



RESEARCH ARTICLE

## Mineral elements in grasses growing in contrasting environmental conditions in southern Patagonia

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### ABSTRACT

The importance of grasses and graminoids for sheep nutrition in Argentinian Patagonia is widely recognized. Focusing on sheep nutrition, we assessed the concentration of mineral elements in grasses growing in three ecological areas of southern Patagonia, representing a vegetation and climate gradient. With the aim of establishing potential relationships, tissue concentrations of several essential and non-essential elements for plants were determined; soil properties were also analysed. Soil and plant tissue mineral element concentrations varied between ecological areas. The results obtained provide new information about the nutritional characteristics of the main feeding source for sheep in southern Patagonia, but further trials will be required to improve understanding of mineral element nutrition in relation to sheep production.

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## Introduction

Domestic livestock grazing is a widespread global activity (Gillson & Hoffman 2007; Järvenranta et al. 2014) that covers more than 60 million km<sup>2</sup> of the Earth's surface (Reid et al. 2008) and plays an important role in providing food for people. For successful livestock production, animals should have access to herbage of acceptable nutritional value throughout the grazing season (Bailey & Ulyatt 1970). In Argentina, there are about 52 million cattle with more than 65% concentrated in the Pampa region, while sheep stock is estimated to be around 15 million, 60% of which are produced in the Patagonian region (SENASA 2015). In southern Santa Cruz province (51°00'–52°20'S), sheep production occupies approximately 3.5 million ha (Sturzenbaum 2012) extending from the mountains to the sea in a west–east gradient with distinct environmental conditions. In the east (near the Atlantic Ocean coast) rainfall does not exceed 150 mm yr<sup>-1</sup>, while in the Andes mountains (west) precipitation may be higher than 1000 mm yr<sup>-1</sup> (Hijmans et al. 2005). Livestock activities in the western section are carried out in a relatively narrow strip of *Nothofagus antarctica* (ñire) forests under silvopastoral use (Peri et al. 2015), with sheep and cattle grazing mostly in the steppe which is dominated by

grasses and shrubs (Oliva et al. 2001). Although livestock production in Patagonia dates from the end of the 19th century (around 1880), no significant management improvements have been made for decades (Quargnolo et al. 2007; Ormaechea et al. 2009); continuous grazing with fixed stocking rates in large paddocks (1000–5000 ha) is the most common practice (Ormaechea & Peri 2015).

A crucial aspect for the success of livestock activities, particularly lamb production, is the nutritional status of the animals (Smith & Cornforth 1982). The nutritive value of a pasture grass species with respect to ruminant production is a function of voluntary intake and feed nutrient utilization (Stone 1994). The period prior to mating (i.e. May) is important for optimal sheep nutrition to ensure that pregnancy occurs and to provide for the higher nutritional demand of the cold winter season (i.e. June–September) which is coincident with the lower offer of food in natural conditions (Ormaechea & Peri 2015).

The importance of grasses and graminoids for sheep nutrition in different environments of Patagonia (forest, wetlands and steppe) is widely recognized, since such species represent the highest proportion (approximately 40%–70% of the total) of sheep diet (Bahamonde 2011; Ormaechea & Peri 2015). However, information about the nutritional value of grasses in Patagonia is limited. There are only a few reports about the crude protein and dry matter digestibility percentage of grasses in silvopastoral systems occurring in *N. antarctica* forests (Bahamonde et al. 2012; Peri & Bahamonde 2012), as well as nutrient storage and digestibility of grasses and graminoids in steppe and wetlands (Peri & Lasagno 2010; Andrade et al. 2015; Ormaechea & Peri 2015). Moreover, existing information about mineral (macro- and micro-elements) nutrient concentrations in forage for extensive livestock production in Patagonia and Argentina is extremely scarce (Peri et al. 2015).

Patagonia is characterized by a distinct longitudinal gradient of precipitation and soil types and a latitudinal gradient of temperature. Forests dominate the more humid, western Patagonian region, predominantly on Andisols (i.e. volcanic soils), from which there is a steady transition eastwards from grasslands to scattered grass and shrub steppes on Aridisols. In the transitional zone between the regions, vegetation consists of shrubs and grasses on xeric Mollisols or Alfisols (Mazzarino et al. 1998). There is only limited information about mineral element concentrations in Patagonian soils, which is largely focused on macro-nutrients (Satti et al. 2007; Diehl et al. 2008). Soils of volcanic origin may be limiting for some elements such as phosphorus (P) (Diehl et al. 2008), but others such as aluminium (Al), arsenic (As), rubidium (Rb), cadmium (Cd), cobalt (Co), copper (Cu), manganese (Mn) and zinc (Zn) (Kiliç et al. 2015) may reach toxic levels for plants and be transferred into the food chain (Dudka & Miller 1999).

Although it is difficult to establish precise values and thresholds of mineral nutrients to meet the requirements for livestock production, due to the interactions between animal factors (race, age, physiological and nutritional status) and consumed forage (nutrient concentrations, chemical form of minerals in the food) (Suttle 2010), there is abundant information about the key role of nutrients in livestock production. For instance, different nutrients (e.g. P, calcium [Ca], magnesium [Mg], iodine [I], Mn, Cu or selenium [Se], among others) have been identified as being crucial for the successful reproduction of dairy cattle (Wilde 2006). Micro-elements such as Cu, Zn or molybdenum (Mo) have also been identified as being important for vital processes (e.g. cellular respiration, DNA and RNA replication, sequestration of free radicals, etc.) (Chan et al. 1998). On

the other hand, there are some trace elements that at high levels may be toxic for livestock (e.g. As, Cd, fluorine [F] or lead [Pb]; Suttle 2010).

Therefore, trace mineral elements should be provided to livestock in a range of concentrations that leads to neither deficiency nor excess of nutrients, although sometimes the minimum level required to overcome deficiency symptoms is not enough to improve cattle productive performance (López-Alonso 2012).

Given the limited information available concerning the mineral element status of Patagonian grazing species and their potential effect on sheep production, the aim of this work is to evaluate the concentration of macro-, micro- and trace elements in leaves of grasses growing under contrasting environmental conditions in Southern Patagonia, focusing on sheep nutrition. For this purpose, three different ecological areas of southern Patagonia representing a vegetation and climate gradient were selected and the following questions addressed: (1) are soil analysis results different between zones and can such results be related to plant tissue mineral element concentrations?; (2) in light of mineral element determinations performed in the different experimental sites, are the quality of pastures sufficient to ensure sheep nutrition and which elements may be limiting or toxic?

## Materials and methods

### Experimental locations

This study was conducted in three different ecological areas under extensive sheep grazing representing a vegetation and climate gradient in Santa Cruz province, southern Patagonia, Argentina.

The first location was an *N. antarctica* forest, a silvopastoral system (SP) located in the ecological area called 'Complejo andino' (46°0'14.4"–50°53'49.2"S, 71°8'46.3"–72°51'14.4"W), with understorey vegetation dominated by grasses and graminoids of the genera *Agrostis* spp., *Bromus* spp., *Carex* spp., *Dactylis* spp., *Deschampsia* spp. and *Festuca* spp. (Bahamonde et al. 2012). In this area, the mean annual precipitation (MAP) is 390 mm yr<sup>-1</sup> with a mean annual temperature (MAT) of 4.9 °C. According to the USDA classification, SP soils are mollisols-haploxerolls of silty loam texture. The pH is slightly acid.

The second location was a thicket zone in the ecological area 'Matorral de Mata negra' (MT) (49°38'22.6"–51°33'25.2"S, 68°29'0.6"–72°23'26.9"W). In this area, the shrub *Mulguraea tridens* covers a large proportion of the surface (35%), which is mostly associated with grasses such as *Jarava chrysophylla*, *Pappostipa ibarii*, *Bromus setifolius* and *Festuca pyrogea* (Peri et al. 2015). The MAP is 155 mm yr<sup>-1</sup> with a MAT of 6.5 °C. The predominant soils are mollisols (USDA soil classification) with silty loam texture.

The third location was a grass steppe (GS) located in the 'Estepa magallánica seca' area (51°2'36.6"–52°7'18.1"S, 68°42'21.2"–71°38'21.8"W) where the dominant grass species is *J. chrysophylla*, which is associated with *Poa spiciformis*, *Carex andina* and *Rytidosperma virescens*. This zone has a MAP of 235 mm yr<sup>-1</sup> and 7.1 °C MAT. Similar to the MT area, soils are mollisols (USDA soil classification) with silty loam texture.

### Sampling and measurements

At each ecological area (never subjected to agricultural activities or fertilization) 10 composite samples (from five subsamples each, using a rectangular frame of 1 × 0.2 m) of

grasses and graminoids (selecting fully expanded leaves) were taken during the period between 20 February and 10 March 2015. Sampling dates were selected because they were prior to mating, a period considered key for the proper nutrition of animals as preparation for reproduction. It has been shown that nutrition during the weeks before mating has an important effect on ovulation and lambing rates (González et al. 1997).

The specific collection of grasses and graminoids in sample areas was based on preliminary trials in which we observed that the majority of such species as described above are consumed by sheep. Andrade et al. (2015) evaluated sheep diet in the MT and GS areas and found that more than 90% of the grass and graminoid species were eaten by sheep. Similarly, Ormaechea & Peri (2015) reported that grass and graminoid species were the major sheep diet components in the SP zone. Plant tissue samples were collected along transects with the following coordinates: (1) SP area: from 51°13'21"S–72°15'34"W to 51°57'22.7"S–71°31'40.7"W; (2) GS area: from 51°17'49.8"S–69°14'56.5"W to 51°56'59.0"S–70°25'10.22"W; and (3) MT area: from 50°57'36.4"S–70°27'56.2"W to 51°05'38.1"S–70°40'53.4"W. Plant tissues were taken to the lab in closed plastic bags and kept at 3 °C until processing. Thereafter, samples were kept at 3 °C in the refrigerator, before discarding the senescent material. Tissue to be analysed was carefully washed with 0.1% detergent (Cif) to remove surface contaminants. Plant tissues were rinsed with abundant tap and then distilled water. They were subsequently oven dried at 70 °C for 2 days, weighed and ground prior to mineral element determination after dry washing. Nitrogen was measured with an elemental analyser (TruSpec). The remaining elements were determined by inductively coupled plasma (ICP) analysis (Optima 3000, PerkinElmer) following the UNE-EN ISO/IEC 17025 standards for calibration and testing laboratories (CEBAS-CSIC Analysis Service).

Additionally, three composite samples (from five subsamples) of the first 20 cm of soils were collected from each ecological area, in places where plant tissues were also sampled. Soil pH was determined for saturated soil pastes (30 mL deionized water per 100 g soil). Organic matter (OM) content was measured according to the loss of ignition method. Samples were dried to eliminate water, subsequently heated for 2 h at 600 °C and the weight loss recorded (Nadal et al. 2004). Soil nutrient concentrations were determined as follows: total N was measured by spectrophotometry; available P by the Olsen method; ammonium acetate-extracted Mg, Ca, potassium (K) and sodium (Na) concentrations were determined by atomic absorption spectroscopy (AAS); while the concentrations of DTPA-extractable iron (Fe) and Mn were also determined by AAS (AQM Laboratories). Values of nutrient requirements and potentially toxic levels for sheep (macro- and micro-elements) were obtained from the literature (e.g. Freer et al. 2007; Suttle 2010) and were used as reference threshold. For this purpose we used the term 'requirements' to refer to the nutrient concentrations in forage that sheep need for their normal development according to their age.

### **Data analysis**

Exploratory testings were carried out to verify the compliance with the assumptions of normality, homoscedasticity and independence of data for each evaluated condition. While the Shapiro-Wilk test was performed to verify the normality of the data, Levene's test was used to verify homoscedasticity. Data independence was verified by analysing

residuals from graphs. Mineral element concentrations were analysed by analysis of variance (ANOVA) with ecological area as a factor. Tukey's tests were performed to test differences among ecological areas when *F* values were significant ( $P < 0.05$ ). Pearson correlation coefficient analyses were evaluated between plant tissue and soil mineral element concentrations to check for potential relationships between them.

## Results

The nitrogen (N), P and sulphur (S) concentrations measured followed a similar trend, with values being significantly higher in the SP area compared with the thicket zone of MT and the GS (Table 1). According to the literature, P (except in the SP area), N and S concentrations were below the minimum values to satisfy sheep requirements in all areas. Calcium concentration in the MT zone was lower than in the other two areas, but all values were above the sheep minimum requirement threshold (Table 1). Potassium and Mg concentrations were observed to follow a gradient from higher values in SP, to intermediate in GS and lower in MT. For these elements, the level required depended on the reference used for comparison (Table 1).

In Table 2 the concentration of micro-elements, which followed different trends depending on ecological area, are shown. For instance, Zn concentrations were lower in MT, whereas Fe concentrations were the highest in the same ecological area. With regard to the nutritional requirements, Zn concentration in the MT area and Cu in the GS area are within the thresholds recommended in the literature (Table 2). On the other hand, the Mn concentration in the SP area was recorded to be above the maximum value permitted in FEDNA (Spanish Foundation for the Development of Animal Nutrition) (2006).

The concentration of Na and trace elements that could be toxic for livestock when present in excess in their diet is shown in Table 3. The levels of Na in all areas are within the recommended thresholds. On the other hand, the concentrations of all the trace elements evaluated are well below the thresholds reported as dangerous in the literature. However, some differences between ecological areas were observed depending on the elements. For instance, Al concentrations are considerably higher in the MT area compared with the two other

**Table 1.** Macro-nutrient concentrations (% DW) in grasses from three different ecological areas in southern Patagonia and range of reference values based on the literature. Data are means  $\pm$  SD.

| Ecological area                         | Macro-nutrients (% DW) |                              |                  |                  |                  |                  |
|---|------------------------|------------------------------|------------------|------------------|------------------|------------------|
|   | N                      | P                            | Ca               | K                | Mg               | S                |
| SP                                      | 1.5 $\pm$ 0.4a         | 0.17 $\pm$ 0.06 <sup>a</sup> | 0.19 $\pm$ 0.06a | 1.38 $\pm$ 0.33a | 0.10 $\pm$ 0.02a | 0.08 $\pm$ 0.03a |
| MT                                      | 1.0 $\pm$ 0.1b         | 0.05 $\pm$ 0.01b             | 0.14 $\pm$ 0.02b | 0.23 $\pm$ 0.04c | 0.04 $\pm$ 0.01c | 0.06 $\pm$ 0.01b |
| GS                                      | 0.9 $\pm$ 0.2b         | 0.05 $\pm$ 0.01b             | 0.19 $\pm$ 0.04a | 0.44 $\pm$ 0.12b | 0.08 $\pm$ 0.02b | 0.06 $\pm$ 0.01b |
| Range of reference values <sup>1†</sup> | 1.7–3.2                | 0.09–0.3                     | 0.14–0.7         | 0.5              | 0.09–0.12        | 0.2              |
| Range of reference Values <sup>2‡</sup> | nd                     | 0.11–0.41                    | 0.14–0.7         | 0.3              | 0.04–0.14        | 0.09–0.21        |

nd, no reference data available.

Different letters in the same columns indicate different levels of significance between ecological areas ( $P < 0.05$ ).

<sup>1</sup>Adapted from Freer et al. (2007). The range represents the concentration in the forage to meet the requirements for sheep of different categories and physiological states, from lambs of 20 kg live weight (LW) to sheep of 50 kg LW pregnant or lactating, assuming that the quantity of forage available is not limiting.

<sup>2</sup>Adapted from Suttle (2010). The range represents the concentration in the forage to meet the requirements for sheep of different categories and weights, from growing lambs of 20 kg LW to pregnant ewes carrying twins of 75 kg LW, assuming that the quantity of forage available is not limiting.

<sup>†</sup>The units of the reference values are the same as those expressed for the plant concentrations.

**Table 2.** Micro-nutrient and Na concentrations (mg kg<sup>-1</sup> DW) in grasses from three different ecological areas in southern Patagonia and range of reference values for adequate sheep nutrition. Data are means  $\pm$  SD.

| Ecological area  | Element concentrations (mg kg <sup>-1</sup> DW) |                |                   |                   |                     |
|--|---|----------------|-------------------|-------------------|---------------------|
|  | Zn  | Cu             | Fe                | Mn                | Na                  |
| SP   | 13.4 $\pm$ 4.9a                                 | 8.2 $\pm$ 2.5a | 39.8 $\pm$ 5.6b   | 218.6 $\pm$ 23.3a | 68.6 $\pm$ 64.9b    |
| MT   | 6.6 $\pm$ 1.6b                                  | 8.5 $\pm$ 4.2a | 104.0 $\pm$ 29.1a | 26.5 $\pm$ 6.7b   | 80.0 $\pm$ 64.6b    |
| GS   | 11.6 $\pm$ 3.7a                                 | 3.5 $\pm$ 1.2b | 33.7 $\pm$ 8.5b   | 23.5 $\pm$ 7.0b   | 134.0 $\pm$ 44.0a   |
| Range of reference values <sup>1#</sup>                            | 9–20  | 4–14           | 40                | 20–25             | nd                  |
| Range of reference Values <sup>2#</sup>                            | 8.8–27  | 4.3–28.4       | 30–50             | 8–20              | 600–1250            |
| Maximum authorized value in Europe according to FEDNA <sup>†</sup> | 150   | 25             | 750               | 150               | 45,000 <sup>‡</sup> |

nd, no reference data available.

Different letters in the same columns indicate different levels of significance between ecological areas ( $P < 0.05$ ).

<sup>1</sup>Adapted from Freer et al. (2007). The range represents the concentration in the forage to meet the requirements for sheep of different ages and physiological states, from lambs of 20 kg LW live weight (LW) to sheep of 50 kg LW pregnant or lactating, assuming that the quantity of forage available is not limiting.

<sup>2</sup>Adapted from Suttle (2010). The range represents the concentration in the forage to meet the requirements for sheep of different categories and weights, from growing lambs of 20 kg LW to pregnant ewes carrying twins of 75 kg LW, assuming that the quantity of forage available is not limiting.

<sup>#</sup>The units of the reference values are the same as those expressed for the plant concentrations.

<sup>†</sup>The values are the maximum amounts of trace elements authorized in feed (or its correctors) for various domestic species, according to European Union.

<sup>‡</sup>Value obtained from NRC (2000).

experimental zones analysed. Lead, boron (B), chromium (Cr), nickel (Ni) and titanium (Ti) also were higher in the MT area, and further differences among other areas were also observed. In all experimental areas, plant tissue As, Cd and vanadium (V) concentrations were low and under the ICP detection limit. With regard to soil mineral element concentrations and remaining properties, only Na and pH were not significantly different between ecological areas, while the remaining variables changed qualitatively and quantitatively depending on the element or ecological zone (Table 4). For example, OM, P and Fe were higher in the SP area, whereas K was higher in the GS zone. Significant correlations between soil and grasses mineral element concentrations were found for N, P, Ca and Fe; the first three were positive and the last one negative (Table 5).

## Discussion

The metabolic and physiological importance of essential nutrients for plant growth is widely recognized. Excess or toxic mineral elements may be detrimental for plant growth (Marschner 2012) and toxic for animal feeding (e.g. Smith & Cornforth 1982; McDowell 1996). Plant tissue nutrient concentrations may be related to factors such as water availability (da Silva et al. 2011), soil characteristics or interactions between mineral elements (Marschner 2012), and this will ultimately influence the nutritional value of pasture for sheep feeding (García-Ciudad et al. 1997; Järvenranta et al. 2014). Plant tissue samples were collected during the period prior to mating, since nutrition during this time has been reported to be critical for ovulation and lambing rates (González et al. 1997). Later during the season (i.e. May and June) the major limiting factor under Southern Patagonian conditions is forage quantity rather than nutritional quality (Hall & Paruelo 2006; Peri 2009). The mineral element concentrations determined are discussed regarding the potential influence of the prevailing water availability regime in the different

**Table 3.** Additional micro-nutrients (B and Ni) and trace element concentrations ( $\text{mg kg}^{-1}$  DW) in grasses from three different ecological areas in southern Patagonia and range of reference values for ruminant toxicity. Data are means  $\pm$  SD.

| Ecological area                         | Micro-nutrient and trace element concentrations ( $\text{mg kg}^{-1}$ DW) |                |                       |      |      |                  |                   |                  |                   |                |         |
|---|---|----------------|-----------------------|------|------|------------------|-------------------|------------------|-------------------|----------------|---------|
|   | B   | Ni             | Al                    | As   | Cd   | Pb               | Cr                | Rb               | Sr                | Ti             | V       |
| SP                                      | 4.0 $\pm$ 1.0c  | 2.1 $\pm$ 0.3b | 17.5 $\pm$ 4.3b       | <0.1 | <0.1 | 1.03 $\pm$ 0.47a | 4.3 $\pm$ 0.8b    | 6.38 $\pm$ 4.45a | 10.1 $\pm$ 1.1b   | 1.0 $\pm$ 0.3c | <0.5    |
| MT                                      | 8.7 $\pm$ 1.1a  | 3.2 $\pm$ 1.1a | 108.0 $\pm$ 34.8a     | <0.1 | <0.1 | 1.24 $\pm$ 0.42a | 6.0 $\pm$ 2.3a    | 0.39 $\pm$ 0.10b | 7.5 $\pm$ 1.3c    | 6.8 $\pm$ 2.3a | <0.5    |
| GS                                      | 7.1 $\pm$ 1.1b  | 1.3 $\pm$ 0.3c | 34.5 $\pm$ 12.0b      | <0.1 | <0.1 | 0.40 $\pm$ 0.18b | 0.7 $\pm$ 0.5c    | 0.22 $\pm$ 0.08b | 15.2 $\pm$ 3.9a   | 2.7 $\pm$ 1.0b | <0.5    |
| Range of reference values <sup>1†</sup> | 800   | 100            | 500–8000 <sup>†</sup> | 2–30 | 1–10 | 5–100            | 1000 <sup>‡</sup> | 250–1000         | 2000 <sup>‡</sup> | nd             | 200–400 |

nd, no reference data available.

Different letters in the same columns indicate different levels of significance between ecological areas ( $P < 0.05$ ).

<sup>1</sup>Adapted from Suttle (2010). The minimum and maximum value of the interval represents the maximum permitted and maximum tolerable, respectively, according to the reference. Value intervals are given for ruminants in general (excepting\*).

\*The units of the reference values are the same as those expressed for the plant concentrations.

<sup>†</sup>According to Jones (2005).

<sup>‡</sup>According to NRC (2000). Reference values specific for cattle tolerance.

**Table 4.** Organic matter, pH and mineral element concentrations in soils from three different ecological areas in southern Patagonia. Data are means  $\pm$  SD.

| Ecological area | OM (%)           | N (%)             | P ( $\text{mg kg}^{-1}$ ) | K (%)            | Ca (%)            | Mg (%)           | Mn ( $\text{mg kg}^{-1}$ ) | Na ( $\text{mg kg}^{-1}$ ) | Fe ( $\text{mg kg}^{-1}$ ) | pH             |
|-----------------|------------------|-------------------|---------------------------|------------------|-------------------|------------------|----------------------------|----------------------------|----------------------------|----------------|
| SP              | 20.7 $\pm$ 12.5a | 0.73 $\pm$ 0.31a  | 41.2 $\pm$ 4.4a           | 0.46 $\pm$ 0.07b | 0.35 $\pm$ 0.22ab | 0.95 $\pm$ 0.54a | 13.5 $\pm$ 6.5a            | 538 $\pm$ 39a              | 185 $\pm$ 62a              | 6.2 $\pm$ 0.3a |
| MT              | 4.4 $\pm$ 1.4b   | 0.53 $\pm$ 0.50ab | 23.1 $\pm$ 6.8b           | 0.48 $\pm$ 0.22b | 0.10 $\pm$ 0.04b  | 0.46 $\pm$ 0.18a | 6.5 $\pm$ 0.3b             | 634 $\pm$ 166a             | 33 $\pm$ 6c                | 6.3 $\pm$ 0.6a |
| GS              | 7.9 $\pm$ 2.5b   | 0.28 $\pm$ 0.02b  | 26.2 $\pm$ 5.4b           | 0.86 $\pm$ 0.51a | 1.00 $\pm$ 0.57a  | 0.08 $\pm$ 0.01b | 16.0 $\pm$ 8a              | 545 $\pm$ 16a              | 97 $\pm$ 31b               | 5.7 $\pm$ 0.3a |

Different letters in the same columns indicate different levels of significance between ecological areas ( $P < 0.05$ ).



**Table 5.** Coefficient of correlation of Pearson (R) between mineral element concentration in soils (0–20 cm) and plant tissues at three different ecological areas in southern Patagonia.

|                                | N (%)    | P (%)    | Ca (%)   | K (%) | Mg (%) | Fe (mg kg <sup>-1</sup> ) | Mn (mg kg <sup>-1</sup> ) | Na (mg kg <sup>-1</sup> ) |
|--------------------------------|----------|----------|----------|-------|--------|---------------------------|---------------------------|---------------------------|
| Coefficient of correlation (R) | 0.67 (+) | 0.86 (+) | 0.40 (+) | ns    | ns     | 0.68 (–)                  | ns                        | ns                        |

Symbols in parentheses indicate if the relationship is positive or negative.  
ns, not significant.

ecological areas under investigation (namely, a more humid silvopastoral *N. antarctica* forest versus a drier grass steppe and especially a thicket), nutrient dynamics, soil properties and nutritional value for cattle feeding.

### Macro-nutrients

Plants growing in the *Nothofagus* forest (SP) appeared to have the highest N, P and K concentrations (Table 1). Such forest soil has significantly higher organic matter, N and P concentrations compared with steppe (GS) and thicket (MT) soils (Table 4). Similar extractable P values to those obtained for SP areas have been reported for Andean-Patagonian forests which have a volcanic origin (Borie & Rubio 2003; Diehl et al. 2008). In general, all soil macro-nutrient concentrations are within the range described for forest and steppe Southern Patagonia soils (e.g. Diehl et al. 2003, 2008; Satti et al. 2007; Peri & Lasagno 2010). The higher soil N concentrations recorded for SP soils may be due to the higher rates of leaf litter decomposition in the more humid SP *N. antarctica* forest compared with steppe zones (Peri et al. 2015). However, the lower precipitation rates in GS and MT may have a strong influence chiefly on soil K but also on Mg and P supply, since in addition to other factors such as soil type, drought is known to limit nutrient availability (da Silva et al. 2011; Marschner 2012). Additionally, the N concentrations determined in this study are similar to those reported by Peri & Lasagno (2010) and Bahamonde et al. (2012) for grasses and graminoids in the steppe and the *N. antarctica* forest in southern Patagonia, respectively. Concerning P concentrations, Peri & Lasagno (2010) determined higher values for grasses sampled in summer in the steppe of southern Patagonia. These values were similar to the ones recorded for the SP ecological area. The P concentrations obtained are also within the range reported by Bertiller et al. (2006) for perennial grasses growing in a broader range of environmental conditions across Patagonia, with an average of 0.15% P. Compared with our results, Peri & Lasagno (2010) recorded higher concentrations of Ca, lower of K, and similar values of Mg and S. This may be due to interannual variations and/or different environmental conditions and should be assessed in future investigations. Differences between ecological areas at least concerning N, P and Ca concentrations could be explained in part due to the existing soil concentrations of these elements, because in some instances we found positive correlations between soil and plant mineral element concentrations. In the case of N, the higher tissue N levels measured in the SP area may be related to the higher precipitation regime (Peri et al. 2015). It is known that higher water availability improves plant root N absorption (Jungk 2002), a fact that has been corroborated in Patagonia by Austin & Sala (2002) who reported higher N concentrations in dominant plants that experienced increased mean annual precipitation. Contrary to our results, Bertiller et al. (2005) found that N concentrations in the



green leaves of perennial grasses growing in Patagonia increased with aridity, while P concentrations did not vary between sites with different precipitation regimes.

Concerning the mineral element requirements for sheep, the apparent deficiency in certain instances as deduced from the plant tissue values recorded may have different implications depending on the element and ecological zone. Nitrogen deficit in diet could reduce the growth rate especially in young animals (Freer et al. 2007). Nevertheless, additional nutritional components of sheep's diet in southern Patagonia, such as herbs and legumes (Ormaechea & Peri 2015), may also be an important source of N and Ca (Suttle 2010). Similarly, temporal variations in grass tissue N concentrations have been reported in southern Patagonia, where values were found to decrease significantly from September to April (Bahamonde et al. 2012). Moreover, it is well recognized that ruminants can recycle N (transfer endogenous N to the rumen) in periods of N scarcity in the feed (Freer et al. 2007), which could compensate for the N shortage when plant tissue N concentrations may be low during the growing season. Similar to N, various reports showed that P, K, Mg and S concentrations in grasses and graminoids in southern Patagonia steppe during the springtime are higher than in summer, reaching the recommended values to satisfy sheep requirements (Peri & Lasagno 2010). Although Ca concentrations are within the recommended range, the importance of vitamin D as a prerequisite for the proper absorption and metabolism of Ca in animals must be noted. Vitamin D levels are additionally related to protection of the skin from solar radiation (Suttle 2010) which is serious limiting factor at high latitudes such as southern Patagonia.

### **Micro-nutrients**

Concerning the concentration of micro-nutrients, no significant correlations between soil and grass tissue element concentrations were generally found with the exception of Fe, where a negative significant correlation was determined. In general, the Zn concentration in grasses has been reported to be lower than that of legumes or herbs (Kabata-Pendias 2011). Nevertheless, the values obtained in this study are lower than those reported for fresh and dry grass tissues in other studies (36 and 33 mg kg<sup>-1</sup>, respectively; e.g. Givens & Moss 1990; Suttle 2010) and are actually within the range of concentrations found in straw. Zinc concentrations are lower in the MT area and may be insufficient to meet sheep requirements according to the thresholds reported, which could, among other detrimental effects, decrease male fertility (Martin & White 1992) and appetite (O'Dell & Reeves 1989). The Cu concentrations in the SP and MT areas are within the range reported for other grasses and legumes in a broad range of conditions (e.g. García-Ciudad et al. 1997; Kabata-Pendias 2011), but concentrations in the GS area were lower and insufficient to meet sheep nutritional requirements. Copper plays a fundamental role in the activity of numerous enzymes, cofactors and reactive proteins for plants and animals (Suttle 2010; Marschner 2012), therefore its low availability for animal feeding could be extremely harmful. Nevertheless, preliminary information indicated that the interaction of Cu with other elements (e.g. Mo, S, Zn or Fe) can affect its bioavailability (Howell & Gawthorne 1987). Hence, the Cu concentration of pastures is a poor indicator of real Cu availability for livestock and the recommendations on dietary requirements should be interpreted with caution (Freer et al. 2007).

The Fe concentrations in soils measured in this work are below the range reported for other silty loam soils (Kabata-Pendias 2011). The Fe concentrations determined in grass tissues were within the range of other grasses growing in different countries (e.g. García-Ciudad et al. 1997; Suttle 2010; Kabata-Pendias 2011) and may not be limiting in terms of meeting sheep nutritional requirements. The soil Mn concentrations recorded are low compared with those reported for soils of different countries by Kabata-Pendias (2011). However, irrespective of the ecological areas, the leaf Mn concentrations determined in this investigation are within the range measured for grasses in various studies (e.g. Suttle et al. 2003; Kabata-Pendias 2011), which is sufficient to meet sheep Mn requirements. A relationship between increased leaf Mn concentrations and the so-called 'carboxylate-releasing P-mobilising strategy' in natural environments where the P availability is low has been suggested (Lambers et al. 2015). The significantly higher grass tissue Mn concentrations recorded in forest species may be, for example, related to P acquisition mechanisms (note the significantly higher leaf P concentrations of SP), improved root Fe acquisition strategies or soil-related factors (Marschner 2012), but further trials will be necessary to explore mineral element absorption and homeostasis in plants native to Patagonia.

The values of soil Na were lower in the experimental zones analysed compared with other temperate zones (e.g. Tisdale et al. 1985), but similar to those reported for other world regions (McGrath & Loveland 1992). Sodium is known to be a beneficial element for plants (Pilon-Smits et al. 2009). The Na values obtained in this study are within the range reported by Kähäri & Nissinen (1978) for timothy grass in Finland. Sodium (together with chlorine [Cl]) plays a fundamental role in maintaining osmotic pressure, regulating acid-base equilibrium and controlling water metabolism in animals (Suttle 2010). The low Na values found in this study (which did not meet sheep requirements) could hence compromise sheep metabolism if Na shortage is prolonged over time, but additional Na inputs may be obtained from other sources (e.g. from soils and/or water; Suttle 2010).

Antagonistic relationships between several micro-nutrients such as Fe, Mn and Zn are well documented in the literature (e.g. Marschner 2012). In the case of Mn, the values recorded will meet sheep requirements, but the concentrations measured in the SP area exceed the permitted thresholds in Europe ( $150 \text{ mg kg}^{-1}$ ). However, there is no information available on the criteria used to establish the maximum limit of  $150 \text{ mg kg}^{-1}$  Mn shown by FEDNA (2006) (Table 2). Some studies indicate that high levels of Mn in the diet of ruminants may be detrimental for their productive performance due to an anorexic effect and metabolic antagonism with other elements such as Fe or Cu (Freer et al. 2007; Suttle 2010). Nevertheless, these negative effects have been found at Mn concentrations higher than  $150 \text{ mg kg}^{-1}$ . Grace (1973) observed that sheep grazing in pastures containing  $140\text{--}200 \text{ mg kg}^{-1}$  dry matter (DM) reduced their growth rate when the animals' diet was supplemented with pellets containing 250 or 500 mg Mn. This supplementation led to lower Fe concentrations in sheep plasma and heart, despite pastures containing  $1100\text{--}2200 \text{ mg Fe kg}^{-1}$  DM. In another study, Black et al. (1985) reported that sheep could tolerate  $3000 \text{ mg Mn kg}^{-1}$  dry weight (DW) during 21 days. On the other hand, Hansen et al. (2006) showed that diets containing  $500 \text{ mg Mn kg}^{-1}$  DW can exacerbate Cu deprivation in heifers.

### **Potentially toxic effects**

In spite of the differences measured between ecological areas, none of the trace elements determined was present at concentrations that could be considered dangerous for sheep and livestock health. In any case, the Al concentrations measured in this study were lower than the ones reported for grasses by Metson et al. (1979) in seven pastures in New Zealand. The authors found mean values of 500 mg Al kg<sup>-1</sup> DW. In addition, extremely low As, Cd and V concentrations were determined in plant tissues, which pose no toxicity risk for grazing sheep. These values are within the normal range determined for grasses growing in soils not contaminated with Pb (280–330 µg kg<sup>-1</sup> DW) as described by Kabata-Pendias (2011). It is assumed that root Pb uptake strongly depends on Pb soil concentrations, but it has been observed that this element is generally poorly taken up by plant roots even when appreciable soil Pb concentrations may be present (Longhurst et al. 2004). Therefore, the main Pb toxicity threat for sheep and livestock production could be related to grazing in Pb contaminated land (Abrahams & Steismajer 2003). Despite B, Cr, Ni, Rb and V potentially being toxic for sheep, these elements can be considered to be beneficial because at low concentrations they may be improve animal performance (Suttle 2010). For instance, an association between poor conception and low serum B concentration has been reported for beef cow herds (Small et al. 1997). Similarly, Spears (1984) reported that supplementation of diets that have a Ni concentration range of 0.26–0.85 mg kg<sup>-1</sup> with an additional 5 mg Ni as NiCl<sub>2</sub>, increased the ruminal urease, growth rate and feed conversion efficiency of lambs and steers. Kabata-Pendias & Pendias (1992) reported similar concentrations of B and V for grasses, lower concentrations of Cr and Ni and higher values of (Rb) compared with the values that we obtained. On the other hand, the strontium (Sr) concentrations determined in this study were within the range of 6–37 mg Sr kg<sup>-1</sup> reported by Kabata-Pendias (2011) for aerial parts of grasses.

Although Ti is not included in the literature as a toxic or beneficial element for sheep (or ruminants in general), we found it interesting to include this information due to the scarcity of plant tissue Ti concentration data. Despite the limited knowledge on plant Ti absorption and metabolism (Carvajal & Alcaraz 1998), this element has been considered as relatively scarce for plants and not readily mobile (Kabata-Pendias 2011). Contradictory effects in plants have been attributed to Ti. For example, Wallace et al. (1977) described toxicity symptoms (chlorotic spots on leaves) in bush bean with Ti concentrations of 200 mg kg<sup>-1</sup>, but there are also studies indicating beneficial effects on yield for several crops (e.g. Pais 1983; Alcaraz-Lopez et al. 2003). Kabata-Pendias (2011) indicated that Ti concentration in plants may vary considerably from 0.15–80 mg kg<sup>-1</sup>.

### **Concluding remarks**

In this study we present new information about the mineral element concentrations of the main feeding source for sheep in southern Patagonia. However, it is necessary to be cautious when interpreting soil properties in relation to the grass and graminoid mineral element tissue results obtained, since an array of additional biotic and abiotic factors may affect such values. Although we provided thresholds for sheep nutritional requirements and toxicity levels gathered from the existing literature, we strongly suggest that they are considered only as reference data since more detailed information is required

to establish a nutritional guide for Patagonia. For instance, it is difficult to accurately determine mineral element requirements for animal feeding due to the interactions between animal absorption and metabolism in relation to the food eaten, the occurrence of net requirement variations due to the productive capacity of each particular species or breed, the rate of production enabled by other key dietary constituents, the effect of the environment (Suttle 2010) and the occurrence of potential antagonistic effects between mineral elements (e.g. Cu uptake may be inhibited by Mo and S; Suttle 2010; López-Alonso 2012). Moreover, in extensive grazing systems, the forage samples collected may not represent the material selected by grazing animals, mainly because the animals may show preferences for different types and parts of plants. Hence, in future investigations it will be necessary to develop methods to assess more accurately the mineral element status of animals to spot potential nutrient deficiencies. For such purposes, blood, urine, saliva and hair analyses (among others) have been carried out to evaluate the mineral status of animals (Suttle 2010) and may be used as a tool to analyse nutrient deficiencies in Patagonian sheep. On the other hand, in extensive systems such as in Patagonia, soil ingestion when animals are grazing can significantly contribute to livestock trace mineral exposure (López-Alonso 2012). In this regard, some reports indicated that soil ingestion in ruminants can represent up to 18% of organic matter in cattle (Herlin & Andersson 1996) and more than 50% in sheep (Thornton & Abrahams 1983). In summary, it is concluded that further trials will be required to improve our understanding of mineral element nutrition as a tool to improve sheep production in Patagonia.

### Disclosure statement

No potential conflict of interest was reported by the authors.

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