

ORIGINAL RESEARCH ARTICLE

Precipitation is the key determinant of topsoil $\delta^{15}\text{N}$ values in southern Patagonia's semiarid rangelands

Pablo L. Peri^{1,2} | Sandra Duarte-Guardia^{1,3} | Wulf Amelung⁴ | Brenton Ladd⁵ 

¹ Univ. Nacional de la Patagonia Austral (UNPA)-CONICET, 9400 Río Gallegos, Argentina

² Instituto Nacional de Tecnología Agropecuaria (INTA), 9400 Río Gallegos, Argentina

³ Laboratorio Nacional de Suelos, Aguas y Foliaves-LABSAF, Instituto Nacional de Innovación Agraria (INIA), Av. La Molina 1981, Lima 15024, Perú

⁴ Institute of Crop Science and Resource Conservation (INRES), Soil Science and Soil Ecology, Univ. of Bonn, Bonn 53115, Germany

⁵ Escuela de Agroforestería, Univ. Científica del Sur, Lima 33, Perú

Correspondence

Brenton Ladd, Escuela de Agroforestería, Univ. Científica del Sur, Lima 33, Perú.
Email: bladd@cientifica.edu.pe

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Abstract

Nitrogen (N) cycling in rangeland soils could potentially be controlled by water supply, stocking rates, or a range of other variables, such as ecosystem N stocks. To gauge the relative importance and elucidate possible interactions among these factors, we measured many abiotic variables to identify first-order controls of $\delta^{15}\text{N}$ for Patagonia's rangeland soils under contrasting historical grazing intensities. The results showed that $\delta^{15}\text{N}$ values declined as water availability increased. The effects of precipitation and stocking rate on soil $\delta^{15}\text{N}$ values were additive, and the effect of precipitation far outweighed the effects of grazing pressure. The soil N stock was a weak predictive variable for modeling variation in $\delta^{15}\text{N}$ of the soil. Earlier assumptions about an inflection point for N cycling and $\delta^{15}\text{N}$ values related to aridity were not confirmed. We conclude that variation in water availability drives variation in $\delta^{15}\text{N}$ values irrespective of grazing intensity. We also conclude that meaningful interpretation of $\delta^{15}\text{N}$ in soil will require a better mechanistic understanding of the interactions between water and N in the vadose zone than we currently possess.

1 | INTRODUCTION

The current misuse of N has increased to a level that exceeds the acceptable planetary boundary (Steffen et al., 2015). Indiscriminate N use has caused serious environmental problems. Nitrogen acts as a potent greenhouse gas, results in the loss of biodiversity, and leads to the eutrophication of water (Craine et al., 2018; Zhao et al., 2020). At the other end of the spectrum, in many regions, farming is practiced with minimal fertilizer inputs. Left behind are soils depleted in nutrients, with reduced capacity to sustain productivity, profitability, and food security (Lal, 2009). The depletion of soil nutrient capital (i.e., soil N) may also limit the potential for sequestration of atmospheric C on farmland (Kirkby et al., 2014; van

Groenigen et al., 2017). A prerequisite to better management of N is a better understanding of the response of ecosystems, including agroecosystems, to changing N fluxes (Craine et al., 2018; van Groenigen et al., 2017). This holds particularly true for semiarid ecosystems where data are comparatively scarce.

The stable isotope composition of N holds potential as an integrative variable that could provide deep insight into both modern and ancient ecosystems, yet incomplete understanding and complex transformations of N limit our ability to interpret N isotope data (Szpak, 2014). $\delta^{15}\text{N}$ values are thought to reflect the balance between supply and demand for N, the so-called openness of the N cycle. Consistent with this idea, there is a tendency to see depleted $\delta^{15}\text{N}$ values with increased N demand in heavily grazed rangeland ecosystems

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(Coetsee, et al., 2011; Golluscio et al., 2009; Xu, et al., 2010). Most processes involved in the N cycle tend to discriminate towards the heavier ^{15}N isotope, and shifts towards heavier $\delta^{15}\text{N}$ values are frequently seen as indicators of more openness in the N cycle (i.e., of reduced N use efficiency) (De Datta et al., 1988), in both native and managed ecosystems (Amundson et al., 2003; Craine et al., 2018; Högberg, 1997; Kriszan et al., 2014). On the other hand, biological N fixation partly shifts $\delta^{15}\text{N}$ values towards zero (Craine et al., 2009). Also, mineral fertilizer application usually adds N, with a $\delta^{15}\text{N}$ value also close to zero (Vitòria, et al., 2004), though overfertilization with synthetic N fertilizers can also lead to the accumulation of heavy $\delta^{15}\text{N}$ (Kriszan, et al., 2009). These different processes, together with the large number of biotic and abiotic factors that can lead to isotopic fractionation in N, make the final interpretation of $\delta^{15}\text{N}$ difficult (Guiry, 2019).

As N transformations are also affected by water, soil $\delta^{15}\text{N}$ values may correlate with water availability, as shown, for instance, in *Nothofagus* forests (Peri et al., 2012), and Hawaii's *Acacia* forests (Chadwick, et al., 2007). The mechanisms are not yet fully resolved, but we know that plant uptake of N is closely linked to transpiration. High rates of water usage and a low water use efficiency may lead to high rates of N uptake and low N use efficiency (McDonald, et al., 2002). A recent analysis of N isotope values in oak tree (*Quercus* spp.) rings from a long-term fire frequency experiment additionally demonstrated that fire suppression leads to enrichment of $\delta^{15}\text{N}$ values (Trumper et al., 2020). It is unclear how combustion could discriminate against "heavy" $\delta^{15}\text{N}$; therefore, we hypothesize that the enrichment of $\delta^{15}\text{N}$ in the study by Trumper et al. (2020) could be related to the growth of the understory in the absence of fire: increased growth of the understory in the absence of fire may have increased evapotranspiration and N uptake with an overall lower N use efficiency at the stand scale.

In summary, research on the distribution patterns of plant and soil $\delta^{15}\text{N}$ values have helped to understand gross changes in N use along gradients of water availability (Amundson et al., 2003; Craine et al., 2018; Högberg, 1997; Kriszan et al., 2014; Wu et al., 2019) and management (Coetsee et al., 2011; Golluscio et al., 2009; Xu et al., 2010). Yet, simultaneous quantification of the interactive effects and relative importance of factors such as management and water availability on $\delta^{15}\text{N}$ values has been limited. Southern Patagonian rangeland is an ideal ecosystem for such an evaluation because land use is relatively homogenous across the region, and because of its low plant diversity, ensuring that confounding factors such as variation in mycorrhizal associations (Craine et al., 2009) do not complicate interpretation of the data. We measured and analyzed many abiotic variables across the Parcelas de Ecología y Biodiversidad de ambientes naturales en

Core Ideas

- Interpretation of $\delta^{15}\text{N}$ values in soils remains a significant and unresolved challenge.
- Precipitation is the main driver of variation in $\delta^{15}\text{N}$ values in Patagonian soils.
- Vadose zone research is needed to better understand the interactive effects of water and N on $\delta^{15}\text{N}$ in soil.

Patagonia Austral (PEBANPA) network of permanent plots in southern Patagonia to identify first-order controls on $\delta^{15}\text{N}$ for Patagonia's semiarid rangeland soils. Because extensive, low-input sheep grazing is the dominant land use in the region, we hypothesized that soil $\delta^{15}\text{N}$ would be low where stocking rates were high relative to ecosystem productivity, consistent with the idea that low $\delta^{15}\text{N}$ values would reflect a mismatch between the demand and supply of N. We also hypothesized that water availability would exert a strong downward pressure on $\delta^{15}\text{N}$ values, consistent with our previous research (Peri et al., 2012).

2 | MATERIALS AND METHODS

2.1 | Data collection

The data used in this analysis correspond to the 108 arid and semiarid rangeland plots in the PEBANPA network of long-term biodiversity plots in southern Patagonian (Peri, Lencinas, et al., 2016), which were comprehensively sampled (for soils) and biodiversity (plants) in the summers of 2003–2004 and 2018–2019 (Figure 1). This cold temperate region possesses mean annual temperatures between 5.5 and 8.0 °C and mean annual precipitation levels between 1,000 and 200 mm yr⁻¹. Temperatures are highest during the short Patagonian summers (December–February). The windiest season occurs from November to March, producing frequent and severe south-southwesterly windstorms reaching over 100 km h⁻¹. The PEBANPA plots were established over 15 yr ago, and since this beginning we have measured and compiled a diverse range of variables for site characterization. At each sampling location, variables have been measured in a 20-m × 50-m quadrat (1,000 m²). This plot size enables regional comparisons in diversity-associated factors for broad vegetation types (e.g., grasslands, shrublands, and forests). A total of 345 plant species from 151 genera and 56 families were recorded in the quadrats surveyed across Santa Cruz province, representing 16% of the flora of Patagonia where local native

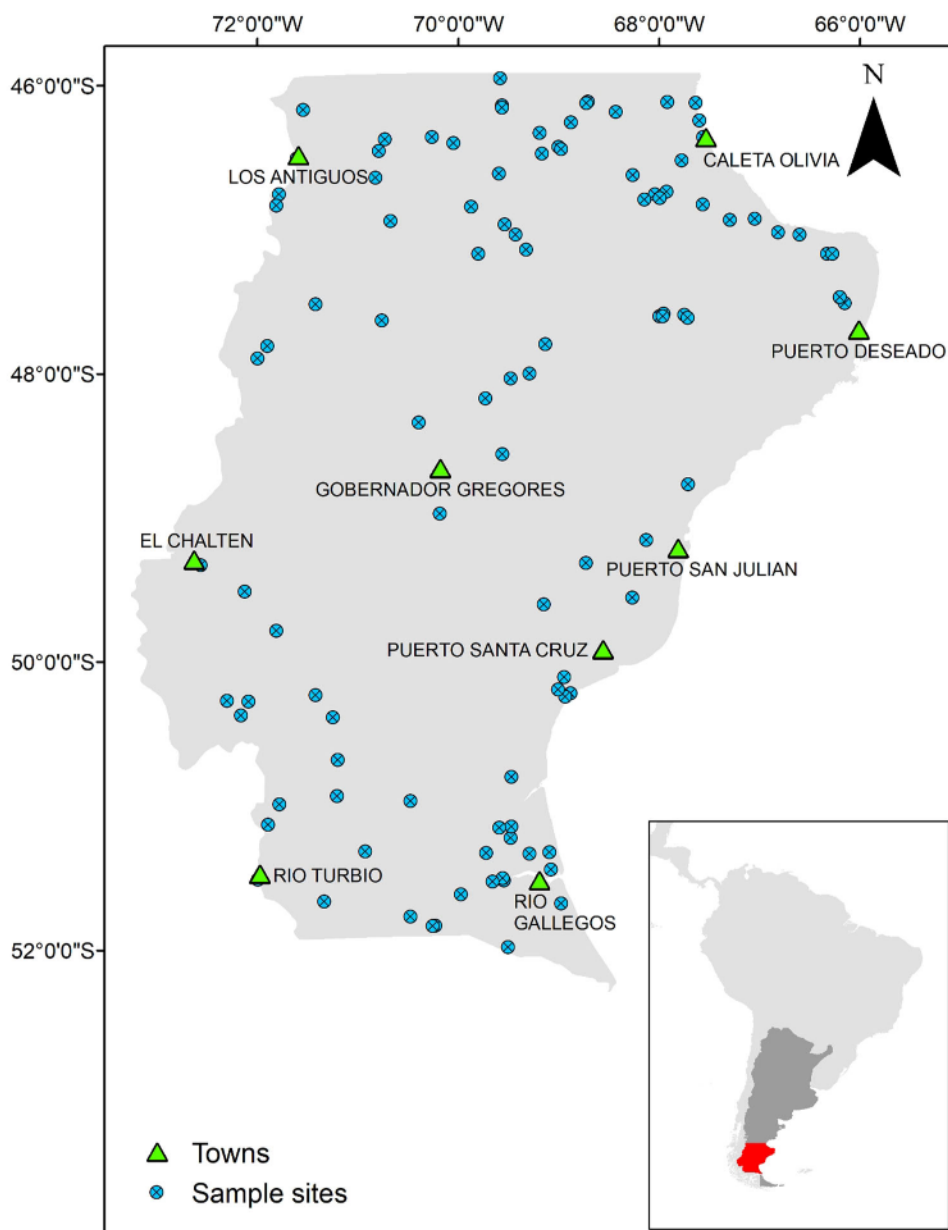


FIGURE 1 The spatial distribution of the 108 Parcelas de Ecología y Biodiversidad de ambientes naturales en Patagonia Austral (PEBANPA) rangeland sites across the southern Patagonian landscape

plants accounted for 92.5% of the total species (47 endemic species) and alien taxa accounted for 24 species.

The steppe ecosystem, mainly characterized by the presence of tussock (*Festuca*, *Stipa*), short grasses (*Poa*, *Carex*), and shrubs, covers 85% of the total area in Santa Cruz province, and the main activity in this ecosystem is extensive sheep grazing. In each quadrat, nine replicate soil samples from the epipedon (0.10-m depth) were collected randomly using a hand auger. Soil samples were pooled into three composite samples that contained an equal proportion of soil from three auger holes ($n = 3$ for each site). From these, information on soil C (Peri et al., 2018) and nutrient stocks

(Peri, Ladd, et al., 2016; Peri, Rosas, et al., 2019), plant biodiversity (Peri, Lasagno, et al., 2019), and GIS-derived variables that characterize climate, topography, soils, geology, and land use (Peri, Lasagno, et al., 2019; Peri, Ladd, et al., 2016) has been obtained.

In addition, we included recently compiled information on aboveground net primary productivity by clipping peak live plant material (current year's green production [excluding woody tissue] obtained from three randomized 0.2-m² quadrats in each site) (Peri, Lencinas, et al., 2016; Peri et al., 2013). Samples were dried for at least 48 h at 55 °C and then weighed. The estimation of carrying capacity (sheep

stocking rates) is based on the biomass production of short grasses and forbs that grow in the space among tussocks of each ecological area and the requirements of 530 kg dry matter yr^{-1} for one Corriedale ewe of 49 kg of live weight, representing a “Patagonian sheep unit equivalent.” Sheep carrying capacity was estimated through a fixed forage allowance at paddock level by using a variable stocking rate allocation model based on the simulation of forage biomass and quality dynamics and the setting of critical residue targets and animal diet at the end of the grazing period (Andrade et al., 2016).

Finally, for the response variable, $\delta^{15}\text{N}$ of soil, samples were collected following the standardized protocols we use for the PEBANPA network (Peri, et al., 2016). Nitrogen isotopes were measured using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon). The gases were separated on a Carbosieve G column (Supelco) before being introduced to the isotope ratio mass spectrometer. Sample isotope ratios were compared with those of pure cylinder gases injected directly into the isotope ratio mass spectrometer before and after the sample peaks, and provisional delta $\delta^{15}\text{N}_{\text{air}}$ values were calculated. Provisional isotope values were adjusted to bring the mean values of working standards samples at intervals in each analytical run to the correct values of the working standards. The ratio of heavy to light isotopes in the sample material (R_{sample}) was measured using mass spectrometry as the deviation from the isotopic ratio of a standard (R_{std}), where R denotes the ratio of stable N ($^{15}\text{N}/^{14}\text{N}$) isotopes, expressed in δ notation; for example, for N:

$$\delta^{15}\text{N}(\text{‰}) = (R_{\text{sample}}/R_{\text{std}} - 1) \times 1,000$$

High values of this parameter indicate the enrichment of N in heavy isotopes, whereas low values indicate the depletion in heavy isotopes relative to the standard. Atmospheric N ($\delta^{15}\text{N}_{\text{air}} = 0$) serves as the international standard for N.

2.2 | Data analysis

To assess the importance of a range of environmental variables on $\delta^{15}\text{N}$ in topsoil, we used random forests, with delta $\delta^{15}\text{N}$ as the response variable. The explanatory variables used in the analysis include descriptors of the plant community, human land use intensity, geology, topography, climate, and soil chemistry (see Supplemental Table S1). The random forest models were created using the *cforest* function in the *party* package for R (Hothorn, et al., 2010; RStudio Team, 2016) with variable importance values calculated via the *varimp* function. The variable importance scores are

mean values developed via running 100 random forest models, each of which contains 500 trees. During each model run, 80% of observations were used for model development, with the remaining 20% of cases used to evaluate model accuracy. Importance values are based on mean decrease measures for model accuracy measurements and were standardized across runs by dividing by the value obtained for the most important variable in the respective model run. Finally, we ran a simple multiple regression using a significance value of .05 and mean annual precipitation and grazing intensity (ewes $\text{ha}^{-1}\text{yr}^{-1}$) as independent variables with $\delta^{15}\text{N}$ as the dependent variable using Xlstat.

3 | RESULTS

The random forest model explained 85% of variance in $\delta^{15}\text{N}$ values, and 9 of the 10 most important predictor variables were moisture related (Figure 2). The soil N stock was only a weak predictor of soil $\delta^{15}\text{N}$ values (Supplemental Figure S1). The only non-water-related variable to appear in the list of important predictive variables was stocking rate (Figure 2), though at rank number 8. The outcome of the random forest model may justify the intention to use moisture related parameters as sole predictors for soil $\delta^{15}\text{N}$ values. Indeed, Figure 3 shows that mean annual rainfall alone already explained 71% of data variability (Figure 3a). A similar trend was also obtained using the aridity index instead of rainfall (Figure 3b). In contrast, the strength of the correlation between stocking rate and $\delta^{15}\text{N}$ was relatively low, although still significant (Table 1; Figure 3c; $R^2 = .14$). Importantly the effects of water and stocking rate were additive (i.e., there was no complex synergistic interaction between the two; see Table 1). Also, it is important to reiterate that soil N stock was only a weak predictor of soil $\delta^{15}\text{N}$ values (see Supplemental Figure S1).

4 | DISCUSSION

The decline in soil $\delta^{15}\text{N}$ values with increasing water availability is in line with previous studies (Amundson et al., 2003; Chadwick et al., 2007; Craine et al., 2015; Peri et al., 2012; Shan et al., 2019; Vitousek, et al., 2019; Wu et al., 2019). This result is also consistent with mechanistic laboratory studies that demonstrate that increasing soil moisture may favor the accumulation of $\delta^{14}\text{N}$ (Yun & Ro, 2014). The correlation between $\delta^{15}\text{N}$ values with rainfall and the aridity index may be attributable to various processes. Firstly, high moisture levels may favor N transformation pathways that lead to the accumulation of isotopically depleted forms of soil N such as nitrate (Kahmen, et al., 2008; Yun & Ro, 2014). Yet, as the latter is easily leached, heavy rather than light

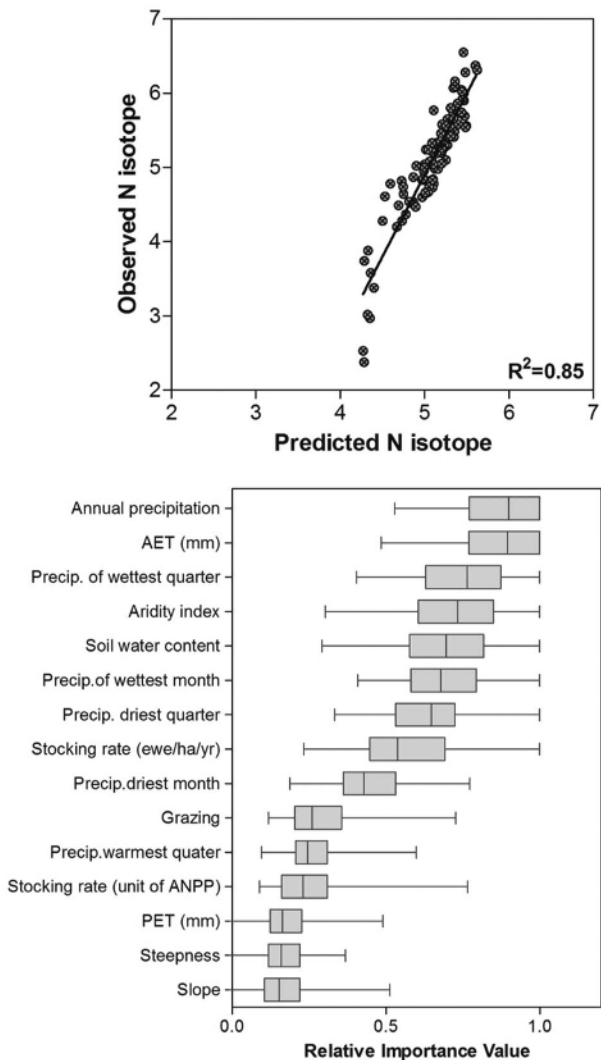


FIGURE 2 Top panel shows the correlation between (random forest) predicted values of $\delta^{15}\text{N}$ vs. observed values. The bottom panel shows the relative importance value of the 15 strongest predictor variables in the random forest analysis of $\delta^{15}\text{N}$ (ran with the *cforest* function of the *party* package, RStudio). AET = annual evapotranspiration, Precip. = precipitation, PET = potential evapotranspiration, Steepness is a topographic variable, Aggmean NDVI-std = the standard deviation of the aggregated mean of normalized difference vegetation index, X bare soil = the % bare soil at the relevant sampling location. See Supplemental Figure S1 for relative importance values of all independent variables included in the random forest analysis. Note that the variable “soil water content” is GIS derived (Trabucco & Zomer, 2009) and uses climatic variables to estimate soil water (monthly values of evaporation and precipitation)

isotope signatures should remain, except if high soil moisture results in significant N losses in the form of dissolved organic N that is ^{15}N -enriched, as described by Koba et al. (2012). Also, other processes like nitrification and denitrification are usually enhanced in humid soils (Davidson et al., 1993), thus potentially accompanying losses of light N (Granger & Wankel, 2016). Enhanced moisture supply may also improve

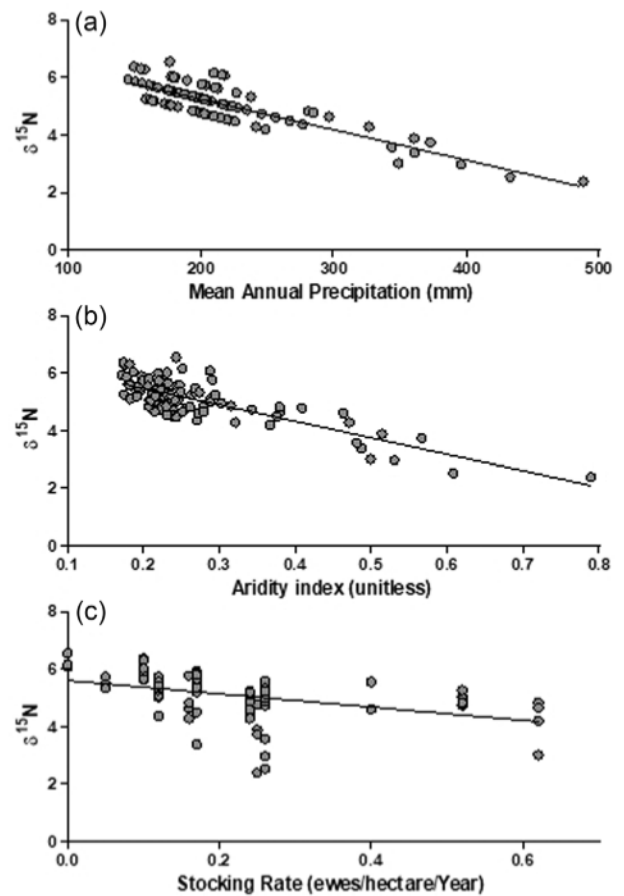


FIGURE 3 Univariate plots of (a) mean annual precipitation vs. $\delta^{15}\text{N}$ ($y = -0.0103x + 7.3041$, $R^2 = .802$, $P < .0001$); (b) the aridity index vs. $\delta^{15}\text{N}$ ($y = -6.0367x + 6.695$, $R^2 = .7367$, $P < .0001$; note that low values indicate aridity), and (c) stocking rate vs. $\delta^{15}\text{N}$ ($y = -1.7772x + 5.4293$, $R^2 = .0871$, $P < .0001$)

water uptake and thus uptake of light N forms such as nitrates by plants. As a result, the overall N use efficiency may be improved at elevated moisture supply, even if water use efficiency is lower (Peri et al., 2012). Finally, at drier sites, plants generally access water from deeper in the soil profile. As soil $\delta^{15}\text{N}$ values tend to increase with soil depth, more heavy $\delta^{15}\text{N}$ might be recycled from deeper soil layers at comparatively dry sites (Peri et al., 2012).

Our data superimposed well onto the global dataset of Craine et al. (2015); however, soil $\delta^{15}\text{N}$ values declined strongly as water availability increased (Figure 4). The slope of the regression line for soil $\delta^{15}\text{N}$ vs. rainfall in the rangeland plots was similar to that of adjacent *Nothofagus* forests (Argentina), but the data clustered much closer along the regression line for the rangeland sites than for the forest sites (Peri et al., 2012). The curves, however, were distinctly different from that of Hawaiian *Acacia* forests (Figure 4), indicating ecosystem-specific relationships.

We also did not find evidence of a threshold for soil $\delta^{15}\text{N}$ values and N cycling at an aridity index value of 0.32, as

TABLE 1 Summary statistics for the multiple linear regression that used mean annual rainfall (MAP) and stocking rate (ewes ha⁻¹ yr⁻¹) as independent variables and $\delta^{15}\text{N}$ values as the dependent variable

| Source | df | SS | F | Pr > F | R ² |
|---------------------|-----|-------|--------|--------|----------------|
| MAP | 1 | 42.52 | 532.07 | 0.00 | 71.74 |
| Stocking rate | 1 | 8.42 | 105.30 | 0.00 | 14.20 |
| MAP × stocking rate | 1 | 0.02 | 0.27 | 0.60 | 0.04 |
| Error | 104 | 8.31 | | | |
| Corrected total | 107 | 59.27 | | | |

Note. df, degrees of freedom; SS, sum of squares; R², the percentage variance in $\delta^{15}\text{N}$ values explained by the relevant factor. $\delta^{15}\text{N} = 7.82 - 1.07 \times \text{MAP} - 2.39 \times \text{stocking rate}$.

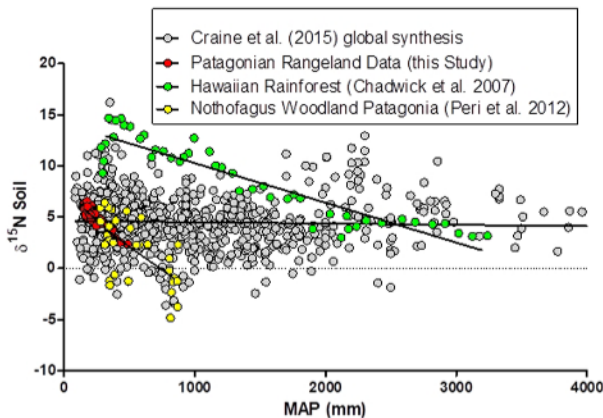


FIGURE 4 $\delta^{15}\text{N}$ values from topsoil samples collected in this study superimposed upon the global compilation of data by Craine et al. (2015), together with data from two regional studies that measured variation in soil $\delta^{15}\text{N}$ values (Chadwick et al., 2007; Peri et al., 2012)

found by Wang et al. (2014). The discrepancy between the Patagonia data and the results of Wang et al. (2014) may be attributable to the influence of groundwater and vadose zone processes. In Patagonia, the influence of these factors was likely minimal because we focused sampling on a single ecosystem type (steppe). In the study of Wang et al. (2014), the sites that had low aridity index values (between 0 and 0.3) were dominated by *Reaumuria reaf* L. (Wang et al., 2014). This plant is adapted to saline conditions, where ground water likely is near the soil surface. Also, Wantzen et al. (2002) demonstrated that hydraulic saturation results in low sediment $\delta^{15}\text{N}$ values in their detailed study of an aquatic food web in Brazil's Pantanal. We acknowledge that using only climatic variables to quantify water availability and aridity disregards the more complex but highly important effects that topography and vadose zone processes can have on site hydrology (Doetterl, et al., 2012; Stallard, 1998). The apparent inconsistencies regarding water effects on $\delta^{15}\text{N}$ values could potentially be resolved if future biogeographical studies and syntheses obtain and use complete geographic coordinates to constrain, when possible, site-scale topographic controls on

water and $\delta^{15}\text{N}$ values (e.g., groundwater, water supply via capillary rise).

5 | CONCLUSION

The decline in soil $\delta^{15}\text{N}$ values as grazing pressure increased provides evidence that N cycling does become tighter and more efficient as N demand, relative to supply, increases. Nevertheless, as stated above, this grazing effect on soil $\delta^{15}\text{N}$ values was small compared with the effect of water. Our data thus indicate that understanding and quantifying the effect of water on N isotope transformations will be critical for developing a better understanding of the underlying reasons for variation in $\delta^{15}\text{N}$ values of soil. Being able to better interpret soil $\delta^{15}\text{N}$ values could in turn increase the value of $\delta^{15}\text{N}$ as an integrative variable generating deeper insight into past and present ecosystems.

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AUTHOR CONTRIBUTIONS

Brenton Ladd: Conceptualization; Formal analysis; Investigation; Writing – original draft; Writing – review & editing. Pablo L. Peri: Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Writing – original draft; Writing – review & editing. Sandra Duarte-Guardia: Formal analysis; Methodology; Writing – original draft; Writing – review & editing. Wulf Amelung: Conceptualization; Writing – original draft; Writing – review & editing.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ORCID

Brenton Ladd  <https://orcid.org/0000-0002-9388-6298>

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