Stable Isotope Tracer Use at RMBL 2016 & 2017

1) Stable isotopes and sources
We purchased 99% ammonium-^{15}N chloride (NH_4Cl) from Cambridge Isotopes Inc (NLM-467) and 99% ammonium-^{15}N chloride (NH_4Cl) from Icon Isotopes Inc (IN 5037). We purchased 99% sodium bicarbonate-^{13}C from Icon Isotopes Inc (IN 4628).

The isotopes are inventoried and stored in Brad Taylor's lab in 106 of the Gothic Research Center. The isotopes are stored in double containment in a secure location in his lab (top drawer of file cabinet inside a large plastic container with a typed warning DO NOT USE and explanation of the contents).

There was some mention of the sodium bicarbonate-^{13}C possibly being a source of ^{14}C contamination. We called or emailed both manufacturers and neither had any information on potential risk as they do not enrich for and are not licensed to sell radioisotopes. Thus, we have no evidence to suggest that our stable isotope of carbon was enriched with ^{14}C.

There was also mention of contamination from sample volatilization in the drying oven. We have no evidence to indicate that carbon or nitrogen would be volatized at 60 ^0C, as this method of drying samples from different sources has been used for decades. However, if there was volatilization, then contamination would not be specific to enriched samples but would also apply to natural abundance samples contaminating one another. This concern seems invalid.

2) Handling protocols (see below for detailed personnel training document)
Brad Taylor was the primary person to weigh tracers from concentrated stock and add them to the mesocosms. Weighing was only done in his lab using his equipment. However, Amanda DelVecchia was also trained by Taylor to weigh and add the isotopes and, on occasion, weighed and added stable isotopes to mesocosms.

3) Enrichment concentrations
^{15}N: To label the microbial communities on sedge detritus, we added ^{15}N to water in tanks (older mesocosms or plastic wading pools) containing a microbial community and pre-weighed sedge detritus bags. Water was enriched at maximum to $\delta^{15}N$ 15,987 ‰ (2016) and 24,057 ‰ (2017). Labeled sedge (90.36 to 121.72 ‰) was transferred from the "labeling tanks" to the 30 large cattle tanks of the experimental array.

^{13}C: We added ^{13}C to the water in each of the cattle tanks of the experimental array daily to enrich the dissolved inorganic carbon pool available for uptake by only algae. Water was enriched at maximum to $\delta^{13}C$ 11 ‰ (2016) and 17 ‰ (2017).

4) Items in contact with ^{13}C and ^{15}N tracers
The bulk of items exposed to ^{13}C and ^{15}N tracers are sole use items of the PIs and are stored in PI-specific storage areas or were discarded in the trash. Any item exposed to tracers is labeled "^{13}C & ^{15}N tracer use only." We do not wash items used for tracer experiments in the common use sinks of the GRC. We rinse items in the field using surface water and wash items with dilute acid (5% acetic acid) at the field site. The only item of common use that was exposed to the tracers is the large convection-oven. This occurred after 4 July 2016 and 5 August of 2016 and after 4 July of 2017.

5) Assessment of contamination of the large convection-drying oven in the GRC
<u>$\delta^{13}C$ and $\delta^{15}N$ of enriched material dried in the large convection-drying oven:</u>

The $\delta^{13}C$ of material dried in the oven ranged from -12.78 to -25.33 ‰. The $\delta^{15}N$ range of material dried in the oven ranged from 90.36 to 121.72 ‰. The natural $\delta^{13}C$ of this material was -26.07 to -27.98 ‰ and the natural $\delta^{15}N$ was 3.90 to 5.71 ‰.

Sampling of drying oven: In September 2017, we collected unknown organic material deposited on the bottom of the large convection-drying oven in the GRC by wiping the bottom with acidified glass fiber filters. We then analyzed the material collected on the filters for ^{15}N and ^{13}C. The results do not indicate contamination of the large convection-drying oven in the GRC, as the values (Table 1) are within the range for natural abundance of ^{13}C (-14 to -28) and ^{15}N (4.9 to 5.5) of plant and animal material dried in the oven. The large convection-drying oven in the GRC was subsequently wiped clean with 5% acetic acid.

Table 1. Unknown material collected from bottom of the large convection-drying oven in the GRC

Sample	$\delta^{13}C$	$\delta^{15}N$
Filter 1	-27.07	4.91
Filter 2	-27.03	5.05

In August 2017, Kate Maher provided a set of unknown samples of organic matter, some of which were dried in the large convection-drying oven in the GRC during and/or after we dried enriched samples in the over, and some of which were NOT dried in the large convection-drying oven in the GRC and therefore were intended to act as a 'natural abundance' reference. Samples 29-1, 29-2, and 005 were all Aspen Pea samples of different plants within the same 1-m^2 plot collected from a hillslope by Bradley Creek. Sample 036 was *Delphenium* collected from the same location. Samples 29-1 or 29-2 were dried in different ovens (one in the large convection oven in the GRC and one in smaller oven that was not used to dry enriched samples); however, it is currently not known which sample (29-1 or 29-2) was in which oven. Nonetheless, additional information enables us to conclude that there is no evidence of enriched ^{13}C or ^{15}N sample contamination in the large drying oven in the GRC. First, among plant variation in $\delta^{13}C$ can span as much as 2 ‰. The differences among 29-1, 29-1 (Table 2), one of which was in the large drying oven in the GRC, and sample 005 are well within the range expected for this among plant variation. Second, our enriched samples dried in the large drying oven in the GRC had higher $\delta^{13}C$ values e.g., -12.78. Thus, if contamination occurred, contaminated samples would be enriched (have higher values) of $\delta^{13}C$. There is no evidence to support this conclusion. For example, interpreting sample 29-2 (-27.16 ‰) as being contaminated relative to sample 29-1 (-28.13 ‰) is invalid given that sample 005 is more 'enriched' (-27.08 ‰) and had no potential contact with our samples in the convection oven. Further, the variation among all 4 samples is within the expected range for natural abundances of $\delta^{13}C$ (-28 to -25 ‰) and $\delta^{15}N$ (-2 to 6 ‰) for plants. Moreover, the absolute values and lack variation in $\delta^{15}N$ among the three Aspen Pea samples are consistent with uncontaminated $\delta^{15}N$ for an N fixing species. It is worth noting that conclusions about contamination from the data above is limited to studies exploring variation > 1‰ given the expected natural variation and limited number of samples. However, it is also important to acknowledge that studies exploring variation < 1‰ could be easily contaminated by other materials (including other 'natural abundance' samples) besides the enriched sedge dried in the large convectional-oven in the GRC. The analytical error for individual $\delta^{13}C$ and $\delta^{15}N$ reported by the UC Davis Stable Isotope Lab is ±0.2.

In summary, there is no evidence that drying enriched samples in the large convection oven resulted in the contamination of the oven itself, or the samples subsequently dried in this oven.

Table 2. Samples provided by Kate Maher in August 2017. Either 29-1 or 29-2 was dried in large convection oven in the GRC, the other was dried in a different oven.

	Sample ID	**$\delta^{13}C$**	**$\delta^{15}N$**
Aspen pea	29-1	-28.13	1.98
Aspen pea	29-2	-27.16	1.68
Aspen pea	005	-27.08	1.74
Delphenium	036	-26.45	2.98

6) Sampling of soils from the research meadow and North Pole sites.

We collected two soil samples from the center of each cattle tank array at each site in late August 2018.

Table 3. Soil samples collected ~10 cm depth in the center of each cattle tank array at each site.

Site	**Sample ID**	**$\delta^{13}C$**	**$\delta^{15}N$**
Research meadow	785	-27.87	7.98
Research meadow	786	-26.19	10.34
North Pole	787	-27.53	3.42
North Pole	788	-27.11	4.76