Freezer storage temperature effects on foliar pigments

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Background

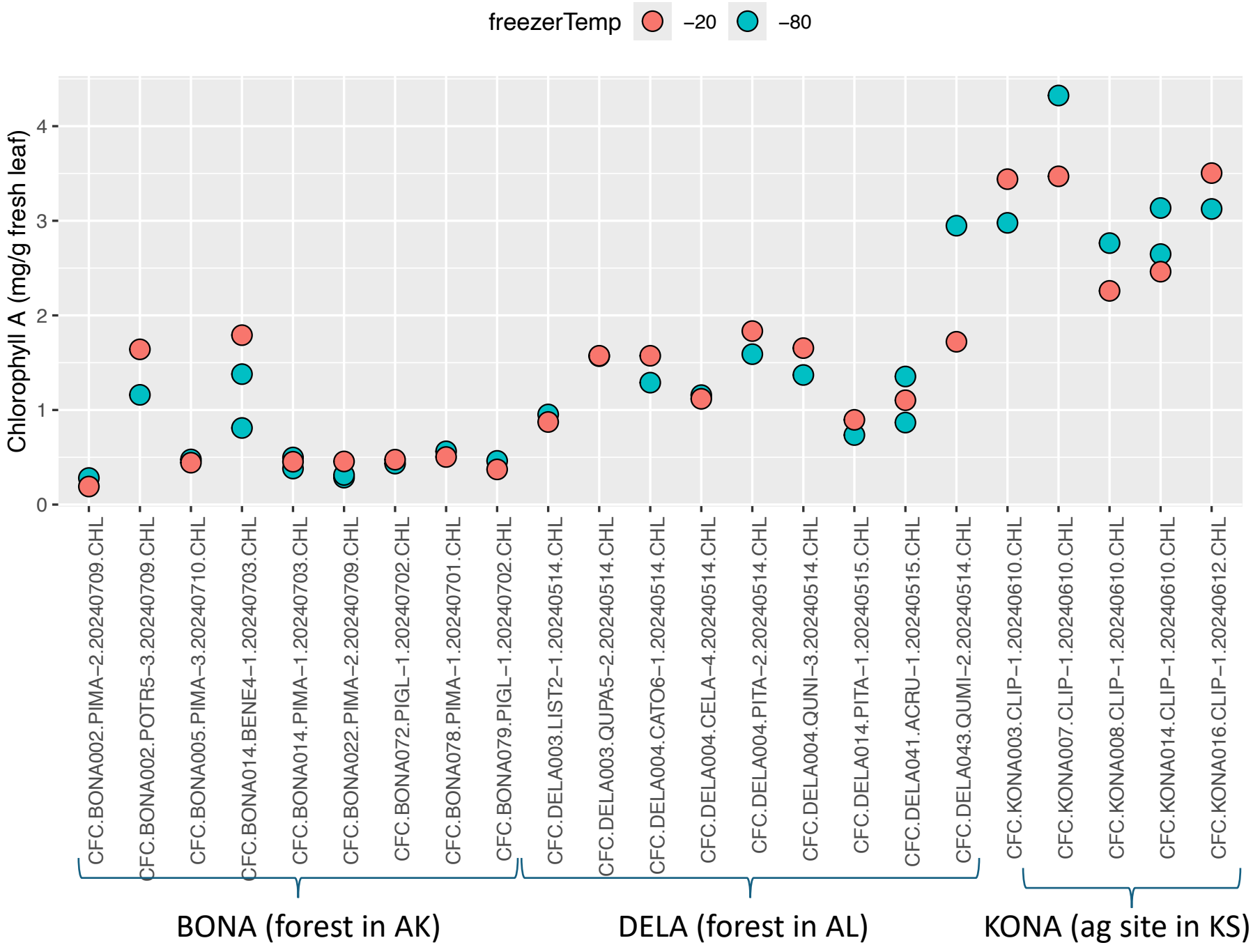
- NEON measures photosynthetic pigments in sunlit plant foliar tissue as part of DP1.10026.001
- In the summer of 2023, the NEON Calibration and Validation division audited a partner lab conducting these foliar chlorophyll and carotenoid analyses for NEON. Unfortunately, two significant issues were observed, as reported in this data notification
- One issue was that foliar samples were being stored at the lab prior to extraction and analysis (range of 1-7 days) at -20°C, instead of -80°C as required by NEON
- All impacted records were flagged in the cfc_chlorophyll table (n = 578) and an issue log was added to the data product information
- NEON leadership authorized a small study to assess how much this handling error may have impacted the data
  - The study was conducted by a new lab in the summer of 2024, and the former lab no longer works with NEON

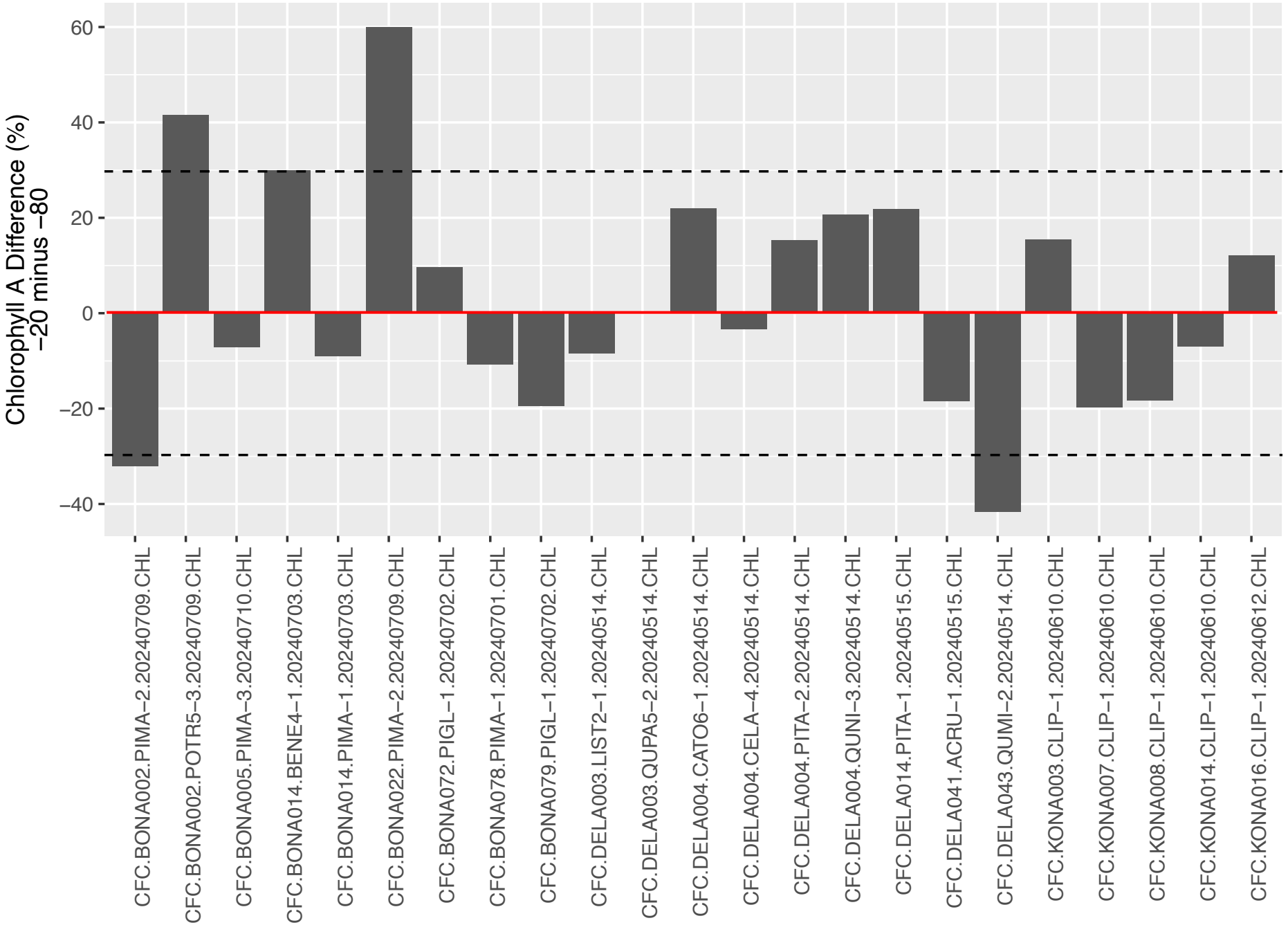
Experiment

- 3 NEON sites were selected in order to test the impacts of storage temperature on different kinds of plant tissue:
  - DEJU (Domain 18/19, AK), mostly conifer samples with some broadleaf (n = 9)
  - DELA (Domain 8, AL), mostly broadleaf samples with some conifer (n = 9)
  - KONA (Domain 6, KS), herbaceous clip strips from agricultural plots (n = 5)
- Requested the field crews create replicate chlorophyll subsamples from the same bulk sunlit tissue. Nothing different until received by the lab
  - flash-freeze subsamples on dry ice > store in -80℃ freezer for up to 1 week > ship to analytical lab overnight on dry ice (e.g., standard NEON procedures)
- Requested that upon receipt, external lab store one replicate at -80℃, the other at -20℃. Vary the time spent in storage at the lab from 1 to 6 days, then analyze replicates together following standard procedures

Results Chl A

- Some samples saw no impact from freezer temp
- Others varied, but direction not consistent

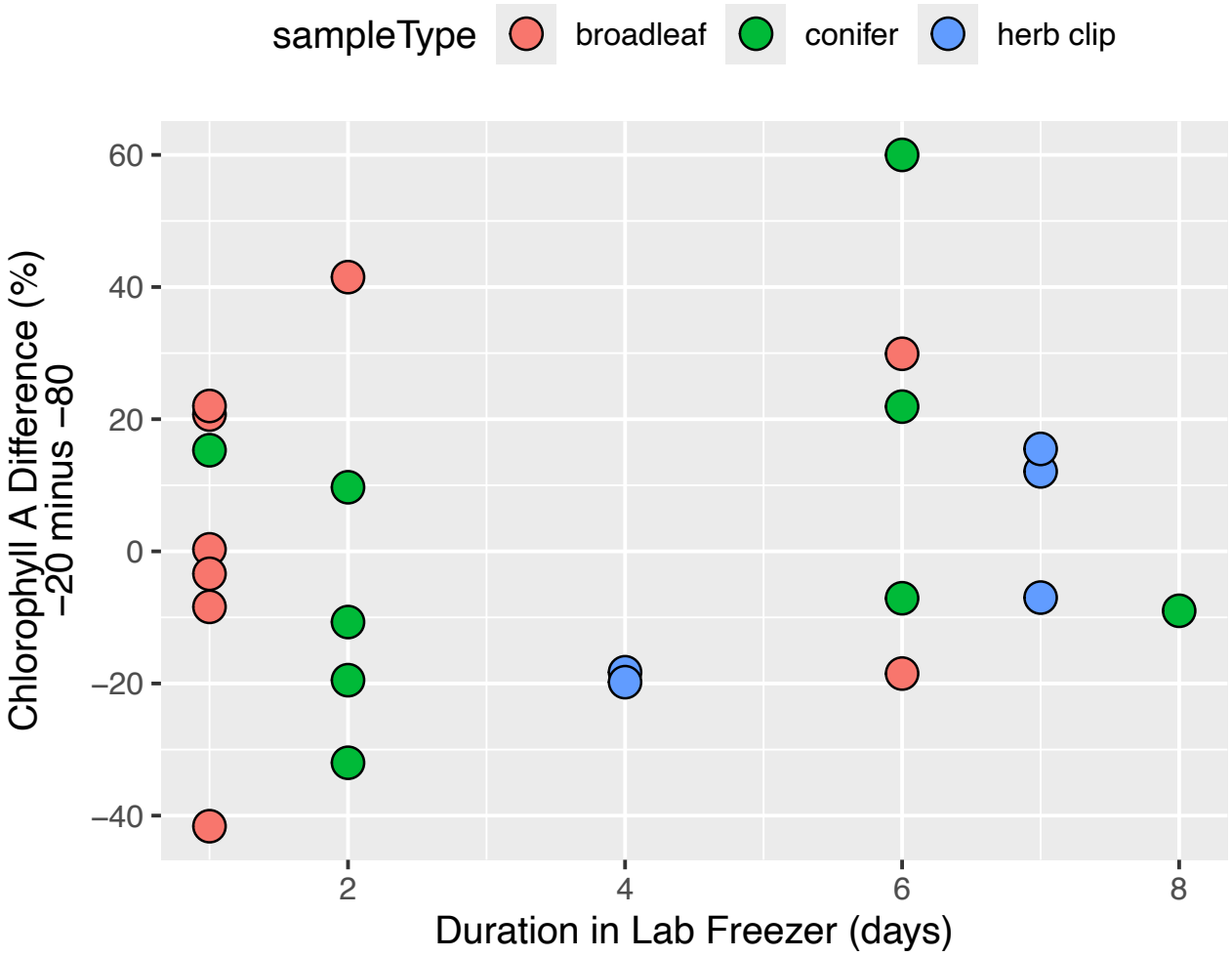




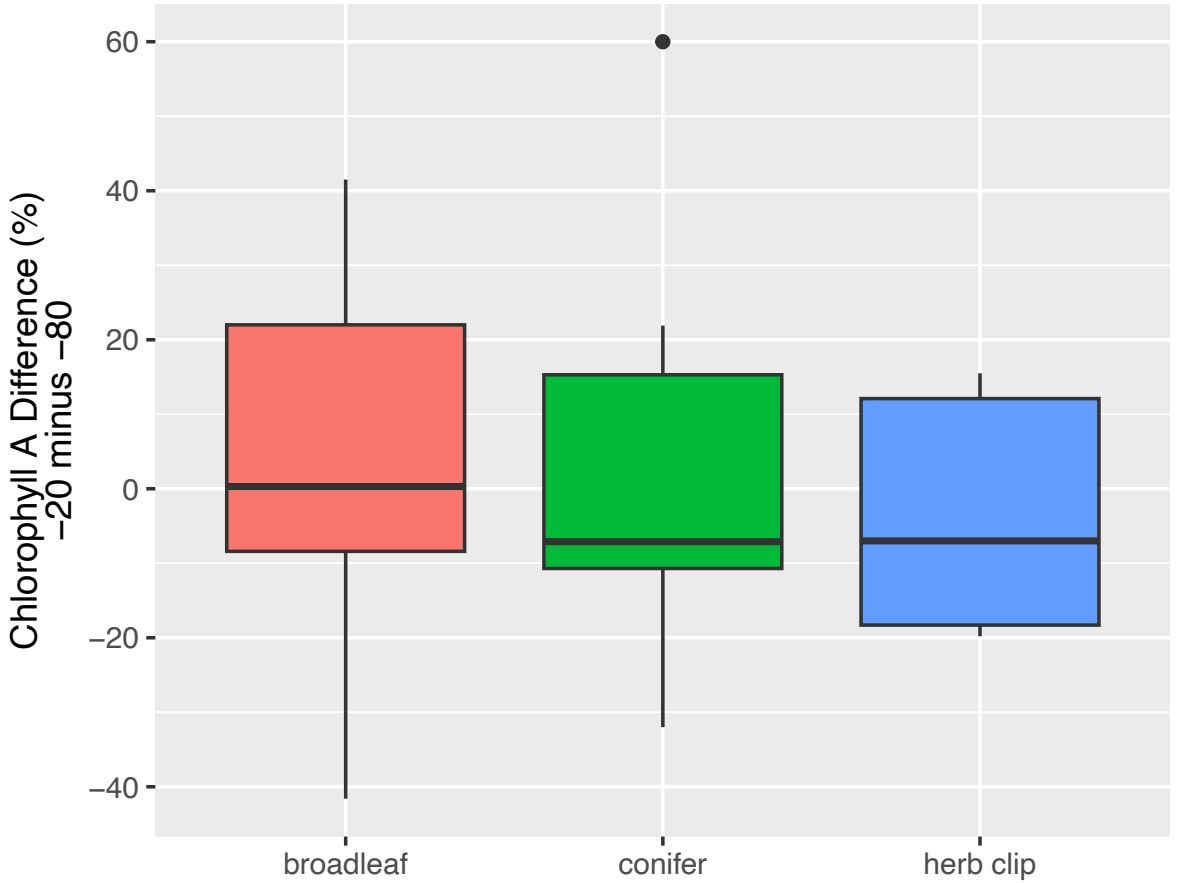
Positive means -20 value was higher

Negative means -20 value was lower

Dashed line is the relative within-sample variance, according to results from analytical reps



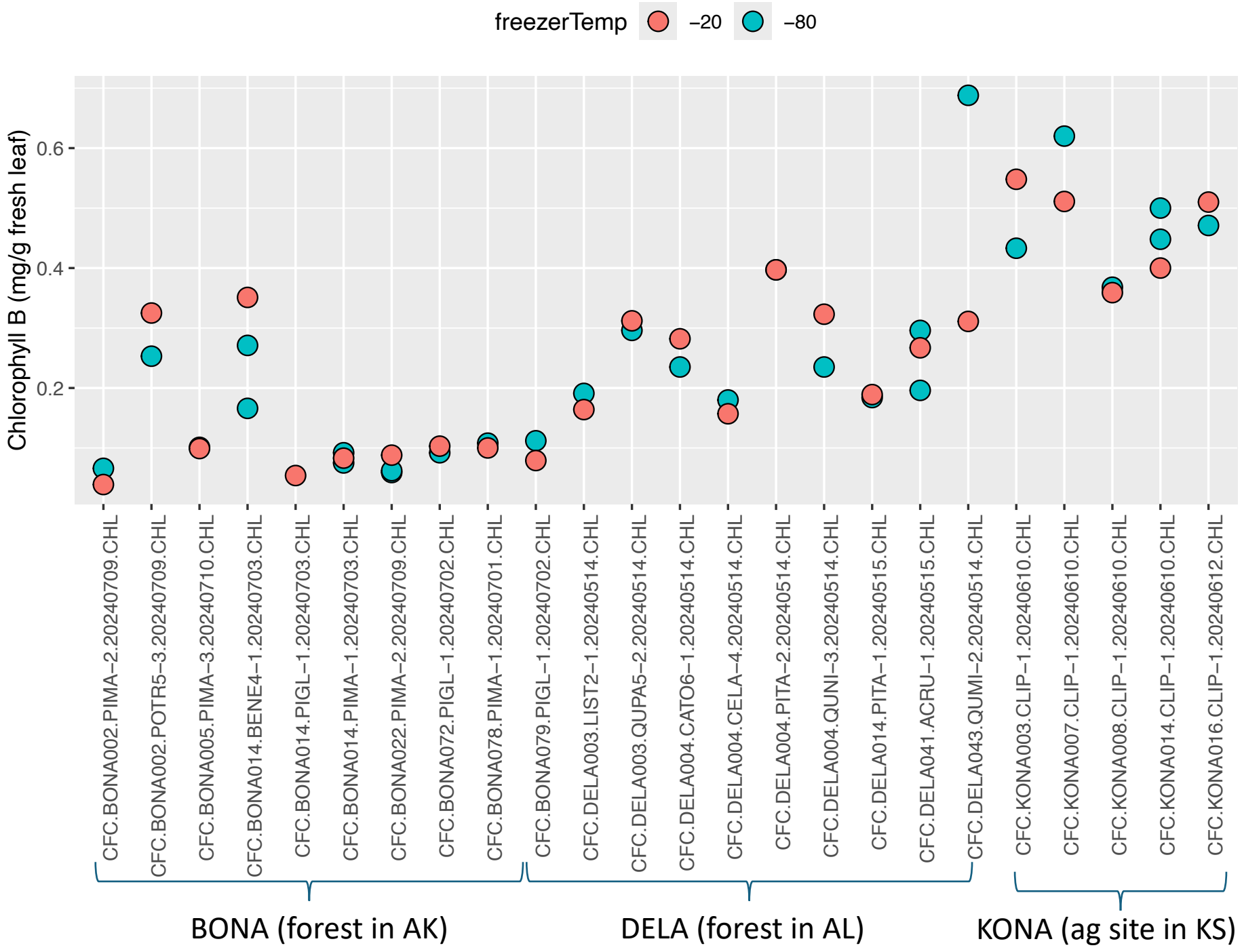
Linear model is not significant (no time effect)

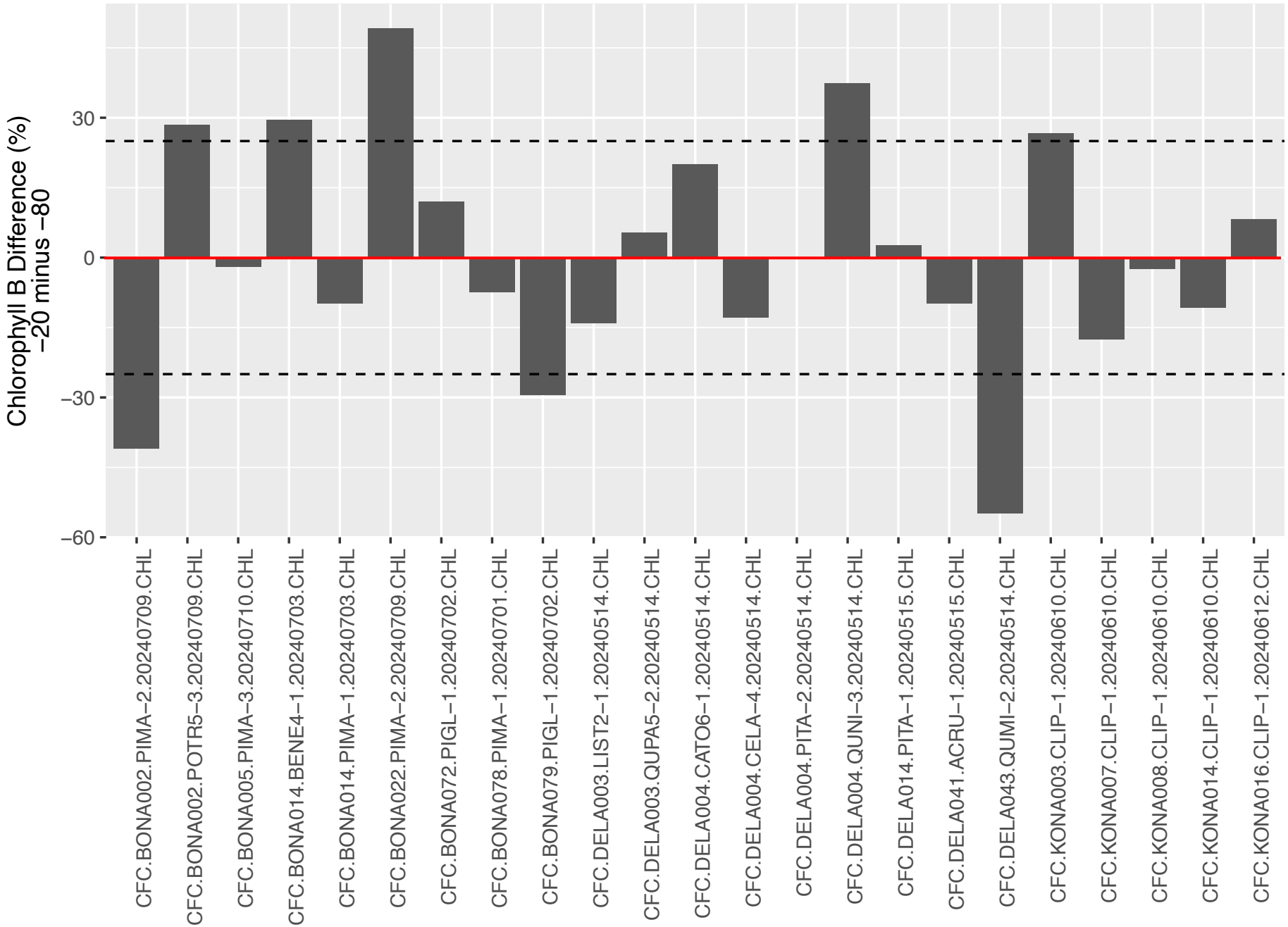


ANOVA is not significant (no sample type effect)

Results - ChlB

Trends are the same as for Chlorophyll A





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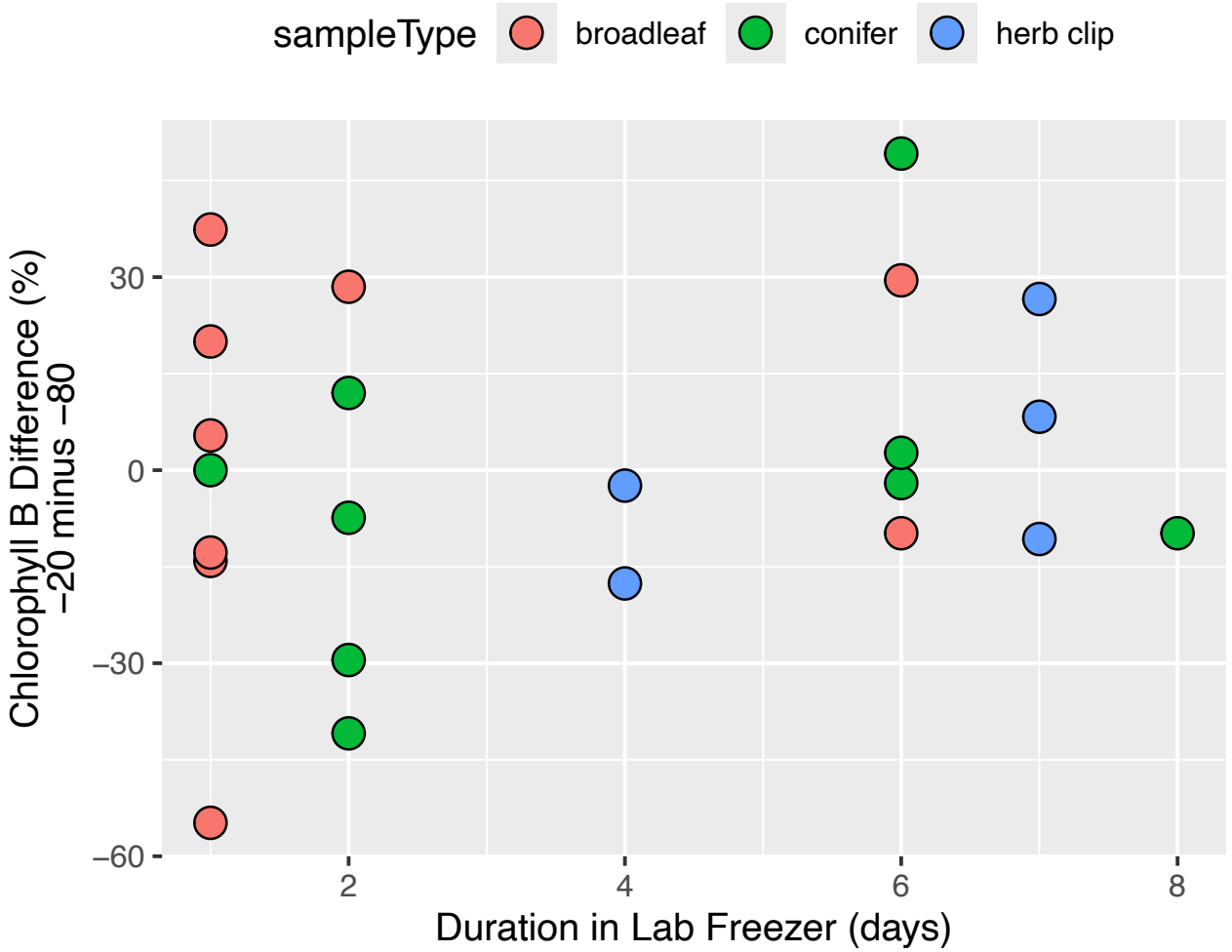
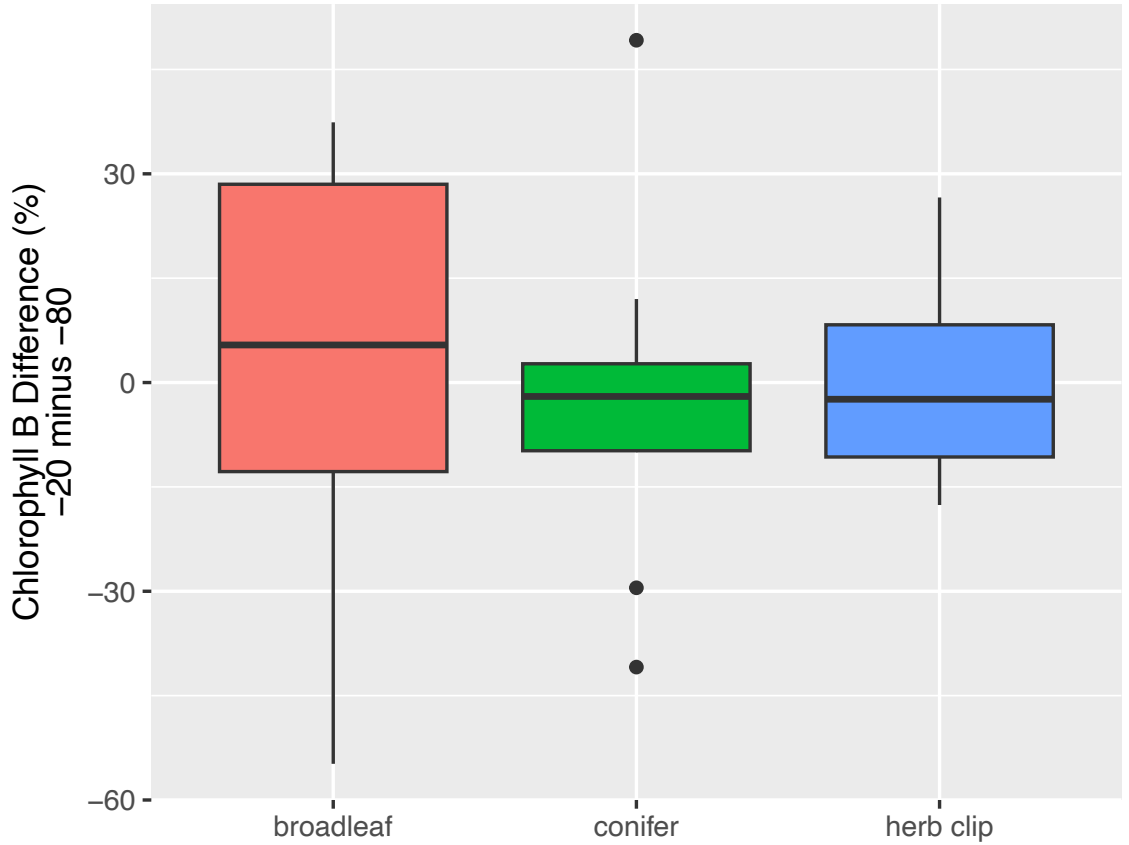


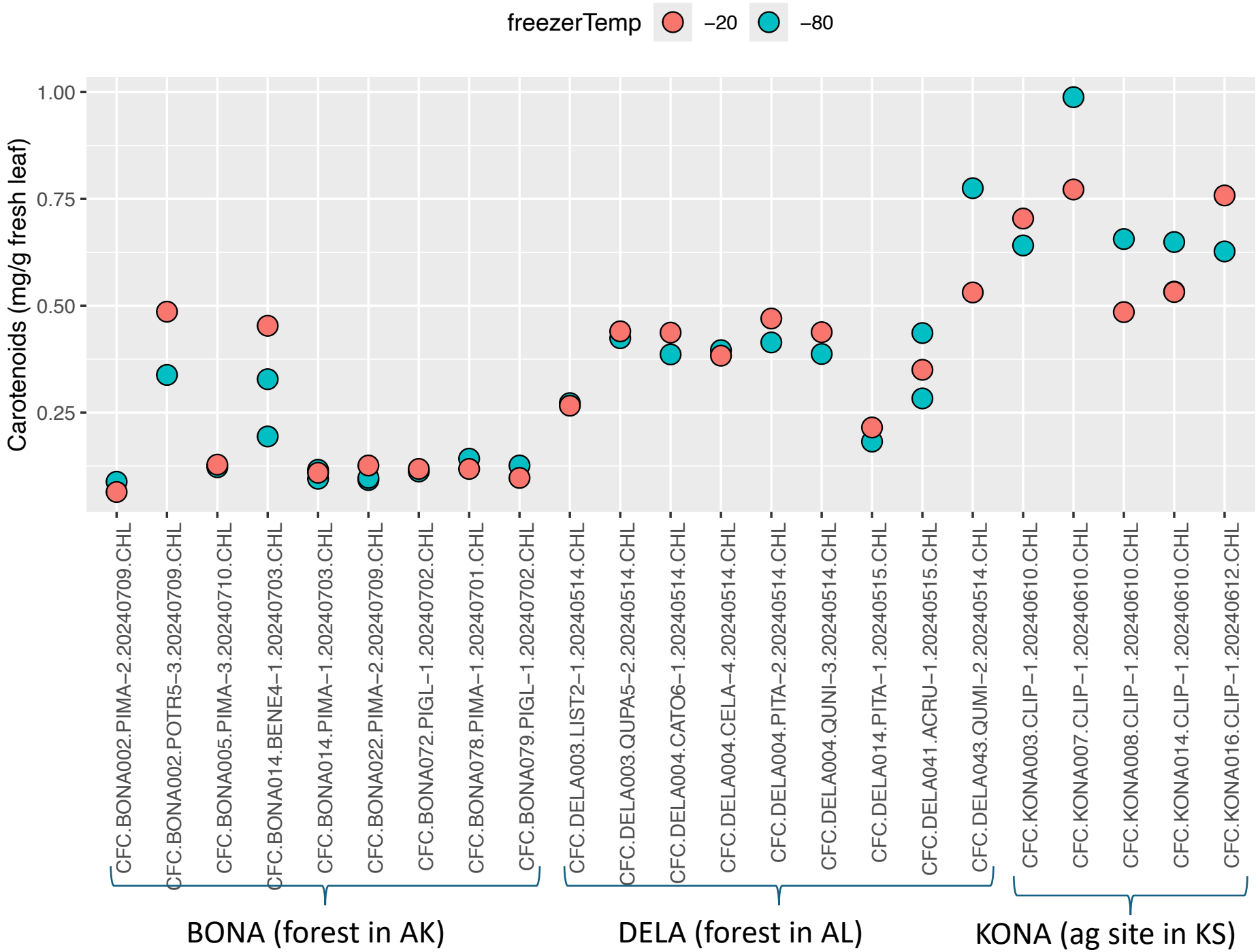
Linear model is not significant (no time effect)

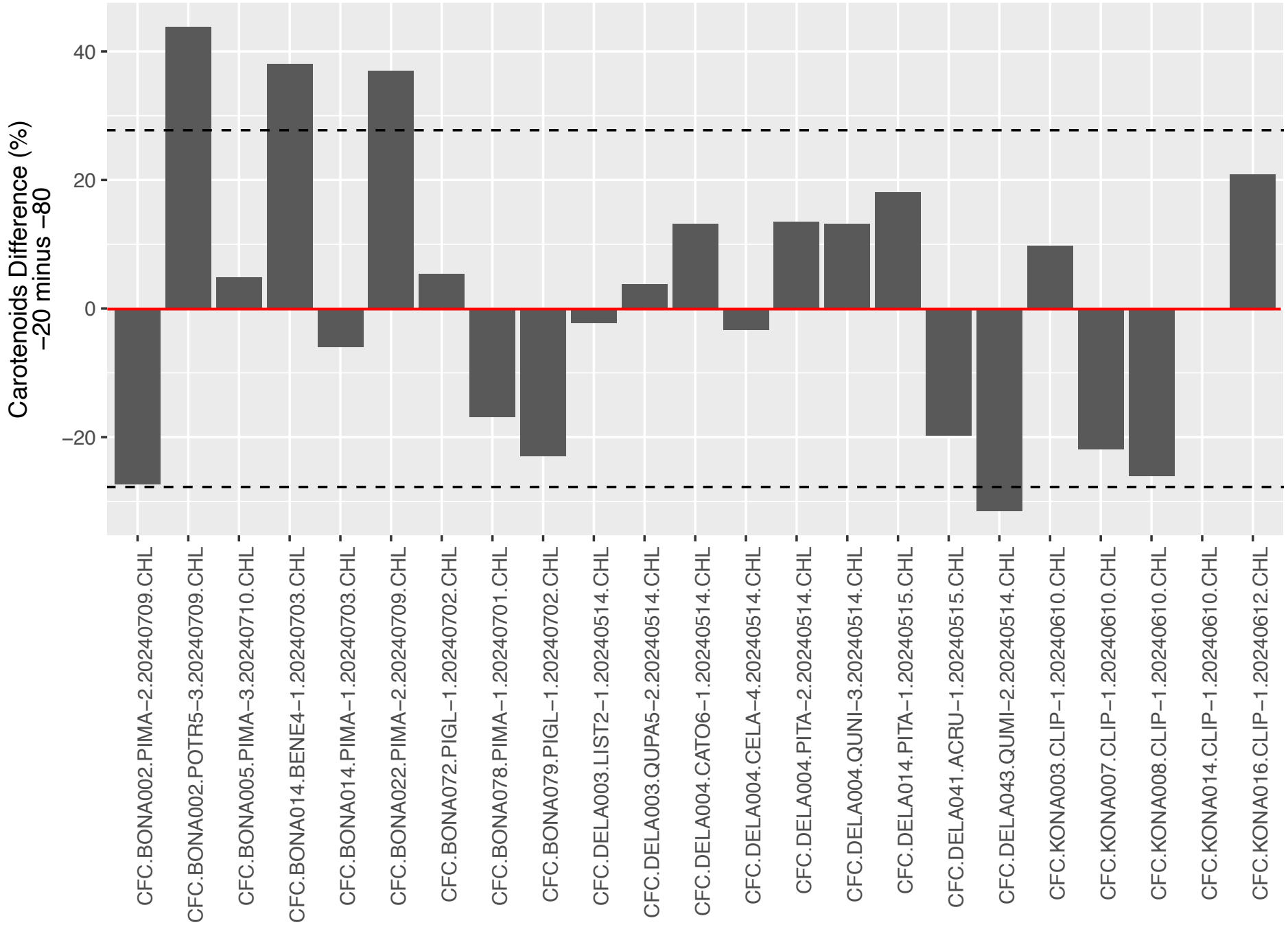


ANOVA is not significant (no sample type effect)

Results - Carotenoids

Same trends as other pigments





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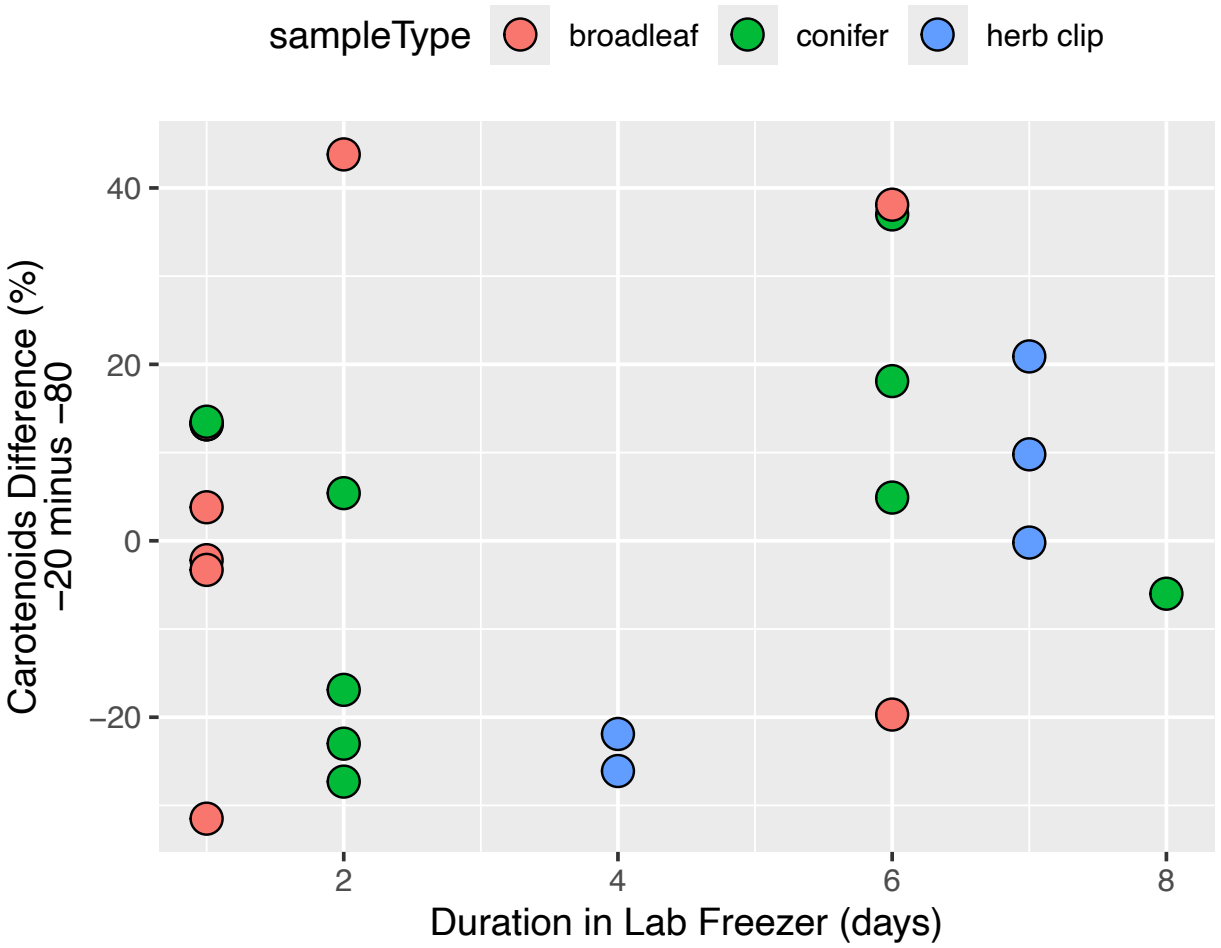
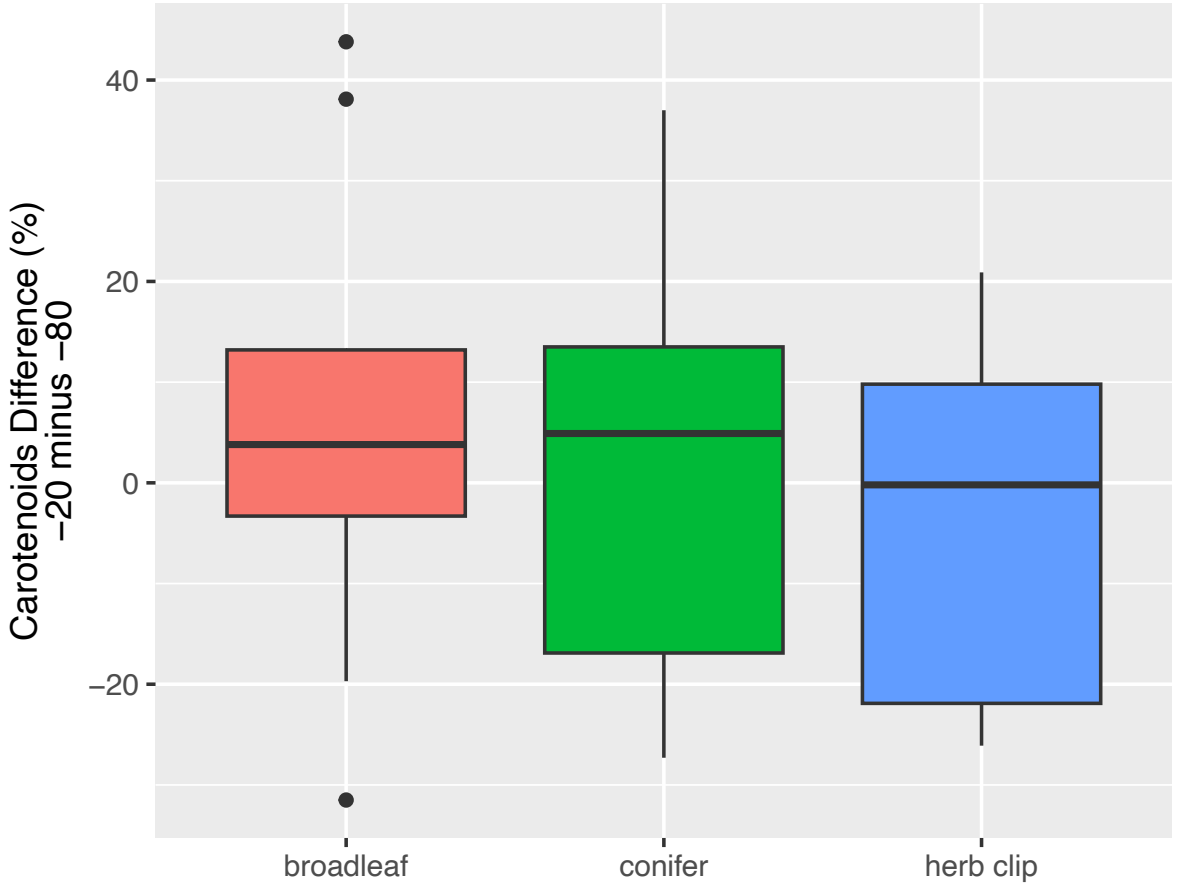


Linear model is not significant (no time effect)



ANOVA is not significant (no sample type effect)

Conclusions

- There is no evidence for a clear directional change in pigment concentration comparing foliar samples stored at -20℃ vs -80℃ for a short number of days
  - Also, there was no effect of time in storage ranging from 1-6 days, or tissue type when comparing broadleaf vs conifer vs herbaceous tissues
- This is good news! Samples collected in 2022 and 2023 that were stored at -20℃ (flagged in the cfc_chlorophyll table) are likely suitable for most analyses, however the flag is there and users can decide
- This was a small sample size (n = 23) and overly strong conclusions should not be drawn. For example, NEON will not be changing required holding temperatures and partner labs will keep storing samples at -80℃ according to community best practice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