Supplementary Material

Supplementary Material for "The mediating role of fungal microbiome in the relation between crop health and soil nutrients" By Sørensen et al.

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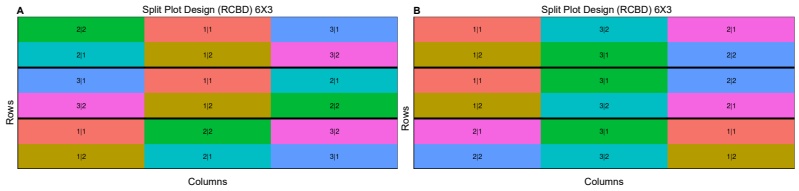
1 Experimental design overview

Supplementary Figure 1: Satellite imagery for conventional (A) and ecological (B) field types. The blue point markers indicate where soil samples were acquired, and the grid lines around the sample points indicate the split-plots.



Supplementary Figure 2: A: Split plot design overview for the conventional field. Rows indicate whole plots (3 replicates, separated by thick black lines), with columns indicating subplots (3 replicates per whole plot). The number inside each subplot refers to the sampling depth set as high (x|2) or low (x|1), with x referring to the subplot number.

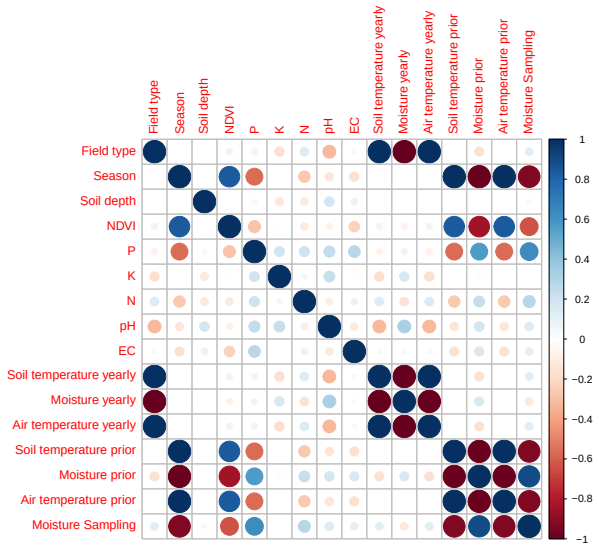
Supplementary Table 1: Overview of the Experimental design

	Time	**Whole plot**	**Split-plot**
Factor	Season	Field type	Depth
Factor levels	2	2	2
Replicates	-	3	6×3

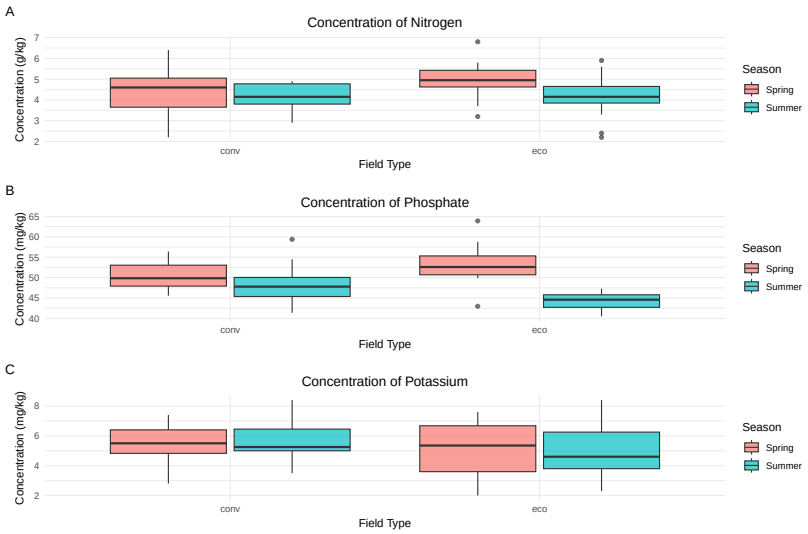
2 Abiotic data description

Supplementary Table 2: Abiotic variables overview

Variable	**Value range**	**Description**
Nitrogen (N)	2.2-6.8	Extractable Nitrogen (g/kg)
Phosphate (P)	40.5 -63.9	Extractable Phosphate (mg/kg)
pH	6.4-7.3	pH index
Potassium (K)	2.0-8.4	Extractable Potassium (mg/kg)
Moisture prior	0.16-0.20	Mean soil moisture from the month before NDVI (m^3)
Moisture Sampling	0.18-0.21	Soil moisture measured at sampling time (m^3)
Moisture yearly	0.17-0.18	Yearly average soil moisture content (m^3)
Air temperature prior	280.13-287.92	Mean air temperature from the month before NDVI (K)
Air temperature yearly	284.17-284.19	Yearly average air temperature (K)
Soil temperature prior	280.42-289.82	Mean soil temperature from the month before NDVI (K)
Soil temperature yearly	284.16- 284.19	Yearly average soil temperature (K)
Electric conductivity (EC)	0.04-0.11	Soil Salinity indicator (mS/m)
Season	0-1	Factor of 2 levels with one level per season with 0 =Spring and 1=Summer
Field type	0-1	Factor of 2 levels with one level per field type with 0= Conventional and 1= Ecological
Soil depth	0-1	Factor of 2 levels at 10 cm and 30 cm depth sampling with 0= 10 cm and 1= 30 cm

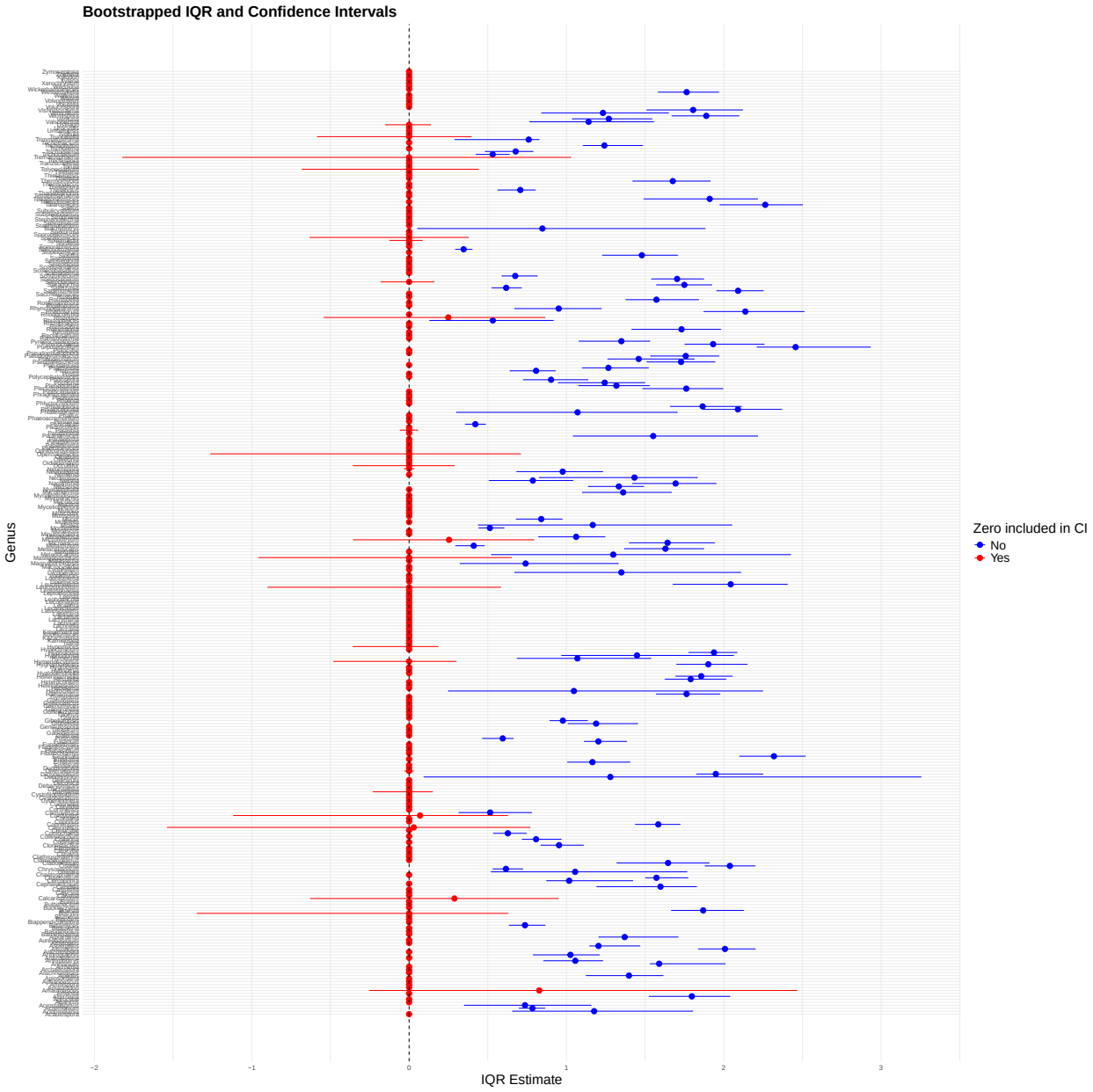


Supplementary Figure 3: Correlation matrix of Abiotic parameters, depicting high Pearson correlation between predictors prior to mixture PCA. Note that the categorical variables have been dummy encoded.

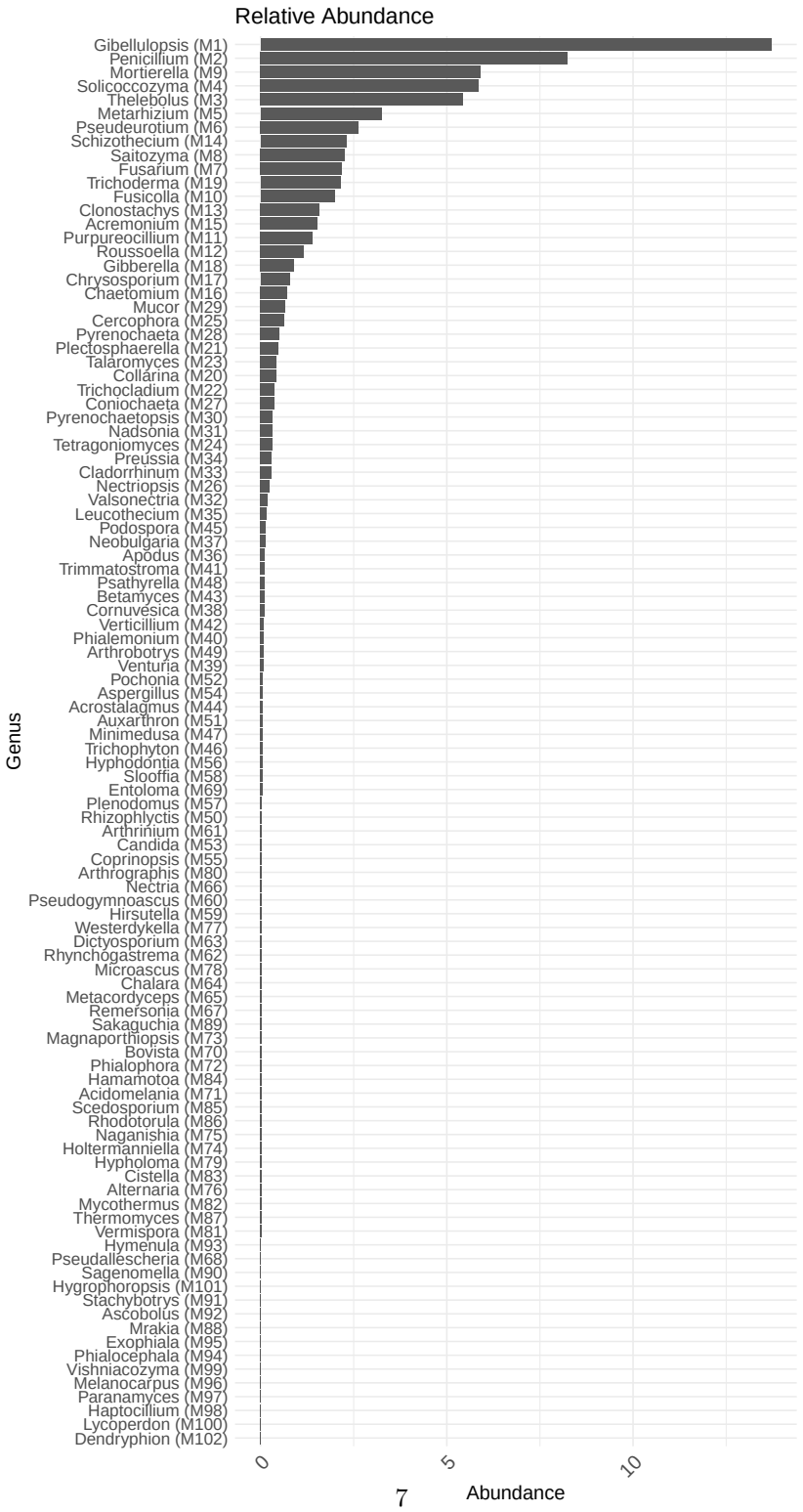


Supplementary Figure 4: (A) concentration of Nitrogen across seasons and field types. (B) Concentration of Phosphate across seasons and field types. (C) Concentration of Potassium across seasons and field types.

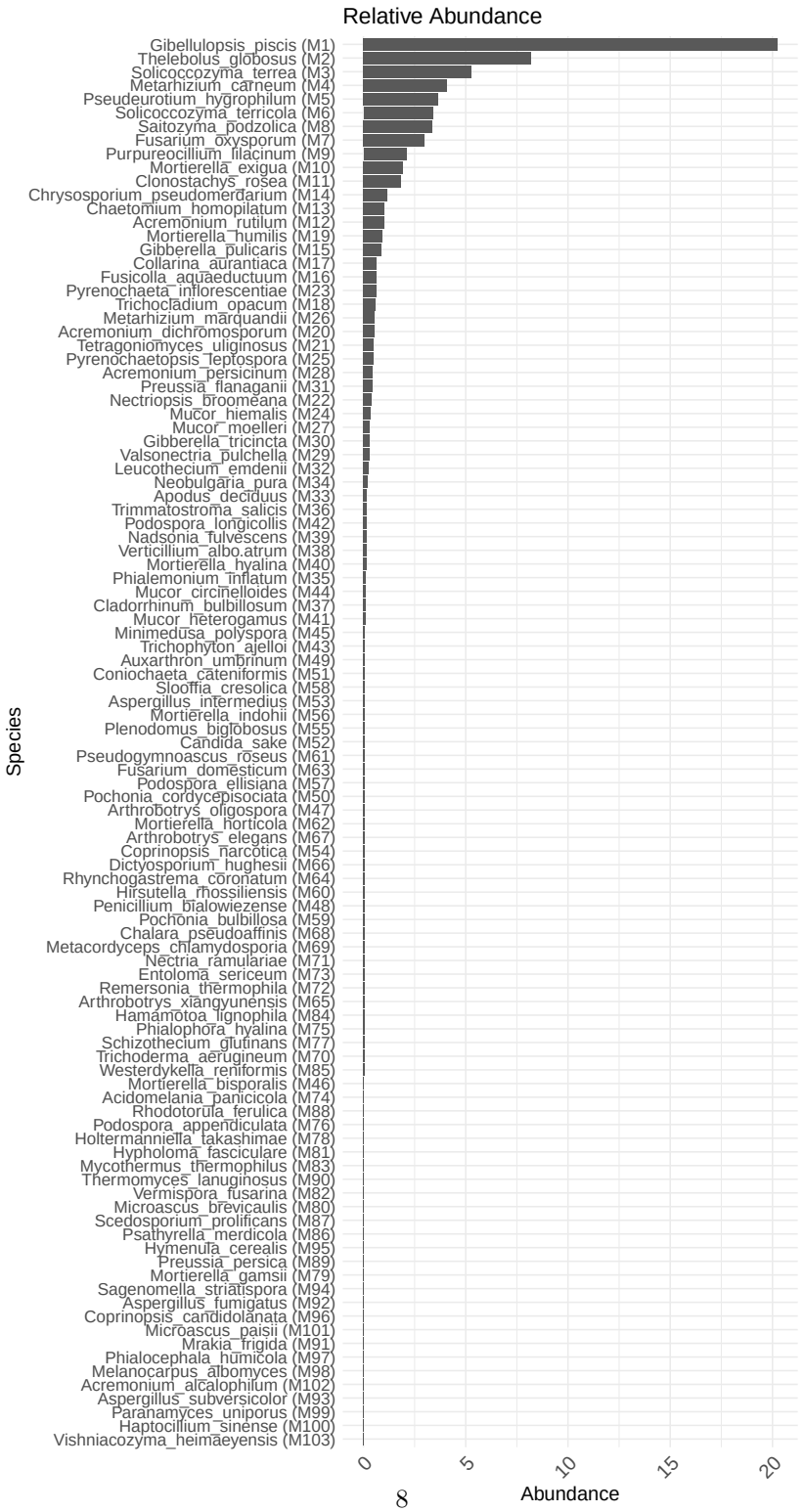
3 OTU outlier taxonomy removal and abundance



Supplementary Figure 5: Interquartile (IQR) bootstrap estimation results, including confidence interval (CI) IQR mean. The CI is marked with straight lines, and the IQR mean with a dot. CIs are marked in red and blue, with blue not containing zero (non-outlier taxonomy), while red contains zero (outlier taxonomy).

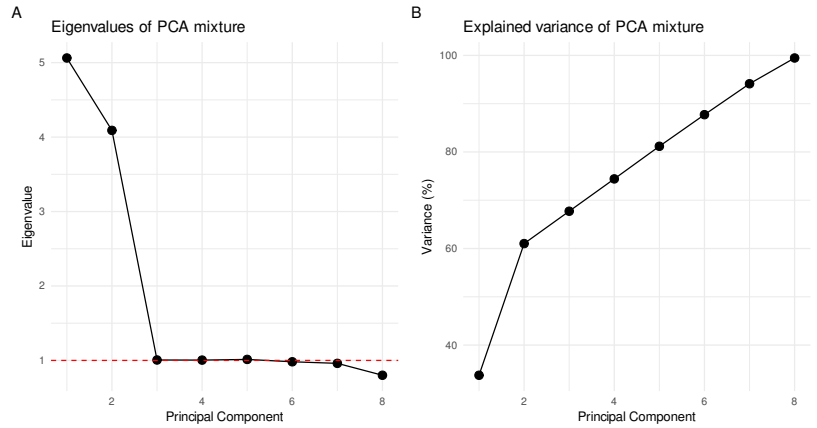


Supplementary Figure 6: Genera total abundance from the IQR filtered data (see figure 5)

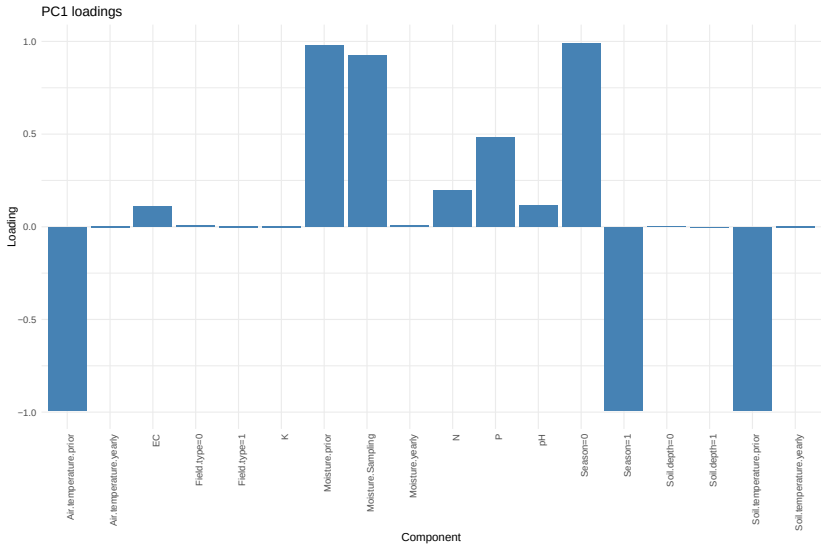


Supplementary Figure 7: Species total abundance from the IQR filtered data (see figure 5).

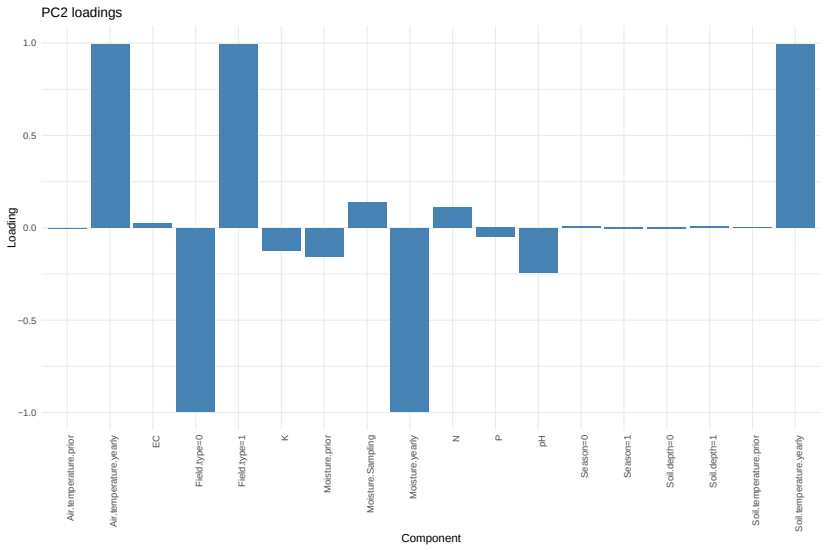
4 Mixture PCA results



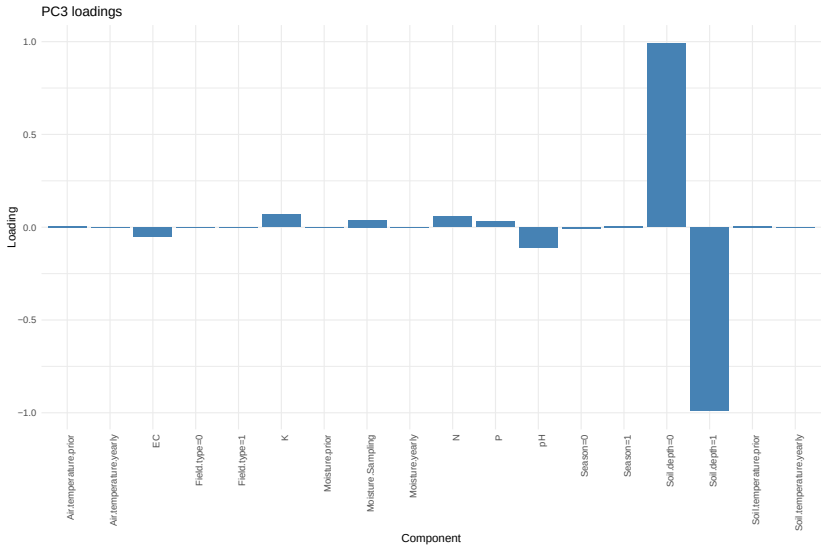
Supplementary Figure 8: (A) PCA component selection applying the Kaiser criterion (standardized data). The dashed red line is set at eigenvalues=1, with components below 1 being removed from further analysis. (B) Explained variance utilizing PCA, at 5 components, approximately 81% of the total variance is explained.



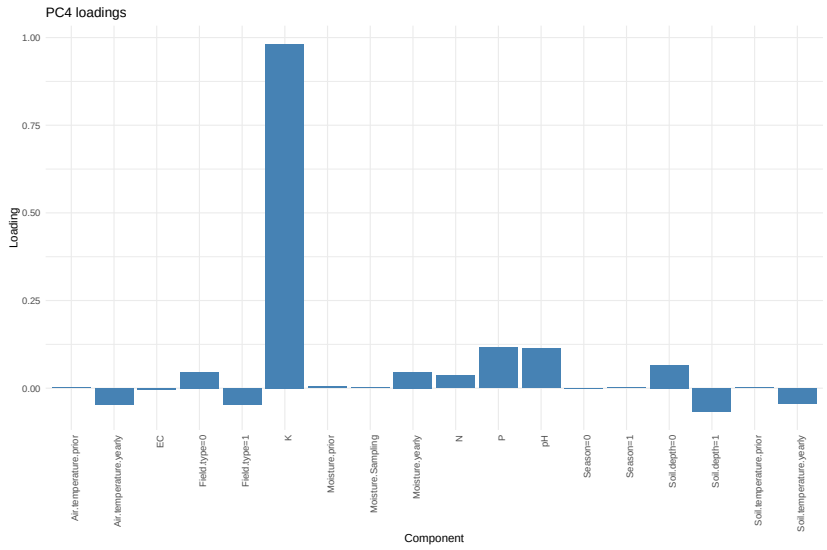
Supplementary Figure 9: Loadings of the first principal component X1. Note that the =1 and =0 signs refer to the factor variable setting - see table 2 for details.



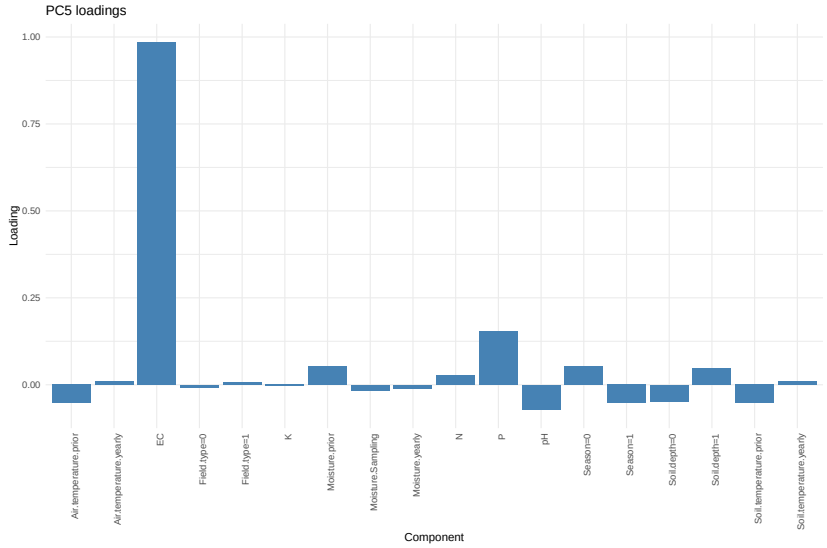
Supplementary Figure 10: Loadings of the second principal component X2.



Supplementary Figure 11: Loadings of the third principal component X3.Note that the =1 and =0 signs refer to the factor variable setting - see table 2 for details.



Supplementary Figure 12: Loadings of the fourth principal component X4.Note that the =1 and =0 signs refer to the factor variable setting - see table 2 for details.



Supplementary Figure 13: Loadings of the fifth principal component X5.Note that the =1 and =0 signs refer to the factor variable setting - see table 2 for details.

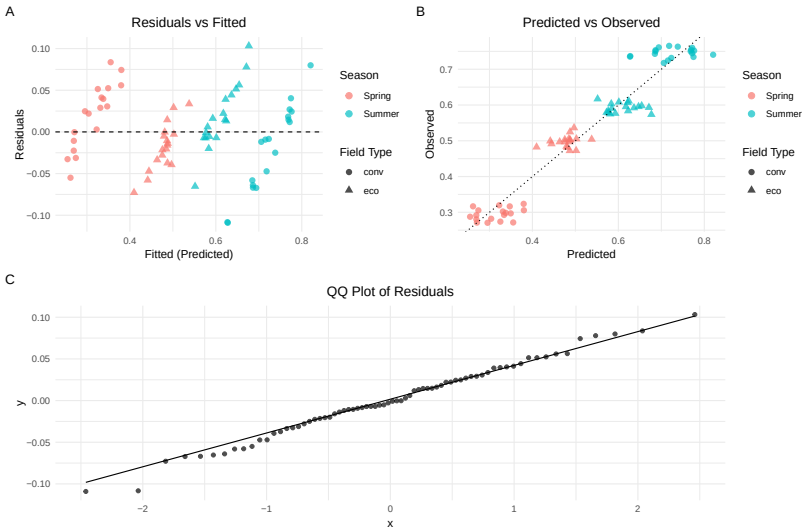
5 SEM diagnostics

Supplementary Table 3: Summary of SEM goodness of fit metrics, including the overall power for the model.

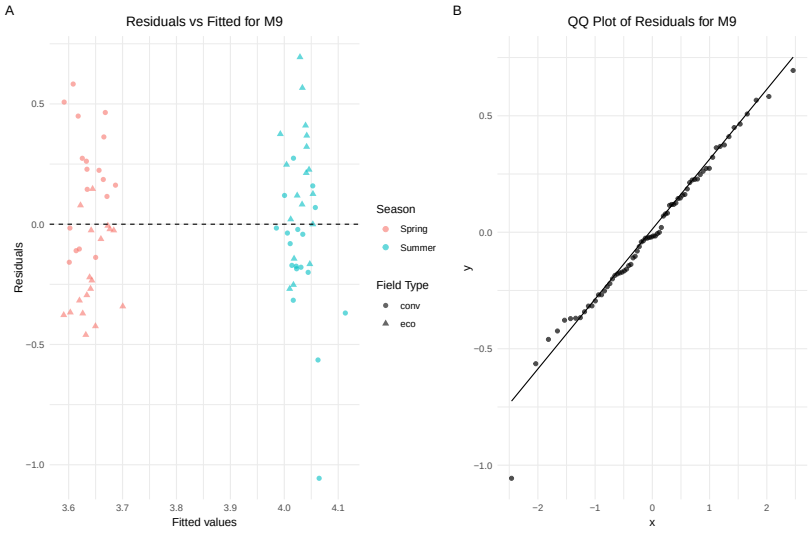
Component	Value
Model Fit (User Model)	
Chi-square (df = 26)	24.098
P-value	0.570
Comparative Fit Indices	
CFI	1.000
TLI	1.000
RMSEA	
Estimate	0.000
90% CI	[0.000, 0.085]
P(RMSEA $\leq$ 0.05)	0.918
P(RMSEA $\geq$ 0.08)	0.066
SRMR	0.054
Overall power	0.812

Supplementary Table 4: Covariance Matrix with Z-score Residuals

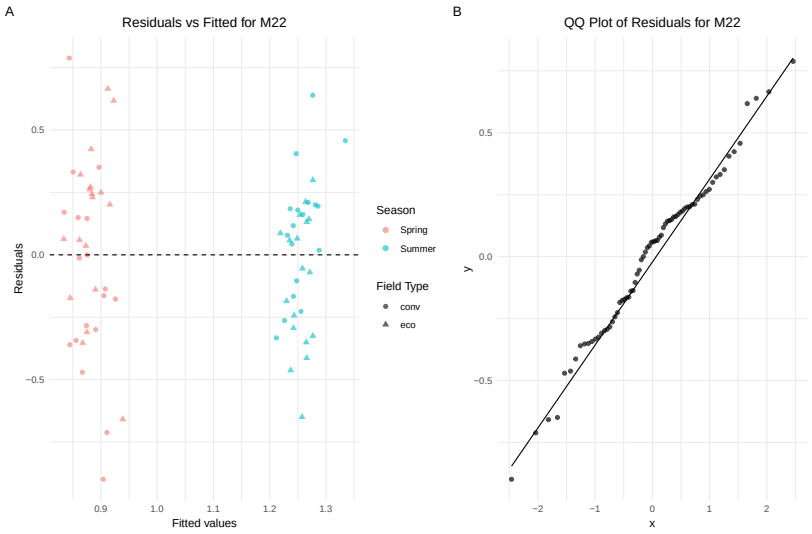
	Y	M9	M22	M35	M53	M87	M40	X1	X2	X5
Y	0.000									
M9	0.659	0.000								
M22	-0.965	-0.139	0.000							
M35	1.443	0.337	-0.410	0.000						
M53	0.942	0.865	0.118	1.787	0.000					
M87	-0.292	-1.931	0.229	-0.533	0.251	0.000				
M40	0.984	-0.395	-1.809	-0.118	0.993	-1.089	0.000			
X1	1.464	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
X2	-0.135	0.475	-0.015	0.391	-0.427	1.360	0.000	0.000	0.000	
X5	-0.165	0.478	1.105	-1.394	0.061	-0.946	0.074	0.000	0.000	0.000



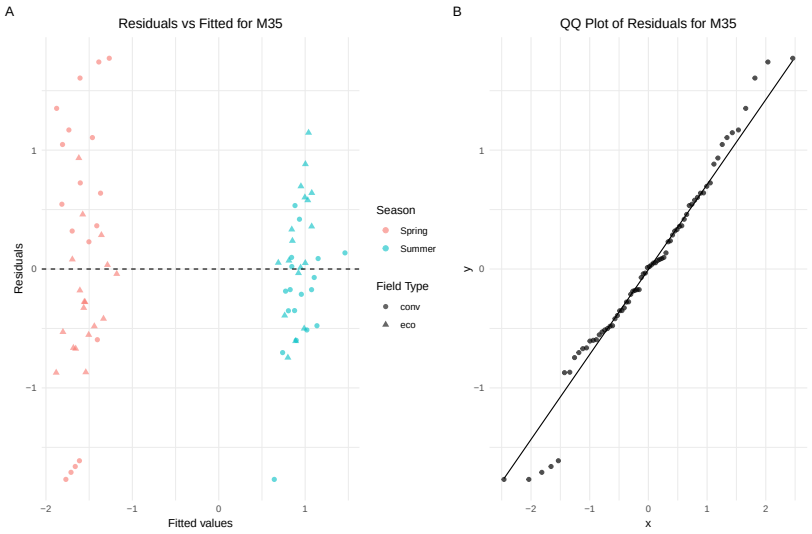
Supplementary Figure 14: The case-wise residual diagnostic plots for the ε_1. (A) case-wise residuals vs fitted with groups of field type and season. (B) Predicted NDVI values vs observed with groupings. (C) Quantile-quantile (QQ) plot assessing normality.



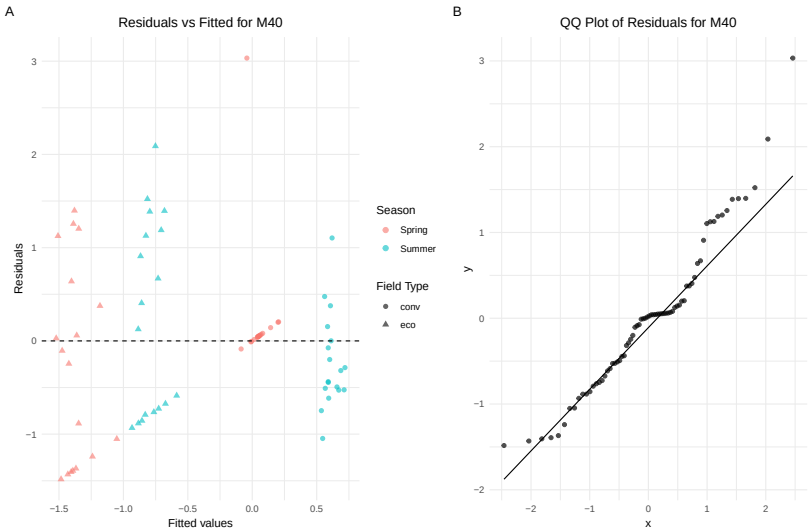
Supplementary Figure 15: The case-wise residual diagnostic plots for the ε_2 for M9. (A) case-wise residuals vs fitted with groups of field type and season. (B) P(C) Quantile-quantile (QQ) plot assessing normality.



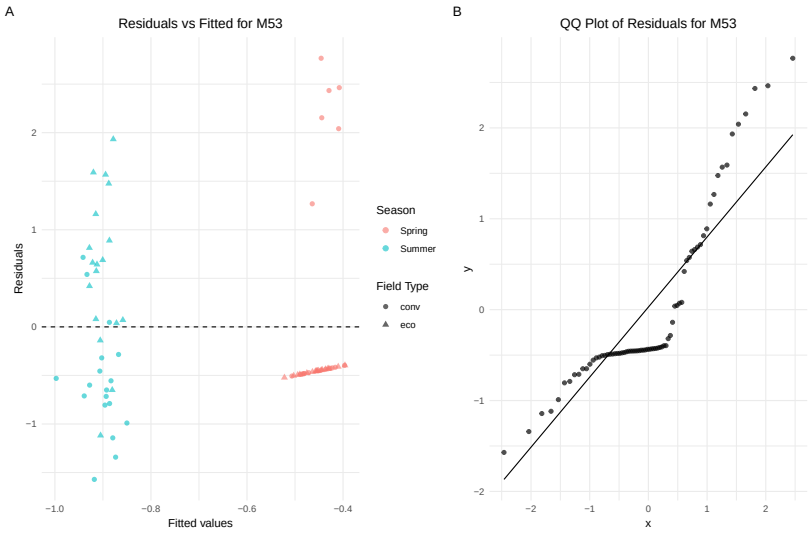
Supplementary Figure 16: The case-wise residual diagnostic plots for the ε_2 for M22. (A) case-wise residuals vs fitted with groups of field type and season. (B) P(C) Quantile-quantile (QQ) plot assessing normality.



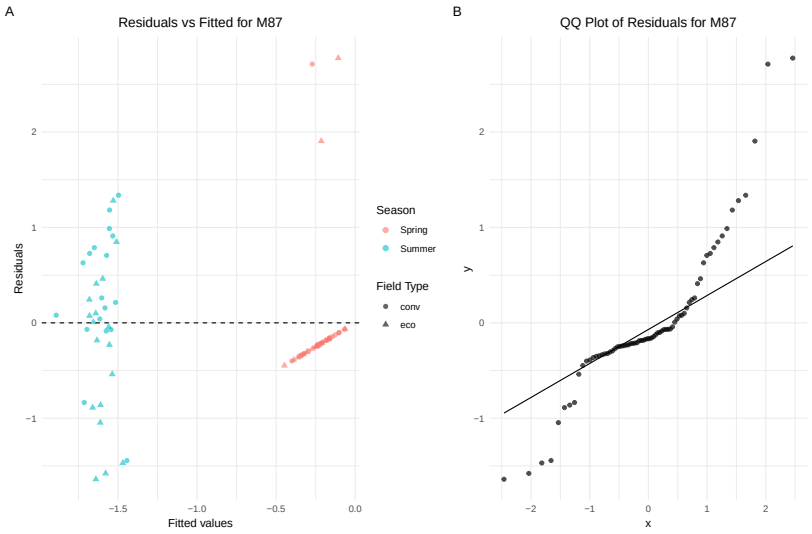
Supplementary Figure 17: The case-wise residual diagnostic plots for the ε_2 for M35. (A) case-wise residuals vs fitted with groups of field type and season. (B) P(C) Quantile-quantile (QQ) plot assessing normality.



Supplementary Figure 18: The case-wise residual diagnostic plots for the ε_2 for M40. (A) case-wise residuals vs fitted with groups of field type and season. (B) P(C) Quantile-quantile (QQ) plot assessing normality.

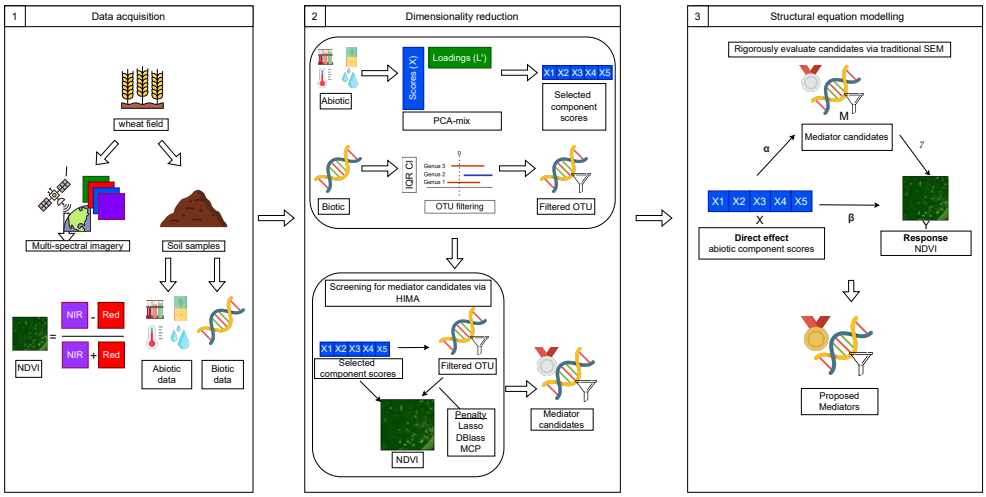


Supplementary Figure 19: The case-wise residual diagnostic plots for the ε_2 for M53. (A) case-wise residuals vs fitted with groups of field type and season. (B) P(C) Quantile-quantile (QQ) plot assessing normality.



Supplementary Figure 20: The case-wise residual diagnostic plots for the ε_2 for M87. (A) case-wise residuals vs fitted with groups of field type and season. (B) P(C) Quantile-quantile (QQ) plot assessing normality.

6 Method workflow



Supplementary Figure 21: Graphical overview of the paper: (1) We sampled soil from wheat fields in Sønderjylland and acquired corresponding satellite imagery from which NDVI values were extracted. The soil samples were sequenced (OTU), and abiotic variables were measured or collected from open sources. (2) We reduced the dimensionality of the Biotic OTU data by removing outlier taxonomy, applying IQR filtering. PCA-mix was applied to the abiotic data, and 5 components were selected. HIMA was applied to screen the 102 fungal mediator candidates based on the selected components (abiotic), filtered OTUs (biotic), and a 10% false discovery rate. (3) The selected mediator candidates (17 genera) were analyzed via structural equation model (SEM).