

Fig. S1. Results of methods comparison between root exudate TOC measured on a Shimadzu TOC analyzer and a UV-VIS spectrophotometer ($p < 0.01$).

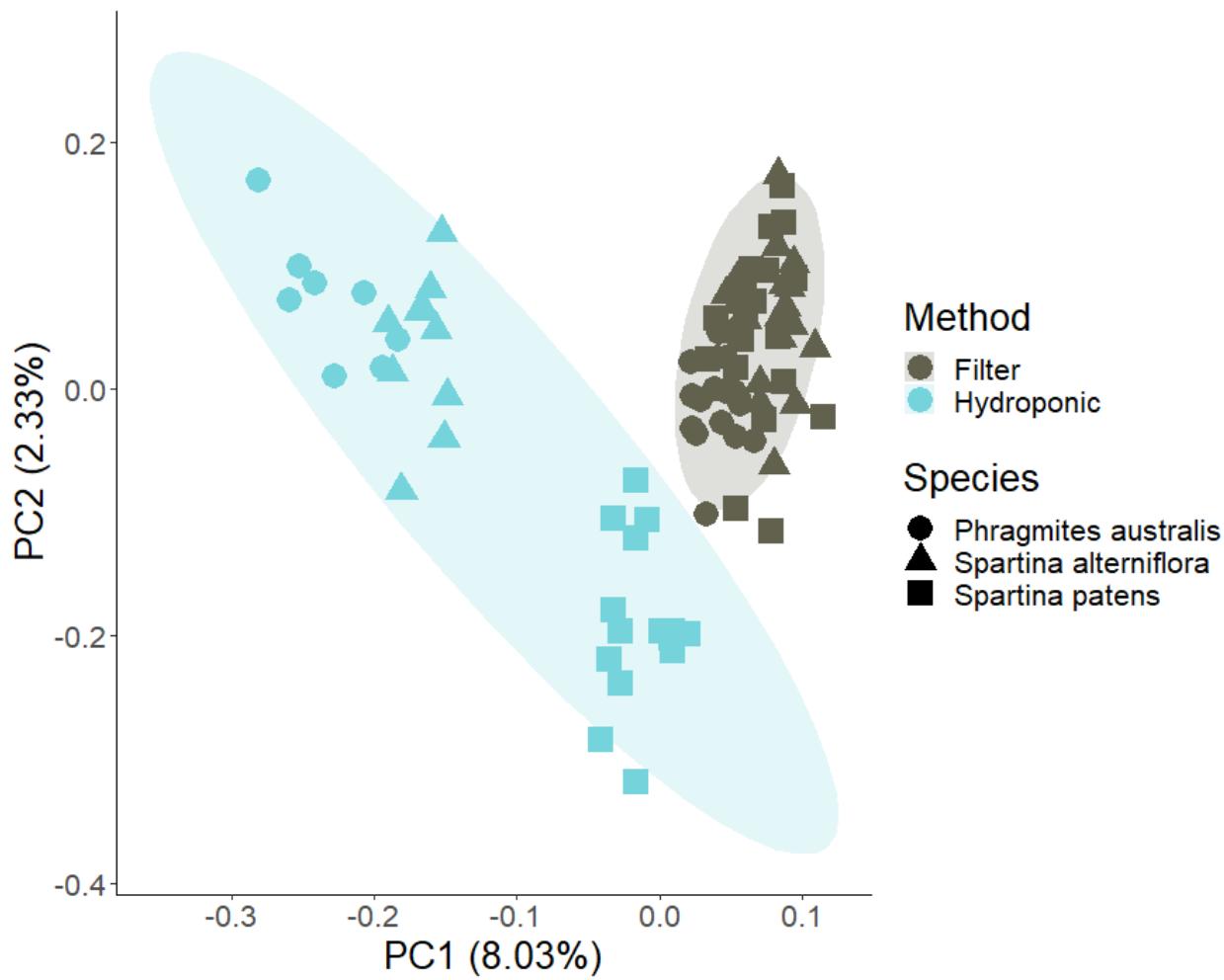


Fig S2. PCA on all identified metabolites assessing methodological comparison between hydroponically collected samples and samples collected using soil filters from 3 species. *S. americanus* is not included due to methodological differences in the filter-collected samples compared to the other 3 species precluding its use in comparisons.

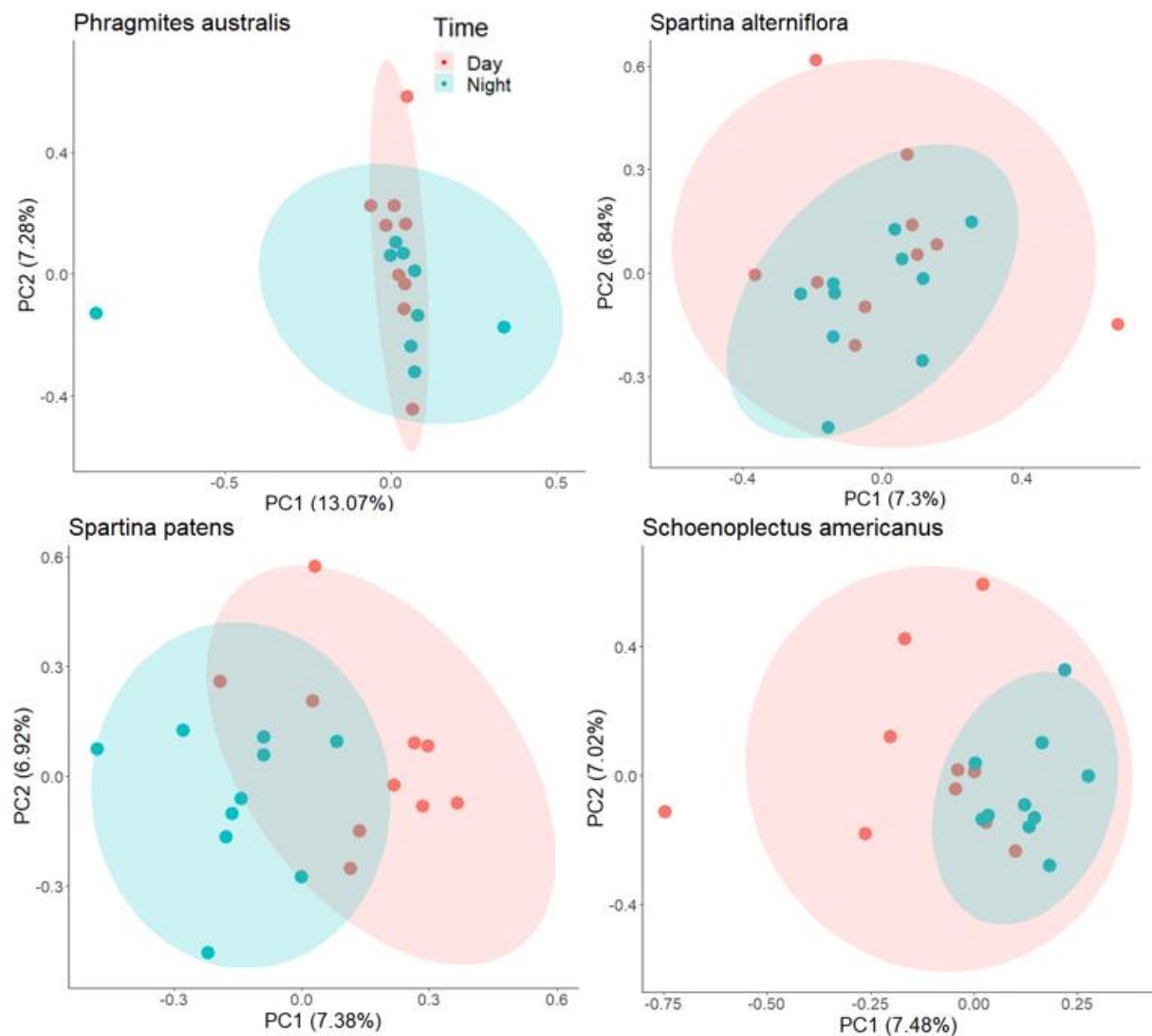


Fig S3. PCAs on all metabolites identified in the light and dark period of 4 species using soil filter collection method. The same root on the same 10 plants was sampled in day and night time. Point size represents relative abundance of all compounds summed.

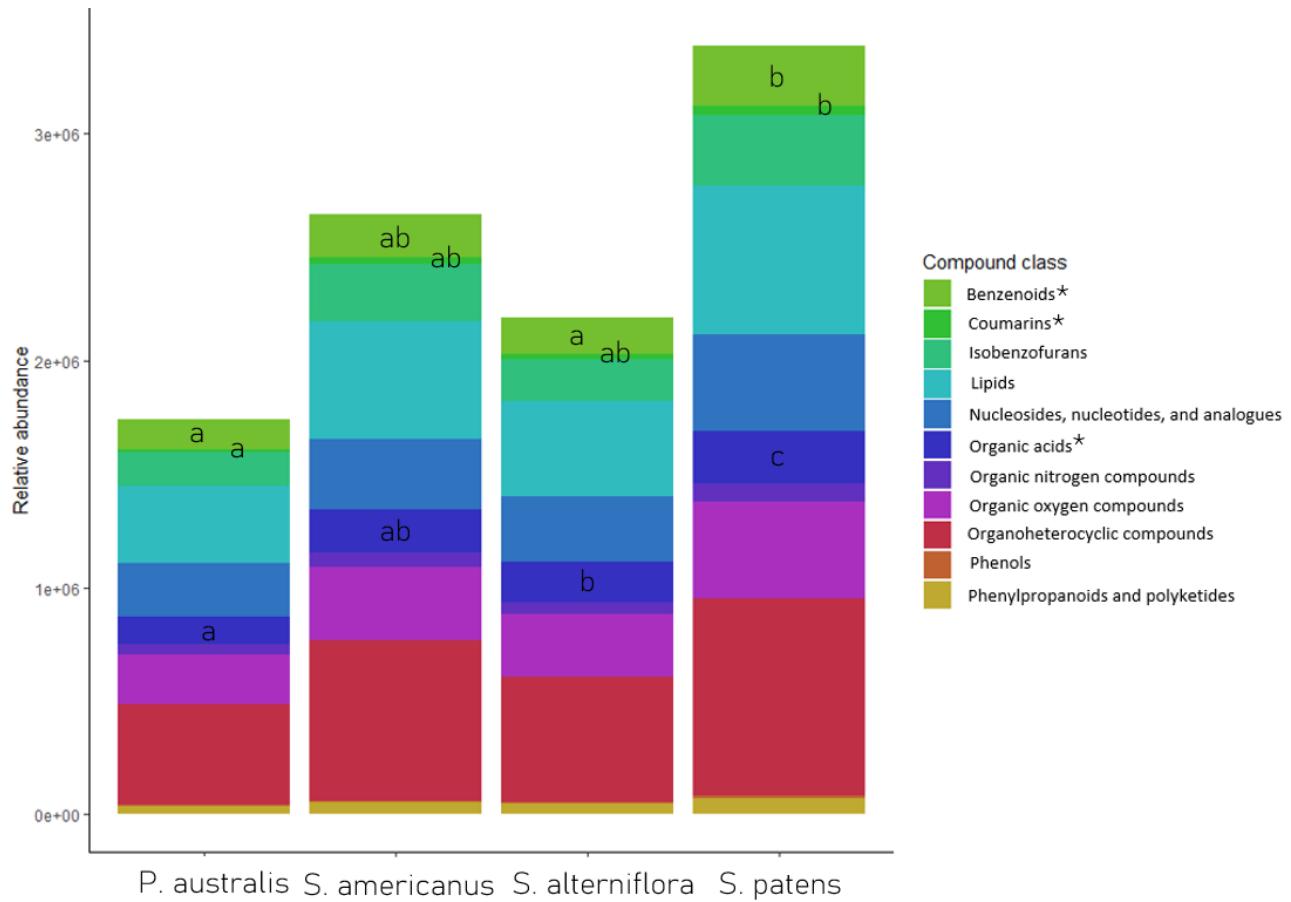


Fig. S4: Average contribution of various compound classes to the overall rhizosphere-associated metabolite pool of each species by relative abundance, using only metabolites previously reported to occur in plants. Relative abundance by compound class was fairly conserved across species, with major differences in compound type scaling to overall relative abundance. There were significant differences ($p < 0.05$, Tukey's test) in relative abundance for benzenoids (*S. patens* > *S. alterniflora* and *P. australis*), coumarins (*S. patens* > *P. australis*), and organic acids (*S. alterniflora* > *P. australis*; *S. patens* > all).

Contribution of variables to Dim-1

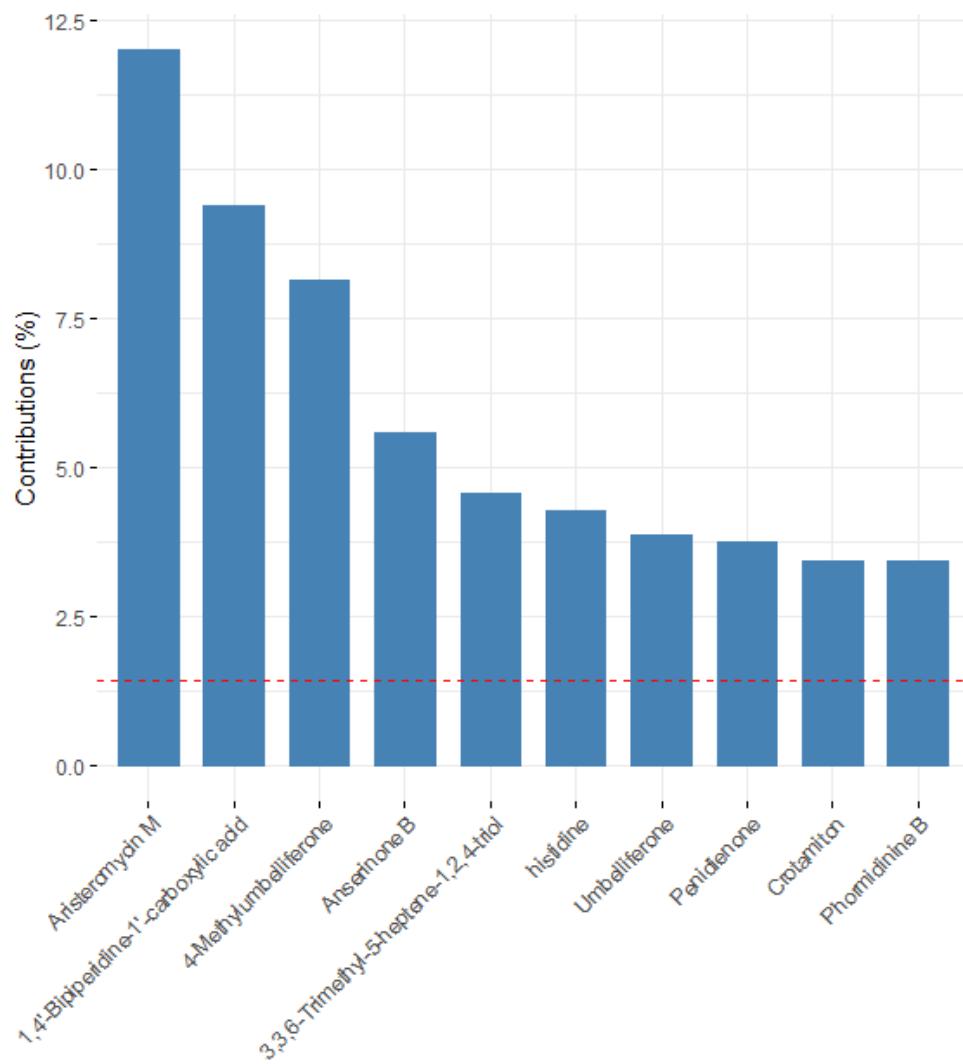


Fig S5. Compounds that differed on a species level (Dimension 1 of PCA).

Contribution of variables to Dim-2

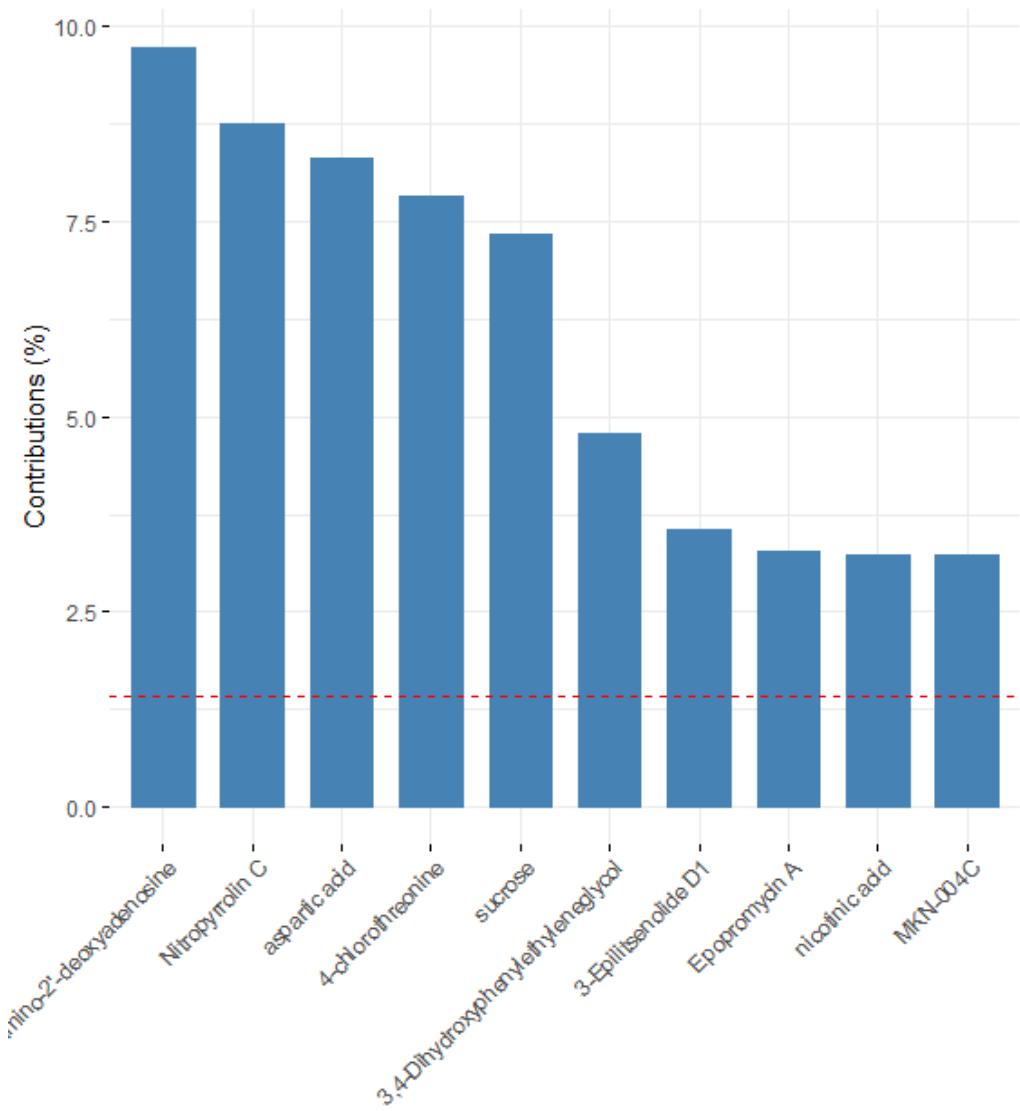


Fig S6. Compounds that differ on a species level (Dimension 2 of PCA).

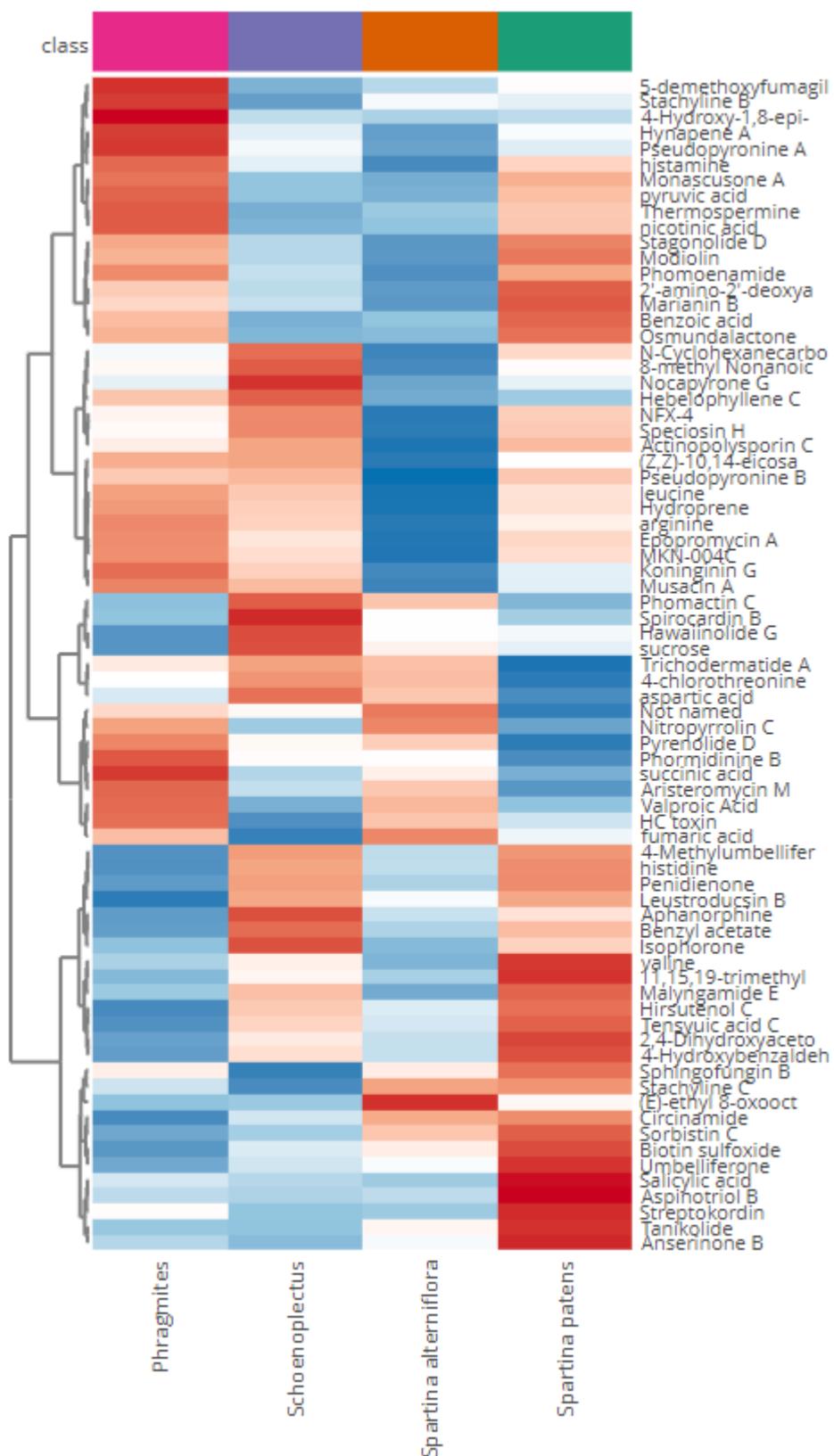


Fig. S7. Relative average abundance of different compounds (only those previously reported as plant metabolites in RMDP) across the 4 species, produced in MetaboAnalyst 6. Blue implies a lower relative abundance, while red implies higher.



Schoenoplectus americanus

Spartina patens

Fig. S8. Typical patterns of oxygenation in the two species demonstrated by methylene blue. Note lack of barrier to ROL in root hairs of *S. patens*, while oxygenated zones in *S. americanus* are limited to the tips of young, adventitious roots.

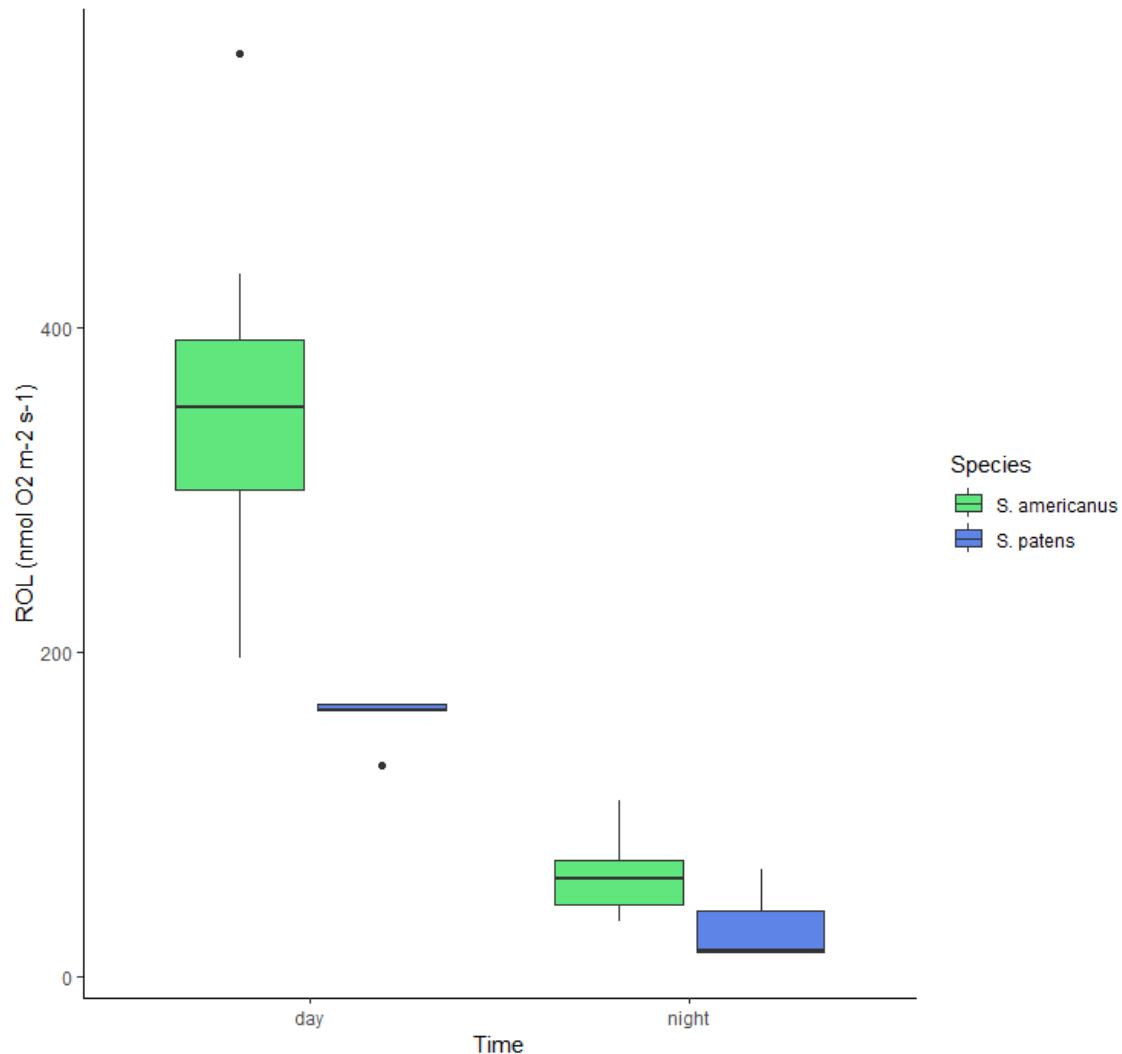


Figure S9. Root oxygen loss rates from *Schoenoplectus americanus* ($n = 7$) and *Spartina patens* ($n = 3$) during the day and night periods.

Table S1: A subset of compounds ($n = 75$) identified using QTOF-MS, of 642 total. Selected based on their matches to compounds previously as plant metabolites in RMDP (<https://www.biosino.org/RefMetaDB/>). Amino acids are shaded in green.

Compound formula	Compound name	Greatest abundance, methodology	Greatest abundance, species
C ₂₂ H ₄₃ NO	13Z-Docosenamide	Hydroponic	<i>S. americanus</i> *
C ₉ H ₆ O ₃	Umbelliferone	Filter	<i>P. australis</i>
C ₈ H ₈ O ₃	Vanillin	Filter	<i>P. australis</i>

C10H8O3	4-Methylumbelliferon	Filter	<i>S. americanus</i> *
C4H7NO4	Aspartic acid	Hydroponic	<i>S. americanus</i> *
C5H9N3	Histamine	Virtually identical	<i>S. americanus</i>
C6H13NO2	Leucine	Hydroponic	<i>S. americanus</i>
C5H11NO2	Valine	Filter	<i>S. patens</i>
C23H44O2	11,15,19-trimethyl-5Z-eicosenoic acid	Filter	<i>P. australis</i>
C17H30O2	Hydroprene	Filter	<i>S. americanus</i>
C19H27NO2	Phormidinine B	Hydroponic	<i>S. americanus</i>
C19H29C1N2O3	Nitropyrrolin C	Hydroponic	<i>S. alterniflora</i>
C34H56NO10P	Leustroducsin B	Filter	<i>S. americanus</i>
C13H17NO	Aphanorphine	Filter	<i>S. americanus</i>
C20H39NO6	Sphingofungin B	Filter	<i>P. australis</i>
C20H36O3	15-HEDE	Filter	<i>P. australis</i>
C20H28O5	Gibberellin	Filter	<i>S. americanus</i> *
C20H36O2	5(Z),14(Z)-Eicosadienoic Acid	Filter	<i>S. americanus</i>
C19H14N4O2	Balantiolide	Hydroponic	<i>S. alterniflora</i> *
C10H12O5	C-veratroylglycol	Hydroponic	<i>S. alterniflora</i> *
C11H14N4O3	Aristeromycin M	Hydroponic*	<i>S. alterniflora</i> *
C6H8O3	5-Hydroxymethylfurfuryl alcohol	Filter	<i>S. patens</i>
C12H16O3	Nocapyrone G	Virtually identical	<i>S. americanus</i> *
C15H22O4	Hebelophyllene C	Filter	<i>S. americanus</i>
C9H10O2	Benzyl acetate	Filter	<i>P. australis</i>
C9H14O	Isophorone	Filter	<i>S. americanus</i>
C11H20O2	Speciosin H	Filter	<i>S. americanus</i>
C15H24O3	5-demethoxyfumagillol	Filter	<i>S. americanus</i>
C15H22O3	Hirsutenol C	Filter	<i>P. australis</i>

C20H32O6	Spirocardin B	Hydroponic	<i>S. americanus</i>
C14H24N2O4	Phomoenamide	Hydroponic	<i>S. patens</i> *
C19H26O3	Marianin B	Filter	<i>S. patens</i>
C14H18O	Penidienone	Virtually identical	<i>S. patens</i>
C20H28O3	Phomactin C	Hydroponic	<i>S. americanus</i> *
C17H30O3	Pseudopyronine B	Hydroponic	<i>S. americanus</i>
C16H30O2	Actinopolysporin C	Filter	<i>S. americanus</i>
C10H14N6O3	2'-amino-2'-deoxyadenosine	Filter	<i>P. australis</i> *
C22H32O7	Trichodermatide A	Filter	<i>S. americanus</i> *
C18H28O5	Hynapene A	Filter	<i>P. australis</i>
C13H18O5	Monascusone A	Filter	<i>P. australis</i> *
C21H36N2O6	Epopromycin A	Hydroponic	<i>S. americanus</i>
C8H9NO2	Streptokordin	Hydroponic	<i>S. patens</i>
C10H14O4	Stagonolide D	Filter	<i>S. patens</i>
C12H22O3	NFX-4	Filter	<i>S. americanus</i>
C6H5NO2	Nicotinic acid	Hydroponic	<i>S. patens</i>
C10H16N2O4S	Biotin sulfoxide	Filter	<i>S. patens</i>
C12H26N2O9	Sorbillin C	Hydroponic	<i>P. australis</i>
C6H14N4O2	Arginine	Hydroponic	<i>S. americanus</i>
C10H20O2	8-methyl Nonanoic acid	Hydroponic	<i>S. americanus</i>
C10H26N4	Thermospermamine	Hydroponic	<i>S. americanus</i>
C18H34N4O5	Circinamide	Hydroponic	<i>S. patens</i>
C6H9N3O2	Histidine	Filter	<i>S. americanus</i>
C8H16O2	Valproic Acid	Hydroponic	<i>S. alterniflora</i>
C12H20O3	Modiolin	Hydroponic	<i>S. americanus</i>
C10H18O3	MKN-004C	Hydroponic	<i>S. americanus</i>
C4H8ClNO3	4-chlorothreonine	Hydroponic*	<i>S. alterniflora</i> *
C11H18O6	Musacin A	Filter	<i>S. americanus</i>

C13H16O4	Stachyline B	Hydroponic	<i>S. alterniflora</i>
C16H28O3	Pseudopyronine A	Filter	<i>S. americanus</i>
C16H30O5	Koninginin G	Hydroponic	<i>S. americanus</i>
C17H32O3	Tanikolide	Filter	<i>S. americanus</i>
C31H55NO6	Malyngamide E	Filter	<i>P. australis</i> *
C12H22O11	Sucrose	Filter*	<i>P. australis</i> *
C13H18O3	Stachyline C	Filter	<i>P. australis</i>
C11H14O4	Anserinone B	Filter	<i>P. australis</i>
C13H20O6	Tensyuic acid C	Hydroponic	<i>S. americanus</i>
C7H6O3	Salicylic acid	Filter	<i>P. australis</i>
C3H4O3	Pyruvic acid	Hydroponic	<i>S. patens</i>
C4H6O4	Succinic acid	Hydroponic	<i>S. alterniflora</i>
C4H4O4	Fumaric acid	Hydroponic	<i>S. alterniflora</i> *
C7H6O2	4-Hydroxybenzaldehyde	Filter	<i>P. australis</i> *
C7H6O2	Benzoic acid	Filter	<i>S. patens</i>
C9H16O3	Aspinotriol B	Filter	<i>P. australis</i>

Additional methodological details:

Service report from UMBC:

Summary: Non-targeted metabolomics analysis was conducted on root exudate samples using LC-MS/MS

Sample Preparation: Samples were stored at -20 °C until use. Prior to analysis, samples were thawed at 4 °C and vortexed for homogeneity. A 1 mL aliquot of each sample was transferred to separate 2 mL autosampler vials and loaded in the HPLC autosampler.

Data Acquisition: Analysis was performed using the Bruker compact Q-TOF-MS equipped with a Dionex UltiMate 3000 UHPLC with a Waters Acquity BEH C18 (1.7 µm, 2.1x50 mm) column. 2 µL of sample was injected onto the column and eluted by the following gradient (A: Water with 0.1% formic acid; B: Acetonitrile with 0.1% formic acid). Flow was held at 2% B for 1 min before increasing to 98% B over a period of 4 min. This was held for 3 min before decreasing to 2% B over 0.1 min, and holding at 2% B for 1.9 min. Flow rate was constant at 0.3 mL/min and column oven was held at 40 °C. The autosampler was chilled to 4 °C. The LC method was saved as “Metabolomics_Acquity”.

Tuning and mass calibration of the mass spectrometer was performed prior to running samples, using 10 mM sodium formate at a flow rate of 3 µL/min. Each sample was run in both positive and negative ionization modes. For positive ion mode, MS/MS data were collected over the mass range of 20-1300 m/z in auto MS/MS mode. For intra-run calibration, 20 uL of sodium formate was injected in the first 0.3 min of the run. The capillary voltage was set at 4500 V with a nebulizer gas flow of 2.2 Bar, dry gas of 10.0 L/min, dry temperature of 220 °C, and collision energy of 20-50 eV. Negative ion mode, MS/MS data was collected with identical conditions, except the capillary voltage was set to -3600 V. MS methods were saved as “Metabolomics_autoMSMS_pos_V2” and “Metabolomics_autoMSMS_neg_V2”.

Data Analysis: MetaboScape 2023b software (Bruker) was used for processing and analyzing the MS/MS data. The following parameters for molecular feature identification and ‘bucketing’ were set in the T-ReX 2D/3D workflow: for peak detection, a minimum intensity threshold of 1000 counts was required, as well as a minimum peak duration of 7 spectra, with feature quantitation determined using peak intensity. Masses were recalibrated based on the external calibrant (sodium formate) injected between 0-0.3 min. Only features found in at least 90% of samples per sample group were considered.

Features were identified by MetaboScape by matching against the Natural Product Atlas, Cell Culture Nutrients, Fatty Acids, and Bruker MetaboBASE Plant Library target lists, as well as by MSMS spectral matching against the NIST 2020 MSMS Library. All analysis results are listed the Supplementary File “KHaviland_Dec2023.xlsx”.