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TECHNICAL NOTE

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Nitrogen rate influences on protein concentration, yield, and NDVI in spring wheat following pea and cover crop mixture

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Abstract

High-protein wheat (*Triticum aestivum* L.) grain is useful for bread flour, and normalized difference vegetation index (NDVI) can be useful in predicting crop yields and assessing overall crop health in response to a wide variety of management systems. However, protein and NDVI responses to nitrogen rates in systems containing cover crops have been understudied in spring wheat. Protein, grain yield, and NDVI measurements were collected during the spring wheat phase of spring wheat–pea (*Pisum Sativum* L.)/cover crop rotations in different plots in 2018 and 2019 at the Northern Great Plains Research Laboratory, Mandan, ND. Grain protein concentration increased with nitrogen (N) rate, but not every level of N rate was significantly different. This result suggests that preceding pea then cover crop mixture can slightly reduce the nitrogen fertilizer requirements of subsequent spring wheat. Spring wheat NDVI did not significantly respond to categorical nitrogen rates, but continuous N rate had a small positive effect. Adding NDVI heterogeneity (as measured by the nugget:sill ratio) improved peak NDVI-based spring wheat grain yield predictions at the relatively small scales that were measured.

Plain Language Summary

High-protein wheat grain is useful for bread flour. Sensors that measure light from plants growing in the field using the normalized difference vegetation index (NDVI) can be useful in predicting crop yields and assessing overall crop health. However, it is not well known how protein and NDVI respond to nitrogen fertilizer applied to spring wheat grown in systems with cover crops. Protein, grain yield, and NDVI were measured in spring wheat in a spring wheat–pea/cover crop rotation in 2018 and 2019 near Mandan, ND. Wheat yield was not affected by nitrogen fertilizer application rate

Abbreviations: N:S, nugget to sill ratio; NDVI, normalized difference vegetation index; NS, nonsignificant; RMSE, root mean square error; SW–P/CC, spring wheat–pea/cover crop.

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(N rate). Grain protein concentration increased with higher N rates, but not every level of N rate was significantly different. This result suggests that nitrogen fertilizer rates could be slightly reduced when spring wheat is grown after peas and a cover crop mix. A yield prediction equation was developed using the peak NDVI measurements, which was improved by including NDVI spatial variability.

1 | INTRODUCTION

Spring wheat (*Triticum aestivum* L.) grain protein increases with nitrogen (N) rate until maximum grain yield and maximum protein concentration is reached (Abedi et al., 2011), in a pattern similar to corn (*Zea mays* L.; Gajula et al., 2023). However, it is unclear if a previous multi-species cover crop mix seeded after a leguminous crop harvest can provide all necessary N for a subsequent spring wheat crop. Similar knowledge gaps exist with vegetation indices following cover crops.

Normalized difference vegetation index (NDVI) has long been used as a proxy for plant phenology (Matongera et al., 2021; Rouse et al., 1974) and is considered an indicator of overall plant health (Huang et al., 2021). The final grain yield of multiple crops can be predicted using NDVI (reviewed by Rembold et al., 2013). Additionally, NDVI can be used to evaluate crop health across growing seasons in response to management treatments. For example, corn NDVI increased with previous radish cover crop relative to no cover crops and decreased with previous annual ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] cover crop compared to no cover crop (Scott et al., 2022). Surprisingly, Scott et al. (2022) found that corn NDVI responded only to cover crop treatment, not N-rate treatment. Spring wheat NDVI has been shown to be a good predictor of final yield (Moriondo et al., 2007; Naser et al., 2020; Sultana et al., 2014), though other predictors such as precipitation during tillering and anthesis can outperform NDVI (Vannoppen & Gobin, 2021). However, spring wheat NDVI response to nitrogen rate treatments in systems with cover crops has been understudied, especially the spatial variability (but see Stoy et al., 2022). Landsat spatial scales (30 m) were found to adequately capture within-field NDVI information but were not a fine enough scale to reflect historical management and edaphic variability (Stoy et al., 2022). The extent to which spring wheat NDVI heterogeneity can improve NDVI-based yield predictions is unknown.

Wheat NDVI is positively correlated with wheat grain yield during wheat phenological stages where NDVI is not saturated, including early growing season, anthesis, and grain fill (Nasser et al., 2020). Wheat NDVI is also positively correlated with grain protein concentration, particularly 1 week prior to harvesting (Du et al., 2021). Our objectives were to (1) determine grain yield and NDVI responses to N rate treatments and (2) develop grain yield predicting equations from NDVI and spatial heterogeneity of NDVI. We hypothesized that N rates could be reduced below the recommended rate without reducing yield or grain protein in plots with cover crops between the pea and spring wheat phases. We predicted that the cover crops would provide sufficient N to offset a reduction in N fertilizer. We also hypothesized that including NDVI heterogeneity (as indicated by the nugget:sill ratio) would increase variance in grain yield explained.

2 | METHODS

This research was conducted in the Bioenergy Cropping Systems of the Northern Great Plains Research Lab near Mandan, ND (46.773° N, 100.904° W, elevation 591 m). This experiment was initiated in 2009 and first described by Archer et al. (2019). This rainfed site is situated on Temvik-Wilton silt loam (fine-silty, mixed, superactive, frigid Typic and Pachic Haplustolls) soils. Residue removal treatments (no removal, harvest and remove wheat straw, harvest and remove residue from each crop, and graze all crop residues) were established in a strip plot randomized complete block design with four replicates. For this study, we focused on the spring wheat phase of a spring wheat–dry pea/cover crops (SW–P/CC) rotation. The cover crop mix consisted of seven species: purple top turnip (*Brassica rapa* var. *rapa* L.), proso millet (*Panicum miliaceum* L.), soybean [*Glycine max* (L.) Merr.], Arvika pea, sunflower (*Helianthus annuus* L.), triticale (× *Triticosecale* Wittm. ex A. Camus), and canola (*Brassica napus* L.). The cover crop mix was planted after dry pea harvest. All treatments were managed in a no-till system. A subset of this experimental design was leveraged to investigate nitrogen fertilizer rate and including cover crop in the rotation effects on spring wheat NDVI.

We utilized a portion of this larger experiment to create a smaller experiment to evaluate wheat responses to N rate following peas and cover crops. In 2018 and 2019, a strip within the selected plots (wheat phase of no residue removal treatment of SW–P/CC rotation) was subdivided into nitrogen fertilizer rate treatments (target N level: A = 235 kg ha^{-1}, B = 202 kg ha^{-1}, C = 168 kg ha^{-1}, D = 135 kg ha^{-1}; previous crop N credit, no-till N credit, and soil N were calculated; then, urea was applied to reach the target N level after accounting for N applied in 56 kg ha^{-1} 11-52-0 starter fertilizer).

The B rate represents a recommended rate for wheat following peas in a long-term no-till system (Franzen, 2022). Due to N credits, target rates and applied urea N rates differed. Actual urea N application rates were A = 115 kg ha^{-1}, B = 88 kg ha^{-1}, C = 50 kg ha^{-1}, D = 20 kg ha^{-1} in 2018; and A = 131 kg ha^{-1}, B = 88 kg ha^{-1}, C = 52 kg ha^{-1}, D = 20 kg ha^{-1} in 2019. Growing season weather in 2018 and 2019 was largely similar, except for a noticeably wetter May and September in 2019 (Figure 1). A randomized complete block design was used to analyze this experiment, where plots of the original experiment were used as blocks. The randomized N rate treatments within a block were applied to 4.5 × 6 m plots.

Wheat protein (% mass at 12% moisture basis) was measured at a subplot level in 2018 and 2019 using a FOSS Infratec NOVA whole grain near-infrared analyzer (FOSS, Hilleroed, Denmark). Wheat grain yield was also measured at a subplot level. Wheat NDVI was measured in the 2018 and 2019 growing seasons using a tractor-mounted Crop Circle ACS-470 (Holland Scientific, Lincoln, NE, USA). The Crop Circle ACS-470 is an active optical sensor for measuring plant canopy reflectance. The instrument was configured to measure three bands, red (670 nm), red edge (730 nm), and near-infrared (760 nm) for this study. NDVI was measured opportunistically throughout the growing season, and peak observed NDVI values were identified for each plot. The peak NDVI values corresponded with the early ripening phenological stage of spring wheat, likely after NDVI saturation. Spatially explicit measurements were viewed in ArcGIS Pro v3.03 (Esri, Redlands, CA, USA), and outlines of plots were defined. NDVI values within a subplot were arithmetically averaged to determine mean NDVI. Additionally, a global (all measurement points) semivariogram was created using ArcGIS Pro. The global NDVI range (~12 m) was smaller than the length of whole plots (24 m), suggesting it was acceptable to interpret whole plot-level semivariograms. Nugget-to-sill ratios of NDVI measurements were derived from plot-level semivariograms. This ratio represents the heterogeneity of NDVI within a plot independent of spatial autocorrelation. It is important to remove the effect of autocorrelation (i.e., points close together are more likely to have similar NDVI values) to not overstate the significance of management effects. The nugget:sill ratio allows the evaluation of heterogeneity in NDVI imparted by N rate and cover crop treatments. The nugget:sill ratio value can range from 0 to 1, with higher values indicating more heterogeneity.

Core Ideas

- Protein concentration generally increased with higher N rate.
- Wheat grain yield did not significantly respond to N rate.
- Categorical nitrogen fertilizer rate had no effect on normalized difference vegetation index (NDVI).
- Continuous nitrogen fertilizer rate had a small positive effect on NDVI.
- Adding NDVI heterogeneity improved NDVI-based yield predictions.

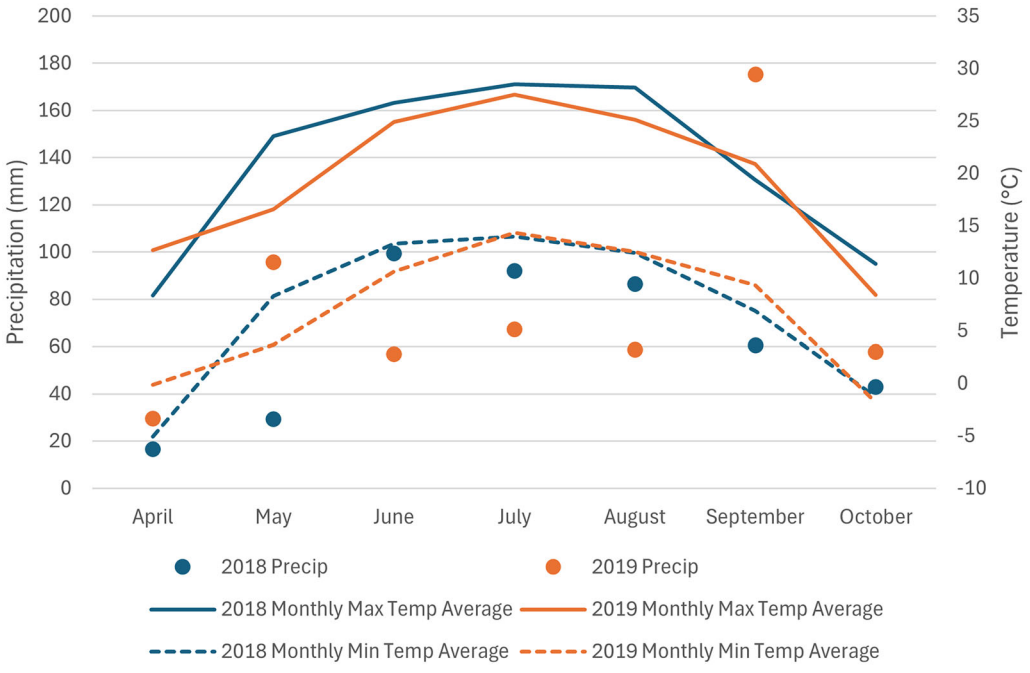


FIGURE 1 Monthly precipitation received and average minimum and maximum temperatures during the 2018 and 2019 growing seasons.

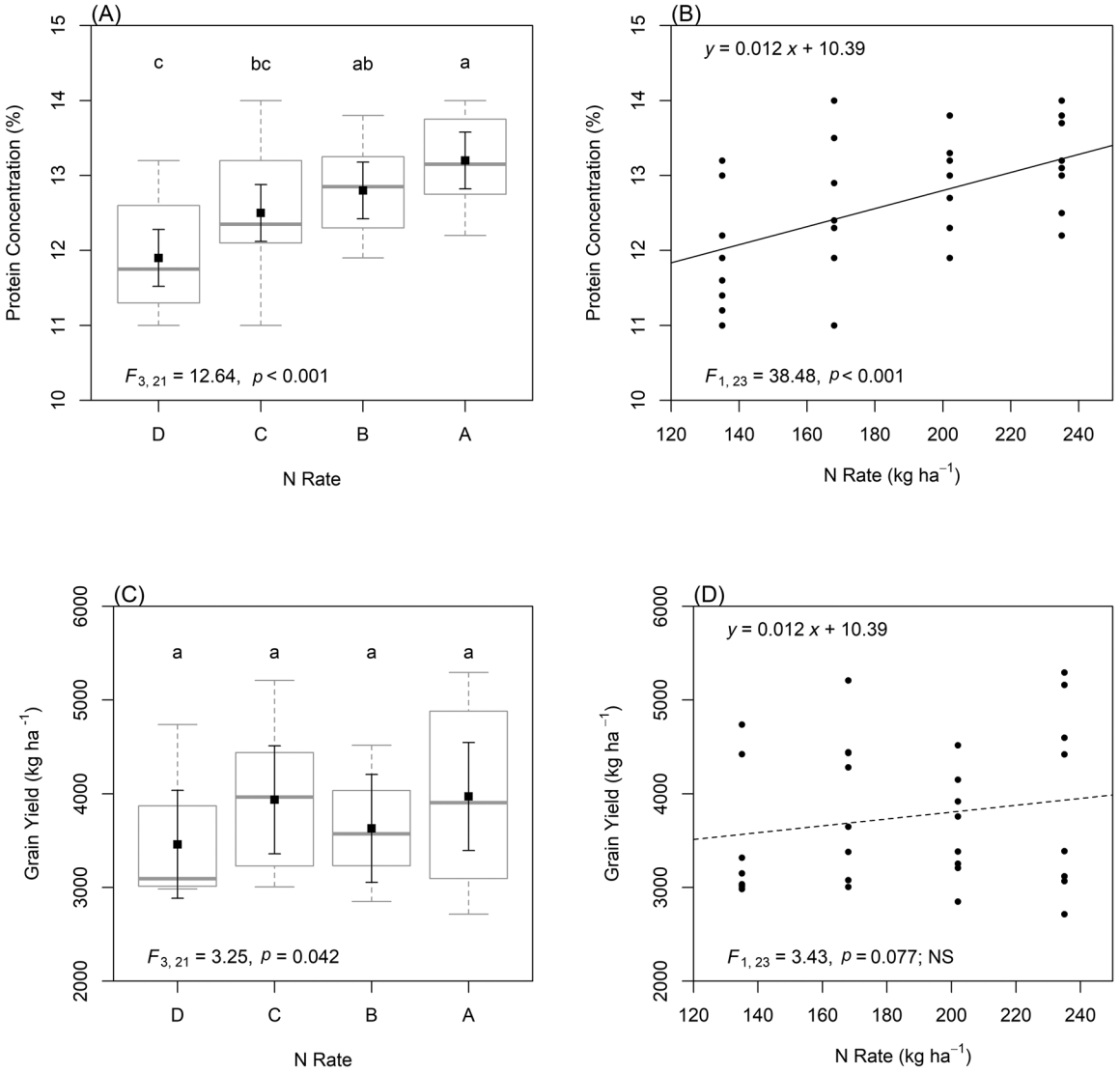


FIGURE 2 (A) Spring wheat protein concentration as measured by near-infrared transmittance response to categorical N rate, (B) spring wheat protein concentration as measured by near-infrared transmittance response to continuous N rate, (C) spring wheat grain yield response to categorical N rate, and (D) spring wheat grain yield response to continuous N rate. Gray box plots indicate median, interquartile range, and maximum and minimum observed values. Black squares indicate estimated marginal means, and black error bars indicate statistical model standard errors. Targeted N rate levels: A = 235 kg ha^{-1}, B = 202 kg ha^{-1}, C = 168 kg ha^{-1}, and D = 135 kg ha^{-1}.

All non-geospatial statistics were conducted with R (version 4.4.1; R Core team, 2024) using an alpha level of 0.05, which is the traditional significance cutoff (Miller & Ulrich, 2019). Mixed model analyses of variance (ANOVAs) were used to statistically model the response of wheat protein concentration (%, at 12% moisture), wheat grain yield, and maximum observed spring wheat NDVI to target N rate, analyzed at the plot level. The fixed effect was N rate with crossed (non-nested) random intercepts of year (to generalize across various growing conditions) and block. N rate was interpreted as both a categorical and a continuous variable using targeted N rates. Block-level predictive equations were also developed, as they may be relevant to producers. The block level also contains variable N from plot to plot, as N rates were applied at the plot level. At the block level using a year random intercept, models with a mean NDVI measurement at plot level (maximum observed NDVI) and with and without the maximum observed nugget:sill ratio of wheat NDVI were compared for predictive ability of yield. Predictive ability was evaluated using psuedo-R^2 as calculated by the r.squared.GLMM function of the MuMIn package (Bartoń, 2024). Marginal pseudo-R^2 ($R^2{}_m$) represents variance explained only by fixed predictors while conditional pseudo-R^2 ($R^2{}_c$) represents variance explained by both fixed and random effects. All statistical models were fit with the lmer function of the lme4 package (Bates et al., 2015). p-values were calculated using Kenward-Roger denominator degrees of freedom correction using the lmerTest package

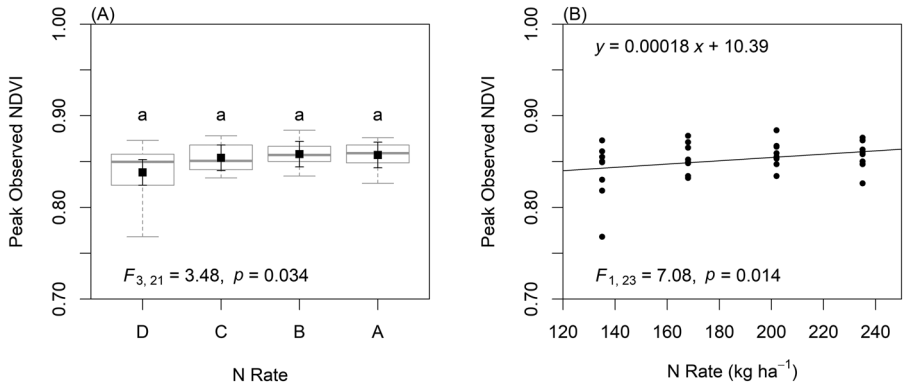


FIGURE 3 Peak observed normalized difference vegetation index (NDVI) response to N rate when considering N rate as (A) categorical and (B) continuous. Gray box plots indicate median, interquartile range, and maximum and minimum observed values. Black squares indicate estimated marginal means, and black error bars indicate statistical model standard errors. Targeted N rate levels: A = 235 kg ha^{-1}, B = 202 kg ha^{-1}, C = 168 kg ha^{-1}, and D = 135 kg ha^{-1}.

(Kuznetsova et al., 2017). In the mixed-model regression with two predictors, maximum observed NDVI and the nugget:sill ratio of max observed NDVI, standardized regression coefficients were calculated by scaling the response and fixed effects. Since this is a limited 2-year dataset, we also performed a leave-one-out cross-validation to indicate if the relationships found are likely to apply to future years. This was done by using the cv package (Fox & Monette, 2025) with full model criterion of leave-one-out cross-validation of year clusters; root mean square error (RMSE) was calculated.

TABLE 1 Normalized difference vegetation index (NDVI)-based prediction of spring wheat yield (kg ha^{-1}) in spring wheat–pea/cover crop rotation measured in 2018 and 2019.

Yield prediction	R^2_m	R^2_c	RMSE
Yield = (4181) NDVI + 189	<0.01	0.81	339.00
Yield = (17,668) NDVI + (−801) NDVI N:S − 10,983	0.06	0.89	269.67

Note: Yield predicting equation based on linear model fit with the lmer function with fitted coefficients rounded to the nearest whole number.
Abbreviations: NDVI N:S, maximum observed nugget:sill ratio of normalized difference vegetation index; RMSE, root mean square error of full model criterion leave one out cross validation of year clusters.

3 | RESULTS

Spring wheat grain protein concentration increased with nitrogen rate, though a one-step increase in N rate treatment did not result in significantly different protein concentrations from the previous level (Figure 2A). When interpreting N rate as continuous, protein concentration increased gradually with N rate (Figure 2B; slope was greater than 0: $t = 6.20$, $p < 0.001$). Spring wheat grain yield responses significantly varied with N rate (i.e., a significant ANOVA result), but no pairwise differences were detected (Figure 2C; model mean = 3749 ± 564 kg ha^{-1}). When N rate was interpreted as a continuous variable, no significant effect on spring wheat yield was detected (Figure 2D; slope was not significantly different from 0: $t = 1.85$, $p = 0.077$, nonsignificant [NS]). Peak observed NDVI varied significantly with N rate, but no pairwise differences were detected (Figure 3A). However, a positive effect of N rate on peak NDVI was observed when considering N rate as a continuous variable (Figure 3B), though the slope was not significantly different from zero ($t = 2.66$, $p = 0.014$, NS). NDVI-based models to predict spring wheat grain yield had a substantially higher conditional pseudo-R^2 than a marginal pseudo-R^2. Including the nugget:sill ratio increased the marginal pseudo-R^2 of the grain predicting model (Table 1). Including the nugget:sill ratio also decreased RMSE (Table 1).

4 | DISCUSSION

Protein results suggest that a slight N rate reduction in spring wheat could be used with cover crops following peas, as protein concentration with N rate C (168 kg ha^{-1}) was not significantly different from the recommended N rate B (202 kg ha^{-1}). However, there is a clear positive relationship of protein concentration with N rate over all N rates evaluated. This is despite the pea/cover crop phase apparently providing sufficient nitrogen to maximize spring wheat yield regardless of applied N, as similar yield values were observed for all N rate treatments.

Observed wheat NDVI did not significantly respond to the N rate in this study when considering N rate as a categorical predictor, much like the lack of NDVI response in corn in a previous study (Scott et al., 2022). However, other studies in wheat found an increase in NDVI with greater N additions

(Ali et al., 2022; Walsh et al., 2020). The relatively small effect on N rate on NDVI in this study might suggest that NDVI responds less to N concentrations when (1) no yield response to N is observed in wheat or (2) when a cover crop precedes wheat.

Variability in NDVI imparted by rotation or year effects should be considered, as NDVI-based yield estimates could be greatly affected. For example, another wheat study found that integral NDVI and peak NDVI of wheat were worse predictors of yield than meteorological variables and tillering rate (Vannoppen & Gobin, 2021). Fitted models conformed to our assumption of a negative heterogeneity–yield relationship (Daynard & Muldoon, 1983; Liu et al., 2004) and positive NDVI-yield relationship (Nasser et al., 2020). The model using NDVI to predict spring wheat grain yield had a similar regression coefficient to the model described by Aranguren et al. (2020; Yield = 4138.7 (NDVI) – 2693.7), though they used the sum of NDVI within a season rather than the max observed NDVI in a season. To our knowledge, we are the first to incorporate a nugget:sill ratio into an NDVI-based prediction of grain yield. The model including NDVI heterogeneity improved the predictive ability of yield, as indicated by the marginal pseudo-R^2. However, in both models, the conditional pseudo-R^2 was substantially higher than the marginal pseudo-R^2, suggesting year effects had substantial influence on yields. This finding is consistent with the finding that meteorological variables can be better predictors than NDVI (Vannoppen & Gobin, 2021). Maximum observed NDVI (0.39) and nugget:sill ratio of max observed NDVI (−0.33) had similar magnitude of effect on yield as indicated by standardized regression coefficients. Additionally, RMSE indicates that future year's yields can be predicted reasonably well, especially the predictive model including NDVI nugget:sill ratio. Incorporating meteorological variables might further improve the yield prediction model. This study demonstrates that mean NDVI and the nugget:sill ratio of NDVI measurements taken with the same multispectral camera can potentially improve yield predictions using fields divided into 24 m × 24 m parcels.

5 | CONCLUSIONS

Protein concentration increased with nitrogen rate, though a one-step increase did not result in significant differences suggesting that the pea followed by cover crop mix previous phase can slightly reduce the nitrogen fertilizer need in spring wheat. Spring wheat grain yield did not significantly respond to N rate, regardless of if N rate was considered a categorical or a continuous predictor. Nitrogen fertilizer rate did not significantly influence spring wheat NDVI values when N rate was considered a categorical predictor and had a shallow but significant positive slope when considered a continuous predictor. This might suggest N rate has a weaker effect on NDVI when there is no significant yield response or when cover crops are included in the system. Incorporating NDVI heterogeneity (as measured by the nugget:sill ratio) improved NDVI-based prediction of spring wheat grain yield. We encourage future yield prediction model development that includes both NDVI-based predictors and micrometeorological-based predictors.

AUTHOR CONTRIBUTIONS

Drew A. Scott: Formal analysis; writing—original draft; writing—review and editing. **David W. Archer**: Conceptualization; Supervision; writing—review and editing. **Holly Johnson**: Investigation; writing—review and editing. **Michael DeGreef**: Formal analysis; writing—review and editing. **Raina Hanley**: Investigation; writing—review and editing. **Billi Jean Petermann**: writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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