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Title:

Harvest interval affects lucerne (*Medicago sativa* L.) taproot total yield, starch,
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24

25 Short title:

26 Harvest interval and lucerne taproot production

27 **Harvest interval affects lucerne (*Medicago sativa* L.) taproot total yield,**
28 **starch, nitrogen and water-soluble carbohydrates**

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37

38

39 **ABSTRACT**

40 Lucerne (*Medicago sativa* L.) has a large taproot to store and release starch,
41 carbohydrates and nutrients during the plant's growth. Recommended
42 management of the lucerne crop aims to keep the taproot stable subject to the
43 demands for feed provision and stand longevity. Field experiments were
44 conducted in Victoria, Australia to examine the effects of recovery period on
45 taproot mass and nutritive status. Both experiments used established SARDI
46 Seven lucerne crops and were either cut every 21-days (short recovery SR) or
47 every 42-days (long recovery LR). At each defoliation taproots were extracted for
48 determination of DM yield and starch, water-soluble carbohydrate (WSC) and
49 nitrogen (N) concentration and DM yield. At both sites, WSC and N DM yields
50 along with total taproot DM yield did not change greatly as the experiment
51 progressed. Starch was responsive to herbage accumulation with both

concentration and DM yield, increasing when herbage accumulation rates were high and decreasing when herbage accumulation rates were low. At both sites, LR taproots had starch levels equal to or higher than those of SR taproots. We conclude that short intervals between defoliations disrupt the energy cycling between shoots and roots. This is likely to reduce lucerne productivity, particularly during periods of rapid growth.

KEYWORDS

Alfalfa, taproots, management, starch, nitrogen, carbohydrate

1. Introduction

Lucerne (or alfalfa) (*Medicago sativa* L.), like any forage species, requires a recovery period following defoliation to allow nutrients stored in taproots to be utilised to promote the growth of new shoots (Bélanger et al., 2006; Pembleton, Cunningham, & Volenec, 2010; Lu et al., 2017). Once shoots have grown sufficiently lucerne plants begin to photosynthesize and nutrients and energy are exported to their storage site - the taproot. The period required to replenish nutrients and energy in taproots after each defoliation depends on the time of the year and seasonal conditions (Teixeira, Moot, & Mickelbart 2007). If the recharge period is too short, especially during the main growing season (spring – early summer in south-eastern Australia) then the productivity of the lucerne crop will be compromised (Clark et al., 2018).

Lucerne is grown throughout the temperate world and there are many published recommendations on how to manage the defoliation frequency to optimise production and persistence. In climates with cold winters (heavy frosts and regular snow) an important recommendation is to allow the lucerne to have a prolonged recovery period from late summer to early autumn to allow taproot reserves to be fully replenished before the cold of winter (Bélanger et al., 2006).

In most areas where lucerne is grown in Australia, the winter periods are not harsh, but the autumn rest period is still commonly recommended (Anon., 2017;

82 Moot et al., 2003). Simple, calendar-based systems have also been recommended
83 (Clinton, 1968; McKinney, 1974).

84 Lucerne plants mobilise carbon (C) and nitrogen (N) reserves from their taproots
85 immediately post defoliation and more generally in spring (Teixeira et al., 2007).
86 They accumulate C and N in their taproots primarily during summer and early
87 autumn if moisture is available. The management system imposed and
88 particularly the timing of defoliation will affect the pattern of accumulation. It is
89 therefore desirable to understand the effect of defoliation interval on the nutrients
90 stored in taproots since it has been reported that the production and nutritive value
91 of the herbage and persistence of the stand are affected by defoliation interval
92 (Avise et al., 1997).

93 Whilst there has previously been research on dry matter and nutrients of lucerne
94 roots in high rainfall and mild temperature environments in Tasmania (Pembleton
95 et al., 2010), there is no research reported from mainland Australia. The research
96 to date has not focused on more extreme environments, like the extensive grazing
97 areas of Victoria and other lucerne growing regions of southern Australia. These
98 environments have high summer temperatures (often above 40°C), annual rainfall
99 of less than 700 mm and high inter and intra seasonal variability of rainfall. In
100 these environments, the potential evapotranspiration can be more than double
101 rainfall, the evaporation component of the water balance accounts for the greatest
102 proportion (> 90%) of the rainfall (eg. Walker, Gilfedder, & Williams, 1999;
103 Zhang, Dawes, & Walker, 2001) and this may have important consequences for
104 the movement of energy and nutrients, in and out of roots. These areas can
105 support grazing for meat and wool and dryland cropping but, without irrigation,
106 usually not dairying and horticulture. In these environments lucerne, with its deep
107 roots, is one of the few pasture species that can provide green feed in summer and
108 autumn.

109 Experiments were established at two contrasting dryland sites within the extensive
110 grazing areas of Victoria with the objective of examining the effects of recovery
111 from different defoliation duration and timing regimes on lucerne herbage DM
112 yield, taproot DM production and nutrient concentrations and DM yields in
113 different environments. Rutherglen in north-east Victoria has a relatively equal

114 contribution of rainfall throughout the year and Hamilton in south-west Victoria
115 has winter dominant rainfall. The experiments were carried out for 18 months
116 during which time the soil wetting/drying cycles and thus the effects of
117 defoliation, manifested themselves. An earlier paper (Clark et al., 2018) presented
118 detailed herbage DM yield and nutritive value and persistence results for four
119 different recovery period treatments. This paper reports on two recovery period
120 treatments (21-day and 42-day) and presents taproot DM yield, starch, water-
121 soluble carbohydrate (WSC) and N concentrations and DM yields, as well as
122 herbage DM yield results presented in a manner similar to the taproot results.

123 It was hypothesised that a 42-day recovery period after defoliation would result in
124 healthier taproots as defined by taproot DM yield, starch, WSC and N
125 concentrations and DM yields. Further, it was hypothesised that, at each sampling
126 occasion, the percentage increase of these taproot resources in a 42-day recovery,
127 compared to a 21-day recovery, would be of a similar order of magnitude for the
128 different resources in the taproot.

129

130 **2. Materials and Methods**

131 **2.1. Site descriptions and treatments**

132 Two sites were established on agricultural research stations at Rutherglen
133 (S36.112, E146.518; AAR 596 mm) and Hamilton (S37.834, E142.086; AAR 681
134 mm) in Victoria, Australia. These are regions with extensive sheep and beef
135 cattle industries. Both sites used established SARDI Seven lucerne pastures –
136 sown in October 2013 at Rutherglen and in November 2011 at Hamilton. At both
137 sites the stands had been managed for commercial lamb grazing in spring-autumn.
138 SARDI Seven has a winter dormancy score of 7 (Kobelt, 2002) and has
139 performed well in experiments in Victoria previously (Clark et al., 2013; Ward et
140 al., 2013, Raeside et al., 2014). Air temperature and rainfall were recorded daily
141 1.1 km from the Rutherglen site and 2.1 km from the Hamilton site. Data for the
142 period of the experiment are compared with the long-term averages in Table 1.
143 Both sites have warm, temperate climates. Rutherglen has greater seasonality of

144 temperatures and Hamilton has greater seasonality of rainfall. Rainfall is more
145 variable at Rutherglen than it is at Hamilton.

146 Both sites experienced periods of low and high rainfall during the experiment. At
147 Rutherglen, the rainfall total for the period July 2015 to April 2016 (395.7 mm)
148 was in the 3rd decile and at Hamilton, the total for the period June 2015 to
149 December 2015 (274.9 mm) was in the 2nd decile.

150

151 Experimental design

152 The designs at each site were identical except for the randomisation. They
153 consisted of four defoliation/recovery period treatments with plots 10 m × 5.5 m.
154 There were four replicates with the design being completely randomised.

155 The two treatments reported here were: Short recovery (SR): Herbage on plots
156 was cut to 50 mm at a 21-day interval; Long recovery (LR): Herbage on plots
157 was cut to 50 mm at a 42-day interval.

158 Short recovery plots were cut at the same time as long recovery plots and then 21
159 days after the long recovery plots. Treatments were imposed from 6 January 2015
160 to 16 June 2016 at Rutherglen and from 2 December 2014 to 20 June 2016 at
161 Hamilton.

162 The Rutherglen site had fertiliser (SuPerfect Pot 1&1) applied in June 2015 (200
163 kg/ha, P 4.4%, K 25%, S 5.5%, Ca 9.5%). The Hamilton site had fertiliser applied
164 in April and September 2015 (superphosphate 100 kg/ha and muriate of potash
165 200 kg/ha) (P 8.8%, K 50.0%, S 11.0%, Ca 20.0%). Soil descriptions are
166 presented in Table 2.

167

168 2.2. Herbage mass

169 Herbage DM yield was assessed at each harvest, i.e. every 21 and 42 days for the
170 SR and LR plots respectively. One to three strips per plot (depending on the
171 amount of herbage available) were cut to a height of 50 mm with a ride-on

172 mower. Each strip was 1.37 m wide $\times$ 10 m long. At Rutherglen the herbage in
173 the mower's catcher was manually weighed. The Hamilton mower was equipped
174 with a self-weighing device. After weighing, a sample was taken, weighed fresh,
175 dried at 100°C to a constant weight and weighed again to determine herbage DM
176 yield. Harvest dates for the SR and LR treatments were fixed, however in autumn
177 2016 at Rutherglen, there was insufficient growth for three consecutive SR
178 harvests and one LR harvest, due to dry seasonal conditions. At Rutherglen, there
179 were 23 SR harvests and 12 LR harvests and at Hamilton, 28 and 14, respectively.

180

181 2.3. Root distribution and taproot mass

182 Following each herbage mass assessment, i.e. every 21 and 42 days for the SR
183 and LR plots respectively, taproots and crowns of any plant were extracted from
184 three 0.01 m² (100 mm $\times$ 100 mm) quadrats per plot. The quadrat locations were
185 chosen on a position basis (the same relative positions in each plot), and not
186 centred on a particular lucerne plant (or plants). Each sample was obtained from
187 digging a 300 mm deep hole. Previous research (Nie et al., 2008, Nie et al., 2015)
188 indicated that over 70% of the lucerne root mass is within 300 mm of the surface
189 and this provided guidance on sampling depth. Soil was sieved to collect all root
190 material which was then washed clean of soil. Any above-crown material was
191 removed and discarded. The remaining crown and taproot was dried at 60°C for
192 72 hours then weighed. No taproot extraction occurred between 5 January 2016
193 and 21 June 2016 at Hamilton due to the difficulty of digging holes during this
194 dry period. At Rutherglen, there were 26 SR crown and taproot extractions and 13
195 LR crown and taproot extractions and at Hamilton, 21 and 11, respectively.

196

197 2.4. Taproot nutrients

198 All dried taproot samples were ground through a 0.5 mm sieve and were analysed
199 by Near Infrared Reflectance Spectroscopy (NIRS). NIRS spectra were collected
200 on all samples using a Bruker MPA FT-NIR instrument (Bruker Optik GmbH,

201 Ettlingen, Germany) in conjunction with OPUS ver 7.5.18 (Bruker Optik GmbH,
202 Ettlingen, Germany).

203 NIRS calibrations for water soluble carbohydrate (WSC), starch (%) and nitrogen
204 (%) were derived by obtaining spectra from 420 lucerne taproot samples covering
205 multiple seasons and conducting reference analysis on a subset of 178 lucerne
206 taproot samples of which 29 samples were used as wet chemistry test spectra.
207 Standards and test samples were chosen by running principal component analysis
208 on all spectra which had been pre-processed using first derivative and
209 multiplicative scatter correction and using OPUS to select a representative set that
210 covered spectral variation using the first 2 principal components.

211 Standard errors of prediction for WSC, starch and N in lucerne taproots were
212 0.60% WSC, 0.09% N, and 1.3% Starch DM respectively. A comparison between
213 NIRS and wet chemistry results was undertaken for ~15% of samples, with
214 resultant correlations (R^2) of 0.94, 0.94 and 0.97 for WSC, starch and N
215 respectively. Reference methods used for NIRS calibrations were as follows:

216 1. WSC using the Alkaline Ferricyanide method and the Lachat 8500 flow
217 injection analyser system (Lachat Instruments, Loveland, Columbia, US)
218 and according to the Australian Fodder Industry Association Official
219 Method – 1.11A.

220 2. Nitrogen determination by AOAC Crude Protein 990.03 using a LECO
221 Trumac combustion Analyser (LECO Corporation, Saint Joseph, Michigan
222 USA)

223 3. Starch digestion using heat stable alpha amylase and amyloglucosidase
224 according to AOAC Method 996.11 with a glucose analytical finish using
225 the Alkaline Ferricyanide method and the Lachat 8500 flow injection
226 analyser system (Lachat Instruments, Loveland, Columbia, US)

227 Any spectral outliers from the calibrations had analysis by NIRS repeated, as well
228 as further analysis by wet chemistry techniques. Outliers in the calibration
229 samples tended to be the result of improper sample preparation for the NIRS
230 system; once analysis was repeated, results were within the acceptable range.

231

232 2.5. Statistical analyses

233 Results from Clark et al. (2018) indicated that LR and SR represented the most
234 and least satisfactory management systems respectively. Statistical analysis was
235 restricted to measurement days, approximately 42 days apart, when taproot
236 samples were obtained from both the SR and the LR plots. For each plot, at each
237 harvest of each site, taproot starch yield, water soluble carbohydrate yield and
238 nitrogen yield were calculated by multiplying DM yield and the corresponding
239 nutrient concentration. The taproot starch yield of one LR plot was excluded from
240 statistical analysis for 22 March 2016 sampling at Rutherglen, because the NIRS
241 reading for starch concentration was negative. This indicated that the true value
242 for the sample was close to zero and the error in the prediction of that particular
243 sample imparted a value slightly below zero.

244 For each of these same measurement days and plots, an associated 42-day, above
245 ground DM cut was calculated for the previous 42-day period as reported in Clark
246 et al. (2018). This was the DM yield from a single cut for LR plots and the sum
247 of the two previous cuts for the SR plots. As there was some variation in the
248 interval length between harvests, the 42-day DM matter cut values were
249 multiplicatively corrected to a 42-day interval using the formula: Corrected DM
250 yield cut = Measured DM yield cut $\times$ (number of days since previous long
251 rotation cut/42). At the Rutherglen site there was insufficient herbage in the LR
252 treatment for the scheduled harvest in March 2016. Thus, the May 2016 readings
253 were taken to be the material grown over an 84-day period since the previous LR
254 harvest, but with the values corrected to a 42-day basis.

255 For each site, measurement occasion and management system (recovery period),
256 42-day above ground DM yield, taproot, starch, WSC and N yields were
257 compared using an unpaired t-test, after the plot data had been logarithmically
258 transformed. The plot was the unit of analysis. The concentrations of starch,
259 WSC and N in the taproots were similarly analysed, but the residuals were
260 homogeneous and not skewed and hence did not require transformation. All
261 analyses had six residual degrees of freedom, except for taproot starch yield on

262 22.03.2016 at Rutherglen which had five residual degrees of freedom. All
263 differences reported were significant at $p < 0.05$.

264 Ninety-five per cent confidence intervals for the percentage change in taproot DM
265 yield and taproot starch yield for the LR treatment, between selected pairs of
266 occasions, were calculated by carrying out two-occasion repeated measures
267 analyses of variance (ANOVA) on logarithmically transformed data. Using the
268 repeated measures ANOVA results, 95% confidence intervals for the difference
269 between the LR means for the two occasions were calculated, and then the limits
270 of these confidence intervals were appropriately transformed to create 95%
271 confidence intervals for percentage change.

272

273 **3. Results**

274 **3.1. Herbage mass**

275 The total herbage DM yield from the SR and LR plots cut over 42-day intervals at
276 each harvest and at each site is presented in Figure 1. At both sites the pattern of
277 herbage production was similar with late winter (Rutherglen) and spring (both
278 sites) being the main growth period. On each date, herbage mass in SR was either
279 less than or not different to that of LR. At Rutherglen, the difference between SR
280 and LR was most pronounced during periods of active growth (late winter-
281 spring), while at Hamilton, differences between SR and LR occurred in spring
282 2015 and early summer 2015/16.

283

284 **3.2. Taproot mass**

285 Taproot DM yield for Rutherglen and Hamilton is presented in Figures 2 and 3
286 respectively.

287 At Rutherglen, the taproot DM yield of LR increased throughout the experiment
288 from 600 kg/ha to 4000 kg/ha by the final measurement (95% CI for percentage
289 increase from 27/1/15 to 16/6/16 in LR = (440%, 830%)), although there were
290 short-term declines in biomass aligned with periods when pasture accumulation

291 was low (Figure 1a). In contrast, at Hamilton, the taproot DM yield of LR ranged
292 from 3000 kg/ha at the time of lowest pasture accumulation to above 6000 kg/ha
293 at the time of highest pasture accumulation (95% CI for percentage increase from
294 1/9/15 to 3/1/16 in LR = (70%, 130%)).

295 At Rutherglen, differences in the taproot DM yield of SR and LR plots could not
296 be detected until the end of the experiment when SR taproots had 55% less DM
297 yield than LR. At Hamilton, SR taproot DM yield was similar to that of LR
298 taproots until spring 2015 after which they were lighter throughout summer 2015-
299 16, with SR taproot DM yields being approximately 60% less than those of LR in
300 late spring/early summer. At the last measurement at Hamilton, in June 2016, the
301 LR and SR taproot DM yields were again similar.

302

303 3.3. Taproot chemistry

304 Starch, WSC and N concentrations in the taproots are presented in Figure 1 and
305 total, starch, WSC and N taproot DM yields for Rutherglen and Hamilton are
306 presented in Figures 2 and 3 respectively. The root DM, starch, WSC and N DM
307 yield results are presented on a 1000-fold logarithmic scale enabling the pattern of
308 temporal changes in the DM yields within SR and within LR to be directly
309 compared between graphs (i.e. a fixed absolute change on each of the graphs
310 indicates the same fixed percentage change in the DM yields, for all eight graphs
311 in Figures 2 and 3). Similarly, the percentage change of SR compared to LR can
312 be directly compared between graphs.

313

314 3.2.1. Starch

315 At both sites, there were large temporal differences in starch yield and
316 concentration of the taproots (Figure 1c and d, Figure 2b, figure 3b). For
317 instance, from early September 2015 to late November 2015, LR starch yield at
318 Hamilton increased by 1295% (97 – 1256 kg/ha DM) (Figure 3b) while taproot
319 starch concentration increased by 630% (3.6 – 22.8 % DM) (Figure 1d).

320 Starch yield changed during the experiment at both sites although the patterns
321 were not the same. At Rutherglen, starch increased from low initial levels (~ 60
322 kg/ha in LR) to a maximum in early summer 2015/16 (~ 700 kg/ha in LR; 95%
323 confidence interval (CI) for percentage increase from 27/1/15 to 22/12/15 in LR =
324 (780%, 3120%)) and then declined through to autumn 2016 (~ 190 kg/ha in LR;
325 95% CI for percentage decline from 22/12/15 to 22/3/16 in LR = (72%, 96%))
326 before rising again towards the end of the experiment (95% CI for percentage
327 increase from 22/3/16 to 16/6/16 in LR = (2%, 150%)) (Figure 2b). At Hamilton,
328 starch yield declined from mid-autumn 2015 (~ 1200 kg/ha for LR) to the end of
329 winter 2015 (~ 100 kg/ha for LR; 95% CI for percentage decline from 27/4/15 to
330 1/9/15 in LR = (86%, 96%)) followed by an increase from early spring 2015 to
331 early summer 2015/16 (~ 1250 kg/ha in LR; 95% CI for percentage increase from
332 1/9/15 to 25/11/15 in LR = (580%, 2370%)) (Figure 3b).

333 In general, at both sites, when SR taproots had high starch yields LR taproots had
334 proportionally higher yields. For instance, at the mid-December 2015 sampling at
335 Rutherglen, the starch yield in LR was about 4.5 times that in SR. At the late
336 November 2015 sampling at Hamilton, starch yield in LR was about 4 times that
337 in SR.

338 At both sites there was greater change in starch yield than in other measured
339 parameters.

340

341 3.2.2. Nitrogen

342 The temporal change in N yield in the LR treatment largely reflected changes in
343 total taproot DM yield at both sites. At Rutherglen, a reduction in the taproot N
344 yield in SR compared to LR was measured from February 2016 onwards ($p < 0.05$
345 on 3 of the 4 sampling occasions; $p < 0.1$ on all 4 occasions, Figure 2c). This
346 compares with total taproot DM yield in which a difference was only detected in
347 the last sampling, in June 2016 ($p = 0.2$). At Hamilton, differences between LR
348 and SR in N yield largely reflected differences in taproot total DM (Figure 3).

349 At Rutherglen there was no difference between SR and LR N concentrations until
350 late summer 2015/16 ($p > 0.1$) (Figure 1e, f). Thereafter LR N concentrations
351 were always higher than the SR N concentrations ($p < 0.05$). At Hamilton, there
352 was no difference between SR and LR N concentrations on any occasion ($p > 0.1$)
353 (Figure 1e, f).

355 3.2.3. *Water-soluble carbohydrate*

356 Temporal changes of root WSC yield in LR, and treatment difference between LR
357 and SR, largely reflected changes and differences in root DM yield (Figures 2 and
358 3).

359 In the LR treatment, taproot WSC concentration ranged from 7% - 14% at
360 Rutherglen (Figure 1g) and 6% - 8% at Hamilton (Figure 1h). At Rutherglen, the
361 LR taproots had higher WSC concentration than did SR on 6 of 10 occasions from
362 June 2015 onwards ($p < 0.05$). At Hamilton, WSC concentrations of the LR and
363 SR taproots were similar throughout the experiment except for one occasion
364 (April 2015) when SR taproots had higher WSC concentrations ($p = 0.006$).

366 4. Discussion

367 The objective of these experiments was to examine the effect of different lengths
368 of recovery between defoliations on lucerne taproot DM yield, starch, WSC and
369 nitrogen in different environments in Victoria, Australia. A key result of this
370 study was that greater recovery periods generally increases dry matter and
371 reserves in the roots. This is consistent with reports from other environments
372 (Bélanger et al., 2006; Teixeira, Moot, & Mickelbart, 2007; Pembleton,
373 Cunningham, & Volenec, 2010).

374 Other key results, that were consistent between the two sites but have not
375 previously been reported elsewhere were that,

376 i. the temporal percentage change in starch levels (both in yield and
377 concentration) of roots is an order of magnitude greater than the temporal
378 changes in dry matter, carbohydrate and nitrogen;

379 ii. The root starch yield with longer recovery interval is often several times
380 greater (often > 250 % change) compared to the root starch yield with
381 short recovery interval (21-day). In contrast the effects on taproot DM,
382 carbohydrate and nitrogen root yield are much smaller; and

383 iii. The relative root starch yield of LR compared to SR was greatest during
384 periods of fastest growth. The relative differences at Rutherglen increased
385 from the commencement of experiment to early summer. In particular,
386 while the effect of defoliation interval increased during periods when
387 starch was accumulating in the roots of LR (presumably good conditions
388 for photosynthesis), the effect (both in absolute and relative terms)
389 decreased during periods when starch was reducing in the roots of LR
390 (presumably poorer conditions for photosynthesis). In fact, at both sites
391 there were times later in the experiment when the relative difference in
392 taproot starch yield, between defoliation treatments, was negligible.

393 The likely reason that these responses have not been reported previously is the
394 environment in which the work was conducted. In particular, the Victorian
395 environment for extensive grazing is one whereby there are strong soil
396 wetting/drying cycles which may affect nutrient and energy cycling between soil
397 and roots and above ground material. Other work has been carried out in more
398 temperate areas (e.g. such as Tasmania, Australia or Canterbury Plains, New
399 Zealand - irrigated) with much greater rainfall or in areas with much colder winter
400 climates (such as Atlantic Canada).

401 The temporal variations in total taproot DM, starch, WSC and N yields reflect the
402 exchange of nutrients between taproots and above ground material. However,
403 since starch is the major energy store in taproots (Reynolds, 1971, Barber et al.,
404 1994), the particularly large temporal changes and defoliation interval effects with
405 root starch yield indicate that the two-way energy exchange between taproots and
406 shoots is an important biological process of lucerne. Avise et al. (1996) showed

407 that most C lost from lucerne after defoliation was due to root respiration and this
408 represented a major expenditure of energy. It follows that the more frequent
409 defoliation of the SR treatment would have disrupted the energy flows between
410 the taproots and the re-growing shoots leading to lower herbage production
411 (Figure 1a, b and Clark et al., 2018) and photosynthesis and ultimately leading to
412 lower taproot mass compared to the LR treatment.

413 Differences in plant persistence between LR and SR are unlikely to have caused
414 the lower herbage or root production in SR, compared to LR, because there were
415 minimal differences in plant persistence between these two harvest treatments
416 (Clark et al., 2018). Whilst shorter cutting intervals have been shown to reduce
417 lucerne persistence (Judd & Radcliffe, 1970; Brink & Marten, 1989; Gramshaw,
418 Lowe & Lloyd, 1993), in this experiment the time period was most likely too
419 short and the dryland growing environment too harsh to fully demonstrate the
420 differences between the SR and LR treatments with regard to persistence.

421 The temporality in the relative reduction of root starch yield in SR, compared to
422 LR, indicates that there are feedback mechanisms in the lucerne plant that limit
423 the effect of defoliation on the taproot energy reserves. Presumably, this protects
424 the integrity of the energy exchange process of the plant during dry periods. This
425 may be another reason why persistence of lucerne in the extensive grazing areas
426 of Victoria, at least for a few years, is not sensitive to a range of grazing or
427 defoliation regimes that provide reasonable intervals between defoliation (Burnett
428 et al., 2018; Clark et al., 2018).

429 The SR treatment was expected to be a test of lucerne's ability to recover from
430 defoliation compared to the less severe LR treatment. This was found to be the
431 case with SR producing less herbage DM yield than LR on multiple occasions at
432 both sites. This was not so clearly the case with taproot DM yield however. At
433 Rutherglen, the SR and LR taproot DM yields were similar for most of the
434 experiment and at Hamilton LR taproots were only heavier from mid-spring 2015
435 to mid-summer 2016. This indicates that for most of the year the SR defoliation
436 interval was sufficient for the replenishment of taproot nutrient and energy stores
437 but during the most active growth period – spring-early summer - a longer interval
438 is desirable.

439 We did not see declines in N concentrations or yield reported by Teixeira, Moot,
440 & Mickelbart (2007) who showed that a grazing interval of 28 days resulted in
441 reductions in taproot N concentrations which reduced leaf growth, leaf size and
442 thus the plants ability to capture light for photosynthesis. This discrepancy is
443 possibly due to the differences between SARDI Seven (dormancy score 7) in our
444 experiments and Kaituna (dormancy score 5) used in the New Zealand experiment
445 of Teixeira et al. (2007), but we do not discount the possibility that the
446 discrepancy is environmental, for example difference in sunlight hours, or because
447 our experiment went for only 18 months. Rutherglen and Hamilton N yields were
448 higher with LR than with SR on three and four occasions respectively. The
449 difference between LR and SR on these occasions was most likely due to the SR
450 treatment using more of the N reserves in the roots to produce vegetative growth
451 than the LR treatment. It is well known that N reserves in the root system are an
452 alternative source of N used by the lucerne plant when N₂ fixation and/or mineral
453 N uptake is reduced (Volenec, Ourry, & Joern, 1996). Hendershot and Volenec
454 (1993) found that N concentrations in taproot bark and wood tissues gradually
455 increased in autumn and declined in spring when growth commenced. The results
456 from Hamilton showed no difference in N concentration between treatments but
457 the N concentration in the taproots did increase at Rutherglen from late summer
458 into autumn thereby supporting the Hendershot and Volenec (1993) finding.

459 Water soluble carbohydrates were higher in the LR treatment compared with the
460 SR treatment at Rutherglen on two occasions in late summer and winter, and at
461 Hamilton during summer, both towards the latter part of the experiment. This
462 result would suggest that the LR treatment, over time, was better at allowing the
463 lucerne to store WSC between harvests than the SR treatment.

464 The taproot DM yield data showed differences at Hamilton, and possibly
465 Rutherglen, by mid spring, 2015. At Rutherglen the low SR taproot DM yield
466 thereafter, suggested plants were under stress to some degree compared to LR
467 taproots. By the end of the experiment the Rutherglen treatments had separated
468 out further with LR taproots heavier. The fact that taproots from both treatments
469 at Rutherglen were heavier at the end of the experiment than they were at the
470 beginning is likely due to the fact that this lucerne pasture was still developing

471 (initial root DM yield ~ 600 kg/ha), despite being sown in October 2013.
472 Rutherglen is a hotter and drier environment than Hamilton and this could affect
473 the time needed for the plants to develop. This also tended to mask any effect due
474 to the seasons as has been reported elsewhere (Teixeira, Moot, & Mickelbart,
475 2007) for a 2-year old stand.

476 At Hamilton the initial high taproot total yield (~ 5000 kg/ha) across both
477 treatments indicated a lucerne pasture that had been conservatively managed
478 before the experiment started. This is a common issue with lucerne as farmers
479 think they are 'saving' feed for later. In reality, they are growing feed that
480 becomes fibrous, loses much of its quality, senesces and is not eaten by livestock
481 (Beauchemin, 1991; McKinney, 1974; Christian, Jones & Freer, 1970). Promotion
482 of a simpler, time-based management system should encourage farmers to better
483 utilise the lucerne they have produced.

484

485 **5. Conclusions**

486 In extensive grazing environments of Victoria, Australia, with very strong soil
487 wetting/drying cycles, starch yield in lucerne taproots is strongly temporal. This
488 indicates that energy cycling between taproots and shoots is also temporally
489 dynamic. It appears to be more temporally dynamic than either N or WSC
490 cycling. In agreement with our first hypothesis that short recovery intervals after
491 defoliation reduces resources in roots, but in contradiction to our second
492 hypothesis where the percentage decrease is similar for different resources, the
493 effects of short recovery intervals after defoliation on productivity appear to
494 primarily disrupt the energy cycling process by reducing the accumulation of
495 starch during periods of rapid growth. These explanations can be used to advise
496 producers of the necessity of avoiding short defoliation intervals, especially when
497 lucerne is actively growing.

498

499 **Conflicts of interest**

500 The authors declare that they have no conflicts of interest in submitting this paper
501 for publication.

502

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507

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628 Table 1. Monthly rainfall totals compared to long term averages (1965-2015) for
629 Rutherglen and Hamilton

Month	Rutherglen		Hamilton	
	Rain (mm)	Long-term average (mm)	Rain (mm)	Long-term Average (mm)
2014				
November	58.8	49.9	24.4	52.8
December	30.8	45.9	32.2	45.8
2015				
January	87.4	40.1	68.8	36.2
February	43.6	39.7	15.8	26.7
March	2.8	37.3	26.0	38.5
April	99.0	39.3	32.0	50.9
May	45.6	51.9	85.8	62.5
June	73.5	51.1	64.4	67.6

July	53.1	64.6	68.6	83.4
August	85.6	63.3	31.2	83.3
September	21.0	57.0	45.5	72.7
October	24.0	55.5	12.0	60.6
November	66.1	49.9	27.4	52.8
December	29.8	45.9	25.8	45.8
<hr/>				
2016				
January	65.6	40.1	43.0	36.2
February	16.6	39.7	22.8	26.7
March	23.6	37.3	37.0	38.5
April	9.8	39.3	48.0	50.9
May	112.2	51.9	99.2	62.5
June	109.4	51.1	86.0	67.9
Total	968.7	855.0	839.3	963.7

Table 2. Soil test results for Rutherglen (samples collected 16 December 2014) and Hamilton (20 November 2014)

Description	Rutherglen	Hamilton
	Brown Chromosol	Ferric-sodic eutrophic brown Chromosol
Total Carbon (g/100g)	1.6	3.8
Total Nitrogen (g/100g)	0.14	0.29

Total organic matter (g/100g)	3.0	7.1
Electrical Conductivity (dS/m)	0.07	0.12
pH(CaCl ₂)	5.4	5.0
pH(water)	6.2	5.7
Total soluble salts (%)	0.02	0.04
Calcium (meq/100g)	4.1	6.2
Calcium as percent (%)	74	77
Calcium:Magnesium ratio	5.0	4.9
Magnesium (meq/100g)	0.83	1.30
Magnesium as percent (%)	15	16
Potassium (meq/100g)	0.57	0.33
Potassium as percent (%)	10	4
Sodium (meq/100g)	<0.05	0.28
Sodium as percent (%)	<1	3
Sum of four cations (meq/100g)	5.6	8.1
Exchangeable Aluminium (KCl) (mg/kg)	<10	<10
Exchangeable Potassium (Skene) (mg/kg)	250	150
Available Phosphorous (Olsen) (mg/kg)	15	20
Available Sulphur (CPC) (mg/kg)	<3	19

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Figure 1. Accumulated herbage mass at 42-day intervals at Rutherglen (a) and Hamilton (b), effect of defoliation frequency on starch concentration (% DM) (c,d), N concentration (% DM) of taproots (e,f), and WSC concentration (% DM) of taproots (g,h) and from early 2015 to mid 2016, for Long Rotation ($\square$) and Short Rotation ($\blacksquare$) (sum of herbage mass at two 21-day harvests) from early 2015 to mid-2016. As intervals were only approximately 42 days, herbage mass values are corrected to a 42-day basis using – Corrected DM yield cut = Measured DM yield cut $\times$ (number of days since previous Long Rotation cut/42). For herbage mass, the y-axis is on a logarithmic scale and the full range of the axis represents a 1000-fold difference. Error bars represent standard error of difference on the logarithmic scale, where * denotes $p < 0.05$, ** denotes $p < 0.01$ and *** denotes $p < 0.001$. Vertical dotted lines represent the official first day of a season (i.e. Dec 1 = summer, Jun 1 = winter).

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661 Figure 2. Effect of defoliation frequency on a) taproot dry matter, b) starch, c) N
 662 yield and d) WSC (kg DM/ha) from early 2015 to mid-2016 at Rutherglen for the

663 Long Rotation (□) and Short Rotation (■) treatments. The Y-axis is on a

664 logarithmic scale and the full range of the axis represents a 1000-fold difference.

665 Error bars represent standard error of difference on the logarithmic scale, where *

666 denotes $p < 0.05$, ** denotes $p < 0.01$ and *** denotes $p < 0.001$. Vertical dotted

667 lines represent the official first day of a season (i.e. Dec 1=summer, Jun 1 =

668 winter).

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Figure 3. Effect of defoliation frequency on a) taproot dry matter, b) starch, c) N yield and d) WSC (kg DM/ha from early 2015 to mid-2016 at Hamilton for the Long Rotation (□) and Short Rotation (■) treatments. The Y-axis is on a logarithmic scale and the full range of the axis represents a 1000-fold difference. Error bars represent standard error of difference on the logarithmic scale, where * denotes $p < 0.05$, ** denotes $p < 0.01$ and *** denotes $p < 0.001$. Vertical dotted lines represent the official first day of a season (i.e. Dec 1=summer, Jun 1 = winter).

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