Structural equation modeling reveals complex relationships in mixed forage swards

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ABSTRACT

Relationships among vegetation components in perennial pastures are complex, particularly those including grasses, weeds, legumes, and other forbs. Where herbicides are used for broadleaf weed control, a trade-off may exist between the benefits of weed removal and legume loss. Few studies have separated the contribution of different vegetation components to total forage yield, either prior to or after spraying. Herein we use Structural Equation Modelling (SEM) to quantify relationships among grasses, legumes (*Medicago sativa* L. or *Trifolium* spp.), a common noxious weed (*Cirsium arvense* (L.) Scop), and other forbs, at two locations within the Parkland region of central Alberta, Canada. After removal of broadleaf vegetation with herbicide, we quantified changes in forage relative yield ratio (RYR) for two years. The SEM approach revealed marked differences in the relationships among sward components between sites. At the more mesic site, abundant thistle biomass had little influence on other sward components and no benefit was observed post-spraying from weed removal. In contrast, even low levels of thistle biomass suppressed grass and legume biomass at the more xeric location, and post-spraying responses revealed benefits from weed removal. Unexpectedly, legumes were found to suppress grass biomass at both sites, suggesting strong interspecific competition between forage types. Subsequent removal of legumes appeared to release grass biomass from competition within sprayed plots, as exemplified by increased forage yields two years after spraying. These results highlight the complexity within temperate perennial pastures, and add clarification to the potential short-term impacts of weeds and legumes to overall sward dynamics and forage production.

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1. Introduction

Pastures are dynamic in composition and prone to invasion by noxious weeds such as Canada thistle (*Cirsium arvense* L.). This particular weed is found worldwide, including temperate regions of Canada and the northern United States (Holm et al., 1997; Wilson and Kachman, 1999). Presence of Canada thistle is known to decrease productivity in annual crops (O'Sullivan et al., 1982; O'Sullivan et al., 1985), as well as the yield of forage in pasture (Grekul and Bork, 2004).

In addition to the competitive nature of Canada thistle, justification for the control of this weed is reinforced through regulations mandating its control (e.g. Province of Alberta (2008)). Options available for controlling this species include mowing (Beck and Sebastian, 2000; Schreiber, 1967), tillage (Lukashyk et al., 2008), burning (Tranicek et al., 2005), biological techniques such as forced ungulate grazing (De Bruijn and Bork, 2006), as well as herbicides (Enloe et al., 2007; Grekul and Bork, 2007). Although the most effective suppression of pasture weeds often requires an integrated approach (Masters and Sheley, 2001), broadleaf herbicides remain a popular and effective method for weed control (DiTomaso, 2000).

Invasion of noxious weeds into pastures results in complex sward dynamics (Tracy and Sanderson, 2004; Sanderson et al., 2007; Bork et al., 2007) and includes negative impacts on

neighboring forage plants. Canada thistle is an effective competitor for available resources and space (Donald, 1990) and reduces forage growth (Reece and Wilson, 1983; Grekul and Bork, 2004), accessibility to livestock (Haggar et al., 1986) as well as species diversity (Stachlon and Zimdahl, 1980). However, the full agronomic, ecologic and economic impacts of noxious weeds within pastures generally remain poorly understood (Lym and Duncan, 2005).

Many studies have examined the production benefits of grass-legume mixtures (Sleugh et al., 2000; Frame and Harkess, 1987; Holt and Jefferson, 1999). Legumes are valued for the ability to directly increase forage yields (Malhi et al., 2002; Popp et al., 2000), but can also lead to facilitation (i.e. improved growth) of neighboring plants (Nyfeler et al., 2009). Presence of legumes within mixed swards can increase the availability of soil nitrogen (N) for neighboring grasses through N fixation in association with *Rhizobium* bacteria (Walley et al., 1996). However, facilitation of growth in pastures may be beneficial to both neighboring grasses and weeds. Previous studies on Canada thistle have demonstrated that this species responds positively to fertilization in the absence of weed control (Grekul and Bork, 2007). In mixed swards that include perennial grasses, weeds and legumes, simultaneous competition for resources and facilitation from enhanced nutrient availability will determine net forage productivity. Outcomes will therefore vary depending on species proximity, competitiveness and abundance.

Weed control with broadleaf herbicides in mixed pastures is likely to lead to a trade-off between the desirable control of competitive weeds and the undesirable loss of beneficial legumes. Although generally assumed that legume removal will reduce net forage yields within mixed pasture swards, this remains untested. As legumes can provide competition against grasses for resources (Hill, 1990), the removal of legumes may allow grasses to increase in growth and this could partly or fully offset the opportunity cost of legume removal, a response that may be further augmented by weed removal. In annual crop rotations legume removal has led to increased grain yield and protein concentration (Cutworth et al., 2010; Jefferson et al., 2013). Thus, testing the ability of grasses to compensate for the removal of legumes, including under variable weed presence, is important for pasture management.

Plant community dynamics are difficult to quantify using conventional yield loss methodology, in part due to the spatial heterogeneity present in pastures, which likely accounts for the variable forage losses found between environments (Grekul and Bork, 2004). However, contemporary methods to evaluate empirical relationships among environmental phenomenon have greatly improved, and now include techniques such as Structural Equation Modeling (SEM). Essentially, SEM aims to generate strong and distinct links between theoretical and experimental ideas (Grace et al., 2010). The ability to disentangle causal relationships and test competing models and theory (as opposed to null hypotheses) are key strengths of SEM methods. Thus, SEM provides a framework to decipher complex networks involving numerous response and predictor variables (Grace et al., 2010). Because of its statistical strength and applicability, SEM approaches have been employed in a wide range of environmental and ecological studies (e.g., Shipley, 2000; Grace, 2006; Jonsson and Wardle, 2010; Lamb et al., 2011a, 2011b; Stewart et al., 2011). To our knowledge SEM methods have not been applied to study weed impacts in pasture.

The goal of this investigation was to quantify relationships among grass, legume and weed (i.e. Canada thistle) abundance in two contrasting perennial pastures, prior to spraying and after spraying with non-selective broadleaf herbicide. Specific objectives were to 1) quantify the competitive or facilitative relationships between grasses, legumes, Canada thistle (CT), and other forbs prior to spraying, and 2) evaluate the net effects of varying levels of

CT removal and legume loss following herbicide application on total forage production in mixed pasture swards.

2. Materials and methods

2.1. Study sites

Two established pastures were selected for this investigation from 2005 to 2007 inclusive, both situated in the Aspen Parkland natural sub-region of central Alberta, Canada. Sites were internally uniform (i.e. slope, aspect, drainage, etc.), and contained a minimum of 30% legume cover, with Canada thistle densities averaging 18.0 and 23.5 stems m^{-2} among plots at the PCF and LI sites, respectively, during the first year. Typical of pastures in the region, legume and thistle populations were not uniformly distributed across each site and this heterogeneity was used to facilitate the assessment of inter-specific relationships among vegetation components.

Lake Isle (LI) is located approximately 70 km NW of Edmonton, Alberta (53° 39′ N; 114° 43′W) on an imperfectly drained riparian floodplain with a Gleyed Black Chernozemic soil. The LI site was an old growth pasture (age >20 years) with a diverse plant community dominated by timothy (*Phleum pratense* L.), smooth brome (*Bromus inermis* Leyss), and substantial amounts of clover (primarily *Trifolium repens* L.). The Parkland Conservation Farm (PCF) site was located near Mundare, Alberta (53° 39′ N; 112° 20′ W), approximately 90 km east of Edmonton, on a well-drained upland with an Orthic Black Chernozemic soil. This sward had been seeded in 1999, six years prior to the initiation of the study, and was dominated by meadow brome (*Bromus riparius* Rehm.), smooth brome, and alfalfa (*Medicago sativa* L.).

Average annual precipitation from the Environment Canada weather stations nearest the LI and PCF sites, was 530 and 403 mm, respectively. While precipitation levels in 2005 were near average, rainfall in 2006 and 2007 at both sites was generally below norms from June through August (Fig. 1). The average annual temperature for the region is 4.3 °C, with a typical frost free period of about 110 days.

2.2. Study design and sampling

Our study design included two complementary components. First, an *in-situ* empirical examination of pasture composition was done prior to spraying in 2005 at each site. This was followed up with the examination of sward responses post-spraying in 2006 and 2007 to assess net forage responses to broadleaf removal with herbicide application in 2005. At each site, 100 permanent 1 m^2 plots were established along a series of linear transects with a minimum 1 m buffer from other plots. Plots were permanently marked to facilitate relocation for repeated measurement. Each plot included a centrally located 0.25 m^2 (i.e. 50 × 50 cm) quadrat within which all biomass measurements were taken.

Biomass was harvested annually from each of the 0.25 m^2 permanent quadrats at peak growth (mid-July to early August) and sorted to perennial grasses, legumes, CT, and other broadleaf forbs. Prior to harvest, the density of CT stems was quantified within each quadrat. Biomass was oven-dried at 60 °C to constant mass and weighed. Both study sites were fenced to prevent grazing prior to sampling in mid-summer. Moderate grazing with cattle occurred on both sites each fall after the first killing frost, which helped prevent excess litter accumulation and allowed sites to remain consistent with typical land use practices in the region.

In the fall of 2005, 80 of the 100 plots at each site were randomly selected and sprayed with the residual broadleaf herbicides aminopyralid (120 g ae ha^{-1}) and 2,4-D (1440 g ai ha^{-1}) using an all-
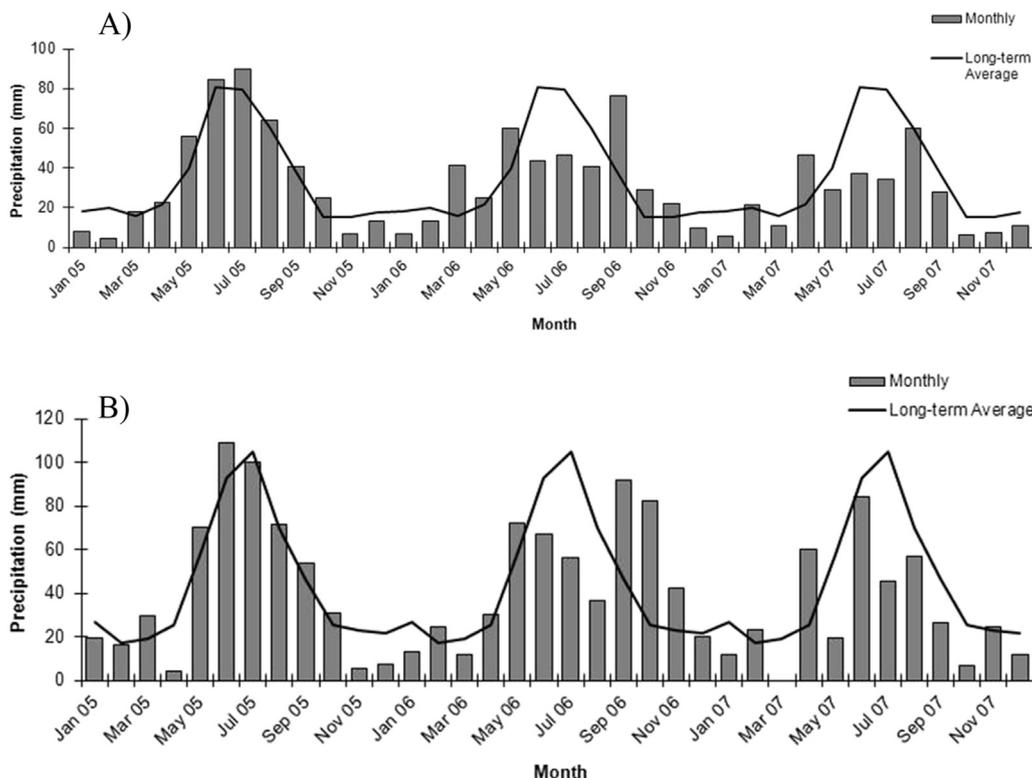
Fig. 1. Average monthly and long-term annual precipitation (mm) at A) the Parkland Conservation Farm site, and B) the Lake Isle site, during the period 2005 through 2007.

terrain vehicle mounted, CO_2 propelled sprayer equipped with Teejet flatfan 8003 nozzles at 32 psi calibrated to deliver $100 \, l \, ha^{-1}$. The remaining 20 unsprayed plots at each site were used to account for natural inter-annual variation in the abundance of broadleaf vegetation components over the balance of the study (described below). Maintenance spraying with the same equipment was used in June 2006 to apply aminopyralid ($120 \, g \, ae \, ha^{-1}$) to remove any remaining Canada thistle and legumes within sprayed plots.

2.3. Data analysis

Data for each study site were analyzed separately due to marked differences in site characteristics, species composition and age of the swards, and separate analyses were conducted for pre-spraying and post-spraying data from 2005 to 2006/2007, respectively. A combination of SEM methods and multiple regression were used to analyze the data and exploit the natural variability in biomass components among plots.

The SEM approach was used to examine the direct and indirect effects of Canada thistle on other sward components, including legumes, other forbs and grasses prior to herbicide application in 2005. SEM is an advanced multivariate statistical tool that allows testing of complex path-relation networks and distinguishes causal relationships from mere correlations (Grace et al., 2010). Given the distinct differences in vegetation composition and ecosite characteristics (climate, soils, etc.) between sites, we developed separate models for each site. A fundamental assumption for estimating models is normality. Prior to constructing models, univariate and multivariate normality were checked using univariate and multivariate Shapiro–Wilk normality tests in R *v.* 3.0.1 statistical package (R Foundation for Statistical Computing, Vienna, Austria). To test multivariate normality the *mvShapirotest* and *mvnormtest* packages were used. Data were log transformed and standardized to achieve

univariate and multivariate normality. In establishing initial relationships within the SEM (Fig. 2), double-headed arrows represent those that are unresolved or uncertain, whereas single headed arrows indicate causal and direct effects of a variable. The first step of SEM methods is to create an initial model based on previous knowledge, site information and background data. A χ^2 test is then conducted to examine if the covariance structure suggested by the model satisfactorily fits the covariance structures (Lamb et al., 2011b); a non-significant χ^2 test (P > 0.05) suggests sufficient model fit. When the initial model has insufficient fit and conditions are not met, model modification indices are considered for data exploration and hypothesis generation (Lamb et al., 2011b).

An initial model was generated using pre-spraying data from 2005 to assess the direct and indirect effects of legumes, Canada thistle, grasses, and forbs on each other (Fig. 2). In this model, both CT density and biomass variables were included. While density may reflect direct physical (i.e. spatial) occupancy of the weed within the plot or community, biomass is generally a stronger proxy for resource (light, water and nutrient) use. We hypothesized that as a
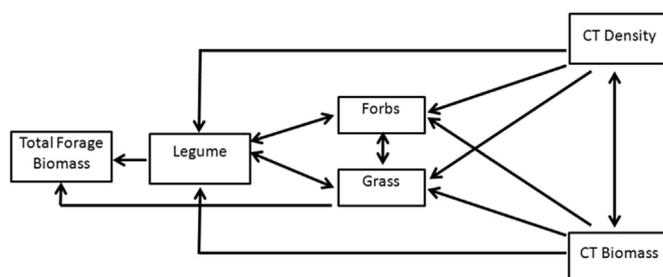

Fig. 2. Initial structural equation model developed to relate pasture sward components to one another, as well as to total forage production.

strong competitor, CT would have a directional impact on the other sward components in our initial model (Fig. 2). Relationships among other variables, including the remaining sward components, were retained as bidirectional in the initial model.

The initial path model was fit to 2005 data from the Lake Isle (LI) and Parkland Conservation Farm (PCF) sites, and had 6 observed variables and 18 parameters to be estimated, including 2 path coefficients, 10 error covariances and 6 error variances. We employed a simple observed variable model to explore causal networks among inter-correlated variables rather than a latent variable model, and thus, information on measurement error was not included in the initial model (Lamb et al., 2011b). Fitness of each model was assessed using 12 different fitness indices. Since the initial model did not provide adequate fit at either site (LI site had $\chi^2 = 169.54$; df $= 6$; P $= 5.61e{-}34$; goodness-of-fit index (GFI) $= 0.774$, whereas the PCF site had $\chi^2 = 40.93$; df $= 6$; P $= 2.99e{-}07$; GFI $= 0.888$), modification indices were used to progressively identify and incorporate new relationship paths to obtain an adequate model fit. Iterative model runs with inclusion of paths having the largest modification indices provided adequate fit to models. The LI model improved slightly ($\chi^2 = 164.35$; df $= 5$; P $= 1.17e{-}33$; GFI $= 0.775$) after incorporation of a (competition) path from CT biomass to forbs, and more substantially as the paths from legume to grass, and forb to grass, were converted to directional from the broadleaf to the grassy component. Broadleaf components, particularly legumes, were hypothesized to impact grasses through changes in nutrient cycling (i.e. N fixation). Inclusion of directional paths improved the model to the point of having adequate fit for acceptance as the final model for the LI site. Similarly, the PCF initial model had inadequate fit initially, which improved ($\chi^2 = 35.42$; df $= 5$; P $= 1.23e{-}06$; GFI $= 0.892$) after incorporation of a direct (competition) path from CT biomass to forb, grass and legume biomass ($\chi^2 = 17.00$; df $= 4$; P $= 0.002$; GFI $= 0.940$). However, the model improved substantially after the inclusion of an additional path from CT density to legume, and directional paths from legume to grass, and forb to grass. This model was accepted as the final model for the PCF site. Paths with P - values greater than 0.05 were considered significant. The final SEM was performed using the *sem* package in R v. 3.0.1, in combination with the *MASS*, *matrix*, *matrixcalc*, and *lattice* packages.

To evaluate post-spraying net forage yield responses, we assessed the relative yield ratio (RYR) post-spraying in 2006 and 2007 for all net forage (grass + legume) biomass. Post-spraying forage yields were relativized to yield data from unsprayed plots (n = 20) within each site to account for natural temporal variation in sward composition (particularly legumes). Considerable inter-annual variation was observed in biomass, including that of legumes within unsprayed plots (data not shown) and could have resulted in erroneous inferences of the 'opportunity cost' from spraying (i.e. natural declines in legume should not be attributed to herbicide application). Forage responses following spraying were therefore adjusted for each sprayed plot by dividing biomass from each sprayed plot by mean forage production for the same year across unsprayed plots (n = 20 per site). Separate RYR values were calculated for every sprayed plot at each site, in each of 2006 and 2007, 1 and 2 years after spraying, respectively, using Equation (1):

Relative Yield Ratio $(RYR_i) =$ Total Forage$_{i,S}$/Average Total Forage$_{US}$ (1)

where Total Forage$_{i,S}$ is the grass production of sprayed plot *i* (n = 80 plots total) within a year (i.e. 2006 or 2007), and Average Total Forage$_{US}$ is the mean total production (grass + legume) of all unsprayed (n = 20) plots within the same year (i.e. 2006 or 2007). RYR values > 1 represent a net increase in total forage relative to

unsprayed plots, while an RYR <1 indicated a net decline.

Initial biomass (kg ha^{-1}) of each vegetation component present within each plot during 2005 was used as the independent variable for all analysis of forage RYR data, and required square root transformation prior to analysis to achieve normality. RYR data for each site and year were analyzed using stepwise regression in SAS (SAS Institute Inc., 1990) and identify those independent broadleaf components (legume, forb or CT) associated with post-spraying relative yield. All variables with P < 0.10 were considered significant in the regression, and results were interpreted using model R^2 values and β coefficients. Vegetation components with positive β values were indicative of net improvements in forage RYR following removal, while negative β values indicated a loss in RYR. To assess the net effect of spraying on total forage yield, a one-way mixed model ANOVA was used to compare biomass in sprayed and non-sprayed treatments during each of 2006 and 2007, separately by site.

3. Results

3.1. Forage sward relationships prior to spraying

Pasture swards at the two sites initially differed from one another, with LI having a large proportion of CT (24% by biomass) relative to legumes (Fig. 3). Other forbs (i.e. excluding CT and legumes) comprised 15% of total forage biomass at this location. At PCF, although CT plants were abundant in number, the weed comprised a relatively small proportion of total biomass (3%) while legumes represented 23% of the sward (Fig. 3).

Final SEMs adequately fit the pre-spraying data at LI (Fig. 4A; $\chi^2 = 5.099$; df $= 5$; P $= 0.403$; GFI $= 0.979$) and PCF (Fig. 4B; $\chi^2 = 4.377$; df $= 3$; P $= 0.223$; GFI $= 0.982$). At LI, the abundance of CT (both density and biomass) had minimal association with the other sward components, with only CT biomass directly reducing the abundance of other (non-CT) forb biomass (z $= -0.225$). In contrast, at PCF, greater levels of CT density and biomass were associated with lower legume (i.e. alfalfa) biomass (z $= -0.326$ and -0.152, respectively), and the same was true for grass biomass (z $= -0.104$), but only in response to CT biomass. Also at this location, forb biomass was positively associated (z $= 0.220$) with CT biomass. Not surprisingly, at both LI and PCF, levels of CT biomass and CT density were directly associated with one another (z = 0.407 and 0.592, respectively).

The nature of additional significant relationships among the remaining sward components were similar at the LI and PCF locations, although those at the PCF site tended to be more pronounced,
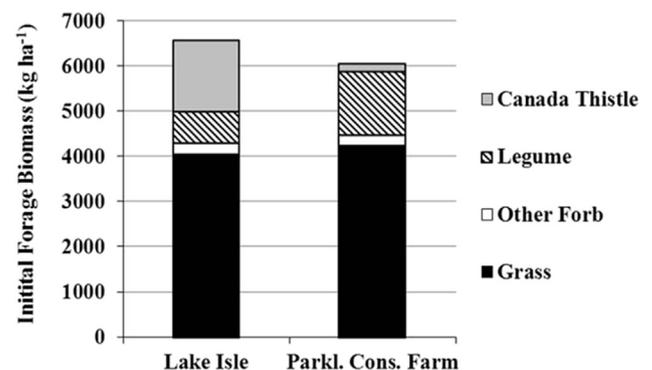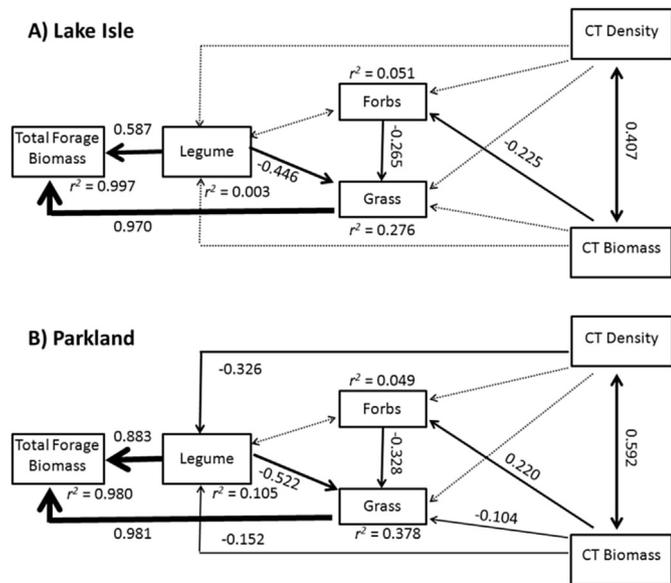

Fig. 3. Initial composition of biomass by vegetation component at each of the Parkland Conservation Farm (PCF) and Lake Isle (LI) study sites. Values are the average from all 100 plots at the start of the study during 2005.

Fig. 4. Summary of the final SEM path analysis of pasture sward components at A) the Lake Isle study site and B) the Parkland Conservation Farm study site, assessed in 2005 prior to herbicide application. Solid arrows show significant pathways, with line thickness representing significance. Progressively greater significance levels are represented by increasing thicknesses of solid lines, from P < 0.05, through P < 0.01, P < 0.001 and P < 0.0001. Dotted pathways indicate non-significant paths (P > 0.05). Coefficients indicate the strength of the association between variables, while arrows indicate directionality. r^2 Values indicate the proportion of total variance for a variable explained by the model.

as represented by the strength of model path coefficients. For example, grass biomass was inversely related to forb biomass ($z = -0.265$ and -0.328, respectively). Similarly, grass biomass demonstrated a strong negative response to legume biomass at both LI ($z = -0.446$) and PCF ($z = -0.522$), which was comprised of primarily clover and alfalfa, respectively. As expected, total forage biomass at both locations was positively associated with legume biomass, and in particular, grass biomass.

3.2. Post-spraying forage dynamics

Post-spraying assessment of aggregate forage responses using RYRs indicated differences between the two sites (Table 1). At LI in 2006, one year after spraying, forage RYR decreased in response to increasing forb removal ($\beta = -0.0082$), although the proportion
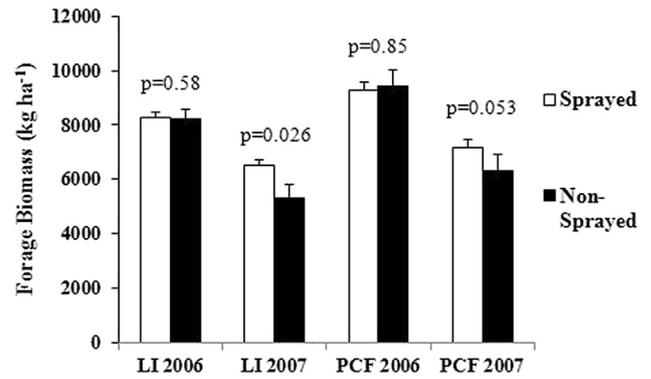
Fig. 5. Comparison of total forage yields in sprayed and non-sprayed plots sampled one and two years after spraying at each of the Lake Isle (LI) and Parkland Conservation Farm (PCF) sites. P − values indicate significance test for paired comparisons of sprayed versus non-sprayed treatments within each location x year combination.

variance in RYR explained remained low ($r^2 = 0.049$). By 2007, forage RYR decreased in response to both the amount of legume and forb biomass previously removed, with a stronger effect size from the latter ($\beta = -0.009$ vs $\beta = -0.021$). No relationship between forage RYR and CT biomass removal was found at the LI site. At PCF, forage RYR decreased in 2006 in response to the amount of legume and forb removed, but increased in response to CT biomass removal (Table 1). Among variables tested at PCF, legumes accounted for the greatest variation in forage RYR ($r^2 = 0.23$). By 2007, only legume removal continued to impact forage RYR, with no continuing effect of CT removal. Finally, RYR data indicate production was generally greater in sprayed than unsprayed plots, as represented by RYR values greater than 1, particularly during the second year (see intercepts in Table 1). This was further corroborated by comparisons of total forage production, which indicated sprayed plots were 22% and 13% greater than unsprayed plots ($P \leq 0.05$) at LI and PCF, respectively, during 2007 (Fig. 5).

4. Discussion

4.1. Sward dynamics prior to spraying

An underlying assumption of research focused on weed control is that weeds negatively influence forage production. Using an SEM approach allowed complex sward relationships to be examined prior to weed control. In this study CT had a larger impact in regulating other sward components, including total forage

Table 1
Stepwise multiple regressions relating forage biomass relative yield ratios (RYR) one (2006) and two (2007) years after spraying to the biomass of broadleaf vegetation components removed at spraying (2005) within each of the Lake Isle (LI) and Parkland Conservation Farm (PCF) study sites.

Site	Dependent	Adjusted R^2	Independent[a]	Partial R^2	Model R^2	β[b]	Prob > |F|[c]
LI	RYR 2006	0.049	Intercept	—	—	+1.1503	<0.0001
			Forb	0.049	0.049	−0.0082	0.048
LI	RYR 2007	0.202	Intercept	—	—	+1.7512	<0.0001
			Legume	0.106	0.106	−0.0092	0.0031
			Forb	0.096	0.202	−0.0208	0.003
PCF	RYR 2006	0.295	Intercept	—	—	+1.3062	<0.0001
			Legume	0.230	0.230	−0.0061	<0.0001
			Forb	0.033	0.263	−0.0131	0.069
			CT	0.032	0.295	+0.0077	0.066
PCF	RYR 2007	0.057	Intercept	—	—	+1.6438	<0.0001
			Legume	0.057	0.057	−0.0056	0.033

[a] All independent variables were square root transformed prior to analysis.

[b] Regression coefficients indicate the direction and magnitude of the change in RYR relative to each broad-leaf component. Intercepts above 1 represent net RYR increases from spraying.

[c] Probability of the F-test for each variable within the model to determine the best overall fit of the regression model. Values of P < 0.10 were considered significant.

availability, at the PCF site rather than at LI. At PCF, despite a lower initial CT infestation (as indicated by biomass), CT had a pronounced negative impact on other sward components, reducing legume, grass and total forage biomass, through the combined effects of CT biomass and density. Legumes, mostly comprised of alfalfa at PCF, appeared to be particularly susceptible to competition from CT, suggesting these plant species may be competing for similar resources within the sward (Georia and Osborne, 2014). The opposite may have been true of other forbs, which were positively associated with CT biomass. As the dominant forb (14% by cover; data not shown), dandelion (*Taraxacum officinale* (L.) Weber) may have been more resistant to competition from CT, or alternatively, benefited from the decline in other forage components under weed competition. The PCF site was also located on an upland where resources such as water and nutrients were likely to be more limited, potentially creating a more competitive environment for sward components (Wilson and Tilman, 1991).

In contrast, the LI site was situated on a floodplain, leading to abundant moisture availability due to the shallow water table, likely coupled with periodic nutrient influx during flooding. At this location, our findings indicate CT suppressed only other forbs (primarily dandelion, averaging 33% cover; data not shown) rather than grasses or legumes (mostly clover). This observation was particularly surprising given the large initial biomass of CT at LI (Fig. 3). As increased resources alone are unlikely to explain the apparent differences in competitive interactions (Wilson and Tilman, 1991), we suggest that CT, and potentially dandelion, may be occupying niches not occupied by other vegetation components, namely grasses and legumes (Georia and Osborne, 2014). Varying CT effects on forage production among sites in northern temperate pastures have been previously reported (Grekul and Bork, 2004).

Forbs negatively impacted grass biomass at both sites. Despite a similar biomass of other forbs at the two locations ($x = 233$–340 kg ha^{-1} per plot), variation in grass biomass in response to forbs was greater (based on SEM path coefficients) at PCF than LI. The prominent role of forbs, comprised largely of dandelion at both sites, suggests broadleaf plants other than CT may be playing a key role in suppressing total forage production in temperate pastures, and in the case of LI, to a greater extent than CT. The competitive impact of dandelion in particular has previously been reported (Stewart-Wade et al., 2002).

An unexpected finding from the SEM analysis was the negative association of legumes on grass biomass, a pattern consistent at both sites. These results suggest there was direct competition between the primary forage components (legume and grass), with the legume being more competitive. Although dominant grasses at the moisture rich LI site included smooth brome and timothy, both of which are relatively competitive species (Otfinowski et al., 2007; Kunelius et al., 2006) and should therefore be resistant to neighbor effects, our SEM results indicate the opposite. With its low creeping growth habit (Frame, 2005), clover is normally susceptible to reduced light (Frame and Harkess, 1987), and removal of defoliation during the spring and early summer would have maximized grass growth (i.e. plant height), which collectively was expected to reduce clover at LI as found elsewhere (Burdon and Turkington, 1983; Evans et al., 1998). The opposite response observed here suggests the favorable growing environment at LI enabled clover to remain competitive against grasses. Similarly, grasses at PCF were also susceptible to competition from legumes, in this case alfalfa. Being a tall-statured plant with a well-developed taproot, alfalfa is known to be a strong competitor (Sleugh et al., 2000; Mortenson et al., 2005; Holt and Jefferson, 1999).

In addition to competition and facilitation effects (Holmgren et al., 1977), many complex factors, including local site characteristics, may have played a role in regulating forage and weedy species abundance. For example, differences between legume species, sward composition, stand age, management practices and environmental conditions, can all influence the abundance of weedy species in pasture (Masters and Sheley, 2001; DiTomaso, 2000). The current results reinforce the variable presence of competitive processes among vegetation components across environments, even within the same agro-climatic zone.

4.2. Forage responses following herbicide application

Post-spraying RYR responses document the net changes in grass yield following the removal of all broadleaf plants. CT removal had little impact on RYR at the LI site, consistent with the earlier conclusion that CT, despite forming a major component of the pasture sward at this location, had limited impact on other vegetation components, presumably occupying otherwise 'vacant' niches within the sward. A markedly different pattern was evident at PCF however, where the removal of CT increased forage RYR the first year after spraying (2006). This outcome corroborates the impact of CT at the PCF site prior to spraying from the SEM, where the weed was observed to reduce biomass of grasses and legumes, and also highlights the benefit of CT removal on forage yields (Bork et al., 2007).

At both study sites, forage RYRs declined from increasing legume removal, though this response at LI was limited to 2007 only. The negative initial relationship between legumes and grasses in the SEM analysis at both locations, suggests the observed forage declines from legume removal may be linked to their direct loss from the sward. The prompt response at the PCF site is not surprising given the abundant initial presence of alfalfa ($x = 1412$ kg ha^{-1}), the removal of which would represent an immediate opportunity cost. Despite the negative effect of legume loss on forage RYRs, the marked increase in forage availability within sprayed plots two year after treatment at both locations suggests that overall forage responses benefited from the collective removal of all broadleaf vegetation. These findings also indicate net forage responses were more complex than simply responding to legume removal, and instead reflected the simultaneous benefits of removing CT and other forb competitors, as well as any associated compensatory responses in grass biomass. In general, it appears the removal of broadleaf components facilitated an increase in grass biomass capable of offsetting legume loss, at least during the first two years after spraying.

Compensatory grass responses to clover removal at LI may be attributed to the latter's abundant fine roots in the shallow soil layer (Caradus, 1990) that contribute to the cycling of N and carbon pools below-ground (Rasmussen et al., 2007). Rapid breakdown of clover roots, together with the removal of competition from clover, could account for why average grass biomass at LI increased sharply from 4070 kg ha^{-1} prior to spraying in 2005–7735 kg ha^{-1} in 2006. Similarly, given the strong initial negative impact of alfalfa on grass biomass at the PCF site based on the SEM analysis, it was again not surprising that grass biomass increased post-spraying at this location with alfalfa removal. Alfalfa has a strong competitive ability (Holt and Jefferson, 1999) and the subsequent release of N from decomposing root systems of the legume (Dubach and Russelle, 1994; Haby et al., 2006) can release as much as 258 kg ha^{-1} of N into the soil (Burity et al., 1989), which would greatly benefit grasses. Parallel results have been observed in rotational cropping systems of western Canada following the termination of alfalfa-based forage mixes, with increases in annual crop yield and quality (i.e. protein concentration) taking place for up to two years (Cutworth et al., 2010; Jefferson et al., 2013). Notably, these same studies indicate the previous presence of alfalfa may decrease water use and water use efficiency of annual crops, and this could

help explain part of the increase in grass and net forage responses post-spraying in the present investigation.

In conclusion, results of this study indicate that pre-spraying pasture sward dynamics and responses to herbicide weed control were complex and inconsistent between locations, with potentially strong influences of species composition and site characteristics. While CT was a factor reducing forage yields at one location, the other study site had little negative impact from CT. Moreover, although benefits of herbicide application on forage production were realized at both sites in the second year after spraying, this occurred despite the negative impact of legume removal. The specific mechanism for these forage responses remain unknown, but may reflect initial releases in grass biomass following the removal of competing legumes and other forbs, and could be related to complex nutrient and moisture dynamics. We caution that long-term sward responses to herbicide application should be explored to fully understand the net impact of herbicide application in mixed forage swards.

Overall, the use of SEM approaches in agricultural applications (Lamb et al., 2011b) and extended here to perennial pastures provided unique insights into understanding sward dynamics in these northern temperate grasslands, including the role of legume and weed presence. The SEM models also provided a useful complement to facilitate interpretation of the more traditional RYR assessment done post-spraying. Collectively, these insights highlight the need for a greater understanding of where, when and how the presence of pasture weeds impact forage availability, with implications for decision making when planning integrated weed control strategies in pasture, particularly those containing legumes.

Acknowledgments

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cropro.2015.08.019.

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