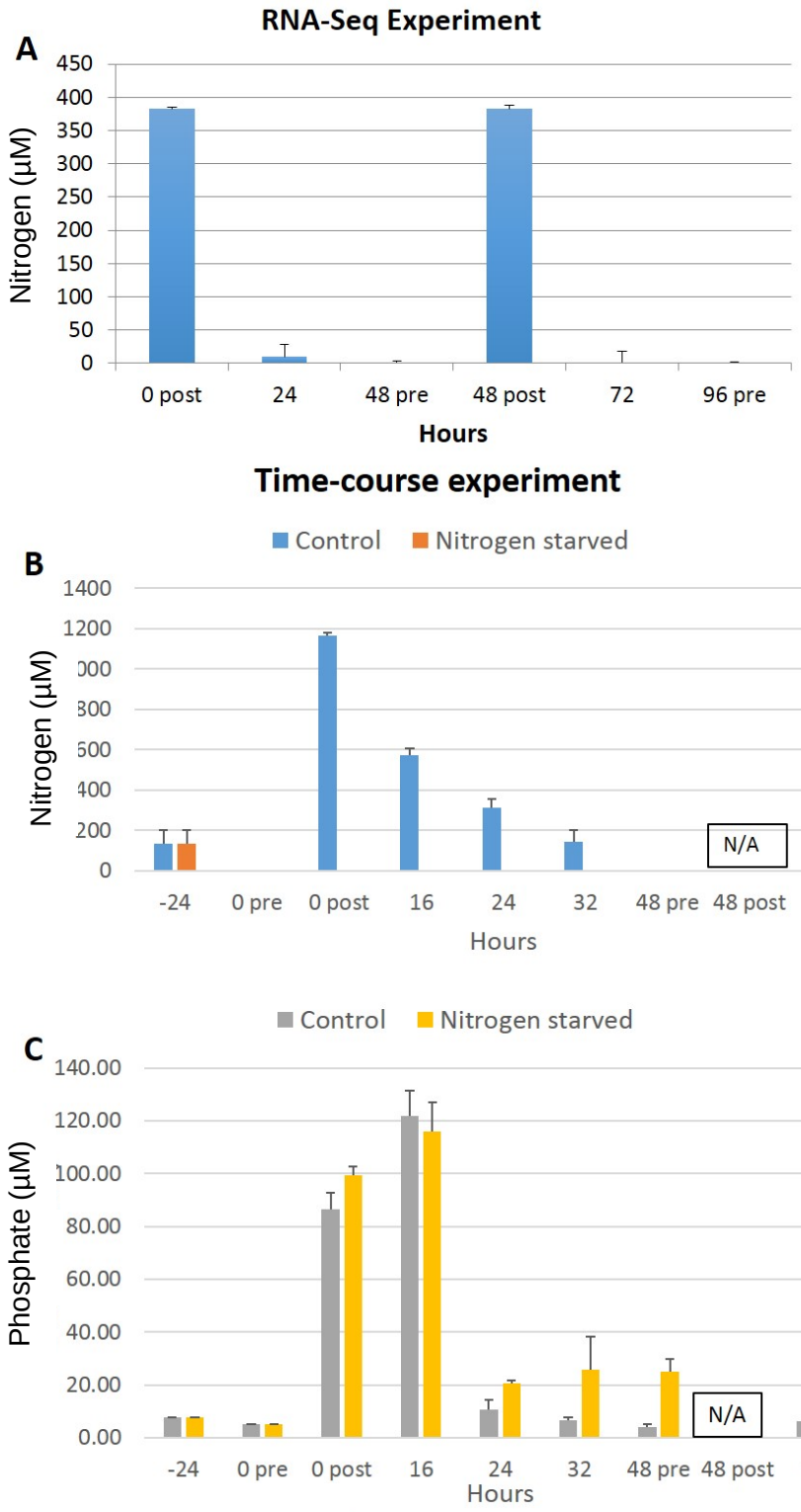
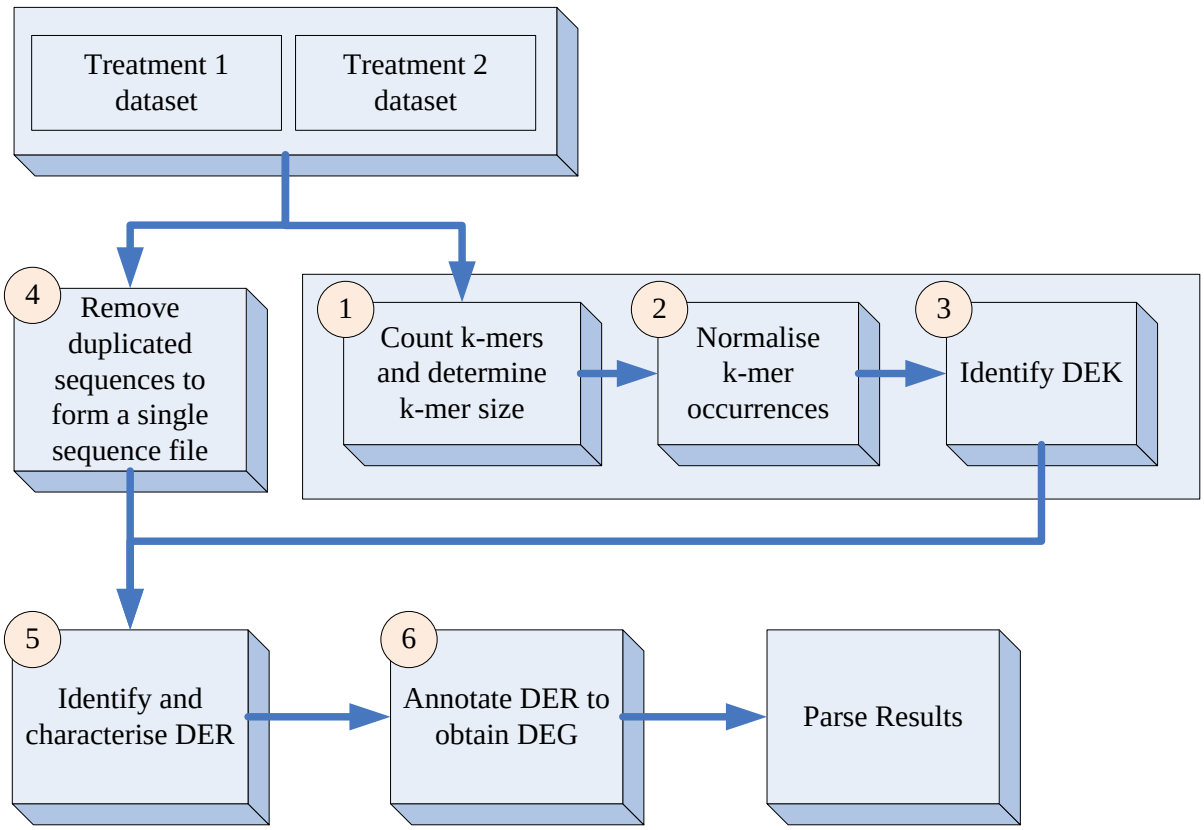
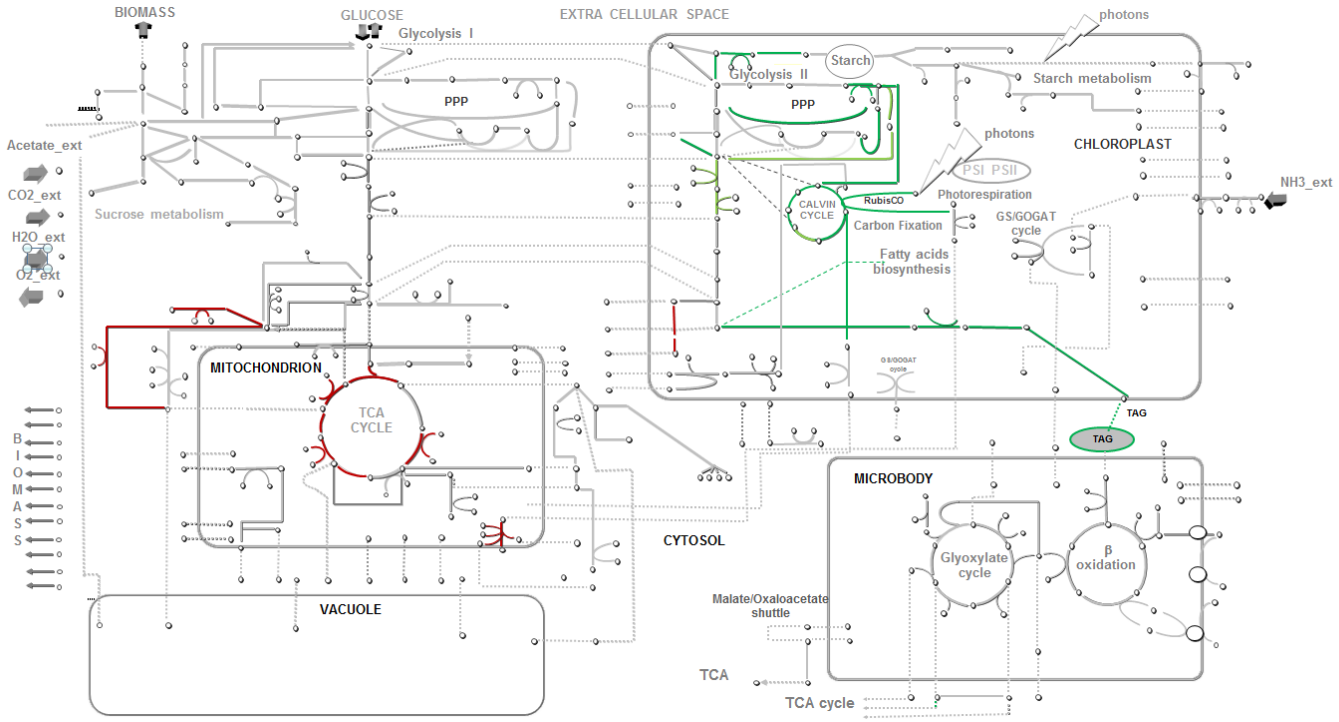


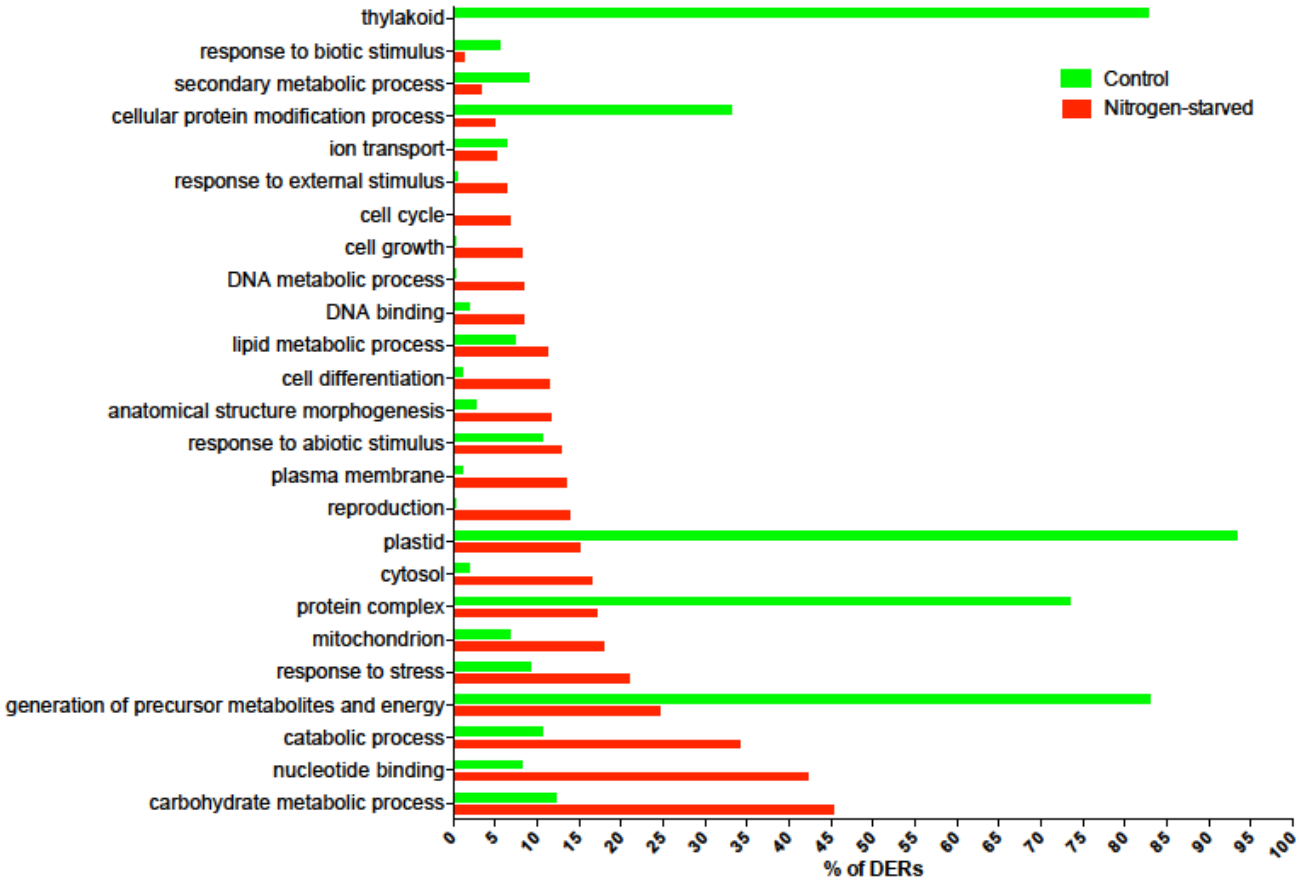
Supplementary Figure 1. Nitrogen (A, B) and phosphate (C) contents of growth medium during *Tetraselmis* sp. M8 cultivation and harvesting for RNA-Seq (A) and qRT-PCR (B, C) experiments. Shown are mean values ± SDs of three separately grown cultures for each treatment. N/A not applicable.



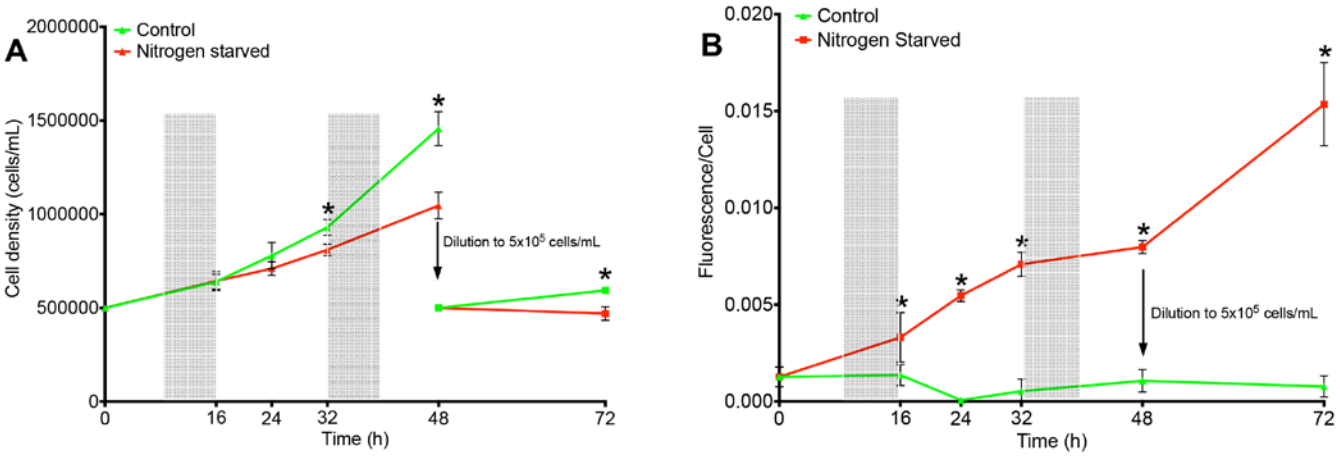
Supplementary Figure 2. DiffKAP dataflow diagram.



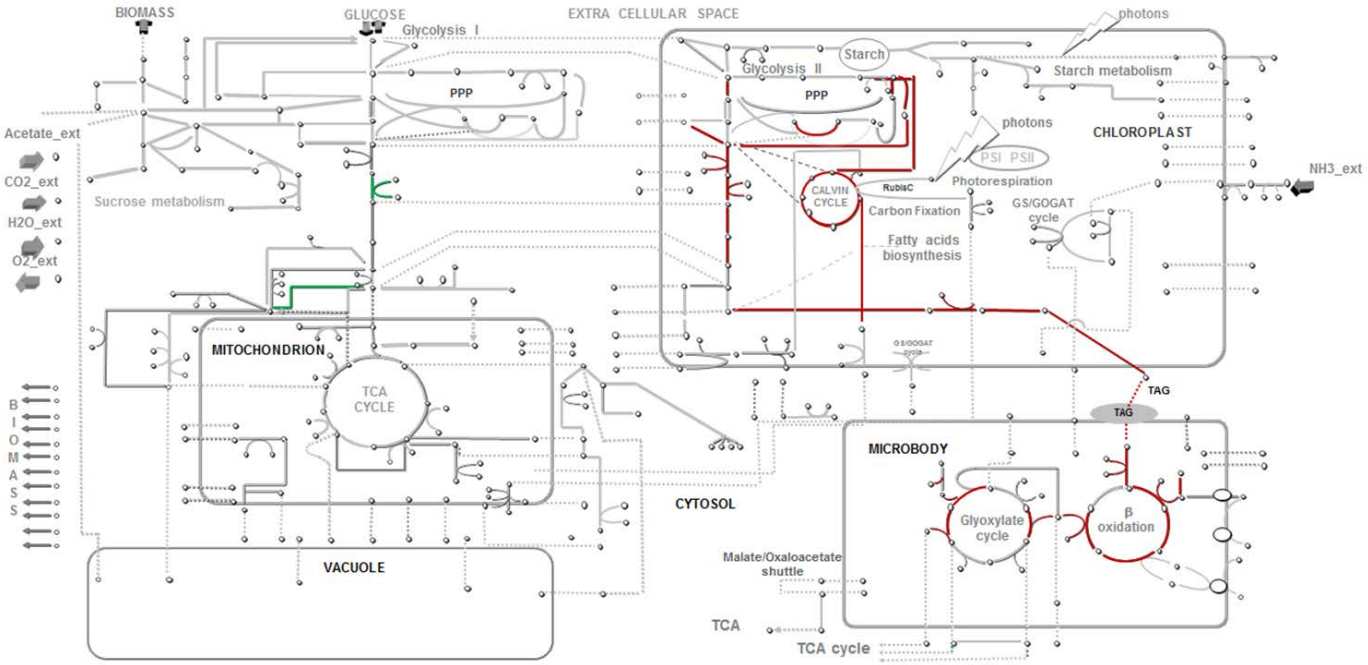
Supplementary Figure 3. Flux map of the central carbon metabolism for *Tetraselmis* sp. M8 under nitrogen limiting conditions at 72 h. Green reactions have a significantly increased flux (>1.5x control), red reactions have significantly reduced flux (<1.5x control) and grey reactions have no significant change under the lipid accumulation conditions.



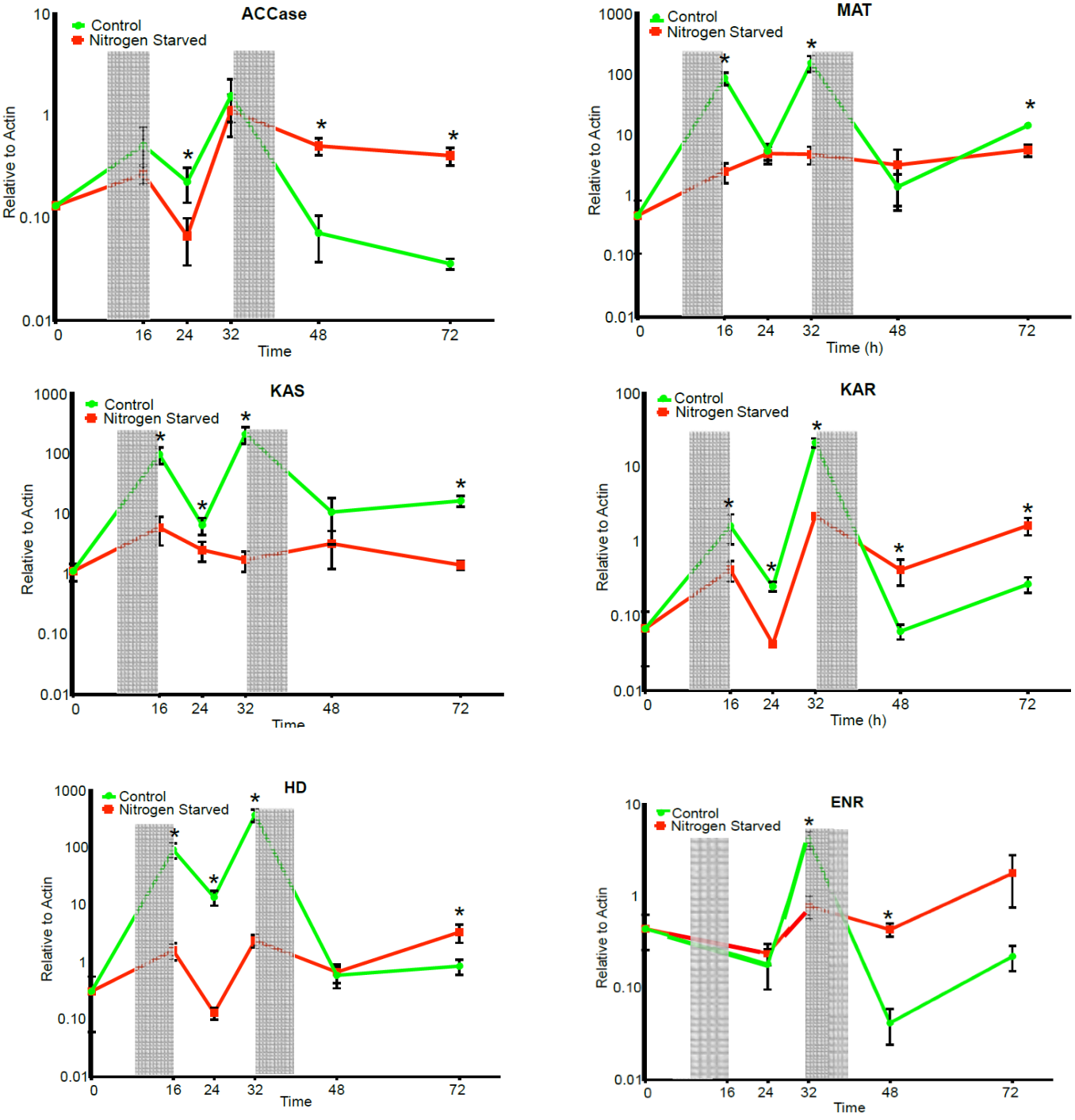
Supplementary Figure 4. Distribution of Gene ontology (GO) terms assigned to annotated differentially expressed reads (DERs) in Control and Nitrogen-starved treatments. Data are shown as a percent of total annotated DERs from each treatment. Only GO terms containing more than 5% of DERs are shown.



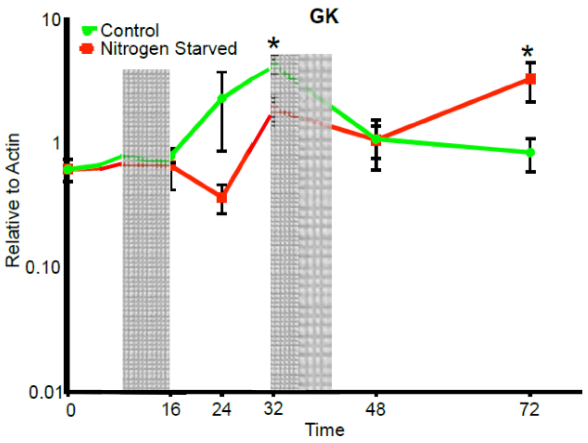
Supplementary Figure 5. Growth and lipid accumulation of *Tetraselmis* sp. M8 in a time-course experiment using control and nitrogen-starved cultures. Grey shaded areas indicate time in dark cycle. A. Growth curve of *Tetraselmis* sp. M8 cultures with significant differences (*) in cell density after 32 h (Student's T-test; $P<0.05$). B. Characterization of lipid accumulation via Nile red fluorescence per cell number with nitrogen-starved cultures displaying significantly higher Nile red fluorescence from 16 h onwards (Student's T-test; $P<0.05$).

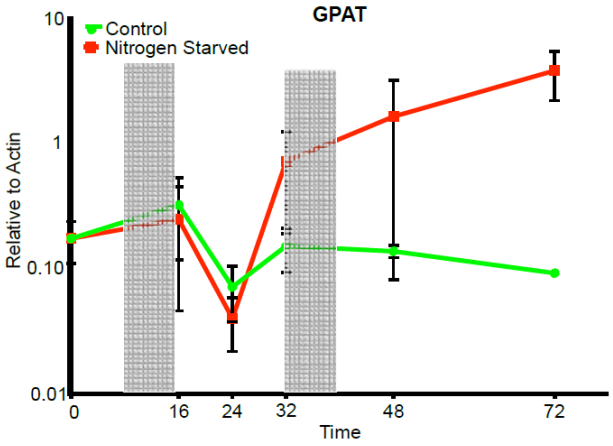


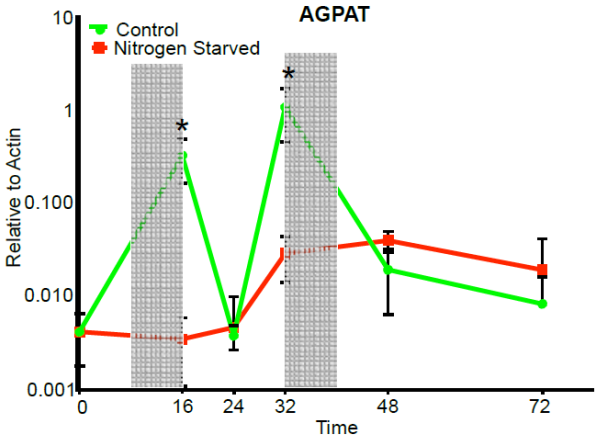
Supplementary Figure 6. Flux map of the central carbon metabolism under the lipid accumulation for *Tetraselmis* sp. M8 under nitrogen limiting conditions at 24 h. Both lipid accumulation and TAG lipase were constrained based on experimental data and assuming regulatory effect at transcriptional level on metabolic fluxes. Green reactions have a significantly increased flux (>1.5x control), red reactions have significantly reduced flux (<1.5x control) and grey reactions have no significant change.

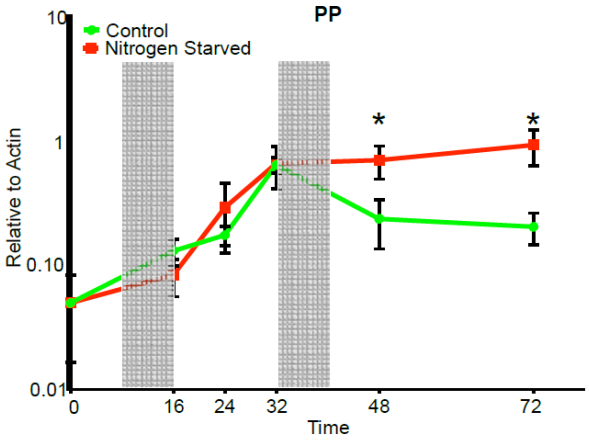


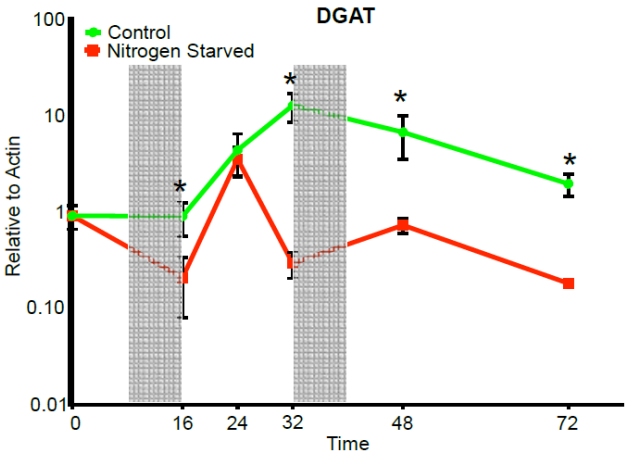
Supplementary Figure 7. qRT-PCR data of fatty acid pathway genes.



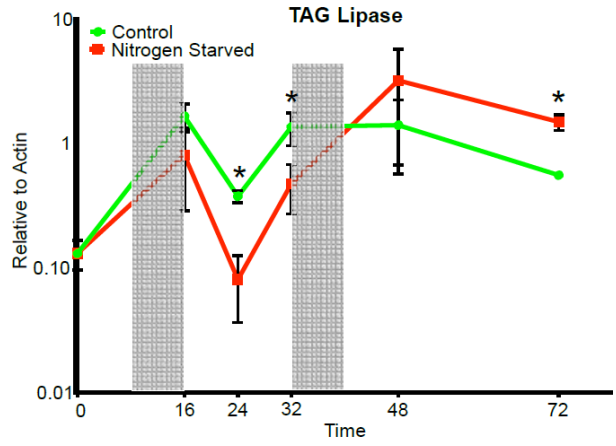


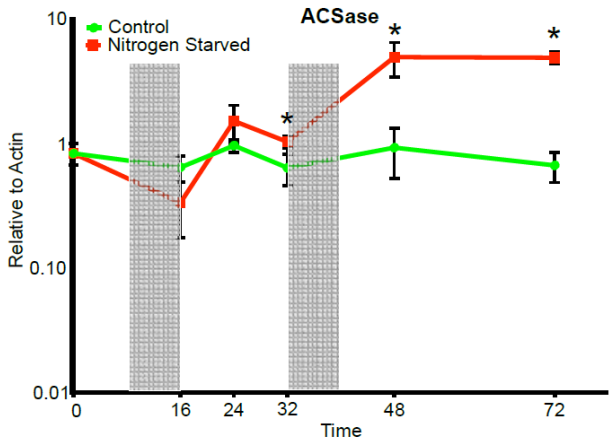


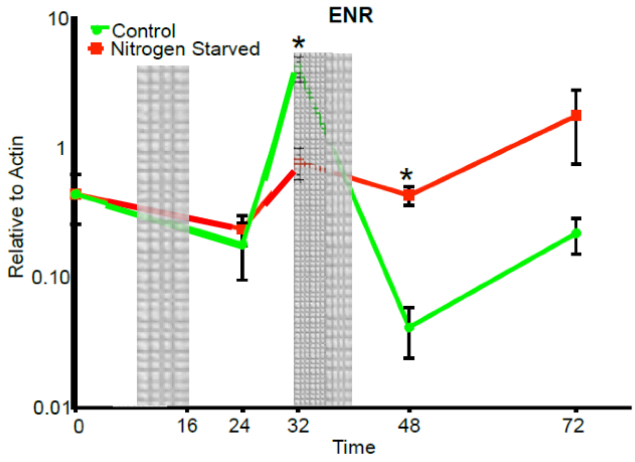


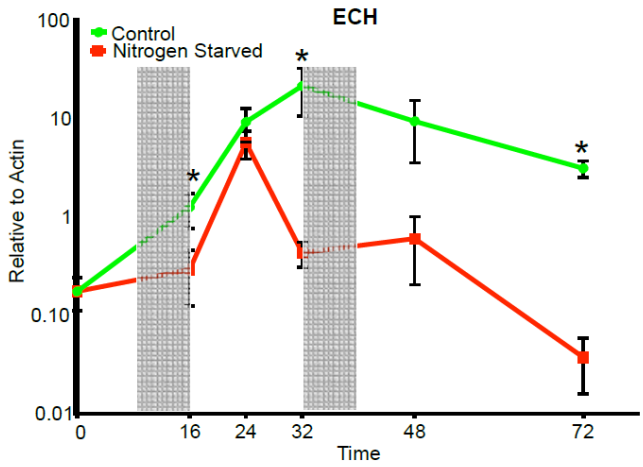


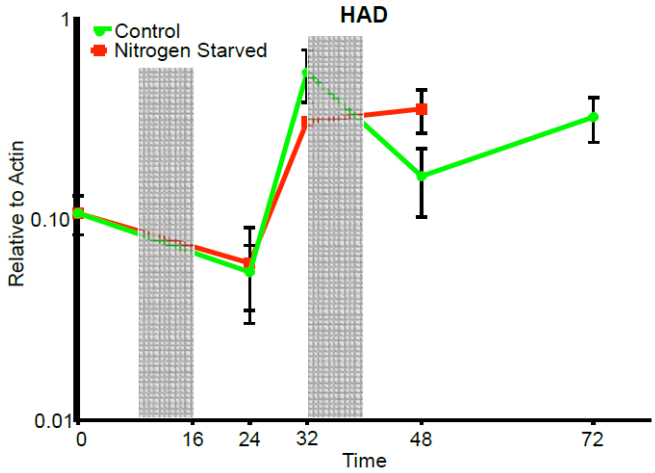
Supplementary Figure 8. qRT-PCR data of TAG pathway genes.











Supplementary Figure 9. qRT-PCR data of lipid catabolism pathway genes.