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Supplementary information

Long-term increased grain yield and soil fertility from intercropping

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Long-term increased grain yield and soil fertility from intercropping

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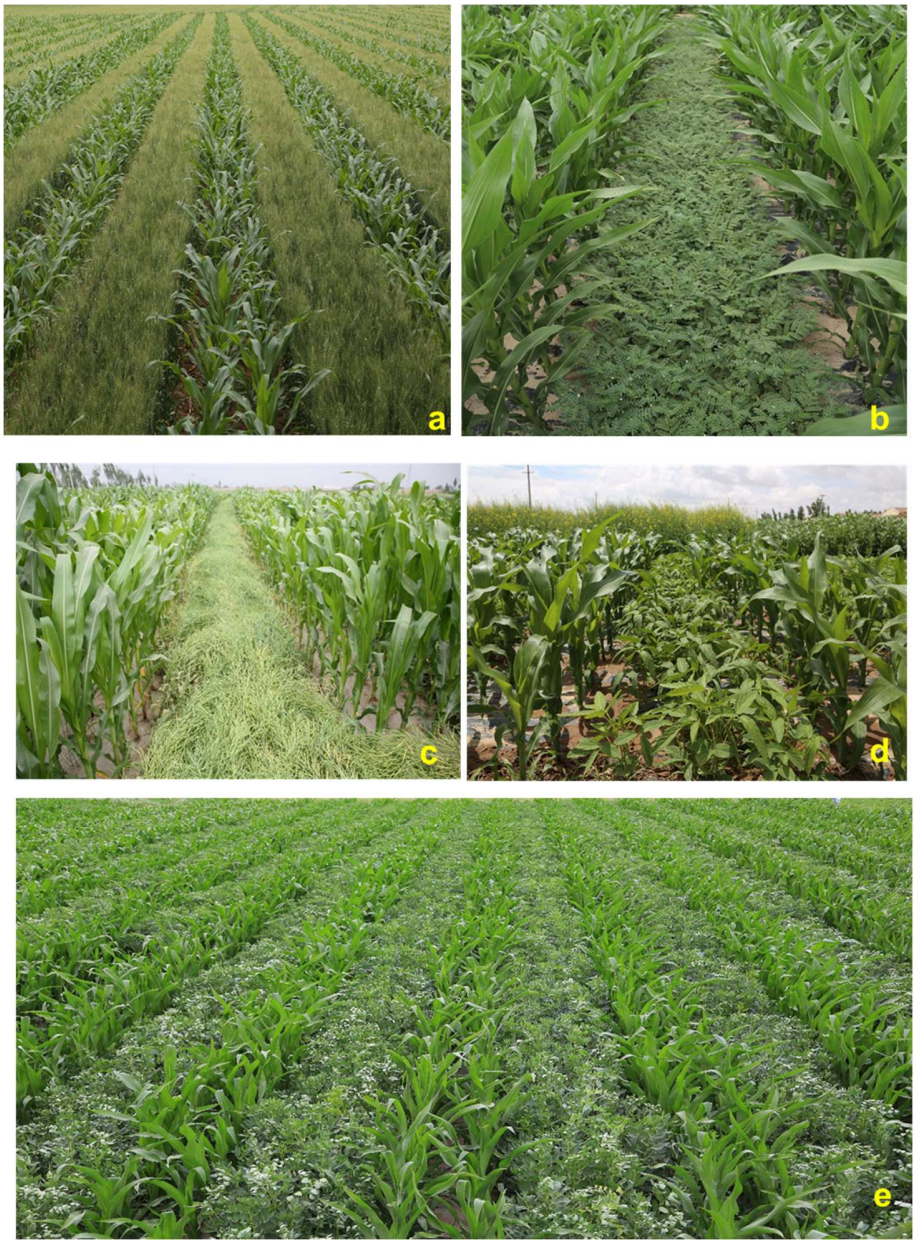
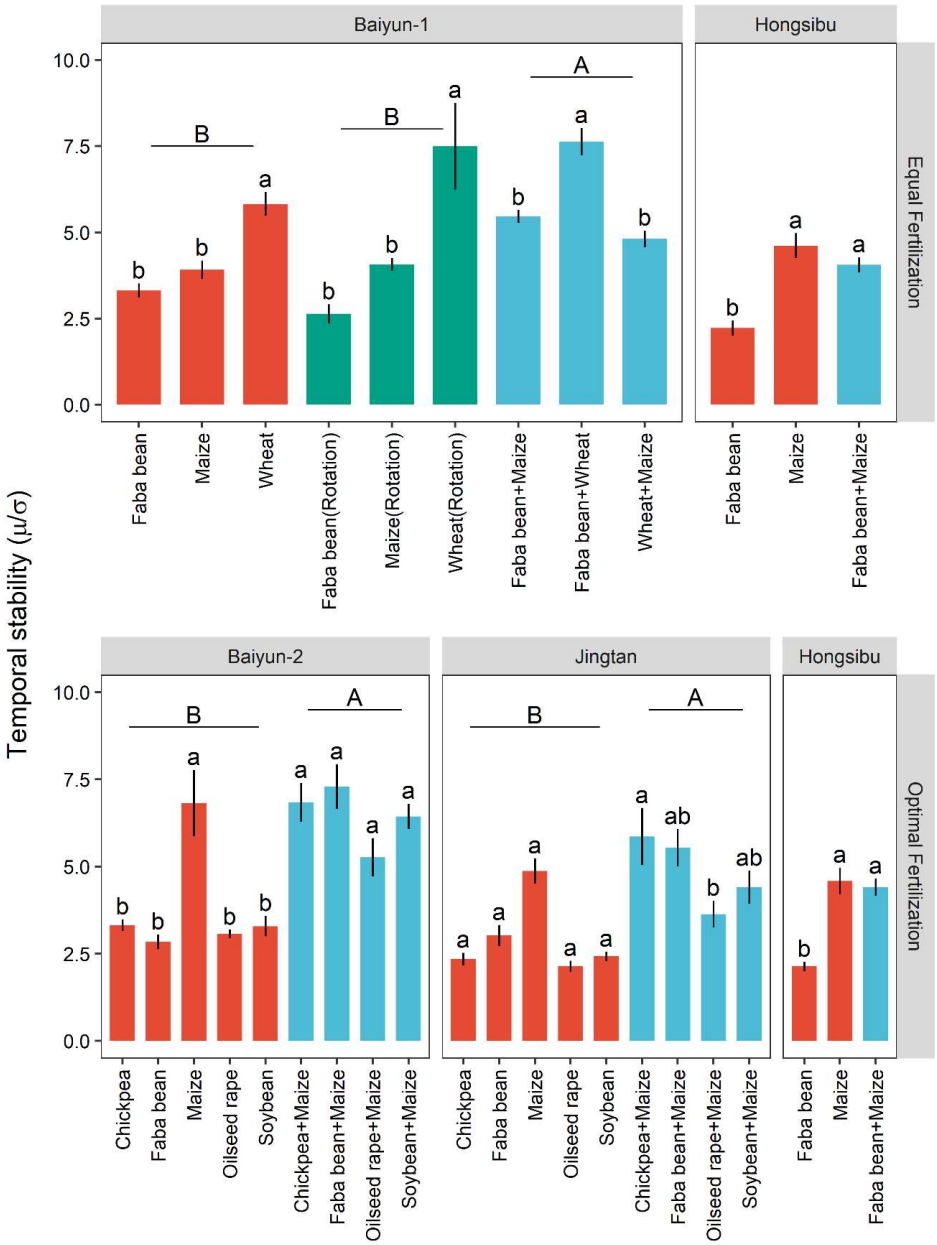
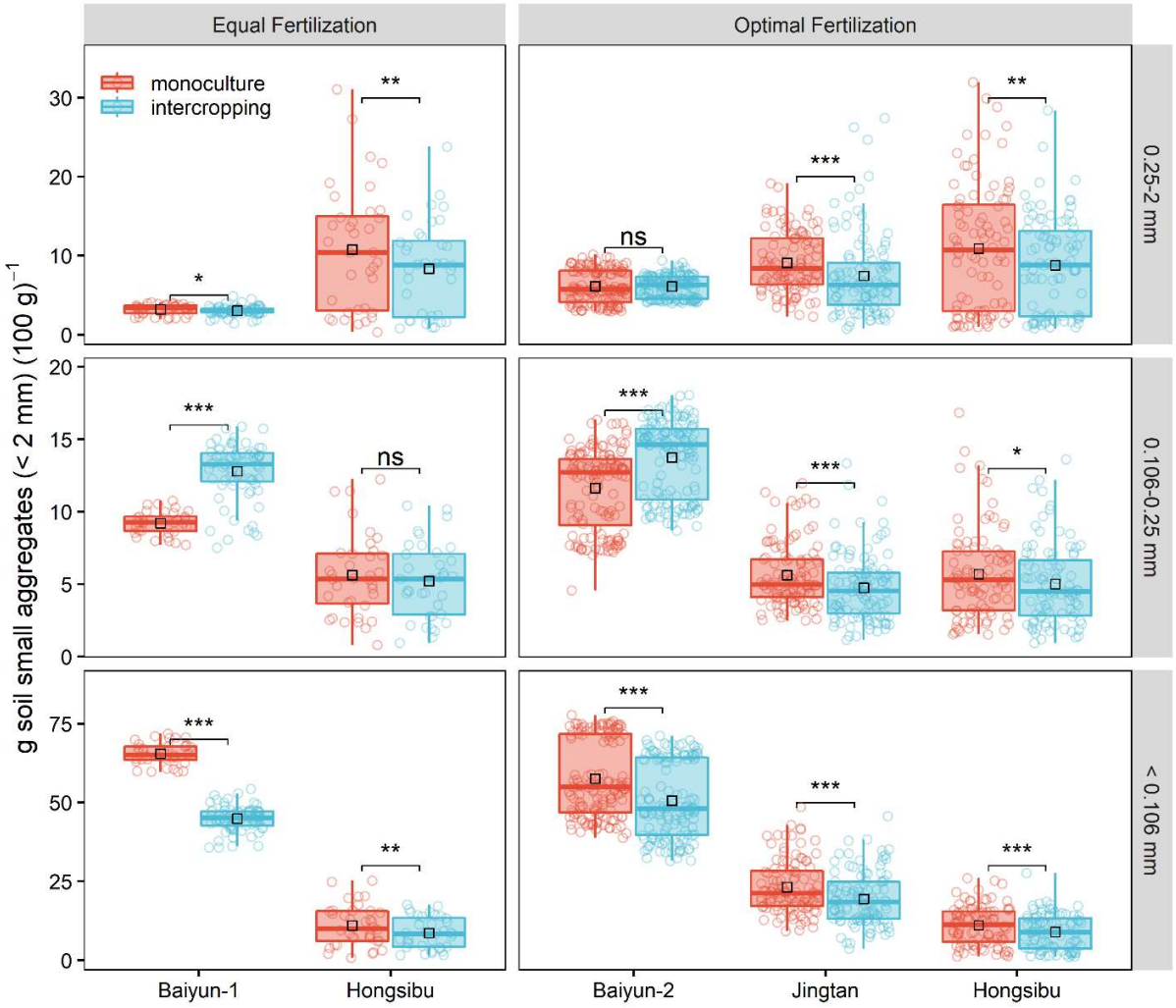


Supplementary Fig. 1. Intercropping of wheat/maize (a), chickpea/maize (b), oilseed rape/maize (c), soybean/maize (d), and faba bean/maize (e) involved in the long-term experiments. Photos credit: Long Li



Supplementary Fig. 2. Temporal stability (μ/σ) comparing intercropping and their respective sole or rotation crops in Equal Fertilization and Optimal Fertilization experiments from 2003/2009 to 2018. The experiment at Baiyun-1 was initiated in 2003, and the remaining of experiments at Baiyun-2, Jingtan, and Hongsibu were initiated in 2009. Equal Fertilization Experiments included Baiyun-1 and subset for Hongsibu at 75 and 150 kg N ha^{-1} yr^{-1} treatments, and Optimal Fertilization Experiments consisted of Baiyun-2, Jingtan and Hongsibu. Data show mean ± SE (n = 3). Temporal stability refers to the ratio of the average grain yield to its temporal standard deviation over the study period, determined after detrending. Different letters indicate significant differences between crop species (lowercase letters) or between cropping systems (capital letters) using Tukey HSD test.



Supplementary Fig. 3. Soil small aggregates (0.25-2, 0.106-0.25 and < 0.106 mm) comparing monoculture and intercropping system in Equal Fertilization and Optimal Fertilization experiments. The experiment at Baiyun-1 was initiated in 2003, and the remaining of experiments at Baiyun-2, Jingtan, and Hongsibu were initiated in 2009. Equal Fertilization Experiments included Baiyun-1 and subset for Hongsibu at 75 and 150 kg N ha^{-1} yr^{-1} treatments, and Optimal Fertilization Experiments consisted of Baiyun-2, Jingtan and Hongsibu. The data number for each aggregate class, each experiment and each site refer to Fig. 3. The solid lines and squares in the boxes, 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 quartiles (Q3), 1.5×Interquartile range (IQR) above the box's Q1 and 1.5×IQR below the box's Q3 of all data, respectively. Asterisks indicate significant differences between monoculture and intercropping in each experiment using Tukey HSD test. *$P < 0.05$; ** $P < 0.01$; ***$P < 0.001$; ns indicates non-significant.

Supplementary Table 1. Soil chemical properties at the beginning of experiments and site information in Equal Fertilization and Optimal Fertilization experiments in northwest China.

Equal or Optimal Fertilization	Experimental site	Location	Year of starting experiment	MAT	MAP	Evaporation capacity	SOM	TN	P	K	pH
				°C	mm	mm	g kg^{-1}	g kg^{-1}	mg kg^{-1}	mg kg^{-1}	
Equal Fertilization	Baiyun-1	38°37′ N, 102°40′ E	2003	7.7	150	2021	19.1	1.18	17.3	233	7.8
Optimal Fertilization	Baiyun-2	38°37′ N, 102°40′ E	2009	7.7	150	2021	18.5	1.08	20.3	220	8.0
Optimal Fertilization	Jingtan	37°05′ N, 104°40′ E	2009	6.6	200	2369	14.7	0.83	9.3	205	7.5
Equal and Optimal Fertilization	Hongsibu	38°37′ N, 104°40′ E	2009	8.9	185	2015	5.70	0.27	2.4	109	7.4

MAT = mean annual temperature; MAP = mean annual precipitation; SOM = soil organic matter; TN = total nitrogen; P = Olsen phosphorus; K = available potassium.

Supplementary Table 2. Information for the experimental design and fertilization treatments in Equal Fertilization and Optimal Fertilization experiments.

Equal or Optimal Fertilization	Experimental site	Crop	Cropping system			N fertilization	P fertilization	K fertilization	Inoculation
			monoculture	intercropping	rotation	kg N ha^{-1}	kg P ha^{-1}	kg K ha^{-1} yr^{-1}	
Equal Fertilization	Baiyun-1	Faba bean (*Vicia faba* L.)	Faba bean (F)	continuous, rotational F/M	W-F	225	40	50 since 2016	–
		Maize (*Zea mays* L.)	Maize (M)	continuous, rotational W/M	W-M				
		Wheat (*Triticum aestivum* L.)	Wheat (W)	continuous, rotational F/W	M-F				
					W-M-F				
Optimal Fertilization	Baiyun-2	Faba bean (*Vicia faba* L.)	Faba bean (F)	continuous F/M		225[1]	0, 40, and 80	50 since 2016	–
		Soybean [*Glycine max* (L.) Merrill]	Soybean (S)	continuous S/M					
		Chickpea (*Cicer arietinum* L.)	Chickpea (C)	continuous C/M					
		Oilseed rape (*Brassica napus* L.)	Oilseed rape (O)	continuous O/M					
		Maize (*Zea mays* L.)	Maize (M)						
Optimal Fertilization	Jingtan	Faba bean (*Vicia faba* L.)	Faba bean (F)	rotational F/M		225[1]	0, 40, and 80	50 since 2016	–
		Soybean [*Glycine max* (L.) Merrill]	Soybean (S)	rotational S/M					
		Chickpea (*Cicer arietinum* L.)	Chickpea (C)	rotational C/M					
		Oilseed rape (*Brassica napus* L.)	Oilseed rape (O)	rotational O/M					
		Maize (*Zea mays* L.)	Maize (M)						
Equal and Optimal Fertilization	Hongsibu	Faba bean (*Vicia faba* L.)	Faba bean (F)	continuous F/M		0, 75, 150, 225, and 300[2]	53	50 since 2016	+, -
		Maize (*Zea mays* L.)	Maize (M)						

Symbols '+' and '−' indicate with and without rhizobium inoculation, respectively. [1]a total of 225 kg N ha^{-1} fertilizer was applied to all treatments, except for monocultures of legumes and oilseed rape, which received 112.5 kg N ha^{-1} from 2009 to 2015 and 150 kg N

ha^{-1} fertilizer since 2016. ^{2}0, 75, 150, 225, and 300 kg N ha^{-1} yr^{-1} were used for monoculture of maize and intercropping; 0, 37.5, 75, 112.5, and 150 kg N ha^{-1} yr^{-1} for monoculture of faba bean.

Supplementary Table 3. Summary of analyses of experimental treatment effects on grain yield in Equal Fertilization and Optimal Fertilization experiments.

Equal or Optimal Fertilization	Experimental site	Source	numDF	denDF	*F*-value	*P*-value
Equal Fertilization	Baiyun-1	Crop combination (Cc)	2	235	655.8	< 0.0001
		Cropping system (Cs)	1	235	104.5	< 0.0001
	Hongsibu	Inoculation (I)	1	182	11.3	0.0009
		N application rate (N)	1	182	26.4	< 0.0001
		Cropping system (Cs)	1	182	26.1	< 0.0001
Optimal Fertilization	Baiyun-2	P application rate (P)	2	667	33.3	< 0.0001
		Crop combination (Cc)	3	667	20.9	< 0.0001
		Cropping system (Cs)	1	667	1066.4	< 0.0001
	Jingtan	P application rate (P)	2	667	0.5	0.6043
		Crop combination (Cc)	3	667	33.7	< 0.0001
		Cropping system (Cs)	1	667	127.5	< 0.0001
	Hongsibu	Inoculation (I)	1	494	3.8	0.0518
		N application rate (N)	4	494	54.1	< 0.0001
		Cropping system (Cs)	1	494	24.2	< 0.0001

numDF and denDF denote the numerator and denominator degrees of freedom, respectively.

Supplementary Table 4. Grain yields, overyielding and net profit of crops under monocultures and intercropping in Equal Fertilization and Optimal Fertilization experiments.

Equal or Optimal Fertilization	Experimental site	Crop	Grain yield (t ha^{-1})					Net profit (US$ ha^{-1}) [3]
			Mono-culture	Inter-cropped	Over-yielding % [1]	*P*	PLER [2]	Mono-culture
Equal Fertilization	Baiyun-1	Faba bean (with maize)	2.52	2.67	5.9	0.092	0.53	158
		Faba bean (with wheat)	2.52	2.39	-5.0	0.115	0.48	
		Maize (with faba bean)	13.46	17.95	33.3	< 0.001	0.69	2360
		Maize (with wheat)	13.46	16.93	25.7	< 0.001	0.64	
		Wheat (with faba bean)	5.49	6.18	12.5	< 0.001	0.57	337
		Wheat (with maize)	5.49	6.08	10.7	< 0.001	0.56	
	Hongsibu	Faba bean	2.25	3.52	56.4	< 0.001	0.66	29
		Maize	11.82	12.87	8.9	0.0013	0.75	1883
Optimal Fertilization	Baiyun-2	Chickpea	3.12	3.69	18.3	< 0.001	0.53	1315
		Faba bean	2.23	2.84	27.3	< 0.001	0.56	18
		Maize	13.14	17.79	35.3	< 0.001	0.75	2266
		Oilseed rape	1.09	1.44	31.2	< 0.001	0.54	-437
		Soybean	2.87	2.05	-28.5	< 0.001	0.35	-9
	Jingtan	Chickpea	2.70	2.32	-13.9	< 0.001	0.39	953
		Faba bean	3.53	4.85	37.7	< 0.001	0.59	968
		Maize	12.45	14.80	18.9	< 0.001	0.70	2064
		Oilseed rape	1.41	1.89	34.1	< 0.001	0.53	-203
		Soybean	3.08	2.26	-26.6	< 0.001	0.35	108
	Hongsibu	Faba bean	2.16	3.43	58.6	< 0.001	0.70	-33
		Maize	11.86	12.57	6.0	0.008	0.76	1894

Notes: 1) The overyielding (%) is calculated as (grain yield in intercropping (t/ha) – grain yield in monoculture (t/ha))/grain yield in monoculture (t/ha) *100 for given a crop or a given crop combination. 2) PLER is partial Land Equivalent Ratio, which is defined as the relative yields per crop species. 3) The profits of intercropped crops are not shown because the total expenditures including NP fertilizer and irrigation are difficult to partition into each crop within intercropping. Calculations for LER and net profit see 'Methods'.

Codes are as follows:

```
rm(list = ls())
library(nlme)
library(AICcmodavg)
library(emmeans)
library(multcompView)
library(purrr)
library(plyr)

# analyze grain yields
df <- read.csv("grain yield.csv", header = T)
head(df); str(df)
vars <- c("year", "N", "P", "block")
df[vars] <- lapply(df[vars], factor)
# Equal Fertilization & Baiyun-1
fit <- lme(yield ~ cs*cc,
        random = list(~1|block, ~1|year),
        data = subset(df, exp == "Equal Fertilization" &
                    site == "Baiyun-1"))
AICc(fit); anova(fit)
Y1_emm <- emmeans(fit, specs = ~ cs, adjust = "tukey",
                data = subset(df, exp == "Equal Fertilization" &
                            site == "Baiyun-1"))
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
remove(fit, Y1_emm)
# Equal Fertilization & Hongsibu-2
fit <- lme(yield ~ inoculation*N*cs,
        random = list(~1|block, ~1|year),
        data = subset(df, exp == "Equal Fertilization" &
                    site == "Hongsibu-2"))
AICc(fit); anova(fit)
Y1_emm <- emmeans(fit, specs = ~ cs, adjust = "tukey",
                data = subset(df, exp == "Equal Fertilization" &
                            site == "Hongsibu"))
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
remove(fit, Y1_emm)
# Optimal Fertilization & Baiyun-2
fit <- lme(yield ~ P*cc*cs,
        random = list(~1|block, ~1|year),
        data = subset(df, exp == "Optimal Fertilization" &
                    site == "Baiyun-2"))
AICc(fit); anova(fit)
Y1_emm <- emmeans(fit, specs = ~ cs, adjust = "tukey",
                data = subset(df, exp == "Optimal Fertilization" &
                            site == "Baiyun-2"))
```

```
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
remove(fit, Y1_emm)
# Optimal Fertilization & Jingtan
fit <- lme(yield ~ P*cc*cs,
          random = list(~1|block, ~1|year),
          data = subset(df, exp == "Optimal Fertilization" &
                        site == "Jingtan"))
AICc(fit); anova(fit)
Y1_emm <- emmeans(fit, specs = ~ cs, adjust = "tukey",
                  data = subset(df, exp == "Optimal Fertilization" &
                                site == "Jingtan"))
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
remove(fit, Y1_emm)
# Optimal Fertilization & Hongsibu
fit <- lme(yield ~ inoculation*N*cs,
          random = list(~1|block, ~1|year),
          data = subset(df, exp == "Optimal Fertilization" &
                        site == "Hongsibu"))
AICc(fit); anova(fit)
Y1_emm <- emmeans(fit, specs = ~ cs, adjust = "tukey",
                  data = subset(df, exp == "Optimal Fertilization" &
                                site == "Hongsibu"))
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
remove(fit, Y1_emm)
# yield trend analysis
subset(df, site %in% c("Baiyun-1", "Baiyun-2", "Jingtan", "Hongsibu-2")) %>%
   split(.$cs) %>%
   map(.,~lm(yield ~ year,.) %>%
         summary)
# ANCOVA
model <- lm(yield ~ year + cs +year:cs,
           data = subset(df, site %in% c("Baiyun-1", "Baiyun-2", "Jingtan", "Hongsibu-2")))
Anova(model, type = "2")
rm(list = ls())
cat("\014")


# analyze temporal stability
df <- read.csv("temporal stability.csv", header = T)
head(df)
# Equal Fertilization
fit <- lme(TSd ~ crop*cs*site,
          random = ~1|replicate,
          data = subset(df, exp == "Equal Fertilization"))
anova(fit); AICc(fit)
Y1_emm <- emmeans(fit, specs = ~ site, adjust = "tukey",
```

```
            data = subset(df, exp == "Equal Fertilization"))
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
remove(fit, Y1_emm)
# Equal Fertilization & Baiyun-1
fit <- lme(TSd ~ crop*cs,
        random = ~1|replicate,
        data = subset(df, exp == "Equal Fertilization" &
                    site == "Baiyun-1"))
anova(fit); AICc(fit)
Y1_emm <- emmeans(fit, specs = ~ crop, adjust = "tukey",
            data = subset(df, exp == "Equal Fertilization" &
                        site == "Baiyun-1"))
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
remove(fit, Y1_emm)
# Equal Fertilization & Hongsibu
fit <- lme(TSd ~ inoculation*crop*N*cs,
        random = ~1|replicate,
        data = subset(df, exp == "Equal Fertilization" &
                    site == "Hongsibu"))
anova(fit); AICc(fit)
Y1_emm <- emmeans(fit, specs = ~ crop, adjust = "tukey",
            data = subset(df, exp == "Equal Fertilization" &
                        site == "Hongsibu"))
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
remove(fit, Y1_emm)
# Optimal Fertilization
fit <- lme(TSd ~ P*crop*site*cs,
        random = ~1|replicate,
        data = subset(df, exp == "Optimal Fertilization"))
anova(fit); AICc(fit)
Y1_emm <- emmeans(fit, specs = ~ site, adjust = "tukey",
            data = subset(df, exp == "Optimal Fertilization"))
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
remove(fit, Y1_emm)
# Optimal Fertilization & Baiyun-2
fit <- lme(TSd ~ P*crop*cs,
        random = ~1|replicate,
        data = subset(df, exp == "Optimal Fertilization" &
                    site == "Baiyun-2"))
anova(fit); AICc(fit)
Y1_emm <- emmeans(fit, specs = ~ crop, adjust = "tukey",
            data = subset(df, exp == "Optimal Fertilization" &
                        site == "Baiyun-2"))
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
remove(fit, Y1_emm)
# Optimal Fertilization & Jingtan
```

```
fit <- lme(TSd ~ P*crop*cs,
        random = ~1|replicate,
        data = subset(df, exp == "Optimal Fertilization" &
                    site == "Jingtan"))
anova(fit); AICc(fit)
Y1_emm <- emmeans(fit, specs = ~ crop, adjust = "tukey",
              data = subset(df, exp == "Optimal Fertilization" &
                          site == "Jingtan"))
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
remove(fit, Y1_emm)
# Optimal Fertilization & Hongsibu
fit <- lme(TSd ~ P*crop*cs,
        random = ~1|replicate,
        data = subset(df, exp == "Optimal Fertilization" &
                    site == "Hongsibu"))
anova(fit); AICc(fit)
Y1_emm <- emmeans(fit, specs = ~ crop, adjust = "tukey",
              data = subset(df, exp == "Optimal Fertilization" &
                          site == "Hongsibu"))
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
rm(list = ls())
cat("\014")


# analyze soil aggregate
df <- read.csv("temporal stability.csv", header = T)
head(df); str(df)
vars <- c("year", "N", "P", "block")
df[vars] <- lapply(df[vars], factor)
# > 2mm
# Equal Fertilization & Baiyun-1
fit <- lme(weight ~ cs*cc,
        random = list(~1|block, ~1|year),
        data = subset(df, exp == "Equal Fertilization" &
                    class == "2" &
                    site == "Baiyun-1"))
AICc(fit); anova(fit)
Y1_emm <- emmeans(fit, specs = ~ cs, adjust = "tukey",
              data = subset(df, exp == "Equal Fertilization" &
                          class == "2" &
                          site == "Baiyun-1"))
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
remove(fit, Y1_emm)
# Equal Fertilization & Hongsibu
fit <- lme(weight ~ cs*inoculation*N,
        random = list(~1|block, ~1|year),
```

```
        data = subset(df, exp == "Equal Fertilization" &
                      class == "2" &
                      site == "Hongsibu"))
AICc(fit); anova(fit)
Y1_emm <- emmeans(fit, specs = ~ cs, adjust = "tukey",
                  data = subset(df, exp == "Equal Fertilization" &
                                class == "2" &
                                site == "Hongsibu"))
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
remove(fit, Y1_emm)
# Optimal Fertilization & Baiyun-2
fit <- lme(weight ~ cs*cc*P,
        random = list(~1|block, ~1|year),
        data = subset(df, exp == "Optimal Fertilization" &
                      class == "2" &
                      site == "Baiyun-2"))
AICc(fit); anova(fit)
Y1_emm <- emmeans(fit, specs = ~ cs, adjust = "tukey",
                  ata = subset(df, exp == "Optimal Fertilization" &
                               class == "2" &
                               site == "Baiyun-2"))
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
remove(fit, Y1_emm)
# Optimal Fertilization & Jingtan
fit <- lme(weight ~ cs*cc*P,
        random = list(~1|block, ~1|year),
        data = subset(df, exp == "Optimal Fertilization" &
                      class == "2" &
                      site == "Jingtan"))
AICc(fit); anova(fit)
Y1_emm <- emmeans(fit, specs = ~ cs, adjust = "tukey",
                  ata = subset(df, exp == "Optimal Fertilization" &
                               class == "2" &
                               site == "Jingtan"))
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
remove(fit, Y1_emm)
# Optimal Fertilization & Hongsibu
fit <- lme(weight ~ cs*inoculation*N,
        random = list(~1|block, ~1|year),
        data = subset(df, exp == "Optimal Fertilization" &
                      class == "2" &
                      site == "Hongsibu"))
AICc(fit); anova(fit)
Y1_emm <- emmeans(fit, specs = ~ cs, adjust = "tukey",
                  ata = subset(df, exp == "Optimal Fertilization" &
                               class == "2" &
```

```
                        site == "Hongsibu"))
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
remove(fit, Y1_emm)
rm(list = ls())
cat("\014")


# analyze soil properties
df <- read.csv("soil properties.csv", header = T)
head(df); str(df)
# Equal Fertilization & SOM
fit <- lme(value ~ cs*site,
        random = list(~1|replicate, ~1|year, ~1|cc, ~1|N, ~1|inoculation),
        data = subset(df, exp == "Equal Fertilization" &
                    variable == "SOM"))
anova(fit); AICc(fit)
Y1_emm <- emmeans(fit, specs = ~ cs, adjust = "tukey",
              data = subset(df, exp == "Equal Fertilization" &
                          variable == "SOM"))
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
# Equal Fertilization & TN
fit <- lme(value ~ cs*site,
        random = list(~1|replicate, ~1|year, ~1|cc, ~1|N, ~1|inoculation),
        data = subset(df, exp == "Equal Fertilization" &
                    variable == "TN"))
anova(fit); AICc(fit)
Y1_emm <- emmeans(fit, specs = ~ cs, adjust = "tukey",
              data = subset(df, exp == "Equal Fertilization" &
                          variable == "TN"))
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
# Equal Fertilization & pH
fit <- lme(value ~ cs*site,
        random = list(~1|replicate, ~1|year, ~1|cc, ~1|N, ~1|inoculation),
        data = subset(df, exp == "Equal Fertilization" &
                    variable == "pH"))
anova(fit); AICc(fit)
Y1_emm <- emmeans(fit, specs = ~ cs, adjust = "tukey",
              data = subset(df, exp == "Equal Fertilization" &
                          variable == "pH"))
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
# change Equal to Optimal to analyze Optimal exps
rm(list = ls())
cat("\014")

# analyze soil properties
df <- read.csv("net profit.csv", header = T)
```

```
head(df); str(df)
# Faba bean+Maize
fit <- lme(value ~ cs*site,
        random = list(~1|replicate, ~1|cc),
        data = subset(df, cc == "faba+maize" &
                site %in% c("Baiyun-1", "Baiyun-2",
                        "Jingtan", "Hongsibu-2")))
anova(fit); AICc(fit)
Y1_emm <- emmeans(fit, specs = ~ cs, adjust = "tukey",
            data = subset(df, cc == "faba+maize" &
                    site %in% c("Baiyun-1", "Baiyun-2",
                            "Jingtan", "Hongsibu-2")))
multcomp::cld(Y1_emm, alpha = 0.05, Letters = letters)
# change faba+maize to chickpea+maize, faba+wheat, wheat+maize...
rm(list = ls())
cat("\014")
```