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# Estimating Nitrogen Harvest Index (NHI) in field crop trials using a LECO analyzer

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Salvador Osuna-Caballero[1], Emily Pearce[1], Ronald A Caicedo-Garcia[1], Derek Wright[1], Kate Congreves[1], Curtis Pozniak[1], Kirstin E. Bett[1]

[1]Department of Plant Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

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Salvador Osuna-Caballero
University of Saskatchewan



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## Abstract

Nitrogen harvest index (NHI) is a ratio of N accumulated in grain to N accumulated in grain plus straw. NHI is an important index in determining crop yield because it is positively associated with grain yield. Furthermore, it is a useful metric for quantifying variability in N allocation in plant populations, offering the possibility of breeding crops to improve the protein content of grain or straw.
This research protocol is for the estimation of NHI in lentil by measuring the nitrogen content in seeds and straw from an alpha lattice design experiment using a LECO analyzer. A LECO analyzer combusts samples at high temperatures (~ 1000 °C ) in an oxygen-rich environment, converting nitrogen to $N_2$ gas, which is measured to determine N concentration.
The procedure encompasses six steps from field sampling to the final NHI calculation for each experimental unit.

## Image Attribution

All rights for the ACTIVATE project logo remain with the researchers who are part of the GenomeCanada ACTIVATE grant.

## Guidelines

**Guidelines for Precise Execution:**

- Standardization: Use consistent row lengths and sampling points across all plots to ensure comparability.
- Sample Integrity: Avoid contamination by cleaning equipment (thresher and grinder) between samples and using separate tools for seeds and straw if possible.
- Calibration: Calibrate the LECO analyzer daily with standards and run blanks/checks every 10-20 samples to maintain accuracy.

## Materials

**Labels:**

- Labels with information with year, location, plot number, and other pertinent information.
- 1 set for paper bags for sample collection.
- 1 smaller set for snap cap vials.
- Barcodes can be included if useful.

**Field Sampling**:

- Meter stick.
- Harvesting knife or shears.
- Labelled sample bags (e.g., paper or breathable cloth bags).

**Sample Processing**:

- Drying oven (capable of maintaining 60 - 70 °C).
- Threshing method (manual or small mechanical).
- Precision balance (0.1 g resolution).
- Grinder or mill (1 mm sieve).
- Plastics snap cap vials (the size depend on the amount sampled).

**Nitrogen Analysis**:

Equipment

| | |
|---|---|
| LECO CN628 | NAME |
| Carbon and Nitrogen Analyzer | TYPE |
| LECO | BRAND |
| 622-000-100 | SKU |
| https://www.leco.com/products/828-series/ | LINK |

PDF

EDTA 50G CALIBRATION SAMPLE **Laboratory Equipment Company Catalog #**502-896

Equipment

Secura® Analytical Balances with Internal Adjustment

NAME

Analytical Balances

TYPE

Sartorius

BRAND

SECURA124-1S

SKU

https://shop.sartorius.com/ca/p/secura-analytical-balances-with-internal-adjustment-01-mg/Analytical-Balance-SEC#

LINK

Equipment

Tin Foil Cups

NAME

36 × 36mm LECO Corp.™ Tin Foil Cups, Non-Sterile, 1000/pack

TYPE

LECO

BRAND

502186100

SKU

https://www.fishersci.com/shop/products/tin-foil-cups-1000-bottle/nc9882067

LINK

## Protocol materials

EDTA 50G CALIBRATION SAMPLE **Laboratory Equipment Company** **Catalog #**502-896

## Safety warnings

Field Sampling:
- Wear gloves and protective clothing to avoid injury from sharp tools.
- Avoid sampling during adverse weather (e.g., high temperatures during summer).

Drying Oven:
- Use heat-resistant gloves when handling hot samples.
- Do not exceed 70°C to prevent thermal decomposition of nitrogen compounds.

Grinding:
- Wear a dust mask and safety goggles to avoid inhaling fine particles or eye irritation.
- Ensure the grinder is unplugged during cleaning to prevent accidental activation.

LECO Analyzer:
- Follow manufacturer safety guidelines.
- Handle oxygen supply with care due to the risk of fire/explosion.

## Before start

- Sampling material: Ensure all the material required for plant sampling is ready; including paper bags, labels, meter stick and harvesting knife or shears. Pre-printed labelled bags with barcode identifiers are encouraged to improve traceability for future sample processing steps.
- Training: Personnel must be trained in field sampling, sample processing, and LECO analyzer operation. Review the LECO manual and safety data sheets (SDS) for equipment and calibration standards.
- Equipment Check: Verify that the drying oven, thresher, grinder, precision balance, and LECO analyzer are functional. Ensure the LECO has adequate oxygen supply and clean combustion tubes.
- Timing: Schedule field sampling for a single day at crop maturity to minimize variability. Confirm weather conditions allowing a safe fieldwork environment.
- Documentation: Prepare a digital spreadsheet to record plot identifiers, sampling details, weights, and nitrogen results.

## Plant Sampling

1 **Identify Plots:**

- Locate each experimental unit (herein after called a *plot*) within your trial design. Take into account all your experimental design levels (block, row, column, replicate and plot) identifiers to sample accurately.

2 **Determine Sampling Points**:

- Avoid plot edges such as outer rows to minimize edge effects.
- Within each plot, use a meter stick to measure the length accurately to identify the plants to be sampled (Figure 1).

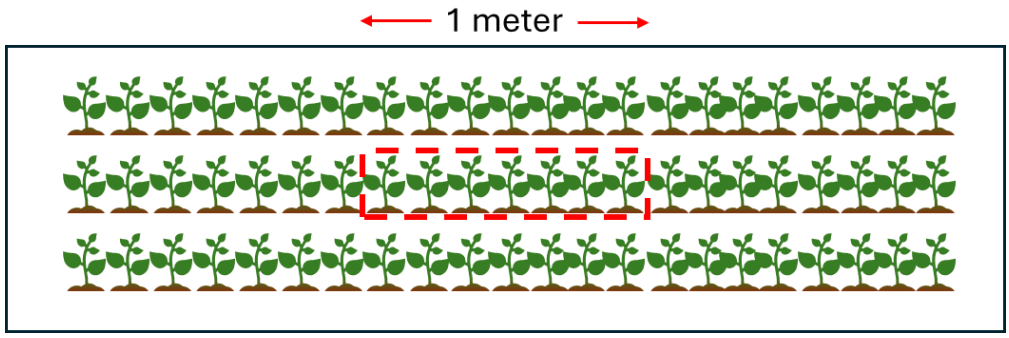


Figure 1. Representation of a plot highlighting the sampled area in dashed red square identified by the meter stick.

3 **Harvest Samples:**

- Harvest a 1 m length of row from the centre rows as highlighted in Figure 1.
- Cut the plants at the base (near soil level) using a harvesting knife or shears to collect the entire above-ground biomass.

4 **Collect and Label:**

- Place the harvested plants within the plot into labelled paper/cloth bags.
- To prevent sample loss, the bags can be closed with a knot or a tie.
- Keep samples separate by plot and record the block, plot number, and sampling point for traceability.

Note

- Pre-printed labelled bags with barcode identifiers are encourage to improve traceability for future sample processing steps.
- Harvest all plots on the same day when the majority of the crop reaches harvest maturity to standardize the growth stage.
- If plot sizes are small, adjust the sampling area (e.g., 0.5 m length ) to ensure feasibility, but always maintain consistency across plots.

## Drying

5 **Dry Samples:**

- Place the harvested plants, in their paper bags, into a drying oven set at 60-70 °C .
- Dry until a constant weight is achieved. Typically 48:00:00 - 72:00:00 , depending on sample size and original moisture content.

Note

- Ensure oven temperature does not exceed 70 °C to avoid N loss or plant material degradation.

## Threshing

6 **Thresh Samples:**

- Thresh the dried plants from each plot to separate seeds from straw (above-ground biomass excluding seeds).
- Manually thresh (e.g., rubbing by hand in a pan) or use a small mechanical thresher, depending on available resources.
- Collect seeds and straw separately from each sample within the plot. Pre-labelled paper envelopes for seeds and pre-labelled paper bags for straw samples are required to safely store each sample type.
- Samples can be stored at Room temperature until weighing them (step 7).

Note

- Ensure no seed or straw material is lost during threshing.
- Clean threshing equipment between samples to prevent cross-contamination.

## Weighing

7 **Weigh Samples:**

- Weigh the seeds and straw from the previous step separately from each sample using a precision balance (with a 0.1 g min resolution).
- The dry weights of seeds and straw within a plot give you the total seed dry weight ($W_{seed}$) and straw dry weight ($W_{straw}$),respectively, in grams.

Note

- Record weights meticulously, associating them with the correct plot identifier.

## Sample Preparation

8 **Sample processing:**

- Grind the seed and straw samples separately (at least 20 g ) into a fine powder using a grinder or mill. Filter the powder using a 1 mm sieve to exclude large, undesired particles.
- Store ground samples in snap cap vials (properly labelled) to prevent moisture uptake. These labelled vials can be stored at Room temperature until LECO analysis.

Note

- Ensure the grinder is cleaned between samples to avoid contamination.
- Fine grinding is critical for LECO analysis to ensure complete combustion.

## Nitrogen Analysis

9 **Calibrate the LECO analyzer:**

- Turn on the LECO analyzer and allow it to stabilize following manufacturer's instructions.
- Open the oxygen, helium and $CO_2$ gas cylinders.
- Run blank samples and check results are zero.
- Run calibration standards using EDTA with known % N to establish a calibration curve:

EDTA 50G CALIBRATION SAMPLE **Laboratory Equipment** **Company** **Catalog #**502-896

- Verify calibration with a check standard; adjust if drift exceeds acceptable limits (e.g., ±0.1 % N).
- After proper calibration the sub-samples (step 10) can be load into the instrument for analysis.

Note

Our calibration standard:

EDTA 50G CALIBRATION SAMPLE **Laboratory Equipment Company Catalog #**502-896

Contains: 40.8 to 41.2 % Carbon, 5.46 to 5.54 % Hydrogen, 9.52 to 9.6 % Nitrogen

10 **Prepare Sub-samples for analysis:**

- Shake by hand the snap cap vial of each sample to ensure homogenization of the ground material.
- Weigh 100 mg from each seed or straw powder sample into a tin foil cup using an analytical balance (Figure 2).

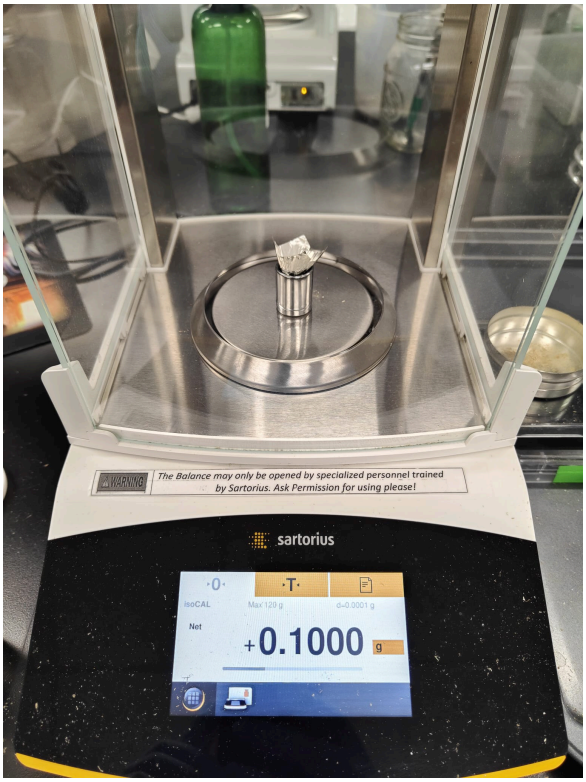


Figure 2. Weighing 100 mg of powdered sample into the aluminum foil capsule using an analytical balance.

- Print the measured value into the LECO software system along with the sample identifier and the carousel position where the sample will be placed.
- Once the sample is weighed into the tin foil cup, gently tap the cup to dislodge any particles adhering to the inner walls, ensuring the entire sample settled at the bottom.
- Close the cup by crimping it into a teardrop shape, and then fold the tip over to secure the seal and prevent any sample leakage (Figure 3).


Figure 3. Example of a processed LECO sample.

- Immediately after, place the sample in that same carousel position (Figure 4).


Figure 4. LECO carousel where samples are loaded.

11 **Analyze Nitrogen Content:**

- Carefully, load the carousel on the LECO analyzer.
- Run the analysis: The LECO combusts the sample, measures $N_2$ gas, and reports nitrogen concentration (% N) on all the samples loaded in the carousel one by one.
- Each output sample is automatically printed in the same spreadsheet on the LECO software where the weighing and the sample identifiers were entered, following same carousel numbering.
- Record nitrogen concentration for seeds (N $\%_{seed}$) and straw (N $\%_{straw}$) for each sample in a different Excel spreadsheet for future data analysis.

12 **Quality Control:**

- Run a blank (empty tin foil cup) and a replicate standard periodically (e.g., every 10-20 samples) to check for contamination or drift.
- Consider analyzing duplicates of 10% of samples to assess precision; then average duplicates for final values.

## Calculations

13 **Calculate Total Nitrogen:**

- Total Nitrogen in seeds ($N_{\text{seed}}$, g):

$$N_{\text{seed}} = \left( W_{\text{seed}} \times \frac{N\%_{\text{seed}}}{100} \right)$$

- Total Nitrogen in straw ($N_{\text{straw}}$, g):

$$N_{\text{straw}} = \left( W_{\text{straw}} \times \frac{N\%_{\text{straw}}}{100} \right)$$

14 **Calculate NHI:**

- Nitrogen Harvest Index:

$$\text{NHI} = \frac{N_{\text{seed}}}{N_{\text{seed}} + N_{\text{straw}}}$$

Note

- NHI is a ratio, so sampled area normalization is unnecessary unless absolute nitrogen uptake is required.

## Protocol references

Citation

Fageria, N. K. (2014). NITROGEN HARVEST INDEX AND ITS ASSOCIATION WITH CROP YIELDS. Journal of Plant Nutrition.

10.1080/01904167.2014.881855 LINK

## Citations

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