Nutrient addition increases insect herbivory in *Nothofagus antarctica* on North-Patagonian forests

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Abstract

The change in nutrient availability in the soil can affect key functions of forest ecosystems, such as insect herbivory. Although insect herbivory is particularly relevant because it can impact on the growth and mortality of forest plant species, the evidence of the impacts of fertilizers addition on herbivory patterns is limited. In this study, we specifically evaluated how herbivory frequency on the timber species *Nothofagus antarctica* is affected by the addition of nitrogen (N), phosphorus (P), potassium (K) and their combinations to the soil, using a long term full-factorial field experiment in a North-Patagonian Forest (Argentina). Our study investigated the effects of fertilizers (N, P, and K) on herbivory frequency and leaf nutrient concentrations of the studied species. We found that the addition of fertilizers to the soil increased herbivory frequency, which was mediated by increases in leaf nutrient concentrations. Furthermore, we observed that interactions between fertilizers in leaf nutrient concentrations generally produced increases in herbivory, both in the amount of leaf consumed and in the foliar damage percentage. These findings suggest that fertilization can have complex effects on plant-herbivore interactions, and that the specific effects may depend on the identity of the nutrients that combined, the plant species, and the level of nutrient availability. Our results provide evidence that changes in soil nutrient availability are important in forest ecosystems, and that should be considered to develop effective management strategies for the sustainable use of natural resources in Patagonian forests.

Keywords: Herbivory; Phosphorus; Nitrogen; Potassium; Nutrient addition

1. Introduction

Nutrient enrichment can promote changes in ecosystem processes, such as the patterns of herbivory (Garibaldi et al., 2010). This is because the addition of fertilizers, such as nitrogen (N), phosphorus (P), and potassium (K), can drive changes in plant growth and tissue quality, and ultimately disturb trophic cascades (Forkner and Hunter 2000; Boyer et al., 2003). Due to increased nutrient limits in the soil, leaf chemical composition may change, impacting leaf palatability and herbivore feeding responses (Garibaldi et al., 2010; Cleland and Harpole 2010; La Pierre and Smith 2016). Insect herbivores are directly influenced by changes in food quality (Throop and Lerdau 2004; La Pierre and Smith 2016) and chemical and physical defences of plants (Chen et al., 2010). The quantity and accessibility of selected food are decisive for the physiological processes and population dynamics of herbivorous insects (Poorter et al., 2004; Boersma and Elser 2006; Zehnder and Hunter 2009). The nutritional content of herbivorous insects is lower than that of the plants they consume, leading to potential shifts in their feeding behaviour in response to changes in the nutritional quality of available food sources (Throop and Lerdau 2004). The herbivorous community can modify their feeding rates because of changes in palatability and other leaf traits, mediated by the addition of fertilizers on forest soils (Poorter et al., 2004). Forest populations may show variable responses to nutrient additions, depending on environmental conditions and plant functional traits (Garibaldi et al., 2010; Menge et al. 2002). Therefore, it is important to understand how native forests respond to different soil management strategies to design sustainable management plans.

The availability of N for plants is a determining factor for their growth, morphology, biochemistry and physiology (Barbour et al., 1991, Rashid et al., 2016). Under conditions of high availability, a plant could invest more in defence metabolites that contain N (Muzika 1993; Gebauer et al., 1998; Mutikainen et al., 2000). In addition, plants can produce volatile substances when subjected to herbivore damage, to repel pests or attract natural enemies, and this can increase under conditions of higher N availability in the soil (Turlings and Erb 2018). This would lead to a decrease in the consumption of leaf matter by herbivorous insects. In contrast, White and collaborators (1984) found that high N availability can lead to an increase in soluble proteins, sugars, and amino acids in leaves, which increases their palatability and, consequently, their consumption by insect herbivores. In this way, the addition of N to the soil could cause an increase in insect herbivory damage. Deciduous plant species, in particular, store N in perennial organs and then mobilize it to the leaves during the growing season, making them more attractive to generalist herbivores that feed on leaves (Millard and Grelet 2010). In contrast, perennial species may contain less N in the leaves because they distribute resources more equitably due to their need for sustainable resource use and N efficiency (González-Paleo et al., 2019). For example, in a previous study conducted under the same experimental conditions, we observed that herbivory decreased with the addition of nitrogen (Pérez-Méndez et al., 2022). This could be due to the lower N content in the leaves of the perennial specie we worked with (*Berberis microphylla*), which would make it less attractive to herbivores. Given the complex interplay between N availability, plant strategies, and herbivore interactions, it is essential to further investigate how life history and leaf traits influence N conservation and use strategies in plants (González-Paleo et al., 2019). Consequently, we decided to conduct a new study using a deciduous species of the genus *Nothofagus* as a model, under same experiment. Also, this genus is more consumed for generalist insect herbivores in our study site (Nacif et al., 2020, Garibaldi et al., 2010).

Producers and consumers use P for key cellular processes such as ATP and nucleic acid (RNA and DNA) synthesis, and protein production (Sterner and Elser 2017). Phosphorus is a key nutrient that enables the maintenance of essential cell functions and, consequently, of organisms. As a result, an increase in soil P availability can enhance plant P use efficiency, ultimately affecting herbivorous consumers (Elser et al., 2000; Sterner and Elser 2017). An increase in available P in the diet can lead to significant alterations in the survival, fecundity, oviposition rate, growth, and population density of herbivorous insects. In this way, insects can increase their feeding rates because of an increment of their populations (Huberty and Denno 2006). Finally, although there are few studies that have evaluated the effect of K on herbivory patterns. In plants, K plays several physiological functions, including the regulation of enzymatic activity, which can alter the palatability of leaves (Wang and Wu 2017; Ibrahim et al., 2012). Additionally, K is involved in the formation of soluble sugars, organic acids, amino acids, and amides in plant tissues, which are ultimately important for the diet of insects that feed on the leaves (Wang and Wu 2017). It is also an important regulator of secondary metabolites which can be used by plants to repel herbivorous insects (Erb and Kliebenstein 2020). Therefore, the direction and strength of the response of herbivores to nutrient addition may vary depending on the specific nutrient, physiological traits of plants, coevolution with their associated herbivores, and the context in which it is added (Kurze et al., 2018; Fischer and Fiedler 2000). Despite the multiple ways through which nutrient addition can propagate towards higher trophic levels, there is little evidence on the potential relationships between nutrient addition and changes in herbivory patterns (Poorter et al., 2004). It is known that these nutrients can interact with each other and thus affect foliar chemistry in different ways (Wu et al., 2020; Güsewell 2004). It is important to note that the interactions between nutrients can be both positive and negative, and can significantly influence the plant nutrition (Güsewell 2004). For instance, nitrogen fertilization can stimulate P mobilization through an increase in phosphatase production or activity increasing their concentration (Treseder and Vitousek 2001). In addition, low K availability could inhibit N uptake and significantly reduce its content in leaves (Hu et al., 2017), although a positive synergistic effect of the two nutrients has also been reported (Ruan et al., 1998; Ruiz and Romero 2002). Understanding these interactions is essential for turn predicting how plant-herbivore interactions may be affected. Because of this, we decided to set up a long-term field experiment of nutrient addition and evaluate their interactive effects on insect herbivory.

In forest systems, herbivory patterns are especially relevant since herbivores can impact on the growth and mortality of plant species with economic interest (Rusch et al., 2017). Patagonian shrublands are used as silvopasture systems, for wood extraction and sheep and cattle production (Collado 2009; Peri and Ormaechea 2013). Despite the fact that the shrublands of *Nothofagus antarctica* (G. Forst.) represent a large part of the native forests in Patagonian woodlands, there remain some important knowledge gaps for their sustainable management

(Peri et al., 2009; Grosfeld et al., 2019). Currently, there are no studies that evaluate how soil management, particularly the addition of fertilizers, can affect plant-herbivore interactions in this ecosystem. In this study, a manipulative field experiment on fertilization was conducted to evaluate how the changes in soil nutrient availability impact on the foliar traits of *N. antarctica* and herbivory frequency. Specifically, we used a full factorial experimental design that allow us to evaluate all potential interacting effects among nutrients (no nutrient addition, N, P, K, NP, NK, PK and NPK) on soil nutrient content, leaf nutrient concentration and insect herbivory. We initially established two competing hypotheses about the relationships between nutrient addition and the cascading effects that ultimately affect herbivory patterns. The first hypothesis (H1) postulates that the addition of nutrients (N, P, and K and their combinations) to the soil would affect the chemical composition of leaves, increasing both the concentration of leaf nutrients and the attractiveness for herbivorous insects, ultimately increasing leaf consumption. On the contrary, the second hypothesis (H2) suggests that insects require a greater amount of leaf material to reach maturity when the material is low in nutrients, leading to an increase in the consumption of leaves by these insects. We expect that: *i*) the addition of N, P, K and their combinations, increases soil nutrient content, *ii*) the increase of soil nutrient content increases foliar nutrient concentration, *iii*) an increase in foliar nutrient concentration enhances the frequency of insect herbivory due to increased food quality (H1), or alternatively, *iv*) a decrease in foliar nutrient concentration increases the frequency of insect herbivory by reducing food quality (H2).

2. Materials and methods

2.1 Study site

The study site is located in "El Foyel", Rio Negro, Argentina (41°38′37″S, 71°26′54″W). The climate of the region is temperate-cold, with an average annual temperature of 3 °C in winter and 15 °C in summer, presenting frequent frosts during the months of June through August. Rainfall is concentrated during autumn and winter with an annual average between 920 – 1300 mm (Reque et al., 2007). The site is located at an altitude between 790 - 880 m asl and its soils are of the Hapludands type (Diehl et al., 2008) limited in nutrients, mainly N (Perakis and Hedin, 2002). The experimental plots are located within a private field composed of native mixed shrublands typical of the northern Patagonian forest (Figure. 1). The vegetation is dominated by shrubs species and some short trees, such as *N. antarctica* (focal species of this study), *Schinus patagonica* (Phil.) I.M. Johnst., *Discaria chacaye* (G. Don) Tortosa, *Berberis microphylla* G. Forst., *Lomatia hirsuta* (Lam.) Diels, *Embothrium coccineum* J.R. Forst. & G. Forst. and *Diostea juncea* (Gillies & Hook. ex Hook.) Miers (Reque et al., 2007). We used *N. antarctica* as our study focal species as *i*) it is one of the most abundant and dominant plant species of North-Patagonian forests, and *ii*) it has a high commercial value as a timber species. *N. antarctica* is a deciduous species that occurs in different morphotypes, from shrubs between 2 to 4 m tall, up to 15 m tall trees under favourable soil conditions (Ramírez et al., 1985). In our study system, the community of herbivorous insects is represented by the orders Lepidoptera (Geometridae, Noctuidae, Saturnidae, Heterobathmiidae), Coleoptera (Curculionidae, Buprestidae), Hymenoptera (sawflies, Cynipidae), Homoptera (Psyllidae), Diptera (Tephritidae) and Hemiptera (McQuillan 1993; Gentili and Gentili 1988).

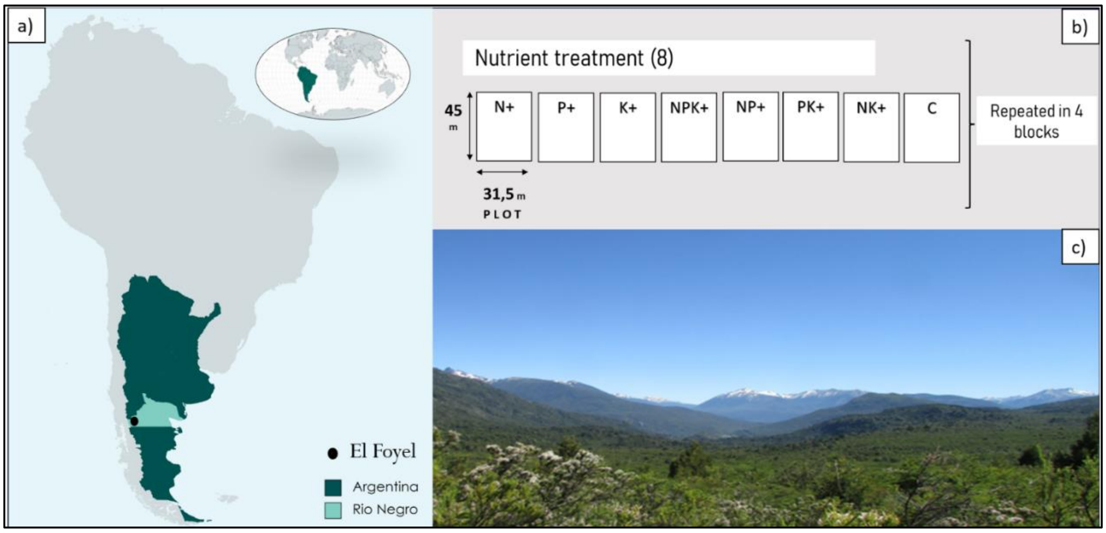

Figure 1**.** Study site and experimental design. (a) Location of the study region, El Foyel, Bariloche, Río Negro, Argentina. (b) The experiment contains 32 plots distributed in four complete random blocks in a full factorial design. The treatments result from addition of N, P and K and all possible combinations and a non-fertilization control (C). (c) Photography of the Patagonian mixed shrublands.

2.2 Experimental design

The nutrient addition experiment was set in 2016, six years prior to our study. The first fertilizations were carried out during November 2016, followed by two additional fertilizations in September 2017 and 2018. After two years, a last fertilization was carried out in October 2021. The experimental design consisted in 32 plots distributed in 4 complete random blocks in a full factorial design. The size of the plots was 31.5 x 45 m and the treatments resulted from addition of N, P and K, and all possible combinations, resulting in a total of 7 experimental treatments (N, P, K, NP, NK, PK, NPK) and a control treatment (i.e., no fertilizer used), replicated in each of the 4 blocks (Figure 1). Fertilizers were applied by hand on the total surface of each experimental plot, and the doses of nutrient addition were calculated based on those used in other similar forest systems. The fertilizers applied were the following: 100 kg ha^{-1} of prolonged release urea for nitrogen (Lindberg and Persson 2004), 75 kg ha^{-1} of triple superphosphate for phosphorus, and 56 kg ha^{-1} of potassium sulphate for potassium (Kim, 2008).

2.3 Soil and leaf sampling for nutrient content analysis

Soil and leaf samples were taken from the 32 experimental plots in February 2022. Soil samples were sieved with a 2 mm mesh and dried at room temperature. For soil samples we measured nitrates (NO_3) using the Bremmer method (Bremmer and Mulvaney 1996), available phosphorus using the Bray-Kurtz method (Bray and Kurtz 1945) and available potassium by extracting with ammonium acetate method (Novozamsky and Houba 1987). To analyse *N. antarctica* nutrient, leaves were taken from eight individuals in each plot (including the six individuals on which HF was measured) and stored in paper bags. A pool of leaves from all the individuals was ground and drying for analysis. We measured nitrogen using the Kjeldahl method (Kjeldahl 1883), total phosphorus and total potassium using the Sommer and Nelson (1973) method.

2.4 Herbivory sampling

During February 2022 (three months after the last fertilization), six individuals of similar size, were selected in each experimental plot. On each individual, four branches of the middle stratum were cut, each oriented towards a different cardinal point, and collected in paper bags. For each branch, we counted the number of both healthy and damaged leaves mediated by different functional groups of herbivores. For this, we utilized the approach proposed by previous researchers (Pérez-Méndez et al., 2022, Nacif et al., 2020; Garibaldi et al., 2010), which included leaf chewers (consumers of leaf area), skeletonizers and miners. However, we found that miners and skeletonizers accounted for less than 1% of the leaf damage so we decided to only use data from leaf chewers. These results later were used to estimate the frequency of damaged leaves for each sampled branch (*n* = 768). Finally, we randomly selected 20 leaves damaged by chewers of four individual per plot and estimated the percentage of damage for leave (*n* = 2560). We used this measure to assess whether the leaf area consumed per leaf changed with the treatments.

2.5 Statistical analysis

To evaluate how the addition of fertilizers modified the composition of nutrients in the soil and leaves we constructed linear mixed models (LMMs). The response variable was the concentration of each nutrient found in soil and leaves. The addition of each nutrient with values of presence or absence was used as interacting fixed effects and the block were included in the model as random effects.

To evaluate the effects of leaf nutrient concentrations on herbivory we build two models, by a generalized linear mixed modelling (GLMM) approach. For the first model the response variable (i.e., herbivory) was represented of healthy leaves/damaged leaves in each plot and we used a binomial distribution of errors that accounts for differences in sample size. For the second model the response variable (i.e., foliar damage percentage) was represented of area consumed by chewers/total area per leaves and we used beta distribution. In both cases the fixed factors were a concentration of nutrients and their interactions (i.e., all possible combinations between the three nutrients) and the experimental block was included in the model as a random effect. We used the *lme4* package (Bates et al. 2014) for LMMs models and *glmmTMB* package for GLMMs models (Brooks et al., 2017) in R statistical program (R Development Core Team 2020). For all models, we used the *DHARMa* package (Hartig 2020) to verify the models' assumptions. To assess the significance of the variables we use Likelihood Ratio Tests (LRT) using the Anova function in *car* r-package (Fox and Weisberg 2018).

3. Results

3.1 Effects of fertilization treatments on soil and leaf nutrient

The results of significance of variables showed that the addition of N to the soil increased the concentrations of nitrates on soil and total nitrogen in leaves (Table 1, Figure 2). The addition of P increased available phosphorus on soil and total phosphorus in leaves (Table 1, Figure 2). Finally, the addition of K to the soil increased the available potassium in the soil and total potassium in the leaves (Table 1, Figure 2). Also, we found a significant interaction between N and P for leaf nitrogen content and leaf potassium content (Table 1, Figure 2). In addition, we found a significant interaction between the addition P and K for soil phosphorus content and leaf potassium (Table 1, Figure 2).

	Soil nutrient			**Leaf nutrient**		
Nutrient Added	N	P	K	N	P	K
N	**38.7*****	2.46	0.74	**11.46****	0.66	0.60
P	0.38	**7.10***	0.53	1.41	**11.87****	0.13
K	4.04	0.42	**17.1 *****	2.20	1.48	**6.60***
N:P	0.18	0.04	1.42	**6.99***	0.01	**7.36***
N:K	2.72	0.26	0.05	1.11	2.03	0.79
P:K	0.07	**4.99***	0.11	0.06	0.57	**4.68***
N:P:K	0.00	3.79	1.31	0.00	1.32	0.03

Table 1. Analysis of deviance for the effects of nutrient addition (N, P, K) on their own and combined on: soil nitrogen, soil phosphorus, soil potassium, leaf nitrogen, leaf phosphorus and leaf potassium. Values reported are F-statistic. Values in boldface indicate significant differences (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$).

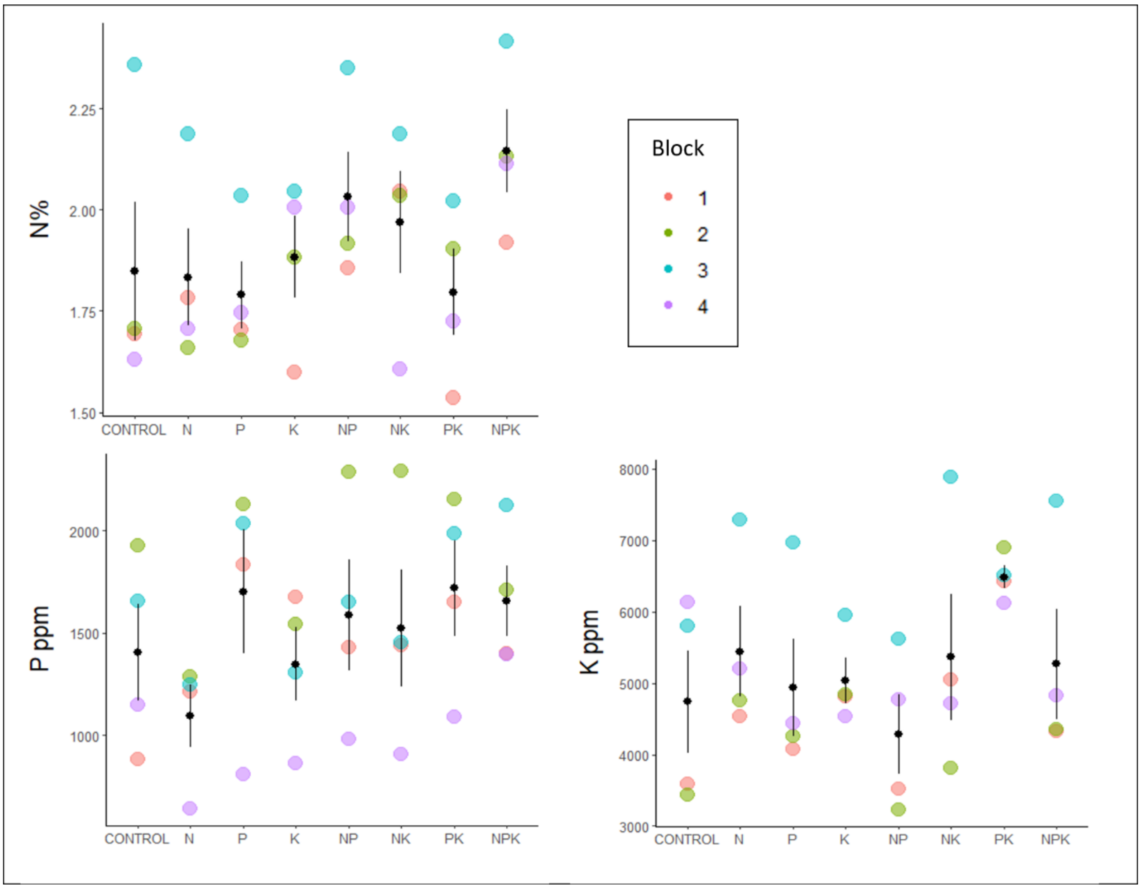

Figure 2. Leaf nutrient content measured in *N. antarctica* under different fertilization treatments (N, P, K, NP, NK, PK and NPK) and control. Data shown are the observations of each block (identified with different colours) and the mean ± SE (point black and line) of each treatment.

3.2 Effects of leaf nutrient content on herbivory

The total number of leaves analysed was 8500, from 192 individuals distributed across 32 experimental plots. We found at least one type of damage in 7016 leaves representing 82% of the analysed material. Both GLMMs results for nutrient concentrations and their interactions on herbivory and foliar damage percentage were significant (Appendix 1, Table A.1; Table A.2). In addition, the deviation analysis for the effects of each variable and their interactions was also significant (Table 2).

Our results showed that herbivory increased as leaf nutrient content increased, with the highest values of herbivory occurring when the three nutrients were at their maximum concentrations

(Figure 3a). However, we observed a reversal in the patterns of N when there was less concentration of K. In this case, when there was a lower concentration of K in the leaves, higher N concentrations decreased herbivory. (Figure 3a). We observed that herbivory generally increased when two nutrients were combined (Figure 3a). For foliar damage percentage, we found that in general it also increased with increasing nutrient concentrations (Figure 3b). The combined N and P increased the percentage of damage in low and medium K conditions. On the contrary, when the three nutrients were in high concentration the percentage of damage tends to be lower (Figure 3b).

LEAF NUTRIENT CONTENT	**HERBIVORY**	FOLIAR **DAMAGE PERCENTAGE**
N	**4.5557 ***	**10.793 ****
P	**4.8617 ***	**10.656 ****
K	**5.7832 ***	**10.786 ****
N:P	**3.9501 ***	**10.916 *****
N:K	**4.8022 ***	**11.063 *****
P:K	**5.2310 ***	**10.928 *****
N:P:K	**4.1655 ***	**11.190 *****

Table 2. Analysis of deviance for the effects of leaf nutrient concentration (N, P and K) on their own and combined on herbivory and foliar damage percentage. Values reported are chisquare tests. Values in boldface indicate significant differences (***p < 0.001; **p < 0.01; *p < 0.05).

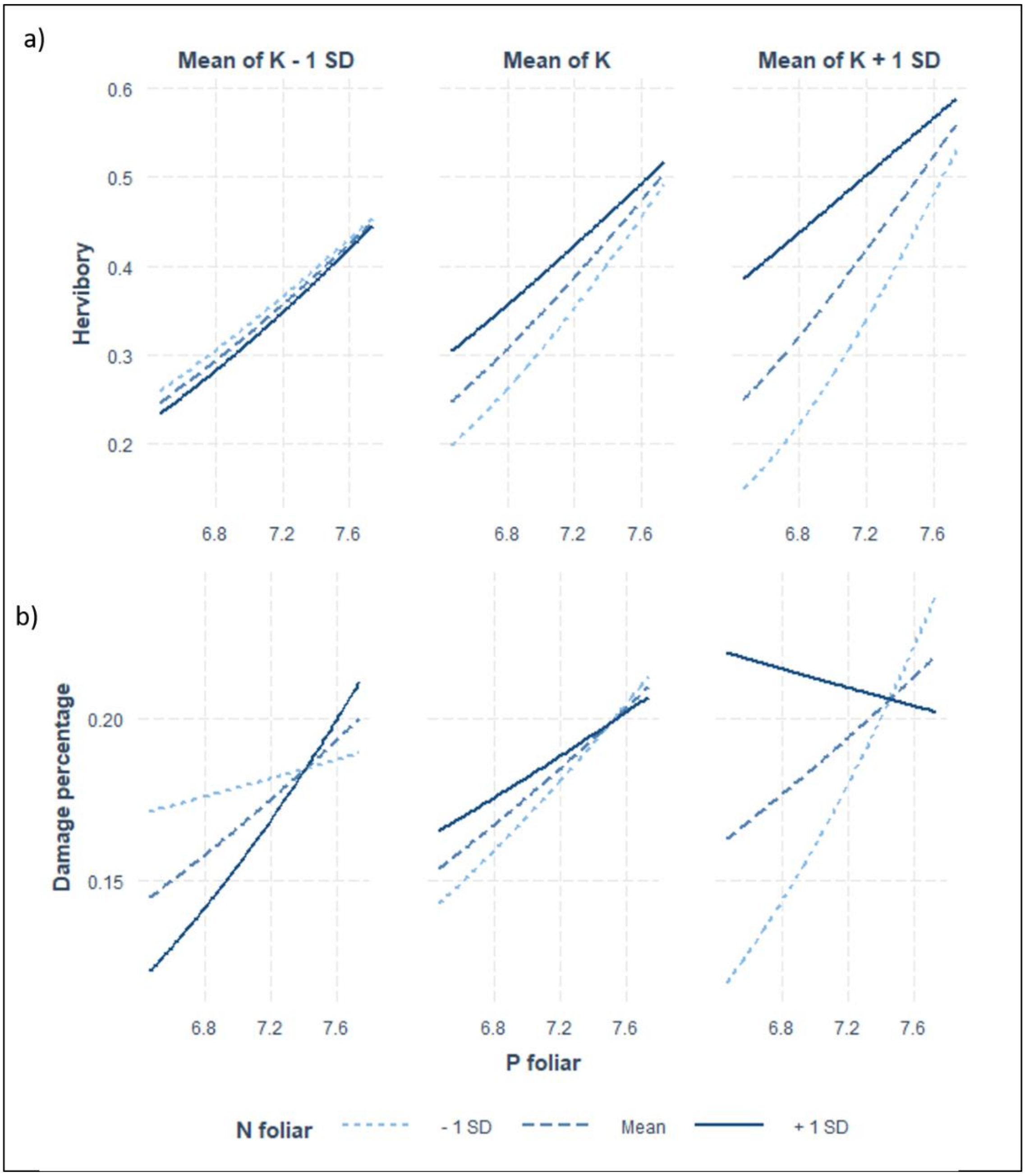

Figure 3. Plots regression lines to explore interactions between leaf nutrient content (N, P and K) on the herbivory (a) and foliar damage percentage (b) of *N. antarctica*. Note that for K and N variable show less content (-1 SD), medium content (mean) and high content (+1 SD). Herbivory and foliar damage percentage (axis Y) and nutrient P (axis X) are shown as a continuous variable.

4. Discussion

Our long-term field experiment provides strong evidence of how changes in soil nutrient availability produce effects that propagate through trophic levels and affects key ecosystem functions, such as herbivory. In this study, we found that the addition of fertilizers to forest soil increased the herbivory on *N. Antarctica* We observed that the increases in leaf nutrient concentrations were related to increases to the patterns of herbivory found and that their combination in general showed a stronger response of herbivory. This supports our initial hypothesis H1, as we expected to find a positive relationship between foliar nutrient concentrations and herbivory. Contrary to the hypothesis H2, our findings revealed a consistent positive correlation between foliar nutrient concentrations and herbivory in the majority of cases, contradicting the expected negative relationship proposed by the hypothesis. In turn, the foliar damage percentage increased with the increase in nutrient concentration, except when the three nutrients were combined in high concentrations. This result highlights the importance of soil nutrient availability in plant-herbivore interactions. Among the various ways nutrients can affect herbivores, our study reveals that food quality strongly influences their food consumption.

The addition of N to the soil modified the concentrations of N in the leaves increasing their concentration. The foliar N content was related in different ways to herbivory, depending on its interaction with other nutrients, but in general was positive relationship. In contrast, when K levels were low, N reversed its effect and showed a negative relationship with herbivory, leading to a reduction in herbivore activity. A previous study conducted on *Berberis microphylla*, a native shrub of the same region and under the same fertilization experiment in previous years, showed the reduced herbivory with N addition, yet this effect was diluted in the presence of K (Pérez-Méndez et al., 2022). This indicates that both species behave differently regarding the interaction between N and K. Leaf physiology or resource allocation within the organism itself could explain the different responses of the two species to fertilization (González-Paleo et al., 2019). Perennial leaf species, such as *B. microphylla,* allocate more nitrogen to photosynthetic tissue and achieve higher overall nitrogen use efficiency, while investing more in defences (Lundgren and Marais

2020). In contrast, deciduous species such as *N. antarctica*, could store nitrogen in other organs (roots or stems). This stored nitrogen can then be utilized during the subsequent growth seasons for the development of new leaves (Muhammad et al., 2020), making them more attractive to herbivores. On the other hand, the addition of N to the soil can lead to a decrease in the diversity of plant species and can subsequently impact in functional diversity of the community of herbivorous consumers (Joseph et al., 1993). Consequently, this scenario may contribute to the dominance of generalist herbivores, which can increase the consumption of foliar matter (Joseph et al., 1993; Blubaugh et al., 2021). As we explained previously, we know that *N. antarctica* is consumed by generalist herbivores which could be taking advantage of these conditions to increase their population density and also their level of food consumption. This mechanism could be occurring within *N. antarctica,* explaining the high herbivory found in this study.

The addition of P to the soil influenced leaf P content, which in turn are positive correlated with increases of herbivory. This may be due to the fact that many physiological processes, such as proteins, DNA and RNA synthesis, depend on the corporal P content, which ultimately will be determined by the composition of the food consumed by herbivores (Poorter et al., 2004; Hrubá et al., 2022). Consequently, it is possible that an increase in P availability could lead to a greater population growth rate or faster development, ultimately resulting in increased food consumption (Schade et al., 2003). Our results are consistent with the findings of Schade and collaborators (2003) who observed a positive relationship between leaf P content and herbivory rates. These authors investigated the Growth Rate Hypothesis (GRH) (Elser et al. 1996), which suggests a positive link between soil P availability, plant tissue P content, and the P and nucleic acid content of herbivores. According to hypothesis, insects growing under high P availability conditions are expected to have higher P and RNA contents, as well as faster growth rates. This mechanism could explain the increase in leaf consumption and the frequency of herbivory observed in our experiment due to the presence of P. In general, we observed a stronger increase in herbivory when N was combined with high levels of P. This could be attributed to the potential of N to enhance the absorption of phosphorus by plant roots (Grunes 1959), thereby intensify the impact of both nutrients. As a result, the food may exhibit a higher content of both nutrients, potentially offering better nutritional quality for herbivores and this increases food consume producing population growth.

Our results showed that the addition of K to the soil resulted in an increase in leaf K concentration and this is positively correlated with herbivory. Potassium plays an important role in plant physiology, such as water osmoregulation and cell turgor (Wang and Wu 2017; Kassen et al., 2022), which could affect the foliar water content and ultimately determine leaf palatability (Poorter et al., 2004). For instance, Poorter (2004) found that foliar traits, such as water content and leaf area, were associated with higher rates of herbivory. This could partly explain our results, which show a positive correlation between the herbivory and the presence of all three nutrients together. Specifically, the high concentration of K in leaves, could enhance the effects of foliar nitrogen and phosphorus content, making the leaves more palatable and attractive for insects. Despite the importance of plant nutrients for herbivores, K has received little attention in previous research, and there are no studies that have explored whether K and their interactions with others nutrients can affect herbivory. We believe that our results provide valuable information, indicating that the addition of K to the soil increased the herbivory on our focal species.

Conversely, when we observed the foliar damage percentage in the presence of all three nutrients in high levels, the foliar damage decreased, despite a high frequency of consumed leaves. This highlights the interactive role of the three nutrients, since their high concentrations in combination determine some mechanism by which insects reduce leaf area consumption. For example, secondary metabolites can function as toxins for consumers (van Genderen et al., 1996), and these increase with N (Vogels et al., 2023) and K additions (Erb and Kliebenstein 2020). The combined high levels of N, P, and K may lead to increased production of toxins, which in turn can cause herbivorous insects to reduce their consumption of leaf material. However, the frequency of herbivory was found to be greater in the presence of high levels of all three nutrients, potentially due to the presence of more abundant populations of generalist insects. Although the percentage of damage to each leaf may decrease, the overall amount of leaves consumed remains high, because the due to population growth. This implies that K likely plays a significant role in influencing aspects of foliar chemistry or physical aspects that can impact herbivorous feedings. Further research is needed to explore the mechanisms underlying the relationship between K, leaf nutrient ratios and herbivory patterns.

Our long-term field experiment is the first to investigate the interactive effects of nutrients N, P, and K on insect herbivory. We observed that an increase in the foliar concentrations of N, P and K was associated with a corresponding increase in herbivory, except in levels lower of K. The fertilizer interaction can have different implications for nutrient uptake by plants, warranting further investigation in future studies. The importance of considering plant functional traits (deciduous vs perennial species) is highlighted in order to potentially forecast the response of forest species to nutrient fertilization and their interactions.

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Appendix A

Herbivory frequency	**Estimate**	**Standar error**	**z**	**Pr(>|z|)**
Intercept	365.932	157.864	2.318	**0.0204 ***
N	-526.458	246.653	-2.134	**0.0328 ***
P	-46.684	21.172	-2.205	**0.0275 ***
K	-44.637	18.561	-2.405	**0.0162 ***
N:P	65.504	32.958	1.988	**0.0469 ***
N:K	63.189	28.835	2.191	**0.0284 ***
P:K	5.694	2.489	2.287	**0.0222 ***
N:P:K	-7.863	3.852	-2.041	**0.0413 ***

Table A.1

Results of the GLMMs evaluating the relationship between herbivory frequency and addition individual fertilizer (N, P, K) and combinations. Values in boldface indicate significant statistical differences (***p < 0.001; **p < 0.01; *p < 0.05)

Foliar damage percentage	**Estimate**	**Standar error**	**z**	**Pr(>|z|)**
Intercept	395.473	122.390	3.231	**0.001232 ****
N	-632.155	192.420	-3.285	**0.001019 ****
P	-53.667	16.440	-3.264	**0.001097 ****
K	-47.232	14.382	-3.284	**0.001023 ****
N:P	85.015	25.732	3.304	**0.000954 *****
N:K	74.804	22.490	3.326	**0.000881 *****
P:K	6.386	1.932	3.306	**0.000947 *****
N:P:K	-10.059	3.007	-3.345	**0.000822 *****

Table A.2

Results of the GLMMs evaluating the relationship between foliar damage percentage and addition individual fertilizer (N, P, K) and combinations. Values in boldface indicate significant statistical differences (***p < 0.001; **p < 0.01; *p < 0.05)