

Active carbon fractions mediate microbial responses to long-term organic–inorganic fertilization and full-film mulching in a Loess Plateau wheat system

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Abstract

Aims

Soil organic carbon (SOC) on the Loess Plateau is widely depleted ($< 1.2\%$), yet how fertilization and full-film mulching jointly regulate carbon quality and microbial functioning over the long term remains unresolved. We partitioned their effects on SOC fractions in dryland winter wheat (*Triticum aestivum* L.) and identified which fractions govern microbial abundance and enzyme activity.

Methods

A six-year (2019–2025) 4×3 factorial experiment crossed four fertilization regimes (CK, M, NPK, MNPK) with three planting–mulching methods (CT, FM + SC, FM – SC). Fulvic acid carbon (FAC), humic acid carbon (HAC), light fraction organic carbon (LFOC), bulk SOC, microbial biomass, culturable community size, enzyme activities and grain yield were analysed by two-way ANOVA, regression, redundancy analysis and structural equation modeling (SEM).

Results

MNPK $\times$ FM + SC produced the greatest gains in LFOC (+ 73.5%) and HAC (+ 61.7%), while M $\times$ FM + SC maximized FAC (+ 58.1%) relative to CK $\times$ CT. Active carbon fractions, particularly LFOC, predicted culturable microbial abundances ($R^2 = 0.593\text{--}0.872$) and carbon-cycling enzyme activities ($R^2 = 0.661\text{--}0.818$), whereas total SOC was unrelated to any biological parameter ($R^2 < 0.11$). Phosphatase was decoupled from all carbon pools, indicating enzyme-specific regulation. SEM identified microbial activity as the dominant pathway driving organic carbon accumulation ($\beta = 0.96$, $R^2 = 0.93$). Grain yield increased by 33.6–38.7% under fertilization combined with full-film mulching.

Conclusions

Carbon quality rather than quantity governs soil biological function in this dryland system. Combined organic–inorganic fertilization with full-film soil-covered hole sowing offers a practical strategy for rebuilding active carbon and sustaining yield on the Loess Plateau.

Introduction

Soil organic carbon (SOC) sits at the intersection of climate regulation, agricultural productivity, and a wide range of ecosystem services (Huang et al. 2025). Roughly one-third of terrestrial carbon is held in soils, and the size and turnover of this reservoir respond strongly to land management decomposition kinetics, and pathways of physical and chemical stabilization (Blanchet et al. 2016). Shifts in SOC

therefore propagate through nearly every functional dimension of soil with downstream consequences for both ecosystem stability and the long-term viability of agriculture (Liu et al. 2018).

Across the Loess Plateau, agricultural performance is increasingly bottlenecked by soil degradation. Topsoil SOC values commonly fall below 1.2%, well under the 2% benchmark associated with healthy biological function (Yan et al. 2021). Compounding this, the soils of the region are markedly alkaline (pH 8.0–8.5); when paired with chronically low organic matter, this combination depresses phosphorus and micronutrient availability, weakens aggregate structure, slows infiltration, and erodes pH buffering (Wang et al. 2017). On top of these intrinsic constraints, precipitation arrives in narrow, unreliable windows, which further hampers the rebuilding of soil organic matter under conventional cropping. Designing management approaches that simultaneously rebuild SOC stocks and sustain yields has accordingly become a central priority for this fragile dryland system (Tao and Liu 2025).

Among the levers available to land managers, fertilization is arguably the most direct way to steer carbon dynamics. Mineral fertilizers act fast on the nutrient side but contribute little carbon themselves; organic amendments, by contrast, deliver carbon and biological substrate together, and so simultaneously alter chemical, biological, and physical fertility (Han et al. 2024). Long-term reliance on mineral inputs alone has been repeatedly shown to draw SOC down, particularly when no organic source is co-applied. Manures and composts maintain a steady carbon flux, sustain microbial populations, and enhance the stability of stored carbon, although their nutrient-release kinetics often lag the demand peaks of vigorously growing crops (Moraes Tavares et al. 2019; Rajkai et al. 2008). Combined organic–inorganic fertilization is now widely viewed as a way to reconcile these two trade-offs simultaneously (Xu et al. 2025). What remains unresolved is how distinct fertilization regimes differentially modulate the various SOC fractions and how those fractions in turn couple to microbial activity in interaction with surface management.

On the surface-management side, full-film soil-covered hole sowing (FM + SC) has been promoted across northwestern China as a way to lift water-use efficiency and stabilize yields under dryland conditions. The technique differs from the more familiar uncovered variant (FM – SC) by adding a 1–2 cm soil layer over the plastic film; this thin overburden slows ultraviolet degradation of the film, dampens surface temperature swings, and improves film–soil contact for moisture retention. The mulching literature has so far emphasized agronomic outcomes while paying comparatively little attention to how different mulching configurations reshape SOC fractions or interact with microbial processes. The shortage of factorial experiments that vary mulching and fertilization within a single design leaves an evident gap in the mechanistic picture.

Functionally, SOC is best understood not as a single pool but as a set of fractions that differ markedly in turnover, stability, and ecological role. The labile end of this continuum responds to management on much shorter timescales than bulk SOC and therefore offers a more sensitive readout of treatment effects (Yang et al. 2024). FAC consists of low-molecular-weight, readily decomposable organic acids that turn over on month-to-year timescales and supply energy to heterotrophic microorganisms. HAC sits

at an intermediate stability level: more humified, contributing to nutrient retention and to the longer-term stabilization of carbon. LFOC, defined operationally as the floatable fraction below 1.7 g cm^{-3} , consists largely of partially decomposed plant residues and acts as a transitional pool between fresh inputs and the more stable matrix; it is widely treated as the most responsive indicator of recent carbon dynamics (Liu et al. 2018). Soil extracellular enzymes complete the picture by mediating the biochemical reactions that transform nutrients and organic matter (Pikula and Ciotucha 2022; Song et al. 2024). Establishing how fertilization and mulching jointly reshape these fractions is a prerequisite for designing carbon-quality-targeted management.

Although fertilization effects on SOC accumulation and persistence have been investigated extensively across the Loess Plateau, most published evidence comes from short-duration trials or studies that vary a single fertilization regime at a time (Wang et al. 2019). Factorial designs that cross fertilization with mulching strategies remain uncommon, which limits our ability to detect synergistic or antagonistic responses that would be invisible to single-factor experiments. A companion single-factor experiment on the same platform showed that full-film mulching buffered winter wheat yield against interannual precipitation variability and kept water use efficiency comparatively stable across wet and dry years (Liu et al. 2026); because that experiment varied mulching alone, it could not establish whether the same configurations interact with fertilization in shaping soil carbon quality and microbial function. The connections among labile carbon fractions, microbial biomass, and enzyme activity under simultaneously varied fertilization and mulching regimes have, in particular, received only piecemeal attention (Carlos et al. 2023).

Against this background, we set up a 4×3 factorial randomized complete block field trial in dryland winter wheat on the Loess Plateau, crossing four fertilization regimes with three planting/mulching configurations. Three specific aims guided the work: (i) to partition the main and interactive effects of fertilization and full-film mulching on FAC, HAC, LFOC, and bulk SOC at multiple soil depths; (ii) to use regression analyses to identify which carbon fractions most directly govern microbial biomass, community size, and enzyme activity; and (iii) to apply redundancy analysis (RDA) together with structural equation modeling (SEM) to recover the multivariate response structure and the causal architecture of the carbon–microbial–enzyme system. Our working hypotheses were two-fold: that combined organic–inorganic fertilization paired with FM + SC would generate a larger response in labile carbon and microbial activity than either practice in isolation; and that microbial biomass would track active carbon fractions far more closely than it tracks bulk SOC. The addition of mulching as a crossed factor and the explicit emphasis on enzyme-specific regulation are the central novel contributions of the present work.

Materials and methods

Study area

Field work was carried out at the Dryland Agricultural Experiment Station in Qinzhou District, Tianshui (Gansu Province), at 34°19'–35°15'N, 105°37'–106°28'E, on the southeastern margin of the Loess Plateau. Climatically the site is temperate continental semi-arid: mean annual precipitation falls between 500 and 700 mm, the bulk of which arrives between June and September, and mean annual temperature is 8–14°C. Local elevations range from roughly 800 to 3000 m a.s.l., and the soils are yellow loams derived from loess. The dominant soil group at the experimental area is Calcic Luvisols, which combine moderate water-holding capacity with pronounced erodibility under high-intensity rainfall. Pre-experiment baseline properties of the 0–30 cm layer were: SOC 10.2 g kg⁻¹, total N 0.85 g kg⁻¹, total P 1.42 g kg⁻¹, available N 15 mg kg⁻¹, available P 20 mg kg⁻¹, available K 85 mg kg⁻¹, and pH 8.3. Agriculture in the surrounding landscape is centred on rainfed winter wheat, traditionally managed with mineral-fertilizer-heavy regimes and limited organic returns (Liu et al. 2022). The site was selected because it is broadly representative of the degraded croplands of northwestern China, where SOC depletion and water-erosion losses jointly constrain productivity; the baseline SOC measured here is well below the 2% threshold typically cited as necessary for healthy soil function (Fig. 1).

Experimental design

The trial was set up in autumn 2019 as a 4×3 factorial in a randomized complete block layout. Factor A (fertilization) had four levels and Factor B (planting method) had three, generating 12 treatment combinations. Each combination was replicated three times across three blocks, yielding 36 plots. Plot size was 2 m × 1 m (2 m²). Because the available land at the station was limited, plots are small relative to large-scale agronomic trials; we accordingly built in three layers of edge-effect control to safeguard data quality. (1) Unfertilized buffer strips were maintained throughout the experiment (0.5 m between adjacent plots within a block and 1.0 m alleys between blocks), and these strips were weeded routinely to suppress lateral nutrient transport and root encroachment. (2) All soil sampling and yield measurements were taken only from the central, edge-excluded portion of each plot (≥ 0.5 m inside every boundary, ≈ 1.0 m² effective area), so that border zones contributed nothing to the reported dataset. (3) Plastic mulching film was installed as discrete, plot-specific sheets to break hydrological connectivity between adjacent treatments and prevent lateral moisture migration. Treatments were applied without interruption in every growing season from 2019 to 2025. Travel and access restrictions imposed during the COVID-19 pandemic prevented our research team from carrying out comprehensive on-site sampling between 2020 and 2023; however, field operations (sowing, fertilizer application, mulching, weeding, harvest) were performed on schedule each year by station-resident staff. The soil and yield observations presented below were therefore taken in the 2024–2025 growing season, and they reflect the cumulative outcome of six consecutive years of uninterrupted treatment. This is the platform described in Liu et al. (2026), where the yield and water use response of the same three planting–mulching configurations is reported; the fractionated carbon, microbial and enzyme measurements presented here have not been published elsewhere.

Factor A (fertilization) consisted of four levels. CK was an unfertilized control providing the baseline reference. M received only organic input and isolated the effect of organic amendment. NPK applied only mineral fertilizer (urea 212 kg ha⁻¹, calcium superphosphate 170 kg ha⁻¹, potassium sulfate 120 kg ha⁻¹) and represented the conventional regional practice. MNPK combined the M and NPK inputs (manure 7500 kg ha⁻¹ + the same mineral fertilizer rates) and represented integrated nutrient management.

Factor B (planting methods) comprised three levels: CT, conventional tillage without mulching, using flat-field hole sowing with 18–20 cm row spacing, 10 cm hole spacing, and 3–5 cm sowing depth; FM + SC (full-film soil-covered hole sowing), involving full-surface full-season plastic film mulching with 1–2 cm of soil cover placed over the film surface, followed by hole sowing 1–2 days after mulching; and FM – SC (full-film no-cover hole sowing), involving full-surface full-season film mulching without soil cover, with hole sowing conducted 1 day after mulching. The soil-covered film layer in FM + SC provides additional benefits including reduced ultraviolet degradation, improved surface insulation through reduced radiative heat loss, enhanced soil–film contact for moisture conservation, and prevention of film displacement by wind. These abbreviations (FM + SC, FM – SC, CT) are used consistently throughout the text.

Sheep manure was sourced from a local farm adjacent to the station, composted, and characterized in-house before each application. Across batches, the manure averaged 11.24 g kg⁻¹ total N, 17.5 g kg⁻¹ total P, 8.6 g kg⁻¹ total K, 35.2% organic matter, and 18.5% moisture, with batch-to-batch variation kept below 8% (CV). Mineral fertilizers were standard commercial products, urea (46% N), calcium superphosphate (7.0% P), and potassium sulfate (41.5% K), all meeting Chinese national standards (GB 2440 – 2017, GB 20413 – 2017, GB 20406 – 2017). Per-treatment nutrient inputs are summarized in Table 1.

Table 1
Nutrient inputs for each fertilization treatment

Treatment	Organic fertilizer (kg ha ⁻¹)	Urea (kg ha ⁻¹)	Ca superphosphate (kg ha ⁻¹)	K ₂ SO ₄ (kg ha ⁻¹)	Total N (kg ha ⁻¹)	Total P (kg ha ⁻¹)	Total K (kg ha ⁻¹)
CK	–	–	–	–	0	0	0
M	7500	–	–	–	84.3	130.9	64.8
NPK	–	212	170	120	97.5	11.9	49.8
MNPK	7500	212	170	120	181.8	142.8	114.6
Note: It is important to recognize that the M and MNPK treatments received substantially higher P inputs (130.9 and 142.8 kg P ha ⁻¹ , respectively) than the NPK treatment (11.9 kg P ha ⁻¹) due to the high phosphorus content of sheep manure. This nutrient input imbalance is an inherent characteristic of the organic fertilizer source and should be considered when interpreting treatment effects.							

Organic fertilizers and all phosphorus and potassium fertilizers were applied as basal fertilizers 10 days before sowing and mechanically incorporated to 15–20 cm depth. Nitrogen fertilizer was split-applied:

80% as basal application 10 days before sowing and 20% as topdressing at jointing stage. This split-application strategy was designed to provide adequate nitrogen supply during tillering while providing supplemental nitrogen during rapid vegetative growth. Fertilization treatments were maintained consistently from 2019 through 2025, with identical application rates and timing in each of the six growing seasons.

Sample collection and processing

We sampled soil at four phenological checkpoints across the 2024–2025 winter wheat season, green-up (early March 2025), jointing (mid-April 2025), heading (mid-May 2025), and maturity (late June 2025). Within each plot, five sub-samples were taken along an S-shaped transect confined to the central, edge-excluded region (≥ 0.5 m inside every plot boundary; see “Experimental design”) and pooled into one composite. Three depth intervals were sampled per plot, 0–10, 10–20, and 20–40 cm, with approximately 500 g of soil collected at each point using a quartering protocol. The composites were transferred on ice (4°C) and processed within 24 h: an aliquot was kept at 4°C and used for microbial biomass within seven days, while the remainder was air-dried, passed through a 2-mm sieve, and reserved for organic-carbon and nutrient assays.

We grouped the measured carbon pools by their characteristic stability and ecological role. The labile pools comprised FAC (low-molecular-weight, rapidly decomposable acidic compounds with month-to-year turnover), HAC (more humified, intermediate-stability compounds), and LFOC (a density-defined fraction $< 1.7 \text{ g cm}^{-3}$ consisting largely of partially decomposed plant residues, bridging fresh inputs and the stable matrix). Bulk SOC was measured in parallel as an integrative descriptor of the total pool.

All four SOC fractions were determined using internationally accepted protocols. For FAC, soil was extracted in 0.1 M KOH for 24 h under N_2 , the extract was then acidified to pH 1.0, and the resulting solution was analyzed by TOC (Gand and Nain 2012). HAC was recovered by sequential alkali–acid extraction following the International Humic Substances Society procedure (Gong et al. 2020). LFOC was isolated as the floating phase during sodium polytungstate density separation (cut-off 1.7 g cm^{-3}). Bulk SOC was determined by dry combustion at 900°C on an Elementar Vario MAX CN analyzer (Germany) after HCl pretreatment to eliminate carbonates (Nie et al. 2012). All carbon assays were run in triplicate.

Microbial biomass C and N (MBC, MBN) were quantified by chloroform-fumigation extraction with conversion factors $k_{\text{EC}} = 0.45$ and $k_{\text{EN}} = 0.54$ (Pires et al. 2022). Culturable microbial community size was estimated by serial dilution plating: bacteria on nutrient agar (NA) at 28°C for 48–72 h; actinomycetes on Gause's No. 1 synthetic medium at 28°C for 5–7 days; and fungi on rose bengal agar (RBA) at 25°C for 3–5 days. Counts were expressed as CFU g^{-1} oven-dry soil. We acknowledge that culture-based plating recovers only a fraction of total soil microbial diversity; the approach was used here because it provides reproducible, comparable estimates of culturable community size suitable for between-treatment contrasts, which is the inferential question of this paper.

Three extracellular enzymes were assayed colorimetrically. Urease activity was measured via the indophenol blue method and expressed as $\text{NH}_3\text{-N}$ released ($\text{mg g}^{-1} 24 \text{ h}^{-1}$); sucrase activity via 3,5-dinitrosalicylic-acid reduction with glucose as the reported product ($\text{mg g}^{-1} 24 \text{ h}^{-1}$); and phosphatase activity via the disodium-phenyl-phosphate method, expressed as phenol released ($\text{mg g}^{-1} 24 \text{ h}^{-1}$). Additional soil chemistry was characterized as follows: available N by alkaline hydrolysis diffusion (Bar-Yosef and Ben Asher 2013), available P by the Olsen method, available K by 1 M NH_4OAc extraction (Wei et al. 2021), total N and total P by $\text{H}_2\text{SO}_4\text{-H}_2\text{O}_2$ digestion, organic matter by potassium dichromate wet oxidation (using the canonical conversion $\text{OM} = \text{SOC} \times 1.724$), and pH by glass electrode at a 1:2.5 soil:water ratio.

Crop management and yield assessment

Winter wheat (*Triticum aestivum* L.) cv. 'Tianxuan 15' was used throughout, a locally adapted cultivar of regional economic importance. The crop was drilled at 225 kg ha^{-1} in early October of each year (2019–2024) under continuous winter-wheat monoculture for six consecutive seasons, targeting roughly 4.5×10^6 plants ha^{-1} . No supplemental irrigation was applied at any point in the experiment; the trial relied entirely on natural precipitation so that the results would reflect authentic dryland conditions. Integrated pest and disease management was applied uniformly to every plot in every season. Disease control combined pre-sowing seed treatment with routine in-season scouting and curative fungicide application as required. Aphids were the principal insect pest and were controlled with 10% imidacloprid WP at 225 g ha^{-1} . All other agronomic operations were held strictly identical across treatments, blocks, and years.

Grain yield was determined by hand-harvesting a 1.0 m^2 area from the center of each plot at physiological maturity, avoiding border effects. Grain yield was reported at 12% standard moisture content for agronomic comparison. Above-ground biomass was determined at maturity by harvesting 0.5 m^2 areas adjacent to the yield sampling zone, drying at 65°C for 72 hours to constant weight. Harvest index was calculated as: $\text{HI} = \text{grain dry weight} / (\text{grain dry weight} + \text{straw dry weight})$, where grain dry weight = grain weight (12% moisture) $\times 0.88$. Thousand-kernel weight was determined by counting and weighing three replicates of 500 kernels per plot. Grain protein content was analyzed using near-infrared spectroscopy (NIRS) calibrated with the Kjeldahl method. Plant height was measured at maturity as the distance from soil surface to the top of the spike.

Statistical analysis

Data were analyzed in R 4.4.3 with $\alpha = 0.05$. Before any inferential test, observations were screened for outliers using the interquartile-range rule and assessed for normality using Shapiro–Wilk tests. The main analysis was a two-way ANOVA partitioning total variance among fertilization (Factor A), planting method (Factor B), and the $A \times B$ interaction, with block included as a random effect. Where the omnibus F-test was significant, Tukey's HSD with family-wise error correction was used for pairwise contrasts. We also report eta-squared (η^2) effect sizes alongside p-values, so that practical magnitude is not obscured by statistical significance alone (Liu et al. 2021).

Linear regression analysis was conducted with soil organic carbon fractions (HAC, FAC, LFOC, SOC) and MBC as independent variables, and microbial community counts (bacteria, actinomycetes, fungi) and enzyme activities (urease, sucrase, phosphatase) as dependent variables. To further investigate the regulation mechanism of phosphatase, additional regression analysis was performed between available phosphorus (AP) and phosphatase activity. Redundancy analysis (RDA) using the vegan package was employed to examine treatment–parameter associations, with statistical significance assessed using Monte Carlo permutation tests (999 permutations) (Zhang et al. 2021). Structural equation modeling (SEM) was conducted using the lavaan package to elucidate causal relationships among fertilization treatments, soil nutrients, microbial activity, organic carbon components, and crop yield. Model fit was evaluated using multiple indices including $\chi^2/\text{df} < 3$, RMSEA < 0.08 , CFI > 0.95 , and SRMR < 0.08 , with bootstrap resampling (1000 iterations) used to generate 95% confidence intervals for path coefficients. All statistical graphics included error bars representing standard errors of treatment means ($n = 3$) with significance levels indicated using standardized notation (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Results

Effects of fertilization and mulching on soil active organic carbon fractions and microbial biomass

Both fertilization and mulching exerted significant effects on every measured carbon fraction and on microbial biomass carbon, and the two factors interacted strongly (Fig. 2). Fulvic acid carbon (FAC) ranged from 1.91 to 3.02 g kg⁻¹ across the 12 treatment cells (Fig. 2(1)). The maximum FAC was reached under M × FM + SC at 3.02 ± 0.10 g kg⁻¹, 58.1% above the CK × CT baseline of 1.91 ± 0.09 g kg⁻¹ ($P < 0.05$). At any fixed mulching level, the two organic-containing fertilization treatments (M and MNPK) consistently held higher FAC than NPK or CK. Independently of fertilization type, FM + SC raised FAC above both FM – SC and CT, suggesting that the soil-covered film configuration generates a microenvironment particularly conducive to forming and retaining low-molecular-weight humic compounds.

HAC values spanned 1.75–2.83 g kg⁻¹ (Fig. 2(2)). The peak occurred under MNPK × FM + SC at 2.83 ± 0.10 g kg⁻¹, 61.7% above the CK × CT level of 1.75 ± 0.04 g kg⁻¹ ($P < 0.01$). Notably, MNPK retained substantial HAC even when paired with conventional tillage (2.43 ± 0.08 g kg⁻¹), implying that combined organic–inorganic inputs drive humification largely independently of the mulching regime. The FM + SC advantage over FM – SC was clearest in the two organic-containing treatments (M: 2.65 vs 2.55 g kg⁻¹; MNPK: 2.83 vs 2.80 g kg⁻¹), consistent with soil-covered mulching promoting microbial transformation of fresh inputs into more stable humic fractions.

Among the four carbon variables, light fraction organic carbon (LFOC) showed by far the largest treatment-driven separation (Fig. 2(3)). LFOC varied from 23.0 to 39.9 g kg⁻¹, with the maximum again under MNPK × FM + SC (39.9 ± 2.2 g kg⁻¹), a 73.5% increase relative to CK × CT (23.0 ± 0.5 g kg⁻¹, $P < 0.001$). The mulching-driven gap in LFOC was substantially wider than the corresponding gaps in FAC or

HAC. Both M and MNPK lifted LFOC across every mulching level (M: +23.5–58.3%; MNPK: +32.6–73.5%), underlining how directly the labile/particulate pool tracks organic carbon input. Bulk SOC, in contrast, varied over only a narrow band (10.4–12.4 g kg⁻¹; Fig. 2(4)), much less proportional movement than the labile fractions showed. The MNPK × FM + SC peak SOC was 12.4 ± 0.9 g kg⁻¹ versus 10.4 ± 0.4 g kg⁻¹ at CK × CT. The contrast between large fractional shifts in labile pools and modest shifts in bulk SOC supports the view that labile fractions function as early-warning indicators of management-driven change long before total SOC moves measurably.

Microbial biomass carbon (MBC) followed a treatment response pattern closely mirroring that of the active carbon fractions (Fig. 2(5)). MNPK×FM + SC achieved the highest MBC (approximately 150–239 mg kg⁻¹ across soil layers), substantially exceeding CK×CT (approximately 77–110 mg kg⁻¹, $P < 0.001$). This parallel response pattern between active carbon fractions and MBC suggests strong functional coupling between carbon substrate availability and microbial biomass dynamics. Across five parameters, a broadly consistent hierarchy emerged: for HAC, LFOC, SOC, and MBC, fertilization effects followed MNPK ≥ M > NPK > CK, while for FAC the ranking was M > MNPK > NPK > CK. Mulching effects uniformly followed FM + SC > FM – SC > CT, with the interactive combination of organic–inorganic fertilization and full-film soil-covered hole sowing creating the most favorable conditions for active organic carbon accumulation and microbial proliferation.

Effects of fertilization and mulching on crop yield and quality

Grain yield varied substantially among treatment combinations, ranging from 2900 to 4541 kg ha⁻¹, reflecting significant effects of both fertilization and mulching on wheat productivity (Fig. 3). NPK×FM + SC achieved the highest yield (4541 ± 215 kg ha⁻¹), followed closely by MNPK×FM + SC (4373 ± 157 kg ha⁻¹) and NPK×FM – SC (4295 ± 204 kg ha⁻¹). Compared with the CK×CT baseline (3274 ± 170 kg ha⁻¹), the MNPK×FM + SC treatment increased grain yield by 33.6%, while NPK×FM + SC achieved a 38.7% increase ($P < 0.001$). The mulching effect was pronounced across all fertilization types, with FM + SC consistently producing the highest yields within each fertilization treatment. Notably, organic fertilizer alone (M) resulted in lower yields than CK under conventional tillage (2900 vs. 3274 kg ha⁻¹), but FM + SC restored M treatment yields to competitive levels (3636 kg ha⁻¹), demonstrating that mulching can partially compensate for the temporal mismatch between organic nutrient release and crop demand.

Above-ground biomass showed similar patterns (Fig. 3(2)), with NPK×FM + SC (10,074 ± 308 kg ha⁻¹) and MNPK×FM + SC (9990 ± 414 kg ha⁻¹) achieving the highest values. Harvest index under MNPK×FM + SC reached 0.47 ± 0.01, substantially higher than CK×CT (0.42 ± 0.01, $P < 0.05$), indicating more efficient photosynthate partitioning to grain under optimized management (Fig. 3(3)). Thousand-kernel weight was highest under NPK×FM + SC (46.0 ± 3.2 g) and MNPK×FM + SC (45.5 ± 0.6 g), compared to CK×CT (37.8 ± 1.0 g) (Fig. 3(4)). Grain protein content was highest under MNPK×FM + SC (13.0 ± 0.4%), representing a 25% increase over CK×CT (10.4 ± 0.8%), correlating with enhanced soil available nitrogen and microbial biomass nitrogen (Fig. 3(5)). Plant height was highest under FM + SC treatments, reaching 121.5 ± 2.7 cm under MNPK×FM + SC compared to 97.2 ± 4.9 cm under CK×CT (Fig. 3(6)).

Regression relationships between carbon fractions, microbial parameters, and enzyme activity

We then ran linear regressions to test which carbon descriptors best predict microbial and enzymatic responses (Fig. 4). For composite microbial biomass, LFOC was the strongest single predictor ($R^2 = 0.217$, $p = 0.004$), followed by HAC ($R^2 = 0.189$, $p = 0.008$) and FAC ($R^2 = 0.175$, $p = 0.011$). Bulk SOC, by contrast, was not significantly related to composite microbial biomass ($R^2 = 0.034$, $p = 0.279$). Together these results imply that variation in microbial biomass is governed by the labile carbon pools rather than by total SOC.

When examining individual microbial community groups, active carbon fractions exhibited even stronger positive correlations. For bacteria, the strongest predictor was LFOC ($R^2 = 0.860$, $p < 0.001$), followed by HAC ($R^2 = 0.774$, $p < 0.001$) and FAC ($R^2 = 0.593$, $p < 0.001$). For actinomycetes, LFOC again showed the strongest relationship ($R^2 = 0.872$, $p < 0.001$), followed by HAC ($R^2 = 0.782$, $p < 0.001$) and FAC ($R^2 = 0.638$, $p < 0.001$). Fungi followed the same pattern with LFOC ($R^2 = 0.816$, $p < 0.001$), HAC ($R^2 = 0.700$, $p < 0.001$), and FAC ($R^2 = 0.595$, $p < 0.001$). In sharp contrast, total SOC showed weak and non-significant relationships with all three microbial groups (bacteria: $R^2 = 0.097$, $p = 0.064$; actinomycetes: $R^2 = 0.082$, $p = 0.091$; fungi: $R^2 = 0.106$, $p = 0.053$). MBC showed the strongest correlations with all microbial groups ($R^2 = 0.848$ – 0.927 , $p < 0.001$), consistent with its role as a direct indicator of microbial community size.

For enzyme activities, urease and sucrase showed strong positive correlations with active carbon fractions and MBC, but not with total SOC. Urease was most strongly predicted by LFOC ($R^2 = 0.818$, $p < 0.001$) and MBC ($R^2 = 0.884$, $p < 0.001$), while SOC showed no significant relationship ($R^2 = 0.089$, $p = 0.076$). Sucrase displayed a similar pattern with LFOC ($R^2 = 0.661$, $p < 0.001$) and MBC ($R^2 = 0.635$, $p < 0.001$), but no significant SOC relationship ($R^2 = 0.107$, $p = 0.052$). Phosphatase activity, however, showed no significant relationship with any carbon fraction, SOC, or MBC ($R^2 \leq 0.086$, $p \geq 0.082$ for all predictors).

Multivariate analysis of treatment effects

Redundancy analysis (RDA) revealed distinct treatment-specific associations with soil parameters, with the first two axes cumulatively explaining 91.9% of total variation (Fig. 5). Along RDA1, a clear gradient of treatment differentiation was observed: CK and NPK treatment centroids occupied the negative direction, while M and MNPK treatments clustered near the origin or slightly toward the positive direction, closely associated with vectors of active carbon fractions (FAC, HAC, LFOC), microbial biomass (MBC, MBN), microbial counts (bacteria, actinomycetes, fungi), and enzyme activities (urease, sucrase). This spatial pattern indicates that fertilization type, particularly the inclusion of organic amendments, is the primary driver governing the soil carbon–microbial–enzyme system. Phosphatase and TN vectors pointed predominantly toward the RDA2 positive direction, perpendicular to the main cluster of carbon and microbial vectors, visually confirming the decoupling of phosphatase from carbon substrates observed in the regression analysis. The AP vector pointed toward the RDA2 negative direction, indicating a distinct nutrient availability axis independent of the carbon–microbial coupling

axis. SOC was positioned intermediate between the carbon fraction cluster and TN, consistent with its weak correlations with microbial parameters.

Structural equation modeling of carbon–microbial–yield pathways

The structural equation model revealed significant pathways linking fertilization treatments to crop yield through multiple soil-mediated mechanisms (Fig. 6). Fertilization treatments demonstrated strong direct effects on soil nutrients ($\beta = 0.84$, $P < 0.001$) and moderate direct effects on microbial activity ($\beta = 0.49$, $P < 0.01$). Fertilization also showed a significant direct effect on crop yield ($\beta = 0.24$, $P < 0.001$).

Numerical values reported in this and the following paragraph are standardized path coefficients (β); R^2 values quantifying the variance explained for each endogenous variable as a whole are summarized at the end of this section.

The model identified soil nutrients as a key mediator, exhibiting a strong positive pathway to microbial activity ($\beta = 0.71$, $P < 0.001$) and to organic carbon components ($\beta = 0.72$, $P < 0.001$). Microbial activity demonstrated the strongest direct pathway to organic carbon components ($\beta = 0.96$, $P < 0.001$), highlighting the predominant role of soil microorganisms in carbon cycling and organic matter formation. Microbial activity also showed a significant direct pathway to crop yield ($\beta = 0.38$, $P < 0.05$), while the indirect pathway (fertilization $\rightarrow$ nutrients $\rightarrow$ microbial activity $\rightarrow$ organic carbon) demonstrated that fertilization influences soil carbon accumulation primarily through cascading effects on nutrient availability and biological activity.

The model explained 93% of organic carbon component variation ($R^2 = 0.93$), 70% of soil nutrient variation ($R^2 = 0.70$), and 54% of microbial activity variation ($R^2 = 0.54$). However, only 24% of crop yield variation was explained ($R^2 = 0.24$), indicating that additional factors not included in the model, such as soil moisture dynamics, root architecture, and temporal nutrient synchrony, contribute substantially to yield determination.

Discussion

Synergistic mechanisms of fertilization and mulching on active carbon accumulation

The pre-eminence of MNPK $\times$ FM + SC in building labile carbon pools is best understood as the outcome of complementary mechanisms acting on different timescales and at different spatial scales. The organic side of MNPK delivers carbon substrate directly: sheep manure, once incorporated, decomposes progressively and feeds carbon into both the rapidly cycling FAC pool and the intermediate-stability HAC and LFOC pools (Beatriz Restovich et al. 2019). At the same time, the mineral side of MNPK supplies immediately available nutrients, which support vigorous crop growth and root development; this in turn raises in-situ carbon return through rhizodeposition and root turnover. The simultaneous operation of

these two carbon-input channels is the most parsimonious explanation for why MNPK lifts most carbon fractions across the board.

For FAC, organic fertilizer alone (M) produced higher values than MNPK (3.02 vs. 2.75 g kg⁻¹ under FM + SC). This likely reflects the direct and abundant organic acid inputs from sheep manure decomposition, which may be more effective for fulvic acid formation than the combined treatment where nutrient-stimulated microbial activity may accelerate the transformation of FAC to more stable forms. This differential response pattern among carbon fractions suggests a hierarchical transformation sequence in which FAC acts as an intermediate node promoting LFOC formation, and indicates that the efficiency of this transformation is modulated by the interaction between fertilization type and the soil hydrothermal conditions created by mulching.

The additional advantage of FM + SC over conventional tillage and FM – SC relates to its distinctive modification of the soil microenvironment. The soil-covered film creates a more stable thermal regime with reduced diurnal temperature fluctuations, maintains higher and more consistent soil moisture levels, and provides a physical barrier that reduces surface evaporation. These conditions sustain continuous microbial activity and organic matter transformation, facilitating the gradual humification of fresh organic inputs into more stable carbon fractions. The particularly large LFOC differences between mulching treatments support the hypothesis that the film–soil interface provides direct physical protection for partially decomposed plant residues through occlusion mechanisms analogous to aggregate protection.

The interactive effect between fertilization and mulching is particularly noteworthy. The MNPK×FM + SC combination produced carbon fraction increments for HAC (+ 61.7%) and LFOC (+ 73.5%) that appeared to exceed the simple additive sum of individual factor effects, suggesting positive interactions between organic–inorganic fertilization and mulching practices. Further confirmation of true statistical synergy would require formal tests of interaction contrast exceeding additivity, which warrants investigation in future studies with expanded replication. In the semi-arid Loess Plateau environment where moisture limitation constrains biological activity, FM + SC effectively alleviates this constraint, allowing fuller expression of organic fertilizer benefits on carbon accumulation. Neither management measure alone achieves the carbon accumulation benefits of their combination, emphasizing the importance of integrated approaches for dryland soil health improvement (Giacometti et al. 2021).

Organic-input treatments also improved soil chemistry indirectly via pH-mediated feedbacks. In the alkaline Loess Plateau soils used here (pH 8.3), the low-molecular-weight organic acids released during manure decomposition contribute meaningful buffering. The accompanying increase in microbial activity stimulates nitrification, which releases H⁺ and gradually nudges soil pH toward ranges that improve P and micronutrient availability (Cui et al. 2022; Iqbal et al. 2025).

Active carbon–microbial coupling and enzyme-specific regulation

The regression analysis results revealed a hierarchical regulation pattern within the soil carbon–microbial–enzyme system. The strong correlations between active carbon fractions and culturable microbial abundances ($R^2 = 0.593\text{--}0.872$), compared to the non-significant relationships with total SOC ($R^2 < 0.11$), highlight the functional importance of carbon quality rather than quantity in driving microbial dynamics. This distinction carries implications for management decisions: practices that enhance active carbon inputs will exert disproportionately greater impacts on soil biological activity than practices that merely increase total carbon stocks without altering carbon quality composition.

The strongest coupling between LFOC and all microbial parameters ($R^2 = 0.816\text{--}0.872$ for microbial counts; $R^2 = 0.818$ for urease; $R^2 = 0.661$ for sucrase) reflects microbial preferential utilization of this fraction. LFOC consists primarily of partially decomposed plant materials with relatively high bioavailability, providing directly utilizable substrates for microbial metabolism (Li et al. 2023; Pikula and Ciotucha 2022). The diminishing but still significant relationships across HAC and FAC provide evidence for a continuum model of carbon–microbial interactions, whereby different carbon pools with varying lability levels support microbial communities at different temporal scales (Schmidt et al. 2015).

The decoupling of phosphatase activity from all carbon fractions, SOC, and MBC ($R^2 \leq 0.086$, $p > 0.05$) represents a mechanistically informative finding. Unlike urease and sucrase, phosphatase appears regulated by distinctly different mechanisms. Several factors contribute to this decoupling. First, phosphatase production is primarily controlled by soil phosphorus dynamics rather than carbon availability (Sainju and Allen 2023). In this study, the positive correlation between phosphatase and available phosphorus ($R^2 = 0.312$, $p < 0.01$) likely reflects the confounding influence of organic fertilizer inputs: the M and MNPK treatments simultaneously introduced high P inputs (130.9 and 142.8 kg P ha⁻¹, respectively, versus 11.9 kg P ha⁻¹ for NPK) and organic substrates that stimulated overall microbial activity, indirectly elevating phosphatase production. This pattern differs from the classic “nutrient mining” response (negative phosphatase–AP correlation) typically observed under phosphorus-limited conditions, and instead suggests that in this phosphorus-enriched system, phosphatase dynamics are co-regulated by both substrate-induced microbial proliferation and mineral phosphorus availability. The observed positive relationship should therefore be interpreted with caution, as it may not generalize to soils with lower phosphorus inputs. Second, in the alkaline Loess Plateau soils (pH 8.0–8.5), phosphorus availability is largely regulated by calcium phosphate precipitation and adsorption to mineral surfaces, creating conditions where phosphatase expression is dominated by soil chemistry rather than carbon substrate supply. Third, phosphatase molecules exhibit adsorptive stabilization on soil mineral surfaces, allowing enzyme activity to persist independently of current microbial biomass levels. The RDA biplot visually supported this decoupling, with the phosphatase vector oriented perpendicular to the main carbon–microbial cluster.

These contrasting enzyme behaviors support a cascade regulation model: active carbon fractions primarily drive microbial biomass and the production of carbon-cycling enzymes (urease, sucrase) through direct substrate-mediated mechanisms, while nutrient-cycling enzymes (phosphatase) are regulated through independent nutrient-demand pathways. This model provides a mechanistic

explanation for why total SOC fails to predict microbial and enzymatic responses: the stable carbon component dilutes the signal from the biologically active fractions that actually drive microbial processes (Cong et al. 2014; Sanchez-Navarro et al. 2021).

Fertilization as the primary driver: evidence from multivariate and path analyses

The RDA results established a hierarchy of factor importance, with the first two axes explaining 91.9% of total variation (RDA1: 75.4%; RDA2: 16.5%). The dominance of the fertilization-related first axis reflects the key role of nutrient and carbon inputs in controlling soil biogeochemical processes. The treatment differentiation follows a gradient from CK and NPK through M to MNPK, corresponding to increasing organic carbon inputs. This gradient indicates that integrated organic–inorganic approaches provide the most comprehensive soil improvement.

The SEM pathway from microbial activity to organic carbon components ($\beta = 0.96$, $R^2 = 0.93$) indicates that soil microorganisms are the primary agents of organic carbon transformation and accumulation, consistent with the “microbial carbon pump” concept (Schmidt et al. 2015). The strong nutrient–microbial pathway ($\beta = 0.71$) indicates that nutrient availability is a key enabler of microbial activity, explaining why combined fertilization outperforms single-source fertilization in enhancing both microbial biomass and carbon accumulation (Lv et al. 2011; Wang et al. 2024).

The relatively modest yield variance explained by the SEM ($R^2 = 0.24$) warrants careful interpretation. This result should not be taken as evidence that the soil carbon–microbial system is unimportant for crop productivity; rather, it indicates that additional factors not included in the model substantially contribute to yield determination. In particular, soil moisture dynamics likely represent a major unmeasured pathway. In dryland systems where water is the primary limiting factor, moisture conservation through FM + SC may directly influence yield through improved water availability during key growth periods, independently of nutrient and carbon pathways. Root architecture, temporal nutrient synchrony, and growing-season weather variability also contribute to unexplained yield variation. Future studies incorporating soil moisture measurements at multiple time points would likely capture a larger proportion of yield variance.

Yield response, nutrient input considerations, and agronomic implications

The yield superiority of NPK×FM + SC (38.7% increase) and MNPK×FM + SC (33.6% increase) over CK×CT shows the practical benefits of integrating fertilization with mulching technologies for dryland wheat production. The slightly higher yield under NPK×FM + SC compared to MNPK×FM + SC reflects the immediate nutrient availability advantage of chemical fertilizers, as organic nutrient release exhibits slower kinetics that may not fully match peak crop demand during key growth stages (Ma et al. 2022). However, the MNPK treatment provided substantially superior soil carbon and microbial benefits,

suggesting that its agronomic value extends beyond single-season productivity to encompass long-term soil health improvement.

The lower yield under M×CT compared to the unfertilized control requires careful interpretation. Several factors may contribute to this counterintuitive result. The temporal mismatch between organic nutrient release and crop demand is a primary factor: sheep manure decomposes slowly in the semi-arid climate, and available nitrogen may not reach sufficient levels during key tillering and jointing stages. Additionally, the high C/N ratio of the organic amendments may have induced temporary microbial nitrogen immobilization, further limiting nitrogen availability during early growth. Furthermore, the M treatment received substantially higher P inputs than the NPK treatment, but lower available nitrogen inputs, creating a nutrient imbalance that may have constrained yield. This nutrient input disparity is an inherent characteristic of the sheep manure source used in this study and should be considered when interpreting the relative effects of organic versus inorganic fertilization on both crop performance and soil carbon parameters. The differential phosphorus inputs may have contributed to some of the observed differences in soil carbon fractions and microbial parameters between the M and NPK treatments that were attributed primarily to organic carbon inputs.

Importantly, FM + SC effectively rehabilitated M treatment yields, demonstrating that the improved hydrothermal conditions created by mulching can partially compensate for the kinetic limitations of organic nutrient sources. This finding has practical implications for smallholder farmers who may have limited access to chemical fertilizers. The mechanism likely involves accelerated microbial decomposition of organic amendments under more favorable moisture and temperature conditions maintained by FM + SC (Lin et al. 2017; Xiang et al. 2020). The concurrent improvements in grain protein content and harvest index (0.47 vs. 0.42) indicate that optimized management simultaneously enhances nutrient allocation efficiency and grain quality (Gauder et al. 2016; Yan et al. 2013).

Study limitations and future directions

Several limitations should be acknowledged. First, although the experiment was established in 2019 and all treatments (fertilization, mulching, sowing, and harvesting) were maintained on schedule in every growing season by station-resident personnel, the COVID-19 pandemic and associated travel and access restrictions prevented the research team from conducting comprehensive soil sampling and yield measurements during the 2020–2023 growing seasons. As a result, the soil and yield data presented in this study were collected during the 2024–2025 growing season and no intermediate monitoring data are available for the intervening years. We emphasize that treatment continuity was never interrupted: identical fertilization rates, mulching operations, and agronomic management were applied each year, and field records maintained by station personnel confirm this unbroken management history. Nevertheless, the absence of inter-annual data means that the carbon pool responses and microbial parameters reported represent a single-season snapshot of cumulative sixth-year effects rather than a temporal trajectory, and the possibility that transient shifts occurred during the unmonitored years cannot be excluded. Multi-year monitoring data would strengthen the evidence for the synergistic mechanisms proposed and allow assessment of temporal trends.

Second, individual plot size (2 m², 2 m × 1 m) is relatively small compared to large-scale agronomic field trials. We acknowledge that smaller plots may increase the potential influence of edge effects on treatment estimates. To address this concern, we adopted three complementary measures as described under “Experimental design”: unfertilized buffer strips, center-only sampling from an effective interior area of approximately 1.0 m² per plot, and independent plot-specific plastic mulching film sheets to break hydrological connectivity between adjacent treatments. We believe these protocols adequately mitigate edge-effect bias for the parameters measured in this study; however, replication at larger spatial scales would further strengthen the generalizability of our conclusions.

Third, microbial community enumeration was conducted using culture-based dilution plate counting, which captures only a fraction of total soil microbial diversity. While this method provides reproducible estimates suitable for comparative treatment assessments, it does not resolve community composition at the taxonomic level. High-throughput sequencing approaches would provide deeper insights into which specific taxa mediate the observed carbon–enzyme coupling and phosphatase decoupling patterns.

Fourth, soil aggregate dynamics were not assessed, limiting our understanding of carbon stabilization through physical protection mechanisms. Given that LFOC showed the most pronounced treatment differences, its accumulation patterns may be closely linked to aggregate formation and stability.

Future research priorities include: (1) multi-year monitoring of soil carbon fractions and microbial parameters to establish temporal dynamics and confirm the persistence of treatment effects; (2) mechanistic studies incorporating soil aggregate fractionation, high-throughput microbial sequencing, and ¹³C isotope tracing to elucidate specific carbon transformation pathways; (3) environmental impact assessment including greenhouse gas monitoring and nutrient leaching quantification; (4) economic analysis considering organic fertilizer availability and labor requirements to facilitate adoption (Francaviglia et al. 2019; Hobley et al. 2018).

Conclusions

After six consecutive years of uninterrupted treatment (2019–2025), our factorial trial in dryland winter wheat on the Loess Plateau demonstrates a clear interaction between organic–inorganic fertilization and full-film soil-covered hole sowing in driving labile carbon accumulation, microbial biomass build-up, and yield. Relative to the unfertilized, conventionally tilled control, the largest gains in HAC (+ 61.7%) and LFOC (+ 73.5%) were achieved under MNPK × FM + SC, while the highest FAC was achieved under M × FM + SC (+ 58.1%). Grain yield reached 4541 and 4373 kg ha⁻¹ under NPK × FM + SC and MNPK × FM + SC, corresponding to 38.7% and 33.6% increases over CK × CT, accompanied by concurrent improvements in harvest index, thousand-kernel weight, and grain protein. Although NPK × FM + SC delivered marginally higher yield, MNPK × FM + SC produced substantially larger gains in active carbon and microbial biomass, pointing to longer-term soil-health benefits that complement its competitive yield performance.

Active carbon fractions, particularly LFOC, rather than total SOC, were identified as the primary predictors of soil biological function. Regression analysis demonstrated strong coupling between active carbon fractions and culturable microbial abundances ($R^2 = 0.593\text{--}0.872$) and carbon-cycling enzymes ($R^2 = 0.661\text{--}0.818$), whereas total SOC showed no significant relationship with any microbial or enzymatic parameter ($R^2 < 0.11$). Phosphatase was decoupled from all carbon fractions and MBC but positively correlated with available phosphorus, supporting a cascade regulation model where carbon-cycling enzymes are substrate-dependent while nutrient-cycling enzymes follow independent demand-driven pathways. Structural equation modeling further indicated that microbial activity drives organic carbon accumulation ($\beta = 0.96$, $P < 0.001$, $R^2 = 0.93$) through cascading nutrient–microbial–carbon pathways.

These findings suggest that management strategies should prioritize active carbon accumulation to maximize biological returns. We recommend the integrated implementation of combined organic–inorganic fertilization with full-film soil-covered hole sowing as a promising sustainable intensification strategy for the Loess Plateau. Future multi-year studies and molecular approaches are needed to confirm the temporal persistence and mechanistic basis of these responses.

Declarations

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Author contributions All authors contributed to the study conception and design. Field management, sampling and laboratory analyses were performed by Qiang Liu, XiangXiang Hu and Xuhui Zhang. Data curation and statistical analysis were performed by Qiang Liu and XiangXiang Hu. The first draft of the manuscript was written by Qiang Liu, and all authors commented on previous versions. All authors read and approved the final manuscript.

Ethics approval This study did not involve human participants or animals, and no permits were required for the field experiment or for the plant material used.

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

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Figures

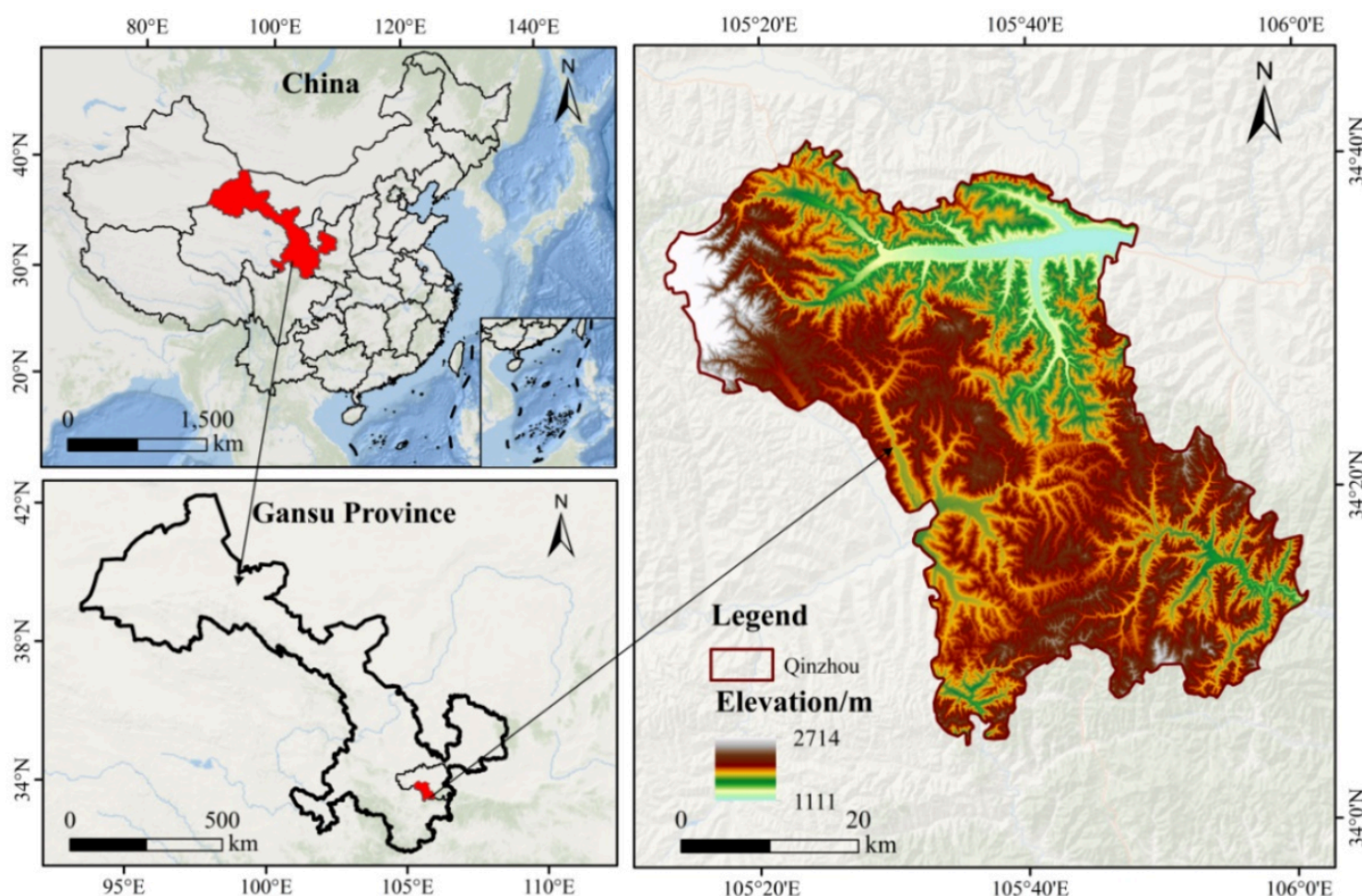


Figure 1

Geographic location of the study area in Qinzhou District, Tianshui City, Gansu Province, northwestern China. Upper left: Location of Gansu Province within China; Lower left: Tianshui City within Gansu Province; Right: Detailed elevation map of Qinzhou District showing the experimental site location.

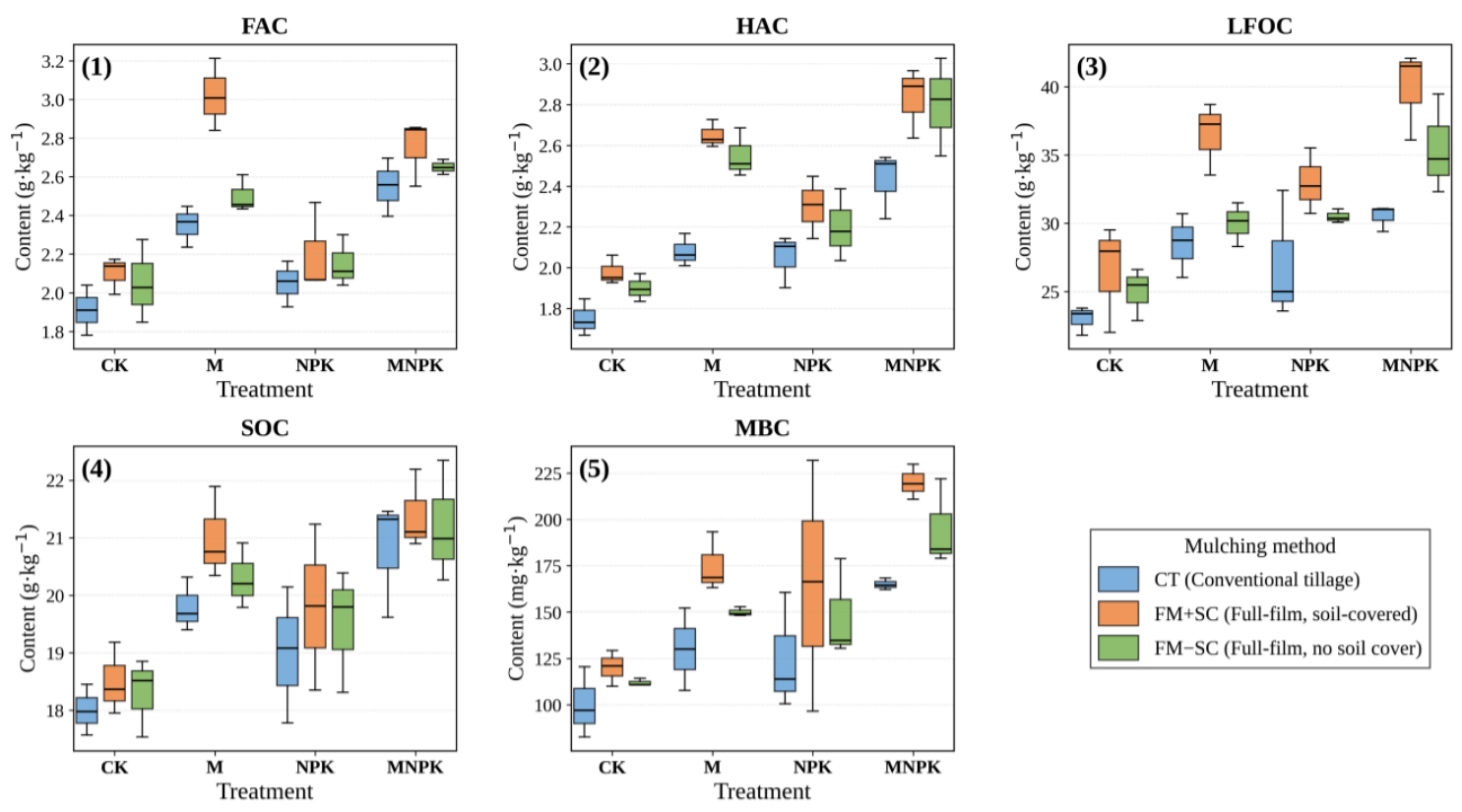


Figure 2

Soil active organic carbon fractions and microbial biomass carbon under different fertilization and mulching treatments. (1) FAC: fulvic acid carbon; (2) HAC: humic acid carbon; (3) LFOC: light fraction organic carbon; (4) SOC: soil organic carbon; (5) MBC: microbial biomass carbon. Treatments: CK = control, no fertilization; M = organic fertilizer only, sheep manure 7500 kg ha⁻¹; NPK = chemical fertilizer only; MNPK = combined organic–inorganic fertilization. Mulching methods: CT = conventional tillage; FM+SC = full-film soil-covered hole sowing; FM–SC = full-film no-cover hole sowing.

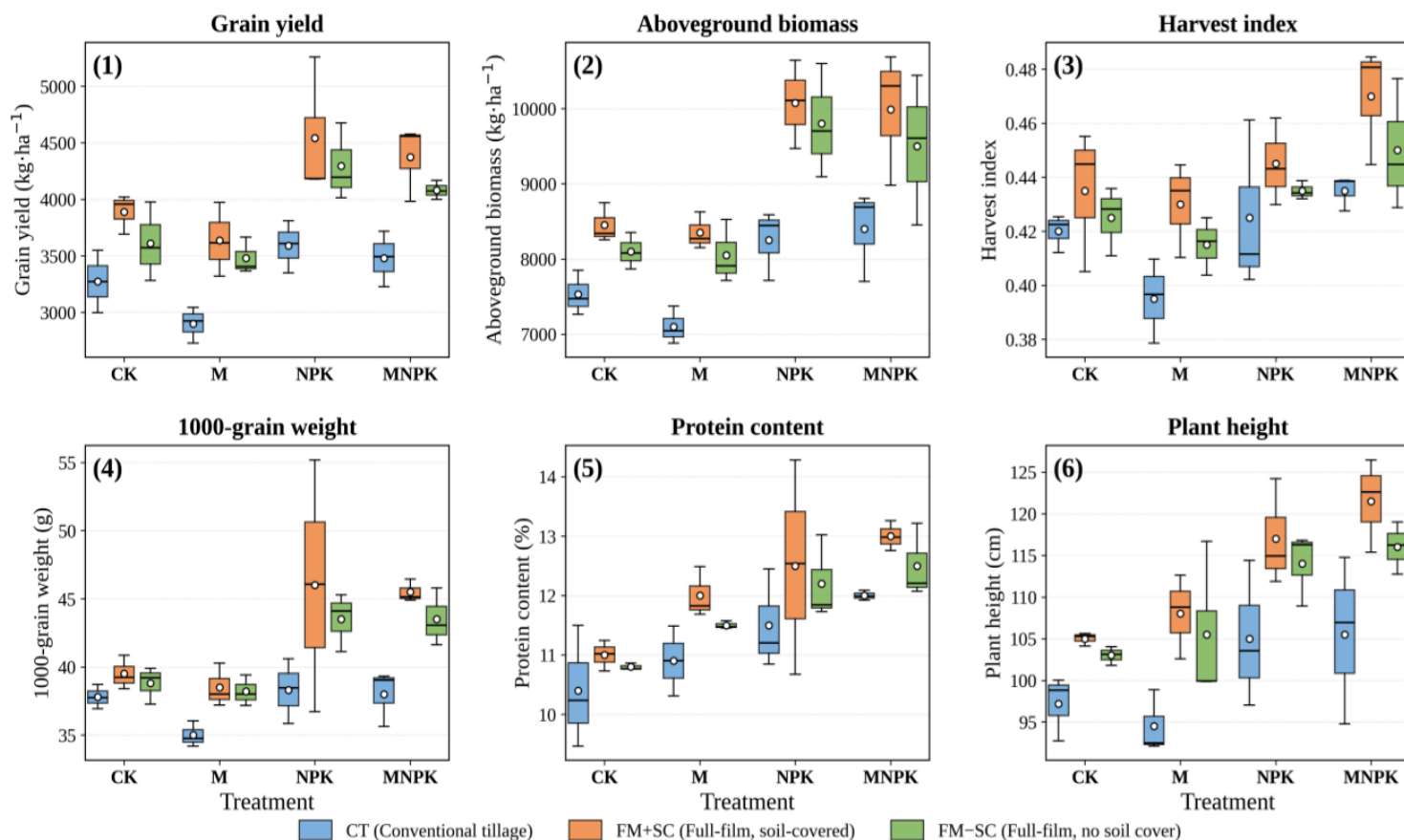


Figure 3

Winter wheat yield components and quality parameters under different fertilization and mulching treatments. (1) Grain yield ($\text{kg}\cdot\text{ha}^{-1}$); (2) Aboveground biomass ($\text{kg}\cdot\text{ha}^{-1}$); (3) Harvest index; (4) 1000-grain weight (g); (5) Protein content (%); (6) Plant height (cm). Fertilization treatments: CK = control (no fertilization); M = organic fertilizer only (sheep manure $7500\text{ kg}\cdot\text{ha}^{-1}$); NPK = chemical fertilizer only; MNPK = combined organic–inorganic fertilization. Mulching methods: CT = conventional tillage; FM+SC = full-film soil-covered hole sowing; FM–SC = full-film no-cover hole sowing.

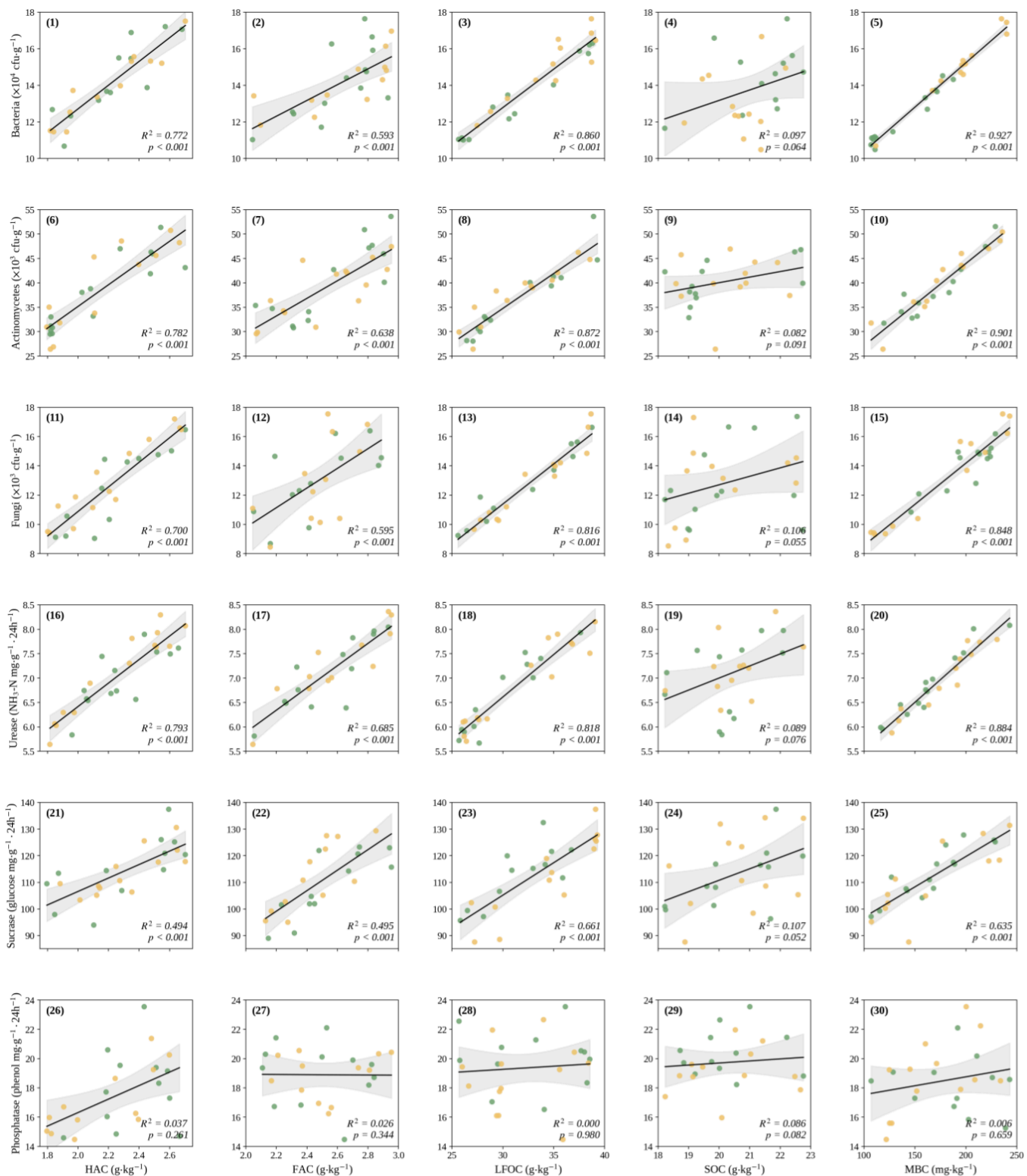


Figure 4

Linear regression relationships among soil active carbon fractions (HAC, FAC, LFOC), total SOC, microbial biomass carbon (MBC), culturable microbial abundances (bacteria, actinomycetes, fungi), and enzyme activities (urease, sucrase, phosphatase). Each panel shows R^2 and p values.

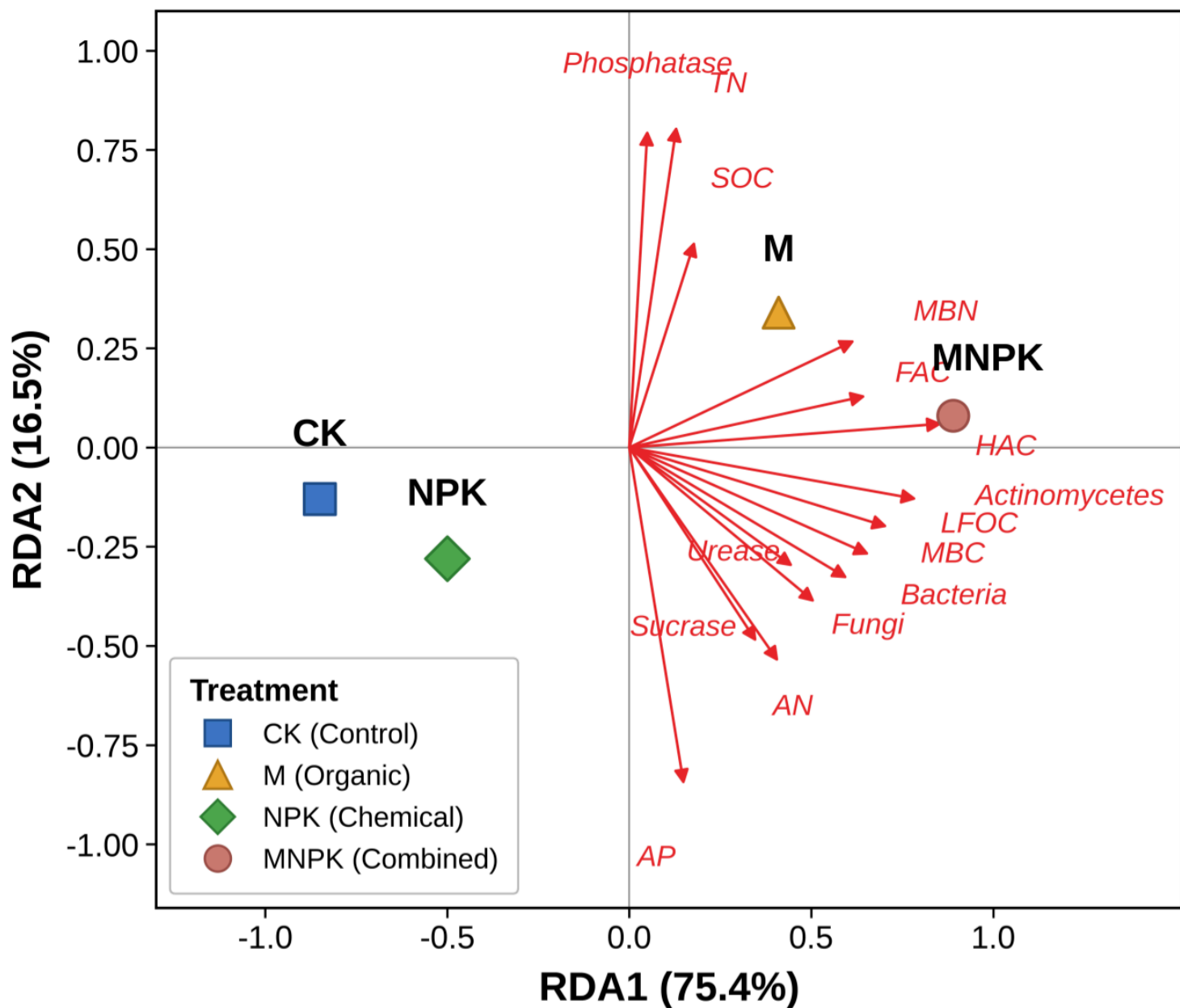


Figure 5

Redundancy analysis (RDA) biplot of soil active carbon fractions, microbial biomass, microbial community counts, enzyme activities, and soil nutrient parameters. Response variables: HAC = humic acid carbon; FAC = fulvic acid carbon; LFOC = light fraction organic carbon; SOC = soil organic carbon; MBC = microbial biomass carbon; MBN = microbial biomass nitrogen; Bacteria, Actinomycetes, Fungi = microbial community counts; Urease, Sucrase, Phosphatase = enzyme activities; TN = total nitrogen; AN = available nitrogen; AP = available phosphorus. Treatment centroids: CK = control; M = organic fertilizer; NPK = chemical fertilizer; MNPK = combined organic–inorganic fertilization.

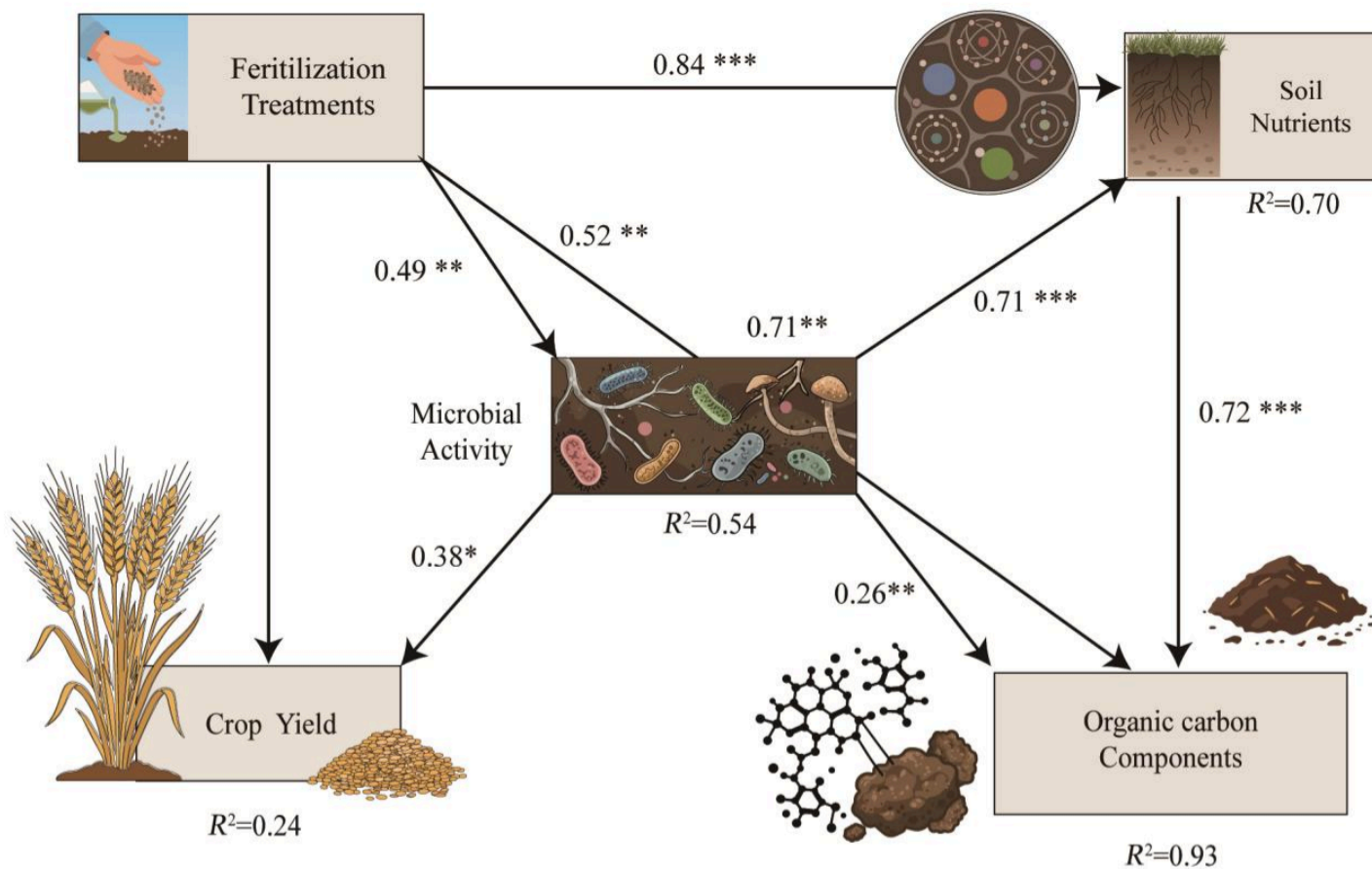


Figure 6

Structural equation model (SEM) of fertilization treatment effects on crop yield through soil nutrient, microbial activity, and organic carbon component pathways. Arrow widths are proportional to standardized path coefficients. Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. R^2 values indicate variance explained for each endogenous variable.