



Soil management–dependent shifts in microbial diversity and nutrient – related functions ultimately shape the nutritional composition of maize and wheat

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Abstract

Background and aims Tillage and fertilization are key soil management practices that influence soil microbial communities, nutrient cycling, and crop performance. However, how management–induced microbial changes relate to crop nutritional performance remains insufficiently understood. Therefore, this study aimed to evaluate the effects of ploughing and deep loosening, as well as contrasting fertilization regimes, on soil microbial community composition and diversity, and to relate these practices and microbial responses to grain nutritional traits, including protein content, antioxidant activity, and carotenoid concentration in maize and wheat.

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Methods Field experiments were conducted within established long–term trial sites, using two tillage methods, ploughing and deep loosening combined with two fertilization regimes (moderate and extensive input) across fifteen nutrient treatments. Sixty–four soil and grain samples were analyzed. Microbial diversity and functions were analyzed by metagenomics, and grain traits by biochemical assays. **Results** Tillage and fertilization significantly altered soil microbial communities and their network organization. Ploughing increased alpha diversity ($p < 0.05$) but favored generalist, stress–tolerant taxa, whereas deep loosening supported more specialized assemblages. Balanced fertilization enhanced network density and modularity (density up to 0.46; modularity 0.108–0.121), while extensive fertilization disrupted network structure. These microbial shifts were mirrored in grain traits. Ploughing increased carotenoid concentrations ($p < 0.001$), lipophilic antioxidants

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showed no treatment differences ($p > 0.05$), and deep loosening was linked to greater accumulation of hydrophilic antioxidants ($p > 0.05$).

Conclusions Soil management shapes crop nutrition: ploughing boosts carotenoids, deep loosening favors hydrophilic antioxidants, while excessive fertilization disrupts these benefits.

Keywords Soil microbiome · Long – term field trials · Tillage–fertilization synergy · Microbial fingerprints · Maize – wheat grain nutritional profile · Microbiome – aware management

Introduction

Crops are the primary source of food for the global population and are both nutritionally and strategically important, particularly during periods of crisis when demand for staple products increases. Recent crises, such as the COVID – 19 pandemic, have highlighted the vulnerability of food systems and the need for resilient, nutritionally adequate crop production.

Soil health underpins this resilience by regulating water and nutrient availability and supporting long – term agricultural sustainability (FAO 2015). Beyond supporting crops, soil is also home to diverse microbial communities that play essential roles in ecosystem functioning. Soil health is a fundamental component of the One Health approach, which recognizes the interconnectedness of environmental, human, and animal health. According to Lehmann et al. (2020), four key ecosystem services provided by soil include: plant production, water quality regulation, the promotion of human health, and climate change mitigation.

To increase productivity, farmers globally apply various soil treatments, often without fully understanding their long – term impacts on soil health. Among these, tillage practices are commonly categorized into conventional and conservation tillage systems. Conservation tillage, which includes no – till, minimum – till, and reduced – till practices, aims to preserve soil structure, moisture, and nutrients. These methods have been shown to improve fertility, water retention, and drought resilience (Lv et al. 2023). Ashworth et al. (2017) reported that no – till systems foster distinct and responsive microbial communities over the long term, shaped by the plant – soil

environment. Consequently, conservation tillage may promote more stable and diverse microbial communities. In contrast, conventional tillage involves deep ploughing and soil inversion, which can degrade structure and fertility over time.

Fertilization is another key driver of soil and crop performance. It is a cornerstone of modern agriculture and, when applied at optimized rates aligned with crop demand and soil conditions, plays a crucial role in sustaining crop productivity and food security. Balanced fertilization improves nutrient availability, enhances nutrient – use efficiency, and can support beneficial soil microbial activity and soil fertility (Xing et al. 2025). Properly managed fertilization therefore contributes not only to yield stability but also to the maintenance of soil functions essential for long – term agricultural sustainability. While high fertilizer inputs are often intended to boost crop growth and nutrient content, extensive use can negatively affect soil health (Wu et al. 2024). These concerns extend beyond soil chemistry to include soil microbial life, which plays a central role in cycling key nutrients such as nitrogen, phosphorus, sulfur, and iron. These processes are fundamental to plant nutrition and broader ecological balance (Banerjee and van der Heijden 2023). Soil microbes decompose organic matter, fix nitrogen, and solubilize phosphorus, all of which are essential for nutrient cycling and improved plant growth (Chen et al. 2024b). In the rhizosphere, functionally important groups such as nitrogen – fixing bacteria (e.g., *Rhizobium*, *Azotobacter*) and nutrient – solubilizing taxa (e.g., *Pseudomonas*, *Bacillus*) contribute to nutrient availability and plant stress tolerance (Ali et al. 2024; Zhao et al. 2023a; Chen et al. 2024b). Conversely, excessive nutrient inputs, particularly imbalanced nitrogen-to-phosphorus ratios, can impair ecosystem functioning and biodiversity (Penuelas et al. 2023). High phosphorus inputs have been shown to reduce nutrient-use efficiency, suppress key enzyme activities, and promote potentially pathogenic microbial taxa, thereby degrading soil microbial health.

Although the effects of tillage intensity and fertilization practices on soil microbial communities and crop performance have been widely investigated, it remains insufficiently resolved how these management factors, when evaluated within the same long – term field context, differentially shape soil microbiomes and how such microbial responses

relate to specific crop nutritional traits. In particular, direct links between management–driven microbial community organization and the accumulation of nutritionally relevant compounds in cereal grains remain limited. Therefore, the aim of this study was to independently evaluate the effects of ploughing and deep loosening, as well as contrasting fertilization regimes, on soil microbial composition, diversity, and functional potential, and to relate these microbial responses to measured grain nutritional parameters, including protein content, hydrophilic and lipophilic antioxidant activity, and carotenoid concentration in maize and wheat. By explicitly linking management–driven microbial patterns to multiple quantified nutritional traits within a long–term field experiment, this study provides novel insight into soil–microbiome–crop relationships relevant to sustainable soil management.

In this study, *crop nutritional profile* refers to measured grain nutritional parameters, including protein content, antioxidant activity, and carotenoid concentration. The term *microbial fingerprints* is used to describe characteristic patterns in soil microbial community composition derived from sequencing data, while *microbiome–aware management* denotes soil management strategies that consider microbial responses when optimizing tillage and fertilization practices.

Materials and methods

Experimental site and sampling process

This field experiment was conducted at the Látókép Experimental Site of the University of Debrecen, Hungary (47°33′ N, 21°26′ E), situated on a chernozem soil (Tamás et al. 2023). The site is located in a region with a long–established tradition of maize and wheat cultivation and has served as a long–term experimental field for over 40 years (University of Debrecen Press, 2017). This long–term setup ensured that observed soil microbial and crop nutritional responses reflected cumulative treatment effects rather than short–term disturbances. The experiment aimed to explore how varying tillage methods and fertilization levels influence the soil microbiome and the nutritional profile of maize and wheat grains (Online resource 1). The experiment followed

a fixed plot field design. Tillage treatments (ploughing and deep loosening) were applied in continuous strips, while fertilization treatments were arranged systematically within each crop type. Due to the fixed layout of the experimental field, treatments were not randomly assigned. Replication was achieved through multiple plots per treatment. The experimental hierarchy consisted of crop type (maize and wheat), tillage system, and fertilization treatment.

To characterize the baseline soil conditions at the experimental site, soil chemical properties were determined prior to the establishment of the treatments at two depths (0–25 and 26–50 cm) (Table 1). Baseline soil chemical properties were measured at deeper intervals to characterize vertical nutrient distribution, whereas microbiome analyses focused on the biologically active topsoil layer where microbial activity and root interactions are greatest. Soil parameters analyzed included pH (KCl), soil texture index (KA), organic matter content (humus), total nitrogen, mineral nitrogen ($\text{NO}_3^- + \text{NO}_2^-$), AL–extractable phosphorus (P) and potassium (K), exchangeable magnesium and sodium, sulfate (SO_4^{2-}), and selected micronutrients (Zn, Cu, Mn). These measurements were conducted to document the initial soil fertility status, chemical composition, and vertical nutrient

Table 1 Soil chemical characteristics of the experimental site

Soil parameters	0–25 cm soil depth	26–50 cm soil depth
pH (KCl)	6.46	6.36
KA (soil texture index)	43.0	44.6
CaCO_3 (%)	0	0
Humus (%)	2.76	2.16
Total N (%)	0.150	0.120
$\text{NO}_3^- + \text{NO}_2^-$ (mg kg ⁻¹)	6.20	1.74
AL–extractable P (mg kg ⁻¹)	133.4	48.0
AL–extractable K (mg kg ⁻¹)	239.8	173.6
Mg (mg kg ⁻¹)	332.4	405.4
Na (mg kg ⁻¹)	38.0	66.2
Zn (mg kg ⁻¹)	2.80	0.80
Cu (mg kg ⁻¹)	5.86	4.54
Mn (mg kg ⁻¹)	438	406
SO_4^{2-} (mg kg ⁻¹)	9.25	9.13

Baseline soil properties were measured at two depths (0–25 cm and 26–50 cm) to document soil fertility and chemical stratification before the application of tillage and fertilization treatments

stratification of the soil profile prior to the evaluation of treatments specific effects related to tillage, fertilization, and crop type.

Two tillage methods were employed: deep loosening (45 cm), representing reduced tillage, and ploughing (35 cm). These tillage practices were specifically selected because they are commonly used by farmers in Hungary and other parts of Europe. In addition to tillage methods, the experiment included two distinct nutrient regimes: a moderate nutrient setting (Setting M) and an extensive nutrient setting (Setting E), which were designed to simulate common farming practices under varying levels of input. Setting M, the moderate nutrient regime, reflects the typical fertilization practices used by farmers in the region, while Setting E represents a more intensive fertilization strategy to assess the effects of higher nutrient inputs on soil health, microbial diversity, and crop yields (Online resource 1). Nitrogen (N), phosphorus (P), and potassium (K) were applied as mineral fertilizers commonly used in long-term field experiments, with nutrient inputs expressed as elemental N, P, and K. The study incorporated 15 different fertilization combinations, ranging from a control (no fertilizer) to individual nutrient applications, dual nutrient combinations, and a full NPK treatment (Online resource 1). These combinations were used to separate the effects of individual nutrients, their interactions, and their combined influence on soil microbial communities and crop nutritional traits.

A total of 64 soil samples were collected, comprising 32 samples from maize-planted plots and 32 samples from wheat-planted plots. Corresponding grain samples were collected from the same plots at harvest maturity, coinciding with soil sampling conducted in mid to late summer for wheat and early autumn for maize, to assess crop nutritional profile (Online resource 1). Soil sampling was conducted shortly before harvest at crop-specific times: wheat soils were sampled in July, while maize soils were sampled in late September. In all cases, soil was collected from the 0–30 cm depth. This sampling depth was selected based on prior knowledge that soil microbes, including bacteria, fungi, and archaea, predominantly thrive within this range due to adequate moisture, nutrient availability, and root activity (Aislabie and Deslippe 2013). Within each experimental plot, soil samples were collected along two parallel transects located near the crop rows to ensure uniform

exposure to tillage and fertilization treatments while avoiding edge effects. At each transect, multiple soil cores were taken using a sterile soil auger and combined into a single composite sample representative of the plot. Each composite sample represented one independent biological replicate corresponding to a single experimental plot. No pooling was performed across plots, and all statistical analyses were conducted at the plot level. The subsamples were thoroughly homogenized, and visible plant residues and stones were removed prior to further processing. Sterile tools and gloves were used throughout sampling to minimize contamination. Immediately after collection, soil samples were placed in sterile containers, transported to the laboratory in insulated coolers under controlled temperature conditions, and stored at -80°C until DNA extraction and microbial analyses.

DNA extraction, library preparation, and sequencing

Microbial DNA was extracted from 0.25 g of well-homogenized soil using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany). Soil samples were subjected to combined chemical and mechanical lysis by incubation with CD1 lysis buffer and bead-beating using a MagNA Lyser instrument (Roche Applied Sciences, Penzberg, Germany) (Feinstein et al. 2009). Following lysis, samples were centrifuged to remove insoluble debris, and the clarified supernatant was used for downstream DNA purification to eliminate humic substances and other PCR inhibitors commonly present in soil (Schrader et al. 2012). DNA purification steps, including binding, washing, and elution, were carried out according to the manufacturer's protocol, resulting in purified DNA eluted in C6 buffer. The concentration of extracted DNA was measured using a Qubit™ 4 Fluorometer (Thermo Fisher Scientific™, Waltham, MA, USA), and DNA purity was assessed using a NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific™, Franklin, MA, USA).

Library preparation and sequencing were performed following the Illumina 16S Metagenomic Sequencing Library Preparation protocol (15044223 Rev. B) and the workflow described by Fidler et al. (2020) with minor modifications. Amplicon libraries were generated from 12.5 ng of soil-derived DNA by amplifying the V3–V4 regions of the

bacterial 16S rRNA gene using the universal primer pair 341F (5′–CCTACGGGNGGCWGCAG–3′) and 785R (5′–GACTACHVGGGTATCTAATC C–3′), both containing Illumina overhang adapters (Klindworth et al. 2013). PCR amplification was carried out using 2×KAPA HiFi HotStart ReadyMix, followed by indexing with the Nextera XT Index Kit and purification with MagSI Pure Beads. Library size and integrity were verified using an Agilent D1000 ScreenTape system, and libraries were quantified by qPCR, normalized, and pooled in equimolar concentrations. The pooled library (4 nM) was denatured, diluted to a final concentration of 8 pM, and sequenced on an Illumina MiSeq platform using the MiSeq Reagent Kit v3 (600 cycles), following standard manufacturer protocols. Detailed laboratory protocols for DNA extraction and library preparation are provided in the Supplementary files (Online resource 1).

Nutritional analysis

Grain samples were analyzed for key nutritional parameters, including protein content, antioxidant activity (both hydrophilic and lipophilic), and carotenoid concentration in maize.

Total protein content was quantified using the bicinchoninic acid (BCA) method with the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific) a bicinchoninic acid–based colorimetric method (Smith et al. 1985), following the manufacturer's instructions, and expressed as mg protein per 100 g of grain. Hydrophilic antioxidants were quantified through the Ferric Reducing Antioxidant Power assay (FRAP) (Brand-Williams et al. 1995; Nemes et al. 2018), while lipophilic antioxidants were assessed using the 2,2–diphenyl–1–picrylhydrazyl (DPPH) assay (Blois 1958; Benzie and Strain 1996). Results were expressed as ascorbic acid equivalents and Trolox equivalents per 100 g of grain. In maize, carotenoid content was determined by high–performance liquid chromatography (HPLC) using a PDA detector and external standards for identification and quantification (Rodriguez-Amaya and Kimura 2004). All nutritional measurements were performed in triplicate for each biological sample. Detailed analytical procedures are provided in the Supplementary files (Online resource 1).

Statistical analysis and data visualization

All statistical analyses and visualizations were conducted in R (version 4.4.0), using RStudio (v2025.09.0+387, Posit Software, 2025) (R Core Team 2024). Analyses were conducted at the plot level, treating composite samples as independent biological replicates. Tillage system, fertilization regime, and crop type were modeled as fixed effects and assessed separately. For univariate analyses, models were specified in the form $\text{response} \sim \text{factor}$, where factor represented tillage, fertilization, or crop type and was tested independently. Interaction terms were not included due to the study design and to focus on the main effects of each management factor. Statistical models excluded interaction terms between factors. Sequencing data were filtered, normalized, and transformed using the phyloseq package prior to downstream analyses (McMurdie and Holmes 2013). Alpha diversity was calculated with vegan, and treatment effects were evaluated using the Kruskal–Wallis and Wilcoxon rank–sum tests after verifying data non–normality with the Shapiro–Wilk test. Beta diversity was assessed using Bray–Curtis and UniFrac distance matrices and visualized through Principal Coordinate Analysis (PCoA) and Multidimensional Scaling (MDS); group differences were tested with PERMANOVA. The relative contribution of tillage, fertilization, and crop type to microbial community variation was quantified using PERMANOVA R^2 values from multivariate community analyses, representing the proportion of variance explained by each fixed effect. Variable importance metrics derived from Random Forest models were additionally used to compare the predictive influence of each factor (Breiman 2001). Differential abundance of microbial taxa and predicted functional genes inferred from 16S rRNA gene data was assessed using DESeq2 (Love et al. 2014). Results were visualized as volcano plots constructed in ggplot2, with $\log_2$ fold change ($\log_2(\text{FC})$) representing effect size and $-\log_{10}(\text{p–value})$ indicating statistical significance. Functional genes were considered significantly differentially abundant when $|\log_2(\text{FC})| > 2$ and $\text{p} < 0.05$. For functional interpretation, significantly enriched genes were grouped based on KEGG–defined functional pathways, and pathway–level summaries were generated to compare maize– and wheat–associated enrichments as

well as differences among fertilization and tillage regimes. Random Forest classification (randomForest package) was used to identify key genera contributing to community differentiation and MDS clustering patterns, with model performance evaluated via the out-of-bag (OOB) error rate and variable importance ranked by Mean Decrease Accuracy and Mean Decrease Gini. Canonical Correspondence Analysis (CCA) was conducted using the vegan package to integrate microbial community composition with tillage, fertilization, and crop nutritional profiles (low, medium, high) and to visualize their joint multivariate relationships (Oksanen et al. 2025). The displayed bacterial genera correspond to the top 15 most abundant genera retained in the ordination dataset and projected onto the CCA space to visualize their relative associations with treatment combinations and nutritional profiles. Correlations between microbial taxa and crop nutritional parameters were assessed using Spearman's rank correlation and visualized as heatmaps and correlation matrices with pheatmap and corrplot. Microbial co-occurrence networks were constructed using NetCoMi from CLR-transformed abundance data based on Pearson correlations (Peschel et al. 2021). Network topology was characterized by modularity, density, and degree centrality, and these metrics were used for descriptive comparison across treatments. Degree centrality values were additionally used to scale node sizes in network visualizations, with larger nodes indicating taxa with higher connectivity within the co-occurrence network. Summary statistics describing node degree distributions for each network are provided in Supplementary files (Online Resource 4). Unless otherwise stated, statistical significance was determined at $p < 0.05$. All graphical outputs, including bar plots, heatmaps, pie charts, line plots, and network visualizations, were produced in ggplot2 and related R packages.

Results

Effects of tillage and fertilization practices on microbial alpha diversity

Ploughing increased microbial alpha diversity relative to deep loosening in both maize and wheat (Fig. 1a and 1b). In maize, Shannon diversity was

higher under ploughing (8.55 ± 0.51) than under deep loosening (8.37 ± 0.33), and this difference was statistically significant according to the Wilcoxon rank-sum test ($W = 75$, $p = 0.047$). In wheat, ploughing also resulted in significantly higher Shannon diversity (8.71 ± 0.33) compared with deep loosening (8.50 ± 0.20 ; Wilcoxon rank-sum test, $W = 70$, $p = 0.029$). Fertilization treatments also had a clear impact on alpha diversity in both maize and wheat, with certain nutrient combinations consistently associated with lower or higher diversity (Fig. 1c and d). In maize, Shannon diversity ranged from 8.17 to 9.01 across fertilization treatments. The lowest mean diversity values were generally observed in treatments with nitrogen or phosphorus alone, particularly under setting M (2 M N: 8.17 ± 0.68 ; 3 M P: 8.18 ± 0.60). In contrast, the highest diversity in maize was found in the treatment combining nitrogen and phosphorus (5 M N+P: 9.01 ± 0.32), followed by the full NPK treatment under setting M (8 M N+P+K: 8.92 ± 0.28). Fertilization treatments showed specific patterns in microbial alpha diversity in wheat (Fig. 1d). Shannon diversity ranged from 7.29 to 8.94 across fertilization treatments. The lowest mean diversity was observed under nitrogen-only fertilization (2 M N: 7.56 ± 0.38), while higher diversity values were recorded under combined or balanced nutrient inputs, including nitrogen with potassium (6 M NK: 8.75 ± 0.22), potassium alone (4E K: 8.72 ± 0.04), and phosphorus alone under setting E (3E P: 8.68 ± 0.37). The full NPK treatment under setting M showed intermediate diversity values (8.11 ± 0.22).

Management-driven shifts in soil microbial fingerprints and structural composition

Variation in soil microbial community composition was closely associated with differences in crop type, tillage intensity, and fertilization regime (Fig. 2). Clear genus-level microbial fingerprints were observed across all management factors.

With respect to crop type, maize and wheat rhizosphere soils exhibited distinct microbial assemblages (Fig. 2a). A substantial proportion of genera was shared between the two crops (62.89%), while notable fractions were uniquely associated with each crop (13.75% for maize and 23.37% for wheat). Maize-associated soils were characterized by higher

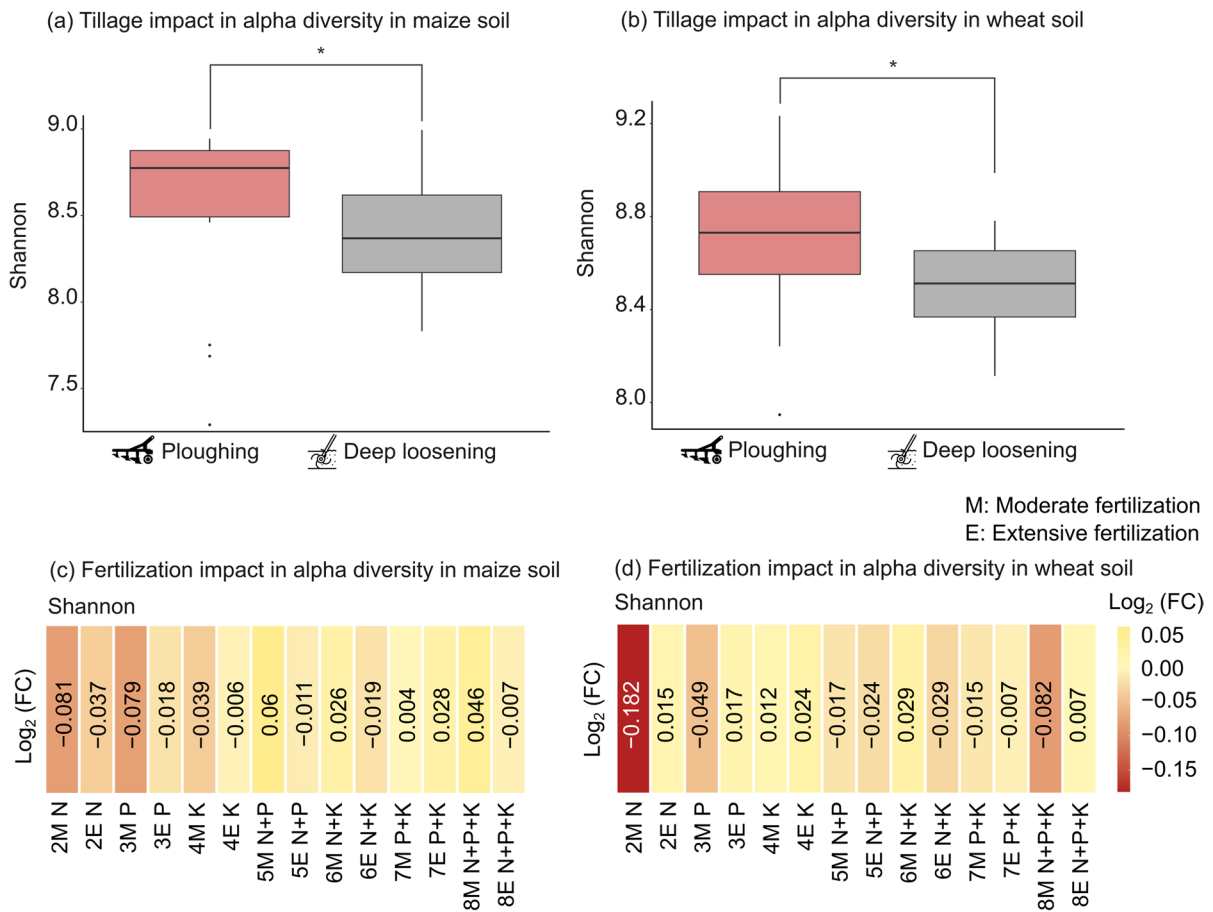


Fig. 1 Alpha diversity under different tillage and fertilization systems. Panels (a) and (b) show Shannon diversity under different tillage settings for maize and wheat, respectively. Panels (c) and (d) present heatmaps of Shannon diversity under different fertilization treatments in maize and wheat. Colors rep-

resent changes relative to the control, where red indicates the lowest values, yellow the highest, and orange corresponds to the control level. M stands for moderate fertilization E stands for extensive fertilization. Asterisks indicate significant differences ($p < 0.05$ *)

relative abundances of genera such as *Luteibacter*, *Methylobacterium*, *Corallococcus*, *Psychrobacillus*, and *Cellulomonas*, whereas wheat soils were enriched in *Geobacter*, *Methylothera*, *Parasegetibacter*, *Luedemannella* and *Dyadobacter*. The shared community was dominated by ubiquitous genera including *Bacillus*, *Rubrobacter*, *Gaiella*, and *Sphingomonas*, which accounted for the majority of the total relative abundance.

Tillage intensity also strongly influenced genus-level community composition (Fig. 2b). Ploughing and deep loosening supported partially distinct microbial assemblages, with a considerable proportion of genera shared between the two systems (61.55%). Ploughed soils were characterized

by genera such as *Luedemannella*, *Micromonospora*, *Pseudonocardia*, *Anaerobaculum* and *Rhodospirillum*, whereas deep-loosened soils were enriched in *Kitasatospora*, *Desertibacter*, *Jatrophihabitans*, *Cystobacter*, and *Marmoricola*. The shared genera, including *Bacillus*, *Tumebacillus*, *Rubrobacter*, *Gaiella*, *Bryobacter*, and *Sphingomonas*, represented the largest fraction of total relative abundance, although their proportional contributions differed between tillage systems.

Distinct genus-level patterns were also evident among fertilization treatments (Fig. 2c). Control, moderate, and extensive fertilization regimes each supported unique subsets of genera, alongside a shared core community (47.85%). Control soils were associated with genera such as *Kitasatospora*,

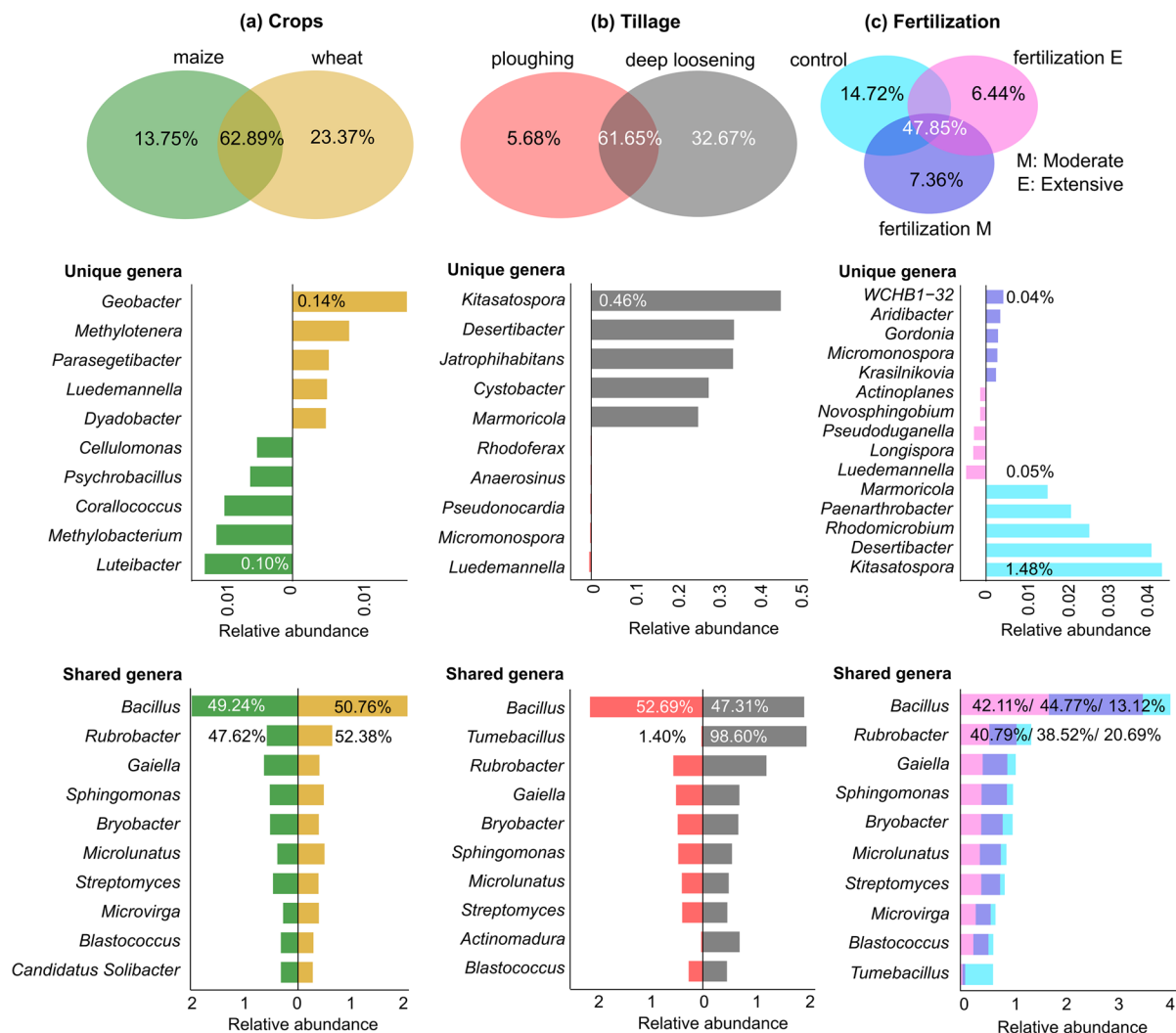


Fig. 2 Microbial fingerprints under different crop, tillage, and fertilization systems. Venn diagrams and relative frequency distributions illustrate the microbial fingerprints associated with key management factors: **(a)** crop type (maize vs. wheat), **(b)** tillage practice (ploughing vs. deep loosening), and **(c)** fertilization settings (control, setting M: moderate fertilization, and setting E: extensive fertilization). Top panels show the proportion of unique and shared microbial genera among treatments, while bottom panels display the relative frequen-

cies of these taxa, highlighting distinct crop-, tillage-, and fertilization-specific microbial fingerprints. For visualization purposes, taxa were ranked by relative abundance, and only the most abundant genera are shown in the figures (top 5 for the unique genera and top 10 for the shared genera). Percentages were calculated relative to the full dataset prior to ranking. The complete list of unique and shared genera, along with their corresponding relative abundances and percentage values, is provided in Online Resource 2

Desertibacter, *Rhodomicrobium*, *Paenarthrobacter*, and *Marmoricola*, whereas moderate fertilization favored genera including *WCHB1-32*, *Aridibacter*, *Gordonia*, *Micromonospora*, and *Krasilnikovia*. Extensive fertilization was characterized by higher abundances of *Luedemannella*, *Longispora*, *Pseudoduganella*, *Novosphingobium*, and *Actinoplanes*.

Across all fertilization regimes, a consistent shared community dominated by *Bacillus*, *Rubrobacter*, *Gaiella*, *Sphingomonas*, and *Bryobacter* persisted.

Following the compositional patterns observed in Fig. 2, multivariate analyses were conducted to explore how management practices structured soil microbial communities. The PCoA plots

(Fig. 3a–3c) show distinct clustering patterns according to crop type, tillage method, and fertilization settings. While fertilization showed no significant effect and produced substantial overlap among samples ($p=0.405$, $R^2=0.03$), crop type ($p=0.001$, $R^2=0.09$) and tillage ($p=0.001$, $R^2=0.07$) resulted in clearer separations. To further explore the influence of soil disturbance, multidimensional scaling (MDS) analyses were performed separately for each tillage practice (Fig. 3d, 3e). Under ploughing, maize and wheat samples partially overlapped, while in contrast, under deep loosening, maize and wheat formed more distinct clusters.

To identify the microbial groups contributing most strongly to the MDS clustering patterns, Random Forest classification was applied, and the key genera were visualized using barcode plots (Fig. 3f, 3g). The resulting models showed out-of-bag (OOB) error rates of 23.08% for ploughing and 3.85% for deep loosening. Under ploughing, genera such as *Bacillus*, *Gaiella*, *Bryobacter*, and *Gemmatimonas* were among the major contributors to the observed variability. Conversely, deep loosening was characterized by *Bacillus*, *Gaiella*, *Nocardioideis*, and *Nitrospira*.

Functional profiling and predicted metabolic potential of soil microbiome

To complement the taxonomic and community-level patterns, functional profiling was conducted to assess differences in predicted functional gene abundance across crop type, tillage, and fertilization settings. This analysis was performed using computational predictions derived from the identified microbial taxa, rather than direct sequencing of functional genes, to estimate the potential metabolic functions associated with each management condition. The analysis revealed distinct variations in the metabolic potential of soil microbial communities under different agricultural practices (Fig. 4). Although most functional genes showed no significant change, specific subsets were differentially regulated depending on the factor considered. In the crop comparison, several KEGG orthologs related to transport and carbohydrate modification were more strongly associated with wheat than with maize (Fig. 4 (a₁)), including orthologs linked to ABC transporters (K11077 [$\log_2FC = -2.50$, $p=0.015$],

K17205 [$\log_2FC = -2.16$, $p=0.016$], K17207 [$\log_2FC = -2.16$, $p=0.016$]), antimicrobial resistance (K19278 [$\log_2FC = -3.98$, $p=0.034$], K19209 [$\log_2FC = -2.45$, $p=2.93 \times 10^{-5}$]), and glycosyltransferases (K16562 [$\log_2FC = -2.62$, $p=0.004$], K18101 [$\log_2FC = -2.02$, $p=3.71 \times 10^{-5}$]). In contrast, maize exhibited a higher relative representation of functions related to biofilm formation and quorum sensing (K14051 [$\log_2FC = 2.59$, $p=0.005$], K18100 [$\log_2FC = 5.08$, $p=0.003$]), peptidases (K09634 [$\log_2FC = 3.84$, $p=0.037$]), and bacterial toxin production (K10954 [$\log_2FC = 2.40$, $p=0.005$]) (Fig. 4 (a₂) and 4 (a₃)).

Tillage practices also induced pronounced functional shifts (Fig. 4 (b₁)). At the KEGG ortholog level, ploughing was associated with a greater number of orthologs involved in lysine biosynthesis (K15792 [$\log_2FC = 2.07$, $p=0.007$]), peptidases (K09650 [$\log_2FC = 2.01$, $p=0.009$]), bacterial toxin production (K10954 [$\log_2FC = 2.16$, $p=0.009$]), and peptidoglycan biosynthesis and degradation (K15792 [$\log_2FC = 2.07$, $p=0.007$]). Conversely, deep loosening showed a higher representation of orthologs linked to transport and metabolic regulation, including ABC transporters (K11953 [$\log_2FC = -4.25$, $p=0.041$], K15495 [$\log_2FC = -2.14$, $p=0.008$]), glycosyltransferases, quorum sensing and biofilm formation (K13061 [$\log_2FC = -3.05$, $p=0.005$], K18100 [$\log_2FC = -2.64$, $p=0.023$]), and cysteine and methionine metabolism (K13061 [$\log_2FC = -3.05$, $p=0.005$]) (Fig. 4 (b₂) and 4 (b₃)).

In contrast, fertilization induced comparatively limited functional differentiation (Fig. 4 (c₁)). Only a small number of KEGG orthologs differed between fertilization settings, with functions related primarily to antimicrobial resistance (K10673 [$\log_2FC = -2.88$, $p=0.005$]) and ABC transporters (K17326 [$\log_2FC = -3.86$, $p=0.017$]) being detected under fertilization E (Fig. 4 (c₂) and 4 (c₃)).

Influence of tillage intensity on crop nutritional profile

Different soil treatments influence not only the soil microbiome but also the nutritional profile of crops (Fig. 5). Regarding tillage, in maize, the distribution of nutritional categories was more balanced under ploughing, with similar proportions of high and moderate levels (37.5% each), while deep loosening produced a more uneven pattern dominated by

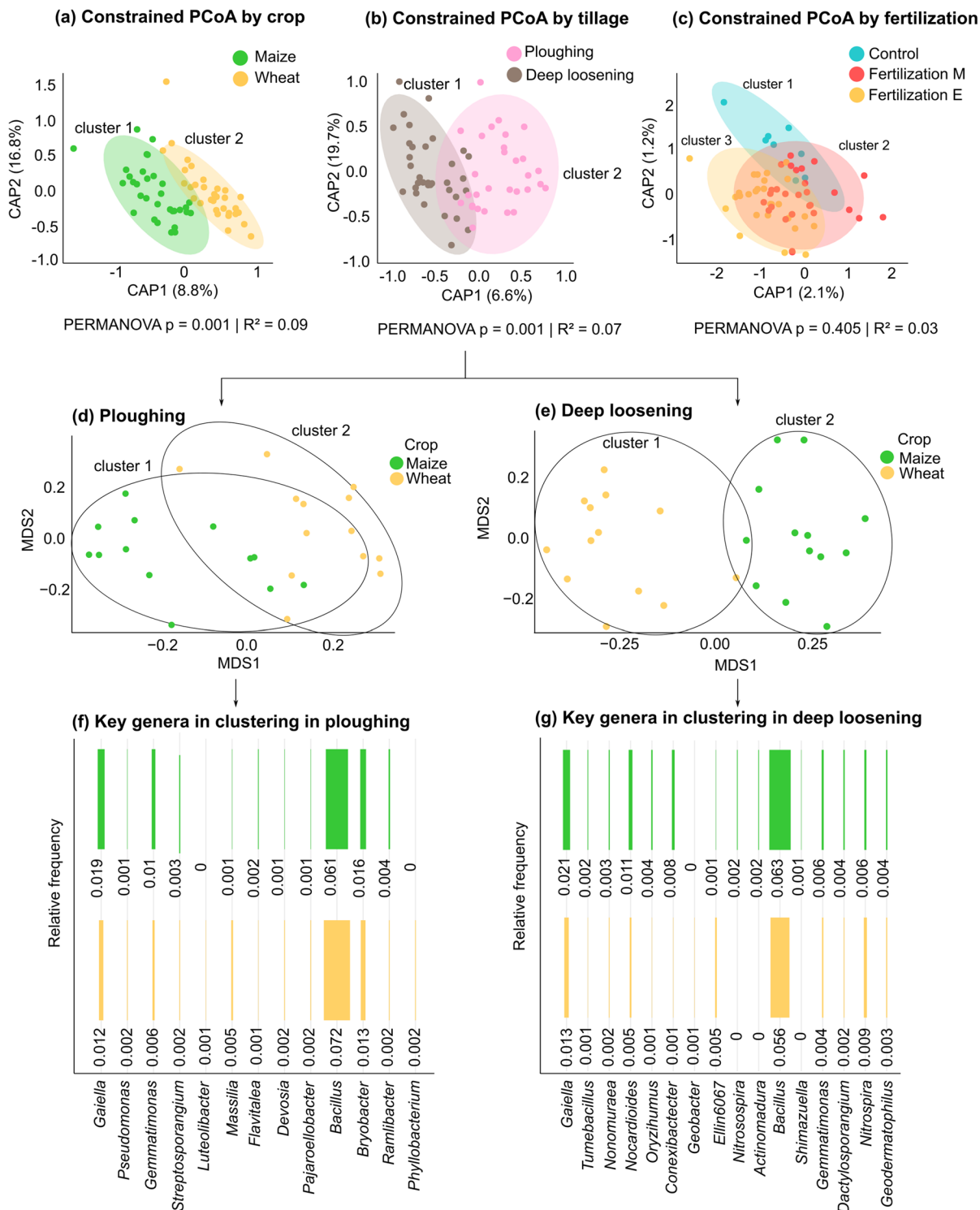


Fig. 3 Constrained ordination and classification of soil microbial communities under different management practices. (a–c) Constrained Analysis of Principal Coordinates (CAP) showing community differentiation by crop type, tillage, and fertilization based on Bray–Curtis dissimilarities. (d–e) Multidimensional Scaling (MDS) plots illustrate crop-specific clustering within each tillage system, with clearer separation under deep loosening. (f–g) Random Forest classification identifying key genera contributing to community differentiation under ploughing and deep loosening. M stands for moderate fertilization E stands for extensive fertilization. The complete list of genera and the corresponding results from Random Forest test are provided in Online Resource 3

low nutritional profiles (43.75% low, 25% medium, 31.25% high nutritional profile) (Fig. 5a). In wheat, a similar pattern was observed for the high nutritional profile, which was also promoted by ploughing (43.75%); however, the medium nutritional profile was more prevalent under deep loosening (50%), while low nutritional values were more common in ploughed soils (43.75%) (Fig. 5c).

Crop-specific nutritional responses to fertilization intensity

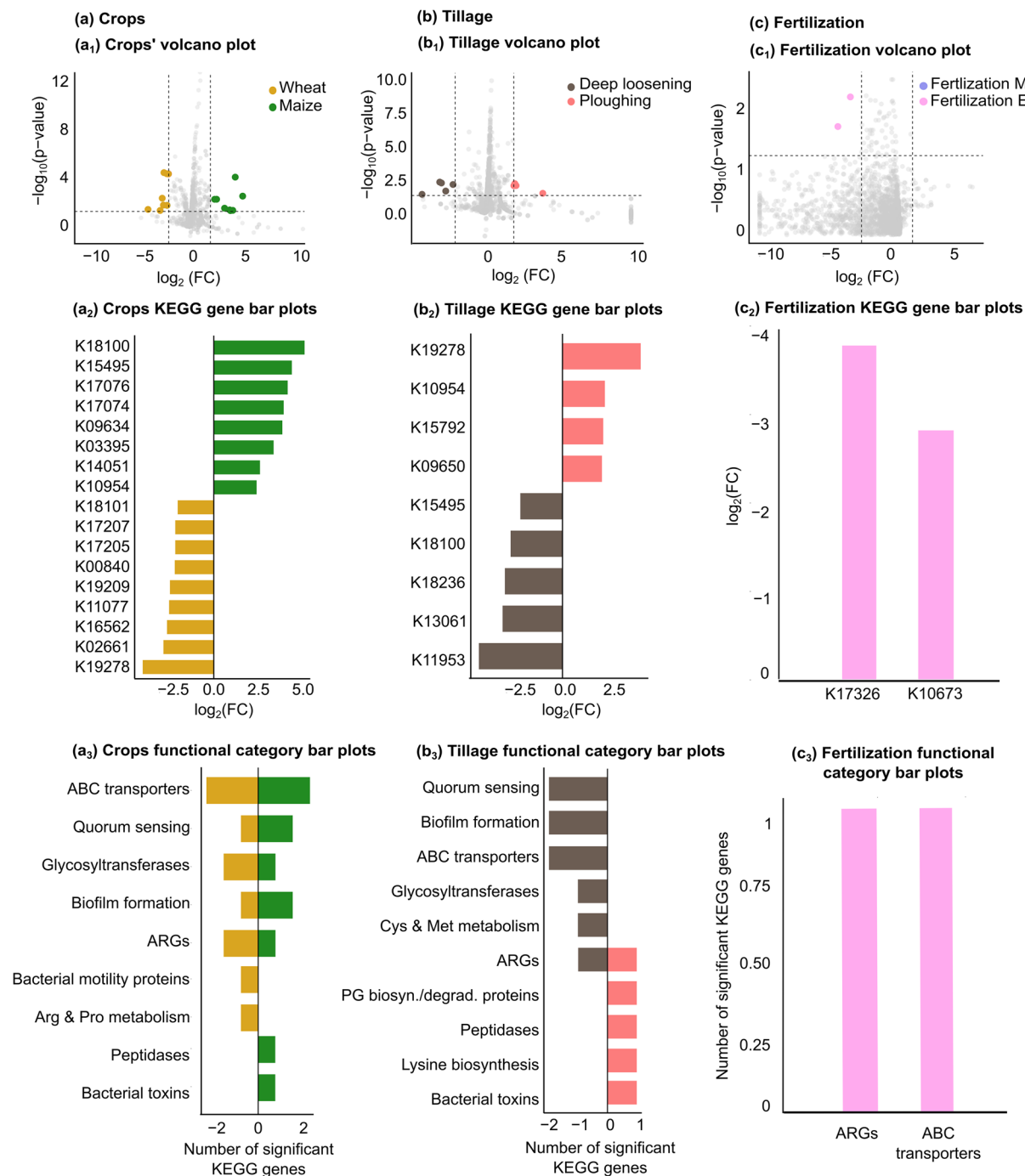
Figure 6 illustrates the influence of different fertilization settings and tillage practices on the nutritional profile and composition of maize and wheat. In maize, the highest nutritional profile was observed under the combined nutrient settings 7 M P+K (7.72%) and 8 M N+P+K (7.53%), while medium nutritional levels were more frequent under the control (6.92%), 5 M N+P (6.78%), and 8E N+P+K (6.70%) treatments. The lowest nutritional profile was predominantly associated with 6E N+K (5.40%), 2 M N (5.95%), and 7E P+K (5.94%) (Fig. 6a). In wheat, the high nutritional profile was mostly found under 2 M N (7.53%), 5 M N+P (7.82%), and 5E N+P (7.57%). Medium nutritional levels occurred under 8E N+P+K (6.40%), 2E N (6.54%), and 4E K (6.50%), while low nutritional values were detected in the control (5.17%), 7E P+K (5.55%), and 8 M N+P+K (5.92%) treatments (Fig. 6b).

At the level of specific nutrients, consistent differences were observed between fertilization settings M and E for both crops. In maize, carotenoid content remained largely unchanged between the two settings. However, both hydrophilic and lipophilic antioxidants were slightly higher under setting E (Fig. 6c).

In maize, antioxidants showed higher occurrence under setting E, with proportional shifts typically ranging from ~1–17%. In wheat, a similar pattern was detected for the antioxidant compounds, with both lipophilic and hydrophilic antioxidants showing higher proportions under setting E, with shifts varying from ~1–14%. Protein content remained comparable between treatments for both maize and wheat, with shifts generally within ~2–15% and a tendency to increase under setting M (Fig. 6c). In line with these findings, Fig. 6c illustrates the proportional contribution of major nutrient classes in maize and wheat. While the overall distribution remained relatively stable across treatments, several crop- and nutrient-specific trends were evident. In maize, the highest relative share of lipophilic antioxidants occurred under fertilization 5E (56.3%), while hydrophilic antioxidants peaked under 4E (59.2%). Protein contribution reached its maximum under 4E (52%), and carotenoids showed their highest proportion under 6E (52.3%). In wheat, the highest proportion of lipophilic antioxidants appeared under 2E (55.9%), whereas hydrophilic antioxidants reached their maximum under 3E (57.2%) and protein levels were highest under 7 M (55.8%).

Microbial correlations with crop nutritional parameters

The correlation analysis revealed distinct linkages between microbial taxa and crop nutritional parameters in both maize and wheat. In maize, the strongest associations were observed for carotenoids and proteins, with several genera showing moderate to strong correlations (Fig. 7a). Carotenoid content exhibited strong positive correlations with *Massilia* ($\rho=0.51$, $p=0.003$) and *Nitrospira* ($\rho=0.44$, $p=0.012$). In contrast, carotenoids were strongly negatively correlated with *Skermanella* ($\rho=-0.47$, $p=0.006$), *Corallococcus* ($\rho=-0.47$, $p=0.006$), *Geodermatophilus* ($\rho=-0.57$, $p=0.001$), *Nocardiodides* ($\rho=-0.46$, $p=0.009$), and *Sorangium* ($\rho=-0.51$, $p=0.003$). Protein content showed its strongest positive correlations with *Pirellula* ($\rho=0.50$, $p=0.003$) and *Hyphomicrobium* ($\rho=0.48$, $p=0.005$), while strong negative correlations were observed for *Terribacillus* ($\rho=-0.43$, $p=0.013$). Hydrophilic antioxidants were generally weakly correlated with most genera, but



notable negative associations were detected for *Oligoflexus* ($\rho = -0.45$, $p = 0.010$) and *Tepidisphaera* ($\rho = -0.42$, $p = 0.017$), whereas positive correlations were limited, the highest recorded for Baia ($\rho = 0.44$, $p = 0.011$). Lipophilic antioxidants showed a strong positive association with *Singulisphaera* ($\rho = 0.46$,

$p = 0.009$) and a strong negative association with *Rhizobacter* ($\rho = -0.45$, $p = 0.010$).

In wheat, the strongest correlations were primarily associated with lipophilic and hydrophilic antioxidants rather than proteins (Fig. 7b). Lipophilic antioxidants were stronger correlated positively with

Fig. 4 Volcano plots illustrate the differential abundance of functional genes under different agricultural settings: (a) crop type, (b) tillage, and (c) fertilization. Each point represents a single enzyme. The x-axis shows the logarithm of fold change ($\log_2(\text{FC})$), while the y-axis shows the negative logarithm of the p-value ($-\log_{10}(p)$), representing statistical significance. Functional genes were considered significantly differentially abundant when $\log_2(\text{FC}) > 2$ or < -2 and $p < 0.05$. Gray points represent functional genes that did not meet these thresholds, whereas colored points indicate significantly enriched genes under the respective condition. In panels (a₂–c₂), significant KEGG genes are displayed as bar plots based on $\log_2(\text{FC})$ values. Panels (a₃–c₃) summarize functional shifts at the mechanism level. Significant enzymes were grouped according to their KEGG-defined functional pathways, and the number of genes within each pathway was aggregated separately for treatment-associated enrichments. Mirrored bar charts illustrate the relative contribution of each functional category, with bars extending in opposite directions to indicate enrichment under the respective contrasting conditions. Abbreviations: M, moderate fertilization; E, extensive fertilization; ARGs, antimicrobial resistance genes; Arg & Pro metabolism, arginine and proline metabolism; PG biosyn./degrad. proteins, peptidoglycan biosynthesis and degradation proteins; Cys & Met metabolism, cysteine and methionine metabolism

Flavisolibacter ($\rho=0.49$, $p=0.004$) and *Parasegetibacter* ($\rho=0.46$, $p=0.008$), while negative correlations were observed with *Sporosarcina* ($\rho=-0.48$, $p=0.006$), *Occallatibacter* ($\rho=-0.47$, $p=0.007$), *Arcticibacter* ($\rho=-0.41$, $p=0.021$). Hydrophilic antioxidants displayed strong positive correlations with *Haliangium* ($\rho=0.54$, $p=0.001$) and *Roseimicrobium* ($\rho=0.45$, $p=0.010$). In contrast, strong negative correlations were observed for *Reyranelia* ($\rho=-0.41$, $p=0.021$), *Occallatibacter* ($\rho=-0.39$, $p=0.027$), and *Hamadaea* ($\rho=-0.39$, $p=0.027$). Protein content in wheat exhibited comparatively weaker correlations overall, with the strongest positive associations observed for *Inquilinus* ($\rho=0.39$, $p=0.029$), and moderate negative correlations for *Edaphobacter* ($\rho=-0.38$, $p=0.033$).

Microbial network responses to management practices

Co-occurrence network analysis revealed clear differences in microbial community structure across crop, tillage, and fertilization settings, with distinct topological patterns corresponding to variations in the nutritional profiles of maize and wheat (Fig. 8).

Across nutritional profiles, wheat consistently exhibits higher modularity than maize, peaking at the medium profile (0.091), while maize remains lower (maximum 0.065) (Fig. 8a). For both crops, density is highest at the medium nutritional profile (maize 0.408; wheat 0.383) and decreases at high. However, both crops show similar trends for modularity and density. In terms of tillage, deep loosening shows very high modularity at the low nutritional profile (0.162), followed by a sharp decline at medium (0.019). In contrast, ploughing displays moderate modularity that increases from low to medium (0.120) and slightly decreases at high (0.073). Density increases toward the high nutritional profile under both practices, particularly under ploughing (up to 0.349) (Fig. 8b). Additionally, we see interesting trends under different fertilization methods (Fig. 8c). Fertilization M maintains relatively stable modularity (0.109–0.121–0.108), whereas fertilization E shows pronounced variability, with very high modularity at low (0.190) and very low at medium (0.048). Density peaks at the medium nutritional profile under both regimes (M=0.460; E=0.406) and declines at high.

Joint effects of tillage and fertilization across nutritional profiles

Canonical correspondence analysis (CCA) integrating tillage practices, fertilization regimes, and nutritional profiles highlighted clear contrasts between management combinations associated with high versus low nutritional outcomes in both crops (Fig. 9). In maize, deep loosening combined with extensive fertilization (DL+E) was positioned in both the high-nutritional (CCA1=-0.16, CCA2=0.54) and low-nutritional regions of the ordination (CCA1=0.55, CCA2=0.01) (Fig. 9a). This variability was reflected in the relative positioning of the displayed bacterial genera within the ordination space. DL+E-associated regions were positioned close to genera such as *Nocardioides* (CCA1=0.48, CCA2=0.37) and *Skermanella* (CCA1=0.22, CCA2=0.05), but also near *Paenibacillus* (CCA1=-0.13, CCA2=0.04) and *Streptomyces* (CCA1=-0.13, CCA2=0.09), which were associated with low-nutritional profiles. In contrast, ploughing combined with moderate fertilization (P+M) clustered primarily with medium

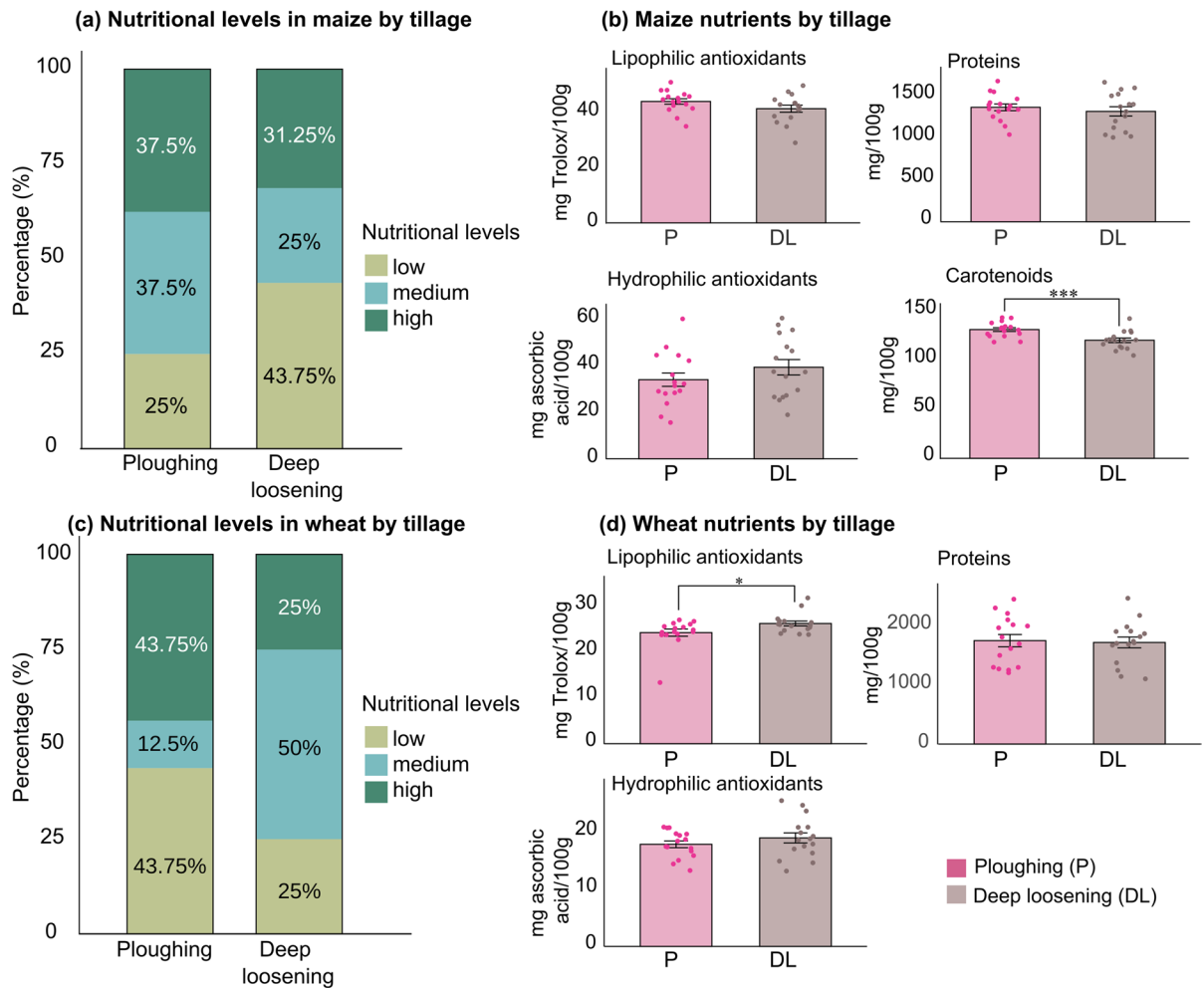


Fig. 5 Nutritional composition of maize and wheat under different tillage practices. Proportions of low, medium, and high nutritional profiles in maize (a) and wheat (c) under ploughing and deep loosening tillage. Concentrations of carotenoids, lipo-

philic antioxidants, hydrophilic antioxidants, and protein in maize (b) and wheat (d) under ploughing and deep loosening tillage. Error bars represent standard errors; significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

(CCA1 = -0.06, CCA2 = -0.51) and high nutritional profiles (CCA1 = -0.44, CCA2 = -0.16). This cluster showed closer alignment with genera such as *Microvirga* (CCA1 = 0.20, CCA2 = -0.16), *Haliangium* (CCA1 = 0.16, CCA2 = -0.22), and *Skermanella* (CCA1 = 0.22, CCA2 = 0.05).

In wheat, deep loosening combined with extensive fertilization (DL + E) showed the strongest association with high nutritional profiles (CCA1 = -0.80, CCA2 = -0.41) (Fig. 9b). This high-nutritional cluster was closely associated with genera such as *Nocardioidea* (CCA1 = 0.37, CCA2 = -0.13), *Skermanella* (CCA1 = 0.28, CCA2 = 0.01), and

Haliangium (CCA1 = 0.23, CCA2 = 0.09), which were positioned away from low-nutritional regions. Conversely, genera such as *Bacillus* (CCA1 = -0.21, CCA2 = 0.01) and *Streptomyces* (CCA1 = -0.05, CCA2 = -0.31), were located closer to low-nutritional wheat samples.

Deep loosening combined with moderate fertilization (DL + M) was predominantly associated with medium nutritional profiles and occupied intermediate ordination space (CCA1 = 0.30, CCA2 = 0.17). Ploughing treatments showed high (CCA1 = -0.19, CCA2 = 0.46) and medium nutritional outcomes (CCA1 = -0.23, CCA2 = 0.48) primarily when

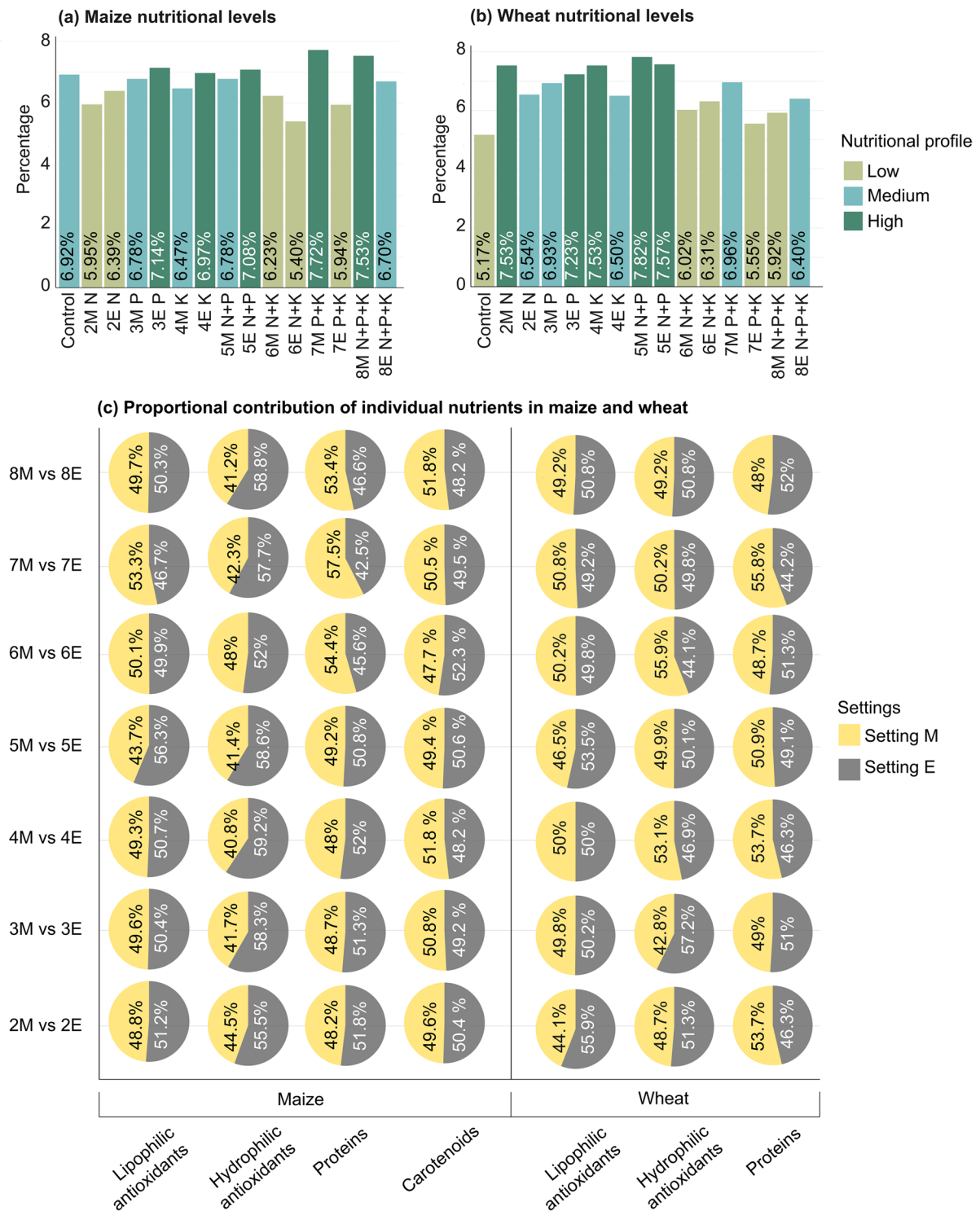


Fig. 6 Effect of different fertilization settings on crop nutritional content. Nutritional levels (high, medium, low) are shown for maize (a) and wheat (b) in bar plots, while the pro-

portional contribution of individual nutrients is illustrated for maize and wheat in pie charts (c). M stands for moderate fertilization E stands for extensive fertilization

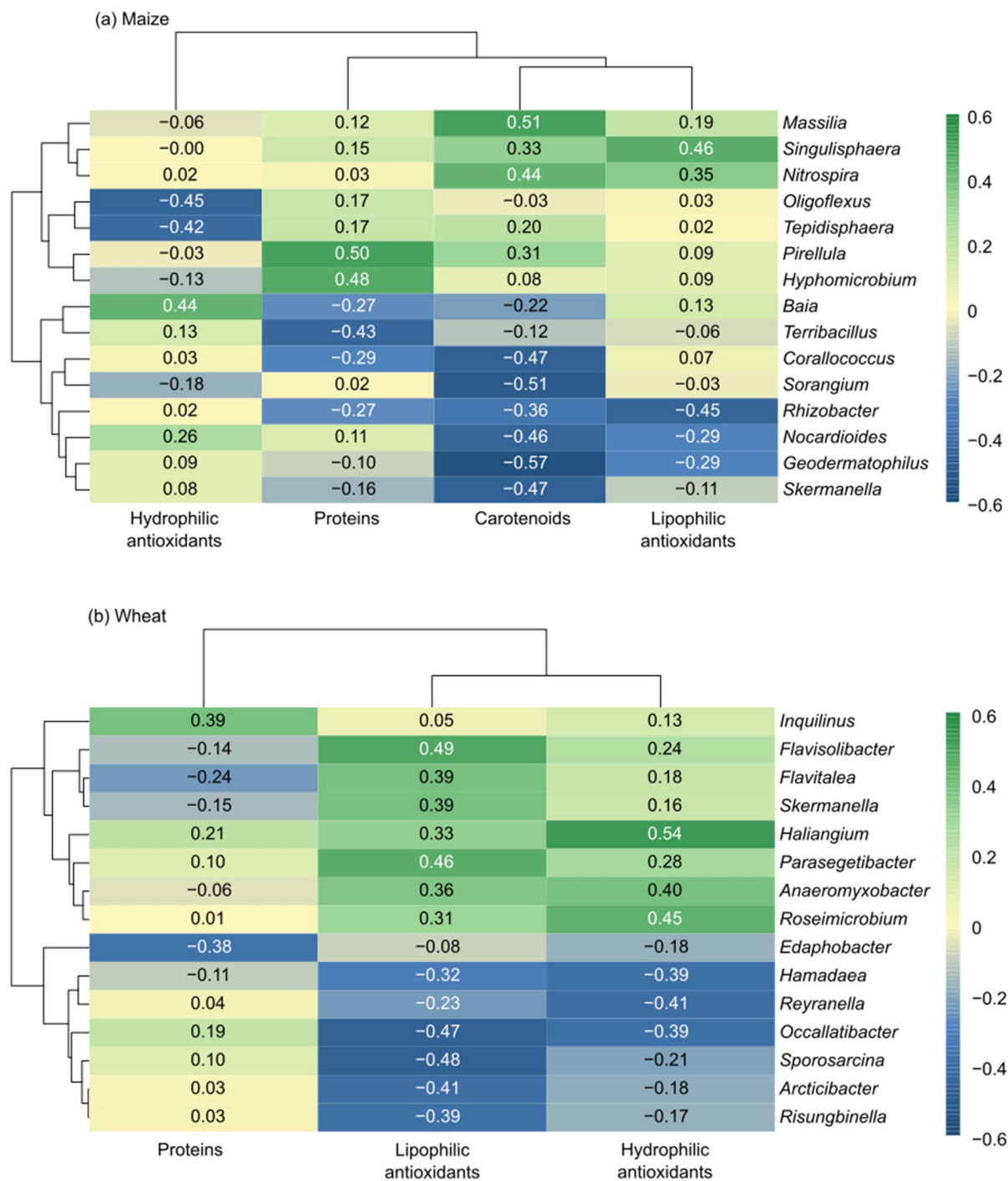


Fig. 7 Correlations between microbial genera and nutrient composition in maize and wheat. Heatmaps show Spearman correlations between the top 15 microbial genera and crops nutritional parameters in (a) maize and (b) wheat. Positive

(green) and negative (blue) correlations indicate the direction and strength of associations between microbial genera and protein, carotenoids (maize only), lipophilic antioxidants, and hydrophilic antioxidants

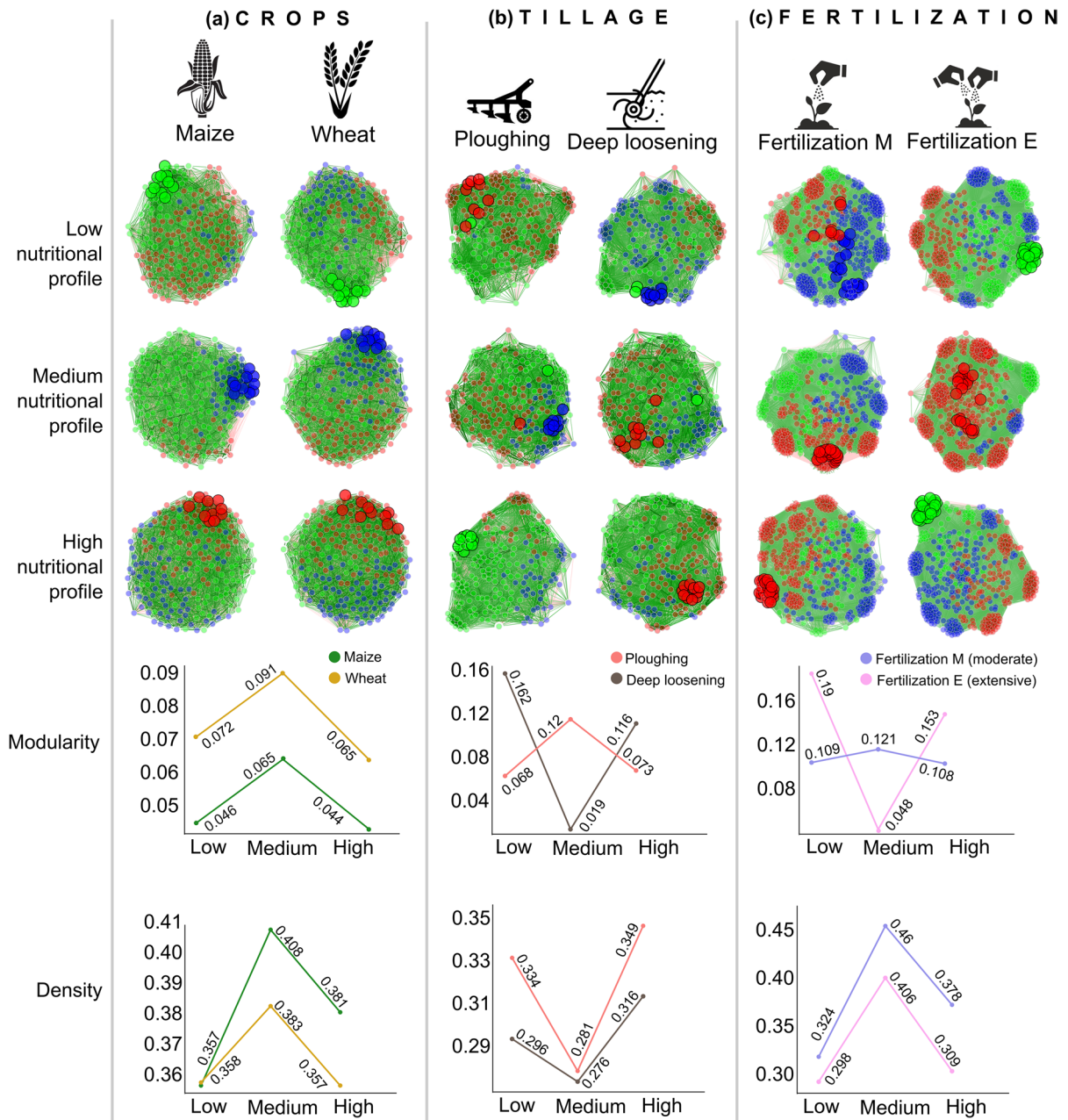


Fig. 8 Co-occurrence network analysis of soil microbial communities across nutritional profiles (low, medium, and high) under different management factors. **(a)** Crops (maize and wheat), **(b)** tillage systems (ploughing and deep loosening), and **(c)** fertilization regimes (M: moderate; E: extensive). For each management category, the upper panels display microbial co-occurrence networks corresponding to low, medium, and high nutritional profiles. Nodes represent microbial taxa, and edges indicate significant positive co-occurrence relationships inferred from Pearson correlations following CLR transformation ($p < 0.05$). Node colors denote distinct co-occurrence

modules identified through hierarchical clustering, while node size reflects degree centrality, indicating the relative connectivity of each taxon within the network. Larger nodes correspond to taxa with higher connectivity and potential hub-like roles within the microbial community. Degree centrality ranges and node-scale summary statistics for each network are provided in Supplementary files (Online Resource 4). Highly connected nodes represent potential hub taxa. The lower panels summarize corresponding trends in network modularity and density across nutritional profiles

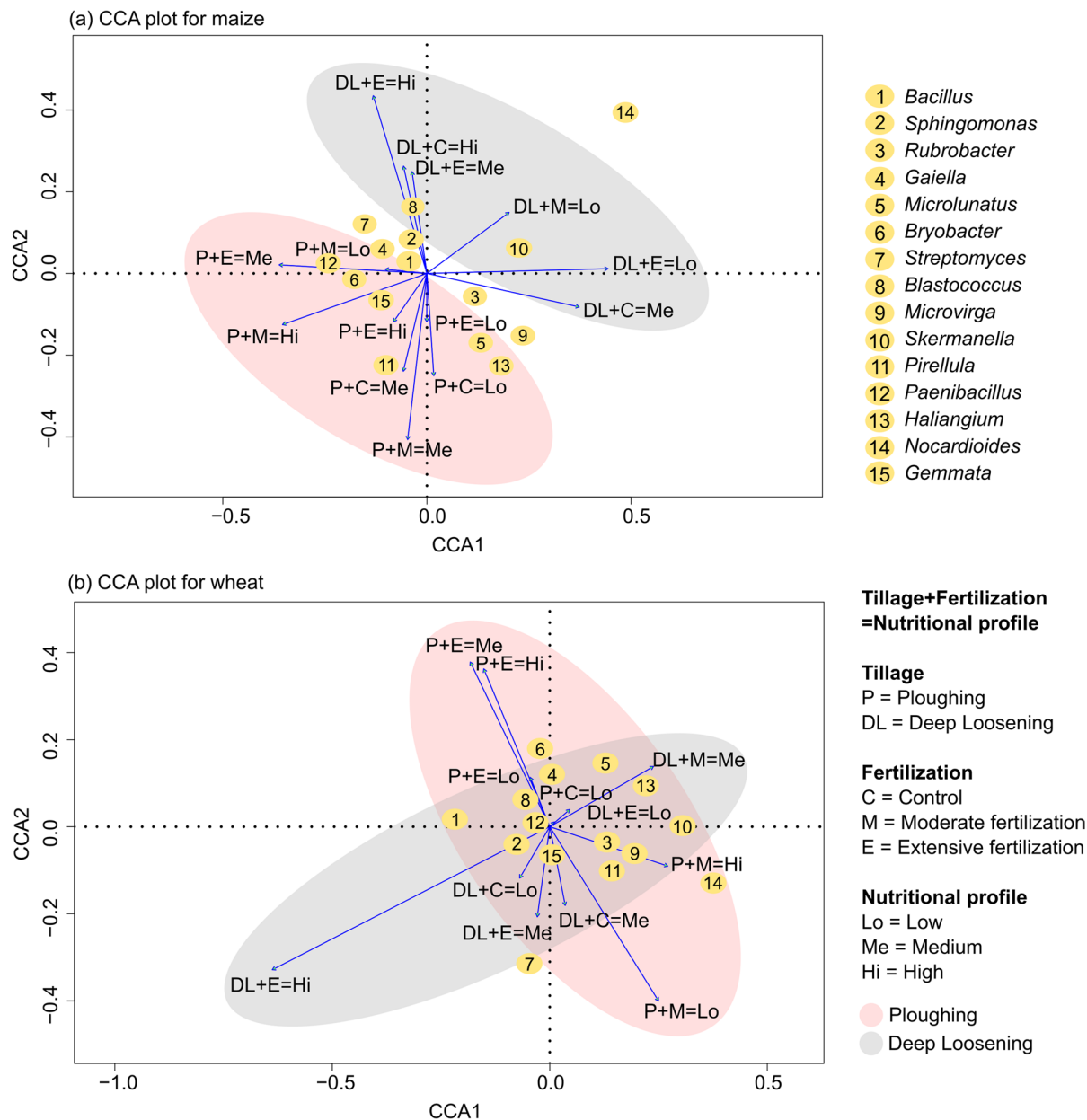


Fig. 9 Canonical Correspondence Analysis (CCA) illustrating the joint effects of tillage, fertilization, and nutritional profile on microbial community composition in (a) maize and (b) wheat. Sample centroids represent combinations of tillage (P=ploughing, DL=deep loosening), fertilization (C=control, M=moderate, E=extensive), and nutritional profile (Lo=low, Me=medium, Hi=high). Ellipses indicate the dispersion of treatment groups based on tillage. Numbered labels represent the top 15 most abundant bacterial genera projected onto the ordination space, with the corresponding genus names

provided in the legend. Taxa positioned closer to specific treatment combinations indicate stronger relative ecological associations with those environmental conditions. Blue arrows represent environmental gradients associated with tillage, fertilization intensity, and nutritional profile. The positions of samples and taxa along CCA1 and CCA2 reflect their relative associations with management practices and crop nutritional outcomes. Underlying numerical data are provided in Supplementary files (Online Resource 5)

combined with extensive fertilization (P+E), whereas ploughing under moderate fertilization displayed weaker alignment with the high–nutritional gradient ($CCA1 = 0.34$, $CCA2 = -0.11$).

Discussion

Tillage intensity and nutrient composition are key drivers of soil microbial diversity but act through different mechanisms. The findings of this study show that both tillage and fertilization substantially shape the diversity and structure of soil microbial communities in maize and wheat. The observed increase in microbial alpha diversity under ploughing suggests that intense soil disturbance creates dynamic microenvironments that stimulate microbial proliferation. Mechanical disturbance likely imposes stress conditions similar to those in disease ecology, where stress often triggers higher microbial activity. However, higher microbial diversity under ploughing does not necessarily imply greater functional stability or sustainability, as disturbance–driven diversity often reflects transient coexistence of stress–tolerant taxa rather than a resilient and functionally integrated soil microbiome (Chen et al. 2024a). This supports the broader idea that stress in microbial communities during disturbance promotes adaptive responses rather than reflecting favorable growth conditions (Rocca et al. 2019). Enhanced aeration and organic–matter exposure following ploughing could further promote microbial growth, although such effects are transient. Galand et al. (2016) found that moderate disturbance increases microbial production and phylogenetic diversity in the short term, promoting substrate turnover and remineralization. Over time, however, extensive disturbance can degrade soil structure and reduce microbial stability, as reported by Sándor et al. (2020).

In maize, higher microbial diversity under balanced multi–nutrient inputs compared with single–nutrient fertilization indicates that complementary resource availability supports broader microbial functionality. In contrast, in wheat, microbial diversity peaked under moderate nutrient availability, suggesting that optimal competition occurs when nutrient supply is neither limiting nor extensive. These contrasting responses indicate that fertilization effects on soil microbial diversity are strongly mediated by crop

identity, likely through differences in root exudation patterns, nutrient uptake strategies, and rhizosphere microenvironments. Similar crop–dependent fertilization effects have been widely reported, emphasizing that balanced nutrient supply enhances microbial diversity by improving soil organic carbon and pH, whereas extensive or unbalanced inputs favor copiotrophic taxa and reduce community heterogeneity (Dai et al. 2018; Kong et al. 2023).

Beyond diversity, distinct microbial fingerprints and community composition revealed clear crop–specific patterns shaped by tillage and fertilization. Ploughing and fertilization E were the main drivers of microbial community disruption under both maize and wheat, with ploughing exerting the strongest disruptive effect. Intensive disturbance weakened the core microbiome, reduced the persistence of unique taxa, and promoted stress–tolerant genera such as *Anaerostipes*, whose presence reflects oxygen–limited microsites and is associated with reduced plant performance, indicating suboptimal soil conditions (Longepierre et al. 2021; Ge and Wei 2023). These patterns are consistent with evidence that conventional tillage acts as chronic disturbance, reducing microbial biomass and impairing nutrient cycling (Domnariu et al. 2025; Řezáčová et al. 2025). In contrast, deep loosening maintained a more balanced and functionally integrated microbiome by preserving habitat heterogeneity, in line with conservation tillage studies (Khan et al. 2023). Beneficial genera such as *Kitasatospora*, *Marmoricola*, *Actinomyces*, and *Tuberacillus* were better sustained under this practice, reflecting enhanced secondary metabolism, phosphate solubilization, carbon turnover, and disease suppression (Bhatti et al. 2017; Jia et al. 2022; Wei et al. 2024b; Chungopast et al. 2025).

Similarly, fertilization M preserved higher functional diversity than fertilization E, which simplified community structure by favoring nutrient–adapted taxa and potentially undermining long–term soil functioning (Zhao et al. 2023b; Cunha-Ferreira et al. 2024). Genera promoted under moderate inputs, including *Gaiella* and *Sphingomonas*, highlight the role of optimized nutrient supply in sustaining multifunctional soils (Asaf et al. 2020; Wei et al. 2024a). Importantly, *Bacillus*, *Bryobacter*, *Sphingomonas*, and *Rubrobacter* persisted across all crops and management regimes, indicating strong ecological resilience and supporting their potential use as microbial

biomarkers for long-term soil health and sustainable management.

Functional profiling indicates that management intensity alters microbial metabolic strategies rather than merely redistributing functional genes. Tillage intensity emerged as the dominant regulator by shaping the physical and biochemical stability of the rhizosphere, thereby controlling whether microbial communities invest in cooperative resource acquisition or in stress-adaptive and competitive behaviors. Deep loosening likely preserves microscale heterogeneity and root-derived resource gradients, favoring coordinated transport and metabolic regulation that support efficient nutrient cycling. Similar stabilization effects of reduced disturbance on microbial functional integration have been reported under long-term conservation practices (Domnariu et al. 2025; Dong et al. 2024). In contrast, ploughing imposes repeated physical disruption that selects rapid turnover, defensive metabolism, and antagonistic interactions, reflecting a shift toward disturbance-tolerant life-history strategies. The enrichment of stress- and defense-related functions under intensive tillage is consistent with evidence that frequent soil disturbance reduces functional redundancy and promotes competitive microbial dynamics (Wang et al. 2023). Crop identity further modulated functional expression by influencing rhizosphere conditions, indicating that plant-driven selection fine-tunes microbial metabolism within the constraints imposed by tillage. Fertilization, however, exerted comparatively minor effects on metabolic potential, suggesting that nutrient inputs primarily adjust microbial activity levels rather than reorganizing functional strategies.

These microbial shifts were reflected in the nutritional profiles of maize and wheat. Ploughing generally promoted higher nutritional values, particularly in maize, where elevated carotenoid and lipophilic antioxidant levels likely resulted from improved aeration and nutrient mineralization stimulating secondary metabolism. Similar outcomes were reported by Xiao et al. (2025), who observed that plough tillage with straw incorporation improved soil nutrients, root growth, and nutrient content. Khan et al. (2024) also emphasized that maize productivity and nutrient status depend on enhanced nutrient turnover and aeration. In contrast, deep loosening favored hydrophilic antioxidants, reflecting enhanced root-microbe interactions and stress-related defense responses

under stable soil conditions. Wheat showed an opposite trend: deep loosening increased lipophilic antioxidants, while ploughing favored hydrophilic ones, reflecting crop-specific differences in nutrient uptake and moisture distribution. The opposite trend in wheat may stem from its finer, shallower root system and higher sensitivity to soil aeration and moisture.

Fertilization affected crop nutritional profile primarily through nutrient balance and intensity. In maize, moderate NPK input (setting M) produced the highest nutritional values, while over-fertilization or single-nutrient applications led to less balanced profiles. Wheat responded best to combined nitrogen and phosphorus inputs; however, high nutrient supply did not consistently enhance the nutritional profile, likely due to metabolic saturation. Across both crops, higher fertilization increased antioxidant activity but not protein content, suggesting that nutrient excess stimulates secondary, stress-related metabolism rather than primary protein synthesis. This pattern highlights the potential of controlled fertilization regimes to modulate the synthesis of bioactive metabolites, which could be valuable for medical or functional food production where specific antioxidant or stress-related compounds are targeted. This agrees also with Ahmadi et al. (2024), who reported that conventional tillage combined with phosphorus fertilization improved wheat yield and quality, while no-till with phosphorus maintained high yields and enhanced bread quality.

Correlations between microbial genera and crop nutritional traits revealed that the links were both taxon- and crop-specific. These relationships are correlative in nature and do not imply direct causal effects but rather indicate ecological associations between rhizosphere community composition and plant nutritional traits. Microbial associations with crop nutritional traits were strongly crop-dependent, indicating that rhizosphere communities influenced nutrient allocation through functional regulation of nitrogen turnover and redox balance rather than direct microbial abundance effects. In maize, positive associations with metabolically active genera such as *Massilia* and *Nitrospira* suggest that efficient nutrient transformation and nitrification supported protein synthesis and carotenoid accumulation (Sun et al. 2021; He et al. 2025), whereas negative relationships with competitive taxa such as *Nocardioides* imply

that microbial competition may limit nutrient availability to plant tissues (Ma et al. 2026; Liu et al. 2025). In wheat, microbial influences were more closely linked to antioxidant compounds, reflecting a stronger role of redox regulation in crop quality. Positive associations with carbon-cycling and stress-responsive genera such as *Haliangium* indicate that stable rhizosphere functioning favored antioxidant investment (Kumar et al. 2023), while negative relationships with disturbance-tolerant taxa such as *Sporosarcina* suggest constrained redox buffering under less favorable microbial conditions (Timmusk et al. 2014).

The co-occurrence network analysis demonstrated that management practices shaped the structural organization of soil microbial communities, and these structural configurations corresponded with differences in crop nutritional profiles. Clear contrasts emerged under different tillage systems. Deep loosening was characterized by a transition toward more integrated but not excessively dense network structures under improved nutritional conditions. This pattern suggests that deep loosening enhances soil physical properties, such as aeration, pore continuity, and root penetration, allowing diverse microbial taxa to interconnect without creating overly compact interaction networks (Tópa et al. 2025). Such moderate structural complexity may represent a balanced state in which cooperation is strengthened while avoiding excessive connectivity that could reduce resilience under environmental stress. In contrast, ploughing appeared to maintain more constrained or rigid interaction structures, potentially limiting broader microbial reintegration across ecological clusters, which is supported by previous research as well (Domnariu et al. 2025; Yuan et al. 2025). Fertilization regime further influenced network organization. Fertilization E showed greater structural variability and shifts in compartmentalization, suggesting that excessive nutrient inputs may disrupt microbial interaction patterns and favor more uneven community organization. Highly connected or tightly structured networks under intensive input showed connection with high nutritional profile; however, this may indicate dominance-driven organization that could reduce long-term resilience and adaptive capacity. This is consistent with the results of Huang et al. (2019) who stated that extensive inputs of fertilizers lead to weaken networks in long-term and reduce the strength of microbial connections. Moderate fertilization, in contrast,

was associated with more flexible and balanced network structures, suggesting adaptive reorganization without excessive centralization or overconnectivity. Such structural balance may support both functional integration and long-term ecological stability. This interpretation is consistent with findings by Zhang et al. (2022) who reported that reducing excessive fertilization improves agricultural efficiency while mitigating environmental impacts, highlighting the ecological benefits of balanced nutrient input.

Additionally, the joint effects of management practices also indicated that nutritional outcomes were shaped by how tillage and fertilization interacted to regulate soil stability and nutrient availability, with crop-specific responses. In maize, deep loosening combined with extensive fertilization produced heterogeneous nutritional outcomes, suggesting that reduced disturbance under high nutrient supply can generate variable rhizosphere conditions, leading to inconsistent nutrient conversion into plant tissues. In contrast, ploughing with moderate fertilization was associated with more stable medium-to-high nutritional profiles, indicating that balanced nutrient input under improved aeration supported more predictable nutrient assimilation. Although ploughing was associated with favorable or stable nutritional outcomes in the short term, the concurrent enrichment of stress-tolerant and potentially pathogenic microbial taxa observed in this study suggests that repeated disturbance may impair soil microbial functioning over time, which could ultimately reduce soil health and compromise nutritional quality in subsequent cropping cycles. Similar results are supported by Domnariu et al. (2025), who reported that 44 years of conventional moldboard ploughing resulted in the lowest total, active, and microbial biomass carbon content. Their findings indicate that repeated physical disturbance leads to a significant degradation of biological and chemical soil fertility, impairing the soil processes that are essential drivers of long-term agroecosystem sustainability.

Collectively, these results demonstrate that balanced nutrient input and minimal physical disturbance enhance microbial cooperation, functional diversity, and soil resilience, while intensive management simplifies network organization and reduces ecological specialization. The consistency between these findings and broader ecological studies reinforces the principle that environmental balance,

through nutrient regulation and limited disturbance, is central to sustaining complex and resilient soil microbial ecosystems.

Conclusions

Soil management practices jointly shaped the ecological and functional organization of the soil microbiome and, consequently, the nutritional composition of maize and wheat. Tillage intensity and fertilization regime were the dominant drivers of microbial network structure and nutrient cycling efficiency. Although ploughing increased microbial diversity, it favored generalist communities associated with lower functional stability, whereas deep loosening maintained more structured and resilient microbial networks that supported efficient nutrient transformation. Moderate, balanced fertilization reinforced these stable configurations, while extensive inputs reduced ecological specialization. Overall, crop nutritional profile in this system emerged from microbiome organization rather than diversity alone. From a management perspective, optimizing tillage intensity and nutrient balance, rather than intensifying disturbance or inputs, appears critical for sustaining soil functionality and improving crop nutritional outcomes.

Limitations

A limitation of this study is that microbial community analyses were restricted to bacteria based on 16S rRNA gene sequencing; fungal communities, which may respond differently to tillage and fertilization, were not assessed and warrant investigation in future studies. In addition, soil physicochemical properties were characterized only to define the soil profile and were not systematically analyzed across treatments, limiting direct linkage between microbial patterns and abiotic drivers. Finally, microbial functional profiles were inferred from 16S rRNA gene data rather than derived from shotgun metagenomics and therefore represent predicted functional potential rather than directly measured gene abundances and should be interpreted with caution.

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Author's contributions Njomza Gashi, Melinda Paholcsek: Writing—original draft; Péter Fauszt: Data curation; Njomza Gashi: Visualization; Njomza Gashi, Péter Fauszt: Formal analysis; Péter Fauszt: Software; Péter Dávid, Péter Fauszt, Zsombor Szőke, Piroska Bíróné Molnár, Andrea Kun—Nemes, Erzsébet Szöllősi: Methodology; Njomza Gashi, László Zsombik, Péter Fauszt, Péter Dávid, Zsombor Szőke, Piroska Bíróné Molnár, Andrea Kun—Nemes, Erzsébet Szöllősi, László Stündl, Ferenc Gál, Judit Remenyik, Melinda Paholcsek: Writing—review & editing, Njomza Gashi, Péter Dávid, Zsombor Szőke, Melinda Paholcsek: Conceptualization, Melinda Paholcsek: Project administration, Judit Remenyik, Melinda Paholcsek: Funding acquisition, Melinda Paholcsek: Supervision.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no conflict of interest.

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