2019

Contrasting soil nitrogen dynamics under Zea mays and Miscanthus × giganteus: A story of complex interactions among site, establishment year, and nitrogen fertilization

Jacob E. Studt
Iowa State University

Contrasting soil nitrogen dynamics under *Zea mays* and *Miscanthus* × *giganteus*:
A story of complex interactions among site, establishment year, and nitrogen fertilization

by

Jacob E. Studt

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Soil Science (Soil Fertility)

Program of Study Committee:
Marshall McDaniel, Co-major Professor
Emily Heaton, Co-major Professor
Michael Thompson

Iowa State University

Ames, Iowa

2019

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

I would like to thank my major professors, Marshall McDaniel and Emily Heaton, for the opportunity to conduct this research, their endless patience, and constant motivation. To Michael Thompson, my third committee member, I believe every conversation we had left me coming away with new, impactful, considerations and for that, thank you. To members of the Heaton lab; Nic Boersma, Mauri Tejera, Valeria Cano-Camacho, Collin De Graaf, Perla Carmenate and members of the McDaniel lab; Morgan Davis, Mriganka De, Rebecca Baldwin-Kordick, Teresa Middleton, Stephen Potter, and Mitchell Roush, thank you all for your support and assistance with many aspects of my research. Finally, I would like to thank my family and friends that have supported me endlessly throughout my journey at Iowa State. Without any of you the experience would not have been the same. Thank you!

ABSTRACT

Perennial cropping systems have been proposed as an alternative to conventional, annual, cropping systems to improve water quality by increasing nitrogen (N) retention in the plant and soil. In this study, I used a staggered-start experimental design to compare a perennial cropping system, miscanthus (*Miscanthus* × *giganteus* Greef et Deu.) at two stand-ages (mature - 3 years old), and juvenile (establishment or first year) with an annual cropping system, continuous corn (*Zea mays* L.), across two N fertility treatments of 0 and 224 kg N ha^{-1}. This experiment was replicated at two locations in Iowa, USA with similar soil parent material, but different background soil fertility due to past fertilizer management. I measured pools and processes associated with N cycling dynamics, including inorganic soil N, net N mineralization, and N leaching. Also measured were soil health indicators, including soil microbial biomass carbon (C) and N, and potentially mineralizable C and N. Measurements were taken at different frequencies over two years. One of the most salient findings in this study was mature miscanthus' ability to alter soil microclimate properties. Mature miscanthus increased soil temperature by 134% in the winter, and decreased it by 16% during the growing season, compared to continuous corn. Also, during the growing season juvenile miscanthus decreased soil moisture by 10% compared to continuous corn. Across both sites and all treatments net soil N mineralization showed large variability, but the juvenile miscanthus treatment, on average, had the greatest cumulative net N mineralization, and mature miscanthus the lowest. Across all sites and N rates, mature miscanthus reduced nitrate-N leaching by 64% compared to continuous corn. Juvenile miscanthus leached the same amount of nitrate-N as continuous corn. Since miscanthus changed soil microclimate properties and N dynamics compared to continuous corn, it was surprising to find very little effect of miscanthus on soil health indicators – microbial biomass or potentially mineralizable C and N. However, the soil aggregates (< 2 mm diameter) under mature miscanthus could hold 11% more water than that under continuous corn. This study suggests that integrating miscanthus into the Midwestern Corn Belt would substantially reduce N leached through the soil profile, potentially preventing it from being lost to surface or groundwater. Miscanthus shows the potential to provide farm income while reducing the impact of agriculture on water quality, and some signs of improving soil health. More research is needed on the underlying mechanisms driving the differences in soil N dynamics between miscanthus and corn.

CHAPTER 1. INTRODUCTION

When integrating a crop into a new landscape there are always questions focused on the economic and environmental benefit, or detriment, that are associated with the new crop. These questions can be economic, such as "*Can this crop make money when grown here?*" or environmental, such as "*How does cultivation and nitrogen fertilization of this crop affect water quality?*". In some cases, the questions from both the economical and the environmental point of view are overlapping. The two questions above are a perfect example of where a single body of research can help shed light on multiple critical agroecological questions. By understanding the soil-plant nitrogen (N) cycling dynamics of a crop we can better identify what causes an increase, or decrease, in nitrogen leaching, as well as how the differentiation in cycling dynamics may affect the need for costly N fertilization. For this study, the C_4 perennial grass miscanthus (*Miscanthus* × *giganteus* Greef et Deu.) is the crop being integrated; Iowa, U.S. is the landscape, and the question is, "*How and why does miscanthus affect water quality when grown in the Midwest?*".

Annual cropping systems with winter fallow periods leave large contiguous portions of the Midwest U.S. landscape barren, making soils susceptible to leaching and erosion (Lark et al., 2015). The combined lack of N uptake by plants during fallow times combined with highly fertile soils and N fertilization leads to large seasonal nitrate fluxes to rivers and groundwater. Nutrient loss from conventional arable crop production throughout the Midwest is a major contributor to hypoxia in the Gulf of Mexico (David et al., 2010). The nutrient transport from field to freshwater is often hastened and amplified by the use of subsurface tile drainage (David et al., 2010; Arenas Amado et al., 2017). There are many options for reducing nitrate leaching from agricultural fields, including better N fertilization practices as well as incorporating edge of field practices, e.g., saturated buffer strips or denitrifying bioreactors (Randall and Sawyer, 2008; Schipper et al., 2010; Jaynes and Isenhart, 2014). Among the most promising option to reduce nitrate is the conversion of entire fields, or portions thereof, to perennial cropping systems to eliminate bare soil over winter and early spring (Iowa Nutrient Reduction Strategy, 2013).

Miscanthus is an increasingly common perennial biomass crop. Besides potentially reducing nitrate leaching, miscanthus has high biomass yields (e.g., 12-40 Mg ha^{-1}) and low input requirements (Christian et al., 1997; Christian et al., 2008; Heaton et al., 2008; Smith et al., 2013). Miscanthus shows potential to reduce nitrate leaching from agricultural systems by about 90% when compared to traditional annual cropping systems (McIsaac et al., 2010; Smith et al., 2013). To date, N fertilization does not consistently increase miscanthus yields: some studies show yield response to N fertilization (Arundale et al., 2014; Shield et al., 2014), while others do not (Christian et al., 2008; Davis et al., 2015; Haines et al., 2015).

The reasons for miscanthus' ability to maintain high yields without N fertilization are poorly understood. When estimating a N budget for miscanthus there are suggestions of a missing N source (Davis et al., 2010; Dohleman et al., 2012). Endophytic or soil-dwelling N-fixing bacteria may be one source of this missing N (Eckert et al., 2001; Davis et al., 2010; Keymer and Kent, 2014). Others have found more complex explanations, however; one plausible explanation is that miscanthus increases soil's potential to mineralize N (Davis et al., 2013; Davis et al., 2015), thereby meeting N demands with inorganic N liberated from soil organic matter in the rich agricultural soils where much of miscanthus research has been conducted. The mechanism by which miscanthus might increase plant-available N from soil organic N could be by either increasing total soil organic matter (Beuch et al., 2000; Kahle et al., 2001; Foereid et al., 2004), or by priming microbial activity and releasing N from organic forms (Zhu et al., 2014). An increase in net N mineralization, combined with the ability to translocate N from above to belowground organs prior to harvest (Strullu et al., 2011), could explain why miscanthus can continually produce high yields with little need for N fertilization.

The aim of this research was to study the N pools, fluxes, and mechanisms behind miscanthus' potential to efficiently cycle N and reduce N leaching in Iowa, U.S., in the heart of the Midwest Corn Belt. With nearly 70% of its land area in row crops (USDA-NASS), Iowa is characterized by both high crop productivity and high rates of nutrient leaching (Iowa Nutrient Reduction Strategy, 2013); miscanthus could help mitigate the nutrient leaching. However, since perennial crops typically take multiple years to reach maturity, there is possibility for higher N leaching early in miscanthus' establishment (Christian and Riche, 1998; Smith et al., 2013). Tejera et al. (2019) a staggered-start field trial to find that newly planted miscanthus crops produce only about 30% of the biomass of mature stands. To understand how miscanthus development and N fertilization rates affect miscanthus soil N cycling dynamics we used the same field trial as Tejera et al. (2019) to conduct an experiment at two sites in Iowa with similar soil types, but differing management history and climate.

The following document includes two major data chapters. Chapter Two focuses on plant-available N, net N mineralization, and inorganic N leaching, in corn (*Zea mays* L.) as well as two stand ages of miscanthus. Chapter Three evaluates the mass of carbon (C) and N in soil microbial biomass and a lab incubation to determine the soil's potential to mineralize C and N in corn and a mature stand of miscanthus. Chapter Four is a conclusion that summarizes the overall impact of this research and discusses future research opportunities.

1.1 References

Arenas Amado, A., K.E. Schilling, C.S. Jones, N. Thomas, and L.J. Weber. 2017. Estimation of tile drainage contribution to streamflow and nutrient loads at the watershed scale based on continuously monitored data. Environ. Monit. Assess. 189(9): 426. doi: 10.1007/s10661-017-6139-4.

Arundale, R.A., F.G. Dohleman, T.B. Voigt, and S.P. Long. 2014. Nitrogen fertilization does significantly increase yields of stands of Miscanthus× giganteus and Panicum virgatum in multiyear trials in Illinois. BioEnergy Res. 7(1): 408–416.

Beuch, S., B. Boelcke, and L. Belau. 2000. Effect of the organic residues of Miscanthus× giganteus on the soil organic matter level of arable soils. J. Agron. Crop Sci. 184(2): 111–120.

Christian, D.G., P.R. Poulton, A.B. Riche, and N.E. Yates. 1997. The recovery of 15N-labelled fertilizer applied to Miscanthus× giganteus. Biomass and Bioenergy 12(1): 21–24.

Christian, D.G., and A.B. Riche. 1998. Nitrate leaching losses under Miscanthus grass planted on a silty clay loam soil. Soil Use Manag. 14(3): 131–135.

Christian, D.G., A.B. Riche, and N.E. Yates. 2008. Growth, yield and mineral content of Miscanthus× giganteus grown as a biofuel for 14 successive harvests. Ind. Crops Prod. 28(3): 320–327.

David, M.B., L.E. Drinkwater, and G.F. McIsaac. 2010. Sources of Nitrate Yields in the Mississippi River Basin. J. Environ. Qual. 39: 1657–1667. doi: 10.2134/jeq2010.0115.

Davis, M.P., M.B. David, and C.A. Mitchell. 2013. Nitrogen Mineralization in Soils Used for Biofuel Crops. Commun. Soil Sci. Plant Anal. 44(5): 987–995. doi: 10.1080/00103624.2012.747607.

Davis, M.P., M.B. David, T.B. Voigt, and C.A. Mitchell. 2015. Effect of nitrogen addition on Miscanthus× giganteus yield, nitrogen losses, and soil organic matter across five sites. Gcb Bioenergy 7(6): 1222–1231.

Davis, S.C., W.J. Parton, F.G. Dohleman, C.M. Smith, S. Del Grosso, et al. 2010. Comparative biogeochemical cycles of bioenergy crops reveal nitrogen-fixation and low greenhouse gas emissions in a Miscanthus× giganteus agro-ecosystem. Ecosystems 13(1): 144–156.

Dohleman, F.G., E.A. Heaton, R.A. Arundale, and S.P. Long. 2012. Seasonal dynamics of above-and below-ground biomass and nitrogen partitioning in M iscanthus× giganteus and P anicum virgatum across three growing seasons. Gcb Bioenergy 4(5): 534–544.

Eckert, B., O.B. Weber, G. Kirchhof, A. Halbritter, M. Stoffels, et al. 2001. Azospirillum doebereinerae sp. nov., a nitrogen-fixing bacterium associated with the C4-grass Miscanthus. Int. J. Syst. Evol. Microbiol. 51(1): 17–26.

Foereid, B., A. de Neergaard, and H. Høgh-Jensen. 2004. Turnover of organic matter in a Miscanthus field: effect of time in Miscanthus cultivation and inorganic nitrogen supply. Soil Biol. Biochem. 36(7): 1075–1085.

Haines, S.A., R.J. Gehl, J.L. Havlin, and T.G. Ranney. 2015. Nitrogen and phosphorus fertilizer effects on establishment of giant Miscanthus. BioEnergy Res. 8(1): 17–27.

Heaton, E.A., F.G. Dohleman, and S.P. Long. 2008. Meeting US biofuel goals with less land: The potential of Miscanthus. Glob. Chang. Biol. 14(9): 2000–2014. doi: 10.1111/j.1365-2486.2008.01662.x.

Iowa Nutrient Reduction Strategy. 2013. A science and technology-based framework to assess and reduce nutrients to Iowa waters and the Gulf of Mexico. Iowa Dep. Agric. L. Steward. Iowa Dep. Nat. Resour. Iowa State Univ. Coll. Agric. Life Sci. Ames, IA.

Jaynes, D.B., and T.M. Isenhart. 2014. Reconnecting Tile Drainage to Riparian Buffer Hydrology for Enhanced Nitrate Removal. J. Environ. Qual. 43: 631–638. doi: 10.2134/jeq2013.08.0331.

Kahle, P., S. Beuch, B. Boelcke, P. Leinweber, and H.-R. Schulten. 2001. Cropping of Miscanthus in Central Europe: biomass production and influence on nutrients and soil organic matter. Eur. J. Agron. 15(3): 171–184.

Keymer, D.P., and A.D. Kent. 2014. Contribution of nitrogen fixation to first year Miscanthus× giganteus. Gcb Bioenergy 6(5): 577–586.

Lark, T.J., J.M. Salmon, and H.K. Gibbs. 2015. Cropland expansion outpaces agricultural and biofuel policies in the United States. Environ. Res. Lett. 10(4): 44003.

McIsaac, G.F., M.B. David, and C.A.M.U. of Illinois. 2010. and Switchgrass Production in Central Illinois: Impacts on Hydrology and Inorganic Nitrogen Leaching. J. Environ. Qual. 39(5): 1790. doi: 10.2134/jeq2009.0497.

Randall, G.W., and J.E. Sawyer. 2008. Nitrogen Application Timing, Forms, and Additives. Final Rep. Gulf Hypoxia Local Water Qual. Concerns Work. St. Joseph, Michigan ASABE: 73–85.

Schipper, L.A., W.D. Robertson, A.J. Gold, D.B. Jaynes, and S.C. Cameron. 2010. Denitrifying bioreactors—An approach for reducing nitrate loads to receiving waters. Ecol. Eng. 36(11): 1532–1543. doi: https://doi.org/10.1016/j.ecoleng.2010.04.008.

Shield, I.F., T.J.P. Barraclough, A.B. Riche, and N.E. Yates. 2014. The yield and quality response of the energy grass Miscanthus× giganteus to fertiliser applications of nitrogen, potassium and sulphur. Biomass and Bioenergy 68: 185–194.

Smith, C.M., M.B. David, C.A. Mitchell, M.D. Masters, K.J. Anderson-Teixeira, et al. 2013. Reduced nitrogen losses after conversion of row crop agriculture to perennial biofuel crops. J. Environ. Qual. 42(1): 219–228.

Strullu, L., S. Cadoux, M. Preudhomme, M.-H. Jeuffroy, and N. Beaudoin. 2011. Biomass production and nitrogen accumulation and remobilisation by Miscanthus× giganteus as influenced by nitrogen stocks in belowground organs. F. Crop. Res. 121(3): 381–391.

Tejera, M., N. Boersma, A. Vanloocke, S. Archontoulis, P. Dixon, et al. 2019. Multi-year and Multi-site Establishment of the Perennial Biomass Crop Miscanthus× giganteus Using a Staggered Start Design to Elucidate N Response. BioEnergy Res.: 1–13.

Zhu, B., J.L.M. Gutknecht, D.J. Herman, D.C. Keck, M.K. Firestone, et al. 2014. Rhizosphere priming effects on soil carbon and nitrogen mineralization. Soil Biol. Biochem. 76: 183–192.

CHAPTER 2. THE INFLUENCE OF NITRGOEN MINERALIZATION AND PLANT AVAILABLE NITROGEN ON LEACHING UNDER *MISCANTHUS* × *GIGANTEUS*

2.1 Introduction

Annual cropping systems, with winter fallow periods, leave large contiguous portions of the Midwest, U.S. landscape barren and susceptible to leaching and erosion. The combined lack of nitrogen (N) uptake by plants during fallow times combined with highly fertile soils and N fertilization leads to large seasonal nitrate fluxes to rivers and groundwater. Nutrient loss from conventional arable crop production throughout the Midwest is a major contributor to hypoxia in the Gulf of Mexico (David et al., 2010). The quantity of nutrient transport from field to freshwater is often hastened and amplified by the use of subsurface tile drainage, which is also common in the area (David et al., 2010; Arenas Amado et al., 2017).

There are many options for reducing N leaching from agricultural fields including better N fertilization practices (Randall and Sawyer, 2008), incorporating edge of field practices like saturated buffer strips or denitrifying bioreactors (Schipper et al., 2010; Jaynes and Isenhart, 2014). Among the most promising is the conversion of entire fields, or portions thereof, to perennial instead of annual crops to eliminate bare soil over winter and early spring (Iowa Nutrient Reduction Strategy, 2013).

Miscanthus (*Miscanthus* × *giganteus* Greef et Deu.) is an increasingly common perennial biomass crop. Besides potentially reducing nitrate leaching, miscanthus has high biomass yields (e.g., 12-40 Mg ha^{-1}) and low input requirements (Christian et al., 1997; Christian et al., 2008; Heaton et al., 2008; Smith et al., 2013). Miscanthus shows potential to reduce nitrate leaching from agricultural systems by about 90% when compared to traditional annual cropping systems (McIsaac et al., 2010; Smith et al., 2013). To date, nitrogen fertilizer does not consistently show to increase miscanthus yields. Some studies show yield response to N fertilization (Arundale et al., 2014; Shield et al., 2014), while others do not (Christian et al., 2008; Davis et al., 2015; Haines et al., 2015). Why is it that miscanthus doesn't always respond to N fertilization, but is almost always capably of high yields?

The reasons miscanthus can maintain high yields without N fertilization are poorly understood. For example, N budget estimates for miscanthus suggest a missing N source (Davis et al., 2010; Dohleman et al., 2012). Endophytic or soil-dwelling N-fixing bacteria may be one source of this missing N (Eckert et al., 2001; Davis et al., 2010; Keymer and Kent, 2014). Others have found more complex explanations, however; and one plausible explanation is that miscanthus increases potentially mineralizable N (Davis et al., 2013; Davis et al., 2015), thereby meeting N demands with inorganic N liberated from soil organic matter. The mechanism by which miscanthus might increase plant-available N from soil organic matter could be by either increasing total soil organic matter (Beuch et al., 2000; Kahle et al., 2001; Foereid et al., 2004), by priming microbial activity and releasing N from organic forms (Zhu et al., 2014), or both. An increase in gross N mineralization (N_{min}), combined with the ability to translocate N from above to belowground organs prior to harvest (Strullu et al., 2011), could also explain why miscanthus can continually produce high yields with little need for N fertilization.

With nearly 70% of its land area in row crops (USDA-NASS), Iowa is characterized by both high crop productivity and high rates of nutrient leaching (Iowa Nutrient Reduction Strategy, 2013). Miscanthus could help mitigate the high nutrient leaching. However, since perennial crops typically take multiple years to establish, this may mean greater N leaching than annual crops during this early establishment phase – typically considered to be 3-4 years for miscanthus (Christian and Riche, 1998; Smith et al., 2013). For instance, Tejera et al. (2019)

found that miscanthus, in its first year, produces only about 30% of aboveground biomass of a mature stand. To understand how miscanthus development and N fertilization rates affect miscanthus soil N dynamics, we used the infrastructure developed by Tejera et al. (2019) to conduct an experiment at two sites in Iowa, U.S. with similar soils, but differing management history and climate. We measured nitrate leaching, plant-available N, and net N_{min} under corn, juvenile miscanthus, and mature miscanthus. We hypothesized: (i) nitrate leaching would be greatest in juvenile miscanthus > corn > mature miscanthus, and (ii) net N_{min} would be greatest in mature miscanthus > corn > juvenile miscanthus. Accordingly, we expected an active but tight N cycle in mature miscanthus. Or in other words, that nitrate leaching would be lowest under mature miscanthus, but that age would also have the greatest net N_{min} rates.

2.2 Methods

2.2.1 Site description and experimental design

Field sites were located in Northwest (NW) Iowa, USA (42.586, -95.012) and Central Iowa, USA (42.013, -93.743). These sites had predominately < 2% slope and had similar soil characteristics but differed in previous management (Table 1 and 2). The NW site had been in corn-soy rotation with annual additions of cattle manure, while the Central site had been in a corn-soy rotation, with parts in perennial grassland; receiving only inorganic fertilizers. The manure caused notably higher phosphorus and potassium concentrations (Table 1).

This study used a subset of plots from a larger staggered-start experiment comparing corn (*Zea mays* L.) and different-aged miscanthus over five N fertilization rates (0, 112, 224, 336, 448 kg N ha^{-1}) (Tejera et al., 2019). Full details of that experiment and its management can be found elsewhere (Tejera et al., 2019). Briefly, the experiment was established in 2015 using a split-plot, randomized complete block design with four replications of miscanthus with adjacent corn check plots that were not randomized. That is, crop was randomized, and N fertilization rate was randomized within miscanthus, but the corn check plots adjacent to the miscanthus plots received the same N rate. Main plots were 24 m by 120 m, with split-plots 24 m by 12 m. Main-plot treatment levels were three planting years (2015, 2016, and 2017) and split-plot treatments were five N fertilization rates (0, 112, 224, 336, and 448 kg N ha^{-1}). Of the main-plot treatments, we only consider here the 2015 (mature miscanthus) and 2017 (juvenile miscanthus) planting years and of the split-plot treatments we consider only the 0 and 224 kg N ha^{-1}; as well as the adjacent 0 and 224 kg N ha^{-1} corn plots. Fertilizer N was applied as banded urea ammonium nitrate (UAN) behind coulter wheel. This resulted in 4 replications × 3 cropping systems × 2 N fertilization rates = 24 experimental units at each the NW and Central sites.

2.2.2 Soil sampling, net N mineralization, and bulk density measurements

Soil samples and soil temperatures were taken approximately monthly over the 2017 and 2018 growing seasons, ranging from May-December. At each sampling event, soil temperature (10 cm) was randomly taken four times per plot with a digital stem thermometer. Ten soil cores (1.75 cm diameter, 15 cm deep) were randomly taken from each plot and thoroughly homogenized for a composite sample. The composite sample was then split, with approximately half designated as initial samples (see below) and placed in a cooler with icepacks for transport to the lab. The other half of the samples were placed in 2.4-mil thick polyethylene bags then buried to a depth of 15 cm to assess net N_{min}.

An in-situ, sequential buried bag method was used to measure net N_{min}. The bags of soil remained buried to incubate for approximately one month before they were replaced during the subsequent sampling event, apart from the bags left to incubate over winter. Once the soils were returned to the lab, whether initial or incubated, they were stored at 4 °C for up to one week.

Soils were sieved (< 2 mm at field moisture), a sub-sample taken and used to determine gravimetric water content, and a separate 5 g of field moist soil was extracted with 25 ml of 2 M KCl for ammonium and nitrate. The remaining soil was set aside to air dry for later analysis of pH, total carbon, and total nitrogen.

Soil bulk density was measured in each Central Iowa plot. Three 5.1-cm diameter cores were taken to a depth of 15 cm. The cores were set aside to air dry followed by sieving through an 0.8-cm sieve to remove rocks and rhizomes. After sieving, the samples were oven dried for 48 hours at 105 °C, and their mass recorded. The mass and volume of all rocks and rhizomes were subtracted from the mass and volume of their respective core for corrections to bulk density. The mass of rocks and rhizomes were measured once oven dried and the corresponding volumes were measured by water displacement.

2.2.3 Measures of inorganic nitrogen leaching – resin and suction lysimeters

To understand how much dissolved nitrate and ammonium was available for leaching to ground water, two resin lysimeters (Susfalk and Johnson, 2002) were installed at a depth of 50 cm in each plot following Davis et al. (2015). The lysimeters consisted of 25 g of ion exchange resin held in place by 153 µm nylon mesh. Above and below the resin a sand layer was used to improve hydraulic connectivity between the resin and soil. The lysimeters were buried in the spring of 2017 and removed approximately a year later in the spring of 2018. To avoid any preferential flow of water, the lysimeters were buried at least 10 cm in side tunnels from the original hole so they would be under an undisturbed soil profile. To extract ammonium and nitrate the resin was agitated for one hour in 200 mL of 2 M KCl.

Soil water samples were collected throughout both growing seasons at the Central Iowa site from the 0 kg N ha^{-1} plots and 336 kg N ha^{-1} using porous cup suction lysimeters installed to a depth of 50 cm. Suction lysimeters were not available in the 224 kg N ha^{-1} or juvenile plots so the 0 and 336 kg N ha^{-1} plots were used in both the corn and mature miscanthus. This is the sole measurement taken from plots other than the ones specified in the site description. To collect the water samples, -50 kPa of vacuum was applied to the lysimeter which were left to draw water into the porous cup for approximately 24 hours. The water samples were pumped out of the lysimeter into bottles that were immediately placed on ice until refrigerated at 4 °C.

2.2.4 Chemical analyses (inorganic N, pH, total C and N)

Extractions from soil samples (initial and incubated), resin beads (lysimeters), and suction lysimeter water samples were analyzed for ammonium using salicylate and ammonia cyanurate reagent packets (Hach Company, Loveland, Colorado, USA), and for nitrate using the single-reagent method (vanadium III, sulfanilamide and *N*-(1-naphthyl)-ethylenediamine dihydrochloride) (Doane and Horwath, 2003). Both ammonium and nitrate extracts were then analyzed using a Synergy™ HTX Multi-Mode Microplate Reader (BioTek Instruments, Winooski, VT, USA) with Gen5™ software. Net N_{min} was calculated by subtracting the initial salt-extractable N (ammonium plus nitrate) values from the post-incubation salt-extractable inorganic N. An annual cumulative net N_{min} value was calculated by summing net N_{min} values over the year.

Soil pH was measured using an HQ430D Laboratory Single Input pH glass electrode probe in a 1:1 soil:water slurry, using deionized water, while being stirred. Total soil C and N were measured by first ball milling soil to a fine powder followed by oven drying at 105 °C for 48 hours. Once oven dried, 5 g of soil was combined with equal parts tungsten oxide catalyst for combustion using an Elementar vario MACRO to provide total soil C and N values.

2.2.5 Statistical analysis

All data was tested for homogeneity of variances and normality. All data met ANOVA assumptions. Statistical analyses were performed using linear mixed models with N, cropping system, and site as fixed effects. For statistical analysis, we considered mature and juvenile miscanthus to be individual cropping systems, resulting in three separate ‘cropping systems’; corn, juvenile miscanthus, and mature miscanthus. A repeated measures ANOVA was used for all data in which sampling occurred at least three times throughout the growing season or if sampling was repeated across 2017 and 2018 growing seasons. All repeated measures ANOVAs were tested for best-fit variance-covariance structure. Using SAS 9.4 differences in means at a probability level of $p < 0.05$ were considered significant.

2.3 Results

2.3.1 Climate, soil temperature, and moisture

In 2017, precipitation at both sites was well below mean annual precipitation: NW received 748 mm and Central received 756 mm (Table 1, Fig. 1). In 2018, the NW site received close to average rainfall, receiving 930 mm while the Central site had a wetter than normal year receiving 1264 mm. Mature miscanthus decreased soil temperatures during the growing season by 16% and increased late fall soil temperatures by 134% compared to corn (Fig. 2). Juvenile miscanthus plots had 10-11% drier soil compared to corn or mature miscanthus, calculated using mean gravimetric water content (Fig. 3). At the NW site, soil in the mature stands had the lowest water content through much of the dry 2017 growing season, but when there was closer to average precipitation, during the 2018 growing season, the mature stands had the highest water content.

2.3.2 Soil inorganic nitrogen

In the unfertilized cropping systems nitrate concentrations ranged from 2 to 40 kg N ha^{-1} and ammonium ranged from 0 to 9 kg N ha^{-1} (Fig. 4 and 5). The fertilized cropping systems had nitrate concentrations ranging from 6 to 101 kg N ha^{-1} and ammonium ranging from 0 to 50 kg N ha^{-1}. The greatest concentrations of soil inorganic N followed N application in the spring. These concentrations were most notable in the corn plots in 2018 and in 2017 at the Central site. The concentrations fell back to expected ranges by the following sampling date. The mature miscanthus at the NW site had high inorganic N concentrations following N fertilization in the spring, but unlike the corn, the N concentration did not return to expected values in the subsequent sampling event.

During both growing seasons, unfertilized mature miscanthus had the lowest nitrate concentrations, never exceeding 20 kg N ha^{-1} (Fig. 4). In the fertilized treatments, mature miscanthus at the Central site had the lowest inorganic N, but at the NW site had the highest inorganic N. In 2017, there was a steady increase in nitrate in the unfertilized juvenile miscanthus with the highest nitrate observed at both sites in August. In the fertilized juvenile miscanthus at the Central site there were high concentrations of plant-available N exceeding 150 kg N ha^{-1} with a large portion of the N being in ammonium form (30 kg NH_4^+-N ha^{-1}) (Fig. 5).

2.3.3 Net N mineralization

Unfertilized soils nearly always mineralized more N than was immobilized, resulting in net N_{min} rates ranging from -0.1 to 1.0 kg N ha^{-1} d^{-1} across all cropping treatments (Fig. 6 and 7). There were two instances where specific treatments and sites showed net immobilization of N: corn in late summer 2018 and miscanthus in the winter between 2017 and 2018. When summed for annual cumulative net N_{min} rates, all sites showed positive mean net N_{min} except for corn at the Central site due to late summer minor immobilization events. Between the two sites, among all unfertilized treatments, cumulative net N_{min} was not significantly different within the same growing season or site (Table 3), although mature miscanthus tended to be lower than juvenile miscanthus and corn most years.

Amongst soils receiving N fertilizer, there were greater extremes of both net mineralization and immobilization, and particularly in 2017. Net N_{min} rates ranged from -3.6 to 2.6 kg N ha^{-1} d^{-1} across all fertilized plots (Fig. 8 and 9). Strong immobilization events of -2.9 and -3.6 kg N ha^{-1} d^{-1} occurred at the NW site in mature miscanthus and Central site in juvenile miscanthus, respectively, during 2017. Also, immobilization events of -2.5 kg N ha^{-1} d^{-1} occurred at Central site in corn during 2018. When summed for annual cumulative net N_{min}, each cropping system had one site-year in which net immobilization occurred. Among all fertilized treatments, cumulative net N_{min} had significant interactions between years, cropping systems, and sites (Table 3). Juvenile miscanthus had the greatest average net N_{min} in three of the four site-years.

2.3.4 Inorganic nitrogen leaching

Across all treatments and both sites, the amount of soil nitrate that leached past 50 cm in 2017 was highly variable. One salient, but unsurprising, trend that emerged across both sites was that the unfertilized treatments had 90 %, 59 %, and 74 % lower N leaching in mature miscanthus, juvenile miscanthus, and corn respectively (Fig. 10). Overall, fertilized corn had a significantly greater N leaching rate (135 kg ha^{-1} yr^{-1}) compared to fertilized mature miscanthus (55 kg ha^{-1} yr^{-1}, Table 4). Averaged across sites, in unfertilized plots, soils under mature miscanthus only leached 6 kg N ha^{-1} yr^{-1}, which was 86% and 73% less than juvenile miscanthus and corn respectively. Juvenile miscanthus leaching rates were not significantly different than corn, but tended to be lower at both sites when fertilizer was added, but greater (54%) than corn in the unfertilized plots only at the Northwest site.

2.4 Discussion

Our main objective was to shed light on the soil N dynamics under miscanthus, but particularly to compare early and later stages of establishment (i.e., juvenile and mature). Changing portions of, or entire, fields to a perennial crop will take a shift in the traditional cropping system paradigm. Here we show that one perennial crop can alter soil conditions just after one to three years after establishment. Soil temperature and moisture are most drastically different when comparing juvenile or mature miscanthus to corn. This alteration of soil microclimate is an undeniably large driver of observed soil N dynamics. However, other mechanisms changing the soil N dynamics appear to be operating in our Midwestern U.S. soils, and will be discussed further. Our results clearly showed that miscanthus can reduce annual leaching when compared to row-crop systems, such as corn, but especially after three years.

2.4.1 Soil net nitrogen mineralization

Across all treatments, soils predominantly showed net N_{min} but especially soils receiving no N fertilization. However, there were instances of strong N immobilization in the fertilized treatments. In the absence of N fertilization juvenile miscanthus tended to mineralize the most N and mature miscanthus the least. The NW site tended to have greater cumulative net N_{min} values which is likely caused, in part, by past manure being mineralized (Lentz and Lehrsch, 2012).

In the unfertilized plots, the juvenile miscanthus had the greatest cumulative net N_{min} apart from the 2018 growing season at the NW site. In this case, all three cropping systems had similar cumulative N_{min}. With the lack of canopy or litter layer in the first few years of growth, the soil associated with juvenile miscanthus was exposed to greater amounts of solar radiation causing higher soil temperatures and lower water. The interaction between soil temperature and water content has shown to have significant effect on N_{min} (Cassman and Munns, 1980; Sierra, 1997). The high C:N ratio of miscanthus residue likely increased gross immobilization ultimately reducing net mineralization. Net N_{min} values from our corn treatments were similar to those found in other in-situ net N_{min} studies (Ma et al., 1999; Fernández et al., 2017).

In this study, we did not measure gross N ammonification, nitrification, or immobilization; instead we measured the net response of gross immobilization and gross mineralization. In forest soils net nitrification has shown to be a poor predictor of the gross nitrification that is occurring do to rapid assimilation of nitrate by microbes (Stark and Hart, 1997). With this in mind, there could have been an array of combinations of gross mineralization and gross immobilization that led to our net N_{min} measurements. It is likely that there are high amounts of gross mineralization in all these soils due to the high total soil N.

Net N_{min} varied drastically by site, year, and N fertilization. Net N_{min} can be influenced by many factors: weather conditions, N fertilization, crop residues, soil characteristics, soil temp, and soil moisture (Cassman and Munns, 1980; Sierra, 1997; Fernández et al., 2017). In this study, we saw a range of all these factors. Our 2017 and 2018 growing seasons had vastly different weather conditions and even within the same growing season, the two sites had different weather patterns. Our treatments included a wide range of N limitation conditions with no N fertilization for 3 years, 224 kg N ha^{-1} y^{-1} for 3 years, as well as a wide range of corn and miscanthus crop residues with likely varying C:N ratios. The immobilization in the fertilized mature miscanthus may be linked to labile C being leached into the soil profile from the high C:N residue decomposing at the surface. The final driving influence, soil characteristics, varied by site in part due to spatial variability, but also because of prior management.

2.4.2 Soil nitrogen leaching

We recorded N leaching 220-310% greater due to N fertilization rates of 224 kg N ha^{-1} y^{-1} at the Central and NW site, respectively. The increase of N leaching with N fertilization rates is well documented (Baker and Johnson, 1981; Jaynes et al., 2001). Even when no fertilizer is applied, it is common to see some N leaching due to net N_{min} with rates reported from 1 to 75 kg N ha^{-1} y^{-1} (Smith et al., 2013). We recorded reduced leaching from miscanthus even when N was applied at a rate likely exceeding any agronomic recommendation.

The fertilized plots in this experiment had 224 kg N ha^{-1} applied annually across multiple miscanthus stand ages and corn. This design differs from similar studies that use a recommended N fertilization rate on the corn (often lower rates than used here) that is different from the experimental N fertilization treatments applied to the miscanthus. Most other studies that have measured N leaching from miscanthus have not had N fertilization treatments greater than 120 kg N ha^{-1} (McIsaac et al., 2010; Behnke et al., 2012; Smith et al., 2013; Davis et al., 2015).

The N leaching fluxes from our unfertilized juvenile miscanthus plots averaged two to four times greater than what was reported in similar studies in unfertilized first-year miscanthus (McIsaac et al., 2010; Behnke et al., 2012; Davis et al., 2015). Smith et al. (2013) measured 79 % greater leaching fluxes from unfertilized first-year miscanthus, but cited poor establishment causing a lack of plant uptake to be the reason. The unfertilized mature miscanthus plots annually leached similar amounts of N as values reported by McIsaac et al. (2010) and Smith et al. (2013) in unfertilized miscanthus. At the Central site we recorded leaching fluxes from unfertilized corn greater than similar studies reported from fertilized corn (McIsaac et al., 2010; Smith et al., 2013). Our fertilized corn treatment leached approximately triple the value reported in similar studies (McIsaac et al., 2010; Smith et al., 2013)

The mechanistic reasons for high leaching from juvenile miscanthus and relatively low leaching while mature are due at least in part to the development of the root system and overall increase in belowground biomass. It is also possible that switching from an annual crop to a perennial grass could be influencing a wide range of soil properties and ultimately reducing N leaching. An increase in soil organic matter and soil microbial biomass are possible results when switching from corn to miscanthus that could be aiding in a tighter, less leaky, N cycle.

What we learned from this study is that planting miscanthus reduces N leaching by 64% in the third growing season, regardless of site and N rate. In other words, if a producer were to transition from corn to miscanthus, there would be a reduction in N leaching and improvement in water quality, but it would take up to the third growing season before this benefit could be realized.

2.5 Conclusion

Miscanthus alters soil conditions and N dynamics; and most importantly showed dramatic reductions in nitrate leaching (past 50 cm depth) across two sites and even with above-average N fertilizer rates. This evidence supports its use as a means of reducing N transport to subs-surface and surface waterways. Additional research is needed, however, to determine how miscanthus (particularly its belowground biomass) alters soils to shift N dynamics. It clearly is not only through changes in soil temperature and moisture. Equipped with a better understanding of how perennial crops, like miscanthus, alter soils, we can select appropriate crops for soil and climatic conditions that are prone to nitrate leaching. Choosing the appropriate soil-plant combination for economic and environmental benefits will be key to reducing Midwestern U.S. N loads to surface waters.

2.7 References

Arenas Amado, A., K.E. Schilling, C.S. Jones, N. Thomas, and L.J. Weber. 2017. Estimation of tile drainage contribution to streamflow and nutrient loads at the watershed scale based on continuously monitored data. Environ. Monit. Assess. 189(9): 426. doi: 10.1007/s10661-017-6139-4.

Arundale, R.A., F.G. Dohleman, T.B. Voigt, and S.P. Long. 2014. Nitrogen fertilization does significantly increase yields of stands of Miscanthus× giganteus and Panicum virgatum in multiyear trials in Illinois. BioEnergy Res. 7(1): 408–416.

Baker, J.L., and H.P. Johnson. 1981. Nitrate-Nitrogen in Tile Drainage as Affected by Fertilization1. J. Environ. Qual. 10: 519–522. doi: 10.2134/jeq1981.00472425001000040020x.

Behnke, G.D., M.B. David, and T.B. Voigt. 2012. Greenhouse Gas Emissions, Nitrate Leaching, and Biomass Yields from Production of Miscanthus × giganteus in Illinois, USA. Bioenergy Res. 5(4): 801–813. doi: 10.1007/s12155-012-9191-5.

Beuch, S., B. Boelcke, and L. Belau. 2000. Effect of the organic residues of Miscanthus× giganteus on the soil organic matter level of arable soils. J. Agron. Crop Sci. 184(2): 111–120.

Cassman, K.G., and D.N. Munns. 1980. Nitrogen Mineralization as Affected by Soil Moisture, Temperature, and Depth1. Soil Sci. Soc. Am. J. 44: 1233–1237. doi: 10.2136/sssaj1980.03615995004400060020x.

Christian, D.G., P.R. Poulton, A.B. Riche, and N.E. Yates. 1997. The recovery of 15N-labelled fertilizer applied to Miscanthus× giganteus. Biomass and Bioenergy 12(1): 21–24.

Christian, D.G., and A.B. Riche. 1998. Nitrate leaching losses under Miscanthus grass planted on a silty clay loam soil. Soil Use Manag. 14(3): 131–135.

Christian, D.G., A.B. Riche, and N.E. Yates. 2008. Growth, yield and mineral content of Miscanthus× giganteus grown as a biofuel for 14 successive harvests. Ind. Crops Prod. 28(3): 320–327.

David, M.B., L.E. Drinkwater, and G.F. McIsaac. 2010. Sources of Nitrate Yields in the Mississippi River Basin. J. Environ. Qual. 39: 1657–1667. doi: 10.2134/jeq2010.0115.

Davis, M.P., M.B. David, and C.A. Mitchell. 2013. Nitrogen Mineralization in Soils Used for Biofuel Crops. Commun. Soil Sci. Plant Anal. 44(5): 987–995. doi: 10.1080/00103624.2012.747607.

Davis, M.P., M.B. David, T.B. Voigt, and C.A. Mitchell. 2015. Effect of nitrogen addition on Miscanthus× giganteus yield, nitrogen losses, and soil organic matter across five sites. Gcb Bioenergy 7(6): 1222–1231.

Davis, S.C., W.J. Parton, F.G. Dohleman, C.M. Smith, S. Del Grosso, et al. 2010. Comparative biogeochemical cycles of bioenergy crops reveal nitrogen-fixation and low greenhouse gas emissions in a Miscanthus× giganteus agro-ecosystem. Ecosystems 13(1): 144–156.

Doane, T.A., and W.R. Horwath. 2003. Spectrophotometric determination of nitrate with a single reagent. Anal. Lett. 36(12): 2713–2722.

Dohleman, F.G., E.A. Heaton, R.A. Arundale, and S.P. Long. 2012. Seasonal dynamics of above-and below-ground biomass and nitrogen partitioning in M iscanthus× giganteus and P anicum virgatum across three growing seasons. Gcb Bioenergy 4(5): 534–544.

Eckert, B., O.B. Weber, G. Kirchhof, A. Halbritter, M. Stoffels, et al. 2001. Azospirillum doebereinerae sp. nov., a nitrogen-fixing bacterium associated with the C4-grass Miscanthus. Int. J. Syst. Evol. Microbiol. 51(1): 17–26.

Fernández, F.G., K.P. Fabrizzi, and S.L. Naeve. 2017. Corn and soybean’s season-long in-situ nitrogen mineralization in drained and undrained soils. Nutr. Cycl. agroecosystems 107(1): 33–47.

Foereid, B., A. de Neergaard, and H. Høgh-Jensen. 2004. Turnover of organic matter in a Miscanthus field: effect of time in Miscanthus cultivation and inorganic nitrogen supply. Soil Biol. Biochem. 36(7): 1075–1085.

Haines, S.A., R.J. Gehl, J.L. Havlin, and T.G. Ranney. 2015. Nitrogen and phosphorus fertilizer effects on establishment of giant Miscanthus. BioEnergy Res. 8(1): 17–27.

Heaton, E.A., F.G. Dohleman, and S.P. Long. 2008. Meeting US biofuel goals with less land: The potential of Miscanthus. Glob. Chang. Biol. 14(9): 2000–2014. doi: 10.1111/j.1365-2486.2008.01662.x.

Iowa Nutrient Reduction Strategy. 2013. A science and technology-based framework to assess and reduce nutrients to Iowa waters and the Gulf of Mexico. Iowa Dep. Agric. L. Steward. Iowa Dep. Nat. Resour. Iowa State Univ. Coll. Agric. Life Sci. Ames, IA.

Jaynes, D.B., T.S. Colvin, D.L. Karlen, C.A. Cambardella, and D.W. Meek. 2001. Nitrate loss in subsurface drainage as affected by nitrogen fertilizer rate. J. Environ. Qual. 30(4): 1305–1314.

Jaynes, D.B., and T.M. Isenhart. 2014. Reconnecting Tile Drainage to Riparian Buffer Hydrology for Enhanced Nitrate Removal. J. Environ. Qual. 43: 631–638. doi: 10.2134/jeq2013.08.0331.

Kahle, P., S. Beuch, B. Boelcke, P. Leinweber, and H.-R. Schulten. 2001. Cropping of Miscanthus in Central Europe: biomass production and influence on nutrients and soil organic matter. Eur. J. Agron. 15(3): 171–184.

Keymer, D.P., and A.D. Kent. 2014. Contribution of nitrogen fixation to first year Miscanthus× giganteus. Gcb Bioenergy 6(5): 577–586.

Lentz, R.D., and G.A. Lehrsch. 2012. Net nitrogen mineralization from past years' manure and fertilizer applications. Soil Sci. Soc. Am. J. 76(3): 1005–1015.

Ma, B.L., L.M. Dwyer, and E.G. Gregorich. 1999. Soil Nitrogen Amendment Effects on Seasonal Nitrogen Mineralization and Nitrogen Cycling in Maize Production ECORC contribution No. 991391. Agron. J. 91: 1003–1009. doi: 10.2134/agronj1999.9161003x.

McIsaac, G.F., M.B. David, and C.A.M.U. of Illinois. 2010. and Switchgrass Production in Central Illinois: Impacts on Hydrology and Inorganic Nitrogen Leaching. J. Environ. Qual. 39(5): 1790. doi: 10.2134/jeq2009.0497.

Randall, G.W., and J.E. Sawyer. 2008. Nitrogen Application Timing, Forms, and Additives. Final Rep. Gulf Hypoxia Local Water Qual. Concerns Work. St. Joseph, Michigan ASABE: 73–85.

Schipper, L.A., W.D. Robertson, A.J. Gold, D.B. Jaynes, and S.C. Cameron. 2010. Denitrifying bioreactors—An approach for reducing nitrate loads to receiving waters. Ecol. Eng. 36(11): 1532–1543. doi: https://doi.org/10.1016/j.ecoleng.2010.04.008.

Shield, I.F., T.J.P. Barraclough, A.B. Riche, and N.E. Yates. 2014. The yield and quality response of the energy grass Miscanthus× giganteus to fertiliser applications of nitrogen, potassium and sulphur. Biomass and Bioenergy 68: 185–194.

Sierra, J. 1997. Temperature and soil moisture dependence of N mineralization in intact soil cores. Soil Biol. Biochem. 29(9): 1557–1563. doi: 10.1016/S0038-0717(96)00288-X.

Smith, C.M., M.B. David, C.A. Mitchell, M.D. Masters, K.J. Anderson-Teixeira, et al. 2013. Reduced nitrogen losses after conversion of row crop agriculture to perennial biofuel crops. J. Environ. Qual. 42(1): 219–228.

Stark, J.M., and S.C. Hart. 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. Nature 385(6611): 61–64. doi: 10.1038/385061a0.

Strullu, L., S. Cadoux, M. Preudhomme, M.-H. Jeuffroy, and N. Beaudoin. 2011. Biomass production and nitrogen accumulation and remobilisation by Miscanthus× giganteus as influenced by nitrogen stocks in belowground organs. F. Crop. Res. 121(3): 381–391.

Susfalk, R.B., and D.W. Johnson. 2002. Ion exchange resin based soil solution lysimeters and snowmelt solution collectors. Commun. Soil Sci. Plant Anal. 33(7–8): 1261–1275.

Tejera, M., N. Boersma, A. Vanloocke, S. Archontoulis, P. Dixon, et al. 2019. Multi-year and Multi-site Establishment of the Perennial Biomass Crop Miscanthus× giganteus Using a Staggered Start Design to Elucidate N Response. BioEnergy Res.: 1–13.

Zhu, B., J.L.M. Gutknecht, D.J. Herman, D.C. Keck, M.K. Firestone, et al. 2014. Rhizosphere priming effects on soil carbon and nitrogen mineralization. Soil Biol. Biochem. 76: 183–192.

2.6 Figures and Tables

Table 1 Locations, soil descriptions, mean annual precipitation, mean annual temperature, management dates, and soil properties at start of the study in 2015. Potassium, phosphorus, pH, and CEC presented as means (± 1 standard error).

Site characteristic	**Central**	**NW**
Location (lat., long.)	42.013° N, 93.743° W	42.586° N, 95.012° W
Soil series	Webster clay loams (fine-loamy, mixed, superactive, mesic Typic Endoaquolls)	Canisteo clay loams (fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls)
30-year mean annual precipitation (mm)	910	881
30-year mean annual temperature (°C)	9.6	8.3
Planting Dates		
Corn	May 31, 2017; May 11, 2018	May 27, 2017; May 18, 2018
Juvenile miscanthus	May 16, 2017	May 9, 2017
Mature miscanthus	May 4, 2015	May 13, 2015
Nitrogen fertilization dates	May 15, 2017; May 9, 2018	April 24, 2017; May 9, 2018
Potassium (ppm)	194 (6)	249 (14)
Phosphorus (ppm)	14.9 (0.7)	81.9 (4.6)
pH	6.9 (0.1)	6.9 (0.1)
Cation Exchange Capacity (cmol kg^{-1})	42.3 (4.1)	22.3 (0.6)

Table 2 Soil pH, mean total soil carbon (± 1 standard error), mean total soil nitrogen (± 1 standard error), and carbon:nitrogen ratio of soil in top 15 cm measured in 2017.

Site	**Depth (cm)**	**pH**	**% Total Carbon**	**% Total Nitrogen**	**C:N**
Central	0-15	6.7 (0.2)	4.02 (0.33)	0.45 (0.06)	8.7
NW	0-15	6.9 (0.1)	3.01 (0.12)	0.44 (0.01)	6.8

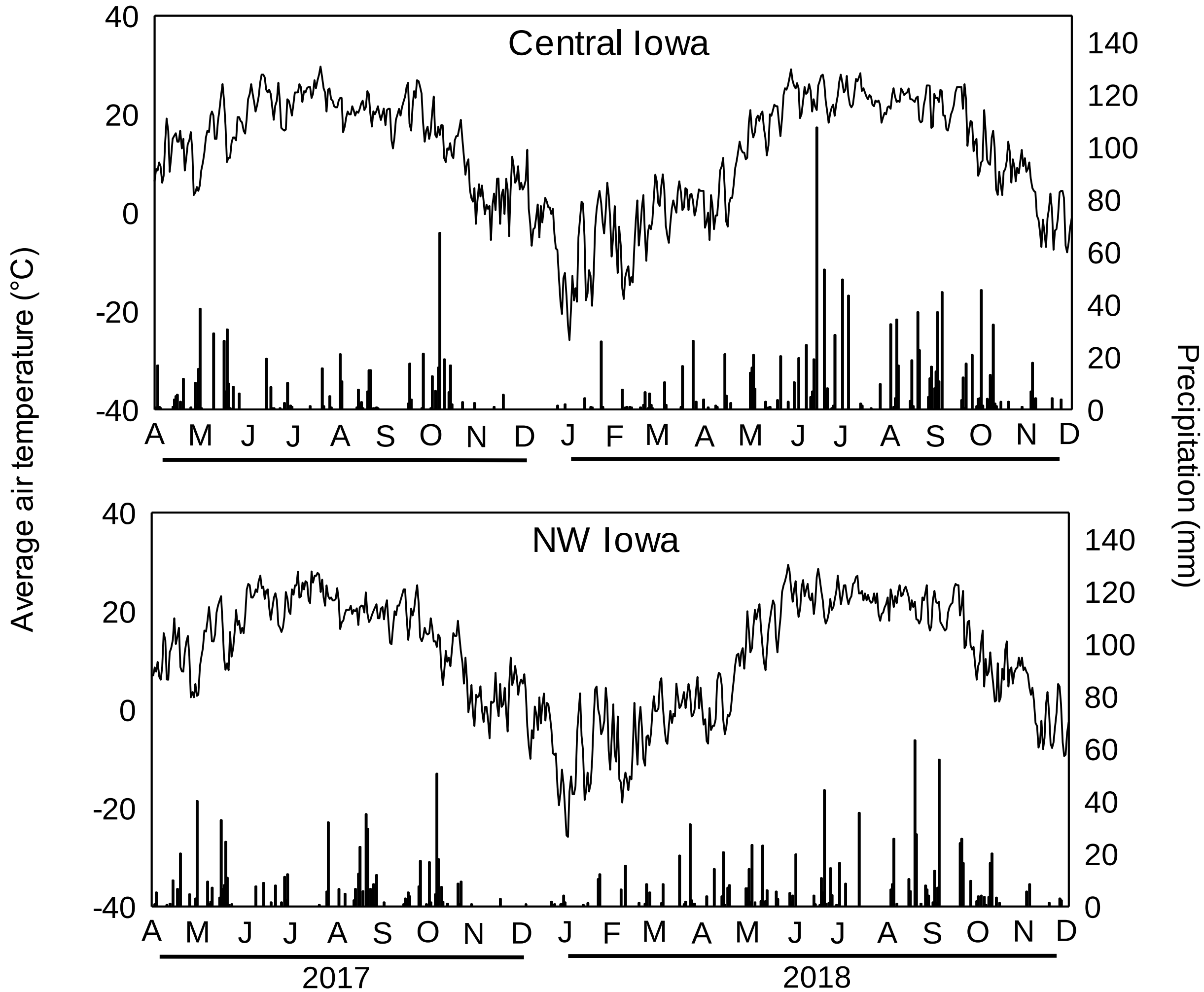

Figure 1 Daily average air temperature as indicated by the line and daily precipitation as indicated by bars for Northwest and Central sites for the duration of the study.

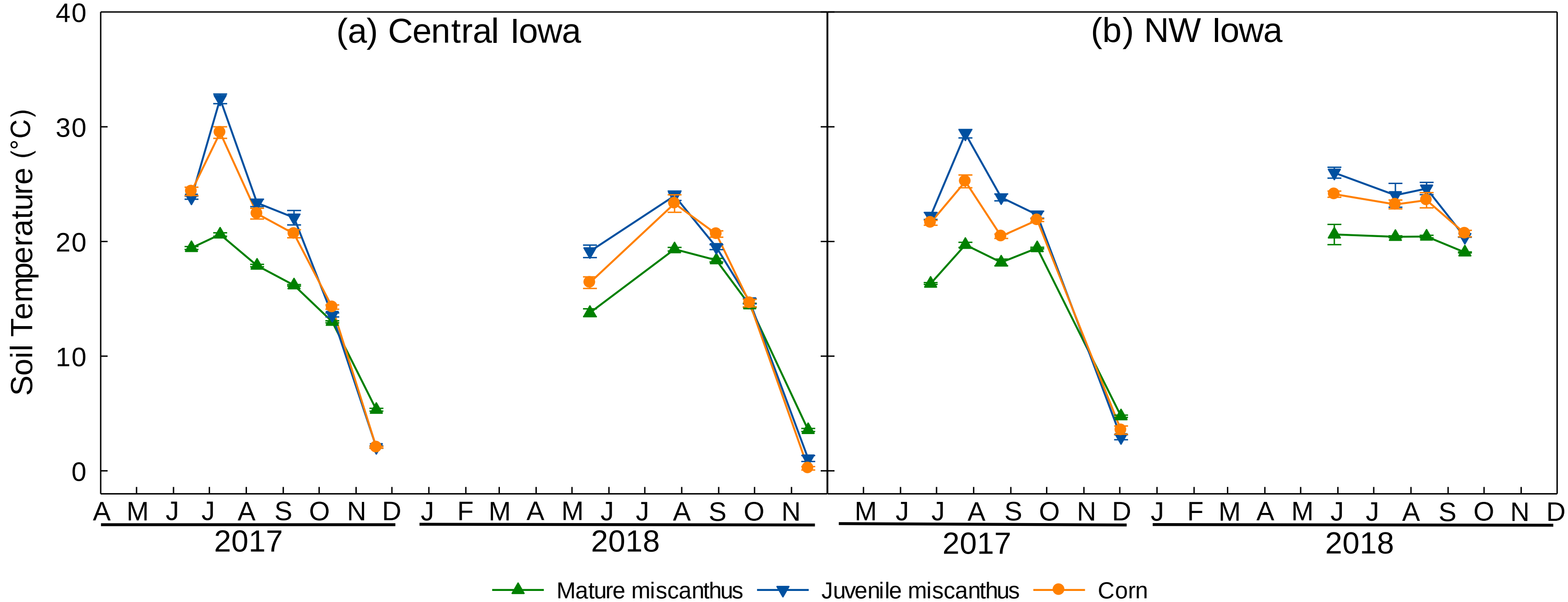

Figure 2 Soil temperature from the top 10 cm of soil during 2017 and 2018 under mature miscanthus, juvenile miscanthus, and corn crops. Data points are means with standard error bars (n=8).

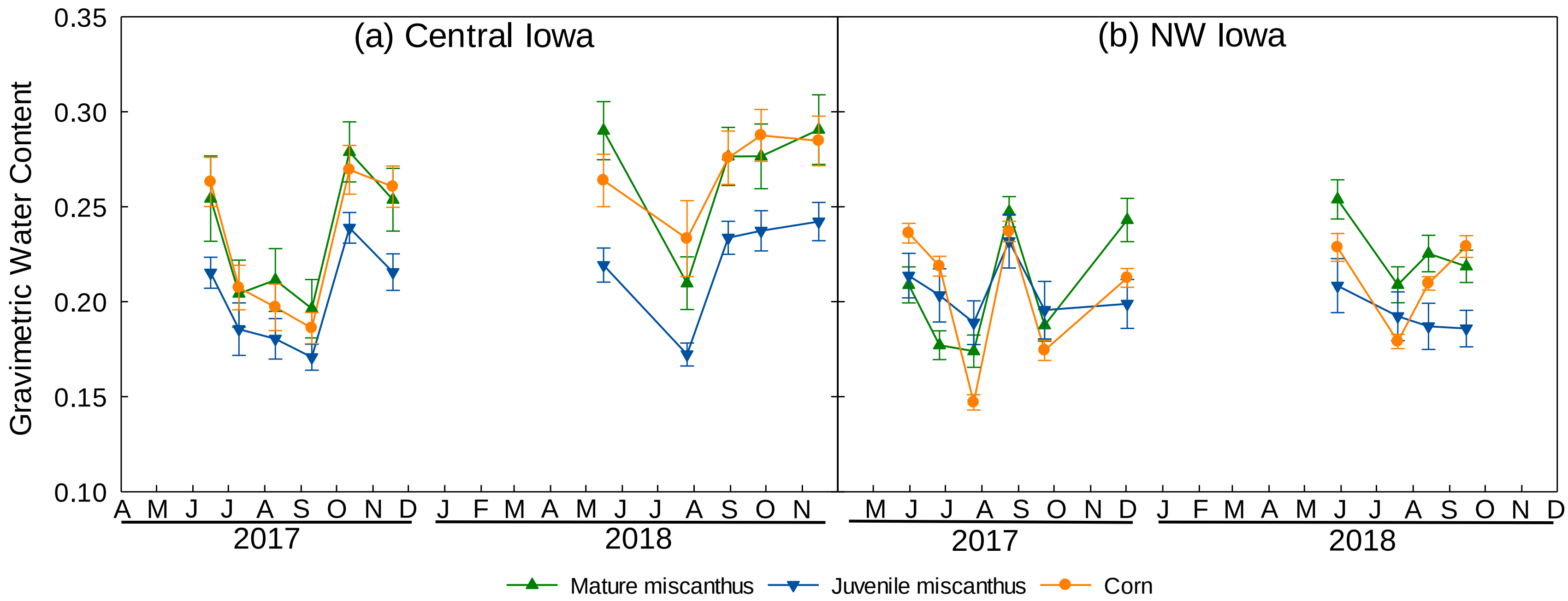

Figure 3 Gravimetric water content in the top 15 cm of soil during 2017 and 2018 under mature miscanthus, juvenile miscanthus, and corn. Data points are means with standard error bars (n=8).

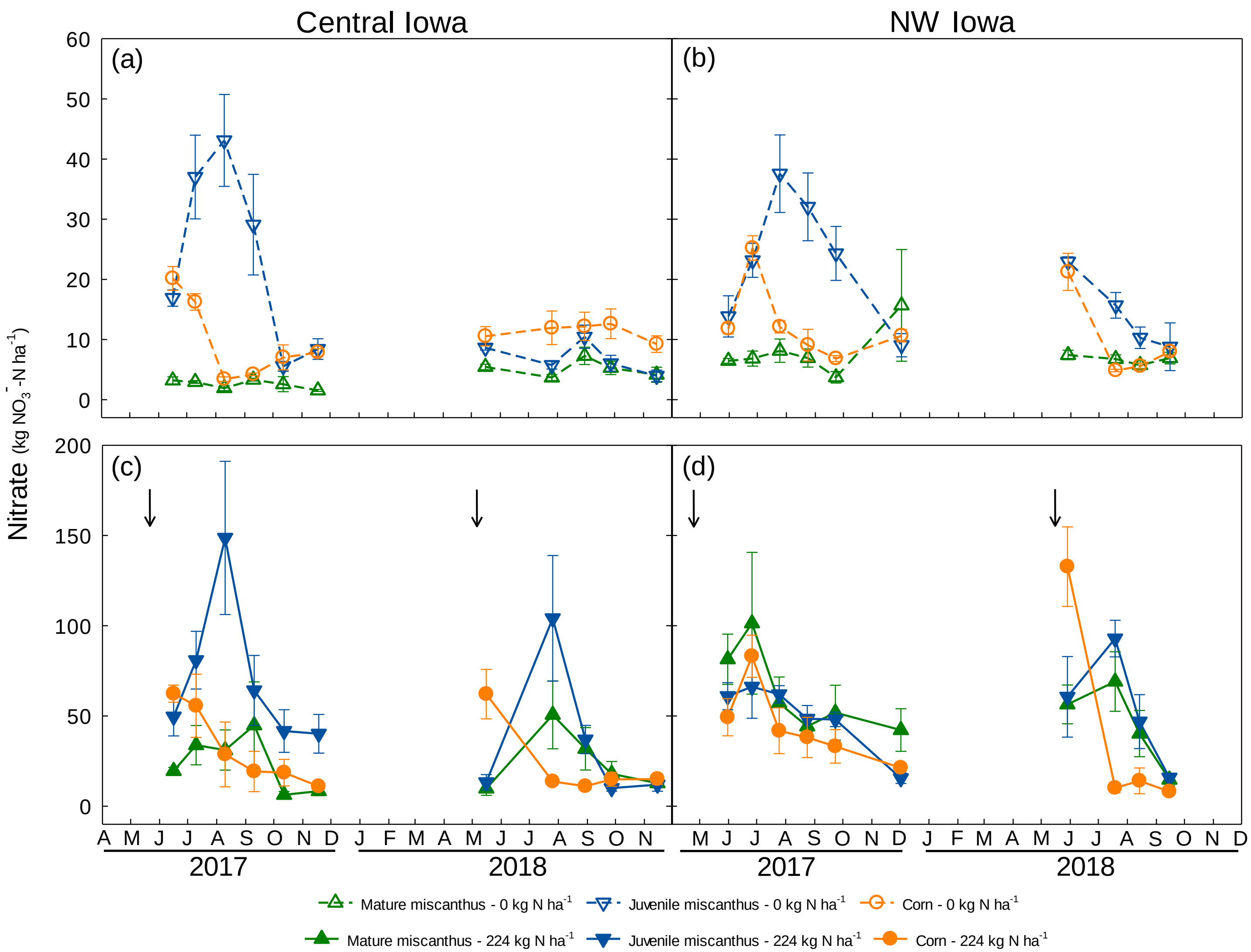

Figure 4 Nitrate concentrations in the top 15 cm of soil during 2017 and 2018 for mature miscanthus, juvenile miscanthus, and corn with nitrogen fertilization rates of 0 kg N ha^{-1} (a,b) and 224 kg N ha^{-1} (c,d). Arrows depict nitrogen fertilization dates. Data points are means with standard error bars (n=4).

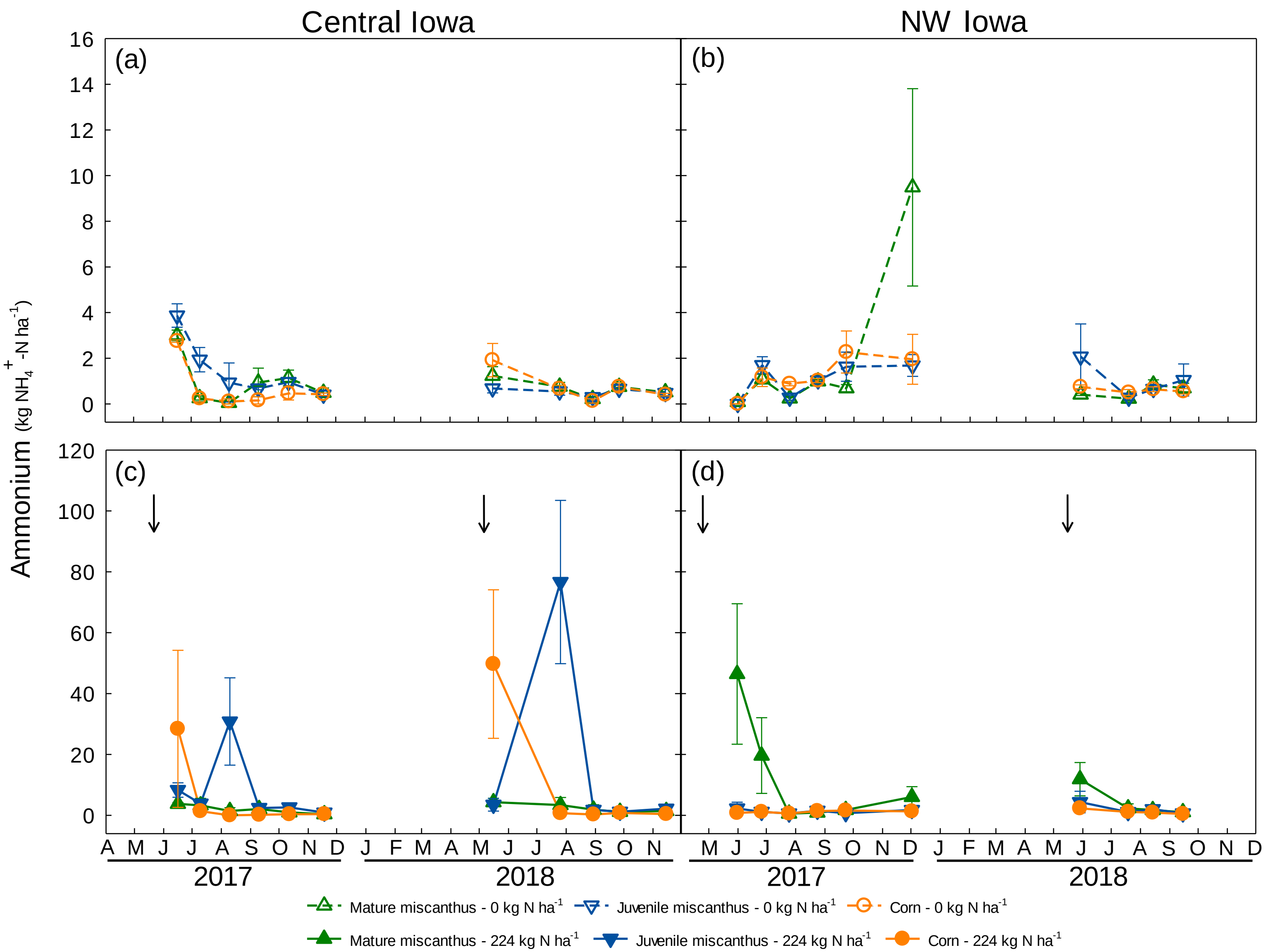

Figure 5 Ammonium concentrations in top 15 cm of soil during 2017 and 2018 for mature miscanthus, juvenile miscanthus, and corn with nitrogen fertilization rates of 0 kg N ha^{-1} (a,b) and 224 kg N ha^{-1} (c,d). Arrows depict nitrogen fertilization dates. Data points are means with standard error bars (n=4).

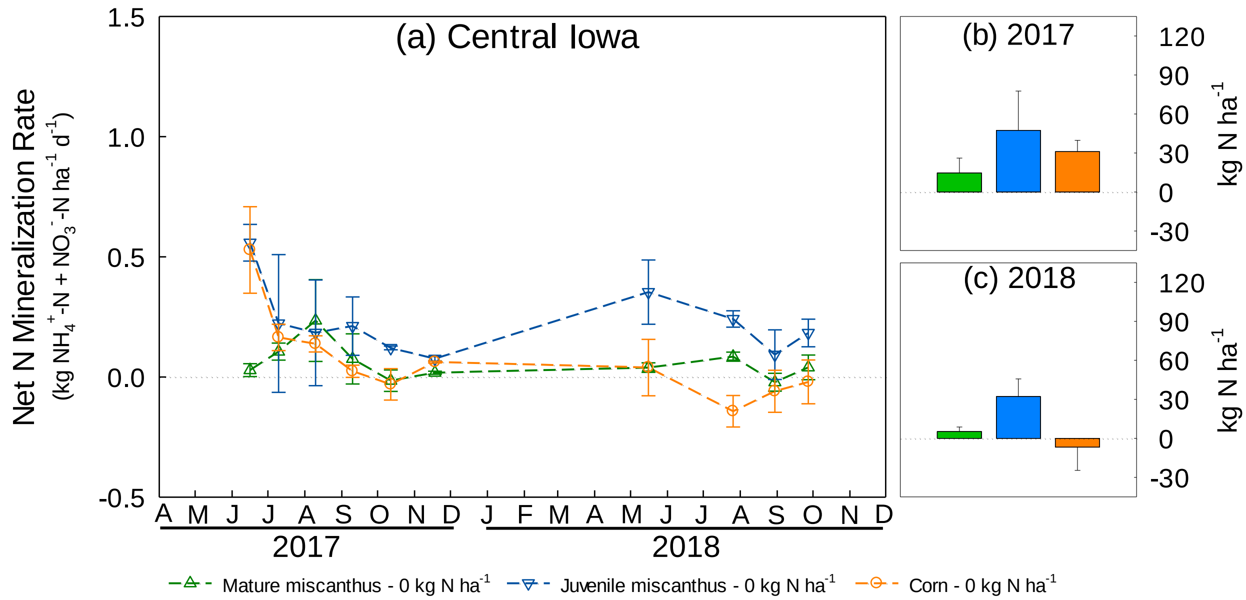

Figure 6 Net nitrogen mineralization rate for unfertilized treatments at the Central, IA site. Net nitrogen mineralization over time (a). Cumulative net nitrogen mineralization summed for individual years (b, c). Data points are means with standard error bars (n=4).

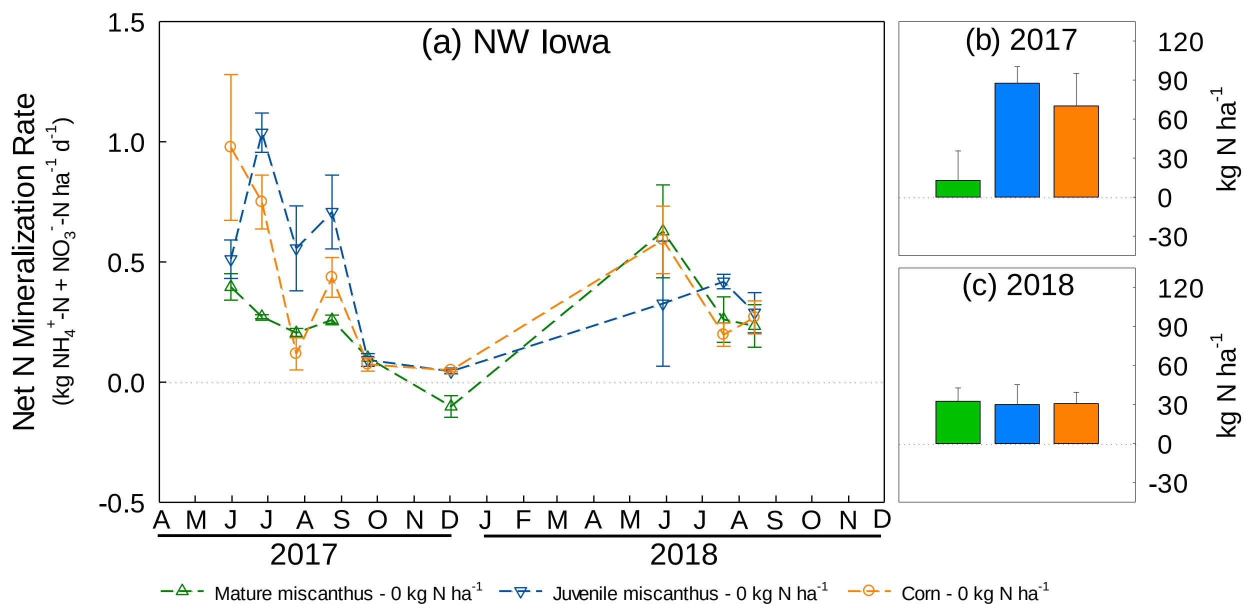

Figure 7 Net nitrogen mineralization rate for unfertilized treatments at the Northwest, IA site. Net nitrogen mineralization over time (a). Cumulative net nitrogen mineralization summed for individual years (b, c). Data points are means with standard error bars (n=4).

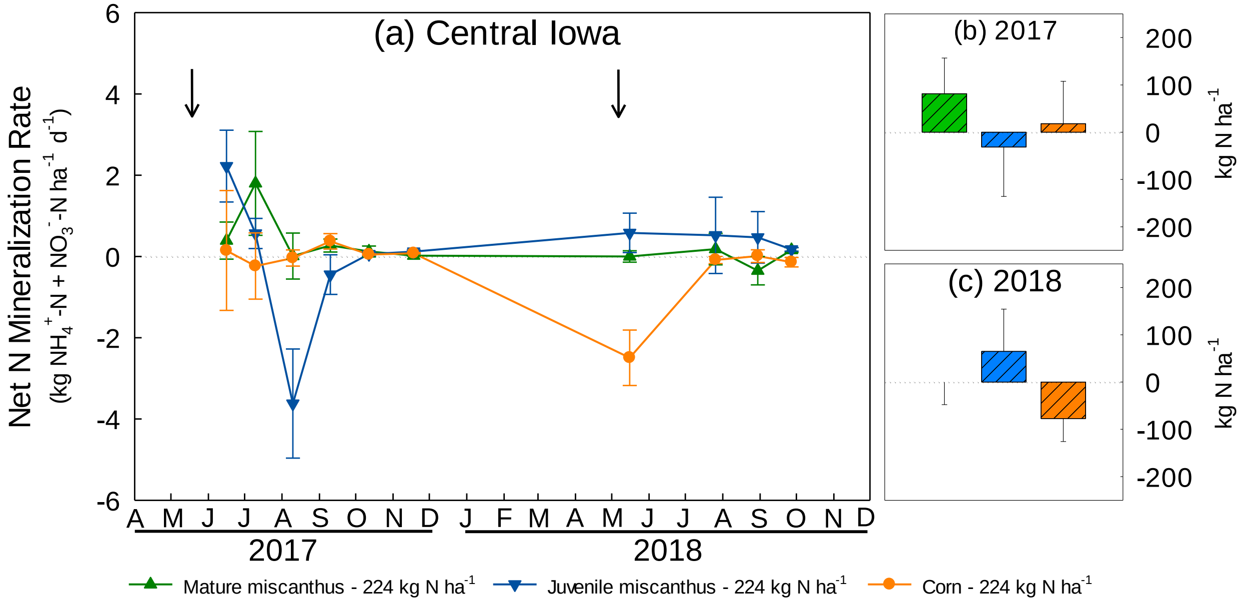

Figure 8 Net nitrogen mineralization rate for fertilized treatments at the Central, IA site. Net nitrogen mineralization over time (a). Cumulative net nitrogen mineralization summed for individual years (b, c). Arrows depict nitrogen fertilization dates. Data points are means with standard error bars (n=4).

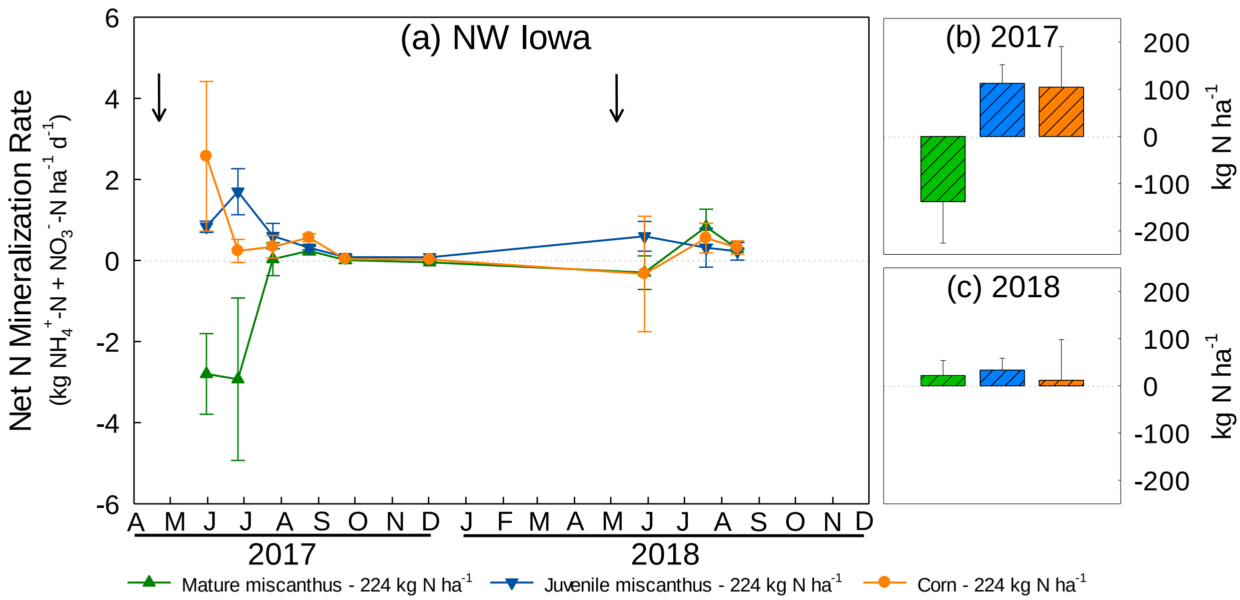

Figure 9 Net nitrogen mineralization rate for fertilized treatments at the Northwest, IA site. Net nitrogen mineralization over time (a). Cumulative net nitrogen mineralization summed for individual years (b, c). Arrows depict nitrogen fertilization dates. Data points are means with standard error bars (n=4).

Table 3 Effects of site, cropping system, and nitrogen fertilization on annual cumulative net nitrogen mineralization

	Cumulative net N min.		
	df	*F*	*P* value
Site (S)	1	2.81	0.1022
Cropping System (C)	**2**	**4.79**	**0.0143**
Nitrogen (N)	1	1.85	0.1825
Year (Y)	1	2.14	0.1518
S × C	**2**	**7.81**	**0.0015**
S × N	1	0.13	0.7183
C × N	2	0.27	0.7618
S × Y	1	0.11	0.7382
C × Y	**2**	**3.76**	**0.0330**
N × Y	1	0.09	0.7656
S × C × N	**2**	**5.11**	**0.0111**
S × C × Y	**2**	**7.11**	**0.0025**
S × N × Y	1	0.27	0.6047
C × N × Y	2	1.43	0.2514
S × C × N × Y	**2**	**3.59**	**0.0377**

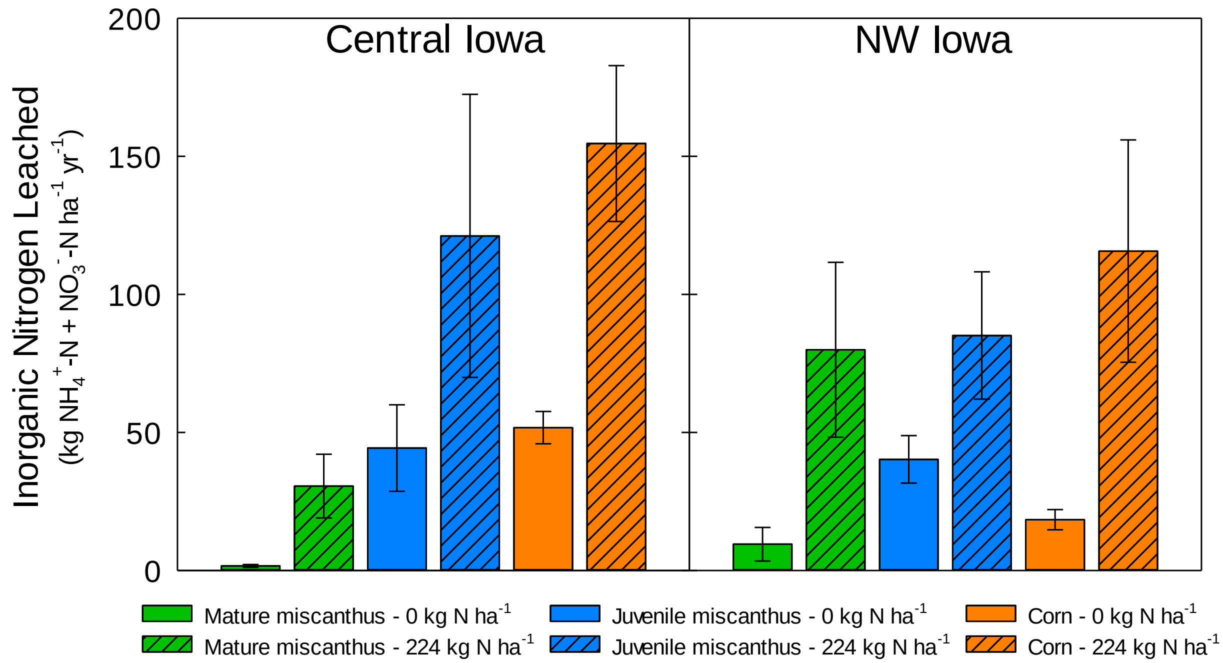

Figure 10 Annual inorganic nitrogen leaching at 50 cm soil depth using resin lysimeters for mature miscanthus, juvenile miscanthus, and corn at 0 and 224 kg N ha^{-1} fertilization. Means with standard error bars (n=4).

Table 4 Effects of site, cropping system, and nitrogen fertilization on annual nitrogen leaching at 50 cm depth

	Nitrate			Ammonium			Total inorganic N		
	df	*F*	*P* value	df	*F*	*P* value	df	*F*	*P* value
Site (S)	1	0.02	0.8863	1	2.04	0.2179	1	0.22	0.6544
Cropping System (C)	**2**	**4.34**	**0.0226**	2	0.98	0.3904	**2**	**4.84**	**0.0161**
Nitrogen (N)	**1**	**23.33**	**<0.0001**	1	0.05	0.8327	**1**	**23.43**	**<0.0001**
S × C	2	2.62	0.0906	2	0.97	0.3917	2	1.7	0.2021
S × N	1	0.14	0.7065	1	1.56	0.2239	1	0	0.9890
C × N	2	0.6	0.5575	2	2.46	0.1056	2	1.04	0.3664
S × C × N	2	1.03	0.3691	**2**	**3.66**	**0.0399**	2	0.58	0.5674

CHAPTER 3. THE INFLUENCE OF *MISCANTHUS* × *GIGANTEUS* ON SOIL HEALTH

3.1 Introduction

Annual cropping systems with winter fallow periods leave large contiguous portions of the Midwest U.S. landscape barren and susceptible to nutrient leaching and soil erosion. The combined lack of nitrogen (N) uptake by plants during fallow times combined with highly fertile soils and N fertilization leads to large seasonal nitrate fluxes to rivers and groundwater. Nutrient loss from conventional arable crop production throughout the Midwest is a major contributor to hypoxia in the Gulf of Mexico (David et al., 2010). There are many options for reducing N leaching from agricultural fields including better N fertilization practices (Randall & Sawyer, 2008) as well as incorporating edge of field practices, but among the most promising is the conversion of entire fields, or portions thereof, to perennial instead of annual crops to eliminate bare soil over winter and early spring (Iowa Nutrient Reduction Strategy, 2013).

Miscanthus (*Miscanthus* × *giganteus* Greef et Deu.) is an increasingly common perennial biomass crop. Besides potentially reducing nitrate leaching, miscanthus has high biomass yields (e.g. 12-40 Mg ha^{-1}) and low input requirements (Christian et al., 1997; Christian et al., 2008; Heaton et al., 2008; Smith et al., 2013). Miscanthus shows potential to reduce nitrate leaching from agricultural systems by about 90% when compared to traditional annual cropping systems (McIsaac et al., 2010; Smith et al., 2013). This could simply be because miscanthus uses more water leaving less to transport nitrate (McIsaac et al., 2010), or it could also be driven by changes in soil aggregate structure, and carbon protection and cycling (Tiemann and Grandy, 2015; Zhu et al., 2018). The extent to which different mechanisms govern observed responses is, yet, unresolved.

To understand the mechanisms behind miscanthus' potential to efficiently cycle N, including enhanced potential to mineralize N and increase microbial biomass, we conducted an experiment at two sites in Iowa, U.S., in the heart of the Midwest Corn Belt. With nearly 70% of its land area in row crops (USDA-NASS), Iowa is characterized by both high crop productivity and high rates of nutrient leaching (Iowa Nutrient Reduction Strategy, 2013). To understand how N fertilization affects miscanthus soil N dynamics we conducted an experiment at two sites in Iowa with similar soil types, but differing management history and climate. We measured microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) as well as potentially mineralizable carbon (PMC) and potentially mineralizable nitrogen (PMN) as indicators of soil health in miscanthus and corn. Soils were sampled at three times during the growing season – spring, summer, and fall. We hypothesized: (i) microbial biomass carbon and nitrogen would be greater in miscanthus than corn, and (ii) potentially mineralizable carbon and nitrogen would be greater in miscanthus than corn.

3.2 Methods

3.2.1 Site description and experimental design

Field sites were located in northwest (NW) Iowa, USA (42.586, -95.012) and Central Iowa, USA (42.013, -93.743). These sites have predominately < 2% slope. The sites had similar characteristics but differed in their previous management (Table 1 and 2). The NW site had been in corn/soy rotations with annual additions of cattle manure, while the Central site had been in corn/soy and some grass and had received only artificial fertilizers. The manure caused notably higher phosphorus and potassium content.

This study used a subset of plots from a larger staggered-start experiment comparing corn (*Zea mays* L.) and different-aged miscanthus over five N fertilization rates (0, 112, 224, 336, 448 kg N ha^{-1}) (Tejera et al., 2019). Full details of that experiment and its management can be found elsewhere (Tejera et al., 2019). Briefly, the experiment was established in 2015 using a split-plot randomized complete block design with four replications of miscanthus with adjacent corn check plots that were not randomized. That is, crop was randomized, and N fertilization rate was randomized within miscanthus, but the corn check adjacent to the miscanthus plot received the same N rate as the miscanthus. Main plots were 24 m by 120 m, with split-plots 24 m by 12 m. Main-plot treatment levels were three planting years (2015, 2016, and 2017) and split-plot treatments were five N fertilization rates (0, 112, 224, 336, and 448 kg N ha^{-1}). Of the main-plot treatments we consider here only the 2015 miscanthus planting year and of the split-plot treatments we consider only the 0 and 224 kg N ha^{-1}; as well as the adjacent 0 and 224 kg N ha^{-1} corn plots. This resulted in 4 replications × 2 crops × 2 N fertilization rates = 16 experimental units at each the NW and Central sites.

3.2.2 Soil sampling

Soil samples were taken three times at each site in 2017, once in each spring, summer, and fall. (May 31, July 25, and December 2 at the NW site and June 16, August 10, and November 18 at the Central site). At each sampling event, ten soil cores (1.75 cm diameter, 15 cm deep) were randomly taken from each plot and thoroughly homogenized for a composite sample. The composite sample was placed in a cooler with icepacks for transport to the lab. Once the soils were returned to the lab, they were stored at 4 °C for up to one week. Soils were sieved (< 2 mm at field moisture), sub-samples were taken to determine gravimetric water content, MBC and MBN, and extracted for ammonium and nitrate. The remaining soil was set aside to air dry for the PMN and PMC incubation.

3.2.3 Measures of microbial biomass carbon and nitrogen

For each sampling event five g of field moist soil was fumigated with ethanol-free chloroform in a desiccator for 24 h in the absence of light. A matching set of samples were not fumigated but left nearby in matching temperature and light conditions. Both the fumigated and non-fumigated samples were extracted with 25 mL of 0.5 M K_2SO_4. The samples were frozen at -20 °C until analysis of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were run simultaneously, for both the fumigated and unfumigated samples, using a Shimadzu TOC-L TNM analyzer. Prior to analysis, phosphoric acid was added to all samples to remove possible carbonates and bicarbonates. Extraction efficiency constants of K_{EN} = 0.54 (Brookes et al., 1985) and K_{EC} = 0.45 (Joergensen, 1996) were applied. The difference between the fumigated and non-fumigated samples for both DOC and TDN were taken to calculate MBC and MBN. Dissolved organic nitrogen (DON) was calculated by taking the difference between the TDN of non-fumigated samples and the measured inorganic N for each sample.

3.2.4 Measures of potentially mineralizable carbon and nitrogen

Potentially mineralizable nitrogen (PMN) and carbon (PMC) were measured using a 14-d aerobic incubation. To prepare for the 14-d incubation, 5 g of air-dried soil for each sample was weighed out into a 50 ml centrifuge tube equipped with a stopcock cap. Water was added to each sample to bring it to 50 % water holding capacity (WHC). WHC was measured by placing 10 g of air-dried soil in moist filter paper and funnel (Whatman #1). Soil was saturated with deionized water, then mass of water retained by the soil 6 h later was measured. WHC was calculated as mass of water retained (g) per g of dry soil.

Potentially mineralizable carbon was measured during the same incubation as PMN, but measuring CO_2 production in the test tube headspace during the 14-d incubation. One-ml gas samples were extracted and injected into an infrared gas analyzer, Li-830 (Li-Cor, Lincoln, Nebraska, USA). For each sampling event, 1 ml of headspace gas was collected immediately after flushing with lab air – initial or time-zero (t_0) sample. A second 1- ml sample was collected 1-3 d after this initial sample, depending on when it occurred during the 14-d incubation (t_1). The difference in CO_2-C mass between two paired sampling points (t_0 and t_1) represents the mass of carbon mineralized during that time interval. After each t_1 sampling the centrifuge tubes were opened and flushed with air to reset the CO_2 to ambient levels to allow for a new t_0 measurement.

Potentially mineralizable nitrogen was calculated by subtracting the initial salt-extractable N (ammonium plus nitrate) values from the post 14-d incubation salt-extractable inorganic N. Extractions from soil samples (initial and incubated) were analyzed for ammonium using salicylate and ammonia cyanurate reagent packets (Hach Company, Loveland, Colorado, USA), and for nitrate using the single-reagent method (vanadium III, sulfanilamide and *N*-(1-naphthyl)-ethylenediamine dihydrochloride) (Doane and Horwath, 2003). Both ammonium and nitrate extracts were then analyzed using a Synergy™ HTX Multi-Mode Microplate Reader (BioTek Instruments, Winooski, VT, USA) with Gen5™ software.

3.2.5 Statistical analysis

All data were tested for homogeneity of variances and normality. All data met ANOVA assumptions. Statistical analyses were performed using linear mixed models with N rate, crop, and site as fixed effects. Repeated measures ANOVAs were used for MBC, MBN, PMC, and PMN. All repeated measures ANOVAs were tested for best-fit variance-covariance structure, using Akaike information criterion, in SAS 9.4 with differences in means at a probability level of $p < 0.1$ considered significant.

3.3 Results

3.3.1 Climate, soil temp, soil moisture, and soil water holding capacity

In 2017, precipitation at both sites was well below the mean annual precipitation (Table 1): NW received 748 mm and Central received 756 mm. At both sites, miscanthus decreased soil temperatures during the growing season by 16 % (Fig. 2). At the NW site, soils in the miscanthus treatment were the drier than corn through much of the growing season. Soil water holding capacity significantly differed by site and by crop (Table 5). Miscanthus increased soil water holding capacity by 15 % on average when compared to corn (Fig. 11).

3.3.2 Microbial biomass carbon and nitrogen

Across all treatments at both sites there were little differences in soil microbial biomass (Fig. 12). There were no significant crop effects on MBC, MBN, or MBC:MBN (Table 6). Microbial biomass N and MBC:MBN significantly responded to fertilizer application causing a decrease in MBN by 29 % and an increase in MBC:MBN by 17 %. Microbial biomass C under miscanthus significantly increased by 6 % between the spring and summer sampling. Across both sites the highest MBC:MBN ratio occurred during the summer at 9.8, while spring and fall were lower at 8.3 and 8.8 respectively.

3.3.3 Dissolved organic carbon and nitrogen

Across both sites the application of fertilizer increased DON concentrations (Table 7). DON increased across the sites by 27-56 % when fertilized (Fig. 13). The two sites had significantly different concentrations of DOC and DON. DOC at the Central site averaged 82 % higher than at the NW site and DON averaged 25 % higher at the NW site than Central site. At both sites, DOC and DON concentrations were 16 and 64 % higher during the spring than during the fall, respectively.

3.3.4 Potentially mineralizable carbon and nitrogen

Potentially mineralizable C was 34 % greater at the NW site than at the Central site (Fig. 14). In the spring and fall, there were no experimental treatment responses for PMC (Table 8). During the summer, the unfertilized miscanthus had 29 and 38 % greater PMC than unfertilized corn and fertilized miscanthus. The unfertilized and fertilized miscanthus had 31 and 27 % greater PMC in the fall than spring. The fertilized miscanthus had significantly lower PMC in the summer than spring and fall.

Similar to PMC, PMN was 30 % higher at the NW site than at the Central site (Fig. 14). The fertilized treatments had 9 % greater PMN than the unfertilized treatments. At the Central site, the fertilized miscanthus had the greatest PMN in both the summer and fall. The fall PMN sample from the fertilized miscanthus had the greatest PMN of all treatments and dates at the Central site at 42 mg N kg^{-1} mineralized. At the NW site, fertilized and unfertilized miscanthus treatments had their greatest PMN in the spring.

3.4 Discussion

Our main objective was to examine alterations in soil health indicators that occur with miscanthus compared to corn. Soil health has been defined as "*The continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal and human health*" (Doran and Safley, 1997). Microbial biomass and microbial activity have been proposed as indicators of soil health and are the engines that drive the N cycle in soils (Falkowski et al., 2008). Switching entire fields, or portions thereof, to perennial cropping systems is thought to increase soil health. We show here that after three years of miscanthus establishment, soil water holding capacity increased but soil MBN, MBC, PMN and PMC were not significantly affected. Our results clearly show that miscanthus alters soil conditions to allow for increased soil water storage.

3.4.1 Microbial biomass carbon and nitrogen

Soil microbial biomass is a small organic pool that drives the decomposition and turnover of all organic matter and has been referred to as the ”*eye of the needle*”, in other words, the pool through which all nutrients must pass (Jenkinson, 1977). Microbial biomass is an important soil health indicator as it is a biologically active fraction of organic matter that is sensitive to management changes. It has also been linked to N mineralization rates (Li et al., 2019). Even though microbial biomass is a sensitive indicator of soil health, we found little difference in MBC or MBN between corn and miscanthus in its third growing season.

3.4.2 Potentially mineralizable nitrogen and carbon

Chapter 2 showed in-situ net N mineralization was highly variable, and though not significant at $\alpha=0.05$, we found it to be 83 % lower under mature miscanthus. Given this reduction, we expected PMN, as measured through laboratory incubation, to also be lower, despite our initial hypothesis that it would increase under mature miscanthus. Miscanthus has shown the ability to increase PMN compared to other biomass crops when in fifth growing season (Davis et al., 2013; Davis et al., 2015). Despite lower in-situ values, we did indeed find increased PMN in fertilized miscanthus during the summer and fall, at one site. There is a potential PMN would increase with additional years of miscanthus growth. With the application of fertilizer there was an increase in overall PMN, likely due to a N priming effect (Jenkinson et al., 1985; Kuzyakov et al., 2000). Priming effects are typically strongest in soils high in C and N, such as those used in this study (Hart et al., 1986).

Potentially mineralizable carbon is a measurement of both microbial activity and labile carbon that has shown to be an indicator of soil N availability as it closely relates to net N_{min} (Franzluebbers et al., 2018). This would suggest that potential soil N availability in corn and miscanthus were similar in this study as there was no significant difference in PMC between the two crops.

3.4.3 Water holding capacity

There was a significant difference in soil water holding capacity in corn soils to miscanthus soils. Miscanthus increased soil water holding capacity by 15 % on average. This increase could be caused, in part, by the transition from conventional tillage to no tillage. Transition to no tillage has shown to increase soil organic matter (Ismail et al., 1994), which has been linked to increased available water capacity (Hudson, 1994). Miscanthus grown in Europe has shown the ability to increase soil organic matter after 4-11 growing seasons (Beuch et al., 2000; Kahle et al., 2001; Foereid et al., 2004). Another possibility is a physical alteration in soil aggregation by a combination of reduced tillage and labile C inputs through rhizodeposition (Bronick and Lal, 2005). The increase in water holding capacity, whether due to shifts in organic matter, aggregate structure, or other reasons, should be an area of future research as water holding capacity alters soil's suitability for sustaining plant growth and biological activity (Doran, 1996).

It is important to remember that these measurements were taken during the third growing season in this study, and with an increase in stand age there could be more measurable shifts in soil aggregation and possibly soil organic matter. Some studies show that it takes 40 years or more for observable increases in SOC from perennial vegetation compared to annual crops – even when changed to a diverse perennial grassland mixture via Conservation Reserve Program

(McLauchlan, 2006; O'Brien and Jastrow, 2013; Rosenzweig et al., 2016). Therefore, we should not expect to see changes in SOC in just three years (Fig. 16). It was, however, unexpected to find very little changes in more active fraction of soil organic matter like microbial biomass and potentially mineralizable pool (Fig. 12 and 14).

3.5 Conclusion

Although we did not find any consistent soil health differences after conversion to miscanthus, we did find a change strong and consistent increases in water holding capacity. The change in water holding capacity is evidence that miscanthus is altering soil properties. Even though there was not an increase in microbial biomass there is still the potential that miscanthus is causing a shift in microbial communities. Additional research is needed, however, on older stands of miscanthus in the Midwest to further understand how miscanthus is driving the change in water holding capacity as well as to investigate possible changes in soil health indicators. A better understanding of how miscanthus alters soil properties will assist in choosing appropriate soil-plant combinations for economic and environmental benefit.

3.7 References

Beuch, S., B. Boelcke, and L. Belau. 2000. Effect of the organic residues of Miscanthus× giganteus on the soil organic matter level of arable soils. J. Agron. Crop Sci. 184(2): 111–120.

Bronick, C.J., and R. Lal. 2005. Soil structure and management: a review. Geoderma 124(1–2): 3–22.

Brookes, P.C., A. Landman, G. Pruden, and D.S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17(6): 837–842. doi: 10.1016/0038-0717(85)90144-0.

Christian, D.G., P.R. Poulton, A.B. Riche, and N.E. Yates. 1997. The recovery of 15N-labelled fertilizer applied to Miscanthus× giganteus. Biomass and Bioenergy 12(1): 21–24.

Christian, D.G., A.B. Riche, and N.E. Yates. 2008. Growth, yield and mineral content of Miscanthus× giganteus grown as a biofuel for 14 successive harvests. Ind. Crops Prod. 28(3): 320–327.

David, M.B., L.E. Drinkwater, and G.F. McIsaac. 2010. Sources of Nitrate Yields in the Mississippi River Basin. J. Environ. Qual. 39: 1657–1667. doi: 10.2134/jeq2010.0115.

Davis, M.P., M.B. David, and C.A. Mitchell. 2013. Nitrogen Mineralization in Soils Used for Biofuel Crops. Commun. Soil Sci. Plant Anal. 44(5): 987–995. doi: 10.1080/00103624.2012.747607.

Davis, M.P., M.B. David, T.B. Voigt, and C.A. Mitchell. 2015. Effect of nitrogen addition on Miscanthus× giganteus yield, nitrogen losses, and soil organic matter across five sites. Gcb Bioenergy 7(6): 1222–1231.

Doane, T.A., and W.R. Horwath. 2003. Spectrophotometric determination of nitrate with a single reagent. Anal. Lett. 36(12): 2713–2722.

Doran, J.W. 1996. Soil health and global sustainability. Soil Qual. is Hands L. Manag.: 45.

Doran, J.W., and M. Safley. 1997. Defining and assessing soil health and sustainable productivity. Biol. Indic. soil Heal. New York CAB Int.

Falkowski, P.G., T. Fenchel, and E.F. Delong. 2008. The microbial engines that drive Earth's biogeochemical cycles. Science (80-.). 320(5879): 1034–1039.

Foereid, B., A. de Neergaard, and H. Høgh-Jensen. 2004. Turnover of organic matter in a Miscanthus field: effect of time in Miscanthus cultivation and inorganic nitrogen supply. Soil Biol. Biochem. 36(7): 1075–1085.

Franzluebbers, A.J., M.R. Pershing, C. Crozier, D. Osmond, and M. Schroeder-Moreno. 2018. Soil-test biological activity with the flush of CO 2: I. C and N characteristics of soils in corn production. Soil Sci. Soc. Am. J. 82(3): 685–695.

Hart, P.B.S., J.H. Rayner, and D.S. Jenkinson. 1986. Influence of pool substitution on the interpretation of fertilizer experiments with 15N. J. Soil Sci. 37(3): 389–403.

Heaton, E.A., F.G. Dohleman, and S.P. Long. 2008. Meeting US biofuel goals with less land: The potential of Miscanthus. Glob. Chang. Biol. 14(9): 2000–2014. doi: 10.1111/j.1365-2486.2008.01662.x.

Hudson, B.D. 1994. Soil organic matter and available water capacity. J. Soil Water Conserv. 49(2): 189.

Iowa Nutrient Reduction Strategy. 2013. A science and technology-based framework to assess and reduce nutrients to Iowa waters and the Gulf of Mexico. Iowa Dep. Agric. L. Steward. Iowa Dep. Nat. Resour. Iowa State Univ. Coll. Agric. Life Sci. Ames, IA.

Ismail, I., R.L. Blevins, and W.W. Frye. 1994. Long-Term No-tillage Effects on Soil Properties and Continuous Corn Yields. Soil Sci. Soc. Am. J. 58: 193–198. doi: 10.2136/sssaj1994.03615995005800010028x.

Jenkinson, D.S., R.H. Fox, and J.H. Rayner. 1985. Interactions between fertilizer nitrogen and soil nitrogen—the so-called 'priming'effect. J. soil Sci. 36(3): 425–444.

Joergensen, R.G. 1996. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k EC value. Soil Biol. Biochem. 28(1): 25–31. doi: 10.1016/0038-0717(95)00102-6.

Kahle, P., S. Beuch, B. Boelcke, P. Leinweber, and H.-R. Schulten. 2001. Cropping of Miscanthus in Central Europe: biomass production and influence on nutrients and soil organic matter. Eur. J. Agron. 15(3): 171–184.

Kuzyakov, Y., J.K. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of priming effects. Soil Biol. Biochem. 32(11–12): 1485–1498.

Li, Z., D. Tian, B. Wang, J. Wang, S. Wang, et al. 2019. Microbes drive global soil nitrogen mineralization and availability. Glob. Chang. Biol. 25(3): 1078–1088.

McIsaac, G.F., M.B. David, and C.A.M.U. of Illinois. 2010. and Switchgrass Production in Central Illinois: Impacts on Hydrology and Inorganic Nitrogen Leaching. J. Environ. Qual. 39(5): 1790. doi: 10.2134/jeq2009.0497.

McLauchlan, K.K. 2006. Effects of soil texture on soil carbon and nitrogen dynamics after cessation of agriculture. Geoderma 136(1–2): 289–299.

O'Brien, S.L., and J.D. Jastrow. 2013. Physical and chemical protection in hierarchical soil aggregates regulates soil carbon and nitrogen recovery in restored perennial grasslands. Soil Biol. Biochem. 61: 1–13.

Rosenzweig, S.T., M.A. Carson, S.G. Baer, and J.M. Blair. 2016. Changes in soil properties, microbial biomass, and fluxes of C and N in soil following post-agricultural grassland restoration. Appl. soil Ecol. 100: 186–194.

Smith, C.M., M.B. David, C.A. Mitchell, M.D. Masters, K.J. Anderson-Teixeira, et al. 2013. Reduced nitrogen losses after conversion of row crop agriculture to perennial biofuel crops. J. Environ. Qual. 42(1): 219–228.

Tejera, M., N. Boersma, A. Vanloocke, S. Archontoulis, P. Dixon, et al. 2019. Multi-year and Multi-site Establishment of the Perennial Biomass Crop Miscanthus× giganteus Using a Staggered Start Design to Elucidate N Response. BioEnergy Res.: 1–13.

Tiemann, L.K., and A.S. Grandy. 2015. Mechanisms of soil carbon accrual and storage in bioenergy cropping systems. GCB Bioenergy 7(2): 161–174. doi: 10.1111/gcbb.12126.

Zhu, X., C. Liang, M.D. Masters, I.B. Kantola, and E.H. DeLucia. 2018. The impacts of four potential bioenergy crops on soil carbon dynamics as shown by biomarker analyses and DRIFT spectroscopy. GCB Bioenergy 10(7): 489–500. doi: 10.1111/gcbb.12520.

3.6 Figures and Tables

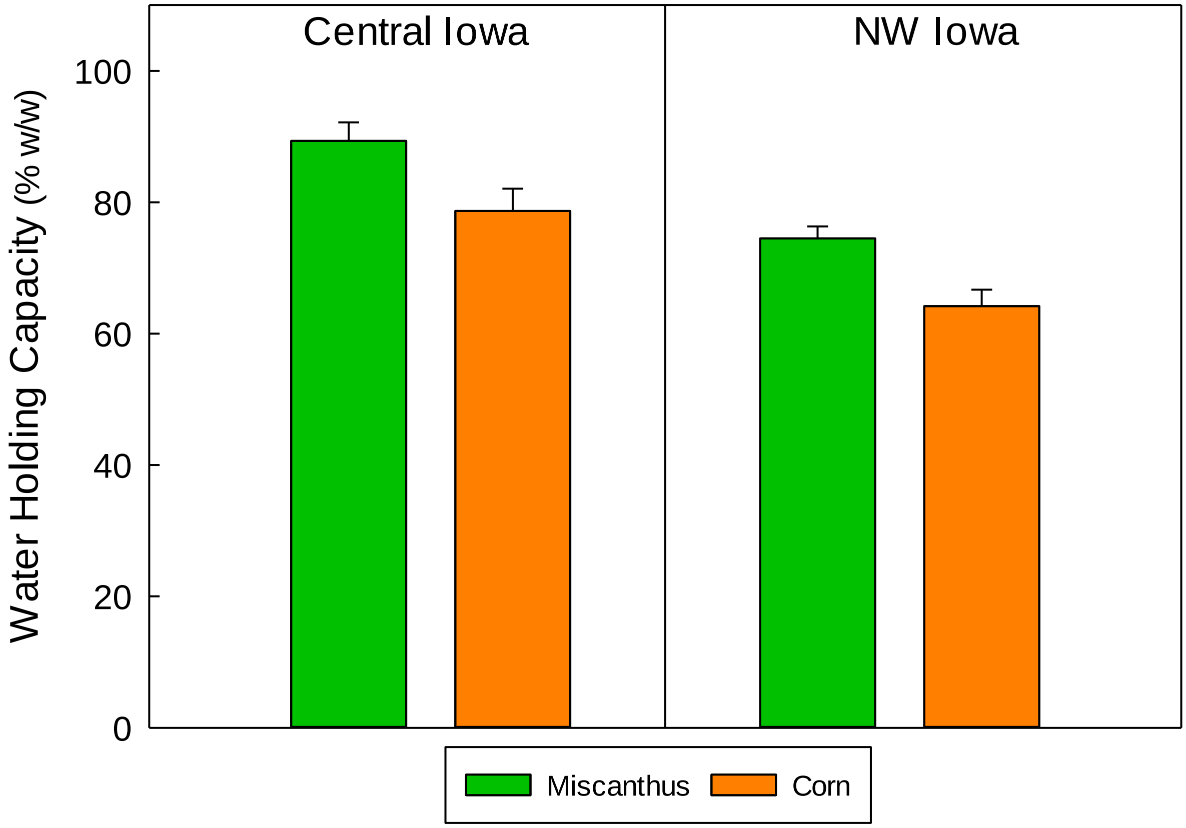

Figure 111 Water holding capacity of the soil for miscanthus and corn. Means with standard error bars (n=8).

Table 5 Effects of site, crop, and nitrogen fertilization on water holding capacity of soil in the top 15cm

	Water Holding Capacity		
	df	*F*	*P* value
Site (S)	**1**	**26.46**	**<0.0001**
Crop (C)	**1**	**13.56**	**0.0012**
Nitrogen (N)	1	0.05	0.8225
S × C	1	0.00	0.9531
S × N	1	0.43	0.5164
C × N	1	0.34	0.5676
S × C × N	1	0.39	0.5377

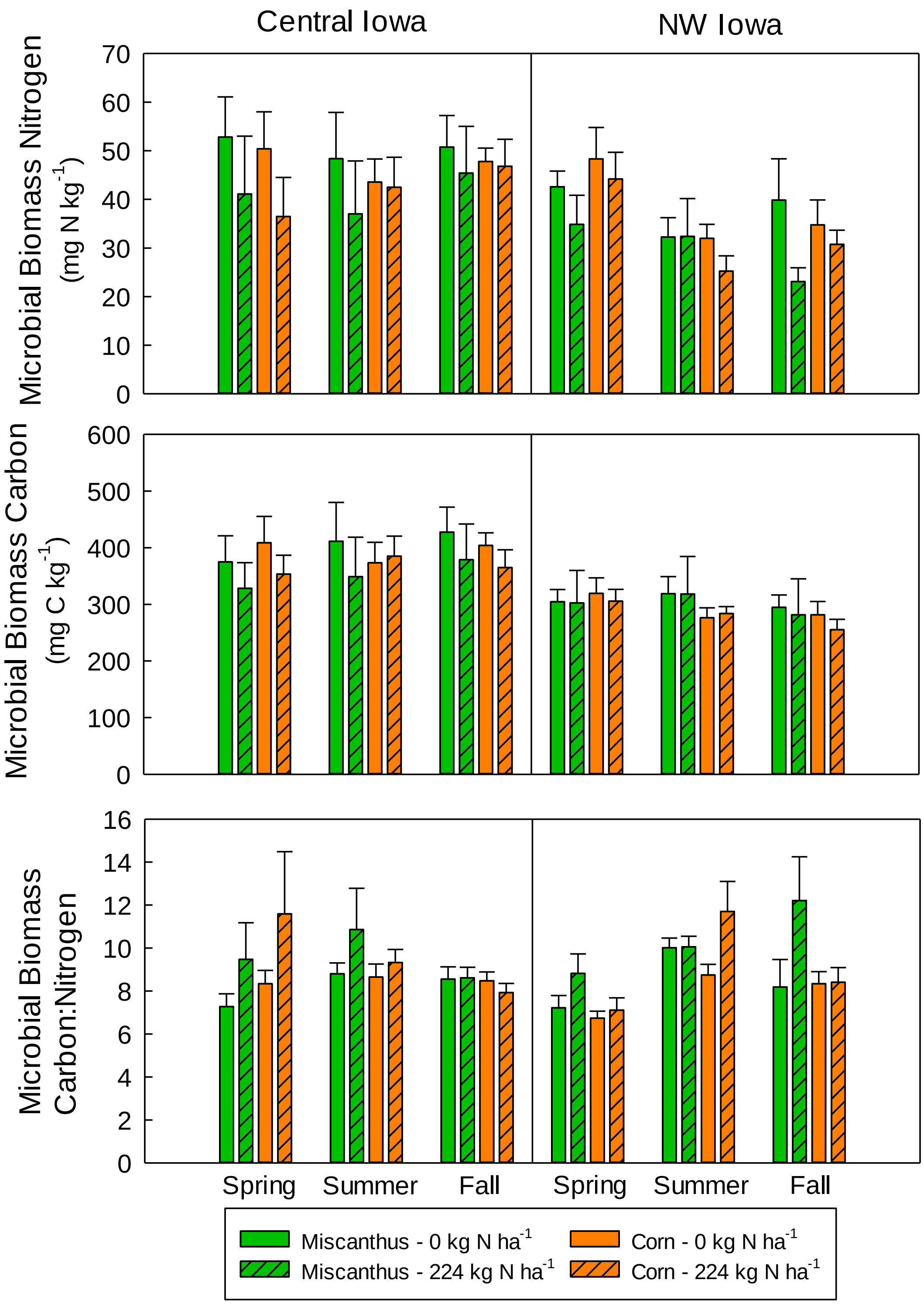

Figure 122 Microbial biomass: carbon, nitrogen, and carbon to nitrogen ratio for miscanthus and corn at 0 and 224 kg N ha^{-1} fertilization. Means with standard error bars (n=4).

Table 6 Effects of site, crop, nitrogen fertilization, and date on microbial biomass carbon, microbial biomass nitrogen, and microbial biomass carbon nitrogen.

	Microbial Biomass Carbon			Microbial Biomass Nitrogen			Microbial Biomass Carbon : Nitrogen		
	df	*F*	*P* value	df	*F*	*P* value	df	*F*	*P* value
Site (S)	**1**	**4.97**	**0.0672**	1	2.5	0.1651	1	0.00	0.9666
Crop (C)	1	0.07	0.7942	1	0	0.9556	1	0.56	0.4659
Nitrogen (N)	1	0.94	0.3450	**1**	**4.19**	**0.0556**	**1**	**6.92**	**0.0170**
Date (D)	2	0.12	0.8892	**2**	**9.01**	**0.0005**	**2**	**5.20**	**0.0090**
S × C	1	0.15	0.6987	1	0.2	0.6632	1	0.95	0.3437
S × N	1	0.42	0.5275	1	0.02	0.8998	1	0.05	0.8299
C × N	1	0.04	0.8463	1	0.29	0.5979	1	0.25	0.6217
S × D	**2**	**7.57**	**0.0014**	**2**	**7.85**	**0.0011**	**2**	**5.06**	**0.0101**
C × D	**2**	**4.51**	**0.0160**	2	0.59	0.5557	2	1.10	0.3413
N × D	2	1.18	0.3153	2	0.93	0.4005	2	0.55	0.5806
S × C × N	1	0.1	0.7605	1	0.01	0.9432	1	0.04	0.8369
S × C × D	2	0.76	0.4743	**2**	**2.5**	**0.0926**	2	2.16	0.1266
S × N × D	2	0.23	0.7993	2	2.31	0.1099	**2**	**2.44**	**0.0977**
C × N × D	2	1.72	0.1908	2	0.8	0.4544	2	1.49	0.2353
S × C × N × D	2	0.6	0.5532	2	2.16	0.1259	**2**	**2.58**	**0.0863**

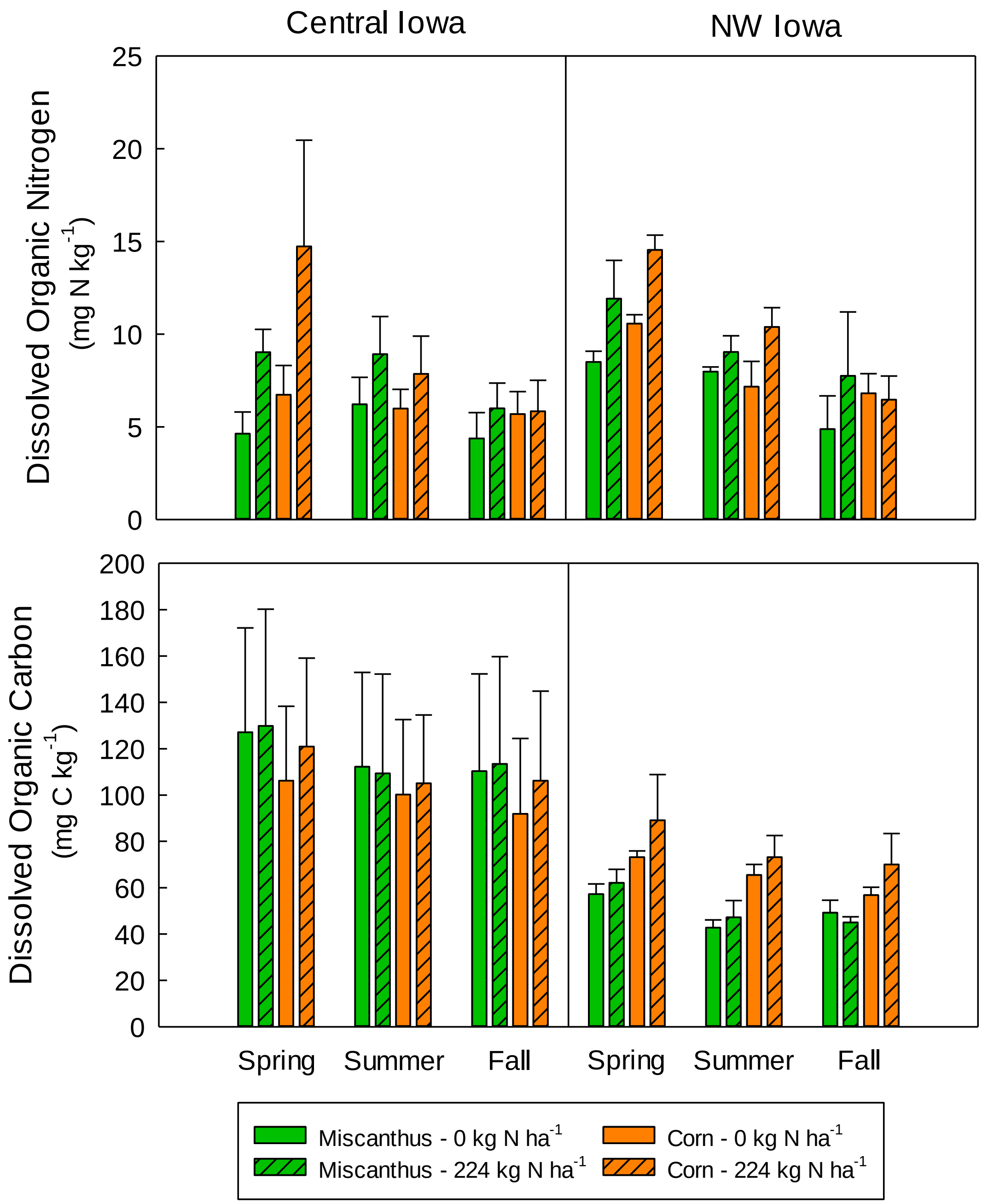

Figure 133 Soil dissolved organic nitrogen and dissolved organic carbon for miscanthus and corn at 0 and 224 kg N ha^{-1} fertilization. Means with standard error bars (n=4).

Table 7 Effects of site, crop, nitrogen fertilization, and date on dissolved organic carbon and dissolved organic nitrogen

	Dissolved Organic Carbon			Dissolved Organic Nitrogen		
	df	*F*	*P* value	df	*F*	*P* value
Site (S)	**1**	**6.46**	**0.0175**	**1**	**4.46**	**0.0448**
Crop (C)	1	0.05	0.8257	1	1.48	0.2357
Nitrogen (N)	1	0.11	0.7416	**1**	**9.85**	**0.0043**
Date (D)	**2**	**28.96**	**<0.0001**	**2**	**6.81**	**0.0044**
S × C	1	0.68	0.4157	1	0.09	0.7629
S × N	1	0.00	0.9827	1	0.34	0.5675
C × N	1	0.07	0.7930	1	0.04	0.8346
S × D	2	0.02	0.9795	2	0.24	0.7921
C × D	2	0.77	0.4742	2	1.71	0.2010
N × D	2	0.67	0.5223	2	1.90	0.1702
S × C × N	1	0.00	0.9973	1	0.00	0.9602
S × C × D	2	0.37	0.6924	2	0.37	0.6942
S × N × D	2	0.49	0.6173	2	0.22	0.8072
C × N × D	2	0.36	0.6994	2	0.45	0.6402
S × C × N × D	2	0.17	0.8432	2	0.46	0.6338

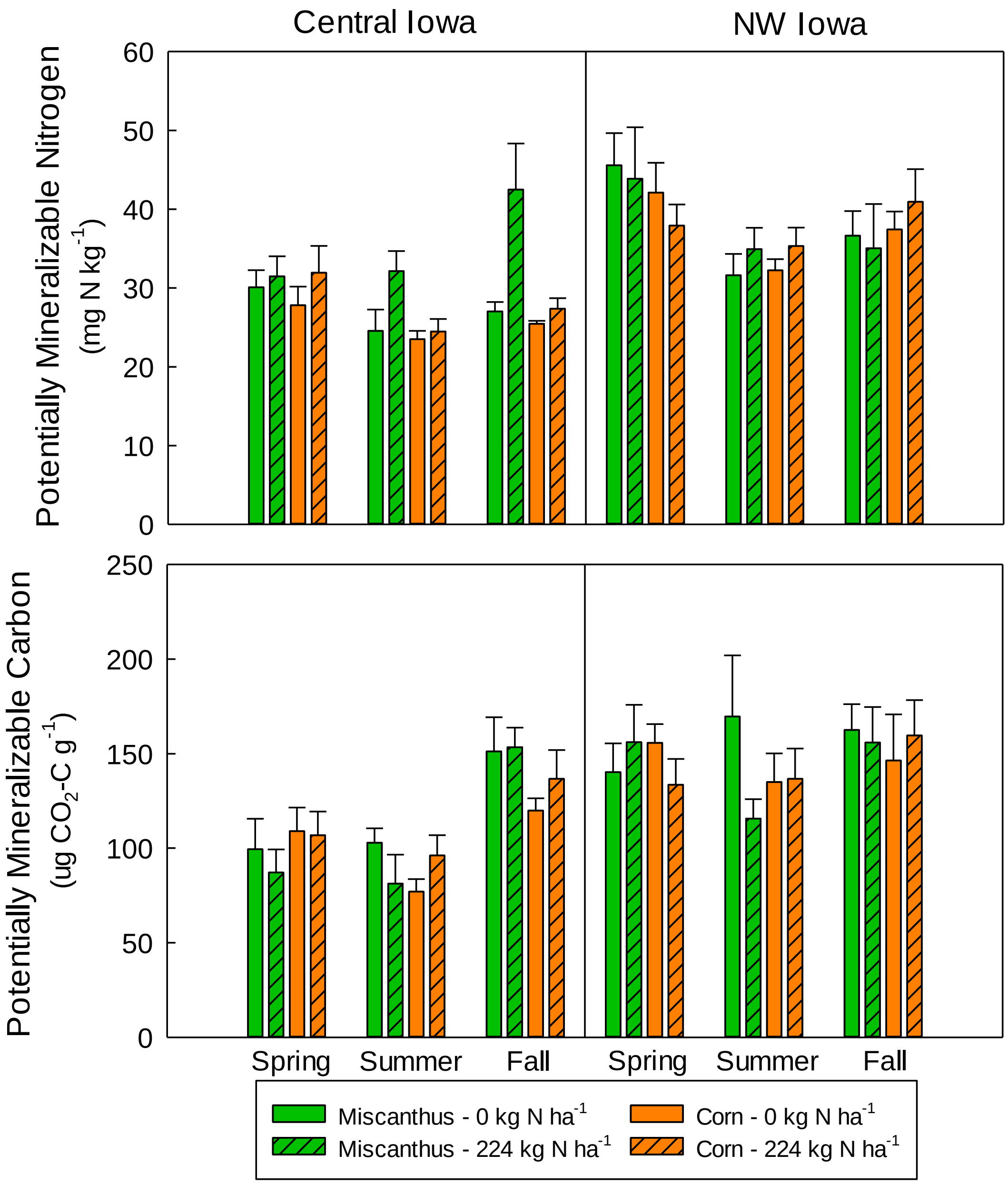

Figure 144 Potentially mineralizable nitrogen and carbon for miscanthus and corn at 0 and 224 kg N ha^{-1} fertilization. Means with standard error bars (n=4).

Table 8 Effects of site, crop, nitrogen fertilization, and date on potentially mineralizable carbon and potentially mineralizable nitrogen

	Potentially Mineralizable Carbon			Potentially Mineralizable Nitrogen		
	df	*F*	*P* value	df	*F*	*P* value
Site (S)	**1**	**19.4**	**0.0002**	**1**	**10.21**	**0.0187**
Crop (C)	1	0.39	0.5398	1	2.96	0.1023
Nitrogen (N)	1	0.25	0.6249	**1**	**4.04**	**0.0596**
Date (D)	**2**	**15.81**	**<0.0001**	**2**	**15.68**	**<0.0001**
S × C	1	0	0.9741	1	2.29	0.1475
S × N	1	0.28	0.5988	1	2.97	0.1022
C × N	1	1.04	0.3191	1	0.8	0.3842
S × D	**2**	**4.43**	**0.0172**	2	2.9	0.0647
C × D	2	1.39	0.2598	2	0.07	0.9311
N × D	2	1.31	0.2801	2	2.41	0.1003
S × C × N	1	0.08	0.7834	1	1.39	0.2535
S × C × D	2	1.04	0.3617	**2**	**5.46**	**0.0073**
S × N × D	2	0.69	0.5043	2	1.04	0.3603
C × N × D	**2**	**3.1**	**0.0543**	2	0.49	0.6177
S × C × N × D	2	0.94	0.3993	**2**	**3.22**	**0.0488**

CHAPTER 4. CONCLUSION

While shifting large portions of the Midwest from annual to a perennial crop, like *Miscanthus* × *giganteus* (Greef et Deu.), would take a paradigm shift, this study joins a larger, growing body of evidence showing consistent environmental benefits of perennial crops compared to annual crops. It is less clear, however, to what extent these benefits manifest on different soil types and climates, and how long after conversion to perennial crops it takes for these benefits to emerge. The novel staggered-start experiment used here allowed me to separate confounding environmental and stand-age effects on soil N dynamics under miscanthus while using continuous corn as a 'baseline'. This experimental design was useful for measuring key soil processes during these biogeochemically sensitive miscanthus establishment phase. Many soil processes are accelerated after any land use change (McDaniel et al., 2014) but eventually these processes stabilize. Soil nitrate leaching is probably the best example of accelerated processes (e.g., mineralization and nitrification), and without large amounts of living roots to take up the ammonium and nitrate, these nutrients are leached. I found that this was the case: both net N_{min} and nitrate leaching were greater in juvenile than mature miscanthus.

It is almost a misnomer to call a 3-year stand of miscanthus mature. Maturation typically occurs within two to five, and stands can perform well for 30 years (Hastings et al., 2009; Arundale et al., 2014; Lewandowski et al., 2016). However, there is enough evidence between the difference in biomass produced and differences in soil temperature and moisture to suggest that the 'juvenile' and 'mature' stands assessed here behaved as biophysically different agroecosystems. High spatial variability, as well as rich organic soils in Iowa, could be masking treatment effects (Kravchenko and Bullock, 2000) that were more observable elsewhere in miscanthus studies (Smith et al., 2013; Davis et al., 2015). Both soils from this study were derived from similar parent material (glacial till), but had very different soil fertility management histories (Table 1).

Cumulative net N_{min} tended to be greatest in the juvenile miscanthus and lowest in the mature miscanthus suggesting that there should be more N available for plant uptake during the first few years of miscanthus establishment compared to mature. Both soil health measurements, soil microbial biomass, and potentially mineralizable C and N had no significant crop effect, but the effect of miscanthus on these indicators of soil health might become more apparent with increased stand age.

Miscanthus increased soil water holding capacity by 15 % compared to corn. At a bulk density of 1.26 g cm^{-3} in the miscanthus and 1.23 g cm^{-3} in the corn, as measured at the Central site, this would increase the water stored in the top 15 cm of soil by 24 mm. This extra water storage could increase average productivity. Or perhaps even more importantly, this enhance water storage would make agroecosystems more resilient to droughts, like that of 2012.

I found that mature miscanthus reduced N leaching regardless of N fertilization. The values of N leaching reported in my research may be higher than what would be found in tile-drained studies as my measurements were at a shallower depth, but I believe the treatment effects to be real and would expect a decline of all N leaching at tile drainage depth. This shows that miscanthus when incorporated in the Midwest will reduce N leaching and improve water quality, although the mechanism for this enhanced uptake remains unclear.

More research, beyond the first few years of establishment, is needed to determine how longer production of miscanthus will further alter soil properties and N dynamics. Combining this research with the work done by Tejera et al. (2019), will be the first steps toward a more comprehensive understanding of miscanthus development and impacts on the soil. In my opinion, revisiting this experiment beyond the establishment years of miscanthus will be key to understanding how stand-age affects soil-plant N cycling dynamics.

4.1 References

Arundale, R.A., F.G. Dohleman, E.A. Heaton, J.M. Mcgrath, T.B. Voigt, et al. 2014. Yields of M iscanthus× giganteus and P anicum virgatum decline with stand age in the Midwestern USA. Gcb Bioenergy 6(1): 1–13.

Davis, M.P., M.B. David, T.B. Voigt, and C.A. Mitchell. 2015. Effect of nitrogen addition on Miscanthus× giganteus yield, nitrogen losses, and soil organic matter across five sites. Gcb Bioenergy 7(6): 1222–1231.

Hastings, A., J. CLIFTON-BROWN, M. Wattenbach, C.P. Mitchell, and P. Smith. 2009. The development of MISCANFOR, a new Miscanthus crop growth model: towards more robust yield predictions under different climatic and soil conditions. Gcb Bioenergy 1(2): 154–170.

Kravchenko, A.N., and D.G. Bullock. 2000. Correlation of corn and soybean grain yield with topography and soil properties. Agron. J. 92(1): 75–83.

Lewandowski, I., J. Clifton-Brown, L.M. Trindade, G.C. van der Linden, K.-U. Schwarz, et al. 2016. Progress on optimizing miscanthus biomass production for the European bioeconomy: Results of the EU FP7 project OPTIMISC. Front. Plant Sci. 7: 1620.

McDaniel, M.D., J.P. Kaye, and M.W. Kaye. 2014. Do “hot moments” become hotter under climate change? Soil nitrogen dynamics from a climate manipulation experiment in a post-harvest forest. Biogeochemistry 121(2): 339–354.

Smith, C.M., M.B. David, C.A. Mitchell, M.D. Masters, K.J. Anderson-Teixeira, et al. 2013. Reduced nitrogen losses after conversion of row crop agriculture to perennial biofuel crops. J. Environ. Qual. 42(1): 219–228.

Tejera, M., N. Boersma, A. Vanloocke, S. Archontoulis, P. Dixon, et al. 2019. Multi-year and Multi-site Establishment of the Perennial Biomass Crop Miscanthus× giganteus Using a Staggered Start Design to Elucidate N Response. BioEnergy Res.: 1–13.

APPENDIX. ADDITIONAL RESULTS

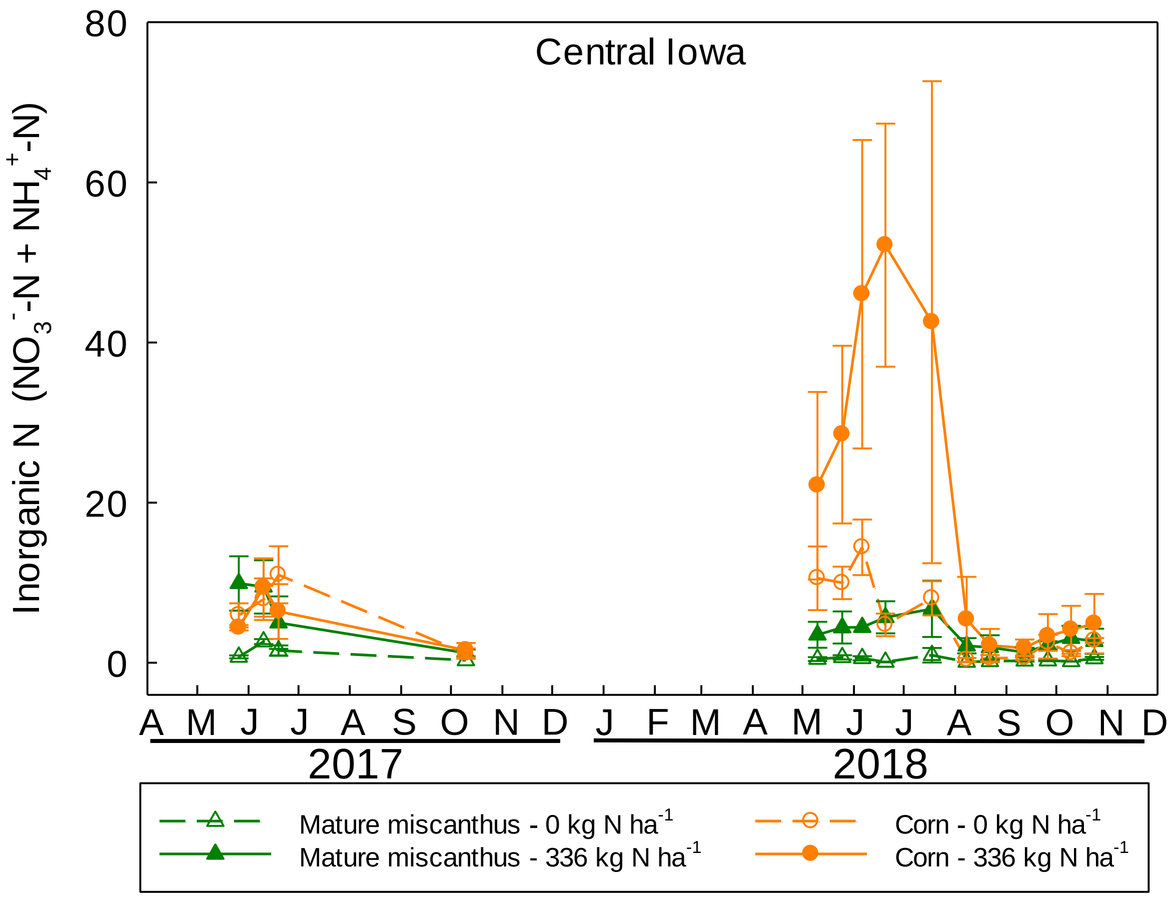

Figure 15 Inorganic nitrogen in soil water at 50 cm depth, as measured by suction cup lysimeters, during 2017 and 2018 for mature miscanthus and corn. Data points are means with standard error bars (n=4).

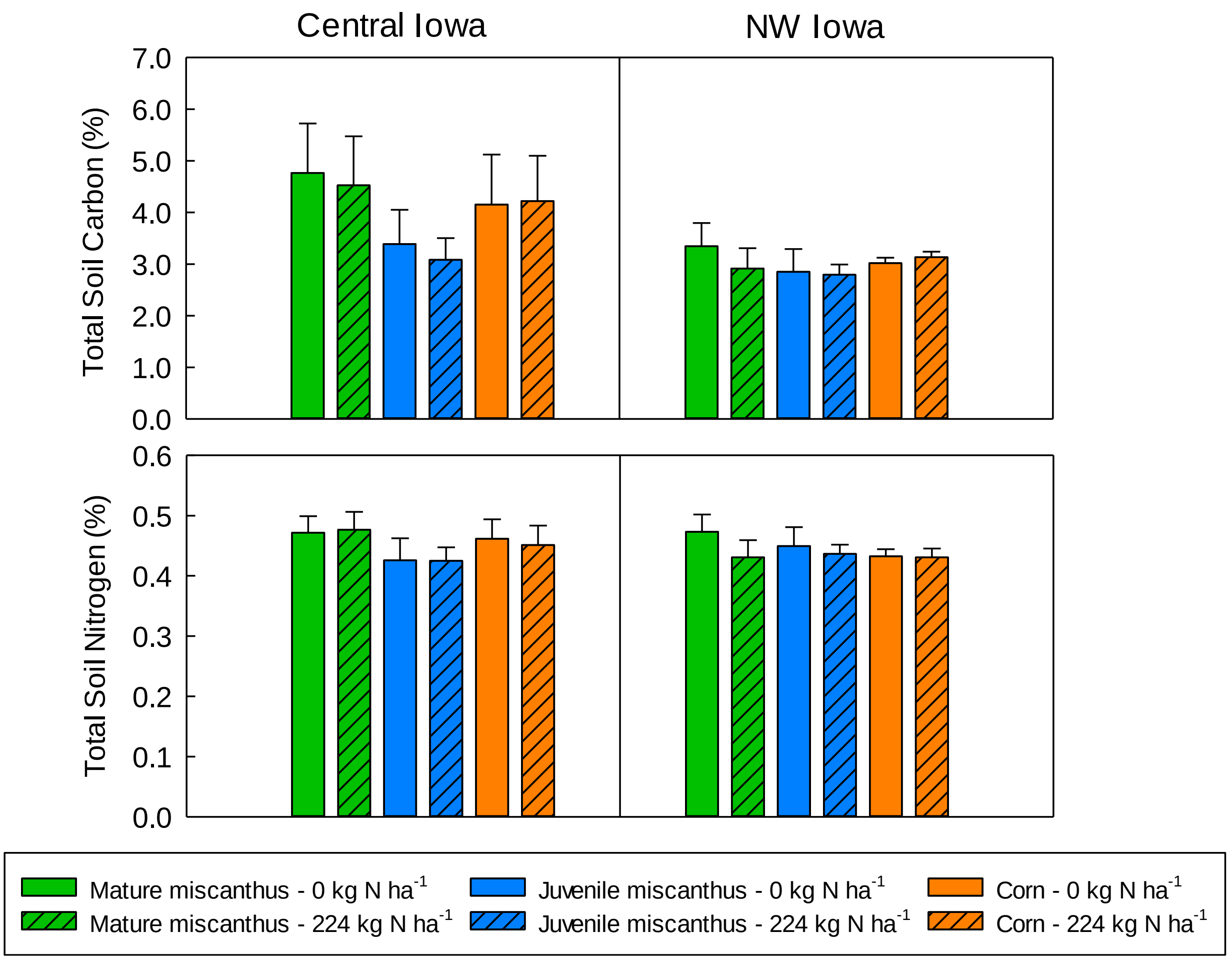

Figure 16 Percent soil carbon and nitrogen in top 15 cm for mature miscanthus, juvenile miscanthus, and corn at 0 and 224 kg N ha^{-1} fertilization. Means with standard error bars (n=4).

Table 9 Effects of site, cropping system, and nitrogen fertilization on total soil carbon and nitrogen.

	Total C			Total N		
	df	*F*	*P* value	df	*F*	*P* value
Site (S)	**1**	**7.33**	**0.0352**	1	0.33	0.5874
Cropping System (C)	2	1.99	0.155	2	1.22	0.3088
Nitrogen (N)	1	0.15	0.698	1	0.48	0.4948
S × C	2	0.79	0.4613	2	0.81	0.4565
S × N	1	0	0.9622	1	0.31	0.5843
C × N	2	0.12	0.8887	2	0.07	0.93
S × C × N	2	0.03	0.9698	2	0.29	0.752

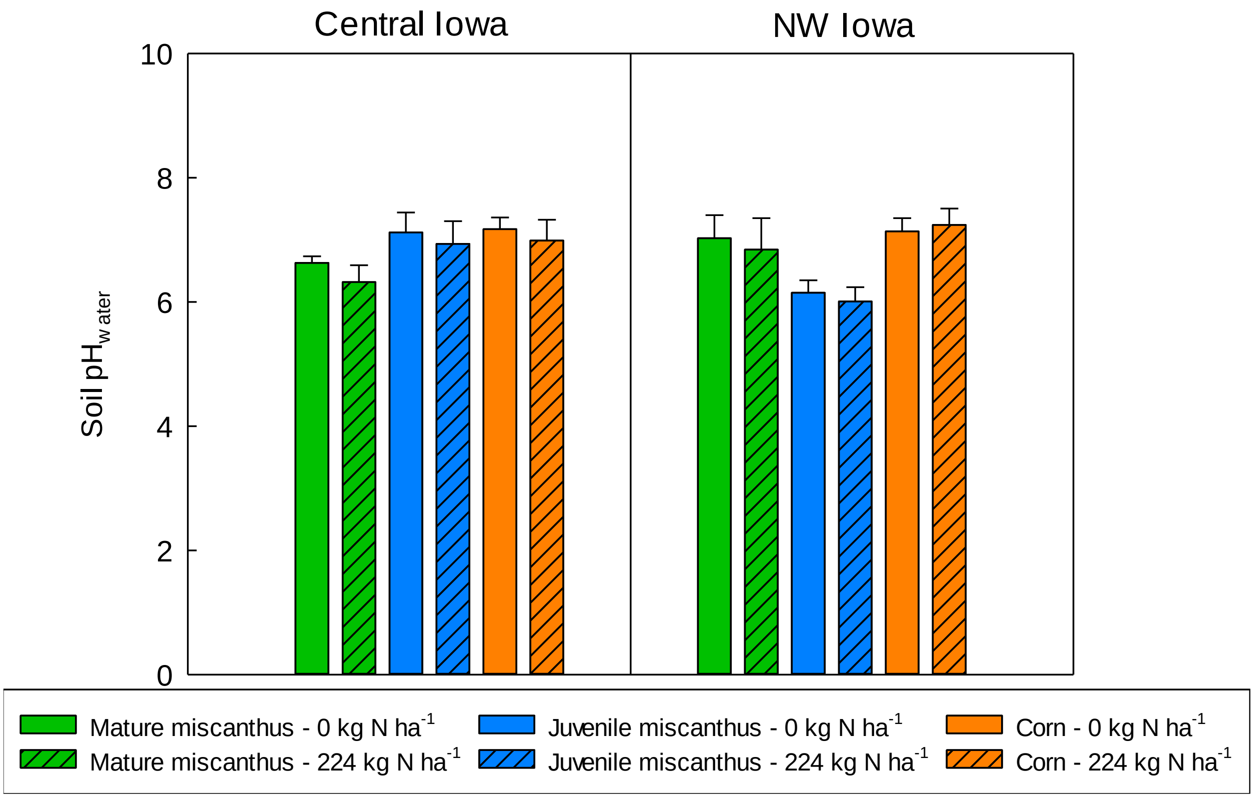

Figure 17 Soil pH in top 15 cm for mature miscanthus, juvenile miscanthus, and corn at 0 and 224 kg N ha^{-1} fertilization. Means with standard error bars (n=4).

Table 10 Effects of site, cropping system, and nitrogen fertilization on soil pH.

	pH		
	df	*F*	*P* value
Site (S)	1	0.2	0.6711
Cropping System (C)	**2**	**6.21**	**0.0055**
Nitrogen (N)	1	1.14	0.2938
S × C	**2**	**9.17**	**0.0008**
S × N	1	0.3	0.591
C × N	2	0.18	0.8345
S × C × N	2	0.06	0.9383

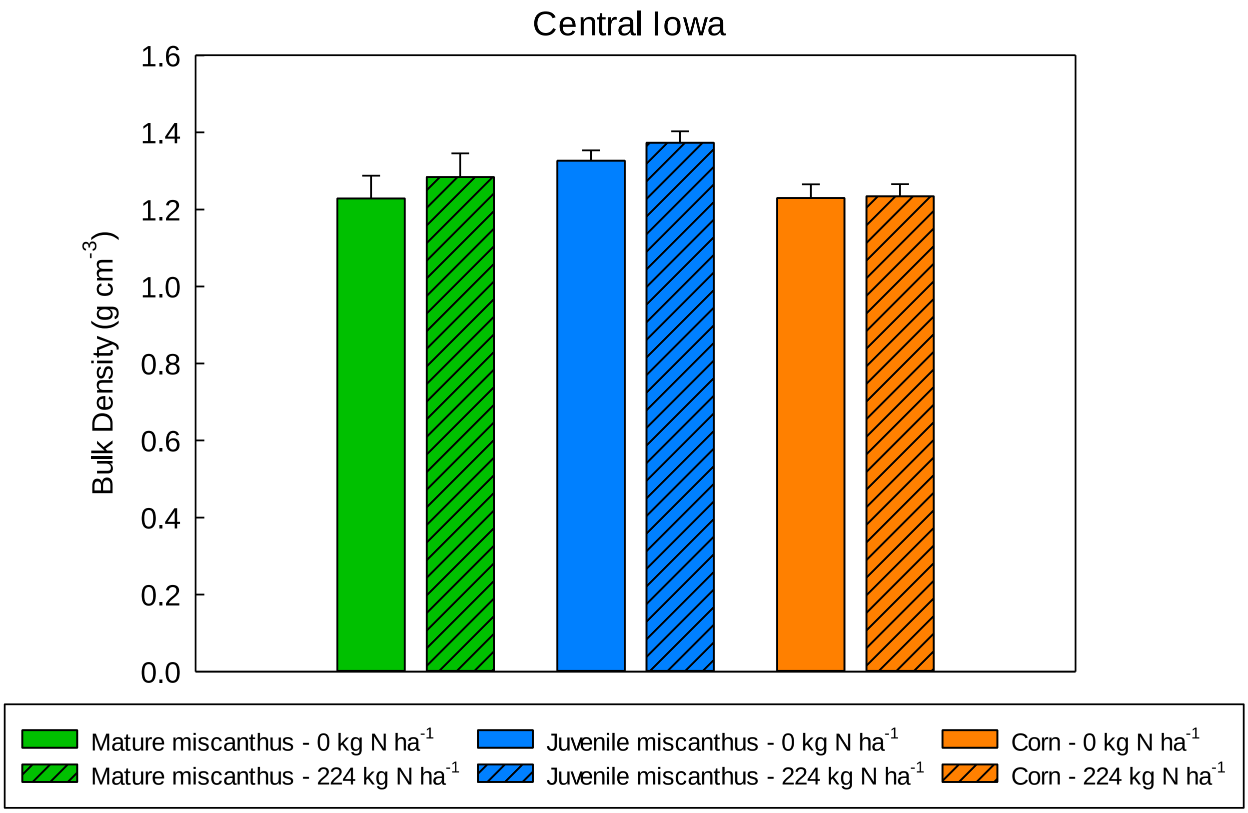

Figure 18 Bulk density of soil in surface 15 cm for mature miscanthus, juvenile miscanthus, and corn at 0 and 224 kg N ha^{-1} fertilization. Means with standard error bars (n=4).

Table 11 Effects of cropping system and nitrogen fertilization on bulk density at the Central Iowa site.

	Bulk Density		
	df	*F*	*P* value
Cropping System (C)	**2**	**4.16**	**0.0327**
Nitrogen (N)	1	1.02	0.3257
C × N	2	0.2	0.8231