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Long-term increased grain yield and soil fertility from intercropping

Xiao-Fei Li^{1,710}, Zhi-Gang Wang^{1,10}, Xing-Guo Bao², Jian-Hao Sun², Si-Cun Yang², Ping Wang³, Cheng-Bao Wang², Jin-Pu Wu¹, Xin-Ru Liu¹, Xiu-Li Tian¹, Yu Wang¹, Jian-Peng Li¹, Yan Wang¹, Hai-Yong Xia^{1,8}, Pei-Pei Mei^{1,9}, Xiao-Feng Wang¹, Jian-Hua Zhao¹, Rui-Peng Yu¹, Wei-Ping Zhang¹, Zong-Xian Che², Lin-Guo Gui³, Ragan M. Callaway⁴, David Tilman^{5,6} and Long Li¹

Population and income growth are increasing global food demand at a time when a third of the world's agricultural soils are degraded and climate variability threatens the sustainability of food production. Intercropping, the practice of growing two or more spatially intermingled crops, often increases yields, but whether such yield increases, their stability and soil fertility can be sustained over time remains unclear. Using four long-term (10-16 years) experiments on soils of differing fertility, we found that grain yields in intercropped systems were on average 22% greater than in matched monocultures and had greater year-to-year stability. Moreover, relative to monocultures, yield benefits of intercropping increased through time, suggesting that intercropping may increase soil fertility via observed increases in soil organic matter, total nitrogen and macro-aggregates when comparing intercropped with monoculture soils. Our results suggest that wider adoption of intercropping could increase both crop production and its long-term sustainability.

he number of crop species used in agriculture has been decreasing globally for 50 years¹. Intensive large-scale monocultures of four crops-maize, rice, wheat and soybeansnow dominate agricultural landscapes around the world. However, intensive agricultural monocultures are also associated with soil degradation, increased risk of crop pest and disease outbreaks, and environmental pollution^{2,3}. We test whether increases in crop diversity via intercropping might help alleviate some of the issues facing agricultural sustainability4-6. Intercropping increases within-field biodiversity by cultivating two or more intermingled crop species7. Intercropping can produce greater yields8 and thus reduce the land area required to produce a given amount of food9. It can also maintain yields while reducing fertilizer⁸⁻¹⁰ and pesticide use¹¹. Because recent analyses have suggested that nations with higher effective crop group and species diversity tend to have greater year-to-year stability of national total agricultural yields⁵, we also ascertain whether the within-field increase in crop diversity generated via intercropping might influence the year-to-year reliability of the total crop production of intercropped fields.

Although the yield benefits of appropriate combinations of two crop species have been well established in intercropping experiments⁸⁻¹⁰, it is not known whether, in the long term, the yield benefits of intercropping might decline, stay the same or increase. In this article, we report the effects of matched sets of long-term

intercropping and monoculture experiments on yields, on the year-to-year temporal stability of crop production, on yield trends and on soil fertility.

To explore the long-term costs and benefits of intercropping relative to monocultures, we conducted four field experiments, one for 16 years and three for 10 years, that spanned a substantial soil fertility and yield production gradient in Gansu Province and Ningxia Hui Autonomous Region in northwest China (Supplementary Table 1). Each of these experiments consisted of both monocultures and intercropped systems of maize (Zea mays L.) combined with one or more of the following five crops: wheat (Triticum aestivum L.), faba bean (Vicia faba L.), soybean [Glycine max (L.) Merrill], chickpea (Cicer arietinum L.) and oilseed rape (Brassica napus L.) (Supplementary Fig. 1 and Supplementary Table 2). For the two 'equal fertilizer' experiments (Baiyun-1 and a portion of Hongsibu), all monoculture and intercropped plots received identical fertilization treatments. For the three 'optimal fertilizer' experiments (Baiyun-2, Jingtan and Hongsibu), monocultures of each crop received agronomically optimal fertilization, and intercrops received the higher rate that is recommended for maize. Other experimental details for the four sites are presented in Supplementary Table 2. In all of these intercropping and monoculture systems, we measured total grain yields, soil nutrient chemistry, soil aggregate size, the year-to-year temporal stability of the intercrops and their monocultures and net farmer profits.

 ¹Key Laboratory of Plant and Soil Interactions, Ministry of Education, Beijing Key Laboratory of Biodiversity and Organic Farming, College of Resources and Environmental Sciences, China Agricultural University, Beijing, China. ²Institute of Soils, Fertilizers and Water-Saving Agriculture, Gansu Academy of Agricultural Sciences, Lanzhou, China. ³Institute of Crop Sciences, Ningxia Academy of Agriculture and Forestry Sciences, Yinchuan, China.
 ⁴Division of Biological Sciences and Institute on Ecosystems, University of Montana, Missoula, MT, USA. ⁵Department of Ecology, Evolution and Behavior, University of Minnesota, Saint Paul, MN, USA. ⁶Bren School of Environmental Science and Management, University of California Santa Barbara, Santa Barbara, CA, USA. ⁷Present address: State Key Laboratory of Cotton Biology, Institute of Cotton Research, Chinese Academy of Agricultural Sciences, Anyang, China. ⁸Present address: Crop Research Institute, Shandong Academy of Agricultural Sciences, Jinan, China. ⁹Present address: College of Life Sciences and Technology, Henan Institute of Science and Technology, Xinxiang, China. ¹⁰These authors contributed equally: Xiao-Fei Li, Zhi-Gang Wang.

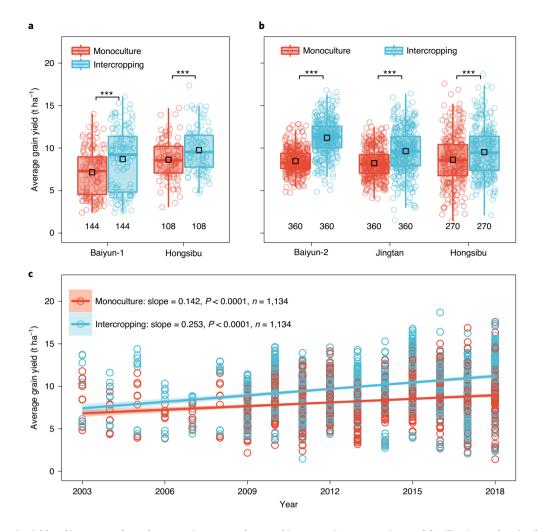


Fig. 1 Average grain yield and its temporal trends comparing monoculture and intercropping systems in equal-fertilization and optimal-fertilization experiments from 2003/2009 to 2018. The experiment at Baiyun-1 was initiated in 2003, and the remaining experiments, at Baiyun-2, Jingtan and Hongsibu, were initiated in 2009. Equal-fertilization experiments included Baiyun-1 and a subset of Hongsibu at 75 and 150 kg ha⁻¹ yr⁻¹ N treatments. Optimal-fertilization experiments consisted of Baiyun-2, Jingtan and Hongsibu. **a,b**, Average grain yield for monoculture and intercropping systems with equal fertilization (**a**) and optimal fertilization (**b**). **c**, Comparison of temporal trends. In box plots, the solid lines and squares in the boxes, the lower and upper edges of the boxes and the lower and upper lines extending from the boxes represent median and mean values, the first (Q1) and third (Q3) quartiles, $1.5 \times$ interquartile range (IQR) above the box's Q1 and $1.5 \times$ IQR below the box's Q3 of all data, respectively. Values under the boxes are the number of data inputs across all treatments, years and replicates. Asterisks indicate significant differences between monoculture and intercropping in each experiment using Tukey's HSD test. ****P* < 0.001. Significant relationships are denoted with solid lines and fit statistics (slopes, *P* values and number of data inputs) for each cropping system. The shaded region is the 95% confidence band for the relationship. Slope for intercropping systems was significantly different from that of monocultures (*F*=12.84, *P*=0.0003) based on analysis of covariance.

Results and discussion

In the following, we discuss our findings, context and implications.

Yield advantage of intercropping. Average grain yields across all four experiments and all six crop combinations were 22.3% greater in intercropped systems than in monocultures of the same crops (Fig. 1a,b and Table 1), and the land equivalent ratio (LER) in intercropping ranged from 1.00 to 1.46 with an average of 1.22. Greater yields mean that a higher proportion of nutrients were removed with the harvested crops in intercropping systems, which has been found to reduce soil nitrate levels¹² and thus should reduce nitrate leaching from soils and its water pollution¹³. During this long-term study, when compared with their matched monocultures, the average intercropping grain yields in the two equal fertilizer experiments were 21.5% greater for Baiyun-1 and 13.0% greater for Hongsibu (Fig. 1a and Table 1). For the three optimal fertilizer experiments,

relative to monocultures, intercropping yields were 32.3% greater for Baiyun-2, 17.2% greater for Jingtan and 10.3% greater for Hongsibu (Fig. 1b and Table 1).

Faba bean and maize intercropping was conducted at all four sites, which differed in soil fertility. Faba bean and maize intercropping had overyielding of 29.0% at Baiyun-1 and 33.9% at Baiyun-2, which had the most fertile soils, 26.3% at Jingtan, which had the next-most fertile soils, and 10.3% at Hongsibu, which had the least fertile soils (Table 1). This is consistent with the finding of a meta-analysis that yield benefits of intercropping were often greater when soil fertility was increased by intensive fertilization⁹.

Increase over time in crop yields. As to the sustainability of the yield benefits of intercropping, our long-term experiments find that intercropping yields increased significantly through time, with intercropping yields increasing by a total of 1.1 tha⁻¹ of grain more than

Table 1 | Grain yields and overyielding of cropping systems under monocultures and intercropping in equal-fertilization and optimalfertilization experiments

Equal or optimal	Experimental site	Crop combination	Grain yield (t ha⁻¹)				
fertilization			Monoculture	Intercropped	Overyielding (%)	Р	LER
Equal	Baiyun-1	Faba bean + maize	7.99	10.31	29.0	<0.001	1.22
fertilization		Faba bean + wheat	4.01	4.29	7.0	<0.001	1.04
		Wheat + maize	9.48	11.51	21.4	<0.001	1.19
		Mean	7.16	8.70	21.5		1.15
	Hongsibu	Faba bean + maize	8.63	9.76	13.0	<0.001	1.41
Optimal	Baiyun-2	Chickpea + maize	8.85	11.66	31.8	<0.001	1.30
fertilization		Faba bean + maize	8.47	11.34	33.9	<0.001	1.34
		Oilseed rape + maize	7.98	10.67	33.8	<0.001	1.32
		Soybean + maize	8.78	11.50	31.0	<0.001	1.00
		Mean	8.48	11.22	32.3		1.25
	Jingtan	Chickpea + maize	8.27	10.11	22.3	<0.001	1.15
		Faba bean + maize	8.62	10.89	26.3	<0.001	1.31
		Oilseed rape + maize	7.72	8.34	8.1	<0.05	1.15
		Soybean + maize	8.46	9.54	12.8	<0.001	1.05
		Mean	8.23	9.64	17.2		1.17
	Hongsibu	Faba bean + maize	8.63	9.52	10.3	<0.001	1.46

Grain yield of monoculture is the expected grain yield, which was calculated as the weighted means of two monoculture crops on the basis of the land proportion occupied by two crops in intercropping. The overyielding (%) is calculated as (grain yield in intercropping (tha⁻¹)-grain yield in monoculture (tha⁻¹)/grain yield in monoculture (t

did monoculture yields (Fig. 1c). In particular, across all four experiments and all crop combinations, intercropped yields increased by an average of 2.52% per year, but monoculture yields of these same crops increased by only 1.67% per year. These increases were based on the slope of yield trends in intercropping and in monoculture from 2009 to 2018, which is the ten-year period that all four sites had in common (Fig. 1c). This result shows that the yield benefits of intercropping were not just sustainable but also increasing.

Greater yield stability in intercropping. On average, the year-to-year temporal stability of intercropping yields was significantly (P < 0.01) higher than that of monocultures for both the equal-fertilization (Fig. 2a) and the optimal-fertilization treatments (Fig. 2b). This field-level result is consistent with the effects of plant diversity on temporal stability observed in grasslands¹⁴⁻¹⁶ and forests¹⁷ and with the greater temporal stability of total national crop production in countries with greater crop diversity⁵. However, some crop monocultures were as stable as some intercropped mixtures (Supplementary Fig. 2), suggesting that the impacts of intercropping on yield stability may depend on the identity of crops and crop combinations.

Increased soil fertility in intercropping. Maintaining and enhancing soil fertility are key goals for agricultural sustainability. Although long-term ecological experiments in grasslands have found that greater diversity of intermingled perennial plant species was associated with increased soil fertility from enhanced soil aggregates¹⁸⁻²⁰ and elevated levels of soil C and N^{21,22}, our long-term field intercropping experiments are able to determine whether intercropping impacts soil fertility when annual crop plants are annually harvested and some of their nutrients are removed from fields. We tested for the impact of intercropping on soil physical structure (macro-aggregate content), soil organic matter (SOM) and total nitrogen content (TN).

The formation of large soil aggregates, with diameters >2 mm, increases soil fertility by improving water infiltration and nutrient cycling and by decreasing erosion^{23–25}. Therefore, we focused

mainly on large soil aggregates. For equal fertilization, large soil macro-aggregates (>2 mm) were 66.5% more abundant in intercropped soils than in their respective monocultures (P < 0.0001) at Baiyun-1 and 9.7% more abundant (P < 0.01) at Hongsibu (Fig. 3a). For optimal fertilization, large soil macro-aggregates were 19.4% (P < 0.0001) more abundant in intercropping than in monoculture treatments at Baiyun-2, 10.1% more abundant (P < 0.0001) at Jingtan and 9.1% more abundant (P < 0.0001)at Hongsibu (Fig. 3b). Moreover, the abundance of the smaller classes of soil aggregates in intercropping was often lower than in monocultures (Supplementary Fig. 3). In particular, intercropping significantly (P < 0.001) reduced the abundance of aggregates of less than 0.106 mm at all sites relative to crop monocultures (Supplementary Fig. 3), suggesting that intercropping may help transform the small soil aggregates into larger ones. Large soil aggregates produced by intercropping might be a mechanism driving the long-term increase in the yields²⁶.

For the equal-fertilization experiments, relative to crop monocultures, intercropping increased SOM by 11.6% (P<0.001) and TN by 9.1% (P<0.01) at Hongsibu, but SOM, TN and pH did not change significantly at Baiyun-1 (Fig. 4a). Moreover, similar analyses for the optimal-fertilization results showed that intercropping increased SOM at Hongsibu (P<0.001) and TN at both Hongsibu and Jingtan (P<0.01), relative to monocultures (Fig. 4b). At Baiyun-2, SOM and TN were not significantly changed by intercropping, but soil pH was slightly reduced (Fig. 4b). Thus, our results show that intercropping enhances soil C and N, especially on infertile soils, much like N fertilization^{27,28}, an effect that is consistent with the effects of higher plant diversity in perennial grasslands^{21,22,29}. In total, our findings show that intercropping enhanced soil aggregates, a physical characteristic of greater soil fertility, at all sites and SOM and TN at some sites.

Enhanced farmer profits in intercropping. Over ten years, our results show that crop diversity had positive and increasing impacts on yields, at least in the context of annually fertilized annual crops

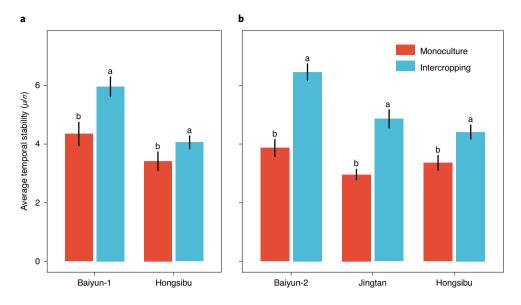


Fig. 2 | Average temporal stability of yields for intercropping and their respective sole cropping across all crop combinations and fertilization treatments in equal-fertilization and optimal-fertilization experiments from 2003/2009 to 2018. a,b, Data show mean \pm standard error at equal fertilization (Baiyun-1: n = 9 for monoculture and n = 18 for intercropping; Hongsibu: n = 24 for monoculture and n = 12 for intercropping) (a) and optimal fertilization (Baiyun-2 and Jingtan: n = 45 for monoculture and n = 36 for intercropping; Hongsibu: n = 60 for monoculture and n = 30 for intercropping) (b). Temporal stability refers to the ratio of the average grain yield (μ) to its temporal standard deviation (σ) over the study period, determined after deternding. Different letters indicate significant differences between cropping systems using Tukey's HSD test.

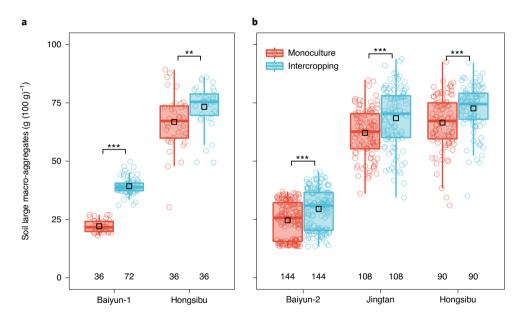


Fig. 3 | Soil large macro-aggregates comparing monoculture and intercropping systems in equal-fertilization and optimal-fertilization experiments. **a**,**b**, Comparison of soil large macro-aggregates (>2 mm) between monoculture and intercropping systems in equal fertilization (**a**) and optimal fertilization (**b**). In box plots, the solid lines and squares in the boxes, the lower and upper edges of the boxes and the lower and upper lines extending from the boxes represent median and mean values, Q1 and Q3, 1.5 × IQR above the box's Q1 and 1.5 × IQR below the box's Q3 of all data, respectively. Values under the boxes are the number of data inputs that are included. Asterisks indicate significant differences between monoculture and intercropping in each experiment using Tukey's HSD test. ***P* < 0.01; ****P* < 0.001.

such as ours. For farmers, economic benefits probably dominate the choice of crop and cropping system³⁰, rather than higher yields, greater stability or environmental benefits. Thus, we used then-current crop, input and labour prices to estimate farmer profits for our monocultures and intercrops. Across the study period and the experimental sites, the majority of maize-based intercropping (except for soybean/maize) increased estimated net farmer profits by 24–75% with a mean value of 47%, equivalent to ~US\$645 ha⁻¹ across four intercropping systems, when compared with the two corresponding monocultures (Table 2). Net profits also varied greatly among cropping system, sites and crop combinations. For example, maize had consistently higher economic profits than other

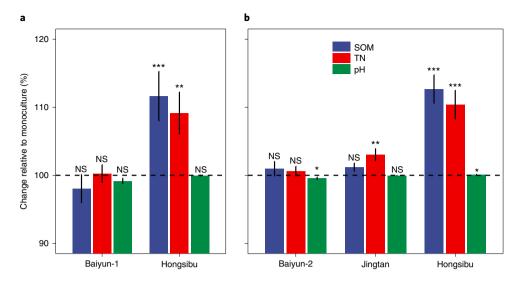


Fig. 4 | Relative changes of soil chemical properties for intercropping compared with corresponding monocultures in equal-fertilization and optimal-fertilization experiments. Analyses were done within the ploughed horizon (0 to 20 cm). Results are presented relative to monocultures (100%, the dashed line). **a,b**, Data show mean \pm standard error for equal fertilization (n = 36 for each) (**a**) and optimal fertilization (n = 72 for Baiyun-2, n = 108 for Jingtan and n = 90 for Hongsibu) (**b**). Asterisks indicate significant differences between monoculture and intercropping. *P < 0.05; **P < 0.01; ***P < 0.001; NS, non-significant.

experiments						
Equal or optimal fertilization	Experimental site	Crop combination	Net profit (US\$ ha ⁻¹)			
			Monoculture	Intercropped	Increase (%)	Р
Equal	Baiyun-1	Faba bean + maize	1,259	1,862	47.9	<0.001
fertilization		Faba bean + wheat	247	323	30.8	0.207
		Wheat + maize	1,348	1,763	30.8	0.001
		Mean	951	1,316	38.3	
	Hongsibu	Faba bean + maize	1,265	1,630	28.9	0.055
Optimal	Baiyun-2	Chickpea + maize	1,858	2,652	42.7	<0.001
fertilization		Faba bean + maize	1,303	2,110	62.0	<0.001
		Oilseed rape + maize	1,108	1,933	74.5	0.005
		Soybean + maize	1,291	1,302	0.8	0.492
		Mean	1,390	1,999	43.8	
	Jingtan	Chickpea + maize	1,588	1,870	17.8	0.094
		Faba bean + maize	1,594	2,364	48.3	0.028
		Oilseed rape + maize	1,092	1,341	22.8	0.152
		Soybean + maize	1,225	1,279	4.4	0.109
		Mean	1,375	1,714	24.6	
	Hongsibu	Faba bean + maize	1,252	1,550	23.8	0.020

Table 2 Net profit of cropping systems under monocultures and intercropping in equal-fertilization and optimal-fertilization
experiments

Net profit is the difference between total income and expenditure. Total income comes mainly from grain yield of crops. Total expenditure includes NP fertilizer, seeds and irrigation costs as well as the salary of workers (used the same wage standard for the four sites and for the all years of experiments) who manage the fields, including weeding, sowing and harvesting during the study period. Net profit in monoculture is the expected, which is the weighted means of two monoculture crops based on the land proportion occupied by two crops in intercropping. US\$1=6.53 RMB. Net profit increase (%) is calculated as (net profit in intercropping – net profit in monoculture)/net profit in monoculture x100 (Methods).

crops. The lowest profits resulted from the cultivation of oilseed rape (Supplementary Table 4).

Increasing global demand for crops is associated with increased land clearing, which is a major cause of extinction risk and the source of about a third of agricultural greenhouse gas emissions. Increases in yields, such as possible through intercropping, could reduce the need for land clearing and thus offer major environmental benefits. Alternatively, intercropping could benefit agroecosystems environmentally through its reported ability to retain current yields while reducing chemical fertilizer application by 19% to 36%⁹, decreasing pesticide use on crop pests, parasitoids³¹ and diseases¹¹, and improving pollination³². Simultaneously, our results suggest that intercropping may help improve the fertility of agricultural soils that have been degraded³³. As such, our results suggest that intercropping has the potential to decrease environmental harm while increasing food supplies and economic returns^{34,35}. Our results suggest that widespread adoption of intercropping in a country also might increase total national crop production on its existing croplands, the long-term fertility of these cropland soils and the stability and security of the national food supply.

Intercropping, although highly effective in labour-intensive agriculture, may be difficult to implement in machine-intensive, large-scale modern agriculture because appropriate large equipment is not commercially available for planting and harvesting various crop mixtures grown with strip intercropping. The potential global benefits of strip intercropping seem unlikely to be attainable until appropriate machinery becomes available.

In summary, our results suggest that, compared with monocultures, intercropping can enhance soil fertility, can provide ~22% more food per unit of land and, in some cases, may help increase the year-to-year reliability of crop production. Previous work has shown that intercropping can enhance biodiversity of soil organisms³⁶ and reduce some agrichemical inputs and the water pollution they can cause^{12,13}. Intercropping is an underused and potentially potent tool for increasing soil fertility and crop production and reducing environmental harm while increasing farmer profits. Because crop demand differs greatly among crops, a balance of intercropped and monoculture production seems likely to be needed to meet demand. However, for those combinations of in-demand crops for which intercropping offers yield, environmental or economic benefits, rapid development of appropriate machinery and adoption of intercropping could offer multiple societal benefits.

Methods

Experimental design and field management. Our four long-term experiments were based on maize (Zea mays L.) intercropped with five crops-wheat (Triticum aestivum L.), faba bean (Vicia faba L.), soybean [Glycine max (L.) Merrill], chickpea (Cicer arietinum L.) and oilseed rape (Brassica napus L.)-in Gansu Province and Ningxia Hui Autonomous Region in northwest China. These crop combinations were selected on the basis of widely practiced combinations of local farmers. The experiment at Baiyun-1 was initiated in 2003, and the remaining of experiments at Baiyun-2, Jingtan and Hongsibu were initiated in 2009. Farming at Baiyun-1 and Baiyun-2 has been active for more than 100 years, at Jingtan for 25 years and at Hongsibu for 3 years. Consequently, initial soil fertility varied greatly among the four sites (Supplementary Table 1). No manure was applied during our study, but all experimental plots have received 50 kg ha⁻¹ yr⁻¹ K as potassium sulfate (K₂SO₄, 46% K) since 2016. Sites varied in some aspect of experimental design, as presented in the following. Aboveground parts (including straw and grain) of all crops for each plot were removed from the field. This is similar to local farmers' practices.

Equal-fertilization experiments. The experiment at Baiyun-1 (38° 37' N, 102° 40' E) was a single-factor, completely randomized block design with three monocultures (maize, wheat and faba bean), three continuous intercropping systems (two crops continuously intercropped on the same strips of land for each crop every year, crop combinations included maize/faba bean, faba bean/wheat and maize/wheat), three rotational intercropping systems (two crops rotationally intercropped with one crop strip in one year and the other crop strips in the subsequent year, crop combinations included maize/faba bean, faba bean/wheat and maize/wheat), and four rotation systems (wheat-faba bean, wheat-maize, maize-faba bean, and wheat-maize-faba bean). Each treatment was replicated three times. Plot size was 4m × 5.6 m for monocultures and 8m × 5.6 m for intercropping systems. All plots received 225 kgha⁻¹ N fertilizer and 40 kg ha⁻¹ P fertilizer annually before sowing.

The experiment at Hongsibu (37° 25′ N, 106° 03′ E) has treatments of faba bean/maize intercropping and faba bean and maize monocultures. Plots received the identical N application rates, with one rate at 75 kg ha⁻¹ yr⁻¹ N and the other at 150 kg ha⁻¹ yr⁻¹ N. Each of three block sets had plots with inoculation by rhizobia (*R. leguminosarum* bv. viciae NM353) and without inoculation. Phosphate fertilizer was applied annually to each plot at a rate of 53 kg ha⁻¹ P before sowing. Plot size was 3.0 m × 1.2 m for monoculture faba bean, 3.0 m × 2.4 m for monoculture maize, and 6.0 m × 3.6 m for faba bean/maize intercropping. The yield data in 2010 were missed; thus, we presented yield data from nine years at the site.

Optimal-fertilization experiments. The optimal-fertilization experiments take into account that maize requires higher rates of nitrogen fertilization to obtain its maximal yields than do faba bean, soybean, chickpea or oilseed rape. For these

experiments, the intercrops received the same amounts of N fertilizer as the maize monoculture.

The experiment at Baiyun-2 (38° 37′ N, 102° 40′ E) was a split-plot completely randomized block design with nine replications of monocultures of maize, faba bean, soybean, chickpea and oilseed rape and nine replications of maize intercropped with faba bean, soybean, chickpea and oilseed rape. These were divided into three replications of each treatment at each of three P application levels (0, 40 and 80 kg ha⁻¹ yr⁻¹ P). Plot size was 4.0 m×5.5 m for monocultures and 5.6 m×5.5 m for the intercropping treatments. A total of 225 kg ha⁻¹ N fertilizer was applied to all treatments, except for monocultures of legumes and oilseed rape, which received 112.5 kg ha⁻¹ N fertilizer from 2009 to 2015 and 150 kg ha⁻¹ N fertilizer since 2016.

The experiment at Jingtan (37° 05′ N, 104° 40′ E) was designed and managed almost identically to the experiment at Baiyun-2. The exception was the application of rotational intercropping rather than continuous intercropping. Each plot size was $6.0 \text{ m} \times 4.2 \text{ m}$.

The experiment at Hongsibu $(37^{\circ}25' \text{ N}, 106^{\circ}03' \text{ E})$ was a split-split-plot completely randomized block design, identical to the Hongsibu equal-fertilization experiment, except faba bean, because it is a legume, received half the N fertilizer of maize. Specifically, each rhizobia treatment was divided into three replications at each of five N levels (0, 75, 150, 225 and $300 \text{ kg} \text{ ha}^{-1} \text{ yr}^{-1} \text{ N}$ for monoculture of maize and intercropping; 0, 37.5, 75, 112.5 and $150 \text{ kg} \text{ ha}^{-1} \text{ yr}^{-1} \text{ N}$ for monoculture of faba bean).

Spatial design of strip intercropping. In the long-term experiments, intercropping used alternating strips of each crop. Each strip had several rows of a crop. There were ten strips in each intercropping plot (five strips of crop A + five strips of crop B) at Baiyun-1, eight strips at Baiyun-2 and six strips at Jingtan and Hongsibu. At Baiyun-1, the row ratio in an intercropping strip was two maize/six wheat, six wheat/four faba bean and two maize/four faba bean. At Baiyun-2 and Jingtan, it was two maize/three soybean, two maize/three chickpea, two maize/three oilseed rape and two maize/three faba bean. At Hongsibu, the ratio was two maize/ two faba bean. The inter-row spacing of maize, wheat, legumes and oilseed rape was 0.4 m, 0.133 m, 0.2 m and 0.2 m, respectively, in both the monoculture and intercropping plots. The inter-plant spacing within the same row was 0.3 m for maize and 0.2 m for legumes and oilseed rape. Wheat was planted at a sowing rate of 450 kg ha-1. Dates of sowing were mid-March of each year for wheat, faba bean, chickpea and oilseed rape and mid-April for maize and soybean. Dates of harvesting were late June to early July for oilseed rape, late July to early August for faba bean and chickpea, mid-July for wheat and late September to early October for sovbean and maize.

Sampling procedures. For all experiments, a central strip of the individual crop species (5.6 m long × 1.6 m wide at Baiyun-1, 5.5 m long × 1.4 m wide at Baiyun-2, $6.0 \text{ m} \log \times 1.4 \text{ m}$ wide at Jingtan and $3.0 \text{ m} \log \times 1.2 \text{ m}$ wide at Hongsibu) was hand harvested in each plot for grain yield after full maturity every year from the starting of experiments through 2018. Grain yields of the two component crops were harvested separately and then summed to determine the total grain yield in intercropping. Soil samples were collected from the top 20 cm of the profile using an auger (35 mm diameter) from 2011 to 2012 at Baiyun-1 and Baiyun-2 and from 2012 to 2014 at Jingtan and Hongsibu. Four soil cores were collected from each plot and combined to give one composite sample per plot for monocultures; there were also four sampling points for each crop strip per plot in intercropping. The composite samples were air dried and sieved through a 2 mm mesh and finally placed in plastic bags for chemical analysis. The undisturbed soil samples were collected using an aluminium box (8.0 cm diameter) from surface soil depth (0-20 cm) of each plot after maize harvest from 2011 to 2014 at Baiyun-1 and Baiyun-2 and from 2012 to 2014 at Jingtan and Hongsibu, air dried under shade for several days and then gently transported to the laboratory for subsequent soil aggregate determination. In soil aggregate sampling, one sample was collected from monoculture plots and two samples from intercropped plots. Sampling for soil aggregate and chemical properties generally was after all crops were harvested in early October.

Soil analysis. SOM and TN were determined using standard protocols³⁷. Soil pH was measured at a ratio of 1/2.5 (dry soil/deionized water, w/v). Soil aggregates were determined with the wet sieving method³⁸ using a Model TTF-100 soil aggregate analyser, in which 100g soil was separated into four aggregate size classes: large macro-aggregates (>2 mm), macro-aggregates (0.25–2.00 mm), micro-aggregates (0.106–0.249 mm) and free silt and clay particles (<0.106 mm).

Calculations. To compare the total productivity of monoculture and intercropping systems at a comparable fertilization level, the weighted means of grain yield in monoculture systems was calculated as follows:

Weighted means of grain yield = $Y_{mono_a} \times O_a + Y_{mono_b} \times O_b$

where $Y_{\text{mono},a}$ and $Y_{\text{mono},b}$ are grain yield of crop a and b in monoculture, respectively. O_s and O_b are the land proportion of crop a and b in intercropping,

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respectively. The preceding formula was also used to calculate the weighted means of soil chemical properties, net profit and soil aggregates by monoculture.

The LER is the sum of the two component crops in the intercrop, each scaled by the monocrop yield³⁹. Partial LER is the relative yields per crop species.

We used the inverse of the coefficient of variation as a measure of temporal stability. In contrast to the coefficient of variation, which approaches zero as stability increases, temporal stability has the advantage that its magnitude increases with stability^{5,14,40}. Temporal stability was defined for each plot as μ/σ , where μ is the temporal mean of grain yield and σ is its temporal standard deviation during the study period. We next removed yield variation attributable to a temporal trend of increasing crop yield by regressing annual crop yields on the year for each experimental site^{5,14}. For each regression, the σ of the residuals provided the measure of detrended variation of yield that was used to calculate the temporal stability of grain yield.

Net profit is the difference between total income and expenditure. Total income comes mainly from grain yield of crops. Total expenditure includes NP fertilizer, seeds and irrigation costs as well as the salaries of workers who manage the fields, including weeding, sowing and harvesting during the study period. The selling prices of grain for maize, oilseed rape, soybean and wheat were CNY1.9, CNY4.9, CNY4.0 and CNY2.2 per kilogram in Gansu Province over the past ten years (2009–2018), respectivelyth. The respective prices of grain were CNY5.6 and CNY4.8 per kilogram for chickpea and faba bean according to the local market. The inputs of N and P fertilizers were, respectively, CNY4.1 and CNY4.6 per kilogram. The conventional dose of N fertilizer used in the study region was 225 kg ha⁻¹ yr⁻¹ for monocultures of wheat and maize crops and all maize-based intercropping systems; the conventional dose of P fertilizer was 40 kg ha⁻¹ yr⁻¹ for all cropping systems. The inputs of seeds, labour cost and irrigation were determined according to the current market prices. Exchange rate was US\$1 ≈ CNY6.53.

Statistical analyses. Linear mixed-effect models were used to determine the main effects of cropping system (intercropping versus monoculture) on grain yield, net profit, soil physio-chemistry and the year-to-year temporal stability in equal-fertilization experiments and optimal-fertilization experiments. Specifically, the preceding responsive variables for each experiment were tested with cropping system and the corresponding experimental treatments (fertilizer application rate, inoculation and crop combination based on each experiment) as fixed effects, and block and year were tested together as random effects. It is important to note that year was excluded in analysis of the year-to-year temporal stability. In the factor 'cropping system', the monoculture value is the weighted means of the two monocultures with the whole system being considered. For each crop or crop combination at each experiment, a similar model was used to analyse the intercropping effect with cropping system and the corresponding experimental treatments (fertilizer application rate and inoculation based on each experiment) as fixed effects, and block and year together as random effects. Moreover, linear regression was applied to examine the relationship between grain yield and experimental year. Then the differences in slopes between intercropped systems and monocultures were tested using analysis of covariance. Means were compared using Tukey's honestly significant difference (HSD) test at P<0.05. All statistical analyses were performed in R version 3.6.242

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data that support the findings of this study are available in Supplementary Data 1 and from the corresponding author upon request. Source data are provided with this paper.

Code availability

The custom code generated for this study is available in the Supplementary Information and from the corresponding author upon request.

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Author contributions

L.L., X.-E.L., Z.-G.W., J.-H.S., X.-G.B. and S.-C.Y. designed the research; X.-F.L., Z.-G.W., X.-G.B., J.-H.S., S.-C.Y., P.W, C.-B.W., J.-P.W., X.-R.L., X.-L.T, Yu Wang, J.-P.L., Yan Wang, H.-Y.X., P.-P.M., X.-F.W., J.-H.Z., R.-P.Y., W.-P.Z., Z.-X.C., L.-G.G. and L.L. performed research; X.-F.L., Z.-G.W., R.-P.Y. and L.L. analysed the data; and X.-F.L., Z.-G.W., R.M.C., D.T. and L.L. wrote the paper.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Long Li.

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Reporting Summary

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		Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about <u>availability of computer code</u>
Data collection
No software was used for data collection. All data were collected from four long-term field experiments based on maize (Zea mays L.)

intercropped with one or more of the following five crops - wheat (Triticum aestivum L.), faba bean (Vicia faba L.), soybean [Glycine max (L.) Merrill], chickpea (Cicer arietinum L.), and oilseed rape (Brassica napus L.) in Gansu and Ningxia Provinces in northwest China.

Data analysis The data analysis was conducted in R version 3.6.2.

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Study description	To explore the long-term costs and benefits of intercropping relative to monocultures, we conducted four field experiments at sites in Gansu and Ningxia Provinces in northwest China that spanned a substantial soil fertility and yield production gradient (Supplementary Table 1). For the two "Equal Fertilizer" experiments (Baiyun-1 and a portion of Hongsibu), all monoculture and intercropped plots received identical fertilization treatments. For the three "Optimal Fertilizer" experiments (Baiyun-2, Jingtan and Hongsibu), monocultures of each crop received agronomically optimal fertilization, and intercrops received the higher rate, that recommended for maize. The experiment at the Baiyun-1 site was a single factor completely randomized block design with three monocultures (maize, wheat and faba bean), three continuous intercropping systems (two crops continuously intercropped on the same strips of land for each crop every year, crop combinations included maize/faba bean, faba bean/wheat, and maize/wheat), three rotational intercropping systems (two crops rotationally intercropped with one crop strips in one year and the other crop strips in subsequent year, crop combination included maize/faba bean, faba bean/wheat, and maize/wheat), and four rotation systems (wheat-faba bean, wheat-maize, maize-faba bean, and wheat-maize-faba bean). There were three replications for each treatment and a total 39 plots. The experiments at the Baiyun-2 and Jingtan sites were a split-plot completely randomized block design with nine replications of monocultures of maize, faba bean, soybean, chickpea and oilseed rape, and nine replications of maize intercropped with faba bean, soybean, chickpea and oilseed rape. These were 81 plots for each experiment. The Equal Fertilization experiment at the Hongsibu has treatments of faba bean/maize intercropping, and faba bean and maize monoculture. Plots received the identical N application rates, with one rate at 75 kg N ha-1 yr-1, and the other at 150 kg N ha-1 yr-1. Each of three block sets
Research sample	Our four long-term experiments were based on maize (Zea mays L.) intercropped with one or more of the following five crops - wheat (Triticum aestivum L.), faba bean (Vicia faba L.), soybean [Glycine max (L.) Merrill], chickpea (Cicer arietinum L.), and oilseed rape (Brassica napus L.). In all of these intercropping and monoculture systems, we measured crop and soil properties, including total grain yields (n = 4332,) soil nutrient chemistry (n = 2169), soil aggregate size (n = 4572), the year-to-year temporal stability (n = 423) of the intercrops and their monocultures, and net farmer profits (n = 126).
Sampling strategy	For all experiments, a central strip of the individual crop species (5.6 m in length × 1.6 m in width at Baiyun-1, 5.5 m in length × 1.4 m in width at Jingtan, and 3.0 m in length × 1.2 m in width at Hongsibu) was hand-harvested in each plot for grain yield after full maturity every year from the starting of experiments through 2018. Grain yields of the two component crops were harvested separately and then summed to determine the total grain yield in intercropping. Soil samples were collected from the top 20 cm of the profile using an auger (35 mm diameter) from 2011 to 2012 at Baiyun-1 and Baiyun-2, and from 2012 to 2014 at Jingtan and Hongsibu. Four soil cores were collected from each plot and combined to give one composite sample per plot for monocultures and there also were four sampling points for each crop strip per plot in intercropping. The composite samples were air-dried and sieved through a 2 mm mesh and finally placed in plastic bags for chemical analysis. The undisturbed soil samples were collected using an aluminum box (8.0 cm diameter) from surface soil depth (0-20 cm) of each plot after maize harvest from 2011 to 2014 at Baiyun-1 and Baiyun-2, from 2012 to 2014 at Jingtan and Hongsibu, air-dried under shade for several days, and then gently transported to the laboratory for subsequent soil aggregate determination. In soil aggregate sampling, one sample was collected from monoculture plots and two samples from intercropped plots. Sampling for soil aggregate and chemical properties generally was after all crops were harvested in early October.
Data collection	In order to compare the total productivity of monoculture and intercropping systems at a comparable fertilization level, we used the weighted means of grain yield in monoculture systems. We also considered that Land Equivalent Ratio (LER) and partial Land Equivalent Ratio (PLER) are relative metrics of intercropping advantage. Temporal stability was defined for each plot as μ/σ , where μ is the temporal mean of grain yield and σ its temporal standard deviation during the study period. We next removed yield variation attributable to a temporal trend of increasing crop yield by regressing annual crop yields on the year for each experimental site. For each regression, the σ of the residuals provided the measure of detrended variation of yield that was used to calculate the temporal stability of grain yield. Net profit is the difference between total income and expenditure. Total income comes mainly from grain yield of crops. Total expenditure includes NP fertilizer, seeds, and irrigation costs as well as the salary of workers who manage the fields, including weeding, sowing and harvesting during study period. The selling prices of grain for chickpea, faba bean, maize, oilseed rape, soybean, and wheat were 5.6, 4.8, 1.9, 4.9, 4.0, and 2.2 CNY per kg, respectively. The inputs of N and P fertilizers were respective 4.1 and 4.6 CNY per kilogram. The conventional dose of N fertilizer used in the study region was 225 kg ha-1 yr-1 for monocultures of wheat and maize crops and all maize-based intercropping systems, and of P fertilizer was 40 kg ha-1 yr-1 for all cropping systems. The inputs of seeds, labor cost, and irrigation were determined according to the current market prices. Exchange rate was 1US\$ \approx 6.53 CNY. All authors of the paper, except for two authors from USA, were involved in the yield data collection from field experiment

in past 16 years (at Baiyun-1) and 10 years (at Baiyun-2, Jingtan, and Hongsibu). Xiao-Fei Li and Zhi-Gang Wang collected data for soil properties in these studies. Timing and spatial scale The time scale of our four field experiments was more than 10 years (one for 16 years from 2003 through 2018 and three for 10 years from 2009 through 2018). These experiments spanned a substantial soil fertility gradient and covered major food and oil crops in northwest China. Sampling for soil aggregate and chemical properties generally was after all crops were harvested in early October. Data exclusions No data were excluded from the analyses. Reproducibility In the long-term field experiments, the yields were measured every year after crops were full mature. The soil properties were repeatedly measured for two or four years. All attempts to repeat the analysis and results were successful. The field arrangement for experimental plots were completely randomized block design. Soil samples were collected randomly within Randomization monocultured plots or within crop strip in intercropping plots. Blinding The collected soil cores and analysis were numbered and only later linked to the different treatments.

Yes Did the study involve field work? No

Field work, collection and transport

Field conditions	The experiment at the Baiyun-1 site was initiated in 2003, and the remaining of experiments at the Baiyun-2, Jingtan, and Hongsibu sites were initiated in 2009. Total average precipitation for these sites is 150, 200 and 185 mm, respectively. The mean annual temperatures are 7.7, 6.6, and 8.9°C.
Location	Experiments were conducted across Gansu and Ningxia Provinces for a total of four sites at Baiyun-1 (38°37′N, 102°40′E), Baiyun-2 (38°37′N, 102°40′E), Jingtan (37°05′N, 104°40′E), and Hongsibu (37°25′N, 106°03′E) where are the Experimental Stations of Gansu Academy of Agricultural Sciences and Ningxia Academy of Agriculture and Forestry Sciences, China.
Access & import/export	The research was carried out under the National Science Foundation of China (31430014), the National Key Research and Development Program of China (2016YFD0300202) and the National Science Foundation EPSCoR Cooperative Agreement OIA-1757351.
Disturbance	There was no disturbance.

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