PLANT COMMUNITY EFFECTS ON ATRAZINE IN GRASSED MESOCOSMS

Kansas Biological Survey Report No. 175

February 2013

by

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Submitted to the Department of Civil, Environmental and Architectural Engineering in partial fulfillment of the requirements for the degree of

Master of Science in Environmental Science

University of Kansas

2012

CE 890 Special Problem Report

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Abstract

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Grassed strips have become a commonly used and inexpensive method for reducing the transport of pesticides from agricultural areas to surface waters. These strips are likely to vary in their capability to reduce non-point pollution depending upon a number of factors including plant community, soil-water interactions, nutrient cycling and rhizobial populations. The objective of this project was to determine if plant community could be related quantitatively to pesticide movement and degradation.

An experiment was conducted to examine the fate of atrazine in two different plant ecosystems commonly used in the Midwest. These plant ecosystems, brome monoculture and a native grass mix, were compared with bare controls in mesocosms to determine the direct and indirect effects of the plant communities on atrazine degradation and mobility in soil. Atrazine was affected to a small but significant degree by cover type. Primarily, the concentration of atrazine and metabolites in all mesocosms was reduced through adsorption to soil particles. Vegetation affected atrazine through increased adsorption, abiotic hydrolysis or plant uptake rather than enhanced microbial degradation.

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INTRODUCTION

Atrazine is a selective herbicide applied primarily in spring for control of broadleaf and grassy weeds. It is also used for season-long weed control in crops and for selective and nonselective weed control for non-crop land. Atrazine has been the most heavily used herbicide in the United States with approximately 77 million pounds applied in 2007 (Thelin *et al.*, 2010). In 2005, two crops, corn and grain sorghum (milo), accounted for over 90% of the atrazine applied (Heri *et al.*, 2008).

The half–life of atrazine has been reported in the literature to be anywhere from 14 days to several months (Koskinen *et al.*, 2008) Atrazine can move into streams or reservoirs either in solution or by adsorbing to eroded soil sediments. Leaching into groundwater may also occur, depending upon soil type, with coarser soils tending to leach and finer soils allowing increased runoff of dissolved and adsorbed atrazine (Regehr *et al.,* 1992). The main risk of atrazine runoff occurs when heavy rainfall follows application of the herbicide (Regehr *et al.*, 1992).

One effective tool for coping with this type of diffuse agricultural pollution is the maintenance of vegetated filter strips – vegetated strips of land between surface waters and areas contributing pollutants from runoff (Fig. 1). A vegetated filter strip (VFS) allows runoff and associated pollutants to be attenuated before reaching surface waters through various mechanisms including filtration and deposition, infiltration, adsorption, chemical or biological decay and plant uptake.

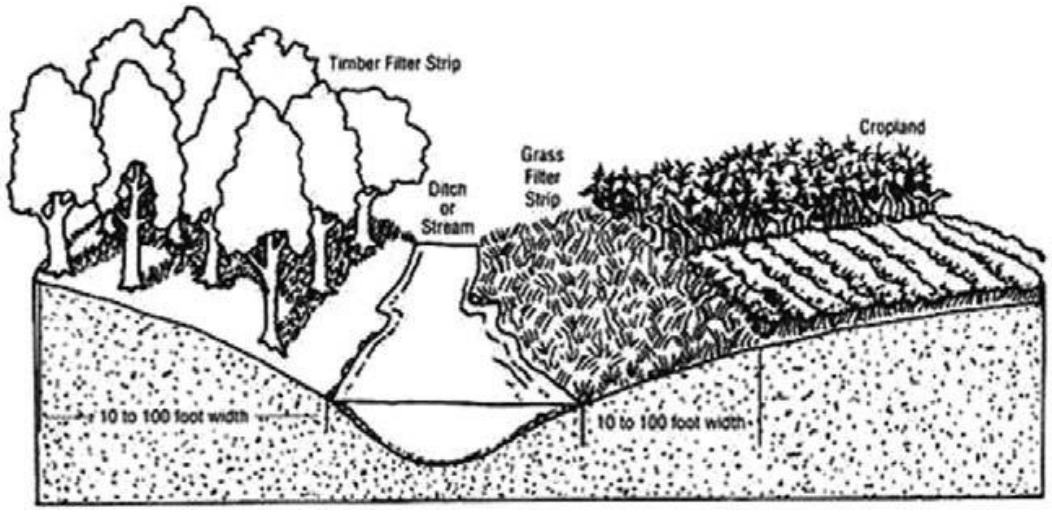


Figure 1: Vegetated filter strips.
Ohio State University Extension, http://fieldforagecropsnutrientmanagement.blogspot.com/2011/05/farming-on-fringes.html

Vegetated filter strips are defined as "a designated strip or area of vegetation for removing sediment, organic material, nutrients, agrochemicals and bacteria from runoff or waste water" (USDA-SCS, 1991). These strips have the capacity to retard or prevent the movement of sediments and agricultural chemicals by slowing runoff and increasing evapotranspiration and adsorption while enhancing biodegradation in the rhizosphere (Nair *et al.*, 1993). Vegetated filters may also function as a sink for pollutants through uptake and storage, which can then be removed as biomass.

Throughout the Midwest, perennial grasses are often employed as inexpensive filter strips. Benefits to farmers include the trapping of sediments, filling rills and gullies, dispersing concentrated flow and the reduction of runoff by temporarily ponding some water thus increasing infiltration (Kemper *et al.*, 1992). Grass strips have been shown to be effective in reducing herbicide runoff (Krutz *et al.*, 2005) which is a major

transport mechanism for soluble pollutants as well as pollutants adsorbed to sediment particles (Bengston *et al.*, 1990).

The most frequently used grasses in the Midwest for filter strips are native grasses or an adapted tame grass that can be established quickly. In Kansas, smooth brome grass (*Bromus inermis*) is commonly used as it has a dense, fibrous root system that resists erosion. Brome also provides management opportunities for the farmer to increase production of forage, seed and pasture. Native grasses are selected for their low maintenance, requiring much lower inputs of fertilizers and herbicides, and for their value as a wildlife habitat.

However, as agricultural pollution occurs through a variety of mechanisms, vegetated filters are likely to vary in their capacities for pollutant uptake and degradation in the soil. These differences may arise from a number of factors including particular soil-water interactions, nutrient cycling and rhizobial populations within each filter. Each factor is unique to specific ecosystems and may influence the effectiveness of vegetated filters.

While several studies have followed the effects of vegetated filters, few have compared the effectiveness of different types of vegetation at removing or degrading herbicides. In a 2005 review VFS literature, Krutz *et al.* (2005) reported only two studies evaluating the effect of vegetation type on the retention of herbicides. No significant differences were found in the retention of fluorometuron and norflurazon in equivalently sized filter strips established in big bluestem, eastern gammagrass,

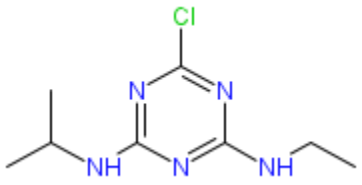
switchgrass or tall fescue (Rankins *et al.*, 2001). Neither were significant differences found for several herbicides in grass vs. mixed grass/shrub strips (Schmitt *et al.*, 1999). One 2008 study did, however, find significant differences in the abilities of orchardgrass, tall fescue, timothy, brome and switchgrass to metabolize and reduce atrazine in soil and leachates (Lin *et al.*, 2008).

Plant community effects on soil nutrients may influence atrazine degradation. Seasonal differences have been reported in the mineralization rates of atrazine in riparian pasture and forest soils (Entry *et al.,* 1994). Forest soils had significantly higher C/N ratios than pasture soils and showed higher rates of mineralization in the summer. Riparian organic litter was found to have higher C/N ratios than both types of soil and showed higher rates of atrazine mineralization in the spring. In a separate study, Entry *et al.* (1993) found that the addition of nitrogen fertilizer suppressed atrazine mineralization in grassland soils.

The objective of this project was to determine if plant community could be related quantitatively to pesticide movement and degradation in filter strip soils. The fate of atrazine in was examined two different plant ecosystems commonly used in the Midwest. These plant ecosystems, brome monoculture and a native grass mix, were compared in mesocosms to determine which was most effective at reducing soil atrazine concentrations. The experiment also measured selected soil parameters to aid in the understanding of reduction mechanisms and develop relationships which would allow grassed filter strips to be optimized for management of agricultural lands.

ATRAZINE IN GRASSED FILTER SOILS

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is a member of the chloro-s-triazines, the term s-triazine meaning symmetrical triazine. Atrazine has the following structure:

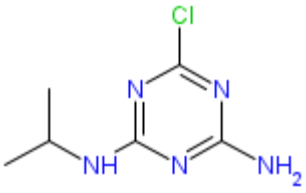


Atrazine

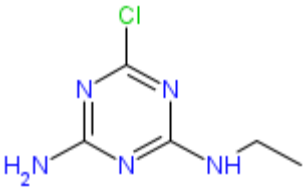
Figure 2: Atrazine structure.
http://www.molecular-networks.com/biopath3/biopath/mols/Atrazine

Atrazine is transported in runoff, and thus into a grassed filter, either adsorbed to sediments or in solution. The removal of atrazine adsorbed to sediments in surface drainage is largely a physical process resulting from the ability of vegetation to slow the transport of water and eroded soil. By increasing the hydraulic resistance to flow, surface drainage velocities are reduced and sediment loads are dropped. Plant roots serve to anchor soil and deposited sediment in place. The density of vegetation and reed stiffness used in controlling surface flow is, therefore, critical. Dissolved atrazine is removed by adsorption to above ground biomass and infiltration of runoff into filter soils.

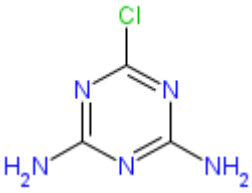
Once retained within a grassed filter, atrazine may be further affected by the particular plant community. The interaction of atrazine with grassed filter soils may be described by basic processes: adsorption, abiotic reactions, plant uptake and microbial degradation. A few of the more common degradation products of these reactions in soils are deethylatrazine, deisopropylatrazine, disopropyldeethylatrazine and hydroxyatrazine:



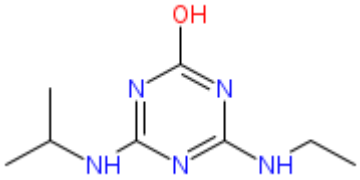
Deethylatrazine



Deisopropylatrazine



Deisopropyldeethylatrazine



Hydroxyatrazine

Figure 3: Common atrazine transformation products.
http://www.molecular-networks.com/biopath3/biopath/mols/deethylatrazine
http://www.molecular-networks.com/biopath3/biopath/mols/deisopropylatrazine
http://www.molecular-networks.com/biopath3/biopath/mols/deisopropydeethylatrazine
http://www.molecular-networks.com/biopath3/biopath/mols/hydroxyatrazine

Adsorption and Abiotic Hydrolysis

Dissolved atrazine is adsorbed to organic matter in the soil, to root exudates or to the roots themselves. Models, field experiments and laboratory studies indicate that

atrazine adsorption is influenced by time, pH, soil organic content, soil moisture, hydraulic conductivity and temperature (Laird *et. al.*, 2008). Atrazine has been shown to adsorb strongly to humic acids (Kalouskova, 1989). Atrazine bound to organics in the soil can accumulate in humin fractions to form bound residues that may not be bioavailable (Andreux *et al.*, 1990). Hydrogen bonding between montmorillonite clays and atrazine has also been suggested (Calvet *et al.*, 1975). A study by Dao *et al.* (1978) showed that decreases in soil moisture led to increased adsorption of atrazine. Baily *et al.* (1970) have suggested that that increased temperature causes a decrease in the adsorption of atrazine due to effects on the atrazine solubility and vapor pressure.

The major abiotic pathway for atrazine degradation in soil is through chemical hydrolysis to hydroxyatrazine (Obien *et al.*, 1969; Skipper *et al.*, 1967) with the mechanism of hydrolysis involving the protonation of a ring nitrogen causing an electron deficiency of the 2-carbon. This carbon is then subjected to nucleophilic attack by water molecules in the soil. Replacement of chlorine then occurs at the 2-carbon (Jordan *et al.*, 1970). Armstrong *et al.* (1967) found that soil pH and organic matter content largely controlled the rate of hydrolysis. Acidic sites on the surfaces of organic matter evidently catalyzed the reaction leading to a more rapid hydrolysis. Because the mechanism is acid hydrolysis, the rate increases with increasing acidity.

Plant Uptake and Metabolism

The principal mode of action of atrazine and other s-triazines in plants is to disrupt the light reaction of photosynthesis (Trebst, 2008). Atrazine is absorbed through plant

roots and is translocated through the xylem to the leaves and meristem where plant death is caused by the inhibition of photosynthesis in the chloroplasts. Atrazine is translocated by apoplastic movement within the free space of the cell walls (Jachetta *et al.*, 1986), a path mainly associated with the uptake of water. Therefore, the transport of atrazine within the xylem is controlled primarily by plant transpiration.

Plant resistance and atrazine selectivity are based on detoxification mechanisms within the plants themselves. Atrazine undergoes benzoxazinone catalyzed hydroxylation to hydroxyatrazine in several resistant species (Raveton *et. al.*, 1997, Roeth *et. al.*, 1971). Atrazine may also undergo n-dealkylation in plant tissues, which conveys partial resistance to some plants in which this n-dealkylation pathway is well developed. (Hassall, 1990).

Binding of atrazine to proteins provides another important pathway for plant resistance. Glutathione is a tripeptide that is important in the degradation of pesticides along a major metabolic pathway involving the enzyme glutathione-S-transferase. Corn and other atrazine resistant crops are well endowed with this enzyme system. Shimabukuro *et al.* (1973) stated that glutathione conjugation of atrazine is a major mechanism for detoxification of atrazine. Of interest is that glutathione-S-transferases from plants are inhibited by the oxirane derivative, tridiphane, a potent synergist of atrazine (Ezra *et al.*, 1985). Its presence increases the potency of atrazine presumably because it prevents the decomposition of atrazine via the glutathione mechanism (Lamoureux *et al.*, 1986).

Biodegradation

Biodegradation of atrazine has been extensively studied and appears to be highly dependent upon the microbial populations present in the soil as well as environmental conditions. Soil characteristics such as moisture, aeration, organic and inorganic components as well as nutrient availability all play key roles in the determination of degradative pathways. Dealkylation appears to be a major mechanism, resulting in one or both of the daughter products deethylatrazine and deisopropylatrazine. However, complete mineralization to carbon dioxide and ammonia is possible under favorable conditions. Adapted soils may, however, be necessary for mineralization.

It is generally accepted that the dechlorination of the parent atrazine proceeds abiotically under most conditions (Obien *et al.*, 1969; Skipper, 1967). Bacterial dechlorination had only been reported using s-triazines with less bulky side chains (Cook, *et al.,* 1984) while only very slow dechlorination of atrazine had been reported by soil fungi (Kaufman *et al.,* 1970). Studies by Mandelbaum et al. (1993), however, have shown that an adapted bacterial mixed culture (LFB6) was capable of rapidly transforming atrazine to hydroxyatrazine under both aerobic and anoxic conditions. Transformation was accomplished when atrazine served as the sole nitrogen source for the mixed culture. Exact environmental conditions for promotion of microbial dechlorination *in-situ* remain unclear and an adapted population may be necessary.

A common atrazine degradation mechanism is n-dealkylation to either deisopropylatrazine or deethylatrazine (Skipper *et al.*, 1972; Goswami *et al.*, 1971). The

mechanisms of n-dealkylation have been well studied and are generally attributed to ubiquitous, naturally occurring enzymes. Alkyl side chains may be utilized as carbon sources by many microorganisms. Kaufman *et al.* (1970) followed the degradation of atrazine by several soil fungi, concluding that fungi were able to cleave atrazine side chains but were unable to degrade the triazine ring. Degradation by the fungus *Aspergillus fumigatus* formed n-dealkylated daughters. Levanon (1993) also determined that side chain cleavage was a result of the enzymatic activity of soil fungi. The preferred dealkylation pathway, however, is highly dependent upon the specific degrading population.

Levanon (1993) elaborated the roles of various microbial populations in the mineralization of atrazine *in-situ*. Soil fungi and bacteria were selectively inhibited and compared to controls. Mineralization of the side chains was found to decrease only in soils treated with fungicide, whereas mineralization of the triazine ring occurred only in controls. Results indicated that stepwise reactions involving the removal of side chains followed by ring degradation were necessary for complete mineralization. This further explains the importance of N-dealkylation reactions to atrazine mineralization. Each step is accomplished utilizing different communities; soil fungi mineralize side chains while soil bacteria catabolize the triazine ring. As previously mentioned, soil bacteria may also be responsible for dechlorination.

Mineralization may generally be subdivided into the following steps: hydrolysis of the 2-carbon, dealkylation of the 4 and 6-carbons, deamination of the 4 and 6-carbons

followed by ring cleavage and mineralization. Thus several key enzymes located in differing species are necessary for degradation and a consortium may be required for complete mineralization. Significant variations from this sequence have, however, been noted (Mandelbaum *et al.*, 2008).

Mineralization of atrazine has also been observed both in-situ and under laboratory conditions. Mandelbaum's LFB6 bacterial mixed culture was found to rapidly mineralize atrazine under aerobic conditions when utilized as a sole nitrogen source (Mandelbaum *et al.*, 1993). Cook *et al.* (1981) isolated a *Pseudomonas* species (strain PSA) capable of aerobically mineralizing atrazine ozonation products as a nitrogen source. Leeson *et al.* (1993) report some success in biomineralization utilizing *Klebsiella terragena* (strain DRS-I). DRS-I was found to utilize dealkylated atrazine as a carbon source with relatively high nitrogen levels.

Studies by Eaton *et al.* (1991) concluded that both *Pseudomonas* and *Klebsiella* strains catabolized melamine to ammonia and CO_2. Melamine is a metabolite of atrazine degradation after the removal of alkyl side chains and replacement of the chlorine with an amine. Melamine catabolization involves the stepwise replacement of the amines with hydroxyl groups to ammeline (2,4-diamino-6-hydroxy-s-triazine), ammelide (2-amino-4,6-dihydroxy-s-triazine) and cyanuric acid (2,4,6-hydroxy-s-triazine). Cyanuric acid is then readily mineralized to CO_2 and NH_3. It may be presumed that atrazine mineralization in both species (and in LFB6 mixed culture) proceeds along this pathway.

EXPERIMENTAL DESIGN AND ANALYTICAL METHODS

An experiment was conducted to determine the direct and indirect effects of plant communities on the degradation and mobility of atrazine retained in grassed filter strips. The experiment utilized mesocosms under greenhouse conditions at the University of Kansas herbarium. Mesocosms were prepared using 20 gallon plastic storage bins purchased from a local supplier. Local topsoil, Martin silty clay loam obtained from land cleared during construction of the herbarium, was sieved to ¼ inch to assure a uniform consistency. This soil was then placed into mesocosms to within 2 inches of the top. With subsequent settling, each mesocosm contained approximately 15 gallons or, assuming a bulk (dry) density of 1.28g/cm^3 (Pedosphere.ca, 2011), approximately 73kg of dry soil.

Three cover types were chosen for the experiment; a brome monoculture, a CRP native grass mix and a bare control, which was mechanically kept free of plants throughout the duration of the experiment. The CRP mix was provided by the Douglas County Soil Conservation Service and is a mix of native tallgrass prairie species (Appendix B). Brome grass seed was provided by a local supplier. Brome grass was chosen as a representative non-native cultivar in the experiment as it is often used by farmers for grassed waterways and buffer strips.

Six replicates for each treatment were prepared for a total of eighteen mesocosms. Planted mesocosms were then sown to a depth of approximately one inch with the chosen plant seeds and watered until soil was saturated. Mesocosms were maintained

by twice a week watering for one year to allow growth of a healthy grass community (Fig. 4). Irrigation volume was not precisely controlled during this time; rather, soil condition was regularly monitored using a soil moisture probe to prevent over saturation. Depending upon soil condition, 1–2 liters were applied weekly, for an estimated total irrigation volume of approximately 80 liters. Mesocosms had no drainage and were irrigated with Lawrence, Kansas tap water, so any salts added with the water accumulated over time. No external nutrients were added other than those present in the tap water, no pesticides were used. During the year of growth dead biomass was culled mechanically to prevent overgrowth of the living material and prevent accumulation on the soil surface.

Figure 4: Planted soil mesocosms.

Atrazine is only moderately soluble in water (~30mg/L), so commercial atrazine flowable concentrate under the brand name Atrazine 4L (Fig. 5) was used for mesocosm application. A flowable concentrate suspends finely ground atrazine (0.5μm – 20μm) in water with proprietary suspension and dispersion agents to stabilize higher concentrations for field application. Atrazine 4L was diluted into 20 liters to a nominal concentration of 100mg/L. One liter of this working solution was applied to each mesocosm using a PVC sprinkler container. This amounted to the addition of 100mg atrazine to the estimated 73kg of soil per mesocosm, for an approximate 1400μg atrazine/kg dry soil. Following application, the watering regime of the plants was carefully controlled to one liter of tap water twice a week applied uniformly over the surface of the pots. A total irrigation volume of 14L was applied to each mesocosm for the remainder of the experiment.

Figure 5: Atrazine 4L labeling

Sampling began 1 day after application. On each sampling date (1d, 9d, 18d, 26d and 51d) a grid overlay of twelve squares was used to select a random location from each mesocosm

and a full-depth soil core was taken from that position. The soil core was collected using a ½-inch-dia. core sampler and placed into a PVC trough. The core was then divided into thirds (upper, middle and lower layers) and each third was placed into a 40-milliliter screw top vial. Immediately following sampling, all samples were refrigerated to <4°C.

Analytical methods utilized in this experiment were carried out in the Kansas Biological Survey (KBS) ecotoxicology laboratory. Procedures were chosen to allow comparative determinations between experimental treatments using a minimum of sample. Total soil amounts for each sample tended to vary but were generally less than 15 grams total. Small subsamples had to be utilized for each determination and in cases where errors occurred in processing of samples, repeat data often could not be obtained due to insufficient sample. Soil blanks, spikes and duplicates were performed when practicable.

Above Ground Biomass

Above ground plant biomass was determined immediately following the last sampling event. Above ground biomass was used as an indirect measure of root biomass in each mesocosm assuming roughly similar relationships exist in both plant species (Niklas, 2005). Grasses were mechanically removed to the level of the soil in each pot, bagged and labeled. Each sample was then oven dried for 48 hours and weighed on a laboratory balance. Above ground biomass was then recorded in grams (g).

Soil Moisture

Soil moisture determinations were made on each sample by placing approximately 0.5g of each sample into an accurately weighed 10mL screw top vial. The vial and sample were weighed on an analytical balance and placed in an oven at 110°C for 48 hours. Samples were then removed and weighed again. Soil moisture was calculated as a percent of the initial weight (%). This value was then used to report all concentration data as dry weight equivalents.

Soluble COD

Soil organic carbon could not be determined directly in the KBS laboratory. Some estimate of bioavailable soil carbon was desirable for the experiment so a procedure measuring soluble carbonaceous oxygen demand (sCOD) was utilized. However, while COD is generally proportional to the concentration of biodegradable material, it is unknown what fraction of the sCOD was actually biodegradable. In this case it was hoped that the uniform soil matrix used in the mesocosms would provide a consistent ratio of sCOD to soluble bioavailable carbon.

Approximately one gram of soil from each sample was weighed out and placed in a 10mL screw top vial. Ten milliliters of deionized water were then added to each vial. Vials were capped and placed in a rotary shaker for one hour. After shaking, samples were vacuum filtered through Whatman 0.45μm filters into a second vial. Filters were not prerinsed and may have contributed COD to the filtrate. Filtered blanks were not analyzed, so the presence or magnitude of any contamination is unknown.

Two milliliters of the filtrate were then pipetted into a Hach COD vial and digested for two hours in a heater block. Chemical oxygen demand was then measured colorimetrically at 440nm on a Milton Roy spectrophotometer. Soluble COD was expressed as milligrams COD per kilogram of dry soil (mg COD/kg).

Extractable Nitrogen

Determinations of ammonia and combined nitrate plus nitrite were made by extraction and colorimetric detection. Approximately one gram of soil was placed into a 10mL screw cap vial followed by 10mL of 2.0N potassium chloride. Samples were then placed into a rotary shaker for one hour then allowed to settle overnight in the refrigerator. The clarified supernatant was then transferred to a 10mL autosampler vial for analysis.

Ammonia was run on a Lachet flow injection analyzer utilizing phenolate colorimetric determination. Nitrate plus nitrite was run on the flow injection analyzer using cadmium reduction (to convert nitrate to nitrite) and nitroprusside colorimetric detection of nitrite. Both ammonia and nitrate/nitrite were expressed in mg as N per kilogram dry soil (mg N/kg).

Kjeldhal N and Total P

Total Kjeldhal nitrogen and total phosphorus were simultaneously determined using a standard Kjeldhal digestion and colorimetric detection. Approximately five grams of sample were transferred to a labeled Kjeldhal digestion tube, followed by a 'Kjeltab'

and 10mL concentrated sulfuric acid. Following the digestion, deionized water was used to bring the samples to 50mL and they were mixed on a vortex mixer. Samples were then run on a Lachet flow injection analyzer set up to simultaneously determine TKN and Total P. Analytes are reported as milligrams nitrogen or phosphorus per kilogram dry soil (mg N/kg or mg P/kg).

Atrazine and Metabolites

Atrazine, deethylatrazine and deisopropylatrazine were extracted using a methanol/solid phase extraction (SPE) procedure and analyzed on a Hewlett Packard 5890 gas chromatograph/5971 mass spectrometer running in selected ion mode. Solid phase extraction was used after the pesticides were extracted from the soil with a water/methanol mixture. Terbuthylazine was used as an internal standard for the analysis.

Following the soil nutrient analysis, the remaining soil sample was placed into a 40-mL screw top vial. Samples were then spiked with 100μL of a 10mg/L solution of terbuthylazine in methanol. To each vial was added 5mL deionized water and 15mL methanol. Vials were then placed in a 75°C water bath and warmed for 20 minutes with frequent shaking. Vials were then allowed to cool and centrifuged. Supernatant was then poured off into a second vial. The process was repeated with the second supernatant being added to the first. The remaining soil was then discarded and the supernatant vials were evaporated under vacuum to less than 10mL. Methanol concentration was reduced sufficiently to allow separation using SPE; however,

recovery was not evaluated using matrix spikes, so it is unknown whether residual methanol may have adversely affected recovery of atrazine and its metabolites.

Samples were then extracted using a Bakerbond quaternary amine anion exchange SPE cartridge on top of a Supelco ENV-18 SPE cartridge. This stacked arrangement was used to aid in the removal of extractable humic acids and other interfering organic compounds. The SPE cartridges were prepared with 3mL methanol followed by 3mL deionized water. Samples were then extracted and eluted with 3mL methanol into conical vials. Sample volume was then reduced to 100μL by rotary vacuum evaporation and low heat. Samples were then transferred to GC autosampler vials for analysis.

Analysis utilized the HP GC/MS system with a HP-35 analytical column (0.25mm ID, 30m, 0.25μm film) and a 2μL-injection volume. The terbuthylazine surrogate added before extraction was used in the manner of an internal standard to help correct for variations in extraction efficiency, analyte loss during the extraction procedure and small differences in the final volume of extract. Terbuthylazine was selected due to its chemical similarity to atrazine and assumes that the extraction efficiency of terbuthylazine is consistent and proportional to that of atrazine, deethylatrazine and deisopropylatrazine. It is likely that variations in extraction efficiency occurred, resulting from differences in time from initial application to extraction and a non-uniform soil matrix.

RESULTS AND DISCUSSION

Data were tabulated for each soil parameter by cover type (CRP mix, bare control and brome), depth (upper, middle and lower soil layers) and sampling date, for a total of 1862 measurements in 327 sets (Appendix A). Each data set ($n \leq 6$) was examined for outliers using a two-tailed Dixon's Q test ($\alpha=0.05$) as described by Rorabacher (1991). A total of 77 data points were determined to be outliers and excluded from the data (4%). Mean and standard error were then calculated and charted for each soil parameter for each data set (Figs. 6-14).

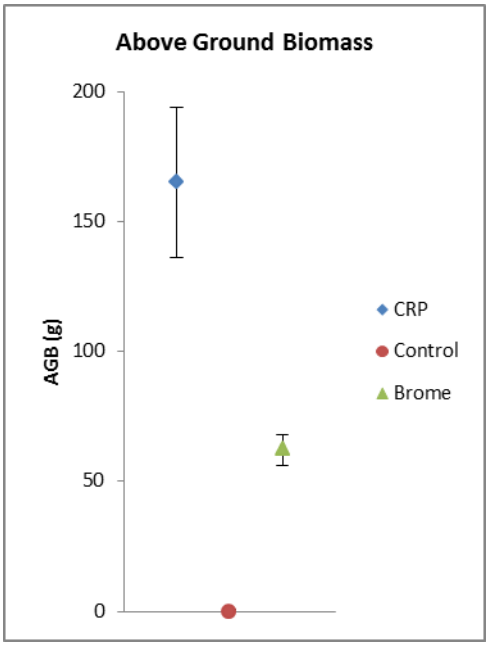


Figure 6: Above ground biomass (g) in mesocosms at 51d. Error bars represent 1 standard error.

Data sets were then checked for normality using the Anderson-Darling test ($\alpha=0.05$). A total of 18 sets of data were found to be non-normal. All data were then logarithmically transformed ($\log_{10}(x+0.001)$) to normalize and reexamined using the A-D test. After excluding outliers and normalizing, 10 sets of data remained non-normal which could not be normalized through transformation. None were atrazine or metabolite data. Two-way analysis of

variance (ANOVA) and Tukey's HSD post-hoc test were used (α=0.05) for comparison of means using cover type and sample depth as qualitative discrete variables. Pearson's correlation analysis was (α=0.05) also used to evaluate trends over time and relationships between atrazine, metabolites and other soil parameters.

Results of ANOVA indicate multiple significant effects of cover type (Tables 1 and 2). Above ground biomass was found to differ significantly among the three treatments with the CRP mix averaging more than twice the mass of brome (Fig. 6). Assuming similar ratios of above to below ground biomass (Niklas, 2005), significant differences would also occur in root mass. Soil moisture was significantly lower in CRP mesocosms compared to other treatments, decreasing in the middle and lower layers over the 51 days (Table 2). The reduced soil moisture is indicative of increased transpiration in the CRP mesocosms and deeper root penetration.

Soluble COD was significantly affected by depth and appeared, in some cases, to increase with time. Highest sCOD concentrations were in the upper soil layers and decreased with depth (Table 2). Depth differences were not, however, related to cover type. Differences in the absence of plants may result from the ability of soils to reduce COD (Larson *et al.*, 2012, von Felde *et al.,* 1997) depending upon soil type and O_2 availability. COD in the upper soil layers may also have reflected the additional oxygen demand of atrazine and adjuvants (stabilizers/dispersants) in the initial dose of Atrazine 4L. Increases in sCOD over time would result from conversion of insoluble

forms of COD by soil microbes or leakage (exudation) of organic matter from plants and microbes.

Ammonia nitrogen was not significantly affected by cover type (Table 1) but did show significant increases over the 51 days in both the CRP and brome mesocosms (Table 3). Chloramines are used by the City of Lawrence for disinfection and the use of chloraminated water for irrigation contributed some ammonia to the mesocosms. City of Lawrence tap water contains approximately 1mg NH_3–N/L. Based on estimates of irrigation volume and mesocosm soil volume, approximately 1.1mg NH_3–N/kg was added during the one year establishment of the mesocossms and approximately 0.2mg NH_3–N/kg was added during the 51 day experiment. Increases in ammonia concentrations of 0.5–2mg NH_3–N/kg were observed, which could not be explained solely through the addition of chloramines. Exudation of ammonia by plants and mineralization of soil organic nitrogen by soil microbes are likely responsible for the remainder of the observed increase. ANOVA and Pearson's correlation analysis of ammonia concentration vs. cover type (Tables 1 and 3) differ as to whether plant exudation is a significant contributor. Neither analysis show a significant reduction of ammonia in planted mesocosms vs. bare soil, indicating plant uptake during the 51 days was minimal. Some utilization or nitrification of ammonia is, however, indicated during the 1 year establishment of the mesocosms, as initial ammonia concentrations are only about one-half the estimated amount added during this time as chloramine.

Nitrate plus nitrite nitrogen was significantly different between cover types, being much higher in the bare controls than in the planted mesocosms (Fig. 10). CRP treatments exhibited slightly (but significantly) lower concentrations than brome. It is likely that much of this nitrogen was utilized by plants for growth, or by microbes associated with plant growth, during the 1 year establishment of the mesocosms. As with ammonia, no significant reduction in nitrate plus nitrite was observed over the 51 days in any treatment, indicating plants had reached a mature stage of slow biomass accumulation (Heckman, 2003) or that remaining nitrate was not readily available to roots.

Some differentiation of nitrate plus nitrite concentrations also occurred through the soil column (Table 2). Although differences in the least squared means were small, the bare control did exhibit large changes in nitrate/nitrite concentration (Fig.10) from the upper soil layer to the middle and lower layers. In the absence of plants, this difference indicates either the presence of nitrates in the irrigation water or increased uptake or denitrification by soil microbes deeper in the soil.

Total phosphorus concentrations were significantly, but only slightly, different among cover types and exhibited no obvious trends over the 51 days (Fig. 12). As with ammonia, polyphosphates used by the City of Lawrence would be present in irrigation water. The City of Lawrence adds approximately 1mg/L of sodium hexametaphosphate to its drinking water. Based on estimates of irrigation volume and mesocosm soil volume, approximately 0.33mg P/kg was added during the one year

establishment of the mesocosms and approximately 0.06mg P/kg was added during the 51 day experiment. Phosphorus and nitrate plus nitrite were correlated and both were negatively correlated with biomass (Table 4), which would be expected if these nutrients were utilized for plant growth. However, phosphorus differences between cover types were small relative to differences between nitrate plus nitrite. Relative removal rates of nitrogen and phosphorus from soil have been found to vary, in switchgrass, from 6:1–9:1 depending upon nutrient availability (Kering, *et al.*, 2012). In this case, phosphorus differences were insufficient to explain reductions of nitrate plus nitrite solely through plant uptake. As previously noted, losses of nitrate plus nitrite appear to be due, in part, to denitrification.

Atrazine concentrations were significantly affected by cover type, sample depth, and their interaction (Table 1). Significant reductions occurred as atrazine moved through the soil column (Fig. 13) and may be attributed to previously discussed adsorption mechanisms. A positive correlation between sCOD and atrazine is apparent (Table 4) and would occur if atrazine is more readily adsorbed by soils with higher amounts organic matter. Initial concentrations in the upper soil layers (Fig. 13) were approximately 1000μg atrazine/kg, which is reasonable based on the estimated 1400μg/kg added to each mesocosm. However, if most applied atrazine remained in the upper third of the mesocosm, the estimated amount added would need to be increased to approximately 4200μg/kg. This appears to indicate significant underreporting of soil atrazine in the data or an overestimation of atrazine applied to

the mesocosms. It may be that the water/methanol soil extraction lacked sufficient solvent strength to fully extract strongly adsorbed atrazine.

Least squared means of atrazine were found to be significantly lower for the CRP mix vs. bare controls (Table 2), however, changes in atrazine concentrations vs. time were not significant at any depth during the 51 day experiment (Table 3). In this case, the CRP mix may modify soil conditions to favor enhanced adsorption (vs. cover types) rather than a slower process of microbial degradation. Least squared means of atrazine in brome and bare controls were not significantly different (Table 2) but both treatments exhibited significant reductions in the upper soil layer (Table 3) implying either slowed adsorption, reduced extraction efficiency, or microbial or abiotic degradation. In each case, cover type effect was small compared to the effect of soil depth on atrazine.

Atrazine metabolites deisopropyl- and deethylatrazine, like the parent, decreased significantly with soil depth (Figs. 14 and 15). No effect of cover type was observed with the metabolites, although significant reductions over time were apparent in the brome mesocosms. Note that, as a result of instrument problems, metabolites were not monitored until day 26, so changes over time would not be as apparent. Deisopropyl- and deethylatrazine were both significantly correlated with decreasing atrazine concentrations (Table 4), indicating a parallel change (decrease) over time. Also, the Atrazine 4L product may contain metabolites as impurities (Fig. 5), so their presence in the soil may be explained, in part, through means other than microbial

degradation. Regardless, reductions of metabolites through soil interactions are significant and meaningful. An accounting of total atrazine (Fig. 13), deisopropyl- and deethylatrazine (Fig. 14 and 15) concentrations (1d vs. 51d) indicate 56–88% of the original atrazine was either metabolized to hydroxyatrazine or was otherwise unaccounted for in the upper soil layers during the 51day experiment. This compares well to the previously discussed half–life of 14 days to several months.

	Biomass	% Moisture	sCOD	NH_4	NO_3+NO_2	TKN	TP	DIA	DEA	ATZ
Cover Type	**<0.0001**	**0.001**	0.752	0.271	**<0.0001**	0.081	**0.010**	0.112	0.151	**0.010**
Depth		0.367	**0.002**	0.121	**0.040**	0.065	0.566	**<0.0001**	**<0.0001**	**<0.0001**
Cover Type*Depth		0.305	0.055	0.816	0.835	0.640	0.555	0.831	0.671	**0.023**

Table 1: Results of ANOVA, showing the probablility of effect (p) of cover type and depth (and their interaction) on atrazine (ATZ), deisopropylatrazine (DIA), deethylatrazine (DEA) and soil parameters. Associations in boldface are significant (α=0.05).

	Biomass (g)	% Moisture	sCOD (mg/kg)	NO_3+NO_2 (mg/kg)	TP (mg/kg)	DIA (µg/kg)	DEA (µg/kg)	ATZ (µg/kg)
Cover Type								
CRP mix	148 *a*	11.5 *a*		0.1 *a*	18.9 *a*			17.9 *a*
Bare control	0.0 *b*	13.6 *b*		101 *b*	22.3 *b*			36.1 *b*
Brome	60.4 *c*	13.8 *b*		8.2 *c*	21.4 *ab*			29.2 *ab*
Depth								
Upper layer			390 *a*	7.8 *a*		56.8 *a*	85.0 *a*	493 *a*
Middle layer			321 *ab*	2.8 *b*		1.5 *b*	1.9 *b*	11.4 *b*
Lower layer			281 *b*	4.1 *ab*		0.6 *c*	0.4 *c*	3.4 *c*
Cover Type*Depth								
CRP mix-upper layer								575 *a*
CRP mix-middle layer								6.8 *bc*
CRP mix-lower layer								1.5 *d*
Bare control-upper layer								406 *a*
Bare control-middle layer								17.9 *b*
Bare control-lower layer								6.5 *bc*
Brome-upper layer								513 *a*
Brome-middle layer								12 *bc*
Brome-lower layer								4.1 *cd*

Table 2: Results of Tukey's HSD test, showing pairwise comparisons of least squares means for cover type and depth (and their interaction) for significant associations from Table 1. Means followed by the same letter do not differ significantly (α=0.05) from others in the same group.

	% Moisture	sCOD	NH_4	NO_3+NO_2	TKN	TP	DIA	DEA	ATZ
CRP mix-upper layer	-0.326	0.357	**0.799**	-0.079	0.057	0.135	-0.404	-0.646	-0.161
CRP mix-middle layer	**-0.594**	0.411	**0.621**	-0.316	-0.051	-0.125	0.609	0.597	0.319
CRP mix-lower layer	**-0.534**	**0.710**	**0.555**	0.062	-0.193	-0.254	0.525	-0.008	0.238
Bare control-upper layer	-0.169	0.421	0.386	0.223	-0.292	-0.378	-0.122	0.496	**-0.385**
Bare control-middle layer	-0.272	**0.436**	**0.418**	-0.028	-0.312	-0.252	-0.503	-0.482	-0.277
Bare control-lower layer	-0.179	0.099	0.349	-0.055	**-0.536**	-0.187	0.132	-0.616	-0.337
Brome-upper layer	0.166	**0.492**	**0.795**	-0.057	-0.034	-0.139	**-0.781**	**-0.754**	**-0.496**
Brome-middle layer	0.158	0.159	**0.674**	-0.033	-0.162	-0.093	-0.297	-0.467	0.195
Brome-lower layer	0.116	0.118	**0.687**	-0.218	0.108	-0.231	**-0.768**	-0.577	0.272

Table 3: Results of Pearson's correlation analysis, showing correlation coefficients (r) vs. time of atrazine (ATZ), deisopropylatrazine (DIA), deethylatrazine (DEA) and soil parameters for each cover type and depth. Correlations in boldface are significantly different (α=0.05) from zero.

	Biomass	% Moisture	sCOD	NH_4	NO_3+NO_2	TKN	TP	DIA	DEA	ATZ
Biomass	**1**	**-0.130**	0.041	0.048	**-0.638**	-0.079	**-0.142**	-0.117	0.053	-0.093
% Moisture		**1**	0.064	**-0.150**	**0.336**	**0.389**	**0.200**	0.000	0.024	-0.024
sCOD			**1**	**0.145**	0.043	**0.152**	0.012	**0.367**	**0.289**	**0.161**
NH_4				**1**	0.030	0.011	0.127	0.068	0.107	0.145
NO_3+NO_2					**1**	0.103	**0.230**	0.168	0.056	0.060
TKN						**1**	**0.464**	0.062	0.027	-0.021
TP							**1**	0.044	-0.003	-0.106
DIA								**1**	**0.935**	**0.923**
DEA									**1**	**0.959**
ATZ										**1**

Table 4: Results of Pearson's correlation analysis, showing correlation coefficients (r) across all samples of atrazine (ATZ), deisopropylatrazine (DIA), deethylatrazine (DEA) and soil parameters. Correlations in boldface are significantly different (α=0.05) from zero.

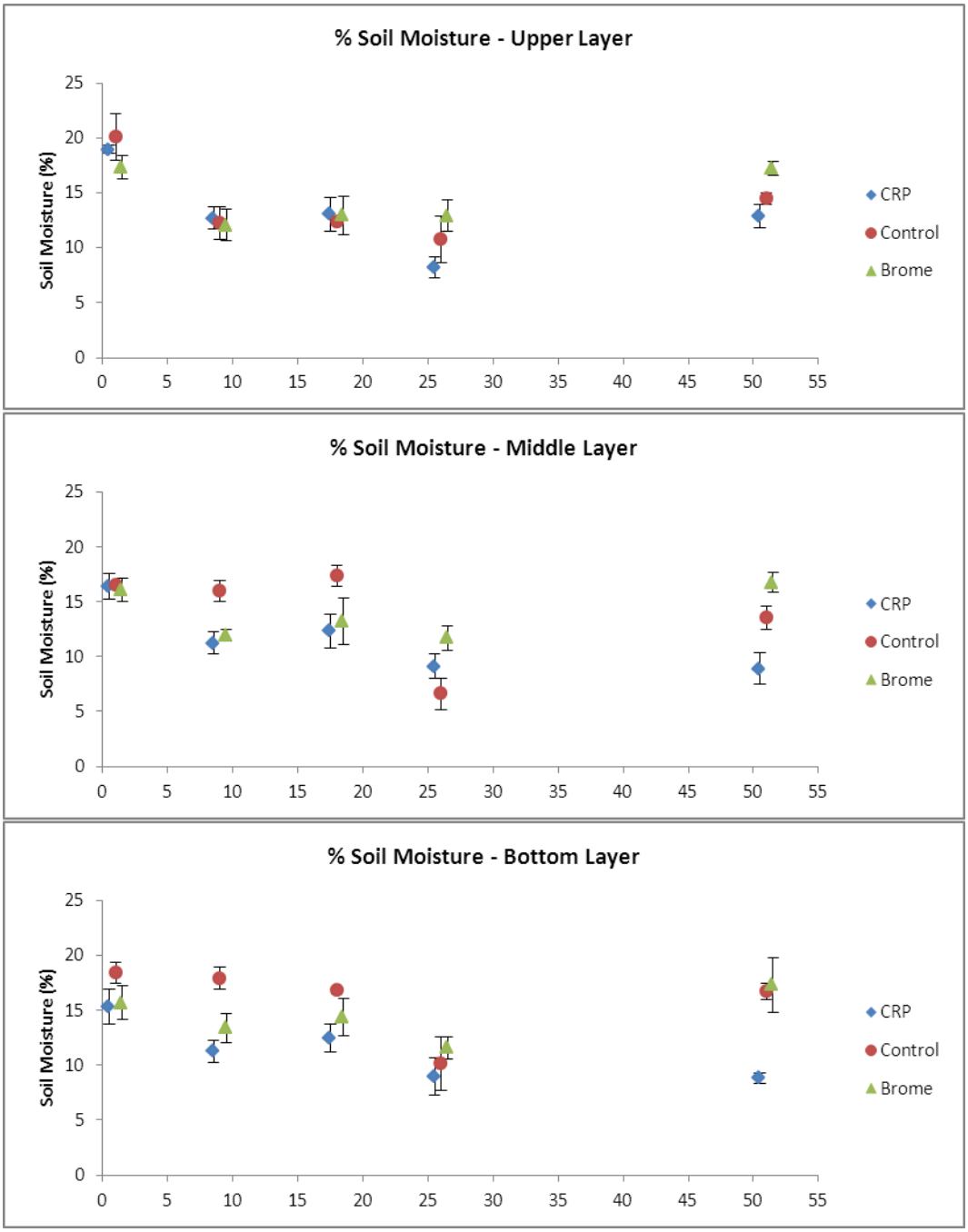


Figure 7: Soil moisture (%) in mesocosms at 1d, 9d, 18d, 26d, and 51d. Error bars represent 1 standard error.

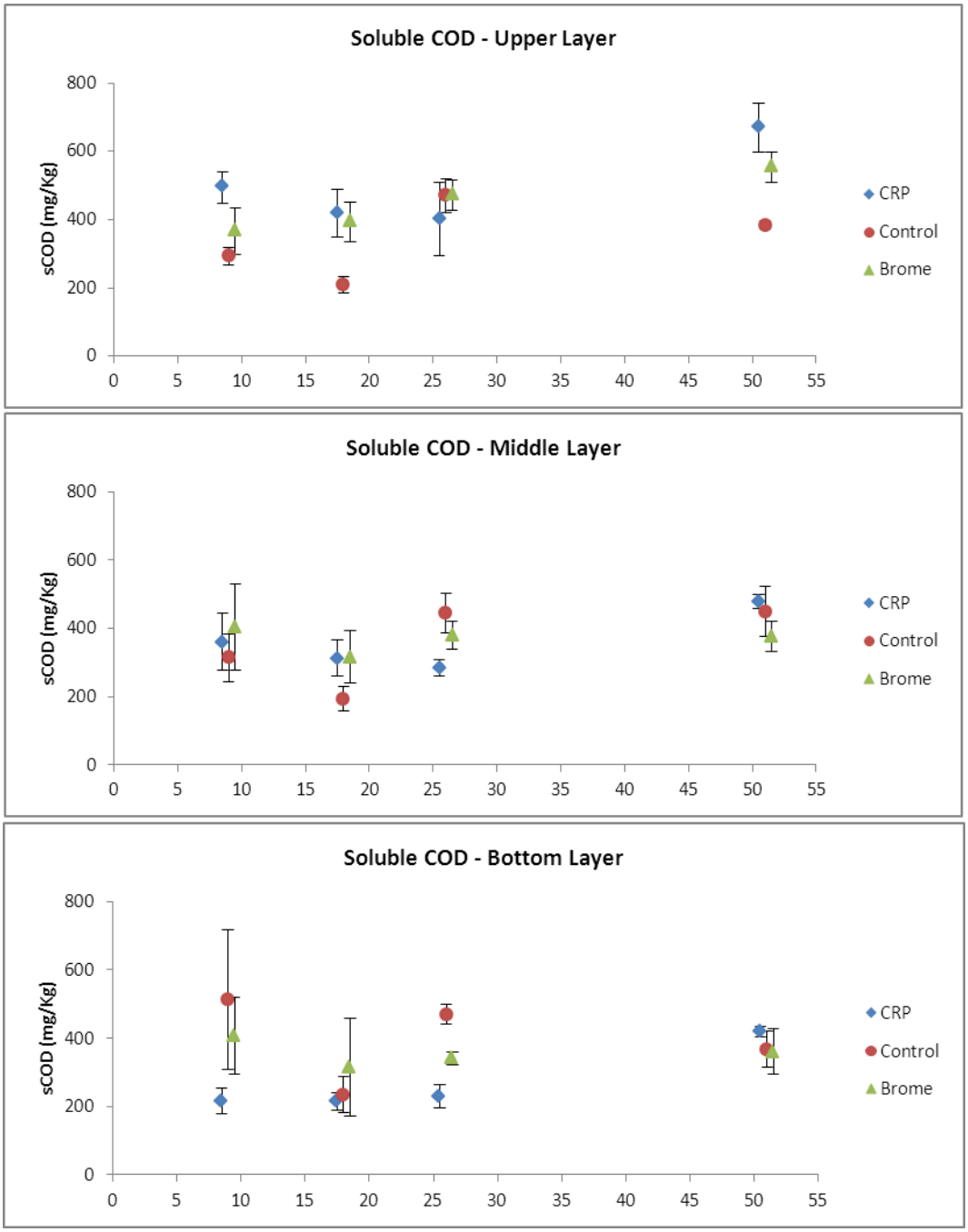


Figure 8: Soluble COD (mg/kg) in mesocosms at 9d, 18d, 26d, and 51d. Error bars represent 1 standard error.

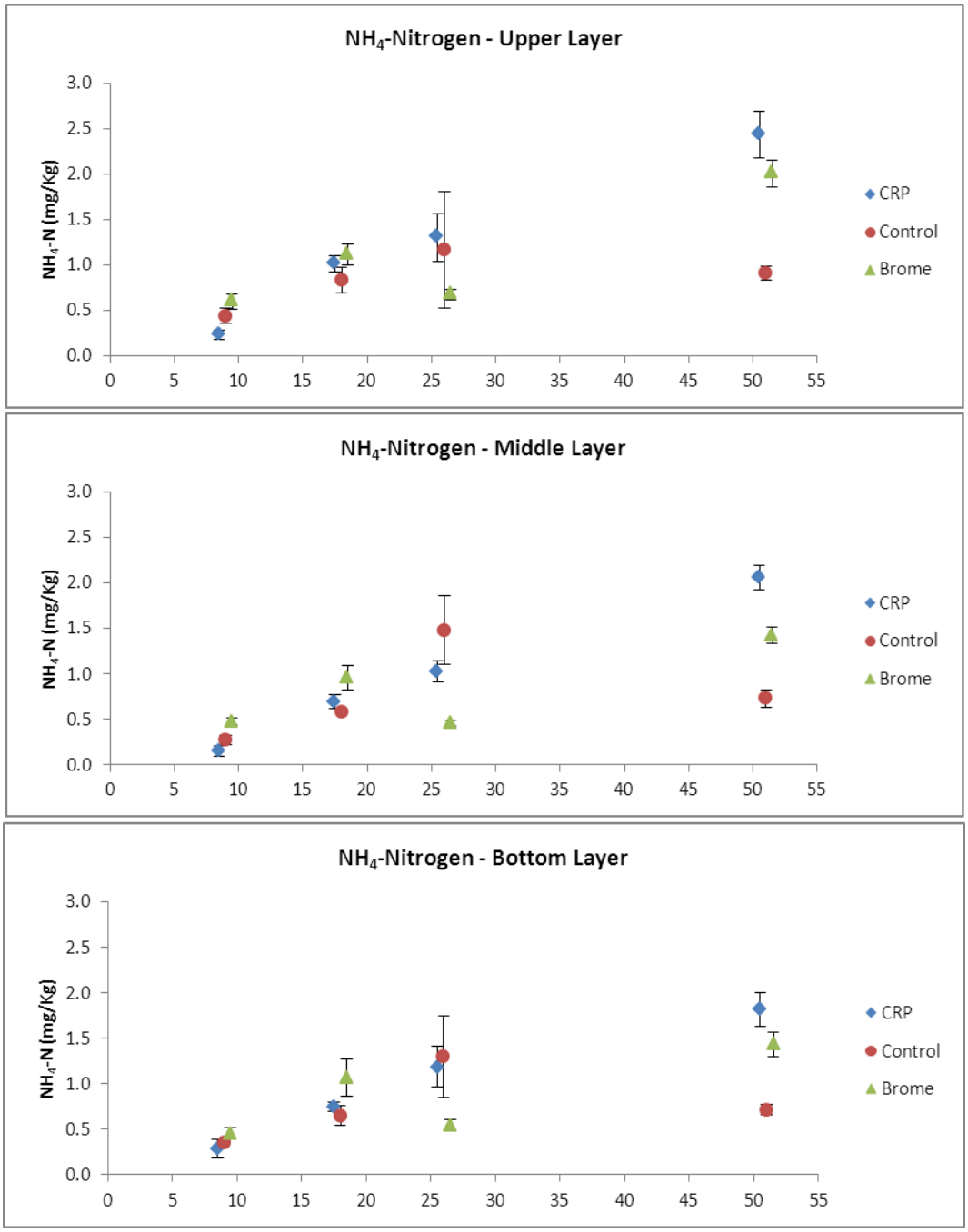


Figure 9: Extractable ammonia (mg N/kg) in mesocosms at 9d, 18d, 26d, and 51d. Error bars represent 1 standard error.

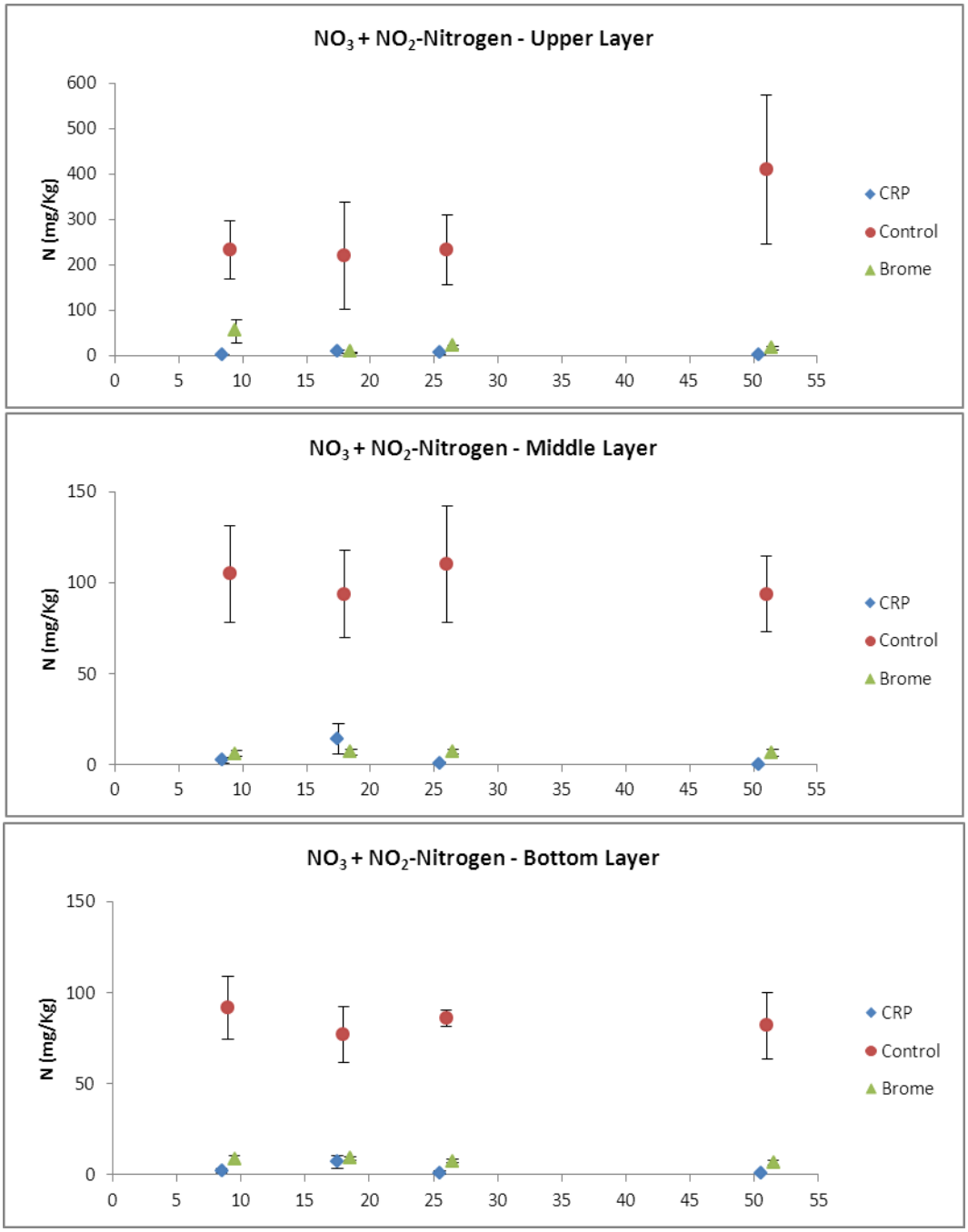


Figure 10: Extractable nitrate and nitrite (mg N/kg) in mesocosms at 9d, 18d, 26d, and 51d. Error bars represent 1 standard error.

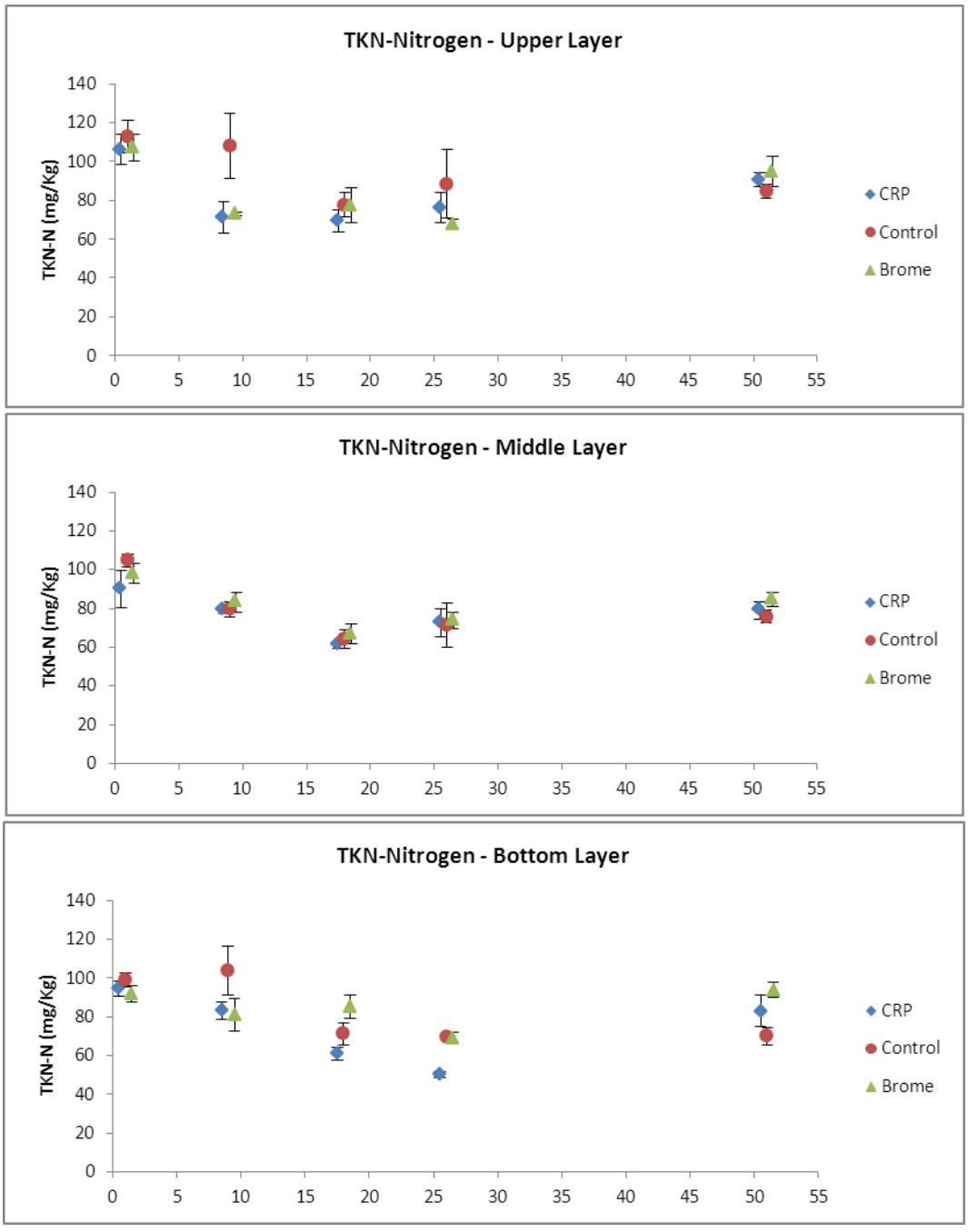


Figure 11: Kjeldhal nitrogen (mg N/kg) in mesocosms at 1d, 9d, 18d, 26d, and 51d. Error bars represent 1 standard error.

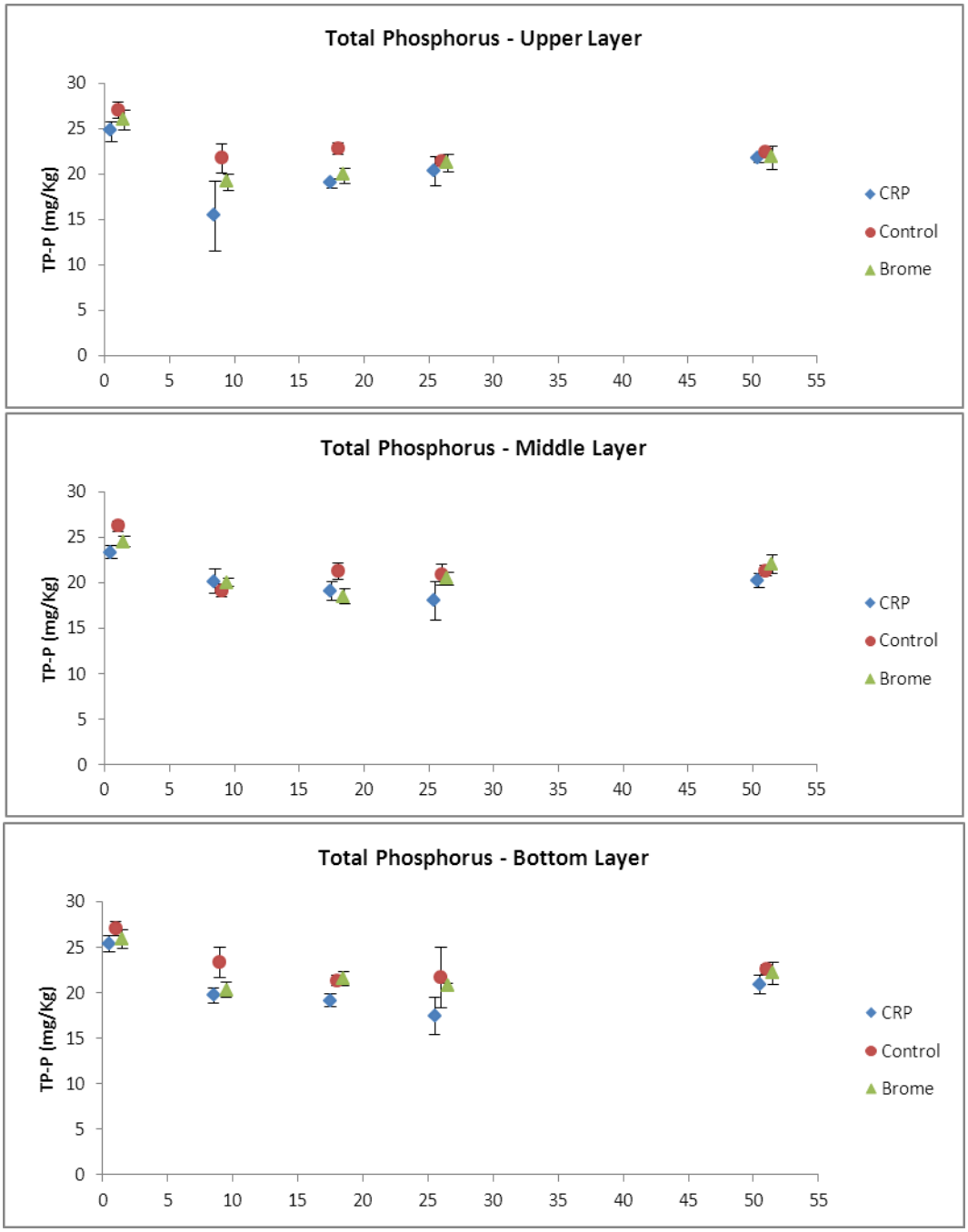


Figure 12: Total (Kjeldhal) phosphorus (mg P/kg) in mesocosms at 1d, 9d, 18d, 26d, and 51d. Error bars represent 1 standard error.

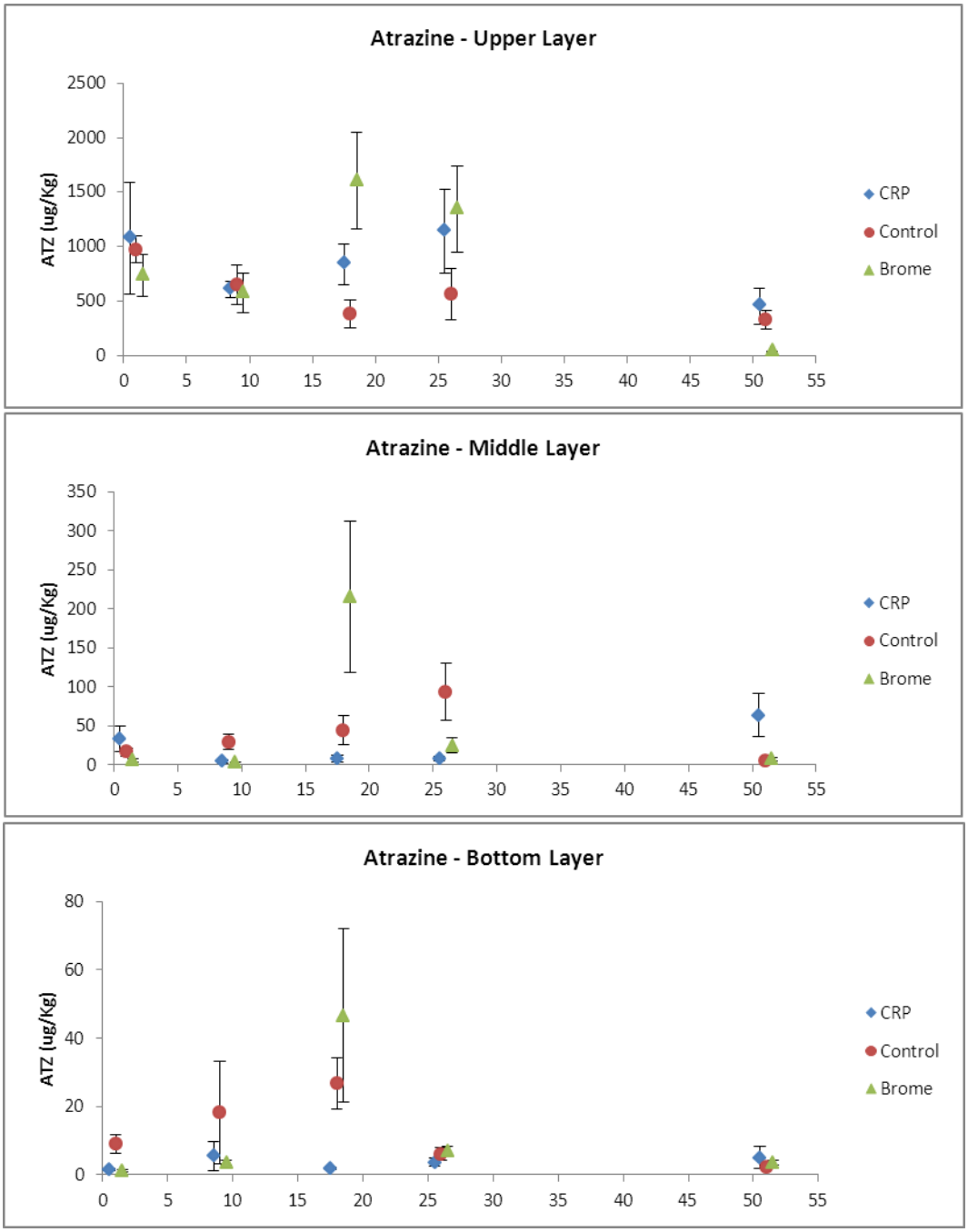


Figure 13: Atrazine (µg/kg) in mesocosms at 1d, 9d, 18d, 26d, and 51d. Error bars represent 1 standard error.

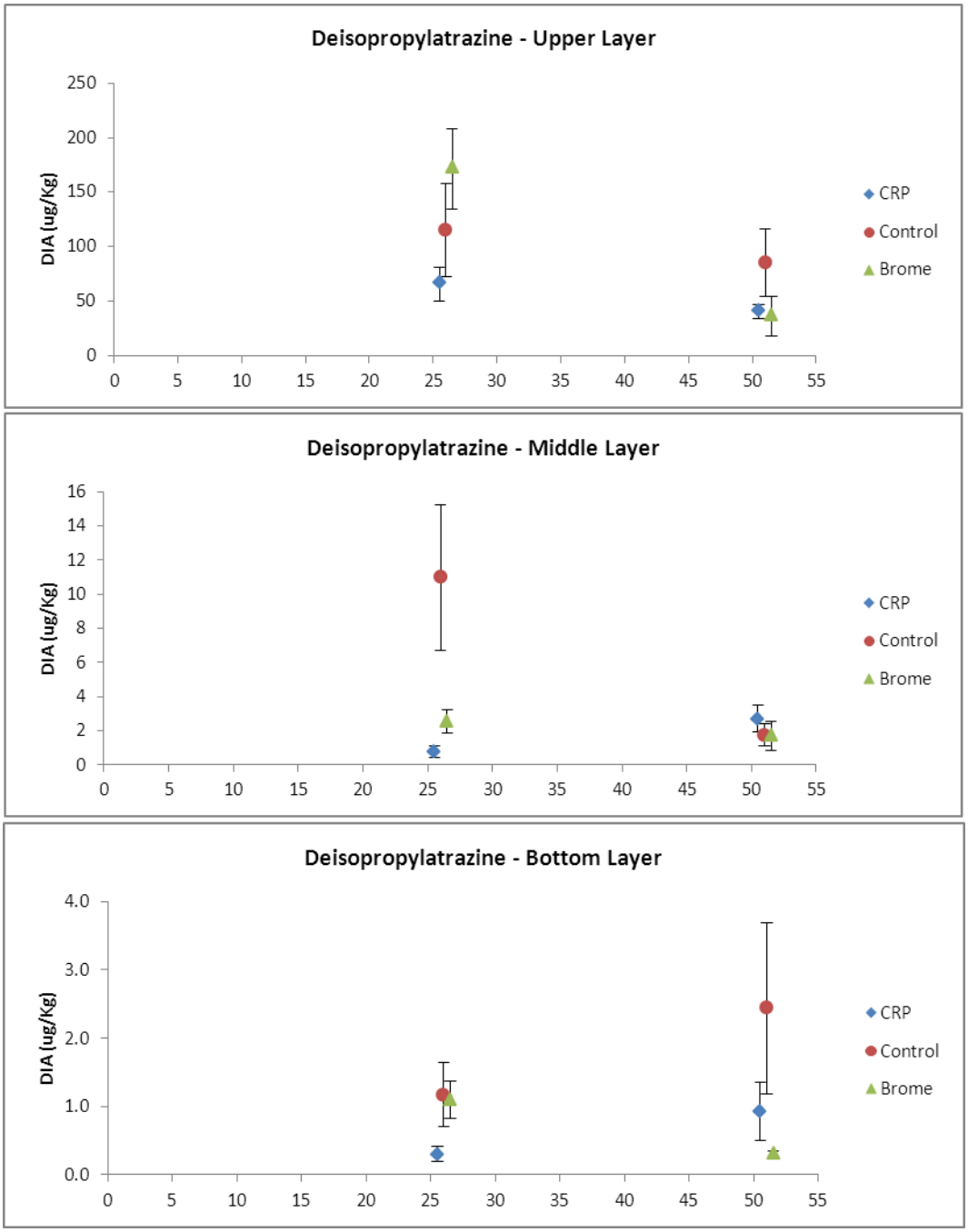


Figure 14: Deisopropylatrazine (µg/kg) in mesocosms at 26d and 51d. Error bars represent 1 standard error.

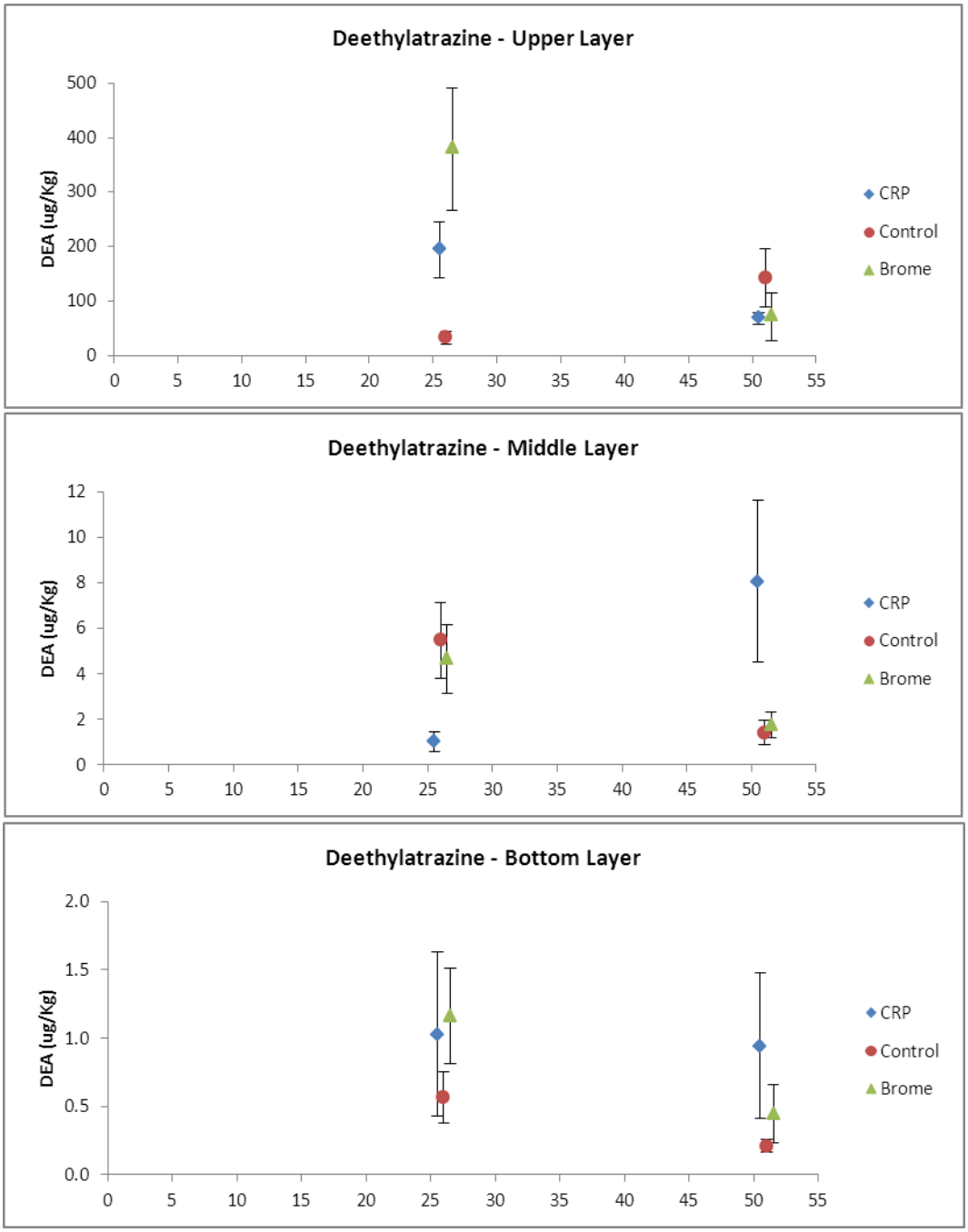


Figure 15: Deethylatrazine (µg/kg) in mesocosms at 26d and 51d. Error bars represent 1 standard error.

CONCLUSIONS

The concentration of atrazine and metabolites in all mesocosms was affected primarily through adsorption to soil particles, as evidenced by the rapid decrease of atrazine concentrations with depth. Atrazine adsorption may have been enhanced by the application of particulate atrazine, which likely dissolved as water was applied and subsequently adsorbed to soil before migrating downward. While vegetative growth was significantly different between all treatments, vegetation had only slight effects on atrazine reduction. Atrazine was affected to a small, but significant, degree by cover type, which may have resulted from increased adsorption, abiotic hydrolysis or plant uptake. Any effect of cover type on microbial degradation of atrazine would presumably have been accompanied by significant differences in deisopropyl- or deethylatrazine concentrations between treatments, which were not observed.

The experiment was unable to differentiate between adsorption, hydrolysis and plant uptake as mechanisms for the observed cover effect(s). Above ground biomass measurements indicate significant differences in the root mass between treatments, however, total soil carbon was not measured. Adsorption of atrazine increases with soil organic carbon and a positive correlation was found between sCOD and atrazine, which would occur if atrazine is more readily adsorbed by soils with higher amounts organic matter. Abiotic hydrolysis also increases with soil organic matter content; catalyzed by hydrogen bonding to the undissociated carboxyl groups of humic and

fulvic acids (Gamble *et al.*, 1985). The experiment did not monitor hydroxyatrazine, the product of abiotic hydrolysis, or atrazine in plant tissues.

Results may have been affected both by the use of tap water for irrigation and the use of a flowable atrazine concentrate. City of Lawrence tap water contained chloramines, polyphosphates and other salts which accumulated in the mesocosms. The flowable concentrate contained particulate atrazine (0.5μm – 20μm) which is unlikely to behave like dissolved atrazine. Fine particles would be filtered by soil reducing dispersion depth. These particles would be dissolved over time into solution, creating localized increases in atrazine concentration, promoting adsorption to soil. Adjuvants in the formulated product may also affect atrazine interactions and deisopropyl- and deethylatrazine may have been present as contaminants. It is unknown whether these factors had significant effects on the outcome of the experiment.

Vegetation clearly plays a role in filter strips, mechanically to retain soil and filter overland flow and biologically to reduce soluble nutrients which are readily taken up during growth phases. Results indicate, however, that atrazine behavior in the soil is controlled largely by soil properties. While it is apparent that vegetation affects soil properties, as evidenced by differences in nitrate plus nitrite concentrations, it does not necessarily create soil conditions greatly enhancing atrazine reduction.

Vegetation may be slow to change preexisting soil conditions and microbial populations may need to be adapted for atrazine utilization. In this case, the presence

of organic nitrogen (TKN) and ammonia from chloramines may have precluded microbial use of atrazine as a nitrogen source. Given sufficient time, and a different source of irrigation water, nutrient ratios may have changed to favor microbial degradation. Time and recurring atrazine exposure may also have caused adaptation in the consortia of soil microbes. It is not clear to what degree adapted microbial populations are necessary for atrazine degradation.

The requirements for filter strips can be met by many types of vegetation. Physical properties which slow surface flow and enhance filtration, such as reed stiffness and density, are required for basic function. Results from this experiment also suggest the use plant communities with high biomass which may increase soil organic carbon. These factors would increase atrazine adsorption, abiotic hydrolysis and plant uptake. Once these requirements are met, selection of vegetation for use in a particular application may be governed by seasonality, ease of maintenance, availability as forage, cost, and aesthetics.

BIBLIOGRAPHY

Andreux, F., Scheunert, I., Adrian, P. (1990) The binding of pesticide residues to natural organic matter. *Preprints, Div. Evrn. Chem., Am. Chem. Soc.* 30(1):501-504.

Armstrong, D., Chesters, G., Harris, R. (1967) Atrazine hydrolysis in soil. *Soil Sci. Soc. Amer. Proc.* 31:61-66.

Bailey, W., White, J. (1970) Factors influencing the adsorption, desorption, and movement of pesticides in soil. *Residue Rev.* 32:29-92.

Bengston, R., Southwick, L., Willis, G., Carter, G. (1990) The influence of subsurface drainage practices on herbicide losses. *J. ASAE.* 33:415-418.

Calvet, R., Terce, M. (1975) Adsorption of atrazine by aluminum saturated montmorillonite. *Annales Agronomique.* 26(6): 693-707

Cook, A., Hütter, R. (1981) S-triazine as nitrogen source for bacteria. *J. Agric. Food Chem.* 29:1135-1143.

Cook, A., Hütter, R. (1984) Deethylsimazine: bacterial dechlorination, deamination and complete degradation. *J. Agric. Food Chem.* 32(3):581-585.

Dao, T., Lavy, T. (1978) Atrazine adsorption on soil as influenced by temperature, moisture content, and electrolyte concentration. *Weed Sci.* 26(3):303-308.

Eaton, R., Karnes, J. (1991) Cloning and comparison of the DNA encoding ammelide aminohydrolase and cyanuric acid amidohydrolase from three s-triazine-degrading bacterial strains. *J. Bacteriology.* 173(3):1363-1366.

Entry, J., Donelly, P., **Emmingham, W. (1994)** Microbial mineralization of atrazine and 2,4-dichlorophenoxyacetic acid in riparian and pasture soils. *Biol Fertil. Soils.* 18:89-94.

Entry, J., Mattson, K., **Emmingham, W. (1993)** The influence of nitrogen on atrazine and 2,4-dichlorophenoxyacetic acid grassland soils. *Biol Fertil. Soils.* 16:179-182.

Ezra, G., Decker, J., Stephenson, G. (1985) Tridiphane as a synergist for herbicides in corn (*Zea mays*) and proso millet (*Panicum miliaceum*). *Weed Sci.* 33:287-290.

Gamble, D., Kahn, S. (1985) Atrazine hydrolysis in soils: catalysis by the acidic functional groups of fulvic acid. *Can. J. Soil Sci.* 65:435-443.

Goswami, K., Green, R. (1971) Microbial degradation of the herbicide atrazine and its 2-hydroxy analog in submerged soils. *Evrn. Sci. Technol.* 5:426-429.

Hassall, K. (1990) *The Biochemistry and Uses of Pesticides –structure, metabolism, mode of action and uses in crop protection, 2nd ed.* MacMillan, London.

Heckman, J. (2003) Soil Nitrate Testing as a Guide to Nitrogen Management for Vegetable Crops. Rutgers Cooperative Extension Service. Rutgers, The State University of New Jersey. Bulletin E285.

Heri, W., Pfister, F., Carroll, B., Parshley, T., Nabors, J. (2008) Production, development, and registration of triazine herbicides. In: *The Triazine Herbicides-50 Years Revolutionizing Agriculture.* Ed. LeBaron, H., McFarland, J., Burnside, O., Elsevier. San Diego.

Jachetta, J., Appleby, A., Boersma, L. (1986) Apoplastic and symplastic pathways of atrazine and glyphosate transport in shoots of seedling sunflower. *Plant Physiol.,* 82(4):1000-1007.

Jordan, L., Farmer, W., Goodin, J., Day, B. (1970) Nonbiological detoxification of s-triazine herbicides. *Residue Rev.* 32:267-286.

Kalouskova, N. (1989) Adsorption of atrazine on humic acids. *J. Evrn. Sci. Health.* B 24(6):599-617.

Kaufman, D., Kearney, P. (1970) Microbial degradation of s-triazine herbicides. *Residue Rev.* 32:235-265.

Kemper, D., Dabney, S., Kramer, L., Dominik, D., Keep, T. (1992) Hedging against erosion. *J. Soil and Water Con.* July-August:284-288.

Kering, M., Biermacher, J., Butler, T., Mosali, J., Guretzky, J. (2012) Biomass yield and nutrient responses of switchgrass to phosphorus application. *Bioenerg. Res.* 5:71-78.

Koskinen, W., Banks, P. (2008) Soil movement and persistence of triazine herbicides. In: *The Triazine Herbicides-50 Years Revolutionizing Agriculture.* Ed. LeBaron, H., McFarland, J., Burnside, O., Elsevier. San Diego.

Krutz, L., Senseman, S., Zablotowicz, R., Matocha, M. (2005) Reducing herbicide runoff from agricultural fields with vegetative filter strips: a review. *Weed Sci.* 53:353-367.

Laird, D., Koskinen, P. (2008) Triazine soil interactions. In: *The Triazine Herbicides-50 Years Revolutionizing Agriculture.* Ed. LeBaron, H., McFarland, J., Burnside, O., Elsevier. San Diego.

Lamoureux, G., Rusness, D. (1986) Tridiphane (2-(3,5dichlorophenyl)-2-(2,2,2-trichloroethyl oxirane) an atrazine synergist: enzymatic conversion to a potent glutathione S-transferase inhibitor. *Pest. Biochem. Physiol.* 26:323-342.

Larson, R., Safferman, S. (2012) Field application of farmstead runoff to vegetated filter strips: surface and subsurface water quality assessment. *J. Evrn. Qual.* 41:592-603.

Leeson, A., Hapeman, C., Shelton, D. (1993) Biomineralization of atrazine ozonation products: application to the development of a pesticide waste system. *J. Agric. Food Chem.* 41(6):983-987.

Levanon, D. (1993) Roles of fungi and bacteria in the mineralization of the pesticides atrazine, Alachlor, malathion and carbofuran in soil. *Soil Biol. Biochem.* 25(8):1097-1195.

Lin, C., Lerch, R., Garrett, George, M. (2008) Bioremediation of atrazine-contaminated soil by forage grasses: transformation, uptake, and detoxification. *J. Evrn. Qual.* 37:196-206.

Mandelbaum, R., Sadowsky, M., Wackett, L. (2008) Microbial degradation of s-triazine herbicides. In: *The Triazine Herbicides-50 Years Revolutionizing Agriculture.* Ed. LeBaron, H., McFarland, J., Burnside, O., Elsevier. San Diego.

Mandelbaum, R., Wackett, L., Allan., D. (1993) Rapid hydrolysis of atrazine to hydroxyatrazine by soil bacteria. *Evrn. Sci. Tech*. 27:1943-1946.

Nair, D., Burken, J., Licht, L., Schnoor. (1993) Mineralization and uptake of triazine pesticide in soil plant systems. *J. Evrn. Eng.* 119(5):842-854.

Niklas, K. (2005) Modelling below- and above-ground biomass for non-woody and woody plants. *Annals of Botony.* 95:315-321.

Obien, S., Green, R. (1969) Degradation of atrazine in four Hawaiian soils. *Weed Sci.* 18:509-513.

Pedosphere.ca (2011) *Soil Texture Triangle Bulk Density Calculator (U.S.).* Pedosphere.ca. /Pedosphere.com http://pedosphere.ca/resources/bulkdensity/triangle_us.cfm

Rankins, A., Shaw, D. (2001) Perennial grass filter strips for reducing herbicide losses in runoff. *Weed Sci.* 49:647-651.

Raveton, M., Ravanel, P., Serre, A., Nurit, F., Tissut, M. (1997) Kinetics of uptake and metabolism of atrazine in model plant systems. *Pesticide Sci.* 49: 157–163.

Regehr, D., Peterson, D., Hickman, J. (1992) Questions and Answers About Atrazine. Cooperative Extension Service. Kansas State University. No. JH2-92-10M.

Roeth, F., Lavy, T. (1971) Atrazine translocation and metabolism in sudangrass, sorghum, and corn. *Weed Sci.* 19:98-101.

Rorabacher, D. (1991) Statistical treatment for rejection of deviant values: critical values of Dixon's "Q" parameter and related subrange ratios at the 95% confidence level. *Anal. Chem.* 63(2):139-146.

Schmitt, T., Dosskey, M., Hoagland, K. (1999) Filter strip performance and processes for different vegetation, widths, and contaminants. *J. Evrn. Qual.* 28:1479-1489.

Shimabukuro, R., Walsh, W., Lamoureux, G., Stafford, L. (1973) Atrazine metabolism in sorghum: chloroform-soluble intermediates in the n-dealkylation and glutathione conjugation pathways. *J. Agr. Food Chem.* 21(6):1031-1036.

Skipper, H., Gilmour, C., Furtick, W. (1967) Microbial verses chemical degradation of atrazine in soils. *Soil Sci. Soc. Amer. Proc.* 31:653-656.

Skipper, H., Volk, V. (1972) Biological and chemical degradation of atrazine in three Oregon soils. *Weed Sci.* 20(4):344-347.

Thelin, G., Stone, W. (2010) Method for Estimating Annual Atrazine Use for Counties in the Conterminous United States, 1992–2007. U.S. Geological Survey Scientific Investigations, Report 2010–5034.

Trebst, A. (2008) The mode of action of triazine herbicides. In: *The Triazine Herbicides-50 Years Revolutionizing Agriculture.* Ed. LeBaron, H., McFarland, J., Burnside, O., Elsevier. San Diego.

USDA-SCS, U.S. Department of Agriculture-Soil Conservation Service (1991) *National Handbook of Conservation Practices.* USDA-SCS. Washington, D.C.

von Felde, K., Kunst, S. (1997) N- and COD removal in vertical-flow systems. *Wat. Sci. Tech.* 35(5):79-85.

APPENDIX A

TABULATED RAW DATA

Above Ground Biomass

sample	AGB (g)
1	61.2
2	202.3
3	241.5
4	93.6
5	203.2
6	187.5
7	0.0
8	0.0
9	0.0
10	0.0
11	0.0
12	0.0
13	47.5
14	78.7
15	64.6
16	47.2
17	78.8
18	54.1

Atrazine and Soil Parameters

date	sample		% Moisture	COD (mg/kg)	NH4-N (mg/kg)	NO3+ NO2-N (mg/kg)	TKN-N (mg/kg)	TP-P (mg/kg)	DIA (µg/kg)	DEA (µg/kg)	ATZ (µg/kg)
8/25	1	U	18.4	-	-	-	-	-	-	-	-
8/25		m	17.1	-	-	-	-	-	-	-	-
8/25		d	17.6	-	-	-	104.0	29.3	-	-	2.02
8/25	2	u	14.2	-	-	-	117.1	25.8	-	-	24.61
8/25		m	12.4	-	-	-	109.1	24.8	-	-	0.94
8/25		d	12.2	-	-	-	101.0	23.4	-	-	0.36
8/25	3	u	18.6	-	-	-	94.2	23.8	-	-	2488.82
8/25		m	13.8	-	-	-	83.5	22.8	-	-	53.13
8/25		d	10.2	-	-	-	87.0	24.4	-	-	-
8/25	4	u	20.6	-	-	-	85.3	21.8	-	-	876.07
8/25		m	20.4	-	-	-	77.1	22.4	-	-	-
8/25		d	21.0	-	-	-	82.5	23.8	-	-	1.91
8/25	5	u	18.6	-	-	-	104.6	23.9	-	-	925.81
8/25		m	17.5	-	-	-	-	-	-	-	-
8/25		d	15.3	-	-	-	105.1	26.3	-	-	1.40
8/25	6	u	18.6	-	-	-	128.7	27.9	-	-	-
8/25		m	17.3	-	-	-	-	-	-	-	44.37
8/25		d	15.6	-	-	-	87.8	24.8	-	-	1.21
8/25	7	u	22.0	-	-	-	148.8	30.7	-	-	974.59
8/25		m	20.4	-	-	-	101.8	28.5	-	-	10.28
8/25		d	22.2	-	-	-	103.6	29.0	-	-	2.57
8/25	8	u	29.7	-	-	-	114.1	27.5	-	-	1212.80
8/25		m	16.3	-	-	-	90.7	24.3	-	-	11.23
8/25		d	16.4	-	-	-	-	-	-	-	12.89
8/25	9	u	16.0	-	-	-	118.5	26.1	-	-	793.68
8/25		m	16.2	-	-	-	112.3	26.6	-	-	31.43
8/25		d	19.7	-	-	-	95.3	26.5	-	-	11.38
8/25	10	u	18.5	-	-	-	111.1	27.5	-	-	1228.81
8/25		m	16.2	-	-	-	113.4	26.8	-	-	24.72
8/25		d	18.2	-	-	-	105.7	27.2	-	-	2.61
8/25	11	u	15.7	-	-	-	93.0	25.2	-	-	462.47
8/25		m	17.0	-	-	-	103.9	25.9	-	-	-
8/25		d	17.4	-	-	-	-	-	-	-	18.90
8/25	12	u	18.6	-	-	-	90.8	25.2	-	-	1169.90
8/25		m	16.7	-	-	-	107.1	25.3	-	-	2.95
8/25		d	16.2	-	-	-	91.3	25.5	-	-	4.39
8/25	13	u	22.0	-	-	-	121.4	27.1	-	-	693.00
8/25		m	20.7	-	-	-	91.1	25.3	-	-	0.83
8/25		D	22.2	-	-	-	98.5	26.4	-	-	0.82

8/25	14	u	14.1	-	-	-	102.5	27.1	-	-	1315.50
8/25		m	13.7	-	-	-	78.7	24.0	-	-	6.41
8/25		d	14.4	-	-	-	99.7	23.8	-	-	1.47
8/25	15	u	17.8	-	-	-	91.1	23.8	-	-	959.86
8/25		m	17.0	-	-	-	97.7	22.0	-	-	105.59
8/25		d	16.6	-	-	-	79.1	22.5	-	-	0.53
8/25	16	u	16.9	-	-	-	87.1	22.7	-	-	147.93
8/25		m	16.1	-	-	-	113.2	25.6	-	-	3.60
8/25		d	15.7	-	-	-	97.2	25.3	-	-	0.53
8/25	17	u	16.5	-	-	-	129.6	30.0	-	-	-
8/25		m	15.4	-	-	-	106.1	25.1	-	-	2.39
8/25		d	14.5	-	-	-	140.7	29.4	-	-	1.70
8/25	18	u	16.6	-	-	-	111.6	24.5	-	-	574.35
8/25		m	13.6	-	-	-	101.9	24.9	-	-	15.24
8/25		d	10.5	-	-	-	83.7	27.4	-	-	1.34
9/3	1	u	15.4	611	0.3	18.9	82.4	22.3	-	-	560.89
9/3		m	12.7	391	0.0	9.1	78.4	21.0	-	-	0.74
9/3		d	12.7	387	0.4	51.2	89.5	22.4	-	-	0.00
9/3	2	u	12.8	654	0.3	0.5	69.2	18.2	-	-	759.25
9/3		m	9.0	319	0.4	0.0	81.4	26.5	-	-	4.74
9/3		d	9.2	204	0.0	0.0	86.8	20.4	-	-	18.28
9/3	3	u	11.4	469	0.4	0.6	83.1	20.6	-	-	868.52
9/3		m	9.8	158	0.3	0.0	75.3	18.9	-	-	9.32
9/3		d	9.8	225	0.7	2.5	83.3	20.3	-	-	70.55
9/3	4	u	16.1	420	0.2	5.3	89.7	19.4	-	-	547.46
9/3		m	15.6	735	0.0	6.6	74.4	18.0	-	-	-
9/3		d	15.7	170	0.3	6.9	63.2	16.3	-	-	-
9/3	5	u	10.0	443	1.7	0.0	33.8	0.1	-	-	593.69
9/3		m	10.3	351	0.2	0.0	78.2	17.3	-	-	131.03
9/3		d	10.3	117	0.0	0.0	81.9	19.8	-	-	1.29
9/3	6	u	10.6	369	0.1	0.0	68.6	18.5	-	-	341.79
9/3		m	9.9	206	0.1	0.0	86.2	19.3	-	-	2.63
9/3		d	9.9	188	0.3	0.0	95.5	18.8	-	-	2.17
9/3	7	u	18.7	294	0.8	251.6	162.8	25.4	-	-	333.11
9/3		m	19.7	1110	0.3	85.1	94.6	17.5	-	-	53.89
9/3		d	22.0	1391	0.4	64.1	150.3	25.9	-	-	63.19
9/3	8	u	13.5	184	0.3	150.6	77.2	18.7	-	-	909.64
9/3		m	14.7	349	0.2	73.0	68.8	18.6	-	-	30.02
9/3		d	15.5	223	0.3	100.4	68.9	17.8	-	-	3.32
9/3	9	u	8.3	367	0.4	237.0	111.8	22.0	-	-	112.96
9/3		m	16.3	449	0.1	47.5	78.7	20.5	-	-	15.94
9/3		d	19.7	842	0.3	36.9	73.7	19.0	-	-	214.97
9/3	10	u	10.1	275	2.5	513.4	119.2	25.1	-	-	654.46
9/3		m	16.6	188	0.3	222.0	82.7	20.5	-	-	4.13
9/3		d	17.1	192	0.2	162.7	108.6	26.8	-	-	1.69

9/3	11	u	11.9	325	0.4	210.4	-	-	-	-	1376.10
9/3		m	12.3	117	0.4	136.5	68.4	16.7	-	-	-
9/3		d	16.8	236	0.4	104.4	95.9	23.2	-	-	-
9/3	12	u	11.1	315	0.4	40.6	68.8	17.6	-	-	543.18
9/3		m	16.1	463	0.3	64.0	84.3	20.8	-	-	43.06
9/3		d	16.2	187	0.4	80.7	125.6	27.3	-	-	4.39
9/3	13	u	18.0	581	0.5	9.6	73.6	16.2	-	-	250.54
9/3		m	20.5	909	0.5	8.6	100.1	20.9	-	-	3.05
9/3		d	17.6	839	0.6	17.0	89.2	20.7	-	-	3.35
9/3	14	u	9.5	430	0.4	8.3	70.1	16.5	-	-	145.72
9/3		m	11.1	603	0.5	5.5	96.2	21.4	-	-	2.82
9/3		d	16.4	607	0.3	5.3	54.9	17.5	-	-	5.68
9/3	15	u	13.3	162	0.5	56.8	72.5	20.1	-	-	1180.81
9/3		m	13.7	139	0.4	3.2	75.2	19.2	-	-	-
9/3		d	14.6	204	0.4	4.8	107.4	20.5	-	-	2.66
9/3	16	u	8.4	300	0.9	166.0	85.3	21.4	-	-	687.51
9/3		m	12.6	217	0.5	12.1	83.0	20.8	-	-	17.85
9/3		d	11.2	347	0.4	13.6	97.3	23.8	-	-	3.36
9/3	17	u	12.7	515	0.8	11.1	75.8	20.3	-	-	-
9/3		m	11.3	414	0.6	4.2	72.4	19.6	-	-	2.56
9/3		d	9.3	356	0.6	6.3	71.2	19.9	-	-	2.28
9/3	18	u	10.4	207	0.4	75.3	73.9	19.7	-	-	629.94
9/3		m	11.1	133	0.4	3.2	72.2	18.3	-	-	2.45
9/3		d	11.3	90.5	0.4	3.8	65.7	19.2	-	-	-
9/15	1	u	17.3	664	0.9	16.3	88.3	24.8	-	-	945.36
9/15		m	16.8	502	0.7	15.1	111.7	23.0	-	-	6.62
9/15		d	15.7	602	0.7	12.2	73.5	21.3	-	-	15.56
9/15	2	u	8.8	295	0.9	0.7	82.5	20.2	-	-	814.36
9/15		m	8.8	273	0.6	3.4	56.8	17.6	-	-	87.32
9/15		d	9.0	253	0.6	18.1	61.1	18.5	-	-	1.88
9/15	3	u	10.1	448	1.3	27.4	56.3	18.0	-	-	-
9/15		m	9.0	173	0.8	45.5	65.5	17.6	-	-	1.07
9/15		d	10.3	217	0.6	0.4	62.0	16.8	-	-	-
9/15	4	u	17.7	552	0.7	6.9	68.5	19.7	-	-	535.39
9/15		m	17.1	421	0.9	6.1	60.6	17.0	-	-	0.84
9/15		d	16.7	283	0.8	48.0	62.5	18.1	-	-	-
9/15	5	u	12.0	336	1.0	3.2	67.9	19.0	-	-	1489.95
9/15		m	10.6	334	0.7	1.0	61.5	17.7	-	-	25.65
9/15		d	11.4	131	0.8	3.8	48.3	20.4	-	-	2.21
9/15	6	u	12.5	209	1.2	0.0	52.9	17.8	-	-	417.38
9/15		m	11.4	173	0.4	205.9	61.2	21.4	-	-	6.91
9/15		d	11.7	189	0.9	0.6	58.9	19.8	-	-	0.99
9/15	7	u	21.4	238	1.3	2.0	66.8	24.1	-	-	-
9/15		m	21.9	141	0.3	160.9	49.6	23.0	-	-	6.77
9/15		d	23.4	310	0.3	101.6	68.1	23.7	-	-	345.38

9/15	8	u	12.8	247	0.6	68.5	52.8	20.0	-	-	92.14
9/15		m	15.5	214	0.6	47.2	55.9	19.0	-	-	27.81
9/15		d	15.9	132	0.7	92.0	57.0	19.7	-	-	26.48
9/15	9	u	12.6	191	0.7	83.8	82.2	21.7	-	-	822.56
9/15		m	17.9	176	0.6	36.3	71.7	20.6	-	-	74.79
9/15		d	17.3	454	0.4	24.6	67.8	21.9	-	-	32.93
9/15	10	u	11.5	272	4.0	736.7	81.3	23.7	-	-	520.33
9/15		m	16.3	155	0.6	117.8	73.9	22.5	-	-	21.30
9/15		d	17.0	212	0.9	43.2	64.0	20.3	-	-	23.10
9/15	11	u	12.1	104	1.1	385.0	97.6	23.6	-	-	222.48
9/15		m	15.6	109	0.6	156.3	77.2	23.7	-	-	8.57
9/15		d	17.3	145	0.5	124.7	74.2	20.7	-	-	2.17
9/15	12	u	12.9	199	0.5	43.5	85.0	23.4	-	-	261.00
9/15		m	16.5	361	0.6	43.9	57.1	18.6	-	-	124.87
9/15		d	16.3	149	0.9	76.2	95.8	21.4	-	-	48.33
9/15	13	u	19.9	565	1.4	6.1	82.1	22.9	-	-	146.85
9/15		m	20.9	652	1.1	5.7	63.7	21.8	-	-	459.27
9/15		d	21.3	827	1.2	8.3	86.5	23.2	-	-	546.20
9/15	14	u	12.3	314	1.2	4.5	68.0	16.9	-	-	1111.69
9/15		m	6.1	270	1.1	6.8	75.1	17.1	-	-	98.33
9/15		d	13.7	29.6	-	-	70.0	20.7	-	-	20.41
9/15	15	u	15.5	387	0.9	11.6	75.6	20.4	-	-	2686.84
9/15		m	14.9	289	0.5	1.1	73.1	18.9	-	-	24.53
9/15		d	15.1	246	1.8	10.0	68.7	19.4	-	-	4.26
9/15	16	u	8.8	535	0.8	12.2	46.2	18.5	-	-	2056.03
9/15		m	15.8	386	1.1	12.5	64.4	19.1	-	-	443.51
9/15		d	16.0	676	0.7	11.3	108.8	23.4	-	-	10.49
9/15	17	u	9.4	175	1.4	49.9	78.7	20.7	-	-	-
9/15		m	10.8	129	1.3	11.4	79.3	17.5	-	-	51.08
9/15		d	10.1	53	1.0	9.8	86.2	20.6	-	-	142.16
9/15	18	u	11.7	381	8.1	0.6	113.4	19.4	-	-	2013.54
9/15		m	10.7	169	0.5	3.9	46.0	16.6	-	-	-
9/15		d	10.0	52.2	0.7	5.2	90.7	41.2	-	-	55.59
9/22	1	u	19.5	789	1.4	13.0	102.6	24.2	103.61	216.88	337.58
9/22		m	12.5	293	1.1	23.9	96.7	25.6	0.04	0.05	0.47
9/22		d	14.9	266	0.7	20.5	46.5	13.5	0.10	0.19	1.86
9/22	2	u	10.1	313	0.9	6.4	74.3	18.7	31.63	88.71	812.34
9/22		m	6.7	348	1.2	0.0	67.4	18.5	1.50	1.95	15.24
9/22		d	5.9	179	1.5	0.0	53.6	14.9	2.31	3.32	37.41
9/22	3	u	9.6	170	0.8	0.0	68.3	19.4	31.38	62.70	512.50
9/22		m	7.4	213	0.8	0.0	75.1	17.3	0.24	0.20	1.58
9/22		d	5.3	320	1.8	0.0	83.7	21.3	0.12	0.10	1.25
9/22	4	u	6.9	279	1.1	4.1	55.3	15.5	64.82	286.96	1726.54
9/22		m	10.9	312	0.7	3.3	51.5	12.5	0.37	0.72	4.42
9/22		d	9.8	257	0.7	4.0	51.9	14.0	0.50	1.09	6.27

9/22	5	u	-	-	-	-	-	-	-	-	-
9/22		m	-	-	-	-	-	-	-	-	-
9/22		d	-	-	-	-	-	-	-	-	-
9/22	6	u	6.3	454	2.3	0.0	81.1	23.6	98.04	317.18	2344.85
9/22		m	8.2	251	1.3	0.0	73.1	16.1	1.72	2.14	16.96
9/22		d	8.9	124	1.3	0.0	48.7	23.2	0.48	0.44	5.06
9/22	7	u	15.8	644	0.3	174.1	113.9	20.7	63.77	20.39	248.27
9/22		m	4.9	461	1.8	61.7	67.0	22.9	18.58	9.29	189.91
9/22		d	5.4	555	1.5	90.1	69.8	20.5	-	-	-
9/22	8	u	4.4	373	3.7	444.6	69.9	20.1	59.51	44.09	479.45
9/22		m	3.9	635	1.5	128.0	33.6	23.7	1.59	1.28	18.39
9/22		d	4.7	508	3.1	104.4	39.3	20.0	0.60	0.26	3.35
9/22	9	u	15.3	1251	14.6	3029.8	159.1	23.2	5.09	1.71	18.04
9/22		m	4.6	557	3.0	1268.9	116.5	23.5	14.05	7.40	105.87
9/22		d	11.7	391	0.5	84.8	71.0	11.0	1.80	0.81	7.60
9/22	10	u	4.9	486	1.0	155.0	37.1	36.2	222.74	70.80	943.23
9/22		m	12.3	414	0.5	222.2	60.6	17.8	26.40	9.96	211.74
9/22		d	5.0	458	1.8	87.9	68.5	25.7	2.10	0.96	10.84
9/22	11	u	14.2	375	0.5	371.6	89.1	20.8	71.65	33.70	171.14
9/22		m	9.4	234	0.5	102.4	89.5	19.8	0.32	0.33	4.46
9/22		d	17.9	431	0.6	71.5	70.2	35.3	0.18	0.22	2.36
9/22	12	u	10.0	472	0.3	18.7	61.7	22.1	269.21	189.68	1548.29
9/22		m	4.5	358	1.5	36.3	61.3	17.7	4.84	4.51	30.03
9/22		d	16.2	983	0.2	76.1	67.7	17.5	248.87	93.02	98.59
9/22	13	u	7.0	2183	4.2	39.1	148.8	37.6	-	-	-
9/22		m	16.3	887	0.6	8.7	88.5	21.1	3.83	2.16	5.64
9/22		d	11.3	982	0.7	8.7	80.8	19.7	1.30	0.29	2.27
9/22	14	u	14.0	579	0.8	5.5	59.6	18.8	164.98	612.08	1951.87
9/22		m	13.3	339	0.5	3.7	65.2	23.1	1.73	4.07	25.49
9/22		d	12.7	348	0.4	4.0	62.1	21.5	2.27	6.67	33.06
9/22	15	u	16.5	557	0.7	10.9	72.1	21.0	275.05	661.50	2488.17
9/22		m	9.7	237	0.4	6.4	70.2	19.6	3.15	10.38	38.41
9/22		d	7.5	290	0.6	5.2	64.7	19.8	0.43	1.17	7.10
9/22	16	u	15.7	346	0.8	22.7	71.0	24.1	218.15	335.14	866.07
9/22		m	11.3	458	0.5	12.1	72.8	19.5	0.89	2.19	9.37
9/22		d	15.1	350	0.4	11.1	69.1	20.9	1.15	2.42	12.51
9/22	17	u	13.4	471	0.6	27.6	64.3	19.8	141.66	187.56	1145.01
9/22		m	10.9	404	0.4	7.9	83.1	21.4	4.76	7.97	63.49
9/22		d	12.0	312	0.7	9.4	68.7	20.8	0.65	0.84	5.80
9/22	18	u	10.9	404	0.5	12.6	72.3	22.2	57.48	101.49	281.12
9/22		m	8.7	452	0.4	2.9	62.2	17.9	0.81	1.08	6.67
9/22		d	10.8	402	0.4	5.8	70.3	21.5	0.76	1.09	5.98
10/23	1	u	15.4	966	1.9	24.1	94.2	23.1	26.89	39.89	67.00
10/23		m	15.2	441	2.1	18.3	84.1	23.6	2.33	0.98	3.88
10/23		d	15.4	368	2.0	12.7	118.4	25.3	0.39	0.54	2.37

10/23	2	u	15.0	593	2.6	0.0	99.5	22.0	138.60	237.82	1007.61
10/23		m	10.6	555	1.7	0.0	96.7	21.1	4.45	11.06	73.37
10/23		d	10.6	472	2.4	1.7	66.7	20.3	139.51	130.48	269.94
10/23	3	u	12.1	765	1.6	0.6	75.1	20.3	-	-	-
10/23		m	6.3	459	1.8	0.0	64.4	18.1	4.47	7.90	90.04
10/23		d	8.5	459	1.5	0.0	61.3	18.4	2.08	2.49	14.62
10/23	4	u	8.2	684	2.3	4.0	86.1	21.1	34.84	80.38	513.23
10/23		m	8.1	466	2.1	0.5	74.3	18.8	0.58	0.63	3.55
10/23		d	8.2	417	2.3	0.4	81.0	20.1	1.03	0.68	2.20
10/23	5	u	12.6	508	2.8	0.0	92.2	22.2	55.43	91.69	505.95
10/23		m	6.6	875	2.1	0.0	79.4	20.1	-	-	-
10/23		d	7.9	411	1.4	0.9	87.3	21.5	0.20	0.06	0.67
10/23	6	u	14.1	503	3.3	2.5	95.8	21.1	46.81	62.66	190.66
10/23		m	6.4	468	2.6	0.0	74.6	19.6	1.59	19.75	146.66
10/23		d	8.7	384	1.4	0.8	83.1	19.6	-	-	-
10/23	7	u	15.9	761	1.1	621.7	86.0	21.4	5.41	11.28	126.78
10/23		m	14.8	781	0.5	52.4	81.8	22.8	0.41	0.36	3.38
10/23		d	19.4	570	0.7	49.1	74.2	22.7	3.49	0.16	1.90
10/23	8	u	13.8	364	1.1	193.9	77.3	21.5	127.68	253.40	631.85
10/23		m	12.7	466	0.4	146.6	75.6	19.7	1.13	3.10	9.87
10/23		d	14.0	253	0.5	155.3	66.7	22.8	0.20	0.11	1.91
10/23	9	u	16.1	425	4.0	1132.7	100.5	23.3	15.04	33.70	305.59
10/23		m	16.6	268	1.0	75.4	72.8	22.7	0.16	0.25	1.87
10/23		d	17.9	357	0.7	44.4	51.7	20.5	0.26	0.25	2.29
10/23	10	u	15.0	380	0.8	211.6	83.7	23.3	96.66	114.53	82.83
10/23		m	15.6	448	0.7	51.2	62.6	20.0	1.25	1.62	4.80
10/23		d	17.2	449	0.9	68.0	80.8	24.4	6.85	0.33	3.04
10/23	11	u	12.9	356	0.8	210.7	82.2	22.3	59.72	95.49	341.70
10/23		m	10.3	356	1.0	168.4	82.8	22.2	3.36	1.73	5.82
10/23		d	15.4	228	0.7	116.6	79.3	22.1	-	-	-
10/23	12	u	13.5	394	0.8	86.1	77.0	27.6	208.13	345.46	500.10
10/23		m	11.1	375	0.7	67.3	79.6	20.4	4.15	10.11	34.47
10/23		d	16.2	342	0.8	56.6	67.0	22.5	1.39	3.25	8.68
10/23	13	u	20.3	578	1.9	11.6	100.0	24.5	-	-	-
10/23		m	19.4	309	1.6	1.3	91.1	23.6	-	-	-
10/23		d	20.7	135	1.3	4.3	84.1	21.9	0.24	0.58	2.81
10/23	14	u	16.0	510	1.5	6.5	65.7	18.1	86.78	201.44	595.13
10/23		m	16.4	498	1.2	2.6	76.6	20.2	18.39	79.89	261.65
10/23		d	26.8	480	1.6	4.3	105.2	27.3	0.39	0.98	3.66
10/23	15	u	16.8	756	1.8	13.9	97.9	21.7	10.07	19.35	31.00
10/23		m	18.6	517	1.7	8.1	92.2	21.3	-	-	-
10/23		d	15.3	386	1.3	5.6	80.1	19.1	-	-	-
10/23	16	u	17.9	481	2.3	24.1	94.9	21.2	37.58	51.01	57.68
10/23		m	17.4	258	1.1	11.7	73.1	21.4	3.31	1.28	5.39
10/23		d	17.9	263	2.0	12.2	94.8	22.4	-	-	-

10/23	17	u	16.4	467	2.5	31.9	87.0	18.9	-	-	-
10/23		m	13.7	354	1.4	10.4	81.9	19.5	1.27	2.90	12.39
10/23		d	13.6	590	1.4	8.9	100.7	22.5	0.29	0.07	5.57
10/23	18	u	16.0	529	2.2	12.9	124.5	26.4	9.46	17.92	34.88
10/23		m	14.9	322	1.5	5.1	93.1	26.0	0.48	1.11	4.49
10/23		d	9.5	308	1.0	4.2	97.7	19.4	1.91	0.14	1.99

APPENDIX B

CRP PRARIE MIX ANALYSIS

* * * * * * * SEED ANALYSIS * * * * * * * *

AGRICULTURAL SEED Sharp Bros. Seed Co., Clinton, Missouri

Lot No.	BBK-3188-M	BIG BLUESTEM KAW			
Pure Seed	55.76%	Germination	88.00%	Origin	MO
Other Crop	0.08%	Firm Seed	0.00%	Tested	03/93
Weed Seed	0.00%	Tot Germ-Frm	88.00%	Noxious	NONE
Inert	44.16%	PLS	49.07	PLS #2.20 Bulk 4.00	

We warrant to the extent of the purchase price, that the seeds sold are as described on the container, within recognized tolerances.
We give no other further warranty, expressed or implied. SHARP BROS. SEED CO.OF MISSOURI, CLINTON, MISSOURI

AGRICULTURAL SEED Sharp Bros. Seed Co., Clinton, Missouri

Lot No.	BLA-7186	LITTLE BLUESTEM ALDOUS			
Pure Seed	76.23%	Germination	87.00%	Origin	KS
Other Crop	0.00%	Firm Seed	0.00%	Tested	01/93
Weed Seed	0.74%	Tot Germ-Frm	87.00%	Noxious	NONE
Inert	23.03%	PLS	66.31	Pls .60	.90 Bulk

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We give no other further warranty, expressed or implied. SHARP BROS. SEED CO.OF MISSOURI, CLINTON, MISSOURI

AGRICULTURAL SEED Sharp Bros. Seed Co., Clinton, Missouri

Lot No.	IYO-3172-M2	YELLOW INDIANGRASS OSAGE			
Pure Seed	92.66%	Germination	87.00%	Origin	MO
Other Crop	0.19%	Firm Seed	4.00%	Tested	02/93
Weed Seed	0.03%	Tot Germ-Frm	91.00%	Noxious	NONE
Inert	7.12%	PLS	84.31	Pls 1.20	Bulk 1.40

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We give no other further warranty, expressed or implied. SHARP BROS. SEED CO.OF MISSOURI, CLINTON, MISSOURI

AGRICULTURAL SEED Sharp Bros. Seed Co., Clinton, Missouri

Lot No.	SWB-7069	SWITCHGRASS BLACKWELL			
Pure Seed	99.32%	Germination	96.00%	Origin	KS
Other Crop	0.00%	Firm Seed	0.00%	Tested	10/92
Weed Seed	0.45%	Tot Germ-Frm	96.00%	Noxious	NONE
Inert	0.23%	PLS	95.34	Pls .30	Bulk .30

We warrant to the extent of the purchase price, that the seeds sold are as described on the container, within recognized tolerances.
We give no other further warranty, expressed or implied. SHARP BROS. SEED CO.OF MISSOURI, CLINTON, MISSOURI

AGRICULTURAL SEED Sharp Bros. Seed Co., Clinton, Missouri

Lot No.	76/5	SIDEOATS GRAMA EL RENO			
Pure Seed	84.38%	Germination	77.00%	Origin	TX
Other Crop	0.00%	Firm Seed	3.00%	Tested	04/93
Weed Seed	0.33%	Tot Germ-Frm	80.00%	Noxious	NONE
Inert	15.29%	PLS	67.50	Pls .60	Bulk .90

We warrant to the extent of the purchase price, that the seeds sold are as described on the container, within recognized tolerances.
We give no other further warranty, expressed or implied. SHARP BROS. SEED CO.OF MISSOURI, CLINTON, MISSOURI

AGRICULTURAL SEED Sharp Bros. Seed Co., Clinton, Missouri

Lot No.	WWB-7021	WESTERN WHEATGRASS BARTON			
Pure Seed	92.85%	Germination	37.00%	Origin	KS
Other Crop	0.00%	Firm Seed	40.00%	Tested	09/92
Weed Seed	0.02%	Tot Germ-Frm	77.00%	Noxious	NONE
Inert	7.13%	PLS	71.48	Pls 1.00	Bulk 1.40

We warrant to the extent of the purchase price, that the seeds sold are as described on the container, within recognized tolerances.
We give no other further warranty, expressed or implied. SHARP BROS. SEED CO.OF MISSOURI, CLINTON, MISSOURI