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Exploring Clover-Based Nurse Cropping for Birdsfoot Trefoil Establishment and Yield

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ABSTRACT

Birdsfoot trefoil (*Lotus corniculatus* L.), renowned for its persistence in challenging soil and climate conditions, serves as a valuable non-bloating forage legume in temperate regions. However, its slow establishment and low initial yields in the establishment year make it vulnerable to competition from companion and weeds species. Therefore, we investigated the effects of three legume species (red clover, *Trifolium pretence* L.; balansa clover, *Trifolium michelianum* L.; and berseem clover, *Trifolium alexandrinum* L.) as nurse crops at three seeding rates (target 60, 120 or 240 plant m^2). These companions increased ($p<0.01$) total forage accumulation by 37%–55% in the establishment year. The number of established birdsfoot trefoil seedlings was similar in monoculture and mixtures with annual legumes but lower ($p<0.01$) when planted with red clover. Neither seeding rate nor legume species impeded birdsfoot trefoil growth in the second season. Notably, binary mixtures of birdsfoot trefoil and red clover outperformed ($p<0.01$) the birdsfoot trefoil monoculture, yielding an additional 3260–5440 kg DM ha^{-1}. These findings highlight a practical strategy for farmers to sow birdsfoot trefoil with a suitable annual or perennial legume species, increasing total forage production without compromising the subsequent birdsfoot trefoil yield or nutritive value.

1 | Introduction

Birdsfoot trefoil (*Lotus corniculatus* L.; BFT) and red clover (*Trifolium pratense* L.) are grown as alternative forage legumes in high cool season rainfall (~1000–2500 mm) coastal regions of Pacific Northwest (PNW; the states of Washington, Oregon, and Idaho between the Rocky Mountains to the east and the Cascade Range to the west). Birdsfoot trefoil (BFT) stands out as a viable legume for the contrasting soil conditions of the region, that alternate between seasonal waterlogging in winter and dry in summer (Blumenthal and McGraw 1999). It enables farmers to produce high quality forage from paddocks with marginal or disturbed soil conditions (e.g., infertile, poorly drained and low pH). It is also a highly valuable legume in organic agricultural systems due to its unique nutritional and agronomic characteristics that include anthelmintic capabilities and secondary metabolites (e.g., condensed tannins). Birdsfoot trefoil has been reported to increase animal performance (Wang et al. 1996; MacAdam et al. 2015), reduce enteric methane emissions (Woodward 2004) and urinary N outputs (Ghelichkhan et al. 2018), and provides a high amount of nectar to pollinators in summer months (Seeno et al. 2023). Despite these attributes, farmers are often reluctant to plant BFT due to its slow establishment, poor seedling vigour, susceptibility to weed invasion, and high cost of seeds (McKersie and Tomes 1982; Chapman et al. 2008). Thus, it is important to maximise forage yield during establishment for the crop to be economically viable and agronomically successful.

Several studies have explored management practices such as seed priming (Beyaz and MacAdam 2023) and planting BFT with annual cereals (Wiersma, Hoffman, and Mlynarek 1999; Hunt, MacAdam, and Griggs 2016) to offset the poor production during the establishment year. Forage legumes have not been extensively investigated as nurse crops, but they may be less aggressive companion crops than cereal or grass species. (Seguin et al. 1999; Ćupina et al. 2011; Cicek et al. 2020). Furthermore, legumes may be a better option than cereals as nurse crops for feeding systems that require high-quality fodder for growing animals or high-yielding cows. For success, binary combinations must increase yield in the establishment year without penalising persistence and productivity of BFT in following years. Nurse crops should be adapted to the same climatic conditions, have compatible growth rates, with no allelopathic effects, and meet the specified forage production goals. One perennial legume that meets these criteria is red clover, which is mainly grown for seed production and silage for dairy farms in the region. It has a summer active growth pattern similar to BFT but it establishes more rapidly (McKersie and Tomes 1982; Moot et al. 2000), so often provides greater forage yield in the year of establishment (Seeno, Naumann, and Ates 2022). Annual clover options include the winter-hardy cultivar 'Frosty' of berseem clover (*Trifolium alexandrinum* L.). This cultivar was bred in Oregon and has been successfully grown in annual pasture and cover crop mixes. It is a summer active annual legume that can remain productive under irrigation until winter. However, it has poor regeneration potential as it predominantly produces soft seeds, which may be advantageous in a nurse crop. In contrast, balansa clover (*Trifolium michelianum* L.) is a winter annual that has excellent waterlogging tolerance and grows more vigorously than berseem clover in early spring (Ross et al. 2001). Balansa clover completes its annual life cycle in early summer with copious amounts of hard seeds as a survival strategy (Nori, Moot, and Mills 2019). Its excellent nutritional value and low risk of bloat make it ideal for grazing programs, particularly in dryland pastures (Moot 2012). It can provide high lamb liveweight gains in spring and is a source of nectar for pollinators (Gultekin et al. 2021; Caudillo et al. 2023).

Thus, in this study, we investigated the effects of sowing BFT in binary mixtures either with red (short-lived perennial), balansa (self-regenerating winter annual) or berseem (non-reseeding, annual) clovers at low (60 plant m^2), medium (120 plant m^2) and high (240 plant m^2) companion clover sowing rates. Measurements included plant populations established, forage yields and nutritive value. The aim was to identify the binary mix and sowing rate that maximised BFT plant populations without compromising persistence and productivity in the following season.

2 | Materials and Methods

2.1 | Site and Weather

The experiment was carried out from 7 September 2018 to 11 November 2020 at Blue Moon Farm (44° 33′ N, 122° 53′ W 100 m a.sl.) in Lebanon, Oregon. The soil at the location is a Malabon silty clay loam (fine, mixed, superactive, mesic, Pachic Ultic Argixerolls). A soil test (0–20 cm) in November 2018 indicated organic matter content of 2.63%, NO_3-N = 24.5 ppm, P (Bray 1) = 35.3 ppm, Ca = 3.7 meq/100 g, Mg = 1.8 meq/100 g, K = 237 ppm and soil pH = 6.7. The experimental location has a warm summer Mediterranean climate (Csb) according to the Köppen-Geiger climate classification system (Peel, Finlayson, and McMahon 2007). Air temperatures during the study period were similar to the long-term mean, whereas rainfall was below average in 2018/19 and 2019/20 by 20% and 7.8%, respectively (Table 1). January and February of 2019 were the coldest months of the 2018/19 growing cycle, with minimum temperatures between 1°C and − 1°C. December 2019, February 2020, and March 2020 were the coldest months during the 2019/20 growing cycle with minimum temperatures between 0°C and 2°C while January 2020 was warmer than is typical for the region.

2.2 | Plot Establishment and Experimental Design

Colonial bentgrass (*Agrostis capillaris* L.) had been grown on the site for seed production until it was left fallow on 22 October, 2016. Lime was applied at 2 t ha^{-1} in July 2017, which was promptly incorporated into the soil using conventional tilling. Glyphosate was applied at a rate of 1.13 kg ai ha^{-1} on 19 May 2018 to eliminate any weeds. This was followed by conventional tilling and harrowing in July 2018 to prepare the seed bed. On 7 September 2018, monocultures of birdsfoot trefoil (cv. VNS, semi-erect and non-rhizomatous), red clover (cv. Raven), balansa clover (cv. Fixation), berseem clover (cv. Frosty) and binary mixtures of BFT with these clovers were seeded in 1.5 × 6.1 m plots with 0.15 m row spacing at a target depth of 10 mm using a Wintersteiger plot seeder (Wintersteiger Inc., Salt Lake City, UT). Across all monoculture and mixture plots the seed rate of BFT was 9 kg ha^{-1} while balansa, berseem and red clover was sown at the seeding rates of 5, 10 and 10.5 kg ha^{-1}, respectively in the monocultures. The seeding rate of the companion legumes in the binary mixtures with BFT was 15% (low), 30% (medium) and 60% (high) of their monoculture sowing rates, aiming to achieve 60, 120 and 240 seedlings per m^2. All legume species were inoculated prior to planting with appropriate Rhizobium strains. Plots were arranged in a randomised complete block design with 4 replicates. Plots were irrigated with 38.1 mm of water on 8 September to facilitate germination of seedlings after planting, but the study was managed as a dryland system thereafter. Major broadleaved species (e.g., *Cichorium intybus*, *Sonchus oleraceus*) were removed by hand weeding in June and July to prevent flowering and seed dispersal, but no herbicide application was realised over the course of the study. Nitrogen (N) was applied to the plot area at a rate of 56 kg ha^{-1} in the form of urea (46–0-0) prior to seeding. Wil-Gro Pro Balance (16–16-16) fertiliser was applied in the following spring at rate of 9 kg ha^{-1} N, 4 kg ha^{-1} P and 7.5 kg ha^{-1} K. The same fertiliser application was repeated in fall 2019 and spring 2020.

2.3 | Sampling Procedure and Field Measurements

Harvests of the plots were made at approximately 6-week intervals during active growth in spring and 7-8-week intervals over summer. All treatments were harvested on the same dates (2019: April 4, May 22, July 8 and 2020: April 16, May 29, July 26). Only red clover and its binary mixtures with BFT produced enough

TABLE 1 | Mean air temperatures and total rainfall per month during the study period.

	Air temperature (°C)			Rainfall (mm)		
Month	**2018/19**	**2019/20**	**LTM***	**2018/19**	**2019/20**	**LTM***
August	20.1	20.3	19.4	0	3	13
September	16.3	16.8	16.8	29	82	34
October	12.8	9.7	11.9	52	64	81
November	7.9	6.7	6.7	108	31	174
December	5.4	5.0	4.5	176	120	194
January	5.1	7.1	4.7	95	239	160
February	3.0	5.9	6.2	176	45	133
March	7.0	6.7	8.2	49	85	111
April	11.1	10.8	10.1	165	47	79
May	14.3	14.1	13.1	49	80	60
June	17.0	16.7	16.1	19	49	36
July	19.2	19.7	19.3	18	0	14

Note: The reported values were obtained from Hyslop farm in Corvallis, 23 km from the study site.
*LTM, long-term mean.

forage to harvest in both fall 2019 (7 November) and 2020 (11 November). Balansa clover was in full bloom at the second harvest on May 22 and it completed its life cycle before the harvest on July 8. At that time, berseem clover was in full bloom and BFT and red clover were at 25% bloom stage.

Forage accumulation (DM kg ha^{-1}) was measured from a random 0.25 m^2 quadrat taken from each plot by cutting with electric shears to a stubble height of 50 mm. Quadrat cuts were sub–sampled for sorting into botanical fractions (BFT, red clover, berseem clover, balansa clover, weeds and dead material) before they were dried. All forage from the quadrat cuts was dried in a forced-air oven at 65°C for 48 h before weighing. Following collection of the samples, the remainder of the plots was mown to a stubble height of ~50 mm, with clippings removed from the site. Mean daily growth rates (kg DM ha^{-1} d^{-1}) were calculated at each harvest by dividing total forage accumulation (kg DM ha^{-1}) by the duration of regrowth since the previous harvest. In both growing seasons, 1st of February was considered to be the starting date of the forage growth.

Seedling and plant numbers of BFT, red clover, balansa clover and berseem clover were counted in two randomly placed 0.028 m^2 quadrats per plot (7 cm × 40 cm) on 17 October 2018 and 10 October 2019. Following the final harvest of the plots, the number of BFT plants in both monoculture and mixture plots were counted in two randomly placed 0.028 m^2 quadrats per plot on 23 November 2020.

2.4 | Land Equivalent Ratio and Competitive Ratio

Partial and total land equivalent ratios (LER) and competitive ratios (CR) were calculated to quantify the yield advantage of mixture vs. monoculture legume stands as described by Dhima et al. (2007). The land equivalent ratio fractions and total were calculated using the following formulae:

$$LER_{BFT} = \frac{Y_{BFT:CL}}{Y_{BFT}}, LER_{CL} = \frac{Y_{CL:BFT}}{Y_{CL}}, LER_{Total} = LER_{BFT} + LER_{CL}$$

where Y_{BFT} is yield of the BFT in monoculture, Y_{CL} is yield of the companion clover in monoculture, and $Y_{\mathrm{BFT:CL,CL:BFT}}$ is the yield of the component (BFT or CL) in mixture with the BFT or companion clover as determined after sorting into botanical fractions. Competitive ratios (CR) for the BFT and clover components of mixtures were calculated as

$$CR_{BFT} = \frac{LER_{BFT}}{LER_{CL}}\left(\frac{Z_{BFT}}{Z_{CL}}\right), CR_{CL} = \frac{LER_{CL}}{LER_{BFT}}\left(\frac{Z_{CL}}{Z_{BFT}}\right)$$

where Z_{BFT} is the sown proportion (based on seed numbers) of BFT in mixture with companion legume and Z_{CL} the sown proportion of clovers in mixture.

$\mathrm{LER_{BFT}}$ and $\mathrm{LER_{CL}}$ values above 0.5 indicate that the component had a competitive advantage in the mixture, and CR values above 1 indicate greater competitive ability compared with the companion legume. Land equivalent ratio and CR were only calculated for BFT-red clover binary mixtures in the 2019/20 growing season because annual legumes did not regenerate.

2.5 | Forage Nutritive Value

Nutritive value of the oven-dried forage samples was determined. Dried bulk samples were ground to pass through a 1-mm screen (MF 10 B; IKA werke, USA). Ground samples were analysed for ash, crude fibre (CF g kg^{-1}), crude protein (CP g kg^{-1}), acid detergent fibre (ADF g kg^{-1}), neutral detergent fibre (aNDF g kg^{-1}), non-fibre carbohydrates (NFC g kg^{-1}), water soluble carbohydrates (WSC g kg^{-1}), lignin (g kg^{-1}) digestible dry matter (DDM g kg^{-1}) NDF digestibility (NDFD g kg^{-1}), metabolizable energy (ME, MJ kg DM), calcium (Ca, g kg^{-1}), phosphorous (P g kg^{-1}), potassium (K g kg^{-1}) and magnesium (Mg g kg^{-1}) by near infra-red spectroscopy (NIRS; FOSS DS2500, Hillerød, Denmark) and NIRS Forage and Feed Consortium (Berea, KY, USA) forage nutritive value prediction equations. Samples were also analysed for total condensed tannins (CT) at Utah State University. Briefly, condensed tannins were quantified from dried, ground samples using a modified iron-HCL-butanol-acetone assay (Grabber, Zeller, and Mueller-Harvey 2013) with isolated BFT CT (Hagerman 2011) as the standard.

2.6 | Statistical Analyses

Plant numbers, forage accumulation, forage growth rates, nutritive value, botanical composition, LER and CR were analysed using a linear mixed model, with treatments (nurse crop, seeding rates) and harvest dates as fixed effects while blocks were treated as random effects. Years were analysed separately. A single degree of freedom contrast was used to compare the control treatment (BFT monoculture) with the nurse crop mixtures and seeding rates. The nine treatments that included nurse crops were analysed as a balanced factorial with three levels of nurse crops (balansa, berseem and red clover), three seeding rate levels (low, medium and high), and the interaction of nurse crops and seeding rates. Differences among treatment means were compared by Fisher's protected least significant difference (LSD) at $\alpha = 0.05$. Computations were carried out using GENSTAT (Payne 2009) statistical software.

3 | Results

3.1 | Effect of Nurse Cropping on Seedling and Established Plant Numbers

The number of BFT seedlings in fall 2018 was not affected ($p = 0.16$) by the companion clover species. However, higher seeding rates of companion clovers resulted in fewer established BFT seedlings ($p < 0.05$) compared with lower seeding rates (Figure 1). Balansa clover had fewer seedlings than red clover, but it had similar seedling numbers as berseem clover when planted with BFT. The combined number of seedlings (BFT + clovers) did not differ across companion clover species ($p = 0.64$) and seeding rates ($p = 0.14$). In fall 2019, the established BFT plant numbers were not different when planted with either annual clover but were lower ($p < 0.01$) when planted with

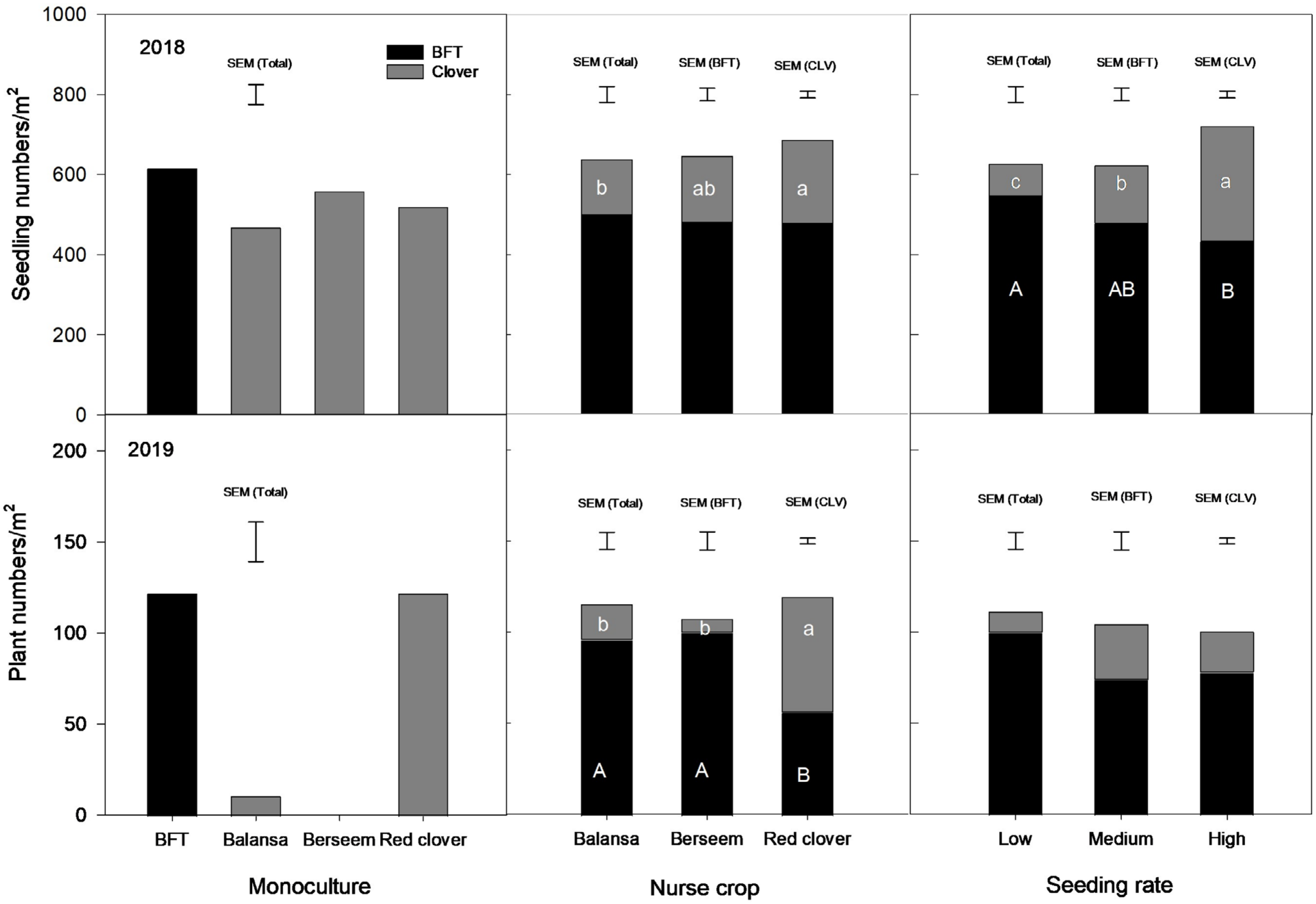

FIGURE 1 | Birdsfoot trefoil and nurse crops plant populations (m^{-2}) at emergence in fall 2018 and following the year of establishment as affected by companion nurse crop and seeding rate. Bar represent maximum standard error of mean (SEM). Capital letters indicate a significant difference in means for birdsfoot trefoil seedling and plant numbers, while lowercase letters indicate a significant difference in means for companion clover seedling and plant numbers ($p < 0.05$).

red clover (Figure 1). Conversely, the number of red clover plants exceeded the annual clovers when mixed with BFT.

Final BFT plant numbers ranged from 48 to 83 m^2 in fall 2020 (Figure 2). The number of birdsfoot trefoil plants in monoculture plots was not different to those planted in mixture with annual clovers but lower ($p < 0.01$) when planted with red clover. There were fewer ($p < 0.01$) BFT plants in medium and high companion clover seeding rates as compared with the lowest seeding rate.

3.2 | Forage Accumulation and Growth Rates of Monocultures

The forage accumulation of BFT reached nearly 4000 kg DM ha^{-1} during its establishment year (2018/2019). In comparison, this yield was lower than the annual clovers and red clover monocultures, by 4000 and 8000 kg DM ha^{-1}, respectively (Figure 3). In the subsequent year (2019/2020), forage accumulation of red clover was consistent with Year 1, whereas BFT yield increased ~2.5 t DM ha^{-1}. However, this was still only about half the forage accumulation of red clover. Neither balansa nor berseem clover regenerated in 2019.

The legume species had different seasonal growth rates ($p < 0.05$) over the two-year period (Figure 4a). Balansa clover had the highest ($p < 0.05$) mean daily growth rate (kg DM ha^{-1} day^{-1}) in the late winter- early spring period in 2019. Growth rates of berseem and red clover were not different but BFT had the lowest growth rate with 5 kg DM ha^{-1} day^{-1} during the same period. The growth rates of companion legumes in late spring ranged from 70to 86 kg DM ha^{-1} day^{-1}. In contrast BFT had the lowest growth rate at 27 kg DM ha^{-1} day^{-1}. Balansa clover completed its life cycle earlier than berseem clover, which grew over 50 kg DM ha^{-1} day^{-1} in summer. The growth rate of BFT was not different to berseem and red clover during summer. In fall, red clover was the only legume that produced measurable growth

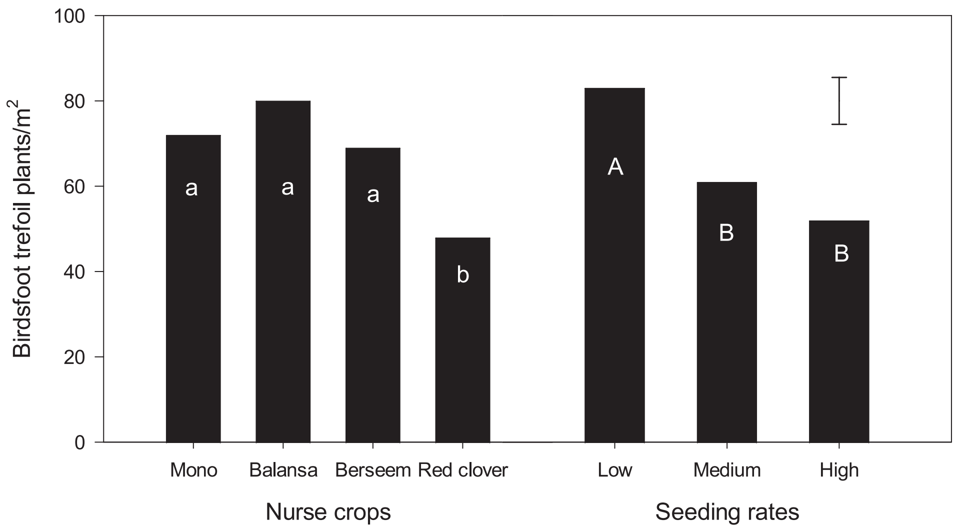

FIGURE 2 | Final birdsfoot trefoil numbers per meter square on November 2020. Bar represent maximum standard error of mean (SEM) for interaction of nurse crops and seeding rates. Capital letters indicate a significant difference in means for seeding rates, while lowercase letters indicate a significant difference in means for companion nurse clovers ($p < 0.05$).

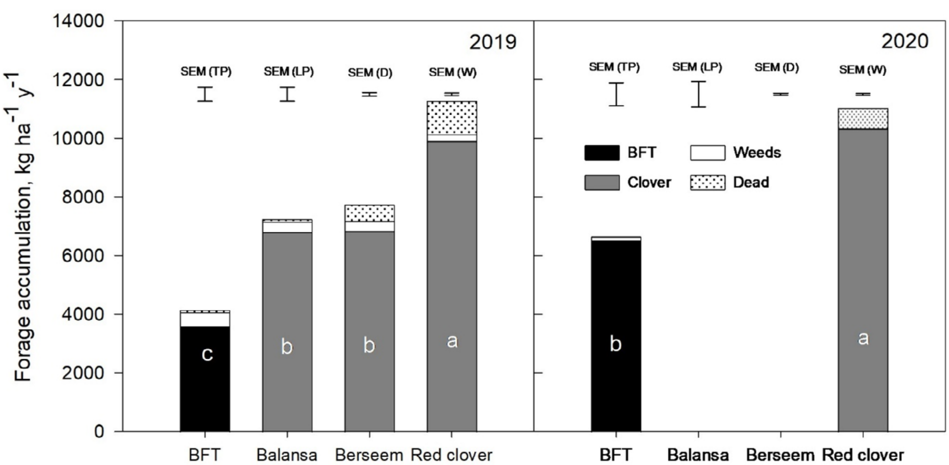

FIGURE 3 | Forage accumulation (kg DM ha^{-1}) of four legume monocultures in 2018/2019 and 2019/2020 growing seasons. Bar represent maximum standard error of mean (SEM). TP: Total production, LP: Legume production, D: Dead material, W: Weeds, Lowercase letters indicate a significant difference in means for forage accumulation ($p < 0.05$).

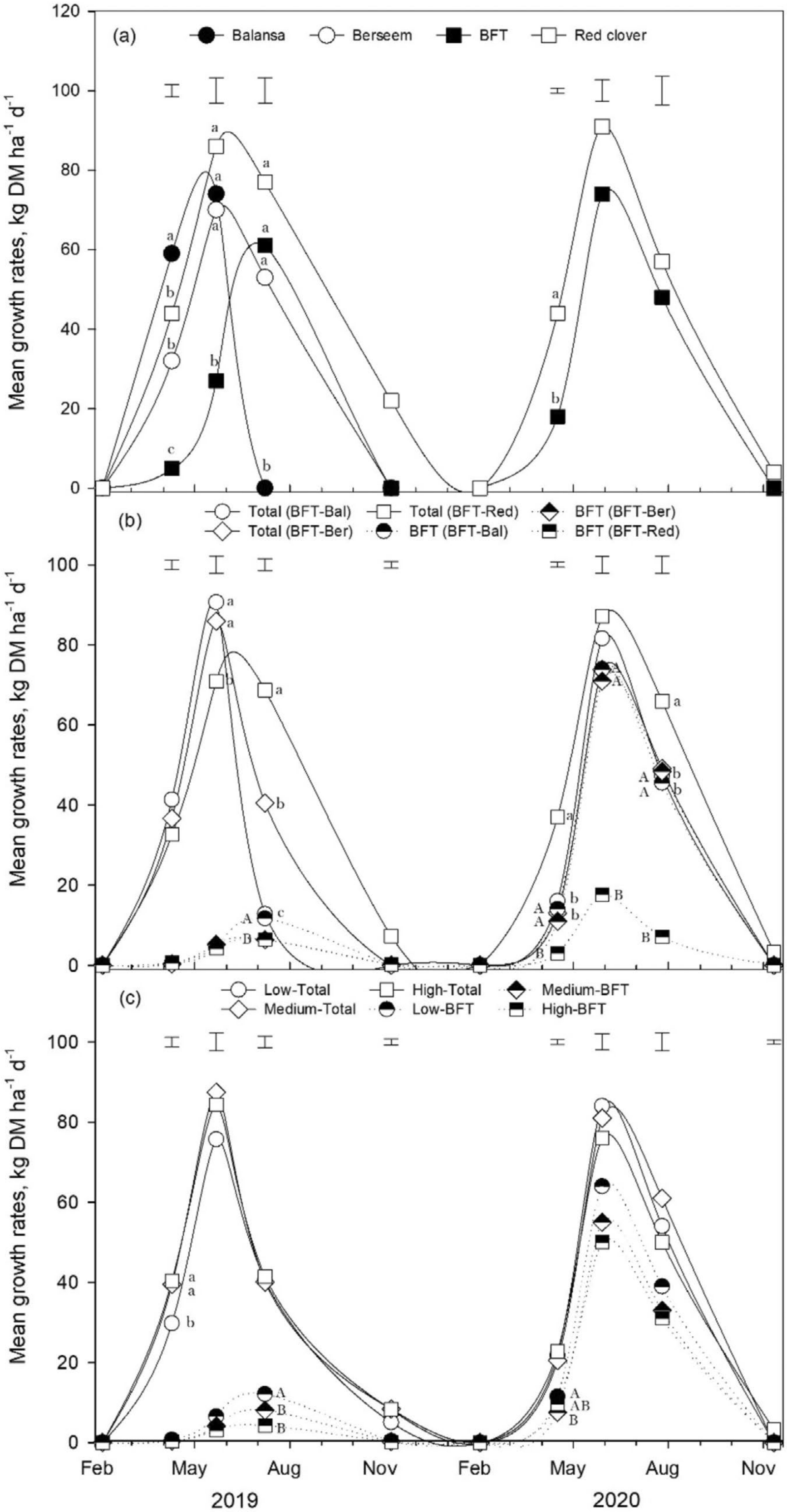

FIGURE 4 | Mean daily growth rates (kg DM ha^{-1} day^{-1}) for four legume monocultures (a), binary mixtures and BFT components of forage stands and (c) sowing rates (low, medium and high) from 2018 to 2020. Bar represent maximum standard error of mean (SEM) for the growth rates. Lowercase letters indicate a significant difference in means for daily growth rates ($p < 0.05$), while capital letters indicate a significant difference in means for daily growth rates of birdsfoot trefoil component of the forage stands.

in 2019. In 2020, red clover and BFT growth rates and patterns were not different except in early spring when red clover grew 21 kg DM ha^{-1} day^{-1} which was higher ($p < 0.01$) than the 8 kg DM ha^{-1} day^{-1} of berseem clover.

3.3 | Effect of Nurse Cropping on Forage Accumulation, Growth Rate, and Botanical Composition

The growth rates of BFT mixtures with balansa and red clover were greater ($p < 0.01$) than for the BFT-berseem clover mixtures in mid-spring, but the growth rates of the BFT component of the mixtures did not differ until summer when balansa clover completed its life cycle (Figure 4b). The growth rates of BFT mixtures with red clover remained over 70 kg DM ha^{-1} day^{-1} and this was greater ($p < 0.01$) than the growth rates of its mixtures with berseem clover berseem and balansa clover. The BFT biomass component of the mixtures was greater ($p < 0.05$) with balansa clover than with berseem and red clovers, but the difference was small. In 2020, growth rate of BFT was lower ($p < 0.01$) in BFT-red clover mixtures than its mixtures with annual legumes throughout the entire growing season. Sowing rates affected ($p < 0.01$) the growth rates only in early spring 2019 when high and medium sowing rates were higher than low seeding rates (Figure 4c).

Forage accumulation increased by 37%–55% in the establishment year (2018/2019) when BFT was sown with a companion legume species. Nurse cropping with red clover had a greater yield increase ($p < 0.01$) compared with nurse cropping with berseem clover, while the yield increase with balansa clover was the lowest (Figure 5). The total yield with medium and high companion clover seeding rates were not different to each other but were greater ($p < 0.01$) than at the low seeding rate. In parallel with the total forage accumulation, contribution of clovers to annual forage accumulation was the greatest ($p < 0.01$) with red clover. Yield of clovers was not different at high and medium seeding rates, but it was lower ($p < 0.01$) at the low seeding rate. Neither the companion legume species ($p = 0.97$) nor the seeding rate ($p = 0.17$) had any effect on contribution of the weeds to total forage accumulation. However, there was a significant interaction between species and seeding rate ($p = 0.05$) where binary mixtures of BFT with red clover had higher dead material compared with other binary combinations and the amount of dead material increased with increasing seeding rates of red clover. Dead material was not affected by seeding rate in the binary combinations with the other two clover species.

In 2020, the binary mixture of BFT with red clover produced 3690 and 4070 kg DM ha^{-1} more ($p < 0.01$) forage than its mixtures with balansa and berseem clovers, respectively (Figure 5). However, the seeding rate did not cause any difference ($p = 0.40$)

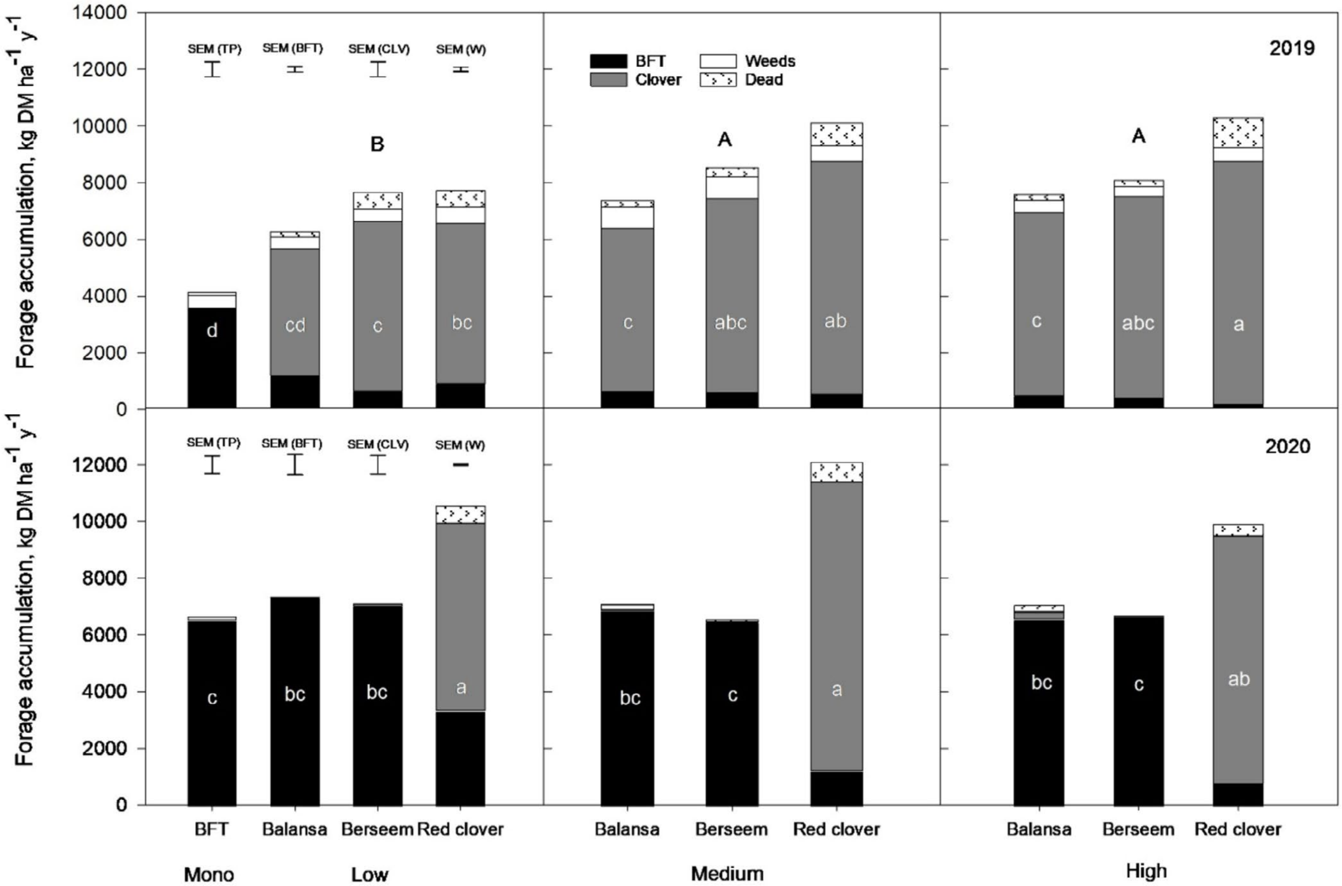

FIGURE 5 | Forage accumulation (kg DM ha^{-1}) of BFT monoculture and mixtures with balansa, berseem and red clover at low, medium and high seeding rates in 2018/19 and 2019/20 growing seasons. TP: Total production, BFT: Birdsfoot trefoil, CLV: Companion clover, W: Weeds, Bar represent maximum standard error of mean (SEM). Lowercase letters indicate a significant difference in means for forage accumulation ($p < 0.05$), while capital letters indicate a significant difference in means for sowing rate ($p < 0.05$).

in forage yield. Similar to Year 1, the contribution of weeds to total annual production did not differ with companion legume species ($p = 0.14$) or seeding rates ($p = 0.33$). Higher proportion ($p < 0.01$) of dead material in BFT mixtures with red clover was also found in 2020, but unlike 2019, the seeding rate did not affect ($p = 0.87$) the proportion of dead material.

3.4 | Land Equivalency Ratio (LER) and Competitive Ratio (CR)

In 2019, partial LER of BFT was not affected ($p = 0.13$) by the companion legume species but it gradually decreased ($p < 0.01$) as the seeding rate of companion legumes increased (Table 2). Partial LER for companion legume was the highest with berseem clover with a 0.96 LER. This was not different from balansa clover but the partial LER for red clover was lower than that of berseem clover. Total LER followed a similar trend to partial LER for companion clover with only berseem and balansa clover having a LER over 1.0. Companion legume × seeding rate interactions ($p < 0.05$) were found for the competitive ratios. The CR for BFT was higher with balansa and red clover at low seeding rate than other seeding rates, whereas CR for BFT did not change for berseem clover at different seeding rates. Competitive ratios for balansa and berseem clover did not differ across seeding rates ($p < 0.01$) but CR of red clover was higher than other clovers at the high seeding rate. In 2020, LER and CR were not different across seeding rates for both BFT and red clover (all $p > 0.05$). Total LER was over 1.0 for medium and low seeding rate.

In 2019, partial LER of BFT was not affected ($p = 0.13$) by the companion legume species but it decreased ($p < 0.01$) as the seeding rate of companion legumes increased (Table 2). Partial LER for companion legume was the highest with berseem clover with a 0.96 LER and not different from balansa clover. Partial LER for red clover was lower than red clover but not different to balansa clover. Total LER followed a similar trend to partial LER for companion clover with berseem and balansa clover having LER over 1.0. A companion legume × seeding rate interaction ($p < 0.05$) was also found for competitive ratio for BFT. The CR for BFT was higher with balansa and red clover at the low seeding rate than at other seeding rates, whereas CR for BFT did not change for berseem clover at different seeding rates. Competitive ratios for companion clovers were comparable across species but lower ($p < 0.01$) at high seeding rate than medium and low seeding rates, which resulted in no differences in CRs. In 2020, LER and CR were not different across seeding rates for both BFT and red clover (all $p > 0.05$). Total LER was over 1.0 for the medium and low seeding rates.

TABLE 2 | Land equivalent (LER) and competitive ratios (CR) for BFT and companion clovers at low medium and high seeding rates in 2018/19 and 2019/20 growing seasons.

			Land equivalency ratio (LER)			Competitive ratio (CR)	
Year	Companion clover	Sowing rate	BFT	Clover	Total	BFT	Clover
2019	Balansa	Low	0.33	0.69	1.02	0.43^{a}	0.25^{b}
	Berseem	Low	0.20	0.80	1.00	0.22^{b}	0.52^{b}
	Red clover	Low	0.25	0.57	0.83	0.39^{a}	0.30^{b}
	Balansa	Medium	0.20	0.86	1.06	0.18^{bc}	1.33^{b}
	Berseem	Medium	0.18	1.02	1.20	0.13^{bc}	1.74^{b}
	Red clover	Medium	0.17	0.84	1.01	0.16^{bc}	1.55^{b}
	Balansa	High	0.13	0.95	1.08	0.08^{bc}	3.63^{b}
	Berseem	High	0.11	1.04	1.15	0.07^{bc}	3.45^{b}
	Red clover	High	0.06	0.88	0.94	0.04^{c}	8.90^{a}
	SEM		0.038	0.082	0.102	0.035	1.025
	$p_{\text{Species (S)}}$		0.13	0.05	0.07	0.01	0.07
	$p_{\text{S rates (SR)}}$		0.01	0.01	0.22	0.01	0.01
	$p_{\text{S×SR}}$		0.50	0.98	0.94	0.05	0.05
2020	Red clover	Low	0.54	0.62	1.15	1.04	0.34
	Red clover	Medium	0.18	1.02	1.20	0.16	2.11
	Red clover	High	0.13	0.84	0.97	0.08	8.23
	SEM		0.130	0.126	0.090	0.299	2.527
	p		0.13	0.16	0.23	0.12	0.14

Note: [a–b] Means within a column with different superscripts differ ($p < 0.05$).
Abbreviation: SEM, standard error of a mean.

3.5 | Nutritive Value of Monoculture Forages

In spring 2019, differences were found across all nutritive value parameters ($p<0.05$ and $p<0.01$), except CP and ash concentrations of forages (Table 3). Notably, BFT had the lowest ADF, aNDF, lignin, P and K concentrations, while it had the highest NFC, DDM, NDFD, ME Ca and Mg concentrations. Conversely, red and berseem clover had higher ADF, aNDF, lignin and lower NFC, DDM, NDFD, ME concentrations than balansa clover and BFT. Balansa clover had the highest WSC and K concentrations but its NDFD and ME concentrations were not different to BFT. In summer 2019, BFT had higher CF, CP, NFC, DDM, NDFD, ME, CT and Mg concentrations but lower lignin and WSC concentrations than red and berseem clover. All ash ($p=0.18$) and Ca ($p=0.31$) concentrations were not different during this period. Similarly, in spring 2020, BFT had lower ($p<0.01$; $p<0.05$) ADF, aNDF, lignin and P concentrations but it had higher ($p<0.01$; $p<0.05$) CP, NFC, DDM, NDFD, ME and Mg concentrations. The differences between two legumes regarding ADF, aNDF, NFC, lignin, DDM, NDFD, ME, CT, P and Mg persisted in summer 2020.

In 2019, red clover had the greatest ($p<0.01$) crude protein (CPP kg ha^{-1} y^{-1}) and metabolizable energy production (MEP, GJ ME ha^{-1} y^{-1}), while the BFT had the lowest production (Table 4). In 2020, red clover also tended to have higher CP production than BFT but MEP did not differ ($p=0.16$) between two legumes. BFT and red clover mixtures had the greatest CPP ($p<0.01$) and MEP ($p<0.05$) in 2019. The CPP and MEP was lower ($p<0.01$) with low seeding than high and medium seeding rates which did not differ from each other. The superiority of BFT and red clover mixture in providing greater CPP and MEP was consistent ($p<0.01$) in 2020 but no difference was observed in sowing rate treatments for CPP and MEP (all $p>0.05$).

4 | Discussion

The challenge of poor seedling vigour in BFT poses a significant obstacle during the establishment phase, especially when competing with companion crops or weeds. The competition for light and nutrients with companion grasses is often more severe for BFT in temperate climates where high rainfall and cool weather conditions are highly conducive for cool-season grass growth (Gultekin et al. 2021). There have been conflicting reports on the benefits of nurse cropping slow establishing legumes with a cereal as a nurse crop on production and persistence of these legumes (Seguin et al. 1999; Wiersma, Hoffman, and Mlynarek 1999; Hunt, MacAdam, and Griggs 2016; Cicek et al. 2020). Wiersma, Hoffman, and Mlynarek (1999) reported no reduction in the number of established BFT seedlings when they were planted either with festulolium (*Festulolium braunii* K.A.), oat (*Avena sativa* L.), or oat + field pea (*Pisum* spp.). In the current study, the germination of BFT in fall 2018 was satisfactory, although high companion legume sowing rate reduced the BFT seedling numbers to 434 plants m^2. It was of note that the BFT seedling numbers at the establishment phase were not affected differentially by companion legume species. Although it is a slow-establishing legume, BFT seedling emergence does not appear to be hindered by companion crops with a range of emergence rates, growth habits, competitiveness and potential allelopathic effects. Although there is a good body of information available regarding the impact of cereals as nurse crops, a paucity of information exists in the literature concerning the effects of legume nurse crops on production and persistence of BFT and other slow establishing legumes. For example, Seguin et al. (1999) reported that another slow-establishing legume Caucasian clover (*Trifolium ambiguum* M. Bieb) population density was not reduced when sown with oats. In contrast, Cicek et al. (2020) noted significant reductions in sainfoin (*Onobrychis viciifolia* Scop.) seedling populations when sown with triticale (× *Triticosecale* Wittm.) or Hungarian vetch (*Vicia pannonica* Crantz.), although this effect was seeding rate and rainfall dependent.

In the current study, established plant numbers were reduced because of self-thinning in BFT monocultures and its binary mixtures with companion legumes following the first growing season in fall 2019. The reduction in plant numbers continued in the following growing season regardless of nurse cropping. This indicates that BFT plants experienced intraspecies and interspecies competition (Stephenson, Johnson, and Winsor 1988), but reduction in BFT plant numbers did not affect the total forage accumulation. Similar reductions in BFT populations were reported in particular under drought conditions in various locations of Australia (Ayres et al. 2006). It is worth noting that red clover caused a more rapid decline, with BFT numbers down to just 56 plants m^2. This suggests that red clover was able to access the most limiting factors (light and soil moisture) more effectively than BFT. This is supported by the reduction in BFT plant numbers in the monocultures being more dramatic than those reported by Hunt, MacAdam, and Reeve (2015) (at 49% vs. 81%.). Their experiment was irrigated, in which case the main competition factor in their study was light. The competition for light was probably also the reason that the effect of seeding rate on BFT populations was more important than companion legume species in the current study. BFT populations decreased at a greater rate as seeding rates of companion clovers increased, although this did not subsequently cause any yield reductions.

Overall, the number of surviving plants (61–100 plants m^2) appears to have been sufficient to provide high yields from BFT stands as 57 (Ayres et al. 2006) to 102 (Taylor, Templeton Jr, and Wyles 1973) plants m^2 have been reported to be agronomically successful for high BFT production. Our results are also consistent with the work of McGraw, Beuselinck, and Ingram (1986), who found that 30 BFT plants m^2 is the minimum number of plants needed to provide optimal yields. In the current study, the relationship between the number of BFT plants (44–122 plants m^2) and total annual forage accumulation of BFT (197–3580 kg DM ha^{-1}) was weak ($r^2=0.25$) during the first growing season. In the monocultures, BFT produced only 3580 kg DM ha^{-1} forage when the plant populations were 122 plant m^2, while it produced 6507 kg DM ha with 72 plants m^2 in the following year. It is likely that the establishing plants partitioned more resources for root than shoot growth, while the priority was shoot growth in the second growing season. However, when compared with the deep-rooted perennial alfalfa, Hunt, MacAdam, and Reeve (2015) suggested that BFT seedlings may have a lower rate of photosynthesis or a higher rate of respiration that reduces the total carbon available. In the subsequent year, at 33–100 plants m^2, the relationship between BFT plant numbers

TABLE 3 | Nutritive value of monoculture forage legumes[1](ME, MJ kg^{-1} DM; CT, mg g^{-1}; others, g kg^{-1}) in 2019 and 2020.

Year	Legume species	Ash	CF	CP	ADF	aNDF	NFC	WSC	Lignin	DDM	NDFD	ME	CT	Ca	P	K	Mg
									Spring 2019								
2019	BFT	92	27^{b}	217	224^{d}	256^{c}	429^{a}	95^{b}	34^{d}	715^{a}	682^{a}	11.3^{a}	8.8^{a}	18.7^{ab}	3.2^{c}	22.9^{c}	4.2^{a}
	Balansa	90	25^{c}	233	266^{c}	302^{b}	371^{b}	115^{a}	41^{c}	682^{b}	674^{a}	11.1^{a}	3.9^{b}	12.7^{c}	4.0^{b}	41.2^{a}	3.4^{b}
	Berseem	98	26^{bc}	224	341^{a}	376^{a}	295^{c}	78^{c}	72^{a}	623^{d}	476^{b}	9.5^{b}	2.5^{c}	19.9^{a}	4.1^{b}	31.8^{b}	3.3^{b}
	Red	102	31^{a}	250	314^{b}	350^{a}	287^{c}	94^{b}	65^{b}	644^{c}	467^{b}	9.7^{b}	3.1^{bc}	17.6^{b}	4.7^{a}	35.7^{b}	3.1^{b}
	SEM	3.0	0.6	8.4	6.5	8.2	10.2	4.3	2.2	5.1	10.4	0.10	0.32	0.5	0.1	1.2	0.1
	p	0.06	0.01	0.16	0.01	0.01	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
									Summer 2019								
	BFT	7.0	29^{a}	213^{a}	242^{b}	273^{b}	436^{a}	91^{b}	36^{b}	701^{a}	588^{a}	11.2^{a}	11.7^{a}	206	3.0^{b}	20.3^{b}	4.8^{a}
	Berseem	66.	$23.^{c}$	166^{c}	341^{a}	396^{a}	368^{b}	109^{a}	72^{a}	624^{b}	428^{b}	9.6^{b}	6.6^{b}	211	3.1^{b}	19.2^{b}	3.8^{b}
	Red	79.	$26.^{b}$	179^{b}	340^{a}	386^{a}	350^{b}	113^{a}	77^{a}	624^{b}	408^{b}	9.4^{b}	3.0^{c}	193	3.5^{a}	25.2^{a}	3.5^{b}
	SEM	4.1	0.6	3.0	9.6	10.6	7.9	3.8	1.7	7.5	12.2	0.09	0.44	0.80	0.10	0.99	0.18
	p	0.18	0.01	0.01	0.01	0.01	0.01	0.05	0.01	0.01	0.01	0.01	0.01	0.31	0.05	0.05	0.01
									Spring 2020								
2020	BFT	80	29	263	207	235	413	87	27	728	721	11.7	14.6	18.1	4.0	27.4	4.1
	Red	89	30	232	318	362	307	100	69	642	428	9.6	2.7	18.7	4.4	29.7	3.1
	SEM	2.2	0.5	2.6	2.8	3.3	7.3	3.1	1.1	2.2	11.3	0.07	0.92	0.27	0.04	0.89	0.04
	p	0.06	0.79	0.01	0.01	0.01	0.01	0.06	0.01	0.01	0.01	0.01	0.01	0.20	0.01	0.17	0.01
									Summer 2020								
	BFT	55	33	175	207	263	494	100	27	728	645	11.9	16.8	173	3.0	18.1	4.4
	Red	75	30	157	359	416	343	100	80	609	427	9.4	5.0	184	3.4	25.0	3.2
	SEM	1.8	0.7	6.6	15.7	18.9	19.0	4.6	3.3	12.2	12.8	0.26	0.28	0.41	0.08	0.81	0.06
	p	0.05	0.05	0.15	0.01	0.05	0.05	0.98	0.01	0.01	0.01	0.01	0.01	0.16	0.05	0.01	0.01

Note: $^{a-c}$ Means within a column with different superscripts differ ($p < 0.05$).
Abbreviations: ADF, acid detergent fibre; aNDF, neutral detergent fibre; CF, crude fibre; CP, crude protein; CT, condensed tannins; DDM, dry matter digestibility; ME, metabolizable energy; NDFD, NDF digestibility; NFC, non-fibre carbohydrates; SEM, standard error of a mean; WSC, water soluble carbohydrates.

TABLE 4 | Total annual crude protein (CPP, kg ha^{-1} y^{-1}) and metabolizable energy (MP, GJ ME ha^{-1} y^{-1}) production of BFT sown with balansa, berseem and red clover at three sowing rates in 2019 and 2020.

		2019		2020	
		CPP	MEP	CPP	MEP
Companion clover	**Sowing rate**	kg ha^{-1} y^{-1}	GJ ha^{-1} y^{-1}	kg ha^{-1} y^{-1}	GJ ha^{-1} y^{-1}
Balansa	Monoculture	1704^{b}	80.4^{b}	—	—
Berseem	Monoculture	1564^{b}	73.2^{b}	—	—
Red clover	Monoculture	2499^{a}	108.9^{a}	2358	107.0
BFT	Monoculture	920^{c}	47.8^{c}	1653	85.7
SEM		104.6	5.10	189.7	8.09
P		0.01	0.01	0.07	0.16
Balansa	Low	1477	75.4	1723	89.0
Berseem	Low	1499	75.2	1579	86.2
Red clover	Low	1572	75.9	2166	105.8
Balansa	Medium	1668	81.1	1666	87.9
Berseem	Medium	1676	81.7	1372	77.7
Red clover	Medium	2152	97.7	2538	118.6
Balansa	High	1691	83.9	1599	83.8
Berseem	High	1560	77.6	1523	81.4
Red clover	High	2130	99.5	1982	95.9
SEM		119.9	5.53	151.1	6.85
$p_{\text{Species (S)}}$		0.01	0.05	0.01	0.01
$p_{\text{S rates (SR)}}$		0.01	0.05	0.42	0.34
$p_{\text{S×SR}}$		0.25	0.34	0.19	0.37

Note: $^{a-c}$ Means within a column with different superscripts differ ($p < 0.05$).
Abbreviation: SEM, standard error of a mean.

and production (751–7301 kg DM ha^{-1}) improved ($r^2 = 0.64$). The implication is that low plant numbers cannot compensate yield by growing a larger canopy (McGraw, Beuselinck, and Ingram 1986), so establishing an adequate initial population is important for successful BFT stands.

The benefit of leguminous nurse crops for increasing the yield of BFT in Year 1 was demonstrated by greater yields (up to 56%) of binary mixtures than the BFT monocultures. This was achieved without penalising productivity in subsequent years. The forage accumulation of BFT during its establishment year was approximately 4.1 t DM ha^{-1}, which constituted only 53% and 37% of what the annual legumes and red clover produced, respectively. The low production of BFT in the establishment year was quantified by its slow growth rate during spring (5–27 kg DM ha^{-1}). This low growth rate suggests the canopy of BFT was slower to close than the other species, but this remains to be quantified. The main difference in growth rates of BFT over the two-year trial period were found in late spring when it grew at 27 kg DM ha^{-1} day^{-1} in 2019 as compared with 74 kg DM ha^{-1} day^{-1} in 2020. In comparison, the difference in its growth rates in early spring (5 vs. 9.6 kg DM ha^{-1} day^{-1}) and summer (48 vs. 61 kg DM ha^{-1} day^{-1}) were much smaller. From this perspective, all nurse clovers had greater spring growth rates than BFT so the total forage accumulation was increased significantly.

At all seeding rates, binary mixtures of BFT either with balansa or berseem clovers provided total LER values greater than 1.0 across all sowing rates. This quantifies the benefit of nurse cropping with these annual legumes when it suits the agronomic and animal feeding programs. Overall, both annual legumes provided similar DM yield (7.7 vs. 7.9 t DM ha^{-1}) in their establishment year but there were differences in seasonality of production. For example, the forage accumulation of balansa clover in early spring was greater than red and berseem clover by approximately 1.0 and 1.86 t DM ha$^{-1,}$ respectively. This meant balansa clover was the most complementary nurse crop during the spring period. However, BFT had a poor recovery after balansa clover had completed its life cycle, which suggests that competition during emergence was critical for the success of the BFT. In effect, the advantage of balansa clover in early spring was offset by the lower yield of the BFT. This was shown in summer, when the BFT monoculture yield was greater than its binary mix with balansa clover (2629 vs. 548 kg DM ha^{-1}). It is of note that the nutritive value of balansa clover was highly comparable to BFT but much greater than berseem and red clover as evidenced by its higher ME, DDM and NDFD than other clovers. High sheep preference (Hyslop, Slay, and Moffat 2003) and lamb liveweight gains (Gultekin et al. 2021) were reported with balansa clover in in situ grazing systems. The superior nutritive value of balansa clover, with its rapid spring growth rates in the BFT-balansa combination may suit grazing systems where high animal performance is desired (e.g., lamb finishing, dairy production). Conversely, the early flowering habit and high moisture content of balansa clover may limit its value if the mix is to be taken as a silage or hay crop (Snowball 1993).

In comparison, berseem clover provided 32% of its total annual forage accumulation in summer when balansa clover had already completed its life cycle. This more spread-out production profile may suit a hay/silage system. The competitive ratio of BFT was lower with berseem than red and balansa clover in particular at low seeding rate. This might be because berseem clover has allelochemicals (Maighany et al. 2007) which helped reduce the holoparasitic plant, *Orobanche crenata* infestation (Fernández-Aparicio, Emeran, and Rubiales 2010). Compared with berseem clover, balansa clover can also be more persistent and prolific in pastures experiencing waterlogging. This assumption is in line with the findings of a recent lamb grazing study where balansa and berseem clover were a part of diverse legume mixture (8 species) grown in a seasonally waterlogged pasture (Caudillo et al. 2023). Balansa clover was the main component of the legume pastures (55%) in spring even at only 2 kg ha^{-1} sowing rate.

It was evident that when red clover was planted with BFT, the biomass and nutrient (CP and ME) production was the greatest and most reliable in both years. This occurred despite red clover stands constantly having more dead material, which would contribute to their poorer nutritional value compared with BFT and balansa clover. High levels of senesced material from red clover plants may be due to plant growth habit. For instance, Buxton et al. (1999) compared nutritive value of perennial legumes and indicated that dead leaves were retained on red clover plants but senesced from alfalfa and birdsfoot trefoil plants, causing differences in vitro dry matter digestibility to be greater in alfalfa and BFT than red clover. While nurse cropping with red clover provided the highest yield, this came at the expense of the BFT component in the mixtures. The proportion of BFT in these mixtures was minimal ($<10\%$) in the establishment year and red clover remained the dominant legume in the composition even at the lowest red clover seeding rate (~65% vs. 35%). This was also the case in a previous dairy cow grazing study where red clover was sown with BFT at a seeding rate close to the low seeding rate of the current study (2 kg DM ha^{-1}). In that study BFT only constituted 13% of the mixture composition (Wilson et al. 2020).

Overall, sowing BFT with any of the three tested legumes proves to be a promising approach for increasing forage production, especially in the establishment year. While it was anticipated that berseem clover may not reestablish in fall 2020, the unexpected failure of balansa clover to regenerate was noteworthy. This setback could be attributed to the timing of balansa clover harvesting during mid-flowering, which likely hampered seed production potential during establishment. Consequently, the BFT and annual legume mix essentially transformed into nearly pure BFT stands in 2020, providing comparable forage production to monoculture BFT stands. This suggests that both annual legumes can serve as effective nurse crops for BFT. The selection of companion species should consider factors such as the intended production system (grazing or conservation) as well as climatic and soil conditions. Legume nurse crops can also be more valuable than the grasses or cereals when high quality feed is desired for high yielding dairy cows or growing young stock. However, when combined with red clover, even at high seeding rates, the resulting sward predominantly favoured red clover growth in 2020. This indicates strong competitive ability of red clover over BFT, particularly evident in its CR at high seeding rates. Notably, this combination did not achieve a LER value exceeding 1.0, indicating inefficacy of mixtures. Nevertheless, incorporating BFT into red clover at lower or moderate seeding rates remains a viable consideration, particularly for its advantages in animal health and environmental benefits, such as in organic production systems (Hunt, MacAdam, and Reeve 2015).

A feature of the results in the current study was the lack of a weed suppression response of nurse cropping in both years. It is of note that the proportion of weeds in forages during the establishment year was reduced by nurse cropping of forbs with balansa clover or subterranean clover (*Trifolium subterraneum* L.) in another trial conducted on adjacent land during the same period (Seeno et al. 2023). However, the discrepancy in weed suppression between the two studies may be due to the lighter weed pressure in the current study and hand weeding of some major broadleaved weed species in the field. This may have affected the results as there is a body of research indicating that nurse cropping is a valid strategy for suppressing weeds, particularly in organic systems (Bilalis et al. 2010; Hunt, MacAdam, and Griggs 2016; Dowling et al. 2021; Gardarin et al. 2022). Ross et al. (2001) reported that both balansa clover and berseem clover suppressed brown mustard (*Brassica juncea* L.) weed in Alberta, Canada. In line with these results, Ćupina et al. (2011) also noted that the proportion of weeds in the botanical composition dropped by 29% when red clover was planted with field pea as compared to its monoculture. Overall, these studies demonstrate the potential benefits of using legumes as nurse crops for sainfoin, alfalfa, and BFT establishment, highlighting their role in improving forage productivity and sustainability.

5 | Conclusions

The results indicated that the legume companion crops used in this study did not compete with BFT even though they increased forage accumulation during the year of establishment without penalising the subsequent yield of BFT. This provides a viable option for farmers wishing to establish BFT for forage production. The yield advantage was 37%–55% in the year of establishment when BFT was sown with a companion legume species. Neither the seeding rate nor the legume species caused any reduction in the forage accumulation in the following growing season. Nurse cropping with red clover had a greater increase in forage accumulation compared with nurse cropping with annual legumes, albeit at the expense of a lower proportion of BFT in the mixtures. A low seeding rate (1.6 kg ha^{-1}) of red clover leads to a more balanced mix of BFT and red clover in the botanical composition. The BFT yield in the second growing season was not penalised at medium and high sowing rates, but the lack of DM yield difference across companion crop seeding rates suggests that both annual legumes can be sown at low seeding rates when establishing a binary mix with BFT.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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