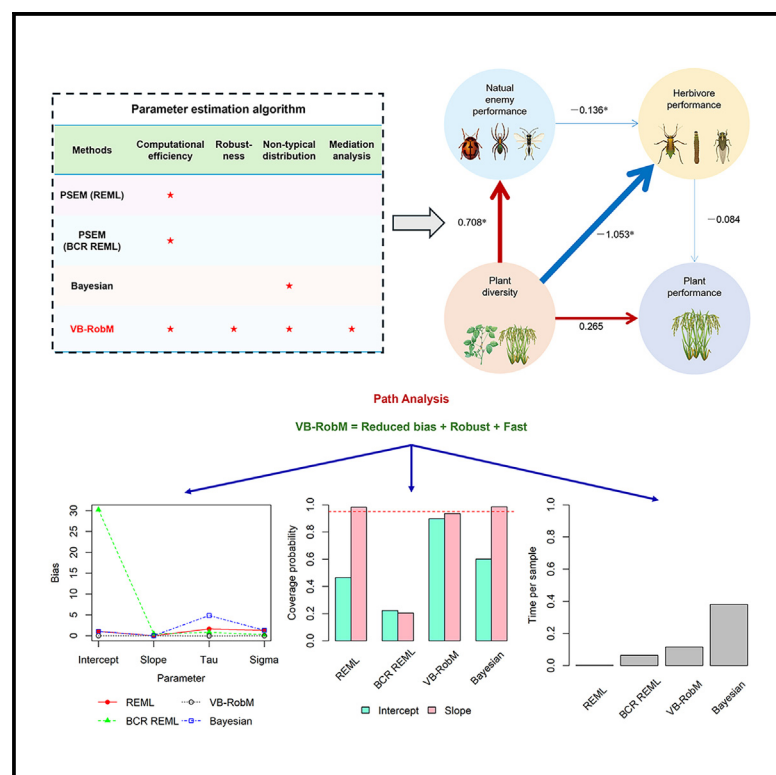


Understanding biodiversity effects on trophic interactions with a robust approach to path analysis

Graphical abstract



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In brief

This paper introduces a novel estimator for path analysis that significantly improves computational efficiency compared with traditional multivariate Bayesian methods. The proposed estimator reduces the time required for estimation while maintaining accuracy. Simulation studies on the effects of plant diversity on ecological trophic interactions demonstrate that it is both unbiased and more robust. This research has broad implications for path analysis across various fields, including natural, medical, and social sciences.

Highlights

- Path analysis is a useful tool to quantify associations among variables
- Frequentist statistical methods for path analysis remain constrained by biased estimates
- We present an efficient estimator to test biodiversity effects on trophic interactions
- Our method is robust while reducing computational time cost for the estimating procedure



Article

Understanding biodiversity effects on trophic interactions with a robust approach to path analysis

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SCIENCE FOR SOCIETY Path analysis is a useful method that helps researchers understand how multiple factors are connected and how they influence each other. However, the increasing volume of data and the complexity of interactions among factors necessitate the development of high-accuracy and high-efficiency statistical methods to enhance our understanding of biological processes and social complexity. Here, we present a benchmark study using simulations and apply the proposed method to investigate biodiversity effects on trophic interactions in ecosystems.

SUMMARY

With its facility to assess causal mechanisms among multiple variables, the application of path analysis in medical, natural, and social sciences has become widespread. Of the many types of path analysis, structural equation modeling (SEM), including Bayesian applications of this method, has gained popularity. However, SEM remains constrained by biased estimates in the case of model misspecification, while Bayesian methods are limited by time consumption and computational requirements. Here, we propose a novel estimator utilizing robust estimating equations combined within a Bayesian framework to improve multilevel path analysis. We apply this method to an ecological trophic interaction case study that assessed the path effects of global plant diversity on the interactions of plants, invertebrate herbivores, and their natural enemies. Using a simulation study, we show that this new estimator is unbiased and more robust. Moreover, the computational time cost for the estimating procedure is reduced compared with multivariate Bayesian analysis.

INTRODUCTION

Path analysis provides insights into direct and indirect pathways between explanatory and outcome variables, allowing a window into what once used to be a “black box” in terms of causal chains of biological or social pathways.¹ High-accuracy and high-efficiency path analysis can facilitate the understanding of biological processes and social complexity. In the past decades, path analysis has become increasingly popular in the natural,^{2,3} medical,^{4,5} and social sciences.⁶ Of the various approaches to path analysis, piecewise structural equation modeling (PSEM)^{7,8} and its implementation within a Bayesian framework^{9–11} has become particularly popular. Typically, SEM

methods use maximum likelihood (ML) to estimate parameters, which can be severely biased in case of poorly specified models.¹² Bayesian methods can achieve more robust estimation using weakly informative priors. However, they may also be biased when the true distribution of the data violates the assumptions of the model; additionally, the Monte Carlo Markov chain sampling algorithm, which is used to obtain converged posterior distributions for parameters, is often time intensive.¹³

Robust estimating equations have been developed to deal with apparent outliers in the data. This type of estimator assesses the influence of outlying observations by substituting the squared loss of residuals with robust loss functions, allowing a reduced weighting to be applied to abnormal observations.¹⁴ The application of



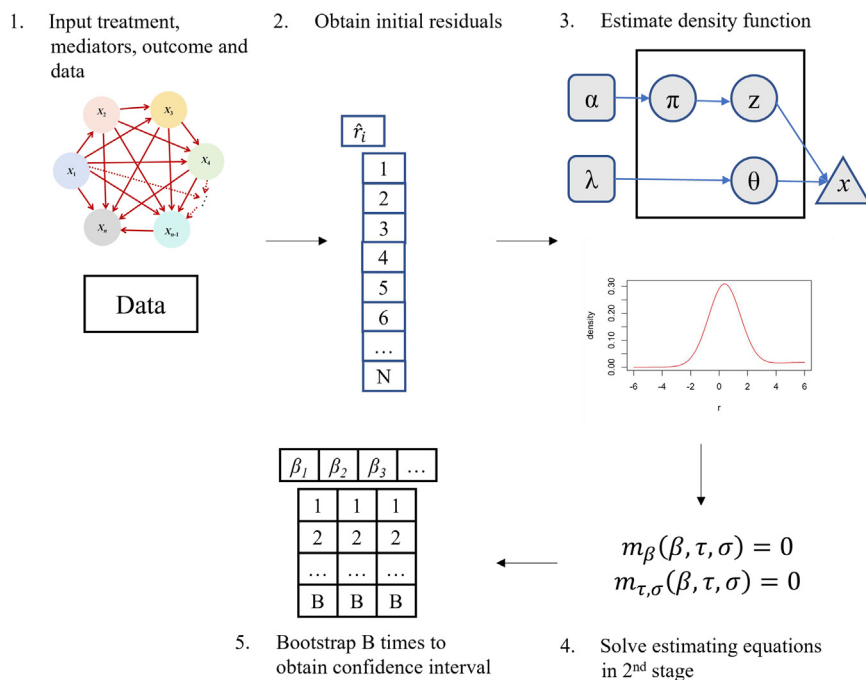


Figure 1. Overview of VB-RobM method

In step 1, VB-RobM specifies the relationships among the variables. In step 2, VB-RobM obtains initial estimates and residuals by robust estimating equations according to the specified relationships. In step 3, VB-RobM estimates the density function for the initial residuals by a Dirichlet process mixture model. The rounded rectangles in step 3 represent hyperparameters for the prior, the circles represent latent variables, and the triangle represents the observations, respectively. In step 4, VB-RobM debiases the initial estimates by integrating the density estimation into the estimating equations. In step 5, VB-RobM obtains confidence intervals for direct, indirect, and total effects by bootstrapping.

supplemental information. Briefly, and as shown in Figure 1, our proposed VB-RobM method consists of five steps: (1) data input and specification of the paths between the variables, (2) obtaining initial estimates of path coefficients and robust residuals for each edge, (3) estimating the density function of the residuals by a Dirichlet process mixture model (DPMM) and the first and second moments of the robust function, (4) obtaining the second stage robust estimation of the path coefficients, and (5) making statistical inferences about the direct, indirect, and total effect of treatments on outcomes by bootstrapping. The fixed-point and coordinate descent algorithm was applied to solve the robust estimating equations with greater stability.¹⁸

robust estimating equations provides a natural progression to improving SEM methods that may also control for the time cost associated with replacing the ML estimations with robust estimation. However, symmetric error terms are a key assumption for robust estimations to be unbiased.¹⁵ Bias-corrected robust estimators based on the linear approximation of the robust estimator have been proposed in generalized estimating equations.^{15,16} These estimators are mainly one-step approximations to the unbiased estimator by expanding the estimating equations and usually depend on the accuracy of correction terms. If the estimation of the correction term is biased, the corrected estimator is also biased.¹⁶ Therefore, there is a need for methods that integrate the advantages of both robust ML estimation and Bayesian methods to address their weaknesses.

In this paper, we develop a novel bias-corrected robust method, Variational Bayesian Robust mediation analysis (abbreviated as “VB-RobM”—see details below). Combined with VB approaches,¹⁷ which approximate the posterior distributions in Bayesian inference using specific distribution families, VB-RobM can obtain a more robust estimate at reduced computational time. We undertake simulation studies to compare VB-RobM with various multilevel analysis methods. We demonstrate this approach using a case study example to illustrate the application of VB-RobM in estimating plant diversity effects on bi-trophic interactions between plants and invertebrate herbivores using a global dataset, expanding this also to tri-trophic interactions that include herbivore natural enemies.

RESULTS

Overview of VB-RobM

The details of the statistical models and algorithms used by VB-RobM can be found in the [methods](#) section and in the

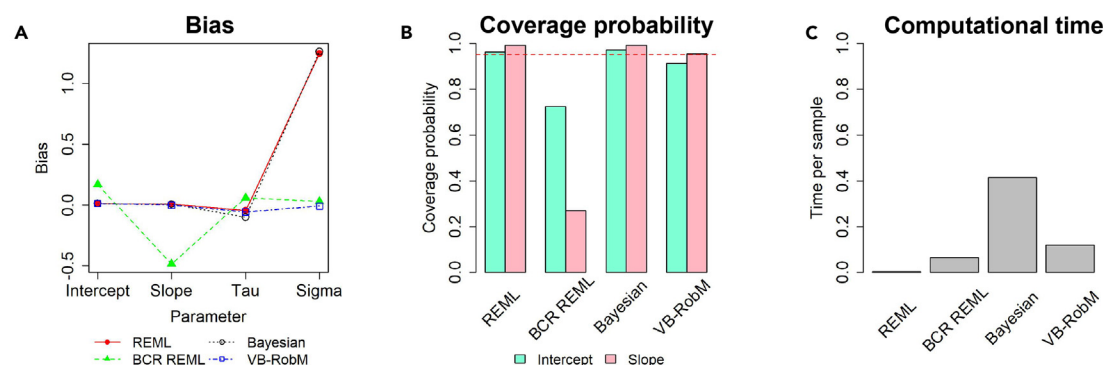
robust estimating equations provides a natural progression to improving SEM methods that may also control for the time cost associated with replacing the ML estimations with robust estimation. However, symmetric error terms are a key assumption for robust estimations to be unbiased.¹⁵ Bias-corrected robust estimators based on the linear approximation of the robust estimator have been proposed in generalized estimating equations.^{15,16} These estimators are mainly one-step approximations to the unbiased estimator by expanding the estimating equations and usually depend on the accuracy of correction terms. If the estimation of the correction term is biased, the corrected estimator is also biased.¹⁶ Therefore, there is a need for methods that integrate the advantages of both robust ML estimation and Bayesian methods to address their weaknesses.

Simulation performance on contaminated datasets

Figure 2 shows the benchmark results of VB-RobM alongside other existing methods, including the restricted maximum likelihood estimator (REML, used by PSEM), bias-corrected robust REML (BCR REML¹⁶), and the multivariate Bayesian estimator (Bayesian). The simulation datasets were generated based on a two-level linear mixed model (LMM) as described in [Equation 12](#). An important question is how these approaches deal with data distributions that do not neatly fit into those widely used in statistical analysis. While this is not always a problem, real-world datasets commonly encounter such issues. Here, we generate “contaminated” datasets that have been deliberately constructed so as not to conform neatly with standard statistical distributions as a means of testing the robustness of parameter estimation between the different methods. Contaminated datasets were generated using a mixture distribution combining a normal and a χ^2 distribution in a 4:1 ratio as an example. Additional simulation results for other mixture distributions with the same 4:1 ratio are provided in [Figures S2 and S3](#). We compared statistical performance under two cases: centered errors, where the mean of the errors is zero (top row of [Figure 2](#)), and uncentered errors (bottom row of [Figure 2](#)).

Overall, VB-RobM proved to be the most accurate and efficient method in both cases, while REML and Bayesian performed reasonably well but were slightly less precise in the

Centered error



Uncentered error

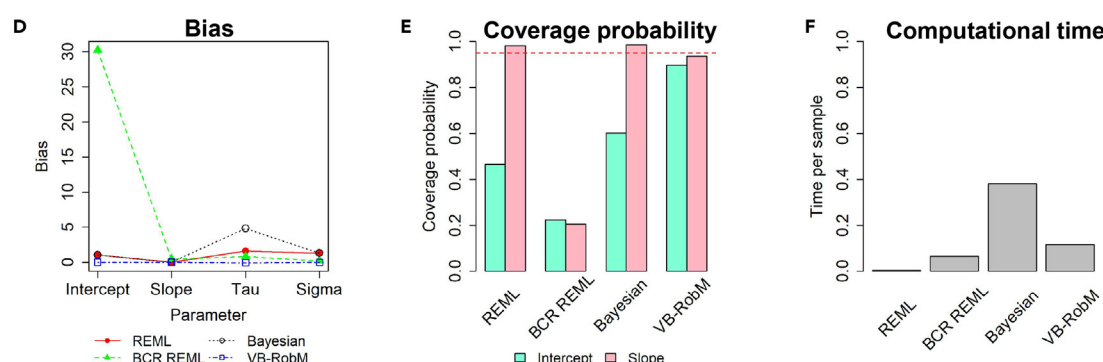


Figure 2. Simulation (or benchmark) results of REML, BCR REML, Bayesian, and VB-RobM under normal- χ^2 mixture model errors

The top row (A–C) and bottom row (D–F) display the bias, CP, and average computational time cost per sample (in seconds) for the four methods under centered and uncentered model errors, respectively. In (B) and (E), the red dashed line indicates the nominal 95% CP, representing the expected level that the methods should achieve. The detailed results are listed in [Tables S1](#) and [S2](#).

case of uncentered errors. BCR REML showed substantial drawbacks in terms of bias and coverage probability (CP). These findings highlight the advantages of VB-RobM over the other three methods, particularly when the normality assumption is violated, in path analysis.

Bias measures the accuracy of the estimator, with a bias of zero indicating that the estimator, on average, provides correct point estimates. In both cases, as shown in [Figures 2A](#) and [2D](#), VB-RobM demonstrates lower biases compared with the other methods, which are nearly zero, for the parameters: intercept (β_0 in [Equation 12](#)), slope (β_1 in [Equation 12](#)), tau (standard deviation of the random effect b_{ij}), and sigma (standard deviation of the error terms ε_{ij}). The biases for the four parameters, for centered and uncentered errors were (0.013, 0.000, -0.058 , and -0.009) and (0.006, -0.000 , -0.065 , and -0.009), respectively (see [Tables S1](#) and [S2](#)). Moreover, REML, BCR REML, and Bayesian exhibited greater bias under uncentered errors, with biases of (1.036, 0.001, 1.616, and 1.284), (30.219, 0.443, 0.787, and 0.213) and (1.033, 0.001, 4.841, and 1.301), respectively, when compared with the centered error case. The comparison of bias reveals that VB-RobM is more accurate and robust than the other three methods, especially in the case of uncentered model errors.

CP refers to the proportion of confidence intervals that contain the true parameter value across repeatedly simulated datasets. A CP close to the nominal 95% typically indicates an accurate estimation of the standard error. Here, we focus only on the CPs of the intercept and slope estimates, as these two parameters represent the path effects in path analysis. [Figures 2B](#) and [2E](#) illustrate that the CP of VB-RobM approaches the nominal 95%, with values of (91.2% and 95.4%) for centered errors and (89.8% and 93.6%) for uncentered errors (see [Tables S1](#) and [S2](#)). By contrast, REML and Bayesian exhibited low CPs for the intercept under uncentered errors, respectively, at 46.6% and 60.2%. BCR REML performed the worst, with CPs consistently falling below the nominal level across both error cases, suggesting severe underestimation of the standard error. This would therefore fail to support robust hypothesis testing.

Finally, the average time cost per sample (in seconds) was recorded in simulation studies to evaluate the computational efficiency of the methods. [Figures 2C](#) and [2E](#) indicate that REML is the most efficient, with a computation time of 0.003 s per sample for both centered and uncentered errors. Due to the additional debiasing procedure, VB-RobM requires slightly more time than REML, taking 0.120 s per sample for centered errors

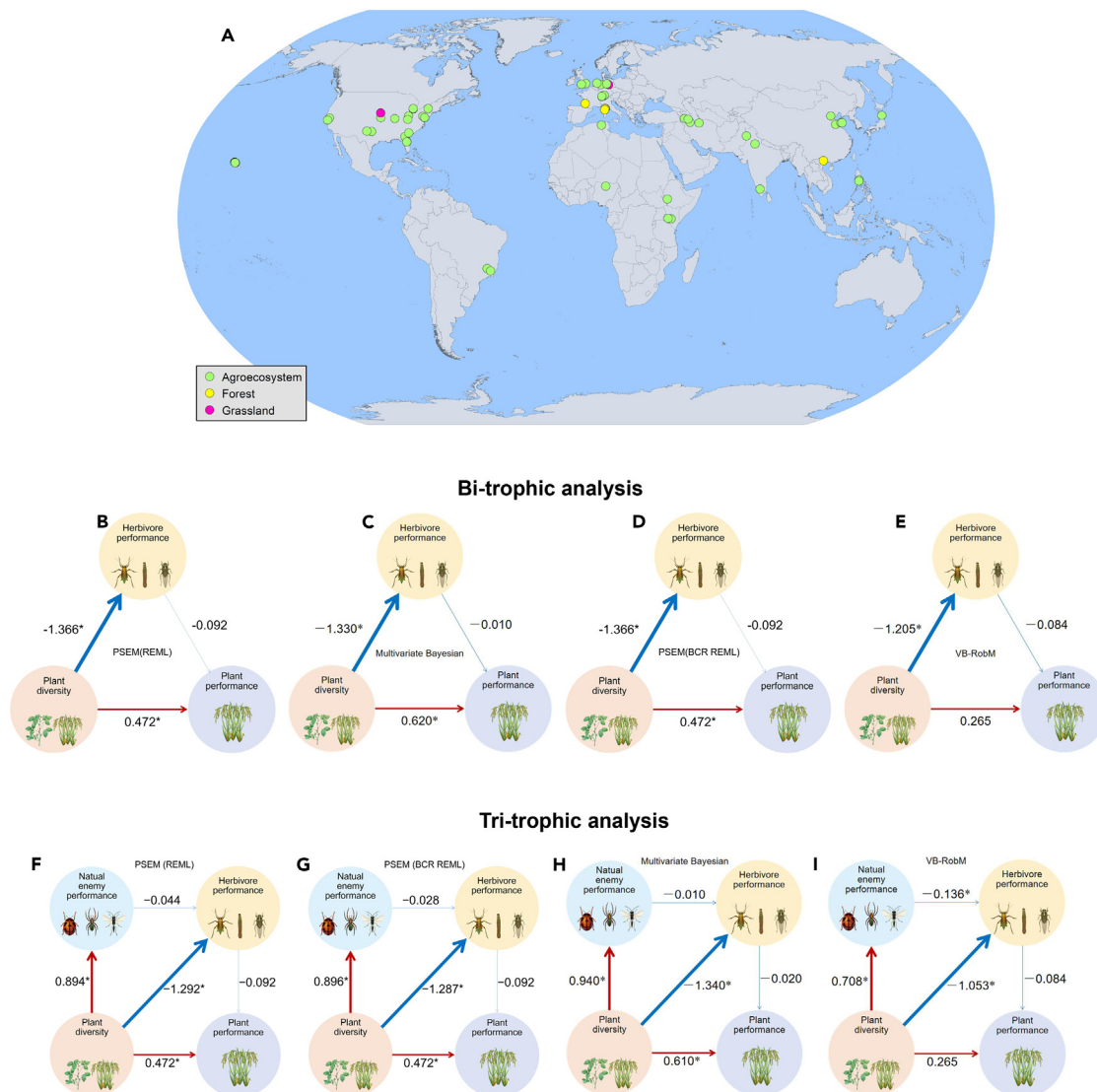


Figure 3. A real data analysis (i.e., global biodiversity experiments in terrestrial ecosystems) to compare the bi-trophic interactions with PSEM (REML), PSEM (BCR REML), Bayesian, and VB-RobM
(A) Locations of 68 global experiments (from 57 articles with 262 paired observations).
(B–E) The results of bi-trophic analyses to test the effects of plant species diversity on the interactions of invertebrate herbivores, and plants, for the four methods.
(F–I) The results of tri-trophic analyses to test the effects of plant species diversity on the interactions of natural enemies, invertebrate herbivores, and plants, for the four methods. Plant performance included the growth, reproduction, and quality of plants, invertebrate herbivore performance included herbivore abundance and damage, and natural enemy performance included the abundance of predators, abundance of parasitoids, predation and parasitism (see [supplemental methods](#)). “***” denotes significance ($p < 0.05$). The red and blue arrows denote positive and negative relationships, respectively, the numbers next to each arrow are the estimates for path effects of the edges, and line width is proportional to the magnitude of the estimates (Tables S3–S7).

and 0.116 s for uncentered errors. Conversely, Bayesian methods are the least efficient, primarily due to the computationally intensive Markov chain Monte Carlo (MCMC) algorithm, requiring 0.416 s per sample for centered errors and 0.382 s for uncentered errors. The comparison of computational efficiency demonstrates that VB-RobM raises accuracy and robustness with reasonable computational demands, making it a preferable choice for path analysis, particularly in situations involving large sample sizes.

Application to a global dataset: The effect of plant diversity on trophic interactions

We applied our method to an ecological dataset to assess the path effects of plant diversity on bi-trophic and tri-trophic interactions among plants, herbivores, and their natural enemies. This dataset, which includes 262 observations from 57 articles, is geographically shown on the world map (Figure 3A).

Figures 3B–3I show the results of bi-trophic (plants and invertebrate herbivores) and tri-trophic (plants, invertebrate herbivores,

and natural enemies) interaction analyses, respectively, using PSEM (REML), PSEM (BCR REML), multivariate Bayesian, and VB-RobM methods. All methods consistently indicated that increasing plant species diversity enhances the performance of natural enemies (respectively effects of 0.894, 0.896, 0.940, and 0.708 for the four methods) and plants (0.472, 0.472, 0.610, and 0.265), while reducing herbivore performance (−1.292, −1.287, −1.340, and −1.053). Additionally, improved natural enemy performance suppresses herbivore performance (−0.044, −0.028, −0.010, and −0.136), which damages plant performance (−0.092, −0.092, −0.020, and −0.084). Detailed information is provided in [Tables S3–S6](#). Similar relationships have been observed in other large-scale meta-analyses,^{2,3} strengthening the credibility of these results.

While the overall relationships predicted by the different approaches were almost identical, VB-RobM yielded results that differed from other methods with respect to statistical significance. While much can be said about the interpretation of significance and its meaning, it remains a key criterion for determining whether observed path effects are due to chance or reflect true underlying relationships in most studies. In summary, these differences appeared in the relationships between natural enemy performance and herbivore performance, as well as plant species diversity and plant performance. For the path effect of natural enemy performance on herbivore performance, only VB-RobM identified a statistically significant result, with a *p* value of less than 0.001 (5.623×10^{-6}). In contrast, the other estimators suggested that these relationships were non-significant (PSEM [REML], *p* = 0.181; PSEM [BCR REML] *p* = 0.365) or for the Bayesian method had confidence intervals overlapping zero (−0.086–0.055). Conversely, for the path effect of plant species diversity on plant performance, VB-RobM was the only method to report a non-significant result, with a *p* value of 0.127 (slightly greater than 0.05). The corresponding *p* values for PSEM (REML) and PSEM (BCR REML) were 0.003 and 0.008, while the multivariate Bayesian method yielded a confidence interval of 0.247 to 0.967. To explain the different results among these methods, we provided diagnostic plots for PSEM (REML), PSEM (BCR REML), Bayesian, and VB-RobM in [Figures S4](#) and [S5](#). The scatter residual points of PSEM (REML), PSEM (BCR REML), and Bayesian methods were either floating above or sinking below the expected line, suggesting potential deviations from normal distribution. Such deviations suggest that the normality assumption underlying these models may not hold, which could lead to biased estimates.

Lastly, as mentioned earlier, VB-RobM can also infer the direct, indirect and total effects of plant species diversity on plant performance, shedding light on the pathways through which the former affects the latter. Briefly, plant species diversity enhances plant performance both directly and indirectly through the pathways: plant species diversity → natural enemy → herbivore → plant performance (path 1) and plant species diversity → herbivore → plant performance (path 2). Statistically, as shown in [Table S7](#), none of the effects were significant, as their confidence intervals included zero: direct effect (−0.068–0.612), path 1 indirect effect (−0.002–0.022), and path 2 indirect effect (−0.020–0.206). However, the total effect, defined as the sum of the direct and indirect effects, was significant with a confidence interval

0.052 to 0.659. In summary, these results provide weak evidence that plant species diversity affects plant performance through herbivore control, while strongly supporting its influence via multiple pathways.

DISCUSSION

In this study, we presented a robust and efficient statistical method to reduce the influence of heterogeneity in path and mediation analysis. Benchmark results indicate that VB-RobM consistently outperforms other methods in terms of bias, CP and time efficiency when handling data with unusual distributions or anomalies, i.e., our contaminated datasets ([Figure 2](#)). Application in trophic analysis demonstrates that VB-RobM is more accurate and robust when the normality assumption of the data is violated (see [Figures S4](#) and [S5](#)).

Previous studies have shown that both LMM and Bayesian approaches lack robustness when the Gaussian distribution assumption for model errors is violated.^{19,20} A notable strength of VB-RobM lies in its capability to handle both centered and uncentered errors, an issue often overlooked in path analysis. Estimating path effects without relying on distributional assumptions is particularly challenging, especially in case of uncentered errors. VB-RobM overcomes these limitations by integrating a robust estimation framework ([Equations 6–8](#)) and leveraging a DPMM ([Equations 9–10](#)) to correct deviations from normality effectively. Another advantage of VB-RobM is its use of variational Bayesian approximation instead of the commonly employed MCMC algorithm for posterior estimation. This substitution substantially reduces computational time ([Figures 2C](#) and [2F](#)).

Despite VB-RobM surpassing the other three methods in the case study, it faces difficulties in specific situations. Simulations with mixture distributions in a 4:1 ratio revealed that the smallest proportion of contamination tolerable by VB-RobM was 20%. To enhance its robustness to higher contamination levels, incorporating an S-estimator into VB-RobM could be a viable strategy.²¹ However, this approach requires equal replication across all studies, a constraint that may limit its practical applicability. Future work could explore integrating the S-estimator into VB-RobM to enhance its robustness and flexibility. Additionally, extending the applied LMM ([Equation 2](#)) to accommodate hierarchical structures with more than two levels could broaden its applicability in complex data.

In conclusion, VB-RobM provides a significant advancement in path analysis, improving accuracy and robustness while maintaining computational efficiency. The method is well suited for ecological research as well as other domains that require path analysis to investigate complex relationships across multiple trophic levels.

METHODS

Literature sources and selection criteria

To compare those different methods mentioned in this study in analyzing biodiversity effects on ecological trophic interactions, we first establish a global dataset (i.e., the effect of plant diversity

on trophic interactions). Studies were selected through a literature search of the Web of Science Core Collection, BIOSIS Previews, Derwent Innovations Index, KCI-Korean Journal Database, MEDLINE, Preprint Citation Index, ProQuest™ Dissertations & Theses Citation Index, and SciELO Citation Index. We used the Boolean search string based on the “TOPIC” searching: [“plant diversity” OR “crop diversity” OR “crop diversification” OR “plant species richness” OR “ground cover vegetation” OR “flower strip” OR “intercrop” OR “interplant”] AND [“herbivor” OR “pest”] AND [“predat” OR “parasit” OR “wasp” OR “natural enem”] AND [“yield” OR “biomass” OR “productivity”]. This literature search was initiated in September 2021 and finalized in late November 2023. The search yielded 196,921 articles (see Figure S1 for a PRISMA diagram), which were screened based on their titles and abstracts. This was done to determine whether the articles measured a response variable relating to the effects of plant species diversity on (1) invertebrate herbivore performance (herbivore abundance and damage); (2) natural enemy performance (the abundance, diversity and parasitism of parasitic wasps, and the abundance, diversity, and predation of predators); and (3) plant performance (the growth, reproduction, and quality of plants).³ In total, 195,939 articles were excluded at this stage. From the remaining 982 papers, we obtained our final selection of 57 articles based on the following criteria for data inclusion: (1) means, standard errors (or standard deviations), and sample sizes could be extracted from tables, figures, text, or [supplemental information](#); (2) the measurements of the treatment (higher plant species diversity, ≥ 2 plant species) and control (single or lowest plant species richness) groups were conducted at the same spatiotemporal scale; (3) tri-trophic groups of plants, invertebrate herbivores and natural enemies were monitored in the same location; and (4) the study included a treatment that increased the number of plant species; (5) any other agricultural practice (e.g., pesticides and fertilizer input) should be the same for the control (single/lowest plant species) and the treatment (diverse plant species).^{2,3} In total, 57 articles with 262 observations were used to test the effects of plant diversity on bi-trophic and tri-trophic interactions of plants, herbivores, and their natural enemies. Measures of plant performance, invertebrate herbivore performance, and natural enemy performance were recorded from these studies.

Two-level LMMs and robust REML

We employ two-level LMMs to acquire the initial estimates for the submodels depicted in Figure 1A. Formally, let $X_{ij,k}$ denotes the j th observation in the i th study for variable X_k , $1 \leq i \leq n$, $1 \leq j \leq n_i$, $1 \leq k \leq K$. For example, we can use $X_{ij,1}$, $X_{ij,2}$, $X_{ij,3}$, $X_{ij,4}$ to denote the plant diversity, natural enemy performance, herbivore performance, and plant performance in Figure 3. In this case: $n = 262$ is the total number of measurements collected in our study, n_i is the number of measurements in i th study, and $K = 4$ is the number of trophic levels. We assume $X_{ij,k}$ and its ancestors or causals $X_{ij,1}$, $X_{ij,2}$, $X_{ij,3}$, $X_{ij,k-1}$ (e.g., the plant diversity is the ancestor of the natural enemy performance, and the herbivore performance has two ancestors, the plant diversity and the natural enemy performance) and that these satisfy the following LMMs:

$$X_{ij,k} = \sum_{l=1}^{k-1} X_{ij,l} \beta_{l,k} + b_{i,k} + \varepsilon_{ij,k}, \quad (\text{Equation 1})$$

where $\beta_{l,k}$ are the coefficients associated with the ancestor variables, $b_{i,k} \sim \mathcal{N}(0, \tau_k^2)$ are the random effects which explain the between-study heterogeneity and $\varepsilon_{ij,k} \sim \mathcal{N}(0, \nu_k^2 \sigma_{ij,k}^2)$ are the error terms with known sampling variances and unknown observation-level heterogeneity. For simplicity, we reformulate model (3) in matrix form below:

$$\mathbf{X}_k = \mathbf{M}_k \boldsymbol{\beta}_k + \mathbf{Z}_k \mathbf{b}_k + \boldsymbol{\varepsilon}_k, \quad (\text{Equation 2})$$

where $\mathbf{X}_k = (\mathbf{X}_{1,k}^T, \mathbf{X}_{2,k}^T, \dots, \mathbf{X}_{n,k}^T)^T$, $\mathbf{M}_k = (\mathbf{X}_1, \mathbf{X}_2, \dots, \mathbf{X}_k)$, $\boldsymbol{\beta}_k = (\beta_{1,k}, \beta_{2,k}, \dots, \beta_{k-1,k})^T$, $\mathbf{b}_k = (b_{1,k}, b_{2,k}, \dots, b_{n,k})^T$, $\boldsymbol{\varepsilon}_k = (\boldsymbol{\varepsilon}_{1,k}^T, \boldsymbol{\varepsilon}_{2,k}^T, \dots, \boldsymbol{\varepsilon}_{n,k}^T)^T$ and $\mathbf{X}_{i,k} = (X_{i1,k}, X_{i2,k}, \dots, X_{in_i,k})^T$, $\boldsymbol{\varepsilon}_{i,k} = (\varepsilon_{i1,k}, \varepsilon_{i2,k}, \dots, \varepsilon_{in_i,k})^T$, $\mathbf{Z}_k$ is the block-diagonal design matrix determined by the clusters (in this study, clusters are the article numbers) of the observations. A general method to estimate the coefficients and variance components in (2) is REML, which takes account of the loss in degrees of freedom that results from estimating $\boldsymbol{\beta}_k$.²² To reduce heterogeneity within the studies, a robust REML estimator was proposed by modifying the estimating equations of REML with a ψ -function¹⁸:

$$\mathbf{M}_k^T \mathbf{V}_k^{-\frac{1}{2}} \boldsymbol{\psi} \left(\mathbf{V}_k^{-\frac{1}{2}} (\mathbf{X}_k - \mathbf{M}_k \boldsymbol{\beta}_k) \right) = \mathbf{0}, \quad (\text{Equation 3})$$

$$\begin{aligned} & \boldsymbol{\psi} \left(\mathbf{V}_k^{-\frac{1}{2}} (\mathbf{X}_k - \mathbf{M}_k \boldsymbol{\beta}_k) \right)^T \mathbf{V}_k^{-\frac{1}{2}} \mathbf{Z}_k \mathbf{Z}_k^T \mathbf{V}_k^{-\frac{1}{2}} \boldsymbol{\psi} \left(\mathbf{V}_k^{-\frac{1}{2}} (\mathbf{X}_k - \mathbf{M}_k \boldsymbol{\beta}_k) \right) \\ & - \text{tr}(\delta \mathbf{P}_k \mathbf{Z}_k \mathbf{Z}_k^T) = 0, \end{aligned} \quad (\text{Equation 4})$$

$$\begin{aligned} & \boldsymbol{\psi} \left(\mathbf{V}_k^{-\frac{1}{2}} (\mathbf{X}_k - \mathbf{M}_k \boldsymbol{\beta}_k) \right)^T \mathbf{V}_k^{-\frac{1}{2}} \hat{\boldsymbol{\Sigma}}_k \mathbf{V}_k^{-\frac{1}{2}} \boldsymbol{\psi} \left(\mathbf{V}_k^{-\frac{1}{2}} (\mathbf{X}_k - \mathbf{M}_k \boldsymbol{\beta}_k) \right) \\ & - \text{tr}(\delta \mathbf{P}_k \hat{\boldsymbol{\Sigma}}_k) = 0, \end{aligned} \quad (\text{Equation 5})$$

where $\delta = E[\psi(e)^2]$, $e \sim \mathcal{N}(0, 1)$, $\mathbf{V}_k = \tau_k^2 \mathbf{Z}_k \mathbf{Z}_k^T + \nu_k^2 \hat{\boldsymbol{\Sigma}}_k$, $\mathbf{P}_k = \mathbf{V}_k^{-1} - \mathbf{V}_k^{-1} \mathbf{M}_k (\mathbf{M}_k^T \mathbf{V}_k^{-1} \mathbf{M}_k)^{-1} \mathbf{M}_k^T \mathbf{V}_k^{-1}$ and $\hat{\boldsymbol{\Sigma}}_k$ is a diagonal matrix whose elements are the corresponding sampling variances $\hat{\sigma}_{ij,k}^2$. The choice of ψ -function is rather loose, we use Tukey ψ -function and fix tuning parameter at 4.685 for location estimation and 5.12 for scale estimation in this study referring to the settings of Richardson and Welsh.¹⁸ These settings could achieve 95% efficiency and 90% efficiency compared with ML estimators for location and scale parameters, respectively.

VB robust estimator

The key assumption for obtaining unbiased estimators for $\boldsymbol{\beta}_k$, τ_k^2 and ν_k^2 is the normality of the scaled residuals

$\mathbf{V}_k^{-\frac{1}{2}}(\mathbf{X}_k - \mathbf{M}_k\beta_k)$.¹⁶ To overcome the normality limitation, we modified the estimating equation as

$$\mathbf{M}_k^T \mathbf{V}_k^{-\frac{1}{2}} \psi \left(\mathbf{V}_k^{-\frac{1}{2}} (\mathbf{X}_k - \mathbf{M}_k \beta_k) \right) = \mathbf{M}_k^T \mathbf{V}_k^{-\frac{1}{2}} \mu_k, \quad (\text{Equation 6})$$

$$\begin{aligned} & \psi \left(\mathbf{V}_k^{-\frac{1}{2}} (\mathbf{X}_k - \mathbf{M}_k \beta_k) \right)^T \mathbf{V}_k^{-\frac{1}{2}} \mathbf{Z}_k \mathbf{Z}_k^T \mathbf{V}_k^{-\frac{1}{2}} \psi \left(\mathbf{V}_k^{-\frac{1}{2}} (\mathbf{X}_k - \mathbf{M}_k \beta_k) \right) \\ & - \text{tr}(\eta_k \mathbf{P}_k \mathbf{Z}_k \mathbf{Z}_k^T) = 0, \end{aligned} \quad (\text{Equation 7})$$

$$\begin{aligned} & \psi \left(\mathbf{V}_k^{-\frac{1}{2}} (\mathbf{X}_k - \mathbf{M}_k \beta_k) \right)^T \mathbf{V}_k^{-\frac{1}{2}} \hat{\Sigma}_k \mathbf{V}_k^{-\frac{1}{2}} \psi \left(\mathbf{V}_k^{-\frac{1}{2}} (\mathbf{X}_k - \mathbf{M}_k \beta_k) \right) \\ & - \text{tr}(\eta_k \mathbf{P}_k \hat{\Sigma}_k) = 0, \end{aligned} \quad (\text{Equation 8})$$

where $\mu_k = E \left[\psi \left(\mathbf{V}_k^{-\frac{1}{2}} (\mathbf{X}_k - \mathbf{M}_k \beta_k) \right) \right]$ and $\eta_k I$

$$= \text{Var} \left[\psi \left(\mathbf{V}_k^{-\frac{1}{2}} (\mathbf{X}_k - \mathbf{M}_k \beta_k) \right) \right]$$

are the expectation and variance of the ψ -transformed standardized residuals, respectively.

However, the two new parameters μ_k and η_k are related to the true distribution of the scaled residuals and needed to be estimated. Therefore, the DPMM is applied to estimate the density and moments of the scaled residuals. Compared with a generally used method, kernel density estimation (KDE), DPMM is free of selecting bandwidth for kernel function.²³ What is more, DPMM is more robust than KDE for we can choose weakly informative priors based on the residuals (further details regarding the methods can be found in the [supplemental methods](#)). To avoid the complicated and time-consuming MCMC sampling algorithm for the convergence of DPMM, we use the VB DPMM following¹⁷:

$$r_i | \mu_i, v_i \sim \mathcal{N}(\mu_i, v_i), \mu_i, v_i | G \sim G, G \sim DP(\alpha, G_0), \quad (\text{Equation 9})$$

where r_i represent the scaled residuals obtained by the first stage estimation using robust REML estimators and the base distribution G_0 has density

$$g(\mu, v) = g_{\mu|v}(\mu|v)g_v(v) = \mathcal{N}(\mu; 0, \tau v)IG(v, s, T). \quad (\text{Equation 10})$$

Here, IG denotes the inverse gamma distribution and the hyperparameters in this model are α, τ, s, T . The posterior distributions for the parameters and the algorithm are given in [supplemental methods](#). Under some regularity conditions, the VB robust estimator $\hat{\beta}_k$ follows normal distribution

$$\hat{\beta}_k \xrightarrow{d} \mathcal{N} \left(\beta_k, \xi_k (\mathbf{M}_k^T \mathbf{V}_k^{-1} \mathbf{M}_k)^{-1} \right) \quad (\text{Equation 11})$$

asymptotically, where $\xi_k = \left(\frac{1}{N} \sum_{i=1}^N \left(\psi(r_i) - \frac{1}{N} \sum_{i=1}^N \psi(r_i) \right)^2 \right) / \left(\frac{1}{N} \sum_{i=1}^N \frac{d\psi(x)}{dx} \Big|_{x=r_i} \right)^2$. Finally, the confidence intervals for the direct and indirect effects are obtained by bootstrapping following Mackinnon.²⁴

Simulation design

We compared the performance of VB-RobM, REML, bias-corrected robust REML and Bayesian LMM by simulation studies. Specifically, the model we used to generate the dataset is

$$Y_{ij} = \beta_0 + \beta_1 X_{ij} + b_j + \varepsilon_{ij}. \quad (\text{Equation 12})$$

We set the slope (β_0) and intercept (β_1) to 0, representing the path effects of plant species diversity and a mediating trophic level (e.g., natural enemy performance) on an outcome trophic level (e.g., herbivore performance), respectively. $X_{ij} \sim \mathcal{N}(-1, 1)$ were fixed in all datasets, representing the mediating trophic level performance. $b_j \sim \mathcal{N}(0, 0.25)$ were the random effects, accounting for heterogeneity across the studies. The error terms $\varepsilon_{ij} \sim \mathcal{N}(0, 0.01)$ were 4:1 mixed by chi-squared distribution, exponential distribution or Poisson distribution. We generated 500 datasets for each distribution. Each dataset had 100 samples and 20 studies, i.e., $1 \leq i \leq 100, 1 \leq j \leq 20$.

Datasets

We compiled a dataset describing 3-factor (plant species diversity, invertebrate herbivores and their natural enemies) and 4-factor interactions (i.e., plant species diversity, plants, invertebrate herbivores and their natural enemies) derived from 262 observations of 57 articles (see [supplemental information](#)). This dataset contained a total of 3/5 trophic feeding links between these species in 3/4-factor interactions. This model dataset was then used to compare the new VB-RobM method with conventional PSEM which uses REML estimator and multivariate Bayesian analysis based on Bayesian LMMs.

Treatments, mediators, and outcome of the case study dataset

To explore how the treatment, i.e., plant diversity, affects the trophic groups (i.e., natural enemy performance, herbivore performance, and plant performance), we took plant diversity as treatment, natural enemy performance (including measures of abundance of predators, abundance of parasitoids, predation, and parasitism) and herbivore performance (including measures of herbivore abundance and damage) as mediators, plant performance (includes growth, quality, and reproduction of plants) as outcomes and latitude, and plant type as covariates, respectively. Further we found that the effects of plant diversity were nonlinear, and the plant diversity increase from 0 to 1 affected the trophic groups majorly. Here, plant species richness is essentially a binary variable (0 or 1), indicating whether plant species richness was increased irrespective of the number of

species added. Specifically, “0” means the control group (i.e., single or lowest plant species richness), and “1” denotes the treatment group (higher plant species richness, ≥ 2). Therefore, to explore the effects of additive plant diversity on the other trophic groups, we defined the added plant diversity D to be the difference between the plant species richness in treatment and control groups minus 1. Here, additive plant diversity is a continuous variable describing the increase in plant species richness between the control (monocultures of the lowest experimental species richness of plants) and the treatment with an increased plant species richness relative to the control. In this case, the intercept terms in the model were interpreted as the treatment effect and the slope of D was interpreted as the additive effect.

Effect size measures in real datasets

The Hedge’s g standardized mean difference (SMD) is applied to measure the effects of plant diversity on the trophic groups, which is calculated as the mean difference between treatment and control groups divided by pooled standard deviation s_p of these two groups, where $s_p = \sqrt{\frac{(n_t - 1)s_t^2 + (n_c - 2)s_c^2}{n_t + n_c - 2}}$, n_t and n_c are the sample sizes, s_t^2 and s_c^2 are the sample variances of the two groups, respectively.

Implementation of the methods compared

We conducted multivariate Bayesian analysis following the priors and settings given in Dainese et al.⁹ Specifically, we used priors $\mathcal{N}(0, 10)$ for β_k and half-Student $\mathcal{T}(3, 0, 5)$ for τ_k^2 and ν_k^2 . We used “rstanarm”²⁵ and “brms”²⁶ package in R to conduct the Bayesian analysis and “piecewiseSEM”²⁷ to conduct SEM analysis, respectively. The VB-RobM analysis was conducted by our developed R package “VB-RobM,” which is available on GitHub (see [data and code availability](#)).

RESOURCE AVAILABILITY

Lead contact

Further information and requests should be directed to the lead contact, Nian-Feng Wan (nfwan@ecust.edu.cn).

Materials availability

This study did not generate new unique materials.

Data and code availability

All data used in this analysis are deposited on Zenodo (<https://zenodo.org/records/10514049>). The open-source R package VB-RobM and code for reproducibility of this analysis is available on GitHub (<https://github.com/YuquanW/VBRobM>). Supplementary code is provided in [Note S2](#).

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AUTHOR CONTRIBUTIONS

N.-F.W. and Y.-Q.H. conceived the idea. N.-F.W. designed the framework of this article. Y.-Q.W. and D.-P.S. collected and analyzed the data. Y.-Q.W., Y.-Q.H., and N.-F.W. drafted this article. Y.-Q.W., D.-P.S., C.S., B.A.W., Y.-Q.H., and N.-F.W. wrote the manuscript. All authors prepared and edited the final drafts.

DECLARATION OF INTERESTS

The authors declare no competing interests.

SUPPLEMENTAL INFORMATION

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