Supplementary Information

Foliar uptake and in-leaf translocation of micro(nano)plastics and their interaction with epicuticular wax

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Text S1. Selection of the optimal spray dosage of PS MNPs

To select the optimal spray dosage for reducing the application dosage error and ensure the same PS MNPs exposure between the two plant species, different volumes of applied spray were tired in the pre-experiment, including 1, 2, 4, 7, 10 and 15 mL per time. When the plants were treated with sprays in volume less than 7 mL per time, the amount of PS MNPs deposited on the leaf surfaces were much low (< 40% of the spray), and it was difficult to observe and quantify the occurrence of PS MNPs in various leaf tissues within 5 days of exposure. This would inevitably reduce the accuracy of statistical results. In contrast, when the volume of sprays exceeded 7 mL per time, high deposition of PS MNPs (58%−63% of the spray) occurred on the leaf surfaces. When the volume of sprays reached 10 mL per time, there was no significant difference in the amount of PS MNPs deposited on the leaves of the two plants (p > 0.05), and the deposition amount of PS MNPs on the two plant leaves was 5.9 ± 1.4 μg per time. Above all, 10 mL per time was chosen as the optimal volume of treatment.

Text S2. Quantification of PS MNPs on crop leaf surfaces

To compare the fluorescence response of PS MNPs on the leaf surfaces, the fluorescence emission spectra of 80 nm PS and 500 nm PS on the leaf surfaces were obtained at different concentrations, including 50, 100, 300, 500, 700, 900 and 1000 ng $spot^{-1}$. When the concentrations of 80 nm PS and 500 nm PS were the same, there was no significant difference in the intensity or shape of the fluorescence emission spectra of the two PS MNPs on the leaf surfaces ($p > 0.05$). Then, the relationship between the fluorescence intensity of PS MNPs on the leaf surfaces and their concentration was obtained. Specifically, nine spots (r = 0.3 cm) that distributed evenly over the front, middle and nether parts per leaf were made using the large circular end of a 5 mL pipette, which served as the measurement unit. Next, different dosage of PS MNPs dispersions were introduced onto the spots slowly and evenly using a 10 μL flat-head microinjector (Zhejiang Lichen Instrument Technology Co., Ltd., China) to produce a series of concentration gradients (0, 10, 50, 100, 200, 400 and 500 ng $spot^{-1}$) on the crop leaf surfaces. After the DI water evaporated completely, the fluorescence spectra of PS MNPs were scanned by a Cary Eclipse fluorescence spectrophotometer equipped with a 2-m fiber-optic (Agilent, Palo Alto, USA) *in situ*. The results demonstrated that the fluorescence intensity of PS MNPs retained on the leaf surfaces strongly depended on the concentration and showed a good linear relationship. Therefore, it is feasible to use fluorescence intensity as an indicator to reflect the amount of PS MNPs retained on the leaf surfaces in this study.

Text S3. Quantification of epicuticular wax

Fresh leaves of maize and soybean were immersed into dichloromethane (150 mL) in beakers. After sealing beakers with using aluminum foil, the epicuticular wax was extracted with dichloromethane (150 mL) in a Branson ultrasonic water bath for 5min. The extraction solution was filtered through a 0.45 μm Millipore membrane, and then the leaves were immersed in dichloromethane (50 mL) for a second extraction for 2 min. Two parts of extracts were collected, mixed, and diluted to 250 mL. All extracts were condensed to approximately 2 mL in a rotary evaporator, subsequently dried under a gentle flow of high-purity nitrogen gas, and finally weighed for the epicuticular wax content.

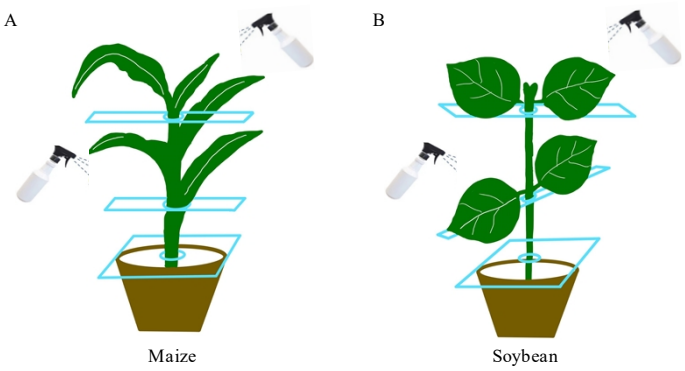

Fig. S1 The schematic diagram for the spraying.

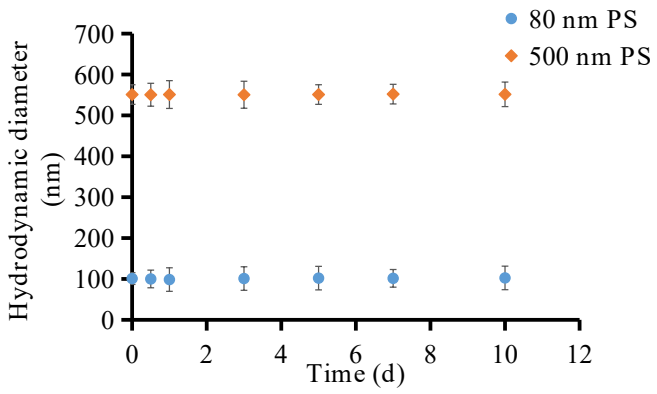

Fig. S2 Hydrodynamic diameter of PS MNPs in DI water at different time.

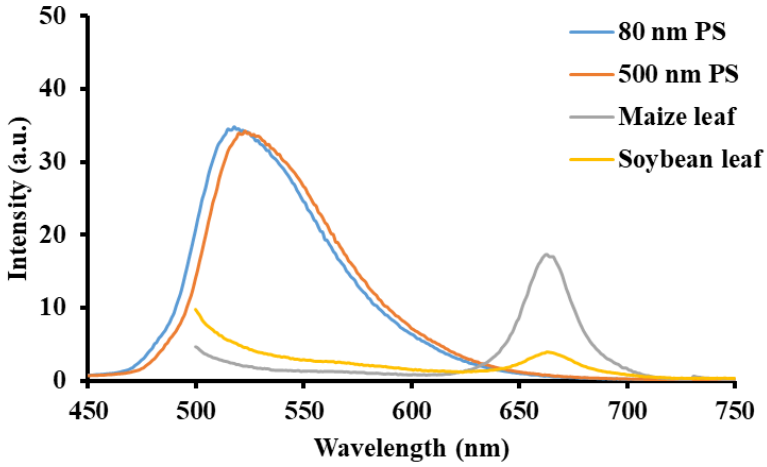

Fig. S3 The fluorescence emission spectra of PS MNPs within the ranges of low background fluorescence emission for maize and soybean leaf at λ_{ex} = 488 nm.

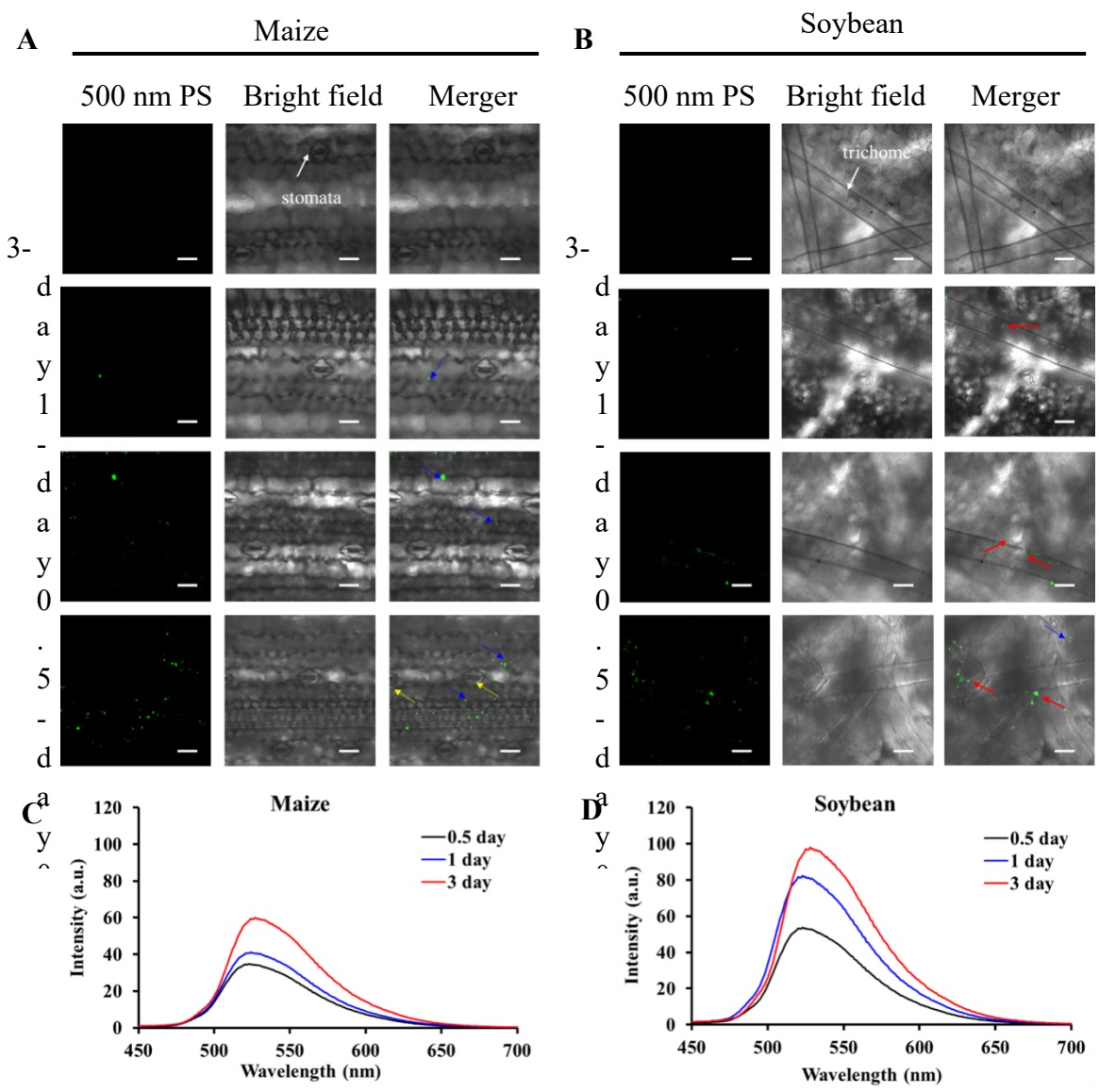

Fig. S4 CLSM images of 500 nm PS distribution on maize (A) and soybean (B) leaf surfaces at different exposure time. Green pixels are 500 nm PS. Scale bar, 40 μm. The blue, red and yellow arrows refer to 500 nm PS distributed in the epicuticular wax, trichomes and stomata, respectively. The changes in fluorescence spectra of 500 nm PS on maize (C) and soybean (D) leaf surfaces at different exposure time. Each fluorescence spectrum is the mean of nine replicates.

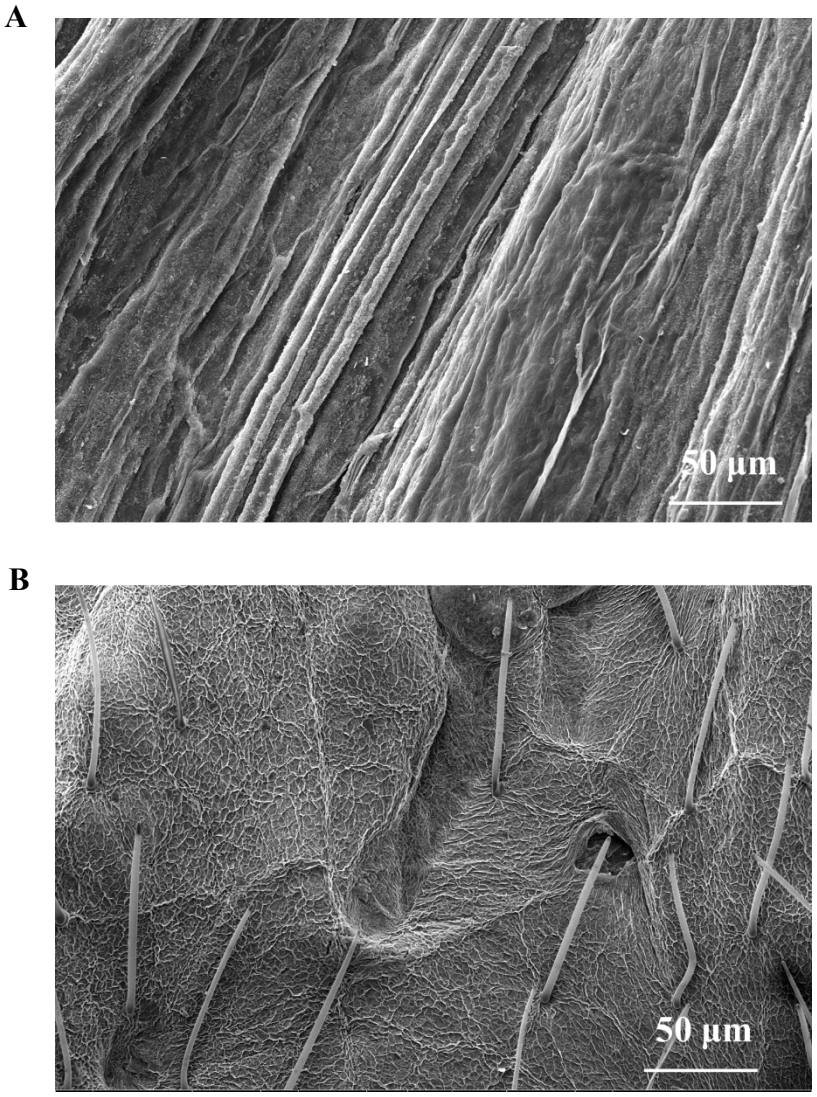

Fig. S5 SEM images of the micromorphology of maize (A) and soybean (B) leaves. Scale bar, 50 μm.

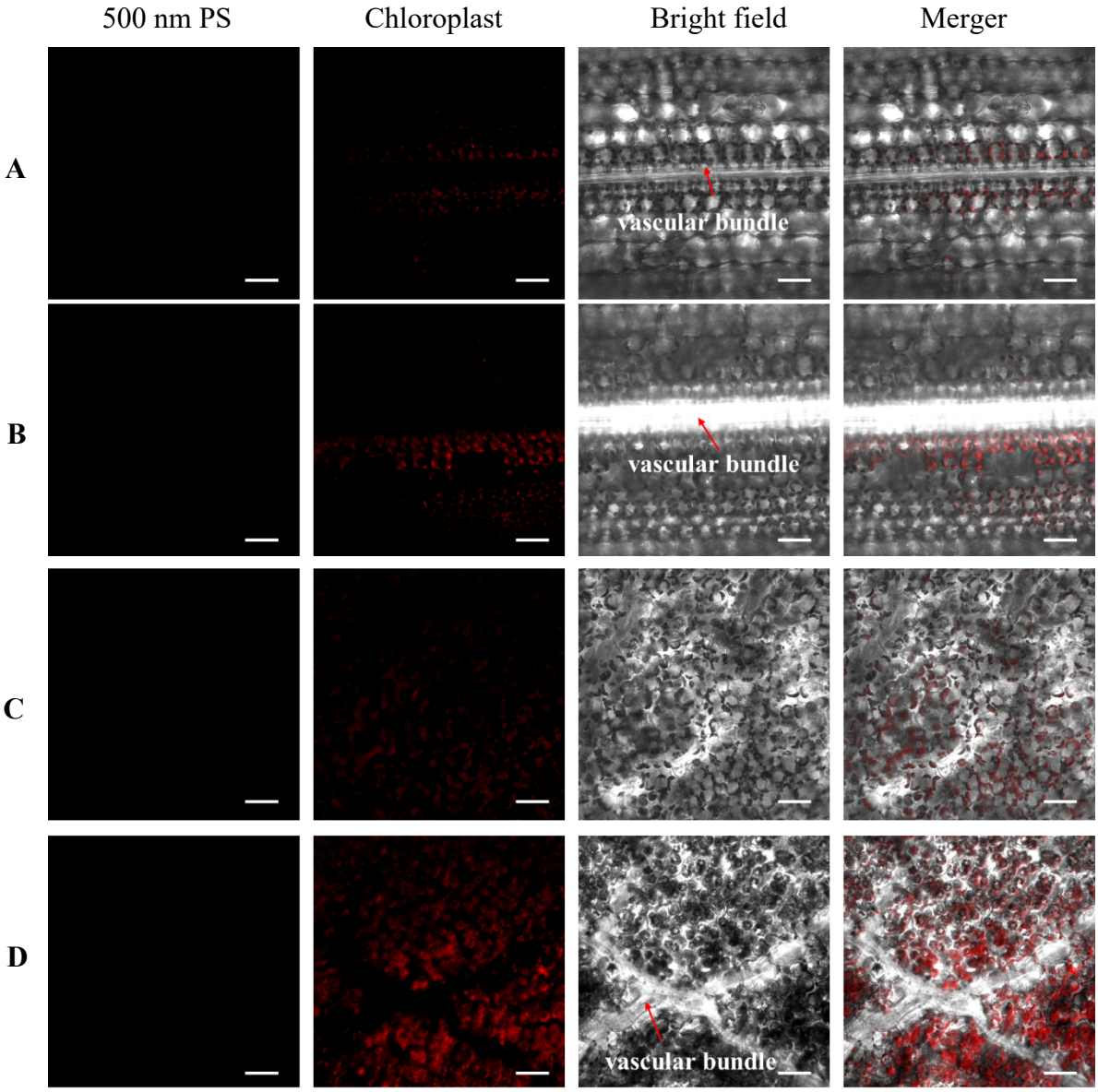

Fig. S6 CLSM images of 500 nm PS in maize (A, B) and soybean (C, D) leaves after 7 (A, C) and 10 (B, D) days of treatment. Red pixels are chloroplasts. Scale bar, 40 μm.

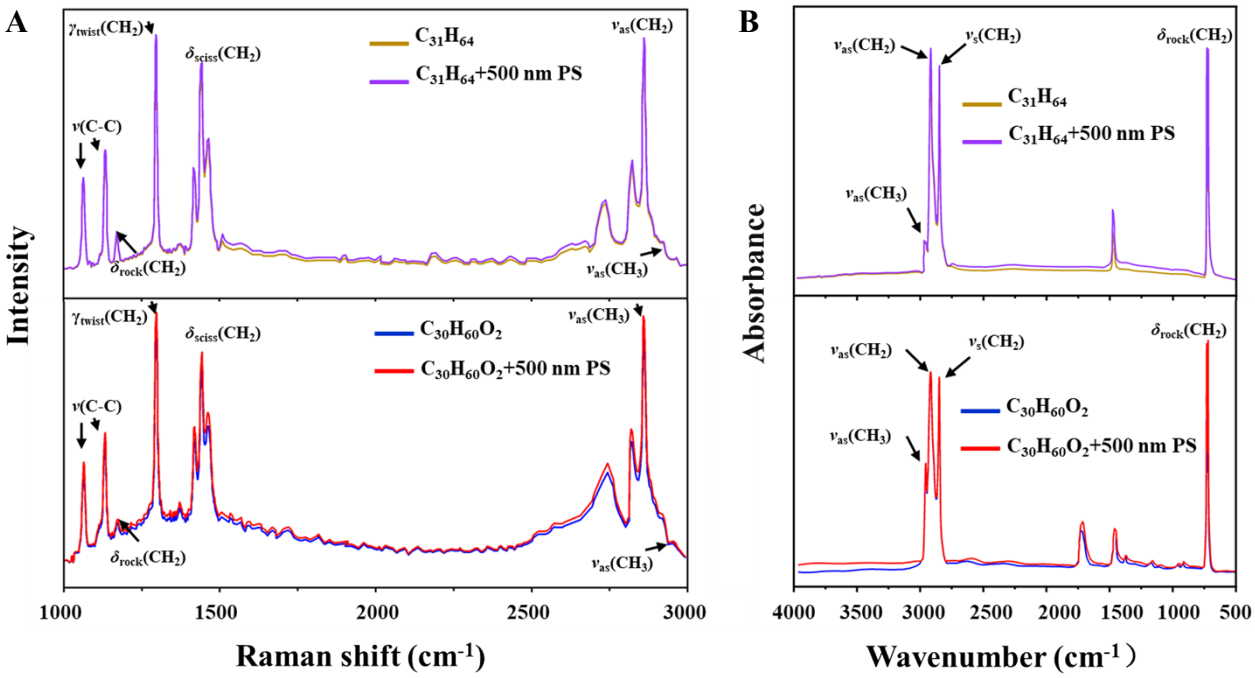

Fig. S7 Raman (A) and FTIR (B) spectra of $C_{31}H_{64}$/$C_{30}H_{60}O_2$ and its mixtures with 500 nm PS. Their intensities are normalized between wax samples and mixtures to quantitatively compare the shifts and intensity in Raman and FTIR spectra, respectively. *ν*, stretching; *δ*, in-plane bending; *γ*, out-of-plane bending; *as*, asymmetric; *s*, symmetric.

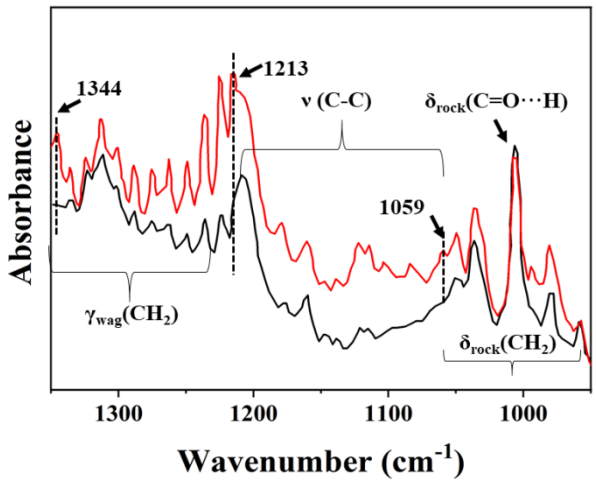

Fig. S8 FTIR spectra of $C_{30}H_{60}O_2$ and its mixtures with 80 nm PS in the conformationally sensitive region. Their intensities are normalized between wax samples and mixtures to quantitatively compare the shifts and intensity. *ν*, stretching; *δ*, in-plane bending; *γ*, out-of-plane bending.

Table S1 The epicuticular wax content of maize and soybean leaves measured at different time points after spraying with PS MNPs.

Time (d)	80 nm PS		500 nm PS	
	Maize (mg g^{-1})	Soybean (mg g^{-1})	Maize (mg g^{-1})	Soybean (mg g^{-1})
0	3.2 ± 0.4^{a}	2.6 ± 0.3^{a}	3.2 ± 0.4^{a}	2.6 ± 0.3^{a}
0.5	3.3 ± 0.5^{a}	2.6 ± 0.6^{a}	3.2 ± 0.4^{a}	2.6 ± 0.3^{a}
1	3.0 ± 0.6^{b}	2.4 ± 0.3^{b}	3.2 ± 0.4^{a}	2.6 ± 0.3^{a}
3	2.8 ± 0.3^{b}	2.3 ± 0.2^{b}	3.3 ± 0.3^{a}	2.5 ± 0.3^{a}
5	2.5 ± 0.2^{b}	2.0 ± 0.4^{b}	3.1 ± 0.4^{a}	2.6 ± 0.5^{a}
7	2.3 ± 0.1^{b}	1.9 ± 0.4^{b}	3.1 ± 0.2^{a}	2.7 ± 0.2^{a}
10	2.0 ± 0.4^{b}	1.7 ± 0.3^{b}	3.2 ± 0.4^{a}	2.6 ± 0.3^{a}

Values were represented as mean ± SD. The different lower-case letters behind the numbers give results of statistical comparison within one column. The letter ‘a’ represents no significant differences between control and treatment groups. The letter ‘b’ indicates significant differences between control and treatment groups ($P < 0.05$).

Table S2 Band positions of PS MNPs, and their mixtures with $C_{31}H_{64}$ and $C_{30}H_{60}O_2$ and the area ratio of I_D/I_G in Raman spectra.

Sample	D band cm^{-1}	G band cm^{-1}	G' band cm^{-1}	I_D/I_G
80 nm PS	1340	1616	2655	1.2
80 nm PS-$C_{31}H_{64}$	1343	1619	2658	1.5
80 nm PS- $C_{30}H_{60}O_2$	1346	1617	2644	1.7
500 nm PS	1342	1613	2640	1.3
500 nm PS-$C_{31}H_{64}$	1342	1613	2640	1.3
500 nm PS-$C_{30}H_{60}O_2$	1342	1613	2640	1.3

Table S3 Crystallinity of the mixtures of $C_{31}H_{64}$ and $C_{30}H_{60}O_2$ with PS MNPs based on the $I_{731\ cm^{-1}}/I_{720\ cm^{-1}}$ ratio in FTIR spectra.

Sample	$I_{731\ cm^{-1}}/I_{720\ cm^{-1}}$	Crystallinity (%)
$C_{31}H_{64}$	1.005 ± 0.005[a]	100
$C_{31}H_{64}$-80 nm PS	0.859 ± 0.009[b]	85.5
$C_{31}H_{64}$-500 nm PS	0.997 ± 0.013[a]	99.2
$C_{30}H_{60}O_2$	1.011 ± 0.007[a]	100
$C_{30}H_{60}O_2$-80 nm PS	0.878 ± 0.004[b]	86.8
$C_{30}H_{60}O_2$-500 nm PS	1.002 ± 0.021[a]	99.1

Values were represented as mean ± SD. The different lower-case letters behind the numbers give results of statistical comparison within one column. The letter 'a' represents no significant differences between control and treatment groups. The letter 'b' indicates significant differences between control and treatment groups ($P < 0.05$).

Table S4 g_{min} (mmol m^{-2} s^{-1}) of maize and soybean leaves measured at different time points after spraying with PS MNPs.

Time (d)	80 nm PS		500 nm PS	
	Maize	Soybean	Maize	Soybean
0	0.48 ± 0.02^{a}	0.44 ± 0.02^{a}	0.48 ± 0.02^{a}	0.44 ± 0.02^{a}
0.5	0.47 ± 0.03^{a}	0.45 ± 0.07^{a}	0.45 ± 0.06^{a}	0.44 ± 0.04^{a}
1	0.51 ± 0.04^{a}	0.45 ± 0.04^{a}	0.47 ± 0.03^{a}	0.42 ± 0.05^{a}
3	0.69 ± 0.06^{b}	0.51 ± 0.05^{b}	0.46 ± 0.05^{a}	0.46 ± 0.03^{a}
5	0.76 ± 0.07^{b}	0.63 ± 0.06^{b}	0.46 ± 0.04^{a}	0.47 ± 0.04^{a}
7	0.89 ± 0.04^{b}	0.69 ± 0.05^{b}	0.47 ± 0.03^{a}	0.44 ± 0.06^{a}
10	0.95 ± 0.02^{b}	0.74 ± 0.03^{b}	0.48 ± 0.06^{a}	0.46 ± 0.03^{a}

Values were represented as mean ± SD. The different lower-case letters behind the numbers give results of statistical comparison within one column. The letter ‘a’ represents no significant differences between controls and treatment groups. The letter ‘b’ indicates significant differences between controls and treatment groups ($P < 0.05$).