Supporting Information for

Comparative physiological and metabolomics analysis reveals that single-walled carbon nanohorns and ZnO nanoparticles affect salt tolerance in *Sophora alopecuroides*†

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The supporting information includes:

Table S1 and Table S2.

Figure S1-S12.

Supporting materials and methods.

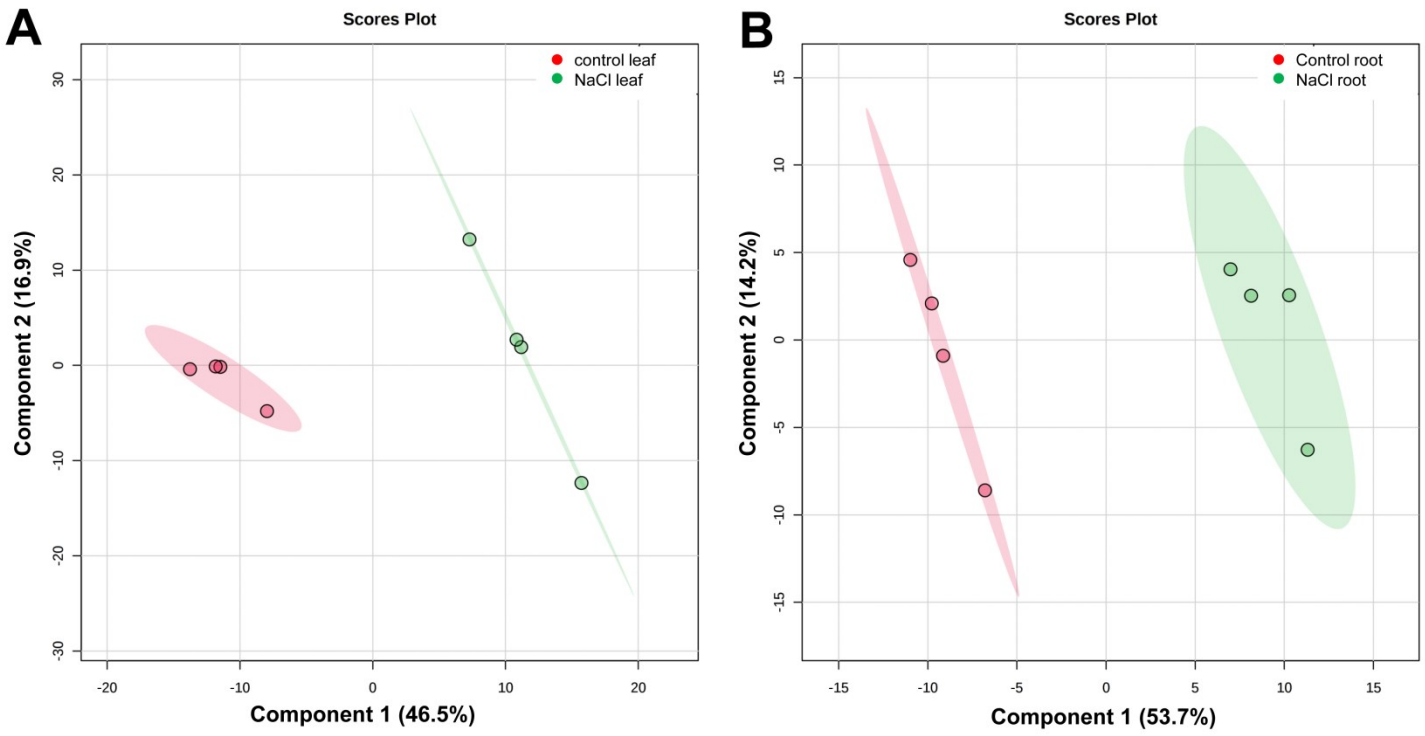

Figure S1. PLS-DA loading plot of metabolites in the leaves (A) and roots (B) of 100 mM NaCl-treated *S. alopecuroides* seedlings.

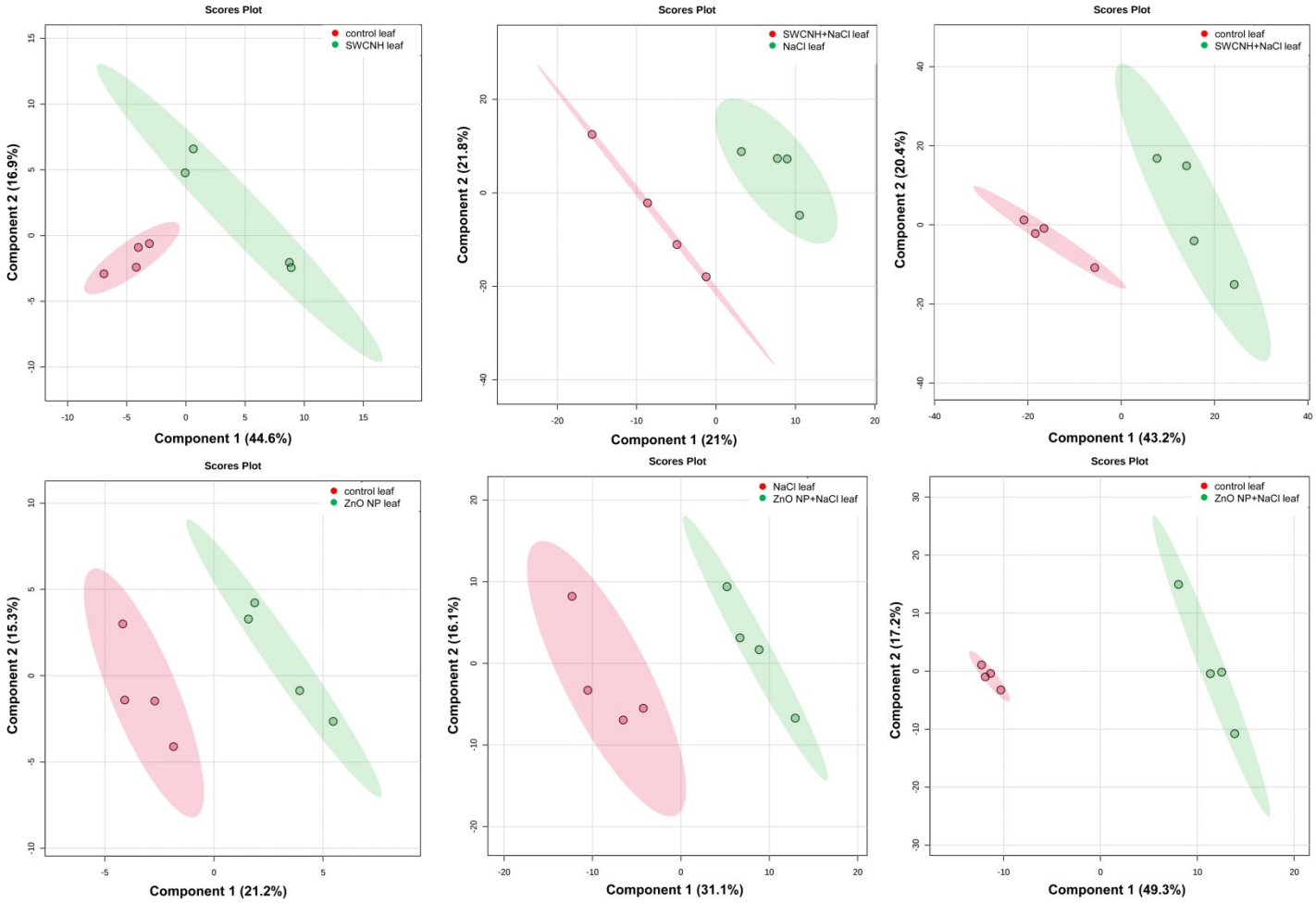

Figure S2. PLS-DA loading plot of metabolites in the leaves of *S. alopecuroides* seedlings in response to salt stress (100 mM NaCl), engineering nanomaterials (50 mg/L SWCNH or 100 mg/L ZnO NP) treatment and their combination.

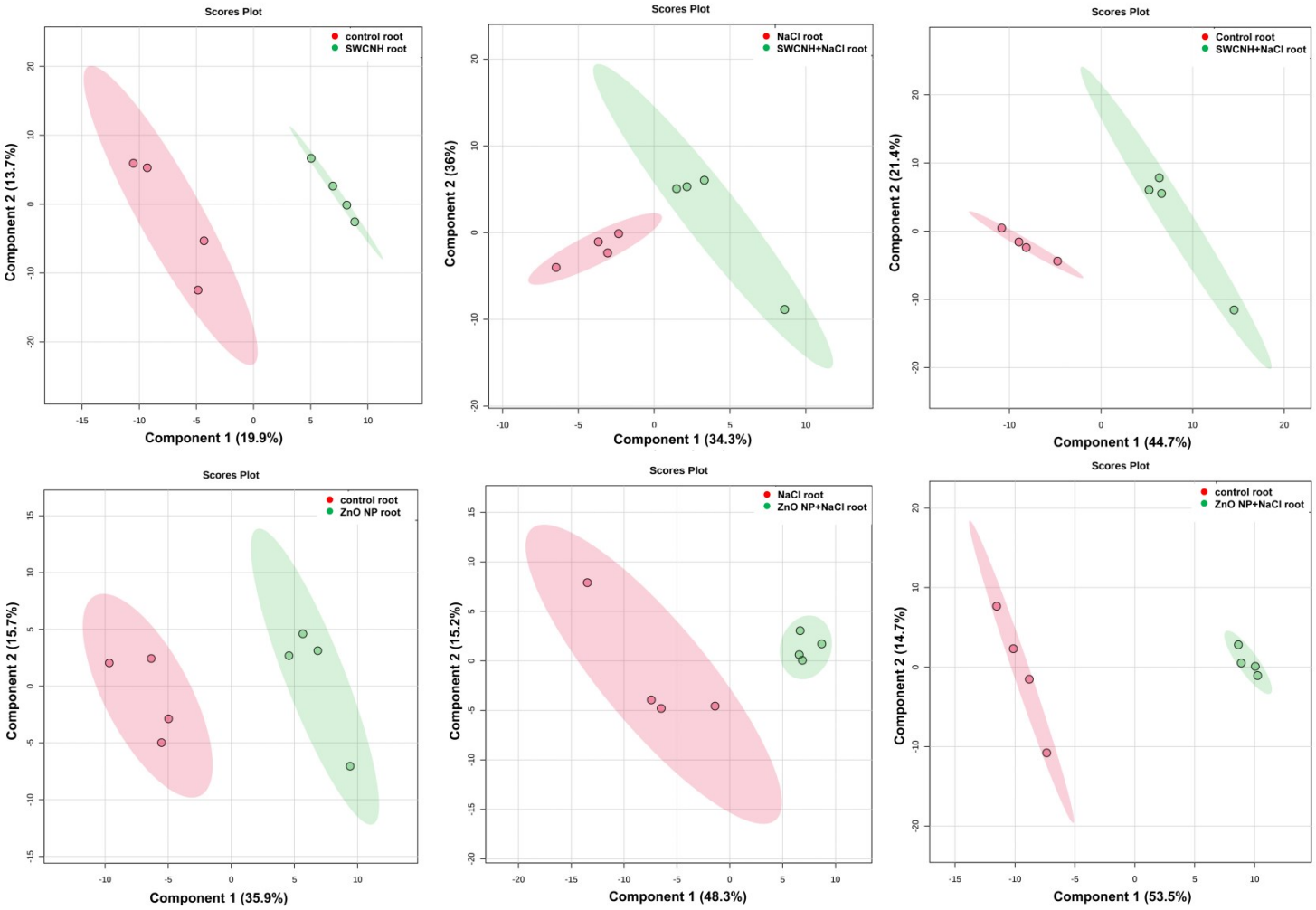

Figure S3. PLS-DA loading plot of metabolites in the leaves of *S. alopecuroides* seedlings in response to salt stress (100 mM NaCl), engineering nanomaterials (50 mg/L SWCNH or 100 mg/L ZnO NP) treatment and their combination.

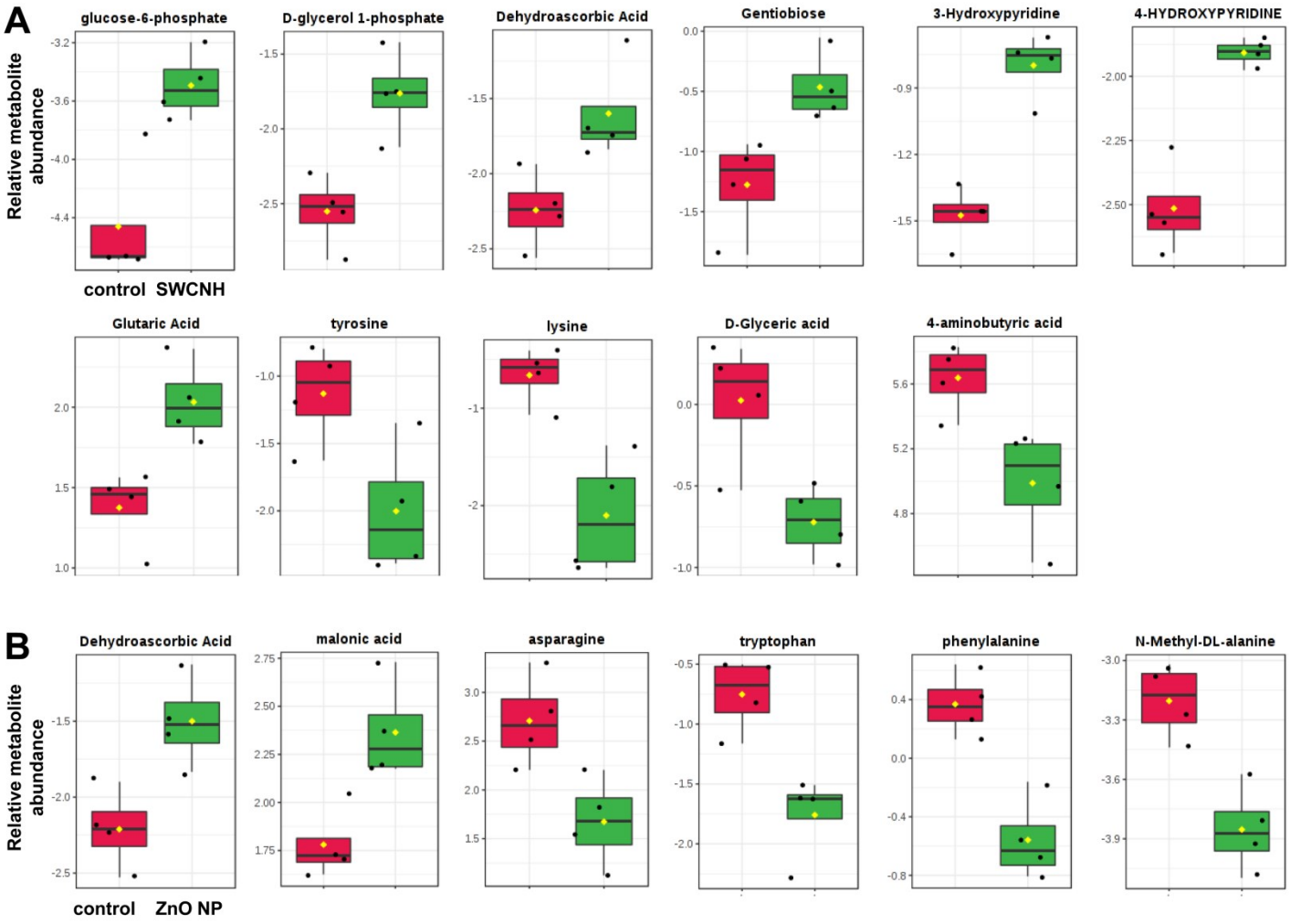

Figure S4. Significantly changed metabolites in the leaves of *S. alopecuroides* seedlings exposed to 50 mg/L SWCNH (A) or 100 mg/L ZnO NP (B). Five-week-old seedlings were transferred to fresh Hoagland solution, and foliar sprayed with 50 mg/L SWCNH or 100 mg/L ZnO NP for 15 days.

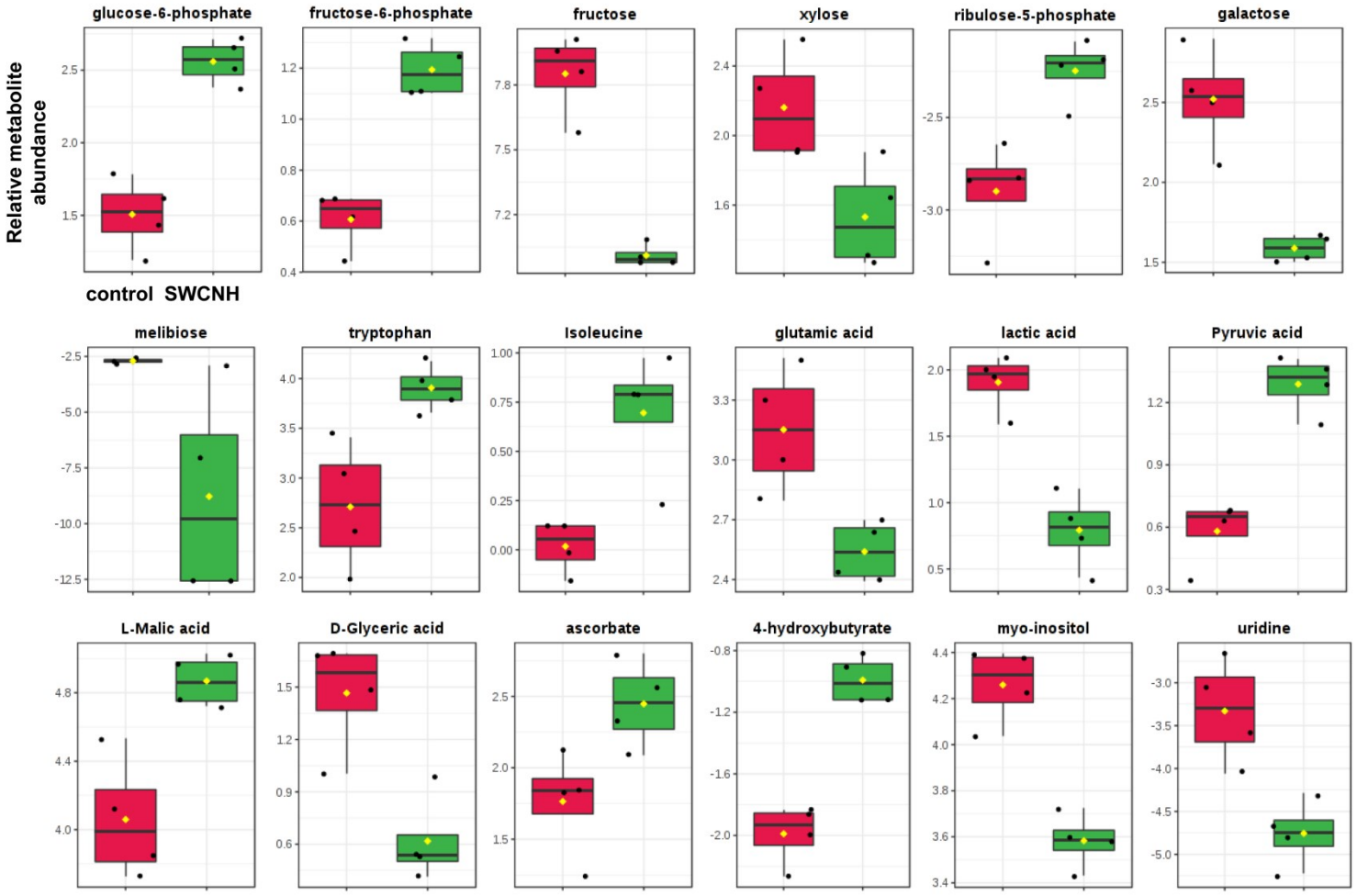

Figure S5. Significantly changed metabolites in the roots of *S. alopecuroides* seedlings exposed to 50 mg/L SWCNH. Five-week-old seedlings were transferred to fresh Hoagland solution, and foliar sprayed with 50 mg/L SWCNH for 15 days.

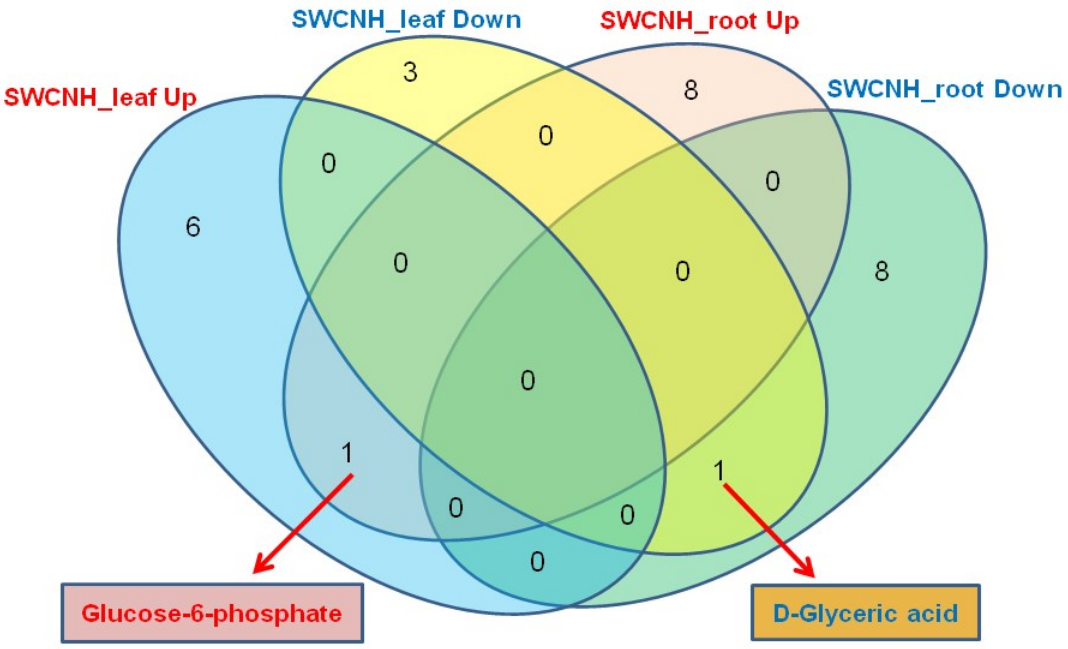

Figure S6. Venn analysis of the significantly changed metabolites in the leaves and roots of *S. alopecuroides* seedlings exposed to 50 mg/L SWCNH. Five-week-old seedlings were transferred to fresh Hoagland solution, and foliar sprayed with 50 mg/L SWCNH for 15 days. UP, upregulated metabolites; DOWN, downregulated metabolites.

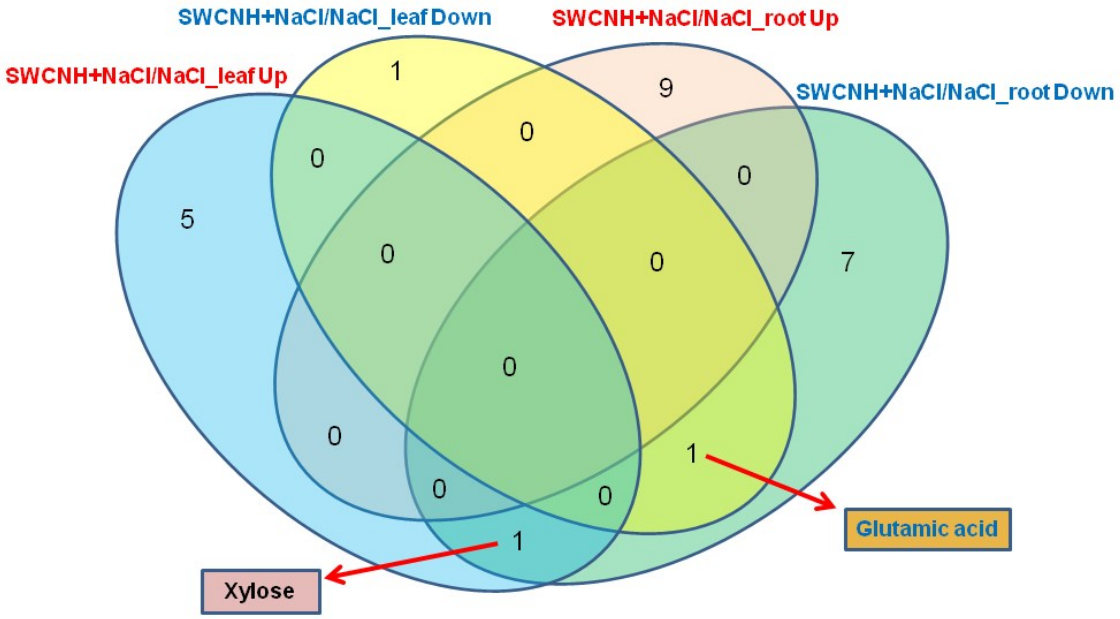

Figure S7. Venn analysis of the significantly changed metabolites in the leaves and roots of salt-treated *S. alopecuroides* seedlings exposed to 50 mg/L SWCNH. Five-week-old seedlings were transferred to fresh Hoagland solution with 100 mM NaCl, and foliar sprayed with 50 mg/L SWCNH for 15 days. UP, upregulated metabolites; DOWN, downregulated metabolites.

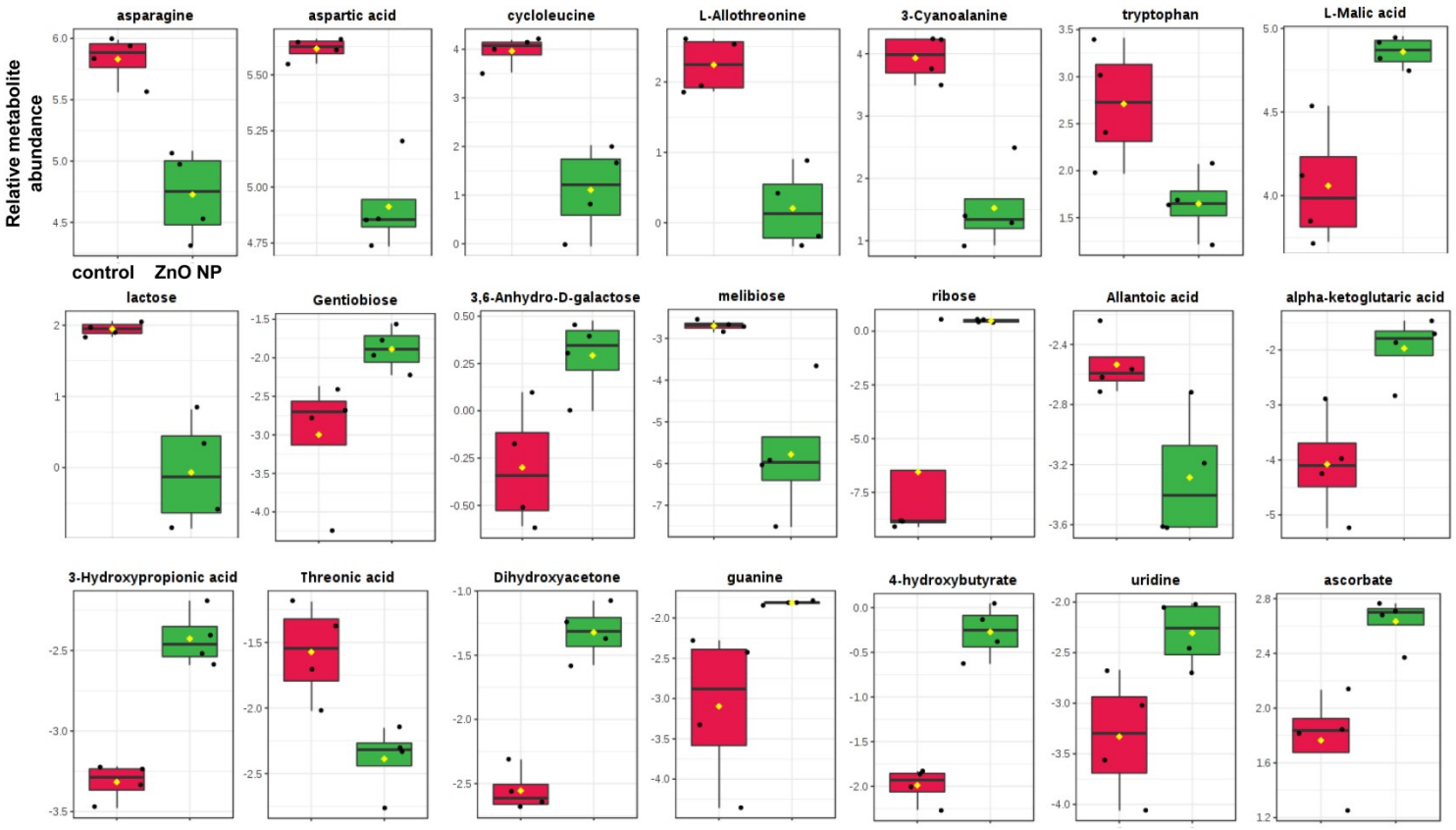

Figure S8. Significantly changed metabolites in the roots of *S. alopecuroides* seedlings exposed to 100 mg/L ZnO NP. Five-week-old seedlings were transferred to fresh Hoagland solution, and foliar sprayed with 100 mg/L ZnO NP for 15 days.

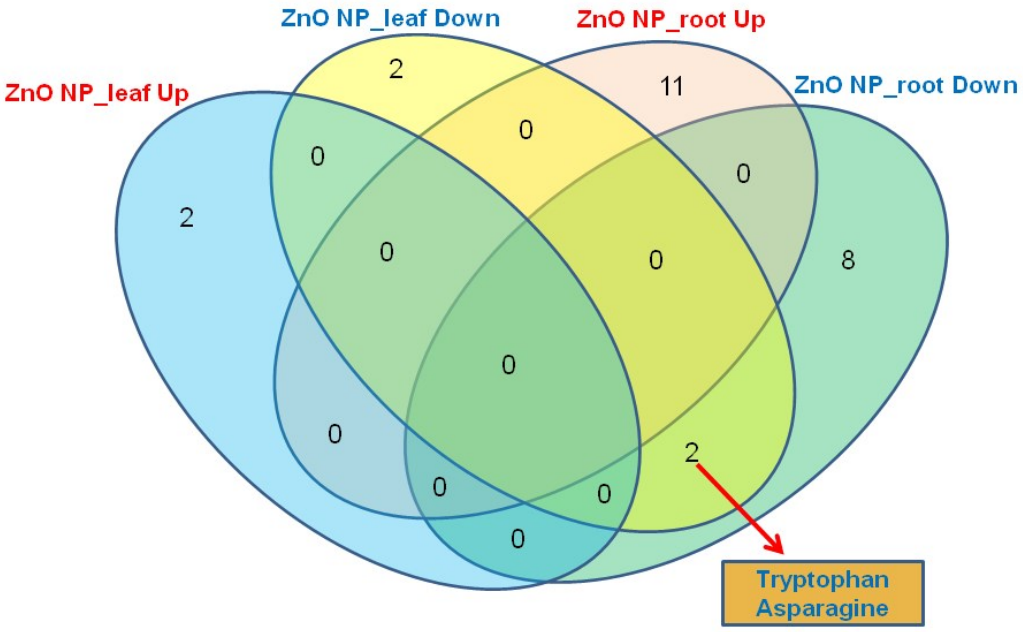

Figure S9. Venn analysis of the significantly changed metabolites in the leaves and roots of *S. alopecuroides* seedlings exposed to 100 mg/L ZnO NP. Five-week-old seedlings were transferred to fresh Hoagland solution, and foliar sprayed with 100 mg/L ZnO NP for 15 days. UP, upregulated metabolites; DOWN, downregulated metabolites.

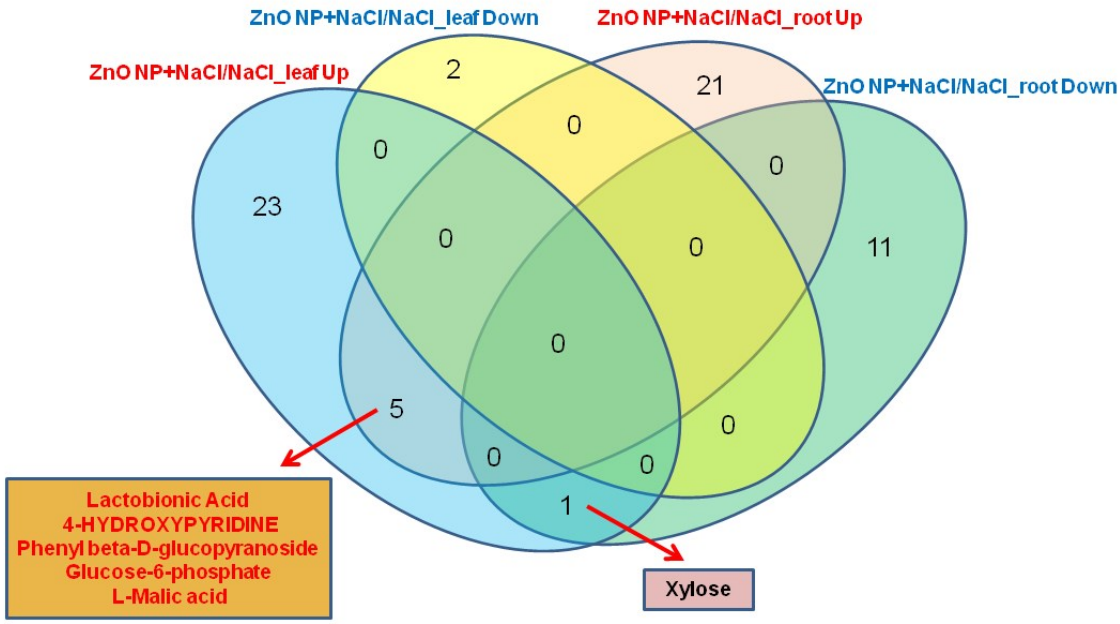

Figure S10. Venn analysis of the significantly changed metabolites in the leaves and roots of salt-treated *S. alopecuroides* seedlings exposed to 100 mg/L ZnO NP. Five-week-old seedlings were transferred to fresh Hoagland solution with 100 mM NaCl, and foliar sprayed with 100 mg/L ZnO NP for 15 days. UP, upregulated metabolites; DOWN, downregulated metabolites.

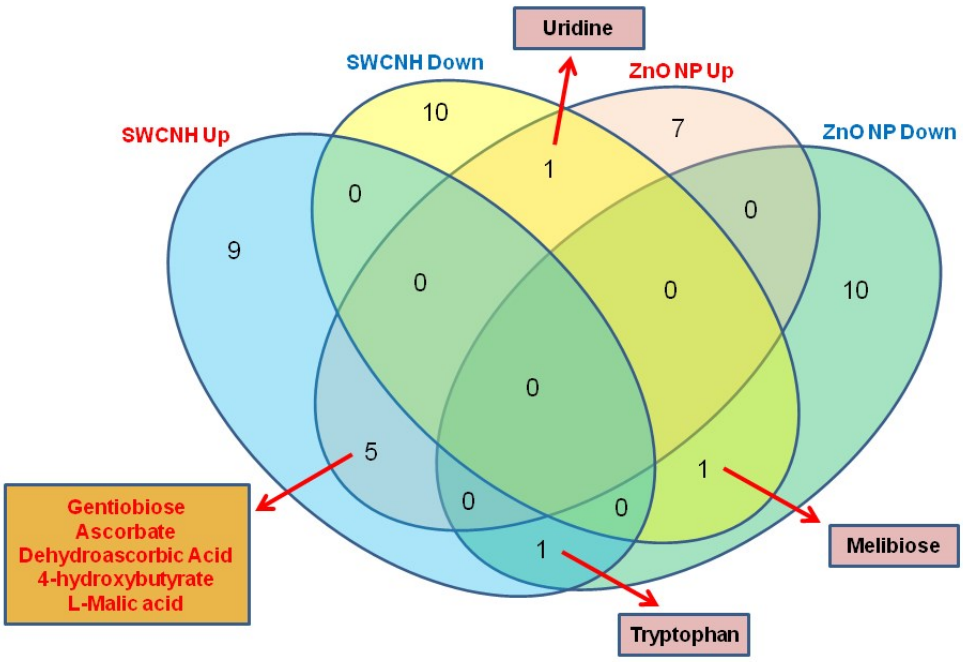

Figure S11. Venn analysis of the significantly changed metabolites in *S. alopecuroides* seedlings (leaves and/or roots) exposed to 50 mg/L SWCNH or 100 mg/L ZnO NP. Five-week-old seedlings were transferred to fresh Hoagland solution, and foliar sprayed with 50 mg/L SWCNH or 100 mg/L ZnO NP for 15 days. UP, upregulated metabolites; DOWN, downregulated metabolites.

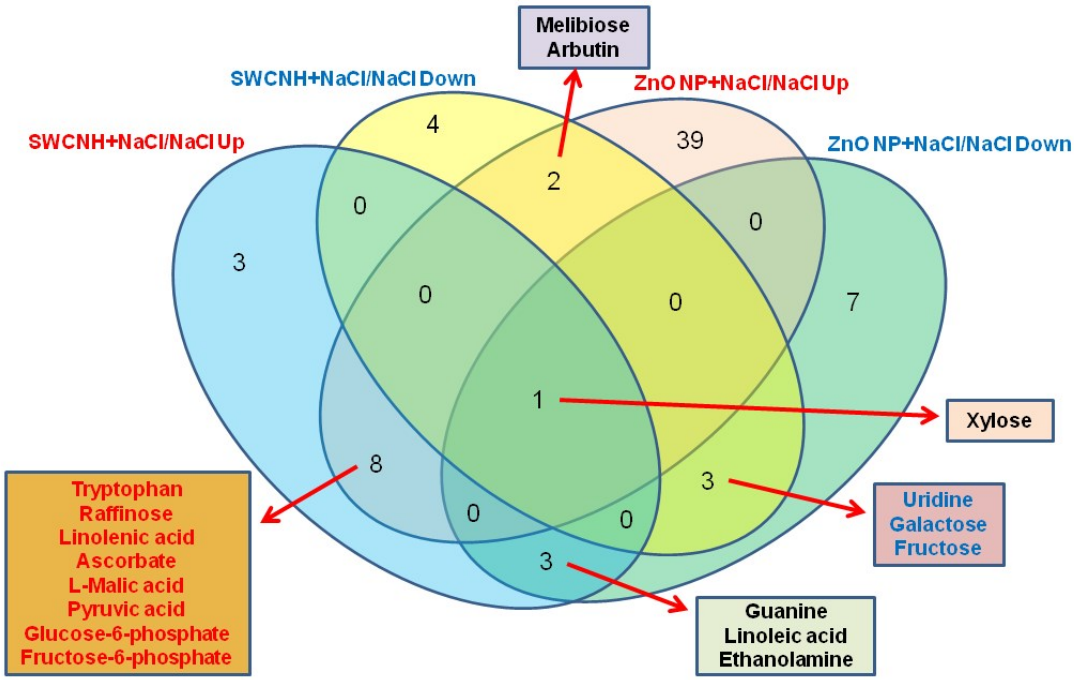

Figure S12. Venn analysis of the significantly changed metabolites in salt-treated *S. alopecuroides* seedlings (leaves and/or roots) exposed to 50 mg/L SWCNH or 100 mg/L ZnO NP. Five-week-old seedlings were transferred to fresh Hoagland solution with 100 mM NaCl, and foliar sprayed with 50 mg/L SWCNH or 100 mg/L ZnO NP for 15 days. UP, upregulated metabolites; DOWN, downregulated metabolites.

Supporting materials and methods

Metabolomic analysis using gas chromatograph coupled with a time-of-flight mass spectrometer

The samples (50 mg) were crushed with 450 μL of cooled extract (methanol:H_2O=3:1) and 10 μL of ribitol in 2 mL Eppendorf tube using a high throughput tissue crusher (-20 °C, 35HZ, 5min). After centrifugation at 12000 g for 15 min at 4 °C, 100 μL of supernatant was transferred to a 1.5 mL Eppendorf tube. After evaporation in a vacuum concentrator, 30 μL of methoxyamination hydrochloride (20mg/mL in pyridine) was added and then incubated at 80 °C for 30 min, then derivatized by 40 μL of BSTFA regent (containing 1% TMCS, v/v) at 70 °C for 1.5 h. The derivatized samples were collected for metabolomic analysis using gas chromatograph coupled with a time-of-flight mass spectrometer (GC-TOF-MS). GC-TOF-MS analysis was performed using an Agilent 7890 gas chromatograph coupled with a time-of-flight mass spectrometer. Samples were separated by a DB-5MS capillary column (30m × 250μm × 0.25μm, Agilent). The front injection temperature was 280 °C and helium was used as the carrier gas. The front inlet purge flow was 3 mL/min and the gas flow rate through the column was 1 mL/min. The oven temperature ramp was: 50 °C for 1min, raised to 310 °C at a rate of 10 °C/min, held on 8min, and then kept for 8 min at 310 °C. The transfer line temperature and ion source temperature were 280 °C and 250 °C, respectively. The energy was -70 eV in electron impact mode. The MS data was obtained in full-scan mode with the m/z range of 50-500 at a rate of 12.5 spectra per second after a solvent delay of 6.3 min.

The raw data analysis of GC-TOF-MS (including peak extraction, baseline adjustment, deconvolution, alignment and integration) was performed by ChromaTOF software (V4.3x, LECO). The metabolites were identified using LECO-Fiehn Rtx5 database by matching the mass spectrum and retention index. After subject to data normalization by sum and data log transformation, the *t*-test was performed ($P < 0.05$). The threshold was set to a fold change > 1.5 and P value < 0.05.