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Research article

## Absorción de Nitrógeno inorgánico en rodales de *Pinus hartwegii* Lindl. en diferentes altitudes y exposiciones

### Inorganic Nitrogen uptake in *Pinus hartwegii* Lindl. stands at different altitudes and exposures

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#### Abstract

To understand the N cycle in *Pinus hartwegii* ecosystems, it is important to investigate the movement of this element in these forests to propose forest management strategies based on knowledge of biogeochemical processes. This paper shows results of an experiment with young *Pinus hartwegii* trees to study the uptake of two N forms, ammonium and nitrate. N uptake was measured using <sup>15</sup>N sources of ammonium sulfate and potassium nitrate, with enrichments of 49.2 and 56.7%, respectively. The variation factors included two elevation levels (3 500 and 3 900 m) and two slope aspects (Northwest and Southwest), composing a 2<sup>3</sup> factorial experiment. In addition, surface soil N mineralization (10 cm) was evaluated over a year in the same sites. Results indicate that the percentage of Nitrogen derived from the labeled source (*NDDF*) in foliage was higher in the Southwest exposure. However, the net mass recovery of <sup>15</sup>N was higher in the Northwest sites ( $P < 0.05$ ), due to higher foliar mass in this aspect. Nitrate uptake exceeded ammonium uptake by 63 % ( $P < 0.05$ ). The mineralization of soil nitrogen, in relation to altitude and aspects, did not show a defined trend. *P. hartwegii* adapts to seasonal conditions for the acquisition of both forms of nitrogen, allowing it to colonize adverse high-mountain sites. *P. hartwegii* stands at low elevations (3 500 m) and with Southwestern exposure are more susceptible to alterations in the N cycle.

**Key words:** Nitrogen absorption, high mountain forests, Nitrogen forms, Nitrogen mineralization, topography, use of <sup>15</sup>N.

#### Resumen

Para comprender el ciclo del N en los ecosistemas de *Pinus hartwegii* es importante investigar sobre el movimiento de este elemento en dichos bosques para proponer estrategias de manejo forestal sustentadas en el conocimiento de procesos biogeoquímicos. Este trabajo muestra resultados de un experimento de fertilización con árboles jóvenes de dicha especie, en el cual se determinó la absorción de dos formas de Nitrógeno: amonio y nitrato. Se usaron dos fuentes con <sup>15</sup>N: sulfato de amonio y nitrato de potasio, enriquecidas a 49.2 y 56.7 %,

respectivamente. Los factores de variación incluyeron dos altitudes (3 500 y 3 900 m) y dos exposiciones (noroeste y suroeste), con un experimento factorial 2<sup>3</sup>. Adicionalmente se evaluó la evolución de la mineralización del N anual del suelo superficial (10 cm). Los resultados indican que el porcentaje de Nitrógeno foliar, derivado de las fuentes con <sup>15</sup>N (*NDDF*), fue mayor al suroeste, pero la recuperación neta en masa de <sup>15</sup>N fue superior al noroeste ( $P < 0.05$ ), debido a la mayor masa foliar en esta exposición. La absorción de nitrato superó a la de amonio en 63 % ( $P < 0.05$ ). La mineralización del N edáfico, con respecto a la altitud y exposición, no mostró una tendencia definida. *P. hartwegii* se adapta a las condiciones estacionales para la adquisición de ambas formas de N, lo que le ha permitido colonizar sitios adversos de alta montaña. Los rodales de *P. hartwegii* en bajas altitudes (3 500 m) y con exposiciones suroeste son más susceptibles a las alteraciones del ciclo del N.

**Palabras clave:** Absorción de Nitrógeno, bosques de alta montaña, formas de Nitrógeno, mineralización de Nitrógeno, topografía, uso de <sup>15</sup>N.

## Introduction

*Pinus hartwegii* Lindl. forests are distributed at altitudes above 3 000 m, and form pure stands associated with grasslands. The biogeochemical cycles in these forests will be affected as temperature increases (Correa-Díaz *et al.*, 2019). In addition, changes in nutrient mobility in this type of ecosystem would compromise the vitality of this kind of forests, which are composed of individuals up to 500 years old (Biondi, 2001) and which represent an important area in the provision of hydrological services (Bolaños-Sánchez *et al.*, 2021).

The effects of climate change in high mountain forests are also reflected in an increase in the loss of vigor and mortality of trees (Sáenz-Romero *et al.*, 2020), which indirectly influence nutrient cycling. For example, as a consequence of the removal of dead trees due to extreme drought and canopy opening, nitrogen mineralization and leaching can be stimulated by changes in soil moisture and temperature. However, the quality of organic residues in the soil is also a determining factor in N mineralization (Prescott *et al.*, 2003).

Due to their location, *P. hartwegii* forests do not have the possibility of migrating to higher altitudes to compensate for increases in temperature, since, as the elevations are higher, the soils are shallower and their ability to provide water and nutrients is reduced (Gómez-Guerrero *et al.*, 2021). In addition, the coarse textures that exist at higher elevations do not favor the incorporation of organic matter and organic N into the soil matrix and its retention against leaching, mainly of nitrate (Gómez-Guerrero and Doane, 2018). In the treeline regions, Nitrogen mineralization is limited by low temperatures that affect soil microbial activity, and this influences the nutritional status of trees (McNown and Sullivan, 2013); however, in addition to altitude, other factors such as the amount of N in the soil and the C/N ratio explain the mineralization rates and the movement of N in forests (Bonito *et al.*, 2003; Baldos *et al.*, 2015).

Although the relationship does not seem so obvious, the constant increase in CO<sub>2</sub> in the atmosphere can indirectly influence the N cycle in forests by stimulating primary productivity and increasing root exudates. The relationship between these processes is that the higher the atmospheric CO<sub>2</sub> concentration, the higher the productivity and stimulation for stomatal closure, which results in lower transpiration, higher soil moisture and N mineralization (Schleppi *et al.*, 2012). These effects were investigated in mixed forests in Switzerland and it was concluded that the amount of nitrates in the soil increased, which was explained by an increase in mineralization rates; it was found that this impact could lead to increased nitrate leaching and N limitation in the medium term (Schleppi *et al.*, 2012).

Johnson *et al.* (2010), when studying some coniferous forests, detected isolated points with disproportionately higher production of nitrate and ammonium in the soil, which would be associated with greater microbial activity or alterations in the N cycle. Although the causes of this high spatial variation were not fully explained, the authors

point out that these greater losses of N from the ecosystem could be the reason for lower availability in the ecosystem, as a result of the increase in temperature.

It is possible to investigate the movement of N between the soil and the plant with tracer of  $^{15}\text{N}$ , analyzing at the same time different forms of inorganic N such as nitrate and ammonium (Gómez-Guerrero and Doane, 2018). At the plant level, studies with  $^{15}\text{N}$  indicate greater absorption of nitrate than of ammonium, possibly due to the greater mobility of nitrate; however, at the ecosystem level, when considering the organic and mineral horizons of the soil, N pools could be similar if the net mass of both forms is added (Gurmesa *et al.*, 2022). Zhou *et al.* (2021) have identified that, at the plant level, conifers are capable of efficiently absorbing both forms, nitrate or ammonium, which indicates their high capacity to adapt to the variation of inorganic forms of nitrogen in the soil.

Therefore, it is relevant to investigate the movement of N in *Pinus hartwegii* forests. In Mexico, studies related to the N cycle and forests are scarce, especially in high mountain forest areas (Torres-Duque *et al.*, 2022a, 2022b). In this work, it was proposed to study the absorption of nitrate and ammonium by trees of the aforementioned species through tracers with  $^{15}\text{N}$ . To include greater variation in the selected sites, different altitude and aspect conditions were considered since both affect soil temperature and moisture and, therefore, N mineralization (Binkley and Fisher, 2019).

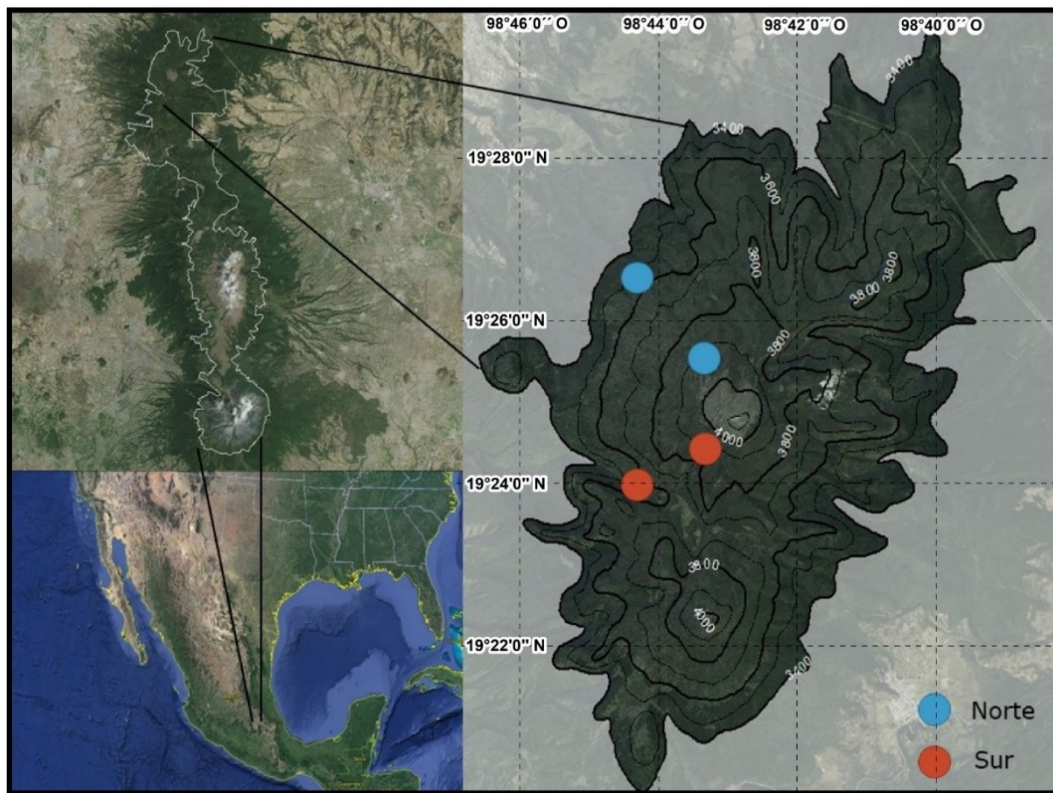
The hypotheses that were tested were that: (1) The absorption of inorganic forms of N varies with the elevation and slope aspects of the site, due to the topographic effects on soil temperature and moisture, (2) *P. hartwegii* trees absorb nitrate and ammonium from the soil in the same proportion, and (3) The variation in N mineralization in the surface soil explains the absorption of sources labeled with  $^{15}\text{N}$ .

The information generated is useful to understand the functioning of high mountain forests and to propose management strategies for this ecosystem in the face of the threat of climate change.

## Materials and Methods

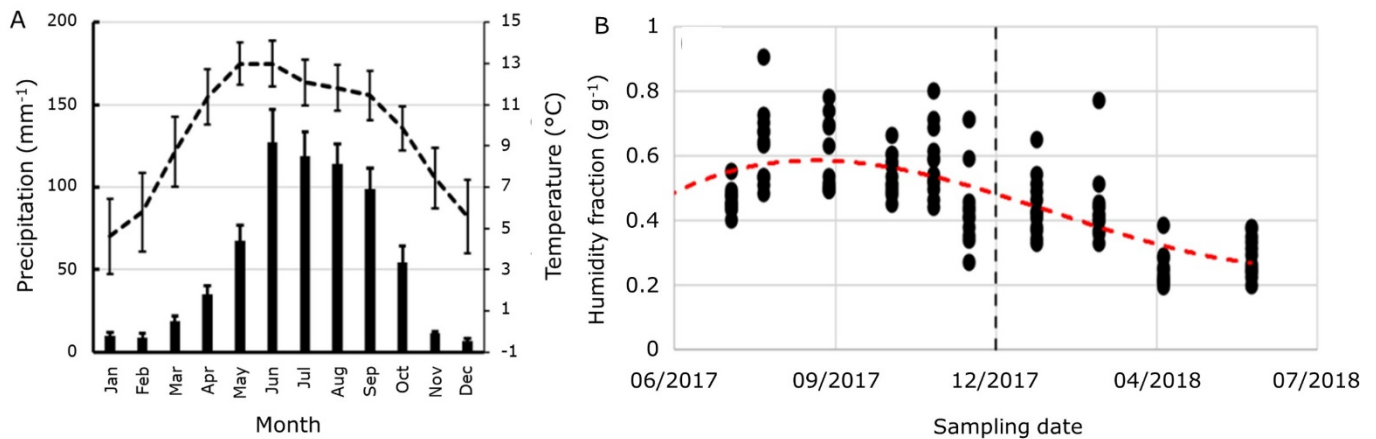
### Study area

The study area was Mount *Tláloc*, State of Mexico (19°23'06" N, -98°44'17" W), belonging to the *Iztaccíhuatl-Popocatepetl* National Park (Figure 1). Mount *Tláloc* has a maximum elevation of 4 125 m and has a semi-cold climate. The average annual temperature and precipitation vary from 7 to 9 °C and from 1 100 to 1 300 mm, respectively (Correa-Díaz *et al.*, 2021). The forest vegetation is dominated by the mixture of *Abies religiosa* (Kunth) Schltdl. & Cham. and *Pinus hartwegii* up to the 3 500 masl, and, above this, pure stands of *Pinus hartwegii* are located up to about 4 000 masl. Figure 2 shows information on the distribution of precipitation during the year and the trend in surface soil moisture (10 cm). On the West side of the mountain, four sites were located with two altitude levels (3 500 and 3 900 m) and two exposures (Northwest and Southwest).



N = North; O = West; *Norte* = North; *Sur* = South. Colored circles indicate study sites according to their exposure and altitude. White line indicates the border of the *Iztaccíhuatl-Popocatepetl* National Park.

**Figure 1.** Location of the study area.



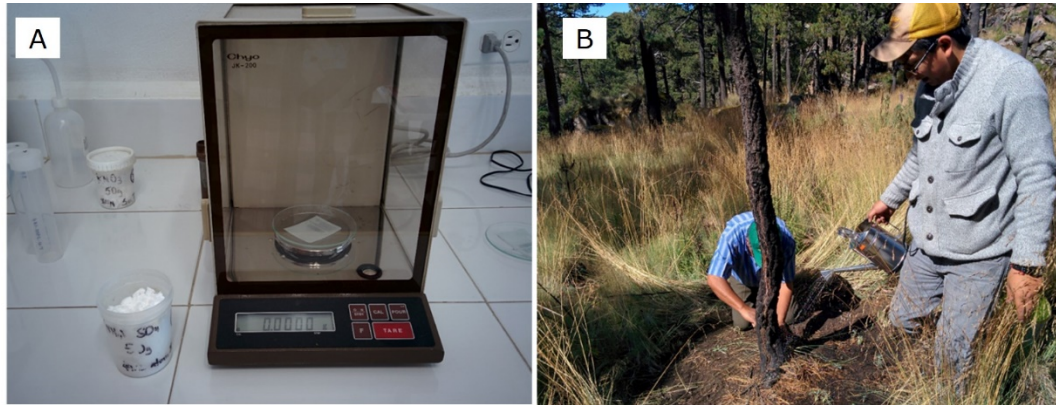
A = Distribution of annual precipitation and temperature variation, constructed with 14 meteorological stations near Mount *Tláloc*; B = Variation in soil moisture at the study sites in the years 2017-2018. The dotted red line indicates the general trend (fitted with a third-order polynomial equation) and the vertical dotted line indicates the separation of the years 2017 and 2018.

**Figure 2.** General climatic information of the study area and surface soil moisture condition (10 cm).

### **<sup>15</sup>N fertilization**

In autumn 2016, trees were fertilized with tracers of <sup>15</sup>N, using two absorbable forms, ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ). For this purpose, ten young trees ( $DN < 15$  cm and total height  $< 4$  m) were selected at each site, considering eight trees for the fertilization treatment (four for each form of N), plus two control trees to which only distilled water was applied; which totaled 40 trees in the experiment.

For fertilization, 1.0 g of ammonium sulfate ( $(^{15}\text{NH}_4)_2\text{SO}_4$ , enrichment of 49.2 %) per tree was used (Figure 3A), applied by dissolving and watering at the base; surrounding vegetation was removed (4 m<sup>2</sup>) and applied at a rate of 5 L per m<sup>2</sup> (Figure 3B). In a similar way, 1.4 g potassium nitrate ( $\text{K}^{15}\text{NO}_3$ , 56.7 % enrichment) was applied per tree. In both cases, the use of amounts higher than the natural inputs of the system (0.27 g N m<sup>2</sup> year<sup>-1</sup>) was avoided to prevent over-fertilization effects in the treated trees (Gomez *et al.*, 2002).



A = Weighing of fertilizers enriched with the Nitrogen isotope ( $^{15}\text{N}$ ); B = Removal of surrounding vegetation prior to fertilization with  $^{15}\text{N}$ .

**Figure 3.** Methodological procedure for the application of marked fertilizer.

### Estimation of N in foliage

To assess the concentration of  $^{15}\text{N}$  absorbed, foliage from the fertilized and control trees in 2016 and 2017 was sampled at each observation site in the summer of 2017. The foliage was dried in an oven (model Fx14-S Sheldon®) for 48 h at 75 °C until constant weight, then ground and placed in tin microcapsules for  $^{15}\text{N}$  analysis ( $\approx 5$  mg). Foliage was sampled from mature trees ( $DN > 30$  cm and total height  $> 10$  m) to obtain additional and confirmatory information on the natural isotopic abundance of  $^{15}\text{N}$  in *P. hartwegii* stands. The analysis of  $^{15}\text{N}$  composition in foliage was performed at the Stable Isotope Laboratory at the University of California, Davis, USA. The  $^{15}\text{N}$  composition and total Nitrogen determinations in the leaf samples were also determined.



## **Estimation of biomass and total foliage of the tree**

The total biomass and foliage of the sampled trees was estimated using the equations developed by Carrillo et al. (2016), which consider a distribution of 65.3% in the stem, 23.8% in branches and 10.9% in foliage of *Pinus hartwegii* trees.

## **Mineralization of N in the surface soil**

To measure the mineralization of the surface soil, mineral soil samples were taken from the first 10 cm deep for 10 dates along the year. The extraction of the N forms, ammonium and nitrate, was done with a 2 M KCl solution; determination was done by the micro-Kjeldahl method, by steam distillation as indicated in the AS-08 method of the NOM-021-SEMARNAT-2000 (Semarnat, 2002) and in Bremner and Keeney (1966).

## **Statistical analysis**

**Experiment with  $^{15}\text{N}$ .** To confirm that there was  $^{15}\text{N}$  absorption in the trees of the experiment, the means of foliar  $^{15}\text{N}$  composition between labelled and unlabelled trees were compared using the Tukey test with a 95 % significance level. Composite samples of current foliage and foliage from the previous year were analyzed, since the leaf mass of *Pinus hartwegii* is mainly composed of the last two leaf cohorts. The percentage of N derived from the enriched source and the net percentage of  $^{15}\text{N}$  recovery in each treatment were considered as main response variables (Cabrera and Kissel, 1989; Chávez-Aguilar *et al.*, 2006).

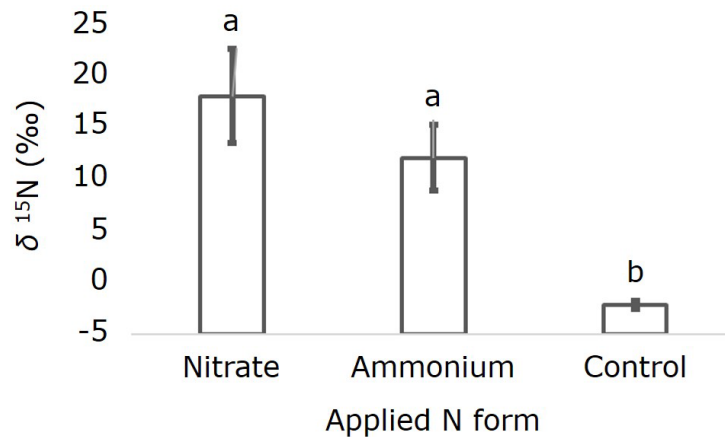
The normal distribution of the variables was verified using the Shapiro-Wilk test and since the data did not show a normal distribution, they were transformed into logarithms. The data were then analyzed as a  $2^3$  factorial design in which the factors of altitude, exposure, and Nitrogen form had two levels.

**N mineralization experiment.** Since repeated samples were taken over time in this case, N mineralization was analyzed by longitudinal analysis (Littell *et al.*, 1998) where each of the four combinations of altitude and exposure had 10 measurement dates during the year.

## **Results and Discussion**

### **Evidence of $^{15}\text{N}$ uptake**

Results on the uptake of  $^{15}\text{N}$  sources showed that trees in the experiment that were fertilized with both forms of N (ammonium or nitrate) recorded higher values of  $^{15}\text{N}$  isotopic composition than control trees (Figure 4). The trees that were fertilized with  $^{15}\text{N}$  showed greater variability in N isotopic composition ( $\delta^{15}\text{N}$ ) compared to unfertilized trees, which is an expected result since the natural abundance of  $^{15}\text{N}$  in foliage is very stable in natural ecosystems (Silva *et al.*, 2015). Likewise, no differences were observed in the  $\delta^{15}\text{N}$  concentrations of foliage of control trees and mature trees taken as an additional reference.



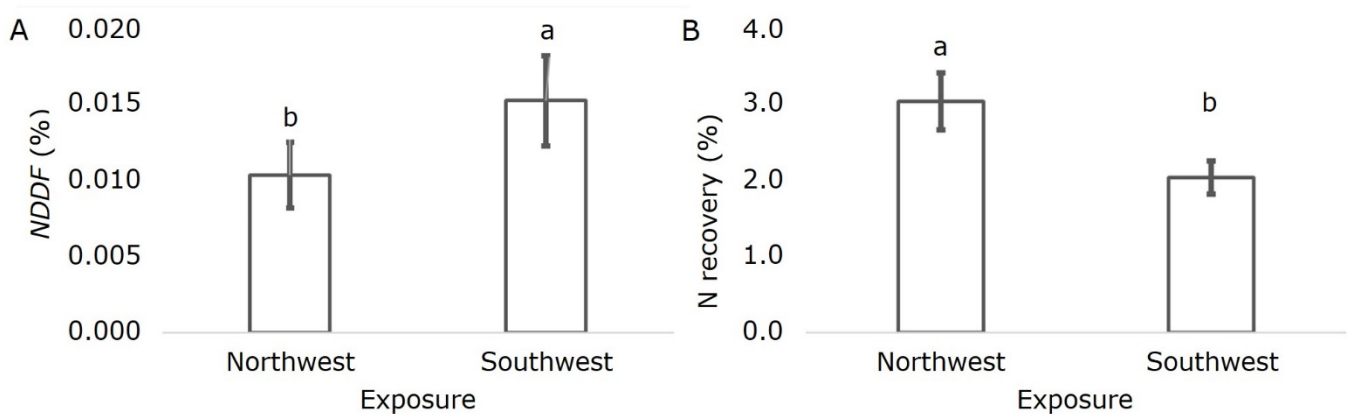
The bars represent the standard error. The equal letters above the bar indicate statistically equal treatments ( $P>0.05$ ).

**Figure 4.**  $^{15}\text{N}$  composition between marked trees and control trees.

### Effect of exposure

Statistical analysis did not indicate significant interactions on  $^{15}\text{N}$  source uptake; however, the effect of exposure and inorganic N forms were significant ( $P<0.05$ ).

Source-derived nitrogen (*NDDF*), which refers to the percentage of N represented by the marked sources integrated into the foliage, was higher in the Southwest exposure (Figure 5A). But the percentage of Nitrogen recovered from the labelled sources was higher in the Northwest exposures. The results indicate that in both aspects, the  $^{15}\text{N}$  applied to the soil was mobilized to the foliage of the trees (Figure 5A), but considering the net amounts with respect to the amount of  $^{15}\text{N}$  initially applied to the soil, a greater mass was recovered in the Northwest aspects (Figure 5B).



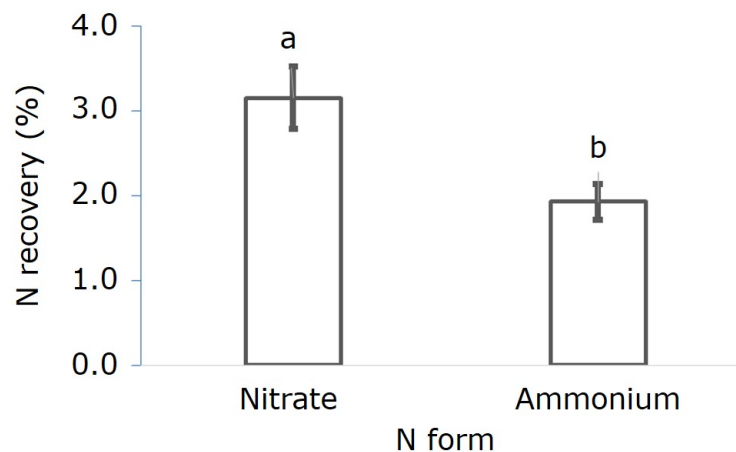
A = Percentage of N derived from labeled fertilizer; B = Percentage of labeled fertilizer recovery. Bars correspond to standard error and statistical differences at 5 %.

**Figure 5.** Identification of N movement from soil to foliage.

Regarding the first hypothesis, this was only partially fulfilled since while aspect was significant, altitude was not. That is, this variable was not relevant for the absorption of the sources labelled with  $^{15}\text{N}$ .

## Differences between N forms

The form of N was statistically different ( $P < 0.05$ ). More nitrate- $^{15}\text{N}$  was recovered than ammonium- $^{15}\text{N}$ , thus rejecting the second hypothesis of the study (Figure 6). This result indicates that nitrate was the form mostly absorbed by *P. hartwegii* trees, and this is consistent with the results of Gurmesa *et al.* (2022). In a study with *Abies religiosa*, Chávez-Aguilar *et al.* (2006) also confirmed the preference for nitrate absorption over ammonium in both foliar application and soil application treatments. However, this result contrasts with that of Liu *et al.* (2017), who concluded that, in both tropical and temperate ecosystems, trees preferentially absorb ammonium or organic forms compared to nitrate. Despite the discrepancies that still exist in the literature regarding the preference between ammonium and nitrate, the results of this study could be explained by the high mobility of nitrate in the soil. Another explanation for a greater net recovery of N towards Northwest exposures is the fact that in these aspects the soil moisture is higher during the year due to receiving less solar radiation, compared to the Southwest aspect.



Bars correspond to standard error.

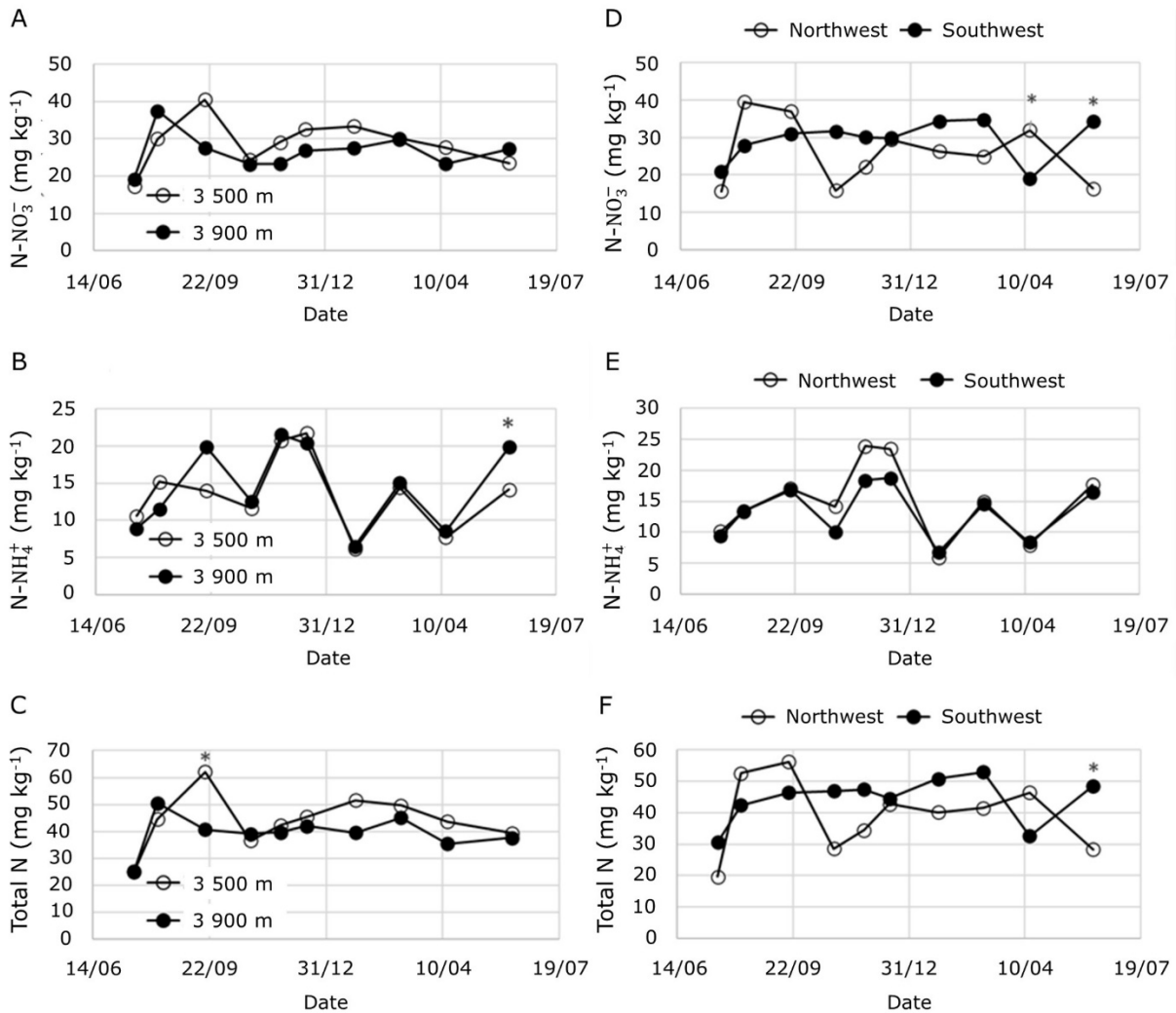
**Figure 6.** Recovery of  $^{15}\text{N}$  by N form.

From an energetic point of view and due to the charge differential, greater assimilation of ammonium in the trees would be expected; in addition, the positive charge in this way limits its mobility in the soil and there is also a great demand for ammonium by microorganisms in forest soils (Gurmesa *et al.*, 2022). Possibly, the explanation for greater nitrate absorption is that the accumulation of ammonium in the plant is a potential risk of toxicity if it accumulates in the plants (Gerendás *et al.*, 1997; Schlesinger and Bernhardt, 2020). Therefore, despite the lower energy cost that ammonium represents for absorption purposes, nitrate was absorbed more efficiently.

### **Nitrogen mineralization**

The variation of nitrate and ammonium, as well as total N (sum of ammonium and nitrate) was similar over time, without showing significant differences by altitude or aspect. However, at the level of specific dates some significant differences were identified. For example, nitrate mineralization was higher in the Northwest in the month of April and the opposite in the month of June when more nitrate was observed in the Southwest aspect (Figure 7D). This alternation in the amount of nitrate could be related to soil humidity and aeration, since the month of April corresponds to the dry season and even in sites with Northwest aspect the soil moisture content decreases. In the case of the Southwest exposure it is likely that the soil was also found with low

levels of humidity, since the measurement corresponds to the beginning of the month of June when the rainy season is establishing.



A, B and C = Show changes with elevation; D, E and F = Show changes with aspect.

**Figure 7.** Mineralization of N over time, nitrate, ammonium, and total N, assessed from July 2017 to June 2018, in the top 10 cm of topsoil in *Pinus hartwegii* Lindl. stands.

June ammonium amounts were significantly higher at 3 900 m elevation compared to 3 500 m elevation (Figure 7B), which is also consistent with higher soil moisture contents at 3 900 m. With respect to total N, mineralization was higher in September and at the low elevation of 3 500 m (Figure 7C). Likewise, the Southwest aspect exceeded the Northwest aspect in total N in June (Figure 7F). Although this difference was only significant for the late June date, the data suggest that the potential for N efflux from the ecosystem is greater in Southwest aspect.

Although significant differences over time were observed, these were not maintained throughout the year evaluated; there was a trend towards higher amounts of nitrate and total N at lower elevations (Figure 7C), which is consistent with the fact that increasing elevation reduces microbial activity and thus mineralization (McNown and Sullivan, 2013; Schlesinger and Bernhardt, 2020). In summary, according to the results of this study, low elevations of the natural distribution of *P. hartwegii* (3 500 m), with Southwest aspects, are the locations most susceptible to showing disturbances in the N cycle.

### **Relationship between N mineralization and <sup>15</sup>N uptake**

No clear relationship was observed between the <sup>15</sup>N uptake experiment and the N mineralization trends during the year. Therefore, the third hypothesis is rejected. One explanation for this lack of correlation is that while the <sup>15</sup>N experiment was conducted in the fall season, the mineralization study represents changes throughout the year. Since active N uptake also depends on the growth phase of the tree, the question for future studies is whether the preference for nitrate is



maintained throughout the year. So far, large-scale studies do support this hypothesis (Gurmesa *et al.*, 2022).

## **Conclusions**

The results suggest that *P. hartwegii* absorbs more nitrate compared to ammonium, since the absorption efficiency is influenced by the forms of N available in the soil and by the seasonal conditions, which has allowed it to colonize high altitude sites with adverse conditions in climate, soil and N availability. The aspect variable has a greater effect on N absorption than the altitude variable.

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## **Conflict of interest**

The authors declare that they have no conflict of interest.

## **Contribution by author**

Arian Correa Díaz: establishment and conduction of field experiment, writing drafts, preparation and handling of experiment samples; Armando Gómez Guerrero: planning of the field experiment, writing of the draft, and analysis and interpretation of results; William R. Horwath: planning of the experiment, analysis of results, and funding of the research.

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