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EDITED BY
Zhou Li,
Guizhou University, China

REVIEWED BY
Zhixin Zhang,
Northwest A&F University, China
Bowen Zhang,
Lund University, Sweden

*CORRESPONDENCE
Xiaoye Gao
✉ gaoxiaoye1220@163.com

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Leguminous green manure amendments improve maize yield by increasing N and P fertilizer use efficiency in yellow soil of the Yunnan-Guizhou Plateau

Xiaoye Gao*, Yan He, Yu Chen and Ming Wang

College of Eco-Environmental Engineering, Guizhou Minzu University, Guiyang, China

The low utilization efficiencies of chemical N and P fertilizers largely threat the sustainability of agriculture. Green manure is conducive to increasing crop yield. This study aimed to explore the effects of leguminous and non-leguminous green manures on the maize production, and N and P fertilizers use efficiency. A green manure-maize rotation experiment was conducted in the Karst region of the Yunnan-Guizhou Plateau. The responses of maize yield, N (NUE) and P (PUE) fertilizer use efficiency to winter fallow fields treated with no fertilizer (CK) and traditional chemical fertilizers (WF), WF with alfalfa (AL), common vetch (CV) and oilseed rape (OR) green manures in 2020 and 2021. The results showed that compared with WF, the maize yield was significantly increased on average by 22% and 15% in AL and CV, respectively, while it was hardly affected in OR. AL significantly increased NUE and PUE by an average of 103% and 66%, and CV increased NUE by an average of 74% and 41%, respectively, while RA had no significant effects on PUE, and decreased NUE by 39% in the second year. Structural equation modeling (SEM) showed that green manures indirectly affected NUE and PUE due to soil available N and P, which directly and indirectly influenced maize N and P uptake, and then enhanced NUE and PUE. Linear regression results showed that maize production had positive relationships with NUE and PUE. Our findings highlight that non-legume green manure would hardly influence grain yield, while legume green manure could be effective for increasing maize production by increasing NUE and PUE, especially for alfalfa in yellow soil of the Yunnan-Guizhou Plateau.

KEYWORDS

alfalfa, legume green manure, crop rotation, NUE, PUE

1 Introduction

Nitrogen (N) and phosphorus (P) are the main factors affecting crop growth (Umar et al., 2020). Crops absorb a large amount of N from soils, and a shortage of N leads to low crop yield by limiting crop photosynthesis (Mu and Chen, 2021). In addition, soil P deficiency and its low availability may constrain crop productivity (Katsalirou et al., 2016), contributing 35%—40% to the restriction of maize yield in China (Cao et al., 2021). Chemical N and P fertilizers are used worldwide to increase crop production and maintain soil fertility; however, only 30%—35% and 18%—20% of mineral N and P fertilizers can be used in the current season for crop growth (Balemi and Negisho, 2012), while the rest are fixed or lost through leaching, runoff and volatilization, leading to the risk of soil degradation and environmental pollution problems (Karamesouti and Gasparatos, 2017). Moreover, chemical P fertilizer is produced from nonrenewable natural phosphate rock, but the input of P fertilizer is increasing to maximize crop production due to the large demand for food (Balemi and Negisho, 2012). Thus, improving N and P fertilizer use efficiency is critical for easing environmental problems and the lack of phosphate ore and developing sustainable agriculture in the long term. The management of organic fertilizers in combination with chemical fertilizers shows positive effects on nutrient use efficiency and environment (Zhang et al., 2023).

Globally, green manuring can reduce soil erosion and promote soil hydraulic properties, which can reduce the loss of chemical fertilizer through runoff and leaching (Lei et al., 2022). Additionally, green manuring has positive effects on soil physicochemical properties and nutrient transformation, such as soil available nutrients, microbial quantity and enzymatic activity (He et al., 2020; Khan et al., 2020; Gao et al., 2021), which further enhance N and P use efficiency and crop yield, especially for legume green manuring (Xie et al., 2016; Yang et al., 2019). For example, common vetch (*Vicia sativa* L.), lupin (*Lupinus*) and lablab (*Lablab purpureus* L.) green manures can increase soil available N (AN) and P (AP), and production in wheat and rice systems (He et al., 2020; Amede et al., 2021). Previous studies have indicated that the combined application of milk vetch (*Astragalus sinicus* L.) green manure and chemical N fertilizer increased NUE by 182%−203% compared with chemical N fertilizer alone (Meng et al., 2019), and the P fertilizer use efficiency increased by 10%−14% with the application of legume green manure in paddy fields (Gao et al., 2022).

There are several mechanisms for enhancing soil nutrients and N and P use efficiency by incorporation of green manure. First, legume plants can fix atmospheric N to improve soil available N, meanwhile arbuscular mycorrhizal fungi and rhizobia in the rhizosphere of legume plants can enhance the N and P uptake of the following crops (Meng et al., 2015; Allito et al., 2020). Second, green manures that exude high rates of organic acids are effective for dissolving soil Fe-P and Al-P to increase the labile P content (Haynes and Mokolobate, 2001). At the same time, organic forms of P released during green manure decomposition are less susceptible to strong adsorption on functional groups of oxides and hydroxides of Fe and Al than inorganic forms (Pavinato et al., 2017). Third, green manure incorporation can improve soil aeration and physical properties (Meena et al., 2018), which is conducive to nutrient retention and water storage in soil. Furthermore, green manure incorporation increases soil enzyme activity and microorganisms, further enhancing nutrient cycling (Zhou et al., 2020). Although many studies have addressed the effect of green manure on crop nutrient cycling, it is as yet not well understood how green manure improves N and P use efficiency through soil available nutrients and whether there is an interaction between N and P use efficiency.

The Karst area in southwest China covers an area of 55 × 10^6 ha, which is one of the largest continuous Karst region in the world (Li et al., 2017). It is particularly susceptible to severe soil degradation due to a complex network of soil pockets, rock matrices, and flow paths with variable hydraulic conductivity (Fu et al., 2015; Li A. et al., 2016). In addition, cultivated land in Karst landforms is highly sensitive and vulnerable due to shallow and thin topsoil (Li S. L. et al., 2020). Yellow clayed soil accounts for 46.2% of soil in this region (Li et al., 2016), which characterized by weathering, erosion and a lack of available N and available P contents (Liu et al., 2017). The yellow soil in Karst areas has been seriously degraded owing to intensive nutrient leaching, which can risk low fertilizer use efficiency, crop productivity and soil N and P losses in agroecosystems (Wang et al., 2019; Li S. L. et al., 2020). The application of sweet pea (*Lathyrus odoratus* L.) green manure led to significantly increased soil available P content and maize yield in northwest China (Ablimit et al., 2022). The application of February Orchid (*Orychophragmus violaceus*) as green manure improved maize grain yield and nitrogen use efficiency and reduced nitrogen losses in northern China (Bai et al., 2015). Therefore, it is essential to reduce N and P losses by increasing chemical fertilizer use efficiency for green manuring management in Karst landforms. Although many studies have been conducted on the effects of green manure incorporation on crop yields and soil fertility around the world (He et al., 2020; Amede et al., 2021), there are still very few available reports regarding the coupling responses of NUE and PUE to legume and non-legume green manure incorporation in Karst maize cultivation systems.

To characterize the combined nutrient use efficiencies and maize yield feedback attributable to different green manures in combination with chemical fertilizers and chemical fertilizers alone, we used alfalfa, common vetch and soilseed rape as green manures in a Karst maize ecosystem in Southwest China. The objectives were (1) to determine the difference in nutrient use efficiency and grain yield between legume and non-legume green manure and (2) to assess the interaction of N and P in a green manure-maize rotation system. We hypothesized that the application of green manures would improve soil fertility and maize yield by increasing N and P use efficiency in karst landforms.

2 Materials and methods

2.1 Experimental site

The experiment was conducted in a typical Karst area in Machang town (26°25′N, 106°27′E), Guiyang, China. The region possesses a subtropical humid monsoon climate. The previous crop was maize, which is fallow in winter. The maize growing season is from April to September, with precipitation of 932.60 and 784.00 mm and mean temperatures of 20.35 and 21.09°C in 2020 and 2021, respectively. The soil type is yellow loam. The basic soil pH was 5.28, and the organic matter, total N and P were 34.94, 1.30, and 1.24 g kg^{-1}, respectively.

2.2 Field treatment and management

In order to choose the suitable green manure to improve the maize yield, NUE, and PUE, different green manure treatments, including (1) winter fallow field + no fertilization (CK); (2) winter fallow field + traditional chemical fertilizer (WF); (3) alfalfa + traditional chemical fertilizer (AL); (4) common vetch + traditional chemical fertilizer (CV); and (5) oilseed rape + traditional chemical fertilizer (OR), were established in randomized plots (3 m × 6 m) with a plot spacing of 0.3 m. There were three replicates (plots) for each treatment. The traditional chemical fertilizer application rate was N 244 kg ha^{-1} and P_2O_5 145 kg ha^{-1}.

Alfalfa, common vetch and oilseed rape were sown after maize harvest in 2019 and 2020 by broadcast seeding, with seeding amounts of 27, 45, and 45 kg ha^{-1}, respectively. No fertilizers were applied during the green manure growing season. All green manures were cut into 5–10 cm pieces and incorporated into the tillage layer (20 cm) 1 week before planting maize. The properties of green manures are shown in Table 1.

The maize varieties were starnuo41 and Yingtai863A in 2020 and 2021, respectively. All the seeds were planted in hill-holes, with three–four seeds per hill-hole, with a distance between the holes of ∼60 cm. After the emergence of seedlings, each hole contained two plants. Monoammonium phosphate fertilizer (including N 11%, P_2O_5 44%) was applied as a base fertilizer on the day of sowing maize, and urea (N 46%) as the top fertilizer was applied at the seedling stage (June 15, 2020, June 2, 2021) and before plucking and silking (July 10, 2020, July 6, 2021), accounting for 35 and 55% of the total N amount, respectively. Other management practices were the same as local conventional field practices.

2.3 Sampling and analyses

Three topsoil (0–20 cm) samples were collected simultaneously with the maize harvest period in each plot, mixed evenly using the quartic method, then divided into two subsamples. One subsample were sieved through 2 mm sieves and stored at −20°C, and another subsample was air dried and sieved through 0.15 and 0.25 mm sieves when relevant soil properties were measured. At the maize harvest stage, ten maize plants were randomly harvested in each plot to measure grain yield and stem and leaf biomasses. The stem and leaf samples were dried for 30 min at 105°C and then dried for 48 h at 60°C. The dried grain, stem, and leaf were sieved (0.15 mm) to determine their N and P contents.

NH_4^--N and NO_3^+-N were extracted with 1 M KCl (1:5 w/v) and measured with a flow analyzer (Cleverchem 380, Germany). Soil AN content contained NH_4^--N and NO_3^+-N contents. The total N content was measured by the Kjeldahl digestion procedure (Bremner and Tabatabai, 1972). The soil total P content was determined by the molybdenum blue method after digestion with concentrated $HClO_4$-H_2SO_4 (1:10 v/v), and the soil AP was determined via the molybdenum blue method after extraction with 0.5 M $NaHCO_3$ at pH 8.5.

2.4 Calculations of nutrient use efficiency

The maize N or P uptakes of grain (Eq. 1), stem (Eq. 2), leaf (Eq. 3), and straw (Eq. 4) were calculated as follows:

$$\text{Grain N or P uptake}\left(\text{kg ha}^{-1}\right) = \text{grain N or P content}\left(\text{g kg}^{-1}\right) \times \text{grain yield}\left(\text{kg ha}^{-1}\right)/1{,}000 \quad (1)$$

$$\text{Stem N or P uptake}\left(\text{kg ha}^{-1}\right) = \text{stem N or P content}\left(\text{g kg}^{-1}\right) \times \text{stem yield}\left(\text{kg ha}^{-1}\right)/1{,}000 \quad (2)$$

$$\text{Leaf N or P uptake}\left(\text{kg ha}^{-1}\right) = \text{leaf N or P content}\left(\text{g kg}^{-1}\right) \times \text{leaf yield}\left(\text{kg ha}^{-1}\right)/1{,}000 \quad (3)$$

$$\text{Straw N or P uptake} = \text{stem N or P uptake} + \text{leaf N or P uptake} \quad (4)$$

NUE (Eq. 5) and PUE (Eq. 6) were determined as follows (Cao et al., 2021):

$$\text{N or P uptake efficiency} = U \times 100/F \quad (5)$$

$$\text{N or P use efficiency} = (U_{+\text{N or P}} - U_{-\text{N or P}}) \times 100/F \quad (6)$$

where F denotes the amount of N and P applied (kg ha^{-1}), U_{+NorP} and U_{-NorP} are the total N and P uptake by grain and straw in the chemical fertilizer treatment and CK (kg ha^{-1}), respectively.

2.5 Statistical analysis

The LSD test was used to test the differences between the yields, N and P concentrations in maize plants, N and P uptake, soil available N and soil available P content among treatments at $P <$ 0.05, and a t-test was used to test the differences in NUE and PUE at $P < 0.05$. Two-way ANOVAs were used to determine the effects of green manure type, year, and their interaction on maize yield and nutrient use efficiency at $P < 0.05$. The "piecewise SEM" package in R (v4.1.1) was used for structural equation modeling (SEM). The a priori model included all possible pathways among these factors. To reduce the complexity of SEM, the representing indices of green manure N and P concentrations were calculated using PCA with the "FactoMineR" package in R (v4.1.1). All relationships were fitted using "lm" linear regression (Xiao et al., 2021).

3 Results

3.1 Grain yield and straw biomass

All the green manure treatments improved maize grain yield, straw and total biomass compared to CK in both years ($P <$ 0.05,

TABLE 1 The nutrient contents and uptake efficiency of green manure in 2020 and 2021.

Items		Alfalfa		Common vetch		Oilseed rape	
		2020	2021	2020	2021	2020	2021
Nutrient contents (g kg^{-1})	C	446.7	482.4	427.4	462.1	422.4	429.4
	N	31.4	36.4	23.2	32.0	14.3	20.8
	P	3.0	2.5	2.2	3.0	3.1	3.1
Nutrient ratios	C/N	14.2	13.2	18.4	14.5	29.6	20.7
	C/P	148.9	178.5	198.3	144.0	138.3	137.2
Nutrient accumulations (kg ha^{-1})	C	616.4	1,413.4	1,767.7	1,693.6	1,573.0	1,514.9
	N	43.3	106.7	96.0	117.3	53.3	73.4
	P	4.1	7.3	9.1	11.0	11.5	10.9
Nutrient uptake efficiency (%)	N	17.8	43.7	39.3	48.1	21.8	30.1
	P	6.5	11.6	14.4	17.4	18.2	17.3

Figure 1), with the highest yields observed in AL. Compared with WF, AL and CV significantly increased maize grain yield by 15 and 21% in 2021 and straw biomass by 44 and 51% in 2020 ($P < 0.05$), and OR decreased grain yield by 9% in 2021 ($P > 0.05$). Compared with WF, AL and CV increased total biomass by 24 and 21% on average over the 2 years ($P < 0.05$), respectively. Compared with the OR treatment, the AL and CV treatments had higher grain yields, straw yields and total yields, which increased by 7%—32%, 21%—27% and 13%—27% in 2020 and 2021, respectively.

Two-way ANOVA showed that year and green manure application had significant effects on grain yield, straw and total yield ($P < 0.05$), but there was no significant interaction effect between them ($P > 0.05$, Table 2).

3.2 Maize N and P concentration and uptake

Green manure incorporation increased the N concentration of maize grains, stems and leaves in 2020 and leaves in 2021 compared to CK ($P < 0.05$, Figure 2A). Both AL and CV increased the grain N concentration compared to WF, but the mean increase intensity differed between AL (23%, $P < 0.05$) and CV (5%, $P > 0.05$). OR decreased the grain N concentration in 2020 ($P > 0.05$) and 2021 ($P < 0.05$, Figure 2A). The P concentration in maize plants was lowest in CK and highest in AL (Figure 2B). The grain, stem and leaf P concentrations in the AL treatment increased by 3%, 20% and 9% on average, respectively, compared with those in the WF treatment.

Both total maize N and P uptake showed consistent changes in 2020 and 2021, with the highest uptake in AL, followed by CV and OR, and the lowest uptake in CK (Figure 3). All fertilizer treatments significantly increased the maize grain, straw, and total N and P uptake compared to CK in 2020 and 2021 ($P < 0.05$, Figure 3). Compared with WF, the maize grain, straw and total uptake in AL and CV increased by 49 and 21%, 87 and 88%, and 63 and 45% on average, while the RA decreased by 20%, 9%, and 11%. The AL treatment significantly increased the maize grain, straw and total N uptake compared with the WF treatment ($P < 0.05$, Figure 3A). AL had the highest grain, straw and total P uptake, and the straw and total P uptake was significantly higher than WF ($P < 0.05$, Figure 3B). Compared with CK, all the treatments significantly increased the maize grain N/P ratio except OR; AL and CV increased the leaf N/P ratio in 2020 and 2021, and OR increased the stem N/P ratio in 2021 ($P < 0.05$, Table 3).

3.3 Maize N and P use efficiency

Legume green manure incorporation showed an overall trend of improving N and P fertilizer use efficiencies, while non-legume green manure (OR) application showed an opposite trend in the second year (Table 4). Compared with the WF treatment, the UEN and NUE increased by an average of 63 and 103% in the AL treatment and by an average of 45 and 74% in the CV treatment, respectively; the UEP and PUE increased by an average of 35 and 66% in the AL treatment and by 22 and 41% in the CV treatment, respectively. AL significantly increased UEP and PUE compared to WF in both years ($P < 0.05$). In addition, the UEP and PUE in AL and CV were higher than those in OR. Two-way ANOVA showed that both N and P fertilizer use efficiencies were significantly affected by green manure type ($P < 0.05$), and there were no significant interactions between green manure type and year ($P > 0.05$, Table 4).

3.4 Soil N and P contents

AL significantly increased the soil available and total N contents compared with the other treatments in 2020 and 2021 (Figures 4A, B, $P < 0.05$). The soil available N of CV and OR was significantly higher than that of CK and WF in 2021 ($P < 0.05$, Figure 4A), and there were no significant differences in total N among CK, WF, CV and OR in both years ($P > 0.05$, Figure 4B). The soil available P in AL and CV was significantly higher than that in CK and WF in 2020 and 2021 ($P < 0.05$, Figure 4C). The soil total P content had no significant changes among fertilizer treatments ($P > 0.05$, Figure 4D).

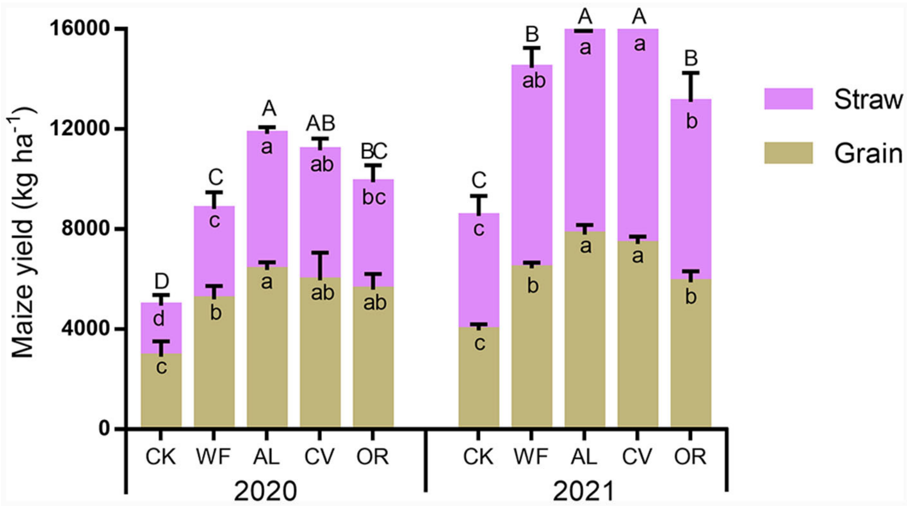

FIGURE 1
Maize grain yield, straw and total biomass in 2020 and 2021 (means ± SEs). Straw biomass including stem and leaf biomass. Different letters in the same item represent a significance level of 0.05 for grain, straw (lowercase) and total biomass (capital).

TABLE 2 The effects of green manure type and year on maize yields.

Treatment	df	Grain		Straw		Total biomass	
		F	*P*-value	*F*	*P*-value	*F*	*P*-value
Green manure (G)	4	30.90	<0.01	182.89	<0.01	150.84	<0.01
Year (Y)	1	42.00	<0.01	30.50	<0.01	52.42	<0.01
G × Y	4	1.16	0.36	1.80	0.17	1.74	0.18

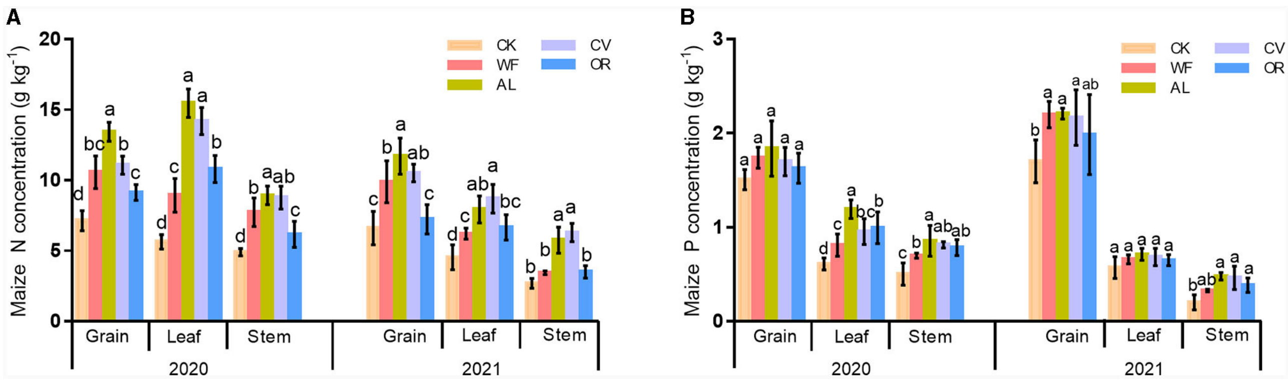

FIGURE 2
The N **(A)** and P **(B)** concentrations in grain, stem and leaf under different treatments in 2020 and 2021 (means ± SEs). Different letters represent a significance level of 0.05 among treatments.

3.5 Controlling factors for NUE, PUE and grain yields

The SEM explained 99 and 96% of the variations in NUE and PUE, respectively (Figure 5). NUE was directly affected by grain and straw N uptake, and PUE was directly affected by grain and straw P uptake. Moreover, green manure indirectly affected NUE due to soil available N and P contents and N uptake in grain and straw, while green manure indirectly affected PUE due to soil available N and P contents and P uptake in grain and straw (Figure 5). Maize total biomass exhibited a significantly positive linear relationship to NUE and PUE (Figures 6A, B), and there were significant positive linear relationships between NUE and PUE, grain yield and grain N/P ratio in 2020 and 2021 ($P < 0.05$, Figures 6C–E).

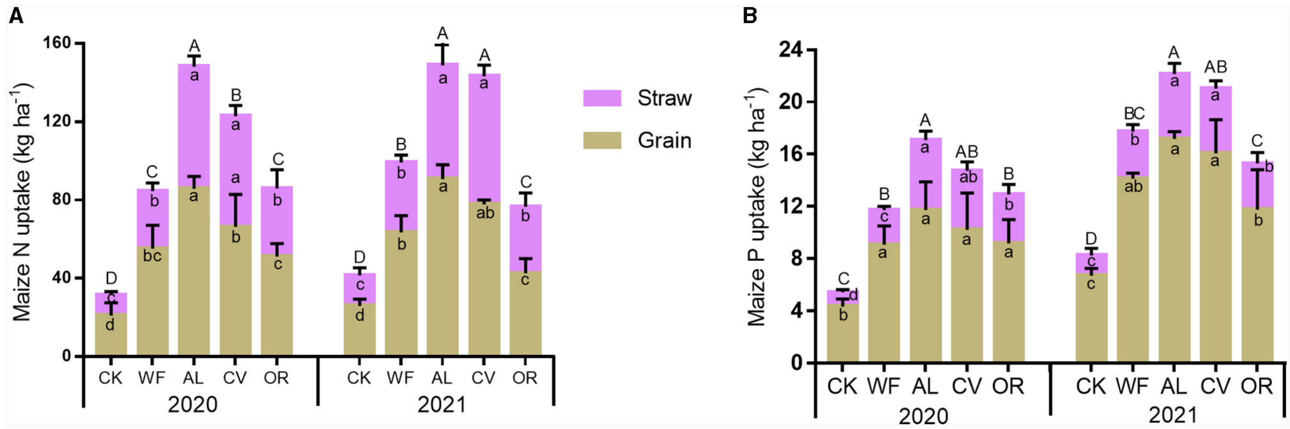

FIGURE 3
The maize N uptake **(A)** and P uptake **(B)** of grain and straw under different treatments (means ± SEs). Different letters in the same item represent a significance level of 0.05 for straw, grain (lowercase) and total uptake (capital).

TABLE 3 The maize N:P ratio in different treatments.

Treatments	Grain		Leaf		Stem	
	2020	2021	2020	2021	2020	2021
CK	4.78 ± 1.37c	3.87 ± 1.99b	9.24 ± 0.51c	7.98 ± 0.27c	10.08 ± 1.37ab	14.22 ± 1.99a
WF	6.07 ± 0.63b	4.50 ± 0.38ab	11.12 ± 0.97bc	9.48 ± 0.76bc	11.00 ± 0.63a	10.53 ± 0.38ab
AL	7.41 ± 1.18a	5.29 ± 0.65a	13.02 ± 0.47ab	11.12 ± 0.2ab	10.68 ± 1.18ab	11.96 ± 0.65ab
CV	6.53 ± 0.84ab	4.92 ± 1.48a	15.03 ± 0.91a	12.81 ± 1.01a	10.85 ± 0.84ab	14.03 ± 1.48a
OR	5.64 ± 0.48bc	3.69 ± 0.75b	11.05 ± 1.00bc	10.26 ± 0.71b	7.88 ± 0.48b	9.25 ± 0.75b

Different letters in the same column represent a significance level of 0.05.

4 Discussion

4.1 Effects of green manures on maize yield

In the present study, alfalfa green manure incorporation significantly increased maize grain and straw yields in 2020 and 2021, and common vetch had a positive effect on grain yield in the second year, while rape green manure reduced grain yield in 2021 (Figure 1). Previous studies showed that the application of milk vetch green manure significantly increased maize yield by 31% in a 2-year study (Tao et al., 2017), while legume hairy vetch application had no significant effect on maize yield, and non-legume annual ryegrass and cereal rye green manure application decreased maize grain yield by 4% in a 5-year study (Qin et al., 2021). A meta-analysis showed that legume and non-legume green manures increased maize yield by 12 and 9% in northern China, respectively (Ma et al., 2021), while non-legume cover crops resulted in a 4% yield decrease (Abdalla et al., 2019). These results are similar to our findings, suggesting that the effects of green manure incorporation on crop yields are closely associated with green manure type and application year.

We found that legume green manures were more effective in promoting maize growth and grain yield than no legume (Figure 1). This may be due to the following mechanisms. First, the soil N content and legume green manure N content were higher than those in the non-legume treatment (Table 1 and Figure 4A). The accumulation of N in leguminous plants is normally higher than that in non-leguminous plants because legumes can fix N_2 from the atmosphere into soils through rhizobia (Zandvakili et al., 2017), which leads to an increase in soil N supplementation and high N concentrations in leguminous plants (Dovrat et al., 2020). Second, the C/N ratio is one of the determining constraints for effective decomposition of green manure. Plant residues contain a lesser proportion of carbon to nitrogen than the 24 perfectly balanced diet soil microorganisms need, the microbes consumed the plant residues and leave the excess nitrogen in the soil. This surplus nitrogen in the soil is available for growing plants. Plant residues with a C/N ratio >24 result in a temporary nitrogen deficit (immobilization), and those with a C/N ratiol <24 result in a temporary nitrogen surplus (mineralization) (United States Department of Agriculture, 2011). The C/N ratios of alfalfa and common vetch were much lower than that of oilseed rape in the present study (Table 1). The decomposition and mineralization rates of legume residues are faster than those of non-legume residues due to the narrow C/N ratio of legume green manures (Calegari et al., 2013; Toom et al., 2019), which release N and P faster, thus improving maize growth and yield. However, non-legume green manure with a high C/N ratio would first stabilize soil available N, which limited the N applied for crop growth (Li et al., 2020).

TABLE 4 Effects of green manure on N and P use efficiency.

Treatments	Uptake efficiency (%)		Nutrient use efficiency (%)	
	N	P	N	P
2020				
WF	34.69 ± 3.73b	18.51 ± 1.48b	21.77 ± 3.73b	9.95 ± 1.48b
AL	60.73 ± 0.32a	26.98 ± 1.67a	47.81 ± 0.32a	18.42 ± 1.67a
CV	50.34 ± 5.18a	23.26 ± 3.18ab	37.42 ± 5.18a	14.70 ± 3.18ab
OR	35.20 ± 1.62b	20.41 ± 1.01b	22.28 ± 1.62b	11.85 ± 1.01b
2021				
WF	40.69 ± 2.86b	28.02 ± 0.48b	23.64 ± 2.86b	14.97 ± 0.48b
AL	61.00 ± 3.33a	35.00 ± 1.23a	43.96 ± 3.33a	21.95 ± 1.23a
CV	58.69 ± 1.48a	33.22 ± 2.78ab	41.65 ± 1.48a	20.17 ± 2.78ab
OR	31.40 ± 3.08c	24.15 ± 3.50b	14.35 ± 3.08c	11.10 ± 3.50b
Green manure (G)	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$
Year (Y)	0.24	$P < 0.01$	0.55	0.04
G × Y	0.20	0.43	0.20	0.48

Different letters indicate a significance level of 0.05 in the same column.

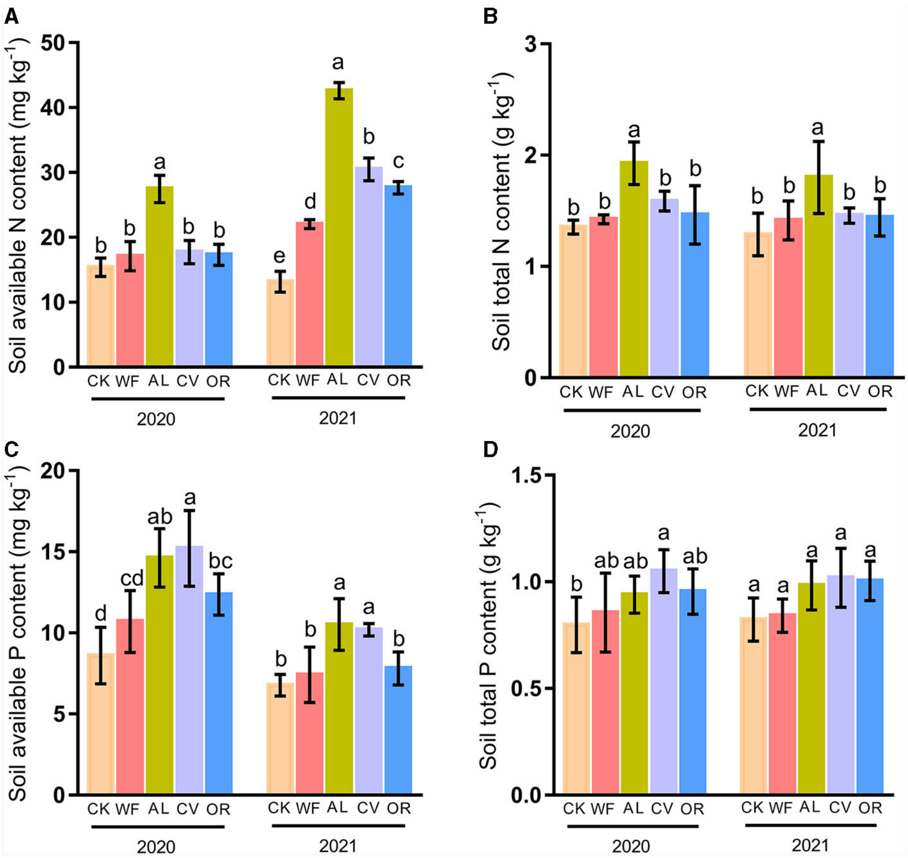

FIGURE 4
Variations in soil available N **(A)**, total N **(B)**, available P **(C)**, and total P **(D)** contents **(D)** among treatments. Different letters in the same item are significantly different at $P < 0.05$.

Furthermore, green manure application may increase available P for succeeding crops (Haynes and Mokolobate, 2001), and net P mineralization from legume green manure is commonly positively correlated with the P concentration in green manure (Pypers et al., 2005). Legume green manure application could increase grain yield by improving a large amount of soil active

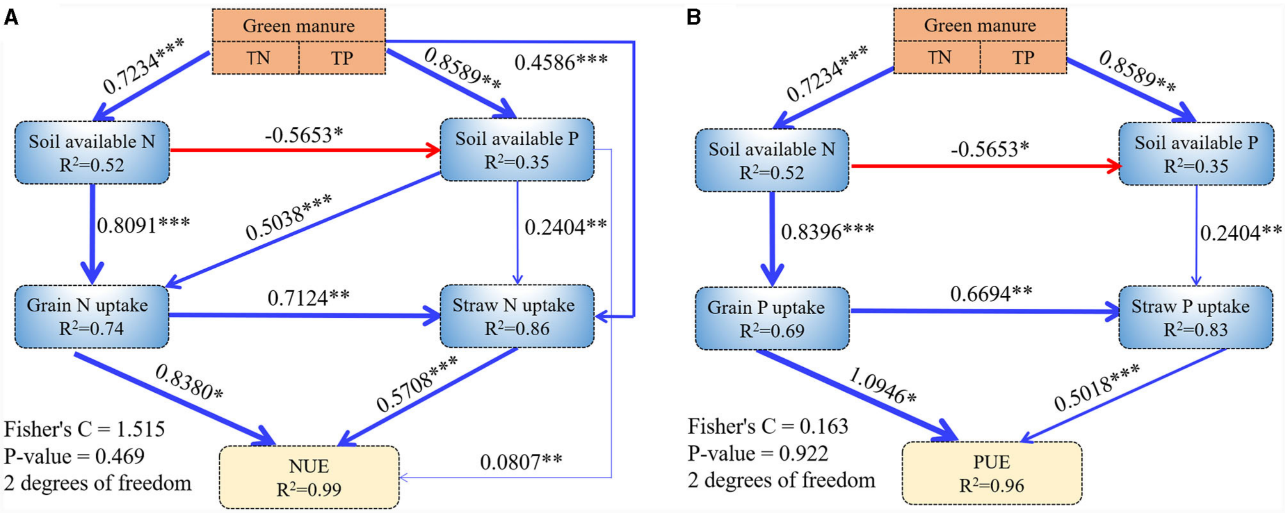

FIGURE 5
The SEM to evaluate the direct and indirect effects of green manure, soil available N content (AN), soil available P content (AP), and N and P uptake on maize NUE **(A)** and PUE **(B)** from 2020 to 2021. Soil available N content including NH_4^+-N and NO_3^--N contents. Continuous arrows represent the significant relationships. Blue and red lines represent positive and negative correlations, respectively. The adjacent number in the same direction as the arrow represents the path coefficients, and the width of the arrow is proportional to the degree of the path coefficients. R^2 is the variance explained by each response variable. ****P* < 0.001, ***P* < 0.01, **P* < 0.05.

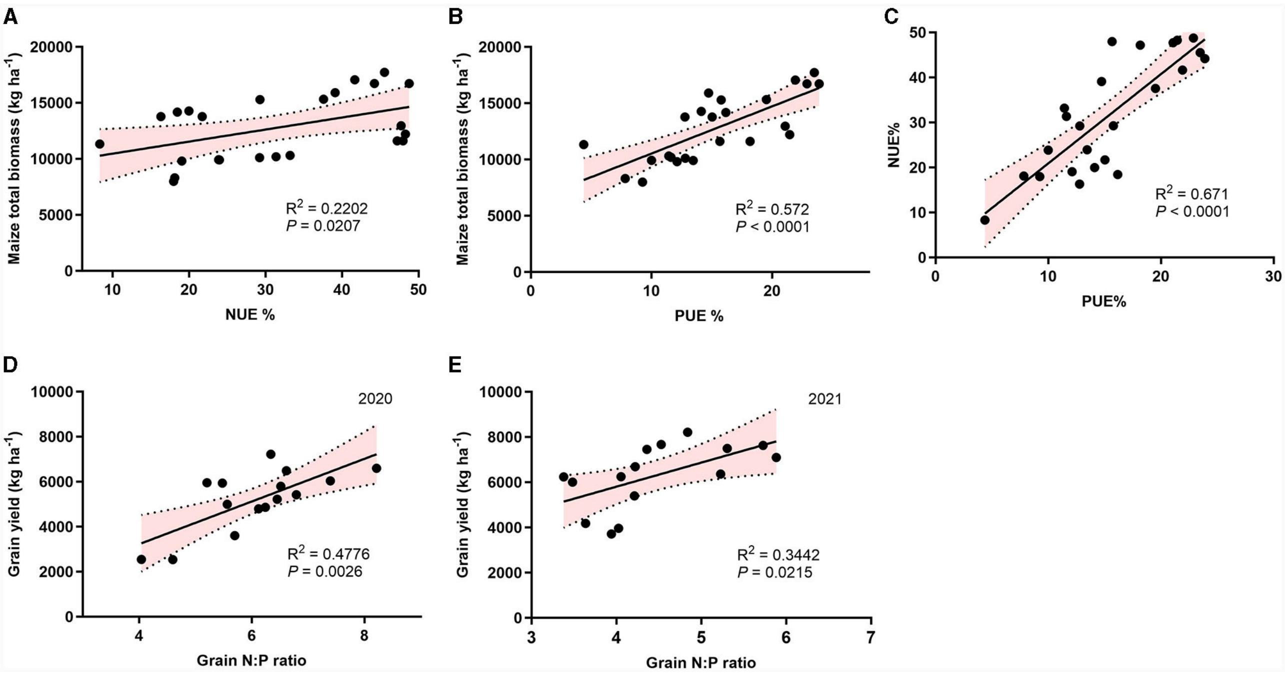

FIGURE 6
Linear relationships between maize above-ground biomass and NUE, and PUE **(A–C)** and between maize grain yield and the grain N:P ratio in 2020 and 2021 **(D, E)**.

P (Gao et al., 2016). Although the P concentration in oilseed rape was the highest in the present study, the soil available P content was lower than those in AL and CV (Figure 4) because the low N concentration and large C/N ratio of oilseed rape limit the decomposition of rape residues and further affect available P release.

4.2 Effects of green manures on maize NUE

Maize has a relatively high nutrient requirement for N, while the average NUE of maize is low (Abbasi et al., 2013). In this study, the NUE ranged from 14 to 48%, and the application of alfalfa and common vetch increased the NUE by 103 and 74% and decreased it by 19% under oilseed rape green manure (Table 4), which coincided with the trends in maize biomass, N uptake, and available N content between fertilization treatments (Figures 1, 3A). Similarly, Murungu et al. (2011) found that the contributions of grazing vetch (*Vicia darsycarpa*) and forage pea (*Pisum sativum*) green manures to maize N uptake were higher than that of oat green manure. Red clover significantly improved maize N availability, but oilseed radish (*Raphanus sativus*), oat and rye green manures had no significant effect on maize N availability (Vyn et al., 2000). Chinese milk vetch increased N uptake and use efficiency by 39%−51% (Zhu et al., 2014). Increasing crop biomass and N concentration are effective ways to increase NUE (Sinclair and Vadez, 2002). The N contributions of alfalfa and common vetch green manures to maize plants were higher than those of rape green manure, causing higher NUE in the AL and CV treatments than in the OR treatment (Figure 2A and Table 4). Legume green manure is not only used as a direct source of available N for plants but also has great potential in increasing the availability of soil N to crops and preserving N (Ashraf et al., 2004). Legume green manure increased NUE mainly by directly increasing N uptake and indirectly improving soil available N and P contents (Figure 5A), suggesting that the improvement in soil available N content may be responsible for the increased crop yields and N uptake and further enhances NUE (Zhang et al., 2020). Therefore, it is suitable for enhancing N supply and NUE in alfalfa green manure-maize rotation systems.

There are several factors affecting soil available N content, which in turn affect crop N uptake and N use efficiency after green manure return to the field. First, organic N in the soil began a rapid initial fixation or mineralization, followed by a slow linear mineralization, and the C/N ratio of green manure determined the decomposition of green manure and N mineralization in soil (Cambardella et al., 2010). In general, green manure with a small C/N ratio mineralizes faster, increasing the availability of mineral N in the soil (Figure 4A), which is the major N source for absorption by crops (Radicetti et al., 2017). Second, the lower C:N ratio of green manure resulted in more rapid N mineralization, and a higher C/N ratio of green manure can prolong the microbial fixation of N available in the intensive cropping system, and its effect is more significant in the early stage of maize growth, thus limiting the N uptake and potential yield in the short term (Radicetti et al., 2017). Moreover, legume and non-legume green manure incorporation exhibited differential responses to the soil microbial community (Khan et al., 2020). Leguminous green manure rich in N easily decomposes following application, probably increasing the soil microbial functional community and soil enzyme activities (Chavarría et al., 2016), which might be attributable to carbon availability in the early stages of maize growth, further enhancing crop nutrient absorption. This was supported by our findings that AL and CV significantly increased soil urease activity and the relative abundance of Proteobacteria (He, 2022). In addition, lower N uptake in maize directly caused the soil available N content to increase with oilseed rape application in 2021. In our study, oilseed rape green manure incorporation reduced the N uptake and NUE in the second year, mainly attributable to maize grain yield reduction (Figures 1A, 3A and Table 4), which may be due to the large C/N ratio of oilseed rape and slow decomposition rate, resulting in the fixation of organic N and little contribution to maize N supplied (Radicetti et al., 2017; Carciochi et al., 2021).

4.3 Effects of green manures on maize PUE

We found that alfalfa and common vetch green manure incorporation increased the P uptake of maize straw, while the effects on maize grain P uptake were insignificant (Figure 3B). Previous studies have found that the P uptake in rice straw with alfalfa and broad bean green manure application was higher than that in chemical fertilizer alone (Gao et al., 2016), and maize P uptake in cobs had no significant changes under the *O. violaceus* green manure-maize system compared with the continuous maize system (Zhang et al., 2022). The PUE of AL and CV increased by 35%−85% in the present study (Table 3), suggesting that short-term application of legume green manure has the potential to improve the maize PUE. Sesbania green manure application increased P uptake and the recovery efficiency of P and PUE in a rice–wheat double system (Mitran and Mani, 2017). Alfalfa and broad bean green manure in combination with chemical fertilizer significantly increased rice PUE by 10%−14% compared with the application of chemical fertilizer alone (Gao et al., 2022). Green manure incorporation can increase soil available P content and crop P uptake and then improve PUE (Pavinato et al., 2017; Gao et al., 2022). Wang et al. (2021) reported that soil properties such as total N, available N, microbial biomass C and N were closely related to PUE. In the present study, SEM analysis showed that the improvement in PUE was attributed to soil available N and P contents and maize P uptake (Figure 6B).

Phosphorus cycling-related microbial and enzyme activities transform soil P through dissolution, mineralization, and absorption, converting soil insoluble P into inorganic P, which is more easily absorbed by plants (Gao et al., 2019). Specifically, the organic instability pool in surface soil was increased through mycorrhizal colonization of green manure, and the arbuscular mycorrhizal fungal abundance of subsequent crops was increased (Arruda et al., 2021). By coincidence, maize plants preferred the mycorrhizal pathway at suboptimal soil available P (Zhang et al., 2021). In addition, organic acids released during the decomposition of green manure can enhance P availability by chelating with aluminum and iron oxides, reducing the number of binding sites and reducing soil P adsorption strength by dissolving soil mineral P (Haynes and Mokolobate, 2001). Moreover, green manure effectively increased the abundances of P-solubilizing bacteria and enhanced phosphatase activity (Wang et al., 2021).

4.4 Implications for green manure maize cropping systems

Balanced nutrient supply is the key factor in nutrient use efficiency and increased crop production (Janssen, 1998; Dash et al., 2015). There was a positive linear correlation between NUE and PUE in our study (Figure 6C). The crop N/P ratio is a direct function of N uptake and an inverse function of P uptake, and its efficiency depends on the range of the N/P ratio. Alfalfa and common vetch green manures increased the maize grain N/P ratio, while oilseed rape had no significant effect on it compared with the winter fallow, and there was a positive relationship between grain yield and grain N/P ratio (Figure 6D), suggesting that lower C/N ratio of legume green manure could enhance maize N and P uptake and consequently increase maize yield owning to the mineralization of green manure (United States Department of Agriculture, 2011). Previous studies showed that crop P uptake and PUE were positively correlated with P concentration in green manure and negatively correlated with the C/P ratio of green manure (Garg and Bahl, 2008; Gao et al., 2022). This study showed the different result that the applicaiton of oilseed rape with the highest P concentration and the lowest C/P ratio had the lowest P uptake and PUE, as well as NUE. The probably reason is that the higher C/N ratio of oilseed rape limited the N supplied and P releasing from oilseed rape residues (Calegari et al., 2013; Toom et al., 2019). Legume green manures rich in N and P contents, in particular N content, may enhance NUE and PUE by improving soil available N and P. In general, legume green manure incorporation generally has positive contributions to N and P in cropping systems.

5 Conclusion

In the 2-year green manure-maize rotation system, alfalfa and common vetch green manure incorporation increased maize yield by 22 and 15%, soil available N content by 77 and 42%, soil available N content by 39 and 40%, NUE by 103 and 74%, and PUE by 66 and 41%, respectively. The improvement in NUE and PUE was attributed to soil available N and P contents, N uptake, and P uptake. Our findings suggest that alfalfa green manure is beneficial for promoting maize NUE and PUE, which will promote the green and sustainable development of agricultural Karst landforms, and that replacing partial chemical N and P fertilizers with alfalfa green manure may be an effective alternative for reducing the application rate of chemical fertilizers and for enhancing maize yields.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

XG: Conceptualization, Writing – review & editing, Writing – original draft. YH: Investigation, Writing – original draft. YC: Investigation, Writing – review & editing. MW: Investigation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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