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# Nitrogen Mineralization in Soils Used for Biofuel Crops

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# Nitrogen Mineralization in Soils Used for Biofuel Crops

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*Perennial biofuel crops such as* Miscanthus *and switchgrass are thought to increase soil organic matter and therefore may increase soil nitrogen (N) mineralization rates. Our objective was to evaluate a range of N-mineralization indices for soils with established biofuel crops and compare these results with soils in a traditional corn and soybean rotation. We sampled surface soil (0–10 cm deep) from switchgrass (6 years after establishment) and* Miscanthus *plots (5 years) in a high-organic-matter Mollisol. The longest potential N mineralization index, a 24-day incubation, was significantly greater in* Miscanthus *soils compared to switchgrass and corn–soybean. In addition, 7-day anaerobic N and potassium chloride–extractable ammonium N were both greater in* Miscanthus *soils compared to switchgrass and corn–soybean. Our results do support our hypothesis that N-mineralization rates are greater in soils under biofuel production.*

**Keywords** Mineralization, *Miscanthus*, nitrogen, switchgrass

## Introduction

Currently, corn is the dominant biofuel crop used for ethanol production in the United States (Hill et al. 2006). However, *Miscanthus* × *giganteus* (*Miscanthus*) and *Panicum virgatum* (switchgrass) are perennial grasses that are possible second-generation biofuel crops that could be used for cellulosic ethanol production. Specifically, these grasses have been shown to have greater biomass production than corn (Heaton, Dohleman, and Long 2008); therefore, it is important to have a better understanding of how these perennial crops may alter soil quality and the environment compared to the traditional row crop system found across much of the Midwest (Heaton, Dohleman, and Long 2008). Some of these critical changes include the ability of *Miscanthus* and switchgrass production systems to sequester greenhouse gases, such as carbon dioxide ($CO_2$), and their much lesser nitrate leaching rates compared to annual row crop systems (Jordan et al. 2007; Heaton et al. 2010; McIsaac, David, and Mitchell 2010). These biological factors have the potential to change current agricultural production systems and the soils beneath the crop.

Nitrogen (N) is a critical element in many aspects of agricultural production and the environment. The amount of potentially mineralizable N plays a large role in the soil, specifically in assessing needs for N applications as fertilizer to agricultural land to maximize crop yields (Schomberg et al. 2009). Fertilizer, when applied in current production systems, typically increases nitrate leaching into streams and rivers (David, Drinkwater,

and McIsaac 2010). Nitrate ($NO_3$) leaching can lead to hypoxia in coastal waters, and large nitrate concentrations are a concern to human health in freshwater systems (USEPA 2007). Mineralization rates of soil N in fields are important to monitor and understand in both traditional and biofuel fields. Many soils in the Midwest with loess parent materials (formerly in prairie vegetation) have large amounts of soil N, a small amount of which mineralizes each year (Gentry et al. 1998). If these soil N mineralization rates were better known, fertilizer applications could be more clearly defined for each field, possibly reducing losses of nitrate.

A single method to calculate potential soil N mineralization during the growing season has not been generally accepted; however, long-term incubation methods are considered to be consistently accurate (Wang, Smith, and Chen 2003). The concern with long-term incubation methods is a factor of time. For samples to be regularly collected and processed, the method for analysis needs to function routinely in a laboratory setting.

Schomberg et al. (2009) assessed a variety of methods for potential soil N mineralization. The group ran a default 41-week long-term incubation method and observed the correlation with a variety of different methods measuring potentially mineralizable soil N. Anaerobic N, N mineralized after a 24-day incubation, and N mineralized by hot potassium chloride (KCl) were found to have the greatest correlations to the 41-week incubation. Total carbon (C), total N, and hot KCl methods were found to be the fastest methods, whereas anaerobic N and the 24-day incubation were found to have the strongest correlations with the 41-week, long-term incubation (Schomberg et al. 2009).

Perennial grass biofuel crops are expected to add C and N to surface soils, because of large belowground organic-matter inputs (Davis et al. 2010). Grasses such as *Miscanthus* have large N requirements in late spring and early summer because of their large leaf biomass, but then they retranslocate much of it belowground before senescence (Heaton, Dohleman, and Long 2009). Nitrogen for the rapidly growing *Miscanthus* can be supplied by stored N belowground in rhizomes or root biomass, from mineralized N released from large organic N pools, or the recently hypothesized biological $N_2$ fixation by bacteria hosted within *Miscanthus* (Davis et al. 2010). *Miscanthus* has typically not been found to respond to fertilization with N (Heaton et al. 2010). One possibility for supplying enough N for growth, therefore, is that recently added organic N to surface soils supporting *Miscanthus* could have greater mineralization rates, providing inorganic N.

Given the possible importance of soil N mineralization rates to biofuel production and the need for new biofuel crops, our objective was to evaluate a range of N-mineralization indices for soils with established *Miscanthus* and switchgrass biofuel crops and compare these results with soils in a traditional corn and soybean rotation. The hypothesis we were testing was that cultivation of *Miscanthus* and switchgrass for 5 to 6 years has changed the surface soil organic-matter quality, increasing mineralization of inorganic N.

## Materials and Methods

### *Site Description*

Soil samples were collected from field plots in early April 2010 at the Crop Science Research and Education Center (South Farms) located south of the University of Illinois at Urbana–Champaign (88.23° W, 40.08° N). These soils are flat (<2% slope), deep loess Mollisols with 4 to 7% organic matter, and they include the soil series Drummer silty clay loam (fine-silty, mixed, superactive, mesic Typic Endoaquolls) and Elburn silt loam (fine-silty, superactive, mesic Aquic Argiudolls). The 1971 to 2000 average or "normal"

annual temperature was 10.8 °C with an average precipitation of 1040 mm. The soils have poor natural drainage but are highly productive when tile drained, as these plots were. The site was arranged in six blocks, with each block having *Miscanthus × giganteus*, switchgrass (Cave-In-Rock variety), and corn–soybean rotation plots. Each plot measured 30.5 m × 61 m (0.19 ha) in area. Within these six blocks three *Miscanthus*, three switchgrass, and three corn–soybean plots were randomly selected for soil sampling. The switchgrass (established 2004) and *Miscanthus* plots (established 2005) had not been fertilized since establishment. The corn–soybean plot was sampled following a corn crop in 2009 (approximately 11 months after being fertilized at 168 kg N ha$^{-1}$). Row crops were harvested for grain, with residues returned to the soil with chisel plowing each fall. Switchgrass and *Miscanthus* were mechanically harvested in the winter when the ground was frozen, with all harvested biomass removed from the plots. More details on the plots can be found in Dohleman and Long (2009) and McIsaac, David, and Mitchell (2010).

### *Field Sampling and Soil Preparation*

Within the nine plots selected for sampling, three composite soil samples were taken diagonally across the plot, sampling the northeast corner, the center, and the southwest corner of each plot. A soil corer with a 1.9-cm diameter was used for sampling the 0- to 10-cm depth. Samples were composited by collecting seven soil cores and combining them into one sample bag. After collection, the samples were returned to the laboratory where they were well mixed and immediately put through a 2-mm sieve (fresh sample). Subsamples were oven dried at 60 °C. The dried subsamples were ground and sieved again through a 2-mm mesh size.

### *Analytical Procedures*

Extractable inorganic N was measured on field-moist samples immediately after sieving and root removal. Approximately 8 g of each field-moist sample was extracted with 40 mL of 2 M KCl following Mulvaney (1996). The extracts were frozen until analyzed for ammonium N (NH$_4$-N) and nitrate N (NO$_3$-N) colorimetrically on a Lachat Quikchem FIA 8000 series (Lachat Instuments, Milwaukee, Wisc.).

A 7-day anaerobic incubation (ActiveN) was conducted following Drinkwater, Cambardella, and Rice (1996). Approximately 8 g of field-moist soil was measured into 50-mL centrifuge tubes, along with 10 mL distilled, deionized water. The samples were purged with N gas for 1 min. The caps to the centrifuge tubes were immediately sealed and electrical tape was applied to ensure that the cap and tube had an airtight seal. The samples were placed into an incubator at 30 °C for 7 days. After the 7 days, the samples were removed from the incubator, and 30 ml of 2.67 M KCl was added to each centrifuge tube. The samples were shaken, extracted, and frozen until analyzed as previously described in the extractable KCl protocol. The total amount of N mineralized was calculated by subtracting the initial KCl NH$_4$-N. We also followed the more traditional 7-day anaerobic N (AnaN) procedure of Kenney and Bremner (1966), using 5 g of air-dried soil incubated at 40 °C.

To help determine short-term potential mineralization rates, a 24-day incubation (24dN) was completed following the protocol of Franzluebbers et al. (2000). Approximately 25 g of dried soil was placed in a 60-ml test tube. Distilled, deionized water was then added to reach 50% water-filled pore space. This was calculated assuming a soil particle density of 2.65 g cm$^{-3}$. The test tubes were placed in 1-L Mason jars along with a 10-mL vial of water to ensure humidity remained constant in the jar. The jars were

sealed and placed in an incubator at 25 °C for 24 days. The jars were opened every 3 days to allow for a sufficient quantity of oxygen to remain constant. After 24 days, the sample was removed from the test tube and placed into a 250-mL bottle. The new weight was recorded for later calculations of N mineralization. To each bottle, 125 mL of 1 M KCl was added and the bottles were placed horizontally onto a reciprocating shaker for 1 h. The samples were removed and the soil was given time to settle out from the solution. The supernatant KCl solution was filtered through Whatman GF/F filters (0.7 µm). The samples were frozen until analyzed for $NH_4$-N and $NO_3$-N, and the amount of N mineralized was calculated by subtracting initial extractable $NH_4$-N and $NO_3$-N.

The Illinois soil nitrogen test (ISNT) was also used to calculate potentially mineralizable N for the samples. The procedure and equipment for the ISNT followed Khan, Mulvaney, and Hoeft (2001), where 1 g of dried soil was placed in a 1-L Mason jar. A Petri dish was attached to each lid apparatus and 5 mL of boric acid indicator solution was pipetted into the Petri dish. The samples were treated with 2 M sodium hydroxide and gently swirled. The lid apparatus was placed on the jar and sealed with a screw band top, and the jar was then placed on a hot plate that had been preheated to 48–50 °C. After 5 h the jar was taken off the hot plate, and the Petri dish was removed. The boric acid indicator solution was then diluted with 5 ml of deionized water before being manually titrated with 0.01 M sulfuric acid ($H_2SO_4$). The endpoint was determined on the basis of color. The potentially mineralizable N was calculated in mg N kg$^{-1}$ by multiplying the milliliters of $H_2SO_4$ titrated by 280 µg N mL$^{-1}$, the titer of 0.001 M $H_2SO_4$.

To determine hot extractable N, 3 ml of previously described dried and sieved soil was weighed into Taylor tubes, and 20 mL of 2M KCl was added to the soil, as described by Gianello and Bremner (1986). Rubber stoppers were placed on the tubes, and the samples were placed on an incubating block for 4 h at 100 °C. After cooling to room temperature the samples were filtered through Whatman GF/F filters. The samples were then frozen until they were analyzed for $NH_4$-N colorimetrically on the Lachat. Hydrolyzable N (HydN) was calculated by subtracting the initial KCl–extractable $NH_4$-N from the $NH_4$-N released during heating.

Total C and total N, soil moisture, and soil pH were analyzed for each soil sample. Soil moisture was calculated by weighing approximately 25 g of fresh soil into beakers. Samples were placed in an oven at 105 °C for 48 h. The soil was weighed after drying and dry mass percentage was calculated. Total C and N were determined using an Elemental Analyzer (EAS 4010, Costech Analytical Technologies, Valencia, Calif.). Soil pH was measured for all samples with a glass electrode (Orion: model 250A; Orion, Espoo, Finland) using a 1:2 ratio of soil to solution with distilled, deionized water on 2-mm sieved samples.

### Statistical Analysis

Relationships among mineralization indices were evaluated using Pearson's correlation coefficients. All soil measurements among treatments were evaluated by analysis of variance (ANOVA), with mean differences evaluated by least significance difference (LSD), with a significance of $P < 0.05$. SAS version 9.2 was used for all statistical analyses (SAS Institute, Cary, N.C.).

## Results and Discussion

Soils under the different biofuel and conventional crops were similar in pH as well as in organic matter measured as total C and N (Table 1). Soil pH was less than is typically found

**Table 1**
Soil characteristics by crop type (n = 9)

| Crop | pH | Total C (%) | Total N (%) | C/N ratio (mass/mass) |
|---|---|---|---|---|
| *Miscanthus* | 5.74*a* | 2.55*a* | 0.203*a* | 12.5*a* |
| Switchgrass | 5.72*a* | 2.21*b* | 0.177*b* | 12.5*a* |
| Corn–soybean | 5.53*b* | 2.39*ab* | 0.196*a* | 12.2*a* |

*Note.* Means by crop with the same letter are not significantly different from each other at $P = 0.05$.

in cultivated soils in this area, with pH varying from 5.53 to 5.74. Corn–soybean had a mean pH significantly less than *Miscanthus* and switchgrass. Total C and N concentrations were typical for a Mollisol (David et al. 2009), indicating a large amount of organic matter in these surface soils. The C/N ratios were all similar as well across treatments, with a value of about 12.5, again, typical for this soil type.

The initial KCl method measured the background concentrations of exchangeable $NH_4$-N and $NO_3$-N in soils sampled in the three different crops (Figure 1). Soils sampled from the *Miscanthus* plots had significantly greater $NH_4$-N compared to soils sampled from switchgrass and corn–soybean. Switchgrass was also found to have greater concentrations of $NH_4$-N compared to corn–soybean. However, the same trend was not seen in the $NO_3$-N concentrations. Corn–soybean plots were found to have the greatest $NO_3$-N followed by *Miscanthus* and switchgrass, both of which were significantly different. One note for these data is that all KCl-extractable inorganic N concentrations were quite low, $<4.5$ mg N $kg^{-1}$. McIsaac, David, and Mitchell (2010) measured inorganic N leaching at 50 cm in these plots during 2005 through 2009 and found much greater leaching of $NO_3$-N from the corn–soybean plots compared to *Miscanthus* and switchgrass. However, McIsaac, David, and Mitchell (2010) did find greater $NH_4$-N leaching under switchgrass compared to *Miscanthus* and corn–soybean, which were about the same.

A correlation analysis was used to examine relationships among the various soil measurements and N indices (Table 2). As expected, total C and N were strongly correlated, as
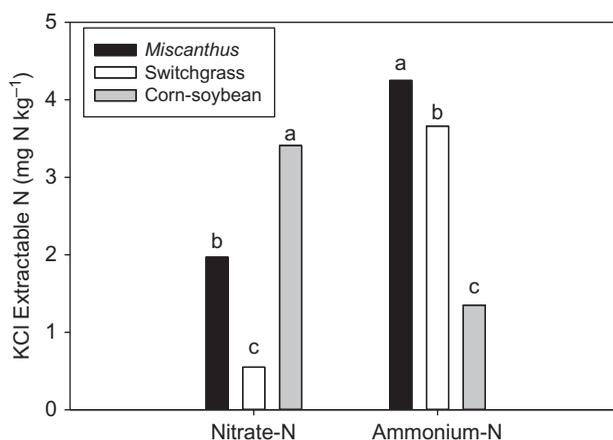


**Figure 1.** KCl-extractable $NO_3$-N and $NH_4$-N by crop type. Within a method, bars with different letters denote a significant difference at the 0.05 level as determined by LSD.

**Table 2**
Pearson correlation coefficients (r) for all soil variables (n = 27)

| Parameter | pH | Total C | Total N | KCl NH$_4$-N | KCl NO$_3$-N | ActiveN | AnaN | 24dN | HydN |
|---|---|---|---|---|---|---|---|---|---|
| Total C | 0.127 | | | | | | | | |
| Total N | 0.059 | 0.983* | | | | | | | |
| KCl NH$_4$-N | 0.518 | 0.260 | 0.178 | | | | | | |
| KCl NO$_3$-N | −0.262 | 0.345 | 0.416 | −0.188 | | | | | |
| ActiveN | 0.329 | 0.163 | 0.124 | 0.689* | −0.322 | | | | |
| AnaN | 0.331 | 0.683* | 0.647* | 0.731* | 0.122 | 0.627* | | | |
| 24dN | 0.111 | 0.333 | 0.349 | 0.443 | 0.177 | 0.676* | 0.613* | | |
| HydN | 0.035 | 0.056 | 0.089 | −0.176 | 0.026 | −0.094 | −0.039 | −0.058 | |
| ISNT | 0.015 | 0.833* | 0.868* | 0.188 | 0.441 | 0.128 | 0.565 | 0.373 | −0.084 |

*Significant correlation at $P < 0.001$.

both are a major component of soil organic matter. Other significant correlations included KCl-extractable $NH_4$-N with ActiveN and AnaN, and ISNT and AnaN with total C and total N. ActiveN was also correlated with AnaN. The 24dN was correlated with both active and AnaN, suggesting that these three indices were measuring a similar pool of soil N. Soil pH was not correlated with any of the other measurements. Two of the N indices (HydN, KCl $NO_3$-N) were not correlated with any other N indices, nor with total C and N. ISNT was correlated only with total C and N and was not correlated with any other N indices. Schomberg et al. (2009) found similar significant relationships among the indices we choose from their larger set, and correlations were likely enhanced by the wide range of soils they used (samples from across seven states).

There were significant differences among crop type for ActiveN, 24dN, AnaN, and ISNT (Figure 2). The soil N index with the greatest difference among the crops was AnaN. *Miscanthus* soils had the greatest concentration (141 mg N $kg^{-1}$), followed by switchgrass (124 mg N $kg^{-1}$) and corn–soybean (107 mg N $kg^{-1}$). *Miscanthus* soil was also found to have the greatest concentration of N in the 24dN procedure, which was significantly greater than switchgrass and corn–soybean. ISNT resulted in switchgrass having significantly lesser concentrations of N when compared to *Miscanthus* and corn–soybean. Finally, HydN was not different in soils among the three different biofuel crops.

Indices that used longer incubations (7 or 24 days) to estimate potential soil N mineralization supported our hypothesis. AnaN has been suggested as a good biological indicator of potentially available N (Bushong et al. 2007, 2008; Soon, Hadq, and Arshad 2007), and Schomberg et al. (2009) also found it to be highly correlated with N release from a long-term incubation. It was also correlated with total C and N and may be a good technique for evaluating potential mineralization differences in these soils.

Effects on soil organic matter from perennial biofuel production are not necessarily straightforward. Woli et al. (2010) used the same site as this work along with two others in Illinois and concluded that the biofuel crops had no effect on the mole fraction of $N_2O$ emissions from the soils, which is thought to be affected by soil organic-matter quality.

Our results suggest that the biofuel crops may have altered soil organic matter so that more N may mineralize from soil organic N pools in surface soils. McIsaac, David, and
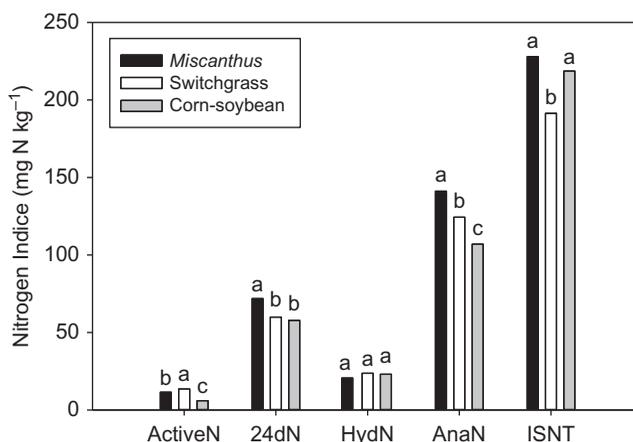


**Figure 2.** Comparison of N indices by crop type. Within an index, bars with different letters denote a significant difference at the 0.05 level as determined by LSD.

Mitchell (2010) found significantly less nitrate leaching from *Miscanthus* and switchgrass compared to a fertilized corn and unfertilized soybean rotation, demonstrating the ability of these perennial grasses to quickly take up mineralized N. Direct measurements of soil N mineralization (i.e., buried soil bags) could provide needed data to further explore possible N biogeochemical changes due to perennial biofuel production.

## Conclusions

We found that longer term N indices showed an increase in soil N-mineralization potential among biofuel crops in a central-Illinois Mollisol after 5 to 6 years of growth. The 7-day anaerobic incubation (AnaN) showed clear differences in mineralizable N, with *Miscanthus* having the greatest release, followed by switchgrass and then corn–soybean. The 24-day potentially mineralizable N (24dN) was also significantly greater in *Miscanthus* soils. Therefore, our hypothesis of this crop enriching soil organic matter, leading to greater N mineralization rates, was supported. AnaN has been suggested to be an excellent biological indicator of potentially available soil N, and our results found it to be most responsive to biofuel production.

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