Article

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 evaluates the difference in %Nc across G x E effects, and directly compares 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 within a given location (i.e., G) had a lesser effect. In addition to using the median value for %Nc (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. Additionally, this study found that the conventional statistical method used to derive CNDCs 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, this study provides 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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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 where the optimal N fertilizer rate is empirically based on the marginal economic returns to yield from N fertilizer (i.e., EONR) under a given set of genotype [G], environment [E], and management [M] factors [1, 5], the NNI framework has conventionally been considered generalizable across E x M effects (e.g., year-to-year or geographic variability) and can be defined for any particular G effect (e.g., crop species or cultivar). In this manner, NNI is considered to represent intrinsic physiological properties [6] rather than a parameter subject to variable environmental conditions (e.g., net soil N supply) or management practices (i.e., rate, source, timing, and placement of N fertilizer).

The NNI approach is defined based on the allometric relationship of declining plant N concentration [%N] with increasing plant biomass, referred to as the critical N dilution curve [CNDC], which defines the critical N concentration [%Nc] below which relative growth rate is reduced [7]:

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| %Nc = a W–b | (1) |

where W represents plant biomass, and *a* and *b* are empirically fitted parameters. Parameter *a* is numerically equivalent to %Nc expressed in units of g N 100 g−1 when W is equal to 1 Mg ha−1, and parameter *b* effectively represents the rate of decline in %Nc as W increases. Using the CNDC, NNI values are then calculated as ratio based on %Nc:

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| NNI = %N / %Nc | (99) |

where %N represents plant N concentration. When NNI is greater than 1.0, crop N status is said to be in excess, and crop growth is not limited by N, while when NNI is less than 1.0, crop N status is deficient, and crop growth is limited by N. At NNI equal to 1.0, crop N status is optimal [8].

A robust theoretical framework has been developed to explain decline in N concentration as biomass increases, but the application of this theory is most commonly restricted to the vegetative period where only metabolic and structural tissues are present [6, 9, 10]. Dilution of N in vegetative tissue occurs in relationship to an increasing proportion of structural biomass, with low N concentration, relative to metabolic (i.e., photosynthetic) biomass, with high N concentration [7, 8].

Multiple previous studies have extended and empirically validated the CNDC relationships beyond its typical applications to describe declining N concentration over the entire crop growth cycle, including periods of reproductive growth, by including consideration of storage tissues in addition to structural and metabolic tissues [11-14]. 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 [11, 12]. Duchenne et al. [12] observed that as an increasing proportion of biomass accumulates in tubers (i.e., as harvest index increases), the rate of decline in N concentration increases with increasing biomass. 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 [12, 15-17]. Despite the validity of this approach, interpretating variation in CNDC observed between cultivars and geographies has been challenging.

However, recent work by Giletto et al. [18] identified a mechanistic relationships underpinning the observed empirical relationships in N dilution for potato. The CNDC based on 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. [18] also observed 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. [18], it is reasonable to expect that variation in CNDC across E (e.g., climate, geography, etc.) and G (e.g., variety) would occur 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 [19].

Previous development of CNDCs for potato [12, 15-17] 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 result from underlying G x E effects or are confounded by the limitations of the statistical approach.

The conventional approach to fit a CNDC, consists of a two-step process: first, the critical 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. [10]. 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. [15] 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. [20] 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 points using the linear-plateau method (e.g., Justes et al. [10]) 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 points. While this approach is newly developed, it has already been used by Ciampitti et al. [21] and Yao et al. [22] 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 [17], Canada [16], and Belgium [15] (from previously published experimental data), 2) extend the implementation of the hierarchical Bayesian framework to compare CNDCs across G x E interactions (i.e., variety, location) based on the uncertainty in %Nc and curve parameters *a* and *b*, 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. Results**

2.1. Fitted Critical N Dilution Curve

The posterior distribution of fitted values for CNDC parameters *a* and *b* are presented below (Figure 1) showing the median value and 90% credible interval (i.e., 0.05 and 0.95 quantile values). For parameter *a*, there was no significant difference for the effect of location at 90% credible interval threshold (Figure 1a). 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* for the variety within location effect is negligible and not statistically significant (Figure 1a).

For parameter *b*, there were significant differences for both the effect of location and variety within location at 90% credible interval threshold (Figure 1b). 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 variety within location 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 higher value for parameter *b* (0.489) than Shepody (0.412).

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| Chart, radar chart  Description automatically generated | |
| **(a)** | **(b)** |

**Figure 1.** Posterior distribution of variety and variety within location effects for **(a)** parameter *a*; and **(b)** parameter *b*. Points represent median value and 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.

There was a positive correlation found between parameter *a* and *b* (Figure 2) which indicates that quantifying differences in these parameter values independently (Figure 1) is not appropriate to describe the uncertainty in %Nc determined by the correlated parameters. Stated alternatively, significant differences between parameters *a* and *b* does not necessarily imply that differences in %Nc are also significant.

Critical N dilution curves for each variety within location and 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 nested within each level of the variety within location effect are presented in the Supplemental Materials (Figure S1).

2.2. Critical N Concentration Uncertainity

The credible region for %Nc varies across variety within location and across levels of biomass (Figure 4). The symmetry of the credible region distribution varies by variety within location with some combinations, such as Argentina x Gem Russet, having a skewed distribution, while other levels, such as Canada x Shepody, having a symmetrical distribution (Figure 4a). There are also differences in the range of the credible region where some varieties within location, such as Argentina x Umatilla Russet, have greater uncertainty in %Nc than others, such as Minnesota x Russet Burbank. The uncertainty in %Nc also varies across the level of biomass for a given CNDC. For example, as the 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.

Diagram, shape

Description automatically generated

**Figure 2.** Distribution of posterior values for parameters *a* and *b* for each location within variety 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*. Data are shown at the level of individual draws (n=28,000).

**Diagram, engineering drawing

Description automatically generated**

**Figure 3.** Critical N dilution curves (i.e., median value of critical N concentration [%Nc]) fitted from the hierarchical Bayesian model for each location with variety is 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 on each individual panel.

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| **(a)** | Diagram, engineering drawing  Description automatically generated |
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**Figure 4.** Comparison of the difference in critical N concentration values [∆%Nc] between the median critical N concentration, represented as a solid black line at constant value of zero, and the various methods to quantify uncertainty in %Nc. In this manner, the magnitude of uncertainty in %Nc is equivalent the ∆%Nc value presented. The grey shaded region represents the 90% credible region (lower bound, 5% quantile; upper bound, 95% quantile) for the fitted Bayesian hierarchical model. The dotted lines represent an estimation of the upper and lower bound of the 90% credible region from using the non-linear regression method (i.e., CNDClo and CNDCup). The dashed lines represent an approximation of the upper and lower bounds of 90% credible region based on the posterior distribution of parameters *a* and *b*. Data are presented for **(a)** all varieties within location, and **(b)** shown in greater detail for Minnesota x Russet Burbank only for individual draws from the Bayesian hierarchical model, for the non-linear regression method, and for the approximation of the 90% credible region based on the posterior distribution of parameters *a* and *b*. For **(b)**, the solid red line represents individual draws (n=15) from the posterior distribution of the fitted Bayesian hierarchical model.

Estimation of the upper and lower boundaries of the 90% credible region using the non-linear regression method (i.e., CNDClo and CNDCup) (Table 1) appears to be reasonable based on graphical evaluation (Figure 4a, 4b). However, these fitted CNDClo and CNDCup curves do not themselves represent a draw directly from the posterior distribution and do not 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) (Figure 4b). While credible regions with boundaries that are non-monotonic (e.g., Argentina x Innovator) have portions of the curve fit approximation that are poorer performing, the credible regions with monotonic boundaries (e.g., Minnesota x Dakota Russet) seem to be satisfactory across the entire range of the curve.

However, the estimation of the credible region for %Nc based directly on uncertainty in CNDC parameters *a* and *b*, using the previously determined credible interval boundaries (Figure 1), were found to contain the entire credible region for all varieties within location evaluated (Figure 4a). Therefore, this estimate approach directly using the uncertainty in CNDC parameters is quite uninformative and should be used as a last resort to estimate %Nc uncertainty when the credible region defined from either the original model fit or from the paired CNDClo or CNDCup curves is unavailable.

Therefore, in the absence of the credible region defined directly from the fitted hierarchical Bayesian model, the CNDClo and CNDCup (Table 1) are a suitable first-order representation of the credible region for %Nc.

**Table 1.** Paired critical nitrogen dilution curve parameters for each variety within location for the median value (CNDC) from the posterior distribution of the fitted hierarchical Bayesian model and the estimates for the credible region lower (CNDClo) and upper (CNDCup) boundaries using the non-linear regression method.

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

2.3. Evaluating Differences between Critical N Concentration

2.3.1. Differences Related to Genotype x Environment Effects

While an evaluation of the pairwise differences between all varieties within location was conducted and is presented in the Supplemental Materials (Figure S2), a subset of the results comparing Minnesota x Russet Burbank to all other varieties within location presented in detail here (Figure 5).

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.

Diagram

Description automatically generated

**Figure 5.** Comparison of the difference in critical N concentration values [∆%Nc] between Russet Burbank x Minnesota and all other varieties within location evaluated in the present study. The grey shaded region represents the 90% credible region (lower bound, 5% quantile; upper bound, 95% quantile) for ∆%Nc. The colored points represent the median value for ∆%Nc at a given Biomass level where blue or red color respectively indicate that credible region for ∆%Nc does or does not contain zero. The solid black line represents a constant value of zero. The range of biomass values for which ∆%Nc is not significantly different (i.e., credible region contains zero) is given in brackets.

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 for all varieties within location evaluated, 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., 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.

2.3.2. 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 varieties within location evaluated (Figure 6). None of the previous CNDCs fall entirely within credible region for the respective CNDC developed in the present study.

**Graphical user interface, diagram

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**Figure 6.** Comparison of the difference in critical N concentration values [∆%Nc] between the conventional statistical methods used in previous studies (i.e., Argentina – Giletto and Echeverría [17]; Belgium – Ben Abdallah et al. [15]; Canada – Bélanger et al. [16]) and the hierarchical Bayesian method used in the present study for each variety within location. The grey shaded region represents the 90% credible region (lower bound, 5% quantile; upper bound, 95% quantile) for %Nc and the black solid line represents the median value for %Nc derived in the present study. Red or blue points respectively indicate that ∆%Nc falls outside of (i.e., is significant) or falls within (i.e., is not significant) the credible region for %Nc. The range of biomass values for which ∆%Nc in not significant is given in brackets.

The %Nc from the previously developed CNDCs for the Argentina varieties [17] was significantly less than that from the present CNDCs across all varieties for biomass levels of greater 5 Mg ha-1 (Figure 6). 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 using the hierarchical Bayesian method in the present study for the Argentina varieties (Figures 5 and S1), 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 [17] 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 [15] were significantly greater than that from the CNDCs developed in the present study (Figure 6). 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 using the hierarchical Bayesian method 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. [15] 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 [16] 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 6). 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 using the hierarchical Bayesian method 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. [16] did not select biased critical points likely due to the minimal bias observed in this experimental dataset.

Because a CNDC using the conventional statistical methods has not been previously published for Minnesota, no comparison across statistical methods is made for this experimental dataset. However, over 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). The bias present in the Minnesota experimental dataset is similar to the bias of the Belgium experimental dataset; therefore, using the conventional statistical methods to derive a CNDC for Minnesota would likely overestimate %Nc relative to the hierarchical Bayesian method.

3. Discussion

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

3.1.1. 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 [19]. The previous findings of Bohman et al. [23] 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]:

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| NUtEc = 1000 (10 a W–b)–1 | (2) |

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. [23] 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.

3.1.2. 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. [18] 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 result in 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. [18], G x E effects that increase the relative proportion of biomass partitioned to tubers will both decrease the %Nc and increase the NUtEc values. Therefore, future efforts to systematically improve NUE in potato through either management practices [M] (e.g., Bohman et al. [23]) or breeding (e.g., Stefaniak et al. [24], Jones et al. [25], Tiwari et al. [26]) should focus on identifying G x E x M 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 varieties within a location (i.e., G) observed in this study (Figure 5, 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.

3.1.3. Comparison to Other Crops

These findings contrasts somewhat with the previous studies evaluating G x E effects on %Nc. Yao et al. [22] found a similar magnitude of effect on %Nc for both G and E effects for wheat in China; however, Yao et al. [22] also reported an E effect where %Nc for wheat in China was significantly difference from that reported by Makowski et al. [20] for wheat in France. Ciampitti et al. [21] 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 [20-22] 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.

3.2. Uncertainity in Critical N Concentration

3.2.1. 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 [20-22] 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 4) suggests 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 1).

3.2.3. 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 [%N] and %Nc. (Eqs. [1] and [2]). 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 = %N / %Nc,up = %N / (aup W–bup) | (4) |
| NNIlo = %N / %Nc,lo = %N / (alo W–blo) | (5) |

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). In contrast, when 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 the effect of various N fertilizer treatments and on yield and biomass (e.g., Bohman et al. [23]) may draw different conclusions when uncertainty in calculated NNI values is explicitly considered.

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] [23]. 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.

3.3. Evaluating Differences between Statistical Methods

While the occurance of difference in CNDCs derived using the Bayesian hierarchical model compared to the conventional statistical methods (Figure 5) is itself notable, the magnitude of these differences found in the present study is especially remarkable. Because of its strong theoretical underpinning, %Nc and NNI are typically considered to be high fidelity measurements of crop N status, not affected by the subjectivity or relativity found in most other methods [2]. However, the findings of the present study strongly suggest that this idealized conception of the NNI framework must be qualified within a particular application by the statistical methods used to derive the CNDC for a given 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 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 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 Bayesian 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.

4. Materials and Methods

4.1. Experimental Data

This study combines experimental data from both newly reported and previously published sources [15, 18]. The data used for analysis in this study are summarized in Table 2 and the relevant methods related to the experimental trials are reported below. All individual experimental observations used in this study are presented in the Supplemental Materials (Table S1).

4.1.1. 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) (Table 3) and were collectively considered in the present study (Table 2). Mean temperature at this station is 7.1 ºC and mean annual precipitation is 809 mm [27]. The soil at this station is characterized as a Hubbard loamy sand (Sandy, mixed, frigid Entic Hapludolls) and excessively well drained with low available water holding capacity [28, 29]. Apart from experimental N and variety treatments, all management and cultural practices were managed by the staff at the SPRF in accordance with common practices for the region [30], nutrients were applied based on soil samples and University recommendations [31, 32], and supplemental irrigation was applied based on the University recommended checkbook method [33, 34]. Additional details on experimental procedures for these studies have been previously reported (Table 3).

**Table 2.** 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. [18] | 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. [15] | Belgium | Bintje | 17 | 49 | 238 |
| Charlotte | 7 | 24 | 114 |

**Table 3.** Summary of newly reported experimental small-plot trials in Minnesota, USA

|  |  |  |
| --- | --- | --- |
| **Experiment** | **Year** | **Reference** |
| MN-1 | 1991-1992 | Errebhi et al. [35], Rosen et al. [36], Rosen et al. [37] |
| MN-2 | 2014-2015 | Sun [38], Sun et al. [39] |
| MN-3 | 2016 | Crants et al. [40] |
| MN-4 | 2018-2019 | Gupta and Rosen [41], Gupta et al. [42] |
| MN-5 | 2019 | Bohman et al. [43] |
| MN-6 | 2020 | Rosen et al. [44] |

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 3). 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 N management practices and varieties evaluated for each of these studies is summarized below (Table 4).

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 [45], 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 [46], or with the salicylic Kjeldahl method [47]. 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.

**Table 4.** 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

Whole-plant samples were also regularly collected during the period of late-May to early-September (Table 5). 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 the same as methods previously described above.

**Table 5.** 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. |

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

Experimental data reported in two previous studies, Giletto et al. [18] and Ben Abdallah et al. [15], was included in the analysis conducted for the present study. The data from Giletto et al. [18] comprises two separate experimental data sets from Argentina [17] and Canada [16, 48, 49].

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 2). 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 2). All data from the Giletto et al. [18] study used in the present analysis was included in this previous publication.

The data from Ben Abdallah et al. [15] 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 2). Only a portion of the data from the Ben Abdallah et al. [15] 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. [15] experiment is reported for the first time in this manuscript.

4.2. Statistical Methods

Based on the general approach outlined by Makowski et al. [20], 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 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 N concentration (Figure 7b) 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 effect level of the hierarchical model (e.g., location, variety) where the critical point of each linear-plateau curve 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. In comparison, the conventional statistical approach fits a negative exponential curve to the subset of critical points (Figure 7c) which are identified via an intermediate statistical analysis (i.e., ANOVA and protected multiple comparisons).

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**Figure 7.** Hypothetical example of statistical methods used showing N concentration [%N] as a function of biomass [W] on five experimental sampling dates for (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. [20], 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. [20] 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 8) and 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* [50], the *brms* package [51, 52] was used to implement the statistical framework outlined by Makowski et al. [20]. The *brms* package, an interface to *Stan* [53], 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) [54]. 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 8.** Flowchart showing nested structure used in the present analysis to fit critical N dilution curves (CNDC) using the Bayesian hierarchical method based on Makowski et al. [20]. 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 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. [20] to parameterize the Bayesian hierarchical model as previously implemented with *rjags* [55] and *JAGS* statistical software [56].

The first expression from Makowski et al. [20] represents the linear-plateau component:

|  |  |
| --- | --- |
| *W* = *min*(*Wmaxi* + *Si* \* (*%NPlant* – *%Nc*), *Wmaxi*) | (6) |

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 N 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. [20] represents the CNDC component:

|  |  |
| --- | --- |
| *%Nc* = *a* (*Wmaxi*) –*b* | (7) |

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 (i.e., for *%Nc*), these two expressions (Eqs. 6 and 7) were combined to produce following non-linear *brms* model formula:

|  |  |
| --- | --- |
| *W* ~ *min*(*Wmaxi* + *Si* \* (*%NPlant* – (*a* \* (*Wmaxi*) –*b*)), *Wmaxi*) | (8) |

Two group-level (i.e., random) effects were specified for this *brms* model to parameterize the nested structure (Figure 8). 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*) | (9) |

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*) | (10) |

where *location* and *location:variety* represents the unique effect 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) [57]. 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 6).

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 [58] was used to document the computing environment utilized while conducting this analysis to ensure code portability and reproducibility.

**Table 6.** 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) | –∞ | ∞ |

4.3. Evaluating Uncertainity

4.3.1. 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 effect levels of *location* and *location:variety* to determine the 90% credible interval for each parameter. The correlation between values for parameters *a* and *b* was determined for each effect level of *location:variety* using the fitted parameter values at the level of the individual draws.

4.3.2. 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 effect level of *location:variety* to determine the 90% credible region for %Nc. This approach makes maximal use of the jointly estimated parameters contained in the posterior distribution.

To develop curves approximating 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* [59] 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 region for %Nc 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 region of %Nc 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.

To compare the various methods described above, the difference in critical N concentration [∆%Nc] was calculated with the 0.50 quantile (i.e., median) value for %Nc designated as the reference values (i.e., ∆%Nc with constant value of zero) and the various methods to quantify uncertainty (i.e., 90% credible region for %Nc, CNDCup & CNDClo, and estimates of credible region for %Nc using 90% credible interval for parameters *a* and *b*). In this manner, the ∆%Nc for each method to quantify uncertainty in %Nc can be directly compared.

4.3.3. Comparing Critical N Concentration across G x E 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 effect level of *location:variety*, the difference between the %Nc for a given comparison and reference CNDC (i.e., ∆%Nc) was calculated at each value of W. From this computed set of ∆%Nc, the 0.05, 0.50 (i.e., median) and 0.95 quantile values were identified for each effect level of *location:variety* to determine the 90% credible region for ∆%Nc. For a given range of W values, the comparison curve considered to be not significantly different from the reference curve if the ∆%Nc values for the 0.05 and 0.95 quantile values of %Nc were respectively less than and greater than zero (i.e., the 90% credible region for ∆%Nc contains zero). In the case where the 0.05 quantile value for ∆%Nc was greater than zero, the comparison curve was considered to have a significantly greater %Nc than the reference curve. In the case where the 0.95 quantile value for ∆%Nc was less than zero, the comparison curve was considered to have a significantly lower %Nc than the reference curve. To evaluate ∆%Nc in the present study, the %Nc for a given effect level of *location:variety* was compared to all other levels, and this approach allows for the direct evaluation of ∆%Nc  across G x E effects.

4.3.4. Comparing Critical N Concentration 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. [15], Giletto et al. [18]). Specifically, the previously published curves were evaluated to see if they fell within the 90% credible region for the corresponding curve fitted with the hierarchical Bayesian method 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.

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 different) 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*), and the %Nc 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 statistical methods. 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 affect 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 variety within location, Table S1: Experimental data used to fit hierarchical Bayesian model.

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**Data Availability Statement:** The full dataset used in this study are available in the Supplementary Materials (Table S1). Additionally, the GitHub repository associated with this paper (<https://github.com/bohm0072/cndc_bayesian_eval>) contains all necessary components to fully reproduce the analysis conducted in this paper.

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References

Morris, T.F.; Murrell, T.S.; Beegle, D.B.; Camberato, J.J.; Ferguson, R.B.; Grove, J.; Ketterings, Q.; Kyveryga, P.M.; Laboski, C.A.M.; McGrath, J.M.; et al. Strengths and Limitations of Nitrogen Rate Recommendations for Corn and Opportunities for Improvement. *Agron. J.* **2018**, *110*, 1, doi:10.2134/agronj2017.02.0112.

Lemaire, G.; Sinclair, T.; Sadras, V.; Bélanger, G. Allometric approach to crop nutrition and implications for crop diagnosis and phenotyping. A review. *Agron. Sustain. Dev.* **2019**, *39*, doi:10.1007/s13593-019-0570-6.

Houlès, V.; Guérif, M.; Mary, B. Elaboration of a nitrogen nutrition indicator for winter wheat based on leaf area index and chlorophyll content for making nitrogen recommendations. *Eur. J. Agron.* **2007**, *27*, 1-11, doi:10.1016/j.eja.2006.10.001.

Morier, T.; Cambouris, A.N.; Chokmani, K. In-Season Nitrogen Status Assessment and Yield Estimation Using Hyperspectral Vegetation Indices in a Potato Crop. *Agron. J.* **2015**, *107*, 1295-1309, doi:10.2134/agronj14.0402.

Nigon, T.J.; Yang, C.; Mulla, D.J.; Kaiser, D.E. Computing uncertainty in the optimum nitrogen rate using a generalized cost function. *Comput. Electron. Agric.* **2019**, *167*, 105030, doi:10.1016/j.compag.2019.105030.

Sadras, V.O.; Lemaire, G. Quantifying crop nitrogen status for comparisons of agronomic practices and genotypes. *Field Crops Res.* **2014**, *164*, 54-64, doi:10.1016/j.fcr.2014.05.006.

Gastal, F.; Lemaire, G.; Durand, J.-L.; Louarn, G. Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency. In *Crop Physiology*, Second ed.; 2015; pp. 161-206.

Lemaire, G.; Gastal, F. N Uptake and Distribution in Plant Canopies. In *Diagnosis of the Nitrogen Status in Crops*, Lemaire, G., Ed.; Springer: Berlin, 1997; pp. 3-43.

Greenwood, D.J.; Lemaire, G.; Gosse, G.; Cruz, P.; Draycott, A.; Neeteson, J.J. Decline in Percentage N of C3 and C4 Crops with Increasing Plant Mass. *Ann. Bot.* **1990**, *66*, 425-436, doi:10.1093/oxfordjournals.aob.a088044.

Justes, E.; Mary, B.; Meynard, J.-M.; Machet, J.-M.; Thelier-Huche, L. Determination of a Critical Nitrogen Dilution Curve for Winter Wheat Crops. *Ann. Bot.* **1994**, *74*, 397-407, doi:10.1006/anbo.1994.1133.

Plénet, D.; Lemaire, G. Relationships between dynamics of nitrogen uptake and dry matter accumulation in maize crops. Determination of critical N concentration. *Plant Soil* **2000**, *216*, 65-82, doi:10.1023/a:1004783431055.

Duchenne, T.; Machet, J.M.; Martin, M. Potatoes. In *Diagonsis of the Nitrogen Status in Crops*, Lemaire, G., Ed.; Springer: Berlin, 1997; pp. 119-130.

Greenwood, D.J.; Neeteson, J.J.; Draycott, A. Quantitative relationships for the dependence of growth rate of arable crops on their nitrogen content, dry weight and aerial environment. *Plant Soil* **1986**, *91*, 281-301, doi:10.1007/BF02198111.

Herrmann, A.; Taube, F. The Range of the Critical Nitrogen Dilution Curve for Maize (Zea mays L.) Can Be Extended Until Silage Maturity. *Agron. J.* **2004**, *96*, 1131-1138, doi:10.2134/agronj2004.1131.

Ben Abdallah, F.; Olivier, M.; Goffart, J.P.; Minet, O. Establishing the Nitrogen Dilution Curve for Potato Cultivar Bintje in Belgium. *Potato Res.* **2016**, *59*, 241-258, doi:10.1007/s11540-016-9331-y.

Bélanger, G.; Walsh, J.R.; Richards, J.E.; Milburn, P.H.; Ziadi, N. Critical Nitrogen Curve and Nitrogen Nutrition Index for Potato in Eastern Canada. *Am. J. Potato Res.* **2001**, *78*, 355-364, doi:10.1007/BF02884344.

Giletto, C.M.; Echeverría, H.E. Critical Nitrogen Dilution Curve in Processing Potato Cultivars. *Am. J. Plant Sci.* **2015**, *06*, 3144-3156, doi:10.4236/ajps.2015.619306.

Giletto, C.M.; Reussi Calvo, N.I.; Sandaña, P.; Echeverría, H.E.; Bélanger, G. Shoot- and tuber-based critical nitrogen dilution curves for the prediction of the N status in potato. *Eur. J. Agron.* **2020**, *119*, doi:10.1016/j.eja.2020.126114.

Lemaire, G.; Ciampitti, I. Crop Mass and N Status as Prerequisite Covariables for Unraveling Nitrogen Use Efficiency across Genotype-by-Environment-by-Management Scenarios: A Review. *Plants* **2020**, *9*, doi:10.3390/plants9101309.

Makowski, D.; Zhao, B.; Ata-Ul-Karim, S.T.; Lemaire, G. Analyzing uncertainty in critical nitrogen dilution curves. *Eur. J. Agron.* **2020**, *118*, doi:10.1016/j.eja.2020.126076.

Ciampitti, I.A.; Fernandez, J.; Tamagno, S.; Zhao, B.; Lemaire, G.; Makowski, D. Does the critical N dilution curve for maize crop vary across genotype x environment x management scenarios? - a Bayesian analysis. *Eur. J. Agron.* **2021**, *123*, 126202, doi:10.1016/j.eja.2020.126202.

Yao, B.; Wang, X.; Lemaire, G.; Makowski, D.; Cao, Q.; Liu, X.; Liu, L.; Liu, B.; Zhu, Y.; Cao, W.; et al. Uncertainty analysis of critical nitrogen dilution curves for wheat. *Eur. J. Agron.* **2021**, *128*, 126315, doi:10.1016/j.eja.2021.126315.

Bohman, B.J.; Rosen, C.J.; Mulla, D.J. Relating nitrogen use efficiency to nitrogen nutrition index for evaluation of agronomic and environmental outcomes in potato. *Field Crops Res.* **2021**, *262*, doi:10.1016/j.fcr.2020.108041.

Stefaniak, T.R.; Fitzcollins, S.; Figueroa, R.; Thompson, A.L.; Schmitz Carley, C.; Shannon, L.M. Genotype and Variable Nitrogen Effects on Tuber Yield and Quality for Red Fresh Market Potatoes in Minnesota. *Agronomy* **2021**, *11*, 255, doi:10.3390/agronomy11020255.

Jones, C.R.; Michaels, T.E.; Carley, C.S.; Rosen, C.J.; Shannon, L.M. Nitrogen uptake and utilization in advanced fresh-market red potato breeding lines. *Crop Sci.* **2021**, *61*, 878–895, doi:10.1002/csc2.20297.

Tiwari, J.K.; Plett, D.; Garnett, T.; Chakrabarti, S.K.; Singh, R.K. Integrated genomics, physiology and breeding approaches for improving nitrogen use efficiency in potato: translating knowledge from other crops. *Funct. Plant Biol.* **2018**, *45*, 587, doi:10.1071/fp17303.

Arguez, A.; Durre, I.; Applequist, S.; Squires, M.; Vose, R.; Yin, X.; Bilotta, R. *NOAA's U.S. Climate Normals (1981-2010)*; NOAA National Centers for Environmental Information: 24 November 2017 2010.

Hansen, B.; Giencke, A.G. *Sand Plains Research Farm Soil Report*; University of Minnesota: St. Paul, MN, 1988.

USDA NRCS. *Soil Series Classification Database – Hubbard Series*; United States Department of Agriculture: 24 November 2017 2013.

Egel, D.S. *Midwest Vegetable Production Guide for Commercial Growers*; University of Minnesota Extension: 2017.

Franzen, D.; Robinson, A.; Rosen, C. *Fertilizing Potato in North Dakota*; North Dakota State University: Fargo, ND, 2018.

Rosen, C.J. *Potato fertilization on irrigated soils*; University of Minnesota Extension: 2018.

Steele, D.D.; Scherer, T.F.; Wright, J.; Hopkins, D.G.; Tuscherer, S.R.; Wright, J. Spreadsheet Implementation of Irrigation Scheduling by the Checkbook Method for North Dakota and Minnesota. *Appl. Eng. Agric.* **2010**, *26*, 983-996, doi:10.13031/2013.35914.

Wright, J. *Irrigation Scheduling Checkbook Method*; University of Minnesota: St. Paul, MN, 2002.

Errebhi, M.; Rosen, C.J.; Gupta, S.C.; Birong, D.E. Potato Yield Response and Nitrate Leaching as Influenced by Nitrogen Management. *Agron. J.* **1998**, *90*, 10-15, doi:10.2134/agronj1998.00021962009000010003x.

Rosen, C.; Birong, D.; Zumwinkle, M. *Nitrogen Fertilization Studies on Irrigated Potatoes: Nitrogen Use, Soil Nitrate Movement, and Petiole Sap Nitrate Analysis for Predicting Nitrogen Needs*; University of Minnesota: St. Paul, MN, 1992; pp. 27–47.

Rosen, C.; Errebhi, M.; Moncrief, J.; Gupta, S.; Cheng, H.H.; Birong, D. *Nitrogen Fertilization Studies on Irrigated Potatoes: Nitrogen Use, Soil Nitrate Movement, and Petiole Sap Nitrate Analysis for Predicting Nitrogen Needs*; University of Minnesota: St. Paul, MN, 1993; pp. 14–33.

Sun, N. Agronomic and Storage Factors Affecting Acrylamide Formation in Processed Potatoes. University of Minnesota, St. Paul, MN, 2017.

Sun, N.; Wang, Y.; Gupta, S.K.; Rosen, C.J. Nitrogen Fertility and Cultivar Effects on Potato Agronomic Properties and Acrylamide-forming Potential. *Agron. J.* **2019**, *111*, 408, doi:10.2134/agronj2018.05.0350.

Crants, J.; Rosen, C.; McNearney, M.; Sun, L. *The Use of Chlorophyll Meters for Nitrogen Management in Potatoes*; Minnesota Area II Potato Research and Promotion Council and Northern Plains Potato Growers Association: Fargo, ND, 2017.

Gupta, S.; Rosen, C.J. *Nitrogen fertilization rate and cold-induced sweetening in potato tubers during storage*; Minnesota Area II Potato Research and Promotion Council and Northern Plains Potato Growers Association: Fargo, ND, 2019.

Gupta, S.K.; Crants, J.; McNearney, M.; Rosen, C.J. *Evaluation of a Promising Minnesota Clone for N Response, Agronomic Traits & Storage Quality*; Minnesota Area II Potato Research and Promotion Council and Northern Plains Potato Growers Association: Fargo, ND, 2020.

Bohman, B.; McNearney, M.; Crants, J.; Rosen, C. *A Novel Approach to Manage Nitrogen Fertilizer for Potato Production Using Remote Sensing*; Minnesota Area II Potato Research and Promotion Council and Northern Plains Potato Growers Association: Fargo, ND, 2020.

Rosen, C.; Crants, J.; Bohman, B.; McNearney, M. *Effects of banded versus broadcast application of ESN, turkey manure, and different approaches to measuring plant N status on tuber yield and quality in Russet Burbank potatoes*; Minnesota Area II Potato Research and Promotion Council and Northern Plains Potato Growers Association: Fargo, ND, 2021.

USDA. *United States Standards for Grades of Potatoes for Processing*; 1997.

Horneck, D.A.; Miller, R.O. Determination of total nitrogen in plant tissue. In *Handbook of reference methods for plant analysis*, Kalra, Y.P., Ed.; CRC Press: Boston, 1998; pp. 75-84.

Horwitz, W.; Chichilo, P.; Reynolds, H. *Official methods of analysis of the Association of Official Analytical Chemists.*, 11th ed.; Association of Official Analytical Chemists.: Washington, DC, 1970.

Bélanger, G.; Walsh, J.R.; Richards, J.E.; Milburn, P.H.; Ziadi, N. Yield response of two potato culivars to supplemental irrigation and N fertilization in New Brunswick. *Am. J. Potato Res.* **2000**, *77*, 11-21, doi:10.1007/BF02853657.

Bélanger, G.; Walsh, J.R.; Richards, J.E.; Milburn, P.H.; Ziadi, N. Tuber growth and biomass partitioning of two potato cultivars grown under different N fertilization rates with and without irrigation. *Am. J. Potato Res.* **2001**, *78*, 109-117, doi:10.1007/BF02874766.

R Core Team *R: A language and environment for statistical computing.*, R Foundation for Statistical Computing: Vienna, Austria, 2021.

Bürkner, P.-C. brms: An R Package for Bayesian Multilevel Models Using Stan. *J. Stat. Softw.* **2017**, *80*, doi:10.18637/jss.v080.i01.

Bürkner, P.-C. Advanced Bayesian Multilevel Modeling with the R Package brms. *R J.* **2018**, *10*, 395-411, doi:10.32614/RJ-2018-017.

Carpenter, B.; Gelman, A.; Hoffman, M.D.; Lee, D.; Goodrich, B.; Betancourt, M.; Brubaker, M.; Guo, J.; Li, P.; Riddell, A. Stan: A Probabilistic Programming Language. *J. Stat. Softw.* **2017**, *76*, doi:10.18637/jss.v076.i01.

McElreath, R. *Staistical Rethinking: A Bayesian Course with Examples in R and Stan*, 2nd ed.; Chapman and Hall/CRC: Boca Raton, 2020.

Plummer, M. *rjags: Bayesian Graphical Models using MCMC*, 2019.

Plummer, M. *JAGS: Just Another Gibs Sampler.*, 2013.

Schad, D.J.; Betancourt, M.; Vasishth, S. Toward a principled Bayesian workflow in cognitive science. *Psychol Methods* **2021**, *26*, 103-126, doi:10.1037/met0000275.

Ushey, K. *renv: Project Environments*, 2021.

R Core Team *"stats": The R Stats Package*, 2021.