



## Productive and nutritional parameters in diverse pastures composed of complementary species (*Lolium perenne* L., *Bromus valdivianus* Phil., *Dactylis glomerata* L., and *Trifolium repens* L.) under the leaf regrowth stage defoliation criterion

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### ABSTRACT

The diversification of plant species within intensively managed pastures has been proposed as an alternative to conventional pure sward systems in an effort to address climatic-driven issues of pasture-based livestock production. In a diverse pasture composed of complementary species (DPCS), individual species fulfil different agroecological niches and complement each other. It is expected that DPCS have a greater capability to tolerate and perform under increasingly extreme climate events; however, appropriate grazing management strategies for diverse pastures still need to be fully elucidated. In the present study, DPCS comprised *Lolium perenne* L., *Bromus valdivianus* Phil., *Dactylis glomerata* L., and *Trifolium repens* L. The leaf regrowth stage [LS; *L. perenne* (2.5–3.0LS), *B. valdivianus* (3.5–4.0LS) and *D. glomerata* (3.5–4.0LS)] is proposed to be an applicable method of defoliation management for DPCS due to a potential overlapping of their optimal defoliation intervals. The productive, morpho-physiological, and nutritional parameters of DPCS and their respective single-grass and *T. repens* pastures were assessed for two years under the LS criterion for defoliation. Within the DPCS, the three grass species presented a temporal overlapping of their respective LS intervals. Significant differences were found in the dry matter seasonal accumulation; DPCS exhibited seasonal asynchronous growth among species and presented an enhanced ability to withstand the ingress of volunteer species. In all pastures, the nutritional parameters were mostly influenced by season and LS criterion. *Lolium perenne*, *B. valdivianus*, and *D. glomerata* were shown to be complementary grass species, presenting growth asynchrony and an overlapping defoliation interval that optimised production. In this study, grazing management that focuses on the morpho-physiological development of grasses within a diverse pasture was successfully applied, with *B. valdivianus* LS optimal interval for defoliation being the most appropriate criterion for determining a grazing regime for DPCS.

### 1. Introduction

Increased extreme climatic events caused by climate change (i.e., higher average temperatures, precipitation variability with concentrated rainfall followed by long periods of rainfed water restriction, and elevated carbon dioxide concentrations) are changing the production dynamics of temperate grasslands (Soussana et al., 2013; Tubiello et al., 2007). New Zealand's livestock production systems are predominantly pasture-based, with 13.3 million hectares (ha) of New Zealand's land area covered in productive pastures, representing 49.6% of the

country's land area (Manaaki Whenua, 2023). The sown pastures in these areas are mostly composed of *Lolium perenne* L. and *Trifolium repens* L. and are intensively managed to achieve high levels of animal productivity (Caradus et al., 2021). Future New Zealand's pastoral systems are expected to display a detrimentally greater degree of inter-annual production variability and seasonal feed surpluses and shortages (Keller et al., 2021; Lee et al., 2013). In addition to the increased climate-driven seasonality of production, in these pasture-based systems, unadjusted rotational grazing interval and over-grazing are a problem causing pugging damage, soil erosion, bare soil areas (Brook

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and Hay, 2001; Brougham, 2011; Greenwood and McKenzie, 2001) and depletion of plant reserves that support regrowth after grazing (Thornton et al., 2000). In the long term, these imbalances can lead to a decrease in the population of sown species, increasing the susceptibility to weed invasion, and reducing productivity and quality (Briske and Richards, 1995).

Species diversity has been proposed as an alternative to conventional and less complex (pure stand or binary) pasture systems that are intensively managed in an effort to address climate-driven issues facing productive grassland systems [see meta-analysis by Isbell et al. (2015)]. Species diversity can contribute to stabilising production due to growth asynchrony and potential facilitation processes between species (Lüscher et al., 2022). For instance, in naturalised and semi-naturalised extensive pastures, the presence of several species is common, and in these ecosystems, each species fulfils specific functional roles (Descalzi et al., 2019; Kemp and López, 2016). However, on a highly-productive pasture-based livestock farm, many challenges emerge with establishing and managing a multi-species system. These challenges originate from the inherited characteristics of forage species when coping with disturbance, competition, and stress (Grime, 1974). For instance, not all species present in extensively managed grasslands can cope with practices of intensive systems, such as fertilisation (Hautier et al., 2009) and grazing frequency and intensity (Barreta et al., 2023; Fischer et al., 2019) - these practices contribute to the competitive exclusion of some less adapted species (Isselstein et al., 2007). Consequentially, intensive management practices may favour or prejudice a given species, resulting in the loss of sown species' population density and increased opportunity for volunteer species to overtake (Tracy and Sanderson, 2004).

The addition *per se* of different species in a pasture can lead to a natural 'drift' in the population of some species caused by inter-species niche competition (Sanderson et al., 2007). Therefore, the choice of species (Komainda et al., 2020; Küchenmeister et al., 2012) and the complementarity of functional groups and specific traits among these chosen species are fundamental for the success of a diverse pasture [see definition of 'complementarity' by Barry et al. (2019)]. Diversification of species based on their complementarity may be appropriate for dealing with climatic-driven problems. Yet, research is needed to determine the progression or degradation of these diverse pastures under intensive grazing management practices.

In a diverse pasture composed of complementary species, individual species fulfil different agroecological niches (Oliveira et al., 2023) and complement each other to enhance ecological diversity through spatial and temporal resource partitioning and abiotic facilitation. The different functional roles (i.e., root exploitation or the season of activity) and the trait-mediate plant responses to disturbance, competition, and stress will dictate species' succession and productivity. In the present study, a diverse pasture is composed of *L. perenne* [shallow rooted, winter-active, waterlogging tolerator, competitor (Crush et al., 2010; Grime et al., 1988; Hoekstra et al., 2014; Stewart, 2006)], *Bromus valdivianus* Phil. [deep rooted, summer-active, drought tolerator, facilitator via hydraulic lift phenomena (García-Favre et al., 2021; López et al., 1997; Meier et al., 2024; Oliveira et al., 2024; Stewart, 1996)], *Dactylis glomerata* L. [drought resistant, year-round-active, competitor and facilitator via hydraulic lift phenomena (Grime et al., 1988; Gross et al., 2007; Oliveira et al., 2024; Volaire, 1995)] and *T. repens* [shallow rooted, high nutritive value, biological N-fixator (Hoekstra et al., 2014; Widdup and Barrett, 2011)].

The challenge of diversification is enhanced by the fact that animal intake requirements often dictate the conventional defoliation management in pasture-based systems (Roche et al., 2017), where pasture is allocated according to availability, which, in turn, is dictated by climatic conditions for growth. Currently, the morpho-physiological development stage of the pasture species is not often taken into consideration when determining the defoliation regime (Donaghy et al., 2021). In this sense, a grazing criterion based on the morpho-physiological maturity of the plants was developed, relating growth stages to the lamina

appearance of individual tillers (Fulkerson and Donaghy, 2001).

The leaf regrowth stage (LS) criterion for defoliation is an interval that ranges from a minimum LS value associated with the replenishment of water-soluble carbohydrate reserves in the stubble and a maximum LS value associated with the onset of leaf senescence (see 'Material and Methods' section for detailed information) (Chapman et al., 2011; Fulkerson and Donaghy, 2001; Fulkerson and Slack, 1994; Gatti et al., 2016; Ordóñez et al., 2021; Turner et al., 2006). Previous studies in Australia, Chile, and New Zealand have confirmed the relation between the water-soluble carbohydrate reserves and the optimal LS targets for *L. perenne* [2.0–3.0 LS (Fulkerson and Donaghy, 2001; Lee et al., 2008)], *B. valdivianus* [3.5–4.0 LS, (García-Favre et al., 2022; Ordóñez et al., 2021)] and *D. glomerata* [around 4.0 LS (Gatti et al., 2016; Turner et al., 2006)]. The LS criterion is endorsed by New Zealand, Chile and Australia's research and advisory institutions (Dairy Australia, n.d.; DairyNZ, n.d.; Proyecto FIA, 2007) and utilised by some farmers as an attempt to mitigate the negative effects of defoliation rotation practices that are purely animal- or timeframe-focused (Clarke et al., 2021; Donaghy et al., 2021).

This investigation advances plant-focused defoliation management as a key factor in the performance of intensively managed diverse pastures and introduces LS as a potentially suitable management criterion. If the individual species present a similar chrono-physiological development regrowth pattern, resulting in a common window of opportunity among species for optimal defoliation in the following grazing cycles, positive outcomes in terms of resilience and short-term persistence are expected to be seen, as defoliation disturbance will not favour or prejudice any species. The three grass species are hypothesised to present an overlap of their individual leaf regrowth stage interval for defoliation, resulting in a common window of opportunity for optimal defoliation of all grass species in diverse pastures comprising *L. perenne*, *B. valdivianus* and *D. glomerata* (see 'graphic abstract'). In contrast to the single-grass pastures, it is expected that the proposed diverse pasture presents complementarity and, consequentially, less seasonality, higher short-term persistence, and similar nutritive value. The current study evaluated the LS of different species present in diverse and single-grass pastures, their seasonal and annual herbage mass accumulation, growth patterns, botanical and morphological composition, and nutritive value parameters compared to single-grass pastures.

## 2. Material and methods

### 2.1. Site and experimental setup

The study was undertaken at Massey University's Dairy 1 (Palmerston North, Manawatu, New Zealand) from 15 June 2021–14 June 2023, with the approval of the Massey University Animal Ethics Committee (Approval number 21/24). The soil type is Manawatu silt loam over sand (Landcare Research National Soil Data Base, Lab. No SB10036). Soil samples were collected at three depths for chemical fertility analysis on 26 February 2021 (Table 1). The area received annual maintenance fertilisation on 1 March 2021 and 17 March 2022, at 500 kg (kg) ha<sup>-1</sup> and 400 kg ha<sup>-1</sup> of superphosphate [0 % nitrogen (N), 22.5 % phosphorus (P), 0 % potassium (K), 27.5 % sulphur (S) and 50 % calcium (Ca)]. Post-grazing N fertilisations were applied as urea (46 % N) in July 2021, November 2021, January 2022, August 2022, November 2022, and February 2023, each at 30 kg N ha<sup>-1</sup>.

The climate is classified as Marine Climate - Cfb [Köppen-Geiger's climate classification (Beck et al., 2018)]. Monthly rainfall, ground minimum temperature, and air minimum and maximum temperature are presented in Fig. 1 [NIWA/AgResearch Weather Station, ~800 metres (m) from the field site].

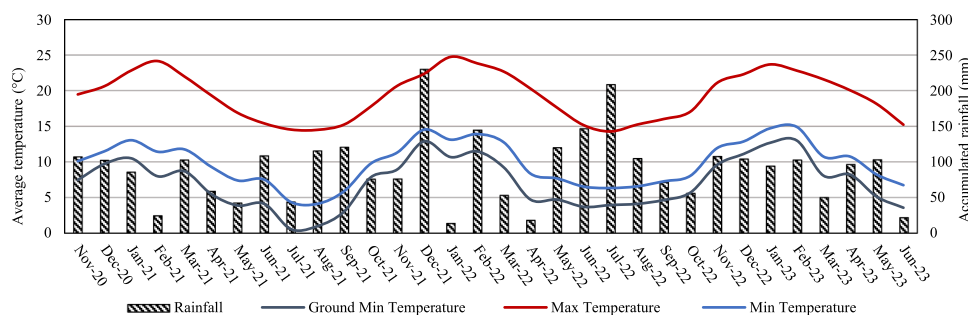
On 24 November 2020, the area was sprayed out with N-(phosphonomethyl) glycine (glyphosate WeedMaster® G360) at 6 litres (L) ha<sup>-1</sup> and Thifensulfuron-methyl (Harmony® 50 SG) at 30 g (g) ha<sup>-1</sup> to control the weed seed population. From 10–18 December 2020, the area

**Table 1**

Soil chemical characteristics at three depths [0–15, 15–30, 50–65 centimetres (cm)] of the experimental area on 26 February 2021.

Soil Layer	pH	OM	Olsen P	K	Ca	Mg	CEC	Tot. Base Sat.	Ext. Org. S	C/N
cm	CaCl <sub>2</sub>	%	mg L <sup>-1</sup>	ME 100 g <sup>-1</sup>				%	mg kg <sup>-1</sup>	
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), Olsen phosphorus (Olsen P), potassium (K), calcium (Ca), magnesium (Mg), cation exchange capacity (CEC), total base saturation (Tot. Base Sat.), extractable organic sulfur (Ext. Org. S), carbon:nitrogen ratio (C/N).



**Fig. 1.** Monthly accumulated rainfall (mm), monthly averaged ground minimum (°C), and monthly averaged air maximum and minimum temperatures (°C) during the establishment and experimental period (November 2020 to June 2023). Bars indicate accumulated rainfall, lines indicate averaged temperatures.

was ploughed, power-harrowed and levelled. On 18 December 2020, mixtures (Mix) of *L. perenne* (Lp), *B. valdivianus* (Bv), and *D. glomerata* (Dg) and their respective single grass pastures (Mono) were established. *Trifolium repens* (Tr) was sown at the same rate in all pastures (Table 2). In December 2020, the area was irrigated for one day with a mobile gun sprinkler to ensure a successful establishment. Following this, the pastures were rainfed.

The study was arranged in a randomised complete block design, with three blocks (n = 3), each with the six experimental treatments in 20 m by 20 m plots. From June 2021 onwards, the seasonal interval for defoliation was determined according to the LS criterion of each species and applied to the single-grass pastures and mixtures (hereafter referred to as diverse pastures of complementary species – DPCS; Table 2). These resulted in six experimental treatments: MonoLp (defoliated at *L. perenne* LS), MonoBv (defoliated at *B. valdivianus* LS), MonoDg (defoliated at *D. glomerata* LS), MixLp (defoliated at *L. perenne* LS), MixBv (defoliated at *B. valdivianus* LS), MixDg (defoliated at *D. glomerata* LS). In the MixLp, for instance, the *L. perenne* plants are the targeted species (α) and thus justified defoliation based on the *L. perenne* LS

interval, whereas the *B. valdivianus*, *D. glomerata*, and *T. repens* neighbouring plants are the companion species.

Massey University’s Dairy 1 herd is mostly comprised of crossbred cows (Jersey with Holstein-Friesian) ranging from 2 to 8 years old (herd average = 4.2 years old), with the whole herd calving season concentrated over late July and August. The farm runs as a year-round pasture-based system, with maize silage being supplied as a complement when pasture growth rates are below herd dry matter (DM) requirements. Within this study’s experimental area, dairy cows grazed the plots during the day from around 07 AM to 05 PM, on a one-day grazing event basis (10–15 animals per plot depending on herbage allowance; animal average = 13 kg DM animal<sup>-1</sup>) until the pasture reached a stubble height of 5–8 centimetres (cm) from ground level (Hodgson, 1990). Pre- and post-grazing data were collected over eight agricultural seasons (winter 2021, spring 2021, summer 2021–22, autumn 2022, winter 2022, spring 2022, summer 2022–23, and autumn 2023), with the first four seasons comprising year 1 and the last four seasons comprising year 2.

**Table 2**

Establishment description of the four pastures species composition and sowing rate, cultivars, resulting experimental treatments, and seasonal leaf regrowth stage (LS) optimal interval for defoliation.

Pasture composition (sowing rates, kg ha <sup>-1</sup> ; seeds m <sup>-2</sup> )	Cultivars	Experimental treatment	Species role*	Winter Autumn (LS interval)	Summer (LS interval)	Spring <sup>a</sup> (LS interval)
Lp + Tr (20 + 6; 936 + 1130)	<i>L. perenne</i> cv. Maxsyn + <i>T. repens</i> cv. Weka	MonoLp	Lp α	2.5 – 3.0		2.25 – 2.75
Bv + Tr (50 + 6; 684 + 1130)	<i>B. valdivianus</i> cv. Bareno + <i>T. repens</i> cv. Weka	MonoBv	Bv α	3.5 – 4.0		3.0 – 3.5
Dg + Tr (15 + 6; 1559 + 1130)	<i>D. glomerata</i> cv. Greenly II + <i>T. repens</i> cv. Weka	MonoDg	Dg α	3.5 – 4.0		3.0 – 3.5
Lp + Bv + Dg + Tr (10 + 15 + 12 + 6; 468 + 205 + 1247 + 1130)	<i>L. perenne</i> cv. Maxsyn + <i>B. valdivianus</i> cv. Bareno + <i>D. glomerata</i> cv. Greenly II + <i>T. repens</i> cv. Weka	MixLp	Lp α	2.5 – 3.0		2.25 – 2.75
		MixBv	Bv α Bv CSpp Dg CSpp Lp CSpp	3.5 – 4.0		3.0 – 3.5
		MixDg	Dg α Lp CSpp Bv CSpp	3.5 – 4.0		3.0 – 3.5

Lp: *L. perenne*, Bv: *B. valdivianus*, Dg: *D. glomerata*, Tr: *T. repens*; α: targeted species that determined each defoliation event; CSpp: companion species; LS: leaf regrowth stage;

\* All pastures have *T. repens* in their composition.

<sup>a</sup> LS interval was slightly anticipated to alleviate detriments in pasture quality due to flowering stems.

## 2.2. Defoliation criterion

The leaf regrowth stage is a practical criterion that requires the assessment of individual tillers in the field, and it is measured as the sum of representative quarters of live leaves in a tiller (Fig. 2). To assess the LS of *L. perenne* tillers, for example, it is necessary to know that this species grows one leaf at a time. Often, the tiller will present one residual leaf (oldest leaf) from the prior defoliation that may be accounted for if representing at least 0.50 LS. Subsequently, any new fully expanded leaves (visible ligule) are accounted individually as 1.00 LS. Lastly, any expanding leaf is accounted as per the relative size it represents in comparison to the newest fully expanded leaf (closest leaf) and recorded as either 0.25, 0.50 or 0.75 LS. The LS values of each leaf are then added, and the resulting sum is the LS for that given *L. perenne* tiller (Fig. 2). In the present study, the LS targets for *L. perenne* are 2.5–3.0 LS in summer, autumn and winter, and 2.25–2.75 LS in spring, and for *B. valdivianus* and *D. glomerata* are 3.5–4.0 LS in summer, autumn and winter, and 3.0–3.5 LS in spring.

## 2.3. Leaf regrowth stage, apparent dry matter accumulation, botanical and morphological composition

The LS of the target species ( $\alpha$ ) and companion species was assessed on nine randomly chosen mature tillers of each grass species per plot on the day before each grazing event (García-Favre et al., 2022). The herbage mass was measured pre- and post-grazing by cutting to ground level three randomly placed 0.1 m<sup>2</sup> quadrats in each plot. Three samples were collected per plot, with quadrats randomly placed in areas representing the average sward status prior to and following defoliation (Frame, 1993). Post-grazing samples were washed when necessary to eliminate residual detritus and soil. The samples were dried for at least 72 h in a forced-air oven at 60 °C and weighed to determine their DM. The apparent accumulated DM (season or year) was the sum of apparent herbage growth of different grazing events over a given period, calculated as the difference between the pre-grazing DM at the current grazing event 'n' and the post-grazing DM of the previous grazing event 'n-1' (Campbell, 2009). The daily growth rate of the pasture was calculated by dividing the apparent accumulated DM by the number of days between consecutive grazing events. The average daily growth rate per season was calculated as a 'weighted mean' balanced by the number of days of each daily growth rate from different grazing events ('n; n + 1; n + 2; etc.') within a given season.

The botanical and morphological composition were assessed at every second grazing event. Two sub-samples of approximately 12.5 % from

two out of three herbage mass samples were randomly taken to be fractionated into individual components of *L. perenne*, *B. valdivianus*, *D. glomerata*, *T. repens*, dead material and volunteer species (every other species that was not the original sown species of each treatment) for the botanical composition; and lamina, sheath (leaf sheath + stem), and flower (above flag leaf) of *L. perenne*, *B. valdivianus*, *D. glomerata* for the morphological composition (Oliveira et al., 2023). The fractionated materials were oven-dried at 60 Celsius degrees (°C) for at least 72 h and weighed to determine the percentage of each botanical and morphological component on a DM basis.

## 2.4. Nutritive value

Throughout the study, the experimental treatments were defoliated on different dates because of the applied defoliation criteria, which were related to each respective targeted species LS. Once a season and within the same grazing rotation time frame of the different treatments, the nutritional parameters of the apparent harvestable herbage were assessed. To reflect the defoliation management that sampling purports to simulate, at least ten sub-samples were cut down to 5 cm residual height in the early morning of the grazing event day (Frame, 1993), avoiding the warmer hours of the day. These sub-samples generated one composite sample of approximately 150 g per plot in each block. The sample was instantly sealed in a zip-lock bag, packed into a thermal bag, refrigerated at 4 °C (Atkin and Tjoelker, 2003) and sent on the same day for analysis at Hill Laboratories Ltd., Hamilton, New Zealand. Hill Laboratories participates in both national (International Accreditation New Zealand - IANZ) and international (Association of American Feed Control Officials - AAFCO) inter-laboratory comparison programmes (ILCP) for the feed test reference methods.

The total nitrogen content was estimated by near-infrared reflectance spectroscopy (NIRS), calibration based on Dumas combustion (Chang and Zhang, 2017) and corrected to a fully dry basis assuming 5 % residual moisture. The crude protein content (CP) was obtained by multiplying N by 6.25. The organic matter digestibility *in-vivo* (OMD) was determined using AFIA (Australian Fodder Industry Association) *in-vitro* Pepsin-Cellulase procedure and derived as *in-vivo* using a linear regression based on calibration samples from Lincoln University, Lincoln, New Zealand. The metabolisable energy content (ME) was calculated from OMD from AFIA Method 7 R (modified), using AFRC (Agriculture & Food Research Council, UK) and Lincoln University's standard formulae. The neutral detergent fibre content (NDF) was estimated by NIRS, calibration based on NDF by NFTA method. The lignin content (LGN) was Estimated by NIRS, calibration based on acid detergent extraction followed by treatment with 72 % sulphuric acid in the Ankom Daisy Incubator. The non-structural carbohydrates content (NSC) was calculated as the difference between 100 % and the sum of CP, ash, crude fat, and NDF percentages.

The results by NIRS are obtained using samples dried at 62 °C and grounded to 1 millimetre-sized particles. Measurement results are calculated on a dry matter basis and calibrated using a multipurpose analyser (MPA II NIR - Bruker Corporation, Billerica, Massachusetts, USA). The algorithm used to construct the calibration from NIRS data (NIR spectra) firstly pre-processes the NIR spectra using a first derivative, the Savitzky-Golay smoothing algorithm (Savitzky and Golay, 1964), vector normalisation and wavelet transformation. Then, the NIR spectra originating from the multipurpose analyser instrument are transformed into a single stream using calibrated transformation matrixes. Following, the transformed NIR spectra associated with each sample pass through a bootstrap re-sampling model, which uses a partial least square data reduction filter with 20 components, a local weighted Euclidean distance (500 nearest neighbours) and a support vector machine model. The bootstrap re-samplings created 20 different random calibrations from the dataset for each on-the-fly prediction. Lastly, the mean of the 20 different predictions is reported as the measurement results.

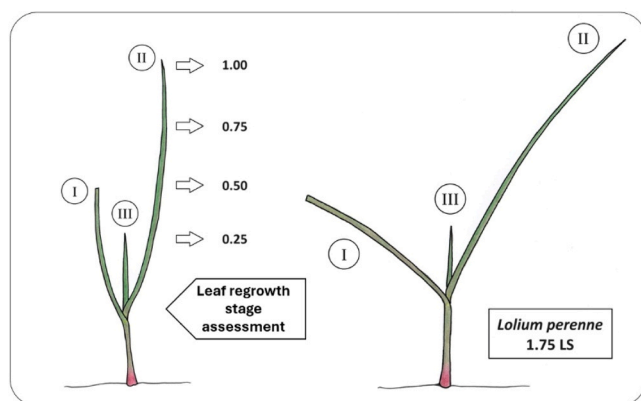


Fig. 2. Conceptual image of the leaf regrowth stage (LS) assessment in the same tiller of *Lolium perenne*. The tiller presents a residue leaf from previous grazing (I), and two new leaves, one fully expanded (II) and one expanding (III). Utilising the concept of quarters to define the stages (indicated by arrows), this tiller is at 1.75 LS, resulting from the sum of leaves I, II and III's LS values.

2.5. Statistical analyses

Statistical analyses were performed using SAS v 9.4 (SAS Institute Inc, Cary, NC, US) and Statistica™ v 14.0.0.15 (TIBCO Software Inc, Hamburg, DE). The data were examined for normal distribution using the Shapiro-Wilk test, finding that all dependent variables followed a normal distribution.

Datasets of annual and seasonal apparent DM accumulation, average daily growth rate, seasonal percentage of individual components in the botanical composition, and seasonal LS per species were analysed with the following mixed model using the GLIMMIX procedure:

$$Y_{ij} = \mu + P_i + B_j + e_{ij} \tag{1}$$

Where  $Y_{ij}$  is the dependent variable,  $P_i$  is the fixed effect of  $i^{th}$  experimental treatment (MonoLp, MonoBv, MonoDg, MixLp, MixBv, MixDg),  $B_j$  is the random effect of the  $j^{th}$  block, and  $e_{ij}$  is the random residual assumed with mean zero and variance  $\sigma_e^2$ .

Least square means and standard errors for the fixed effect were obtained and used for multiple mean comparisons using Fisher's least significant difference test. Significant differences between the least squares means were declared at  $p \leq 0.05$ . Datasets composed of percentage values were transformed into arcsine. Results related to the transformed variables were subsequently back-transformed to the original scale and presented in the results section.

The nutritive value of each pasture treatment was modelled across successive seasons for two years using polynomial regression models of the nutritive value on the day of the year. The choice of polynomial order was guided based on the goodness of fit between the regression curve and the data points, with considerations of the R-squared ( $R^2$ ) and the relative prediction error (RPE; Eq. 2). The RPE was used to evaluate the fitness of the predicted nutritive value and defined as the positive square root of the mean square prediction error (MSPE; Eq. 3) and expressed as a percentage of the mean of the actual values (O'Neill et al., 2012):

$$RPE = \frac{\sqrt{MSPE}}{\mu_{A_i}} \times 100 \tag{2}$$

$$MSPE = \frac{1}{n} \sum_{i=1}^n (A_i - P_i)^2 \tag{3}$$

Where A represents the actual observed values for nutritive value, and P represents the predicted values. An RPE value lower than 10 % indicates

satisfactory prediction, whereas an RPE between 10 % and 20 % indicates a relatively acceptable prediction, and an RPE greater than 20 % indicates poor prediction (Fuentes-Pila et al., 1996).

3. Results

3.1. Leaf regrowth stage

In the first year, the defoliation criteria applied resulted in 11 grazing events for MonoLp and MixLp, 10 for MonoBv and MixBv, and 9 for MonoDg and MixDg, and in the second year, the same defoliation criteria resulted in 10 grazing events for all six treatments. During these two years, the targeted seasonal LS intervals for defoliation according to each  $\alpha$  species were achieved in the six treatments. In the MixLp, the companion *B. valdivianus* was defoliated earlier than recommended in winter 2021 and summer 2021–22 (< 3.5 LS in Table 3; see Table 2 and 'defoliation criteria' section for recommended LS intervals), while the companion *D. glomerata* was mostly defoliated earlier than recommended during the four seasons of the first year, and in the second-year winter (winter 2022) (< 3.5 LS). During both years, the MixBv had most companion species defoliated within their respective LS intervals, except for autumn and winter 2022, when the companion *D. glomerata* was defoliated slightly earlier than recommended. In the MixDg, the companion *L. perenne* was defoliated later than its recommended LS in both winters (> 3.0 LS), while the companion *B. valdivianus* was defoliated later than recommended during the first-year winter (winter 2021) (> 4.0 LS) and just at its LS interval maximum point limit (4.0 LS) in the second-year winter (winter 2022).

Significant differences in the LS of the same species within each different mixture (*L. perenne* in MixLp, MixBv, and MixDg, *B. valdivianus* within MixLp, MixBv, and MixDg and *D. glomerata* within MixLp, MixBv, and MixDg) were found in both winters and summers (Table 3;  $p < 0.05$ ). Overall, when differences were found, higher LS values of all species were present in MixDg. In winter 2021 and 2022, the *L. perenne* LS was higher in MixDg than in MixLp and MixBv, and the *B. valdivianus* LS was higher in MixDg than in MixLp and MixBv. In winter 2021, the *D. glomerata* LS was higher in MixDg and MixBv than in MixLp, while in winter 2022, it was higher in MixDg than MixLp and MixBv. In summer 2021–22, *L. perenne* LS was higher in MixDg than in MixLp and MixBv, while in summer 2022–23, MixDg and MixLp were higher than MixBv. In summer 2021–22, *B. valdivianus* LS was higher in MixDg and MixBv than MixLp. In summer 2021–22, the *D. glomerata* LS was higher in MixDg than in MixLp and MixBv, while in summer 2022–23, it was

Table 3

Seasonal average leaf regrowth stage (LS) of the same species within each of the three mixtures prior to defoliation (*L. perenne* in MixLp, MixBv and MixDg; *B. valdivianus* in MixLp, MixBv and MixDg; *D. glomerata* in MixLp, MixBv and MixDg) in winter 2021, spring 2021, summer 2021–22, autumn 2022, winter 2022, spring 2022 and summer 2022–23.

Species within each mixture	Winter 2021	Spring 2021	Summer 2021-22	Autumn 2022	Winter 2022	Spring 2022	Summer 2022-23	Autumn 2023
<i>L. perenne</i> LS in								
MixLp	2.6c (±0.006)	2.5 (±0.041)	2.6 b (±0.034)	2.7 (±0.021)	2.6c (±0.048)	2.5 (±0.075)	2.8 a (±0.029)	2.7 (±0.042)
MixBv	3.0 b (±0.017)	2.5 (±0.032)	2.6 b (±0.019)	2.6 (±0.045)	2.8 b (±0.023)	2.6 (±0.035)	2.7 b (±0.042)	2.9 (±0.039)
MixDg	3.3 a (±0.035)	2.7 (±0.057)	2.9 a (±0.068)	2.7 (±0.061)	3.1 a (±0.048)	2.5 (±0.039)	2.9 a (±0.021)	2.7 (±0.060)
Significance	***	NS	*	NS	**	NS	*	NS
<i>B. valdivianus</i> LS in								
MixLp	3.3c (±0.051)	3.1 (±0.021)	3.2 b (±0.052)	3.6 (±0.024)	3.5c (±0.056)	3.3 (±0.041)	3.7 (±0.074)	3.6 (±0.102)
MixBv	4.0 b (±0.008)	3.0 (±0.037)	3.6 a (±0.029)	3.6 (±0.027)	3.8 b (±0.016)	3.4 (±0.042)	3.7 (±0.000)	3.7 (±0.012)
MixDg	4.2 a (±0.028)	3.2 (±0.077)	3.6 a (±0.141)	3.7 (±0.038)	4.0 a (±0.037)	3.4 (±0.008)	3.9 (±0.107)	3.6 (±0.127)
Significance	****	NS	*	NS	**	NS	NS	NS
<i>D. glomerata</i> LS in								
MixLp	3.2 b (±0.003)	2.9 (±0.044)	3.2c (±0.038)	3.4 (±0.075)	3.0c (±0.034)	3.2 (±0.065)	3.5 b (±0.055)	3.5 (±0.049)
MixBv	3.7 a (±0.158)	3.1 (±0.070)	3.5 b (±0.021)	3.4 (±0.025)	3.3 b (±0.084)	3.4 (±0.028)	3.7 a (±0.003)	3.5 (±0.075)
MixDg	3.7 a (±0.029)	3.2 (±0.088)	3.6 a (±0.044)	3.5 (±0.052)	3.6 a (±0.046)	3.1 (±0.033)	3.5 b (±0.016)	3.5 (±0.013)
Significance	*	NS	**	NS	**	NS	*	NS

Letters that differ within columns for the same species indicate values that are significantly different; NS=non-significant,

\*  $p < 0.05$ ,

\*\*  $p < 0.01$ ,

\*\*\*  $p < 0.001$ ; following each species LS least square mean is the (±) standard error of the mean.

**Table 4**  
Effect of defoliation criteria on experimental treatments (MonoLp, MonoBv, MonoDg, MixLp, MixBv, MixDg) on seasonal (winter 2021, spring 2021, summer 2021–22, autumn 2022, winter 2022, spring 2022, summer 2022–23 and autumn 2023) and annual (1st and 2nd year) dry matter accumulation (t DM ha<sup>-1</sup>).

Treatment	Apparent DM accumulation (t DM ha <sup>-1</sup> )										
	Winter 2021	Spring 2021	Summer 2021–22	Autumn 2022	1st Year	Winter 2022	Spring 2022	Summer 2022–23	Autumn 2023	2nd Year	
MonoLp	3.35 a (±0.32)	5.45 bc (±0.37)	6.90c (±0.36)	2.98 ab (±0.49)	18.67 (±1.08)	2.56 abc (±0.16)	6.30c (±0.26)	6.52 (±0.47)	4.25 (±0.39)	19.62c (±0.21)	
MonoBv	1.86c (±0.19)	4.75c (±0.54)	9.23 ab (±0.34)	1.99 b (±0.19)	17.82 (±0.79)	2.23 bc (±0.21)	6.57c (±0.34)	7.98 (±0.81)	4.30 (±0.56)	21.12 b (±0.30)	
MonoDg	3.50 a (±0.24)	6.54 ab (±1.16)	7.40c (±0.69)	2.24 b (±0.18)	19.68 (±2.15)	1.69c (±0.25)	8.07 a (±0.29)	6.61 (±0.35)	5.86 (±0.68)	22.22 ab (±1.10)	
MixLp	3.67 a (±0.18)	7.71 a (±0.49)	7.86 abc (±0.06)	4.11 a (±0.35)	23.35 (±0.47)	2.81 ab (±0.16)	7.81 ab (±0.12)	6.72 (±0.73)	3.66 (±0.24)	21.00 bc (±0.72)	
MixBv	3.15 ab (±0.14)	5.68 bc (±0.74)	9.44 a (±0.90)	2.86 ab (±0.34)	21.13 (±1.80)	3.21 a (±0.11)	6.67 bc (±0.44)	6.73 (±0.18)	5.45 (±0.13)	22.07 ab (±0.29)	
MixDg	2.68 b (±0.07)	6.72 ab (±0.38)	7.55 bc (±0.27)	4.00 a (±0.81)	20.94 (±0.70)	2.90 ab (±0.55)	7.96 a (±0.48)	7.20 (±0.29)	4.88 (±0.68)	22.94 a (±0.39)	
Signific.	***	*	*	*	NS	*	*	NS	NS	**	
SEM season	0.20	0.50	0.55	0.42	1.17	0.29	0.36	0.31	0.53	0.45	

Letters that differ within columns indicate values that are significantly different; NS=non-significant.

\* p < 0.05,

\*\* p < 0.01,

\*\*\* p < 0.001;

following each experimental treatment, the least square mean is the (±) standard error of the mean; SEM season: standard error of the mean per season.

higher in MixBv than MixLp and MixDg.

### 3.2. Annual and seasonal apparent dry matter accumulation and seasonal growth rates

In the first year, the total accumulated pasture herbage mass did not differ (p > 0.05) between all treatments, with an average annual accumulation of 20.26 (±1.17) tonnes (t) DM ha<sup>-1</sup>. Nonetheless, in the first year, significant differences were found in the seasonal accumulated DM (Table 4). In the second year, significant differences in the total herbage mass accumulation were found (p < 0.01) - MixDg was greater than MonoBv, MixLp, and MonoLp but similar to MonoDg and MixBv; MonoLp accumulated 15 % less than MixDg. Significant seasonal differences were found in all first-year seasons and the second year, in winter 2022 and spring 2022 (Table 4 and Fig. 3). The MixLp had greater growth rates than MonoLp in spring 2021 and spring 2022 (41.5 % and 22.8 %, respectively; p < 0.05), summer 2021–22 (13.9 %; p < 0.05), and autumn 2022 (37.7 %; p < 0.05) (Fig. 3). The MixBv had greater growth rates than MonoBv in winter 2021 (69.6 %; p < 0.05), winter 2022 (41.2 %; p < 0.001), and autumn 2022 (43.8 %; p < 0.05). MixDg presented a greater growth rate in autumn 2022 (78.7 %; p < 0.05) and winter 2022 (71.8 %; p < 0.05) and lower growth in winter 2021 than MonoDg (23.6 %; p < 0.001).

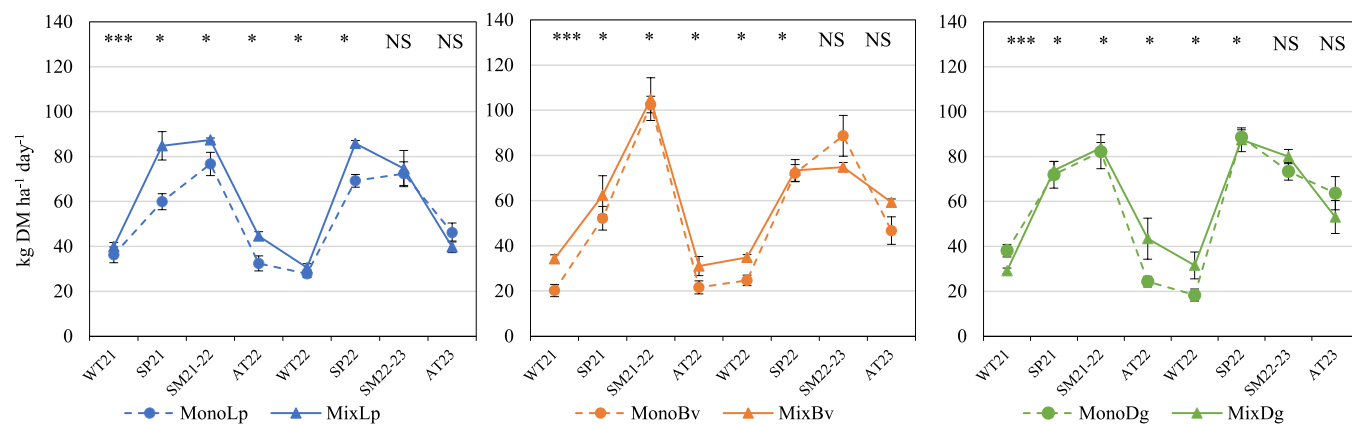
### 3.3. Botanical and morphological composition

In both years, the *L. perenne* percentage within the MonoLp and the *B. valdivianus* percentage within the MonoBv treatment presented a consistent and marked decrease from winter to summer (Fig. 4). In MonoLp, from winter to summer, the *L. perenne* percentage dropped from 77 % to 22 % in year 1 and from 73 % to 44 % in year 2. In MonoBv, *B. valdivianus* percentage dropped from 61 % to 18 % in year 1 and from 65 % to 23 % in year 2. Conversely, in summer 2021–22, MonoLp had a significant peak at 33 % of volunteer plant species' proportion in the sward, followed by MonoBv peak of 24 % (p < 0.001). MonoBv also had significant peaks of volunteer plant species in the sward (p < 0.05) in spring 2022 (19 %) and summer 2022–23 (30 %). In MonoDg, the *D. glomerata* percentage presented variations with no apparent seasonal consistency.

In MixLp, the *L. perenne* percentage was significantly higher than in the other two mixtures in both springs, when it dominated approximately 46 % of the sward, compared to approximately 30 % in MixBv and MixDg (p < 0.001). Throughout seasons, the proportion of *L. perenne* in the mixtures remained overall around 30 %. The *B. valdivianus* proportion in the mixtures markedly decreased from 30 % in winter 2021–5 % in summer 2022–23, remaining between 5 % and 10 % for the rest of the studied period. In contrast, the *D. glomerata* proportion consistently increased from winter 2021 until autumn 2023. In MixDg throughout the study, the average *D. glomerata* percentage in the sward became eight times greater, MixBv six times, and MixLp five times.

Across seasons, MixLp, MixBv, and MixDg had at most 4 % of the sward dominated by volunteer species. In summer 2021–22, MonoLp and MonoBv presented the highest proportion of volunteer species in the sward (average of 28 %; p < 0.001). In winter 2022, MonoDg presented the highest proportion of volunteer species in the sward (20 %; p < 0.001). In spring 2022, summer 2022–23, and autumn 2023, MonoBv presented the highest proportion of volunteer species in the sward (19 %, 30 %, and 14 %, respectively; p < 0.05). In both winters, MonoDg had the highest *T. repens* presence in the sward (average of 20 %; p < 0.01), and in summer 2021–22, MonoBv had the highest *T. repens* presence in the sward (36 %; p < 0.001).

The morphological composition also varied with season. In MonoLp, the lamina: sheath ratio remained stable throughout the eight seasons, with the *L. perenne* plants comprising approximately 70 % of laminas. In MonoBv, the morphological composition significantly changed from



**Fig. 3.** Average daily growth rate ( $\text{kg DM ha}^{-1} \text{ day}^{-1}$ ) of the treatments (MonoLp - dashed blue line and blue circles, MixLp - solid blue line and blue triangles, MonoBv - dashed orange line and orange circles, MixBv - solid orange line and orange triangles, MonoDg - dashed green line and green circles, and MixDg - solid green line and green triangles) in winter 2021 (WT21), spring 2021 (SP21), summer 2021–22 (SM21–22), autumn 2022 (AT22), winter 2022 (WT22), spring 2022 (SP22), summer 2022–23 (SM22–23) and autumn 2023 (AT23). The figure was split according to the  $\alpha$ LS of the same species to facilitate the visualisation of growth rate lines. Levels of significance are indicated in the graphs per season as NS=non-significant, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ . The vertical bars indicate ( $\pm$ ) S.E.M. within the same season for the six treatments.

winter to spring in both years, when the lamina proportion dropped from 73 % to 26 % in the first year and from 75 % to 47 % in the second year, and the sheath and flower proportion increased from 27 % to 74 % in the first year and from 25 % to 53 % in the second year. In MonoDg, a similar fluctuation in the morphological composition was also observed towards spring, with an increased presence of sheath and flowers (Fig. 5). Within the mixtures, a more significant presence of sheath and flowers during spring 2021 was observed in MixDg (71 %), followed by MixBv (52 %) and then MixLp (39 %).

### 3.4. Nutritive value

In all treatments, the polynomial regression models for ME and OMD provided a good fit to the data ( $R^2$ -values  $> 0.7$ ) and a satisfactory prediction of the model (RPE values  $< 10$  %). In MonoLp, MonoBv, MixLp, and MixBv, ME peaked at an average of  $12 \text{ MJ kg}^{-1} \text{ DM}$  in winter and dropped to approximately  $10 \text{ MJ kg}^{-1} \text{ DM}$  in the transition from spring to summer. In MonoDg and MixDg, ME values were lower than  $11 \text{ MJ kg}^{-1} \text{ DM}$  in summer, spring, and most of autumn. For OMD, values lower than 80 % were observed in the transition from spring to summer in MonoLp, MonoBv, MixLp, and MixBv. In MonoDg, the OMD values were overall lower than 80 % during the four seasons and in MixDg during spring, summer, and autumn (Fig. 6).

The polynomial regression models for CP in MonoLp, MonoBv, MixLp, and MixBv provided a good fit to the data ( $R^2$ -values  $> 0.7$ ) and satisfactory prediction of the model (RPE values  $< 10$  %). For MonoDg and MixDg, the polynomial regression models did not present a desirable fit to the CP data ( $R^2$ -values  $< 0.7$ ) and only provided a relatively acceptable prediction of the model (RPE values between 10 % and 20 %). Overall, the numerically lowest values of CP were found during spring, with the lowest values in MonoLp. The highest predicted values of CP were found on the transition from autumn to winter when MonoLp, MonoBv, MixLp, and MixBv presented CP values higher than 25 %. The polynomial regression models for NDF of MonoLp, MonoDg, MixLp and MixDg provided a good fit to the data ( $R^2$ -values  $> 0.7$ ) and satisfactory prediction of the model (RPE values  $< 10$  %). For MonoBv and MixBv, although the RPE values provided a relatively satisfactory prediction, the polynomial regression model could not explain the variation in the data ( $R^2$ -values  $< 0.7$ ). In MonoDg, an NDF above 50 % was observed most of the year, while in MixDg and MixLp, this level was only recorded during spring and summer.

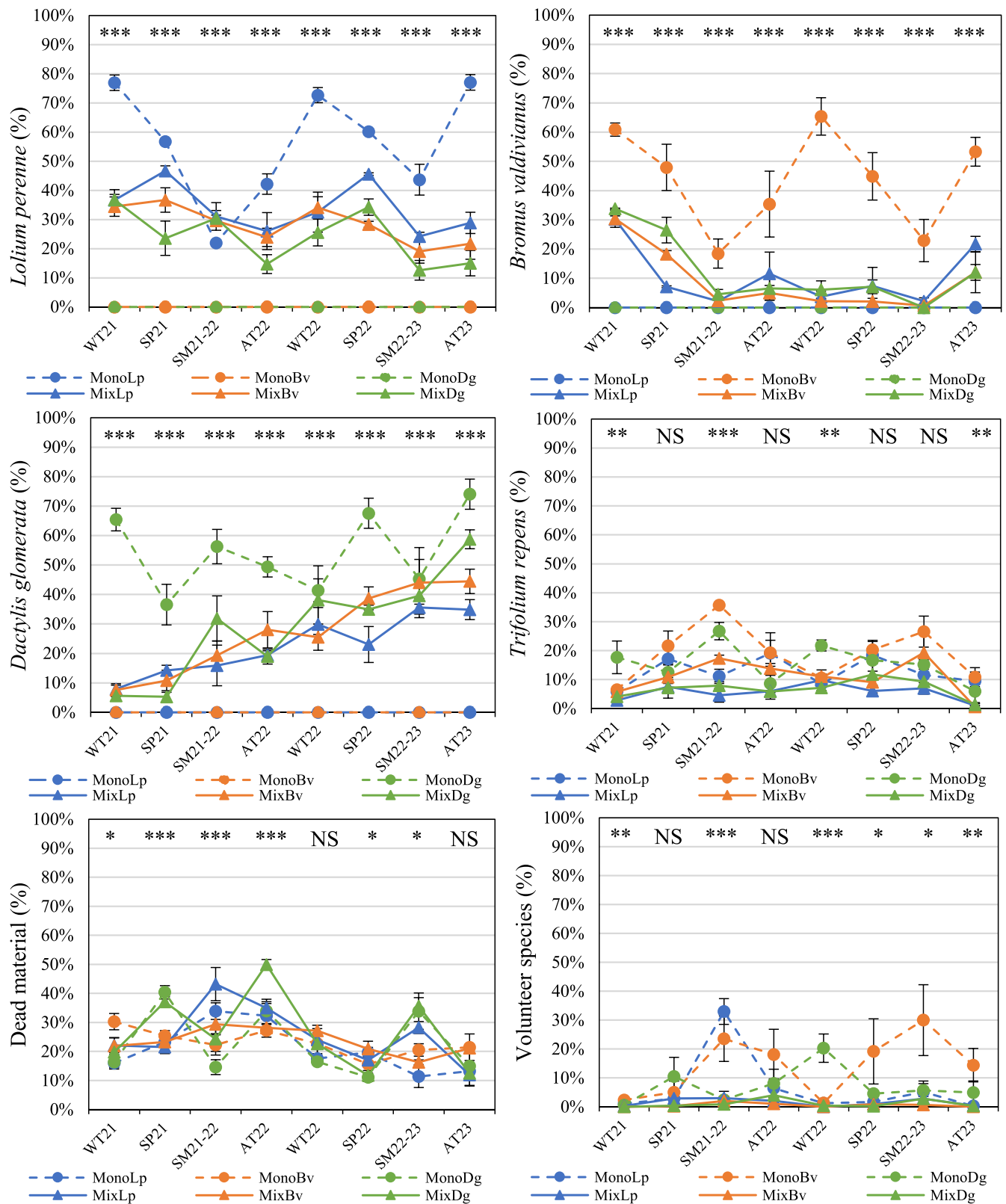
The polynomial regression models for LGN did not present a desirable fit to the data ( $R^2$ -values  $< 0.7$ ), although presenting a relatively

acceptable prediction of the model (RPE values between 10 % and 20 %); therefore, the models only explained relative trends of the LGN content dataset. Overall, MonoBv, MonoDg, and MixDg presented higher LGN contents than the other treatments. The polynomial regressions for the NSC in MonoLp, MixLp, MixBv and MixDg provided a good fit to the data ( $R^2$ -values  $> 0.7$ ) and a relatively acceptable prediction of the model (RPE values between 10 % and 20 %). However, for the NSC in MonoBv and MonoDg, the polynomial regression did not provide a good fit to the dataset nor a satisfactory prediction of the models. In MonoLp, MonoBv, MixLp, and MixLp, a peak of NSC was observed at the end of winter, remaining high during spring and summer and dropping towards autumn and early winter; meanwhile, MonoDg and MixDg presented different NSC patterns, with a high NSC value in late autumn and early winter, then dropping during spring and in the case of MonoDg, presenting a secondary peak in early summer (Fig. 7).

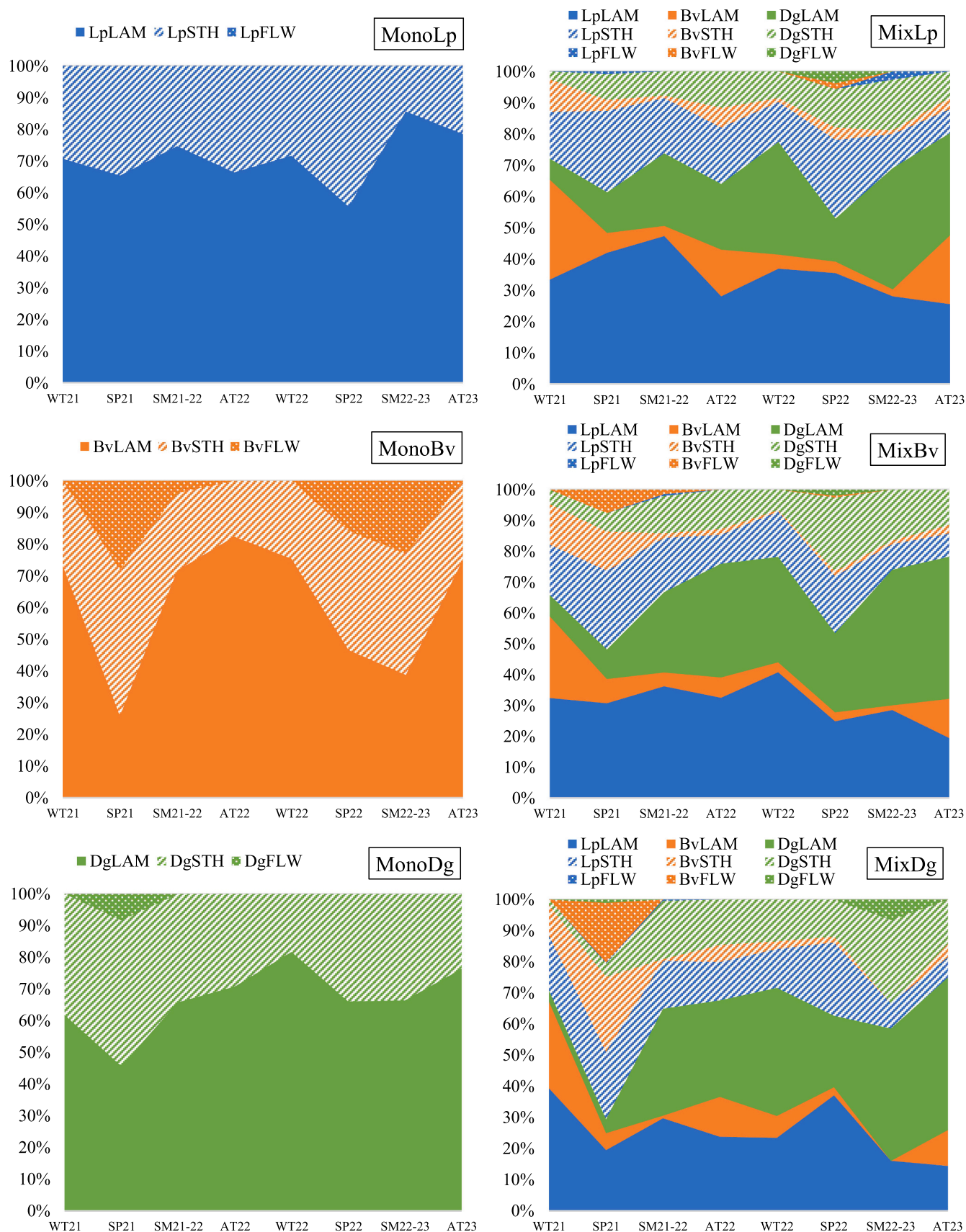
## 4. Discussion

The challenge associated with selecting species or cultivars that comprise a diverse pasture is 'situation-specific' and depends on the climate, soil type, and pasture utilisation (Pembleton et al., 2015). Moreover, it requires a strategic approach for appropriate defoliation management 'rules' that prevent the pasture from being dominated by one or two species (Donaghy et al., 2021). These aspects underpin the successful performance and persistence of a diverse pasture under intensive pastoral systems, enabling diversity to become a viable alternative to adapt to climatic changes.

In the present study, the information acquired at a pasture level (annual and seasonal DM accumulation, average daily growth rates per season, and nutritive value parameters) and at an individual species level (botanical and morphological composition) allowed us to: (i) infer the suitability of the optimal LS interval as a criterion to defoliate DPCS and define the temporal overlapping among different species that result in a common window of opportunity for defoliation; (ii) characterise the seasonal patterns of yield, fluctuations in botanical and morphological composition and susceptibility to volunteer species ingression in DPCS and single-grass pastures; (iii) identify complementary effects (e.g., herbage growth asynchrony, LS intervals synchronicity) underlying the combination of *L. perenne*, *B. valdivianus* and *D. glomerata*; and (iv) understand the seasonal fluctuations of nutritive parameters in DPCS and single-grass pastures. Further research is needed to test DPCS in years with longer drought periods.



**Fig. 4.** The average percentage of individual components in the botanical composition of MonoLp (dashed blue line and blue circles), MonoBv (dashed orange line and orange circles), MonoDg (dashed green line and green circles), MixLp (solid blue line and blue triangles), MixBv (solid orange line and orange triangles), and MixDg (solid green line and green triangles) during winter 2021 (WT21), spring 2021 (SP21), summer 2021–2022 (SM21–22), autumn 2022 (AT22), spring 2022 (SP22), winter 2022 (WT22), spring 2022 (SP22), summer 2022–23 (SM22–23) and autumn 2023 (AT23). Levels of significance are indicated in the graphs per season as NS = non-significant, \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001. The vertical bars indicate (±) S.E.M. within the same season for the six treatments.



**Fig. 5.** Seasonal fluctuations of the average percentage of individual components in the morphological composition [lamina (LAM - solid area), sheath (STH - dashed area) or flower (FLW - pointed area)] of each species (Lp = *Lolium perenne* - in blue, Bv = *Bromus valdivianus* - in orange and Dg = *Dactylis glomerata* - in green) per treatment (MonoLp, MonoBv, MonoDg, MixLp, MixBv, MixDg) winter 2021 (WT21), spring 2021 (SP21), summer 2021–2022 (SM21–22), autumn 2022 (AT22), spring 2022 (SP22), winter 2022 (WT22), spring 2022 (SP22), summer 2022–23 (SM22–23) and autumn 2023 (AT23).

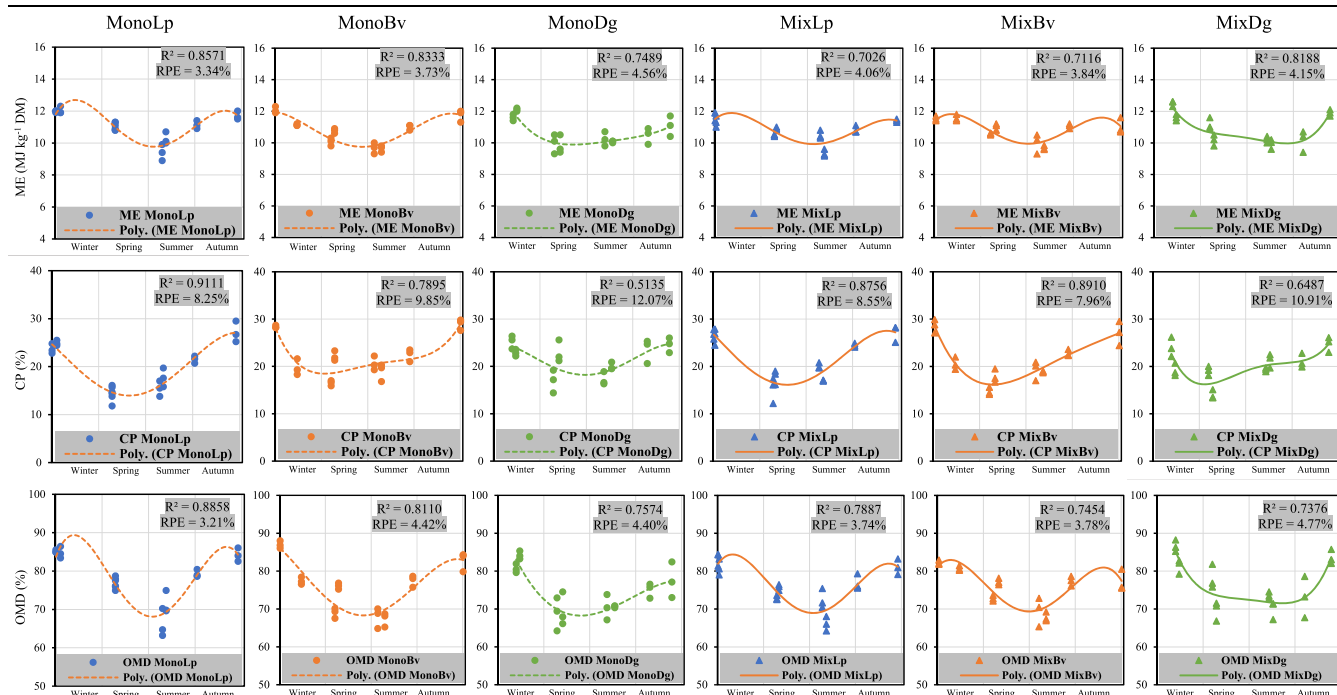


Fig. 6. Metabolisable energy content (first row - ME, MJ kg DM<sup>-1</sup>), crude protein percentage (second row - CP, %) and organic matter digestibility *in-vivo* percentage (third row - OMD, %) in the dry matter of treatments (MonoLp - blue points; MonoBv - orange point; MonoDg - green points; MixLp - blue triangles, MixBv - orange triangles, MixDg - green triangles) per season (winter, spring, summer, and autumn). Polynomial regressions (Poly.) of annual predicted values are estimated based on sampled data points (MonoLp - blue dashed line; MonoBv - orange dashed line; MonoDg - green dashed line; MixLp - blue solid line, MixBv - orange solid line, MixDg - green solid line). R<sup>2</sup>=r-squared value. RPE=relative prediction error (%).

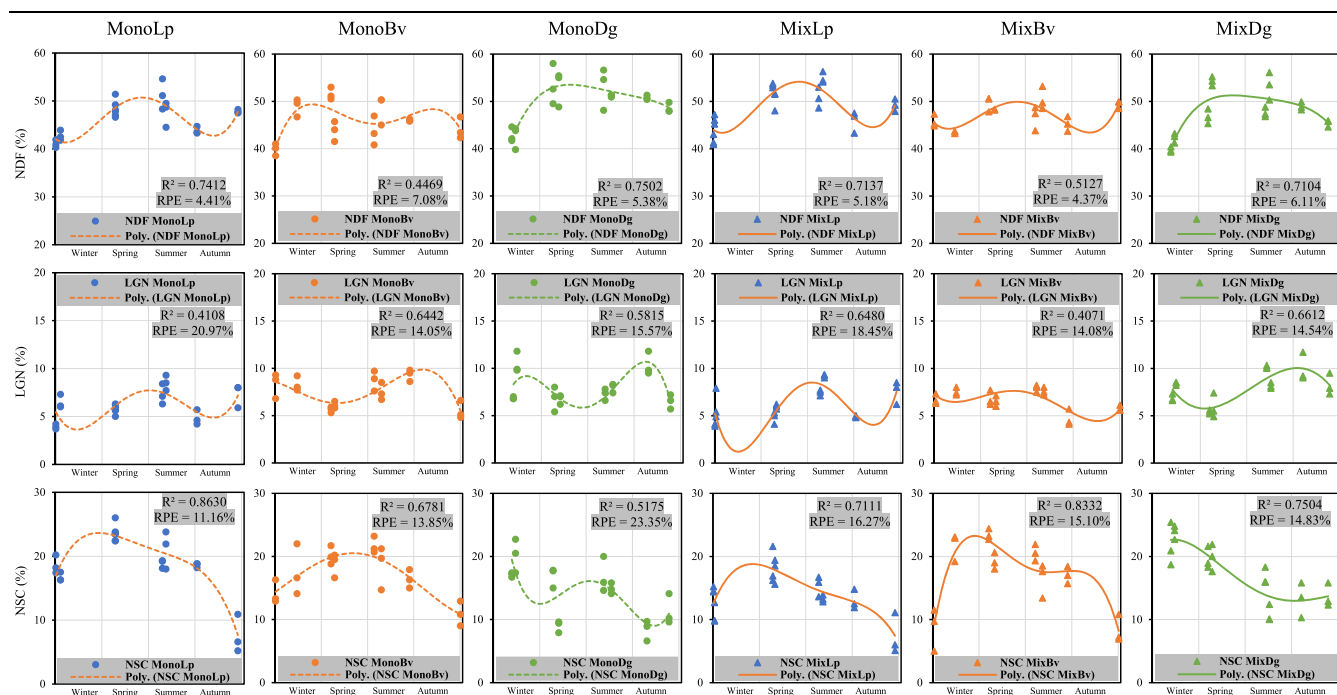


Fig. 7. Neutral detergent fibre percentage (first row - NDF, %) and lignin percentage (second row - LGN, %) non-structural carbohydrates percentage (third row - NSC, %) in the dry matter of treatments (MonoLp - blue points; MonoBv - orange point; MonoDg - green points; MixLp - blue triangles, MixBv - orange triangles, MixDg - green triangles) per season (winter, spring, summer, and autumn). Polynomial regressions of annual predicted values are estimated based on sampled data points (MonoLp - blue dashed line; MonoBv - orange dashed line; MonoDg - green dashed line; MixLp - blue solid line, MixBv - orange solid line, MixDg - green solid line). R<sup>2</sup>=r-squared value. RPE=relative prediction error.

#### 4.1. Diverse pastures of complementary species and the use of leaf regrowth stage

According to the present study, a diverse pasture comprising *L. perenne*, *B. valdivianus*, *D. glomerata*, and *T. repens* is a viable alternative to the conventional ryegrass-based pastures in temperate humid climates. All treatments recorded a high yield in the first two years, yielding on average 20.27 t DM ha<sup>-1</sup> ( $\pm 1.17$ ) in Year 1 and 21.50 t DM ha<sup>-1</sup> ( $\pm 0.45$ ) in Year 2. This overall performance can be associated with the intensified management practices implemented, including maintenance fertilisation and regular application of N (Whitehead, 2000), and the use of optimal post-grazing residuals [e.g. stubble heights of 5–8 cm, and post-grazing targets of 1200–1700 kg DM ha<sup>-1</sup> (Chapman et al., 2011; Lee et al., 2008)]. Further, the climatic conditions during the experimental period were favourable to herbage growth due to a prevailing La Niña pattern [warmer summers and milder winters with precipitation around 900–1300 mm year<sup>-1</sup>, reliable and distributed throughout the year in the Manawatu region (Mosley, 2000)]. In New Zealand, pastoral growth is often constrained during the summer, which is a dry and warm season - average maximum temperatures at mid-20s °C across the country - (Matthew et al., 2012; McCahon et al., 2021) and is sometimes subjected to periods of low soil moisture and heat that can cause temperate pastures to cease growth completely (Norton et al., 2008). However, under La Niña's influence (summer 2021–22 and 2022–23), the present study registered pastures growing all year round, with summer growth rates of approximately 80 kg DM ha<sup>-1</sup> day<sup>-1</sup>. This is higher than commercial dairy farms in the Lower North Island (the same region as the current experiment), which also reportedly registered high pasture growth rates during both summers, ranging from approximately 40 kg DM ha<sup>-1</sup> day<sup>-1</sup>–70 kg DM ha<sup>-1</sup> day<sup>-1</sup> (DairyNZ, 2024).

Additionally, the LS criteria for defoliation also contributed to the high yielding found in all treatments. The LS optimal interval for defoliation, which is based on the leaves' lifespan, allows the replenishment of plant energy reserves, resumption of root growth and increased tillering, and consequently, maximum growth rates are achieved (Donaghy et al., 2021; Lemaire et al., 2009). Extension studies with farmers have shown increased pasture accumulation when the grazing rotation is realigned to fit the LS interval of *L. perenne* in ryegrass-based pastures (Clarke et al., 2021). In the present study, the seasonal optimal LS intervals of targeted ( $\alpha$ ) species were achieved in all single-grass and diverse pastures. Meanwhile, the companion species were mostly defoliated within, and sporadically defoliated out of their optimal LS intervals. In MixBv (see Table 2 for the LS target in  $\alpha$  species), most grazing events had companion species defoliated within their optimal LS interval, except *D. glomerata*, which was defoliated slightly earlier than recommended in autumn and winter 2022 (<3.5 LS; Table 3). Overall, this indicates that when *B. valdivianus* LS is the grazing criterion, the three grass species present a recurrent overlapping of their optimum defoliation intervals, showing that they present a common window of opportunity for defoliation. In winter, summer, and autumn, these overlapping intervals were found to be 2.6–3.0 LS for *L. perenne*, 3.6–4.0 LS for *B. valdivianus* and 3.5–3.7 LS for *D. glomerata*, and in spring they were 2.5–2.6 LS for *L. perenne*, 3.0–3.4 LS for *B. valdivianus* and 3.1–3.4 LS for *D. glomerata*. These findings corroborate those of García-Favre et al. (2022), who found that mixed pastures of *L. perenne* and *B. valdivianus* have overlapping optimal LS intervals.

However, in the other two DPCS (MixLp and MixDg), the defoliation interval based on the  $\alpha$  species (*L. perenne* and *D. glomerata*, respectively) did not always match the optimal LS interval of the other two companion species. In the MixLp (*L. perenne* as  $\alpha$ ), *B. valdivianus* was defoliated earlier than recommended in winter 2021 and summer 2021–22 (<3.5 LS; Table 3), and *D. glomerata* in winter 2021, spring 2021, summer 2021–22, autumn 2022 and winter 2022 (<3.5 LS in summer, autumn, and winter; <3.0 LS in spring; Table 3). Overall, in MixLp, the three species' LS were closer to the lower limit within their LS interval (*L. perenne* closer to 2.5 LS, and *B. valdivianus* and *D. glomerata* closer to

3.5 LS). Conversely, in MixDg (*D. glomerata* as  $\alpha$ ), *L. perenne* was defoliated later than recommended in winter 2021 and winter 2022 (>3.0 LS) and *B. valdivianus* in winter 2021 (>4.0 LS; Table 3). Overall, in the MixDg, the three species' LS were closer to the upper limit within their LS interval (*L. perenne* closer to 3.0 LS, and *B. valdivianus* and *D. glomerata* closer to 4.0 LS; Table 3). From these findings, it is possible to conclude that *D. glomerata* takes slightly longer to reach its optimal LS interval for defoliation compared to *L. perenne* and *B. valdivianus*. In a mini-sward study with mixtures of *D. glomerata* and *Bromus willdenowii* Kunth., it was demonstrated that *D. glomerata* took 19 winter days longer than *B. willdenowii* to get to 4 LS (Gatti et al., 2016).

Considering the theoretical background of the LS criteria, for the grazing events in which the companion species were defoliated slightly earlier or later than the recommended LS intervals (MixLp or MixDg), it is possible to infer that there were trade-offs at a species level between water-soluble carbohydrate reserves for regrowth and optimising feed productivity and quality. In other words, a farmer may choose to utilise a faster rotation with pasture being defoliated just before the companion species' minimum interval limit to maximise feed quality or, alternatively, a longer rotation with pastures defoliated just after the maximum interval limit to maximise herbage yield and full replenishment of WSC reserves. In the present study, the species in the DPCS were mostly grazed within their optimal LS interval for defoliation, which may also encourage pasture short-term persistence. More research and extension promoting the use of LS as a defoliation criterion is needed to evaluate on-farm long-term effects on diverse pasture persistence and resilience.

#### 4.2. The effects of complementarity in diverse pastures: growth asynchrony and overyielding

By combining summer- and winter-active species, the DPCS showed that growth asynchrony can result in seasonal overyielding, exhibited as greater seasonal growth rates of DPCS compared to their respective (same  $\alpha$  LS species) single-grass pastures. For example, MixLp consistently presented significantly higher daily growth rates in the warmer seasons (both spring periods and summer 21–22) than MonoLp (Fig. 3). Similarly, MixBv consistently presented significantly higher daily growth rates than MonoBv in the cooler seasons (Fig. 3). The single-grass pastures presented significant season-to-season variation, highlighted by production peaks in one season followed by a trough in the next (Table 4). For instance, MonoLp presented relatively high accumulated DM masses in winter 2021 and 2022 and in autumn 2021, followed by relatively low accumulated DM masses in spring 2021 and 2022 and in summer 2021–22. MonoBv was among the treatments that presented the lowest accumulated masses in winter 2021 and 2022, spring 2021 and 2022, and autumn 2022, and a peak of production in summer 2021–22. Lastly, MonoDg had variations in accumulated herbage masses, however, with no apparent seasonal consistency.

Previous studies under different conditions (glasshouse, plot, farm, up to continental scale) have registered growth asynchrony among species in diverse pastures (Descalzi et al., 2019; Finn et al., 2013; García-Favre et al., 2022; Komainda et al., 2020; Küchenmeister et al., 2012). However, these same studies have not always found seasonal or annual overyielding of diverse pastures in relation to less complex pastures (pure stand or binary). For example, in the main production year of two different glasshouse experiments utilising pure stands, three-species diverse pastures, and five-species diverse pastures (*L. perenne*, *D. glomerata*, *T. repens*, *Plantago lanceolata* L., and *Taraxacum officinale* Agg.), it was found that *T. repens* pure stands presented the highest accumulated DM in relation to all other possible combinations of species (Komainda et al., 2020; Küchenmeister et al., 2012). The diverse treatments containing the legume presented medium to high accumulated biomass, and the forb and grass pure stands were among the lowest accumulated DM values. Both studies suggested that these findings were primarily driven by the legume N supply, and secondarily driven by forbs or grass species alternatively experiencing optimal temperatures

for growth across seasons.

A field study by Descalzi et al. (2019) demonstrated the growth asynchrony in fertilised naturalised pastures composed mostly of *Agrostis capillaris* L., *Holcus lanatus* L., *L. perenne*, *Vulpia dertonensis* All., *Anthoxanthum odoratum* L., and a diverse pasture of *L. perenne*, *B. valdivianus*, *H. lanatus*, *D. glomerata* and *T. repens*, albeit not presenting overyielding in relation to the control *L. perenne* and *T. repens*. On the other hand, field studies by Nyfeler et al. (2009) observed overyielding in diverse pastures compared with pure stands, up to the point of ‘transgressive’ overyielding, where diverse pastures produced more than the best pure stand treatment. In a plot-scale experiment with sheep grazing pure stands and mixed swards of *B. valdivianus* and *L. perenne*, García-Favre et al. (2022) found growth asynchrony in the mixtures that, in this case, resulted in 15 % higher accumulated DM (overyielding) when compared to the pure stands. Moreover, in an intercontinental scale study with the same experimental design across 31 different locations, Finn et al. (2013) found that four species mixtures containing two grass and two legume components presented ‘transgressive’ overyielding in relation to pure stands in 97 % of the comparisons over multiple years.

Although not all studies have reported the overyielding of diverse pastures in relation to less complex pastures, one common factor was consistently reported: diverse pastures presented a clear effect of annual or seasonal yield stability as a buffering ability to respond to varied abiotic and biotic disturbances. Finally, due consideration to species identity and functional roles must be given when selecting species to comprise a diverse pasture (Sanderson et al., 2007). The succession and persistence of a diverse pasture depend not upon including more species but on the causal relationship of combining all these species.

#### 4.3. The effects of complementarity in diverse pastures: volunteer species suppression and botanical composition dynamics

In intensive dairy pastoral systems such as New Zealand’s, susceptibility to weed invasion is associated with a decline in pasture resilience caused by various issues, such as overgrazing (Hernández-Garay et al., 1997), pugging damage (Drewry et al., 2008) and lower persistence traits of perennial-ryegrass cultivars (Chapman et al., 2015), amongst others (McCahon et al., 2021). The reduced presence of volunteer species in diverse pastures is an ecological side effect, resulting in enhanced niche competitiveness and consequent higher resource exploitation and utilisation (Knops et al., 1999; Naeem et al., 2000), termed by Tracy and Sanderson (2004) as “resource use complementarity”. A probable secondary explanation for this reduction is the “sampling effect” which describes the higher probability that at least one of the pasture species is at the peak of its competitiveness and actively growing, which in turn reduces the opportunities for non-desired species ingress (Aarssen, 1997; Huston, 1997). Tracy and Sanderson (2004) suggest that both processes occur concomitantly in diverse pastures, which may also be the case in this study.

The present study found that DPCS have an enhanced ability to withstand the ingress of volunteer species in the sward. Seasonal peaks of volunteer plant species presence (~20 %) were often registered in the single-grass pastures (summer 2021–22, winter 2022, spring 2022, summer 2022–23, and autumn 2023), while the DPCS had at most 4 % presence of volunteer plant species. These significant differences in volunteer species proportion were, anecdotally, associated with a significant decrease in the sown species proportion (MonoLp and MonoBv in summer 2021–22, MonoDg in winter 2022, and MonoBv in spring 2022), up to an extreme in which MonoLp had significantly less *L. perenne* percentage (22 %) than in MixLp, MixBv and MixDg (average of 30 % among DPCS). These events were driven by the aggressive invasion of volunteer spring- and summer-active species (*Rumex obtusifolius* L., *Paspalum dilatatum* Poir.) and volunteer winter-active species (*Poa annua* L.). These findings agree with other studies comparing pure stands and different mixed pastures in Northern Europe and Canada

[*Phleum pratense* L., *Poa pratense* L., *Trifolium pratense* L. and *T. repens* (Sturludóttir et al., 2013)] and in Europe [*L. perenne*, *D. glomerata*, *T. repens* and *T. pratense* L., or *L. perenne*, *P. pratense* L., *T. repens* and *Trifolium ambiguum* M. Bieb. (Connolly et al., 2009)]. Furthermore, the seasonal fluctuations in botanical composition found in this study are probably related to competition (for *T. repens*), environmental constraints (for *B. valdivianus*), opportunistic overtake (for *D. glomerata*), and defoliation criteria (for *L. perenne*).

Many factors can compromise the persistence of legumes in pastures, including establishment issues, livestock grazing selection, treading, and resource competition for light, water, and nutrients (Brock and Hay, 2001). The higher *T. repens* proportion in single-grass pastures than in DPCS may have resulted from severe interspecific plant competition (Annicchiarico et al., 2014) by the three grass species that present characteristics of competitors (Grime et al., 1988). In both summers, the higher rainfall events caused by La Niña [especially December 2021, with 229.8 mm recorded, compared with the long-term (1981–2010) average of 87.5 mm (NIWA, n.d.)] resulted in long-term saturated soil conditions, and the *B. valdivianus* proportion drastically dropped in all treatments. *Bromus* spp are recommended for well-drained soils as they do not tolerate low oxygen levels in soils (López et al., 1997; Stewart, 1996). Such sensitivity is probably due to small-sized aerenchyma, contrasting with *L. perenne*, which presents well-developed aerenchyma (Zhang et al., 2023).

As the *B. valdivianus* proportion in the mixtures significantly decreased, the *D. glomerata* proportion concomitantly increased (Fig. 4), showing an opportunistic overtake of one species by another. *Dactylis glomerata* plants have larger tillers and longer leaves than both *L. perenne* and *B. valdivianus*, which may result in a competitive advantage, especially at longer grazing rotations. The ‘umbrella effect’ is a phenomenon that occurs in diverse pastures when species that are taller than their companion species shade out the neighbouring tillers, giving them an advantage in capturing light (Bryant et al., 2015). However, such phenomena can be manipulated in diverse pastures by introducing faster grazing rotations. Carlen et al. (2002) assessed the competitive ability of *D. glomerata* in mixtures with *Festuca pratensis* and reported *D. glomerata*’s overtake by the second growing season under infrequent defoliation treatment. In the present study, MixLp and MixBv, the treatments in which the LS targets resulted in faster grazing rotations, showed a more even proportion between the morphological components of *L. perenne* and *D. glomerata* (Fig. 5). In MixDg, the longer grazing rotations resulted in *D. glomerata* components representing a greater proportion of the sward, favouring its spatial dominance over other species.

#### 4.4. Grazing management and diversity effects on the pastures’ nutritional parameters

In New Zealand’s dairy pastoral systems, the recommended CP content for lactating cows is in the order of 16–18 % during early and mid-lactation (spring and summer), 14 % during late lactation (autumn), and 12 % during the dry period (winter) (DairyNZ, 2023). In the present study, although all experimental treatments experienced a trough in CP content during spring and early summer, pastures still presented adequate CP for early and mid-lactation cows during that period (except for MonoLp with CP ~14 % in spring). The trough in CP content may be associated with an increased pasture growth rate during spring that led to a dilution effect on a percentage basis. Conversely, all experimental treatments presented excessively high CP content during autumn and winter (above 25 % DM), which can result in environmental issues and, potentially, be toxic to animals (Pacheco and Waghorn, 2008). In pastures containing CP content higher than dairy cattle requirements, the ingested N surplus (~70 %) is excreted predominantly as a waste product (Marshall et al., 2021), which can ultimately result in nitrate leaching into the water catchments and reservoirs (Selbie et al., 2015). In the present study, the high CP values in winter and autumn

were associated with the presence of legumes in the pastures (ranging from 5 % to 20 %), and also probably the result of years of breeding programs focusing on higher nutritive value (Wilkins, 1991).

In pasture-based dairy systems, ME is usually the first limiting factor of production (van Vuuren and Meijjs, 1987). Normally, the minimum threshold for lactating dairy cows to maintain milk yield is around 11 MJ kg<sup>-1</sup> DM (AFRC, 1993). All pastures presented slightly low ME values (~10 MJ ME<sup>-1</sup> DM) in late spring and summer, a well-known problem in pasture-based systems caused by the onset of the reproductive phase in grasses, where lower lamina:stem ratios result in higher fibre levels (Delagarde et al., 2000). In a broad study with C<sub>3</sub> and C<sub>4</sub> grasses, Fulkerson et al. (2007) concluded that, overall, the nutritional parameters are higher during cool seasons (winter and autumn) and are often followed by a decline in quality over warmer seasons, a fact associated with reproductive development. This clear pattern was also found in New Zealand by Roche et al. (2009a), (2009b), suggesting that, at that time of the year, feed supplementation may be necessary to maintain milk production.

In the current study, MonoDg and partially MixDg did not record the post-summer recovery in ME content, as observed in MonoLp, MonoBv, MixLp, and MixBv. As previously discussed, the *D. glomerata* LS treatments were defoliated fewer times throughout the study period. In some seasons (spring 2021, autumn 2022, and summer 2022–23), MonoDg and MixDg recorded a higher proportion of dead material than the other treatments (over 35 %), along with a higher proportion of sheaths and flowers (35–40 %), up to an extreme in spring 2021 when flowers and sheaths comprised 70 % of MixDg and 55 % of MonoDg. This higher proportion of dead material, along with sheaths and flowers, resulted in higher NDF and LGN contents because fibre levels are high in sheaths and flowers (Griggs et al., 2007). MonoBv also contained high levels of LGN and NDF throughout spring and summer (30 % and 5 % in year 1, and 15 % and 20 % in year 2, respectively), which can be associated with a relatively longer reproductive phase than the other two grass species (Balocchi et al., 2001).

Despite significantly increased biomass, Suter et al. (2021) found that forage quality was generally similar between mixtures and pure stands. According to their study, this results in greater units of CP, ME, or OMD per area (i.e., kg CP ha<sup>-1</sup>) in mixtures. Therefore, it is possible that mixtures can maintain a higher stocking capacity than pure stands, which is an area requiring further research. In this study, the differences in nutritive parameters were mostly driven by the defoliation criteria and, to a lesser extent, by the diversity of the pasture. Therefore, the *B. valdivianus* LS criteria can be proposed as the most suitable defoliation criteria for DPCS because it (i) covers the optimal LS interval for the defoliation of all three grass species more often, (ii) results in adequate nutritive quality, and (iii) it also resulted in overyielding and reduced susceptibility to volunteer species compared to the single-grass pastures. In addition, on some occasions during spring and summer when herbage growth was faster, the *L. perenne* LS criteria can be a suitable defoliation criterion since it (i) resulted in a more balanced species proportion, (ii) adequate nutritive quality, and (iii) it also resulted in overyielding and reduced susceptibility to volunteer species compared to the single-grass pastures. The LS can be used as a flexible management tool to optimise production, quality, and utilisation of diverse pastures.

## 5. Conclusions

The LS criterion was a suitable management tool for DPCS and can be considered successful due to an overall high annual yield, seasonal overyielding in relation to single-grass pastures, low susceptibility to ingression of volunteer species, and satisfactory levels of crude protein and metabolisable energy. It is possible to conclude that DPCS have growth asynchrony among different species DM accumulation and development synchrony among species LS. Within DPCS, a common window opportunity for optimal defoliation of *L. perenne*, *B. valdivianus* and *D. glomerata* was achieved mostly when utilising the *B. valdivianus*

LS target. The *L. perenne* LS could be a target alternative when aiming to buffer the competition by *D. glomerata*. The *D. glomerata* LS is not the most appropriate grazing criterion since the resulting longer grazing rotation causes an increase in dead material, sheath and flower proportions, overall lower nutritive quality and promotes the overtake of *D. glomerata* over the other species.

Finally, the hypothetical bases of the study (DPCS defoliated according to the LS criterion) can become an important alternative for temperate grazing and have the potential to be applied to diversified pastoral ecosystems of other climates and soil types; nonetheless, the combined species must present complementary traits. Further studies are necessary to assess the production and quality parameters of DPCS defoliated according to the LS criterion in years with longer or more extreme drought periods.

## Ethical approval

Massey University Animal Ethics Committee (Approval number 21/24).

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## CRedit authorship contribution statement

**Anchão Oliveira Bia:** Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Donaghy Daniel Joseph:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Nicolas López-Villalobos:** Writing – review & editing, Validation, Methodology. **Kemp Peter David:** Writing – review & editing, Methodology, Conceptualization. **López Ignacio Fernando:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Cranston Lydia Margaret:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis, Conceptualization.

## Declaration of Competing Interest

The authors declare no conflict of interest

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## Data availability

Data will be made available on request.

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