**Background / References:**

This method documents the data manipulations used to produce estimates of nitrogen balances in various cropping systems at Michigan State University’s W.K. Kellogg Biological Station (KBS). Nitrogen mass balance is a useful approximation of the rate of change of surplus N in a system, the accumulation of which can lead to harmful environmental impacts like nitrate leaching and the emission of nitrous oxide gas. The following calculations use data collected at KBS from 1989 to the present, including dry-weight biomass measurements, fertilizer additions, and plant tissue carbon and nitrogen concentrations. Additionally, these calculations use literature-derived parameters like root-shoot ratios and proportions of N delivered from the atmosphere (Ndfa). See:

Blesh, J., & Drinkwater, L. E. (2013). The impact of nitrogen source and crop rotation on nitrogen mass balances in the Mississippi River Basin. *Ecological Applications*, *23*(5), 1017–1035. https://doi.org/10.1890/12-0132.1

Gelfand, I., & Philip Robertson, G. (2014). A reassessment of the contribution of soybean biological nitrogen fixation to reactive N in the environment. *Biogeochemistry*, *123*(1-2), 175–184. https://doi.org/10.1007/s10533-014-0061-4

**Objective**:

Use dry-weight harvest biomass, fertilizer additions, and plant tissue nitrogen concentration data to calculate yearly N balances for different experimental treatments and replicate blocks in KBS, on an areal basis. N mass balance is calculated as the difference between N inputs, including fertilizer application and biological N fixation, and N outputs through grain harvest. N mass balance is calculated on an areal basis. Positive mass balances indicate net N inflow and negative mass balances indicate net N outflow.

**Procedure**:

*A. Prepping for analyses:*

1. Download the following datasets from the KBS MSCE online database:
   1. Annual Crops and Alfalfa Biomass (1990 to present)
      1. Convert these values from g/m2 to kg/ha by multiplying the “Biomass” column by a factor of 10.
   2. Expanded Agronomic Log (1988 to present)
      1. Import only rows where “observation\_type” is designated as “Fertilizer application”.
   3. Cover Crop Biomass (1990 to present)
      1. Convert these values from g/m2 to kg/ha by multiplying the “biomass\_g” column by a factor of 10.
   4. Fertilizer Application Report for the MCSE (1989 to 2019)
   5. Tissue Carbon and Nitrogen (1989 to 2019)
2. Install and load the packages ‘tibble’ and ‘tidyverse’ in R to help with analyses.
3. Define the following parameters from literature, including a measure of variance (standard deviation) when available:
   1. Proportions of N derived from the atmosphere (Ndfa)
      1. Soybean, fertilized Ndfa\_soy\_bean\_fert = 0.34 (Gelfand, 2015)
      2. Soybean, unfertilized Ndfa\_soy\_bean\_unfert = 0.84 (Gelfand, 2015)
      3. Soybean, general Ndfa\_soy\_bean = 0.58
      4. Soy stover Ndfa\_soy\_stover = 0.8
      5. Soy root Ndfa\_soy\_root = 0.9
      6. Red clover Ndfa\_clover = 0.7 (Wilke, 2010)
      7. Vetch Ndfa\_vetch = 0.7
   2. Root-to-shoot ratios
      1. Soy Soy\_RS\_ratio = 0.17 +/- 0.05 (s.d.)
      2. Clover Clover\_RS\_ratio = 0.4
      3. Vetch Vetch\_RS\_ratio = 0.25
   3. Proportion of soy tissue N not from the atmosphere

Soy\_N\_nonBNF = 0.43

* 1. Soy root N concentration

Soy\_root\_N\_conc = 0.009

*B. Calculating tissue N concentrations:*

1. Using the Tissue Carbon and Nitrogen (1989 to 2019) dataset, calculate mean tissue N content for each crop species and fraction category.
   1. Sort the observations into the following crop species and fraction categories using the “species” and “type” columns:
      1. Corn – seed
      2. Corn – stover
      3. Soy – seed
      4. Soy – stover
      5. Wheat – seed
      6. Wheat – stover
      7. Rye
      8. Red clover
      9. Vetch
      10. Unsorted (includes any observation that doesn’t fall in an above category)
   2. Use the values in the “percent\_N” column to calculate mean (+/- standard deviation) tissue N content (%) for each crop species and fraction category.
   3. Multiply each mean and standard deviation value by 0.01 to convert from percentages to decimal values, for ease of future computation.
   4. Calculate the coefficient of variation (CV) for mean tissue N content for each crop species and fraction category using Equation 1.
      1. CV is the standard deviation divided by the mean.
2. Using the Tissue Carbon and Nitrogen (1989 to 2019) dataset, calculate yearly mean tissue N content values for the three main crops (Corn, Soy, and Wheat).
   1. Sort the observations into the following categories using the “species” and “type” columns:
      1. Corn – seed
      2. Corn – stover
      3. Soy – seed
      4. Soy – stover
      5. Wheat – seed
      6. Wheat – stover
   2. Disregard observations that do not fall in the above categories.
   3. Sort the observations by year using the “year” column.
   4. Use the values in the “percent\_N” column to calculate mean (+/- standard deviation) tissue N content (%) for each crop species and fraction category and year.
      1. Each year will have at most one “Seed” mean and one “Stover” mean.

*C. Calculating N inputs from fertilizer applications:*

1. Join the Expanded Agronomic Log (1988 to present; Fertilizer Application rows only) dataset with the Fertilizer Application Report for the MCSE (1989 to 2019) dataset to create a combined fertilizer dataset.
   1. Join by “Date”, “Plot”, “Material”, and “Treatment”. Ensure these columns have the same name prior to joining.
   2. Remove extraneous rows from combined dataset.
      1. Seed and herbicide applications
      2. “Custom mix” applications
      3. NA rows
      4. Rows where no nitrogen was added (N\_Rate = 0)
      5. Microplot observations
2. Add year-crop to the combined fertilizer dataset.
   1. We are defining “year-crop” as the crop harvested in a designated calendar year.
      1. e.g. 2021 is a soy year because soy was the crop harvested that year.
   2. Create a year-crop key.
      1. Create a tibble with each year present in the combined fertilizer dataset.
      2. Designate the crop that was harvested in each treatment for each calendar year.
         1. You can use the Expanded Agronomic Log dataset, with rows where “observation\_type” was “Harvest”.
   3. Join the crop year key into the combined fertilizer dataset by “Year”..
   4. Manually fill in the crop years for 1991, when corn was harvested from T1 and T2, and soy was harvested from T3 and T4.
3. Using the combined fertilizer dataset, calculate the total fertilizer N input in each year, treatment, and replicate, in Kilograms per hectare.
   1. Sort by “Year”, “Treatment”, and “Replicate”.
   2. Sum the values in the “N\_Rate” column.

*D. Calculating N inputs from biological N fixation:*

1. Using the Annual Crops and Alfalfa Biomass (1990 to present) dataset, calculate N additions due to **soybean** biological fixation for each year, treatment, and replicate using Equations 2 and 3.
   1. Calculate seed biomass.
      1. Sort observations in the Annual Crops and Alfalfa Biomass (1990 to present) dataset by year, treatment, and replicate.
      2. Select only observations where “Species” is “Glycine max L. (\*)”.
      3. Sort observations where “Fraction” is “SEED”.
      4. Calculate mean, standard deviation, and CV of values in the “Biomass” column.
   2. Calculate seed biological N fixation rates.
      1. Calculate soy seed N content by multiplying seed biomass by the soybean N concentration value calculated in Section B (seed%N).
      2. Calculate soy seed BNF rates by multiplying the soy seed N content by the correct soy Ndfa value.
         1. For all treatments, multiply by the general Ndfa value for soy.
         2. Additionally, multiply by the Ndfa value for fertilized soy in T1, T2, and T3. Multiply by the Ndfa value for unfertilized soy in T4.
   3. Calculate stover biomass.
      1. Sort observations in the Annual Crops and Alfalfa Biomass (1990 to present) dataset by year, treatment, and replicate.
      2. Select only observations where “Species” is “Glycine max L. (\*)”.
      3. Sort observations where “Fraction” is “WHOLE”.
      4. Calculate mean, standard deviation, and CV of values in the “Biomass” column.
      5. Calculate the difference between whole-plant biomass and seed biomass mean values to get mean stover biomass. Propagate error to find standard deviation and CV of stover biomass.
   4. Calculate stover biological N fixation rates.
      1. Calculate stover N content by multiplying stover biomass values by the soy stover N concentration value calculated in Section B (stover%N).
      2. Calculate stover BNF rates by multiplying stover N content by the stover Ndfa value.
   5. Calculate root biomass.
      1. Multiply whole-plant aboveground biomass (calculated in Step c.) by the root-to-shoot ratio for soy.
   6. Calculate root biological N fixation rates.
      1. Calculate root N content by multiplying root biomass values by the soy stover N concentration value defined in Section A.
      2. Calculate root BNF rates by multiplying root N content by the root Ndfa value.
   7. Calculate overall biological N fixation rates using Equation 3
      1. For our purposes, we will be focusing on non-seed BNF rates. These are calculated by summing stover and root BNF rates.
2. Using the Cover Crop Biomass (1990 to present) dataset, calculate N additions due to **red clover and vetch** biological fixation for each year, treatment, and replicate using Equations 3 and 4.
   1. Sort observations by year, treatment, and replicate.
   2. Calculate mean, standard deviation, and CV values for aboveground biomass of red clover and vetch.
   3. Multiply by tissue N concentrations calculated in Section B.
   4. Multiply by Ndfa values defined in Section A.

*E. Calculating N exports from grain harvest:*

1. Using the Annual Crops and Alfalfa Biomass (1990 to present) dataset, calculate mean N content in agricultural harvest for each crop species and year, treatment, and replicate.
   1. Calculate yield biomass.
      1. Sort observations by year, treatment, and replicate.
      2. Filter only observations where “Fraction” is “SEED”.
      3. Calculate mean and CV values of the “Biomass” column for each crop species (corn, soy, and wheat).
   2. Calculate N export of corn and wheat yield using Equation 4.
      1. For corn and wheat, multiply mean biomass values with the corresponding seed tissue N concentration values calculated in Section B.
   3. Calculate N export of soy using Equation 5.
      1. Multiply mean biomass values with the corresponding soybean tissue N concentration calculated in Section B.
      2. Also, multiply by a factor of 0.43, which is the proportion of soy tissue N not from biological N fixation.

*F. Calculating N balances:*

1. Calculate N balances for each year, treatment, and replicate combination using Equation 6.
   1. All above calculations can be joined together into one large tibble to enable ease of data viewing and processing.
   2. Make sure all NA values are replaced with zeroes before summing components.

**Calculations**:

Equation 1:

Equation 2:

Equation 3:

Equation 4:

Equation 5:

Equation 6: