SUPPLEMENTRY FIGURES

Modelling metabolic fluxes of tomato stems reveals that nitrogen shapes central metabolism for defence against Botrytis cinerea

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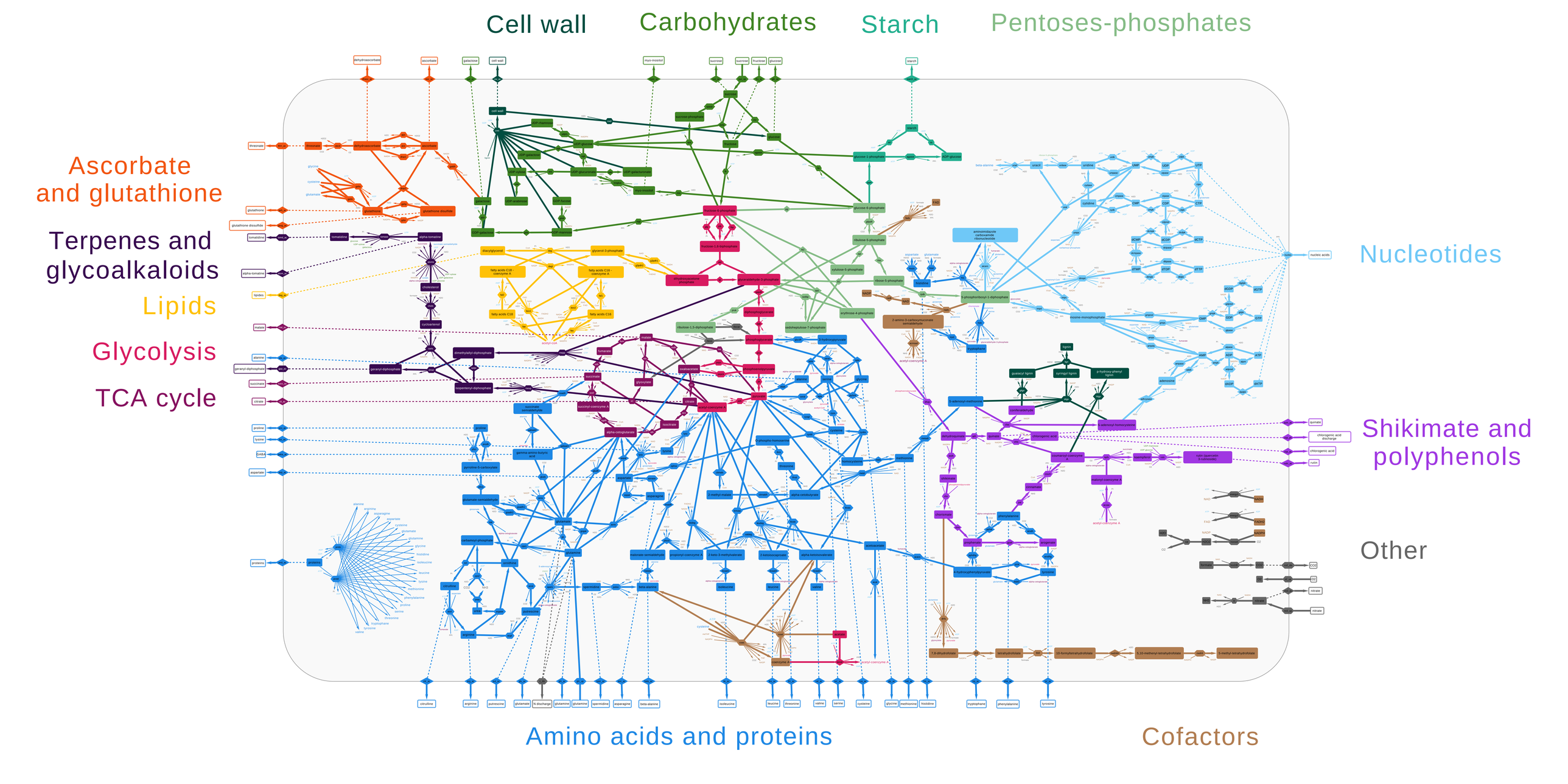
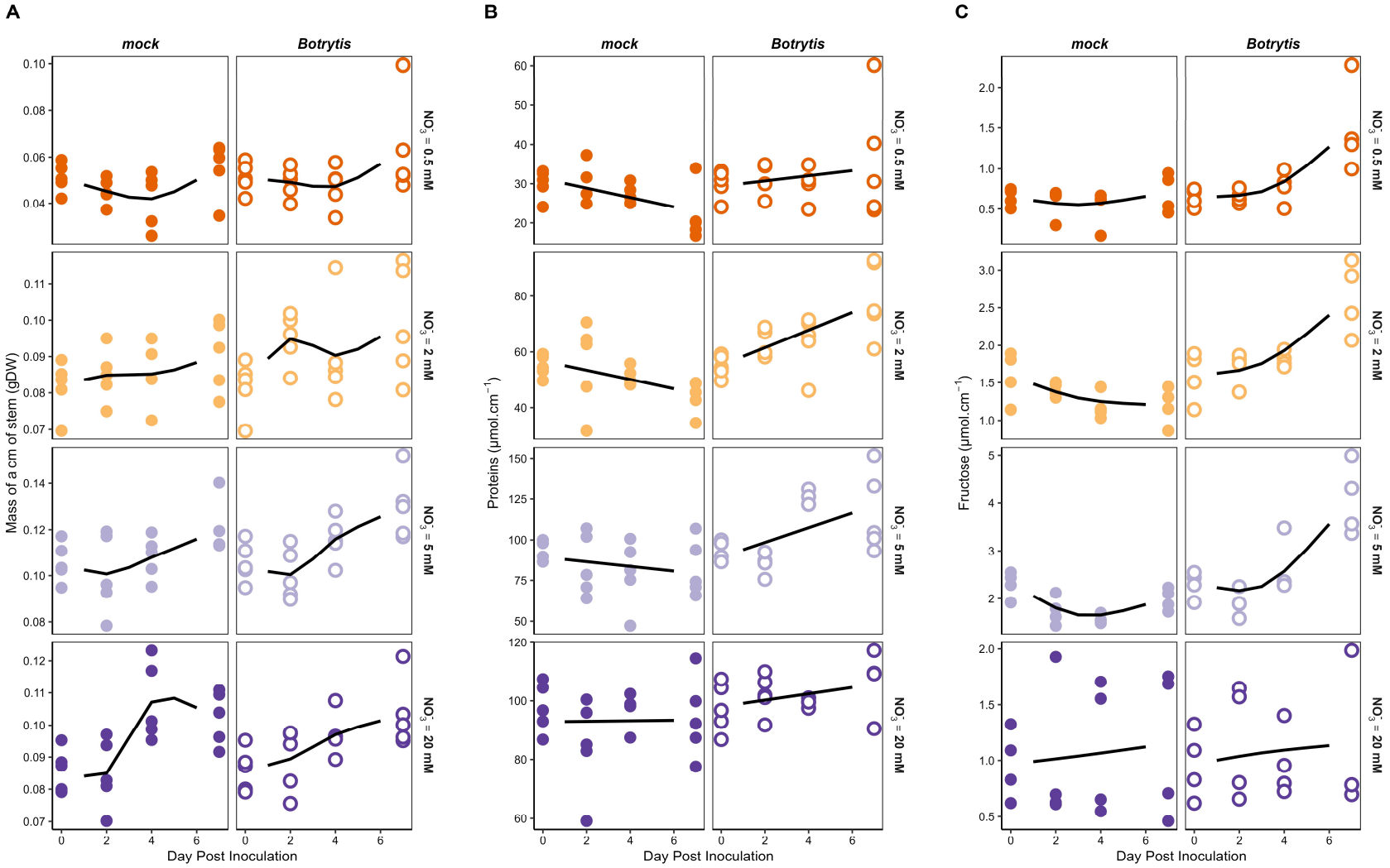
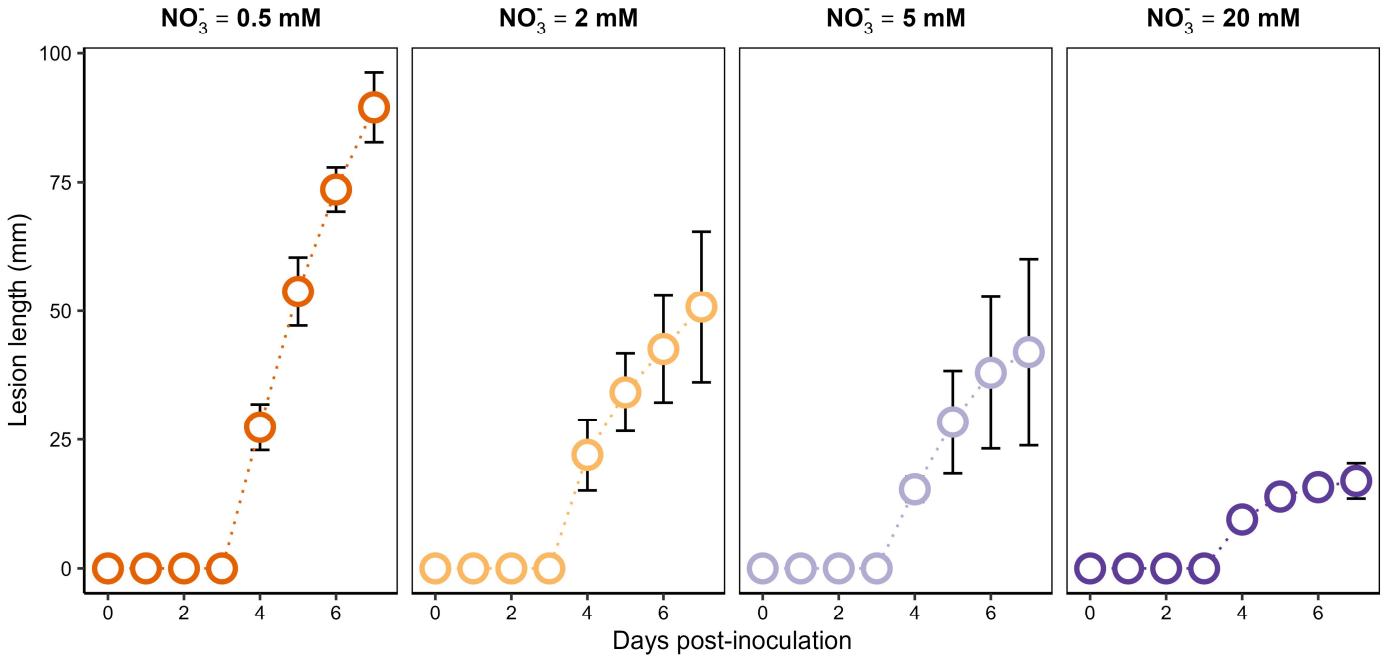
Supplementary Figure S1: Modelled stem metabolic network. The system is an average tomato stem cell of one cm length with biomass growth capabilities. Complete representation of the model with symbolic cell boundary; empty rectangles indicate external compounds and solid rectangle unbounded compounds. The side compounds of the reactions are noted in smaller size. Each colour indicates a pathway. Irreversible reactions are represented by hexagons and reversible reactions by diamonds. Arrows indicate the direction in which the reactions are written in the model.


Cell wall
Carbohydrates
Starch
Pentoses-phosphates
Ascorbate and glutathione
Terpenes and glycoalkaloids
Lipids
Glycolysis
TCA cycle
Nucleotides
Shikimate and polyphenols
Other
Amino acids and proteins
Cofactors

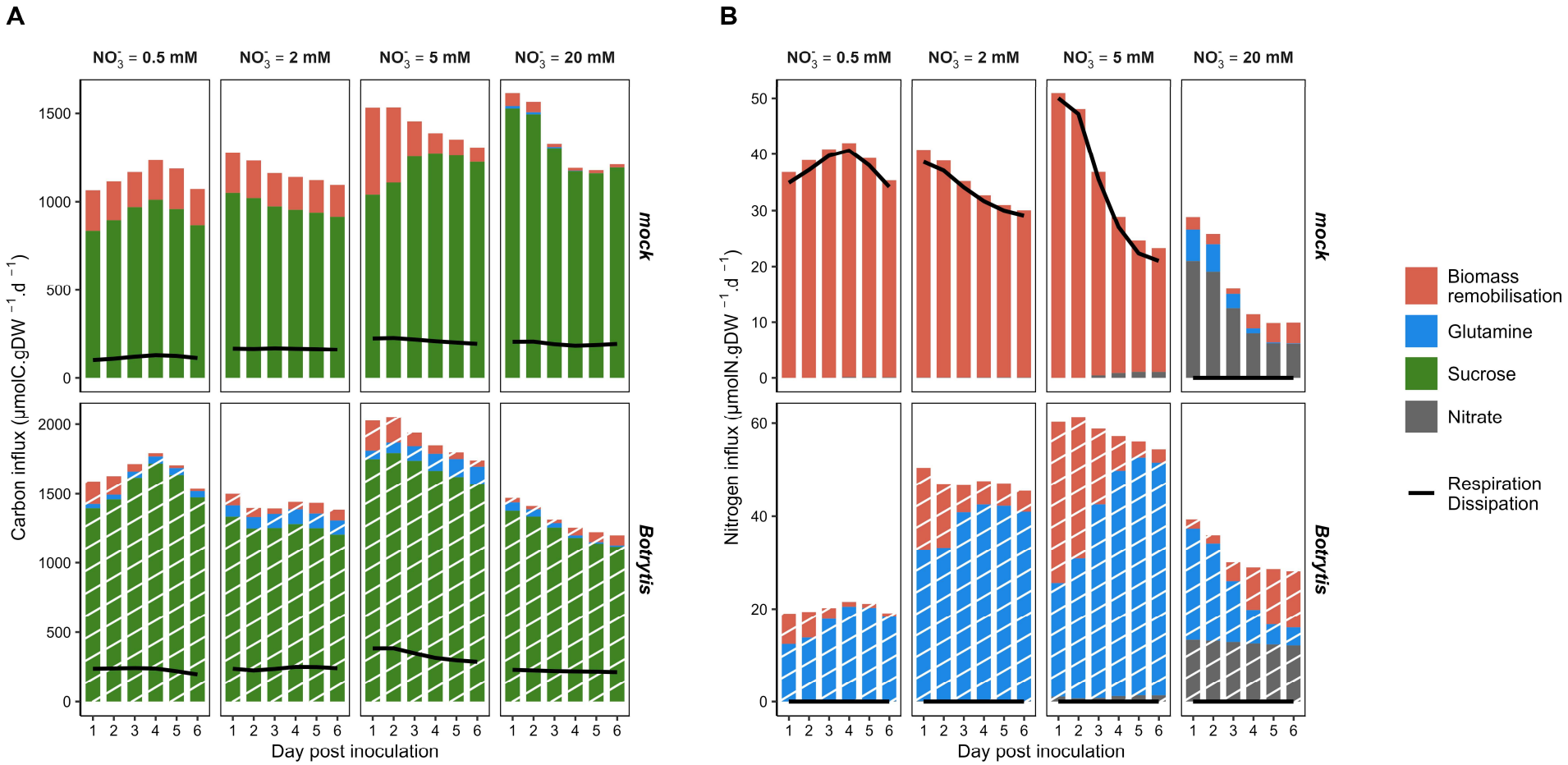
<u>Supplementary Figure S2:</u> Time course of stem mass and protein and fructose contents after inoculation. Data were measured in stems of tomato plants grown under four levels of N supply (0.5 mM NO_3^- in orange, 2 mM NO_3^- in yellow, 5 mM NO_3^- in light purple and 20 mM NO_3^- in dark purple) and inoculated with *mock* (closed circles) or *B. cinerea* (open circles). **A** Time course of the mass of one centimetre of stem in gDW (dry weight) with a regression line from spline fits (black lines). **B** Time course of the concentrations of total proteins in µmol.cm^{-1} (MW = 130 g.mol^{-1}) with a regression line from linear fits (black lines). **C** Time course of the concentrations of accumulated fructose in µmol.cm^{-1} with a regression line from spline fits (black lines).



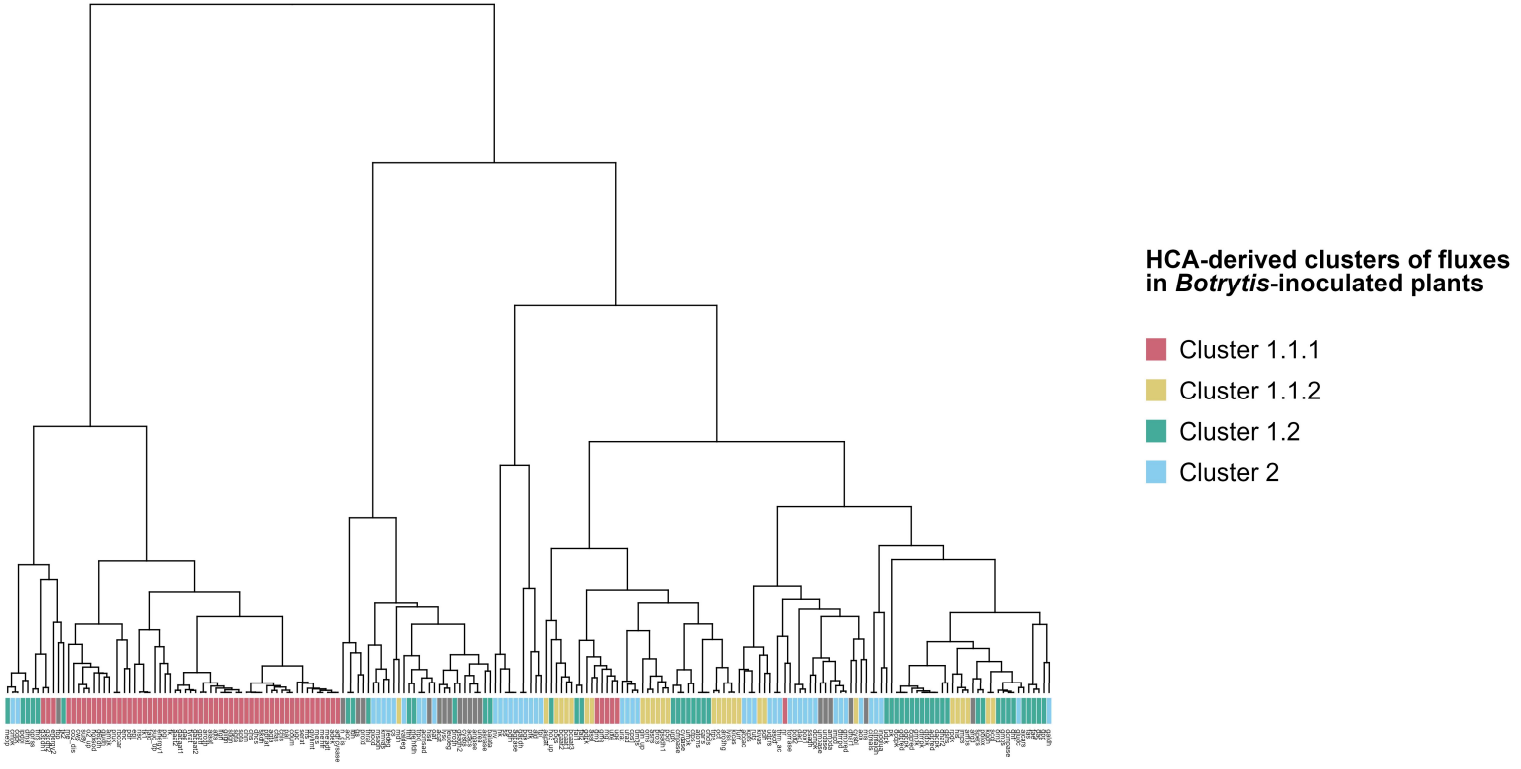
<u>Supplementary Figure S3:</u> Disease severity caused by *B. cinerea*. Lesion sizes were mesured on the stems of tomato plants grow upon four levels of N supply (0.5 mM NO_3^- in orange, 2 mM NO_3^- in yellow, 5 mM NO_3^- in light purple and 20 mM NO_3^- in dark purple). Each dot represents the mean of five biological replicates, and error bars represents the standard deviation.



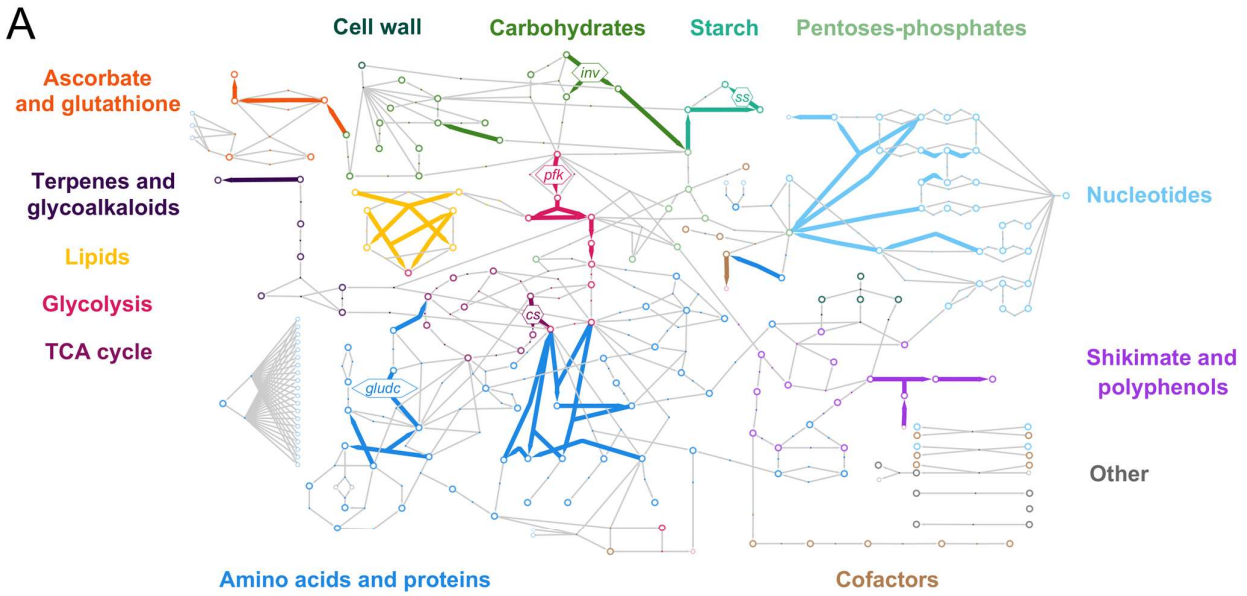
<u>Supplementary Figure S4:</u> Carbon and nitrogen influx. Carbon (**A**) and nitrogen (**B**) metabolised by stems of tomato plants grown upon four levels of N supply (0.5, 2, 5 and 20 mM NO_3^-) and inoculated with *mock* or *Botrytis cinerea*. Influxes are expressed as the sum of sucrose (*Vsuc_up*, green), glutamine (*Vgln_up*, blue) and nitrate (*Vno3_up*, grey) influxes, and biomass remobilisation fluxes (pink) which reflect a decrease over time in the pools of certain accumulated metabolites and biomass constituents, weighted by the number of carbon and nitrogen atoms in each molecule. The black lines represent the flux of carbon respired as CO_2 (*Vco2_dis*) or nitrogen released in the phloem as glutamate and glutamine (*Vn_dis*).

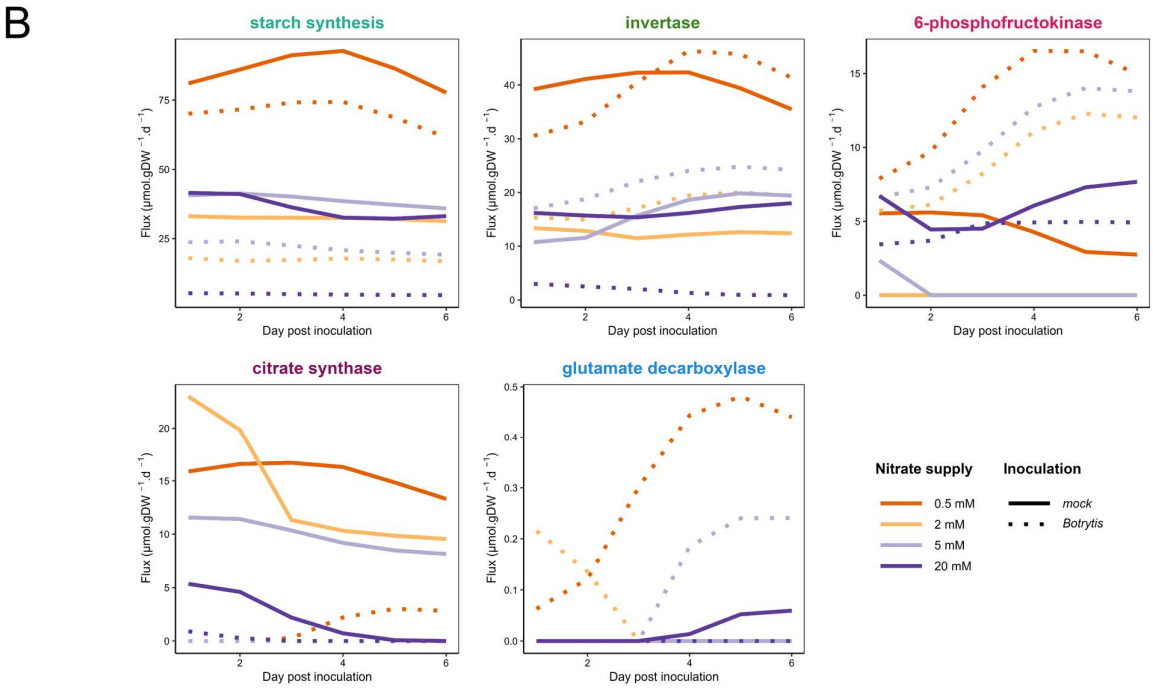


<u>Supplementary Figure S5:</u> Coordination within the metabolic network of *mock*-inoculated plants. Hierarchical clustering analysis (HCA) performed on unbounded fluxes greater than 0.001 µmol.gDW^{-1}.d^{-1} (in absolute value) in *mock*-inoculated plants. Data were cube-root-transformed and Pareto-normalised (mean-centred and divided by the root-square of standard deviation). HCA was performed using the Euclidean distance and the Ward algorithm. Each reaction was coloured according to HCA-derived clusters of fluxes of *Botrytis*-inoculated plants presented in Figure 5, to allow comparisons.



<u>Supplementary Figure S6:</u> Detail of flux cluster 2 in *Botrytis*-inoculated plants. A Simplified metabolic network highlighting reactions from HCA-derived Cluster 2 of unbounded fluxes greater than 0.001 µmol.gDW^{-1}.d^{-1} (in absolute value) of *Botrytis*-inoculated plants (see Figure 5). The open circles represent metabolites, the arrows highlight the reactions contained in the cluster, and the lines represent the other reactions of the network. Each color refers to a pathway. The reactions shown in hexagons (irreversible reactions) correspond to those whose fluxes are illustrated in panel B: *ss*, starch synthesis; *inv*, invertase; *pfk*, 6-phosphofructokinase; *cs*, citrate synthase; *gludc*, glutamate decarboxylase. The complete network with all the names of the metabolites and reactions is presented in Supplementary Figure S1. **B** Time course of the five most representative fluxes of the cluster. Fluxes of symptomless stems of tomato plants grown under four levels of N supply (0.5 mM NO_3^- in orange, 2 mM NO_3^- in yellow, 5 mM NO_3^- in light purple and 20 mM NO_3^- in dark purple) and inoculated with *mock* (solid lines) or *B. cinerea* (dashed lines) were expressed in µmol.gDW^{-1}.d^{-1}.





<u>Supplementary Figure S7:</u> Detail of flux cluster 1.2 in *Botrytis*-inoculated plants. A Simplified metabolic network highlighting reactions from HCA-derived Cluster 1.2 of unbounded fluxes greater than 0.001 µmol.gDW^{-1}.d^{-1} (in absolute value) of *Botrytis*-inoculated plants (see Figure 5). The open circles represent metabolites, the arrows highlight the reactions contained in the cluster, and the lines represent the other reactions of the network. Each color refers to a pathway. The reactions shown, in hexagons for irreversible reactions and in diamonds for reversible reactions, correspond to those whose fluxes are illustrated in panel B: *idh*, isocitrate dehydrogenase; *pk*, pyruvate kinase; *gshs*, glutathione synthesis; *mepp*, MAP/DOXP pathway; *gmps*, GMP synthase. The complete network with all the names of the metabolites and reactions is presented in Supplementary Figure S1. **B** Time course of the five most representative fluxes of the cluster. Fluxes of symptomless stems of tomato plants grown under four levels of N supply (0.5 mM NO_3^- in orange, 2 mM NO_3^- in yellow, 5 mM NO_3^- in light purple and 20 mM NO_3^- in dark purple) and inoculated with *mock* (solid lines) or *B. cinerea* (dashed lines) were expressed in µmol.gDW^{-1}.d^{-1}.