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Weed control and weed biomass influenced first cutting forage accumulation and nutritive value of spring-seeded alfalfa

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Abstract

Weeds can influence the economics of alfalfa (*Medicago sativa* L.) production by reducing forage yield and nutritive value or by contaminating hay. Field studies were conducted in Idaho in 2021 and 2022 to evaluate the effect of weed control treatments on alfalfa forage accumulation, weed biomass, and nutritive value. In addition, the relationship between the proportion of individual weed species biomass and alfalfa nutritive value was assessed. These studies included eight different herbicide and herbicide combination treatments, including the untreated check. Treatments were comprised of preemergence, early postemergence (after 80% alfalfa emergence), and postemergence (third trifoliate alfalfa) herbicide applications. Data collection included weed control efficacy, weed and alfalfa biomass, and alfalfa nutritive value. Additional samples were collected and combined in these alfalfa to weed biomass proportions (percentage by weight): 0/100, 20/80, 40/60, 60/40, 80/20, and 100/0, for wet chemistry analysis of forage nutritive value to evaluate the relationship between the proportion of individual weed species biomass and alfalfa nutritive value. The acetochlor-only treatment provided less than 50% weed control, while the EPTC (*S*-ethyl-*N*,*N*-dipropylthiocarbamate)-only treatment provided 54%–81% weed control. The control provided by acetochlor and EPTC was less than that provided by treatments containing imazamox and imazamox plus bromoxynil. Weed biomass in forage (23%–55% of total biomass) due to poor or no weed control reduced crude protein, increased fiber concentrations, and reduced the relative feed value. The relationship between the proportion of individual weed species biomass and alfalfa nutritive value was linear for all weed species evaluated.

1 | INTRODUCTION

Weed management is one of the most important practices in alfalfa (*Medicago sativa* L.) production, particularly in newly established alfalfa (Bradley et al., 2010; Dillehay et al., 2011). Established alfalfa is able to tolerate different herbicides and therefore, there are multiple herbicide options (e.g.,

Abbreviations: ADF, acid detergent fiber; CP, crude protein; DDM, digestible dry matter; EPTC, S-ethyl-N,N-dipropylthiocarbamate; MCPA, 2-methyl-4-chlorophenoxyacetic acid; NDF, neutral detergent fiber; RFV, relative feed value; TDN, total digestible nutrients.

carfentrazone, diuron, flumioxazin, hexazinone, imazethapyr, 2-methyl-4-chlorophenoxyacetic acid (MCPA), metribuzin, pendimethalin, paraquat, saflufenacil, and terbacil) for effective weed control in alfalfa after the first harvest or established stands (Adjesiwor & Prather, 2022). Thus, weed control before the first harvest remains one of the critical practices in alfalfa production. Weeds tend to be more problematic in spring-seeded alfalfa compared to summer or fall-seeded alfalfa, as summer annuals often emerge at the same time as the alfalfa (Bradley et al., 2010). Although late-summer or fall seeding often reduces competition from summer annual weeds, winter annual weeds and late-emerging weeds can still be problematic in newly established alfalfa (Adjesiwor et al., 2017; Hall et al., 1995). Weed control is, therefore, important in newly seeded alfalfa to reduce weed competition, increase establishment success, and improve subsequent alfalfa forage accumulation and nutritive value (Bradley et al., 2010; Hall et al., 1995; Roberts et al., 2023). For example, weed control using herbicide application increased alfalfa forage accumulation by 36%–39% compared to the nontreated plots (Roberts et al., 2023). Weed control has also been found to increase alfalfa stand persistence and productivity over the life of the stand (Dowdy et al., 1993). Dillehay et al. (2011) reported that under severe weed infestations, weed control must be initiated before the seven trifoliate growth stage of alfalfa to prevent economic yield loss (Hall et al., 1995). However, because weeds nearly always produce harvestable aboveground biomass, poor weed control or the absence of weed control tends to increase total forage (alfalfa + weeds) biomass under heavy weed pressure (Cosgrove & Barrett, 1987; Moyer & Acharya, 2006; Temme et al., 1979). Studies have found that in some instances, effective weed control reduces forage accumulation of the whole stand due to herbicide injury to the alfalfa or the absence of weed biomass (Bradley et al., 2010; Dowdy et al., 1993; Moyer & Acharya, 2006). Nonetheless, when weeds are present in large quantities in alfalfa, there is a trend of reduced alfalfa dry matter and reduced alfalfa yields overall as the weeds tend to take up more of the biomass (Pike & Stritzke, 1984; Temme et al., 1979). This may affect the nutritive value of hay, depending on the kind of weed present and the proportion of weed biomass in the hay. Studies have shown that weeds vary greatly in their nutritional composition (Bosworth et al., 1986; Frost et al., 2008; Khan et al., 2013). For example, Temme et al. (1979) found that weeds like *Chenopodium album* L. and *Ambrosia artemisiifolia* L. had similar or greater crude protein (CP) and digestibility than alfalfa. This tendency of some weed species to contribute to the biomass of the whole stand without significantly reducing forage nutritive value has led to arguments that it may be time to change attitude and view weeds as friends of the agroecosystem rather than as foes (Gholamhoseini et al., 2013). While arguments like this are important for sustainable weed management, no thresholds have been established for weed biomass or compositions that optimize forage accumulation without reducing forage nutritive value. In addition, weeds from certain genera, such as *Amaranthus*, *Chenopodium*, *Solanum*, and so forth, may accumulate compounds such as nitrates, which may be toxic to livestock if the nitrate levels exceed certain thresholds (Bolan & Kemp, 2003; Ekwealor et al., 2019). The objectives of this study were to (1) evaluate the effect of weed control treatments on alfalfa forage accumulation, weed biomass, and nutritive value of the first cutting of spring-planted alfalfa and (2) assess the relationship between the proportion of individual weed species biomass on nutritive value components and nitrate accumulation of the forage mixture.

Core Ideas

- Poor weed control increased weed biomass and reduced forage nutritive value.
- Effective weed control reduced forage accumulation due to reduced weed control and herbicide injury to alfalfa.
- A linear relationship between weed biomass proportion and mixed stand nutritive value is described.
- The risk of nitrate poisoning may increase when alfalfa hay has 60% or more biomass from certain weeds.

2 | MATERIALS AND METHODS

2.1 | Study #1: Forage accumulation and nutritive value as influenced by weed control treatments

Field experiments were conducted at the University of Idaho Kimberly Research and Extension Center, Kimberly, ID (42.549877, −114.349615) in 2021 and 2022 to evaluate the effect of weed control treatments on alfalfa forage accumulation, weed biomass, and nutritive value. The soil was a Portneuf silt loam (coarse-silty, mixed, superactive, mesic Durinodic Xeric Haplocalcids) with 23% sand, 58% silt, and 19% clay. In both 2021 and 2022 study years, the soil had a pH of 8.0, an organic matter content of 2.4%, and a cation exchange capacity of 19.8 meq/100 g soil. The average field location air temperature and relative humidity from planting to harvest in 2021 and 2022 were retrieved from the AgriMet Cooperative Agricultural Weather Network Database (https://www.usbr.gov/pn/agrimet/agrimetmap/twfida.html) and presented in Figure 1.

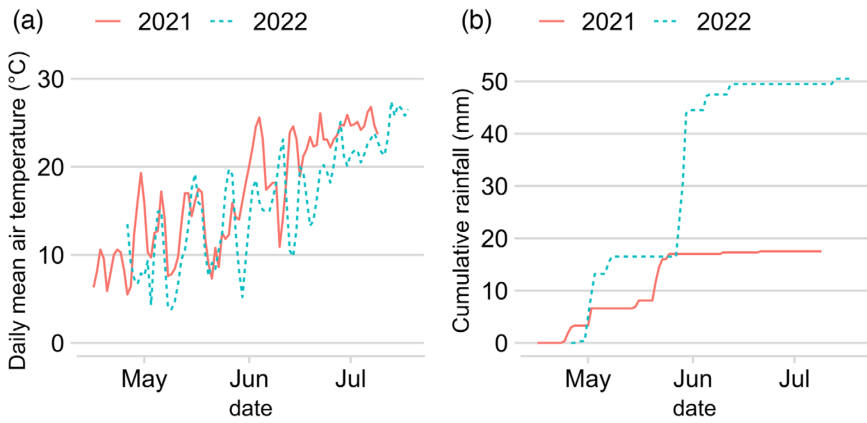

FIGURE 1 Mean daily air temperatures (a) and cumulative precipitation (b) from planting to harvest in 2021 and 2022, Kimberly, ID. Data from the AgriMet Cooperative Agricultural Weather Network Database (https://www.usbr.gov/pn/agrimet/agrimetmap/twfida.html).

TABLE 1 Weed control treatments used in the experiments in study #1 in 2021 and 2022, Kimberly, ID.

Treatment	Rate (g ai/ha)	Commercial product
Untreated	–	–
EPTC[a]	2940	Eptam 7E
Acetochlor[b]	1260	Warrant
Imazamox	44	Raptor
Imazamox[c] + bromoxynil[c]	44 + 420	Raptor + Maestro 2EC
EPTC *fb* imazamox	2940 *fb* 44	Eptam fb Raptor
EPTC *fb* imazamox + bromoxynil	2940 *fb* 44 + 420	Eptam *fb* Raptor + Maestro 2EC
Acetochlor *fb* imazamox	1260 *fb* 44	Warrant *fb* Raptor

Abbreviations: EPTC, *S*-ethyl-*N*,*N*-dipropylthiocarbamate; *fb*, followed by.
[a]Applied pre-plant incorporated (with 2.5 cm of irrigation).
[b]Early postemergence (80% alfalfa emergence).
[c]Postemergence (3rd trifoliate alfalfa). Postemergence applications included urea ammonium nitrate (2.5% v/v) and nonionic surfactant (0.25%v/v).

Alfalfa ("WL354") was planted into a well-prepared seedbed at a rate of 22 kg ha^{-1} on April 16, 2021, and April 26, 2022, using a Great Plains 3P806NT no-till drill (Great Plains Ag). Plots were uniformly irrigated using a sprinkler irrigation system.

These studies included eight different herbicide and herbicide combination treatments, including the untreated check. In both years, treatments were arranged in a randomized complete block design with four replications. Treatments comprised of herbicide treatments applied preemergence and incorporated, early postemergence (after 80% alfalfa emergence), or postemergence (third trifoliate alfalfa; Table 1). Individual plot size was 3.0 × 9.1 m. Herbicides were applied using a CO_2-pressurized bicycle sprayer delivering 144 L ha^{-1} at 207 kPa with TeeJet 11002DG nozzles and a swath width of 3 m.

2.2 | Weed control efficacy, herbicide injury, forage accumulation, and weed control cost

Immediately before plot harvest each year, weed control efficacy (by weed species) was visually assessed in each plot on a scale of 0%–100%, with 0% being no weed control and 100% being complete weed control. A quadrat (0.5 m^2) was randomly placed within each plot, and aboveground biomass (alfalfa and weeds) within the quadrat area was clipped using rice knives, leaving a stubble of about 12 cm. This was hand separated into weed and alfalfa biomass to enable evaluation of alfalfa and weed contribution to total forage accumulation.

The 2021 and 2022 seedings were harvested on July 9, 2021, and July 18, 2022, respectively. A 1.5 × 7.6 m area was harvested at about 10% bloom using the Wintersteiger Cibus F forage plot harvester, and fresh weight was recorded. Forage was harvested only once each year because, in newly established alfalfa, the first harvest often has the highest weed density (Renz, 2015). Subsamples were collected from the harvester, weighed, and oven-dried to a constant weight at 60˚C for 72 h to quantify dry harvestable weight and dry matter. Estimated moisture from the subsamples was used to adjust plot weights, and forage accumulation was expressed in kg dry matter ha^{-1}.

Oven-dried subsamples were ground in a Wiley Mill (Model 4, Thomas Wiley, Laboratory Mill, Thomas Scientific) to pass through a 1-mm mesh. Samples were scanned for CP, acid detergent fiber (ADF), and neutral detergent fiber (NDF) using near-infrared reflectance spectroscopy (NIRS, Foss InfraXact analyzer) that was calibrated using reference samples from wet chemistry analyses. Relative feed value (RFV) was calculated from the following relation (Equation 1; Belyea et al., 1993):

$$\mathrm{RFV} = \frac{\mathrm{DDM} \times \mathrm{DMI}}{1.29} \quad (1)$$

where DDM = digestible dry matter and is calculated from the relation: DDM = 88.9 – 0.779 × %ADF, and DMI = dry matter intake and is calculated from the relation: DMI = 120/%NDF (Belyea et al., 1993).

The DDM is an estimate of the total digestibility of the feed, and it is calculated from percentage ADF. The DMI is an estimate of the amount of feed an animal will consume in percentage of its body weight, and this is calculated from percentage NDF.

The cost of weed control programs was calculated using average unit herbicide cost from local agrochemical dealers as follows: \$14.53 L^{-1} of Eptam 7E (*S*-ethyl-*N*,*N*-dipropylthiocarbamate [EPTC]), \$10.14 L^{-1} of Warrant (acetochlor), \$154.53 L^{-1} of Raptor (imazamox), and \$14.9 L^{-1} of Maestro 2EC (bromoxynil).

2.3 | Study #2: Relationship between selected weed species biomass contribution and overall mixed stand nutritive value

To assess the relationship between the proportion of individual weed species biomass and nutritive value of the mixed stand (alfalfa + weeds), a second field study was established in 2022. Four plots of alfalfa (WL354, 9 × 18 m) were planted into a well-prepared seedbed at a rate of 22 kg ha^{-1} on April 26, 2022, using a Great Plains 3P806NT no-till drill (Great Plains Ag.). Each plot was considered a replica. Plots were uniformly irrigated using a sprinkler irrigation system. No herbicide was applied in this study to permit adequate weed biomass production. On July 20, 2022, a quadrat (0.5 × 1 m) was randomly placed at 10 locations within the midportion of each strip (replicate), and aboveground biomass in the quadrat area was clipped using rice knives. Clipped samples were hand separated into alfalfa and the dominant and uniform weed species: common lambsquarters, kochia [*Bassia scoparia* (L.) Schrad.], field bindweed (*Convolvulus arvensis* L.), shepherd's-purse [*Capsella bursa-pastoris* (L.) Medik], and green foxtail [*Setaria viridis* (L.) P. Beauv.]. At sampling on July 20, 2022, alfalfa was 43 ± 1.8 cm tall (± standard error of the mean) and 10% bloom, common lambsquarters was 73 ± 1.8 cm tall and 15% bloom, kochia was 83 ± 1.8 cm tall and 5% bloom, field bindweed was 27 ± 3.2 cm tall and 5% bloom, shepherd's-purse was 58 ± 1.2 cm tall and 95% bloom, and green foxtail was 36 ± 2.9 cm tall and 5% bloom. Harvested samples were oven-dried and ground as previously described. Dried and ground samples were weighed and combined for these alfalfa to weed biomass (individual weed species) proportions (percentage by weight): 0/100, 20/80, 40/60, 60/40, 80/20, and 100/0, and sent to Ward Laboratories Inc. for wet chemistry analysis of forage nutritive value following standard forage testing procedures.

2.4 | Data analysis

2.4.1 | Study #1: Forage accumulation and nutritive value as influenced weed control treatments

All data analyses were performed in R statistical language version 4.0.2 using the *lme4, lmerTest*, and *emmeans* packages (Bates et al., 2015; Kuznetsova et al., 2017; Lenth, 2022; R Core Team, 2022). Effects of weed control treatments on alfalfa, weed and total biomass, and nutritive values of the whole stand were estimated using a mixed-effects model, herbicide treatment identified as a fixed effect and year, year × herbicide treatment, and block as random parameters. Estimated marginal means were calculated from the model, and post hoc Tukey-adjusted pairwise treatment comparisons were performed at $\alpha = 0.05$ using the *emmeans* and *multcomp* packages (Hothorn et al., 2008; Lenth, 2022). To evaluate the relationship between weed biomass proportion and whole stand nutritive value, linear regression analyses were performed using the *lm* function in R. In addition, polynomial regression (using the *lm* function) and nonlinear regression using the *drm* function from the *drc* package (Ritz et al., 2015) were fitted for model comparisons. The best model was selected by comparing various models using the Akaike Information Criterion function in R. The linear regression equation for each whole-stand nutritive value was obtained from the linear regression model.

2.4.2 | Study #2: Relationship between selected weed species biomass contribution and overall mixed stand nutritive value, and nitrate concentration

The relationship between weed biomass proportion of selected weed species and forage nutritive value parameters and nitrate concentration was estimated through linear regression analyses using the *lm* function in R (R Core Team, 2022). In addition, polynomial regression (using the *lm* function) and nonlinear regression using the *drm* function from the *drc* package (Ritz et al., 2015) were fitted for model comparisons. The best model was selected by comparing various models

TABLE 2 Broadleaf and grassy weed control and alfalfa injury ratings from herbicide treatments and approximate cost of herbicide programs from study #1 in 2021 and 2022, Kimberly, ID.

Factor/treatment[a]	Common lambsquarters	Kochia	Redroot pigweed	Shepherd's-purse[b]	Green foxtail	Alfalfa injury	Cost of control (USD ha^{-1})
	p-Value						
Year	0.25	0.99	0.27	–	0.16	0.23	–
Herbicide	<0.001	<0.001	<0.001	<0.001	<0.001	0.18	–
Year × herbicide	0.10	0.99	0.01	–	0.09	0.07	–
Herbicide	(%)						
Untreated	0d	0e	0c	0d	0c	0	0
EPTC	59bc	59c	66ab	81b	64ab	2	50.98
Acetochlor	29cd	33d	43b	48c	43b	1	35.58
Imazamox	84ab	73bc	88a	95ab	90a	4	56.46
Imazamox + bromoxynil	94a	92a	95a	95ab	88a	8	82.61
EPTC *fb* imazamox	91a	83ab	92a	97a	87a	6	107.44
EPTC *fb* imazamox + bromoxynil	95a	92a	95a	96a	88a	8	133.58
Acetochlor *fb* imazamox	85ab	81ab	88a	93ab	87a	5	92.05

Note: Within a column, means followed by the same letters are not different at 0.05 probability level according to Tukey's HSD.
Abbreviations: EPTC, *S*-ethyl-*N*,*N*-dipropylthiocarbamate; *fb*, followed by.
[a]Herbicide treatment was identified as a fixed effect, and year, year × herbicide treatment as random parameters in the data analysis.
[b]Shepherd's-purse was only evaluated in 2022 because it was not uniformly present at the study site in 2021.

using the Akaike Information Criterion function in R. The linear regression equation for each weed species and whole stand nutritive value parameters (CP, ADF, NDF, total digestible nutrients [TDN], and RFV) and nitrate concentration were obtained from the linear regression model.

3 | RESULTS AND DISCUSSION

The Kimberly, ID, area is semiarid, characterized by cold winter and spring and warm and dry summer (Figure 1). Production of alfalfa is heavily reliant on irrigation to supplement precipitation. Air temperatures were slightly warmer in 2021 than in 2022 (Figure 1a). Although precipitation in 2022 was greater than in 2021 (Figure 1b), the difference in moisture was negated through irrigation.

3.1 | Study #1: Weed control efficacy, alfalfa herbicide injury, forage accumulation, and weed control cost

Herbicide treatment significantly affected weed control ratings but had no impact on alfalfa injury ratings (Table 2). The most dominant weeds were common lambsquarters > kochia > green foxtail > shepherd's-purse > redroot pigweed. The year × treatment interaction was significant for redroot pigweed control ratings because of a greater density of redroot pigweed in 2021 compared with 2022 (Table 2). Herbicide treatments explained more of the variance in weed control ratings compared to year or year × herbicide treatment (data not shown). The acetochlor-only treatment provided less than 50% weed control, while the EPTC-only treatment provided 54%–81% weed control. The control provided by acetochlor and EPTC treatments was less than for treatments containing imazamox and imazamox plus bromoxynil (Table 2). Acetochlor is a preemergence herbicide that controls imbibed seeds that are germinating (Adjesiwor & Prather, 2022). Thus, delayed application (after 80% alfalfa emergence) is likely to be less effective since a significant proportion of the weeds would have germinated or emerged at the time of application. The poor weed control from acetochlor-only and EPTC-only treatments increased the amount of weed biomass in the forage at harvest (Table 3). Forage accumulation from the acetochlor-only treatment was comprised of 53% weed biomass, which was similar to that of the untreated check (55%; Table 3). Weed biomass was 23% of the forage accumulation in the EPTC-only treatment (Table 3). In contrast, weed biomass was less than 9% of the forage accumulation in treatments containing imazamox and imazamox plus bromoxynil (Table 3). Treatments containing imazamox plus bromoxynil were highly effective at controlling weeds, but they resulted in nearly 20% alfalfa injury within 2 weeks after application (data not shown). Although the alfalfa recovered from injury (leaf chlorosis) caused by these herbicide treatments by the time of harvest, this resulted in stunting and reduced alfalfa forage accumulation (Table 3). This confirms a previous observation that effective weed control may

TABLE 3 Forage accumulation and whole stand nutritive value (crude protein [CP], acid detergent fiber [ADF], neutral detergent fiber [NDF], total digestible nutrients [TDN], digestible dry matter [DDM], and relative feed value [RFV]) from study #1 in 2021 and 2022, Kimberly, ID.

Factor/treatment[a]	Alfalfa	Weed (percentage by weight of total biomass)	Total	CP	ADF	NDF	TDN	DDM	RFV
	p-Values								
Year	0.04	0.86	0.002	0.99	0.99	0.03	0.99	0.99	0.16
Herbicide	0.006	0.04	0.01	<0.001	0.05	<0.001	0.05	0.05	0.001
Year x herbicide	0.06	<0.001	0.30	0.58	0.99	0.99	0.99	0.99	0.99
Herbicide effect	(kg ha^{-1})			(g kg^{-1})					
Untreated	1535c	1895 (55)a	3430a	197b	342	451a	607	623	148b
EPTC	2086abc	624 (23)ab	2711ab	237ab	308	402abc	643	649	172ab
Acetochlor	1640bc	1816 (53)a	3456a	199b	332	440ab	618	630	155b
Imazamox	2381a	115 (5)b	2496b	249a	310	383abc	641	647	182ab
Imazamox + bromoxynil	1566c	126 (7)b	1692c	277a	283	346c	670	669	214a
EPTC *fb* imazamox	2272a	49 (2)b	2321bc	261a	305	368abc	647	652	188ab
EPTC *fb* imazamox + bromoxynil	2177ab	178 (8)b	2355bc	271a	293	353bc	659	661	205a
Acetochlor *fb* imazamox	2254a	107 (5)b	2361bc	256a	310	379abc	642	648	183ab

Note: Within a column, means followed by the same letters are not different at 0.05 probability level according to Tukey's HSD.
Abbreviations: EPTC, *S*-ethyl-*N*,*N*-dipropylthiocarbamate; *fb*, followed by.
[a]Herbicide treatment was identified as a fixed effect, and year, year × herbicide treatment as random parameters in the data analysis.

reduce forage accumulation due to alfalfa injury from herbicides and the absence of weed biomass (Moyer & Acharya, 2006). Although effective weed control was obtained at a cost of \$56 ha^{-1} by applying imazamox only (Table 2), this herbicide must be combined with one or more effective herbicide sites of action to reduce the chances of herbicide resistance evolution (Beckie, 2006; Kniss et al., 2022). As observed in this study, the addition of other herbicides to imazamox substantially increased the cost of weed control (Table 2). For example, the addition of bromoxynil increased the cost of weed control by \$26 ha^{-1} while the addition of EPTC increased the cost of weed control by \$51 ha^{-1} (Table 2).

3.2 | Study #1: Whole stand nutritive value as influenced by weed control treatments and weed biomass

Herbicide treatments affected whole-stand forage CP, NDF, and RFV (Table 3). Whole-stand nutritive value was not affected by year or year × treatment interaction (Table 3). Herbicide treatments explained more of the variance in whole stand nutritive value compared to year or year × herbicide treatment. Poorer weed control and increased weed biomass reduced whole-stand forage CP and RFV and increased NDF. Weed biomass did not increase ADF, and thus, DDM and TDN were not different among treatments (Table 3). The linear model of the relationship between percentage weed biomass and forage nutritive value showed that a percentage unit increase in weed biomass reduced forage CP by 1 g kg^{-1}, DDM by 0.34 g kg^{-1}, TDN by 0.47 g kg^{-1}, and RFV by 0.58 g kg^{-1} (Figure 2). The reduction in DDM, TDN, and RFV were due to increased ADF and NDF with an increase in weed biomass (Figure 1). Previous studies have shown that weeds such as shepherd's-purse, green foxtail, and redroot pigweed tend to have lower CP concentrations compared to alfalfa (Bosworth et al., 1980; Temme et al., 1979). Thus, high density of these weeds may reduce CP of the whole stand.

3.3 | Study #2: Relationship between weed biomass proportion and forage nutritive value of the artificial mixtures

From the nutritive value analyses of individual weed species, we observed that kochia and common lambsquarters had similar CP concentrations as alfalfa (Figure 3a). Thus, increasing proportions of kochia and common lambsquarters biomass did not decrease the CP of the forage mixture. Conversely, an increasing proportion of field bindweed, shepherd's-purse, and green foxtail biomass decreased CP of the forage mixes because these species contained significantly lower CP concentrations compared to alfalfa (Figure 3a). In a previous study, Temme et al. (1979) reported that shepherd's-purse

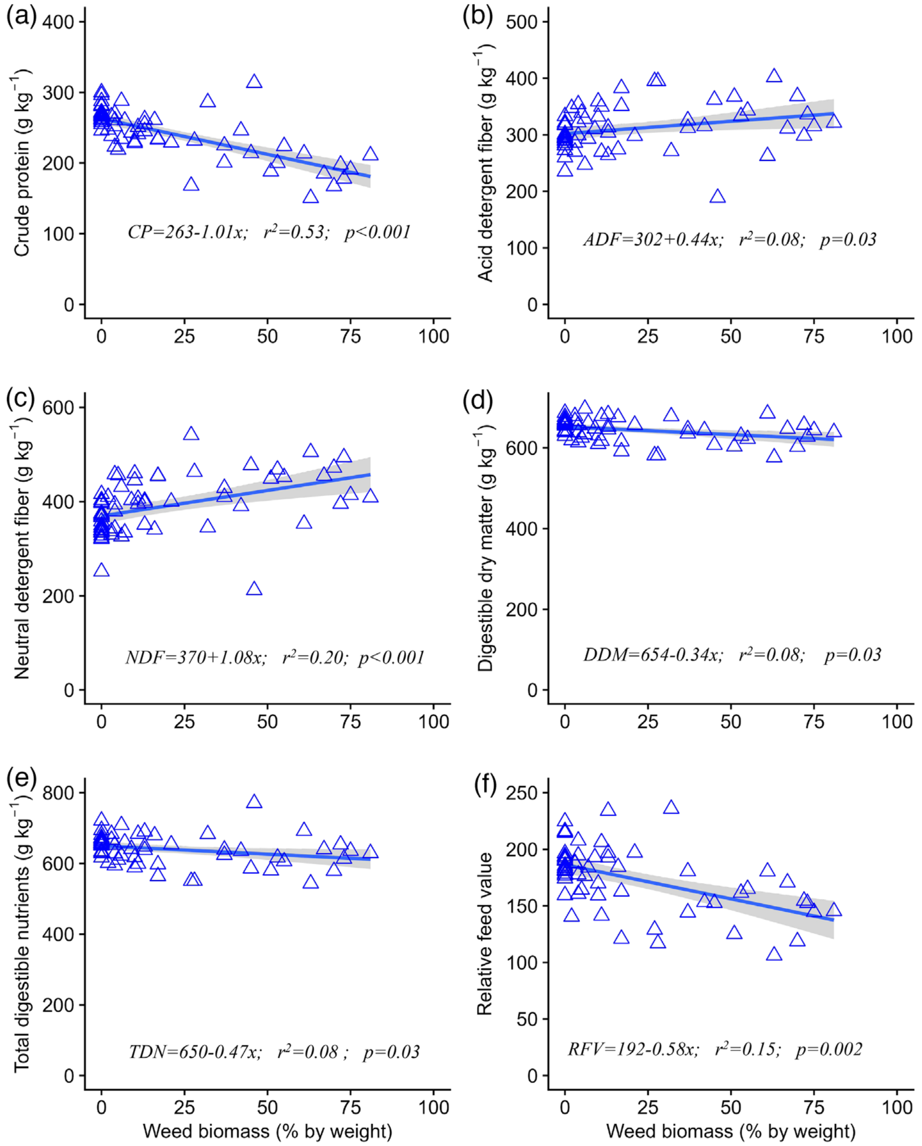

FIGURE 2 Linear relationships between weed biomass proportion (% by weight) and nutritive value of the whole stand forage at first harvest in 2021 and 2022 from study #1, Kimberly, ID. Shading around the regression line are the 95% confidence intervals. Values are the means of 2 years, each a separate planting, and four replicates in each planting.

harvested at green seed stage had 6 g kg^{-1} less CP than alfalfa at early bloom stage and 45 g kg^{-1} less CP when shepherd's-purse harvested at the seed stage compared to alfalfa at early bloom stage. Similarly, CP concentration in yellow foxtail [*Seteria pumila* (Poir.) Roem. & Schult], a grassy weed closely related to green foxtail, was 35 g kg^{-1} less harvested at early seed stage, and alfalfa was harvested at the early bloom stage. The difference in CP concentration was 63 g kg^{-1} when yellow foxtail harvest was delayed until the seed stage (Temme et al., 1979). This suggests that delaying alfalfa harvest may result in further reduction in forage nutritive value due to a faster decline in the nutritive value of some weed species.

ADF concentration was lower in common lambsquarters compared to alfalfa, thus increasing the proportion of common lambsquarters decreased ADF concentration of the forage mixture (Figure 3b). However, increasing proportions of kochia and field bindweed biomass did not affect the ADF of the forage as these weed species had similar ADF concentrations to alfalfa (Figure 3b). Only shepherd's-purse and green foxtail increased ADF concentration with an increasing proportion of biomass (Figure 3b). Kochia, common lambsquarters, and field bindweed had similar NDF concentrations as alfalfa, and therefore, increasing the biomass proportion of these weed species did not affect NDF concentration of the forage mixture (Figure 3c). In contrast, shepherd's-purse and green foxtail contained significantly greater NDF concentrations than alfalfa, and thus, increasing the biomass proportion of these weed species linearly increased NDF concentration in the forage mixture (Figure 3c). This was expected as weeds

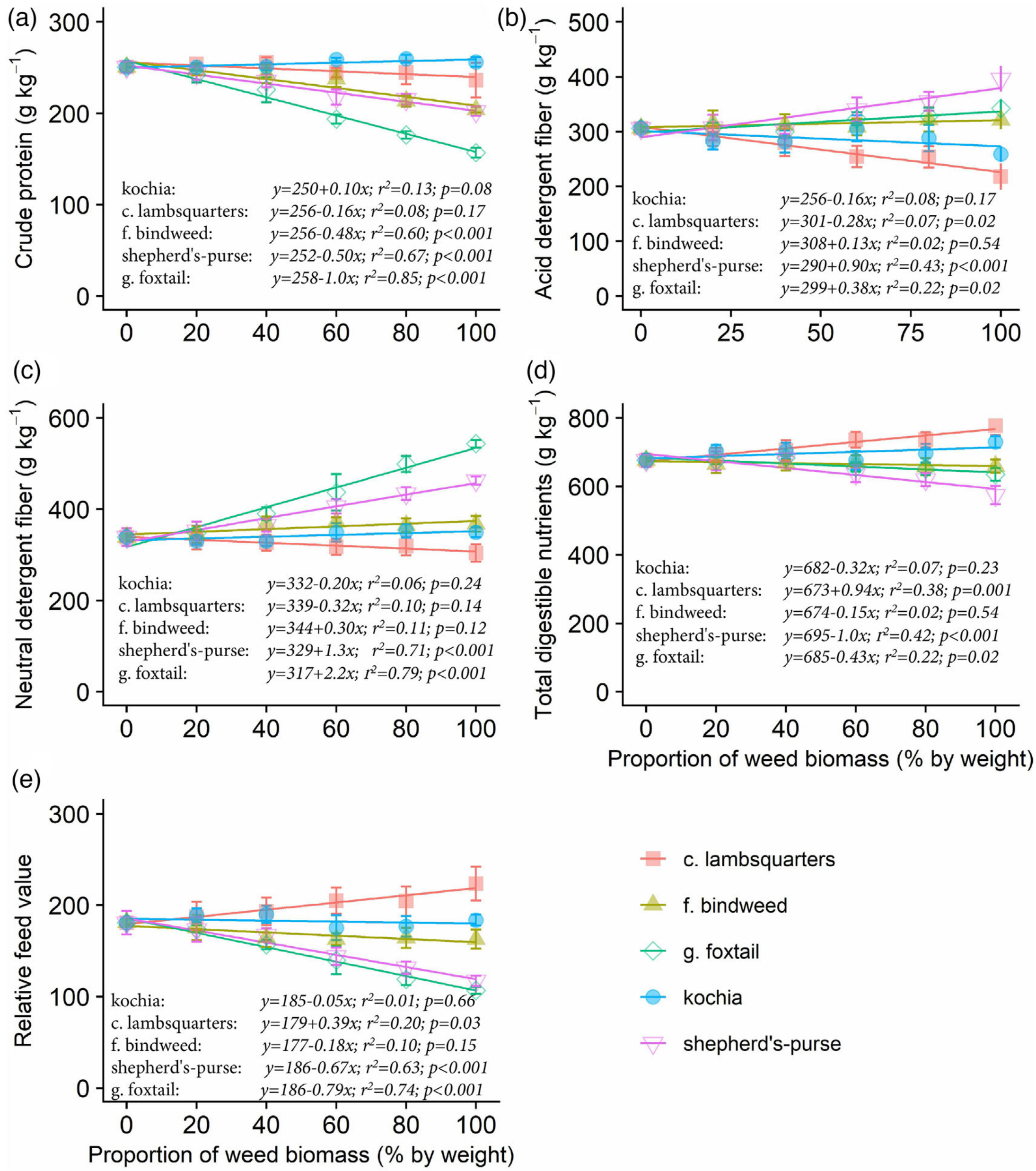

FIGURE 3 Linear relationships between the biomass proportion (% by weight) of individual weed species (kochia, common lambsquarters, field bindweed, shepherd's-purse, and green foxtail), and nutritive value of artificially created forage mixtures at first harvest in 2022 from study #2, Kimberly, ID. The 0% represents the nutritive value of pure alfalfa. Each point is the mean of four replicates.

such as shepherd's-purse and foxtail (*Setaria* spp.) have been shown to have greater fiber concentrations compared to alfalfa (Cosgrove & Barrett, 1987; Temme et al., 1979). In a previous study, it was reported that grassy weeds such as foxtails can dramatically increase mixed forage NDF and thus have the most potential to reduce forage nutritive value when present in high density (Becker et al., 1998). In these instances, weed control may increase overall forage nutritive value in the first harvest of the establishment year (Becker et al., 1998; Cosgrove & Barrett, 1987).

Common lambsquarters had a greater concentration of TDN than alfalfa, and therefore, increasing the proportion of common lambsquarters linearly increased the TDN concentration of the mixed alfalfa forage (Figure 3d). Kochia and field bindweed had similar TDN concentrations as alfalfa, and therefore, increasing the biomass proportion of these weed species did not affect TDN concentration of mixed alfalfa forage (Figure 3d). Shepherd's-purse and green foxtail, on the other hand, linearly decreased alfalfa TDN with increasing biomass proportions. Like TDN (Figure 3d),

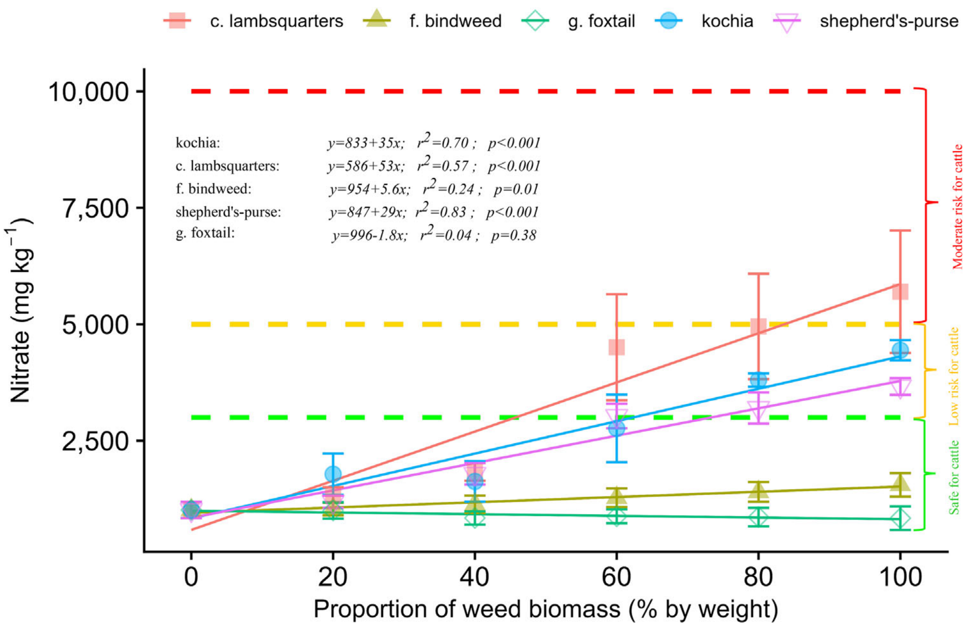

FIGURE 4 Linear relationships between the biomass proportion (% by weight) of individual weed species (kochia, common lambsquarters, field bindweed; shepherd's-purse, and green foxtail) and nitrate concentration of the artificially created forage mixtures at first harvest in 2022 from study #2, Kimberly, ID. The 0% represents the nitrate concentration of sole alfalfa. Each point is the mean of four replicates.

only common lambsquarters had greater RFV than alfalfa, resulting in a linear increase in RFV with an increasing proportion of the mixed forage biomass (Figure 3e). Kochia and field bindweed had similar RFV as alfalfa, and thus, increasing the biomass proportion of these weed species did not affect the RFV of mixed alfalfa forage (Figure 3e), whereas an increasing proportion of shepherd's-purse and green foxtail linearly decreased alfalfa RFV (Figure 3e).

3.4 | Study #2: Relationship between weed biomass proportion and nitrate accumulation of the artificial mixtures

Nitrate in hay may persist after harvest and drying and can result in poisoning and mortalities in livestock (Costagliola et al., 2014). Generally, forage with a nitrate concentration of 0–3000 mg kg^{-1} (parts per million, on dry matter basis) is safe for cattle; 3000–5000 mg kg^{-1} is safe for nonpregnant cattle but at low risk for pregnant cattle (Puschner, 2017; Strickland et al., 2017). Hay with 5000–10,000 mg kg^{-1} nitrate concentration presents a moderate risk of toxicity to cattle and may cause mid- to late-term abortions, reduce milk production, and weaken calves (Puschner, 2017; Strickland et al., 2017). Nitrate concentrations > 10,000 mg kg^{-1} is potentially toxic for all cattle and could lead to acute toxicity, abortions, and even death (Puschner, 2017; Strickland et al., 2017). In this discussion, we chose the threshold of 3000 mg kg^{-1} because below this threshold, no health effects would be expected for any class of cattle. Above 3000 mg kg^{-1} nitrate, the forage could potentially be unsafe for some livestock. The pure alfalfa biomass without any weed biomass contained nitrate concentration of 1014 mg kg^{-1}. However, common lambsquarters contained nitrate concentration of 5700 mg kg^{-1}. Thus, the nitrate concentration of the forage mixture increased as the proportion of common lambsquarters in the mixture increased (Figure 4). At 60% or greater proportion of common lambsquarters biomass in the forage mixture, nitrate concentration increased above the 3000 mg kg^{-1} threshold (Figure 4), presenting some toxicity risk to some classes of cattle if consumed in high quantities. Up to 15,000 mg kg^{-1} nitrate concentration was observed in common lambsquarters in a previous study (Davison et al., 1965). Thus, under certain conditions, common lambsquarters may accumulate significantly greater amounts of nitrate. Kochia also contained nitrate concentration of 4400 mg kg^{-1} and thus, the nitrate concentration of the forage mixture increased in proportion to the amount of kochia in the mixed forage (Figure 4). At more than 60% proportion of Kochia biomass in the forage mixture, nitrate concentration increased above the 3000 mg kg^{-1} threshold (Figure 4), which presents some toxicity risk to some livestock if consumed in high enough quantities. Shepherd's-purse had nitrate concentration of about 3700 mg kg^{-1} and therefore, the nitrate concentration of the forage mixture increased when the proportion of Shepherd's-purse in the mixture increased (Figure 4). However, the nitrate concentration of the forage mixture only increased above the 3000 mg kg^{-1} threshold when the proportion of shepherd's-purse biomass in the forage mixture exceeded 80% (Figure 4).

Field bindweed and green foxtail had nitrate concentrations of 1500 mg kg^{-1} and 840 mg kg^{-1}, respectively. Thus, the presence of these weeds in the forage mixture did not increase nitrate concentrations to toxic levels (Figure 4). Although no stress conditions that would be expected to increase nitrate concentrations were observed in this study, it must be noted that conditions that may reduce plant growth (e.g., drought) can increase nitrate accumulation and the risk of livestock poisoning (Bolan & Kemp, 2003; J. O. Hall, 2018; Olson et al., 2002). Research has also shown that nitrate accumulates more in the vegetative tissue, particularly in the stems (Bedwell et al., 1995). Delaying harvest may increase stem tissue and possibly increase the nitrate concentration of the forage mixture.

4 | CONCLUSIONS

Alfalfa hay producers who manage their fields for high nutritive value forage will benefit from the application of effective herbicides before the first harvest to reduce weed competition during alfalfa establishment and the proportion of weed biomass at harvest. The effect of weeds on total forage accumulation and nutritive value is dependent on the weed species present and their proportion of the stand at harvest. Early-maturing weeds like shepherd's-purse and grassy weeds such as foxtails can dramatically increase forage fiber concentration and reduce CP, and thus, they have the most potential to reduce forage nutritive value. Although weeds like common lambsquarters and kochia can add to whole-stand forage accumulation without reducing forage nutritive value drastically, these weeds can accumulate significant amounts of nitrate at levels that can be toxic to livestock. Thus, it is highly recommended to control these weed species in alfalfa to reduce the amount of biomass from these weed species.

AUTHOR CONTRIBUTIONS

Chandra L.-M. Montgomery: Data curation; investigation; writing—original draft. writing review and editing. **Albert O. Kwarteng**: Data curation; investigation. writing—review and editing. **Albert T. Adjesiwor**: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; supervision; validation; writing—original draft; writing—review and editing.

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