

Supplementary Data File 1

Instructions for preparing leaf images for spatial mapping of carbon isotope patterns

STEP #1: For each leaf, create three text files containing x-y information about the leaf outline, scale bar, and sampling locations.

A. Exporting x-y coordinates of leaf outline in ImageJ

- Image→type→8-bit
- clip petiole off: use brush tool at high magnification with black ink
- repair any damage: use brush tool at high magnification with white ink
- Image→transform→flip vertically (this is necessary because R flips the image)
- Image→adjust→threshold: move the two sliders until the leaf outline is correctly rendered
- Click on Wand tool and select leaf so that outline is selected
- File→save as→xy coordinates
- Next, open the text file and paste the first row into a new last row. This is necessary because the shape needs to be closed off.

B. Exporting x-y coordinates of scale bar

- Optional: in Photoshop (or equivalent), use rectangle tool to reproduce 1 cm scale and move to an appropriate place on the image
- In ImageJ: Image→type→8-bit
- Image→transform→flip vertically (this is necessary because R flips the image)
- Click on rectangle tool and draw rectangle directly on top of scale bar
- File→save as→xy coordinates
- Next, open the text file and paste the first row into a new last row. This is necessary because the shape needs to be closed off.

C. Exporting x-y coordinates of isotope samples in ImageJ

- Image→type→8-bit
- Image→transform→flip vertically (this is necessary because R flips the image)
- Click on multi-point tool button and click on the sampling spots **in the correct order** (and remember the image is flipped); that is, the ordering of these coordinates should match the ordering of your carbon isotope inputs in "Intra-leaf d13C variation.csv"
- File→save as→xy coordinates

STEP #2: Create spatial maps of isotope data in R.

For instructions, see the first few lines of the R file "Leaf carbon isotope maps".