantly influenced the stomatal expansion behavior of *L. serricorne* larvae. These results indicate that NPR-99 treatment may induce adaptive structural changes in stomata through a synergistic effect of nitrogen anoxia and air pressure regulation, ultimately impacting the respiratory efficiency of the larvae.

The utilization of transcriptomics in investigating the mechanisms underlying insect mortality holds substantial importance. Through the examination of gene expression in insects subjected to varying conditions, the molecular processes governing their responses are elucidated. For example, research on *Bemisia tabaci* has identified nuclear receptor genes as pivotal in insect metamorphosis, with RNA interference technology markedly impacting their survival rates and developmental timelines[36]. Additionally, *Monochamus alternatus* exhibited notable thermotolerance under high-temperature stress, with transcriptomic analysis indicating significant upregulation of genes associated with protein folding, immune response, and signal transduction[37]. This suggests that transcriptomics not only aids in identifying potential pesticide targets but also offers a novel perspective for studying the mechanisms underlying insect mortality. Following NPR-99 treatment, the differentially expressed genes in adult *L. serricorne* were predominantly enriched at the molecular functional level, specifically in genes associated with molecular functions such as unfolded protein binding, response to heat, and protein folding. Previous research has demonstrated that mechanisms preventing protein expansion, including the induction of heat shock proteins, are crucial for anoxia tolerance. These findings imply that genes involved in responding to protein unfolding may play a significant role in intraspecific variations in anoxia tolerance, aligning with our transcriptomic findings[38,39]. Furthermore, Quan et al. have highlighted that alterations in the expression of genes associated with the heat stress response are frequently accompanied by regulatory changes within the endoplasmic reticulum protein processing pathway[40]. This aligns with our KEGG enrichment analysis, which revealed a significant enrichment of adult differential genes in the "endoplasmic reticulum protein processing" pathway. Additionally, genes involved in protein folding and unfolded protein binding are pivotal in various biological stress processes. Research by Amanda et al. suggests that these genes typically exhibit marked expression differences during stress responses[41]. The binding function of unfolded proteins is essential for maintaining intracellular homeostasis and is intricately linked to cell death and stress response[42]. For example, studies on grapevines' response to salt stress have demonstrated a significant

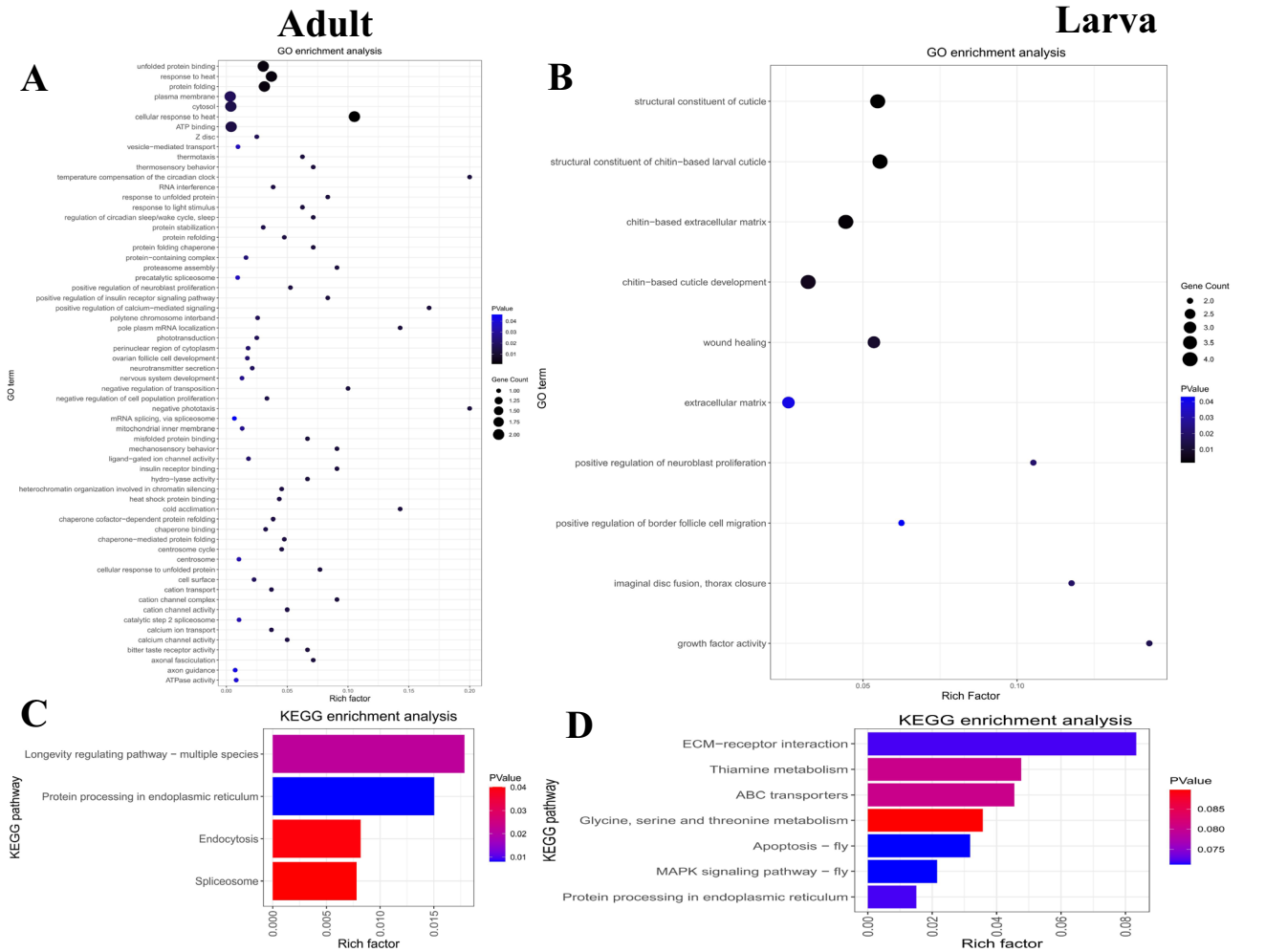

Fig. 7 | Functional enrichment analysis of differentially expressed genes treated by Control and NP-99. GO database functional enrichment analysis of (**A**) *L. sericorn* adults and (**B**) *L. sericorn* larvae. Functional enrichment analysis of KEGG database for (**C**) *L. sericorn* adults and (**D**) *L. sericorn* larvae. Values are mean ± SD from three independent experiments ($n = 20$ per group per experiment).

upregulation in the expression of genes related to unfolded protein binding[43]. In this study, the expression of genes related to unfolded protein binding exhibited a significant upregulation following NPR-99 treatment, suggesting that the treatment may have disrupted the homeostasis of *L. serricorne* adult cells. This disruption potentially initiated a cascade of stress responses, which could ultimately result in cell death (Figs. 6, 7).

Following treatment with NPR-99, significant alterations were observed in the expression of multiple genes associated with the epidermal structure in *L. serricorne* larvae. These genes are involved in cuticular structural constituents, larval cuticle formation, chitin-based extracellular matrix, and cuticle development. Epidermal structural components are critical for insect development and adaptation, forming an integral part of the exoskeleton. They not only provide mechanical support but also perform protective functions. The epidermis of insect larvae is predominantly composed of chitin, and its structural integrity is essential for withstanding external environmental pressures and successfully completing developmental stages such as molting[44,45]. Thus, NPR-99 treatment likely accelerates larval mortality by disrupting cuticular structure, thereby inhibiting growth and development. This finding aligns with the study by Ze et al. which also reported a 100% mortality rate in larvae following damage to the cuticular structure[46] (Fig. 7).

Paper-based cultural artifacts possess substantial historical and cultural significance. Consequently, when selecting pest control methods, it is imperative to prioritize the preservation of their original material characteristics. Prior research has demonstrated that nitrogen anoxic/hypoxic treatment does not significantly affect the structural stability of cellulose[47]. Additionally, this treatment has been shown to have no substantial impact on various types of paper, including heat-aged single Xuan paper, mulberry bark paper, typing paper, bamboo paper, and dictionaries[48]. Therefore, this study employs NPR treatment methods with varying N2 concentrations to assess their effects on the integrity of paper. The findings indicate that the NPR method does not significantly alter the mechanical strength, color difference, pH value, or fiber crystallinity of the paper, as illustrated in Fig. 8. This suggests that the NPR method exerts a negligible impact on the paper substrate at the macroscopic level. Future research should prioritize examining the effects of NPR treatment on the microscopic chemical structure of various paper types. This can be achieved by monitoring key indicators, such as the distribution of cellulose chain length and characteristic functional groups, to comprehensively evaluate the long-term safety of this technology for cultural heritage materials.

This study systematically evaluated the efficacy of NPR treatment technology in the pest management of paper-based cultural artifacts. The findings indicate that NPR-99 treatment significantly reduces the time required to exterminate *L. serricorne* larvae and adults, while preserving the mechanical properties and color stability of the paper. Molecular biology experiments further elucidated that this treatment disrupts the stress

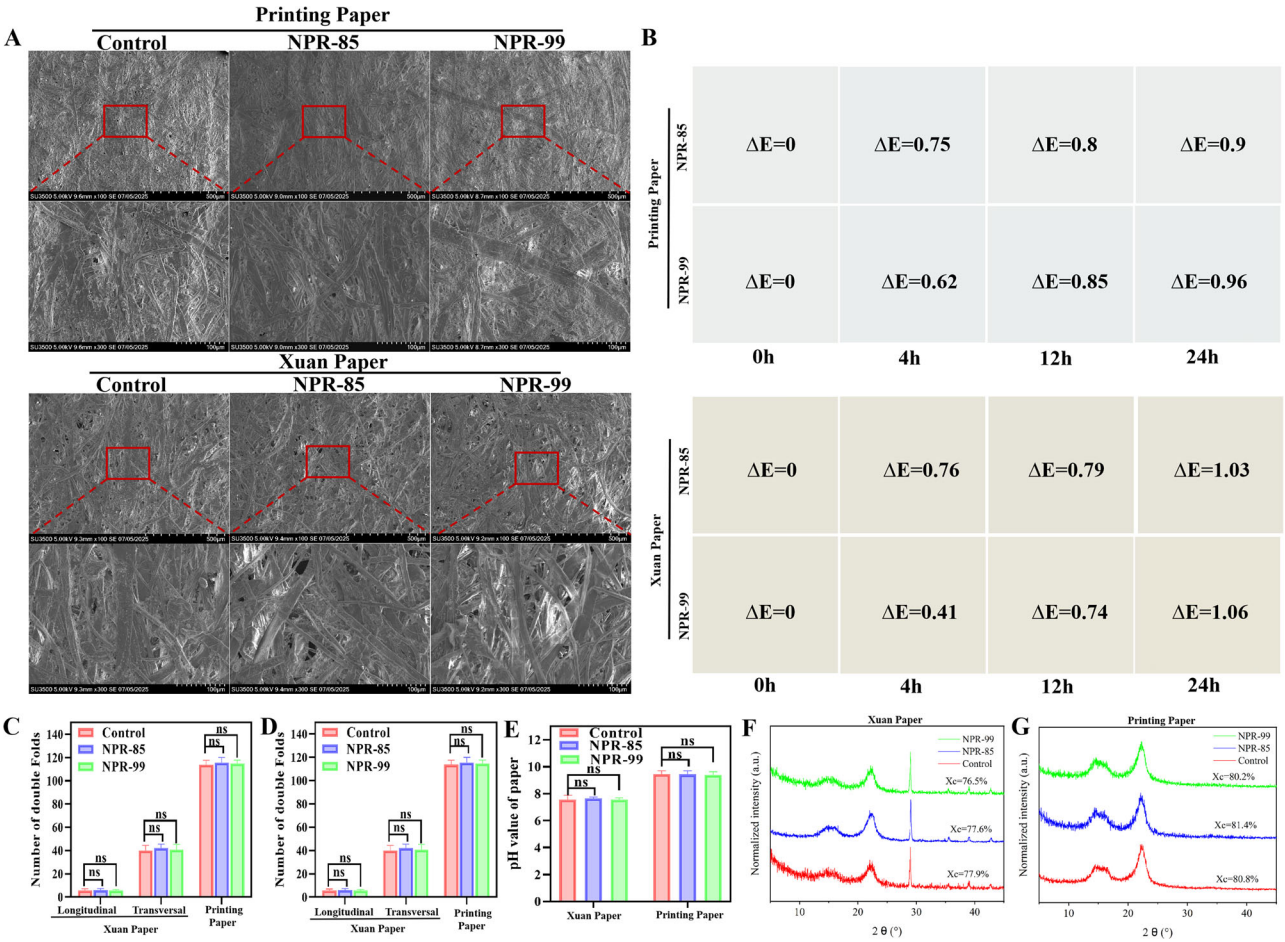

Fig. 8 | Characterization of paper properties. A Microscopic morphology characterization. **B** colorimetric degree. **C** Folding endurance. **D** Tensile strength. **E** pH value. **F** Printing paper and (**G**) Xuan paper XRD spectra. nsP å 0.05, compared with control. Values are mean ± SD from three independent experiments ($n = 6$ per group per experiment).

protection mechanisms of pests by markedly inhibiting the expression of the heat shock protein gene family. Concurrently, it down-regulates the expression of chitin synthase genes, thereby interfering with the development of the larval epidermal structure and effectively impeding the normal growth and developmental processes of the pests. It is important to note that these conclusions are derived from studies on laboratory-standard paper samples, and the safety of this treatment for application to actual cultural relics requires further validation through specialized evaluations, particularly for large and composite material artifacts. Future research endeavors will concentrate on optimizing the parameters of this technology within real-world settings to enhance the preservation of cultural artifacts. This will involve the development of safety operation protocols and an investigation into the technology's applicability to sensitive materials, including painted paper and bound documents. Such efforts aim to furnish reliable technical support for the practice of cultural heritage conservation.

Data availability

The datasets generated and analyzed during the current study are not publicly available due to privacy or ethical restrictions, but are available from the corresponding author, Bingjie Mai, upon reasonable request. Requests for data access should be submitted via email to [maibingjie@shu.edu.cn] and will be evaluated based on the purpose of the request and compliance with ethical and privacy guidelines.

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Author contributions
Xin Liu, Yuhu Li, Bingjie Mai, and Guangtao Zhao designed the experiment, performed the experiment, analyzed the data, and wrote the manuscript. Jiayi Zhang, Kezhu Han, Xujia Dong, Huimin Li, and Mantang Ge assisted in figure format, data analysis, and visualization. All authors have given approval to the final version of the manuscript.

Competing interests
The authors declare no competing interests.

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