Short-term native grassland compositional responses following liquid hog manure application

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Bork, E. W. and Blonski, L. J. 2012. **Short-term native grassland compositional responses following liquid hog manure application**. Can. J. Plant Sci. **92**: 55–65. Intensive livestock operations (ILOs) are becoming more common in remote regions of the Canadian prairies in an effort to reduce conflict with other land uses. This has led to ILOs situated where the typical sink for manure application, cultivated land, is not available, leading to growing interest in using native grasslands for manure disposal. Significant opposition exists to this practice, in part due to limited information available on the impact of manure application to native grassland diversity and species composition. We examined plant species composition changes over two growing seasons following varying rates (~9.5, 19, 38, 75 and 150 kg ha^{-1} available N), methods (surface broadcast vs. coulter injected) and timing (fall vs. spring) of one-time liquid hog manure (LHM) application. Our results revealed divergent responses between study sites, with metrics of plant diversity declining in mixed prairie but increasing in fescue grassland with increases in manure application rate. Both communities also demonstrated minor changes in plant species composition, primarily in response to LHM rate and manure application method. Responsive plant species included a mix of native grasses and dicots, some of which may be regarded as undesirable (e.g., *Artemisia frigida*): however, most vegetation responses were temporary and disappeared by the second year. Although invasion of non-native plant species was not observed, suggesting these communities are tolerant of LHM application, changes in the reproductive effort of dominant grasses (negative in *Hesperostipa curtiseta*; positive in *Pascopyrum smithii* and *Festuca hallii*) suggest LHM application could alter long-term grassland composition. Thus, despite exhibiting resilience to one-time LHM application, future use of native grasslands for manure disposal should be done cautiously.

Key words: Best management practices, biodiversity, hog manure disposal, inflorescence density, native grassland, weed invasion

Bork, E. W. et Blonski, L. J. 2012. **Réaction à court terme des prairies naturelles à l'application de lisier de porc**. Can. J. Plant Sci. **92**: 55–65. L'élevage intensif gagne en popularité dans les régions reculées des Prairies canadiennes, l'espoir étant qu'on réduise ainsi les conflits avec d'autres vocations des terres. Cette tendance a pour conséquence que les élevages intensifs se retrouvent maintenant où n'existent pas de terres cultivées, sur lesquelles est habituellement épandu le fumier. C'est pourquoi on s'intéresse de plus en plus à la fumure des prairies naturelles. Malheureusement, cette pratique suscite une vive opposition, en partie parce qu'on sait peu de choses des répercussions de l'application du fumier sur la diversité et sur la composition des espèces dans ces prairies. Les auteurs ont examiné l'évolution de la composition des espèces végétales pendant les deux périodes végétatives qui ont suivi l'application unique d'une quantité variable de lisier de porc (~9,5, 19, 38, 75 et 150 kg de N disponible par hectare), selon diverses méthodes (épandage à la volée en surface c. injection par coutre) et le moment de l'application (automne c. printemps). Les résultats indiquent que la réaction varie avec le site, la diversité des plantes diminuant dans les prairies mixtes et augmentant dans les prairies à fétuque avec la hausse de la quantité de fumier épandue. Des changements mineurs au niveau de la composition des espèces ont été observés dans les deux cas, principalement en réaction au taux et à la méthode d'application du lisier. Parmi les espèces sensibles figure un mélange de graminées et de dicotylédones indigènes, certaines pouvant être considérées comme nuisibles (par ex., *Artemisia frigida*). Néanmoins, dans la majorité des cas, la réaction s'avère temporaire et disparaît dès la deuxième année. Quoiqu'on n'ait pas observé l'envahissement par des espèces non indigènes, signe que les populations tolèrent l'application du lisier, les modifications relevées au niveau de la reproduction des graminées dominantes (négatives pour *Hesperostipa curtiseta*; positives pour *Pascopyrum smithii* et *Festuca hallii*) laissent croire qu'une telle pratique pourrait altérer la composition des prairies à long terme. Par conséquent, en dépit d'une résilience évidente à une seule application de lisier de porc, on devrait faire preuve de prudence en recourant aux prairies naturelles pour l'élimination du fumier dans l'avenir.

Mots clés: Pratiques exemplaires, biodiversité, élimination du fumier de porc, densité des inflorescences, prairies naturelles, envahissement par les mauvaises herbes

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Abbreviations: **FG**, fescue grassland; **ILO**, intensive livestock operations; **LHM**, liquid hog manure; **MP**, mixed prairie; **MRPP**, multi-response permutation procedure; **NMS**, non-metric multidimensional scaling; **perMANOVA**, permutations based multiple analysis of variance

Intensive livestock operations (ILOs) have been moving towards increased confinement feeding and greater densities of livestock (McKenna and Clark 1970; Jackson et al. 2000), in turn increasing the potential for conflict with adjacent land users (Jongbloed and Lenis 1998). In an attempt to circumvent this, many ILOs across western Canada, including large-scale hog facilities, have been establishing in sparsely populated semi-arid regions (Canada-Alberta Environmentally Sustainable Agriculture Agreement 1991). While such activities may limit conflict over aesthetics and odor, this expansion remains dependent on the effective disposal of waste such as liquid hog manure (LHM).

Manure applications must be conducted in a manner that does not contribute to soil, water or air pollution, while remaining compatible with efficient and sustainable crop production (Evans et al. 1977). Profit maximization from the use of manure has led large-scale producers to the concept of manure management at minimum cost, while remaining below certain "nuisance" levels (McKenna and Clark 1970). While traditional sinks for LHM in the Canadian prairies have been cultivated lands, these are routinely not available in semi-arid regions. Given that it is unrealistic to transport manure long distances, alternative lands must be considered for LHM disposal. Although less productive relative to agronomic croplands (Blonski et al. 2004), native rangelands are often abundant adjacent to these operations and have been considered as an alternative sink for LHM.

Previous research has documented the impact of fertilization (e.g., Kowalenko and Bittman 2000) and manure (e.g., Bittman et al. 1997) on tame pastures, but less information exists on the effects of nutrient addition, particularly LHM, to native rangelands (Power and Alessi 1971). Of those that have, they generally indicate that application of nitrogen (N) can increase production and forage quality (Blonski et al. 2004). Little is known, however, about the influence of manure application on plant composition of native grasslands. As growth of semi-arid grasslands is limited primarily by moisture (Willms and Jefferson 1993), community responses to nutrients are often tied to rainfall (e.g., Read 1969), and may therefore limit vegetation responses.

Improper nutrient application may alter communities to the detriment of their long-term sustainability, including undesirable changes in vegetation attributes. There is strong experimental evidence documenting reductions in community diversity with increasing nutrients, particularly N (Wilson and Tilman 1991), and manure applications are known to lead to increased weed biomass (Singh and Ghoshal 2010). Other studies suggest nutrient addition can alter community composition: for example, N application can favor graminoids over dicots (Dougherty and Rhykerd 1985). Moreover, nutrient application can eliminate flora or increase invasive plants (Goetz 1969; Berg 1995). While some species appear capable of rapidly capitalizing on nutrients (e.g., *Artemisia frigida*; Goetz 1969), others express long-term residual responses to nutrient addition, in part due to increased seed production in response to fertility (Loeppky et al. 1999). For example, Berg (1995) found warm-season plantings in Oklahoma took 2 yr to demonstrate vegetation responses. Finally, biomass increases known to occur from manure (Blonski et al. 2004) are likely to increase litter, which in turn, is known to influence species diversity in temperate grasslands (Lamb 2008).

The method of manure application may also impact native grasslands. Physical disturbances associated with directly incorporating LHM into rangeland soils could encourage compositional changes by promoting competitive release of disturbance adapted plants (Huston 1979), conserving N through reduced volatilization (Webb et al. 2010), and ultimately favoring plants with more direct access to N placed near roots. While Olson and Papworth (1999) found no concerns with manure injection on tame pastures in Alberta, parallel studies on native grassland have not been done. Similarly, the timing (e.g., season) of manure application is another factor seldom addressed when applying nutrient amendments as manure to native grasslands. Applying N fertilizers in late fall or early spring has been observed to promote cool-season species (Samuel and Hart 1998) over short-statured, warm-season grasses such as *Bouteloua gracilis*, which instead are known to respond better to applications of N later in the growing season (Whiteman 1980).

As environmental factors are key drivers of management decisions on native grasslands, particularly public rangelands, and may influence government regulations of associated land uses (Neeteson 2000), the objective of this research was to assess plant community responses within two native grasslands following LHM application, including varying rates, methods and seasons of application over 2 yr. A secondary objective was to determine the potential effects of LHM application on inflorescence development.

MATERIALS AND METHODS

Study Sites

Field trials were conducted in southeastern Alberta, approximately equidistant from the municipal centers of Hanna and Drumheller (lat. 51°22N, long. 112°13′W), between October 1998 and August 2000. This region represents the juxtaposition of the Mixed-grass Prairie and Aspen Parkland natural sub-regions, the latter of which includes northern Fescue Grasslands. The area has a gently undulating landscape and experiences a continental climate. The 30-yr average precipitation was 394 mm, while growing season precipitation (May to August, inclusive) averaged 217 mm. However, there is considerable inter-annual variation in growing conditions.

Rainfall during 1999, the first year of treatment response, was favorable for plant growth, with precipitation from May 27 to Aug. 24, totaling 284 mm (averaged across sites), which was approximately 30% greater than the long-term norm. In contrast, dry conditions prevailed in 2000, with rainfall from May 03 through Aug. 15 of only 106 mm. Although this total represents a conservative estimate for growing season precipitation, 2000 was below the long-term norm (e.g., ~–50%).

Manure application trials were conducted within two native grasslands representing the dominant communities of the region: more xeric Mixed Prairie (MP) and relatively mesic Fescue Grasslands (FG). Specific range types on the MP and FG sites were the *Stipa*-*Agropyron* and *Festuca*-*Stipa* faciations, respectively (Coupland 1961). The latter community was situated within a shallow but broad valley dominated by plains rough fescue [*Festuca hallii* (Vasey) Piper], while the MP site was on an upland bench and dominated by western porcupine grass [*Hesperostipa curtiseta* (Hitchc.) Barkworth], Junegrass [*Koeleria macrantha* (Ledeb.) Schult.], and dryland sedges (*Carex* spp.), with minor amounts of western wheatgrass [*Pascopyrum smithii* (Rydb.) A. Love], Hookers oatgrass [*Avenula hookeri* (Scribn.) Holub] and blue grama grass [*Bouteloua gracilis* (HBK) Lag.] (Table 1).

Soil conditions ranged from a Brown Chernozem with 3.9% organic matter at the MP site, to a Dark Brown Chernozem with 4.9% organic matter at the FG site. Total available soil N levels in September prior to the study were 1.94 and 1.48 mg kg^{-1} in the MP and FG sites, respectively, and pH levels were 6.6 and 6.3 for the MP and FG sites. Soils at both sites were loamy in texture. Study sites were 150 by 50 m in size and were selected on the basis of homogeneity of slope, aspect, topographic position and associated drainage, as well as vegetation. Both sites had a history of mid to late summer grazing at light stocking rates (~1.6 ha AUM^{-1}), with all vegetation considered to be in late seral status at the time of the study.

Table 1. Summary of mean cover values for all plant species with greater than 1% cover during the study period

	Mixed Prairie Grassland		Fescue Grassland	
Species	1999	2000	1999	2000
Achillea millefolium	0.2	0.1	1.9	1.3
Anemone patens	6.8	2.4	–	–
Artemisia frigida	11.2	2.2	0.8	0.5
Artemisia ludoviciana	0.1	<0.1	0.9	1.3
Astragalus spp.	1.8	0.1	1.2	<0.1
Avenula hookeri	4.8	2.4	1.6	1.0
Bouteloua gracilis	1.7	1.2	<0.1	<0.1
Carex spp.	15.8	8.8	7.8	6.7
Elymus trachycaulus	0.1	0.1	1.2	0.8
Festuca hallii	4.8	4.8	42.8	29.1
Hesperostipa curtiseta	15.3	9.5	4.0	7.2
Koeleria macrantha	13.4	4.6	0.2	0.2
Pascopyrum smithii	5.0	0.8	1.5	0.6
Rosa arkansana	0.3	<0.1	1.1	0.6
Vicia americana	–	–	3.1	0.4

Manure Application

Treatments were conducted within each site using a randomized experimental design with 20 plots, each 7 by 50 m in size, consisting of combinations of five different target rates of LHM (10, 20, 40, 80 and 160 kg NH_3-N ha^{-1}), applied with one of two methods (dribble broadcast vs. subsurface injection), at each of two times. Fall applications of LHM were conducted from 1998 Oct. 05 to 07, while spring applications were done from 1999 Apr. 12 to 13, after frost thaw but prior to the onset of plant growth.

All manure applications were conducted using the Greentrac liquid injection system. This technology was used because of its capability to achieve accurate LHM application rates. The Greentrac employs a pressurized tank and distributer to deliver manure through hoses to injector shanks that lie behind vertical coulters spaced 25 cm apart. Manure was injected to a maximum depth of 10 cm. Surface applications were made using the same equipment, with the applicator raised 30 cm enabling manure to be deposited on the ground surface. This technique differs from traditional splash-plate application, which generates greater agitation but applies manure at less accurate rates.

All manure came from an open lagoon storage system associated with a 4000 head farrowing sow operation situated within 10 km of the study sites. Manure samples were taken from the storage lagoon and analyzed for nutrient content 2 wk prior to each application period (fall and spring) so that the applicator could be calibrated to obtain the appropriate rates. Orifice diameters within the manure distributor on the Greentrac applicator and machine speeds were adjusted to obtain the required rates. During treatment, each tank-load of manure was sampled and subsequently tested for nutrient content to ensure consistency among loads (Blonski 2001). Available nitrogen, primarily as NH_4-N, was the main constituent of the applied LHM, and averaged 0.17% in fall and 0.21% in spring. Volumes applied depended on seasonal manure content, but ranged from 11 000 to 107 000 L ha^{-1} (Table 2), with actual manure N values from each load used to derive final N application (Table 2). A 1-m buffer was maintained between all plots.

Vegetation Sampling

Both study sites were fenced in spring 1999 to prevent livestock grazing during summer. The composition of plant species was determined by canopy cover (to the nearest percent) in each plot using a series of 0.1-m^2 quadrats systematically located along a randomly located transect. Preliminary sampling and assessment of cover-area curves indicated that 20 quadrats (2 m^2) and 15 quadrats (1.5 m^2) were sufficient for providing stable cover values for each plant species in plots of the MP and FG sites, respectively (Blonski 2001). Species canopy cover was assessed at peak vegetative growth in each plot and site during 1999 and 2000, between Jul. 26 and Aug. 18. Total species richness, diversity and evenness were determined for each plot to assess aggregate community responses to the treatments.

Inflorescence density of *Festuca hallii* on the FG site and *Hesperostipa curtiseta, Koeleria macrantha*, and *Pascopyrum smithii* at the MP site, were sampled in each year to gain an understanding of how treatments may alter reproductive fitness. Inflorescences were counted within four, randomly placed 0.5-m^2 quadrats in each plot.

Analysis

Species composition data within each plot and site-year were initially summarized to richness (species number, or s) and Shannon's diversity. Diversity was quantified using the formula

$$H' = \sum (Pi \times \ln Pi)$$

where Pi is the proportion of total species cover comprised of each individual species i. Additionally, evenness (J) was quantified by dividing H' by lns, the natural log of species richness. Effects of season and method of manure treatment on richness, diversity and evenness, together with the density of inflorescences of various grass species, were assessed using single-degree freedom contrasts ($P<0.05$) within each site-year, while the effect of manure rate (9.5 to 150 kg ha^{-1} N) was examined using linear least squares regression ($P<0.05$) with Proc Reg in SAS 9.1.3 (SAS Institute, Inc., Cary, NC).

Table 2. Target and actual LHM application rates for fall and spring applications

Season	Target manure application rate (kg ha^{-1} NH_4-N)	Actual manure application rate (kg ha^{-1} NH_4-N)	LHM application rate volumes (L ha^{-1})
Fall	10[a]	9.3	13 000
	20	18.6	13 000
	40	37.1	27 000
	80	74.2	53 000
	160	148.4	107 000
Spring	10[z]	9.5	11 000
	20	19.0	11 000
	40	37.9	21 000
	80	75.8	42 000
	160	151.6	84 000

[z]Application volumes for the 10 and 20 rates are the same because the 10 rate was achieved by mixing LHM and water at a 1:1 ratio due to equipment limitations.

Detailed plant species compositional responses to the experimental treatments at each site were assessed with multivariate analysis and ordination using PC-Ord v.5 (MJM Software Design, Gleneden Beach, OR). Multivariate techniques allow assessment of detailed changes in composition to be examined in relation to external treatments (McCune and Grace 2002), in this case LHM rate, application method and season of application. A permutations based MANOVA (perMANOVA) and a multi-response permutation procedure (MRPP) were performed using the Sorensen distance measure to directly test the impact of each fixed treatment on community composition. Significance in the MRPP ($P<0.05$) was based on the probability of the observed delta statistic being smaller or equal to the expected delta based on 100 randomized trials. For the perMANOVA, 4999 randomizations were used and significance was based on the proportion of randomized trials with an indicator cover greater or equal to the observed cover value, calculated as follows:

$$P = [(1 + \text{number of runs} \geq \text{observerd})/(1 + \text{number of randomized runs})].$$

Separate analyses were performed for data from 1999 and 2000 to examine immediate and residual second year responses to a single manure application. An Indicator Species Analysis using 4999 permutations was done on each site-year of plant community data to identify those plant species that responded directly to the treatments ($P<0.05$).

Finally, data were examined using non-metric multidimensional scaling (NMS) ordinations to assess patterns among plots in relation to the treatments. Real data were run 250 times, as were the randomized data for the Monte Carlo test. A total of 500 iterations were used to obtain the final stable solution with instability of 0.00001. Axes scores were not rotated, and were interpreted based on Pearson correlations with all treatments and indicator species (min $r>|0.30|$).

RESULTS

Mixed Prairie Responses

We documented a total of 40 plant species across the MP site in 1999 but only 29 in 2000. While total richness, diversity and evenness in either year did not demonstrate any relation with method ($P\geq0.10$) or season ($P\geq0.08$) of manure application, species richness decreased at the MP site in 1999 with increasing rates of manure ($P=0.03$; Table 3). Neither diversity nor evenness exhibited any response to manure rate.

Inflorescence densities of *Koeleria macrantha* were greater ($P<0.01$) in fall treated plots (33.0 ± 4.5 stems m^{-2}) than spring plots (9.5 ± 4.5 stems m^{-2}) during 1999, with no residual effects in 2000. While *Hesperostipa curtiseta* did not respond to season or method of manure application, inflorescence densities of this species declined in 2000 with increasing manure application rates ($P=0.02$; Table 3). In contrast, inflorescence densities of *Pascopyrum smithii* increased with manure rates ($P<0.0001$; Table 3), but did not respond to season or method.

The MRPP analysis of data from the MP site in 1999 indicated this community did not respond to season (A $=-0.00038$, $P=0.40$), method (A $=-0.00009$, $P=0.39$) or rate (A $=0.020$, $P=0.27$) of manure application. The perMANOVA provided similar results for all treatments ($P\geq0.16$). Indicator analysis revealed that fall applied manure favored the dicot *Phlox hoodii* ($P=0.04$), and that greater rates of manure were associated with increases in the native forb *Artemisia frigida* ($P=0.03$). Similarly, the grass *Avenula hookeri* increased with manure rates ($P=0.02$), but peaked at 75 kg ha^{-1} of N. No species were associated with method of application.

Ordination with NMS of the data from 1999 provided a three-dimensional solution (all axes $P=0.004$), with final stress of 8.56. Axis 1 represented 15.0% of species variance while axes 2 and 3 represented 10.1 and 66.4%, respectively (see Fig. 1A). Correlation of the treatments with ordination axes indicated that manure application method was correlated with axes 1 and 2, while manure rate was correlated with axes 2 and 3 (Table 4). Season was poorly correlated with all axes.

During the second growing season, no effects of season, method, or rate of manure application were evident at the MP site based on the MRPP ($P\geq0.23$) and perMANOVA ($P\geq0.18$). This was further supported for manure rate with a separate MRPP test using only the 9.5 and 150 kg ha^{-1} N treatments ($P=0.15$). Ordination of the 2000 data using NMS indicated a two-dimensional solution with final stress of 9.69, where axes 1 ($P=0.03$) and 2 ($P<0.01$) explained 8.7 and 84.0% of species variance, respectively (Fig. 1B). Manure application method and rate were both correlated with axis 1 (Table 4). Indicator species analysis revealed a number of relationships between treatments and plant species. While *P. pratensis* remained positively associated with the spring application of manure ($P=0.03$), the dicot *Androsace septentrionalis* ($P=0.03$) was associated with fall application. The grass *Avenula hookeri* remained greater on injected plots ($P=0.04$), and in contrast to the year before, *Artemisia frigida* ($P=0.03$) became negatively associated with greater rates of manure in 2000.

Fescue Grassland Responses

We documented a total of 42 plant species (or groups) at the FP site in 1999, but only 32 in 2000. Similar to the MP site, no impacts of application method or season were evident on total richness, diversity or evenness at the FP site in either year ($P\geq0.21$). However, both species diversity and evenness at the FP site increased with manure application rate in 1999 ($P<0.01$; Table 3), relationships that remained 1 year later ($P\leq0.02$; Table 3). These responses were paralleled by weak positive responses in richness at the FP site ($P=0.06$ and $P=0.08$ in 1999 and 2000, respectively, data not shown). Although inflorescence densities of *Festuca hallii* did not respond to season or method of manure application in either year ($P\geq0.58$), inflorescence density of this species responded positively to manure rate in 2000 ($P=0.02$; Table 3).

Table 3. Summary of the empirical relationship between manure application rate and plant species richness, Shannon's diversity, species evenness, and the inflorescence density (stems m^{-2}) of several grasses, at each of the MP and FG sites during each of 1999 and 2000

Site	Year	Response	F stat	P value	Adj R^2	Relationship[z]
Mixed Prairie Grassland	1999	Spp. richness	5.29	0.03	0.18	$18.2-0.015x$
		Spp. diversity	0.01	0.94		–
		Spp. evenness	2.53	0.13		–
		K. macrantha inflorescences	0.02	0.89		–
		H. curtiseta inflorescences	0.55	0.47		–
		P. smithii inflorescences	23.4	<0.001	0.54	$-0.47+0.030x$
	2000	Spp. richness	3.78	0.07		–
		Spp. diversity	2.16	0.16		–
		Spp. evenness diversity	0.00	0.95		–
		K. macrantha inflorescences	0.38	0.54		–
		H. curtiseta inflorescences	6.79	0.02	0.23	$25.3-0.112x$
		P. smithii inflorescences	2.38	0.14		–
Fescue Grassland	1999	Spp. richness	3.94	0.06		–
		Spp. diversity	11.46	<0.01	0.36	$0.54+0.0015x$
		Spp. evenness diversity	0.982	<0.01	0.32	$0.204+0.00049x$
		F. hallii inflorescences	2.98	0.10		–
	2000	Spp. richness	3.50	0.08	0.12	$12.6+0.014x$
		Spp. diversity	14.6	<0.001	0.42	$0.57+0.00094x$
		Spp. evenness diversity	6.28	0.02	0.26	$0.228+0.00027x$
		F. hallii inflorescences	17.4	<0.001	0.46	$33.1+0.23x$

[z]Equations and r^2 are provided only for those relationships with $P<0.05$. The independent (x) variable is kg ha^{-1} of N applied in LHM.

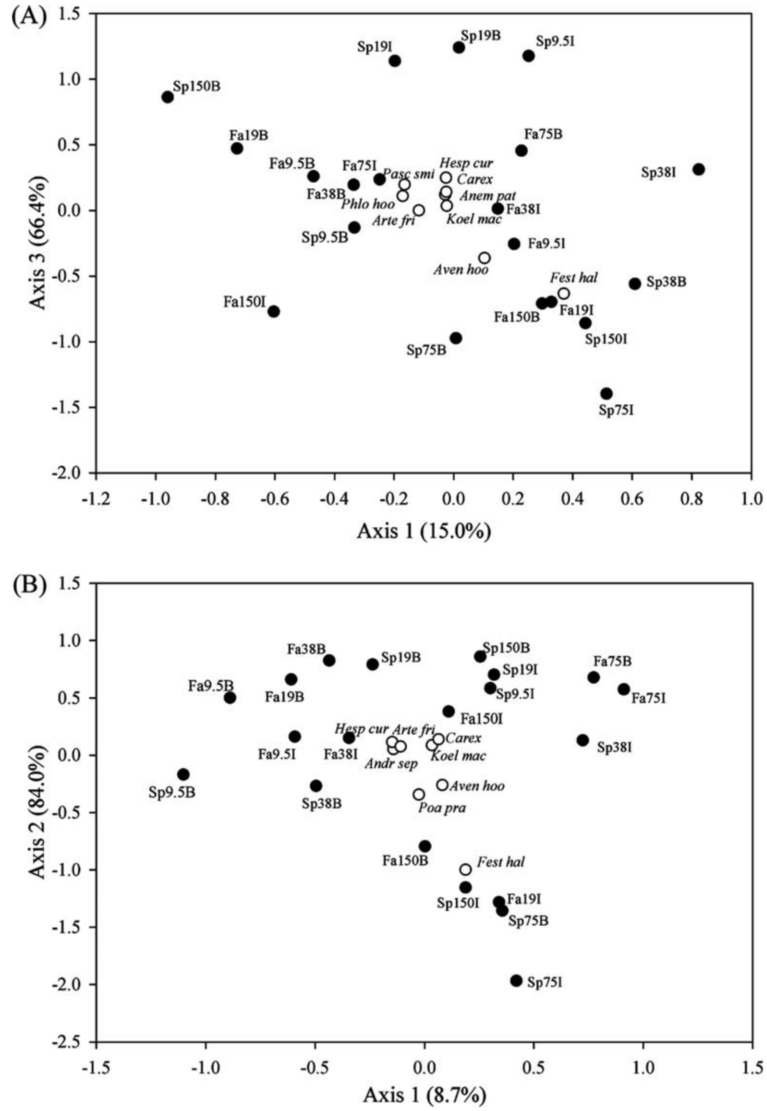

Fig. 1. Results of the NMS ordination analysis of plant composition data from the Mixed Prairie site in each of (A) 1999 and (B) 2000. The first year ordination shows the first and third axes, which accounted for the most species variance. Plot labels (solid symbols) indicate season of application, manure rate and method, respectively. Species shown (open symbols) include indicator species and those plants with at least 4% cover in the community.

First year responses in species composition at the FP site were again limited primarily to manure application method (A = 0.039, $P = 0.04$) rather than season (A = −0.007, $P = 0.57$) or rate (A = −0.018, $P = 0.65$) based on the MRPP, with similar findings from the perMANOVA (e.g. method, $P = 0.04$). However, comparison of LHM rates in the MRPP indicated that plots receiving the 9.5 and 150 kg ha^{-1} N rates differed in composition ($P = 0.04$). Ordination of data from 1999 using NMS indicated a two-dimensional solution ($P < 0.01$) with final stress of 11.35, where axes 1 and 2 accounted for 22.5 and 70.2% of variance in species (Fig. 2A). Method and rate of manure application were both correlated primarily with axis 1 (Table 4). Indicator analysis revealed *Hesperostipa curtiseta* was associated with spring applied manure ($P = 0.02$), and both *Pascopyrum smithii* ($P = 0.02$) and *Koeleria macrantha* ($P = 0.05$) were positively associated with manure rate. Additionally, several other species remained positively associated ($P \leq 0.04$) with either the injection (*Vicia americana*, *Artemisia ludoviciana*) or broadcast application (*Carex spp*., *Anemone multifida*) of manure.

No effects of season, method or rate were evident on species composition within the FG site during 2000 based on the MRPP ($P \geq 0.10$), although the perMANOVA suggested a residual effect remained of manure application method (F = 1.87, $P = 0.08$). The absence of any residual rate effect was further confirmed

Table 4. Summary of Pearson correlations between individual axes scores and treatment factors or indicator plant species following non-metric multi-dimensional scaling (NMS) analysis of species composition at each of two sampling sites and two sampling years following LHM application. Bolded correlations indicate those with $|r| > |0.30|$

			NMS ordination axes		
			Axis 1	Axis 2	Axis 3
Site	Year	Treatment/species	*r*	*r*	*r*
Mixed Prairie Grassland	1999	Plant spp. variance explained	15.0%	10.1%	66.4%
		Season	0.256	0.016	0.107
		Method	**0.361**	**−0.300**	−0.148
		Rate	−0.112	**0.474**	**−0.390**
		Artemisia frigida	−0.249	**−0.797**	0.103
		Avenula hookeri	0.180	0.135	**−0.623**
		Phlox hoodii	**−0.445**	−0.126	0.161
	2000	Plant spp. variance explained	8.7%	84.0%	NA
		Season	0.133	−0.221	
		Method	**0.434**	−0.206	
		Rate	**0.397**	−0.238	
		Androsace septentrionalis	−0.140	0.034	
		Artemisia frigida	**−0.594**	0.271	
		Avenula hookeri	0.219	**−0.457**	
		Poa pratensis	−0.024	−0.211	
Fescue Grassland	1999	Plant spp. variance explained	22.5%	70.2%	NA
		Season	−0.050	−0.102	
		Method	**0.347**	0.276	
		Rate	**0.300**	−0.172	
		Anemone multifida	0.175	**−0.460**	
		Artemisia ludoviciana	**0.708**	0.271	
		Carex spp.	−0.132	**−0.886**	
		Hesperostipa curtiseta	−0.061	**−0.505**	
		Pascopyrum smithii	**0.519**	**−0.554**	
		Vicia americana	**0.715**	0.113	
	2000	Plant spp. variance explained:	48.1%	47.1%	NA
		Season	−0.039	−0.009	
		Method	**−0.359**	**0.343**	
		Rate	**−0.519**	**−0.359**	
		Achillea millefolium	**−0.561**	**0.417**	
		Anemone multifida	0.119	−0.190	
		Artemisia ludoviciana	**−0.550**	**0.453**	
		Elymus trachycaulus	**−0.723**	−0.282	
		Pascopyrum smithii	**−0.691**	−0.235	
		Penstomen procerus	0.042	0.269	
		Solidago missouriensis	**−0.411**	**0.540**	
		Vicia americana	0.005	**0.472**	

with a separate MRPP test on manure rates using only the 9.5 and 150 kg ha^{-1}-N treatments ($P=0.17$). Results of the perMANOVA were further supported by the finding that several species were correlated with method in the indicator analysis: specifically the abundance of *Penstomen procerus* ($P=0.04$), *Artemisia ludoviciana* ($P=0.05$), *Achillea millefolium* ($P=0.05$), *Vicia americana* ($P=0.02$), *Elymus trachycaulus* ($P=0.04$), *Solidago missouriensis* ($P=0.02$) and *Pascopyrum smithii* ($P=0.04$), were all associated with the injection rather than broadcasting of manure. *Pascopyrum smithii* was also positively correlated with manure rate ($P=0.05$), and only *Anemone multifida* was correlated ($P=0.01$) with application season (i.e., fall application). The NMS ordination indicated a two-dimensional solution with stress of 9.03, where axes 1 and 2 accounted for 48.1 and 47.1% of variance in species composition ($P<0.02$) (Fig. 2B). Method and rate were both associated with axes 1 and 2 (Table 4).

DISCUSSION

Overall Diversity Responses

Application of LHM generally had weak effects on plant composition within both study sites. Additionally, most responses occurred during the first year, and disappeared by the second growing season, suggesting these grasslands are relatively tolerant to one-time LHM application, and resilient following short-term changes in species composition. However, increased responses during 1999 also coincided with high rainfall, which may have been a critical prerequisite enabling these grasslands to respond to added nutrients, as vegetation

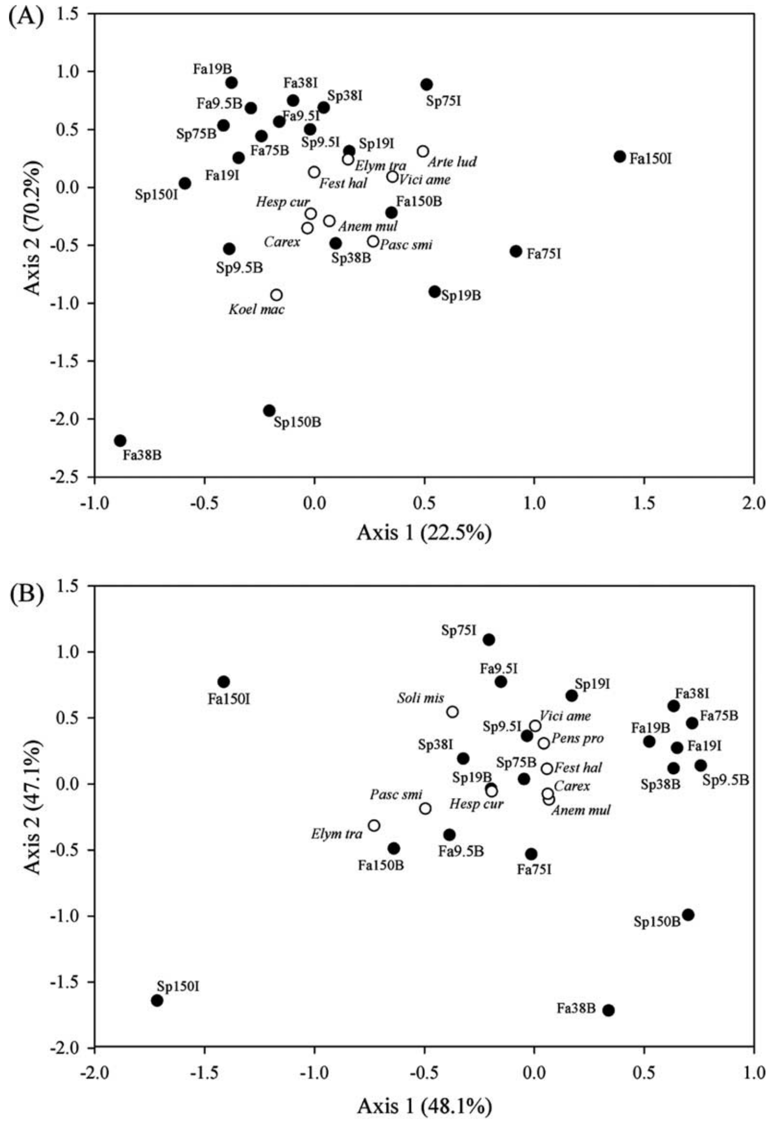

Fig. 2. Results of the NMS ordination analysis of plant composition data from the Fescue Grassland site in each of (A) 1999 and (B) 2000. Plot labels (solid symbols) indicate season of application, manure rate and method, respectively. Species shown (open symbols) include indicator species and those plants with at least 4% cover in the community.

growth in this region is limited primarily by soil moisture (Willms and Jefferson 1993). Although residual effects of the manure treatments remained in 2000, they were weak and expressed largely through individual species responses. Although low rainfall in the second year could have limited the expression of vegetation responses to manure, another explanation is that observed depletion of available soil N by spring 2000 (Lambert 2002) may have limited vegetation responses to manure the year prior. Reduced moisture in year 2 may also account for the overall reduction in species richness at both sites. Nevertheless, our findings reinforce the ability of native grasslands to respond to N in LHM (Blonski et al. 2004), particularly when moisture is abundant.

Contrasting responses in overall diversity were observed between the MP and FG sites to manure application. Decreases in species richness with increasing manure rates at the MP site are attributed to a large increase in the size and abundance of opportunistic plant species adapted to high N, particularly dominant grasses, which, in turn, may have suppressed neighboring species, primarily dicots that had a low initial abundance. This pattern has been observed in other studies following nutrient addition including prairie grasslands (Dougherty and Rhykerd 1985). In contrast, diversity, specifically evenness, increased with greater rates of manure at the FG site in 1999, a pattern that persisted into the following year. As richness was generally similar between sites, LHM high in N appears to have allowed species with normally minor abundance in the FG community to assume greater relative abundance. Moreover, this effect persisted into the following year despite depletion of measured soil N (Lambert 2002).

Plant Species Responses

Only a small number of plant species were explicitly affected by manure application. *Avenula hookeri* exhibited peak abundance at intermediate LHM rates of 75 kg ha^{-1}, suggesting it was either less competitive under, or intolerant of, high rates of N, or may have had a limited capacity to exploit surplus N given its low initial abundance (<4% cover). *Avenula hookeri* also appears to be disturbance tolerant or even dependent given its positive response to manure injection at the FG site. Peak *K. macrantha* and *P. smithii* abundance were found at maximum rates of LHM within the FG site, with *P. smithii* responses persisting into the second year. Increases in *P. smithii* have been found in other studies that assessed nutrient addition on native prairie (e.g., Samuel and Hart 1998).

Heavy applications of nutrients have been associated with an increase in the abundance of annual forbs (Todd et al. 2004), and the maintenance of species-rich communities has been suggested as a mechanism to defend against invasion of undesirable plant species (Milbau et al. 2005). In the current study, increasing LHM was associated with increases in the abundance of the undesirable native plant *A. frigida* at the MP site, suggesting this species is well positioned to exploit added nutrients. However, the reversal of this pattern 1 yr later, when maximum levels of this species were found under the lowest rate of LHM, indicates that the initial response was transient. The likely mechanism responsible for the marked decline in *A. frigida* is strong interspecific competition imposed by the threefold greater biomass generated by high rates of LHM in 1999 (Blonski et al. 2004), and is supported by studies elsewhere indicating *A. frigida* is an opportunistic species, but is also susceptible to competition from grasses (Gao et al. 2005).

The limited overall changes in abundance of undesirable plant species under low application rates of manure suggest this practice can be consistent with native grassland conservation. These results are similar to studies examining dairy manure application to warm season grassland in the southwestern US (Stavast et al. 2005). These findings are also not surprising given that long-term studies show N inputs to agro-ecosystems from biological fixation or atmospheric sources can be as high as 30 kg ha^{-1} even in the absence of fertilization (Jenkinson 1991). To our knowledge the current study is the first examining native grassland responses to hog manure. Menalled et al. (2005) assessed weed seed bank responses to composted swine manure in Montana, but on cropland, and Pleasant and Schlather (1994) assessed the potential for weed spread from cow manure: both concluded manure application posed limited risk of increasing the weed seed bank.

Several plant species responded to the method of LHM application, particularly at the FG site. During the first growing season following treatment, two species were favored by injection, while two others were negatively impacted by this treatment. These results suggest divergent tolerance among native plants to coulter injection of manure. Species like *Carex* and *A. multifida* appear to have been negatively impacted by injection despite its low associated soil impact (i.e., less than 15% of the soil surface was disturbed). In contrast, many other species were favored by manure injection, potentially due to reduced N loss to the atmosphere (Lambert and Bork 2003; Webb et al. 2010) or improved nutrient placement within the immediate rooting zone of these species (Chen et al. 2001).

Finally, although season of manure application had limited impacts on plant species in this investigation, those species responding were generally greater following fall rather than spring manure application. Plants likely benefited under fall application from the extended dormant season that followed, which we hypothesize may have allowed for earlier use of applied nutrients. Nevertheless, both *H. curtiseta* and the naturalized species *P. pratensis* preferred spring applications of LHM. Previous studies have shown that *P. pratensis* responds better to spring fertilizer applications (Oral and Acikgoz 2001).

Inflorescence Responses in Dominant Grasses

Many perennial native grass species are long-lived and rely proportionately more on vegetative reproduction than seed to survive. Despite this, the application of LHM to these perennial grasslands resulted in some notable changes to inflorescence production of dominant grasses. Changes in seed production can lead to altered community composition over time, or alternatively provide a buffer for the existing community against future environmental change.

Increased flowering of grasses has been observed in response to fertilization, particularly with N (Loeppky et al. 1999). In the current study, increased inflorescences of both *F. hallii* (FG site) and *P. smithii* (MG site) under increasing LHM rates may be associated with their extensive root mass (Coupland and Johnson 1965), which would enhance the nutrient uptake capacity of these species relative to neighboring plants. The 2-yr delay in the increase of *F. hallii* is consistent with studies from other regions of Alberta where marked inflorescence responses in *Festuca* have occurred the second growing year after disturbance (Gerling et al. 1995; Bork et al. 2002). The latter response has been linked to the timing of floral induction (i.e., meristem specialization) that occurs in the fall prior to dormancy (Johnston and MacDonald 1967). In the current study, LHM application in the late fall of 1998 and spring of 1999 was likely after floral induction, which instead occurred in the fall of 1999, thereby accounting for the delayed but large increase in inflorescences during 2000. As *F. hallii* is a species whose seed is frequently in demand for reclamation of disturbed native grasslands, these results suggest that LHM may be a practical tool to increase seed availability.

In contrast to *F. hallii*, *H. curtiseta* declined in inflorescence density, but not until the second growing season after LHM application. Although the exact cause of this response is unknown, we hypothesize this may be in response to the increased growth of neighboring plants the year prior. Increased competition or associated environmental stress can lead to reduced inflorescence production in *H. curtiseta*, similar to that observed by Bailey and Anderson (1978) following prescribed fire. Decreased inflorescence production in some grasses has also been linked with reduced light availability (Willms 1988), and may have occurred here due to the large increase in herb biomass under maximum rates of LHM (Blonski et al. 2004).

CONCLUSION

Application of increasing rates of LHM high in N led to relatively minor but nonetheless detectable changes in species diversity and associated plant composition in both Fescue and Mixed Prairie grasslands. Although observed responses were relatively transient and had largely disappeared by the second growing season, suggesting these communities remained relatively tolerant to a one-time application of LHM, below-normal rainfall may also have limited vegetation responses in the second year. Observed individual species responses to treatments, primarily rate and method, were varied and likely reflected differences in their adaptation to nutrient addition, coupled with changes in inter-specific growth among neighbors within the plant community. Although no invasion by new plant species was found, temporary increases in the abundance of the undesirable forb *Artemisia frigida* were observed. Overall, these findings highlight the tolerance of these grasslands to periodic nutrient addition, but also identify the need for longer-term studies to assess the role of growing conditions in mediating vegetation responses, the implications of manure rates greater than those tested here as well as repeated applications over time. We also note that we did not assess environmental concerns outside of vegetation responses in this investigation, such as impacts on air and water quality, which should be examined as well.

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