WINTER BIOMASS YIELD, YEAR-ROUND

ELEMENTAL CONCENTRATIONS OF 'KANLOW'

SWITCHGRASS, AND ASSOCIATED SOIL

NUTRIENTS IN A ZERO INPUT ENVIRONMENT

By

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CHAPTER I

INTRODUCTION

Switchgrass (*Panicum virgatum* L.) is a warm-season perennial species native to

North America (McLaughlin and Kszos, 2005). It was selected by the U.S. Department of

Energy (DOE) as the model herbaceous species for development as a cellulosic feedstock

crop for biofuels production. Switchgrass is one of the best cellulosic plant materials for

future biofuels production. In recent years, the focus of study on switchgrass has shifted

from its traditional forage value to a bioenergy perspective. Modern plant communities

like switchgrass can be cultivated and managed as renewable resources for biofuels

production in order to reduce our over-dependence on fossil fuels (Parrish and Fike,

2005). Long term cropping sustainability and optimum yield are the key factors in any

crop production endeavors. Maximum biomass yields in switchgrass can be produced

with one harvest by mid-September (Sanderson et al., 1999; McLaughlin and Kszos,

2005). However, harvest after the first frost can help maximize retranslocation of carbon

and energy to root systems (McLaughlin and Kszos, 2005). Delaying the harvest past

October with one cut system can help to maximize the switchgrass biomass production

the next year (Sanderson et al., 1999) as well.

Correct procedure of harvest management can maximize long-term biomass yield

and maintain appropriate level i.e., low level of moisture and ashes in the biomass for

biofuels (Monti et al., 2008). Although some farmers have forage harvest machines and equipment for use, specific harvest machines, storage facility, and transportation infrastructure are yet to be developed (Mapemba et al., 2008). Harvest machines may not be available in sufficient quantities if a large quantity of lignocellulosic biomass field is to be operated at a narrow harvest period (Mapemba et al., 2008). The yield losses in spring harvested biomass were mostly contributed by harvest losses (Adler et al., 2006). Adler et al. (2006) stated that the spring harvested biomass with decreased mineral concentrations could make good combustion quality of the biomass compared to the fall harvested biomass. Delayed harvest can help preserve carbohydrate reserves and maintain stand stability (Casler and Boe, 2003). Delayed harvest increased dry matter and NDF concentration, while the ash concentration was decreased (Casler and Boe, 2003). Biomass with higher NDF content and lower ADF and lignin content would be beneficial for increasing fermentable sugars and decreasing unfermentable and/or uncombustible residues (Casler and Boe, 2003).

A reasonable estimate of available harvest days is required to calculate cost of using harvest machines for lignocellulosic biomass (LCB) refinery (Hwang et al., 2009). Hwang et al. (2009) studied two potential harvest seasons (short harvest season and extended harvest season) that could be utilized for switchgrass mowing and baling. They found that an extended harvest season (October – February in Oklahoma) could reduce the cost of harvest machines and the feedstock delivery compared to the short harvest season (October – December in Oklahoma) (Hwang et al., 2009). The short harvest season includes delaying of harvesting to allow plants to undergo transition from a non-dormant to a dormant state (Hwang et al., 2009). This period is useful for plants to

mobilize nutrients from above-ground plant parts to below-ground plant parts and to let above-ground tissues to initiate senescence (Hwang et al., 2009). By delaying harvest until this period, biomass yield can be maximized and the amount of nutrients removed in the biomass can be reduced (Hwang et al., 2009). On the other hand, the extended harvest season includes harvest window from July through the following February. This extended harvest season is especially beneficial for more economical use of harvest machines and is useful to reduce biomass quantity required for storage (Hwang et al., 2009).

One way to increase economic return from any crop is to reduce its input requirements such as fertilizer application. Depletion of soil nutrients with biomass harvest can be reduced by selection of genotypes and change of harvest time (Yang et al., 2009). Yang et al. (2009) found Kanlow switchgrass as efficient in terms of nutrient use (less loss of nutrient per unit biomass) for N and P contents in senescent shoots. N is a major fertilizer input and holds a major share in cost of production. Therefore the production practices to reduce N requirement may be profitable; specific economic analysis is needed to confirm the profitability of the particular production practice (Vogel et al., 2002). Haque et al. (2009) found maximum expected net return from switchgrass biomass yield at N fertilization at 65 kg N ha^{-1} yr^{-1} and one time post-senescence harvest per year for most biomass price and N price combinations for Oklahoma. Yield and nitrogen outputs from the biomass in winter in a zero input scenario can be useful for future economic analysis studies.

Remobilization of N from the aboveground biomass to stem bases, crowns, or roots implies a reduction in input of costly N fertilizer although the amount of stored N reused in the next year was not known (Vogel et al., 2002). The keeping of organic

matter abundantly in the soil and liberal application of nitrogenous fertilizers are considered important to maintain soil N content (Donahue, 1961). Organic matter from leftover trash biomass in the field after harvest can help maintain N in the soil. The switchgrass harvested plots are found to be covered with trash leftover biomass (about 1-2 inch thick) which can act as mulch and upon degradation provide organic matter to the soil. It can work as a sort of natural manure fertilization.

Harvest delay until winter (November through the following March) is useful in terms of economic use of harvest machines and in reducing storage quantity of biomass. Winter harvest can provide a wider harvest window which is needed to harvest large areas, to provide producer an opportunity to gain from potential off-season market price, and to enable a large scale industrial biorefinery plants to have assured year-round supply of feedstocks. Information is limited on winter biomass yield, elemental composition of standing cured biomass harvested, and associated soil nutrient status. Therefore, the objectives of this study were to evaluate changes in winter biomass yield, year-round elemental composition of Kanlow switchgrass, and associated year-round soil nutrient status in a zero input environment.

CHAPTER II

REVIEW OF LITERATURE

At present we are mainly using non-renewable fossil based gasoline and diesel fuels to run our vehicles and farm equipments. Biomass is a renewable resource which can be used to produce biofuels (U.S. DOE, 2009). The U.S. Department of Energy (DOE) Biomass Program has focus towards a viable and sustainable domestic biomass industry to produce renewable biofuels, bioproducts and biopower in order to enhance energy security, reduce dependence on fossil oil, provide environmental benefits including reduction in greenhouse gas emissions, and create economic opportunities. Biomass Program of the Office of Energy Efficiency and Renewable Energy in the U.S. DOE is carrying out research, development, and demonstration efforts to develop integrated biorefineries and make cellulosic ethanol cost competitive by this year (2010).

At present, bioethanol is mostly made from starch and sugar crops. In United States, corn is the major feedstock, and in Brazil, sugarcane is the major feedstock. Now the advanced technology research and development have gained momentum to use cellulosic biomass such as trees and grasses for bioethanol production. Plants are the feedstocks for bioethanol. Through photosynthesis, plants convert water and carbon dioxide to sugars utilizing energy from sun light. These sugars are stored in various plant parts.

These plant sugars can be converted into energy forms such as fuels and electricity. Plants like sugar cane and sugar beets, store the energy in the form of simple sugars while the plants such as corn store the energy in the form of starch, a complex sugar form. Both sugar and starch crops are used for human food. Cellulosic biomass from plants is very complex sugar polymer, and this is not used as human food. This kind of biomass is the ideal feedstock for bioethanol production.

The U.S. ethanol industry

At present, the U.S. ethanol industry is a maturing corn ethanol industry with technology development capable to accommodate cellulosic feedstocks in the near future. Corn ethanol industry is expected to stand at a capacity leveling out at 15 bgpy by year 2015, and the advanced biofuels, including cellulosic ethanol, are expected to begin their outcomes in the next several years (U.S. DOE, 2008). 2007 Renewable Fuel Standard has set a target production of 36 billion gallon ethanol per year by year 2022 (U.S. DOE, 2008). Thus we have a tremendous potential for development of cellulosic biofuel industry and cellulosic feedstocks such as switchgrass in near future.

The United States has envisioned a 30 percent replacement of the US petroleum consumption with biofuels by 2030. In order to achieve this goal, approximately 1 billion dry tons of biomass feedstock per year is required. This requirement can be fulfilled by forest and agricultural resources. We can produce 368 million dry tons of biomass from forest resources and 998 million dry tons of biomass from agricultural resources. Together it makes about 1.3 billion dry tons per year. Out of 998 million dry tons biomass per year from agricultural resources, 377 million dry tons is from perennial

crops, 446 million dry tons is from crop residues, 87 million dry tons is from process

residues, and 87 million dry tons is from grains (for ethanol) (Perlack et al., 2005).

Botanical aspects of switchgrass

Switchgrass (*Panicum virgatum* L.) belongs to Kingdom Plantae and Division

Magnoliophyta which includes flowering plants (NRCS, USDA, 2009). Within this

Division it comes under Liliopsida class which includes Monocotyledonous plants.

Within the Liliopsida class, it comes under Family Poaceae, which is also known as grass

family. The genus is *Panicum* and the species is *virgatum*.

Switchgrass belongs to tallgrass prairies, and grows particularly in mesic to wet

prairies, on dry slopes, open oak or pine woodlands, shores, river banks, and brackish

marshes (Barkworth et al., 2007). Switchgrass plants are green or glaucous with large

bunches and numerous scaly creeping rhizomes; erect, tough and hard culms; 1 to 2 m.,

rarely to 3 m., tall; glabrous sheaths; 10 to 60 cm. long blades, 3 to 15 mm. wide, flat,

glabrous, or sometimes pilose above near the base, rarely pilose all over; 15 to 50 cm.

long, open and sometimes diffuse panicle; 3.5 to 5 mm. long, acuminate spikelets. Its first

glume is clasping, two-thirds to three-fourths as long as the spikelet, acuminate or

cuspidate, fruit is narrowly ovate, the margins of the lemma inrolled only at base

(Hitchcock and Chase, 1951). Seeds are very small which remains dormant after harvest.

Aging, treatment with water, chilling temperatures or storing it in warm condition can

help break dormancy (Bransby, 2009). Because of small seed size, the seedlings are slow

to develop and susceptible to weed competition in the beginning. Only from the third

year, the plants reach full yield potential; the yield in the second year is about two thirds of the full yield (Bransby, 2009).

It is a C4, warm-season, perennial species from North America with considerable morphological diversity and wide range of adaptation (Parrish and Fike, 2005). The adaptation of switchgrass ranges primarily on the eastern side of Rocky Mountains, from southern Canada through the United States to Mexico, Cuba, Bermuda, and Costa Rica, and, possibly an introduction in Argentina (Barkworth et al., 2007). Switchgrass has two ecotypes: the upland ecotype occurs in tallgrass prairie, and the lowland ecotype in riverine grasslands (Brunken and Estes, 1975).

Field populations of switchgrass were different in clonal habit, clone, and in the morphology of the vegetative parts. Variations were observed within both upland and lowland cultivars; however, lowland plants were in general larger in size (Porter, 1966). The two ecotypes also exhibited difference in physiological water requirements and nitrogen requirements. The lowland cultivars performed well under flooded conditions, whereas the upland cultivars preferred more moderate soil water conditions. The lowland cultivars have comparatively lower nitrogen requirement than the upland cultivars (Porter, 1966). The genetic makeup differences indicated that the lowland cultivars were tetraploids and the upland cultivars were hexaploids and octoploids, with most common being the hexaploids (Porter, 1966). However, ploidy levels ranging from diploid (2n = 2x = 18) to duodecaploid (2n = 12x = 108) have been found in switchgrass accessions across a broad geographic range of the USA (Nielson, 1944; McMillan and Weiler, 1959; Henry and Taylor, 1989; Hopkins et al. 1996; Das et al. 2004, Hultquist et al. 1996).

Breeding

Switchgrass propagates sexually by seed and outcrossing results from cross-pollination enforced by strong self-incompatibility (Taliaferro, 2002). The new cultivars are expected to be either: broad genetic base plant populations, synthetics consisting of 2 to 12 selected plants, or F1 hybrids (Taliaferro, 2002). It is technically feasible to produce F1 hybrid switchgrass cultivar although no commercial F1 hybrid has been produced yet (Taliaferro, 2002). Martinez-Reyna and Vogel (2002) have developed the procedure to hybridize switchgrass plants which was successfully used to obtain hybrid plant between 'Summer', an upland tetraploid and 'Kanlow', a lowland tetraploid ecotype. Alexandrova et al. (1996a and 1996b) developed micropropagation procedure of switchgrass by node culture and in vitro development of inflorescences from switchgrass nodal segments as the maintenance of genotype is difficult through sexual reproduction. This tissue culture technique propagation can be used for maintenance of hybrid genotype. Ploidy levels ranging from diploid ($2n = 2x = 18$) to duodecaploid ($2n = 12x = 108$) have been reported by past studies (Nielson, 1944; McMillan and Weiler, 1959; Henry and Taylor, 1989; Hopkins et al. 1996; Das et al. 2004, Hultquist et al. 1996).The crosses of plants with different ploidy levels resulted in very low frequencies of hybrid progeny indicating that gene flow occurs at low levels among cytotypes (Taliaferro, 2002).

Switchgrass became the best candidate for cellulosic feedstock for ethanol production as it offers good pest and disease resistance, high yields of cellulose, low fertility needs, local adaptation and relatively availability, excellent wildlife habitat, carbon sequestration because of its extensive and very deep root system, tolerance of poor soils and wide variations of soil pH, drought and flood tolerance (depending on the

ecotype and variety), and water use efficiency in grassland ecosystem (Rinehart, 2006). Long term maintenance of these qualities will be important to avoid yield loss and quality deterioration of the biofuel feedstock crop.

In recent years, many of the breeding programs are aimed to develop new switchgrass cultivars with increased biomass yield. Identification of cultivars with high yield potential and acceptable biofuels quality will determine success in development of a bioenergy industry (Lemus et. al, 2002). Bouton (2007) indicates that production of high yielding hybrids and the use of genomic and transgenic biotechnologies are the future research areas to enhance both yield and chemical composition and projects an example of future research as reducing bioconversion recalcitrance via reduction of lignin content.

Although cultivar, year, and location determine variation in biomass yield of switchgrass, breeding and biotechnology research can potentially improve yield, as well as other traits that should add value to its use as a biofuel feedstock (Bouton, 2007). Development of switchgrass cultivars with increased yield, improved quality, and wider geographic and ecological adaptation can be important contributions in the development of future biofuels industry.

Water issues

Agriculture already uses significant amount of fresh water and the expansion of biofuels crop production would require additional water. There is possibility of converting natural habitat, forest, grass- and peat-lands into biofuels crop production with negative consequences on biodiversity, greenhouse gas balances, and water availability (Bringezu et. al., 2009). With growing of biofuels crops, there is potential for change in

irrigation water use, and consequently the availability of local water with the replacement of existing crops with biofuels crops. The excess loading of nitrogen in the Northern Gulf of Mexico (NGOM) has resulted in hypoxia affecting aquatic life. The Chesapeake Bay and other coastal water bodies were also reported to have the same problem (Schnoor et al., 2008). The study by Costello et al. (2009) estimated the impact of the increased agricultural activities from the estimated production target of 36 billion gallons (Bgal) of biofuels and population growth. They found a reduction in hypoxia by 20 percent through adoption of cellulosics in place of corn and recommended extensive nutrient management to further reduce the hypoxia in the NGOM.

Efficient irrigation technique can be one the promising ways to mitigate effects of the increased biofuels production on water resources. Irrigation techniques should reduce the amount of water applied per unit of biomass produced. Subsurface drip irrigation systems can minimize the amount of water lost from evaporation and runoff as the polyethylene tubing is buried directly beneath the crop, apply water directly to the crop root zone, and keep the soil surface dry (Payero et al., 2005; Schnoor et al., 2008). Irrigation scheduling can be improved by the help of real-time soil moisture monitoring with microwave remote sensing and weather monitoring technologies. Harvesting of rainfall, efficient transport of irrigation water, and use of reclaimed water are some of the measures for efficient water utilization for biofuels. Efficiency measures taken can lead to less water being withdrawn from an aquifer, thus leaving more water in long-term groundwater storage for future use by crops (Schnoor et al., 2008). The practice of rain-fed agriculture, wherever possible, would be beneficial to conserve water in aquifers. Control of soil erosion can help to maintain water quality of streams and rivers and

prevent nutrient pollution. Conservation buffers are important in reducing sediment in runoff and limiting soil erosion.

Use of precision agriculture (PA) tools can be useful for efficient application of irrigation and other inputs. PA is beneficial to reduce wastage, increase profits, and maintain environmental quality. Efficient fertilizer application can be achieved using spectral radiometers (Scharf et al., 2001). Perennial feedstocks such as switchgrass or other native grasses can be more beneficial to apply PA technologies as these crops stay in the field for longer period (Schnoor et al., 2008). For example, switchgrass can stay in the field for fifteen to twenty years. The use of biotechnology to increase ethanol production efficiency would be helpful. We can optimize biomass feedstock to have a better water-use efficiency, higher nitrogen-use efficiency, increased drought and water-logging tolerance, and improved root distribution characteristics through biotechnology for biofuels feedstocks. The knowledge of molecular genetics can be utilized for weather-sensitive crop models to help design matching crop varieties with the climatic conditions and to determine optimal management of crop to a particular climate. Biotechnology tools can also be utilized to improve lignocellulosic, microbial, bioconversion, and thermochemical conversions (Schnoor et al., 2008).

At present, U.S. biofuel facilities consist primarily of corn ethanol production and minor biodiesel production from soybeans, and the pilot / demonstration-scale production of cellulosic ethanol. In regard to biofuels production, the factors such as energy return on energy invested including consideration of production of pesticides and fertilizers, running farm machinery and irrigating, harvesting and transporting the crop, the overall carbon footprint of biofuels, and the food vs. fuel concern with the possibility of farmers

worldwide shifting to biofuels production should be considered seriously (Schnoor et al., 2008). The policies should focus on decreasing total nutrient and sediment loadings in waters. The practice of feedstock production requiring low nutrient inputs is better from water quality perspective (Schnoor et al., 2008). Cellulosic feedstocks possess less expected impact on water quality. Based on per unit energy gain, cellulosic biofuels will have less impact on water quality. Therefore, it will be beneficial to shift from corn based ethanol production to cellulosic and other advanced biofuels. However the transition will be easier when the cost-effective cellulosic technologies are developed, policies directed to favor cellulosic feedstock and commercial viability is assured to the producers.

Cultivars

Released lowland switchgrass cultivars in the U.S. include Kanlow, Alamo, Performer , BoMaster, and Cimarron. Similarly, released upland cultivars in the U.S. include Grenville, Blackwell, Bebraska 28, Caddo, Summer, Pathfinder, Cave-In-Rock, Sunburst, Trailblazer, Shelter, Forestburg, Dacotah, Shawnee, and Carthage (Caddel et al. 2009).

The established switchgrass fields produced annual average yield of 5.2-11.1 $Mg.ha^{-1}$ which resulted in net energy yield (NEY) of 60 $GJ.ha^{-1}.y^{-1}$ (Schmer et al., 2008). The ratio of output (renewable energy ethanol) produced and input (non-renewable petroleum energy) consumed in the production cellulosic switchgrass biomass is > 5 (Schmer et al., 2008). The yield, persistence and profitability of the switchgrass depend on cultivar selection (Alexopoulou et al. 2008). Alexopoulou et al. (2008) found the

lowland varieties (Cathage, Kanlow, SL 93-2 and SL 93-3) more productive compared to the upland varieties.

Kansas Agricultural Experiment Station and the Crops Research Division, ARS/USDA developed and released Kanlow switchgrass. Parent seeds were collected from a low land site near Wetunka, OK in 1957. The population obtained was isolated and increased on the Agronomy Farm at Kansas State University. In the beginning, it was identified as Kansas Strain 2218. Kanlow has special adaptation to wet conditions and hence can supplement upland switchgrass. It is a good soil conserving plant with excellent pasture and hay quality as well (Oklahoma Crop Improvement Association, 2010).

Harvest management

Little bluestems (*Andropogon scoparius* Michx.), big bluestems (A. gerardi Vitman), indian grass [*Sorghastrum nutans* (L.) Nash], and switchgrass (*Panicum virgatum* L.) are the most important native grass species in the eastern prairie region of Oklahoma (Dwyer et al., 1963). A study conducted near Stillwater, OK on grass species of El Reno side-oats, Tucson side-oats, big bluestems, indiangrass, switchgrass, little bluestems, and King Ranch bluestem by Dwyer et al. (1963) showed that frequent clipping reduced stand density and plant vigor, and increased broadleaved weeds and annual bromes. Their study on native grass species indicated that switchgrass was not well adapted to regular mowing or clipping. Removal of apical meristems caused more decline in total non-structural carbohydrate (TNC) in aboveground biomass in clipped plants than uncut switchgrass plants (Anderson et al., 1989). At the end of growing

season, Anderson et al. (1989) observed 22 and 40% more TNC in above- and belowground tissue of uncut switchgrass compared to the clipped ones.

Sanderson et al. (1999) showed total seasonal yield reduction from 12% to 19% depending on location when the final autumn harvest was taken in November compared with September. Similarly, they showed more than 50% total seasonal yield reduction upon increasing harvest frequency from one to four cuts per year in Texas. Similar yield reduction response was obtained by Balasko et al. (1984) in 'Blackwell' switchgrass produced under marginal fertility and without addition of fertilizer or lime in Virginia.

Delay in the final autumn harvest of switchgrass was associated with decrease in yield as reported by Parrish et al. (1997) as well. In their view, the decrease in biomass yield is partially contributed by the remobilization and translocation of C and N reserve compound from above-ground biomass to underground root portion (Parrish et al., 1997; Sanderson et al., 1999). The leaf loss might be the reason for remaining yield reduction (Parrish et al., 1997; Sanderson et al., 1999). Although mid-September harvest as recommended by Sanderson et al. (1999) can maximize yield (15-20 Mg ha^{-1}), there may be various reasons for need of a wider harvest window and hence a delayed harvest. Therefore, it becomes imperative to study yield performance in delayed and/or offseason harvests such as winter month harvests.

For a single harvest of bioenergy feedstock switchgrass, late summer or early autumn was the optimum harvest date (Casler and Boe, 2003). Earlier harvest date can increase biomass yield for the short term but it will reduce the stands in the long term (Casler and Boe, 2003). Therefore, the delayed harvest and preservation of carbohydrate

reserves can help to reduce plant mortality (Casler and Boe, 2003). Delay in harvesting until spring showed decrease in yield and mineral concentrations in upland switchgrass cultivars and the yield losses in spring harvested biomass were mostly contributed by harvest losses (Adler et al., 2006). The harvest machines and equipments used as of recent days were mostly the ones used for traditional hay harvest. In future, harvest machines specifically designed for biofuels switchgrass might be developed which might help to reduce harvest losses. The decrease in minerals in the switchgrass above-ground biomass will be beneficial in two respects – suitable for biomass processing for biofuels and soil fertility maintaining. The study by Adler et al. (2006) suggested the spring harvested biomass were better for combustion quality because of decreased mineral concentrations especially ash content. Increased amount of dry matter and NDF, and decrease in ash content upon delay in harvest were considered good for increasing fermentation of sugar by Calser and Boe (2003). The development of switchgrass germplasm with higher NDF content and lower ADF and lignin content would be beneficial for increasing fermentable sugars and decreasing unfermentable and/or uncombustible residues (Casler and Boe, 2003). Lower crude protein (CP) and higher neutral detergent fiber (NDF) were observed in switchgrass regrowth compared to initial growth by Anderson et al. (1989).

Sanderson et al. (1999) observed significant effect of year and location on tiller density of 'Alamo' switchgrass in Texas. Study of Casler and Boe (2003) on upland switchgrass found biomass yield varied by harvest date, location, year, and cultivar. On the other hand, Garten et al. (2010) found no significant difference ($P>0.05$) among cultivars and no significant cultivar x time interaction for dry biomass, C stocks, and N

stocks in aboveground biomass and surface litter. The study by Gartern et al. (2010) revealed belowground biomass (90 cm soil depth) increased from April to October with net production of total belowground biomass occurring in the last half of the growing season.

Harvesting after a killing frost produced lower yield of switchgrass; however, a significant amounts of N were found remobilized from the aboveground biomass to stem bases, crowns, or roots. It implied reduction in input of costly N fertilizer but the amount of stored N reused in the next year was not known (Vogel et al., 2002). According to Vogel et al. (2002), the benefit of harvest after a killing frost should be evaluated in terms of whether the value of yield loss is compensated or is less than the value of reduced input cost. They also state that the harvest timing conflicts with grain and oilseed crop harvest, which should also be taken into consideration. Based on a study by Sanderson et al. (1999) in the south central U.S., the highest yields (15-20 Mg ha-1) in lowland cultivar Alamo was obtained with a single harvest in the autumn (about mid-September).

Any large-scale industrial biofuel production requires a long-term storage of feedstocks for year-round supply availability (Agblevor et al., 1995). Long-term storage may result in losses due to mechanical loss and biochemical reactions (Agblevor et al., 1995). One alternative to a long-term storage of feedstocks is delayed harvest i.e., winter harvest. However the rainfall, wind and other weather factors may affect the winter yield.

Switchgrass is compatible with current agricultural equipments and hence can be harvested with hay harvester and baler at plant height above 4 – 6 inches (Blade Energy

Crops, 2009). The stubble of about that height is considered to capture snow and provide insulation to reduce crown injury in cold climates.

Biomass elements

The study by Yang et al. (2009) on 31 accessions of *Panicum virgatum* found significant differences in the concentration of 20 elements (N, P, K, Na, Mg, Ca, Mn, Fe, Co, Ni, Cu, Zn, Mo, B, Li, As, Se, Rb, Sr, and Cd) in the aboveground biomass between harvest at maturity and harvest after senescence. They observed that the concentration of nitrogen (N), phosphorus (P), potassium (K), and rubidium (Rb) decreased in the shoots of all accessions during senescence.

Yang et al. (2009) found remobilization efficiency (RE) ranging from 20% to 61% for N, 31% to 65% for P, 25% to 84% for K, and 33% to 84% for Rb using formula [(M−S)/M] where M and S denote elemental concentrations at maturity (M) and senescence (S) stages respectively. On the other hand, their study indicated negative RE for Ca, Mg, and Na. They also found compositional differences of elements in upland and lowland ecotypes. A greater average RE was obtained for N and P contents in lowland accessions compared to upland accessions. The reason for greater RE in lowland accessions was contributed by significantly lower N and P contents in post-senescence stage for lowland accessions than upland accessions, and the N content in the maturity stage were not significantly different. The average K content was higher in lowland accessions at both maturity and post-senescence stages, and the average RE of the two ecotypes was similar. The lowland tillers were significantly lower in average Ca and Mg contents than upland tillers harvested at maturity, and no significant differences were

observed in the case of post-senescence harvested tillers. At both maturity and post-senescence stages, the lowland tillers were significantly higher in Na content compared to upland tillers (Yang et al., 2009).

The cultivars with nutrient use efficiency during growth phase and remobilization efficiency during the senescent phase will possibly be the better choices for breeding programs for having low level of macronutrients in harvested above-ground biomass (Yang et al., 2009). Identification of accessions with efficient N use in their growth phase and remobilization in the root-soil system after harvest will be beneficial (Yang et al., 2009). Lowland cultivars are more efficient in N remobilization than upland cultivars during senescence (Yang et al., 2009). Similarly they observed significantly lower P content in senescent tillers of lowland cultivars indicating more efficient export of the breakdown products of proteins, nucleic acids, phospholipids, and other organic macromolecules during senescence.

Soil nutrients

Nitrogen (N), phosphorus (P) and potash (K) are the primary nutrients required by plants, calcium (Ca), magnesium (Mg) and sulfur (S) are considered secondary nutrients, and zinc (Zn), molybdenum (Mo), iron (Fe), copper (Cu), boron (B), manganese (Mn) and chlorine (Cl) are considered minor plant nutrients (Donahue, 1961).

N is found mostly in topsoil organic matter or plow layer (Donahue, 1961). The keeping of organic matter abundantly in the soil and liberal application of nitrogenous fertilizers are keys to maintain soil N content (Donahue, 1961). In our study, we observed organic matter contribution from leftover trash biomass in the field after harvest. The

switchgrass harvested plots were found to be covered with trash leftover biomass which is about 1-2 inch thick which acted as mulch and upon degradation provided organic matter to the soil. It helped as a sort of natural fertilization. N in the form of nitrate (NO_3) or ammonium (NH_4) nitrogen is available to plants. Biological action converts N in organic matter into the ammonium and nitrate forms (Donahue, 1961). Soil microorganisms convert all N in the soil into nitrate nitrogen (NO_3-N), the form which is quickly available to plants (Donahue, 1961).

Phosphate in plants helps in cell division and formation of fats and proteins (Donahue, 1961). The soil phosphate in native form is often bound or fixed in some unavailable form and the phosphate fertilizers in the form of calcium and magnesium compounds are used (Donahue, 1961).

Potassium is available in adequate supply (about 2.5 %) in the surface soil almost everywhere, although more humid areas of the United States may have low available supply of potassium (Donahue, 1961). Our study was designed as a zero input design in terms of use of fertilizers, pesticides and any form of cultural operations except the final harvest operation. Therefore we did not apply any fertilizers in the study.

Maximum expected net return from switchgrass biomass yield was obtained with N fertilization at 65 kg N ha^{-1} yr^{-1} and one time post-senescence harvest per year for most biomass price and N price combinations (Haque et al., 2009). In another study, optimum biomass yields were obtained when upland switchgrass was harvested at full panicle emergence from boot to post anthesis at 120 kg N ha^{-1} applied fertilizer (Vogel et

al., 2002). N is a major fertilizer input and holds a major share in cost of production. Therefore the production practices that reduces N input can be profitable.

Even without the application of mineral fertilizers, N yield of the perennial fields were similar compared to the conventional high-input wheat fields (Glover et al., 2009). Perennial fields were also superior in providing harbor for number and/or diversity of insect pollinators, herbivores and detrivores (Glover et al., 2009). The same study also showed belowground maintenance of 43 Mg ha[1] more soil carbon and 4 Mg ha[1] more soil N than annual crop fields in the surface 1m. In addition, the study showed positive correlation of increased annual cropland with increased riverine nitrate-nitrogen levels.

Heggenstaller et al. (2009) found that switchgrass shoot biomass was affected by N rate, and year. They obtained highest yield at 220 kg N ha^{-1}, however, biomass yield return on incremental N beyond 140 kg N ha^{-1} was negligible. Hence, they recommended about 140 kg N ha^{-1} or slightly greater as the optimum N input for Central Iowa with precise recommendation to be dependent on N and biomass costs (Heggenstaller et al., 2009). Their study also found that active shoot to root translocation of P and K was negligible compared to N at the time of harvest.

Switchgrass is input efficient crop. Annual rate of nitrogen use for switchgrass (70-100 kg/ha) is about half of the amount required by annual row crops like corn (140-280 kg/ha) (McLaughlin et al., 1994). Switchgrass require herbicide application only during the establishment year unlike that of corn which requires herbicide application annually (McLaughlin et al., 1994). For erosive soils, perennial crops can be the best alternative to annual row crops to reduce depletion of soil nutrients and organic matter

(McLaughlin et al., 1994). The deep and vigorous rooting system of perennial grasses like switchgrass helps improve soil structure, increases water-holding capacity and infiltration through changes in soil structure and porosity, improves conservation and availability of nutrients, and decreases soil erosion (McLaughlin et al., 1994). Switchgrass enriches soil organic matter which improves availability of existing and added water and nutrients to the plants (McLaughlin et al., 1994).

CHAPTER III

METARIALS AND METHODS

Experimental site and treatments

This study was conducted at an experimental field at Efaw located at Stillwater, Payne County, Oklahoma. According to Oklahoma Ag Experiment Station Field and Research Service Unit (FRSU) website, the soil type was easpur loam (fine-loamy, mixed, superactive, thermic Fluventic Haplustoll) with 0 to 1 percent slopes and occasional flooding. The Payne County has a climate characterized by mild temperatures, hot summer, cool weather and occasional sharp drops due to cold surges (Henley et al., 1987). The county has a relatively uniform precipitation throughout the year with a slight peak in spring and an infrequent snowfall (Henley et al., 1987).

An unfertilized 'Kanlow' switchgrass planting was used in the study. The plant stands were established in 1998 and were used for this study from November 2007 through October 2009. The experimental design used was a split plot randomized complete block design (split plot RCBD) with 6 replications, each replication being a large plot of 200 m^2. The variable year was subplot in main plot month. For the evaluation of biomass yield over winter months, the experimental treatments were monthly harvests of standing

biomass beginning in November and ending in March of the two test years completed. For the evaluation of year-round biomass elemental composition, the experimental treatments were months from November through March of the following year for winter and May through October for growing season. Similarly, for the study of year-round soil nutrient changes in the 'Kanlow' switchgrass field, the experimental treatments were months from November through March for winter and May through October for growing season. The month of April was not included in the treatments as the plants in the field just begin to grow. The months November through the following March were categorized as winter season months and the months from May through October were categorized as growing season months.

The growing season biomass samples and soil samples were taken from the middle alley. The middle alley was divided into six blocks corresponding to the harvest blocks . The samples were taken from the large block plot each month (total 6 blocks each month).

The GPS coordinates and the layout map of the field were shown in the Figures 31 and 32 respectively.

Measurements and chemical analyses

The yield, biomass and soil data were collected towards the end of each harvest/sampling month. In each winter months, fresh biomass yields of six replications were collected after harvest. Swathing and baling operations were carried out using John Deere company's swather MoCo – Model 630 and Baler – Model 568 respectively. Fresh biomass weight was recorded after each plot was swathed and baled. Digital Load Cell

System machine was used for weighing the bales. At the same time, random hand grabbed biomass samples (about 500 grams each) were also collected from each plot. Biomass samples were then weighed (fresh weight), dehydrated at 130^oF $(55^o$C) in an air forced oven for 3 to 7 days, and again weighed (dry weight). Data for dry matter and moisture contents were thus obtained. The dry matter percent was multiplied with fresh biomass weight to get the dry matter yields for the respective plots, which were used to calculate the dry biomass yield per hectare.

In each growing months (from May through October), six above ground biomass samples were randomly hand clipped from the 90 feet alley between the winter harvest plots. Fresh weight of each sample taken was about 1000 grams. The biomass samples were then dehydrated at 130^oF $(55^o$C) in an air forced oven for 3 to 7 days. Data of dry matter and moisture contents were obtained and used to calculate the dry matter percentage of the sample.

The dry biomass samples thus obtained in winter and growing seasons were then taken for further processing for analysis of elemental composition. At first the dry biomass samples were chopped into small pieces of size about 5 mm in Thomas Wiley Laboratory Mill-Model 4, and then powdered in Cyclone Sample Mill manufactured by Udy Corporation, USA. The powdered samples (approximately 50 g) were then put into paper sampling bags for forage quality analyses of P, Ca, K, Mg, Na, S, Fe, Zn, Cu, Mn, Ni, total N, total C, ash, acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL).

Soil samples were collected both in winter and growing seasons. During winter months, soil samples were collected from the winter harvest plots immediately after harvest and biomass sample collection. During growing season months, soil samples were taken from the 90 feet alley between the winter harvest plots. Each soil sample was collected by mixing 15 to 20 random sub-samples from 6-inch surface soil profile with a soil probe. In summer when soil in the field was very dry, soil drill was used to obtain the samples. The collected soil samples were then allowed to dry at room temperature to get rid of excessive moisture. The samples were pulverized after excess moisture was dried. The pulverized soil samples were then bagged in small soil sampling bags for soil analyses of soil pH, NO_3-N, soil tested P, K, SO_4, Ca, Mg, Fe, Zn, B, Cu, and organic matter (OM).

Monthly records of daily rainfall data were retrieved from Oklahoma Mesonet website for November, 2007 to March, 2008 and November, 2008 to March, 2008. Monthly accumulated rainfall up to the date of winter harvest month was calculated for each of the months December, January, February, and March. Therefore, for the month of December, we used daily rainfall data after November harvest date up to the December harvest date. The same procedure was followed for remaining months as well. The yield decrease in each successive winter month was calculated using yield difference of prior month and the present month.

Forage quality analyses

Forage samples were collected and dried at 85°C over night and ground to pass 1 mm screen. The moisture content of plant sample was determined by drying ground

sample at 105 ^{o}C. Total nitrogen (TN) was determined using a dry combustion Nitrogen Analyzer (Undersander, 1993). Acid detergent fiber and acid detergent lignin were determined using the Ankom Fiber Analyzer (Ankom Technology). Mineral contents of the forage were analyzed by a Spectro CirOs ICP following digestion (Undersander, 1993).

Soil analyses

Soil samples were dried at 60 ^{o}C overnight and ground to pass a 2mm sieve. All samples were analyzed for pH, buffer index (BI), NO_3-N, plant available P and K index, and soil organic C. Soil pH and BI were measured by glass electrode in a 1:1 soil:water suspension and SMP buffer solution, respectively (Sims, 1996). Soil NO_3-N was extracted with 1 M KCl solution and quantified by the cadmium reduction method on a Lachat QuikChem 8000 (LACHAT, 1994). Soil available P, K, Ca and Mg were extracted using Mehlich 3 solution (Mehlich, 1984). Mehlich 3 P was quantified colorimetrically using a Lachat, while K, Ca, and Mg analyzed by a Spectro CirOs ICP (Soltanpour et al., 1996). Soil organic carbon was determined using a LECO Truspec dry combustion carbon analyzer (Nelson and Sommers, 1996). Soil sulfate was extracted by 0.008 M $Ca_3(PO_4)_2$ and analyzed by a Spectro CirOs ICP. Plant available Zn, Fe, Cu, and were extracted by DTPA-Sorbitol and quantified by ICP (Procedures for Western States Laboratory Proficiency Testing Program).

Soil texture was determined using the Hydrometer method (Gee and Bauder, 1996).

Statistical analyses

The data obtained on yield, biomass chemical composition, and soil nutrients were analyzed using Statistical Analysis Software (SAS) version 9.1. Analysis of variance (ANOVA) was carried out using PROC MIXED procedure with replication as a random effect in a SAS program according to split plot RCBD. The data for decrease in yield and accumulated rainfall for the month were utilized to examine if any correlation existed between the two.

CHAPTER IV

RESULTS

Yield

The dry biomass yields of different winter months for two years have been shown in Table 1 and Figure 1. Based on analysis of data pooled over two winters, there were no yield differences among different winter harvest months (P=0.7730). The yield, however, was different between the two winters (2007-2008 and 2008-2009) (P<0.0001). We observed higher yield in the second year winter. The interaction of year and harvest month was not significant (P=0.1029). Yearwise analysis of monthly yield data showed different monthly yield in the first year winter (P=0.0008) and constant yield in the second year winter (P=0.6754). In general the yield trend was decreasing in the first year with highest value in December. The correlation analysis of yield decrease and total monthly accumulated rainfall indicated no linear association of monthly yield decrease and accumulated monthly rainfall in winter for both the first year winter (r =-0.35367, P=0.6463, and $\alpha = 0.05$) and the second year winter (r=0.90516, P=0.6463, and $\alpha = 0.05$).

Table 1. The effect of winter harvest months on dry biomass yield of switchgrass.

Harvest month	Yield (t/ha)		
	2 years average	Year 1 (2007-2008)	Year 2 (2008-2009)
November	5.20	4.99	5.42
December	5.43	4.46	6.41
January	5.38	3.88	6.89
February	4.92	3.14	6.70
March	4.71	2.95	6.48
Winter average (Nov. through Mar.)	5.13	3.88	6.38
Effect			
Month	NS	***	NS
Year	***		
Year*month	NS		

NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

Biomass elements

The concentrations of phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), sulfur (S), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), nickel (Ni), total nitrogen (N), total carbon (C), ash, acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL) have been shown in Table 2 and Figures 2 – 18.

Total nitrogen (N)

There were no differences of biomass N concentrations among different winter months (P=0.0550). However, the differences were observed between winters of the two test years (P<0.0001). The concentration of N was higher in the second year. The interaction of year and month was not significant (P=0.1728). Yearwise analysis showed no differences of N concentrations among winter months in both the first year (P=0.5895) and the second year (P=0.0660).

There were differences of biomass N concentrations among different growing months (P<0.0001) and between growing seasons of the two test years (P<0.0001). The concentration of N was higher in the second year. The interaction of year and month was also significant (P=0.0072). Yearwise analysis showed differences of N concentrations among growing months in both the first year (P<0.0001) and the second year (P<0.0001). The N concentration was decreased as the growth progressed in growing season.

Phosphorus (P)

There were differences of biomass P concentrations among different winter months (P<0.0001). However, the P concentrations were not different between the winters of two test years (P=0.1209). The interaction of year and month was significant (P=0.0030). Yearwise analysis indicated the differences of biomass P concentrations among different winter months in the first year (P<0.0001) as well as the second year (P<0.0001).

There were differences of biomass P concentrations among different growing months (P<0.0001) and between the growing seasons of the two test years (P<0.0001). The concentration was higher in the second year. The interaction of year and month was also significant (P<0.0001). Yearwise analysis indicated the differences of biomass P concentrations among different growing months in the first year (P<0.0001) as well as the second year (p<0.0001). In general, a decreasing trend of P concentration was observed for growing season as well as winter season.

Potassium (K)

There were differences of biomass K concentrations among different winter months (P<0.0001). However, the concentrations of K were not different between the winters of two test years (P=0.4657). The interaction of year and month was significant (P<0.0001). Yearwise analysis showed differences of K concentrations among winter months in both the first year (P<0.0001) and the second year (P<0.0001).

There were differences of biomass K concentrations among different growing months (P<0.0001). However the concentrations were not different between the winters of two test years (P=0.2518). The interaction of year and month was significant (P=0.0001). Yearwise analysis showed differences of K concentrations among winter months in both the first year (P<0.0001) and the second year (P<0.0001).

Calcium (Ca)

There were no differences of biomass Ca concentrations among different winter months (P=0.1815). However, the concentrations were different between winters of the two test years (P<0.0001). The interaction of year and month was not significant (P=0.9804). Yearwise analysis showed no differences of P concentrations among winter months in both the first year (P=0.5131) and the second year (P=0.2357).

There were differences of biomass Ca concentrations among different growing months (P=0.0114) and between winters of the two test years (P<0.0001). The concentration was higher in the second year. However, the interaction of year and month was not significant (P=0.2514). Yearwise analysis showed differences of P

concentrations among growing months in the first year (P=0.0014) but showed no differences in the second year (P=0.2256).

Magnesium (Mg)

There were differences of biomass Mg concentrations among different winter months (P=0.0006). Similarly, the Mg concentrations were different between the winters of the two test years (P=0.0008). The Mg concentration in the second year was lower. The interaction of year and month was also significant (P=0.0420). Yearwise analysis showed differences of Mg concentrations among winter months for both the first year (P=0.0004) and the second year (P=0.0150).

There were differences of biomass Mg concentrations among different growing months (P=0.0003) and between the growing seasons of the two test years (P=0.0174). The Mg concentration in the second year was higher. The interaction of year and month was not significant (P=0.5876). Yearwise analysis showed differences of Mg concentrations among growing months for both the first year (P=0.0195) and the second year (P=0.0031). K concentration decreased with the progressing of age of plant in the growing season.

Sulfur (S)

There were differences of biomass S concentrations among different winter months (P=0.0004), and between winters of the two test years (P=0.0003). The sulfur concentration in the second year winter was lower. The interaction of year and month was not significant (P=0.2521). Yearwise analysis showed differences of S

concentrations among winter months in both the first year (P=0.0020) and the second year (P=0.0156).

There were differences of biomass S concentrations among different growing months (P<0.0001), and between growing seasons of the two test years (P<0.0001). The sulfur concentration in the second year growing season was higher. The interaction of year and month was also significant (P=0.0022). Yearwise analysis showed differences of S concentrations among growing months in both the first year (P<0.0001) and the second year (P<0.0001).

Sodium (Na)

There were differences of biomass Na concentrations among different winter months (P=0.0367), and between winters of the two test years (P=0.0007). The concentration of Na was lower in the second year. The interaction of year and month was also significant (P=0.0141). Yearwise analysis showed differences of Na concentrations among winter months in the first year (P=0.0047) but no differences were observed in the second year (P=0.3268).

There were no differences of biomass Na concentrations among different growing months (P=0.0719). However, the concentrations were different between growing seasons of the two test years (P=0.0007). The concentration of Na was lower in the second year. The interaction of year and month was also significant (P=0.0002). Yearwise analysis showed differences of Na concentrations among growing months in both the first year (P=0.0051) and the second year (P<0.0079).

Iron (Fe)

There were no differences of biomass Fe concentrations among different winter months (P=0.0918). However, the concentrations were different between the winters of the two test years (P=0.0159). The Fe concentration in the second year was lower. The interaction of year and month was not significant (P=0.2414). Yearwise analysis showed no differences of S concentrations among winter months in the first year (P=0.1628) but showed significant differences in the second year (P=0.0011).

There were differences of biomass Fe concentrations among different growing months (P<0.0001) and between the growing seasons of the two test years (P<0.0001). The concentration was higher in the second year. The interaction of year and month was also significant (P<0.0001). Yearwise analysis showed differences of S concentrations among growing months in the first year (P<0.0001) as well as the second year (P<0.0001).

Zinc (Zn)

There were no differences of biomass Zn concentrations among different winter months (P=0.1964). Similarly, no differences of Zn concentrations were observed between winters of the two test years (P=0.0634). The interaction of year and month was also not significant (P=0.2901). Yearwise analysis showed no differences of Zn concentrations among winter months in the first year (P=0.1256) as well as the second year (P=0.1384).

There were differences of biomass Zn concentrations among different growing months (P<0.0001) and between the growing seasons of the two test years (P<0.0001).

Table 2. Elemental concentrations of switchgrass biomass for different months.

Month	Total N (%)			P (%)			K (%)		
	2 years average	Year 1 (2007-08)	Year 2 (2008-09)	2 years average	Year 1 (2007-08)	Year 2 (2008-09)	2 years average	Year 1 (2007-08)	Year 2 (2008-09)
Winter season									
November	0.38	0.30	0.46	0.09	0.10	0.07	0.26	0.31	0.21.
December	0.49	0.34	0.64	0.07	0.07	0.07	0.17	0.15	0.19
January	0.40	0.32	0.48	0.06	0.05	0.06	0.13	0.12	0.15
February	0.39	0.31	0.48	0.05	0.05	0.05	0.10	0.08	0.12
March	0.37	0.30	0.45	0.05	0.05	0.05	0.07	0.06	0.08
Average	0.41	0.31	0.50	0.06	0.07	0.06	0.15	0.14	0.15
Effect									
Month	NS	NS	NS	***	***	***	***	***	***
Year	***			NS			NS		
Year*Month	NS			**			***		
Growing season									
May	1.26	1.10	1.43	0.23	0.23	0.23	1.78	1.87	1.69
June	0.92	0.68	1.16	0.18	0.15	0.22	1.31	1.24	1.38
July	0.78	0.51	1.04	0.15	0.12	0.19	0.90	0.77	1.03
August	0.51	0.36	0.67	0.11	0.10	0.13	0.58	0.53	0.64
September	0.46	0.35	0.58	0.11	0.11	0.11	0.52	0.59	0.46
October	0.34	0.25	0.44	0.08	0.08	0.09	0.36	0.38	0.34
Average	0.71	0.54	0.88	0.14	0.13	0.16	0.91	0.89	0.92
Effect									
Month	***	***	***	***	***	***	***	***	***
Year	***			***			NS		
Year*Month	**			***			***		

NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

Table 2. Elemental concentrations of switchgrass biomass for different months (contd.).

Month	Ca (%)			Mg (%)			S (%)		
	2 years average	Year 1 (2007-08)	Year 2 (2008-09)	2 years average	Year 1 (2007-08)	Year 2 (2008-09)	2 years average	Year 1 (2007-08)	Year 2 (2008-09)
Winter season									
November	0.19	0.21	0.16	0.16	0.18	0.14	0.05	0.06	0.04
December	0.21	0.24	0.19	0.14	0.15	0.13	0.05	0.05	0.05
January	0.19	0.22	0.16	0.13	0.13	0.13	0.04	0.04	0.04
February	0.19	0.21	0.16	0.13	0.13	0.12	0.04	0.04	0.04
March	0.18	0.21	0.16	0.10	0.10	0.09	0.04	0.04	0.04
Average	0.19	0.22	0.17	0.13	0.14	0.12	0.05	0.05	0.04
Effect									
Month	NS	NS	NS	***	***	*	***	**	*
Year	***			***			***		
Year*Month	NS			*			NS		
Growing season									
May	0.21	0.21	0.21	0.19	0.19	0.19	0.11	0.11	0.11
June	0.21	0.19	0.22	0.19	0.18	0.19	0.09	0.08	0.10
July	0.23	0.19	0.27	0.22	0.19	0.24	0.08	0.07	0.10
August	0.18	0.15	0.20	0.17	0.17	0.18	0.06	0.05	0.07
September	0.18	0.16	0.21	0.15	0.13	0.16	0.06	0.05	0.06
October	0.17	0.14	0.20	0.14	0.13	0.15	0.05	0.04	0.05
Average	0.20	0.17	0.22	0.17	0.16	0.19	0.07	0.07	0.08
Effect									
Month	*	**	NS	***	*	**	***	***	***
Year	***			*			***		
Year*Month	NS			NS			**		

NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

Table 2. Elemental concentrations of switchgrass biomass for different months (contd.).

Month	Na (%)			Fe (ppm)			Zn (ppm)		
	2 years average	Year 1 (2007-08)	Year 2 (2008-09)	2 years average	Year 1 (2007-08)	Year 2 (2008-09)	2 years average	Year 1 (2007-08)	Year 2 (2008-09)
Winter season									
November	0.01	0.02	0.01	51.60	55.17	48.02	20.88	24.02	17.74
December	0.01	0.01	0.02	75.61	86.00	65.22	21.12	21.42	20.82
January	0.02	0.01	0.02	64.61	75.33	53.89	16.41	15.95	16.88
February	0.01	0.01	0.01	72.02	77.33	66.70	18.03	18.48	17.57
March	0.01	0.00	0.01	101.17	140.33	62.01	20.29	22.50	18.07
Average	0.01	0.01	0.01	73.00	86.83	59.17	19.34	20.47	18.22
Effect									
Month	*	**	NS	NS	NS	**	NS	NS	NS
Year	***			*			NS		
Year*Month	*			NS			NS		
Growing season									
May	0.03	0.03	0.04	69.08	69.17	68.99	27.32	26.13	28.51
June	0.04	0.05	0.03	74.09	58.00	90.18	26.87	22.78	30.95
July	0.05	0.09	0.02	83.38	63.67	103.10	24.50	18.35	30.65
August	0.05	0.07	0.02	59.36	47.17	71.56	20.41	16.85	23.96
September	0.03	0.05	0.02	50.79	35.67	65.92	20.64	19.52	21.77
October	0.04	0.05	0.02	51.35	26.50	76.20	16.36	12.97	19.76
Average	0.04	0.06	0.02	64.68	50.03	79.33	22.68	19.43	25.93
Effect									
Month	NS	**	**	***	***	***	***	***	***
Year	***			***			***		
Year*Month	***			***			*		

NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

Table 2. Elemental concentrations of switchgrass biomass for different months (contd.).

Month	Cu (ppm)			Mn (ppm)			Ni (ppm)		
	2 years average	Year 1 (2007-08)	Year 2 (2008-09)	2 years average	Year 1 (2007-08)	Year 2 (2008-09)	2 years average	Year 1 (2007-08)	Year 2 (2008-09)
Winter season									
November	20.21	28.63	11.78	64.60	79.90	49.31	0.54	0.83	0.25
December	20.93	28.28	13.58	71.88	91.38	52.38	1.06	1.83	0.28
January	15.03	19.97	10.09	56.97	70.45	43.48	0.68	1.17	0.20
February	14.89	18.08	11.69	70.86	89.83	51.88	0.77	1.33	0.21
March	12.98	16.20	9.76	62.80	79.50	46.09	1.00	1.83	0.17
Average	16.81	22.23	11.38	65.42	82.21	48.63	0.81	1.40	0.22
Effect									
Month	***	***	***	NS	NS	NS	NS	NS	NS
Year	***			***			***		
Year*Month	***			NS			NS		
Growing season									
May	23.35	30.53	16.17	83.88	67.23	100.53	1.97	2.50	1.43
June	21.75	21.23	22.26	68.38	60.68	76.07	1.41	1.67	1.15
July	23.69	22.17	25.21	66.69	64.68	68.69	0.99	1.00	0.98
August	15.50	16.43	14.57	57.54	51.92	63.15	0.91	1.33	0.49
September	15.30	17.65	12.94	58.70	50.02	67.39	0.99	1.67	0.32
October	13.56	12.55	14.57	59.16	43.87	74.46	1.05	1.83	0.26
Average	18.86	20.09	17.62	65.72	56.40	75.05	1.22	1.67	0.77
Effect									
Month	***	***	***	**	NS	**	***	***	***
Year	**			***			***		
Year*Month	***			NS			***		

NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

Table 2. Elemental concentrations of switchgrass biomass for different months (contd.).

Month	Total C (%)			Ash (%)			ADF (%)		
	2 years average	Year 1 (2007-08)	Year 2 (2008-09)	2 years average	Year 1 (2007-08)	Year 2 (2008-09)	2 years average	Year 1 (2007-08)	Year 2 (2008-09)
Winter season									
November	48.44	48.32	48.56	2.97	3.15	2.80	51.28	49.53	53.03
December	48.38	48.17	48.59	3.12	3.38	2.86	50.85	49.65	52.04
January	48.52	48.35	48.70	2.84	3.12	2.57	53.23	51.66	54.80
February	48.60	48.31	48.89	3.09	3.70	2.47	51.74	51.35	52.14
March	47.55	48.33	46.77	2.65	2.65	2.66	51.78	52.50	51.07
Average	48.30	48.30	48.30	2.94	3.20	2.67	51.78	50.94	52.61
Effect									
Month	NS	NS	NS	NS	NS	NS	NS	*	NS
Year	NS			**			**		
Year*Month	NS			NS			*		
Growing season									
May	46.61	46.48	46.74	4.11	5.87	2.35	37.75	37.86	37.65
June	47.23	47.14	47.31	3.69	4.93	2.44	39.92	41.39	38.46
July	47.51	47.71	47.30	3.16	3.92	2.40	43.72	49.54	37.90
August	47.75	47.61	47.90	2.93	3.27	2.59	45.43	49.47	41.40
September	47.67	47.56	47.77	2.97	3.50	2.44	46.68	49.79	43.56
October	48.20	48.21	48.19	2.74	2.93	2.55	50.43	51.69	49.16
Average	47.49	47.45	47.54	3.27	4.07	2.46	43.99	46.62	41.35
Effect									
Month	***	***	**	***	***	NS	***	***	***
Year	NS			***			***		
Year*Month	NS			***			***		

NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

Table 2. Elemental concentrations of switchgrass biomass for different months (contd.).

Month	NDF (%)			ADL (%)		
	2 years average	Year 1 (2007-08)	Year 2 (2008-09)	2 years average	Year 1 (2007-08)	Year 2 (2008-09)
Winter season						
November	71.84	63.42	80.26	8.81	8.72	8.91
December	72.40	64.77	80.04	8.78	9.17	8.40
January	74.43	67.91	80.95	9.23	9.45	9.02
February	73.74	67.11	80.37	9.25	9.84	8.66
March	72.73	67.30	78.16	8.10	9.08	7.13
Average	73.03	66.10	79.96	8.83	9.25	8.42
Effect						
Month	NS	*	NS	NS	NS	NS
Year	***			*		
Year*Month	NS			NS		
Growing season						
May	62.12	55.60	68.63	5.79	8.69	2.90
June	64.16	58.38	69.95	5.45	6.77	4.12
July	65.45	63.37	67.54	6.95	9.41	4.48
August	66.54	63.63	69.45	6.82	7.68	5.96
September	67.86	64.42	71.29	7.37	8.25	6.49
October	71.53	65.09	77.96	7.20	8.20	6.20
Average	66.28	61.75	70.80	6.60	8.17	5.02
Effect						
Month	***	***	***	**	*	***
Year	***			***		
Year*Month	***			**		

NS,*,**,*** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

The concentration was higher in the second year. The interaction of year and month was also significant (P=0.0309). Yearwise analysis showed differences of S concentrations among growing months in the first year (P=0.0002) as well as the second year (P<0.0001).

Copper (Cu)

There were differences of biomass Cu concentrations among different winter months (P<0.0001). Similarly, the Cu concentrations were different between winters of the two test years (P<0.0001). The concentration of Cu in the second year was lower. The interaction of year and month was also significant (P=0.0001). Yearwise analysis showed differences of Zn concentrations among months in both the first year (P<0.0001) and the second year (P=0.0007).

There were differences of biomass Cu concentrations among different growing months (P<0.0001) and between growing seasons of the two test years (P=0.0010). The concentration of Cu in the second year was lower. The interaction of year and month was also significant (P<0.0001). Yearwise analysis showed differences of Zn concentrations among growing months in both the first year (P<0.0001) and the second year (P<0.0001).

Manganese (Mn)

There were no differences of biomass Mn concentrations among different winter months (P=0.3282). However, the concentrations were different between winters of the two test years (P<0.0001). The concentration of Mn was lower in the second year. The interaction of year and month was not significant (P=0.7482). Yearwise analysis showed

no differences of Zn concentrations among winter months in both the first year (P=0.4439) and the second year (P=0.4072).

There were differences of biomass Mn concentrations among different growing months (P=0.0046) and between growing seasons of the two test years (P<0.0001). The concentration of Mn was higher in the second year. The interaction of year and month was not significant (P=0.0552). Yearwise analysis showed no differences of Zn concentrations among growing months in the first year (P=0.0511) but showed differences in the second year (P=0.0032).

Nickel (Ni)

There were no differences of biomass Ni concentrations among different winter months (P=0.4256). However, the concentrations were different between winters of the two test years (P<0.0001). The concentration of Ni in the second year was lower. The interaction of year and month was not significant (P=0.3999). Yearwise analysis showed no differences of Zn concentrations among winter months in both the first year (P=0.3359) and the second year (P=0.0501).

There were differences of biomass Ni concentrations among different growing months (P<0.0001) and between growing seasons of the two test years (P<0.0001). The concentration of Ni in the second year was lower. The interaction of year and month was also significant (P=0.0002). Yearwise analysis showed differences of Zn concentrations among growing months in both the first year (P=0.0003) and the second year (P<0.0001).

Total carbon (C)

There were no differences of biomass C concentrations among different winter months (P=0.4515) and between winters of the two test years (P=0.9885). The interaction of year and month was also not significant (P=0.3488). Yearwise analysis showed no differences of C concentrations among winter months in both the first year (P=0.8421) and the second year (P=0.3969).

There were differences of biomass C concentrations among different growing months (P<0.0001). However, the concentrations were not different between growing seasons of the two test years (P=0.4778). The interaction of year and month was also not significant (P=0.5410). Yearwise analysis showed differences of C concentrations among growing months in both the first year (P<0.0001) and the second year (P=0.0076).

Ash

There were no differences of biomass ash concentrations among different winter months (P=0.4714). However, the concentrations were different between winters of the two test years (P=0.0066). The concentration of ash is lower in the second year. The interaction of year and month was not significant (P=0.3121). Yearwise analysis showed no differences of C concentrations among winter months in both the first year (P=0.4107) and the second year (P=0.2404).

There were differences of biomass ash concentrations among different growing months (P<0.0001) and between growing seasons of the two test years (P<0.0001). The concentration of ash is lower in the second year. The interaction of year and month was also significant (P<0.0001). Yearwise analysis showed differences of C concentrations

among growing months in the first year (P<0.0001) but showed no differences in the second year (P=0.6900).

ADF

There were no differences of biomass ADF concentrations among different winter months (P=0.2990). However, the concentrations were different between winters of the two test years (P=0.0018). The concentration of ADF was higher in the second year. The interaction of year and month was also significant (P=0.0197). Yearwise analysis showed differences of ADF concentrations among winter months in the first year (P=0.0283) but showed no differences in the second year (P=0.2160).

There were differences of biomass ADF concentrations among different growing months (P<0.0001) and between growing seasons of the two test years (P<0.0001). The concentration of ADF was lower in the second year. The interaction of year and month was also significant (P<0.0001). Yearwise analysis showed differences of ADF concentrations among growing months in both the first year (P<0.0001) and the second year (P<0.0001).

NDF

There were no differences of biomass NDF concentrations among different winter months (P=0.4467). However, the NDF concentrations were different between winters of the two test years (P<0.0001). The concentration of NDF was higher in the second year. The interaction of year and month was not significant (P=0.2576). Yearwise analysis showed differences of NDF concentrations among winter months in the first year (P=0.0277) but showed no differences in the second year (P=0.7950).

There were differences of biomass NDF concentrations among different growing months (P<0.0001) and between growing seasons of the two test years (P<0.0001). The concentration of NDF was higher in the second year. The interaction of year and month was also significant (P<0.0001). Yearwise analysis showed differences of NDF concentrations among growing months in both the first year (P<0.0001) and the second year (P<0.0001).

ADL

There were no differences of biomass ADL concentrations among different winter months (P=0.1780). However, the concentrations were different between winters of the two test years (P=0.0142). The ADL concentration was lower in the second year. The interaction of year and month was not significant (P=0.2949). Yearwise analysis showed no differences of ADL concentrations among winter months in both the first year (P=0.4060) and the second year (P=0.1535).

There were differences of biomass ADL concentrations among different growing months (P=0.0076) and between growing seasons of the two test years (P<0.0001). The ADL concentration was lower in the second year. The interaction of year and month was also significant (P=0.0016). Yearwise analysis showed differences of ADL concentrations among growing months in both the first year (P=0.0258) and the second year (P=0.0007).

Soil properties

The concentrations of soil pH, nitrate nitrogen (NO_3-N), soil tested phosphorus (P), potassium (K), sulfate (SO_4), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn),

boron (B), copper (Cu), and organic matter (OM) have been shown in Table 3 and Figures 19 - 30.

pH

There were no soil pH differences in winter months (P=0.5927). However, the pH values were different between the winter seasons of the two test years (2007-2008 and 2008-2009) (P<0.0001). The pH value in the second year winter was higher than pH value in the first year winter. There was no significant interaction of year and month (P=0.1608). Yearwise analysis showed that there were no monthly pH differences in both the first year winter (P=0.4422) and the second year winter (p=0.5378).

There were no soil pH differences in growing season months (P=0.7541). However, the pH values were different between the growing seasons of the two test years (2007-2008 and 2008-2009) (p<0.0001). The pH value in the second year growing season was higher. There was no significant interaction of year and month (P=0.2361). Yearwise analysis showed that there were no monthly pH differences in both the first year growing months (P=0.1290) and the second year growing months (P=0.6918).

Nitrate nitrogen (NO_3-N)

There were no differences in soil N concentration in different winter months (P=0.8769). However, the concentrations were different between the winters of the two test years (P=0.0345). The N concentration in the second year was higher. No significant interaction of year and month (P=0.6211) was observed. Yearwise analysis showed no significant differences of N concentrations in the first year winter months (P=0.5514) as well as the second year winter months (P=0.8402).

There were significant differences in soil N concentrations in different growing season months (P<0.0001). However, the concentrations were not different between the growing seasons of the two test years (P=0.6700). A significant interaction of year and month (P=0.0436) was observed. Yearwise analysis showed significant differences of N concentrations in the first year growing months (P<0.0001) as well as the second year growing months (p=0.0042).

Soil tested phosphorus (P)

There were no differences in soil P concentrations in different winter months (P=0.6745). However, the soil P concentrations were different between the winter seasons of the two test years (P<0.0001). There was significant interaction of year and month (P=0.0341). Yearwise analysis indicated no differences of soil P concentrations in the winter months of both the first year (P=0.6220) as well as the second year (P=0.5127).

There were differences in soil P concentrations in different growing months (P=0.0344). Similarly, the soil P concentrations were different between the growing seasons of the two test years (P<0.0001). However, the interaction of year and month (P=0.6828) was not significant. Yearwise analysis indicated no differences of soil P contents in the growing months of the first year (P=0.6836) but showed significant differences in the second year (P=0.0012).

Potassium (K)

There were no differences in soil K concentrations in different winter months (P=0.4938) based and between growing seasons of two test years (P=0.2273). However,

there was significant interaction of year and month (P=0.0177). Yearwise analysis

indicated no differences in winter months in both the first year (P=0.2044) and the second

year (P=0.5282).

There were no differences in soil K concentrations in different growing months

(P=0.1221). However, the K concentrations were different between growing seasons of

the two test years (P=0.0001). The significant interaction of year and month (P=0.0356)

was also present. Yearwise analysis indicated no differences of K concentrations in the

growing months of the first year (P=0.1062) but showed significant differences in the

second year growing months (P=0.0007).

Sulfate (SO_4)

There were no differences in soil SO_4 concentrations in different winter months

(P=0.1484). However, the concentrations were different between winters of two test years

(P<0.0001). The interaction of year and month (P=0.3940) was not significant. Yearwise

analysis indicated no differences in both the first year (P=0.0927) and the second year

(P=0.2187).

There were no differences in soil SO_4 concentrations in different growing months

(P=0.1853). However, the soil SO_4 concentrations were different between growing

seasons of the two test years (P<0.0001). The interaction of year and month (P=0.0005)

was also significant. Yearwise analysis indicated differences in both the first year

(P=0.0102) and the second year (P=0.0013).

Table 3. pH, OM and soil chemicals in switchgrass field for different months.

Month	pH			NO3-N (kg/ha)			Soil tested P (kg/ha)		
	2 years	Year 1	Year 2	2 years	Year 1	Year 2	2 years	Year 1	Year 2
Winter season									
November	6.1	6.1	6.2	2.1	2.1	2.1	65	67	62
December	6.2	6.1	6.2	2.1	1.7	2.4	66	70	61
January	6.2	6.1	6.2	2.1	1.5	2.6	60	66	55
February	6.0	6.0	6.1	1.6	1.5	1.7	66	73	60
March	6.1	6.0	6.2	1.9	1.5	2.2	62	68	56
Average	6.1	6.1	6.2	1.9	1.6	2.2	64	69	59
Effect									
Month	NS	NS	NS	NS	NS	NS	NS	NS	NS
Year	***			*			***		
Year*Month	NS			NS			*		
Growing season									
May	6.1	6.0	6.3	1.6	1.3	1.9	66	71	61
June	6.2	6.1	6.2	1.8	2.2	1.3	64	69	59
July	6.1	6.0	6.3	1.5	1.3	1.7	62	67	58
August	6.1	6.0	6.3	1.2	1.1	1.3	61	68	54
September	6.1	6.0	6.2	2.6	2.6	2.6	63	70	57
October	6.1	6.0	6.2	2.7	3.0	2.4	60	66	54
Average	6.1	6.0	6.2	1.9	1.9	1.9	63	69	57
Effect									
Month	NS	NS	NS	***	***	**	*	NS	**
Year	***			NS			***		
Year*Month	NS			*			NS		

NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

Table 3. pH, OM and soil chemicals in switchgrass field for different months (contd.).

Month	K (kg/ha)			SO4 (kg/ha)			Ca (kg/ha)		
	2 years	Year 1	Year 2	2 years	Year 1	Year 2	2 years	Year 1	Year 2
Winter season									
November	262	251	273	27	29	25	3088	3276	2899
December	276	282	270	27	30	24	3496	3700	3292
January	248	250	245	25	28	22	3215	3370	3060
February	266	273	259	25	29	21	3345	3516	3174
March	250	256	245	23	26	20	3043	3157	2929
Average	260	263	258	25	28	23	3237	3404	3071
Effect									
Month	NS	NS	NS	NS	NS	NS	NS	*	NS
Year	NS			***			***		
Year*Month	*			NS			NS		
Growing season									
May	275	273	276	23	26	20	3290	3410	3170
June	295	341	249	23	28	19	3443	3744	3143
July	298	303	293	22	24	20	3333	3480	3187
August	305	321	289	22	22	22	3348	3695	3002
September	317	340	294	23	27	20	3389	3653	3124
October	294	315	274	22	24	19	3258	3384	3131
Average	297	316	279	23	25	20	3344	3561	3126
Effect									
Month	NS	NS	***	NS	*	**	NS	NS	NS
Year	***			***			***		
Year*Month	*			***			NS		

NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

Table 3. pH, OM and soil chemicals in switchgrass field for different months (contd.).

Month	Mg(kg/ha)			Fe(ppm)			Zn(ppm)		
	2 years	Year 1	Year 2	2 years	Year 1	Year 2	2 years	Year 1	Year 2
Winter season									
November	636	649	623	47.69	46.27	49.12	1.09	1.10	1.07
December	721	744	698	50.40	47.73	53.06	1.07	1.03	1.10
January	642	662	622	48.12	49.50	46.74	1.01	1.02	1.01
February	668	689	648	57.22	57.25	57.19	1.15	1.23	1.06
March	624	642	606	49.50	51.02	47.99	1.04	1.05	1.02
Average	658	677	639	50.59	50.35	50.82	1.07	1.09	1.05
Effect									
Month	NS	*	NS	NS	NS	NS	NS	NS	NS
Year	***			NS			NS		
Year*Month	NS			NS			NS		
Growing season									
May	682	698	667	65.07	61.95	68.18	1.19	1.17	1.21
June	703	775	632	64.11	68.53	59.68	1.17	1.28	1.06
July	702	736	668	62.01	62.67	61.35	1.10	1.18	1.01
August	693	752	633	60.73	66.63	54.82	1.04	1.05	1.04
September	695	745	646	71.23	76.10	66.37	1.10	1.15	1.04
October	674	723	625	66.67	69.60	63.74	0.90	0.90	0.91
Average	692	738	645	64.97	67.58	62.36	1.08	1.12	1.04
Effect									
Month	NS	NS	NS	*	NS	*	***	***	**
Year	***			**			*		
Year*Month	NS			NS			NS		

NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

Table 3. pH, OM and soil chemicals in switchgrass field for different months (contd.).

Month	B (ppm)			Cu (ppm)			OM (%)		
	2 years average	Year 1 (2007-08)	Year 2 (2008-09)	2 years average	Year 1 (2007-08)	Year 2 (2008-09)	2 years average	Year 1 (2007-08)	Year 2 (2008-09)
Winter season									
November	0.34	0.39	0.29	1.33	1.28	1.38	1.21	1.4	1.0
December	0.35	0.38	0.32	1.47	1.33	1.61	1.47	1.7	1.3
January	0.32	0.34	0.30	1.39	1.37	1.42	1.18	1.5	0.9
February	0.34	0.39	0.29	1.57	1.50	1.64	1.31	1.6	1.0
March	0.31	0.32	0.31	1.44	1.40	1.49	1.10	1.4	0.8
Average	0.33	0.36	0.30	1.44	1.38	1.51	1.25	1.5	1.0
Effect									
Month	NS	*	NS	NS	NS	NS	*	*	*
Year	***			**			***		
Year*Month	NS			NS			NS		
Growing season									
May	0.34	0.35	0.32	1.55	1.42	1.69	1.40	1.8	1.0
June	0.35	0.41	0.28	1.52	1.53	1.50	1.47	1.9	1.1
July	0.31	0.34	0.29	1.49	1.40	1.59	1.44	1.9	1.0
August	0.34	0.37	0.31	1.47	1.45	1.48	1.34	1.7	1.0
September	0.35	0.39	0.31	1.52	1.42	1.62	1.55	2.0	1.1
October	0.32	0.34	0.31	1.48	1.35	1.62	1.36	1.7	1.1
Average	0.34	0.37	0.30	1.51	1.43	1.58	1.43	1.8	1.0
Effect									
Month	NS	NS	NS	NS	NS	*	NS	NS	NS
Year	***			***			***		
Year*Month	*			NS			NS		

NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

Calcium (Ca)

There were no differences in soil Ca concentrations in different winter months (P=0.0744). However, the soil Ca concentrations were different between the winters of two test years (P<0.0001). The Ca concentration in the second year winter was lower. There was no significant interaction of year and month (P=0.7301). Yearwise analysis indicated differences in the first year winter months (P=0.0320) but showed no difference in the second year winter months (P=0.2346).

There were no differences in soil Ca concentrations in different growing months (P=0.7171). However, the soil Ca concentrations were different between the growing seasons of the two test years (P<0.0001). The Ca concentration in the second year growing season was lower. There was no significant interaction of year and month (P=0.3085). Yearwise analysis indicated no differences growing months in the first year (P=0.4176) as well as the second year (P=0.5354).

Magnesium (Mg)

There were no differences in soil Mg concentrations in different winter months (P=0.0730). However, significant differences were observed between winters of the two test (P<0.0001). The Mg concentration in the second year winter was lower. The month and year interaction was not significant (P=0.6262). Yearwise analysis indicated differences among months in the first year (P=0.0459) but no significant difference in the second year (P=0.1272).

There were no differences in soil Mg concentrations in different growing months (P=0.7420). However, significant differences were observed between growing seasons of

the two test years (P<0.0001). The Mg concentration in the second year growing season was lower. The month and year interaction was not significant (P=0.1883). Yearwise analysis indicated no differences among growing months in the first year (P=0.4461) as well as the second year (P=0.1173).

Iron (Fe)

There were no differences in soil Fe concentrations in different winter months (P=0.4278) and between winters of the two test years (P=0.8366). The month and year interaction was also not significant (P=0.7167). Yearwise analysis indicated no differences in soil Fe concentrations among winter months in the first year (P=0.6399) as well as the second year (P=0.2542).

There were differences in soil Fe concentrations in different winter months (P=0.0439). Similarly, there were differences of soil Fe concentrations between the growing seasons of the two test years (P=0.0085). However, the month and year interaction was not significant (P=0.0843). Yearwise analysis indicated no differences in soil Fe concentrations in the first year growing months (P=0.1132) but showed significant differences in the growing months of the second year (P=0.0279).

Zinc (Zn)

There were no differences in soil Zn concentrations in different winter months (P=0.6626) and between winters of the two test years (P=0.5005). The month and year interaction was also not significant (P=0.6653). Yearwise analysis indicated no differences among winter months in the first year (P=0.4392) as well as in the second year (P=0.9016).

There were differences in soil Zn concentrations in different growing months (P=0.0001) and between growing seasons of the two test years (P=0.0130). However, the month and year interaction was not significant (P=0.0679). Yearwise analysis indicated significant differences among growing months in the first year (P=0.0009) as well as the second year (P=0.0027).

Boron (B)

There were no differences in soil B concentrations in different winter months (P=0.3740). However, B concentrations were different between winters of the two test years (p<0.0001). The B concentration was lower in the second year. The month and year interaction was not significant (P=0.1558). Yearwise analysis indicated differences among winter months in the first year (P=0.0109) while no differences in the second year (P=0.9050).

There were no differences in soil B concentrations in different growing months (P=0.4870). However, B concentrations were different between growing seasons of the two test years (P<0.0001). The B concentration was lower in the second year. The month and year interaction was also significant (P=0.0493). Yearwise analysis indicated no differences among growing months in both the first year (P=0.2416) and the second year (P=0.0850).

Copper (Cu)

There were no differences in soil Cu concentration in different winter months (P=0.2902). However, Cu concentrations were different between the winters of the two test years (P=0.0073). The Cu concentration of soil was higher in the second year. The

month and year interaction was not significant (P=0.5580). Yearwise analysis indicated no differences among months in both the first year (P=0.6295) and the second year (P=0.0661).

There were no differences in soil Cu concentrations in different growing months (P=0.7420). However, Cu concentrations were different between the growing seasons of the two test years (P=0.0001). The Cu concentration of soil was higher in the second year. The month and year interaction was not significant (P=0.0800). Yearwise analysis indicated no differences among growing months in the first year (P=0.6136) but showed differences in the second year (p=0.0358).

Organic matter (OM)

There were differences in soil OM contents in different winter months (P=0.0297) and between winters of the two test years (P<0.0001). The OM content of soil was lower in the second year winter. The month and year interaction was not significant (P=0.1159). Yearwise analysis indicated differences among winter months in both the first year (P=0.0330) and the second year (P=0.0384).

There were no differences in soil OM content in different growing months (P=0.2573). However, the soil OM contents were different between growing seasons of the two test years (P<0.0001). The OM content of soil was lower in the second year growing season. The month and year interaction was not significant (P=0.5543). Yearwise analysis indicated no differences in soil OM contents among growing months in the first year (P=0.1740) as well as the second year (P=0.9405).

CHAPTER V

DISCUSSIONS AND CONCLUSIONS

Yield

Dry biomass yield of 'Kanlow' switchgrass responded differently to the different winter years. Yield variation in response to year was also observed in the study of Sanderson et al. (1999) on 'Alamo' switchgrass. According to Sanderson et al. (1998), yield was positively affected by growing season rainfall. However, in our study the yield was lower in the first year winter although rainfall was higher. The rainfall (in mm) for Stillwater Mesonet station has been shown in Table 4. In a study by Gunderson et al. (2008) at Oak Ridge National Laboratory, maximum potential yield of switchgrass was observed in the precipitation range of 300 mm to 700 mm although yield response to rainfall was highly variable and no strong correlation of rainfall and yield were observed. Growing season rainfall of 796 mm might have been in excess and caused yield decrease in the first year winter. The rainfall in the second year winter (433 mm) was in the favorable range of 300 mm to 700 mm. However, rainfall is one of many reasons affecting yield through soil moisture availability in switchgrass field and time, size, and critical water requirement periods during growing season have modifying role in yield response to rainfall (Gunderson et al., 2008).

Table 4. Rainfall in mm for Stillwater Mesonet station

Year	Annual total	Growing season total
30-Year Normal	933	338
2007	1440	796
2008	960	433

Source: Oklahoma Mesonet website
Note: Growing season includes months from April to September.

After senescence, there would be no further plant growth and any yield decrease can be expected to be related to effect of weathering process. A partial contribution in yield reduction can be from translocation of C and N reserve compound towards root portion (Parish and Wolf, 1993) and remaining contribution can be from leaf loss (Parish and Wolf, 1993). In our study, a decreasing trend of yield was observed in the first year winter while a constant yield was observed in the second year winter. The reduction of yield can be up to a maximum of 40.88 % as in the first year winter and while yield may not be affected in other winters. However, the clear-cut reasons remain unknown.

There is not much studies that involve winter season rainfall and yield response to it. In our study, no significant correlation was observed between yield decrease and the total monthly accumulated rainfall for both the first year winter and the second year winter.

Biomass elements

The concentration of total N was constant in the winter while decreasing in the growing season. In general, the concentrations of P, K, Mg, S, and Cu were decreasing in both the winter and growing seasons. These elements were highest in the beginning of the growing season and lowest near the end of the winter season. Constant biomass

concentrations of Ca, Zn, Mn, Ni, total C, ash, and ADL were observed for winter. The

concentration of Ca was decreasing in the first year growing season while constant in the

second year. The concentration of Zn was in general decreasing during the growing

season. The concentration of Na was decreasing in the first year winter while constant in

the second year. The concentration of Fe was constant in the first year winter while

different in the second year. The growing season Na and Fe concentrations were varied

for both years. The concentrations of Mn and Ni were different in the growing season.

The total C was increasing in the growing season. The concentration of ash was

decreasing in the first year growing season while constant in the second year. ADF and

NDF were increasing in the growing season. In general, ADF and NDF were increasing

in the first year winter while constant in the second year. The ADL content was

increasing throughout the growing season. The growth of biomass might have dilution

effect in percentage composition for many of the elements that decreased during growing

season.

Soil properties

Soil pH was constant for both winter and growing seasons. In winter, NO_3-N, soil

tested P, K, SO_4, Fe, Zn and Cu were constant among different months. Ca, Mg and B in

winter were constant in the second year winter while different in the first year. OM

contents in the winter were different among months. In growing season, NO_3-N was

observed increasing after August up to October. P and K contents were constant in the

first year growing season while varied in the second year. Ca, Mg, B, and OM contents

were constant in growing season. SO_4 contents were varied during growing season. Soil

Fe contents were constant in the first year growing season while varied in the second

year. In general Zn concentration was decreasing during growing season. The Cu was constant in the first year growing season while varied in the second year.

From the observation on biomass chemicals and soil properties, we observed that biomass quality was not affected by harvesting 'Kanlow' switchgrass from November to March as nutrient, ash, and cell wall components were not affected.

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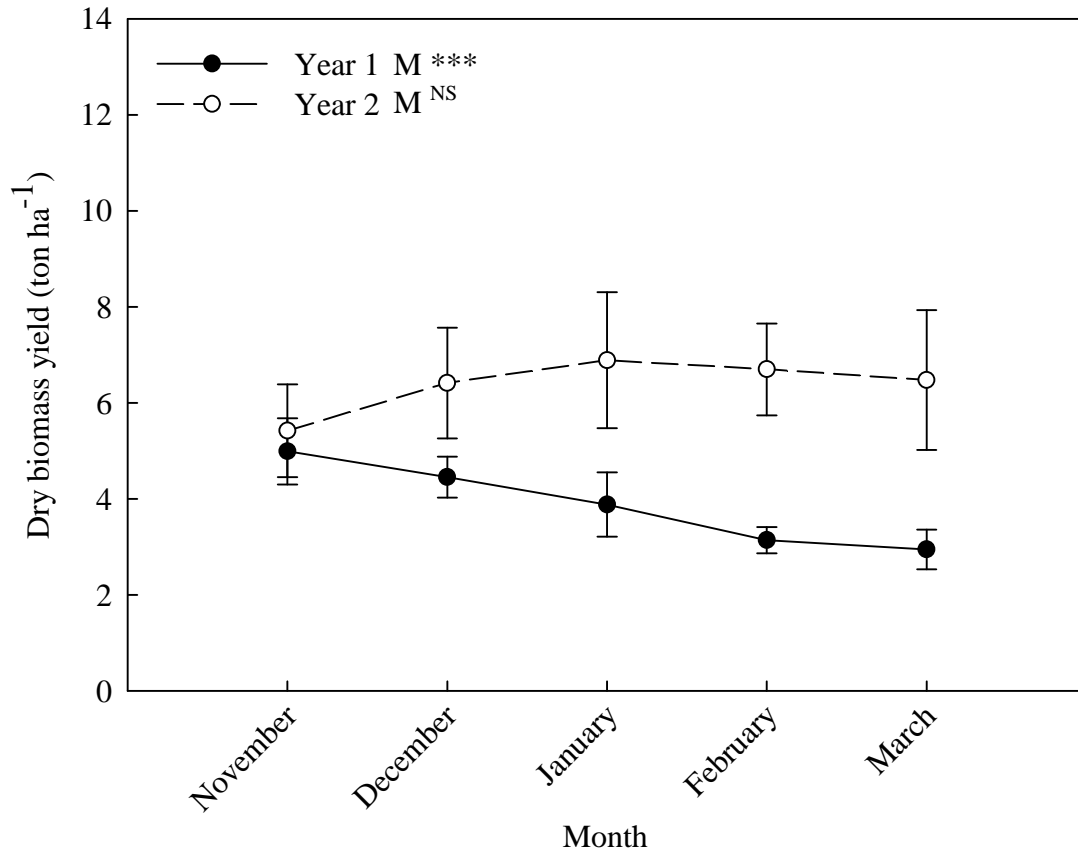
Fig. 1. Effect of winter harvest month on dry biomass yield of 'Kanlow' switchgrass. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.
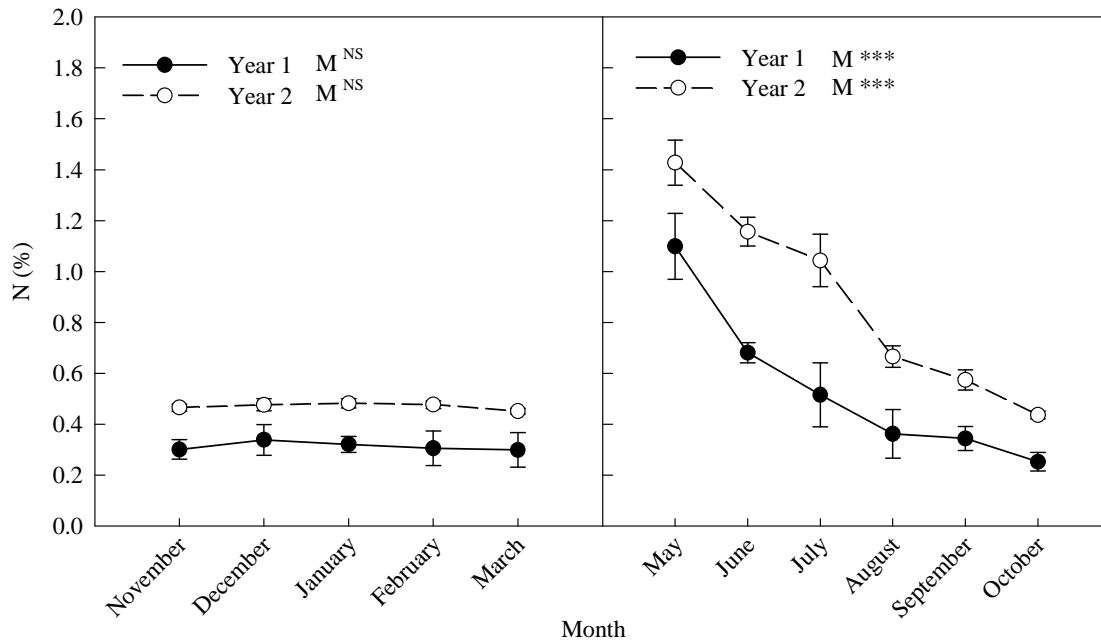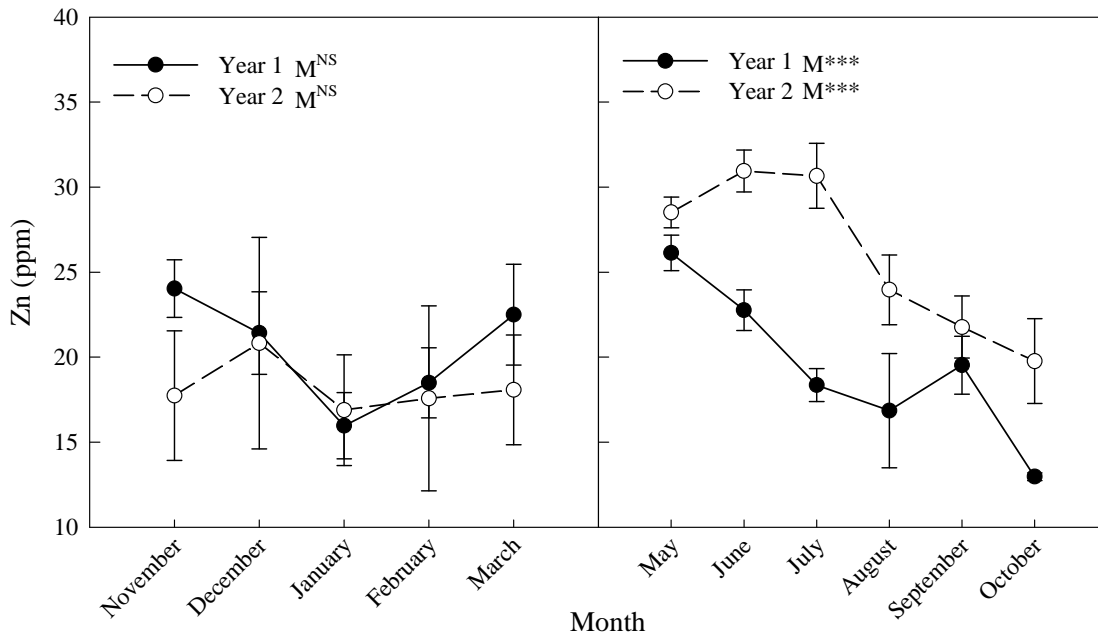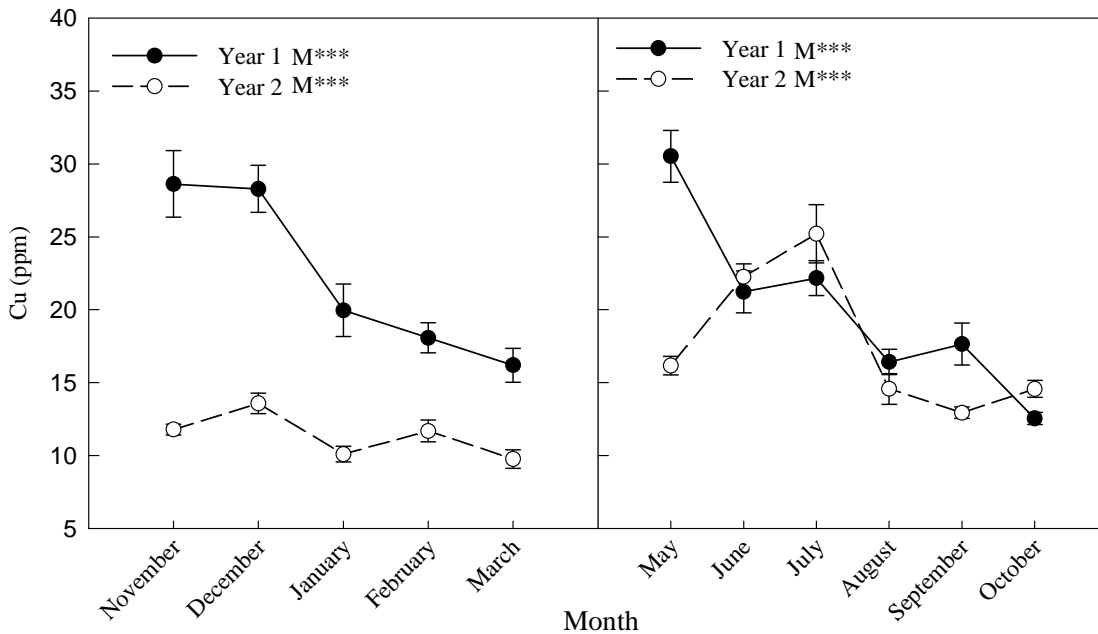
Fig. 2. Effect of sampling month on total N concentration of 'Kanlow' switchgrass. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.
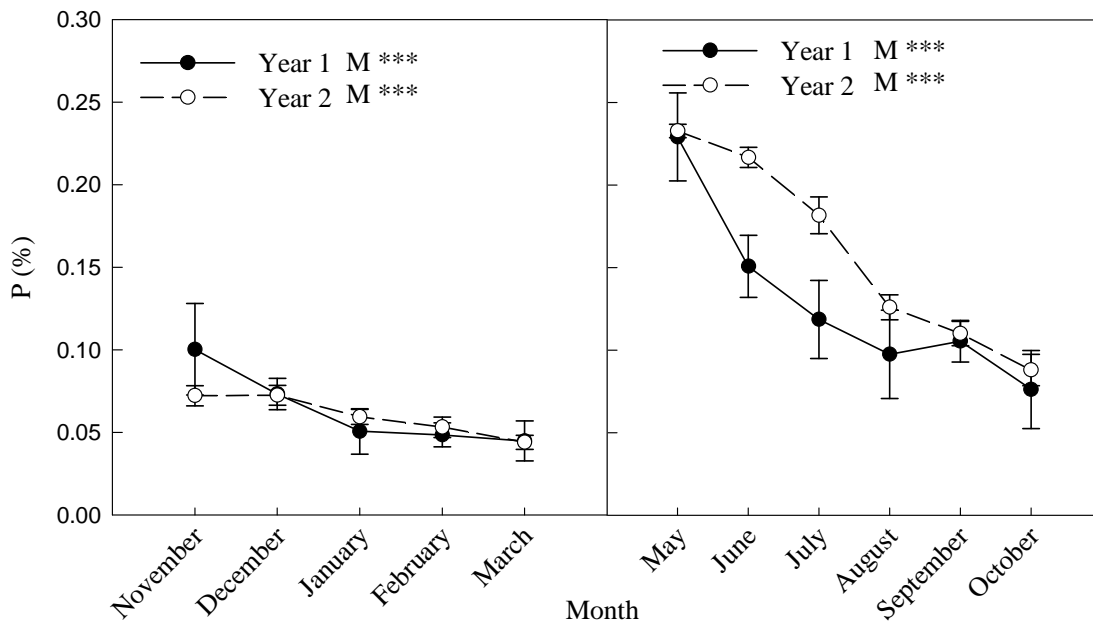
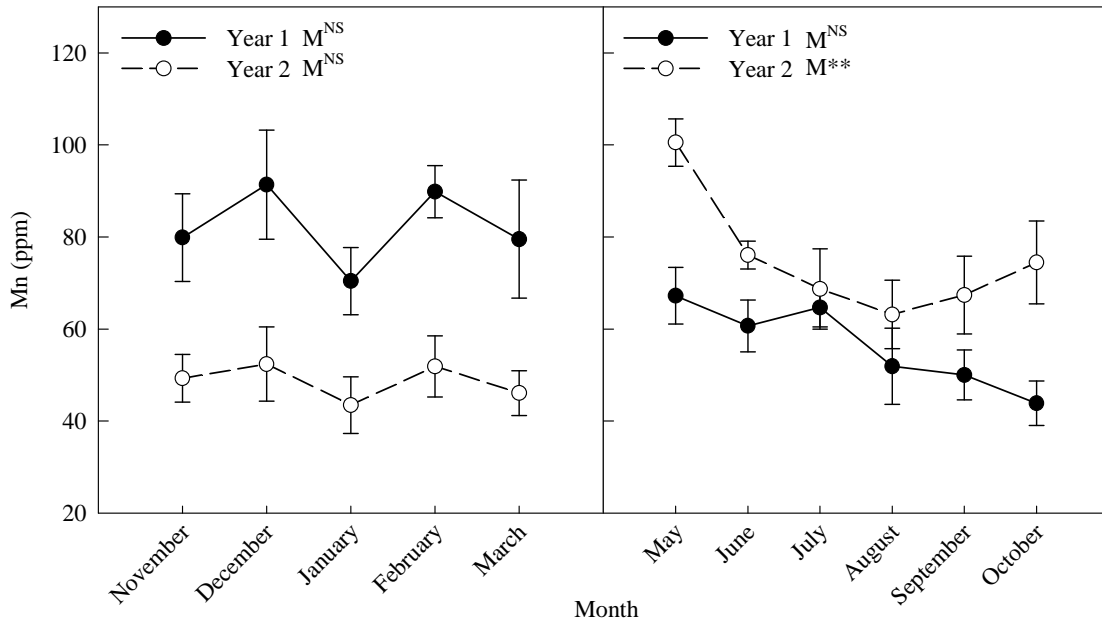Fig. 3. Effect of sampling month on P concentration of 'Kanlow' switchgrass. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.
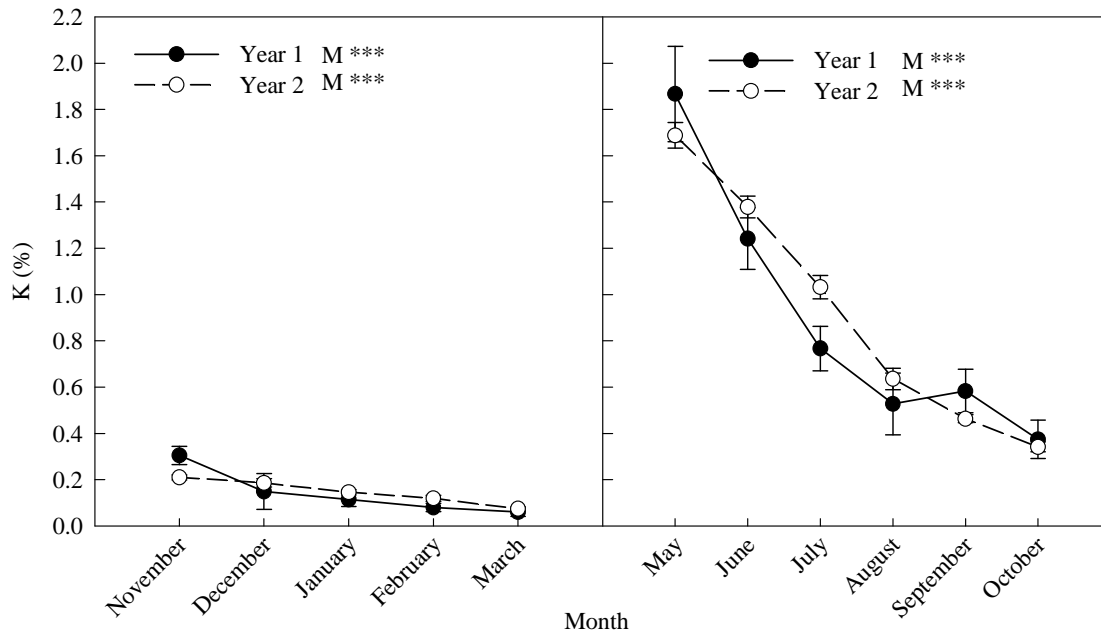
Fig. 4. Effect of sampling month on K concentration of 'Kanlow' switchgrass. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.
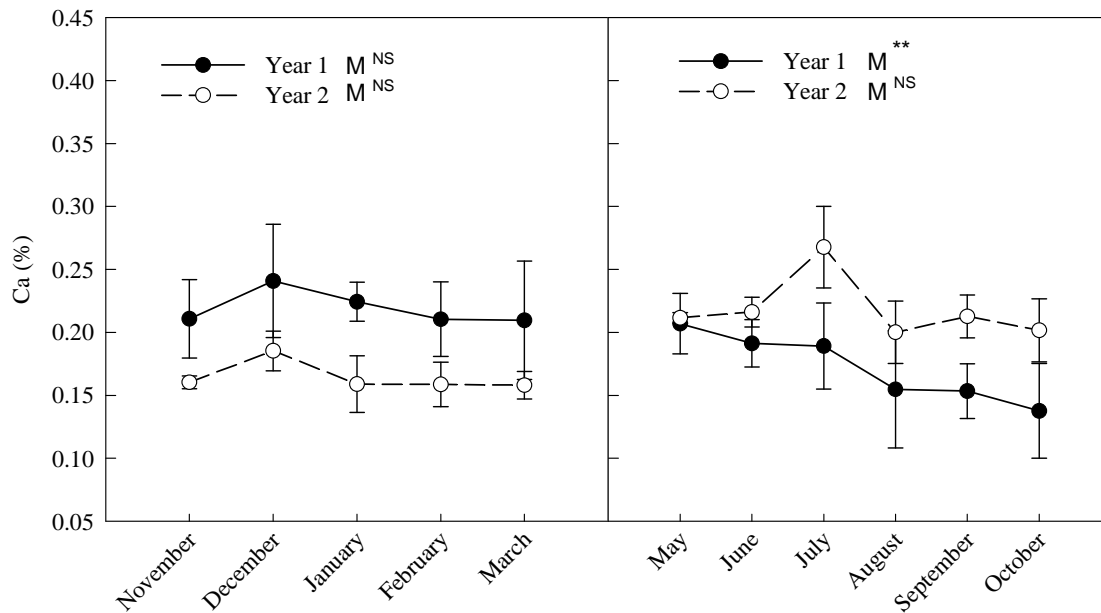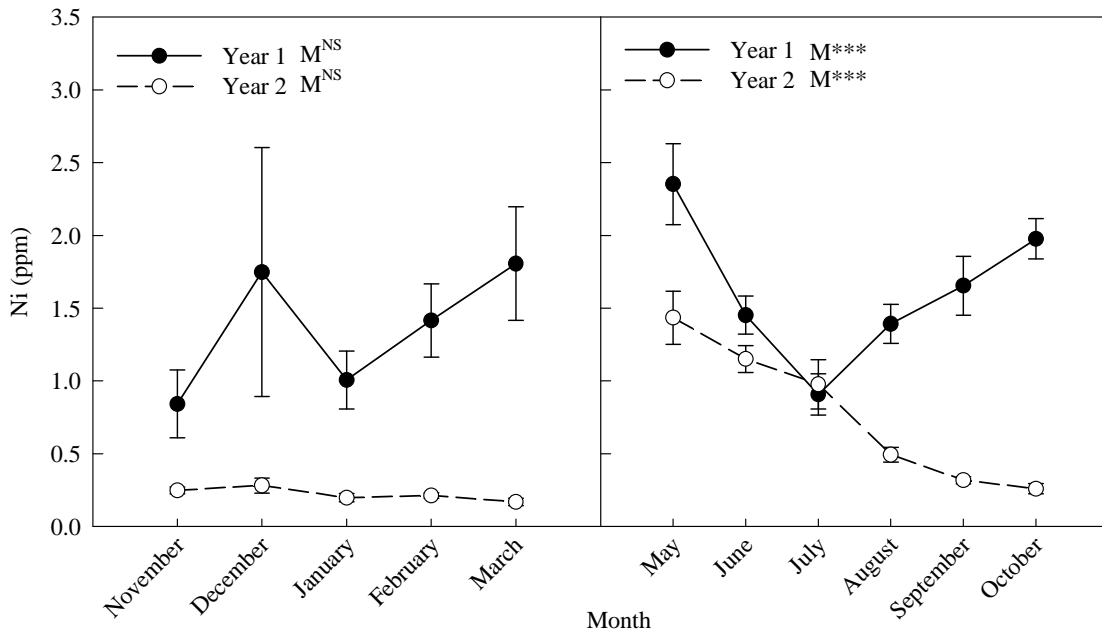

Fig. 5. Effect of sampling month on Ca concentration of 'Kanlow' switchgrass. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.
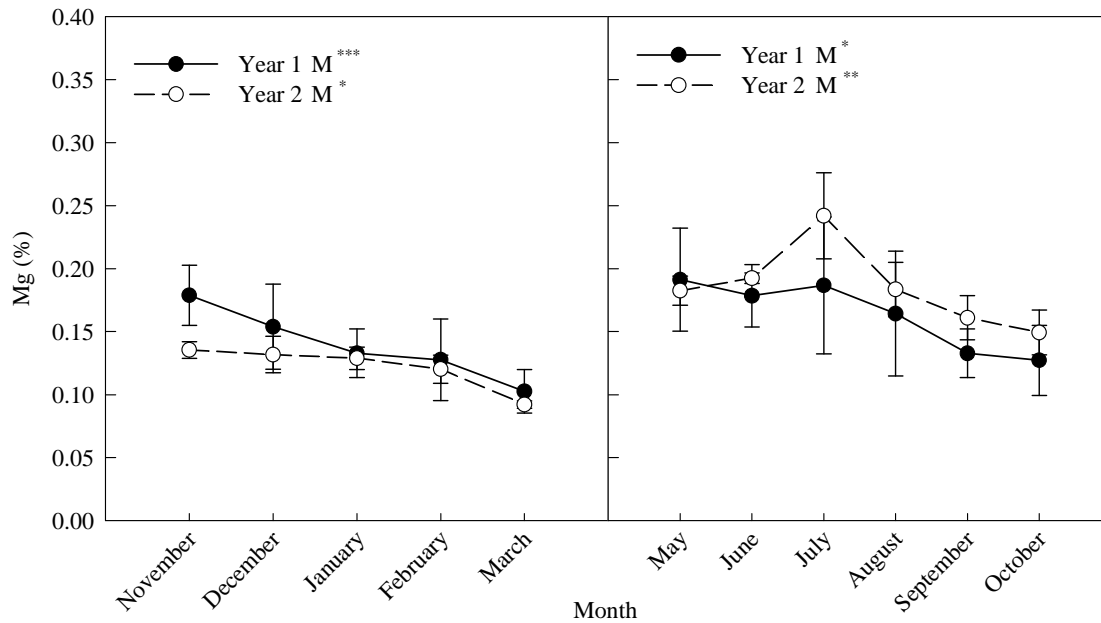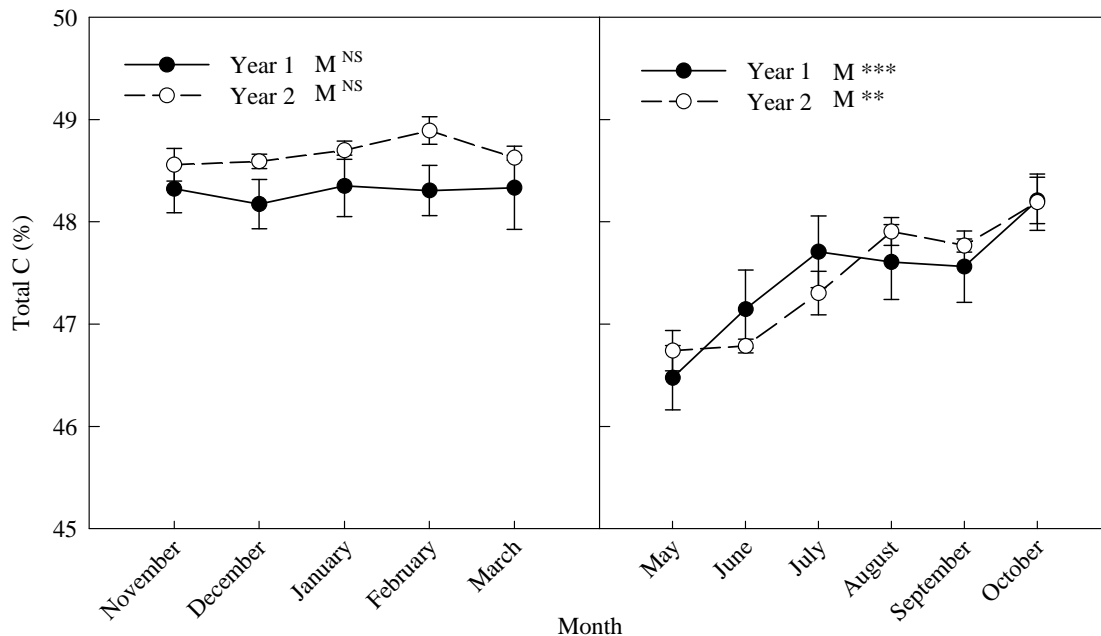
Fig. 6. Effect of sampling month on Mg concentration of 'Kanlow' switchgrass. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.
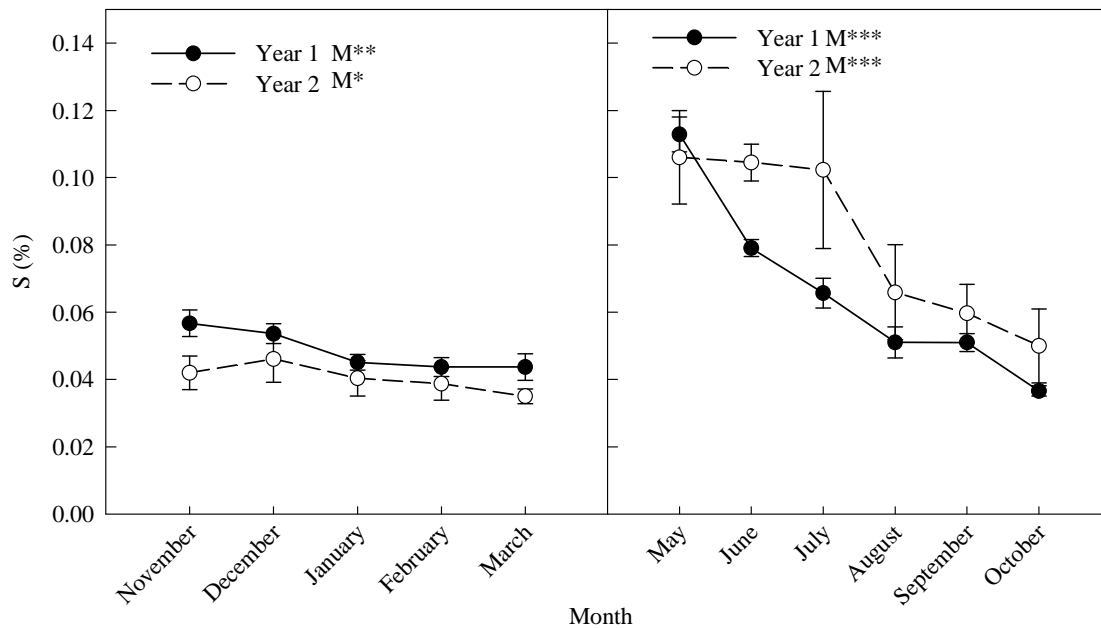
Fig. 7. Effect of sampling month on S concentration of 'Kanlow' switchgrass. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.
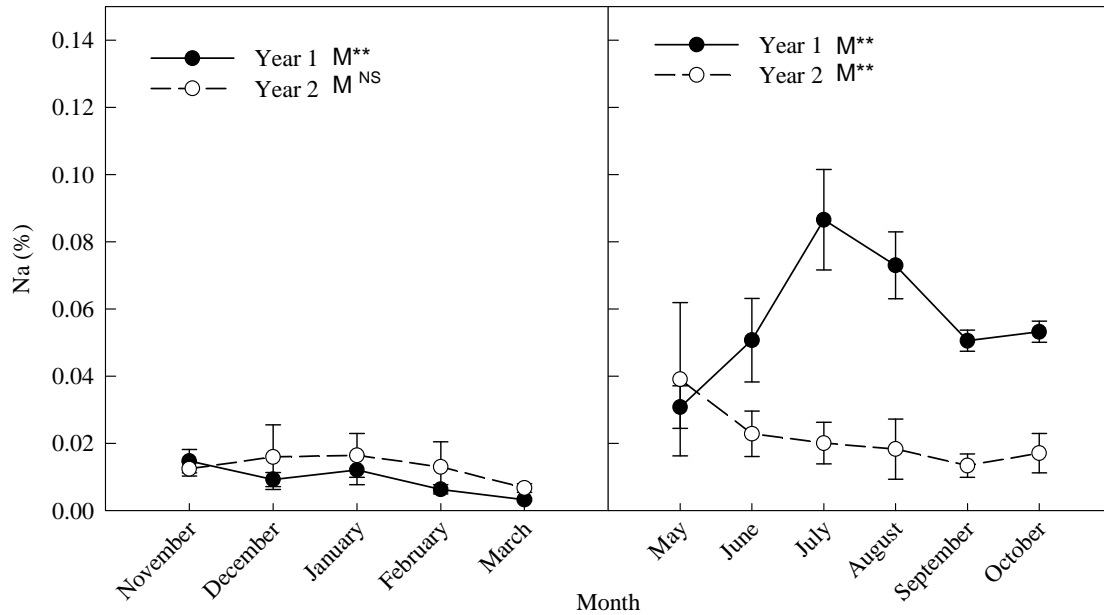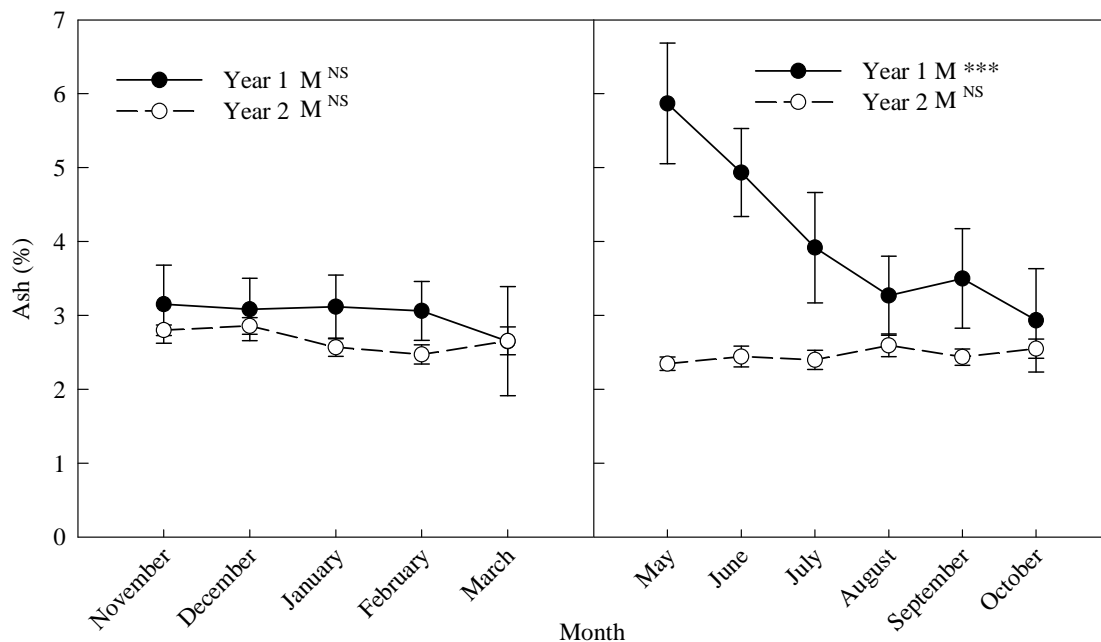
Fig. 8. Effect of sampling month on Na concentration of 'Kanlow' switchgrass. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.
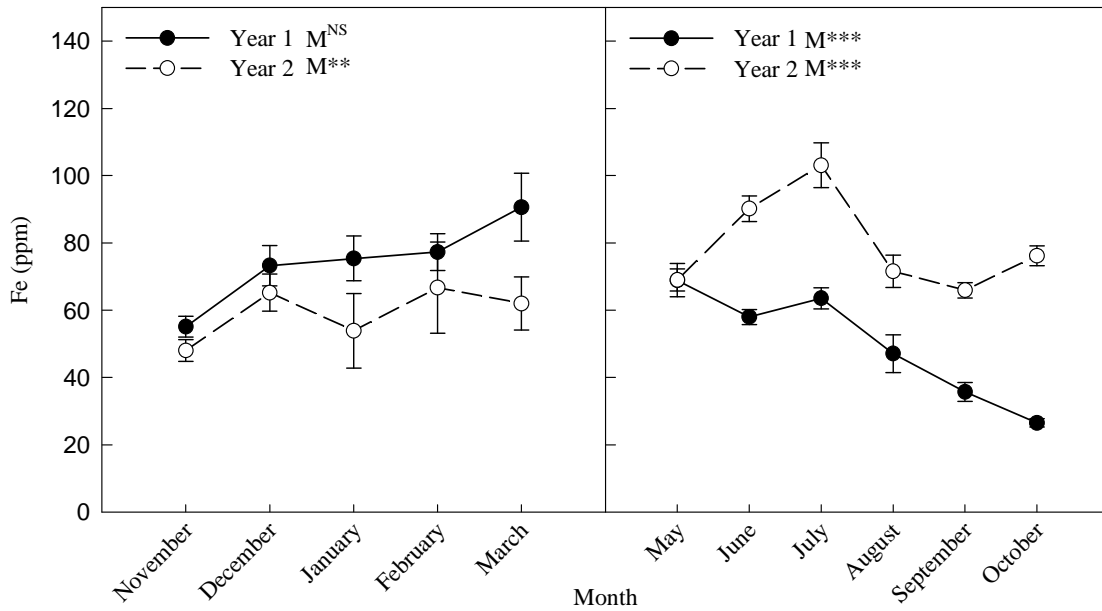
Fig. 9. Effect of sampling month on Fe concentration of 'Kanlow' switchgrass. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.
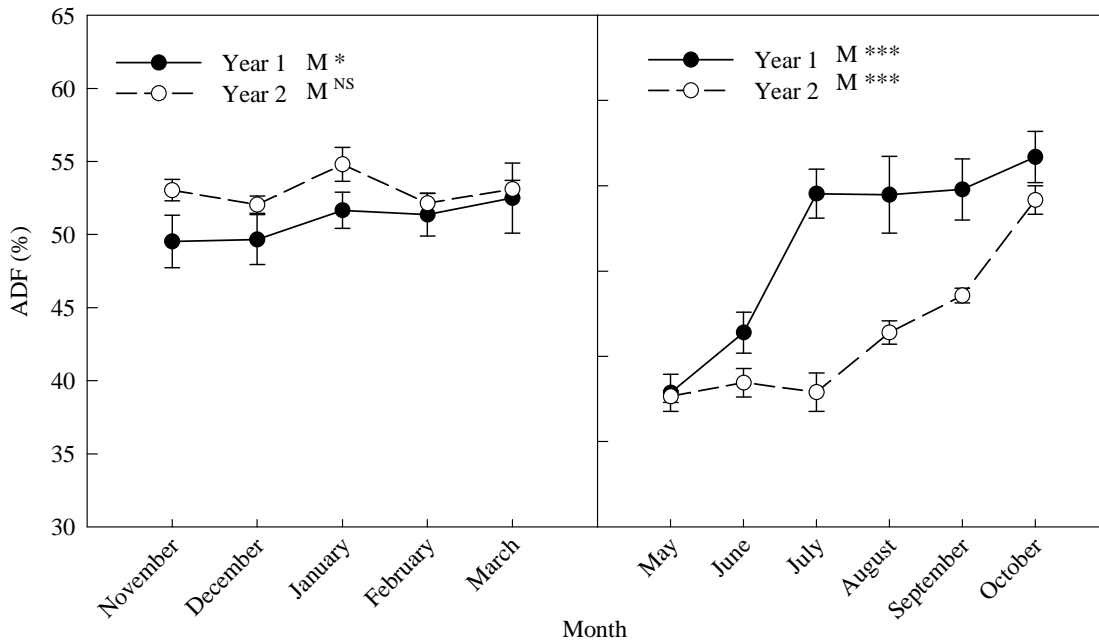
Fig. 10. Effect of sampling month on Zn concentration of 'Kanlow' switchgrass. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

Fig. 11. Effect of sampling month on Cu concentration of 'Kanlow' switchgrass. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.
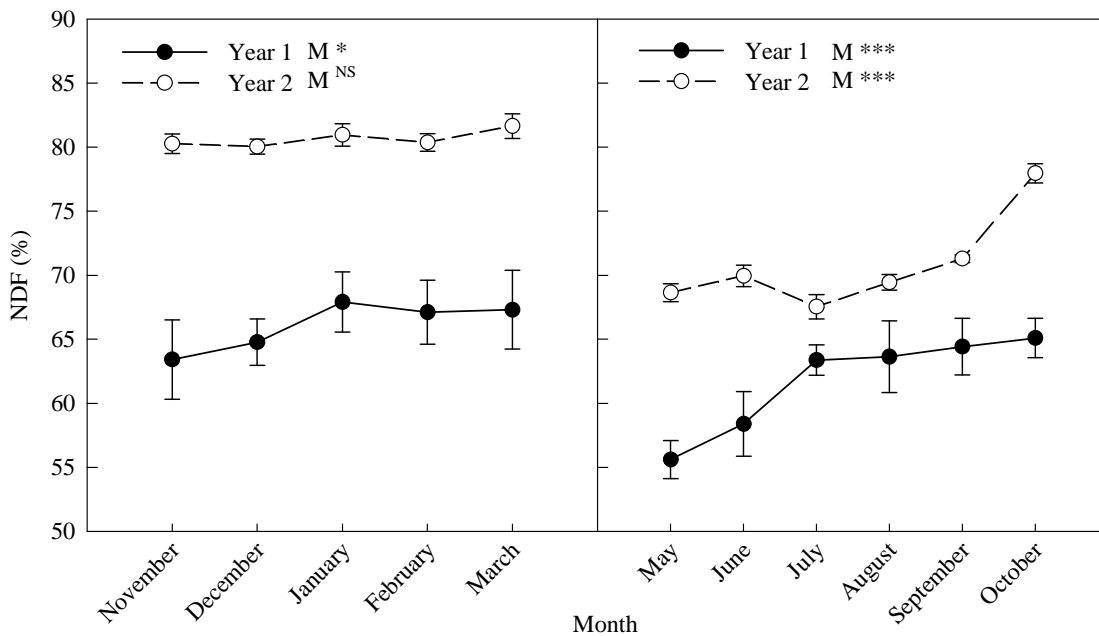
Fig. 12. Effect of sampling month on Mn concentration of 'Kanlow' switchgrass. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.
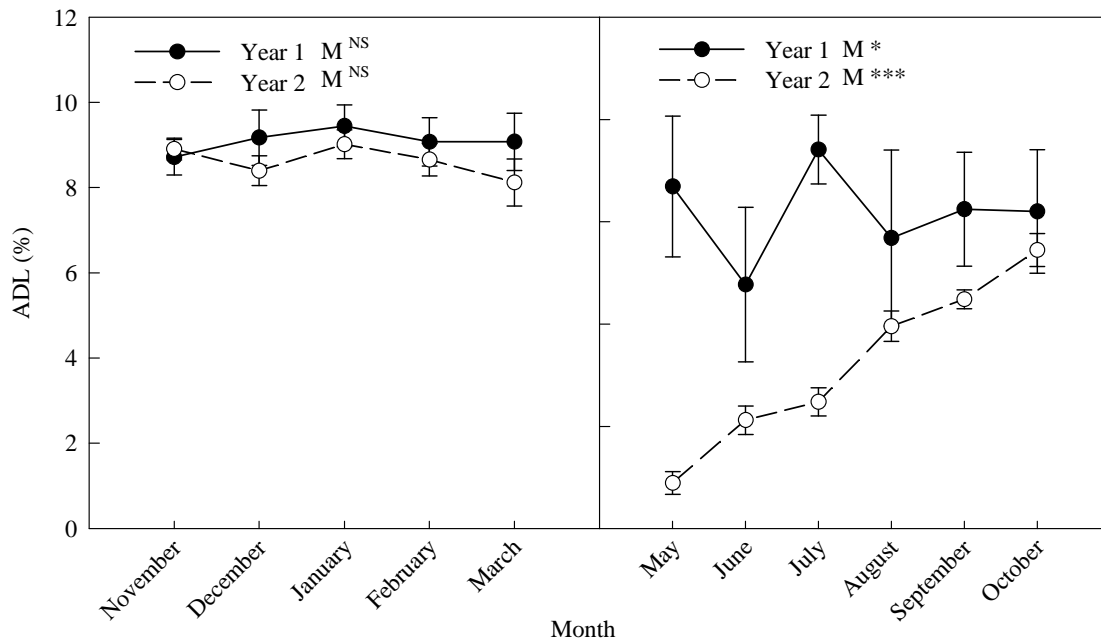

Fig. 13. Effect of sampling month on Ni concentration of 'Kanlow' switchgrass. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

Fig. 14. Effect of sampling month on total C concentration of 'Kanlow' switchgrass. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

Fig. 15. Effect of sampling month on ash concentration of 'Kanlow' switchgrass. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

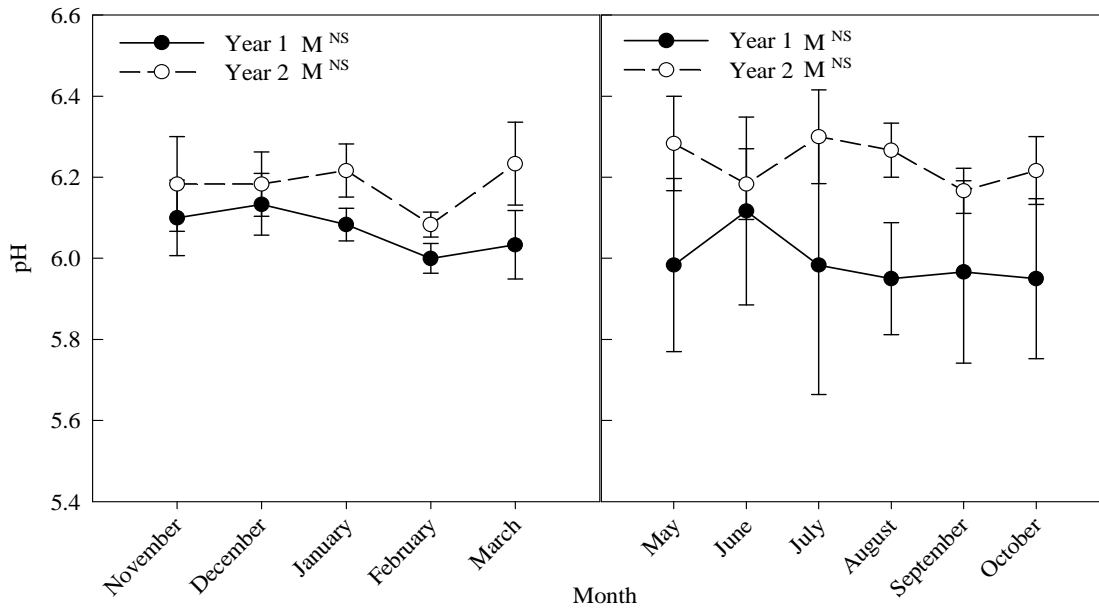Fig. 16. Effect of sampling month on ADF concentration of 'Kanlow' switchgrass. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.
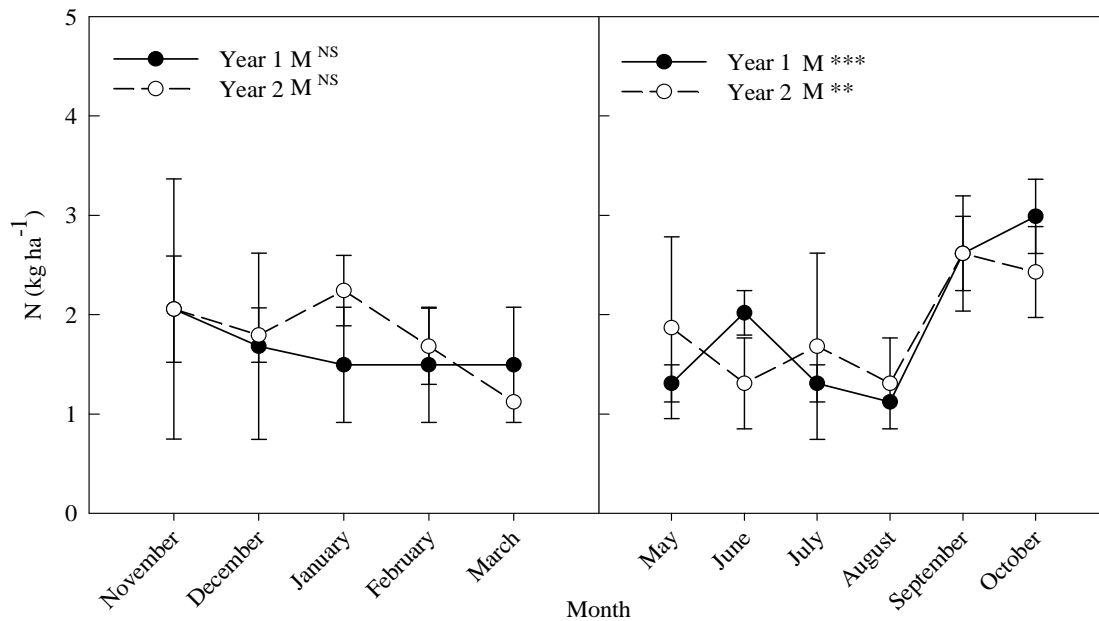

Fig. 17. Effect of sampling month on NDF concentration of 'Kanlow' switchgrass. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

Fig. 18. Effect of sampling month on ADL concentration of 'Kanlow' switchgrass. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.
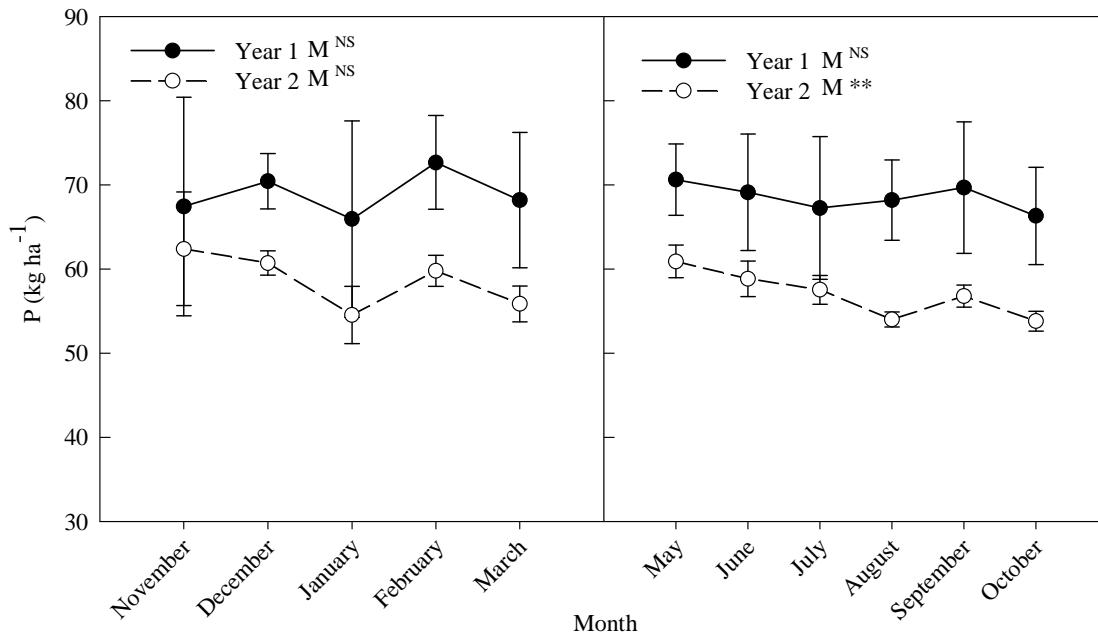
Fig. 19. Effect of sampling month on soil pH in 'Kanlow' switchgrass field. Vertical bars represent standard errors of mean. Significance level of month (M) is shown.
NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

Fig. 20. Effect of sampling month on soil NO_3-N content in 'Kanlow' switchgrass field. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

Fig. 21. Effect of sampling month on soil tested P content of 'Kanlow' switchgrass field. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.
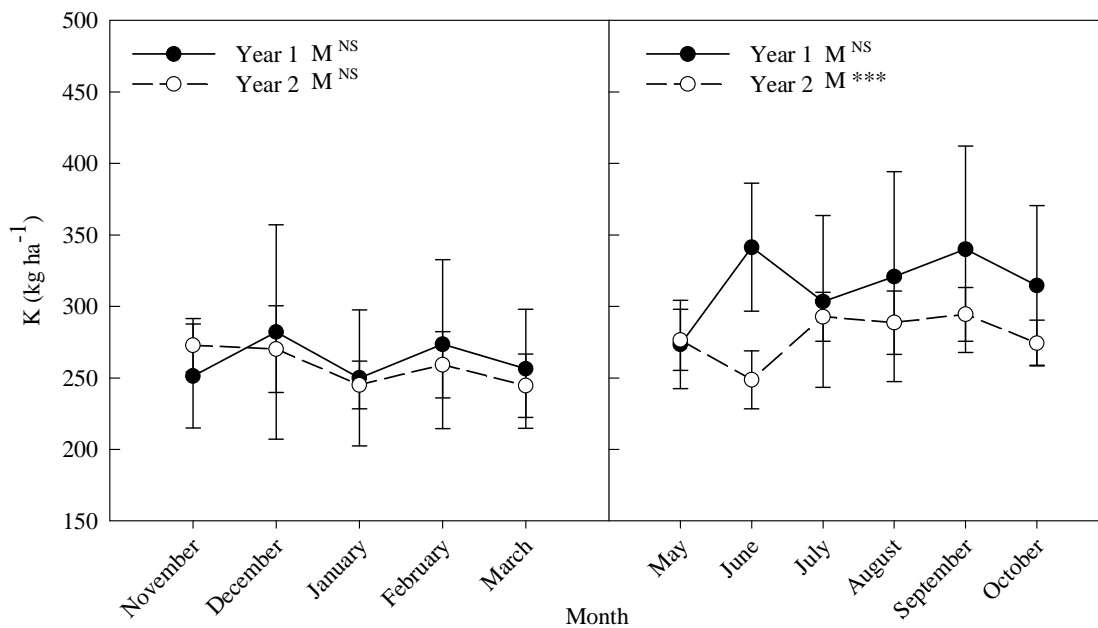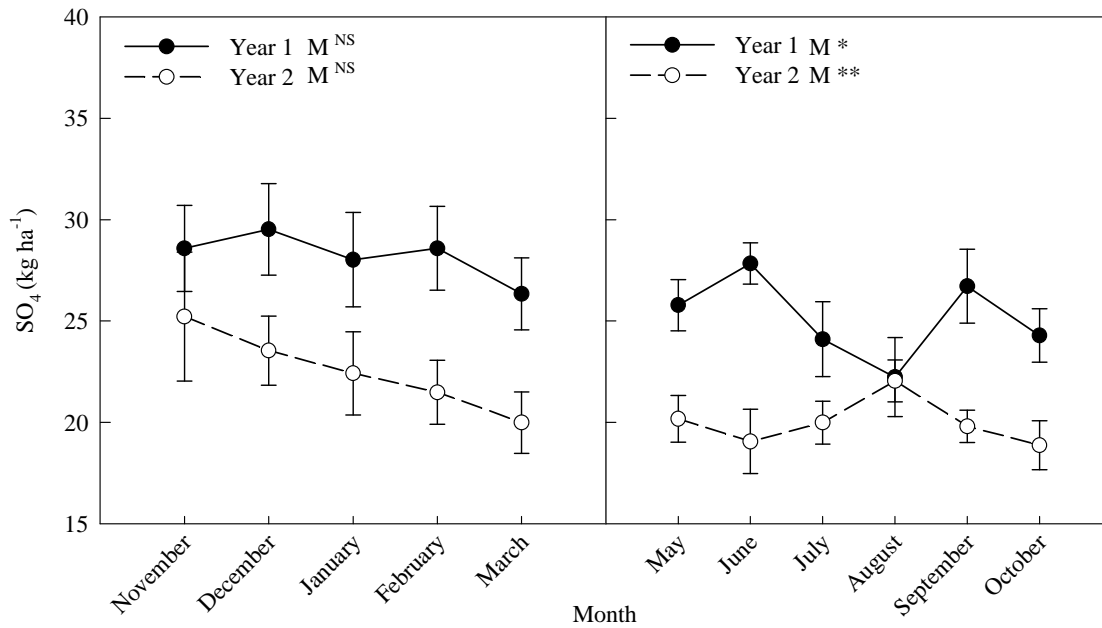

Fig. 22. Effect of sampling month on soil K content of 'Kanlow' switchgrass field. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

Fig. 23. Effect of sampling month on soil SO_4 content of 'Kanlow' switchgrass field. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.
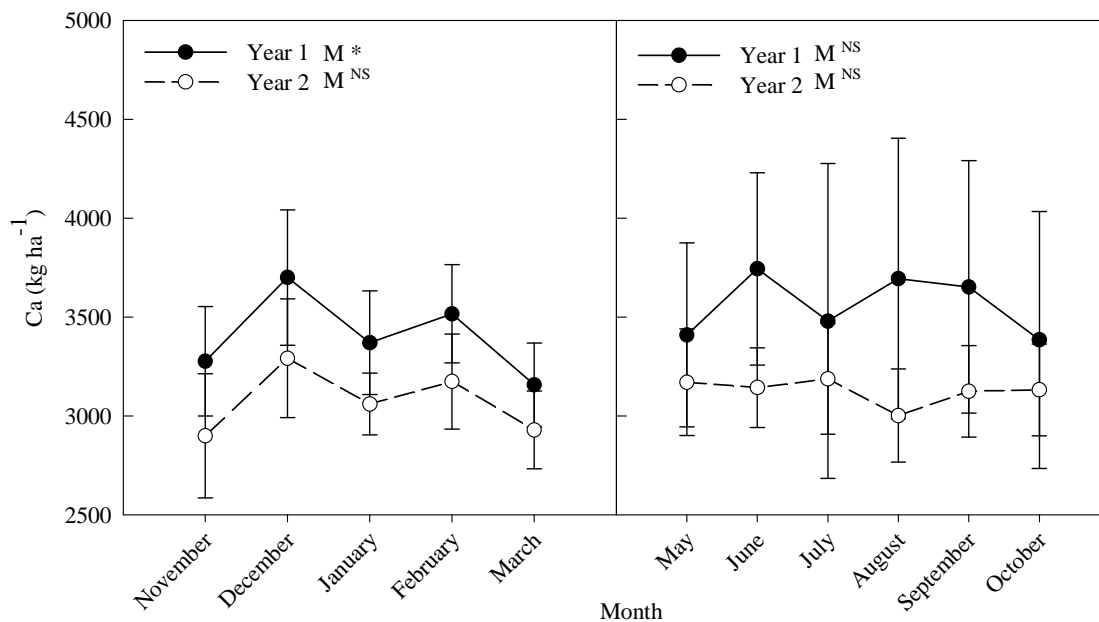

Fig. 24. Effect of sampling month on soil Ca content of 'Kanlow' switchgrass field. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.
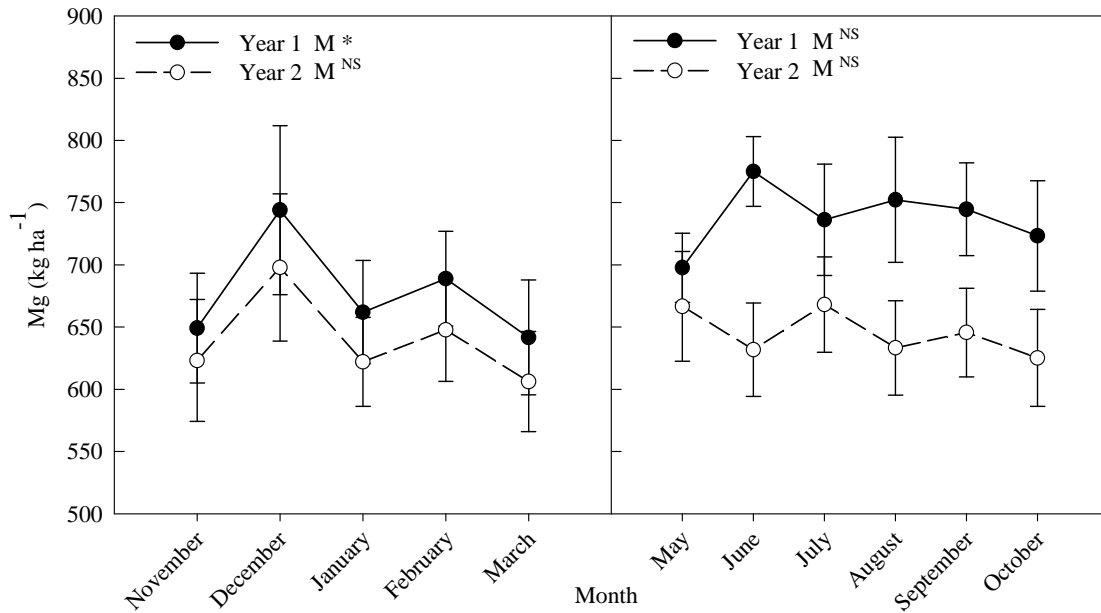
Fig. 25. Effect of sampling month on soil Mg content of 'Kanlow' switchgrass field. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.
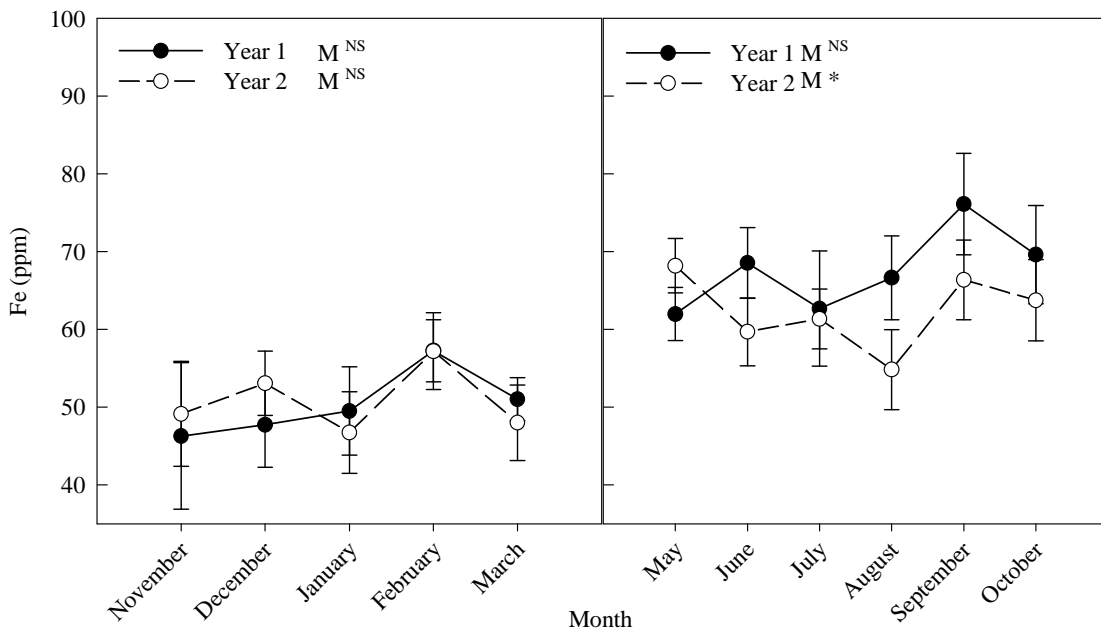

Fig. 26. Effect of sampling month on soil Fe content of 'Kanlow' switchgrass field. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

Fig. 27. Effect of sampling month on soil Zn content of 'Kanlow' switchgrass field. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.
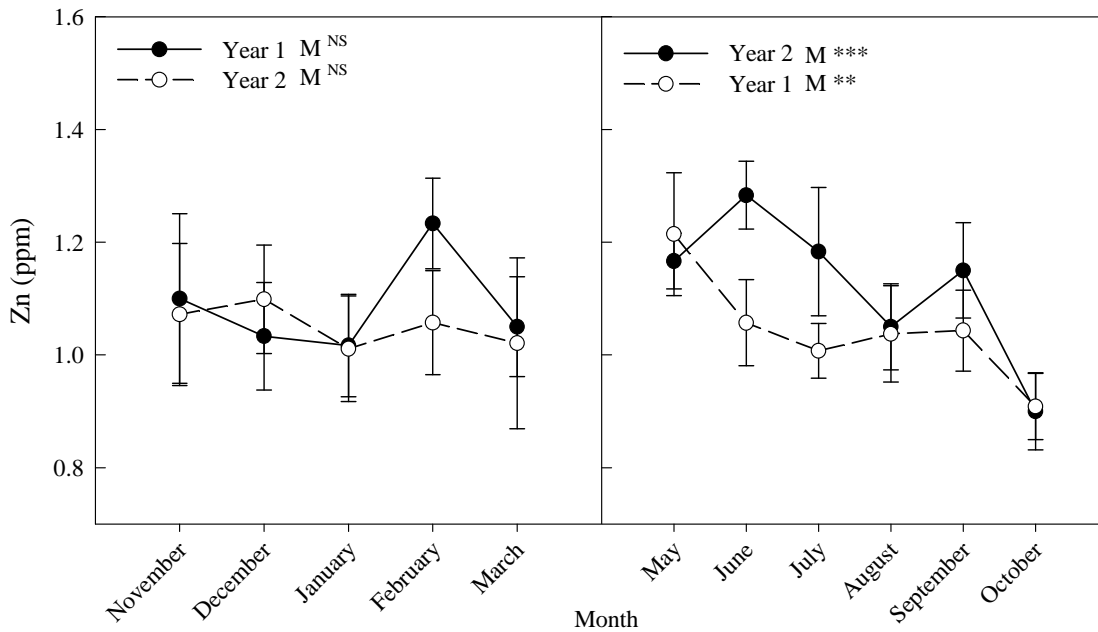

Fig. 28. Effect of sampling month on soil B content of 'Kanlow' switchgrass field. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

Fig. 29. Effect of sampling month on soil Cu content of 'Kanlow' switchgrass field. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.
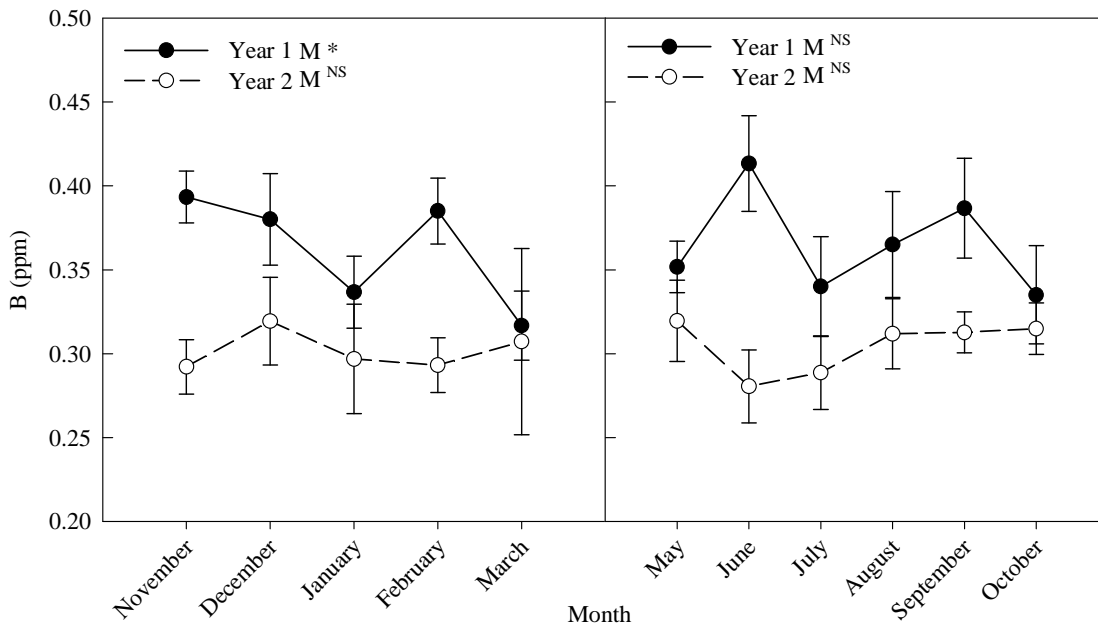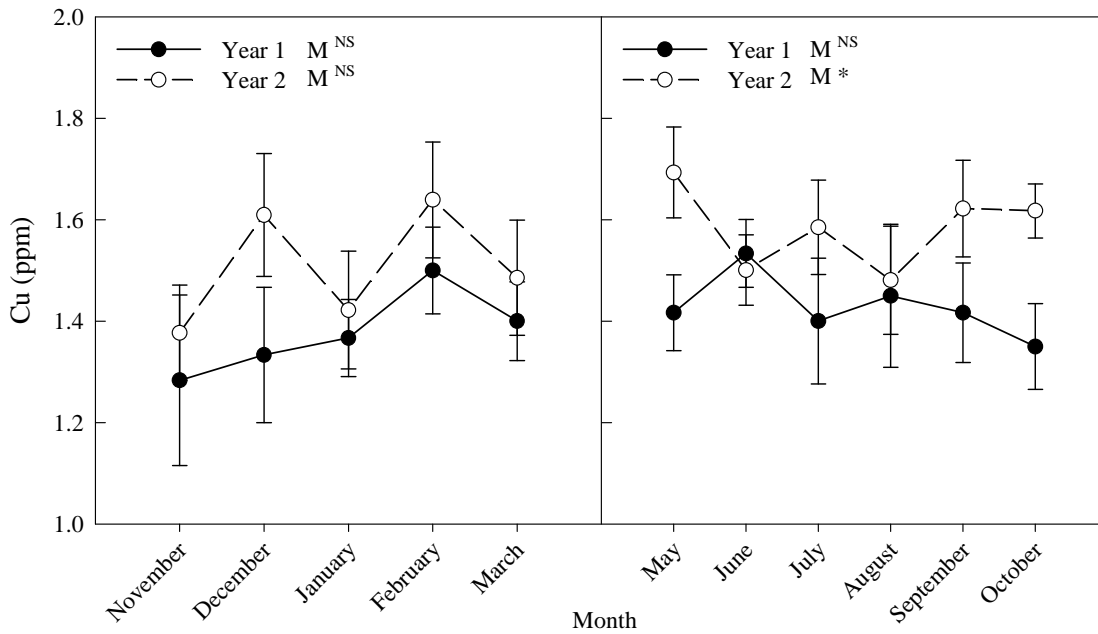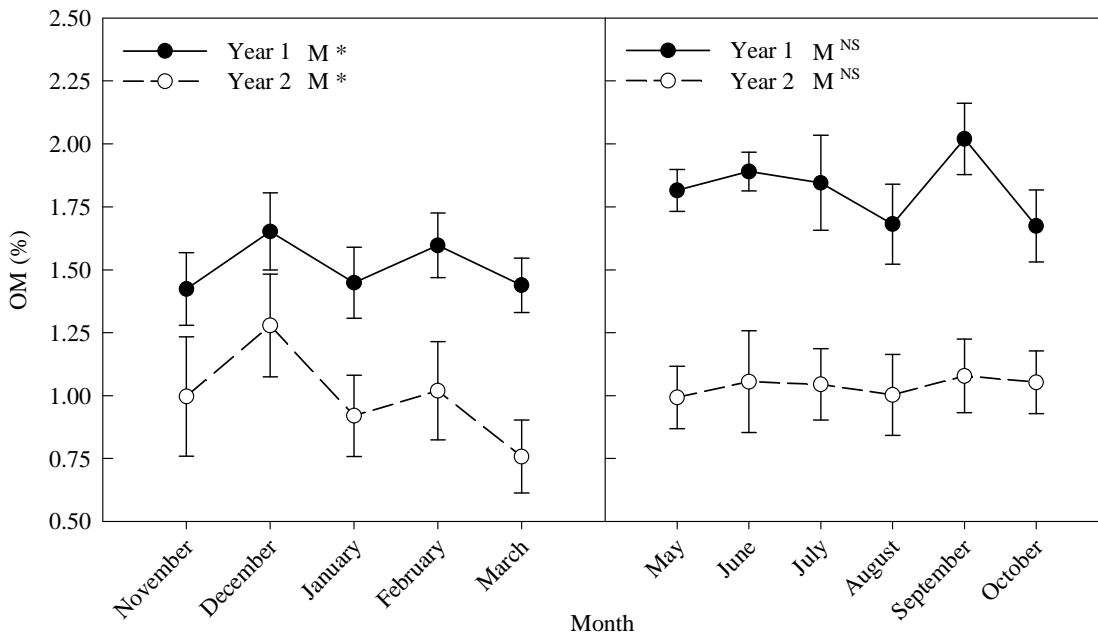

Fig. 30. Effect of sampling month on soil OM content of 'Kanlow' switchgrass field. Vertical bars represent standard errors of mean. Significance level of month (M) is shown. NS,*,**,*** Nonsignificant or significant at P≤0.05, 0.01, or 0.001, respectively.

N 36° 07.996 ft. W 097° 06.299 ft. 9ft. (+/-)   N 36° 07.996 ft. W 097° 06.204 ft. 11ft. (+/-)

N 36° 07.981 ft. W 097° 06.299 ft. 9 ft. (+/-)   N 36° 07.981 ft. W 097° 06.200 ft. 12ft. (+/-)

N 36° 07.966 ft. W 097° 06.298 ft. 9 ft. (+/-)   N 36° 07.965 ft. W 097° 06.201 ft. 11 ft. (+/-)

N 36° 07.951 ft. W 097° 06.297 ft. 8ft. (+/-)   N 36° 07.951 ft. W 097° 06.200 ft. 10 ft.(+/-)
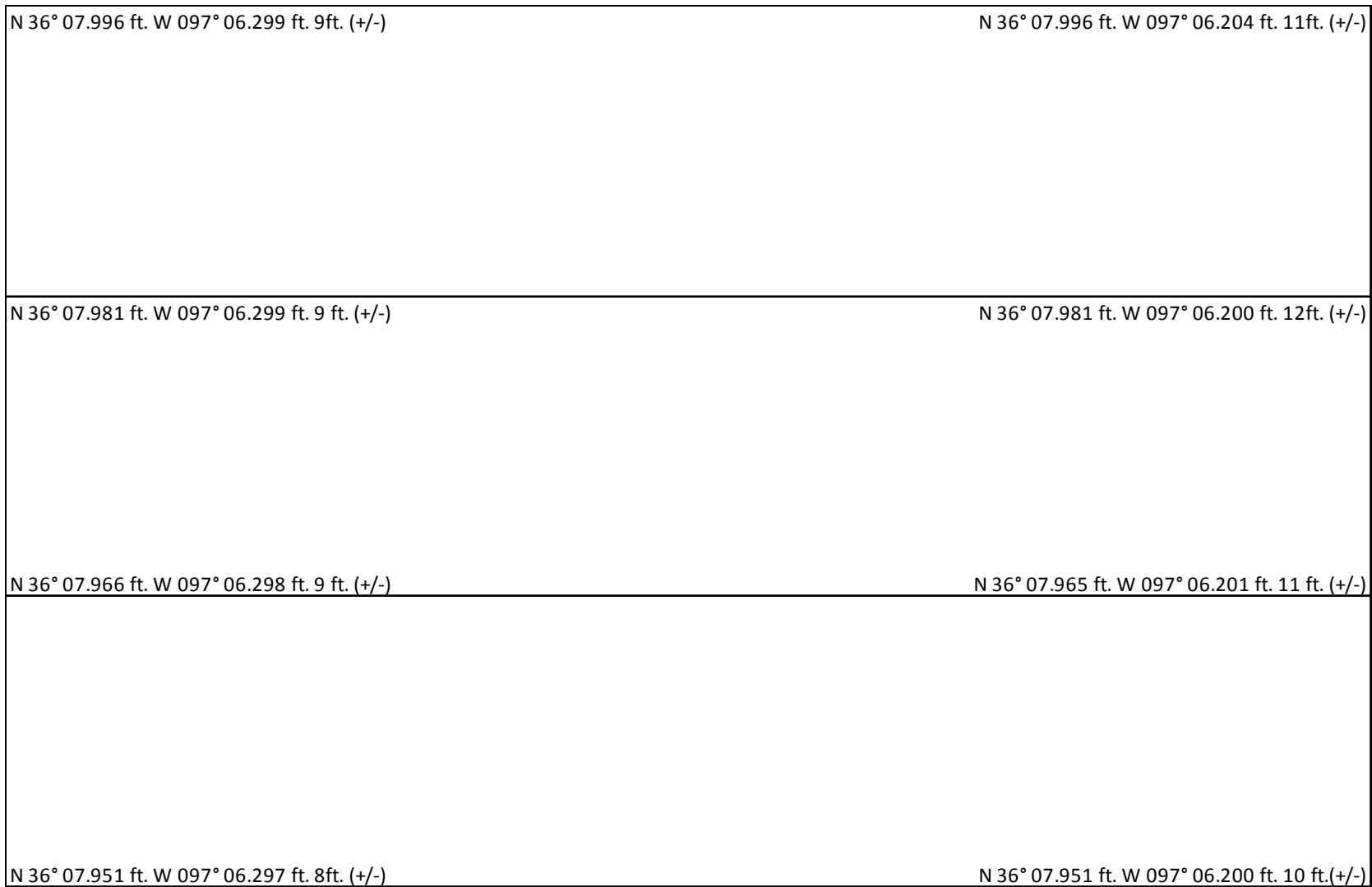
Fig. 31 Geographic coordinates of the experimental field.

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90'x11'	D-R1	90'x8'	J-R1	F-R1	J-R2	F-R2	J-R3	N-R3	M-R3	M-R4	N-R4	J-R5	D-R5	M-R5	D-R6	90'x8'	M-R6	90'x11'
90'x11'	90'x24'	90'x8'	90'x24'	90'x8'												90'x8'	90'x24'	90'x8'	90'x24'	90'x11'
90'x11'	N-R1	90'x8'	M-R1	D-R2	M-R2	N-R2	F-R3	D-R3	D-R4	F-R4	J-R4	F-R5	N-R5	F-R6	J-R6	90'x8'	N-R6	90'x11'

1x11'+15x24'+14x8'+1x11'=494'

Fig. 32. Field layout map of the experimental field.

N-November   Plot size: 90'x24'
D-December   Rep: 6
J-January   Design: Split plot randomized complete block design
F-February   Alley: 8'
M-March

N

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VITA

Shiva Om Makaju

Candidate for the Degree of

Master of Science

Thesis: WINTER BIOMASS YIELD, YEAR-ROUND ELEMENTAL CONCENTRATIONS OF 'KANLOW' SWITCHGRASS, AND ASSOCIATED SOIL NUTRIENTS IN A ZERO INPUT ENVIRONMENT

Major Field: Plant and Soil Sciences

Biographical:

Education:

Completed the requirements for the Master of Science in Plant and Soil Sciences at Oklahoma State University, Stillwater, Oklahoma in May, 2010.

Completed the requirements for the Bachelor of Science in Agriculture at Tribhuvan University, Chitwan, Nepal in 2003.

Experience: Technical Officer, Nepal Agricultural Research Council (NARC), Nepal, January 2004 to December 2006.

Professional Memberships: American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, April 2009 to present.

Name: Shiva Om Makaju   Date of Degree: May, 2010

Institution: Oklahoma State University   Location: Stillwater, Oklahoma

Title of Study: WINTER BIOMASS YIELD, YEAR-ROUND ELEMENTAL
                        CONCENTRATIONS OF 'KANLOW' SWITCHGRASS, AND
                        ASSOCIATED SOIL NUTRIENTS IN A ZERO INPUT
                        ENVIRONMENT

Pages in Study: 86   Candidate for the Degree of Master of Science

Major Field: Plant and Soil Sciences

Scope and Method of Study: Switchgrass (*Panicum virgatum* L.) is a warm-season
        perennial species native to North America. It was selected by the U.S. Department
        of Energy (DOE) as the model herbaceous species for the development as a
        cellulosic feedstock crop for biofuels production. Maximum biomass yields in
        switchgrass can be harvested with one-cut system by mid-September. However,
        information is limited on winter biomass yield, elemental composition of standing
        cured biomass, and associated soil nutrient status. Therefore, the objectives of this
        study were to evaluate changes in winter biomass yield, year-round elemental
        composition of Kanlow switchgrass, and associated year-round soil nutrient
        dynamics in a zero input environment. An unfertilized Kanlow switchgrass
        planting established in 1998 was used in the study. The experimental design was a
        split plot randomized complete block design with 6 replications. The experimental
        treatment was monthly harvest from November to March in winter and year-
        round monthly sampling of biomass and soil for chemical analyses. The variable
        year was sub-plot within the main plot month. Each replication was on a large
        plot of 200 m^2.

Findings and Conclusions: The 2-yr mean dry matter yield of winter harvests was 5.13
        t/ha, ranging from 3.88 t/ha in 2007-2008 to 6.38 t/ha in 2008-2009. Biomass
        yield decreased as winter progressed, statistically significant in first winter but not
        in the second winter. Concentrations of biomass elements and soil nutrients
        changed with various degrees over the two years. Biomass quality was not
        affected by harvesting 'Kanlow' switchgrass from November to March as
        nutrient, ash, and cell wall components were not affected.

ADVISER'S APPROVAL: Dr. Yanqi Wu