

SUPPLEMENTARY MATERIAL

TABLES

Table S1. Summary of biochemical biomarkers evaluated in *Tenebrio molitor* larvae fed with newly emerged *Musca domestica* adults previously exposed or not to polylactic acid (PLA) microplastics.

Table S2. Summary of biochemical biomarkers evaluated in *Tenebrio molitor* larvae fed with newly emerged *Musca domestica* adults previously exposed or not to polylactic acid (PLA) microplastics.

Table S3. Topological properties of systemic biochemical networks in *Tenebrio molitor* larvae following trophic exposure to polylactic acid microplastics (PLA-MPs)⁽¹⁾.

FIGURES

Figure S1. Behavioral parameters of *Tenebrio molitor* larvae in the open field test following trophic exposure to polylactic acid microplastics (PLA-MPs). (A) Distance traveled (mm), (B) time spent in center (s), (C) percentage of time spent in the periphery, (D) time spent immobile (s), (E) velocity standard deviation (cm/s), and (F) linearity index across baseline (dark) and aversive light phases. Bars represent mean + standard deviation. Statistical summaries for each behavioral outcome are presented at the top of each panel and were obtained through a two-way repeated measures ANOVA, considering the effects of treatment (Control vs. PLA-MP), phase [baseline (dark) vs. light], and animal ID (a within-subject factor). MP: microplastic; PLA-MP: polylactic acid microplastic.

Figure S2. Behavioral parameters of *Tenebrio molitor* larvae in the open field test following trophic exposure to polylactic acid microplastics (PLA-MPs). (A) Spatial recurrence index, (B) directional persistence index, (C) number of direction reversals, (D) spatial entropy, (E) fractal complexity, (F) locomotor irregularity index, and (G) efficient exploration index, measured during baseline (dark) and aversive light phases. Bars represent mean + standard deviation (parametric data). Statistical summaries for each behavioral outcome are shown at the top of each panel and were obtained using two-way repeated measures ANOVA, considering the effects of treatment (Control vs. PLA-MP), phase [baseline (dark) vs. light], and animal ID (as a within-subject factor). MP: microplastic; PLA-MP: polylactic acid microplastic.

Figure S3. Morphometric assessment and multivariate discrimination of *Tenebrio molitor* larvae following trophic exposure to polylactic acid microplastics (PLA-MPs). (A) Instar classification, (B) total body length, (C) head capsule width, (D) delta body biomass, (E) mass conversion index, (F) body density, (G) length-to-mass ratio, (H) cephalo-corporeal ratio, and (I) cephalic allometry index. Bars represent the mean ± standard deviation for normally distributed variables (unpaired t-test) or the median ± interquartile range for non-parametric data (Mann–Whitney U-test), and the estimation plots display the difference between group medians with 95% confidence intervals. Statistical test results are presented at the top of each panel. (J) Partial Least Squares–Discriminant Analysis (PLS-DA) score plot showing group separation based on morphometric traits. (K) Variable Importance in Projection (VIP) scores from the PLS-DA indicate the relative contribution of each trait to the discrimination. C: control group; PLA: PLA-MP-exposed group. The abbreviations shown in (K) correspond to the initials of the morphometric parameters depicted in panels A–I.

Figure S4. Bioenergetic reserve capacity assessment and multivariate discrimination of *Tenebrio molitor* larvae following trophic exposure to polylactic acid microplastics (PLA-MPs). Concentrations of (A) total protein, (B) triglycerides, and (C) total soluble carbohydrates; relative allocation of energy to (D) total protein, (E) triglycerides, and (F) total soluble carbohydrates; (G) rapid energy allocation index, (H) lipid-to-protein index, (I) protein-to-carbohydrate metabolic index, and (J) bioenergetic condition index. Bars

represent the mean \pm standard deviation for normally distributed variables (unpaired t-test) or the median \pm interquartile range for non-parametric data (Mann–Whitney U–test), and the estimation plots display the difference between group medians with 95% confidence intervals. Statistical summaries for each variable are shown at the top of the respective panels. (K) Partial Least Squares–Discriminant Analysis (PLS-DA) score plot illustrating group separation based on bioenergetic traits. (L) Variable Importance in Projection (VIP) scores derived from the PLS-DA model, indicating the relative contribution of each bioenergetic variable to group discrimination. C: control group; PLA: PLA-MP-exposed group. The abbreviations shown in (L) correspond to the initials of the bioenergetic variables presented in panels A–J.

Figure S5. Systemic biochemical responses of *Tenebrio molitor* larvae following trophic exposure to polylactic acid microplastics (PLA-MPs). (A–D) Activities of trypsin (TRY), chymotrypsin (CHY), lipase (LIP), and alkaline phosphatase (ALP), respectively. (E–H) Levels of reactive oxygen species (ROS), malondialdehyde (MDA), and activities of superoxide dismutase (SOD) and catalase (CAT). Bars represent mean \pm standard deviation for normally distributed variables (unpaired t-test) or median \pm interquartile range for non-parametric data (Mann–Whitney U test). Estimation plots display the differences between group medians with 95% confidence intervals. Statistical results are presented at the top of each panel. C: control group; PLA: PLA-MP-exposed group.

Figure S6. Systemic biochemical responses of *Tenebrio molitor* larvae following trophic exposure to polylactic acid microplastics (PLA-MPs). (A–B) Activities of glutathione S-transferase (GST) and cytochrome P450-type oxidase (CYP450). (C–F) Levels of nitrite (an indirect marker of nitric oxide), dopamine (DA), serotonin (5-HT), and acetylcholinesterase (AChE) activity. Bars represent mean \pm standard deviation for normally distributed variables (unpaired t-test) or median \pm interquartile range for non-parametric data (Mann–Whitney U test). Estimation plots display the differences between group medians with 95% confidence intervals. Statistical results are shown at the top of each panel. C: control group; PLA: PLA-MP-exposed group.