Herbage Accumulation and Quality of Alfalfa-Grass Intercropping Systems in Response to Two Irrigation Regimes

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Luana Quirino Souza Dayoub Zagato

Approved by:
Major Professor: Xi Liang, Ph.D.
Committee Members: Juliet Marshall, Ph.D.; Zachary Kayler, Ph.D.
Department Administrator: Juliet Marshall, Ph.D.

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Abstract

Alfalfa-hay is frequently produced for its excellent yield and quality. Due to the extended growing season, irrigation is the most challenging practice in alfalfa. Incorporating grasses into alfalfa cropping systems may shorten crop water use, refine water use efficiency, and increase fiber digestibility. This experiment compares the herbage accumulation and quality of hay for pure alfalfa to mixed and interrow cropping in response to full and deficit irrigation. A field experiment was set at the Aberdeen Research & Extension Center, University of Idaho, in 2020 and 2021. There were ten alfalfa-grass planting configurations: pure alfalfa, 75% alfalfa + 25% grass in mixture with three grass species (i.e., tall fescue, orchardgrass, and meadow bromegrass), 50% alfalfa + 50% grass in mixture with the three grass species, and 50% alfalfa + 50% grass in alternate rows with the three grass species. Results indicated that the herbage accumulation of the first year was greater than the second year; the first cuts yielded more than the other two cuts for both years; there were no differences between intercrops within cuts during 2021; concerning the irrigation regimes within cuts, the differences were observed during the third cut of 2020 and the second cut of 2021, being the herbage accumulation of the full irrigation regime greater than that of deficit irrigation. The differences between intercrops within cuts happened mainly in the first cut, especially for acid detergent fiber and total digestible nutrients; the highest crude protein content was observed in the third cut of the two years; in general, the neutral detergent fiber amount was greater for the intercrops in alternate rows. There was a negative correlation between crude protein and neutral detergent fiber; the same was observed for acid detergent fiber; however, the correlation with crude protein was positive for the total digestible nutrients. Therefore, both the irrigation regime and intercropping system significantly affect herbage accumulation and hay quality.

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Dedication

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Forage crops

Alfalfa

Alfalfa (Medicago sativa L.) is referred to as the "Queen of Forages" due to its great yield and excellent nutritive value; it is mainly used as hay and haylage. It originated in south-central Asia (modern Iran) (El-Ramady et al., 2020; Lei et al., 2017). It can be cultivated in monoculture and intercropping systems with grasses and other legumes.

According to El-Ramady et al. (2020) and Patra and Paul (2018), alfalfa is a dicotyledonous, leguminous forage with trifoliate leaves, flowers grouped in a raceme, reniform seeds, and an axial, long, and robust root system. Alfalfa is insect pollinated; the flowers can be yellow, blue, white, and purple.

Alfalfa is adapted to grow in various soil types in the western U.S.: sandy, clayey, and organic soils, as well as deserts and cool mid-valleys. Most western areas demand irrigation, but there are also substantial areas of rainfed alfalfa (Putnam et al., 2000). Alfalfa drought resistant is because its roots optimize soil penetration. For example, alfalfa can continue growing even with irrigation's end. When soil moisture is depleted, alfalfa begins a period of "drought-induced dormancy," which allows its survival (Orloff et al., 2014).

Among all crops grown for high-protein feed in the United States, alfalfa is one of the most important. According to the Crop Production released in August 2021 by the National Agricultural Statistics Service (NASS), Agricultural Statistics Board, and the United States Department of Agriculture (USDA), alfalfa and alfalfa mixtures for hay production were 53, 067 million tons in 2020, with Idaho being the state with the highest production: 4, 545 million tons, which represents 8.6% of this total alfalfa and alfalfa mixtures for hay production in 2020.

The price of alfalfa hay is lower during the harvest season and higher during the winter (Ward, 1985). Another factor that influences the price of alfalfa hay is quality. High-quality alfalfa implies a reduced quantity of alfalfa produced and increased production costs (Ward, 2004). Most alfalfa hay production in the US comes from irrigated areas, and these regions face restrictions regarding water use (Putnam et al., 2018).

Monocropping can increase rain runoff, plant diseases, pathogens, and weeds (Altieri, 2009). In an alfalfa monoculture, the resources are only partially used, especially the water resources, resulting in the loss of agricultural sustainability (Niu et al., 2020).

Orchardgrass

Orchardgrass (*Dactylis glomerata* L.) is a grass native to Europe that has been acclimatized in North America for over 200 years, making it important for grazing and hay production in the Northeast, Mid-North, and Pacific Northwest of the U.S. (Jensen et al., 2001). Its cultivation is easy to manage due to its good forage accumulation (balance between production and forage senescence (Weiss & Shockey, 1991).

The main limitation of orchardgrass is the high-water demand during all growth stages (Hoveland, 1992). On the other hand, orchardgrass can last from 5 to 8 years being well adapted to intense tillage and having a very expressive root system in the soil depth of 0-30 cm; it also can resist to shading caused by intercropped species, in addition to being a high feed value grass (Lopes and Santos (2002). During the growing seasons, the life cycle of orchardgrass and alfalfa are very similar (Miller, 1984). In addition, to yield and quality, orchardgrass-alfalfa mixtures have proven to be an alternative to alfalfa and grass in monoculture (Aponte et al., 2019).

Meadow bromegrass

Meadow bromegrass (Bromus riparius Rehm.) is native to southeastern Europe, the Caucasus, Turkey, and Central Asia (de Araujo et al., 2002). It is a cold-tolerant grass; in addition to having fast germination, it can also propagate vegetatively. Most of the leaves are found at the base of the plant, so the seed stalks are above them. In addition to producing lots of roots, it produces short and robust rhizomes, which may provide some drought tolerance. (Hanson, 1972a). A characteristic of the bromegrass meadow is the high number of small tillers below harvest height, contributing to good regrowth. Compared to other bromegrasses, the meadow, having smaller rhizomes, does not overrun alfalfa as readily. (Pearen & Baron, 1996). According to Knowles et al. (1993), as for all forages, the feed quality of meadow bromegrass also varies at different growth stages, with the vegetative stage being more nutritional than flowering.

Meadow bromegrass is suitable for intercropping with alfalfa. It grows upright, allowing vegetables to do well in mixtures, and intercropping can lower nitrogen fertilization costs and increase feed nutritive value compared to grass monoculture (Ashilenje & Islam, 2018).

Tall fescue

Tall fescue (*Festuca arundinacea* Schreb.) is a perennial grass initially utilized for hay or pasture in Europe. However, it is the most common cold-climate grass in the U.S. (Ball et al., 1991). The three most desirable agronomic characteristics of tall fescue are low requirements for nutrients, resistance to grazing, and staying green for most of the cold season; despite that, tall fescue is not as palatable as other grasses (Yates, 1962). Tall fescue is a good choice for intercropping with alfalfa because it can tolerate high temperatures and drought (Tracy et al., 2016; Carrow, 1996). In addition, intercropping tall fescue with alfalfa can improve weed competition compared to monocropping alfalfa or tall fescue (Tracy et al., 2016).

Meadow fescue

Meadow fescue (*Festuca pratensis* Huds), a wind-pollinated and self-incompatible species, is a perennial grass native to Europe with strong and long roots (Vinall, 1909). With grass genetic improvement in the last ten years, meadow fescue has returned to be cultivated in temperate regions of the USA (Brink et al., 2010). According to Brink et al. (2007) and Nieman et al. (2019), besides being compatible with alfalfa and maintaining good alfalfa:grass ratio, meadow fescue generally provides a more significant amount of nutrients than the vast majority of other temperate grasses. However, the palatability of meadow fescue is higher than that of tall fescue (Casler et al., 1998).

Forage Nutritional Parameters

Hay quality is measured by livestock performance. The main factors that influence cattle performance are the palatability of the hay (it must be ingested in satisfactory amounts), digestibility, and nutritional value (Jensen et al., 2006).

Wet chemistry is a widely-used laboratory procedures to assess the nutritive value of forages; it involves different chemical, biochemical, dehydrating, and combustion steps (Schroeder, 2004). A more rapid alternative to wet chemistry is the near-infrared reflectance (NIRS) method; it does not require massive sample collections and allows for broad sampling (Stuth et al., 2003). According to Stuth et al. (2003), the main advantages of NIRS over wet chemistry are that it is not necessary to use reagents or destroy samples, and it is possible to get multiple results at once. When using the NIR approach, a specified amount of near-infrared light is emitted, and the sample's reflectance is recorded in response to the quivering of CH, NH, OH, CO, and CC bonds (Stuth et al., 2003).

One of the feed's most critical nutritional characteristics is protein content. Crude protein (CP) is quantified from the amount of nitrogen, for calculating CP, nitrogen (g) is multiplied by 6.25 (1 part nitrogen for every 6.25 parts protein in most forages), representing the protein potential of the forage (Salo-väänänen & Koivistoinen, 1996).

Structural polysaccharides (e.g., cellulose and hemicellulose), waxes, and lignin are the main components of fiber. Because lignin impairs the nutritional quality of plant fiber, it is classified as an “anti-quality” constituent in forages, preventing the structural polysaccharides from being digested and absorbed (Hatfield et al., 1999).

The purpose of evaluating forage quality for neutral detergent fiber (NDF) is to evaluate digestibility. Silica, cellulose, lignin, and hemicellulose are components of NDF (Schroeder, 2004). Forage NDF digestibility (NDFD) is measured as the percentage of total NDF of the biomass and used to determine the energy rating of forages. Several factors can affect NDF digestibility: plant maturity (as the xylem tissues lignify, the NDF digestibility decreases), genetics, and the growing environment of the plant (plants from tropical climates have a lower NDF digestibility than those from temperate and cold climates) (Hoffman et al., 2001; Hoffman & Combs, 2004). Another way to assess digestibility is the in vitro true dry matter digestibility (IVTDMD), which considers the feed's actual digestibility and includes nutrient losses during digestion (Mertens & Grant, 2020). Acid detergent fiber (ADF) is a portion of the cell wall that consists of cellulose, lignin, and silica. ADF-rich forages are generally lower in energy and digestibility; the difference between NDF and ADF provides an estimate of hemicellulose. The relative feed value (RFV) is an indicator for temperate climate grass and legume forages that considers the digestibility from ADF and intake potential from NDF. This indication is handy for determining the characteristics of hay and silage of legumes or legumes intercropping with grass. (Allen et al., 2011).

The three components comprising the feed's energy value are carbohydrates, fat, and proteins. Total digestible nutrients (TDN) are the nutritional value of a feed computed from the ingestion of digestible nutrients (protein, fat, fiber, and non-structural carbohydrate) (Allen et al., 2011). TDN intake and fiber digestibility calculate the relative forage quality (RFQ) (Jeranyama & Garcia, 2004). Net energy (NE) is the energy produced by assimilation processes subtracted from the energy lost due to fermentation and nutrition consumption heat. The two best and most used methods to measure sugar levels in feeds are ethanol- and water-soluble carbohydrates. Water- and ethanol-soluble carbohydrates include glucose, fructose, sucrose, and fructans, with the fructan concentrations being much smaller for ethanol-soluble carbohydrates than for water-soluble ones (Kagan, 2022; Kagan et al., 2018; Waite & Boyd, 1953).

Ash is all the forage mineral and non-organic content (Villalba et al., 2021; Allen et al., 2011). Ruminants obtain most of their essential minerals from forages. Two factors are necessary for the forage to supply the animal's mineral demand: the quantity of mineral present in the forage and the nature of the mineral's bioavailability; some of the main minerals required by livestock are calcium, phosphorus, potassium, and magnesium (Spears, 2015).

According to Livestock, Poultry, and Grain Market News (LPGMN), Agricultural Marketing Service, and the United States Department of Agriculture (USDA) (n.d.), these are the quality guidelines for alfalfa hay with less than 10% grass and grass hay:

Table 1.1. Quality guidelines for alfalfa hay with less than 10% grass.

Quality	ADF	NDF	RFV	TDN-100%	TDN-90%	CP
Supreme	<27	<34	>185	>62	>55.9	>22
Premium	27-29	34-36	170-185	60.5-62	54.5-55.9	20-22
Good	29-32	36-40	150-170	58-60	52.5-54.5	18-20
Fair	32-35	40-44	130-150	56-58	50.5-52.5	16-18
Utility	>35	>44	<130	<56	<50.5	<16

Table 1.2 Quality guidelines for grass hay.

Quality	CP (%)
Premium	>13
Good	9-13
Fair	5-9
Utility	<5

According to Marsalis et al. (2009), the hay quality classifications are:

Table 1.3 Hay quality designations (Marsalis et al., 2009).

	Maturity stage	**Bloom stage**	**Leaf-stem ratio**	**Nutrient content**	**Appearance**
Supreme	Very early	Pre-bloom	Very leafy	Very high	No damage; Very good color
Premium	Early	Pre-bloom (legumes); Pre-head (grass)	Very leafy	High	No damage; Green
Good	Early to mid	Early to mid-bloom (legume); Early head (grass)	Leafy	Good	No damage; Some discoloration
Fair	Late	Mid to late-bloom (legume); Head-in (grass)	Average to below leafy	Average	Light damage
Utility	Very late	Seeds (legume); Mature head (grass)	Below leafy	Poor	Significant damage; Weeds; Mold

Alfalfa-grass systems

Advantages of intercropping

Intercropping is when two or more crops are grown concurrently and on the same field with a long period of overlap (Gomez & Gomez, 1983). The main advantage of intercropping is better resource use efficiency (Gebru, 2015). For example, in a three-year alfalfa-grass system experiment, the total yield of the 50-50% and 70-30% meadow bromegrass mixtures with alfalfa were more outstanding when compared to alfalfa and meadow bromegrass in monoculture. In addition, compared to the CP percentage of sole meadow bromegrass (12%), there was an improvement of 20% for the mixture of 50-50% meadow bromegrass and alfalfa and 19% for the mixture of 70-30% of meadow bromegrass and alfalfa. Intercropping alfalfa and grass can increase yield and feed quality (Ashilenje & Islam, 2018). Also, without intercropping with grass, alfalfa can be more affected by low temperatures during winter and thus persist less (Malhi et al., 2002).

Grass can be intercropped with a legume in hay fields to benefit forage quality and reduce costs (Brown & Munsell, 1943). The legume can complete the amount of protein lacking in grass feed and also fix nitrogen from the atmosphere and convert it so that it can also be used by the grass (Cinar & Hatipoglu, 2015). According to Tomm (1994), the amount of N in meadow bromegrass obtained from N_2 fixation improved from 13% in the first year to 32% in the second and 34% in the third year, showing that the alfalfa-grass yield can be similar to monocultures of grasses well supplemented with nitrogen (Sollenberger et al., 1984). Therefore, adding grasses to alfalfa can be a cultural control to lower alfalfa weevil levels in legume forage fields, since it is the main pest in alfalfa (Roda et al., 1996).

Two most advantages of intercropping alfalfa and grass are improving the feed's nutritional content and animal performance (Aponte et al., 2019). Because alfalfa is more digestible than grasses, cattle-fed alfalfa tends to consume more forage and water than tall fescue and orchardgrass (Warren et al., 1974). Additionally, alfalfa-grass systems can guarantee a better seasonally distributed forage supply than grass monoculture. The fiber digestibility of the mixture might be higher or close to that of sole alfalfa hay, and the crude protein can be close to alfalfa monoculture, thus decreasing the need for supplementation in the diet (Veira et al., 2010); (Aponte et al., 2019).

It is important that the grass species used in the intercropping with alfalfa regrows rapidly to reduce the bloating risk and maintain a balanced legume-to-grass ratio (Kopp et al., 2003). Bloating arises when the animal is not able to eliminate the excess gas produced during the digestion process. In diets where most of the forage offered is leguminous, there is a higher production of foamy rumen tenors, which hinders or prevents the elimination of gas (Majak et al., 2003).

Even if the proportion of alfalfa in the stand decreases, it is still possible that there is an increase in feed quality. For example, in a 4-years experiment, the alfalfa proportion on the stand decreased from 84% to 40% (Kopp et al., 2003). However, it was still possible to improve by 28% the amount of forage available for grazing compared to bromegrass meadow-only pasture and meet the nutritional requirements for the beef cows during the lactation phase. Therefore, intercropping can be a viable and economical alternative to pure alfalfa (Kopp et al., 2003).

Factors influencing the intercropping

Grasses are less drought tolerant than alfalfa because they have hairy roots and do not access deep soil moisture (Orloff et al., 2014). Alfalfa, on the other hand, can yield more than tall fescue in a water deficit regime (Lazaridou et al., 2012). Furthermore, grasses cannot recover vigorously after a long drought season (Orloff et al., 2014; Lazaridou et al., 2012).

Yield may vary depending on the soil. Data showed that the smooth bromegrass and alfalfa mixture had a higher hay yield than the intercrop with meadow bromegrass in rich soils; however, the opposite was observed in poorer soils with less moisture (Pearen et al., 1995). Therefore, soil fertility can influence the choice of the best grass species for the alfalfa intercrop (Pearen et al., 1995).

Another important characteristic of alfalfa is that it can be overly competitive, interfering with the balance in an alfalfa-grass intercropping. Light absorption is the leading competitive factor in an alfalfa-grass intercrop; the vegetative capacity and the plant's architectural aspects are related to interspecific competition (Annicchiarico et al., 2014). Grasses have a lower yield when planted with tall legumes than intercropped with short legumes (Cooper 1979). So, the contribution of the grass to the yield depends on the height of the legume growth (Cherney et al., 2020). Also, the percentage of grass in alfalfa-grass intercrops can significantly be influenced by characteristics of the grass species. Donald (1963) asserted that grasses with fewer horizontal leaves and legumes with more parallel leaves can contribute to a favorable interaction between grass and legume. In legume and grass mixtures, the grass ratio reduces while the legume proportion improves in the year following initiation. In addition, the grass competitiveness moderately declines as the establishment is extended (Liu et al., 2022).

Each grass species has different forage nutritional parameters. According to Allen et al. (2013), for a two-year rainfed grazing experiment conducted in Minnesota, with two nitrogen applications of 56 kg/ha per year, during the first year, in spring, summer, and fall, crude protein values for meadow bromegrass, orchardgrass, and tall fescue were, respectively 22.4, 18.3, and 19.2%, 17.3, 16.7, and 19.3%, 21.6, 17.9, and 20.6%; the amounts for the second were: 21.3, 20.1, 21.1%, 22.9, 21.5, and 22.6%, 23, 19.4, and 20%, respectively. The NDF estimates in the first year for the same seasons and species, respectively, were: 44.8, 47.5 and 45.9%, 59.4, 58.9 and 55.3%, 46.3, 47.3 and 46.7%, for the next year following the same order of seasons and species, the NDF values were: 41, 42.2 and 45.9%, 48.2, 48.5 and 50.4%, 48, 64.8 and 51.4%. Therefore, the choice of grass species influences the quality of the alfalfa-grass feed.

As time passes, the amount of grass in the alfalfa grass stands can increase. According to Aponte et al. (2019), this happens because the amount of alfalfa plants decreases as the alfalfa becomes more vigorous, allowing grass growth in these spaces. In contrast, Chamblee and Collins (1988) state that most alfalfa cultivars in North America tend to dominate the stand when intercropped with grass.

There is not always a balance between yield and feed quality. For example, a study with grass and legume monocultures for grazing showed that the average yield of meadow bromegrass was approximately 60% of the average yield of orchardgrass and tall fescue (MacAdam et al., 1997). In contrast, the mean crude protein of orchardgrass (14.6%) and tall fescue (13.9%) was less than that of meadow bromegrass (18.8%), highlighting that in late July, the amount of CP of meadow bromegrass (20.3%) was close to that of alfalfa (20%) for the same period of harvest; the average NDF of tall fescue (55.8%) and orchardgrass (53.9%) was higher than that of meadow bromegrass (50.4%) and alfalfa (39.6%) during the entire crop cycle (39.6%); the same happened for the ADF, the total mean ADF of orchardgrass (31.7%) and tall fescue (33.0%) was higher than that of meadow bromegrass (27.3%) and alfalfa (29.5%) (MacAdam et al., 1997). In an irrigated experiment in the southern interior of British Columbia, combinations of tall fescue and orchardgrass with alfalfa seem to have correspondent yields, but those with tall fescue differ positively in terms of nutritive value; therefore, decrease in yield can be compensated with an increase in feed quality (Thompson, 2013).

Yield and feed quality vary according to the prevailing intercropping crop. According to Adjesiwor and Islam (2015), in general, mixtures of 50-50% and 30-70% of alfalfa and meadow bromegrass had a lower dry matter (0.467kg/m^2 and 0.479 kg/m^2, respectively) than alfalfa in monoculture (0.521 kg/m^2) for the first year after establishment. However, the opposite happened the following year, with 0.682 kg/m^2 for the 50-50% mixture and 0.822 kg/m^2 for the 30-70% mix compared to 0.610 kg/m^2 of alfalfa sole. Furthermore, in the same study (Adjesiwor & Islam, 2015), mixtures of 50-50% and 30-70% of alfalfa and meadow bromegrass had a lower crude protein during the first year after establishment (214% and 206%, respectively) than during the second year (187% and 181%, respectively), being lower than the crude protein value for alfalfa during the two years (286% and 292%, respectively). Therefore, in the first year after establishment, it is possible that alfalfa was the predominant crop and that grass predominated in the second year due to its increase in yield and decrease in crude protein.

The harvest season can interfere with the yield of alfalfa and grass intercrops, since the regrowth periods are different for each season, temperature and soil moisture differences can interfere with forage maturation. A two-year study in which alternate rows of alfalfa-reed canarygrass and alfalfa-orchardgrass were planted in two different patterns showed that alfalfa and grass intercrops yielded more than sole alfalfa during only one development period in the spring. Moreover, the intercrops were less advantageous in the summer than the alfalfa monoculture. In intercropping, the season can benefit one species more than the other (Mooso & Wedin, 1990). The harvest season can also influence the nutritional value. As Bagley et al. (1983) reported, for tall fescue harvested in summer (May and July), crude protein and ADF were higher than for winter cuts (November and February).

When alfalfa or perennial grasses are cut, the plant must start regrowing from axillary basal stem buds; the stem replaces itself and may expand the crown too (Collins et al., 2017). The cutting frequency can influence the alfalfa ratio in the mix. In a three-year experiment, for alfalfa mixed with tall fescue, in the third year, the percentage of alfalfa in the mix was around two times greater for the 4- and 6-week cutting frequency than for the 3-week cutting frequency (Hoveland et al., 1995). The grass species also influences the regrowth. Three-year research of alfalfa binary intercrops with orchardgrass, smooth bromegrass, and ryegrass at a frequency of 3 cuts per year reported that orchardgrass intercropping seemed to have the best vigor, ground cover, and regrowth performance when compared to the other two mixes (Casler et al., 1988). In other words, it is necessary to consider the regrowth capacity of alfalfa and grass to determine the harvesting frequency of intercropping.

Hay quality can vary with the harvest and stand age. With the passage of time and plant maturity, a reduction in crude protein degradability was observed from 85 to 80% in alfalfa and from 78% to 69% in orchardgrass; the degradability of dry matter also decreased for both species, from 73 to 62% in alfalfa and from 69 to 56% in orchardgrass; therefore, legume and grass maturity stages can affect crude protein and dry matter breakup (Balde et al., 1993).

With increasing harvesting intensity, irrigation seems to have a lessening impact on alfalfa-orchardgrass yield. In a irrigated field of alfalfa and orchardgrass mixture, the yield improvement happened only at a schedule of four cuttings per year, when cut six times a year, the yield was not affected; showing that a high cutting frequency can impair the re-establishment of the carbohydrate reserve between harvests (Ward et al., 1966).

Forage is evaluated by total production, thus not considering the differences between species (Mooso & Wedin, 1990). The alfalfa-grass system follows the same cultivation recommendations for alfalfa monoculture, and the choice of grass species for the intercrop is still based on the sole performance of these species. According to Rhodes (1976) and Haynes (1980), grass characteristics that influence the competition with alfalfa in intercropping are grass plant morphology, defoliation severity, growth rate, and lateral shoot production aspects. When a grass species is too aggressive, it can reduce the alfalfa stand and the nutritional value (Knowles et al., 1993). Still, the best solo performance is not always the best choice for intercropping with alfalfa (Casler, 1988).

Methods of irrigation

Evapotranspiration (ET) is the sum of the evaporation (from the soil surface and a wet canopy) and the plant transpiration. According to Allen et al. (1998), the crop evapotranspiration (ET_c) “is determined by the crop coefficient approach whereby the effect of the various weather conditions are incorporated into ET_0 and the crop characteristics into the K_c coefficient." This method is based on plant water requirements and simply indicates the amount of water to be applied (Jones, 2004).

In order to calculate the evapotranspiration rate for alfalfa (ET_c), it is first necessary to estimate the alfalfa reference crop evapotranspiration (ET_0) (the ET from a large area of well-watered alfalfa that completely shades the soil) and also the applicable crop coefficient (K_c) (Wright, 1998). Crop properties and the typical impacts of soil evaporation are considered in the K_c coefficient (Allen et al., 1998). irrigation and water management are applied following alfalfa evapotranspiration, $ET_c = K_c \times ET_0$ (Allen et al., 1998).

The irrigation demand for alfalfa is constrained by precipitation and soil water holding capacity; because it has dense foliage, is not a short-season crop, and does not have a shallow root system, alfalfa requires high water use (Shewmaker et al., 2011). Temperature, wind, moisture, and luminosity all impact the amount of water required to grow alfalfa. The quantity of water exported with alfalfa hay is less than other crops. For a typical alfalfa hay crop, and if the hay is harvested at 12% moisture content, it can take about 109 kg water/ton of hay per year (Shewmaker et al., 2011).

Alfalfa has a proportional yield return to water input, and forage water output is often higher during the first cut than other harvests (Shewmaker et al., 2011). Experiments of non-stressed alfalfa near Kimberly, Idaho, indicated that each ton of alfalfa would require around 127 mm of water (Wright, 1988).

Nowadays, alfalfa growers primarily use four irrigation methods: flood, sprinkler, center pivot, and subsurface drip irrigation. The benefits and drawbacks of these four irrigation methods are listed in the table below (Sanden et al., 2011; Fortier, 1940; Almarshadi & Ismail, 2011):

Table 1.4 The advantages and disadvantages of flood, sprinkler, center pivot and subsurface drip irrigation according to Sanden et al. (2011) Fortier (1940) and Almarshadi & Ismail (2011).

Irrigation method	**Advantages**	**Disadvantages**
Flood	• No water filtration; • The infiltration rate may vary according to the season; • Tailwater can improve stand quality; • Energy cost is low or zero	• Drought stress may occur between irrigation cycles; • The crop land must be flat; • The soil can be saturated, and anoxia may occur;
Sprinkler	• No borders and planting levels; • The water use is more effective during the germination; • It is possible to fertigate	• High cost of energy and labor
Pivot	• Quick area coverage; • More standardized than hand-move and side-roll; • Pesticide application and fertigation are possible; • Acceptable cost; • Less expensive in terms of power and labor than other sprinklers	• Water filtration; • The rates of prompt flow are elevated; • Increased evaporation losses and waste on the edges
Subsurface drip irrigation	• Daily watering; • Attainable maximum crop transpiration; • The wind does not affect uniformity; • Potentially superior P and K application	• Sprinklers are necessary for starting; • Salinity may cause problems; • Root clogging; • Monitoring of pressure and soil moisture is essential

Water use efficiency

Water use efficiency (WUE) shows the relationship between crop yield and water use (Briggs & Shantz, 1913), another common way to define water use efficiency is the quantity of biomass produced per unit of crop evapotranspiration (Irmak et al., 2011). In addition, efficient water use creates environmental and economic benefits by protecting water resources (Fink, 2021). Alfalfa-grass intercropping systems allow for better soil and water use than alfalfa in monoculture; due to the morphological differences in the root system of alfalfa and grass, there is a better use of water, since the roots have different depths and therefore the use of water is more efficient (Mousavi & Eskandari, 2011).

Evapotranspiration occurs according to atmospheric conditions, canopy temperature and light absorption capacity, the plant itself, and the weather (Carlesso, 1995). The depth of extraction is the most significant factor in determining the total amount of water removed from the soil, and this amount of extracted water is associated with crop yield. (Squire et al., 1987).

Under drought conditions, although a grass monoculture may have a higher water use efficiency than an alfalfa-grass mixture, the monoculture system also has a higher soil water depletion (Hendrickson et al., 2013). Soil water depletion comprises the volume of water taken out of the system; significant soil water depletion can be a problem during prolonged droughts.

In the western states, water restriction for irrigation can be a concern (Orloff & Putnam, 2010). Once the water reserve for sole alfalfa is restricted, there are three ways forward: not irrigating some areas and thoroughly irrigating the other fields, deficit irrigating all areas, and fully irrigating all fields during the first few harvests and then suspending irrigating when alfalfa ET demands are high (usually mid-season) (Orloff et al., 2014). In a rainfed experiment in Texas, alfalfa-grass mixtures had a 25% higher water use efficiency than sole grasses during two years, demonstrating that the increment in forage mass due to intercropping with alfalfa was more significant than the soil water removal rise (Dhakal et al., 2020a). Alfalfa monoculture and intercropping with grasses (orchardgrass and bromegrass) were more effective at using water for the entire harvest year under non-irrigated conditions than the other two irrigation regimes (irrigation was applied when the 10-cm depth reached 85% and 30% of its available water) (Powell & Kardos, 1968). Intercropping grass with alfalfa can increase soil water depletion and improve water use efficiency. For example, according to Adjesiwor and Islam (2015), in a two-year experiment, the water use efficiency was better for the mixes of 50-

50% and 30-70% of alfalfa and meadow (60 kg DM/mm and 74 kg DM/mm respectively) than for the alfalfa monoculture (50 kg DM/mm).

Depending on the temperature, variety, number of cuts per year, latitude, elevation, and winter season, alfalfa can require anywhere between 508 and 1168 mm of water per year (Shewmaker et al., 2011). Alfalfa-native grass systems outperformed sole grass systems in terms of forage biomass, nitrogen output, and water use efficiency; also, interseeding alfalfa at a wide row spacing in native pasture would be one approach to reduce soil water loss (Dhakal et al., 2020a; Dhakal et al., 2020b).

The consistency of the water application process is also related to quality and yield (Montazar, 2010). In addition to limiting crop respiration, growth rate, and yield, over-irrigation can lead to soil saturation and root waterlogging, stimulating pest outbreaks. Therefore, the linkage between irrigation supply, uniformity, alfalfa-grass yield, and quality should be established to help farmers manage their irrigation systems more effectively. Also, more information is needed on alfalfa-grass intercropping systems and how water stress can affect the stand yield and hay quality across cuts and years.

Chapter 2: Herbage accumulation

Introduction

Alfalfa hay

Alfalfa (*Medicago sativa* L.) was initially grown on a few acres in the eastern USA and well adapted to the west during the 19th century; today, alfalfa is a “top crop” in the Western US (Putnam et al. (2000). The west “Gold Rush” was of great importance for expanding alfalfa cultivation since, at that time, almost everything was moved by animals, and hay appeared with the need to conserve forage for animal feed in unfavorable periods for grazing. Because alfalfa hay is a crucial part of the feed proportion for dairy cows, it is difficult to substitute it with other forages (Blank et al., 2001).

Hay is not only alfalfa; hay is all forage that goes through haying. The three haying processes are: cutting, drying, and baling. After cutting, the forage is dried in the field until it reaches between 12 and 18% moisture (Bonato, 2004).

Farmers must pay attention to the harvest timing for hay production since the hay's nutritive value is related to the crop's growth stages. Another essential factor would be to avoid cutting on rainy and dewy days since this can affect the hay drying and viability (Bonato, 2004).

The yield may vary according to the number of cuts per year for alfalfa hay production. For example, according to Blank et al. (2001), the yield decreased by about 0.75 tons with four cuts per year, compared to three cuts per year.

Hay price varies according to the hay type, quality, and location; a bale of grass is much cheaper than a bale of alfalfa. Alfalfa is a high-water use crop because it is perennial with dense canopy coverage, and in the western states, water restriction for irrigation can be an obstacle (Orloff & Putnam, 2010). According to Aponte et al. (2019) and Sleugh et al. (2020), the first harvest is the highest-yielding cutting. So, the recommendation would be to irrigate thoroughly during this period (Orloff et al., 2014). Another concern about pure alfalfa hay is the bloating risk; bloating is common when ruminants graze legume forages but can also occur with high-quality hay feed (Collins et al., 2017). There is a positive relationship between the amount of alfalfa protein and bloating (Majak et al., 1995).

Alfalfa-grass intercropping systems

By excessive use of water and soil, monocropping can cause ecology and environmental problems (Reganold, 1992). Intercropping can be an alternative to monocropping, since intercropping, in addition to increasing diversity in an agricultural ecosystem, also allows for better soil and water use (Mousavi & Eskandari, 2011). Intercropping is when two or more crop species are planted at the same time during the growing season (Mousavi & Eskandari, 2011).

According to Vandermeer (1992), there are four types of intercropping: row intercropping, mixed intercropping, strip intercropping, and relay intercropping. Row-intercropping is when crops are planted in regular lines, while in mixed-intercropping, there is no distinction between rows. In strip-intercropping, crops are planted in different wide strips. Finally, relay-intercropping is when crops are not grown at the same time throughout the entire growing season. The main advantages of intercropping are increased yield, better use of environmental resources, improved soil fertility, increased nitrogen, and decreased pests, diseases, and weeds (Mousavi & Eskandari, 2011).

It is possible to intercrop alfalfa and grass to obtain better hay yields; orchardgrass, tall fescue, and meadow bromegrass are cool-season perennials that perform well when intercropped with alfalfa and are drought-tolerant (Lopes & Santos 2002); (Hanson, 1972b); (Pearen & Baron, 1996) (Tracy et al., 2016; Carrow, 1996).

Some factors can influence the quality and yield of alfalfa-grass hay: planting design, height cutting, maturity stage, grass species, and irrigation management. As a result, the yield and nutritive value of the alfalfa-grass intercropping system can be similar to pure alfalfa and higher than monocropping grasses (McDonald et al., 2021). For example, according to Aponte et al. (2019), alfalfa-grass mixtures had a higher yield and nutritive value than grasses in monoculture; they produced a feed with similar crude protein contents to pure alfalfa.

Companion grasses

According to Brougham (1959), in an intercrop, the species' annual growth curves must be complementary; due to different optimum growth temperatures, the contribution of each species to the total yield varies. Other variables that can influence the botanical composition of an intercrop are the growth vigor and the yield potential of each species (Haynes, 1980). Grass species with high yield potential in monocultures also tend to yield well when intercropped with legumes since the environmental conditions do not favor only the grass (Cowling & Lockyer 1967). Another crucial factor is the speed at which seeds emerge and grow: alfalfa tends to be aggressive in the seeding period, while some grasses might become aggressive once it has been established (Blaser et al., 1956).

Competition for light in a legume and grass intercropping encompasses interactions with water and nutrient availability (Blackman & Black, 1959). Still, according to Donald (1963), in an intercrop, if a species obtains a more significant amount of the limiting nutrient, consequently, it will grow more and shade the other species. According to Chamblee and Collins (1988), the vast majority of alfalfa cultivars grown in North America are inclined to overgrow their intercropped grasses, perhaps because the way alfalfa leaves are arranged favors the light to penetrate even the lower leaves (Leach, 1978). Grasses compete with alfalfa by shading the "crown buds," which can prevent them from developing (Chamblee & Lovvorn, 1953).

According to Leach (1978), legumes are less efficient in water use than grasses; grasses have better stomatal control and therefore lose less water by transpiring. On the other hand, legumes have approximately twice as much root cation exchange capacity as grasses, which results in a better absorption of divalent cations and an impoverishment in P and K competitiveness by legumes (Asher & Ozanne 1961).

The main difference during water stress between alfalfa and perennial grasses (tall fescue, orchardgrass, and bromegrass) is that the yield of grasses drops significantly as soon as irrigation is suspended. In contrast, for alfalfa, the effect is not that immediate. This distinction happens because alfalfa has deeper roots and can take better advantage of deep soil water (Orloff et al., 2014).

Still according to Orloff et al. (2014), bromegrass species may perform better under water stress than tall fescue and orchardgrass. However, they do not endure full-season irrigation, because bromegrasses are more adapted to drier conditions, full-season irrigation can cause excessive vegetative growth, leading to a yield reduction. Orchardgrass can persist for at least one season under deficit irrigation but performs worse than tall fescue. Tall fescue best fit full and deficit irrigation regimes.

In a grass-legume intercropping, although the legumes fix nitrogen, they still compete with the grasses for soil mineral nitrogen (Simpson, 1965). However, the dynamics of nitrogen are not consistent. They depend on which species are intercropped, the percentage of legume in the intercrop, the age of the stand, and the cultivation management (Vallis & Wilson 1978; Whitehead, 1970). According to Parsons (1958), the yield and composition of alfalfa-bromegrass were not affected by nitrogen fertilization, while the yield of the first alfalfa-orchardgrass harvest was. This response is because orchardgrass can dominate the mixture with nitrogen fertilization.

More information is needed on how alfalfa-grass intercropping systems and how water stress can affect the stand yield across cuts and years. We hypothesize that if the right grass species and mixing ratio are selected, alfalfa-grass intercropping systems can produce a similar yield to pure alfalfa stands. This experiment aims to compare the herbage accumulation in pure alfalfa stands to mixed and alternate-row intercropping systems in response to full and deficit irrigation.

Material and methods

Experimental Design

A two-year field experiment was conducted in 2020 and 2021 at Aberdeen Research & Extension Center of the University of Idaho in Aberdeen, ID. The experimental field was planted with alfalfa (cultivar 'FSG 415BR'), meadow bromegrass (cultivar 'Cache'), orchardgrass (cultivar 'Pawnee'), and Tall Fescue (cultivar 'FSG 402TF') on August 19, 2019. Ten alfalfa-grass intercropping systems (Table 2.1) and two irrigation regimes were arranged following a split-plot design with four replicates. The irrigation treatment was the main plot, and the alfalfa-grass intercropping system was the sub-plot.

Irrigation was applied following alfalfa crop Evapotranspiration (ET_c) described by Allen et al. (1998):

$$ET_c = ET_0 \times K_c$$

Daily reference ET (ET_0) was retrieved from the AgriMet Cooperative Agricultural Weather Network in the Columbia-Pacific Northwest Region. The crop coefficient (K_c) is determined experimentally; it accounts for the changes in leaf area, canopy, plant height, development, irrigation method, and soil and weather conditions (Pereira & Alves, 2005; Irmak, 2008). In this experiment, to simplify the ET_c, for each cut, a crop coefficient (K_c) of 0.5 was employed before full canopy coverage, and 1.0 was used at canopy closure in pure alfalfa under full irrigation.

There were three cuts during each growing season. For the full irrigation treatment, 100% ET_c was applied throughout the growing season. For the deficit irrigation treatment, 100% ET_c was applied during the first cut and 60% ET_c during the second and third cuts. In 2020, the total for full irrigation was 560 mm and 432 mm for deficit irrigation, while for the year 2021, the total for total irrigation was 747 mm and 533 mm for deficit irrigation.

Table 2.1The ten alfalfa-grass intercropping systems and the seeding rates.

	Seeding rate of alfalfa (kg/ha)	Seeding rate of grass (kg/ha)
100% Alfalfa	22.4	---
75% Alfalfa + 25% Orchardgrass in mixture	16.8	2.8
75% Alfalfa + 25% Meadow brome in mixture	16.8	4.2
75% Alfalfa + 25% Tall Fescue in mixture	16.813	4.203
50% Alfalfa + 50% Orchardgrass in mixture	11.209	5.604
50% Alfalfa + 50% Meadow brome in mixture	11.209	8.406
50% Alfalfa + 50% Tall Fescue in mixture	11.209	8.406
50% Alfalfa + 50% Orchardgrass in alternate rows	22.417	11.209
50% Alfalfa + 50% Meadow brome in alternate rows	22.417	16.813
50% Alfalfa + 50% Tall Fescue in alternate rows	22.417	16.813

Table 2.2 Water input during 2020 and 2021.

	2020			2021		
	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
			mm			
Full irrigation	245	123	192	213	299	235
Deficit irrigation	245	74	115	213	179	141
Rainfall	39	43	5	17	1	36

An area equivalent to 0.975 m^2 (1.067 m × 0.914 m) was harvested from each plot during each year's cuts; in 2020, the first cut was harvested on June 9th, the second on July 16th and the third on August 22nd; in 2021, the first cut was harvested on June 7th, the second on July 19th, and the third on August 31st. The entire harvested biomass was weighed as fresh weight, and a subsample was separated for alfalfa and grass composition and measuring dry matter concentration. After drying in an oven at 60ºC for three days, the herbage accumulation was estimated. The herbage accumulation is the biomass that grows above ground and degrades over time, including senescence (Allen et al., 2011).

Data Analysis

Analyzing the data separately for each year allows it to account for the variation between the years and observe how the factors affect herbage accumulation over time. All response variables were analyzed using linear mixed model procedures as implemented in RStudio (R version 4.2.2). Intercropping systems (sub-plot), cuts, irrigation regimes (main plot), and their interactions were considered fixed effects. Block was considered a random effect, and cut was considered repeated measure. The CLD function (compact letter display) (multcomp and multcompView packages) (Bonferroni method) was used to provide the contrasts within interactions. Differences were considered significant at $P \leq 0.05$.

Results

Herbage accumulation

In 2020, the herbage accumulation was significantly affected by the intercropping system (P<0.001), irrigation regime (P=0.043), cut (P<0.001), and the interactions between the intercropping system and cut (P<0.001), and between the irrigation regime and cut (P<0.001) (Table 2.3). Among the three cuts, the first was the only one that showed differences between the averages of herbage accumulation for the intercropping systems (Table 2.5). The 50A+50F(AR) and 50A+50M(AR) intercrops showed a higher herbage accumulation than the others, while the 100A intercrop differed inferiorly from the others, except in comparison with the 75A+25O (Table 2.5). The intercrops planted in mixtures, except for 75A+25O, did not differ (Table 2.5). Among the intercrops with 50% alfalfa, the grass species orchardgrass showed no difference when mixed or in alternate rows (Table 2.5). Regarding the irrigation regime, the herbage accumulation in the full irrigation treatment was greater than in the deficit irrigation regime only in the third cut (Table 2.7).

In 2021, the herbage accumulation was significantly affected by the intercropping system (P=0.0002), cut ((P<0.001), and the interaction between the irrigation regime and cut (P<0.001) (Table 2.3). The only difference observed between the irrigation regimes occurred in the second cut, where the full irrigation regime had a more significant herbage accumulation than the deficit irrigation (Table 2.9).

Alfalfa herbage accumulation

During 2020, the alfalfa herbage accumulation was significantly affected by the intercropping system (P<0.001), irrigation regime (P =0.034), cut (P <0.001), and the interactions between the intercropping system and cut (P <0.001), and between irrigation regime and cut (P <0.001), (Table 2.3). In the first cut, the intercrop with the highest alfalfa herbage accumulation were 100A and 75A+25F, while the lowest was 50A+50O (Table 2.6); the intercrops with 75% alfalfa did not differ among each other, except for the treatment with tall fescue (Table 2.6); among the intercrops with 50% alfalfa, the grass species orchardgrass was the only one that showed a difference when mixed or in alternate rows (Table 2.6); intercrops with 50% alfalfa were lower than those with 75% alfalfa, except 75A+25F (Table 2.6). In the second cut, the 100A intercrop differed positively from all others, except 75A+25F and 75A+25M (Table 2.6); intercrops with 75% alfalfa did not differ among themselves, those with 50% alfalfa in the mixture and those with 50% alfalfa in alternate rows did not differ among themselves either (Table 2.6); among the intercrops with 50% alfalfa, there was no difference between the grass species in the mixture or alternating rows (Table 2.6). In the third cut, the intercrop 100A differed positively only about the intercrops in alternate rows and 50A+50O (Table 2.6); among the intercrops with 50% alfalfa, there was no difference between the grass species in the mixture or alternating rows (Table 2.6); intercrops with 75% alfalfa treatments did not differ, those with 50% alfalfa in the mixture and those with 50% alfalfa in alternate rows did not differ either (Table 2.6). With regards to the irrigation regime, the alfalfa herbage accumulation was greater in full irrigation than the deficit irrigation only in the third cut (Table 2.8).

In 2021, the intercropping system ($P<0.001$), cut ($P<0.001$), and the interaction between the irrigation regime and cut ($P<0.001$) significantly affected the alfalfa herbage accumulation (Table 2.3). The only difference observed between the irrigation regimes occurred in the second cut, where the full irrigation regime showed a more significant alfalfa herbage accumulation than the deficit irrigation (Table 2.10).

Total herbage accumulation

The total herbage accumulation was significantly affected by the intercropping system ($P=0.0002$), irrigation regime ($P=0.031$), year ($P<0.001$), and the interaction between the intercropping system and year ($P<0.001$) (Table 2.4).

In 2020, the 100A intercrop was significantly lower than all other intercrops, except for those with 75% alfalfa and 50A+50O (Table 2.11). Intercrops in alternating rows with tall fescue and meadow bromegrass were only more significant than intercrops 75A+25O, 75A+25F, and 100A (Table 2.11). Intercrops with 75% alfalfa did not differ (Table 2.11); those with 50% alfalfa in the mixture and those with 50% alfalfa in alternate rows did not differ either (Table 2.11). Among the intercrops with 50% alfalfa, there was no difference between the grass species in the mixture or alternating rows (Table 2.11).

Concerning 2021, the intercrop 100A was significantly higher than the intercrops in alternating rows with orchargrass and meadow bromegrass (Table 2.11). Intercrops with 75% alfalfa did not differ (Table 2.11); those with 50% alfalfa in the mixture and those with 50% alfalfa in alternate rows did not differ either (Table 2.11). Among the intercrops with 50% alfalfa, there was no difference between the grass species in the mixture or alternating rows (Table 2.11). Mixed treatments do not differ from each other (Table 2.11).

Total alfalfa herbage accumulation

The intercropping system (P=0.001), irrigation regime(P=0.039), and the interaction between the intercropping system and year (P=0.0003) significantly affected the total alfalfa herbage accumulation (Table 2.4).

During 2020, intercropping 100A showed more significant alfalfa herbage accumulation than all except 75A+775F (Table 2.12). Intercrops with tall fescue showed higher alfalfa herbage accumulation in a mixture than in AR (Table 2.12). Intercrops in alternate rows did not differ from each other. Among the mixtures, those with orchardgrass had less alfalfa (Table 2.12).

During 2021, the 100A intercrop was higher than all other intercrops except tall fescue mixes (Table 2.12). The 50A+50O intercrop was smaller than all others except compared to those in alternate rows (Table 2.12). The intercrops with 50% alfalfa in the mixture and those with 50% alfalfa in alternate rows did not differ from each other (Table 2.12). Among the intercrops with 75% alfalfa, the one with tall fescue was higher than the one with orchardgrass, with no difference between the one with meadow bromegrass (Table 2.12). Among the intercrops with 50% alfalfa, there was a difference concerning the grass species when mixed and in alternating rows (Table 2.12).

Table 2.3 P-value of intercropping system, irrigation regime, cut, and their interactions for total herbage accumulation and alfalfa herbage accumulation of three cuts during 2020 and 2021.

	2020		2021	
	Herbage accumulation	Alfalfa herbage accumulation	Herbage accumulation	Alfalfa herbage accumulation
Intercropping	<0.001	<0.001	0.0002	<0.001
Irrigation	0.043	0.034	0.054	0.177
Cut	<0.001	<0.001	<0.001	<0.001
Intercropping*Irrigation	0.321	0.209	0.608	0.722
Intercropping*Cut	<0.001	<0.001	0.574	0.266
Irrigation*Cut	<0.001	<0.001	<0.001	<0.001
Intercropping*Irrigation*Cut	0.720	0.189	0.281	0.223

Table 2.4 P-value of intercropping system, irrigation regime, year, and their interactions for total herbage and total alfalfa herbage accumulation.

	Total	
	Herbage accumulation	Alfalfa herbage accumulation
Intercropping	0.0002	0.001
Irrigation	0.031	0.039
Year	<0.001	0.108
Intercropping*Irrigation	0.393	0.389
Intercropping*Year	<0.001	0.0003
Irrigation*Year	0.513	0.504
Intercropping*Irrigation*Year	0.865	0.916

Table 2.5 Mean herbage accumulation affected by the interaction of intercropping system and cut during 2020.

	First cut	Second cut	Third cut
Intercropping		kg/m^2	
100A	0.915a	0.489a	0.330a
75A+25O	1.017ab	0.465a	0.322a
75A+25F	1.134bc	0.462a	0.334a
75A+25M	1.143bc	0.497a	0.312a
50A+50O	1.193cd	0.447a	0.321a
50A+50F	1.195cd	0.462a	0.328a
50A+50M	1.198cd	0.450a	0.339a
50A+50O(AR)	1.277d	0.420a	0.292a
50A+50F(AR)	1.410e	0.430a	0.331a
50A+50M(AR)	1.436e	0.406a	0.303a

Different letters indicate significant differences within each cut.

Table 2.6 Mean alfalfa herbage accumulation affected by the interaction of intercropping system and cut during 2020.

	First cut	Second cut	Third cut
Intercropping		kg/m^2	
100A	0.915f	0.489d	0.330d
75A+25O	0.608de	0.348abc	0.247abcd
75A+25F	0.870f	0.415cd	0.295cd
75A+25M	0.648e	0.432cd	0.287bcd
50A+50O	0.304a	0.273ab	0.221abc
50A+50F	0.539cd	0.372bc	0.259abcd
50A+50M	0.447bc	0.352abc	0.271bcd
50A+50O(AR)	0.442bc	0.250a	0.166a
50A+50F(AR)	0.516bcd	0.286ab	0.202abc
50A+50M(AR)	0.424b	0.270ab	0.187ab

Different letters indicate significant differences within each cut.

Table 2.7 Mean herbage accumulation affected by the interaction of irrigation regime and cut during 2020.

	First cut	Second cut	Third cut
Irrigation		kg/m^2	
Full	1.175a	0.449a	0.429a
Deficit	1.209a	0.456a	0.213b

Different letters indicate significant differences within each cut.

Table 2.8 Mean alfalfa herbage accumulation affected by the interaction of irrigation regime and cut during 2020.

	First cut	Second cut	Third cut
Irrigation		kg/m^2	
Full	0.557a	0.345a	0.335a
Deficit	0.586a	0.353a	0.158b

Different letters indicate significant differences within each cut.

Table 2.9 Mean herbage accumulation affected by the interaction of irrigation regime and cut during 2021.

	First cut	Second cut	Third cut
Irrigation		kg/m^2	
Full	0.555a	0.531b	0.458a
Deficit	0.596a	0.389a	0.434a

Different letters indicate significant differences within each cut.

Table 2.10 Mean alfalfa herbage accumulation affected by the interaction of irrigation regime and cut during 2021.

	First cut	Second cut	Third cut
Irrigation		kg/m^2	
Full	0.417a	0.453a	0.374a
Deficit	0.484a	0.318b	0.354a

Different letters indicate significant differences within each cut.

Table 2.11 Mean herbage accumulation affected by the interaction of irrigation regime and year.

	2020	2021
Intercropping	kg/m^2	
100A	1.730a	1.620c
75A+25O	1.800ab	1.390abc
75A+25F	1.870ab	1.620c
75A+25M	1.950abc	1.580bc
50A+50O	1.960abc	1.450abc
50A+50F	1.990bc	1.620c
50A+50M	1.990bc	1.570bc
50A+50O(AR)	1.990bc	1.210a
50A+50F(AR)	2.170c	1.410abc
50A+50M(AR)	2.140c	1.360ab

Different letters indicate significant differences within each cut.

Table 2.12 Mean alfalfa herbage accumulation affected by the interaction of irrigation regime and year.

	2020	2021
Intercropping	kg/m^2	
100A	1.734f	1.624e
75A+25O	1.202cd	1.188bc
75A+25F	1.526ef	1.481de
75A+25M	1.367de	1.309cd
50A+50O	0.799a	1.153cd
50A+50F	1.170cd	1.382cde
50A+50M	1.070bc	1.293cd
50A+50O(AR)	0.858ab	0.856a
50A+50F(AR)	0.881ab	0.951ab
50A+50M(AR)	1.003abc	0.802a

Different letters indicate significant differences within each cut.

Discussion

The results indicate that, as the cuts progress, there are fewer significant differences between the intercropping systems and the alfalfa monoculture (Tables 2.3; 2.5 and 2.6). Grass tends to be less competitive with alfalfa over time (Liu et al. (2022), which may have contributed to reducing the differences between intercropping systems. Chamblee and Collins (1988) found most of the alfalfa cultivars adapted for the US, when intercropped, are likely to be more competitive and dominate the grass. How alfalfa leaves are arranged favors the penetration of light even in the lowest leaves (Leach, 1978), alfalfa can also shade the grass and reduce the incidence of light on them. In general, intercrops with 75% alfalfa had the least grass permanence; in contrast, those in alternate rows had the best grass permanency, which means that since alfalfa and grass are in alternate rows the competition for light is attenuated. (Tables 2.6 and 2.12). Regarding alfalfa herbage accumulation, during the first two cuts of 2020, among the mixtures with 75% alfalfa, orchardgrass is the only one that differed from alfalfa in monoculture, showing some resistance to alfalfa dominance (Tables 2.6 and 2.12). This could be because the orchardgrass can benefit from shading as a consequence of intercropping with alfalfa (Chamblee, 1958; Lopes & Santos, 2002; Mercier 2020).

In 2020, in the first cut, tall fescue and meadow bromegrass in alternate rows had the highest herbage accumulation (Table 2.5); they also differed in alfalfa herbage accumulation from monocropping alfalfa during the three cuts (Table 2.6). The tall fescue's high tillering capacity (Zarrough et al.,

1983) and the meadow bromegrass's number of tillers below harvest height, (Pearen & Baron, 1996) can be advantages concerning herbage accumulation.

There is a significant difference in total herbage accumulation between 2020 and 2021 ($P < 0.001$) (Table 2.4). During the establishment year (2019-2020), crops establish their root system and accumulate their biomass, so much of the plant's energy is focused on developing roots and leaves. After this period, the density of alfalfa and grass is high since the stand was established based on a dense seeding rate. The same was reported by Aponte et al. (2019) and Sleugh et al. (2000).

In conclusion, both irrigation regimen and intercropping system have significant effects on herbage accumulation. Concerning the irrigation regimes within cuts, in the third cut of 2020, the full irrigation regime had a more significant herbage accumulation than the deficit irrigation regime (Table 2.7). In the second year, the same happened during the second cut (Table 2.11). The periods with less rainfall were the third harvest of 2020 and the second harvest of 2021, which increased the water input difference between the full irrigation and deficit irrigation regimes (approximately 84 mm in 2020 and 119.4 mms in 2021). Water deficit can affect the plant in different ways; it depends on how long this stress lasts, the amount of water reduced, and the rate of reduction. For example, water stress can limit the amount of carbon dioxide absorbed due to stomatal closure and reduces photosynthetic activity; water stress can also cause a reduction in nutrient uptake (Bray, 1997). Another important point would be that, when experiencing water restrictions, the focus should be on the new fields since the first year had greater herbage accumulation than in 2021.

It seems possible that the herbage accumulation of alfalfa-grass intercropping systems is similar to pure alfalfa. In the first cut of 2020, the differences in herbage accumulation favored the intercropping systems over alfalfa in monoculture (Table 2.5). For the other cuts and 2021, there was no difference in the herbage accumulation of pure alfalfa and intercropping systems (Tables 2.3 and 2.5). Therefore, regarding the total herbage accumulation per year, in 2020, alfalfa-grass intercropping systems would be recommended over alfalfa monoculture due to variations in herbage accumulation (Table 2.11). Moreover, in 2021 the treatments in alternating rows with orchardgrass and meadow bromegrass were the only ones with a lower herbage accumulation than pure alfalfa (Table 2.11).

Tall fescue in alternate rows would be a good option for intercropping with alfalfa since, in the first year and the first cut (the most productive period), it presented an excellent herbage accumulation (Table 2.5); concerning the total herbage accumulation per year; in 2020, it also showed significant herbage accumulation and, in 2021, its herbage accumulation was similar to pure alfalfa (Table 2.11).

Chapter 3: Hay quality

Introduction

Alfalfa hay quality

In most places with a temperate-cold climate, hay is the staple food for livestock; the quality of the hay defines whether or not there is a need for supplementation. Generally, higher-quality hay is low in fiber and high in protein (Ullrey et al., 1997). Forage quality is related to the animal's performance, whether the animal's response to the diet will be as expected (Ball et al., 2001).

The environmental elements that affect hay quality are soil type, temperature, distribution and intensity of light throughout the day, fertilization, and available water. However, the plant species and the maturation stage at harvest are the main factors that interfere with the animal's composition, taste acceptance, and amount of nutrients absorbed (Ullrey et al., 1997).

Of all the factors that affect hay quality, the growth stage at harvest is the most important; during the reproductive stage, the plant is richer in fiber and poorer in protein, which affects the digestibility and palatability of the hay (Lacefield et al., 1999; Ullrey et al., 1997). Therefore, alfalfa (*Medicago sativa* L.), in the first cut, must be harvested at late bud to first flower and first flower to 1/10 bloom for the other cuts; the temperate climate grasses, in the first cut, must be harvested until the boot to early head stage, keeping 4-6 weeks between the subsequent cuts (Lacefield et al., 1999). When alfalfa is cut to 1/10 bloom, it contains 58% total digestible nutrients (TDN), 17.2% crude protein (CP), and 34% acid detergent fiber (ADF) contrasting with 52% TDN, 13.6% CP, and 42% ADF when cut at the mature stage (Lacefield, 1988).

Another factor that can interfere with hay quality is the presence of weeds, in general, because weeds are low-feed quality plants. According to Temme et al. (1979), reasonable weed control during the establishment of the alfalfa stand contributes to higher quality hay compared to the stand where no weed control was carried out.

Forage nutritional parameters

Neutral detergent fiber (NDF) is the total plant fiber amount of a feed: cellulose, hemicellulose, and lignin; it determines the feed digestibility. ADF measures the feed's cellulose and lignin content, including only the more indigestible plant fiber; it indicates the feed energy content. NDF and ADF are important because they determine how much the cattle can eat (Schroeder, 2004; Allen et al., 2011). Leaves have significantly less fiber than stems, so stems are lower forage quality than leaves. In addition, the number of leaves decreases with plant maturation.

Carbohydrates are the primary source of energy in the livestock diet. They formed two groups: non-neutral detergent fiber (non-NDF) and detergent fiber (NDF), with non-NDF being more digestible than NDF. Non-neutral detergent fiber or non-fiber carbohydrates (NFC) are inside cells except for soluble fiber and fructans (NASEM, 2016). Hemicellulose, cellulose, and lignin sets form NDF. However, lignin is not a carbohydrate, but a polymer developed from monolignols obtained from a pathway called phenylpropanoid (Moore & Jung, 2001). Organic acids are not carbohydrates either, but in digestion, they are more similar to carbohydrates than proteins or lipids. They are, therefore, also included in the NFC fraction (NASEM, 2016).

Due to their symbiosis with nitrogen-fixing bacteria, legumes have a higher CP content and a lower presence of NDF and cellulose than grasses. When massively fertilized with nitrogen, nonmature grasses may have CP levels close to legumes. However, legumes are more accepted and consumed in more significant quantities than grasses, in addition to being fermented more quickly in the digestive tract (Ullrey et al., 1997).

Proteins are complex for ruminants to digest; this is due to the fermentation that occurs in the pre-stomach. (NASEM, 2016). The protein used by the cattle can come from the diet or the "microbes washed from the rumen." The cattle's primary protein source is the rumen microbes, which convert the rumen degradable protein into amino acids and then into ammonia, and then they use that ammonia to grow. These microbes are digested when they get to the abomasum. The protein derived from the diet of cattle, and that which is directly available (undegradable dietary protein) is also digested in the abomasum. Amino acids are part of a series of vital processes, such as tissue growth, enzymatic activity, transport of molecules, and cell differentiation (Moran, 2005).

Total digestible nutrients (TDN) do not account precisely for all types of nutrients; it explains the feed energy better; the greater the TDN content is, the more energy concentration the forage has. There are two ways to calculate TDN; one way is using the nitrogen-free extract, crude fiber, crude protein, and ether extract, and the other way is using ADF (TDN=96.35(ADFx1.15)) (Allen et al., 2011).

Alfalfa-grass hay

Alfalfa hay is a significant irrigated crop in the western United States. However, alfalfa is a high-water use crop, which generates concern about the water supply and a search for better use of resources (Orloff & Putnam, 2010; Breazeale et al., 2000). Due to their root morphological differences in an alfalfa-grass intercropping system, the species have attenuated competition for water, which contributes to better water use efficiency (Mousavi & Eskandari, 2011). Additionally, bloating is a problem associated with pure alfalfa and is more likely to happened when the ruminants graze legume, but it can also happen with pure alfalfa hay, since the bloating risk and the alfalfa protein intake are positively correlated (Collins et al., 2017; Majak et al., 1995).

It is possible to produce alfalfa-grass hay with a similar quality to alfalfa hay. For example, the hay quality of alfalfa-grass can be higher than pure alfalfa hay after the first cut (Spandl & Hesterman, 1997). Perennial cool-season grasses such as orchardgrass (*Dactylis glomerata* L.), meadow bromegrass (*Bromus riparius* Rehm.), and tall fescue (*Festuca arundinacea* Schreb.) are widely cultivated for hay production in the western US. The intercropping of alfalfa with perennial cool season grasses can contribute to better cropland water and an improvement in hay quality since alfalfa can increase protein in mixed hay and, with the increase in fiber by the grass, bloating can be prevented (Cinar & Hatipoglu, 2015; Kopp et al., 2003).

The interrelations between alfalfa and intercropped grasses as a crop field are complicated; therefore, more research is needed on how alfalfa-grass intercropping systems and water stress can affect the forage quality over the cuts. However, we hypothesize that if the suitable grass species and mixing ratio are selected, alfalfa-grass intercropping systems can produce hay similar in forage quality to pure alfalfa hay. Therefore, this experiment compares the forage quality in pure alfalfa stands to mixed and alternate-row intercropping systems in response to full and deficit irrigation.

Material and methods

Experimental Design

A two-year field experiment was conducted in 2020 and 2021 at Aberdeen Research & Extension Center of the University of Idaho in Aberdeen, ID. The experimental field was planted with alfalfa (cultivar ‘FSG 415BR’), meadow bromegrass (cultivar ‘Cache’), orchardgrass (cultivar ‘Pawnee’), and Tall Fescue (cultivar ‘FSG 402TF’) on August 19, 2019. Ten alfalfa-grass intercropping systems (Table 2.1) and two irrigation regimes were arranged following a split-plot design with four replicates. The irrigation treatment was the main plot, and the alfalfa-grass intercropping system was the sub-plot.

Irrigation was applied following alfalfa crop Evapotranspiration (ET_c) described by Allen et al. (1998):

$$ET_c = ET_0 \times K_c$$

Daily reference ET (ET_0) was retrieved from the AgriMet Cooperative Agricultural Weather Network in the Columbia-Pacific Northwest Region. The crop coefficient (K_c) is determined experimentally; it accounts for the changes in leaf area, canopy, plant height, development, irrigation method, and soil and weather conditions (Pereira & Alves, 2005; Irmak, 2008). In this experiment, to simplify the ET_c, for each cut, a crop coefficient (K_c) of 0.5 was employed before full canopy coverage, and 1.0 was used at canopy closure in pure alfalfa under full irrigation.

There were three cuts during each growing season. For the full irrigation treatment, 100% ET_c was applied throughout the growing season. For the deficit irrigation treatment, 100% ET_c was applied during the first cut and 60% ET_c during the second and third cuts. In 2020, the total for full irrigation was 560 mm and 432 mm for deficit irrigation, while for the year 2021, the total for total irrigation was 747 mm and 533 mm for deficit irrigation. The ten alfalfa-grass intercropping systems and the seeding rates are described in Table 2.1. The water input during 2020 and 2021 is described in Table 2.2.

An area equivalent to 0.975 m^2 (1.067 m × 0.914 m) was harvested from each plot during each year's cuts; in 2020, the first cut was harvested on June 9th, the second on July 16th and the third on August 22nd; in 2021, the first cut was harvested on June 7th, the second on July 19th, and the third on August 31st. First, the entire harvested biomass was weighed as fresh weight, and a subsample was separated for alfalfa and grass composition and measuring dry matter concentration. Then, after drying in an oven at 60ºC for three days, grass and alfalfa samples were ground separately to 1 mm and then mixed following the proportion determined by the alfalfa and grass composition of each plot; finally, the forage nutritional parameters (crude protein, acid detergent fiber, neutral detergent fiber, total digestible nutrients, in vitro true dry matter digestibility, relative feed value, lignin, fat, non-fiber carbohydrates and water-soluble carbohydrates) of those mixed samples was determined using the NIRS method. The analyses were conducted by Ward Laboratories, Inc. in Kearney, NE.

Data Analysis

Analyzing the data separately for each year allows it to account for the variation between the years and observe how the factors affect herbage quality over time. All response variables were analyzed using linear mixed model procedures as implemented in RStudio (R version 4.2.2). Intercropping systems (sub-plot), cuts, irrigation regimes (main plot), and their interactions were considered fixed effects. Block was considered a random effect, and cut was considered repeated measure. The CLD function (compact letter display) (multcomp and multcompView packages) (Bonferroni method) was used to provide the contrasts within interactions. The COR.TEST (Spearman method) function was used to provide the correlations. Differences were considered significant at $P \leq 0.05$.

Results

Crude protein (CP)

During the year 2020, crude protein was significantly affected by the intercropping system ($P<0.001$), cut ($P<0.001$), and the interaction between the intercropping system and cut ($P<0.001$) (Table 3.1). In the first cut, alfalfa in monoculture presented higher crude protein than all others, except for 75A+25F; the intercrop 50A+50M had lower crude protein than the others, except for 50A+50O and intercrops in alternate rows; there was no difference between intercrops with 75% alfalfa, and between those in alternate rows; among intercrops with 50% alfalfa in the mixture, there were differences between those with tall fescue and meadow bromegrass (Table 3.4). In the second cut, there was no difference between the crude protein of alfalfa monoculture between intercrops with 75% alfalfa and 50% alfalfa with tall fescue and meadow bromegrass; the intercrop 50A+50O(AR) had a lower crude protein amount than all except 50A+50O; there was no difference between intercrops with 75%; within those with 50% alfalfa, the ones with orchardgrass had less crude protein (Table 3.4). In the third cut, 100A had the highest crude protein compared to 50A+50O and the intercrops in alternate rows; 50A+50O(AR) had the lowest crude protein compared to all other intercrops except 50A+50O and 50A+50F(AR); there was no difference between those with 75% alfalfa; within the intercrops with 50% alfalfa in the mixture, the only one that differed was the one with orchardgrass, while within the intercrops in alternate rows, the only difference was between the one with orchardgrass and meadow bromegrass; tall fescue is the only grass that differed in terms of planting design (Table 3.4).

During the year 2021, crude protein was significantly affected by the intercropping system ($P<0.001$), cut ($P=0.0001$), the interaction between intercropping system and cut ($P<0.001$) and the interaction between intercropping system, irrigation, and cut ($P=0.05$) (Table 3.1). In the first cut with full irrigation, 100A showed higher crude protein than the intercrops 50A+50M(AR), 50A+50O(AR), 75A+25M (Table 3.5); the intercrop 50A+50M(AR) had lower crude protein than the others, except when compared with 50A+50O(AR); there was no difference between intercrops in the mixture; within the intercrops in alternate rows, the only difference was between those with tall fescue and meadow bromegrass; meadow bromegrass was the only grass that differed in terms of planting design (Table 3.5). In the first cut with deficit irrigation, the only difference observed was compared to the 50A+50M(AR) intercrop, which was smaller than all the others, except in comparison with the 50A+50O(AR); meadow bromegrass was the only grass that differed in terms of planting design (Table 3.5). There was no difference between the intercrops in the second cut with full irrigation (Table 3.5). In the second cut with deficit irrigation, alfalfa in monoculture and intercrop 50A+50O are the only ones that differ (Table 3.5). In the third cut with full irrigation, intercrops with 50%

alfalfa mixed with tall fescue and orchardgrass had higher crude protein only compared to intercrops in alternating rows with meadow bromegrass and orchardgrass; the intercrop 50A+50O(AR) had lower crude protein than all others except 50A+50O, 50A+50F, and 75A+25F; there was no difference between mixtures, and there was no difference between alternate rows; orchardgrass was the only grass that differed in terms of planting design (Table 3.5). Finally, in the third cut with deficit irrigation, intercrops 50A+50F, 50A+50M, and 100A only had more crude protein than 50A+50O(AR) and 50A+50F(AR); there was no difference between the mixtures and also between the intercrops in alternate rows; tall fescue was the only grass that differed in terms of planting design (Table 3.5).

Acid detergent fiber (ADF)

During the year 2020, ADF was significantly affected by the intercropping system (P=0.008), irrigation (P=0.011), cut (P<0.001), the interaction between the intercropping system and cutting (P<0.001), and the interaction between irrigation and cut (P<0.001) (Table 3.1). In the first cut, 50A+50M was greater than all but 75A+25M; all intercrops did not differ from alfalfa in monoculture except 50A+50M; meadow bromegrass was the only grass that differed in terms of planting design (Table 3.6). There were no differences between the intercrops in the second cut (Table 3.6). In the third cut, 50A+50F(AR) and 50A+50O(AR) were only higher than 100A, 75A+25M, 50A+50F, and 50A+50O; there was no difference between the mixtures, and there was also no difference between those in alternate rows; tall fescue was the only grass that differed in terms of planting design (Table 3.6). Regarding the irrigation and cutting regimen, full irrigation was greater than deficit irrigation in the third cut (Table 3.8).

During the year 2021, ADF was significantly affected by the intercropping system (P=0.0001), cut (P<0.001), and the interaction between the intercropping system and cut (P=0.011) (Table 3.1). In the first cut, the 50A+50M(AR) was greater than 50A+50F(AR), 50A+50O, 75A+25O, and 100A; there was no difference between the mixtures; about the intercrops in alternate rows, the only difference was between those with tall fescue and meadow bromegrass (Table 3.7). In the second cut, 50A+50O was greater than alfalfa in monoculture, and 100A was less than 50A+50O (Table 3.7). In the third cut, 50A+50M(AR), 75A+25M, and 75A+25F were only greater than 100A, and pure alfalfa was only greater than 50A+50M(AR), 75A+25M, and 75A+25F (Table 3.7).

Neutral detergent fiber (NDF)

During the year 2020, NDF was significantly affected by the intercropping system (P<0.001), irrigation (P=0.027), cut (P<0.001), the interaction between the intercropping system and cutting (P<0.001), and the interaction between irrigation and cut (P<0.001) (Table 3.1). In the first cut, 50A+50M showed higher NDF than all others except 50A+50O, 50A+50O(AR), and 50A+50M(AR);

100A had lower NDF than all but 75A+25F; there was no difference between intercrops in alternating rows; within the intercrops with 75% alfalfa, the only difference was between those with tall fescue and meadow bromegrass, the same happened between the intercrops with 50% alfalfa (Table 3.9). In the second cut, 50A+50O was greater than all others except 75A+25O and 50A+50O(AR); 100A was less than 50A+50O(AR), 50A+50M(AR), 50A+50O and 75A+25O; there was no difference between intercrops with 75% alfalfa; within those with 50% alfalfa, there were differences concerning those with orchardgrass (Table 3.9). In the third cut, the 100A was only not smaller than the 75A+25F, 75A+25M, and 50A+50M; 50A+50O(AR) was higher than 50A+50M, 50A+50F, 75A+25M, 75A+25F, 75A+25O, and 100A; among the intercrops with 75% alfalfa, the only difference was about the one with orchardgrass, the same happened within those with 50% alfalfa in mixtures; there was no difference between intercrops in alternate rows; meadow bromegrass and tall fescue differed in terms of planting design (Table 3.9). Regarding the irrigation regime and cut, full irrigation was greater than deficit irrigation in the third cut (Table 3.11).

During 2021, NDF was significantly affected by the intercropping system ($P<0.001$), cut ($P<0.001$), and the interaction between the intercropping system and cut ($P<0.001$) (Table 3.1). In the first cut, 50A+50M(AR) was higher than all other cuts; meadow bromegrass is the only one that presents differences concerning planting design (Table 3.10). In the second cut, 50A+50O was only greater than 100A, 75A+25F, and 50A+50M; there were no differences between intercrops with 75% alfalfa, the same happened between those in alternate rows; among the intercrops with 50% alfalfa in mixtures, the only difference was between those with orchardgrass and meadow bromegrass (Table 3.10). Finally, in the third cut, 50A+50O(AR) was greater than 100A and 50A+50F; 100A was only smaller than the intercrops in alternate rows; there was no difference between intercrops in mixtures; there was no difference between intercrops in alternate rows; tall fescue is the only one that presents differences about planting design (Table 3.10).

Total digestible nutrientes (TDN)

In 2020, TDN was significantly affected by the intercropping system ($P<0.001$), cut ($P<0.001$), and the interaction between the intercropping system and cut ($P<0.001$) (Table 3.1). In the first cut, the 75A+25O was only greater than the 100A and the 50A+50M; 100A was less than 75A+25O, 75A+25F, 50A+50O, and 50A+50F(AR); there was no difference between intercrops with 75% alfalfa, the same within those in alternate rows; within the intercrops with 50% alfalfa in the mixture, there was a difference between those with orchardgrass and meadow bromegrass (Table 3.12). In the second cut, the 100A exceeded intercrops (Table 3.12). In the third cut, there was no difference between intercrops (Table 3.12).

During 2021, TDN was significantly affected by the intercropping system (P=0.001), irrigation (P=0.034), cut (P<0.001), the interaction between the intercropping system and cutting (P=0.002), and the interaction between irrigation and cut (P=0.023) (Table 3.1). In the first cut, 50A+50F(AR) was only greater than 50A+50M(AR); 50A+50M(AR) was only less than 75A+25O, 100A, and 50A+50F(AR); there was no difference between the mixtures; among the intercrops in alternate rows, the only difference was between those with tall fescue and meadow bromegrass (Table 3.13). In the second cut, 100A was only greater than 50A+50O; 50A+50O was only less than 50A+50M and 100A; there was no difference among intercrops with 75% alfalfa, the same within those in alternate rows; among those with 50% alfalfa in the mixture, the only difference was between those with orchardgrass and meadow bromegrass (Table 3.13). In the third cut, the 100A and 50A+50F(AR) were only greater than the 50A+50M(AR) and vice versa; there is no difference between the mixtures; among intercrops in alternate rows, there was a difference only between tall fescue and meadow bromegrass (Table 3.13). Regarding the irrigation regime and cut, the deficit irrigation was higher than the full irrigation in the second cut (Table 3.14).

Relative feed value (RFV)

In 2020, RFV was significantly affected by the intercropping system (P<0.001), cut (P<0.001), the interaction between the intercropping system and cut (P<0.001), and the interaction between irrigation regimen and cut (P<0.001) (Table 3.2). In the first cut, 100A was higher than all other intercrops except 75A+25F; 50A+50M was less than all others except 50A+50O and 50A+50M(AR); there was no difference between those with 75% alfalfa, the same within those in alternate rows; among the intercrops with 50% alfalfa in the mixture, there was a difference between those with tall fescue and meadow bromegrass (Table 3.15). In the second cut, 100A was only greater than 50A+50O and 50A+50O(AR); 50A+50O was only not less than 75A+25O, 50A+50O(AR), and 50A+50M(AR); there was no difference among intercrops with 75% alfalfa, the same among those in alternate rows; there was a difference between those with orchardgrass concerning those with tall fescue and meadow bromegrass for the intercrops in a mixture with 50% alfalfa (Table 3.15). In the third cut, 100A was not greater than 75A+25F, 75A+25M, 50A+50F, and 50A+50M; 50A+50O(AR) was only not less than 50A+50F(AR), 50A+50M(AR) and 50A+50O; there was no difference among the intercrops with 75% alfalfa, the same happened within those in alternate rows; there was a difference between those with orchardgrass and those with tall fescue and meadow bromegrass among those with 50% alfalfa mixture (Table 3.15). Regarding the irrigation regime and cut, the deficit irrigation was higher than the full irrigation in the third cut (Table 3.17).

During 2021, RFV was significantly affected by the intercropping system (P<0.001), cut (P<0.001), the interaction between the intercropping system and cut (P=0.0004), and the interaction between intercropping system, irrigation regime, and cut (P=0.043) (Table 3.2). In the first cut with full irrigation, 100A was greater than 50A+50O(AR) and 50A+50M(AR); 50A+50M(AR) was lower than 50A+50F(AR), 50A+50F, 50A+50O and 100A; there was no difference between the mixtures; among those in alternate rows, there was a difference only between those with tall fescue and meadow bromegrass (Table 3.16). In the first cut with deficit irrigation, 100A was only greater than 50A+50M(AR), whereas 50A+50M(AR) was only smaller than 100A and 75A+25O; there were no differences between the mixtures; there were no differences between those in alternate rows (Table 3.16). There was no difference between the intercrops in the second cut with full irrigation (Table 3.16). In the second cut with deficit irrigation, the 75A+25F and 100A were only higher than the 50A+50O, and the opposite is also true; there was no difference between intercrops with 75% alfalfa; there was no difference between intercrops with 50% alfalfa (Table 3.16). In the third cut with full irrigation, 100A was only greater than 50A+50O(AR) and 50A+50M(AR), while 50A+50M(AR) was only smaller than 100A, 50A+50O and 50A+50F; there was no difference between the mixtures; there was no difference between the ARs (Table 3.16). Finally, in the third cut with deficit irrigation, 100A was not only greater than 50A+50F and 50A+50M; there was no difference between mixtures and those in alternate rows (Table 3.16).

In vitro true dry matter digestibility (IVTDMD)

During 2020, IVTDMD was significantly affected by the intercropping system (P=0.0001), irrigation (P=0.024), cut (P<0.001), the interaction between the intercropping system and cutting (P<0.001), and the interaction between irrigation and cut (P<0.001) (Table 3.2). In the first cut, 100A and 50A+50O(AR) were only not greater than 50A+50O, 50A+50O(AR), and 75A+25O; the 50A+50M was only not lower than the 75A+25M; within the intercrops with 75% alfalfa, there was a difference concerning the ones with meadow bromegrass; among the intercrops with 50% of alfalfa in the mixture, there was a difference in comparison with the one with meadow bromegrass; among those in alternating rows there was a difference compared with orchardgrass (Table 3.18). There are no differences between the intercrops in the second and third cuts (Table 3.18). Regarding the irrigation regime and cut, the deficit irrigation was higher than the full irrigation in the third cut (Table 3.18). During the year 2021, IVTDMD was significantly affected by the intercropping system (P=0.049) and cut (P<0.001) (Table 3.2).

Lignin

In 2020, lignin was significantly affected by the intercropping system (P<0.001), irrigation (P=0.034), cut (P<0.001), the interaction between the intercropping system and cutting (P=0.0003), and the interaction between irrigation and cut (P<0.001) (Table 3.2). In the first cut, 100A was higher than all other intercrops except 75A+25F; 50A+50O was less than 100A, all intercrops with 75% alfalfa, 50A+50F, and 50A+50M; among those with 75% alfalfa, there was a difference between tall fescue and orchardgrass; among the mixtures with 50% alfalfa, the one with orchardgrass was the only one that had some difference; there was no difference between those in alternate rows (Table 3.20). In the second cut, the 100A was not greater than the 75A+25F, 75A+25M, and 50A+50F; 50A+50O(AR) was just not smaller than those in alternate rows and 50A+50O; there was no difference between the mixtures; there was no difference among those in alternate rows; intercrops with tall fescue and meadow bromegrass differ in terms of planting design (Table 3.20). In the third cut, 100A was greater than 50A+50O, 50A+50F, 50A+50O(AR), and 50A+50M(AR); 50A+50M(AR) was smaller than 100A, 75A+25F, and 75A+25M; there was no difference between the mixtures; there was no difference between those in alternate rows (Table 3.20). Regarding the irrigation regime and cut, full irrigation was greater than deficit irrigation in the third cut (Table 3.22).

During 2021, lignin was significantly affected by the intercropping system ($P=0.001$), cut ($P<0.001$), the interaction between the intercropping system and cutting ($P=0.002$), and the interaction between irrigation and cutting ($P=0.002$) (Table 3.2). There was no difference between the intercrops during the first and second cuts (Table 3.21). In the third cut, 75A+25F was higher than 50A+50O, 50A+50O(AR), and 50A+50F(AR); 50A+50F(AR) was lower than 50A+50M, 75A+25M, and 75A+25F; there was no difference among intercrops with 75% alfalfa, the same happened among those in alternate rows and among those with 50% alfalfa in the mixture (Table 3.21). Regarding the irrigation regime and cut, full irrigation was greater than deficit irrigation in the second cut (Table 3.23).

Fat

During 2020, fat was significantly affected by the intercropping system ($P<0.001$), cut ($P<0.001$), the interaction between the intercropping system and irrigation ($P=0.008$), the interaction between irrigation and cut ($P=0.0001$) and the interaction between intercropping system, irrigation and cut ($P=0.031$) (Table 3.2). In the first cut with full irrigation, 50A+50O(AR) was greater than 100A, all intercrops with 75% alfalfa, 50A+50M, and 50A+50F; 100A was less than 50A+50O and all ARs; there was no difference among those with 75% alfalfa, the same occurred among those in alternate rows; among those with 50% alfalfa in the mixture, only the one with orchardgrass is different (Table 3.24). In the first cut with deficit irrigation, 50A+50O(AR) was not greater than 50A+50M(AR), 50A+50O, and 75A+25O; 75A+25F was less than 75A+25O, 50A+50O, and all ARs; among the intercrops with 75% alfalfa, the only one that differed was the one with orchardgrass, the same occurred among the mixtures with 50% alfalfa; among those in alternate rows, the only difference was between tall fescue and orchardgrass; meadow bromegrass is the only grass species that differed in terms of planting design (Table 3.24). In the second cut with full irrigation, 50A+50O(AR) was higher than the mixtures with tall fescue and meadow bromegrass; 75A+25F was lower than intercrops with 50% alfalfa with orchardgrass; there was no difference among those with 75% alfalfa, the same happened among those with 50% alfalfa in the mixture and among the ARs (Table 3.24). In the second cut with deficit irrigation, the 50A+50O(AR) was only not greater than 50A+50M(AR) and 50A+50O; 100A was only less than 50A+50M(AR) and 50A+50O(AR); there was no difference among intercrops in the mixture; among the ARs, the only difference was between the one with orchardgrass and tall fescue; meadow bromegrass was the only species that differed among planting designs (Table 3.24). In the third cut with full irrigation, 50A+50O was only higher than 75A+25F; 75A+25F was only lower than 50A+50O(AR); there was no difference among the intercrops with 75% alfalfa, the same among the ARs and those with 50% alfalfa in the mixture (Table 3.24). In the third cut with deficit irrigation, the 50A+50O(AR) was not greater than the 50A+50M(AR); 100A was smaller than all intercrops with orchardgrass and 50A+50M(AR); there was no difference among those with 75% alfalfa, the same among those with 50% alfalfa in the mixture; among the ARs, the only difference was about the tall fescue; about the planting design, the orchardgrass and bromegrass meadow were different (Table 3.24).

In 2021, fat was significantly affected by the intercropping system ($P<0.001$), cut ($P<0.001$), the interaction between the intercropping system and cut ($P=0.001$), the interaction between irrigation and cutting ($P=0.012$) (Table 3.2). There was no difference between the intercrops during the first and second cuts (Table 3.25). In the third cut, 50A+50O(AR) was only not greater than 50A+50F(AR) and 50A+50O; 75A+25F was lower than all orchardgrass mixtures and all ARs; there was no difference between mixtures with 50% alfalfa; among those with 75% alfalfa there was a difference only between orchardgrass and tall fescue; among those in alternate rows, there was a difference only between orchardgrass and meadow bromegrass (Table 3.25). Regarding the irrigation regime and cut, deficit irrigation was greater than full irrigation in the second cut (Table 3.26).

Water-soluble carbohydrates (WSC)

During 2021, WSC was significantly affected by the intercropping system ($P=0.0002$), cut ($P<0.001$), and the interaction between the intercropping system and cut ($P=0.0004$) (Table 3.3). In the first cut, 50A+50O(AR) was only greater than 75A+25M and vice-versa (Table 3.27). In the second cut, 75A+25O was only greater than 75A+25O and vice-versa (Table 3.27). In the third cut, 50A+50O(AR) was greater than 50A+50F, 50A+50M, and 50A+50M(AR); 50A+50F was smaller than 50A+50F(AR) and 50A+50O(AR); there was no difference among the mixtures, among the ARs there was a difference only concerning the one with meadow bromegrass; tall fescue is the only species that differed in terms of planting design (Table 3.27).

Non-fiber carbohydrates (NFC)

In 2021, NFC was significantly affected by the intercropping system (P<0.001), cut (P<0.001), and the interaction between the intercropping system and cut (P=0.006) (Table 3.3). In the first cut, 50A+50M(AR) was lower than all intercrops except 75A+25M; 100A was only greater than 50A+50M(AR); there was no difference among the mixtures, among those in alternate rows, there was a difference about meadow bromegrass; the only grass that differed in terms of planting design was meadow bromegrass (Table 3.28). In the second cut, 100A was only greater than 50A+50O and vice-versa; there was no difference between mixtures and alternating rows (Table 3.28). In the third cut, 100A was greater than all ARs, 50A+50O, and 75A+25O; 50A+50M(AR) was only not lower than 75A+25F and 100A; there was no difference between the mixtures; no difference between the ARs (Table 3.28).

Correlations

In 2020, there were no non-significant correlations (Table 3.29). In 2021, the non-significant correlations were: crude protein (CP) and herbage accumulation (HA), crude protein (CP) and lignin, alfalfa herbage accumulation (AHA) and neutral detergent fiber (NDF), alfalfa herbage accumulation (AHA) and acid detergent fiber (ADF), alfalfa herbage accumulation (AHA) and in vitro true dry matter digestibility (IVTDMD), herbage accumulation (HA) and neutral detergent fiber (NDF), relative feed value (RFV) and in vitro true dry matter digestibility (IVTDMD) (Table 3.30).

Results

Table 3.1 P-value of intercropping system, irrigation regime, cut, and their interactions for crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), and total digestible nutrients (TDN) of three cuts during 2020 and 2021.

	2020				2021			
	CP	ADF	NDF	TDN	CP	ADF	NDF	TDN
Intercropping	<0.001	0.008	<0.001	<0.001	<0.001	0.0001	<0.001	0.001
Irrigation	0.409	0.011	0.027	0.449	0.590	0.085	0.495	0.034
Cut	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001
Intercropping*Irrigation	0.749	0.096	0.471	0.081	0.544	0.700	0.463	0.878
Intercropping*Cut	<0.001	<0.001	<0.001	<0.001	<0.001	0.011	<0.001	0.002
Irrigation*Cut	0.138	<0.001	<0.001	0.021	0.199	0.062	0.644	0.023
Intercropping*Irrigation*Cut	0.626	0.373	0.601	0.956	0.050	0.173	0.215	0.164

Table 3.2 P-value of intercropping system, irrigation regime, cut, and their interactions for relative feed value (RFV), in vitro true dry matter digestibility (IVTDMD), lignin, and fat of three cuts during 2020 and 2021.

	2020				2021			
	RFV	IVTDMD	Lignin	Fat	RFV	IVTDMD	Lignin	Fat
Intercropping	<0.001	0.0001	<0.001	<0.001	<0.001	0.049	0.001	<0.001
Irrigation	0.048	0.024	0.034	0.056	0.342	0.172	0.093	0.092
Cut	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Intercropping*Irrigation	0.346	0.132	0.268	0.008	0.598	0.558	0.728	0.471
Intercropping*Cut	<0.001	<0.001	0.0003	0.082	0.0004	0.051	0.002	0.001
Irrigation*Cut	<0.001	<0.001	<0.001	0.0001	0.890	0.311	0.002	0.012
Intercropping*Irrigation*Cut	0.635	0.744	0.500	0.031	0.043	0.498	0.395	0.147

Table 3.3 P-value of intercropping system, irrigation regime, cut, and their interactions for water-soluble carbohydrates (WSC) and non-fiber carbohydrates (NFC) of three cuts during 2021.

	2021	
	WSC	NFC
Intercropping	0.0002	<0.001
Irrigation	0.081	0.217
Cut	<0.001	<0.001
Intercropping*Irrigation	0.154	0.229
Intercropping*Cut	0.0004	0.006
Irrigation*Cut	0.079	0.462
Intercropping*Irrigation*Cut	0.695	0.088

Table 3.4 Mean crude protein (CP) affected by the interaction of intercropping system and cut during 2020.

	First cut	Second cut	Third cut
Intercropping		%	
100A	17.9e	22.2c	23.9d
75A+25O	15.5cd	20.4c	22.1bcd
75A+25F	16.7de	21.7c	23.1cd
75A+25M	15.0bcd	21.8c	24.1d
50A+50O	14.0abc	18.3ab	20.6ab
50A+50F	15.6cd	21.7c	23.2cd
50A+50M	12.9a	21.7c	23.4cd
50A+50O(AR)	13.2ab	17.7a	19.4a
50A+50F(AR)	14.2abc	20.2bc	20.6ab
50A+50M(AR)	13.9abc	20.6c	21.6bc

Different letters indicate significant differences within each cut.

Table 3.5 Mean crude protein (CP) affected by the interaction of intercropping system, irrigation and cut during 2021.

	First cut		Second cut		Third cut	
	Full irrigation	Deficit irrigation	Full irrigation	Deficit irrigation	Full irrigation	Deficit irrigation
Intercropping			%			
100A	25.5d	23.4b	22.4a	22.9b	22.4abc	25.1b
75A+25O	21.8bcd	24.4b	21.3a	19.7ab	22.5abc	23.5ab
75A+25F	23.6cd	22.7b	19.9a	22.9b	23.4bc	22.2ab
75A+25M	20.7bc	21.8b	21.8a	21.4ab	22.1abc	23.6ab
50A+50O	22.2bcd	21.6b	19.2a	18.0a	24.4c	21.2ab
50A+50F	22.3bcd	21.8b	21.8a	21.2ab	24.1c	24.7b
50A+50M	21.1bcd	21.7b	23.1a	21.4ab	22.6abc	24.3b
50A+50O(AR)	18.4ab	20.7ab	19.8a	19.9ab	18.5a	19.3a
50A+50F(AR)	21.3bcd	22.4b	21.2a	19.8ab	20.3abc	19.8a
50A+50M(AR)	15.3a	17.1a	21.6a	21.1ab	18.9ab	21.0ab

Different letters indicate significant differences within each cut.

Table 3.6 Mean acid detergent fiber (ADF) affected by the interaction of intercropping system and cut during 2020.

	First cut	Second cut	Third cut
Intercropping		%	
100A	38.4a	33.5a	25.3a
75A+25O	38.7a	33.5a	27.2ab
75A+25F	39.0a	33.0a	26.3ab
75A+25M	40.5ab	33.5a	25.5a
50A+50O	38.9a	35.5a	28.2ab
50A+50F	39.4a	32.7a	25.3a
50A+50M	42.8b	33.2a	25.9a
50A+50O(AR)	38.5a	34.4a	29.3b
50A+50F(AR)	39.3a	32.4a	29.2b
50A+50M(AR)	39.7a	32.6a	27.7ab

Different letters indicate significant differences within each cut.

Table 3.7 Mean acid detergent fiber (ADF) affected by the interaction of intercropping system and cut during 2021.

	First cut	Second cut	Third cut
Intercropping		%	
100A	30.5a	32.4a	26.6a
75A+25O	31.9a	34.5ab	30.0ab
75A+25F	32.8ab	34.4ab	31.8b
75A+25M	33.9ab	34.7ab	32.0b
50A+50O	32.2a	37.4b	29.8ab
50A+50F	32.8ab	34.5ab	28.9ab
50A+50M	33.4ab	33.7ab	31.1ab
50A+50O(AR)	32.8ab	34.6ab	30.9ab
50A+50F(AR)	30.5a	34.0ab	29.2ab
50A+50M(AR)	36.9b	34.0ab	32.9b

Different letters indicate significant differences within each cut.

Table 3.8 Mean acid detergent fiber (ADF) affected by the interaction of irrigation regime and cut during 2020.

	First cut	Second cut	Third cut
Irrigation		%	
Full	39.8a	33.2a	29.4b
Deficit	39.3a	33.6a	24.6a

Different letters indicate significant differences within each cut.

Table 3.9 Mean neutral detergent fiber (NDF) affected by the interaction of intercropping system and cut during 2020.

	First cut	Second cut	Third cut
Intercropping		%	
100A	45.3a	38.9a	29.7a
75A+25O	52.5bc	44.0bcd	36.6cde
75A+25F	49.8ab	40.7ab	32.9abc
75A+25M	54.7cd	41.6ab	31.6ab
50A+50O	57.4de	48.6d	39.9ef
50A+50F	53.2bcd	41.1ab	34.4bcd
50A+50M	60.1e	41.9ab	33.5abc
50A+50O(AR)	56.1cde	48.2cd	43.0f
50A+50F(AR)	55.3cd	43.1ab	39.9ef
50A+50M(AR)	57.5de	43.8bc	38.6def

Different letters indicate significant differences within each cut.

Table 3.10 Mean neutral detergent fiber (NDF) affected by the interaction of intercropping system and cut during 2021.

	First cut	Second cut	Third cut
Intercropping		%	
100A	34.2a	37.3a	32.5a
75A+25O	36.6a	43.8ab	37.8abc
75A+25F	39.1a	40.5a	36.8abc
75A+25M	42.1a	43.8ab	38.6abc
50A+50O	38.9a	49.6b	39.1abc
50A+50F	38.9a	42.6ab	34.8ab
50A+50M	40.5a	41.2a	37.5abc
50A+50O(AR)	42.3a	45.2ab	44.7c
50A+50F(AR)	37.5a	42.9ab	41.1bc
50A+50M(AR)	52.8b	41.7ab	44.8c

Different letters indicate significant differences within each cut.

Table 3.11 Mean neutral detergent fiber (NDF) affected by the interaction of irrigation regime and during 2020.

	First cut	Second cut	Third cut
Irrigation		%	
Full	54.6a	43.0a	38.2b
Deficit	53.8a	43.5a	33.8a

Different letters indicate significant differences within each cut.

Table 3.12 Mean total digestible nutrients (TDN) affected by the interaction of intercropping system and cut during 2020.

	First cut	Second cut	Third cut
Intercropping		%	
100A	53.5a	57.2a	64.7a
75A+25O	58.4c	64.4b	63.1a
75A+25F	58.0c	64.8b	62.5a
75A+25M	56.3abc	64.3b	64.5a
50A+50O	58.2c	62.1b	62.2a
50A+50F	57.6abc	65.3b	62.8a
50A+50M	53.7ab	64.7b	64.2a
50A+50O(AR)	57.2abc	63.3b	61.3a
50A+50F(AR)	57.8bc	65.5b	62.4a
50A+50M(AR)	57.2abc	65.5b	62.6a

Different letters indicate significant differences within each cut.

Table 3.13 Mean total digestible nutrients (TDN) affected by the interaction of intercropping system and cut during 2021.

	First cut	Second cut	Third cut
Intercropping		%	
100A	59.4b	58.0b	61.6b
75A+25O	58.4b	56.5ab	59.9ab
75A+25F	57.8ab	56.5ab	58.5ab
75A+25M	56.9ab	56.3ab	58.4ab
50A+50O	58.2ab	54.3a	60.9ab
50A+50F	57.8ab	56.4ab	60.6ab
50A+50M	57.3ab	57.8b	59.0ab
50A+50O(AR)	57.7ab	56.3ab	60.2ab
50A+50F(AR)	59.5b	56.8ab	61.6b
50A+50M(AR)	54.7a	57.5ab	57.6a

Different letters indicate significant differences within each cut.

Table 3.14 Mean total digestible nutrients (TDN) affected by the interaction of irrigation regime and cut during 2021.

	First cut	Second cut	Third cut
Irrigation		%	
Full	57.8a	55.8a	59.1a
Deficit	57.8a	57.5b	60.5a

Different letters indicate significant differences within each cut.

Table 3.15 Mean total relative feed value (RFV) affected by the interaction of intercropping system and cut during 2020.

	First cut	Second cut	Third cut
Intercropping		%	
100A	121.6d	150.3c	218.7e
75A+25O	104.0bc	133.1abc	173.2bcd
75A+25F	109.4cd	144.3c	195.0de
75A+25M	97.4bc	140.5c	203.6de
50A+50O	95.1ab	117.4a	157.0ab
50A+50F	101.8bc	143.6c	187.0cde
50A+50M	86.1a	140.2c	191.6cde
50A+50O(AR)	97.8bc	119.6ab	143.0a
50A+50F(AR)	98.2bc	137.2bc	162.3abc
50A+50M(AR)	93.7ab	135.0abc	162.1abc

Different letters indicate significant differences within each cut.

Table 3.16 Mean total relative feed value (RFV) affected by the interaction of intercropping system, irrigation regime and cut during 2021.

	First cut		Second cut		Third cut	
	Full irrigation	Deficit irrigation	Full irrigation	Deficit irrigation	Full irrigation	Deficit irrigation
Intercropping			%			
100A	188.2c	169.2b	150.2a	168.2b	201.0c	219.0b
75A+25O	139.0abc	182.5b	135.0a	132.2ab	163.0abc	162.8a
75A+25F	150.2abc	156.5ab	124.8a	132.2b	168.8abc	157.2a
75A+25M	140.0abc	142.5ab	134.8a	132.2ab	157.0abc	153.0a
50A+50O	159.5bc	149.5ab	114.2a	112.8a	176.0bc	143.8a
50A+50F	157.8bc	147.5ab	134.8a	136.8ab	176.2bc	181.8ab
50A+50M	146.2abc	149.2ab	151.0a	135.8ab	149.2abc	177.0ab
50A+50O(AR)	135.0ab	146.5ab	126.5a	132.8ab	138.0ab	137.0a
50A+50F(AR)	161.0bc	165.2ab	139.0a	137.5ab	153.5abc	148.5a
50A+50M(AR)	99.5a	116.8a	138.0a	135.5ab	122.5a	147.8a

Different letters indicate significant differences within each cut.

Table 3.17 Mean total relative feed value (RFV) affected by the irrigation regime and cut during 2020.

	First cut	Second cut	Third cut
Irrigation		%	
Full	98.9a	136.9a	161.5a
Deficit	101.3a	134.5a	196.1b

Different letters indicate significant differences within each cut.

Table 3.18 Mean total in vitro true dry matter digestibility (IVTDMD) affected by the interaction of intercropping system and cut during 2020.

	First cut	Second cut	Third cut
Intercropping		%	
100A	76.8e	79.8a	84.7a
75A+25O	75.5cde	80.7a	85.3a
75A+25F	73.9bcd	79.8a	84.5a
75A+25M	72.2ab	79.8a	85.4a
50A+50O	75.8de	79.3a	85.4a
50A+50F	73.8bcd	80.7a	86.3a
50A+50M	69.6a	80.6a	85.9a
50A+50O(AR)	76.8e	79.4a	85.3a
50A+50F(AR)	73.5bcd	80.1a	84.7a
50A+50M(AR)	73.0bc	80.5a	85.9a

Different letters indicate significant differences within each cut.

Table 3.19 Mean total in vitro true dry matter digestibility (IVTDMD) affected by the interaction of irrigation regime and cut during 2020.

	First cut	Second cut	Third cut
Irrigation		%	
Full	73.9a	80.6a	83.8a
Deficit	74.3a	79.5a	86.9b

Different letters indicate significant differences within each cut.

Table 3.20 Mean lignin affected by the interaction of intercropping system and cut during 2020.

	First cut	Second cut	Third cut
Intercropping		%	
100A	8.03e	6.95d	5.48c
75A+25O	6.50bc	5.77bc	4.82abc
75A+25F	7.46de	6.33cd	5.25bc
75A+25M	6.84cd	6.17cd	5.11bc
50A+50O	5.45a	5.58abc	4.45ab
50A+50F	6.52bc	6.18cd	4.65ab
50A+50M	6.48bc	6.04c	4.72abc
50A+50O(AR)	5.30a	4.88a	4.23a
50A+50F(AR)	5.96ab	5.04ab	4.65abc
50A+50M(AR)	5.80ab	5.02ab	4.21a

Different letters indicate significant differences within each cut.

Table 3.21 Mean lignin affected by the interaction of intercropping system and cut during 2021.

	First cut	Second cut	Third cut
Intercropping		%	
100A	6.35a	7.04a	5.74bcd
75A+25O	5.85a	6.25a	5.17abcd
75A+25F	6.28a	6.95a	6.38d
75A+25M	6.04a	6.11a	5.87cd
50A+50O	6.07a	6.15a	4.72abc
50A+50F	6.32a	6.23a	5.26abcd
50A+50M	6.30a	6.10a	5.72bcd
50A+50O(AR)	6.25a	6.21a	4.37ab
50A+50F(AR)	6.08a	6.48a	4.09a
50A+50M(AR)	5.50a	5.93a	5.01abcd

Different letters indicate significant differences within each cut.

Table 3.22 Mean lignin affected by the interaction of irrigation regime and cut during 2020.

	First cut	Second cut	Third cut
Irrigation		%	
Full	6.48a	5.76a	5.19b
Deficit	6.39a	5.84a	4.33a

Different letters indicate significant differences within each cut.

Table 3.23 Mean lignin affected by the interaction of irrigation regime and cut during 2021.

	First cut	Second cut	Third cut
Irrigation		%	
Full	6.00a	6.64b	5.47a
Deficit	6.21a	6.06a	5.00a

Different letters indicate significant differences within each cut.

Table 3.24 Mean fat affected by the interaction of intercropping system, irrigation regime and cut during 2020.

	First cut		Second cut		Third cut	
	Full irrigation	Deficit irrigation	Full irrigation	Deficit irrigation	Full irrigation	Deficit irrigation
Intercropping			%			
100A	1.87a	2.03ab	1.24abc	2.13a	2.59ab	2.45a
75A+25O	2.08abc	2.46bcd	2.48abc	2.43ab	2.77ab	2.91bc
75A+25F	1.89ab	1.89a	2.13a	2.24a	2.42a	2.64ab
75A+25M	2.08abc	1.97a	2.30ab	2.19a	2.58ab	2.67ab
50A+50O	2.56d	2.56cd	2.56bc	2.54abc	2.93b	3.02bc
50A+50F	2.08abc	2.05ab	2.24ab	2.24a	2.56ab	2.86abc
50A+50M	2.03abc	1.97a	2.27ab	2.27a	2.77ab	2.83abc
50A+50O(AR)	2.67d	2.70d	2.70c	2.91c	2.85b	3.49d
50A+50F(AR)	2.30bcd	2.19abc	2.44abc	2.41ab	2.56ab	2.75ab
50A+50M(AR)	2.37cd	2.40bcd	2.62bc	2.67bc	2.65ab	3.20cd

Different letters indicate significant differences within each cut.

Table 3.25 Mean fat affected by the interaction of intercropping system and cut during 2021.

	First cut	Second cut	Third cut
Intercropping		%	
100A	2.22a	2.04a	2.13ab
75A+25O	2.44a	2.23a	2.35b
75A+25F	2.24a	2.01a	1.93a
75A+25M	2.35a	2.22a	2.10ab
50A+50O	2.39a	2.30a	2.46bc
50A+50F	2.22a	2.08a	2.29ab
50A+50M	2.21a	2.04a	2.12ab
50A+50O(AR)	2.41a	2.29a	2.82c
50A+50F(AR)	2.31a	2.06a	2.45bc
50A+50M(AR)	2.38a	2.29a	2.38b

Different letters indicate significant differences within each cut.

Table 3.26 Mean fat affected by the interaction of irrigation regime and cut during 2021.

	First cut	Second cut	Third cut
Irrigation		%	
Full	2.32a	2.06a	2.25a
Deficit	2.31a	2.25b	2.35a

Different letters indicate significant differences within each cut.

Table 3.27 Mean water-soluble carbohydrates (WSC) affected by the interaction of intercropping system and cut during 2021.

	First cut	Second cut	Third cut
Intercropping		%	
100A	8.75ab	8.47ab	9.40abc
75A+25O	8.79ab	8.62b	9.21abc
75A+25F	8.43ab	8.41ab	9.25abc
75A+25M	8.40a	7.90ab	9.19abc
50A+50O	8.78ab	7.97ab	9.25abc
50A+50F	8.68ab	8.29ab	9.04a
50A+50M	8.68ab	8.34ab	9.10ab
50A+50O(AR)	9.21b	7.99ab	9.94c
50A+50F(AR)	8.99ab	7.78a	9.84bc
50A+50M(AR)	8.43ab	8.10ab	8.88a

Different letters indicate significant differences within each cut.

Table 3.28 Mean non-fiber carbohydrates (NFC) affected by the interaction of intercropping system and cut during 2021.

	First cut	Second cut	Third cut
Intercropping		%	
100A	32.7b	32.0b	34.5c
75A+25O	30.2b	27.7ab	29.9ab
75A+25F	29.8b	30.0ab	31.8bc
75A+25M	28.1ab	26.5ab	29.9abc
50A+50O	30.2b	23.8a	28.7ab
50A+50F	30.1b	27.0ab	31.1abc
50A+50M	29.7b	28.1ab	30.2abc
50A+50O(AR)	29.2b	26.8ab	26.6ab
50A+50F(AR)	30.8b	27.3ab	27.0ab
50A+50M(AR)	21.8a	26.8ab	25.8a

Different letters indicate significant differences within each cut.

Table 3.29 Spearman correlation matrix for 2020.

	CP	AHA	HA	NDF	ADF	TDN	RFV	IVTDMD	Lignin
AHA	-0.4*	--							
HA	-0.7*	0.8*	--						
NDF	-0.9*	0.5*	0.8*	--					
ADF	-0.9*	0.7*	0.9*	0.9*	--				
TDN	0.7*	-0.6*	-0.6*	-0.7*	-0.8*	--			
RFV	0.9*	-0.5*	-0.8*	-0.9*	-0.9*	0.7*	--		
IVTDMD	0.8*	-0.7*	-0.9*	-0.9*	-0.9*	0.7*	0.9*	--	
Lignin	-0.4*	0.8*	0.6*	0.4*	0.7*	-0.6*	-0.5*	-0.7*	--
Fat	0.3*	-0.8*	-0.6*	-0.4*	-0.7*	0.5*	0.5*	0.7*	-0.9*

Crude protein (CP); alfalfa herbage accumulation (AHA); herbage accumulation (HA); neutral detergent fiber (NDF); acid detergent fiber (ADF); total digestible nutrientes (TDN); relative feed value (RFV); in vitro true dry matter digestibility (IVTDMD); lignin; fat; $*P \leq 0.05$

Table 3.30 Spearman correlation matrix for 2021.

	CP	AHA	HA	NDF	ADF	TDN	RFV	IVTDMD	Lignin	Fat	WSC
AHA	0.4*	--									
HA	0.1	0.8*	--								
NDF	-0.9*	-0.4	-0.1	--							
ADF	-0.7*	0.01	0.2*	0.8*	--						
TDN	0.7*	-0.03	-0.2*	-0.8*	-0.9*	--					
RFV	0.9*	0.3*	0.1	-0.9*	-0.9*	0.8*	--				
IVTDMD	0.5*	-0.1	-0.2*	-0.5*	-0.8*	0.8*	0.5*	--			
Lignin	0.1	0.5*	0.4*	-0.1*	0.4*	-0.4*	0.03	-0.7*	--		
Fat	-0.2*	-0.4*	-0.2*	0.2*	-0.2*	0.2*	-0.1*	0.5*	-0.7*	--	
WSC	0.3*	-0.1*	-0.2*	-0.5*	-0.7*	0.7*	0.65*	0.7*	-0.4*	0.2*	--
NFC	0.8*	0.4*	0.1*	-0.9*	-0.6*	0.6*	0.9*	0.2*	0.4*	-0.3*	0.5*

Crude protein (CP); alfalfa herbage accumulation (AHA); herbage accumulation (HA); neutral detergent fiber (NDF); acid detergent fiber (ADF); total digestible nutrientes (TDN); relative feed value (RFV); in vitro true dry matter digestibility (IVTDMD); lignin; fat; water-soluble carbohydrates (WSC); non-fiber carbohydrates (NFC); $^{*}P \leq 0.05$

Discussion

In 2020 and 2021, the amount of crude protein was significantly affected by the cut (P<0.001 and P=0.0001, respectively) (Table 3.2), with the third cut being the one with the greatest crude protein amount during both years; the herbage accumulation also seems to decrease during the third cut in both years. Since the yield improves as the shoots grow, crude protein and other nutrients usually decline (Collins et al., 2017). There was no difference in the crude protein amount between the grass species when with 75% alfalfa (Table 3.4 and 3.5); the differences between the grass species were neither frequent nor uniform but happened among the 50% mixtures and those in alternating rows (Table 3.4 and 3.5); thus, the design of planting and percentage of alfalfa in the intercrop affect the crude protein amount more than the grass species. In general, mixtures with a higher percentage of alfalfa had a higher crude protein, especially concerning intercrops in alternate rows (Table 3.4 and 3.5); this is because the planting design favors the permanency of the grass. Grass has less protein content than legumes because legumes can fix nitrogen from the atmosphere, and nitrogen plays a crucial role in protein synthesis, in addition to a deeper root system and a better leaf-to-stem ratio (Sengul, 2003; Dhakal et al., 2020a; Dhakal et al., 2020b).

The ADF amount was uniform during the cuts in the two years without significant differences compared to the alfalfa in monoculture (Tables 3.6 and 3.7). This may have resulted over time since the alfalfa herbage accumulation differed less and less from the alfalfa in monoculture, so the alfalfa percent in the intercrops could be high enough to be similar to pure alfalfa.

Simarily, Spandl and Hesterman (1997) found differences in forage quality, including ADF amount, between alfalfa-grass (65% of alfalfa and 35% of grass) and alfalfa in monoculture in the first harvest; this may have been because the grass accounted for 13 % or less of the total biomass in the second harvest and 8% or less in the third, so the similarity in forage quality in the second and third harvests can be explained by the lower presence of grass and higher presence of alfalfa. Another point would be that, when at the same maturity stage, cool-season grasses and cool-season legumes usually have similar ADF amounts (Collins et al., 2017).

In the first year, intercrops with 75% alfalfa had NDF content more similar to pure alfalfa, especially in comparison with intercrops in alternate rows (Table 3.9). In the second year, during the first and second cuts, the NDF values were more uniform; however, in the third cut, it was evident that the NDF was higher for the intercrops in alternate rows (Table 3.10). Grasses tend to have more fiber than legumes because grasses have more cell walls and legumes have more cell contents; cell walls are fibrous portions (cellulose, hemicellulose, lignin), and cell contents are nonfibrous constituents (lipids, sugars, proteins, starch) (Buxton and Redfearn (1997), this may explain why intercrops in alternate rows had higher NDF. The irrigation regime was only significant for ADF and NDF in the third cut of the first year (Tables 3.8 and 3.11), which was the time with less rainfall and more difference (84 mm) between full irrigation and deficit irrigation; during water stress, the stem may grow less, which would improve the leaf-stem ratio. resulting in lower fiber content (Putnam & Orloff, 2016).

The irrigation regime was only significant for ADF and NDF in the third cut of the first year (Tables 3.8 and 3.11), which was the time with less rainfall and more difference (84 mm) between full irrigation and deficit irrigation. During water stress, the stem may grow less, which would improve the leaf-stem ratio, resulting in lower fiber content (Putnam & Orloff, 2016).

The differences in lignin content between pure alfalfa and intercrops appeared to decrease with time because the differences between intercrops and alfalfa in monoculture in terms of alfalfa herbage accumulation also decreased with time (Tables 3.20 and 3.21). Legumes have more lignin than grasses; the difference is where it is located, in grasses it can be found in many cell types, and in the legumes, it is restricted to xylem and tracheary cells; this more spread lignin dispersion can make it easier for digestive enzymes to access the cell walls components which results in better digestibility (Wilson & Mertens, 1995; Moore & Jung, 2001; Buxton & Redfearn, 1997). Regarding the irrigation and cutting regime, in 2020, full irrigation was higher in the third cut, and in 2021, the same happened for the second cut (Tables 3.22 and 3.23); these were the cuts with less rainfall in each year and consequently with more difference between the irrigation regime and full and deficit irrigation, for these cuts, herbage accumulation was higher in full irrigation than in deficit irrigation. Therefore, content lignin was higher in full irrigation; this may explain the positive correlation between lignin and herbage accumulation in both years (Tables 3.29 and 3.30)

The TDN content and the ADF amount are reasonably uniform during the two-year cuts without significant differences concerning alfalfa in monoculture (Tables 3.12 and 3.13). However, the more indigestible plant fiber the forage has, the less dense energy the forage is.

Correlation shows the power and direction of the relationship between variables, but nothing clarifies the causality between them or the reason for this relationship. For example, during the year 2020, the herbage accumulation and the alfalfa herbage accumulation decreased during the cuts; the same does not occur in the year 2021(Tables 3.29 and 3.30); the herbage accumulation and the alfalfa herbage accumulation do not differ between the second and third cuts; this makes it difficult to establish a correlation with the other variables (Table 3.30). However, in both years, it is possible to observe a negative correlation between the crude protein content and NDF and crude protein and ADF, this is in accordance with Coleman et al., (2003) and Du et al., (2016), for TDN, the correlation was positive (Tables 3.29 and 3.30), the total digestible nutrients account for how dense-energy the forage is (Allen et al., 2011) and crude protein is also a source of energy to the cattle.

In conclusion, irrigation regime and intercropping system significantly affected hay quality; also, the quality of alfalfa-grass can be comparable to pure alfalfa. Intercrops with 75% alfalfa were more consistently similar to alfalfa in monoculture; the intercrop with 75% of alfalfa and 25% of tall fescue was the only intercrop similar to pure alfalfa about crude protein and neutral detergent fiber in the first cut of 2020. Therefore, 75A+25F would be an alternative to alfalfa in monoculture in terms of quality. Concerning the irrigation regime and the cuts, the differences were between the two periods of less rain: the third cut of 2020 and the second cut of 2021, where deficit irrigation presented better values than full irrigation to forage quality. However, the visual quality of the hay can be lower than the one under full irrigation, and the leaves in water-stressed regime hay may detach more easily (Ottman & Putnam, 2017).

Chapter 4: Conclusions

The experiment was designed to understand how the herbage accumulation and quality of different alfalfa-grass intercropping systems under two irrigation regimes can be compared to alfalfa in monoculture. Results indicated that the irrigation regime and intercropping system significantly affected herbage accumulation and hay quality. Furthermore, alfalfa-grass intercropping systems' herbage accumulation and hay quality can be similar to pure alfalfa. However, herbage accumulation and quality are inversely related; obtaining high herbage accumulation or hay quality seems possible, but usually not both. It happens because as the alfalfa plant matures, the herbage accumulation increases but the hay quality decreases. Water deficit can affect the plant in different ways; it depends on how long this stress lasts, the amount of water reduced, and the rate of reduction. When facing water restrictions, the focus should be on the new fields since the first year had greater herbage accumulation than the second. Another important point would be that even that deficit irrigation presented better values for hay quality than full irrigation, the visual quality of the hay can be lower than the one under full irrigation, and the leaves in water-stressed regime hay may detach more easily. Further economic studies are necessary to investigate whether the gain in herbage accumulation offsets the drop in hay quality, whether the gain in hay quality offsets the drop in herbage accumulation, and finally, if the changes in hay quality and the loss of herbage accumulation under the deficit irrigation regime compensate for the water save.

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