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Thirty-three years of plastic film mulching does not leave a negative legacy for subsequent maize growth and yield

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Abstract:

Plastic pollution in croplands has the potential to threaten long-term food security. Plastic mulching film is widely used in agricultural ecosystems, and its long-term use may leave a net negative legacy on maize growth and yield, due to deleterious effects of plastic and microplastic accumulation in soil. Here, we stopped covering soil with plastic film in an experimental site that was previously covered for 33 years, and compared soil properties and subsequent maize growth and yield between plots that were previously and never covered with plastic film. Maize growth and yield were generally similar between previously and never-mulched plots. Maize had an earlier dough stage (6~10 days) in previously mulched compared to never-mulched plots. Although plastic film mulching did add substantial amounts of film residues and microplastic accumulation into soils, it did not leave a net negative legacy (given the positive effects of the mulching practice in the first place) for soil structure, and subsequent maize growth and yield, at least not as an initial effect in our experiment. Our data add long-term information on this important form of plastic pollution in agricultural systems.

Synopsis: Our study indicate that the practice of plastic film mulching and its plastic residues in croplands do not pose an unsurmountable threat to food security, at least as gauged against the legacy of the positive effects of the mulching practice.

Keywords: Plastic pollution; Microplastic; Legacy effect; Soil health; Crop performance

1. Introduction

In the Anthropocene, human activities and products profoundly change the earth. Plastics, a group of artificially synthesized compounds, are now ubiquitous on the earth even in remote places such as near the top of Mount Everest [1]. In recent decades, plastic pollution has attracted great attention due to its potential ecological and environmental implications on a global scale [2]. Consequently, plastic pollution was recently listed as one of the top 10 global environmental problems by the United Nations Environment Program [3]. Compared with plastic pollution of oceans and freshwater, little is known about plastic pollution of terrestrial ecosystems [4, 5]. Due to the widespread use of plastic mulch, shed plastic film, and biosolids [6-8], croplands have been identified as a major source of plastic debris [9]. Due to the plastic film residue accumulation negatively impacting soil health, plastic pollution in croplands has the potential to threaten long-term food security [10].

Polyethylene (PE) plastic film mulching (PFM) is widely used in global agricultural ecosystems to improve plant growth because it increases soil temperature and moisture [11-14]. A recent meta-analysis showed that PFM increased crop yields by

24% on average [15]. However, increased adoption and time of soil contact results in greater soil accumulation of plastic residues because plastic films often cannot be completely removed, especially the thin films (i.e., 5~8 μm thick) used in countries such as China [16]. Our recent study showed that macro-residues of plastic film (diameter > 5 mm) were as high as 360 kg ha^{-1} and film-derived microplastics (< 5 mm) exceeded 8000 items per kg soil in the 0~10 cm layer after 32 years of plastic film mulching [17]. Excessive residual plastic accumulated in soil could decrease pore connectivity and porosity [18], thus affecting the movement of nutrients and water in the soil [19]. Thus, the germination of crop seeds and the development of roots would be also compromised by the residual film [20, 21]. Moreover, PFM, or polyethylene film-derived plastic fragments or microplastic accumulation may induce soil water repellency [22] and increase water evaporation [23]. Therefore, long-term PFM is expected to leave a negative legacy for crop growth and yield.

Studies exploring the effect of plastic residual film or PE microplastic accumulation in soil on crop performance show inconsistent results [24]. Hu, et al. [20] found that maize yield was decreased by 15~18% and 23~25%, when adding plastic film residues into the tillage layer at levels of 300 and 600 kg ha^{-1}, respectively. A meta-analysis showed a reduction of yield by 3% for cotton but little effect on potato and maize at 100 kg ha^{-1} of residual film, as estimated through regression relationships between yield and soil residual film [10]. Negative [25], and no [26-28] impacts of PE microplastic on crop performance effect have both been reported for different types of crop, such as maize. However, those previous studies were based on the artificial addition of plastic into soils, which may not fully reflect reality. The reason is that plastic film in the field passes through a complex fragmentation and degradation process, which requires appreciable time. To our knowledge, there is no evaluation of the legacy of long-term PFM on subsequent crop growth and yield.

Our study evaluated the legacy effects of 33 years of PFM on soil properties, maize growth, and yield in a continuous plastic film mulching and urea fertilization experiment initiated in 1987. To investigate the legacy effect, previous mulching plots were not covered with polyethylene film in 2021 and never-mulched plots served as a control. Maize aboveground and belowground growth indices (stem diameter, height, leaf chlorophyll and flavonoid contents, root-associated phosphatase activity, root P, root morphological parameters, and biomass) and soil basic physical and chemical properties were measured at the six leaf stage, tasseling stage, and physiological maturity stage. Maize yield and maturation time were measured at the end of the growing season. Our aim was to test the hypothesis that long-term PFM leaves a net negative legacy on maize growth and yield, due to deleterious effects of plastic and microplastic accumulation in soil outweighing any positive legacy effects of the mulching practice (such as increased soil moisture). We also expect that long-term nitrogen (N) fertilization with urea would have a negative effect on maize growth, due to soil acidification and its induced plant phosphorus limitation.

2. Materials and methods

2.1 Study site and experiment design

The experimental field site was the long-term polyethylene film mulching (colorless and transparent, 8 μm thick) and fertilization station (built in 1987) at Shenyang Agriculture University (41°49'N, 123°34'E) in Shenyang, Liaoning Province, China. This site has a temperate continental monsoon climate, with a mean annual temperature of 7.9 °C and average annual rainfall of about 705 mm. The soil is a brown earth according to Chinese Soil Taxonomy (a Haplic-Udic Alfisol according to US Soil Taxonomy). The experiment was arranged in a split-plot design with two levels of plastic film mulching (with and without) as main plots and two levels of N fertilizer as subplots that produces a combination of 4 treatments with three plot replicates by treatment. The fertilizer levels included (i) zero N fertilizer (N_0) and (ii) 135 kg N ha^{-1} $year^{-1}$ application (N_{135}). Each plot had an area of 69 m^2. The N fertilizer was urea powder, applied as basal fertilizer in spring. The crop type is monoculture maize (*Zea may* L.) with a conventional tillage system and does not change since 1987. A detailed description of agricultural operations at this field can be seen in Ding, et al. [14].

In order to investigate the legacy effect of previous PFM, two ridges (5 m×2 m) were randomly selected within previous PFM plots to cease covering with plastic film in 2021: this is referred to previous PFM (PrevPFM). Plots that never possessed PFM were set as the control i.e. never-PFM plots (NeverPFM). Soil properties and maize growth at the N_0 and N_{135} plots under previous and never-plastic film mulching treatments (called N_0-PrevPFM, N_{135}-PrevPFM, N_0-NeverPFM, N_{135}-NeverPFM, respectively) were measured during the growing season in 2021.

2.2 Sampling and measurements

Soil moisture, plant height, and stem diameter were measured every 7 days from June to July, every 14 days from July to August, and every 21 days from August to September in 2021. Soil moisture was measured at a depth of 10 cm using a moisture probe (Trime ®-Pico 64/32, IMKO GmbH, Ettlingen, Germany). Three plants were randomly selected from each plot. Plant height was measured from the base to the tip with steel tape, and stem diameter, defined as the middle diameter of the second aboveground section, was measured with a vernier caliper.

Leaf pigments, above- and below-ground biomass, root morphological properties, root phosphorus concentration, and associated phosphatase activity were measured at the sixth leaf stage (V6, the key period from vegetative to reproductive growth, about 48 days after seeding), tasseling stage (VT, the period when the plant reaches its full height and begins to shed its pollen, about 90 days after seeding), and physiological maturity stage (R6, about 149 days after seeding). The sampling dates for each of the three stages occurred when more than 80% of the plants were in that respective stage. Chlorophyll and flavonoid contents were measured for the third fully expanded mature leaf from top to bottom for a selected plant at 9:00~11:30 in the morning using a Dualex Scientific + device (Force-A, Orsay, France). Two plants were sampled from each plot, and then divided into aboveground and belowground tissues by cutting the first section of the stem with a sickle. Plant tissues were oven-dried at 60℃ to constant weight. Within each plot, two plants were randomly sampled by excavating the soil adjacent to the main trunk up to a radius of 15 cm and a depth of 40 cm, and collecting all scattered roots. Roots were washed with tap water to remove soil and then rinsed with ultrapure water 3~5 times. Roots from a single plant were cut into parts, and measured using a root scanner (EPSON Expression 11000XL) and an image analyzer (the WinRHIZO software, Regent Instr., QC, Canada) for root morphology, including total root length, total surface area, total volume. Scanned roots were dried to a constant mass at 60℃ and then weighed. Dry roots were ground and passed through a 0.25 mm sieve and then digested with a combination of H_2SO_4 and H_2O_2 (8:5) to determine root phosphorus concentrations [29]. The remaining root was used to determine root-associated phosphatase activity (APase) [30].

Soil samples were collected at 0~20 cm layer for the measurements of pH, plant-available soil phosphorus (Olsen-P), soil acid phosphatase (AcP), ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) contents, bulk density, total porosity, and water holding capacity at corresponding crop stages. Three soil cores were randomly sampled using an auger (4 cm in diameter) and then composited for each plot. Soil samples were passed through a 2-mm sieve to remove plant debris and gravel. One part was air-dried to determine soil pH and Olsen-P, and the field-moist soil was used to determine soil acid phosphatase (AcP), NH_4^+-N and NO_3^--N (values were expressed on a dry weight basis). Soil pH was measured by a glass electrode in a 1:2.5 soil/distilled water suspension after shaking. Olsen-P concentration was measured after extraction with 0.5

M $NaHCO_3$ according to the colorimetric method [29]. Soil NH_4^+-N and NO_3^--N were extracted with 10 m*M* $CaCl_2$ (soil: water = 1:10) and measured using a continuous flow analyzer (Bran-Luebbe AA3, Germany). Soil bulk density, total soil porosity, and soil water holding capacity were determined according to the cutting-ring method in Chen [31]. After crop harvest in autumn, soil compaction was measured using a soil compaction meter (Spectrum SC 900in, United States). The conical head was pushed down at a constant speed and inserted into the soil to 45 cm depth, and data were automatically read and recorded.

Soil acid phosphatase activity and root-associated phosphatase activity were measured following the spectrophotometer method in Lin, et al. [30]. 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 °C 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). Four analytical replicates were used for each root sample, including a blank. For the blank, *p*NPP was added after NaOH and $CaCl_2$ stopped the reaction. The concentration of *p*NP is obtained by the standard curve between the configured *p*NP concentration and the absorbance value. Soil phosphatase activity is expressed by *p*NP produced in the above reaction divided by reaction time and dry weight. Root-associated phosphatase activity is expressed by *p*NP produced in the above reaction divided by reaction time and fresh weight.

Moreover, we observed and recorded the time when maize entered into dough stage, which is defined as the time when most kernels are becoming a consistency similar to dough and accumulate almost 50% of the dry mass [32]. At the physiological maturity stage, the yield was measured through randomly selecting four plants in the middle of each plot. The 100-seed dry weight (randomly chosen 100 maize seeds) and the length of the maize cob were recorded. Maize ears were dried at 60 °C to constant weight in an oven and then used to obtain the yield.

2.3 Statistical analyses and calculations

The effects of PFM (PrevPFM and NeverPFM, whole-plot factor), N fertilization (N_0 and N_{135}, subplot factor) and their interactions on soil and crop parameters were assessed by split-plot ANOVA at each sampling time. Normality of residuals and homogeneity of the variances of the residuals across groups were checked through the Shapiro–Wilk test and Levene's test, respectively [33]. When necessary, the data were logarithmically transformed. Pearson correlation analyses were conducted between plant growth parameters and three soil parameters (i.e., pH, moisture, and Olsen-P concentrations) at the sixth leaf stage, tasseling stage, and physiological maturity stage, respectively.

To understand how the treatments (PrevPFM v.s. NeverPFM and N_0 v.s. N_{135}) influence total maize performance and their relations with soil properties, redundancy analysis (RDA) was conducted based on crop performance data (stem diameter, height, aboveground biomass, belowground biomass, total root length, root surface area, chlorophyll, root P, and APase) and soil properties (pH, soil moisture, Olsen-P, bulk density, soil porosity, water holding capacity and AcP). Monte Carlo permutations were used to test significance of relationships between selected soil factors and plant growth ($P < 0.05$), and we then tested the significance of the difference between each soil factor and plant growth through the envfit function in vegan package. RDA was performed using R. 4.1.3. The other statistics analyses were conducted using SPSS version 22.0. All reported differences are significant at $P < 0.05$.

3. Results

3.1 Soil properties

Soil moisture was about 5-16% higher for previous plastic film mulching than for never-mulching (most $P < 0.05$, df = 1, Fig. 1a, Table S1). Soil pH had a higher value at previous plastic film mulching plot than at never plastic film mulching plot only at N_{135} level (Fig. 1b). Soil NH_4^+-N concentrations were similar between previous and never plastic film mulching ($P > 0.05$, df = 1, Fig. 1c), but NO_3^--N concentrations were lower for previous plastic film mulching than never plastic film mulching at the sixth leaf stage and tasseling stage for N_{135} treatment ($P = 0.03$ and $P < 0.001$, df = 1, Fig. 1d). Soil Olsen-P concentrations and phosphatase activity were both similar between previous and never plastic film mulching in all the growth stages ($P > 0.05$, df = 1, Fig. 1e and 1f). Both NH_4^+-N and NO_3^--N concentrations were lower at tasseling and physiological maturity stages than at sixth leaf stage (Fig. 1c and 1d). Soil phosphatase activity were higher at tasseling and physiological maturity stages than at sixth leaf stage (Fig. 1f), although Olsen-P changed little across growth stages (Fig. 1e).

Soil moisture was about 5-21% lower at N fertilized plots than at non-fertilized plots for most of the growing season (Fig. 1a, Table S1). Average soil pH was about 12~15% lower in N fertilized plots than in non-fertilized plots across growth stages ($P < 0.001$, df = 1, Fig. 1b). Soil NO_3^--N concentrations were about 4 and 35 times higher at N fertilized plots than at non-fertilized plots during the sixth leaf stage and tasseling stage, respectively ($P < 0.001$, df = 1, Fig. 1d), but these two plots had similar NH_4^+-N ($P > 0.05$, df = 1, Fig. 1c). Soil Olsen-P concentrations were lower in N fertilized than in non-fertilized plot, especially at the sixth leaf stage (i.e., 17.21 mg kg^{-1} v.s. 9.89 mg kg^{-1}) ($P = 0.004$, df = 1, Fig. 1e). Soil phosphatase activity did not differ between the contrastingly fertilized plots ($P > 0.05$, df = 1, Fig. 1f).

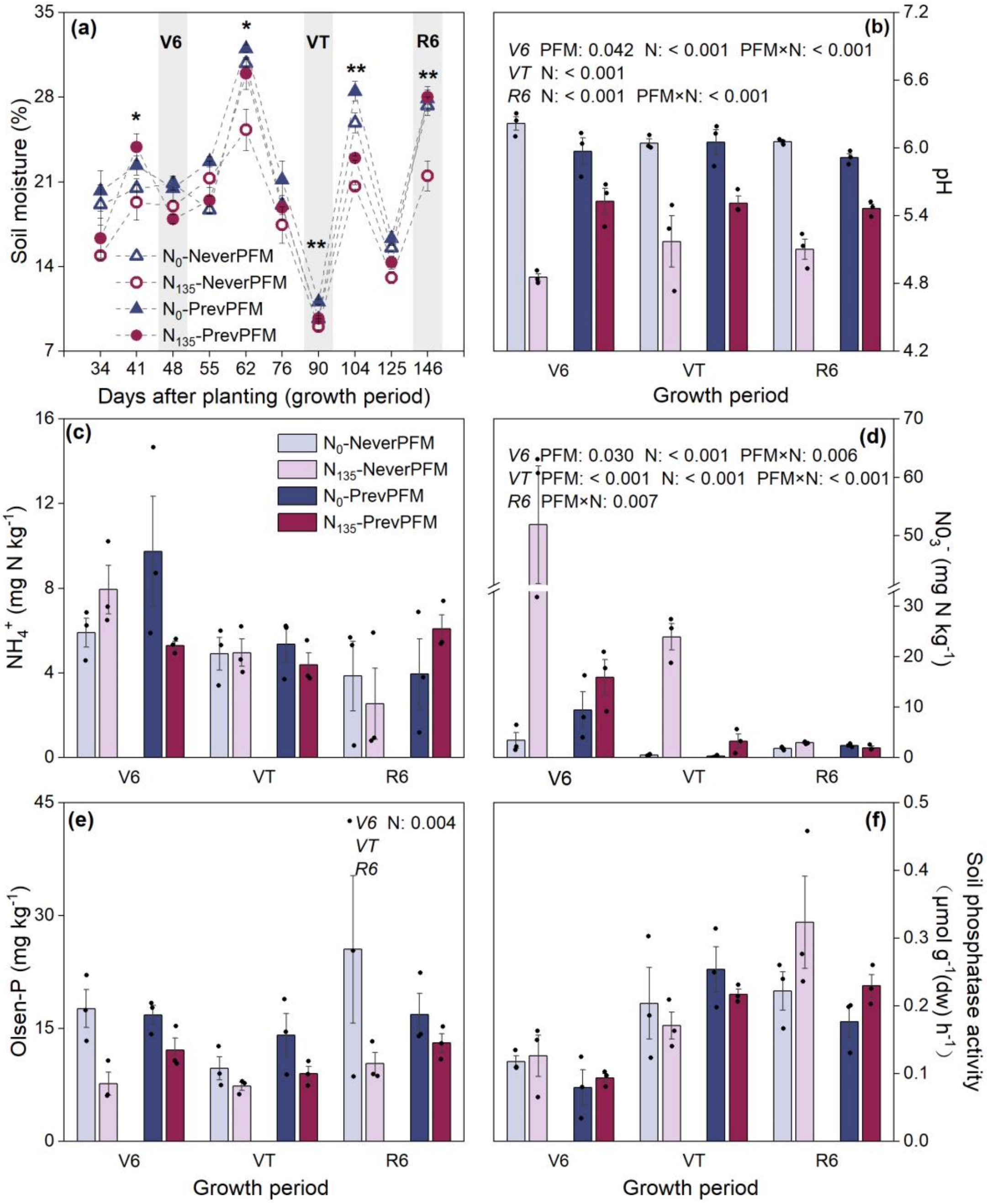

Fig.1 Soil moisture (a), pH (b), NH_4^+-N (c), NO_3^--N (d), Olsen-P (e) concentrations and phosphatase activity (f) during growth seasons. V6: sixth leaf stage, VT: tasseling stage, R6: physiological maturity stage. N_0: zero N fertilizer, N_{135}: 135 kg N ha^{-1} yr^{-1}, PrevPFM: previous plastic film mulching, NeverPFM: never plastic film mulching. Bars represent ± standard errors of the replicates ($n = 3$) and individual data points are shown as black opaque circles. The symbols "**", and "*" in panel (a)

denote main effects of plastic film mulching from ANOVA results at $P < 0.01$, and $P < 0.05$, respectively. The values behind 'PFM', 'N' or 'PFM × N' represent the P values for main effects of plastic film mulching, N fertilization, and their interaction, respectively. Only *P* values less than 0.05 are shown in panels.

3.2 Maize above- and below-ground parameters

Long-term plastic film mulching did not have a negative legacy for subsequent maize, and promoted maize growth in some cases. Stem diameter and height were generally greater for previous plastic film mulching than for never mulching across the whole growing season, especially at N_{135} level (most $P<0.05$ or $P<0.01$, df = 1, Fig. 2a, 2b). Correspondingly, aboveground biomass was larger for previous plastic film mulching than for never mulching, but these differences only occurred at the sixth leaf stage ($P = 0.039$, df = 1, Fig. 2f) and disappeared at tasseling and maturity stages ($P > 0.05$, df = 1, Fig. 2f). Both leaf chlorophyll and flavonoid concentrations and NBI were similar between previous and never plastic film mulching ($P > 0.05$, df = 1, Fig. 2c, 2d, 2e). Total root length was 46% higher in previous plastic film mulching than in never mulching treatment at the sixth leaf stage ($P = 0.018$, df = 1, Fig. 3a), but this trend was reversed at physiological maturity stage ($P = 0.019$, df = 1). Similarly, root total surface area was about 30% smaller for previous plastic film mulching than for never mulching only at physiological maturity stage ($P = 0.017$, df = 1, Fig. 3b). However, other root properties, i.e., total volume, biomass, root-associated phosphatase activity, and root P were all similar between previous plastic film mulching and never mulching ($P > 0.05$, df = 1, Fig. 3b, 3c 3d, 3e, 3f).

Long-term N fertilization inhibited maize growth, especially at the seedling stage. Specifically, stem diameter and height were much lower in N fertilized plots than in non-fertilized plots during the whole growing season (Fig. 2a and 2b). Correspondingly, aboveground biomass was much smaller in N fertilized plots than in non-fertilized plots, but these differences only occurred at the sixth leaf stage ($P < 0.001$, df = 1, Fig. 2f) and disappeared at tasseling and maturity stages ($P > 0.05$, df = 1). At the sixth leaf stage, plants from N fertilized plots had lower chlorophyll concentrations and NBI but higher flavonoid contents in leaves than non-fertilized plots, especially for never plastic film mulching ($P < 0.001$, Fig. 2c; $P = 0.002$ Fig. 2e and $P = 0.003$, Fig. 2d. respectively). By contrast, at tasseling and maturity stages, chlorophyll concentrations were higher in N fertilized plots, especially for never plastic film mulching ($P < 0.001$, df = 1, Fig. 2c). Roots generally followed similar trends to aboveground biomass in response to N fertilization. Root biomass, total root length, total surface area, and total volume were much smaller in N fertilized than in non-fertilized plots at the sixth leaf stage (all $P < 0.01$, df = 1, Fig.3 a, b, c, d), but the difference disappeared at tasseling and maturity stages ($P > 0.05$). In response to Olsen-P deficiency induced by N fertilization (Fig. 1e), root-associated phosphatase activities were about 20~100% higher in N fertilized plot than in non-fertilized plots during the whole growing season (all $P <0.05$ or $P < 0.001$, df = 1, Fig. 3e). Accordingly, root P concentrations were lower in N fertilized plots, especially for the physiological maturity stage ($P < 0.001$, df = 1, Fig. 3f).

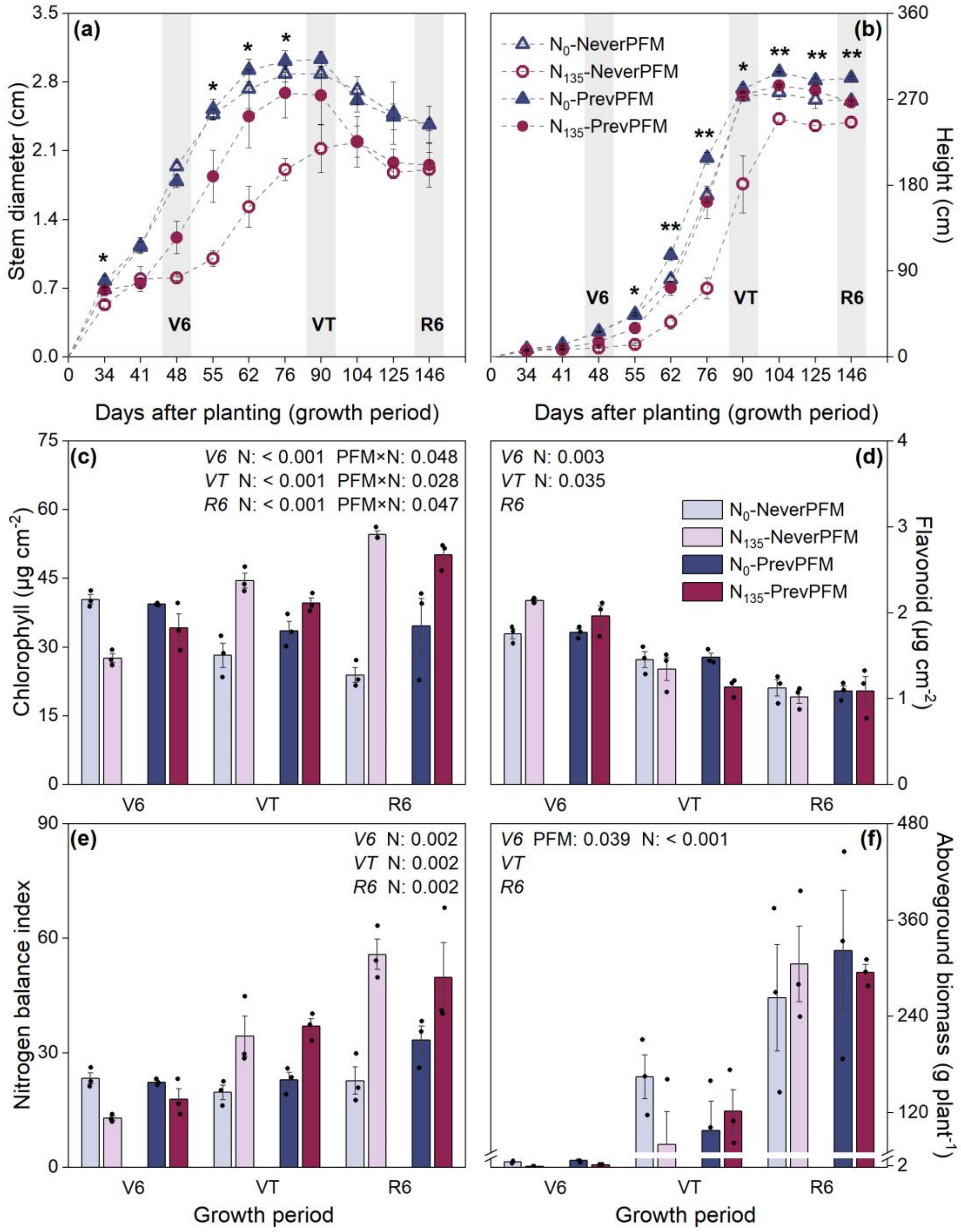

Fig.2 Maize above-ground parameters during various growth stages. Stem diameter (a), height (b), leaf chlorophyll (c), flavonoid(d), nitrogen balance index (e), and aboveground biomass (f). Nitrogen balance index was calculated by chlorophyll/flavonoid. V6: sixth leaf stage, VT: tasseling stage, R6: physiological maturity stage. N_0: zero N fertilizer, N_{135}: 135 kg N ha^{-1} yr^{-1}, PrevPFM: previous

plastic film mulching, NeverPFM: never plastic film mulching. Bars represent ± standard errors of the replicates ($n = 3$) and individual data points are shown as black opaque circles. The symbols “**”, and “*” in panel (a) denote main effects of plastic film mulching from ANOVA results at $P < 0.01$, and $P < 0.05$, respectively. The values behind ‘PFM’, ‘N’ or ‘PFM × N’ represent the P values for main effects of plastic film mulching, N fertilization, and their interaction, respectively. Only P values less than 0.05 are shown in panels.

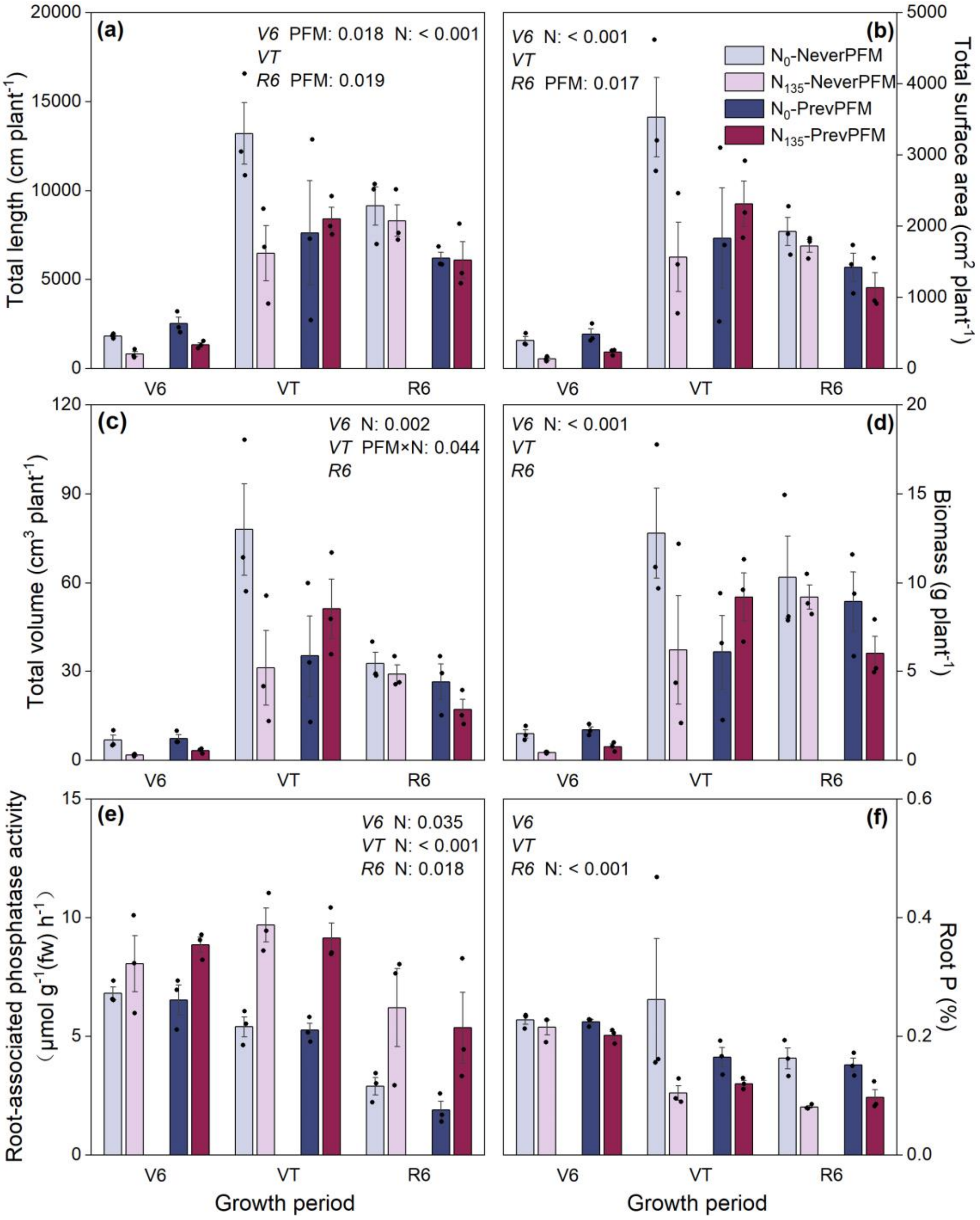

Fig.3 Maize below-ground (root) parameters during various growth stages. Total length (a), total surface area (b), total root volume (c), biomass (d), root associated phosphatase activities (e), P concentration (f). V6: sixth leaf stage, VT: tasseling stage, R6: physiological maturity stage. N_0: zero N fertilizer, N_{135}: 135 kg N ha^{-1} yr^{-1}, PrevPFM: previous plastic film mulching, NeverPFM: never plastic film mulching. Bars represent ± standard errors of the replicates (n = 3) and individual data

points are shown as black opaque circles. The values behind ‘PFM’, ‘N’ or ‘PFM × N’ represent the P values for the main effects of plastic film mulching and N fertilization, or their interaction, respectively. Only P values less than 0.05 are shown in panels.

3.3 Maize yield and maturation time

Maize yields were similar between previous and never plastic film mulching ($P > 0.05$, df = 1, Fig. 4a), and also yield parameters (100-seed mass and spike length) ($P > 0.05$, df = 1, Fig. 4b, 4c). However, maize in previous plastic film mulching plots had an earlier dough stage (6~10 days) than those in never mulching plots (Fig. 4d). Maize yield was similar between fertilized and non-fertilized plots ($P > 0.05$, Fig. 4a). This was also the case for spike length, but 100-seed mass was larger at fertilized than at non-fertilized plots ($P = 0.003$, df = 1, Fig. 4b). At seeding stage, plants in N fertilized plots displayed symptoms of serious P deficiency, indicated by purple leaf and obvious growth inhibition, whereas plants at non-fertilized plot did not have these symptoms (Fig. 4d). The symptoms in fertilized plots were a litter lighter for previous plastic film mulching than never plastic film mulching. Although P deficiency symptoms were no longer present at tasseling stage and maturity stage (Fig. 4d), the time of dough stage was delayed in fertilized plot for 10~15 days.

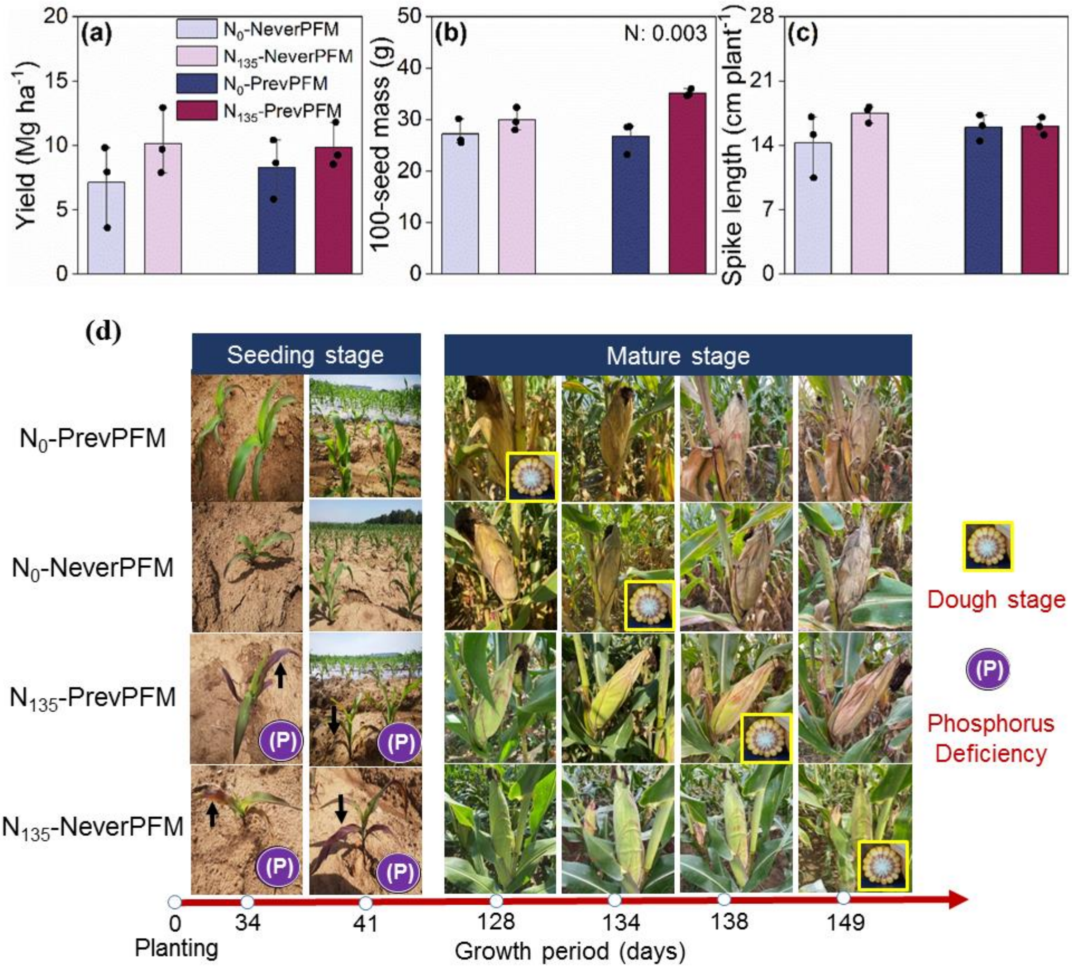

Fig.4 Maize yield (a), 100-seed mass (b), spike length (c), and growth process and maturation time (d) under the combined plastic film mulching and fertilization with urea-nitrogen (N) treatments. N$_0$: zero N fertilizer, N$_{135}$: 135 kg N ha^{-1} yr^{-1}, PrevPFM: previous plastic film mulching, NeverPFM: never plastic film mulching. Bars represent ± standard errors of the mean (n = 3) and individual data points are shown as black opaque circles. The values behind 'PFM', 'N' or 'PFM × N' represent the P values for the main effects of plastic film mulching and N fertilization, or their interaction, respectively. Only P values less than 0.05 are shown in panels.

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

Redundancy analysis (RDA) results showed that axis 1 and axis 2 together explained 91%, 88.49% and 86.01% of the variance between soil proporties and maize performance at the sixth leaf stage, tasseling stage, and physiological maturity stage, respectively (Fig. 5a, 5b, 5c). The groups of PrevPFM and NeverPFM generally clustered together, both for N_0 and N_{135} levels. By contrast, the groups N_{135} and N_0 were positioned at opposite ends of the first canonical axis, and the data points of N_0 stood generally in the positive direction of all the maize growth parameters (except for leaf chlorophyll content and root-associated phosphatase activity) during all growth stages. Soil pH and moisture were the two most important soil factors influencing maize performance during all growth stages, and were positively correlated with most crop growth parameters. Soil Olsen-P content was also a key factor for maize growth at the sixth leaf stage, but did not play an important role after this period.

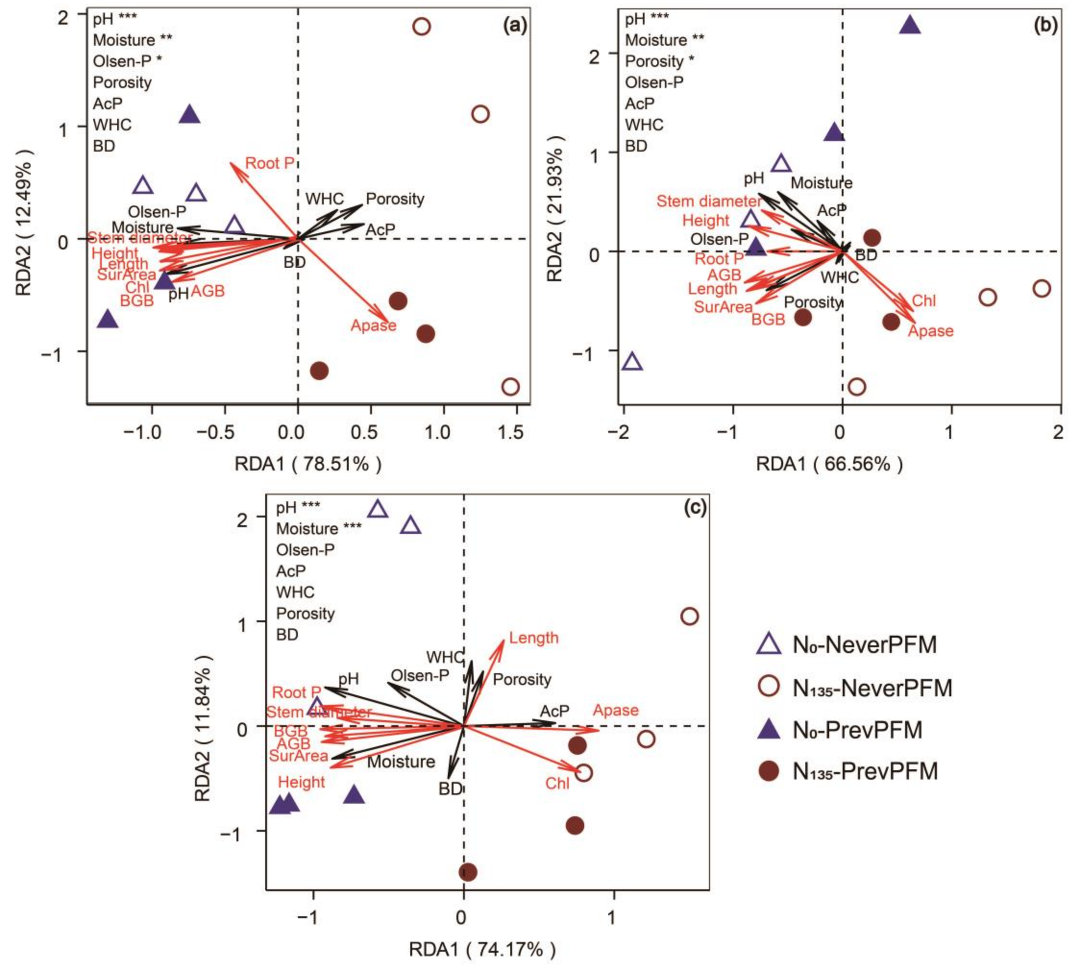

Fig.5 Redundancy analysis of plant growth impacted by soil properties at sixth leaf stage (a), tasseling stage (b) and physiological maturity stage (c). Red and black arrows indicate plant growth parameters and soil properties, respectively. SurArea: total root surface area; AGB: aboveground biomass; BGB: belowground biomass; Chl: chlorophyll; APase: root-associated phosphatase activity; BD: soil bulk density; WHC: water holding capacity; AcP: soil phosphatase activity. On top, the soil properties were fitted to the ordination plots using a 999 permutations test (*P*-values). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

4. Discussion

4.1 Legacy effects of long-term plastic film mulching

In contrast to our hypothesis, 33 years of plastic film mulching did not appear to leave a net negative legacy on maize growth and yield, although root total length and surface area were inhibited at previously mulched plots at physiological maturity stage (Fig. 3a and 3b). This occurred despite the high levels of macro-plastic residues (diameter >5 mm) present in the mulched plots in surface soil: plastic film residues had accumulated to 360 kg ha^{-1} or 6796 pieces m^{-2}, of which about 80% were < 4 cm^{2} and 20% were 4~25 cm^{2} in area [17]. Plastic film residues accumulation may reduce maize yield through inhibiting root growth and development [15, 20, 34]. Xie, et al. [35] found that the yield of maize was only decreased when the residual film amount was above 720 kg ha^{-1}. Hu, et al. [20] showed that maize yield was decreased by 15~18% and 23~25%, when adding plastic film residues at 300 and 600 kg ha^{-1}, respectively. Chen, et al. [34] found that the threshold when maize yield started to decrease was 180 kg ha^{-1} plastic film residues. However, all these studies were conducted by artificially adding plastic film residues to soil, in which the plastic residue is fresh and does not experience a long-term aging process. Aged plastic residues may affect crop growth less than fresh residue, because it is more brittle and easy to form holes, and may thus not interfere with root growth as fresh ones. Fresh plastic film residues have high tensile strength and are thus difficult to be torn, due to containing high molecular weight polymers with high hydrophobicity and semi-crystalline structures [36]. Contrastingly, aged plastic film residues after ultraviolet radiation are easy to be fragmented into microplastics, accompanied by the formation of cracks and cavities on the mulch film surface and an increase in crystallinity and hydroxyl index [37]. Pflugmacher, et al. [38] found that the adverse effects on the germination and seedling growth of *Lepidium sativum* were reduced as a function of the aging time applied to the polycarbonate. Accordingly, we did not observe negative legacy on maize growth and yield though the amounts of plastic film residues are close to or exceed the calculated thresholds. Similarly, a recent meta-analysis did not observe a decrease in maize yield with increasing amounts of residual films and more than half of their collected data points even showed an increase in maize yield to plastic film residue [10].

Apart from macro-residues of plastic film, the accumulation of film-derived microplastic reached as high as 8318 particles per kg soil in the 0~10 cm layer, 436 particles per kg soil in the 80~100 cm layer, and a total of 3.7×10^6 particles m^{-2} soil in 0~100 cm soil profile in our mulched plots [17]. In the literature, numerals studies reported that microplastic had caused inhibitory effects on higher plants (e.g., Qi, et al. [26] and Colzi, et al. [27]). However, the microplastic accumulation in our plot seems to have no net negative impact on maize growth and yield. The reason could be that polyethylene (PE) film-derived microplastic is not as toxic as other types of microplastic [24]. Many studies did not observe negative impact of PE microplastic on plant growth but observed the negative impact for polyvinyl chloride (PVC) or polylactic acid (PLA) microplastic [26-28]. This may result from the minor effect of PE plastic on soil structure and microbial activities, as compared to polyester and polyacrylic microplastics [39]. Nevertheless, several studies observed the negative impact of PE microplastic on maize growth in pots [25] and hydroponic conditions [40], suggesting that our explanation needs to be further verified.

On the contrary, 33 years of plastic film mulching even had a positive legacy for maize at the seedling stage, as maize aboveground biomass and root length were larger for previous plastic film mulching than for never mulching at the sixth leaf stage (P =

0.018, Fig. 2f, 3a). This may be driven by higher soil moisture for previous plastic film mulching than for never-mulching (Fig. 1a, Table S1). The RDA result showed soil moisture was a key soil property controlling crop growth performance and was positively correlated with most growth parameters (Fig. 5a, S1). Higher soil moisture was attributed to a higher degree of compaction of surface and subsurface soils for previous plastic film mulching than for never-plastic film mulching ($P < 0.05$, Fig. S2), which slowed down water evaporation. Accordingly, we observed deeper tracks from tractors at previous plastic film mulching plots than at never-plastic film mulching plots when planting in spring of 2021. This is supported by Sun and Ma [41] who observed film mulching promoted the movement of clay particles to the subsurface soil resulting in obvious deposition and cementation. The reason could be linked with the diurnal internal water cycle under the mulch, i.e., plastic mulch traps evaporative water, and condensed water drops underneath the mulch during the daytime can be returned to soil during the nighttime [42]. Frequent alternation of wet and dry changes the composition of soil particles, and more clay particles move and deposit with water, thereby blocking the pore space and increasing soil compaction [41]. In our study, we observed the lower soil porosity under previous PFM than under never PFM, although it only occurred at the seedling stage ($P = 0.03$, Table S3). A soil incubation study also observed the decrease of soil porosity after addition of plastic film residues [43]. Although a soil incubation study observed the accelerated water evaporation from soil amended with plastic film residues [23], the plastic doses were 0.5% and 1% relative to soil mass and much larger than the reality in our mulched plots. However, positive impacts of previous plastic film mulching on maize growth did not occur at tasseling and maturity stages. This suggests that soil moisture was a limiting factor for maize growth only at the seedling stage but not later stages.

4.2 Impacts of long-term N fertilization

In our experiment, 33 years of only N fertilization induced severe P limitation for maize growth, confirming our previous study [14]. Soil Olsen-P (available for plant) concentrations were lower in N fertilized plot than non-fertilized plots (Fig. 1e), indicating a decline of soil P supply capacity following N fertilization. Accordingly, maize root P concentrations were lower at fertilized plots (Fig. 3f). To alleviate this situation, maize roots at fertilized plots secreted larger amounts of phosphatase compared to non-fertilized plots (Fig. 3e). This is in line with previous studies which have shown that long-term application of N fertilizer exacerbated P deficiency [30]. Ultimately, long-term N applications reduce soil pH, which has a major impact on soil P solubility. Soil acidification following urea fertilization occurs due to the nitrification process [44]. This acidification then increases the solubility of iron and aluminum minerals [45], which can decrease soil P availability through re-precipitation of P with free Fe^{3+} and Al^{3+} and also increase the ability of Fe and Al oxy-hydroxide minerals to strongly adsorb P by ligand exchange [46]. A 10-year N fertilized grassland experiment also observed the increase of Al-P and Fe-P amounts with the decrease of pH [47]. In our study, although we did not measure Al-P and Fe-P, this mechanism is supported by the decrease of soil pH by about 1 unit (Fig. 1b) and the increase DTPA-Fe (Table S4)following 32 years of N fertilization. This is likely occurring in our case because the pH dropped from above 6 to below 5.5, which is the pH zone in which P solubility dramatically decreases due to the increase in Al solubility [46].

However, urea-induced P deficiency only inhibited maize growth at the sixth leaf stage (Fig. 4). At this stage, maize leaves had lower chlorophyll concentration but higher flavonoid concentration at fertilized plot at non-fertilized plot, also suggesting plant growth suffering from stress following fertilization (Fig. 2c, d). In contrast at middle (tasseling stage) and late stages (physiological maturity stage), maize growth rates were greater on fertilized plots, indicated by its higher chlorophyll concentration than non-fertilized plot. Maize above- and below-ground biomass at fertilized plots eventually recovered to equal those at non-fertilized plots (Fig. 2f, 3f). Seedling stage is the most vulnerable period when crops are sensitive to various environmental stresses [48]. At tasseling and maturity stages, maize may have multiple strategies to relieve P deficiency. For example, the difference of root-associated phosphatase between fertilized and non-fertilized plots (fertilized > non-fertilized) increased from the sixth leaf stage to tasseling and maturity stages (Fig. 3e), suggesting that maize root at fertilized plots was stimulated to secrete phosphatase at later stages to increase P sources for uptake. In addition, the difference in root P content between fertilized and non-fertilized plots (fertilized < non-fertilized) increased from at the sixth leaf stage to tasseling and R6 (full maturity) (Fig.3b), suggesting that maize in fertilized plots may have transferred large amounts of P from root to aboveground biomass at later stages.

5. Conclusion

Our study evaluated the impacts of long-term plastic film mulching-derived film residues and microplastic accumulation on crop performance. We demonstrate that 33 years of plastic film mulching did not leave a net negative legacy for subsequent maize growth and yield. Although plastic film mulching can add substantial amounts of macroplastic residues (360 kg ha^{-1}) and microplastic (3.7×10^{6} particles m^{-2}) accumulation into soils, it did not negatively impact soil structure and maize growth. Plastic mulching is a management strategy that intentionally induces positive effects on soils; our study therefore could only assess the net effects resulting from these positive effects of the mulching practice (moisture legacy) and the presumed negative effects of plastic accumulation. Our data showed that, at least in the short term, there are no strong net negative effects on the parameters measured. Such negative effects may materialize in the future, as the positive effects subside with the absence of plastic cover, while negative effects become more apparent, for example by increasing fragmentation of plastic to micro- or nanoplastic size. Future research should determine if there are delayed negative effects of plastic pollution that develop with time after ceasing plastic use, and also address whether the microplastics at this site have potential to become nanoplastics and impact organisms. On the other hand, long term N fertilization resulted in a pH decrease of about one unit, which likely led to decreased P solubility; this manifested itself as a temporary maize P deficiency occurring in early stages of growth.

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Ethics declarations

The authors declare no competing interests.

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