




# Plant Species and Defoliation Effects on Soil Nitrogen Mineralization in a Semiarid Rangeland of Argentina

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## Abstract

The objective of this study was to evaluate the effects of moderate defoliation and grass species on soil nitrogen (N) mineralization in a semiarid grassland of northeastern Patagonia, Argentina. Studied species were *Poa ligularis* and *Nassella tenuis* (desirable/preferred by cattle) and *Amelichloa ambigua* (undesirable/non preferred). Two defoliations were made to a 5 cm stubble height during the growing season. Hypotheses were that (1) net N mineralization and N availability in the soil are increased by a moderate defoliation and greater species forage quality, and (2) potential N mineralization is higher in the soil beneath the desirable than undesirable species. In 2013 and 2014, in situ net N mineralization was estimated using the tube incubation technique under field conditions. Potential N mineralization was estimated by long-term laboratory incubations. Defoliation treatments did not affect the soil inorganic N dynamics. The soil under *A. ambigua* showed a greater in situ net N mineralization than other species, but only in 2013. *Poa ligularis* presented the highest initial inorganic N and potentially mineralizable N values. However, the opposite was recorded in this specie for the mineralization constant rate. These results demonstrated that moderate defoliations did not affect soil N availability, and presence of the desirable perennial grasses increased the potential N mineralization pool. Sustainable management practices that promote the persistence of these species in plant community are important to maintain soil fertility on semiarid grasslands.

**Keywords** *Poa ligularis* · *Nassella tenuis* · *Amelichloa ambigua* · Perennial grasses · Inorganic soil N

## 1 Introduction

Nitrogen (N) is an essential nutrient that limits primary production in terrestrial ecosystems (Yahdjian et al. 2011). Soil organic matter (SOM) regulates N-dynamics because of its key role in N mineralization and availability to plants (Martínez et al. 2018). In arid and semiarid areas, N availability is dynamic and varies annually in relation to water availability (Reichmann et al. 2013). In some areas, increased water availability does not affect net N mineralization. However, there is less available N due to the higher N uptake by plants than under drought conditions (Yahdjian et al. 2006; Reichmann et al. 2013).

Plant litter has a substantial effect on soil N dynamics (Moretto and Distel 2002). N release from litter varies with its quality, and this is mostly associated with the initial tissue nutrient concentration, regardless of climate, edaphic conditions, or soil biota (Parton et al. 2007). High litter quality (high N concentration, low C:N ratio, and lignin contents) results in rapid decomposition and net N mineralization.

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Low litter quality (low N concentration, high C:N ratio, and lignin contents) produces a lower decomposition rate and greater N immobilization than high litter quality (Aber and Melillo 1991).

In arid and semiarid grasslands, there are desirable (i.e., preferred) species for cattle that produce high litter quality, with a high net N mineralization rate (Moretto and Distel 2002). Selective grazing induces their replacement by undesirable (i.e., non preferred) species (Busso and Fernández 2018), affecting the quality and quantity of the above- and belowground biomass, the quality and accumulation of the litter, and it may imply a reduction in soil fertility (Moretto and Distel 2002). Nevertheless, Oñatibia et al. (2015) reported that forage supply and C and N storage were higher under moderate grazing than ungrazed and intensively grazed areas of semiarid ecosystems.

In Argentina, cattle production system is based on grazing of native vegetation on arid and semi-arid grasslands (Busso and Fernández 2018). The Phytogeographical Province of the Monte constitutes the most arid region in the country, and it has been degraded by overgrazing mainly of cattle and sheep in the last 100 years (Busso and Fernández 2018). Therefore, studies that involve the combined action of defoliation and plant species characteristics (i.e., forage and litter quality) on soil N mineralization are important to maintain soil fertility in these semiarid environments with low resilience levels.

This study aimed to evaluate the effect of grazing on different perennial grass species on soil fertility, estimated as N mineralization, in a semiarid rangeland. We hypothesized that (1) soil net N mineralization and N availability are increased by moderate defoliation and greater species forage quality, and (2) potential N mineralization is higher in the soil beneath the desirable than the undesirable species. To test these hypotheses, we performed a defoliation experiment and we estimated soil N mineralization under the dominant plant species in a semiarid rangeland of Argentina.

## 2 Material and Methods

### 2.1 Study Site and Grass Species

This study was conducted in a 16-year-old enclosure (1.12 ha) to domestic herbivores in the Chacra Experimental Patagones (40° 39' S, 62° 54' W; 40 m above sea level), located in the south of Buenos Aires province, northeastern Patagonia, Argentina. The area was under long-term continuous grazing by cattle and sheep until 1951. Thereafter, it was cleared of woody vegetation and undergrowth and cropped from 1951 until 1975. It was then excluded from domestic herbivores from 1975 to 1994, and then exposed to a controlled grazing by cattle and sheep.

Finally, it was excluded from cattle grazing since 1996 to date. Desertification in this region is evident by a loss of plant cover, decrease in soil fertility, and increase in soil compaction and salinization (SAyDS 2011).

The soil is classified as Aridisol (i.e., Typic Haplocalcid) of 15 cm in depth with a sandy clay loamy texture in the A horizon (Soil Survey Staff 2014). The annual precipitation values were 422 mm in 2013 and 597 mm in 2014.

This study was performed with three perennial C<sub>3</sub> grass species: *Poa ligularis* Nees ex Steud, *Nassella tenuis* (Phil.) Barkworthand, and *Amelichloa ambigua* (Speg.) Arriaga and Barkworth. These cool-season species show differences in litter quality, successional stage, and above- and belowground litter decomposition rates (Distel and Bóo 1996; Andrioli and Distel 2008; Ambrosino et al. 2019). *Poa ligularis* is a highly desirable species, available in areas where there is rotational grazing and a low stocking rate (Giorgetti et al. 2006). With moderate, continuous grazing, this species is replaced by other desirable grasses, such as *N. tenuis*. *Amelichloa ambigua* is an undesirable grass only consumed when desirable forages are unavailable (Ithurrart 2015).

### 2.2 In Situ Net N Mineralization

In situ net N mineralization beneath the studied species was estimated following Raison et al. (1987).

In January 2013 (early summer), 10 plants per species were selected and clipped to 5 cm stubble height to remove senescent material accumulated in previous years. In the vegetative stage of developmental morphology (early winter: July 2013), an initial soil sample set (0–15 cm depth) was collected using PVC tubes (15 cm long, 3.5 cm in diameter) under the foliage of each individual plant. At the same time, another PVC tube of the same size was buried for incubation during 1 month and half of the plants of each species were defoliated to 5 cm stubble height while the other half remained undefoliated (control). These same plants were again defoliated after the differentiation of the growth apex from vegetative to reproductive (early spring: September 2013), and the tube incubation technique was once again conducted. The aim was to simulate a moderate grazing intensity and defoliation frequency common at the study area (Giorgetti et al. 2006). Apical and some active, intercalary meristems remained on the plants after the defoliation treatments (Giorgetti et al. 2000). This study was repeated in 2014 using a different plant set (initial sampling and first defoliation: July 2014, second defoliation: August 2014). Mineralized inorganic N was calculated by subtracting initial soil inorganic N concentrations from the final concentrations after each incubation.

## 2.3 Potential N Mineralization

In July 2013, additional composite soil samples (4 plants per species) (0–15 cm) were collected below the species canopy at the same site as for the in situ net N mineralization experiment. Several (4) unvegetated sites (controls) were selected at random in the different vegetation patches. The following soil chemical properties were initially determined: (1) soil organic carbon (SOC) by dry combustion with a Leco automatic analyzer (Leco Corporation, St Joseph, MI), and (2) soil organic N (SON) by the Kjeldahl method (Bremner 1996). Then, the C:N ratio of soil was calculated.

Potential N mineralization was estimated by a long-term aerobic incubation of soil following the procedure proposed by Griffin et al. (2008). Following the incubation period, N mineralized was determined by non-linear regression (Eq. 1), assuming that the cumulative mineralized N ( $N_{min}$ ) fitted an exponentially first-order kinetic model (Stanford and Smith 1972):

$$N_{min} = N_0 (1 - e^{-kt}) \quad (1)$$

where  $N_{min}$  was the cumulative amount of N mineralized at time  $t$ ,  $N_0$  was the potentially mineralizable N, and  $k$  was the rate constant.

## 2.4 Statistical Analysis

Data were analyzed using the INFOSTAT software (Di Rienzo et al. 2016). In situ net N mineralization data were transformed to  $\sqrt{x+0.5}$  to fulfill assumptions of normality and homoscedasticity (Sokal and Rohlf 1984). Data corresponding to the initial analysis of the soil inorganic N for each year was analyzed using a one-way analysis of variance (ANOVA), taking plant species as the fixed factor. Subsequently, variables were analyzed with multi-factorial ANOVA considering (1) species, (2) defoliation treatments, (3) field incubations, and (4) years as fixed factors. Since data corresponded to repeated measures, mixed linear models were used with regressive correlation of first order and homoscedastic variances. Potential N mineralization data were analyzed using a non-linear regression to obtain  $N_0$  and  $k$  from Eq. 1. Then, data were transformed to  $\ln x$  to fulfill assumptions of normality and homoscedasticity (Sokal and Rohlf 1984). Estimated variables and data corresponding to the initial soil chemical properties were analyzed using a one-way ANOVA, with species-unvegetated site as the fixed factor.

A comparison of means was conducted using the protected test of Fisher (i.e., LSD), with a significance level of 0.05. All reported data correspond to untransformed values.

## 3 Results and Discussion

### 3.1 In Situ Net N Mineralization

In 2013, the initial inorganic N differed between species ( $F = 4.69$ ,  $P = 0.0179$ ). The highest values were recorded underneath the desirable species ( $P. ligularis = 53.6 \pm 6.6 \text{ mg kg}^{-1}$ ,  $N. tenuis = 54.2 \pm 3.9 \text{ mg kg}^{-1}$ ) and the lowest under  $A. ambigua$  ( $35.4 \pm 3.8 \text{ mg kg}^{-1}$ ). In 2014, the values of the initial inorganic N were higher in  $P. ligularis$  ( $96.9 \pm 5.3 \text{ mg kg}^{-1}$ ) than in  $A. ambigua$  ( $73.5 \pm 4.8 \text{ mg kg}^{-1}$ ), whereas the soils under  $N. tenuis$  presented intermediate values to those of the other species ( $83.4 \pm 5.5 \text{ mg kg}^{-1}$ ) ( $F = 5.06$ ,  $P = 0.0136$ ). Differences in the successional stage of the studied species might explain these results: late-seral species that produce high above-ground litter quality, such as  $P. ligularis$  (Moretto and Distel 2003), show faster decomposition rates and higher soil available N values than earlier seral species (Aber and Melillo 1991). In both years, no effects of defoliation treatments on net N mineralization were detected ( $P > 0.05$ ); these results are in contrast to the first proposed hypothesis and other studies, where greater (Oñatibia et al. 2015) or lower (Wang et al. 2020) mineralization was detected on grazed sites. The difference between these results and those of other studies may be due to the grazing characteristics (intensity, frequency, and timing), and the fact that the plants were clipped rather than grazed in our research (Heady and Child 1994). Use of a domestic herbivores enclosure allowed us to analyze closely the effects on soil N dynamics in relation to plant physiological changes after defoliation, such as litter production (above and below-ground) and nutrient availability necessary for regrowth. Grazing by cattle involves other (additional) aspects, such as selection of plant species and trampling (which can damage vegetation), and incorporation of feces and urine to the soil (which modifies the nutrient availability: Sirotnak and Huntly 2000). However, Andrioli et al. (2010) demonstrated that the contents of mineralized N and potential soil N mineralization associated with  $N. tenuis$  plants were not affected when comparing sites exposed to high grazing pressures with those excluded from herbivory grazing. Moreover, Ithurrart (2015) working with the same species did not detect any effect of moderate defoliations after fire on soil N availability. In our study, it might be that the defoliation treatments did not remove enough photosynthetic tissue to affect the soil N dynamics associated with the roots of the studied species.

Regarding the perennial grasses, a significant interaction was detected between species and field incubations in 2013 ( $F = 3.44$ ,  $P = 0.0401$ ). The soil under plants of  $A. ambigua$  shows a higher net N mineralization than

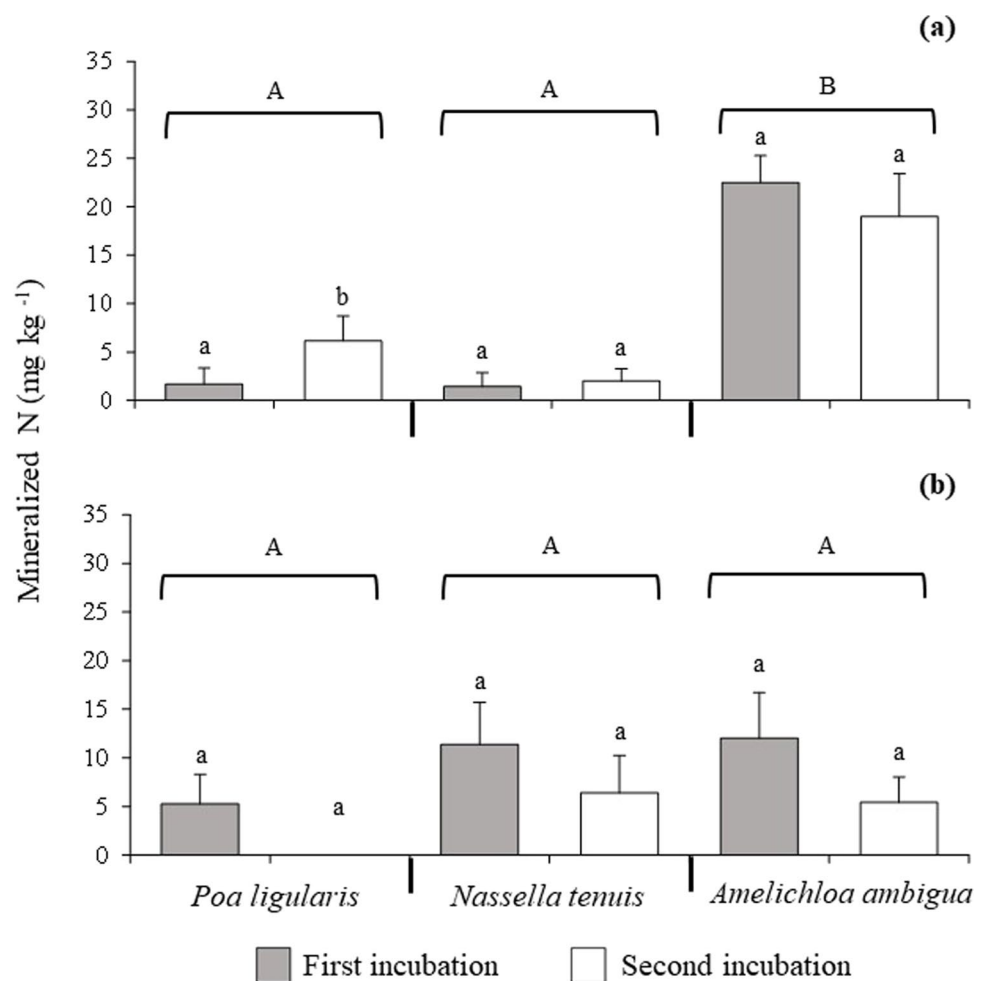
that under the desirable species in contrast to the proposed hypothesis (Fig. 1a). This could be attributed to a higher N content and decomposition rates of the belowground litter in *A. ambigua* than in the other two desirable species (Ambrosino et al. 2019). Also, Ithurrart (2015) demonstrated that *P. ligularis* and *N. tenuis* show a more advanced biological cycle than *A. ambigua*. This suggests that much of the available N might have been used to form reproductive structures in the desirable species, thus leaving less soil inorganic N associated with their roots. In 2014, the analysis did not detect any significant differences between species (Fig. 1b). The first incubation averaged over the species ( $9.56 \pm 2.35 \text{ mg kg}^{-1}$ ) had higher values of inorganic N than the second incubation ( $3.95 \pm 1.59 \text{ mg kg}^{-1}$ ;  $F = 6.92$ ,  $P = 0.0114$ ). The annual precipitation values were higher in 2014 than 2013, and rainfall concentrated in August, September, and October (Fig. S1). It means that part of the mineralized N may have been lost by leaching (Yahdjian et al. 2006).

### 3.2 Potential N Mineralization

*Poa ligularis* presented the highest  $N_0$  value (Table 1;  $F = 52.07$ ,  $P < 0.0001$ ). This result supports the second hypothesis, and coincides with Moretto and Distel (2002). These authors reported that soil under *P. ligularis* showed a higher potential N mineralization than that under the foliage of the undesirable *Stipa tenuissima*. Nevertheless, Andrioli and Distel (2008), performing long-term incubations, found no differences between species. Aboveground litter of the desirable species had a higher N content and decomposed faster than that of *A. ambigua*; the opposite occurred with the belowground litter of these species (Ambrosino et al. 2019). However, the highest aboveground litter contribution (Ambrosino et al. 2019), and soil microbial activity of the soil under *P. ligularis* (Ambrosino et al. 2021) are important factors that might contribute to determine the greatest  $N_0$ .

The lowest  $N_0$  values were in the unvegetated sites (Table 1). The fact that  $N_0$  was positively correlated with SON (data not shown), and that unvegetated sites had lower organic N contents (Table 2;  $F = 3.84$ ,  $P = 0.0421$ ), might explain these results. Also, differences in moisture

**Fig. 1** In situ net soil N mineralization (means and standard error of  $n = 10$ ) below the canopies (both defoliated + undefoliated plants) of species in **a** 2013 and **b** 2014. For each year, different upper and lower case letters indicate significant differences ( $P \leq 0.05$ ) between species and incubations within each species, respectively



**Table 1** Aerobic potentially mineralizable N ( $N_0$ ) in the long-term, and mineralization constant rate ( $k$ ) in the unvegetated sites and under the species canopies with different characteristics

	Unvegetated sites	<i>Poa ligularis</i>	<i>Nassella tenuis</i>	<i>Amelichloa ambigua</i>
Forage quality*	-	Highly preferred	Preferred	Non preferred
Aboveground litter quality*	-	High N content, low C:N ratio, and lignin contents	High N content, low C:N ratio, and lignin contents	Low N content, high C:N ratio, and lignin contents
$N_0$ (mg kg <sup>-1</sup> )	56.34 ± 3.18 <b>a</b>	159.5 ± 9.98 <b>c</b>	86.22 ± 5.09 <b>b</b>	99.7 ± 5.9 <b>b</b>
$k$ (week <sup>-1</sup> )	0.0189 ± 0.0028 <b>c</b>	0.0045 ± 0.0004 <b>a</b>	0.0086 ± 0.001 <b>b</b>	0.0084 ± 0.0007 <b>b</b>

Data are means ± standard error ( $n=4$ ). Different letters within a row indicate significant differences ( $P \leq 0.05$ ). \*Distel and Boó 1996; Andrioli and Distel, 2008; Ambrosino et al. 2019

**Table 2** Soil organic carbon (SOC), soil organic nitrogen (SON), and carbon:nitrogen ratio (C:N) under three grass species and in unvegetated sites (0–15 cm depth)

Soil property	Unvegetated sites	<i>Poa ligularis</i>	<i>Nassella tenuis</i>	<i>Amelichloa ambigua</i>
SOC (g kg <sup>-1</sup> )	20.42 ± 1.38 <b>a</b>	23.11 ± 1.98 <b>a</b>	22.53 ± 0.9 <b>a</b>	24.7 ± 1.91 <b>a</b>
SON (g kg <sup>-1</sup> )	1.06 ± 0.09 <b>a</b>	1.43 ± 0.18 <b>b</b>	1.26 ± 0.04 <b>ab</b>	1.56 ± 0.13 <b>b</b>
C:N	19.32 ± 1.45 <b>a</b>	16.3 ± 0.77 <b>a</b>	17.91 ± 0.25 <b>a</b>	17.33 ± 1.36 <b>a</b>

Data are means ± standard error ( $n=4$ ). Different letters within a row indicate significant differences ( $P \leq 0.05$ )

and temperature under the species canopies versus bare spaces allow microorganisms associated with plants to have a greater activity during the year (Whitford 2002). This in turn will allow for a higher N mineralization (Austin et al. 2004). The data analysis did not detect significant differences in SOC ( $F = 1.27$ ,  $P = 0.3409$ ) and C:N ratio ( $F = 1.53$ ,  $P = 0.272$ ) between grasses species and unvegetated soils (Table 2). These sites are the result of topographic characteristics and previous grazing mismanagement. Moreover, studies performed in the Central Monte Desert (Argentina) have reported the presence of biological soil crusts in disturbed areas by grazing (García et al. 2015; García et al. 2021). The presence of biocrusts in the unvegetated sites may contribute to mitigate the SOC differences between unvegetated soils and grasses species, despite the SON variations under these treatments.

The highest  $k$  values were recorded in the unvegetated soils (Table 1;  $F = 24.00$ ,  $P < 0.0001$ ). The mineralization process in these sites occurred from native SOM, with N that is immediately available to microorganisms (Blagodatsky et al. 2010; Yanardağ et al. 2017). The easily mineralizable N fractions of the soils under the grass species depend on the labile SOM content produced from litter degradation (Martínez et al. 2017). The fact that the organic fractions entering the system must first be transformed into labile forms available for mineralization (Martínez et al. 2018) might explain these results.

## 4 Conclusions

Moderate and early defoliations of perennial grasses species (i.e., those which did not remove the active meristems) did not affect soil N availability. Under these conditions, the presence of *P. ligularis* increased the potential N mineralization pool. Sustainable management practices that promote the persistence of desirable perennial grasses in the plant community are important to maintain soil fertility in semiarid grasslands. We suggest that further studies should address the effects of cattle grazing at different intensities and frequencies on soil N mineralization.

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## Declarations

**Competing Interests** The authors declare no competing interests.

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