The Rufford Foundation
Final Report

Congratulations on the completion of your project that was supported by The Rufford Foundation.

We ask all grant recipients to complete a Final Report Form that helps us to gauge the success of our grant giving. The Final Report must be sent in **word format** and not PDF format or any other format. We understand that projects often do not follow the predicted course but knowledge of your experiences is valuable to us and others who may be undertaking similar work. Please be as honest as you can in answering the questions – remember that negative experiences are just as valuable as positive ones if they help others to learn from them.

Please complete the form in English and be as clear and concise as you can. Please note that the information may be edited for clarity. We will ask for further information if required. If you have any other materials produced by the project, particularly a few relevant photographs, please send these to us separately.

Please submit your final report to jane@rufford.org.

Thank you for your help.

Josh Cole, Grants Director

Grant Recipient Details	
Your name	Ramiro Ruben Ripa
Project title	Adaptations to recurrent fires in Patagonia foster the invasion of the exotic conifer *Pinus radiata*
RSG reference	24894-1
Reporting period	July 2018 – July 2019
Amount of grant	£5000
Your email address	ramiro.ripa@comahue-conicet.gob.ar
Date of this report	29 August 2019

1. Please 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
Serotiny levels and aerial seed bank size				Samples of cones and seeds were collected and variables that could explain serotiny levels were measured: a) morphometric characteristics as total height, diameter at breast height, crown diameter, and canopy area; b) stand luminosity, represented by the percentage of canopy openness and total solar radiation, which were estimated by hemispherical photography. This objective is finished, and in this context, we also studied the role of cones as a fuel component. These results are part of a publication that is under review in Basic and Applied Ecology.
Germination response to fire				Serotinous cones were collected, dried and heated at 150 Celsius for 10 minutes using a laboratory stove, to promote their opening and the release of the seeds. Percent seed viability was determined by the pressure method. Germination assay was carried out under controlled light, temperature and irrigation conditions. We studied post-fire germination experimentally by testing the effects of aqueous ash solution and two heat shocks (90° and 120° C for 5 min). Control treatments were not treated with heat or ash. After germination, seedlings were cultivated under a naturally lighted greenhouse. The germination and vigor experiments have been completed and we are currently performing data analysis and interpretation of results.
Effect of fire on the genetic structure and				Fresh foliage from 100 randomly selected individuals was sampled

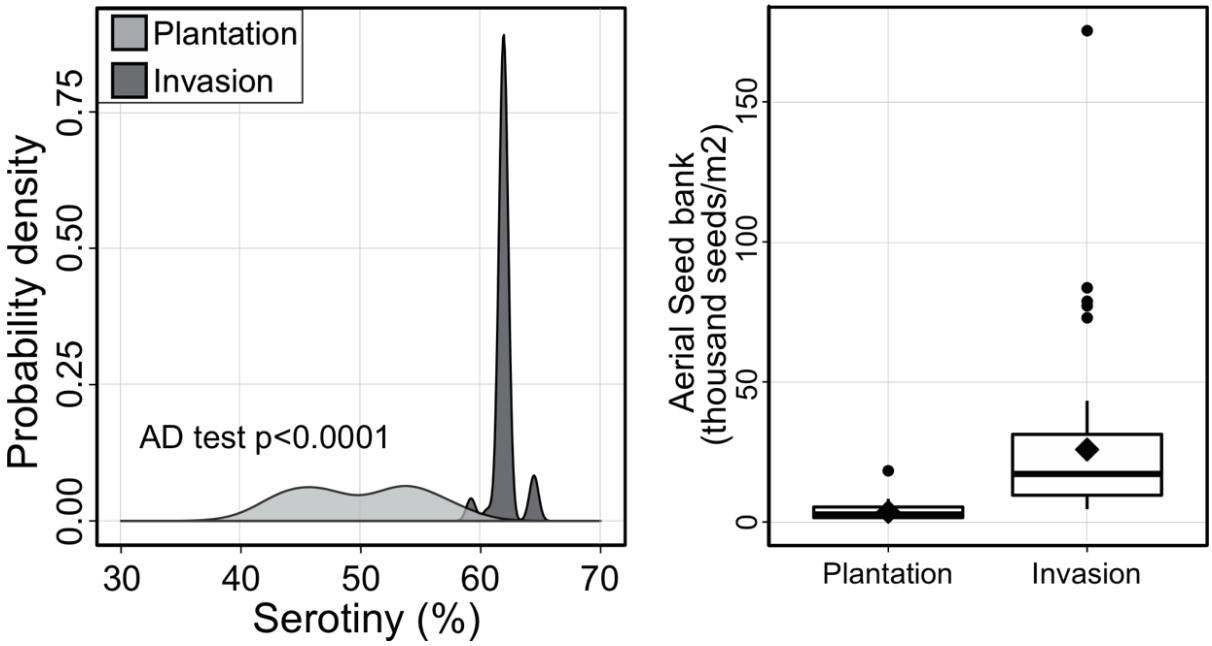
diversity of *P. radiata* invasions				from each of four cohorts: plantation and three post-fire invasion cohorts (from fires that occurred in the years 1987, 2012 and 2015). Protein homogenates were extracted which were analysed by isozyme electrophoresis using 12 enzymatic systems. Our data were compared to published information from the native range and other invaded regions (i.e. Australia). These results are already being analysed for a manuscript to be sent for publication during September 2019. We are currently working on genomic techniques using genotype by sequencing (GBS) to identify single nucleotide polymorphisms (SNPs). These markers permit us to analyse the association between genotypic variation and adaptive characters that could promote post-fire invasion. Extraction of high-quality DNA has been accomplished already and samples will be analysed by GBS as part of an internship at Memorial University of Newfoundland, Canada during October-December 2019.
Dissemination activities				We organised a participative workshop carried out on 7 December 2018 in the village of El Hoyo. We also elaborated a technical report about the ecological situation of the study site derived from the pine invasion. In addition, we participated in a space for scientific dissemination organised by the Biodiversity and Environment Research Institute (INBIIOMA) at Bariloche.
Communication to the scientific community.				We presented our results at two scientific meetings (see below). The results of the aerial seed bank estimation have been sent to a scientific journal for publication. We are currently working on a manuscript about isozyme results.

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

In order to improve the significance level of the findings we decided to increase the number of trees analysed in the objective related to aerial seed bank (from the 60 proposed in the project to 100 that were finally analysed), and in the genetic analyses (from the 30 per cohort proposed to 100 per cohort). This increased the estimated costs for some of the proposed objectives.

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

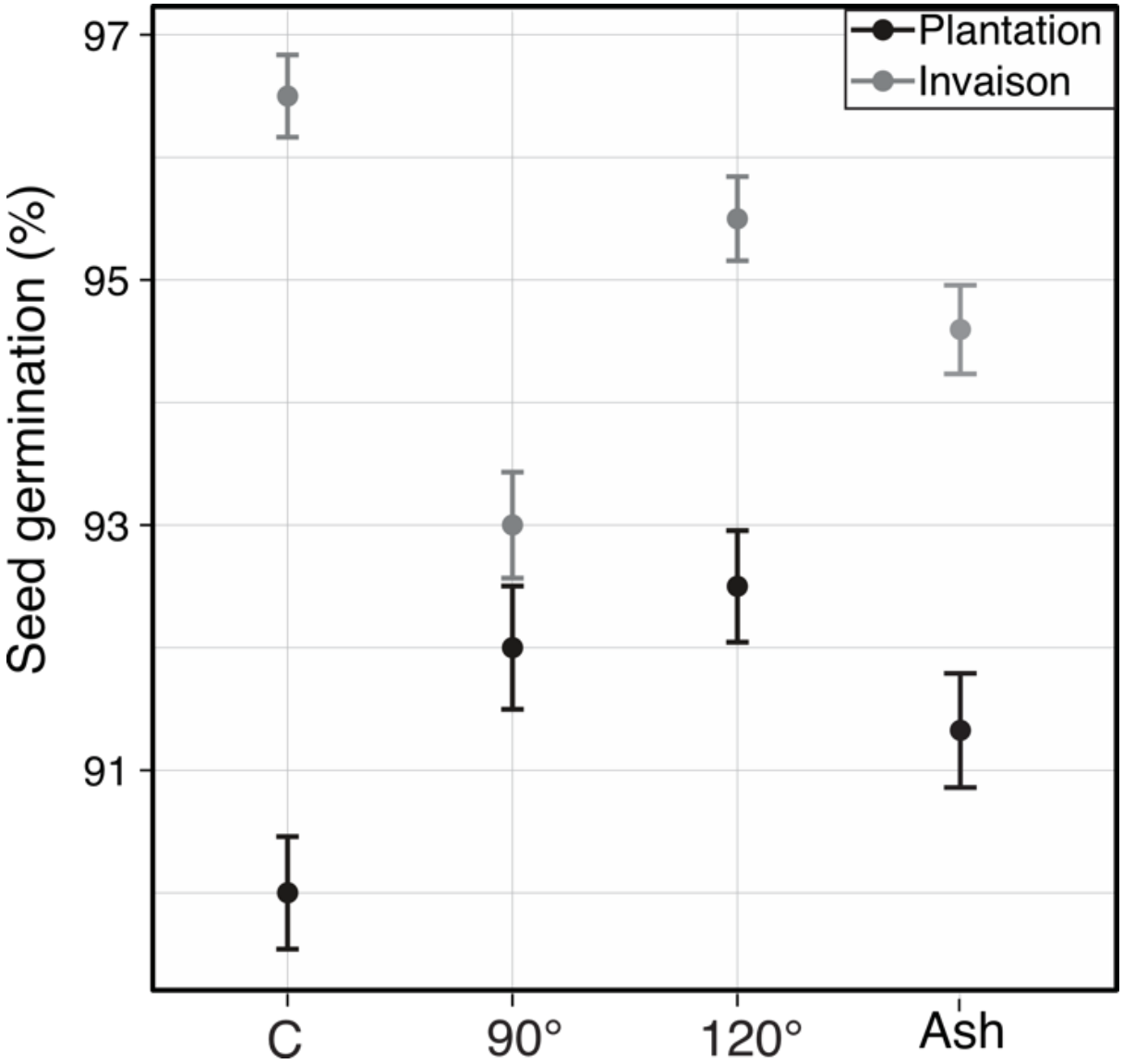
i). We found that fire acts as a selection factor on an adaptive character, such as serotiny, in *P. radiata* in Patagonia. The increase of the level of serotiny (Left panel) and the size of the aerial seed bank (Right panel), together with the high tree density in post-fire conditions, could be fostering a cycle of fire-invasion feedback.



Left panel- Probability density polygon of the serotiny level, estimated by kernel (the x axis indicates the level of serotiny classes in percent) for plantation (light gray) and post-fire invasion (dark gray) trees. The Anderson Darling K-Sample test (AD) reported a significant difference between the frequency distributions of plantation and post-fire invasion. **Right panel-** *Pinus radiata* seed density comparison (as number of viable seeds per square meter) between plantation and post-fire invasion. Boxplots indicate the median (horizontal line), the mean (black diamond), the first and third quartiles (box), the range that excludes outliers (vertical line), and the outliers (black circles).

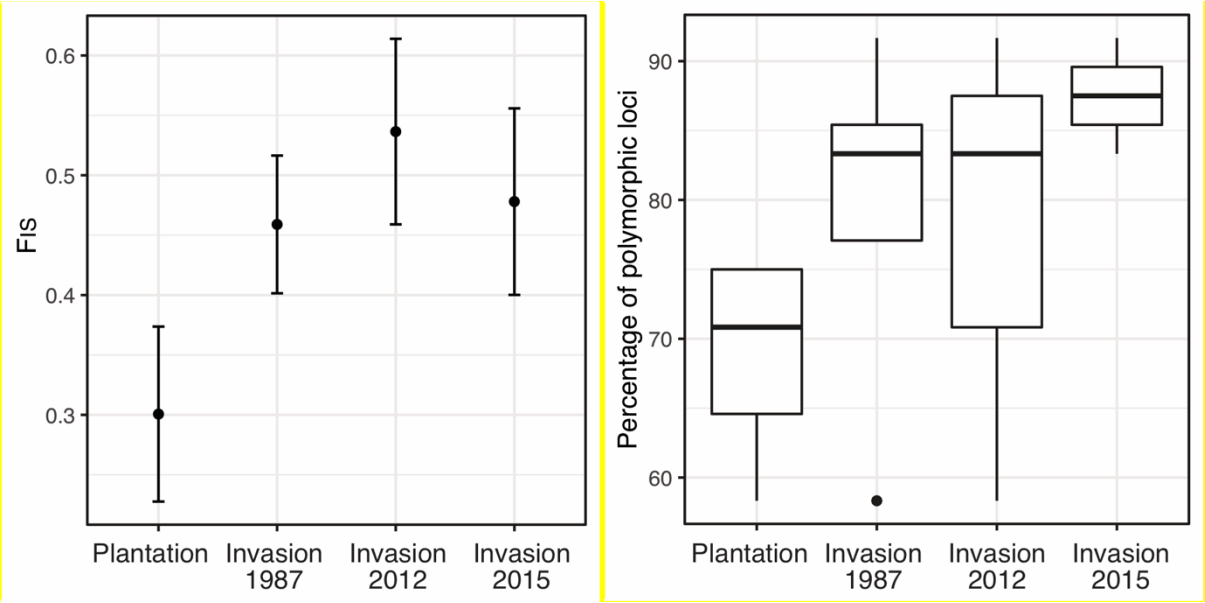
ii). Overall high germination capacity (>90%) was obtained for seeds collected from original plantation and post fire recruited pines. The slightly higher germination percentage for invasion was maintained under distinct temperatures and ash treatments. These preliminary results suggest an increase in the recruitment capacity

of this species in stands recruited after fires, but not mediated by fire related factors such as heat shock or ash, this increase may be associated with a process of rapid selection mediated fire.



Percentage of germinated seeds (Mean ± SE) for invasion and plantation in function of fire treatments: control (C), 90 ° Celsius, 120 ° Celsius and ash.

iii). An increase in inbreeding (left panel) was observed in the cohorts recruited after fire, which could be due to bottlenecks and the effects of genetic drift that occurred during each fire event. Another possible explanation could be that those remnant individuals that survived each fire event and therefore with greater aptitude for this disturbance could leave more offspring, thus producing an adaptive selection process and an increased inbreeding. The three post-fire cohorts presented higher levels of polymorphism than plantation (right panel).



Left panel- Temporal changes in the within-population inbreeding by means of the fixation index (FIS) for trees from original plantations and post-fire invasions recruited after 1987, 2012 and 2015 fires. **Right panel-** Temporal changes in levels of polymorphism (%P) for original plantations and post-fire invasions recruited after 1987, 2012 and 2015 fires. The polymorphic level was represented as the percentage of polymorphic loci (%P) *sensu stricto*, i.e. all those loci with more than one allele regardless of the frequency of the least common allele were considered polymorphic.

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

During our field work we had the opportunity to work very tightly with people from the Secretary of Forests of the Chubut Province and the Service of Prevention and Fight against Forest Fires (SPLIF) who have helped us to understand the historical land use, and current landscape of our study site at Reserva Forestal Lago Epuyén. We also had the opportunity to organise and participate at a discussion workshop carried out on 7 December 2018 in the village of El Hoyo (15 km from the reserve). With different attendants as: local people, Secretariat of Forests of Chubut, the Service of Prevention and Fight against Forest Fires (SPLIF), National Parks, forest producers, etc.

The results related to genetics, aerial seed bank and germination derived from this project, in conjunction whit the results about flammability derived from the research of colleagues help us to understand the cycle of fire invasion that is taking place on the site. It is therefore of great importance in future management plans, for example the detection and control of seed trees or thinning to reduce the probability of fires. The recovery of the site would bring great benefits to the local inhabitants, since the currently high tree densities of pine invasion have negative consequences for livestock and tourism (as well as negative effects on biodiversity and ecosystem dynamics).

5. Are there any plans to continue this work?

We are planning to continue with this work. First of all, we will write and publish in scientific journals the results corresponding to the germination experiment and genetic analyses. We will also continue to be linked to the site as we participate in the pilot site of the National Observatory of Land Degradation and Desertification that is currently operating in the area (http://desertificacion.gob.ar/sitiospiloto/?sp=122).

Our next aim is working with the Secretariat of Forests of Chubut and the Service of Prevention and Fight against Forest Fires on the use of the information, obtained in this project, in invasion control and management plans and future decision-making activities. For this, and with the information obtained in this project, we want to develop invasion models against different fire frequency scenarios that help us predict the course of the invasion in the area. Also, we plan to study the effects of silvicultural practices (such as pruning or thinning) on serotiny levels and the size of the aerial seed bank.

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

The participative discussion workshop permitted us to communicate our results directly to local residents, tourism providers, forest producers, and members of the Chubut Forest and Fire Department. As result of the workshop, we prepared a technical report about the current environmental problems in the study site [1]. Results related to the aerial seed bank and its implication in the pine invasion of the area were included in the discussion. This technical report contains important information about different aspects of pine invasion and can be useful for the management and conservation of the reserve. In addition, we participate in a space for scientific divulgation called "The Week of Biodiversity and Environment", organised by the Biodiversity and Environment Research Institute (INBIOMA) at Bariloche. This event was attended by many primary schools from Bariloche and through diverse activities we were able to teach them about our research.

These results are shared in the National Observatory of Land Degradation financed by CONICET and in the Advisory Council of the Lake Epuyen Reserve. This council is a space of periodic discussion in which different actors of the community intervene (Intendents, councillors, settlers, chamber of tourism, etc.).

A manuscript including the aerial seed bank estimation have been sent to a scientific journal for publication under the title "Increased serotiny in post-fire pine invasion: Evidence of rapid selection mediated by fire". Currently we working on a manuscript about isozyme results comparing data on individuals from original plantations, successive post fire invasions, native range and other invaded regions (i.e. Australia). Other manuscript will include the results of the germination response to fire. We also hope to write a manuscript about genomic studies, and another about the implications of rapid adaptations in the process of invasion of this species.

We already presented some of the results of this project in two scientific meetings: Reunion Argentina de Ecología - RAE (Argentine meeting of ecology) and the conference "Adapting forest ecosystems and wood products to biotic and abiotic stress". In the RAE I presented results corresponding to the estimation of the aerial seed bank [2] as well as those related to the cones as fuel [3]; and in the Adapting forest ecosystems conference, the preliminary results of the genetic studies corresponding to isoenzymes were presented [4].

[1] E. Raffaele, J. Franzese, R. Ripa, Clara Pissolito, M. Blackhall. Technical report: Problemática ambiental actual en la Reserva Forestal de Usos Múltiples Lago Epuyén (Current environmental problems in the Lake Epuyén Multiple Use Forest Reserve).

[2] Ripa R.R., Franzese J., Premoli A.C., Raffaele E; Banco de semillas aéreo de pino radiata en áreas invadidas post-fuego de la Patagonia Andina. XXVIII Reunión Argentina de Ecología; Mar del Plata, Buenos Aires. 2018.

[3] Ripa R.R., Franzese J., Blackhall, M., Raffaele E; La contribución de los conos de pino radiata como combustible en incendios de copa: implicancias en la invasión post-fuego. XXVIII Reunión Argentina de Ecología; Mar del Plata, Buenos Aires. 2018.

[4] Ripa R.R., Franzese J., Raffaele E, Premoli A.C.; Genetics of *Pinus radiata*, the most widely planted forestry species, in its original and invaded range related to fire. International Conference TOPWOOD + LIA Forestia: Adapting forest ecosystems and wood products to biotic and abiotic stress. San Carlos de Bariloche, Argentina. 2019.

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

The RSG was used from July 2018 to the end of June 2019, according to what we planned. Fieldwork was conducted in spring, summer and part of autumn in the southern hemisphere. Laboratory assays were performed as samples were collected. The germination experiment was conducted in December 2018 and January 2019. The Rufford funding covered all the expenses for fieldwork during the complete duration of the project. This funding also covered the costs of materials, inputs and assistance services in the field, greenhouse and laboratory.

8. Budget: Please 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.

Item	Budgeted Amount	Actual Amount	Difference	Comments
Field tools and materials (measuring tapes,	950	1100	150	Adjustments to original costs were made based on

diameter tapes, vernier caliper, flagging, paper and plastic bags, etc.)				necessities of the project for the different activities (see point 2).
Laboratory and green house tools and materials (plastic pots, petri dishes, filter paper, gloves, etc)	500	800	300	
Transportation and fuel cost	1400	1800	400	
Other expenses (meals during field work, library items, etc)	700	800	100	
Haglöf Vertex IV Hypsometer	800	0	-800	After the adjustment of costs (see above), we decided to prioritize field and laboratory work and did not buy these pieces of equipment. Instead we borrowed one from a colleague and the GPS was bought whit another grant.
Garmin GPSMAP 64s GPS	500	0	-500	
Chainsaw	150	0	-150	
Field and laboratory assistant	0	500	500	Although we didn't have it stipulated, we had to contract technical assistance at various stages of the project.
Total	**5000**	**5000**		

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

An important next step is to detect priority areas for pine invasion control and estimate the cost and effort of control actions. The prioritisation of the areas to be controlled should be done in an integral manner, considering technical aspects (flammability of the stands, probability of recruitment and re-invasion, etc.), estimating management costs (in terms of personnel and economic resources) and including the different social sectors present in the reserve (inhabitants, tourist providers, forestry managers, etc.). In this regard, we consider it important to control seed trees to eliminate the source of propagules for future invasions; this would also reduce the density of future invasion stands, and therefore could decrease the occurrence of fires and positive feedback from the fire invasion cycle.

Although the effects of invasive species on biodiversity loss have already been extensively studied, we believe that an important next step is to conduct studies in the reserve where the effects of pine invasion on local fauna and flora can be quantified. This will allow the development of conservation plans adapted to this specific situation, allowing a more comprehensive and effective management.

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

Yes, we used the logo in the technical report about the current environmental problems in the Lake Epuyén Multiple Use Forest Reserve, and in the talks, we gave in scientific meetings. We will also acknowledge The Rufford Foundation in a manuscript that is under review.

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

The core team is composed of **Ramiro Ripa (RR)**, **Jorgelina Franzese (JF)**, **Estela Raffaele (ER)** and **Andrea Premoli (AP)**.

RR led the project. All members participated in the experimental design of the study. **RR, JF** and **ER** conducted fieldwork. **RR, JF** and **AP** performed experiments and run genetic analyses. **RR** conducted the data analysis. **RR** led the writing of aerial seed bank manuscript whit the help and guide of **JF, ER** and **AP. RR, JF** and **ER** organized the participative workshop and communicate the results to the attendants. All members in different stages shared the results whit the local and scientific community.

In addition, many people participated in various parts of the development of the project and made it possible, among them **Gerardo Finster** (Secretary of Forests of Chubut), **Sandro Lobos** (inhabitant of the site and member of the Service of Prevention and Fight Against Forest Fires) and the assistants of field and laboratory: **Ariel Mayoral, Pablo Alvear, Victoria Campopiano, Dayana Diaz and Priscila Edwards**.

12. Any other comments?

We would like to thank the Rufford Foundation for the grant received, as it was key to achieve our goals. I consider it highly enriching for my career to have had the experience of leading a project like this. I would also like to point out that thanks to the support of the Rufford Foundation, I have not only initiated this project (which I consider of great relevance for the conservation of various sectors of Patagonian forest invaded by pines), but it has also given me the opportunity to lay the basis for future studies, new collaborations and contact with people from various entities such as the secretary of forests or the Service of Prevention and Fight Against Forest Fires.

Landscapes dominated by the exotic conifer Pinus radiata in Puerto Patriada – Chubut – Patagonia - Argentina.

Top: Radiata pine plantation burned in January 2019. Bottom: Serotinous cones opened after fire, resulting in the release of seeds.

Ramiro taking hemispheric photos, used to characterize the canopy structure of the stands.