Estimation of Botanical Nitrate Concentration Using Spectroscopic Analysis and Modeling

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Introduction

Nitrogen is one of the most important nutrients in biomass feedstock crop production, such as native warm-season grasses (NWSG), yet one of the most difficult to manage. Due to its complex chemistry and behavior in soils and plants, rapidly assessing botanical N concentration and monitoring its dynamics within a cropping system are of paramount importance. Insufficient N application can cause low biomass production and reduce economic profitability. In contrast, excessive N application can lead to accumulation of nitrate, a polyatomic anion of N, in feedstock material that can reduce hydrocarbon yield during the thermochemical process, increase NO_x emissions and cause nitrate toxicity problems once grazed by livestock.

Rapidly and accurately monitoring crop nitrate concentration is difficult. Traditional methods that involve harvesting the plant are laborious and require destructive plant samplings as well as chemical analyses; thus, alternative methods are warranted. The objective of our research was to compile a rapid nitrate assessment and evaluation method using both remote sensing-based precise spectroscopic instruments and regression-based mathematical models. We anticipate the results from this study can help develop an N management tool that can guide producers to reach high NWSG biomass production with low N input.

Materials and Methods

This experiment was conducted in the MTSU Plant and Soil Science Greenhouse in Murfreesboro, Tennessee from May 25th to July 24th 2015, and repeated from May 5th to September 14th 2016. Two NWSG species, including 'Alamo' switchgrass (Panicum virgatum L.) and 'Cheyenne' indiangrass [Sorghastrum nutans (L.) Nash] were planted in 46-cm diameter flower pots with standard potting soils. Urea (46-0-0) fertilizer was applied one month after planting at four levels: control (0 kg N ha^{-1}), low (65 kg N ha^{-1}), medium (130 kg N ha^{-1}), and high (260 kg N ha^{-1}). Each species and fertilizer level combination was replicated four times accounting for sixteen pots per species for a total of 32 pots. Foliar chlorophyll concentration was measured weekly using a SPAD 502 Plus Chlorophyll meter (Spectrum Technologies, Inc., Aurora, IL) for five weeks immediately following fertilization. Meanwhile, plant height (cm) was measured using a ruler. *In-vivo* foliar reflectance data was measured weekly following fertilization using the ASD FieldSpec®4 Standard-Resolution Spectroradiometer (ASD Inc., Boulder, CO 80301), which records spectral digital count data between the 350-to-2,500-nm range, yielding a 1.4-nm sampling interval in the 350-1,000 nm spectral range and 2.0-nm in the 1,000-2,500 nm range.

Three representative leaves were randomly selected from each pot for spectral measurements. All spectral data were optimized, white-referenced, recorded, and converted to reflectance values using the ASD RS3 Spectral Acquisition Software. Then, reflectance data was converted to ASCII format using the ViewSpecPro Software for further analysis using Matlab Programming Language. After the leaf reflectance measurements, all above-ground biomass was immediately cut and dried at 60˚C to a constant weight, and sent to the University of Tennessee Soil, Plant, and Pest Center for forage and plant tissue analysis.

Plant height, SPAD readings, and botanical nitrate were analyzed as a completely randomized design with four replications and repeated measure effect to control for autocorrelation of observations over time using the MIXED procedure in SAS (SAS Institute Inc., Cary, NC). All ASCII spectral data were analyzed using machine learning algorithms implemented using the Matlab Programming Language based on a generalized regression neural network (GRNN) model to recognize the spectral pattern differences and predict the nitrate concentration across two grass species. A standard leave-one-out cross-validation procedure was used to evaluate the prediction performances.

Results and Discussion

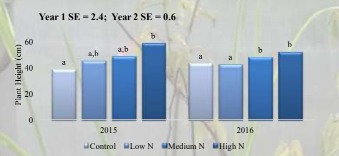

Fig. 1. Plant height of indiangrass affected by different N treatments, including control (0 kg N ha^{-1}), low (65 kg N ha^{-1}), medium (130 kg N ha^{-1}), and high (260 kg N ha^{-1}) in two years (2015 and 2016). Letters separate means based on P < 0.05 level by pair-wise comparisons.

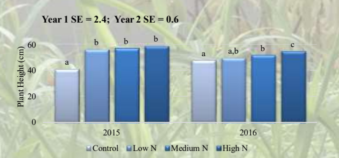

Fig. 2. Plant height of switchgrass affected by different N treatments, including control (0 kg N ha^{-1}), low (65 kg N ha^{-1}), medium (130 kg N ha^{-1}), and high (260 kg N ha^{-1}) in two years (2015 and 2016). Letters separate means based on P < 0.05 level by pair-wise comparisons.

Plant height

Plant height responses were presented by years and species for consistency purposes (Fig. 1 and 2). For indiangrass, the high-rate of N fertilization increased plant height by more than 15-cm compared to control in both years (Fig. 1). In year two, both medium and high rates increased plant height more than the control and low rates. For switchgrass, all N fertilization treatments increased average plant height by almost 18-cm compared with no-N control during year one (Fig. 2). In year two, medium N fertilization rate increased height compared with no-N control and high N rate indicated even greater responses when compared to the medium rate. Plant height is one of the key indicators of plant growth and nutrient responses. The results from this study agreed well with other plant phenotyping studies involving crop canopy height measurements under different N management regimes (Yin *et al.*, 2011).

Foliar chlorophyll concentration

In year one, medium and high N treatments increased SPAD readings of indiangrasses (Fig. 3), meanwhile, fertilized switchgrasses indicated higher SPAD readings compared with no-N control (Fig. 4). Additionally, high N treatment increased SPAD readings when compared with low N and no-N control (Fig. 4). In year two, both medium and high N treatments increased SPAD readings when compared to no-N control of indiangrass and switchgrass. Results presented in this study provide strong evidence that SPAD readings could be used for quickly assessing N responses of NWSG.

Fig. 3. Foliar SPAD readings of indiangrass affected by different N treatments, including control (0 kg N ha^{-1}), low (65 kg N ha^{-1}), medium (130 kg N ha^{-1}), and high (260 kg N ha^{-1}) in two years (2015 and 2016). Letters separate means based on P < 0.05 level by pair-wise comparisons.

Fig. 4. Foliar SPAD readings of switchgrass affected by different N treatments, including control (0 kg N ha^{-1}), low (65 kg N ha^{-1}), medium (130 kg N ha^{-1}), and high (260 kg N ha^{-1}) in two years (2015 and 2016). Letters separate means based on P < 0.05 level by pair-wise comparisons.

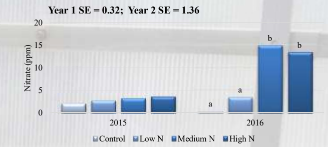

Fig. 5. Average botanical nitrate concentration of indiangrass and switchgrass affected by different N treatments, including control (0 kg N ha^{-1}), low (65 kg N ha^{-1}), medium (130 kg N ha^{-1}), and high (260 kg N ha^{-1}) in two years (2015 and 2016). Letters separate means based on P < 0.05 level by pair-wise comparisons.

Botanical Nitrate Concentration

In year two, botanical nitrate concentration was strongly affected by treatment (Fig. 5; $P < 0.01$). No species effect ($P = 0.09$) nor two-way interaction was present ($P = 0.41$). Both medium and high levels of fertilization treatment increased botanical nitrate concentration when compared with no-N control and low N treatments (Fig. 5).

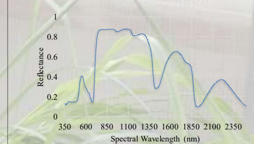

Fig. 6. Foliar reflectance pattern of switchgrass across 350 to 2,500 nm with an average leaf nitrate concentration of 12 ppm.

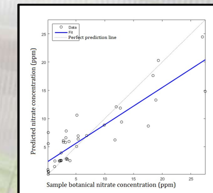

Fig. 7. Prediction performances of botanical nitrate concentration using a generalized regression neural network (GRNN) model.

Spectral reflectance responses

Spectral reflectance differences were detected between two grass species and between high nitrate and low nitrate concentrations within the same species (e.g. Fig. 6). We implemented computational algorithms using Matlab (The MathWorks Inc., Natick, MA) based on a generalized regression neural network (GRNN) model to recognize the spectral pattern differences and predict the nitrate concentration across two grass species. A standard model training, testing, and validation paradigm was followed. Particularly, four hundred spectral samples with nitrate concentration ranging from 0.1 to 27.5 ppm were selected for building the prediction model. The final model yielded great performances (Fig. 7; $R^2 = 0.88$ and RMSE = 0.358).

Conclusions

Nitrogen is one of the most important nutrients for plant production, however, one of the most difficult to manage (Cui *et al.*, 2013). Precisely applying N fertilizer on bioenergy feedstock grasses has great impact on hydrocarbon yield efficiency (Foster *et al.*, 2013). This study verified the biomass production responses of switchgrass and indiangrass affected by different N treatments. A preliminary nitrate monitoring tool was successfully developed by integrating hyperspectral spectroscopy analysis and mathematical modeling, which can accurately predict botanical nitrate concentration even at its low level.

Literature Cited

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