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

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

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Abstract:

1. Introduction

In the Anthropocene, human activities and artificial products profoundly change the earth. Plastic, as an artificially synthesized compound, is now ubiquitous on the earth even near the top of Mount Everest, the highest peak in the world (Napper et al., 2020). In the recent decades, plastic pollution has attracted great attention due to its potential ecological and environmental implications on global scale (Jambeck et al., 2015). Consequently, plastic pollution was recently listed as one of the top 10 global environmental problems by the United Nations Environment Programme (UNEP, 2014). Compared with plastic pollution of oceans and freshwater, little is known about plastic pollution of terrestrial ecosystems (Bläsing & Amelung, 2018, Rillig & Lehmann, 2020). Due to the widespread use of plastic mulch, shed plastic film, and biosolids (Duis & Coors, 2016, John & Wang, 2021, Ng *et al.*, 2018), croplands have been identified as a major reservoir of plastic debris (Nizzetto et al., 2016). Due to the plastic film residues accumulation negatively impacting soil health, plastic pollution in croplands has potential to threaten long-term food security (Zhang et al., 2020).

Plastic film mulching (PFM) is widely used in global agricultural ecosystems to improve plant growth because of increasing soil temperature and moisture (Ma *et al.*, 2018, Wang *et al.*, 2021, Zhou *et al.*, 2009). A recent meta-analysis showed that PFM increased crop yields by 24% on average (Gao et al., 2019). However, as the use time of mulch film increases, there are more and more plastic residues remaining in the soil, because plastic films are not possible to be removed clearly, especially for thin films (i.e., 5~8 μm thick) used in some countries, e.g., China (Ding et al., 2021). Our recent study showed that the residues of plastic film (size > 5 mm) were as high as 360 kg hm^{-2} and microplastics (< 5 mm) exceeded 10000 items by 1 kg soil in 0~20 cm layer after 32 years of plastic film mulching (Li et al., 2022b). The residual plastic accumulated over a certain value in the soil could decrease soil pore connectivity and porosity (Yan et al., 2006), thus affecting the movement of nutrients and water in the soil (Li et al., 2020). Thus, the germination of crop seeds and the development of roots would be also seriously affected by the residual film (Hu *et al.*, 2020a, Hu *et al.*, 2020b). Moreover, polyethylene (PE) film-derived plastic fragments and microplastic accumulation in soil may change soil water retention, water evaporation, or soil water repellency (Machado *et al.*, 2018, Steinmetz *et al.*, 2016, Wan *et al.*, 2019). Therefore, long-term PFM is expected to leave a negative legacy for crop growth and yield.

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

Our study evaluated the legacy effects of 33 years of PFM on soil properties, succeeding maize growth, and yield in a continuous plastic film mulching and fertilization experiment initiated in 1987. To investigate the legacy effect, previous mulching plots were not covered with polyethylene film in 2021 and never mulching plots were set as control. Maize aboveground and belowground growth indexes (stem thick, height, chlorophyll, flavonoid, root-associated phosphatase activity, root P, root morphological characteristics parameter, 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 ripening time were measured at the end of the growing season. The aim is to test the hypothesis: long-term PFM would have a negative legacy on maize growth and yield, due to large amounts of plastic and microplastic accumulation in soil.

2. Materials and methods

2.1 Study site and experiment design

The experimental field site was the long-term plastic film mulching 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 factorial design with two levels of plastic film mulching (with and without) and two levels of N fertilizer, that produces a combination of 4 treatments with three 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 has an area of 69 m^2. The N fertilizer was urea powder, applied as basal fertilizer in spring. There was no fertilization with other nutrients (e.g., phosphorus) in any of the plots. Maize (*Zea may* L.) was selected for this experiment because it is one of the major crops grown in northeast China. Seeds of maize were sown at an approximate density of 50000 plants per hectare. A detailed description of the experiment can be found in Ding et al. (2019). In order to investigate the legacy effect of previous PFM, two ridges (5 m×2 m) were randomly selected at the plots with PFM to stop covering with plastic film in 2021, which are called as previous PFM (PrevPFM). The plots without PFM were set as control, which are called as never PFM (NeverPFM). Soil properties and maize growth at the N_0 and N_{135} plots under previous and never plastic film mulching treatments (called as N_0-PrevPFM, N_{135}-PrevPFM, N_0-NeverPFM, N_{135}-NeverPFM, respectively) were measured during the growth season in 2021.

2.2 Sampling and measurements

Soil moisture, plant height, and stem thick were measured every 7 days from June to July, every 14 days from July to August, and every 21 days from August to September. 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 highest with steel tape, and stem thick which was the middle diameter of the second aboveground section was measured with a vernier caliper.

Leaf pigment, above- and below-ground biomass, root morphological properties, root phosphorus concentration, and its-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 the three stages were the time when more than 80 % of the plants are in that 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°C to constant weight. At each plot, two plant roots in each plot were randomly sampled by digging up the soil adjacent to the main trunk up to a radius of 15 cm and a depth of 40 cm and collected all scattered roots. The roots were washed with tap water to remove soil and then wash it with ultrapure water for 3~5 times. One plant root was cut into parts, and measured by a root scanner (EPSON Expression 11000XL) and an image analyzer (EPSON Expression 11000XL) for root morphology, including total root length, total surface area, total volume. Scanned roots were dried to a constant mass at 60°C and then weighed. Dry roots were crushed pulverizer and passed through a 0.25mm mesh for the determination of root phosphorus (root P), which was digested by combination of H_2SO_4 and H_2O_2 (8:5). The other root was used to determined root-associated phosphatase activity (APase).

Meanwhile, soil samples were collected at 0~20 cm layer for the measurements of pH, soil phosphorus, soil acid phosphatase (AcP), ammonium (NH_4^+-N) and nitrate nitrogen (NO_3^--N) contents, bulk density, total porosity, and water holding capacity at the three corresponding crop stages. Three soil cores were randomly sampled using an auger (4 cm in diameter) and then mixed into one sample at each plot. Soil samples were passed through a 2-mm sieve to remove plant debris and gravel. One part was air dried under natural conditions to determine the soil pH and plant-available soil phosphorus (Olsen-P), and the other part of fresh soil is used to determine soil acid phosphatase (AcP), ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N). 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 being extracted with 0.5 *M* $NaHCO_3$ according to the colorimetric method (Bao, 2000). Soil NH_4^+-N and NO_3^--N were extracted with 10 m*M* $CaCl_2$ (soil: water = 1:10) and measured by a continuous flow analyzer (Bran-Luebbe AA3, Germany). Soil bulk density, total soil porosity, and soil water holding capacity were determined according to the methods in Chen (2005). After crop harvest in autumn, soil compactness 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 with 45 cm depth, and data was automatically read and recorded.

Soil acid phosphatase activity and root-associated phosphatase activity were measured following the methods in Lin et al. (2020). Briefly, 1g fresh soil or 0.2g 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 cultured in the dark at 20°C for 1h, and then the reaction was stopped by 0.5 *M* NaOH and 0.5 *M* $CaCl_2$. Then, the absorbance of *p*-nitrophenol (*p*NP) in the supernatant was measured at 410 nm by Unic-7200 Spectrophotometer (Shanghai, China). Four analytical replicates were used for each root sample, including a blank. For 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 (Guo et al., 2004). At the physiological maturity stage, the yield was measured through randomly selecting four plants at the middle position at each plot. The 100-seed fresh weight 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), N fertilization (N_0 and N_{135}) and their interactions on soil and crop parameters were assessed by two-way ANOVA. Normality of residuals and homogeneity of the variances of the residuals across groups were checked for each ANOVA. Pearson correlation analyses were conducted between plant growth parameters and soil properties at the sixth leaf stage, tasseling stage, and physiological maturity stage, respectively. We found the three soil parameters (i.e., pH, moisture, and Olsen-P concentrations) were well correlated with most plant growth parameters, especially at six leaf stage.

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 the data of crop performance (stem thick, 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 then tests 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 always higher for previous plastic film mulching than for never mulching (most $P < 0.05$, Fig. 1a). 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 ($P > 0.05$, 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 ($P < 0.05$ and $P < 0.001$, Fig. 1d). Soil Olsen-P concentrations and phosphatase activity were both similar between previous and never plastic film mulching) in all studied stages ($P > 0.05$, Fig. 1e and 1f).

Soil moisture was lower at N fertilized plot than at no fertilized plot for most time in growth season (Fig. 1a). Soil pH was dramatically lower at N fertilized plot than at no fertilized plots ($P < 0.001$, Fig. 1b). As expected, N fertilized plot had larger soil NO_3^--N concentrations than at no fertilized plot, especially for never plastic film mulching ($P < 0.001$, Fig. 1d), but these two plots had similar NH_4^+-N ($P > 0.05$, Fig. 1c). Soil Olsen-P concentrations were lower at N fertilized plot than no fertilized plot, especially at the sixth leaf stage ($P = 0.004$, Fig. 1e). Soil phosphatase activity did not have the difference between the contrasting fertilized plots ($P > 0.05$, Fig. 1f).

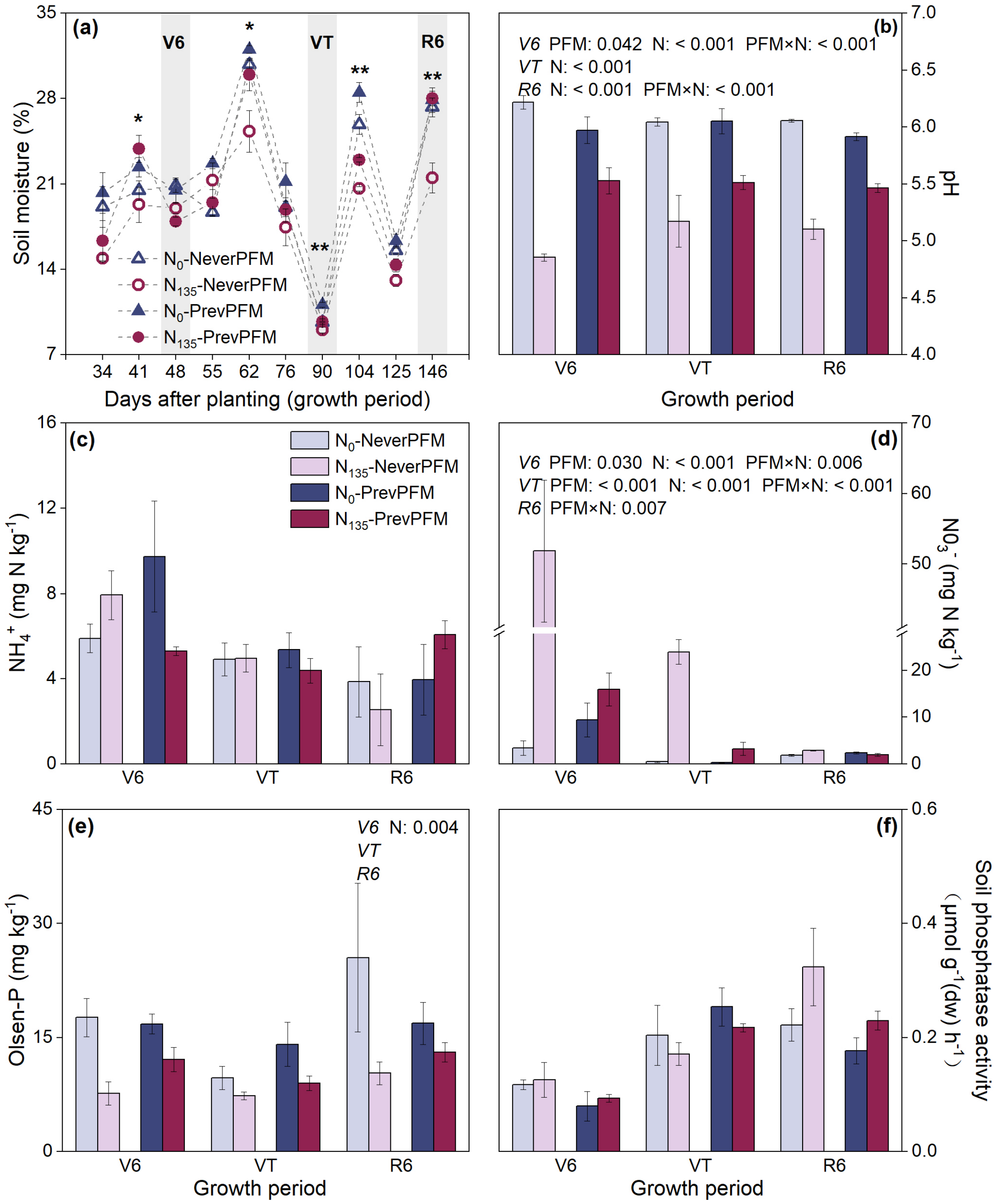

Fig.1 Soil moisture (a), pH (b), NH_4^+-N (c), NO_3^--N (d), Olsen-P (e) concentrations and soil phosphatase activity (f) during growth seasons. V6: sixth leaf stage, VT: tasseling stage, R6: physiological maturity stage. N_0: zero N fertilizer, N_{135}: 135 kg N ha^{-1} yr^{-1}, PrevPFM: previous plastic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

(a) denote main effects of plastic film mulching within the 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 were showed in panels.

3.2 Maize above- and below-ground parameters

Long-term plastic film mulching did not have a negative legacy for succeeding maize, but even promoted maize growth sometimes. Maize stem was generally thicker and height was greater for previous plastic film mulching than for never mulching, especially at N_{135} level (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 ($P < 0.05$, Fig. 2f) and disappeared at tasseling and maturity stages ($P > 0.05$). Both leaf chlorophyll and flavonoid concentrations and NBI were similar between previous and never plastic film mulching ($P > 0.05$, Fig. 2c, 2d, 2e). Total root length was higher for previous plastic film mulching than for never mulching at the sixth leaf stage ($P < 0.05$, Fig. 3a), but this trend was reversed at physiological maturity stage ($P < 0.05$). However, other root properties, i.e., total surface area, total volume, biomass, root-associated phosphatase activity, and root P were all similar between previous plastic film mulching than for never mulching ($P > 0.05$, Fig. 3b, 3c 3d, 3e, 3f), except for total surface area at physiological maturity stage ($P < 0.05$).

Long-term N fertilization inhibited maize growth, especially at the seedling stage. Specifically, maize stem was finer and height was greater at N fertilized plot than at no fertilized plot during the whole growing season (Fig. 2a and 2b). Correspondingly, aboveground biomass was much smaller at N fertilized plot than at no fertilized plot, but these differences only occurred at the sixth leaf stage ($P < 0.05$, Fig. 2f) and disappeared at tasseling and maturity stages ($P > 0.05$). At the sixth leaf stage, N fertilized plot had lower chlorophyll concentrations and NBI but higher flavonoid contents in leaves than no fertilized plot, especially for never plastic film mulching (both $P < 0.01$, Fig. 2c, 2d, 2e). Oppositely, at tasseling and maturity stages, chlorophyll concentration was higher at N fertilized plot, especially for never plastic film mulching (Fig. 2c). Root generally had similar trends toward N fertilization with aboveground biomass. Root biomass, total root length, total surface area, total volume were much smaller at N fertilized plot than at no fertilized plot at sixth leaf stage (all $P < 0.01$, 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 activity was higher at N fertilized plot than no fertilized plot during the whole growing season ($P < 0.05$, Fig. 3e). Accordingly, root P concentrations were lower at N fertilized plot, especially for maturity stage ($P < 0.001$, Fig. 3f).

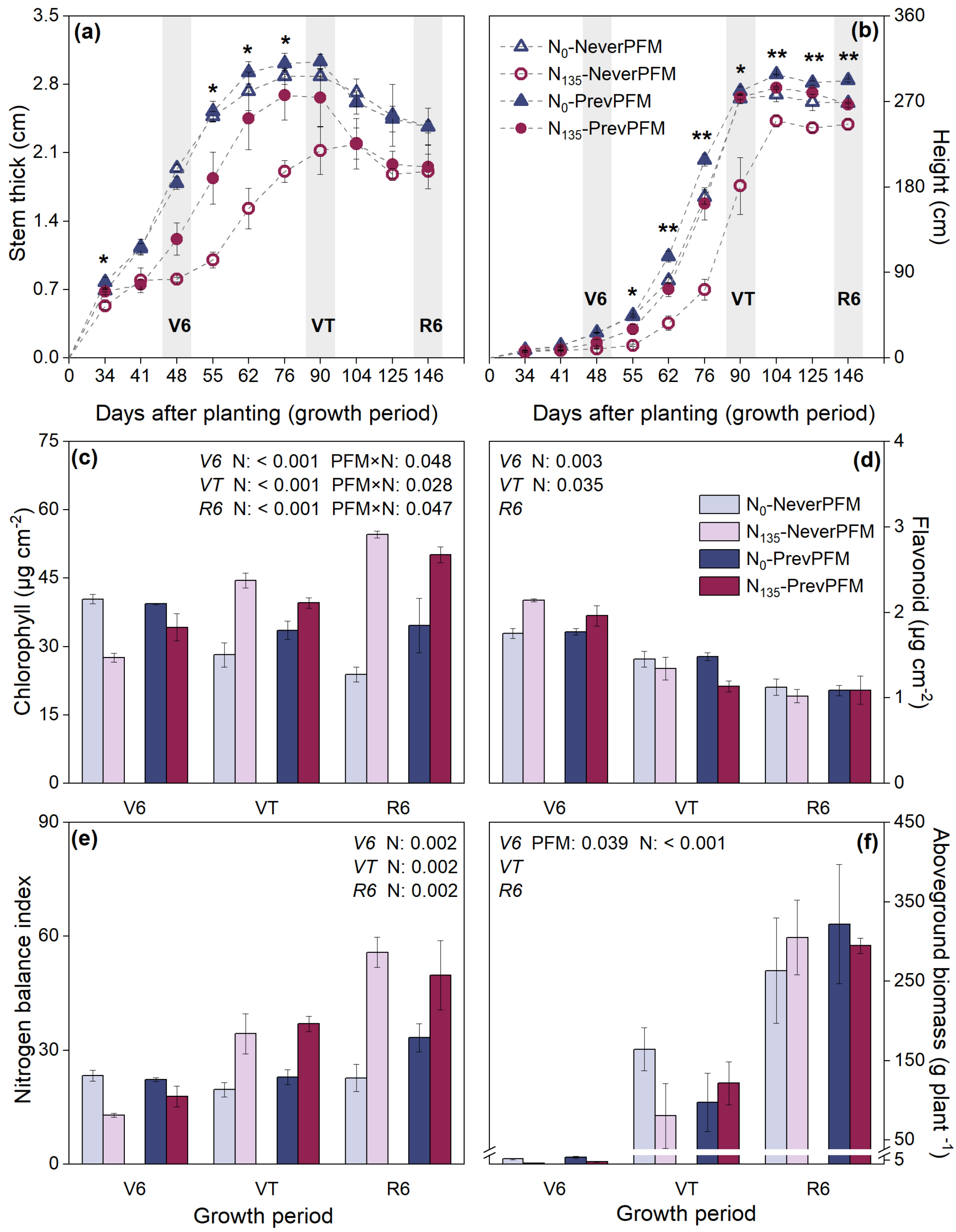

Fig.2 Maize above-ground parameters during various growth stages. Stem thick (a), height (b), chlorophyll (c), flavonoid(d), nitrogen balance index (e), and aboveground biomass (f). Nitrogen balance index was calculated by chlorophyll/flavonoid. V6: sixth leaf stage, VT: tasseling stage, R6: physiological 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). The symbols “**”, and “*” in panel (a) denote main effects of plastic film mulching within the 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 were showed in panels.

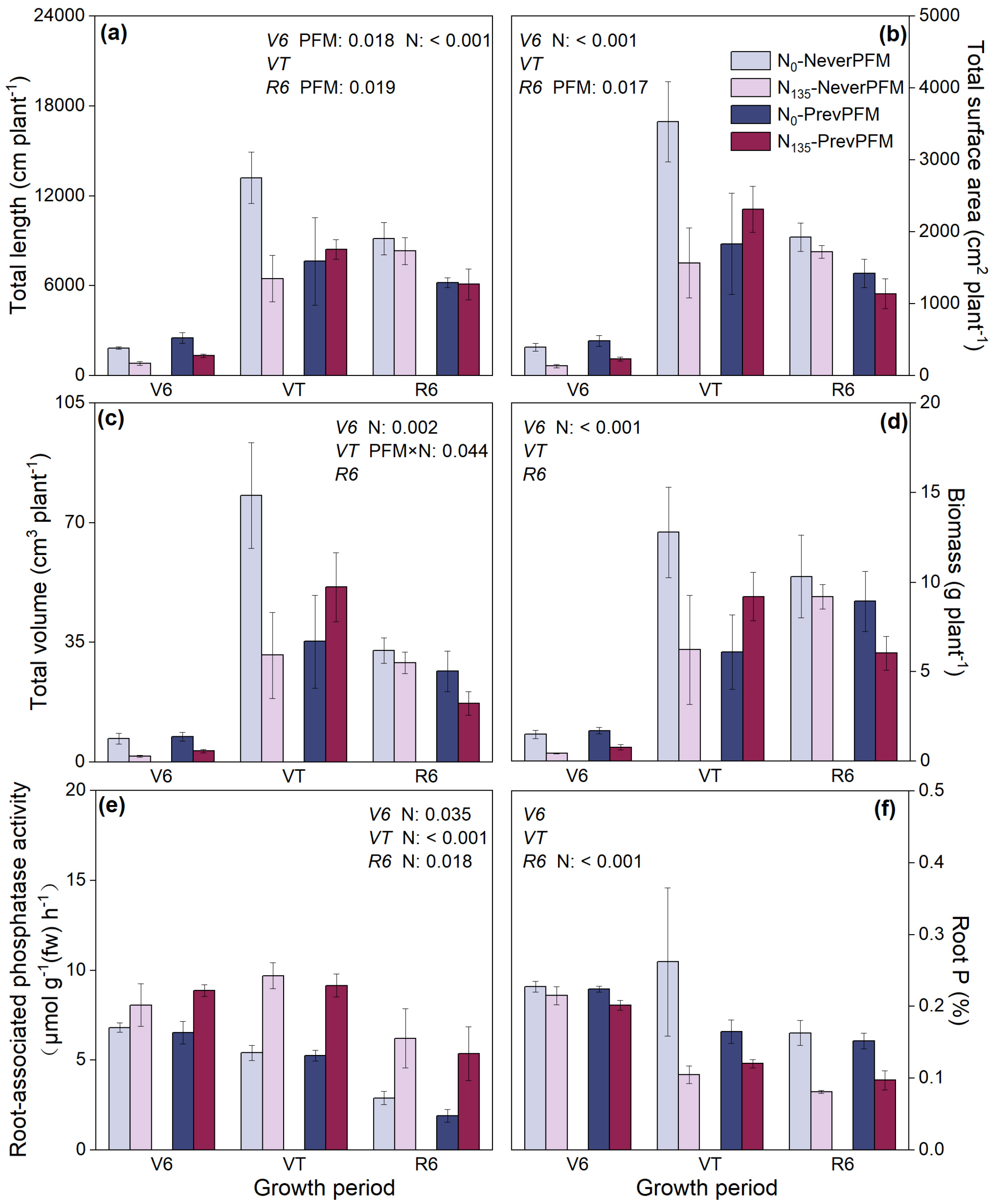

Fig.3 Maize below-ground (root) parameters during various growth stages. Total length (a), total surface area (b), total root volume (c), biomass (d), root associated phosphatase activities (e), P concentration (f). V6: sixth leaf stage, VT: tasseling stage, R6: physiological maturity stage. N_0: zero 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). 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 were showed in panels.

3.3 Maize yield and ripening time

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

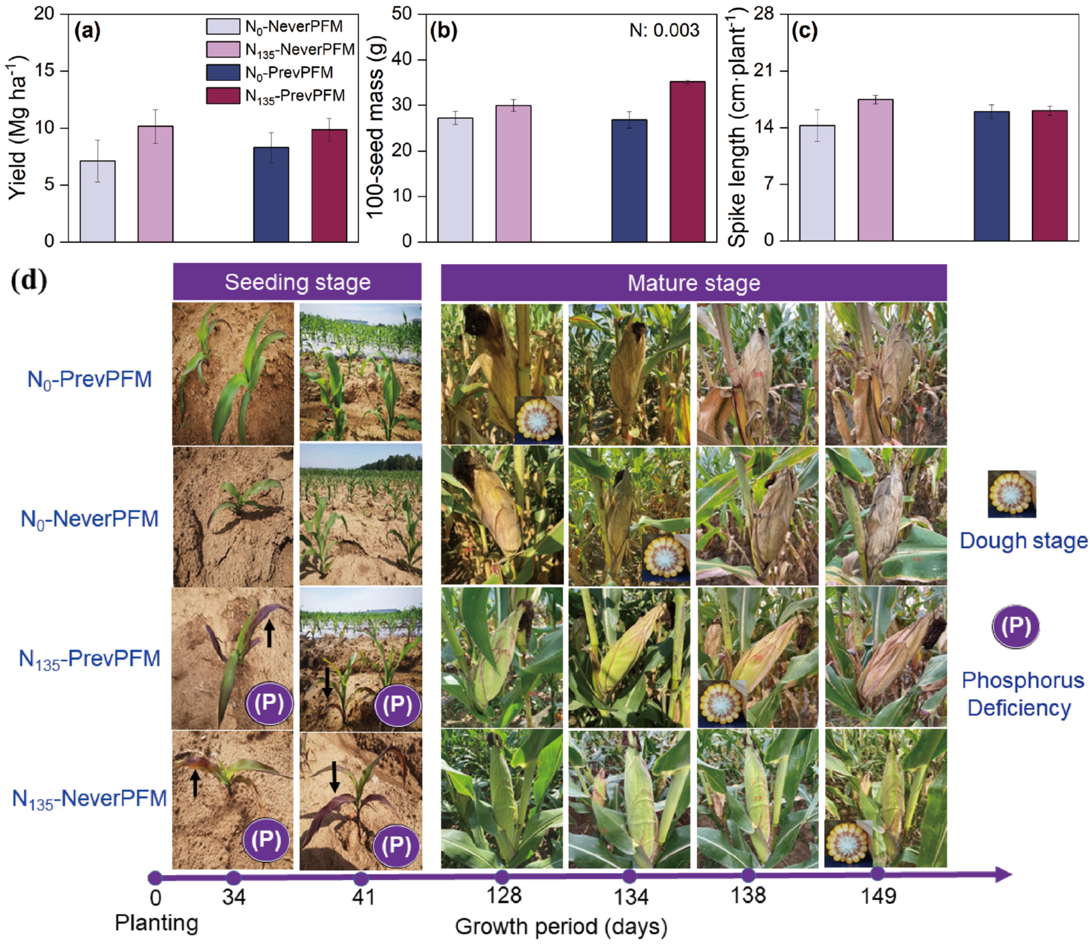

Fig.4 Maize yield (a), 100-seed mass (b), spike length (c), and growth process and ripening time (d) under the combined plastic film mulching and fertilization with urea-nitrogen (N) treatments. N_0: zero N fertilizer, N_{135}: 135 kg N ha^{-1} yr^{-1}, PrevPFM: previous plastic film mulching, NeverPFM: never plastic film mulching. Bars represent ± standard errors of the mean (n = 3). 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 were showed 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. Contrastingly, the groups N_{135} and N_0 were completely opposed in the factorial plan and factor N_0 stood generally in the positive correlation with all the maize growth parameters (except for leaf chlorophyll content and root-associated phosphatase activity) during all the growth stage. Soil pH and moisture were two most important determining soil fractors on maize performance during all the growth stage, and were positively correlated with most crop growth parameters. Soil Olsen-P content was also a key factor on maize growth at sixth leaf stage, but did not play an important role after this period.

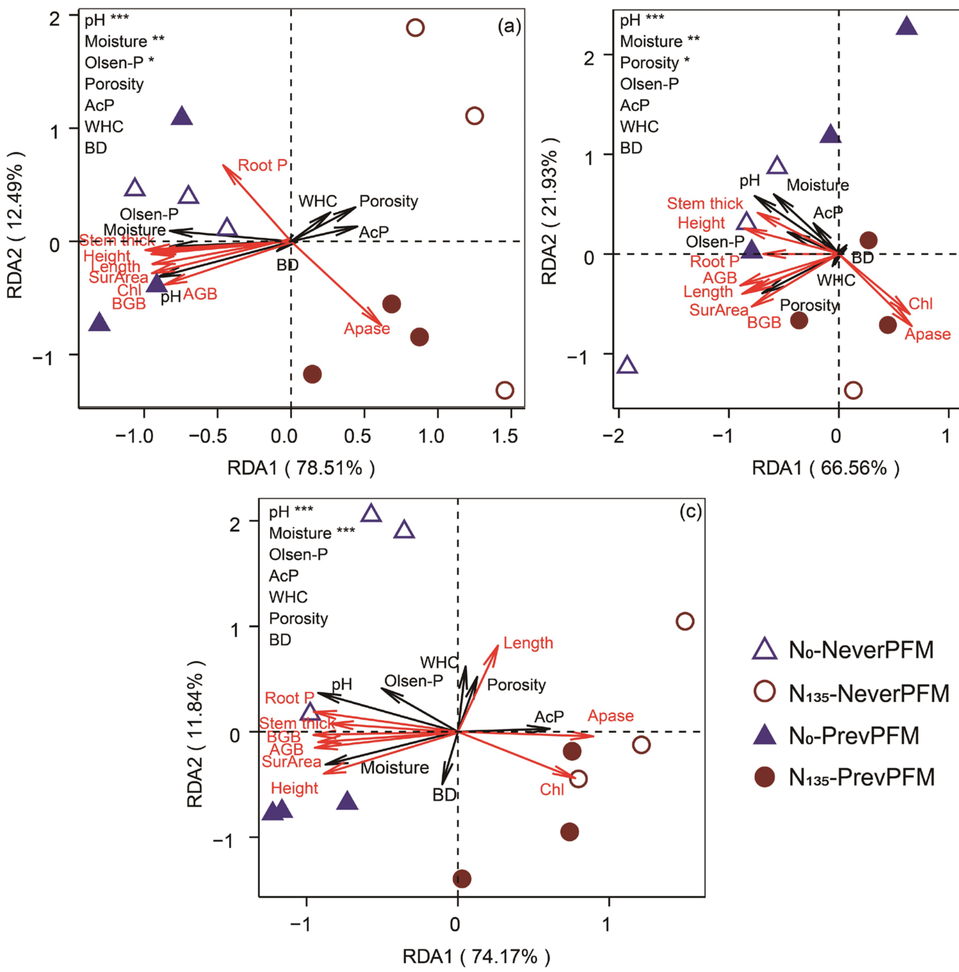

Fig.5 Redundancy analysis of plant growth impacted by soil properties at sixth leaf stage (a), tasseling stage (b) and physiological maturity stage (c). Red and black arrows indicate plant growth parameters and soil properties, respectively. SurArea: total root surface area; AGB: aboveground biomass; 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

Not supporting our hypothesis, 33 years of plastic film mulching does not have a negative legacy on maize growth and yield. In our mulching plots, plastic film residues accumulated high to 6796 pieces m^{-2} or 360 kg ha^{-1} in surface soil (Li et al., 2022b). Plastic film residues accumulation may reduce maize yield through inhibiting root growth and development (Chen *et al.*, 2022, Gao *et al.*, 2019, Hu *et al.*, 2020b). Xie et al. (2007) found that the yield of maize was only decreased when the residual film amount was above 720 kg ha^{-1}. Hu et al. (2020b) showed that maize yield was decreased by 15~18% and 23~25%, when added plastic film residues with 300 and 600 kg ha^{-1}, respectively. Chen et al. (2022) found the threshold of maize yield starting to decrease was 180 kg ha^{-1} plastic film residues. However, all these studies are conducted by artificial 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 less affect crop growth than fresh residue, as it is fragile and may not hinder root growth. Pflugmacher et al. (2021) 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 above 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 residues (Zhang et al., 2020).

Apart from plastic film residues, the accumulation of film-derived microplastic reached as high as 3.7×10^6 particles m^{-2} soil in 0~100 cm soil profile in our plots (Li et al., 2022b). In the literature, numerals studies reported that microplastic had caused inhibitory effects on higher plants (e.g., Qi et al. (2018) and Colzi et al. (2022)). However, the microplastic accumulation in our plot seems to have no negatively 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 (Li *et al.*, 2022a). 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 (Colzi *et al.*, 2022, Qi *et al.*, 2018, Wang *et al.*, 2020). This may result from the minor effect of PE plastic on soil structure and microbial activities, as compared to polyester and polyacrylic microplastics (Machado et al., 2018). Nevertheless, several studies observed the negative impact of PE microplastic on maize growth in pots (Pehlivan & Gedik, 2021) and hydroponic condition (Urbina et al., 2020), suggesting that our explanation needs to be further affirmed.

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 ($P < 0.05$ Fig. 2f, 3a). This may be driven by higher soil moisture for previous plastic film mulching than for never mulching (Fig. 1a). The RDA result showed soil moisture was a key soil property controlling crop growth performance and positively correlated with most growth parameters (Fig. 5a and S1). Higher soil moisture was attributed to higher degree of compaction at surface soil for previous plastic film mulching than never plastic film mulching ($P < 0.05$, Fig. S2), which slowed down water evaporation. We observed deeper track of tractors at previous plastic film mulching plots than at never plastic film mulching plots when planting in spring of

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

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 (Ding et al., 2019). Soil Olsen-P (available for plant) concentrations were lower at N fertilized plot than no fertilized plot (Fig. 1e), indicating the 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 root 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 (Lin *et al.*, 2020, Tian *et al.*, 2019). Two mechanisms may explain P deficiency following N fertilization. Firstly, soil acidification following urea fertilization increases the solubility of non-base cations (e.g., Fe^{3+}, Al^{3+}) (Tian & Niu, 2015, Zarif *et al.*, 2020), which may decrease soil P availability by the precipitation of P with free Fe^{3+} and Al^{3+}. This conjecture is supported by an incubation experiment Meyer et al. (2021) who found that the decrease of soil pH increased solubilization of soil Al and the precipitation of Al-P minerals, and hence markedly decreased potential P availability in non-calcareous soils. A 10-year N fertilized grassland experiment also observed the increase of Al-P and Fe-P amounts with the decrease of pH (Wang et al., 2022). In our study, although we did not measure Al-P and Fe-P, this mechanism was partly supported by the decrease of soil pH by about 1 unit (Fig. 1b) and the increase DTPA-Fe (unpublished data) following 32 years of N fertilization. Another possible reason is that N fertilization promoted the uptake of P from soil by plants, due to increasing yield and plant biomass (Deng *et al.*, 2017, Pasley *et al.*, 2019, Rowe *et al.*, 2008). Year by year harvest would take away larger amounts of P from soil, which can reduce the pool of soil P and lead to P deficiency (Qu et al., 2009). This explanation was supported by that soil total P was lower at N fertilized plot than non-fertilized plot in our experiment (Ding et al., 2019).

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). Contrastingly at middle (tasseling stage) and late stages (physiological maturity stage), maize growth rates were faster at fertilized plot, indicated by its higher chlorophyll concentration than this at non-fertilized plot. Maize above- and below-ground biomass at fertilized plot were recovered to the same as those at non-fertilized plot (Fig. 2f, 3f). Seedling stage is the most vulnerable period when crops are sensitive to various environmental stresses (Jisha et al., 2013). 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 plant. Phosphatase can activate soil organic P and make them available for root uptake (Weil & Brady, 2017). In addition, the difference of root P between at fertilized and non-fertilized plots (fertilized < non-fertilized) increased from at the sixth leaf stage to at tasseling and maturity stages (Fig.3b), suggesting that maize at fertilized plot may transfer large amounts of P from root to aboveground growth at later stages.

5. Conclusion

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

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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 (μm cm^{-2})	Flv (μm 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 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 (*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).

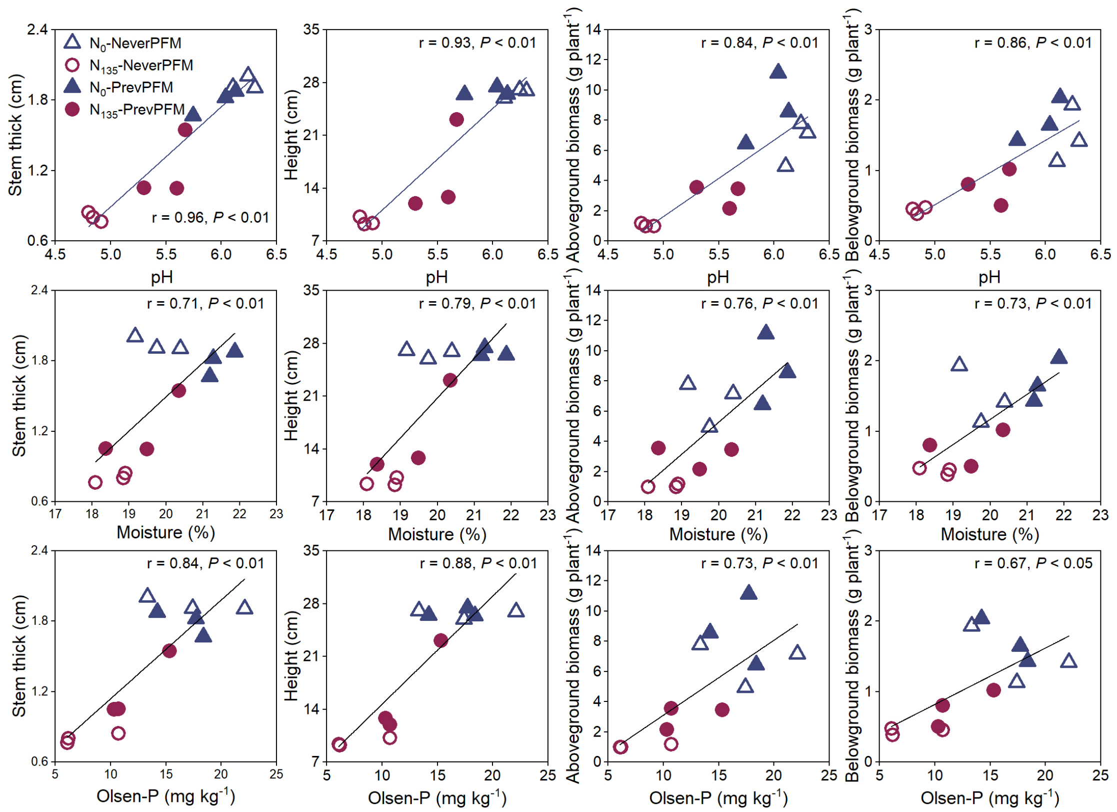

Fig. S1 The correlations of maize above- and below-ground growth parameters with soil pH, soil moisture and Olsen-P at the sixth leaf stage.

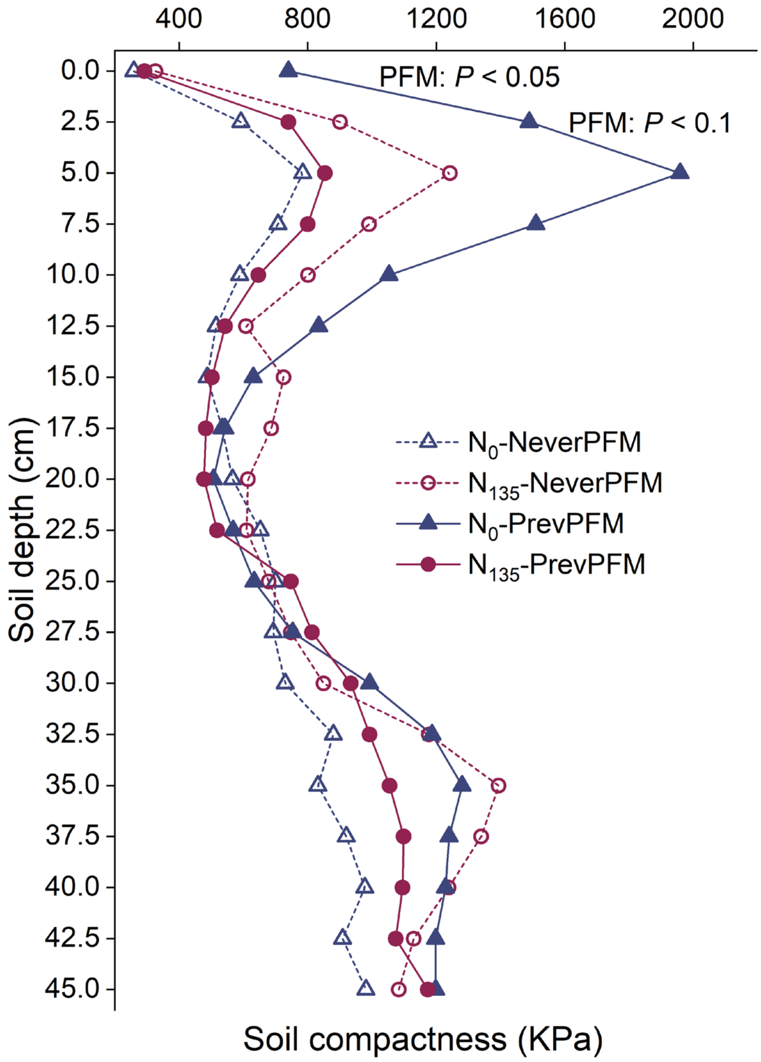

Fig. S2 Soil compactness through soil profile under the combined plastic film mulching and fertilization with urea-nitrogen (N) treatments. The *P* values behind PFM indicated the significance of main effect of plastic film mulching on soil compactness.

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