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

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**Abstract:** Multiple critical N dilution curves [CNDCs] have been previously developed for potato; however, attempts to directly compare differences in CNDCs 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 [%Nc]. This study implements a hierarchical Bayesian framework to develop CNDCs for previously published and newly reported experimental data, systematically evaluate the difference in %Nc across G x E effects, and compare CNDCs from the Bayesian framework to CNDCs from conventional statistical methods. Differences in %Nc were primarily the result of differences in location (i.e., E) while variety (i.e., G) within a given location had a lesser effect. In addition to the median value (i.e., CNDC), the boundary values for the credible region (i.e., CNDClo and CNDCup) should be used in subsequent calculations (e.g., N nutrition index) to propagate and account for uncertainty in %Nc. Additionally, this study found that the conventional statistical method is subject to greater inferential bias resulting from biased experimental datasets (i.e., unbalanced distribution of N limiting and non-N limiting observations) than the Bayesian hierarchical method. Overall, the findings of this study provide additional evidence that %Nc is dependent upon G x E interactions; therefore, evaluation of crop N status or N use efficiency must account for variation in %Nc across G x E interactions.

**Keywords:** critical nitrogen dilution curve; nitrogen nutrition index; nitrogen use efficiency; potato; Bayesian; genotype-by-environment interactions

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| **Citation:** Lastname, F.; Lastname, F.; Lastname, F. Title. *Plants* **2021**, *10*, x. https://doi.org/10.3390/xxxxx  Received: date  Accepted: date  Published: date  **Publisher’s Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.    **Copyright:** © 2021 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). |

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 [1]. 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 [2]. Typically, NNI is used to determine crop N status using whole plant analysis and to direct adaptive N management within a growing season [3, 4]. Unlike the yield-goal or rate-response approach, NNI is generalizable across environmental (e.g., year-to-year or geographic) variability [5].

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 critical N concentration [%Nc] below which relative growth rate is reduced [6]. A robust theoretical framework has been developed to explain decline in N concentration as biomass increases, but the application of this theory is commonly restricted to the vegetative period where only metabolic and structural tissues are present [5, 7, 8]. 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 [6, 9].

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, including periods of reproductive growth [10-13]. Dilution of N beyond the vegetative period primarily 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 [10, 11]. Duchenne, et al. [11] 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 [11, 14-16]. Despite the validity of this approach, interpretating of variation in CNDC observed between cultivars and geographies has been challenging.

However, recent work by Giletto, et al. [17] 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. [17] 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, where parameter *b* of the CNDC represents the relative rate of decline in %Nc as biomass increases.

Based on this framework developed by Giletto, et al. [17], it is reasonable to hypothesize 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 (i.e., relative partitioning of biomass to tubers) across these G x E gradients. Understanding the effects of G x E interactions on crop N requirements and status is critical to improving agronomic outcomes and N use efficiency [NUE] within cropping systems [18].

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 %Nc values or the fitted parameter values themselves. This makes it difficult to ascertain whether observed differences in CNDCs are resulting from underlying G x E effects or confounded by the limitations of the statistical approach.

The conventional approach to fit a CNDC, consists of a two-step process: first, the critical N points are selected using statistical criteria; second, a negative exponential curve is fit to the subset of critical points using non-linear regression. There are two commonly used statistical approaches to identify critical points: (1) linear-plateau curve fit and (2) ANOVA and protected multiple comparison.

Using a linear-plateau curve to derive critical points was originally suggested by Justes, et al. [8]. This approach is rigorous and requires sufficient empirical data such that a linear-plateau curve can be identified (i.e., at least two N limiting and at least two non-N limiting data points) for each observation date. Therefore, this approach can be difficult or impossible to implement due to potential limitations of the experimental data used such as insufficient levels of N treatments (i.e., fewer than 5 treatment levels) or interactions with environmental conditions (i.e., all observations are either N limiting or non-N limiting).

In contrast, many studies use methods similar to Ben Abdallah, et al. [14] where critical points are determined using a simplified statistical method. In this approach, ANOVA is first used to identify experimental dates where variation in biomass is statistically significant. Subsequently, a protected multiple comparisons analysis is used to identify which experimental treatments had the highest level of biomass – the treatment level with the significantly greatest level of biomass is then defined as the critical point. While this statistical method is more flexible to implement, it cannot resolve deficiencies in the underlying empirical data (i.e., insufficient level of N treatments, interactions with environmental conditions) that the linear-plateau method was designed to discriminate against. Therefore, the critical points selected using the simplified method may be biased when implemented using biased empirical data (e.g., without sufficient quantity of both N limiting and non-N limiting observations).

New statistical methods developed first by Makowski, et al. [19] 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. [8]) while fitting the negative exponential curve which defines %Nc. The advantage of this method is that it fits the CNDC from the entire 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 Ciampitti, et al. [20] and Yao, et al. [21] to evaluate differences in CNDCs across G x E interactions for maize and wheat cropping systems, respectively. 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.

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) extend the hierarchical Bayesian framework to compare CNDCs across G x E interactions based on the uncertainty in %Nc and curve parameters, 3) identify the optimal methods to determine uncertainty in %Nc 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 [14, 17]. 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 Supplemental Materials (Table S1).

**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. [17] | 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, et al. [14] | 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 [22]. 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 [23, 24]. 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 [25], nutrients were applied based on soil samples and University recommendations [26, 27], and supplemental irrigation was applied based on the University recommended checkbook method [28, 29]. 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. [30], Rosen, et al. [31], Rosen, et al. [32] |
| MN-2 | 2014-2015 | Sun [33], Sun, et al. [34] |
| MN-3 | 2016 | Crants, et al. [35] |
| MN-4 | 2018-2019 | Gupta and Rosen [36], Gupta, et al. [37] |
| MN-5 | 2019 | Bohman, et al. [38] |
| MN-6 | 2020 | Rosen, et al. [39] |

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 experimental treatments evaluated in 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 [40], 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 [41], 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.** In-season and harvest sampling dates for the 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. [17] and Ben Abdallah, et al. [14], was included in the analysis conducted for the present study. The data from Giletto, et al. [17] comprises two separate experimental data sets from Argentina [16] and Canada [15, 42, 43].

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 under non-water limiting conditions 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 under non-water limiting conditions 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. [17] study used in the present analysis was included in this previous publication.

The data from Ben Abdallah, et al. [14] 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. [14] study used in the present analysis was included in this previous publication – while the dry weight biomass data were previously reported, the nitrogen concentration data from the Ben Abdallah, et al. [14] experiment is reported for the first time in this manuscript.

2.2. Statistical Methods

Based on the general approach outlined by Makowski, et al. [19], 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 %Nc, and compare fitted CNDCs across the effects of location and variety.

In summary, this statistical approach uses the entire set of experimental data (Figure 1a) 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 1b) and the join point of the linear-plateau curve is used to define the %Nc. Simultaneously, a negative exponential curve (i.e., CNDC) is fit across all experimental sampling dates for a given level of the hierarchical model (i.e., location, variety within location) where the critical N point of each linear-plateau curves lies exactly upon the negative exponential curve (Figure 1b). 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. In comparison, the conventional statistical approach fits a negative exponential curve to the subset of critical points (Figure 1c) which are identified via an intermediate statistical analysis (i.e., ANOVA and protected multiple comparisons).

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**Figure 1.** 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., 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 Bayesian hierarchical framework outlined by Makowski, et al. [19] was extended to explicitly include E (e.g., location) and G (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 2). 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* [44], the *brms* package [45, 46] was used to implement the statistical framework outlined by Makowski, et al. [19]. The *brms* package, an interface to *Stan* [47], 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 (i.e., data from all other levels of an effect influence the inference for a particular level) [48]. The *brms* package includes a user-friendly modeling language, robust documentation, and a diverse set of tools to analyze and assess models.

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**Figure 2**. 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. [19]. Linear-plateau curves and critical points (i.e., the fitted join point of each linear-plateau curve) 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.

A non-linear *brms* model was defined by combining the two separate expressions used by Makowski, et al. [19] to parameterize the Bayesian hierarchical model as previously implemented with *rjags* [49] and *JAGS* statistical software [50].

The first expression from Makowski, et al. [19] represents the linear-plateau component:

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

where *Si* and *Wmaxi* are the slope of the linear-plateau curve and the maximum value of biomass (i.e., plateau) for a given date [*i*], respectively, *min* represents the minima function (i.e., the plateau component), and *W*, *%NPlant,* and *%Nc* 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. [19] represents the CNDC component:

|  |  |
| --- | --- |
| *%Nc* = *a* (*Wmaxi*) –*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*(*Wmaxi* + *Si* \* (*%NPlant* – (*a* \* (*Wmaxi*) –*b*)), *Wmaxi*) | (3) |

Two group-level (i.e., random) effects were specified for this *brms* model to parameterize the nested structure (Figure 2). First, the parameters *S* and *Wmax* included group-level effects to fit a linear-plateau curve to each experimental sampling date:

|  |  |
| --- | --- |
| *Wmax* + *S* ~ 1 + (1|*index*) | (4) |

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

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

where *location* and *location:variety* represents the unique level for location and variety within location, respectively.

The *brms* model was fitted using 4 chains and 10000 iterations with 3000 warmups per chain. The priors for this model were chosen based on expert knowledge (i.e., previously reported values), empirical observations (i.e., summary values from the data set), and the joint prior predictive distribution (i.e., if a set of relatively uninformative priors led to biologically or physically impossible predictions, the prior ranges were narrowed) [51]. This is particularly important for hyperparameters dealing with the standard deviation between groups in a hierarchical model. A summary of the prior values used in this model is given below (Table 5).

The entire workflow used to generate this analysis is reproducible and available via GitHub repository (<https://github.com/bohm0072/cndc_bayesian_eval>). The *renv* package [52] was used to document the computing environment utilized while conducting this analysis to ensure code portability and reproducibility.

**Table 5.** Priors used in fitting the *brms* model.

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Distribution** | **Bounds** | |
| **Lower** | **Upper** |
| *a* | Normal (5.3, 0.1) | 0 | ∞ |
| *σ(alocation)* | Normal (0.10, 0.02) | –∞ | ∞ |
| *σ(alocation:variety)* | Normal (0.05, 0.01) | –∞ | ∞ |
| *b* | Normal (0.40, 0.01) | 0 | 1 |
| *σ(blocation)* | Normal (0.05, 0.02) | –∞ | ∞ |
| *σ(blocation:variety)* | Normal (0.02, 0.01) | –∞ | ∞ |
| *Wmax* | Normal (8.0, 0.1) | 1 | ∞ |
| *σ(Wmaxindex)* | Normal (7.0, 1.0) | –∞ | ∞ |
| *S* | Normal (6.0, 0.1) | 0 | ∞ |
| *σ(Sindex)* | Normal (1.0, 0.1) | –∞ | ∞ |
| *σ* | Student\_T (3.0, 1.0, 0.1) | –∞ | ∞ |

2.3. Evaluating Uncertainity

2.3.1. Critical N Dilution Curve Parameter Uncertainty

After the statisticalmodel was successfully fit to the data (n=28,000 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 90% credible region. The correlation between values for parameters *a* and *b* was determined for each level of variety within location using the fitted parameter values for individual draws.

2.3.2. Critical N Concentration Uncertainty

The %Nc for a set of discrete 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 %Nc 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 90% credible region. This approach makes maximal use of the jointly estimated parameters contained in the posterior distribution.

To approximate curves defining the upper and lower boundaries of the 90% credible region for %Nc (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* [53] to the set of data previously identified as defining the boundaries of the 90% credible region (i.e., 0.05 and 0.95 quantile values). These curves approximating the upper and lower boundaries of the credible region are respectively referred to as CNDCup and CNDClo, where parameters *aup* and *bup* correspond to CNDCupand parameters *alo* and *blo* correspond to CNDClo.

Additionally, an estimate of the 90% credible region was calculated by using the boundary values of the 90% credible interval of 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*. This approach does not account for the joint estimation of parameters offered by the Bayesian approach, so the 0.95 quantile value for parameter *a* and the 0.05 quantile value for parameter *b* might not actually occur in the posterior distribution.

2.3.3. Comparing Critical N Dilution Curves across Genotype x Environment Effects

Similar to the above methods, the %Nc for each draw was calculated across a set of discrete values of W over the range of 1 Mg ha-1 and the maximum observed value of W in the experimental data set. At the level of *location:variety*, the difference between the %Nc for a given comparison and reference CNDC was calculated at each value of W. From this computed set of difference in %Nc, the 0.05, 0.50 (i.e., median) and 0.95 quantile values were identified for each level of *location:variety* to determine the 90% credible region. For a given range of W values, the comparison curve considered to be not significantly different from the reference curve if the 0.05 and 0.95 quantile value were respectively less than and greater than zero (i.e., the 90% credible interval contains zero). In the case where the 0.05 quantile value was greater than zero, the comparison curve was said to have a significantly greater %Nc than the reference curve. In the case where the 0.95 quantile value was less than zero, the comparison curve was said to have a significantly lesser %Nc than the reference curve. To evaluate the differences between curves fit in the present study, the CNDC for a given level of *location:variety* was compared to all other levels This approach allows for the direct evaluation of differences in %Nc across G x E effects.

2.3.4. Comparing Critical N Dilution Curves across Statistical Methods

An analogous 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. [14], Giletto, et al. [17]). Specifically, the previously published curves were evaluated to see if they fell within the 90% credible interval for the corresponding curve fitted in the present study. Using the determined credible region for %Nc, it is possible to identify the range for which two CNDCs are significantly different. If the previously identified %Nc value falls outside of the credible region for %Nc identified in this study, then the two curves are determined to be significantly different over the range for which the previous value falls outside of the credible region. This approach allows for direct evaluation of differences in %Nc for CNDCs developed from the same set of data 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 *location:variety*. The experimental data, median linear-plateau curve for each experimental sampling date, and median value of %Nc are presented below (Figure 3). The individual linear-plateau curves fitted for each experimental sampling date of each level of *location:variety* is presented in the Supplemental Materials (Figure S1).

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 4) showing the median value and 90% credible interval (i.e., 0.05 and 0.95 quantile values).

Diagram, engineering drawing

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**Figure 3.** Median fitted critical N dilution curve for each level *location:variety* shown as solid black line. Biomass and nitrogen concentration [%N] data are displayed as points with the median linear-plateau curve for each sampling date shown as grey line. The number of samples [n] and the number of sampling dates [i] are displayed for each *location:variety* level.

|  |  |
| --- | --- |
| Chart, radar chart  Description automatically generated | Chart, scatter chart  Description automatically generated |

**Figure 4.** Posterior distribution of *location* and *location:variety* effects 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 with the figures are the median value with the 90% credible interval boundaries (i.e., 0.05 and 0.95 quantiles) displayed within the parentheses.

For parameter *a*, there was no significant difference for the effect of either *location* or *location:variety* at 90% credible interval threshold (Figure 4a). 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 *location:variety* level is negligible.

For parameter *b*, there were significant differences at both the levels of *location* and *location:variety* at 90% credible interval threshold (Figure 4b). For *location*, Argentina had the lowest value for parameter *b* (0.175), while Canada had a greater value for parameter *b* (0.448)than Argentina but lower than either Belgium (0.561) or Minnesota (0.582). The difference between parameter *b* for Belgium and Minnesota was not significant.

For the *location:variety* effect, 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 (0.212), followed by Gem Russet, Umatilla Russet, Markies Russet, and Bannock Russet (0.178, 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 *location:variety* (Figure 5) 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 independently is not sufficient to describe the combined uncertainty in %Nc described by the correlated parameters. Stated alternatively, non-significant differences in parameters *a* and *b* does not ensure that differences in %Nc are non-meaningful or not significant.

Diagram, shape

Description automatically generated

**Figure 5.** Distribution of posterior values for parameters *a* and *b* for each *location:variety* level 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:variety* level. Data are shown at the level of individual draws (n=28,000)

3.2. Critical N Concentration Uncertainity

The credible region for %Nc varies across levels of *location:variety* and across levels of biomass within a given level of *location:variety* (Figure 6). The symmetry of the credible region distribution varies across levels of *location:variety* 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 *location:variety*, such as Argentina x Umatilla Russet, have greater uncertainty in %Nc than other levels, such as Minnesota x Russet Burbank. The uncertainty in %Nc 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.



**Figure 6.** Comparison of the difference in critical N concentration values [%N Difference] between the median critical N concentration, represented by the solid black line at constant value of zero and various methods to represent uncertainty in critical N concentration. The grey shaded region represents the 90% credible region (lower bound, 5% quantile; upper bound, 95% quantile) for the critical nitrogen concentration values fitted by the Bayesian hierarchical model. The dotted lines represent an estimation of the upper and lower bound of the 90% credible region using non-linear regression to fit a negative exponential curve of the same equation form as the critical nitrogen dilution curve (i.e., CNDClo and CNDCup). The dashed lines represents a conservative approximation of the upper and lower bounds of 90% credible region 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*.

Approximations of the upper and lower boundaries of the 90% critical region using non-linear regression (i.e., CNDClo and CNDCup) (Table 6) appears to be reasonable based on graphical evaluation (Figure 6). However, these fitted CNDClo and CNDChi curves do not themselves represent a draw directly from the posterior distribution and don’t necessarily represent the most extreme possible curves (e.g., it is plausible to have an individual draw that goes from the lower left to upper right corner of the interval, or vice versa).

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 credible region defined directly from the fitted model, the CNDClo and CNDCup method based on non-linear regression is a suitable first-order representation of the credible region.

**Table 6.** Paired critical nitrogen dilution curve parameter values for each *location:variety* level defining the estimate values for the credible region lower (CNDClo) and upper (CNDCup) boundaries, and the median value (CNDC) from the posterior distribution for critical nitrogen concentration from the fitted Bayesian hierarchical model.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Location** | **Variety** | **CNDClo** | | **CNDC** | | **CNDCup** | |
| Argentina | Bannock Russet | *alo* | 4.82 | *a* | 4.96 | *aup* | 5.10 |
| *blo* | 0.146 | *b* | 0.140 | *bup* | 0.135 |
| Gem Russet | *alo* | 4.80 | *a* | 4.96 | *aup* | 5.07 |
| *blo* | 0.190 | *b* | 0.178 | *bup* | 0.152 |
| Innovator | *alo* | 4.83 | *a* | 4.94 | *aup* | 5.06 |
| *blo* | 0.241 | *b* | 0.212 | *bup* | 0.193 |
| Markies Russet | *alo* | 4.82 | *a* | 4.96 | *aup* | 5.08 |
| *blo* | 0.167 | *b* | 0.155 | *bup* | 0.135 |
| Umatilla Russet | *alo* | 4.85 | *a* | 4.95 | *aup* | 5.06 |
| *blo* | 0.195 | *b* | 0.165 | *bup* | 0.143 |
| Belgium | Bintje | *alo* | 4.52 | *a* | 4.72 | *aup* | 4.90 |
| *blo* | 0.606 | *b* | 0.579 | *bup* | 0.567 |
| Charlotte | *alo* | 4.56 | *a* | 4.74 | *aup* | 4.89 |
| *blo* | 0.607 | *b* | 0.559 | *bup* | 0.531 |
| Canada | Russet Burbank | *alo* | 4.53 | *a* | 4.74 | *aup* | 4.93 |
| *blo* | 0.498 | *b* | 0.489 | *bup* | 0.480 |
| Shepody | *alo* | 4.55 | *a* | 4.77 | *aup* | 4.95 |
| *blo* | 0.416 | *b* | 0.412 | *bup* | 0.406 |
| Minnesota | Clearwater | *alo* | 4.56 | *a* | 4.75 | *aup* | 4.93 |
| *blo* | 0.622 | *b* | 0.585 | *bup* | 0.558 |
| Dakota Russet | *alo* | 4.54 | *a* | 4.75 | *aup* | 4.94 |
| *blo* | 0.619 | *b* | 0.599 | *bup* | 0.588 |
| Easton | *alo* | 4.54 | *a* | 4.75 | *aup* | 4.91 |
| *blo* | 0.608 | *b* | 0.592 | *bup* | 0.567 |
| Russet Burbank | *alo* | 4.51 | *a* | 4.74 | *aup* | 4.95 |
| *blo* | 0.562 | *b* | 0.566 | *bup* | 0.567 |
| Umatilla Russet | *alo* | 4.56 | *a* | 4.75 | *aup* | 4.92 |
| *blo* | 0.631 | *b* | 0.588 | *bup* | 0.546 |

However, the estimate based directly on uncertainty in CNDC parameters *a* and *b*, using the previously determined credible interval boundaries (Figure 4), were found to contain the entire credible region and for all *location:variety* levels (Figure 6). Therefore, this estimate approach is quite uninformative and should be used as a last resort to estimate %Nc uncertainty is required and the credible region defined from the original model fit or from the CNDClo or CNDCup curves is unavailable.

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 *location:variety* was conducted and is presented in the Supplemental Materials (Figure S2), a subset of the results comparing Minnesota x Russet Burbank to all other levels is presented in detail here (Figure 7).

Diagram

Description automatically generated

**Figure 7.** Comparison of the difference in critical N concentration values [%N Difference] between Russet Burbank x Minnesota and all other levels of *location:variety* evaluated in the present study. The grey shaded region represents the 90% credible region (lower bound, 5% quantile; upper bound, 95% quantile) and the colored points represent the median value for the difference in critical N concentration values between the reference and comparison critical N dilution curves computed from the difference of the individual draws from the posterior distribution of the fitted Bayesian hierarchical model. Red or blue points for a given Biomass value respectively indicate that the difference in critical N concentration between the reference and comparison curves at that point is significant (i.e., does not contains the value zero) or is not significant (i.e., contains the value zero). The solid black line represents a constant value of zero. The range of biomass values for which the difference in critical N concentration between the comparison and reference curve is not significantly is given in brackets.

For Minnesota x Russet Burbank, there were no significant differences in %Nc for any level of W evaluated with any of the other varieties in Minnesota (i.e., Clearwater, Dakota Russet, Easton, and Umatilla Russet) or with the Belgium varieties (i.e., Bintje, and Charlotte). The %Nc for both of the Canadian varieties (i.e., Russet Burbank, and Shepody) were significantly greater than that for Minnesota x Russet Burbank when biomass values were greater than 2 Mg ha-1. The %Nc for Canada x Russet Burbank and Canada x Shepody were up to 0.3 and 0.6 g N 100g-1 greater than that for Minnesota x Russet Burbank, respectively. The %Nc for the Argentina varieties (i.e., Bannock Russet, Gem Russet, Innovator, Markies Russet, and Umatilla Russet) were significantly greater than for Minnesota x Russet Burbank, except for at a biomass value of 1.0 Mg ha-1, with a difference in value depending on variety of up to 2.4 g N 100 g-1.

There are two notable findings to point out here. First, there were no significant differences between Minnesota x Russet Burbank and any other varieties evaluated in Minnesota (i.e., when controlling for E, no significant differences due to G). This finding did not hold across all levels of *location:variety*, however; while there was no significant difference between the varieties evaluated in Belgium, there were significant differences between the varieties evaluated in Canada and some of the varieties evaluated in Argentina (Figure S2). Second, the Minnesota x Russet Burbank and Canada x Russet Burbank curves were significantly different (i.e., when controlling for G, a significant difference due to E). The only other comparison controlling for G across E, Minnesota x Umatilla Russet and Argentina x Umatilla Russet, conducted in this study was also significantly different (Figure S2).

Taken together, these findings provide evidence that the effect of E (i.e., *location*), even when controlling for G (i.e., *location:variety*), can result in significantly different %Nc; additionally, this provides evidence that differences in G within a given E do not necessarily result in significant different %Nc. Therefore, these findings suggest that E is relatively more important than G in determining %Nc.

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 levels of *location:variety* (Figure 8). None of the previous CNDCs fall entirely within credible interval for the respective CNDC developed in the present study.

Graphical user interface, diagram

Description automatically generated

**Figure 8.** Comparison of the difference in critical N concentration values [%N Difference] from the critical N dilution curves reported in previous studies (i.e., Argentina – Giletto and Echeverría (2015); Belgium – Ben Abdallah et al. (2016; Canada – Bélanger et al. (2001)) and from the critical N dilution curve developed in the present study for each *location:variety* pair. The grey shaded region represents the 90% credible region (lower bound, 5% quantile; upper bound, 95% quantile) and the black solid line represents the median value for the posterior distribution of the Bayesian hierarchical model fitted in the present study. Red or blue points for a given Biomass value respectively indicate that the difference in critical N concentration between the previous critical N dilution curve and median value at that point is significant (i.e., falls outside the credible region) or is not significant (i.e., falls within the credible region). The range of biomass values for which the difference in critical N concentration between the critical N dilution curves from the present and previous studies in not significantly is given in brackets.

The %Nc from the previously developed CNDCs for the Argentina varieties [16] was significantly less than that from the present CNDCs across all varieties for biomass levels of greater 5 Mg ha-1 (Figure 8). The magnitude of this difference was relatively large with the %Nc from the previous method ranging up to 0.6 to 1.1 g N 100 g-1 less than median %Nc from the present method, depending on variety. Based on the CNDCs fitted in the present study for the Argentina varieties (Figure 3), more than 60% of the observed data fall below the CNDC (i.e., represent N limiting conditions) with over 40% of sampling dates having exclusively N limiting conditions observed (Figure S1).Therefore, it appears that the statistical methods used by Giletto and Echeverría [16] selected biased critical points due to a overrepresentation of N limiting observations in the experimental dataset leading to a systematic underestimation of the %Nc.

The %Nc from the previously developed CNDCs for Belgium [14] were significantly greater than that from the CNDCs developed in the present study (Figure 8). While the magnitude of this difference in %Nc was 0.7 g N 100 g-1, there was no level of biomass for which the %Nc from the previous and present methods were not significantly different. Based on the CNDCs fitted in the present study for the Belgium varieties (Figure 3), more than 80% of the observed data fall above the CNDC (i.e., represent non-N limiting conditions) with almost 30% of sampling dates having exclusively non-N limiting conditions observed (Figure S1). Therefore, it appears that the statistical methods used by Ben Abdallah, et al. [14] selected biased critical points due to overrepresentation of non-N limiting observations in the experimental dataset leading to a systematic overestimation of the %Nc.

The %Nc from the previously developed CNDCs for Canada [15] 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 greater than 6 Mg ha-1, respectively (Figure 8). Relative to the other locations, however, the CNDCs for Canada were the most similar between statistical methods, with magnitude of difference in %Nc of 0.2 g N 100 g-1. Based on the CNDCs fitted in the present study for the Canada varieties (Figure 3), over 60% of observed data represented non-N limiting conditions but less than 10% of sampling dates had exclusively non-N limiting conditions observed (Figure S1). Therefore, it appears that the statistical method used by Bélanger, et al. [15] did not select biased critical points likely due to the minimal bias observed in this experimental dataset.

4. Discussion

4.1. Implication of G x E Variataion on N Use Efficiency

4.1.1. Critical N Utilization Efficiency

Understanding and properly interpreting the impact of G x E effects on NUE is a critical goal necessary to improve N fertilizer use; however, this must be done while controlling for the effect of crop N status [18]. The previous findings of Bohman, et al. [54] demonstrated that interpreting NUE and its constituent component of N utilization efficiency [NUtE] is directly related to the parameters of the CNDC through the critical N utilization efficiency curve [CNUtEC] which defines the critical value of NUtE [NUtEc]:

|  |  |
| --- | --- |
| NUtEc = 1000 (10 a W–b)–1 | (9) |

where parameters *a*, *b*, and *W* have the same meaning and units as previously defined in the present study. When NUtE is greater than NUtEc, crop N status is deficient (i.e., NNI less than 1); conversely, when NUtE is less than NUtEc, crop N status is excessive (i.e., NNI greater than 1).

The finding in the present study that the CNDC can vary across G x E effects and the finding from Bohman, et al. [54] of the intrinsic relationship between NUE and the CNDC together lead to the conclusion that the CNUtEC must also vary across the same G x E effects as the CNDC. Therefore, the effect of G x E on variation of NUtEc is one of the multiple set of factors that ultimately control NUE. Understanding and accounting for the G x E effect on the CNUtEC is therefore critically important to understand the impacts of G x E interactions on NUE.

4.1.2. Physiological Mechanisms

While the present study presents direct evidence of significant differences between CNDCs for potato across G x E effects, other previous studies help describe the potential physiological mechanisms for this source of this variation. The findings of Giletto, et al. [17] suggest that variation in CNDCs for potato across G x E effects is primarily due to differences in the relative rate of partitioning of biomass to tubers. For example, G x E effects that result in greater partitioning of biomass from vines (i.e., high N metabolic and structural tissue) to tubers (i.e., low N storage tissues) will have resultingly greater N dilution (i.e., lower %Nc) at the same level of total plant biomass.

Following from the above discussion of the CNUtEC and the findings of Giletto, et al. [17], G x E effects that increase the relative proportion of biomass partitioned to tubers will both decrease the %Nc and increases the NUtEc value. Therefore, efforts to systematically improve NUE in potato should focus on identifying G x E interactions that result in an increased proportion of biomass partitioned to tubers.

Additionally, based on the larger magnitude of differences in %Nc between *locations* (i.e., E) compared to differences between *location:variety* (i.e., G) observed in this study (Figure 7, Figure S2), it is reasonable to conclude that increases in NUE for potato resulting from decreasing %Nc will be of a greater magnitude from E rather than G effects.

4.1.3. Comparison to Other Crops

These findings contrasts somewhat with the previous studies evaluating G x E effects on %Nc. Yao, et al. [21] found a similar magnitude of effect on %Nc for both G and E effects for wheat in China; however, Yao, et al. [21] also reported an E effect where %Nc for wheat in China was significantly difference from that reported by Makowski, et al. [19] for wheat in France. Ciampitti, et al. [20] found variation as a result of G x E interactions, but did not independent report either G or E effects. In any case, the magnitude of the difference in %Nc for any effect (i.e., G, E) or interaction (i.e., G x E) reported by the previous studies [19-21] is less than that observed for E in the present study.

Therefore, the impact of E on %Nc is not just significant for potato, but is also of much greater relative importance compared to other major crops (e.g., wheat, maize). Due to the magnitude of this variability, accounting for variation in %Nc due to G x E interactions when evaluating NUE is relatively more important for potato. In order to improve the understanding of this relationship between NUE and %Nc, future work should continue to better characterize the relative partitioning of potato biomass to tubers across G x E effects.

4.2. Uncertainity in Critical N Concentration

4.2.1. Communicating Uncertainty in Critical N Concentration

The findings of this present study as well as those of other previous studies which have implemented Bayesian statistical methods to derive critical N dilution curves [19-21] clearly indicate that there is meaningful uncertainty in %Nc values. Therefore, the use of %Nc in subsequent calculations should include this inherent uncertainty. However, the direct use of the credible region defined from posterior distribution of the fitted Baysian hierarchical model in subsequent calculations is impractical and a method to concisely and accurately communicate the credible region remains necessary.

Our finding that the credible region can be satisfactorily estimated using an equation of the same form as the CNDC (Figure 6) suggest that an additional pair of negative exponential curves representing the upper and lower boundary of the credible region for %Nc (i.e., CNDClo and CNDCup) should be reported in future studies. In this manner, the median value and credible region for %Nc is defined by a set of three, two-parameter curves (i.e., CNDC – *a*, *b*; CNDCup – *aup*, *bup*; CNDClo – *alo*, *blo*) which can be easily communicated and used in subsequent computations (Table 6).

4.2.1. Computing Uncertainty of Derived Parameters

Critical N concentration and the associated CNDC parameters are commonly used to derive and calculate other related parameters. For example, the calculation of NNI depends on both the actual plant N concentration [%NPlant] and %Nc:

|  |  |
| --- | --- |
| NNI = %NPlant / %Nc = %NPlant / (a W–b) | (6) |

However, to properly account for the uncertainty in %Nc when computing NNI, the upper [%Nc,up] and lower [%Nc,lo] bounds of the credible region should also be used to determine the upper [NNIup] and lower [NNIlo] bounds of NNI, where %Nc,up and %Nc,lo are calculated using the CNDCup and CNDClo, respectively:

|  |  |
| --- | --- |
| NNIup = %NPlant / %Nc,up = %NPlant / (aup W–bup) | (7) |
| NNIlo = %NPlant / %Nc,lo = %NPlant / (alo W–blo) | (8) |

This has important practical implications for interpreting NNI values. For example, in a case where NNI is less than 1 but NNIup is greater than 1, then it follows that crop N status would not be considered deficient (i.e., NNI is not significantly different from 1); comparingly, in a case where both NNI and NNIlo are greater than 1, then it follows that crop N status would be considered surplus (i.e., NNI is significantly greater than 1). The conclusions of a small-plot trial evaluating various N fertilizer treatments and using NNI to interpret the effects of those treatments on yield and biomass (e.g., Bohman, et al. [54]) may draw different conclusions in light of considering uncertainty in calculated NNI values.

Additionally, the parameters of the CNDC (i.e., *a*, *b*) are also used to parameterize other related curves such as the critical N uptake curve [CNUC] or the critical N utilization efficiency curve [CNUtEC] [54]. When computing the critical N uptake [Nc] or critical N utilization efficiency [NUtEc] values defined by these curves, respectively, the parameters from the CNDClo (i.e., *alo, blo*) and CNDCup (i.e., *aup, bup*) should be used to calculate the upper and lower bounds of these derived values. In general, any calculation depending on either %Nc or any equation that uses the parameters of the CNDC, should also additionally use the CNDClo and CNDCup to account for uncertainty in %Nc.

4.3. Evaluating Differences between Statistical Methods

While the observation of difference in CNDCs derived using the Bayesian hierarchical model compared to the conventional statistical methods used in previous studies (Figure 7) is not necessarily remarkable, the magnitude of these differences as found in the present study is, however. Because of its strong theoretical underpinning, %Nc and NNI are typically considered to be high fidelity measurements of crop N status, absent of the subjectivity or relativity found in most other methods [2]. However, the findings of the present study strongly suggest that idealized conception of the NNI framework must be qualified upon application by the statistical methods used to derive the CNDC for a particular experimental dataset.

Unfortunately, the direct evaluation of different statistical methods to calculate the CNDC from the same experimental dataset cannot directly answer the question of which statistical method or resulting CNDC is “correct” (i.e., most accurate, least biased). However, we can reasonably conclude from both deduction and from the findings of the present study that a Bayesian hierarchical model utilizing the linear-plateau method and leveraging partial pooling across effect levels will result in an inference that is less subjected to potential bias in the experimental data set compared to the conventional statistical methods. Additionally, it extracts the greatest amount of information from a given dataset, as no data are excluded from the fitting of the total model.

Therefore, it appears preferable for the future development of CNDCs to utilize the Bayesian hierarchical method to both quantify uncertainty and reduce bias in %Nc. Without addressing these limitations (i.e., bias and uncertainty), both directly resulting from the statistical methods used, the NNI framework cannot not fulfill its core objective of providing an absolute reference of crop N status.

Additionally, with the further development adequate tools for this scientific computing task, the implementation of the Bayseian hierarchical framework for deriving the CNDC can be made trivial and may enable the development of CNDCs from existing but unutilized experimental datasets. Therefore, the development of a dedicated software library to implement the Bayesian hierarchical method is a priority for future research efforts.

5. Conclusions

First, this study demonstrated that there are significant differences between CNDCs developed across G x E effects for potato. Therefore, any application of %Nc must use an appropriate CNDC (i.e., not significantly difference) for the G x E interaction being considered. Second, this study developed an approach to communicate uncertainty in %Nc through the concise set of six parameters defined by the CNDC (i.e., *a*, *b*), CNDClo (i.e., *alo*, *blo*), and CNDCup (i.e., *aup*, *bup*). The critical N concentration value computed from these three curves should be used in all subsequent computations to propagate uncertainty. Third, this study demonstrated that the statistical method used to derive CNDCs has an impact on the inferred %Nc values, and that the hierarchical Bayesian framework is less susceptible to bias due to biased experimental data than the conventional method. Therefore, future efforts to derive CNDCs should utilize the hierarchical Bayesian framework whenever possible. Fourth, the findings of this study suggest that variation in %Nc across G x E interactions necessarily extends to NUE, via the relationship between the CNDC and the CNUtEC. Therefore, NUE is dependent on the mechanisms that control N dilution (i.e., biomass partitioning), and future efforts to improve NUE should explicitly consider how G x E interactions effect N dilution.

**Supplementary Materials:** The following are available online at www.mdpi.com/xxx/s1, Figure S1: Linear-plateau curves fitted for each experimental sampling date, Figure S2: Pairwise comparisons of differences in critical N concertation for all levels of *location:variety*, Table S1: Experimental data used to fit hierarchical Bayesian model.

**Author Contributions:** Conceptualization, B.B. and C.R.; methodology, B.B. and M.C.M.; software, B.B. and M.C.M; validation, B.B., and M.C.M.; formal analysis, B.B. and M.C.M.; investigation, C.R., F.B.A.; resources, C.R.; data curation, B.B., F.B.A.; writing—original draft preparation, B.B.; writing—review and editing, M.C.M, C.R., D.M.; visualization, B.B., M.C.M.; supervision, C.R., D.M.; project administration, C.R.; funding acquisition, C.R. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded in part by Minnesota Area II Potato Growers Research and Promotion Council.

**Data Availability Statement:** The data presented in this study are available in Supplementary Materials (Table S1) as well as the GitHub repository associated with this manuscript (<https://github.com/bohm0072/cndc_bayesian_eval>).

**Acknowledgments:** The authors acknowledge the contribution of open source data from previously published papers, which made this paper possible.

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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