Supplementary information

Consequences of thirty-three years of plastic film mulching and nitrogen fertilization on maize growth and soil quality

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Summary:

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Section S1. Root P measurement

Dry roots were ground and passed through a 0.25 mm sieve. 0.1 g dry roots were digested with a combination of H_2SO_4 and H_2O_2 (8:5) to make the various forms of phosphorus in the digested plant roots were converted into phosphate. 2mL digestion solution were transferred into volumetric flask. Then, dinitrophenol indicator, NaOH and H_2SO_4 were successively added into the bottles. 5 mL Mo-Sb-Vc colorize the solution. Then the absorbance was measured at 700 nm by Unic-7200 spectrophotometer (Shanghai, China) [1].

Section S2. Soil and root phosphatase measurements

Soil acid phosphatase activity (AcP) and root-associated phosphatase activity (APase) were measured following the spectrophotometer method in Lin, et al. [2]. Briefly, 1 g fresh soil or 0.2 g fresh roots (< 2mm) were transferred into a centrifuge tube containing 50 m*M* acetate buffer (pH = 5.0). Then, 5 m*M* *p*-nitrophenyl phosphate (*p*NPP) was added to the centrifuge tube as the reaction substrate. The centrifuge tube was kept in the dark at 20 ℃ for 1 hour, until stopping the reaction by adding 0.5 *M* NaOH and 0.5 *M* $CaCl_2$. Absorbance of *p*-nitrophenol (*p*NP) in the supernatant was then measured at 410 nm by a Unic-7200 Spectrophotometer (Shanghai, China).

Section S3. Soil Olsen-P measurement

2.5 g dry soil were extracted with 0.5 M $NaHCO_3$ and then 5 mL Mo-Sb-Vc were added to colorize the solution. Then the absorbance was measured at 700 nm by Unic-7200 spectrophotometer (Shanghai, China) [1].

Section S4. Soil bulk density, total soil porosity, and soil water holding capacity measurements

Soil from 0-5 cm soil layer were collected with cutting-rings. Cutting-rings were placed in a flat bottomed container filled with water and absorbed water for 12 hours. Afterwards, the cutting-rings containing water were placed in a flat bottomed container filled with dry sand and drained for 2 hours. After continuing to drain for days and nights, finally all the soil was placed in an aluminum box and dried at a 105 ℃ to constant weight in an oven. Bulk density is calculated as the mass of dried soil per unit of soil volume. Field water capacity is calculated the water loss during the drying process. Total porosity is calculated through the transformation from bulk density [3]

Table S1 Soil moisture and *P* values for effect of plastic film mulching, nitrogen fertilization, and their interactions by two-way ANOVA.

Treatments	Days after planting (growth period)									
	Day 34	Day 41	Day 48	Day 55	Day 62	Day 76	Day 90	Day 104	Day 125	Day 146
N_0-NeverPFM	19.11±1.67	20.45±0.80	20.85±0.53	18.67±0.30	30.8±0.33	19.09±0.76	9.68±0.07	25.88±0.82	15.56±0.28	27.28±0.77
N_{135}-NeverPFM	14.89±0.29	19.29±1.47	19.01±0.44	21.28±1.43	25.30±1.71	17.44±1.52	9.03±0.24	20.61±0.18	13.09±0.49	21.49±1.22
N_0-PrevPFM	20.28±1.02	22.37±1.28	20.47±1.04	22.67±0.41	32.03±0.62	21.16±1.58	11.09±0.19	28.49±0.81	16.33±0.58	27.87±1.03
N_{135}-PrevPFM	16.34±1.65	23.89±1.10	17.92±0.36	19.47±1.06	29.96±1.3	18.87±0.99	9.71±0.25	22.98±0.17	14.33±0.55	28.01±0.59
Sig (*P* value)										
N	0.01	0.88	< 0.01	0.76	0.01	0.16	< 0.01	< 0.01	< 0.01	0.02
PFM	0.34	0.03	0.29	0.27	0.03	0.20	< 0.01	< 0.01	0.07	< 0.01
N*PFM	0.92	0.29	0.60	0.01	0.17	0.81	0.10	0.84	0.65	0.01

N_0: zero N fertilizer, N_{135}: 135 kg N ha^{-1} yr^{-1}, PrevPFM: previous plastic film mulching, NeverPFM: never plastic film mulching.

Data are mean ± standard errors of the replicates (n = 3).

Table S2 *P* values for effect of plastic film mulching, nitrogen fertilization, and their interactions on crop and soil properties by two-way ANOVA.

	Aboveground biomass (g plant^{-1})	Belowground biomass (g plant^{-1})	Chl (μg cm^{-2})	Flv (μg cm^{-2})	pH	NH_4^+ (mg N kg^{-1})	NO_3^- (mg N kg^{-1})	AcP (μmol g^{-1}(dw) h^{-1})	Olsen-P (mg kg^{-1})	APase (μmol g^{-1}(fw) h^{-1})	Root P (%)
Sixth leaf stage											
N	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.43	< 0.01	0.60	< 0.01	0.04	0.07
PFM	0.04	0.14	0.13	0.28	0.04	0.69	0.03	0.13	0.35	0.72	0.34
N*PFM	0.96	0.72	0.05	0.18	0.00	0.06	0.01	0.90	0.18	0.47	0.54
Tasseling stage											
N	0.40	0.48	< 0.01	< 0.03	0.00	0.54	< 0.01	0.32	0.06	< 0.01	0.09
PFM	0.71	0.45	0.91	0.32	0.22	0.93	< 0.01	0.18	0.12	0.54	0.46
N*PFM	0.15	0.07	0.03	0.24	0.24	0.49	< 0.01	0.96	0.45	0.72	0.32
Physiological maturity stage											
N	0.90	0.23	< 0.01	0.65	< 0.01	0.79	0.20	0.09	0.10	0.02	< 0.01
PFM	0.67	0.18	0.36	0.90	0.06	0.25	0.44	0.12	0.59	0.44	0.84
N*PFM	0.55	0.57	0.05	0.63	0.00	0.28	0.01	0.56	0.30	0.95	0.30

Chl: chlorophyll, Flv: flavonoid, AcP: soil acid phosphatase, APase: root-associated phosphatase activity.

Table S3 Soil physical properties and *P* values for effect of plastic film mulching, nitrogen fertilization, and their interactions by two-way ANOVA.

Treatments	Bulk density (g cm^3)	Porosity (%)	Water holding capacity (%)
Sixth leaf stage			
N_0-NeverPFM	1.28 ±0.00	48.36 ±0.13	35.32 ±0.19
N_{135}-NeverPFM	1.25 ±0.03	49.78 ±1.34	37.32 ±1.62
N_0-PrevPFM	1.28 ±0.03	46.98 ±0.24	34.69 ±0.78
N_{135}-PrevPFM	1.32 ±0.01	46.89 ±0.06	33.12 ±0.43
Sig (*P* value)			
N	0.77	0.36	0.83
PFM	0.11	0.01	0.03
N*PFM	0.09	0.30	0.09
Tasseling stage			
N_0-NeverPFM	1.26 ±0.03	51.26 ±1.14	31.61 ±0.25
N_{135}-NeverPFM	1.27 ±0.05	48.30 ±1.30	32.61 ±2.21
N_0-PrevPFM	1.28 ±0.02	49.90 ±1.96	33.06 ±0.27
N_{135}-PrevPFM	1.24 ±0.01	51.66 ±0.26	33.44 ±0.38
Sig (*P* value)			
N	0.69	0.66	0.56
PFM	0.87	0.47	0.35
N*PFM	0.41	0.11	0.79
Physiological maturity stage			
N_0-NeverPFM	1.28 ±0.02	47.18 ±3.53	33.11 ±3.46
N_{135}-NeverPFM	1.26 ±0.04	46.03 ±1.16	31.38 ±1.38
N_0-PrevPFM	1.35 ±0.04	43.28 ±1.55	28.94 ±0.65
N_{135}-PrevPFM	1.37 ±0.02	43.91 ±0.75	28.82 ±0.50
Sig (*P* value)			
N	0.88	0.90	0.64
PFM	0.03	0.18	0.12
N*PFM	0.51	0.68	0.69

N_0: zero N fertilizer, N_{135}: 135 kg N ha^{-1} yr^{-1}, PrevPFM: previous plastic film mulching, NeverPFM: never plastic film mulching. Data are mean ± standard errors of the replicates (n = 3).

Table S4 The data of extractable soil Fe (DTPA-Fe (mg kg^{-1})) concentrations and *P* values for effect of plastic film mulching, nitrogen fertilization, and their interactions by two-way ANOVA.

Treatments	Depth		
	0-20 cm	20-40 cm	40-60 cm
N_0-NeverPFM	14.60 ±0.21	10.89 ±0.39	9.53 ±1.21
N_{135}-NeverPFM	36.37 ±2.80	14.72 ±1.94	9.08 ±1.25
N_0-PFM	16.68 ±1.82	8.07 ±0.38	6.96 ±0.11
N_{135}-PFM	28.01 ±2.46	10.70 ±0.71	8.51 ±0.78
Sig (*P* value)			
N	< 0.01	0.02	0.58
PFM	0.17	0.01	0.14
N*PFM	0.04	0.59	0.33

N_0: zero N fertilizer, N_{135}: 135 kg N ha^{-1} yr^{-1}, PFM: plastic film mulching, NeverPFM: never plastic film mulching. Data are mean ± standard errors of the replicates (*n* = 3).

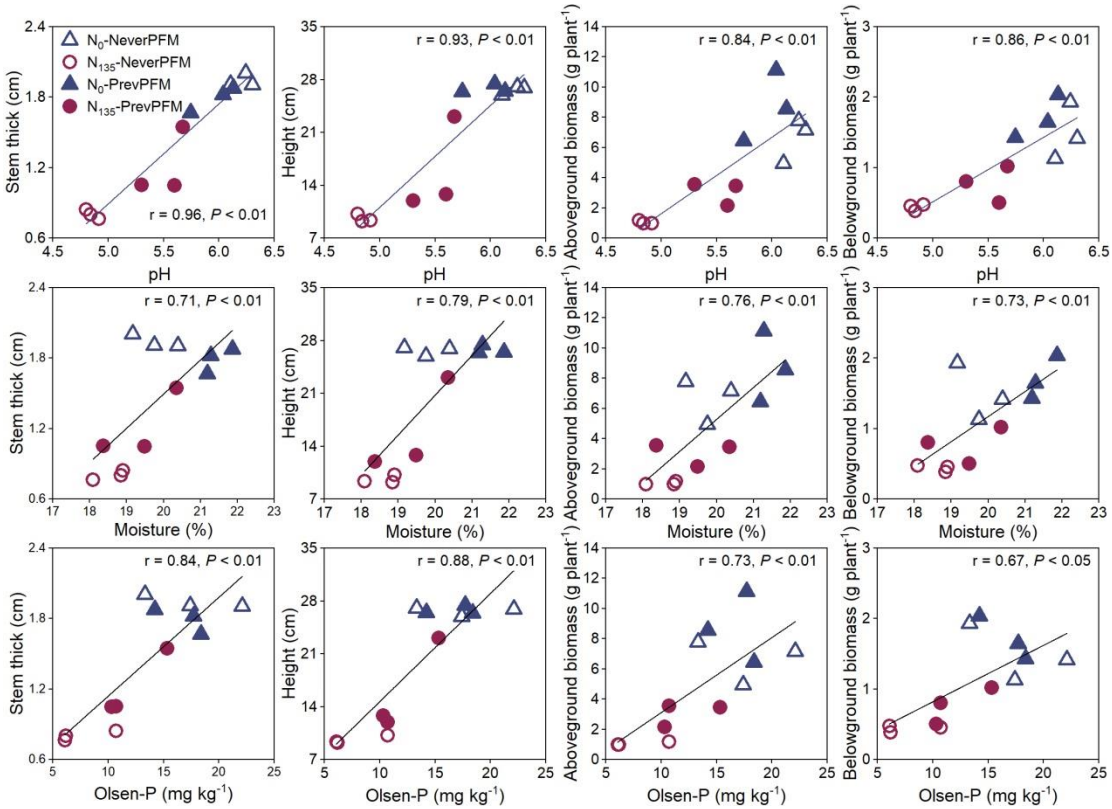

Fig. S1 The correlations of maize above- and below-ground growth parameters with soil pH, soil moisture and Olsen-P at the sixth leaf stage.

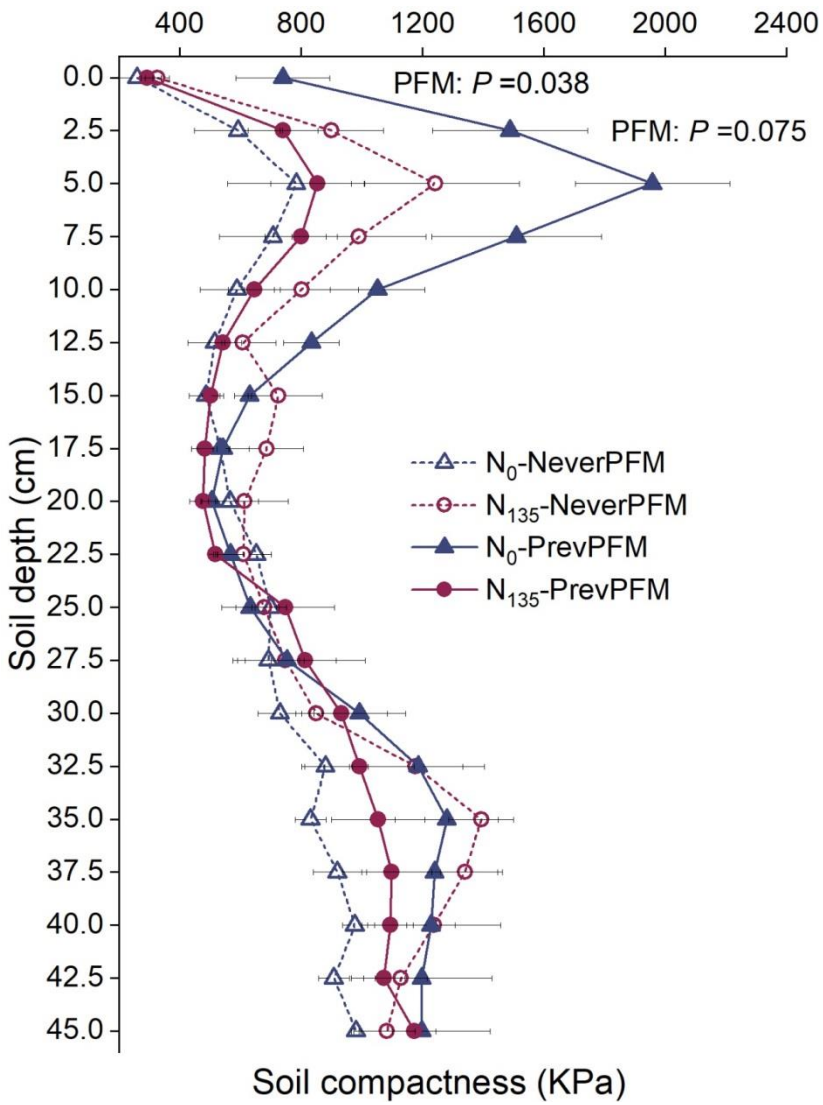

Fig. S2 Soil compactness through soil profile under the combined plastic film mulching and fertilization with urea-nitrogen (N) treatments. The *P* values behind PFM indicated the significance of main effect of plastic film mulching on soil compactness.

References

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