

Final Evaluation Report

Your Details	
Full Name	Gabriela Juri
Project Title	Climate-induced tree dieback of ecosystem dominants, guidelines for future persistence
Application ID	29211-1
Grant Amount	£5,970
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1. Indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
Identify sensitive (partial dieback) and resistant (healthy) trees in an area recently affected by extreme drought				<i>Nothofagus dombeyi</i> trees are dominant components of humid forests in northern Patagonia. In the last 20 years, humid forests have suffered from massive tree decay due to extreme droughts. In the same stand we identified pairs of partially affected and non-affected trees by drought that were used in dendrogenomic analyses.
Study individual-level tree growth patterns before and after the stress event using wood cores				Dendrochronological techniques on <i>Nothofagus dombeyi</i> individuals were applied to analyse the impact on growth patterns of partially affected and non-affected trees by drought. We estimated distinct recovery parameters that yielded significant differences between them.
Evaluate potential adaptive value of distinct provenances at the extreme of precipitation gradients by means of common garden and water stress experiments				Forests on the eastern slopes of the Andes are subjected to one of the steepest precipitation gradients of the world. This is due to the rain shadow effect of the southern Andes. <i>Nothofagus dombeyi</i> and <i>Nothofagus pumilio</i> are dominant tree species that occur as almost pure stands at low and high elevation forests, respectively, in northern Patagonia. Using common garden and water-stress manipulative experiments using seedlings from the extremes of the precipitation gradient we found species-specific responses. While <i>N. dombeyi</i> developed genetically based adaptation to drought, <i>N. pumilio</i> showed greater plasticity to desiccation.
Find genotypes adapted to drought using genotyping-by-sequencing (GBS)				Genomic differences by means of Single Nucleotide Polymorphisms (SNPs) between contrasting precipitation regimes were analysed in <i>Nothofagus</i>

<p>analysis of sensitive and resistant trees that inhabit precipitation extremes</p>			<p><i>dombeyi</i>. Adaptive SNPs yielded similar divergence than quantitative traits in common garden-grown seedlings. Five adaptive loci had a significant association to whole plant and leaf traits.</p>
<p>Identify and map vulnerable populations prone to be affected by future climate conditions (drought and heat) with species distribution models (SDMs)</p>			<p>Populations of <i>Nothofagus pumilio</i> are distributed along steep precipitation gradients in northern Patagonia. We analysed if eastern-most locations are long-term relict forests of a once widespread range that survived past dryness with conserved chloroplast DNA sequences and ecological niche models (ENMs). Divergence between eastern marginal and western central areas dated at least from the early Pleistocene and demographic analyses yielded stable populations over time. Future ENMs showed population decay in both central and marginal regions with a stronger effect in the latter.</p>
<p>Provide scientifically based information to apply to climate-adjusted restoration where signs of forest decay have been detected</p>			<p>Results on <i>Nothofagus dombeyi</i> show that in spite of the small climatic differences between sites and continuous gene flow, populations from the driest extreme yielded adaptive adjustments and differed significantly in growth, ecophysiology, and genetically. This information can be used in climate-oriented restoration efforts.</p> <p>Information on <i>Nothofagus pumilio</i> shows that marginal populations are as genetically diverse and demographically stable as central ones, and their genetic makeup may assure their long-lasting persistence. Unique variants and genetically based adaptive traits of the former may be used as germplasm sources for restoration under forecasted droughts.</p>
<p>Based on the detection of locally adapted gene pools, promote the in-situ protection of areas towards the eastern</p>			<p>We already detected some locally adapted gene pools, but we have to work together with the national park authorities and landowners to promote new areas for the protection of the species towards its marginal</p>

<p>(driest) distribution margin which are currently outside protected areas and could serve as valuable seed sources for restoration and management programs</p>			<p>distributions.</p>
<p>Organize workshops with local people and conservation authorities of the National Parks Administration at Nahuel Huapi National Park to increase awareness on the effects of climate change producing massive forest decay in northern Patagonia</p>			<p>The workshop was organised for May 2020 but, due to the Covid-19 pandemic we had to postpone it.</p>

2. Please explain any unforeseen difficulties that arose during the project and how these were tackled.

Due to Covid restrictions, field and laboratory work was interrupted for a long period. As soon as the activities could be restarted, we were able to make progress on the objectives. Unfortunately, we were only able to sample the *Fitzroya* population in Blest and samples are currently being analysed. The only objective we were not able to do was the workshop (which was organised for May 2020). The workshop will be conducted at a later date, when meetings with large numbers of people can be arranged.

3. Briefly describe the three most important outcomes of your project.

Tree populations inhabiting abrupt precipitation and thermal gradients have species-specific adaptive characteristics including responses to drought and temperature. Despite potential between-species gene flow within subgenus *Nothofagus*, sympatric species as *Nothofagus dombeyi* and *N. pumilio* maintain their genetic identity by phenological barriers that result in distinct ecophysiological tolerances and thermal niches which are of adaptive value as yielded by genomic analyses (in progress).

Mesic *Nothofagus dombeyi* geographically restricted to low-elevation humid forests can be considered vulnerable to desiccation given that drought-resistant traits have been removed by stabilising selection towards moister areas. Nonetheless, local populations at the driest end occupying riparian environments show ecotypic variation with genotypes adapted to water stress that can be used as seed sources

for restoration under forecasted scenarios of increased drought in Patagonia. In addition, variation in humid areas also highlight the high polymorphism in drought tolerance at the individual level in this species.

High elevation *Nothofagus pumilio* inhabiting steep precipitation extremes were similarly diverse for neutral genetic markers and showed signals that they have been long-term isolated. In addition, they show similar amounts of phenotypic plasticity for adaptive traits measured under common gardens. Such environmentally driven controls may be advantageous in such drought tolerant species.

4. Briefly describe the involvement of local communities and how they have benefitted from the project.

This aspect will be developed once restrictions due to the Covid pandemic are alleviated.

5. Are there any plans to continue this work?

Barriers to gene flow between dominant taxa in forests of Patagonia as *Nothofagus dombeyi* and *N. pumilio* as well as native conifers within the Cupressaceae as *Pilgrodendron uviferum* and *Fitzroya cupressoides* are currently being analysed including sympatric and allopatric populations of these species. Similarly, genomic analyses are needed of humid and dry populations of species inhabiting abrupt precipitation gradients as *Nothofagus pumilio*, *Pilgrodendron uviferum* and *Fitzroya cupressoides*.

6. How do you plan to share the results of your work with others?

Many scientific publications have resulted from this project. See list at the end of Final Report. Also, once restrictions due to the Covid pandemic are alleviated we will organise the workshops with local people and conservation authorities of the National Parks Administration at Nahuel Huapi National Park to increase awareness on the effects of climate change producing massive forest decay in northern Patagonia.

7. Timescale: Over what period was the grant used? How does this compare to the anticipated or actual length of the project?

The anticipated length of the project was 1 year but due to the pandemic the duration was of 2 years.

8. Budget: Provide a breakdown of budgeted versus actual expenditure and the reasons for any differences. All figures should be in £ sterling, indicating the local exchange rate used. It is important that you retain the management accounts and all paid invoices relating to the project for at least 2 years as these may be required for inspection at our discretion.

The following tables show the original budget and the approved modifications

Item	Budgeted Amount	Actual Amount	Difference	Comments
GPS	246		-246	Redirected to new item (1)
Haglöf Increment borer 16" (2)	483	333	-150	Redirected to new item (1)
Haglöf Increment borer 18" (2)	775	402	-373	Redirected to new item (1)
Travel expenses	600	326	-274	Redirected to new item (2)
Genomic data	2266	2089	-177	Redirected to new item (1)
Greenhouse work	300		-300	Redirected to new item (3)
Sample collection	100		-100	Redirected to new item (3)
Transportation and fuel expenses	1200	483	-717	Redirected to new item (3)
SUB-TOTAL	5970	3633	-2337	
Whole Chloroplast Genome Sequencing of 6 individuals (1)		946	+946	Agreed by RF
Chloroplast PCR fragment sequencing (1 plate of 96 individuals) (2)		274	+274	Agreed by RF
CPU - intel core i7 11700 s1200 – Motherboard gigabyte B560M DS3H s1200 DDR4 – RAM 32 GB DDR4 – SSD 480GB (3)		1117	+1117	Agreed by RF
TOTAL	5970	5970		

9. Looking ahead, what do you feel are the important next steps?

Nuclear genome sequencing and functional annotation would be an important next step. With this information we could further analyse and comprehend the underlying mechanisms of drought tolerance in our study species. With a reference genome it would be possible to identify metabolic pathways relevant to the process. This allows the development of cheaper genetic markers that can be used to describe the structure and geographic distribution of drought resistance alleles. This information can be useful to review the conservation status of particular populations that may reveal as diversity hotspots.

10. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Foundation receive any publicity during the course of your work?

Yes, in presentations at scientific conferences, workshops and doctoral seminars.

11. Please provide a full list of all the members of your team and briefly what was their role in the project.

Gabriela Juri, coordinator, analysis of genomic data, phenological studies.

Griselda Ignazi, PhD student, ecophysiological, genetic, and ecological niche models on *Nothofagus pumilio*.

Dayana G. Diaz, Bachelor's thesis: ecophysiological and genomic analyses of *Nothofagus dombeyi* and PhD thesis: genomic analyses of *Fitzroya cupressoides*.

Andrea C. Premoli, senior researcher, provided background theory, design of experiments, data interpretation.

Mariana Fasanella, researcher, fieldwork and analysis of genomic data on *Nothofagus dombeyi*.

Laura Suarez, researcher, fieldwork and analysis of dendrochronological data on *Nothofagus dombeyi*.

Paula Mathiasen, researcher, analysis of genetic data and ecological niche models on *Nothofagus pumilio*.

All participants contributed equally to writing scientific articles.

12. Any other comments?

List of publications that resulted from the project:

Fasanella, M., P. Mathiasen, G. Juri, D.G. Díaz, R. Hasbún & A.C. Premoli. Ancient vicariance driven by geology reinforced by adaptive divergence in *Nothofagus*: contributions from Geogenomics. *Journal of Biogeography*, in review.

Juri, G. & A.C. Premoli. 2021. Allochrony of neighbour ecological species: Can isolation by time maintain divergence? The natural experiment of sympatric *Nothofagus*.. *Forest Ecology and Management*, doi: 10.1016/j.foreco.2021.119466

Mathiasen P., G. Ignazi, & A.C. Premoli. 2021. Biogeographically marginal: Source of evolutionary novelties and future potential. *Forest Ecology and Management*, doi: 10.1016/j.foreco.2021.119596

Diaz, D.G., G. Ignazi, P. Mathiasen & A.C. Premoli. 2021. Climate-driven adaptive responses to drought of dominant tree species from Patagonia. *New Forests*, doi: 10.1007/s11056-021-09843-4

Fasanella, M., M.L. Suárez, R. Hasbún & A.C. Premoli. 2021. Individual-based dendrogeomic analysis of forest dieback driven by extreme drought. *Canadian Journal of Forest Research* 51: 420-432, doi: 10.1139/cjfr-2020-0221

Diaz, D.G., P. Mathiasen & A.C. Premoli. 2020. Subtle precipitation differences yield adaptive adjustments in the mesic *Nothofagus dombeyi*. *Forest Ecology and Management* 461: 117931, doi: 10.1016/j.foreco.2020.117931

Ignazi, G., S.J. Bucci & A.C. Premoli. 2020. Stories from common gardens: Water shortage differentially affects *Nothofagus pumilio* from contrasting precipitation regimes. *Forest Ecology and Management* 458: 117796, doi: 10.1016/j.foreco.2019.117796

Ignazi G., P. Mathiasen & A.C. Premoli. 2019. Gradientes climáticos modelan la diversidad genética en especies leñosas de amplia distribución: el caso de *Nothofagus pumilio* en los Andes del sur. *Ecosistemas* 28: 35-47 doi: 10.7818/ECOS.1547

List of attended scientific conferences that resulted from the project:

Diaz, D.G., G. Ignazi, P. Mathiasen, A.C. Premoli. Respuestas adaptativas a la sequía en especies leñosas dominantes de Patagonia. XXIX Reunión Argentina de Ecología. 4-6 Agosto 2021, Córdoba Argentina. Modalidad Virtual.

Diaz, D.G., P. Mathiasen, T., Kitzberger, T., J. Bannister Hepp & A.C. Premoli. ¿Cuán distintos son los nichos ecológicos de *Fitzroya* y *Pilgerodendron*?. XXIX Reunión Argentina de Ecología. 4-6 Agosto 2021, Córdoba Argentina. Modalidad Virtual.

Mathiasen P., M. Fasanella, D.G. Diaz, G. Juri, R. Hasbún & A.C. Premoli. Phylogeography of *Nothofagus dombeyi* reflects past geological and climatic changes in southern South America. II Virtual Meeting of Systematics, Biogeography, and Evolution, Brasil. 2021.

Fasanella M., P. Mathiasen, G. Juri, D.G. Diaz, R. Hasbún & A.C. Premoli. Vicarianza antigua reforzada por divergencia adaptativa en *Nothofagus dombeyi*. XXIX REUNION ARGENTINA DE ECOLOGIA, Tucumán, Argentina. 2021.

Fasanella M., C.P. Souto, T. Kitzberger & A.C. Premoli. Refugios rocosos: reservorios de variantes adaptativas al cambio climático. Argentina. XXIX REUNION ARGENTINA DE ECOLOGIA, Tucumán, Argentina. 2021.

Juri, G. & A.C. Premoli. Aislamiento fenológico en poblaciones simpátricas de especies emparentadas. XXIX REUNION ARGENTINA DE ECOLOGIA, Tucumán, Argentina. 2021.

Mathiasen P., M. Fasanella, D.G. Diaz, G. Juri, R. Hasbún & A.C. Premoli. Phylogeography of *Nothofagus dombeyi* reflects past geological and climatic changes in southern South America. II Virtual Meeting of Systematics, Biogeography,

and Evolution SBE Meeting: The Research of Biodiversity and the Diversity of Researchers. Poster Session 4: Phylogeography, June 20th, 2021, 5:00 PM doi: 10.5281/zenodo.4904236

Fasanella, M., P. Mathiasen P, G. Juri, D. Díaz, R. Hasbún & A.C. Premoli. Eventos antiguos de vicarianza reforzados por divergencia adaptativa en *Nothofagus dombeyi*. III Reunión de Biología Evolutiva, FCEyN UBA, CABA, 5-7 agosto 2019.

Fasanella, M., M.L. Suárez, R. Hasbún & A.C. Premoli. Marcadores genómicos como indicadores potenciales de adaptación a la sequía en bosques de *Nothofagus dombeyi* del norte de la Patagonia. III Reunión de Biología Evolutiva, FCEyN UBA, CABA, 5-7 agosto 2019.

Juri, G., M. Fasanella & A.C. Premoli. Divergencia simpátrica versus alopátrica: barreras al flujo génico intraespecífico e interespecífico en tres especies del género *Nothofagus* (*N. pumilio*, *N. antarctica* y *N. dombeyi*). III Reunión de Biología Evolutiva, FCEyN UBA, CABA, 5-7 agosto 2019.

Mathiasen, P. G. Ignazi, D. Diaz & A.C. Premoli. Relictos climáticos y su relevancia en la conservación del potencial evolutivo y adaptativo de bosques dominados por *Nothofagus*. III Reunión de Biología Evolutiva, FCEyN UBA, CABA, 5-7 agosto 2019.