SUPPLEMENTARY MATERIAL

TROPHIC TRANSFER OF POLYLACTIC ACID MICROPLASTICS INDUCES MULTISYSTEMIC DYSFUNCTION IN *Tenebrio molitor* AND CHALLENGES THE PERCEIVED SAFETY OF BIODEGRADABLE PLASTICS

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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.

Measured metrics	Calculation	Interpretation
Distance traveled (cm) (DT)	Sum of the Euclidean distances between consecutive (x, y) positions across the time series	Reflects total locomotor activity during the observation window. Higher values indicate greater displacement; lower values suggest hypoactivity or behavioral suppression.
Time spent in center (s) (TSC)	Total time (in seconds) that each larva spent within the central zone, defined as a circular area with radius = 4.0 cm, corresponding to 27.7% of the arena's total area (Petri dish: 13.5 cm diameter). Presence in the center was determined frame-by-frame by verifying whether the larva's (x, y) coordinate fell within the central radius.	Increased time in the center is typically associated with reduced thigmotaxis (edge-following behavior) and lower anxiety-like behavior; reduced center time may indicate avoidance.
Percentage of time spent in the periphery (PTSP)	Percentage of total trial time spent in the peripheral zone	Higher PTSP values reflect increased thigmotactic behavior, which may be interpreted as anxiety-like or defensive behavior. Lower values indicate greater central exploration.
Time spent immobile (s) (TSI)	Cumulative duration of time intervals with null or negligible displacement	Elevated immobility time may reflect lethargy, stress, or suppression of exploratory drive.
Velocity standard deviation (cm/s) (VSD)	Standard deviation of instantaneous velocity values computed across all frames	Higher values indicate greater fluctuations in movement speed, which may reflect erratic or inconsistent locomotion patterns.
Linearity index (LI)	Ratio between the net displacement (straight-line distance from the starting point to the endpoint) and the total distance traveled across the trajectory	Higher values indicate more goal-directed and linear movement patterns; lower values reflect erratic, meandering, or exploratory locomotion with frequent changes in direction.
Spatial recurrence index (SRI)	Ratio between the number of revisits to previously occupied spatial bins and the total number of unique bins visited	Higher values suggest repetitive exploration or spatial hesitation; lower values indicate extensive and non-redundant space use.
Directional persistence index (DPI)	Mean cosine of angles between successive displacement vectors throughout the trajectory	Values closer to 1 indicate strong directional consistency and straight movement; values near 0 suggest random or undirected movement; negative values reflect frequent reversals or backward orientation.
Number of direction reversals (NDR)	Total number of occurrences in which the angle between two successive displacement vectors exceeds 90°	Higher values indicate more frequent shifts in orientation and erratic locomotion; lower values reflect more stable, directional movement with fewer corrections or reversals.
Spatial entropy (SE)	Shannon entropy computed from the probability distribution of spatial occupancy within a 10 × 10 grid over the arena.	Higher values reflect more uniform and widespread spatial exploration, while lower values indicate restricted,

Fractal complexity (FC)	Fractal dimension of the larval movement trajectory, estimated using the box-counting algorithm applied to the spatial path recorded during each behavioral phase (dark and light).	stereotyped, or confined movement patterns. Higher values indicate more complex, space-filling, and exploratory trajectories, potentially reflecting disorganized or hyperactive locomotion. Lower values suggest simpler, more linear or stereotyped movement patterns.
Locomotor Irregularity Index (LII)	Composite metric integrating velocity variability, directional inconsistency, and trajectory non-linearity. The index is computed as: $LII = \left(\frac{Velocity\ SD\ (\frac{cm}{s})}{Mean\ velocity\ (\frac{cm}{s})}\right) \times (1 - DPI) \times (1 - LI)$	Higher LII values reflect greater locomotor irregularity, characterized by high speed variability, low directional consistency, and meandering paths. Lower values indicate more stable, directed, and regular locomotion.
Efficient Exploration Index (EEI)	Composite measure that integrates total displacement and spatial complexity, adjusted by recurrence to previously visited locations. The index is computed as: $EEI = \frac{TD \times FC}{SRI + 1}$	Higher EEI values indicate more efficient exploration, characterized by extensive spatial coverage with minimal redundancy. Lower values indicate inefficient locomotion, characterized by high spatial recurrence and limited exploratory effectiveness despite the distance traveled.

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.

FA[(1)]	Measured biomarkers	Abbreviation	Method used	Reference basis
Redox and detoxification capacity	Reactive oxygen species (mM eq. H_2O_2/mg protein)	ROS	ROS levels were estimated in *T. molitor* larval homogenate supernatants using 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), adapted from Zhao et al. (2013) and Maharajan et al. (2018). Briefly, 60 µL of sample supernatant, H_2O_2 standard, or PBS blank were mixed with PBS and 25 µL of DCFH-DA solution under light-protected conditions. After vortex homogenization, reactions were incubated at 37 °C for 30 min in the dark and centrifuged at 15,000 rpm for 5 min at room temperature. Then, 150 µL of the clarified supernatant were transferred to a microplate in duplicate, and fluorescence was measured at 492 nm. ROS levels were calculated relative to the H_2O_2 standard and expressed as mM equivalents of H_2O_2, normalized to total protein content in the corresponding sample volume.	Zhao et al.[1] and Maharajan et al.[2]
	Malondialdehyde levels (nM/mg protein)	MDA	MDA levels were estimated in *T. molitor* larval homogenate supernatants using a colorimetric assay based on the reaction of malondialdehyde with 1-methyl-2-phenylindole (MPI) under acidic conditions, adapted from Islayem et al. (2022). Briefly, 100 µL of sample supernatant, PBS blank, or MDA standard were mixed with 100 µL of MPI solution and 50 µL of 37% HCl. The reaction mixtures were incubated at 100 °C for 3 min, immediately cooled on ice for 10 min, and centrifuged at 13,000 rpm for 5 min at room temperature. Then, 150 µL of the clarified supernatant were transferred to a microplate, and absorbance was measured at 630 nm. MDA concentration was calculated from a standard curve prepared with 1,1,3,3-tetramethoxypropane as the MDA equivalent and corrected for the dilution factor. Values were normalized to total protein content in the corresponding sample volume.	Islayem et al.[3]
	Superoxide dismutase activity (% inhibition/mg protein)	SOD	SOD activity was determined in *T. molitor* larval homogenate supernatants using a microplate colorimetric assay based on the inhibition of MTT reduction by superoxide radicals generated through riboflavin photoreduction, adapted from Madesh and Balasubramanian (1998), Das et al. (2000), and Paoletti et al. (1986). Briefly, 45 µL of sample supernatant were mixed with Tris-HCl buffer (50 mM, pH 8.2), MTT, riboflavin, EDTA, and ascorbic acid. Control wells without sample and dark blanks with or without sample were included to correct for background absorbance. Plates were incubated for 15 min under fluorescent light at room temperature, while dark blanks were protected from light. After incubation, DMSO was added to solubilize the formazan product, and absorbance was measured at 450 nm. SOD activity was calculated as the percentage inhibition of formazan formation	Paoletti et al.[4] and Das et al.[5]

		relative to the light-exposed control and normalized to total protein content in the corresponding sample volume.	
Catalase activity (U/mg protein)	CAT	Catalase activity was determined in *T. molitor* larval homogenate supernatants using a spectrophotometric molybdate-based assay, adapted from Hadwan and Abed (2016). Briefly, diluted sample supernatants were incubated with 20 mM H_2O_2 in PBS for 5 min at 25 °C. The reaction was stopped by adding ammonium molybdate solution, which reacts with residual H_2O_2 to form a yellow complex. After 3 min at room temperature, 150 µL of the reaction mixture were transferred to a microplate and absorbance was measured at 405 nm. Residual H_2O_2 was calculated from a standard curve prepared with known H_2O_2 concentrations, and catalase activity was estimated from the amount of H_2O_2 consumed during the reaction. Values were normalized to total protein content in the corresponding sample volume.	Hadwan & Abed[5]
Glutathione S-transferase activity [(nM/min)/mg protein]	GST	GST activity was determined in *T. molitor* larval homogenate supernatants using a kinetic spectrophotometric assay based on the conjugation of reduced glutathione (GSH) with 1-chloro-2,4-dinitrobenzene (CDNB), adapted from Vontas et al. (2000). Briefly, 60 µL of sample supernatant were mixed with PBS buffer (pH 6.0) and 5 mM GSH in a microplate, and the reaction was initiated by adding 1 mM CDNB. Absorbance was measured at 405 nm immediately after CDNB addition and again after 5 min of incubation at room temperature in the dark. GST activity was calculated from the change in absorbance per minute using the molar extinction coefficient of the GS-DNB conjugate and normalized to total protein content in the corresponding sample volume.	Vontas et al.[6]
CYP450-type oxidase activity (µU/mg protein)	CYP450	CYP450-type oxidase activity was estimated in *T. molitor* larval homogenate supernatants using a TMBZ/H_2O_2 colorimetric assay, adapted from El-Samad et al. (2024). Briefly, 3 µL of sample supernatant, cytochrome C positive control, or acetate buffer blank were mixed with PBS, 0.2 mg/mL 3,3′,5,5′-tetramethylbenzidine (TMBZ), and 3% H_2O_2. After gentle mixing, reactions were incubated for 5 min at room temperature, and absorbance was measured at 630 nm. Activity was calculated by subtracting the blank absorbance and expressing the sample signal relative to the cytochrome C positive control, adopted as an empirical oxidase activity reference, and normalized to total protein content in the corresponding sample volume.	El-Samad et al.[7]

	Nitrite levels (μM/mg protein)	NO	Nitrite levels, used as an indirect indicator of nitric oxide production, were quantified in *T. molitor* larval homogenate supernatants using the Griess reaction, adapted from Grisham et al. (1998). Briefly, 30 μL of sample supernatant were added to 150 μL of freshly prepared Griess reagent in 96-well microplates. The reagent consisted of equal volumes of N-(1-naphthyl)ethylenediamine dihydrochloride solution and sulfanilic acid solution prepared in 5% phosphoric acid. After 5 min of incubation at room temperature, absorbance was measured at 540 nm. Nitrite concentrations were calculated from a sodium nitrite standard curve and normalized to total protein content in the corresponding sample volume.	Grisham et al.[8]

[1]FA: functional axis.

Table S2. *Continuation*

FA[(1)]	Measured biomarkers	Abbreviation	Method used	Reference basis
Bioenergetic reserve capacity	Total protein levels (µg/mg body biomass)	TP	Total protein levels were determined in *T. molitor* larval homogenate supernatants using a commercial colorimetric biuret-based kit (Biotécnica®, Total Protein, BT1000900), adapted to microplate format. The assay is based on the reaction of Cu^{2+} ions with peptide bonds under alkaline conditions, forming a colored complex measured at 550 nm. Briefly, sample supernatants, standard, and blank were incubated with the ready-to-use reagent at room temperature for 10 min, using reduced reaction volumes while maintaining the manufacturer-recommended reagent-to-sample proportion. Absorbance was measured at 550 nm, and protein concentration was calculated from the kit standard/calibration factor and expressed relative to body biomass.	Gornall et al.[9] and Biotécnica® kit instructions
	Triglyceride levels (µg/mg body biomass)	TAG	Triglyceride levels were quantified in *T. molitor* larval homogenate supernatants using a commercial enzymatic-colorimetric kit (Biotécnica®, Triglycerides, BT1001000), adapted to microplate format. The assay is based on enzymatic hydrolysis of triglycerides to glycerol, followed by glycerol phosphorylation/oxidation and peroxidase-mediated formation of a quinonimine chromogen. Briefly, sample supernatants, standard, and blank were incubated with the ready-to-use reagent at 37 °C for 10 min using reduced reaction volumes while maintaining the manufacturer-recommended reagent-to-sample proportion. Absorbance was measured at 505 nm, and triglyceride concentration was calculated from the kit standard/calibration factor and expressed relative to body biomass.	Bucolo & David[10] and Biotécnica® kit instructions
	Total soluble carbohydrates (µg/mg body biomass)	TSC	Total soluble carbohydrates were quantified in *T. molitor* larval homogenate supernatants using the phenol–sulfuric acid colorimetric method, adapted to microplate format. Briefly, 50 µL of sample supernatant, glucose standard, or blank were added to microplate wells, followed by 50 µL of 5% phenol solution and 250 µL of concentrated sulfuric acid. After gentle homogenization, the reaction was incubated for 30 min at room temperature to allow color development. Absorbance was measured at 490 nm, and carbohydrate concentration was calculated from a glucose standard curve and expressed relative to body biomass.	DuBois et al.[11]
	Index	**Abbreviation**	**Description**	**Interpretation**

Relative Allocation of Energy (RAE)	RAETP, RAETAG, and RAETSC	$RAEi = \frac{Ci}{\sum_{n=1}^{3} Cn}$ where: Ci: concentration of macromolecule *i* (i.e., TP, TAG, or TSC); ∑Cn = total concentration of all three macromolecules (TP + TAG + TSC); and RAE: Relative Allocation of Energy for macromolecule *i*.	Represents the proportion of each macromolecule (TP, TAG, and TSC) relative to the total bioenergetic reserve. Higher values for a given macromolecule indicate preferential energy allocation: elevated TAG suggests energy storage, higher TP reflects structural maintenance, and increased TSC indicates readiness for immediate metabolic use.
Rapid Energy Allocation Index	REAI	Calculated as the ratio between TSC and TAG levels.	High RAEI values indicate reliance on immediate energy (TSC), while low values suggest long-term energy storage (TAG).
Protein-to-Carbohydrate Metabolic Index	PCI	Calculated as the ratio between TP and TSC levels.	Represents the relative balance between structural mass (TP) and readily available energy reserves (TSC); high values may indicate tissue building or catabolic states, while low values suggest energetic prioritization.
Lipid-to-Protein Index	LPI	Calculated as the ratio between TAG and TP levels.	High values indicate predominant energy storage (TAG), while low values suggest investment in structural or functional biomass (TP).
Bioenergetic Condition Index	BCI	Calculated as the sum of TP, TSC, and TAG, all expressed in µg per mg of body mass.	Higher values indicate a robust physiological condition and a favorable energetic balance, while lower values may reflect stress, nutrient depletion, or compensatory energy expenditure.

[1]FA: functional axis.

Table S2. *Continuation*.

FA[(1)]	Measured biomarkers	Abbreviation	Method used	Reference basis
Digestive enzymatic activity	Trypsin activity [(µg/mL)/µg protein]	TRY	Trypsin- and chymotrypsin-like proteolytic activities were determined in *T. molitor* larval homogenate supernatants using fluorescein isothiocyanate-labeled casein (FITC-casein) as a fluorogenic protein substrate, adapted from Twining (1984). Briefly, 10 µL of sample or enzyme standard were mixed with 20 µL of assay buffer (100 mM Tris-HCl, pH 7.8, containing 10 mM $CaCl_2$) and 20 µL of FITC-casein, followed by incubation at 37 °C for 1 h. The reaction was stopped with 120 µL of 5% trichloroacetic acid (TCA), and non-hydrolyzed proteins were precipitated for 1 h at room temperature or overnight at 4 °C. After centrifugation at 10,000 rpm for 5 min, 120 µL of the supernatant were transferred to a microplate and neutralized with 80 µL of 500 mM Tris-HCl buffer (pH 8.5). Fluorescence was measured at 492 nm excitation, and activities were calculated from independent standard curves prepared with trypsin or chymotrypsin standards and normalized to total protein content.	Twining[12]
	Chymotrypsin activity [(µg/mL)/µg protein]	CHY		
	Lipase activity [(U/mL)/µg protein]	LIP	Lipase activity was determined in *T. molitor* larval homogenate supernatants using p-nitrophenyl palmitate (pNPP) as a chromogenic substrate, adapted from Yel (2021). Briefly, 200 µL of sample supernatant, blank, or lipase standard were mixed with 200 µL of pNPP substrate solution and incubated at 40 °C for 30 min in the dark. After incubation, the reaction mixtures were centrifuged at 15,000 rpm for 5 min at 4 °C, and 200 µL of the clarified supernatant were transferred to a microplate. The release of p-nitrophenol was quantified spectrophotometrically at 492 nm. Lipase activity was calculated from a standard curve prepared with lipase type VII standards and expressed as U/mL normalized to total protein content in the corresponding sample volume.	Yel[13]
	Alkaline phosphatase activity [(U/L)/mg protein]	ALP	Alkaline phosphatase activity was determined in *T. molitor* larval homogenate supernatants using a commercial kinetic DGKC kit (Biotécnica®, Alkaline Phosphatase-DGKC, BT1106400), adapted to microplate format. The working reagent was prepared according to the manufacturer's instructions by mixing R1 and R2 at a 4:1 ratio and pre-warmed to 37 °C. Sample supernatants were incubated with the working reagent, and the enzymatic hydrolysis of p-nitrophenyl phosphate to p-nitrophenol was monitored by absorbance at 405 nm. Activity was calculated from the mean change in absorbance per minute using the kit factor and normalized to total protein content in the corresponding sample volume.	Rosalki et al.[14] and Biotécnica® kit instructions
Neuroch	Dopamine levels (µM/µg protein)	DA	Dopamine levels were determined in *T. molitor* larval homogenate supernatants using an indirect colorimetric assay based on dopamine-mediated inhibition of the	Liang et al.[15] and Wang et al.[16]

			oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) by ceric ammonium nitrate under acidic conditions, adapted from Liang et al. (2021) and Wang et al. (2017). Briefly, 12 µL of sample supernatant were incubated with ceric ammonium nitrate, TMB, and sodium acetate/acetic acid buffer (pH 3.5) in a microplate at room temperature for 3 min. Absorbance was measured at 630 nm, and dopamine concentration was calculated from the percentage inhibition of TMB oxidation using a dopamine standard curve. Results were normalized to total protein content in the corresponding sample volume.	
emical activity	Serotonin levels [(mg/mL)/µg protein]	5-HT	Serotonin levels were determined in *T. molitor* larval homogenate supernatants using a colorimetric assay based on the reaction of serotonin with ammonium metavanadate under acidic conditions, adapted from Pathak and Shukla (1979). Briefly, 150 µL of sample supernatant, blank, or serotonin standard were mixed with 15 µL of 0.3 M ammonium metavanadate prepared in 10% H_2SO_4. The reaction mixtures were vortexed and incubated at 100 °C for 10 min in sealed microtubes. After cooling, 120 µL were transferred to a microplate, and absorbance was measured at 492 nm. Serotonin concentration was calculated from a standard curve prepared with serotonin standards and normalized to total protein content in the corresponding sample volume.	Pathak & Shukla[17]
	Acetylcholinesterase activity [(nmol/min/mL)/µg protein]	AChE	Acetylcholinesterase activity was determined in *T. molitor* larval homogenate supernatants using the Ellman colorimetric method, adapted to microplate format. Briefly, 50 µL of sample supernatant or PBS blank were mixed with 100 µL of acetylthiocholine iodide and 100 µL of DTNB [5,5′-dithiobis-(2-nitrobenzoic acid)] under light-protected conditions. Absorbance was measured at 405 nm at 30 and 180 s, and the increase in absorbance was corrected against the blank. Enzymatic activity was calculated from the corrected ΔAbs using the assay conversion factor and expressed as µmol of substrate hydrolyzed min^{-1} mL^{-1}, normalized to total protein content in the corresponding sample volume.	Ellman et al.[18]

[1]FA: functional axis.

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

Biomarkers	Control group		PLA group		Δ Grau	Δ betweenness
	Grau	Betweenness	Grau	Betweenness		
Serotonin (5-HT) levels	12	0.025	2	0.001	-10	-0.024
Malondialdehyde (MDA) levels	4	0.003	11	0.025	7	0.022
Catalase (CAT) activity	10	0.007	13	0.096	3	0.089
Superoxide dismutase (SOD) activity	6	0.008	3	0.003	-3	-0.005
Triglycerides (TAG) levels	8	0.016	5	0.002	-3	-0.014
Acetylcholinesterase (AChE) activity	11	0.048	9	0.003	-2	-0.045
Cytochrome P450-type oxidase (CYP450) activity	12	0.042	10	0.047	-2	0.005
alkaline phosphatase (ALP) activity	12	0.069	11	0.021	-1	-0.048
Dopamine (DA) levels	11	0.012	10	0.012	-1	0
Glutathione S-transferase (GST) activity	10	0.016	9	0.051	-1	0.035
Lipase (LIP)	11	0.023	12	0.039	1	0.016
Reactive oxygen species (ROS) levels	13	0.046	12	0.027	-1	-0.019
Total soluble carbohydrates (TSC) levels	7	0.011	6	0.014	-1	0.003
Chymotrypsin (CHY) activity	8	0.007	8	0.004	0	-0.003
Nitrite levels [an indirect marker of nitric oxide (NO)]	13	0.036	13	0.104	0	0.068
Total protein (TP) levels	12	0.056	12	0.027	0	-0.029
Trypsin (TRY) activity	8	0.007	8	0.031	0	0.024

[(1)]The table presents the degree and betweenness centrality values for each biochemical biomarker in the control and PLA-MP-exposed groups, as well as the absolute differences between these groups (Δ Grau and Δ Betweenness). "Grau" represents the number of direct connections (edges) a node has within the network. At the same time, "Betweenness" denotes the extent to which a node lies on the shortest paths between other nodes, reflecting its topological influence. Positive Δ values indicate increased connectivity or centrality in the PLA group, while negative values represent losses relative to the control group.

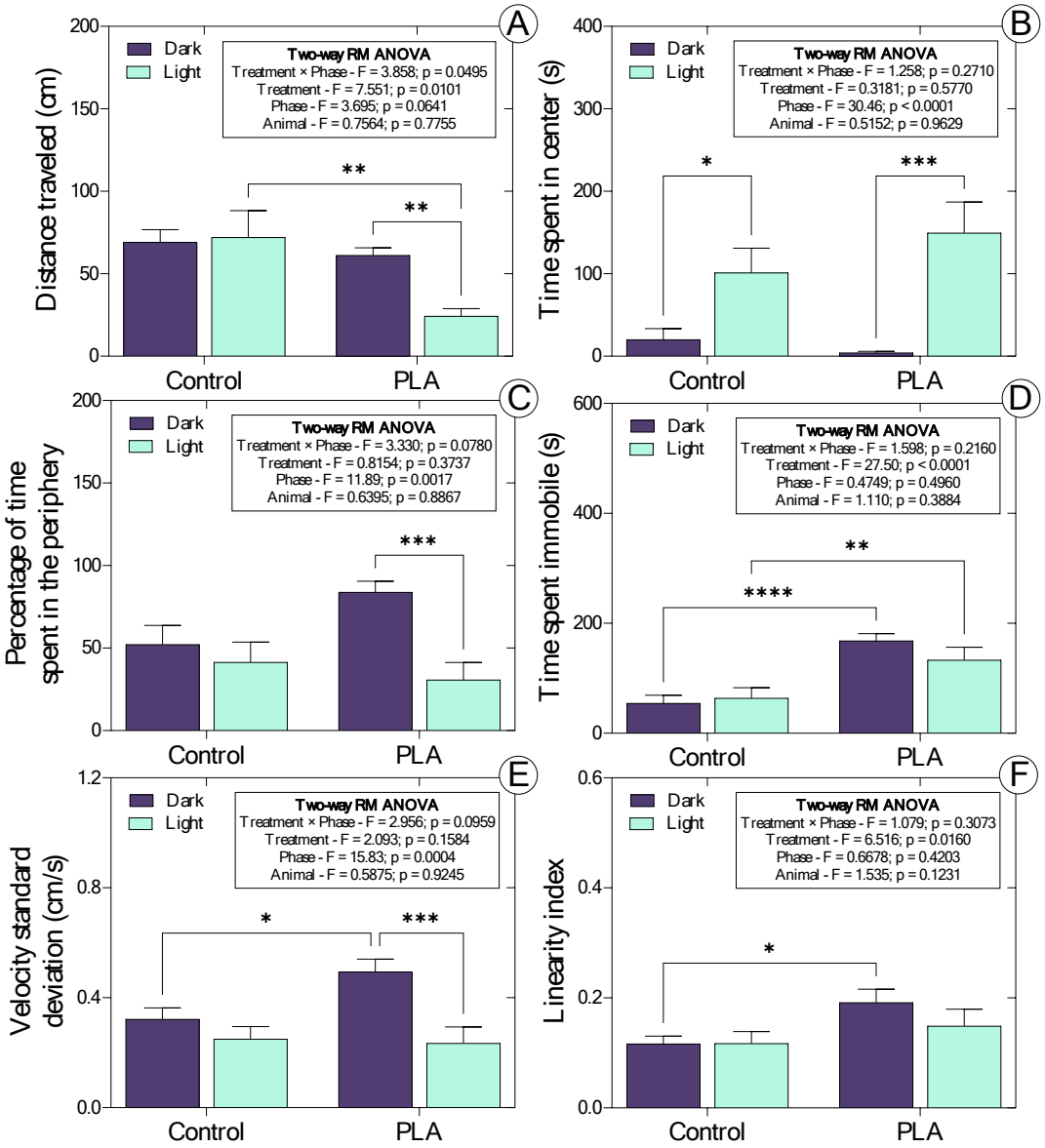

Figure S1. Behavioral parameters of *Tenebrio molitor* larvae in the open field test following trophic exposure to polylactic acid microplastics (PLA-MPs). (A) Distance traveled (mm), (B) time spent in center (s), (C) percentage of time spent in the periphery, (D) time spent immobile (s), (E) velocity 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.

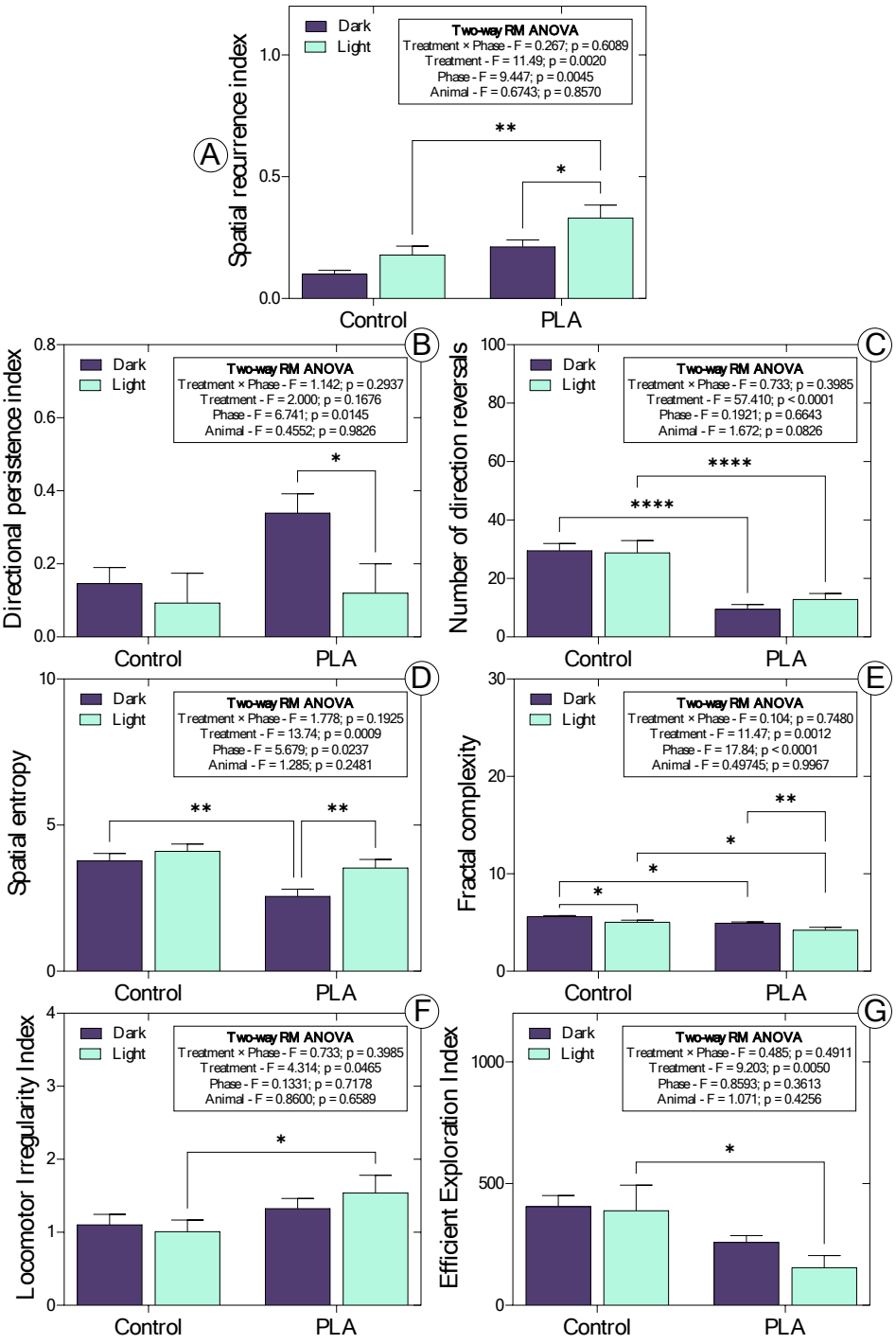

Figure S2. Behavioral parameters of *Tenebrio molitor* larvae in the open field test following trophic exposure to polylactic acid microplastics (PLA-MPs). (A) Spatial recurrence index, (B) directional persistence index, (C) number of direction reversals, (D) spatial entropy, (E) fractal complexity, (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.

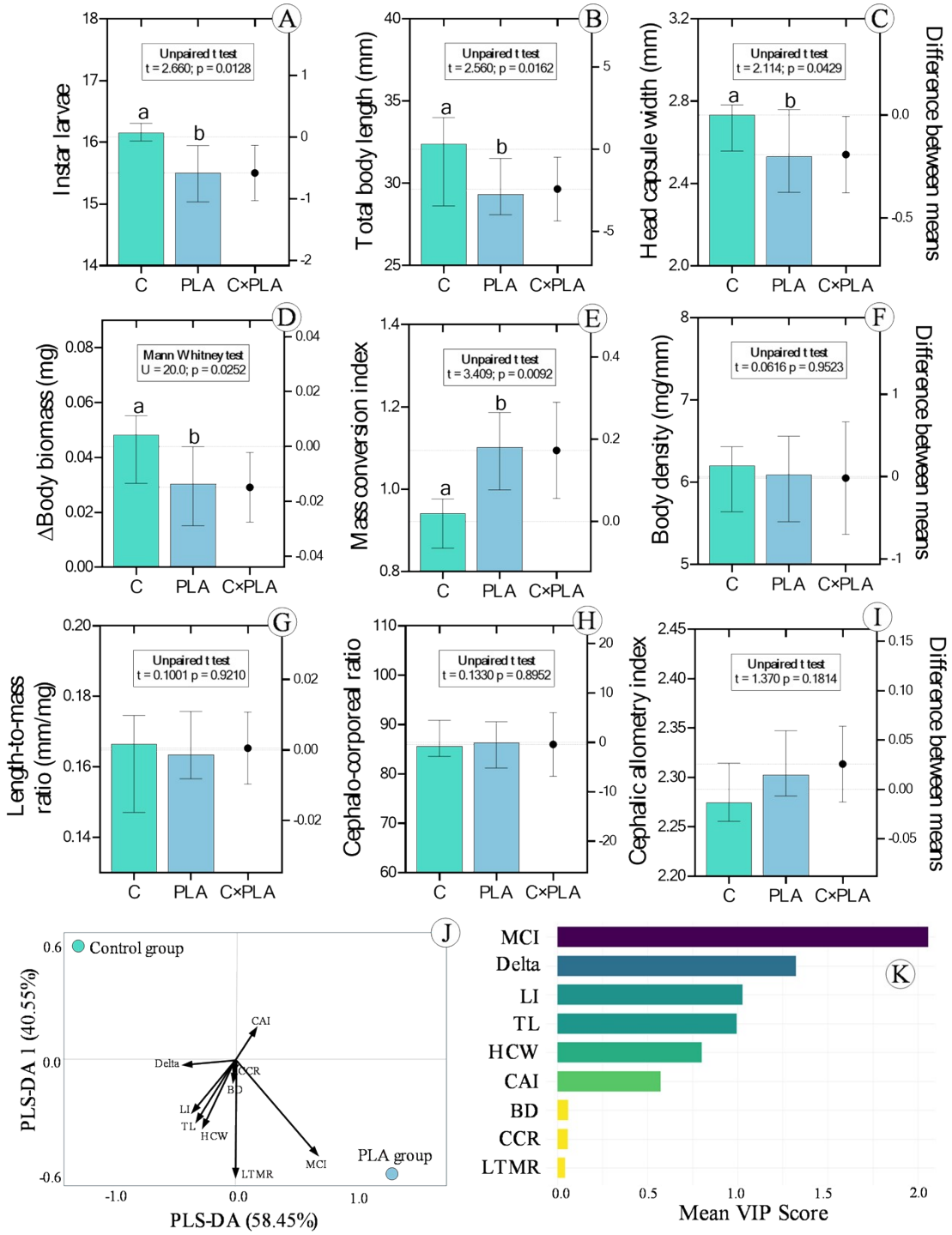

Figure S3. Morphometric assessment and multivariate discrimination of *Tenebrio molitor* larvae following trophic exposure to polylactic acid microplastics (PLA-MPs). (A) Instar classification, (B) total body length, (C) head capsule width, (D) delta body biomass, (E) mass conversion index, (F) body 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.

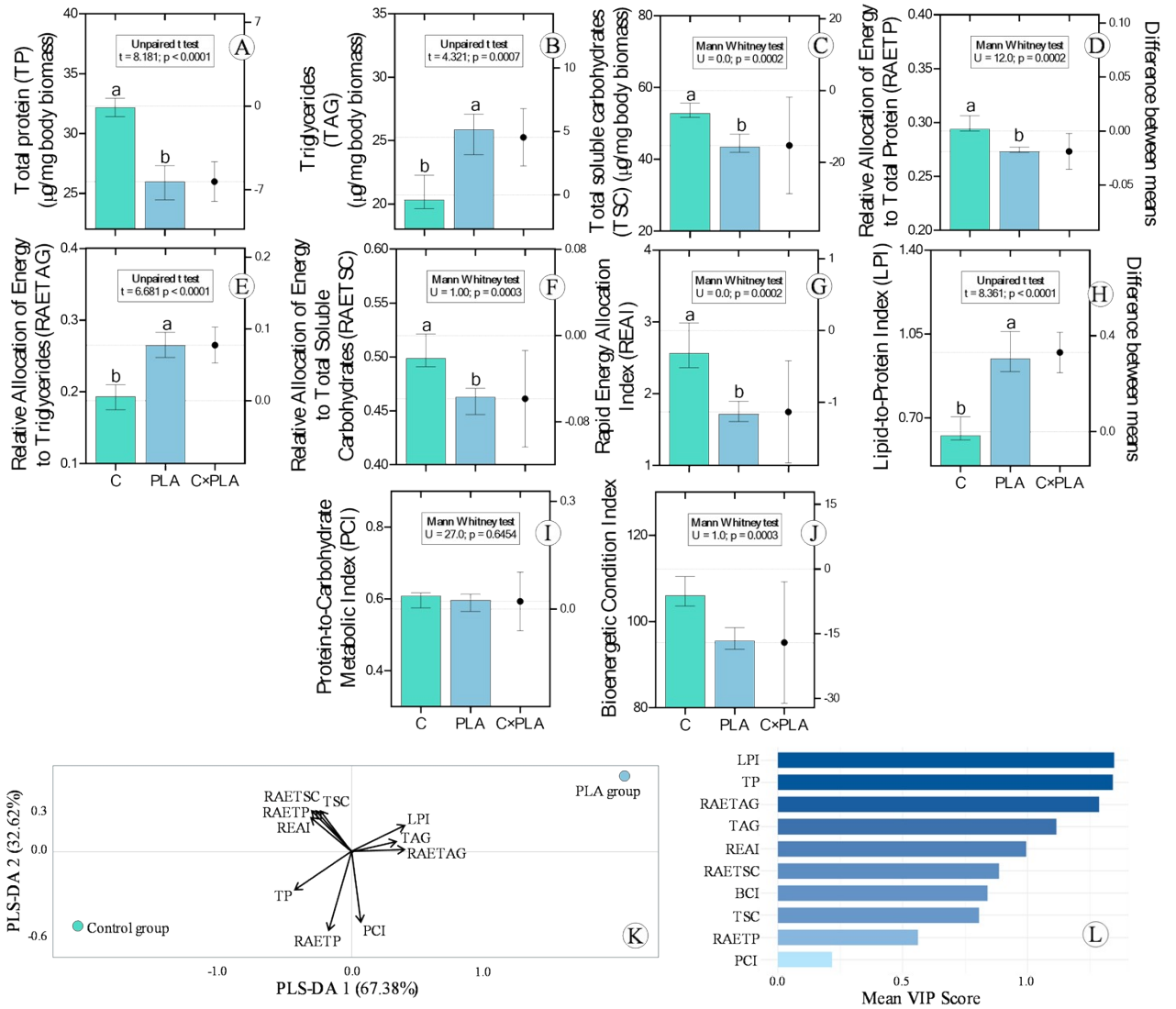

Figure S4. Bioenergetic reserve capacity assessment and multivariate discrimination of *Tenebrio molitor* larvae following trophic exposure to polylactic acid microplastics (PLA-MPs). Concentrations of (A) total protein, (B) triglycerides, and (C) total soluble carbohydrates; relative allocation of energy 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 ± 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 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.

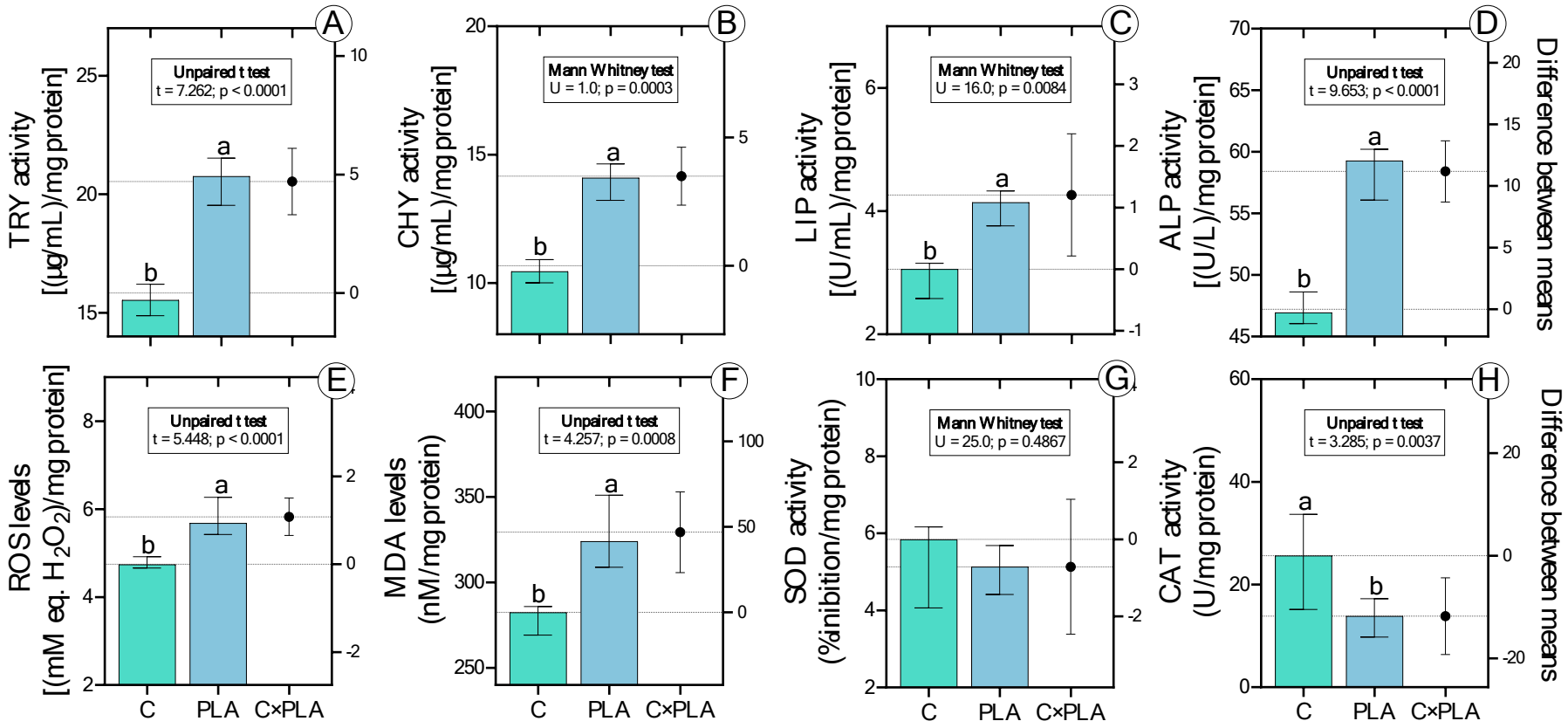

Figure S5. Systemic biochemical responses of *Tenebrio molitor* larvae following trophic exposure to polylactic acid microplastics (PLA-MPs). (A–D) Activities of trypsin (TRY), chymotrypsin (CHY), lipase (LIP), and alkaline phosphatase (ALP), respectively. (E–H) Levels of reactive oxygen species (ROS), malondialdehyde (MDA), and activities of superoxide dismutase (SOD) and catalase (CAT). Bars represent mean ± standard deviation for normally distributed variables (unpaired t-test) or median ± 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.

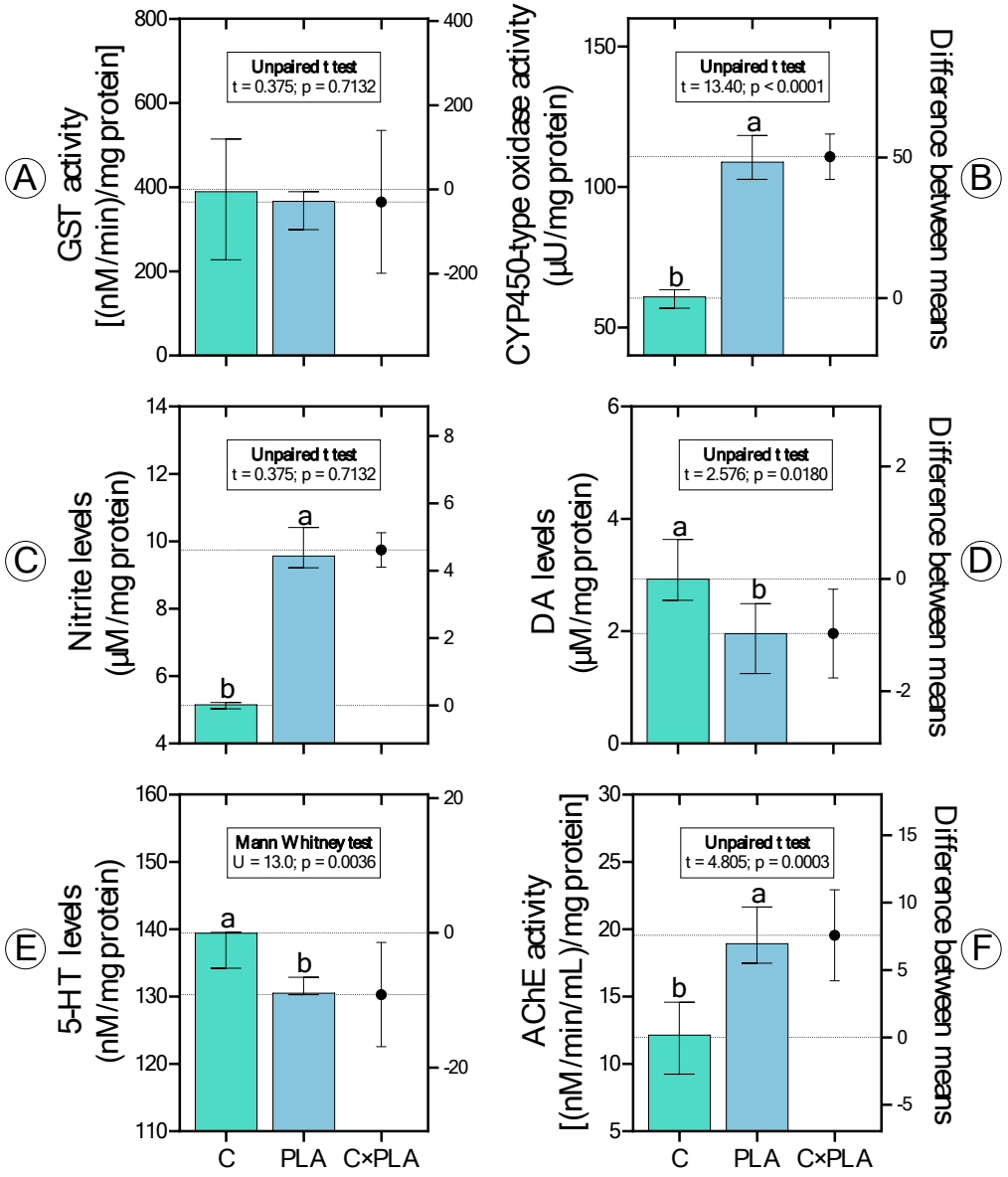

Figure S6. Systemic biochemical responses of *Tenebrio molitor* larvae following trophic exposure to polylactic acid microplastics (PLA-MPs). (A–B) Activities of glutathione S-transferase (GST) and cytochrome P450-type oxidase (CYP450). (C–F) Levels of nitrite (an indirect marker of nitric oxide), dopamine (DA), serotonin (5-HT), and acetylcholinesterase (AChE) activity. Bars represent mean ± standard deviation for normally distributed variables (unpaired t-test) or median ± 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.

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