The Use of Remote Sensing to Determine Nitrogen Status in Perennial Ryegrass (*Lolium perenne* L.) for Seed Production

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Abstract: Sufficient nitrogen (N) supply is decisive to achieve high grass seed yields while overfertilization will lead to negative environmental impact. From the literature, estimation of N rates taking into account the crop’s N status and its yield potential, seems promising for attaining high yields and averting adverse environmental impacts. This study aimed at an evaluation of remote sensing to predict final seed yield, N traits of the grass seed crop and the usability of nitrogen nutrition index (NNI) to measure additional N requirement. It included four years’ data and eight N application rates and strategies. Several reflectance measurements were made and used for the calculation of 18 vegetation indices. The predictions were made using partial least square regression and support vector machine. Three different yield responses to N fertilization were noted; one with linear response, one with optimum economic nitrogen (EON) at ~188 kg N ha$^{-1}$, and one with EON at ~138 kg N ha$^{-1}$. We conclude that although it is possible to make in-season predictions of NNI, it does not always portray the differences in yield potential; thus, it is challenging to utilize it to optimize N application.

Keywords: critical nitrogen dilution curve; nitrogen nutrition index; precision agriculture; nitrogen uptake

1. Introduction

Nitrogen (N) is an essential plant nutrient strongly related to the growth, yield, and quality of agricultural crops. In grass seed crops, adequate N uptake is decisive to achieve high seed yields [1,2]. Its positive seed yielding effects imply a risk of overfertilization, commonly named “insurance fertilization”. The reason for overfertilization is, of course, to avoid that N becomes a yield-limiting factor. Unfortunately, there are numerous environmental problems related to excess N applications. A fraction of the applied N is lost via ammonia (NH$_3$) volatilization, worsening air quality [3]. Nitrate (NO$_3^-$) accumulates as a result of N leaching below the root zone, and it can cause groundwater pollution and surface water eutrophication [4,5]. In addition, nitrous oxide (N$_2$O) emissions are reported to increase with N fertilization [6]. Consequently, it is an important challenge for modern agronomy to mitigate these effects by predicting and applying the required amount of N at the right time to N requirement while ensuring high yields.

Restrictions in the use of N for agricultural crops have been one of the consistent topics in the Danish political action plans since 1987 and are described in detail in Dalgaard et al. 2014 [7]. Restrictions have been based on an ‘N application rate’ for each crop species with some variability according to soil type and management. The benchmark for maximum N application rates was ‘economically optimum N rates’ (EON) based on actual prices of product (here seed), and N. Calculation of EON is, of course, an *ex-ante* calculation on a national level as the actual prices for product and N are unknown at the time where N is bought, and the seed is sold. The use of EON as the benchmark for N restrictions is problematic as N will limit yield in crops with a high yield potential due to, e.g., optimum
management or favorable climatic growing conditions, whereas crops with a low yield potential may be overfertilized. The use of restrictions based on maximum N application rates, either based on EON or N rates, to achieve maximum biological yield might also be considered discriminative against skilled farmers as they can have a yield potential that is not realized due to a N shortage. Consequently, it is of great importance to test if precision agriculture based on the use of new technology is useful to define the optimum N application rate in a specific year.

It is a complex task to develop novel methods that will define the optimum N application rate in a specific year. Not only due to the spatial and temporal variability of nutrient dynamics in cropping systems [8], crop dynamics but also to the large impact that the weather has on the final seed yield. Besides, it is vital that the last N application before harvest has been applied at a time where the crop still takes up soil N. Nitrogen uptake (N_{up}) has been described as a helpful tool to depict crop N condition and synchronize N demand with supply [8–10]. Another assistive plant feature is the N nutrition index (NNI) that has been reported as one plant N trait that accurately indicates crop N status [11–15]. NNI is the ratio of actual N concentration (N_a) to the critical N concentration (N_c), where the N_c is defined as the minimum N concentration in plants needed for maximum growth rate [16]. NNI was created in a way that integrates N dilution in crop canopies—a process where N_a drops with increasing biomass [17]—and thus, it is considered a useful tool to evaluate the crop N status [8]. This dilution process, combined with the N_c, is described by the critical N dilution curve (CNDC) using an equation that relates N_c with above-ground biomass [18]. Plant’s metabolic activity and above-ground biomass are heavily associated with N during vegetative growth, as mentioned by Hardwick, 1987 [19]. However, a part of N is placed in storage tissues during reproductive growth and, thus, is unavailable for biomass production. Under this perspective, Hardwick’s findings seem unsound [20]. Based on the difference in the N storage during vegetative and reproductive growth, Lemaire et al., 1997 [18], suggested that the CNDC equation should be determined individually for each kind of crop. For perennial ryegrass, CNDC has been developed by Gislum et al., 2009 [17], and it has been utilized to predict seed yield response to N fertilization by Vleugels et al. [1]. Other methods to identify optimum N application rates have been published. Sicard, 1995 [21], utilized N uptake at full ear emergence as a method to identify sufficient uptake and found that 130 kg N ha^{-1} was necessary to achieve maximum seed yield. Total plant uptake of 150 kg N ha^{-1} was required to achieve maximum seed yield in the US and New Zealand [22].

Determining NNI, N_a, and N_{up} is usually a complex procedure that requires time-consuming laboratory work and can be a barrier for timely and accurate N fertilizer management. Thus, several attempts have been made to estimate these traits remotely, using optical sensing [8,9,13,23–25]. This includes proximal canopy sensors that make measurements of the reflected light at selected wavelengths, which are later used for calculating vegetation indices (VI) [8]. The sensitivity of VI and their performance in predicting plant N traits are affected by the combination of wavelengths that is used for their calculation as well as by the time of sensing [26,27]. NNI can be predicted in a direct or indirect method. The direct method is founded on a regression model from measured NNI and VI. The indirect method is based on two regression models; one regression model developed for measured N_a and VI and one regression model developed for measured DM and VI, NNI is then calculated based on predictions from these two models [26]. Besides estimating plant N status, VI can be used to make in-season predictions of the final yield as shown in corn, cotton, and wheat [24,25,28,29].

Developing an N application strategy based on measurements during the spring growing season ensuring a high yield is not a trivial task. Crop growth and utilization of the yield potential are not only based on accurate N application. Our perception is, that we are able to explain some of the variations in the seed yield based on measurements during the spring growth; however, several other factors have similar large effects on the final
seed yield. Consequently, our goal must be to secure that N is not limiting for establishing the potential seed yield.

Based on the available literature and in combination with our data, the objectives of the current study were to: (i) Evaluate the use of remote sensing to estimate final seed yield, NNI, and other relevant crop parameters during the spring growing season and (ii) evaluate the use of NNI as a method to quantify if additional N should be applied perennial ryegrass for seed production.

2. Materials and Methods

2.1. Field Trial Conditions

The field experiments were conducted in Flakkebjerg, Denmark (55°32′ N and 11°39′ E) during the period 2001–2004. The soil is formed from mixed glacial deposits. The soil classifies as a sandy loam (haplic Luvisol (FAO)/Typic Hapludalf (USDA)) with clay illuviation below the plow layer. There was a plow sole in roughly 30 cm depth, and the subsoil was typically clayey, although sand lenses did occur. The monthly mean temperature (°C) and accumulated precipitation (mm) are presented in Figure 1.

Perennial ryegrass was sown in spring barley (cover crop) in spring 2000, 2001, 2002, and 2003 in 4 different fields. Space between rows was 12 cm, seed rate was 6 kg ha⁻¹, and the seeds were sown at approximately 1 cm depth. The spring barley crop received 90 kg N ha⁻¹. The net plot size was 2.5 × 8 m. Weeds and diseases were controlled when necessary, in accordance with good experimental practice in both spring barley and the following grass seed crop. The cover crop was harvested with a trial combiner, and the straw was removed immediately after harvest. The stubble was cut to 8–10 cm height. In the following seed production year, the grass seed crop was swathed before seed shedding or harvested directly (in years with full lodging at crop maturity) with a trial combine harvester, and seeds were air-dried to 12% moisture before determining the seed yield. Seed yields were expressed as 100% pure seed.

A total of 8 different N application rates and application strategies (40, 80, 120, 160, 200, 40 + 80, 80 + 40, and 40 + 40 + 40 kg N ha⁻¹) were applied as treatments. The first 5 N treatments were single N applications at the initiation of spring growth (N40, N80, N120, N160, and N200—numbers refer to application rate in kg N ha⁻¹). Two N treatments (N40 + 80 and N80 + 40) consisted of 40 or 80 kg N ha⁻¹ applied at the initiation of spring growth followed by an additional 80 or 40 kg ha⁻¹ after approximately 30 days, respectively. The last N treatment (N40 + 40 + 40) was 40 kg N ha⁻¹ applied at the initiation of spring growth, 40 kg N ha⁻¹ approximately 30 days later, and an additional 40 kg N ha⁻¹ roughly 40 days after the 2nd application (Figure 2). Nitrogen was applied as ammonium nitrate.
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Accumulated growing-degree-days (GDD) were used in order to describe the physiological process based on heat accumulation. The accumulated GDD for a given date $t$ was calculated as shown in Equation (1):

$$GDD(t) = \sum_{\tau=t_0}^{t} \frac{\text{Tmax}(\tau) + \text{Tmin}(\tau)}{2} - \text{Tbase,} \ 0$$

where $\text{Tmax}$ and $\text{Tmin}$ are the daily maximum and minimum air temperature at 2 m, respectively. $\text{Tbase}$ is the base temperature below which the process of interest does not progress. Hence, days where the average of $\text{Tmax}$ and $\text{Tmin}$ did not contribute to the accumulated GDD. For perennial ryegrass $\text{Tbase}$ was set to 2 °C. The starting date $t_0$ was set on 1st January in the seed harvesting year.

2.2. Plant N Status

Nitrogen concentration in the ground samples ($N_a$) was measured using an elemental analyzer (Vario EL III, Langenselbold, Germany), and N uptake was subsequently calculated by multiplying dry matter DM by N concentration. The critical N concentration

Figure 2. Accumulated growing-degree-days for N application (circle), plant sampling (triangle), and reflectance measurements (cross) for 2001, 2002, 2003, and 2004. The starting date for GDD was set on 1st January in the seed harvesting year, and $\text{Tbase}$ was set at 2 °C.

Stem lodging was a displacement of the crop from its upright position and early and severe lodging can be detrimental for the seed yield. Severe lodging can be a derived effect from excess N application, and lodging scores were obtained to secure that this was not the case in the present study. Visual lodging scores of the full plots were obtained at flowering and at harvest using a visual score ranging from 0 to 100, where 0 was no lodging, and 100 was a fully lodged plot. The number of fertile tillers was calculated on a sample taken from a 0.5 m × 0.5 m area within the plot a few days before harvest and extrapolated to numbers per hectare.

Plant samples per plot were taken on 3 occasions during the spring growing season (Figure 2) by cutting the plants approximately 1 cm above ground level, covering an area of 0.0625 m$^2$. All samples were oven-dried for 24 h at 80 °C, and the weight of the total dry matter content was calculated as tons of dry matter per ha. All samples were ground through a 1 mm sieve and stored in small glass bottles (5.5 cm × 2.5 cm, height × diameter) before the subsequent measurement of N concentration.

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was calculated based on CNDC of perennial ryegrass for seed production (Gislum et al., 2009 [17]). The CNDC was calculated based on Equation (2):  

\[ N_c = 6.36 \times W^{-0.71} \]  

(2)

where \(N_c\) is the N critical concentration and \(W\) is shoot dry matter (t ha\(^{-1}\)). The NNI was calculated using the Equation (3):

\[ \text{NNI} = \frac{N_a}{N_c} \]  

(3)

where \(N_a\) is the actual N concentration and \(N_c\) is the critical N concentration. The nitrogen use efficiency (NUE) was calculated as the ratio of N concentration in seed yield to the N application.

2.3. Canopy Reflectance and Calculation of Vegetation Indices

Canopy reflectance was measured with a hand-held spectroradiometer (CropScan MSR87, CropScan Inc., Rochester, MN, USA). Solar radiation and canopy reflectance were measured at 8 wavelengths: 460, 560, 610, 660, 710, 810, 905, and 950 nm (bandwidth at 10 nm). A data acquisition device (DLC Model 2000, CropScan Inc., Rochester, MN, USA) equipped with sun angle cosine correction capacity was used to record reflectance data. Measurement times varied from year to year (Figure 2). Average values of the 3 measurements within each plot in 2001, 2002, 2004, and of 5 measurements in 2003 were used for further analysis. The reflectance values were corrected for irradiation by subtraction of the irradiation values. A total of 18 vegetation indices were calculated based on these reflectance measurements, as described in Table 1.

| Index | Formula | Reference |
|-------|---------|-----------|
| Normalized Difference Vegetation Index (NDVI) | \(\frac{\rho_{810} - \rho_{660}}{\rho_{810} + \rho_{660}}\) | [30] |
| Normalized Difference Red Edge (NDRE) | \(\frac{\rho_{810} - \rho_{710}}{\rho_{810} + \rho_{710}}\) | [31] |
| Ratio Vegetation Index I (RVI I) | \(\frac{\rho_{810} - \rho_{660}}{\rho_{810} \cdot \rho_{660}}\) | [32] |
| Ratio Vegetation Index II (RVI II) | \(\frac{\rho_{810}}{\rho_{560}}\) | [33] |
| Modified Chlorophyll Absorption Ratio Index (MCARI) | \(\frac{1.5(2\rho_{810} - \rho_{710} - 2.5(\rho_{660} - \rho_{560}))}{(\rho_{710} - 0.2(\rho_{710} - \rho_{560}))}\) | [34] |
| Modified Triangular Vegetation Index 2 (MTVI 2) | \(\sqrt{\frac{(\rho_{710} - \rho_{660} - 5\sqrt{\rho_{660}}) - 0.5}{\rho_{710} - 0.2(\rho_{710} - \rho_{560})}}\) | [35] |
| MCARI / MTVI 2 | \(\frac{1.5}{\rho_{810} - \rho_{560} - 5\sqrt{\rho_{660}} + 0.5}\) | [36] |
| Transformed Chlorophyll Absorption Reflectance Index (TCARI 1) | \(3\left(\frac{\rho_{710} - \rho_{660}}{\rho_{810} - \rho_{660}}\right)\) | [37] |
| Optimized Soil Adjusted Vegetation Index (OSAVI) | \(\frac{1.6\left(\rho_{810} - \rho_{660}\right)}{\rho_{810} + \rho_{660} + 0.16}\) | [38] |
| TCARI / OSAVI | \(3\left(\frac{\rho_{710} - \rho_{660} - 0.2(\rho_{710} - \rho_{560})}{\rho_{810} - \rho_{660}}\right)\) | [39] |
| Modified Soil-Adjusted Vegetation Index (MSAVI) | \(\frac{2\rho_{810} + 1}{\sqrt{(2\rho_{810} + 1)^2 - 8(\rho_{810} - \rho_{660})}}\) | [40] |
| Green Normalized Difference Vegetation Index (GNNDVI) | \(\frac{\rho_{810} - \rho_{660}}{\rho_{810} + \rho_{660}}\) | [41] |
| Red Edge Soil-Adjusted Vegetation Index (RESAVI) | \(\frac{1.5(\rho_{810} - \rho_{710})}{\rho_{810} + \rho_{710}}\) | [42] |
| Difference Vegetation Index (DVI) | \(\frac{\rho_{810} - \rho_{660}}{\rho_{810} + \rho_{660}}\) | [43] |
| Red Edge Ratio Vegetation Index (RERVI) | \(\frac{\rho_{810}}{\rho_{710}}\) | [44] |
| Red Edge Difference Vegetation Index (REDVI) | \(\rho_{810} - \rho_{710}\) | [30] |
| Optimized Vegetation Index 1 (VIopt 1) | \(100(\ln \rho_{810} - \ln \rho_{710})\) | [44] |
2.4. Data Analysis

The experiment design was a Latin square design with 8 treatments and 8 replications, turning out 64 plots in total. A total of 320 plots were used during the 4 years, 128 for 2001 (double experiment) and 64 for each of the rest. Data on the measured characters: lodging at flowering, lodging at harvest, number of fertile tillers, and seed yield were analyzed within each using a generalized linear model. The effects of N treatment were treated as fixed effects. Replicates were treated as random effects.

The model for lodging at flowering, lodging at harvest, the number of fertile tillers, and seed yield within years is shown in Equation (4):

\[ Y_{ar} = \mu + \alpha + E_r \]  

(4)

where \( Y_{ar} \) is the lodging score at flowering, lodging score at harvest, number of fertile tillers or seed yield at the \( r \)th replicate and at the \( a \)th N treatment. \( \mu \) is the general mean of the response variable, and \( \alpha \) is the main effect of N treatment. \( E_r \) is the random effect of replicate that is assumed to be independently and normally distributed with mean zero and variance \( \sigma^2_E \), respectively.

To test the hypothesis of no N treatment effects on lodging score at flowering, lodging score at harvest, the number of fertile tillers, or seed yield, the denominator in the F-tests was calculated according to the random effects of the model. Means for N treatments were separated by pairwise comparison at the 5% level of significance.

To calculate the biological and economical optimum N application rate within each year the following Equation (5) was used:

\[ Y = \mu + aN + \gamma N^2 + E \]  

(5)

where \( Y \) is the seed yield in kg ha\(^{-1} \), \( N \) is the N application rate in kg ha\(^{-1} \) for treatment one to five and \( E \) is the random effect. \( \mu, \alpha \) and \( \gamma \) are systematic effects estimated using least quadratic methods.

The economical optimum N application rate (EON) is subsequently calculated using Equation (6):

\[ EON = \frac{\rho - \alpha}{2\gamma} \]  

(6)

where \( \rho \) is the price relation between 1 kg N and 1 kg seed. The price for N and seeds were both 1.08 € kg\(^{-1} \), hence \( \rho = 1 \).

The analyses were performed using the PROC MIXED and PROC IML within the Statistical Analysis System version 9.4, software package (SAS, Cary, NC, USA).

Partial least squares regression (PLSR) models [45] and support vector machine (SVM) models [46] were developed on the independent explanatory auto-scaled wavelengths and VIs and the dependent response variables \( N_a, N_{up}, \) DM, NNI, and seed yield. Auto-scaling was a combination of mean-centering and a division of each variable by its standard deviation. Validation of the model was performed using venetian blinds cross-validation with 8 splits. In venetian cross, validation was each test set determined by selecting every \( s \)th object in the data set, starting at object numbered 1 through \( s \). Root mean square error of cross-validation (RMSECV) plotted against the number of PLSR components was used to select the optimum number of components in the PLSR model. The optimum number of PLSR components was chosen as the 1st local minimum in the smooth declining RMSECV curve or the point where this curve flattened. An epsilon-SVR type of SVM was used, and the kernel type was set at a radial basis function. The performance of the PLSR and SVM models was evaluated using the root mean square error of cross-validation (RMSECV) and root mean square error of prediction (RMSEP).

All data analyses were carried out using MATLAB version 9.9. (R2020b) (The Mathworks, Inc., Natick, MA, USA) along with the PLS toolbox 8.9.1 (Eigenvector Research, Inc., Manson, WA, USA).
3. Results

3.1. Growing Conditions

The accumulated precipitation in 2002 was higher compared with precipitation in 2001, 2003, and 2004, which showed no mutual differences and was similar to the average from 1984 to 2004 (Figure 1). The average monthly temperatures in the four years were comparable with the averages from 1984 to 2004 (Figure 1). An average temperature of 5 °C was reached during March and the average temperature at harvest was above 15 °C in all four years.

Lodging at flowering was lower in 2001 compared with the other years, which showed no mutual difference (Table 2). The lodging score increased at higher N application rates independent of year. Splitting the N application in two did not affect lodging at flowering while three split applications (N40 + 40 + 40) caused a lower lodging score. The lodging score at harvest in 2001 and 2004 was considerably lower than in 2002 and 2003 (Table 2). There was still an increase in the score at increased N application, however, there was no difference between the application of 160 or 200 kg N ha⁻¹ in all years.

Table 2. Lodging at flowering and at harvest within years and nitrogen (N) treatments and as an average of years. Different letters within each year show significant differences at a 5 percent level.

| N Treatment | Lodging at Flowering | Lodging at Harvest |
|-------------|----------------------|--------------------|
|             | 2001 2002 2003 2004 | 2001–2004 2001 2002 2003 2004 2001–2004 |
| N40         | 1c 20e 1d 1e        | 6 65c 85c 68b 26d 61 |
| N80         | 3c 28d 33bc 22d     | 22 80b 89b 97a 59c 81 |
| N120        | 16b 36bc 38ab 35bc  | 31 82ab 93ab 98a 71ab 86 |
| N160        | 21a 39ab 40a 43ab   | 36 85a 93ab 98a 83a 90 |
| N200        | 25a 44a 35abc 46a   | 38 86a 98a 96a 81a 72 |
| N40+80      | 14b 38abc 40a 31c   | 31 80b 94ab 98a 75ab 87 |
| N80+40      | 13b 38ab 39a 35bc   | 31 81b 92ab 98a 68bc 85 |
| N40+40+40   | 5c 31cd 31c 19d     | 22 79b 94ab 99a 60c 83 |
| Average     | 12 34 32 29         | - 80 92 94 65 - |

3.2. N Application Rates and Strategies’ Effects on Yield

Seed yield increased with an increasing N rate from 40 to 200 kg N ha⁻¹ of single applications in 2001, 2002, and 2003, whereas there was a drop in seed yield from 160 kg N ha⁻¹ to 200 kg N ha⁻¹ in 2004. This drop in seed yield in 2004 was consistent for all replicates and indicated that another factor than N was limiting yield (Table 3). N40 + 80 and N80 + 40 applications performed similarly on average and they did not differ from N120. Seed yield at N40 + 40 + 40 was low and only similarly to N80. In 2002 and 2003 the maximum seed yield was achieved between 160 kg N ha⁻¹ and 200 kg N ha⁻¹, whereas in 2004, 160 kg N ha⁻¹ or the N40 + 80 application strategy had the highest yields.

Table 3. Seed yield in kg ha⁻¹ within years and nitrogen (N) treatments and as an average of years. Different letters within each year show significant differences at a 5 percent level.

| N Treatment | Seed Yield (kg ha⁻¹) 2001 2002 2003 2004 2001–2004 |
|-------------|-----------------------------------------------------|
| N40         | 856e 933e 1162e 937f 949d 949d |
| N80         | 1001d 1253d 1379d 1335de 1194c |
| N120        | 1154bc 1500bc 1574bc 1485bc 1373b |
| N160        | 1250ab 1593ab 1795a 1566ab 1498a |
| N200        | 1332a 1688a 1752a 1409cd 1508a |
| N40+80      | 1182b 1536bc 1607b 1580a 1417ab |
| N80+40      | 1204bc 1452c 1533bc 1470c 1373b |
| N40+40+40   | 1135c 1319d 1471cd 1303e 1275c |
A similar trend was noticed in Table 4 where the biological optimal N rate defined as maximum yield using the modeled N response curve was estimated at 125 kg N ha\(^{-1}\) in 2004, while in 2002 and 2003, the N rate was over 200 kg N ha\(^{-1}\). Likewise, the economical optimum N rate was lower in 2004 than in 2002 and 2003. The reason was a general lower seed yield for N200 in 2004, which of course, has a large effect on the response model. In 2001 the response curve was linear and, therefore, neither biological nor economical optimum N rates can be estimated.

Table 4. Biological (BO) and economical optimum N (EON) application rate (kg N ha\(^{-1}\)) and appurtenant estimated seed yield (kg ha\(^{-1}\)) for the four years and as an average of the four years, and nitrogen use efficiency (NUE) calculated for BO and EO within years and as an average of years. The nitrogen response curve was linear for 2001, and consequently, it was not possible to calculate BO nor EON.

| Year          | Biological Optimum | Economical Optimum | NUE  |
|---------------|--------------------|--------------------|------|
|               | N Rate             | Seed Yield         | N Rate | Seed Yield | BO  | EON  |
| 2001–2004     | 196                | 1514               | 175   | 1503       | 0.16 | 0.18 |
| 2001          | -                  | -                  | -     | -          | -   | -    |
| 2002          | 206                | 1678               | 187   | 1669       | 0.12 | 0.13 |
| 2003          | 211                | 1783               | 188   | 1773       | 0.02 | 0.05 |
| 2004          | 125                | 1487               | 138   | 1550       | 0.26 | 0.25 |

3.3. N Application Rates and Strategies’ Effects on \(N_{up}\), DM, \(N_a\), NNI

The impact of N application rates on \(N_{up}\), \(N_a\), and DM is illustrated in Figure 3. \(N_{up}\) remained at low levels in N40 and N80, had an upturn from N80 to N120, but there were only slight differences between N120, N160, and N200. Regarding \(N_{up}\), there seems to be a peak between 900 and 1000 GDD. On the contrary, \(N_a\) had its highest value early in the growing season, reducing with increasing GDD due to dilution, while it was positively affected by increasing N rates. DM was developing through the growing season, while the effect of N rates was not noticeable.

Measured and predicted NNI values for N treatments N40, N80, N120, N160, and N200 using SVM models are shown in Figure 4. Predicted values illustrated were calculated using the direct method as it performed better in the statistical analysis than the indirect method (data not shown). Measured NNI at N40 was below 1 for most of the plots, and, therefore, they were characterized as a N-deficit. At N80, the amount of the N-deficit to the N-sufficient plots was balanced. At N120 and N160 the NNI values increased considerably, while at N200 almost all of the plots were N-sufficient. Predicted NNI values developed similarly to the measured NNI values for N40 and N80. For N120, N160, and N200 there seems to be more observations with NNI below 1 than for the measured NNI method.

Measured and predicted NNI values of split N applications are in Figure 5. As in Figure 4, predicted values were calculated using the direct method. Measured NNI increased with increasing GDD until approximately 900 to 1000 GDD after which the values seemed to drop. In general, most of the measured NNI values were above one. Results for the predicted NNI were scattered around NNI = 1.
affected by increasing N rates. DM was developing through the growing season, while the effect of N rates was not noticeable.

Figure 3. Boxplot showing the mean and the standard deviation for N_{up}, N_a and DM measurements for 2001, 2002, 2003, 2004 per GDD for each N treatment.

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Figure 4. Measured and predicted NNI for 2001, 2002, 2003, 2004 per GDD for each N treatment. A red horizontal line is drawn at NNI = 1.

3.4. Models’ Performance on Na, DM, N_{up}, NNI and Seed Yield Predictions

The performance of PLSR and SVM models on dependent variables (raw wavelengths and VIs) and the independent response variables (N_a, DM, N_{up}, and NNI) are shown in Table 5. Overall, there was no difference between the performance of PLSR and SVM. The prediction error (RMSEP) was, respectively, PLSR and SVM 0.47 and 0.65 %N for N_a, 3.9 and 6.7 t DM ha^{-1} for DM, 37 and 39 kg N ha^{-1} for N_{up}, and 0.26 and 0.28 for NNI. The models for DM, N_{up}, and NNI showed low performance with relatively high error for the predictions.
Figure 4. Measured and predicted NNI for 2001, 2002, 2003, 2004 per GDD for each N treatment. A red horizontal line is drawn at NNI = 1.

Figure 5. Measured and predicted NNI for 2001, 2002, 2003, 2004, per GDD for each split N treatment. A red horizontal line is drawn at NNI = 1.

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Table 5. Calibration and validation statistics of PLSR and SVM models on a combination of raw wavelengths and vegetation indices for determination of N concentration (Na), dry matter (DM), N uptake (Nup), and N nutrition index (NNI). Units for RMSECV and RMSEP are similar to the units defined for each response variable.

| Calibration | Test Set Validation |
|-------------|---------------------|
| **PLSR**    |                     |
| Response Variable | n | #PLSR comp | R\(^2\) | RMSECV | n | R\(^2\) | RMSEP | Bias |
| N\(_{a}\), % | 582 | 7 | 0.71 | 0.77 | 236 | 0.67 | 0.65 | 0.26 |
| DM, t ha\(^{-1}\) | 594 | 7 | 0.55 | 2.8 | 164 | 0.43 | 3.9 | 3.5 |
| Nup, kg ha\(^{-1}\) | 572 | 5 | 0.28 | 48 | 284 | 0.38 | 39 | –2.2 |
| NNI | 582 | 6 | 0.33 | 0.34 | 236 | 0.39 | 0.28 | 0.09 |

| SVM |                     |
| --- | -------------------|
| Response Variable | n | #SVs | R\(^2\) | RMSECV | n | R\(^2\) | RMSEP | Bias |
| N\(_{a}\), % | 583 | 405 | 0.84 | 0.56 | 241 | 0.77 | 0.47 | 0.12 |
| DM, t ha\(^{-1}\) | 600 | 454 | 0.69 | 2.4 | 288 | 0.52 | 6.7 | 5.4 |
| Nup, kg ha\(^{-1}\) | 589 | 520 | 0.31 | 48 | 288 | 0.46 | 36.7 | –3.03 |
| NNI | 586 | 582 | 0.36 | 0.34 | 243 | 0.40 | 0.26 | 0.07 |
3.5. Prediction of Seed Yield Based on Reflectance Measurements

Prediction of seed yield within years using raw wavelengths and VIs in combination with PLSR and the final seed yield is presented in Table 6 and Figure 6. Overall, it was possible to develop good calibration models with a low number of PLSR components. The $R^2$ values for the validation set of 0.51, 0.87, 0.79, and 0.58 in 2001, 2002, 2003, and 2004, respectively. The RMSECV were 156, 112, 117, and 161 kg seed ha$^{-1}$ in 2001, 2002, 2003, and 2004, respectively. The validation statistics were close to the calibration statistics, which indicated a lower possibility for overfitted models.

Table 6. Calibration and validation statistics of PLSR models on raw wavelengths and vegetation indices for determination of seed yield in 2001 to 2004. Units for RMSEC and RMSECV are kg seed ha$^{-1}$.

| Year | Calibration | Cross Validation |
|------|-------------|------------------|
|      | $n$ | #PLSR comp. | $R^2$ | RMSEC | $R^2$ | RMSECV |
| 2001 | 77  | 2            | 0.63  | 1354  | 0.51  | 156    |
| 2002 | 40  | 3            | 0.93  | 81    | 0.87  | 112    |
| 2003 | 40  | 1            | 0.81  | 112   | 0.79  | 117    |
| 2004 | 40  | 1            | 0.63  | 151   | 0.58  | 161    |

Figure 6. Measured vs. predicted seed yield for the four years, including the best linear fit and a 1:1 line.

4. Discussion

4.1. Response Curves

Precision agriculture is motivated by the fact that growing conditions and optimal management differ between seasons. The fact that our N response models fall into three categories, namely a) linear response where it is not possible to define EON, b) a category with a high EON at ~188 kg ha$^{-1}$, and c) a category with a low EON at 138 kg ha$^{-1}$ is interesting and useful for our aims.

Danish regulations state that perennial ryegrass growers should apply 170 kg ka$^{-1}$. This is almost equal to the average EON in the present study. Therefore, it can be implied that the regulations are well balanced and can be quite efficient from an economical point of view in the long term. Nevertheless, in 2002 and 2003, the regulations’ suggested rate would be slightly lower than the EON, while in 2004 it would result in 32 kg ha$^{-1}$ excess of N. Precision agriculture aims on minimizing those differences by focusing on the specific

\[ \text{Predicted seed yield, kg ha}^{-1} \]

\[ \text{Measured seed yield, kg ha}^{-1} \]
field in the specific year. To do so, it is important to derive information about the crops’ growth and variety type from the plant traits to regulate the N application rates accordingly.

However, in this study, the variability in the response models is not depicted in the $N_{up}$, $N_a$, DM or NNI results (Figures 3–5), where all the years seem to perform similarly within each treatment. Consequently, N does not seem to be the critical factor that caused these differences in yield responses. It is evident that although it is possible to determine these crucial plant traits during the growing season, it remains a very challenging task to utilize them to discover the optimum N rate for each year and each field. Analogous difficulties in detecting the optimum N rate by taking advantage of the remote sensing were presented in a case where optimum N varied enormously between different fields [24]. Still, this remains a debatable issue as there are a lot of studies suggesting that the relationship between remote sensing and defining optimum N is very strong [13,27,47].

As weather and lodging data cannot justify the underperformance in 2001 or the lower optimum N rate in 2004, it is more likely that these results are based upon the variability of the soil properties and the nutrient reserves of the fields. This variability can considerably affect crop growth and fertilizers performance, as shown by Argento et al. (2020) [23]. Still, it remains unclear which exact soil characteristics (texture, moisture, soil fauna, or others) or N-related processes (mineralization, immobilization) created this inconsistency.

It is also meaningful to evaluate the study’s performance in predicting $N_{up}$, $N_a$, DM, and NNI. Several studies evaluated the use of VIs as predictors for those plant traits [9,15,23,26]. Argento et al. [23] reported a considerably better relationship between $N_{up}$ and VI ($R^2 = 0.8$) than the present study; however, this is not represented in the RMSE (= 30 kg N ha$^{-1}$), which is comparable to ours. Nigon et al. [9] managed to reduce this amount to only 13.6 kg N ha$^{-1}$ using the random forest method, but in an experiment with a remarkably lower dataset ($n = 148$). The present study shows good results in correlating VIs and $N_a$ with an $R^2 = 0.72$. Numerous studies present a weaker estimation accuracy [14,15,23], while some have similar results [26]. DM estimation seems to be the most challenging one, according to relevant literature, and if lodging appears, it is even more challenging. Wang et al. [11] suggest that due to the N dilution in the crop, high correlations between VIs that focus on chlorophyll information and DM should not be expected. Nevertheless, our estimations for DM performed at an acceptable level, comparable with other studies [15]. This study also tried to evaluate the direct and indirect methods for predicting NNI based on VI. On the one hand, some studies utilize the indirect method to produce decent predictions of NNI [14]. On the other hand, good predictions were also achieved using the direct method [11]. Zhao et al. [26] suggest that although both methods can be used to estimate NNI, the combination structure and the calculation method for newly developed VI were easier in the direct method. In this study, the direct method was the one that resulted in better prediction performance.

Regarding split applications, N40 + 80 and N80 + 40 resulted in similar yields on average of the four years (Table 4). In addition, they both resulted in significantly higher yields than N40 + 40 + 40. This can be interpreted to a certain degree by Figure 4, where the addition of 80 or 40 kg N ha$^{-1}$ as a second N application increased measured NNI values to a point above the NNI = 1 line, whereas the third N application of 40 kg N ha$^{-1}$ did not have any clear effect. Still, this is not apparent in the predicted NNI values. The overall conclusion on split N application is not in favor of this application strategy in perennial ryegrass for seed production. Application of 80 kg N ha$^{-1}$ followed by an additional 40 kg N ha$^{-1}$ at stem elongation was expected to be a strategy that fits perfectly well into the use of in-season NNI measurements. The idea was that 80 kg N ha$^{-1}$ is sufficient for crop growth and development until stem elongation and NNI should be used to identify the need for additional N. Unfortunately, this was not the case and emphasize the fact that perennial ryegrass is dynamic in its growth and ability to adapt according to current growing conditions and furthermore without a large impact on the final seed yield.
4.2. Yield Models Performance

One approach to optimize N fertilization could be to predict final seed yield and, based on this estimate, the total harvested N amount in seeds and straw, or simply assume that a high estimated yield has a larger N demand. Calculation of N application rate should include the utilization of the applied N and N released from the soil. The unknown parameter in this method is the soil N pool, and especially the amount of N released and being plant available during the growing season. However, this unknown soil N parameter is also present and equally important when aiming to use NNI during the vegetative growth phase of the crop. The first step is, therefore, to predict seed yield using canopy reflectance measurements. Year largely affected seed yields exemplified for N160 where the difference was 545 kg seeds ha$^{-1}$ between 2001 and 2003. Surprisingly, this large variance did not influence the possibility to develop yearly PLSR models based on raw wavelengths and VI to predict seed yields. The idea of predicting seed yield based on VI is not novel. Astaoui et al. [25] estimated a 1 to 21% difference between predicted and actual wheat yield in a single year using the random forest technique. Gislum et al. [48] estimated an RMSECV between 49 and 113 kg seed ha$^{-1}$ in perennial ryegrass using the same spectroradiometer as in the current study. Our average RMSECV was 137 kg seed ha$^{-1}$, equivalent to 11% based on the average seed yields for N40, N80, N120, N160, and N200 in all years. Our prediction error is thus in or close to the range published by Astaoui et al. [25] and Gislum et al. [48].

5. Conclusions

Different VIs and raw wavelengths were used in combination with PLSR and SVM to predict final seed yield, NNI, Na, DM, and N$\uparrow$ in the four-year field experiment. The best models were obtained for the prediction of the final seed yield, while prediction of especially DM and NNI was challenging. Prediction of NNI and the possibility to utilize this method to quantify if additional N should be applied perennial ryegrass for seed production is challenging. Based on this, we conclude that although it is possible to make in-season predictions of NNI, it does not always portray the differences in yield potential; thus, it is challenging to utilize it to optimize N application.

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