ASSESSING THE IMPACTS OF TANNIN DIETS ON LAND APPLICATIONS OF DAIRY MANURE

By

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Before I started the journey into graduate school, I was told that “graduate school is the most selfish thing you’ll ever do,” and now I truly have come to understand what that means. Graduate school is a community endeavor with one goal: to let me do what I love; in a word, learn.

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Dedication

I dedicate my thesis in loving memory to Tim Myrick, my mentor. Tim was a lifelong environmental engineer and through volunteering as a guest speaker in my high school environmental science classes, he became the first to introduce me to the fascinating world of soil. Tim helped me land my first job, steered me towards environmental work, and was an integral part in my decision to come to graduate school. Tim lost his cancer battle in December 2014, but remained my mentor through it all.

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Chapter 1

Greenhouse Gas Abatement and Nutrient Management Potential from Tannin Enhanced Dietary Trials in Dairy Production Systems

Agricultural greenhouse gas (GHG) emissions are responsible for 10% of total US emissions by economic sector (EPA 2012). A primary concern for the US dairy industry is the mitigation of GHG emissions during manure management, which is responsible for significant emission loads during manure handling and land application. In an effort to reduce GHG emissions from dairy farms, new technologies and practices are used to mitigate methane (CH_4), nitrous oxide (N_2O), carbon dioxide (CO_2) and ammonia (NH_3). Although NH_3 is not a direct GHG, after deposition in terrestrial ecosystems it becomes an indirect source of N_2O and is responsible for acid rain. One of the most promising techniques to mitigate GHG emissions from dairy systems is enhancing the nutritional quality of the rations fed to dairy cows. Previous studies have found that reducing crude protein and increasing fiber digestibility in dairy forages can reduce CH_4 emissions during rumination and subsequent manure handling (Aguerre et al. 2012, Montes et al. 2014).

Other feed additives to dairy cow rations can be used to change the availability of N sources, including polyphenol compounds like tannins. Polyphenol compounds naturally occur in most plants at concentrations from 1-25% of total organic matter depending on the type of tissue and plant species. The word tannin is derived from Tannenbaum, and the compounds are commonly found in conifer trees. Tannins occur in higher concentrations in broadleaf plants typically found in warmer climates, including oak

(*Quercus spp.*), chestnut (*Castanea spp.*), and quebracho (*Schinopsis spp.*). Lespedeza (*Sericea lespedeza*) and birdsfoot trefoil (*Lotus corniculatus*), common forages in the southern United States, can have concentrations as high as 18% of dry matter (MacAdam et al. 2013). Polyphenols are astringent compounds that bind to plant protein during digestion and can protect nitrogen (N) through rumination in the gut of the cow, essentially working against microorganism herbivory (Hättenschwiler and Vitousek 2000, Powell et al. 2011).

The effects of polyphenol compounds from plant materials to terrestrial carbon (C) and N cycling are not well known especially at the field scale (Hättenschwiler and Vitousek 2000). Studies have shown that polyphenols in plant litter are capable of inhibiting soil nitrifiers, which simultaneously slows N mineralization rates and increases organic N concentrations in soils (Kuiters et al. 1990, Millar and Baggs 2004). This mechanism protects protein sources against microorganism herbivory, which is particularly effective at protecting N sources in N limited ecosystems, including tropical forests and coniferous forests, by maintaining N concentrations in organic forms (Hättenschwiler and Vitousek 2000, Mutabaruka et al. 2007, Millar and Baggs 2004). Mutabaruka et al. (2007) found that polyphenol compounds with high protein binding capacities were initially rapidly degraded in agricultural soils, but over time microbial communities became less effective at degrading plant tissues with higher polyphenol concentrations, suggesting that polyphenols can influence N and C cycling over extended periods of time. The combined effects of protein binding and nitrification inhibition suggest that polyphenols are an under studied N management strategy, particularly in agricultural systems where reactive N is quickly lost through leaching, volatilization, or gaseous means.

There are still major gaps in understanding the roles of polyphenols and tannins in nutrient cycling, especially in agricultural soils. Because of the volatility of polyphenols, little is known about how their chemical characterization in agricultural settings, because as organic matter decomposes, the tannins rapidly change composition and relative abundance (Hättenschwiler and Vitousek 2000). Because of this volatility, very little is known about how much of the tannin added to dairy cow rations remains in manure after being fed to dairy cows, and manure tannin subsequent effects on soil nutrient cycles. Tracing the impact of tannin manures on nutrient cycling in dairy forages is also unknown, and warrants further study.

Because of tannin's ability to affect nitrification and mineral N availability in soils, tannins have high potential to mitigate GHG emissions from soil (Hättenschwiler and Vitousek 2000, Osterholtz et al. 2014, Powell et al. 2009). Previous research shows that at high concentrations in plant tissues, polyphenol compounds delay plant residue decomposition in soils, which slows CO_2 respiration especially in ecosystems having low pH soils (Mutabaruka et al. 2007). Particularly of interest for this study, tannin, like other polyphenols, may reduce reactive N and therefore total N_2O flux from soils. Nitrous oxide, the most potent GHG emitted from soils in terms of global warming potential, is the most common end product of denitrification, and represents 2.8 Tg N_2O-N annually (IPCC 2007). As land applied manure is the largest source of agriculture borne N_2O

emissions, mitigation strategies aimed at manure management could reduce overall emissions from animal production systems.

Studies found that plant tissues incorporated into tropical soils with high polyphenol concentrations resulted in lower N_2O emissions from controlled experimental treatments, even when N concentrations in plant tissues were the same (Millar and Baggs 2004). Across several species, strong negative relationships have been established relating N_2O emissions to total polyphenol content and protein binding capacity (Millar and Baggs 2004; and Baggs et al. 2001).

Although there is extensive research on the effects of polyphenol compounds on the N and C cycles of terrestrial ecosystems, only few studies have specifically looked specifically at tannins as a livestock feed additive. Tannin additions to Holstein dairy cow diets did not affect milk production but increased milk protein (Nx 6.36) while decreasing urea-N concentrations in milk (Aguerre et al. 2010). The same tannin trials also revealed decreased concentrations of urea-N in urine, suggesting that tannin may impede the activity of urease enzymes in dairy cows, which helps reduce overall ammonia emissions from barn floors (Powell et al. 2011a). In laboratory studies, tannin extracts from quebracho and chestnut tree additions to diets fed to Holstein dairy cows resulted in increased feed N efficiency (more feed N secreted as milk N, rather than manure N) and reduced soil born NH_3 emissions by as much as 23-49% in silt loam soils (Powell et al. 2011b). This suggests that tannins may inhibit rumen microbial functions providing more recalcitrant N residues in manure which impacts soil N cycles after manure application.

These findings corroborate that tannin incorporation into dairy cow nutrition may slow N cycling throughout dairy production systems which could maximize N utilization in forage cropping systems while also providing a better quality milk product.

Previous research has studied tannin feeding trials and implications to GHG emissions and soil N at the laboratory scale, but manures from nutrition feeding trials have not been land applied to understand GHG emissions and plant N response at the field scale.

The objectives of this study are to determine the effect of tannin concentration in feed on: (i) GHG emissions after land application of manure to soil, (ii) plant production and N response to manure application, and (iii) seasonal plant available N concentrations from soil. Three experiments were conducted to address these objectives. Initially, a field-level application of manure was conducted to assess GHG emissions, soil N, and plant response to tannin manure applications. Following this experiment, a greenhouse experiment was completed with manure applications to pots over a 6 month period to assess possible long-term effects of tannin manures to plant production and soil with multiple application frequencies of manure over time. To more closely examine soil N mineralization following the field experiment, an incubation experiment was conducted to determine the effects of tannin on soil N mineralization in a temperature and soil moisture controlled setting. All three studies were conducted using the same soil, to assess the impact of tannin manures on Wisconsin dairy forage production systems.

Chapter 2

Assessing the Impact of Tannin Diets on Land Applications of Dairy Manure

Abstract

Growing concerns about environmental impacts of dairy farms has driven producers to address greenhouse gas (GHG) emissions and N losses from soil following land applications of manure. Tannin dietary additives have proved to be a successful technique to mitigating NH_3 emissions in barns and result in higher N concentrations in less reactive manure solids. Tannin diets at three levels (0, 0.4, and 1.8% of dry matter intake) were assessed in manures applied to soils at two N rates (240 and 360 kg N ha^{-1}) to determine if diet tannin level and manure N rate had GHG mitigation potential, affected soil N concentrations, or impacted corn silage production at the field level. Through the first growing season from May to September 2014, there were no significant differences in GHG emissions from tannins or N application rate. Soil NH_4^+-N concentrations were significantly lower with tannin manure in the first 14 days following manure application, and NO_3^- -N concentrations were significantly less 20 days after manure application, suggesting that there was a delay in N mineralization with tannin manures. Pre-plant soil samples the year following manure applications showed higher total soil inorganic N, suggesting that tannins may provide a N credit during the second growing season. Tannin did not negatively impact corn silage production in the first growing season, and in the second growing season, no differences were observed between manure treatments and control plots, suggesting that there was not significant N carryover into the second

growing season. Overall, tannin manures show potential to slow N mineralization, although this did not significantly impact corn silage yield or feed quality.

Introduction

Agricultural greenhouse gas (GHG) emissions are responsible for 10% of total anthropogenic emissions, or 652.6 million metric tons of carbon dioxide (CO_2) equivalents annually, based on gross production in the United States (EPA 2012). The dairy and beef industries are responsible for 65% of total animal agricultural GHG emissions, particularly in the forms of methane (CH_4) and nitrous oxide (N_2O) (Anderweg et al. 2014). Though these GHGs are usually emitted in smaller quantities than CO_2, CH_4 and N_2O have a global warming potential 25 and 298 times higher than CO_2 respectively (IPCC 2007). Thus per unit of emission (e.g., kg) CH_4 and N_2O gases are longer lived with greater impact to atmospheric warming. Across the livestock industry, CH_4 represents 44% of total emissions, N_2O 29%, and CO_2 27% (Gerber et al. 2013). Soil borne GHG emissions, mostly in the form of N_2O from manure land applications, represent a significant amount of total GHG emissions from the dairy industry.

In order to mitigate GHG emissions, nutritional changes for dairy cows, including changes to silage digestibility and crude protein levels, have resulted in lower overall enteric CH_4 from rumen fermentation and changes to manure chemistry, especially C:N ratio and manure pH, which influence total GHG losses (Anderweg et al. 2014, Powell et al. 2011a). However, few studies have determined the impact of nutritional feeding trials on soil borne GHG emissions from applications of various manures. Studies have shown that polyphenol compounds commonly found in higher order plants and forages have the ability to slow nitrification, improve N use efficiency of the manures derived from these feeds, and potentially mitigate GHGs (Hättenschwiler and Vitousek 2000, Mutabaruka et al. 2007, Powell et al. 2011b). Tannin, a polyphenol compound that binds to and protects protein during rumen fermentation, is a feed additive that has many positive results. Previous studies have found that increasing tannin additives to feed reduces bloat in cattle and can improve milk quality in dairy cows, with higher protein concentrations in milk (MacAdam et al. 2013, Aguerre et al. 2010). Feeding tannin also has shown results of reduced gaseous emissions from dairy manure, especially ammonia (NH_3) volatilization from barn floors and soil incubations (Powell et al. 2011a, Powell et al. 2011b). Because feed protein is protected during digestion, more N is concentrated in manure solids than urine, and there are some studies that suggest that tannin may inhibit activity of the urease enzyme, thus lowering overall urea-N in urine, and decreasing overall ammonia volatilization (Powell et al. 2011a).

To date, no studies have been done to investigate tannin impacts as a feed additive on manure N levels and on soil borne GHG emissions that arise from tannin manure applications at the field level. Additionally, no studies have been conducted to determine how tannin manure influences soil N mineralization and plant response. The objectives of this study are to determine soil GHG mitigation potential from tannin derived manures, assess soil N availability following land applications of tannin manures, and determine the effects on plant N uptake and production from manure applications over two growing seasons at the field level.

Materials and Methods

Site Description and Experimental Design

The study was conducted at the U.S. Dairy Forage Research Center in Prairie du Sac, WI, (43.33° N, 89.72° W) during the 2014 and 2015 growing seasons. The experimental design was a randomized complete block, with two whole-plot factors, with each plot 3 x 4 m. The first whole-plot factor was manure derived from three dietary tannin concentrations: 0, 0.4, and 1.8% tannin as dry matter intake of the total mixed rations that was fed to lactating Holstein dairy cows. The second whole-plot factor was target manure N rate, at two target levels: 240 and 360 kg N ha^{-1}. The soil type throughout the field was a St. Charles silt loam (Fine-silty, mixed, superactive, mesic Typic Hapludalfs). The plots were situated on a south-west facing slope of 2%. Field plots were blocked together as treatment replicates based on their location along the slope, with 7 plots in each block and 6 total blocks. All blocks were randomized, and each treatment was present in every block along with a control plot with no manure applied. The field was planted to field corn for silage on 19 May 2014 and 1 May 2015 and harvested for silage on 17 September 2014 and 08 September 2015.

Manure Collection and Field Application

Manure was collected from a dairy cow nutrition trial conducted at the USDA Dairy Forage Research Center in Prairie du Sac, WI. The tannin extract (Bypro; Silvateam, Indunor S.A., Argentina) added to the cow diets was comprised (by weight) of approximately one third chestnut tannin extract and two thirds quebracho tannin extract having the following chemical properties (polyvinylpyrrolidone powder method; Tempel, 1982): tannin concentration of 792 g kg^{-1} and pH of 3.72. Thirty six mid-lactation cows were randomly assigned to one of three diets, 0% tannin (0T), 0.45% tannin (LT), and 1.8% tannin (HT) of total dry matter intake (DMI). The tannin was added to a normal total mixed rations (TMR) diet containing a mix of 21% alfalfa silage, 29% corn silage, 50% concentrate (ground corn, solvent soybean meal, roasted soybeans, soy hulls, and cottonseed) on a total dry matter basis. Manure collection was staggered so that manure was collected from all dairy cows on days 95 and 96 of the 105 day nutrition trial. The feeding trial had a staggered start for four replicates of each treatment such that manure collection occurred on 10 and 11 March 2014, 24 and 25 March 2014, 31 March and 1 April 2014, and 7 and 8 April 2014. Manure was stored in 1800 L sealed bins until 5 May 2014, when manure was mixed by tannin level and transferred into buckets, which were sealed with lids, and stored for ten more days, until field application on 15 May 2014 by pouring the bucket manure contents between eventual corn rows.

To achieve the targeted N application rates, samples from each manure storage bin corresponding to the 3 tannin levels were collected and analyzed on 8 April 2014 to determine the total N content of the manures. At the time of manure application, 40 days after the original collection and analysis, manure N was analyzed again to determine the actual manure N application rates.

Manure was field applied on 15 May 2014. Immediately before application, the manure for each inter-corn row band was homogenized within a 18.9 liter bucket using a drill powered mixer. Two, 100 mL manure subsamples were collected from each bucket with a stainless steel ladle. The first sample was stored on ice in coolers in the field, and the second sample was acidified to pH 2.0 for later mineral N determination. Manure application was applied by block, and within each block the treatments were applied by increasing manure tannin concentrations and N rate. The tractor tires and roto-tiller were cleaned between treatments by tilling up untreated soil adjacent to the research plots and then spinning off excess soil between applications of manures at the different tannin levels. Each plot received manure in bands between corn rows, with three manure bands in each plot, extending the entire length (4 m) of the plot. The manure bands were further spread with a stainless steel shovel, so that each band had a width of 30 cm. Within approximately 15 minutes of manure application, manure was incorporated 10 cm below the soil surface with a tractor mounted roto-tiller. No additional fertilizer was applied.

Soil Measurements

Soil samples were taken at 0-10 and 10-20 cm depths using a 2 cm diameter soil probe on days 1, 2, 4, 13, 18, 62, 75, 89, 103, 142, 347, 389, and 483 of the experiment (here Day

1 corresponds to the day of manure application). Soil samples were taken in between corn rows, in the end two meters of the outside rows of each plot in order to cause the least disturbance on soil and corn plants within the interior of the plot from which gas measurements and plant harvests would be taken. In 2015, soil samples were taken in the same manner one month prior to planting on 27 April 2015, one month after planting on 8 June 2015, and at harvest 9 September 2015. Soil samples were dried in a forced air drier for 48 hours and ground to pass through a 2 mm sieve. Soil nitrate-N and ammonium-N concentrations were determined using a 2.0 M KCl extraction procedure, shaking extracts for 15 minutes, and filtering with #18 Whatman filters (Doane and Horwath 2003) and analyzed colormeterically using a Lachat (Lachat Instruments, Loveland, CO, 1996). Soil pH (1:1 water) was determined on all soil samples for all soil sampling days using an Accumet AB 150 pH analyzer (Fisher Scientific, Pittsburg, PA, 1998).

Greenhouse Gas Measurements

Greenhouse gas measurements were taken intensively at 0 (immediately following manure incorporation), 6.5, 10, 23, 30, 47, 53, and 76 hours following manure application, and then sampled at 7-10 day intervals throughout the growing season. At 0, 6.5, and 10 hours, sampling occurred in three blocks, but occurred in all six blocks for all subsequent measurements. Gas was measured on days 1, 2, 3, 4, 13, 18, 25, 39, 47, 54, 61, 68, 80, 87, 94, 101, 111, and 125 (here Day 1 corresponds to the day of manure application).

All greenhouse gas measurements were taken using a GasMet DX 4030 Fourier Transform Infrared Spectrometer gas analyzer (FTIR) (GasMet Technologies, Helsinki, Finland). At the beginning of each measurement day, the FTIR was flushed with high purity N_2 gas and run for at least 20 minutes to equilibrate to ambient air temperature. The portable FTIR was operated using a generator in the field, and covered with reflective insulation to reduce heating from solar radiation. If the ambient air temperature increased by 10° C or more during a gas measurement day, the system was re-flushed with N_2 gas mid-day and allowed to equilibrate to the new ambient air temperature.

Closed static gas chambers constructed of stainless steel following the USDA GraceNet protocol were used in all 42 field plots (Parkin and Venterea et al. 2010). Each chamber lid was 91.4 cm long, 40.6 cm wide, and 15.2 cm high. Anchor dimensions were 91.4 cm long, 40.6 cm wide, and were inserted approximately 15.2 cm into the soil. Chamber anchors were deployed immediately following manure application, and were only removed once for corn planting on 19 May 2014. After planting, anchors were installed permanently for the rest of the growing season and inserted flush with the soil surface using a tractor mounted Giddings probe and a stainless steel plate. Chamber lids were wrapped in reflective insulation for temperature control and vented on the side to allow air mixing within the chamber headspace. The chamber lip was fitted with 0.5 cm thick window insulation to create an air tight seal, and 2 inch metal binder clips were used to hold the lid in place during all measurements. Teflon tubing connected the chamber headspace to the FTIR in a closed system, allowing gas to be pumped into the FTIR for measurement then returned to the chamber headspace. To insure the least possible disturbance to soil and corn, the Teflon tubing was at least 3 m in length, allowing the measurement operator to enter the plot with as little impact on the plot as possible. This tubing was routinely checked for damage, breaks, and kinking in the sample lines.

Greenhouse gas flux was determined using linear regression fitting following Collier et al. (2014), and GraceNet protocol standards (Parkin and Venterea et al. 2010). Greenhouse gas emission loads over the growing season were calculated using trapezoidal interpolation between sampling time points.

Plant Measurements

In 2014 and 2015, all field plots were planted in SmartStax Refuge Advanced ® corn for silage (DuPont Pioneer, Johnston, IA). Manure was applied in May of 2014, and no further fertilizer additions were made throughout the study. Corn was grown for 120 days in 2014 and 122 days in 2015. Plots were harvested by hand, taking the interior two meters of the 2 middle corn rows of each plot to determine treatment yield. Corn stalks were harvested by hand with machetes, cutting the stalks at approximately 15 cm above the soil surface. Harvested corn biomass was weighed immediately, then a sub-sample of 4 corn plants from each plot were coarsely chopped in the field, then dried at 100°C for 1 week and weighed again to determine moisture content. Sub-samples were then ground to pass through a 2 mm sieve, and analyzed for forage quality using near infrared spectroscopy for DM, crude protein (CP), acid detergent fiber (ADF), ADF-CP, neutral detergent fiber (NDF), NDF-CP, Fat, Ash, NDFD, and starch according to procedures at the University of Wisconsin Soil and Forage Analysis Laboratory (Peters 2013). Total N was calculated by dividing CP concentration by 6.25 and total N uptake was calculated as TN* DM from total plot yield.

Calculations and Statistics

Manure N use efficiency was calculated as the percentage of silage total N uptake, in treatment plots (kg) minus silage N uptake in control plots (kg) divided by total manure N applied. Partial factor productivity was calculated as total plant N (kg) divided by manure N applied (kg). To determine differences from control treatments, whole treatment factors, as a combination of tannin level and manure N rate, were compared to control treatments which received no manure additions using a pairwise Dunnett's test. Without the control, analysis of variance was conducted using Proc MIXED in SAS 9.4 to determine the effect of manure treatments on corn silage yield (dry matter), N uptake, forage quality, and partial factor productivity (SAS Institute Inc., 2012). Because treatments were different for each year (i.e. Year 1 was application year and Year 2 was carry over year), years were analyzed separately. Tannin level and N rate were treated as fixed effects and analyzed together for an overall treatment effect to determine difference from control, no manure plots. Block was treated as a random effect, and plot (tannin*rate*block) was a repeated measure for all GHG emission and soil N analysis. The variation attributed to each treatment was calculated as the percent of variability from the total sum of squares. Contrast statements were used to determine differences between N rates and between tannin concentrations. All analyses were conducted using a Tukey least significant difference adjustment, and used a threshold of $\alpha = 0.10$ to determine statistical significance.

Results and Discussion

Tannin Manures

Between the initial manure sampling and field application, TN concentrations decreased in each manure type, resulting in no significant differences ($P<0.1$) across tannin levels in manure TN concentrations at the time of field application. The differences between N concentrations from initial sampling and field applications are assumed to be lost from NH_3 volatilization during manure storage, mixing, and preparations for field application. Total N losses during storage at the 0, 0.4, and 1.8% tannin level were 3.7%, 13.5%, 16.0% of total manure N, respectively. These results contrast with previous studies with trends showing that manure from tannin diets were able to retain more N during manure storage than manure from non-tannin diets (Aguerre et al. 2010).

Because of N loss during storage, applied total manure N levels to each field plot varied from the originally planned N rates of 240 kg N ha^{-1} and 360 kg N ha^{-1}. Table 1.0 summarizes actual manure N application rates. Manure N rates within the target 240 N and 360 N rate were on average 212 and 321 kg N ha^{-1} across manure tannin diet level respectively.

Previous studies showed that the pH of the environment in which tannin is introduced can have significant impact on the effectiveness of tannin to slow N mineralization and microbial activity (Mutabaruka et al. 2007). In agricultural soils where pH change was relatively small with the introduction of quebracho and chestnut tannins to soil, microbial communities were not impacted as strongly as they were in more acidic environments, including forests and sugarcane production (Mutabaruka et al. 2007). Manures used for this study had pH of 7.85, 7.84, and 7.63 for 0, 0.4, and 1.8% DMI tannin respectively. These high pH manures can negate the effects of adding more acidic tannin to soils, which can impede the effectiveness of tannin to bind to slow microbial N mineralization.

Greenhouse Gas Emissions following Tannin Manure Applications

There were no significant treatment effects on NH_3 emissions during the first 48 hours following manure application and subsequent tillage. During and after the first 48 hours, all NH_3 emissions were negligible and were not analyzed beyond the first 48 hours. NH_3 emissions were likely low because the manure was immediately tilled in following manure application, resulting in less overall NH_3 volatilization. Previous studies conducted at the same field sites and soils have shown that most NH_3 emissions from manure occur in the first 48 hours following land application (Pfluke et al. 2011, Powell et al. 2011b).

During the 2014 growing season, no differences were observed in CO_2, N_2O, or CH_4 flux among all treatments on any given sampling day. CO_2, N_2O, and CH_4 emissions peaked

14 days following manure application for all treatments, including control plots that received no manure (Figure 1). Within 40 days of the initial application, all soil GHG emissions had dropped to negligible levels.

All manure and control treatments showed similar patterns of CO_2 flux over the growing season. CO_2 emissions were driven predominantly by a tannin*day interaction effect; immediately following manure application, 0% tannin treatments had the highest CO_2 flux, while later on, treatments with tannin had the higher flux. Significant CO_2 emission peaks occurred 4 days following manure application, which is likely linked to warming soil temperatures at this time. High emission levels immediately after application were not observed, likely due to low soil temperatures and therefore reduced soil microbial activity resulting in reduced levels of labile carbon decay and lower CO_2 flux early in the growing season.

CH_4 emissions were fairly low throughout the growing season, with the highest flux events occurring on days 14 and 84, at a rate of 580 g CH_4-C m^{-2} h^{-1}. Total emission loads for CH_4 were highest for the high tannin, high N rate treatment, at 1468 g CH_4-C over the growing season. Soil is often considered a sink for CH_4 emissions, especially in the presence of chamber-based greenhouse gas measurements, which may bias emission concentrations for methane over significant periods of time (Lai et al. 2012, Wood et al. 2013). More CH_4 flux measurements were negligible and near zero flux values over the growing season, with no significant trends established across all measurements.

Nitrous oxide emissions were most closely related to timing of manure application, with the most N_2O emissions occurring on day 14 following manure application. Because of low temperatures and low soil mineral N concentrations, N_2O emissions were not significant in the first 72 hours after application. It is possible that the highest peak emission event was missed because of delayed GHG sampling following the first 72 hours. Manure applications resulted in reduced, oxygen poor environments, which can create ideal conditions for denitrification and N_2O emissions from soil (Henault et al 2012). Depending on the C:N ratio of the manure, this can drive further overall emission loads. When C:N ratios are high in manure, the higher carbon concentrations result in more CH_4 and CO_2 emissions, with less N_2O emissions from manure storage (van der Weerden et al. 2014). Once land applied, manures with high C:N ratios result in higher soil N immobilization, resulting in higher N stores in soil organic matter and less in mineralized forms, resulting in lower N_2O emissions loads. Because of relatively higher C:N ratios of the manures applied for this study of 14.1, 14.3, and 13.4 for 0, 0.4, and 1.8% DMI tannin concentrations respectively, lower N_2O emissions were observed, making it difficult to discern treatment differences. Tannin applications in Wisconsin soils at this location may not have been as effective at GHG mitigation as other studies, due in part to the high soil pH in this ecosystem. Other studies have found that polyphenol additions to soil result in decreased pH, closer to pH of 4.00, which can result in slowing of soil microbial activity (Mutabaruka et al. 2007). With soils from these systems averaging a soil pH of 5.4; the tannin compounds are significantly less effective at reducing N mineralization and associated emissions than in acidic soils (Mutabaruka et al. 2007, Millar and Baggs 2004).

Manure N rate was not a significant factor that changed emission flux for N_2O or NH_3 emissions within a given sampling day. Previous research has shown that with added N inputs to soil, and in systems that incorporate liquid or digested manures, total emission fluxes increase significantly, which was not observed in this field study (Collins et al. 2011, Osterholtz et al. 2010). The peak N_2O emissions in the present study, 14 g N_2O-N m^{-2} h^{-1}, were significantly higher than another study on similar silt loam soils in Wisconsin, 4.2 g N_2O-N m^{-2} h^{-1} (Collins et al. 2011).

An interaction effect was observed with N rate*day; early in the growing season emissions were higher for target 360 kg N ha^{-1} rates, but later in the growing season the 240 kg N ha^{-1} rate had significantly higher N_2O emissions. Overall, variability in flux measurements was likely responsible for no significant differences between treatments in the field, which is likely associated with soil heterogeneity, with soil moisture, inorganic N concentrations, and microbial activity varying significantly within soil microsites, which are significantly smaller than the area of the 1.03 m^2 measurement chamber (Henault et al. 2012).

Emission loads show no significant differences between treatments for the 115 day sampling period in 2014 (Table 2). Trends show that for CO_2, N_2O, CH_4, and NH_3, the no tannin, high N rate diet had the highest overall emission load. The 0.4% tannin level resulted in the second highest emissions loads.

Soil Nitrogen Following Tannin Manure Applications

Soil characteristics prior to manure application show that compared to other Wisconsin soils, soil N concentrations were relatively low (Table 3). Significant effects of tannin were observed in total soil NH_4+-N at a depth of 0-10 cm on day 4 following manure application, with the 0.4% tannin as DMI having significantly higher ($P<0.1$) NH_4+-N concentrations in soil than the no tannin and high tannin diet manure treatments. By day 40, all NH_4+-N concentrations in soil samples were negligible and equivalent to the control plot levels (Figure 1).

Soil NO_3^--N were also impacted by manure tannin level. The no tannin manure treatment had significantly higher soil NO_3^- -N in the 0-10 cm depth on day 20 following manure application than tannin manure treatments. With the exception of peak N mineralization on day 20, during the growing season no differences were observed between tannin diet concentrations higher in soil NO_3^- -N than the control or no tannin levels. On all other days there were no significant differences in treatment effect. By day 60, all soil NO_3^--N concentrations were negligible, at levels below 5 mg NO_3^--N kg soil^{-1} (Figure 1).

Soil samples from 10-20 cm depth showed no significant effects of tannin or N rate on soil NH_4^+ -N or soil NO_3^- -N at any point during the 2014 growing season. Control treatments were not significantly different than treatments that received manure for both NH_4^+-N and NO_3^- -N concentrations, except in the cases previously mentioned.

Total soil inorganic N concentrations (NH_4^+-N + NO_3^- -N) from control plots were not significantly different from manured treatments after 64, which had total IN concentrations of 14 mg N kg soil^{-1} without added N additions prior to manure applications in spring 2014. It can be assumed that N losses from soil are accounted for in plant N uptake, gaseous losses, and nitrate leaching—which were not determined for this study. Other factors, including the variability between treatments associated with natural soil heterogeneity and N loss during manure storage, could result in less significant differences in soil inorganic N.

During the 2015 growing season, no differences were observed in soil NH_4+-N concentrations from 0-10 cm with respect to tannin or N rate across all sampling days, with all NH_4+-N concentrations negligible and no differences from control treatments that had received no manure. While no differences were observed by tannin or N rate at the 10-20 cm depth across the growing season, on 27 April 2015 pre-plant soil samples showed that the 1.8% tannin concentration had significantly higher NH_4+-N concentrations than no tannin manure treatments, suggesting that tannin may have impeded N mineralization during the previous growing season, leaving more NH_4^+-N available for the second growing season.

Extractable NO_3^- -N concentrations in the top 10 centimeters were not different from each other on any sampling day across all treatments, including control plots in 2015. At the 10-20 cm depth, NO_3^- -N concentrations were different by tannin rate (P=0.08) with significantly higher nitrate concentrations in the high tannin diet when compared to no tannin manure from pre-plant soil samples taken 27 April 2015 (Figure 3). This result suggests that manures containing tannin were able to retain more N over winter and suggests that N mineralization was delayed compared to the low and no tannin manures, resulting in more NO_3^- -N in soil at the onset of the second growing season.

Corn Silage Yield and Quality

In the 2014 growing season, manure treatments yielded significantly higher corn silage than the control, no manure treatments. There were no significant effects of manure N rate or tannin level on yield among treatments that received manure in 2014 (Table 4). This was likely influenced by the high natural soil N concentrations at the research site. The grand mean for all corn silage harvested in 2014 at 65% moisture was 38.8 Mg ha^{-1}. In 2015, corn silage yields were not significantly different between any treatments, and were not significantly different from control plots (Table 5). The grand mean for all corn silage harvested in 2015 at 65% moisture was 21.0 Mg ha^{-1}. The significant drop in yields during the second growing season was driven by less precipitation, cooler summer temperatures, and no additional N applications in the second year. No differences between treatments in the 2015 harvest suggest that there was no carry over N credit of the tannin manure in soil for agronomic production.

Subsampled treatments show that there was no significant effect ($P<0.1$) of tannin level or N rate on corn silage crude protein, ADIN, and NDIN. N rate significantly influenced N uptake, with higher N uptake in plants that had received target 360 kg N ha^{-1} rate applications.

Manure N rate and tannin level significantly influenced partial factor productivity (PFP), which was significantly higher ($P<0.0001$) at the 240 N rate (3.7 g DM kg N applied^{-1}) than the 360 N rate (2.5 g DM kg N applied^{-1}). This result is expected because the lower N rate corresponded to the agronomic recommended rate while the high N rate was 1.5 times the recommended rate (Laboski and Peters 2012). PFP was also significantly higher ($P< 0.01$) in treatments with 0.4 and 1.8% tannin than no tannin manure treatments, suggesting that tannin may improve N efficiency in corn silage production. Given that no differences were observed in N concentrations in manure at the end of manure storage, the differences observed in PFP suggest that tannin may improve N efficiencies by delaying N mineralization and plant available N following land application.

In 2014, corn silage subsamples showed interaction effects of tannin and rate in neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and starch. For NDF and ADF the 0% tannin, 240 N rate resulted in higher estimates as percent of DM, while at the low and high tannin levels, the 240 N rate resulted in lower estimates than the 360 N rate, however, for NDF and ADF, this interaction effect did not result in significant differences between treatments. ADL results saw the same trend as NDF and ADF, but differences were significant, with the 0% tannin, 240 N rate having significantly higher ADL (3.65% of DM) than 0.4 and 1.8% tannin at the 240 N rate, at 3.13 and 3.12% of DM respectively. Starch concentrations were higher at the 240 N rate at the 0.4% DMI tannin level, while at 0 and 1.8% tannin levels, the opposite was observed, though there were no significant differences.

In 2015, corn silage subsamples showed no significant differences in forage quality between plots which received manure and control, non-manured plots. A significant effect of tannin was observed on NDF and ADL ($P<0.1$) without comparisons to the control treatments. In both cases, the low tannin treatment had higher NDF and ADL than high tannin treatments. Manure treatments without tannin were not different from low or high tannin treatments.

Conclusions

Soil borne GHG measurements were highly variable following applications of tannin manures; there were no tannin or N rate effects on any given sampling day, and no differences in seasonal emission loads. This suggests that there is no GHG mitigation potential from tannin manures. Tannin manures also did not affect plant available N concentrations 20 d after application. Corn silage yields where not diminished from tannin manures, suggesting no impediment to plant production on dairy farms. Tannin manures did not negatively affect dairy farm production in anyway, and did not increase the overall GHG emissions from manure land applications. Since other studies have shown a positive effect on GHG emission reduction at the cow and barn phases of production, the non-negative effects quantified here corroborate that tannins are overall positive to the production system. Because positive environmental and N management benefits were observed in earlier stages during dairy production at the cow and barn level, tannin diets are still have excellent potential to benefit dairy producers.

Tables and Figures

Table 1. Manure characteristics 40 days before and the day of field application (2014).

Diet Tannin Level	——N Application Rate—		N lost†	————TN————		TC	pH
	Target	Actual		Pre-application	At Application	At Application	
g kg DMI^{-1}	kg N ha^{-1}	kg N ha^{-1}	%	g N kg wet manure^{-1}		g C kg wet manure^{-1}	
0	240	230c‡	4.3 b	4.32†	4.14	58.4	7.85
	360	347a	3.7 b				
0.4	240	204d	15.2 a	4.69	4.07	58.1	7.84
	360	317b	12.1 ab				
1.8	240	203d	15.74 a	4.93	4.13	55.4	7.63
	360	301b	16.61 a				
ANOVA							
Treatment	*P*	***	***	--	ns	ns	ns
<u>Contrasts</u>							
N Rate	240 vs 360	***	ns	--	ns	--	--
Tannin	0 vs 0.4	***	***	--	ns	ns	--
	0 vs 1.8	***	***	--	ns	ns	--
	0.4 vs 1.8	ns	ns	--	ns	ns	--

* Signficant at $P< 0.1$

** Significant at $P<0.05$

*** Significant at $P< 0.001$

† Abbreviations: N lost during 40 day manure storage, as % of manure N that went into storage, TN, total nitrogen, TC, total carbon

‡ Values within columns followed by the same lower case letters are not significantly different at the 0.10 probability using Tukey's HSD test.

† Only one sample for each tannin level for pre-application sampling within column.

Table 2. Manure N rate and tannin level impacts on GHG and ammonia emissions during the 2014 growing season.

Tannin	Rate	CO_2	N_2O	CH_4	NH_3
g kg DMI^{-1}	kg N ha^{-1}		kg m^{-2}		
0	240	227	0.69	2.8	1.5
	360	334	0.92	3.6	1.8
0.4	240	405	0.91	4.4	1.6
	360	280	0.64	2.9	1.9
1.8	240	280	0.67	3.3	1.6
	360	258	0.68	3.4	1.8
Control	0	248	0.70	3.5	1.7
		ANOVA			
Treatment	*P*	0.63 ns	0.19 ns	0.12 ns	0.97 ns
<u>Contrasts</u>					
N Rate	240 vs 360	0.99 ns	0.86 ns	0.58 ns	0.33 ns
Tannin	0 vs 0.4	0.92 ns	0.72 ns	0.27 ns	0.77 ns
	0 vs 1.8	0.42 ns	0.16 ns	0.68 ns	0.86 ns
	0.4 vs 1.8	0.48 ns	0.29 ns	0.48 ns	0.91 ns

***** Signficant at $P < 0.1$

****** Significant at $P < 0.05$

******* Significant at $P < 0.001$

Table 3. Soil characteristics of St. Charles silt loam in Prairie du Sac, WI, prior to manure applications, May 2014.

Depth	pH	Bulk Density	Soil Inorganic N	CEC	Texture
cm		g cm^{-3}	g N kg $soil^{-1}$	cmol kg $soil^{-1}$	
0-10	5.2	0.84	0.15	8	Silt loam
10-20	5.0	0.81	0.12	8	Silt loam

Table 4. Fixed effects of tannin and manure N application rate on corn silage yield and quality at 2014 harvest.

Source of Variation		Yield	PFP†	CP	TN	N Uptake	NDF	ADF	ADL	ADL-NDF	NDFD	Starch
	DF						*P value*					
Tannin (T)	2	NS	0.001	NS	NS	NS	NS	NS	0.1	0.03	0.03	NS
Rate (R)	1	NS	<.0001	NS	0.06	0.07	NS	NS	NS	NS	NS	NS
T * R	2	NS	NS	NS	NS	NS	0.02	0.04	0.01	NS	0.1	0.04
Block	1	NS	0.05	NS	0.1	0.1	0.09	0.1	NS	NS	0.1	0.06
Residual	29	--	--	--	--	--	--	--	--	--	--	--

†Abbreviations: PFP, partial factor productivity, CP, crude protein, TN, total nitrogen, ADF, acid detergent fiber, NDF, neutral detergent fiber, ADL, acid detergent lignin, ADL-NDF, acid detergent lignin-neutral detergent fiber, NDFD, neutral detergent fiber digestibility after in vitro 48 h assay.

‡ Treatment variability attributed to each fixed effect, calculated as % of variation in sum of squares.

Table 5. Fixed effects of tannin and N rate on corn silage yield and quality at 2015 harvest.

Source of Variation	DF	Yield	CP†	TN	N Uptake	NDF	ADF	ADL	ADL-NDF	NDFD	Starch
		P value									
Tannin (T)	2	0.19	0.63	0.59	0.58	0.09	0.16	0.09	0.23	0.55	0.24
Rate (R)	1	0.52	0.22	0.23	0.21	0.38	0.36	0.12	0.27	0.25	0.70
T * R	2	0.31	0.84	0.84	0.81	0.99	0.95	0.63	0.31	0.30	0.92
Block	1	0.65	0.99	0.96	0.93	0.21	0.26	0.62	0.19	0.67	0.16

†Abbreviations: CP, crude protein, TN, total nitrogen, ADF, acid detergent fiber, NDF, neutral detergent fiber, ADL, acid detergent lignin, ADL-NDF, acid detergent lignin-neutral detergent fiber, NDFD, neutral detergent fiber digestibility after in vitro 48 h assay

‡ Treatment variability attributed to each fixed effect, calculated as percent of variation in sum of squares.

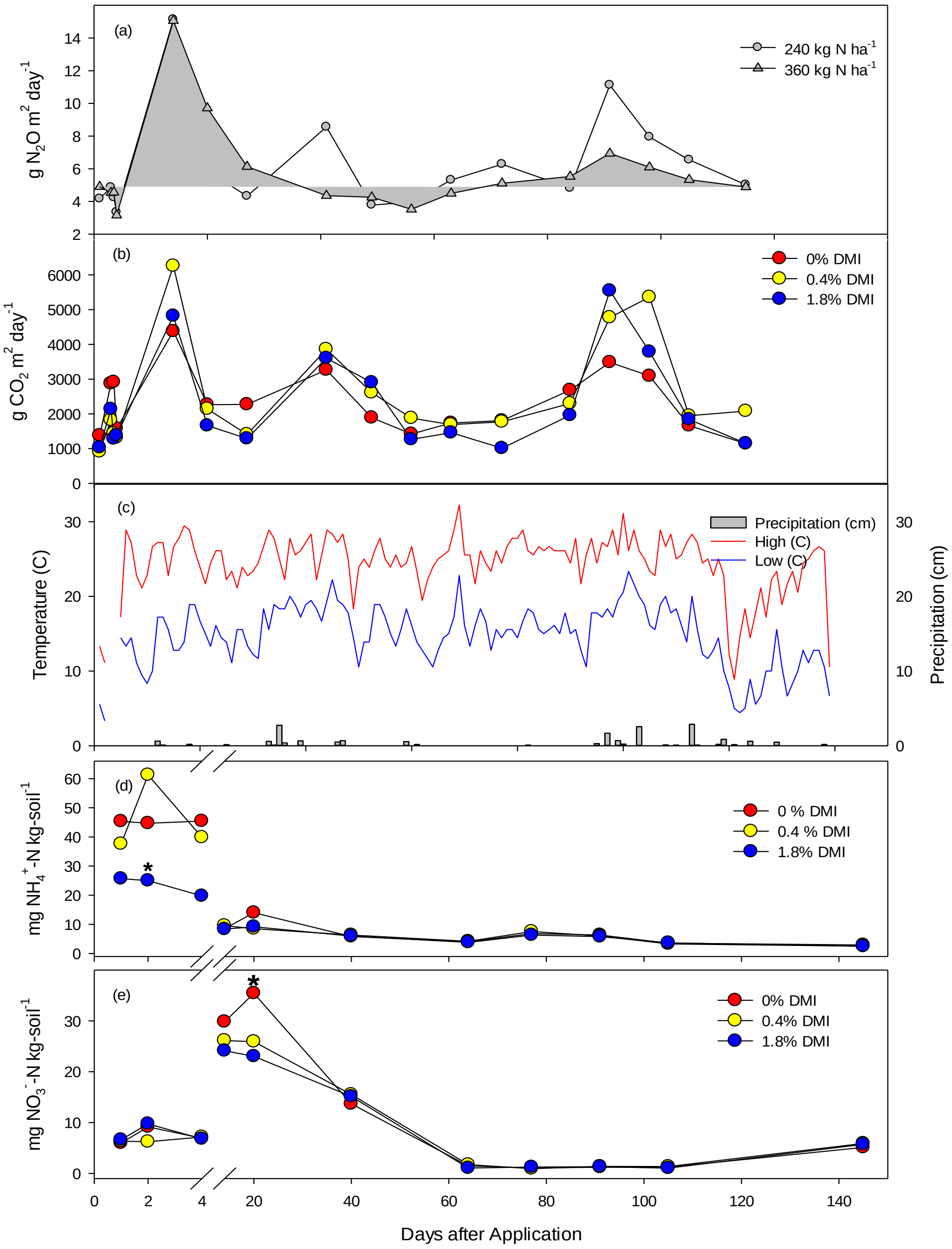

Figure 1. (a)Nitrous oxide and (b)carbon dioxide emissions during the 2014 growing season, shown with (c) 2014 weather, and soil (d) NH_4^+-N and (e) NO_3^- -N concentrations.

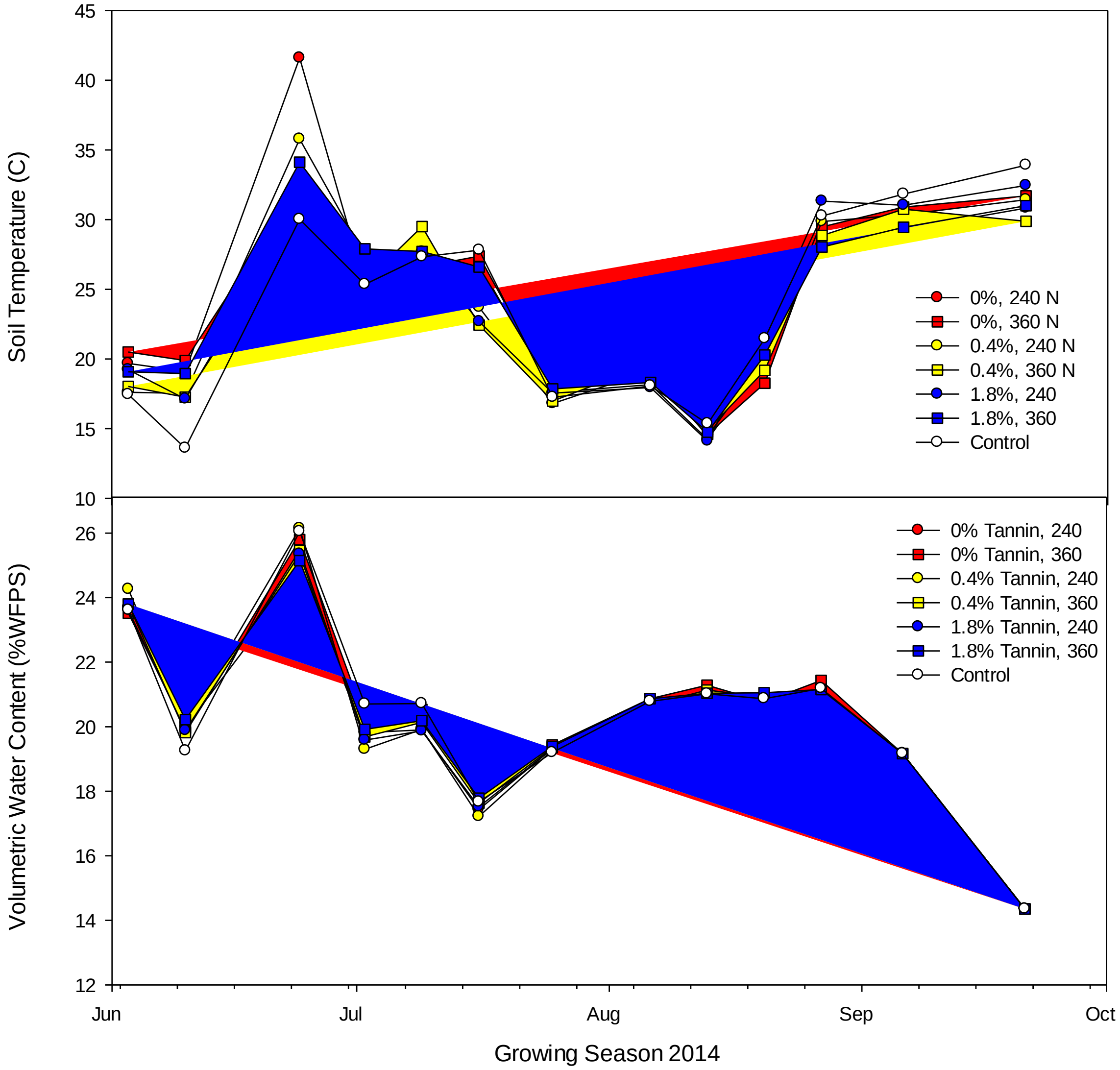

Figure 2. Soil temperature and moisture, shown as water filled pore space, during the 2014 growing season. Soil temperature and moisture, influential factors on GHG emissions, were consistent between treatments across the season, with soil temperature peaking at the same time as soil GHG emissions, as shown in Figure 1.

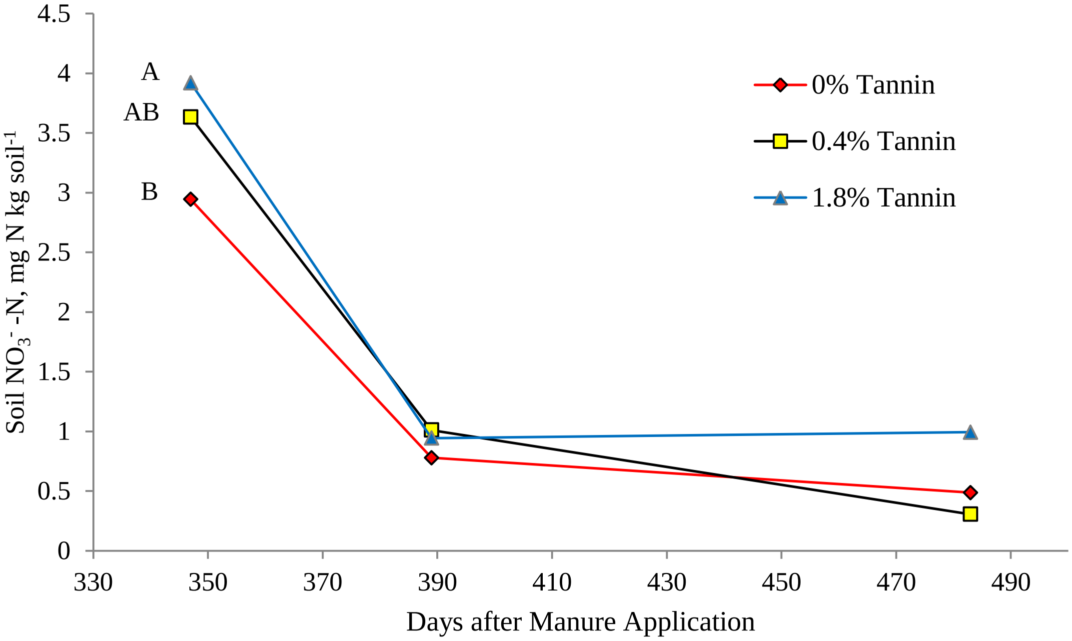

Figure 3. Extractable NO_3^- -N in soil from the 2015 growing season, representing days 347, 389, and 483 following manure application. Letters show significant differences within sampling day ($P<0.05$).

SAS Code

For Manure and Corn Yield and Quality:

```
proc mixed data=a;
class trt;
model {input} = trt / solution;
lsmeans trt/ diff adjust=Tukey;
estimate 'LowN vs HighN' trt 1 -1 1 -1 1 -1 0;
estimate 'OT vs LT' trt 1 1 -1 -1 0 0 0;
estimate 'OT vs HT' trt 1 1 0 0 -1 -1 0;
estimate 'LT vs HT' trt 0 0 1 1 -1 -1 0;
contrast 'LowN vs HighN' trt 1 -1 1 -1 1 -1 0;
contrast 'OT vs LT' trt 1 1 -1 -1 0 0 0;
contrast 'OT vs HT' trt 1 1 0 0 -1 -1 0;
contrast 'LT vs HT' trt 0 0 1 1 -1 -1 0;
run;


proc glm data=a;
      class plot block trt tannin rate;
      model {input} = trt;
        random block;
      means trt/ LSD LINES alpha=0.1;
      lsmeans trt/ PDIFF adjust=Tukey alpha=0.1;
run;
```

For Greenhouse Gas Emissions and Soil N:

```
data c; set a;
time=day;
if day=1 then delete;

proc glm data=c; class  day trt block;
model n2o = trt day day*trt;
random block block*trt;
repeated / subject=block*trt type=sp(pow)(time);
means trt trt*day/LSD lines;
lsmeans trt*day / slice=day adjust=tukey alpha=.1;
run;
```

Chapter 3

Assessing the Impact of Tannin Diets for Dairy Cattle on Multiple Applications of Dairy Manure in a Greenhouse

Abstract

Dietary choices for dairy cows can be a critical part of manure N management. When dairy cows are fed tannins, studies show that more N is excreted in manure solid components, making N less reactive and more available for plants in subsequent land applications. Manure from experimental dairy cow diets containing three tannin levels, 0, 0.4, and 1.8% tannin of total mixed ration as dry matter intake was applied at two target N rates, 240 and 360 kg N ha^{-1} at three frequencies (F1, F2, and F3) to a winter wheat-sorghum-sorghum ratoon cropping sequence. Plant dry matter yields were influenced by tannin level, manure N rate, and manure application frequency. Following F1 and F2 manure applications, winter wheat and sorghum shoot biomass was significantly less at the 1.8% tannin level than the 0 and 0.4% tannin level, while winter wheat root biomass was significantly higher with tannin manure than with non-tannin containing manure. Increased root N uptake with increasing tannin manure concentrations suggest that tannins may delay manure N mineralization in soils and potentially provide subsequent cropping systems with an N credit. After the F3 manure application, biomass yields were influenced by N rate and frequency, with greater higher N rates and manure frequencies resulting in higher yields and N uptake. These findings suggest that tannin may delay N mineralization, potentially allowing better synchronization between soil N availability and plant N needs.

Introduction

Dairy farms are often considered inefficient users of nitrogen throughout manure management, from manure collection, storage, and land application (Montes et al. 2013, De Vries et al. 2015). Manure N losses come in three significant forms from dairy systems, (1) as immediate ammonia (NH_3) volatilization from urine-N sources in dairy barns , manure storage lagoons, and after land application (2) through leaching and runoff of manure sources associated with manure land applications—resulting in pollution and eutrophication of water resources, and (3) through gaseous nitrous oxide (N_2O) emissions, which is a potent greenhouse gas (Montes et al. 2014, Bundy and Jackson 2004). These losses can greatly decrease manure N recycling, with a return efficiency as low as only 10% of the total N from manure reaching plants in the subsequent cropping application (Montes et al. 2013).

Because of these N use inefficiencies, producers are very interested in finding cost effective means of preserving N in more stable forms to be better utilized and efficiently recycled across all aspects of manure management on dairy farms. Manure N excretion and loss are directly related to how dairy cows are handled, including diet, housing, and manure collection processes (Chadwick et al. 2011). One effective method of preserving N in manure is through feeding dairy cows tannins, a polyphenol compound that binds to feed proteins during ruminal digestion processes, resulting in more efficient feed N use and stabilized N sources in manure feces (Aguerre et al. 2010). Tannin trials conducted by Powell et al. (2011) suggest that when tannin is added in small quantities of 0.4 to 1.8% to total mixed rations, it can inhibit the urease enzyme, resulting in less urea-N and therefore less immediate N volatilization as NH_3. In laboratory incubation studies, tannins reduced NH_3 volatilization from concrete barn floors and from soil suggesting mitigation potential of NH_3 from dairy systems (Powell et al. 2011a, Powell et al. 2011b). These experiments suggest that feeding tannins may not only be an effective strategy to stabilize manure N in solid manure feces, rather than gaseous losses, but also enhance overall N use efficiency in dairy production systems.

Polyphenol compounds are important regulators of carbon and nitrogen cycling in terrestrial ecosystems, which can have important implications on nutrient responses in agricultural crop rotations, though the effectiveness of polyphenols in field level experiments is not well understood (Hättenschwiler and Vitousek 2000). Tannins, a class of polyphenol compounds analyzed for their ability to bind to proteins, are found in many higher order plants, but certain species have much higher concentrations of these compounds, including species like quebracho (*Schinopsis spp.*), chestnut (*Castanea spp*.), oak (*Quercus spp.*), many conifers, and forage species, birdsfoot trefoil (*Lotus corniculatus*) and lespedeza (*Sericea lespedeza*), where tannin concentrations are as high as 18% of total dry matter (MacAdam et al. 2013). Studies have shown that tannins from quebracho and chestnut in plant litter are capable of inhibiting soil nitrifiers, which simultaneously slows N mineralization rates and increases organic N concentrations in soils (MacAdams et al. 2010, Mutabaruka et al. 2007). This mechanism protects plant proteins against microorganism herbivory, which is particularly effective at protecting N sources in N limited ecosystems, including tropical forests and coniferous forests, by maintaining N concentrations in organic forms (Hättenschwiler and Vitousek 2000, Mutabaruka et al. 2007, Millar and Baggs 2004). However, there are still significant gaps in understanding the role of tannins in nutrient cycling, especially in agricultural soils.

Field-based trials showed that greenhouse gas emissions, soil N concentrations, and plant response were not impacted by land applied tannin containing manures (Chapter 2). This agrees with previous field studies at the same location, showing that land applications of manure from various forage types, including birdsfoot trefoil, had no significant impacts on corn yield, soil N, and corn N uptake (Powell and Grabber 2009). Differences due to manure in these trials may have been diminished because of field level variability and only one application of manure in the spring to determine results from these dietary trials. Comparatively, Mutabaruka et al. 2007, found that polyphenol compounds, including quebracho and chestnut tannins, with high protein binding capacities were initially degraded rapidly in agricultural soils, but over 2 years, microbial communities were not as effective at degrading plant tissues with higher polyphenol concentrations. This suggests that tannins can influence N and C cycling over extended periods of time when multiple inputs of tannin are added to a soil ecosystem. No previous studies have looked at long term effects on soil N and plant response with multiple applications of tannin manures.

Results from a field-based tannin manure experiment suggest that tannins have a positive carryover effect on soil inorganic N, with more soil N available in the second growing season following applications of manure with tannin concentrations of 1.8% of dry matter intake (Chapter 2). Though no significant differences were observed during the first growing season following tannin manure applications, second year observations suggest that more study is needed to assess the long term effects of tannins in soil. In the present study, tannin manure was applied through three consecutive cropping sequences, to assess the influence of tannin level, N rate, and multiple manure frequency inputs over a six month period in a greenhouse trial. The primary goal of this study was to assess the long term effects of tannin manures to plant and soil response, and determine if the same soils as the field study could result in significant effects of tannin level, N rate, and frequency of manure application.

Materials and Methods

Soil Description

Representative soil was excavated from the USDA Dairy Forage Research Center farm in Prairie du Sac, WI, 53578 (43.332899, -89.716275), of St. Charles silt loam (Fine-silty, mixed, superactive, mesic Typic Hapludalfs) in August 2014. Soil was removed with a stainless steel shovel from the A horizon of the soil profile (0-15 cm). All soil was homogenized, air dried and ground to pass through a 2 mm sieve and stored in plastic containers until the study began 16 December 2014.

Prior to study initiation, soil pH was analyzed using a 1:1 soil: water weight ratio with an Accumet AB 150 pH analyzer (Fisher Scientific, Pittsburg, PA, 1998). Inorganic soil N was extracted from bulk soil samples before the start of the experiment using 2.0 M KCl, shaken for 15 minutes, following Doane and Horwath (2003), and analyzed for NO_3^- and NH_4^+ on an automated Lachat N analyzer (Lachat Instruments, Loveland, CO, 1996).

Manure Collection

Manure for this study was collected during a nutrition feeding trial conducted at the USDA Dairy Forage Research Center farm in Prairie du Sac, WI, 53578. Manure was collected from 36 lactating Holstein cows that were fed a diet with supplemental crude tannin extracts at three experimental levels, 0, 0.4, and 1.8% tannin of dry matter intake. The tannin extract (Bypro; Silvateam, Indunor S.A., Argentina) was comprised (by weight) of approximately one third chestnut tannin extract and two thirds quebracho tannin extract having the following chemical properties (polyvinylpyrrolidone powder method; Tempel, 1982): tannin concentration of 792 g kg−1 and pH of 3.72. Thirty six cows were randomly assigned to one of three diets, 0% tannin (0T), 0.4% tannin (LT), and 1.8% tannin (HT) added to total mixed rations by weight. Manure collection was staggered so that manure was collected from the nutrition trial on days 95-96 of the 105 day nutrition trial. Nine cows were randomly assigned to each of four staggered start dates for the trial, and manure collection occurred four times on days 95 and 96 into the dietary trial for that group of 9 cows. This allowed manure to be collected from cows fed tannins for the same number of days. Collection occurred 10-11 March, 24-25 March, 31 March 31 to 1 April, and 7-8 April 2014. Raw manure, including feces, urine, and small amounts of urine soaked bedding, was collected by hand with large plastic scoops used to scrape out catchment pans fitted in the manure gutters behind each cow. Manure was stored in 1.2 m^3 bins with lids until 5 May. The manure used for this greenhouse trial was collected as subsamples from a field-based land application experiment that were frozen immediately on 15 May 2014, and were thawed 24 hour prior to each application event in this study.

Before application to the potted soil, the manure slurries were sampled in triplicate by tannin level and analyzed for pH (1:2 manure: water), dry matter (at 100° C after 24 hours) and sub-samples were freeze-dried, ground to pass a 1 mm sieve, and analyzed for total N and C by combustion assay on a Elementar Variomax using the Glutamic acid method (Elementar 2005) (Table 1). Samples were also analyzed for neutral detergent fiber with an Ankom 200 fiber analyzer (Ankom Technology, Inc 2015). The N contained in the resultant NDF was analyzed using the Elementar Variomax. A complete analysis of manure characteristics is including in Chapter 2, Table 1.

Experimental Design

The greenhouse study was designed as a complete block design with three factors, tannin level (0 %, 0.4%, or 1.8% tannin of dry matter intake), target N rate (240 kg N ha^{-1} or 360 kg N ha^{-1}), and manure application frequency (F) during the cropping sequence (one, two, or three applications). At each manure application frequency, a new cropping sequence was started, with winter wheat planted one week after the first manure application (F1), sorghum planted one week after the second manure application (F2), and sorghum re-growth (ratoon) continued at the third cropping sequence with a third manure application (F3). Each tannin level by N rate treatment combination received all possible manure application frequency events, with all tannin by rate by frequency treatments replicated three times. Therefore, each tannin-rate treatment combination received the F1 manure application (n=9), then two-thirds of the pots received the F2 frequency (n=6 for F2, n =3 for F1), and then one third of pots received the final F3 manure application (n=3 for F1, F2, and F3). Therefore at the end of the study, F1 treatments had received manure only once, 150 days prior to harvest, F2 treatments had received manure 150 days and 105 days before, and F3 had received manure three times, at 150, 105, and 60 days prior to the final harvest. In addition to all manure treatments, three control pots received no manure throughout the study. Pots were watered every 1 to 2 days to maintain approximately 60% water filled pore space (WFPS) and pot locations were randomized each week on the greenhouse bench. The greenhouse was kept at approximately 27° C and received a combination of natural or artificial greenhouse lighting for 12 hours a day. Pots were weeded as needed.

Eight hundred grams of dried, ground soil was placed in 700 mL non-draining pots. Phosphorus was applied at a rate of 40 kg P ha^{-1} as KH_2PO_4 and watered with distilled water to achieve 60% WFPS. Pots were then left fallow for one week before manure application. Manure treatment rates of 240 and 360 kg N ha^{-1} were added to soil based on pre-determined manure TN concentrations from manure samples taken immediately following manure collection on 10 April 2014 (Chapter 2) and the total surface area of the potted soil. The F1 application was applied on 10 December 2014. To apply manure, approximately 25% of the soil was removed from each pot. After the manure application, the removed soil was then replaced and repacked to a bulk density of 1.20 g cm^{-3}. F2 manure applications occurred in the same manner as F1 on 6 February 2015. F3 manure was applied on 14 April 2015 in bands, by cutting 2 cm deep slots into the soil surface to simulate manure injection.

The F1 (winter wheat) and F2 (sorghum) cropping sequences were grown from seed starting one week after its paired manure applications. Nine seeds were started with each cropping sequence, and thinned 10 days after planting, keeping the 5 most robust seedlings for the rest of that cropping sequence. Winter wheat was planted on 16 December 2014, grown for 45 days (F1), and then harvested. Sorghum was planted on 13 February 2015 and grown for 60 days (F2), then shoots were harvests and sorghum was allowed to ratoon for 45 days (F3). Above ground biomass was cut 2 cm above the soil surface, weighed, and dried for dry matter content. Root biomass was also harvested after winter wheat to diminish the possible impact of root N mineralization on subsequent soil N dynamics. Soil from each pot was replaced and repacked to a bulk density of 1.20 g cm^{-3}. Roots were also harvested after the final sorghum ratoon crop sequence. Roots were removed by hand and washed over a 2 mm sieve. All plant and root biomass samples were then ground to pass through a 2 mm sieve on a Udy grinder (Udy, Fort Collins, CO), and analyzed for total N and C using the Glutamic acid method (Elementar CN Analyzer, Elementar Inc., Hanan, Germany).

Statistics

All statistical analyses were performed using the SAS statistical package (SAS Institute, 1990). Two statistical approaches with ANOVA were used. First, all tannin level by N rate by manure application frequency combinations were compared to the control using a generalized linear model (Proc GLM) ANOVA with a Dunnett's t-test at an $\alpha=0.05$ level for all effects on biomass yield and N uptake. Each cropping sequence was then analyzed separately without the control to compare manure tannin level, application rate, frequency, and their interactions as fixed effects using a proc mixed approach, $\alpha = 0.05$. The same approach was used to determine the treatment and interaction effects on cumulative shoot and root biomass yield and N uptake, and soil analyses for each crop.

Results and Discussion

Winter Wheat Yield, Root Biomass, and N Uptake

There was a significant interaction effect of tannin level and N rate ($P<0.1$) in winter wheat shoot and root yield (Table 2). Control pots which received no manure had significantly higher shoot and root yields than pots that received manure for all treatments. Root biomass at the no tannin and low tannin level were significantly lower than control treatments. The control soil used from this study had lower soil inorganic N concentrations of 14 mg N kg soil^{-1} compared to other Wisconsin soils, such as control Plano silt loam and Rosholt sand loam which have total soil plant available N (PAN) of 40.2 and 20.2 mg N kg soil^{-1} (Wu and Powell 2007). Because of this lower PAN and high C:N ratio of manure, more N was initially immobilized from manure applications, resulting in less N availability to plants.

There was a significant interaction effect on winter wheat shoot yield and N uptake of tannin level and N rate ($P<0.1$) (Figure 1). The no tannin and low tannin manures had a higher yields and N uptake than the high tannin manure, though there was not any significant difference between shoot N uptake due to the application of the low tannin and no tannin manures. Field-level studies showed no significant effects of tannin containing manures on yield or N uptake in corn for silage following one manure application (Chapter 2; Powell and Grabber 2009). Research studying manure management with forages containing polyphenol compounds in Uganda showed that compared to control treatments, DM yields were diminished in treatments with higher concentrations of tannin and lignin, suggesting that more N is bound in manure and less plant available, resulting in lower yields (Kato et al. 2010).

In the winter wheat root harvest, root biomass was impacted by tannin level ($P< 0.01$) and N rate ($P <0.05$), with tannin level and N rate accounting for 15.8 and 7.4% of the total root yield variability (Table 2) (Figure 2). Winter wheat root biomass from the no tannin manure treatments and low tannin manure (240 N rate only), were significantly higher than root biomass from control pots. Tannin level was also a significant factor in root N uptake, with tannin manures having significantly greater root N uptake than the no tannin manure, though root N uptake was not significantly different between the low and high tannin levels (Figure 3). For winter wheat, therefore, tannin positively affected root N uptake, while the opposite occurred in aboveground biomass with shoot N uptake decreasing with increasing tannin manure level. This increasing N uptake by roots due to tannin level suggests that there could be long-term tannin effects on soil N mineralization and subsequently crop N availability when roots decompose. This agrees with an observation from the field study that showed higher available inorganic N in soils the spring following tannin manure applications, an increase that was likely related to N mineralization from roots (Chapter 2).

Sorghum Shoot Yield and N Uptake

In harvest 2, sorghum aboveground shoot yield and N uptake was significantly affected by tannin level ($P<0.0001$) and N rate ($P<0.1$), with the 0 and 0.4% tannin manures providing significantly higher yields and N uptake than 1.8% tannin level treatments (Table 3 and Figure 4). High tannin manure treatments were not significantly different from control plots in shoot yield and N uptake regardless of application frequency ($P<0.05$).

Both winter wheat and sorghum did not show significant effects from N rate ($P<0.05$), in aboveground biomass yield or N uptake. Manure N rates were determined based on the bulk manure samples used for the 2014 field application, where manure N results were highly variable and lower than target N application rates (Chapter 2). Treatment differences from manure N rate were not observed which may be due to high N application rates, with agronomic N needs of 77 and 89 kg N ha^{-1} as the most efficient fertilizer N application rates for winter wheat and sorghum respectively (Laboski and Peters 2012). Models for corn N responses on similar soils in Wisconsin predicted that when fertilizer N application rates are reduced, there would be no long term negative effects on forage and grain yield, while environmental concerns were ameliorated, with less NO_3^- leaching from soils and reduced denitrification losses (Powell and Rotz 2015).

Sorghum Ratoon Shoot and Root Yields and N Uptake

Sorghum ratoon yield and N uptake were not significantly influenced by manure tannin levels, likely because all systems that received multiple manure applications had ample PAN, resulting in no differences due to tannin level. Previous studies by Millar and Baggs (2004) of tannin additions to soil found that tannins are more effective at slowing down N cycling in N limited ecosystems. In the present greenhouse trial, N concentrations in manures and soils were likely too high to discern tannin effects.

The sorghum ratoon harvest was influenced significantly by the frequency of manure application, with mixed results on how shoot and root yield and N uptake differed from the control (Figure 5 and Table 4). Shoot yields were significantly greater with three manure applications (F3) than with one or two manure applications, but significant differences were not detected between F1 and F2 manure frequencies (Figure 6). Shoot yield and N uptake showed the same trends, and were significantly greater ($P<0.05$) at the 360 target N rate compared to the 240 target N rate (Figure 7), though N rate did not have a significant effect on yield after F1 and F2 manure applications.

Root yield and root N uptake were increased significantly ($P<0.0001$) by N rate and frequency of manure application (Figure 8) (Table 4). This agrees with previous findings from Wu and Powell (2007), which showed that as manure N rate and application frequency increased, root organic matter and N uptake increased. A significant interaction effect of tannin, rate, and frequency was observed ($P<0.05$) for root N uptake, with increasing tannin level, manure frequency, and N rate all impacting root N uptake resulting in higher root N uptake (Figure 9). No differences were observed between F1 and F2 manure frequencies, regardless of tannin level and N rate; however, at the F3 frequency, the low tannin manure did not increase between the 240 and 360 N rates, while the no tannin and high tannin treatments did. Though tannins did not impact root yield, previous studies have found that tannin can impact mycorrhizal fungi communities in soil, which adapt to the presence of increased polyphenols in soil over time, and therefore mineralize N from tannins more effectively after long term treatment exposure (Mutabaruka et al. 2007).

Winter Wheat-Sorghum-Sorghum Ratoon Combined Sequence Effects

Cumulative yield, as the sum of both above and belowground, was significantly influenced by manure tannin level, N rate, and frequency of manure application (Table 5). All treatments that received F3 manure frequencies had significantly higher shoot yield than the control treatments. This agrees with findings from a previous greenhouse study, whereby manure application frequency and N rate positively influenced yields (Wu and Powell 2007). The application of tannin manure, at F1 or F2 did not result in shoot or root responses, which were significantly different from the control, suggesting that tannin may delay shoot growth by affecting soil N availability. The exception to this trend was the shoot yield at high N rate, low tannin level, which was significantly greater than control pots. This was likely due to very high shoot yields in these pots in the second harvest. These greenhouse results contrast with results from field studies, where the effects of tannin level on corn silage biomass was not detected (Chapter 2, Powell and Grabber 2009).

All root yields were significantly higher than the control, regardless of N rate, tannin level, or manure application frequency. Root yield was impacted by tannin (*P*<0.02), rate (*P*<0.0005), and frequency (P<0.0001) (Table 5). The high tannin treatments had significantly higher root yields than no tannin manure treatments. The increase in root yield in high tannin manure pots could be related to a plant stress response from N limitation associated with delayed N mineralization, though previous studies have not analyzed root N responses with tannin manures. Pots which received the targeted 360 N ha^{-1} equivalent rate were significantly higher than the 240 N ha^{-1} equivalent rate, with root yields of 15.2 g pot^{-1} and 12.8 g pot^{-1} respectively.

For total biomass, only the low and high tannin manures at the 360 N rate and F3 manure application frequency were significantly different from the control (*P*<0.05). This is likely due to the low winter wheat biomass yields from the first harvest, which were not significantly different from the control. Without considerations to the control, rate (*P*<0.0001), tannin (*P*<0.05), and frequency (*P*<0.0001), were all significant factors that influenced overall biomass production (Table 5). The same trends observed in the root yield were observed in total biomass, with the F3 frequency having significantly higher biomass (21.9 g pot^{-1}) than F1 (17.3 g pot^{-1}) or F2 (17.8 g pot^{-1}), which were not different from each other. Biomass yield was significantly higher from the 360 N rate (20.4 g pot^{-1}) than the 240 N rate (17.6 g pot^{-1}). The low tannin treatments resulted in the highest biomass yield (20.4 g pot^{-1}), which was significantly higher than high tannin (18.8 g pot^{-1}) and no tannin (18.1 g pot^{-1}) treatments, which were not different from each other.

Nitrogen uptake was not different from control pots for all treatments, except the low tannin, 360 N rate, F3 pots ($P<0.05$). Without the control, the effects of rate ($P<0.05$), frequency ($P<0.0001$), and the interaction effect of rate and frequency ($P=0.01$) significantly influenced N uptake. Nitrogen uptake was significantly higher for F3 (0.24 g N pot^{-1}) than treatments that received F1 only (0.19 g N pot^{-1}) or F2 only (0.19 g N pot^{-1}). Nitrogen uptake was also significantly higher at the 360 N rate (0.21 g N pot^{-1}) than the 240 N rate (0.19 g N pot^{-1}). When divided into shoot and root N uptake, the same trends appear, with no differences from the control in roots for all treatments, except for the greater shoot N uptake in the low tannin manure, 360 N rate, F3 as well as the no tannin level, 360 N rate, F3 treatment.

Manure Application Effects on Soil

At the end of the greenhouse trial, soil pH was significantly affected by tannin concentration ($P < 0.005$), N rate ($P<0.0001$), and manure frequency ($P<0.0001$) (Table 6). Frequency was the most influential factor representing 54% of the total variability; treatments that only received F1 applications showed no significant difference ($P<0.05$) in pH from control plots, with an average soil pH of 6.09. Except for the high tannin level, 360 target N rate at F1, soil pH (6.26) which was significantly greater than the pH of the control. At the low tannin, 240 target N rate, with F1+F2 frequencies, treatments were also not significantly different from the control ($P<0.05$). Following F2 or F3 manure frequencies, pH increased to 6.45, with no significant differences between F2 and F3 manure applications. At the 360 target N rate, pH was 6.41, which was significantly greater than the pH of 6.29 at 240 target N rate. Tannin concentration also influenced pH, with the soil from no tannin and high tannin manure applications having significantly greater pH than low tannin manure soils (*P*<0.05).

Plant available N in soil was significantly influenced by N rate (*P*<0.1) and manure application frequency (*P*<0.05) (Table 5). The high, 360 target N rate resulted in significantly higher PAN than the lower 240 target N rate. This agrees with Wu and Powell (2007), found that higher manure N rate applications and frequencies resulted in higher PAN in soil systems. These higher PAN levels were likely observed because the 360 target N rate was greater than crop requirements and therefore used N less efficiently than the 240 target N rate. Manure frequency also influenced plant available N, with PAN increasing significantly with each manure application. This result contrasts with field level observations where manure N rate did not impact PAN, likely because of soil heterogeneity and nitrate leaching from the soil system (Chapter 2). Observations of NO_3^- -N concentrations with the same manure N rate and tannin levels following an incubation trial assessing N mineralization rates of tannin manures showed that tannin is a more limiting factor on N mineralization because NO_3^- -N concentrations in soil were significantly lower following manure application (Appendix 1). The field and incubation trials were conducted with only a signle manure application, suggesting that in systems that are more N limited, differences may be driven by manure tannin level rather than manure N application.

Polyphenol compounds are more commonly measured in specialty herbs, fruits, and vegetable crops, including teas, grapes, artichokes and medicinal products, where research has shown that tannins added to soils reduce yields (Jurgenson et al. 2012). In tropical Uganda, where forages have inherently high tannin concentrations, similar observations were noted, with less dry matter in cropping systems and pastures after tannin additions to soil (Kato et al. 2010). In soils with higher tannin concentrations, N mineralization is often limited which allows for plants with higher tolerance for limited N to grow, which is commonly associated with lower biomass production (Hättenschwiler and Vitousek 2000). The results of the greenhouse study corroborate this finding, that with F1 or F2 applications, manure tannin level decreased shoot biomass, while the opposite was observed in roots, suggesting that tannins may be delaying PAN, while storing N in roots. The results differed from this trend following the F3 manure application, with higher tannin manure showing increased biomass production, especially at the 240 manure N application rate.

Conclusions

Applications of tannin manure led to yield and N uptake differences in winter wheat and sorghum. Manure from high tannin diets resulted in decreased aboveground yield but increased root yield, with no significant impacts on N uptake. The effective increase in root yield has potential for long term benefits to soil N cycles and crop N availability, with delayed N mineralization over time potentially enhancing N use efficiency. Manure N rate and manure application frequency were significant factors only after three manure applications, suggesting that potential benefits of tannin manures on soil N cycles and crop yields may be available. These results show that, though tannin does not have a short term impact following a single manure application as observed at the field-level, tannins can result in significant yield and N uptake implications to dairy production systems which remains unquantified at the field level or in pasture based systems.

Though differences were observed, the effects of tannin manures were not consistent between winter wheat and sorghum, and overall there are no conclusive agronomic recommendations from tannin manure applications. Given positive observations in previous research at the cow level, with decreased urea-N concentrations in urine, and at the barn level with lower NH_3 emissions, tannin diets for dairy cows still show overall positive benefits for dairy farms, with no negative effects observed in manure land applications with respect to crop production.

Tables

Table 1. Characteristics of manure applied to Winter Wheat.

Tannin Level	TN Pre-Application	TN Lost	pH
% DMI	g N kg wet manure	% N of Pre-Application	
0	4.3	3.7	7.85
0.4	4.7	13.5	7.84
1.8	4.9	16.0	7.63

Table 2. ANOVA for manure tannin level and N rate on winter wheat, harvest 1 following one manure application.

Source of Variation	df	Shoot Yield	N Uptake Shoots	Root Yield	Root N Uptake
		P	*P*	*P*	*P*
Tannin (T)	2	0.131	0.059	0.009	0.016
Rate (R)	1	0.365	0.134	0.031	0.153
T x R	2	0.054	0.051	0.162	0.132

Table 3. ANOVA for manure tannin level, N rate, and frequency effects on sorghum harvest following either F1 or F2 manure applications.

Source of Variation	df	Shoot Yield	N Uptake Shoots
		P	*P*
Tannin (T)	2	<.0001	<.0001
Rate (R)	1	0.082	0.066
Freq (F)	1	0.501	0.118
T*R	2	0.456	0.189
T*F	2	0.629	0.287
R*F	1	0.479	0.203
T*R*F	2	0.773	0.644

Table 4. ANOVA for manure tannin level, N rate, and frequency effect on sorghum ratoon harvest 3 following either F1, F2, or F3 manure applications.

Source of Variation	df	Shoot Yield	N Uptake Shoots	Root Yield	Root N Uptake
		P	*P*	*P*	*P*
Tannin (T)	2	0.224	0.551	0.613	0.078
Rate (R)	1	0.0032	<.0001	<.0001	<.0001
Freq (F)	2	<.0001	<.0001	<.0001	<.0001
T*R	2	0.057	0.279	0.404	0.018
T*F	2	0.585	0.733	0.469	0.607
R*F	1	0.031	0.001	0.069	0.005
T*R*F	2	0.853	0.785	0.528	0.036

Table 5. Means and ANOVA for manure tannin level, N rate, and frequency effect on additive effects across the winter wheat-sorghum-sorghum ratoon system.

Treatment		Shoot Yield	Root Yield	Shoot N Uptake	Root N Uptake
Tannin		g pot^{-1}	g pot^{-1}	g N g tissue^{-1}	g N g tissue^{-1}
0		5.4a†	12.8b	0.0923a	0.105b
0.4		5.4a	14.8a	0.0891a	0.126a
1.8		4.5b	14.4ab	0.0767b	0.122ab
Rate					
240		4.9b	12.8b	0.0864	0.109b
360		5.3a	15.2a	0.0856	0.126a
Frequency					
F1		4.7b	12.8b	0.081b	0.106b
F2		4.8b	13.1b	0.079b	0.107b
F3		5.9a	16.1a	0.098a	0.139a
ANOVA					
Source of Variation	df	*P*	*P*	*P*	*P*
Tannin (T)	2	0.0001	0.024	0.003	0.032
Rate (R)	1	0.036	0.001	0.823	0.016
Freq (F)	2	<.0001	<.0001	0.001	0.001
T*R	2	0.289	0.430	0.118	0.376
T*F	2	0.929	0.906	0.357	0.955
R*F	1	0.221	0.281	0.022	0.135
T*R*F	2	0.826	0.209	0.338	0.332

† Letters within columns are significantly different within treatment level, i.e. different within tannin, rate, or frequency, but not across treatment factors.

Table 6. Means and ANOVA for manure tannin level, N rate, and frequency effect on soil pH and inorganic N following the sorghum ratoon harvest.

Treatment		Soil pH	Plant Available N
Tannin			g N kg soil^{-1}
	0	6.37a†	1.16
	0.4	6.27b	1.45
	1.8	6.36a	1.47
Rate			
	240	6.26b	1.16b
	360	6.41a	1.56a
Frequency			
	F1	6.13b	0.99b
	F2	6.42a	1.36ab
	F3	6.42a	1.72a

——————————ANOVA——————————

Source of Variation	df		
		P	*P*
Tannin (T)	2	0.003	0.454
Rate (R)	1	<.0001	0.076
Freq (F)	2	<.0001	0.043
T*R	2	0.005	0.368
T*F	2	0.046	0.756
R*F	1	0.039	0.426
T*R*F	2	0.829	0.338

† Letters within columns are significantly different within treatment level, i.e. different within tannin, rate, or frequency, but not across treatment factors.

Figures

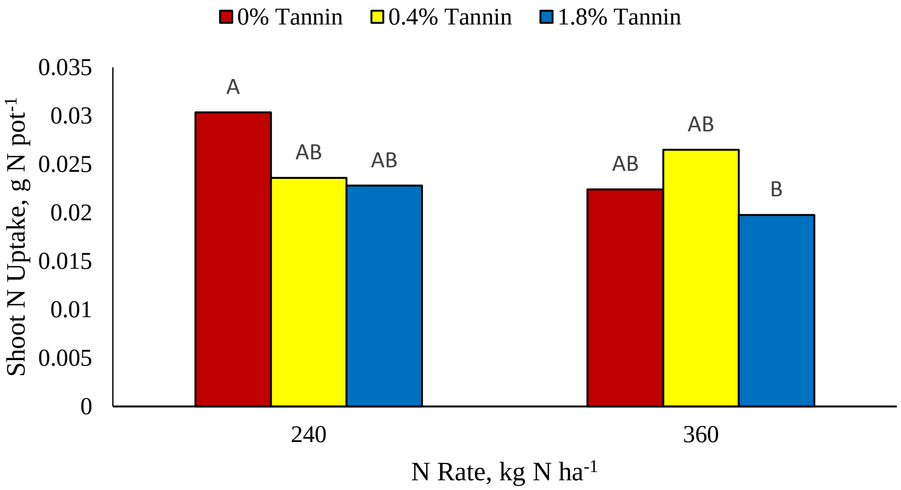

Figure 1. N uptake in winter wheat following F1 manure application. Letters show significant differences between tannin and N rate interactions (P<0.05).

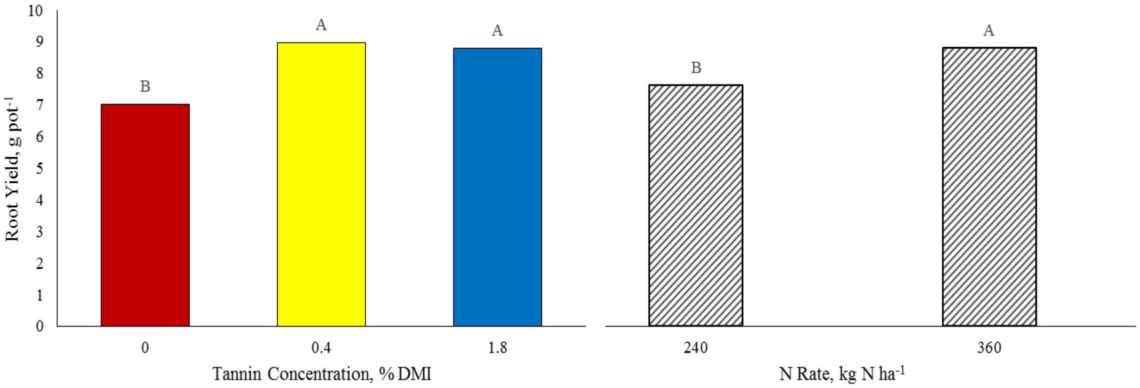

Figure 2. Winter wheat root yield, shown by tannin concentration and N rate. Letters show significant differences within tannin level and N rate (P<0.05)

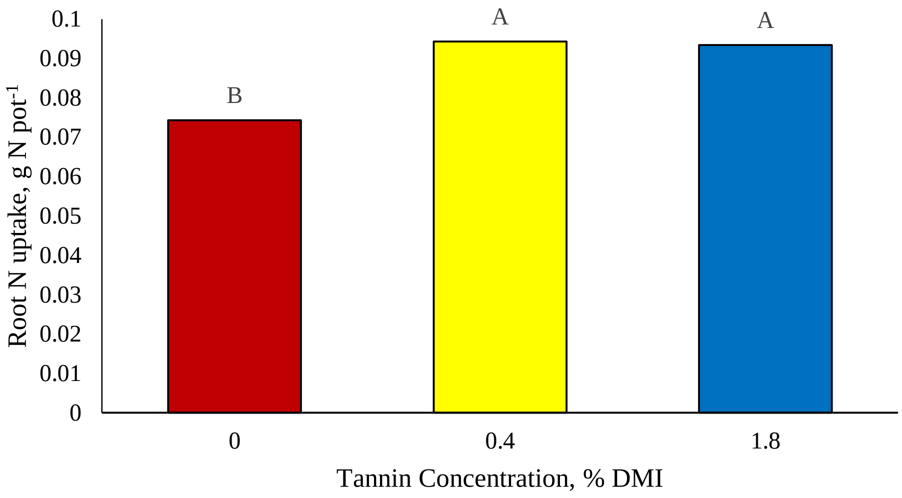

Figure 3. Winter wheat root N uptake, shown by tannin concentration from F1 manure application. Letters show significant differences within tannin level ($P<0.05$).

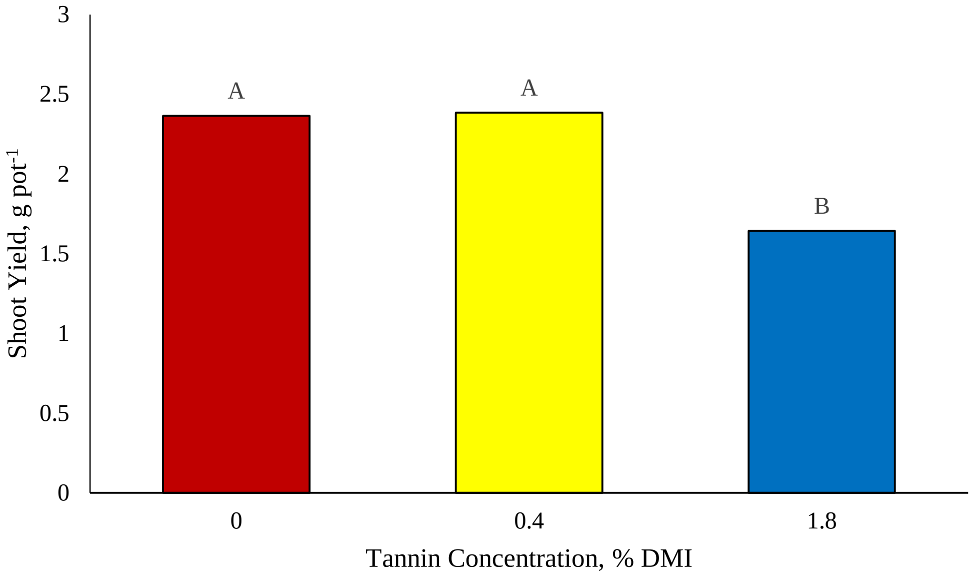

Figure 4. Sorghum shoot yield following harvest 2, shown by tannin diet concentration in manure. Letters show significant differences within tannin level (*P*<0.05).

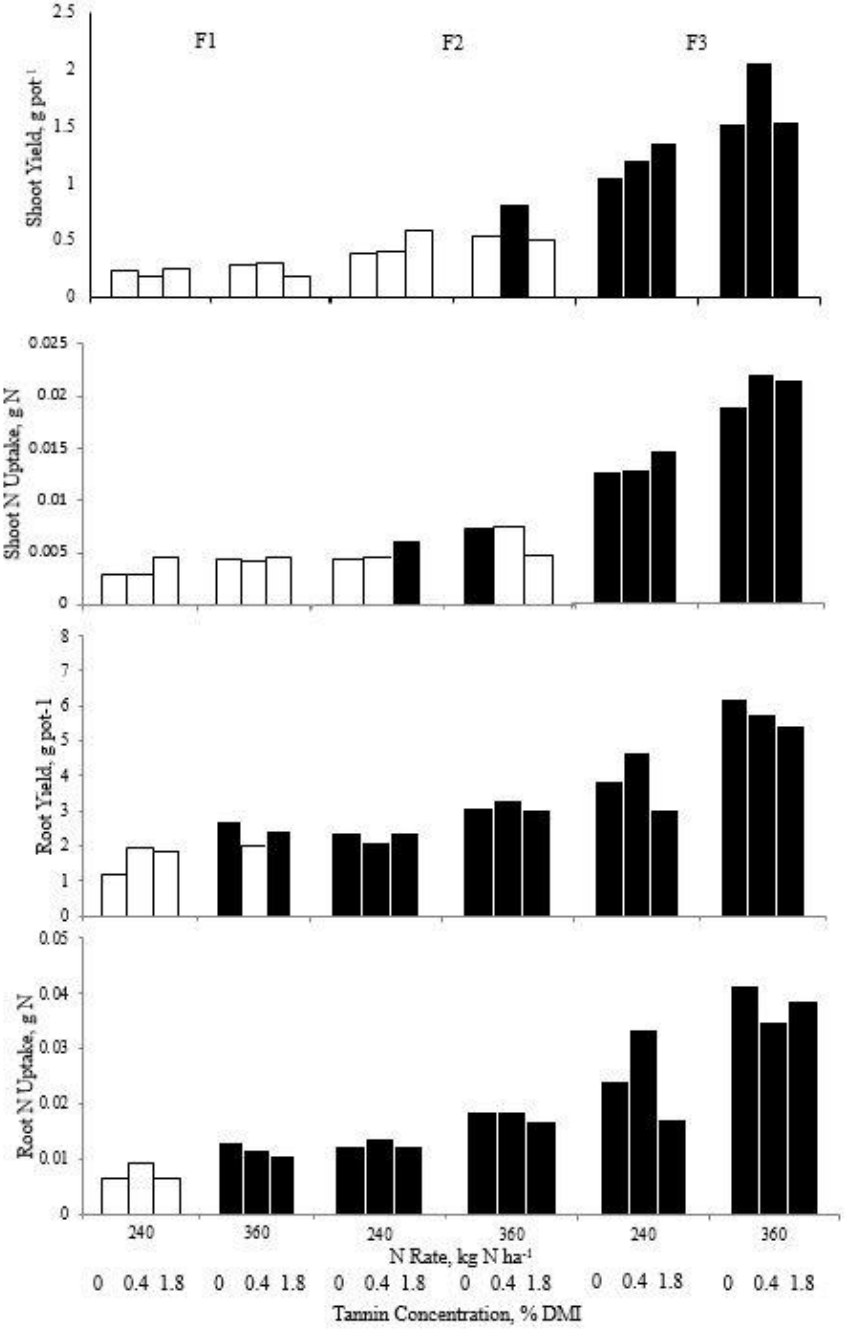

Figure 5. Sorghum ratoon (a) shoot yield, (b) root yield, (c) shoot N uptake, and (d) root N uptake shown as difference from control, following F1, F2, or F3 manure applications at either 240 or 360 kg N ha^{-1} manure application rates. Within each frequency and N application rates, tannin level increases from left to right at levels 0, 0.4, and 1.8% DMI. White bars indicated that yields were not significantly different from the control, while black bars were significantly different (P<0.05).

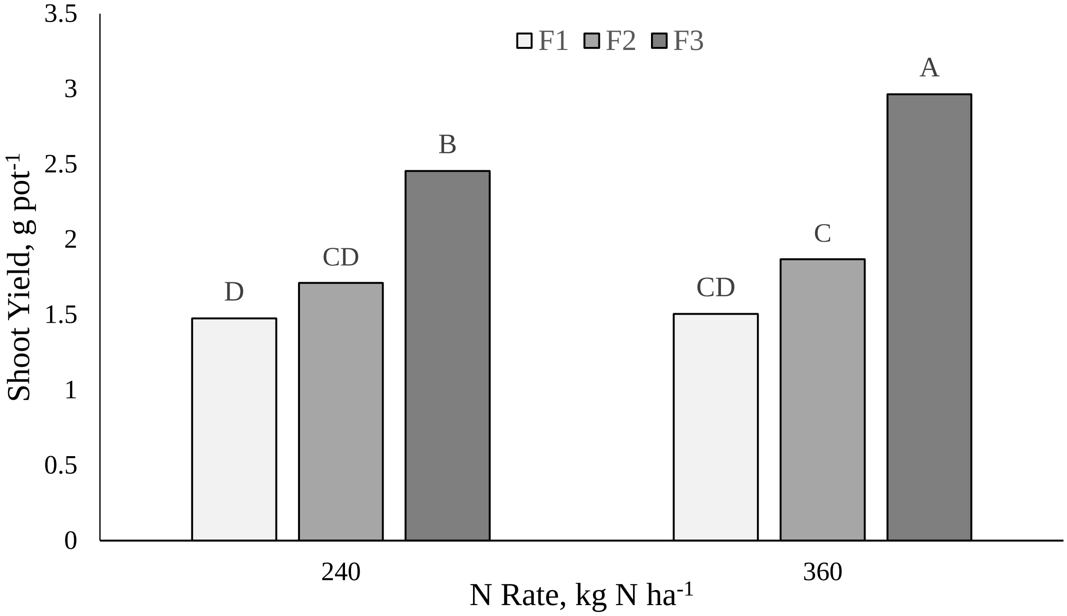

Figure 6. Sorghum ratoon shoot yield by N rate and manure application frequency. With increasing frequency, yield increased, with significantly higher yields at the F3 level for both N rates. Letters designate significant differences across N rate and frequency (P<0.05).

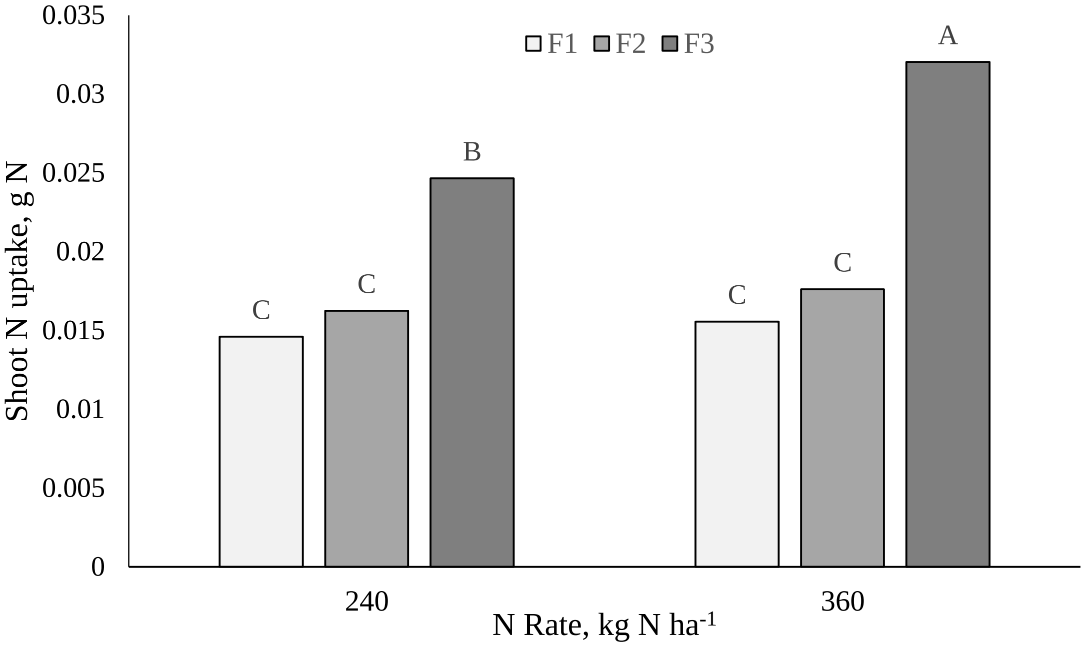

Figure 7. Sorghum ratoon shoot N uptake by N rate and manure application frequency. Letters show significant differences within N rate and frequency (*P*<0.05).

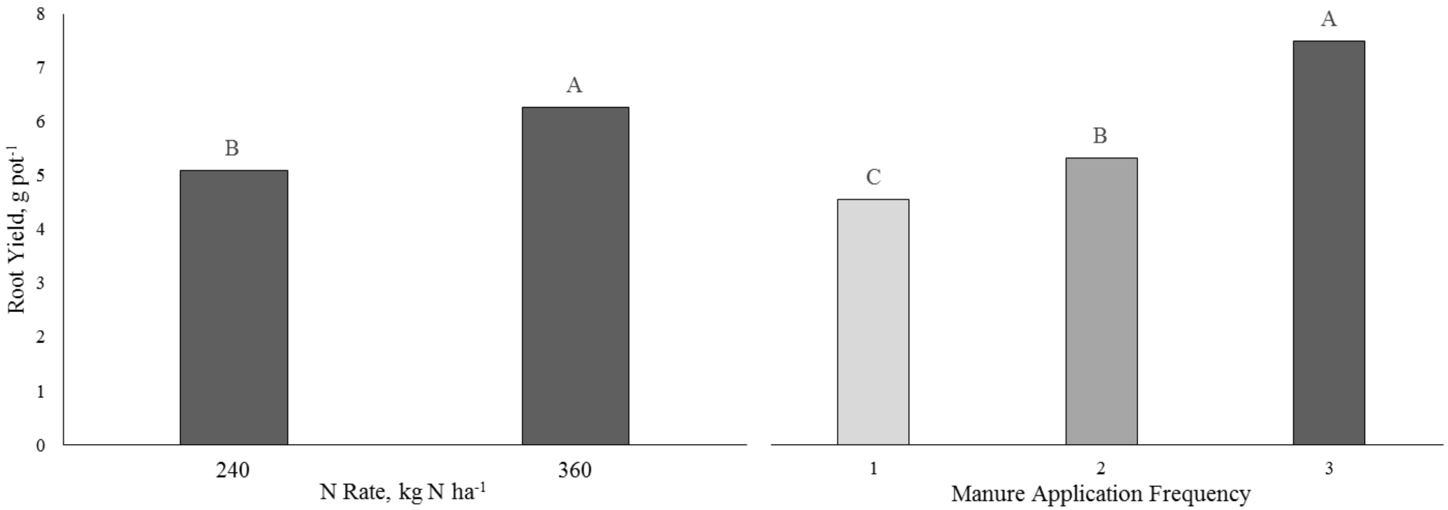

Figure 8. Sorghum ratoon root yield, shown by N rate and manure application frequency. Letters show differences between N rates and between manure application frequency separately (P<0.05).

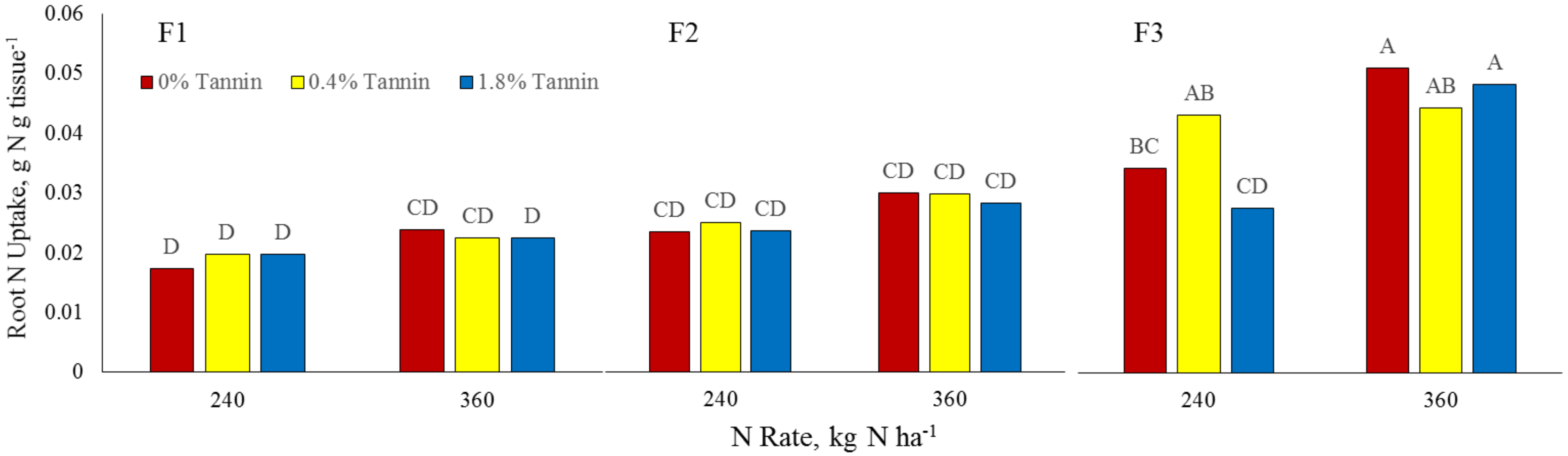

Figure 9. Sorghum ratoon root N uptake, following harvest 3, shown by N rate and tannin level, at F1, F2, and F3 manure application frequencies. Interaction effects of tannin, N rate, and frequency are shown, with the highest root N uptake occurring in pots which received the 360 N rate at the F3 level. Letters show significant differences across frequency, N rate, and tannin level (P<0.05).

SAS Code

Winter Wheat, Harvest 1:

```
proc glm data=a;
class trt;
model abvyield = trt / solution;
means trt/ dunnett ('7');
/*where trt=7 is the control*/
contrast 'LowN vs HighN' trt 1 -1 1 -1 1 -1 0;
contrast 'OT vs LT' trt 1 1 -1 -1 0 0 0;
contrast 'OT vs HT' trt 1 1 0 0 -1 -1 0;
contrast 'LT vs HT' trt 0 0 1 1 -1 -1 0;
contrast 'freq 1 vs freq 2' trt 1 1 1 1 1 1 1;
contrast 'freq 1 vs freq 3' trt 1 1 1 1 1 1 1;
contrast 'freq 2 vs freq 3' trt 1 1 1 1 1 1 1;
run;

data b; set a; if trt=7 then delete;
proc mixed data=a method=type3;
      class pot trt tannin rate freq;
      model abvyield = tannin|rate|freq ;
        lsmeans tannin|rate|freq / PDIFF adjust=Tukey alpha=0.05;
        slice tannin*rate*freq/ sliceBy freq;
run;
```

SAS Code for Sorghum, Harvest 2:

```
proc glm data=b;
class trt;
model Nuptake = trt / solution;
means trt/ dunnet('13');
/*where trt=13 is the control*/
contrast 'LowN vs HighN' trt 1 1 -1 -1 1 1 -1 -1 1 1 -1 -1 0;
contrast 'OT vs LT' trt 1 1 1 1 -1 -1 -1 -1 0 0 0 0 0;
contrast 'OT vs HT' trt 1 1 1 1 0 0 0 0 -1 -1 -1 -1 0;
contrast 'LT vs HT' trt 0 0 0 0 1 1 1 1 -1 -1 -1 -1 0;
contrast 'freq 1 vs freq 2' trt 1 -1 -1 1 -1 -1 1 -1 -1 1 -1 -1 0;
contrast 'freq 1 vs freq 3' trt 1 1 1 1 1 1 1 1 1 1 1 1 1;
contrast 'freq 2 vs freq 3' trt 1 1 1 1 1 1 1 1 1 1 1 1 1;
run;

data b; set b; if trt=13 then delete;
proc mixed data=b method=type3;
      class tannin rate freq;
      model Nuptake = tannin|rate|freq;
      lsmeans tannin|rate|freq / PDIFF adjust=Tukey alpha=0.05;
        ods output diffs=ppp lsmeans=mmm;
        ods listing exclude diffs lsmeans;
run;

%include 'c:\pdmix800.sas';
```

```
%pdmix800(ppp,mmm,alpha=0.05,sort=yes);
run;
```

SAS Code for Sorghum Ratoon, Harvest 3:

```
proc glm data=a;
      class trt;
      model  pH = trt / solution;
      means trt/ dunnett('19') alpha=0.05;
/*where trt=19 is the control*/
      contrast 'LowN vs HighN' trt 1 1 1 -1 -1 -1 1 1 1 -1 -1 -1 1 1 1 -1 -1 -1
0;
      contrast 'OT vs LT' trt 1 1 1 1 1 1 -1 -1 -1 -1 -1 -1 0 0 0 0 0 0 0;
      contrast 'OT vs HT' trt 1 1 1 1 1 1 0 0 0 0 0 0 -1 -1 -1 -1 -1 -1 0;
      contrast 'LT vs HT' trt 0 0 0 0 0 0 1 1 1 1 1 1 -1 -1 -1 -1 -1 -1 0;
      contrast 'freq 1 vs freq 2' trt 1 -1 0 1 -1 0 1 -1 0 1 -1 0 1 -1 0 1 -1 0
0;
      contrast 'freq 1 vs freq 3' trt 1 0 -1 1 0 -1 1 0 -1 1 0 -1 1 0 -1 1 0 -1
0;
      contrast 'freq 2 vs freq 3' trt 0 1 -1 0 1 -1 0 1 -1 0 1 -1 0 1 -1 0 1 -1
0;
run;

data a; set b; if trt=19 then delete;
proc print;
proc mixed data=a;
      class tannin rate freq;
      model pH = tannin|rate|freq;
      lsmeans tannin|rate|freq / PDIFF adjust=Tukey alpha=0.05;
        ods output diffs=ppp lsmeans=mmm;
        ods listing exclude diffs lsmeans;
%include 'C:\pdmix800(3).sas';
%pdmix800(ppp,mmm,alpha=0.05,sort=yes);
run;
```

Chapter 4
Conclusions

Manure management through changes to dairy cow diets is an under studied technique to improve whole farm N management and mitigate GHG emissions for dairy farms. Research has shown that polyphenol compounds have potential to reduce gaseous N losses from dairy barns and manure application to soil, particularly from tannin, and improve manure N characteristics and plant availability after manure application to soil. Through field (Chapter 2), greenhouse (Chapter 3), and incubation (Appendix 1) studies, the benefits of tannin diets were analyzed to assess: (i) N losses in manure from storage, (ii) GHG mitigation potential from tannin manure at the field level, (iii) soil inorganic N concentrations over time to determine N mineralization and (iv) agronomic production potential through manure land applications to corn, winter wheat, and sorghum.

Manure N Losses during Storage

From the end of manure collection in the barn until land applications, manures from tannin diets had higher N losses during a 40 day storage period, though manure total N was higher in tannin manures at the time of collection. This result was contradictory to previous findings, where tannin manures were able to retain more reactive N through storage (Aguerre et al. 2010). Research has shown that tannins are most effective at binding to N (proteins) and slowing the N cycle when pH is more acidic—and dairy manure, a high pH environment, may diminish the overall effectiveness of tannins in these systems. Nitrogen is lost through manure storage as NH_3 gas at high pH. When manure is handled in association with research practices such as the stirring, transfer to buckets and spreading during land application as occurred in the field study, reactive N is lost, which has implications on volatile N loss and subsequent N availability to crops. This study showed that, tannin manures lost more reactive N during handling and storage, which altered N application rate and subsequent N availability.

Greenhouse Gas Mitigation Potential

No differences in GHG flux were observed between treatments following field applications of manures in the 2014 growing season. Though it was not significant, lower GHG emission flux was observed in treatments at the lower manure N application rates and in treatments with tannin. This suggests potential for GHG mitigation, though field level variability can cause large ranges in GHG flux, even within the same field system associated with soil moisture, temperature, and inherent heterogeneity of soil N pools.

Soil N Dynamics following Tannin Manure Applications

Tannin manures showed potential to delay N mineralization in field, greenhouse, and incubation soils, perhaps allowing for enhanced synchronization of soil N availability and plant N requirements. In the first 20 days following manure application, soil N differences were observed due to tannin treatments at the field level, while differences were observed by N rate in incubated pots during the first week after manure application. At the field level, tannin manures delayed NO_3^- -N formation, while in a controlled study the amount of N applied had increased NH_4^+-N concentrations. Pre-plant field soil samples in 2015 corroborated that tannin delays N mineralization, with higher inorganic N concentrations in treatments at the high tannin level compared to treatments without tannin. Thus, there is potential for added N credit in a second growing season from tannin or when tannin manures are added over multiple cropping sequences, as shown in the greenhouse study.

Corn Silage, Winter Wheat, and Sorghum Production

Tannins may benefit dairy producers as a soil fertility amendment to improve yields, boost agronomic production, and influence root N uptake in corn silage, winter wheat, and sorghum. Across all dairy forage cropping sequences studied, tannin diets did not negatively impact yields of any crop when compared to other treatments that received manure. In the field corn silage system, all manure treatments had significantly higher yields than control treatments in the first growing season, while no differences were observed in the second season, suggesting that no manure N credit was available. In the greenhouse trial, with multiple applications of manure over three cropping sequences, tannin manures positively influenced sorghum yield following two or three manure applications. Root N uptake was higher due to tannin manure applications to winter wheat and sorghum, which suggests that a potential N credit exists with tannin manure.

Overall, tannins manures showed positive impacts when added to dairy production systems in Wisconsin. Though GHG mitigation potential was not statistically significant, trends show that there are less overall emissions from soil following tannin manure applications, which is corroborated by findings from previous incubation trials studying tannin mitigation potential. Tannin manure appeared to slow soil N mineralization during the incubation trials, suggesting that more N is available at optimum growth periods for forage cropping systems, which was corroborated in the field, with inorganic N still available approximately one year after tannin manure application. Yields were not negatively impacted in the presence of a single tannin manure application, and after multiple applications, actually increased forage shoot and root yields.

Results of these field, greenhouse, and incubation trials shed light on possible directions of future research assessing tannin impacts on N cycles of dairy systems. In manure storage, it may be useful to assess how tannins influence N loss during other techniques for manure handling, including liquid-solid separation and digestion, with or without additives lower manure pH. To assess impacts on milk production, soil N, and agronomic production, further study could be conducted to determine an optimum tannin feeding rate that maximized N use in the various production components. Lastly, in order to assess the long-term benefits of tannins, multiple applications of manure are needed at the field level, to assess how tannin manures impact soil N cycle dynamics, including crop yields and environmental N loss.

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Appendix 1

Soil Nitrogen Mineralization with Tannin Manures in an Incubation

Introduction

Livestock manure land applications are an important source of nitrogen (N) for agricultural crops. However, manure N is often utilized inefficiently, with N lost throughout the manure collection, storage, and eventually land application of manure to soils (Chadwick et al. 2011, Montes et al. 2013). Nitrogen losses occur through gaseous losses, as ammonia and nitrous oxide emissions, and through nitrate leaching through soils, which can account for up to 30% of total agricultural N loss in Wisconsin, resulting in higher concentrations of nitrates in groundwater and agricultural watersheds (Montes et al. 2013, Aguirre-Villegas et al. 2014). Research has shown that one way to improve manure N management is by adjusting the nutrient contrent of diets fed to dairy cows (Powell et al. 2009). Changes to dietary crude protein, fiber digestibility, and feed additives have reduced manure N excretions thereby mitigating greenhouse gas emissions and reducing N losses from soils (Montes et al. 2013, Aguerre et al. 2010, Powell et al. 2009). One particular feed additive that has shown success in improving N management in dairy farms is tannin, a polyphenol compound that binds to protein (Aguerre et al. 2010, Powell et al. 2009).

Previous research using tannins as feed additives to total mixed rations (TMR) has shown significant positive effects at the cow and farm level, with enchancements in milk protein, reduced ammonia emissions from manure during collection and storage, and increased N concentrations in feces, which can enhance manure N recycling and subsequent crop N availability (Aguerre et al. 2010, Powell et al. 2011). A study assessing the nutrient response from the tannin containing forage birdsfoot trefoil showed however, no differences in manure N availability to soils when applied at the field level (Powell and Grabber 2009). Though not discernible at the field level, manures from tannin feeding trials may have potential to have higher fertility when applied to soils based on higher N concentrations at the time of manure collection, which can influence N mineralization and plant available N in soils.

Tannins can act as regulators of N cycling in soils, particularly when N is limited in an ecosystem (Mutabaruka et al. 2007, Hättenschwiler and Vitousek 2000). In forests with acidic soil, research has shown that tannins can significantly slow soil N mineralization rates, which can allow for higher efficiencies in plant N uptake and less nitrate leaching (Kuiters et al. 1990). In many plants containing polyphenols, as cell tissue dries out in the fall, tannin is preserved in dried leaf tissue, accounting for as much as 60% of leaf N. Because tannins are tightly bound to protein, these materials are preserved until they are needed when soil microbial activity is higher, in the spring and summer (Kuiters et al. 1990).

Tannin is able to regulate soil N because microbial communities cannot as effectively utilize tannin N compounds. Tannins protect plant tissues from herbivory, resulting in more N remaining in organic forms in soils (MacAdams et al. 2013). Research has shown that when applied to agricultural fields, tannin was initially degraded more rapidly, but after a 480 day incubation of polyphenol materials, the maize system decreased overall microbial N biomass, resulting in decreased N mineralization in agricultural soils (Mutabaruka et al. 2007). The slowing of N cycling is attributed to the presence of polyphenols over several growing seasons, and studies suggest that this allows a slower release of N at times when it is most needed for plant production (Kuiter et al. 1990, Millar and Baggs 2004). In a greenhouse, tannin manures resulted in significantly lower aboveground biomass yield and higher root yields, showing that tannin influences plant production in N limited soils (Chapter 3). However, particularly in forested areas that have received tannin inputs for several seasons, tannin tissues can more effectively be metabolized, and N is mineralized more rapidly, suggesting that systems with long term exposure to polyphenol materials are likely to stimulate N cycling and drive higher N mineralization (Mutabaruka et al. 2007). There are still significant gaps in understanding of polyphenols and the role of tannins in N cycling, especially in agricultural soils, where N is more readily managed so as not to be limiting (Hättenschwiler and Vitousek 2000, Millar and Baggs 2004).

Because tannins hydrolyze to organic compounds, which change rapidly as plant tissues and forages dry and decay, they are often difficult to quantify in soils and manure. However, previous research suggests that tannin addition to soil suggest reduces N losses through leaching and denitrification, and result in potentially higher soil fertility from these systems (Millar and Baggs 2004, MacAdams et al. 2013, Hättenschwiler and Vitousek 2000, Kato et al. 2010). The complexities of the senescence and eventual mineralization of tannins is further obscured by the fact that at the field level, abiotic factors, including temperature, soil moisture, and soil disturbance can further influence tannin behavior in agricultural systems. To assess this behavior without the influence of field level variability, the objective of this trial was to determine how the presence of tannin manures from feeding trials influence soil N.

Materials and Methods

Soil Description

Representative soil samples were taken from the USDA Dairy Forage Research Center farm in Prairie du Sac, WI, 53578 (43.332899, -89.716275), on a St. Charles silt loam (Fine-silty, mixed, superactive, mesic Typic Hapludalfs) in August 2014. Soil samples were removed with a stainless steel shovel from the A horizon of the soil profile (0-15 cm). All samples were homogenized, air dried and sieved to pass through a 2 mm sieve and stored in plastic containers until the study began in December 2014. Phosphorus was applied at a rate of 40 kg P ha^{-1} as KH_2PO_4 and watered with deionized water to achieve 60% water filled pore space (WFPS). Pots were then left fallow for one week before manure application.

Soil for this experiment was also used in a paired greenhouse study, with physical and chemical characteristics analyzed for these samples prior to the start of the study (Table 1).

Experimental Design

This study was designed as a randomized complete factoral design, with two whole plot factors; tannin level from a paired feeding trial, at 3 levels (0, 0.4, and 1.8% tannin as dry matter intake) (Aguerre et al. 2015 *in review*). The second factor was target N application rate, at two levels (240 and 360 kg N ha^{-1}). Each treatment was replicated three times, along with 3 control incubation soil cups, which received no manure. After application, manure cups were randomly placed inside a 22° C incubator and watered weekly to maintain 60% WFPS throughout the trial.

Manure Application and Measurements

Dietary feeding trials were conducted at the Dairy Forage Research Center farm in Prairie du Sac, WI in association with a paired manure application study (Chapter 2). To mimic a field manure injection, approximately 25% of soil was removed from the incubation cup, manure was added by weight, based on the TN concentration of the manure assumed from bulk manure sample analysis, and the top 25% of the soil was replaced. The soil and manure were then mixed with a stainless steel spatula and packed to an approximate bulk density of 1.20 g cm^{-3}, and watered immediately. Cups were then capped and placed in an incubator.

Manure characteristics were determined at the time of the initial manure application for this study with samples from 6 February 2015 (Table 2). TN and TC was determined using the Glutamic acid method on a Variomax automated analyzer (Elementar Variomax CN Analyzer, Elementar Inc., Hanan, Germany). Manure pH was analyzed using a 2:1 manure to water mixture and analyzed with a portable pH probe at the time of the initial incubation manure application.

Soil Measurements

To determine soil N mineralization and nitrification, soil cores were taken on days 2, 3, 7, 28, 56, 112, and 168, with day 1 representing the day that manure was applied. Cores were taken using a size 8 rubber cork stopper hole borer throughout the entire soil profile within the specimen cup. Soil samples were oven dried for 24 hours at 100° C and weighed to determine dry matter. Soil inorganic N was determined using a 2 M KCl extraction following the methods of Doane and Horwath (2003), and analyzed for extractable NO_3^- -N and NH_4^+ -N on an automated Lachat N analyzer (Lachat Instruments, Loveland, CO, 1996).

Calculations and Statistics

Plant available N (PAN) concentrations were determined as the sum of extractable ammonium and nitrate concentrations at each sampling time point. Net PAN was determined as PAN at a sampling time point minus PAN of the control at the same sampling time. Net nitrogen mineralization (NNM) was determined following Griffen et al. (2008), where NNM was calculated as the change in inorganic N between the control at the time of manure application and PAN concentrations at each sampling point, using the following formula:

$$NNM = [\, NH_4^+\text{-}N_{(t)} + NO_3^-\text{-}N_{(t)} \,] - [NH_4^+\text{-}N_{(0)} + NO_3^-\text{-}N_{(0)}] \qquad (1)$$

Where NH_4^+-$N_{(t)}$ and NO_3^- -$N_{(t)}$ were sampled at time (t), subtracted from control levels at the beginning of the incubation trial at time zero (0). Net nitrogen mineralization rate was calculated as NNM divided by total number of days following manure application at that sampling time (t), and was thus calculated for each sampling day based on new cumulative PAN concentrations. NNM rate was calculated two ways, (1) averaged over all sampling days and (2) determined between sampling days.

Statistical analysis was completed using analysis of variance in SAS 9.4 (SAS Institute 1990). Treatments, defined as a combination of tannin and rate, were compared to control plots which received no manure using a general linearized model (Proc GLM) and a Dunnett's t- test, where incubation cup was treated as a repeated measure across all sampling days. A slice statement was used to determine differences between treatments within a given sampling day. To assess differences in tannin level and N rate, a separate analysis of variance was conducted without the control treatment using Proc Mixed. Without the control, each incubation cup was treated as a repeated measure over the six sampling days, with a slice statement used to determine effects of tannin and rate within day.

Results and Discussion

Manure

Between the initial sampling on 10 April 2014 and the time of manure application for the incubation trial on 6 February 2015, TN concentrations decreased in each manure treatment, resulting in no significant differences across tannin levels in manure TN concentrations at the time of application, with the tannin manures losing more N during storage than the non-tannin manure (Chapter 2). At the time of manure application for the incubation study, NH_4^+-N concentrations in manure were 1.05, 0.91, and 0.73 g N kg^{-1} wet manure for 0, 0.4, and 1.8% tannin levels respectively. Dry matter content of manure was 10.3, 11.7, and 11.5% of total manure weight at the time of application for 0, 0.4, and 1.8% tannin levels respectively, with the rest of the manure in liquid components.

Manure pH at the time of the first manure application for this trial was 7.85 for no tannin, 7.84 for 0.4% tannin, and 7.63 for 1.8% tannin. Previous research has shown that polyphenol compounds are more effective at delaying N mineralization in soil when pH is low (MacAdams et al. 2013, Mutabaruka et al. 2007). Because manure has a neutral, and sometimes high pH, the environment may lead to diminished effectiveness of tannin in protecting N sources through storage and soil application. Additionally, at lower pH, studies have found that microbial activity is significantly diminished, which could slow mineralization of organic materials in soil (Mutabaruka et al. 2007).

Soil Nitrogen

Following manure application, all treatments that received manure had significantly higher soil NH_4^+ -N concentrations than control treatments for the first seven days (*P*<0.0001), but by day 28, NH_4^+ -N concentrations decreased to negligible levels and no statistical differences were observed between any manure treatments and control, no manure soil (Figure 1). Within sampling day, there was a significant effect of target N rate on NH_4^+ -N concentrations, with the target 360 N rate applications resulting in higher NH_4^+ -N concentrations than samples at the 240 target N rate in the first 7 days following manure application. Interaction effects of rate*tannin*day were observed in the first 7 days following application. While other treatments immediately began to decrease NH_4^+ -N concentrations following application, soil NH_4^+ -N increased in treatments that received 0% tannin-360 N rate and 0.4% tannin, 240 N rate. The NH_4^+ -N concentrations observed in the incubation were the opposite of what was seen at the field, where tannin concentration was the influencing factor on NH_4^+ -N concentrations in the first 20 days following field application, with tannin decreasing NH_4^+ -N concentrations (Chapter 2).

The differing results of the incubation and field results could also be due to higher soil N variability at the field level, and the differences in the total volume of soil exposed to the manure application—with a significantly higher manure to soil weight ratio used in the incubation trial.

Soil nitrate concentrations were significantly different from control plots that received no manure when sampled day 3 following application. Throughout the experiment, control pots that had received no manure had significantly higher NO_3^- -N concentrations compared to treatments with manure (Figure 2). This is due to the handling of soil during preparations for the experiment, including soil grinding, sieving, and watering prior to manure application, which promoted soil mineralization at rates above normal field levels. Previous incubation trials of soil have seen similar results, with very high concentrations of NO_3^- -N early in incubation periods as the result of soil handling (Cusick et al. 2006). High NO_3^- -N observed in the 72 hours following manure application could be the result of rapid soil mineralization, given that soil microorganisms had already been stimulated by soil processing for the experiment. Therefore, the most significant N mineralization that occurred following manure application was observed in the first hours following manure application, with nitrate concentrations declining in manured treatments following the immediate mineralization. Comparatively the control, which did not receive manure, showed a more standard soil N mineralization response, with increasing NO_3^- -N on days 28 and 56. The decline in NO_3^- -N in control incubation cups between days 56 and 128 is assumed to be the result of denitrification following incubation watering on day 80, which promoted more anaerobic conditions in the incubation cups, though there was no significant increase in soil moisture in any incubation cups (Figure 3).

The combined effects of denitrification and immobilization resulted in lower nitrate concentrations from soil. Without the control, pots were also analyzed to determine the impacts of manure N rate and tannin treatments. Results show that while manure N rate was not significantly different between manure treatments over time, there was a significant effect of tannin on nitrate ($P < 0.05$). On day 7, the highest nitrate concentrations were observed in treatments without tannin added to feeding trials at the target 360 N rate, while nitrate concentrations were significantly less in systems that had received tannin in feed. This suggests that tannins are impeding manure nitrification in soils immediately following manure applications. Similar results were observed in the field study, with significantly less NO_3^- -N in soil 20 days following manure applications in treatments that had received tannin in manures compared to manure without tannin (Chapter 2). A study by Kuiter et al. (1990), found similar results, with the presence of higher concentrations of polyphenol compounds in soils reducing nitrification rates in forest stands. Upon examination of the bacterial communities in these systems, the study concluded that *Nitrosomonas*, bacteria responsible for nitrification, was more susceptible to than *Nitrobacter*, bacteria responsible for ammonification, to the inhibiting effects of polyphenols in soils, which explains why tannins influence nitrate but not ammonium concentrations (Kuiters et al. 1990). These results differ from previous tannin feeding trial manure applications, which showed no effects of tannin on PAN in soils following feeding trials of birdsfoot trefoil, a forage with naturally high tannin concentrations (Powell and Grabber 2009).

The decline in treatment differences in NO_3^- -N concentrations over time could be caused by the degradation of tannin compounds over time—something that is not well understood once tannin compounds reach soil surfaces (Mutabaruka et al. 2007, Hättenschwiler and Vitousek 2000). An interaction effect of rate*tannin*day was also observed; a significantly higher soil NO_3^- -N concentration peaked on day 7 for the no tannin, 360 N rate treatment, and then declined to similar levels as all other treatments. A study by Mutabaruka et al. (2007) found that tannin applications to soil as plant compounds have shown that when tannins are first introduced to agricultural soils, there is a significant microbial respiration response, suggesting that the tannins are readily taken up, which agrees with the data found in this study. With very low nitrate concentrations, much of the nitrogen was immediately immobilized or denitrified.

Total PAN concentrations were highest in the first week following manure application, and dropped off quickly over time, with the highest total PAN concentrations observed on day 3 after application (Figure 4). Manure N rate significantly affected total PAN in the first 7 days ($P<0.05$), with higher PAN in 360 N rate treatments than 240 N rate treatments. The decline in PAN is associated with the rapid N mineralization in the first 3 days following manure application, with NO_3^- -N concentrations being denitrified, resulting in decreasing PAN concentrations.

At the end of the incubation period, all treatments that had received target 360 N rate manure applications had significantly higher cumulative soil PAN concentrations than control treatments. This cumulative PAN suggests that over the growing season, without losses from nitrate leaching, N immobilization, or denitrification plants had access to more total N that was released during N mineralization over the entire growing season. This suggests that high N rate manure resulted in a manure N credit, associated with the higher total PAN available compared to control and 240 target N application rate levels.

Soil N mineralization rates, calculated over the entire growing season and between sampling days of the incubation trial were significantly higher from the control for all treatments on days 2 and 3 following manure application. On day 7, soil N mineralization rates were significantly different for all treatments except the high tannin, 240 N rate at $P<0.05$. No statistical differences were observed between the two methods of NNM rate calculation; all results presented below are based on background levels of soil N calculations for mineralization rates.

When the control was not considered, differences in NNM rate across treatments were influenced significantly by manure N rate and tannin level ($P<0.0001$). Nitrogen mineralization rate was significantly higher at the 360 N rate compared to the 240 N rate on days 2, 3, and 7 following application, with the highest NNM rates observed on day 1 (Figure 4). After day 7, no differences were observed.

Tannin level significantly influence N mineralization rate in the first week following manure application, with significantly less N minerlization occurring in treatments that received tannin (Figure 5).

On days 28, 56, and 128, there were no significant differences between treatments, suggesting that there was a delay in N mineralization caused by tannin, however, this delay did not inhibit N availability over the entire 128 incubation and the total PAN concentrations were not limited by tannin. The same observations were observed in forest systems, where phenolic substances impacted rates of N fixation and nitrification in stands of balsam and fir trees (Kuiters et al. 1990). Further studies agreed with this conclusion, but found that soil N nitrification was only delayed when soil systems had N in excess of plant needs (Turtura et al. 1989), which can have a significant effect on N cycling. This delay in mineralization could result in higher N efficiency in soils; where more soil N would become plant available later into an agricultural growing season. Additional benefits, including reduced nitrous oxide emissions and nitrate leaching could be observed in systems with delayed nitrification in agricultural settings.

Conclusions

Applications of dairy manure to soils had a significant impact on cumulative soil PAN and N mineralization over time based on N rate. Nitrate concentrations were significantly influenced by tannin concentrations in the first week following manure applications, suggesting there is potential for delayed N availability and thus better plant N synchronization and higher N efficiencies in dairy systems. These findings suggest there are potential mitigation benefits for environmental concerns including groundwater nitrate leaching and nitrous oxide greenhouse gas emissions. Temporary delays in N cycling observed in this study could result in more significant impacts when applied at the field level, in forages containing tannin, or with higher concentrations of tannins above the 1.8% DMI level. Further study is needed to determine what the environmental benefits of tannins are with respect to nitrate leaching and overall denitrification, and if these benefits are available in over multiple growing seasons.

Tables and Figures

Table 1. Soil characteristics of St. Charles silt loam in Prairie du Sac, WI, prior to manure applications.

Depth	pH	Bulk Density	Plant Available N	CEC	Texture
cm		g cm^{-3}	g N kg soil^{-1}	cmol kg $^{-1}$	
0-15	5.2	0.84	0.15	8	Silt loam

Table 2. Manure characteristics from tannin feeding trials from bulk manure samples on 10 December 2014.

Tannin Level	NH_4^+-N	TN	TC	pH
% DMI	g N kg^{-1} wet manure	g N kg^{-1} wet manure	g C kg^{-1} wet manure	
0	1.041	4.4	58.4	7.85
0.4	0.910	4.2	58.1	7.84
1.8	0.733	4.1	55.4	7.63

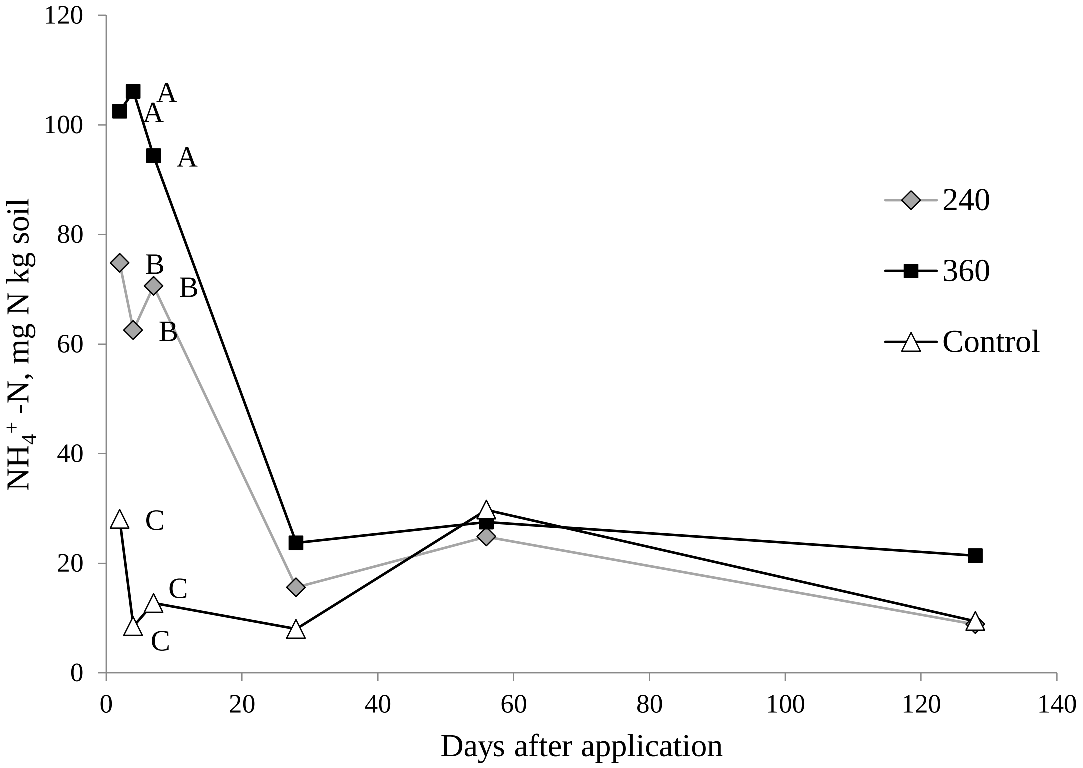


Figure 1. Extractable ammonium nitrogen from soil samples collected on days 2, 3, 7, 28, 56, and 128 following manure application. Letters show significant differences between treatments within sampling day (*P*<0.05).

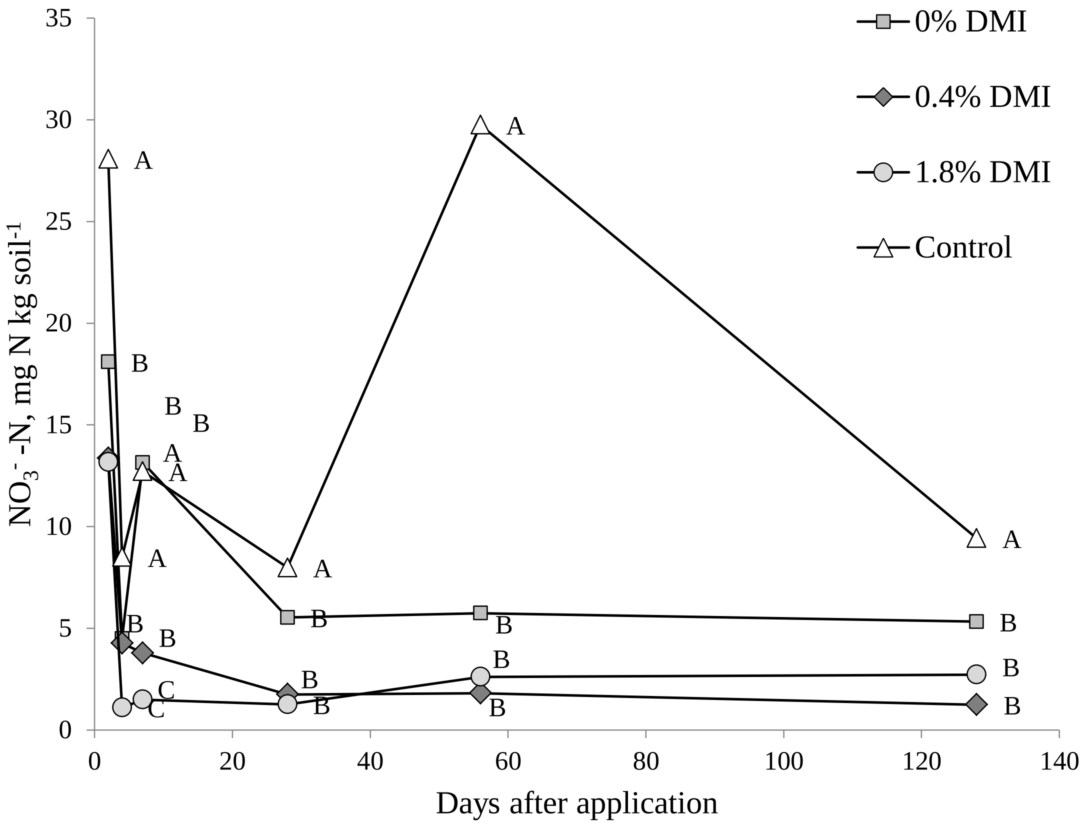


Figure 2. Extractable nitrate nitrogen from soil samples collected on days 2, 3, 7, 28, 56, and 128 following manure application. Letters indicate differences within sampling day.

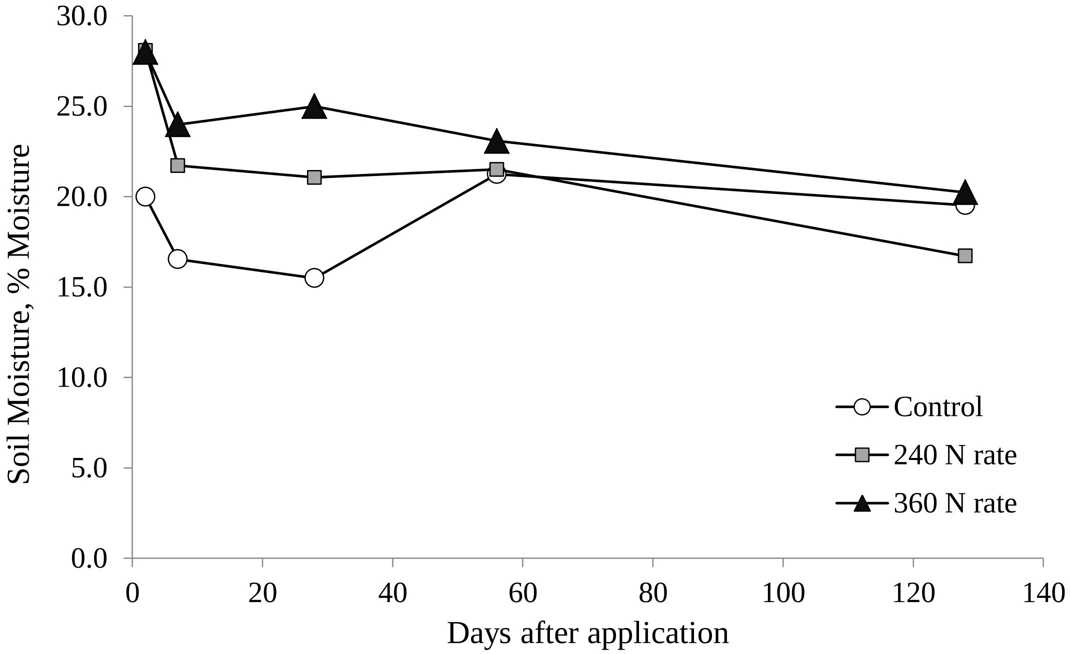


Figure 3. Soil moisture from incubation samples take on day 0, 7, 28, 56, and 128.

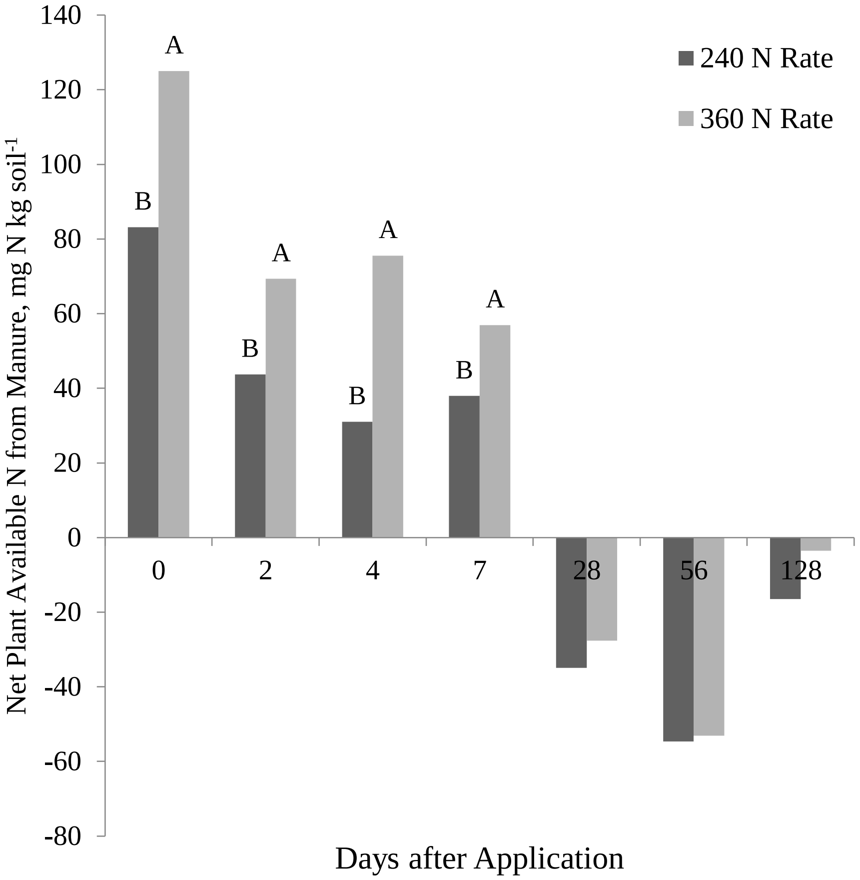


Figure 4. Net plant available N concentrations following manure applications at 240 and 360 kg N ha^{-1}. Letters indicate differences within a sampling day (*P*<0.05). On days 2, 4, and 7, positive net PAN concentrations are the result of rapid N mineralization, while on days 28, 56, and 128, negative net PAN suggests N was lost from the system compared to control cups.

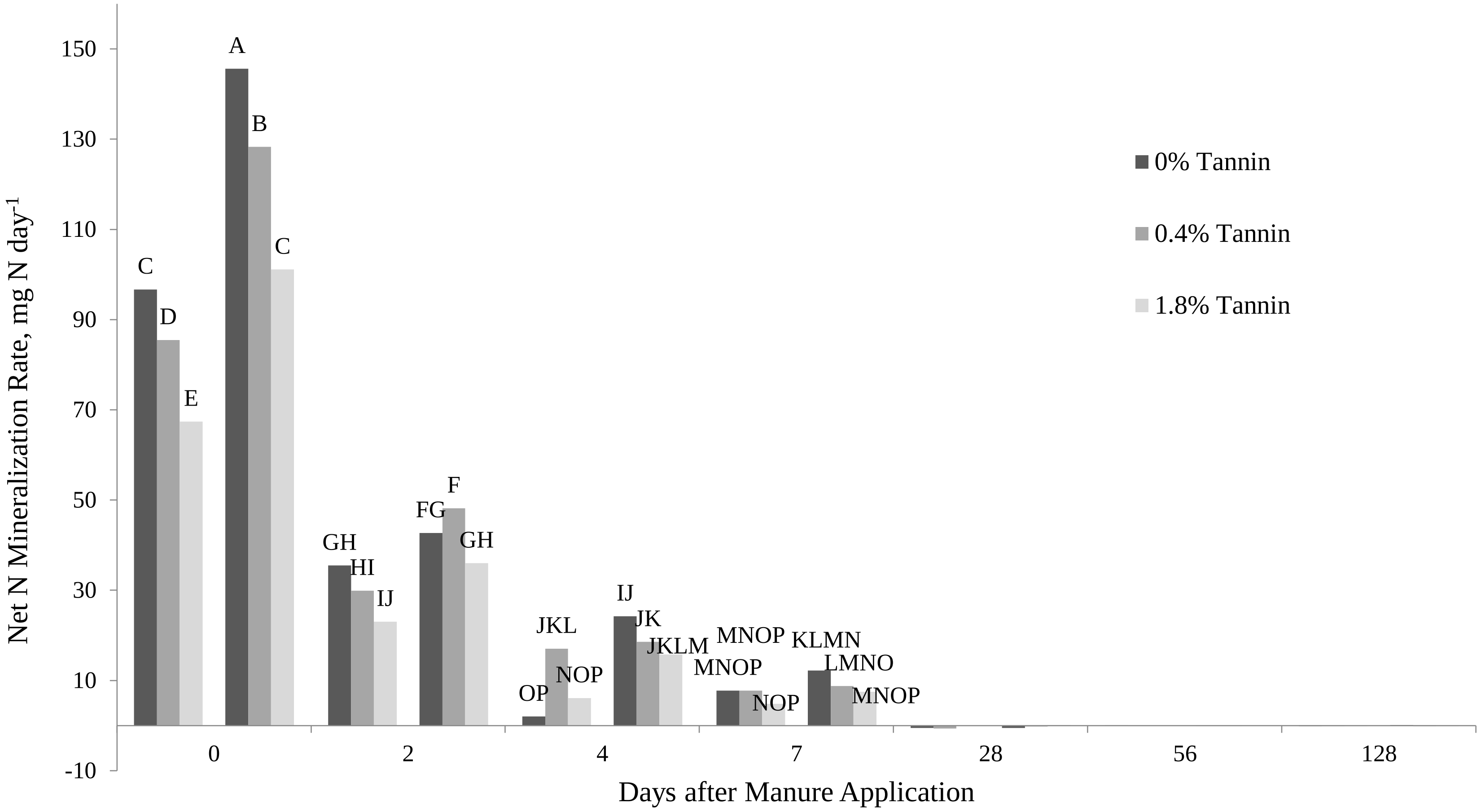


Figure 5. Net N mineralization rate, calculated on days 1, 2, 4, 7, 28, 56, and 128 following manure application. Letters indicate differences between tannin level and N rate treatment combinations across all sampling days (*P*<0.05). After day 7, no differences were observed between treatments.

SAS Code for Incubation Trial

```
data d; set a;
if trt=7 then trt=0;
d=day;

proc sort; by plot rate tannin trt;

proc mixed; class rate tannin trt days plot;
model {dependent variable} =  trt days trt*days;
random plot(trt);
repeated / subject=plot(trt) type=sp(pow)(d);
lsmeans trt  days trt*days / slice=days adjust=dunnett diff;
lsmeans trt  days trt*days / slice=days diff;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;

%include 'C:\pdmix800 (3).sas';
%pdmix800(ppp,mmm,alpha=0.05,sort=yes);
run;
;



data b; set a;
if trt=7 then delete;
proc print;
proc mixed;  class plot rate tannin days trt;
model {dependent variable} = rate tannin rate*tannin days rate*days
tannin*days rate*tannin*days / ddfm=kr;
random plot(rate*tannin);
repeated / subject=plot(rate*tannin) type=sp(pow)(d);
lsmeans rate tannin rate*tannin days rate*days tannin*days
rate*tannin*days/ slice=days diff;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;

%include 'C:\pdmix800 (3).sas';
%pdmix800(ppp,mmm,alpha=0.05,sort=yes);
Run;
```

Appendix 2: Greenhouse Trial Weekly Photos

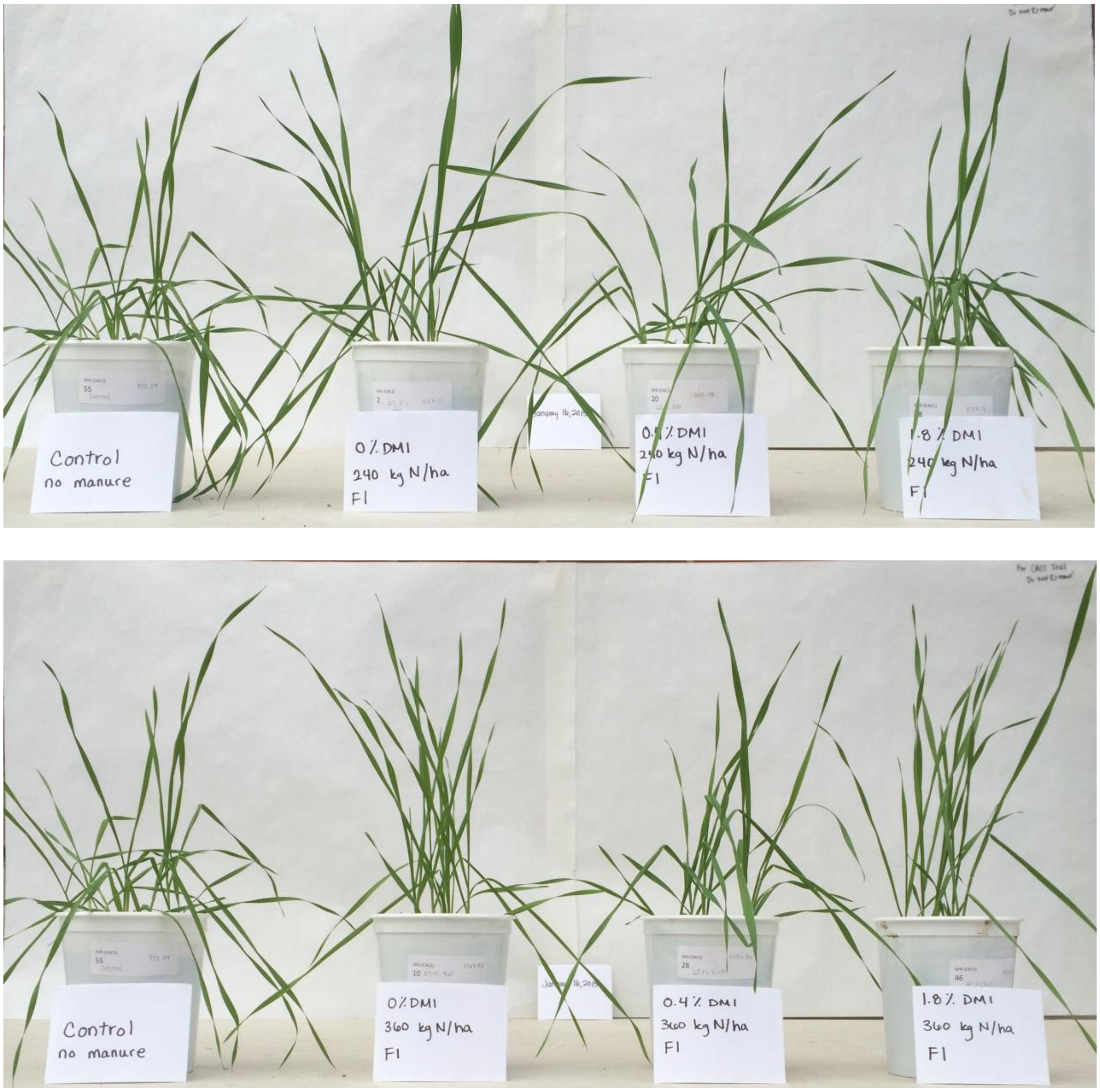


Figure 1. Winter wheat plants on 16 January 2015, with increasing tannin concentrations (left to right), of 0, 0.4, and 1.8% tannin of dry matter intake at (a) 240 kg N/ha target application rates and (b) 360 kg N/ha target N rates. In winter wheat, differences were not observed by tannin concentrations, but visually, differences were observed in winter wheat, with less lodging occurring at the higher N rate.

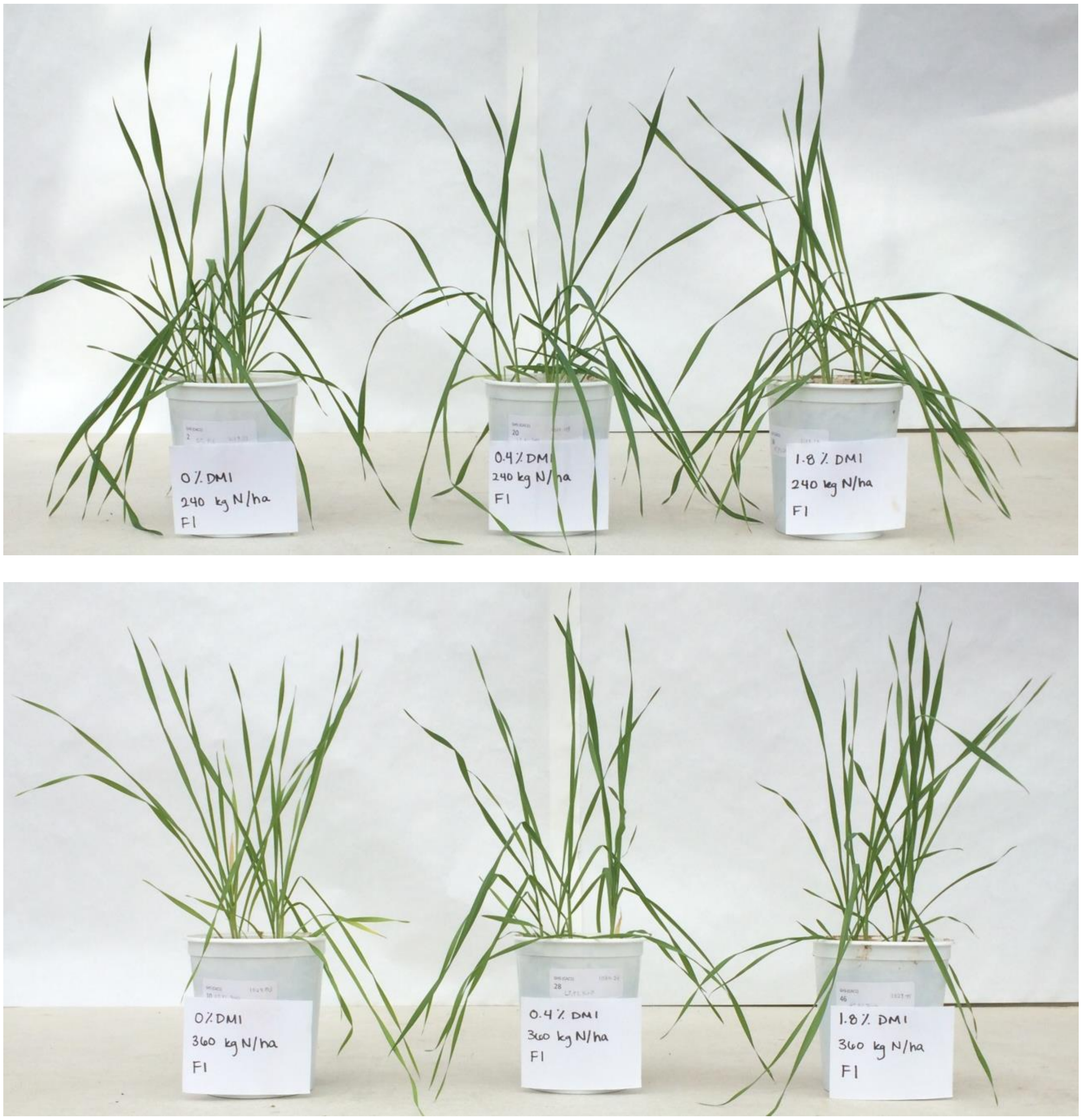


Figure 2. Winter wheat plants on 23 January 2015, with increasing tannin concentrations (left to right), of 0, 0.4, and 1.8% tannin of dry matter intake at (a) 240 kg N/ha target application rates and (b) 360 kg N/ha target N rates. In winter wheat, differences were not observed by tannin concentrations, but visually, differences were observed in winter wheat, with less lodging occurring at the higher N rate.

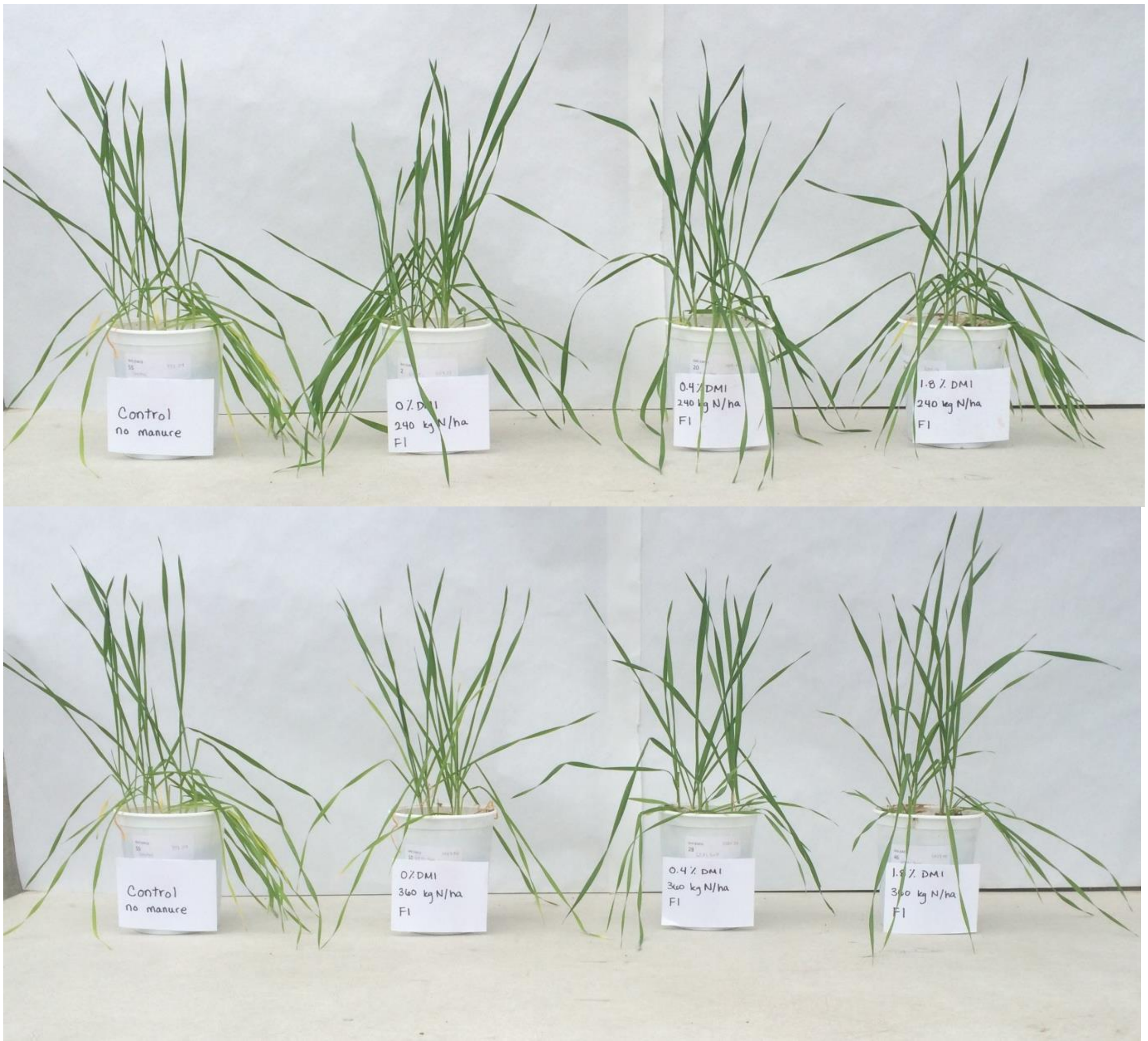


Figure 3. Winter wheat plants on 29 January 2015, with control pots and pots with increasing tannin concentrations (left to right), of 0, 0.4, and 1.8% tannin of dry matter intake at (a) 240 kg N/ha target application rates and (b) 360 kg N/ha target N rates. In winter wheat, differences were not observed by tannin concentrations, but visually, differences were observed in winter wheat, with less lodging occurring at the higher N rate. The control plots and low N rate began to show signs of chlorosis by 29 January 2015.

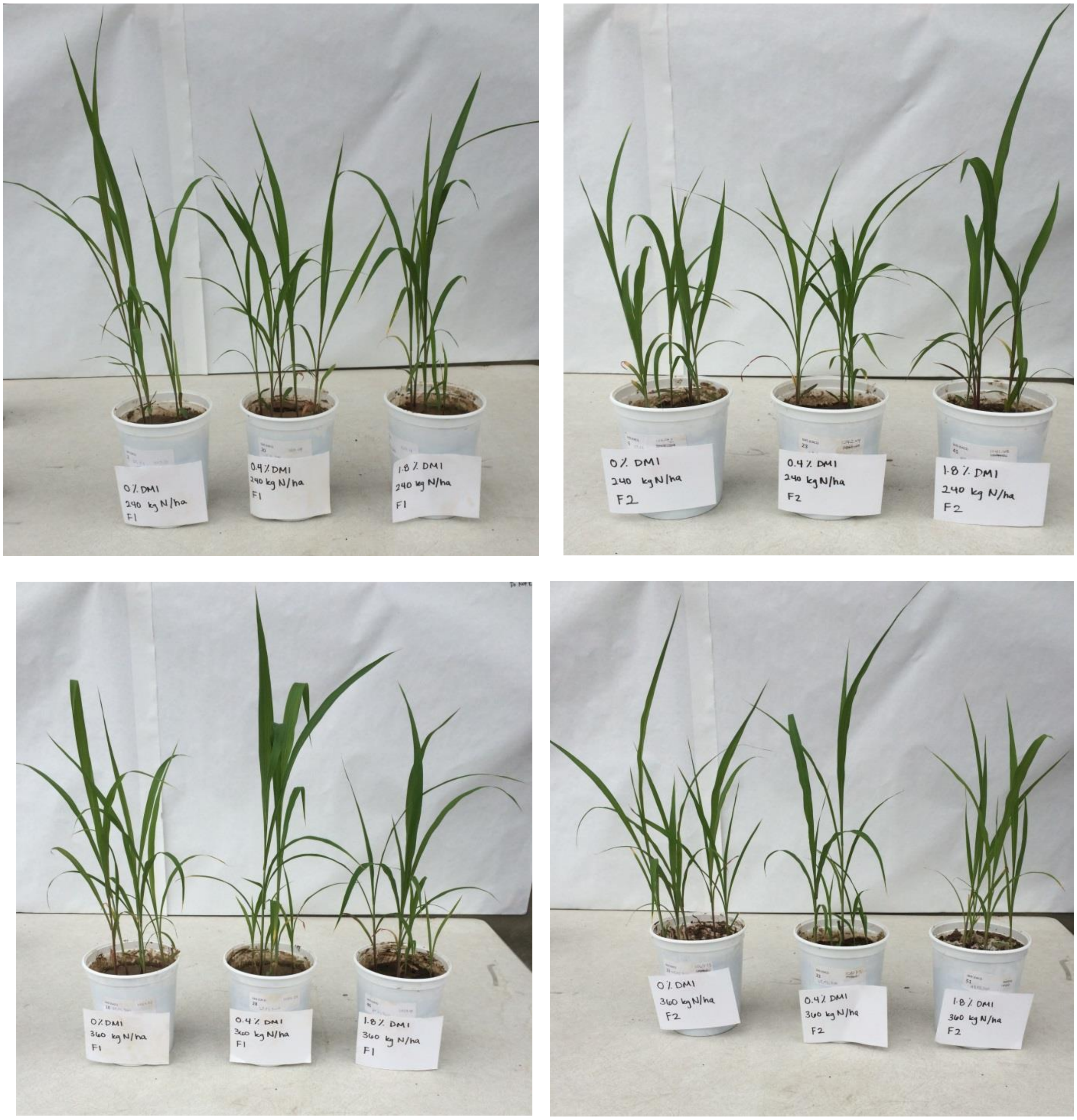


Figure 4. Sorghum plants on 16 March 2015, with increasing tannin concentrations (left to right), of 0, 0.4, and 1.8% tannin of dry matter intake at (a and b) 240 kg N/ha target application rates and (c and d) 360 kg N/ha target N rates. Manure was applied in two frequencies, with one application (a and c) and two applications (b and d). In sorhgum

differences were not observed by tannin concentrations, but visually, differences were observed in winter wheat, with less lodging occurring at the higher N rate.

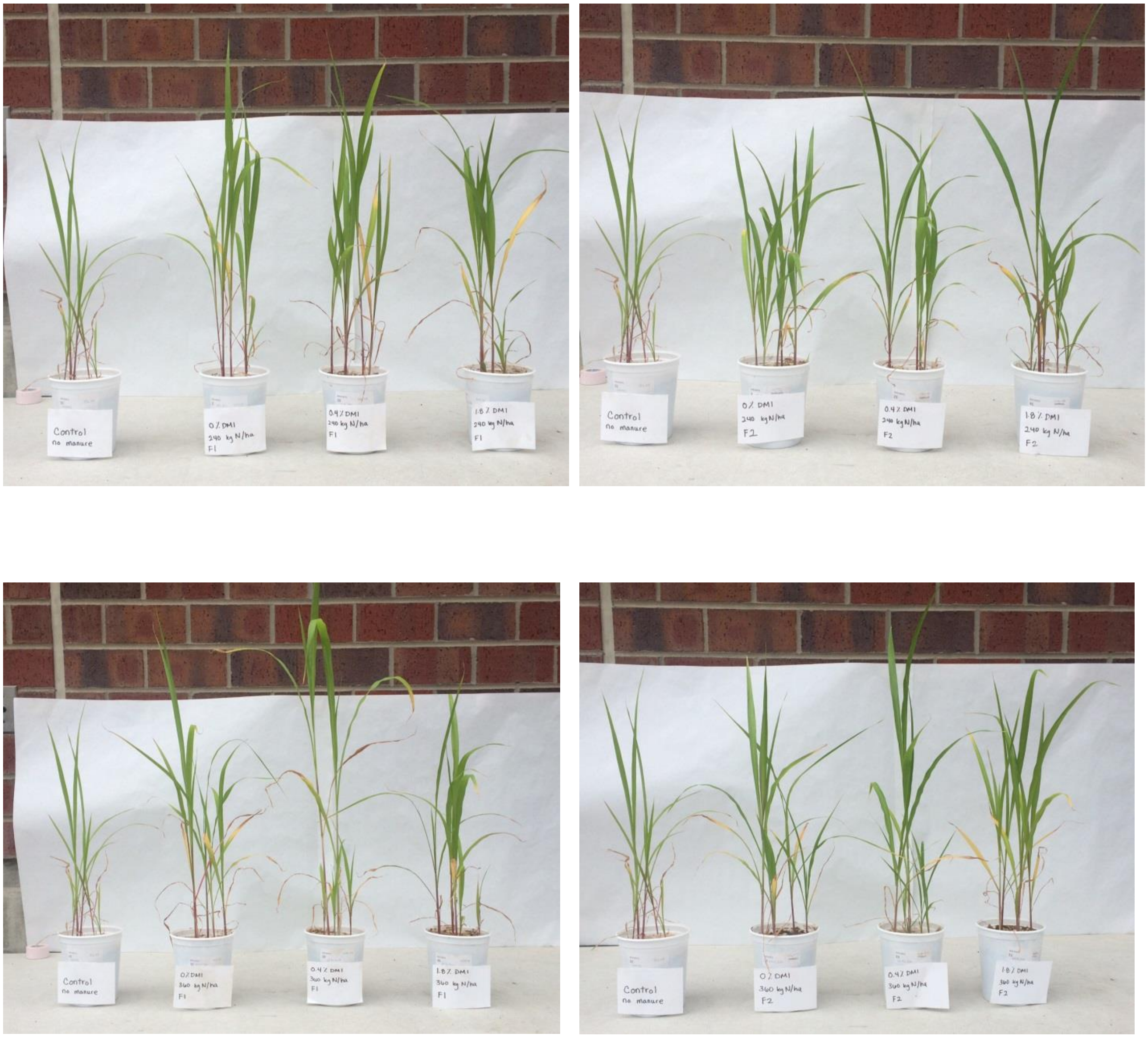
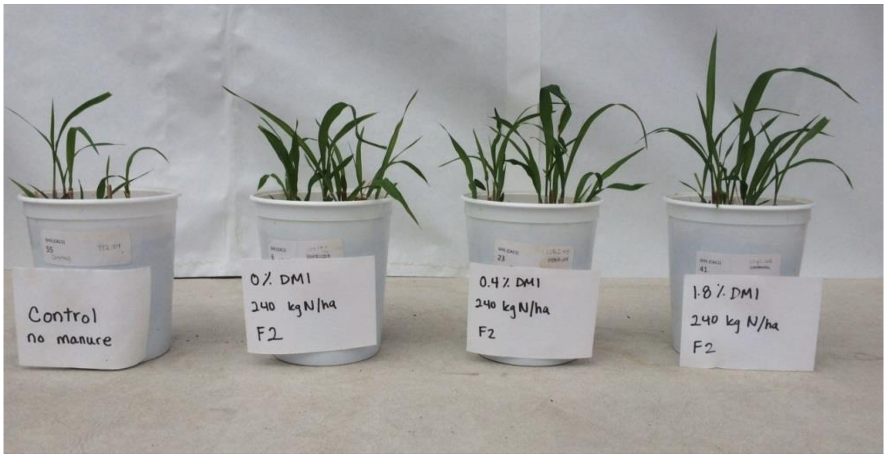


Figure 5. Sorghum plants on 7 April 2015, with control pots and pots with increasing tannin concentrations (left to right), of 0, 0.4, and 1.8% tannin of dry matter intake at (a and b) 240 kg N/ha target application rates and (c and d) 360 kg N/ha target N rates. Manure was applied in two frequencies, with one application (a and c) and two applications (b and d). In sorhgum differences were observed by tannin concentrations, by N rate, and by frequency of manure application.


Control
no manure
0% DMI
240 kg N/ha
F1
0.4% DMI
240 kg N/ha
F1
1.8% DMI
240 kg N/ha
F1


Control
no manure
0% DMI
240 kg N/ha
F2
0.4% DMI
240 kg N/ha
F2
1.8% DMI
240 kg N/ha
F2

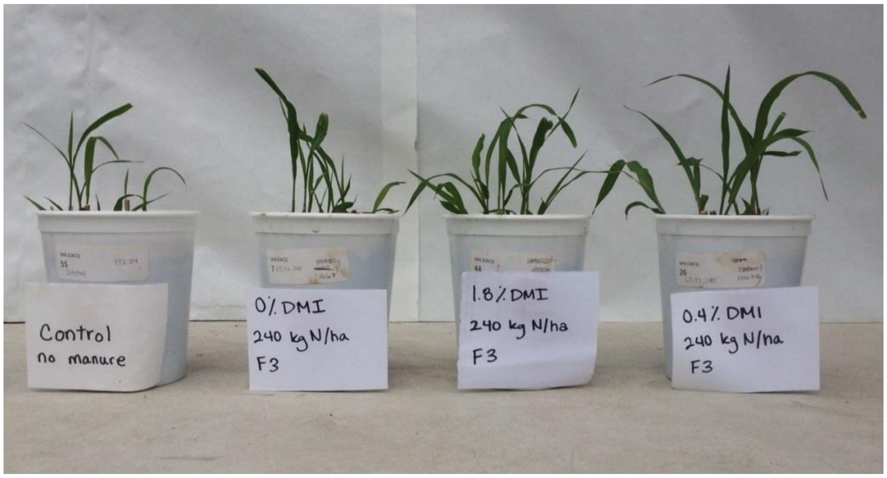

Control
no manure
0% DMI
240 kg N/ha
F3
1.8% DMI
240 kg N/ha
F3
0.4% DMI
240 kg N/ha
F3

Figure 6. Ratoon of sorghum plants on 21 April 2015, with control pots and pots with increasing tannin concentrations (left to right), of 0, 0.4, and 1.8% tannin of dry matter intake at 240 kg N/ha target application rates, with increasing frequencies of manure application with (a) one, (b) two, and (c) three applications. Visual observations suggest that as tannin concentrations increased, above ground biomass also increased in treatments that received two or three manure applications.

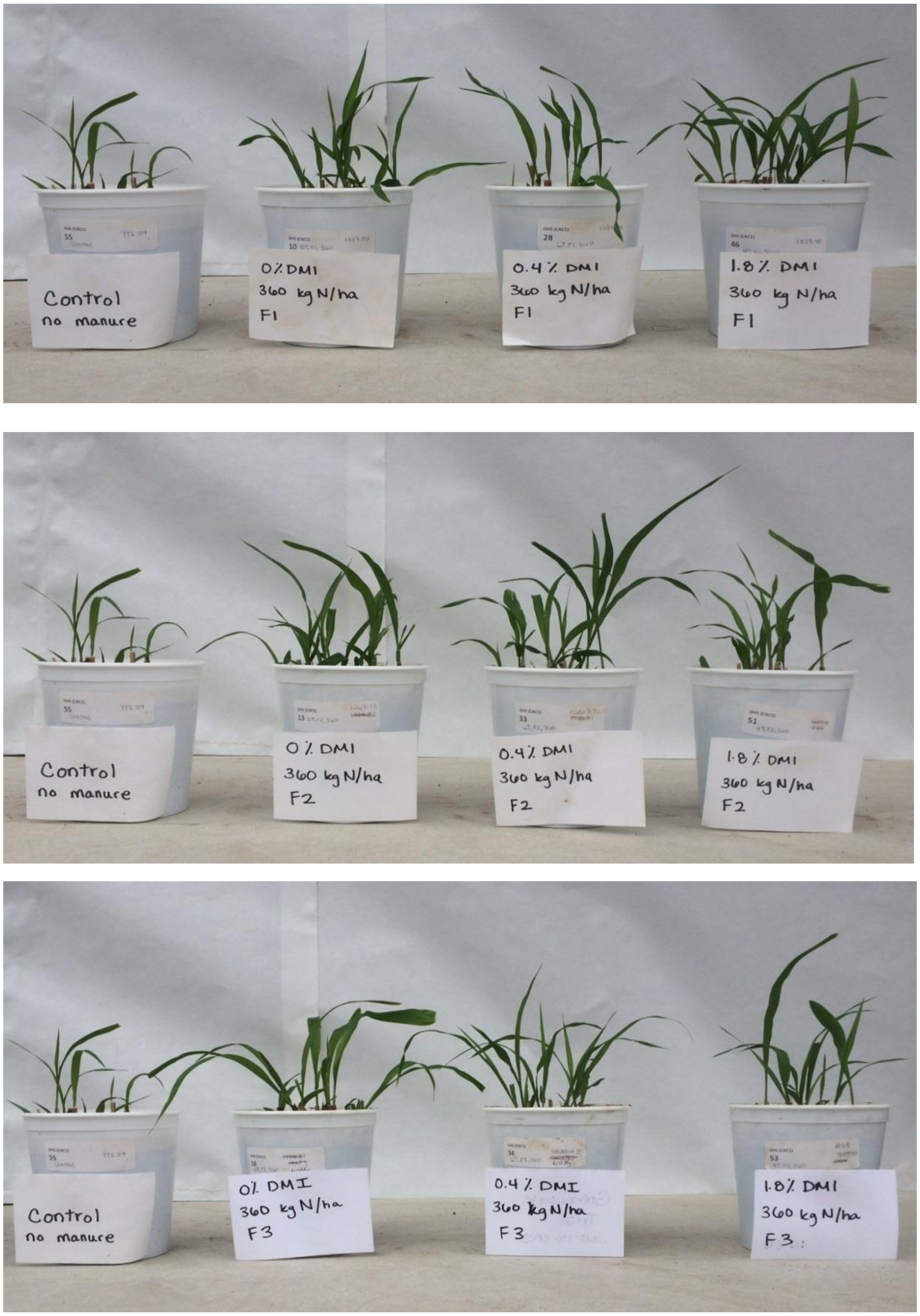


Figure 7. Ratoon of sorghum plants on 21 April 2015, with control pots and pots with increasing tannin concentrations (left to right), of 0, 0.4, and 1.8% tannin of dry matter intake at 360 kg N/ha target application rates, with increasing frequencies of manure application with (a) one application, (b) two, and (c) three applications. Visual observations suggest that as tannin concentrations increased, above ground biomass also increased in treatments that received two or three manure applications.

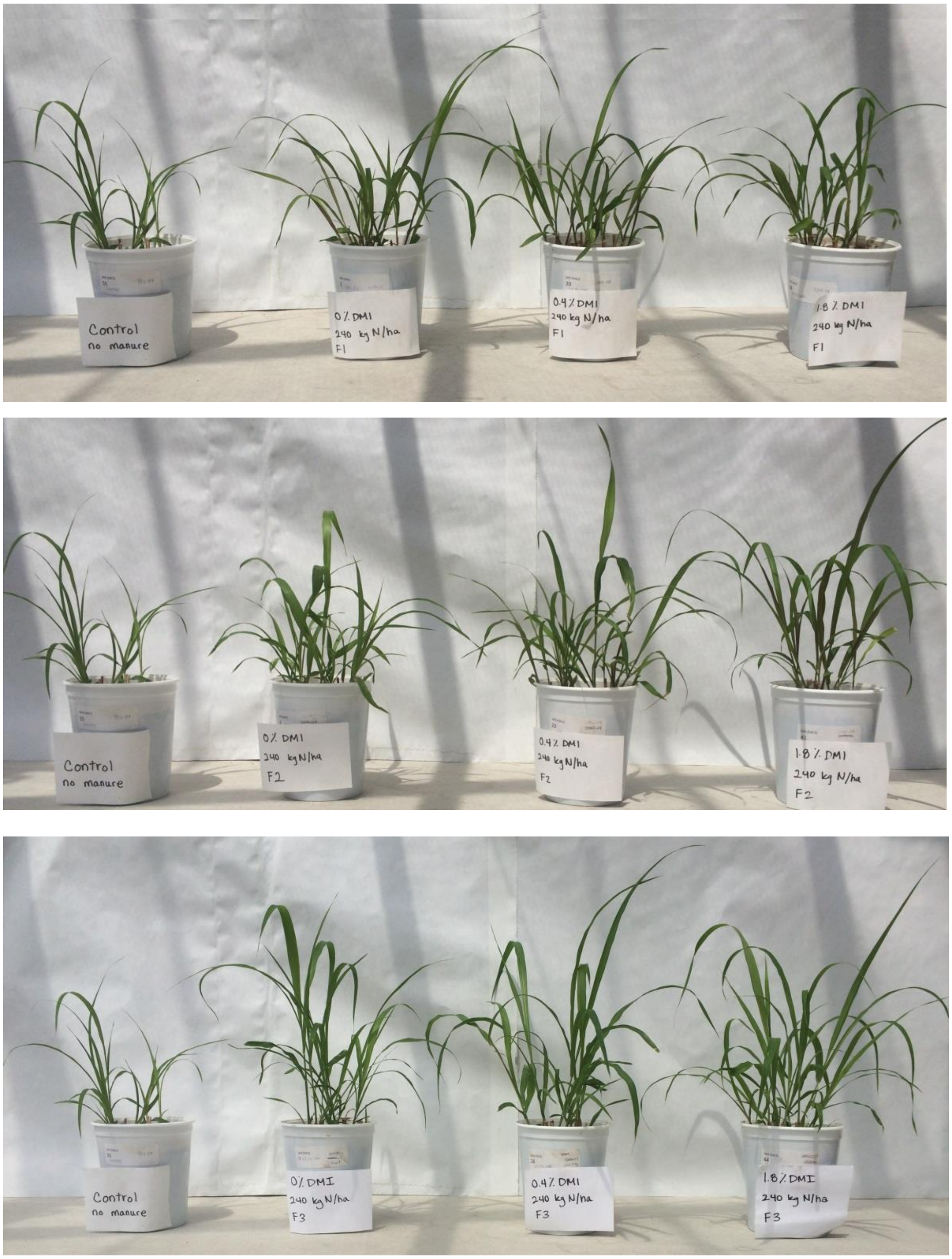


Figure 8. Ratoon of sorghum plants on 7 May 2015, with control pots and pots with increasing tannin concentrations (left to right), of 0, 0.4, and 1.8% tannin of dry matter intake at 240 kg N/ha target application rates, with increasing frequencies of manure application with (a) one application, (b) two, and (c) three applications. Visual observations suggest that as tannin concentrations increased, above ground biomass also increased in treatments that received two or three manure applications.

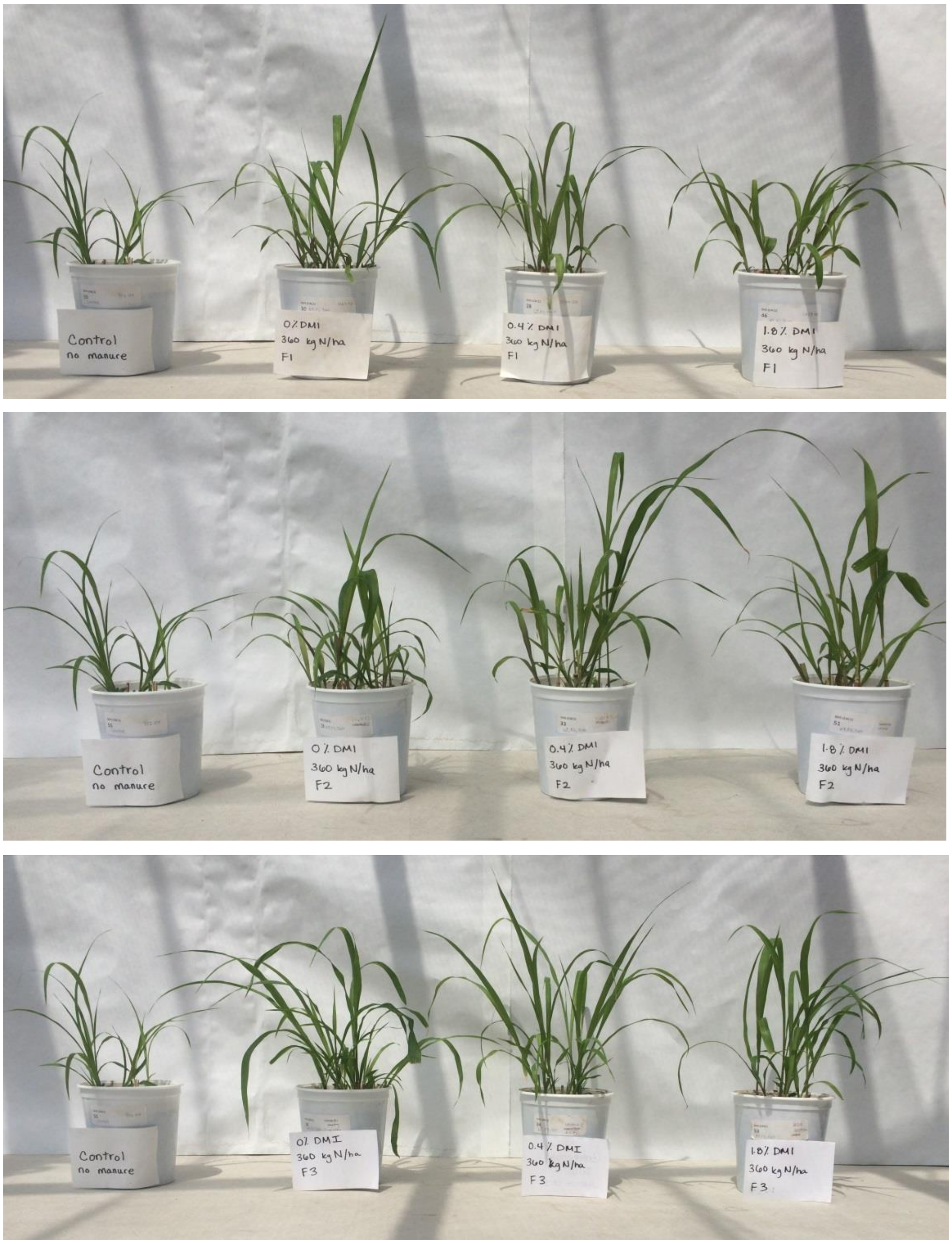


Figure 9. Ratoon of sorghum plants on 7 May 2015, with control pots and pots with increasing tannin concentrations (left to right), of 0, 0.4, and 1.8% tannin of dry matter intake at 360 kg N/ha target application rates, with increasing frequencies of manure application with (a) one application, (b) two, and (c) three applications. Visual observations suggest that as tannin concentrations increased, above ground biomass also increased in treatments that received two or three manure applications.

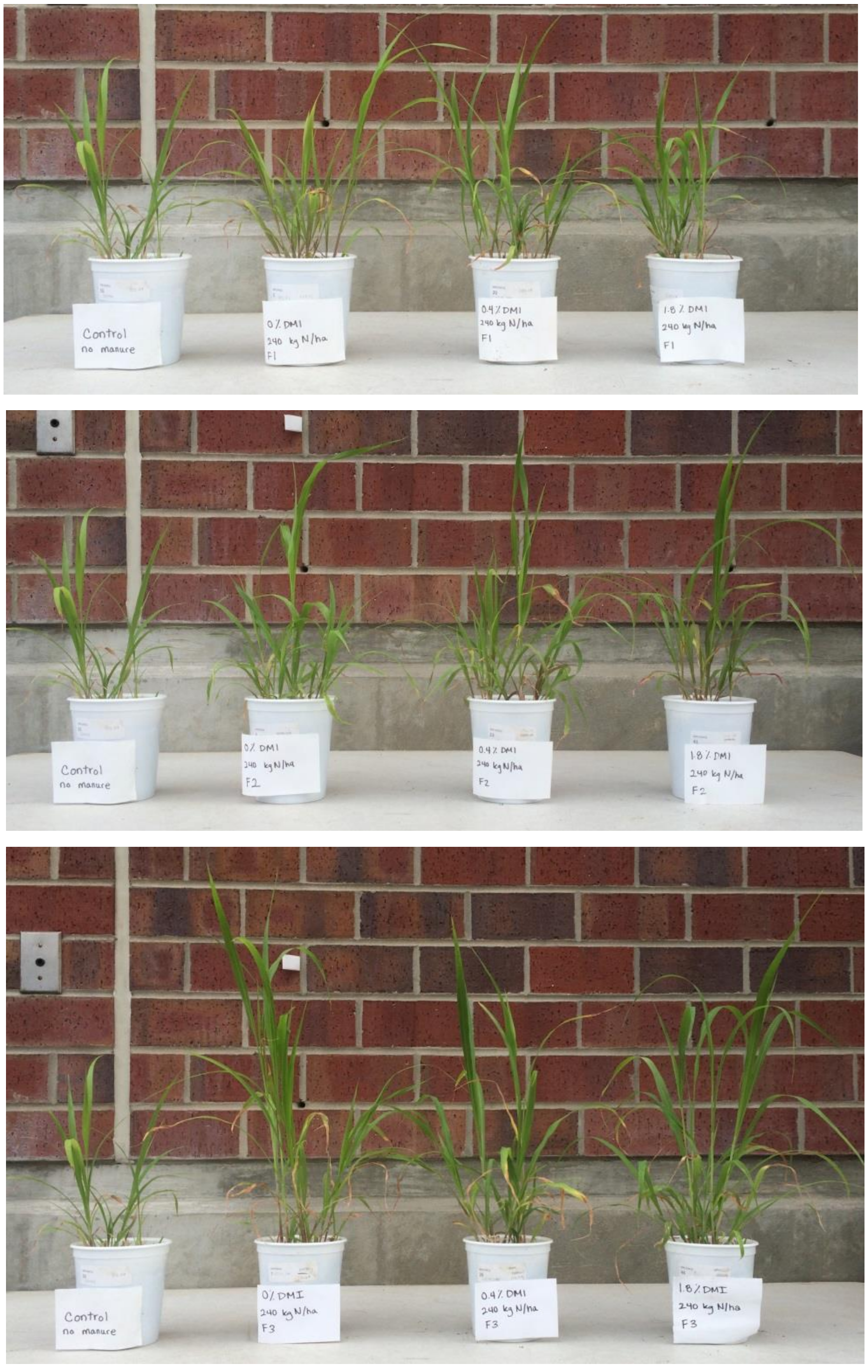


Figure 10. Ratoon of sorghum plants on 29 May 2015, with control pots and pots with increasing tannin concentrations (left to right), of 0, 0.4, and 1.8% tannin of dry matter intake at 240 kg N/ha target application rates, with increasing frequencies of manure application with (a) one application, (b) two, and (c) three applications. Visual observations suggest that as tannin concentrations increased, above ground biomass also increased in treatments that received two or three manure applications.

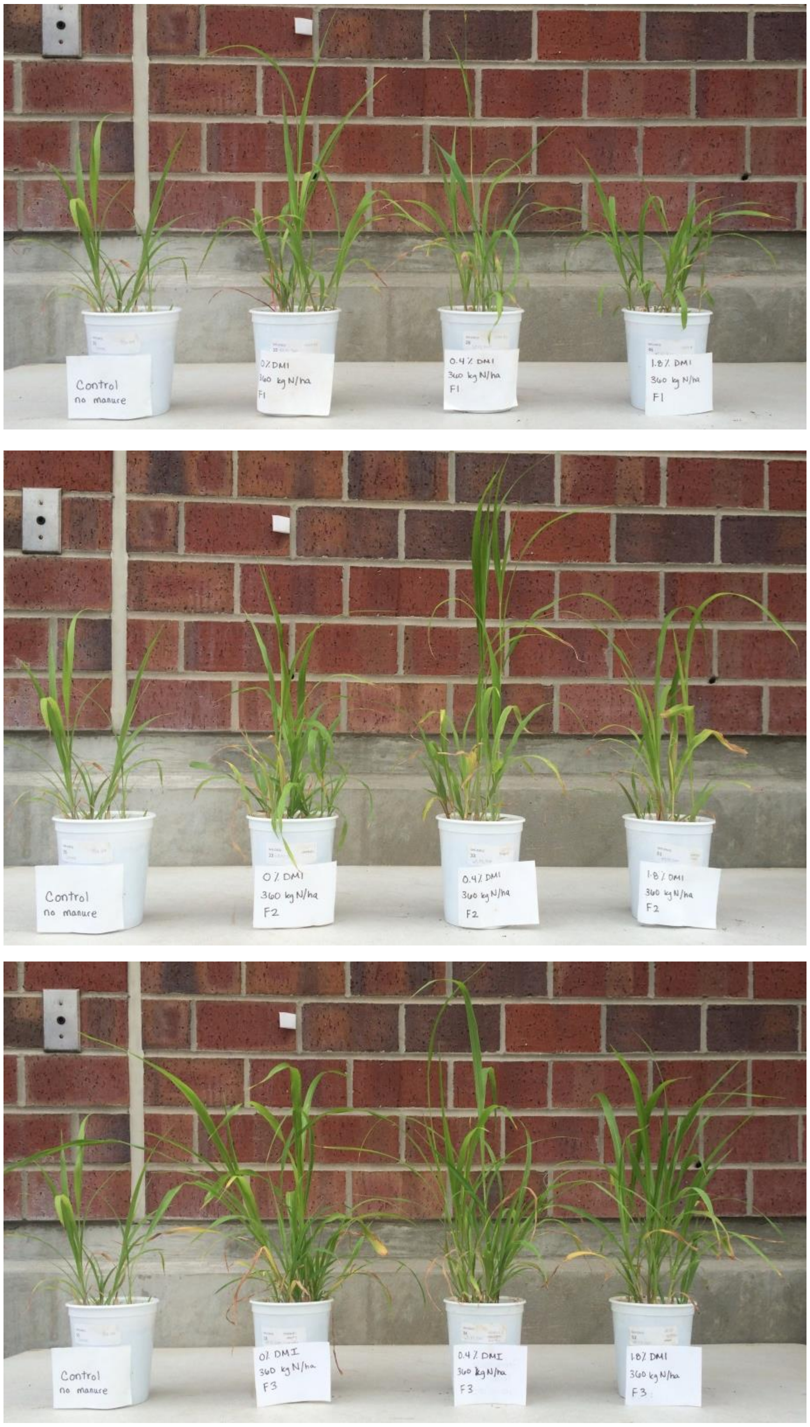


Figure 11. Ratoon of sorghum plants on 29 May 2015, with control pots and pots with increasing tannin concentrations (left to right), of 0, 0.4, and 1.8% tannin of dry matter intake at 240 kg N/ha target application rates, with increasing frequencies of manure application with (a) one application, (b) two, and (c) three applications. Visual

observations suggest that as tannin concentrations increased, above ground biomass also increased in treatments that received two or three manure applications.

Appendix 3: Greenhouse Trial Protocol

Greenhouse trial (JMP-15-GH-05)
November-2014

Objectives

Determine the rate and frequency of tannin manure applications on plant yield, plant N and soil inorganic N

Treatments

St Charles Silt Loam (taken from alleyways of JMP-15 field trial of Claire Campbell)
3 tannin manures (0T, LT and HT)
2 application rates
    Agronomic rate (R1=240 kg N/ha)
    1.5 times agronomic rate (R2=360 kg N/ha)
3 application frequencies
    Single (F1, applied to all pots day=0)
    Second application (F2, applied to all pots day=70)
    Third application (F3, day=130)
3 replications per treatment

Set-up

Each pot contains 800 g of soil
Pots required = 3 manures x 2 rates x 3 freq. x 3 reps = 54 + 3 controls = 57 pots

Pot preparation

Eight hundred grams of air-dried soil placed in non-draining plastic 700 ml pots, phosphorus (P) applied at the rate of 40 kg P ha^{-1} as KH_2PO_4, and distilled water added to achieve soil moisture contents of approximately 60% water-filled pore space (WFPS).
Allow 1 week fallow period before manure application

Manure analyses

The day prior to manure application to pots, the frozen subsamples of slurry are thawed. Take triplicate slurry samples per tannin level, analyze for pH (1:2 manure:water mixture), DM (100 ºC, 24 h), and sub-samples freeze dried, ground to pass a 1 mm screen and analyzed for total N and total C, total ammonium (KCl (5 g slurry in 50 mL 2 *M* KCl, shaken for 2 h and filtered through Whatman no. 42) and analyzed on the Lachat), NDF, ADF, NDIN, ADIN

First manure application (F1)

Remove approx. ¼ of soil volume from each pot, add manure (F1), replace soil, mix, and re-pack to approximate soil bulk density (1.20 g cm3), followed by immediate watering. A 5 d initial fallow period follows F1 manure application

1st Planting and harvest

Nine oat seeds are sown in each pot, and seedlings thinned at 10 d to keep the 5 most robust plants per pot.

Pots watered every 2 to 3 d to maintain 60% WFPS and pot locations on the greenhouse bench relocated randomly each week.

After a 45 d growth period, oat shoots and roots are harvested. Oat roots are removed from pots to minimize their impact on N mineralization and crop growth during the next cropping phases. Soil and organic debris were washed from roots and wash water returned to pots.

Second manure application (F2) and 2nd cropping cycle

After oat harvest, one-third of the pots received no manure (F1 treatment) and F2 manures were applied in the same manner as F1 to the remaining two-thirds of the pots (one-third of which would eventually receive F3 manure application just prior to the third cropping phase). The F2 manure application followed by 20 d fallow period, after which 7 sorghum seeds are planted per pot and grown in the same manner as oats for 60 d. Sorghum shoots are harvested.

Third manure application (F3) and 3rd cropping cycle

After sorghum harvest, F3 manure applied within 4 slots, approximately 2.5 cm deep, to one-third of the pots (one-third of the remaining pots kept as F1 and the other one-third as F2) and sorghum plants allowed to ratoon (re-grow) for an additional 45 d, then sorghum ratoon shoots were harvested.

Plant analyses

Total N in oat and sorghum shoots and roots are determined using the same methods as outlined for the manure. Nitrates in oat and sorghum shoots were extracted with 2% glacial ascetic acid and analyzed using QuickChem Methods 13-107-04-1-A on a Lachat automated N analyzer.

Ash residues were used to determine possible soil contamination of oat and sorghum roots. Approximately 0.5 g root sub-samples were combusted at 600°C for 24 h, and soil contamination is subtracted from root DM to calculate root organic matter (OM) production per pot. After oat harvest and at trial’s end, representative soil samples are taken from each pot and analyzed for pH and total soil inorganic N (IN)

Appendix 4: Incubation Trial Protocol

Incubation trial (JMP-15-INC-07)
November-2014

<u>Objectives</u>
Determine the rate of tannin manure applications on soil inorganic N

Treatments
St Charles Silt Loam (taken from alleyways of JMP-15 field trial of Claire Campbell)
3 tannin manures (0T, LT and HT)
2 application rates
    Agronomic rate (R1=240 kg N/ha)
    1.5 times agronomic rate (R2=360 kg N/ha)
3 replications per treatment

Set-up
Each cup contains 100 g of soil
Cups required = 3 manures x 2 rates x 3 reps = 18 + 3 controls = 21 cups

Pot preparation
One hundred grams of air-dried soil placed in non-draining urine specimen cups (screw tops with pin holes),
Phosphorus (P) applied at the rate of 40 kg P ha^{-1} as KH_2PO_4, and distilled water added to achieve soil moisture contents of approximately 60% water-filled pore space (WFPS).
Allow 1 week fallow period before manure application

Manure analyses
The day prior to manure application to cups, the frozen subsamples of slurry are thawed.
Use same sampling and chemical analyses as with JMP-16-GH-05 (no need for extra samples)

Manure application
Remove approx. ¼ of soil volume from each cup, add manure, replace soil, mix, and re-pack to approximate soil bulk density (1.20 g cm3), followed by immediate watering. A 5 d initial fallow period follows manure application

<u>Sampling sequence</u>
Take single soil cores day 0 then 7, 21, 28, 56, 112, and 168 days after manure application

<u>Soil analyses</u>
Divide single core taken at each sampling period into 2 approx. equal parts, take wet weights
    Oven dry ½ core
KCl-extractable N on ½ core.