

Biodiversity Challenge Funds Projects Darwin Initiative, Illegal Wildlife Trade Challenge Fund, and Darwin Plus

Half Year Report

It is expected that this report will be a **maximum of 2-3 pages** in length.

If there is any confidential information within the report that you do not wish to be shared on our website, please ensure you clearly highlight this.

Submission Deadline: 31st October 2024

Please note all projects that were active before 1 October 2024 are required to complete a Half Year Report.

Submit to: BCF-Reports@niras.com including your project ref in the subject line.

Project reference	<i>31-001</i>
Project title	Quinoa-associated fungi and bacteria in Bolivia: conservation and sustainable use
Country(ies)/territory(ies)	Bolivia
Lead Organisation	CABI
Partner(s)	Permaculture Association [UK]; PROINPA [Bolivia]; Universidad Autónoma del Estado de Morelos [Mexico]; Universidad Mayor de San Simón [Bolivia]
Project leader	<i>D.W. Minter</i>
Report date and number (e.g. HYR1)	<i>HYR1</i>
Project website/blog/social media	

1. Outline progress over the last 6 months (April – September) against the agreed project implementation timetable (if your project started less than 6 months ago, please report on the period since start up to end of September).

Although we are not looking for specific reporting against your indicators, please use this opportunity to consider the appropriateness of your M&E systems (are your indicators still relevant, can you report against any Standard Indicators, do your assumptions still hold true?). The guidance can be found on the resources page of the relevant fund website.

This project began on 1 July 2024, its scheduled starting date. The current report thus covers only three months.

- Activities General (1) & General (2). The gender-equal character of this project and its M&E procedures have been explained to each core staff member. An M&E spreadsheet has been designed and made accessible on-line, with guidance for its use. In one section, each core staff member is asked to confirm they understand the project's gender-equal character, and its M&E procedures. It also records progress in each project activity.
- Project staff have been asked collectively to prepare a document describing in detail the scientific procedures for sampling, processing, storage of collections, and their use for project purposes. In effect, this represents the Materials & Methods section of the project's work.

- The format for recording and storing collection information has been discussed, and a draft version of a spreadsheet for this purpose has been prepared. The spreadsheet is a convenient tool for data gathering. While not expected to be the final repository of collection data, its format is compatible with long-established standards already in use by the Project Leader.
- A questionnaire has been circulated to project staff to review what is currently known about Bolivian fungi and bacteria, and to determine what cultures and specimens are already held. The purpose of this is to establish a baseline against which project work can be measured.
- The taxonomic systems to be adopted by this project and the sources providing them have been discussed and agreed. They cover fungi, bacteria and their associated organisms.
- Work has begun to locate and gather existing literature on Bolivian fungi and bacteria, particularly those associated with quinoa. Several hundred publications have already been identified.
- Work to locate existing digitized records of Bolivian fungi and bacteria has begun. Several thousand have so far been found.
- Planning of field trips has begun.
- Two papers have been published which relate to this project and include core project staff among the authors:
 - YARZÁBAL RODRÍGUEZ, L.A., ÁLVAREZ GUTIÉRREZ, P.E., GUNDE-CIMERMAN, N., CIANCAS JIMÉNEZ, J.C., GUTIÉRREZ-CEPEDA, A., FERNÁNDEZ OCAÑA, A.M. & BATISTA-GARCÍA, R.A. Exploring extremophilic fungi in soil mycobiome for sustainable agriculture amid global change. *Nature Communications* **15**: no. 6951, 11 pp. (2024) [DOI: 10.1038/s41467-024-51223-x].
 - ZENTENO-ALEGRÍA, C.O., YARZÁBAL RODRÍGUEZ, L.A., CIANCAS JIMÉNEZ, J., ÁLVAREZ GUTIÉRREZ, P.E., GUNDE-CIMERMAN, N. & BATISTA-GARCÍA, R.A. Fungi beyond limits: the agricultural promise of extremophiles. *Microbial Biotechnology* **17**: e14439, 24 pp. (2024) [DOI: 10.1111/1751-7915.14439].

2. Give details of any notable problems or unexpected developments/lessons learnt that the project has encountered over the last 6 months. Explain what impact these could have on the project and whether the changes will affect the budget and timetable of project activities.

- There have been several unexpected problems in the processing of funds, none on its own serious, but collectively sufficient to cause delays, particularly in getting support to non-UK partners. Some of the problems have been resolved. The others are being addressed. We are hopeful they will not recur. The impacts of these problems are that contact with village communities has been hindered, and survey and sampling fieldwork has not yet begun. These are not insurmountable problems, but they add additional challenges to an already tight schedule.

3. Have any of these issues been discussed with NIRAS and if so, have changes been made to the original agreement?

Discussed with NIRAS:	No
Formal Change Request submitted:	No
Received confirmation of change acceptance:	No

Change Request reference if known: *If you submitted a financial Change Request, you can find the reference in the email from NIRAS confirming the outcome*

4a. Please confirm your actual spend in this financial year to date (i.e. from 1 April 2024 – 30 September 2024)

Actual spend:

4b. Do you currently expect to have any significant (e.g. more than £5,000) underspend in your budget for this financial year (ending 31 March 2025)?

No

4c. If you expect and underspend, then you should consider your project budget needs carefully. Please remember that any funds agreed for this financial year are only available to the project in this financial year.

If you anticipate a significant underspend because of justifiable changes within the project, please submit a re-budget Change Request as soon as possible. There is no guarantee that Defra will agree a re-budget so please ensure you have enough time to make appropriate changes to your project if necessary. Please DO NOT send these in the same email as your report.

NB: if you expect an underspend, do not claim anything more than you expect to spend this financial year.

5. Are there any other issues you wish to raise relating to the project or to BCF management, monitoring, or financial procedures?

No.

6. Please use this section to respond to any feedback provided when your project was confirmed, or from your most recent annual report. If your project was subject to an Overseas Security and Justice Assistance assessment please use this space to comment on any changes to international human rights risks, and to address any additional mitigations outlined in your offer letters. Please provide the comment and then your response. If you have already provided a response, please confirm when.

- The scientific research rationale and evidence for microbial yield/resilience improvements in quinoa could be stronger.

Response. A substantial and growing body of peer-reviewed published research (several thousand hits in Google Scholar) provides this evidence. Rather than re-invent the wheel, our research rationale builds on those elements of preceding work judged relevant to this project. The following are selected recent publications:

Research on quinoa mycorrhizal fungi

- ALQUICHIRE-ROJAS, S., ESCOBAR, E., BASCUÑÁN-GODOY, L. & GONZÁLEZ-TEUBER, M. Root symbiotic fungi improve nitrogen transfer and morpho-physiological performance in *Chenopodium quinoa*. *Frontiers in Plant Science* **15**: 1386234 (2024).
- BENAFFARI, W., BOUTASKNIT, A., ANLI, M., AIT-EL-MOKHTAR, M., AIT-RAHOU, Y., BEN-LAOUANE, R., BEN AHMED, H., MITSUI, T., BASLAM, M. & MEDDICH, A. The native arbuscular mycorrhizal fungi and vermicompost-based organic amendments enhance soil fertility, growth performance, and the drought stress tolerance of quinoa. *Plants* **11** (3): p. 393 (2022).
- BENAFFARI, W., BOUTASKNIT, A., ANLI, M., NASRI, N. & MEDDICH, A. Application of arbuscular mycorrhizal fungi alone or combined with different composts to improve physiological and biochemical attributes related to drought stress tolerance in quinoa. *Journal of Soil Science and Plant Nutrition* **23**: 4250–4266 (2023) [DOI: 10.1007/s42729-023-01345-w].
- GARCÍA-PARRA, M., CUELLAR-RODRÍGUEZ, L.Á. & BALAGUERA-LÓPEZ, H.E. Arbuscular mycorrhiza

symbiosis in quinoa (*Chenopodium quinoa* Willd.): a systematic review. *Revista Facultad Nacional de Agronomía Medellín* **75** (1): 9853-9865 (2022).

- KELLOGG, J.A., REGANOLD, J.P., MURPHY, K.M. & CARPENTER-BOGGS, L.A. A plant-fungus bioassay supports the classification of quinoa (*Chenopodium quinoa* Willd.) as inconsistently mycorrhizal. *Microbial Ecology* **82** (1): 135-144 (2021).
- TOUBALI, S., AIT-EL-MOKHTAR, M., BOUTASKNIT, A., ANLI, M., AIT-RAHOU, Y., BENAFFARI, W., BEN-AHMED, H., MITSUI, T., BASLAM, M. & MEDDICH, A. Root reinforcement improved performance, productivity, and grain bioactive quality of field-droughted quinoa (*Chenopodium quinoa*). *Frontiers in Plant Science* **13**: 860484 (2022).

Research on quinoa fungal endobionts

- GONZÁLEZ-TEUBER, M., VILO, C. & BASCUÑÁN-GODOY, L. Molecular characterization of endophytic fungi associated with the roots of *Chenopodium quinoa* inhabiting the Atacama Desert, Chile. *Genomics Data* **11**: 109-112 (2017). [DOI: 10.1016/j.gdata.2016.12.015].
- GONZÁLEZ-TEUBER, M., URZÚA, A., PLAZA, P. & BASCUÑÁN-GODOY, L. Effects of root endophytic fungi on response of *Chenopodium quinoa* to drought stress. *Plant Ecology* **219**: 231-240 (2018).
- LI, L., JIANG, Z., YANG, X., ZHANG, Y., HUANG, J., DAI, J., NOOR, H., WU, X., REN, A., GAO, Z. & SUN, M. Effects of nitrogen accumulation, transportation, and grain nutritional quality and advances in fungal endophyte research in quinoa (*Chenopodium quinoa* Willd.) plants. *Journal of Fungi* **10** (7): p.504 (2024).
- PITZSCHKE, A. Molecular dynamics in germinating, endophyte-colonized quinoa seeds. *Plant and Soil* **422**: 135-154 (2018).

Research on bacteria beneficial for quinoa

- PITZSCHKE, A. Development peculiarities and seed-borne endophytes in quinoa: omnipresent, robust bacilli contribute to plant fitness. *Frontiers in Microbiology* **7**: 2 (2016) [DOI: 10.3389/micb.2016.00002].
- RAFIQUE, E., MUMTAZ, M.Z., ULLAH, I., REHMAN, A., QURESHI, K.A., KAMRAN, M., REHMAN, M.U., JAREMKO, M. & ALENEZI, M.A. Potential of mineral-solubilizing bacteria for physiology and growth promotion of *Chenopodium quinoa* Willd. *Frontiers in Plant Science* **13**: 1004833 (2022).
- YANG, A., AKHTAR, S.S., IQBAL, S., AMJAD, M., NAVEED, M., ZAHIR, Z.A. & JACOBSEN, S.E. Enhancing salt tolerance in quinoa by halotolerant bacterial inoculation. *Functional Plant Biology* **43** (7): 632-642 (2016).
- YAÑEZ-YAZLLE, M.F., ROMANO-ARMADA, N., ACRECHE, M.M., RAJAL, V.B. & IRAZUSTA, V.P. Halotolerant bacteria isolated from extreme environments induce seed germination and growth of chia (*Salvia hispanica* L.) and quinoa (*Chenopodium quinoa* Willd.) under saline stress. *Ecotoxicology and Environmental Safety* **218**: 112273 (2021).

- You could have included more information about the pilot study results to show the importance of fungi to plant fitness.

Response. The meaning of this comment is unclear. For evidence of the importance of fungi to quinoa plant fitness, please see our response to the previous comment. In our project proposal, the word “pilot” occurs only four times. In every instance it refers to altiplano bacteria, not fungi. If we have not addressed this comment satisfactorily, please re-phrase it and we will try again.

- Is it realistic to screen quinoa plants for fungi and bacteria, isolate the microbial symbionts, test them, and develop bioproducts in just three years?

Response. Developing an agricultural bioproduct from inception to commercialization usually involves several stages: research and development, tests, regulatory approval, and commercialization. Collectively, these take between three and ten years, depending on complexity of the product and the regulatory environment. PROINPA, with its extensive field and laboratory experience, has in the past completed the entire process in three years.

All project partner representatives are aware that the schedule is very ambitious, something made clear in our proposal. In the response to Q13b of the application form, development of bioproduct(s) is described as an “aspiration”. The response to Q15 refers to bioproduct(s) “developed by, or after the end of this project”. The text in Q20, Risk 4 recognizes that additional resources may be needed, particularly to ensure strong stakeholder rights protection. Project partners recognize that, supposing a suitable source organism can be found, roll-out of the developed bioproduct - the commercialization stage - may occur after the end of the project. Bolivian partners are ready for this contingency.

- How will any potential risks to human health from bioproducts be identified, and what are the licensing regulations in Bolivia?

Response. Potential risks to human health from bioproducts will be identified using guides, lists of dangerous pathogens and similar resources, including (but not only) those listed as examples below. Relevant professional experience of partners will also contribute to the process. The perceived risk to human health from this work is currently low.

- ANON. [ADVISORY COMMITTEE ON DANGEROUS PATHOGENS]. *The Approved List of biological agents*. Edn 5. UK, Health and Safety Executive, 44 pp. (2023) [www.hse.gov.uk/pubns/misc208.pdf].
- COLOMBI, A., MARONI, M. & FOÀ, V. Gesundheitsrisiken in der biotechnologischen Industrie [Health risks in the biotechnological industry]. *Zeitschrift für die Gesamte Hygiene und ihre Grenzgebiete* **35** (8):451-454 (1989) [text in German; PubMedID: 2815865].
- ANON. [INTERNATIONAL LABOUR ORGANIZATION]. *Advice Page on Harmful Chemical and Biological Agents / Substances* [www.ilo.org/topics/labour-administration-and-inspection/resources-library/occupational-safety-and-health-guide-labour-inspectors-and-other/harmful-chemical-and-biological-agentssubstances].

Licensing regulations for bioproducts in Bolivia are managed by the National Service of Agricultural Health and Food Safety [SENASAG]. All bioproducts must obtain a sanitary registration issued by SENASAG [see www.senasag.gob.bo/index.php/requisitos-para-la-obtencion-o-renovacion-de-registro]. This ensures products comply with established safety and quality requirements. To obtain this registration, several documents must be submitted. These include certificates of free sale, certificates of analysis, and documents demonstrating good manufacturing practice. Companies which produce, fractionate, package or import bioproducts must be categorized and comply with the specific regulations for each category (industrial, semi-industrial, artisanal etc.). SENASAG conducts evaluations and technical audits to ensure compliance with current regulations. This includes reviewing labels and planning technical audits.

- The training of villagers in bioproduct use appears to happen in advance of bioproduct development. Are there existing bioproducts already on the market?

Response. Training is planned during bioproduct development, not in advance of it. Roll-out (commercialization) of the bioproduct is expected to occur after the end of the project, so as much training as possible is needed beforehand. Both Bolivian partners have excellent outreach schemes, and further training is anticipated after project completion. Project partner PROINPA has already developed several bioproducts for use with crops in the Bolivian Andes. These include “Tricobal L” (a growth regulator), “VigorTop Plus” (a plant growth stimulant), “Bacterial Mix” (a growth regulator), “Bio Max” (an eco-insecticide) and “Energy Top” (a microbial inoculant promoting availability of soil phosphorus). They are all advertised on-line and available from PROINPA. All are generic, and none of them explicitly mention quinoa as a potentially beneficiary crop.

- Further information on the Pathway to Change would be useful, beyond explaining the potential end point.

Response. We are puzzled by the suggestion that our answer to Q18 (Pathway to Change) on the application form merely explained “the potential end point”. The project’s key components (poverty alleviation and biodiversity) were both identified, each with a description of the current position. They were followed by a series of “if this... ..then that” statements providing the logic for change, and those led to the anticipated outcomes and intended impact. Each step required by the “Theory of Change” was covered, but the order recommended by the theory was reversed for compatibility with the wording of Q18 (i.e., impact came last in our answer, where the “Theory of Change” places it first).

Having discussed this comment, we wonder whether we may somewhere have missed an important conceptual point relating to the “Theory of Change”. If so, and if this answer is not sufficient, we would appreciate more guidance so that a satisfactory response can be made.

- The methodology has outreach and soil analysis steps, but it is not clear how all of this information will be used to develop a route to market.

Response. For this project, outreach means ensuring local people are involved and sympathetic. Without this, field work and field trials could be vulnerable. Assessing

characteristics of plant and soil samples is an important step in establishing the environment from which potential sources for bioproducts have come. That provides a reference point against which tests and roll-out in other environments can be compared. Outreach and analysis are not there to help develop a route to market, rather, a marketable product may be impossible without them.

- The logframe could be strengthened: output 2 is not particularly clear. How will training schools help?

Response. This question is unclear. In the text of Output 2, the phrase “training schools” does not occur; the word “training” occurs once, in SMART indicator 2.5, which relates to villagers learning about bioproduct use; the word “schools” occurs once, in SMART indicator 2.4, which relates to provision of resources for local schools. Project partners consider educating local children about the value of their environment is important for their long-term welfare, particularly given that the saline areas of the altiplano where this project is located are coming under intense pressure from industries wanting to extract lithium for car batteries. If we have not answered this comment satisfactorily, please re-phrase it and we will try again.

- The logframe could be strengthened: the wording of the Outcome could be revised as it is unclear what exactly "Conserving Bolivia's fungi and bacteria made realistic" means.

Response. At the time of proposal submission, resources and advice for conserving Bolivia's fungi and bacteria were virtually non-existent. Without them, conservation of these organisms, in the sense of legislation, policy and on-the-ground action, was completely unrealistic. For fungi, this situation is changing very rapidly, particularly in the light of the “pledge” in support of fungi being promoted by the governments of Chile and the UK at October 2024's COP16 in Colombia. To coincide with presentation of that “pledge”, the collective Fungal Specialist Groups of the IUCN Species Survival Commission, with other fungal conservation specialists, have issued a document entitled “Contribution of Fungi to the Global Biodiversity Framework”. This contains a point by point commentary about fungi and each goal of the Kunming-Montreal Global Biodiversity Framework. Information and advice generated by the present project and specifically relating to Bolivia will dovetail with that document to provide a template which can be used to address the country's challenges in conserving its fungi. For bacteria, resources and advice remain almost completely non-existent. Our project aims to provide a pioneering pilot document as a first step to filling that gap.

- The logframe could be strengthened: output 3: it is unclear how the project plans to measure the “enhanced capability and capacity” of the CBD “national focus point”. An indicator could be added to specifically measure this.

Response. The CBD document “Role of the CBD National Focus Point” [www.cbd.int/doc/training/nbsap/a2-train-role-nfp-v2-2009-02-en.pdf] defines national focus points, their terms of reference, and what they do. This includes advice on the type and level of expertise required (item 4 of the document), identification of experts (item 5 of the document), and co-ordination of input for CBD National Reports and National Biodiversity Strategies and Action Plans (item 6 of the document). SMART indicators for output 3 of this project measure the amount of information about fungi and bacteria which will become available to Bolivia's National Focus Point, where currently there is more or less nothing. The downloadable assessments for Bolivia's fungi and bacteria (SMART indicator 3.3), will include names and contact information of its authors, thereby helping the National Focus Point to identify expertise. To make that even clearer, we propose a new fourth SMART indicator for the output: “3.4. A list of individuals and institutions in Bolivia able to provide expert advice about conservation of the country's fungi and bacteria”.

Checklist for submission

For New Projects (i.e. starting after 1st April 2024)

Have you responded to any additional feedback (other than caveats) received in the letter you received to say your application was successful which requested response at HYR (including safeguarding points)? You should respond in section 6, annexes other requested materials as appropriate.	
If not already submitted, have you attached your risk register ?	
For Existing Projects (i.e. started before 1st April 2024)	
Have you responded to feedback from your latest Annual Report Review ? You should respond in section 6, annexes other requested materials as appropriate.	
For All Projects	
Include your project reference in the subject line of submission email.	
Submit to BCFs-Report@niras.com .	
Have you clearly highlighted any confidential information within the report that you do not wish to be shared on our website?	
Have you reported against the most up to date information for your project ?	
Please ensure claim forms and other communications for your project are not included with this report.	