Supplemental Material and Methods

Gas Chromatography- Mass spectrometry (GC-MS)

Preparation

Freeze-dried exudate pellets were resuspended in 1 mL of LC-MS grade water (Sigma-Aldrich, Gillingham, UK) and transferred into new 2.5 mL microcentrifuge tubes. At this point, equal aliquots (5 µL) from all samples were combined in a new tube, for each sample type (leachates, and hydroponics), to be used as quality control (QC) sample. This was followed by addition of 100 µL of internal standard (0.2 mg mL^{-1} of succinic-d_4 acid, and glycine-d_5) to all samples before being lyophilised overnight using a speed vacuum concentrator (Concentrator 5301, Eppendorf, Cambridge, UK). All dried extracts were derivatized by oximation followed by a silylation step, using methoxyamine-hydrochloride in pyridine and *N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide, respectively (Fiehn, Kopka, Trethewey, & Willmitzer, 2000; Wedge et al., 2011).

GCMS technical and run specifics

Metabolomics data were acquired using a 7890B GC coupled to a 5975 series MSD quadrupole mass spectrometer, equipped with a 7693 auto-sampler and piloted by Chemstation software (Agilent, Technologies, UK). Samples were randomly positioned on the sample tray along with blank and pooled quality control (QC) samples, which were run after every 6^{th} sample injection. There were two types of QCs; the first were pooled from leachate samples only (5 µL per sample), the second consisted of a pooled proportion of all other samples. Samples were run in three randomised batches, each with QCs, allowing correction for between-batch variation.

For analysis, 1 µL of derivatized sample was loaded into a VF-5MS column (30 m x 0.25 mm x 0.25 µm; Agilent Technologies). The injector was operated in split mode, with a ratio of 20:1, and the inlet temperature was held at 280°C. Helium was used as the carrier gas with a flow rate of 1 $mL^{-1}.min^{-1}$. The oven injection temperature was set at 70°C for 4 min, followed by a 14°C.min^{-1} oven temperature gradient to a final 350°C, and then held for 4 min at 300°C. The system was then equilibrated at 70°C for 15 s before subsequent sample injection. Ions are generated by a 70 eV electron beam at an ionization current of 35 µA and spectra were recorded at 2.91 scans per second with an m/z scanning range of 50–550 amu with a scan speed of 3125 (*N*=1). The total run time of the analysis was 24.43 min, the EI ion source was kept at 230°C and the MS QUAD at 150°C. Retention time correction was applied using a retention index, as performed in Begley et al., (2009).

Data pre-processing

Raw output folders obtained from Chemstation (Agilent MassHunter) were converted to mzXML format using MSConvert software (Adusumilli & Mallick, 2017), with peak picking enabled with the Vendor algorithm. These mzXML files were then deconvolved and aligned using the eRah package in R (Domingo-Almenara et al., 2016). A missing compound recovery step was taken to ensure a complete representation of all metabolites that were present in at least 10 samples. Two datasets were obtained this way, one containing only leachate samples, one containing hydroponic samples. These datasets each consisted of relative metabolite intensities with 395 and 320 unique metabolite features, respectively. To correct for drift, batch and GC-MS injection order for each dataset, QC correction was implemented according the procedure described in Dunn *et al.* (2011) using an inhouse script for MATLAB (Mathwork, MA; freely available at https://github.com/Biospec/cluster-toolbox-v2.0). Finally, to account for variation of root biomass between species, metabolomics data were standardised per mass of root dry weight root.

References

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