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Quantifying the uncertainty in critical N concentration for potato using Bayesian methods.

Brian J. Bohman1, Michael J. Culshaw-Maurer2, Carl J. Rosen1\*, David J. Mulla1, Feriel Ben Abdallah3, Claudia Gilletto4, and ???...

1 Affiliation 1; e-mail@e-mail.com

2 Affiliation 2; e-mail@e-mail.com

\* Correspondence: e-mail@e-mail.com; Tel.: (optional; include country code; if there are multiple corresponding authors, add author initials)

**Abstract:** Multiple critical N dilution curves [CNDCs] have been previously developed for potato; however, attempts to directly compare differences across genotype [G] and environment [E] interactions have been confounded by non-uniform statistical methods and lack of proper quantification of uncertainty in critical N concentration and CNDC parameters. This study implements a hierarchical Bayesian framework to develop CNDCs for previously published and newly reported experimental data to systematically evaluate the difference between critical N concentration across G x E interactions. Differences in critical N concentration are primarily the result of differences in environmental factors (i.e., location) while genotype (i.e., variety) can cause differences within a given location. Additionally, the uncertainty range in critical N concentration should be used in subsequent, dependent calculations (e.g., N nutrition index) to propagate and account for uncertainty. The findings of this study provide additional evidence that critical N concentration is dependent upon G x E interactions.

**Keywords:** critical N dilution curve, nitrogen nutrition index, potato, Bayesian statistics

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1. Introduction

Identifying optimal crop N status to maximize growth and yield production is an elusive goal. Traditionally, either the yield-goal approach or rate-response curves have been used to identify optimal N fertilizer application rate (Morris, et al., 2018). The N nutrition index [NNI] is an alternative approach to the current paradigm and comprises a well-developed framework to determine optimal crop N status (Lemaire, et al., 2019). Typically, NNI is used to determine crop N status using whole plant analysis and to direct adaptive N management within a growing season (Houlès, et al., 2007, Morier, et al., 2015). Unlike the yield-goal or rate-response approach, NNI is generalizable across environmental (e.g., year-to-year or geographic) variability (Sadras and Lemaire, 2014).

The NNI approach is defined based on the allometric relationship of declining N concentration with increasing biomass, referred to as the critical N dilution curve [CNDC], which defines the level of N concentration below which relative growth rate is reduced (Gastal et al., 2015). A robust theoretical framework has been developed to explain decline in N concentration as biomass increases, but the application of this theory is restricted to the vegetative period (Sadras and Lemaire, 2015; Greenwood et al., 1990; Justes et al., 1994). Dilution of N in vegetative tissue occurs in relationship to an increasing proportion structural biomass, with low N concentration, relative to metabolic (i.e. photosynthetic) biomass, with high N concentration (Lemaire and Gastal, 1997; Gastal et al., 2015).

Multiple previous studies have extended and empirically validated the CNDC relationships beyond its original theoretical justification to describe declining N concentration in relationship to whole plant biomass over the entire crop growth cycle (Plénet and Lemaire, 2000; Duchenne et al., 1997; Greenwood et al., 1986; Herrmann and Taube, 2004). Dilution of N beyond the vegetative period occurs as low N biomass (i.e. starch) accumulates in storage tissues such as grain or tubers, and the rate of decline is determined by the relative N concentration in storage biomass compared to vegetative biomass (Plénet and Lemaire, 2000; Duchenne et al., 1997). Duchenne et al. (1997) observed that as an increasing proportion of biomass accumulates in tubers (i.e. as harvest index increases), the rate of N decline with increasing biomass is also increased. Certain crops, such as potato, exclusively use a CNDC based on whole plant biomass due to the complex relationship between vine growth and tuber production (Duchenne et al., 1997; Bélanger et al., 2001; Ben Abdallah et al., 2016). Despite the empirical validity of this approach, the lack of a theoretical explanation makes interpretation of variation in CNDC observed between cultivars and geographies challenging.

However, recent work by Giletto et al. (2020) identified the theoretical relationships underpinning the observed empirical relationships in N dilution for potato. The CNDC on the basis of whole plant biomass reflects dilution in both the tuber and vine biomass, individually, and the increasing proportion of biomass allocated to low concentrations of N in biomass (i.e., tubers) as whole plant biomass increases. Giletto et al. (2020) also identified that varieties and locations with a greater proportion of biomass allocated to tubers have a greater value for parameter *b* of the CNDC. Parameter *b* of the CNDC represents the relative rate of decline in critical N concentration as biomass increases.

Based on this framework developed by Giletto et al. (2020), it is reasonable to conclude that variation in CNDC across environments [E] (e.g., climate, geography, etc.) and genotypes [G] (e.g., variety) would be expected due to known variation in total biomass and harvest index across these GxE gradients. Understanding the effects of GxE interactions on crop N requirements and status is critical to improving agronomic outcomes and nitrogen use efficiency within cropping systems (Lemaire and Ciampitti, 2020).

Previous development of CNDCs for potato has been conducted using a non-uniform set of statistical methods and with limited quantification of uncertainty in either the range of plausible critical N concentration values or the fitted parameter values themselves. This makes it difficult to ascertain whether observed differences in CNDCs are resulting from underlying GxE effects or confounded by the limitations of the statistical approach.

New statistical methods developed first by Makowski et al. (2020) provide a framework which allows for standardization in statistical approach, quantification of uncertainty, and a means to evaluation differences in CNDCs for various G x E interactions. In short, this novel framework implements a hierarchical Bayesian model which simultaneously identifies critical N points using the linear-plateau method (e.g., Justes et al., 1994) while fitting the negative exponential curve which defines critical N concentration. The advantage of this method is that it fits the CNDC from the whole set of experimental data and removes the arbitrary intermediate step of separately identifying critical N points. While this approach is newly developed, it has already been used by Ciampiti et al. (2021) to evaluate differences in CNDCs across G x E interactions for maize cropping systems.

Building upon the previous work, the objectives of this paper are to 1) develop CNDCs using the hierarchical Bayesian framework for potato varieties in Minnesota (from both previously published and unpublished experimental data) and for potato varieties in Argentina, Canada, and Belgium (from previously published experimental data), 2) compare CNDCs across G x E interactions based on the uncertainty in critical N concentration and curve parameters identified with the hierarchical Bayesian framework, 3) identify the optimal methods to determine uncertainty in critical N concentration for use in propagation to secondary computations (e.g., NNI), and 4) compare CNDCs developed with the hierarchical Bayesian framework methods to previously published CNDCs for the same data with different statistical methods.

2. Materials and Methods

2.1. Experimental Data

This study combines experimental data from both newly reported and previously published sources (Giletto et al., 2020; Ben Abdallah et al., 2016). The data used for analysis in this study is summarized in Table 1 and the relevant methods related to the experimental trials is reported below. All individual experimental observations used in this study are presented in the Appendix (Appendix Table 1).

**Table 1.** Summary of experimental data used in this study

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Study** | **Location** | **Variety** | **Site-Years** | **Dates** | **Samples** |
| Present Study | Minnesota | Clearwater | 2 | 10 | 30 |
| Dakota Russet | 2 | 14 | 70 |
| Easton | 2 | 14 | 70 |
| Russet Burbank | 9 | 52 | 329 |
| Umatilla Russet | 2 | 10 | 30 |
| Giletto et al. (2020) | Argentina | Bannock Russet | 3 | 13 | 52 |
| Gem Russet | 4 | 18 | 72 |
| Innovator | 4 | 18 | 72 |
| Markies Russet | 2 | 9 | 36 |
| Umatilla Russet | 3 | 14 | 56 |
| Canada | Russet Burbank | 4 | 30 | 120 |
| Shepody | 4 | 30 | 120 |
| Ben Abdallah (2016) | Belgium | Bintje | 17 | 49 | 238 |
| Charlotte | 7 | 24 | 114 |

2.1.1. Newly Reported Data – Minnesota

Six individual plot-scale field experiments were conducted over a total of eight years (1991–1992, 2014–2016, 2018-2020) on irrigated plots at the Sand Plain Research Farm [SPRF] in Becker, MN (45º 23’ N, 93º 53’ W). Mean temperature at this station is 7.1 ºC and mean annual precipitation is 809 mm (Arguez et al., 2010). The soil at this station was characterized as a Hubbard loamy sand (Sandy, mixed, frigid Entic Hapludolls) and excessively well drained with low available water holding capacity (Hansen and Giencke, 1988; USDA NRCS, 2013). Apart from experimental nitrogen and variety treatments, all management and cultural practices were managed by the staff at the SPRF in accordance with common practices for the region (Egel, 2017), nutrients were applied based on soil samples and University recommendations (Franzen et al., 2018; Rosen, 2018), and supplemental irrigation was applied based on the University recommended checkbook method (Steele et al., 2010; Wright, 2002). Additional details on experimental procedures for these studies have been previously reported (Table 2).

**Table 2.** Summary of newly reported experimental small-plot trials in Minnesota, USA

|  |  |  |
| --- | --- | --- |
| **Experiment** | **Year** | **Reference** |
| MN-1 | 1991-1992 | Errebhi et al. (1998); Rosen et al. (1992); Rosen et al. (1993) |
| MN-2 | 2014-2015 | Sun (2017) |
| MN-3 | 2016 | Crants et al. (2017) |
| MN-4 | 2018-2019 | Gupta and Rosen (2019); Gupta and Rosen (2020) |
| MN-5 | 2019 | Bohman et al. (2020) |
| MN-6 | 2020 | Crants et al. (2021) |

A randomized complete block design with three or four replicates was used in each field experiment. All studies evaluated at least 3 nitrogen rates (0 – 400 kg N ha-1) for Russet Burbank potato [Solanum tuberosum (L.)], with some studies evaluating additional potato varieties (Table 2). Those studies that evaluated multiple varieties had either a factorial design, or split-plot design with variety treatment as the whole-plot and nitrogen treatment as the split-plot. Plots in these studies were between 5.4 – 6.4 m wide (6 or 7 x 0.9 m rows) and 6.1 – 9.1 m long. Planting density ranged between 36,000 – 48,000 plants ha-1, depending on year and variety. Experiments were planted each year in late-April to early-May and were mechanically harvested in mid-September with vines terminated one to two weeks prior to harvest. A summary of nitrogen management practices and varieties evaluated for each of these studies is summarized below (Table 3).

**Table 3.** Summary of N treatments and varieties evaluated in the newly reported experimental small-plot trials in Minnesota, USA

|  |  |  |  |
| --- | --- | --- | --- |
| **Experiment** | **N treatments**1 | **N rates** | **Varieties** |
| MN-1 | 10 | 0, 135, 180, 225, 270 | Russet Burbank |
| MN-2 | 5 | 135, 200, 270, 335, 400 | Russet Burbank, Dakota Russet, Easton |
| MN-3 | 4 | 45, 180, 245, 335 | Russet Burbank |
| MN-4 | 3 | 135, 270, 400 | Russet Burbank, Clearwater, Umatilla Russet |
| MN-5 | 8 | 45, 155, 245, 290, 335 | Russet Burbank |
| MN-6 | 8 | 55, 155, 245, 270, 290, 335 | Russet Burbank |

1 Including N source, timing, and placement combinations occurring at an equivalent N rate

Samples of vine biomass were harvested immediately prior to mechanical termination for determination of fresh weight vine yield. Harvested tubers were mechanically sorted into weight classes and graded (USDA, 1997), and fresh weight tuber yield was determined as the sum of all weight classes and tuber grades. Harvested biomass was oven dried at 60ºC to determine dry matter content of vines and tubers. Dry weight tuber and vine biomass was calculated as the product of fresh weight and dry matter content for each tissue respectively. Total N concentration of vines and tubers was determined from subsamples of plant tissues with either combustion analysis (Elementar Vario EL III, Elementar Americas Inc., Mt. Laurel, NJ) using standard methods (Horneck and Miller, 1998), or with the salicylic Kjeldahl method. Total N content of vines and tubers was calculated as the product of N concentration and dry weight biomass for each tissue respectively. Total plant N content [NPlant] (kg N ha-1) was calculated from the sum of tuber and vine N content. Total plant dry weight biomass [W] (Mg dry wt. ha-1) was calculated from the sum of vine and tuber dry weight biomass. Plant N concentration [%NPlant] (g N 100 g-1) was calculated as the ratio of NPlant to W.

Whole-plant samples were also regularly collected during the period of late-May to early-September (Table 4). Two to three plants were harvested from each plot on four to six dates each year with vines, roots, and tubers each measured separately. Dry weight biomass, N concentration, and N content for vines and tubers were determined for these in-season plant tissue samples using the methods described above. Calculations for W, NPlant, and %NPlant were also equivalent to the methods previously described.

**Table 4.** List of in-season whole plant sampling dates and harvest sampling date in the newly reported experimental small-plot trials in Minnesota, USA

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Experiment** | **Year** | **In-Season** | | | | | | **Harvest** |
| **1** | **2** | **3** | **4** | **5** | **6** |
| MN-1 | 1991 | 12 June | 24 June | 2 July | 16 July | 30 July | 13 Aug | 10 Sept. |
| MN-1 | 1992 | 10 June | 25 June | 17 July | 5 Aug. | 26 Aug. |  | 15 Sept. |
| MN-2 | 2014 | 30 June | 15 July | 24 July | 11 Aug. | 26 Aug. | 8 Sept. | 15 Sept. |
| MN-2 | 2015 | 23 June | 7 July | 21 July | 4 Aug. | 17 Aug. | 1 Sept. | 16 Sept. |
| MN-3 | 2016 | 28 June | 13 July | 26 July | 3 Aug. | 10 Aug. |  | 13 Sept. |
| MN-4 | 2018 | 26 June | 10 July | 18 July | 1 Aug. |  |  | 13 Sept. |
| MN-4 | 2019 | 26 June | 11 July | 24 July | 7 Aug |  |  | 16 Sept. |
| MN-5 | 2019 | 25 June | 9 July | 23 July | 6 Aug | 21 Aug |  | 16 Sept. |
| MN-6 | 2020 | 24 June | 7 July | 22 July | 4 Aug |  |  | 16 Sept. |

2.1.2. Previously Published Data – Belgium, Argentina, and Canada

Experimental data reported in two previous studies, Giletto et al. (2020) and Ben Abdallah et al. (2016), was included in the analysis conducted for the present study. The data from Giletto et al. (2020) comprises two separate experimental data sets from Argentina (Giletto and Echeverría, 2015) and Canada (Bélanger et al., 2001, Bélanger et al., 2000).

In the Canadian study, two varieties (Russet Burbank and Shepody) and four N fertilization rates (0, 50, 100, and 250 kg ha-1) were evaluated with each variety having 4 site-years of experimental data and 10 sampling dates per site year (Table 1).

In the Argentina study, five varieties (Bannock Russet, Gem Russet, Innovator, Markies Russet, and Umatilla Russet) and four N fertilization rate (0, 80, 150, 250 kg N ha-1) were each evaluated for between 2 to 4 site-years with between 4 to 5 sampling dates per site year (Table 1).

All data from the Giletto et al. (2020) study used in the present analysis was included in this previous publication.

The data from Ben Abdallah et al. (2016) represents multiple experimental data set from Belgium. In the Belgium studies, three to six N rates (ranging from 0 to 250 kg N ha-1) were evaluated for two varieties (Bintje and Charlotte) for 17 and 7 site-years, respectively, and with 1 to 8 sampling dates per site year (Table 1).

Only a portion of the data from the Ben Abdallah et al. (2016) study used in the present analysis was included in this previous publication. While the dry weight biomass data was previously reported, the nitrogen concentration data from the Ben Abdallah et al. (2016) experiment is reported in this manuscript for the first time.

2.2. Statistical Methods

Based on the approach outlined by Makowski et al. (2020), this study implemented a Bayesian hierarchical framework to infer CNDC parameters for each location and variety within location, assess the uncertainty in model parameters and critical N concentration, and compare fitted CNDCs across location and variety effects.

In summary, this statistical approach uses the entire set of experimental data (Figure 7a) and does not require any preliminary or intermediary statistical analysis. At the level of each experimental sampling date, a linear-plateau curve is fit for biomass as a function of nitrogen concentration (Figure 7b) and the join point of the linear-plateau curve is used to define the critical N concentration. Simultaneously, a negative exponential curve (i.e., CNDC) is fit across all experimental sampling dates where the critical N point of each linear-plateau curves lies exactly upon the negative exponential curve (Figure 7b). In this manner, the linear-plateau curve fitted for any given date is influenced by the data from all other experimental sampling dates through the fitting of the negative exponential curve.

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**Figure 7.** Hypothetical example of statistical methods used showing **(a)** raw experimental data, **(b)** linear-plateau curves (solid colored lines) fitted for each experimental sampling date (points with each date distinguished by color) and the critical N dilution curve (solid black line) fitted using the hierarchical Bayesian method based on Makowski et al. (2020), and **(c)** critical points (opaque) and non-critical points (transparent) selected using conventional statistical analysis (i.e., ANOVA and protected multiple comparison) with critical N dilution curve (dotted line) fitted using conventional methods (i.e., non-linear regression using only the critical points).

The conventional approach to fit a CNDC, which contrasts with the Bayesian hierarchical method, consists of a two-step process: first, the critical N points are selected using statistical criterial; second, a negative exponential curve using non-linear regression was fit to the subset of critical points (Figure 7c). There are two commonly used statistical approaches to identify critical points: linear-plateau curve fit and protected multiple comparison.

Using a linear-plateau curve to identify the critical points was originally suggested by Justes et al. (1994); however, while this approach is methodologically superior and follows directly from N dilution theory, it is difficult to implement in practice due to potential limitations of the experimental data used (e.g., insufficient levels of N treatments, interactions with environmental conditions, etc.). In many cases, these limitations prevented the implementation of this method to identify a sufficient number of critical points requiring the use of alternative methods.

Most studies identifying a CNDC, therefore, use methods similar to Belangér et al. (2001) where critical points are determined using a simplified statistical method. In this general approach, the critical points are first identified using 1) ANOVA to identify experimental dates where variation in biomass is statistically significant and 2) protected multiple comparison to identify which experimental treatments had the highest level of biomass. An experimental treatment with a significantly greater level of biomass than all other treatments is then defined as the critical point. However, this method does not necessarily ensure that an identified critical points represents a true point and may be biased.

Through a single-step process, the Bayesian hierarchical method both eliminates the need to separately identify critical points and implements the theoretically preferred method (e.g., linear plateau curve) to select critical points.

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**Figure 8**. Flowchart showing nested structure used in the present analysis to fit critical N dilution curves using the Bayesian hierarchical method based on Makowski et al. (2020). Linear-plateau curves and critical points are identified at the level of each experimental sampling date and pooled at various levels of location and variety within location to determine the critical N dilution curve (CNDC) for that level. The structure of the model fits all individual levels for location and variety within location, as well as the global level of all experimental data, simultaneously which allows for direct comparison across levels.

The Bayesian hierarchical framework outlined by Makowski et al. (2020) was extended to explicitly include Environmental (e.g., location) and Genotype (e.g., variety) interactions within the fitted model. This was implemented through the nesting of experimental data according to location and variety within location (Figure 8). The linear-plateau curve fitted for each experimental sampling date can be pooled at various nested levels of location or variety within location.

Using *R* (R Core Team, 2020), the *brms* package (Bürkner 2017; Bürkner, 2018) was used to implement the statistical method outlined by Makowski et al. (2020). The *brms* package, an interface to *Stan* (Carpenter et al., 2017), was chosen due to the ability to include group-level (i.e., random effects) which allows for the fit of a single model for all of the experimental data and improves model performance through the inclusion of partial pooling (CITATION). The *brms* package includes a user-friendly modeling language, robust documentation, and a diverse set of tools to analyze and assess models.

A non-linear *brms* model was defined by combining the two separate expressions used by Makowski et al. (2020) to parameterize the Bayesian hierarchical model as implemented with *rjags* (Plummer, 2019) and *JAGS* statistical software (Plummer, 2013).

The first expression from Makowski et al. (2020) represents the linear-plateau component:

|  |  |
| --- | --- |
| W = *min*(Wmax,i + Si \* (%NPlant – %Nc), Wmax,i) | (1) |

where %Nc is the critical N concentration, Si and Wmax,i are the slope of the linear-plateau curve and the maximum value of biomass (i.e., plateau) for a given date, respectively, *min* represents the minima function (i.e., plateau), and W and %NPlant have the same meaning as previously defined in this present study. This linear-plateau curve is defined with nitrogen concentration as the independent variable and biomass as the dependent variable and is written in point-slope form where the reference point used is the critical point.

The second expression from Makowski et al. (2020) represents the CNDC component:

|  |  |
| --- | --- |
| %Nc = *a* (Wmax,i) –*b* | (2) |

where *a* and *b* are the parameters that define the negative exponential curve and %Nc and Wmax,i have the same meanings as defined above.

Using algebraic substitution (for %Nc), these two expressions were combined to produce following non-linear *brms* model formula:

|  |  |
| --- | --- |
| W ~ *min*(Wmax,i + Si \* (%NPlant – (*a* \* (Wmax,i) –*b*)), Wmax,i) | (3) |

Two group-level (i.e., random) effects were specified for this *brms* model to parameterize the nested structure (Figure 8). First, the parameters Si and Wmax,i included group-level effects to fit an linear-plateau curve to each experimental sampling date:

|  |  |
| --- | --- |
| Wmax,i + Si ~ 1 + (1|index) | (4) |

where index represents the unique level of each experimental sampling date nested within the variety x location. Second, the parameters *a* and *b* included group-level effects to fit the CNDC for each level of location and variety within location:

|  |  |
| --- | --- |
| *a* + *b* ~ 1 + (1|location) + (1|location:variety) | (5) |

The *brms* model was fit using 4 chains and 10000 iterations with 3000 warmups per chain. The priors for this model were chosen both based on expert knowledge (i.e., previously reported values), empirical observations, and the joint prior predictive distribution (CITATION) (i.e., if a set of relatively uninformative priors led to biologically or physically impossible prior predictions, the prior ranges were narrowed). This is particularly important for hyperparameters dealing with the standard deviation between groups in a hierarchical model. A summary of the prior model values and their sources is given below (Table 5).

**Table 5.** Model priors…

|  |  |  |
| --- | --- | --- |
| **Title 1** | **Title 2** | **Title 3** |
| entry 1 | data | data |
| entry 2 | data | data |

The entire workflow used to generate this analysis is reproducible and available via associated GitHub repository (https://github.com/bohm0072/cndc\_bayesian\_eval).

2.3. Evaluating Uncertainity

2.3.1. Critical N Dilution Curve Parameter Uncertainty

After the statisticalmodel was successfully fit to the data (n=12,000 draws), values for parameters *a* and *b* of the CNDC were reported at the 0.05, 0.50 (i.e., median) and 0.95 quantiles for the levels of location and variety within location to determine the credible interval. The correlation between values for parameters *a* and *b* was determined for each level of variety within location using the fitted values for individual draws.

2.3.2. Critical N Concentration Uncertainty

The critical N concentration for all values of W between 1 Mg ha-1 and the maximum observed value of W in the experimental data set was calculated for each individual draw based on the fitted values of parameters *a* and *b* for that draw. From the distribution of critical N concentration values, the 0.05, 0.50 (i.e., median) and 0.95 quantile values were identified for each level of variety within location to determine the credible region.

To approximate the upper and lower boundaries of the credible region for critical N concentration (i.e., the 0.05 and 0.95 quantile values, respectively), a negative exponential curve of the same form as the CNDC (i.e., y = a \* x ^ (–b)) was fit using *nls* (source?).

Additionally, an estimate of the credible region was calculated by using the boundary values of the credible interval for parameters *a* and *b*. The estimate for the upper boundary of the credible interval was determined from the 0.95 quantile value for parameter *a* and 0.05 quantile value for parameter *b*; the estimate for the lower boundary of the credible interval was determined from the 0.05 quantile value for parameter *a* and 0.95 quantile value for parameter *b*.

The approximation and estimation methods were compared to the true credible region for each level of variety within location. This true credible region was calculated by generating predicted %N values along a sequence of discrete biomass values for a given location x variety. For each discrete biomass value, X predictions were made using draws from the posterior distribution, allowing for the calculation of 0.05, 0.5, and 0.95 quantiles for %N at that biomass value. By connecting the points for each quantile across the range of biomass values, we can draw the median curve with a 90% credible interval.

2.3.3. Comparing Differences Between Critical N Dilution Curves

Using the previously identified credible interval for critical N concentration, it is possible to identify the range for which two CNDCs are significantly different. If the median critical N concentration value of one curve falls outside of the credible interval for critical N concentration of another curve, then the two curves are determined to be significantly different over the range for which the median value falls outside of the credible interval.

To evaluate the differences between curves fit in the present study, the CNDC for a given level of variety within location was compared to all other levels using this method. This approach allows for the direct evaluation of differences in critical N concentration across G x E effects.

This same method was also used to compare the CNDCs fitted in the present study to the CNDCs published in previous studies (i.e., Ben Abdallah et al., 2016; Giletto et al., 2020). In this case, the previously published curve was evaluated to see if it fell within the credible interval for the corresponding curve fitted in the present study. This approach allows for direct evaluation of differences in critical N concentration across various statistical methods.

**3. Results**

3.1. Fitted Parameter Values and Uncertainity

3.1.1. Overall Model Fit

Critical N dilution curves were fit for each level of variety within location. The experimental data, median linear-plateau curve for each experimental sampling date, and median value of critical N concentration are presented below (Figure 3). The individual linear-plateau curves fitted to each experimental sampling date for each variety within location is presented in the Appendix.

3.1.2. Critical N Dilution Curve Parameter Fit and Uncertainty

The distribution of fitted values for CNDC parameters *a* and *b* are presented below (Figure 1) showing the median value and 90% credible interval (0.05 and 0.95 quantile values).

For parameter *a*, there was no significant difference for the effect of either location or variety within location at 90% credible interval threshold (Figure 1). Although Argentina has a numerically greater value of parameter *a* (4.95) than the other three locations (4.74-4.77), these differences are not significant. Additionally, the variation in parameter *a* at the variety within location level is negligible.

For parameter *b*, there were significant differences at both the levels of location and variety nested within location at 90% credible interval threshold (Figure 1). For location, Argentina had the lowest value for parameter *b* (0.174), while Canada had a greater value for parameter *b* (0.448)than Argentina but lower than either Belgium (0.561) or Minnesota (0.581). The difference between parameter *b* for Belgium and Minnesota was not significant.

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**Figure 3.** Median fitted critical N dilution curve for each location x variety interaction shown as solid black line. Biomass and nitrogen concentration data used for fitting the statistical model are displayed as points with the median fitted linear-plateau curve for each sampling date shown as grey line. The number of samples [n] and the number of sampling dates [i] used to fit the linear-plateau curves are displayed for each location x variety interaction.

For variety within location, parameter *b* significantly varied for varieties in Argentina and Canada while there were no significant differences in parameter *b* within either Belgium or Minnesota. For Argentina, Innovator had the greatest value for parameter *b* (0.211), followed by Gem Russet, Umatilla Russet, Markies Russet, and Bannock Russet (0.179, 0.165, 0.155, and 0.140, respectively). The difference between Innovator and Umatilla Russet, Markies Russet, and Bannock Russet was significant, while all other differences between varieties was not significant. For Canada, Russet Burbank had a significantly greater value for parameter *b* (0.489) than Shepody (0.412).

3.1.3. Correlations between Critical N Dilution Curve Parameters

There was a positive correlation found between parameter *a* and *b* for all levels of variety within location (Figure 2) and the Pearson correlation coefficient ranged from 0.40 to 0.80. The positive correlation of parameter *a* and *b* indicates that quantifying uncertainty and differences in these parameter values alone is not sufficient to describe the combined uncertainty in critical N concentration. This is because the *a* and *b* values jointly define the CNDC curve, so while the independent parameters might not differ significantly, the combination of differences in *a* and *b* may generate curves that are different. Additionally, due to the shape of the curves, the differences between curves may vary along the range of biomass. This means that two curves may be similar to each other at one biomass value, but different from each other at another biomass value.

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**Figure 1.** Posterior distribution of variety x location interaction effect and of location effect for (**a**) parameter *a*; and (**b**) parameter *b*. Points represent median value and thin line represents 0.05 and 0.95 quantile range. Values displayed adjacent to distributions are for the median value with the values for the 0.05 and 0.95 quantile range displayed within the parentheses.

3.2. Critical N Concentration Uncertainity

The credible region for critical N concentration varies across levels of variety within location and across levels of biomass within a given level of variety within location (Figure 4). The symmetry of the credible interval distribution varies across levels of variety within location with some levels, such as Argentina x Gem Russet, having a skewed distribution, while other levels, such as Canada x Shepody, having a symmetrical distribution. There are also differences in the range of the credible region where some levels of variety within location, such as Argentina x Umatilla Russet, have greater uncertainty in critical N concentration than other levels, such as Minnesota x Russet Burbank. The uncertainty in critical N concentration also varies across the level of biomass for a given CNDC. For example, as level of biomass increases, Argentina x Umatilla Russet has increasing credible region range, Minnesota x Russet Burbank has decreasing credible region range, and Argentina x Bannock Russet has nearly constant credible region range.

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**Figure 2.** Distribution of posterior values for parameters *a* and *b* for each location x variety interaction shown as a scatterplot with marginal density distribution given for each parameter. Pearson correlation coefficient [R] is displayed for the relationship between parameters *a* and *b* for each location x variety interaction. Data are shown at the level of individual draws (n=28,000)

Approximations of the upper and lower boundaries of the critical region by fitting a negative exponential curve to the 0.05 and 0.95 quantile data were successfully made (Table 6) and this method appears to be reasonable based on graphical evaluation (Figure 4). While critical regions with boundaries that are non-monotonic (e.g., Argentina x Innovator) have portions of the curve fit approximation that are poorer performing, the critical regions with monotonic boundaries (e.g., Minnesota x Dakota Russet) seem to be satisfactory across the entire range of the curve. Therefore, in the absence of the critical region defined directly from the fitted model, this approximation is an appropriate first-order representation of the credible region.

However, the estimate based directly on uncertainty in CNDC parameters *a* and *b* (Table 6) contains the entire credible region and for all variety within location levels (Figure 4). Therefore, this estimate approach is more conservative than the curve fit approximation approach and should be used if a more restrictive definition of critical N concentration uncertainty is required and the credible region defined from the original model fit is unavailable.

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**Figure 4.** Comparison of methods to quantify uncertainty in critical nitrogen dilution curve values. Percent N (%N) Difference represents the difference between the median critical nitrogen concentration value and the various boundary estimates as previously described above. The solid black line (at constant value of zero) represents the median critical nitrogen concentration from the fitted distribution of critical nitrogen concentration values which was the reference used

Grey shaded region represents the 90% credible region (lower bound, 5% quantile; upper bound, 95% quantile) for the critical nitrogen concentration values derived from the distribution of critical nitrogen concentration values as computed from posterior distribution of paired values for parameters *a* and *b*.

Dotted lines represents non-linear regression estimate for the upper and lower bound of the credible interval based on subsequent fit of negative exponential curve using the same equation form as the critical nitrogen dilution curve.

Dashed lines represent more conservative estimates of the upper and lower bounds of critical nitrogen concentration uncertainty based on the posterior distribution of parameters *a* and *b* where the estimated lower bound is defined using the 95% quantile value of parameter *a* with the 5% quantile value of parameter *b* and the estimated upper bound is defined using the 5% quantile value of parameter *a* with the 95% quantile value of parameter *b*.

Percent N (%N) Difference represents the difference between the median critical nitrogen concentration value and the various boundary estimates as previously described above.

**Table 6.** Paired critical nitrogen dilution curve parameter values for each variety x location interaction defining a conservative estimate for the lower (Conserv. Low) and upper (Conserv. High), the estimate values for the credible region lower (Cred. Est. Low) and upper (Cred Est. High), and the Median value from the posterior distribution for critical nitrogen concentration.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Location** | **Variety** |  | Conserv. Low | Cred. Est. Low | **Median** | Cred. Est. High | Conserv. High |
| Argentina | Bannock Russet | *a* | 4.72 | 4.82 | **4.95** | 5.10 | 5.20 |
| *b* | 0.163 | 0.146 | **0.140** | 0.135 | 0.118 |
| Gem Russet | *a* | 4.71 | 4.79 | **4.95** | 5.07 | 5.19 |
| *b* | 0.206 | 0.190 | **0.179** | 0.153 | 0.140 |
| Innovator | *a* | 4.70 | 4.83 | **4.94** | 5.05 | 5.18 |
| *b* | 0.252 | 0.241 | **0.211** | 0.191 | 0.177 |
| Markies Russet | *a* | 4.72 | 4.82 | **4.96** | 5.08 | 5.20 |
| *b* | 0.183 | 0.168 | **0.155** | 0.135 | 0.121 |
| Umatilla Russet | *a* | 4.71 | 4.85 | **4.95** | 5.06 | 5.19 |
| *b* | 0.206 | 0.195 | **0.165** | 0.143 | 0.131 |
| Belgium | Bintje | *a* | 4.42 | 4.52 | **4.71** | 4.90 | 4.99 |
| *b* | 0.640 | 0.606 | **0.579** | 0.568 | 0.531 |
| Charlotte | *a* | 4.45 | 4.56 | **4.74** | 4.90 | 5.00 |
| *b* | 0.636 | 0.606 | **0.559** | 0.531 | 0.499 |
| Canada | Russet Burbank | *a* | 4.46 | 4.53 | **4.74** | 4.93 | 5.01 |
| *b* | 0.531 | 0.498 | **0.489** | 0.480 | 0.447 |
| Shepody | *a* | 4.48 | 4.55 | **4.76** | 4.95 | 5.03 |
| *b* | 0.447 | 0.416 | **0.412** | 0.406 | 0.376 |
| Minnesota | Clearwater | *a* | 4.45 | 4.54 | **4.75** | 4.93 | 5.01 |
| *b* | 0.646 | 0.619 | **0.584** | 0.557 | 0.53 |
| Dakota Russet | *a* | 4.45 | 4.53 | **4.74** | 4.94 | 5.01 |
| *b* | 0.646 | 0.617 | **0.598** | 0.587 | 0.557 |
| Easton | *a* | 4.45 | 4.53 | **4.74** | 4.91 | 5.01 |
| *b* | 0.636 | 0.608 | **0.591** | 0.566 | 0.541 |
| Russet Burbank | *a* | 4.45 | 4.50 | **4.74** | 4.94 | 5.00 |
| *b* | 0.595 | 0.561 | **0.564** | 0.565 | 0.532 |
| Umatilla | *a* | 4.46 | 4.55 | **4.75** | 4.91 | 5.01 |
| *b* | 0.655 | 0.628 | **0.586** | 0.545 | 0.521 |

3.3. Evaluating Differences between Critical N Concentration

3.3.1. Differences Related to Genotype x Environment Effects

While an evaluation of the differences between all levels of variety within location was conducted and is reported in the Appendix, a subset of the results comparing Minnesota x Russet Burbank to all other levels is presented here (Figure 5). For Minnesota x Russet Burbank, there were no significant differences in critical N concentration with either Belgium x Bintje, Belgium x Charlotte, or Minnesota x Clearwater. However, there were significant differences found with the other levels of variety within location. At biomass levels greater than approximately 5 Mg ha-1, the critical N concentration for Minnesota x Dakota Russet, Minnesota x Easton, and Minnesota x Umatilla Russet was significantly less than that for Minnesota x Russet Burbank; however for biomass values less than the threshold specified above, the critical N concentration was not significantly different. The critical N concentration for Canada x Russet Burbank and Canada x Shepody were significantly greater than that for Minnesota x Russet Burbank at biomass values of approximately 2 Mg ha-1. All of the Argentina varieties had critical N concentration values significantly greater than Minnesota x Russet Burbank, except for at a biomass value of 1.0 Mg ha-1. The difference in critical N concentration between Minnesota x Russet Burbank and the varieties in Argentina was in some cases greater than 2 g N 100 g-1.

There are three notable findings here to callout. First, while the values for both parameters *a* and *b* between all varieties within Minnesota were not significantly different, there were significant differences in critical N concentration between 4 of the 5 varieties. This indicates that evaluation of uncertainty at the level of CNDC parameter may lead to erroneous conclusions. Second, the Minnesota x Russet Burbank and Canada x Russet Burbank curves were significantly different. This provides evidence that the effect of Environment (i.e., location), even when controlling for Genotype (i.e., variety), can result in significantly different critical N concentration. Third, the findings that within a constant Environment (i.e., Minnesota) that there are significant differences between some Genotypes (e.g., Russet Burbank and Dakota Russet) while there are not significant differences between other Genotypes (e.g., Russet Burbank and Dakota Russet) suggests that Genotype is of secondary importance in determining critical N concentration relative to Environment.

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**Figure 5.** Comparison of critical nitrogen concentration values between Russet Burbank x Minnesota and all other location x variety levels fitted in the present study. Blue points indicate that the median critical nitrogen concentration is within the credible region for the critical nitrogen concentration for Russet Burbank x Minnesota; red points are those which fall outside of the credible region for critical nitrogen concentration.

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**Figure 6.** Comparison of critical nitrogen concentration from previous studies using conventional methods to derive the critical nitrogen dilution with the method used in the present study. Blue points indicate critical nitrogen concentration values calculated using the critical nitrogen dilution curve from previous studies that is within the credible region for the critical nitrogen concentration identified in the present study; red points are those which fall outside of the credible region for critical nitrogen concentration.

3.3.2. Differences related to statistical methods

Comparing the curves fit in the present study with the Bayesian hierarchical method to the curves fit in the previous studies using conventional statistical methods, there were significant differences between statistical curve fit methods for all variety within location levels (Figure 6). None of the previous CNDCs fall entirely within credible interval for the respective CNDC developed in the present study.

The critical N concentration from the previously developed CNDCs for Canada (Bélanger et al., 2001) was significantly greater for both Canada x Russet Burbank and Canada x Shepody than the present CNDCs for biomass levels of less than 3 Mg ha-1 and less than 5 Mg ha-1, respectively. Relative to the other locations, the CNDCs for Canada were the most similar between statistical methods.

The critical N concentration from the previously developed CNDCs for the Argentina varieties (Giletto and Echeverría, 2015) was significantly less than that from the present CNDCs for biomass levels of greater than approximately 5 Mg ha-1. For biomass levels less than 5 Mg ha-1, the critical N concentration is either significantly greater than or not significantly different than that from the present study. The magnitude of this difference is relatively large with the critical N concentration from the previous method as much as 1 g N 100 g-1 less than from the present method.

The critical N concentration from the previously developed CNDCs for Belgium (Ben Abdulla et al., 2016) were significantly greater than the than from the CNDCs developed in the present study. While the magnitude of this difference in critical N concentration was approximately 0.5 g N 100 g-1, there was no value of biomass for which the critical N concentration from the two methods was not significantly different.

While this evaluation of different statistical methods to calculate a CNDC from the same set of data cannot direct answer the question of which statistical method is correct to use, it does provide direct evidence that the statistical approach itself can confound the interpretation of differences in critical N concentrations across G x E interactions. Therefore, it is essential to know the statistical method used to develop a CNDC when evaluating and interpreting differences between various curves.

4. Discussion

4.1. Propogating Uncertainity in Critical N Concentration

* Calculations of NNI should be based on a credible range of critical N concentration values to produce NNI values with associated uncertainty range
* This uncertainty can also be applied to other derivative/related constructs including critical N uptake curve and critical N utilization efficiency curve (Bohman, 2021)
* Even for a given variety within a location, there is no true value of critical N concentration without some level of uncertainty. This should be quantified whenever possible, and this statistical approach is one method to do so.
* The best way to do this is using the “approximation” approach for the credible region from Table 6. Report the median value, with range based on the upper and lower “approximated” boundaries of the critical region.

4.2. Implication of G x E effects of Critical N Concentration

* Location appears to be a more important factor for determining N dilution in potato than variety
* Based on the work of Giletto et al. (2020) this would be due to differences in bulking across Environmental conditions
* Even controlling for variety across locations, Genotype is less important than Environment for determining critical N concentration
* Extending this to critical N utilization efficiency, this implies that NUtE (and NUE subsequently) is primarily a function of Environment rather than Genotype.
* Crop N demands in potato appear to be driven by Environmental factors, rather than Genotypic ones…

4.3. Understanding Differences between Statistical Methods

* The resulting CNDCs between this method and the previous methods is quite striking for some locations (i.e., Belgium and Argentina)
* One plausible rationale here is that the previous methods were selecting incorrect critical points due to the limitations of both the experimental and statistical procedures.
  + For example, in cases like Argentina where even the highest N rate was not N limiting but significantly greater than all other N rates (i.e., significantly greatest based on ANOVA and multiple comparisons) the critical N point selected is less than the “true” critical N point.
  + In other case, like Belgium, perhaps this has to do with more clustering and less spread in experimental? Some bias in the selection criteria that results in upwardly biased critical N points?
* The inclusion of all of the data rather than *a priori* selection of some of the data removes the arbitrary step of selecting the critical N points and rather identifies these values from all of the experimental data
* Not clear if this method is empirically “better” at reflecting agronomic outcomes (i.e., relative yield/biomass response as function of NNI), but from a deductive standpoint this method is “better” at inferring differences between G x E interactions and making the most of expensive experimental data to do so.

5. Conclusions

**Appendix 1.**

**Appendix 2.**