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# The South African perennial legume *Lebeckia ambigua* sustainably supports livestock production on infertile soils in rain-fed agro-ecosystems

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## Abstract

*Lebeckia ambigua* is a perennial legume endemic to rangeland South Africa that is adapted to dry (<400-mm annual rainfall), infertile and acidic (below pH 5.5<sub>Ca</sub>) soils. Although it is considered that the legume was grazed by mammalian ungulates during its evolution, little is known about its production, nutritive value for grazing animals or optimal management in an agricultural context, in either its centre of the origin or in Australia. Experiments were conducted to test the hypotheses that sheep would graze *L. ambigua* in autumn without adverse effects on either the animal or the plant. *Lebeckia ambigua* was established at two sites in Western Australia. In experiment 1, three grazing intensities were imposed by exclusion cages introduced during a single intensive grazing period, to provide differing residual pasture heights. The metabolisable energy and crude protein content of *L. ambigua* were more than 11.4 MJ/kg dry matter and 21%, respectively for all treatments. Un-grazed *L. ambigua* produced 1740 kg DM/ha while a single moderate or severe grazing reduced total biomass production by about 50% over 10 weeks post-grazing ( $P < 0.001$ ). In experiment 2, continuous grazing of *L. ambigua* for 64 days had no negative impacts on biochemical health indicators of merino wethers. Grazing had no effect on plant density in either experiment indicating that *L. ambigua* is adapted to substantial grazing pressure in autumn. *Lebeckia ambigua* could become a valuable plant for sustainably increasing livestock production on infertile soils in rain-fed agro-ecosystems where other legumes fail.

**Keywords** *Lebeckia ambigua* · Nutrition · Grazing · Perennial · Autumn feed gap · Legume · Acidic sand

## 1 Introduction

Perennial forage species are important for the sustainable management of rain-fed grazing ecosystems because they contribute to hydrological stability by preventing rising water tables (Le Houerou 1984), managing irregular rainfall events (Oba et al. 2000) and providing a more even feed supply for browsing animals while spreading defoliation

patterns throughout the year (Pickup 1994). Because of their generally higher nutritive value than grasses and forbs and their capacity to improve the N-fertility of soils, perennial legumes are sought for improving the dryland grazing systems of Western Australia. However, there are no legumes native to this region that are suitable for grazing, and no domesticated legumes available that are adapted to the acidic, coarse-textured soils (Edwards et al. 2019). Therefore, perennial legumes from other regions of the globe with similar edaphic properties are being evaluated to fill this role. In particular, perennial plants that provide green feed during the summer-autumn feed gap are being sought to grow on soils which currently support only the dried residues of annual weeds with little agricultural value at this time, which severely limits ruminant production.

The Cape Floristic Region (CFR) of southern Africa contains a wide range of indigenous leguminous herbs and shrubs (Manning and Goldblatt 2012; Trytsman et al. 2016) that have a potential role in the development of global forage resources

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for rain-fed rangelands (Müller et al. 2017a, 2017b, 2019; Chimphango et al. 2020, 2021). While the interaction between these legumes and the native ungulates of the Fynbos Biome has largely been understudied, it is considered that they were historically grazed and/or browsed by diverse mammalian herbivores prior to European settlement in the seventeenth century. The herbivores included eland (*Tragelaphus oryx*), Cape mountain zebra (*Equus zebra zebra*), plains zebra/quagga (*Equus quagga*), ostrich (*Struthio camelus*), red hartebeest (*Alcephalus buselaphus*) and grey rhebuck (*Pelea capreolus*) (Radloff 2009). The plant species composition and spatial distribution of CFR vegetation have almost certainly been influenced by the grazing behaviour of these herbivores, as well as plant palatability and reproductive strategies, soil fertility and rainfall. The Khoekhoen introduced sheep (*Ovis aries*) and later cattle (*Bos Taurus*) to the Fynbos Biome around 2000 years ago, again with a presumed impact on the vegetation. Subsequent to European settlement in 1652, grazing pressures on the fynbos vegetation were altered, when additional stressors such as summer burning to increase the palatability of grasses were introduced (Treydte et al. 2013).

In terms of its direct impact upon the plants, grazing behaviour by herbivores was considered more destructive to CFR vegetation than was browsing behaviour, with recent studies indicating that prolonged and intensive grazing resulted in the replacement of more palatable plant species by less palatable ones (Seymour et al. 2010; Treydte et al. 2013). Modern studies in this region show that intensive grazing favours plant species which reproduce by stolons rather than by seed, because seedlings have less capacity to tolerate grazing than mature plants, and hence, recruitment is compromised (Seymour et al. 2010). However, there is evidence from carbon isotope analysis that prior to 1652 the large herbivores largely avoided the sandstone fynbos in favour of the more nutritious Renosterveld vegetation (Radloff et al. 2010). We postulate that the grazing pressures upon the sandstone fynbos in its history were not as intensive as they are under contemporary conditions with domesticated herbivores.

The fynbos legumes are becoming targets for the domestication of new forage plants because of their adaptation to severely infertile soils in water-limited agro-ecosystems (Trytsman et al. 2019; Howieson et al. 2008; Müller et al. 2017a). If domestication is successful, they may provide feed for farmed ruminants whose production is limited by the autumn-summer feed gap (Moore et al. 2009). A particular focus of some plant breeding programmes has been the development of perennial herbaceous plants adapted to aridity and soil acidity (Howieson et al. 2008; Sprent et al. 2010). Concomitant with the adaptation to these edaphic stresses, domestication of legumes from the fynbos must consider their tolerance to grazing by farmed herbivores so that sensible decisions are to be made on which of the hundreds of fynbos legume species to target.

One fynbos legume that holds promise for domestication is *Lebeckia ambigua* E. Mey. This plant is a deep-rooted perennial legume endemic to the CFR of South Africa (le Roux and Van Wyk 2007). It is well adapted to acidic, infertile, deep sand soils, and grows in regions with less than 400-mm annual rainfall (Howieson et al. 2013; Edwards et al. 2019). However, while there is anecdotal evidence of *L. ambigua* being grazed by sheep in the CFR (B.E. van Wyk pers comm. 2007), there is nothing reported about its response to intensive grazing in the near monoculture conditions experienced in many modern agricultural systems. As well, many perennial legumes produce secondary compounds to deter grazing. The African plant tribe Crotalariaeae, to which *L. ambigua* belongs, is known to produce pyrrolizidine alkaloids, including ammodendrine, lebeckianine and lupanine (Van Wyk and Verdoorn 1990; Van Wyk et al. 2002). The presence of these compounds may affect palatability, intake and perhaps animal health. Many of the perennial shrubs native to Western Australia produce fluoroacetate, which is highly toxic to mammals (Mead et al. 1985).

There is little to no published data on the growth and persistence of *L. ambigua* when grazed by sheep, its nutritive value and intake, nor effects of grazing this legume upon animal health. Hence, the experiments reported in this paper were designed to test two hypotheses. The first, that sheep would graze *L. ambigua* during the summer-autumn feed gap and that a single intensive grazing of 6–18-month-old plants would reduce their rate of re-growth without compromising plant survival. Secondly, it was hypothesised that sheep grazing *L. ambigua* in autumn in Western Australia would not suffer adverse health effects as indicated by blood biochemistry analysis.

## 2 Materials and methods

Experiment 1 examined the response of a 7-month-old stand of *L. ambigua* to a short period of grazing by sheep during autumn in Western Australia, and experiment 2 examined the impact of grazing an 18-month-old stand of *L. ambigua* on animal health. All procedures reported were conducted following the guidelines of the Australian Code of Practice for the Use of Animals for Scientific Purposes and received approval from the Murdoch University Animal Ethics Committee.

### 2.1 Experiment 1: Impact of grazing on the plant

#### 2.1.1 Experimental site

The first experiment was conducted between the 2nd of April and the 13th of June 2014 at Romily Hills farm near Beverly (32°21'33.5"S, 116°34'02.0"E), in the mixed farming zone of Western Australia where crops and grazed pastures are

rotated frequently. Rainfall was recorded weekly and average temperatures were recorded daily at the experimental site for the period of this experiment. Rainfall and temperature data during the 6 months prior to the experimental period were accessed from a weather station located about 45 km from the experimental site. The topsoil (0–10 cm) at the experimental site was acidic, with a  $\text{pH}_{\text{Ca}}$  of 4.5, phosphorus buffering index (PBI) in the range 40–80, phosphorus (Colwell) of 24 mg/kg, potassium (Colwell) of 102 mg/kg and sulphur (KCL40) of 7.1 mg/kg (Rayment and Lyons 2010).

### 2.1.2 Plant establishment and management

One hectare of *L. ambigua* was sown on the 10th of September 2013, following an application of 2 L/ha of glyphosate (360 g/L active ingredients) 12 days before sowing to remove the existing pasture. *Lebeckia ambigua* was sown as a monoculture in 100-m-long rows with spacing of 0.6 m between the rows. *Lebeckia ambigua* seed (200 g/ha) was direct drilled with a modified Aitchison (AT-SM4020C, Reese Group, New Zealand) precision seeder immediately after rhizobial inoculation with WSM4184, which is a specific strain of *Burkholderia sprentiae* (De Meyer et al. 2013). The average weight of *L. ambigua* seed is 1.8 mg, and thus, this seeding rate delivered approximately 111,000 seeds/ha. With the 0.6-m row spacing of the seeder, this translated to 15 seeds/m row or one seed per 6.7 cm of row. Counts at emergence revealed 11 plants established per linear m. Superphosphate/potash fertiliser (6.8%P, 12.4%K, 7.9%S, 15%Ca) was applied at 150 kg/ha at sowing.

### 2.1.3 Experimental areas and timing of grazing

The autumn period was selected for the first grazing experiment because it represents the critical feed gap in the mixed farming zone of Western Australia and is thus the time when *L. ambigua* is likely to be utilised for grazing on-farm. We chose to graze plants in the first autumn after establishment as this is acknowledged as the period in their life cycle when perennial plants are most susceptible to damage from grazing animals (Taddese et al. 2002).

Seven months after seeding, eight uniform areas in the 1 ha block of *L. ambigua* were chosen within which to impose the three grazing treatments. Each area allocated to the experiment was 100 m<sup>2</sup> in dimension. Sheep (Merino ewe weaners, 9 months of age, 30 kg average weight) grazed across the eight areas randomly at a stocking rate of 50 sheep/ha and the grazing severity was achieved by placing grazing exclusion cages into each of the eight regions on three dates: before the commencement of grazing on the 2nd of April 2014 for the un-grazed treatment, after plants had been grazed down to a height of 9 cm for the moderately grazed treatment (6th April 2014) and after grazing to

4 cm for the severely grazed treatments (7th April 2014). The grazing exclusion cages were placed randomly when the target height was achieved. They were constructed from a 6-mm steel bar welded into a mesh, with dimensions of 100 cm (w) × 100 cm (l) × 25 cm (h), giving an effective exclusion area of 1 m<sup>2</sup>. When placed across the established plants, two complete rows each of 100 cm, carrying a plant on average every 8.8 cm, were protected from (further) grazing.

### 2.1.4 Measurements of pasture re-growth, density and nutritive value following exposure to three grazing intensities

At the start of the re-growth period, the number of plants in a 30-cm portion of a row from each of the eight replicates of each grazing severity was counted to estimate plant density. The height of the longest vertical stem of each plant was then measured with a handheld ruler, and the total weight of plant material was assessed by cutting the plants to ground level. This plant material was then dried (60 °C oven for 3 days) and weighed to determine the dry weight per plant. The height of five adjacent plants from each replicate of each treatment was then measured weekly during the assessment period. Survival of these five plants was also recorded each week. After 10 weeks of pasture re-growth, between five and 11 plants (according to original plant density within the cages) including those monitored weekly were cut to ground level and the material dried and weighed. The total amount of the plant material present at the commencement and end of the re-growth period for each grazing severity, expressed as kg dry matter (DM)/ha, was calculated from the plant density per linear metre, the average weight of individual plants and the space between rows.

### 2.1.5 Calculations of feed on offer and pasture growth

Feed on offer (FOO), at different times during the experiment, was calculated from plant density and weight per plant. A measure of plant structure (g/cm) was derived from the weight and height of individual plants cut from each replicate of each treatment at the end of the experimental period. Plant structure was also measured prior to the start of the experimental period on plants within the un-grazed treatment, and it was assumed that the structure of plants in the other treatments would have been similar as the grazing treatments were allocated at random within each replicate which were also selected to be uniform. The structure of plants at different times during the experiment was then predicted assuming that the changes in structure during the 10-week period were linear, and the weight of each plant was calculated from the predicted plant structure and the actual height of the 5 plants per replicate which

were measured weekly. Feed on offer for each replicate was then calculated using the following equation: FOO (kg DM/ha) =  $((1/0.6) \times \text{density of plants per lineal m} \times \text{g per plant} \times 10,000) / 1000$ , where (1/0.6) is the row spacing. Plant growth rate (PGR) was calculated from the change in FOO and the number of days between two points in time.

### 2.1.6 Nutritive value

The plant material harvested at the end of the 10-week treatment period was used to estimate the nutritive value of *L. ambigua*. Samples of whole plants from each replicate were ground through a 1-mm sieve and analysed by a commercial laboratory. Digestibility and crude protein (CP) content were determined by near-infrared reflectance (NIR) using calibrations developed from the following wet chemistry methods; digestibility (digestible organic matter (dry matter basis): DOMD) determined by modified Tilley and Terry (1963) in vitro technique; nitrogen (N) determined using Dumas combustion method with a Leco CNS 2000® analyser (Leco, St Joseph MI USA) (AOAC 1997) and CP content calculated as  $N \times 6.25$ . Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined as described within the Australian Fodder Industry Association – Laboratory Methods Manual (2011).

### 2.1.7 Statistical treatment

All statistical analyses were performed using GENSTAT (18<sup>th</sup> Edition: VSN International 2017, Hemel Hempstead, UK). The statistical method of restricted maximum likelihood was used to model plant height over time. Various variance–covariance structures were examined with treatment, time of measurement and interaction thereof as fixed effects. The selection was via a sequence of likelihood ratio tests. A power model in which the correlation between observations from the same plot decays as the time delay between the observations increases was considered the most appropriate. All other analysis including plant density, biomass production and nutritive value, utilised analysis of variance (ANOVA) with the block structure being plot nested within replicates. For ANOVA, differences between means were assessed using least significant differences ( $P = 0.05$ ).

## 2.2 Experiment 2: Impact of grazing on the animals

The second experiment to determine the impact on animal health associated with grazing *L. ambigua* was conducted on a farm near Harrismith in the mixed farming zone of Western Australia (32° 56' 10.2"S, 117° 51' 19.5"E). At the start of the experiment in March 2016, the *L. ambigua* had been established for 18 months. We chose this period to conduct the experiment (rather than after 6 months of growth as in

experiment 1) as it would likely have allowed sufficient time for the accumulation of any anti-nutritional compounds in the plant (Van Wyk and Verdoorn 1990). The soil at the site is an infertile deep white sand with (0–20 cm) organic carbon of 0.2%; Colwell P and K of 10 and 15 mg/kg respectively; PBI < 10; sulphur, nitrate N and ammonium N of 1, 1 and 3 mg/kg respectively and pH<sub>Ca</sub> of 5.3. The average annual rainfall is 370 mm. The *L. ambigua* had been sown in September 2014 with a modified Aitchison precision seeder at a rate of 200 g/ha. The seed was inoculated with the same specific strain of *B. sprentiae* as in the first experiment. The establishment was successful, and the stand had produced 1900 kg DM/ha of biomass after 16 months of growth. Biomass was assessed by cutting eight quadrats each 1 m<sup>2</sup> across the site and drying the material at 60 °C for 48 h. However, there was a loss of biomass through the 2016 autumn due to an infestation of *Uresiphita ornithopteralis* (tagasaste moth) which reduced the amount of plants that survived through the summer from 1.9 to 0.5 plants/m<sup>2</sup>.

At the start of the experiment, 10-month-old Merino wethers (*Ovis aries*) were allocated into two groups of ten animals based on their live weight (34–46 kg) to ensure a similar average weight distribution per treatment. To acclimatise to the experimental conditions and diets, these sheep initially grazed a small field of dry annual pasture (control group) or a field with a low density of *L. ambigua* and dry annual pasture (treatment group), and both groups were supplemented with 450 g/day of a commercial pellet (11.0 MJ/kg DM and 14.5% protein) for 21 days. The sheep were then split into two replicates of five and allocated to control plots or treatment plots containing *L. ambigua* for another 43 days. All plots contained dry residues of *Lolium rigidum* Gaud. and *Arctotheca calendula* L. at 910 kg DM/ha, of which there was 570 kg DM/ha remaining at the completion of the experiment (data not shown). All plots were 0.19 ha and provided with fresh water in a trough. The sheep in the control plots continued to be supplemented with 450 g/day of the commercial pellet, whereas they were not offered to sheep grazing *L. ambigua*.

Sheep were weighed (in field, unfasted) and blood samples collected at the start of the acclimatisation period and then on days 32, 50 and 64. The blood was collected via jugular venipuncture using a 21-gauge needle into serum and lithium heparin vacutainer tubes. Haematological and biochemical parameters indicative of ovine health were measured and compared with the normal ranges for wethers including red blood cell count, white blood cell count, haemoglobin concentration, haematocrit, creatinine kinase, alanine transaminase, gamma-glutamyltransferase, glutamate dehydrogenase, creatinine, urea, glutathione peroxidase, methylmalonic acid, copper and zinc. The plasma clinical chemistry panel was processed on a AU480 Clinical Chemistry Analyser (Beckman Coulter Australia Pty



Ltd., Lane Cove West, NSW). Beckman Coulter clinical chemistry kits were used for the following panel components: alanine aminotransferase (ALT; OSR6007), creatine kinase (CK; OSR6179), creatinine (OSR6178), gamma-glutamyltransferase (GGT; OSR6020), iron (OSR6186), cations (OSR6189), total protein (OSR6132) and urea (OSR6134) while Randox kits (Randox Australia Pty Ltd., Parramatta, NSW) were used for glutamate dehydrogenase (GLDH; GL 441). Plasma haptoglobin was in-house and performed using the method of Eckersall et al. (1999).

### 2.3 Statistical treatment

All statistical analyses were performed using GENSTAT (17<sup>th</sup> Edition: VSN International 2014, Hemel Hempstead, UK). A linear mixed model was fitted to blood parameter data using restricted maximum likelihood. The fixed effects of the model included the effects of treatment, sample date and the treatment by sample date interaction. The random effects of the model included terms for replicate, animal and animal by sampling date. Various variance–covariance structures were examined to take account of possible correlations between measurements made on the same animal. The final structure for each parameter was selected via a sequence of likelihood ratio tests. Fixed effects were assessed as significant if  $P < 0.05$  and parameters were transformed if plots of residuals indicated that this was necessary.

## 3 Results

### 3.1 Experiment 1

#### 3.1.1 Rainfall data

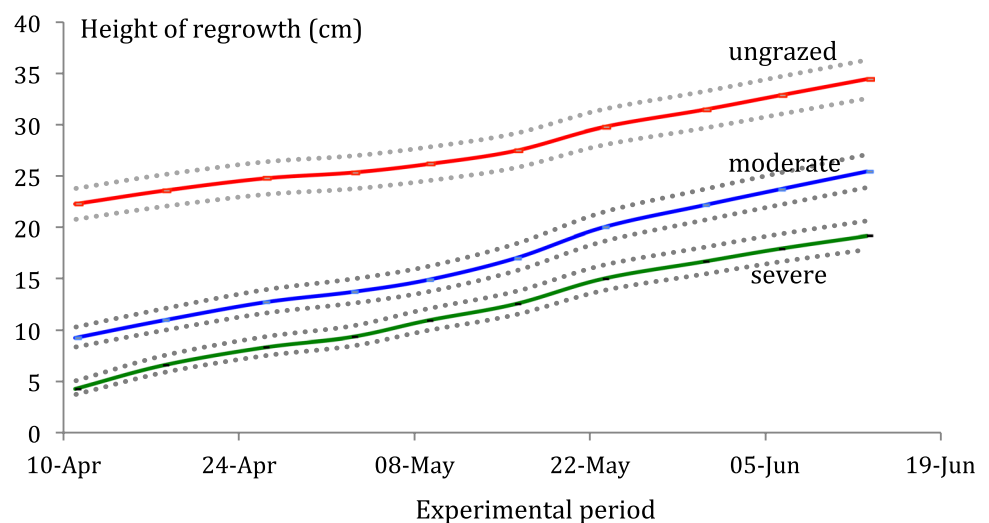
Spring rainfall, from sowing in September until November 2013, was 97 mm, and this allowed sown seeds to germinate and establish. Summer rainfall from November until the commencement of grazing in early April 2014 was less than 10 mm. Autumn/winter rainfall during the experimental period (April–June) was 137 mm.

#### 3.1.2 Effect of grazing intensity on plant height

The sheep took 3 days to acclimatise before starting to consume the *L. ambigua*, and the target pasture heights were achieved by day 5 for the moderate treatment and day 6 for the severe grazing treatment. The average plant height for each treatment immediately after grazing was 4.4, 9.3 and 22.3 cm for the severely, moderately and un-grazed treatments respectively ( $P < 0.001$ , Fig. 1).

The change in height over time after grazing was close to linear for all treatments and at all times during the experiment, there were significant differences ( $P < 0.001$ ) in plant height between treatments (Fig. 1). The gain in height over the 10-week period post-grazing was greater for the moderately grazed treatment than the un-grazed treatment (16.1 vs 12.1 cm;  $P < 0.05$ ) and tended to be greater for the severely grazed compared to and the un-grazed treatment (15.1 vs 12.1 cm;  $P < 0.1$ ).

**Fig. 1** Average height of *L. ambigua* for plants that were un-grazed or grazed at two levels of intensity over 6 days (moderate and severe) from 2 April 2014. Values are treatment means with dotted lines representing 95% confidence intervals.



### 3.1.3 The effect of grazing intensity on plant density, structure and biomass

Grazing pressure had no effect on plant density with 100% survival recorded during the re-growth period for all treatments (Table 1). The height, weight and weight per unit of height of individual plants at the end of the re-growth period declined as the intensity of grazing increased ( $P < 0.001$ , Table 1). The final ratio of weight to height of *L. ambigua* plants in the un-grazed treatment was more than double that of plants in both grazed treatments ( $P < 0.001$ ), indicative of a more dense plant structure (Fig. 2). Re-growth of more severely grazed plants was often characterised by one or two dominant shoots.

At the beginning of the re-growth assessment period, the average biomass was 80, 170 and 490 kg DM/ha for the

severely, moderately and un-grazed treatments, respectively ( $P < 0.001$ ). At the completion of the 10-week re-growth period, the un-grazed treatment had the greatest biomass of 1740 kg DM/ha compared to 520 kg DM/ha for the moderately grazed and 300 kg DM/ha for the severely grazed treatment ( $P < 0.001$ ). The rates of accumulation of biomass were 3.1, 5.0 and 17.8 kg DM/ha/d over the 10-weeks for the severely, moderately and un-grazed treatments, respectively.

### 3.1.4 The effect of grazing on plant growth rate

Average PGR was positively related to the residual feed on offer after grazing ( $P < 0.001$ ), but there was an additional negative effect of grazing on PGR independent of FOO during the initial 6 weeks. The prediction of PGR over weekly intervals from the same level of FOO indicated that

**Table 1** Density, average height, weight and weight per unit height of individual *L. ambigua* plants after 10 weeks of re-growth for severely grazed, moderately grazed and un-grazed treatments in experiment

Plant characteristic	Severely grazed	Moderately grazed	Un-grazed	LSD ( $P=0.05$ )	Significance
Density (linear m)	11.6	10.7	11.6	2.7	NS
Height (cm)	19.4 <sup>a</sup>	25.5 <sup>b</sup>	34.4 <sup>c</sup>	3.3	***
Weight (g)	1.7 <sup>a</sup>	3.1 <sup>b</sup>	9.6 <sup>c</sup>	1.4	***
Weight to height (g/cm)	0.08 <sup>a</sup>	0.12 <sup>b</sup>	0.28 <sup>c</sup>	0.04	***

1. Means within columns with different superscripts are significantly different. NS, not significant; \*\*\*  $P < 0.001$ . LSD is the least significant difference.



**Fig. 2** The upper photographs, taken on 18 April 2014, illustrate the post-grazing appearance of *L. ambigua* in the un-grazed, moderately grazed and severely grazed treatments of experiment 1 respectively.

The lower images, taken on 6 June 2014, illustrate the post-re-growth appearance of *L. ambigua* in the un-grazed, moderately grazed and severely grazed treatments respectively.

the negative impact of grazing disappeared after week six (Table 2). In other words, the differences in actual PGR after week six were attributed entirely to differences in FOO rather than grazing treatment.

### 3.1.5 The effect of grazing intensity on nutritive value

There were no significant effects of grazing treatments on the digestibility and metabolisable energy content of *L. ambigua* plants at the end of the experimental period (Table 3). However, the crude protein content diminished with decreasing grazing intensity from 26.4 to 21.7%, whereas the neutral detergent fibre content was highest for the un-grazed plants ( $P < 0.01$ ). The content of ash was highest in the severely grazed compared to the moderately and un-grazed treatments ( $P < 0.001$ ).

**Table 2** Weekly plant growth rate (kg DM/ha/day) of *L. ambigua*, with FOO (food on offer) as a covariate, for the severely, moderately and un-grazed treatments. Values presented are treatment means predicted at the average FOO across all treatments. Means within rows with different superscripts are significantly different. NS, not significant; \* at  $P < 0.05$ , \*\* at  $P < 0.01$ .

Week	Severely grazed	Moderately grazed	Un-grazed	Significance
1	8.0 <sup>a</sup>	5.8 <sup>a</sup>	12.7 <sup>b</sup>	*
2	6.9 <sup>a</sup>	6.5 <sup>a</sup>	11.1 <sup>b</sup>	*
3	4.6 <sup>a</sup>	4.8 <sup>a</sup>	10.4 <sup>b</sup>	**
4	8.2	7.0	10.8	NS
5	7.5 <sup>a</sup>	9.1 <sup>a</sup>	13.2 <sup>b</sup>	*
6	10.0 <sup>a</sup>	12.1 <sup>a</sup>	19.6 <sup>b</sup>	*
7	9.5	10.6	13.4	NS
8	8.7	10.2	16.1	NS
9	12.0	12.5	12.4	NS

**Table 3** Average nutritive value of *L. ambigua* plants (above ground) including digestibility of organic dry matter (DOMD), metabolisable energy (ME), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and water-soluble carbohydrate (WSC) sam-

Nutritive value	Severely grazed	Moderately grazed	Un-grazed	LSD ( $P = 0.05$ )	Significance
DOMD (%DM)	70.9	72.9	71.0	2.46	NS
ME (MJ/kg DM)	11.4	11.8	11.4	0.48	NS
CP (%DM)	26.4 <sup>a</sup>	24.0 <sup>b</sup>	21.7 <sup>c</sup>	1.10	***
NDF (%DM)	30.3 <sup>b</sup>	29.8 <sup>b</sup>	34.1 <sup>a</sup>	2.22	**
ADF (%DM)	19.5	19.6	20.6	1.55	NS
ASH (%DM)	9.2 <sup>a</sup>	8.4 <sup>b</sup>	8.6 <sup>b</sup>	0.41	***
WSC (%DM)	6.5	7.4	6.5	1.56	NS

## 3.2 Experiment 2: Impacts on animal health

There was 133 mm of rainfall over the 6-month period from the start of November to the end of April in 2015 enabling excellent plant establishment. At the commencement of the grazing period in March 2016, when plants were 18 months old, there was an average of 790 kg DM/ha of *L. ambigua* in the treatment plots, as well as dry residues of low-quality annual herbs and grasses. At the completion of the 43-day grazing period, there was 250 kg DM/ha of *L. ambigua* remaining in the treatment plots, 570 kg DM/ha of dry residues, and the plant density of *L. ambigua* remained at 0.5 plants per m<sup>2</sup> throughout the experiment (data not shown).

It is estimated that sheep grazing the *L. ambigua* consumed a minimum of 0.5 kg DM/day between day 21 and day 64, being the difference between the initial biomass of *L. ambigua* and the final biomass, and not accounting for growth during this period. The control group consumed approximately 0.45 kg/day of feed pellets. Both groups also potentially consumed up to 0.3 kg DM/day of dry residues, although these largely disintegrated under trampling. There was no significant difference in live weight between the two groups at any of the time points, with the average live weight decreasing from 43 kg on day 1 to 40 kg on day 64 for the control group and from 44 to 39 kg for animals grazing *L. ambigua* ( $P > 0.05$ ).

There was no difference ( $P > 0.05$ ) in the analysis of the blood samples between sampling dates (days 32, 50 and 64), and hence, data from samples taken on day 64 are presented (Table 4). However, analysis of blood samples indicated some differences between the two groups ( $P < 0.05$ ). The group grazing *L. ambigua* returned nearly 50% higher levels of alanine transaminase (16.5 vs. 11.2 u/L) and elevated creatinine and methylmalonic acid, but lower levels for all other blood parameters and the trace elements copper and zinc (Table 4). There were no treatment  $\times$  sampling day interactions for any of the blood parameters ( $P > 0.05$ ) and no visible evidence of problems in animals grazing *L. ambigua*.

pled 10 weeks after a severe or moderate grazing in comparison to un-grazed plants in experiment 1. Means within rows with different superscripts are significantly different. NS, not significant; LSD least significant difference; \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .



**Table 4** Blood parameters haematocrit (HCT), alanine transaminase (ALT), glutathione peroxidase (GSPx), methylmalonic acid (MMA), gamma-glutamyl transferase (GGT), glutamate dehydrogenase (GLDH), red blood cells (RBC) for young sheep grazed on dry annual pasture supplemented with commercial pellets (control) or *L. ambigua* (treatment) for 63 days in experiment 2. Values represent the mean of four sampling times and are compared with the normal range for ovine wethers. NS, not significant; \*  $P < 0.05$ , \*\*\*  $P < 0.001$ .

Parameter	Control	Treatment	Significance	Normal
HCT (%)	44.4	42.3	*	35.0–45.0
ALT (U/L)	11.2	16.5	***	11.0–40.0
Creatinine ( $\mu\text{mol/L}$ )	104	123	***	50–150
GSPx (U/gHb)	444	362	***	< 500
MMA ( $\mu\text{mol/L}$ )	0.9	1.1	***	< 2.0
Copper (mg/L)	0.9	0.8	***	1–1.5
Zinc (mg/L)	0.9	0.7	***	0.8–1.2
GGT (U/L)	42.9	41.7	NS	23.0–67.0
GLDH (U/L)	17.5	21.1	NS	< 20.0
RBC	11.4	11.3	NS	10–14.0
Urea (mmol/L)	7.4	7.4	NS	3.3–8.0

## 4 Discussion

The hypothesis that sheep would graze *L. ambigua* when in near monoculture conditions and that intensive grazing of young plants would reduce their rate of re-growth was supported by the results. Plants that were heavily grazed over 5 to 6 days in experiment 1 produced up to five times less biomass than the un-grazed plants over the following 10 weeks. Un-grazed *L. ambigua* produced 1740 kg DM/ha mostly during summer and autumn, and even after accounting for the consumption of *L. ambigua* for the grazed treatments, the severe and moderate grazing treatments reduced total biomass production during summer and autumn by about 50%. When correcting for FOO as a covariate, the weekly PGR of the grazed treatments was less than the un-grazed, until week seven when FOO became the only limiting factor to PGR. This suggested that by the end of the experimental period, the growth rate across all treatments was the result of increased FOO, rather than differences caused by additional effects of grazing. Initially, the reduced PGR was likely to have been caused by the stressed state of the grazed plants (Gaspar et al. 2002), their consequent reduced leaf area index (Parsons et al. 1988; Frame and Laidlaw 2011; Lemaire and Chapman 1996) and their depleted resources in the roots (Belsky 1986; Smith 1972).

Similar findings have been reported for other plant species, where defoliation has had a negative impact on the subsequent growth (Sternberg et al. 2006; Ferraro and Oosterheld 2002; Taddese et al. 2002), but the magnitude of these effects measured in the current study is larger than other studies. It is likely that the severity of the defoliation,

which removed up to 83% of the aboveground biomass over a few days, and the extremely dry period from the start of November until late April contributed to these results. It is also worth considering that the grazing severity imposed in experiment 1 (approximately 30 DSE/ha) far exceeds the normal stocking rates of 5–10 DSE/ha in this farming region.

Despite the severe grazing of juvenile plants (7 months old) in experiment 1, it did not compromise their survival. Similarly, there was also no loss of 18-month-old plants that were continuously grazed for 43 days, with 25 sheep per hectare, down to 250 kg DM/ha in experiment 2. This strongly indicates that *L. ambigua* is well adapted to intensive grazing by sheep for a short period in summer and autumn in a Mediterranean environment. While the species may respond differently to continuous grazing at a lower DSE, it is anticipated that stands of *L. ambigua* will be fenced off and protected from grazing until a period that offers the most value to animal production and welfare over a given year.

In terms of the evolution of *L. ambigua* in the fynbos, the largely accepted opinion is that the sandstone vegetation did not support large resident herbivore populations as these animals were concentrated in the renosterveld shrubland, on more nutrient-rich shale substrates (Johnson 1992; Rebelo 1996, 1992; Owen-Smith and Danckwerts 1997). Beukes and Smit (1987) assessed the habitat and diet selection of the browsing ungulate grey rhebuck in the fynbos biome. He reported that grey rhebuck predominantly fed on dicotyledonous plants (on average 97% of rumen content) with some grasses also grazed during the cool and wet winter months of the growing season. Studies such as this allow us to infer that *L. ambigua* was exposed to at least some browsing and/or grazing during its evolution and that it has developed traits which provide tolerance to defoliation. Our data indicate that grazing of *L. ambigua* with sheep is possible in the summer and autumn, both immediately after sowing, and also in the second year after sowing. However, if *L. ambigua* is grazed in the first summer and autumn, it should be grazed to a residual height greater than 9 cm to avoid significant penalties in subsequent pasture growth.

In contrast to the treatment effects on biomass production, grazing treatments had relatively small effects on the height of *L. ambigua* re-growth. If anything, the height of re-growth was greater for the grazed plants, indicating responses to grazing were reflected mostly in changes to plant structure or density of the canopy. The rapid increase in height of one or two shoots post-grazing would allow plants to intercept the maximum amount of light and, in turn, increase their capacity to undergo photosynthesis (Detling et al. 1979; Caldwell et al. 1981; Belsky 1986). This is an important result in the context that autumn grazed *L. ambigua* in the mixed farming zone of Western Australia will need to re-grow in winter to compete for light against the winter growing annuals. This response may also be explained by the natural environment

of *L. ambigua* where it must grow quickly in response to fire disturbance when in competition with other plant species in the fynbos (Edwards et al. 2019).

The nutritional value of *L. ambigua* plants was extremely high regardless of grazing severity, especially given the samples represented total aboveground dry matter. This is consistent with contemporary analyses of the fynbos and renosterveld vegetation of the CFR (Chimphango et al. 2020). The overall average in vitro values for metabolisable energy (11.6 MJ/kg), crude protein (24%), NDF (32%) and ADF (20%) is comparable to or better than values in the literature reported for subterranean clover dominant pastures (Heard et al. 2006), irrigated white clover (Stockdale 1999) or chicory (Muir et al. 2014) during the growing season and much higher than the nutritive value of lucerne (Finlayson et al. 2012) or annual pastures during autumn in the mixed farming zone of Western Australia (Schut et al. 2009). The nutritive value of *L. ambigua* in autumn was well in excess of that required by grazing ruminants, regardless of its physiological state (Freer et al. 2007).

The absence of an effect of grazing treatment on digestibility is consistent with the content of acid detergent fibre and probably indicated that there had been little maturation and lignification of the 11 to 28% of dry matter produced prior to the grazing treatments, which was still only 7 to 9 months of age. The apparent effects of grazing on the content of neutral detergent fibre but not digestibility of organic matter probably reflects inaccuracies in using the Ankon method for the determination of neutral detergent fibre in perennial legumes (Norman et al. 2019). The nutritive value of *L. ambigua* that was un-grazed for 9 months was not reduced to levels that would adversely influence productivity. This may enable greater flexibility in the time the plants are grazed to complement the energy needs of the sheep production system.

Grazing *L. ambigua* did not negatively impact the health of animals when it was a major component of the diet, which supports our second hypothesis. The dry residues on the field surface in experiment 2 were considered of little value and disintegrated somewhat with trampling (Ballard et al. 1990). While there were some significant differences in essential trace elements and blood biochemical concentrations detected between the groups, the predicted mean values for each treatment were all within the normal reference ranges for grazing ruminants (Puls 1994; Suttle 2010). Alanine transaminase levels are often indicators of liver or kidney damage in ruminants, and although animals grazing *L. ambigua* had elevated alanine transaminase, the levels were well within the expected range for wethers of 11–40 u/L (Cristaldi et al. 2005). The trend towards lower concentrations of Cu and Zn in the animals grazing *L. ambigua* might indicate a potential issue with long-term grazing of *L. ambigua* monocultures, although concentrations in the blood

were similar to those reported in Dorper sheep of the same age grazing fynbos vegetation (Van Niekerk et al. 1990). The renosterveld vegetation of this biome is characterised as having sub-optimal levels of Cu and Zn for grazing animals, probably as a consequence of the infertile soils they inhabit (Chimphango et al. 2020). However, for mineral deficiencies in sheep, amelioration strategies are well defined and include the use of fertilisers containing Cu and Zn, and mineral supplements.

The loss of weight by sheep grazing *L. ambigua* in experiment 2 has been noted in other studies of ruminants grazing perennial forages at this time of the season, especially when available biomass falls below 700 kg DM/ha (e.g. Gutman and Seligman 1979). Based on the measured characteristics of intake and nutritive value of *L. ambigua* in experiment 1, GrazFeed (Freer et al. 1997) predicted a weaner growth rate of 236 g/day when 500 kg DM/ha of biomass of *L. ambigua* was available (data not shown). This was clearly not achieved in Experiment 2 perhaps because available biomass fell to 250 kg DM/ha by the end of the grazing period. Further analysis of the foliage of *L. ambigua* may reveal temporal features of alkaloid accumulation and other chemistry that might be important in grazing management.

## 5 Conclusions

Autumn grazing of young plants of *L. ambigua* by Merino wethers damaged neither the plant nor the animal. The grazed plants maintained density and displayed an ability for re-growth after the stock was removed, and the blood biochemistry of the grazing animals was within normal ranges for ruminants on all parameters, on all sampling dates.

This study is the first to quantify plant and animal effects from grazing stands of *L. ambigua* in an agricultural production context. *Lebeckia ambigua* was grazed in late autumn at two different sites, beginning 7 months or 18 months after planting, without detriment to plant survival. The ability to provide grazing within 12 months of sowing, in dry regions (< 400-mm annual rainfall) on deep sandy soils, sets *L. ambigua* apart from other perennial forage legumes available in commerce.

It is concluded that the high nutritional value of *L. ambigua* during the summer and autumn period, when grown on infertile, sandy soils that otherwise support plants of little agricultural value, indicates it could improve production efficiency and husbandry of sheep enterprises in mixed farming systems. Further work is required to quantify the potential holistic economic value of *L. ambigua* into mixed farm enterprises within rain-fed agro-ecosystems, particularly in the novel “perennial ley” system envisaged by Edwards et al. (2019). This will necessitate a more intensive evaluation of animal

performance and improved agronomy of the plant, including management of the dormant seed behaviour of this legume identified by Harrison et al. (2021). While many plant improvement programmes in southern Australia have attempted to identify deep-rooted forages to extend the growing season, until now no perennial legume has been successful in the low rainfall, acidic and coarse-textured soils of the mixed farming zone of Western Australia, after three decades of research (Revell et al. 2013). The adaptation of *L. ambigua* to these challenging soils presents a rare opportunity to improve pasturage without compromising other land usages.

There are many rain-fed agro-ecosystems which support extensive herbivory, but in which the natural vegetation is degraded and of low nutritive value (Gintzburger et al. 1990; Gintzburger and Saïdi 2010; Le Houerou 1984), and where productivity (Schoknecht 2015) and sustainability of the ecology (Crews et al. 2016) is uncertain. Problematically, some of these regions have significant edaphic constraints to plant success, such as acidic soils, variable and changing rainfall patterns, prolonged summer drought and extreme temperatures, which when combined are hostile to plant survival and soil biology (Howieson and Ballard 2004). It is now evident that *L. ambigua* can tolerate these stresses and, together with other legumes of the fynbos, may have value in the sustainable management of these regions around the globe.

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**Data availability** The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Declarations

**Ethics approval** Australian Code of Practice for the Use of Animals for Scientific Purposes was followed and the project received approval from the Murdoch University Animal Ethics Committee.

**Consent for publication and participation** N/A.

**Conflict of interest** The authors declare no competing interests.

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