Hazards of Organic Carbon: Sample Pretreatment Effects on Analysis by EA-IRMS

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Desired Measurement(s)

- Total organic carbon (**TOC**)
- Total nitrogen (**TN**)
- Stable Isotope Composition of Organic Carbon ($\boldsymbol{\delta^{13}C_{org}}$)
- Stable Isotope Composition of Nitrogen ($\boldsymbol{\delta^{15}N}$)

All at once!

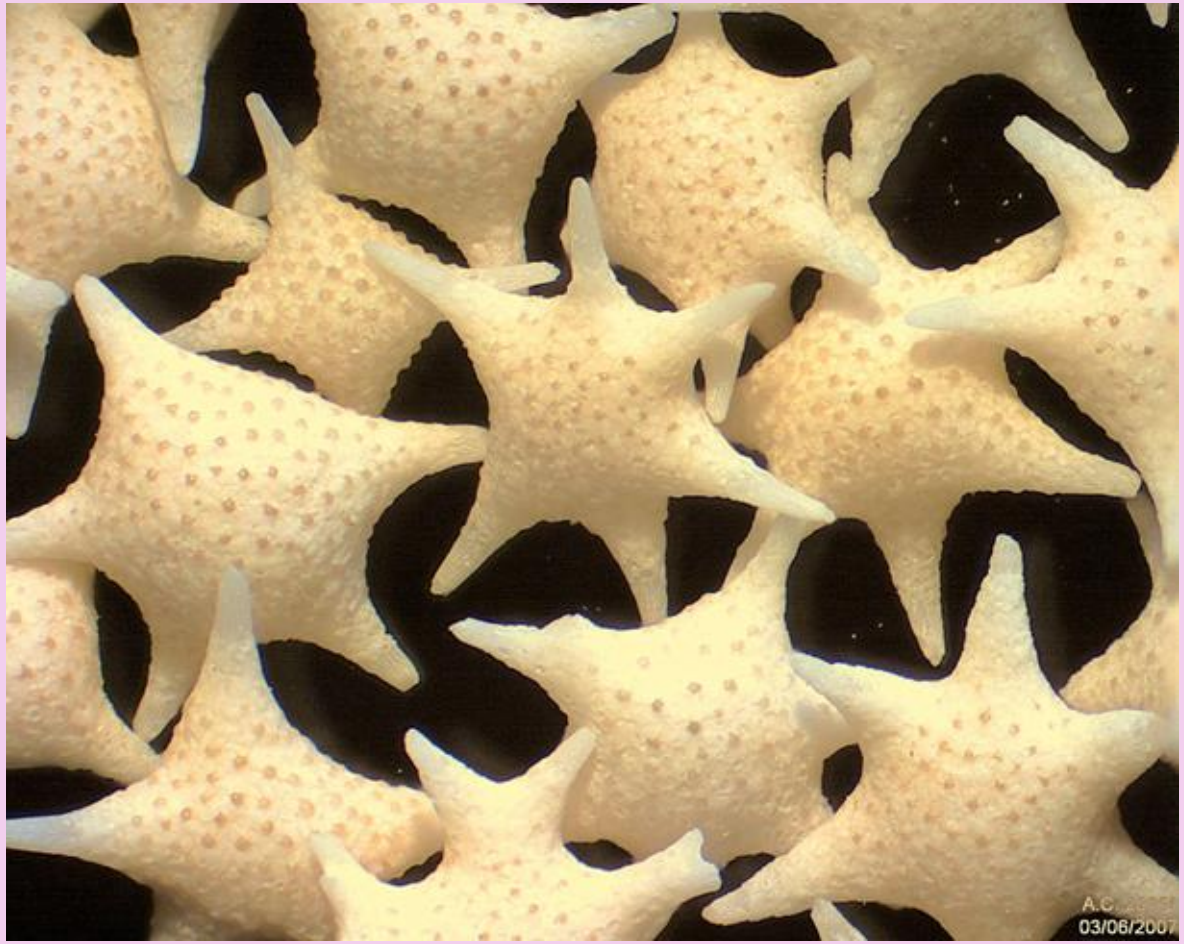
Thermofisher, 2019

Total *Organic* Carbon

- … but also inorganic carbon (**TIC**)!
- Strictly speaking, the EA measures total carbon (**TC**)
- Consider:

$$TC = TIC + TOC$$

- How to account for or remove TIC?

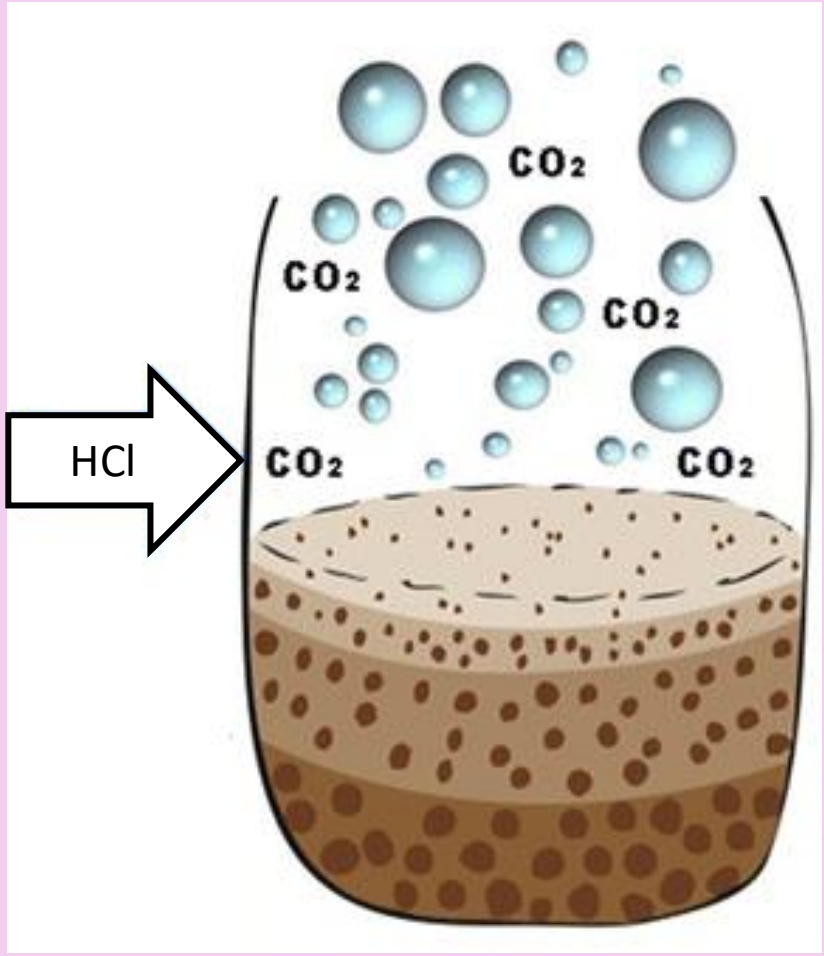


Wikimedia, 2019

Carbonate Removal

- Two popular methods:
  - Direct Acidification
  - Vapor-Phase Acidification

- Problems?
  - Direct: Sample loss due to soluble C and N, suspended material, **alteration of N**
  - Vapor: Accumulation of salts, **alteration of N**



Vinci et al., 2019

All at once? ☹

- Any acid treatment appears to alter TN and $\delta^{15}N$
- Direct acid treatment may also alter TOC
- High C:N values alone can be problematic for simultaneous C & N determination
- Acid fumigated samples accelerate reactor aging and possibly impact performance

Analytical Approach

- 4 sample sources: freshwater wetland, carbonate lake, coastal seagrass, & acidic tundra soils
- 4 samples from each setting with varying TIC (0 - 10%), TOC, and C:N were measured 3x for:
    - TIC via acid digestion & coulometry
    - EA-IRMS (TC, TN, $\delta^{13}C$, $\delta^{15}N$) using the following pretreatments:
        - Untreated
            - Separate runs for C-optimized and N-optimized weights
        - Direct Acidification
        - Vapor Phase Acidification for 8, 16, and 24 hours

Method Details: Direct Acidification

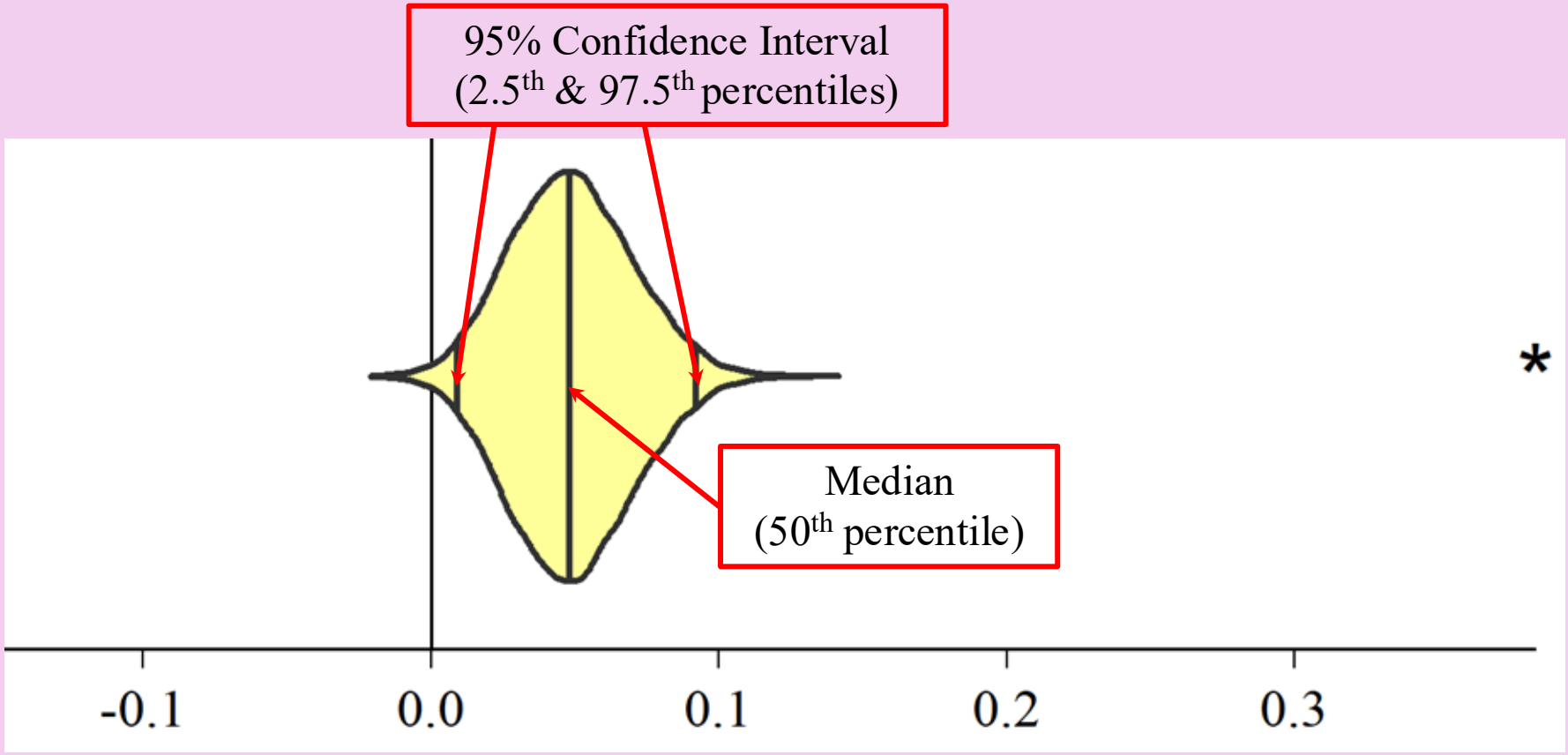
- A targeted amount of dry, homogenized sample was added to a plastic centrifuge tube
- Acidified with 2N HCl following by 3 x rinses of DI water prior to oven drying at 50 °C
- Mass of original vs acidified samples ‘enables’ a TIC estimate

Method Details: Acid Fumigation

- Target weight of each sample based on previous analysis added to silver capsules in a 96-well tray
- Samples were pre-wetted with 10 μL of DI water
- Tray placed in a glass desiccator along with an open 60 mL beaker of 6N HCl and allowed to react
- After, trays dried in oven at 50 °C and the silver capsules placed in tin capsules and wrapped for analysis

Statistical Tests

- ‘Violin plots’ show balanced bootstrap distributions of mean, paired differences, with significance identified by an asterisk based on the 95% confidence interval.

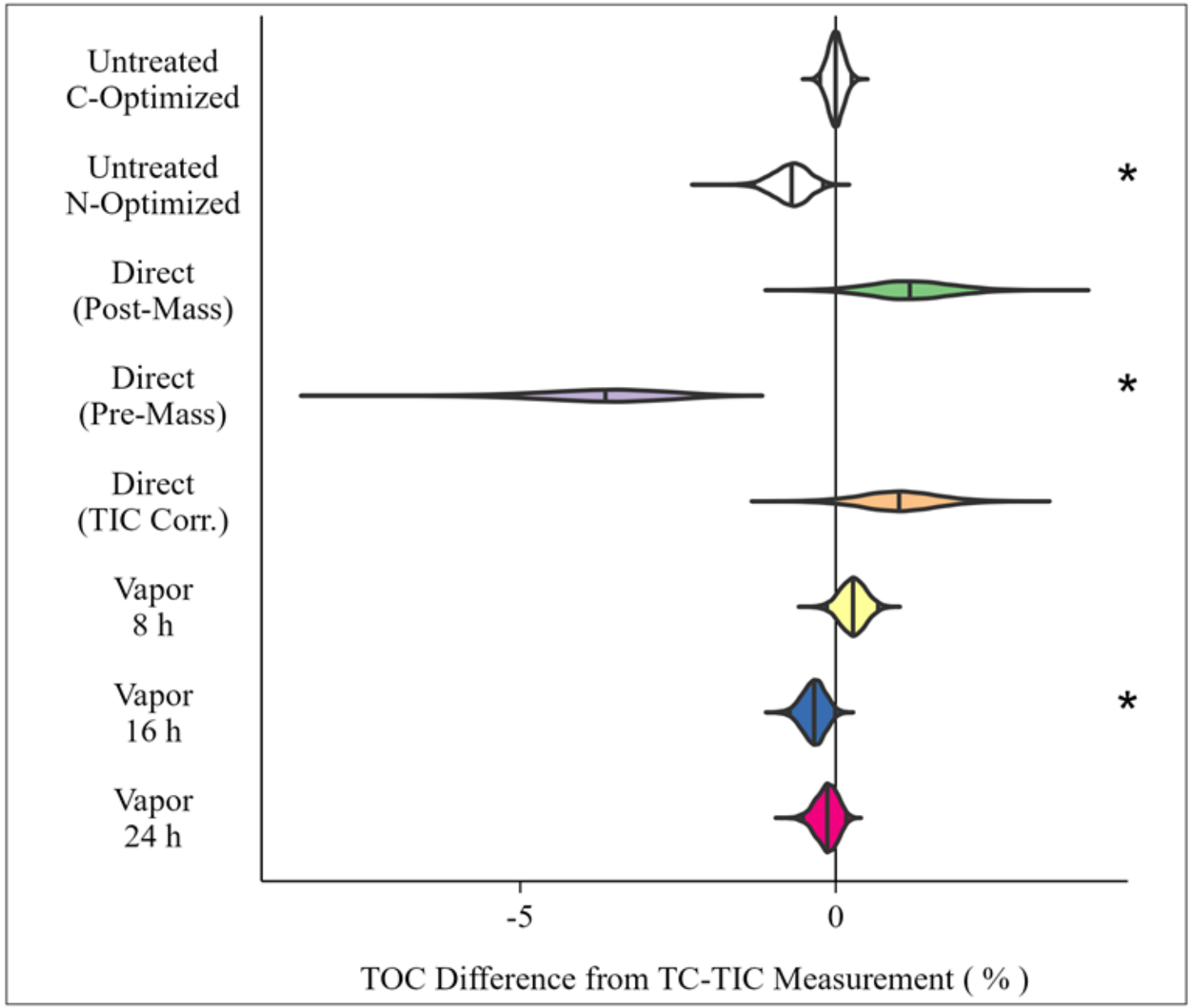


Research Questions

- Can any acid treatment yield comparable TOC to the TC-TIC two-step analysis?
- Does optimizing sample weights for C or N affect results?
- Can coulometric TIC be estimated by acid treatments?
- Do acid fumigated samples degrade instrument performance?

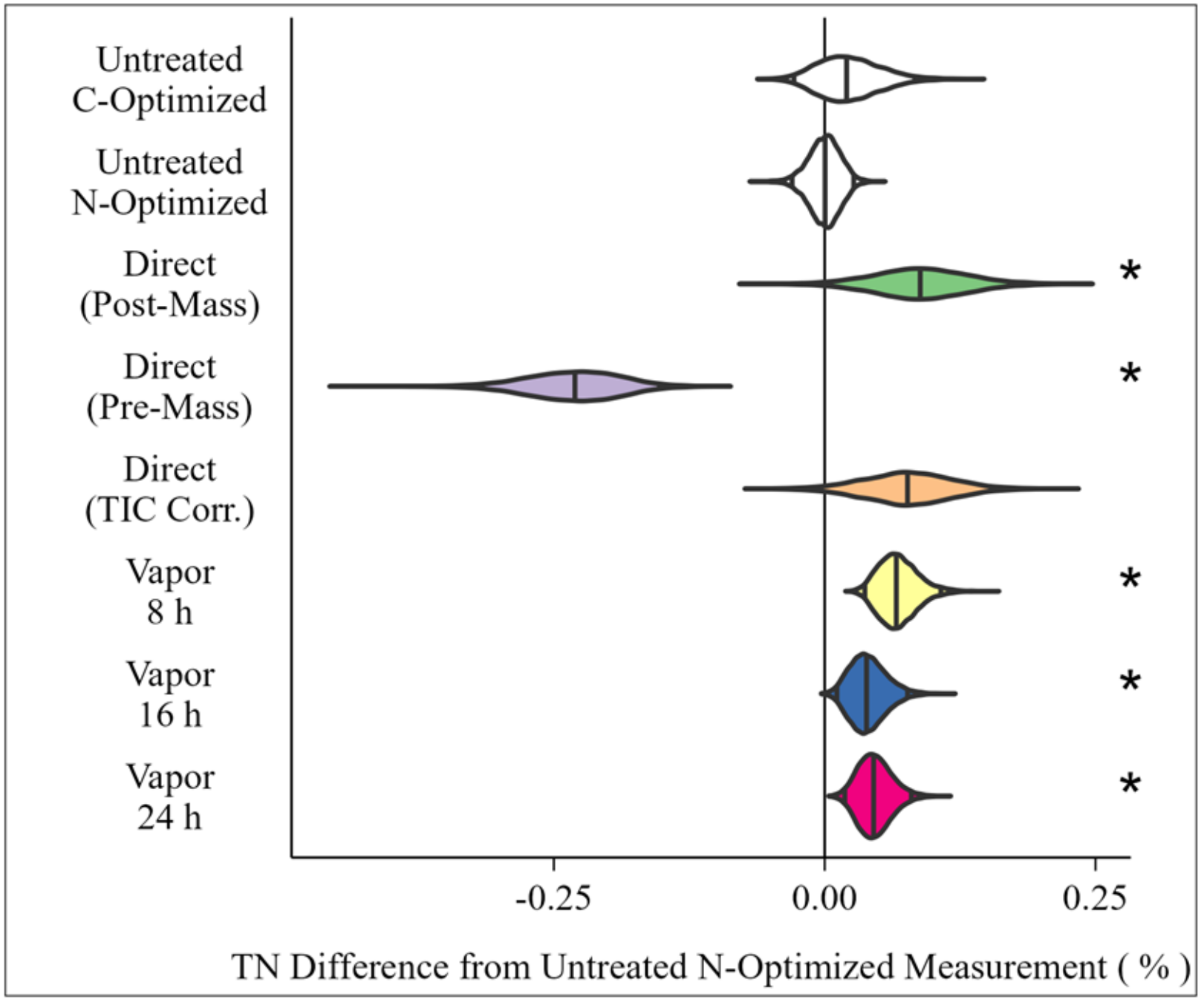
TOC

- Difference in expected yield from the ‘best’ method
- Direct methods…
  - (Post-Mass) final sample weight
  - (Pre-Mass) pre-sample weight
  - (TIC Corr.) final weight divided by (1 – TIC)



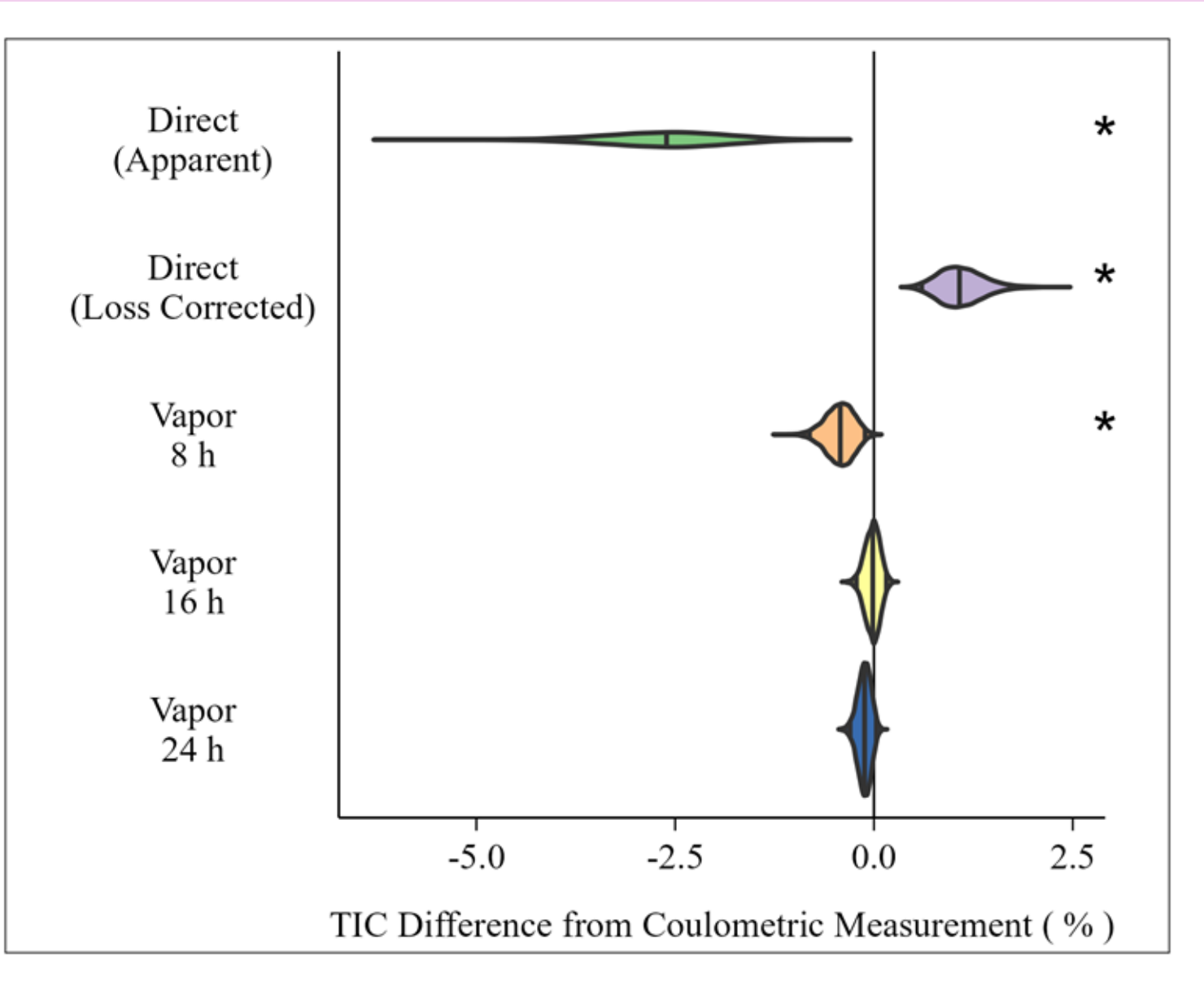
TN

- ‘Best’ method is now N-optimized
- Target N mass of 50 µg

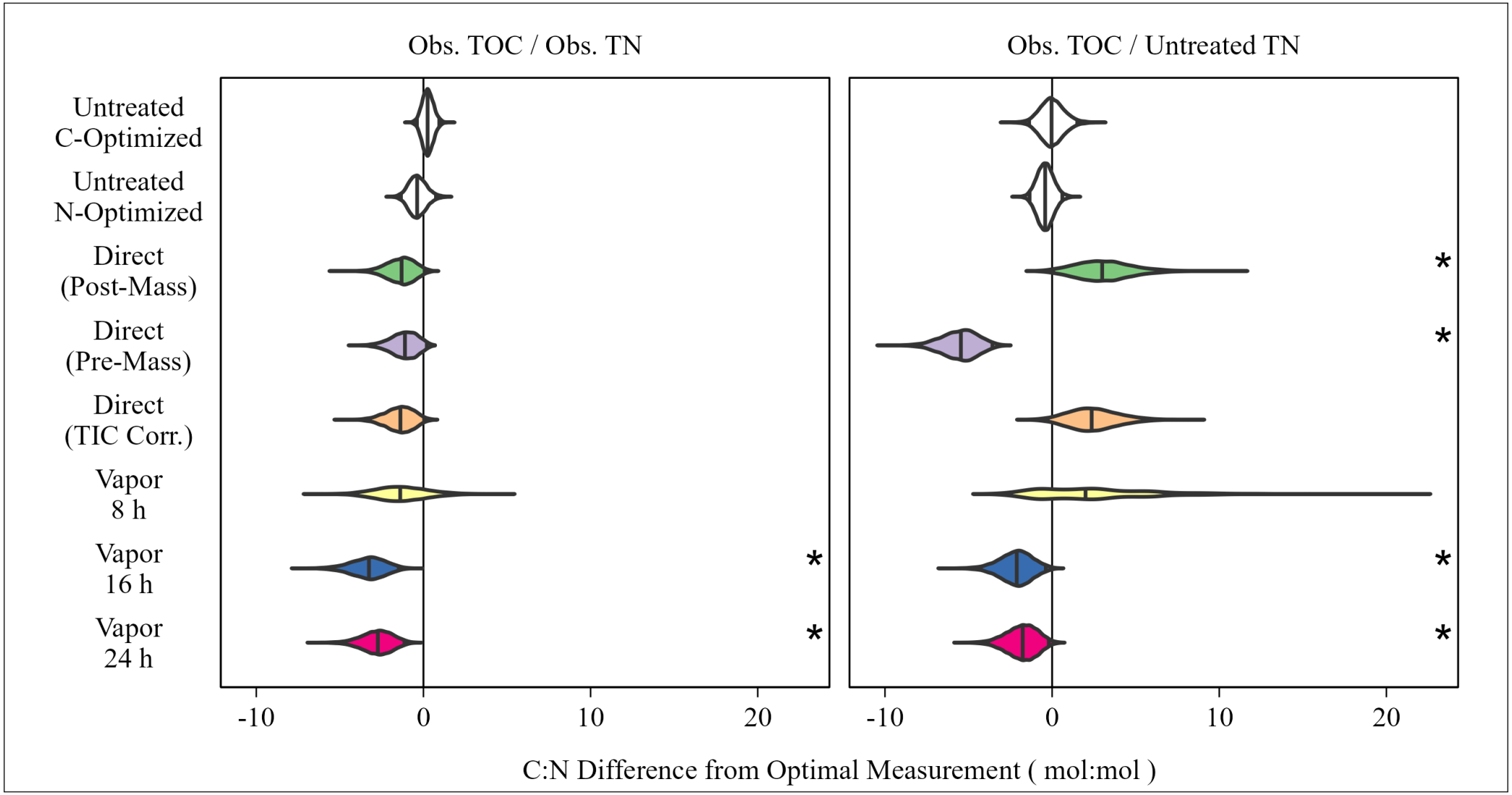


TIC Estimation

- TIC estimated from acid treatments by TC - TOC

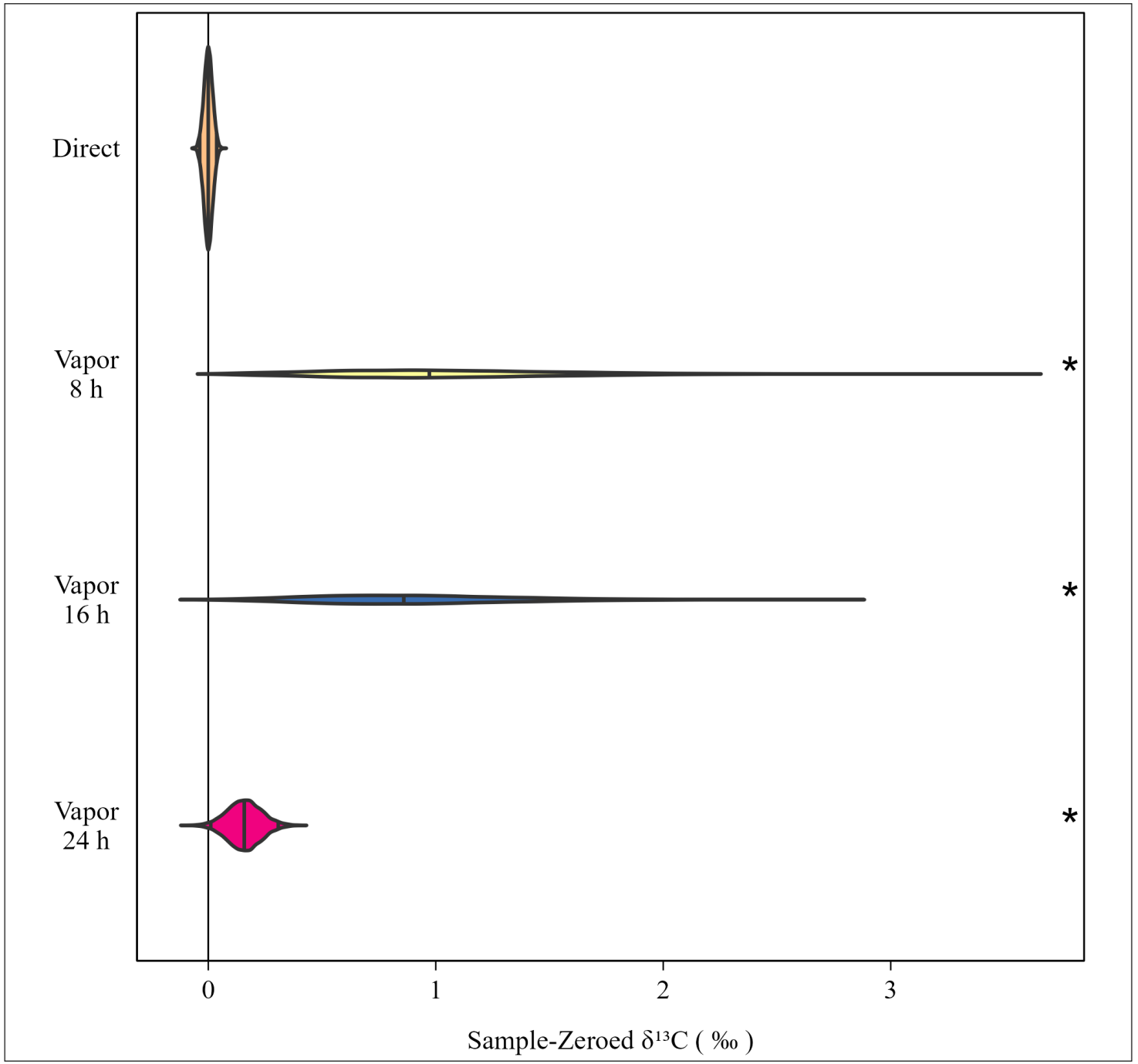


C:N


Obs. TOC / Obs. TN
Obs. TOC / Untreated TN
Untreated C-Optimized
Untreated N-Optimized
Direct (Post-Mass)
Direct (Pre-Mass)
Direct (TIC Corr.)
Vapor 8 h
Vapor 16 h
Vapor 24 h
*
-10
0
10
20
C:N Difference from Optimal Measurement (mol:mol)

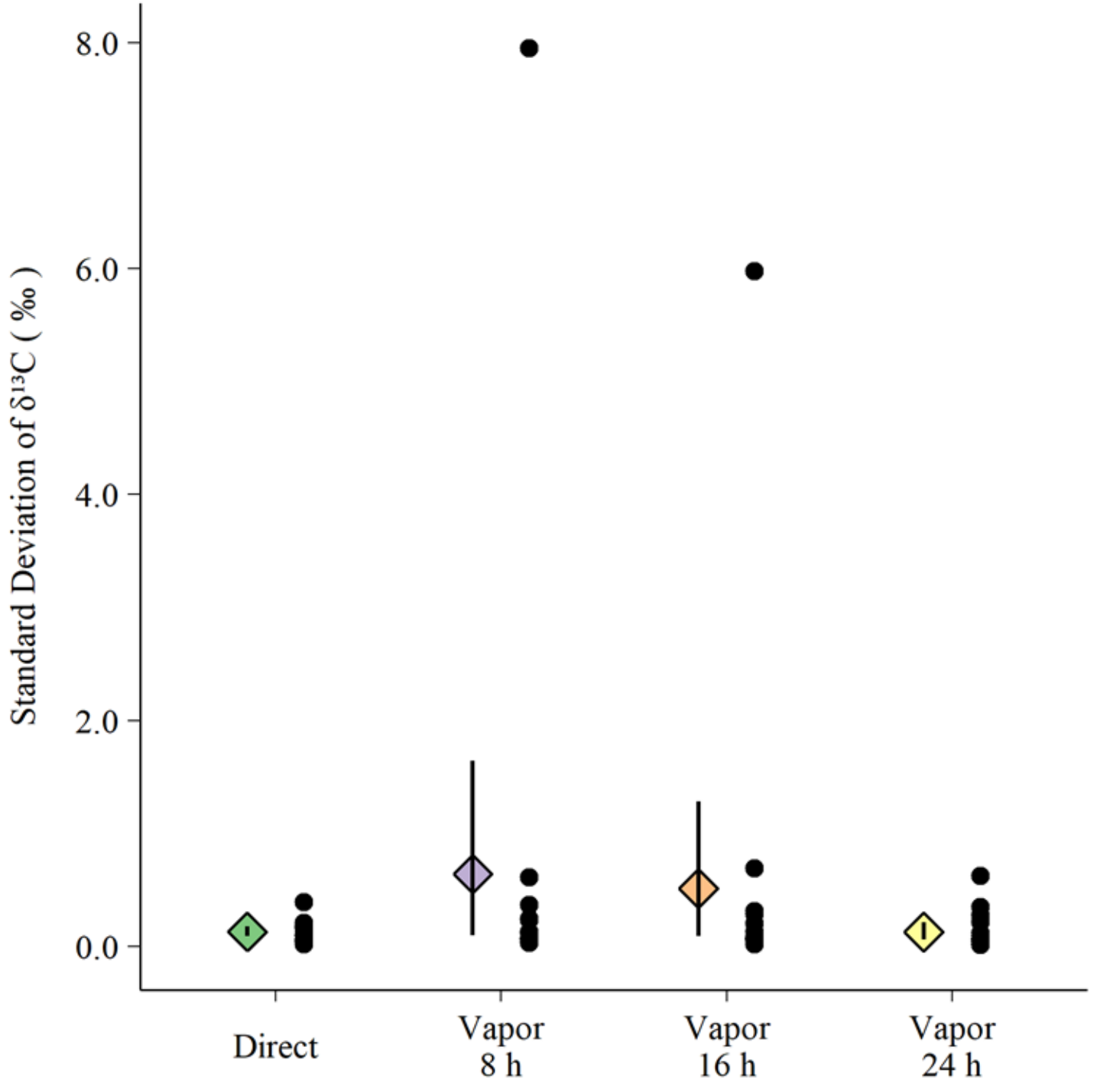
$\delta^{13}C_{Org}$

- Calculated as deviation from the mean Direct result
- No obvious ‘true’ result
- Some incomplete TIC-removal in some of the 8 and 16 h vapor samples



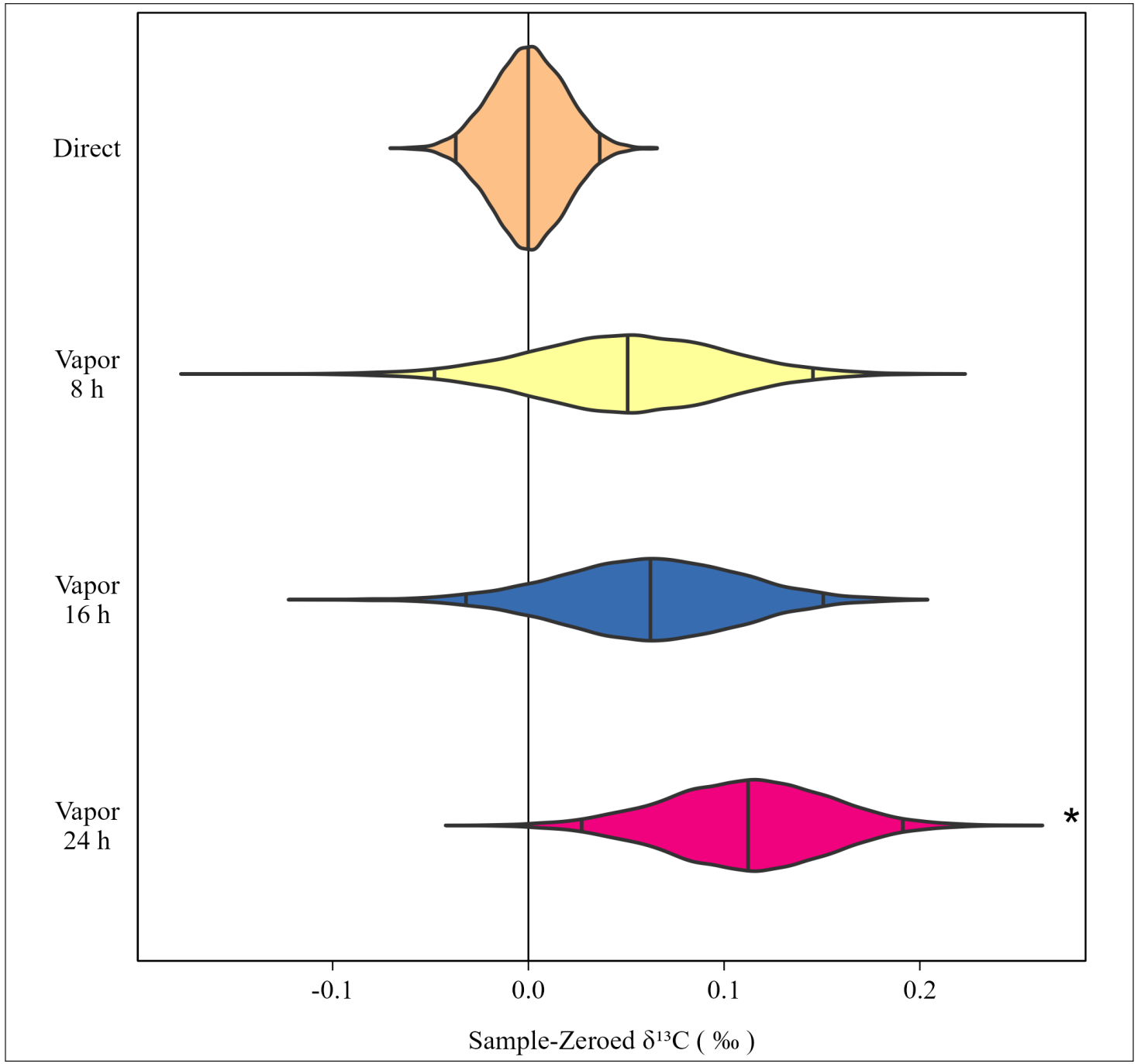
$\delta^{13}C_{Org}$: Outliers

- Depiction of sample-level standard deviations.
- Some replicates had incomplete fumigation



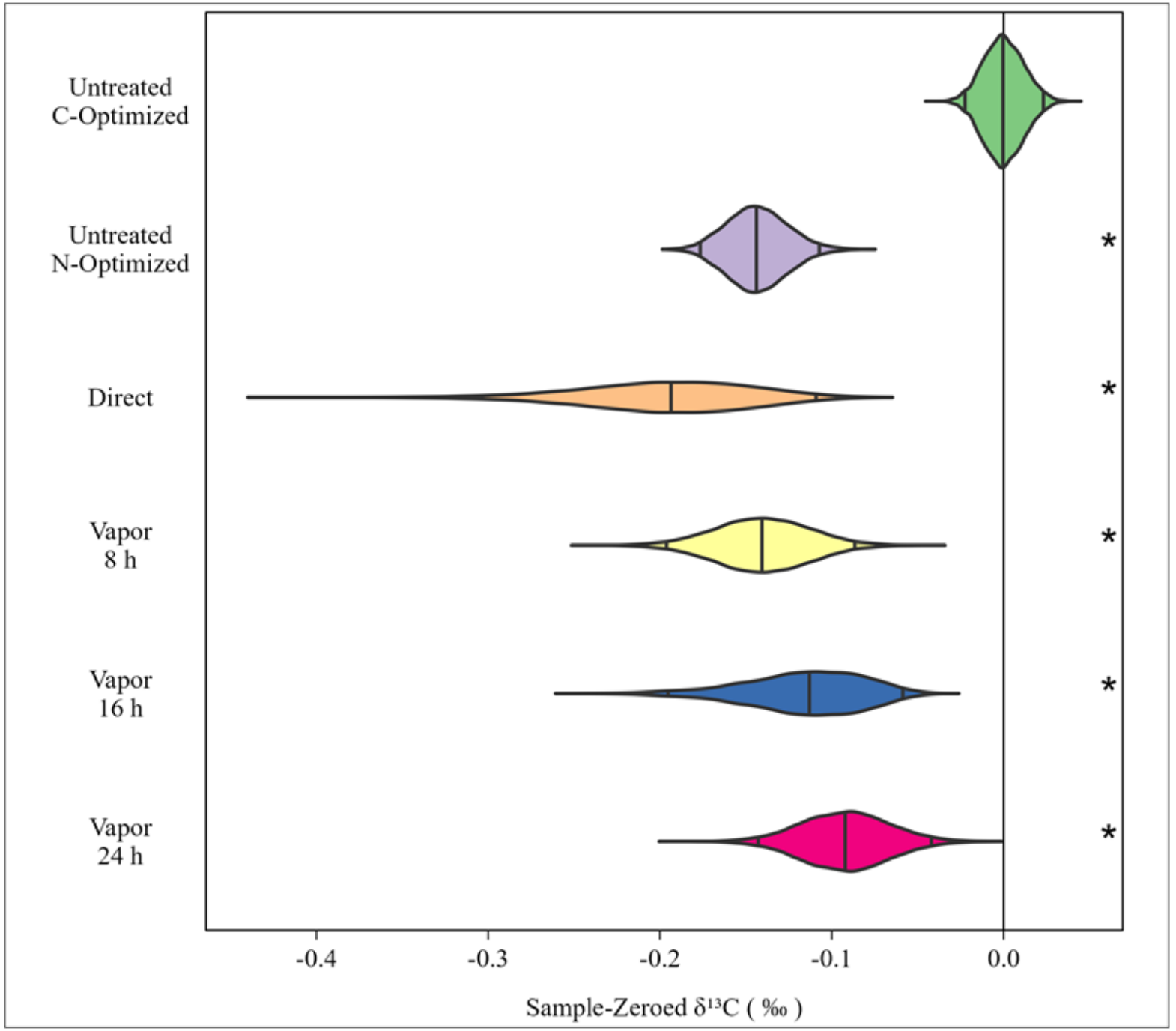
$\delta^{13}C_{Org}$: Major Outliers Removed

- Defined as > 1‰ deviation from Direct
- No practical way to identify these in practice



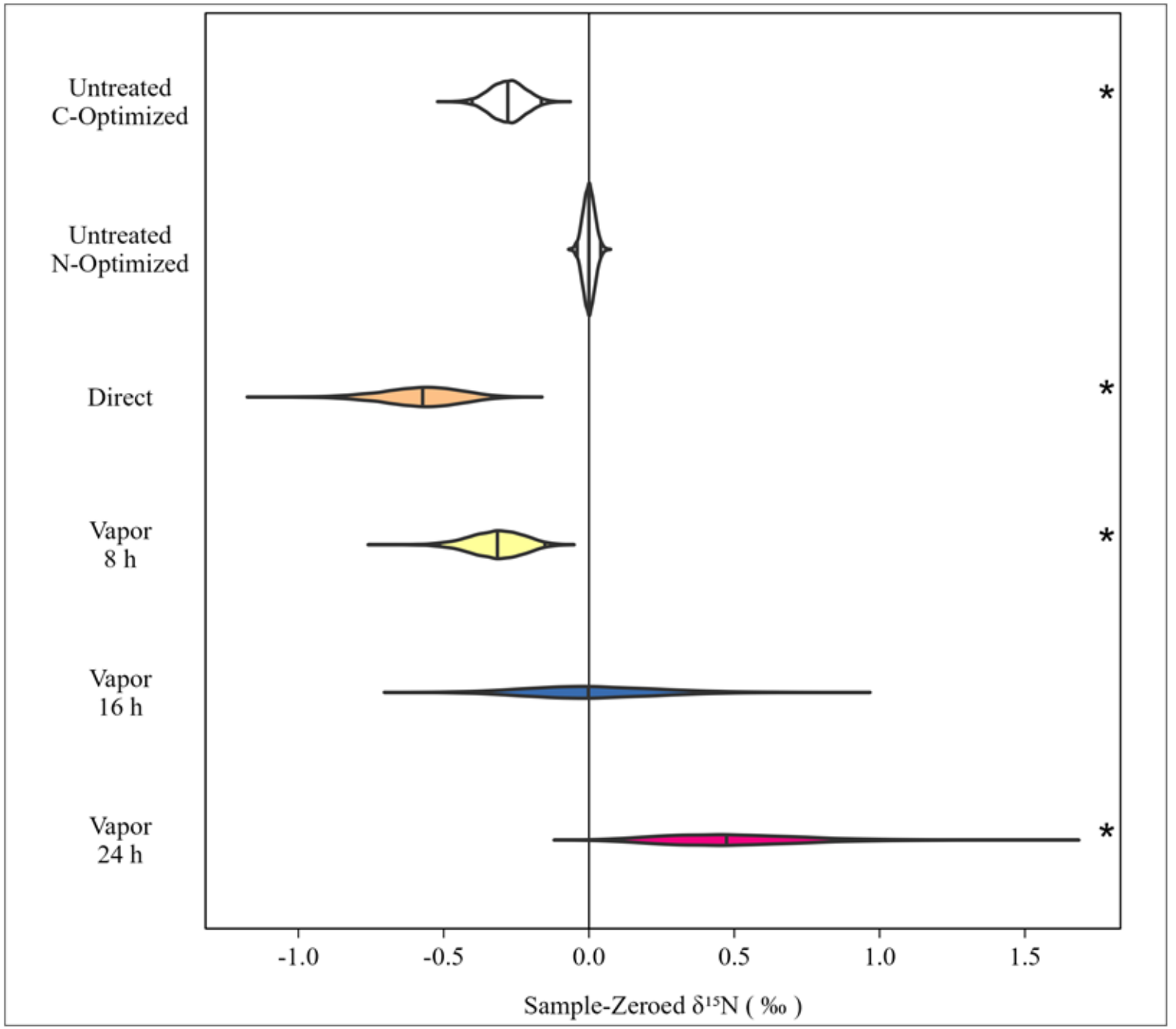
$\delta^{13}C$ TIC-Free Samples

- Untreated, C-optimized is clearly ‘best’ and used as the center
- Same patterns of direct versus vapor as before, but all of them have a small, negative bias

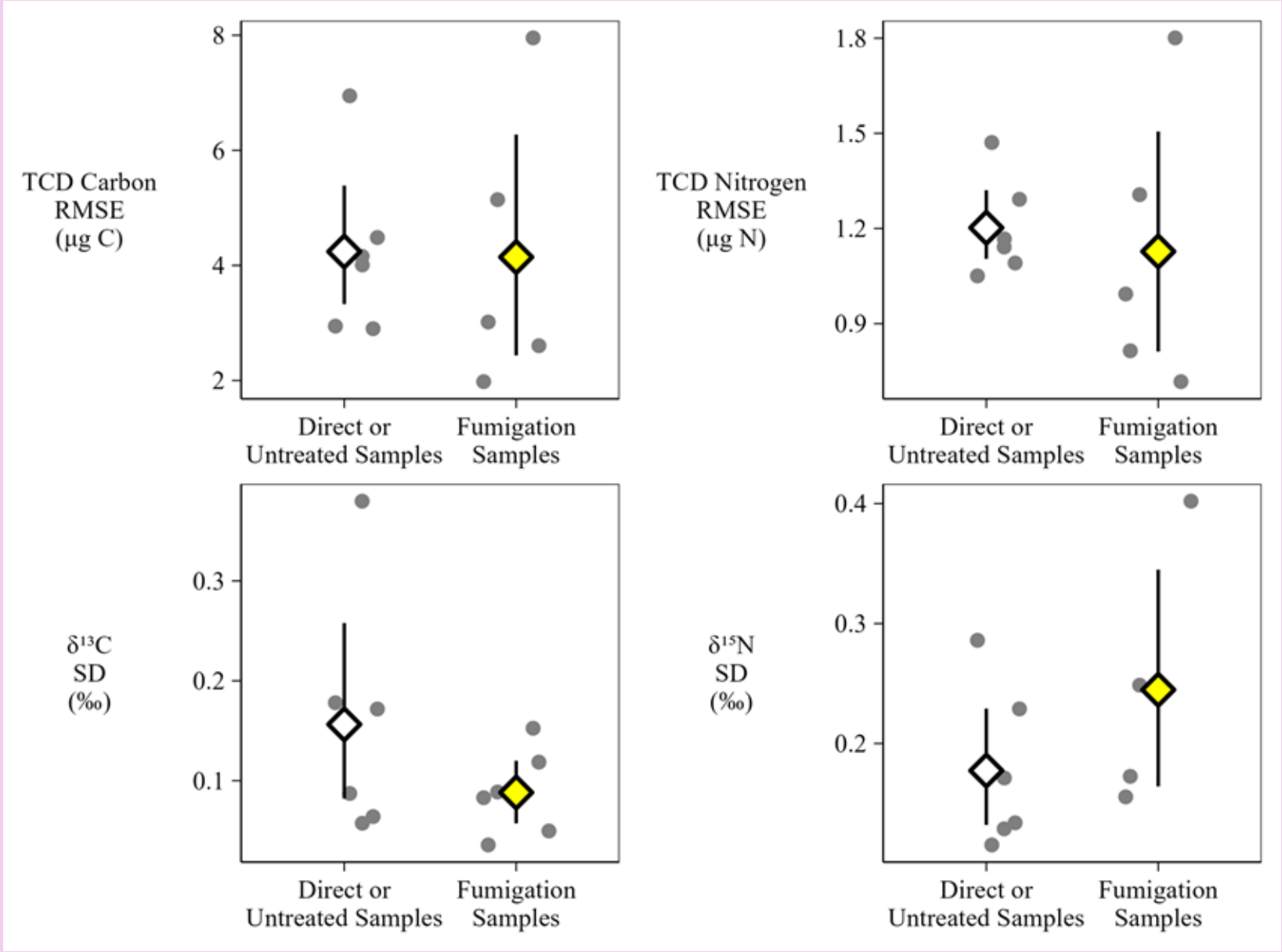


$\delta^{15}N$

- Untreated, N-optimized is clearly ‘best’ and used as the center
- Acid treatments clearly impact the measurement with a trend visible in the fumigation series that happens to center the 16 h treatment.



Instrument Performance



Conclusions

- **TOC**: Incomplete fumigation can cause issues. Direct acidification demands a large sample excess. N-optimized bias driven by high (>20 C:N) samples
- **TIC**: Reasonably correct TIC can be backed out from (complete) fumigation.
- **TN**: Acid is bad!
- **C:N**: Subtle differences result in some significant effects for acid treatments. If C:N is a critical result, it should probably be calculated using TC/TN and TIC
- $\boldsymbol{\delta^{13}C_{Org}}$: Small, curious biases introduced by acid treatments.
- $\boldsymbol{\delta^{15}N}$: Substantial issues with both acid treatments and small IRMS N2 peaks.