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Field Crops Research

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https://doi.org/10.1016/j.fcr.2024.109262

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Field Crops Research 307 (2024) 109262

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Diversification of wheat-maize double cropping with legume intercrops improves nitrogen-use efficiency: Evidence at crop and cropping system levels

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ARTICLE INFO

Keywords:
Simultaneous intercropping
Three-quadrant diagram
Apparent recovery efficiency
EU Nitrogen Expert Panel
Nitrogen surplus

ABSTRACT

Context or problem: The sustainability of traditional maize-wheat (M-W) double cropping in the North China Plain (NCP) is threatened by excessive nitrogen (N) input and surplus. Meanwhile, there is strong market demand of more protein and oil crops, such as soybean or peanut. Incorporation of legumes into M-W via intercropping with maize is emerging in the NCP to foster China's self-sufficiency for edible oils and proteins.
Objective or research question: It is unknown how such a change in cropping system affects the required annual fertilizer N input, and the resulting N-use efficiency (NUE) and N surplus (Ns).
Methods: We conducted a four-year field experiment involving four N fertilizer rates and rotations of winter wheat with six different summer crops: maize (conventional and density-increased), peanut (P), soybean (S), and intercrops of density-increased maize and peanut or soybean (MP, MS). The "three-quadrant diagram" and NUE proposed by the EU Nitrogen Expert Panel (EUNEP) were used to assess NUE at crop and cropping system level, respectively.
Results: Land equivalent ratios of N uptake in intercrops averaged 1.02–1.07 while N fertilizer equivalent ratios averaged 1.15–1.20, indicating more efficient N uptake and more yield per unit fertilizer than sole crops. Intercropped maize exhibited greater N acquisition efficiency than sole maize. Inclusion of intercrops lowered required annual N inputs and Ns and increased the apparent recovery efficiency (RE) of applied N and EUNEP-NUE. Soybean was a more productive and N-use efficient companion species for maize than peanut. Increasing N decreased RE and EUNEP-NUE while elevated Ns of all rotation systems. N productivity responses of each rotation system to increasing N followed a "linear-plateau" model. Compared to M-W with an optimal 240–360 kg N/ha, MS-W with 210–320 kg N/ha saved 11.1–12.5% fertilizer, increased N uptake by 12.4–16.0%, augmented RE from 36.0–37.0% to 47.8%, increased EUNEP-NUE from 0.50 to 0.67 kg/kg (within the target of 0.50–0.90 kg/kg), lowered Ns by 38.8–39.2%, and reduced N emission by 48.6–49.3%.
Conclusions: Therefore, here we show for the first time, using multiple N performance indicators, that MS-W with moderate N provides diversified products, higher N productivity, NUE and lower N loss than M-W, thus being a suitable option for sustainable intensification of agricultural production.
Implications or significance: Such a diversified rotation approach with legume intercropping aligns with the principles of agricultural green development and has a global relevance for countries with sequential double cropping or rotation systems.

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https://doi.org/10.1016/j.fcr.2024.109262
Received 6 October 2023; Received in revised form 9 December 2023; Accepted 9 January 2024

1. Introduction

Double cropping refers to the practice of cultivating two consecutive crops on the same land within a single year (Papendick et al., 1976). Various double cropping systems are practiced worldwide. In Brazilian maize production, double cropping stands out as the most significant form (Elobeid et al., 2019). In Southern Europe, particularly in Mediterranean-type areas, a quite long cold-free period allows double-annual cropping systems to be practiced, involving a summer crop (sorghum or maize) and a winter cereal such as barley or triticale (Ovejero et al., 2016; Simon-Miquel et al., 2023). Rice-based double cropping is popular in South Asia (Bhatt et al., 2021; Timsina et al., 2010). The summer maize-winter wheat (M-W) double cropping is the dominant in North China Plain (NCP) (Zhao et al., 2022). This production system accounts for nearly 30% of maize and 45% of wheat production in China (Lu et al., 2021). However, intensive large-scale sole cropping of wheat and maize, and excessive nitrogen (N) inputs, led to soil degradation, environmental pollution, and impoverished landscapes with low crop diversity (Bélanger and Pilling, 2019; Rockström et al., 2017; Zhao et al., 2022).

Chinese farmers apply 550–600 kg N/ha annually for maize and wheat, significantly surpassing the combined N demand of both crops, which is about 330 kg N/ha (Zhao et al., 2015). Comparable sustainability concerns were raised regarding rice-maize and rice-wheat cropping in South Asia (Bhatt et al., 2021; Nayak et al., 2022) and barley-maize cropping in the Mediterranean region (Maresma et al., 2019). N overapplication diminishes crop N-use efficiency (NUE) and increases nitrate leaching into surface water, and emissions of NH_3, N_2O, and NO into the air (Congreves et al., 2021; Erisman et al., 2013; Liu et al., 2022; Steffen et al., 2015; Yin et al., 2021). To minimize N pollution while maintaining food security at both national and global scales, it is imperative to decrease N inputs without compromising crop yields (Davidson et al., 2015; Gu et al., 2023).

Many legume species can fix atmospheric N_2, generating significant interest in including legumes in the cropping systems via rotation or intercropping to lower the need for artificial fertilizers (Chen et al., 2019; Gao et al., 2020; Li et al., 2020; Martin-Guay et al., 2018; Nemecek et al., 2008; Rose et al., 2019; Thierfelder et al., 2012; Xiao et al., 2022; Xu et al., 2022). Moreover, legumes, owing to their rich protein and vegetable oil content, can contribute to meet the market demand for healthy and nutritious diets (Huang et al., 2022; Semba et al., 2021). Legume incorporation not only aids in reducing N inputs but also enhances the production of edible oils and proteins, resulting in a win-win situation (Simon-Miquel et al., 2023).

Currently, China maintains a self-sufficiency rate of over 95% for cereal grains, but its self-sufficiency in edible oils falls < 35%. The country imports > 80% of its soybean proteins, as reported in The China Agricultural Sector Development Report (2020). The Chinese government is actively encouraging the diversification of traditional wheat-maize double cropping systems by incorporating legumes, either through crop rotation or intercropping (China's No. 1 Central Document, 2023; General Office of the State Council of China, 2015). One potential avenue for diversification involves introducing double cropping systems where winter wheat is succeeded by a concurrent intercropping summer maize with peanut or soybean. Actually, maize is usually harvested after a legume crop, as known as the relay strip intercropping method (Brooker et al., 2015; Li et al., 2013; Raza et al., 2020), especially in temperate climate areas with a growing season longer than necessary for one crop, but too short for two consecutive crops as in double cropping. These relay strip intercrops have higher productivity and NUE than sole cropping mainly due to the interspecific complementarity in resource use (Bedoussac et al., 2015; Jensen et al., 2020; Justes et al., 2021; Li et al., 2020; Liu et al., 2018; Stomph et al., 2020; Xu et al., 2020). However, very few research has investigated the simultaneous intercropping of maize and legumes, which involves sowing and harvesting maize and legumes concurrently. Moreover, there is currently no available data on the impacts of preceding intercropping summer maize and legumes on NUE of winter wheat. Similarly, the impacts of such diversified double cropping systems with maize/legume intercrops on required annual N input and the resulting NUE and N surplus (Ns) remain unexplored in the NCP.

When assessing NUE at crop level, distinguishing acquisition efficiency (the fraction of available N captured or net taken up) and conversion efficiency (the ratio of biomass or yield to the acquired N amount) is informative. NUE encompasses both aspects. The "three-quadrant diagram", initially introduced by Van Keulen (1982), serves as a valuable method for intuitively dissecting the implications of nutrient management on nutrient acquisition and conversion efficiency in sole crops. Surprisingly, three-quadrant diagrams have been underutilized for analyzing the relative contributions of N acquisition and conversion efficiency in intercropping systems (Stomph et al., 2020).

The EU Nitrogen Expert Panel (EUNEP) introduced a robust and consistent protocol for benchmarking N performance of cropping and farm systems (EUNEP, 2015). The EUNEP allows the evaluation of NUE at the level of rotations consisting of multiple crops (Silva et al., 2021), whereas the three-quadrant diagram is valid only for a single crop. The EUNEP framework defines several key indicators, including EUNEP-NUE and Ns. The former represents the ratio of N output in harvested products to N input from sources like fertilizer and atmospheric deposition, while the latter indicates the difference between N input and output. Therefore, both elaborate the entire N budget of a plot, not solely "plant available" N. However, the EUNEP guidelines have not been previously used to evaluate N management in double cropping systems comprising intercrops.

This study executed a four-year field investigation of permanent plots to compare different double cropping systems, which combined wheat and maize, peanut, or soybean and intercropped or excluded legumes, under different annual N application levels in the NCP. The study had three objectives: (1) unveil the impacts of simultaneous intercropping maize and legumes on NUE in comparison to sole crops, especially the relative contribution of N acquisition and conversion efficiency using the three-quadrant diagram; (2) determine how intercropping maize and legumes influences the NUE of the subsequent wheat crop; and (3) benchmark N performance of different annual cropping systems, with or without intercropping, according to the EUNEP guideline. The integration of aforementioned indicators is required for a comprehensive assessment of NUE at crop and cropping system levels. We hypothesized that incorporating maize/legume intercrops into the traditional wheat-maize rotation would improve the environmental sustainability due to reduced N inputs, improved NUE, and decreased Ns. This study presents a comprehensive NUE assessment of a more diversified cropping system, differing from previous reports on relay strip intercropping and traditional wheat-maize rotations. The insights derived from this analysis will shed light on cleaner and sustainable food production with lower environmental impacts and more diversified products and have a worldwide relevance for regions or countries with cereal-based double cropping systems.

2. Materials and methods

2.1. Study location

The four-year field experiment was executed at Jiyang Experimental Station (36°58′N116°58′E) of Shandong Academy of Agricultural Sciences, Jinan, China, from mid-June 2017 to mid-June 2021. The area experiences a mild-temperate monsoon continental climate characterized by dry, cold springs and winters and hot, rainy summers, with an annual average temperature of 12.0–13.6 °C. The region accumulates 4000–4500 growing days above a growing base temperature of 10 and 0 °C, respectively. The frost-free period spans 195–210 days, with annual sunshine totaling 2400–2700 h. Annual precipitation typically ranges from 500–700 mm, while potential evapotranspiration reaches

1800–2100 mm. Detailed weather data for the four-year experiment duration was sourced from the Shandong Meteorological Bureau (Table S1). The experimental soil was classified as Aquic Inceptisol (a calcareous yellow fluvo-aquic soil; Soil Survey Staff, 2014), with a sandy loam texture, a bulk density of 1.49 g/cm^3 and a pH level of 7.6 (1:2.5 *w/v* in water). Prior to the experiment in the early summer of 2017, the soil contained 13.3 g/kg organic matter content, 0.95 g/kg total N, 82.6 mg/kg alkaline hydrolysable N, 15.4 mg/kg Olsen-phosphorus (P), and 107.1 mg/kg NH_4OAC-exchangeable potassium (K) in the top 30 cm layer.

2.2. *Experimental design and crop management*

The dominant maize variety “Xianyu no. 335″, peanut variety “Huayu no. 25″, soybean variety “Qihuang no. 34″, and wheat variety “Jimai no. 22″ were employed in the study. N fertilizer was urea, with an N content of 46.4%, P fertilizer was calcium superphosphate, and K fertilizer was potassium sulfate.

The four-year investigation was conducted at a single site with permanent plots (Fig. 1) following a split-plot design with four N fertilizer levels as the primary factors and six different double cropping systems as sub-factors, which resulted in a total of 4 × 6 = 24 treatments. Each treatment was triplicated, totaling of 72 plots. The six double cropping systems shared a common structure: a summer crop was initially cultivated from mid-June to mid-October, followed by the sowing of winter wheat from mid-October to mid-June. The summer crops included conventional sole maize, labeled M30; a density-increased sole maize, labeled M20; sole peanut, labeled P; sole soybean, labeled S; maize/peanut intercropping, labeled MP; and maize/soybean intercropping, labeled MS.

Conventional sole maize M30 was planted at a 50 cm row spacing with an inter-plant distance of 30 cm in the row, resulting in a density of 6.7 plants/m^2 (Fig. 1), following local farmers’ practice. The density-increased sole maize M20 had the same row distance as M30 but with

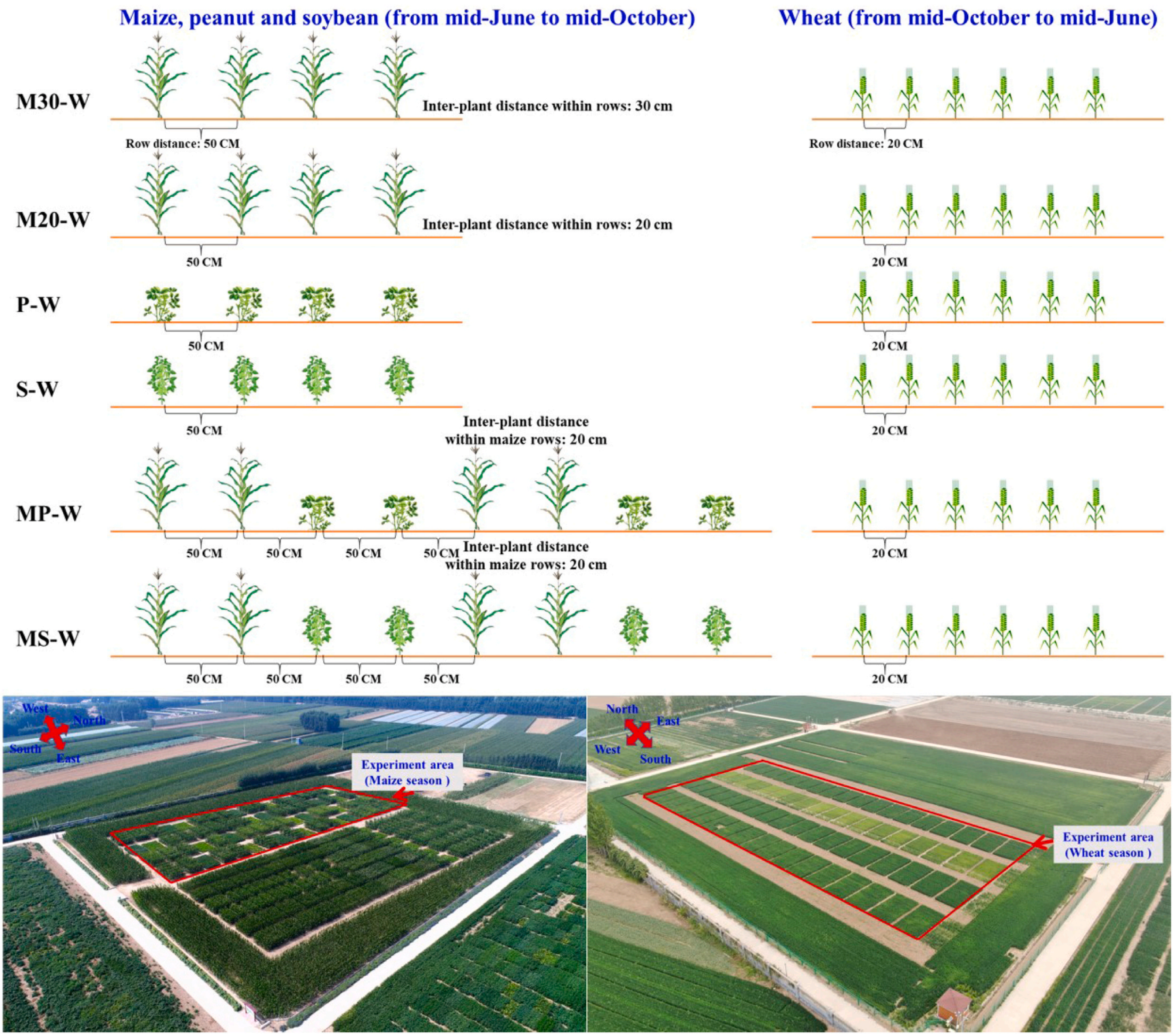

Fig. 1. Schematic diagram of six different double cropping systems with wheat (W) as a winter crop and maize (M), peanut (P), soybean (S), maize/peanut intercropping (MP), or maize/soybean intercropping (MS) during summer, and aerial photograph of the experiment area during the maize and legume growing season in summer 2019 and during the wheat growing season in the spring of 2021. Note that the photos made during the spring and summer seasons were made from a different direction (see direction markers).

an inter-plant distance of 20 cm, resulting in a density of 10 plants/m^2. Sole peanut and soybean were sown in holes at a 50 cm distance and a 20 cm inter-hole distance in the row, resulting in 10 holes/m^2 and 20 plants/m^2 as each hole had two seedlings. In strip intercropping, two rows of maize were interspaced with two rows of peanut or soybean (Fig. 1). Row and inter-plant distances of maize and peanut/soybean in intercropping followed that in M20 and in the sole peanut/soybean crops. The space of maize to the neighboring legume was 50 cm in intercropping (Fig. 1), with each occupying half of the intercropped area. The relative densities of intercropped maize and peanut or soybean in comparison to M20 and sole legume were all 0.5, indicative of a replacement intercropping strategy. The density of intercropped maize relative to M30 was 0.75, a value greater than that would have been employed in a replacement intercropping. Here, density was the number of plants per unit area of the whole cropping system (van der Werf et al., 2021) and the relative density was considered as the ratio of intercropping density to sole cropping density.

Each mono- or intercropped plot was 8.0 m wide and 5.0 m long (40 m^2), with rows aligned in a north-south orientation. A sole crop plot consisted of 16 rows of maize, peanut, or soybean, while an intercrop plot featured 8 rows of maize and 8 rows of peanut or soybean, arranged in alternating strips, with each strip containing two rows of the same species. The positioning of maize and legume strips in the intercrop plots remained consistent year after year. These permanent plots allow the occurrence of potential cumulative effects over the 4 years.

During the summer crop growing season, four basal N application rates were employed (0, 60, 80, 100 kg N/ha), which were uniformly broadcast to all plots before sowing (Table S2). Equal quantity of N fertilizer was top-dressed via broadcasting at the maize's pre-tasseling (V12-VT) stage for maize plots and strips within the intercrop plots, but not for legumes. Thus, the N input to peanut or soybean was half of that to maize, while the intercrop plot received an intermediate level of N fertilizer compared to sole crop plots of maize and the legume crop (Table S2).

From mid-October to mid-June, covering the winter and spring seasons, all plots were planted with the locally dominant winter wheat variety "Jimai no. 22″ at a row spacing of 20 cm and a seeding rate of 225 kg/ha (Fig. 1). A basal N application of 0, 60, 100 and 120 kg/ha (in the form of urea) was provided before sowing via broadcast fertilization. Additionally, a topdressing of the same dose was applied during wheat regreening-jointing (GS25-GS30, Zadocks) in spring (Table S2).

N fertilizer levels were categorized as N0, N1, N2, and N3, with N0 representing plots with no applied N, N1 representing plots with N levels below the recommendation, N2 representing plots with standard/adequate N levels, and N3 representing the plots with high N levels, but still lower than the practice commonly followed by farmers. Lower N inputs were administered to legumes than maize and wheat, while maize/legume intercrops received an intermediate level of N fertilizer (Table S2). The N input levels N1, N2, and N3 thus corresponded to different N quantities for different crops. Depending on the species composition of the double cropping system, featuring a winter crop (wheat: W) and a summer crop (M30, M20, P, or S) or a mixed species summer crop (MP or MS), the total annual N fertilizer input in N1, N2, and N3 plots ranged between 180–240, 280–360, and 340–440 kg/ha, respectively (Table S2).

P fertilizer (150 kg P_2O_5/ha) and K fertilizer (120 kg K_2O/ha) were uniformly applied as basal fertilizers to the upper 20 cm of soil before sowing winter and summer crops. Given that two full crops were sown each year, the annual P input was totaled 300 kg P_2O_5/ha and the annual K input amounted 240 kg K_2O/ha. Organic manure was not applied. Peanut and soybean straws were removed during the grains/pod harvest, while maize and wheat straws were kept using a harvesting machine. This harvesting method was partly due to the plot design not being conducive to mechanization and reflecting the traditional approach employed by smallholders in the region. The residue removal was also expected to relieve the continuous cropping obstacle caused by soil-borne diseases (Li et al., 2022; Tan et al., 2021).

All plots received sufficient irrigation. During each year, wheat was irrigated three times, with each 75 mm. The first broad irrigation was applied immediately after wheat sowing, the second one before-wintering (around GS 20), and the third during wheat regreening-jointing (GS25-GS30). As 60–70% of the yearly precipitation generally occurs from June to September, the summer crops received only one irrigation of 75 mm immediately after sowing. All irrigation water came from groundwater, and each irrigation event was precisely controlled by an electronic water meter.

A pre-emergence application of (S)-metolachlor was conducted to control weeds in maize, legumes, and intercropped plots, and after emergence these plots were weeded manually. Wheat weeds were controlled by normal herbicides containing tribenuron-methyl or fluroxypyr. The omethoate (2-dimethoxyphosphinoylthio-N-methylacetamide) (Dazhou Xinglong Chemical Co., Ltd., Dazhou, China) was sprayed to control aphids during wheat booting stage.

2.3. *Plant sampling and nutrient analysis*

Maize and soybean grain and peanut pod yields were assessed by harvesting two adjacent rows (5 m long × 2 rows). Wheat grain yield was assessed in a 1 m^2 area (1 m × 1 m) at the center of each plot. To measure the harvest index, aboveground biomass, and straw yield, samples were collected from a 0.6 m long × 1.0 m wide area for maize and legumes and 0.5 m long × 0.4 m wide area for wheat in each pot, manually separated into grain/pod and straw, and oven-dried to constant at 65–70 °C. Additionally, dried sub-samples were ground and digested using concentrated H_2SO_4 and H_2O_2 solution, and subjected to micro-Kjeldahl procedure to measure N concentrations. N uptake in the grain/pod or straw was measured as the product of yield and N mass concentration, and N uptake in aboveground biomass was determined as the sum of N uptake in grain/pod and straw components.

2.4. *Calculations*

2.4.1. *Land equivalent ratio for N uptake (NLER)*

The land equivalent ratio (LER) was defined as the advantage of intercropping over monoculture in land use (Rao and Willey, 1980). It is a dimensionless marker of relative grain or biomass yields in intercropping versus sole cropping and calculated as the sum of partial LERs (relative yields) per intercropped species ($\mathrm{pLER_M}$ and $\mathrm{pLER_L}$):

$$\mathrm{LER} = \mathrm{pLER_M} + \mathrm{pLER_L} = \mathrm{Y_M/M_M} + \mathrm{Y_L/I_L} \quad (1)$$

where Y represents the yield per unit of the intercrop in intercropping, M is the yield in sole cropping, and subscripts "M" and "L" denote maize and legume (peanut or soybean), respectively. An LER > 1.0 indicates that intercropping saves land, while LER ≤ 1.0 suggests either no advantage or a disadvantage of intercropping over sole cropping.

To assess N acquisition efficiency in intercropping over sole cropping, LER was extended to evaluate N acquisition advantage in intercropping (Gao et al., 2020). NLER and NpLER are metrics based on N yields in the grain or shoot biomass (grain + straw).

2.4.2. *N fertilizer equivalent ratio (NFER)*

In a manner analogous to LER, we used a relative index, NFER, to assess fertilizer use efficiency in intercropping systems in comparison to sole cropping. NFER is considered as the relative N fertilizer quantity needed to produce the equivalent component yield in intercropping versus sole cropping (Li et al., 2020; van der Werf et al., 2021; Xu et al., 2020). It is measured as the ratio of N fertilizer quantity needed for sole crops to achieve the same component yield per unit area as an intercrop:

$$\mathrm{NFER} = (\mathrm{Nfert_M} \times \mathrm{Y_M/M_M} + \mathrm{Nfert_L} \times \mathrm{Y_L/M_L})/\mathrm{Nfert_{IC}} = \mathrm{pLER_M} \times (\mathrm{Nfert_M}/\mathrm{Nfert_{IC}}) + \mathrm{pLER_L} \times (\mathrm{Nfert_L}/\mathrm{Nfert_{IC}}) \quad (2)$$

where $Nfert_{IC}$ represents the quantity of N fertilizer applied per unit area (in kg/ha) in the intercropping system, $Nfert_M$ and $Nfert_L$ are the N fertilizer input for maize and legumes in the sole cropping system, and "M", "L", and "IC" indicate maize, legume (peanut or soybean), and intercropping, respectively. $NFER > 1.0$ indicates that intercropping saves N fertilizer. NFER=LER implies that N fertilizer savings from intercropping can be attributed to the concentration of production on a smaller land area (Xu et al., 2020). When N fertilizer application in intercropping is intermediate compared to the sole crops, NFER tends to be $>$ LER. Conversely, when intercrop application rate is greater, NFER tends to be $<$ LER (Li et al., 2020). NFER of grain and biomass yields were calculated to assess the relative N fertilizer use efficiency in intercropping compared to sole cropping.

2.4.3. NUE at the crop level

Five agronomic indicators were used to evaluate the NUE at crop level (Table 1). These indicators included partial factor productivity (PFP), agronomic efficiency (AE), apparent recovery efficiency (RE), internal efficiency (IE), and N requirement for producing 100 kg grain (100 kg grain N), which could capture various features of N use (Gao et al., 2020; Stomph et al., 2020; Wu et al., 2022; Xia et al., 2019a).

Fertilizer is not the only plant-available N source in crop production. Other sources, such as organic matter mineralization, atmospheric deposition, and biological N_2 fixation by legumes, can also contribute to plant-available N. N input from these other sources was not quantified. Therefore, here the recovery efficiency of N fertilizer is "apparent" (Gao et al., 2020).

RE refers to the slope of the relationship between N applied and N uptake, denoting the overall N fertilizer acquisition efficiency, i.e., the quantity of N acquired per unit applied. This metric serves as a valuable tool for assessing NUE not only at the individual crop level but also at the cropping system level, which comprises multiple crops, such as rotations and intercropping systems. Additionally, 100 kg grain N, particularly the IE, which inversely relates to N mass concentration in plant biomass, serves as the indicator of N conversion efficiency, illustrating the connection between the acquired N and crop biomass or yield. N in roots was not quantified and thus not considered into calculations presented in this study. Finally, PFP and AE delineate the resulting relationship between N applied and crop yield (Table 1).

2.4.4. N use indicators at cropping system level

Five indicators, including N output, N input, NUE, Ns and N emission intensity (NEI), were calculated for each double cropping system per year and then averaged across four years, following the EUNEP (2015) guidelines (Table 1). N inputs from capillary rise or irrigation water were not considered due to uncertainties regarding the volume of capillary supply from groundwater, the quantity of irrigation water supplied, and the corresponding N concentrations. For wheat and maize, N content in the straw was not factored when calculating N output because the straw was left in the field. However, for peanut and soybean, the straws were removed after harvest and thus included in calculating N output (Table 1).

The EUNEP framework assumes a mass balance principle for N and a steady equilibrium between annual net soil N mineralization and overall annual N input into the soil N pool. Soil N mineralization, being an internal process, does not factor into the N balance sheet (EUNEP, 2015; Quemada et al., 2020). As reported in most field experimental situations, the soil organic matter (SOM) changed very little especially in a short period of time or even in long-term (Nascente et al., 2013; Varvel et al., 2002). Eventually, both the SOM content and N mineralization rate were not determined in our study, and our study aligns with the established practice in prior research employing the EUNEP framework, where the actual net N mineralization from SOM is not considered (Quemada et al., 2020; Silva et al., 2021). We did not quantify biological N_2 fixation by legumes in this study, and we thus did not include this in the calculation of all EUNEP indicators, which can be justified as follows: (1) the apparent recovery efficiency (RE) of fertilizer N, which is widely used in the evaluation of N-use efficiency of various crops including intercrops, does not consider biological N_2 fixation by legumes (Congreves et al., 2021; Gao et al., 2020); (2) the isotope-based ^{15}N

Table 1
Nitrogen-use efficiecy (NUE) indicators at crop and cropping system levels.

NUE indicator		Calculation	What it represents	Explanation of abbreviations or terms in the calculation formula
Crop level	PFP (kg/kg): Partial factor productivity	Y/F	Yield of crop harvested per unit of fertilizer N applied	(1) Y and Y_0 are the grain yield with or without N fertilizer, respectively; (2) F is the applied N fertilizer quantity; (3) U and U_0 is the N uptake in the crop shoot biomass with and without N fertilizer, respectively; (4) The shoot biomass comprises the grains/pods and straw.
	AE (kg/kg): Agronomic efficiency	$(Y-Y_0)/F$	Increase in yield per unit of fertilizer N applied	
	RE^a (%): Recovery efficiency	$(U-U_0)/F$	Increase in N in crop shoot biomass per unit applied	
	IE (kg/kg): Internal efficiency	Shoot biomass/shoot N uptake	Shoot biomass accumulation per unit of N uptake	
	100 kg grain N (kg)	N uptake in shoot biomass/ grain yield$\times$ 100	N requirement for producing 100 kg grain	
Cropping system level	N output (kg N/ha/y)	N uptake in cereal grains+ N uptake in legume biomass (grain/pod+straw)	The total quantity of N that leaves the field as harvested products	(1) Total N_{APPL} indicates the cumulative amount of N applied with mineral fertilizers (no organic fertilizer was applied in the current study); (2) N_{SEED} is N content in planting material and is determined using the product of the quantity of seed sown and the average N concentration in harvested grains; (3) N_{DEPO} refers to the atmospheric N deposition, which is 85 kg N/ha each year in the study region (Bellarby et al., 2018; Wang et al., 2020).
	N input (kg N/ha/y)	Total $N_{APPL}+N_{SEED}+N_{DEPO}$	The total of all N inputs, including fertilizer, atmospheric deposition, and N input in seeds	
	NUE (kg N/kg N): N-use efficiency	N output/N input	A measure of how efficiently N is utilized, quantifying the N output generated per unit N input	
	Ns (kg N/ha/y): N surplus	N input-N output	The potential N loss to the environment, representing the gap between N input and output	
	NEI (kg N/kg N): N emission intensity	Ns/N output	To gauge the environmental impact stemming from Ns per unit N output (van Groenigen et al., 2010)	

[a] The metric can serve as a valuable tool for assessing NUE not only at the individual crop level but also at the cropping system levels, which comprises multiple crops, such as rotations and intercropping systems.

technique for calculation of N_2 fixation by legumes is expensive and complicated to undertake (Bedoussac and Justes, 2010); (3) this is the first study to apply the EUNEP-NUE framework to estimate the N budget of double cropping systems comprising maize/legume intercrops; and (4) in practice, the calculation of the system-level NUE can vary considerably among studies, since some N inputs can be assumed to be small, and some are not measured (Scientific Panel on Responsible Plant Nutrition, 2023). Therefore, the EUNEP indicators in this study are also “apparent”. Indicators mentioned in Section 2.4.3 are referred to as conventional NUE indicators to distinguish them from the EUNEP indicators. We discuss in Section 4.2 the feasibility/plausibility and limitations of the apparent EUNEP method by comparison with the conventional RE.

The EUNEP (2015) proposed a target range for NUE (0.5–0.9 kg/kg) and a threshold for Ns (80 kg/ha) based on European averages (Oenema et al., 2009). NUE > 0.90 kg/kg indicates potential long-term N mining, while NUE < 0.50 kg/kg signifies inefficient N use. Ns exceeding 80 kg/ha is often associated with substantial N losses to the environment, including nitrate leaching and greenhouse gas emissions. NEI lacked a specific target value and was primarily used for comparative purposes across various double-cropping systems.

2.5. Statistical analysis

Statistical analyses were executed using SAS 9.4 (SAS Institute, Cary, NC, USA). A two-factor split-plot analysis of variance (ANOVA) was performed to explore the impact of N level and cropping system. A one-way ANOVA was applied to assess the interaction effect of N level and cropping system. A three-way ANOVA was used to measure the influences of N level, cropping system, and year on the pooled index/parameter from the four-year experiment. After the ANOVA tests, means were compared using the Fisher’s protected least significant difference (LSD) analysis with $p \leq 0.05$ as the significance level. Response curves of N uptake to N application rate in various double cropping systems were plotted with fitted linear-plateau models following the NLIN procedure (Yan et al., 2014).

3. Results

3.1. NUE in summer maize

N application rate had a pronounced and statistically significant impact on various NUE indicators for summer maize (Table 2). Specifically, it exhibited a significant positive influence on maize N uptake and the production of 100 kg grain N while simultaneously exerting a significant negative effect on plant internal N-use efficiency, and the resultant AE and PFP of applied N. However, N application rate did not show any significant impact on RE of applied N or N harvest index of summer maize. It’s noteworthy that these trends held true across all years and cropping systems. On average, when considering data from all years and cropping systems, key parameters such as maize N uptake, 100 kg grain N, IE, and AE all plateaued at N2. In contrast, the four-year

Table 2
Effects of nitrogen (N) fertilization level and cropping system (C) on the N-use efficiency of summer maize, averaged across four years (Y).

Category	Parameter	N level	Maize (M)					ANOVA			
			M30	M20	IMP	IMS	Mean	Variable	*P*	Variable	*P*
Acquisition efficiency	N uptake (kg/ha)	N0	114.7ab	125.1a	92.7ab	79.3b	102.9C	Y	< 0.0001	Y × N	0.0366
		N1	133.0ab	148.7a	112.5bc	110.3c	126.1B	N	< 0.0001	Y × C	0.3655
		N2	150.3ab	153.7a	120.4b	121.0ab	136.3A	C	< 0.0001	N × C	0.9845
		N3	147.6a	154.9a	118.4b	119.1b	135.0AB			Y × N × C	0.6902
		Mean	136.4A	145.6A	111.0B	107.4B	125.1				
	RE (%)	N0	-	-	-	-	-	Y	< 0.0001	Y × N	0.7726
		N1	15.3	19.6	33.1	51.7	29.9	N	0.4525	Y × C	0.1094
		N2	22.3	17.9	34.7	52.1	31.7	C	< 0.0001	N × C	0.9923
		N3	16.5	14.9	25.7	39.8	24.2			Y × N × C	0.9618
		Mean	18.0B	17.5B	31.2B	47.8A	28.6				
Conversion efficiency	IE (kg/kg)	N0	172.7	175.9	162.4	172.5	170.9A	Y	< 0.0001	Y × N	< 0.0001
		N1	158.0	158.7	156.4	149.8	155.7B	N	< 0.0001	Y × C	0.0332
		N2	146.1	155.2	141.3	149.9	148.1C	C	0.1155	N × C	0.7929
		N3	149.4a	147.2a	143.1ab	137.6b	144.3C			Y × N × C	0.2477
		Mean	156.6	159.2	150.8	152.4	154.8				
	NHI (%)	N0	0.60	0.61	0.63	0.63	0.62	Y	< 0.0001	Y × N	0.1710
		N1	0.66a	0.623ab	0.64ab	0.615b	0.63	N	0.0576	Y × C	0.5124
		N2	0.63	0.61	0.58	0.61	0.61	C	0.7270	N × C	0.4475
		N3	0.60	0.60	0.61	0.58	0.60			Y × N × C	0.7970
		Mean	0.62	0.61	0.62	0.61	0.61				
	100 kg grain N (kg)	N0	1.28	1.20	1.29	1.20	1.24B	Y	< 0.0001	Y × N	0.0013
		N1	1.30	1.31	1.28	1.35	1.31B	N	< 0.0001	Y × C	0.3264
		N2	1.38	1.39	1.49	1.44	1.42A	C	0.6141	N × C	0.5972
		N3	1.37b	1.41ab	1.40ab	1.46a	1.41A			Y × N × C	0.4343
		Mean	1.33	1.33	1.36	1.36	1.35				
Resultant efficiency	AE (kg/kg)	N0	-	-	-	-	-	Y	0.1333	Y × N	0.9074
		N1	13.0ab	6.3b	25.9a	27.5a	18.2A	N	0.0369	Y × C	0.3683
		N2	11.9ab	3.9b	10.2ab	24.4a	12.6AB	C	< 0.0001	N × C	0.5845
		N3	10.0ab	1.8b	13.0ab	15.7a	10.1B			Y × N × C	0.7613
		Mean	11.6B	4.0C	16.4AB	22.5A	13.6				
	PFP (kg/kg)	N0	-	-	-	-	-	Y	< 0.0001	Y × N	0.3405
		N1	85.8b	93.8b	144.7a	136.0a	115.1A	N	< 0.0001	Y × C	0.1997
		N2	66.5b	69.6b	99.3a	105.7a	85.3B	C	< 0.0001	N × C	0.0118
		N3	53.7b	54.3b	84.2a	80.8a	68.2C			Y × N × C	0.5981
		Mean	68.7B	72.5B	109.4A	107.5A	89.5				

Values followed by the same lowercase letters among different cropping systems (horizontal comparison) and values followed by the same capital letters among different N levels (vertical comparison) or among different cropping systems are not significantly different at the 5% level according to Fisher’s protected LSD. ANOVAs give the probabilities (*P* values) of the source of variation. IMP and IMS indicate maize in the intercrops with peanut (P) and soybean (S), respectively. The inter-plant distance within the row of maize is 30 cm in M30, and 20 cm in M20 and intercropping. AE is agronomic efficiency, IE is internal use efficiency, PFP is partial factor productivity, RE is apparent recovery efficiency of applied N, NHI is harvest index for N, and 100 kg grain N is N-requirement for producing 100 kg grain.

average PFP decreased significantly from N1 to N2, and further from N2 to N3 (Table 2).

Cropping system substantially affected the acquisition efficiency and resultant efficiency of applied N, but not the conversion efficiency of maize (Table 2). On average, considering all years and N levels, intercropping led to an increase in maize RE, which ranged from 17.5–18.0% in sole maize (M30 and M20) to 31.2–47.8% in maize/peanut and maize/soybean plots. Additionally, intercropping increased AE from 4.0–11.6 to 16.4–22.5 kg/kg and PFP from 68.7–72.5 to 107.5–109.4 kg/kg, but decreased N uptake from 136.4–145.6 to 107.4–111.0 kg/ha. Maize intercropped with soybean exhibited a dramatic higher average RE compared to maize intercropped with peanut, but there were no obvious differences in other NUE indicators between maize intercropped with peanut or soybean. Increasing plant density significantly decreased AE of sole maize from 11.6 kg/kg in M30 to 4.0 kg/kg in M20. However, planting density displayed no significant effect on other parameters of sole maize.

Intercropped maize had a greater yield and N acquisition than sole maize, when yield and N acquisition were expressed per plant or per unit area planted in the intercrop (Fig. 2 and S1). With greater N fertilizer input, maize biomass N reached a plateau at N2 (Fig. 2, quadrant I) while grain yield plateaued already at N1 (Fig. 2, quadrant III). The association of N acquisition with maize yield was linear with a generally common slope in all systems, indicating the conversion efficiency of N into maize yield hardly changed across cropping systems (Fig. 2 and S1, quadrant II).

3.2. NUE of legumes

N application rate did not have significant impact on the acquisition efficiency (uptake and RE) and AE of applied N in peanut and soybean when averaged across years and cropping systems. However, it significantly affected IE and PFP (Table S3). Compared with N0, N application significantly decreased the four-year average IE of legumes, except for N1 in soybean. Notably, no significant variation was identified among N1, N2, and N3 for peanut and between N2 and N3 for soybean. As N input increased, PFP decreased gradually from an average of 29.5 kg/kg at N1 to 16.3 kg/kg at N3 for peanut, and from 69.4 to 34.8 kg/kg for soybean. N application rate didn't significantly affect the N harvest index of peanut and the 100 kg grain N of soybean. The N-requirement for producing 100 kg pod of peanut was significantly increased from an average of 2.86 kg at N0 to 3.45–3.77 kg at N1, N2, and N3. Compared to N0, the N harvest index of soybean decreased at N1 and increased at N2 and N3 (Table S3).

When considering data average across multiple years and N application rates, intercropping significantly lowered N uptake, harvest index, and PFP of both peanut and soybean while it significantly increased the N-requirement for 100 kg pod of peanut. Intercropping had no significant effect on other NUE-related parameters of peanut and soybean (Table S3).

Intercropped legumes had much lower N uptake (Fig. 2 and S1, quadrant I) and yield (Fig. 2 and S1, quadrant III) than sole legumes per unit area. This was consistent at all N levels. Soybean showed a positive link between N uptake and yield (Fig. 2 and S1, quadrant II), however,

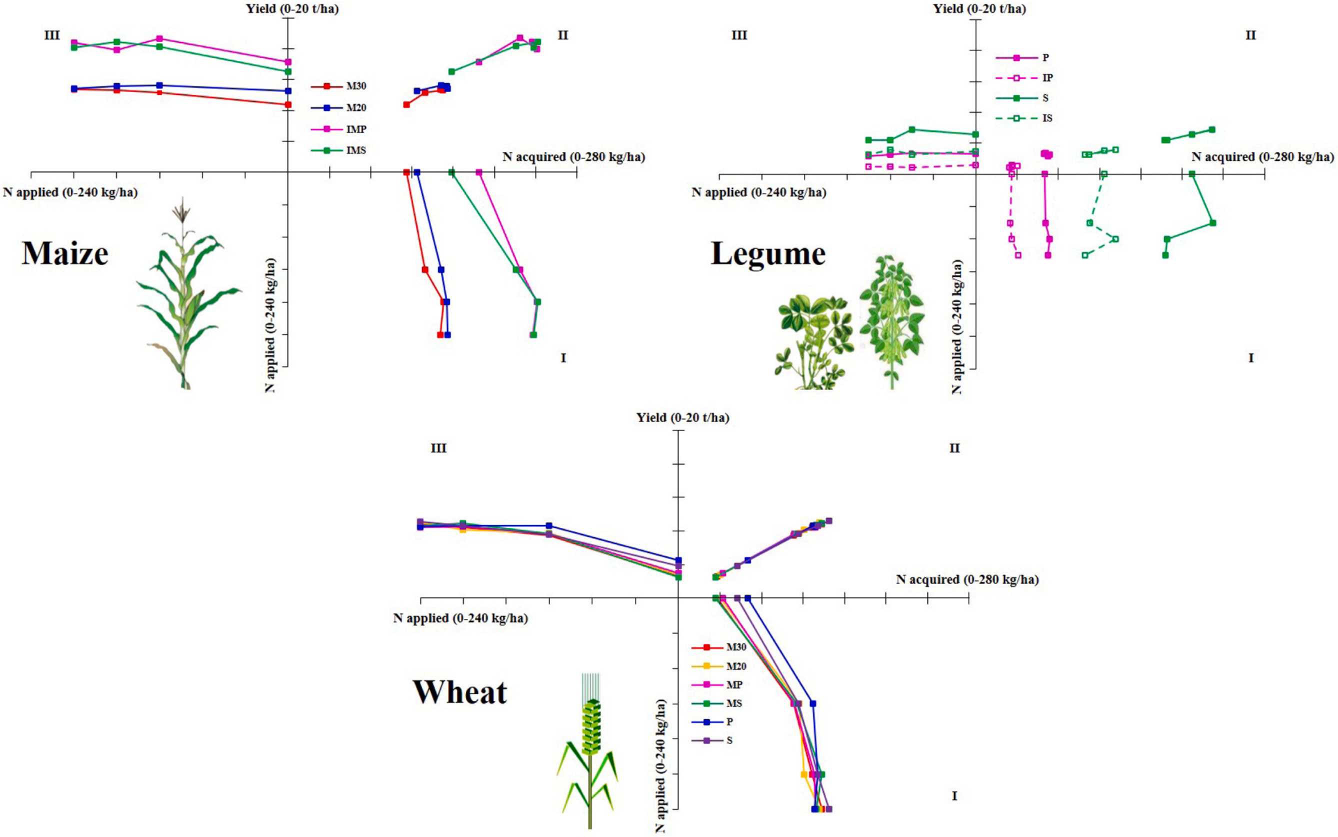

Fig. 2. Three-quadrant diagrams summarizing the uptake, conversion and overall nitrogen (N) use efficiency of maize, legumes and wheat in six double cropping systems. Data represent averages over four experimental years. In each panel, quadrant I shows the response of N uptake in the above-ground biomass to applied N while quadrant II shows the conversion of acquired N into grain yield (maize and soybean) or pod yield (peanut). Quadrant III shows the overall relationship between N applied and grain or pod yield. In intercrops, the grain/pod yield and N uptake of total biomass (without roots) are given per unit area occupied by the species, excluding the area of the companion crop. Meaning of abbreviations: maize (M), peanut (P), soybean (S), maize/peanut intercropping (MP) and maize/soybean intercropping (MS), intercropped maize with peanut (IMP), intercropped maize with soybean (IMS), intercropped peanut (IP) and intercropped soybean (IS). The inter-plant distance within the row is 30 cm in maize treatment M30, and 20 cm in M20 and in intercropped maize.

for peanut such a relationship was not evident due to an overall lack of response of yield and N uptake to N applied in sole and intercropped peanut. Soybean showed an erratic response to N applied (Fig. 2 and S1, quadrants I and III).

3.3. NLER and NFER of intercropping

N fertilizer application had no significant effect on partial NLER for grain and biomass of legumes and maize in intercropping systems. Similarly, it did not significantly affect the overall NLER and NFER of intercropping (Fig. 3). The averaging NLER of 1.02 over years and N levels in maize/peanut intercropping was close to one and only marginally increased to 1.07 by intercropping maize and soybean, indicating that the overall efficiency of N acquisition was not or only marginally improved by intercropping. However, the NFER was significantly greater than one for both intercrops at N1, N2 and N3 (on average over N levels 1.17 for maize/peanut and 1.18 for maize/soybean), indicating that intercrops use applied fertilizer N more efficiently to generate yield than sole crops do. The partial NLERs for grain and biomass of intercropped soybean averaged 0.32–0.33, which were dramatically higher than those of intercropped peanut (0.23–0.27), but lower than the ratio of area occupied by intercropped species of 0.5, indicating a substantial N acquisition disadvantage for intercropped legumes compared to sole legumes. The partial NLERs for grain and biomass of intercropped maize with peanut and with soybean averaged 0.74–0.78, which were much higher than 0.5 (i.e., the ratio of area occupied by maize in the whole intercropping system), indicating a substantial N yield advantage of intercropping for maize.

3.4. N uptake and RE of different cropping systems during the summer season

Considering M30, M20, MP, MS, P, and S, during the summer season, the N uptake averaged 68.7–202.0 kg/ha and RE averaged −7.4–28.7% (Fig. 4). The averaged N uptake over years and cropping systems was significantly increased from 127.9 kg/ha at N0 to a plateau (ranging 144.4–147.9 kg/ha) starting at N1. However, no significant changes in RE were found among various N application rates. Cropping systems significantly affected N uptake and RE across years and N application rates. On average, MS had the highest RE and comparatively higher N uptake than M30, M20, P and MP. P exhibited the lowest N uptake, and S had the largest N uptake but lowest RE. N uptake of MP was significantly lower than M20 (Fig. 4).

3.5. NUE of winter wheat

When considering data spanning all years and cropping systems, N application had a significant effect on all NUE indicators of winter wheat (Table S4; Fig. 4). Increased N fertilizer enhanced N uptake and 100 kg grain N while lowering AE, IE, and RE of applied N, and N harvest index of winter wheat, and the plateaus occurred at N2. On average over all years and cropping systems, PFP of wheat decreased significantly from N1 to N2, and further from N2 to N3.

The previous-season sole legume treatment increased N uptake by wheat, especially at zero and/or low N supply environments, but lowered RE and AE at N1, N2 and N3 compared to most treatments involving sole maize and maize intercropping (Table S4; Fig. 4). Interestingly, cropping systems did not significantly affect wheat N harvest index, 100 kg grain N, and PFP across years and N application rates. When averaged across multiple years and N application rates, although there were indeed significant variations in IE among different cropping

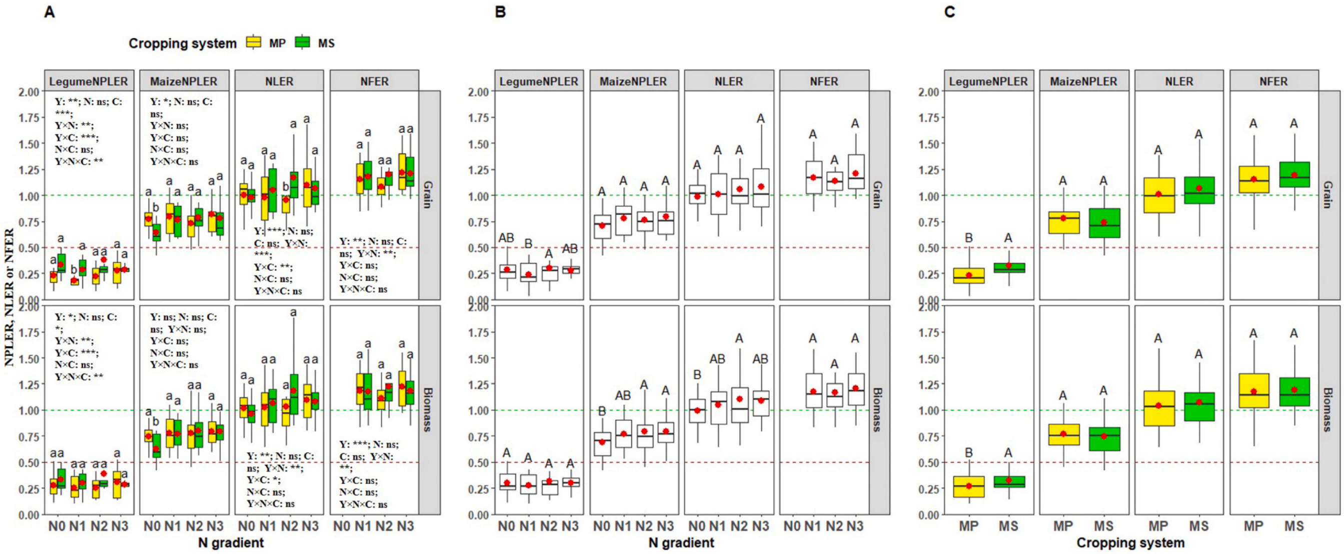

Fig. 3. Partial and total land equivalent ratios for grain and biomass nitrogen (N) uptake (NPLERs, NLERs) of legume and maize crops in intercropping and N fertilizer equivalent ratios (NFERs) of grain and biomass yields in intercropping as affected by different N application rates and cropping systems across four experimental years (Y). Panels A, B, and C represent boxplots of averages over years, averages over years and cropping systems, and averages over years and N application rates, respectively. MP indicates maize/peanut intercropping and MS indicates maize/soybean intercropping. Four nitrogen application levels were compared: no N supply (N0), below the recommended or standard rate (N1), standard or adequate rate (N2), and a high rate (N3). NFER indicates the relative amount of N fertilizer that would be required if sole crops were used to produce the same yields as a unit area of intercrop. Boxplot elements are defined as follows: the center line represents the median, box limits represent the upper and lower quartiles, whiskers represent 1.5 times the interquartile range, and the red point represents the mean. The same lowercase letters above boxes in panel A indicate no significant difference among different cropping systems with each N application level; the same capital letters above boxes in panel B indicate no significant difference among different N levels; same capital letter above boxes in panel C indicate no significant difference between cropping systems. All significance tests were carried out using Fisher's protected LSD at 5% level. ANOVA results indicating the probabilities (P values) of different sources of variation are shown in A. * , * *, * ** and ns indicate $P < 0.05$, 0.01, 0.001 and no significance, respectively.

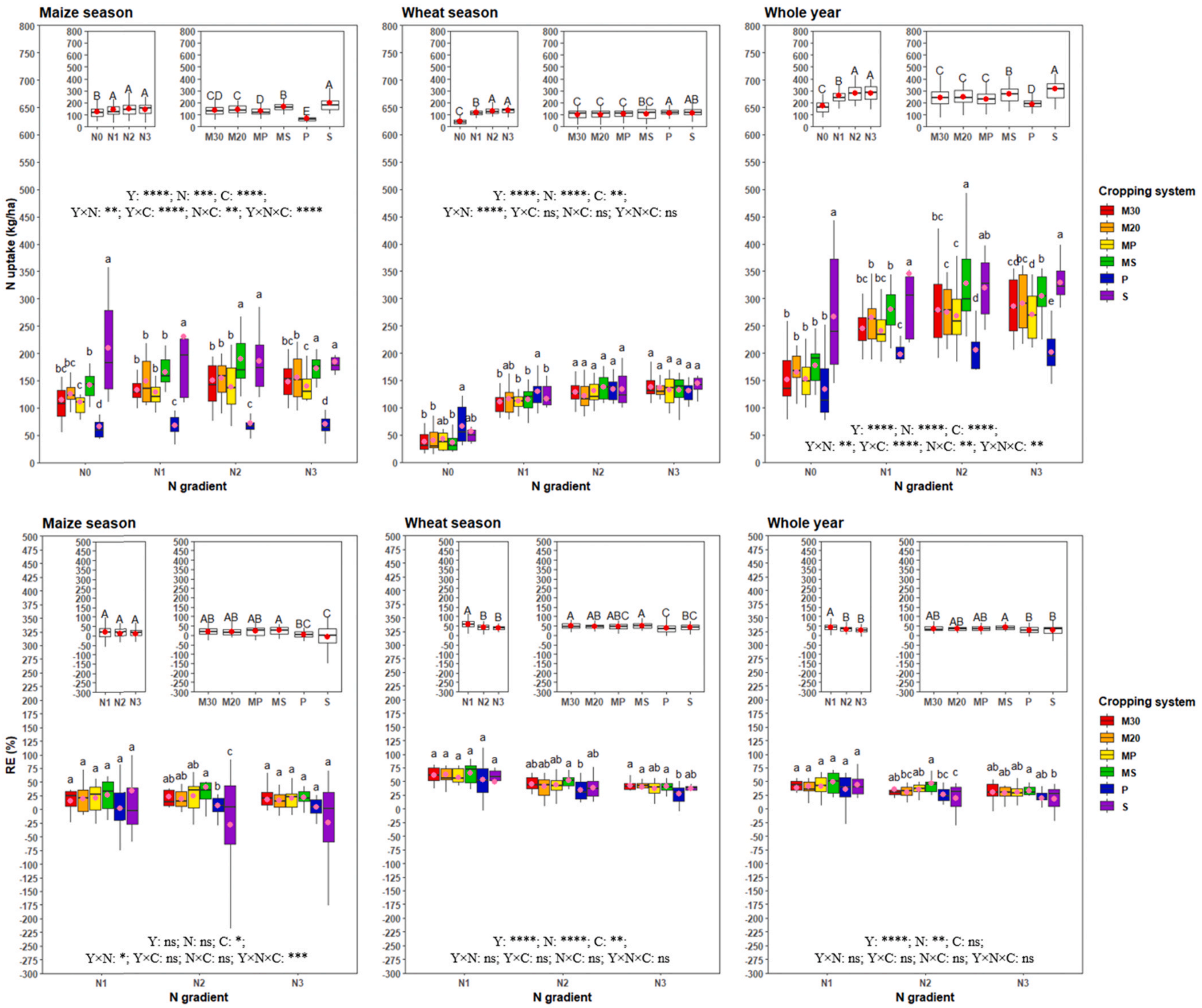

Fig. 4. Total nitrogen (N) uptake and apparent recovery efficiency (RE) as affected by N application rates and cropping systems (C) across four experimental years (Y). Main panels represent plot averages over years, while insets represent averages across years and cropping systems (left) and across years and N application rates (right). M, P, S, MP and MS indicate maize, peanut, soybean, maize/peanut intercropping and maize/soybean intercropping, respectively. The inter-plant distance within the row of maize is 30 cm in M30, and 20 cm in M20 and intercropping. Four nitrogen application levels were compared: no N supply (N0), below the recommended or standard rate (N1), standard or adequate rate (N2), and a high rate (N3). Boxplot elements are defined as follows: the center line represents the median, box limits represent the upper and lower quartiles, whiskers represent 1.5 times the interquartile range, and the pink point represents the mean. The same lowercase letters above boxes indicate no significant difference among different cropping systems with each N application level; same capital letters above boxes indicate no significant difference between different N levels or cropping systems. All significance tests were carried out using Fisher's protected LSD at 5% level. ANOVA results indicating the probabilities (P values) of different sources of variation are shown in each main panel. * , * *, * ** , * ** * and ns indicate $P < 0.05$, 0.01, 0.001, 0.0001 and no significance, respectively.

systems, the variation was very small, ranging between 118.5–122.0 kg/kg. As shown in Fig. 2 and S1, all observations in quadrant II fall on the same line, indicating a minimal variation in the efficiency of converting acquired N to wheat grain yield. Consequently, any disparities in yield can be primarily attributed to differences in N acquisition, not N conversion.

3.6. *Annual N uptake and RE in double cropping systems*

As the supply of fertilizer N increased, the annual total N uptake showed an upward trend, rising from 174.4 kg/ha at N0 to 280.2 kg/ha at N3. However, this increase in N supply led to a decrease in RE of applied N, which dropped from 41.6% at N1 to 26.7% at N3. These trends were observed when averaging data over multiple years and across various double cropping systems (Fig. 4). Notably, non-significant differences were identified in N uptake and RE between N2 and N3. The response of total N uptake in annual cropping systems to augmented N fertilizer application rates followed a "linear-plateau" model (Fig. S2). The plateau for N uptake occurred before N1 for P-W and S-W and between N1 and N2 for the other cropping systems (Fig. S2). The four-year average annual total N uptake in the six rotation systems, averaged over N levels, varied from 184.2 to 314.8 kg/ha, with a ranking of S-W > MS-W > M20-W > M30-W > MP-W > P-W (Fig. 4). Significant differences were observed among S-W, MS-W, and P-W and between wheat-maize double cropping (or MP-W) and any other systems. RE averaged between 27.0% and 42.7%, with the order being MS-W > MP-W > M30-W > M20-W > P-W > S-W. There were marginally significant differences between MS-W and P-W or S-W.

With the course of experimental duration, varying N application rates led to increasingly pronounced gaps in total N uptake (Table S5). From the first to the fourth year, it became evident that maintaining productivity without N fertilization proved to be less effective compared to cropping systems with N supply. In the first year, N uptake in each annual cropping system at N1 was comparable to that at N2 and N3. However, by the fourth year, significant differences emerged between N1 and N2 for M20-W and S-W and between N1 and N3 for M30-W, M20-W, and S-W (Table S5). However, during these four years no significant differences in total N uptake were observed between N1 and N3 for MP-W, MS-W, and P-W, indicating a higher resilience to low N supply than other systems. Additionally, for each annual cropping system, except M30-W in the fourth year, no significant differences were detected between N2 and N3, suggesting that moderate N supply can sustain high N productivity without a yield penalty.

3.7. N performance indicators of annual cropping systems according to EUNEP

Averaged over years and cropping systems, N application significantly decreased the NUE from 1.54 kg/kg at N0 to 0.46 kg/kg at N3, while significantly increasing the Ns from −49.5 to 264.6 kg/ha and the NEI from −0.22 to 1.34 kg/kg (Table 3). NUE averaged 0.62–1.37 kg/kg across the six double cropping systems tested, with a ranking of S-W > MS-W > P-W > MP-W > M20-W > M30-W, while the reverse order was observed for Ns (with values ranging between −1.3 and 180.7 kg/ha) and for NEI (with values ranging 0.04–0.99 kg/kg). In terms of these three N indicators, values of the double-cropping system with intercropping were intermediate between double cropping systems comprising wheat and maize (low NUE, high Ns and NEI) and wheat and a legume crop (high NUE, low Ns and NEI) (Table 3).

The average NUE of three replicates fell within the proposed EUNEP target of 0.50–0.90 kg/kg for 38 out of 96 samples (39.6%) ($n = 4$ years × 4 N levels × 6 cropping systems = 96 observations) (Fig. 5). Additionally, 35.4% of samples registered values below 0.5 kg/kg, while 25.0% exceeded 0.9 kg/kg. In particular, the N output of wheat-maize rotation (M30-W and M20-W) across all the four experimental years at an N input amount of ~530 kg/ha (i.e., 440 kg/ha fertilizer N) consistently fell below the NUE of 0.50 kg/kg, with a mean NUE of 0.38 kg/kg, indicating inefficient N use (Table 3; Fig. 5). Consistently over all four years, MS-W exhibited higher N outputs compared to MP-W, and S-W outperformed P-W (Fig. 5). The responses of total N output to increasing N input in the six annual cropping systems were well-fitted by the "linear-plateau" model (Fig. S2). Compared to M-W, the response curves of MS-W and S-W reached the plateau with lower N inputs and higher N outputs, while that of MP-W and P-W reached the plateau with lower N input but also lower N output. The total N input required to reach the plateau and the N output at the plateau in each diversified rotation with intercropping (MS-W or MP-W) fell between monoculture rotations of M-W and wheat with the corresponding legume (Fig. S2). However, Ns exceeded 80 kg/ha for 63 out of 96 samples (65.6%), indicating the

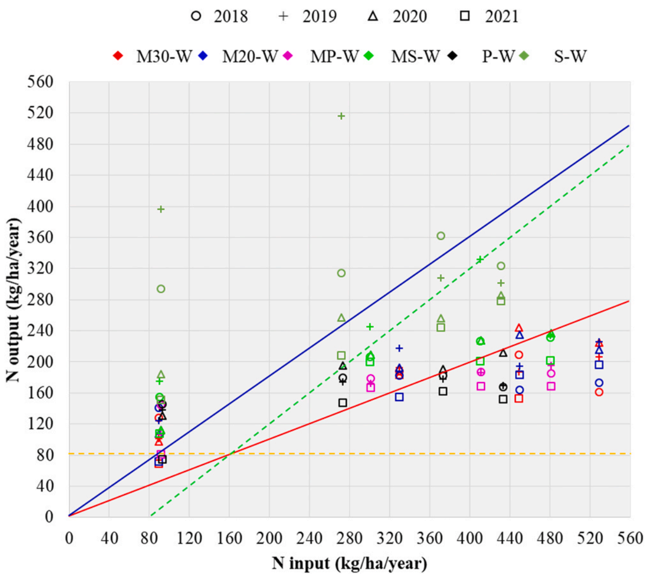

Fig. 5. Relationships between N input (x-axis) and N output (y-axis) in six cropping systems. Data points represent means of three replicates of a treatment in a year. Different symbols indicate different years and different colors indicate different cropping systems. For different double cropping systems, W, M, P, S, MP and MS indicate wheat, maize, peanut, soybean, maize/peanut intercropping and maize/soybean intercropping, respectively. The inter-plant distance within the row of maize is 30 cm in M30, and 20 cm in M20 and intercropping. Solid red and blue lines mark the target range for NUE of 0.50–0.90 kg/kg, the dashed green line marks the N surplus threshold value of 80 kg/ha/yr, and the dashed yellow line marks the desired minimum N productivity (80 kg/ha/yr).

Table 3
Effects of nitrogen (N) fertilization level and cropping system (C) on the EU N Expert Panel proposed N-use efficiency, N surplus and N emission intensity, averaged across four years (Y).

Parameter	N level	Cropping system							ANOVA			
		M30-W	M20-W	MP-W	MS-W	P-W	S-W	Mean	Variable	*P*	Variable	*P*
N-use efficiency (kg/kg)	N0	1.11c	1.25bc	1.22bc	1.57b	1.31bc	2.81a	1.54A	Y	< 0.0001	Y × N	< 0.0001
	N1	0.55b	0.57b	0.60b	0.72b	0.64b	1.19a	0.71B	N	< 0.0001	Y × C	< 0.0001
	N2	0.44c	0.43c	0.47c	0.61b	0.48c	0.79a	0.54C	C	< 0.0001	N × C	< 0.0001
	N3	0.37d	0.38cd	0.41c	0.48b	0.40c	0.69a	0.46D			Y × N × C	< 0.0001
	Mean	0.62D	0.66CD	0.68CD	0.84B	0.71C	1.37A	0.81				
N surplus (kg/ha)	N0	-9.8a	-21.9ab	-20.3ab	-51.1b	-29.0ab	-165.2c	-49.5D	Y	< 0.0001	Y × N	< 0.0001
	N1	149.3a	142.6a	119.3ab	83.1b	99.0ab	-52.9c	90.1C	N	< 0.0001	Y × C	< 0.0001
	N2	251.0a	255.1a	218.7b	160.2c	194.9b	78.7d	193.1B	C	< 0.0001	N × C	0.0438
	N3	332.2a	326.6a	284.9b	252.1c	257.7c	134.1d	264.6A			Y × N × C	0.0003
	Mean	180.7A	175.6A	150.7B	111.1D	130.7C	-1.3E	124.6				
N emission intensity (kg/kg)	N0	0.00035a	-0.13ab	-0.13ab	-0.34b	-0.14ab	-0.59c	-0.22D	Y	< 0.0001	Y × N	< 0.0001
	N1	0.85a	0.81ab	0.68bc	0.41d	0.60c	-0.017e	0.55C	N	< 0.0001	Y × C	< 0.0001
	N2	1.36a	1.37a	1.19a	0.71b	1.13a	0.31c	1.01B	C	< 0.0001	N × C	< 0.0001
	N3	1.73a	1.65ab	1.50c	1.13d	1.54bc	0.46e	1.34A			Y × N × C	0.0005
	Mean	0.99A	0.93A	0.81B	0.48C	0.78B	0.04D	0.67				

Values followed by the same lowercase letters among different cropping systems (horizontal comparison) and values followed by the same capital letters among different N levels (vertical comparison) or among different cropping systems are not significantly different at the 5% level according to Fisher's protected LSD. ANOVAs give the probabilities (*P* values) of the source of variation. For different double cropping systems, W, M, P, S, MP and MS indicate wheat, maize, peanut, soybean, maize/peanut intercropping and maize/soybean intercropping, respectively. The inter-plant distance within the row of maize is 30 cm in M30, and 20 cm in M20 and intercropping.

potential for high N losses to the environment (Fig. 5). Ns values lower than 80 kg/ha were mainly observed for cropping systems with a total N input of approximately 90 kg/ha (i.e., no fertilizer N) and for MS-W and S-W with low to moderate N input.

4. Discussion

4.1. Optimal N management maintained high N productivity without greatly reducing NUE

Increasing N supply inevitably decreased metrics related to NUE (i.e., RE, IE, AE, and PFP) of maize, peanut, soybean, and wheat (Table 2, S3-S4; Fig. 4), and at cropping systems level (Table 3; Fig. 5). These findings align with the conclusions of a previous study (Liu et al., 2022). In this study, regardless of cropping systems and years, there was an observable plateau in the average N uptake of maize, wheat, and double cropping systems that commenced at N2 supply level. Further increases in fertilizer N did not yield significant additional increases in N uptake (Table 2; Fig. 4). These results collectively suggest that totaling 160 kg N/ha for sole maize, 120 kg N/ha for intercropped maize, 200 kg N/ha for wheat, and yearly 280–360 kg N/ha for all double-cropping systems is enough to keep high productivity without incurring a yield penalty while greatly improving NUE. Similar N amounts were reported for maize (Liu et al., 2022; Xu et al., 2022), wheat (Yin et al., 2021), and the wheat-maize system (Yin et al., 2021; Zhao et al., 2015).

4.2. Introducing intercropping to the wheat-maize rotation led to reduced N fertilizer requirement and increased NUE

In our study, the conventional NUE of legumes did not respond to N applied (for AE and RE) nor to intercropping (for AE, IE, and RE). Accordingly, the results suggest that fertilizer N inputs to legumes, which can acquire N through biological N_2 fixation (Masson-Boivin and Sachs, 2018), can be limited to a starter fertilization attuned to the soil N content, and only such a tailored starter N would avoid yield penalties and reduce environmental impacts (Huang et al., 2017).

Intercropping with maize resulted in a notable decrease in the N harvest index of peanut, dropping from a mean of 0.67 in monoculture to 0.55 in intercropping systems (Table S3), confirming a previous study (Xia et al., 2019b). As a result, intercropping increased the N requirement for 100 kg pod of peanut. The detrimental effect of shading by maize on peanut performance was also described by Xia et al. (2019b) and Gao et al. (2020) and may be related to the low vigor of the shaded peanut plant, resulting in difficulties for young pods, known as pegs, to pierce the soil. On the other hand, the N harvest index of soybean was less influenced by intercropping, possibly because the pods are located above the ground. Due to its comparatively better performance under shade, soybean is a more suitable companion legume for intercropping with maize than peanut.

Due to the shading effect of maize on intercropped legumes, PFP and N uptake of peanut and soybean in intercropping were all greatly reduced (Table S3), and the NPLER of peanut and soybean were much lower than 0.5 (Fig. 3), indicating a substantial disadvantage in N productivity. This is closely associated with the growth suppression on the short-stemmed legume caused by the tall maize plants due to shading (Xue et al., 2016). In contrast, maize intercropped with peanut or soybean had much greater RE, AE and PFP of N than sole maize, and the NPLER for maize grain and biomass yield was much higher than 0.5 (ranging 0.74–0.78, on average) (Table 2; Fig. 3), underscoring how integrating legumes into intercropping systems significantly enhances N acquisition and resultant efficiency for the intercropped maize relative to sole maize.

The present study showed that intercropping had a relatively modest impact on IE and 100 kg grain N of maize (Table 2). However, our prior investigation, spanning ten years and encompassing six experimental sites, demonstrated that intercropping significantly reduced the internal P-use efficiency of maize by 4.9–16.0% while increasing the amount of P required to produce 100 kg grain by 7.0–17.4% (Xia et al., 2019a). The underlying mechanisms responsible for these contrasting responses in the conversion efficiency of applied N and P in maize to intercropping warrant further research.

The NLER for grain and biomass in the intercropping system averaged 1.02 for maize with peanut and 1.07 for maize with soybean, indicating a slight advantage in total N uptake. On the other hand, an average of 15–20% less N input could be used in intercropping to achieve an equivalent yield to sole crops, as indicated by the NFER for grain and biomass (Fig. 3). These findings align with a meta-analysis (Li et al., 2020), which found that intercropped systems with maize had an average NFER of 1.33, while those without maize had an average NFER of 1.19. This suggests that sole crops require 19–33% more N fertilizer compared to intercropped systems for the same level of production. Additionally, the RE values in intercropping averaged 21.1–28.7%, surpassing those of sole maize (17.5–18.0%) and legumes (−7.4–3.5%) (Fig. 4).

Our study thus demonstrates that the advantage in N use observed in simultaneous intercropping primarily stems from the performance of intercropped maize. Maize, as the primary crop species in cereal/legume intercropping, possesses superior light capture capabilities, contributing to increased yields with the same N input as sole maize (Liu et al., 2018). Furthermore, the belowground roots of maize plants can access more N in the vicinity of itself and neighboring legume rows, leading to enhanced overall N uptake and apparent fertilizer N recovery (Xia et al., 2013).

Gao et al. (2020) showed a RE of only 7.2% for the entire intercropping system, which was considerably less than 12.4% for sole peanut and 27.2% for sole maize, indicating a low NUE in intercropping. Our findings, however, differ from those of Gao et al. (2020), possibly due to differences in the N application methods between the two studies. In our research, N was applied to each species strip based on the specific N demand of each species, whereas in Gao et al. (2020)'s study, N was uniformly applied based on the demand per unit area of maize. Consequently, in their study, the N applied to the peanut exceeded its actual requirements, resulting in a significant portion of the fertilizer N within the peanut area (especially the inner rows) likely remaining unabsorbed and, consequently, lost. In contrast, in our intercropped area, only the maize plants received topdressing N, while the legumes did not. This fertilization approach allowed us to save 25% of the N fertilizer applied compared to sole maize cultivation. Considering the specific N requirements of each crop and placing fertilizer where it is needed can optimize supply-demand matching, aligning with proposals by Snyder et al. (2014) and Gao et al. (2020). Given that most AE and RE values for peanut and soybean were negative and extremely low in our study (Table S3), it's possible that the basal fertilizer N applied within the peanut or soybean area can be further reduced, although this warrants further investigation.

The influence of preceding sole legumes on the yield and N uptake of subsequent wheat, compared to wheat following sole maize, was positive at zero N input but not evident at adequate and high N supply (Table S4; Figs. 2 and 4). This aligns with most other studies showing that residual effects of legumes are most noticeable at low fertilizer N rates (Guinet et al., 2020; Muschietti-Piana et al., 2020). This residual effect of legumes may open the possibility of reducing N input during the wheat season after legume cultivation to enhance fertilizer NUE in practice. However, intercropping peanut or soybean with maize did not yield such a positive effect. It's possible that the simultaneous presence of maize in intercropping systems could diminish the residual effect of intercropped legumes on wheat. Maize, being a strong competitor for available soil N in intercropping systems, might effectively utilize the N resources, reaping the benefits of the N_2-fixing legume (Hauggaard-Nielsen et al., 2009b; Jensen et al., 2020; Li et al., 2011; Rodriguez et al., 2020). In line with this, Hauggaard-Nielsen et al. (2009a) demonstrated that intercropping barley and pea had no discernible impact on depleting soil mineral N in subsequent wheat compared to wheat following sole barley.

In terms of the conventional NUE, the annual P-W or S-W system consumed 22.2–25.0% less fertilizer N than the M-W system while they had lower NUE as indicated by the RE of applied N (Fig. 4). The MP-W and MS-W systems consumed 11.1−12.5% less fertilizer N than the M-W system and had average RE increased from 33.5–34.8% to 36.0–42.7%. However, regarding EUNEP-NUE, the incorporation of legume crops into the double cropping system (P-W, S-W, MP-W, and MS-W) all led to improved NUE with lower Ns and emission intensity compared to the traditional wheat-maize double cropping (Table 3). It's important to note that the EUNEP-NUE approach used in this study did not account for the N inputs resulting from biological N_2 fixation by legumes. Incorporating N from biological N_2 fixation would increase N input and consequently reduce NUE while increasing Ns (EUNEP, 2015; Quemada et al., 2020). To our knowledge, this is the first study applying the NUE framework proposed by EUNEP to assess annual cropping systems with legumes, and the first time to use the EUNEP-NUE in combination with the conventional NUE to evaluate the NUE of different cropping systems. Notably, both methods confirmed that the inclusion of intercropping maize with peanut or soybean in the traditional rotation system of wheat-maize can save N input while improving NUE with lower environmental impacts, and MS-W is more resource-efficient and environmentally friendly than MP-W (Table 3; Figs. 4–5). Further research is needed to account for biological N_2 fixation in the calculation of the EUNEP indicators to verify the performance of rotations with intercrops versus sole crops (Bedoussac and Justes, 2010).

4.3. Synergies between appropriate intercropping with rotation and optimal N management

As summarized in Fig. 6, for each of the six annual cropping systems, increasing N applied would inevitably decrease the RE and EUNEP-NUE, and increase Ns. Different from the linear responses of NUE and N loss, responses of N productivity (uptake and output) of each cropping system followed a linear-plateau model. As N application increases, the incorporation of legumes into the M-W annual system results in either positive (Type 1) or negative (Type 2) N productivity responses. The positive effect signifies that incorporating legumes, such as soybean in this study, through intercropping or monoculture, results in N savings, and maintains a higher plateau of N productivity compared to double cropping with wheat and maize. However, inappropriate selection of legume crop species and management practices, as seen with peanut in this study, may reduce total N uptake and output at the plateau, resulting in a negative effect. Consequently, the favorable N performance is achieved only through a combination of suitable intercropping within a rotation system and optimal N management (Fig. 6). Compared to the M-W system with a total of 240–360 kg N/ha application rate, the diversified rotation of MS-W with 210–320 kg N/ha achieved a saving of 11.1−12.5% N fertilizer. Simultaneously, it enhanced N uptake by 12.4–16.0%, increased RE of N from 36.0–37.0% to 47.8%, increased the EUNEP-NUE from 0.5 to 0.67 kg/kg, reduced Ns by 38.8–39.2% and NEI by 48.6–49.3%. At 210 kg/ha N application rate, MS-W achieved EUNEP-NUE of 0.72 kg/kg and Ns of 83.1 kg/ha. This nearly simultaneous achievement of two crucial targets - high NUE (0.50–0.90 kg/kg) and low Ns (80 kg/ha) as suggested by EUNEP (2015) - underscores its potential.

A recent field study showed that 225.0 kg N/ha application along with nitrification and urease inhibitors in wheat-maize rotation systems could lower N-related environmental pollution and provide optimal economic returns in the NCP (Liu et al., 2022). At national level, 319 kg N/ha for wheat and maize (averaged across 3824 counties in China) could decrease N fertilizer use by 21.0–28.0% and mitigate reactive N losses by 23.2–28.9%, while keeping or increasing yields by 6.0–7.0% and N productivity (yield/N fertilizer) by 26.0–33.2% (Yin et al., 2021). In addition to optimizing N usage and implementing advanced fertilization techniques, innovative cropping systems, conservation tillage, improvements in seed quality/nutrients, manure inputs, and pest management also play a crucial role in preserving or enhancing productivity while reducing N losses (Li et al., 2018; Morris et al., 2021; van Kessel et al., 2013; Zhang et al., 2020). Our findings indicate that the optimal N rate could be lowered to 210 kg N/ha/year, primarily through the diversification of rotations via legume intercropping. Consequently, this study demonstrates, for the first time, through multiple N performance indicators, that maize/soybean intercropping with wheat rotation along with appropriate fertilizer N management increases N productivity, improves NUE, and reduces potential N losses to the environment compared to the traditional wheat-maize double cropping system. Adoption of this diversified double cropping system can therefore contribute towards sustainable agricultural production with lower environmental impacts in the NCP.

5. Conclusions

This study compared for the first time the N performance of two

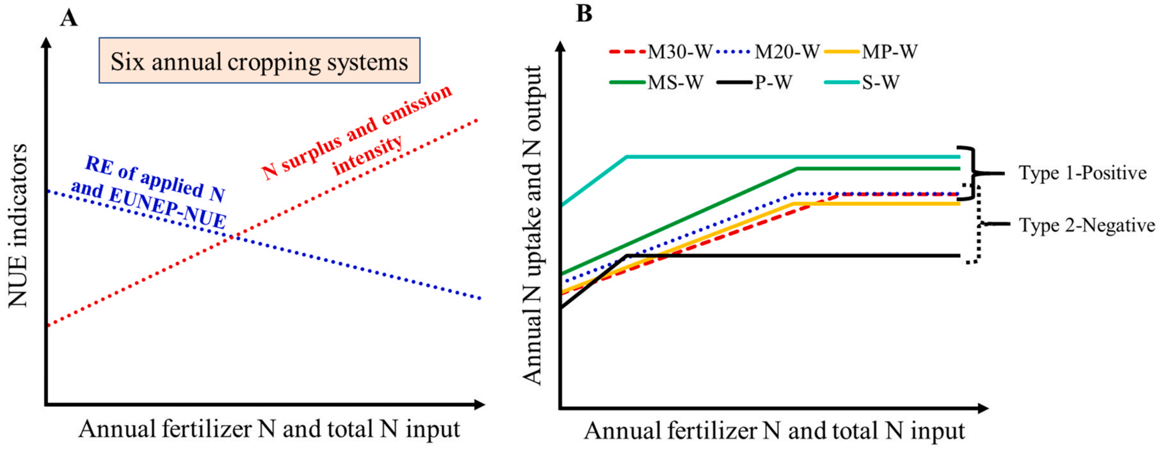

Fig. 6. Schematic diagram showing nitrogen-use efficiency (NUE), N surplus and emission intensity of six annual cropping systems (A) and annual N uptake and N output of each double cropping system (B) in response to annual fertilizer N and total N input. RE indicates the apparent recovery efficiency. EUNEP-NUE indicates the NUE proposed by the EU N Expert Panel. For different double cropping systems, W, M, P, S, MP and MS indicate wheat, maize, peanut, soybean, maize/peanut intercropping and maize/soybean intercropping, respectively. The inter-plant distance within the row of maize is 30 cm in M30, and 20 cm in M20 and intercropping.

approaches of integrating legumes into wheat-maize double cropping at both the crop and cropping system levels. In one approach, the legumes were integrated as sole crops, replacing the summer crop maize. In the other approach, legumes were intercropped with maize simultaneously. In all tested cropping systems, wheat was grown as a winter crop. In the intercrops, maize, as the dominant species, achieved a pLER of 0.74–0.78 for N uptake, indicating higher N acquisition efficiency of intercropped maize than sole maize. This determined the overall advantages of the intercropping system over the monoculture system regarding N uptake (NLER) and yield per unit N applied (NFER). Meanwhile, including legumes via monoculture or intercropping would increase the production of edible oils and proteins, thus diversifying products. Integration of legumes allowed a reduction in total annual N input by approximately 22.2–25.0% when sole crops were used, and by 11.1–12.5% when intercrops were used.

Total N input, N uptake, N output, EUNEP-NUE, N surplus, and N emission intensity in each of the diversified rotations with intercropping (MS-W or MP-W) generally fell between the values observed in monoculture rotations M-W and wheat with the corresponding legume, indicating a neutralizing or modulating effect of intercropping. Diversifying the traditional wheat-maize rotation with legume intercropping improved both the conventional NUE and the EUNEP-NUE with lower N surplus, and intercropping with soybean had a better performance than with peanut. However, rotations of wheat-sole legumes exhibited lower recovery efficiency of applied N than the traditional wheat-maize rotation, indicating further potential to reduce fertilizer N input into such cropping systems. The response of total N productivity (uptake and output) of each annual cropping system to elevated N input followed a "linear-plateau" model. MS-W and S-W showed a plateau with lower N input and higher N productivity than M-W, while MP-W and P-W showed a plateau with lower N input and lower N productivity. Therefore, compared to the traditional M-W, the integration of legumes via intercropping with maize could save fertilizer N input, increase NUE, and decrease environmental N losses. However, N productivity decreased if an inappropriate legume crop species was used, i.e., peanut in this study.

Considering various factors, including N input, N productivity, conventional and EUNEP-NUE, N losses, and diversified products, our findings strongly support the integration of legumes into cereal-based cropping systems with rational N application in the NCP. Overall, these results provide robust evidence for the adoption of diversified cropping systems that include legumes intercropped with maize, with potential global relevance for countries with double cropping or rotation systems, especially if legumes are not currently included. This approach aligns with the principles of agricultural green development.

CRediT authorship contribution statement

Xue Yanfang: Funding acquisition, Resources. **Cui Zhenling:** Funding acquisition, Resources. **Silva João Vasco:** Methodology, Writing – review & editing. **van der Werf Wopke:** Conceptualization, Methodology, Writing – review & editing. **Xia Haiyong:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Writing – original draft, Writing – review & editing. **Li Xiaojing:** Investigation. **Qiao Yuetong:** Investigation. **Xue Yanhui:** Investigation. **Yan Wei:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The study was funded by the National Key R&D Program of China (2021YFD1901003, 2016YFD0300202, 2017YFD0300407), the Taishan Scholarship Project of Shandong Province (TS201712082), the Project of Science and Technology Commissioners in Shandong Province (2022DXAL0125), the International Science and Technology Cooperation Program of Shandong Academy of Agricultural Sciences of China (CXGC2023G23), and the Study Abroad Program by the Government of Shandong Province. Special thanks are due to Dr. Tjeerd-Jan Stomph from Wageningen University for his expert support in designing the three-quadrant diagram.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fcr.2024.109262.

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