

RESEARCH ARTICLE

Effect of three defoliation frequency treatments and drought on perennial ryegrass herbage and root growth and water-soluble carbohydrate reserves

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Handling editor: Charlie Brummer

Funding information

Strategic Science Investment Fund, Grant/Award Number: C10X1702

Abstract

Background: Grazing approaches are needed to increase the resilience of perennial ryegrass (*Lolium perenne* L.)-based pastures subject to increasing drought stress. One opportunity has focused on seedhead management in late spring. Paddock-level studies demonstrated increased pasture resilience when ryegrass seedheads are allowed to mature, but knowledge is lacking on how defoliation management affects plant carbohydrate status and hence resilience in the sward.

Methods: A glasshouse study was conducted from spring to autumn using 1 m deep root tubes. Plant growth and water-soluble carbohydrate (WSC) reserves were measured every 4–6 weeks. Defoliation treatments comprised “VEGETATIVE”—regular defoliation based on leaf stage and trimmed to 4 cm; “FLOWERING”—no defoliation spring to anthesis; and “SENESCENT”—no defoliation spring to reproductive tiller senescence. Thereafter, regular defoliation was carried out for all treatments until the end of the study. From spring to the end of summer, plants were watered daily in WET (no drought, well watered) and on four occasions in DRY (drought) treatments, with daily watering thereafter.

Results: Herbage mass, tillering, root depth, root mass, and WSC were generally higher in SENESCENT than VEGETATIVE with FLOWERING intermediate ($p < 0.05$). Nutritive values were similar in VEGETATIVE and FLOWERING, but in SENESCENT, metabolizable energy and crude protein declined and neutral detergent fiber increased ($p < 0.05$). Soil moisture effects were small, with the DRY treatment resulting in moderate suppression of herbage growth and a minor reduction in WSC reserves ($p < 0.05$).

Conclusions: Results were consistent with field studies and recommendations to allow perennial ryegrass tillers to set seed to improve pasture resilience.

KEYWORDS

deferred grazing, grazing management, moisture deficit stress, multiple stresses, pasture persistence, pasture quality, pasture resilience

INTRODUCTION

When the climate is conducive for perennial ryegrass (*Lolium perenne* L.) growth, pastures can be grazed intensively throughout the year, with a focus on maintaining high-quality leafy herbage particularly during spring (e.g., Tallowin, 1981). In these situations, the “2-leaf-stage” grazing management principle can be

applied, where an average minimum of two new leaves have appeared on perennial ryegrass tillers since the last grazing. This is considered sufficient to allow water-soluble carbohydrate (WSC) reserves to be replenished and tiller populations to be maintained (Donaghy & Fulkerson, 1998). However, in temperate and subtropical regions, such as in New Zealand, Australia, and South Africa, drought stress over late spring and summer, and

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associated summer-active weed ingress, is a major contributor to the collapse of perennial ryegrass populations (Donaghy et al., 2021; McKenzie, 1997; Tozer et al., 2011). Given increasing climatic variability in many temperate regions, and particularly increasing summer temperatures and drought stress, the 2-leaf-stage principle may no longer be sufficient to restore reserves (Tozer et al., 2022); thus, new strategies are required to ensure persistence of tiller populations.

Swards have traditionally been managed to maintain a green, leafy vegetative state to increase pasture quality and minimize the production of lower nutritive value seedhead (Lambert & Litherland, 2000). Intensive grazing in spring resulting in a short, leafy sward can also increase tiller densities (Boswell & Crawford, 1978). However, the leaf area may be inadequate to maintain the high population of small tillers (Matthew et al., 1996), and persistence may decline. Severe repeated defoliation can also reduce root growth (Evans, 1971, 1973), which may contribute to a decline in persistence.

Alternative extended spring rotation defoliation strategies, which allow increased late spring seedhead development and improve persistence, have been explored (Matthew et al., 2000). This involved lax grazing during spring to enable reproductive tiller development, followed by intensive grazing of the pasture to remove the reproductive tillers (Matthew et al., 2000). Although energy reserves within the plant are prioritized for reproductive tiller development, a small portion (e.g., 3%–4%) of the reserves present in a flowering tiller are translocated to the base for daughter tiller development (Matthew, 2002). While this is a small amount of the total carbon, it would provide a biologically significant input for the development of the small daughter tillers and is likely to be a fundamental physiological plant process that underpins the daughter tillering response. Once the apical meristems are removed by grazing, and light can reach the base of the plants, dormant daughter tillers develop, resulting in increased tillering and leaf growth (Garay et al., 1997). These new tillers are more likely to survive and contribute to the tiller population in the following year than tillers produced from adult vegetative tillers (Waller et al., 2003). Da Silva et al. (2004) found a significant increase in herbage and milk production in simulated farm systems using “late control” of seedheads compared to standard grazing. However, while anthesis may be easier for farmers to assess visually than the leaf stage, it only allows a narrow window for grazing. There were also negligible benefits of this strategy for tiller populations or herbage production, when compared to standard rotational grazing, in summer wet or summer dry hill country in the upper North Island of New Zealand (Tozer, Müller, et al., 2021).

Another approach is that of *deferred grazing*, where perennial ryegrass is allowed to undergo full reproductive development and produce mature seeds, after which livestock graze the accumulated herbage in late summer/early autumn and daughter tiller growth is promoted (Tozer, Müller, et al., 2021). This is dependent on there being a spring pasture surplus and the ability of farmers to meet the feed demand of livestock from the pastures that are not deferred. This strategy is well suited, for example, to many New Zealand sheep

and beef hill country pastures when spring pasture growth is abundant (Tozer, Howarth, et al., 2021).

The livestock exclusion (i.e., “deferred”) period typically extends from mid-spring, when stem elongation begins, through to late summer, when seed fall has occurred. During perennial ryegrass seed development and maturation, WSC in the reproductive tillers is translocated to vegetative tillers (Clemence & Hebblethwaite, 1984). It has also been demonstrated that the yield of perennial ryegrass seed is proportional to the amount of new tiller growth (Rolston et al., 2007), especially in mature seedhead (Clemence & Hebblethwaite, 1984).

Additionally, when compared to standard rotational grazing and to the extended spring rotation strategies, deferred grazing enables a longer period of uninterrupted root growth in the absence of grazing defoliation and a longer period for WSC accumulation, both of which benefit pasture regrowth and persistence (Donaghy & Fulkerson, 1998; Tozer, Douglas, et al., 2021).

No published studies are available that explore the impact of these strategies on perennial ryegrass herbage growth, tillering, and root growth in relation to WSC reserves. We therefore conducted a glasshouse study, using simulated grazing defoliation and drought treatments, to generate a data set that integrated plant physiological parameters with agro-ecological plant traits. The hypothesis tested was as follows: “Herbage accumulation, tillering, root growth, and WSC accumulation would be lowest in a standard rotational grazing, intermediate in an extended spring rotation, and greatest in a deferred grazing treatment, in both summer wet and summer dry conditions.” Further, in view of the inherent heterogeneity in field swards, it was considered that a controlled glasshouse experiment was the ideal way to test the impact of defoliation strategies on WSC reserves.

MATERIALS AND METHODS

Glasshouse conditions

The experiment was conducted over 25 weeks (September, 2017–April, 2018), incorporating a 16-week moisture stress period and a 9-week recovery period (Table 1), in a glasshouse at the Ruakura Research Centre in Hamilton, New Zealand (37°47′ S, 175°19′ E; 40 m a.s.l.). The mean maximum and minimum temperatures in the glasshouse were 35°C and 13°C during the stress period and 31°C and 12°C during the recovery period, respectively. Maximum temperatures, averaged over each week, exceeded 30°C during all but one of the weeks of the stress period and one of the weeks in the recovery period. The average daylength during the months of October 2017, November 2017, December 2017, January 2018, and February 2018 was 13.1, 14.1, 14.4, 14.3, and 13.4 h, respectively.

Plant material

The study used cv. Grasslands Samson diploid perennial ryegrass infected with the fungal endophyte *Epichloë* sp.

TABLE 1 Timing of moisture stress and defoliation treatment application, trimming, and measurements for Samson perennial ryegrass.

Item	Moisture stress period (Weeks 1–16)																Recovery period (Weeks 17–25)								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Trim harvests	T1		T2			T3					T4					T5				T6					T7
Destructive harvests	DH1					DH2					DH3					DH4									DH5
Date	October 24		November 13			December 6					January 8					February 12				March 14					April 17
Defoliation treatments																									
VEGETATIVE	X		X			X					X					X				X					X
FLOWERING						X					X					X				X					X
SENESCENT																X				X					X
Drought treatments																									
DRY	W					W					W					W	W	W	W	W	W	W	W	W	W
WET	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
Measurements																									
Leaf morphology ^a	Y		Y			Y					Y					Y				Y					Y
Other plant traits ^b	Y					Y					Y					Y									Y

Note: Destructive harvest 1 (DH1) occurred and stresses were applied on Day 1 (October 24) of the stress period; destructive harvest 2 (DH2) occurred in Week 6 (December 6); destructive harvest 3 (DH3) occurred on Week 11 (January 8); and destructive harvest 4 (DH4) occurred in Week 16 on the last day of the stress period (February 12). Plants in the WET treatment were watered daily. During the stress period, plants in the DRY treatment were watered once in October, December, and January and watered daily from T5 onwards. All plants were well watered on the first day of the recovery period, and destructive harvest 5 (DH5) occurred on the last day of the recovery period (April 17). A single cohort, comprising eight replicates of each of the six treatment combinations, was destructively sampled on each harvest occasion. The VEGETATIVE treatment only was trimmed to 4 cm on November 13 (T2) during the stress period when at least 50% of tillers had produced more than 2.5 new leaves since the previous defoliation event and all treatments were trimmed to 4 cm on March 14 (T6) during the recovery period when at least 50% of tillers had produced more than 2.5 new leaves since the previous defoliation event. The FLOWERING treatment was defoliated seven times, the FLOWERING treatment was defoliated five times, and the SENESCENT treatment was defoliated three times during the experiment. Blue bars: no defoliation occurred between September 15 and T3, which allowed at least 50% of the reproductive tillers to flower (flowering treatment), or T5, which allowed 50% of the reproductive tillers to senesce and drop seed (senescent treatment). “X” denotes defoliation events; “W” denotes weeks when pots were watered in the drought treatments (“WET” [no drought] and “DRY” [drought]; and “Y” denotes measurements taken).

^aNo herbage harvested from the beginning of the study until T3 for the Flowering Treatment or T5 for the Senescent treatment.

^bLeaf biomass and number of new leaves.

^cOther plant traits: Survival, tiller number, leaf biomass, stubble biomass, crown biomass, root biomass, root length, and water-soluble carbohydrate.

LpTG-3 strain AR37. Samson contains Spanish germplasm and is a mid-heading variety, with a standard heading date of October 22 (Lee et al., 2012).

Stock plants for the experiment were obtained by sowing Samson ryegrass seeds into trays containing sand on November 23, 2016, to establish over 750 plants with an endophyte infection frequency of 88%. To assess the endophyte infection frequency, a single tiller from each of 100 plants was cut at the soil surface in December 2016. The cut end was immediately placed on nitrocellulose blotting paper, and the blotted paper was processed to determine the presence/absence of endophyte (Simpson et al., 2012). The stock plants were maintained in a glasshouse with an average daily temperature range of 16°C–22°C, between November 23, 2016 and January 5, 2017, after which they were maintained outdoors, with periodic trimming.

Ramets ($n = 800$) with 3–4 tillers were excised from the stock plants on September 15, 2017, the shoots were trimmed to 4 cm, and the roots were trimmed to 1 cm and then propagated in fine-sieved river sand in the experimental glasshouse. Plants were watered daily to drip point and fertilized on September 25, 2017 with Yates Thrive All Purpose soluble fertilizer applied at label rates of 8 g fertilizer m^{-2} in 4.5 L of water.

The study system was based on Nichols et al. (2014), comprising root tubes held within a wooden frame. Each of the 240, 15 cm diameter $\times$ 1 m deep root tubes was filled with fine washed river sand (bottom third), 50:50 washed river sand : sieved sandy loam soil (middle third), and sieved sandy loam soil (top third) on September 7, 2017. Soil was obtained from Florida Ltd., Cambridge, New Zealand. All soil nutrient levels were adequate for plant growth (e.g., Olsen $P = 31 \text{ mg L}^{-1}$, other data not shown, Hill Laboratories Limited, Hamilton, New Zealand). To increase the pH from 5.2 to 5.8, 10 g of lime was added to each tube and mixed into the top centimeter of soil. All-purpose 12-month slow-release fertilizer (Osmocote Total All Purpose + trace elements) was added to the topsoil, along with the lime, at a rate of 400 $g m^{-2}$. Water-holding capacity of the soil was determined by saturating four 1 kg samples of each of the three growing media, allowing them to drain for 24 h at room temperature, followed by subsample weighing, oven drying at 105°C for 48 h, and re-weighing.

The tubes were positioned within the wooden frame, encased with aluminized insulation paper to minimize solar heating of the external rows of tubes. The filled tubes were watered daily for 2 weeks to ensure that the topsoil was moist so that any weed seeds would germinate, and emerged seedlings were removed.

Three randomly selected perennial ryegrass ramets were transplanted at equal spacings into each tube on September 26, 2017. Ramets had 3 or 4 tillers and averaged $4.02 \pm 0.26 \text{ g}$ ($\pm$ standard error). No trimming occurred when ramets were initially transplanted into the tubes. Yates Thrive All Purpose soluble fertilizer was applied immediately after transplanting and immediately after each harvest, at a rate of 50 g in 12 L of water and 60 mL per pot, to all treatments. Osmocote Total All Purpose + trace elements fertilizer was reapplied to the soil surface at a rate of 400 $g m^{-2}$ at the beginning of the recovery period to all treatments.

Treatments

Six treatments were applied, comprising three defoliation frequency treatments (“VEGETATIVE” [vegetative], “FLOWERING” [flowering], and “SENESCENT” [senescent]) and two drought treatments (“DRY” [drought] and “WET” [no drought, well watered]), in a factorial combination. These treatments were applied over a 16-week period between October 24, 2017 and February 12, 2018.

VEGETATIVE was trimmed to 4 cm when at least 50% of tillers had produced more than 2.5 new leaves per tiller since the previous defoliation. Plants in VEGETATIVE were checked weekly for the presence of reproductive tillers, with the beginning of the “reproductive” growth stage defined by the inflorescence being present and enclosed in the uppermost leaf sheath, based on the description of Moore et al. (1991). Reproductive tillers were excised at the base, separated into leaf lamina and stubble components, the components were weighed, oven-dried for 48 h at 65°C, and reweighed. This was done before sampling for WSCs and nutritive values.

FLOWERING was not trimmed during the stress period until December 6, to allow anthesis to occur for the reproductive tillers. Any tillers that became reproductive after December 6 (T3/DH2) and before the end of the study were excised at the soil surface and processed as above.

SENESCENT was not trimmed during the stress period until February 12 (T5/DH4), by which time all reproductive tillers were shedding seed and senescing; no reproductive tillers were produced subsequently.

VEGETATIVE was trimmed in Weeks 1, 3, 6, 11, 16, 20, and 25, FLOWERING was trimmed in Weeks 6, 11, 16, 20, and 25, and SENESCENT was trimmed in Weeks 16, 20, and 25 of the measurement period (Table 1).

In the “WET” treatment (WET), plants were watered at the rate of 65 mL tube⁻¹ daily for Weeks 1–5, 105 mL tube⁻¹ daily for Week 6, and 200 mL tube⁻¹ daily for Weeks 7–16 (end of the stress period) (Table 1). In the “DRY” treatment (DRY), during the stress period, plants were watered at the rate of 4000 mL tube⁻¹ at trim 3 (T3) and trim 4 (T4) (Weeks 6 and 11) and 2000 mL tube⁻¹ at trim 5 (T5) at the end of the stress period (end of Week 16) (Table 1). In total, during the 16-week stress period, plants in WET received 17 010 mL of water and plants in DRY received 10 000 mL of water. This is equivalent to 962 and 566 mm of rainfall, respectively.

The stress period was followed by a 9-week recovery period (Weeks 17–25, February 13–April 17, 2018) in which all plants in all treatments were well watered with 100 mL tube⁻¹ daily and trimmed when >2.5 new leaves per tiller had emerged (as per the VEGETATIVE treatment) (Table 1). No tillers became reproductive during the recovery period.

Trimming (T) and destructive harvests (DH)

During T and DH, all plants were trimmed to 4 cm above the soil surface, and the cut herbage was dried and weighed, at varying times during the treatment and recovery periods, depending on defoliation treatment (Table 1). Vegetation

above the 4 cm cutting height comprised mainly leaf lamina, hereafter referred to as “leaf,” while vegetation between the soil surface and 4 cm cutting height comprised mainly pseudostem, hereafter referred to as “stubble.” In the FLOWERING and SENESCENT treatments, vegetation above the 4 cm cutting height included leaf, sheath, reproductive stem, and inflorescence, when present. The “crown” was defined as the plant portion between the soil surface and beginning of the roots, which emerged from beneath the crown, and was typically 20 mm of shoot.

During the 16-week stress period, plants were trimmed on five occasions (T1–T5), three occasions (T3–T5), or one (T5) occasion for the VEGETATIVE, FLOWERING, and SENESCENT treatments, respectively. All treatments were trimmed twice in the 9-week recovery period (T6, T7).

Eight of the 40 replicate tubes for each of the six treatment combinations (hereafter referred to as plant cohorts) were destructively harvested every 5–6 weeks, with four destructive harvests during the stress period (DH1–DH4; Weeks 1, 6, 11, and 16), and one destructive harvest at the end of the recovery period (DH5; Week 25) (Table 1). A cohort comprised eight replicate tubes, with each tube containing up to three live plants, for each of the six treatments.

Plant shoot and root measurements

Plant survival, leaf emergence, the number of live and dead adult tillers and new tillers, the biomass of leaf, stubble, crown and roots, root length, and concentrations of WSC were measured for each cohort when it was destructively harvested. For trims 2 and 6 (T2, T6), when no destructive harvest occurred, only the leaf emergence and trimmed leaf mass were measured.

To assess plant survival, the number of live plants in each tube was counted. To assess leaf emergence, the number of new leaves was counted on one randomly selected tiller from each plant in each tube. To assess tiller appearance and mortality rates, one plant in each tube was randomly selected at the start of the stress period and each tiller was tagged by placing a narrow (ca. 2 mm) band of split 3 mm diameter polyolefin heat shrink tubing around its base, with tweezers. During each successive destructive harvest (DH2–DH5), all new tillers in each of the selected plants were tagged, with a different tag color used for each harvest. The numbers of live and dead adult tillers and new tillers in the tagged plants were counted.

To assess WSC concentrations and mass, two randomly selected adult tillers from each of the three plants in each tube were cut at the soil surface within 3–5 h of sunrise to limit the confounding effects of diurnal variation on WSC concentrations of plants (Fulkerson et al., 1994). The excised tillers were bulked and trimmed to 4 cm to separate the leaf from the stubble. Both components were immediately frozen in liquid N and stored at -80°C until freeze-drying and grinding to a fine powder and subsequent WSC and nutritive value analyses. In the cohort being destructively sampled, the remaining vegetation within each tube was similarly trimmed to 4 cm and then excised at the soil surface during each destructive harvest.

The remaining plant material (crown and roots) in the tubes was immediately processed on most occasions. When this was not possible, tubes were stored at 4°C to minimize respiration and processed within 24 h of destructive harvest. For processing, tubes were opened longitudinally, and the length from the soil surface to the deepest root was measured to determine rooting depth (i.e., the extended root depth, occasionally longer than the depth of a root tube if roots had reached the bottom of the tube and continued to grow and spread sideways). The growth medium was separated into three equal layers (top [0–33 cm deep], middle [33–66 cm], and bottom [66–100 cm] of the 1 m deep root tube) and ~ 75 g of growth media was removed from each layer to determine its moisture content by weighing, oven-drying at 105°C for 24 h, and reweighing. The growth media were then removed by gentle washing with tap water. Roots in the top layer were excised from the crown. Each root-depth sample was then frozen in liquid N and stored at -80°C until freeze-drying and grinding to a fine powder for subsequent WSC analyses (Figure S1).

Low- and high-molecular-weight (HMW) WSC concentrations for all plant components were determined using the anthrone method (Jermyn, 1956; Thomas, 1977), adapted as described by Parsons et al. (2004), using a water- and ethanol-based extraction procedure. HMW WSC (e.g., fructans) are used for storage, while low-molecular-weight (LMW) carbohydrates (e.g., sucrose) are more easily transported within the plant to provide energy for growth (Pollock & Cairns, 1991). Data were expressed as concentrations, total amounts, and percentages, to gain an understanding of the total reserve capacity and distribution within the plant.

The nutritive value of leaf was determined at Hill Laboratories Ltd, Hamilton, New Zealand, using near-infrared spectroscopy (Corson et al., 1999).

The herbage trimmed from plants (>4 cm) at Trims 1 to 7 (T1–T7) was weighed, oven-dried at 65°C for 48 h, and reweighed. For each destructive harvest date, cumulative leaf mass was calculated by summing the mass of all leaf removed before and on that destructive harvest date. Total stubble mass was the stubble mass summed over destructive harvests and total shoot biomass was the sum of the total leaf mass, total stubble mass, and total crown mass.

Statistical analyses

All factorial combinations ($n = 6$) of three levels of defoliation frequency (VEGETATIVE, FLOWERING, and SENESCENT) $\times$ two levels of drought (DRY and WET) treatments were arranged in a randomized block design with eight replicates for each treatment for each of five destructive harvests (DH1–DH5 [$n = 240$ root tubes]). Data for each harvest were analyzed using Genstat for Windows (2021) by analysis of variance, fitting eight replicate blocks, three defoliation treatments, two drought treatments, and the interaction. Mean separation was achieved using Fisher's protected least significant difference.

Graphs were produced from an analysis using a multidimensional mixed-model smoother (Upsdell, 1994).

This is a nonlinear regression analysis that overcomes the problems of nonexact confidence intervals for predicted y values by treating each fitted y value as a separate parameter (Upsdell, 1994). A full model:

$$y \text{ var} = \text{Date}/(\text{Drought Treatment} \\ \times \text{Defoliation Treatment}) + \text{Date} \times \text{Across} \\ \times \text{Along} \times \text{Initial Weight}$$

was searched to find a suitable submodel with the minimum Bayesian Information Criterion. “Across” and “Along” indicate the positions of each experimental root tube “across” and “along” the glasshouse. The x -axis (date) origin was set on October 24, 2017, the beginning of the treatment and measurement period. There were no differences between Drought and Defoliation treatments at this point. The position of each tube in each rectangular block (as described by “Across” and “Along”) was incorporated into the analyses.

The model: $Y \text{ Variate} = \text{Date}/(\text{Drought Treatment} + \text{Defoliation Treatment}) + \text{Across}$ was used for all graphed variables, except for soil moisture, which was measured in all three layers of the growth media. For soil moisture, the full model tested was as follows:

$$Y \text{ Variate} = \text{Date}/(\text{Drought Treatment} \\ \times \text{Defoliation Treatment}) \times \text{Layer} \\ + \text{Date} \times \text{Across} \times \text{Along} \times \text{Layer} \\ \times \text{Initial Weight},$$

$$\text{Soil Moisture} = \text{Date}/(\text{Drought Treatment} \\ + \text{Defoliation Treatment} \\ + \text{Layer}) + \text{Layer} + \text{Across}.$$

WSC concentration data were log-transformed; back-transformed data are presented. General trends over time are described for the data in figures, focusing on the calendar seasons (summer: December–February; autumn: March–May; winter: June–August; and spring: September–November). Treatments are significantly different where the intervals do not overlap ($p < 0.05$). Data from specific time points (i.e., trims, harvests) are presented in the tables.

RESULTS

Interactions

There were few first-order interactions between defoliation and drought ($p < 0.05$). Interactions with consistent trends are reported in the appropriate sections below.

Soil moisture content

The gravimetric moisture content of the growing media remained below 40% in the top layer, 25% in the middle layer, and 10% in the bottom layer, depending on the

treatment (Figure S2). The trends over time were similar for each of the layers and for the average moisture content. The moisture content was greater in WET than DRY between mid-spring and mid-autumn for each layer and for the average moisture content ($p < 0.05$, Figure S2).

Plant survival

Nearly all plants survived up to DH3 (Table S1). Survival was greater in FLOWERING and SENESCENT than in VEGETATIVE at DH4 ($p = 0.002$), and greatest for SENESCENT, intermediate for FLOWERING, and lowest for VEGETATIVE for DH5 ($p < 0.001$). There was no effect of drought on plant survival throughout the study ($p > 0.05$, Table S1).

Tiller characteristics

There was an average of 13 live tillers per plant at DH1 and there were no differences between treatments in the number of live tillers in November (Figure 1a,b).

The number of live tillers per plant was similar for SENESCENT and FLOWERING, both of which had more live tillers per plant than VEGETATIVE, during early summer (Figure 1a). Thereafter, live tiller number per plant was greatest in SENESCENT, intermediate in FLOWERING, and lowest in VEGETATIVE ($p < 0.05$, Figure 1a). Live tiller number per plant was greater in WET than DRY from early summer until the end of the study ($p < 0.05$, Figure 1b).

The production of new tillers (“tillering”) for all treatments increased through spring, declined over summer, and then increased in autumn (Figure 1c,d). Peak tillering was almost two times greater for FLOWERING and SENESCENT than for VEGETATIVE. The production of new tillers was similar for all three treatments between mid-summer and early autumn, but by mid-autumn, it was greater for SENESCENT than for FLOWERING and VEGETATIVE ($p < 0.05$, Figure 1c). There was no effect of drought on tillering until mid-summer, after which it was greater in WET than DRY for the duration of the study ($p < 0.05$, Figure 1d).

Tiller death remained low throughout late spring and early summer, after which it increased until early autumn (March, Figure 1e,f). Tiller death was similar for all three defoliation treatments until the end of summer ($p > 0.05$), after which it was greater in VEGETATIVE and FLOWERING than in SENESCENT. There were more dead tillers in DRY than WET from the end of summer onwards ($p < 0.05$, Figure 1e,f).

No reproductive tillers were present before or at DH1 (Table S1). Fewer reproductive tillers were produced in VEGETATIVE and/or FLOWERING than SENESCENT for DH2, DH3, and DH4 ($p < 0.01$, Table S1). At DH5, more reproductive tillers were produced in VEGETATIVE than in FLOWERING and SENESCENT ($p < 0.001$, Table S1). There was no effect of drought on reproductive tiller production ($p > 0.05$, Table S1). Up to 5%, 7%, and 10% of tillers became reproductive, in VEGETATIVE, FLOWERING, and SENESCENT

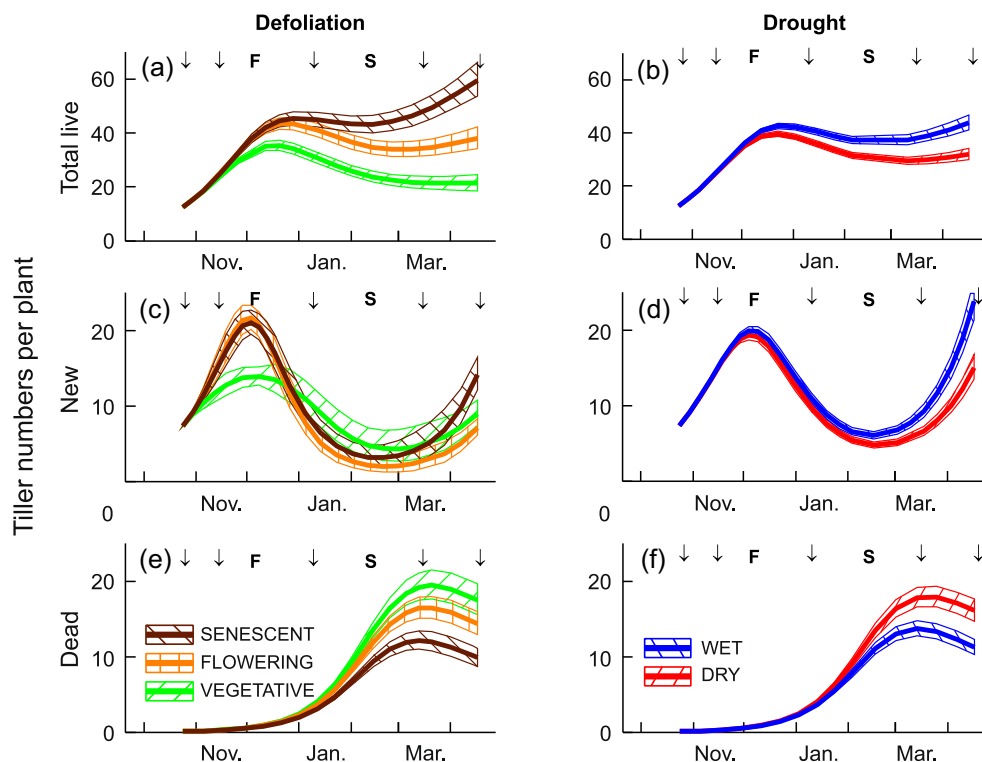


FIGURE 1 Live tillers per plant (a, b), new tillers per plant (c, d), and dead tillers per plant (e, f) for the defoliation treatments (VEGETATIVE, FLOWERING, SENESCENT) averaged over drought treatments, and the drought treatments (WET, DRY), averaged over defoliation treatments for Samson perennial ryegrass. VEGETATIVE—simulated rotational grazing, FLOWERING—no herbage removal from September 15 until flowering (December 6, T3 [F]), and SENESCENT—no herbage removal from September 15 until seed set and senescence (February 12, T5 [S]). Drought treatments were applied from October until mid-February, after which all plants were well watered. The band represents the mean and 95% confidence interval. Arrows indicate trims (T), “F” indicates flowering (anthesis), and “S” indicates seed fall. VEGETATIVE and FLOWERING were also trimmed at “F” and “S,” and SENESCENT at “S.”

respectively, with the percentage depending on the assessment date (Table S1).

The number of new leaves per tiller (“leaf emergence”) for DH2, DH3, DH4, and DH5 was higher for SENESCENT than VEGETATIVE, with FLOWERING intermediate but not always significantly different from either ($p < 0.001$, Table S1). The number of new leaves per tiller was greater for WET than DRY for DH3 ($p = 0.004$) and DH4 ($p = 0.010$) but similar in DRY and WET for DH2 and DH5 ($p > 0.05$, Table S1).

Shoot and root mass

Cumulative leaf mass and stubble mass were greater in SENESCENT than VEGETATIVE, with FLOWERING intermediate, or similar to SENESCENT, for DH2, DH3, DH4, and DH5 ($p < 0.001$, Table S2). There was a similar trend for crown mass for DH3 and DH5 ($p < 0.01$, Table S3), but no effect of defoliation on tiller mass when measured for DH1 or DH5 ($p > 0.05$, Table S2).

Cumulative leaf mass was greater in WET than DRY for DH2, DH3, DH4, and DH5 ($p < 0.05$, Table S2), but there was no effect of drought on stubble mass, or tiller mass, except for crown mass, which was greater in WET than DRY for DH3 (Table S2, $p < 0.05$). There were no interactions between defoliation and drought for any of these variables ($p > 0.05$).

Cumulative shoot (cumulative leaf + stubble + crown), root, and total plant mass were similar for FLOWERING

and SENESCENT until early summer ($p < 0.05$, Figure 2). Thereafter, values for all three parameters were highest for SENESCENT, intermediate for FLOWERING, and lowest for VEGETATIVE ($p < 0.05$, Figure 2). Cumulative shoot mass and total plant mass were approximately two times greater in SENESCENT than in VEGETATIVE, and root mass was three times greater in SENESCENT than in VEGETATIVE, by the end of the study ($p < 0.05$, Figure 2). The same pattern occurred for root mass in the top layer, with even greater differences between treatments occurring in the middle and bottom layers ($p < 0.001$, Table S2). This resulted in root mass in the bottom layer being five times greater in SENESCENT than in VEGETATIVE for DH5 ($p < 0.001$, Table S3).

Cumulative shoot mass and cumulative total plant mass were similar in DRY and WET in mid-spring to late spring, after which they were higher in WET ($p < 0.05$, Figure 2b,d). There was no effect of drought on total root mass ($p > 0.05$, Figure 2f) and no effect on root mass in the top, middle, or bottom layers of the growing media on nearly all occasions ($p < 0.05$, Table S2). The only exception was for the bottom layer in DH3, when root mass was greater in DRY than WET ($p = 0.030$, Table S2).

Root distribution and root length

Depending on the defoliation treatment, 75%–95% of the total root mass was present in the top layer, 5%–15% in the middle layer, and 0–15% in the bottom layer (Figure 3). In

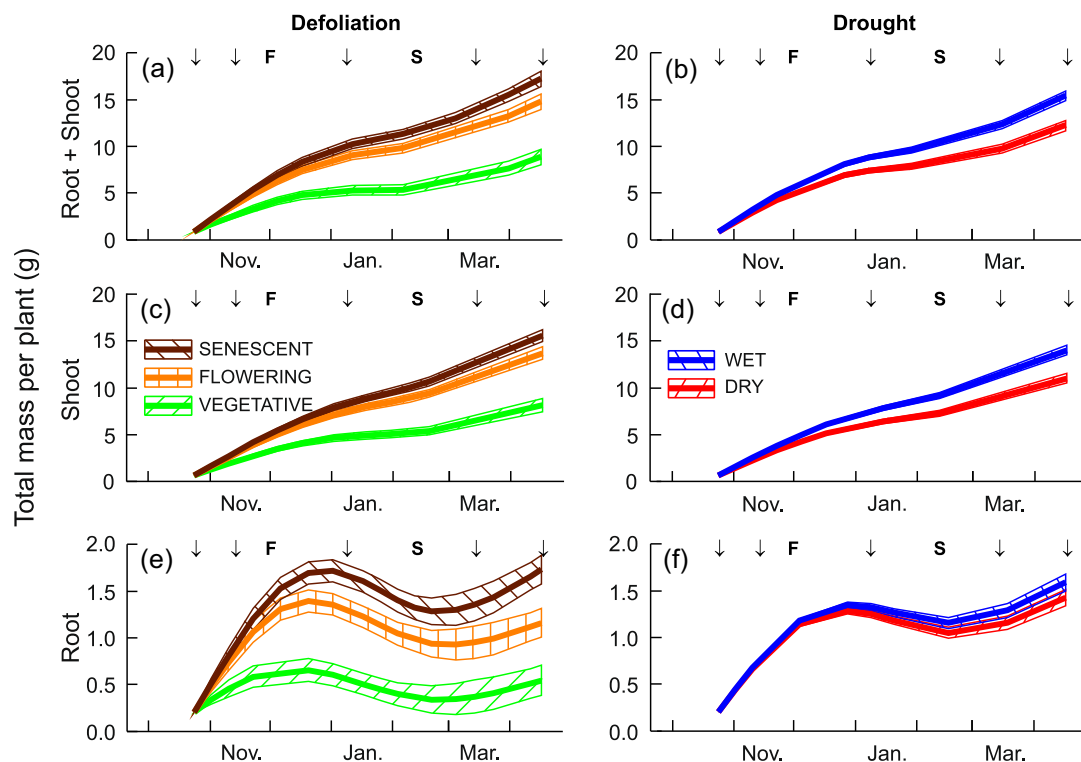


FIGURE 2 Cumulative total (shoot + root) mass per plant (a, b), shoot mass per plant (c, d), and root mass per plant (e, f) for the defoliation treatments (VEGETATIVE, FLOWERING, SENESCENT) averaged over drought treatments, and the drought treatments (WET, DRY), averaged over defoliation treatments for Samson perennial ryegrass. VEGETATIVE—simulated rotational grazing, FLOWERING—no herbage removal from September 15 until flowering (December 6, T3 [F]), and SENESCENT—no herbage removal from September 15 until seed set and senescence (February 12, T5 [S]). Drought treatments were applied from October until mid-February, after which all plants were well watered. The band represents the mean and 95% confidence interval. Arrows indicate indicates trims (T), “F” indicates flowering (anthesis), and “S” indicates seed fall. VEGETATIVE and FLOWERING were also trimmed at “F” and “S,” and SENESCENT at “S.”

the top layer, there was a higher proportion of roots in VEGETATIVE than SENESCENT and FLOWERING from mid-spring onwards, while in the middle layer, the reverse occurred and there was a higher proportion of roots in SENESCENT and FLOWERING than VEGETATIVE from mid-summer onwards ($p < 0.05$, Figure 3a,c). In the bottom layer, the proportion of roots was highest in SENESCENT, intermediate in FLOWERING, and lowest in VEGETATIVE, between early summer and early autumn ($p < 0.05$), after which VEGETATIVE and FLOWERING were similar ($p > 0.05$, Figure 3e).

The proportion of roots was greater in WET than DRY in the top layer from early summer onwards, with the opposite occurring in the middle and bottom layers ($p < 0.05$, Figure 3b,d,f).

Root depth averaged 59 cm at DH1 (Table S2). Thereafter, there was a consistent trend of root depth being greater in SENESCENT than VEGETATIVE, with FLOWERING intermediate, although differences between treatments were only significant for DH4 and DH5 ($p < 0.001$, Table S2). No effect of drought on root depth was detected ($p > 0.05$, Table S2).

Cumulative shoot mass to root mass ratio

The cumulative shoot mass: root mass ratio was greatest in VEGETATIVE and least in SENESCENT, with FLOWERING intermediate from January onwards (Figure 4a). The ratio was approximately two times greater in

VEGETATIVE than SENESCENT between late summer and mid-autumn. There was no effect of drought on the shoot mass: root mass ratio until mid-autumn, when it was greater in WET than DRY ($p < 0.05$, Figure 4b).

Nutritive values

The contents of metabolizable energy and crude protein were higher in VEGETATIVE and FLOWERING than SENESCENT from early summer until the end of the study ($p < 0.05$, Figure 5a,c). The content of NDF was higher in SENESCENT than in VEGETATIVE and FLOWERING during early and mid-summer ($p < 0.05$), but otherwise similar for all defoliation treatments ($p < 0.05$, Figure 5e). The content of soluble sugars was higher in SENESCENT and FLOWERING than in VEGETATIVE during early summer ($p < 0.05$), but otherwise similar for all defoliation treatments ($p > 0.05$, Figure 5g).

There was no effect of drought on the contents of metabolizable energy, crude protein, or soluble sugars ($p > 0.05$, Figure 5b,d,h). Levels of NDF were higher in WET than DRY in mid-summer and late summer ($p < 0.05$), but similar at other times ($p > 0.05$, Figure 5f).

Mass of WSC

The mass of total WSC per plant was greatest in SENESCENT, intermediate in FLOWERING, and

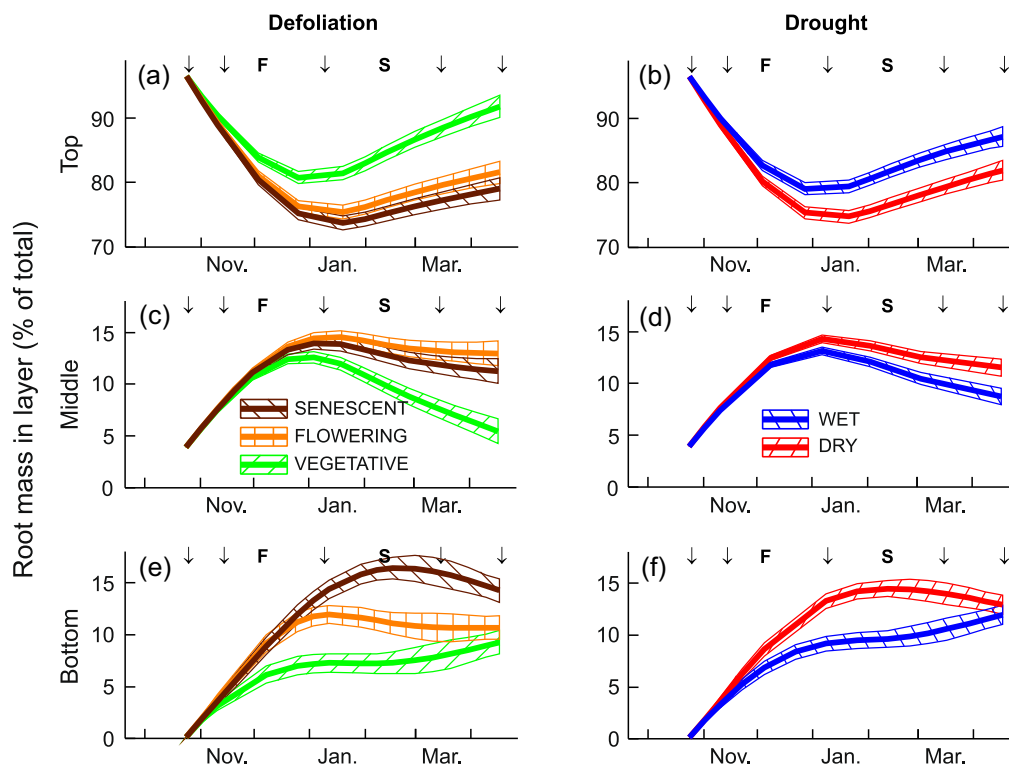


FIGURE 3 Percentage of roots in the top layer (a, b), the middle layer (c, d), and the bottom layer (e, f) of growing media for the defoliation treatments (VEGETATIVE, FLOWERING, SENESCENT) averaged over drought treatments, and the drought treatments (WET, DRY), averaged over defoliation treatments for Samson perennial ryegrass. VEGETATIVE—simulated rotational grazing, FLOWERING—no herbage removal from September 15 until flowering (December 6, T3 [F]), and SENESCENT—no herbage removal from September 15 until seed set and senescence (February 12, T5 [S]). Drought treatments were applied from October until mid-February, after which all plants were well watered. The band represents the mean and 95% confidence interval. Arrows indicate “T” indicate trims (T), “F” indicates flowering (anthesis), and “S” indicates seed fall. VEGETATIVE and FLOWERING were also trimmed at “F” and “S,” and SENESCENT at “S.”

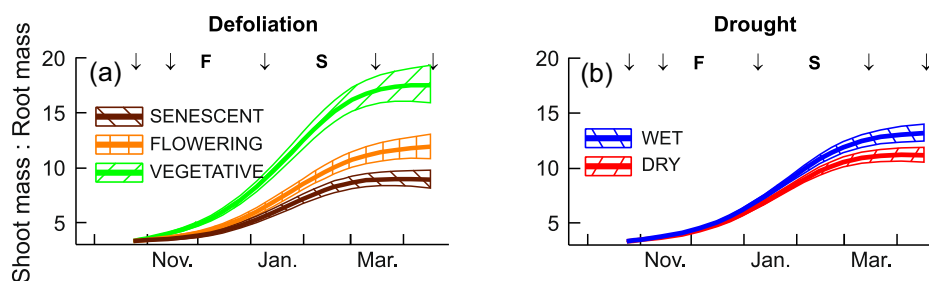


FIGURE 4 Cumulative shoot (cumulative leaf + stubble + crown) mass: root (root) mass ratio for the defoliation treatments (VEGETATIVE, FLOWERING, SENESCENT) averaged over drought treatments (a), and the drought treatments (WET, DRY), averaged over defoliation treatments (b) for Samson perennial ryegrass. VEGETATIVE—simulated rotational grazing, FLOWERING—no herbage removal from September 15 until flowering (December 6, T3 [F]), and SENESCENT—no herbage removal from September 15 until seed set and senescence (February 12, T5 [S]). Drought treatments were applied from October until mid-February, after which all plants were well watered. The band represents the mean and 95% confidence interval. Arrows indicate trims (T), “F” indicates flowering (anthesis), and “S” indicates seed fall. VEGETATIVE and FLOWERING were also trimmed at “F” and “S,” and SENESCENT at “S.”

lowest in VEGETATIVE until the end of the study, by which time treatments were converging but still greater in SENESCENT than in FLOWERING and VEGETATIVE ($p < 0.05$, Figure 6a).

Up to 45% of the total WSC content was present in the leaf, 55% in the stubble, 15% in the crown, and 15% in the roots, depending on the treatment and time of year (Figure 6c–j).

The percentage of total WSC in the leaf was greater in SENESCENT than FLOWERING and VEGETATIVE from early summer until the end of the study, with the reverse pattern occurring for stubble ($p < 0.05$, Figure 6c,e). The percentage of total WSC in the crown and in the roots

was similar for all three treatments throughout the study ($p > 0.05$, Figure 6g,i).

The mass of total WSC per plant and percentage of total WSC in the crown and in the root were similar in WET and DRY throughout the study ($p < 0.05$, Figure 6b,h,j). The percentage of total WSC in the leaf was greater in WET than DRY during summer, with the reverse occurring in the stubble ($p < 0.05$, Figure 6d,f).

The mass of HMW, WSC per plant and LMW per plant and their distributions within the plant (i.e., the percentage of the total amount within the leaf, stubble, crown, and root) are shown in Figures S3 and S4.

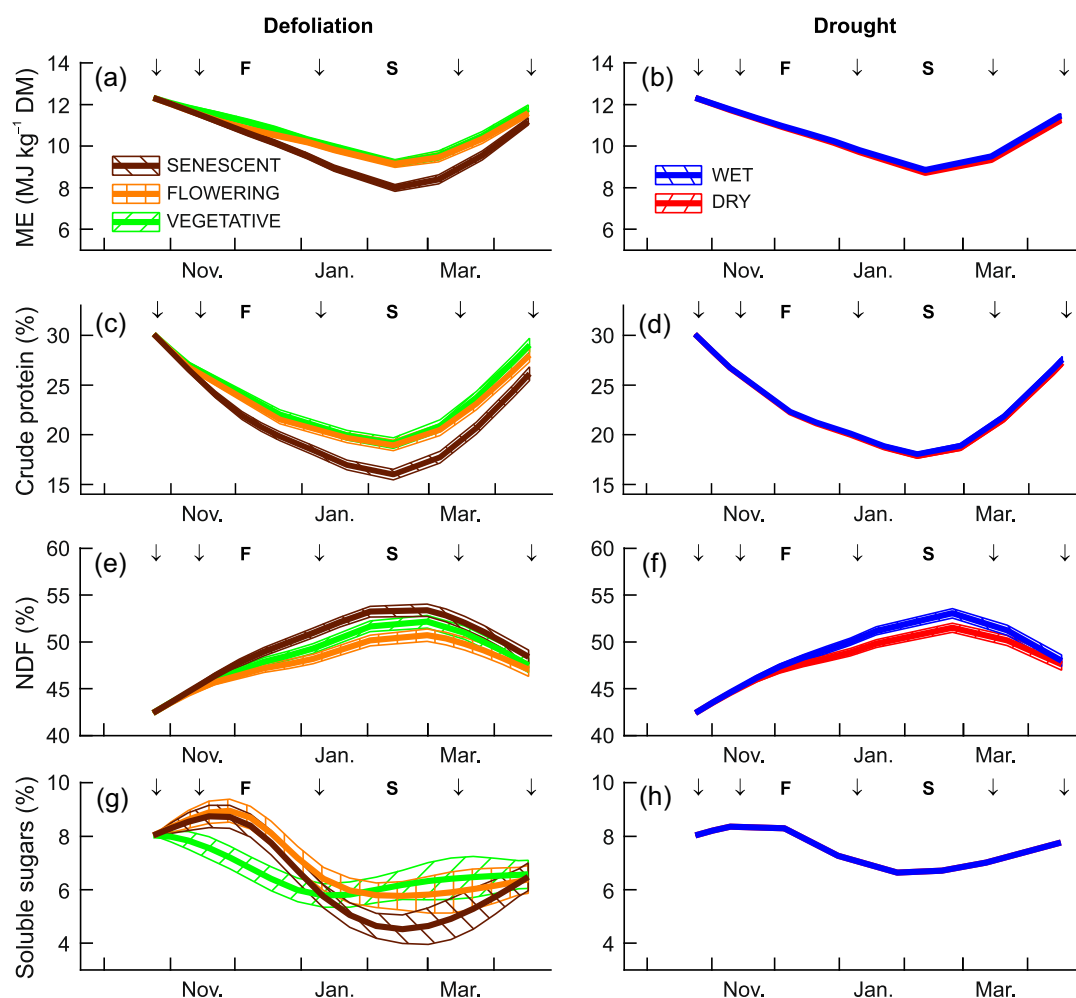


FIGURE 5 Content of metabolizable energy (ME) (a, b), crude protein (c, d), neutral detergent fiber (NDF) (e, f), and soluble sugars (g, h) in the herbage above trimming height for the defoliation treatments (VEGETATIVE, FLOWERING, SENESCENT) averaged over drought treatments, and the drought treatments (WET, DRY), averaged over defoliation treatments for Samson perennial ryegrass. VEGETATIVE—simulated rotational grazing, FLOWERING—no herbage removal from September 15 until flowering (December 6, T3 [F]), and SENESCENT—no herbage removal from September 15 until seed set and senescence (February 12, T5 [S]). Drought treatments were applied from October until mid-February, after which all plants were well watered. A single line means that the treatments were not different ($p > 0.05$). The band represents the mean and 95% confidence interval. Arrows indicate trims (T), “F” indicates flowering (anthesis), and “S” indicates seed fall. VEGETATIVE and FLOWERING were also trimmed at “F” and “S,” and SENESCENT at “S.”

WSC concentrations

Concentrations of LMW, HMW, and total WSC for the leaf, stubble, crown, and roots are presented in Tables S3–S5. They followed similar patterns as for the mass of WSC (i.e., SENESCENT > FLOWERING > VEGETATIVE, Tables S6–S8). However, as the physical biomass and the concentration of WSC in each of the plant parts generally increased with increasing defoliation interval (SENESCENT > FLOWERING > VEGETATIVE), the effect on the plant total mass of WSC was magnified.

Total WSC: Crude protein ratio

The ratio of total WSC (leaf + stubble) to crude protein (leaf, Figure 7) was higher in SENESCENT than in VEGETATIVE, with FLOWERING intermediate, for DH2 ($p = 0.001$) and DH3 ($p < 0.001$), by which time it was two times greater in SENESCENT than in VEGETATIVE.

The same trend continued for DH4, although there was no difference between the defoliation treatments for DH1, DH4, or DH5 (Tables S9). The ratio was also greater in DRY than in WET for DH2 ($p = 0.042$), DH3 ($p = 0.003$), and DH4 ($p = 0.002$), but similar in DRY and WET for DH1 and DH5 ($p > 0.05$, Tables S9).

DISCUSSION

Defoliation effects

In this controlled deep pot experiment with defoliation designed to be representative of contrasting farm grazing management options, results support our hypothesis that herbage production, tillering, root growth, and accumulation of carbohydrate reserves would be greatest in the senescent treatment, intermediate in the flowering treatment, and lowest in the vegetative treatment, regardless of drought.

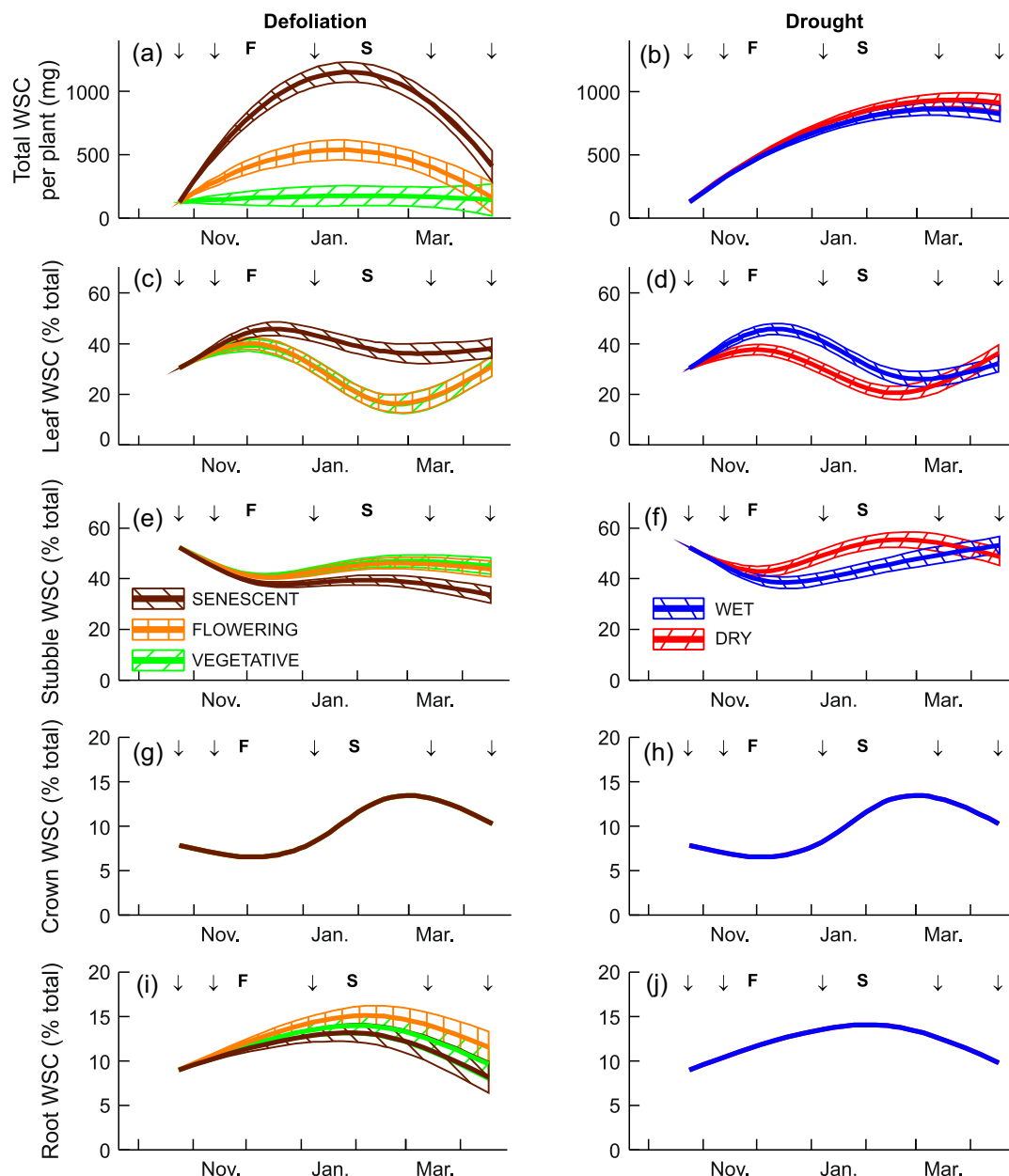


FIGURE 6 Content of total water-soluble carbohydrate (WSC) per plant (a, b), and distribution (%) between leaf (vegetation above trimming height) (c, d), stubble (e, f), crown (g, h), and roots (i, j) for the defoliation treatments (VEGETATIVE, FLOWERING, SENESCENT) averaged over drought treatments, and the drought treatments (WET, DRY), averaged over defoliation treatments for Samson perennial ryegrass. VEGETATIVE—simulated rotational grazing, FLOWERING—no herbage removal from September 15 until flowering (December 6, T3 [F]), and SENESCENT—no herbage removal from September 15 until seed set and senescence (February 12, T5 [S]). Drought treatments were applied from October until mid-February, after which all plants were well watered. The band represents the mean and 95% confidence interval. Arrows indicate trims (T), “F” indicates flowering (anthesis), and “S” indicates seed fall. VEGETATIVE and FLOWERING were also trimmed at “F” and “S,” and SENESCENT at “S.” A single line indicates that there was no difference between treatments ($p > 0.05$).

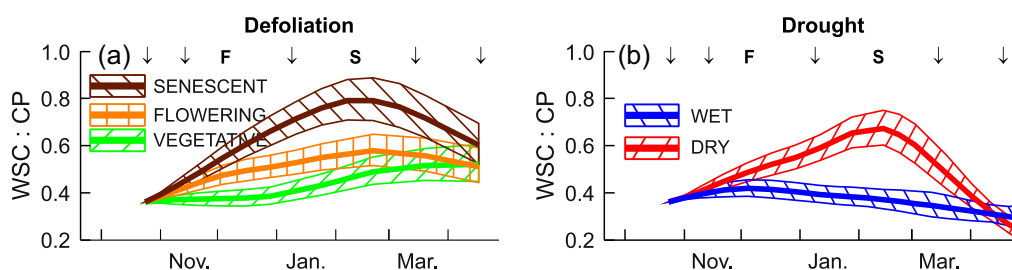


FIGURE 7 WSC:CP ratio for the defoliation treatments (VEGETATIVE, FLOWERING, SENESCENT) averaged over drought treatments (a), and the drought treatments (WET, DRY), averaged over defoliation treatments (b) for Samson perennial ryegrass. WSC data were of the combined leaf and stubble, and CP data were of leaf. VEGETATIVE—simulated rotational grazing, FLOWERING—no herbage removal from 15 September until flowering (December 6, T3 [F]), and SENESCENT—no herbage removal from September 15 until seed set and senescence (February 12, T5 [S]). Drought treatments were applied from October until mid-February, after which all plants were well watered. The band represents the mean and 95% confidence interval. Arrows indicate trims (T), “F” indicates flowering (anthesis), and “S” indicates seed fall. VEGETATIVE and FLOWERING were also trimmed at “F” and “S,” and SENESCENT at “S.”

Results clearly highlighted the importance of reproductive development for tiller production, with close to double the number of new tillers in the flowering and senescent treatments than in the vegetative treatment, in late spring. Tiller production was also greater in late autumn in the senescent treatment than in both of the other treatments. During the seedhead development stage, a small amount of carbon is translocated from various parts of the plant to the tiller bases, which provides a significant input of carbon to fuel the growth of new tillers, as has been demonstrated in controlled studies (Matthew, 2002). Defoliation of plants during reproductive development can therefore reduce the flow of energy reserves to the tiller bases and this can reduce the extent of new tiller development, as occurred in this study. Extending the defoliation interval to the end of summer to allow seed production had a further benefit of reducing tiller mortality. When temperatures are high, such as occurred during late summer in the glasshouse, and plants are subjected to intensive defoliation, the loss of reserves from respiration can exceed the rate of replenishment of reserves through photosynthesis and cause tiller death (Donaghy & Fulkerson, 1997; Fulkerson, 1994; Fulkerson & Donaghy, 2001; Jung et al., 1976). This occurred especially in the vegetative treatment, which was subjected to the most intensive defoliation frequency, and which had the lowest levels of LMW and HMW and total WSC concentrations and content. The low soluble sugar content at the end of summer (February, Figure 5g) is consistent with this explanation.

High rates of tiller birth and death have been observed in grazed swards between late spring and mid-summer (L'Huillier, 1987), as occurred in our study. There is variation between studies in the timing of tiller appearance. For example, Korte (1986) only observed the production of new tillers from the base of reproductive tillers after apical meristems were decapitated. Before decapitation, there was minimal tiller growth, possibly due to the developing inflorescence providing a strong sink for carbohydrate (Matthew, 2002). Plant hormones may also be involved in the regulation of source/sink relations and the demand for carbohydrates (Roitsch & Ehneß, 2000). In Korte's study, there was a high proportion of reproductive tillers (e.g., up to 38% of the total tiller number, depending on the tiller age category). In our study, fewer than 10% of the total number of tillers became reproductive. This may reflect the low number of tillers in each plant vernalized in winter and therefore able to undergo reproductive development (Fjellheim et al., 2014).

This low number of reproductive tillers would presumably limit the extent to which daughter tiller production was suppressed. The extended defoliation interval enabled carbohydrate reserves to accumulate, which presumably fueled the growth of new tillers. The extent to which tillers emanated from reproductive tillers or vegetative tillers in this study is unknown. However, the importance of flowering for tiller persistence has clearly been demonstrated in other research (e.g., Waller et al., 2003).

Summer drought can also delay tiller production until autumn, when temperatures are cooler and soil moisture is replenished (Silsbury, 1961). This was not the case

in our study, possibly because the drought was not sufficiently severe. Regardless of differences between the studies in the mechanisms involved in determining the timing of new tiller development, results demonstrate the importance of allowing perennial ryegrass to flower and set seed, to increase WSC reserves, increase the number of new tillers, and reduce tiller mortality.

Tillers produced during flowering and seed set are likely to play a critical role in pasture persistence. In perennial ryegrass-based pastures in south eastern Australia, Waller et al. (2003) found that the number of tillers produced from the base of reproductive tillers that had flowered and died was 10 times greater than the number of tillers that had survived summer in a vegetative state. Similarly, in perennial ryegrass-based pastures grazed by beef cattle in Waikato in the upper North Island of New Zealand, the production of new tillers and seedlings combined was over 1.5 times greater in swards that were deferred and in which seed set had occurred than in swards that were either allowed to flower (and then grazed) or that were rotationally grazed to maintain herbage in a vegetative state (Tozer, Müller, et al., 2021). Benefits for tiller populations of allowing the perennial ryegrass to flower, followed by grazing, were minimal. In contrast, higher tiller densities were observed for up to 18 months after perennial ryegrass was allowed to flower and produce mature seed (Tozer, Müller, et al., 2021). In particular, there was an increase in tiller populations in autumn after the grazing exclusion period, as occurred in this study.

Plant biomass accumulation mirrored tillering patterns, with the highest herbage mass and rates of leaf emergence occurring in the senescent treatment. Given that tiller mass did not differ between treatments, increased biomass in the senescent treatment was due to the increases in the number of live tillers. Increased growth rates and herbage accumulation in response to increased tiller densities in deferred pastures have also been observed by McCallum et al. (1991) in dairy pastures and by Tozer, Douglas, et al. (2021) in sheep and beef pastures.

The three times greater root mass in the senescent than the vegetative treatment by the end of the study was most likely related to fewer grazing events in the senescent treatment, given that at each defoliation, root growth stops and WSC is prioritized for herbage regrowth and light capture, after which root growth resumes (Donaghy & Fulkerson, 1997; Evans, 1971).

The highest proportion of roots was in the top layer regardless of treatment, although the senescent treatment increased root mass in all layers. Increased root growth will have benefits for exploring the soil volume and accessing soil moisture (Evans, 1978), which may enable plants to better survive and recover from drought. The ability of plants in the senescent treatment to explore the soil profile was demonstrated by the lower soil moisture content in the senescent than the vegetative treatment for most of the study duration. Greater root mass, and particularly root mass at depth, may also provide an opportunity for increased carbon sequestration at depth (Whitehead, 2020).

Plant bases are considered to be the main storage organ for carbohydrate reserves in grasses (White, 1973),

with the highest concentration of HMW and total WSC occurring in the stubble. These carbohydrates provide a source of carbon for regrowth, particularly in the days immediately following defoliation (Evans, 1978; Prud'homme et al., 1992). Short-term studies, using radio-labeled carbon, have been used to determine the role of remobilized carbon, and carbon produced from photosynthesis after defoliation, in leaf and root growth (De Visser et al., 1997).

The accumulated shoot to root ratio was altered substantially by extending the defoliation interval. With increased grazing frequency, root mass declined and the accumulated shoot to root ratio decreased. This may be because the shoot to root ratio in grazed pastures is suboptimal in terms of pasture resilience or the mass and depth of roots present are insufficient to maintain herbage growth and tiller populations (e.g., Evans, 1972). In the upper North Island of New Zealand, pastures are subjected to multiple stresses, including frequent drought and invertebrate pests (Beukes et al., 2021; Zydenbos et al., 2011). Perennial ryegrass is particularly susceptible to repeated defoliation, as rapid leaf growth is favored at the expense of root growth (Cullen et al., 2006). This study shows how deferred grazing can be a useful tool to increase pasture persistence, through replenishing carbohydrate reserves, increasing tiller populations and seedling recruitment, and increasing root mass and particularly root mass at depth.

By the end of the study, differences in carbohydrate reserves between the three defoliation treatments had mostly disappeared, but agronomic benefits, such as increased herbage growth and tillering, continued into autumn (March and April). This is consistent with field results, where an extended spring rotation treatment improved pasture growth in the summer and autumn following the decapitation of seedheads (Da Silva et al., 1994) and deferred grazing improved pasture production for up to 18 months after deferment in summer wet and summer dry perennial ryegrass-based hill country pastures (Tozer, Müller, et al., 2021). Benefits were less in the summer dry environment than the summer wet environment in the above field study. However, the same rankings were observed at both sites, with greater growth benefits occurring in response to deferred grazing than the extended spring rotation, which enables ryegrass to undergo reproductive development and anthesis (Da Silva et al., 2004; Tozer, Müller, et al., 2021).

Nutritive values in this study were as expected, with similar values for metabolizable energy, crude protein, NDF, and soluble sugars in grazed field studies comparing standard rotational grazing, a long spring rotation, and deferred grazing (Tozer, Müller, et al., 2021). The low metabolizable energy in the senescent treatment in late summer occurred as reproductive tillers reached full maturity and began to senesce, and mirrors the accumulation of rank herbage that can occur towards the end of the deferred period in grazed pastures (Tozer, Müller, et al., 2021). However, animals are more likely to select green, more palatable material of higher nutritive value (Dumont et al., 2007), so the differences between the treatments do not necessarily reflect the animal diet.

Nutritive values were improving by mid-autumn, which is also consistent with what occurs in grazed pastures after the deferred period (Tozer, Müller, et al., 2021). The loss of nutritive value can have a negative impact on the performance of livestock grazing the deferred pasture at the end of the deferred period. However, excluding paddocks from grazing can lead to increased stocking pressure and improved pasture quality on the rest of the farm, leading to an overall increase in livestock production and farm systems' profitability (Tozer, Howarth, et al., 2021).

The ratio of total WSC to crude protein can be a useful indicator of nitrogen use efficiency (NUE) for milk production, especially when the ratio exceeds 0.75 (Pacheco et al., 2007). The ratio was two times greater in the senescent treatment than in the vegetative treatment and approached the value of 0.75 in DH3 and DH4 in the senescent treatment. Plants were sampled using herbage cut to ground level but the crude protein content was only available from leaf samples. Pacheco et al. (2007) used pluck samples, which would include a mixture of leaf and some stem. A similar technique to Pacheco et al. (2007) needs to be used in the field when sampling deferred pasture, with WSC and CP measured in the same plant parts, to draw robust conclusions. Despite these sampling limitations, preliminary results indicate that further investigation is warranted on the effect of deferred grazing on the ratio of total WSC to crude protein.

Drought effects

In this study, the drought treatment had less impact on plant growth than the defoliation treatment, despite a statistically significant separation in soil water content between the watering treatments. There were also few consistent interactions between defoliation and drought stress. Drought reduced some aspects of plant growth, including leaf emergence and mass, tiller numbers per plant, shoot mass, and total plant mass. Other drought stress studies have also shown reductions in these characteristics for perennial ryegrass (Barker et al., 1985; Hofer et al., 2017; Korte & Chu, 1983; Tozer et al., 2017).

The mass (and/or concentration) of WSC per plant was higher in drought-stressed than well-watered plants for all plant parts on some occasions. This is consistent with other studies (e.g., Hofer et al., 2017; Thomas & James, 1999) and occurs because the rate of assimilation of carbohydrate is greater than the rate at which assimilates are being utilized for plant growth.

While drought in our study resulted in a change in the distribution of roots in the soil profile (a lower proportion in the top third), there were no effects on total root mass or maximum root depth. This is in contrast to Wedderburn et al. (2010), who found in a microcosm study that there was an initial increase in root growth of perennial ryegrass in response to drought, followed by rapid root death in the top 15 cm of soil and to a lesser extent deeper in the re-constituted soil profile. Drought stress was likely more severe in the microcosm study as irrigation was withheld between December and March. However, in our study, low amounts of irrigation were applied to drought-stressed plants throughout the experimental period. Given the

increasing importance of drought under a changing climate, further studies should investigate the effects of several severities of drought, and this might reveal important interactions with defoliation interval.

Applications to the field

Our indoor study used mechanical defoliation to simulate grazing by livestock. In the field, grazed plants are subjected to trampling, and dung and urine deposition (Skarpe, 2001), along with invertebrate pest pressure, a biotic stress that can severely reduce pasture production (Zydenbos et al., 2011). Despite the absence of these stresses, simulated, indoor studies are useful for understanding the impacts of grazing defoliation in pastures, with Cashman et al. (2016) concluding that simulated grazing in plot studies was a “useful indicator of DM yield performance of animal-grazed swards.” Furthermore, the aboveground plant responses observed in our study are consistent with those observed in the field at both plot and paddock scales, in grazed pastures (e.g., Da Silva et al., 1994, 2004; Tozer, Müller, Craven, et al., 2020; Tozer, Müller, et al., 2021). This gives confidence that the experimental approach adopted was valid and that the knowledge generated on eco-physiological processes is useful for interpreting results of field studies investigating a long spring rotation and/or deferred grazing.

The extended defoliation intervals of the flowering and senescent treatments in this study began earlier (mid-September) than would typically occur in grazed field studies (e.g., from late October, Tozer, Müller, et al., 2021). For the first trim harvest in October (T1), we chose not to defoliate the flowering and senescent treatments to avoid the risk of decapitating the apical meristems of developing reproductive tillers. Subsequent data analyses demonstrated that this would have occurred if we had defoliated all treatments at T1 (see Table S1, which shows that fully reproductive tillers were present by DH2 and would therefore have been undergoing early reproductive development between DH1 and DH2).

Both the flowering and senescent treatments demonstrated substantial benefits for perennial ryegrass growth and tiller populations in this study. Defoliation at anthesis also provides a repeatable and objective marker that can be used to quantify the impact of phenological development on eco-physiological responses, as was successfully demonstrated by Da Silva et al. (2004) in their simulated dairy farm study. Further, Da Silva et al. (2004) achieved a significant increase in herbage and milk production in a simulated dairy farm system on implementing a grazing management strategy akin to the flowering treatment applied here. This involved a fast rotation to maintain intake, with high postgrazing residuals. However, in pastures grazed by beef cattle, benefits for perennial ryegrass tiller populations and ground cover were minimal for an extended spring rotation (i.e., flowering treatment) but lasted for up to 15 months after a deferred period (i.e., senescent treatment), when compared to standard rotational grazing (Tozer, Müller, et al., 2021). Benefits for perennial ryegrass tiller populations and ground cover were also greater in response

to deferred grazing than an extended spring rotation in pastures grazed by dairy cattle (Griffiths et al., 2021).

It has been demonstrated from field research that benefits of deferred grazing for pasture rejuvenation occur due to an increase in tiller densities of existing perennial ryegrass plants, as well as from natural seeding and establishment of new seedlings (Tozer, Müller, et al., 2021). Our study also showed increased tiller densities, although we were unable to investigate reseeding within the confines of the glasshouse study. A further benefit includes improvement of pasture quality at the farm scale (Tozer, Douglas, et al., 2021). Often, there is a surplus of feed in late spring, when feed supply exceeds livestock demand. As seedhead is produced, pasture quality drops, leading to a decline in livestock performance. However, if the late spring surplus feed is deferred, pastures on the majority of the farm are kept short and leafy in the paddocks that are not deferred. This can increase pasture quality, livestock productivity, and profitability of beef and sheep farms (Tozer, Müller, & Tarbotton, 2020; Tozer, Douglas, et al., 2021; Tozer, Howarth, et al., 2021) and milk production and profitability of dairy farms (McCallum et al., 1991). Given these factors, and that it may be difficult to implement an extended spring rotation so that it corresponds with anthesis (Donaghy et al., 2021), deferred grazing may be the more preferable option of the two.

CONCLUSIONS

The results of this study support our hypothesis that allowing perennial ryegrass to undergo reproductive development had significant benefits for herbage production, tillering, root production, and WSC reserves. The benefits for plant growth and reserves increased with increasing phenological development, that is, they were greatest for the senescent treatment. The results of this glasshouse study were largely consistent with those observed in field studies grazed by livestock, thereby increasing our understanding of the eco-physiological responses of ryegrass to variation in the grazing interval during the spring–summer reproductive growth period. A greater understanding of these responses increases certainty in recommending these grazing strategies as alternatives to standard rotational grazing for improved pasture resilience, in both summer wet and drought-stressed environments. Field research is required to quantify the impacts of extended defoliation intervals during the reproductive period on root growth and effectiveness under greater drought stress. This will increase our understanding of factors associated with pasture resilience and possible co-benefits such as increased carbon sequestration.

AUTHOR CONTRIBUTIONS

Katherine N. Tozer: Conceptualization; data curation; funding acquisition; investigation; methodology; project administration; supervision; writing—original draft; and writing—review and editing. **Rose M. Greenfield:** Data curation and investigation. **Catherine A. Cameron:** formal analysis and writing—original draft. **Martin P. Upsdell:** Formal analysis. **David E. Hume:** Writing—original draft; writing—review and editing.

ACKNOWLEDGMENTS

Thanks are due to Natalie Mapp, Tony Craven, Libby North, Tracy Dale, Bridget Wise, and Grant Rennie for technical assistance and Wendy Griffith for discussions on the experimental design and assistance with WSC data collection. This project was funded from the AgResearch Strategic Science Investment Fund (C10X1702).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data are available on request from the authors.

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How to cite this article: Tozer, K. N., Greenfield, R. M., Cameron, C. A., Upsdell, M. P., & Hume, D. E. (2024). Effect of three defoliation frequency treatments and drought on perennial ryegrass herbage and root growth and water-soluble carbohydrate reserves. *Grassland Research*, 3(4), 347–363. <https://doi.org/10.1002/glr2.12105>