agronomy

MDPI

Article

LANZA® Tedera Is Strongly Suppressed by Competition from *Lolium multiflorum* and Is Best Adapted to Light-Textured Soils

Jesús Fernández-Habas [1], Daniel Real [2], Tom Vanwalleghem [3] and Pilar Fernández-Rebollo [1,*]

[1] Department of Forest Engineering, University of Cordoba, 14071 Cordoba, Spain
[2] Department of Primary Industries and Regional Development (DPIRD), Perth, WA 6151, Australia
[3] Department of Agronomy, Hydraulic Engineering Area, University of Cordoba, 14071 Cordoba, Spain
* Correspondence: ir1ferep@uco.es

Abstract: *Bituminaria bituminosa var. albomarginata*, known as Tedera, is a promising forage for Mediterranean climates. An improved variety named LANZA® has been developed. Previous research suggests that soil water saturation in heavy-textured soils might affect its initial development. Competition from grasses could also compromise its successful establishment and persistence. We investigated the effects of soils with contrasting textures (loamy sand vs. clay) with a high soil water content and the competition from *Lolium multiflorum* on the development of LANZA® in a pot experiment. Dry mass (DM) production of LANZA® was strongly reduced (86%) when grown with *L. multiflorum*. LANZA® shoot DM was 60% higher than the total shoot DM production of the mixture of LANZA® and *L. multiflorum*. Soil type did not significantly affect the shoot and total root DM. However, a 44% reduction of the DM of thin roots and slower development was observed in clay soils, which may indicate a preference for light-textured soils. This study provides further information on the factors limiting the establishment and persistence of LANZA®. Future research should confirm these results at field scale and investigate measures aimed at reducing early competition in monocultures and functional complementarity with partner species in mixtures to successfully establish LANZA®.

Keywords: competitive ability; establishment; mixtures; forage legumes; *Bituminaria bituminosa*

check for updates

Citation: Fernández-Habas, J.; Real, D.; Vanwalleghem, T.; Fernández-Rebollo, P. LANZA® Tedera Is Strongly Suppressed by Competition from *Lolium multiflorum* and Is Best Adapted to Light-Textured Soils. *Agronomy* **2023**, *13*, 965. https://doi.org/10.3390/agronomy13040965

Academic Editor: Steven R. Larson

Received: 1 March 2023
Revised: 17 March 2023
Accepted: 22 March 2023
Published: 24 March 2023

1. Introduction

Mediterranean grasslands and their rich provision of ecosystem services are strongly associated with livestock grazing in extensive and semi-extensive systems, in which small ruminants and beef cattle graze rain-fed grasslands and croplands [1]. These systems are challenged by scarce summer rainfall, droughts, and high inter-/intra- annual rainfall variability, resulting in feed gaps and irregular feed availability [2,3]. This leads to poor animal performance, inefficient farm management, and often, insufficient revenues to maintain the enterprise [3,4]. Climate change is expected to exacerbate this situation due to an overall reduction of mean annual rainfall, its increased inter-/intra- annual variability, and more frequent and severe droughts [5–7]. Among the options to cope with these issues and reduce their effects on farming systems in Mediterranean regions, the use of drought-resistant legumes has been proposed, especially hard-seeded annual legumes and perennial legumes [3,8–10], as well as their integration in ley-farming systems [11].

Research efforts have focused on the search for species that could meet the requirements of Mediterranean livestock systems [12,13]. One of the most promising species is the perennial legume *Bituminaria bituminosa* C.H. Stirton, especially *var. albomarginata* from the Canary Islands, where it is known by the common name Tedera [14]. Previous research has demonstrated that this species is productive under very low rainfall (<200 mm), is highly drought-resistant [15–17], has good forage quality [18–20], and it is suitable for feeding livestock [14,21–23]. Recently, an improved variety has been developed as a result of a collaborative breeding programme led by Australian and Spanish researchers [9]. This new variety is registered under the trademark of LANZA® and protected by the Plant Breeder's Right Act 1994 in Australia [24]. It was bred by the Department of Primary

Industries and Regional Development (DPIRD) as part of an initiative by the Future Farm Industries Cooperative Research Centre. Seed from LANZA® became marketable in Australia in 2019. The breeding program combined attributes from elite plants of var. *crassiuscula* and var. *albomarginata* to seek ideotypes with drought and cold tolerance [9].

Recently, Real [25] demonstrated that this species could be established using machinery commonly used for cereal establishment and provided information about fertilization requirements [26] and herbicide tolerance [27].

There is still research to be carried out to clarify the potential adaptation of this species to the wide range of environmental conditions of the Mediterranean Basin [21]. Two factors that could potentially affect the adaptation of *B. bituminosa* are soil texture and the ability to deal with competition during the establishment phase. Previous research suggested that soil water saturation could be unfavourable for *B. bituminosa var. albomarginata* [18] and that it is unlikely to persist in environments with cold wet winters [28]. Real et al. [9] found *B. bituminosa* to be sensitive to waterlogging overall, although it showed high variability in waterlogging tolerance. One of the essential characteristics for new perennial legumes to ensure successful adaptation in Mediterranean environments is having a broad range of soil and climatic adaptations [29]. Additionally, the slow early vigour of this plant coupled with competition might compromise its successful establishment in temporary or permanent Mediterranean grasslands, where annual grasses and forbs might outcompete *B. bituminosa*. Low competitiveness has been reported as a major limiting factor in establishing perennial legumes [30–32]. Field observations of *B. bituminosa* plantations in Australia have reported failure due to competition by weeds [28]. The cost of establishment and unreliable production in variable environments are the main barriers hindering the adoption of pasture legumes [32,33]; therefore, its study is essential for the successful introduction of new forage legumes such as LANZA® [10].

In light of the potential of *B. bituminosa* as a new forage legume to fulfil the needs of Mediterranean livestock systems, we studied the effects of soil texture and competition. This study aimed at investigating the development of cv. LANZA® in (i) interspecific competition with *Lolium multiflorum* and (ii) in two types of soils of contrasting texture (loamy sand and clay) in a pot experiment in controlled conditions.

We hypothesise that:

Hypothesis 1: *The interspecific competition of LANZA® with Lolium multiflorum will reduce LANZA® development.*

Hypothesis 2: *Heavy-textured clay soils in conditions of high soil water content could limit the development of the root system of LANZA® due to its water retention capacity, and hence reduce the biomass production of this species.*

2. Materials and Methods

2.1. Plant Material

LANZA® seeds were provided by the Department of Primary Industries and Regional Development (DPIRD), Australia. To achieve fast and effective germination, the seeds were mechanically scarified by nicking the outer seed coat with a surgical scalpel [34]. After scarification, the seeds were germinated in flat trays with a wet filter paper [16,35]. Once germinated, the seeds were planted in seedlings trays and grown for approximately 30 days. When the seedlings had three leaves, they were transplanted into six litre, free draining, individual plastic cylindrical pots that were 37 cm high, with a 16.8 cm and 12.5 cm diameter at the top and bottom of the pot, respectively. *L. multiflorum* var. Tambo seeds were provided by FERTIPRADO (http://www.fertiprado.pt/es/ (accessed on 15 January

2023)). *L. multiflorum* seeds were directly planted in seedling trays until they produced three leaves, at which point they were transplanted into the same pots described previously.

2.2. Experimental Design

The experiment design was a completely randomised factorial design with two factors, two treatments per factor and six replicates. The factors were: level of competition and soil texture. For competition, the treatments were single LANZA® plants grown alone in pots (control) and single plants of LANZA® grown in mixture with two plants of *L. multiflorum* (competition). The soil textures were loamy sand (S) and clay (C) (Table 1). S soil was collected at an experimental farm located at Hinojosa del Duque, Cordoba, Spain (38°29′46″ N and 05°06′55″ W). This area is characterised by shallow Eutric Cambisols devoted mainly to livestock grazing of permanent grasslands in rangelands of *Dehesa* ecosystems [36]. This soil was fertilised with 50 kg P_2O_5 ha^{-1} two years before collecting the soil. C soil was collected at the experimental farm of University of Cordoba in the Campus of Rabanales, Cordoba, Spain (37°55′01″ N and 04°42′57″ W). These soils are deep Vertisols from the Guadalquivir valley typically devoted to cereal crops [37]. The agronomic characteristics of both soils are described in Table 1. Both soils were mixed at 1/5 $v\ v^{-1}$ with soil collected from natural populations of *B. bituminosa* to promote *Rhizobium* inoculation (soil data correspond to the soil already mixed). The water retention curves of both soils were determined in the laboratory to obtain the saturated volumetric water content (θ_s) (Table 1). Three undisturbed samples were directly extracted from the three different pots per soil type using 100 cm^3 volume core rings. The sandbox apparatus (Sand/Kaolin box, Eijkelkamp, Giesbeek, ZG, The Netherlands) was used to obtain five pressure head points between saturation and −63.1 cm [38]. The sand/kaolin box was used to obtain two pressure head points at −100 and −199.5 cm [39]. Pressure head points between -1×10^3 cm and -1.5×10^6 cm were determined using the WP4-T Dew Point Potentiometer (Decagon Devices, Inc., NE Hopkins Court, Pullman, WA, USA) [40]. The resulting points were fitted to the Van Genuchten [41] Equation (1) with the RETC software version 1.0 (https://www.pc-progress.com/en/Default.aspx?retc (accessed on 15 November 2022)) [42].

$$\theta = \theta_r + \frac{(\theta_s - \theta_r)}{[1 + |\alpha\psi|^n]^m} \quad (1)$$

where θ is the volumetric water content ($cm^3\ cm^{-3}$), θ_s is the saturated volumetric water content ($cm^3\ cm^{-3}$), θ_r is the residual volumetric water content ($cm^3\ cm^{-3}$), ψ is the matric potential (-cm), α, n and m are fitting parameters that determine the shape of the curve, being $m = 1 - 1n^{-1}$.

Table 1. Soil analysis of the two soil types used in the experiment.

Parameters	Units	Loamy Sand (S)	Clay (C)
Sand	%	73	29
Silt	%	13	25
Clay	%	14	46
Bulk density	$g\ cm^{-3}$	1.48	1.02
Soil pH in water (12.5)	U. pH	7.1	8.12
Cation exchange capacity	Meq 100 g^{-1}	11	31
Electrical conductivity 25 °C (1:5)	$mS\ cm^{-1}$	0.16	0.22
Oxidable Organic matter content	%	1.97	3.21
N (Kjeldahl)	%	0.11	0.19
Available P (Olsen)	$mg\ kg^{-1}$	44	7
Exchangeable K (NH_4Cl)	$mg\ kg^{-1}$	138	319
Exchangeable Ca (NH_4Cl)	$mg\ kg^{-1}$	1.12	4.91
Exchangeable Mg (NH_4Cl)	$mg\ kg^{-1}$	114	371
Exchangeable Na (NH_4Cl)	$mg\ kg^{-1}$	102	118
Saturated volumetric water content	$cm^3\ cm^{-3}$	0.33	0.62

The resulting water retention curves of both soils and the corresponding fitting parameters of Van Genuchten's model are shown in Figure S1.

During the experiment, the soil water content of the pots was maintained between θ_s and 70% of θ_s (on average) by weighing the pots and watering periodically (with a watering cadence that fluctuated from three days at the beginning of the experiment to every day at the end). The experiment was conducted from 8 March to 21 July 2021 (135 days) in a shade house at the experimental farm of the University of Cordoba in the Campus of Rabanales, Cordoba, Spain (37°55′01″ N and 04°42′57″ W). The net of the shade house had a nominal shading factor of 60%. The mean of the daily mean temperatures, mean of the daily maximum, and mean of the daily minimum temperature during the experiment were 20.6, 28.3, and 12.9 °C, respectively (Figure S2).

2.3. Phenology

From day 65 (12 of May) to the end of the experiment on day 135 (21 July), the phenology stage of LANZA® in both soil types with and without competition was recorded. The phenological stages were recorded following the general scale of the Biologische Bundesanstalt Bundessortenamt und Chemische Industrie (BBCH) [43]. The phenology of *L. multiflorum* at key phenological stages (from inflorescence emergence to senescence) was also recorded. The phytovolume of LANZA® plants was estimated by calculating the cylinder volume using the average diameter of two perpendicular measurements and plant height. Phytovolume was calculated at day 65 (12 May) and day 135 (21 July).

2.4. Dry Mass, Morphological Traits, and Leaf Nutrients Content

At the end of the experiment, plants were cut to ground level. The shoot biomass of LANZA® was separated into stems and leaves. Roots were separated from the soil using a 2 mm sieve and divided into thin (<2 mm) and thick roots (>2 mm). Thin root separation was not possible in competition treatment because the roots of LANZA® and *L. multiflorum* were strongly intertwined (Figure S3). Therefore, in competition treatment, only thick roots of LANZA® were separated because *L. multiflorum* did not produce roots of >2 mm. Biomass was oven-dried at 60 °C for 72 h and then weighed. Leaf to stem ratio was measured as leaf dry mass/stem dry mass in LANZA® plants grown in both treatments. The mean leaf area for LANZA® plants was calculated by scanning the leaves (EPSON 1640 XL, Epson America, Inc., Long Beach, CA, USA) before drying and then measuring the leaf area using the image analyser software ImageJ (https://imagej.nih.gov/ij/ (accessed on 28 July 2021)). When the leaf biomass of a plant was higher than 1.5 g, a random representative sample of leaves was scanned. The sample was always at least 15% of the total leaves' dry mass. From the ratio between the leaf area and its dry mass, the specific leaf area (SLA) ($cm^2\ g^{-1}$) was also calculated for LANZA®. Finally, for LANZA® plants grown without competition (control) in both soil types, the proportion of thin roots was estimated as thin root dry mass/total root dry mass.

Samples of leaves of LANZA® plants of all treatments were ground and passed through a 1 mm sieve and then analysed for the following nutrients: *N*, *P*, *K*, *Ca*, *Mg* and *S*. The ratios of *C*/*N* and *N*/*P* were also calculated. Concentrations of *N*, *C*, and *S* were analysed at the Central Service for Research Support (SCAI) of the University of Cordoba using a LECO macro combustion instrument, model CNS928 (LECO Corporation, Lakeview Ave, St. Joseph, MI, USA). The concentrations of *P*, *K*, *Mg* and *Ca* were analysed at the Soil Science Unit of the University of Cordoba. *K* was analysed using a flame photometer Jenway™ PFP7 (Cole-Parmer Ltd., Staffordshire, UK). *Ca* and *Mg* were measured with an Atomic Absorption Spectrometer Perkin Elmer AAnalyst 200 (PerkinElmer, Inc., Waltham, MA, USA). *P* was analysed using an absorbance microplate reader BioTek PowerWave HT Microplate (BioTek® Instruments, Inc., Winooski, VT, USA).

2.5. Physiological Measurements

Physiological measurements were taken the day before harvesting the plants, on day 134 (20 July). Net photosynthesis per area (A_{area}) and stomatal conductance per area (g_{Sarea}) were measured between 09:00–12:00 h in fully expanded and undamaged leaves of mid-height using a portable infrared CO_2 gas analyser (LiCor Li6400XT, Li-Cor, Inc., Lincoln, NE, USA) fitted with a 2 cm^2 leaf cuvette. The settings of the gas analyser were: flow rate at 500 µmol s^{-1} PAR set at 1500 µmol photon m^{-2} s^{-1}, [CO_2] to 350 ppm, and block temperature set at 25 °C. Water use efficiency (WUE) was calculated as the ratio A_{area} $g_{Sarea}{}^{-1}$. After recording A_{area} and g_{Sarea}, six leaves per plant of the same characteristics were cut to estimate leaf relative water content (RWC). RWC was calculated as RWC = ((FW − DW)/(SFW − DW)) × 100, where FW is fresh leaf weight, DW is dry leaf weight and SFW is saturated fresh leaf weight. After being weighed to obtain FW, the leaves were immersed in deionised water in 90 mm Petri dishes. The leaves were weighed again after 24 h at 20–25 °C to record SFW. DW was obtained after weighing the oven-dried leaves at 60 °C for 72 h.

2.6. Statistical Analysis

The shoot dry mass production, leaf: stem ratio, SLA, phytovolume, mean leaf area, thick roots dry mass and leaf nutrients content of LANZA® without competition (control) and with competition (competition) in both soil types (S and C) were tested by two-way ANOVAs. The effect of soil type and competition on the physiological measurement of LANZA® was also tested by two-way ANOVAs. Total root dry mass, thin root dry mass production and their proportion in the total root dry mass were further investigated for LANZA® in each soil type for control plants only by one-way ANOVAs. The differences in shoot dry mass, root dry mass, and total dry mass production by pot between competition (*L. multiflorum* plus LANZA®) and LANZA® grown alone (control) in both soil types (S and C) were also tested by two-way ANOVAs. Variables were log-transformed when necessary to meet normality and homoscedasticity assumptions. When differences were significant ($p < 0.05$), a post hoc Tukey's test at the 0.05 level was carried out to separate homogeneous groups. The differences in phenology between treatments were investigated by cumulative link mixed models [44]. Cumulative link models, also known as ordinal regressions models [45], can be used to test the effects on a response variable following an ordered finite set of categories. In this case, the different BBCH stages were considered as the response variable following an ordinal scale, and soil type, level of competition, and date of observation as predictor categorical variables to account for the main effects. The interactions of soil type x competition, soil type x date of evaluation and competition x date of evaluation were also tested. To account for dependence among observations on different dates over the same plant, the plant identity was considered as a random effect. Models were fitted using the "ordinal" package of R [46], following Christensen [44] and Mangiafico [47]. The significance of the main effects and their interactions were tested by an analysis of deviance (ANODE) using the "Anova.clm" function from the "RVAideMemoire" package [48]. All statistical analyses were performed using the software R v. 3.6.1 [49].

3. Results

3.1. Effect of Competition and Soil Type on the Development of LANZA®

Figure 1 schematises the phenology of LANZA® in each treatment. In these conditions of culture in the shade house, none of the LANZA® plants reached reproductive stages (inflorescence emergence which corresponds to stage five in BBCH). Therefore, only the main growth stage corresponding to stem elongation was recorded (from 3.1, meaning that 10% of the final biomass has been reached, to 3.9, where 90% has been reached). All *L. multiflorum* plants reached the reproductive stage of seed ripening (stage eight). The phenology of LANZA® reported significant differences by soil type, level of competition and by the interaction of level of competition and date of evaluation (Table S1). LANZA® plants reached more advanced phenological stages when grown in S soil compared to plants grown in C soil (df = 1; LR Chisr:15.15; $p < 0.001$). The interaction of level of competition by date of evaluation (df = 18; LR Chisr:124.93; $p < 0.001$) revealed that plants grown without the competition of *L. multiflorum* also reached higher phenological stages and that their growth did not follow the same pattern over time as the growth of plants under competition. The advance in the phenological stage three of control plants plateaued from 15 July onwards at stage 3.39, whereas the advance of plants in competition seems to have been subject to the phenology of *L. multiflorum* (Figure 1).

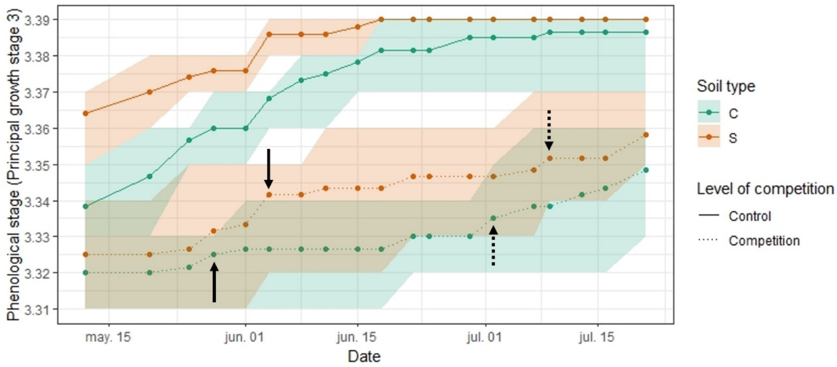

Figure 1. Phenology of LANZA® in each treatment from 12 May 2021 to 21 July 2021 according to the BBCH system. Shaded area indicates range of the phenological stage (from plant with the lowest phenological stage to the plant with the highest). Solid arrows indicate the date at which all *L. multiflorum* plants reached stage 5 (inflorescence emergence) in each soil type. Dotted arrows indicate the date at which all *L. multiflorum* plants reached stage 8 (ripening or maturity of fruit and seed) in each soil type. C: clay soil; S: loamy sand soil.

This is in agreement with the results of the analysis of the phytovolume of LANZA®. Significant differences were found by level of competition (df = 1; F = 44.04; $p < 0.001$) and soil type (df = 1; F = 7.71; $p < 0.05$) for the phytovolume measured at day 65. Control plants showed significantly higher phytovolume than their counterparts grown under competition (averaged values by level of competition: 9160 ± 1003 SE cm^3 in control and 2396 ± 607 SE cm^3 in competition). Plants grown in S soil also had higher phytovolume than plants grown in C soil (averaged values by soil type: 7193 ± 1456 SE cm^3 in S and 4362 ± 984 SE cm^3 in C). At harvesting time on day 135, the differences by level of competition were also significant (df = 1; F = 86.04; $p < 0.001$), but no significant differences were found by soil type (df = 1; F = 2.68; $p = 0.117$). Figure 2 shows an example of plants at the two dates of measurement in each treatment.

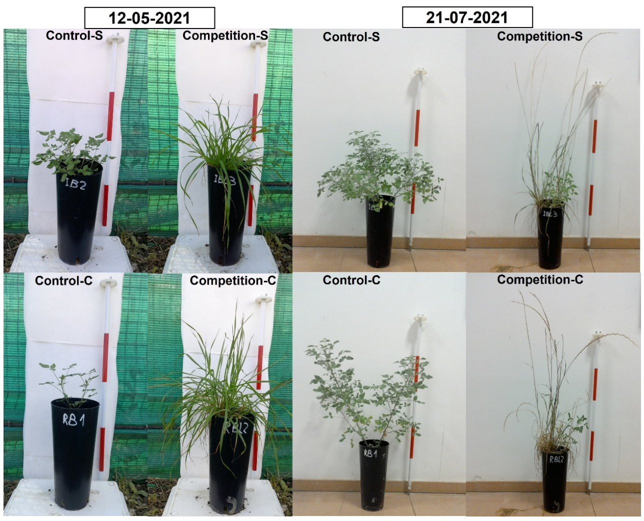

Figure 2. Example of plants under each treatment on two dates, 12 May 2021 and 21 July 2021. Control-S: LANZA® plants grown without competition in loamy sand soil, Control-C: LANZA® plants grown without competition in clay soil, Competition-S: LANZA® plants grown with competition of *L. multiflorum* in loamy sand soil, Competition-C: LANZA® plants grown with competition of *L. multiflorum* in clay soil. Red and white marks on scale bar are 20 cm intervals.

3.2. Effects of Competition and Soil Type on Dry Mass and Morphological Traits

There was a significant effect (df = 1; F = 137.6; $p < 0.001$) of competition on shoot dry mass production of LANZA® (Figure 3A). The shoot dry mass of LANZA® decreased by 86% when grown with competition of *L. multiflorum* (Figure 2). Although a slower development of LANZA® in clay soil was observed during the initial stages (Figures 1 and 2), at the end of the experiment, only a trend of lower shoot dry mass production was observed (df = 1; F = 3.97; $p = 0.060$) (Figure 3A and Table S2).

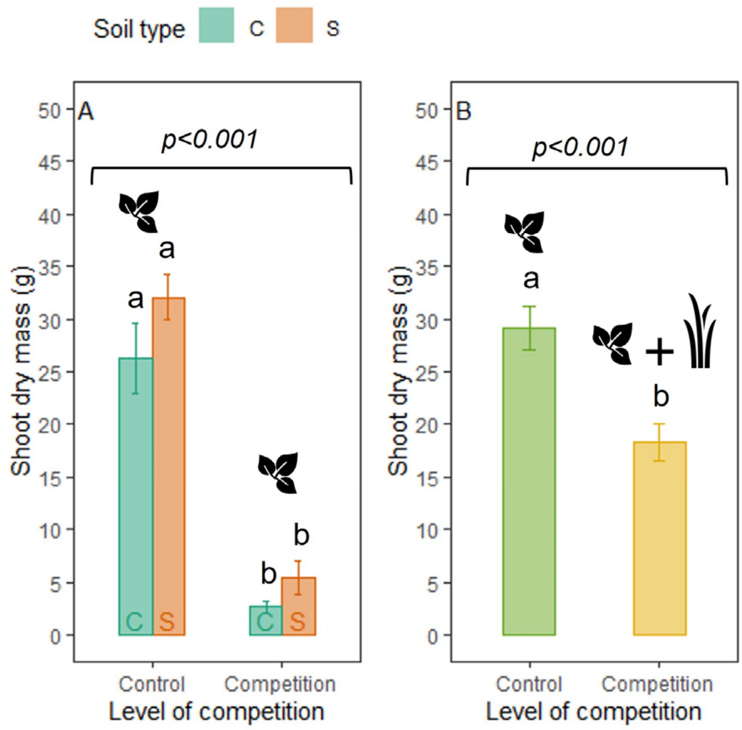

Figure 3. (**A**): Mean shoot dry mass of LANZA® by soil type and level of competition. (**B**): Mean shoot dry mass averaged by soil type of LANZA® grown without competition (control) compared to total shoot dry mass in competition treatment (*L. multiflorum* plus LANZA®). The *p* values indicate the main effect of competition; the main effect of soil was not significant. Mean values ($n = 6$) not sharing a common letter differ significantly ($p < 0.05$) according to Tukey's test. Error bars show standard errors. C: clay soil; S: loamy sand soil.

The leaf: stem ratio was not affected by soil type (Table S2). It significantly increased by 30% (df = 1; F = 14.46; $p < 0.001$) in LANZA® plants grown in competition with *L. multiflorum* (0.86 ± 0.3 SE) compared to LANZA® control plants (0.66 ± 0.3 SE). SLA was not affected by either soil type or competition (Table S2), showing a mean value of 170.3 $cm^2\ g^{-1}$ ± 6 $cm^2\ g^{-1}$. The mean leaf area of LANZA® plants grown in competition (2.6 cm^2 ± 0.3 SE) was 28% lower (df = 1; F = 9.53; $p < 0.01$) than plants without the competition of *L. multiflorum* (3.6 ± 0.1 SE cm^2) with no significant differences by soil type (Table S2).

When the total shoot dry mass production by pot (*L. multiflorum* plus LANZA®), was compared to the shoot dry mass of LANZA® grown alone, significant differences were found by level of competition (df = 1; F = 16.78; $p < 0.001$) but no differences were found by soil type (df = 1; F = 0.33; $p = 0.571$). The average LANZA® shoot dry mass was 60% higher (29.2 ± 2.1 SE) than the average total shoot dry mass production of the mixture of LANZA® and *L. multiflorum* (18.3 ± 1.7 SE) (Figure 3B). LANZA® contributed to 23% of the shoot dry mass of the mixed culture of LANZA® and *L. multiflorum*. There was a trend (df = 1; F = 4.85; $p = 0.052$) towards a lower proportion of LANZA® in the total shoot dry mass of the mixture when grown in C soil (15.1% ± 3.8 SE) compared to plants grown in S soil (30.9% ± 6.1 SE).

Table 2 shows the mean values of thick and thin roots dry mass and thin roots proportion of LANZA® plants grown in both soil types and level of competition. The dry mass of thick roots showed significant differences according to level of competition (df = 1; F = 91.22; $p < 0.001$), being 90% lower in plants under competition (Figure S3). No significant differences in thick roots dry mass were observed between plants grown in different soils (Table 2). However, significant differences by soil type were found for thin roots dry mass and their proportion of the total root dry mass when only control plants were analysed (Table S4). A significant decrease (df = 1; F = 5.57; $p < 0.05$) of 44% in thin roots dry mass of plants grown in clay soil was observed compared to plants grown in sandy soil. Similarly, the proportion of thin roots in clay soil was also lower than in sandy soil (Table 2). See Figure S4 for an example of the root system of LANZA® in clay and sandy soil. As for the shoot dry mass, when the total root dry mass per pot was analysed (*L. multiflorum* plus LANZA®), there were significant differences by level of competition only (df = 1; F = 7.35; $p < 0.05$). The average LANZA® root dry mass was 13.9 ± 1.5 SE, which is 70% higher than the average total root dry mass production of the mixture of LANZA® and *L. multiflorum* (8.2 ± 1.6 SE). All LANZA® plants presented nodules of *Rhizobium* at the moment of harvesting (after 135 days of culture).

Table 2. Effect of level of competition and soil type (loamy sand and clay) on thick roots, thin roots and thin roots proportion of LANZA®.

Level of Competition	Soil Type	Thick Roots Dry Mass (g)	Thin Roots Dry Mass (g)	Thin Roots Proportion (g)
Control	S	7.1 ± 0.9 a	9.0 ± 1.5 a	0.55 ± 0.04 a
	C	6.8 ± 1.1 a	5.0 ± 0.8 b	0.42 ± 0.01 b
Competition	S	1.0 ± 0.3 b	-	-
	C	0.4 ± 0.1 b	-	-

Note: Mean values ($n = 6$) not sharing a common letter differ significantly ($p < 0.05$) according to Tukey's test. Standard error is shown. S: Pots with sandy soil; C: Pots with clay soil. Thin root separation was not possible in competition.

3.3. Leaf Nutrients Content

LANZA® plants under competition had higher *N* content (df = 1; F = 7.44; $p < 0.05$) than control plants with 2.20% ± 0.08 SE and 2.72% ± 0.18 SE, respectively, but the soil type did not affect *N* concentration (Table 3). This resulted in a lower $C\ N^{-1}$ ratio in plants under competition (16.90% ± 0.97 SE) compared to control plants (20.1% ± 0.59 SE). The *P* content was affected by soil type (df = 1; F = 66.20; $p < 0.001$), being higher in plants grown in S (0.37% ± 0.03 SE) soil than in *C* soil (0.13% ± 0.01 SE). The $N\ P^{-1}$ ratio was lower in plants grown in *S* soil, as affected by the higher *P* concentration of these plants. No significant differences were found for *K* and *Ca* by level of competition or soil type (Table S5). *Mg* content showed a significant interaction (df = 1; F = 10.49; $p < 0.01$) by soil type and level of competition (Table 3). Control plants grown in C soil had lower *Mg* content than control plants grown in S soil and plants under competition in C soil but no differences were found in plants grown in S soil with or without competition. Soil type (df = 1; F = 4.98; $p < 0.05$) and competition (df = 1; F = 18.94; $p < 0.001$) had a significant effect on the content of *S*, being higher in plants under competition and in C soil (Table 3).

Table 3. Leaf content (%) of macronutrients, C N^{-1} and N P^{-1} ratios of LANZA® plants.

Level of Competition	Soil Type	*N*	*P*	*C N⁻¹*	*N P⁻¹*
Control	S	2.10 ± 0.07 b	0.42 ± 0.03 a	20.7 ± 0.67 a	5.16 ± 0.45 b
Control	C	2.30 ± 0.14 b	0.12 ± 0.02 b	19.4 ± 0.95 a	21.4 ± 2.58 a
Competition	S	2.58 ± 0.16 a	0.33 ± 0.06 a	17.5 ± 0.97 b	9.03 ± 1.46 b
Competition	C	2.89 ± 0.37 a	0.13 ± 0.01 b	16.1 ± 1.86 b	22.8 ± 2.36 a
Level of competition	**Soil type**	***K***	***Ca***	***Mg***	***S***
Control	S	2.69 ± 0.22 n.s.	1.29 ± 0.09 n.s.	0.85 ± 0.04 a	0.24 ± 0.01 d
Control	C	2.16 ± 0.10 n.s.	1.24 ± 0.17 n.s.	0.52 ± 0.08 b	0.27 ± 0.01 c
Competition	S	2.63 ± 0.18 n.s.	1.23 ± 0.13 n.s.	0.68 ± 0.05 ab	0.30 ± 0.02 b
Competition	C	2.60 ± 0.18 n.s.	1.19 ± 0.14 n.s.	0.78 ± 0.08 a	0.32 ± 0.01 a

Note: Mean values (*n* = 6) not sharing a common letter differ significantly ($p < 0.05$) according to Tukey's test. Standard error is showed. S: Pots with loamy sand soil; C: Pots with clay soil. n.s.: no significance.

3.4. Physiological Response of LANZA® to Soil Type and Competition

There was a significant interaction between soil type and competition in net photosynthesis per area (A_{area}) (df = 1; F = 4.3; p = 0.05) and stomatal conductance per area (g_{Sarea}) (df = 1; F = 5.4; $p < 0.05$). Plants grown under competition in sandy soils did not differ in A_{area} values from the rest of the treatments (Figure 4A). However, plants under competition in clay soils showed significantly higher A_{area} than plants in pure culture (in clay and sandy soils). For g_{Sarea}, plants grown in competition in both soil types showed higher g_{Sarea} values than plants in pure culture in clay soils (Figure 4B). Plants grown under competition in clay soil showed the highest g_{Sarea} values (0.35 mol H_2O m^{-2} s^{-1} ± 0.02 SE), which were also significantly higher than the g_{Sarea} values of plants without competition in sandy soil. These interactions were not detected for water use efficiency (WUE) (Table S3). WUE was affected by competition only, showing a significant decrease (df = 1; F = 18.9; $p < 0.001$) of 18% in LANZA® plants grown with *L. multiflorum* competition compared to those grown in pure culture (Figure 4C). The leaf relative water content (RWC) was not affected by either soil type or competition (Table S3) with a mean value of 85.6% ± 0.4 SE.

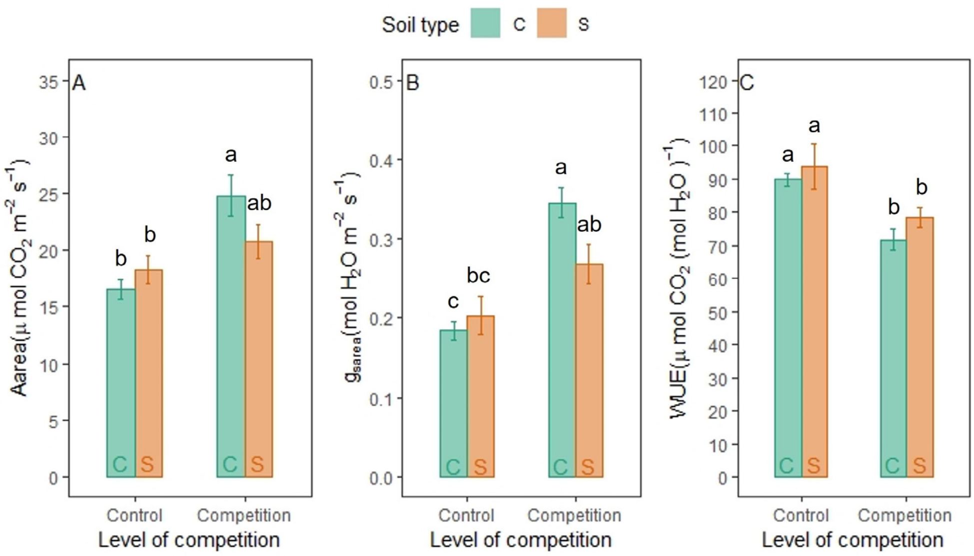

Figure 4. Effect of soil type and competition on net photosynthesis per area (A_{area}) (**A**), stomatal conductance per area (g_{Sarea}) (**B**), and water use efficiency (WUE) (**C**) of LANZA® measured at the end of the experiment. Mean values (*n* = 6) not sharing a common letter differ significantly ($p < 0.05$) according to Tukey's test. Error bars show standard errors. C: clay soil; S: loamy sand soil.

4. Discussion

The results previously reported are discussed and interpreted below, providing comments on the effects associated with short-term pot experiments, the need for field-scale trials, and identification of research gaps for future studies.

4.1. Effect of Competition

The first hypothesis, *"The interspecific competition of LANZA® with Lolium multiflorum will reduce LANZA® development"* was confirmed. The results reported in this study showed that LANZA® had low competitive ability. Competition with *L. multiflorum* had a strong effect on the dry mass production of LANZA®. Therefore, this species would not be suitable for use in mixtures with grasses such as *L. multiflorum* because its production is greatly reduced and the mixture is less productive than the pure culture of LANZA®. Bell et al. [50] also reported legume biomass reductions of 80–98% when co-sown with forage cereals, making this reduction more severe in perennial legumes (*Hedysarum coronarium* L. and *Medicago sativa* L.).

The higher A_{area} of plants under competition did not compensate for their higher g_{Sarea}, leading to a lower WUE (Figure 2) at the same RWC (Table S3), which is in line with the lower biomass production of plants under competition. Thus, the establishment, production, and persistence of LANZA® can be challenged when competing with annual grasses. This might be a major limitation for this species concerning its introduction into Mediterranean permanent grasslands where annual grasses are abundant [51]. It also highlights the importance of avoiding competition in the initial phases of development to achieve a proper establishment in temporary grasslands. The analysis of leaf nutrients content suggests that competition for nutrients was not the reason limiting the development of LANZA® because the only significant differences were the higher *N* and *S* content of plants under competition. The content values of *K*, *P* and *S* were similar to values reported in previous studies for LANZA® [26]. The higher *N* and *S* content could be related to a dilution effect due to the higher biomass of the control plants. The higher *N* content of plants in competition can explain the larger A_{area} of plants under competition due to the known relationship between photosynthesis and nitrogen content in leaves [52]. Water limitation was also not the reason for competition because a high soil water content was maintained. Therefore, the causes for the strong suppression of LANZA® when grown with *L. multiflorum* might be competition for light (shoot competition) and/or competition for space belowground (root competition) [53]. It is likely that the fibrous and more vigorous root system of *L. multiflorum* might impose strong competition for space belowground on LANZA®. However, the specific effects of root and shoot competition on LANZA® are out of the scope of this study and should be addressed in future research with a specific experimental design for such purpose [53].

Further research is needed to confirm if these results apply to field conditions, and in combination with species of less aggressive development than *L. multiflorum*. For example, it has been demonstrated that the competition effect on herbaceous plants is highly dependent on the identity of its neighbours, being least suppressed by their frequent neighbours [54,55]. In addition, sowing density and spatial aggregation are expected to affect competitive ability, for example, Semchenko et al. [55] showed that weaker competitors tend to form aggregated spatial distribution patterns as a possible strategy to aid their persistence in the community. Multi-species mixtures including LANZA® could increase the ecosystem's resistance and resilience to drought; however, the effect of interspecific competition must be minimised by targeting functional traits complementarity among species [56,57]. Malisch et al. [56] demonstrated that the proportion and persistence of *Onobrychis viciifolia* (a forage legume of low competitive ability) in mixtures can be improved through the selection of suitable partner species. Although our experiment clearly proves the low competitive ability of LANZA® with *L. multiflorum*, the afore-mentioned aspect of competitive ability needs to be further investigated in long-term field-scale trials testing different partner species and sowing densities. The competition effect might be greater in field conditions as root and shoot competition tend to be underestimated in pot experiments [53].

These results open a gap for future research on the options to reduce early competition of grasses and forbs to successfully establish LANZA® in monocultures. The overall main alternatives could be (i) crop rotations to reduce weed burden, (ii) leaving the fields fallow during the season before sowing to avoid seed production of forbs and grasses, (iii) strategic grazing management, and (iv) the use of herbicides. Recently, Real et al. [27] have provided options for a herbicide package for weed control in LANZA®. They found that propyzamide and carbetamide as pre- or post-emergent applications and butroxydim, clethodim, and haloxyfop for post-emergent applications can be recommended to control grasses such as annual ryegrass (*Lolium rigidum*) [27]. Because Mediterranean permanent grasslands have been labelled as a biodiversity hotspot [58], are commonly associated with High Natural Value (HNV) farming systems such as *Dehesas* and *Montados* from the Iberian Peninsula [59] and are greatly valued for their plant diversity, the use of herbicides could be inappropriate. Therefore, the feasible alternatives may be found in other options or combinations of them. For example, some experiences in Australia have shown that sheep without previous knowledge of *B. bituminosa* avoided this species during the first days of grazing [60], which may be used to relieve LANZA® from competition. However, this technique could be difficult for farmers to implement practically, as the beneficial effect of relief from competition against grazing damage depends on careful grazing management. The sowing time might also play an important role in the competitive ability of LANZA® with weeds. Real et al. [25] reported that LANZA® performs best when sown just before or early after the start of the rainy season which might also have a beneficial effect due to lower competition from grasses and forbs. The option of leaving the fields fallow the season before sowing might be of limited effectiveness in Mediterranean grasslands due to their rich seed bank [61,62]. Therefore, this management option may be more effective if combined with early sowing to favour asynchrony with competing species and to allow LANZA® to develop before winter as it is less vigorous than grasses.

The high phenological asynchrony between *L. multiflorum* and LANZA® together with the high drought resistance of LANZA® [16] might have an interacting effect on the performance of LANZA® under competition. Because *L. multiflorum* shows earlier senescence than LANZA® due to their contrasting strategies to face summer drought (dehydration escape vs. avoidance) (see [63]), LANZA® could take advantage of the time after *L. multiflorum* senescence to develop without the competition of *L. multiflorum*. This would greatly depend on previous successful establishment, development, and the biomass produced until this moment which could determine the ability to respond to the cessation of competition. It will also depend on the soil water content, although *B. bituminosa* has the advantage of a deep root system and high drought resistance [16], which enables it to maintain shoot biomass under drought (especially var. *albomarginata*) [17]. In this context, late spring rainfall, after the senescence of grasses, could also benefit LANZA® by allowing a greater after-competition period of development. This is supported by the increase in development in stage three of the phenology of LANZA® plants under competition (especially in soil C) after the maturity of *L. multiflorum* fruit (Figure 2). This study investigated the effect of competition and soil type at high soil water content (between θ_s and 70% of θ_s). However, interspecific competition can vary along environmental gradients [57,64]; therefore, studies incorporating water shortage and its possible interaction with the competitive ability of LANZA® are necessary to fully understand the response of LANZA® to competition in different scenarios. Especially during the establishment phase, LANZA® could show higher competitive ability under drought conditions due to its drought resistance even at the seedling stage [15]. Future research should also investigate the competitive ability of LANZA® plants of different ages, in order to clarify if competition from annual grasses has a limiting effect on LANZA® culture after the first year of establishment.

It is worth mentioning that the shade created by the shade house (nominal shading factor of 60%) where the plants were grown might have affected the phenology of LANZA®, probably delaying the flowering of this species. This effect could also be caused by the shade of tree canopies in agroforestry systems such as *Dehesas* and *Montados*, where it could negatively affect the persistence of LANZA®, in fact, the absence of shade-tolerant legumes for agroforestry systems has been pointed out by Hernández-Esteban et al. [8].

4.2. Effect of Soil Type

Successful inoculation with *Rhizobium* proves that LANZA® roots can be inoculated by *Rhizobium* from the natural populations of the Iberian Peninsula. This is crucial to ensure the successful establishment, development, and ecosystem services provision by LANZA® as a new forage for Mediterranean livestock systems.

The second hypothesis, "*Heavy-textured clay soils in conditions of high soil water content could limit the development of the root system of LANZA® due to its water retention capacity, and hence reduce the biomass production of this species*" was rejected. LANZA® was established successfully with similar root and aerial dry mass production at harvesting time in both soils with a soil water content close to saturation. This proves that *B. bituminosa* is well adapted to a wide range of environmental conditions. In fact, the use of the *crassiuscula* and *albomarginata* varieties to obtain LANZA® could have provided this new variety with a wider aptitude for different soils [9]. However, although it did not translate into reduced shoot dry mass production at harvesting time, the soil type did affect the dry mass and proportion of thin roots (<2 mm), which was lower in plants grown in clay soil with a soil water content close to saturation (Table 2), and which also presented a slower development compared to plants grown in loamy sand soil. Oxygen depletion in heavy clay soil with slow drainage is a well-known phenomenon [65–67] that might induce deficient root function and thin root dry mass reduction [68,69]. Clay soil has two-fold the water content of loamy sand soil at saturation (Table 1). Fernández-Habas et al. [18] pointed out that the root system development of *B. bituminosa var albomarginata* could be negatively affected by high soil water content in soils with elevated soil water retention capacity such as clay/silty soils, which could explain the reduction in thin root dry mass of plants grown in clay soil. Differences in soil *P* content could also affect root biomass [70,71]. Previous studies have reported a positive response of *P* addition to root and shoot growth in *B. bituminosa* reaching a maximum of shoot and root dry mass and root length to an optimum concentration of 12 $\mu g\ P\ g^{-1}$ soil followed by a plateau with higher *P* concentrations, although they did not report the extractable *P* of the tested concentration [72,73]. Real et al. [26] also reported that LANZA® reached its peak of productivity in soils at 3–19 $mg\ kg^{-1}$ *P* Colwell. However, these studies did not analyse the distribution of biomass between fine and thick roots. In this study, C soil had 7 $mg\ kg^{-1}$ *P* Olsen, which is within the range of *P* Colwell values and *P* concentrations [74–76] indicated by these authors at which maximum root biomass and peak of productivity can be achieved. The differences in leaf nutrients in *P*, *Mg* and *S* were in relation to the differences in concentration of these nutrients in both soils. The *P*, *K* and *S* leaf content was above the critical shoot nutrient concentration at which LANZA® reached 90% of the peak biomass in Real et al. [26] in both soils with no symptoms of deficiency or toxicity for these or the rest of the nutrients analysed.

This result might indicate a LANZA® 's preference for and suitable adaptation to light sandy soils. This is in agreement with the distribution of *B. bituminosa* in the Canary Islands, where the genetic material to produce LANZA® was collected [9,14]. Mendez et al. [77] studied the agronomic traits and soils of nineteen populations of *B. bituminosa* from the Canary Islands and the Iberian Peninsula. They found that soils from most of the populations (14 out of 19) were soils with more than 50% sand with just one population of clay soils [77]. The fact that *B. bituminosa* is usually found in poor sandy and rocky soils might be related to its adaptation to this soil type, but could also be due to a lower competition with plant species that might outcompete *B. bituminosa* in other soil types. In addition to its application as a new forage legume in Mediterranean climates, *B. bituminosa* could also be used for the revegetation and improvement of disturbed and poor soils (low competition environments) where it could increase the fertility of the soil by fixing atmospheric N_2. Previous studies have demonstrated tolerance of *B. bituminosa* to heavy metals [78,79] and suggested its use as suitable species in the initial stages of revegetation of non-saline soils with a pH between 6.5–8.0 and moderate levels of heavy metal contamination [80].

5. Conclusions

This study investigated the effects of contrasting texture soils (loamy sand vs. clay) at high soil water content and the competition from *Lolium multiflorum* on the development of LANZA®. As expected, LANZA® showed low competitive ability and strong dry mass reduction when grown with *L. multiflorum*. LANZA® developed in both soils, clay, and loamy sand at high soil water content, with no significant effect on shoot and total root dry mass production. However, the lower thin root dry mass production in clay soil might denote LANZA® 's preference for and suitable adaptation to light sandy soils. The low competitive ability of LANZA® with annual grasses might compromise the successful establishment and persistence of this novel perennial legume in Mediterranean grasslands. Further research should investigate measures aimed at reducing early competition from grasses to establish LANZA® as a monoculture. Functional complementarity among species must be considered and further explored to avoid interspecific competition when designing multi-species mixtures including LANZA®.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13040965/s1, Figure S1: Water retention curves for loamy sand (S) and clay (C) soils adjusted to Van Genuchten equation. Van Genuchten parameters for S soil: θ_s = 0.33, θ_r = 0.01, α = 0.013, n = 1.47. Van Genuchten parameters for C soil: θ_s = 0.62, θ_r = 0.05, α = 0.186, n=1.26; Figure S2: Daily mean (black), maximum (red) and minimum (blue) temperatures during the experiment; Figure S3: Example of roots of LANZA® and *L. multiflorum* grown in the same pot (Competition); Figure S4: Example of roots after separation from soil in two plants of LANZA® grown in clay soil (left) and grown in loamy sand soil (right); Table S1: Cumulative link mixed model fitted for phenology analysis; Table S2: Summary results of the two-way ANOVA evaluating the effects of Competition, soil type and their interaction on shoot mass production and leaf to stem ratio of LANZA®; Table S3: Summary results of the two-way ANOVA evaluating the effect of soil type and Competition on net photosynthesis per area (A_{area}), stomatal conductance per area (g_{Sarea}), water use efficiency (WUE), specific leaf area (SLA) and mean leaf area of LANZA measured at the end of the experiment; Table S4: Summary results of the one-way ANOVA evaluating the effect of soil type on thin roots, thick roots and thin roots proportion of LANZA®; Table S5: Summary results of the two-way ANOVA evaluating the effect of soil type and Com-petition on leaf content of macronutrients and C N^{-1} and N P^{-1} ratios.

Author Contributions: Conceptualization, J.F.-H. and P.F.-R.; methodology, J.F.-H., P.F.-R. and T.V.; formal analysis, J.F.-H. and P.F.-R.; investigation, J.F.-H. and P.F.-R.; resources, P.F.-R., T.V. and D.R.; data curation, J.F.-H.; writing—original draft preparation, J.F.-H.; writing—review and editing, J.F.-H., P.F.-R., T.V. and D.R.; visualization, J.F.-H.; supervision, P.F.-R.; funding acquisition, T.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the European Union Horizon 2020 research and innovation programme, under grant agreement 774124, project SUPER-G (Developing Sustainable Permanent Grassland Systems and Policies) and Regional Government of Andalusia (Consejería de Universidad, Investigación e Innovación, Junta de Andalucia) Proy-Excel 00465 PASTOS-SECOS.

Data Availability Statement: Not applicable.

Acknowledgments: The study was developed thanks to a PhD fellowship FPU (code FPU18/02876) of the Spanish Ministry of Education awarded to J. Fernández-Habas. We thank José Ramón Leal-Murillo for his invaluable help to conduct the experiment, and data collection, Mª Teresa Hidalgo-Fernández for her help in the nutrient analysis and Rafael Sánchez-Cuesta for his assistance during the data collection. The authors would also like to thank the lab members of the Soil science Unit of the Agronomy Department at the University of Cordoba for offering their facilities for the leaf and soil analyses and for their assistance. We are grateful to Vanesa García-Gamero and Ester Anta Domínguez for their assistance in determination of the water retention curves. We would like to acknowledge the effort and dedication of all the researchers that have contributed to increasing knowledge on *Bituminaria bituminosa*.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Cosentino, S.; Porqueddu, C.; Copani, V.; Patanè, C.; Testa, G.; Scordia, D.; Melis, R. European grasslands overview: Mediterranean region. In Proceedings of the 25th General Meeting of the European Grassland Federation: The Future of European Grasslands, Aberystwyth, UK, 7–11 September 2014; pp. 41–56.
2. Bell, L.W.; Moore, A.D.; Thomas, D.T. Feed-base strategies that reduce risk of feed-gaps in livestock systems across Australia's mixed farming zone. In *Animal Production in Australia, Proceedings of the 31st Biennial Conference of the Australian Society of Animal Production, Adelaide, Australia, 5–7 July 2016*; Australian Society of Animal Production (ASAP): Adelaide, Australia, 2016; pp. 1–2.
3. Moore, A.D.; Bell, L.W.; Revell, D.K. Feed gaps in mixed-farming systems: Insights from the Grain & Graze program. *Anim. Prod. Sci.* **2009**, *49*, 736–748.
4. Bell, L.W.; Robertson, M.J.; Revell, D.K.; Lilley, J.M.; Moore, A.D. Approaches for assessing some attributes of feed-base systems in mixed farming enterprises. *Aust. J. Exp. Agric.* **2008**, *48*, 789–798. [CrossRef]
5. Giannakopoulos, C.; Le Sager, P.; Bindi, M.; Moriondo, M.; Kostopoulou, E.; Goodess, C.M. Climatic changes and associated impacts in the Mediterranean resulting from a 2 °C global warming. *Glob. Planet. Chang.* **2009**, *68*, 209–224. [CrossRef]
6. Giorgi, F.; Lionello, P. Climate change projections for the Mediterranean region. *Glob. Planet. Chang.* **2008**, *63*, 90–104. [CrossRef]
7. Ma, Z.; Liu, H.; Mi, Z.; Zhang, Z.; Wang, Y.; Xu, W.; Jiang, L.; He, J.-S. Climate warming reduces the temporal stability of plant community biomass production. *Nat. Commun.* **2017**, *8*, 15378. [CrossRef] [PubMed]
8. Hernández-Esteban, A.; López-Díaz, M.L.; Cáceres, Y.; Moreno, G. Are sown legume-rich pastures effective allies for the profitability and sustainability of Mediterranean dehesas? *Agrofor. Syst.* **2019**, *93*, 2047–2065. [CrossRef]
9. Real, D.; Oldham, C.M.; Nelson, M.N.; Croser, J.; Castello, M.; Verbyla, A.; Pradhan, A.; Van Burgel, A.; Mendez, P.; Correal, E.; et al. Evaluation and breeding of tedera for Mediterranean climates in southern Australia. *Crop Pasture Sci.* **2014**, *65*, 1114–1131. [CrossRef]
10. Thomas, D.T.; Flohr, B.M.; Monjardino, M.; Loi, A.; Llewellyn, R.S.; Lawes, R.A.; Norman, H.C. Selecting higher nutritive value annual pasture legumes increases the profitability of sheep production. *Agric. Syst.* **2021**, *194*, 103272. [CrossRef]
11. Edwards, T.; Howieson, J.; Nutt, B.; Yates, R.; O'Hara, G.; Van Wyk, B.E. A ley-farming system for marginal lands based upon a self-regenerating perennial pasture legume. *Agron. Sustain. Dev.* **2019**, *39*, 13. [CrossRef]
12. Moore, G.A.; Sanford, P.; Dolling, P.J.; Real, D. The challenges of developing resilient perennial pastures for a Mediterranean environment—A review for Western Australia. *Crop Pasture Sci.* **2021**, *72*, 613–633. [CrossRef]
13. Rognli, O.A.; Pecetti, L.; Kovi, M.R.; Annicchiarico, P. Grass and legume breeding matching the future needs of European grassland farming. *Grass Forage Sci.* **2021**, *76*, 175–185. [CrossRef]
14. Méndez, P.; Fernández, M.; Santos, A. Variedades de Bituminaria bituminosa (L.) Stirton (Leguminosae) en el archipélago canario. *Pastos* **1991**, *20–21*, 157–166.
15. Foster, K.; Ryan, M.H.; Real, D.; Ramankutty, P.; Lambers, H. Drought resistance at the seedling stage in the promising fodder plant tedera (Bituminaria bituminosa var. albomarginata). *Crop Pasture Sci.* **2012**, *63*, 1034–1042. [CrossRef]
16. Foster, K.; Lambers, H.; Real, D.; Ramankutty, P.; Cawthray, G.R.; Ryan, M.H. Drought resistance and recovery in mature Bituminaria bituminosa var. albomarginata. *Ann. Appl. Biol.* **2015**, *166*, 154–169. [CrossRef]
17. Martínez-Fernández, D.; Walker, D.J.; Romero, P.; Martínez-Ballesta, M.C.; Correal, E. The Response of the Leguminous Fodder Plant Bituminaria bituminosa to Water Stress. *J. Agron. Crop Sci.* **2012**, *198*, 442–451. [CrossRef]
18. Fernández-Habas, J.; Hidalgo-Fernández, M.T.; Leal-Murillo, J.R.; Méndez, P.; Quero, J.L.; Vanwalleghem, T.; Fernández-Rebollo, P. Effects of two water regimes on morphological traits, nutritive value and physiology of three Bituminaria bituminosa varieties from the Canary Islands. *J. Agron. Crop Sci.* **2021**, *208*, 413–426. [CrossRef]
19. Ventura, M.R.; Castanon, J.I.R.; Mendez, P. Effect of season on tedera (Bituminaria bituminosa) intake by goats. *Anim. Feed Sci. Technol.* **2009**, *153*, 314–319. [CrossRef]
20. Ventura, M.R.; Méndez, P.; Flores, M.P.; Rodriguez, R.; Castañon, J.I.R. Energy and protein content of Tedera. *Analysis* **1991**, *82*, 1988–1990.
21. Finlayson, J.; Real, D.; Nordblom, T.; Revell, C.; Ewing, M.; Kingwell, R. Farm level assessments of a novel drought tolerant forage: Tedera (Bituminaria bituminosa C.H. Stirt var. albomarginata). *Agric. Syst.* **2012**, *112*, 38–47. [CrossRef]
22. Oldham, C.M.; Wood, D.; Milton, J.; Real, D.; Vercoe, P.; Van Burgel, A.J. An animal house study on utilisation of fresh tedera (Bituminaria bituminosa var. albomarginata and crassiuscula) by Merino wethers. *Anim. Prod. Sci.* **2015**, *55*, 617–624. [CrossRef]
23. Oldham, C.; Real, D.; Bailey, H.J.; Thomas, D.; Van Burgel, A.; Vercoe, P.; Correal, E.; Rios, S. Australian and Spanish scientists are collaborating in the domestication of tedera: Young Merino sheep grazing a monoculture of tedera in autumn showed preference for certain accessions but no signs of ill health. *Crop Pasture Sci.* **2013**, *64*, 399–408. [CrossRef]

24. Real, D. Tedera (Bituminaria bituminosa). *Plant Var. J.* **2016**, *29*, 97.
25. Real, D. Critical Agronomic Practices for Establishing the Recently Domesticated Perennial Herbaceous Forage Legume Tedera in Mediterranean-like Climatic Regions in Western Australia. *Agronomy* **2022**, *12*, 274. [CrossRef]
26. Real, D.; Bennett, R.G.; Nazeri, N.K.; Weaver, D.M. Critical P, K and S Concentrations in Soil and Shoot Samples for Optimal Tedera Productivity and Nodulation. *Agronomy* **2022**, *12*, 1581. [CrossRef]
27. Real, D.; Dhammu, H.; Moore, J.; Clegg, D.; van Burgel, A. Herbicide Tolerance Options for Weed Control in Lanza® Tedera. *Agronomy* **2022**, *12*, 1198. [CrossRef]
28. Raeside, M.C.; Nie, Z.N.; Clark, S.G.; Partington, D.L.; Behrendt, R.; Real, D. Evaluation of tedera [(*Bituminaria bituminosa* (L.) C.H. Stirton var. *albomarginata*)] as a forage alternative for sheep in temperate southern Australia. *Crop Pasture Sci.* **2012**, *63*, 1135–1144. [CrossRef]
29. Real, D.; Li, G.D.; Clark, S.; Albertsen, T.O.; Hayes, R.C.; Denton, M.D.; Dantuono, M.F.; Dear, B. Evaluation of perennial forage legumes and herbs in six Mediterranean environments. *Chil. J. Agric. Res.* **2011**, *71*, 357–369. [CrossRef]
30. Bell, L.W.; Moore, G.A.; Ewing, M.A.; Bennett, S.J. Establishment and summer survival of the perennial legumes, Dorycnium hirsutum and D. rectum in Mediterranean environments. *Aust. J. Exp. Agric.* **2005**, *45*, 1245–1254. [CrossRef]
31. Häring, D.A.; Scharenberg, A.; Heckendorn, F.; Dohme, F.; Lüscher, A.; Maurer, V.; Suter, D.; Hertzberg, H. Tanniferous forage plants: Agronomic performance, palatability and efficacy against parasitic nematodes in sheep. *Renew. Agric. Food Syst.* **2008**, *23*, 19–29. [CrossRef]
32. Hogg, N.; Davis, J.K. What is hindering the adoption of new annual pasture legumes? Extension requirements to overcome these barriers. *Ext. Farming Syst. J.* **2009**, *5*, 29–38.
33. Lewis, C.; Farquharson, R.; Leury, B.; Behrendt, R.; Clark, S. Economic analysis of improved perennial pasture systems. *Aust. Farm Bus. Manag. J.* **2012**, *9*, 37–56.
34. Carruggio, F.; Onofri, A.; Impelluso, C.; Del Galdo, G.G.; Scopece, G.; Cristaudo, A. Seed dormancy breaking and germination in *Bituminaria basaltica* and *B. bituminosa* (fabaceae). *Plants* **2020**, *9*, 1110. [CrossRef] [PubMed]
35. Pecetti, L.; Tava, A.; Pagnotta, M.; Russi, L. Variation in forage quality and chemical composition among Italian accessions of *Bituminaria bituminosa* (L.) Stirt. *J. Sci. Food Agric.* **2007**, *87*, 985–991. [CrossRef]
36. Reyna-Bowen, L.; Fernandez-Rebollo, P.; Fernández-Habas, J.; Gómez, J.A. The influence of tree and soil management on soil organic carbon stock and pools in dehesa systems. *Catena* **2020**, *190*, 104511. [CrossRef]
37. García, M.C.; García, A.D.; Gavilán, A.G.; Castellet, J.T. *Estudio Edafológico de la Finca 'Rabanales' Córdoba, Spain*; Departamento de Ciencias y Recursos Agrícolas y Forestales, Universidad de Córdoba: Córdoba, Spain, 1993.
38. Eijkelkamp. *Sandbox for pF-Determination. User Manual*; Eijkelkamp: Giesbeek, The Netherlands, 2019.
39. Eijkelkamp. *08.02 Sand/Kaolin Box. Operating Instructions*; Eijkelkamp: Giesbeek, The Netherlands, 2016.
40. Decagon. *WP4 Dewpoint PotentialMeter Operator's Manual Version 5*; Decagon Devices, Inc.: Pullman, WA, USA, 2007.
41. Van Genuchten, M.T. A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. *Soil Sci. Soc. Am. J.* **1980**, *44*, 892–898. [CrossRef]
42. Van Genuchten, M.T.; Leij, F.J.; Yates, S.R. The RETC Code for Quantifying the Hydraulic Functions of Unsaturated Soils. In *Version 1.0. EPA Report 600/2-91/065*; U.S. Salinity Laboratory: Riverside, CA, USA, 1991.
43. Bleiholder, H.; Weber, E.; Lancashire, P.; Feller, C.; Buhr, L.; Hess, M.; Wicke, H.; Hack, H.; Meier, U.; Klose, R. *Growth Stages of Mono-and Dicotyledonous Plants, BBCH Monograph*; Federal Biological Research Centre for Agriculture and Forestry: Berlin/Braunschweig, Germany, 2001.
44. Christensen, R.H.B. A Tutorial on Fitting Cumulative Link Mixed Models with clmm2 from the Ordinal Package. 1-18. Available online: https://cran.r-project.org/web/packages/ordinal/vignettes/clmm2_tutorial.pdf (accessed on 15 December 2022).
45. Agresti, A. *Categorical Data Analysis*; John Wiley & Sons: Hoboken, NJ, USA, 2003.
46. Christensen, R.H.B. Ordinal—Regression Models for Ordinal Data, R package version 2022.11-16. 2022. Available online: https://cran.r-project.org/web/packages/ordinal/index.html (accessed on 15 December 2022).
47. Mangiafico, S.S. One-Way Repeated Ordinal Regression with CLMM. Available online: https://rcompanion.org/handbook/G_08.html (accessed on 7 June 2022).
48. Hervé, M. Package 'RVAideMemoire': Testing and Plotting Procedures for Biostatistics. 2022. Available online: https://cran.r-project.org/web/packages/RVAideMemoire/index.html (accessed on 15 December 2022).
49. R Development Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2019; Available online: https://www.R-project.org/ (accessed on 7 November 2022).
50. Bell, L.; Lawrence, J.; Jasper, S.; Guppy, C. Pasture legume production severely reduced when co-sown with winter forage cereals. Capturing Opportunities and Overcoming Obstacles in Australian Agronomy. In Proceedings of the 16th ASA Conference, Armidale, Australia, 14–18 October 2012; Australian Society of Agronomy Inc.: Armidale, Australia, 2012.
51. Olea, L.; San Miguel-Ayanz, A. The Spanish dehesa. A traditional Mediterranean silvopastoral system linking production and nature conservation. In *Sustainable Grassland Productivity, Proceedings of the 21st General Meeting of the European Grassland Federation, Badajoz, Spain, 3–6 April 2006*; Lloveras, J., González-Rodríguez, A., Vázquez-Yáñez, O., Piñeiro, J., Santamaría, O., Olea, L., Poblaciones, M.J., Eds.; Sociedad Española para el Estudio de los Pastos: Badajoz, Spain, 2006; pp. 1–13.
52. Evans, J. Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia* **1989**, *78*, 9–19. [CrossRef]

53. Kiær, L.P.; Weisbach, A.N.; Weiner, J. Root and shoot competition: A meta-analysis. *J. Ecol.* **2013**, *101*, 1298–1312. [CrossRef]
54. Lüscher, A.; Connolly, J.; Jacquard, P. Neighbour specificity between Lolium perenne and Trifolium repens from a natural pasture. *Oecologia* **1992**, *91*, 404–409. [CrossRef]
55. Semchenko, M.; Abakumova, M.; Lepik, A.; Zobel, K. Plants are least suppressed by their frequent neighbours: The relationship between competitive ability and spatial aggregation patterns. *J. Ecol.* **2013**, *101*, 1313–1321. [CrossRef]
56. Malisch, C.S.; Suter, D.; Studer, B.; Lüscher, A. Multifunctional benefits of sainfoin mixtures: Effects of partner species, sowing density and cutting regime. *Grass Forage Sci.* **2017**, *72*, 794–805. [CrossRef]
57. Volaire, F.; Barkaoui, K.; Norton, M. Designing resilient and sustainable grasslands for a drier future: Adaptive strategies, functional traits and biotic interactions. *Eur. J. Agron.* **2014**, *52*, 81–89. [CrossRef]
58. Myers, N.; Mittermeler, R.A.; Mittermeler, C.G.; Da Fonseca, G.A.B.; Kent, J. Biodiversity hotspots for conservation priorities. *Nature* **2000**, *403*, 853–858. [CrossRef] [PubMed]
59. Plieninger, T.; Flinzberger, L.; Hetman, M.; Horstmannshoff, I.; Reinhard-Kolempas, M.; Topp, E.; Moreno, G.; Huntsinger, L. Dehesas as high nature value farming systems: A social-ecological synthesis of drivers, pressures, state, impacts, and responses. *Ecol. Soc.* **2021**, *26*, 23. [CrossRef]
60. Real, D.; Department of Primary Industries and Regional Development (DPIRD), Perth, WA, Australia. Personal communication, 2021.
61. Lavorel, S.; McIntyre, S.; Grigulis, K. Plant response to disturbance in a Mediterranean grassland: How many functional groups? *J. Veg. Sci.* **1999**, *10*, 661–672. [CrossRef]
62. Levassor, C.; Ortega, M.; Peco, B. Seed bank dynamics of Mediterranean pastures subjected to mechanical disturbance. *J. Veg. Sci.* **1990**, *1*, 339–344. [CrossRef]
63. Volaire, F. A unified framework of plant adaptive strategies to drought: Crossing scales and disciplines. *Glob. Chang. Biol.* **2018**, *24*, 2929–2938. [CrossRef] [PubMed]
64. Foxx, A.J.; Fort, F. Root and shoot competition lead to contrasting competitive outcomes under water stress: A systematic review and meta-analysis. *PLoS ONE* **2019**, *14*, e0220674. [CrossRef]
65. Ben-Noah, I.; Friedman, S.P. Aeration of clayey soils by injecting air through subsurface drippers: Lysimetric and field experiments. *Agric. Water Manag.* **2016**, *176*, 222–233. [CrossRef]
66. Drew, M.C.; Lynch, J.M. Soil Anaerobiosis, Microorganisms, and Root Function. *Annu. Rev. Phytopathol.* **1980**, *18*, 37–66. [CrossRef]
67. Friedman, S.P.; Naftaliev, B. A survey of the aeration status of drip-irrigated orchards. *Agric. Water Manag.* **2012**, *115*, 132–147. [CrossRef]
68. Bhattarai, S.P.; Huber, S.; Midmore, D.J. Aerated subsurface irrigation water gives growth and yield benefits to zucchini, vegetable soybean and cotton in heavy clay soils. *Ann. Appl. Biol.* **2004**, *144*, 285–298. [CrossRef]
69. Irving, L.J.; Sheng, Y.B.; Woolley, D.; Matthew, C. Physiological effects of waterlogging on two lucerne varieties grown under glasshouse conditions. *J. Agron. Crop Sci.* **2007**, *193*, 345–356. [CrossRef]
70. Haling, R.E.; Yang, Z.; Shadwell, N.; Culvenor, R.A.; Stefanski, A.; Ryan, M.H.; Lambers, H.; Simpson, R.J. Root morphological traits that determine phosphorus-acquisition efficiency and critical external phosphorus requirement in pasture species. *Funct. Plant Biol.* **2016**, *43*, 815–826. [CrossRef] [PubMed]
71. Hill, J.O.; Simpson, R.J.; Moore, A.D.; Chapman, D.F. Morphology and response of roots of pasture species to phosphorus and nitrogen nutrition. *Plant Soil* **2006**, *286*, 7–19. [CrossRef]
72. Pang, J.; Ryan, M.H.; Tibbett, M.; Cawthray, G.R.; Siddique, K.H.M.; Bolland, M.D.A.; Denton, M.D.; Lambers, H. Variation in morphological and physiological parameters in herbaceous perennial legumes in response to phosphorus supply. *Plant Soil* **2010**, *331*, 241–255. [CrossRef]
73. Pang, J.; Tibbett, M.; Denton, M.D.; Lambers, H.; Siddique, K.H.M.; Bolland, M.D.A.; Revell, C.K.; Ryan, M.H. Variation in seedling growth of 11 perennial legumes in response to phosphorus supply. *Plant Soil* **2010**, *328*, 133–143. [CrossRef]
74. Edmeades, D.C.; Metherell, A.K.; Waller, J.E.; Roberts, A.H.C.; Morton, J.D. Defining the relationships between pasture production and soil P and the development of a dynamic P model for New Zealand pastures: A review of recent developments. *N. Z. J. Agric. Res.* **2006**, *49*, 207–222. [CrossRef]
75. Moody, P.W.; Speirs, S.D.; Scott, B.J.; Mason, S.D. Soil phosphorus tests I: What soil phosphorus pools and processes do they measure? *Crop Pasture Sci.* **2013**, *64*, 461–468. [CrossRef]
76. Saggar, S.; Hedley, M.J.; White, R.E.; Perrott, K.W.; Gregg, P.E.H.; Cornforth, I.S.; Sinclair, A.G. Development and evaluation of an improved soil test for phosphorus, 3: Field comparison of Olsen, Colwell and Resin soil P tests for New Zealand pasture soils. *Nutr. Cycl. Agroecosyst.* **1999**, *55*, 35–50. [CrossRef]
77. Mendez, P.; Santos, A.; Correal, E.; Ríos, S. Agronomic traits as forage crops of nineteen populations of Bituminaria bituminosa. In Proceedings of the 21st General Meeting of the European Grassland Federation, Badajoz, Spain, 3–6 April 2006; Sociedad Española para el Estudio de los Pastos (SEEP): Madrid, Spain, 2006; pp. 300–302.

78. Martínez-Fernández, D.; Walker, D.J.; Romero-Espinar, P.; Flores, P.; del Río, J.A. Physiological responses of Bituminaria bituminosa to heavy metals. *J. Plant Physiol.* **2011**, *168*, 2206–2211. [CrossRef]
79. Walker, D.J.; Bernal, M.P.; Correal, E. The influence of heavy metals and mineral nutrient supply on Bituminaria bituminosa. *Water Air Soil Pollut.* **2007**, *184*, 335–345. [CrossRef]
80. Martínez-Fernández, D. Respuestas Fisiológicas de Bituminaria Bituminosa Frente a Sequía y Metales Pesados. Ph.D. Thesis, Universidad Politécnica de Cartagena, Cartagena, Spain, 2012.