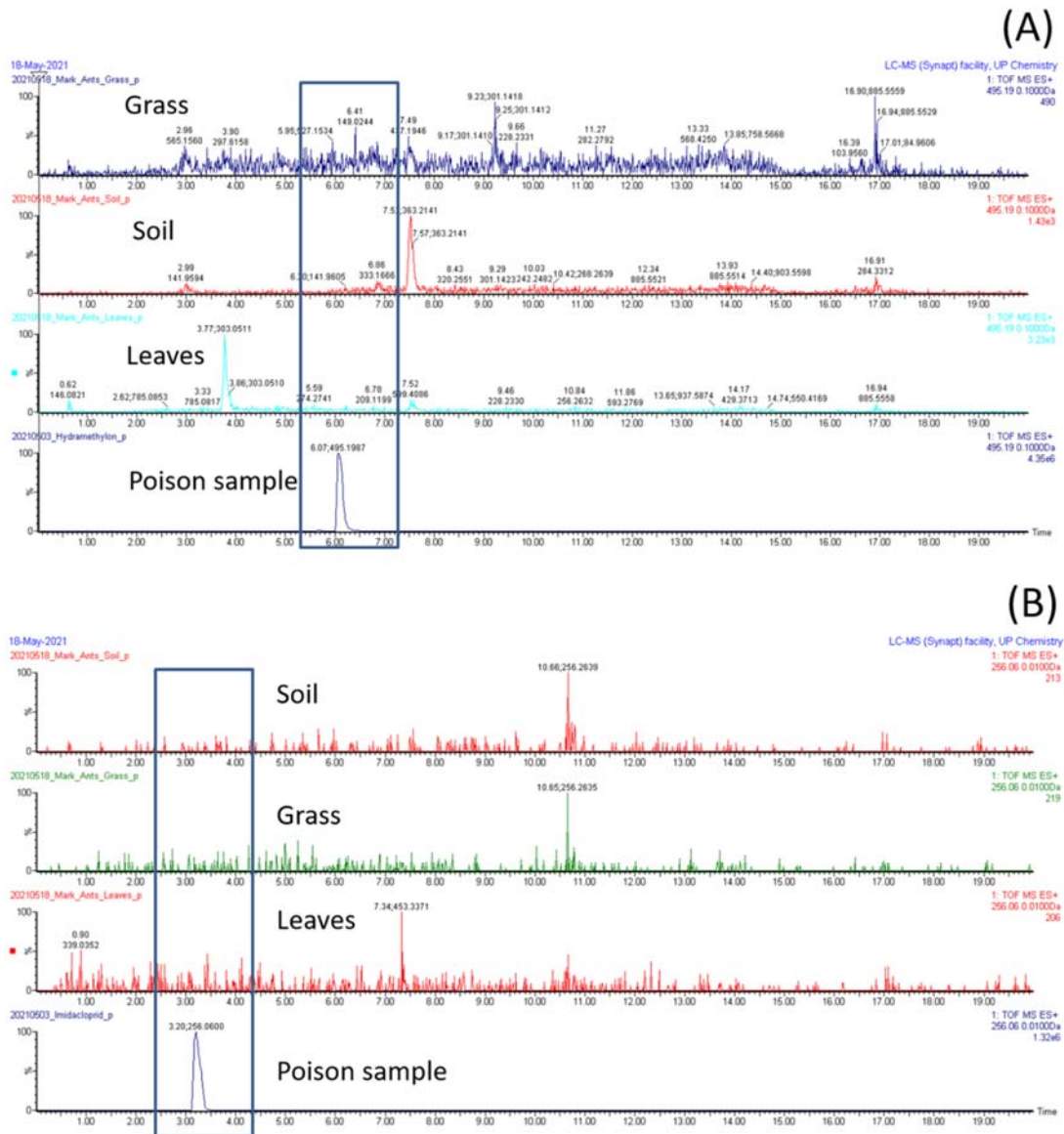


Functional compensation in a savanna scavenger community

S2. Poison residue analyses of soil, grass and tree leaves in ant suppression plots.



Poison residue analyses

Extraction method: Soil samples, finely grounded grass samples and finely grounded leave samples were all individually extracted using 20 ml Acetonitrile:MeOH (1:1) (ultra purity lc methanol/ acetonitrile (Romil-UpS™, Microsep, South Africa)) and sonicated for 15 minutes. The samples were filtered using 0.2µm Nylon filters (Agilent, Captiva) and placed in sample vials for analyses with UPLC.

Instrumentation: Compound separation and detection were performed using a Waters® Synapt G2 [high definition mass spectrometry](#) (HDMS) system (Waters Inc., Milford, Massachusetts, USA). The system comprises of a Waters Acquity Ultra Performance Liquid Chromatography (UPLC®) system hyphenated to a quadrupole-time-of-flight (QTOF) instrument. The system was operated with MassLynx™ (version 4.1) software (Waters Inc., Milford, Massachusetts, USA) for data acquisition and processing. An internal lock mass control standard, 2 ng/µL solution leucine enkephalin (m/z 555.2693), was directly infused into the source through a secondary orthogonal electrospray ionisation (ESI) probe allowing intermittent sampling. The internal control was used to compensate for instrumental drift, ensuring good mass accuracy, throughout the duration of the runs. The instrument was calibrated using sodium formate clusters and Intellistart functionality (mass range 112.936 – 1 132.688 Da). Resolution of 20 000 at m/z 200 (full width at half maximum (FWHM)) and mass error within 0.4 mDa were obtained.

LCMS method: The source conditions were as follows: the capillary voltage for ESI was 2.6 kV and 2.0 kV for positive and negative mode ionisation. The source temperature was set at 120 °C, the sampling cone voltage at 25 V, extraction cone voltage at 4.0 V and cone gas (nitrogen) flow at 10.0 L/Hr. The desolvation temperature was set at 300 °C with a gas (nitrogen) flow of 600.0 L/Hr. Mass spectral scans were collected every 0.3 seconds. The raw data was collected in the form of a continuous profile. Mass to charge ratios (m/z) between 50 and 1 200 Da were recorded.

Quantitative data-independent acquisition (DIA) was done using two simultaneous acquisition functions with low and high collision energy (MSE approach) with a QTOF instrument. The high energy MS scan can be time aligned with the low energy scan in order to predict which fragment ions belong to which precursor ions, consequently the full mass spectrum is acquired. Fragmentation patterns can thus be used for qualitative confirmation. Fragmentation was performed using high energy collision induced dissociation (CID). The fragmentation energy was set at 2 V and 3 V for the trap and collision

energy, respectively. The ramping was set from 3 to 4 V and 20 to 40 V for the trap and transfer collision energy, respectively.

Separation was completed using a reverse phase step gradient elution scheme from 97% H₂O (0.1% formic acid) to 100% acetonitrile (0.1% formic acid). Formic acid (99+% purity) (Thermo Scientific, South Africa) was added to the solution as buffer (pH correction), preservative and proton source for ionisation. The gradient started with an isocratic flow (hold 0.1 min) followed by a linear increase to 100% ACN; subsequently the column was washed for 1 min followed by conditioning and re-establishing of initial conditions to allow for equilibration before the start of the next run for the complete elution scheme. The column temperature was kept constant at 40 °C and the flow rate was set at 0.4 mL/min for the entire run giving a total run time of 20 min. Injection volumes were set at 5 µL. A Kinetex® 1.7 µm EVO C18 100 Å (2.1 mm ID x 100 mm length) column was used. The positive and negative ion mass spectra were collected in separate chromatographic runs (employing the same separation conditions).

All analyses were performed by Ms Madelien Wooding, University of Pretoria, Department of Chemistry.