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ORIGINAL PAPER

Differential Effects of Nitrogen, Phosphorus and Potassium Soil Enrichment on Mycorrhization, Nutritional Status and Nutrient Resorption in *Nothofagus Antarctica*

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Abstract

To understand how changing environmental conditions affect forest ecosystems it is crucial to explore how soil nutrient availability influences plant nutrient dynamics. However, it remains unclear the extent to which the availability of various nutrients in the soil jointly influences the dynamics of plant nutrition, including nutrient acquisition strategies (e.g., reliance on mycorrhizal symbiosis), nutritional status, and nutrient resorption during senescence. We investigated the interactive effects of N, P, and K soil enrichment on the nutritional dynamics of *Nothofagus antarctica* using a factorial design. The experiment included 32 plots with eight fertilization treatments (N, P, K, NP, NK, PK, NPK, and control) across four blocks. We collected root samples to measure mycorrhizal colonization, and leaf samples for specific leaf area (SLA), nutrient content, resorption efficiency and proficiency. Fertilization increased soil nutrient availability, and decreased mycorrhizal colonization. This suggests that plants may no longer need to invest in mycorrhizas, as nutrients are more readily accessible. Fertilization improved nutritional status and, at senescence, N resorption was reduced by nitrogen addition, but not P and K, indicating that *N. antarctica* might only be limited by N. Nutrient addition, a predicted consequence of global change, facilitated plants nutrient uptake, either alleviating nutritional needs (N) or promoting luxury consumption (P and K), and disrupted the forest's mycorrhizal networks. This could potentially disrupt long-term ecosystem services such as carbon sequestration and nutrient cycling. Our results highlight the need for targeted nutrient management strategies in forest ecosystems to mitigate the effects of global change.

Keywords Plant nutrition · Ectomycorrhizas · Soil eutrophication · Forest ecology

1 Introduction

Forests are essential for global ecosystem functioning, and are responsible for a wide range of essential dynamics that contribute to the Earth's overall health and stability. One of their most important roles is acting as carbon sinks, absorbing and storing atmospheric carbon dioxide through photosynthesis, and therefore mitigating the effects of climate change (Heinrich et al. 2021; Pan et al. 2011). Forests also sustain a huge biodiversity by providing habitats

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for countless plant and animal species (Buotte et al. 2020; Matos et al. 2020). Moreover, they provide numerous additional ecosystem services to humans, including timber and fruit production, protection against natural hazards such as landslides and avalanches, and recreation spaces, all of which hold an important cultural, spiritual and economic value (Cooper et al. 2016; Häyhä et al. 2015). However, these services are being increasingly threatened by climate change and human-induced disturbances (Runting et al. 2017; Weiskopf et al. 2020).

One of the main drivers of global change is soil nutrient enrichment, or terrestrial eutrophication, which is mostly a consequence of human activities (Vitousek et al. 1997). Practices associated with forest management, such as the application of fertilizers, play a significant role in increasing soil nutrient levels (Li et al. 2022). Industrial activities also contribute to soil eutrophication, by augmenting nutrient deposition (Goll et al. 2023; Penuelas et al. 2009; Vitousek et al. 1997). This rise in soil nutrient availability, particularly of N (nitrogen), P (phosphorus) and K (potassium), is leading to nutrient cycles alterations (Geremew 2021; Li et al. 2019) as well as biodiversity loss, changes in species interactions and overall disrupting ecological stability (Harpole et al. 2016; Hautier et al. 2020; Pahalvi et al. 2021). Given the severity of these changes, it is crucial to develop forest management strategies that mitigate their impacts, which requires a comprehensive understanding of the mechanisms driving these ecological shifts. A critical aspect of this understanding lies on the impact of eutrophication on plant nutritional dynamics, such as how plants acquire nutrients, their overall nutritional status, and how they shift strategies for nutrient conservation and recycling during senescence.

Plant nutrition begins at the roots, where plants often rely on the assistance of soil microorganisms, particularly mycorrhizal fungi, to enhance nutrient uptake. These fungi form symbiotic relationships with plants, providing support for plant health and resilience, especially under nutrient-limited conditions (Azcón-Aguilar et al. 2009; Smith and Read 2008). Ectomycorrhizas (EcM), a distinct type of mycorrhiza, develop a mantle over root tips and extend an hyphal network through the soil, enhancing the plant's access to water and soil nutrients, in exchange for photosynthetically derived carbon from the host (Azcón-Aguilar et al. 2009; Becquer et al. 2019; Bowles et al. 2016; Bücking and Kafle 2015; Santander et al. 2017; Smith and Read 2008). Ectomycorrhizal fungi are also essential for broader ecosystem functions, beyond the benefits for an individual plant, such as carbon sequestration, soil organic matter formation, and nutrient retention, reinforcing their ecological importance (Bahr et al. 2015; Högberg et al. 2020; Wallander and Ekblad 2015).

Plants tend to allocate carbon to EcM fungi mainly for their role on nutrient acquisition. When plants can access nutrients directly from the soil, however, this carbon investment in EcM may decline, resulting in decreased colonization rates (Read 1991; Stock et al. 2021; Wang et al. 2017). The differential impact of soil enrichment with only one or several nutrients is less studied, but there is evidence of an additive effect of various nutrients (Lin et al. 2020; Wang et al. 2020). However, the effect of soil eutrophication has predominantly been investigated in the context of arbuscular mycorrhizas, primarily through pot experiments. These studies are mostly focused on N and P but not on other nutrients such as K, and their applicability to EcM through field experiments and real forest ecosystems remains insufficiently addressed, with limited and scattered studies presenting contradicting results (Nagy et al. 2009; Nouri et al. 2014; Pu et al. 2022).

Once nutrients have been acquired by the roots and fungal symbionts, they are transported to leaves and other tissues, where they play a fundamental role in plant nutritional status. This status, often studied through indicators like foliar nutrient content and specific leaf area (SLA), provides insights into a plant's capacity to optimize nutrient use and maintain physiological functions under varying environmental conditions (Lambers and Oliveira 2019; Wright et al. 2005). Elevated foliar nutrient concentrations reflect an optimal nutritional status, promoting growth and potentially enhancing competitive ability, whereas SLA —a measure of leaf area relative to mass— serves as an indicator of resource allocation and photosynthetic efficiency, and both parameters are also associated with higher water use efficiency (Lambers and Oliveira 2019; Poorter et al. 2009; Querejeta et al. 2022; Reich 2014; Zangani et al. 2021). Both indicators (SLA and foliar nutrient concentrations) have been shown to increase under higher soil nutrient availability (Daou et al. 2021; de Oliveira Carvalho et al. 2022; Feng et al. 2023; Yu et al. 2019), but the specific effects of different nutrient combinations (especially in multifactorial NPK experiments) remain poorly understood.

Finally, nutrient resorption, the process by which plants retrieve essential elements from senescing leaves before they are shed, is a key component of nutrient conservation strategies that reduce the plant's dependency on soil nutrient availability (Aerts 1996; Hill 1980; Killingbeck 1996; Urbina et al. 2021). Under nutrient-rich conditions, resorption efficiency often declines, influencing nutrient cycling within the ecosystem by altering litter quality and affecting microbial activity in the soil (Hobbie 2015; Urbina et al. 2021). However, the effect of specific nutrients and the underlying mechanisms are a matter of debate. Some studies suggest that N resorption is negatively affected by soil N, but P resorption is not related to soil P (Yan et al. 2018), while others propose the exact opposite (negative association between P resorption and soil P content, while no association for N resorption and soil N; Urbina et al. 2021). The role of other macronutrients such as K has been investigated only on a few occasions (Urbina et al. 2021; Vergutz et al. 2012; Yan et al. 2018). Some authors, in contrast with previous evidence, have even suggested that nutrient resorption is largely independent of soil nutrient availability (Urbina et al. 2021; Yan et al. 2018). This variability in results across studies and even meta-analyses highlights the nutrient-specific and context-dependent nature of resorption. For example, in northern Patagonia, *Nothofagus* woodlands are characterized by soils with low N, and high P that is mostly retained in the soil due to active aluminum and iron associated with organic and mineral fractions (such as aluminum-humus complexes, allophanes and ferrihydrite; Satti et al. 2007). In consequence, plants might be limited by both N and P, but previous studies had shown that mycorrhizal plants are only limited by N (Diehl et al. 2008). Potassium has been less studied, although no K limitation has been documented in these forests (Diehl et al. 2003). Furthermore, most of these studies have been only observational, while there is a lack in manipulative experiments to test the implications of nutrient limitation (or eutrophication) on these dynamics. Thus, examining the individual and combined impacts of specific nutrients in nutrient resorption is essential for gaining a more nuanced understanding of these dynamics, and predicting how soil nutrient enrichment could reshape forest ecosystems.

Comprehending all these effects is pivotal for understanding of the evolving ecology within forest ecosystems, especially in the context of global change and the trend of increasing nutrient content in forest soils. This study seeks to investigate the impact of the increment of several individual nutrients and their combinations on various aspects of plant nutrition. We addressed this through a full factorial field experiment involving the fertilization with N, P and K, and their respective combinations. Our study focused on the effects of these treatments on: EcM colonization as a nutrient acquisition strategy, leaf nutrient content and SLA as indicators of plant nutritional status during the growth period, and nutrient resorption to better understand the dynamic of recycling nutrients during senescence. We selected *Nothofagus antarctica* (G.Forst.) Oerst. as our study species, a prominent tree in Patagonian forests and shrublands. Recognized for its remarkable plasticity, *N. antarctica* is a key species shaping the entire ecosystem, influencing biodiversity from understory plants to insect and bird communities (Peri and Ormaechea 2013).

We hypothesize that soil eutrophication reduces the reliance of *N. antarctica* on mycorrhizal symbionts by alleviating nutritional limitations, resulting in decreased mycorrhizal colonization. Since plants in these woodlands are mostly limited by N however, we expect N additions to have a greater impact on this symbiotic relationship compared to P or K. Furthermore, we expect this alleviation of nutrient limitations to enhance the overall nutritional status of the trees, reflected in increased foliar nutrient content and SLA. Finally, as soil nutrients become readily available and plant nutritional status improves, we hypothesize a reduced necessity for nutrient conservation during senescence, evidenced by a decrease in nutrient resorption due to nutrient-specific reductions in nutritional constraints. We expect a greater effect of N, compared to P and K, since the plants in this region are mostly limited by this nutrient.

2 Materials and Methods

2.1 Experimental Design and Study Site

We conducted a field experiment with a factorial design and four randomized blocks containing each the 8 treatment plots separated by a > 5 m buffer area. Plots measured 31.5 × 45 m and were randomly designated for fertilizer treatments: N, P, K, NP, NK, PK, NPK, and a control (C) plot devoid of any added fertilizers. Over recent years these plots underwent four rounds of fertilization (in summer 2016 and springs 2017, 2018 and 2021), following a modified protocol derived from the Nutrient Network (Nut Net network: A Global Research Cooperative, https://nutnet.org/). The fertilizer quantities applied mirrored those utilized in prior experiments conducted in temperate forests: 100 kg ha^{-1} of N, 75 kg ha^{-1} of P, and 56 kg ha^{-1} of K (Kim 2008; Lindberg and Persson 2004; Prescott et al. 1995), with the final aim to cause a marked enrichment of each nutrient. The fertilizers applied were urea, triple superphosphate and potassium sulfate, as indicated by the Nutrient Network protocol. Although urea is an organic form of N, in contrast with P and K fertilizers, this fertilizer is recommended for field experiments to delay absorption and prevent loss due to volatilization.

We established the experiment in “ConCiencia” property in El Foyel, Río Negro (northwestern Patagonia, Argentina). The regional climate is characterized as temperate-cold, maintaining an average temperature of 9 °C, and an annual precipitation of 920 mm (Reque et al. 2007). This is a mountainous region of the Patagonian Andes, characterized by uneven topography with marked elevation changes. The study area includes slopes with both northern and southern exposures. Experimental blocks were distributed across slopes with different exposures (north- and south-facing) and inclinations, to account for this topographic variability. The site is characterized as a shrubland, primarily dominated by *N. antarctica*, *Berberis* sp., *Lomatia hirsuta*, *Fabiana imbricata*, and *Schinus patagonica* (Coulin et al. 2019). The soils of the region are identified as young Andisols (Haplulands), which typically exhibit P retention and are poor in N (Diehl et al. 2008). Previous studies in the region have reported values of approximately 0.3% of total N and 6–7 mg kg^{-1} of available P (Diehl et al. 2008). In the study area Goldenberg et al. (2020) found 0.5% of total N and 7.7 mg/kg of available P. In the exact control plots of this experiment, in prior years, we have found 43.8 mg kg^{-1} of NO_3-N, and 14 mg/kg and 146 mg kg^{-1} of available P and K, respectively (approximately a 3:1:10 -N: P:K- ratio; Fioroni et al. 2024).

2.2 Sample Collection

In each of the 32 plots, we sampled between five and ten *N. antarctica* trees. Trees were sampled along the central area of each plot to avoid edge effects and to ensure they were well within the treated zone. Additionally, we maintained a minimum distance of 5 m between sampled individuals to reduce the likelihood of sampling clonal or closely interacting trees, and to minimize spatial autocorrelation. During the 2021/2022 summer, we collected leaves from the tree canopies, specifically those directly exposed to sunlight, as well as soil samples surrounding the roots of each tree. A soil corer was employed to extract three equidistant sub-samples per tree from the uppermost 20 cm of soil. Soil cores were taken at a distance of approximately 50 cm from the base of each tree trunk, and we used a soil corer with a diameter of 10 cm. These sub-samples were then combined to create a single representative sample for each tree. Additionally, in the fall of 2022, we collected senescent leaves from the same trees. Senescent leaves were collected by placing clean plastic sheets on the ground beneath the canopy and gently shaking the tree trunk to dislodge leaves that were naturally detaching but still attached. This method has been used in previous studies (Urbina et al. 2021; Hu et al. 2023) and allowed us to collect leaves representative of the natural abscission process. Although light exposure was controlled for mature leaves but not for senescent ones, which may introduce variability in traits such nutrient concentrations due to canopy position, this limitation applies equally across all treatments and therefore did not compromise the validity of treatment comparisons. For subsequent analyses, we excluded any leaves that showed visible damage or were not fully senescent. Only leaves displaying uniform orange/red coloration were retained. Leaves were stored in paper bags at room temperature, while soil samples were preserved in plastic bags and kept in a freezer (−18 °C) until further processing. During the sampling process, we also recorded the height of the tallest tree in each plot, which was designated as the dominant height (DH).

3 Roots and Soil Processing and Analysis

Roots were separated manually from the soil cores mentioned previously, and those corresponding to *N. antarctica* were visually identified by morphology (this species roots are the only ectomycorrhizal in the study site and easily distinguishable), and isolated. Random root segments were selected and observed under a stereoscopic microscope. We counted a total of 300 root tips per sample, except when sample size was smaller, in which case all root tips from the sample were measured. Using both stereoscopic and optical microscope, we identified colonized root tips by EcM following the morphological criteria described by Agerer (1987), including changes in color, shape, and branching patterns of the root tips, as well as the presence and structure of the fungal mantle and extraradical mycelium. To calculate colonization percentages, the total number of EcM root tips was divided by the overall number of quantified tips (Taniguchi et al. 2007). Soil samples were then dried at room temperature and sieved through a 2 mm mesh. Subsequently, a single sample pool was created per plot. For these pooled samples we determined pH, electrical conductivity, the nitrate concentration (NO_3-) in the soil (N_S, using the Bremner method; Bremner and Mulvaney 1982), soil available phosphorus (P_S, with the Bray-Kurtz method; Bray and Kurtz 1945), and soil available potassium (K_S, extracted using the ammonium acetate method; Novozamsky and Houba 1987).

3.1 Leaves Processing and Analysis

We scanned twelve green leaves from each plant and measured their one side area (including petioles) using the open-source software ImageJ (https://imagej.net/ij/index.html). Subsequently, we dried the leaves at 60 °C and weighed them. This process facilitated the calculation of their SLA.

We pooled leaves from individual trees to de plot level (green and senescent separate) after drying them at room temperature (final $n=32$). We determined their N content (N_F) using the Kjeldahl method (Kjeldahl 1883), and their total P (P_F) and total K (K_F) using the Sommer and Nelson method (Nelson and Sommers 1973). We also determined the mass loss correction factor (MLCF), calculated as:

$$MLCF = 1 - \frac{green\ leaves\ mass - senescent\ leaves\ mass}{green\ leaves\ mass}$$

We multiplied the MLCF to the nutrient content of senescent leaves. This was done to overcome the overestimation of nutrient content in senescent leaves and, in consequence, the underestimation of resorption, following Zhang et al. (2022). These analyses enabled us to calculate the percentage of each nutrient resorbed before leaf senescence. Nutrient resorption proficiency (N_{RP}, P_{RP}, K_{RP}) was defined as the nutrient content remaining in senescent leaves, and nutrient resorption efficiency (N_{RE}, P_{RE}, K_{RE}) was calculated as follows:

$$\frac{Green\ leaves\ nutrient\ content - senescent\ leaves\ nutrient\ content}{Green\ leaves\ nutrient\ content} \times 100$$

3.2 Data Analysis

To evaluate the impact of fertilization, we employed various statistical models with N, P, K, and their interactions as fixed effects. All statistical analyses were conducted in R software (R Core Team 2022). We used the *lme4* package (Bates et al. 2015) to estimate model parameters, the *DHARMa* package (Hartig 2020) to verify the models' assumptions (when analyzing the models fitted with *gls*, since *DHARMa* does not support this models, we verified the assumptions visually with Q-Q and residuals vs. fitted plots), and the *emmeans* package for post hoc comparisons (Lenth et al. 2018).

We used linear mixed-effects models (LMM, *lmer* function) for soil nutrient content, foliar nutrient content, foliar nutrient resorption, and SLA as response variables, except N_S, for we used a generalized linear mixed-effects model (GLMM, *glmer* function) with a log-link function to meet model's assumptions. For P_S we applied a log transformation to meet the model's (LMM) assumptions as well. For EcM colonization, we used a GLMM with a binomial family distribution. For the latter, we utilized the cbind function (in particular, cbind(number of colonized root tips, total number of root tips - number of colonized root tips)) to ensure that the model accurately represented the nature of the data. Experimental blocks and plant ID (nested within the blocks) were included as random effects for mycorrhizal colonization to account for the non-independence of root tips counted within the same plant. Although root sampling with soil cores does not allow us to determine with certainty whether the sampled roots belong to the same tree, including plant ID as a random factor in the model helps account for the potential lack of independence due to the spatial proximity of the samples. For the LMMs, only the experimental block was added as a random effect.

The results of the ANOVA for all response variables are presented in Table 1, as values are Wald X^2 statistic ("analogous" to F-statistic) and significance indicated with asterisks. Firstly, we evaluated whether the interaction terms between the fertilization factors (N, P, K) were statistically significant. When a significant interaction is detected, the main effects should not be interpreted in isolation, as their meaning changes depending on the levels of the other factors. Instead, the focus must shift to the interaction effects, which capture how the influence of one factor depends on the state of the others. Accordingly, for each significant interaction detected in Table 1, we performed a 'interaction analysis', through multiple comparisons with the *emmeans* package, to examine the effect of each nutrient within the specific combinations of interest. The results of these two- and three-way interactions are presented in Tables 2 and 3, accordingly. This approach ensures that the interpretation is based on context-dependent effects rather than potentially misleading main-effect summaries.

Additionally, we constructed not-factorial linear models (LMs, *lm* function), with continuous explanatory variables. In these models, the explanatory variables were N_S, P_S and K_S, as well as DH. The inclusion of DH is justified as it provides an indication of site productivity beyond just soil nutrient content. Notably, DH was not correlated with the content of any of the nutrients in the soil, allowing us to assess its independent effect (Fig. S1). We also evaluated the variance inflation factor for each model to ensure the lack of multicollinearity. The response variables were both related to foliar nutrient content and resorption. Also, we fitted similar LMs for SLA and EcM colonization, both variables averaged at plot level (with a final $n = 32$) so that each value from the response variables match the data corresponding to the explanatory variables. To meet the model's assumptions, we applied a log transformation to K_S in the K_F model, to K_{RP}, and to all the explanatory variables in the EcM model. Furthermore, we fitted the LMs for N_{RE}, K_F, and SLA using the *gls* (instead of *lm*) function from the *nlme* package (Pinheiro et al. 1999) to allow the variance to differ between blocks and meet the model's assumptions. The results of these models are presented in Figs. 4, 5, 6 and 7 and text, but not in the previously mentioned tables, since the analysis for these models was not factorial as the previously described.

4 Results

4.1 Soil Chemistry

The addition of fertilizers increased the soil nutrient content and altered soil pH and conductivity (Fig. 1; Table 1). N application resulted in a 201.61% increase in terms of N_S ($p < 0.001$), while K application reduced N_S by 27.93% ($p = 0.04$). We found a triple interaction when analyzing P_S ($p = 0.002$, Tables 1 and 3). The application of P increased P_S by 405.75% on average when applied alone and in

Table 1 Analysis of deviance to assess the impact of nutrient addition treatments on N -nitrogen-, P -phosphorus-, and K -potassium- content in the soil, and *Nothofagus Antarctica* N, P and K content in green leaves, senescent leaves, the specific leaf area (SLA) and ectomycorrhizal colonization (EcM). Rows correspond to response variables, and columns indicate the main effects of N, P, and K addition and their two- and three-way interactions. Further analyses of the significant interactions are presented in Tables 2 and 3. Values reported are Wald X^2-statistic. Values in boldface indicate significant differences (p-value: 0–0.001 = '***', 0.001–0.01 = '**', 0.01–0.05 = '*', 0.05–0.1 = '°'). N_S: soil nitrate concentration (mg kg^{-1}); P_S: soil available phosphorus (mg kg^{-1}); K_S: soil available potassium (mg kg^{-1}); EC: electrical conductivity (µS); N_F: foliar nitrogen (%); N_{RE}: nitrogen resorption efficiency; N_{RP}: nitrogen resorption proficiency (%); P_F: foliar phosphorus (%); P_{RE}: phosphorus resorption efficiency; P_{RP}: phosphorus resorption proficiency (%); K_F: foliar potassium (%); K_{RE}: potassium resorption efficiency; K_{RP}: potassium resorption proficiency (%); SLA: specific leaf area (cm^2 g^{-1}); EcM: ectomycorrhizal colonization (%). ^{1}: *n*=32 (plot level, 4 per treatment); ^{2}: *n*=258 (individual plant level, ~8 per plot, ~32 per treatment). The model for N_S was a generalized linear mixed model (GLMM) with a log-link function, for EcM we used a GLMM with binomial family, and for the remaining variables we applied linear mixed models. In all cases, the application of N, P and/or K were the factors of analysis, the experimental blocks were the mixed factor

Variable	*N*	*P*	K	*N*: *P*	*N*: K	*P*: K	*N*: *P*:K
N_S^1	__23.66***__	0.68	__6.34**__	0.01	0.37	0.02	0.02
P_S^1	0.12	__82.29***__	__5.83*__	0.18	1.85	1.23	__9.62**__
K_S^1	0.74	0.53	__17.11***__	1.42	0.05	0.12	1.31
pH^1	__45.05***__	0.57	3.22	0.69	0.96	0.04	__5.51*__
EC^1	__22.49***__	0.09	0.33	0.39	2.87	0.77	3.04
N_F^1	__11.47***__	1.42	2.21	__7**__	1.12	0.07	0
N_{RE}^1	0.01	0.01	1.72	35	__4.54*__	0.15	0.69
N_{RP}^1	1.47	0.44	0.74	0.08	__3.96*__	0	0.66
P_F^1	0.67	__11.88***__	1.48	0.02	2.03	0.57	1.32
P_{RE}^1	0.19	1.77	1.64	0.42	0.29	0	2.11
P_{RP}^1	1.42	__8.75**__	1.41	0.15	0	0.34	1.57
K_F^1	0.6	0.13	__6.6*__	__7.37**__	0.79	__4.68*__	0.03
K_{RE}^1	3.26°	2.39	0.64	0.01	0.52	0.46	0.01
K_{RP}^1	1.45	2.49	2.72°	0.4	0.22	0.15	0
SLA^2	3.32°	1.42	__12.71***__	__5.98*__	__6.96**__	0.14	1.45
EcM^2	__5.66*__	__10.77**__	1.07	__4.36*__	__34.54**__	__12.85***__	1.99

Table 2 Effect of fertilization treatments obtained from an interaction analysis (two-way interactions) on foliar measurements in *Nothofagus antarctica*. Response variables are listed in columns, while rows show the effect of each nutrient under the presence or absence of the other nutrient ('Condition'), as derived from the interaction analysis. For example, the 'N effect' on 'Condition P-' indicates the effect of N in plots not fertilized with P (i.e., the average of control and K plots vs. the average of N and NK plots); the 'N effect' on 'Condition P+' indicates the effect of N in plots fertilized with P (i.e., the average of P and PK plots vs. the average of NP and NPK plots). Values reported are t-ratios. Values in boldface indicate significant differences (p-value: 0–0.001 = '***', 0.001–0.01 = '**', 0.01–0.05 = '*', 0.05–0.1 = '°'). N: nitrogen; P: phosphorus; K: potassium; N_F: foliar nitrogen (%); N_{RE}: nitrogen resorption efficiency; N_{RP}: nitrogen resorption proficiency (%); K_F: foliar potassium (%); SLA: specific leaf area (cm^2 g^{-1}). Arrows indicate the directionality of the nutrient addition effect on the variable of interest. When there was not a significant interaction between two factors (e.g., N and K for N_F), there was no analysis needed and values were left blank ('-'). Only variables with significant two-way interactions (from Table 1) were added

	Condition	N_F	N_{RE}	N_{RP}	K_F	SLA
N effect	P-	0,52	-	-	1,37	↑ __3,05**__
	P+	↑ __4,26***__	-	-	↓ __−2,47*__	−0,44
	K-	-	−1,57	↑ __2,27*__	-	−0,57
	K+	-	1,45	−0,55	-	↑ __3,19**__
P effect	N-	−1,03	-	-	↑ __2,18*__	↑ __2,57*__
	N+	↑ __2,71*__	-	-	−0,11	−0,89
	K-	-	-	-	−1,27	-
	K+	-	-	-	1,79°	-
K effect	N-	-	−0,58	0,8	-	0,66
	N+	-	↑ __2,43*__	−2,02°	-	↑ __4,39***__
	P-	-	-	-	0,29	-
	P+	-	-	-	↑ __3,34**__	-

Table 3 Effect of fertilization treatments obtained from an interaction analysis (three-way interactions) on soil measurements and colonization by ectomycorrhizas in *Nothofagus antarctica*. Response variables are listed in columns, while rows show the effect of each nutrient under the presence or absence of the remaining two nutrients ('Condition'), as derived from the interaction analysis. For example, the 'N effect' on 'Condition P-, K-' indicates the effect of N in plots not fertilized with P nor K (i.e., control vs. N); the 'N effect' on 'Condition P+, K+' indicates the effect of N in plots fertilized with P and K (i.e., PK vs. NPK). Values reported are t-ratios. Values in boldface indicate significant differences (p-value: 0–0.001 = '***', 0.001–0.01 = '**', 0.01–0.05 = '*', 0.05–0.1 = '°'). N: nitrogen; P: phosphorus; K: potassium; P_S: available phosphorus in soil (mg kg^{-1}); EcM: ectomycorrhizal colonization (%). Arrows indicate the directionality of the nutrient addition effect on the variable of interest. Only variables with significant three-way interactions (from Table 1) were added, except for EcM where all three two-way interactions were significant, which also means that the effect of each factor is dependent on the remaining two

	Condition	P_S	pH	EcM
N effect	P-, K-	↓ **−2.27***	↓ **−2.26***	↓ **−5.93*****
	P+, K-	1.26	↓ **−5.44*****	↓ **−2.34***
	P-, K+	↑ **2.2***	↓ **−3.62****	1.42
	P+, K+	−0,48	↓ **−2.11***	↑ **2.083***
P effect	N-, K-	↑ **3.33****	1.11	↓ **−5.15*****
	N+, K-	↑ **6.85*****	−1.03	−1.69°
	N-, K+	↑ **5.32*****	−2.07°	−0.19
	N+, K+	↑ **2.64***	0.48	0.5
K effect	N-, P-	↓ **−2.88****	1.48	↓ **−5.97*****
	N+, P-	1.57	0.11	1.35
	N-, P+	−0.89	−0.66	−0.95
	N+, P+	↓ **−2.63***	↑ **2.66***	↑ **3.49*****

combination with N and/or K ($p < 0.015$ in all cases). On the other hand, plots fertilized only with K or N had less P_S than control plots ($p < 0.034$, Table 3). Finally, K application increased K_S by 91.44% ($p < 0.001$, Table 1). Soil pH decreased by 4% on average with N addition ($p < 0.047$, Table 3), but increased with K application by 3.33% when comparing NP vs. NPK ($p = 0.015$). Soil electrical conductivity increased by 74.96% on average with N addition ($p < 0.001$).

5 Ectomycorrhization

Roots analyzed presented EcM colonization (Fig. 2a), which was negatively affected by fertilization, especially by N (Tables 1 and 3; Fig. 2b). Since the effect of each nutrient was dependent on the remaining two due to multiple significant interactions (Table 1), we analyzed the effect of each nutrient on its own and in different combinations (Table 3). All three fertilizers reduced EcM colonization when applied on their own ($p < 0.001$ in all cases, Table 3). Furthermore, N alone and in combination with P resulted in a lower colonization than NPK and P alone (Table 3). Surprisingly, EcM colonization (averaged at the plot level) was not correlated with any of the soil nutrient content variables, and neither with DH ($p > 0.05$ for all cases).

5.1 Foliar Nutrient Content and Resorption

In terms of nitrogen dynamics, N_F exhibited a significant increase with the combined application of N and P (Tables 1 and 2; Fig. 3). N_{RE} significantly increased with the application of K in plots that also received N fertilization ($p = 0.02$, Tables 1 and 2; Fig. 3). N_{RP} values increased with the application of N in plots not fertilized with K ($p = 0.03$, Tables 1 and 2; Fig. 3). Examining the relationships between these variables and soil nutrient content as well as DH, we found that N_F increased with dominant height ($p = 0.024$, Fig. 4a), but not with soil nutrients ($p < 0.2$ in all cases). Conversely, N_{RE} decreased with N_S ($p = 0.036$ Fig. 4b) and increased only slightly with K_S ($p = 0.024$, Fig. 4c), while N_{RP} values increased with N_S ($p < 0.001$, Fig. 4d). Neither N_{RE} nor N_{RP} showed significant associations with dominant height ($p = 0.138$ and $p = 0.202$, respectively).

For P, both P_F and P_{RP} values increased with the application of P fertilizer ($p < 0.001$ and $p = 0.012$, respectively), while P_{RE} remained unchanged across treatments (Tables 1 and 2; Fig. 3). Only P_F showed a significant, yet very slightly, association with P_S (Fig. 5a). However, P_F and P_{RP} values were positively linked with DH ($p < 0.001$ for both, Fig. 5b and d), while P_{RE} was negatively linked ($p = 0.03$, Fig. 5c).

In the case of potassium, only in plots fertilized with P, the addition of K increased K_F, and the addition of N reduced it, ($p = 0.003$ and $p = 0.022$, respectively, Tables 1 and 2; Fig. 3). Conversely, P addition increased K_F in plots fertilized with N but not K (N vs. NP, $p = 0.041$, Tables 1 and 2; Fig. 3), and marginally increased it in plots fertilized with K but not N (K vs. PK, $p = 0.088$, Tables 1 and 2; Fig. 3). No significant differences were found for K_{RE} and K_{RP} concerning the fertilization treatments (Table 1; Fig. 3). K_F increased with K_S but not with DH ($p = 0.037$ and $p = 0.43$, respectively, Fig. 6a). On the other hand, K_{RE} and K_{RP} values decreased and increased, respectively, with DH ($p = 0.012$ and $p = 0.009$, respectively, Fig. 6b and c). These variables did not change with soil nutrient content ($p > 0.143$ in all cases).

5.2 Specific Leaf Area

Fertilization affected SLA as well. Multiple interactions were significant (Table 1), and our results showed that the addition of P alone, N alone and N and K in combination, increased tree SLA (Table 2; Fig. 7a). The average of SLA in control plots was 110.23 cm^2 g^{-1}, and it increased up to 18.5% with the fertilization treatments. However, SLA was

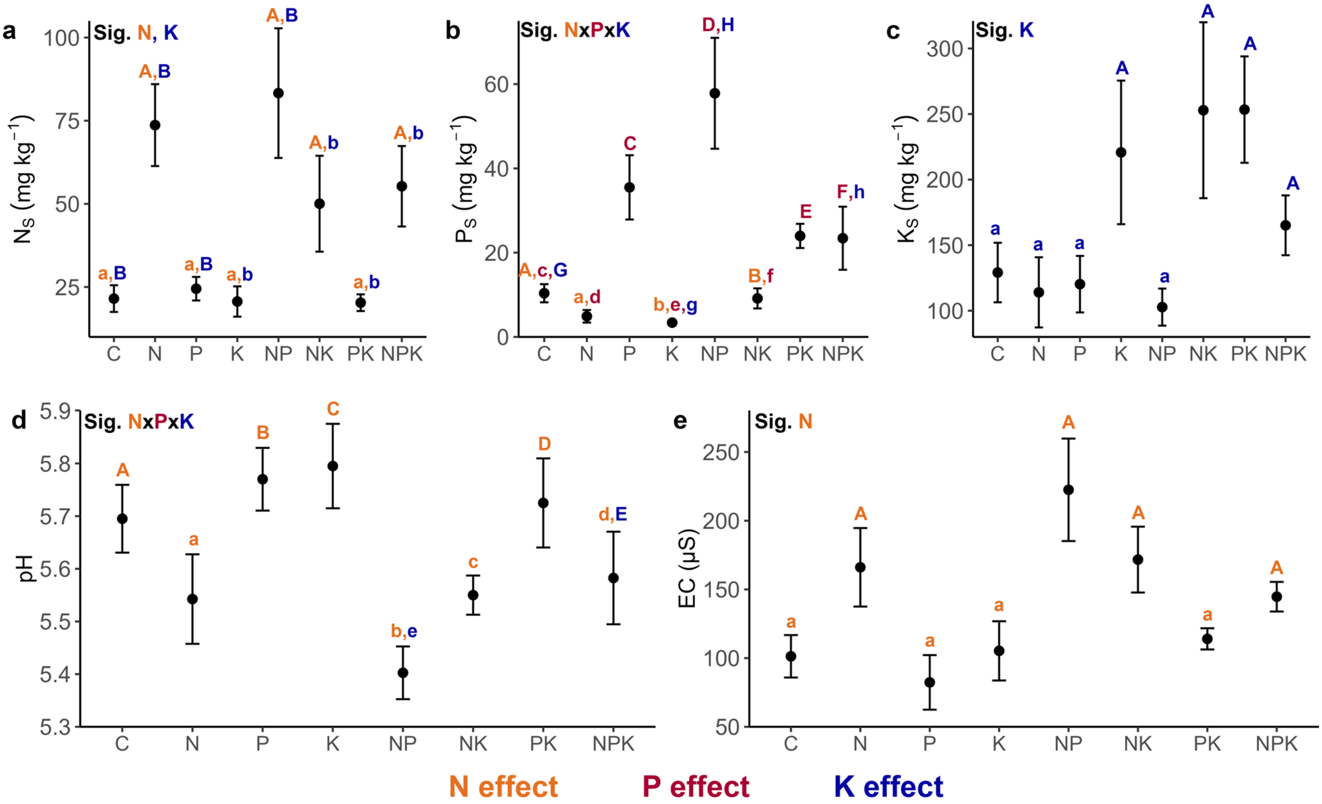

Fig. 1 Soil chemical analyses following fertilization treatments with nitrogen (N), phosphorus (P) or potassium (K) and their combinations in a *Nothofagus antarctica* shrubland in El Foyel, Río Negro, Argentina. "C", in the horizontal axis, indicate the control plots (without fertilization). (**a**) soil nitrate concentration; (**b**) soil available phosphorus; (**c**) soil available potassium; (**d**) soil pH; (**e**) soil electrical conductivity. Values plotted are means and standard errors. Different letters denote significant differences between fertilization treatments. Uppercase indicates significantly higher values and lowercase significantly lower values within each comparison. Letters are color-coded according to the nutrient tested (N: orange; P: pink; K: blue). Labels "Sig. X" or "Sig. X × Y" indicate significant main effects or interactions from the ANOVA. Treatments without letters indicate no significant differences relative to others. All variables had a $n=32$ (plot level, 4 per treatment). The model for N_S was a generalized linear mixed model with a log-link function, and for the remaining variables we applied linear mixed models. In all cases, the application of N, P and/or K were the factors of analysis, the experimental blocks were the mixed factor. N_S: soil nitrate concentration (mg kg^{-1}); P_S: soil available phosphorus (mg kg^{-1}); K_S: soil available potassium (mg kg^{-1}); EC: Electrical conductivity (µS)

positively associated only with K_S and DH ($p=0.031$ and $p<0.001$, respectively, Fig. 7b and c).

6 Discussion

Overall, our findings indicate that fertilization altered nutrient acquisition strategies, improved plant nutritional status, and influenced nutrient conservation during senescence, as shown in Fig. 8, although the effects varied depending on the specific nutrients added and on which combinations were applied.

6.1 Soil Chemistry

The soil content of each nutrient increased through the application of fertilizers, as expected (Yahaya et al. 2023), but we found interactions between specific nutrients for soil N and P. While N addition mainly increased soil N content, plots treated with both N and K had lower N levels than those treated only with N, regardless of P. Although K typically reduces soil pH, which can hinder nitrification (Li et al. 2016), in this study we found that only N lowered pH. A likely explanation is that sulfates in potassium sulfate could be reducing nitrification rates (McCarty 1999). Conversely, P_S was lower in plots receiving N and K compared to non-fertilized plots. This outcome may be attributed to a reduction in pH associated with the application of these fertilizers, especially N (Meyer et al. 2020). An alternative explanation is that the application of N and K may have stimulated plant growth and nutrient uptake, thereby increasing P acquisition and leading to lower P_S concentrations in the soil.

In concordance with most literature, N addition caused soil acidification. Acidification was lower than that reported in other studies (Hao et al. 2019; Hong et al. 2019; Tian et al. 2018). However, there is evidence of low values reported

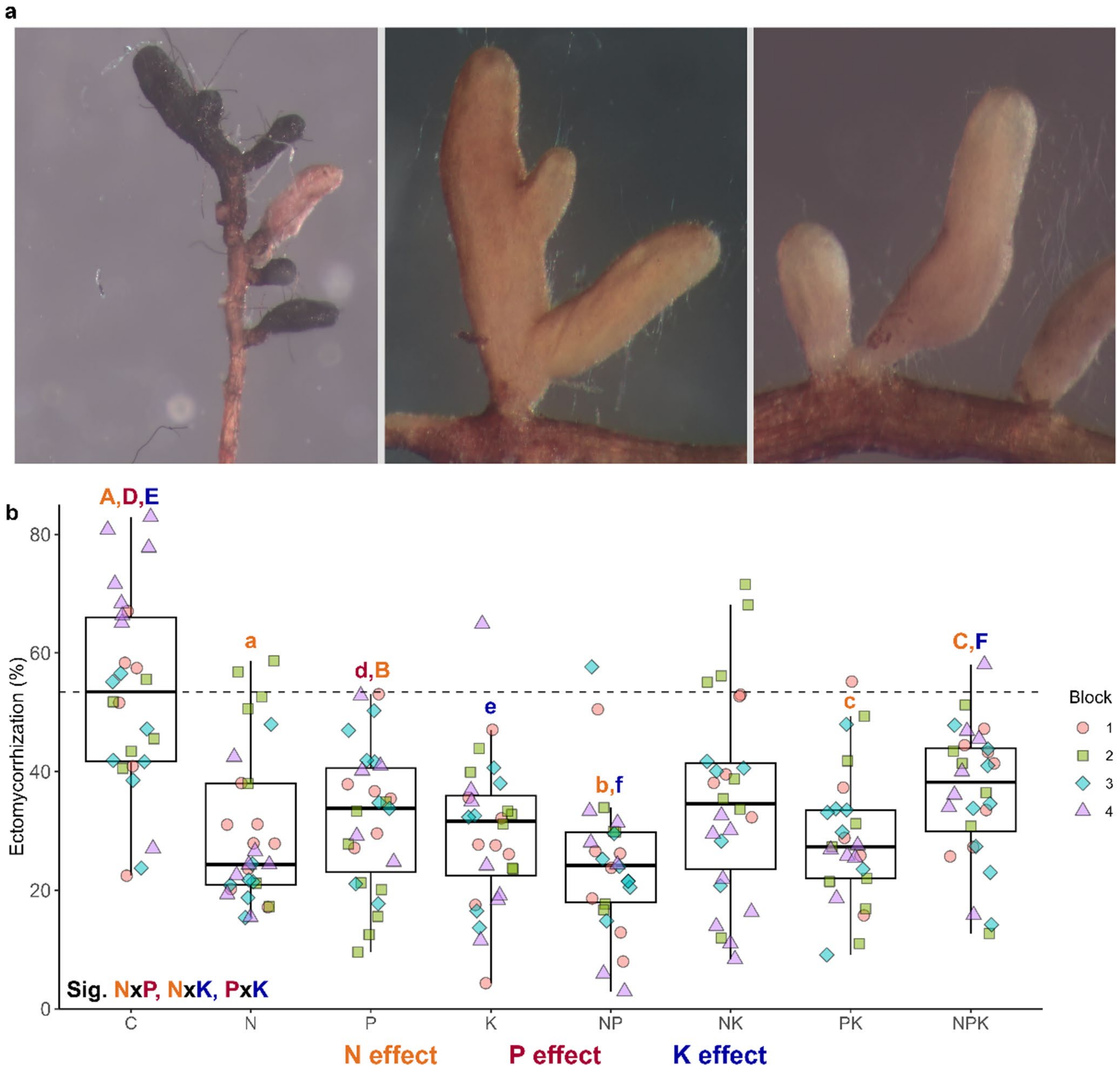

Fig. 2 Ectomycorrhizal colonization of *Nothofagus antarctica* decreases with nutrient addition. (**a**) examples of ectomorphotypes found in *N. antarctica* roots. (**b**) ectomycorrhizal colonization in nutrient addition treatments (N -nitrogen-, P -phosphorus-, K -potassium- and combinations, C: control), with each point representing the data of roots from a single tree. Points are differentiated by experimental blocks. The dashed line indicates the control median. Different letters denote significant differences between fertilization treatments. Uppercase indicates significantly higher values and lowercase significantly lower values within each comparison. Letters are color-coded according to the nutrient tested (N: orange; P: pink; K: blue). Labels "Sig. X" or "Sig. X × Y" indicate significant main effects or interactions from the ANOVA. Treatments without letters indicate no significant differences relative to others. The analysis had a $n=258$ (individual plant level, ~8 per plot, ~32 per treatment). The model was a generalized linear mixed model with binomial family. The application of N, P and/or K were the factors of analysis, the experimental blocks and plant ID were the mixed factor

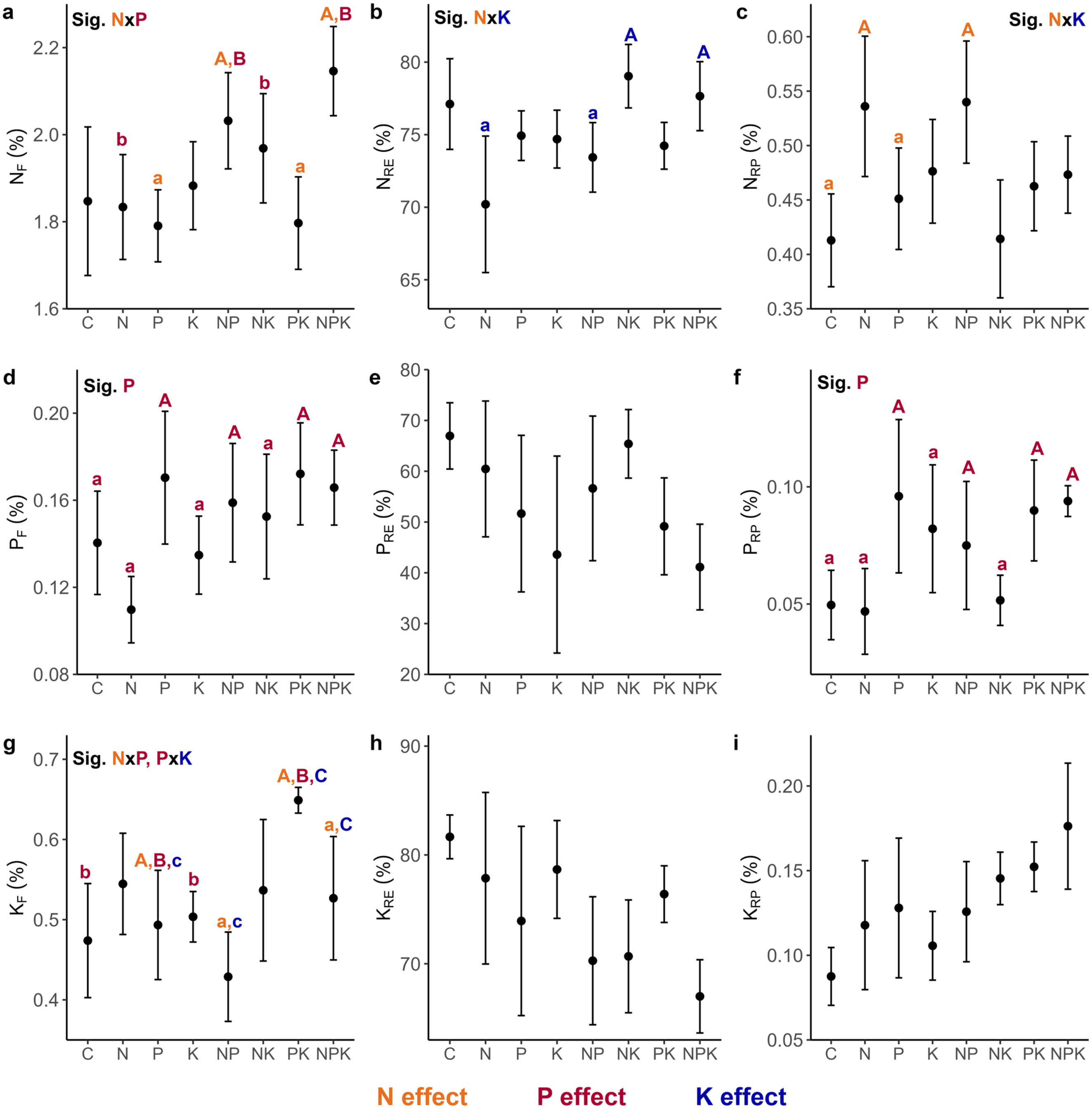

Fig. 3 Foliar chemical analyses following fertilization treatments with nitrogen (N), phosphorus (P) or potassium (K) and their combinations in a Nothofagus antarctica shrubland in El Foyel, Río Negro, Argentina. "C", in the horizontal axis, indicate the control plots (without fertilization). a. Foliar nitrogen; b. Nitrogen resorption efficiency; c. Nitrogen resorption proficiency; d. Foliar phosphorus; e. Phosphorus resorption efficiency; f. Phosphorus resorption proficiency; g. Foliar potassium; h. Potassium resorption efficiency; i. Potassium resorption proficiency. Values plotted are means and standard errors. Different letters denote significant differences between fertilization treatments. Uppercase indicates significantly higher values and lowercase significantly lower values within each comparison. Letters are color-coded according to the nutrient tested (N: orange; P: pink; K: blue). Labels "Sig. X" or "Sig. X × Y" indicate significant main effects or interactions from the ANOVA. Treatments without letters indicate no significant differences relative to others. For all models n = 32 (plot level, 4 per treatment), and we applied linear mixed models. In all cases, the application of N, P and/or K were the factors of analysis, the experimental blocks were the mixed factor. N_F: foliar nitrogen (%); N_{RE}: nitrogen resorption efficiency; N_{RP}: nitrogen resorption proficiency (%); P_F: foliar phosphorus (%); P_{RE}: phosphorus resorption efficiency; P_{RP}: phosphorus resorption proficiency (%); K_F: foliar potassium (%); K_{RE}: potassium resorption efficiency; K_{RP}: potassium resorption proficiency (%)

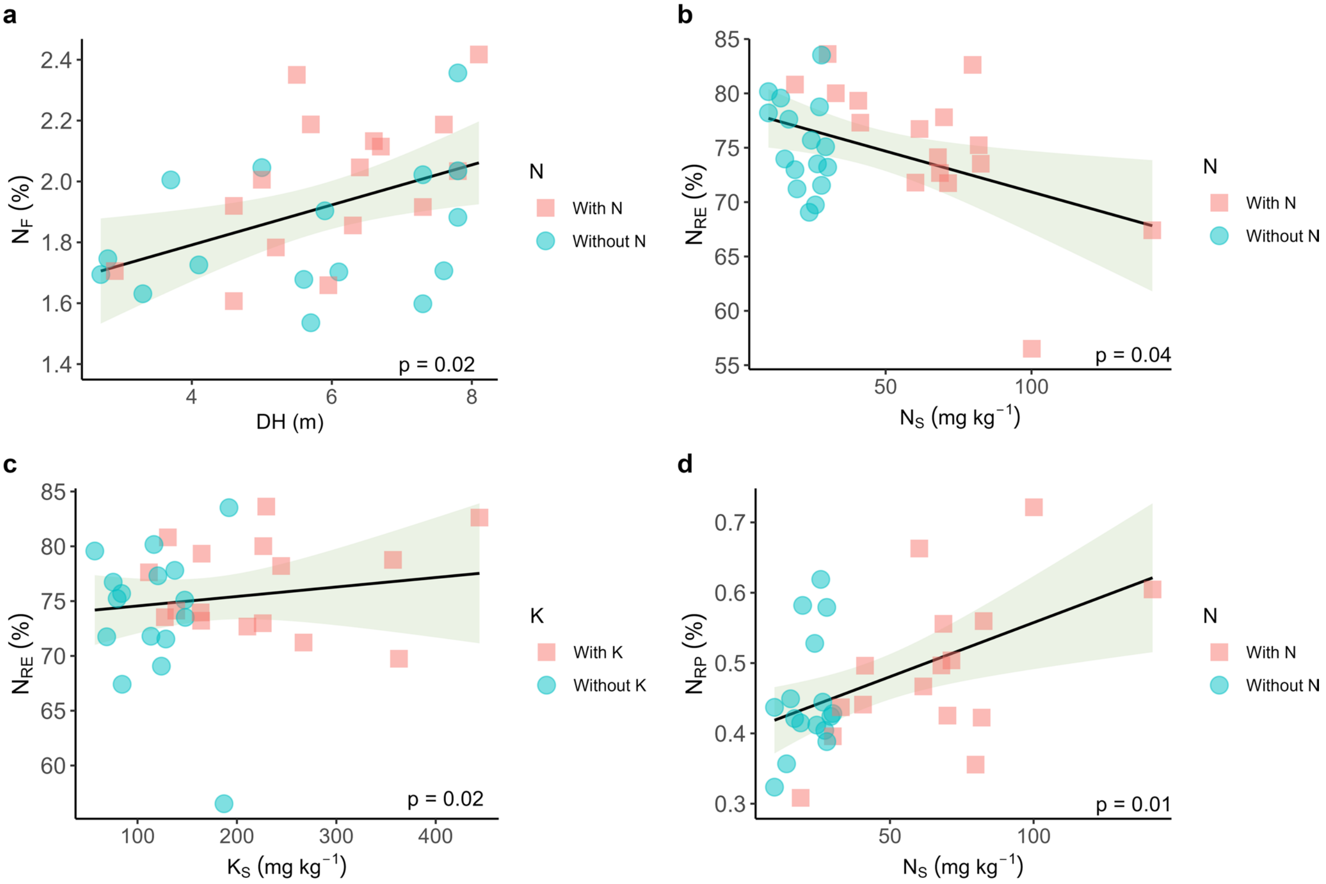

Fig. 4 *Nothofagus antarctica* foliar N -nitrogen- increases with the plot's dominant height, while soil N decreases N resorption efficiency and increases N resorption proficiency. Soil K -potassium- increases slightly N resorption efficiency (a) foliar nitrogen content as a function of dominant height. (b) nitrogen resorption efficiency as a function of soil nitrate concentration. (c) nitrogen resorption efficiency as a function of soil available K. (d) nitrogen resorption proficiency as a function of soil nitrate concentration. Pink squares represent data from plots fertilized with nitrogen (with or without P -phosphorus- and/or K), and blue circles represent data from plots not fertilized with nitrogen (with or without P and/or K), except for c., where pink squares represent data from plots fertilized with potassium (with or without N and/or P), and blue circles represent data from plots not fertilized with potassium (with or without N and/or P). 'p' indicates the p-value. Shaded area indicates 95% confidence intervals. For all models *n*=32 (plot level, 4 per treatment). DH: Dominant height (m); N_F: foliar nitrogen (%); N_{RE}: nitrogen resorption efficiency; N_{RP}: nitrogen resorption proficiency (%); K_S: soil available potassium (mg kg^{-1})

in temperate forests (Tian et al. 2017). On the other hand, the increase in electrical conductivity in plots fertilized with N could be attributed to a higher soil nitrate concentration, since conductivity is a measure of the ions present in solution (Carmo et al. 2016; Kekane 2015). These results further supported by the significant correlation found between N_S and pH and conductivity (Fig. S1).

6.2 Ectomycorrhization

Consistent with previous studies, we found that N and P addition reduced EcM colonization (Baum and Makeschin 2000; Gandini et al. 2015; Hawkins et al. 2015; Sun et al. 2010), even though we did not find a direct correlation between soil nutrient content and EcM colonization. Interestingly, K addition also led to a reduction in EcM colonization as well, a result that, to our knowledge, is undocumented in the literature. Previous studies examining K's role in EcM interactions generally report no significant influence of this nutrient on EcM fungal communities, and some findings suggest that increased K may even promote the colonization of specific EcM fungal species (Suz et al. 2010; Wang et al. 2016). Our results also indicate that, even if phosphorus and potassium also contribute to colonization loss, ectomycorrhizal colonization responds especially to nitrogen addition. As seen in Table 3; Fig. 2, while P alone caused a decrease in EcM compared to controls, adding P to plots already fertilized with N did not further reduce colonization, suggesting a dominant effect of N. Conversely, N consistently reduced EcM, both compared to controls and in combination with P or K. This suggests that nitrogen has a particularly strong influence on EcM, consistent with the idea that these symbioses evolved as adaptations to nitrogen-limited environments (Read 1991).

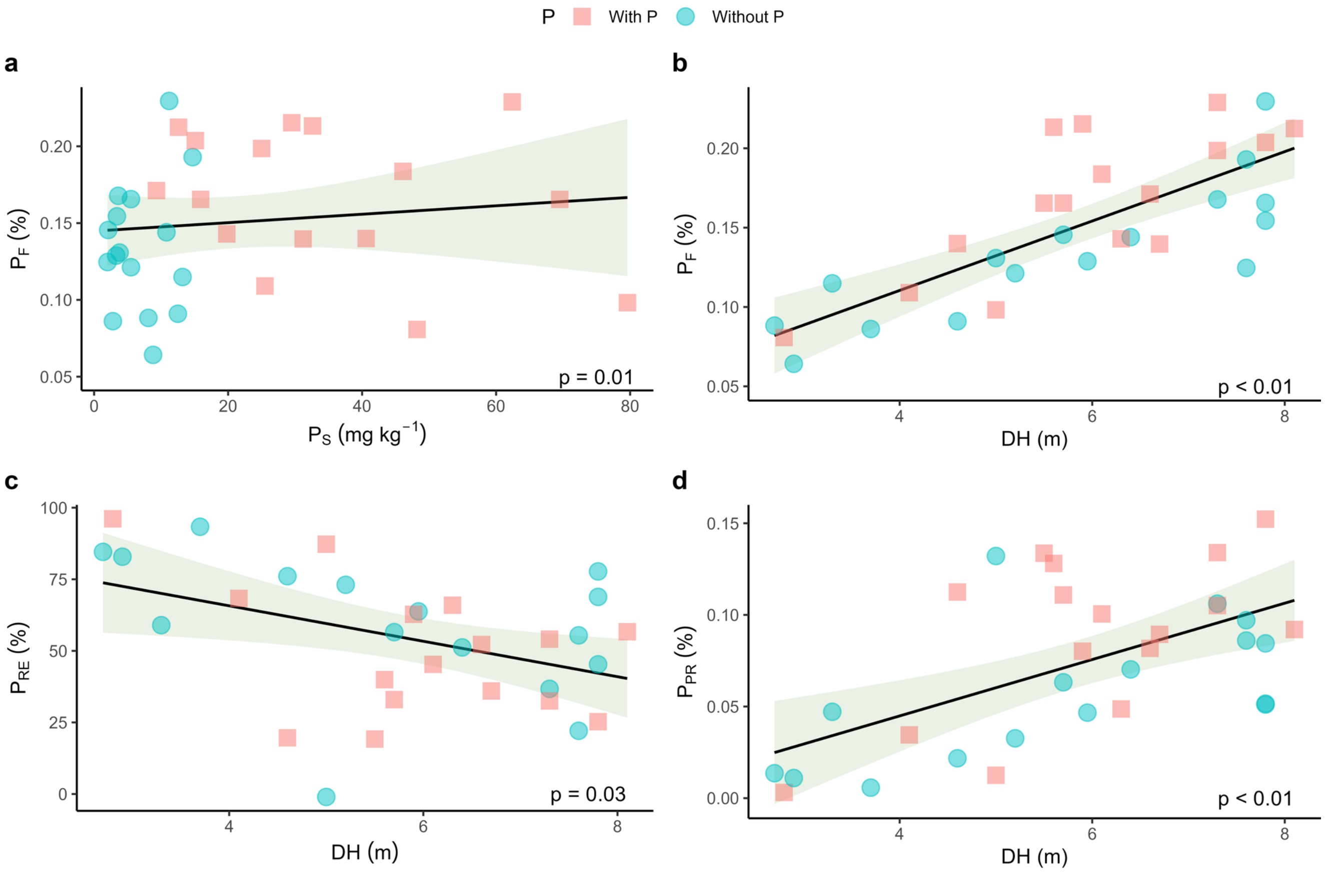

Fig. 5 Nothofagus antarctica foliar P -phosphorus- and P resorption proficiency increases, and P resorption efficiency decreases with the plot's dominant height, but neither is largely affected by soil available P. (a) Foliar phosphorus content as a function of soil available phosphorus. (b) Foliar phosphorus content as a function of dominant height. (c) Phosphorus resorption efficiency as a function of dominant height. (d) Phosphorus resorption proficiency as a function of dominant height. Pink squares represent data from plots fertilized with phosphorus (with or without N -nitrogen- and/or K -potassium-), and blue circles represent data from plots not fertilized with phosphorus (with or without N and/or K). 'p' indicates the p-value. Shaded area indicates 95% confidence intervals. For all models n = 32 (plot level, 4 per treatment). DH: Dominant height (m); P_F: foliar phosphorus (%); P_{RE}: phosphorus resorption efficiency; P_{RP}: phosphorus resorption proficiency (%); P_S: soil available phosphorus (mg kg^{-1})

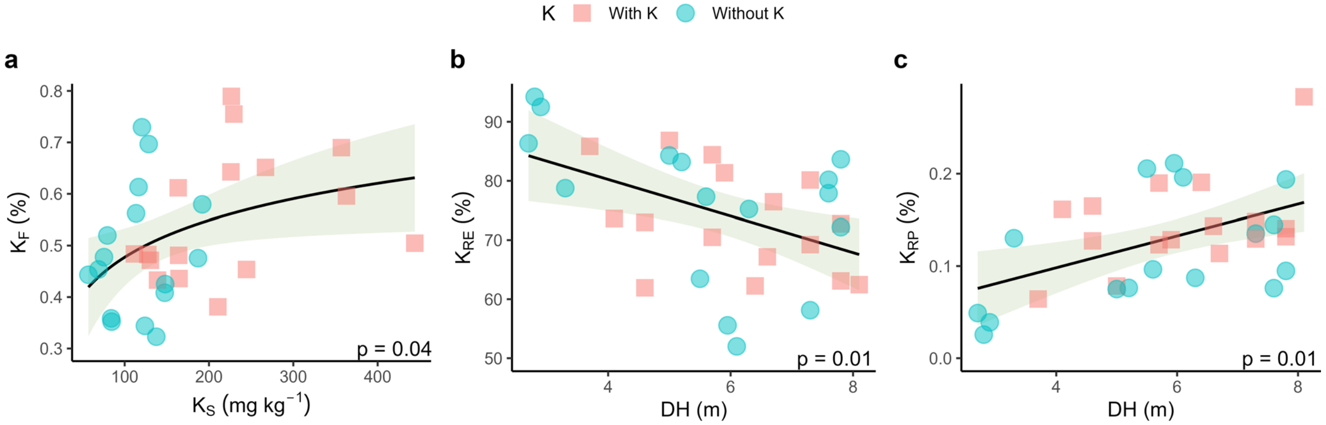

Fig. 6 Soil K -potassium- increases *Nothofagus antarctica* foliar K content, while resorption efficiency decreases and resorption proficiency increases with the plot's dominant height. (**a**) foliar potassium content as a function of soil available potassium. (**b**) potassium resorption efficiency as a function of dominant height. d. potassium resorption proficiency as a function of dominant height. Pink squares represent data from plots fertilized with potassium (with or without N -nitrogen- and/or P -phosphorus-), and blue circles represent data from plots not fertilized with potassium (with or without N and/or P). 'p' indicates the p-value. Shaded area indicates 95% confidence intervals. N: nitrogen; P: phosphorus; K: potassium. For all models $n = 32$ (plot level, 4 per treatment). DH: Dominant height (m); K_F: foliar potassium (%); K_{RE}: potassium resorption efficiency; K_{RP}: potassium resorption proficiency (%); K_S: soil available potassium (mg kg^{-1})

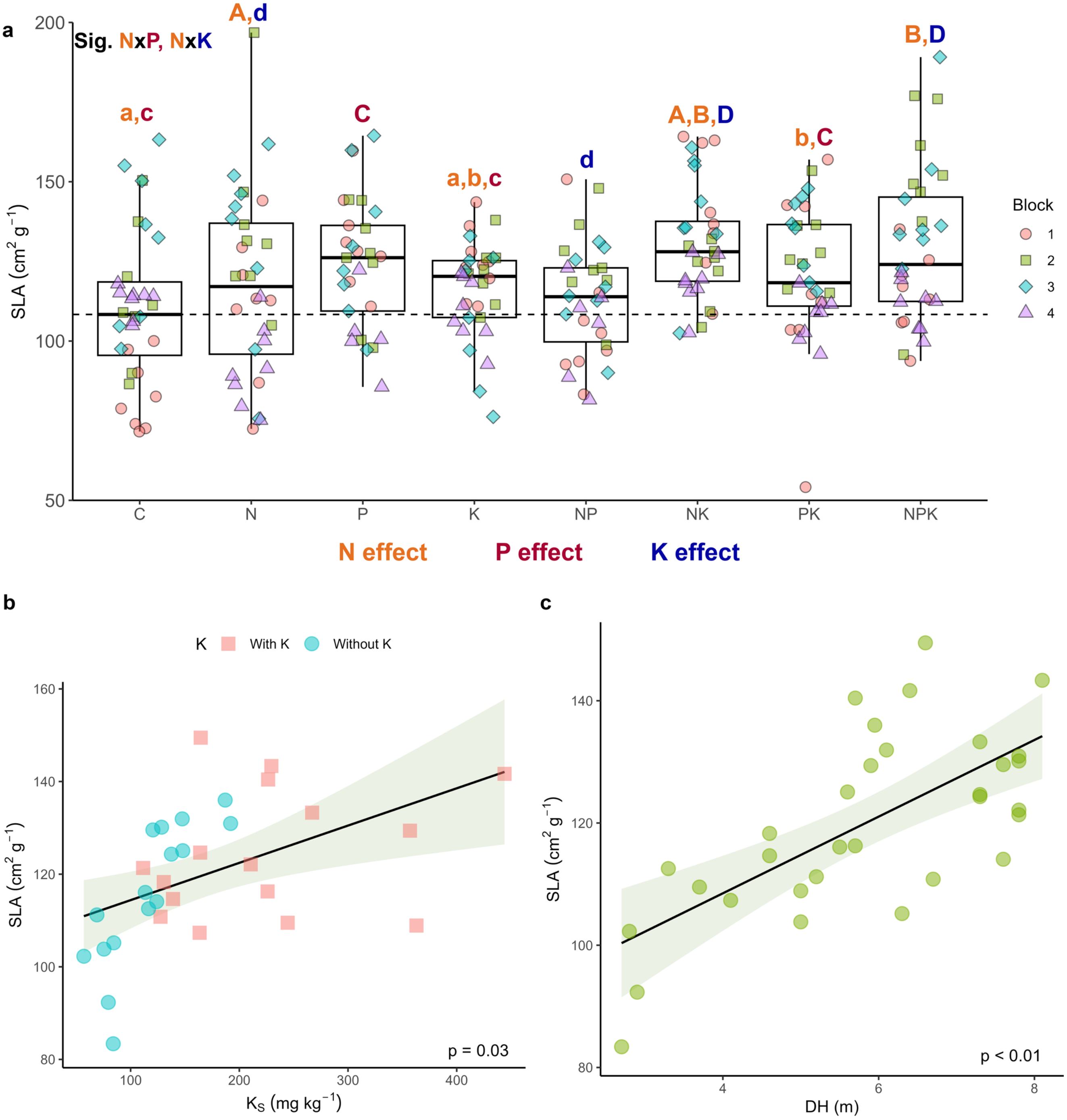

Fig. 7 *Nothofagus antarctica* specific leaf area increases with fertilization, especially with K -potassium- soil content, and it increases as well with plot dominant height. Plots represent specific leaf area as a function of: (**a**) nutrient addition treatment, with each point representing the data of leaves from a single tree, the dashed line indicates the control median; (**b**) soil available potassium with each point representing the average specific leaf area from each plot (pink squares represent data from plots fertilized with potassium (with or without N -nitrogen- and/or P -phosphorus-), and blue circles represent data from plots not fertilized with potassium (with or without N and/or P)); and (**c**) dominant height, where each point represents the average specific leaf area from each plot. 'p' indicates the p-value. Shaded area indicates 95% confidence intervals. For (**a**) $n = 258$ (individual plant level, ~8 per plot, ~32 per treatment), and for (**b**) and (**c**) $n = 32$ (plot level, 4 per treatment). The model for a was a linear mixed model. The application of N, P and/or K were the factors of analysis, the experimental blocks were the mixed factor. SLA: specific leaf area ($cm^2\ g^{-1}$); DH: Dominant height (m); K_S: soil available potassium ($mg\ kg^{-1}$)

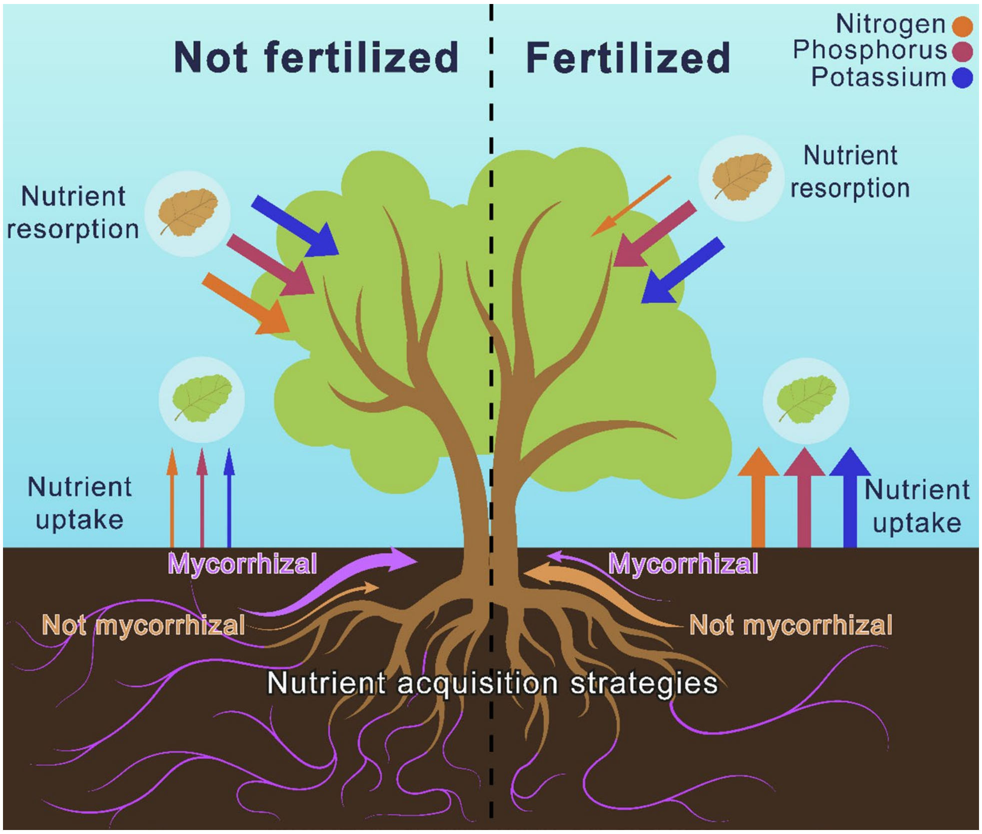

Fig. 8 Summary of nutrient fluxes (arrows) in *Nothofagus antarctica* under natural conditions (non-fertilized) and with nutrient addition (fertilized). Under natural conditions, *N. antarctica* primarily relies on its mycorrhizal associations for nutrient acquisition. However, when nutrients are added to the soil, the plant reduces its investment in this symbiosis, shifting toward a strategy of more direct nutrient uptake via 'naked' roots. Foliar nutrient uptake is higher under nutrient addition compared to natural conditions. At the onset of senescence, nitrogen resorption is reduced with higher soil nitrate concentration, whereas phosphorus and potassium resorption remain unchanged in response to varying availability of soil P and K, indicating that nitrogen is more limiting for *N. antarctica* than phosphorus or potassium

However, the significant effects of P and K support the notion that EcM contribute to the uptake of multiple nutrients. This is further supported by the fact that, despite low soil P availability in control plots, trees do not appear to be phosphorus-limited, suggesting a functional role of EcM in P acquisition, which has been extensively reported in previous studies (Becquer et al. 2019; Plassard and Dell 2010; Smith et al. 2015). It is also noteworthy that N was applied as an organic form, urea, while P and K were added in inorganic forms together with other macronutrients (Ca and S, respectively), which could be influencing the differences found between the three nutrients evaluated in this study. Overall, the observed reduction in EcM colonization suggests that, in nutrient-rich soils, plants may allocate fewer resources to sustaining these symbiotic relationships, shifting instead toward direct root-soil N, P and K uptake pathways (Bicharanloo et al. 2019; Konvalinková et al. 2017; Stock et al. 2021).

6.3 Plant Nutritional Status

Our results indicate that nutrient enrichment impacts not only nutrient acquisition strategies but also the overall nutritional status of *N. antarctica*. Fertilization generally enhanced the plant's nutritional status, as evidenced by elevated foliar nutrient concentrations and SLA. However, the addition of each nutrient had different effects, and sometimes only in specific combinations.

Foliar N, for example, was higher when N and P were added in combination, but not on their own, suggesting that other factors beyond soil N availability play a role in N uptake (Firn et al. 2019). This was supported by the positive correlation between N_F and DH. This correlation is an indicator of overall site productivity and not particularly of soil nutritional content, which could indicate that N_F is dependent on other factors such as water availability (Schlesinger et al. 2016). In the study site, for example, DH is closely and positively associated with data corresponding to soil water content from previous studies on the same field experiment (Fig. S1). Something similar was found for P_F where, even if P fertilized plots presented increased P_F, soil available P predict weakly the foliar content of this nutrient. P_F was, however, closely linked to DH. Interestingly, K_F responded differently, being directly associated with K_S but not with DH. This could be related to the fact that anions as phosphates are more retained in the study site soil than cations as K, due to its content of active Al and Fe, and therefore their availability is more limited, rather than its quantity (Satti et al. 2007).

Overall, nutrient uptake in *N. antarctica* varies not only with the soil content of individual nutrients but also with their interaction and broader site productivity factors, such as water availability, as seen in other studies (Schlesinger et al. 2016). The distinct responses of foliar N, P, and K suggest that each nutrient has a unique uptake pathway and dependency on specific environmental conditions, being K more directly dependent on its availability in soil, and N and P on other environmental factors.

Specific leaf area also demonstrated an increase with overall fertilization, a phenomenon that has been previously documented and associated with foliar nutrient content and photosynthetic rates (Firn et al. 2019; Funk et al. 2017; Poorter et al. 2009). Interestingly, SLA increased together with K_S and DH, which could be linked with water availability and trees' hydric stress. On the other hand, it is widely reported that K improves plant water use efficiency (whether it's through its role on stomatal control or by modulating biomass allocation patterns; Almeida et al. 2015; Asensio et al. 2020; Turcios et al. 2021). Therefore, and considering that SLA normally decreases under hydric stress (Liu and Stützel 2004; Lozano et al. 2020; Wellstein et al. 2017), an increase in SLA could be expected in plots where there is more water available (high DH plots) and in plots where soil K is more available and plants are consequently more efficient in their water use.

6.4 Nutrient Resorption During Senescence

As for EcM colonization and overall plant nutritional status, our results indicate a differential response of nutrient resorption depending on the nutrients applied and their combinations. The negative association between N_S content and N_{RE}, coupled with the positive association with N_{RP} values, aligns with the concept that plants reduce their dependence on internal N recycling when soil N is more abundant (Aerts 1996; Killingbeck 1996). However, the absence of similar correlations for P and K indicates that *N. antarctica* may be primarily limited by nitrogen in this ecosystem. This goes in concordance with previous studies that state that mycorrhizal plants, such as those of the *Nothofagus* genus, are principally limited by N (Diehl et al. 2008). The observed increase in P and K uptake without a subsequent increase in resorption suggests that the trees might not immediately require phosphorus for growth, indicating a potential luxury consumption scenario (Hommels et al. 1989).

Phosphorus and potassium resorption was, however, associated with the overall site productivity (particularly, with DH). This is not surprising, since numerous studies report an association between resorption and climatic factors (Brant and Chen 2015; Yuan and Chen 2009). We believe this dynamic could stem from differences in soil nutrient availability under varying water availability conditions (correlated with DH). In our study, for example, water availability is associated with K availability, which could have modulated to a certain extent the response of K resorption (Fig. S1). Moreover, it is possible that greater site productivity is linked to a more efficient nutrient use (Prieto et al. 2023; Sathongkaen et al. 2024), which might reduce the necessity for nutrient resorption prior to senescence. However, more in depth studies are needed to elucidate the mechanisms behind this association between resorption and site productivity.

6.5 Ecological Implications

The alterations in *N. antarctica* nutritional dynamics seen in this study could translate to broader implications for forest ecosystems. Firstly, EcM fungi rely on plant-transferred carbon to thrive (Smith and Read 2008). Reduced carbon allocation from host trees could decrease fungal growth, diminishing the presence of EcM fungal derived organic matter in soil, which is highly recalcitrant and contributes to carbon sequestration (Fernandez et al. 2016; Schweigert et al. 2015). This effect could be further aggravated by a reduction of mycorrhizal carbon exudates, and, consequently, of soil aggregates formed by these fungal symbionts (Agnihotri et al. 2022; Jeewani et al. 2021; Sae-Tun et al. 2022). Furthermore, reductions in EcM fungal biomass could lead to long-term soil nutrient losses and water contamination, as they are, for example, associated with increased nitrogen leaching (Bahr et al. 2015; Fang et al. 2020).

Even though foliar nutrient concentrations and SLA suggest that fertilized plants would produce litter that is richer in N, P, and K, this was not the case for all three nutrients after resorption during senescence. Reduced N_{RE} and increased N_{RP} values in fertilized soils (as seen in Fig. 4) could likely lead to higher N returns via litterfall, increasing soil N availability and promoting faster N cycling (Hou et al. 2021; Pei et al. 2019). Conversely, sustained P and K resorption, even in nutrient-rich conditions (as seen in Figs. 5 and 6), implies that these nutrients may remain limited (Sun et al. 2023), maintaining a balanced nutrient cycle that prevents overaccumulation of P and K. This could be due a limitation not in the amount of these nutrients, but their availability, which is supported by these variables associated with DH, as previously described. This unbalanced effect for each nutrient could lead to changes in soil dynamics, for example, reducing C: N ratios, which have been shown to favor bacterial over fungal communities in the soil (Hou et al. 2021). Such shifts in microbial community composition can, in turn, negatively impact broader ecosystem services such as carbon sequestration, due to changes in soil structure (e.g., less aggregates) and reductions in soil organic matter (Malik et al. 2016; Six et al. 2006). Such shifts underscore how differential resorption of several nutrients affects not only immediate nutrient cycling but also broader ecosystem resilience and functioning through alterations and imbalances in soils and litter nutrients.

7 Conclusions

This study demonstrates that soil nutrient enrichment has complex and interacting effects on plant nutritional dynamics in *N. antarctica* forests. While certain aspects of plant nutrition improved with fertilization, the potential disruption of mycorrhizal networks and alterations in nutrient conservation strategies, especially under N enrichment, highlight the necessity for careful consideration of nutrient management in forest ecosystems. Developing targeted strategies that balance the potential benefits of improved plant nutrition with the preservation of crucial ecological processes will be essential for maintaining forest health and ecosystem services in the context of global change.

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Data Availability The datasets generated during and analyzed during the current study are available in the Figshare repository, https://figshare.com/s/c6b5c5db9b1d75409aa0.

Declarations

Conflict of interest and competing interests The authors have no conflict of interests nor competing interests to declare that are relevant to the content of this article.

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