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The Degradability of Microplastics May Not Necessarily Equate to Environmental Friendliness: A Case Study of Cucumber Seedlings with Disturbed Photosynthesis

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Abstract: In the environment, degradable plastics are decomposed into biodegradable microplastics (Bio-MPs), but there is limited study on the impact on plant growth and development. Therefore, this study aimed to investigate biodegradable polylactic acid microplastics (PLA-MPs) and nonbiodegradable polystyrene microplastics (PS-MPs) with different concentrations (0.02%, 0.2%, and 2% *w*/*w*) to explore their short-term toxic effects on cucumbers. The results of this study showed that PLA-MPs significantly ($p < 0.05$) reduced the aboveground and belowground biomass of cucumber seedlings compared to the control. At the level of 2% MPs, the chlorophyll content and PRI vegetation index of cucumber plants decreased significantly, anthocyanin content increased, and the photosynthetic system was disturbed. Likewise, the antioxidant defensive system of cucumber was affected after exposure to MPs stress, especially under 2% levels. The hyperspectral image is a novel technique which analyzed the chlorophyll content and absorption under MPs treatment; there was still a high correlation between chlorophyll content, anthocyanin content, and MCARI vegetation index, so a single vegetation index could be used for rapid detection of plant physiological status. Our study suggests that Bio-MPs have potential ecological toxicity that could affect the growth of cucumber seedlings through deactivation of the PSII reaction center. Therefore, biodegradable plastics do not seem to be the optimal solution, and there is an urgent need for long-term monitoring and evaluation of the biological toxicity of biodegradable MPs.

Keywords: biodegradable microplastics; cucumber; photosynthesis; hyperspectral image; plant growth

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1. Introduction

Microplastics (MPs) pollution is one of the crucial concerns in the environment [1]. Annually, a survey reported that the MPs pollution in soil is 4 to 23 times greater than that in the ocean [2]. It is estimated that the amount of MPs imported into farmland through sludge is around 63,000~43,000 t in Europe and 44,000~300,000 t in North America [3]. Additionally, the sources of MPs pollution in soil are organic fertilizer and agricultural film residues [3,4]. Recent studies found that the presence and accumulation of MPs cause an array of toxic effects in plants [5]. In addition, MPs can complete their migration through the food chain at various trophic levels [6] as well as via circulatory systems in organisms [7,8].

In addition, degradable and ecofriendly plastics are used to control environmental pollution [9]. However, limited reports were published in this area, and question still arise as to whether biodegradable plastics are a positive solution for the long term in ecosystems [10]. With reference to the escalating global issue of microplastics (MPs) pollution affecting the total ecological system, the development of biodegradable plastics has emerged as a solution [11]. Biodegradable plastics are easily mineralized with the help of microorganisms into less harmful substances in a short time period [12]. Undoubtedly, this environmentally friendly material has become a focal point for significant advancements within the plastics market across different countries [13,14]. Nowadays, biodegradable plastics are used widely in ecosystems [15]. Therefore, as a new biodegradable MP prominently used in agriculture and daily activities, compound polylactic acid (PLA) has gradually entered people's field of vision [16]. Biodegradable microplastics (Bio-MPs) gradually decrease in molecular weight after entering the soil and with increasing degradation time [17]. Recent studies revealed that many biodegradable plastics presently on the market tend to break down into smaller MPs particles rather than completely biodegrading [18]. This could give rise to the accumulation of MPs in the environment and have multiple impacts on ecological systems [18]. Similar to nonbiodegradable plastics, biodegradable plastics might include chemical additives such as plasticizers, flame retardants, and antimicrobial agents to meet processing and practical needs. These additives can potentially release harmful substances during weathering and degradation, disrupting the biodegradation process and posing environmental risks [10,19]. In contrast, the few studies on Bio-MPs do not have firm conclusions about their ecological risks [20,21]. For example, Qi et al. [22] reported that MPs residues from biodegradable plastic exhibited many more negative effects on wheat plants than polyethylene MPs. Butyleneadipate-co-tere-phthalate (PBAT)-derived MPs significantly reduced rice plant growth and physiological response [23]. Under the combined pollution of PBAT and Bio-MPs with heavy metal arsenic (As), this combination significantly decreased the content of As in maize seedlings along with the total chlorophyll content and photosynthetic rate compared with the same concentration of polyethylene microplastics (PE-MPs); it showed greater toxic effects [24]. In contrast, the latest study by Chu et al. [25] indicated that the Bio-MPs did not affect the growth of oats and soybeans or the in-soil quality index. Bio-MPs are more likely to hydrolyze in soil, which will increase the content of soil-soluble organic matter (DOM) but reduce the humification degree of soil DOM, increase the contribution of microbial sources of soil DOM, and stimulate the utilization of easily decomposed carbon sources and energy sources by microorganisms [26]. The study by Zhang et al. [27] also found that aging Bio-MPs affect the organic carbon balance of grassland and farmland by influencing the content of phthalates in soil, microbial biomass, nutrients, and extracellular enzyme activity. In addition, Bio-MPs have different effects on soil organisms; for example, PLA Bio-MPs exposure can induce oxidative stress and gene expression in earthworms, increasing the ecological risk to earthworms [28]. Considering this, the environmental risk of Bio-MPs is still unclear; therefore, it is required to assess the effect of toxicity in plants and develop rapid detection techniques.

The presence of Bio-MPs in soil has been demonstrated to reduce soil pH, elevate soil's redox potential, and enhance the population of fungi and bacteria in soil. Most previous studies focused on the direct effects of biodegradable microplastics on soil. However, whether Bio-MPs can affect plant growth by affecting photosynthesis is largely unclear, and further studies are urgently needed. We conducted a case study to used oil-based plastic polystyrene microplastics (PS-MPs) and biodegradable microplastics (PLA-MPs) as test materials, and cucumber (*Cucumis sativus* L.), an important vegetable crop cultivated under plastic film mulching in China, was used as a test plant. The aim of this study was to evaluate the response of cucumber seedlings to microplastics (MPs) by investigating the effects of PS-MPs and PLA-MPs on the growth and physiological response of cucumber and to evaluate the potential of biodegradable plastics to replace conventional plastics in greenhouse agriculture. We hypothesized that (1) the biodegradable microplastic PLA-MPs would have adverse effects on cucumber seedlings in the short term and cause biomass changes; (2) PLA-MPs were used to control the biomass of cucumber seedlings by directly or indirectly inhibiting plant photo-cooperation; (3) the application of hyperspectral technology in plant response to microplastic stress also has a rapid and accurate effect. This study could be beneficial regarding the accumulation, physiology, and positive effect of MPs in crops.

2. Material and Methods

2.1. Charectrization of Microplastic

PS-MPs and PLA-MPs were purchased from Jiecheng Plasticizing Materials Co., Ltd. (Dongguan, Guangdong, China). The morphological characteristics of PLA-MPs and PS-MPs are shown in Figure 1. The PLA-MPs are mostly spherical with a rough surface (Figure 1A), and the average particle size is about 28.1 μm; PS-MPs contained a large number of flake and block irregular fragments (Figure 1B), with an average particle size of about 26.82 μm.

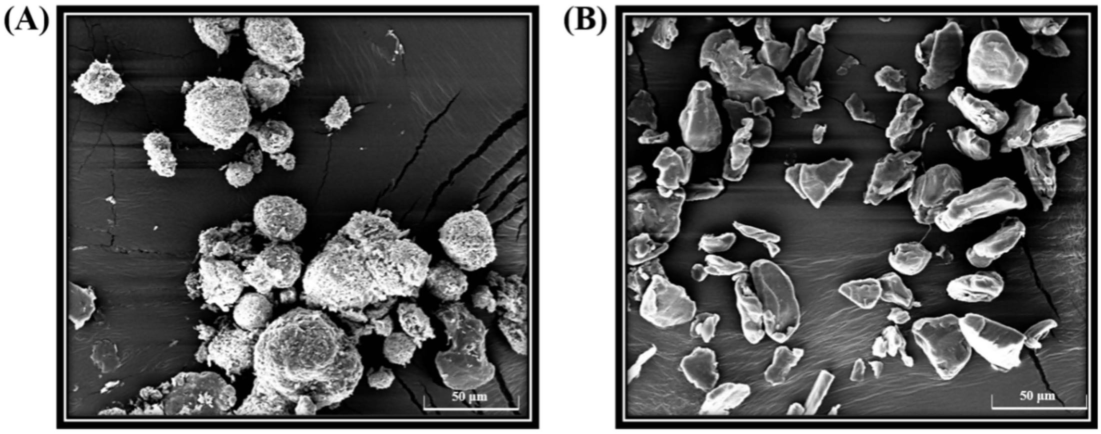

Figure 1. Scanning electron micrographs of polylactic acid microplastics (PLA-MPs) (**A**) and polystyrene microplastics (PS-MPs) (**B**).

2.2. Cultivated Plants and Soil

Cucumber (*Cucumissativus* L.) seeds were purchased from Zhongnong 26, Beijing Zhongshu Dasen Seed Co. Ltd. (Beijing, China) Cucumber seeds were first soaked in 2% (*v*/*v*) hydrogen peroxide (H_2O_2) solution for 30 min and then washed with distilled water 3–5 times to remove the residual H_2O_2 solution. Seeds of the same size were soaked in pure water for 5 min and placed in a seedling tray to germinate in a dark and humid environment until the true leaves of the seedlings were fully expanded. The nutrient soil (organic matter content > 25%) used in the experiment was purchased from Xinyang Guotong E-commerce Co., Ltd. (Xinyang, Henan, China). The collected soil was dried at room temperature, and the litter, debris, roots, and rocks in the soil were removed with a 5 mm sieve and preserved at room temperature for further study.

2.3. Experimental Design

This study set up 7 different treatments along with the control, indicated as CK (without any MPs): 0.02% PS-MPs (soil amended with 0.02% PS-MPs); 0.2% PS-MPs (soil amended with 0.2% PS-MPs); 2% PS-MPs (soil amended with 2% PS-MPs); 0.02% PLA-MPs (soil amended with 0.02% PLA-MPs); 0.2% PLA-MPs (soil amended with 0.2% PLA-MPs); and 2% PLA-MPs (soil amended with 2% PLA-MPs), with eight replicates. We selected the MPs concentrations from previous studies in soil [17,29]. The seedlings were kept in an experimental chamber, 14 h light and 10 h dark photoperiod, 22–26 °C, and 80% humidity. We renewed the water twice a week. After three weeks, plants were harvested, and we analyzed different phenotypical and physiological parameters.

2.4. Plant Physiological Analysis

Reactive oxygen species (ROS) were determined by grinding and extracting 0.01 M (pH = 7.2) phosphate buffer, centrifuging at 5000 rpm (4 °C) for 15 min, and finally taking the supernatant and using an Elisa kit (Wuhan Saipei Biotechnology Co., Ltd. (Wuhan, China)) for quantitative analysis of ROS. The crude enzyme solution was also extracted by grinding with 0.5 M (pH = 7.8) phosphate buffer and centrifuged at 10,000 rpm (4 °C). The supernatant was obtained using the NBT photoreduction method to determine the activity of superoxide dismutase (SOD) so as to inhibit the photoreduction of NBT to 50% of the control as an enzyme activity unit (U) [30]. Peroxidase (POD) activity was measured using the guaiacol method, that is, an increase of 0.01 per gram of fresh OD_{470} per minute was defined as one activity unit (U). Catalase (CAT) activity was measured using the UV spectrophotometric method, and a decrease of 0.01 per gram of fresh OD_{240} per minute was defined as one activity unit (U). Similarly, leaf nitrogen was detected using a plant nutriometer (SPAD-502 PLUS; Konica Minolta). The total chlorophyll, flavonol, and anthocyanins contents were measured using a multipigment meter (MPM-100) and an AP-C 100 (Photo Subsystem Instrument; Drásov, Czech Republic) fluorometer AquaPen-C. The symbols, definitions, and meaning of these chlorophyll parameters are described in Table S1.

The SpecimCamera with a range of 400–1000 nm of IQ hyperspectral image (Specim, Spectral Imaging Ltd., Oulu, Finland) was used to collect hyperspectral data. The hyperspectral image size of plant leaves was in the range of [1900 × 1024]–[2200 × 1024] pixels in 448 bands. Each image reflection was calculated with Formula (1).

$$\text{Reflectance} = \frac{I - D}{W - D} \tag{1}$$

The hyperspectral data were processed in ENVI 5.3, and the related vegetation index was calculated. The linear interpolation model was used to measure the red edge position of the plant [31]:

$$\text{REP} = 700 + 40 \times \frac{\rho_{red} - R700}{R740 - R700} \tag{2}$$

where $\rho_{red} = \frac{1}{2} \times (R670 + R780)$, and R represents the original reflectivity. The normalized difference vegetation index (NDVI) and photochemical reflectance index (PRI) are widely used in vegetation detection [32]. The modified chlorophyll absorption in reflectance index (MCARI) sum of vegetation was calculated to help analyze the change in leaf chlorophyll content [33,34]. The calculation methods of NDVI, PRI, and MCARI are shown in Equations (3)–(5):

$$\text{NDVI} = \frac{(R800 - R680)}{(R800 + R680)} \tag{3}$$

$$\text{PRI} = \frac{(R531 - R570)}{(R531 + R570)} \tag{4}$$

$$\text{MCARI} = [(R700 - R670) - 0.2 \times (R700 - R550)] \times \left(\frac{R700}{R670}\right) \tag{5}$$

2.5. Software Validation and Statistical Analysis of the Data

The data of this study were indicated as a mean value with standard deviation in RStudio software version 2022.07.0-548 (2009–2022 RStudio, PBC). The Kolmogorov–Smirnov test (K-S test) was used to check the normality of all data, and the Bartlett test was used to check the homogeneity of variance of the data. Nonparametric tests were performed on data that did not meet the homogeneity and normality of variance. And we used Tamhane's T2 for post hoc comparison. The data were analyzed with the analysis of one-way ANOVA performed on all calculated means using the "Agricolae" package. Similarly, multiple comparisons were performed using the Duncan statistical method. Statistical significance was accepted for p values below 0.05. The Pearson correlation test assessed the relationship between plant morphology, plant biomass, and physiological changes and photosynthesis. In order to explore the contribution of factors affecting the biomass of cucumber seedlings, a structural equation model (SEM) was constructed using the software IBM. SPS. AMOS (version 24). Before modeling, each variable was standardized by a Z-score transformation. The goodness of fit of the model was determined with the χ^2 test (χ^2/df ratio was near 0–2, $p > 0.05$) and a comparison fitting index (CFI), where CFI $\geq$ 0.9, $p > 0.05$ was considered a good fit.

3. Results

3.1. Effects of Microplastics on Plant Growth

Different types and levels of MPs had different effects on plant growth (Figure 2A). Compared with the control, PS-MPs and PLA-MPs did not significantly affect the plant height (Figure 2B) but significantly reduced the aboveground biomass by 33.49% at 2% of PLA-MPs (Figure 2C). MPs treatments significantly reduced the leaf area with 33.22% and 42.09%, respectively, at 0.2 and 2% treatment (Figure 2D). Similarly, no significant effect was observed in root length among the treatments (Figure 2E). The dry biomass of root in cucumber decreased significantly in all treated groups except the 2% treatment group (Figure 2F). MPs significantly reduced the width of the plant basal stem at 0.2% PLA-MPs and 2% PS-MPs treatments (Figure 2G).

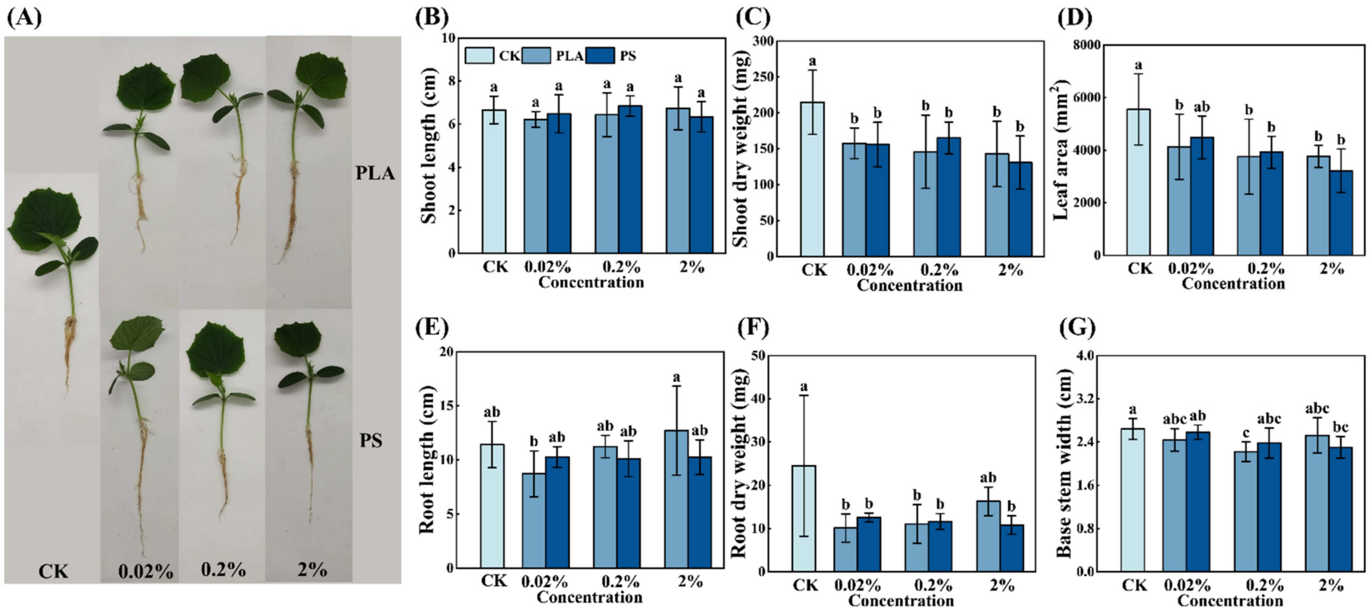

Figure 2. Phenotypic growth of cucumber seedlings (**A**), the shoot length (**B**), shoot dry weight (**C**), leaf area (**D**), root length (**E**), root dry weight (**F**), and base stem width (**G**) of cucumber seedlings exposed to microplastics (MPs). The data were all mean (n = 5) ± standard deviation. Different letters indicate a significant increase or decrease between the mean values of different treatments ($p < 0.05$). PLA, polylactic acid microplastics; PS, polystyrene microplastics.

3.2. Effects of Microplastics on Physiological Properties of Cucumber Seedlings

With the increase in PLA-MPs concentration, the chlorophyll content showed a decreasing trend (Figure 3A). Under the 2% PLA treatment, the chlorophyll content was significantly decreased by 19.1% compared to the control group but significantly increased by 13.6% in MPs treatment compared to PLA (Figure 3A). Under different levels of PLA-MPs treatment, leaf nitrogen content decreased gradually with the increase in concentration of PLA-MPs (Figure 3B). No significant difference occurred in anthocyanin content at all levels of PS-MPs but significantly increased under 2% PLA-MPs treatment (Figure 3D).

Anthocyanin content increased significantly by 20.6% and 7.8%, respectively, under the PLA condition.

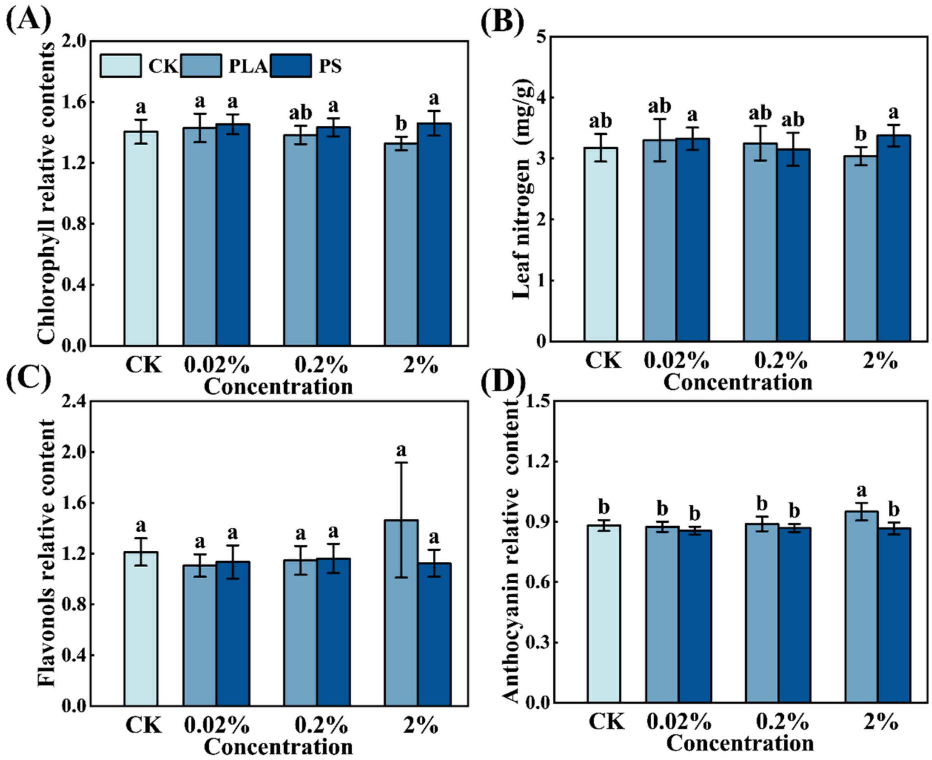

Figure 3. The relative contents of chlorophyll (**A**), leaf nitrogen (**B**), flavonol (**C**), and anthocyanin (**D**) in cucumber seedling leaves exposed to microplastics (MPs). The data were all mean (n = 8) ± standard deviation. Different lowercase letters represent significant ($p < 0.05$) difference of polylactic acid (PLA) and polystyrene (PS) microplastics.

3.3. Antioxidant Defense System of Cucumber under MPs

The ROS level of cucumber seedlings was significantly affected by MPs treatment (Figure 4A). The ROS activity was higher by 40.9% in PLA-MPs 2% treatment and significantly higher at PS-MPs 2% treatment (Figure 4A). No significant difference was observed in SOD and POD activity of cucumber under MPs treatments (Figure 4B,C). With the increase in MPs concentration, the CAT activity of seedlings showed a trend of increasing first and then decreasing, such as under 2% PLA-MPs, which showed decreased CAT activity by 79% compared to the control (Figure 4D).

3.4. Effects of Microplastics on Chlorophyll Fluorescence of Cucumber

Fv/Fm represents the net fluorescence activity under different treatments (Table S1). With the increase in PLA-MPs, the Fv/Fm gradually decreased (Figure 5A). Under the 2% PLA-MPs treatment, the rate of Fv/Fm was reduced by 7.9% compared with the control (Figure 5A). Similarly, under 2% PS-MPs treatment, the Fv/Fm decreased by 8.0% compared to the control. ABS/RC indicates the light energy absorbed per unit reaction center (Table S1). With the increase in PLA-MPs concentration, ABS/RC first increased and then decreased, while with the increase in PS-MPs concentration, ABS/RC first increased and then decreased and then increased, but there were differences between ABS/RC treated with different levels of MPs and the control (Figure 5B). TRo/RC is the energy captured per unit of the reaction center to reduce the first quinone electron acceptor of Photosystem II (QA). Under the PLA-MPs treatments, the TRo/RC was increased by 21.8%, 22.1%, and 12.6%, respectively, compared with the control (Figure 5C). While on the other hand, the TRo/RC increased by 25.1% only under high levels of PSMPs treatment. ETo/RC represents the electron transfer energy captured per unit of the reaction center. The ETo/RC increased under the first two levels but decreased under 2% PLA-MPs but increased under the PS-MPS treatment (Figure 5D). DIo/RC is the energy dissipated per unit of the reaction center. With the increase in MPs concentration, DIo/RC increased compared to the control (Figure 5E).

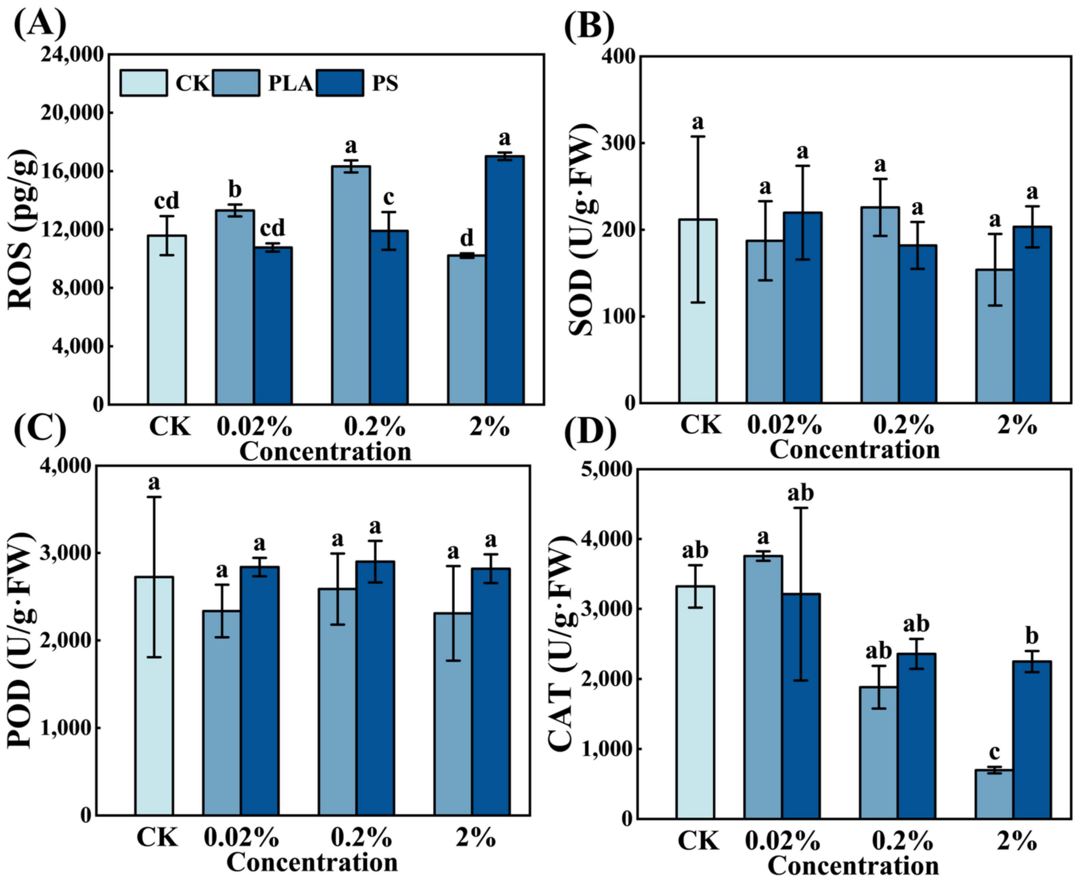

Figure 4. The activities of ROS and antioxidant enzyme system, i.e., SOD, POD, and CAT, in cucumber seedlings under microplastic stress. (**A**) reactive oxygen species (ROS); (**B**) superoxide dismutase (SOD); (**C**) peroxidase (POD); (**D**) catalase (CAT). Different lowercase letters represent a significant ($p < 0.05$) difference of polylactic acid (PLA) and polystyrene (PS) microplastics.

3.5. Hyperspectral Response of Cucumber to Microplastics

The vegetative index calculated by the region of targeted interest (ROI) of the hyperspectral image of cucumber seedlings is displayed (Figure 6A); the spectral reflectance of plants changed in the green (±530 nm), red (±700 nm), and near-infrared (>700 nm) regions of the spectrum. Light-use efficiency and narrowband and broadband greenness are estimated in these regions, respectively. The green peak reflectance of cucumber seedlings varied with the type of MPs stress (Figure 6A). The green peak reflectance of cucumber seedlings increased with the increase in PLA-MPs stress levels (Figure S1A), although it decreased at the lowest concentration. The linear interpolation model was used to calculate the red edge position of the plant. It was found that with the increase in PLA-MPs concentration, the red edge gradually moved in the short-wave direction (Figure S1B), although the red edge shift is very small (±2 nm), but the Duncan test showed that there were significant differences between CK and 2% PLA-MPs plants. For NDVI and PRI, there was no significant change in the NDVI vegetation index of cucumber seedlings between the two MPs treatments (Figure 6B,C). The PRI value of the plants were significantly decreased under middle and high concentrations of MPs stress (Figure 6C).

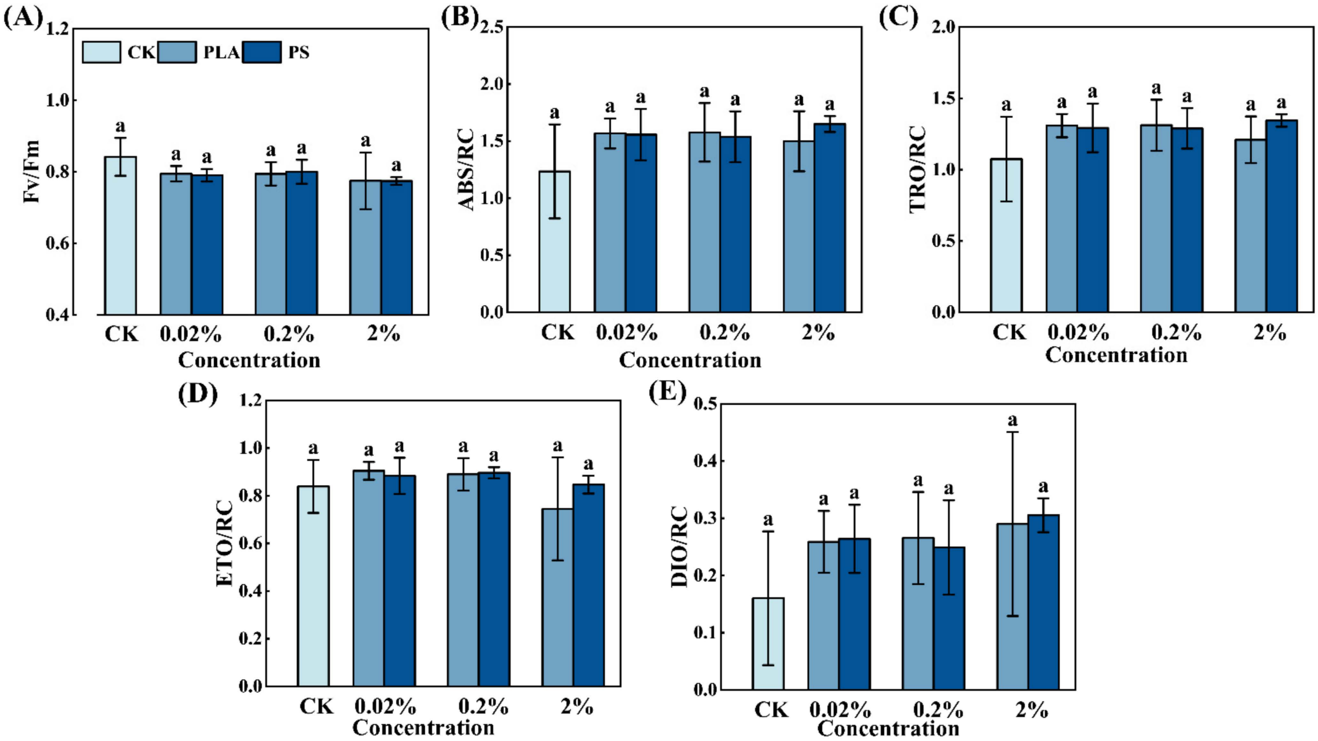

Figure 5. Changes in chlorophyll fluorescence parameters of cucumber seedlings exposed to microplastics. The data were all mean (n = 8) ± standard deviation. (**A**) Fv/Fm, maximum photochemical efficiency; (**B**) ABS/RC, light energy absorbed by the unit reaction center; (**C**) TRO/RC, energy captured by the unit reaction center for reducing Q_A; (**D**) ETO/RC, energy captured by a unit reaction center for electron transfer; (**E**) DIO/RC, energy dissipated in the unit reaction center. Different lowercase letters represent a significant ($p < 0.05$) difference of polylactic acid (PLA) and polystyrene (PS) microplastics.

3.6. The Relationship between Plants and Physiological and Biochemical Parameters

The Mantel test and Pearson correlation analysis were used to evaluate the relationship between vegetation morphology and physiological traits (Figure 7A). The results showed that Fv/Fm was negatively correlated with ROS, CAT, and other plant antioxidant indices. Pearson's r = 0.42, $p > 0.05$, and Pearson's r = 0.38, $p > 0.05$. It was positively correlated with plant biomass (Mantel's r = 0.81, $p < 0.01$). Plant biomass had significant effects on ABS/RC and DIO/RC (Mantel's r = 0.84, $p < 0.05$, and Mantel's r = 0.87, $p < 0.01$). The SEM results are basically consistent with the above correlation, further determining the direct and indirect effects between the variables (Figure 7B). Our study revealed that the Bio-MPs may directly or indirectly affect changes in plant biomass through changes in photosynthesis. In addition, we found no significant correlation between NDVI vegetation index and physiological indices ($p < 0.05$), but MCARI was negatively correlated with chlorophyll content (Pearson's r = −0.92, $p < 0.01$) and positively correlated with anthocyanins (Pearson's r = 0.95, $p < 0.01$). A positive correlation was observed between PRI and CAT activity (Pearson's r = 0.96, $p < 0.05$) and a positive correlation was observed between PRI and Fv/Fm (Pearson's r = 0.41, $p > 0.05$). We found a significant correlation between Fv/Fm and aboveground biomass and underground biomass ($p < 0.05$).

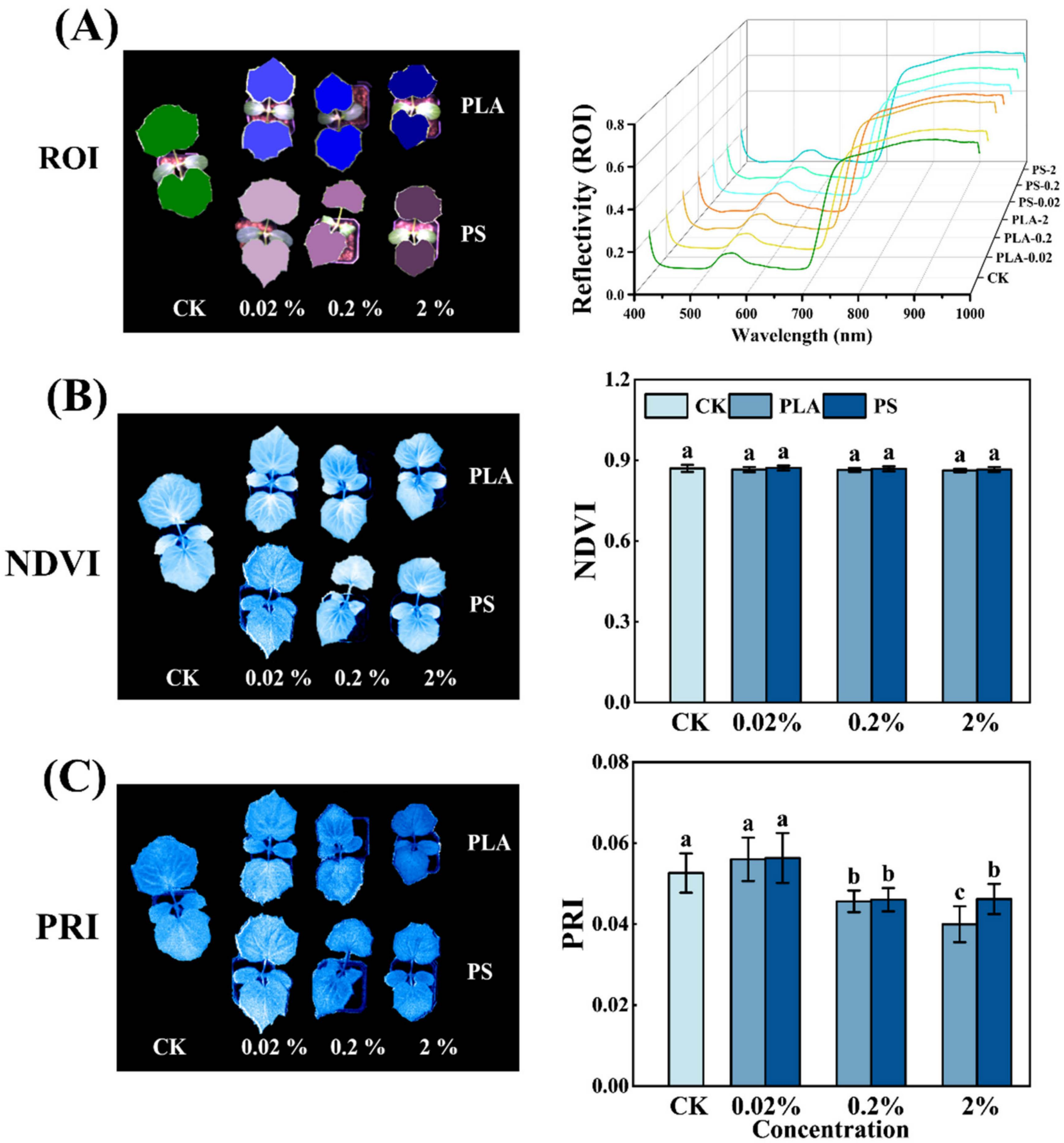

Figure 6. Spectral response of cucumber seedlings to microplastics exposure. (**A**) the spectral curve of region of interest (ROI); (**B**) the normalized difference vegetation index (NDVI); (**C**) the photochemical reflectance index (PRI); (**B**,**C**) are expressed as mean (n = 8) ± standard deviation, and the different letters indicate significant differences among the different treatments at the 0.05 level. PLA, polylactic acid microplastics; PS, polystyrene microplastics.

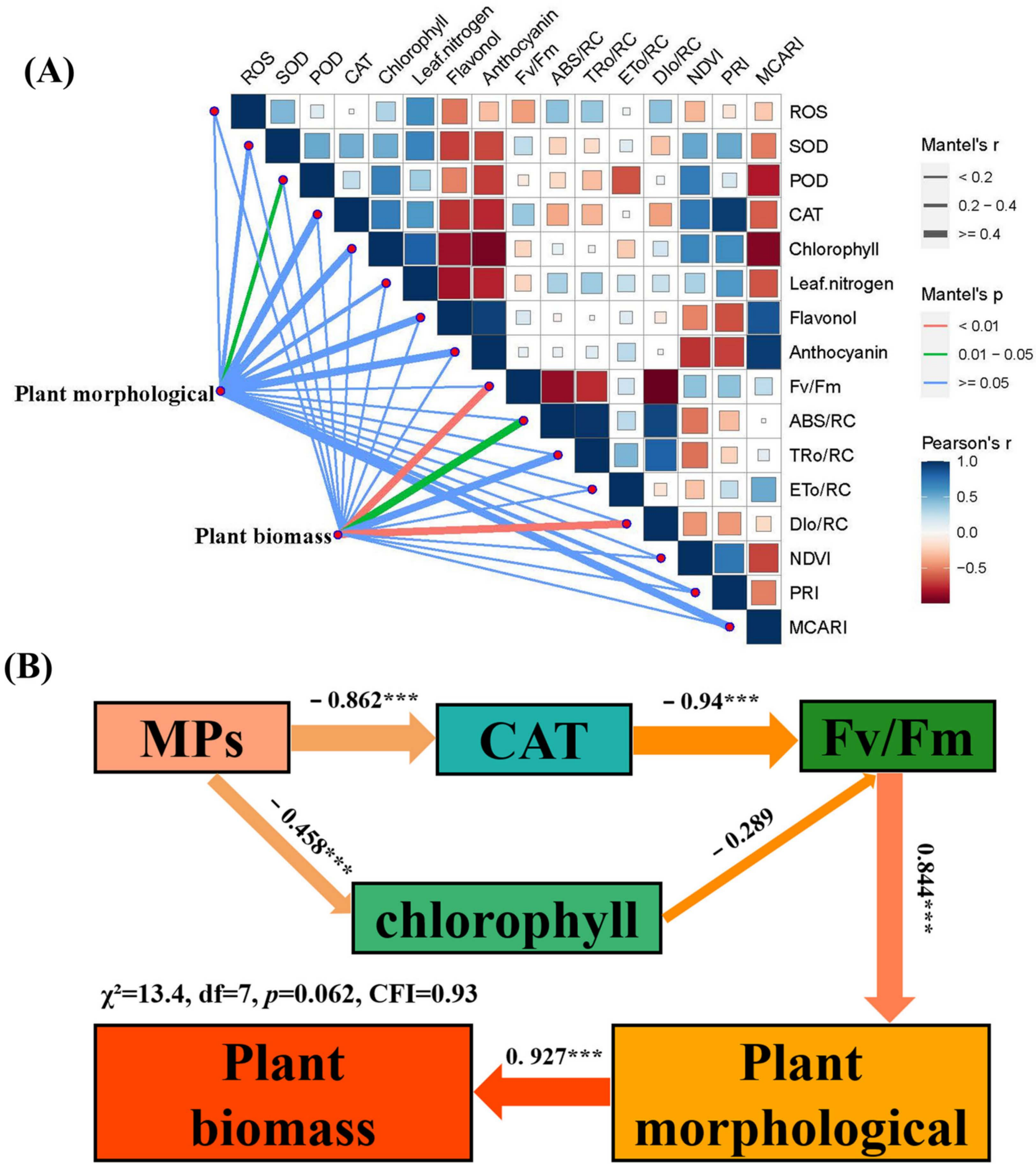

Figure 7. The relationship between plants and physiological and biochemical parameters. (**A**) Correlation and Mantel test between cucumber seedlings and vegetation monitoring parameters under microplastic treatment; the pair comparison of environmental factors is shown in the upper-right corner, and the color gradient represents the Pearson correlation coefficient. On the right, there is a significant correlation between the single variables, and the color gradient represents the Pearson correlation coefficient. The figure on the left shows the Mantel test between multivariate variables and each single variable. The edge width corresponds to the value of Mantel's r, and the edge color indicates statistical significance. (**B**) The structural equation model (SEM) shows the relationship between the expectation-based experimental treatment and the response variables under the prior model. The number next to the arrow is the path coefficient (*** $p < 0.001$), and the width of the arrow is proportional to the strength of the path coefficient. The goodness of fit of the model is displayed next to the model CFI, comparative fit index. PLA, polylactic acid microplastics; PS, polystyrene microplastics.

4. Discussion

4.1. Biodegradable Microplastics Effect on Morphology and Biomass of Cucumber Seedlings

Various type of MPs have different effects on plant growth, which was observed previously [35–37]. In our study, MPs treatment did not significantly affect the plant height and root length of cucumber seedlings but significantly reduced the aboveground and belowground biomass of cucumber seedlings (Figure 2). Leaves are the main energy converter for the producers in ecosystems well as important constituent for plants' photosynthesis. The properties of leaves directly affect the basic behavior and important functions of plants. In addition to the 0.02% PS-MPs treatment group, the leaf area of crops was significantly reduced due to MPs pollution, and the effects of biodegradable MPs like PLA-MPs at low and medium concentrations (0.02% and 0.2%) were more serious than those at high concentrations of PS-MPs. The decrease in leaf area leads to an effective area of light energy and the decrease in productivity, which may be the direct cause of cucumber biomass. Chlorophyll is considered to be the most principal pigment in photosynthesis, and it accomplishes crucial processes of harvesting solar energy in the antenna systems and actuates electrons to transfer light energy to the photosynthetic reaction center [38,39]. Under 2% PLA-MPs treatment, the chlorophyll content of plants decreased significantly while anthocyanins increased significantly, but PS-MPs had no significant effect on plants at the same concentration (Figure 3A,D). The decrease in the chlorophyll content in plants affects photosynthesis and inhibits plant growth [40,41]. In addition, anthocyanins, as a kind of flavonoid, play an important role in plant growth and development and stress response. They have also been shown in many plants to reduce the frequency and severity of light suppression to accelerate light repair [42], and anthocyanin accumulation in plants is used to resist the adverse effects brought by MPs stress. Although MPs treatment did not cause changes in plant root length, each treatment caused a decrease in root biomass (Figure 2E,F), which may be due to the addition of MPs to change the physicochemical characteristic in the soil. The introduction of PLA-MPs into the soil environment reduces the contents of soil organic matter (SOM) and soil available nitrogen [18,26]. At the same time, the addition of PLA can change the mycorrhizal symbiosis, such as changing the community structure and diversity of arbuscular mycorrhizal fungi (AMF) [43], which may be another reason for this change. Similar to our results, Meng et al. observed that at 2%, Bio-MPs significantly decreased the aboveground biomass and root biomass of the common bean, while the same concentration of LDPE-MPs had no significant effect on aboveground biomass and belowground biomass [44]. In general, Bio-MPs exhibit ecotoxicity different from conventional MPs due to their biodegradability.

4.2. Effects of Biodegradable Microplastics on Active Oxygen Species and Antioxidant System of Cucumber Seedlings

ROS is a key signaling molecule for plant cells to respond to various environmental stresses. The positive response of ROS to bio-/abiotic stress occurs via the activation of a special type of antioxidant enzymes. In order to respond to the adverse effects of excessive ROS, plants have developed a series of antioxidant defensive mechanisms to eliminate ROS [45]. Previous studies confirmed that microplastics in soil can lead to the rapid accumulation of ROS in plants [23]. In this experiment, with the increase in PLA-MPs concentration, the ROS level gradually increased, suggesting that the antioxidant mechanism may play a key role in alleviation. Also, the flavonoid content can affect the growth and development of plants and help them to resist biotic and abiotic stresses [46]. The amount of flavonoid content increased under 2% PLA-MPs concentration, suggesting a decrease in the negativity of ROS under MPs treatments. In this study, antioxidant activity was activated through SOD, POD, and CAT induced by MPs in cucumber seedlings (Figure 4B–D). Similar to this study, Lian et al. reported that the CAT activity of soybean seedlings increased after PLAMPs treatment, but there was no significant effect on SOD activity [47]. Exposure to PE-MPs stimulated the antioxidant system reaction of buckwheat and caused an increase in POD and SOD [48]. Changes in the antioxidant defense system induced by MPs vary largely according to plant species, MPs size and type, and exposure time [49,50].

4.3. Biodegradable Microplastics Effect on Photosynthesis

The maximum photochemical efficiency of PSII is the most significant process that positively responds to abiotic stress. However, in this study, chlorophyll fluorescence parameters did not show significant differences. With the increase in MPs concentration, Fv/Fm gradually decreased (Figure 5A), indicating that MPs caused the inactivation of some PSII reaction centers in plant leaves due to photoinhibition, and the efficiency of PSII photochemistry in plants was inhibited. The kinetic parameters induced by chlorophyll fluorescence can reflect the absorption, utilization, and distribution of light energy by PSII. With the comparison of the "apparent" gas exchange index, the chlorophyll fluorescence parameters also act as an "intrinsic" factor [51]. By analyzing the kinetic parameters of the chlorophyll fluorescence induction, we further understand the effect of MPs on the structure and function of PSII in cucumber [52]. The light energy absorbed by the PSII reaction center unit (ABS/RC), which determines the energy utilized for the reduction of Q_A (TRo/RC) and the dissipated energy (DIo/RC), of cucumber leaves treated with PLA-MPs increased, while the energy captured by the unit PSII reaction center for electron transfer (ETo/RC) decreased (Figure 5D). ABS/RC is generally used to measure the size of antenna pigments. This study suggested that MPs promoted the size of antenna pigments due to light energy absorbed and captured by antenna pigments. However, the amount of energy used for electron transfer was reduced, resulting in more light energy being lost as heat energy rather than being transferred to capacity. This result is consistent with [53], who found that MPs significantly reduced the light conversion efficiency (Fv/Fm) of algae and increased the high dissipation of the reaction center. MPs pollution deactivates the PSII reaction center, and more light energy is dissipated as heat rather than through photochemical use to promote the electron transfer rate, which affects the normal growth of plants. Previous studies showed that different plant PSIIs in different environments have different vulnerabilities due to complex interactions between pollutants and cellular systems [54,55].This may be the reason why the fluorescence parameters in this paper are not a good indicator of the toxicity of microplastics.

4.4. Rapid Hyperspectral Response to Plant Physiology and Photosynthesis under Microplastic Treatment

Recently, a novel biotechnology technique offered a reliable and less time-consuming way to detect plant toxicity under stressful conditions; this technique is known as the hyperspectral imaging (HSI) technique [56]. Since chlorophyll fluorescence did not show sensitivity to microplastic stress, we used hyperspectral imaging as a probe to evaluate the effects of microplastics on cucumber seedlings. The spectral absorption of pigments, water, and other dry matter in the vegetation formed by blue edge, yellow edge, red edge, and other characteristic spectral change areas were visible in the visible–near-infrared band [34,57]. Therefore, the spectral positions/wavelengths can be used to invert plant physiological and biochemical parameters. Among them, the red edge position indicated the changes in chlorophyll content, biomass, and leaf internal structure parameters. When plants are "chlorotic" due to infection of pests or pollution, then the "red edge" moves towards the blue light direction (called "blue shifts of red edge"). In this experiment, with the increase in PLA-MPs concentration, the chlorophyll content of cucumber seedlings decreased, and the red edge gradually shifted to the blue edge (Figure S1B), indicating that the internal structure of leaves may change. The normalized vegetative index (NDVI) measures the chlorophyll absorption at red wavelengths and leaf scattering at near-infrared (NIR) wavelengths and is often used to estimate of vegetation productivity [58–60]. The current study result did not find any significant effect in the NDVI of cucumber under MPs treatments (Figure 6B). Therefore, this study provided another vegetation index known as MCARI, which helped to analyze the leaf chlorophyll content. MCARI showed a significant negative correlation with chlorophyll content and a positive correlation with anthocyanins (Figure 7A). It is feasible to use a vegetation index to rapidly evaluate plant physiological changes under MPs stress. The lutein cycle releases excess light energy absorbed by leaves in the form of heat dissipation [61]. The photochemical vegetation index (PRI) is a widely used index of the lutein cycle, which can be used to detect nonoptical fluorescence quenching (NPQ) and is often used to identify photosynthetic light energy utilization efficiency or carbon absorption efficiency. Except for the lowest concentration, the PRI values of cucumber under MPs treatment were significantly reduced, indicating that the light energy utilization rate of the plants decreased and the heat dissipation increased (Figure 6C). It seems to be more sensitive than chlorophyll fluorescence induction kinetics under microplastics. The PRI of PLA-MPs was significantly lower than that of PS-MPs at high concentrations. We speculate that PLA-MPs may have higher ecological risks or are at least not as environmentally friendly as previously reported.

5. Conclusions

In this study, we indicated the intricate relationship between the degradability of MPs and their environmental impact, focusing on the case of cucumber seedlings with impaired photosynthesis. The biodegradable MPs have potential ecological toxicity that could affect the growth of cucumber seedlings through a disturbance in the photosynthesis system. Hyperspectral imaging technology may play a greater role in the rapid detection and identification of plant response to MPs. This study clearly indicated the toxicity of PLA-MPs on plant growth and in entire ecosystems. Hence, it is necessary to regulate the quantity and frequency of usage when employing degradable plastics in lieu of traditional plastics for agricultural purposes. Also, the interactive mechanism between Bio-MPs and microbial–soil–plant systems is still unclear, and further investigations are urgently required to explore the interaction between MPs and soil–plant systems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14010053/s1, Table S1: Definition of chlorophyll fluorescence parameters in this study. Figure S1. Spectral response of cucumber seedlings to MPs exposure.

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