Metabolite extraction:

Fifty mg of root tissue was excised from 10 day old seedlings of WT or *gat1_2.1*, grown using the treatment types specified in the study design, collected in 2 mL Eppendorf tubes and flash frozen in liquid N_2. Frozen tissue was homogenized using a tissue lyser and metabolites were isolated using 1 mL of methanol:water (4:1) with incubation in an ultra-sonication bath for 20 min followed by shaking for 30 min at 4 ˚C. The mixture was centrifuged at 12,000 × *g* for 10 min at 4 ˚C and 700 µl of the supernatant was transferred into fresh tubes and evaporated to dryness using a Vacufuge at ambient temperature. The residue was re-dissolved in 500 µl of 1:1 methanol:water and the samples were filtered using a 0.2 µm PTFE microfuge filter (Cytiva Whatman). Five µl of 1 µg/mL $^{13}C_6$ Phe was added to the samples for monitoring the quality of LC-MS runs.

MS data acquisition and analysis:

MS data were obtained from individual replicates of WT or *gat1_2.1* on a Q-Exactive Quadrupole Orbitrap mass spectrometer (Thermo Fisher Scientific) coupled to an Agilent 1290 high performance liquid chromatography (HPLC) system. Compounds were resolved using a SeQuant® ZIC®-HILIC column; 3.5µm, 100 Å, 100 × 2.1 mm (Sigma-Aldrich) with mobile phase 5 mM ammonium acetate, pH = 4.00 (A); 90% acetonitrile, 0.1% formic acid (B) and the following gradient: 87% B for 5 min, decreased to 55% over 8 min and held for 4 min before returning to 87% over 3 min. The following heated electrospray ionization (HESI) conditions were optimized for the analysis of amino and organic acids: spray voltage, 3.9 kV (ESI+), 3.5 kV (ESI-); capillary temperature, 250 °C; probe heater temperature, 450 °C; sheath gas, 30 arbitrary units; auxiliary gas, 8 arbitrary units; and S-Lens RF level, 60%. Injections of 5 µl were used with a flow rate of 0.3 mL min^{-1}. Compounds were detected and monitored using targeted MS/MS, spectra were collected at 17,500 resolution, AGC target 1e6, maximum IT 65 ms, isolation window of 1 m/z, normalized collision energy of 30, intensity threshold of 1.6e5 and 10s dynamic exclusion. Data analysis and calculation of all theoretical masses was carried out using Xcalibur™ software. Compounds were identified and quantified using commercial standards.