Effects of soil nutrient enrichment on trophic interactions between herbivorous insects and foliage spiders in Patagonian forests

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Abstract

The addition of nutrients to soil can induce changes through "bottom-up" effects in soil-plant-animal interactions, impacting higher levels in food chains. Changes in foliar nutrient content may influence diets of herbivorous insects, subsequently altering their feeding rates and abundance. Spiders established in foliage rely on these insects for sustenance; therefore, fluctuations in the abundance of herbivorous insect communities ultimately determine the population dynamics of their spider predators. We established a long-term field experiment to investigate the effects of nutrient addition of N (nitrogen), P (phosphorus), K (potassium) and their combinations on plant-arthropod interactions. We carried out sampling in 32 plots under different nutrient addition treatments, where we captured herbivorous insects and spiders on *Nothofagus antarctica* plants, the most abundant plant species in the Patagonian scrubland. Our results showed that foliar nutrient concentrations had cascading effects throughout the forest food chain. Herbivorous insects (Diptera, Coleoptera, Hemiptera and Lepidoptera orders) exhibited a positive response to the increased foliar phosphorus content, resulting in higher population densities. Additionally, we found a strong positive relationship between herbivorous insect abundance and spider abundance. Our findings indicate that variations in the availability of soil nutrients play a crucial role in defining the structure of predator-prey interactions in forest ecosystems.

Key words

Nitrogen; Phosphorous; Potassium; *Nothofagus antarctica*; arthropods.

Introduction

Nutrient enrichment of forest soils drive 'bottom-up' cascading effects that propagate throughout the trophic food web (Penuelas et al. 2020; Cuff et al. 2022; Zhu et al. 2023). Numerous studies have demonstrated that both plants (Garibaldi et al. 2010; Harpole et al. 2011; Hautier et al. 2020) and animals (Sperfeld et al. 2012; Kaspari and Powers 2016; Toft et al. 2021) are often limited by multiple nutrients, including nitrogen (N), phosphorus (P) and potassium (K). However, responses can vary depending on ecological factors such as local biogeochemistry and species life history traits, making it difficult to forecast the effects of nutrient enrichment on plants and animals (Elser et al. 2000, 2007; Zhu et al. 2023). Arthropods are critical components of terrestrial ecosystems, as they support the life of many other animal groups (Rosenberg et al. 2023). As an extremely abundant group, changes in the composition of insect communities may directly impact the entire food chain that is supported by them (Senior et al. 2016; Timberlake et al. 2022). While several studies have linked nutrient supply to changes in arthropod abundance, biomass, and diversity, few have successfully identified the specific mechanisms or processes responsible for these community-level responses (Gruner and Taylor 2006; Poelman et al. 2008; Wimp et al. 2010; Rzanny et al. 2013; Nessel et al. 2023). Understanding how arthropod communities and predator-prey interactions respond to nutrient enrichment is crucial in the face of ongoing changes in land use, as these shifts can have profound impacts on biodiversity and ecosystem functioning (Cuff et al. 2022; Rosenberg et al. 2023).

Herbivorous arthropods are strongly dependent on both the quantity and quality of foliar nutrient content to develop their life cycles, thus responding to alterations in nutrient stoichiometry (i.e., N, P, K) and other nutrient-related foliar traits (Elser et al. 2000, 2007; Fagan et al. 2002). Arthropods depend on N and P as vital nutrients for synthesizing proteins, nucleic acids and several biomolecules, which are essential for their growth, development, and reproductive processes (Elser et al. 2000). For instance, herbivorous insects may modulate their feeding behaviour by selectively consuming plant species or tissues with higher content of limiting nutrients (Awmack and Leather 2002; Zhu et al. 2023). Therefore, plants with greater levels of limiting nutrients are expected to experience higher rates of herbivory (Garibaldi et al. 2010; Martínez et al. 2024) and host more abundant populations of herbivores than individual plants with a poorer nutrient content (Haddad et al. 2000). Although the literature on this topic is growing, more experimental data is needed to understand how the abundance of herbivorous insects in nature is causally linked to changes in plant nutrient content and how the addition of nutrients to the soil may affect this process (Haddad et al. 2000; Zhu et al. 2023; Nessel et al. 2023).

Predatory arthropods, such as spiders, are also impacted by the availability of food (Mayntz et al. 2005; Gruner and Taylor 2006; Michalko et al. 2019; Cuff et al. 2022). Several studies have found that the abundance and diversity of spiders can vary with the abundance of their prey (Horváth et al. 2005; Carvalho et al. 2015; Betz and Tscharntke 2017). This would indicate that individuals that have a more diverse diet and more abundant prey availability could have higher reproduction rates and therefore higher population densities (Uetz et al. 2002; Cuff et al. 2022). Furthermore, higher prey availability may reduce intraguild predation pressures and emigration, and therefore, their populations would remain constant or increasing (Vance-Chalcraft et al. 2007). Given that these predators feed on herbivores, changes in the abundance and quality of plant resources at lower trophic levels can propagate upwards, ultimately affecting the predators (Elser et al. 2000, 2007; Rzanny et al. 2013). In this way, disruptions of soil nutrient availability can trigger bottom-up effects across the entire food web, influencing diverse consumer levels (Elser et al. 2000; Zhu et al. 2023). Despite the importance of understanding how nutrient enrichment affects primary producers, primary consumers, and predators, in predicting potential effects on forest food web structure, there is currently very limited evidence available on this topic (Zhu et al. 2023; Nessel et al. 2023).

Here, we conducted a long-term field experiment involving the addition of multiple nutrients in a forest system in northern Patagonia, Argentina. By using a field-scale experimental study, we evaluated whether the addition of nutrients (i.e., N, P, K) to the soil affects the abundance of herbivorous insects and, consequently, the abundance and family diversity of the foliage spider community. We hypothesize that the addition of fertilizers to the soil increases the concentration of nutrients in plant leaves, enhancing the nutritional quality of the available food for herbivorous insects. This improvement in resource quality is expected to translate into an increase in the abundance of different groups of herbivorous insects, which ultimately increases the abundance and diversity of foliage spiders, as they respond to the greater availability of prey. Previous research found that soil nutrient enrichment increases leaf nutrient content (Martínez et al. 2024), we therefore predict that: *i*) fertilized plots will have a greater abundance of herbivorous insects than control plots and *ii*) the greater abundance of herbivorous insects will lead to an increase in the total abundance and diversity of foliage spiders.

Materials and Methods

Experimental site

The study was conducted in El Foyel, Río Negro province, Argentina (41°38′37″S and 71°26′54″W; Fig. 1). The experimental site is located within the framework of the project "CONCIENCIA" in a private area dedicated to the conservation of biodiversity, education, and the development of scientific projects (https://www.proyectoconciencia.org/). The region experiences a temperate-cold climate, with average annual temperatures ranging from 3°C in winter to 15°C in summer, and frequent frost events during the June to August period. Precipitation is concentrated predominantly in autumn and winter, with an annual average between 920 and 1300 mm (Reque et al. 2007). The study site lies at an elevation between 790 and 880 m above sea level, and its soils are classified as Hapludands (Diehl et al. 2008), characterized by nutrient limitations, particularly in N and P (Perakis and Hedin 2002).

The experimental plots are situated in a field of native mixed scrubland, typical of the Andean-Patagonian region. The vegetation is primarily composed of shrubs and short-sized trees, including characteristic species such as *Schinus patagonicus* (Phil.) I.M. Johnst., *Discaria chacaye* (G. Don) Tortosa, *Berberis microphylla* a G. Forst, *Lomatia hirsuta* (Lam.) Diels, *Embothrium coccineum* J.R. Forst. and G. Forst., and *Diostea juncea* a (Gillies and Hook. ex Hook.) Miers (Reque et al. 2007).

Nothofagus antarctica (G. Forst) was selected as the focal species for our study since it is the most dominant species of these scrublands. Furthermore, as a genus widely distributed throughout the Andean-Patagonian regions, it serves as a suitable model for investigating plant-animal interactions in this region (Peri et al. 2009). This deciduous tree exhibits a variable morphology, ranging from shrubs 2 to 4 meters in height to trees potentially reaching up to 15 meters (Ramírez et al. 1985).

Experimental design

The experimental design consists of 32 plots distributed across 4 complete randomized blocks, following a full factorial design. Each plot measures 31.5 x 45 m, and the treatments result from all possible combinations of N, P, and K. The initial fertilizations were carried out during the spring of 2016, 2017, and 2018. After a three-year interval, the final fertilization was applied in October 2021. The fertilizers were manually applied to the entire surface of each experimental plot, generating a total of 7 treatments (N, P, K, NP, NK, PK, NPK) and a control treatment (i.e., without fertilizer application), with each treatment replicated in each of the 4 blocks (Fig. 1) (Pérez-Méndez et al. 2022; Martínez et al. 2024). The nutrient addition doses were calculated based on those used in other similar forest systems. The applied fertilizers were as follows: 100 kg/ha of extended-release urea for N (Lindberg and Persson 2004), 75 kg/ha of triple superphosphate for P, and 56 kg/ha of K sulfate for K (Kim 2008).

Soil and leaf data collection

In our previous study, we showed that soil nutrient additions increased foliar N, P and K levels in *N. antarctica* (Martínez et al. 2024) (Fig. S1). Here, and based on these findings, we investigated the potential impact of elevated foliar nutrients on the herbivorous insect community. Soil and leaf samples were collected in February 2022, coinciding with the same sampling season as the arthropod collection. In the soil samples, we measured nitrates (NO_3) using the "Bremner method" (Bremner and Mulvaney 1982), available phosphorus using the "Bray-Kurtz method" (Bray and Kurtz 1945), and available potassium with the "Ammonium acetate method" (Novozamsky and Houba 1987). Leaf samples of *N. antarctica* were collected from eight individuals in each plot and combined into a single sample (1 g). We measured nitrogen using the "Kjeldahl method" (Kjeldahl 1883) and total phosphorus and total potassium using the "Sommer and Nelson method" (Nelson and Sommers 1973).

Arthropod sampling

The collection of foliage arthropods was conducted during February 2022 (fourth month after the last fertilization). We selected six *N. antarctica* individual trees per experimental plot and applied 10 strokes per tree with an entomological net over the canopy. During each sweep, we collected all the specimens that fell into the net and placed them in 100-ml jars containing 70% alcohol for subsequent classification. During the sampling, the number of leaves on each sampled tree was visually estimated to determine the average biomass per plot. In the laboratory, the spiders were separated from the rest of the collected arthropods and identified, with individuals classified according to family, sex, life stage and hunting behaviour. For spiders we calculated abundance and Shannon diversity index for each plot. We used the Shannon diversity index because it combines the richness of families found with their relative abundance, providing a synthetic measure of diversity. For the collected insects, classification was performed at the order level (i.e., Diptera, Hemiptera, Coleoptera, Hymenoptera and Lepidoptera). The individuals were counted to estimate total abundance for each insect order.

Statistical Analysis

Statistical analyses were conducted in in the R statistical software (Core, 2021).We constructed models using a generalized linear mixed model (GLMM) approach to assess the effects of foliar nutrient concentrations and their interactions on the abundance of herbivorous insects (Prediction *i*). Separate models were made for each of the insect orders (i.e., Diptera, Hemiptera, Coleoptera, Hymenoptera and Lepidoptera) and the response variable was their abundance in each plot, considering negative binomial error. The fixed factors included the concentrations of foliar nutrients (N, P and K) and their interactions, while the experimental block was incorporated as a random factor. The total abundance for each order of herbivorous insects was calculated as the sum of all individuals collected from the six trees sampled in each plot. To account for the influence of biomass (average of the total number of leaves of all the trees sampled per plot) on insect responses, it was included as a fixed factor in each model.

Additionally, we built another model in which we used the Shannon diversity of spiders as the response variable (with Gaussian error distribution) and the abundance of insects of each order for fixed factor, following the same approach as in the previous models. Shannon diversity index was calculated by the R Vegan package (Oksanen et al., 2019). In order to estimate possible direct effects that soil nutrient addition could have on the spider community, we built a GLMM to evaluate the interactive effects of adding N, P, and K to the soil on spider abundance. The response variable was the abundance of spiders on each plot, considering negative binomial error distribution. The addition of each nutrient with values of presence or absence were used as interacting fixed effects, and the blocks were included in the model as random factor. We used *glmmTMB* package for GLMMs models (Brooks et al. 2017) and *DHARMa* package (Hartig 2020) to verify the assumptions of the models. We used Likelihood Ratio Tests (LRT) with the

Anova function in the *car* package (Fox and Weisberg 2018) to evaluate the significance of the variables.

Finally, to evaluate how spiders were affected by the abundance of their potential prey (Prediction *ii*) we conducted a structural equation model (SEM) in R-package *piecewise* (Lefcheck 2016). We applied a set of models in which the abundance of the insect orders (Diptera, Hemiptera, Coleoptera, Hymenoptera, and Lepidoptera) was used as the response variable. The predictor variables identified as significant in the previous GLMMs were incorporated as fixed factors in these models (foliar P content and biomass, see results). In all the component models, we included block as random variable, and used Poisson error distributions. The final SEM included sub models in which we evaluated the response of spider abundance in relation to insect orders and biomass. We evaluated the overall goodness of fit of the model using directed separation tests (Shipley 2000; Lefcheck 2016).

Results

Effects of nutrient foliar on insects

Using the entomological net on the tree foliage, a total of 1,709 insects that were potential spider prey was collected. The most abundant order was Diptera, representing 53% of the specimens, followed by Hemiptera (25%), Coleoptera (10%), Hymenoptera (8%) and Lepidoptera (4%) (Table S1). The models revealed that insect abundance was positively related to foliar P content for Diptera, Hemiptera, Coleoptera and Lepidoptera and to biomass for Hemiptera and Lepidoptera (Table 1, Fig. 2). We did not find significant differences for the Hymenoptera in relation to foliar P content (Table 1). On the other hand, no significant differences were found for the other nutrients and their combinations on insect abundance (Table 1).

Effects of abundance of insect on spider diversity and abundance

Overall, 3,518 spider individuals were collected, of which 3,477 were juveniles. Of the remaining 41 adults, 32 were females and 9 were males. The most abundant family was Anyphaenidae representing 78% of the collected individuals. The second most abundant family was Theridiidae with 13% followed by Araneidae with 4% (Table S2). The remaining families each accounted for less than 1% (Table S2). We did not find an effect of insect abundance on the diversity of spiders (Table 2; Table S3). Finally, the addition of nutrients (N, P, K and combinations) to the soil was not related to the abundance of spiders (Table 3).

The relationships between spider abundance and the predictors variables were adequately represented by the piecewise SEM (global goodness of fit: Fisher's C = 7.664, df = 12, p = 0.811; tests of directed separation in Table S4). This causal model indicates that the effect of foliar nutrient content on spider abundance was indirect, through an effect on abundance of herbivorous insects (Fig. 3, Table S5). The relationship between foliar P content and orders of insects was positive from Coleoptera, Hymenoptera and Lepidoptera, in keeping with the result from GLMMs, (Fig. 3, Table S5). In turn, spider abundance was positively related to the orders Coleoptera, Hymenoptera, Hemiptera and Lepidoptera (Fig. 3, Table S5). Thus, the positive effect of foliar P content on herbivorous insects cascaded into an increased abundance of spiders. Notice also that an additional effect negative of biomass on the spider abundance and positive on order Hemiptera (Fig. 3, Table S5). All non-significant relationships are presented in Table S5 of the supplementary material.

Discussion

We found a strong relationship between the supply of limiting nutrients to the leaves and the change in the abundance of herbivorous insects and foliage spiders. Our long-term field experiment allowed us to test the central hypothesis of our research and partially corroborate our predictions. While we confirmed that increased foliar P levels lead to higher herbivore abundance on four insect orders (Diptera, Hemiptera, Coleoptera and Lepidoptera), supporting our first prediction, we found no effect of foliar nutrients on the order Hymenoptera. The second prediction was only partially upheld, as we found that spider abundance correlates positively with herbivore abundance, but no effect was detected on spider diversity. Likewise, we did not find evidence that soil nutrient addition had a direct impact on the spider community.

Populations of herbivorous insects exhibited higher densities in P-fertilized plots compared to non P-fertilized plots, suggesting that they are more limited by P than by other nutrients. This is interesting because this nutrient is not typically considered as limiting for insects like N, and there are few studies that have been conducted on this topic (Huberty and Denno 2006; Butler et al. 2012; Nessel et al. 2023). The P content in terrestrial insects is more variable than the N content, both in leaf-chewing species, such as lepidopteran larvae, and those that feed on phloem, such as aphids (Elser et al. 2000, 2007). Therefore, the variation in the nutritional content of the food may cause insects to consume more of the tissues with a higher P content (Elser et al. 2000). Phosphorus is particularly important for animals since RNA and protein molecules are synthesized from it (Schade et al. 2003; Huberty and Denno 2006). Therefore, higher availability of this element in their food sources could lead to improved individual fitness and population growth (Schade et al. 2003), as we observed in our experiment. Fecundity, defined as the number of viable offspring produced, is directly affected by the quality of food consumed by females (Awmack and Leather 2002). For example, it has been studied that in lepidopterans (Stern and Smith 1960; Boggs and Ross 1993), coleoptera (Hopkins and Ekbom 1999) and aphids (Dixon et al. 1982) the quality of the host plant determines fecundity during the larval and adult stages (Awmack and Leather 2002). This could explain why we found a greater abundance of insect populations in plots with higher P levels. It is important to note that not all insects analysed are herbivorous at some stage of their life. Many could be carnivorous or parasitic, which could distort our results. This is particularly true for Hymenoptera (mainly wasp species), which often act as predators or parasitoids and may not show a relationship with foliar nutrient levels (Huber 2017). However, they can be affected by other factors such as changes in plant species richness, plant productivity, plant composition, as well as the quality and quantity of resources such as nectar (Haddad et al. 2000).

Plots where P was combined with other nutrients did not show any effect on the herbivore abundance. This could be due to the fact that the positive effect of P was seen to be diminished by other nutrients (i.e. N and K). For example, the addition of nitrogen can increase plant defensive compounds against herbivorous insects while also reducing leaf digestibility, which may lead to a decline in populations feeding on these leaves in N-fertilized plots (Chen et al. 2010). Moreover, N enrichment can result in smaller, high-density leaves that are less palatable to herbivorous insects, leading to a decrease in their populations (Stiling and Moon 2005). On the other hand, it has been observed that plants with higher potassium availability are less susceptible to herbivorous insect attacks and disease incidence (Wang et al. 2013). This could explain why, in plots with the addition of K and N, the positive effect of P in the populations of insects, they can be reduced.

Multiple studies in forests have shown that mature trees reaching the canopy support a greater richness of herbivorous insects compared to smaller trees (Basset et al. 2001; Neves et al. 2014). In our study, we found that Hemiptera and Lepidoptera were the orders that were positively affected by biomass. This may be because bigger trees may offer greater availability of resources to herbivores (Stiling and Moon 2005; Neves et al. 2014). Furthermore, insect herbivores that live in larger host trees decrease their risk of being encountered by predators (Boege 2005; Rijhimäki et al. 2006). Additionally, different feeding guilds will respond differently to changes in the forest canopy structure (Vehviläinen et al. 2008; Sobek et al. 2009). We found that changes in biomass also affected certain herbivorous insect orders, including sap-feeders and chewers, consistent with Neves et al. (2014), who observed increased abundance of these groups with larger tree canopies.

Spider abundance was positively associated with the abundance of four orders of herbivorous insects, although we did not find an effect on spider diversity. Most of spiders captured in our experiments were generalist predators, which feed mainly on insects such as dipterans, coleopterans, hemipterans and hymenopterans (Michalko and Pekár 2019), as well as other spiders (Pekár et al. 2012). This is congruent with the strong positive relationship that we found among spider abundance and the abundance of the above-mentioned groups of insects. Additionally, we observed a negative relationship between biomass and total spider abundance, which we interpret as a dilution effect resulting from the increased amount of foliage. This could be attributed to a sampling effect, for instance, in canopies with a greater quantity of leaves, spiders were more difficult to capture using an entomological net.

Only Diptera did not affect spider abundance, which could be attributed to several factors. Generalist predators, such as most of the captured spiders, can adjust their prey consumption based on the nutritional quality, relative availability of the prey, size and mobility (Mayntz et al. 2005; Schmidt et al. 2012; Cuff et al. 2024). Spiders have been shown to be highly selective regarding their food sources and to make precise decisions about their prey choices. Some studies indicate that the availability of prey does not always correlate positively with the selection of those prey by predators (Schmidt et al. 2012). In scenarios with multi-taxon prey abundance, like our P-fertilized plots, spiders may have a wider variety of prey and choose those that are nutritionally superior (Cuff et al. 2024). This could explain why Diptera were not selected as prey by spiders and why no relationship was found between their abundances. On the other hand, hunting spider species, such as Anyphaenidae spiders (representing more than 70% of our samples) are typically more effective at capturing sedentary prey (Kuusk and Ekbom 2012; Sweeney et al. 2013), which could explain the avoidance of dipterans by predators, as they are highly mobile and difficult to capture.

Although predators are expected to act as insect controllers, which would mean that a greater number of predators (spiders), would produce a decrease in insects, we found a positive relationship between both groups. This may be because, at the time of sampling, most of the spiders captured were juveniles, indicating that the feeding peak was just beginning. If sampling had been done later, the number of insect individuals might have started to decrease due to the predator pressure exerted by spiders.

Through the analysis of the direct effects of adding N, P and K to the soil on spider abundance, we were able to rule out the influence of other mechanisms on the spider community in our experiment. This finding contrasts with a meta-analysis by Nessel et al. (2023), which reported a decrease in arachnid abundance following the addition of N and the combination of N and P. Therefore, this effect may be attributed to multiple factors and may not be related to foliar nutrient content. It is known that nutrient addition can affect predators through various mechanisms, such as increasing plant biomass, which in turn is positively correlated with the biomass of herbivores and predators (Kempel et al. 2023). In contrast, other studies have found that increased productivity and improved nutrition of host plants lead to higher densities of herbivorous insects, which in turn enhances the abundance of their predators (Hartvigsen et al. 1995; Fraser and Grime 1998; Polis et al. 1998; Forkner and Hunter 2000). For example, Denno et al. (2002) found in a laboratory experiment that increasing the N content of plants resulted in increased reproduction of spiders, associated with increased populations of their prey. These results support instead the mechanism proposed by our hypothesis. Here, we show that the abundance of prey insects is influenced by nutrient additions (particularly P), and this has a direct relationship with spider abundance, thereby revealing a strong bottom-up effect.

We were able to determine that prey availability is a factor that influences spider abundance but does not affect spider diversity. We did not find significant results for spider family diversity; which could be due to the fact that we obtained very few representatives from spider families other than juvenile of Anyphaenidae. Also, the Anyphaenidae family was dominant in all treatments, representing more than 70% of the spiders found in all plots. Working at the family level rather than the species level may have limited our ability to detect changes in the diversity index. This limitation was due to the impossibility of determining the juvenile spiders at the species level. Therefore, we propose conducting more exhaustive sampling using methods that capture a greater number of spider families to obtain a more comprehensive diversity index in future studies. Additionally, sampling should be repeated at various stages of the spiders' life cycle to capture a greater number of adult individuals, thereby enhancing the specificity of species identification. Although our study is limited to a single, temporary sampling event, we believe that the data collected are representative of the arthropod community. This is supported by the fact that the sampling was conducted during the summer months, which correspond to the peak period of arthropod activity in our study system.

Our study represents a first step towards understanding how food webs function in forest ecosystems through soil management induced changes. These findings are valuable for predicting future changes in arthropods population dynamics and interactions under the pressures of anthropogenic global change, such as soil nutrient enrichment. Overall, our findings have important implications for the management plans for native mixed scrublands in Patagonian forest.

Declarations

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Conflicts of interest

The authors declare there are no conflicts of interest.

Ethics approval

(Not applicable)

Consent to participate

(Not applicable)

Consent for publication

(Not applicable)

Availability of data and material

Data generated or analyzed during this study are available from the corresponding author upon reasonable request.

Code availability (Not applicable)

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Lucía C. Martínez, Justina Panchuk, Joana P. Haedo, Néstor Pérez-Méndez, Gabriel Pompozzi, Facundo Fioroni, Lucas A. Garibaldi and Hugo J. Marrero. The first draft of the manuscript was written by Lucía C. Martínez and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Results of GLMMs evaluating the relationship between insect orders (Diptera, Hemiptera, Coleoptera, Hymenoptera, and Lepidoptera), foliar nutrient content (N, P, K, and their combinations) and biomass. For each variable that appear in the models, the estimate, standar error (SE), value Z and p-value are shown. Variance and standard deviation (Std. Dev) belong to the random effects (block). The table also includes the analysis of deviance for the models, reporting chi-square test statistics (Chisq) and degrees of freedom (Df). Significant differences are highlighted in bold (***p < 0.001; *p < 0.01; p < 0.05).

Order Diptera								
Foliar nutrient content	Estimate	SE	z	Pr(>|z|)	Variance (block)	Std.Dev (block)	Chisq	Df
Intercept	1.6e+00	1.2e+00	1.33	0.18	3.9e-10	1.9e-05	1.79	1
N	1.0e-02	1.2e-01	0.08	0.93			0.00	1
P	4.3e-01	1.4e-01	3.13	**0.001**			**9.80*****	1
K	1.3e-01	1.3e-01	1.03	0.30			1.06	1
N:P	5.8e-05	1.4e-01	0.00	0.99			0.00	1
N:K	2.1e-01	1.3e-01	1.60	0.10			2.58	1
P:K	-2.3e-01	1.3e-01	-1.83	0.06			3.37	1
N:P:K	-2.1e-01	1.3e-01	-1.59	0.11			2.54	1
Biomass	2.0e-01	1.7e-01	1.15	0.24			1.33	1
Order Hemiptera								
Intercept	-0.18	1.18	-0.15	0.87	4.8e-10	2.2e-05	0.02	1
N	0.00	0.11	0.02	0.97			0.00	1
P	0.31	0.12	2.61	**0.008**			**6.83****	1
K	-0.14	0.13	-1.08	0.27			1.17	1
N:P	-0.12	0.13	-0.95	0.33			0.91	1
N:K	0.08	0.13	0.63	0.52			0.40	1
P:K	0.08	0.12	0.68	0.49			0.46	1
N:P:K	-0.09	0.12	-0.74	0.45			0.55	1
Biomass	0.37	0.16	2.28	**0.02**			**5.22***	1
Order Coleoptera								
Intercept	0.14	1.39	0.10	0.91	7.7e-10	2.7e-05	0.01	1
N	0.02	0.15	0.16	0.87			0.02	1
P	0.52	0.14	3.71	**0.0002**			**13.8*****	1
K	0.01	0.16	0.06	0.94			0.00	1
N:P	-0.15	0.18	-0.87	0.38			0.76	1
N:K	0.13	0.16	0.83	0.40			0.69	1
P:K	0.21	0.14	1.49	0.13			2.22	1
N:P:K	-0.26	0.15	-1.66	0.09			2.77	1
Biomass	0.20	0.14	1.03	0.30			1.06	1

Order Hymenoptera								
Intercept	3.12	1.52	2.04	**0.0**	0.34	0.58	**4.18***	1
N	0.05	0.14	0.36	0.71			0.13	1
P	-0.10	0.23	-0.43	0.66			0.13	1
K	-0.08	0.16	-0.50	0.61			0.25	1
N:P	0.009	0.16	0.05	0.95			0.003	1
N:K	-0.15	0.16	-0.91	0.35			0.84	1
P:K	0.06	0.13	0.44	0.65			0.20	1
N:P:K	-0.02	0.15	-0.18	0.85			0.03	1
Biomass	-0.24	0.21	-1.13	0.25			1.29	1
Order Lepidoptera								
Intercept	-2.46	1.45	-1.69	0.08	8.1e-07	0.0009	2.88	1
N	-0.18	0.15	-1.17	0.24			1.38	1
P	0.36	0.16	2.27	**0.02**			**5.18***	1
K	0.15	0.15	0.96	0.33			0.93	1
N:P	-0.08	0.20	-0.43	0.66			0.19	1
N:K	0.19	0.17	1.12	0.25			1.27	1
P:K	0.08	0.14	0.54	0.58			0.29	1
N:P:K	-0.11	0.16	0.54	0.49			0.46	1
Biomass	0.42	0.20	2.11	**0.03**			**4.46***	1

Table 2. Results of GLMMs assessing the relationship between spider diversity and insect orders (Diptera, Hemiptera, Coleoptera, Hymenoptera, and Lepidoptera). For each variable that appear in the models, the estimate, standar error (SE), value Z and p-value are shown. Variance and standard deviation (Std. Dev) belong to the random effects (block). The table also includes the analysis of deviance for the models, reporting chi-square test statistics (Chisq) and degrees of freedom (Df). Significant differences are highlighted in bold (***p < 0.001; *p < 0.01; p < 0.05).

Orders of insects		**Estimate**	**SE**	**z**	**Pr(>|z|)**	**Variance (block)**	**Std.Dev (block)**	**Chisq**	**Df**
Diptera	diversity	0.002	0.002	1.032	0.302			1.065	1
	intercept	0.932	0.090	10.256	**2e-16**	0.011	0.105		
Hemiptera	diversity	-0.005	0.006	-0.960	0.337			0.921	1
	intercept	1.068	0.124	8.614	**2e-16**	0.027	0.164		
Coleoptera	diversity	0.007	0.015	0.455	0.649			0.207	1
	intercept	0.954	0.110	8.610	**2e-16**	0.013	0.117		
Hymenoptera	diversity	0.0009	0.016	0.062	0.951			0.003	1
	intercept	0.986	0.106	9.309	**2e-16**	0.019	0.139		
Lepidoptera	diversity	0.010	0.037	0.293	0.769			0.086	1
	intercept	0.968	0.111	8.680	**2e-16**	0.017	0.131		

Table 3. Results of the GLMM for the effects the relationship between nutrient added to soil (N, P and K) and their combinations on abundance of spiders. The estimate, standar error (SE), value Z and p-value are shown. Variance and standard deviation (Std. Dev) belong to the random effects (block). The table also includes the analysis of deviance for the models, reporting chi-square test statistics (Chisq) and degrees of freedom (Df). Significant differences are highlighted in bold (***p < 0.001; *p < 0.01; p < 0.05).

	Abundance of spiders							
Soil nutrient added	Estimate	SE	z	Pr(>|z|)	Variance (block)	Std.Dev (block)	Chisq	Df
Intercept	4.89	0.22	21.46	**2e-16**	0.09	0.31	**460.85*** **	1
N	-0.32	0.24	-1.30	0.19			1.69	1
P	-0.26	0.24	-1.08	0.27			1.16	1
K	-0.45	0.25	-1.78	0.07			3.17	1
N:P	0.20	0.36	0.56	0.57			0.31	1
N:K	0.19	0.38	0.50	0.61			0.25	1
P:K	0.69	0.35	1.94	0.05			3.79	1
N:P:K	-0.08	0.51	-0.17	0.86			0.02	1

Figure captions

Figure 1. Study site and experimental design. (a) El Foyel, Río Negro, Argentina. (b) Experimental design with 32 plots distributed across four blocks. Each plot contains a nutrient addition treatment (N, P, K, NP, NK, PK and NPK) and a control (Co). (c) Photograph of the study site in the Patagonian forest.

Figure 2. Relationship between the abundance of herbivorous insects (Diptera, Hemiptera, Coleoptera and Lepidoptera) and foliar phosphorus content (P). The points represent the total number of individuals found for each order across the experimental plots. The p-value of the GLMM is shown for each order.

Figure 3. Structural equation model (SEM) testing the direct and indirect effects of orders of insects (Diptera, Hemiptera, Coleoptera, Hymenoptera and Lepidoptera) on abundance of spiders and biomass. Boxes represent measured variables. Continuous arrows show significant ($p < 0.05$) unidirectional relationships among variables, whereas dashed arrows show not significant correlated, being positives marked in black and negatives in red.

Figures

Figure 1

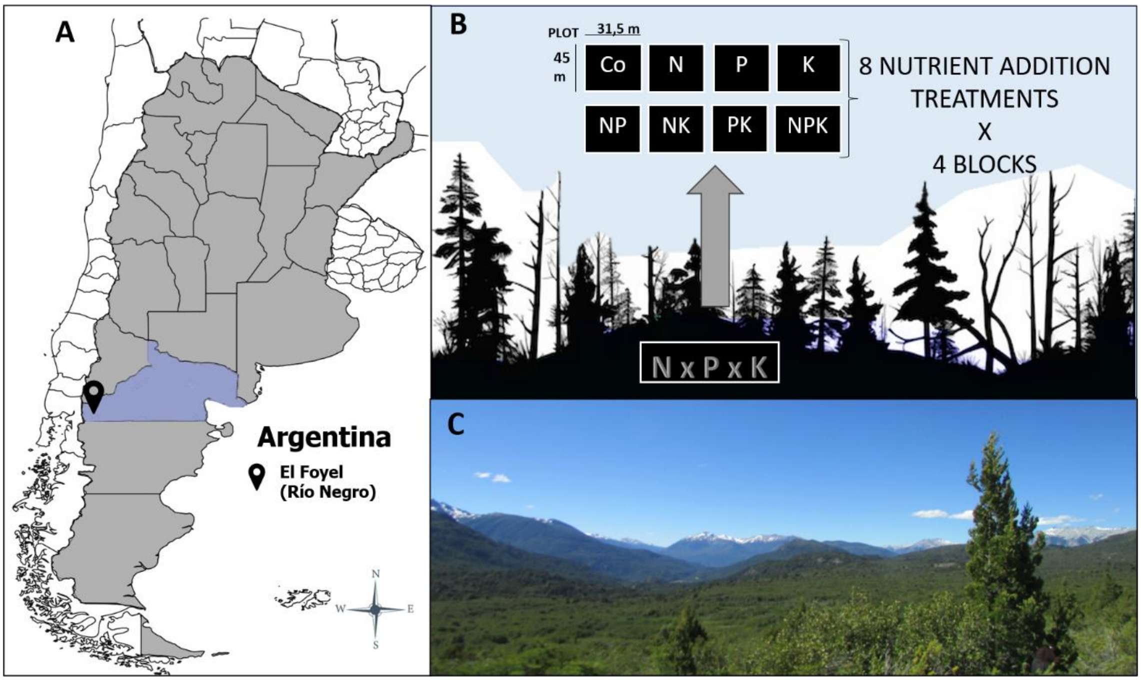


Figure 2

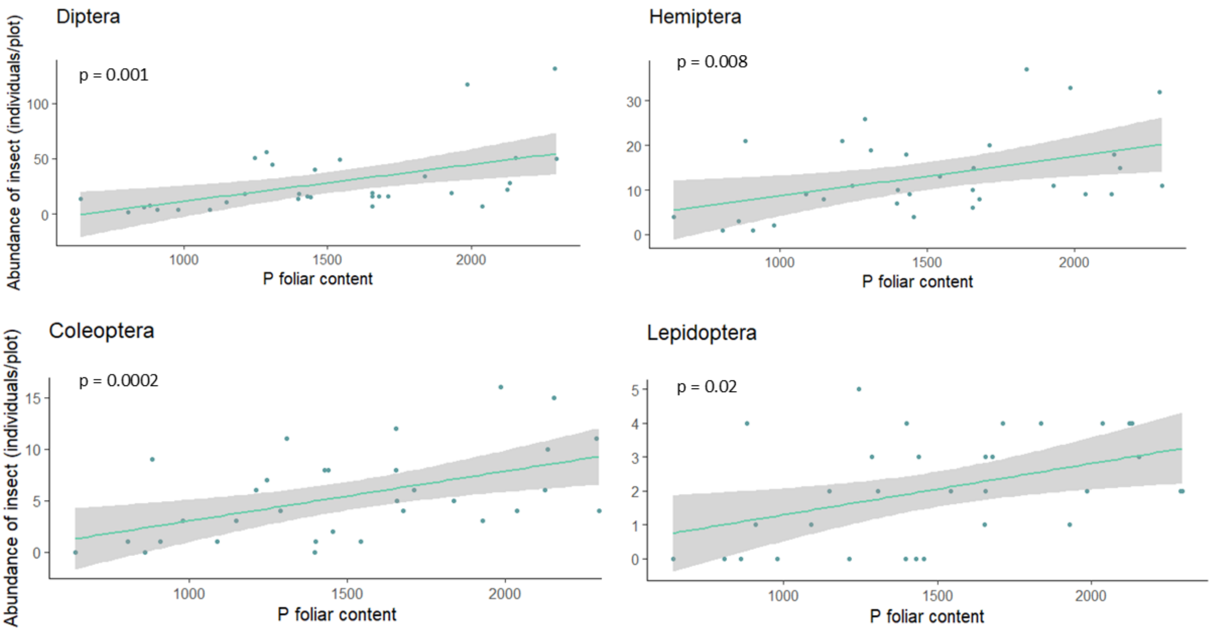
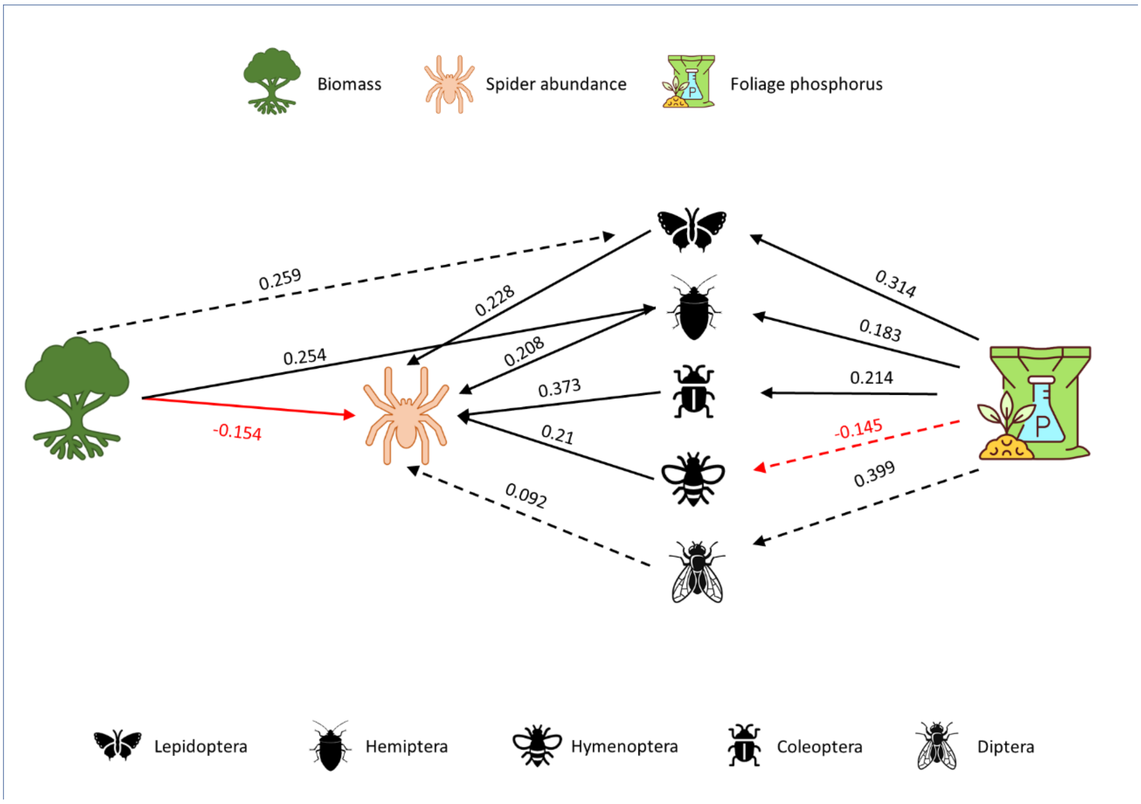

Diptera
p = 0.001
Abundance of insect (individuals/plot)
0
50
100
1000
1500
2000
P foliar content
Hemiptera
p = 0.008
0
10
20
30
1000
1500
2000
P foliar content
Coleoptera
p = 0.0002
Abundance of insect (individuals/plot)
0
5
10
15
1000
1500
2000
P foliar content
Lepidoptera
p = 0.02
0
1
2
3
4
5
1000
1500
2000
P foliar content

Figure 3


Biomass
Spider abundance
Foliage phosphorus
0.259
0.228
0.254
0.208
-0.154
0.373
0.21
0.092
0.314
0.183
0.214
-0.145
0.399
Lepidoptera
Hemiptera
Hymenoptera
Coleoptera
Diptera