Convocatoria AEET-SIBECOL de ayudas a proyectos de investigación ERC en ecología (11ª ed., 2021)

1. Datos de identificación.

Título de la propuesta	Comprehensive assessment of Darwin's Naturalization Conundrum: exploring the role of herbivory, phylogenetic and functional similarity in the success of non-native species.
Categoría	Ganando independencia
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2. Memoria Técnica. Actividades y resultados de investigación

2.1. Introducción (Planteamiento, objetivos y justificación)

Non-native species are one of the main drivers of global change. Understanding the ecological mechanisms that enable non-native species to succeed in novel environments is a central goal in ecology. Phylogenetic relatedness has been proposed as an important factor explaining invasion successes and failures. Based on Darwin's Naturalization Conundrum, being closely phylogenetic related to native species would be positive for the non-natives to overcome abiotic filtering but negative to overcome biotic competition. In order to disentangle the ecological mechanisms driving the success of non-native species in a novel environment is important to consider the following aspects:

i) Different mechanisms may be working at different environmental scales. This can be tested by the use of elevational gradients as tools to evaluate the relative role of biotic competition and environmental filtering.

ii) When exploring the factors determining the success of non-native species there is a lack of assessment of the role of biotic interactions of plants with antagonistic and mutualistic organisms. In this sense, herbivore sharing may complement competitive exclusion in closely related species

iii) Taxonomic relatedness is not always a good proxy of functional similarity. Functional diversity (like phytochemical diversity) can be a better proxy of the interactions of plants with the environment and other organisms.

To test for these factors, I studied plant communities with non-native and native species along elevational gradients. For the studied plant species, I collected leaves to estimate herbivory and conduct metabolomic analyses (focusing on compounds related to anti-herbivory defenses and to the nutritional status of the plants). In addition, I generated a phylogenetic tree of the species to calculate the phylogenetic distance of non-natives to natives. With this project I want to i) test for the effect of elevation and plant status (i.e. native or non-native) on herbivory level, ii) test for elevational gradients in phylogenetic and functional similarities of non-native to native species and iii) check whether the patterns of herbivory of non-native species are underlined by their phylogenetic or functional similarity to natives.

2.2. Descripción de la ejecución- Metodología

2.2.1. Sampling

To conduct this study, I sampled plant communities including native and non-native species along elevational gradients in the Island of Tenerife (Canary Islands, Spain). During the growing season, from late March to early April, I selected 18 plant communities (sampling locations) along two elevational gradients expanding 1600m and 1700m each, from the coast to the central plateau of Teide, until the maximum elevation where non-natives plants were present. I selected the sampling locations based on the information on species composition provided by the MIREN network (www.mountaininvasions.org). This network periodically conducts vegetation surveys in different locations on the island. This allowed me to know the identification and floristic status (native or non-native) of the species included in this study. Each sampling location was at least 100 meters separated from each other (more in the cases there were human populations between sites). Within each location, I defined a community of 50x100m. At each community, I selected all species covering 80% of the total cover and all the species with at least 10 individuals (including grasses, forbs and shrubs). I sampled five plants of each species, for each plant I collected 5 leaves in the case of grasses and forbs and 10 leaves for the shrubs. To account for the phenological differences along elevation, I began the sampling in the lowest plots and moved up the gradient over the sampling.

Leaves were oven-dried for 72 h at 40°C.

2.2.2. Herbivory

For each leaf, I visually estimated the percentage of leaf area removed by insect herbivores (leaf chewers) using the following scale: 0= no damage; 1= 1–5% damaged;2 = 6–10% damaged; 3 = 11–25% damaged; 4 = 26–50%damaged; 5 = 51–75% damaged; 6 ≥ 75% damaged ('leaf herbivory' hereafter). I also accounted for the presence of damage caused by miners and galls when present on the leaves.

2.2.3. Metabolomic analyses by Mass Spectrometry

After measuring herbivory, I selected a subset of three to six leaves (depending on the species) per plant and pooled the leaves from the same species for each community. These leaves were grounded to powder and reserved to conduct the metabolomic analyses.

The metabolomic analysis where conducted at the Platform of Analytical Chemistry at the Université de Neuchâtel (Switzerland).

The analyses were performed as targeted metabolomics to quantify and identify phytochemicals related to plant defenses and nutritional status for all plant species collected in the field (n = 124 species). We extracted 15 mg of each of the dry ground tissue with 0.5 mL of extraction solvent (MeOH: MilliQ water: formic acid; 80:20:0.1) following Defossez et al. (2021). The extracted samples were analyzed via ultrahigh performance liquid chromatography (UHPLC) coupled to a quadrupole time-of-flight mass spectrometry (QTOFMS) and using an Acquity UPLC C18 column (50 mm × 2.1 mm, 1.7 µm; Waters). For more specifications on the program see Defossez et al. (2021). Phytochemicals were identified based on their MS/MS spectra and using the NIST Standard Reference Database 1A v17.

The final steps of integration of all the different compounds are taking place and the final results will be obtained by the beginning of February. Thus, the results from the effects of functional diversity are not included in this report yet only some preliminary results are shown. Once the final data is ready, I will test for i) functional similarity in the metabolomic profile between native and non-native species and ii) for the association between the functional diversity in metabolomics for each plant species and the levels of herbivory.

2.2.4. Statistical analyses

Phylogenetic tree

In order to calculate the phylogenetic distances of the non-native species to the native species in the community I generated a phylogenetic tree of the 124 species sampled for this study. I used the phylo.maker function in 'V.PhyloMaker' R-packages by matching the family, genus and species epithet from my study with those in the backbone using the GBOTB.extended phylogeny (i.e., the mega-tree implemented in the 'V.PhyloMaker' R package). These analyses were performed in R version 4.02 (R Core Team, 2021).

Patterns of variation in leaf herbivory across environmental gradients and between species status

To analyze the effect of elevation and species status (i.e. native or non-native) on the level of herbivory at the community level, I fitted, for each of the three measurements of herbivory (chewers, miners and galls) a linear mixed-effects model ('lmer' in the R package 'lmerTest'). I included each measure of herbivory as the response variable and, as predictor variables, elevation, species status and their interaction as fixed effects. As random factor, I added sample sites nested within road to account for the nested structure of the sampling design.

Phylogenetic distance of non-natives to natives and its patterns of variation with elevation

In order to quantify the phylogenetic relatedness between non-native and native species, I calculated the weighted mean distance (WMDNS) of the non-native species relative to all native species for each community. The WMDNS is a phylogenetic dissimilarity metric developed by Thuiller et al. (2010) adapting metrics that are commonly used to depict the phylogenetic structure of natural communities. I averaged the mean value across all non-native species per plot.

To analyze the effect of elevation on the phylogenetic distance of non-native to natives, I fitted a mixed model including WMDNS as the response variable and elevation as the predictor. I included sample sites nested within road as random factors to account for the nested structure of the sampling design.

Effect of phylogenetic distance on herbivory levels

I repeat the initial mixed model using a subset of the dataset only for non-native species and including the phylogenetic distance of non-natives to natives instead of species status to check if phylogenetic distance varies with elevation and if phylogenetic distance underlies the variability in herbivory.

Multivariate analyses of phytochemical diversity and plant status

The final step of compound identification of the metabolomic dataset obtained is not finished yet. But using the entire metabolomic dataset (untargeted metabolomics) I ran a redundancy analysis (RDA) to see differences in the metabolomic profiles of non-native and native species. Specifically, I performed a PCA test with the rda function in the Vegan R-package.

1.1. Resultados obtenidos (cumplimiento de objetivos)

1.1.1. Patterns of variation in leaf herbivory across environmental gradients and between species status (Objective 2 in the initial proposal)

I found that the levels of herbivory vary across elevations and differ for natives and non-natives. The interaction of elevation and species status had a significant effect on herbivory (p-value= 0.02). Surprisingly, herbivory levels increased with increasing elevation and were greater for non-native species at low and mid elevations but similar to the levels for natives at high elevations (Figure 1 in Appendix A).

1.1.2. Phylogenetic distance of non-natives to natives and its patterns of variation with elevation (Objective 3 in the initial proposal)

I found a significant effect of elevation on the phylogenetic distance of non-natives to natives (p-value= 0.01). The phylogenetic distance of non-natives to natives increased towards mid-elevations (Figure 2 in Appendix A).

1.1.3. *Effect of phylogenetic distance on herbivory levels (Objective 1 in the initial proposal)*

Although phylogenetic distance varies with elevation, I did not find an effect of the phylogenetic distance of non-natives to natives on the levels of herbivory experienced by non-native species, suggesting that phylogenetic distance is not driving the patterns of herbivory.

1.1.4. Functional similarity between native and non-native species (preliminary results prior to phytochemicals identification) and its patterns of variation with elevation (Objective 3 in the initial proposal)

In total, I quantified more than 100.000 metabolomics on the leaves of the studied species. These compounds still need to be identified in functional groups in order to test for the effect of plant defences and nutrients in the different patterns of herbivory found for native and non-native species. However, is important to highlight that the redundancy analysis (RDA) showed that the metabolomic profiles of non-native and native species are significantly different (p-value= 0.01; Figure 3).

1.1.5. *Functional relatedness explains herbivory? (Objective 1 in the initial proposal)*

To be answer

1.2. Conclusiones y valoración de la ejecución

Surprisingly, I found an elevational gradient in herbivory levels where herbivory increase with increasing elevation. This goes against the idea that herbivory pressure should be greater towards low elevations because of stronger biotic interactions. In addition, I found that the levels of herbivory were different for non-natives and natives and I found non-parallel patterns of variation in herbivory between non-natives and natives along elevation. Specifically, I found that non-natives had greater levels of herbivory than natives at low and mid-elevations. This result contradicts the Enemy Release Hypothesis according to which non-native species should present lower levels of herbivory when arriving at novel environments. However, the levels of herbivory of non-natives are similar to those of natives towards higher elevations. This might indicate that environmental filtering and biotic interactions act differently along the gradient. It could be that, because of strong biotic competition, the non-native species that are successful at low elevations are those that present different characteristics to natives (including different phytochemical composition), these non-native species might not present the defensive compounds needed to escape the native enemies. While at harsher conditions at high elevations, only non-native species with more similar characteristics to natives are able to establish. If this is the case, at high elevations non-native species might present similar phytochemical composition to the natives and are recognized by local herbivores in a similar way. Further analyses of the phytochemical composition might help to better understand what drives these patterns of variability in herbivory.

In conclusion, antagonism interactions with herbivores have an impact on the establishment of non-native species in novel environments. The response of local herbivores might be more determined by the functional similarity to the native species than by phylogenetic relatedness.

Although further analyses are needed, the results presented in this report show important insights for a better understanding of the factors and mechanisms affecting the establishment of non-native species and how these factors and mechanisms vary with different environments.

1.3. Publicaciones resultantes

The manuscript including the results of this study is under preparation.

2. **Informe de gastos del proyecto.** Relación de partidas de gastos y sus importes. Se deberán aportar justificantes originales de los pagos realizados (tickets, recibos o facturas).

The table below summarizes the final expenses of the project. The tickets and receipts are attached in Appendix B.

Concept	**Total (€)**
Rental apartment in Tenerife for 2 weeks	936
Rental car for two weeks to access the field sites	443
Metabolomic analyses	1200
	2579

Fdo: Andrea Cortegoso Galmán

en Haale (Saale), a 30 de Enero de 2023

REFERNCES

Defossez, E., Pitteloud, C., Descombes, P., Glauser, G., Allard, P. M., Walker, T. W., ... & Rasmann, S. (2021). Spatial and evolutionary predictability of phytochemical diversity. *Proceedings of the National Academy of Sciences*, *118*(3), e2013344118.

Thuiller, W., Gallien, L., Boulangeat, I., De Bello, F., Münkemüller, T., Roquet, C., & Lavergne, S. (2010). Resolving Darwin's naturalization conundrum: a quest for evidence. *Diversity and distributions*, *16*(3), 461-475.

Appendix A: Main results

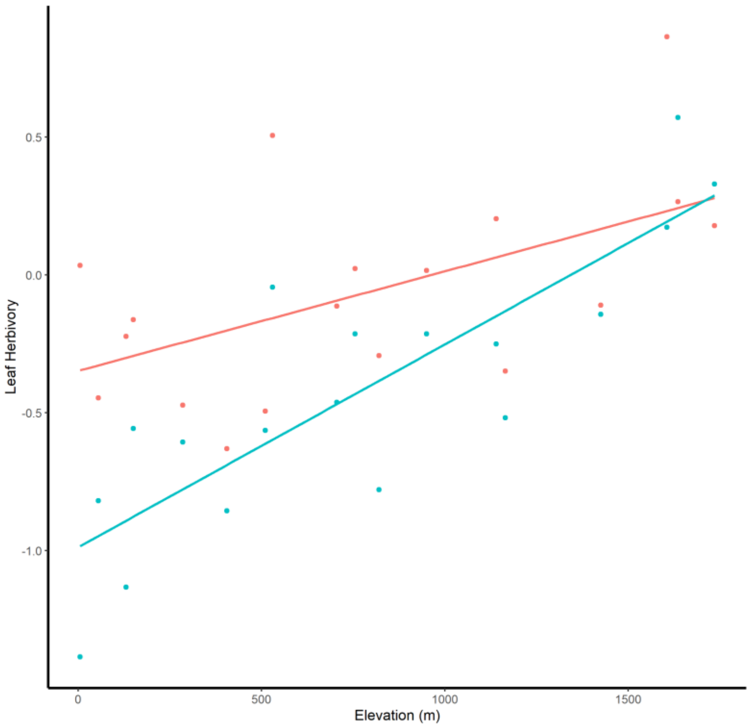

Figure 1: Variability in leaf herbivory with elevation and species status. Red dots and line represent values for non-native species and blue dots and line represent values for native species.

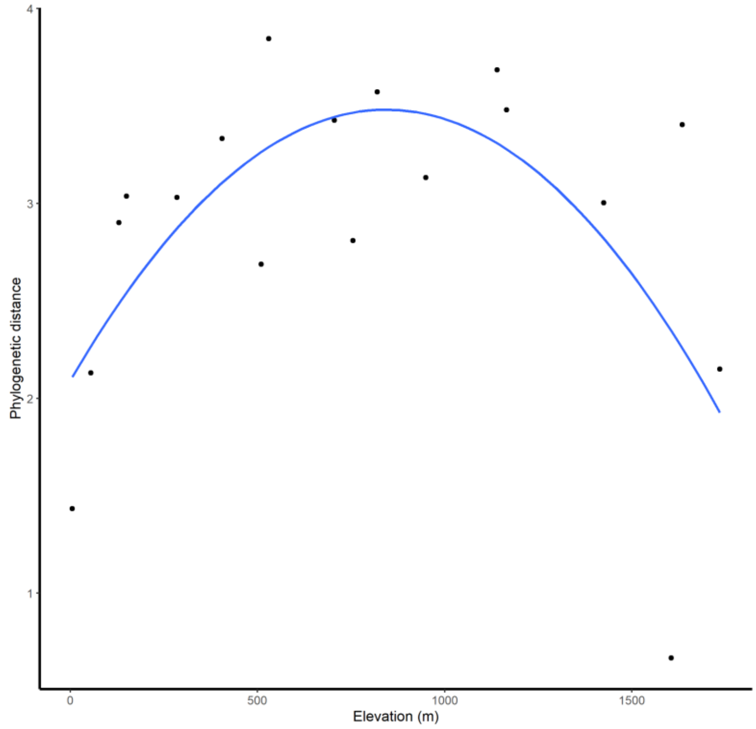

Figure 2: Variability in the phylogenetic distance of non-native species to the natives with elevation. A quadratic term was fitted for elevation.

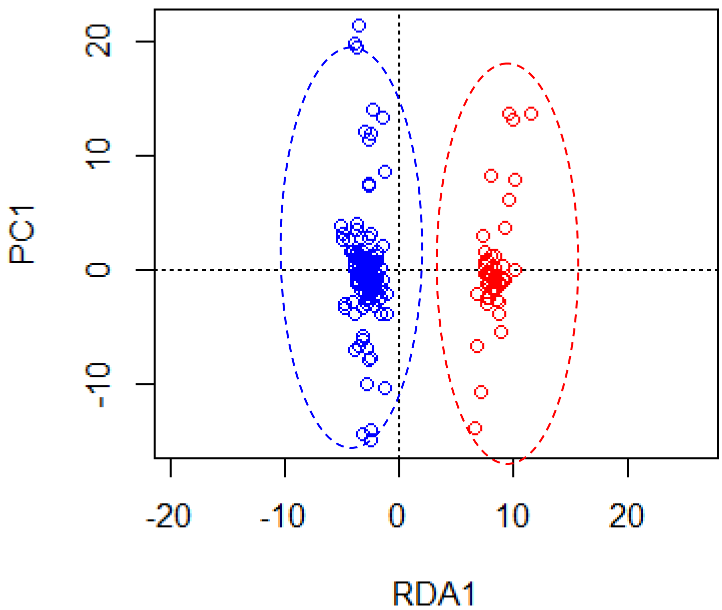

Figure 3: Redundancy analysis (RDA) showing the results of untargeted metabolomic dissimilarity. The figure shows the separation in the metabolomic profiles of natives (in blue) and non-native species (in red).

Appendix B: Tickets and receipts

Tu recibo de Airbnb

Identificador del recibo: RCHP3E2ZB4 · 28 de enero de 2022

El Médano

16 noches en El Médano

mié, 23 mar. 2022 → vie, 8 abr. 2022
Casa/Apt. entero · 2 camas · 1 huésped

Anfitrión: Patricia Ripoll

Código de confirmación: HMAYJ3T24A
Ir al itinerario · Ir al anuncio

Viajero: Andrea Cortegoso

Política de cancelación

Cancela antes del 22 mar. a las 15:00 y consigue un reembolso completo. Pasado este plazo, cancela antes del 23 mar. a las 15:00 y consigue un reembolso completo, menos la primera noche y la comisión de servicio.

Las horas límites se basan en el huso horario local del anuncio

Desglose del precio

50,00€ x 16 noches	800,00€
Comisión de servicio	136,66€
Total (EUR)	**936,66€**

Pago

Transferencia bancaria con Sofort 28 de enero de 2022, 11.43.43 CET	936,66€
Cantidad abonada (EUR)	**936,66€**

¿Tienes alguna pregunta?
Visita el Centro de ayuda

Tarifa de servicio de Airbnb
Incluye los cargos relativos al IVA. Entra en Información del pago para ver la factura con IVA.

Airbnb Payments Luxembourg S.A.
Airbnb Payments es una agencia de cobro limitada de tu Anfitrión. Esto significa que, después de abonar la Tarifa Total a Airbnb Payments, habrás satisfecho tu obligación de pago al Anfitrión. Las solicitudes de reembolso se tramitarán de acuerdo con lo siguiente: (i) La política de cancelación del anfitrión (disponible en el anuncio). (ii) La Política de Reembolso al Huésped, que puedes consultar en www.airbnb.com/terms.

Pago procesado por:
Airbnb Payments Luxembourg S.A.
4 Rue Henri Schnadt
2530 Luxemburgo

Airbnb Ireland UC
The Watermarque Building
South Lotts Road, Ringsend, Dublin 4
Ireland
VAT Number: IE 9827384L
www.airbnb.es

unine
Université de Neuchâtel
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Neuchâtel Platform of Analytical Chemistry
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Andrea Cortegoso Galmán
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CHE-343.042.942 TVA

Neuchâtel, le 17 novembre 2022

Invoice Nr. 90/22

Number	Description	Unit price (EUR)	Total amount (EUR)
1	Contract type: collaboration Analysis type: metabolomics metabolomics analysis Period: 28.09.2022-17.11.2022	1200,00	1200,00
			1 200,00

Amount to be paid within the next 30 days on the following account, please make sure that the full amount is being paid without any costs on our side:

Bank	UBS SA, CH-1002 Lausanne
Beneficiary name	Université de Neuchâtel Bureau des Fonds de Tiers Faubourg de l'Hôpital 106 CH-2000 Neuchâtel
Account number	290-500080.60L
IBAN	CH49 0029 0290 5000 8060 L
BIC/SWIFT	UBSSWCHZH80A

Do not forget to mention: U.01101

Contact: Dr. Gaétan Glauser
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Fax +41 (0)32 718 2511

Canary Islands Car, S.L.U
B35051820
Avda.Mamerto Cabrera Medina S/N
35509 San Bartolomé

Copia para el cliente

Ticket	Centro	Pos
1139599		

COPIA PARA EL CLIENTE

COBRO

Comercio: **0049:066753575**
Resp.Aut.: **00**
HCP: **REDSYS**
Aplicación: **A0000000041010**
MC CONTACTLESS (CONT

C 15349330
Tarjeta: ************3692
NºOper PAYTEF: **437069868**
Autorización: **N5HMUX**
Importe: **443,39 €**

Operación con PIN. Firma no necesaria

Fecha: **23/03/2022 16:18:00**