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Crop metrics for nutrient management research in corn-based cropping systems

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Abstract

This protocol was used by a collaborative research team referred to as the “NutriNet Project” in corn-based cropping systems of the U.S. Midwest and Canada. Plant measurements were collected from field-based experiments to determine aboveground biomass and nutrient concentration at different crop development stages. Corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.) are the cash crops and cereal rye (*Secale cereale* L.) is the cover crop described in this protocol. The total amount of nutrient uptake is calculated based on the plant biomass and nutrient concentration measurements. Nutrient analysis was conducted using dried, ground plant samples, separated into grain and non-grain components when applicable. Ancillary measurements, such as chlorophyll meter readings and corn stalk nitrate concentrations, are also described as these provide important context for informing nutrient management decisions. Corn was the dominant crop and focus of the project team when collecting measurements; this is evident by the detail describing corn in this protocol.

Troubleshooting

Random plant sampling

1 For measurements that require a subset of plants to be collected from a plot, a method for random plant sampling is recommended. This method will reduce unintentional bias when selecting plants, as those who are sampling may inadvertently select the larger or healthier plants, skewing the results.

1.1 Generally, a subset of plants is collected to comprise the random sample.

1.2 The main goal of random sampling is to remove decision-making by field personnel. To this end, practitioners predetermine the plants to sample:

- A random number generator can be used to determine the plant selection.
- Alternatively, an established sequence of numbers can be followed. For example: *(1) if a 6-plant sample is desired, collect plants numbered 7, 14, and 21 from rows 2 and 3, or (2) if a 20-plant sample is desired, collect the even numbered plants such as 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 from row 2 and #30, 32, 34, 36, 38, 40, 42, 44, 46, 48 from row 3.*
- Alternatively, drop a rod in a random location in the field and sample all plants in the row along the length of the rod. One experimental site used a rod 5.2 m long.
- Exceptions exist when following these systematic, random approaches such as skipping severely stunted plants whose height is less than half of neighboring plants or plants that prematurely died.

1.3 For area-based plant sampling, plants are randomly sampled from (1) a predetermined number of rows along a specified row length within the plot or (2) within a 0.5–1 m^2 frame (quadrat) dropped or placed at random in 2–5 locations in the plot to identify areas to be sampled. This area-based approach is generally used when rows cannot easily be identified such as solid-seeded soybean. Most importantly, when collecting samples from a smaller area, ensure it can be appropriately scaled to a meaningful area basis (such as hectare).

Note

At one location, aboveground biomass was randomly collected from a 1-m^2 area at five locations in each plot. This result was then scaled up to represent the whole plot on a hectare basis.

Note

At another location, plants were harvested from one meter of a row at two locations within each plot. The number of plants harvested was counted to calculate plants per m^2 based on the 0.76-m row spacing.

Note

When sampling cover crops, another location clipped all cover crops at the soil surface within a 0.76 m × 0.50 m frame placed at three random locations within each plot. This frame was centered on the row of the previous crop with the long side perpendicular to the row direction. Samples were dried at 60 °C and weighed; cover crop shoot biomass dry weights were then scaled up to the per-plot or per-hectare value using the size of the sampling frames.

Collection of corn measurements

2 Use the standard reference for corn development staging (Abendroth et al., 2011).

3 Record the seedling emergence date as the day when 50% of plants have emerged through the soil surface (Anderson et al., 2025a).

4 **Corn measurements at vegetative stage 6 (V6)**

4.1 Collect aboveground dry biomass at V6 +/- 1 stage.

Note

This sampling can be performed during the same period when sampling is carried out for the “Late Spring Nitrate Test” (LSNT) (Sawyer and Mallarino, 2017). The LSNT occurs before sidedress or topdress applications of nitrogen fertilizer are made.

4.2 Use a random sampling method as described in Section 1 above.

4.3 Collect 5–10 plants per row except in scenarios where more plants may be needed to best represent the spatial variability. If possible, do not remove plants from rows that will later be harvested for grain. The removal of plants from harvest rows must be accounted for later when calculating yield.

4.4 Cut plants at the soil surface using a sharp tool. Remove residual soil from the plants. Place plant samples into properly labeled paper or cloth bags of the appropriate size.

4.5 Oven dry samples at 60 °C as soon as possible. Forced-air dryers are preferred. Open the bags to expedite drying. Large samples should be mixed during drying. Samples should be dried until constant weight is reached and weighed to determine dry weight. Record the number of plants in the bag so results can be expressed per plant if desired.

4.6 After drying, each biomass sample is ground in a mill (e.g., Wiley mill, Arthur H. Thomas Co., Philadelphia, PA) until it can traverse a 1-mm sieve or smaller, homogenized, and a subsample collected for nutrient analysis.

4.7 Prepare samples for nutrient analysis of corn biomass. Only the concentration of nitrogen in the sample is needed at this time.

- Nitrogen analysis is performed with a dry combustion CN analyzer using certified plant standards and the concentration expressed as g N kg^{-1} dry biomass.

5 For each treatment, record the silking date as when one or more silks have emerged from the uppermost ear of 50% of plants. Be aware that in modern hybrids, tasseling (VT) may not occur before the silks appear (R1) (Abendroth et al., 2011). Document the developmental stage based on silk appearance, not the emergence of the tassel.

6 **Corn measurements at reproductive stage 1 (R1, silking)**

6.1 Record chlorophyll meter readings of ear leaves at R1 (silking). An ear leaf is the leaf attached to the node where the ear shoot is developing. This measurement is ancillary and will help inform nutrient status of the crop (Scharf et al., 2006).

- In the sampled rows of each plot, collect one reading from 20 plants. Use a random sampling method as described in Section 1 above.
- A handheld "SPAD" meter is used in-field for measuring the chlorophyll content (Konica Minolta, Inc.)
- Collect the SPAD meter readings midway between the tip and base of the leaf blade centered between the midrib and leaf edge.

6.2 Collect ear leaves at R1.

- Randomly collect 20 ear leaves from the plot. The best approach is to collect the same leaves used for SPAD readings.
- Place the leaves in a labeled paper bag of the appropriate size.

6.3 Oven dry the samples at 60 °C as described in Section 4.5.

6.4 After drying, process the samples as described in Section 4.6.

6.5 Nutrient analysis of ear leaves at R1. The concentration of nitrogen, phosphorus, and potassium should be determined on these samples.

- Nitrogen analysis is performed with a dry combustion CN analyzer using certified plant standards and the concentration expressed as g N kg^{-1} dry biomass. Cavigelli and Strickland (2024) provide a step-wise methodology.
- Phosphorus analysis is performed with ICP after microwave digestion (or another acceptable analytical method) and the concentration expressed as g P kg^{-1} dry biomass. Kovar and Fortuna (2024) provide a step-wise methodology.
- Potassium analysis is performed with ICP after microwave digestion (or another acceptable analytical method) and the concentration expressed as g K kg^{-1} dry biomass.

7 Corn measurements at physiological maturity (R6)

7.1 Collect aboveground biomass

Plants should be sampled as soon as possible after reaching R6 (Abendroth et al., 2011) to minimize loss of biomass as well as loss of nutrients out of the biomass via leaching. Loss of non-grain biomass can occur due to senescence, high-wind events, and so forth that cause the necrotic plant material to separate from the stalk.

Note

Physiological maturity (R6) occurs at approximately 350 g kg^{-1} (35%) grain moisture while the visible “black layer” occurs at ~280 g kg^{-1} (28%) grain moisture at approximately 2 weeks after physiological maturity. You can predict the approximate calendar date when R6 will occur by monitoring the progression of R5 (“kernel denting”). Approximately ~30 days from the start of R5 is needed to reach R6 (see Table 3 on page 38 in *Corn Growth and Development,* Abendroth et al., 2011).

- Use a random sampling method as described in Section 1 with the following exception: avoid very small plants or barren plants.
- Collect 5–10 plants except in scenarios where more plants may be needed to best represent the spatial variability. If possible, do not sample from rows that will later be harvested for grain yield. Otherwise, you will need to subsequently account for these removed plants (grain) when calculating yield. Make sure to note how many plants were sampled and whether or not they come from harvest rows.
- Cut plants at the soil surface using a sharp tool. Remove residual soil from the plants. Place plant samples into properly labeled paper or cloth bags of the appropriate size.

7.2 Separate plant components

- All non-grain components will constitute the vegetative fraction: leaves, stalk, tassel, ear husks, ear shank, and cob.
- Grain is shelled from the cob with the cob then added to the non-grain fraction. Depending on the grain moisture, it may be necessary first to dry the ear at 60 °C and remove the grain from the cob.
- Cobs are difficult to grind and to homogenize in the right proportion with the remaining non-grain fraction. They can be kept separate during initial processing if needed.

7.3 Dry plant samples at 60 °C as described in Section 4.5.

- Depending on lab and oven space, groups may modify sample processing by:
  - Drying non-grain components and collecting a subsample
  - Obtaining a representative subsample in the field before drying and processing.

The fresh weight of the sample and the fresh weight of the subsample need to be reported as this will be necessary to calculate the dry weight of the sample later. Also, if the subsample is collected in the field, it is important to note that cobs are not included in the sample. The cob fraction will need to be accounted for later.

- Anytime that cobs are not included in the non-grain fraction, it is important to know the dry weight of the cobs relative to the dry weight of the rest of the non-grain components. This will help you in summing total dry weight and also the nutrient content.

- Collect the dry weight of the grain fraction and the non-grain fraction.

7.4 Prepare samples for nutrient analysis of non-grain plant components (leaves, stalk, tassel, ear husks, ear shank, cob).

- After drying, process the samples as described in Section 4.6.
- Nitrogen, phosphorus, and potassium analysis is performed as described in Section 6.5 above.

- Cobs mill differently than other tissues, making it challenging to obtain a representative sample. If the cob is not included in the non-grain subsample, an estimate of nutrient content can be obtained to estimate nutrient per hectare. The NutriNet project used the values in Table 1 in a few instances although these are unpublished values. For published values, refer to the following papers: see Table 1 in Karlen et al. (2015), Sindelar et al. (2015), and Jansen et al. (2012).
- Because cobs are difficult to grind and did not comprise a large percent of the aboveground biomass, some sites chose to exclude them from the total vegetative nutrient values reported.

	A	B	C	D
		Nitrogen (N)	Phosphorus (P)	Potassium (K)
	Concentration in cob (g kg-1)	3.45	0.23	5.7
	Content in cob (kg ha-1)	5.6	0.35	9.4

Table 1. Nutrient concentration and content estimations for the amount of nitrogen, phosphorus, and potassium within the cob fraction (John Sawyer, unpublished data). These cob values need to be combined with the non-grain component to arrive at a final value that represents the total non-grain biomass.

7.5 Prepare samples for nutrient analysis of grain component

- Use the grain from the plants sampled at the R6 stage (physiological maturity).
- In addition, a subsample from the whole-plot, machine-harvested grain can also be collected for nutrient analysis if desired. Harvest methods are described in Section 7.7 *below*.
- Oven dry samples at 60 °C as described in Section 4.5.
- After drying, process the samples as described in Section 4.6.
- Nitrogen, phosphorus, and potassium analysis is performed as described in Section 6.5.

7.6 Collect lower stalk subsamples

A corn stalk nitrate test is conducted on the lower stalk. Approximately 8 inches of stalk immediately above the brace roots can be collected after physiological maturity but before machine harvest. Collect 12–15 stalks for nitrate analysis (Sawyer and Mallarino, 2018; Brouder 2003).

- If the stalks come from the harvest row, save the ears for use as an R6 grain sample. Make sure to add their grain mass into the plot grain yield results obtained from the combine or other grain harvesting equipment.
- If the stalks come from a non-harvest row, the ear does not need to be retained.

7.7 Harvest the grain from a predetermined area using a combine or manually (hand) harvesting and record the grain fresh weight and moisture content. Moisture content can be obtained using sensors mounted within the combine or a hand-held moisture sensor. Calculate grain yield at a standard moisture basis or dry weight basis as outlined in Section 15.2, see Calculations 4 and 6.

7.8 Record plant population

Count the number of plants from each harvest row for small plots. For large plots, determine plants per hectare in several representative areas and calculate the average.

Note

This measurement will provide an estimate of plant population used for the calculations in Section 14, Calculation 1 (plants ha^{-1}).

7.9 Measuring corn stover removal

For the NutriNet project, corn stover is not removed from the field except in a few instances. When it is removed, this is to simulate its use for silage or cellulosic (bioenergy) markets. In these cases, record as the percent of stover removed.

Collection of soybean measurements

8 Use the standard reference for soybean development staging (Pederson, 2014). Notice that eight reproductive stages occur in soybean compared to six for corn.

9 Record the seedling emergence date as the date when 50% of plants have emerged through the soil surface (Anderson et al., 2025b).

10 **Soybean measurements at reproductive stage 6 (R6, full seed, not mature)**

Note

Although peak plant N accumulation occurs by the late R6 development stage, biomass accumulation and partitioning of dry weight into the seed is not complete at R6. Therefore, sampling at R8 is needed in tandem with the R6.

The methodology described here for R6 will provide biomass and nutrient content for the whole plant. There is no separation of the non-grain and grain fractions described here. If detailed measurements of soybean biomass and nutrient allocation are desired, we recommend users separate the fractions by drying and threshing plants to separate the green seeds from their pods at R6. This approach will enable a more complete understanding of biomass and nutrient allocation between the non-grain and grain fractions in soybean.

10.1 Aboveground dry biomass is collected at R6 (full seed, not mature). Plants should be sampled as soon as possible after reaching R6 to minimize losses of dry matter and nutrients that occur as plants senescence and leaf fall to the soil ensues.

10.2 Use a systematic random sampling method to collect multiple subsamples per plot as described in Section 1. We recommend two or three 0.3-m row lengths per plot (minimum) or an equivalent area when plots are seeded with a drill or broadcasted. If possible, do not sample from final harvest rows; otherwise, make sure to note how many plants were sampled. You will subsequently account for this quantity when calculating yield.

10.3 Cut plants at the soil surface using a sharp tool. Remove residual soil from the plants. Place plant samples into properly labeled paper or cloth bags of the appropriate size.

10.4 Oven dry samples at 60 °C as described in Section 4.5 above.

10.5 Prepare samples for nutrient analysis of all plant components (stem, leaves, pods)

- After drying, do not separate stems, leaves, and pods unless the nutrient concentrations of these tissue components are of interest.
- Process the samples as described in Section 4.6.
- Nitrogen, phosphorus, and potassium analysis is performed as described in Section 6.5.

11 Soybean measurements at reproductive stage 8 (R8, physiological maturity)

11.1 Harvest the grain

Harvest the grain from a predetermined area using a combine or manually (hand) harvesting and record the grain fresh weight and moisture content. Determine moisture content using a grain moisture sensor. Calculate grain yield at a standard moisture basis or dry weight basis as outlined in Section 15.2, see Calculations 5 and 6.

11.2 Collect a grain sample

- Oven dry component samples at 60 °C as described in Section 4.5.
- After drying, process the samples as described in Section 4.6. A flour mill may be helpful to grind the grain.
- Nitrogen, phosphorus, and potassium analysis is performed as described in Section 6.5.

Collection of cover crop measurements

12 Aboveground biomass of cover crop samples are collected before a killing frost (applicable for non-winter hardy cover crops), termination, or harvest for sale. One biomass measurement per season is the minimum requirement.

12.1 Collect multiple subsamples per plot. Area-based sampling is most likely used for cover crops given they were broadcast seeded or drilled in narrow rows. Area-based sampling is described in Section 1.

12.2 Cut plants at the soil surface using a sharp tool. Remove residual soil from the plants. Place plant samples into properly labeled paper bags of the appropriate size.

12.3 Oven dry samples at 60 °C as described in Section 4.5 above.

12.4 Weigh dry samples and use the area sampled to calculate the dry biomass of cover crop as kg ha^{-1}. See Calculation 2.

12.5 Nutrient concentration of aboveground biomass

- After drying, process the samples as described in Section 4.6.
- Nitrogen, phosphorus, and potassium analysis is performed as described in Section 6.5.

12.6 If the cover crop or cash crop happens to be removed for use as hay or silage, record the proportion of the aboveground biomass removed. Also record cover crop stubble height

after cutting.

Calculations

13 This section includes helpful calculations for grain yield, non-grain biomass, and nutrient content.

> Note
>
> When these calculations are performed, the weights of any plants removed from harvest rows in previous sampling events must be accounted for.

14 **Calculate aboveground biomass (non-grain and grain) to an area basis (kg ha^{-1})**

14.1 For corn when collected as a subset of plants randomly:

Calculation 1.
Biomass (dry, kg ha^{-1}) = Oven-dried biomass (kg $plant^{-1}$) * Plant population (plants ha^{-1})

14.2 For soybean and cover crops when collected as a subset using a quadrat:

Calculation 2.
Biomass (dry, kg ha^{-1}) = (Oven-dried biomass (kg) / sampling area (m^2)) * 10,000 m^2 ha^{-1}

15 **Calculate grain yield and moisture from machine-harvested samples**

15.1 Calculate moisture content at time of harvest using wet (fresh) and dry weights

If the moisture of grain is not known, this can be obtained at the time of harvest manually. Collect the wet weight of the grain subsample in the field, measure instantly or store in air-tight container and measure later, dry at 60 °C, and then collect the dry weight of the same grain subsample.

Note

When we use the term "dry weight", it is important to mention that it is not possible to get all moisture out of the grain unless incinerated at very high temperatures. The reference to "dry" in Calculation 3 and those that follow are widely accepted and used in the scientific community but with the knowledge that grain is not truly at 0.0% moisture.

Enter these weights into Calculation 3 to determine the moisture content at time of harvest. This will be needed to modify the grain yields to a standard moisture basis and/or dry weight.

Calculation 3.
Grain moisture = [(Grain weight $_{wet}$ - Grain weight $_{dry}$) / Grain weight $_{wet}$]

15.2 Determine if you want to report grain yield adjusted to the standard moisture basis (MB) set by the market for corn and soybean or reported at dry weight. When grain yields are reported, it is important to explicitly state the moisture content.

Note

The term "grain yield" is equivalent to "grain weight" when working in the metric system. In metric, we are concerned only with the weight of grain coming from an area. This is evident based on the units used, which are kg ha^{-1}. All calculations included below are for the metric system.

However, the terms "grain yield" and "grain weight" are not equivalent when working in the US system as this is based on a volume basis. In this system, crops are reported as pounds per bushel. For example, corn is defined as 56 pounds per bushel while soybean is defined as 60 pounds per bushel. For further explanation on this system, refer to Nielsen (2024).

Calculate grain yield to the standard moisture basis (MB). Corn is reported at 15.5% MB (155 g kg^{-1}) while soybean is at 13.0% MB (130 g kg^{-1}).

Calculation 4 (for corn).
Grain yield (kg ha^{-1}) at 15.5% MB = [Grain yield (kg ha^{-1}) $_{wet}$ * (1 - Grain moisture (g kg^{-1}))] / (1 - 0.155)

Calculation 5 (for soybean).
Grain yield (kg ha^{-1}) at 13.0% MB = [Grain yield (kg ha^{-1}) $_{wet}$ * (1 - Grain moisture (g kg^{-1}))] / (1 - 0.130)

Calculate grain yield to dry weight basis. This step will result in grain yield reported at 0.00 g kg^{-1} (0%) moisture ("dry").

Calculation 6.
Grain yield (dry, kg ha^{-1}) = Grain yield (kg ha^{-1}) $_{wet}$ / (1 - Grain moisture (g kg^{-1}))

16 **Calculations for nutrient content (kg nutrient ha^{-1})**

Analyses for nitrogen, phosphorus, potassium, and other nutrients will typically be reported as a concentration, such as grams per kilogram of dry biomass. The amount of that nutrient on an area basis requires the biomass (grain or non-grain) to be multiplied by the nutrient concentration.

16.1 Nutrient content for non-grain fraction or whole plant

For this calculation, you will need the dry weight of the non-grain component.

Calculation 7.
Non-grain nutrient content (dry, kg nutrient ha^{-1}) = Non-grain weight (dry, kg ha^{-1}) * Nutrient concentration (g kg^{-1}) * (1 kg / 1000 g)

16.2 Nutrient content for grain fraction

For this calculation, you will need the grain yield reported at dry weight.

Calculation 8.
Nutrient content (dry, kg nutrient ha^{-1}) = Grain yield (dry, kg ha^{-1}) * Nutrient concentration (g kg^{-1}) * (1kg / 1000 g)

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