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Full Length Article

Transplanted Tussock Grasslands Related to Altitude, Climate and Application of Natural Fertilizers

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Abstract

Andean grasslands are providers of multiple ecosystem services for humanity and biodiversity conservation; however, since they are not part of livestock feed, they are very vulnerable to anthropogenic action of phytomass renewal through fires that decimate the vegetation cover in these ecosystems. In response to this negative action, alternative uses are being sought as a supplier of plant fiber; but for this to be sustainable, it is necessary to generate basic knowledge regarding the biology of the species and their relationship with environmental and anthropogenic factors, oriented to design appropriate management strategies for the grasslands to optimize phytomass production. With this criterion, the objective was to evaluate the development of cuttings at transplanting, in canopy cover, canopy height and inflorescence height, in altitudinal gradients, and application of cattle manure and rock phosphate to the soil. The results obtained show acceptable viability, with significant differences between species, location of plots and response to the application of natural fertilizers. The result obtained determines the feasibility of optimizing the plant density per m^2, which will make it possible to increase the production of aerial biomass.

Keywords: Andean grassland species; Transplantation; Development factors; Fertilization

Introduction

The Andean tussock grasslands in the central Andes of Peru are located in the headwaters of river basins from 3800 meters above sea level, covering the topographically more rugged areas with steep slopes and unstable soils, very prone to erosion; however, it is the most important natural plant formation, for fulfilling ecological functions such as the regulation of carbon sequestration, regulation of rainwater infiltration at that altitudinal level (Sarmiento *et al.* 2014). These grasslands are not considered important in the economy of local populations because they are not very popular with domestic livestock such as sheep, cattle and Andean camelids (alpacas and llamas), which reduces the opportunity to be conserved or regenerated, but on the contrary, they are burned with the sole intention of causing a green shoot that is temporarily consumed by livestock (Yaranga *et al*. 2019).

The burning of grasslands has an adverse effect not only on the sustainability of plant formation with degradation of surface organic matter stored in the soil for millions of years but also on the loss of Andean biodiversity (Grigulis and Lavorel 2020), decreased ability to protect the soil against erosion and the protection of the most vulnerable grassland species (Sarmiento *et al*. 2014) that manage to produce seed and spread in the environment by not being consumed by animals. In this situation, there is the alternative of using the plant fiber of these grasslands in the elaboration of construction materials (Veláquez *et al*. 2016) that, the current ecological industry is undertaking around the world. This study is part of another project that studies this possibility, to give the tussock grasslands the use with a local economic interest, which could change the attitude of local ranchers in taking care of the tussock as a source of additional income to Andean livestock. The interest in recovering degraded Andean grasslands by transplanting seedlings in areas with low density requires the need to study the viability of transplanting and its behavior in the face of fertilization treatments with natural inputs and microclimatic factors.

There are many studies on the use of natural fertilizers for crop growth and production; however, there are few studies on the application of these inputs on natural grasslands, especially cattle feces and rock phosphate. Andean soils are characterized by being acidic and potassium deficient (Zapata and Roy 2007). However, phosphate rock has become an effective alternative to highly soluble industrial phosphates (Jouany *et al*. 2021); meanwhile, the low solubility of phosphate rock makes P remain available in

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the soil for a longer time (Ojeda *et al*. 2019). In addition to the fact that this input provides important secondary minerals such as calcium and magnesium, from other microelements such as calcite and dolomite, this increase the pH by reducing the saturation of aluminum in the soil (Zapata and Roy 2007).

On the side of the use of cattle manure in agriculture, studies have been conducted on the effect of the application of cattle manure as a source of organic matter, to achieve long-term stable yields, maintaining optimal soil properties (Menšík *et al*. 2018), in addition, they carry a large amount of germinal seed (Wang and Hou 2021), which is also beneficial in the case of application on natural pastures because it helps the restocking of useful species in the animal diet. Livestock manure application favors carbon sequestration in plants, and also increases soil organic carbon content and total nitrogen (Ozlu and Kumar 2018), which induces higher phytomass production even in soils contaminated by mining (Elouear *et al*. 2016). It has been reported that the C:N ratio in manure depends on the animal species, the diet consumed by the livestock (Wang *et al*. 2018) and the geographical location (Aricha *et al*. 2021); however, N mineralization is higher in cattle manure than sheep manure despite the higher N concentration in the latter (Wang *et al*. 2018). The availability of labile C and N is relatively higher in cattle feces because of the cellulose/hemicellulose content, which promotes microbial growth that accelerates the decomposition of feces, directly influencing the higher mineralization rate, with approximately twice as much N as sheep feces (Wang *et al.* 2018).

On the side of climatic effect on plant development, the link of plant behavior with climate is important, to obtain a deeper understanding of the function: stability and sustainability of grassland ecosystems (Gao *et al*. 2017), with precipitation and temperature being the most important climatic factors in the ecosystemic process of grasslands (Jiang *et al*. 2017). It is well known that rainfall favors plant growth, but excess rainfall mainly in autumn can impair growth, thus an increase of 10 mm can cause a delay of 0.2 to 4 days in the mean senescence date of grasslands (An *et al*. 2020). On the other hand, temperature elevation affects soil N and C reserve, thus also growth, flowering duration of plants in combination with altitude (Arroyo *et al.* 2021) and other phenological characteristics, through disturbance in respiration, assimilation, photosynthesis, and plant metabolism (Getabalew and Alemneh 2019); However, an irregular topography in the Andes also maintains a diversity of local microclimates that differ in soil temperatures, to which various plant species have adapted, which would buffer the abrupt effect of general climate change (Ohler *et al*. 2020).

A similar experience was carried out in the Cordillera Blanca of Huaraz – Peru, revegetating a degraded area by transplanting *Festuca dolichophylla* and *Calamagrostis macrophylla*, obtaining good results with 28% of revegetation, through the application of sheep manure (Tacuna *et al*. 2015). Taking into account these considerations, the general objective was to evaluate the viability and level of growth of Andean grassland species related to altitudinal gradient, the application of natural fertilizers (cattle manure and rock phosphate) to the soil, and the behavior of temperature and local precipitation (Tacuna *et al*. 2015).

Materials and Methods

Study area

The study was conducted in the territory of the Acopalca community in the province of Huancayo and Junín region, in the central Andes of Peru, located between UTM coordinates L18 S: 481880, E 8672695 at 3498 m altitude and 4941157, E 8683594 at 5510 m altitude. The local population is mainly dedicated to livestock raising, consisting of cattle, sheep and alpacas, on grazing areas ceded to each registered family as active community members. The specific study areas are located between 4012 and 4333 meters above sea level, so the average seasonal temperature varies from -8°C at dawn to 16.2°C during the day during the dry season (May to September) and from 4°C to 12°C during the rainy season (October to April), with an average daily seasonal rainfall of 0.56 mm and 2.88 mm respectively, accumulating an annual average of 1170 mm.

Data collection

The study areas were selected for the convenience of the research, considering the dominance of grassland species (Fig. 1a), in them were fenced five plots of 900 m^2 according to the method suggested by Otzen and Manterola (2017), with wooden posts and barbed wire, each plot was separated between 0.8 and 3 km away. Within each plot, five subplots of 64 m^2 were located and each of them was divided into two halves, to apply two natural fertilizers: cattle manure and phosphoric rock; in each fertilized plot 25 seedlings were transplanted, of different species taking into account the species present in them, such as *C. intermedia*, *F. rigidifolia*, *C. antoniana*, *Festuca spp* and *C. tarmensis*.

After having marked the subplots, the natural fertilizer was applied in each area of fertilization, on the left side was applied the cattle manure, previously dried and crumbled, spreading over the area, broadcast and uniformly at the rate of 4000 kg/ha (Zapata and Roy 2007). In the same way, but on the right side was spread ground phosphate rock (P_2O_5: 18–22%, CaO: 28–30%, SO_4: 3–5%) at a rate of 1500 kg/ha (Elouear *et al.* 2016). The transplanting procedure was carried out in stages as follows: (a) plot fencing followed by subplot marking and division of composting areas, (b) estimation of the average density of the grassland species present, using the "nearest neighbor" method (Pieper 1973), measuring the distance to the nearest plant of the same species in cm, in the form of a cross starting from the epicenter of a plant, (c) identification and marking of transplanting points in those empty spaces between 3 or 4 plants, with space greater than the average distance between neighboring plants, (d) from the contour of the plot were selected the plants of good development, enough to be divided into more than 5 cuttings, (e) the leaves and stems were cut leaving between 3 to 5 cm in height, then they were extracted taking care that the root biomass is covered soil, (f) division of the extracted plant in rectangular sections with approximately 10 to 15 cm of side, as far as possible maintaining the soil covering the root system, using a metal machete and (g) digging of holes in the transplanting points and transplanting of the cuttings.

Data collection began 30 days after the transplants were installed (October 2020), in the case of non-viable cuttings, these were exchanged for new ones; 10 transplants were also marked in each specific area of fertilization. The height of the shoot was measured in cm, using a pleximeter graduated in mm (two measurements per plant to record the resulting average), recording 100 data for each plot. The foliage cover of the shoot was evaluated by double measurement of the projection to the ground in a cross from the flag leaves. The total data collected amounted to 200 monthly records per plot, accumulating 18000 data in 9 months of monitoring. From the last 5 months (February 2021) the beginning of inflorescence formation of some plants was observed, whose height was also measured, as additional data.

Data analysis

The collected data were arranged in double-entry matrices, the factors and variables (canopy cover, canopy height and inflorescence height) and physical and (climatic, etc.) factors in rows and monthly data in columns, in an Excel sheet. Foliage cover was calculated by the ellipsoidal area formula, using the equation A= r1*r2*л, where: A is the area covered by foliage, r1 is the radius of axis 1 in cm, r2 is the radius of axis 2 and л is the ratio between the length of a circumference and its diameter as a constant element with a value of 3.1416 (Martínez-Encino *et al*. 2013; Yaranga *et al*. 2021). To contrast the study hypotheses on foliage cover, foliage height considering the average leaf flag and, inflorescence height. The data generated were analyzed using the "Generalized linear mixed model" method recommended for biological studies by Dicovskiy Riobóo and Pacheco (2018), using the Rstudio vs 4.1.2, using the following equation:

$$Yijkl = \mu + \Omega\, i + \beta j + \lambda k + \varepsilon ijkl$$

Where Yijkl: Plant characteristic evaluated; Ωi: The effect of the plot on the evaluated plant characteristic; βj: The effect of the species; λk: The random effect of the evaluated plant characteristic and εijkl: the random effect of variation.

A canonical correlation analysis was also performed between the biological variables under study and the environmental variables: minimum temperature and maximum temperature in °C, rainfall in liters per m2 in each plot, using PAS vs 3.14 software, under the multiple linear correlation model: $X=(X_1, X_2, X_p) and Y=(Y1, Y2,..., Yq)Y=(Y1, Y2,..., Yq) recommended by Trendafilov and Gallo (2021).

Results

Foliage cover, leaf height and inflorescence height

Foliage cover and growth height were considered important morphological characteristics for monitoring transplanted plants, according to plant species, plot location, and fertilizer applied. Regarding foliage cover, it was observed that the species *C. intermedia* showed the fastest response in the second month of control from 226.91 to 271.8 cm^2 to experience a gradual reduction in the following months up to 195.57 cm^2 in the last month of control (Fig. 2a); *F. rigidifolia* showed a continuous increase until the fourth month from 232.71 to 281.71 cm^2 and ended with 231.62 cm^2 (Fig. 2b). Third species *C. tarmensis* showed maximum development in the second month from182. 64 to 276.3 cm^2 and was reduced to 120.16 cm^2 in the 9th month of control (Fig. 2c); while, in *Festuca spp* the increase was observed in the third month from 320.63 to 390.4 cm^2 being reduced at the end with 195. 5 cm^2 (Fig. 2d); finally, the species *C. antoniana* showed the greatest increase in the third month from 250.3 to 326.05 cm^2 then decreased in the eighth month to 122 cm^2 and recovered in the ninth month with 269.67 cm^2 (Fig. 2e).

In the statistical analysis of foliage cover, significant differences were observed for $P \leq 0.001$, resulting in the species *C. antoniana* with the highest cover of 873 ± 165.7 cm^2, followed by *Festuca spp*. with 620±143.3 cm^2, then *F. rigidifolia* with 301±63.3 cm^2 and *C. intermedia* with 278±83.2 cm^2 and finally *C. tarmensis* with 227±42.6 cm^2 (Fig. 3a). At the plot level, differences were also observed for $p \leq 0.001$, resulting in the plot located in Gerbacio (P5) with 747±231.2 cm^2, followed by the plot in Sillapata Baja (P3) with 714±113. 9 cm^2, then in Sillapata Alta (P2) with 388±69.1 cm^2, then in Aylli (P1) with 205±67.9 cm^2 and finally in Otushpalla (P4) with 197±82.3 cm^2 (Fig. 3b).

Leaf height in the 5 species showed a certain homogeneity even with small seasonal variations, thus in C. intermedia increased from 8.425 cm in the first month of control to 16.23 in the final control; in *F. rigidifolia* from 9.121 to 20.11 cm, in *C. tarmensis* from 8.035 to 14.19 cm, in *Festuca spp*. from 8.938 to 22.23 cm and finally in *C. antoniana* from 10.748 to 19.55 cm. In a statistical analysis, no significant difference was observed for $p \leq 0.05$, with averages of *Festuca spp*. with 22.2±1.15 cm; *F. rigidifolia* with 20.1±0.77 cm, *C. antoniana* with 19.50±1.07 cm; *C. intermedia* with 16.2±0.815 and *C. tarmensis* with 14.2±0.865 cm (Fig. 3c). In the comparison by plot location effect, a significant difference was observed for $p \leq 0.05$, being the highest in P3 with 21.9±0.843 cm, followed by P5, P2, and P1 with 18.9±084 cm, 18.5±0.84 cm and 17.8±.83 cm, finally the lowest for P4 with 14.4±084 cm.

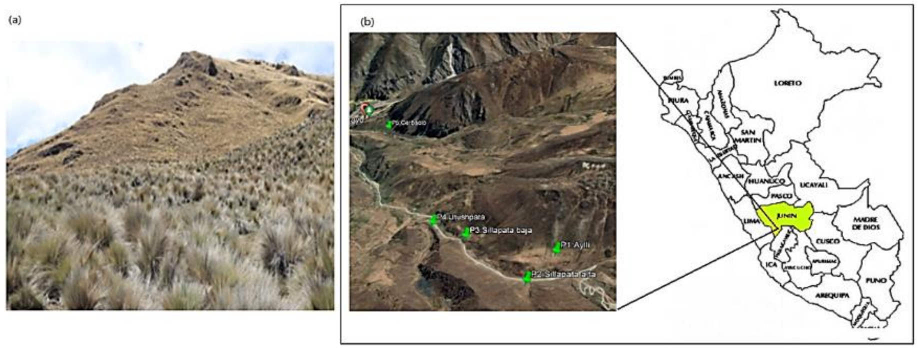

Fig. 1: a) Andean tussock grasslands, b) location of the study area in the central Andes of Peru

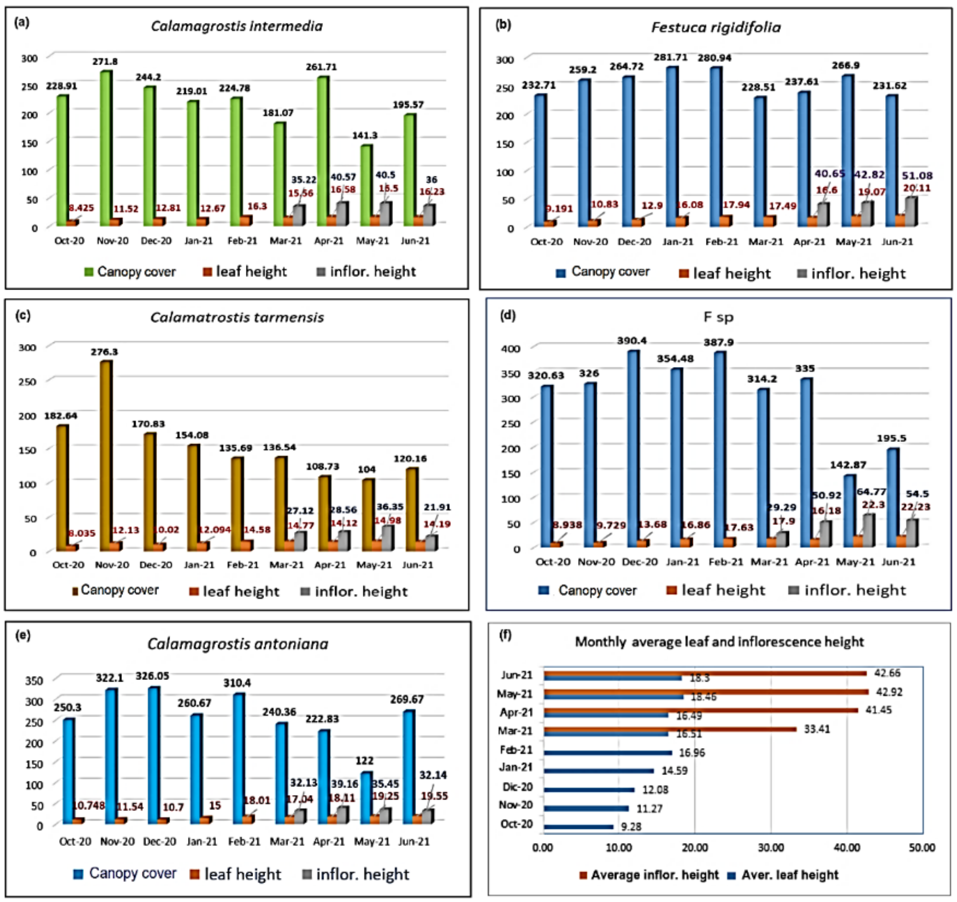

Fig. 2: The behavior of aerial coverage, leaf flag height, of five Andean grassland species: a) *Calamagrostis intermedia*, b) *Festuca rigidifolia*, c) *C. tarmensis*, d) *Festuca* sp., e) *C. antoniana*, f) monthly average leaf and inflorescence height

In addition, the height to the apex of the inflorescence of the plants was evaluated (Fig. 2f), which on average started at 33.41 cm and reached 42.92 cm in May and was reduced to 42.66 cm in June, due to the effect of the night frosts on the first inflorescences. The same data shows the evolution of the monthly average height including the five species, which at the first control started with 9.28 cm, reached 18.46 cm in May and by June was reduced to 18.3 cm due to the effect of the low temperature during the period. In the statistical analysis between species, no difference was observed for $p \leq 0.05$, whose averages were, for *C. antoniana* 50.0±14.29 cm, *F. rigidifolia* 54.4±5.91 cm; for *Festuca spp*. 54.3±12.31 cm; for *C. intermedia* 46.3±7.48 cm (Fig. 3c). In the statistical analysis by the effect of plot location, no difference was observed for $p \leq 0.05$, whose averages were for P3 57.7±9.99 cm, for P2 55.8±6.55 cm, for P1 45.9±6.42 cm, for P5 34.5±19.49 cm and finally P4 52.3±7.34 cm (Fig. 3d).

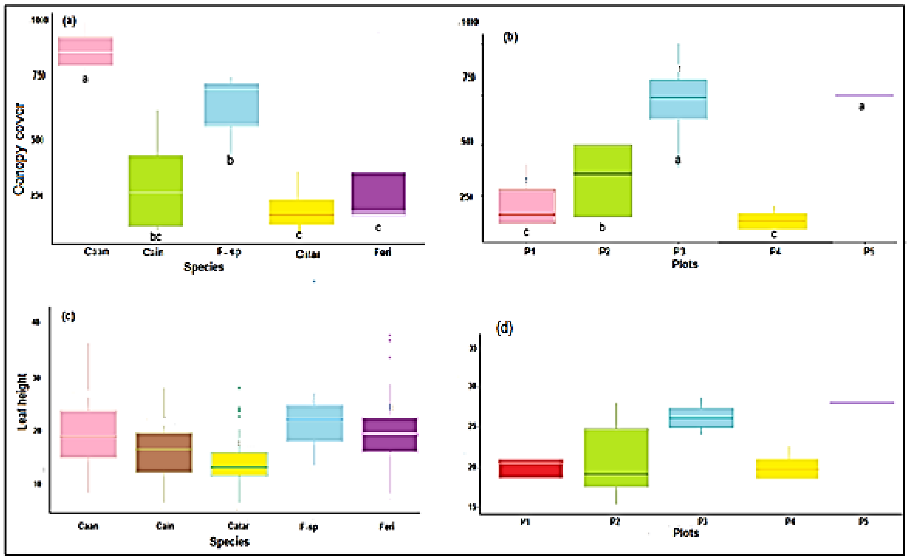

Fig. 3: Least significant difference (LSD) of canopy cover: a) between species and b) at plot level; LSD of leaf height: c) between species and d) at plot level

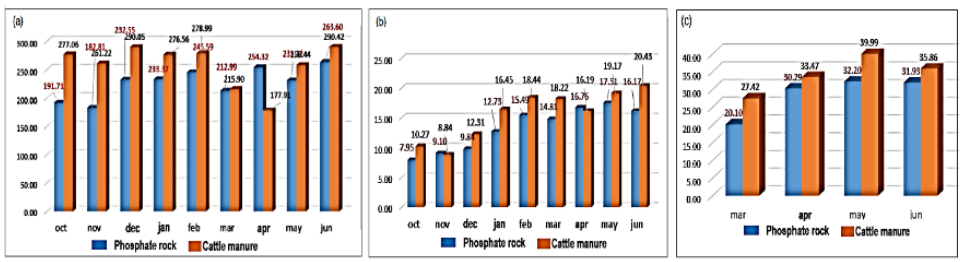

Fig. 4: Monthly growth trend of species: a) canopy cover, b) leaf height and inflorescence height, by the effect of fertilization with cattle manure and phosphate rock

Effect of natural fertilization on canopy cover, leaf height and inflorescence height

Foliage cover due to the effect of the application of cattle manure was better than in those applied with rock phosphate, In the first case, after 30 days it reached 227.06 cm^2 and at the end of the evaluation period, it reached 290.42 cm^2 (Fig. 4a), while in the second case, it started with 191.71 cm^2 and at the end, it reached 263.60 cm^2 (Fig. 4b). Statistical analysis showed differences for $p \leq 0.05$ in favor of cattle manure with 413±38.2 cm^2 and rock phosphate with 258±100.8 cm^2 (Fig. 5b).

The evolution of the average height of the leaf tray was different between natural fertilizers applied at the study site (Fig. 4a) it was observed that the transplants fertilized with cattle manure developed from 10.27–20.43 cm, while those fertilized with rock phosphate developed from 7.95–16.17 cm. In both cases, it was observed that the greatest development occurred between the third and fifth months coincided with the beginning of the rainy season, and then slowed its growth until the last month of control. In the statistical analysis, no difference was found for $p \leq 0.05$, whose averages were 54.5±4.59 cm for the transplants fertilized with cattle manure and 45.4±6.76 cm for those fertilized with rock phosphate (Fig. 5a).

Canonical correlation between the biological and environmental variables

Among the environmental variables, the monthly accumulated rainfall (recorded in each plot), showed that in November 2020 there was no rainfall, which marked an irregular event during the rainy season; likewise, February 2021 did not correspond to the peak of rainfall. On the other hand, precipitation was also not uniform for the five plots during the observation period (Fig. 6). Otherwise, the monthly precipitation averages varied from 1.59 to 154.67 L/m^2 and during the evaluation period from 457.57 to 533.96 L/m^2 in 9 months of record (Table 1).

The CCA with the environmental variables (monthly average of minimum temperature, the maximum temperature and rainfall analyzed at 95% probability, showed that the biological variables (foliage cover, foliage height, and inflorescence height) maintained the highest correlation in

Table 1: Monthly rainfall recorded on each plot (P)

Months	Aylli (P1)	Sillapata alta (P2)	Sillapata baja (P3)	Otush palla (P4)	Gerbacio (P5)	Monthly average
Oct-20	47.75	35.81	3.98	35.81	35.81	31.83
Nov-20	0.00	0.00	0.00	0.00	0.00	0.00
Dec-20	109.42	89.13	99.47	92.51	92.51	96.61
Jan-21	158.76	157.96	171.89	167.11	167.11	164.57
Feb-21	67.84	39.39	48.94	25.86	25.86	41.58
Mar-21	79.58	71.62	79.58	79.58	79.58	77.99
Apr-21	64.66	59.68	67.64	85.55	85.55	72.61
May-21	3.98	1.99	15.92	5.97	5.97	6.76
Jun-21	1.99	1.99	3.98	0.00	0.00	1.59
Total plots	533.96	457.57	491.39	492.38	492.38	493.54

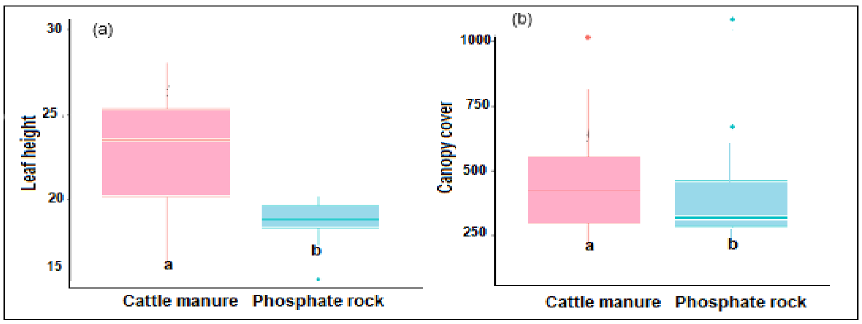

Fig. 5: LSD of: a) aerial cover of Andean grassland species and b) height of flag leaves, both due to the effect of fertilization with cattle manure and rock phosphate

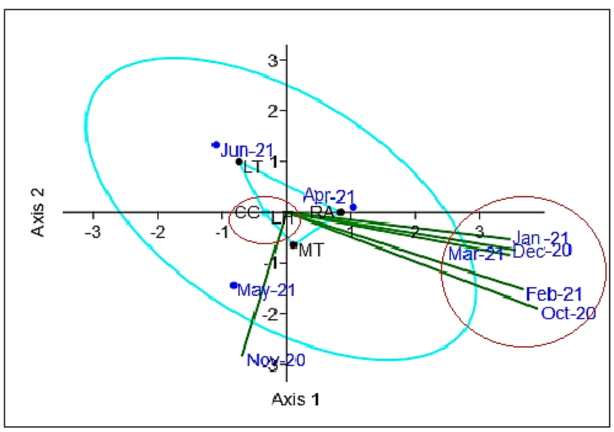

Fig. 6: Canonical correlation of canopy cover, canopy height, and inflorescence height, with climatic variables: minimum temperature, maximum temperature and rainfall

the second quadrant, with the months from October to March (rainy period) and the maximum temperature; on the contrary, the months of April, May and June (dry period) were not correlated with the biological variables (Fig. 6). The description of occurrences was in 90.65% of the data.

Discussion

Rapid response observed in the initial growth of the shoot of the transplanted cuttings was due to the solidity of the root architecture that was protected by the soil loaf that accompanied and provided nutrients necessary for the growth of the grassland plants, avoiding water stress (Fry *et al.* 2018); even though the transplanting was done at the end of the dry seasonal period (September 2021), allowing to continue the good development in some until the third month and in others as in *F. rigidifolia* until the sixth month of transplanting, to then move to a period of slowing growth despite the rainy period that should influence the greater development. This aspect indicated that the nutritional reserve of the soil loaf was depleted, therefore, the plants had to consolidate the fixation of their roots to the surrounding soil to assimilate the nutrients of the new edaphic layer (Lepik *et al.* 2021), which was achieved in the ninth month of transplanting to show new growth acceleration in June, except for *F. rigidifolia*. This biological behavior indicates that the transplanting of grassland species including the soil bread surrounding the roots was necessary and reduced the effects that should be negative by the extraction of the plant from the soil and segmentation into cuttings; on the other hand, it was revealed the indication that root fixation under the form as the cuttings have been obtained is consolidated from the ninth month of transplanting, which coincides with results obtained in the asexual propagation of native grasses evaluated in Brazil by Figueiredo *et al.* (2018).

The variation of response based on the location of the plots is due to differences in soil physical-chemical characteristics such as structure, compaction, erosion susceptibility, mineral contents, moisture content and other properties; according to these criteria the study plots varied in altitude (4012 and 4333 masl), precipitation received by plots (491 and 533 L/m^2) and soils that varied in pH (4. 6 to 5.9), in OM (7.3 to (15.2%), in P (3.8 to 24.2 ppm); this was also found by Andueza *et al*. (2021) when evaluating growth and maturity stage and chemical composition in 6 perennial types of grass, about altitudinal gradient and climatic variables during 2 years in 3 different locations; also topography influences plant development, through a regulatory phenomenon of respiration, being higher in flat areas than in sloping ones (Zhang *et al*. 2021).

The incorporation of organic matter and minerals to the soil, enrich the availability of nutritional elements for plants (Elouear *et al*. 2016); however, the effect on natural fertilizers could not be perceived immediately because the mineralization process is slow due to several factors: the decomposition time of cattle manure, the climatic characteristics and the altitude of the location of the plots; on the other hand, the poor solubility of rock phosphate in water and the acid condition of Andean soils (Rolando *et al*. 2017); in this context, transplants fertilized with cattle manure had greater response in the expansion of canopy cover versus those fertilized with phosphate rock, because cattle manure carries with it parts of the urine that is a source of nitrogen plus the labile carbon that is released in the decomposition period of cellulose and hemicellulose, plus those released by the microorganisms in the rumen of cattle (Wang *et al*. 2018) and these when washed by rain is integrated into the soil in less time, therefore assimilated by the plant and by soil microorganisms; meanwhile, the phosphate rock did not contribute N or C which, are the main promoters of growth and leaf elongation in plants in the first instance.

The regret is shared that inter-annual changes in precipitation are not visualized in their real dimension, because the data available in the long term are statistically managed for large areas, which hides the real changes that occur and vary over small areas where there is no recording equipment (Djebou *et al*. 2021). These irregularities of precipitation create space of scarcity or lack of rainfall that affects the maintenance of soil moisture; however, the lack of water in the soil can be mitigated if the vegetation protects the soil against rapid evaporation through the shade formed by the abundance of its leaves (De Jesus *et al.* 2021). These criteria are very important, to note that the Andean grasslands, being populated by tall grass species with many tillers, maintain soil moisture, which allows maintaining a continuous growth of the transplanted cuttings, at least until completing their vegetative development (Muñoz 2017; Padilla *et al*. 2019).

The correlation of canopy cover, canopy height, and high inflorescence height from October 2020 to March 2021 was due to the higher rainfall that occurred in these months by which the soils were maintained with higher humidity, in addition to the less abrupt temperature, mainly in the maximum temperature. These variables, being the main climatic factors influenced the production of leaves, stems, and the development of the different phenological phases of production and reproduction of the plants (Muñoz 2017). Meanwhile, the dry months: April, May and June did not maintain the level of correlation observed for that period, which contrarily reduced the expansion of canopy cover and the growth of leaves, stems, and inflorescence, because of the scarcity of water in the soil, the reduction of soil microorganisms and their association with minimum temperatures (Li *et al*. 2021); however, the continued growth even at the lower level was due to the change of the structure with increased vertical development of roots to fulfill the function of searching for water in the subsoil (Padilla *et al*. 2019). Since longer-lasting rainfall has allowed the greater accumulation of water deep in the subsoil and was not strongly affected by the phenomenon of evapotranspiration (Muñoz 2017; Chen *et al*. 2021).

Conclusion

The transplantation of cuttings in Andean pasture proved to be feasible despite having started the research in the dry season (April to August). The development of the cover and height of foliage was different due to effect of the application of natural fertilizers, as well as according to altitude, average temperature, and monthly rainfall, measured for nine months in each control site.

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Author Contributions

RMY planned the research, performed the data analysis, and directed the writing of the article. KM, MYR, and DHC participated in research planning, and control area installation and was responsible for data collection.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

The original data can be seen in the attached file.

Ethical Approval

Not applicable in this paper.

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