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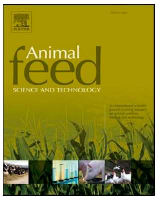

Animal behaviour and dietary preference of dairy cows grazing binary and diverse pastures under the leaf regrowth stage defoliation criterion

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ABSTRACT

In New Zealand, intensively managed pasture-based dairy systems rely on binary pastures mostly comprised of *Lolium perenne* L. and *Trifolium repens* L.. More frequent and extreme climatic events have been negatively affecting the persistency and production of these pastures, which now present increased seasonality, with marked peaks and troughs of production throughout the year. Diversification of plant species offers a solution to deal with increased seasonality. However, little is known about animal behaviour and dietary preferences of dairy cows grazing diverse pastures. The present study aimed to assess the grazing preferences of dairy cows when unrestrictedly offered binary (*L. perenne and T. repens*; Bi) and diverse pastures (*L. perenne, Bromus valdivianus* Phil., *Dactylis glomerata* L. and *T. repens*; Mix) subjected to three different leaf regrowth stage (LS) defoliation criteria. Secondarily, the study aimed to determine the main plant-related drivers for any potential animal preference. The treatments were MixLp (defoliated at *L. perenne* LS), BiLp (defoliated every time MixLp was defoliated), MixBv (defoliated at *B. valdivianus* LS) and BiBv (defoliated every time MixBv was defoliated), MixDg (defoliated at *D. glomerata* LS) and BiDg (defoliated every time MixDg was defoliated). Dairy cattle were evaluated over five agricultural seasons. The response variables were grazing time and location, bite rate, animal behavioural activity, pre-grazing herbage mass, undisturbed sward height, lamina:stem ratio, crude protein, metabolisable energy, organic matter digestibility, non-structural carbohydrates, neutral detergent fibre and lignin. Where significant differences were found, binary pastures presented lower sward height and higher non-structural carbohydrate content in comparison to the diverse pastures under the same LS defoliation criteria. However, no significant differences were found in the percentage of time that cows spent grazing both pastures. Season was the greatest contributor to

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Abbreviations: AM, morning; Bi, binary pasture *Lolium perenne* L. and *Trifolium repens* L.; BiBv, defoliated every time MixBv was defoliated; BiDg, defoliated every time MixDg was defoliated; BiLp, defoliated every time MixLp was defoliated; BR, bite rate; Bv, *Bromus valdivianus* Phil.; CP, crude protein; CVA, canonical variate analysis; Dg, *Dactylis glomerata* L.; F, Holstein Friesian; GT, grazing time; HM, pre-grazing dry matter herbage mass; HT, pre-grazing undisturbed sward height; J, Jersey; LGN, lignin; Lp, *L. perenne* L.; LS, leaf regrowth stage defoliation criteria; LSR, lamina:stem ratio; ME, metabolisable energy; Mix, diverse pasture *L. perenne*, *B. valdivianus*, *D. glomerata* and *T. repens*; MixBv, defoliated at *B. valdivianus* LS; MixDg, defoliated at *D. glomerata* LS; MixLp, diverse pasture defoliated at *L. perenne* LS; NDF, neutral detergent fibre; NIRS, near-infrared spectroscopy; NSC, non-structural carbohydrates; OMD, organic matter digestibility *in-vivo*; Tr, *T. repens*; V, variation proportion; WSC, water-soluble carbohydrates.

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the proportion variation found in all response variables, with values ranging from 47.55 % up to 88.77 %. In winter and spring, cows modulated their grazing behaviour (proportional time spent grazing, ruminating, or idling), investing more time actively grazing pastures under *L. perenne* LS interval of defoliation (2.5–3.0 LS), the criterion which resulted in shorter grazing rotations. This study allowed us to understand the suitability of diverse pastures from an animal perspective, and highlighted that independent of the pasture type, the positive productive and nutritional effects of defoliation management based on the LS may also extend themselves to positive outcomes in animal preference, interpreted as the percentage of time dairy cows spend grazing rather than ruminating or idling across and within seasons.

1. Introduction

The strategic advantage of low-cost and efficient dairy farming in New Zealand has been largely explained by its reliance on pasture-based systems (Caradus et al., 2023; Macdonald and Roche, 2023; Roche et al., 2017), specifically, pastures comprising *Lolium perenne* L. and *Trifolium repens* L. (Caradus et al., 2021). These two species have been a recurrent subject of study in pastoral research for almost a century (Blackman and Templeman, 1940; Brougham, 1959, 1960; Da Silva et al., 2004; Hernández-Garay et al., 2010a; Hernández-Garay et al., 2010b; Macdonald and Roche, 2023). The interest in these two species is mostly due to their adaptability to intensively managed dairy systems since both species present high levels of productivity, great nutritive value and dietary acceptability by dairy cows (Brock and Hay, 2001; Cosgrove, 2011; Hunt and Easton, 1989). However, these two species are highly sensitive to soil water stress and high temperatures (Cosgrove, 2011), and under the current climatic scenario, the persistence and continued high productivity of *L. perenne* and *T. repens* binary pastures are threatened by more frequent and intense extreme weather events (Keller et al., 2021; Lee et al., 2013).

One of the main advantages of pasture-based dairy systems utilising *L. perenne* and *T. repens* binary pasture is that, for most of the year, the pasture annual growth curve matches dairy cows' feed requirements. Firstly, by aligning the peak of herbage production and animal calving period in spring and, secondly, by the lower herbage production when cows dry off during late autumn and early winter (Garcia and Holmes, 2010; Holmes and Roche, 2007). However, due to a more marked seasonality of production as an unfolding situation triggered by climate change (Kalaugher et al., 2017; Keller et al., 2021), herbage production of these binary pastures is no longer sufficient to meet cows' feed requirements throughout the year, and farmers are increasingly using external feed sources to maintain milk production (Clark, 2011; Holmes et al., 2002). In New Zealand, a review on pasture resilience showed that the amount of non-pasture feeds (harvested or imported supplements) utilised in dairy farms increased from an average of 4.0–18.8 % per cow from 1990 to 91–2017–18 (Rys et al., 2021). Although the inclusion of external source feed may result in the production of extra milk solids, it does not necessarily result in increased profitability as it consequentially increases the cost of production (NZD $0.30 to $0.40 per extra kg of dry matter) (Silva Villacorta et al., 2005).

A large body of research has been done on the inclusion of new forage species within New Zealand pasture-based dairy farms as an attempt to buffer the increasing seasonality of production in *L. perenne* and *T. repens* binary pastures. The inclusion of more species within a pasture-based system conceives the idea behind diverse pastures and could occur as the addition of either herbs or legumes such as *Cichorium intybus* L. (Cranston et al., 2015; Li and Kemp, 2005), *Plantago lanceolata* L. (Bryant et al., 2019; Kemp et al., 2013; Nobilly et al., 2013), *Trifolium pratense* L. (Brock et al., 2003), *Medicago sativa* L. (Moot et al., 2016), or other grasses, such as *Dactylis glomerata* L. and *Bromus valdivianus* Phil. (García-Favre et al., 2022; Oliveira et al., 2023). In these studies, positive outcomes related to plant production (e.g. overyielding and growth asynchrony), environmental solutions (e.g. reduced nitrate leaching), and improved nutritive value (e.g. nutritive contribution over summer) were found. Yet, little is known about these diverse pastures' dietary acceptability over common binary pastures of *L. perenne* and *T. repens* and, moreover, their effects on dairy cows' grazing behaviour.

The complex animal-plant dietary relationship in experimental conditions is often conceived as a two-step process composed of animal grazing 'preference' and dietary 'selection' (Gregorini et al., 2015; Hodgson, 1979; Parsons et al., 1994). Preference has been defined as relative feed intake or relatable measures when access to different feeds is unrestricted under grazing conditions (Allen et al., 2011; Hodgson, 1979). Meanwhile, dietary selection is a behavioural expression of preference when access to feed is affected by the environment or opportunity for selection, resulting in the removal of some sward components (plant patches or plant parts) (Allen et al., 2011; Hodgson, 1979). When animals are unrestrictedly offered a diverse array of forage types and have the opportunity to exhibit their preference fully, different layers of processes or features occurring at a plant level will affect the animal's preferences (Poli, 1998). For example, seasonal or diurnal variability (e.g. plant phenological state), morphological and structural characteristics of the sward (e.g. sward height or lamina:stem ratio), and lastly, nutritive parameters of the forages (e.g. energy and carbohydrates content or fibrous composition) (Poli, 1998). Several grass species, such as *L. perenne*, *B. valdivianus*, *Bromus willdenowii* Kunth, *D. glomerata*, *Festuca arundinacea* Schreb., have been accessed as monocultures in preferential grazing trials (Garcia-Favre et al., 2023; Horadagoda et al., 2009; Villalba et al., 2015). In general, for grasses within these aforementioned studies, *Bromus* spp. were the most preferred species, followed by *L. perenne* and secondarily by *D. glomerata,* while *F. arundinacea* was the least preferred species. To our knowledge, although a wide range of theoretical models have been proposed explaining the reason behind the dietary preference of animals for mixing different sources of nutrition in the diet (Rutter, 2006), very little research has been done on animal preference when grazing diverse pasture mixes (Soder et al., 2007).

Considering the adoption of plant species diversification in farming systems (Cranston et al., 2015; Isbell et al., 2015; Sanderson et al., 2007) and given that grazing preference of dairy cows is commonly driven by nutritive value, sward structure and plant morphology, the present study hypothesised that dairy cows prefer binary pastures (*L. perenne* and *T. repens*) due to their potentially higher nutritive value and greater morphological structure of the sward in comparison to diverse pastures (*L. perenne*, *B. valdivianus*, *D. glomerata* and *T. repens*). Therefore, this study aimed to assess the grazing preference of dairy cows when they were unrestrictedly offered binary (*L. perenne* and *T. repens*) and diverse pastures (*L. perenne*, *B. valdivianus*, *D. glomerata* and *T. repens*) subjected to three different leaf regrowth stage (LS) defoliation criteria. The LS is a plant-focused defoliation criterion which consists of an optimal interval for defoliation based on a minimum point set by the replenishment of water-soluble carbohydrate (WSC) reserves in the tillers and a maximum point by the onset of senescence in the older parts of the tiller (see material and methods section for further details) (Fulkerson and Slack, 1994). Secondarily, the study aimed to determine what were the main plant-related attributes that may lead to preferential grazing.

2. Material and methods

2.1. Experimental site

The present study was conducted over five agricultural seasons between February 2022 and March 2023 within a wider pastoral study undertaken at Massey University's Dairy 1 (Palmerston North, Manawatu, New Zealand; 40°22'36.1"S 175°36'40.2"E), 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). The results of soil chemical analysis (0–15 cm soil depth) collected on 26 February 2021 indicated 5.60 pH [$CaCl_2$ 0.01 M (1:2.5)], 3.33 % organic matter content, 29.0 Olsen P (mg/L), 0.21 exchangeable K (me/100 g), 6.80 exchangeable Ca (me/100 g), 1.35 exchangeable Mg (me/100 g), 13.0 cation exchange capacity (me/100 g), 64.0 % total base saturation content, 3 extractable organic sulphur (mg/kg) and 10.03 carbon:nitrogen ratio. The area received annual maintenance fertilisation in the form of superphosphate on 1 March 2021 at 112.5 kg P/ha, 137.5 kg S/ha and 250 kg Ca/ha, and on 17 March 2022 at 90 kg P/ha, 110 kg S/ha and 200 kg Ca/ha. Post-grazing nitrogen fertilisations were applied at a rate of 30 kg N/ha in the form of urea (46 % N) in July 2021, November 2021, January 2022, August 2022, November 2022, and February 2023. 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 m from the field site).

On 24 November 2020, the area was sprayed out with N-(phosphonomethyl) glycine (glyphosate WeedMaster® G360) at 6 L/ha and thifensulfuron-methyl (Harmony® 50 SG) at 30 g/ha to control weed seed bed and population. From 10–18 December 2020, the area was ploughed, power harrowed and levelled. On 18 December 2020, the two types of pastures, diverse (Mix) and binary pastures (Bi) (see pasture composition and sowing rates in Table 1), were established side-by-side (see Fig. 3 for plot details) with a roller drill, and the area was lightly irrigated for one day with a mobile gun sprinkler to ensure a successful establishment. Following this, the pastures were rainfed.

2.2. Experimental design and treatments

The binary and diverse pastures were regularly grazed for two production years according to the methodology utilised in a previous wider pastoral study (Oliveira et al., 2023). The wider study had its grazing protocol started in June 2021 with dairy cows grazing on a one-day event basis until the pasture reached an undisturbed post-grazing height of 5–8 cm from ground level. The defoliation criteria to define different treatments were based on the LS interval of each grass species, *L. perenne* (2.5–3.0 LS), *B. valdivianus* (3.5–4.0 LS) and *D. glomerata* (3.5–4.0 LS) (Fig. 2). Detailed information about the LS defoliation criteria for diverse pastures utilised in the present study can be found in Oliveira et al. (2023).

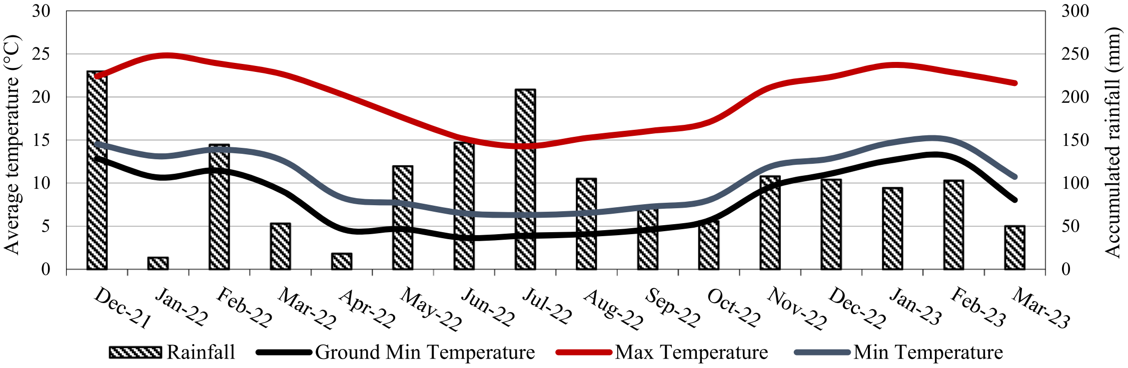

Fig. 1. Monthly accumulated rainfall, monthly averaged ground minimum, and monthly averaged air maximum and minimum temperatures during the experimental period (five agricultural seasons, from December 2021 to March 2023). Bars indicate accumulated rainfall; lines indicate average temperatures.

Table 1
Establishment description of the two pastures species composition and sowing rates, cultivars, resulting experimental treatments, and defoliation parameter.

Pasture species (acronym)	Cultivars and sowing rates (kg/ha)	Experimental treatment	Defoliation parameter
Lp + Bv + Dg + Tr (Mix)	*L. perenne* cv. Maxsyn (10) + *B. valdivianus* cv. Bareno (15) + *D. glomerata* cv. Greenly II (12) + *T. repens* cv. Weka (6)	MixLp MixBv MixDg	*L. perenne* LS *B. valdivianus* LS *D. glomerata* LS
Lp + Tr (Bi)	*L. perenne* cv. Maxsyn (20) + *T. repens* cv. Weka (6)	BiLp BiBv BiDg	Following MixLp Following MixBv Following MixDg

Lp: *L. perenne*, Bv: *B. valdivianus*, Dg: *D. glomerata*, Tr: *T. repens*; Mix: diverse pasture, Bi: binary pasture; LS: leaf regrowth stage.

The present study was arranged in a randomised complete block design, with three blocks (n=3), each with the six experimental treatments in 20 m x 7 m plots (140 m^2) (Fig. 3). The six treatments are detailed in Table 1 and defined as follows: MixLp (defoliated at *L. perenne* LS), BiLp (defoliated every time MixLp was defoliated), MixBv (defoliated at *B. valdivianus* LS) and BiBv (defoliated every time MixBv was defoliated), MixDg (defoliated at *D. glomerata* LS) and BiDg (defoliated every time MixDg was defoliated). For both MixBv and BiBv, for instance, the *B. valdivianus* plants present in the mixture were the targeted species (α), thus justifying defoliation based on *B. valdivianus* LS interval. This was done so that both pastures (diverse and binary) sown side-by-side could be offered simultaneously for cows (Fig. 3). As a result of the grazing protocol applied in the wider study, pastures targeting *L. perenne* LS were more frequently defoliated than the pastures defoliated according to *B. valdivianus* LS, and lastly, *D. glomerata* LS. Detailed information about the agricultural performance of these diverse pastures can be found in Oliveira et al. (2023).

All response variables were assessed once a season, for five seasons [summer 2022 (summer 1), autumn 2022 (autumn), winter 2022 (winter), spring 2022 (spring), and summer 2023 (summer 2)]. The animal-related dependent variables were grazing time (GT; %) and location, bite rate (BR; bites per minute), and animal behavioural activity (percentage of time spent grazing, ruminating, and idling). The plant-morphology dependent variables were pre-grazing dry matter herbage mass (HM; ton DM/ha), pre-grazing undisturbed sward height (HT; cm), and lamina:stem ratio (LSR). On a dry matter basis, the plant nutritive parameters were crude protein (CP; g/kg), metabolisable energy (ME; MJ/kg DM), organic matter digestibility *in-vivo* (OMD; %), non-structural carbohydrates (NSC; g/kg), neutral detergent fibre (NDF; g/kg) and lignin (LGN; g/kg).

For summer 1, the grazing events and experimental assessments occurred on 16 February 2022 (MixLp versus BiLp), 10 February 2022 (MixBv versus BiBv) and 02 March 2022 (MixDg versus BiDg); for autumn on 21 April 2022 (MixLp versus BiLp), 14 April 2022 (MixBv versus BiBv) and 11 May 2022 (MixDg versus BiDg); for winter on 13 July 2022 (MixLp versus BiLp), 11 July 2022 (MixBv versus BiBv) and 16 August 2022 (MixDg versus BiDg); for spring 2022 in 08 November 2022 (MixLp versus BiLp), 07 November 2022 (MixBv versus BiBv) and 22 November 2022 (MixDg versus BiDg); and for summer 2 on 08 February 2023 (MixLp versus BiLp), 09 February 2023 (MixBv versus BiBv), and 22 February 2023 (MixDg versus BiDg).

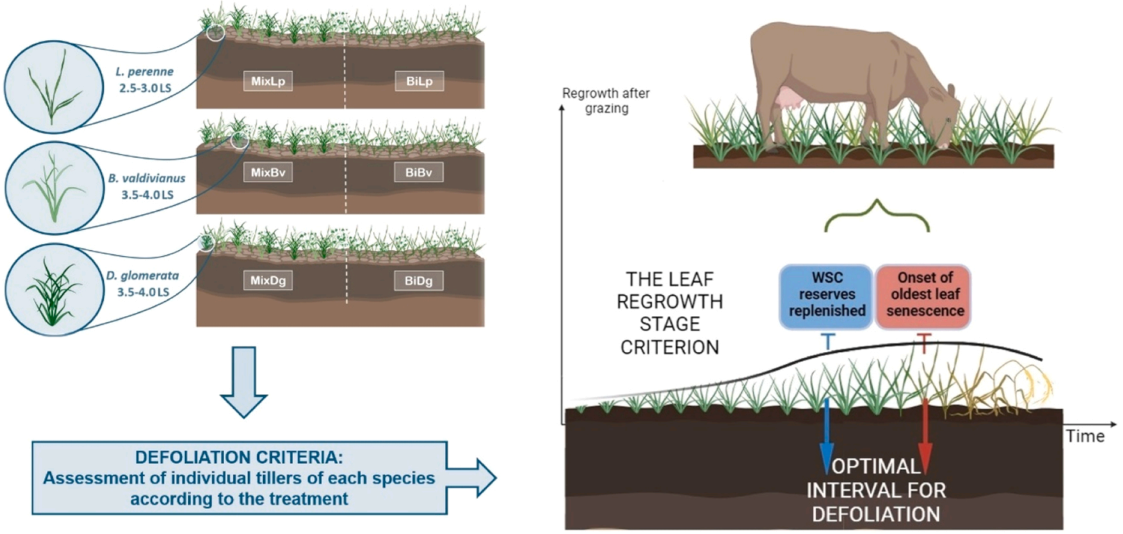

Fig. 2. Conceptual diagram of the defoliation criteria methodology, which displays the leaf regrowth stage (LS) assessment of individual tillers of each species within diverse pastures for defining the experimental treatments based on the optimal interval for defoliation for *L. perenne* (2.5–3.0 LS), *B. valdivianus* (3.5–4.0 LS) and *D. glomerata* (3.5–4.0 LS). This image was partially created in BioRender.com. COLOURED IMAGE.

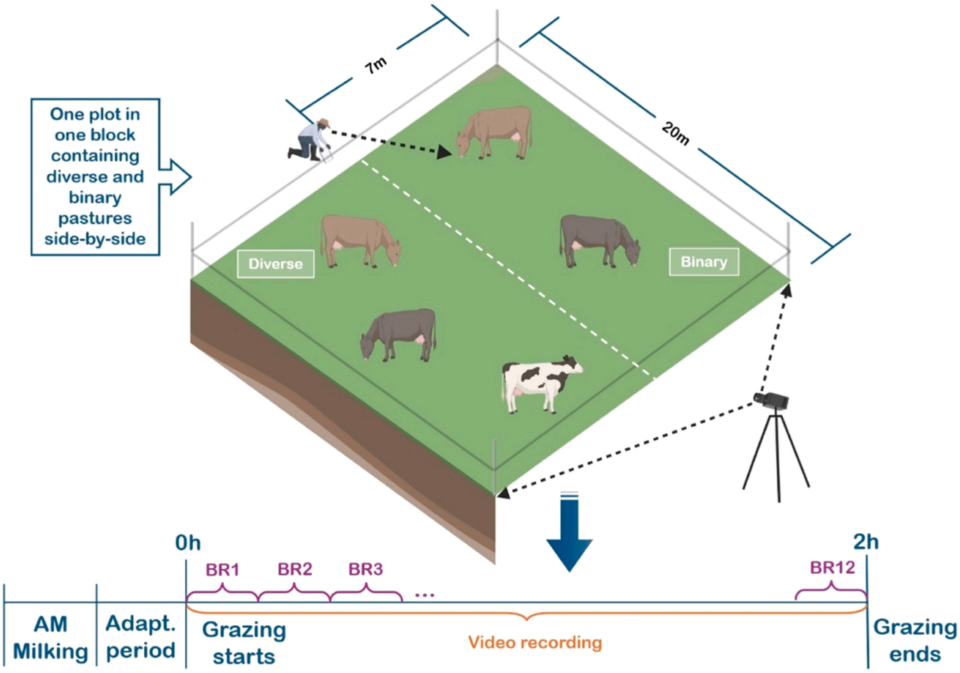

Fig. 3. Conceptual diagram of the preferential grazing assessment day. The image depicts one plot within one experimental block containing diverse and binary pastures side-by-side (7 m x 20 m each), five dairy cows, one observer and one video camera. The timeline depicts the activities during the day, from morning (AM) milking and adaptation (adapt.) period, through to the beginning of preferential grazing assessment, and the recording of 12 bite rate (BR) rounds concomitantly to the video recording, until 2 hours later, when the preferential grazing assessment period ends. This image was partially created in BioRender.com. COLOURED IMAGE.

2.3. Animal measurements

Twenty dairy cows from Massey University's Dairy 1 herd were selected according to their attributes, aiming for a homogeneous and representative average group of New Zealand dairy farm cows. The farm has adopted once-a-day milking for the entire season since 2013. Out of the twenty dairy animals selected, fifteen were continuously utilised in the experiment, and five were in the replacement group, utilised in case one of the original animals was unavailable for one specific grazing event. The dairy cows' attributes utilised to select the experimental group of fifteen animals were breed [Holstein Friesian × Jersey (F × J), namely, kiwi-cross], breed proportion ($0.375 \leq F \leq 0.625$ and $0.375 \leq J \leq 0.625$), age (3–5 years) and average daily milk production (15–23 L/day). The dairy cows' attributes utilised to select the experimental group of five replacement animals were most of the same as the original animals, except for one cow being seven years old and the other two cows with a daily milk production of approximately 10.5 L/day. The cows were predominantly pasture-fed but were supplemented with maize silage (2 kg of dry matter per cow) in autumn and winter.

Following a 6 AM milking (spring, summer, autumn) or at 7 AM in winter (dry-off season), the fifteen cows were randomly subdivided into groups of five animals, accounting for one group per block (Fig. 3). All cows had previously grazed all the tested pastures so there was no novel feed (Horadagoda et al., 2009). Even though, aiming to reinforce the experiment's robustness, before every grazing event, each group was allocated for 20 minutes into an external adaptation area containing the diverse pasture treatment of the given day. Following the adaptation period, the cows were allocated to the treatment areas containing, side-by-side, 140 m^2 of a diverse pasture and 140 m^2 of a binary pasture, offering both pastures simultaneously (Fig. 3). The animals were then evaluated for two hours during their first post-milking grazing (Fig. 3), the moment in which the grazing is generally more active (Gibb et al., 1998).

Three cameras (GoPro HERO8 Black – GoPro Inc, San Mateo, California, USA) were placed at a strategic point close to each experimental plot in such a way that each camera covered the whole evaluated area within each block, thus the five cows' behavioural activity could be posteriorly assessed, and the two different pasture treatments could be thoroughly distinguishable (Fig. 3). As soon as the cows were transferred from the adaptation area to the experimental plot, the recording started and proceeded for the next two hours. Afterwards, a group of five trained people analysed the video footages captured during the five seasons of the study. Briefly, each person would follow one of the five animals within a plot and detect which type of activity the animal was performing every 30 seconds timeframe - grazing (actively grazing or selecting forage with its head down), ruminating (while standing up or lying down) or idle (every other behaviour that not grazing or ruminating) (Penning et al., 1984; Sheahan et al., 2013). For the grazing activity, the location in which each animal was grazing (mix or binary) was also recorded. When an animal was grazing within 0.5 m from the plot's edges, it would be accounted as a lost data point. In this study, over 50,000 data points were collected to assess the behavioural activity of the cows. The resulting data of this evaluation is presented as the seasonal GT in each pasture type and seasonal percentage fluctuation of the three behavioural activities.

The counting of bites was performed in the field for optimal accuracy. A group of three trained observers were distributed within the experimental area and were responsible for assessing five animals, each within the same block. The measurement was recorded every 10 minutes, following the same randomly defined order of cows, one by one assessed as the time it took to perform 30 bites (Forbes and Hodgson, 1985) and in which pasture type it occurred (Fig. 3). If the animal changed the pasture type in which it was grazing, or if the time between two consecutive bites in the same pasture type exceeded 15 seconds, the measurement would be reset, and the counting started again from the new particular position (Balocchi et al., 2002). The animals were assessed for two hours, within the same time frame as the recording for animal behavioural activity, resulting in a total of twelve rounds of bite counting measurement per animal. The resulting data from this evaluation is presented as seasonal BR in each pasture type.

2.4. Plant measurements

The HM was measured by cutting to ground level the herbage within three quadrats (0.1 m^2) randomly placed in each plot. The samples were dried for at least 72 hours in a forced-air oven at 60 °C and weighed to determine their dry matter content. The HT was obtained through the average of 40 zig-zagged random points per plot taken with a sward stick the day before each grazing event (Barthram, 1986).

The nutritive parameters of the apparent harvestable herbage were assessed by randomly harvesting down to 5 cm residual height at least ten sub-samples per plot (Frame, 1993) in the early morning of the previous day to the grazing event, avoiding the warmer hours of the day. These sub-samples generated one composed sample of approximately 150 g per 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 spectroscopy (NIRS), calibration based on Dumas combustion (Chang and Zhang, 2017), and corrected to a fully dry basis assuming 5 % residual moisture. The CP was obtained by multiplying N by 6.25. The OMD *in-vivo* 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 ME content was calculated from OMD from AFIA Method 7 R (modified), using AFRC (Agriculture & Food Research Council, UK) and Lincoln University's standard formulae. The NDF was estimated by NIRS, calibration based on NDF by NFTA method. The LGN was Estimated by NIRS, calibration based on acid detergent extraction followed by treatment with 72 % sulphuric acid in the Ankom Daisy Incubator. The 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 mm-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, 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.

2.5. Statistical analysis

All statistical analyses were performed using SAS v 9.4 (SAS Institute Inc, Cary, NC, USA). The data were examined for normal distribution using the Shapiro-Wilk test, finding that all dependent variables followed a normal distribution using the UNIVARIATE procedure. The homogeneity of variance between pasture treatments was examined with Bartlett's test using the GLM procedure.

Analyses of variance, within seasons of the year, for the dependent variables (GT, BR, HM, HT, LSR, CP, ME, OMD, NDF, LFN and NSC were performed using the GLIMMIX procedure with the following mixed model:

$$\mathrm{Y}_{ijk} = \mu + \mathrm{P}_i + \mathrm{B}_j + \mathrm{e}_{ij} \tag{1}$$

Where P_i is the fixed effect of i^{th} pasture type (MixLp, BiLp, MixBv, BiBv, MixDg, BiDg), B_k is the random effect of the j^{th} block and e_{ij} is the random residual assumed with mean zero and variance σ_e^2.

Least square means and standard errors for the pasture treatments were obtained and used to perform pair-wise comparisons (MixLp and BiLp; MixBv and BiBv; MixDg and BiDg) using Fisher's least significant difference (LSD post hoc test). Significant differences between the least squares means were declared at $P \leq 0.05$.

Out of the eleven dependent variables analysed via analysis of variance and the post-hoc test, nine were selected to undergo a multivariate statistical analysis, allowing the characterisation of relationships between them. Two dependent variables were excluded: OMD due to its direct relationship with ME values and LGN due to its direct relationship with NDF. The dataset of the nine selected dependent variables was standardised (Gittins, 1985). The chosen multivariate analysis was the canonical variate analysis (CVA), performed to determine the extent to which variables explained most of the differences between pasture types and seasons of the year, and at the same time, to explore the relationships between the measured variables in the pastures and grazing cows through the seasons (Garcia-Favre et al., 2023; Jobson, 1992). The CVA was performed using the CANDISC procedure with the following mixed model:

$$Y_{ijk} = \mu + P_i + S_j + (P * S)_{ij} + B_k + e_{ijk} \tag{2}$$

Where P_i is the fixed effect of i^{th} pasture type (MixLp, BiLp, MixBv, BiBv, MixDg, BiDg), S_j is the fixed effect of the j^{th} season (summer 1, autumn, winter, spring and summer 2), $P * S_{ij}$ is the fixed effect of the interaction between the i^{th} pasture type and the j^{th} season, B_k is the random effect of the k^{th} block and e_{ijk} is the random residual assumed with mean zero and variance σ_e^2.

Posteriorly, utilising the same model as for the CVA, the fixed factors of pasture type and season, their interaction and the random effect of the block were analysed with the GLM procedure to obtain the proportion of contribution (V) in the variation of each response variable within the experiment, utilising the following equation:

$$V = \frac{F_e}{\sum F} \tag{3}$$

Where V is the variation proportion explained by one of the effects (random = block; fixed = pasture, season and interaction between pasture and season), and F_e is the F ratio value of the same one of the effects (F-value).

3. Results

3.1. Pair-wise comparisons of dependent variables

The percentage of time spent grazing did not differ between pairs of treatments within seasons ($P > 0.05$; Table 2). The bite rate differed between MixLp and BiLp in summer 1 and autumn ($P \leq 0.05$), with BiLp presenting the highest rates in both seasons. The bite rate also differed between MixBv and BiBv in summer 2 ($P = 0.04$), with BiBv presenting the highest rate (Table 2).

Overall, the HM did not differ between pairs of treatments within seasons ($P > 0.05$), with the exception of MixBv and BiBv in autumn ($P = 0.02$), when MixBv accumulated 0.6 t DM/ha more than BiBv (Table 3). The HT significantly differed between MixLp and BiLp in summer 1, autumn and summer 2 ($P \leq 0.02$), between MixBv and BiBv in summer 1, autumn and summer 2 ($P \leq 0.02$), and between MixDg and BiDg in summer 1 and summer 2 ($P \leq 0.01$), with Mix treatments consistently presenting greater heights when compared to their respective Bi treatments (Table 3). In winter and spring, the HT did not differ between any treatment pairs ($P > 0.05$). Overall, the LSR did not differ between pairs of treatments within seasons ($P > 0.05$), with the exception of MixDg and BiDg in spring ($P = 0.03$), with MixDg presenting a ratio 47 % higher than BiDg (Table 3).

The CP content differed between MixLp and BiLp in summer 2 ($P = 0.04$), with BiLp presenting 22 g/kg DM more CP than MixLp (Table 4). The CP content also differed between MixDg and BiDg in autumn and winter ($P \leq 0.01$), with BiDg presenting the highest content in autumn and MixDg the highest content in winter. The ME content differed between MixLp and BiLp in summer 1 ($P = 0.03$), when MixLp presented 0.3 MJ/kg DM more than BiLp (Table 4). Also, the ME content differed between MixBv and BiBv in winter ($P =$

Table 2

Effect of pasture type on bite rate and percentage of the time allocated grazing in each treatment during summer 1, autumn, winter, spring, and summer 2 as a pair-wise comparison (MixLp and BiLp; MixBv and BiBv; MixDg and BiDg) followed by respective *P-values.*

	Summer 1	Autumn	Winter	Spring	Summer 2
Grazing time (%) *					
MixLp	39.6 (±5.5)	39.8 (±6.7)	42.0 (±3.82)	35.3 (±0.78)	39.5 (±4.8)
BiLp	54.4 (±7.9)	39.7 (±2.0)	41.3 (±2.33)	40.0 (±7.29)	53.3 (±5.9)
P-value	*0.38*	*0.99*	*0.72*	*0.62*	*0.31*
MixBv	49.6 (±7.8)	51.1 (±2.2)	36.1 (±5.3)	21.0 (±5.9)	43.6 (±8.1)
BiBv	48.6 (±7.0)	46.4 (±3.7)	48.9 (±0.5)	33.8 (±0.6)	54.0 (±6.1)
P-value	*0.96*	*0.63*	*0.32*	*0.30*	*0.66*
MixDg	45.1 (±2.7)	45.7 (±2.7)	31.8 (±6.7)	26.5 (±4.1)	45.6 (±3.8)
BiDg	49.5 (±1.2)	52.1 (±4.2)	27.9 (±4.7)	34.0 (±3.0)	52.8 (±3.8)
P-value	*0.26*	*0.45*	*0.51*	*0.19*	*0.44*
Bite rate (bites/min)					
MixLp	57.38 (±0.41)	57.90 (±1.30)	56.20 (±2.92)	47.56 (±0.81)	49.04 (±3.44)
BiLp	65.45 (±1.89)	63.35 (±1.38)	56.20 (±4.25)	56.76 (±3.37)	54.35 (±2.68)
P-value	*0.04*†	*0.03*†	*0.97*	*0.08*	*0.17*
MixBv	58.20 (±3.32)	58.75 (±2.46)	55.18 (±1.10)	52.51 (±1.12)	52.72 (±4.54)
BiBv	60.78 (±2.03)	60.66 (±3.26)	56.59 (±0.19)	51.39 (±4.54)	59.92 (±3.32)
P-value	*0.43*	*0.38*	*0.35*	*0.78*	*0.04*†
MixDg	50.61 (±3.38)	61.98 (±2.35)	63.10 (±2.36)	51.27 (±1.87)	57.80 (±2.12)
BiDg	55.75 (±4.63)	64.38 (±2.68)	62.27 (±2.35)	54.38 (±1.50)	60.42 (±2.04)
P-value	*0.08*	*0.45*	*0.37*	*0.15*	*0.31*

Following each least square mean value is the (±) standard error of the mean. P-values refer to the pair-wise comparison between Mix and Bi pasture types under the same LS criteria within the same seasons. *The grazing time does not add to 100 % due to time spent idling or ruminating. † Significant P-values.

Table 3
Effect of pasture type on the pre-grazing herbage mass, pre-grazing undisturbed sward height and lamina:stem ratio in each treatment during summer 1, autumn, winter, spring, and summer 2 as a pair-wise comparison (MixLp and BiLp; MixBv and BiBv; MixDg and BiDg) followed by respective *P-values.*

	Summer 1	Autumn	Winter	Spring	Summer 2
Pre-grazing herbage mass (ton DM/ha)					
MixLp	3.50 (±0.14)	3.65 (±0.19)	2.30 (±0.12)	3.92 (±0.07)	4.18 (±0.41)
BiLp	3.14 (±0.06)	3.26 (±0.26)	1.98 (±0.31)	3.44 (±0.47)	3.73 (±0.08)
P-value	*0.13*	*0.45*	*0.45*	*0.42*	*0.44*
MixBv	4.40 (±0.35)	2.94 (±0.14)	2.24 (±0.13)	3.52 (±0.22)	3.61 (±0.19)
BiBv	3.56 (±0.06)	2.34 (±0.11)	2.26 (±0.24)	3.30 (±0.35)	3.02 (±0.07)
P-value	*0.18*	*0.02*†	*0.95*	*0.62*	*0.15*
MixDg	5.37 (±0.20)	3.46 (±0.46)	2.21 (±0.27)	3.70 (±0.18)	3.38 (±0.20)
BiDg	4.90 (±0.70)	2.34 (±0.22)	2.32 (±0.05)	3.32 (±0.32)	3.02 (±0.58)
P-value	*0.55*	*0.08*	*0.76*	*0.21*	*0.54*
Pre-grazing undisturbed sward height (cm)					
MixLp	21.5 (±0.3)	23.1 (±1.5)	19.4 (±0.7)	31.6 (±2.3)	34.5 (±1.3)
BiLp	15.7 (±1.0)	17.6 (±1.8)	19.5 (±2.3)	30.0 (±1.1)	22.6 (±1.2)
P-value	*0.02*†	*0.02*†	*0.93*	*0.35*	*0.01*†
MixBv	19.6 (±0.5)	25.6 (±0.4)	21.6 (±2.3)	34.0 (±1.3)	36.1 (±1.6)
BiBv	13.0 (±0.7)	20.1 (±0.8)	21.1 (±0.5)	29.1 (±0.9)	21.9 (±0.6)
P-value	*0.01*†	*0.01*†	*0.86*	*0.10*	*0.02*†
MixDg	31.7 (±0.7)	17.2 (±0.9)	13.2 (±1.1)	36.9 (±2.5)	33.7 (±1.3)
BiDg	19.6 (±0.7)	16.0 (±0.4)	14.2 (±0.9)	33.3 (±1.2)	22.5 (±1.4)
P-value	*0.01*†	*0.16*	*0.28*	*0.27*	*0.002*†
Lamina:stem (ratio)					
MixLp	2.46 (±0.46)	1.87 (±0.13)	3.72 (±0.17)	1.31 (±0.04)	2.10 (±0.30)
BiLp	3.38 (±0.32)	2.02 (±0.11)	2.49 (±0.41)	1.31 (±0.19)	3.10 (±0.16)
P-value	*0.30*	*0.55*	*0.16*	*0.96*	*0.09*
MixBv	2.15 (±0.33)	3.07 (±0.09)	4.47 (±0.35)	1.36 (±0.08)	2.96 (±0.47)
BiBv	2.04 (±0.29)	3.49 (±0.39)	2.73 (±0.21)	1.37 (±0.09)	3.54 (±0.77)
P-value	*0.83*	*0.38*	*0.08*	*0.97*	*0.57*
MixDg	1.97 (±0.15)	2.30 (±0.31)	2.59 (±0.12)	0.57 (±0.08)	1.76 (±0.10)
BiDg	1.54 (±0.26)	1.98 (±0.12)	2.43 (±0.01)	0.84 (±0.04)	2.58 (±0.22)
P-value	*0.11*	*0.21*	*0.29*	*0.03*†	*0.06*

Following each least square mean value is the (±) standard error of the mean. P-values refer to the pair-wise comparison between Mix and Bi pasture types under the same LS criteria within the same seasons. † Significant P-values.

0.04), when BiBv presented 0.5 MJ/kg DM more than MixBv. Lastly, the ME content differed between MixDg and BiDg in summer 2 ($P = 0.04$), when BiDg presented 0.8 MJ/kg DM more than MixDg. The OMD percentage differed between MixLp and BiLp in spring ($P = 0.01$), with BiLp presenting the highest percentage. Also, the OMD percentage differed between MixDg and BiDg in summer 1 ($P = 0.04$), with BiLp presenting 6.1 % more OMD than MixDg (Table 4).

The NDF content differed between MixLp and BiLp and MixBv and BiBv in summer 2 ($P \leq 0.05$) and between MixDg and BiDg in autumn ($P = 0.01$), with all Mix treatments presenting higher values than Bi treatments (Table 5). The LGN content did not differ between pairs of treatments within seasons ($P > 0.05$; Table 5). The NSC content differed between MixLp and BiLp in autumn and spring ($P \leq 0.04$;), between MixBv and BiBv also in autumn and spring ($P \leq 0.03$), and between MixDg and BiDg in autumn, winter and spring ($P \leq 0.02$; Table 5). In all events where statiscally significant differences were found, the Bi treatments presented greater NSC content than the Mix treatments.

3.2. *Canonical variate analysis and proportions of variation*

Out of the eleven dependent variables of this study analysed via the univariate procedure, nine were selected to undergo a CVA (animal-related: GT and BR; plant morphology: HM, HT and LSR; plant nutritive parameters: CP, ME, NDF and NSC). These variables had a significant Wilk's Lambda value ($p < 0.0001$) and explained 71.1 % (CAN 1 = 37.9 %; CAN 2 = 33.2 %; Fig. 4) of the differences between treatments when the interaction of pasture treatments and seasons were analysed (MixLp, BiLp, MixBv, BiBv, MixDg and BiDg in summer 1, autumn, winter, spring and summer 2; Fig. 5). Along CAN 1 and CAN 2, the error is declared at ±0.3, in such a way that CAN 1 has four contributing response variables in the positive direction (GT, BR, CP, LSR) and two in the negative direction (HM, HT), while CAN 2 has four contributing variables in the positive direction (GT, HM HT, NDF), and three in the negative direction (BR, ME, NSC).

Within the pasture treatments, groupings due to the seasonal effect were found for spring and autumn, with winter highlighted by a circle in blue (Fig. 5). The pasture treatment grouping formed in spring was mostly explained by CAN 1, closely associated with HT and HM, and oppositely associated with GT, BR, CP and LSR. The pasture treatment grouping formed in autumn and winter was explained by CAN 1 and CAN 2, oppositely associated with HT and HM (CAN 1) and NDF (CAN 2), and neutrally to closely associated with GT, BR, CP and LSR (CAN 1) and with NSC and ME (CAN 2). For both summers, no grouping containing all treatments was found; however, along CAN 1, it was possible to observe that Mix treatments were always more closely associated with HT and HM (towards the left

Table 4
Effect of pasture type on the crude protein, metabolisable energy and organic matter digestibility content in each treatment during summer 1, autumn, winter, spring, and summer 2 as a pair-wise comparison (MixLp and BiLp; MixBv and BiBv; MixDg and BiDg) followed by respective *P-values.*

	Summer 1	Autumn	Winter	Spring	Summer 2
Crude protein (g/kg DM)					
MixLp	197 (±7.2)	245 (±2.3)	264 (±10.1)	179 (±8.5)	170 (±0.5)
BiLp	187 (±2.0)	206 (±30.7)	259 (±18.5)	153 (±3.0)	192 (±2.0)
P-value	*0.20*	*0.64*	*0.78*	*0.10*	*0.04*†
MixBv	184 (±2.9)	228 (±4.2)	286 (±8.1)	179 (±8.3)	189 (±0.9)
BiBv	169 (±5.2)	211 (±1.1.4)	254 (±16.3)	151 (±2.9)	197 (±2.8)
P-value	*0.19*	*0.36*	*0.13*	*0.09*	*0.17*
MixDg	191 (±4.5)	211 (±8.7)	240 (±11.9)	191 (±5.5)	214 (±8.4)
BiDg	175 (±0.8)	230 (±8.6)	187 (±9.2)	182 (±13.6)	233 (±10.6)
P-value	*0.07*	*0.002*†	*0.01*†	*0.43*	*0.42*
Metabolisable energy (MJ/kg DM)					
MixLp	10.3 (±0.08)	10.8 (±0.13)	11.3 (±0.20)	10.9 (±0.06)	9.3 (±0.13)
BiLp	10.0 (±0.08)	11.2 (±0.31)	11.6 (±0.13)	11.1 (±0.06)	9.8 (±0.22)
P-value	*0.03*†	*0.49*	*0.42*	*0.08*	*0.21*
MixBv	10.1 (±0.24)	11.1 (±0.09)	11.5 (±0.09)	11.0 (±0.12)	9.7 (±0.10)
BiBv	10.1 (±0.26)	11.7 (±0.20)	12.0 (±0.03)	11.2 (±0.15)	9.9 (±0.08)
P-value	*0.96*	*0.35*	*0.04*†	*0.55*	*0.26*
MixDg	10.3 (±0.09)	10.2 (±0.39)	12.5 (±0.10)	11.2 (±0.22)	9.9 (±0.18)
BiDg	9.9 (±0.15)	11.2 (±0.19)	12.8 (±0.03)	11.8 (±0.12)	10.7 (±0.01)
P-value	*0.18*	*0.12*	*0.13*	*0.07*	*0.04*†
Organic matter digestibility (%)					
MixLp	71.5 (±0.72)	76.9 (±1.19)	80.9 (±1.22)	75.7 (±0.44)	66.0 (±1.10)
BiLp	70.2 (±0.44)	78.9 (±2.74)	83.4 (±0.30)	78.5 (±0.28)	70.0 (±1.23)
P-value	*0.05*†	*0.65*	*0.21*	*0.01*†	*0.16*
MixBv	70.0 (±1.74)	77.4 (±0.72)	82.2 (±0.34)	77.2 (±0.50)	67.8 (±0.72)
BiBv	70.5 (±1.23)	81.2 (±1.11)	84.9 (±0.38)	79.4 (±0.79)	70.3 (±0.77)
P-value	*0.83*	*0.18*	*0.06*	*0.22*	*0.05*
MixDg	73.7 (±1.21)	73.2 (±3.15)	86.6 (±0.88)	78.1 (±1.86)	70.0 (±1.38)
BiDg	72.0 (±0.86)	79.5 (±0.63)	88.7 (±0.47)	82.5 (±0.79)	76.1 (±0.33)
P-value	*0.30*	*0.20*	*0.19*	*0.09*	*0.04*†

Following each least square mean value is the (±) standard error of the mean. P-values refer to the pair-wise comparison between Mix and Bi pasture types under the same LS criteria within the same seasons. † Significant P-values.

side) than their respective Bi treatment, which were more oppositely associated to HT and HM (towards the right side) (Fig. 5).

The same nine variables in the CVA were used in an F-value test to obtain the proportion of contribution in a variation of each response variable within the experiment (Table 6). The season was the only fixed effect that was significant ($Pr<0.0001$) for all tested variables, consistently presenting the highest proportion of contribution in variation (highest V values). The season factor contributed to 69 % of variation occurring in GT, 48 % in BR, 72 % in HM, 77 % in HT, 74 % in LSR, 87 % in CP content, 89 % in ME, 72 % in NDF and 42 % in NSC. Therefore, season was the main source of variation for all tested response variables in the present study. In addition to that, following the season effect of variation, pasture type presented a relatively high percentage of contribution in the variation occurring for BR (18 %), NDF (22 %) and NSC (38 %).

3.3. *Animal behaviour activity*

Because the fixed effect ‘season’ was the greatest contributor to the variation found in the response variables, a graph with the seasonal fluctuations in animal behaviour (grazing, rumination or idleness) was plotted to depict changes across seasons according to each of the three different defoliation criteria (*L. perenne* LS, *B. valdivianus* LS and *D. glomerata* LS) utilising an average between both binary and diverse pastures values (Fig. 6). Animals grazing the pasture treatments defoliated according to the *L. perenne* LS interval presented little fluctuation in GT time across seasons, spending over 70 % of their time grazing in autumn, winter and spring and over 90 % in both summers. Still, within these treatments, the time spent by the animal in rumination and idleness was often around 10 % and did not present major fluctuations within seasons. On the other hand, animals grazing pastures defoliated according to the *B. valdivianus* LS and *D. glomerata* LS presented much more seasonal fluctuation in their behavioural activity. For *B. valdivianus* LS, the most striking feature in animal behaviour changes is a major shift starting in winter and peaking in spring, in which, at the expense of grazing time, animals begin to spend more time idling and secondarily ruminating. Similarly, but more markedly, the shift in the behavioural activity of the animals grazing pastures, according to *D. glomerata* LS, also began in winter and remained during spring, both seasons in which animals spent around 60 % of their time grazing, and from 10 % to 30 % of their time idling or ruminating.

4. Discussion

The univariate analysis showed recurrent significant differences in HT and NSC within the pair-wise comparison of binary and diverse pastures, which could have driven cows to exhibit preference. However, no significant differences were found in the percentage

Table 5

Effect of pasture type on the neutral detergent fibre, lignin and non-structural carbohydrates content in each treatment during summer 1, autumn, winter, spring, and summer 2 as a pair-wise comparison (MixLp and BiLp; MixBv and BiBv; MixDg and BiDg) followed by respective *P-values.*

	Summer 1	Autumn	Winter	Spring	Summer 2
Neutral Detergent Fibre (g/kg DM)					
MixLp	517 (±3.1)	459 (±13.1)	461 (±5.8)	504 (±11.8)	549 (±6.8)
BiLp	520 (±7.0)	435 (±20.4)	449 (±12.7)	488 (±05.8)	487 (±8.0)
P-value	*0.68*	*0.55*	*0.55*	*0.83*	*0.05*†
MixBv	471 (±16.7)	452 (±9.0)	458 (±7.8)	482 (±0.3)	547 (±9.2)
BiBv	477 (±6.9)	422 (±8.3)	420 (±13.3)	477 (±15.9)	488 (±4.9)
P-value	*0.80*	*0.20*	*0.04*†	*0.78*	*0.01*†
MixDg	480 (±10.4)	490 (±5.0)	398 (±3.3)	468 (±9.0)	533 (±16.9)
BiDg	481 (±9.2)	439 (±3.8)	383 (±4.7)	433 (±5.8)	467 (±5.8)
P-value	*0.96*	*0.01*†	*0.21*	*0.14*	*0.09*
Lignin (g/kg DM)					
MixLp	84 (±3.1)	49 (±0.6)	61 (±9.3)	60 (±1.5)	92 (±0.9)
BiLp	86 (±5.8)	61 (±4.5)	69 (±8.1)	52 (±2.5)	84 (±6.3)
P-value	*0.86*	*0.09*	*0.49*	*0.12*	*0.34*
MixBv	78 (±3.2)	47 (±5.0)	67 (±3.1)	65 (±3.2)	85 (±4.0)
BiBv	89 (±6.1)	53 (±4.9)	67 (±6.5)	52 (±0.9)	79 (±2.8)
P-value	*0.09*	*0.51*	*0.90*	*0.06*	*0.34*
MixDg	88 (±6.6)	99 (±8.7)	69 (±2.2)	54 (±1.2)	82 (±1.7)
BiDg	93 (±12.2)	67 (±6.3)	60 (±3.4)	60 (±3.2)	73 (±1.9)
P-value	*0.59*	*0.06*	*0.10*	*0.10*	*0.12*
Non-structural carbohydrates (g/kg DM)					
MixLp	151 (±9.9)	131 (±8.8)	108 (±9.7)	178 (±11.5)	133 (±3.2)
BiLp	147 (±10.1)	208 (±22.1)	118 (±35.3)	210 (±14.3)	162 (±9.4)
P-value	*0.83*	*0.02*†	*0.91*	*0.04*†	*0.11*
MixBv	209 (±12.2)	170 (±7.8)	187 (±19.4)	192 (±7.6)	123 (±6.5)
BiBv	214 (±2.8)	227 (±7.5)	163 (±31.0)	221 (±22.6)	164 (±2.1)
P-value	*0.64*	*0.02*†	*0.46*	*0.03*†	*0.41*
MixDg	170 (±5.4)	132 (±15.9)	217 (±19.7)	196 (±10.1)	108 (±8.0)
BiDg	168 (±26.0)	176 (±11.8)	297 (±12.1)	243 (±7.9)	147 (±4.3)
P-value	*0.92*	*0.02*†	*0.01*†	*0.52*	*0.01*†

Following each least square mean value is the (±) standard error of the mean. P-values refer to the pair-wise comparison between Mix and Bi pasture types under the same LS criteria within the same seasons. † Significant P-values.

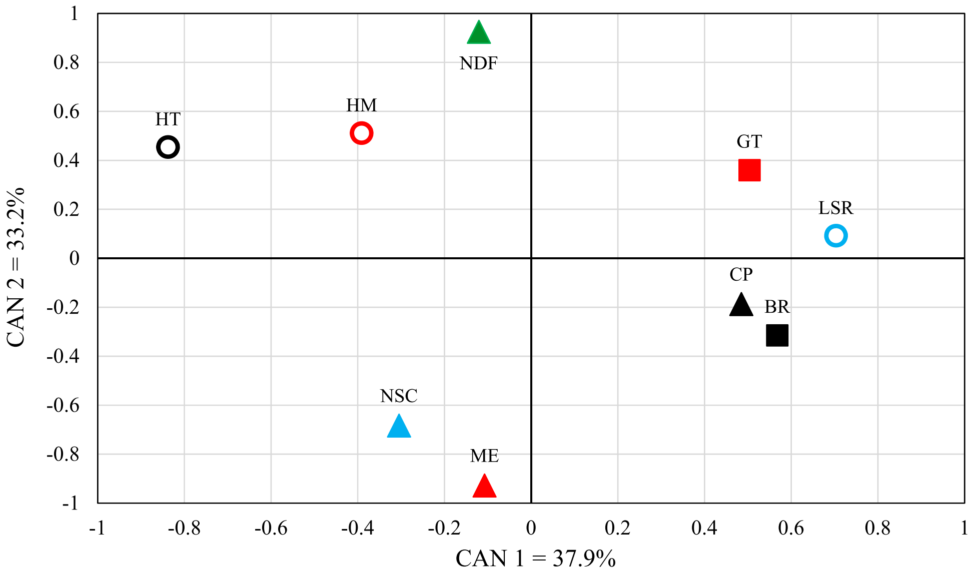

Fig. 4. Distribution of the original response variables according to their weight on the first two canonical variates (CAN 1 and CAN 2). Animal-related variables: Grazing time (GT; black triangle), bite rate (BR); Plant morphology variables: pre-grazing herbage mass (HM), pre-grazing undisturbed sward height (HT), lamina:stem ratio (LSR); Plant nutritive parameters: crude protein (CP), metabolisable energy (ME), non-structural carbohydrates (NSC) and neutral detergent fibre (NDF). COLOURED IMAGE.

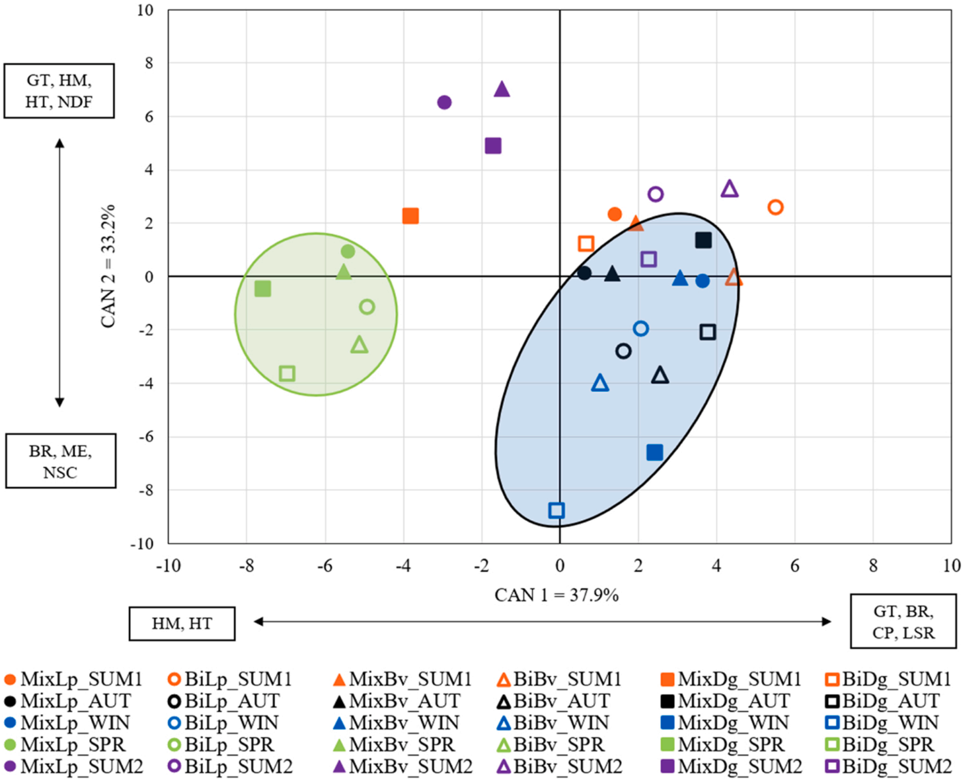

Fig. 5. Canonical scores for the interaction of fixed effect pasture (MixLp, BiLp, MixBv, BiBv, MixDg, BiDg) and season (SUM1: summer 1, AUT: autumn, WIN: winter, SPR: spring, SUM2: summer 2) averaged by the random effect of the block. Arrows transpose the significant (>0.3 or <-0.3) response variables for CAN 1 (x-axis) and CAN 2 (y-axis). Response variables abbreviations: Grazing time (GT), bite rate (BR), pre-grazing herbage mass (HM), pre-grazing undisturbed sward height (HT), lamina:stem ratio (LSR), crude protein (CP), metabolisable energy (ME), non-structural carbohydrates (NSC) and neutral detergent fibre (NDF). COLOURED IMAGE.

Table 6

F ratio values (F Value), statistical significance (Pr > F), and variation proportion (V) explained by the effects (random = block; fixed = pasture type, season and interaction pasture type x season) utilised in the model for canonical multivariate analysis per each response variable (grazing time, bite rate, pre-grazing herbage mass, pre-grazing undisturbed sward height, lamina:stem ratio, crude protein, metabolisable energy, neutral detergent fibre and non-structural carbohydrates).

	Grazing time			Bite rate			Pre-grazing herbage mass		
	F Value	***Pr > F***	**V**	**F Value**	***Pr > F***	**V**	**F Value**	***Pr > F***	**V**
Block	1.61	*0.2103*	9.44 %	5.42	*0.0069*	25.49 %	8.68	*0.0005*	13.27 %
Pasture type	2.22	*0.0671*	13.02 %	3.9	*0.0041*	18.34 %	5.52	*0.0003*	8.44 %
Season	11.82	*<.0001*	69.33 %	10.11	*<.0001*	47.55 %	47.34	*<.0001*	72.40 %
Pasture*Season	1.4	*0.1713*	8.21 %	1.83	*0.038*	8.61 %	3.85	*<.0001*	5.89 %
	Pre-grazing undisturbed sward height			Lamina:stem ratio			Crude protein		
	F Value	***Pr > F***	**V**	**F Value**	***Pr > F***	**V**	**F Value**	***Pr > F***	**V**
Block	2.61	*0.0818*	1.32 %	0.62	*0.543*	1.14 %	0.7	0.502	1.08 %
Pasture type	30.32	*<.0001*	15.31 %	9.51	*<.0001*	17.46 %	2.45	0.0444	3.80 %
Season	152.85	*<.0001*	77.20 %	40.26	*<.0001*	73.93 %	56.39	<.0001	87.40 %
Pasture*Season	12.22	*<.0001*	6.17 %	4.07	*<.0001*	7.47 %	4.98	<.0001	7.72 %
	Metabolisable energy			Neutral detergent fibre			Non-structural carbohydrates		
	F Value	***Pr > F***	**V**	**F Value**	***Pr > F***	**V**	**F Value**	***Pr > F***	**V**
Block	1.15	*0.3253*	0.65 %	0.06	*0.9423*	0.07 %	0.45	*0.6399*	1.21 %
Pasture type	13.14	*<.0001*	7.37 %	20.19	*<.0001*	22.18 %	14	*<.0001*	37.61 %
Season	158.19	*<.0001*	88.77 %	65.9	*<.0001*	72.40 %	15.52	*<.0001*	41.70 %
Pasture*Season	5.73	*<.0001*	3.22 %	4.87	*<.0001*	5.35 %	7.25	*<.0001*	19.48 %

of time that cows spent grazing binary or diverse pastures under the same LS criteria for defoliation within the same season. Further multivariate and univariate analysis highlighted that season was the greatest contributor to the proportion of variation found in all response variables. Within the same season, LS defoliation criteria were responsible for modulating cows' grazing behaviour

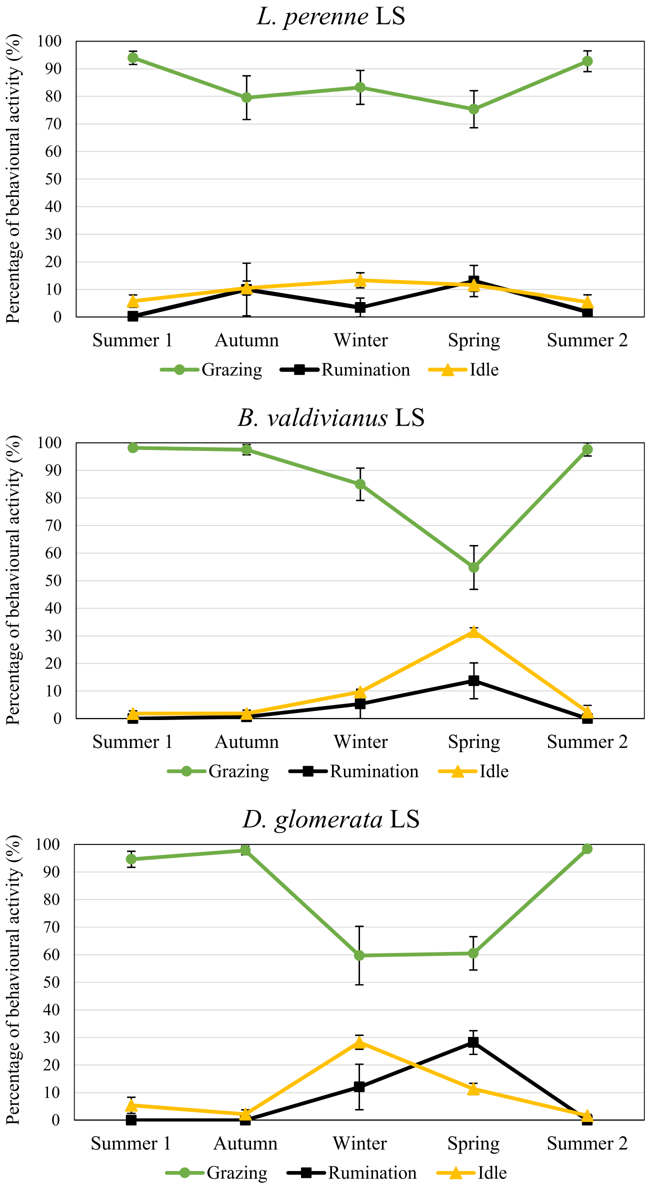

Fig. 6. Seasonal percentage fluctuation (summer 1, autumn, winter, spring and summer 2) of the behavioural activity (grazing, ruminating or idle) exerted by dairy animals during their first two hours in a pasture sward after the morning milking period. Graphs were done using an average between binary and diverse pasture behaviour values according to each LS interval criterion. The vertical bars indicate the (±) standard error of the mean per activity within each season.

(proportional time spent grazing, ruminating, or idling).

5. The role of pasture productivity and morphology on the dietary preference of dairy cows

The utilisation of plant-focused criteria (LS) to define the most appropriate time for defoliation is an effective alternative to enhance pasture productivity and persistence (Donaghy et al., 2021) (Fig. 2). However, different grass species will present different intervals for optimal defoliation (Fulkerson and Donaghy, 2001; Ordóñez et al., 2021; Turner et al., 2006). Consequently, in a rotational grazing system containing multispecies mixes, not always all species will be coincidently and simultaneously 'prepared' for defoliation. For the case of this study, this temporal mismatching between species is noteworthy in such a way that pasture treatments following *L. perenne* LS were under a more frequent defoliation basis, followed by *B. valdivianus* LS and, ultimately, *D. glomerata* LS (less frequent defoliation events).

Grazing frequencies directly affect plant growth accumulation, morphological and structural characteristics, and nutritive parameters (Gordon, 2000; Hodgson, 1990). Thus, clarifying the consequences of grazing frequencies on animal dietary preference is essential to understanding the role and potential suitability of the LS defoliation interval as a criterion for diverse pastures.

In the present study, the herbage mass offered did not differ between the pairs of treatments (Bi versus Mix), with only one exception out of fifteen grazing events (MixBv versus BiBv in autumn; Table 2). Several studies have found that the herbage on offer strongly influences dietary preference (Demment et al., 1993; Illius et al., 1992; Kenney and Black, 1986), process related to potential dry matter intake and plant density within an area and the consequent energetic efficiency for the animal once it invests its time grazing the given area (Kacelnik and Bernstein, 1988). Since no significant differences in HM were found in the pair-wise comparisons, it is possible to affirm that, for the present study, herbage allowance was not a factor of influence. Such results enhanced the robustness of this study because animals had the chance to choose and show preference, if any, without external interferences caused by herbage on offer and potential intake.

Unlike HM, the HT presented several significant differences between Bi and Mix treatments over time (Table 2). In the literature, the influence of sward height on animal dietary preference is contradictory. Forbes and Hodgson (1985) found significant differences in the time spent grazing (625 ± 15 minutes/day against 580 ± 16 minutes/day) two different *L. perenne* treatments with statistically similar sward heights (~ 18.3 cm). Anecdotally, Smit et al. (2006) found that there were no significant relationships between cattle preference and pre-grazing surface sward heights when testing six cultivars of *L. perenne* in three different experimental arrangements (experiment 1: ~ 18.6 cm; experiment 2: ~ 14.0 cm; experiment 3: ~ 25.4 cm). Other studies, however, suggest that cattle tend to prefer higher swards (Bailey, 1995; Gibb et al., 1997; Griffiths et al., 2003a, b). Griffiths et al. (1997) carried out a study in which *L. perenne* pasture treatments presented progressively increasing targets for pre-grazing sward height (8.9–19.6 cm). They found that cows regularly sampled from all but the shortest swards (8.9 cm). These contradictions may be explained by potential minimum thresholds limiting animal preference so that, at pre-grazing conditions, only the extremely short swards [i.e. 8.9 cm in Griffiths et al. (1997)] represent a non-preferred area for cattle. In the present study, although differences in HT between binary and diverse pastures were found, they did not affect the time that cows spent grazing (Tables 1 and 2), likely because all HT were relatively high and above a potential minimum threshold.

6. The role of pasture nutritive value on the dietary preference of dairy cows

Beyond the structural characteristics of the sward, plant nutritive parameters can also affect animal preference for species (Horadagoda et al., 2009) or cultivars (Smit et al., 2006). Plant nutritive parameters are highly affected by biotic factors, such as the choice and proportion of each species in a mix (Rutter et al., 2004), the defoliation intensity and the frequency of grazing (Waghorn and Clark, 2004). Because nutritive parameters can drive animals' preference and selection (Provenza et al., 2007; Waghorn and Clark, 2004), the statistical approach of this study adopted two analyses. Firstly, the univariate analysis between a pair of treatments focused on the comparison between treatments under the same LS defoliation criterion. Secondly, the multivariate analyses (see next section) embraced broader differences among treatments occurring due to seasonal fluctuations and different LS defoliation criteria.

Among nutritive parameters, WSC is a factor of great influence on grazing preference and selectivity. In a cafeteria study carried out over eight seasons, Horadagoda et al. (2009) found that cows spent more time grazing *B. willdenowii*, *Pennisetum clandestinum* Chiov., *T. repens*, *M. sativa*, and less time grazing *F. arundinacea, Paspalum dilatatum* Poir., *C. intybus* and *P. lanceolata*. Horadagoda et al. (2009) concluded that animal preference can be reasonably well predicted in pastures comprised of plants with higher WSC content and lower nitrate-nitrogen content. Similarly, Smit et al. (2006), in a study with six cultivars of *L. perenne*, also found that cows preferred the pastures containing higher concentrations of WSC.

In the present study, significant differences in NSC (for temperate pastures, analogous to WSC) content were found between Mix and Bi treatments (Table 5). However, they did not appear to greatly impact cows' preferences, as seen in Table 6, where the pasture type is non-significant Pr = 0.0671) for the proportion of variation (V = 13.02 %) in grazing time. This may be associated with the fact that WSC values were still relatively high, with an average of 16.11 % for Mix and 21.22 % for Bi in grazing events where the treatments differed, as opposed to the rejected species (*F. arundinacea, Paspalum dilatatum* Poir., *C. intybus* and *P. lanceolata*) in Horadagoda et al. (2009) presenting a WSC mostly below 10 %, and to the rejected cultivars (*L. perenne* cv. Agri, cv. Barezane, cv. Herbie, cv. Respect) in Smit et al. (2006) presenting WSC ranging from 10.46 % to 8.17 %.

The fibre content in grasses, although necessary to maintain rumen function (AFRC, 1993; SCA, 1990), has often been associated with a negative impact on dairy cows' selection and preference (Horadagoda et al., 2009; Jacobs et al., 1999; Smit et al., 2006). In particular, the lignin fraction of fibre content in grasses has a direct impact on tensile strength (Baumont et al., 2000), which makes difficult feed prehension and utilisation (Inoué et al., 1994). In the present study, no differences between LGN content were found between Mix and Bi treatments within the same season, which corroborates the cows not exhibiting preference.

7. Seasonal fluctuations and defoliation criteria influencing dairy cows' behavioural activity

Within seasons, the pair-wise comparisons (Bi versus Mix) did not show significant differences in the time cows spent grazing. In this study, cows' preference was mostly driven by seasonal effects rather than pasture type. Changes in the patterns of grazing behaviour (GT and BR) according to the seasons were found in the CVA (Figs. 4 and 5) and F-value analysis (Table 5). For all the response variables shown in Table 5, 'season' presents the greatest percentage contributing to variation.

Seasonal fluctuations in the nutritive value occur due to changes in plant physiology, phenology and growth stages (O'Reagain and Schwartz, 1995). As an active response to these seasonal fluctuations in pasture quality, cows modulate their grazing behaviour. In a three-year farm-level study conducted by Iqbal et al. (2022) with cows continuously monitored by automated collar devices, the season was the second most influential source of variance (after individual cow choice) and explained 5–12 % of the variation in time spent grazing. In a study by Cullen et al. (2017), the nutritive value of vegetative and reproductive tillers of four grass pure stands (*L. perenne, D. glomerata, B. wildenowii* and *F. arundinacea*) were assessed in winter (only vegetative) and spring. For all four species, the nutritional drop in quality is explained by the growth of reproductive tillers, when, in spring, CP and ME significantly decreased while fibre content significantly increased. These studies corroborate the CVA 'green' grouping in Fig. 5, when the morphological and nutritional characteristics of the plants '*per se*' became less preferable to the animals. That grouping depicts a close association of spring with HM and HT and an opposite association with GT, BR, CP and LSR.

Fluctuations in dietary behaviour can also be attributed to nutritive quality and morphological structure as affected by grazing management criteria. In the present study, grazing based on the *D. glomerata* LS resulted in less frequent grazing events [see Oliveira et al. (2023)]. In spring, Mix and Bi pastures under *D. glomerata* LS presented extremely low LSR, respectively 0.57 and 0.84 (Table 2). These are similar to the results found in a study by Griffiths et al. (1997), showing that animals prefer swards with a high LSR (2.53 ± 0.0004), deliberately avoiding the swards with a low LSR (0.74 ± 0.0004). In that respect, it is possible to conclude that changes in behavioural activities within spring were partially driven by changes in plant morphological characteristics caused by different LS criteria grazing intervals.

Furthermore, these changes in behavioural activities as affected by grazing management criteria can be well exemplified by two results of this present study. Firstly, by the marked shift in the behavioural activity when grazing pastures defoliated according to *D. glomerata* LS, in which cows spent 60 % of their time grazing and 40 % idling or ruminating during winter and spring (Fig. 6). Secondly, by the 'green' grouping in spring (Figs. 4 and 5), where Mix and Bi pastures defoliated according to *D. glomerata* LS are located the furthest from GT and BR.

According to the results presented in this study and corroborating previous results in the same experimental area by Oliveira et al. (2023), grazing management is critical to maintaining high-quality nutritive parameters in pastures as a continuous attempt to buffer inherent and detrimental seasonal effects. In addition, this study shows that the positive effects of defoliation management based on LS may also extend itself to positive outcomes in animal preference, interpreted as the percentage of time dairy cows spend grazing rather than ruminating or idling.

Finally, the initial study hypothesis is rejected since, within the same seasons, dairy cows did not exhibit preference - as in time spent grazing - when unrestrictedly offered binary (*L. perenne* and *T. repens;* Bi) or diverse pastures (*L. perenne, B. valdivianus, D. glomerata*, and *T. repens;* Mix) that had been subjected to the same LS defoliation criteria.

8. Conclusion

Most of the differences detected by pair-wise comparisons showed that HT and WSC often differ between binary (*L. perenne* and *T. repens*) and diverse pastures (*L. perenne, B. valdivianus, D. glomerata* and *T. repens*) under the same LS interval of defoliation within the same season. However, these differences did not influence the actual time that animals spent grazing either binary or diverse pastures. Therefore, the present study provided evidence that dairy cows did not exhibit any grazing preference for pastures subjected to the same grazing management.

In this study, pastures that were less frequently defoliated (*D. glomerata* LS) had animals spending less time actively grazing during winter and spring, increasing the time spent idling and ruminating. Furthermore, seasonal effects contributed the greatest to the variation occurring in the response variables. These findings show that grazing management and seasonal effects are more relevant than plant species diversity for modulation of grazing behaviour. Diverse pastures comprising *L. perenne, B. valdivianus, D. glomerata* and *T. repens* do not affect dairy cows' grazing preference and can, therefore, be considered a reliable option for dairy farmers that wish to diversify their pasture-based systems.

Ethical approval

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

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CRediT authorship contribution statement

Bia Anchão Oliveira: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ignacio F. López:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Lydia M. Cranston:** Writing – review & editing, Supervision, Investigation, Formal analysis, Conceptualization. **Cesar H. E. C. Poli:** Writing – review & editing, Methodology, Conceptualization. **Peter D. Kemp:** Writing – review & editing, Conceptualization. **Daniel J. Donaghy:** Writing – review & editing, Funding acquisition, Conceptualization. **Ina Draganova:** Writing – review & editing, Conceptualization. **Nicolas López-Villalobos:** Writing – review & editing, Validation, Software, Methodology, Formal analysis.

Declaration of Competing Interest

The authors declare no conflict of interest

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