



Macro- and microplastic accumulation in soil after 32 years of plastic film mulching

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1 **33 years of plastic film mulching does not have negative legacy for succeeding**
2 **maize growth and yield**

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15

16 **Abstract:**

17

18 **1. Introduction**

19 In the Anthropocene, human activities and artificial products profoundly change
20 the earth. Plastic, as an artificially synthesized compound, is now ubiquitous on the
21 earth even near the top of Mount Everest, the highest peak in the world ([Napper et al.,](#)

22 [2020](#)). In the recent decades, plastic pollution has attracted great attention due to its
23 potential ecological and environmental implications on global scale ([Jambeck et al.,](#)
24 [2015](#)). Consequently, plastic pollution was recently listed as one of the top 10 global
25 environmental problems by the United Nations Environment Programme ([UNEP,](#)
26 [2014](#)). Compared with plastic pollution of oceans and freshwater, little is known
27 about plastic pollution of terrestrial ecosystems ([Bläsing & Amelung, 2018,](#) [Rillig &](#)
28 [Lehmann, 2020](#)). Due to the widespread use of plastic mulch, shed plastic film, and
29 biosolids ([Duis & Coors, 2016,](#) [John & Wang, 2021,](#) [Ng et al., 2018](#)), croplands have
30 been identified as a major reservoir of plastic debris ([Nizzetto et al., 2016](#)). Due to the
31 plastic film residues accumulation negatively impacting soil health, plastic pollution
32 in croplands has potential to threaten long-term food security ([Zhang et al., 2020](#)).

33 Plastic film mulching (PFM) is widely used in global agricultural ecosystems
34 to improve plant growth because of increasing soil temperature and moisture ([Ma et](#)
35 [al., 2018,](#) [Wang et al., 2021,](#) [Zhou et al., 2009](#)). A recent meta-analysis showed that
36 PFM increased crop yields by 24% on average ([Gao et al., 2019](#)). However, as the use
37 time of mulch film increases, there are more and more plastic residues remaining in
38 the soil, because plastic films are not possible to be removed clearly, especially for
39 thin films (i.e., 5~8 μm thick) used in some countries, e.g., China ([Ding et al., 2021](#)).
40 Our recent study showed that the residues of plastic film (size > 5 mm) were as high
41 as 360 kg hm^{-2} and microplastics (< 5 mm) exceeded 10000 items by 1 kg soil in
42 0~20 cm layer after 32 years of plastic film mulching ([Li et al., 2022b](#)). The residual
43 plastic accumulated over a certain value in the soil could decrease soil pore

44 connectivity and porosity ([Yan et al., 2006](#)), thus affecting the movement of nutrients
45 and water in the soil ([Li et al., 2020](#)). Thus, the germination of crop seeds and the
46 development of roots would be also seriously affected by the residual film ([Hu et al.,
47 2020a](#), [Hu et al., 2020b](#)). Moreover, polyethylene (PE) film-derived plastic fragments
48 and microplastic accumulation in soil may change soil water retention, water
49 evaporation, or soil water repellency ([Machado et al., 2018](#), [Steinmetz et al., 2016](#),
50 [Wan et al., 2019](#)). Therefore, long-term PFM is expected to leave a negative legacy
51 for crop growth and yield.

52 There are many studies exploring the effect of plastic residual film or PE
53 microplastic accumulation in soil on crop performance, and the results are
54 inconsistent ([Li et al., 2022a](#)). [Hu et al. \(2020b\)](#) showed that maize yield was
55 decreased by 15~18% and 23~25%, when added plastic film residues with 300 and
56 600 kg ha⁻¹, respectively. A meta-analysis showed a reduction of yield by 3% for
57 cotton but hardly any effect for potato and maize when an increase of 100 kg ha⁻¹ of
58 residual film, through building regression relationships between yield and the amount
59 of residual film ([Zhang et al., 2020](#)). Negative ([Pehlivan & Gedik, 2021](#)), and no
60 ([Colzi et al., 2022](#), [Qi et al., 2018](#), [Wang et al., 2020](#)) impacts of PE microplastic on
61 crop performance effect have all been reported. However, all the above results are
62 based on the artificial addition of plastic into soils, which may be not in line with the
63 actual situation. The reason is that plastic film in the field has a complicated
64 fragmentation and degradation process, which needs a relatively long time. To our
65 knowledge, there is no evaluation for the legacy of long-term PFM on succeeding

66 crop growth and yield.

67 Our study evaluated the legacy effects of 33 years of PFM on soil properties,
68 succeeding maize growth, and yield in a continuous plastic film mulching and
69 fertilization experiment initiated in 1987. To investigate the legacy effect, previous
70 mulching plots were not covered with polyethylene film in 2021 and never mulching
71 plots were set as control. Maize aboveground and belowground growth indexes (stem
72 thick, height, chlorophyll, flavonoid, root-associated phosphatase activity, root P, root
73 morphological characteristics parameter, and biomass) and soil basic physical and
74 chemical properties were measured at the six leaf stage, tasseling stage, and
75 physiological maturity stage. Maize yield and ripening time were measured at the end
76 of the growing season. The aim is to test the hypothesis: long-term PFM would have a
77 negative legacy on maize growth and yield, due to large amounts of plastic and
78 microplastic accumulation in soil.

79 **2. Materials and methods**

80 **2.1 Study site and experiment design**

81 The experimental field site was the long-term plastic film mulching and
82 fertilization station (built in 1987) at Shenyang Agriculture University (41°49'N,
83 123°34'E) in Shenyang, Liaoning Province, China. This site has a temperate
84 continental monsoon climate, with a mean annual temperature of 7.9 °C and average
85 annual rainfall of about 705 mm. The soil is a brown earth according to Chinese Soil
86 Taxonomy (a Haplic-Udic Alfisol according to US Soil Taxonomy). The experiment

87 was arranged in a factorial design with two levels of plastic film mulching (with and
88 without) and two levels of N fertilizer, that produces a combination of 4 treatments
89 with three replicates by treatment. The fertilizer levels included (i) zero N fertilizer
90 (N_0) and (ii) $135 \text{ kg N ha}^{-1} \text{ year}^{-1}$ application (N_{135}). Each plot has an area of 69 m^2 .
91 The N fertilizer was urea powder, applied as basal fertilizer in spring. There was no
92 fertilization with other nutrients (e.g., phosphorus) in any of the plots. Maize (*Zea*
93 *may* L.) was selected for this experiment because it is one of the major crops grown in
94 northeast China. Seeds of maize were sown at an approximate density of 50000 plants
95 per hectare. A detailed description of the experiment can be found in [Ding et al.](#)
96 [\(2019\)](#). In order to investigate the legacy effect of previous PFM, two ridges ($5 \text{ m} \times 2$
97 m) were randomly selected at the plots with PFM to stop covering with plastic film in
98 2021, which are called as previous PFM (PrevPFM). The plots without PFM were set
99 as control, which are called as never PFM (NeverPFM). Soil properties and maize
100 growth at the N_0 and N_{135} plots under previous and never plastic film mulching
101 treatments (called as N_0 -PrevPFM, N_{135} -PrevPFM, N_0 -NeverPFM, N_{135} -NeverPFM,
102 respectively) were measured during the growth season in 2021.

103 **2.2 Sampling and measurements**

104 Soil moisture, plant height, and stem thick were measured every 7 days from
105 June to July, every 14 days from July to August, and every 21 days from August to
106 September. Soil moisture was measured at a depth of 10 cm using a moisture probe
107 (Trime ®-Pico 64/32, IMKO GmbH, Ettlingen, Germany). Three plants were

108 randomly selected from each plot, plant height was measured from the base to the
109 highest with steel tape, and stem thick which was the middle diameter of the second
110 aboveground section was measured with a vernier caliper.

111 Leaf pigment, above- and below-ground biomass, root morphological properties,
112 root phosphorus concentration, and its-associated phosphatase activity were measured
113 at the sixth leaf stage (V6, the key period from vegetative to reproductive growth,
114 about 48 days after seeding), tasseling stage (VT, the period when the plant reaches its
115 full height and begins to shed its pollen, about 90 days after seeding), and
116 physiological maturity stage (R6, about 149 days after seeding). The sampling dates
117 for the three stages were the time when more than 80 % of the plants are in that stage.
118 Chlorophyll and flavonoid contents were measured for the third fully expanded
119 mature leaf from top to bottom for a selected plant at 9:00-11:30 in the morning using
120 a Dualex Scientific + device (Force-A, Orsay, France). Two plants were sampled from
121 each plot, and then divided into aboveground and belowground tissues by cutting the
122 first section of the stem with a sickle. Plant tissues were oven-dried at 60°C to
123 constant weight. At each plot, two plant roots in each plot were randomly sampled by
124 digging up the soil adjacent to the main trunk up to a radius of 15 cm and a depth of
125 40 cm and collected all scattered roots. The roots were washed with tap water to
126 remove soil and then wash it with ultrapure water for 3~5 times. One plant root was
127 cut into parts, and measured by a root scanner (EPSON Expression 11000XL) and an
128 image analyzer (EPSON Expression 11000XL) for root morphology, including total
129 root length, total surface area, total volume. Scanned roots were dried to a constant

130 mass at 60°C and then weighed. Dry roots were crushed pulverizer and passed
131 through a 0.25mm mesh for the determination of root phosphorus (root P), which was
132 digested by combination of H₂SO₄ and H₂O₂ (8:5). The other root was used to
133 determined root-associated phosphatase activity (APase).

134 Meanwhile, soil samples were collected at 0~20 cm layer for the measurements
135 of pH, soil phosphorus, soil acid phosphatase (AcP), ammonium (NH₄⁺-N) and nitrate
136 nitrogen (NO₃⁻-N) contents, bulk density, total porosity, and water holding capacity at
137 the three corresponding crop stages. Three soil cores were randomly sampled using an
138 auger (4 cm in diameter) and then mixed into one sample at each plot. Soil samples
139 were passed through a 2-mm sieve to remove plant debris and gravel. One part was air
140 dried under natural conditions to determine the soil pH and plant-available soil
141 phosphorus (Olsen-P), and the other part of fresh soil is used to determine soil acid
142 phosphatase (AcP), ammonium nitrogen (NH₄⁺-N) and nitrate nitrogen (NO₃⁻-N). Soil
143 pH was measured by a glass electrode in a 1:2.5 soil/distilled water suspension after
144 shaking. Olsen-P concentration was measured after being extracted with 0.5 M
145 NaHCO₃ according to the colorimetric method ([Bao, 2000](#)). Soil NH₄⁺-N and NO₃⁻-N
146 were extracted with 10 mM CaCl₂ (soil: water = 1:10) and measured by a continuous
147 flow analyzer (Bran-Luebbe AA3, Germany). Soil bulk density, total soil porosity,
148 and soil water holding capacity were determined according to the methods in [Chen](#)
149 [\(2005\)](#). After crop harvest in autumn, soil compactness was measured using a soil
150 compaction meter (Spectrum SC 900in, United States). The conical head was pushed
151 down at a constant speed and inserted into the soil with 45 cm depth, and data was

152 automatically read and recorded.

153 Soil acid phosphatase activity and root-associated phosphatase activity were
154 measured following the methods in [Lin et al. \(2020\)](#). Briefly, 1g fresh soil or 0.2g
155 fresh roots (< 2mm) were transferred into a centrifuge tube containing 50 mM acetate
156 buffer (pH = 5.0). Then, 5 mM *p*-nitrophenyl phosphate (*p*NPP) was added to the
157 centrifuge tube as the reaction substrate. The centrifuge tube was cultured in the dark
158 at 20°C for 1h, and then the reaction was stopped by 0.5 M NaOH and 0.5 M CaCl₂.
159 Then, the absorbance of *p*-nitrophenol (*p*NP) in the supernatant was measured at 410
160 nm by Unic-7200 Spectrophotometer (Shanghai, China). Four analytical replicates
161 were used for each root sample, including a blank. For blank, *p*NPP was added after
162 NaOH and CaCl₂ stopped the reaction. The concentration of *p*NP is obtained by the
163 standard curve between the configured *p*NP concentration and the absorbance value.
164 Soil phosphatase activity is expressed by *p*NP produced in the above reaction divided
165 by reaction time and dry weight. Root-associated phosphatase activity is expressed by
166 *p*NP produced in the above reaction divided by reaction time and fresh weight.

167 Moreover, we observed and recorded the time when maize entered into dough
168 stage, which is defined as the time when most kernels are becoming a consistency
169 similar to dough and accumulate almost 50% of the dry mass ([Guo et al., 2004](#)). At
170 the physiological maturity stage, the yield was measured through randomly selecting
171 four plants at the middle position at each plot. The 100-seed fresh weight and the
172 length of the maize cob were recorded. Maize ears were dried at 60 °C to constant
173 weight in an oven and then used to obtain the yield.

174 **2.3 Statistical analyses and calculations**

175 The effects of PFM (PrevPFM and NeverPFM), N fertilization (N₀ and N₁₃₅) and
176 their interactions on soil and crop parameters were assessed by two-way ANOVA.
177 Normality of residuals and homogeneity of the variances of the residuals across
178 groups were checked for each ANOVA. Pearson correlation analyses were conducted
179 between plant growth parameters and soil properties at the sixth leaf stage, tasseling
180 stage, and physiological maturity stage, respectively. We found the three soil
181 parameters (i.e., pH, moisture, and Olsen-P concentrations) were well correlated with
182 most plant growth parameters, especially at six leaf stage.

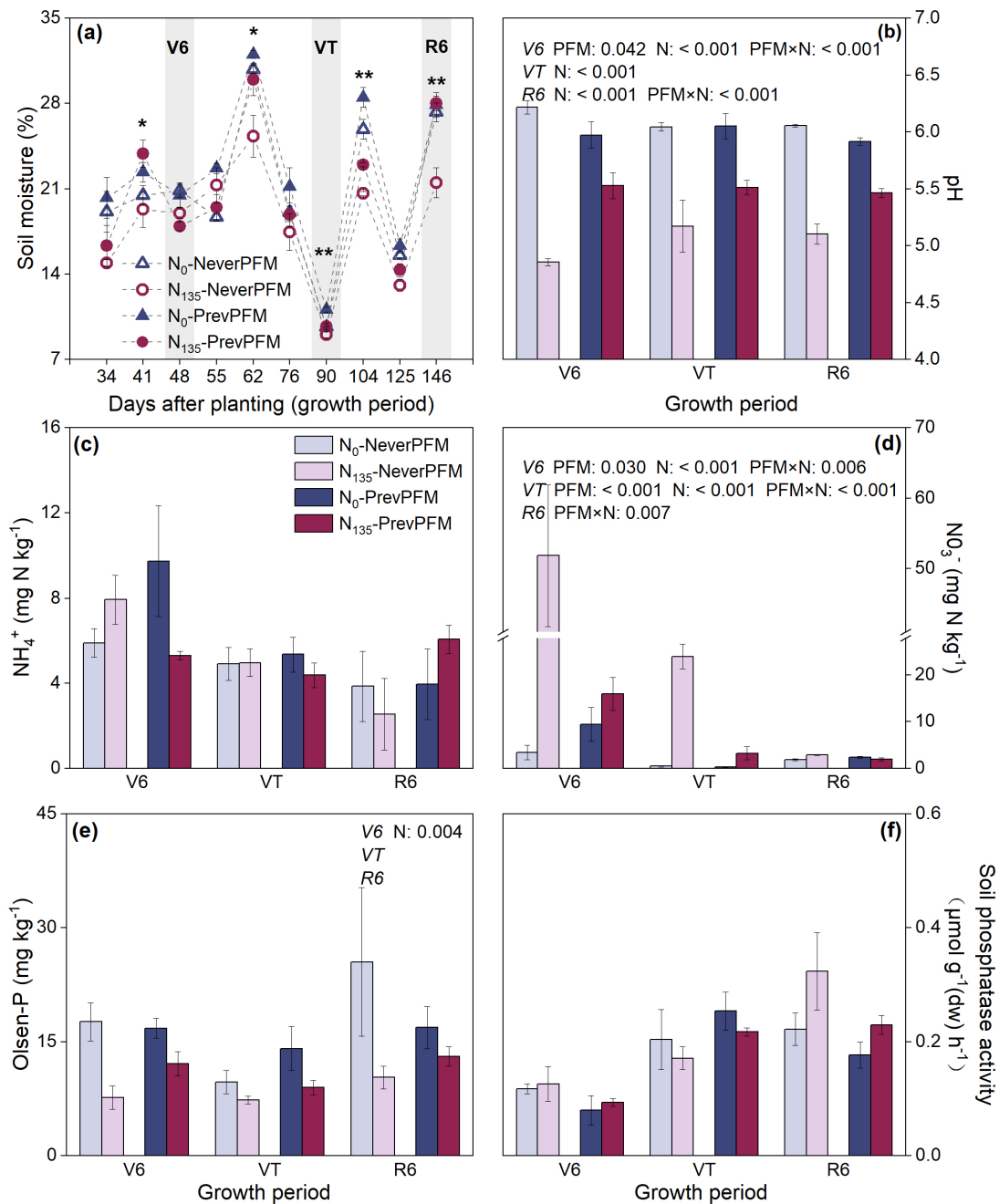
183 To understand how the treatments (PrevPFM v.s. NeverPFM and N₀ v.s. N₁₃₅)
184 influence total maize performance and their relations with soil properties, redundancy
185 analysis (RDA) was conducted based on the data of crop performance (stem thick,
186 height, aboveground biomass, belowground biomass, total root length, root surface
187 area, chlorophyll, root P, and APase) and soil properties (pH, soil moisture, Olsen-P,
188 bulk density, soil porosity, water holding capacity and AcP). Monte Carlo
189 permutations were used to test significance of relationships between selected soil
190 factors and plant growth ($P < 0.05$), and then tests the significance of the difference
191 between each soil factor and plant growth through the envfit function in vegan
192 package. RDA was performed using R. 4.1.3. The other statistics analyses were
193 conducted using SPSS version 22.0. All reported differences are significant at $P <$
194 0.05.

195 3. Results

196 3.1 Soil properties

197 Soil moisture was always higher for previous plastic film mulching than for
198 never mulching (most $P < 0.05$, Fig. 1a). Soil pH had a higher value at previous
199 plastic film mulching plot than at never plastic film mulching plot only at N₁₃₅ level
200 (Fig. 1b). Soil NH₄⁺-N concentrations were similar between previous and never
201 plastic film mulching ($P > 0.05$, Fig. 1c), but NO₃⁻-N concentrations were lower for
202 previous plastic film mulching than never plastic film mulching at the sixth leaf stage
203 and tasseling stage ($P < 0.05$ and $P < 0.001$, Fig. 1d). Soil Olsen-P concentrations and
204 phosphatase activity were both similar between previous and never plastic film
205 mulching) in all studied stages ($P > 0.05$, Fig. 1e and 1f).

206 Soil moisture was lower at N fertilized plot than at no fertilized plot for most
207 time in growth season (Fig. 1a). Soil pH was dramatically lower at N fertilized plot
208 than at no fertilized plots ($P < 0.001$, Fig. 1b). As expected, N fertilized plot had
209 larger soil NO₃⁻-N concentrations than at no fertilized plot, especially for never plastic
210 film mulching ($P < 0.001$, Fig. 1d), but these two plots had similar NH₄⁺-N ($P > 0.05$,
211 Fig. 1c). Soil Olsen-P concentrations were lower at N fertilized plot than no fertilized
212 plot, especially at the sixth leaf stage ($P = 0.004$, Fig. 1e). Soil phosphatase activity
213 did not have the difference between the contrasting fertilized plots ($P > 0.05$, Fig. 1f).



214

215 **Fig.1** Soil moisture (a), pH (b), $\text{NH}_4^+\text{-N}$ (c), $\text{NO}_3^-\text{-N}$ (d), Olsen-P (e)

216 concentrations and soil phosphatase activity (f) during growth seasons. V6:

217 sixth leaf stage, VT: tasseling stage, R6: physiological maturity stage. N_0 :

218 zero N fertilizer, N_{135} : $135 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, PrevPFM: previous plastic film

219 mulching, NeverPFM: never plastic film mulching. Bars represent \pm

220 standard errors of the replicates ($n = 3$). The symbols “***”, and “**” in panel

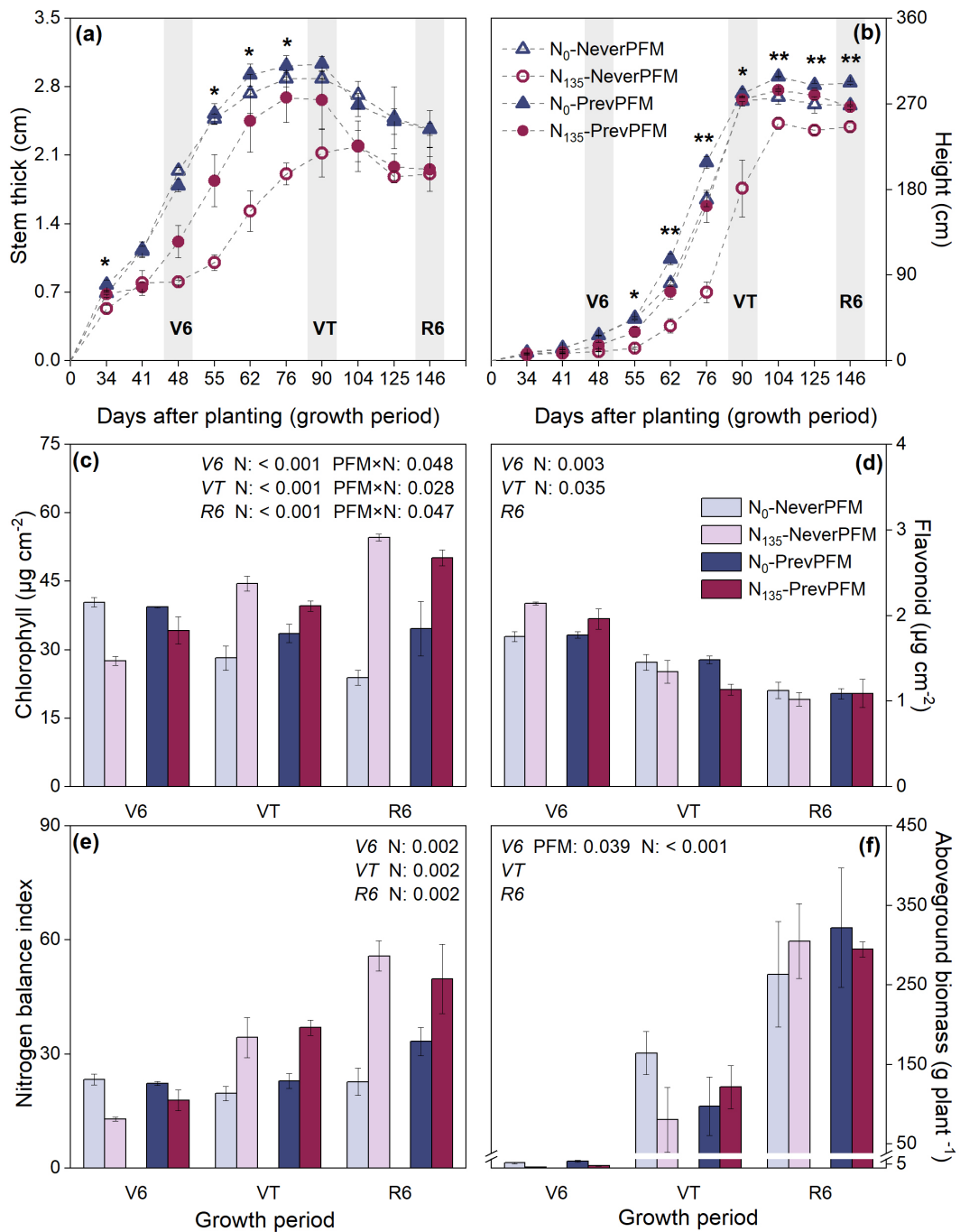
221 (a) denote main effects of plastic film mulching within the ANOVA results
222 at $P < 0.01$, and $P < 0.05$, respectively. The values behind ‘PFM’, ‘N’ or
223 ‘PFM × N’ represent the P values for main effects of plastic film mulching,
224 N fertilization, and their interaction, respectively. Only P values less than
225 0.05 were showed in panels.

226 **3.2 Maize above- and below-ground parameters**

227 Long-term plastic film mulching did not have a negative legacy for succeeding
228 maize, but even promoted maize growth sometimes. Maize stem was generally thicker
229 and height was greater for previous plastic film mulching than for never mulching,
230 especially at N₁₃₅ level (Fig. 2a, 2b). Correspondingly, aboveground biomass was
231 larger for previous plastic film mulching than for never mulching, but these
232 differences only occurred at the sixth leaf stage ($P < 0.05$, Fig. 2f) and disappeared at
233 tasseling and maturity stages ($P > 0.05$). Both leaf chlorophyll and flavonoid
234 concentrations and NBI were similar between previous and never plastic film
235 mulching ($P > 0.05$, Fig. 2c, 2d, 2e). Total root length was higher for previous plastic
236 film mulching than for never mulching at the sixth leaf stage ($P < 0.05$, Fig. 3a), but
237 this trend was reversed at physiological maturity stage ($P < 0.05$). However, other
238 root properties, i.e., total surface area, total volume, biomass, root-associated
239 phosphatase activity, and root P were all similar between previous plastic film
240 mulching than for never mulching ($P > 0.05$, Fig. 3b, 3c 3d, 3e, 3f), except for total
241 surface area at physiological maturity stage ($P < 0.05$).

242 Long-term N fertilization inhibited maize growth, especially at the seedling stage.
243 Specifically, maize stem was finer and height was greater at N fertilized plot than at
244 no fertilized plot during the whole growing season (Fig. 2a and 2b). Correspondingly,
245 aboveground biomass was much smaller at N fertilized plot than at no fertilized plot,
246 but these differences only occurred at the sixth leaf stage ($P < 0.05$, Fig. 2f) and
247 disappeared at tasseling and maturity stages ($P > 0.05$). At the sixth leaf stage, N
248 fertilized plot had lower chlorophyll concentrations and NBI but higher flavonoid
249 contents in leaves than no fertilized plot, especially for never plastic film mulching
250 (both $P < 0.01$, Fig. 2c, 2d, 2e). Oppositely, at tasseling and maturity stages,
251 chlorophyll concentration was higher at N fertilized plot, especially for never plastic
252 film mulching (Fig. 2c). Root generally had similar trends toward N fertilization with
253 aboveground biomass. Root biomass, total root length, total surface area, total volume
254 were much smaller at N fertilized plot than at no fertilized plot at sixth leaf stage (all
255 $P < 0.01$, Fig.3 a, b, c, d), but the difference disappeared at tasseling and maturity
256 stages ($P > 0.05$). In response to Olsen-P deficiency induced by N fertilization (Fig.
257 1e), root-associated phosphatase activity was higher at N fertilized plot than no
258 fertilized plot during the whole growing season ($P < 0.05$, Fig. 3e). Accordingly, root
259 P concentrations were lower at N fertilized plot, especially for maturity stage ($P <$
260 0.001 , Fig. 3f).

261



262

263

Fig.2 Maize above-ground parameters during various growth stages.

264

Stem thick (a), height (b), chlorophyll (c), flavonoid(d), nitrogen balance

265

index (e), and aboveground biomass (f). Nitrogen balance index was

266

calculated by chlorophyll/flavonoid. V6: sixth leaf stage, VT: tasseling

267

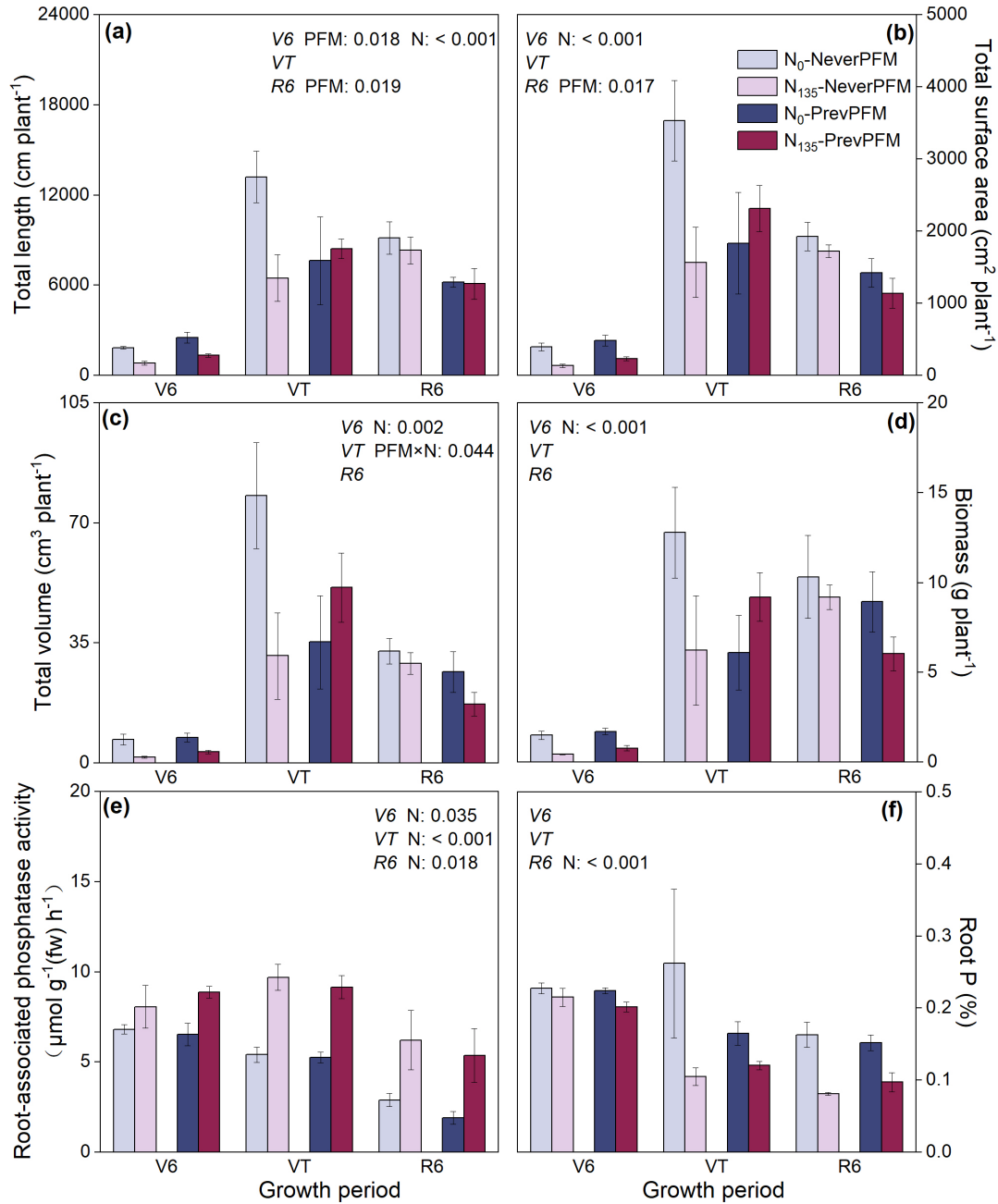
stage, R6: physiological maturity stage. N₀: zero N fertilizer, N₁₃₅: 135 kg

268

N ha⁻¹ yr⁻¹, PrevPFM: previous plastic film mulching, NeverPFM: never

269 plastic film mulching. Bars represent \pm standard errors of the replicates (n
270 = 3). The symbols “***”, and “*” in panel (a) denote main effects of plastic
271 film mulching within the ANOVA results at $P < 0.01$, and $P < 0.05$,
272 respectively. The values behind ‘PFM’, ‘N’ or ‘PFM \times N’ represent the P
273 values for main effects of plastic film mulching, N fertilization, and their
274 interaction, respectively. Only P values less than 0.05 were showed in
275 panels.

276



277

278 **Fig.3** Maize below-ground (root) parameters during various growth

279 stages. Total length (a), total surface area (b), total root volume (c), biomass (d),

280 root associated phosphatase activities (e), P concentration (f). V6: sixth leaf stage,

281 VT: tasseling stage, R6: physiological maturity stage. N₀: zero N fertilizer,

282 N₁₃₅: 135 kg N ha⁻¹ yr⁻¹, PrevPFM: previous plastic film mulching,

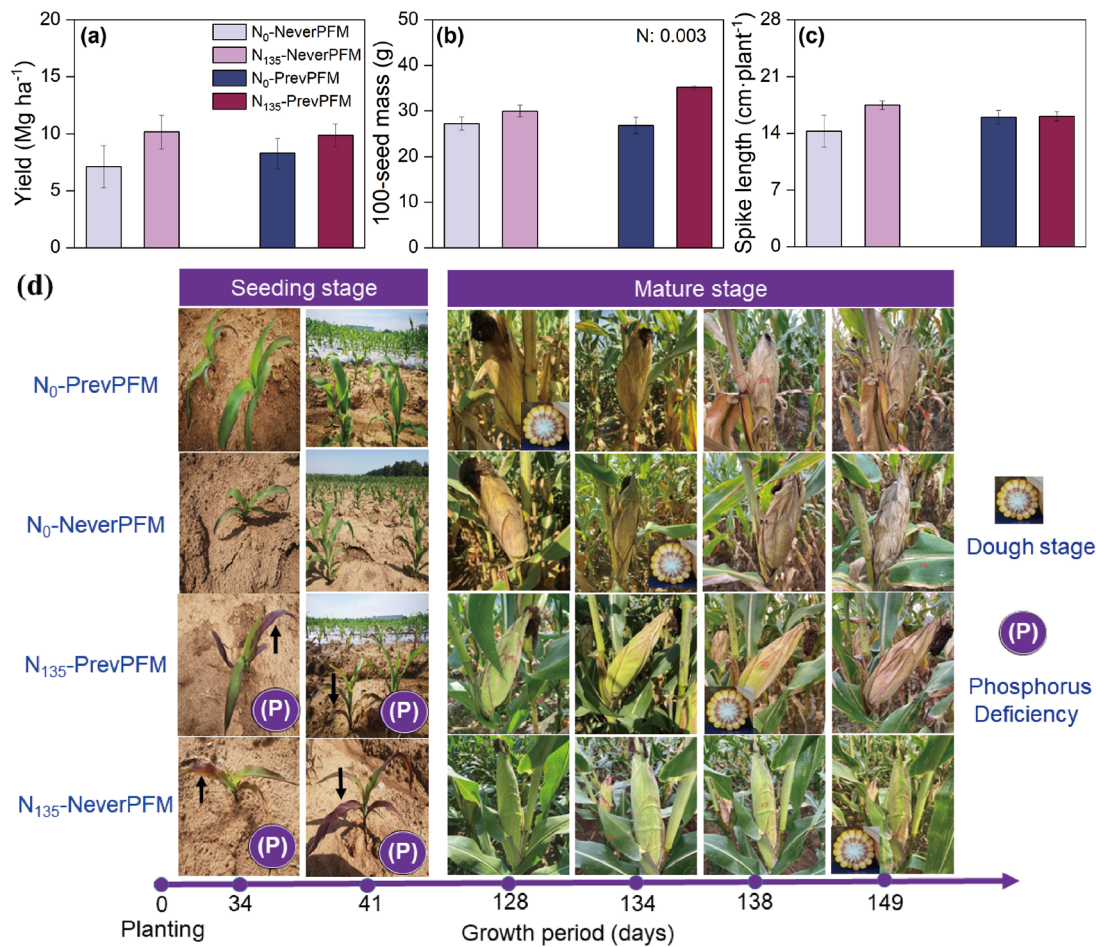
283 NeverPFM: never plastic film mulching. Bars represent ± standard errors of the

284 replicates ($n = 3$). The values behind ‘PFM’, ‘N’ or ‘PFM \times N’ represent the P
285 values for the main effects of plastic film mulching and N fertilization, or
286 their interaction, respectively. Only P values less than 0.05 were showed
287 in panels.

288

289 **3.3 Maize yield and ripening time**

290 Maize yields were similar between previous and never plastic film mulching ($P >$
291 0.05, Fig. 4a). Yield parameters (100-seed mass and spike length) were also the case
292 ($P > 0.05$, Fig. 4b, 4c). However, maize at previous plastic film mulching plots had an
293 earlier dough stage (6~10 days) than those at never mulching plots (Fig. 4d). Maize
294 yield were similar between at fertilized and non-fertilized plots ($P > 0.05$, Fig. 4a).
295 This was also the case for spike length, but 100-seed mass was larger at fertilized than
296 at non-fertilized plot ($P < 0.05$, Fig. 4b). At seeding stage, plants at N fertilized plots
297 experienced the symptom of serious P deficiency, indicated by purple leaf and
298 obvious growth inhibition, whereas plants at non-fertilized plot did not have this
299 symptom (Fig. 4d). The symptom at fertilized plot was a litter lighter for previous
300 plastic film mulching than never plastic film mulching. Although the symptom of P
301 deficiency was recovered at tasseling stage and maturity stage (Fig. 4d), the time of
302 dough stage was delayed at fertilized plot for 10~15 days.



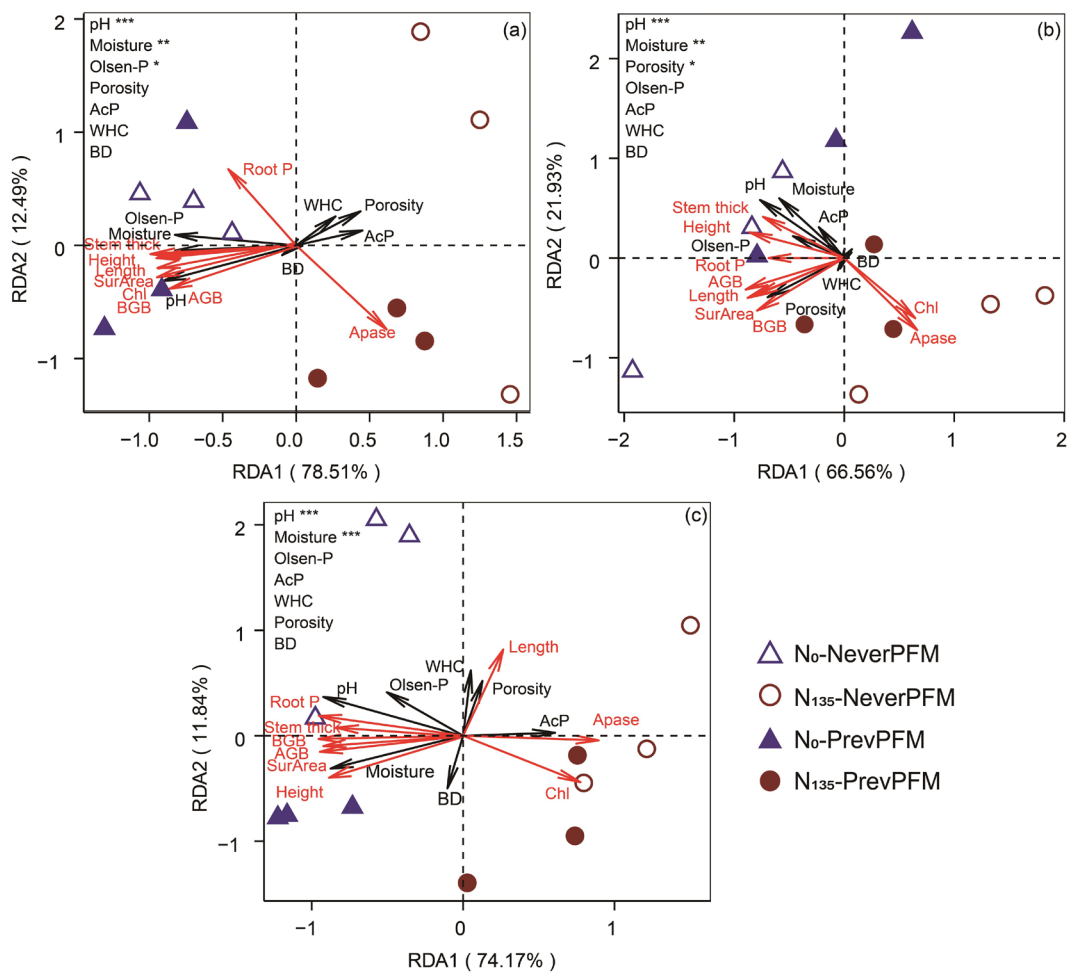
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306 **Fig.4** Maize yield (a), 100-seed mass (b), spike length (c), and growth
 307 process and ripening time (d) under the combined plastic film mulching and
 308 fertilization with urea-nitrogen (N) treatments. N₀: zero N fertilizer, N₁₃₅:
 309 135 kg N ha⁻¹ yr⁻¹, PrevPFM: previous plastic film mulching, NeverPFM:
 310 never plastic film mulching. Bars represent ± standard errors of the mean (*n* = 3).
 311 The values behind 'PFM', 'N' or 'PFM × N' represent the *P* values for the
 312 main effects of plastic film mulching and N fertilization, or their
 313 interaction, respectively. Only *P* values less than 0.05 were showed in
 314 panels.

315 **3.4 The influence of PFM and N treatments on total maize performance**
316 **and their relations with soil properties**

317 Redundancy analysis (RDA) results showed that axis 1 and axis 2 together
318 explained 91%, 88.49% and 86.01% of the variance between soil properties and
319 maize performance at the sixth leaf stage, tasseling stage, and physiological maturity
320 stage, respectively (Fig. 5a, 5b, 5c). The groups of PrevPFM and NeverPFM generally
321 clustered together, both for N₀ and N₁₃₅ levels. Contrastingly, the groups N₁₃₅ and N₀
322 were completely opposed in the factorial plan and factor N₀ stood generally in the
323 positive correlation with all the maize growth parameters (except for leaf chlorophyll
324 content and root-associated phosphatase activity) during all the growth stage. Soil pH
325 and moisture were two most important determining soil factors on maize
326 performance during all the growth stage, and were positively correlated with most
327 crop growth parameters. Soil Olsen-P content was also a key factor on maize growth
328 at sixth leaf stage, but did not play an important role after this period.



329

330 **Fig.5** Redundancy analysis of plant growth impacted by soil properties at sixth

331 leaf stage (a), tasseling stage (b) and physiological maturity stage (c). Red and black

332 arrows indicate plant growth parameters and soil properties, respectively. SurArea:

333 total root surface area; AGB: aboveground biomass; BGB: belowground biomass; Chl:

334 chlorophyll; APase: root-associated phosphatase activity; BD: soil bulk density; WHC:

335 water holding capacity; AcP: soil phosphatase activity. On top, the soil properties

336 were fitted to the ordination plots using a 999 permutations test (*P*-values). * *P* < 0.05,

337 ** *P* < 0.01, *** *P* < 0.001.

338

339 4. Discussion

340 4.1 Legacy effects of long-term plastic film mulching

341 Not supporting our hypothesis, 33 years of plastic film mulching does not have a
342 negative legacy on maize growth and yield. In our mulching plots, plastic film
343 residues accumulated high to 6796 pieces m⁻² or 360 kg ha⁻¹ in surface soil ([Li et al.,](#)
344 [2022b](#)). Plastic film residues accumulation may reduce maize yield through inhibiting
345 root growth and development ([Chen et al., 2022](#), [Gao et al., 2019](#), [Hu et al., 2020b](#)).
346 [Xie et al. \(2007\)](#) found that the yield of maize was only decreased when the residual
347 film amount was above 720 kg ha⁻¹. [Hu et al. \(2020b\)](#) showed that maize yield was
348 decreased by 15~18% and 23~25%, when added plastic film residues with 300 and
349 600 kg ha⁻¹, respectively. [Chen et al. \(2022\)](#) found the threshold of maize yield
350 starting to decrease was 180 kg ha⁻¹ plastic film residues. However, all these studies
351 are conducted by artificial adding plastic film residues to soil, in which the plastic
352 residue is fresh and does not experience a long-term aging process. Aged plastic
353 residues may less affect crop growth than fresh residue, as it is fragile and may not
354 hinder root growth. [Pflugmacher et al. \(2021\)](#) found that the adverse effects on the
355 germination and seedling growth of *Lepidium sativum* were reduced as a function of
356 the aging time applied to the polycarbonate. Accordingly, we did not observe negative
357 legacy on maize growth and yield though the amounts of plastic film residues are
358 close to or exceed the above thresholds. Similarly, a recent meta-analysis did not
359 observe a decrease in maize yield with increasing amounts of residual films and more
360 than half of their collected data points even showed an increase in maize yield to
361 plastic film residues ([Zhang et al., 2020](#)).

362 Apart from plastic film residues, the accumulation of film-derived microplastic

363 reached as high as 3.7×10^6 particles m^{-2} soil in 0~100 cm soil profile in our plots ([Li](#)
364 [et al., 2022b](#)). In the literature, numerous studies reported that microplastic had caused
365 inhibitory effects on higher plants (e.g., [Qi et al. \(2018\)](#) and [Colzi et al. \(2022\)](#)).
366 However, the microplastic accumulation in our plot seems to have no negatively
367 impact on maize growth and yield. The reason could be that polyethylene (PE)
368 film-derived microplastic is not as toxic as other types of microplastic ([Li et al.,](#)
369 [2022a](#)). Many studies did not observe negative impact of PE microplastic on plant
370 growth but observed the negative impact for polyvinyl chloride (PVC) or polylactic
371 acid (PLA) microplastic ([Colzi et al., 2022](#), [Qi et al., 2018](#), [Wang et al., 2020](#)). This
372 may result from the minor effect of PE plastic on soil structure and microbial
373 activities, as compared to polyester and polyacrylic microplastics ([Machado et al.,](#)
374 [2018](#)). Nevertheless, several studies observed the negative impact of PE microplastic
375 on maize growth in pots ([Pehlivan & Gedik, 2021](#)) and hydroponic condition ([Urbina](#)
376 [et al., 2020](#)), suggesting that our explanation needs to be further affirmed.

377 On the contrary, 33 years of plastic film mulching even had a positive legacy for
378 maize at the seedling stage, as maize aboveground biomass and root length were
379 larger for previous plastic film mulching than for never mulching at the sixth leaf
380 stage ($P < 0.05$ Fig. 2f, 3a). This may be driven by higher soil moisture for previous
381 plastic film mulching than for never mulching (Fig. 1a). The RDA result showed soil
382 moisture was a key soil property controlling crop growth performance and positively
383 correlated with most growth parameters (Fig. 5a and S1). Higher soil moisture was
384 attributed to higher degree of compaction at surface soil for previous plastic film
385 mulching than never plastic film mulching ($P < 0.05$, Fig. S2), which slowed down
386 water evaporation. We observed deeper track of tractors at previous plastic film
387 mulching plots than at never plastic film mulching plots when planting in spring of

388 2021. This is also supported by larger bulk density and lower total porosity for the
389 soils at previous plastic film mulching plots at most time (Table S3). The higher
390 compaction and lower porosity of the soil under previous mulching plots may be due
391 to the higher surface water content of the long-term mulching. The appropriate water
392 content promoted the cohesive force between soil particles, thus increasing the
393 compactness of the soil. However, the positive impact of previous plastic film
394 mulching on maize growth did not occur at tasseling and maturity stages. This
395 suggested that soil moisture was a limiting factor for maize growth only at seedling
396 stage but not at later stage.

397 **4.2 Impacts of long-term N fertilization**

398 In our experiment, 33 years of only N fertilization induced severe P limitation for
399 maize growth, confirming our previous study ([Ding et al., 2019](#)). Soil Olsen-P
400 (available for plant) concentrations were lower at N fertilized plot than no fertilized
401 plot (Fig. 1e), indicating the decline of soil P supply capacity following N fertilization.
402 Accordingly, maize root P concentrations were lower at fertilized plots (Fig. 3f). To
403 alleviate this situation, maize root at fertilized plots secreted larger amounts of
404 phosphatase compared to non-fertilized plots (Fig. 3e). This is in line with previous
405 studies which have shown that long-term application of N fertilizer exacerbated P
406 deficiency ([Lin et al., 2020](#), [Tian et al., 2019](#)). Two mechanisms may explain P
407 deficiency following N fertilization. Firstly, soil acidification following urea
408 fertilization increases the solubility of non-base cations (e.g., Fe^{3+} , Al^{3+}) ([Tian & Niu,](#)
409 [2015](#), [Zarif et al., 2020](#)), which may decrease soil P availability by the precipitation of
410 P with free Fe^{3+} and Al^{3+} . This conjecture is supported by an incubation experiment
411 [Meyer et al. \(2021\)](#) who found that the decrease of soil pH increased solubilization of

412 soil Al and the precipitation of Al-P minerals, and hence markedly decreased potential
413 P availability in non-calcareous soils. A 10-year N fertilized grassland experiment also
414 observed the increase of Al-P and Fe-P amounts with the decrease of pH ([Wang et al.,
415 2022](#)). In our study, although we did not measure Al-P and Fe-P, this mechanism was
416 partly supported by the decrease of soil pH by about 1 unit (Fig. 1b) and the increase
417 DTPA-Fe (unpublished data) following 32 years of N fertilization. Another possible
418 reason is that N fertilization promoted the uptake of P from soil by plants, due to
419 increasing yield and plant biomass ([Deng et al., 2017](#), [Pasley et al., 2019](#), [Rowe et al.,
420 2008](#)). Year by year harvest would take away larger amounts of P from soil, which can
421 reduce the pool of soil P and lead to P deficiency ([Qu et al., 2009](#)). This explanation
422 was supported by that soil total P was lower at N fertilized plot than non-fertilized
423 plot in our experiment ([Ding et al., 2019](#)).

424 However, urea-induced P deficiency only inhibited maize growth at the sixth leaf
425 stage (Fig. 4). At this stage, maize leaves had lower chlorophyll concentration but
426 higher flavonoid concentration at fertilized plot at non-fertilized plot, also suggesting
427 plant growth suffering from stress following fertilization (Fig. 2c, d). Contrastingly at
428 middle (tasseling stage) and late stages (physiological maturity stage), maize
429 growth rates were faster at fertilized plot, indicated by its higher chlorophyll
430 concentration than this at non-fertilized plot. Maize above- and below-ground biomass
431 at fertilized plot were recovered to the same as those at non-fertilized plot (Fig. 2f, 3f).
432 Seedling stage is the most vulnerable period when crops are sensitive to various
433 environmental stresses ([Jisha et al., 2013](#)). At tasseling and maturity stages, maize
434 may have multiple strategies to relieve P deficiency. For example, the difference of
435 root-associated phosphatase between fertilized and non-fertilized plots (fertilized >
436 non-fertilized) increased from the sixth leaf stage to tasseling and maturity stages (Fig.

437 3e), suggesting that maize root at fertilized plots was stimulated to secrete
438 phosphatase at later stages to increase P sources for plant. Phosphatase can activate
439 soil organic P and make them available for root uptake ([Weil & Brady, 2017](#)). In
440 addition, the difference of root P between at fertilized and non-fertilized plots
441 (fertilized < non-fertilized) increased from at the sixth leaf stage to at tasseling and
442 maturity stages (Fig.3b), suggesting that maize at fertilized plot may transfer large
443 amounts of P from root to aboveground growth at later stages.

444

445 **5. Conclusion**

446 Our study first evaluated the impact of real long-term plastic film
447 mulching-derived film residues and microplastic accumulation on crop performance.
448 We demonstrate that 33 years of plastic film mulching does not have a negative legacy
449 for succeeding maize growth and yield. Although plastic film mulching can bring
450 substantial amounts of film residues and microplastic accumulation in soils, it seems
451 to not destruct soil structure and negatively impact soil fertility and maize growth. It
452 proved that plastic film mulching is a sustainable agricultural technology for maize
453 production on a relatively long-term scale. Certainly, we still need to reduce plastic
454 residues accumulation in plastic film mulched filed.

455

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Supplementary materials

Table S1 *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 ($\mu\text{m cm}^{-2}$)	Flv ($\mu\text{m cm}^{-2}$)	pH	NH ₄ ⁺ (mg N kg ⁻¹)	NO ₃ ⁻ (mg N kg ⁻¹)	AcP ($\mu\text{mol g}^{-1}(\text{dw})$ h ⁻¹)	Olsen- P (mg kg ⁻¹)	APase ($\mu\text{mol g}^{-1}(\text{fw})$ 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 S2 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 (<i>P</i> 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 (<i>P</i> 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 (<i>P</i> value)			
N	0.88	0.90	0.64
PFM	0.03	0.18	0.12
N*PFM	0.51	0.68	0.69

N₀: zero N fertilizer, N₁₃₅: 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).

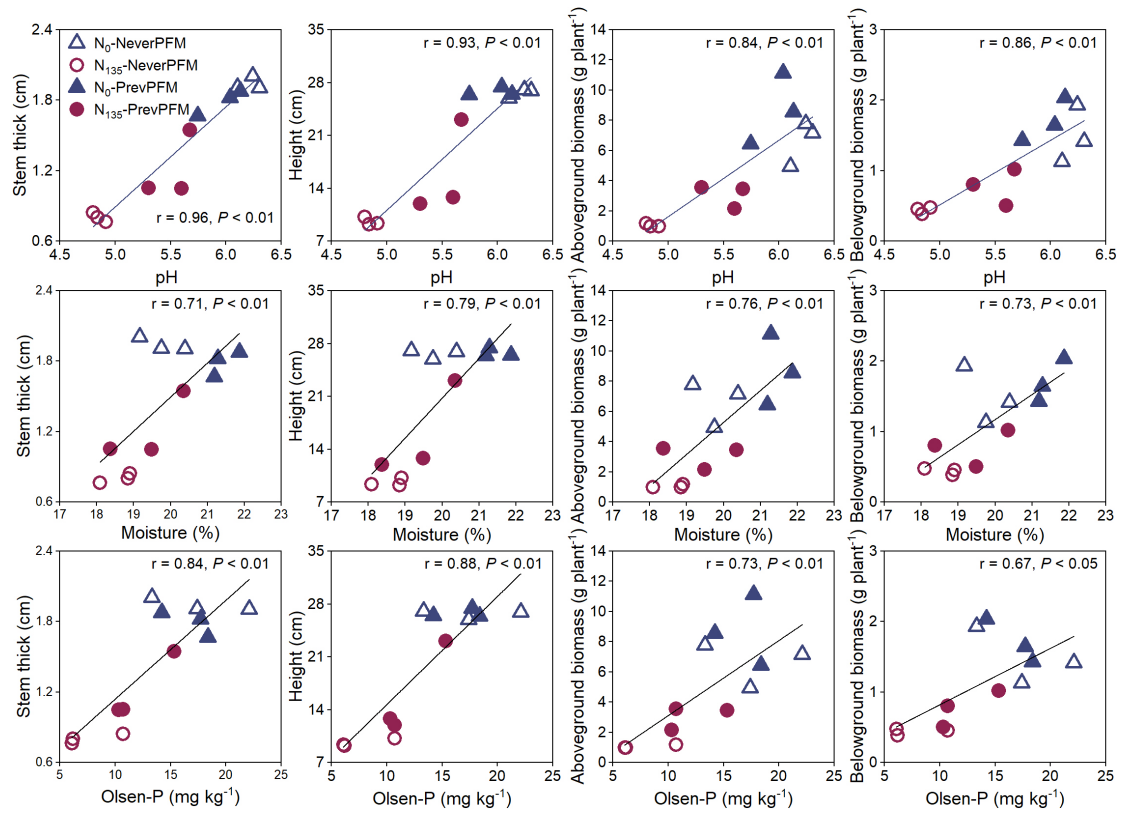


Fig. S1 The correlations of maize above- and below-ground growth parameters with soil pH, soil moisture and Olsen-P at the sixth leaf stage.

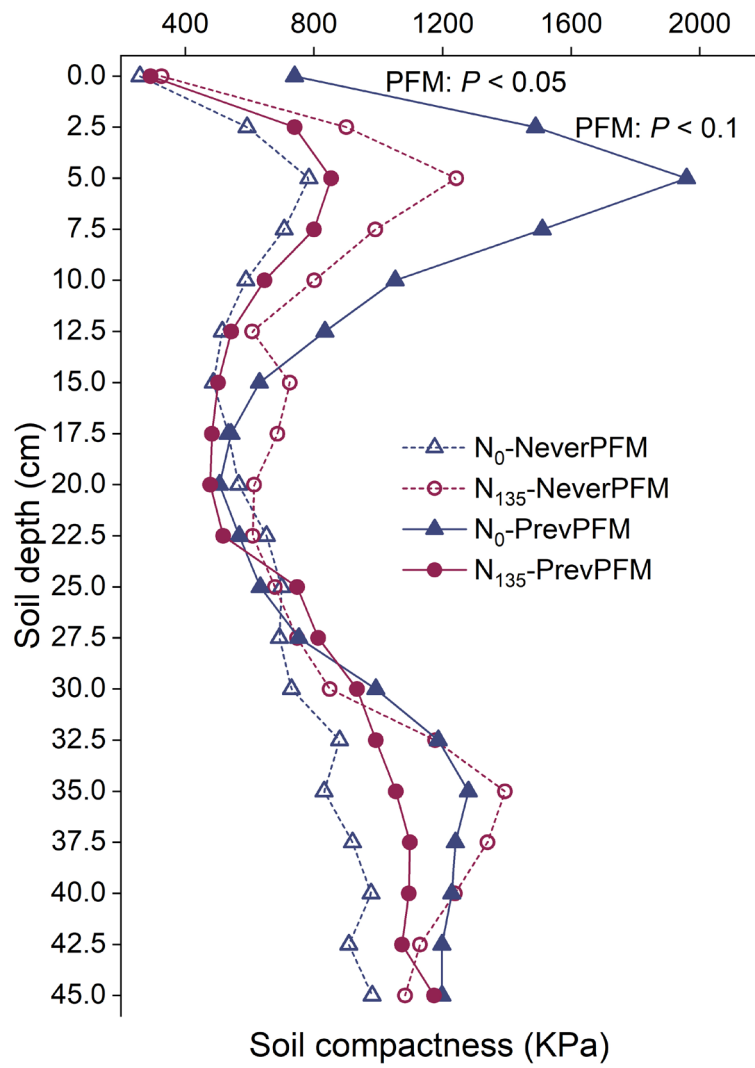


Fig. S2 Soil compactness through soil profile under the combined plastic film mulching and fertilization with urea-nitrogen (N) treatments. The P values behind PFM indicated the significance of main effect of plastic film mulching on soil compactness.

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