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Nutritive value of pastures along an elevation gradient

Scope statement (200 words)

To our knowledge, this study is the first one to show how the nutritional traits vary across an elevation gradient in tropical conditions as well as within the elevation ranges for each grass genus analyzed. With this study, we show that how the nutritive value of grass genera changes within the range of elevation at which they are grown in tropical conditions.

Our study may be of interest to readers with backgrounds in agronomy, animal science, and ecology, among others.

This study is unique as it includes C_3 and C_4 perennial grasses with different adaptation requirements, that are used in grass-based livestock systems. Because there are many countries with similar conditions located in the tropics (Central and South America and Africa), we consider this study to be relevant to start analyzing agroecological conditions and comparing nutritional value at different elevations.

Nutritive Value of Perennial Pastures along an Elevation Gradient in Tropical Conditions

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Abstract

The nutritive value of forages is one of the main drivers of productivity for livestock. In many tropical regions, same grass species occur at different elevations, but few studies have evaluated nutritive value changes within elevation gradients. The objective of this study was to analyze the changes in nutritive value of six grass genera across and within elevation gradients in Costa Rica. We synthesized elevation and nutritive data for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and *in-vitro* dry matter digestibility (IVDMD) in a database (n = 1,192) containing five C_4 grasses (*Urochloa*, *Cynodon*, *Digitaria*, *Megathyrsus,* and *Cenchrus*) and one C_3 grass (*Lolium*). *Urochloa*, *Megathyrsus,* and *Digitaria* are grasses grown primarily at low elevation (0–999 masl), and *Lolium* at high elevation (>2,000 masl). *Cynodon* and *Cenchrus* overlap low to mid, and mid to high elevations, respectively. Greater CP and lower NDF concentrations were found for grasses grown at high elevation compared to those grown at low elevation (CP = 18.2–22.4 vs. 7.8–15.2 %, NDF = 48.9–49.3 vs. 64.6–67.3 %, and ADF = 32.2–33.2 vs. 37.4–44.3 %). Consequently, IVDMD was greater for grasses grown at high than at low elevation (80.9–86.0 vs. 61.4–71.1 % of DM). CP increased with elevation, especially for *Lolium*, while NDF and ADF tended to decrease for *Megathyrsus*, *Urochloa*, and *Cenchrus*. The groups of grasses classified by nutritive value in this study, provide a baseline for potential nutrient supply to livestock and rations adjustments accordingly.

1. Introduction

Grasses are the staple feedstuff in ruminant diets in tropical environments (Thornton and Herrero 2010)(Thornton and Herrero 2010)(Thornton and Herrero 2010)(Thornton and Herrero 2010). Costa Rica is a tropical country (latitude 10° 00' N and longitude 84° 00' W) with a wide range of elevation [0–3,800 meters above sea level (masl)]. The varying environmental conditions in the tropics have shown to influence the adaptation of both C_4 and C_3 grasses along elevation gradients(Angelo and Daehler 2015). In tropical conditions, the relatively consistent temperatures year-round (Angelo and

Daehler 2015) and the lower transition temperatures between C_4 and C_3 grasses (Chazdon, 1978)(Chazdon, 1978)(Chazdon, 1978)(Chazdon, 1978), create temperature ranges closely related to elevation gradients where C_4 grasses are not adapted due to physiological limits (Angelo and Daehler 2015; Chazdon 1978b)(Angelo and Daehler 2015; Chazdon 1978b)(Angelo and Daehler 2015; Chazdon 1978b)(Angelo and Daehler 2015; Chazdon 1978b).

Using controlled experiments, previous studies have evaluated the distribution of C_3 and C_4 grasses along elevation gradients in temperate (Cavagnaro 1988)(Cavagnaro 1988)(Cavagnaro 1988)(Cavagnaro 1988), subtropical (Rundel 1980)(Rundel 1980)(Rundel 1980)(Rundel 1980), and tropical environments (Chazdon, 1978)(Chazdon, 1978)(Chazdon, 1978)(Chazdon, 1978). These studies have analyzed the physiological mechanisms allowing for adaptation to varying weather conditions. In Costa Rica, Chazdon (1978)Chazdon (1978)Chazdon (1978)Chazdon (1978) found that C_4 grasses are mostly adapted at low elevations with high temperatures and relatively low rainfall and C_3 grasses at high elevations (> 2000 masl) with low temperatures, high rainfall, high humidity, and low atmospheric O_2 conditions.

At low elevations (<1,000 masl), C_4 grasses such as *Urochloa* (formerly *Brachiaria*) and *Megathyrsus* (formerly *Panicum)* are intensively cultivated in the lowlands of Costa Rica (Villarreal 1994; Andrade et al. 2008; Vallejos et al. 1989; Villanueva et al. 2008)(Villarreal 1994; Andrade et al. 2008; Vallejos et al. 1989; Villanueva et al. 2008)(Villarreal 1994; Andrade et al. 2008; Vallejos et al. 1989; Villanueva et al. 2008)(Villarreal 1994; Andrade et al. 2008; Vallejos et al. 1989; Villanueva et al. 2008). Also, *Digitaria* is a versatile grass genus (Cook et al. 2005; Vega and de Agrasar 2007), adapted at low elevations and suitable both for grazing (Blydenstein et al. 1969) and hay production (Rojas and Dormond 1994). At mid elevation (1,000-2,000 masl), grass species with adaptation ranges from sea level to higher elevations overlap (Chazdon, 1978), with Stargrass (*Cynodon nlemfuensis* Vanderyst) being the most cultivated grass species in both specialized dairy (Villalobos et al., 2013; Villalobos and Arce, 2013)(Villalobos et al., 2013; Villalobos and Arce, 2013)(Villalobos et al., 2013; Villalobos and Arce, 2013)(Villalobos et al., 2013; Villalobos and Arce, 2013) and dual-purpose (milk and weaning calves) operations at low and mid elevations (González et al., 1996; Sanchez et al., 1998; Sánchez and Soto, 1996)(González et al., 1996; Sanchez et al., 1998; Sánchez and Soto, 1996)(González et al., 1996; Sanchez et al., 1998; Sánchez and Soto, 1996)(González et al., 1996; Sanchez et al., 1998; Sánchez and Soto, 1996).

Between 2,000 and 3,000 masl, the C_4 grass Kikuyu (*Cenchrus clandestinus*, formerly *Pennisetum clandestinum*) is the main species due to its adaptation to cooler environments (optimal temperature of 15°C) (Kaiser et al. 2000; Retana 2006), thus mostly becoming the most used grass in dairy farms at higher elevations in Costa Rica (Castillo et al. 1983; Andrade 2006; Villalobos et al. 2013). Additionally, the climatic conditions in high elevations in tropical environments can emulate those of temperate regions (Boudon et al., 2002; Donaghy and Fulkerson, 2002),(Boudon et al., 2002; Donaghy and Fulkerson, 2002),(Boudon et al., 2002; Donaghy and Fulkerson, 2002),(Boudon et al., 2002; Donaghy and Fulkerson, 2002), with lower temperatures (8 and 19 °C average minimum and maximum, respectively) (Retana 2006) suitable for adaptation of temperate (C_3) grasses such as ryegrass (*Lolium* spp., Villalobos and Sánchez 2010a) showing greater persistence than in temperate regions (Donaghy and Fulkerson, 2002).

Despite documented differences in nutritive characteristics between C_3 and C_4 grasses (Capstaff and Miller 2018, Jung et al. 1997) and their impact on grass-fed livestock production (Bohnert et al. 2011; García et al. 2014; Lee et al. 2017), there are not studies that have assessed the nutritive value of C_3 and C_4 grasses through a wide elevation gradient such as it is the case in Costa Rica. In this research, we studied the nutritive value of six grass genera at different elevations (15–2850 masl) in tropical conditions in Costa Rica. By using average values of nutritive parameters, we illustrate general trends across forage species but, direct comparisons among different grass genera should be interpreted with caution, as factors other than elevation may influence such values. Our hypothesis was that elevation influences not only the adaptation of grass species in tropical conditions, but their nutritive value. We aimed to understand how the elevation affects the nutritive value within each species in tropical conditions.

2. Materials and Methods

2.1 Databases and nutritive variables

Two databases from the National Institute for Agricultural Technology (INTA) and the Research Center for Animal Nutrition (CINA) from Costa Rica, were compiled into a single database that comprised 33 years (1986–2019) of samples that were collected by researchers from both institutions assessing the nutritive value of grasses in Costa Rica. Grass samples were randomly taken, comprising different stages of regrowth, stubble heights, and different months throughout the year. The database includes samples from all over the country with biennial updates for data cleaning (Figure 1). The initial database comprised 1,429 records of samples of 16 genera that included 27 grass species., which was reduced due to data points outside their elevation range of adaptation as well as nutritive values outside of normal ranges reported by Martínez (2020). In order to have sufficient replicates per grass genus and repeatability within each variable, only grass genera with at least 24 samples per nutritive trait were considered in the database (Table S1 in supplemental material), which allowed us to increase the total number of samples per grass genus. Each sample analyzed included at least one nutritive trait and the location (latitude and longitude), resulting in a final database with 1,192 records of six grass genera with a variety of days of regrowth and annual nitrogen rates: *Urochloa brizantha* (Hochst. ex A. Rich.) Stapf. (1—120 d; 50–100 kg.ha^{-1}), *Cynodon nlemfuensis* Vanderyst (14–60 d; 100–350 kg.ha^{-1}), *Digitaria decumbens* Stent (14–120 d; 200–400 kg.ha^{-1}), *Lolium spp.* (33–46 d; 250–300 kg.ha^{-1}), *Megathyrsus maximus (Jacq.)* (10–120 d; 50–300 kg.ha^{-1}), and *Cenchrus clandestinus* Hochst. ex Chiov (25–120 d; 100–300 kg.ha^{-1}).

Although the forage mass was not included within the variables analyzed in this study, it has been (J. L. M. González and Redondo 2009)(J. L. M. González and Redondo 2009)(J. L. M. González and Redondo 2009)(J. L. M. González and Redondo 2009)previously evaluated in Costa Rica. At low and mid elevation, the grass genera *Urochloa, Megathyrsus* and, *Digitaria* have shown values between 14–23 t DM.ha^{-1}.yr^{-1} (Andrade et al., 2008; Villanueva et al., 2008)(Andrade et al., 2008; Villanueva et al., 2008)(Andrade et al., 2008; Villanueva et al., 2008)(Andrade et al., 2008; Villanueva et al., 2008), 17–108 t DM.ha^{-1}.yr^{-1} (Núñez-Arroyo et al., 2022; Villalobos and WingChing-Jones, 2019) and 17–52 t DM.ha^{-1}.yr^{-1} (Cerdas and Vallejos, 2012; González and Redondo, 2009; Morales and Acuña, 2018; Morales et al., 2006; Murillo-Benavides, 2013), respectively. *Cynodon* has been evaluated at mid elevation with forage mass ranging from 40–78 t DM.ha^{-1}.yr^{-1} (Villalobos and Arce, 2013; Villalobos et al., 2013a; Villalobos and WingChing-Jones, 2019; Villalobos and WingChing-Jones, 2023)(Villalobos and Arce, 2013; Villalobos et al., 2013a; Villalobos and WingChing-Jones, 2019; Villalobos and WingChing-Jones, 2023)(Villalobos and Arce, 2013; Villalobos et al., 2013a; Villalobos and WingChing-Jones, 2019; Villalobos and WingChing-Jones, 2023)(Villalobos and Arce, 2013; Villalobos et al., 2013a; Villalobos and WingChing-Jones, 2019; Villalobos and WingChing-Jones, 2023). At high elevation, *Cenchrus* and *Lolium* may yield 38–92 t DM.ha^{-1}.yr^{-1} (Andrade, 2006; Núñez-Arroyo et al., 2022; Villalobos et al., 2013) and 29–47 t DM.ha^{-1}.yr^{-1}

(Villalobos et al., 2013; Villalobos and Sánchez, 2010a; Villalobos and WingChing-Jones, 2023), respectively.

The grass samples were analyzed in the INTA and CINA laboratories following the methods of the Association of Analytical Chemists (AOAC 2000) for crude protein (CP), Van Soest et al. (1991) for neutral and acid detergent fiber (NDF and ADF, respectively), and Van Soest and Robertson (1985) for *in-vitro* DM digestibility (IVDMD). The latter was only available for 349 samples analyzed by the CINA laboratory. Elevation was included for some of the samples, and the rest were obtained from Google Earth based on farm location. Descriptive characteristics of the database are reported in Table S1.

2.2 Statistical analyses

The statistical analyses were conducted in R v3.6.1 (R Core Team, Vienna, Austria). Tests applied prior to the use of the statistical models showed normality for the histograms and qqplots for CP, NDF, ADF, and IVDMD. Linear models were used to analyze the database with the lm function in the R package. Factors were elevation, grass genus and their interaction (Table 1), and response variables were the nutritive values (CP, NDF, ADF, and IVDMD). No random effects were considered in the model as some factors (year, fertilization, and days of regrowth) were not available for all species. The nutritive value was analyzed using three elevation ranges (0–999 [low], 1,000–1,999 [mid], and 2,000–2,999 [high] masl) aimed to provide a straightforward arrangement of results that are closely related with the specific adaptations ranges of grass species in tropical conditions. In addition, the association of elevation and nutritive value was analyzed with a linear regression using the elevation range available for each species (individual altitude values for each sample) and estimating their respective coefficients and intercepts (Table 2). Backward selection and the likelihood ratio test were used to select the models. Multiple comparisons were made using Tukey test with the HSD procedure. The P-values < 0.05 and < 0.10 were declared as significant and trend, respectively.

Multivariate statistics methods were applied to the grass database by using a principal component analysis (PCA) and a correspondence analysis (CA). The PCA and CA were post-hoc analyses based on a subset (n=337) of the database created by culling samples with missing values for at least one of the nutritive variables (CP, DM, NDF, and IVDMD) considered in the PCA. PCA was based on a correlation matrix and scree plots were used to determine the number of principal components retained as well as to indicate the contributions of the nutritive variables in each component.

The final database (n = 1,192) was also analyzed by using a correspondence analysis (CA) in Jamovi version 2.3 for four variables that were converted into categorical (low, medium, and high). The latter were defined for each variable as follows: elevation (0–999, 1,000–1,999, and 2,000–2,999 masl), crude protein (<10, 10–20, and >20 %), neutral detergent fiber (<50, 50–60, and >60 %), and *in-vitro* dry matter digestibility (<50, 50–60, and >60 %). Contingency tables were designed for each variable and biplots were used to visualize the relationships among row and column pairs. The biplots were interpreted based on the distance from each grass genus and the category given in the same region of space (quadrant). Both the PCA and CA aimed to visually support our results and differences in nutritive value among the grass genera evaluated.

3. Results

The frequency across elevations (Figure 2) indicated that *Urochloa*, *Cynodon*, *Digitaria*, and *Megathyrsus* were primarily found below 1,500 masl, however, *Digitaria* was only found below

1,000 masl. *Cenchrus* and *Lolium* were more common between 1,500 and 3,000 masl, with *Cenchrus* sometimes as low as 1,000 masl (Table S1 and Figure 2).

3.1 Nutritive composition

Lolium and *Cenchrus* had similar average CP concentrations ($p < 0.05$), showing the highest values among the analyzed species (Table S1). Our results showed that in the tropical highlands (>2000 masl), *Lolium* and *Cenchrus* have greater protein concentration than the grass species in the tropical lowlands (<1000 masl) (Figure 3A).

At high elevation, *Lolium* had CP concentration 4.2 percentage points (pp) greater ($p < 0.05$, Figure 3A) than *Cenchrus,* but at mid-elevation the latter had 5.7 pp greater CP concentration than *Lolium* ($p < 0.05$). *Cynodon* had 6.3 pp greater CP concentration than *Urochloa* (Figure 3A), and the latter and *Megathyrsus* had similar CP concentrations at mid elevation (average 7.2 % of DM). At low elevation, *Cynodon* had on average 7.1 pp greater CP concentration than *Digitaria*, *Megathyrsus* and *Urochloa* ($p < 0.05$, Figure 3A).

Megathyrsus had on average 2.1 pp greater NDF concentrations than *Digitaria, Urochloa*, and *Cynodon* at low elevation ($p < 0.05$, Figure 3B). At mid elevation, *Megathyrsus*, *Urochloa* and *Cynodon* had similar NDF concentrations (average ± SD = 63.8 ± 1.4%) and, on average, 8.9 and 16.7 pp greater NDF concentrations than *Cenchrus* and *Lolium,* respectively ($p < 0.05$). While *Cenchrus* had 7.8 pp greater NDF concentration than *Lolium* at mid elevation ($p < 0.05$), no difference was found between the two genera at high elevations (average ± SD = 49.1 ± 0.3 %; Figure 3B).

Megathyrsus and *Digitaria* had on average 4.9 pp greater ADF concentrations than *Urochloa* and *Cynodon* at low elevation (average ± SD = 43.9 ± 0.6 % vs. 39.0 ± 2.3 %, respectively: $p < 0.05$; Figure 3C). In contrast, *Megathyrsus* and *Urochloa* had similar and significantly greater ADF concentrations at mid elevation than *Cenchrus* and *Lolium* (average ± SD = 39.8 ± 0.9 % vs. 33.9 ± 0.1 %, respectively: $p < 0.05$; Figure 3C). The ADF concentration of *Cynodon* at mid-elevation was intermediate and not different from any other species found at mid elevation. *Cenchrus* and *Lolium* had similar ADF concentrations at both mid and high elevation (average ± SD = 33.3 ± 0.1 %, Figure 3C).

In-vitro DMD of *Urochloa* was 9.0 pp lower than *Megathyrsus* and *Cynodon* at low elevation (61.4 % vs. average ± SD = 70.4 ± 1.0 %; $p < 0.05$; Figure 3D). *Cenchrus* had 15.3 and 5.1 pp greater IVDMD than *Cynodon* and *Lolium* at mid and high elevation, respectively. Average IVDMD values of *Cenchrus* and *Lolium* at high elevation were greater (83.4 % ± 2.8 %) than those of *Megathyrsus, Urochloa,* and *Cynodon* at low elevation (67.4 % ± 5.1 %; Figure 3D).

3.2 Effect of elevation on nutritive composition

CP concentration increased significantly with elevation for *Megathyrsus*, *Urochloa*, *Cenchrus* and *Lolium* (Table 2 and Figure 4A). The association between elevation and CP was 3-fold greater for *Lolium* than the average for *Urochloa*, *Megathyrsus* and *Cenchrus* (0.85 %/100 m vs. 0.28 %/100 m; $p < 0.05$).

The NDF concentration of *Megathyrsus* and *Cenchrus* decreased on average by 0.35 ± 0.01 %/100 m increase in elevation and NDF concentration of *Urochloa* tended to decrease by 0.21 %/100 m increase (Table 2 and Figure 4B). In *Megathyrsus, Urochloa* and *Cenchrus*, ADF concentrations decreased on average by 0.54 ± 0.7 %/100 m increase in elevation (Table 2 and Figure 4C). No effect of elevation on ADF and NDF concentrations was observed for *Lolium*.

The IVDMD of *Cenchrus* and *Lolium* tended to increase with increasing elevation (Table 2 and Figure 4D), while elevation had no effect on IVDMD of *Cynodon*. For *Digitaria*, *Urochloa* and *Megathyrsus,* no regression analysis was carried out due to low number of samples available at multiple elevations.

3.3 Principal Components Analysis (PCA)

Four components were obtained from the PCA but only two were retained, explaining 84.9% of the variability of the data (Table 4). Factor loadings of each nutritive variable indicated greater correlations for NDF concentration (positive) and IVDMD (negative) with the PC1 (Table 5). Thus, PC1 is the factor related to fiber accumulation, with negative impact on the digestibility of the grasses evaluated in this study (Figure 5A). Although the eigen value of PC2 was less than 1 (Table 4), this factor correlated with DM and CP (Table 5), which can be interpreted as the factor related to DM accumulation in the grasses with CP contributing positively to its increase (Figure 5B).

Each grass sample included in the PCA was grouped by genus and overlapped in a second biplot (Figure 6B), showing their position on PC1 and PC2 as an indicator of their nutritive value. The PC1 indicated lower NDF concentrations and greater digestibility for *Cenchrus* and *Lolium* while *Urochloa* and *Megathyrsus* had greater NDF concentrations and lower digestibility. *Cynodon* varied with respect to the other genera, some samples being more comparable to *Urochloa* and *Megathyrsus* and some overlapping with *Cenchrus* and *Lolium*.

Dry matter and crude protein concentrations were the nutritive variables with more impact on PC2 (Table 5), indicating how *Cynodon* was the genus with greater DM concentrations and, the opposite was true for *Cenchrus* and *Lolium* (Figure 6B). These two genera had greater protein concentrations than the other three, with *Urochloa* and *Megathyrsus* having the lowest protein concentrations in the subset of data used for the PCA.

3.4 Correspondence Analysis (CA)

The elevation biplot (Figure 7A) for CA indicated that *Lolium* was the genus furthest from the average in our database, and it is only adapted to high elevations. The genera *Digitaria*, *Megathyrsus*, and *Urochloa* are adapted to low elevations and, *Cynodon* and *Cenchrus* have wider ranges of adaptation from low to medium and medium to high elevations, respectively. Low concentrations of crude protein were commonly associated with the genera *Digitaria*, *Urochloa* and, *Megathyrsus* (Figure 7B). *Cynodon* had medium CP concentrations, followed by *Cenchrus*, while *Lolium* was the genus with greatest CP concentrations in the database.

Megathyrsus was the genus with greatest NDF concentrations (Figure 7C), followed by *Urochloa*, *Digitaria* and *Cynodon*; with the latter having medium and high fiber concentrations. *Cenchrus* had NDF concentrations considered medium to low and *Lolium* was categorized as a low fiber grass as well as the largest deviation from the rest of the genera analyzed. *Lolium* and *Cynodon* were the genera with greatest deviances for *in-vitro* digestibility with respect to the other grass genera (Figure

7D). *Digitaria, Megathyrsus* and, *Urochloa* were associated with low digestibility, and *Cenchrus*, despite having medium to low NDF concentrations, was categorized as a medium digestibility genus.

4. Discussion

Previous studies have indicated that the tropical highlands (> 2,000 masl) are suitable for temperate grasses as well as tropical species adapted to lower temperatures (Villalobos and Sánchez 2010b; Villalobos et al. 2013).

4.1 Nutritive composition

Lolium had similar CP concentration to *Cynodon* and *Urochloa* at mid-elevation, which could be attributed to lack of adaptation of C_3 grasses below 2,000 masl in tropical conditions (Retana 2006; Chazdon 1978). The intermediate CP concentration of *Cynodon* in the lowlands as well as the lowest CP concentrations of *Urochloa*, *Digitaria* and *Megathyrsus* (Figure 3A) is in line with findings by Bohnert et al. (2011) that grazing animals in the tropical lowlands are more likely to be deficient in protein and might need supplementation (NRC 2000). Although the average CP concentrations found for all four C_4 species grown at mid and low elevations (Table S1) indicate that they could meet the requirements for ruminants (NRC 2000), *Cynodon* was, on average, the only genus with greater values across the elevation gradients included in our study.

Our study found consistent patterns for NDF and ADF concentrations, with both fractions being greater in *Megathyrsus*, *Digitaria*, *Urochloa* and *Cynodon* than in *Cenchrus* and *Lolium* (Figure 3B and 3C). NDF and ADF represent the amount of total cell wall in forages and the cell wall minus the hemicellulose, respectively (Van Soest et al. 1991). Because fiber is the most prominent nutrient fraction in grasses, it has been extensively analyzed in previous research, consistently showing a tendency of being greater in perennial tropical grasses than temperate species (Capstaff and Miller 2018), which are typically regarded as low fiber forages (Jung et al. 1997). However, in this study NDF and ADF concentrations of *Cenchrus* and *Lolium* were not different at mid and high elevation (Figure 3B and 3C).

In-vitro DM digestibility (IVDMD) is an indicator of the nutritive quality in forages and indicates the potential nutrient utilization through the digestion process (Van Soest and Robertson 1985). In our database, *Lolium* and *Cenchrus* were the most digestible grasses, which showed at the same time the lowest NDF and ADF concentrations (Table S1 and Figure 3B, 3C, and 3D). This is in line with the expected negative correlation between fibrous fractions and dry matter digestibility (Jung et al. 1997). *Cenchrus* showed greater digestibility than *Lolium* at high elevation (Figure 3D), which coincides with results from similar environmental conditions (Andrade 2006; Escobar et al. 2020). Thus, the tropical highlands provide suitable ecological conditions for both C_3 and C_4 grasses with the potential to meet nutritive requirements of highly productive cattle (Villalobos and Sánchez 2010b; Villalobos et al. 2013; NRC 2001).

4.2 Effect of elevation on nutritive composition

Few studies have reported the effect of elevation on the nutritive composition of grasses. For *Urochloa,* Wassie et al. (2018) found no effect from 1,200 to 1,800 masl and a decrease in CP above 1,800 masl. In other studies, CP decreased above 1,700 masl for *Cenchrus* (Asmare et al. 2017; Escobar et al. 2020). No studies on the effect of elevation on CP concentrations were found for *Megathyrsus and Lolium*.

The greatest increase in CP concentrations ($p < 0.05$) with elevation found for *Lolium* could be attributed to physiological differences between C_3 and C_4 grasses (Bohnert et al. 2011; Deinum, 1966). Increasing elevation creates environmental conditions more suitable for *Lolium*, emulating those of temperate regions (Donaghy & Fulkerson, 2001). However, the extent to which CP concentration increases with elevation might not only be the result of physiological differences but also of management in the farms, where practices like fertilization or grazing intensity are likely important but were not taken into consideration in this analysis. For instance, *Lolium* and *Cenchrus* are grown at high elevations (Table S1) in specialized dairy farms where nitrogen fertilization is a common practice all year-round (Andrade 2006; Villalobos et al. 2013). At mid elevation, *Cenchrus* had greater CP concentration than *Lolium*, but at high elevation greater CP concentrations were found in the latter (Figure 3A). This contrasting shift in CP concentrations might indicate that the differences in the ranges of elevation where both genera are grown in Costa Rica (from 1,023 to 2,773 vs. from 1,583 to 2,850 masl; Table 2), impose adaptation limits to be considered by livestock producers. For example, *Cenchrus* might be more resilient than *Lolium* at lower elevations under tropical conditions (Cook et al. 2005).

Although fibrous fractions in grasses are largely determined by the number of days of regrowth (Johnson et al. 2001), the lower temperatures at high elevations (Retana 2006) seem to favor lower NDF and ADF in the grasses evaluated in this study (Moore and Jung 2001) (Figure 4B and 4C). The significant decreases in NDF and ADF concentrations found for *Cenchrus* with increasing elevation might indicate that the wider range of elevation where it is grown in Costa Rica (1,750 m) with respect to those of *Megathyrsus* (1,098 m) and *Urochloa* (1,223 m), seems to allow for a significantly greater reduction in fiber deposition with increasing elevation (Table 2). The lower concentrations of fiber with increasing elevation may impact the potential dry matter intake of livestock species (Van Soest et al. 1991; Moore and Jung 2001)(Van Soest et al. 1991; Moore and Jung 2001) as well as it imposes challenges for ration balancing (Bohnert et al. 2011; Capstaff and Miller 2018)(Bohnert et al. 2011; Capstaff and Miller 2018). The fiber fractions of *Lolium* were not affected by elevation, which could be attributed to its adaptation at merely higher elevations resulting in limited lignin accumulation (Boudon et al. 2002, Moore and Jung 2001) compared with tropical grasses (Johnson et al. 2001).

The negative correlation between elevation and temperature, has supported the results of various authors where lower temperatures have been generally associated with increasing CP and decreasing NDF concentrations (Deinum 1966; Lee et al. 2017), thus increases in IVDMD were expected as a result of greater CP and lower NDF concentrations. This study found increased CP concentration and IVDMD with increasing elevation for *Cenchrus* and *Lolium* and a significant decrease in NDF for *Cenchrus* with increasing elevation. The effect of elevation on NDF, ADF, and IVDMD concurred with the literature data at similar elevations for *Cenchrus* and *Urochloa* (Table 3), but not for CP concentration.

In our study, the regression equations estimated where single genera of grasses were evaluated along elevation gradients were inconclusive. Besides elevation, the vast array of management practices applied on Costa Rican livestock operations (Vargas-Leitón et al. 2013; Villalobos et al. 2013), likely influence the grass nutritive value.

4.3 Principal Components Analysis (PCA)

The two-dimensional plot of the PCA characterized how the four nutritive variables interact with each other in the two components retained for the analysis (Figure 6A). NDF concentration negatively affects the IVDMD, having both arrows pointing towards opposite directions on the PC1, which concurs with previous studies where digestibility is significantly reduced in highly fibrous feeds (Jayanegara et al. 2009; Jung et al. 1997)(Jayanegara et al. 2009; Jung et al. 1997).

Cynodon had nutritive variables that overlapped with grass genera grown at low and high elevations, which could be related to the wide range of elevation where this genus is grown in Costa Rica (1755 m, Table S1). Higher temperatures tend to increase both intensity and partitioning of plant dry matter to more lignified tissues (Moore and Jung 2001), which might partly explain the greater fiber concentrations found for the genera grown at low elevation.

Days of regrowth of pastures in Costa Rica vary accordingly across the elevation range. *Lolium* and *Cenchrus* are typically grazed after 30 to 45 days (Andrade 2006; Villalobos et al., 2013; Villalobos and Sánchez, 2010a), whereas *Megathyrsus* and *Urochloa* are grazed after 16 to 30 days of regrowth, respectively (Villalobos and WingChing-Jones, 2019) and *Cynodon* after 25 days (Villalobos and Arce, 2014). Consequently, the PCA visually supported the characterization of grasses with relation to their nutritive value, thus, showing three groups comprised by *Urochloa* and *Megathyrsus*, *Cenchrus* and *Lolium* and, *Cynodon* (Figure 6B).

4.4 Implications for Livestock Producers

Our results showed that the genera *Urochloa*, *Megathyrsus*, and *Digitaria* are mostly adapted to low elevations, whereas the genus *Lolium* is only adapted to cooler temperatures found at high elevation (≥ 2,000 masl) in tropical environments. The width and similarity in the elevation ranges for *Cynodon* (1755 m) and *Cenchrus* (1750 m) indicate their successful adaptation to varying environmental conditions in the tropics.

Although one might recommend that livestock enterprises requiring forage of high nutritive value are better suited to higher elevations than to lowlands, the upper ranges of nutritive values found for the grass genera analyzed indicate that, in appropriate seasons and applying adequate management practices, forage of high nutritive value can also be produced in the tropical lowlands.

5. Conclusions

Costa Rica's wide range of elevations creates climatic conditions suitable for C_3 and C_4 grasses. We acknowledge that elevation alone does not determine nutritive value of grasses and that other biotic and abiotic factors can affect the nutritive indicators included in this study. However, we found evidence that, for most species, crude protein tends to increase within their specific elevation range.

Pasture species adapted to high elevations (*Lolium* and *Cenchrus*) had greater CP concentrations and IVDMD, and lower NDF and ADF concentrations than species adapted to low elevation (*Urochloa*, *Digitaria* and *Megathyrsus*).

Crude protein concentrations increased within the elevation ranges for the genera *Megathyrsus*, *Urochloa*, *Cenchrus* and, *Lolium* while NDF and ADF concentrations decreased for *Cenchrus*, *Megathyrsus* and *Urochloa*. IVDMD tended to increase for *Cenchrus* and *Lolium* at mid and high elevations.

Although our results indicate that elevation may be used by livestock producers in the tropics as an indicator of the grass genera with potential to be grown in their operations, we acknowledge that factors such as pasture management and environmental conditions will also determine the nutritive value of pastures.

6. Figures

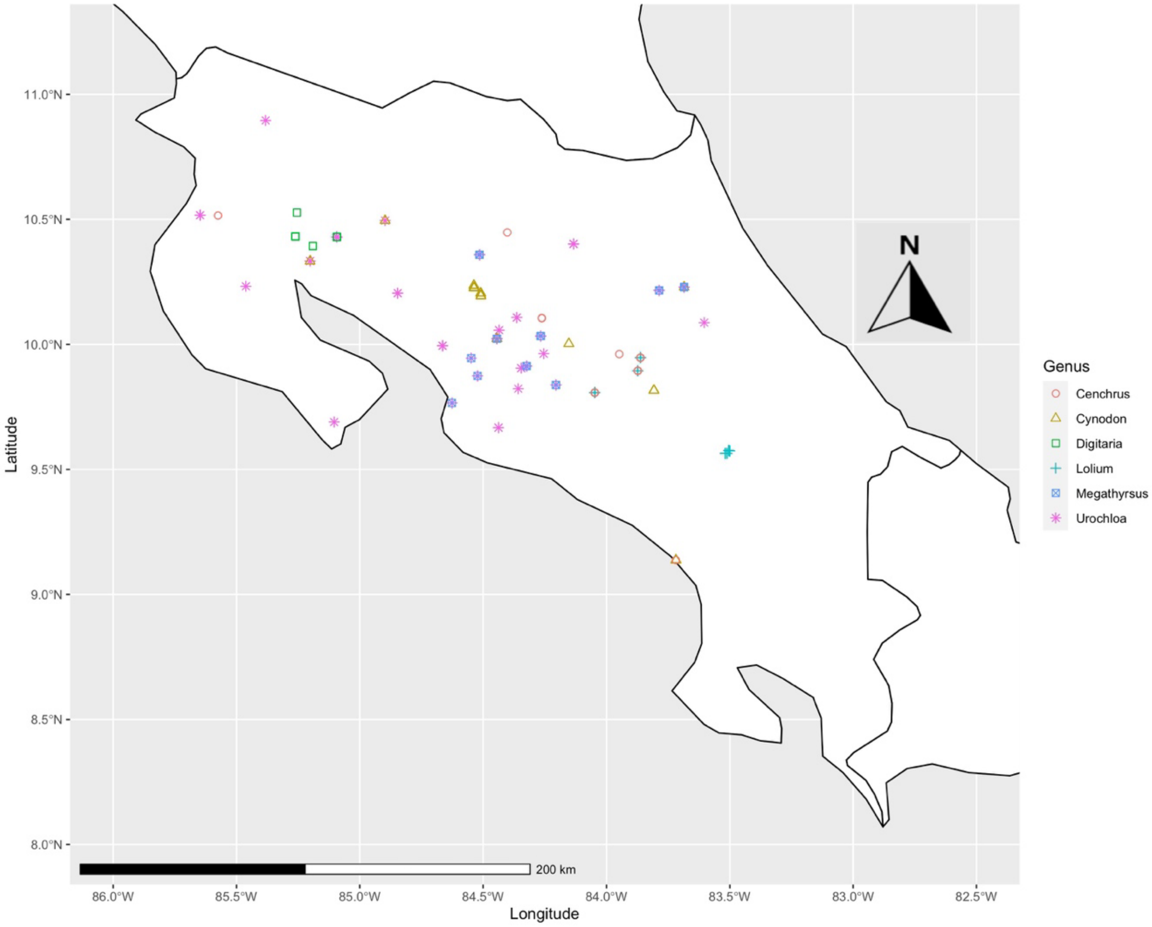

Figure 1. Datapoints of locations sampled in Costa Rica.

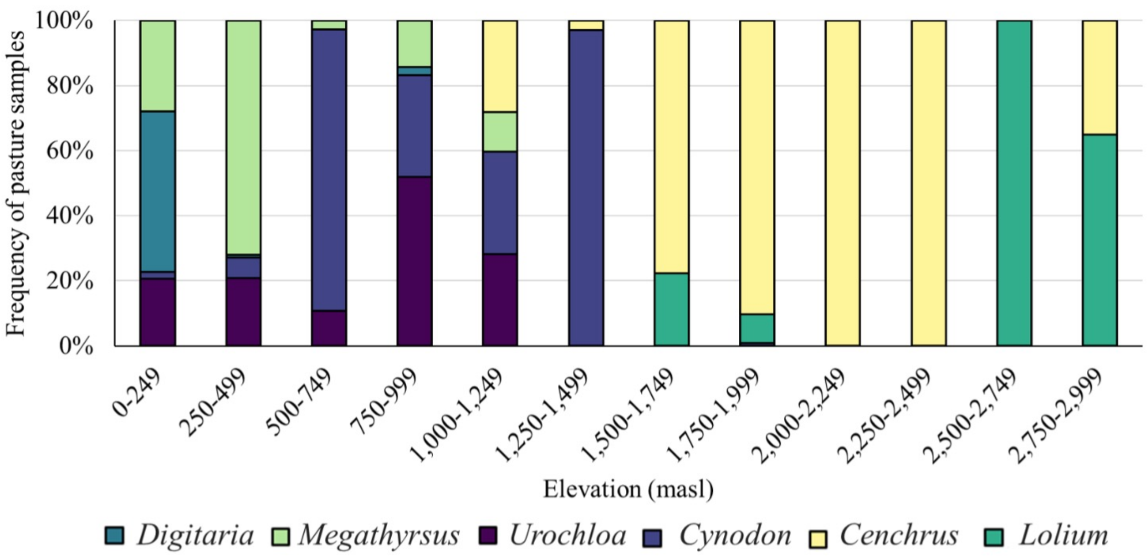

Figure 2. Frequency of pasture species by elevation (meters above sea level; masl).

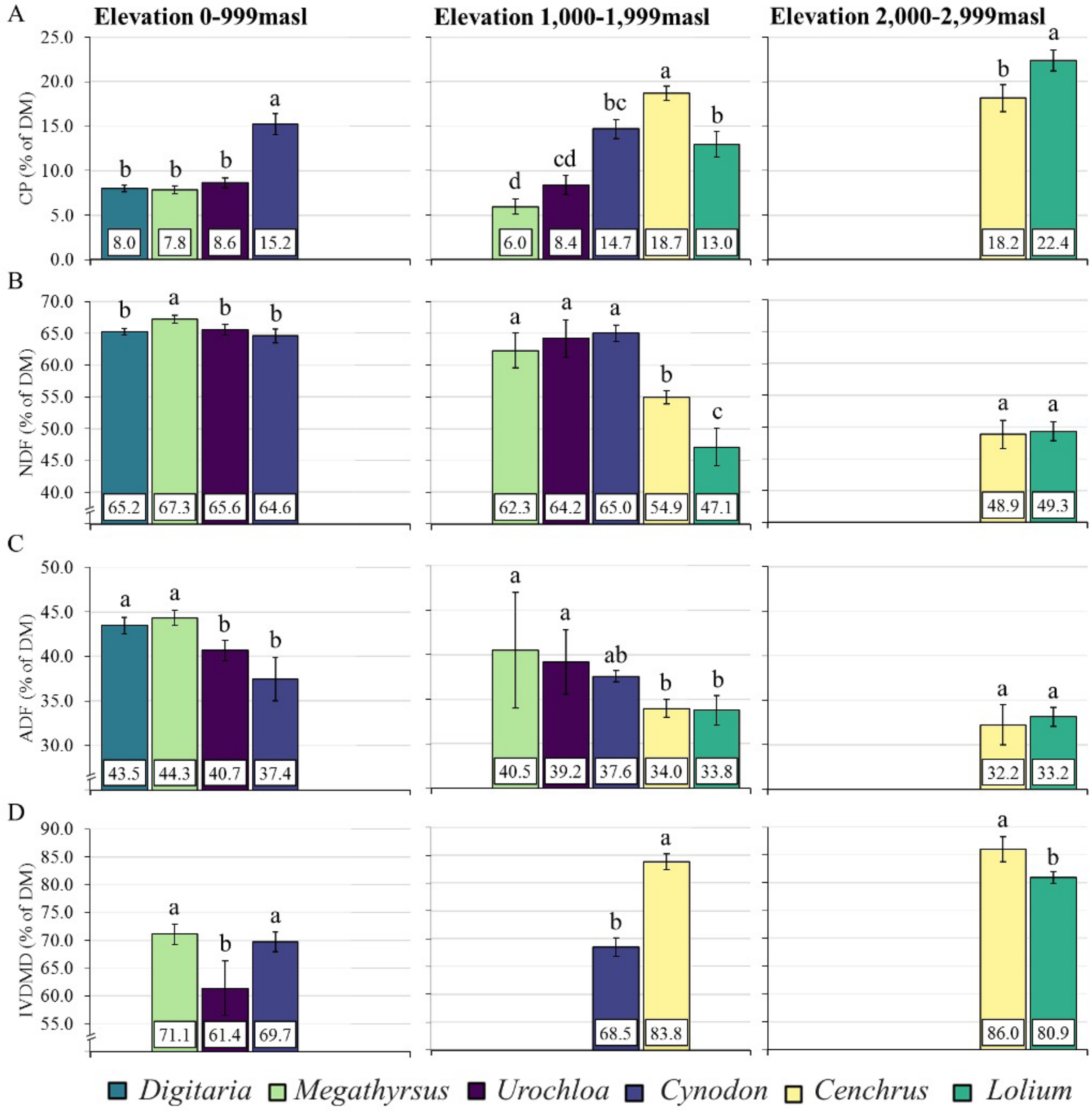

Figure 3. Least square means, 95% confidence interval and number of observations (n) by genus and for three ranges of elevation for A. Crude protein (CP), B. Neutral detergent fiber (NDF), (C) Acid detergent fiber (ADF), and (D) *In-vitro* dry matter digestibility (IVDMD). Different letters denote significant differences among grass genera within each range of elevation ($p \leq .05$).

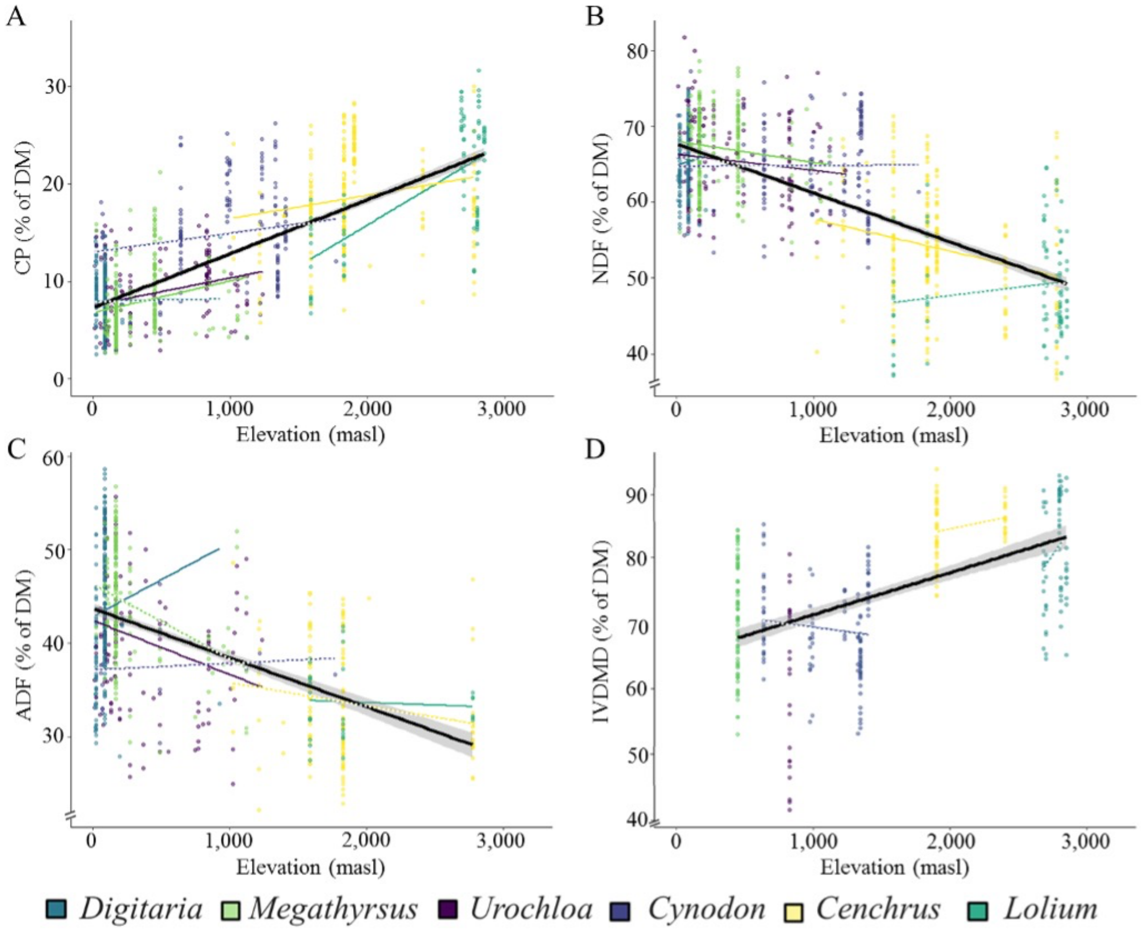

Figure 4. The effect of elevation on (A) Crude protein (CP), (B) Neutral detergent fiber (NDF), (C) Acid detergent fiber (ADF), and (D) *In-vitro* dry matter digestibility (IVDMD) on six pasture genera (*Urochloa*, *Cynodon*, *Digitaria*, *Lolium*, *Megathyrsus*, and *Cenchrus*). An uninterrupted trend line for individual genera indicates a significant effect of elevation ($p < .05$) while an interrupted trend line indicates a non-significant effect of elevation ($p > .05$). The black trend line shows the effect of elevation across genera.

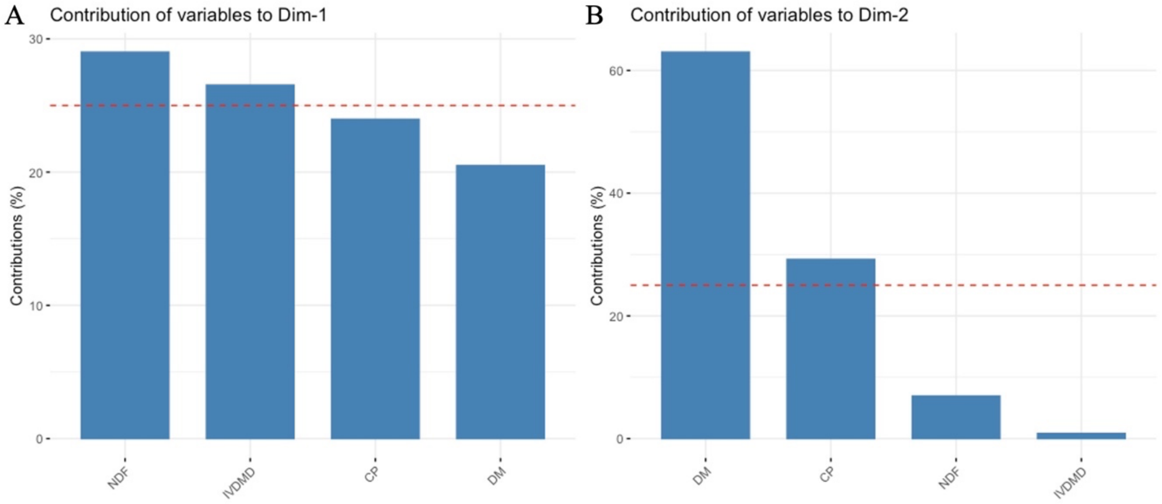

Figure 5. Scree plots indicating the contributions of the nutritive variables within PC1 (A) and PC2 (B).

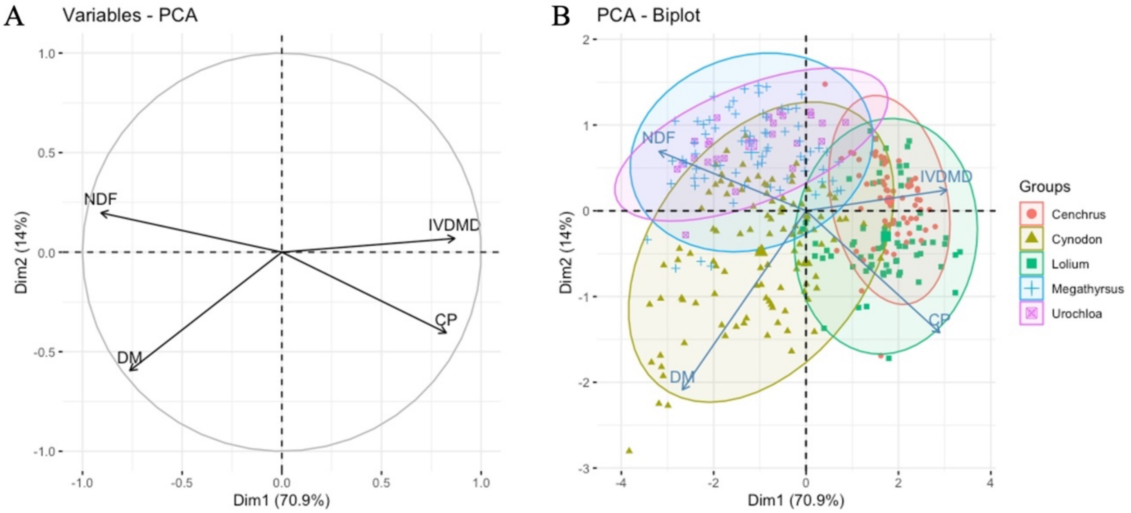

Figure 6. (A) Principal component analysis (PCA) biplots of the nutritive variables Dry matter (DM), Crude protein (CP), Neutral detergent fiber (NDF), and *In-vitro* dry matter digestibility (IVDMD) and (B) overlapped by pasture genus (*Urochloa*, *Cynodon*, *Lolium*, *Megathyrsus*, and *Cenchrus*).

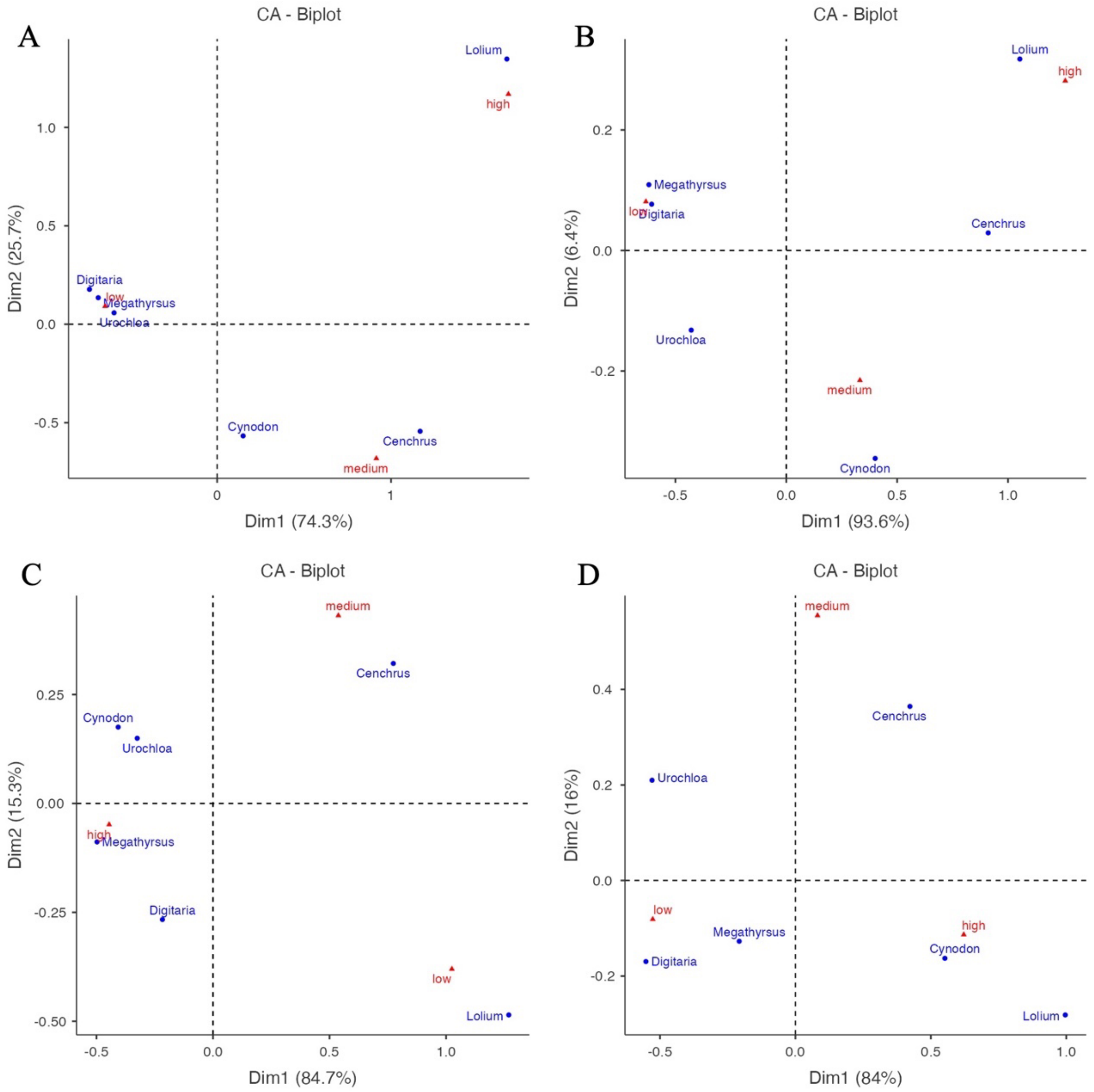

Figure 7. Correspondence analysis biplots for (A) Elevation, (B) Crude protein (CP), (C) Neutral detergent fiber (NDF), and (D) *In-vitro* dry matter digestibility (IVDMD) on six pasture genera (*Urochloa*, *Cynodon*, *Digitaria*, *Lolium*, *Megathyrsus*, and *Cenchrus*).

7. Tables

Table 1. Analysis of variance of elevation, grass genus and their interaction for crude protein, neutral detergent fiber, acid detergent fiber, and *in-vitro* dry matter digestibility.

Nutritive variable	F value	p-value
CP		
Elevation	1585.13	< .001
Genus	22.77	< .001
Elevation x Genus	8.47	<.001
NDF		
Elevation	1068.22	< .001
Genus	45.86	< .001
Elevation x Genus	3.53	<.01
ADF		
Elevation	334.40	< .001
Genus	7.83	< .001
Elevation x Genus	3.49	<.01
IVDMD		
Elevation	188.53	< .001
Genus	32.87	< .001
Elevation x Genus	3.59	<.05

n.s.= non-significant

Table 2. Intercept (β_0) and coefficient (β_1) of elevation with SE for crude protein, neutral detergent fiber, acid detergent fiber, and *in-vitro* dry matter digestibility by genus

Genus	β_0 (SE)	β_1 (SE)[1]	p-value for β_1
Crude protein (% of DM[2])			
All genera	7.3 (0.1)	0.54 (0.01)	<.001
Digitaria	8.0 (0.3)	0.02 (0.26)^{ab}	.94
Megathyrsus	6.8 (0.4)	0.33 (0.10)^{b}	< .01
Urochloa	7.5 (0.4)	0.28 (0.07)^{b}	< .01
Cynodon	13.0 (1.1)	0.19 (0.10)^{b}	.06
Cenchrus	14.0 (1.6)	0.24 (0.08)^{b}	< .01
Lolium	-1.1 (2.6)	0.85 (0.10)^{a}	< .01
Neutral detergent fiber (% of DM)			
All genera	67.7 (0.3)	-0.64 (0.02)	<.001
Digitaria	64.8 (0.6)	0.59 (0.74)^{ab}	.43
Megathyrsus	68.0 (0.5)	-0.28 (0.13)^{ab}	.04
Urochloa	66.3 (0.6)	-0.21 (0.12)^{ab}	.07
Cynodon	64.7 (1.2)	0.01 (0.11)^{a}	.93
Cenchrus	62.0 (2.3)	-0.42 (0.12)^{b}	<.01
Lolium	43.4 (3.7)	0.21 (0.15)^{a}	.14
Acid detergent fiber (% of DM)			
All genera	43.7 (0.3)	-0.52 (0.03)	<.001
Digitaria	42.9 (0.7)	0.79 (0.68)^{a}	.25
Megathyrsus	46.3 (0.6)	-0.79 (0.16)^{a}	<.01
Urochloa	42.4 (0.7)	-0.58 (0.16)^{a}	<.01
Cynodon	37.1 (1.3)	0.07 (0.18)^{a}	.69
Cenchrus	38.2 (2.0)	-0.25 (0.11)^{a}	.02
Lolium	34.7 (3.1)	-0.05 (0.16)^{a}	.74
In-vitro **dry matter digestibility (% of DM)**			
All genera	0.064 (0.1)	0.64 (0.54)	<.001
Digitaria	---	---	---
Megathyrsus	---	---	---
Urochloa	---	---	---
Cynodon	72.2 (2.3)	-0.29 (0.20)^{a}	.15
Cenchrus	75.5 (4.7)	0.44 (0.23)^{a}	.06
Lolium	-3.7 (42.8)	3.05 (1.54)^{a}	.05

$^{a–b}$ Elevation coefficients (β_1) for a given output variable not sharing common superscripts differ significantly ($p < .05$).

[1] Units expressed as %/100 m increase in elevation.

[2] DM = dry matter.

Table 3. Comparison of the association of elevation and crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and *in-vitro* dry matter digestibility (IVDMD) % of dry matter over 100 m (%/100 m) of different genera in studies.

Reference	Genus	Elevation range (masl)[1]	CP	NDF	ADF	IVDMD
				(%/100 m)		
This research	*Digitaria*	15-927	+0.02	+0.59	+0.79	---
This research	*Megathyrsus*	23-1,121	**+0.33***	**-0.28***	**-0.79***	---
This research	*Urochloa*	11-1,234	**+0.28***	-0.21	**-0.58***	---
Wassie et al. (2018)[2]	*Urochloa*	1,230-1,770	-0.23	-0.39	-0.15	---
Wassie et al. (2018)	*Urochloa*	1,770-2,650	**-0.20***	+0.28	+0.14	---
This research	*Cynodon*	13-1,768	+0.19	+0.01	+0.07	-0.29
This research	*Cenchrus*	1,023-2,773	**+0.24***	**-0.42***	**-0.25***	+0.44
Escobar et al. (2020)	*Cenchrus*	2,550-2,914	**-0.22***	-0.14	-0.16	+0.08
Asmare et al. (2017)	*Cenchrus*	1,730-2,650	**-0.22***	**-0.27***	**-0.26***	---
This research	*Lolium*	1,583-2,850	**+0.85***	+0.21	-0.05	+3.05

Slopes in bold and with * were significantly different from zero ($p < .05$).

[1] masl = meters above sea level.

[2] Average of three ecotypes.

Table 4. Standard deviation, proportion of variance and cumulative proportion of variance of four components analyzed for nutritive variables of six genera of grasses.

	PC1	PC2	PC3	PC4
Standard deviation	1.6843	0.7495	0.6287	0.4539
Proportion of Variance	0.7092	0.1404	0.0988	0.0515
Cumulative Proportion	0.7092	0.8496	0.9484	1.0000
Eigen values	2.837	0.562	0.395	0.206

Table 5. Factor loading of the nutritive variables analyzed within each principal component.

	Factor loading of each variable			
Variable	PC1	PC2	PC3	PC4
DM	0.4526	**0.7937**	0.4034	0.0481
CP	-0.4895	**0.5405**	-0.5608	0.3919
NDF	**0.5386**	-0.2635	-0.1789	0.7800
IVDMD	**-0.5151**	-0.0917	0.7004	0.4854

8. Conflict of Interest

The authors declare no conflict of interest. The funders (Research Provost Office at the University of Costa Rica and the Global Challenge Research Fund) had no role in the design of the study nor the collection of data, analyses performed, interpretation of the results, writing of the manuscript, or in the decision to publish the results.

9. Author Contributions

LV: Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing
CA: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing
RVDH: Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing
AM: Conceptualization, Data curation, Formal analysis, Investigation, Software, Validation
DC: Conceptualization, Funding acquisition, Project administration, Resources, Supervision

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13. Supplementary Material

14. Data Availability Statement

The datasets analyzed for this study can be shared upon request to the corresponding author.