UC Davis
UC Davis Previously Published Works

Title

Microbiome-enabled genomic selection improves prediction accuracy for nitrogen-related traits in maize.

Permalink

https://escholarship.org/uc/item/17w4m496

Journal

G3: Genes, Genomes, Genetics, 14(3)

Authors

Yang, Zhikai
Zhao, Tianjing
Cheng, Hao
et al.

Publication Date

2024-03-06

DOI

10.1093/g3journal/jkad286

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

G3, 2024, **14(3)**, jkad286
https://doi.org/10.1093/g3journal/jkad286
Advance Access Publication Date: 19 December 2023
Genomic Prediction

Microbiome-enabled genomic selection improves prediction accuracy for nitrogen-related traits in maize

Zhikai Yang ,[1,2] Tianjing Zhao,[3,4] Hao Cheng ,[4] Jinliang Yang [1,2,*]

[1]Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68588, USA
[2]Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
[3]Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
[4]Department of Animal Science, University of California Davis, Davis, CA 95616, USA

*Corresponding author: Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68588, USA; Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE 68583, USA. Email: jinliang.yang@unl.edu

Root-associated microbiomes in the rhizosphere (rhizobiomes) are increasingly known to play an important role in nutrient acquisition, stress tolerance, and disease resistance of plants. However, it remains largely unclear to what extent these rhizobiomes contribute to trait variation for different genotypes and if their inclusion in the genomic selection protocol can enhance prediction accuracy. To address these questions, we developed a microbiome-enabled genomic selection method that incorporated host SNPs and amplicon sequence variants from plant rhizobiomes in a maize diversity panel under high and low nitrogen (N) field conditions. Our cross-validation results showed that the microbiome-enabled genomic selection model significantly outperformed the conventional genomic selection model for nearly all time-series traits related to plant growth and N responses, with an average relative improvement of 3.7%. The improvement was more pronounced under low N conditions (8.4–40.2% of relative improvement), consistent with the view that some beneficial microbes can enhance N nutrient uptake, particularly in low N fields. However, our study could not definitively rule out the possibility that the observed improvement is partially due to the amplicon sequence variants being influenced by microenvironments. Using a high-dimensional mediation analysis method, our study has also identified microbial mediators that establish a link between plant genotype and phenotype. Some of the detected mediator microbes were previously reported to promote plant growth. The enhanced prediction accuracy of the microbiome-enabled genomic selection models, demonstrated in a single environment, serves as a proof-of-concept for the potential application of microbiome-enabled plant breeding for sustainable agriculture.

Keywords: genomic prediction; intermediate omics; microbiomes; mediation analysis; maize

Introduction

An increasing number of studies have suggested that plant-associated microbial communities, especially the microbial species colonizing in the plant roots, can stimulate plant growth (Saleem *et al.* 2019), enhance nutrient availability in soils (Zhu *et al.* 2016; Gomes *et al.* 2018), and decrease abiotic stress responses (Roquigny *et al.* 2017; Xu *et al.* 2018). Harnessing these beneficial microbes in crop production provides a promising opportunity for crop improvement to fight against climate challenges, reduce dependency on chemical fertilizers, and boost genetic gain. Indeed, from the beginning of plant domestication, rhizosphere microbes were reported to be involved in crop performance (Soldan *et al.* 2021). For example, studies have shown that domesticated plants exhibited distinct microbial compositions as compared to the wild ancestors and showed a reduced ability to establish symbiotic relationships with beneficial microbes (Abdullaeva *et al.* 2021; Abdelfattah *et al.* 2022). Recent crop improvement further reduced the microbial diversity in a number of different crop species, including wheat (Hetrick *et al.* 1992), maize (Sangabriel-Conde *et al.* 2014), and soybean (Kiers *et al.* 2007). Realizing the importance of microbiomes in contributing to crop production, recently, efforts have been made to screen for beneficial microbes as potential seed additives to promote plant performance (Singer *et al.* 2021; Yee *et al.* 2021). Though promising results were found in controlled environments (Eida *et al.* 2017; Sessitsch *et al.* 2019; Kaur *et al.* 2020), it is difficult for many microbial inoculants to survive for a long period of time under field conditions (Piromyou *et al.* 2011). Considering the collective genomes of microbial species colonized in plants as the secondary genome of a plant (Berendsen *et al.* 2012), a hologenome (i.e. plant genome with its associated endocellular or extracellular microbiome) approach to incorporate the naturally occurring genotype-specific microbiomes into the genomic selection (GS) protocol provides an alternative strategy to improve tomorrow's crops.

GS, an innovative plant and animal breeding technology, enables the selection of promising individuals (and the associated heritable microbes) to advance to the next generation before or without phenotyping, therefore reducing the generation interval and increasing the genetic gain per unit of time. After the initial introduction of the landmark GS research conducted by Meuwissen *et al.* (2001), animal and plant breeders embraced the unprecedented acceleration of GS with the development of statistical methods and computational tools (de Koning 2016), including genomic best linear unbiased prediction (G-BLUP), ridge regression best linear unbiased prediction (RR-BLUP), and the Bayesian regression models (BayesA, BayesB,

Received on 19 May 2023; accepted on 05 December 2023
© The Author(s) 2023. Published by Oxford University Press on behalf of The Genetics Society of America.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

BayesCπ, Bayes least absolute shrinkage and selection operator [LASSO], etc.) (Gianola *et al.* 2009; Habier *et al.* 2011; Burgueño *et al.* 2012; Wang *et al.* 2015, 2018; Krishnappa *et al.* 2021). The Bayesian regression models (with different assumptions for prior distributions of marker effects) allow genome-wide markers to have different effects and variances and usually achieve high prediction accuracy (Wang *et al.* 2018). However, Bayesian regression models might be more sensitive to the number of QTLs as compared to the RR-BLUP and G-BLUP methods (Wang *et al.* 2015). In addition to these parametric methods, non-parametric methods, such as reproducing kernel Hilbert space (Gianola *et al.* 2006) and deep learning-based methods (Gianola *et al.* 2011), are likely to be more efficient for capturing non-additive genetic effects than conventional methods, while there is no clear superiority in terms of prediction power (Montesinos-López *et al.* 2021). Albeit the rapid development of the GS methods in the past 20 years, little attention has been paid to incorporating host-associated microbiomes into the prediction protocols (but see a recent simulation study in dairy cattle; Pérez-Enciso *et al.* 2021).

For microbiome-enabled GS (MEGS) modeling, it is straightforward to consider different microbes in a linear mixed model as the random variables, similar to the SNP markers used in conventional GS. However, unlike SNPs, microbiomes are dynamic and profoundly affected by soil microenvironments and root exudates, composed of organic acids, polysaccharides, and other metabolites (Canarini *et al.* 2019). These differentially recruited microbiomes by different genotypes may, in turn, impact soil physicochemical characteristics and nutrient bioavailability for plants, such as nitrogen (N) availability, ultimately regulating plant physiological processes and leading to phenotypic variation. In such a scenario, microbiomes can be modeled as an intermediate process bridging the host genotype and phenotype. In human genetics studies, using Mendelian randomization analysis by treating the microbiome as an exposure, results revealed potential causal effects of the human gut microbiome on blood metabolites (Liu *et al.* 2022), ulcerative colitis, rheumatoid arthritis (Kurilshikov *et al.* 2021), abdominal obesity (Xu *et al.* 2021), or even colorectal cancer (Ni *et al.* 2022). However, Mendelian randomization assumes that there is no other pathway through which the instrumental variables (i.e. host genotypes) affect the outcome (i.e. host phenotypes) other than the exposure itself. Violation of this assumption can introduce bias (Hemani *et al.* 2018). Similar to the concept of Mendelian randomization analysis but without the assumption that the effect is solely through the exposure, either directly or indirectly through the host genotypes, genome-wide mediation analysis enables the identification of significant intermediate mediators with large effects (Yang *et al.* 2022).

In this study, by leveraging the GS methods and our recently developed mediation models, we conducted integrative analyses using our previously published datasets (Meier *et al.* 2022; Rodene *et al.* 2022) collected on the maize diversity panel—a panel representing maize genetic diversity in temperate latitudes (Flint-Garcia *et al.* 2005). The rhizosphere microbiomes were collected under high N (HN) and low N (LN) field conditions (Meier *et al.* 2022). From the same fields, phenotypic data were collected using an unmanned aerial vehicle (UAV) in a time-series manner (Rodene *et al.* 2022). In our MEGS analysis, a linear mixed model was used to predict maize phenotype by including both host genotype and rhizosphere microbiome, where SNPs and amplicon sequence variants (ASVs) (from plant rhizobiomes) were treated as random effects with different variance components. Additionally, we modeled a presumed causal chain from host genotype to host-associated microbe to host phenotype using our previously developed high-dimensional mediation analysis method (Yang *et al.* 2022). These methods serve as an initial trial to integrate genome and microbiome data, aiming to enhance prediction accuracy and identify beneficial microbes for use as seed additives in field conditions. Overall, our study highlights the potential of beneficial microbes in enhancing crop performance and emphasizes the importance of considering plant-microbe interactions in plant breeding.

Materials and methods

Microbiome and phenotype data in the maize diversity panel

The rhizosphere microbiome (or rhizobiome) data were obtained from our previously published study collected from a subset of the maize diversity panel ($n = 230$ genotypes) eight weeks after planting in both HN and LN field conditions in 2019 (Meier *et al.* 2022). The rhizobiome data included $n = 3,626$ ASVs that can be clustered into 154 microbial groups. These microbial groups spanned 19 major classes of rhizosphere microbiota. In summary, the relative abundances of $n = 3,626$ ASVs were collected for $n = 795$ observations.

Meanwhile, high throughput phenotyping data were collected from the same field in a time-series manner using UAV (Rodene *et al.* 2022). After image analysis at the plot level, a number of vegetation indices (VIs) were obtained, some of which showed a high correlation with conventional agronomic traits, such as leaf N content and 20-kernel weight. Of the eight VIs assessed, the Visible Atmospherically Resistant Index (VARI) showed the highest overall correlations with both leaf N content and 20-kernel weight; therefore, we will use VARI as a representative trait for illustrating our theme. The environmental conditions under which VIs were collected varied, leading to differences in data quality. Here, we selected the data from 11, 21, and 35 days after rhizobiome data sampling, which shared similar high quality, for our analysis. More details of phenotypic data can be found in Rodene *et al.* (2022).

Genotypic data processing and linkage disequilibrium pruning

The genotypic data for maize HapMap V3.2.1 (with imputation, AGPv4) were obtained from the Panzea database (https://www.panzea.org/genotypes; Bukowski *et al.* 2017). Using the PLINK software (Purcell *et al.* 2007), we merged the variants on different chromosomes and retained the bi-allelic SNPs only. We then performed SNP filtration by discarding variants with the missing rate >0.3 across lines and a minor allele frequency <0.05, resulting in a subset of 22.5 million SNPs. Subsequently, linkage disequilibrium (LD)-based SNP pruning was performed by calculating LD between each pair of SNPs in the window of 10 kb, and one of a pair of SNPs was removed if the LD (R^2) was greater than 0.1. We then shifted the window 10 bp forward and repeated the procedure, resulting in a final subset of 770k SNPs.

Linear mixed model for microbiome-enabled genomic prediction

We conducted the MEGS using the rrBLUP software (Endelman 2011), where $n = 50,000$ randomly selected SNPs and $n = 3,626$ reproducible ASVs were included simultaneously (Meier *et al.* 2022). Below is the model used for genomic prediction with both maize SNPs and rhizobiome ASVs:

$$y_{ijkuv} = \mu + f_i + b_j + sp_u + spb_v + \sum_{l=1}^{n} \alpha_l g_{kl} + \sum_{t=1}^{s} \gamma_t m_{ijkt} + \epsilon_{ijk},$$

$$\alpha_l \overset{iid}{\sim} N(0, \sigma_\alpha{}^2) \qquad \gamma_t \overset{iid}{\sim} N(0, \sigma_\gamma{}^2) \qquad \epsilon_{ijk} \overset{iid}{\sim} N(0, \sigma_\epsilon{}^2),$$

where y_{ijk} is the observation of phenotype for the kth genotype in the jth block uth split plot and vth split plot block with the ith N treatment level, and there are 795 observations in total; μ is the intercept; f_i is the fixed effect of the ith N treatment ($i = 1, 2$); b_j is the fixed effect of the jth block ($j = 1, 2$); sp_u is the fixed effect of the uth split plot ($u = 1, 2, 3, 4$); spb_v is the fixed effect of the vth split plot block ($v = 1, 2, 3$); α_l is the random coefficient of the lth SNP; g_{kl} is the value of the lth SNP for kth genotype ($l = 1, \ldots, n$, where n is the total number of SNPs); γ_t is the random coefficient of the tth ASV ($t = 1, \ldots, s$, where s is the total number of ASVs); m_{ijkt} is the value (log relative abundance from 16S sequencing) of the tth ASV for kth genotype in jth block with the ith N level; and ϵ_{ijk} is the residual error.

In the model, we assumed that the random coefficients of the lth SNP (α_l), the tth ASV (γ_t), and the residual error (ϵ) are independent variables following normal distributions with a mean of zero and estimated variances of σ_α^2 (i.e. $\sigma_\alpha^2 = 2.6 \times 10^{-8}$ for VARI trait at 11 days after rhizobiome sampling), σ_γ^2 (i.e. $\sigma_\gamma^2 = 6.5 \times 10^{-6}$ for VARI trait at 11 days after rhizobiome sampling), and σ_ϵ^2, respectively. To estimate the marker effect variance σ_α^2, we only fitted SNPs with random effects without ASVs in an RR-BLUP model. Similarly, to get an estimate for ASV effects variance σ_γ^2, we only fitted ASVs with random effects with the first three principal components (PCs) of SNPs (fixed effects) to control for the genomic background effect in an RR-BLUP model.

Finally, we assessed the predictive accuracy through randomized five-fold cross-validation. During this process, we conducted randomization at the plot level and ensured that maize genotypes were distinct and non-overlapping between folds. And, to exclude that the possibility of just adding more variables will increase prediction accuracy, we also used shuffled microbiome as control. We kept the rows of the microbiome matrix constant, with each row assigned a randomly non-repetitive sampled ID within the same N-treatment, either within or not within a quadrant.

Conventional GS model by considering ASVs as microenvironmental factors

We conducted the following analyses to account for the microenvironmental effects on the rhizobiome. We fitted a conventional mixed linear model to obtain the BLUP value for each genotype by considering both with ASVs and without ASVs and conducted the analyses under HN and LN conditions separately.

$$y_{ijkl} = \mu + g_i + b_j + sp_k + spb_l + \epsilon_{ijkl},$$

$$y_{ijkl} = \mu + g_i + b_j + sp_k + spb_l + \sum_{t=1}^{154} \gamma_t m_{ijt} + \epsilon_{ijkl},$$

$$g \sim MVN(0, A\sigma_g{}^2) \quad \epsilon_{ijkl} \overset{iid}{\sim} N(0, \sigma_\epsilon{}^2),$$

where y_{ijkl} is the observation of phenotype for ith genotype in jth block, kth split plot, and lth split plot block; μ is the intercept; g_i is the ith genotype effect, $i = 1, \ldots, 230$; A is the additive relationship matrix calculated from SNPs using "A.mat" function from the rrBLUP package; b_j is the jth block effect, $j = 1, 2$; sp_k is the kth split plot effect, $k = 1, 2, 3, 4$; spb_l is the lth split plot block effect; γ_t is the random coefficient of the tth taxonomic group, $t = 1, \ldots, 154$; m_{ijt} is the value of the tth taxonomic group for ith genotype in jth block; $t = 1, \ldots, 154$; and ϵ_{ijkl} is the residual error. We employed the randomized five-fold cross-validation to obtain the prediction accuracy.

Mediation analysis by considering microbes as intermediate variables

Mediation analysis introduces a type of variable called mediator to infer the underlying mechanism of the relationship between an independent variable and a dependent variable (Baron and Kenny 1986). Similar to our previous study (Zhang 2021; Yang *et al.* 2022), here we conducted genome-wide mediation analysis using SNPs as exposures, ASVs as microbe mediators, and the first three PCs with other experiment and treatment factors as confounders. The mediation analysis consists of two models: the mediator model and the outcome model. Specifically, the mediator model is shown as follows:

$$\mathbf{M_j} = \mathbf{QA_j} + \mathbf{ZB_j} + \mathbf{e_j},$$

where $\mathbf{M_j}$ (a $n \times 1$ vector) represents the abundance of the jth ASV; $\mathbf{Q}$ (a $n \times (s + f + b + u + v)$ matrix) is the design matrix for first s PCs of genotypes, f nitrogen treatments b blocks, u split plots, and v split plot blocks ($s = 3, f = 2, b = 2, u = 4, v = 3$ in our analysis); $\mathbf{A_j}$ (a $(s + f + b + u + v) \times 1$ vector) is the coefficient of the first s PCs, f nitrogen treatments, b blocks, u split plots, and v split plot blocks to the jth ASV; $\mathbf{Z}$ (a $n \times q$ matrix) represents the SNP set of the population composed of n individuals with q number of SNPs; $\mathbf{B_j}$ (a $q \times 1$ vector) is the coefficients of the q SNPs to the jth ASV ($j = 1, \ldots, p$); and $\mathbf{e_j}$ is the vector of the residual errors with $\mathbf{e_j} \sim N(\mathbf{0}, \sigma_j^2 \mathbf{I_n})$.

Additionally, we fitted the SNPs and the microbe mediators (i.e. ASVs) in the outcome model, which uses phenotype as the response variable, as shown below:

$$\mathbf{y} = \mathbf{Qv} + \mathbf{Za} + \mathbf{Mc} + \mathbf{e},$$

where $\mathbf{y}$ (a $n \times 1$ vector) represents the phenotype; $\mathbf{Q}$ (a $n \times (s + f + b + u + v)$ matrix) and $\mathbf{Z}$ (a $n \times q$ matrix) are the same matrices as the above mediator model; $\mathbf{v}$ (a $(s + f + b + u + v) \times 1$ vector) is the coefficients of the first s PCs, f nitrogen treatments, b blocks, u split plots and v split plot blocks; $\mathbf{a}$ (a $q \times 1$ vector) denotes the coefficients of the SNPs to the phenotype; $\mathbf{M}$ (a $n \times p$ matrix) is the abundance of the ASVs (i.e. a matrix combines all the vectors of $\mathbf{M_j}$); $\mathbf{c}$ (a $p \times 1$ vector) is the coefficient of ASVs to the phenotype; $\mathbf{e}$ is the vector the residual errors with $\mathbf{e} \sim N(\mathbf{0}, \sigma^2 \mathbf{I_n})$.

Results

Rhizobiome incorporation into the GS model improves prediction accuracy for host phenotype

Tens of thousands of microbial species colonize plant roots, many of which are heritable (Peiffer *et al.* 2013; Meier *et al.* 2021). To assess if rhizosphere microbiomes (hereafter referred to as rhizobiomes) could be leveraged to predict the plant phenotypic performance, we analyzed the composition of rhizobiomes quantified in a diversity panel of 230 maize inbred lines under HN and LN field conditions (Meier *et al.* 2022). Additionally, we obtained the image-extracted VI traits collected from the same field in 2019 (Rodene *et al.* 2022). Since most of the VI traits exhibit high correlation (see Supplementary Fig. S1), we focused on one of the most representative VIs—VARI—collected on three dates, i.e. 11, 21, and 35 days after microbiome sampling (DAMS) in the field. We fitted the host rhizobiomes (ASVs) at varying levels of resolution—3,626 ASVs, 154 taxonomic groups, and 19 classes—along with host genotypes (SNPs) as explanatory variables, by

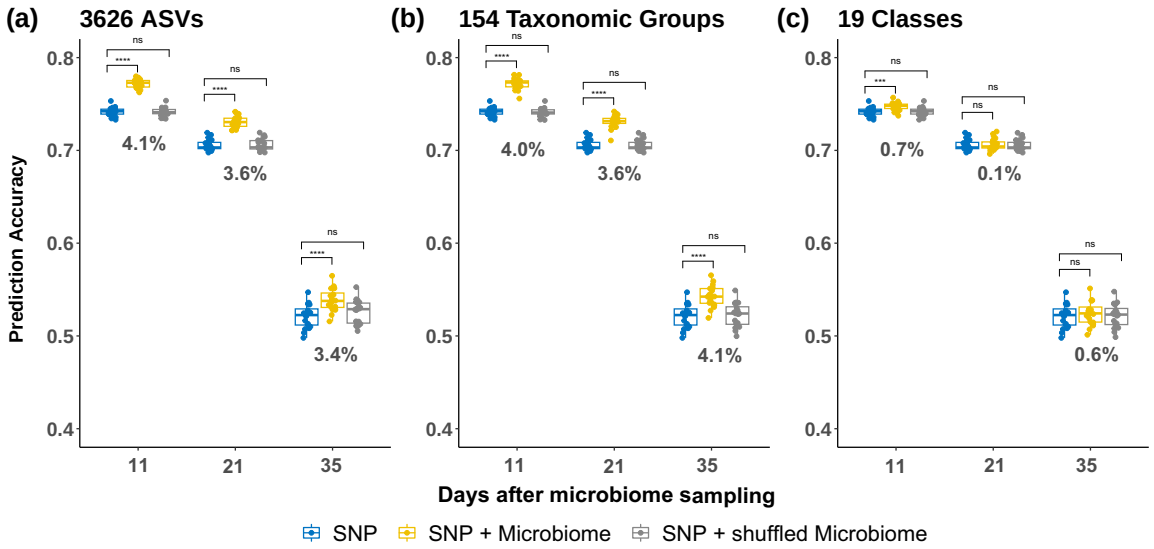

Fig. 1. Comparison of prediction accuracy for an image-extracted trait using different variables in GS models across gradient rhizobiome resolutions. Genomic prediction results using SNPs only (blue), both SNPs and rhizobiomes (yellow), and both SNPs and shuffled rhizobiomes (gray) at different resolutions: a) 3,626 ASVs, b) 154 taxonomic groups, and c) 19 classes. Asterisks indicate the statistical significance of the difference in accuracy between the models: ns (not significant), *(0.01 < P−value ≤ 0.05), **(0.001 < P−value ≤ 0.01), ***(0.0001 < P−value ≤ 0.001), and ****(P−value ≤ 0.0001). The numbers below the boxplots are the improvement of prediction accuracy ($(r_{SNP+ASV} - r_{SNP})/r_{SNP}$, where $r_{SNP+ASV}$ is the prediction accuracy for the SNP and ASV models and r_{SNP} is the accuracy for the SNP-only model) on different dates.

incorporating them as random effects into a linear mixed model (see Materials and Methods). After conducting 20 randomized five-fold cross-validations, our results indicate that including ASVs in the model significantly enhances the GS prediction accuracy compared to the traditional SNP-only model for all three time points when using either 3,626 ASVs (Fig. 1b) or 154 taxonomic groups (Fig. 1b). For the 19 classes (Fig. 1c), a slight increase in prediction accuracy was observed on Day 11 post-microbiome sampling, which was an anticipated result given the substantial decrease in microbiome resolution from 3,626 ASVs to 19 classes. Interestingly, the 154 taxonomic groups are as informative as the 3,626 ASVs, particularly for the 35 DAMS. As the days post-microbiome sampling increased, we noted a decline in overall prediction accuracy, likely due to the decreased heritability of VARI traits as plants close to senescence. To verify that the improved prediction accuracy was not merely a consequence of a larger number of explanatory variables, we generated a comparable set of dummy variables by randomly shuffling the ASVs using different shuffling strategies (see Materials and Methods). Results showed that the additional shuffled variables either did not significantly influence the prediction accuracy (Fig. 1) or improved prediction accuracy but less than the original variables (Supplementary Fig. S2, this shuffling strategy within the quadrant is closer to original order). Compared to the conventional GS model utilizing only SNPs, the addition of ASVs resulted in accuracy improvements of 4.1%, 3.6%, and 3.4% at 11, 21, and 35 DAMS, respectively (Fig. 1a). Even when measured against results from the randomly shuffled ASVs, an average improvement of 3.7% was observed. Using 154 taxonomic groups, the resulting improvement is similar or even slightly higher, with an average improvement of 3.9% (Fig. 1b). Similar results were also observed for other image-extracted traits (see Supplementary Fig. S3).

Prediction improvement is predominantly observed under LN conditions

To investigate the impact of N treatment on MEGS results, we fitted the ASV-based model separately for HN and LN conditions. Under HN conditions, the model did not outperform the conventional SNP-only model (Fig. 2a). However, under LN conditions, significant improvements were observed on all three days (Fig. 2b). Specifically, prediction accuracy increased from 55.9% to 59.7% at 11 DAMS (6.8% improvement), from 52.3% to 56.7% at 21 DAMS (8.4% improvement), and from 24.1% to 33.8% at 35 DAMS (40.2% improvement). While the rhizobiome is likely influenced by microenvironment, to account for its effects, we fitted ASVs as environmental factors in a conventional GS model to obtain the BLUP values (see Materials and Methods). As shown in Supplementary Figure S4, considering ASV in BLUP calculation yielded consistent results with the MEGS model (except for the 11 DAMS under HN conditions), and the results were more substantial and statistically significant under LN conditions. These findings suggest that rhizobiomes are not entirely confounded by microenvironments, as evidenced by the consistent experimental layout under both N conditions. To further test rhizobiome's effect on host phenotype prediction, we conducted a parallel analysis using four yield component traits (cob length, cob weight, cob width, and 20-kernel weight) collected from the same field (Palali Delen *et al.* 2023). MEGS results revealed significant improvements for two yield-related traits (cob length and cob weight) under HN and three traits (cob length, cob weight, and cob width) under LN conditions (Supplementary Fig. S5). Similar to VI traits, the enhancements for cob length and cob weight were more pronounced under LN than HN conditions (Supplementary Fig. S5).

Microbiomes exhibit more pronounced effects in the early DAMS

To better understand the impact of specific microbes on prediction, we evaluated the effect sizes of the ASVs and SNPs included in the prediction model (Fig. 3). As expected, the overall SNP effects remained largely consistent across different dates (Fig. 3b). In contrast, the effects of ASVs diminished with the increasing interval between the initial microbiome sampling and the subsequent collection of plant phenotypes (Fig. 3a). The investigation into the relationship between the effect sizes of ASVs and their respective taxonomic groups revealed highly significant results

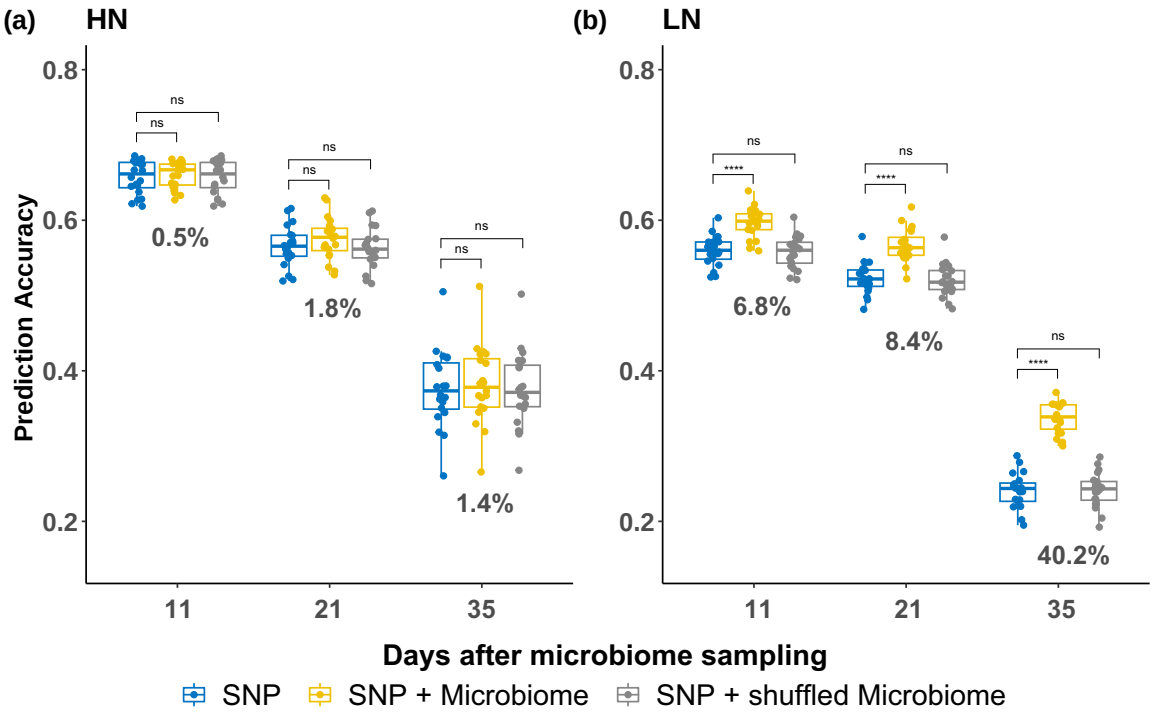

Fig. 2. Prediction accuracy of incorporating rhizobiome under different N conditions. The prediction accuracy of using SNPs only (blue), using both SNPs and rhizobiomes (yellow), and using both SNPs and randomly shuffled rhizobiomes (gray) in a) HN and b) LN conditions. Asterisks indicate the statistical significance of the difference in accuracy between the models: ns (not significant), *(0.01 < *P*–value ≤ 0.05), **(0.001 < *P*–value ≤ 0.01), ***(0.0001 < *P*–value ≤ 0.001), and ****(*P*–value ≤ 0.0001).

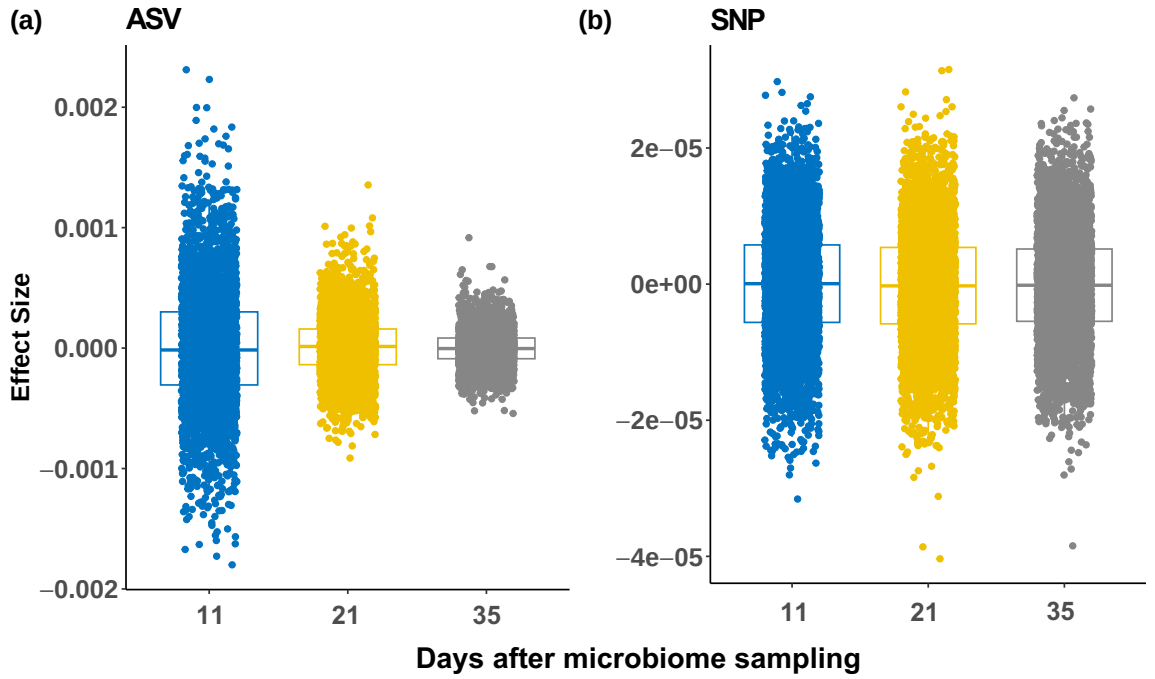

Fig. 3. The effect sizes of ASVs and SNPs on the VARI phenotype. The distribution of effect size of a) ASVs and b) SNP at different DAMS.

(Kruskal–Wallis test, *P*–value < 2.2×10^{-16}) for both N conditions on all three days, suggesting a disproportionate enrichment of large effect ASVs within certain taxonomic groups. Notably, among the top 1% large effect ASVs (Supplementary Table S1), four ASVs from the taxonomic groups *Massilia niabensis*, *Microbacterium testaceum*, *Pseudomonas parafulva*, and *Sphingomonas limnosediminicola* consistently exhibited relatively large effects on VARI trait across all dates in both N conditions. Additionally, we identified one ASV belonging to *P. parafulva* that showed a consistently large effect under HN conditions and another ASV associated with *Solirubrobacter* that consistently showed a large effect under LN conditions.

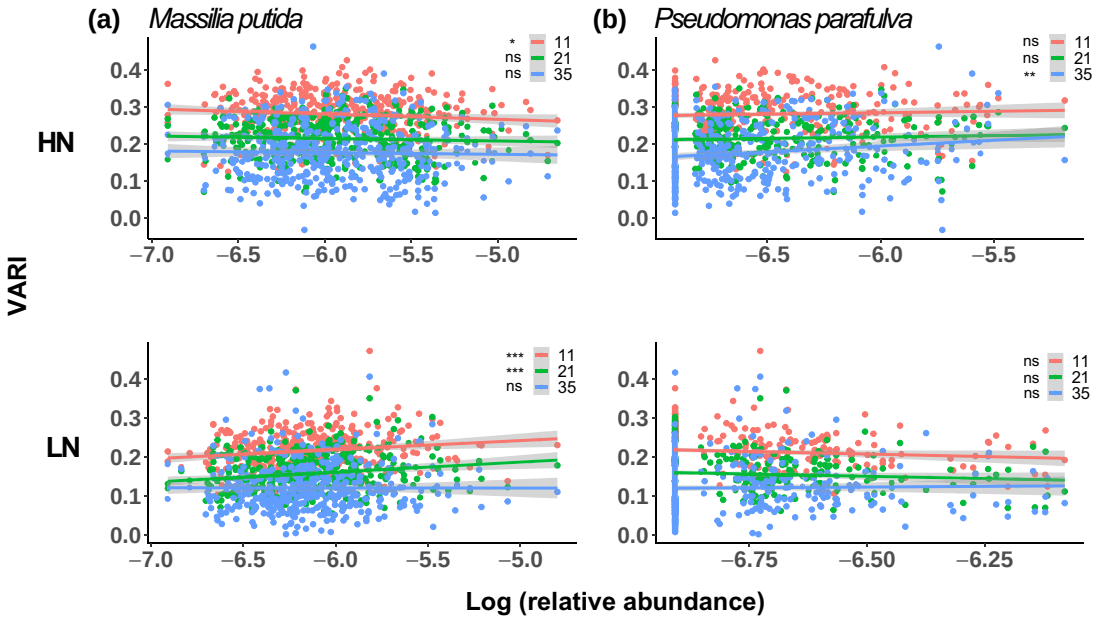

Fig. 4. The relative abundance of *M. putida* and *P. parafulva* and their relationships with the VARI phenotype. The Spearman's rank correlations between the relative abundance of *M. putida* a) and the VARI phenotype collected on 11, 21, and 35 DAMS under HN and LN conditions. The Spearman's rank correlations between the relative abundance of *P. parafulva* b) and the VARI phenotype collected on 11, 21, and 35 DAMS under HN and LN conditions. Asterisks indicate the statistical significance of the difference in accuracy between the models: ns (not significant), *(0.01 < *P*−value ≤ 0.05), **(0.001 < *P*−value ≤ 0.01), ***(0.0001 < *P*−value ≤ 0.001), and ****(*P*−value ≤ 0.0001).

To identify shared features of ASVs with the largest effects, we compared the top 1% of ASVs against all other ASVs in terms of their heritability (the degree to which that ASV abundance is determined by host genetics as compared to the surrounding environmental factors) and selection scores (the magnitude of effect of ASV to the phenotypic trait of interest), as calculated previously (Meier *et al.* 2022). The results showed that the top 1% ASVs were more heritable under LN field conditions (Wilcoxon rank sum test, *P*−values = 1.2×10^{-4}, 0.0015, and 0.0071 for Day 11, 21, and 35, respectively, under LN; *P*−values = 0.99, 0.15, and 0.14 for Day 11, 21, and 35, respectively, under HN), and they had significantly higher selection scores under LN conditions on 11 and 21 DAMS (Wilcoxon rank sum test, *P*−values = 0.0019, 3.5×10^{-6}, and 0.291 for Day 11, 21, and 35, respectively, under LN; *P*−values = 0.90, 0.12, and 0.62 for Day 11, 21, and 35, respectively, under HN) (Supplementary Fig. S6). These findings align with the observed enhancement in prediction accuracy in the LN field attributed to the rhizobiome and imply that plant hosts may possess a genetic mechanism that promotes the recruitment of specific microbes to alleviate LN stress.

Microbiome mediation analysis reveals promising microbial mediators

In order to establish a causal chain from plant genotype to microbiome to plant phenotype, we sought to model ASVs as the intermediate mediators that are selectively recruited by different plant genotypes and have a significant effect on plant phenotype. Therefore, we supplemented the MEGS with our previously developed genome-wide mediation analysis (Yang *et al.* 2022). This approach (see Materials and Methods) enabled us to identify 17 unique ASVs acting as mediators for eight VI traits (Supplementary Table S2), with 8/17 ASVs mediating the VARI trait. Notably, ASVs in taxonomy groups of *Enterobacter*, *Massilia putida*, *P. parafulva*, *TM7a sp* 2 were also the top 1% with the largest effect sizes in the MEGS model under both N conditions. Additionally, the same ASVs annotated as *M. putida* and *P. parafulva* were also in the top 1% large effect ASVs.

To investigate the effects of *M. putida* and *P. parafulva* on plant phenotype, we performed Spearman's rank correlation test to determine if there was a significant correlation between the abundance of ASVs and the VARI phenotype. For *M. putida* (Fig. 4a), under HN conditions, we found barely negative correlations on 11 ($r = -0.10$, *P*−value = 0.044), 21 ($r = -0.048$, *P*−value = 0.34), and 35 ($r = -0.014$, *P*−value = 0.78) days. Under LN conditions, results showed significantly larger and positive correlations on days 11 ($r = 0.17$, *P*−value = 9.7×10^{-4}) and 21 ($r = 0.17$, *P*−value = 3.4×10^{-4}), yet the correlation on 35 DAMS was insignificant ($r = 0.015$, *P*−value = 0.78). These results are consistent with the previous finding that *M. putida* promotes plant growth under LN conditions (Yu *et al.* 2021). In terms of *P. parafulva* (Fig. 4b), an opposite trend was observed. There were larger and more significant correlations under HN conditions: Day 11 ($r = 0.079$, *P*−value = 0.11), 21 ($r = 0.058$, *P*−value = 0.25), and 35 ($r = 0.13$, *P*−value = 0.0083) than LN conditions: Day 11 ($r = -0.063$, *P*−value = 0.21), 21 ($r = -0.046$, *P*−value = 0.37), and 35 ($r = 0.054$, *P*−value = 0.29), aligning with the fact that *P. parafulva* was top 1% ASVs under HN conditions.

Discussion

In this study, we developed a MEGS method and provided empirical evidence, albeit from only one location, that incorporating microbiome data led to a significant increase (about 4%) in prediction accuracy for most traits extracted from UAV images across different dates. We acknowledged that there might be correlations between SNPs (host genome) and ASVs (microbiome) or microenvironments and ASVs, leading to multicollinearity, but this will not affect the screen for beneficial ASVs as seed additives for improving crop production. Besides, the spatial and temporal stability of the microbiome affects the efficacy of prediction models

(Pérez-Enciso *et al.* 2021). Several studies suggest that the microbiome residing in the roots undergo alterations throughout the lifespan of an individual plant (Mougel *et al.* 2006; Houlden *et al.* 2008; Yu *et al.* 2012; Chaparro *et al.* 2014). However, research has also indicated that the microbiome could potentially reach a relatively stable condition after the initial two weeks of growth (Ibekwe and Grieve 2004; Edwards *et al.* 2015; Aleklett *et al.* 2022). Consequently, to enhance the accuracy of predictions, incorporating microbial data collected during a pre-determined stable stage would be recommended. This preference for stable stage significantly restricts the application of microbiome in prediction within breeding programs when compared to genomic data, which remains unchanged (Pérez-Enciso *et al.* 2021). Nonetheless, significant potential applications remain, given the observed increase in prediction accuracy (about 4% on average) after incorporating microbiome data into prediction models. This improvement is noteworthy when compared to the approximately 1% yearly genetic gain achieved using traditional breeding approaches. As the cost of obtaining ASV data through 16S rRNA sequencing decreases with advances in sequencing technology, MEGS offers an unprecedented opportunity to predict complex traits, such as N or water usage efficiency, by including ASVs as additional explanatory variables.

We analyzed prediction accuracy separately under HN and LN conditions and found that microbiome data were more beneficial under the latter (improvement can be up to 40% under N deficit field conditions). This observation is consistent with the idea that the symbiotic relationship between plants and their associated microbiomes has evolved over a long history of coexistence. However, recent changes in farming practices, especially modern crop production under N-sufficient field conditions with inorganic N fertilizer, may have a homogenizing effect on the root-associated microbiome and reduce plants' reliance on rhizosphere microbial species for assistance in N uptake. This change is reflected in our results, where microbiome data were not as strong predictors under HN conditions compared to LN conditions. It suggests that plants may selectively recruit specific microbes to enhance nutrient absorption when soil N levels are insufficient. However, bulk soil data were not collected; therefore, a comparison of the homogeneity between the HN and LN fields cannot be conducted in this study.

Our mediation analysis, which considered the microbes as intermediaries between plant genotype and plant phenotype, pinpointed several microbe mediators. Among these, two mediator microbes, namely *M. putida* and *P. parafulva*, played crucial roles in predicting the plant phenotype in the MEGS analysis. Notably, *M. putida* was enriched in the rhizosphere and is thought to enhance plant growth and N uptake by inducing lateral root formation under LN conditions (Yu *et al.* 2021). And, *P. parafulva* have previously been reported to promote plant growth (Preston 2004; Oteino *et al.* 2015). We also detected several other large effect ASVs, such as *Bacillus fumarioli*, some of which have been linked to plant development (Kumar *et al.* 2012). Other top 1% large effect ASVs in the prediction model, like strains belonging to *Bacillus* which were only detected on certain dates, may also be important candidates for seed additives. However, further phenotypic and functional validation of these identified microbe mediators and large effect microbes is necessary to reveal their effects on the host plants.

Due to computational constraints, we randomly sampled a subset of SNPs in low LD across the whole genome to fit the MEGS models. To determine the influence of SNP size on prediction accuracy, we performed a sensitivity test and found no significant difference in prediction accuracy when random sampling 10k, 25k, and 50k SNPs out of the 770k low LD SNPs. The prediction accuracy was reasonably high, ranging from 52.1–76.4% for both SNP-only and SNP-ASV models across different dates (Supplementary Fig. S7). To maintain high fidelity under the current computation resource, we chose 50k SNPs for all traits and dates in the study. However, the use of more computationally efficient methods is necessary to handle larger SNP datasets in the future. Although the present study was able to achieve reasonable prediction accuracy with a limited set of SNPs and the whole set of ASVs, the use of more comprehensive datasets with more host diversity, more environmental conditions, and enhanced microbiome sampling would undoubtedly improve the accuracy of the MEGS models.

There are several caveats to microbiome-enabled prediction. One limitation is the transferability of models, as microbiomes collected in one environment at a particular plant developmental stage may differ significantly from other microbiome datasets. In fact, our previous study has demonstrated that collection date, crop rotation, and N treatment all exert significant effects on microbiome composition (Meier *et al.* 2021). In the current dataset, our microbiome data were collected within a three-day window at a single location. In the analysis, we controlled for microenvironment effects using the experimental design factors and also compared models with and without including ASV in calculating BLUP values. From the results, we could not determine whether the rhizobiome is simply associated with the microenvironment or actively recruited by the host genotype. Nevertheless, under both scenarios, incorporating root-associated microbiome data consistently improved prediction accuracy, especially under LN field conditions. Another foreseeable caveat is the method implementation. One of the most significant advantages of GS is the ability to predict phenotypes without planting, but microbiome data have to be collected from plant roots, which limits the extent to which predictions can be made before observations. While it may not scale up the breeding process as effectively as SNP-based GS, it still offers valuable applications, i.e. making crossing decisions before observing the phenotypes. Despite these limitations, the proof-of-concept of this study positions MEGS as a possible alternative for sustainable crop improvement. Our results suggest that microbial effects are substantial when considered close to the date of collection, but these effects diminish over time. To successfully implement this approach in a breeding program, meticulously designed experiments are essential for in-field microbiome data collection. This is crucial to mitigate microenvironmental effects and ensure uniform data collection within a specified timeframe. In addition to root-associated microbiome data, collecting the microbiome in bulk soil, along with other environmental factors such as soil type, temperature, and precipitation, is important.

Data availability

The data and code used for the analyses can be accessed through GitHub (https://github.com/ZhikaiYang/GP_microbiome).

Supplemental material is available at G3 online.

Acknowledgments

This work is conducted using the Holland Computing Center of the University of Nebraska-Lincoln Start-up, which receives support from the Nebraska Research Initiative. We appreciate the constructive suggestions from anonymous reviewers.

Funding

This work is supported by the Agriculture and Food Research Initiative (Grant Numbers 2019-67013-29167 and 2022-67013-3656)

from the USDA National Institute of Food and Agriculture and by the U.S. Department of Energy under Award Number DE-SC0023138.

Conflicts of interest

The authors declare no conflicts of interest.

Literature cited

Abdelfattah A, Tack AJ, Wasserman B, Liu J, Berg G, Norelli J, Droby S, Wisniewski M. 2022. Evidence for host–microbiome co-evolution in apple. New Phytol. 234(6):2088–2100. doi:10.1111/nph.v234.6

Abdullaeva Y, Ratering S, Manirajan BA, Rosado-Porto D, Schnell S, Cardinale M. 2021. Domestication impacts the wheat-associated microbiota and the rhizosphere colonization by seed-and soil-originated microbiomes, across different fields. Front Plant Sci. 12:806915. doi:10.3389/fpls.2021.806915

Aleklett K, Rosa D, Pickles BJ, Hart MM. 2022. Community assembly and stability in the root microbiota during early plant development. Front Microbiol. 13:826521. doi:10.3389/fmicb.2022.826521

Baron RM, Kenny DA. 1986. The moderator–mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. J Pers Soc Psychol. 51(6): 1173–1182. doi:10.1037/0022-3514.51.6.1173

Berendsen RL, Pieterse CM, Bakker PA. 2012. The rhizosphere microbiome and plant health. Trends Plant Sci. 17(8):478–486. doi:10.1016/j.tplants.2012.04.001

Bukowski R, Guo X, Lu Y, Zou C, He B, Rong Z, Wang B, Xu D, Yang B, Xie C, *et al.* 2017. Construction of the third-generation *Zea mays* haplotype map. GigaScience. 7:gix134.

Burgueño J, de los Campos G, Weigel K, Crossa J. 2012. Genomic prediction of breeding values when modeling genotype× environment interaction using pedigree and dense molecular markers. Crop Sci. 52(2):707–719. doi:10.2135/cropsci2011.06.0299

Canarini A, Kaiser C, Merchant A, Richter A, Wanek W. 2019. Root exudation of primary metabolites: mechanisms and their roles in plant responses to environmental stimuli. Front Plant Sci. 10: 157. doi:10.3389/fpls.2019.00157

Chaparro JM, Badri DV, Vivanco JM. 2014. Rhizosphere microbiome assemblage is affected by plant development. ISME J. 8(4): 790–803. doi:10.1038/ismej.2013.196

de Koning DJ. 2016. Meuwissen et al. on genomic selection. Genetics. 203(1):5–7. doi:10.1534/genetics.116.189795

Edwards J, Johnson C, Santos-Medellín C, Lurie E, Podishetty NK, Bhatnagar S, Eisen JA, Sundaresan V. 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. Proc Natl Acad Sci U S A. 112(3):E911–E920. doi:10.1073/pnas.1423603112

Eida AA, Hirt H, Saad MM. 2017. Challenges faced in field application of phosphate-solubilizing bacteria. In: Mehnaz S, editor. Rhizotrophs: Plant Growth Promotion to Bioremediation. Singapore: Springer. p. 125–143.

Endelman JB. 2011. Ridge regression and other kernels for genomic selection with R package rrBLUP. Plant Genome. 4(3):250–255. doi:10.3835/plantgenome2011.08.0024

Flint-Garcia SA, Thuillet AC, Yu J, Pressoir G, Romero SM, Mitchell SE, Doebley J, Kresovich S, Goodman MM, Buckler ES. 2005. Maize association population: a high-resolution platform for quantitative trait locus dissection. Plant J. 44(6):1054–1064. doi:10.1111/j.1365-313X.2005.02591.x

Gianola D, De Los Campos G, Hill WG, Manfredi E, Fernando R. 2009. Additive genetic variability and the Bayesian alphabet. Genetics. 183(1):347–363. doi:10.1534/genetics.109.103952

Gianola D, Fernando RL, Stella A. 2006. Genomic-assisted prediction of genetic value with semiparametric procedures. Genetics. 173(3):1761–1776. doi:10.1534/genetics.105.049510

Gianola D, Okut H, Weigel KA, Rosa GJ. 2011. Predicting complex quantitative traits with Bayesian neural networks: a case study with Jersey cows and wheat. BMC Genet. 12(1):1–14. doi:10.1186/1471-2156-12-87

Gomes E, Lana U, Quensen J, de Sousa S, Oliveira C, Guo J, Guimarães L, Tiedje J. 2018. Root-associated microbiome of maize genotypes with contrasting phosphorus use efficiency. Phytobiomes J. 2(3): 129–137. doi:10.1094/PBIOMES-03-18-0012-R

Habier D, Fernando RL, Kizilkaya K, Garrick DJ. 2011. Extension of the Bayesian alphabet for genomic selection. BMC Bioinformatics. 12(1):1–12. doi:10.1186/1471-2105-12-186

Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, Laurin C, Burgess S, Bowden J, Langdon R, *et al.* 2018. The MR-base platform supports systematic causal inference across the human phenome. elife. 7:e34408. doi:10.7554/eLife.34408

Hetrick B, Wilson G, Cox T. 1992. Mycorrhizal dependence of modern wheat varieties, landraces, and ancestors. Can J Bot. 70(10): 2032–2040. doi:10.1139/b92-253

Houlden A, Timms-Wilson TM, Day MJ, Bailey MJ. 2008. Influence of plant developmental stage on microbial community structure and activity in the rhizosphere of three field crops. FEMS Microbiol Ecol. 65(2):193–201. doi:10.1111/fem.2008.65.issue-2

Ibekwe A, Grieve C. 2004. Changes in developing plant microbial community structure as affected by contaminated water. FEMS Microbiol Ecol. 48(2):239–248. doi:10.1016/j.femsec.2004.01.012

Kaur T, Rana KL, Kour D, Sheikh I, Yadav N, Kumar V, Yadav AN, Dhaliwal HS, Saxena AK. 2020. Microbe-mediated biofortification for micronutrients: present status and future challenges. In: Rastegari AA, Yadav AN, Yadav N, editors. New and Future Developments in Microbial Biotechnology and Bioengineering. Amsterdam: Elsevier. p. 1–17.

Kiers ET, Hutton MG, Denison RF. 2007. Human selection and the relaxation of legume defences against ineffective rhizobia. Proc R Soc B: Biol Sci. 274(1629):3119–3126. doi:10.1098/rspb.2007.1187

Krishnappa G, Savadi S, Tyagi BS, Singh SK, Mamrutha HM, Kumar S, Mishra CN, Khan H, Gangadhara K, Uday G, *et al.* 2021. Integrated genomic selection for rapid improvement of crops. Genomics. 113(3):1070–1086. doi:10.1016/j.ygeno.2021.02.007

Kumar P, Dubey R, Maheshwari D. 2012. Bacillus strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. Microbiol Res. 167(8): 493–499. doi:10.1016/j.micres.2012.05.002

Kurilshikov A, Medina-Gomez C, Bacigalupe R, Radjabzadeh D, Wang J, Demirkan A, Le Roy CI, Raygoza Garay JA, Finnicum CT, Liu X, *et al.* 2021. Large-scale association analyses identify host factors influencing human gut microbiome composition. Nat Genet. 53(2):156–165. doi:10.1038/s41588-020-00763-1

Liu X, Tong X, Zou Y, Lin X, Zhao H, Tian L, Jie Z, Wang Q, Zhang Z, Lu H, *et al.* 2022. Mendelian randomization analyses support causal relationships between blood metabolites and the gut microbiome. Nat Genet. 54(1):52–61. doi:10.1038/s41588-021-00968-y

Meier MA, Lopez-Guerrero MG, Guo M, Schmer MR, Herr JR, Schnable JC, Alfano JR, Yang J. 2021. Rhizosphere microbiomes in a historical maize-soybean rotation system respond to host species and nitrogen fertilization at the genus and subgenus levels. Appl Environ Microbiol. 87:e03132–20. doi:10.1128/AEM.03132-20

Meier MA, Xu G, Lopez-Guerrero MG, Li G, Smith C, Sigmon B, Herr JR, Alfano JR, Ge Y, Schnable JC, *et al.* 2022. Association analyses of host genetics, root-colonizing microbes, and plant phenotypes

under different nitrogen conditions in maize. eLife. 11:e75790. doi:10.7554/eLife.75790

Meuwissen TH, Hayes BJ, Goddard ME. 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics. 157(4):1819–1829. doi:10.1093/genetics/157.4.1819

Montesinos-López OA, Montesinos-López A, Pérez-Rodríguez P, Barrón-López JA, Martini JW, Fajardo-Flores SB, Gaytan-Lugo LS, Santana-Mancilla PC, Crossa J. 2021. A review of deep learning applications for genomic selection. BMC Genomics. 22:1–23. doi:10.1186/s12864-020-07350-y

Mougel C, Offre P, Ranjard L, Corberand T, Gamalero E, Robin C, Lemanceau P. 2006. Dynamic of the genetic structure of bacterial and fungal communities at different developmental stages of *Medicago truncatula* Gaertn. cv. Jemalong line J5. New Phytol. 170(1):165–175. doi:10.1111/nph.2006.170.issue-1

Ni JJ, Li XS, Zhang H, Xu Q, Wei XT, Feng GJ, Zhao M, Zhang ZJ, Zhang L, Shen GH, *et al.* 2022. Mendelian randomization study of causal link from gut microbiota to colorectal cancer. BMC Cancer. 22(1):1371. doi:10.1186/s12885-022-10483-w

Oteino N, Lally RD, Kiwanuka S, Lloyd A, Ryan D, Germaine KJ, Dowling DN. 2015. Plant growth promotion induced by phosphate solubilizing endophytic pseudomonas isolates. Front Microbiol. 6:745. doi:10.3389/fmicb.2015.00745

Palali Delen S, Xu G, Velazquez-Perfecto J, Yang J. 2023. Estimating the genetic parameters of yield-related traits under different nitrogen conditions in maize. Genetics. 223(4):iyad012. doi:10.1093/genetics/iyad012

Peiffer JA, Spor A, Koren O, Jin Z, Tringe SG, Dangl JL, Buckler ES, Ley RE. 2013. Diversity and heritability of the maize rhizosphere microbiome under field conditions. Proc Natl Acad Sci U S A. 110(16):6548–6553. doi:10.1073/pnas.1302837110

Pérez-Enciso M, Zingaretti LM, Ramayo-Caldas Y, de Los Campos G. 2021. Opportunities and limits of combining microbiome and genome data for complex trait prediction. Genet Sel Evol. 53(1):1–20. doi:10.1186/s12711-021-00658-7

Piromyou P, Buranabanyat B, Tantasawat P, Tittabutr P, Boonkerd N, Teaumroong N. 2011. Effect of plant growth promoting rhizobacteria (PGPR) inoculation on microbial community structure in rhizosphere of forage corn cultivated in Thailand. Eur J Soil Biol. 47(1):44–54. doi:10.1016/j.ejsobi.2010.11.004

Preston GM. 2004. Plant perceptions of plant growth-promoting pseudomonas. Philos Trans R Soc Lond B Biol Sci. 359(1446):907–918. doi:10.1098/rstb.2003.1384

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, De Bakker PI, Daly MJ, *et al.* 2007. Plink: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 81(3):559–575. doi:10.1086/519795

Rodene E, Xu G, Palali Delen S, Zhao X, Smith C, Ge Y, Schnable J, Yang J. 2022. A UAV-based high-throughput phenotyping approach to assess time-series nitrogen responses and identify trait-associated genetic components in maize. Plant Phenome J. 5(1):e20030. doi:10.1002/ppj2.20030

Roquigny R, Novinscak A, Biessy A, Filion M. 2017. Rhizotrophs: plant growth promotion to bioremediation. In: Egamberdieva D, Ahmad P, editors. Pseudomonadaceae: From Biocontrol to Plant Growth Promotion, vol. 2. Singapore: Springer. p. 39–68.

Saleem M, Hu J, Jousset A. 2019. More than the sum of its parts: microbiome biodiversity as a driver of plant growth and soil health. Annu Rev Ecol Evol Syst. 50(1):145–168. doi:10.1146/ecolsys.2019.50.issue-1

Sangabriel-Conde W, Negrete-Yankelevich S, Maldonado-Mendoza IE, Trejo-Aguilar D. 2014. Native maize landraces from Los Tuxtlas, Mexico show varying mycorrhizal dependency for P uptake. Biol Fertil Soils. 50(2):405–414. doi:10.1007/s00374-013-0847-x

Sessitsch A, Pfaffenbichler N, Mitter B. 2019. Microbiome applications from lab to field: facing complexity. Trends Plant Sci. 24(3):194–198. doi:10.1016/j.tplants.2018.12.004

Singer E, Vogel JP, Northen T, Mungall CJ, Juenger TE. 2021. Novel and emerging capabilities that can provide a holistic understanding of the plant root microbiome. Phytobiomes J. 5(2):122–132. doi:10.1094/PBIOMES-05-20-0042-RVW

Soldan R, Fusi M, Cardinale M, Daffonchio D, Preston GM. 2021. The effect of plant domestication on host control of the microbiota. Commun Biol. 4(1):1–9. doi:10.1038/s42003-021-02467-6

Wang X, Xu Y, Hu Z, Xu C. 2018. Genomic selection methods for crop improvement: current status and prospects. Crop J. 6(4):330–340. doi:10.1016/j.cj.2018.03.001

Wang X, Yang Z, Xu C. 2015. A comparison of genomic selection methods for breeding value prediction. Sci Bull. 60(10):925–935. doi:10.1007/s11434-015-0791-2

Xu L, Naylor D, Dong Z, Simmons T, Pierroz G, Hixson KK, Kim YM, Zink EM, Engbrecht KM, Wang Y, *et al.* 2018. Drought delays development of the sorghum root microbiome and enriches for monoderm bacteria. Proc Natl Acad Sci U S A. 115:E4284–E4293. doi:10.1073/pnas.1717308115

Xu Q, Zhang SS, Wang RR, Weng YJ, Cui X, Wei XT, Ni JJ, Ren HG, Zhang L, Pei YF. 2021. Mendelian randomization analysis reveals causal effects of the human gut microbiota on abdominal obesity. J Nutr. 151(6):1401–1406. doi:10.1093/jn/nxab025

Yang Z, Xu G, Zhang Q, Obata T, Yang J. 2022. Genome-wide mediation analysis: an empirical study to connect phenotype with genotype via intermediate transcriptomic data in maize. Genetics. 221(2):iyac057. doi:10.1093/genetics/iyac057

Yee MO, Kim P, Li Y, Singh AK, Northen TR, Chakraborty R. 2021. Specialized plant growth chamber designs to study complex rhizosphere interactions. Front Microbiol. 12:507. doi:10.3389/fmicb.2021.625752

Yu P, He X, Baer M, Beirinckx S, Tian T, Moya YAT, Zhang X, Deichmann M, Frey FP, Bresgen V, *et al.* 2021. Plant flavones enrich rhizosphere oxalobacteraceae to improve maize performance under nitrogen deprivation. Nat Plants. 7(4):481–499. doi:10.1038/s41477-021-00897-y

Yu L, Nicolaisen M, Larsen J, Ravnskov S. 2012. Succession of root-associated fungi in *Pisum sativum* during a plant growth cycle as examined by 454 pyrosequencing. Plant Soil. 358(1–2):225–233. doi:10.1007/s11104-012-1188-5

Zhang Q. 2021. High-dimensional mediation analysis with applications to causal gene identification. Stat Biosci. 14:432–20.

Zhu S, Vivanco JM, Manter DK. 2016. Nitrogen fertilizer rate affects root exudation, the rhizosphere microbiome and nitrogen-use-efficiency of maize. Agric Ecosyst Environ Appl Soil Ecol. 107:324–333. doi:10.1016/j.apsoil.2016.07.009

Editor: K. Verhoeven