Lab effect on carbon and nitrogen concentration and stable isotope values of NEON vegetation samples

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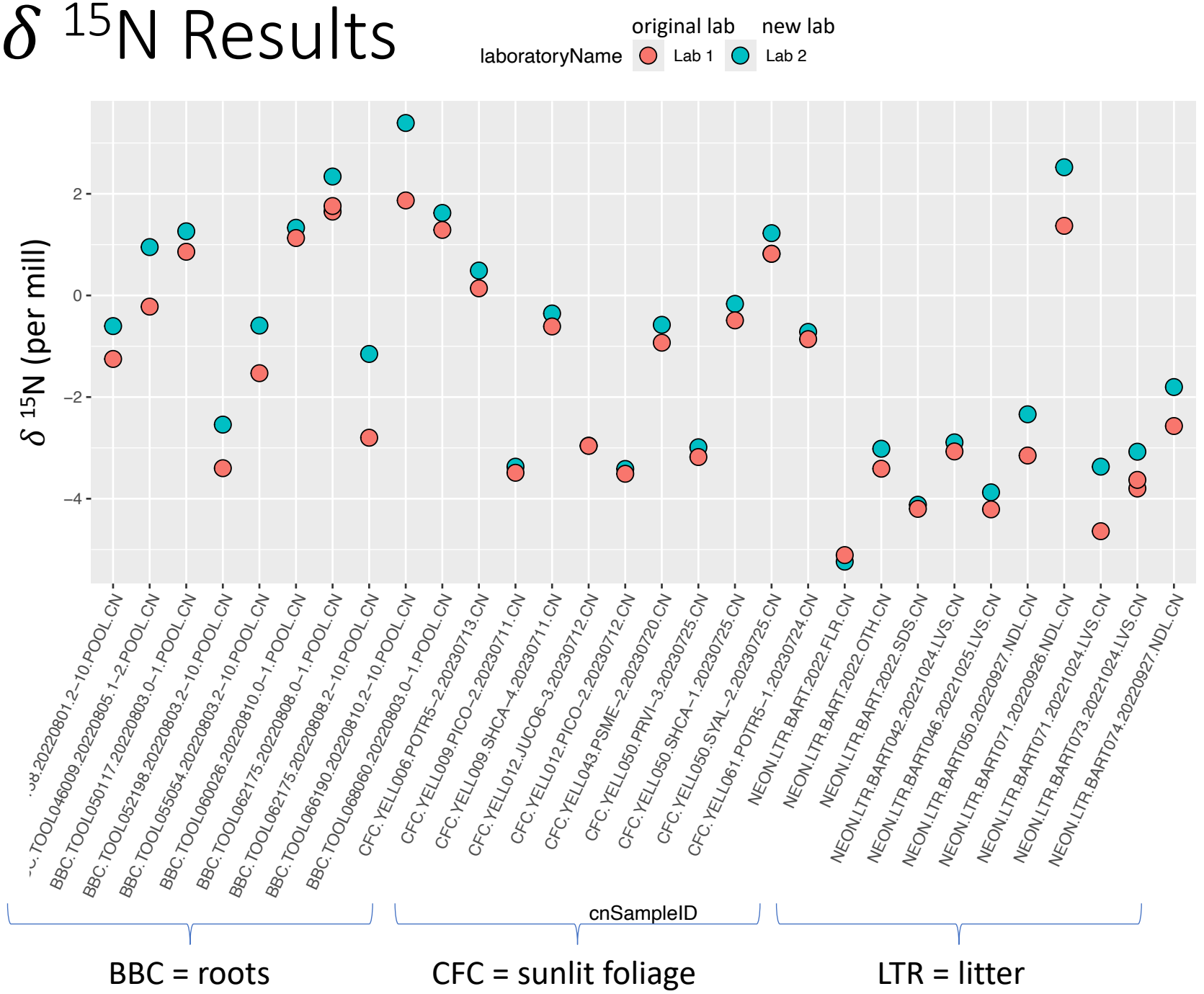
Background

- NEON conducts no chemistry analyses in house, all analytical work is contracted out
- Every five years, NEON hosts a competitive process to identify which labs to work with. Criteria for selection is based on rankings for technical merit and price
- Based on results of a recent competition, a new lab was selected to analyze **carbon and nitrogen concentrations and stable isotopes in vegetation**. Impacted sample types and their data product IDs include:
  - Sunlit canopy foliage (CFC), DP1.10026.001 Plant Foliar Traits
  - Fine roots (BBC), DP1.10067 Root biomass and chemistry, periodic
  - Litterfall (LTR), DP1.10033 Litterfall and fine woody debris production and chemistry
- The method involves elemental analysis coupled with isotope ratio mass spectrometry (EA-IRMS) on finely ground samples
- The analytes measured include δ^{15}N, δ ^{13}C, %N, %C, and C:N ratio

Background (continued)

- Before awarding a contract, the NEON Calibration and Validation department conducts a lab audit
- As part of the audit, and since dried and ground vegetation samples do not expire, NEON sought to evaluate the potential impact of a lab shift by having the candidate new lab re-analyze 30 samples, 10 from each vegetation type, recently analyzed by the previous lab
- The following slides display results of these tests for each analyte. Along with visual examination and summary tables, paired t-tests were used to determine if there was a significant difference by lab for each analyte
- For context, the 2-sigma uncertainty for each analyte and lab is shown. This value is often used as a criteria when analyzing standard reference materials as unknowns, the expectation is that variance should be within these levels.
- Lab 1 = original lab, analyzed vegetation samples for NEON from 2016-2023
- Lab 2 = new lab, selected to analyze NEON samples starting in 2024

δ ^{15}N Results



diff = Lab 2 - Lab 1

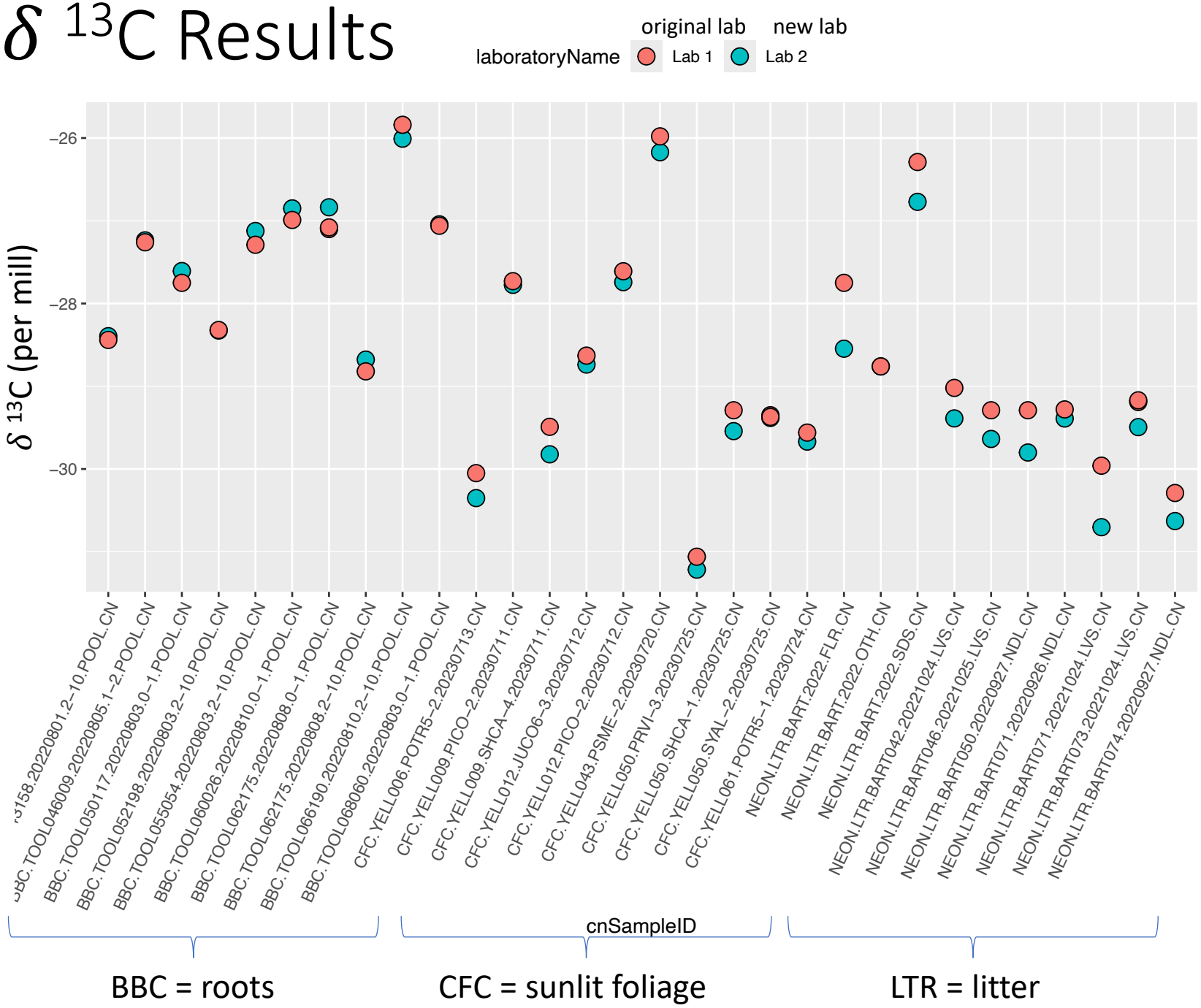
Sample type	δ ^{15}N diff mean	δ ^{15}N diff sd
Litter (LTR)	0.55	0.46
Roots (BBC)	0.84	0.49
Sunlit foliage (CFC)	0.22	0.13

Lab 2 is higher overall compared to Lab 1, most pronounced for roots

$P < 0.0001$ for paired T-test
mean difference = 0.54 per mill

2 sigma uncertainty Lab 1 = 0.4 per mill
2 sigma uncertainty Lab 2 = 0.4 per mill

δ ^{13}C Results



diff = Lab 2 - Lab 1

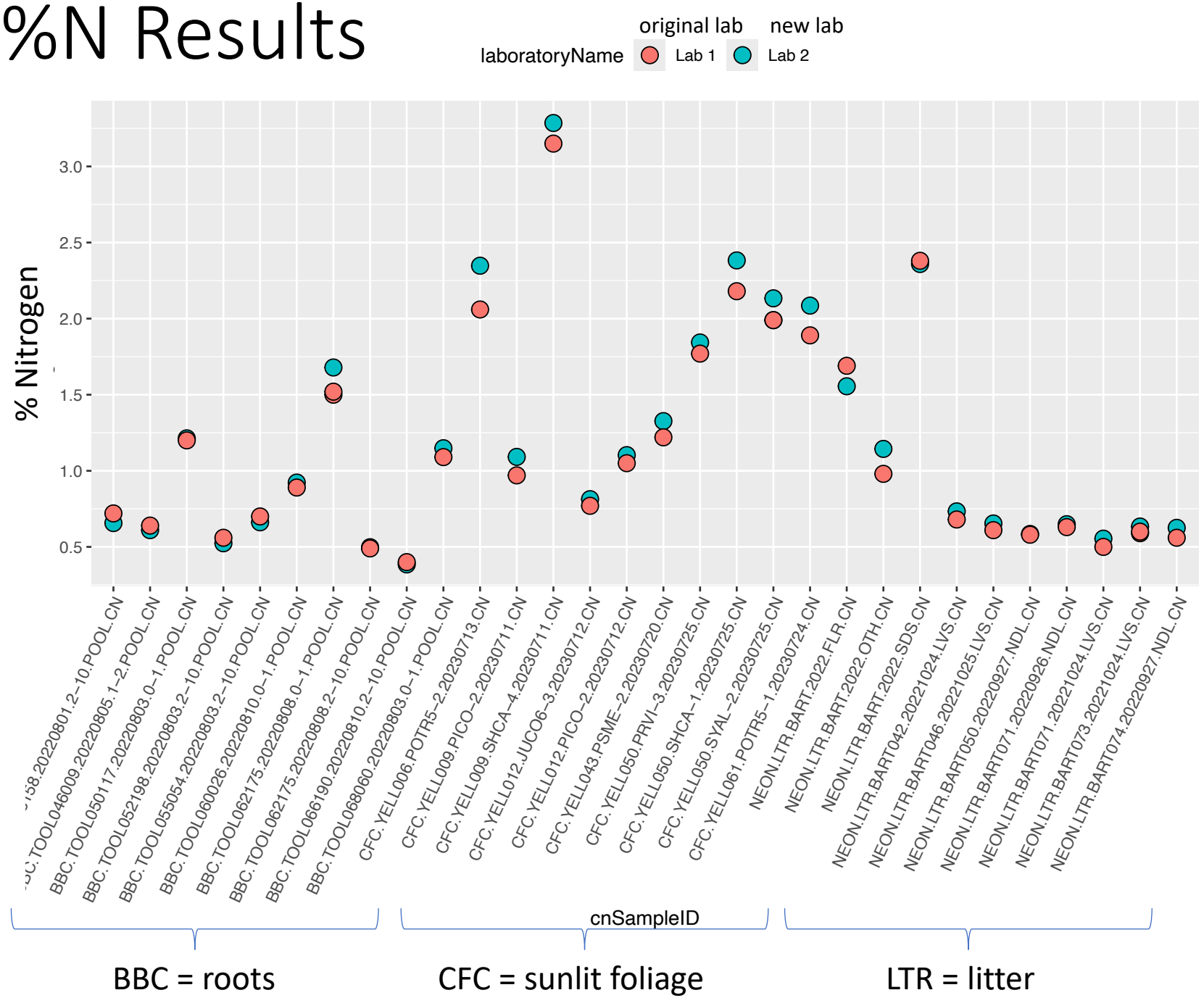
Sample type	δ ^{13}C diff mean	δ ^{13}C diff sd
Litter (LTR)	-0.4	0.25
Roots (BBC)	0.08	0.12
Sunlit foliage (CFC)	-0.16	0.1

Lab 2 is lower compared to Lab 1 for litter and sunlit foliage, slightly higher for roots

P = 0.00157 for paired T-test
mean difference = -0.16 per mill

2 sigma uncertainty Lab 1 = 0.3 per mill
2 sigma uncertainty Lab 2 = 0.4 per mill

%N Results



diff = Lab 2 - Lab 1

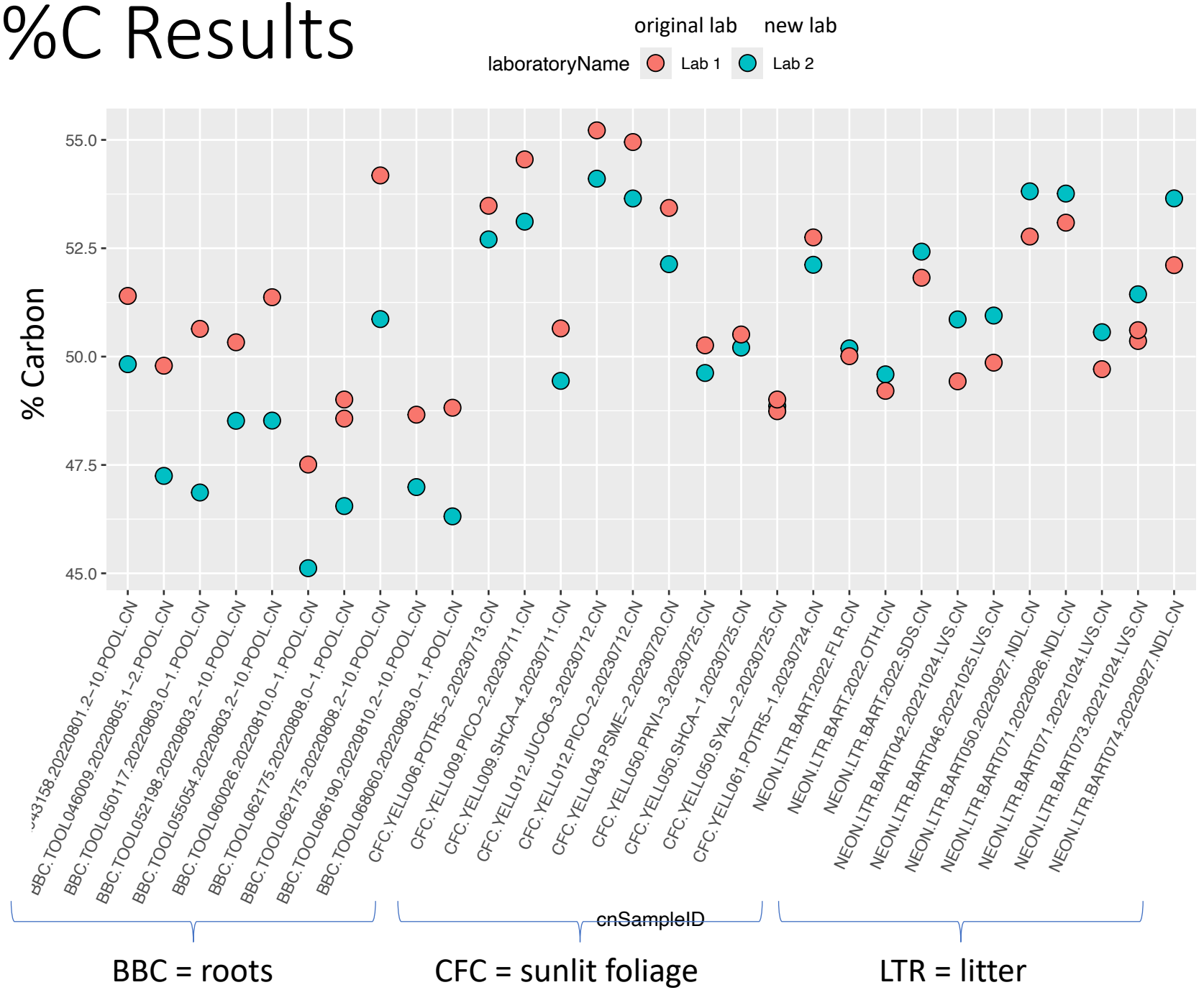
Sample type	%N diff mean	%N diff sd
Litter (LTR)	0.03	0.08
Roots (BBC)	0.01	0.07
Sunlit foliage (CFC)	0.14	0.08

Lab 2 is higher overall compared to Lab 1, most pronounced for sunlit foliage

P = 0.00136 for paired T-test
mean difference = 0.06 %

2 sigma uncertainty Lab 1 = 0.1%
2 sigma uncertainty Lab 2 = 0.6 %

%C Results



diff = Lab 2 - Lab 1

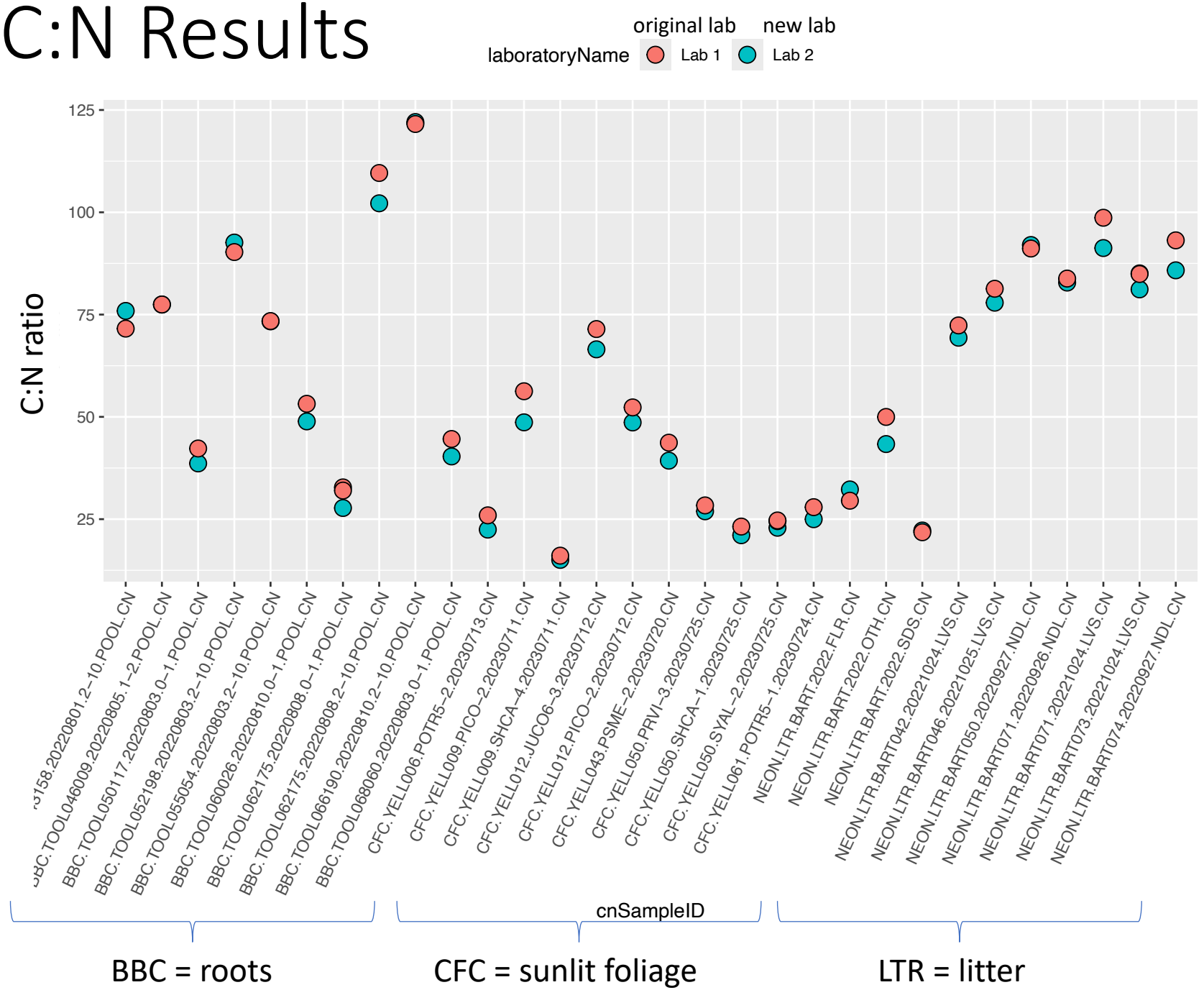
Sample type	%C diff mean	%C diff sd
Litter (LTR)	0.87	0.43
Roots (BBC)	-2.47	0.71
Sunlit foliage (CFC)	-0.87	0.48

Lab 2 is lower compared to Lab 1 for roots and sunlit foliage, higher for litter

P = 0.00512 for paired T-test, mean difference = -0.82 %

2 sigma uncertainty Lab 1 = 2%
2 sigma uncertainty Lab 2 = 0.6 %

C:N Results



BBC = roots

CFC = sunlit foliage

LTR = litter

diff = Lab 2 - Lab 1

Sample type	C:N diff mean	C:N diff sd
Litter (LTR)	-2.85	3.59
Roots (BBC)	-1.72	3.67
Sunlit foliage (CFC)	-3.33	1.98

Lab 2 is lower overall compared to Lab 1

$P < 0.0001$ for paired T-test

mean difference = -2.64

Results from analysis of lab reference materials treated as unknown, Lab 2 test sample run

	δ ^{15}N	δ ^{13}C	%N	%C
mean	-0.44	-27.38	6.13	40.36
known	-0.40	-27.41	5.95	40.53
accuracy	-0.04	0.03	0.18	-0.17
SD	0.17	0.14	0.18	0.54

Criteria for accuracy & SD based on 2 sigma = within 0.4 per mill (isotopes) or 0.6% (concentration)
This run passes according to the criteria

Standard deviation of sample replicates, based on ~ 1,000 samples run in duplicate by Lab 1 over the last 8 years across all three vegetation types

δ ^{15}N sd	δ ^{13}C sd	N % sd	C % sd	C:N sd
0.16	0.12	0.03	0.52	2.36

Discussion

- There is in fact a significant lab effect for all analytes.
  - δ ^{15}N and %N values are higher with the new lab
  - δ ^{13}C, %C, and C:N ratios are lower with the new lab
- However, only the differences in δ ^{15}N are larger than the long-term analytical uncertainty (e.g., 2 sigma). Differences in %N were consistent directionally but small, and differences in δ ^{13}C and %C were inconsistent in their direction across sample types.
- Both labs passed NEON Calibration and Validation audits, meaning they follow best practices and use appropriate standards to calibrate and validate their data. As such, it is unclear what the ‘true’ value for these samples is. Both labs produce trustworthy data.
- Given these results, NEON determined it was appropriate to proceed with the change of labs and a new contract was awarded.

Conclusion

- While NEON has confidence in the results generated by the new lab, end users should be aware that the lab switch may have introduced subtle differences in vegetation chemistry. The only difference that rises above long-term uncertainty is $\delta\ ^{15}N$, which may increase ~ 0.5 per mill with the new lab. Users should interpret NEON timeseries data in this context.