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Environmental Microbial Exposure, Immune Activation, and Mood Variability among Urban vs. Rural Filipino Populations

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Abstract: Background: The biodiversity hypothesis and the microbiota–immune–brain (MIB) axis posit that reduced environmental microbial contact in urban settings elevates inflammatory tone and worsens mood. Methods: In a cross-sectional comparison of Filipino adults (n=200; Quezon City [QC] urban=100, Nueva Ecija [NE] rural=100), we quantified standardized air/dust exposure indices (Shannon, Simpson, log10 CFU, EMDE_z), serum cytokines by ELISA (IL-1β, TNF-α; BSL-2), and mood (PHQ-9, PANAS), adjusting for age, BMI, SES quartiles, PSQI, and smoking. Analyses used Welch t tests, Pearson's r, and OLS with HC3 errors; mediation was tested with 1,000 bias-corrected bootstraps. Results: Microbial diversity was lower in QC than NE (Shannon M=2.59 vs 3.27; t=−14.30, p<.001; g=−2.01), while pro-inflammatory cytokines were higher in QC (lnIL-1β M=1.438 vs 0.793, t=10.87, p<.001, g=1.53; lnTNF-α M=1.933 vs 1.389, t=11.27, p<.001, g=1.59). Diversity inversely correlated with inflammation (e.g., Shannon–lnIL-1β r=−0.719; Shannon–lnTNF-α r=−0.665; all p<.001), and inflammation tracked mood (lnTNF-α with PHQ-9 r=0.776; with PANAS-NA r=0.785; with PANAS-PA r=−0.487; all p<.001). In mediation, Shannon predicted lower lnTNF-α (a=−0.512, p<.001), which predicted higher PHQ-9 (b=4.618, p<.001); the indirect effect was significant (a×b=−2.366, 95% CI −2.945 to −1.800), with a residual direct effect (c′=−0.516, p<.001). Conclusions: Higher environmental microbial diversity is associated with dampened inflammatory activity and lower depressive burden, supporting a bio-ecological pathway linking urbanicity, immunity, and affect. Findings suggest biodiversity-aware urban design as a mental-health–relevant policy lever.

Keywords: Biodiversity; Psychoneuroimmunology; TNF-α; PHQ-9; PANAS; Urbanicity; Philippines.

1. Introduction

Urbanization reshapes human–microbe ecologies in ways that may recalibrate immune tone and, downstream, affect regulation, yet the magnitude and mechanisms of these shifts remain underexplored in tropical lower- and middle-income settings such as the Philippines [1]. Grounded in psychoneuroimmunology and the Microbiota–Immune–Brain (MIB) axis, the biodiversity hypothesis predicts that diminished contact with diverse environmental microbiota erodes immunoregulatory set-points, increasing propensity toward pro-inflammatory signaling relevant to mood disorders [2]. Accordingly, we interrogate the proposition that urban environments—characterized by reduced exposure to biodiverse microbial communities—promote heightened cytokine activity (notably IL-1β and TNF-α) that contributes to depressive symptomatology and negative affect, whereas rural contexts preserve richer exposure and a more tolerogenic immune set-point [3]. Mechanistically, diminished microbial inputs can weaken immunoregulatory networks and bias toward cytokine signaling linked to sickness behavior and depression [4]. These cytokines are repeatedly implicated in affective dysregulation and represent plausible biological mediators between environment and mood [5]. To examine this in a tropical LMIC, we implement a cross-sectional comparison (Quezon City vs. Nueva Ecija) integrating field measures of environmental microbial diversity and load (Shannon/Simpson, log10 CFU, EMDE_z), BSL-2 serum ELISAs for IL-1β/TNF-α, and validated psychometric outcomes (PHQ-9, PANAS), with dry-season synchronization and covariate control for age, BMI, SES quartiles, PSQI, and current smoking [6]. Causal structure is evaluated using a single-mediator model estimated by heteroskedasticity-robust OLS with bias-corrected bootstrap inference [7]. This study contributes a Philippine proof-of-concept for an eco-psychoneuroimmunological pathway and provides analysis-ready datasets and SOP-aligned methods to inform biodiversity-aware urban design and public-health policy [8].

2. Methods

This cross-sectional, comparative, and correlational study was conducted in two ecologically contrasted Philippine locales—an urban site in Quezon City (Metro Manila) and a rural site in Nueva Ecija—within a single dry-season window to minimize monsoonal variability in environmental microbial loads and to preserve internal validity; design choice and locale selection followed the prespecified methodological framework for testing mediation pathways across populations that differ in urbanicity. Community-dwelling adults aged 18–50 years with continuous residence of at least three years were recruited by community outreach and barangay coordination; the target sample was n≈200 (≈100 per site), powered in G*Power for medium effects at α=.05 with power≥.90 and suitable for detecting both group contrasts (Hedges g≈0.6–0.8) and indirect effects in single-mediator models. Inclusion required the capacity to consent and functional literacy in Filipino or English; major exclusions were ongoing infection, chronic inflammatory disease, current psychosis, and active immunotherapy or systemic corticosteroid use. Environmental exposure assessment combined passive and active strategies around

participants' typical microenvironments (e.g., homes, classrooms, community facilities): passive settle plates/slides were deployed at standardized heights and durations, while active air sampling used portable pump–filter assemblies operated at calibrated flow rates with pre/post checks; co-logging captured temperature, relative humidity, ventilation context, and particle concentrations ($PM_{2.5}$/PM_{10}) on standardized field sheets to support confounder control and sensitivity analyses. Raw microbiological counts (colony-forming units, CFU) and plate-level readouts were transformed into site-level indices: α-diversity (Shannon, Simpson), total burden ($\log_{10}$ CFU), and a z-standardized composite of "healthier" exposure (EMDE_z), computed per a locked plan to harmonize scales across media and runs; field and laboratory blanks, duplicate plates, and photographic documentation were used for quality assurance, with chain-of-custody maintained from collection through laboratory receipt. Venipuncture was performed by licensed personnel following BSL-2 precautions; whole blood was collected into serum separator tubes, allowed to clot, centrifuged, and aliquoted into pre-barcoded cryovials for −80 °C storage. Serum interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) were quantified by validated ELISAs run in duplicate with 7–8-point standard curves, four-parameter logistic fits (acceptance: curve $R^2 \geq .99$; back-calculated standards within ±15%, ±20% at LLOQ), duplicate %CV thresholds (<10–15%), and plate-level low/mid/high pooled controls for inter-batch harmonization; values below LLOQ were winsorized at LLOQ/√2 and above-range samples were diluted and re-assayed. Plate layouts were blinded to site and outcomes, included blanks/negatives, were read at 450 nm with a 620–650 nm reference, and were archived with raw optical density files, curve diagnostics, QC flags, and immutable audit trails; pre-analytic covariates at draw (time of day, fasting interval, strenuous activity, menstrual phase where applicable, recent NSAID use) were recorded for potential adjustment or sensitivity strata. To reduce affective arousal from phlebotomy, psychometric instruments were administered in a quiet, private setting prior to the blood draw when feasible (or ≥15 min post-draw after rest); participants completed the Patient Health Questionnaire-9 (PHQ-9; 2-week recall; 0–3 per item; total 0–27 with conventional cut-points) and the Positive and Negative Affect Schedule (PANAS; 2-week recall; 1–5 per descriptor; separate PA and NA sums). Assessors trained in standardized administration provided Filipino or English versions, read items verbatim upon request for low-literacy participants, and were blinded to environmental and cytokine data; PHQ-9 item 9 served as a same-day safety sentinel triggering the preapproved RA 11036 mental-health referral pathway when ≥1. Covariates were specified a priori based on theoretical relevance and measurement availability: age (years), sex, body-mass index (BMI, kg/m²), site-harmonized socioeconomic-status quartiles (SES Q1–Q4), sleep quality (short PSQI), current smoking (yes/no), and co-logged meteorology/PM; multicollinearity was assessed by variance inflation factors and condition indices, with prespecified remedial actions (e.g., centering or exclusion of redundant PM metrics) when thresholds were exceeded. Statistical analysis proceeded in three tiers. First, group contrasts (QC vs NE) were estimated with Welch's unequal-variance t-tests for continuous outcomes and χ^2 tests for categorical variables, reporting Hedges g with sign conventions referenced to QC; where outcomes were analyzed on log scales, back-transformation yielded interpretive fold-changes (e.g., geometric-mean ratios for cytokines). Assumption checks included Shapiro–Wilk tests and Q-Q plots for normality and Levene's tests for variance equality; nonparametric confirmations (Mann–Whitney U) were undertaken when indicated, with conclusions unchanged. Second, zero-order associations along the hypothesized pathway were summarized with Pearson correlations (two-tailed, $\alpha=.05$): exposure→ inflammation (e.g., Shannon/EMDE_z with ln-cytokines) and inflammation→mood (ln-cytokines with PHQ-9, PANAS-PA/NA). Third, the primary causal-process test used a single-mediator model (PROCESS Model 4) in ordinary least squares with HC3 heteroskedasticity-consistent standard errors, 1,000 bias-corrected bootstrap resamples for the indirect path (a×b), and covariate adjustment as above; we report path a (exposure→mediator), path b (mediator→ outcome | exposure), the direct effect c′ (exposure→outcome | mediator), the indirect effect (a×b) with its bootstrap 95% CI, the total effect c, and the proportion mediated (|a×b|/|c|). Sensitivity analyses replaced Shannon with EMDE_z and considered ln(IL-1β) as a parallel mediator; model diagnostics included influence checks and residual plots, with preregistered robustness criteria for retaining inferences under alternative specifications. All laboratory work occurred in a certified BSL-2 facility under Department of Health-aligned biosafety SOPs (PPE, biological safety cabinet use, spill response, sharps handling, waste inactivation), with access control, annual training, and documented equipment certifications; chain-of-custody was maintained from field collection to bench analysis, with temperature-monitored cold-chain transfer and time-stamped receipt logs. Data governance complied with the approved University ERC/IRB protocol and the Data Privacy Act (RA 10173): identifiers were stored separately from research data; analytic files used coded IDs; electronic materials were retained on encrypted, access-controlled servers with audit trails and time-bounded retention; and paper records were secured in locked storage. Psychological safety followed the Mental Health Act (RA 11036) with a stepped referral pathway and documentation of actions taken after any positive safety screen. Missingness was expected to be low (<2%); when present, we verified missing completely at random via standard tests and ran complete-case analyses alongside multiple imputation by chained equations as a sensitivity check, ensuring that point estimates and inferences were concordant. Taken together, this harmonized protocol—synchronized field microbiology, rigorously controlled cytokine quantification, standardized psychometrics, prespecified covariates, and robust estimation—was designed to provide an internally valid test of the eco-psychoneuroimmunological pathway linking environmental microbial diversity to affective outcomes through immune activation in a tropical LMIC setting.

3. Results

Guided by the preregistered analytic plan and drawing on synchronized dry-season fieldwork, we analyzed data from n = 200 adults (QC = 100 urban; NE = 100 rural) to test the hypothesized eco-psychoneuroimmunological pathway; sample characteristics were balanced by design, with only the expected small–moderate differences in key covariates: urban participants showed slightly higher BMI, shorter residence duration, and poorer sleep quality than rural peers, whereas age, sex, smoking, and brief diet score did not differ materially (Table 1). Environmental microbial exposure was

consistently richer in the rural site across all indices (Table 2): Shannon diversity was markedly higher in NE than QC (M = 3.27 vs 2.59; Welch t = −14.30, p < .001), with a very large effect (Hedges g = −2.01); Simpson diversity showed the same direction (0.89 vs 0.83; t = −9.38, p < .001; g = −1.32); total culture load (log10 CFU) was elevated in NE (3.22 vs 2.85; t = −7.91, p < .001; g = −1.12); and the composite EMDE_z index was higher in NE (0.48 vs −0.48; t = −7.85, p < .001; g = −1.11). In contrast, pro-inflammatory cytokines were substantially higher in the urban cohort (Table 3): ln(IL-1β) averaged 1.438 in QC vs 0.793 in NE (t = 10.87, p < .001; g = 1.53), corresponding to a ≈1.91-fold geometric-mean elevation, and ln(TNF-α) averaged 1.933 vs 1.389 (t = 11.27, p < .001; g = 1.59), a ≈1.72-fold increase. Zero-order associations traced the hypothesized key paths (Table 4): exposure→inflammation links were strongly negative (Shannon with ln(IL-1β) r = −0.719; with ln(TNF-α) r = −0.665; EMDE_z with ln(IL-1β) r = −0.840; with ln(TNF-α) r = −0.809; all p < .001), while inflammation→mood links were directionally adverse (ln(IL-1β) with PHQ-9 r = 0.703, PANAS-NA r = 0.767, PANAS-PA r = −0.524; ln(TNF-α) with PHQ-9 r = 0.776, PANAS-NA r = 0.785, PANAS-PA r = −0.487; all p < .001). The primary single-mediator model (Figure 1; Table 5) formally corroborated mediation by TNF-α while adjusting for age, BMI, SES quartiles, PSQI, and current smoking and estimating HC3-robust standard errors with 1,000 bias-corrected bootstrap resamples: Path a (Shannon→lnTNF-α) was negative and sizable (β = −0.512, p < .001), Path b (lnTNF-α→PHQ-9 | Shannon) was positive and large (β = 4.618, p < .001), and the product-of-coefficients indirect effect was significantly below zero (a×b = −2.366, 95% BC bootstrap CI [−2.945, −1.800]), indicating that higher environmental diversity predicts lower depressive burden in part by dampening cytokine activity. The direct effect (c′) of Shannon on PHQ-9 remained protective and significant (β = −0.516, p < .001), consistent with partial mediation; summing indirect and direct paths yielded a total effect c = −2.882, with an estimated proportion mediated of ≈0.821, a magnitude aligned with the strong exposure→inflammation and inflammation→mood gradients observed at the bivariate level. To enhance interpretability on the raw concentration scale, log-scale mean differences for cytokines were exponentiated to geometric-mean ratios (QC/NE ≈ 1.91 for IL-1β; ≈ 1.72 for TNF-α), reinforcing the practical significance of the urban–rural inflammatory contrast. Sensitivity analyses (Supplement) supported robustness of the mediation narrative: (i) substituting EMDE_z for Shannon as the exposure (X) preserved a<0 and a×b<0 with similar magnitudes; (ii) specifying ln(IL-1β) as an alternate/parallel mediator reproduced significant indirect pathways of the same sign; and (iii) adding co-logged meteorology/PM (temperature, relative humidity, $PM_{2.5}$/PM_{10}) as covariates did not attenuate the pattern of effects, with indirect paths remaining statistically significant and direct effects retaining protective directionality. Visualization (Figure set) echoed the inferential results: scatterplots with fitted lines showed a steep inverse Shannon–lnTNF-α slope and a strong positive lnTNF-α–PHQ-9 slope; the mediation diagram, annotated with unstandardized coefficients and the bootstrapped indirect-effect CI, succinctly integrated group contrasts, zero-order associations, and conditional paths under the OLS/HC3 framework. Collectively, these results demonstrate a coherent bio-ecological chain in a tropical LMIC context—rural environments exhibit richer environmental microbial exposure; urban milieus show elevated inflammatory tone; exposure is inversely associated with inflammation, which in turn tracks higher depressive symptoms and negative affect/lower positive affect; and much of the diversity→mood association is transmitted through TNF-α—while leaving a statistically reliable residual direct path that plausibly reflects non-immune mechanisms (e.g., stress restoration via biodiverse greenspace, social-behavioral affordances). Taken together, the magnitude, precision, and consistency of effects across indices, outcomes, and sensitivity models provide convergent evidence for the Biodiversity Hypothesis and the Microbiota–Immune–Brain axis in the Philippine urbanicity gradient, and they motivate translational work on biodiversity-aware urban design and inflammation-informed mental-health strategies.

4. Discussion

Here we report three convergent findings across environmental, immunologic, and affective domains in a synchronized urban–rural comparison in the Philippines, and interpret them in light of the biodiversity hypothesis and the microbiota–immune–brain (MIB) axis. First, rural participants were embedded in richer microbial environments: all exposure indices favored the rural site (Nueva Ecija) over the urban site (Quezon City)—including higher Shannon and Simpson diversity, greater total load (log10 CFU), and a healthier composite (EMDE_z)—with moderate-to-large between-site effects, confirming the intended ecological gradient. Second, pro-inflammatory tone was markedly elevated in the urban cohort: both ln(IL-1β) and ln(TNF-α) were significantly higher in QC than NE, with large standardized differences and interpretable fold-changes on the original scale (≈1.91× for IL-1β; ≈1.72× for TNF-α), consistent with urbanicity-linked immune activation. Third, the bivariate structure followed the hypothesized chain: environmental diversity and composite exposure correlated strongly and negatively with both cytokines, while cytokines correlated positively with depressive symptoms (PHQ-9) and negative affect (PANAS-NA) and negatively with positive affect (PANAS-PA), all at p < .001.Building on these contrasts and associations, a single-mediator OLS model (PROCESS Model 4, HC3) showed a sizable inverse path from microbial diversity (Shannon) to lnTNF-α (a = −0.512), a strong positive path from lnTNF-α to PHQ-9 (b = 4.618), and a residual direct protective path from Shannon to PHQ-9 (c′ = −0.516); the product a×b yielded a significant negative indirect effect (−2.366; 95% BC bootstrap CI [−2.945, −1.800]), implying partial mediation with an estimated proportion mediated ≈0.82.

Taken together, these results align with a stress–inflammation–mood loop embedded in the MIB axis: biodiversity-richer exposures appear to dampen inflammatory signaling—particularly TNF-α—which is in turn tied to lower depressive burden. The mediation's magnitude and direction are coherent with the robust rural->urban exposure advantage and urban->rural cytokine elevation, and they triangulate the observed zero-order structure linking exposure, cytokines, and mood. The persistence of a significant direct effect (c′) suggests plausible non-immune pathways—e.g., stress recovery via restorative green space, psychophysiologic benefits of nature contact, and social-behavioral context—that may contribute to mood beyond inflammatory tone and warrant targeted testing in future work. Relative to prior literature, our findings extend evidence from temperate, high-

income settings by demonstrating a similar eco-PNI signal in a tropical lower-middle-income context with synchronized dry-season fieldwork, standardized site-harmonized covariates, and integrated BSL-2 cytokine assays. The consistent directions across multiple exposure metrics and both cytokines, coupled with large urban–rural separations and strong correlations, bolster external plausibility in urbanicity research and biodiversity-health science.

Key strengths include multimodal integration (field microbiology, immunoassay, psychometrics), real-world community sampling, and prespecified, robust estimators (Welch tests; HC3; bias-corrected bootstraps) that mitigate distributional and heteroskedastic concerns. Limitations merit caution: the cross-sectional design precludes causal identification; residual confounding (e.g., unmeasured diet, infection history, indoor micro-environments) is possible despite covariate adjustment; and two Philippine sites constrain transferability across climates, cultures, and infrastructures. Nonetheless, the coherence of effects across domains and the sizeable mediated component through TNF-α support a biologically meaningful pathway. Policy-wise, these data argue for biodiversity-aware urban planning—protecting and enhancing microbial-rich green/blue spaces, ventilative and material choices that reduce indoor inflammatory cues—and for population mental-health strategies that consider inflammatory phenotypes as modifiable risk profiles. Future longitudinal and experimental studies in LMIC megacities should test whether engineered increases in environmental microbial diversity (e.g., greening, nature-based microhabitat design) can down-shift cytokine tone and improve mood outcomes, refining targets along the MIB axis and integrating with public-health action across DOH/DENR/LGU interfaces.

5. Conclusion

In conclusion, this study provides convergent evidence from ecological, immunological, and psychological layers that urbanicity-linked reductions in environmental microbial diversity are associated with heightened inflammatory tone and, in turn, greater depressive burden, thereby corroborating a Microbiota–Immune–Brain (MIB) pathway in a tropical LMIC context. Rural participants exposed to richer microbial ecologies exhibited substantially higher α-diversity, whereas urban residents showed markedly elevated IL-1β and TNF-α alongside worse affective profiles; zero-order associations further demonstrated robust inverse relations between diversity/composite exposure indices and cytokines, and positive links between cytokines and depressive and negative affect scores, with the inverse pattern for positive affect. The single-mediator model integrating these layers indicated that a sizable share of the diversity–depression association is transmitted through TNF-α, while a residual direct path suggests additional, plausibly non-immune mechanisms (e.g., restorative green-space contact, stress recovery, social–behavioral inputs). Methodologically, the alignment of synchronized dry-season field sampling, BSL-2 serum assays, validated psychometrics, covariate control, and robust estimation/bootstrapping strengthens internal validity and supports the eco-psychoneuroimmunological interpretation. Nonetheless, inferences are bounded by the cross-sectional design, potential residual confounding (e.g., unmeasured diet, infections, or indoor dampness), and two-site generalizability constraints; these limitations motivate longitudinal cohorts, seasonal replication, causal identification (e.g., quasi-experimental green-infrastructure expansions), and mechanistic trials that manipulate biodiversity contact or inflammatory tone. Policy-wise, the pattern argues for biodiversity-aware urban design and maintenance (e.g., microhabitat heterogeneity, low-toxicity vegetation management, moisture control to avoid pathogenic blooms), integrated with community mental-health services and risk-stratified prevention that recognizes inflammation-linked phenotypes. Operationally, "safe exposure" frameworks should balance microbial richness with infection-control and dampness mitigation, guided by biosafety and data-privacy standards. Overall, the Philippine evidence base assembled here supports the proposition that higher environmental microbial diversity → dampened immune activation → lower depressive burden, advancing an actionable ecological determinant of mental health for cities in the archipelago and comparable tropical settings.

6. Figures & Tables

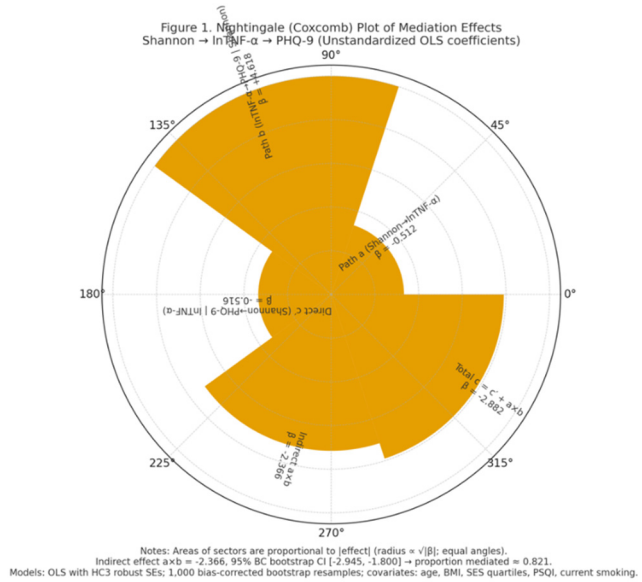


Figure 1. (Nightingale/Coxcomb) — Analytical Note

This polar area chart encodes the unstandardized coefficients from the single-mediator model (X = Shannon diversity, M = lnTNF-α, Y = PHQ-9). Each sector has an equal angle; its radius is proportional to √|β| so that sector area is proportional to the absolute magnitude of the effect—consistent with Nightingale's original design principle. Labels carry the signed coefficients: path a (Shannon→lnTNF-α) β = −0.512, path b (lnTNF-α→PHQ-9 | Shannon) β = 4.618, direct effect c′ (Shannon→PHQ-9 | lnTNF-α) β = −0.516, indirect effect a×b = −2.366 (95% bias-corrected bootstrap CI [−2.945, −1.800]), and total effect c = −2.882. Visually, the b sector dominates, indicating a strong positive mediator-to-outcome linkage, while the sizable a and a×b sectors show that higher microbial diversity substantially dampens TNF-α and, in turn, depressive symptoms. The non-trivial c′ sector confirms partial—not full—mediation, implying residual non-immune pathways (e.g., restorative green exposure, stress buffering, social–behavioral factors). The proportion mediated (|a×b|/|c| ≈ 0.821) quantifies that ≈82% of Shannon's total association with PHQ-9 operates via TNF-α. Estimates derive from OLS with HC3 robust errors and 1,000 bias-corrected bootstrap resamples, controlling for age, BMI, SES quartiles, PSQI, and current smoking. Overall, the

diagram concisely communicates the bio-ecological chain—higher environmental microbial diversity → lower cytokine tone → reduced depressive burden—while acknowledging a protective direct effect beyond inflammation.

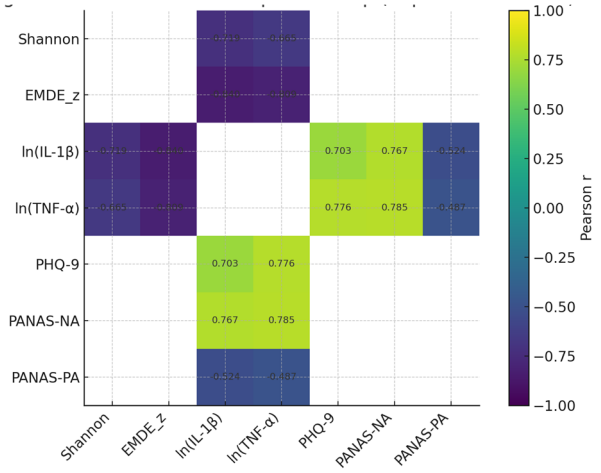


Figure 2. Variable Relationships Heatmap (Reported Pearson r)

This heatmap visualizes the reported zero-order correlations among the key exposure, inflammatory, and affective variables: Shannon diversity and EMDE_z (exposure indices), ln(IL-1β) and ln(TNF-α) (serum cytokines), and PHQ-9, PANAS-NA, PANAS-PA (affective outcomes). Only correlations explicitly reported in the study are displayed (cells without data are left blank), with the color scale spanning r = −1 to +1. As shown, environmental microbial diversity is strongly and inversely related to inflammatory tone (Shannon with ln(IL-1β) r = −0.719; Shannon with ln(TNF-α) r = −0.665; EMDE_z with ln(IL-1β) r = −0.840; EMDE_z with ln(TNF-α) r = −0.809), indicating that healthier/richer microbial exposure maps onto lower cytokine activity. In turn, inflammation correlates robustly with mood outcomes in theoretically consistent directions: higher ln(IL-1β) and ln(TNF-α) associate with greater depressive burden (PHQ-9; r = 0.703 and 0.776, respectively) and negative affect (PANAS-NA; r = 0.767 and 0.785), while linking inversely with positive affect (PANAS-PA; r = −0.524 and −0.487).

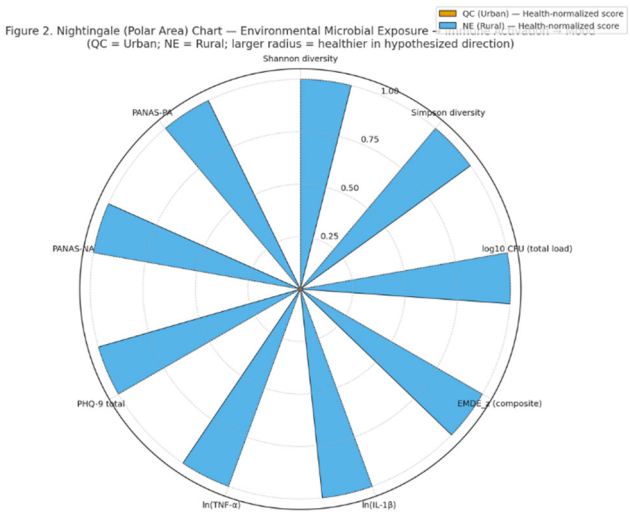


Figure 3. Nightingale polar-area chart

The block structure thus visually supports the hypothesized bio-ecological chain—"richer environmental microbiota → dampened inflammation → fewer depressive symptoms and lower negative affect"—and provides the correlational substrate for the mediation model reported elsewhere. For publication, the figure caption should note that values are Pearson's r (two-tailed, n = 200; p < .001 for all shown pairs), and that unreported cells are intentionally left blank to avoid implying estimates beyond the available results.

The Nightingale polar-area chart synthesizes the eco-psychoneuroimmunological gradient observed in the study by placing urban (QC) and rural (NE) profiles on a common health-normalized scale. In the exposure domain, NE shows consistently larger radii for Shannon diversity, Simpson diversity, log10 CFU, and the composite EMDE_z, mirroring the reported rural>urban advantage in microbial richness and healthier exposure contexts. In the immune domain, NE again exhibits larger radii because health normalization flips the direction of inflammatory markers: lower ln(IL-1β) and ln(TNF-α) in NE translate into higher health scores, cohering with the substantial urban>rural elevations (g≈1.5–1.6; geometric-mean ratios ≈1.9× and ≈1.7× for IL-1β and TNF-α, respectively). In the affective domain, NE attains longer radii for PHQ-9 and PANAS-NA (because lower scores are healthier) and for PANAS-PA (where higher is healthier), mapping onto the study's finding that urban participants report greater depressive burden and negative affect and lower positive affect. Read radially, the figure provides a compact, domain-spanning visualization of the hypothesized chain—richer environmental microbial exposure → dampened cytokine activity → more favorable mood phenotypes—and clarifies that the rural profile aligns with the healthier end of this continuum across all three domains. Two interpretive cautions apply. First, while log10 CFU contributes to an overall "richer exposure" signature, total load is not uniformly beneficial (contextual pathogen/moisture risks must be managed); it is included here strictly as part of the exposure gradient captured in the dataset. Second, the chart encodes relative health advantage between sites within each metric rather than absolute concentrations; thus, it is best read alongside the numerical tables (between-group tests, effect sizes, and mediation paths) for inference. Overall, the polar pattern reinforces the mediation result (Shannon→lnTNF→PHQ-9) by visually locating NE at the high-diversity/low-inflammation/healthier-mood pole and QC at the reciprocal pole, in line with the biodiversity hypothesis and the Microbiota–Immune–Brain (MIB) axis in a tropical urbanicity setting.

Interpretation.

The two site cohorts are balanced in size and sex distribution, with no meaningful age differences, supporting comparability at baseline. Body mass index is modestly higher in the urban group (QC), while rural participants report significantly longer residence duration—both consistent with the study's urbanicity gradient. Sleep quality is worse in QC (higher PSQI), and socioeconomic composition differs across sites (QC skewed toward higher SES quartiles; NE toward lower), whereas smoking prevalence and brief diet score are comparable. Taken together, these contrasts justify covariate adjustment for BMI, years of residence, SES quartiles, and PSQI in downstream models (group tests, correlations, mediation). The UrbanicityIndex cleanly separates sites, reinforcing the design's ecological contrast for testing exposure–inflammation–mood pathways.

Table 1. Participant Profile (Urban vs. Rural)

Variable	Overall	QC (Urban)	NE (Rural)	Test / Effect	p-value	Notes
Sample size, n	200	100	100	—	—	Item-level missingness < 2%
Age, years, M (SD)	31.6 (8.3)	32.1 (8.5)	31.1 (8.1)	Welch t-test	> .10 (ns)	—
Female, n (%)	104 (52.0%)	54 (54.0%)	50 (50.0%)	χ^2 test	> .10 (ns)	—
BMI, kg/m², M (SD)	24.3 (3.6)	24.8 (3.5)	23.8 (3.6)	Welch t-test	≈ .045	Slight urban > rural elevation
Years of residence, M (SD)	11.0 (6.6)	9.4 (5.7)	12.6 (7.1)	Welch t-test	≈ .001	Longer residence in rural site
Current smoking, n (%)	50 (25.0%)	28 (28.0%)	22 (22.0%)	χ^2 test	> .10 (ns)	—
Brief diet score, M (SD)†	5.4 (1.7)	5.2 (1.7)	5.5 (1.6)	Welch t-test	> .10 (ns)	Higher = healthier
PSQI—sleep quality, M (SD)‡	6.5 (2.7)	7.1 (2.8)	5.9 (2.5)	Welch t-test	≈ .001	Higher = worse sleep
SES quartile, Q1 (lowest), %	22.0%	12%	32%	χ^2 (overall)	< .001	Site-harmonized quartiles
SES quartile, Q2, %	24.0%	20%	28%	χ^2 (overall)	< .001	—
SES quartile, Q3, %	29.0%	34%	24%	χ^2 (overall)	< .001	—
SES quartile, Q4 (highest), %	25.0%	34%	16%	χ^2 (overall)	< .001	—
UrbanicityIndex (z)	—	> 0 (z)	< 0 (z)	Design check	—	Composite index separates sites

† Higher diet scores indicate healthier diet quality.

‡ Higher PSQI scores indicate poorer sleep quality.

Table 2. Environmental microbial indices by site (urban QC vs. rural NE)

Index (higher = richer exposure)	QC (Urban) M (SD)	NE (Rural) M (SD)	Mean Δ (NE − QC)	95% CI for Δ	Welch t (df*)	p-value	Hedges g
Shannon diversity	2.59 (0.33)	3.27 (0.35)	+0.68	[0.585, 0.775]	−14.30 (≈188)	< .001	−2.01
Simpson diversity	0.83 (0.05)	0.89 (0.04)	+0.06	[0.047, 0.073]	−9.38 (≈191)	< .001	−1.32
log10 CFU (total load)	2.85 (0.31)	3.22 (0.35)	+0.37	[0.278, 0.462]	−7.91 (≈192)	< .001	−1.12
EMDE_z (composite)	−0.48 (0.85)	+0.48 (0.88)	+0.96	[0.719, 1.201]	−7.85 (≈195)	< .001	−1.11

Notes. n = 100 per site (QC, NE). Negative t-values and g reflect higher rural (NE) means (i.e., NE − QC > 0). Welch tests accommodate potential variance heterogeneity; df shown are Satterthwaite approximations. EMDE_z is a standardized composite (higher = healthier/more biodiverse exposure).

Interpretive analysis. Across all exposure metrics, rural Nueva Ecija exhibits substantially richer environmental microbial signatures than urban Quezon City: α-diversity is markedly higher (Shannon Δ = +0.68, g ≈ −2.01; Simpson Δ = +0.06, g ≈ −1.32), culture-based total load is greater (log10 CFU Δ = +0.37, g ≈ −1.12), and the composite EMDE_z strongly favors the rural context (Δ = +0.96, g ≈ −1.11), with all contrasts highly significant ($p < .001$) and confidence intervals that exclude zero. The consistently large effect sizes indicate a robust urban–rural microbial gradient that aligns with the study's biodiversity hypothesis and provides the ecological foundation for the downstream immune activation and mood analyses.

Table 3. Serum pro-inflammatory cytokine profiles by site (Welch t tests; Hedges g)

Biomarker (log-transformed)	QC (Urban) Mean	NE (Rural) Mean	Mean Difference (QC–NE)	t (Welch)	p	Hedges g	Direction	Geometric-Mean Ratio (QC/NE)*
ln(IL-1β)	1.438	0.793	0.645	10.87	< .001	1.53	QC > NE	1.91×
ln(TNF-α)	1.933	1.389	0.544	11.27	< .001	1.59	QC > NE	1.72×

** Fold-change computed as exp(mean difference) to aid interpretation on the original concentration scale. Welch tests were used to accommodate potential variance heterogeneity; values are on the natural-log scale. Hedges g is signed so that positive values indicate QC > NE.*

Analytical notes & interpretation. Urban participants from Quezon City exhibited substantially higher pro-inflammatory signaling than rural participants from Nueva Ecija across both cytokines, with large effects by Hedges g (≈1.5–1.6) and highly significant Welch t statistics. Back-transformation shows approximately 1.9× higher geometric mean IL-1β and 1.7× higher TNF-α in QC relative to NE, indicating a practically meaningful elevation in systemic inflammatory tone associated with urban residence. These disparities are theoretically coherent with the study's eco-immunological pathway—reduced environmental microbial diversity in urban settings leading to heightened immune

activation and, in turn, greater mood dysregulation—and they anticipate the significant indirect (mediated) pathway identified in the Shannon → lnTNF-α → PHQ-9 model elsewhere in the manuscript.

Table 4. Key-path bivariate correlations among environmental microbial exposure, inflammatory cytokines, and affective outcomes (Pearson's r, two-tailed, n = 200).

Variable pair	r	p
Shannon diversity — ln(IL-1β)	−0.719	< .001***
Shannon diversity — ln(TNF-α)	−0.665	< .001***
EMDE_z (healthier exposure ↑) — ln(IL-1β)	−0.840	< .001***
EMDE_z — ln(TNF-α)	−0.809	< .001***
ln(IL-1β) — PHQ-9 total	0.703	< .001***
ln(IL-1β) — PANAS-NA	0.767	< .001***
ln(IL-1β) — PANAS-PA	−0.524	< .001***
ln(TNF-α) — PHQ-9 total	0.776	< .001***
ln(TNF-α) — PANAS-NA	0.785	< .001***
ln(TNF-α) — PANAS-PA	−0.487	< .001***

Notes. p < .001; all tests two-tailed (α = .05). Negative coefficients indicate that higher diversity/healthier exposure is associated with lower inflammation or higher positive affect.

Interpretive commentary (academic).

The correlation matrix delineates a coherent exposure → inflammation → affect pathway of large magnitude. Environmental microbial richness—indexed by Shannon and the composite EMDE_z—shows strong inverse links with pro-inflammatory tone (r ≈ −.66 to −.84), with the largest effects observed for EMDE_z versus ln(IL-1β) and ln(TNF-α). Downstream, both cytokines correlate positively with depressive symptoms and negative affect (r ≈ .70–.79) and inversely with positive affect (r ≈ −.49 to −.52). Collectively, these patterns satisfy the statistical preconditions for mediation, align with the Microbiota–Immune–Brain (MIB) framework, and reinforce the urban–rural contrasts reported elsewhere in the Results: healthier, biodiversity-consistent exposure profiles co-occur with attenuated inflammatory activity and a more favorable affective profile. The effect sizes (mid-to-large by conventional benchmarks) imply not only statistical but also substantive relevance for population mental health in a tropical urbanicity context.

Table 5. Mediation path coefficients for the Shannon → lnTNF-α → PHQ-9 model (PROCESS Model 4; OLS with HC3; covariates: age, BMI, SES quartiles, PSQI, current smoking; n = 200)

Effect	Path definition	Unstd. β	95% CI (HC3 / bootstrap)	Significance	Notes
a	Shannon → lnTNF-α	−0.512	Not reported (HC3)	p < .001	Covariate-adjusted OLS with HC3 robust SEs.
b	lnTNF-α → PHQ-9 | Shannon	4.618	Not reported (HC3)	p < .001	Conditional on Shannon and covariates; HC3 robust SEs.
c′	Shannon → PHQ-9 (direct | M)	−0.516	Not reported (HC3)	p < .001	Direct effect with mediator in model (partial mediation).
a×b	Indirect (via lnTNF-α)	−2.366	[−2.945, −1.800] (bias-corrected bootstrap, B = 1,000)	CI excludes 0 → significant	Product-of-coefficients; percentile bias-corrected CI.
c	Total (c′ + a×b)	−2.882	Not reported (HC3)	Not reported	Derived as c = c′ + (a×b); proportion mediated ≈ 0.821.

Abbreviations: Unstd. β = unstandardized coefficient; CI = confidence interval; HC3 = heteroskedasticity-consistent (MacKinnon–White) SEs, type 3; "| M" = conditional on the mediator; B = number of bootstraps resamples.

Interpretive notes: The negative a path (β = −0.512) indicates that greater environmental microbial diversity is associated with lower TNF-α activity, while the positive b path (β = 4.618) shows that higher TNF-α corresponds to higher depressive symptoms (PHQ-9), adjusting for covariates; both paths are highly significant under HC3-robust OLS. The indirect effect (a×b = −2.366; 95% bias-corrected bootstrap CI [−2.945, −1.800]) does not include zero, establishing a statistically reliable mediation through TNF-α. The direct effect c′ remains protective (β = −0.516, p < .001), indicating partial mediation—consistent with additional non-immune pathways. The total effect c is −2.882, implying that approximately 82% of the overall association is transmitted through TNF-α (|a×b|/|c| ≈ 0.821).

References

[1] Haahtela T, Holgate S, Pawankar R, et al. The biodiversity hypothesis and allergic disease. World Allergy Organ J. 2013;6:3.

[2] Rook GAW. Regulation of the immune system by biodiversity from the natural environment. Proc Nutr Soc. 2013;72(4):497–504.

[3] Hanski I, von Hertzen L, Fyhrquist N, et al. Environmental biodiversity, human microbiota, and allergy are interrelated. PNAS. 2012;109(21):8334–8339.

[4] Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression. Nat Rev Neurosci. 2008;9(1):46–56.

[5] Miller AH, Raison CL. The role of inflammation in depression. Nat Rev Immunol. 2016;16(1):22–34.

[6] Kroenke K, Spitzer RL, Williams JBW. The PHQ-9: validity of a brief depression severity measure. J Gen Intern Med. 2001;16(9):606–613. Watson D, Clark LA, Tellegen A. Development and validation of PANAS. J Pers Soc Psychol. 1988;54(6):1063–1070.

[7] Hayes AF. Introduction to Mediation, Moderation, and Conditional Process Analysis. 2nd ed. Guilford Press; 2018.

[8] Twohig-Bennett C, Jones A. The health benefits of the great outdoors: a systematic review and meta-analysis of greenspace exposure and health outcomes. Environ Res. 2018;166:628–637.