Agricultural Performance of Diverse Pastures of Complementary Species and Monoculture Pastures Defoliated According to the Leaf Regrowth Stage Window of Opportunity Criterion

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Abstract. In a diverse pasture of complementary species (DPCS), individual species fulfil agro-ecological functions that confer growth asynchrony and complementarity of ecosystem functions. These attributes provide yield consistency with a more even forage supply pattern across the year compared to monocultures. A common leaf regrowth stage window opportunity (LSWO) for the diverse species enables pasture defoliation that stimulates growth and persistence. The study assessed seasonal and annual growth traits of *Lolium perenne* (Lp), *Bromus valdivianus* (Bv) and *Dactylis glomerata* (Dg) as single grass species (Mono) sown with *Trifolium repens* (Tr) and as DPCS with the four species (Lp+Bv+Dg+Tr=Mix). The defoliation criteria applied (LSWO of a target species: Lp, Bv or Dg) resulted in eleven grazing events for MonoLp and MixLp, ten grazing events for MonoBv and MixBv, and nine grazing events for MonoDg and MixDg in a year. MixBv and MixDg displayed synchronized overlaps of the three species LSWOs during the seasons and across the year. MixLp had Bv and Dg being grazed slightly earlier than their LSWOs. There were no significant differences in annual herbage accumulation for all treatments. Significant differences were found within seasons, and the seasonality of the pasture growth was reduced in the DPCS when compared to their respective Mono establishment. This resulted in a more evenly distributed pasture feed resource throughout the year and can mitigate the negative impacts of extreme climatic events (longer periods of soil water restriction or saturation). The LSWO criterion enabled the successful management of monocultures and DPCS.

Introduction

Recent extreme climatic events have been shown to restrict the persistence of monoculture pastures (Lüscher et al., 2022). Diverse pastures of complementary species (DPCS) are pastures that combine species of asynchronous growth and different functional roles that complement each other within the same pasture. These are presented as an alternative for farmers to deal with climate change (Brophy et al., 2017). The DPCS enable a greater pastoral ecosystem resilience and stability (Suter et al., 2021). When aiming for the persistence of DPCS as a functional ecosystem, the defoliation regime of species with different morpho-physiological traits is a challenge to be overcome. Morpho-physiological traits of individual species, such as the leaf regrowth stage window opportunity (LSWO), can be a criterion to define the defoliation moment. This is well-established for monocultures of *Lolium perenne* (Lp) (Fulkerson & Donaghy, 2001), binary mixtures of Lp and *Bromus valdivianus* (Bv) (García-Favre et al., 2022), and *Dactylis glomerata* (Dg) (Gatti et al., 2017). In DPCS, the different species are expected to present a temporal overlap of optimal defoliation moment across the year. The study objective was to assess agricultural traits of *L. perenne* (Lp), *B. valdivianus* (Bv) and *Dactylis glomerata* (Dg) defoliated according to their respective LSWOs as DPCS and monocultures and determine the growth asynchrony between these species across and over seasons.

Methods

The study was at Massey University's Dairy 1 (Palmerston North, Manawatu, New Zealand) from 15 June 2021 to 14 June 2022. The soil type is Manawatu silt loam over sand (Landcare Research National Soil Data Base, Lab. No SB10036). Soil fertility samples were collected on 26 February 2021 (Table 1). The area received annual maintenance fertilisation on 1 March 2021, and 17 March 2022, at 500 kg ha^{-1} and 400 kg ha^{-1} of Superphosphate + Se (0% N, 22.5% P, 0% K, 27.5% S and 50% Ca). Post-grazing nitrogen fertilisations were applied in July 2021, November 2021 and January 2022, each one at 30 kg ha^{-1}. The climate is classified as Marine West Coast climate - Cfb (Köppen climate classification). The annual rainfall was 1214.7 mm (winter: 334.8 mm; spring: 356.5 mm; summer: 225 mm; autumn: 328.4 mm). Monthly rainfall, ground minimum temperature, and air minimum and maximum temperature are presented in Figure 1 (NIWA/AgResearch Weather Station, ~800 m from the field site). On 18 December 2020, mixtures (Mix) of *L. perenne* cv. Maxsyn (Lp), *B. valdivianus* cv. Bareno (Bv) and *D. glomerata* cv. Greenly (Dg), and their respective single grass pastures (Mono) were established. *Trifolium repens* cv. Weka (Tr) was sown at the same rate in all plots. From June 2021 onwards, the defoliation criterion was applied according to the LSWO of Lp, Bv and Dg for each Mix and their respective Mono. This resulted in six experimental treatments: MonoLp, MonoBv, MonoDg, MixLp (defoliated at Lp LSWO), MixBv (defoliated at Bv LSWO), MixDg (defoliated at Dg LSWO). In winter, summer, and autumn the LSWO were: Lp (2.5-3.0LS), Bv (3.5-4.0LS) and Dg (3.5-4.0LS); in spring: Lp (2.25-2.75LS), Bv (3.0-3.5LS) and Dg (3.0-3.5LS). The defoliation criteria applied resulted in 11 grazing events for MonoLp and MixLp, 10 grazing events for MonoBv and MixBv, and 9 grazing events for MonoDg and MixDg in a year. The six pasture treatments were arranged in a randomised complete block design, with three blocks (n=3), each block with six experimental units (plots) of 20m x 20m. The plots were grazed by dairy cows (10-15 animals/plot) in one-day grazing events. The LS of the target species (*α*) and complementary species (CSp) was assessed on 9 tillers per species per plot the day before each grazing event. The herbage mass was measured both pre- and post-grazing by cutting three 0.1m^2 quadrats to ground-level per plot. The samples were dried for at least 72 hours in a forced-air oven at 60°C then weighed. The apparent accumulated dry matter (DM) was the sum of apparent herbage removal, calculated as the difference of the pre-grazing DM at the current grazing event minus the post-grazing DM of the previous grazing event for a certain period (season or year).

Table 1. Soil chemical characteristics at three depths (0-15, 15-30, 50-65 cm) of the experimental area on 26th of February 2021.

Soil Layer	pH	OM†	P	K	Ca	Mg	CEC	Tot. Base Saturation	Ext. Organic Sulphur	C/N
cm	$CaCl_2$	%	------------------ME 100 g^{-1}------------------					%	mg kg^{-1}	
0-15	5.60	3.33	29.0	0.21	6.80	1.35	13.00	64.00	3.00	10.03
15-30	5.53	2.53	23.0	0.20	6.33	1.30	12.70	63.30	3.00	9.57
50-65	5.67	1.30	5.33	0.16	6.10	1.15	10.70	71.00	2.30	9.70

†Organic matter (OM), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), cation exchange capacity (CEC), carbon:nitrogen ratio (C/N).

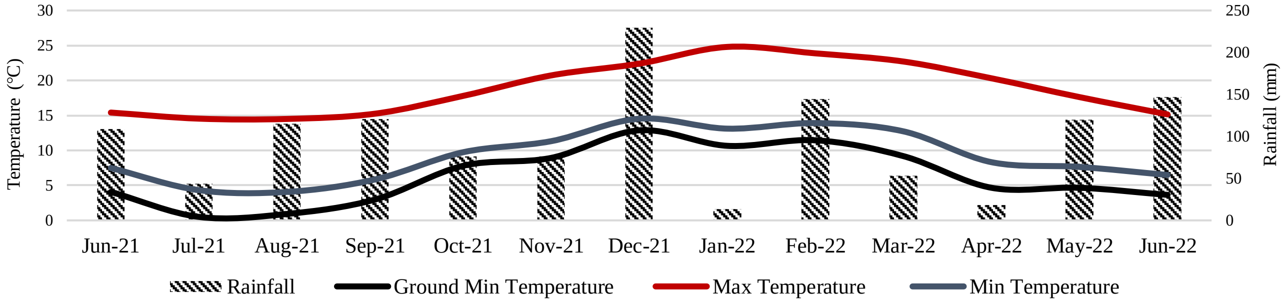

Figure 1. Monthly rainfall, ground minimum, air maximum and minimum temperatures during the experimental period (June 2021 to June 2022). Bars indicate rainfall; lines indicate temperatures.

Data distribution was verified for the residual normality and variance of homogeneity, using the Shapiro-Wilk test and Chi-square test (PROC Univariate), respectively. Subsequently, data were analysed by a one-way analysis of variance (ANOVA), using LSD test to define differences among treatments (PROC Glimmix) on Statistical Analysis System (SAS 9.4). The statistical model had the fixed effect of defoliation treatment and the random effect of block. Statistically significant differences were considered at $p \leq 0.05$.

Results and Discussion

The target seasonal LSWO for the *α* species was achieved in the six pasture treatments. Across the year, in the MixLp, Bv was defoliated earlier than recommended in winter and summer, and Dg during the whole year; thus, this defoliation frequency did not suit the target LSWO for Dg, and partially Bv. The MixBv had all species being grazed within their target LSWO. The MixDg had the three species being grazed within its targeted LSWO in spring, summer, and autumn; in winter, Lp and Bv (CSp) were defoliated later than recommended, at 3.3 and 4.2 LS, respectively (Table 2). Overall, the only mixture that showed an overlap of LSWOs for the three species throughout the year was the MixBv. The MixLp and MixDg had the most grazing events with the three species LSWOs overlapping. The few events in which the CSp were slightly earlier or later than their target LSWOs suggested a flexibility in management, with a trade-off amongst the productivity and plant reserves. García-Favre et al. (2022) found similar patterns of asynchronous growth using LSWO in mixtures of Lp and Bv in a two-year study, such that in the mixture with Lp LSWO as target, the Bv was defoliated slightly earlier (3.2LS average) with significant lower values of root water-soluble carbohydrates storage, but still allowing the species to persist.

Table 2. Pasture treatments (MonoLp, MonoBv, MonoDg, MixLp, MixBv, MixDg), pasture species composition (Spp), seasonal leaf stage window opportunity (LSWO) for pasture defoliation according to the α species (winter, summer, and autumn; spring), and seasonal average leaf stage of each pasture species at the defoliation event.

	Spp *	Seasonal leaf-stage window opportunity	Seasonal average of species LS

Pasture treatments		Winter, summer, and autumn	Spring	Winter 2021	Spring 2021	Summer 2021-22	Autumn 2022
MonoLp	Lp α	2.5 – 3.0	2.25 – 2.75	2.7	2.4	2.6	2.7
MonoBv	Bv α	3.5 – 4.0	3.0 – 3.5	3.8	3.2	3.7	3.7
MonoDg	Dg α	3.5 – 4.0	3.0 – 3.5	3.8	3.2	3.5	3.7
MixLp	**Lp $\boldsymbol{\alpha}$**	**2.5 – 3.0**	**2.25 – 2.75**	**2.6_C**	**2.5_C**	**2.6_C**	**2.7_C**
	Bv			3.3_E	3.1_C	3.2_E	3.6_C
	Dg			3.2_E	2.9_E	3.2_E	3.4_E
MixBv	Lp			3.0_C	2.5_C	2.6_C	2.6_C
	Bv $\boldsymbol{\alpha}$	**3.5 – 4.0**	**3.0 – 3.5**	**4.0_C**	**3.0_C**	**3.6_C**	**3.6_C**
	Dg			3.7_C	3.1_C	3.5_C	3.4_C
MixDg	Lp			3.3_L	2.7_C	2.9_C	2.7_C
	Bv			4.2_L	3.2_C	3.6_C	3.7_C
	Dg $\boldsymbol{\alpha}$	**3.5 – 4.0**	**3.0 – 3.5**	**3.7_C**	**3.2_C**	**3.6_C**	**3.5_C**

α: target species that determined each defoliation event in monocultures (defoliation criterion = α_{LSWO}); $\boldsymbol{\alpha}$ in bold: targeted species in mixtures (defoliation criterion = $\boldsymbol{\alpha}_{LSWO}$); C: correct LSWO of the species according to the season; E: earlier than the correct LSWO of the species according to the season; L: later than the correct LSWO of the species according to the season. *All plots had *T. repens* in their composition.

For all mixtures, the seasonal average LS of Lp, Bv and Dg did not differ (p>0.05) in spring and autumn, but significantly differed during winter (Lp: p=0.0001, Bv: p=0.0001 and Dg: p<0.05) and summer (Lp: p<0.05, Bv: p<0.05 and Dg p<0.01). An overall pattern occurred, in which the three species presented the lowest LS in the MixLp and the highest in the MixDg (Table 3). Further assessments are necessary to better understand the flexibility of LSWO as a tool for pasture defoliation management, and the phenotypic plasticity of the species to cope with sporadic early and/or late defoliation events over and across seasons.

Table 3. Leaf-stage of the species within each mixture at the moment of defoliation (Lp on MixLp, MixBv and MixDg; Bv on MixLp, MixBv and MixDg; Dg on MixLp, MixBv and MixDg) in winter, spring, summer, and autumn.

	Seasonal average LS per species			
Species within each mixture	Winter 2021	Spring 2021	Summer 2021-22	Autumn 2022
Lolium perenne				
MixLp	2.6 c (±0.006)	2.5 (±0.041)	2.6 b (±0.034)	2.7 (±0.021)
MixBv	3.0 b (±0.017)	2.5 (±0.032)	2.6 b (±0.019)	2.6 (±0.045)
MixDg	3.3 a (±0.035)	2.7 (±0.057)	2.9 a (±0.068)	2.7 (±0.061)
Significance	****	NS	*	NS
Bromus valdivianus				
MixLp	3.3 c (±0.051)	3.1 (±0.021)	3.2 b (±0.052)	3.6 (±0.024)
MixBv	4.0 b (±0.008)	3.0 (±0.037)	3.6 a (±0.029)	3.6 (±0.027)
MixDg	4.2 a (±0.028)	3.2 (±0.077)	3.6 a (±0.141)	3.7 (±0.038)
Significance	****	NS	*	NS
Dactylis glomerata				
MixLp	3.2 b (±0.003)	2.9 (±0.044)	3.2 c (±0.038)	3.4 (±0.075)
MixBv	3.7 a (±0.158)	3.1 (±0.070)	3.5 b (±0.021)	3.4 (±0.025)
MixDg	3.7 a (±0.029)	3.2 (±0.088)	3.6 a (±0.044)	3.5 (±0.052)
Significance	*	NS	**	NS

Letters that differ within columns for the same species indicate values that are significantly different; ** p<0.05, ** p<0.01, *** p<0.001, ****p=0.0001, NS = non-significant;* following each species leaf-stage least square mean, is the (±) standard error of the mean (S.E.M.).

The annual accumulated pasture herbage mass was similar for all treatments (p>0.05), with an average annual accumulation of 20.26 t DM ha^{-1}. Forage production was considered high, peaking between December to March, mostly explained by the combination of great pluviosity in the period (wettest summer since 2004) and optimal temperatures for herbage growth. The average daily growth rate of the pasture treatments showed significant differences in all seasons (p<0.05). Herbage growth rates are shown for the pairs that have the same $\boldsymbol{\alpha}$ species LSWOs (Figure 2). MixLp had greater growth rates than MonoLp in spring (41.5%), summer (13.9%) and autumn (37.7%). MixBv had greater growth rates than MonoBv in winter (69.6%) and autumn (43.8%). MixDg presented a greater growth rate than MonoDg only in autumn (78.7%). All mixtures had a better post-summer recovery represented by a slower decline in growth rate towards autumn. The presence of winter- and summer-active species within the same pasture explained the over yielding of the mixtures in relation to their respective monocultures. Annual increments in production were also reported for mixtures of Lp and Bv, being 15% higher than their monocultures, mainly due to an increase in summer and spring production (García-Favre et al., 2022). Overall, the growth rate of monocultures presented a greater fluctuation than the mixtures from one season to the next (i.e., spring 2021 and summer 2021-22 for MonoLp and MixLp), which depicts a more even distribution of the herbage accumulated during the year (Figure 2). Even though there were no significant differences in annual accumulation, the asynchronous growth of the species in the mixtures resulted in a more consistent herbage mass accumulation with a less notable seasonality.

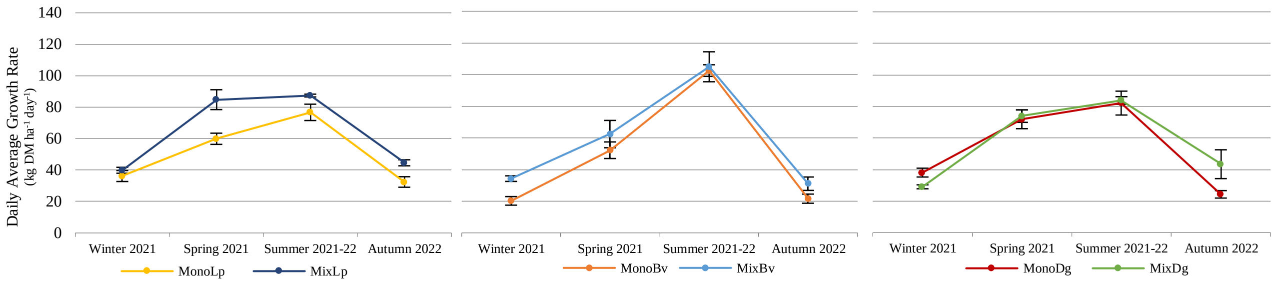

Figure 2. Average daily growth rate of MonoLp, MixLp, MonoBv, MixBv, MonoDg and MixDg in winter 2021, spring 2021, summer 2021-22 and autumn 2022. The figure was split according to the a_{LSWO} of the same species to facilitate the visualization of growth rate lines. The vertical bars indicate (±) S.E.M. within the same season for the six treatments (*p<0.05*).

Conclusions and/or Implications

The LSWOs results within mixtures showed that Lp, Bv and Dg have an overlapping LSWO for defoliation, which was wide enough to provide flexibility, and allowed the three species to be together as a functional ecosystem. The LSWO criterion allowed the monocultures and DPCS to be successfully managed. Due to the complementary traits of the studied species (i.e., winter- and summer-active spp), the mixtures presented less seasonality than monocultures. The consistency of production is of great value to farmers, since a more evenly distributed annual pasture feed resource can mitigate the negative impacts of longer periods of soil water restriction or saturation). DPCS defoliated according to LSWO can become an important alternative for grazing systems based on perennial ryegrass and clover of Temperate Humid climate as those in New Zealand.

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