



OPEN Influence of reduced nitrogen fertilizer combined with sheep manure on root-soil interaction in Korla fragrant pear orchards in Xinjiang, China

Wei Li¹, Xuemei Wang³, Linseng Yan¹, Xiuxiu Liu¹, Zhanyi He¹, Kailong Wang¹, Xing Shen^{1,2}✉ & Zhongping Chai^{1,2}✉

The experiment aimed to address the issue of excessive nitrogen fertilizer application, explore an optimal model for combining reduced nitrogen fertilizer with organic fertilizer (sheep manure), and investigate their relationships with soil properties and pear tree physiology while assessing impacts on Korla fragrant pear yield. During the period from 2021 to 2023, 10–12-year-old pear trees at Awati Farm in Korla were selected for the study. Six treatments were established: no fertilization (CK), no nitrogen fertilizer application (N_0), conventional fertilization (N), nitrogen fertilizer reduction treatment (N_2 with a 20% reduction in nitrogen compared to conventional fertilization), and two combined treatments of reduced nitrogen fertilizer with two application gradients of organic fertilizer (F_1 and F_2 , applying 22500 kg·hm⁻² and 33750 kg·hm⁻² of sheep manure, respectively), denoted as N_2F_1 and N_2F_2 . Results showed that reducing nitrogen fertilizer application effect on soil physical and chemical properties and nutrient content, inhibited enzyme and root activities, increased root cortex thickness, and reduced the proportion of cellulose cell wall cells in phloem and xylem, as well as the total area of pear root vessels and slice area in the 20–40 cm soil layer. However, combining sheep manure improved these effects. Compared to full nitrogen application, N_2F_1 treatment significantly increased alkali-hydrolyzable nitrogen content in the 0–60 cm soil layer, soil organic matter in the 0–20 cm layer, total nitrogen in the 20–40 cm layer, protease activity in the 20–40 cm layer, and the number and proportion of phloem cellulose cell wall cells, while maintaining catalase and urease activities at levels similar to full nitrogen application. The N_2F_2 treatment significantly increased total nitrogen in the 0–20 cm soil layer, organic matter in the 40–60 cm layer, protease activity, root cortex thickness, and conduit and slice areas, with no significant change in root activity and total yield compared to full nitrogen application.

Keywords Korla fragrant pear, Reduced nitrogen fertilizer application, Sheep manure, Soil nutrients, Enzyme activity, Root, Yield

Fertilization is a basic means for crops to improve productivity. The application of chemical fertilizers could increase the per unit yield of crops by 55–65% and was one of the important measures to increase crop yield¹. According to the calculation of the China Nitrogen Fertilizer Industry Association, in 2023, the apparent consumption of nitrogen fertilizer in China was 38.4 million tons (in terms of pure nitrogen), with a year-on-year increase of 5.6%. The apparent consumption of urea was 58.668 million tons, with a year-on-year increase of 7.1%. China has already become a major country in nitrogen fertilizer consumption in the world. At the same time, the statistical results of the National Bureau of Statistics on the application amount of chemical nitrogen fertilizer in various regions of the country in 2022 showed (Fig. 1) that the application amount of nitrogen fertilizer in Xinjiang region reached 1,038 thousand tons, ranking third in China. Upon reviewing the statistics

¹College of Resources and Environment, Xinjiang Agricultural University, Urumqi 830052, China. ²Xinjiang Key Laboratory of Soil and Plant Ecological Processes, Xinjiang Agricultural University, Urumqi 830052, China. ³College of Geographical Science and Tourism, Xinjiang Normal University, Urumqi 830052, China. ✉email: shenxing@xjau.edu.cn; czp@xjau.edu.cn

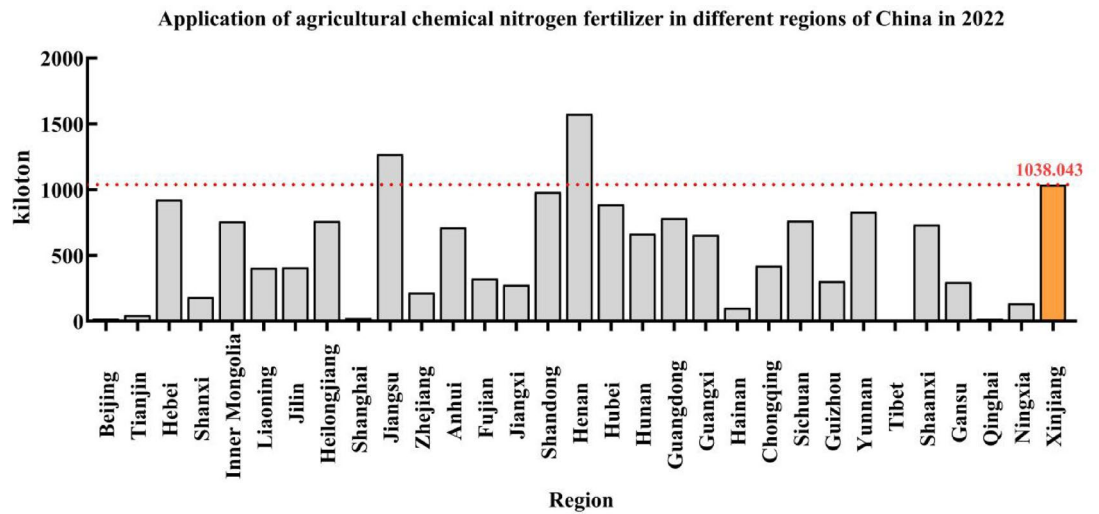


Fig. 1. Application of agricultural chemical nitrogen fertilizer in different regions of China in 2022. Source: National Bureau of Statistics of China.

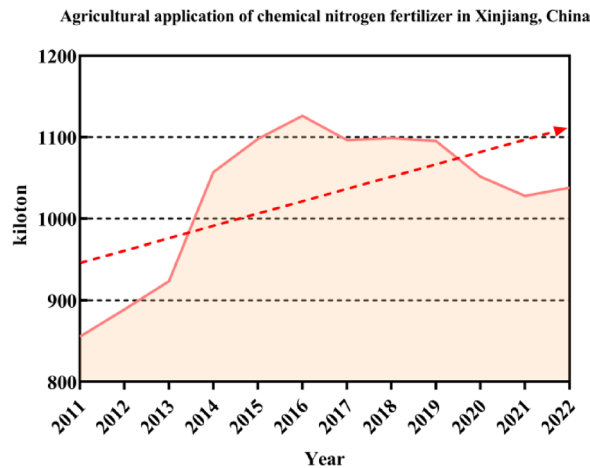


Fig. 2. Agricultural application of chemical nitrogen fertilizer in Xinjiang, China. Source: National Bureau of Statistics of China.

of nitrogen fertilizer application in Xinjiang over the past 12 years(2011–2022), it was found that the historical peak of nitrogen fertilizer application in the Xinjiang region was 1,126 thousand tons in 2016. Overall, there was an upward trend, yet with fluctuations (Fig. 2).

Excessive nitrogen fertilizer use by fruit growers, driven by yield goals, raises costs, contributes to greenhouse gas emissions² (e.g., nitrous oxide, a potent GHG with 265 times the global warming potential of CO₂), pollutes aquatic environments through leaching, and fails to boost crop yields or quality^{3–5}. Reducing nitrogen inputs while maintaining productivity is critical for sustainable agriculture⁶. However, abrupt fertilizer cuts risk crop nutrient deficits. Organic fertilizers offer a solution by slowly releasing nutrients, improving soil structure, modulating microbial communities to reduce nitrification-denitrification processes (a major source of N₂O emissions)⁷ and enhancing carbon sequestration through stable organic matter inputs. Studies on crops like citrus, pitaya, and rice demonstrate organic fertilizers improve soil properties and enzyme activity (urease, phosphatase, etc.), supporting nutrient cycling and long-term fertility^{8–10}. Unlike chemical fertilizers, organic amendments introduce beneficial microbes and enzymes directly into soils while reducing environmental impacts^{11,12}. Combined use of organic and chemical fertilizers balances rapid nutrient availability with sustained soil health, optimizing agricultural sustainability.

Korla fragrant pear (*Pyrus sinkiangensis* Yü), a key crop in Xinjiang’s forestry-fruit industry, represents over 80% of plantations in the Tarim Basin region. To address improper fertilization practices, local orchards increasingly use sheep manure to enhance soil fertility. However, growers’ belief that “reducing fertilizer reduces yield” hinders effective integration of nitrogen reduction and organic amendments. This study evaluates reduced nitrogen application (based on prior optimal rates) combined with sheep manure, analyzing their

effects on soil nutrients, enzyme activity, root vitality, and yield during fruit expansion. The goal is to determine optimal nitrogen-organic ratios, providing strategies to improve nitrogen efficiency, balance inorganic-organic fertilization, and enhance orchard sustainability.

Materials and methods

Study site

This experiment was carried out in Awati Farm (41°40′28″N, 86°07′12″E) in Korla City, Xinjiang. The area where the experimental field is located is in southern Xinjiang (Fig. 3). It has a temperate inland arid desert natural ecological climate. The annual average temperature is around 11 °C. The annual precipitation is 50–56 mm (Fig. 4). The annual maximum evaporation is about 2800 mm. The average annual sunshine hours reach 2800–3000 h. The total solar radiation is 5700–6500 mJ·cm⁻². The effective accumulated temperature is 4100–4400 °C. The frost-free period is 210–239 days. Before the experiment was started, the background values of the contents of various nutrients in the initial soil of the fragrant pear orchard, including organic matter, alkali-hydrolyzable nitrogen, available phosphorus and available potassium, were 17.26 g·kg⁻¹, 54.11 mg·kg⁻¹, 49.99 mg·kg⁻¹ and 180.0 mg·kg⁻¹ respectively, and the pH value was 7.98.

Experimental design

During the period from 2021 to 2023, 10–12-year-old Korla fragrant pears were taken as test materials by us to conduct field experiments with different treatments of reducing nitrogen fertilizer and applying organic fertilizer. The plant spacing and row spacing were set to be 2 × 4 m, with 1125 plants per hectare (Fig. 5A). Thirty fruit trees that were in similar growth conditions and free from pest and disease infestations were selected and marked with tags by us. Six treatments were designed in the experiment. Five Korla fragrant pear trees were selected for each fertilization treatment, and each individual fragrant pear tree was regarded as one repetition. Semi-decomposed sheep manure (with the contents of total nitrogen, total carbon, total phosphorus and total potassium being 0.76%, 18.52%, 0.52% and 0.45% respectively) was chosen and applied in a ring ditch before the budding stage, and the whole amount was applied at one time. Urea containing 46% nitrogen was selected as the nitrogen fertilizer. 60% of it was applied as a base fertilizer before the budding stage, and 40% was top-dressed before the fruit expansion stage. Triple superphosphate containing 46% P₂O₅ was chosen as the phosphorus fertilizer, and potassium sulfate containing 51% K₂O was selected as the potassium fertilizer. Both of them were applied in a ring ditch and the whole amount was applied at one time before the budding stage (Fig. 5B). Other field management measures were the same as those in the local area. The specific fertilization amounts are shown in Table 1.

Collection and determination of samples

Sample collection

Soil Sample Collection: On August 3 (fragrant pear fruit expansion period), soil samples were collected from the orchard. For each treatment, three trees were sampled. At 5–10 cm from both sides of fertilization ditches (after removing surface litter), soil from three layers (0–20, 20–40, 40–60 cm) was collected. Same-layer samples from

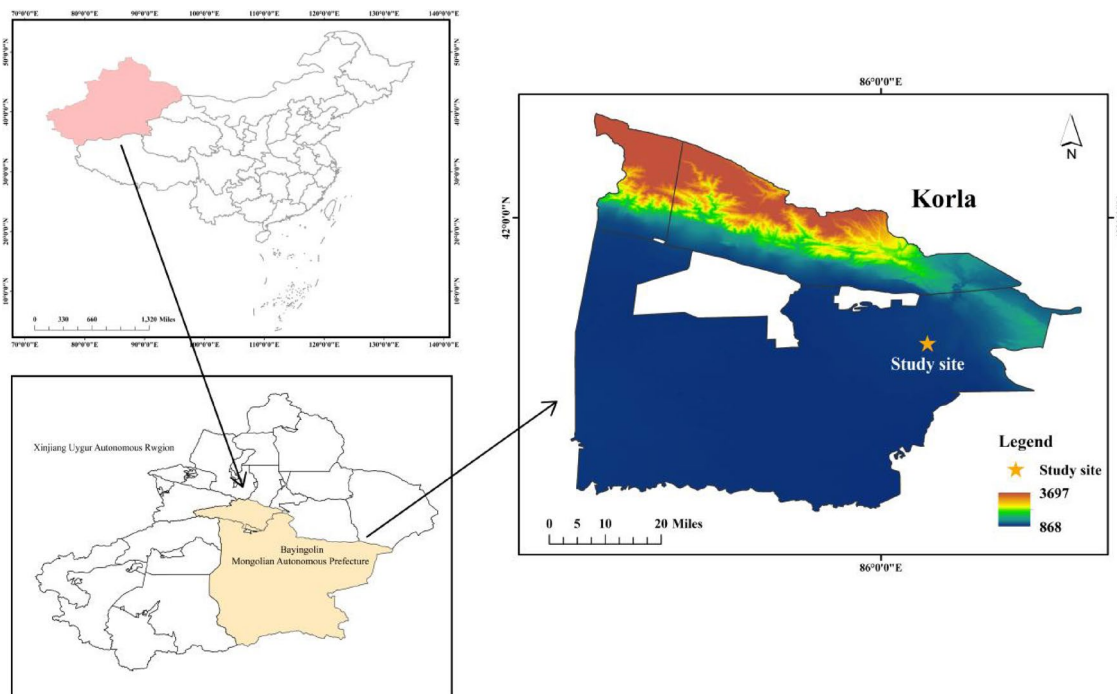


Fig. 3. Map of the experimental site in Korla, Xinjiang Uygur Autonomous Region, China.

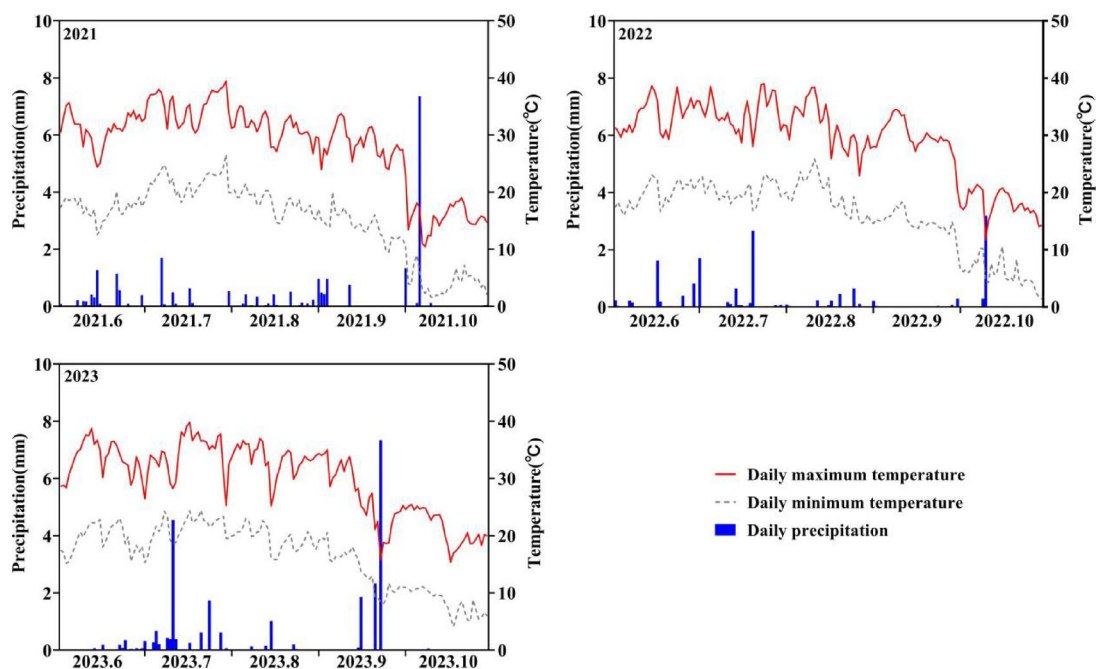


Fig. 4. Daily rainfall, highest temperature, and lowest temperature during the fruit expansion period of fragrant pears from 2021 to 2023.

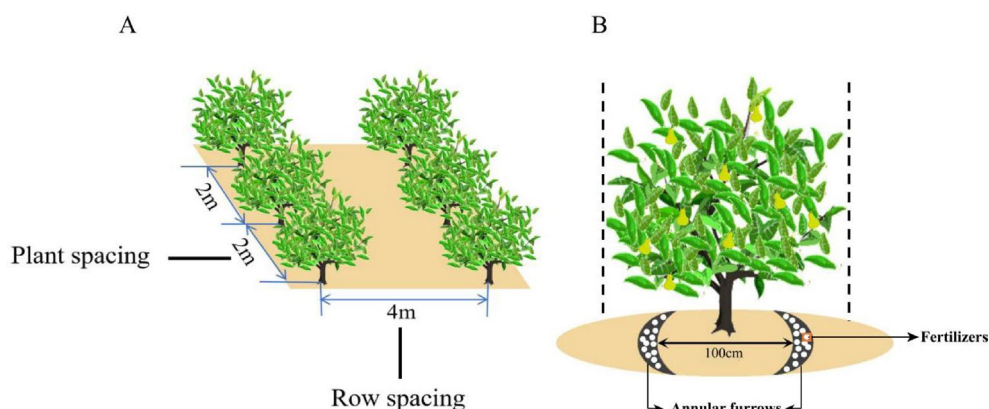


Fig. 5. Schematic diagram of Korla fragrant pear planting and fertilization.

Treatment	Amount fertilizer rate							
	kg·hm ⁻²				kg·plant ⁻¹			
	N	P ₂ O ₅	K ₂ O	Sheep manure	N	P ₂ O ₅	K ₂ O	Sheep manure
1	CK	0	0	0	0	0	0	0
2	N ₀	0	300	75	0	0	0.267	0.067
3	N	300	300	75	0	0.267	0.267	0.067
4	N ₂	240	300	75	0	0.213	0.267	0.067
5	N ₂ F ₁	240	300	75	22,500	0.213	0.267	0.067
6	N ₂ F ₂	240	300	75	33,750	0.213	0.267	0.067

Table 1. Nitrogen fertilizer reduction with sheep manure experimental program.

both sides were combined. After preliminary crushing and mixing, samples were stored in self-sealing bags, transported in dry ice-cooled insulated boxes to the lab. In the lab, roots and stones were removed, and samples were sieved (2 mm), homogenized, and split: one subset (dry ice-stored) was analyzed for urease, protease, catalase, and nitrate reductase activities; the other was air-dried, sieved (1 mm and 0.25 mm), and used for physicochemical analysis.

Root Sample Collection: Roots were collected on the same date using a root drill ($\phi=7$ cm). For each treatment, three trees were sampled along fertilization ditch inner sides and four directions (east, south, west, north) under tree crowns (after litter removal). Roots from three soil layers (as above) were collected, rinsed with deionized water, homogenized by layer, and split into two cryotubes with FAA fixative. Tubes were stored in dry ice-cooled boxes: one subset was sent to Chengdu Baihui Biotechnology Co., Ltd. for paraffin sectioning; the other was used for root vitality analysis.

Measurement and calculation

Soil properties were analyzed as follows: organic matter (potassium dichromate-sulfuric acid oxidation), total nitrogen (sulfuric acid digestion, Kjeldahl method), alkali-hydrolyzable nitrogen (alkali hydrolysis diffusion), available phosphorus (sodium bicarbonate extraction, molybdenum-antimony colorimetry), and available potassium (ammonium acetate extraction, flame photometry). Soil pH (5:1 water-soil ratio) and electrical conductivity were measured using an FE28 pH meter and DDS11A conductivity meter, respectively.

Enzyme activities were measured using: Urease: Sodium phenolate colorimetry (activity = $\text{mg NH}_4^+ \cdot \text{N} \cdot \text{g}^{-1} \text{ soil} \cdot 24 \text{ h}^{-1}$). Protease: Casein colorimetry (activity = $\text{mg amino-N} \cdot \text{g}^{-1} \text{ soil} \cdot 24 \text{ h}$ at 30°C). Catalase: Potassium permanganate titration (activity = $\text{mL } 0.1 \text{ M KMnO}_4 \text{ consumed} \cdot \text{g}^{-1} \text{ soil}$). Nitrate reductase: Phenol disulfonic acid colorimetry (activity = $\text{mg NO}_3^- \cdot \text{N change} \cdot \text{g}^{-1} \text{ soil}$).

Determination of root vitality: Root vitality was determined by the triphenyltetrazolium chloride (TTC) method¹³.

Statistical analyses

Soil physicochemical properties and enzyme activity data were preprocessed in Excel. Statistical analysis (Pearson/Duncan tests, $P < 0.05$) and mean \pm SE calculations were performed using SPSS 27.0. Plots were generated in Origin. Spearman correlations (R linkET package) and Mantel tests were conducted on the GenesCloud platform (<https://www.genescloud.cn>). PLS-EM modeling (SmartPLS 4) and figure refinement were completed in Adobe Illustrator. The location map of the study area was drawn using the software ArcGIS 10.8 (Data source: https://datav.aliyun.com/portal/school/atlas/area_selector).

Results

Effects of different fertilization treatments on soil physical and chemical properties

In the 0–20 cm layer (Fig. 6), nitrogen reduction (N_2) significantly increased soil electrical conductivity (+43.27%) and pH (+5.67%) compared to full nitrogen (N) ($P < 0.05$), while total nitrogen, alkali-hydrolyzable nitrogen, and available phosphorus decreased. N_2F_1 elevated organic matter (+5.25%) and alkali-hydrolyzable nitrogen (+9.86%) ($P < 0.05$), whereas N_2F_2 raised total nitrogen (+6.94%) without altering other parameters. At 20–40 cm, N_2 increased available nitrogen (+8.79%), conductivity (+16.31%), and pH (+2.35%) versus N, while N_2F_1 enhanced total nitrogen (+7.39%) and available nitrogen (+8.79%) ($P < 0.05$). In the 40–60 cm layer, N_2 boosted available potassium (+16.28%) but reduced organic matter, total nitrogen, alkali-hydrolyzed nitrogen, and available phosphorus ($P < 0.05$). N_2F_1 increased alkali-hydrolyzed nitrogen (+9.39%) but decreased available phosphorus, while N_2F_2 improved organic matter (+24.93%) and reduced conductivity (-6.45%) and pH (-6.45%) ($P < 0.05$). The integration of reduced nitrogen with sheep manure ($\text{N}_2\text{F}_1/\text{N}_2\text{F}_2$) mitigated adverse pH and conductivity increases observed under N_2 alone, particularly in deeper soil layers, demonstrating the potential for optimized nutrient management to reduce salinity risks while enhancing soil fertility. (Significant Difference Analysis as Shown in Table S3–S5)

Effects of different fertilization treatments on soil enzyme activity

The activities of urease, protease, catalase, and nitrate reductase exhibited no obvious variations with increasing soil depth (0–60 cm) (Fig. 7), suggesting that changes in soil layer depth exerted limited effects on soil enzyme activity. In the 0–20 cm layer, nitrogen reduction (N_2) significantly reduced all soil enzyme activities compared to full nitrogen (N) ($P < 0.05$). While combined sheep manure application ($\text{N}_2\text{F}_1/\text{N}_2\text{F}_2$) maintained lower enzyme levels than N, N_2F_1 partially reversed this trend by increasing urease (+5.56%), protease (+21.15%), and catalase (+12.20%) activities versus N_2 ($P < 0.05$). At 20–40 cm, N_2 similarly suppressed all enzymes, though N_2F_1 enhanced protease (+28.79%) and urease (+2.25%) compared to N_2 ($P < 0.05$), despite persistent reductions in nitrate reductase. In the 40–60 cm layer, N_2 decreased protease (-12.80%) and nitrate reductase (-21.53%) ($P < 0.05$), with no significant recovery under $\text{N}_2\text{F}_1/\text{N}_2\text{F}_2$ treatments except stabilized catalase activity. These results demonstrate that organic amendments partially mitigate nitrogen reduction-induced enzyme inhibition in surface soils but exhibit limited efficacy in deeper strata, highlighting the need for stratified nutrient management to sustain soil biochemical functions. (Significant Difference Analysis as Shown in Table S6–S8)

Effects of different fertilization treatments on root activity of Korla fragrant pears

Root vitality of Korla fragrant pear exhibited a depth-dependent decline across soil profiles (0–60 cm), with maximum activity observed in the surface layer (0–20 cm) (Fig. 8A). All nitrogen fertilization treatments significantly enhanced root vitality compared to non-fertilized (CK) and nitrogen-free (N_0) controls in all layers ($P < 0.05$), confirming nitrogen's critical role in root function. In the 20–40 cm layer, nitrogen reduction (N_2) and combined N_2 + manure treatments ($\text{N}_2\text{F}_1/\text{N}_2\text{F}_2$) reduced vitality versus full nitrogen (N), though N_2F_2 partially

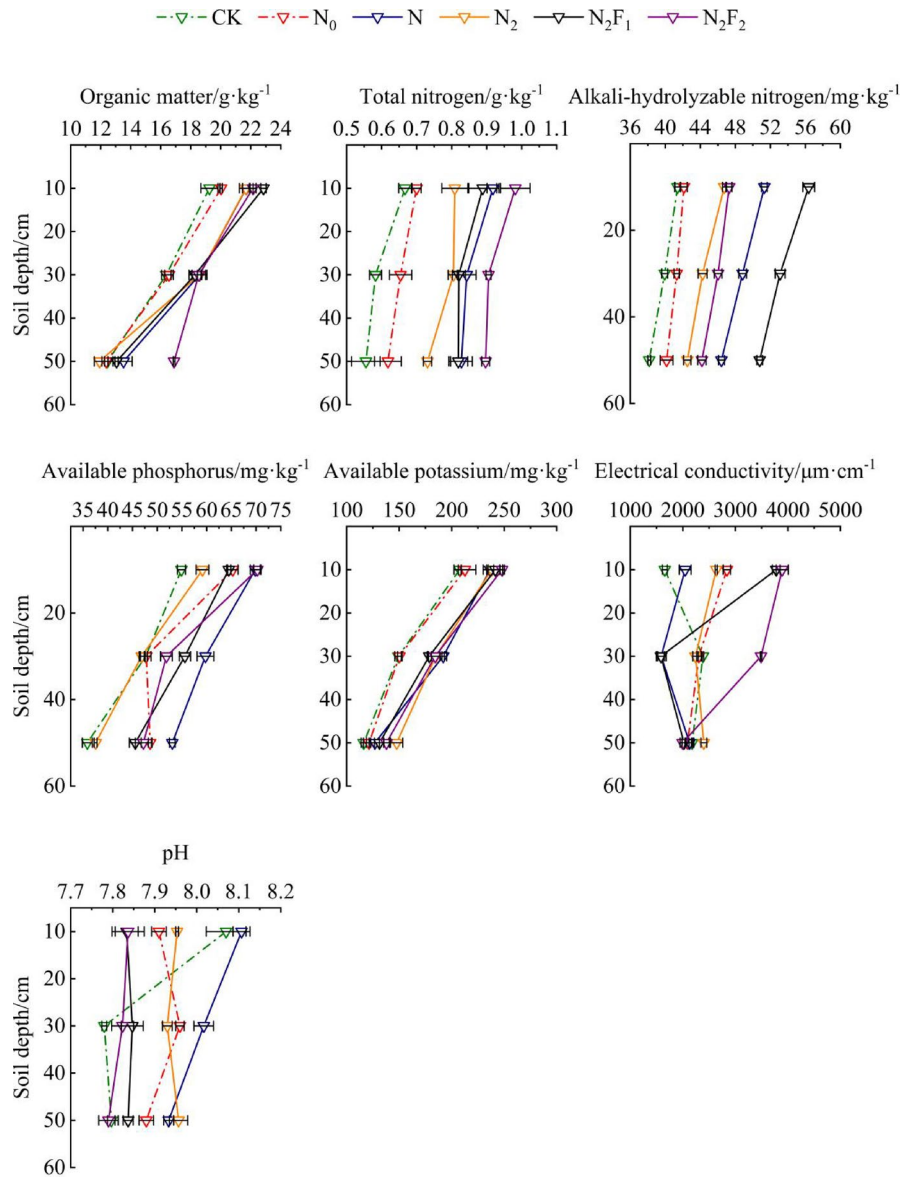


Fig. 6. Effects of reduced nitrogen fertilizer combined with sheep manure on soil nutrient content. Different colors represent different fertilization treatments.

offset this decline (+20% vs. N₂, $P < 0.05$). No significant differences were detected in the 40–60 cm layer between N₂, N₂F₂, and N treatments. These findings underscore nitrogen fertilization's necessity for sustaining root activity in Korla fragrant pear, while strategic organic supplementation (N₂F₂) may alleviate nitrogen-reduction impacts in intermediate soil horizons.

Effects of different fertilization treatments on anatomical structure of fragrant pear roots

In the root anatomical structure, we focused primarily on the indicators related to root cortex thickness, the proportion of different cell types in the root xylem and phloem, catheter area, and section area (Fig. 9).

Effects on root cortex thickness

As shown in Fig. 8B, compared with the N treatment, the thickness of the root cortex had no significant change under the nitrogen reduction N₂ treatment. It was significantly increased ($P < 0.05$) under the nitrogen reduction combined with sheep manure N₂F₂ treatment, increasing by 105.31%. And there was no significant change under the N₂F₁ treatment. In the 20–40 cm soil layer, compared with the full application of nitrogen, no significant change was found in the cortex thickness under the nitrogen reduction N₂ treatment. Under the treatments of nitrogen reduction combined with sheep manure, namely N₂F₁ and N₂F₂, the cortex thicknesses of the roots were significantly increased by 266.28% and 289.32% respectively ($P < 0.05$).

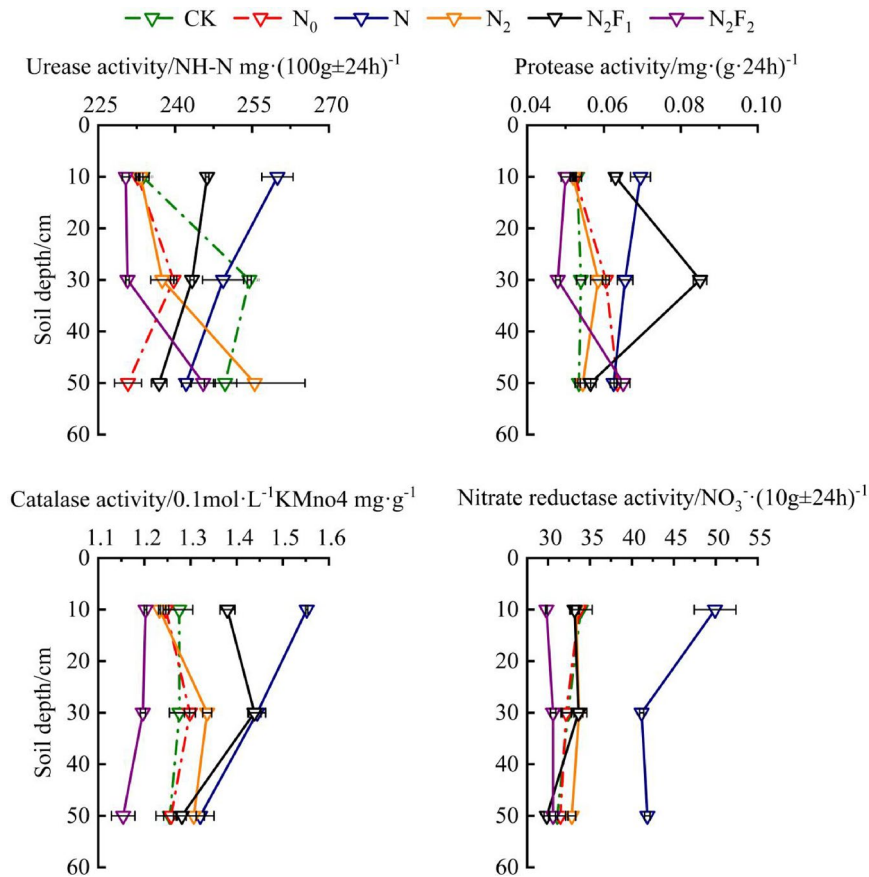


Fig. 7. Effects of reduced nitrogen fertilizer combined with sheep manure on soil enzyme activity. Different colors represent different fertilization treatments.

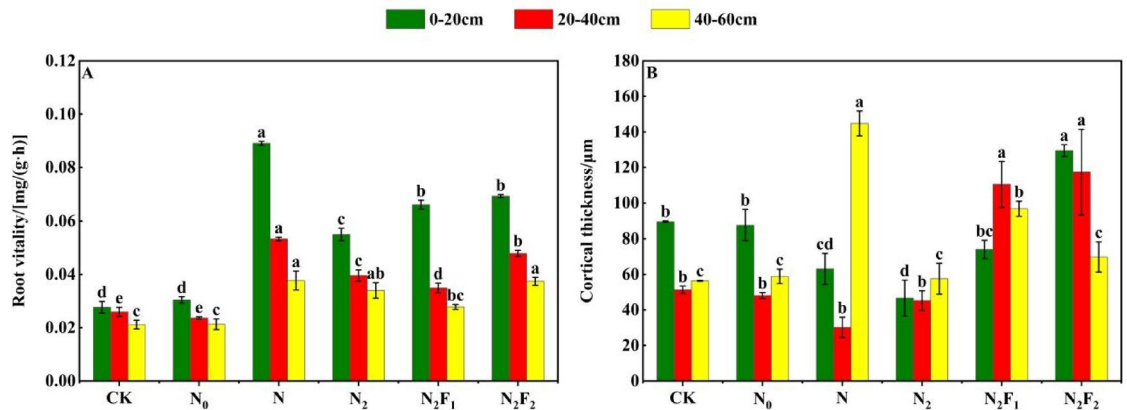


Fig. 8. Effects of reduced nitrogen fertilizer combined with sheep manure on root vitality and cortex thickness of fragrant pears. Diverse letters indicated significant difference among treatments at the same growth stage at 0.05 level, the same below.

Effects on the proportion of different cell types in root xylem and phloem

As shown in Fig. 10, compared with the N treatment, in the 0–20 cm soil layer, the proportion of phloem cellulose cell wall cells was decreased by 4.96% under the nitrogen reduction N₂ treatment. Under the treatments of nitrogen reduction combined with sheep manure, the proportions were decreased by 8.04% and 32.57% respectively. In the 20–40 cm soil layer, compared with the N treatment, the proportion of phloem cellulose cell wall cells was increased by 7.3% under the N₂ treatment. Under the nitrogen reduction combined with sheep manure treatments, namely N₂F₁ and N₂F₂, the proportions were decreased by 6.31% and 21.22% respectively. In the 40–60 cm soil layer, under the nitrogen reduction N₂ treatment, the proportion of cellulose cell wall cells

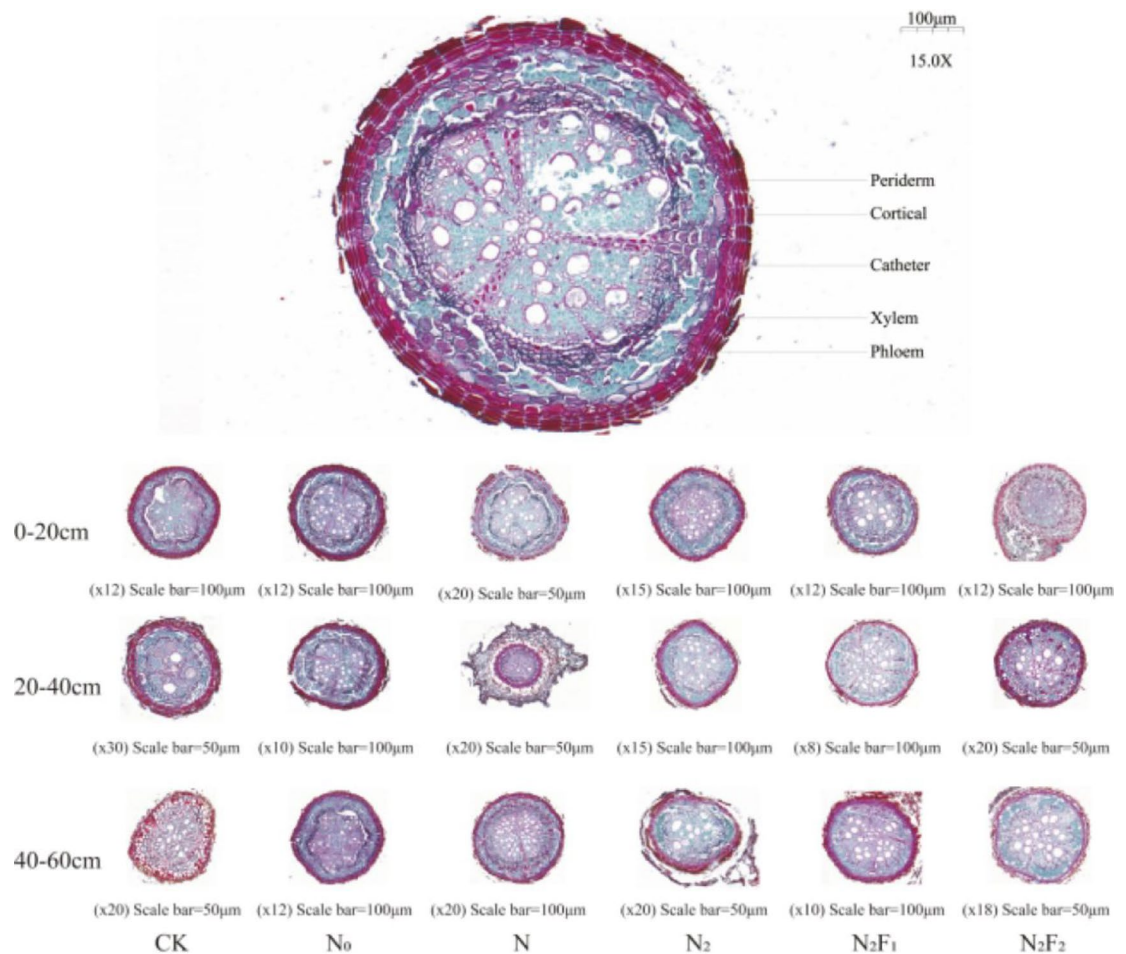


Fig. 9. Paraffin sections of roots under different fertilization treatments.

was increased by 13.95% compared with the full nitrogen application N treatment. Under the N_2F_1 treatment after combined application of sheep manure, it was increased by 1.96% compared with the N treatment. Under the N_2F_2 treatment, it was decreased by 9.22%.

It could be found from Fig. 10 that in the 0–20 cm soil layer, compared with the N treatment, the proportion of xylem cellulose cell wall cells under the reduced nitrogen N_2 treatment decreased by 9.34%. And under the N_2F_1 and N_2F_2 treatments with reduced nitrogen combined with sheep manure application, it decreased by 15.66% and 4%. In the 20–40 cm soil layer, compared with the N treatment, the proportion was increased by 8.1% under the N_2 treatment. Under the N_2F_2 treatment with reduced nitrogen combined with sheep manure application, it was increased by 2.13%. However, under the N_2F_1 treatment, it was decreased by 7.65%. In the 40–60 cm soil layer, the activity of xylem cells as a whole was lower than that of the upper roots. Compared with the N treatment, the proportion of xylem cellulose cell wall cells was reduced by 19.35% under the N_2 treatment. Under the N_2F_1 treatment with reduced nitrogen combined with sheep manure application, it was increased by 1.15%. However, under the N_2F_2 treatment, it was decreased by 24.59%.

Effects on changes and slice area

As shown in Fig. 11, the changes in the catheter area and total slice area of the root of fragrant pears under the treatment of reduced nitrogen fertilizer combined with sheep manure were as follows. In the 0–20 cm soil layer, compared with the N treatment, both the catheter area and the slice area showed no significant change under the reduced nitrogen N_2 treatment. Under the N_2F_2 treatment with reduced nitrogen combined with sheep manure application, they were significantly increased ($P < 0.05$), increasing by 58.84% and 327.46% respectively. In the 20–40 cm soil layer, compared with the N treatment, both the catheter area and the slice area were significantly reduced under the reduced nitrogen N_2 treatment ($P < 0.05$). Under the treatment of reduced nitrogen fertilizer combined with sheep manure, the catheter area showed no significant change compared with the complete nitrogen application N treatment. The slice area was significantly increased under the N_2F_1 and N_2F_2 treatments ($P < 0.05$), increasing by 11.56% and 82.38% respectively. In the 40–60 cm soil layer, compared with the N treatment, under both the reduced nitrogen treatment and the reduced nitrogen combined with sheep manure treatment, both the catheter area and the slice area showed a significant downward trend ($P < 0.05$).

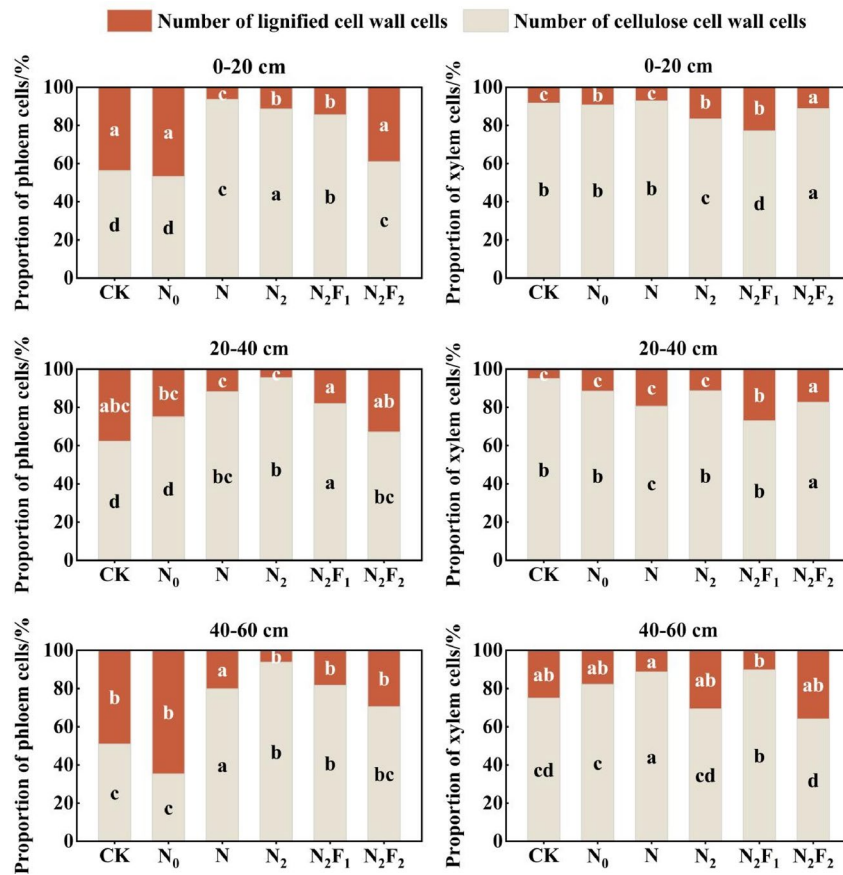


Fig. 10. Effects of different fertilization treatments on phloem and xylem cells of roots.

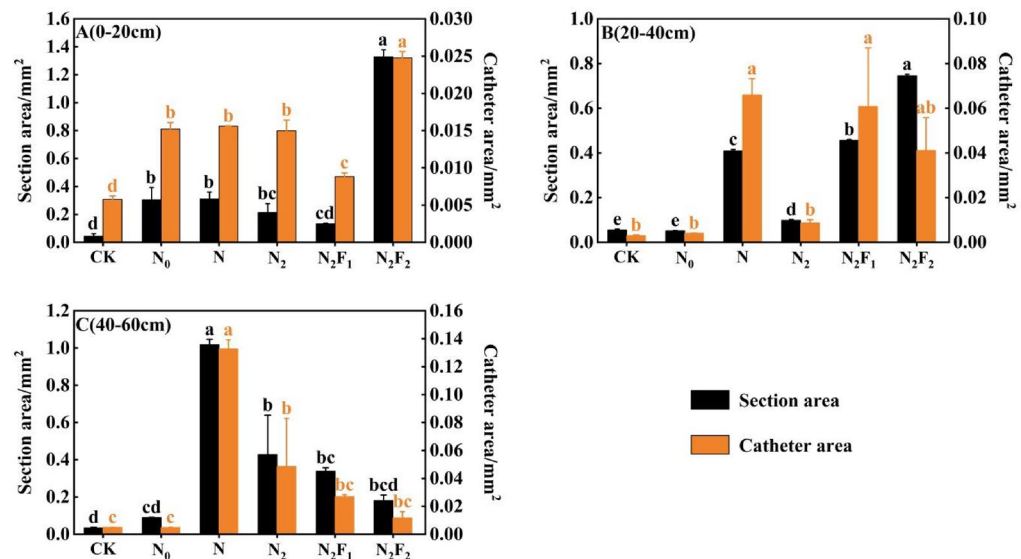


Fig. 11. Effects of different treatments on catheter and section area of fragrant pear roots.

Effects of different fertilization treatments on fragrant pear yield

The yield indexes of Korla fragrant pears are shown in Fig. 12. Compared with the N treatment, the number of single fruits, yield per plant, and total yield were all significantly reduced under the reduced nitrogen N₂ treatment ($P < 0.05$), while there was no significant change in the single fruit weight. Under the treatment of reduced nitrogen fertilizer combined with sheep manure, there was no significant change in the number of single

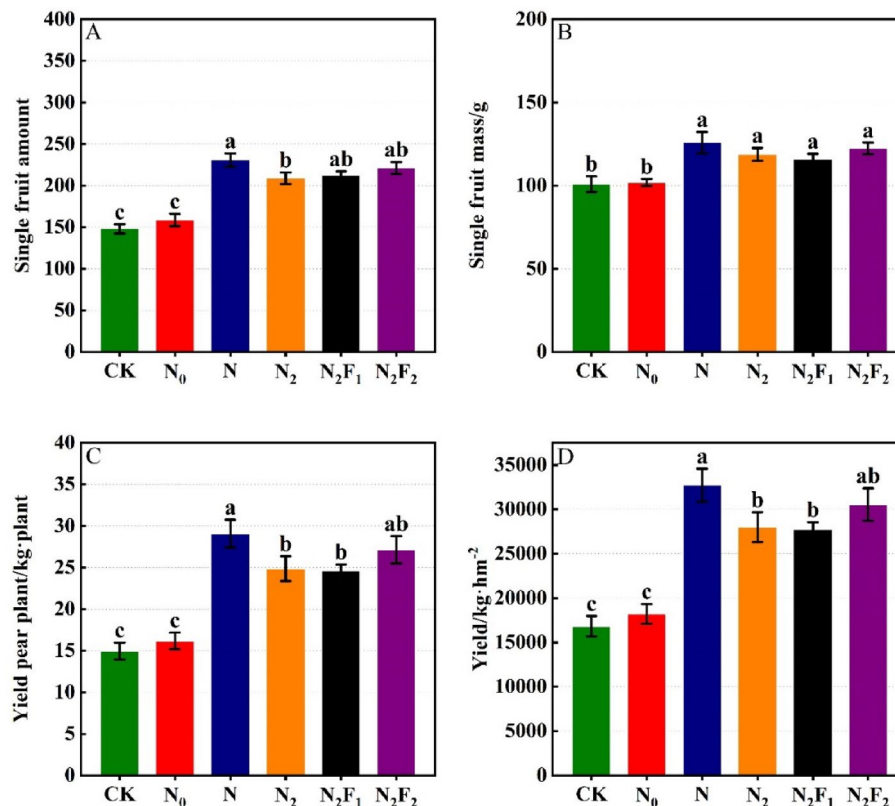


Fig. 12. Effects of different fertilization treatments on fragrant pear yield.

fruits and single fruit weight of fragrant pears under the N₂F₁ and N₂F₂ treatments. There was also no significant change in yield per plant and total yield under the N₂F₂ treatment.

Correlation among soil nutrients, enzyme activities, root activity and yield under different fertilization treatments

Mantel test was conducted using soil nutrients, enzyme activities, root activity and fragrant pear yield (Fig. 13). It could be found that in the 0–20 cm soil layer, the root activity of fragrant pears was extremely significantly correlated with soil organic matter, total nitrogen and available potassium content ($P < 0.01$), and there was a significant correlation with soil urease, protease, catalase activity and available phosphorus content ($P < 0.05$). The yield of fragrant pears was extremely significantly correlated with soil organic matter, total nitrogen and available potassium content ($P < 0.01$), and there was a significant correlation with soil available phosphorus content ($P < 0.05$). In the 20–40 cm soil layer, the root activity of fragrant pears was extremely significantly correlated with the contents of soil organic matter, total nitrogen and available potassium ($P < 0.01$), and it was significantly correlated with the activity of soil nitrate reductase and the content of available phosphorus ($P < 0.05$). The yield of fragrant pears was extremely significantly correlated with the contents of soil organic matter, total nitrogen and available potassium ($P < 0.01$). In the 40–60 cm soil layer, the root activity of fragrant pears was only extremely significantly correlated with the total nitrogen content in the soil ($P < 0.01$), and was significantly correlated with the alkali-hydrolyzable nitrogen content ($P < 0.05$). The yield of fragrant pears was extremely significantly correlated with the total nitrogen and alkali-hydrolyzable nitrogen contents in the soil ($P < 0.01$).

The partial least squares path model (PLS-PM) integrating fertilization methods, soil properties, enzyme activity, root vitality, (Fig. 14; Tables S1-S2) and yield demonstrated robust predictive accuracy ($R^2 > 0.26$, exceeding the “good” threshold)^{14,15}. Fertilization strategies exerted strong positive direct effects on soil physicochemical properties ($P < 0.01$) and yield ($P < 0.01$), with moderate positive impacts on root vitality ($P < 0.05$). However, nitrogen reduction combined with organic amendments significantly suppressed soil enzyme activity ($P < 0.01$). Soil physicochemical parameters positively correlated with enzyme activity ($P < 0.01$) and root vitality ($P < 0.05$) but negatively influenced yield ($P < 0.05$). Enzyme activity showed strong positive associations with yield ($P < 0.01$) and root vitality ($P < 0.05$), while root vitality directly enhanced yield ($P < 0.01$). This systemic analysis reveals that nitrogen-organic co-management enhances yield through coordinated root-soil interactions despite enzyme inhibition, emphasizing the need for balanced strategies to optimize orchard productivity and ecological functions.

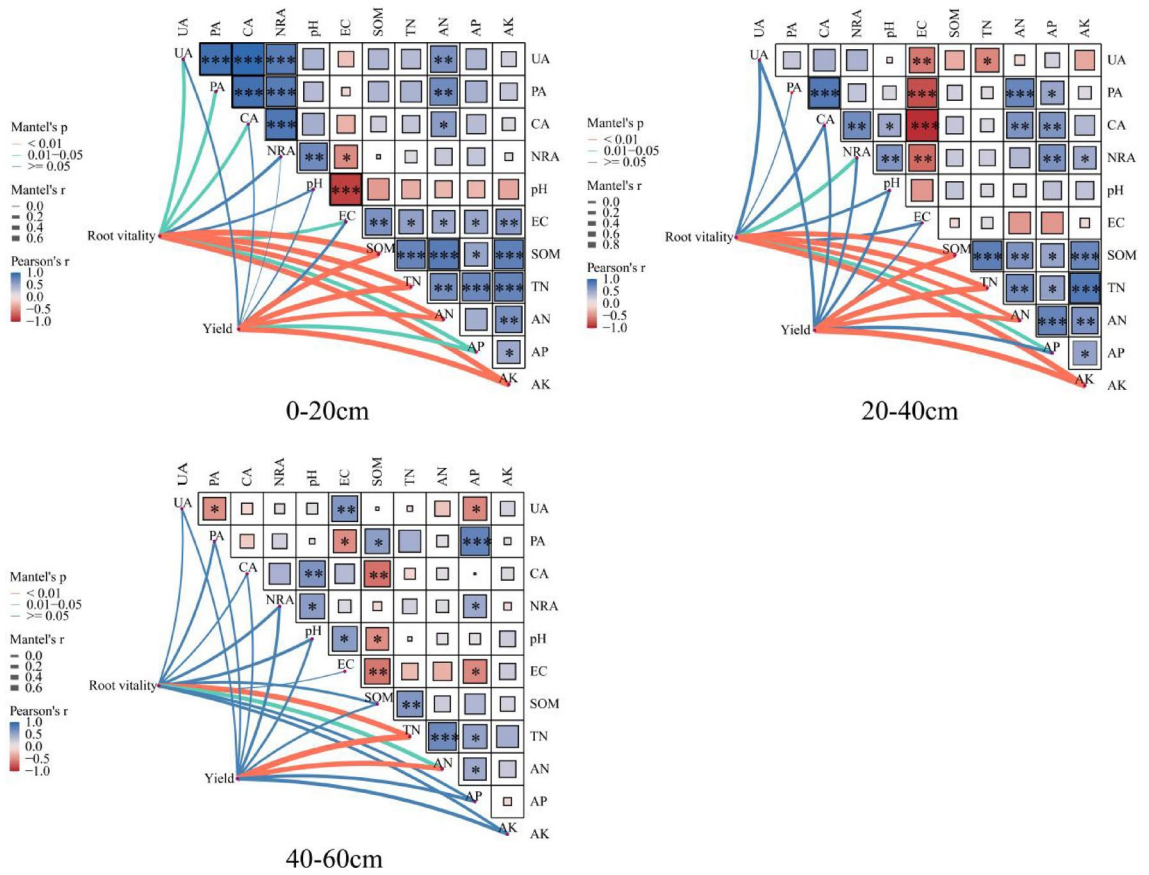


Fig. 13. Correlation among soil nutrients, enzyme activities, root activity and yield under different fertilization treatments.* means a significant difference at $P < 0.05$ level. ** means a significant difference at $P < 0.01$ level.

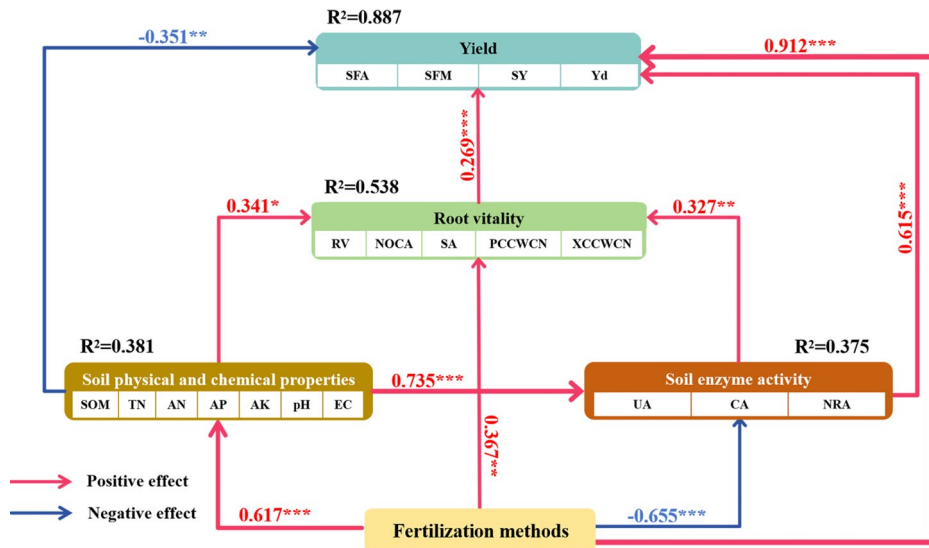


Fig. 14. Partial least squares path model (PLS-PM) illustrates the direct and indirect effects among fertilization methods, soil nutrients, soil enzyme activities, root activity and yield. The PLS path model demonstrated the impact of fertilization on soil quality, root system development, and fragrant pear yield. The red and blue arrows indicate positive and negative flows of causality, respectively. * means a significant difference at $P < 0.05$ level. ** means a significant difference at $P < 0.01$ level. *** means a significant difference at $P < 0.001$ level. R^2 represents the total interpretation rate of all independent variables to the dependent variable.

Discussion

Effects of reduced nitrogen fertilization combined with sheep manure on soil physical and chemical properties

Organic fertilizers contained the essential nutrient elements for plant growth. While improving soil quality, they didn't increase the environmental burden. In recent years, the fertilization method of reducing chemical fertilizers combined with organic fertilizers had been vigorously advocated by people¹⁶. Previous researchers¹⁷ found that compared with applying chemical nitrogen fertilizers alone, the combined application of organic fertilizers could improve soil structure better, enhance the ability of soil nutrient supply, slow down soil acidification and increase the number of soil microorganisms. For improving soil fertility and enhancing soil quality, similar results were also obtained in this experiment. This was because the application of organic fertilizers brought organic matter into the soil and increased the content of soil organic matter¹⁸. Meanwhile, the combined application of chemical nitrogen fertilizers and organic sheep manure could make good use of their respective advantages. The nutrients in chemical fertilizers could be quickly decomposed to meet the nutrient requirements of crop growth. After being combined with sheep manure, the substances contained in it were able to effectively improve the soil environment and increase the activity of soil microorganisms. As a result, the release of nutrients in sheep manure was promoted and the utilization rate was increased¹⁹. The content of soil available nutrients could be effectively increased to some extent by applying organic fertilizers²⁰. In this experiment, after reducing nitrogen by 20% on the basis of complete nitrogen application, certain negative impacts were brought to various soil nutrient indexes and physical and chemical properties to some extent. However, after applying sheep manure on the basis of reducing nitrogen fertilizer, the contents of soil organic matter, total nitrogen and alkali-hydrolyzable nitrogen showed a significant increasing trend. And compared with the N treatment, there was no significant change in the other nutrient indexes either. Meanwhile, there was no significant change in the soil pH of different soil layers under the treatment of applying sheep manure. The best effect was achieved under the N₂F₂ treatment.

Effects of reduced nitrogen fertilization combined with sheep manure on soil enzyme activities

The decomposition of organic matter was a complex process, in which the combined action of multiple organisms was required. Soil enzymes, as the main power source for soil metabolism, had their activities that could reflect the vitality of microorganisms and the level of nutrient availability in the soil. They were the indicators for measuring soil quality²¹. Urease was an enzyme related to the nitrogen cycle in the soil, and its main function was to catalyze the decomposition of urea into NH₃ and CO₂. In this study, it was found that in the 0–40 cm soil layer, the activity of urease was significantly reduced compared with the treatment of complete nitrogen application N, whether it was under the reduced nitrogen N₂ treatment or under the N₂F₁ and N₂F₂ treatments combined with sheep manure application, but compared with the N₂ treatment, the activity of urease could be significantly increased under the N₂F₁ treatment. It was shown that nitrogen addition had a very significant impact on soil urease activity, especially chemical nitrogen fertilizers. This differed from the research results of previous researchers²². The main reason might be that in previous studies, organic fertilizers were used to replace part of nitrogen fertilizers under the condition that the total nitrogen amount remained unchanged. In this experiment, however, simply applying different amounts of organic fertilizers after reducing nitrogen could not supplement the part of nitrogen that had been subtracted, thus reducing the substrate concentration of urease. Protease can promote the hydrolysis of proteins and peptides into amino acids. It participates in soil nitrogen transformation together with urease and is an important indication of the soil's nitrogen supply ability²³. In this experiment, it was found that in the 0–40 cm soil layer, the activities of the four enzymes were all significantly lower under the N₂ treatment than under the complete nitrogen application N treatment. In the 0–20 cm soil layer, the activity of protease was significantly lower compared with that under the complete nitrogen application N treatment, both under the treatment of reducing nitrogen fertilizer and under the treatment of combining reduced nitrogen fertilizer with sheep manure application. However, under the N₂F₁ treatment, the activity of protease was significantly higher than that under the N₂ treatment. In the 20–40 cm soil layer, compared with complete nitrogen application, the activity of protease was significantly increased under the N₂F₁ treatment. This shows that applying F₁ (22,500 kg·hm⁻²) sheep manure after reducing nitrogen by 20% is more beneficial for improving soil protease activity than simply reducing nitrogen fertilizer. This is because reducing nitrogen fertilizer would inhibit the activity of soil protease, while after applying a certain amount of sheep manure, the content of soil organic matter is increased, thus increasing the activity of protease. Catalase was an important kind of redox enzyme in the soil. Its function was to decompose hydrogen peroxide, which was toxic to organisms in the soil. To some extent, its activity could reflect the intensity of soil microbial processes and the level of soil toxic effects^{24,25}. After reducing nitrogen fertilizer and applying sheep manure, there was no significant increase in the activity of soil catalase. In fact, in most treatments, it showed a significant inhibitory effect. This was because, after reducing nitrogen fertilizer, compared with complete nitrogen application, the concentration of available nitrogen substrate for catalase in the soil began to decrease. As a result, its activity would also be significantly reduced compared with the N treatment. Soil nitrate reductase was able to catalyze the production of NO₂⁻ from NO₃⁻^{26,27}. It was the main enzyme participating in soil denitrification and the allosteric assimilation of NH₄⁺ by NO₃⁻-N^{28,29}. In this study, it was found that there was a highly close relationship between soil nitrate reductase and the amount of nitrogen applied. Compared with complete nitrogen application, a significant decreasing trend was observed under the treatments of nitrogen reduction and the combination of nitrogen reduction with sheep manure application. The reason might be that in this experiment, after reducing nitrogen based on the optimal nitrogen application amount obtained from previous experiments^{30,31} the contents of soil nitrate nitrogen and ammonium nitrogen that could originally be supplied were decreased. And the applied sheep manure could not fully supplement the nutrient amounts missing after reducing nitrogen fertilizer, thus inhibiting the production of nitrate reductase and reducing its activity³².

Effects of reduced nitrogen fertilization combined with sheep manure on root activity of Korla fragrant pears

Roots could sense the water and fertilizer conditions of the soil and undergo adaptive development. When the availability of soil nutrients was limited, crop roots could adapt to the surrounding environment through self-regulation³³. Root vitality was an important indicator of the physiological characteristics of roots³⁴. An increase in its value could reflect that the growth of roots was accelerated, the absorption of nutrients was enhanced and the redox ability was improved. Different nutrient supplies were an important cause for the vertical and horizontal changes in root vitality. The uneven distribution of nutrients would strongly affect root growth³⁵. The results of this experiment indicated that the application of nitrogen fertilizer could significantly increase the root vitality of fragrant pears, which was consistent with the research findings of Tiantian Hu and others³⁶. For the treatments involving only nitrogen fertilizer reduction, after reducing nitrogen fertilizer, compared with complete nitrogen application, the root vitality in the surface layer (0–40 cm) of the soil was significantly inhibited. However, a 20% reduction in nitrogen fertilizer had no significant impact on the root vitality in the deep layer (40–60 cm) of the soil. After reducing nitrogen fertilizer and applying sheep manure, the root vitality in the 0–40 cm soil layer was also significantly inhibited compared with complete nitrogen application. Nevertheless, it was significantly increased compared with the N₂ treatment. In the deep soil layer (40–60 cm), the root vitality under the treatment of reducing nitrogen by 20% and applying sheep manure F₂ showed no significant change compared with the N treatment of complete nitrogen application. This result indicated that reducing nitrogen fertilizer on the basis of complete nitrogen application had a significant inhibitory effect on root vitality. While after reducing the amount and applying a certain amount of sheep manure, the root vitality could be maintained at the same level as in the case of complete nitrogen application. However, the root vitality in the surface soil still could not be effectively maintained or improved. This might be because the fertilization method adopted in this experiment was annular ditch application, and the fertilization position was around 30 cm soil layer. At the same time, it was affected by factors such as irrigation and rain leaching, so the impact on root vitality in the lower soil layer was more significant.

The Mantel Test results indicated that the root vitality of fragrant pears was significantly influenced by soil nutrients and enzyme activities. Under the treatments of nitrogen reduction and nitrogen reduction combined with sheep manure application, there was an extremely strong correlation between the root vitality of fragrant pears and the nutrients and soil enzyme activities in the soil. Particularly, it was evidently affected by the contents of soil organic matter, total nitrogen, alkali-hydrolyzable nitrogen, available phosphorus, available potassium and the activities of urease, protease, and catalase. This demonstrated that the improvement of soil nutrients and physicochemical properties could effectively enhance the root vitality of fragrant pears and thereby directly affect the growth and development of the aboveground part. Ban Chunguo³⁷ also found that as the content of soil organic matter increased, the root vitality of young apple trees generally showed an increasing trend. This might be due to the fact that after applying organic fertilizers such as sheep manure, the soil layer became loose and the soil structure was good. This enhanced the soil's ability to retain water and fertilizers and improved the soil's nutrition and aeration conditions³⁸. As a result, a more suitable growth environment was created for the roots, making the correlation between root vitality and soil nutrients and enzyme activities even stronger.

Effects of reduced nitrogen fertilization combined with sheep manure on root anatomical structure

Plants' fine roots played an important role in terrestrial ecosystems³⁹. The anatomical structure of roots is the most intuitive manifestation of the level of root development. Previous studies have found that the relationship between the diameter of fine roots and the thickness of the cortex is quite significant⁴⁰. The cortical tissue is an important tissue for roots to absorb nutrients⁴¹. As an important indicator related to the absorption function of fine roots, it is rather sensitive to the response of soil nutrients and physicochemical environment⁹. In previous studies, there were still disagreements regarding the results of changes in the thickness of the cortex of fine roots after nitrogen fertilizer application. Some studies found that short-term nitrogen addition would increase the cortical thickness⁴². On the other hand, Hong Ziming⁴³ and others discovered that long-term nitrogen addition would significantly inhibit the cortical thickness. The results of this experiment were similar to those of Hong Ziming and others. The cortical thickness of the roots of fragrant pears was influenced by the application of nitrogen. Compared with the treatment without nitrogen fertilizer application, the cortical thickness under complete nitrogen application was significantly reduced. This might be due to the fact that under the stimulation of high-concentration nitrogen from the outside, the roots would decrease their cortical thickness to better absorb nutrients, this was to reduce the transport resistance of the roots and make the absorption rate faster⁴⁴. After reducing nitrogen and applying sheep manure in combination, compared with complete nitrogen application, the cortical thickness could be reduced under some treatments. For instance, the cortical thickness of the surface layer (0–20 cm) was reduced under the N₂F₁ treatment. Meanwhile, the cortical thickness of the roots in the bottom layer (40–60 cm) all showed a decreasing trend under the treatments of reducing nitrogen and applying sheep manure in combination. This indicates that after applying sheep manure in combination, the cortical thickness of fragrant pear roots can be reduced more effectively, which is more beneficial for the tree to absorb and utilize soil nutrients and moisture.

In the anatomical structure characteristics of roots, the characteristics of xylem and phloem and their responses to nitrogen were rarely paid attention to^{45,46}. However, in this study, it was found that the phloem cells of roots were significantly affected by nitrogen addition. In different soil layers, the number of cellulose cell walls of phloem in each nitrogen application treatment gradually increased compared with the treatment without fertilization (CK). For the soil layer of 0–40 cm, compared with the complete nitrogen application treatment (N), the proportion of cellulose cell walls in the treatments of reducing nitrogen and reducing nitrogen combined with sheep manure application showed a decreasing trend. As for the soil layer of 40–60 cm, the proportion of

cellulose cell walls of phloem under the treatments of N_2 and N_2F_1 was significantly increased compared with the treatment of N. Under the treatments of reducing nitrogen and reducing nitrogen combined with sheep manure application, the proportion of cellulose cell walls of xylem in the 0–40 cm soil layer was significantly lower than that under the treatment of N. In the 40–60 cm soil layer, the proportion of cellulose cell walls of xylem under the treatment of N_2F_1 was significantly lower than that under the treatment of N. However, the number of cellulose cell walls of xylem in the 0–40 cm soil layer was significantly increased under the treatment of N_2F_2 compared with that under the treatment of N. This shows that after reducing nitrogen and applying a certain amount of sheep manure, the number of cellulose cell walls of xylem in the roots of fragrant pears can be effectively increased.

Previous studies found that an increase in the number of catheters, average catheter diameter, and total catheter area may thicken the stele diameter and thereby transport nutrients faster⁴⁷. In contrast, the results of this experiment revealed that the catheter characteristics of fine roots of fragrant pears, such as catheter area, showed a very obvious response to the application of nitrogen fertilizer. Specifically, in different soil layers, compared with the CK treatment, the catheter area under each nitrogen application treatment exhibited a significant increasing trend. In the 0–20 cm soil layer, the catheter area under the N_2F_2 treatment was significantly increased compared with the N treatment. In the 20–40 cm soil layer, there was no significant change in the catheter area under the N_2F_1 and N_2F_2 treatments compared with the N treatment. However, in the 40–60 cm soil layer, the catheter area was significantly reduced under the treatments of reducing nitrogen and reducing nitrogen combined with sheep manure application. The size of the root slice area of fragrant pears reflects the transportation capacity of nutrients and moisture. The roots can sense soil moisture regulation and adjust the transportation rate by changing their own diameters. Previous researchers⁴⁸ found that nitrogen application could significantly increase the diameter of fine roots. In this experiment, it was found that the application of nitrogen fertilizer had a very significant impact on the slice area. In the 0–20 cm soil layer, the slice area under the N_2F_2 treatment was significantly increased compared with the N treatment. In the 20–40 cm soil layer, the slice areas under the N_2F_1 and N_2F_2 treatments both showed a significant increasing trend compared with the N treatment. In the 40–60 cm soil layer, it was consistent with the trend of the conduit area and the slice area was significantly reduced under the treatments of reducing nitrogen and reducing nitrogen combined with sheep manure application. It was shown that complete nitrogen fertilizer N had a highly significant influence on the slice area and conduit area of fine roots of fragrant pears. Reducing nitrogen application by 20% would restrain the increase in conduit area and slice area of roots. Nevertheless, after the combined application of sheep manure, this inhibitory effect would be mitigated. Likewise, due to the slow release and migration of fertilizer efficacy, the response of deep roots (40–60 cm) was relatively sluggish and had minimal impact.

Effects of reduced nitrogen fertilization combined with sheep manure on the yield of fragrant pears

Crop yield can objectively reflect its growth situation⁴⁹. Currently, excessive application of nitrogen fertilizer is widespread in the management and observation of Korla fragrant pears. This is unfavorable for increasing crop yield. This experiment found that applying nitrogen fertilizer could effectively increase the yield of fragrant pears. However, after reducing nitrogen fertilizer application, the number of single fruits, yield per plant, and total yield in the yield components would be significantly reduced. Under the N_2F_2 treatment with combined application of sheep manure, the number of single fruits, single fruit weight, yield per unit area, and total yield of fragrant pears could be maintained at the same level as under the N treatment. This clearly indicates that reducing nitrogen fertilizer by 20% has a significant inhibitory effect on the yield of fragrant pears. Meanwhile, after the F_2 treatment with combined application of sheep manure, the yield of fragrant pears can be maintained at the same level as under the complete nitrogen application (N treatment), thus achieving the effect of reducing the application of chemical nitrogen fertilizer. Additionally, correlation analysis found that there is a highly significant correlation between the yield of fragrant pears and the content of total nitrogen and alkali-hydrolyzable nitrogen in the soil.

Conclusion

To achieve the goals of “double reduction” and “zero growth” of nitrogen fertilizer, attention was paid to improving the absorption and transportation ability of fragrant pear roots to water and fertilizers in the soil and improving the soil nutrient status while ensuring and increasing production, reducing the harm caused by excessive chemical nitrogen fertilizer to the environment of fragrant pear orchards, and realizing high-yield, high-efficiency and sustainable development of agriculture. In this study, it was recommended that sheep manure of $33,750 \text{ kg}\cdot\text{hm}^{-2}$ be applied in combination after reducing nitrogen by 20% ($240 \text{ kg}\cdot\text{hm}^{-2}$) on the basis of complete nitrogen application ($300 \text{ kg}\cdot\text{hm}^{-2}$). This could be used as a reference basis for optimizing the organic-inorganic combined application mode in Korla fragrant pear orchards.

Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

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Author contributions

Wei Li: Investigation, Data curation, Formal analysis, Visualization, Writing - original draft. Xuemei Wang: Conceptualization, Experimental design. Linsen Yan&Xiuxiu Liu: Investigation, Data curation, Formal analysis, Writing - original draft. Zhanyi He & Kailong Wang: Methodology, Experimental design, Resources. Zhongping Chai: Conceptualization, Experimental design. Xing Shen: Experimental design, Writing - Review & Editing, Supervision.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to X.S. or Z.C.

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