P. celeri strains TG1, TG2, and TG1-MYB99 were adapted to HL intensity (1000 µmol m⁻² s⁻¹ PAR) by ensuring chlorophyll concentration was maintained below 0.75 µg/mL for several days prior to any transient labeling experiment. A 2 L volume was inoculated on the day prior to the transient labeling experiment in the SAGE photobioreactor and total organic carbon (TOC) measurements were taken 2 hours before and at the start of the experiment to determine the specific growth rate. OD₇₅₀ of the cultures were always maintained <0.3 for the transient labeling experiment. The culture was then split equally into 6 photobioreactor positions, each of which served as a time point for the transient labeling experiment. Transient labeling was achieved by turning off the pH control (CO₂ supply) and by the addition of NaH¹³CO₃ (Cambridge Isotope Laboratories, Andover, MA) up to a final concentration of 11.76 mM simultaneously with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (Sigma Aldrich, St. Louis) up to a final concentration of 5 mM. NaH¹³CO₃ was preferred to ¹³CO₂ to avoid gas-liquid mass transfer limitations and achieve rapid equilibrium. Preliminary experiments were performed to determine the concentration of NaH¹³CO₃ and HEPES buffer that need to be added simultaneously to achieve a step change from ¹²C to ¹³C, maintain pH <7.5, as well as be able to minimize contribution of unlabeled carbon in the labeling dynamics.

Once the ¹³C pulse was introduced, each identical culture was harvested at different time points, namely 0 (no label pulse), 30, 60, 180, 300, and 600s by rapidly filtering on a 9 cm Fisherbrand glass microfiber filters followed by washing with 15 mL of 0.2 M ammonium bicarbonate in 5% methanol kept in an ice bath and quenching in liquid nitrogen. These time points were chosen to capture the complete labeling dynamics of the rapidly labeling CBB cycle metabolites. It was consistently observed that quenching was achieved not more than 10s after the desired time point. Filters were stored in -8°0 C freezer prior to extraction. Experiments were performed in biological triplicates to determine standard deviation in labeling dynamics.