Supplementary information for the article:

Metabolism of Ibuprofen by *Phragmites australis*: Uptake and Phytodegradation

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List of Support information

Text S1. Chemicals and reagents

1) Chemicals used in enzyme extraction

Sucrose (Sigma), Ethylenediaminetetraacetic acid (EDTA, Sigma), ascorbic acid (Fluka), phenylmethanesulfonyl fluoride (PMSF, Fluka), Dithioerythritol (DTE, Roth), glycerine (Roth), sodium phosphate (Roth), $MgCl_2$ (Roth), polyvenylpyrrolidone PVP K90 (Fluka), polyvenylpyrrolidone PVP K30 (Fluka), Tris(hydroxymethyl) aminomethan (Merck), nonidet P40 substitude (Fluka).

2) Chemicals used in enzyme activity analysis

Cinanamic acid (Fluka), glucose 6-phosphate (Fluka), glucose-6-phosphat dehydrogenase (Simga), nicotinamide adenine dinucleotide phosphate (NADPH, Fluka), uridine-5'-diphosphoglucose (Fluka), nitrophenyl-ß-D-glucopranoside (4-PNG, Fluka), D-(−)-salicin (Fluka), quercetin dihydrat 98% (Fluka), chlor(1-) 2,4 dinitrobenzol (CDNB, Merck), glutathion reduced (GSH, Fluka), guajacol (Fluka), hydrogen peroxide solution (35%, Sigma), glutathion oxidized (GSSG, Fluka).

Text S2. Batch experiments of IBP sorption on perlite

Batch experiments were conducted to investigate the IBP sorption on perlite in triplicate. In batch, 100 µg/l IBP solution was added to perlite in flasks as the same liquid/perlite ratio (200 g perlite/500 ml solution) in the exposure experiment. Flasks were shaken at 100 rpm at room temperature for 8 days. To explore the desorption potential, on day 3 IBP solution was replaced with deionized water to mimic IBP removal by other mechanisms (e.g. biodegradation) in blank groups. Results are shown in Figure S2.

Text S3. Validation of IBP extraction from plant tissue

Roots and rhizomes (RR tissue, 5 g), stems and leaves (SL tissue, 5 g) were cut into pieces and cultivated in 100 times diluted Murashige and Skoog medium spiked with a known amount of IBP (20 mg/l). Cultivation bottles were vacuumed so that air bubbles could not block transpiration channels of tissue. After 24 h cultivation, medium samples were collected. Tissue were washed with demi water and frozen with liquid nitrogen.

Tissue samples were then extracted according to the method described in section 2.3. Recovery of extraction was calculated by the ration of IBP mass extracted from tissue to IBP loss in medium. Experiments were performed in triplicate. Recovery of the extraction method was 110-123%.

Text S4. Q-Exactive method for chemical analysis

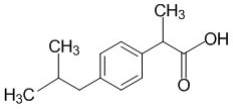
HESI (Heated Electrospray) ion source was used for the ionization. The HESI parameters were optimized as follows: sheath gas flow rate 53 units; aux. gas flow rate 14 units; sweep gas flow rate 3 units; spray voltage 2.5 kV; capillary temperature 269 °C; aux gas heater temperature 438 °C; and S lens RF level 50. Detection of the compounds was performed using a Q-Exactive mass spectrometer. Full scan data in both positive and negative was acquired at a resolution of 17500 FWHM (full width half maximum at m/z 200). For the compounds of interest, a scan range of m/z 100-600 was chosen; the automatic gain control (AGC) was set at 3e6 and the injection time was set to 200 ms. Scan-rate was set at 1 scan/s. For confirmatory purpose, a targeted MS/MS analysis was performed using the mass inclusion list. List of target precursor ions is shown in Table S2. The Orbitrap spectrometer was operated in negative mode at 17,500 FWHM. The AGC target was set to 2e5, with the maximum injection time of 100 ms. The precursor ions are filtered by the quadrupole which operates at an isolation window of m/z 4. Collision energy was optimized at 20% NCE (normalized collision energy) by injected working mix standard solution at a concentration of 10 µg/l.

Text S5. HPLC method for GT enzyme activity

First, 10 µl of 0.1 mM quercetin (substrate) was incubated with 20 µl of 2 mM uridine diphosphate glucose, 10 µl of 3.125 mM N-nitrophenyl-d-glucopyranoside and 10 µl of 3.125 mM salicin in 50 µl of 200 mM Tris/HCl buffer (pH 7.3). Second, the reaction was initiated by adding 100 µl GT enzyme extract. After 30 min incubation at 30 °C the reaction was stopped by protein-precipitation with 10 µl of H_3PO_4 and centrifuged at 15,000 × g for 2 min. Finally, the supernatant was diluted 1:4 (v/v) with solvent A

(water, 0.1% trifluoroacetic acid, TFA) of HPLC before analysis. Reverse phase HPLC was performed using a Varian Pro-Star M215 HPLC system and a C18 Prontosil Spheribond column (Bischoff Chromatography, Leonberg). Mobile phases consisted of 0.1% TFA (solvent A) and acetonitrile with 0.1% TFA (solvent B) run at a constant flow rate of 0.85 ml min^{-1}: 0-0.5 min 10% B; 0-8 min linear increased from 8 % to 40% B and hold 1.5 min; 9.5-12.5 min decreased to 8% B and hold until 15 min. The injection volume was 20 µl.

Table S1. Physico-chemical properties of ibuprofen

therapeutic use	CAS number	Chemical structure	molecular weight (g/mol)	pKa	Log K_{ow}	Log D_{ow}
nonsteroidal anti-inflammatory drug	15687-27-1		206.3	4.91	3.97	1.88 (pH=7)

Notes: Pka, log Kow From database provided by Syracuse Research Corporation:

http://www.syrres.com/esc/physdemo.htm

Table S2. List of target precursor ions of parent ibuprofen and related metabolites

Compounds	Formula	Mass $(M-H)^-$	Product ion $(M-H)^-$	RT (min)
IBP	$C_{13}H_{18}O_2$	205.1234	159.1179	10.18
hydroxy-IBP	$C_{13}H_{18}O_3$	221.1183	177.1287	9.52
1,2-dihydroxy-IBP	$C_{13}H_{18}O_4$	237.1132	193.1232	5.74
carboxy-IBP	$C_{13}H_{16}O_4$	235.0976	NM	9.32
glucopyranosyloxy-IBP	$C_{19}H_{28}O_7$	367.1762	ND	ND
glucopyranosyloxy-hydroxy-IBP	$C_{19}H_{28}O_8$	383.1711	NM	6.56
glucopyranosyloxy-carboxy-IBP	$C_{19}H_{27}O_{10}$	414.1532	ND	ND
IBP-glutathione conjugate	$C_{23}H_{33}N_3O_8S$	510.1916	ND	ND

Notes: ND means not detected; NM means not measured.

Figure S1. Growing plants under greenhouse conditions. Photos were taken by author Yujie He.

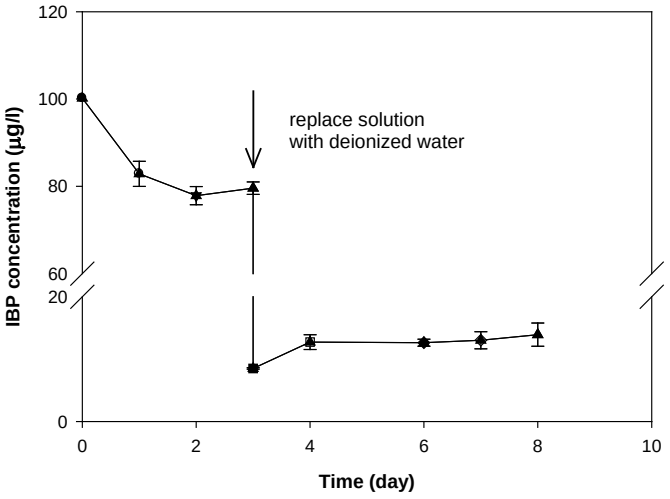
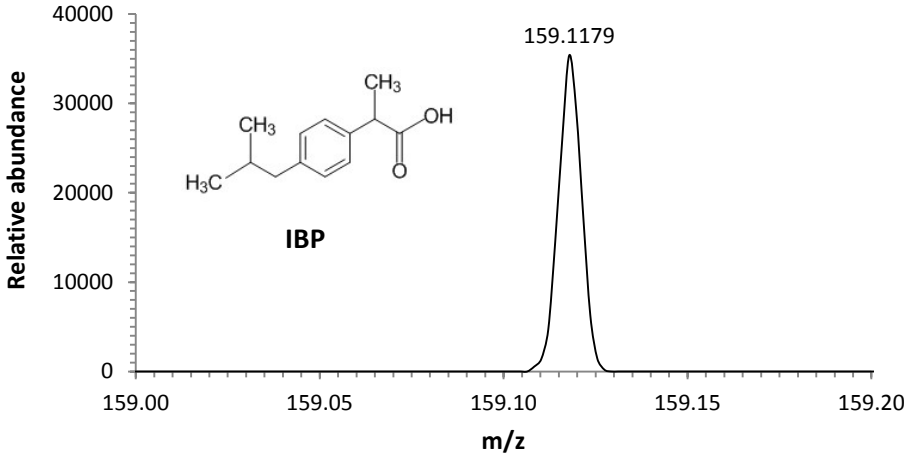
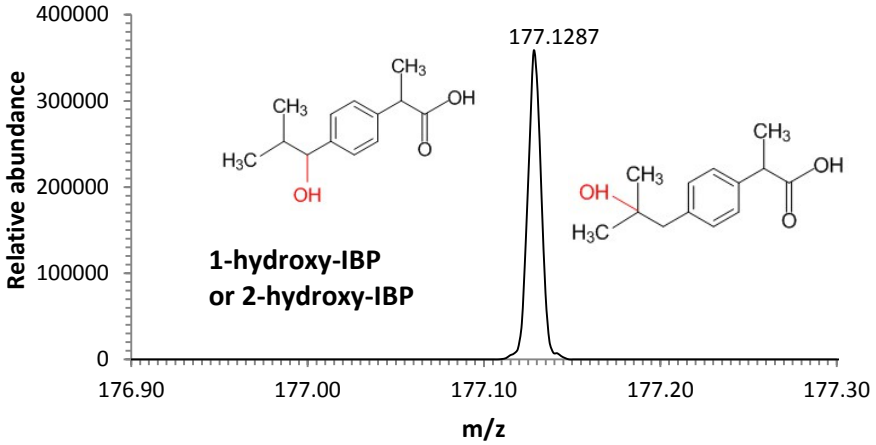


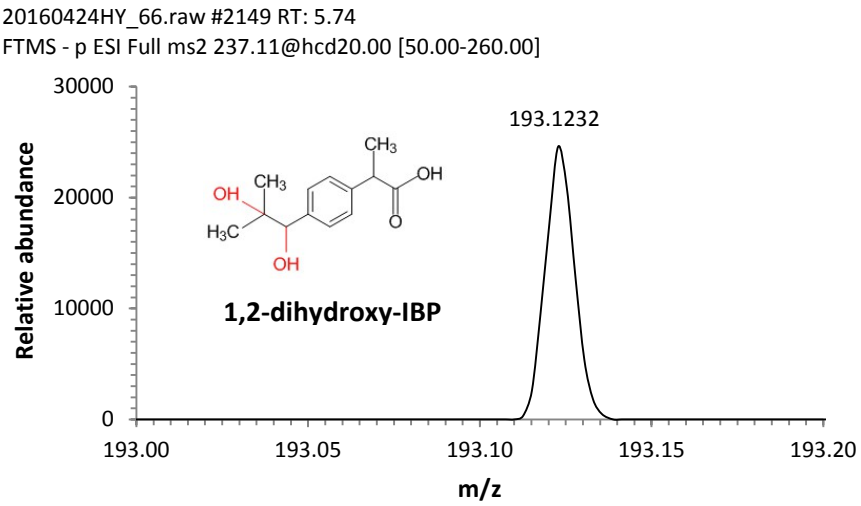
Figure S2. Sorption of IBP on perlite Sorption of IBP on perlite. At day 3, IBP solution was replaced with deionized water to mimic IBP removal by other mechanisms (e.g. biodegradation) in blank groups. After 3 days sorption, IBP sorption reached equilibrium. The results showed that 20% of IBP was absorbed on perlite until day 3 and desorption occurred after solution replacement.

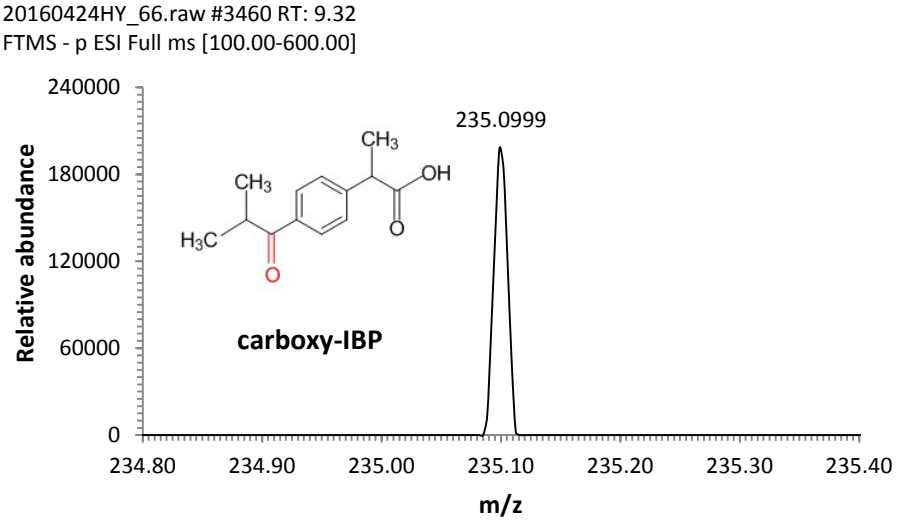
20160424HY_66.raw #3849 RT: 10.18
FTMS - p ESI Full ms2 205.12@hcd20.00 [50.00-230.00]



20160424HY_66.raw #3547 RT: 9.52
FTMS - p ESI Full ms2 221.12@hcd20.00 [50.00-245.00]]







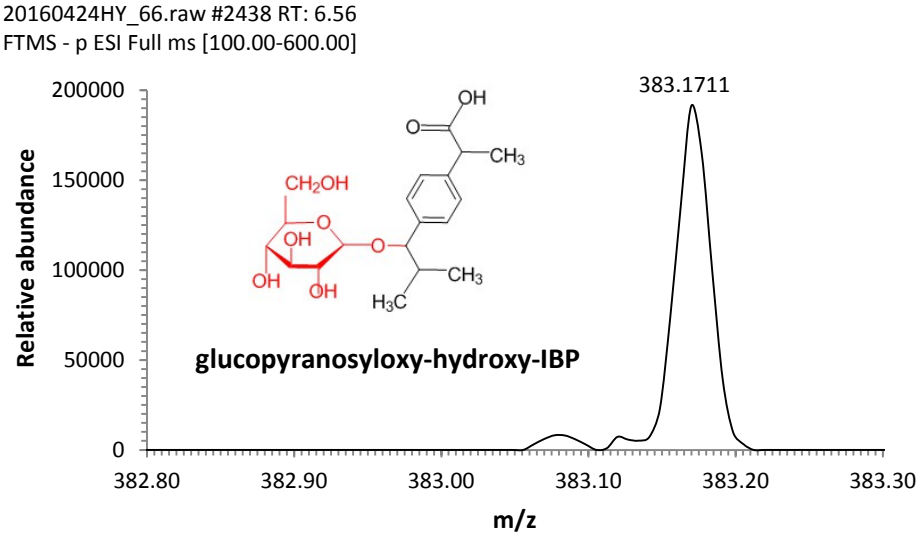


Figure S3. Major product ions of parent ibuprofen and related metabolites which were used to tentative identifictaion

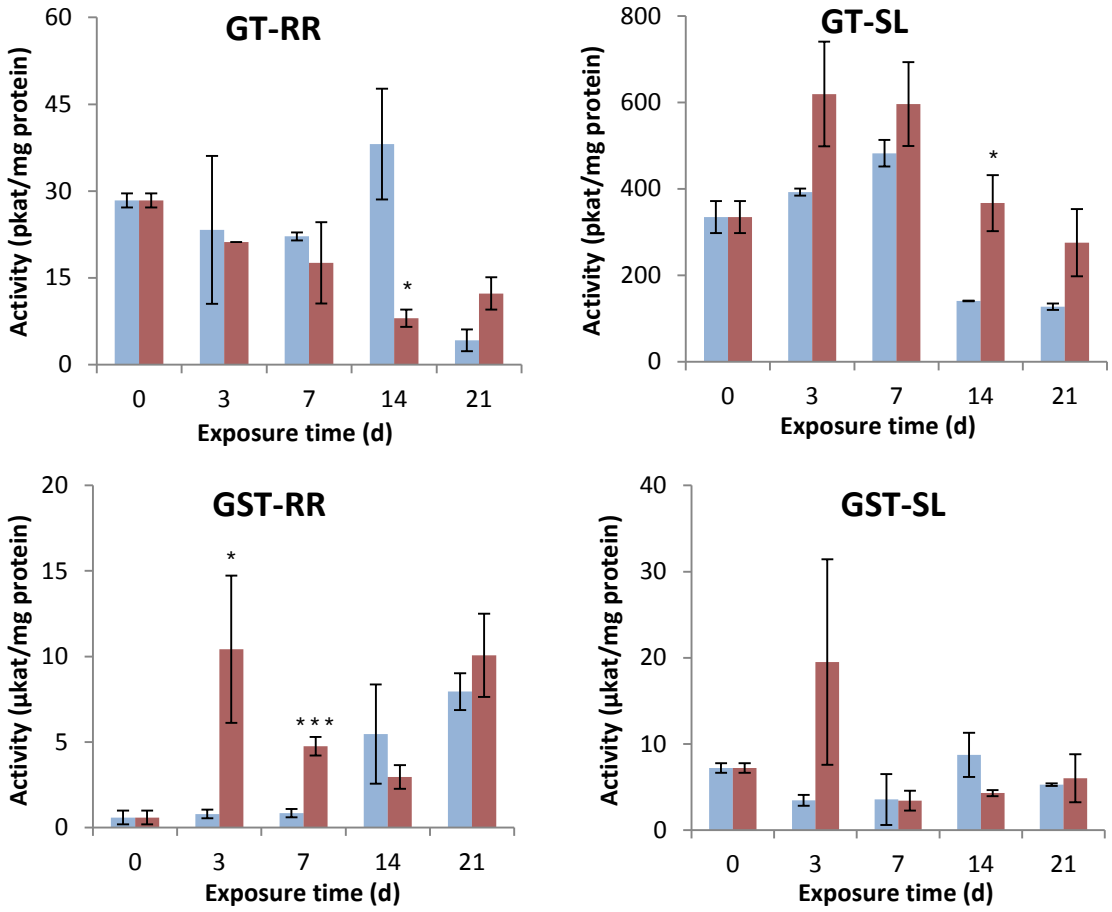


Figure S4. Enzyme activities of GT and GST in plant tissues (RR, roots and rhizomes; SL, stems and leaves) in untreated and treated groups. Data are mean activity ± standard error (n = 3).

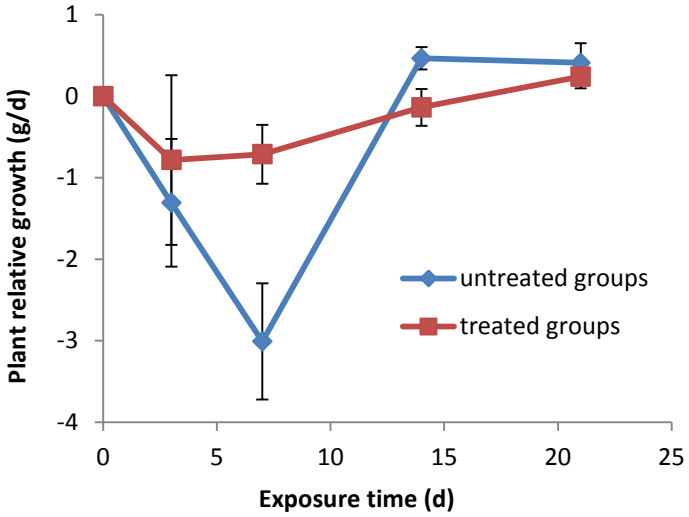


Figure S5. Relative growth rates of plants during the exposure period. Data are mean growth ± standard error (n = 3).

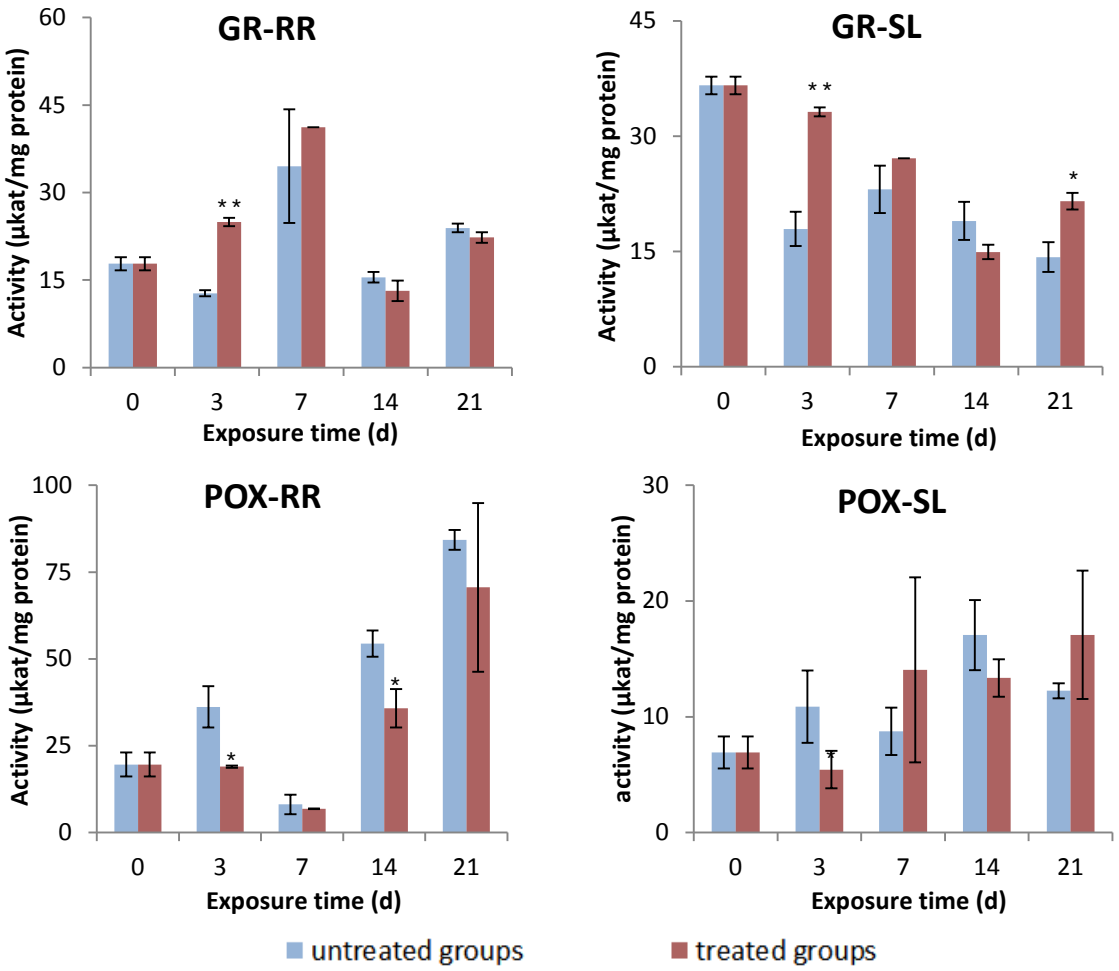


Figure S6. Stress enzyme activities (POX, GR) in plant tissues (RR, roots and rhizomes; SL, stems and leaves) in untreated and treated groups. Data are mean activity ± standard error (n = 3).