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Modeling agroecosystem allelopathic soil legacy effects using generalized and mixed linear models

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ABSTRACT

This study addresses the methodological and ecological challenges of evaluating soil legacy and allelopathic residual effects in agroecosystems. The allelopathic activity of edaphotopes from five agroecosystems (fallow with mixed herbage, apricot, walnut, *Echinacea purpurea* and an abiotic sand control) was evaluated with 16 test crops in a three-level hierarchy: 8000 seeds, 320 trays and 80 crop-by-agroecosystem cells. Because the soil of each agroecosystem was collected as a single composite sample, the four trays are technical subsamples; inference on the agroecosystem factor was therefore drawn at the crop-block level, with the 16 test crops acting as independent bioassays of the same soils. Morphometric measurements were modelled with binomial generalized linear mixed models for germination and viability and with linear mixed models of log-transformed root length, shoot length and their ratio, and were summarised by the normalized response index, the stress effect index and a composite vigor index. After correction for multiple comparisons across all ten pairwise contrasts, one difference between agroecosystems was supported: fallow with mixed herbage exceeded the *Echinacea purpurea* soil in composite vigor ($\Delta = 1.440$; 95% CI 0.572 to 2.308; p[Holm] = 0.030), with the same contrast supported for root length, shoot length and the normalized response index; germination, viability and the stress effect index showed no differences at this level. The response was strongly crop-specific: Kendall's coefficient of concordance across the 16 crops was 0.170, so the ranking of agroecosystems obtained with one test species is largely not transferable to another. Variance partitioning attributed 43.3% to 91.8% of the variation to the biology of the test crop and at most 7.4% to the agroecosystem, with 23.1% of the variation of composite vigor residing in the crop-by-agroecosystem interaction. In the multivariate analysis at the replicate level the test crop accounted for 70.7% of the variation, the interaction for 16.7% and the agroecosystem for 3.1% (PERMANOVA, 9999 permutations). The study demonstrates that a multi-level mixed modelling framework with explicit attention to the level of replication yields a defensible, if narrower, description of the soil allelopathic regime, and that bioassays based on a single test species are not a reliable instrument for ranking agroecosystems.

Keywords: allelopathy, soil legacy effect, generalized linear mixed models, mixed-effects ordination, normalized response index, phytotoxicity, bioindication, regenerative farming systems.

INTRODUCTION

Allelopathy represents a fundamental mechanism regulating the productivity and stability of agrophytocenoses. Conceptual studies by Kohli et al., [2006] demonstrate the synergistic effect of allelochemicals on seed germination, root system development, and the transformation of soil microbial composition. The soil allelopathic regime is defined as a complex network of chemical

interactions within the "plant-microorganisms-edaphotope" system, which is established during the emission, biochemical transformation, and detoxification of exometabolites in the rhizosphere Kohli et al., [2006]. The foundational principles of allelopathy as a physiological process with ecological implications have been synthesized by Blum [2006], while a review by Singh et al. [2001] systematizes its role in agroecosystem functioning and crop rotations. Inderjit et al. [2001] emphasizes that allelopathic interactions are realized both through the direct impact of volatile and water-soluble allelochemicals and indirectly via alterations in the structure of microbial communities.

The methodological foundation of contemporary allelopathic research in Ukraine is established by works edited by Blanco [2007], which unified the protocols for phytotoxicity bioassays, chemical analysis of allelochemicals (phenols, tannins), and evaluation of the functional status of acceptor plants. Based on these approaches, Moskalik and Legeta [2019] determined that the inhibition level of test crops by aqueous extracts of rhizospheric soil serves as a reliable integrated indicator of the allelopathic regime. In turn, Korkhova and Mykolaichuk [2021] identified significant cultivar-specific differentiation in the allelopathic potential of winter wheat under Steppe conditions, reinforcing the necessity of accounting for species-specific responses when designing crop rotations. Cheng and Cheng [2015] systematized the physiological mechanisms of allelopathy (alterations in membrane permeability, inhibition of enzymatic activity, disruption of mineral nutrient uptake) and demonstrated that the practical application of allelopathy could significantly decrease reliance on synthetic herbicides. Vashishth et al. [2025] summarized emerging directions for utilizing allelopathy in sustainable agriculture – ranging from breeding allelopathically active cultivars to developing plant extracts as bioherbicides.

The global body of research on soil legacy effects is anchored in several fundamental studies. Specifically, Ens et al. [2009] identified allelopathy as a primary driver of plant community transformation under the influence of the invasive shrub *Chrysanthemoides monilifera*. Grove et al. [2012], applying a factorial experimental design with activated carbon, differentiated the chemical and trophic components of the soil legacy left by *Cytisus scoparius*.

The negative impact of the edaphotope beneath *Pteridium esculentum* on the regeneration of woody species was statistically verified by Maidana-Tuco et al. [2025]. A comprehensive synthesis of the composition of allelochemicals within the *Poaceae* family and the methodologies for their detection was presented by Sahrir et al. [2023]. An important aspect is also the additive and synergistic nature of the combined action of multiple invasive species identified by Lenda et al. [2023], the specificity of which depends on the biology of the acceptor species. The allelopathic potential of invasive woody species as a threat factor to natural forest communities was described by Glogov [2025]. Practical aspects of exploiting allelopathic effects for weed suppression in agroecosystems were systematized by Ferreira and Reinhardt [2016], while the application of plant extracts as natural herbicides in organic farming was reviewed by Uludağ et al. [2017].

Despite a substantial body of data, classical analysis of variance (ANOVA) still dominates most allelopathic studies, ignoring the hierarchical structure of samples and the non-Gaussian nature of morphometric responses. A standard completely randomized design (CRD) involving multiple test crops and agroecosystems generates a three-level hierarchy (seed → tray → treatment variant), where residuals are interdependent due to the nestedness of the levels.

The germination rate (GERMINATION) is inherently a proportion, while root and shoot lengths typically exhibit right-skewed asymmetry, which severely violates the ANOVA assumption regarding the normality of residual distributions [Bolker et al., 2009; Zuur et al., 2009]. Gbur et al. [2012] emphasize that for agronomic and ecological experiments with proportional and count responses, the application of generalized linear mixed models (GLMM) constitutes the methodological standard. Under such conditions, implementing GLMMs is statistically rigorous. They allow for a flexible choice of response distributions (Binomial, Gamma, etc.) combined with explicit modeling of the random effects structure [Lee et al., 2006; Ruíz et al., 2023; Hryhoriv et al., 2025].

The objective of this study is to perform a comparative analysis of the allelopathic activity of edaphotopes across five agroecosystems. The study is based on the application of contemporary mixed modeling frameworks (LMM, GLMM) to verify hierarchical data, alongside multivariate

analysis techniques (PERMANOVA, db-RDA, NMDS) to visualize and statistically evaluate structural discrepancies in the allelopathic response. The object of the study was the allelopathic activity of edaphotopes from five agroecosystems as a factor shaping residual effects (soil legacy) in agrophytocenoses. The subject of the study comprised the morphometric parameters of seedlings (root length – ROOT, shoot length – SHOOT) and germination rates (GERMINATION) of 16 test crops from various families under the influence of soil allelochemicals. To quantitatively assess the allelopathic effect, the Normalized Response Index (NRI) was calculated according to the methodology of Williamson and Richardson [1988]:

$$NRI = (T - C)/C \quad (1)$$

where: T is the mean value of the parameter in the experimental treatment; C is the mean value in the control (quartz sand).

MATERIAL AND METHODS

The research on soil allelopathic activity targeted a typical medium-loamy, medium-humic Chernozem (formed on loess) from the Myrhorod district, Poltava region, situated within the Ukrainian Left-Bank Forest-Steppe. The substrate featured the following parameters: humus content – 3.47%, hydrolytic acidity – 1.10 mg 100^{-1} g of soil, easily hydrolysable nitrogen – 61.6 mg kg^{-1} of dry soil, available phosphorus – 313.5 mg kg^{-1}, exchangeable potassium – 145.2 mg kg^{-1}, alongside an EC of 64 $\mu S \cdot cm^{-1}$ determined in a 1:5 soil-to-water suspension.

The test crops represent the following families: Fabaceae (shell pea – *Pisum sativum*, perennial lupine – *Lupinus polyphyllus*), Brassicaceae (radish – *Raphanus sativus*, white mustard – *Sinapis alba*, garden cress – *Lepidium sativum*), Poaceae (popcorn – *Zea mays*, winter wheat – *Triticum aestivum*), Amaryllidaceae (sand onion – *Allium scorodoprasum*, Welsh onion – *Allium fistulosum*), Amaranthaceae (beetroot – *Beta vulgaris*, amaranth – *Amaranthus spinosus*), Cucurbitaceae (pumpkin – *Cucurbita pepo*), Lamiaceae (purple basil – *Ocimum basilicum*), Apiaceae (dill – *Anethum graveolens*), Polygonaceae (common buckwheat – *Fagopyrum esculentum*) and Asteraceae (sunflower – *Helianthus annuus*). To calculate the relative indices and to evaluate the baseline effect of allelochemicals, washed quartz sand served as the abiotic control. The English names used here are those used throughout the tables and figures.

The study was conducted according to a completely randomized design with respect to the allocation of soils to trays. The total sample comprises 16 test crops × 5 agroecosystems × 4 trays × 25 seeds = 8000 primary observation units, organised in a three-level hierarchy: L1, the seed (n = 8000); L2, the tray (n = 320); L3, the crop-by-agroecosystem cell (n = 80). The physical arrangement of the trays was not randomised, as described below.

The classification of each observation unit (seed) was performed using a three-category ISTA scheme adapted to morphometric thresholds: “Normal”: critical organ > threshold / 10 mm; “Abnormal”: critical organ > 0 mm, but < threshold/10 mm; “Dead”: critical organ = 0 mm. The choice of the critical organ was determined by the biological characteristics of the species: root (ROOT) – for popcorn (*Zea mays*), perennial lupine (*Lupinus polyphyllus*), winter wheat (*Triticum aestivum*), Welsh onion (*Allium fistulosum*) and sand onion (*Allium scorodoprasum*); shoot (SHOOT) – for shell pea (*Pisum sativum*), white mustard (Sinapis alba), radish (*Raphanus sativus*) and garden cress (*Lepidium sativum*); total length (ROOT + SHOOT) – for the remaining seven species. The complete assignment is listed in Supplement S1d. Threshold values varied from 15 mm (*Triticum aestivum*) to 50 mm (*Helianthus annuus*). For statistical analysis, a binary viability variable VIABBIN was introduced: VIABBIN = 1 for the “Normal” and “Abnormal” categories, and VIABBIN = 0 for “Dead”. At the replicate level (tray, n = 320), 10 descriptors of the allelopathic profile were calculated. The primary metrics were germination (GERMINATION, %) and the mean length of organs. Additionally, the root-to-shoot ratio (RSR) was computed as:

Unit of replication and level of inference

The soil of each agroecosystem was obtained as a single composite sample: material collected from five points of one 0.15 ha plot, 10 kg per point, was pooled, homogenised and distributed among four trays. The four trays of an agroecosystem are consequently technical subsamples of one composite sample and not independent field replicates; at the field scale the agroecosystem

factor is replicated once. Treating trays as replicates for this factor would constitute pseudoreplication in the sense of Hurlbert [1984]. Two levels of analysis were therefore distinguished, and every quantity reported below is explicitly attributed to one of them.

Replicate level (tray, n = 320). All mixed models, their residual diagnostics, the variance partitioning, the full coefficient tables and the multivariate analyses were fitted at this level, which retains the crop-by-agroecosystem structure of the design. Estimated marginal means, coefficients of determination, intraclass correlation coefficients, AICc values and effect sizes obtained here describe the five composite soils that were actually sampled and are reported as descriptive quantities.

Crop-block level (n = 16). Each of the 16 test crops constitutes an independent bioassay of the same five soils. The agroecosystem factor was therefore tested in a randomised complete block design in which the test crop is the block. The four trays of every cell were averaged, yielding a 16 × 5 matrix; all ten pairwise contrasts between agroecosystems were evaluated by paired t-tests on the within-crop differences, with Wilcoxon signed-rank tests as a distribution-free check, and the p-values were adjusted by the Holm step-down procedure across the family of ten contrasts. The compact letter displays given in Tables 5 and 7 and in Figure 1 originate from this level. The transferability of the agroecosystem ranking between crops was quantified by the Friedman test and by Kendall's coefficient of concordance.

All inference concerning the agroecosystem factor is drawn from the crop-block level. Replicate-level p-values are reported alongside in Table 5 for a single purpose: the point estimate of a contrast is identical at both levels, so the ratio of the two p-values quantifies how far the degrees of freedom are inflated when technical subsamples are treated as replicates.

Experimental conditions, watering and arrangement of trays

Soil for all agroecosystems was collected in mid-April from the 0–10 cm layer by the envelope method: five points per 0.15 ha plot and 10 kg per point, pooled into one composite sample per agroecosystem and homogenised. The soil was used fresh and was not stored before the bioassay. The abiotic control consisted of quartz sand washed with 0.1 N hydrochloric acid and subsequently rinsed with distilled water.

The bioassay was carried out in transparent polyethylene terephthalate punnets of 1 L capacity, each filled with 400 g of soil and sown with 25 seeds. All trays were sown on the same day and watered once, on the first day, with distilled water to 60% of full water capacity; no further water was applied at any point of the experiment. For the first five days the trays were covered with transparent cling film, which kept evaporation low enough for the initial moisture content to be maintained without additional watering; the film was removed for the final two days so that seedlings could develop freely. All trays were measured on day 7. The temperature ranged from 20 to 25 °C and illumination was natural daylight from a window; photoperiod and photon flux density were not recorded.

Trays were arranged haphazardly on the shelves of a rack standing beside the window and were not re-positioned during the experiment. The arrangement was therefore neither a formal randomisation nor a rotation scheme, and this is the most plausible source of the systematic tray effect reported below ($F(3, 237) = 7.28$, $p < 0.001$): shelves differ in their distance from the window and in their angle to it, so trays received slightly different amounts of daylight. Because the tray identifier is confounded with shelf position, the effect cannot be partitioned into a positional and a handling component. Its consequences are examined in the sensitivity analysis of Supplement S7, where the ranking of agroecosystems proves identical whether the tray is modelled as a random intercept, as a fixed block, or omitted.

$$RSR = mean\left(\frac{ROOT}{SHOOT}\right), at\ SHOOT > 0 \quad (2)$$

The allelopathic effect (AE, cm) was calculated as: $AE = MeanCritT - CtrlCrit$, where *MeanCritT* is the mean length of the critical organ in the treatment, and *CtrlCrit* is that in the control (sand).

The laboratory record also contains a set of relative indices computed at the tray level (seedling vigour indices I and II, the critical growth index, the relative difference and growth indices, the stimulation, tolerance and relative performance indices). They are supplied with the data and their derivations are documented and numerically verified in Supplement S1e, but they are not used in the analyses reported here, which rest on germination, viability, root and shoot length, their

ratio, the normalized response index, the stress effect index and composite vigor.

Because the class depends on the critical organ alone, a seed is recorded as dead when that organ measures zero even if another organ has developed. Twelve seeds, 0.150% of the data set, fall into this category; ten of them are popcorn, in which the critical organ is the root, so a seed that produced a coleoptile without a radicle is classified as dead. The classification rule is recomputed from the raw measurements in the analysis script and reproduces the recorded class for all 8000 seeds without a single mismatch. The species-specific assignment of the critical organ and the thresholds are listed in Supplement S1d.

No observation was excluded from the analysis and no value was imputed. The data set is complete and balanced: 25 seeds in each of the 320 trays, four trays in each of the 80 cells, and no missing values in any variable. These properties are verified by assertions at the start of the analysis script, which halts if any of them is violated.

For multivariate analysis (NMDS, db-RDA) and mixed modeling, data transformation was executed to compute the Stress Effect Index (SEI):

$$SEI_{raw} = ln(max(RG,0.001)) + ln(max(RRG,0.001)) \quad (3)$$

where: RG is relative germination, and RRG is relative root growth.

The final EI was standardized using z-scores. The integrated vegetative power (CV) was formalized as:

$$CV = z(ln(ROOT)) + z(ln(SHOOT)) + z(NRI) \quad (4)$$

During pre-modeling preparation for the binomial models, percentage rates were converted into event counts: k = round(P/100 × 25). For ROOT, SHOOT and RSR a lower bound x = max(x, 0.001) was enforced so that the logarithmic transformation is defined for seedlings with a zero measurement. Statistical processing was carried out in the R language, version 4.6.1 [R Core Team, 2026], within the RStudio integrated development environment [Posit team, 2026], with a fixed pseudo-random number generator seed, set.seed(2026). The analytical complex relied on the following packages: lme4 2.0.1 [Bates et al., 2015] and lmerTest 3.2.1 [Kuznetsova et al., 2017] for mixed-effects modelling, blme 1.0.7 [Chung et al., 2013] for penalized estimation under complete separation, glmmTMB 1.1.14 as an alternative estimation engine, emmeans 2.0.3 and multcompView 0.1.11 for marginal means and compact letter displays, DHARMa 0.4.7 [Hartig, 2026] and performance 0.16.0 [Lüdecke et al., 2021] for diagnostics, MuMIn 1.48.19 [Bartoń, 2026] for information criteria, MCMCglmm 2.36 [Hadfield, 2010] with coda 0.19.4.1 for the Bayesian multi-response model, vegan 2.7.3 [Oksanen et al., 2026] for PERMANOVA, db-RDA and NMDS, betareg 3.2.4 for the beta-regression sensitivity check and boot 1.3.32 for the parametric bootstrap.

Generalized linear mixed models (GLMM). Seed germination (GERMINATION) and seedling viability (VIABILITY) parameters were modeled as aggregated binomial responses (number of successes k out of n = 25 trials) via GLMM with a logit link function [Lee et al., 2006; Ruíz et al., 2023]. Gianinetti [2020] substantiated that GLMM with a binomial distribution represents the most rigorous approach for analyzing germination data within hierarchical designs. Onofri et al. [2022] proposed a unified framework for the analysis of germination and time-to-event metrics compatible with the GLMM approach. López-Almansa [2023] confirmed the efficacy of GLMM for evaluating germination factors in woody species. The hierarchical structure of the experiment (nestedness of tray replicates, n = 320) was accommodated by introducing a random intercept for the replicate factor (Replicate). To statistically test hypotheses, two competing models were constructed. Main effects model (main): 1. main effects model (main): R^2m.

This model assumes an additive influence of factors without specific responses of individual crops to concrete ecosystems. 2. Full interaction model (full):

$$Logit\,(p_{ij}) = \beta_0 + \beta_1 \bullet Agroecosystem_i + \beta_2 \bullet Test\text{-}Crop_j + u_{rep} + \varepsilon_{ij} \quad (5)$$

$$Logit\,(p_{ij}) = \beta_0 + \beta_1 \bullet Agroecosystem_i + \beta_2 \bullet Test\text{-}Crop_j + \beta_3, \quad (6)$$

where: u_{rep} (random intercept) is the random effect of the replicate, $u_k \sim N(0, q_u^2)$, accounting for tray-to-tray variability; e_{ij} is the model residual noise.

The selection between the additive and interaction models was based on the corrected Akaike information criterion (AICc), where a lower value indicated a superior fit to the empirical data.

Linear mixed models (LMM). The normalized response index (NRI), the stress effect index (SEI) and composite vigor (CV) are continuous, unbounded and approximately symmetric, and were therefore modelled by classical linear mixed models [Zuur et al., 2009; Bates et al., 2015]. Their residual behaviour is examined in the diagnostic section rather than assumed: the simulated residuals show a detectable but small departure from uniformity at n = 320, together with underdispersion, which makes the reported standard errors conservative.

$$Y_{ijk} = \beta_0 + \beta_1 \bullet Agroecosystem_i + \beta_2 \bullet Test\text{-}Crop_j + \\ + \beta_3 \bullet (Agroecosystem_i \bullet Test\text{-}Crop_j) + u_k + \varepsilon_{ijk} \quad (7)$$

Statistical assumptions and estimation procedure – random effects: $u_k \sim N(0,\ q_u^2)$ – random intercept of the replicate (tray), accounting for intraclass correlation. Residual error – $varepsilon_{ijk} \sim N(0,\ q_u^2)$ – independent and identically distributed error. Parameter optimization – competing models (with and without interaction) were compared using the maximum likelihood (ML) method, whereas the final model was re-estimated using the restricted maximum likelihood (REML) method to obtain unbiased variance component estimates [Bates et al., 2015]. Significance verification – p-values for fixed effects were computed using the Satterthwaite approximation of degrees of freedom (lmerTest package) [Kuznetsova et al., 2017], which ensures high accuracy for unbalanced or complex hierarchical designs.

The generalized linear mixed model (GLMM) describes the relationship between the response and predictors via a linear predictor η in the scale of the link function g(μ) [Lee et al., 2006; Ruíz et al., 2023]:

$$\eta = X\beta + Zu + \varepsilon \quad (8)$$

where: η is the vector of linear predictors in the link function scale; X and Z are the design matrices for fixed and random effects, respectively; β is the vector of fixed effects; $u \sim N(0,\ G)$ is the vector of random effects; and ε represents the residuals.

An LMM is a special case of a GLMM under a Gaussian distribution and an identity link function ($g(\mu)$=) [Lee et al., 2006; Ruíz et al., 2023]. This unified approach allows for a mathematically valid comparison of results obtained for different biometric data types (germination, growth, indices) within a single statistical paradigm.

To evaluate the quality of the final models, three primary statistical criteria were utilized: the marginal coefficient of determination (R^2m), the conditional coefficient of determination (R^2c), the intraclass correlation coefficient (ICC), and the corrected Akaike Information Criterion (AICc). The marginal and conditional coefficients of determination were calculated according to the approach of Shinichi Nakagawa and Holger Schielzeth [2013]:

$$R^2m = \frac{Var(X\beta)}{Var(X\beta) + Var(u) + Var(e)} \quad (9)$$

$$R^2c = \frac{Var(X\beta) + Var(u)}{Var(X\beta) + Var(u) + Var(e)} \quad (10)$$

where: $Var(X\beta)$ is the variance explained by fixed effects; $Var(u)$ is the variance of random effects; $Var(e)$ is the residual variance (which includes the distribution-specific component for GLMMs).

R^2m characterizes the contribution of fixed effects (Test-Crop × Agroecosystem), while the difference ($R^2c - R^2m$) quantitatively reflects the tray-level effect contribution. These were computed using the r.squaredGLMM function from the MuMIn package [Bartoń, 2026].

The intraclass correlation coefficient (ICC, evaluated via the performance package) [Lüdecke et al., 2021] is defined as:

$$ICC = \frac{Var(u)}{Var(u) + Var(e)} \quad (11)$$

The intraclass correlation coefficients obtained in this study range from 0.063 to 0.379 across the seven models for which they are estimable (Table 1). The random effect of the tray is therefore modest for most responses and appreciable only for germination; it is nevertheless systematic, which justifies the use of mixed models. For comparing alternative specifications of the same response, the corrected Akaike Information Criterion (AICc) was utilized:

$$AICc = AIC + \frac{2K(K+1)}{n - K - 1} \quad (12)$$

where: K is the number of estimated parameters and n is the number of observations [Bolker et al., 2009; Bartoń, 2026]. Models with $\Delta AIC > 2$ were considered significantly distinct [Bolker et al., 2009].

For the analysis of binomial responses, specifically germination rates, the logit link function was applied:

$$logit(\pi) = \frac{\pi}{1-\pi} = \eta \quad (13)$$

where: π is the probability of germination, and η is the linear predictor of the model.

This transformation maps the probability space onto the log-odds scale, ensuring a linear relationship between the response and the predictor system [Jardim Amorim et al., 2021; Ruíz et al., 2023]. The logit link guarantees that predicted values remain strictly within the interval $0 < \pi < 1$. Results presented by Jardim Amorim et al. [2021] showed that for maize and soybean germination data, the logit function provided superior modeling quality compared to probit and complementary log-log (CLL) links, verified by lower AIC and BIC values.

For the log-transformed length responses a linear mixed model was used, $\log(y) = X\beta + Zu + \varepsilon$. The logarithmic transformation is appropriate for strictly positive, right-skewed variables and gives coefficients a multiplicative interpretation: an exponentiated coefficient is a response ratio relative to the reference level. A lower bound of 0.001 cm was applied before transformation to accommodate seedlings with a zero measurement; the sensitivity of the results to this constant is examined in Supplement S7.

GLMM adequacy was verified using simulated quantile residuals (DHARMa package) [Hartig, 2026]. For each GLMM, nsim = 1000 simulations were generated; for each observation i, the quantile of the actual value within the simulated distribution was calculated: ri = Fsimi(yi) \~ Uniform(0, 1) if the model specification is correct. The diagnostics evaluated: (1) conformance to a uniform distribution via the Kolmogorov-Smirnov test; (2) overdispersion/underdispersion; (3) the presence of outliers. Overdispersion in binomial GLMMs was additionally assessed by the ratio of Pearson residuals sum of squares to residual degrees of freedom:

$$log(\mu) = \pi \quad (14)$$

The statistic was computed for the binomial models only – in a Gaussian mixed model the residual variance is a free parameter, so the ratio is not interpretable, and the dispersion behaviour of the linear models is assessed instead by the simulated residuals of the DHARMa procedure. No observation-level random effect was required, both binomial models showing dispersion ratios below unity. Multicollinearity among fixed effects was assessed via the variance inflation factor: $VIF = 1/(1 - R^2j)$; values above 10 were deemed critical (performance package) [Lüdecke et al., 2021].

To simultaneously analyze the log(ROOT) and log(SHOOT) parameters while accounting for their joint covariation at the replicate level, a multivariate Bayesian generalized linear mixed model was deployed via the MCMCglmm package (Hadfield, 2010). The model was specified using the response vector cbind(logROOT, logSHOOT) as dependent variables, while the fixed effect's structure was set as trait: TestCrop + trait: Agroecosystem − 1, enabling the estimation of residual correlation between the ROOT and SHOOT parameters. For the random effect's matrices G and residual variance R, uninformative Inverse-Wishart prior distributions were implemented: V = 0.5 × I2, ν = 2, where I2 is a 2 × 2 identity matrix and ν is the degrees of freedom parameter.

The Markov Chain Monte Carlo (MCMC) parameters were defined as follows: total iterations = 50000; burn-in period = 10000; thinning interval = 20. Under these settings, the effective posterior sample size was stable. The posterior correlation between ROOT and SHOOT responses was computed as:

$$r_{post}(ROOT,SHOOT) = \frac{COVpost(ROOT,SHOOT)}{\sqrt{VARpost(ROOT) \bullet VARpost(SHOOT)}} \quad (15)$$

where: Cov_{post} is the posterior covariance, and Var_{post} denotes the posterior variances of the respective responses.

Gamma generalized linear mixed models with a logarithmic link were applied initially to root length, shoot length and their ratio. The model of the ratio failed to converge (max|grad| = 0.881), and the dispersion behaviour of the Gamma fits for the two lengths was not satisfactory. All three responses were consequently modelled as linear mixed models of the log-transformed response, log(y) ~ TestCrop × Agroecosystem + (1 | Replicate), which preserves the multiplicative interpretation of the coefficients while avoiding the dispersion misfit. The random effect of the log-ratio model was estimated as zero (singular fit), which is reported as a substantive result: the tray factor explains no part of the variance of that response, and the corresponding conditional R^2 and ICC are not estimable.

Four cells for germination and five for viability contained no variation, producing complete

separation in the binomial models. Rather than excluding these cells, the binomial GLMMs were re-estimated with weakly informative priors on the fixed effects [blme package; Chung et al., 2013]. After the correction the largest standard error on the logit scale was 2.13 for germination and 2.00 for viability, and no coefficient approached the degeneracy threshold.

The p-values of fixed effects were obtained by the Satterthwaite approximation (lmerTest). Estimated marginal means and their contrasts were computed with emmeans using the Kenward–Roger approximation of the denominator degrees of freedom. Confidence intervals for the five marginal means were adjusted by the Šidák method and pairwise comparisons at the replicate level by the Tukey method; at the crop-block level the Holm procedure was applied over the ten pairwise contrasts. Underdispersion detected by the simulated residuals of the linear models makes the reported standard errors conservative rather than anticonservative. The Pearson chi-square dispersion statistic was computed for the binomial models only, since in a Gaussian mixed model the residual variance is a free parameter and the statistic is not interpretable.

The multivariate analyses were carried out at the replicate level on a matrix of n = 320 trays and p = 7 column-standardised metrics. The aggregated level of n = 80 treatment combinations used in the previous version cannot support the full factorial model: with 79 model degrees of freedom and no residual degrees of freedom the decomposition is an identity and returns $R^2 = 1$ by construction. PERMANOVA was run on Euclidean distances with 9999 permutations, homogeneity of multivariate dispersions was tested by betadisper with the same number of permutations, constrained ordination was obtained by distance-based redundancy analysis, non-metric multidimensional scaling used two dimensions and up to 200 random starts, and principal component analysis was applied to the standardised matrix.

Seven sensitivity checks were performed and are reported in Supplement S7: an alternative estimation engine (glmmTMB against lme4; maximum absolute difference between coefficients 1×10^{-5}), beta regression for germination, three specifications of the tray effect, variation of the bounding constant across five orders of magnitude, leave-one-out over test crops, a parametric bootstrap with 1000 replicates, and four-fold cross-validation stratified by cell.

All computations were performed in the R language, version 4.6.1 [R Core Team, 2026], within the RStudio integrated development environment [Posit team, 2026], with a fixed seed (2026). The principal packages were lme4 2.0.1, lmerTest 3.2.1, blme 1.0.7, glmmTMB 1.1.14, emmeans 2.0.3, multcompView 0.1.11, DHARMa 0.4.7, performance 0.16.0, MuMIn 1.48.19, MCMCglmm 2.36, coda 0.19.4.1, vegan 2.7.3, betareg 3.2.4 and boot 1.3.32. The Bayesian multiresponse model was run for 50 000 iterations with a burn-in of 10 000 and a thinning interval of 20, giving 2000 posterior samples; convergence was assessed by the Geweke diagnostic (95.0% of parameters with $|z| < 2$), by effective sample sizes (minimum 1605) and by trace and density plots (Supplement S5). The complete session record, the analysis scripts, the seed-level and replicate-level data and all supplementary files are archived at Zenodo. The SHA-256 checksums of the primary workbook and of every exported data file are computed and printed by the pipeline at run time.

RESULTS AND DISCUSSION

Statistical tool selection and substantiation. Mixed models were applied for three reasons. First, the data are hierarchical (seed → tray → cell), and the tray contributes a systematic component of variation ($F(3, 237) = 7.28$, $p < 0.001$) that a fixed-effects analysis of variance cannot represent. The intraclass correlation coefficients estimated at the tray level range from 0.063 to 0.379 across the seven models for which it is estimable (Table 1), so the random component is modest for most responses and appreciable only for germination. Second, the responses violate the assumptions of a Gaussian linear model: germination and viability are proportions of 25 binary events, and root and shoot lengths are right-skewed. Binomial generalized linear mixed models were therefore used for the two proportions and linear mixed models of the log-transformed response for the two lengths and their ratio. Third, the specification of the tray as a random intercept isolates micro-environmental variation from the systematic effect of the agroecosystem, which matters here because the physical arrangement of the trays was not randomised.

The structural nature of the investigated responses explicitly violates the core assumptions of standard Gaussian models. The GERMINATION

Table 1. Model fit, explained variance, and random-effect contribution

Model	R^2 marginal	R^2 conditional	ICC	AICc
GLMM G	0.936	0.960	0.379	1342.534
GLMM V	0.944	0.954	0.188	1251.091
LMM log(RL)	0.938	0.943	0.086	204.584
LMM log(SL)	0.929	0.933	0.063	289.672
LMM log(RSR)	0.977	–	–	-112.429
LMM NRI	0.805	0.818	0.064	-1.362
LMM SEI	0.722	0.753	0.111	701.944
LMM VIGOR	0.837	0.849	0.073	975.872

Note: R^2 marginal = fixed effects; R^2 conditional = fixed and random effects; ICC = intraclass correlation coefficient. – = not estimable because the random effect is singular. AICc is comparable only within a response variable; values from binomial, log-linear and index models are not competing fits.

metric is bounded as a proportion generated from 25 binary events, while the SHOOT and ROOT variables display a marked right-skewed distribution. Under these constraints, classical ANOVA or ordinary linear regression becomes statistically invalid. Deploying generalized linear mixed models with a binomial family for the proportions, and linear mixed models of the log-transformed lengths, respects the nature of each response while keeping the coefficients interpretable on a multiplicative scale [Lee et al., 2006; Ruíz et al., 2023; Dehodiuk et al., 2025].

For the linear mixed models AICc is computed under REML and is not comparable with the maximum-likelihood AICc used for specification selection in Supplement S5. ICC is estimated from a random effect with four levels; its robustness is assessed in Supplement S7.

Third, incorporating Replicate as a random intercept allowed the model to explicitly isolate variation stemming from experimental micro-environmental noise from the systematic effects of the agroecosystem. This is particularly valuable given the design constraints of four replicates across 16 test crops. In such setups, mixed models yield vastly more reliable parameter estimates compared to single-level approaches. Bolker et al. [2009] and Zuur et al. [2009] recommend generalized models for proportional and count responses, and linear mixed models where the transformed response behaves acceptably. Here the two proportions are modelled by binomial generalized mixed models, while root length, shoot length and their ratio are modelled on the logarithmic scale, which keeps the multiplicative interpretation of the coefficients and avoids the dispersion misfit of the Gamma specification. The three integrated indices are continuous and unbounded and are modelled directly; the adequacy of every model is examined by simulated residuals rather than assumed.

Interpretation of marginal and conditional R^2. The marginal R^2 reflects the variance explained by the fixed crop-by-agroecosystem combination, and the difference between the conditional and the marginal value quantifies the tray-level component. Because the fixed part of every model is the full factorial of 80 cells estimated by 80 parameters, the models reproduce the cell means almost exactly and the marginal R^2 is correspondingly high; this property of the design is examined separately below. Variance partitioning between the crop and the agroecosystem was obtained from the factorial decomposition of sums of squares at the replicate level.

Marginal R^2 ranges from 0.722 for the Stress Effect Index to 0.977 for the log-ratio of root to shoot, and conditional R^2 from 0.753 to 0.960; for the log-ratio model the conditional value and the intraclass correlation are not estimable because the random effect is singular. The difference between the conditional and the marginal coefficient does not exceed 0.031, indicating that tray-level variation, although systematic, is small in magnitude relative to the fixed structure. AICc values are comparable only within a response variable; the values of the binomial, log-linear and index models are not competing fits, and for the linear mixed models AICc is computed under REML and is not comparable with the maximum-likelihood values used for specification selection in Supplement S5.

The biology of the test crop is the dominant source of variance for every metric, from 43.3% for the Stress Effect Index to 91.8% for root length. The independent contribution of the

agroecosystem does not exceed 7.4% and is below 2% for both lengths. The crop-by-agroecosystem interaction accounts for 5.6% to 31.5% of the variation, reaching 23.1% for Composite Vigor; that is, the effect of a soil depends substantially on which species is used to measure it, and this is the quantitative counterpart of the low concordance reported below (Table 2).

Model diagnostic adequacy and stability. Simulated quantile residuals were examined with the DHARMa package for the six models to which the procedure applies; for the two binomial models with informative priors the separation and dispersion controls are reported separately. Full diagnostic output, including quantile-quantile plots, quantile deviation tests and homogeneity checks by group, is provided in Supplement S5.

The mean and median of the scaled residuals lie close to 0.5 for all six models, and the proportion of extreme residuals ranges from 3.4% to 8.4%. The Kolmogorov-Smirnov uniformity test is significant in every case; at n = 320 this test detects departures that are statistically visible but small in magnitude, and the corresponding deviations are apparent from the quantile plots in Supplement S5. Dispersion ratios of 0.750 to 0.770 indicate underdispersion, which follows from the aggregation of 25 seeds into a tray mean and makes the reported standard errors conservative. Levene's test of variance homogeneity is not significant across agroecosystems but is significant across test crops, so heterogeneity resides in the blocking factor rather than in the factor under test (Table 3).

To construct a comprehensive, integrated assessment of the allelopathic footprint of the agroecosystems, standardized biological response indices, absolute morphometric shifts, and the mean composite index of viability were synthesized. Descriptive statistics of the primary biometric data confirmed a distinct dependence of seedling growth parameters on the agroecosystem type. The most consistent biostimulatory impact was observed in the fallow system with mixed herbage (Fallow), where most test crops displayed an increase in mean root and shoot length relative to the control. For example, in pumpkin, mean root length increased from 4.34 to 6.54 cm, and shoot

Table 2. Factorial variance partitioning for early-growth metrics

Metric	Test-crop (%)	Agroecosystem (%)	Interaction (%)	Residual (%)
Germination	71.7	2.5	14.5	11.3
Viability	72.5	1.5	13.0	13.0
Root length	91.8	0.7	5.6	1.9
Shoot length	88.8	1.9	8.0	1.4
Normalized response index	45.7	7.4	31.4	15.4
Stress effect index	43.3	3.9	30.4	22.4
Composite vigor	59.4	4.8	23.0	12.8

Note: Values are percentages of the total sum of squares in the factorial fixed-effects model.

Table 3. Residual diagnostics and dispersion assessment

Model	KS p-value	Dispersion ratio	Dispersion p-value	Outlier p-value	Diagnostic conclusion
GLMM G	–	–	–	–	Not applicable to bglmerMod
GLMM V	–	–	–	–	Not applicable to bglmerMod
LMM log(RL)	<0.001	0.765	0.002	0.020	Departure from uniformity; see Supplement S5
LMM log(SL)	<0.001	0.761	<0.001	0.020	Departure from uniformity; see Supplement S5
LMM log(RSR)	<0.001	0.750	<0.001	<0.001	Departure from uniformity; see Supplement S5
LMM NRI	<0.001	0.761	<0.001	<0.001	Departure from uniformity; see Supplement S5
LMM SEI	<0.001	0.770	0.006	0.040	Departure from uniformity; see Supplement S5
LMM VIGOR	<0.001	0.763	<0.001	<0.001	Departure from uniformity; see Supplement S5

Note: DHARMa diagnostics are not applicable to bglmerMod models; their separation and overdispersion checks are reported separately. DHARMa uniformity tests are sensitive to sample size; at n = 320 a significant KS statistic indicates a detectable, not necessarily material, departure. Dispersion ratios below unity indicate underdispersion, which makes the reported standard errors conservative rather than anticonservative.

length rose from 4.40 to 5.42 cm. For shell pea, shoot length expanded from 8.47 to 10.03 cm, and for sunflower, it shifted from 10.36 to 12.19 cm. Conversely, the *Echinacea purpurea* edaphotopic matrix exerted a predominantly inhibitory effect, which was particularly severe for amaranth (shoot length reduction from 2.81 to 1.88 cm) and winter wheat (reduction from 16.27 to 11.38 cm). For individual cultures, notably perennial lupine, the most pronounced suppression of shoot growth was recorded, plunging to 57.8% relative to the control baseline. The coefficient of variation indicated high measurement stability for the majority of cereal and brassica crops (CV < 5%), whereas perennial lupine, pumpkin, and beet exhibited elevated intra-group variance, reflecting an unstable biological response. For a clear visual rendering of the integrated influence of agroecosystems on the growth and index profiles of the test crops, refer to Figure 1.

The upper panel shows the mean of four trays for each of the 16 test crops in every agroecosystem, together with the mean across crops and its confidence interval; letters denote agroecosystems that could not be shown to differ at the crop-block level (Table 4). Fallow and *Echinacea* are separated, while the three intermediate soils share letters with both extremes. The lower panel resolves the fallow–*Echinacea* contrast by crop: the difference is positive in 12 of 16 crops and negative in four, and its magnitude ranges from about −1 to about +5 units, which illustrates directly why the overall contrast is supported while the ranking of the intermediate soils is not.

Ordered by Composite Vigor, the five soils form the sequence fallow (+0.794), apricot (+0.292), sand control (−0.101), walnut (−0.338) and *Echinacea* (−0.647); the Normalized Response Index and the Stress Effect Index follow the same order. These values are descriptive: they are arithmetic means at the tray level, and the statistical support for the differences between them is assessed in Table 5. ΔRL and ΔSL are arithmetic differences on the original scale against the sand control of the same test crop, whereas Table 7 reports back-transformed

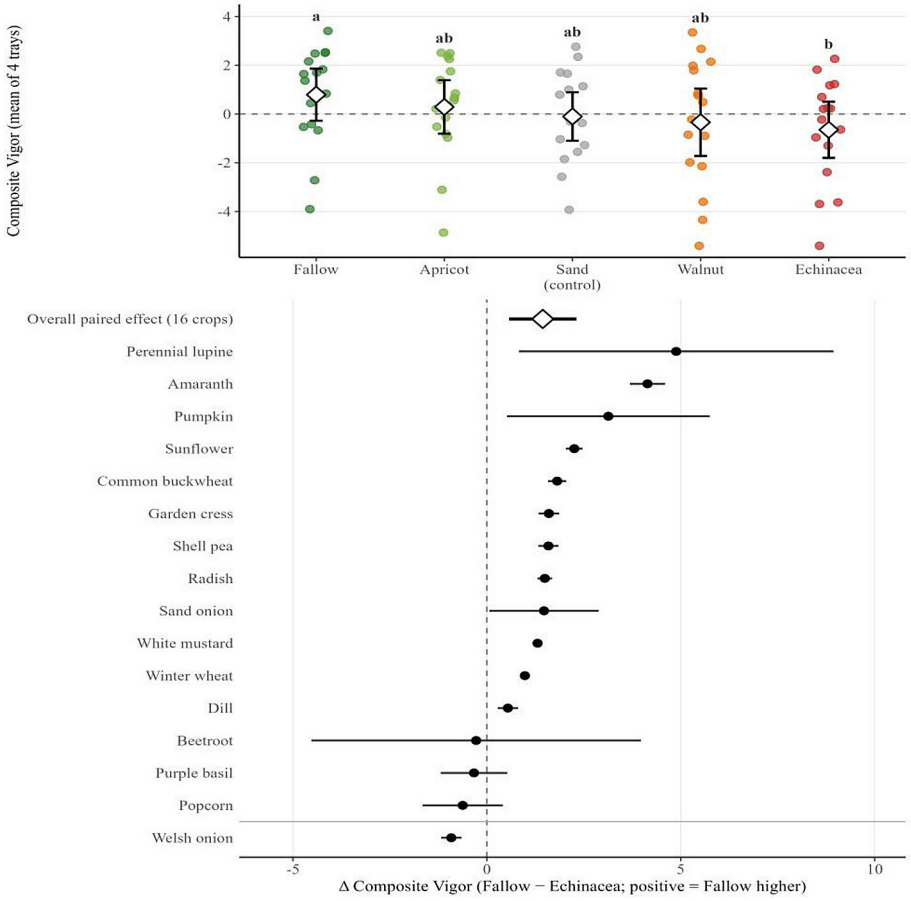

Figure 1. Composite vigor across five agroecosystems (top) and crop-specific fallow – *Echinacea* differences (bottom). Points are test-crop means of four trays; diamonds with whiskers are means across the 16 crops and their 95% confidence intervals. Letters denote agroecosystems that could not be shown to differ at the crop-block level

Table 4. Agroecosystem effects on early growth and composite vigor

Agroecosystem	NRI	SEI	ΔRL	ΔSL	Mean CV	General phytotoxic conclusion
Sand (control)	-0.006	-0.092	+0.000	+0.000	-0.101	Baseline control level
Apricot	+0.056	+0.180	+0.165	+0.061	+0.292	Weak positive stimulation
Walnut	-0.030	-0.160	+0.080	-0.336	-0.338	Moderate growth suppression
Echinacea purpurea	-0.086	-0.214	-0.241	-0.668	-0.647	Pronounced phytotoxic inhibition
Fallow (herbage)	+0.130	+0.286	+0.693	+0.856	+0.794	Strong growth stimulation

Note: NRI – normalized response index; SEI – stress effect index; ΔRL – root-length shift; ΔSL – shoot-length shift; CV – composite vigor index. ΔRL and ΔSL are arithmetic differences on the original scale against the sand control of the same test crop; Table 7 reports back-transformed marginal means of log-scale models (geometric means), so numerical identity between the two tables is not expected.

Table 5. Post-hoc comparisons of composite vigor at the replicate and crop-block levels

Agroecosystem	EMM	Letter (n = 16)	Key contrast	Δ (95% CI)	p tray (n = 320)	p block (n = 16)
Fallow (herbage)	0.794	a	vs *Echinacea purpurea*	+1.440 (0.572 to 2.308)	<0.001	0.030
Apricot	0.292	ab	vs Fallow (herbage)	-0.502 (-1.291 to 0.287)	0.018	0.977
Sand (control)	-0.101	ab	vs Fallow (herbage)	-0.895 (-1.690 to -0.100)	<0.001	0.239
Walnut	-0.338	ab	vs Fallow (herbage)	-1.132 (-2.259 to -0.005)	<0.001	0.344
Echinacea purpurea	-0.647	b	vs Fallow (herbage)	-1.440 (-2.308 to -0.572)	<0.001	0.030

Note: Estimated marginal means are from the replicate-level model (n = 320). Letters, Δ and its 95% confidence interval treat each of the 16 test crops as an independent block (paired t-test, Holm correction across all 10 pairwise contrasts). Replicate-level p-values are reported for comparison only: the four trays per agroecosystem are technical subsamples of one composite soil sample and therefore inflate the degrees of freedom for the agroecosystem factor (Hurlbert, 1984). Point estimates are identical at both levels; only the standard error differs. Inference on the agroecosystem factor is drawn from the crop-block column.

marginal means, so numerical identity between the two tables is not expected.

The contrast between fallow and *Echinacea* is supported at both levels of analysis (Δ = 1.440; 95 % CI 0.572 to 2.308; p[Holm] = 0.030 at the crop-block level). No other contrast survives correction: the difference between fallow and apricot, which reaches p = 0.018 when the 320 trays are treated as replicates, has p[Holm] = 0.977 when the 16 crops are treated as blocks, and the differences of fallow against the sand control and against walnut give p[Holm] = 0.239 and 0.344. The point estimates are identical at both levels; only the standard error differs, and the comparison quantifies how far the degrees of freedom are inflated when technical subsamples are treated as replicates. Applying a false-discovery-rate correction instead of the Holm procedure does not alter the outcome, the second-ranked contrast reaching p = 0.100.

Allelopathic profiles of the agroecosystems. Comparative analysis of the five soils shows differentiation of their allelopathic profiles, but with a magnitude and a certainty that differ from the previous version of this manuscript. The walnut soil is characterised by the widest dispersion of the normalized response index (SD = 0.334), which is consistent with the specialised action of juglone, an allelochemical with a documented mechanism of inhibiting mitochondrial respiration [Kohli et al., 2006]; several test crops nevertheless showed stimulation in this soil, which may reflect hormesis or species-specific detoxification. The *Echinacea purpurea* soil gives the lowest values of every integrated index (mean NRI = −0.086), in line with the phytochemical activity of this species, whereas the fallow soil gives the highest (NRI = +0.130), which is plausibly related to a more mature soil microbiota capable of degrading allelochemicals or of synthesising growth-promoting substances, in agreement with the concept of indirect trophic components of soil legacy proposed by Grove et al. [2012]. The principle of species-specific allelopathic interaction validated by Lenda et al. [2023] is reflected quantitatively in our data: Kendall's coefficient of concordance across the 16 crops is 0.170 (Friedman $\chi^2 = 10.85$, df = 4, p = 0.028), the fallow soil being ranked first by six crops and last by none, and the *Echinacea* soil last by seven and first by

Table 6. Key parameter estimates and interaction terms of fixed effects

Term	Odds ratio (OR) G	Odds ratio (OR) V	Response ratio (RR) RL	Response ratio (RR) SL	Log coefficient (RSR)	Multiplicative RR
Shell pea	2.476	2.001	7.204	3.009	0.873	2.394
Popcorn (maize)	2.712	1.249	5.437	1.494	1.292	3.639
Fallow (herbage)	4.559	2.366	1.067	1.783	-0.513	0.599
Shell pea x fallow	2.296	7.674	1.164	0.665	0.560	1.752
Popcorn x fallow	0.327	0.770	1.040	0.571	0.600	1.822

Note: Multiplicative RR = exp(Log coefficient RSR). The reported terms are the pre-specified key effects. Estimates come from the interaction model and are therefore simple effects at the reference agroecosystem; the Bayesian multivariate model in Figure 2 is specified additively and its coefficients are main effects, so the two sets are not directly comparable. Standard errors, confidence intervals and p-values for all terms are given in Supplement S6.

Table 7. Estimated marginal means of early-growth metrics across agroecosystems

Agroecosystem	Germination	Viability	Root length	Shoot length	NRI	SEI	Composite vigor
Fallow (herbage)	0.894a	0.933a	4.345a	5.049a	0.130a	0.286a	0.794a
Apricot	0.870a	0.921a	4.044ab	4.505ab	0.056ab	0.180a	0.292ab
Sand (control)	0.792a	0.884a	3.778ab	4.219ab	-0.006ab	-0.092a	-0.101ab
Walnut	0.830a	0.902a	3.579ab	3.957ab	-0.030ab	-0.160a	-0.338ab
Echinacea purpurea	0.758a	0.860a	3.465b	3.756b	-0.086b	-0.214a	-0.647b

Note: Cell values are estimated marginal means from the replicate-level models; root and shoot lengths are back-transformed from the log scale. Letters are assigned at the crop-block level (each of the 16 test crops is one block; paired t-test with Holm correction across all 10 pairwise contrasts) and are identical to those in Table 5 and Figure 1. Agroecosystems sharing a letter were not shown to differ.

two. No agroecosystem inhibited or stimulated all 16 test crops to a comparable degree, which is precisely why multivariate frameworks are required and why a bioassay based on a single test species cannot rank agroecosystems reliably.

Among the main effects, shell pea and popcorn show the largest response ratios for root length, 7.204 and 5.437 respectively, and the fallow soil the largest odds ratios for germination and viability, 4.559 and 2.366 (Table 6). The interaction terms confirm that the response of an individual crop is modified by the soil: the term popcorn × fallow gives an odds ratio of 0.327 for germination, and shell pea × fallow an odds ratio of 7.674 for viability. These estimates come from the interaction model and are simple effects at the reference agroecosystem; the Bayesian multivariate model of Figure 2 is specified additively and its coefficients are main effects, so the two sets are not directly comparable. Standard errors, confidence intervals and p-values for all 79 terms of the eight models are given in Supplement S6.

The fallow soil gives the highest marginal means for every metric: germination 0.894, viability 0.933, root length 4.345 cm, shoot length 5.050 cm, NRI 0.130, SEI 0.286 and composite vigor 0.794. The lowest values belong to the *Echinacea* soil, 0.758, 0.860, 3.470 cm, 3.760 cm, −0.086, −0.214 and −0.647 respectively. At the crop-block level the two extremes are separated for root length, shoot length, the normalized response index and composite vigor, while germination, viability and the stress effect index show no differences among agroecosystems; the three intermediate soils share letters with both extremes for every metric. The letters are identical to those of Table 5 and of Figure 1, all three originating from the same crop-block analysis.

The largest posterior mean effects for root length belong to shell pea (2.306; 95 % HPD 2.110 to 2.475), popcorn (1.900; 1.715 to 2.086) and winter wheat (1.793; 1.619 to 1.983); for shoot length, to winter wheat (1.454; 1.249 to 1.667), common buckwheat (1.134; 0.914 to 1.322) and radish (1.019; 0.805 to 1.211), with perennial lupine showing the largest negative effect (−1.338; −1.544 to −1.132). The posterior residual correlation between root and shoot length is 0.762 (95 % HPD 0.715 to 0.808), that is, strongly positive: after the fixed effects are accounted for, trays

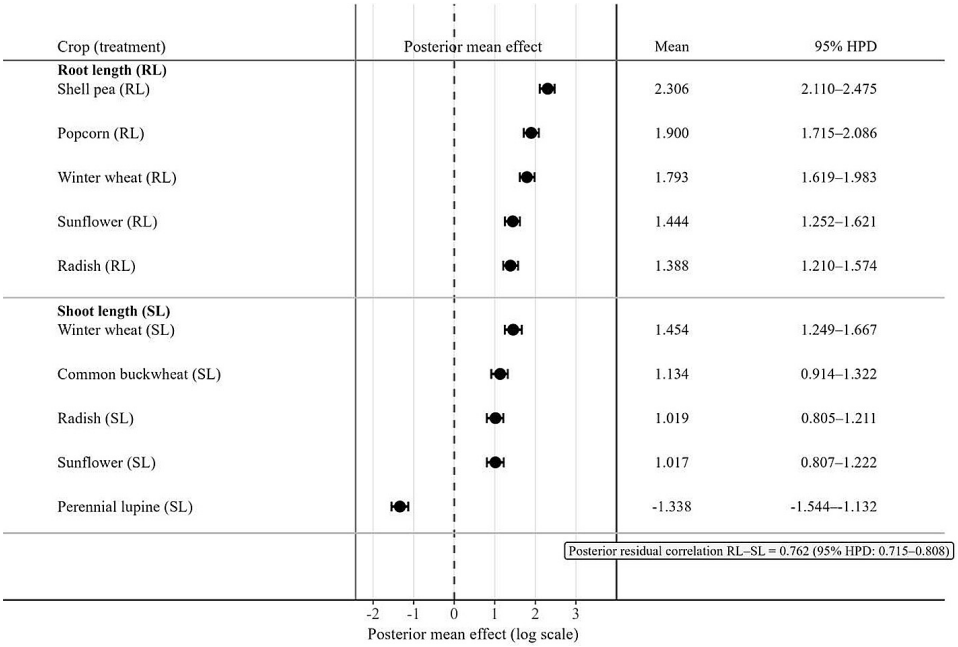

Figure 2. Posterior effects of the Bayesian multivariate model for root and shoot length

with longer roots also have longer shoots, which is the expected allometric signature. To evaluate the spatial structure of the integrated biological response, an array of multivariate ordination methods was applied, spanning unsupervised and constrained techniques (Table 8).

The first two principal components explain 51.8% and 23.0% of the variance, 74.8% cumulatively (Figure 3). The first axis is formed by the integrated indices together with root and shoot length and represents a general morphofunctional gradient; the second axis separates the test crops and reflects their species-specific biology. Non-metric multidimensional scaling in two dimensions converged with a stress of 0.132, which is within the range conventionally regarded as a usable representation, and reproduced the configuration obtained by the principal component analysis. Distance-based redundancy analysis constrained by the test crop and the agroecosystem explained 73.7% of the inertia.

To construct a comprehensive multi-dimensional assessment of the data structure, a distance-based permutational multivariate analysis

Table 8. Ordination structure of the integrated biological response

Method	Axis coordinates	Axis 1 range	Axis 2 range	Core structural conclusion
PCA	Dim1–Dim2	-3.00 to 7.09	-3.19 to 2.90	Linear gradient; 74.8% cumulative variance
NMDS	MDS1–MDS2	-7.73 to 3.22	-2.96 to 3.00	Non-linear ordination; stress = 0.132
db-RDA	dbRDA1–dbRDA2	-1.36 to 0.82	-1.64 to 0.75	Constrained ordination; 73.7% explained inertia

Note: db-RDA was used as the constrained distance-based ordination; conventional CCA was not fitted.

Table 9. Multivariate tests of crop and agroecosystem effects (PERMANOVA, BETADISPER, db-RDA)

Method	Source factor	Df	F-value	p-value	Structural interpretation
PERMANOVA	Test crop	15	118.519	<0.001	Dominant multivariate differentiation
PERMANOVA	Agroecosystem	4	19.312	<0.001	Significant edaphic legacy effect
PERMANOVA	Test crop × agroecosystem	60	7.007	<0.001	Crop-specific edaphic response
BETADISPER	Agroecosystem	4	2.354	0.053	Homogeneity of dispersion assessed
BETADISPER	Test crop	15	7.528	<0.001	Dispersion differs among test crops
db-RDA	Test crop	15	53.838	<0.001	Dominant constrained effect
db-RDA	Agroecosystem	4	8.773	<0.001	Significant secondary constrained effect

Note: PERMANOVA and db-RDA p-values are permutation-based.

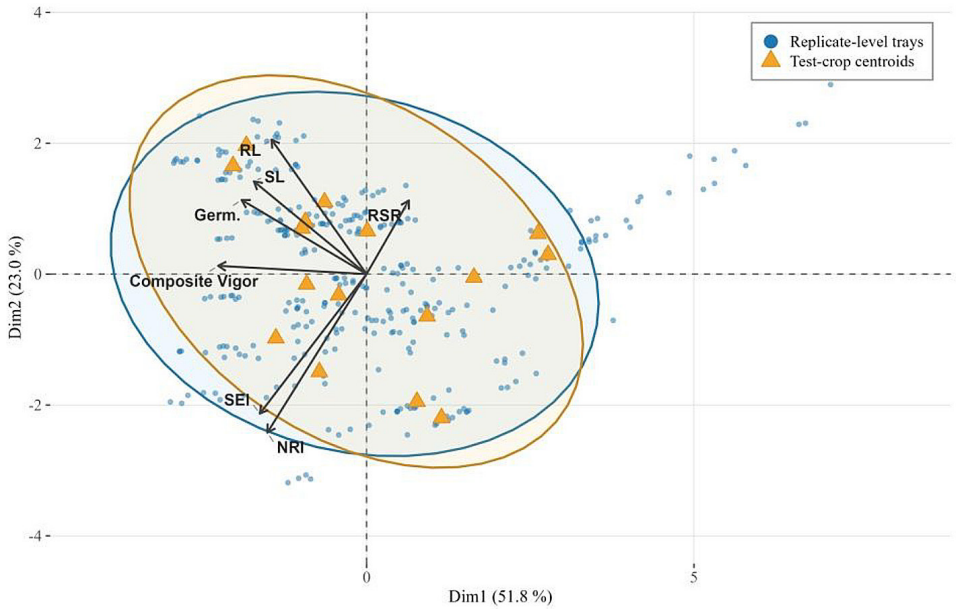

Figure 3. Principal component biplot of replicate-level early-growth responses and test-crop centroids

of variance (PERMANOVA), homogeneity of multivariate dispersions testing (BETADISPER), and distance-based redundancy analysis (db-RDA) were executed. The combined statistical outputs are consolidated in Table 9. All multivariate analyses are conducted at the replicate level (n = 320) and are therefore descriptive with respect to the agroecosystem factor, for which the biological replication is one composite sample per agroecosystem. The significant BETADISPER result for test crop indicates that part of the crop effect reflects heterogeneous within-group dispersion rather than centroid displacement alone.

At the replicate level the test crop accounts for 70.7% of the multivariate variation (F = 118.5), the crop-by-agroecosystem interaction for 16.7% (F = 7.01) and the agroecosystem for 3.1% (F = 19.3), with 9.5% unexplained; all three terms are significant at p = 0.0001 with 9999 permutations. Homogeneity of multivariate dispersions is not rejected for the agroecosystem factor (F = 2.354, p = 0.053) but is rejected for the test crop (F = 7.528, p = 0.0001), so part of the crop effect reflects heterogeneous within-group dispersion rather than displacement of centroids, and the two results are interpreted jointly. Distance-based redundancy analysis confirms both factors, with a far larger F-ratio for the test crop (53.8) than for the agroecosystem (8.77).

Correlation analysis of the seven metrics at the replicate level shows predominantly positive relationships. The strongest association is between the normalized response index and the stress effect index (r = 0.927; 95% CI 0.909 to 0.941), which confirms that the two indices capture convergent aspects of early seedling response and are partly redundant. Composite vigor correlates with germination (r = 0.791; 0.746 to 0.829), shoot length (r = 0.733; 0.678 to 0.780) and root length (r = 0.668; 0.602 to 0.724), that is, germination and elongation jointly determine the integrated index. Germination is associated with shoot length (r = 0.709; 0.650 to 0.760) more closely than with root length (r = 0.600; 0.525 to 0.666). The root-to-shoot ratio behaves differently from the remaining variables, correlating negatively with shoot length (r = −0.408; −0.495 to −0.312) and with the Normalized Response Index (r = −0.225; −0.326 to −0.118) and positively with root length (r = 0.296; 0.192 to 0.392), which reflects its function as a measure of allocation rather than of size. All coefficients except the association between root length and the normalized response index remain significant after Holm correction across the 21 pairs; the complete matrix with confidence intervals is given in Supplement S7b.

Deploying PERMANOVA and db-RDA on a matrix of seven metrics (GERMINATION, ROOT, SHOOT, RSR, NRI, SEI, CV) effectively determines whether the global allelopathic profile shifts systematically across agroecosystems – a resolution that single univariate GLMMs may miss due to multiplicity adjustment constraints. Maidana-Tuco et al. [2025] implemented a parallel strategy to map the comparative

allelopathy of Pteridium esculentum across multiple woody species, verifying the suitability of this methodology. The Bayesian multi-response model implemented via MCMCglmm [Hadfield, 2010] uniquely isolates the true posterior correlation between ROOT and SHOOT parameters – a critical allometric signature of seedling development under chemical stress. The endophytic microbiome of donor plants can profoundly alter soil legacy: Minás et al. [2021] showed that endophytes shape a specialized allelopathic footprint, driven by differential impacts of above- and below-ground litter on acceptor performance. For the systematic deployment of allelopathic potential within Chernozem management architectures, the approach outlined by Boincean and Dent [2020] is paramount: the deliberate integration of crops with verified allelopathic activity into rotations as an instrument of sustainable soil fertility management.

Ward clustering of the 80 crop-by-agroecosystem combinations on Euclidean distances between standardised metrics separates three groups of 11, 34 and 35 combinations; the ten combinations closest to the cluster centroids are displayed (Figure 4). The first group collects combinations with markedly depressed values of the length and germination metrics, the second an intermediate tier, and the third combinations with elevated values. Full cluster membership is supplied as a supplementary file.

Model performance and its independent verification

The high coefficients of determination reported in Table 1 follow from the structure of the design rather than from overfitting, and this deserves an explicit statement. The fixed part of every model is the full factorial of 16 test crops and five agroecosystems, that is, 80 cells estimated by 80 parameters, so the model reproduces the mean of each cell almost exactly. What remains as residual variation is only the dispersion among the four trays within a cell, and each tray value is itself an average over 25 seeds. Averaging suppresses seed-level variability by design; consequently a large proportion of the variance of tray means is explained by the cell structure. The same mechanism produces the underdispersion of the simulated residuals, and underdispersion makes the reported standard errors conservative rather than liberal, so it cannot generate spurious significance.

The value of $R^2 = 1.00$ obtained for PERMANOVA in the previous version of this manuscript has a different and purely technical origin, and it has been corrected. The analysis had been performed on the 80 aggregated treatment combinations, for which the full factorial model consumes 79 degrees of freedom and leaves none for the residual; the decomposition is then an algebraic identity that returns unity irrespective of the

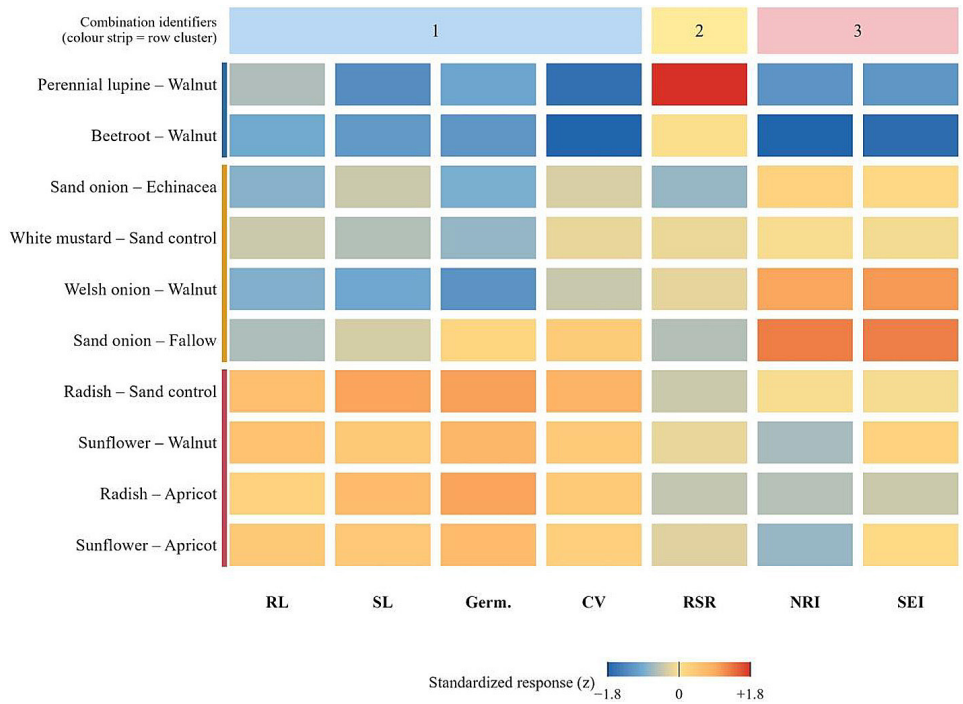

Figure 4. Compact heatmap of standardized early-growth responses grouped by metric clusters

data. All multivariate analyses are now conducted at the replicate level, where 240 residual degrees of freedom remain.

Independent verification of the model fit was carried out. Four-fold cross-validation stratified by cell gave a shrinkage of the coefficient of determination between 0.020 and 0.072, that is, the out-of-sample fit is nearly identical to the in-sample fit; a model that merely reproduced noise would lose far more. Estimation with an alternative engine changed no coefficient by more than 1×10^{-5}. Re-analysis of germination by beta regression, a different distributional family, reproduced the same ordering of agroecosystems. Leave-one-out over test crops, a parametric bootstrap with 1000 replicates and three alternative specifications of the tray effect are reported in Supplement S7; the bootstrap intervals exclude zero for the fallow and *Echinacea* soils only, in agreement with the crop-block analysis.

Limitations

The principal limitation is the level of replication of the agroecosystem factor. The soil of each agroecosystem originates from a single 0.15 ha plot and was processed as one composite sample; the four trays are technical subsamples. The study therefore describes the five soils that were actually sampled and cannot, by itself, establish that the differences observed are properties of the agroecosystem types in general.

The response is markedly crop-specific. Kendall's coefficient of concordance across the 16 crops is 0.170, so the ranking of agroecosystems obtained with one test species is largely not transferable to another. Leave-one-out over crops changes the marginal means by 0.45 to 0.58 units, an amount comparable to the effect itself. Any practical recommendation derived from a single test species should be treated as provisional.

A ceiling effect constrains the germination endpoints. Winter wheat reached complete germination in four of the five soils, and four cells for germination and five for viability contained no variation at all. The penalized estimation described in the Methods keeps these cells in the analysis, but no bioassay can resolve differences among soils in a species that germinates completely everywhere.

The physical arrangement of the trays was not randomised. Trays were placed haphazardly on the shelves of a rack beside a window and were not rotated during the seven days of the bioassay, so shelf position is confounded with tray identity and with the amount of natural daylight received. This is the most plausible explanation of the systematic tray effect, in which the first tray performed consistently below the others. Specifying the tray as a random intercept, as a fixed block or omitting it altogether leaves the ranking of agroecosystems unchanged, so the conclusions are not driven by this effect; nevertheless a rotation scheme or a randomised position assignment would remove this source of variation in future work, and illumination should be recorded rather than assumed uniform.

Finally, the design measures a soil legacy effect and not allelopathy in the strict sense. The composite samples differ in their entire history, so nutrient status, physical structure and the soil microbial community vary together with any allelochemical residue, and no chemical characterisation of phenolic or terpenoid compounds was performed. Attributing the observed differences specifically to allelochemicals would require chemical quantification and activated-carbon or sterilisation controls, neither of which was part of this experiment. The bioassay was conducted indoors under natural daylight on a single sampling occasion in April, with temperature between 20 and 25 °C and with photoperiod and photon flux density unrecorded; the extrapolation of its outcome to field emergence therefore remains to be tested.

CONCLUSIONS

1. Early seedling development is controlled jointly by the biology of the test crop and by the soil legacy of the agroecosystem, with the crop dominating. Variance partitioning attributes 43.3% to 91.8% of the variation to the test crop, at most 7.4% to the agroecosystem and 5.6% to 31.5% to their interaction; in the multivariate analysis the corresponding shares are 70.7%, 3.1% and 16.7%.
2. At the level of replication appropriate to the design, with the 16 test crops as blocks, one difference between agroecosystems is supported after correction for multiple comparisons: fallow with mixed herbage exceeds the *Echinacea purpurea* soil in composite vigor ($\Delta = 1.440$; 95% CI 0.572 to 2.308; p[Holm] = 0.030), and the same contrast is supported for

root length, shoot length and the Normalized response index. Germination, viability and the stress effect index show no differences among agroecosystems at this level, and the ordering of the three intermediate soils is descriptive.

3. The effect of a soil depends on the species used to measure it. Kendall's coefficient of concordance across the 16 test crops is 0.170, and the crop-by-agroecosystem interaction accounts for 23.1% of the variance of composite vigor. A bioassay based on a single test species is therefore not a reliable instrument for ranking agroecosystems, and multi-species designs are required.
4. The integrated indices are informative but partly redundant: the normalized response index and the stress effect index capture convergent aspects of early seedling response, and composite vigor summarises them together with the two length metrics. Their use as rapid biodiagnostic instruments is justified, provided that the level of replication of the factor under test is respected.
5. Explicit attention to the hierarchy of the design changes the conclusions materially. Treating the four trays as replicates yields four supported contrasts out of ten; treating the test crops as blocks yields one. The framework combining generalized and linear mixed models with multivariate ordination remains appropriate, but its output must be read at the level at which the factor of interest is replicated.
6. For agricultural practice the results indicate that a mixed-herbage fallow constructs a more favourable soil legacy for early crop development than an *Echinacea purpurea* stand, while the position of apricot, walnut and the abiotic control cannot be resolved by the present design.

REFERENCES

1. Anderson, M.J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology, 26*, 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x
2. Anderson, M.J. (2006). Distance-based tests for homogeneity of multivariate dispersions. *Biometrics, 62*(1), 245–253. https://doi.org/10.1111/j.1541-0420.2005.00440.x
3. Balah, M.A., Al-Andal, A., Radwan, A.M., Donia, A.E.R.M. (2024). Unveiling allelopathic dynamics and impacts of invasive *Erigeron bonariensis* and *Bidens pilosa* on plant communities and soil parameters. *Sci Rep., 14*, 10159. https://doi.org/10.1038/s41598-024-57552-7
4. Bartoń, K. (2026). *MuMIn: Multi-Model Inference* (Version 1.48.19) [Computer software]. Comprehensive R Archive Network (CRAN). https://CRAN.R-project.org/package=MuMIn
5. Bates, D., Mächler, M., Bolker, B., Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01
6. Ben Saad, R., Ben Romdhane, W., Wiszniewska, A., Baazaoui, N., Taieb Bouteraa, M., Chouaibi, Y., Alfaifi, M. Y., Kačániová, M., Čmiková, N., Ben Hsouna, A., Garzoli, S. (2024). *Rosmarinus officinalis* L. essential oil enhances salt stress tolerance of durum wheat seedlings through ROS detoxification and stimulation of antioxidant defense. *Protoplasma, 261*(6), 1207–1220. https://doi.org/10.1007/s00709-024-01965-8
7. Blanco, J. A. (2007). The representation of allelopathy in ecosystem-level forest models. *Ecological Modelling*, *209*(2–4), 65–77. https://doi.org/10.1016/j.ecolmodel.2007.06.014
8. Blum, U. (2006). Allelopathy: A soil system perspective. *Allelopathy*, 299–340. https://doi.org/10.1007/1-4020-4280-9_14
9. Boincean, B., Dent, D. (2020). Land use, soil quality and management of soil organic matter. *Sustainable management of chernozem soils. Springer,* 29–51. https://doi.org/10.1007/978-3-030-22533-9
10. Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., White, J. S. S. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution*, *24*(3), 127–135. https://doi.org/10.1016/j.tree.2008.10.008
11. Cheng, F., Cheng, Z. (2015). Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Frontiers in Plant Science*, *6*, 1020. https://doi.org/10.3389/fpls.2015.01020
12. Chung, Y., Rabe-Hesketh, S., Dorie, V., Gelman, A., Liu, J. (2013). A nondegenerate penalized likelihood estimator for variance parameters in multilevel models. *Psychometrika*, *78*(4), 685–709. https://doi.org/10.1007/s11336-013-9328-2
13. Dehodiuk, S., Davydiuk, H., Butenko, A., Litvinova, O., Shkarivska, L., Klymenko, I., Tonkha, O., Litvinov, D. (2025). Chemical composition of bottom silts as an indicator of environmental contamination in a small river basin. *Agriculture and Forestry, 71*(3), 189–213. https://doi:10.17707/AgricultForest.71.3.12
14. Ens, E. J., French, K., Bremner, J. B. (2009). Evidence for allelopathy as a mechanism of community composition change by an invasive exotic shrub, *Chrysanthemoides monilifera* spp. rotundata.

Plant and Soil, *316*(1–2), 125–137. https://doi.org/10.1007/s11104-008-9765-3

15. Ferreira, M. I., Reinhardt, C. F. (2016). Allelopathic weed suppression in agroecosystems: a review of theories and practices. *African Journal of Agricultural Research*, *11*(6), 450–459. https://doi.org/10.5897/AJAR2015.10580
16. Gbur, E. E., Stroup, W. W., McCarter, K. S., Durham, S., Young, L. J., Christman, M., West, M., & Kramer, M. (2012). Analysis of generalized linear mixed models in the agricultural and natural resources sciences. *Madison: American Society of Agronomy*, 304. https://doi.org/10.2134/2012.generalized-linear-mixed-models.index
17. Gianinetti, A. (2020). Basic features of the analysis of germination data with generalized linear mixed models. *Data, 5*(1), 6. https://doi.org/10.3390/data5010006
18. Glogov, P. (2024). Role of allelopathy in the ecological strategy of some invasive alien tree species posing a threat to the natural forests in Bulgaria. *The Scientific Heritage*, *132*, 6–10. https://doi.org/10.5281/zenodo.10795924
19. Grove, S., Haubensak, K.A., Parker, I.M. (2012). Direct and indirect effects of allelopathy in the soil legacy of an exotic plant invasion. *Plant Ecol., 213*, 1869–1882. https://doi.org/10.1007/s11258-012-0079-4
20. Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *Journal of Statistical Software*, 33(2), 1–22. https://doi.org/10.18637/jss.v033.i02
21. Hamburdă, S. B., Munteanu, N., Stoleru, V., Teliban, G. C., Galea, F. M., Sava-Pavăl, S., Cojocaru, A. (2015). The allelopathy relations' importance in developing technologies for intercropping cultivation system. *Lucrări Ştiinţifice: Seria Horticultură*, 58(1), 87–94. https://www.uaiasi.ro/revista_horti/files/Nr1_2015/Vol%2058_1_2015(14).pdf
22. Harrison, X. A. (2014). Using observation-level random effects to model overdispersion in count data in ecology and evolution. *Peer J., 2*, e616. https://doi.org/10.7717/peerj.616
23. Hartig, F. (2026). *DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models* (Version 0.5.0) [Computer software]. Comprehensive R Archive Network (CRAN). https://cran.r-project.org/web/packages/DHARMa/index.html
24. Hryhoriv, Y., Butenko, A., Karbivska, U., Solovei, H., Datsko, O., Tymchuk, D.S., Krokhin, S., Kriuchko, L., Tokman, V. (2025). Qualitative and quantitative assessment of soils in the Western forest-steppe of Ukraine. *Agriculture and Forestry*, *71*(2), 23–38. https://doi:10.17707/AgricultForest.71.2.02
25. Hurlbert, S.H. (1984). Pseudoreplication and the design of ecological field experiments. *Ecological Monographs, 54*(2), 187–211. https://doi.org/10.2307/1942661
26. Inderjit, Weiner, J., (2001). Plant allelochemical interference or soil chemical ecology? Perspectives in Plant Ecology, *Evolution and Systematics*, *4*(1), 3–12. https://doi.org/10.1078/1433-8319-00011
27. Jardim Amorim, D., Pereira dos Santos, A. R., Nunes da Piedade, G., Quelvia de Faria, R., Amaral da Silva, E. A., Pereira Sartori, M. M. (2021). The use of the generalized linear model to assess the speed and uniformity of germination of corn and soybean seeds. *Agronomy, 11*(3), 588. https://doi.org/10.3390/agronomy11030588
28. Jiang L., Zheng Z., Fang H., Yang J. (2021). A generalized linear mixed model association tool for biobank-scale data. *Nature Genetics, 53*(11), 1616–1621. https://doi.org/10.1038/s41588-021-00954-4.
29. Kohli, R. K., Batish, D. R., Singh, H. P. (2006). Allelopathic interactions in agroecosystems. Allelopathy: A physiological process with ecological implications. *Springer*, 465–493. https://doi.org/10.1007/1-4020-4280-9_21
30. Korkhova, M., Mykolaichuk, V. (2021). Allelopathic properties of winter wheat varieties in the phase of full maturity in the Steppe Zone of Ukraine. *Ukrainian Black Sea Region Agrarian Science*, *25*(3), 46–53. https://doi.org/10.31521/2313-092X/2021-3(111)-6
31. Kuznetsova, A., Brockhoff, P. B., Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software, 82*(13), 1–26. https://doi.org/10.18637/jss.v082.i13
32. Lee, Y., Nelder, J.A., Pawitan, Y. (2006). Generalized Linear Models with Random Effects: Unified Analysis via H-likelihood (1st ed.). *Chapman and Hall/CRC*, 416. https://doi.org/10.1201/9781420011340
33. Legendre P., Anderson M. J. (1999). Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs, 69*(1), 1–24. https://doi.org/10.1890/0012-9615(1999)069[0001:DBRATM]2.0.CO;2.
34. Lenda, M., Steudel, B., Skórka, P., Zagrodzka, Z. Moroń, D., Bączek-Kwinta, R., Janowiak, F., Baran, A., Possingham, H. P., Knops, J. M. H. (2023). Multiple invasive species affect germination, growth, and photosynthesis of native weeds and crops in experiments. *Scientific Reports, 13*(1), 22146. https://doi.org/10.1038/s41598-023-48421-w
35. Lenth, R. V., Piaskowski, J. (2026). emmeans: *Estimated Marginal Means, aka Least-Squares Means* (Version 2.0.3) [Computer software]. Comprehensive R Archive Network (CRAN). https://doi.org/10.32614/CRAN.package.emmeans
36. López-Almansa, J. C. (2023). Ecological factors

related to seed germination and early seedling establishment in *Ulmus minor* Mill., an endangered riparian tree species. *Forestry: An International Journal of Forest Research, 96*(5), 775–786. https://doi.org/10.1093/forestry/cpad014

37. Lüdecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P., Makowski, D. (2021). An R package for assessment, comparison and testing of statistical models. *Journal of Open Source Software*, *6*(60), 3139. https://doi.org/10.21105/joss.03139
38. Maidana-Tuco, Y., Pacheco, L.F., Hensen, I., Gallegos, S. C. (2025). Weak allelopathic effects of bracken fern (*Pteridium esculentum subsp. arachnoideum*) on early life stages of native tree species in a Neotropical montane forest. *Plant Ecol., 226*, 251–263. https://doi.org/10.1007/s11258-025-01488-z
39. McCulloch, C. E., Searle, S. R. (2001). *Generalized, Linear, and Mixed Models*. New York: Wiley. https://doi.org/10.1002/0471722073
40. Minás, A., García-Parisi, P. A., Chludil, H., Omacini, M. (2021). Endophytes shape the legacy left by the above- and below-ground litter of the host affecting the establishment of a legume. *Functional Ecology, 35*(12), 2870–2881. https://doi.org/10.1111/1365-2435.13938
41. Mohammed, A. A. (2025). Allelopathy of *Rumex* spp.: A review. *Aswan University Journal of Environmental Studies*, *6*(4), 322–334. https://doi.org/10.21608/aujes.2025.367558.1335.
42. Moskalik, H. G., Legeta U. V. (2019). Allelopathic properties of species of the genus *Acer L. Scientific Bulletin of Chernivtsi University. Biology (Biological Systems)*, *11*(2), 70–75. https://doi.org/10.31861/biosystems2019.02.154. [in Ukrainian].
43. Nakagawa S., Schielzeth H. (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution, 4*(2), 133–142. https://doi.org/10.1111/j.2041-210x.2012.00261.x
44. Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., De Caceres, M., Durand, S., Evangelista, H. B. A., Hannigan, G., Weedon, J. (2026). *Vegan: Community Ecology Package* (Version 2.7–5) [Computer software]. Comprehensive R Archive Network (CRAN). https://CRAN.R-project.org/package=vegan
45. Posit team. (2026). RStudio: Integrated development environment for R. *Posit Software,* PBC. http://www.posit.co/.
46. Onofri, A., Mesgaran, M. B., Ritz, C. (2022). A unified framework for the analysis of germination, emergence, and other time-to-event data in weed science. *Weed Science, 70*(3), 259–271. https://doi.org/10.1017/wsc.2022.8
47. R Core Team. (2024). R: *A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/
48. Ruíz, J.S., Montesinos López, O.A., Ramírez, G.H., Hiriart, J.C. (2023). Generalized linear mixed models with applications in agriculture and biology. *Cham: Springer International Publishing*, 427. https://doi.org/10.1007/978-3-031-32800-8
49. Sahrir M. A. S., Yusoff N., Azizan K. A. (2023). Allelopathy activity under laboratory, greenhouse and field conditions: A review. *AIMS Agriculture and Food*, *8*(1), 78–104. https://doi.org/10.3934/agrfood.2023004
50. Scavo, A., Mauromicale, G. (2021). Crop allelopathy for sustainable weed management in agroecosystems: Knowing the present with a view to the future. *Agronomy, 11*(11), 2104. https://doi.org/10.3390/agronomy11112104
51. Singh, H. P., Batish, D. R., Kohli, R. K. (2001). Allelopathy in agroecosystems: An overview. *Journal of Crop Production*, *4*(2), 1–41. https://doi.org/10.1300/J144v04n02_01
52. Uludağ, A., Uremis, İ., Rusen, M., Tursun, N. (2017). Possible uses of allelopathy in weed control in organic farming in Turkey. *Acta Herbologica*, *26*(2), 87–93. https://doi.org/10.5937/actaherb1702087u
53. Valcheva, E., Popov, V., Marinov-Serafimov, P., Golubinova, I., Nikolov, B., Velcheva, I., Petrova, S. (2019). A case study of allelopathic effect of parsley, dill, onion and carrots on the germination and initial development of tomato plants. *Ecologia Balkanica, 11*(1), 167–177. http://web.uni-plovdiv.bg/mollov/EB/2019_vol11_iss1/167-177_eb.19112.pdf.
54. Vashishth, D. S., Yadav, R., Bachheti, R.K., Arya, A.K., Bachheti, A. (2025) Exploring the applications of allelopathy in agriculture: A review. *Biologica Nyssana, 16*(2), 1371–1387. https://doi.org/10.46793/BiolNyss.16.2.19V
55. Vitanov, O. D., Zelendin, Y. D., Chefonova, N. V., Melnyk, O. V., Ivanin, D. V. (2020). Allelopathic properties of associated tomato plants. *Vegetable and Melon Growing, 67*, 39–50. https://doi.org/10.32717/0131-0062-2020-67-39-50
56. Williamson G. B., Richardson D. (1988). Bioassays for allelopathy: measuring treatment responses with independent controls. *Journal of Chemical Ecology, 14*(1), 181–187. https://doi.org/10.1007/BF01022540
57. Zuur A. F., Ieno E. N., Walker N. J., Saveliev A. A., Smith G. M. (2009). Mixed effects modelling for nested data. *Mixed Effects Models and Extensions in Ecology with R. New York: Springer*, 101–142. https://doi.org/10.1007/978-0-387-87458-6