



Environment and Natural Resources Trust Fund

2024 Request for Proposal

General Information

Proposal ID: 2024-248

Proposal Title: Rapid Restoration of Soil Functions Using Algal Crusts

Project Manager Information

Name: Roger Ruan

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Project Basic Information

Project Summary: Select suitable desert algal species through artificial intelligence-powered virtual screening and use a biological in-situ resource utilization-based approach to establish artificial algal crusts for rapid restoration of soil functions.

Funds Requested: \$200,000

Proposed Project Completion: June 30, 2027

LCCMR Funding Category: Small Projects (H)

Secondary Category: Methods to Protect, Restore, and Enhance Land, Water, and Habitat (F)

Project Location

What is the best scale for describing where your work will take place?

Statewide

What is the best scale to describe the area impacted by your work?

Statewide

When will the work impact occur?

During the Project and In the Future

Narrative

Describe the opportunity or problem your proposal seeks to address. Include any relevant background information.

Minnesota's northeast region contains thousands of abandoned mine pits and rock quarries, which present a significant challenge for the restoration of soil functionality and ecological diversity. Global warming and climate change further exacerbate soil degradation and desertification, which could threaten environmental resources and the livelihoods of millions of local residents if not adequately addressed. Simply increasing plant growth is insufficient to restore soil functionality due to the low availability of micronutrients and poor water-holding potential. Soil biocrusts restoration is regarded as a crucial approach for mitigating desertification and promoting ecosystem recovery. However, restoring naturally degraded soils can take several decades.

The use of desert algae, such as Chlorophyceae, Trebouxiophyceae, and Charophyceae, which have evolved to survive in drought and other extreme environments, demonstrates the promising potential to address this challenge. However, the application of desert algae still faces various challenges, such as slow growth rates, unstable algal communities, and vulnerability to external perturbations. Therefore, significant efforts are necessary to form artificial algae-crusts for rapidly restoring degraded lands in Minnesota.

What is your proposed solution to the problem or opportunity discussed above? Introduce us to the work you are seeking funding to do. You will be asked to expand on this proposed solution in Activities & Milestones.

The proposed solution to address the aforementioned problem is to develop and establish artificial algae crusts for rapid restoration of soil functions, utilizing an artificial intelligence-based algal species screening, inoculation-based soil restoration, and biological in-situ resource utilization (Bio-ISRU) based strategy to ensure successful implementation.

The detailed procedures are listed as follows:

First, artificial intelligence-based genomic comparison and virtual screening will be utilized to screen ideal microalgal species with essential survival and soil-restoring capabilities. Second, selected microalgal species will be cultivated under simulated microcosm conditions to assess three typical features for soil restoration, namely the expression of drought/cold/high irradiation-induced late embryogenesis abundant (LEA) proteins with crucial cell protective functions, bio-safety of biomass as feedstocks for secondary producers, and the capabilities to utilize nutrients from in situ natural resources for algae crust formation. Third, the algae crusts-based bio-ISRU strategy will be applied to form artificial algal biocrusts for soil restoration through optimizing biological and material variables in laboratory-scale soil inoculation experiments, finally facilitating the reclamation of degraded lands in Minnesota and statewide.

What are the specific project outcomes as they relate to the public purpose of protection, conservation, preservation, and enhancement of the state's natural resources?

The northeast region of Minnesota has many abandoned mine pits and quarries that are at risk of aridification and desertification due to climate change. Artificial algal crusts are proposed as a restoration solution to stabilize soil, reduce erosion, and increase fertility. Successfully implementing this solution is crucial for restoring degraded lands, protecting local biodiversity, and promoting environmental sustainability and socio-economic development. Using artificial algal crusts is a promising approach to combatting environmental degradation and climate change.

Activities and Milestones

Activity 1: Artificial intelligence-based virtual screening of ideal desert algal species

Activity Budget: \$60,000

Activity Description:

Desert algal species possess extremophilic properties and the ability to form (1) LEA proteins (resistance to drought, cold, and high UV irradiation), (2) soil enzymes (capabilities of soil resource utilization), and (3) bio-safe biomass without toxin production. To select ideal algal species, an artificial intelligence-based virtual screening will be developed based on these three criteria. This process will involve:

- (1) Data set preparation: Targeted genes responsible for expressing LEA proteins, soil enzymes, and toxin synthetic enzymes will be identified first. Genome and transcriptome data of algal species from NCBI online databases will be obtained and further processed for artificial intelligence analysis.
- (2) Genome mining for identifying targeted gene clusters: The genome mining tools such as ClustScan, ClusterFinder, and antiSMASH will be compared and then used for identifying targeted gene clusters, assessing the presence of the three kinds of enzyme-coding genes in algal genomes.
- (3) Machine learning-based virtual screening: Different machine learning algorithms (e.g., logistic regression, random forest, and gradient-boosted trees classifier) will be compared and trained. The optimal machine learning approach will be used to predict targeted features and identify discriminative markers, eventually leading to the selection of ideal algal species with soil restoration capabilities.

Activity Milestones:

Description	Approximate Completion Date
Genome and transcriptome data for artificial intelligence training and analysis obtained and processed for experiments	September 30, 2024
Advanced genome mining tools compared and used to identify targeted gene clusters in algal genomes	December 31, 2024
Microalgal species identified, the abundance of targeted genes quantified for investigating of algal biocrusts functions	June 30, 2025

Activity 2: Cultivate selected algal species under simulated microcosm conditions for biomass amplification and characteristic evaluation

Activity Budget: \$70,000

Activity Description:

Selected algal species will be purchased from public culture collections (or isolated from soil samples) and cultivated in agar tubes, plates, and flasks under laboratory-scale microcosm conditions. Cultivation parameters will be optimized for biomass amplification and feature assessment.

- (1) Cultivation parameter optimization: Micronutrients, pH, and external phytohormones will significantly influence enzyme activities and then benefit algal growth. Thus, these crucial cultivation variables, including nutrient availability, pH, desiccation, and irradiation, will be investigated and optimized.
- (2) Characteristic evaluation: The algal growth characteristics will be investigated, and the obtained biomass will be further evaluated to determine if selected algal species have protective proteins such as LEA proteins and dehydrins, and also ensure they do not produce toxins.
- (3) Soil enzyme activities: Algal species will secrete different soil enzymes with multifunctionality. Thus, different algal species as well as culture conditions will be optimized to enhance soil enzyme activities.

(4) Exopolysaccharides (EPS) and compatible solutes: EPS and compatible solute production are additional algal strategies to protect against environmental stresses, which favors the formation of soil aggregates and then promotes soil fixation and restoration. Therefore, the effects of different environmental variables on the synthesis of these organic substances and their compositions will also be investigated.

Activity Milestones:

Description	Approximate Completion Date
Desert algal species purchased from algae banks or isolated from fields, and enriched in lab	December 31, 2025
Microalgae cultivation for desert algae amplification and the according optimization of culture conditions are evaluated	March 31, 2026
Morphological patterns and growth characteristics of microalgal species evaluated, culture conditions optimized for soil restoration	March 31, 2026

Activity 3: Inoculate algae onto degraded soils in laboratory to evaluate formation of algal biocrusts and their effects on restoring soil function

Activity Budget: \$70,000

Activity Description:

Laboratory-scale soil inoculation experiments will be conducted to evaluate the feasibility of using artificial algal crusts for soil restoration. Native soil from mined lands or drylands will be added to identical culture dishes (diameter: 10 cm) or pots (10 cm × 10 cm), and culturing conditions similar to those in native dryland ecosystems will be applied for soil inoculation experiments.

(1) Synthetic microbial communities: Single algal inoculation will be investigated and used to form artificial algal crusts, followed by the evaluation of their effects on soil restoration. Synthetic communities (SynComs) will be used as a powerful tool to assemble different algal communities and evaluate their capabilities for soil restoration.

(2) Bio-ISRU strategy: Rapid restoration of soil functions via desert algal species requires leveraging in situ natural resources to a large extent. Thus, the biological (e.g., algal species and growth features) and material (e.g., growth substrates and operation units) optimization of the bio-ISRU strategy will be considered together and integrated with above-advanced approaches to address these constraints of soil composition and extreme conditions.

Ultimately, artificial algal crusts will be developed and then efficiently used in situ available resources on degraded lands, facilitating the rapid restoration of soil functions.

Activity Milestones:

Description	Approximate Completion Date
The laboratory-scale cultivation system associated with extreme culture conditions established for soil inoculation experiments	September 30, 2026
The effects single algal species and synthetic microbial communities investigated and optimal culture conditions optimized	December 31, 2026
The bio-ISRU approach investigated and applied to develop artificial algal biocrusts for soil restoration	March 31, 2027
Further R&D and commercialization strategy will be proposed and recommended for future use	June 30, 2027

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Paul Chen	University of Minnesota	Co-PI	No

Long-Term Implementation and Funding

Describe how the results will be implemented and how any ongoing effort will be funded. If not already addressed as part of the project, how will findings, results, and products developed be implemented after project completion? If additional work is needed, how will this work be funded?

Following the research project completion, the artificial algae crusts-based ISRU system will be developed and established for future application on mine lands and drylands. Additionally, we will establish advanced infrastructure and research techniques to promote the protection and utilization of dryland resources. We will seek additional funding to facilitate field-scale implementation and demonstration of our approach for restoring degraded lands and ecosystems while also contributing to climate change mitigation in the long run.

Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Methods to Destroy PFAS in Landfill Leachates	M.L. 2022, , Chp. 94, Art. , Sec. 2, Subd. 04a	\$200,000

Project Manager and Organization Qualifications

Project Manager Name: Roger Ruan

Job Title: Professor and Director

Provide description of the project manager’s qualifications to manage the proposed project.

Dr. Ruan, Professor and Director of Graduate Studies of Bioproducts and Biosystems Engineering Department, and Director of Center for Biorefining at University of Minnesota, is a Fellow of ASABE, IFT, Vebleo, and IAAM, and has received many other awards, including CAFS Professional Achievement and Scientist of IAAM, etc. He is a top cited author in engineering and technology with an h-index of 88, i10-index of 443, and has over 30,000 citations. Dr. Ruan’s research include renewable energy and environment technologies for sustainable development. He has published over 500 referred journal articles, two books, 24 book chapters, and holds 20 US patents in the areas of municipal, agricultural, and industrial liquid and solid waste including biomass and waste plastics treatment and utilization through novel anaerobic digestion, microalgae and hydroponic cultivation, pyrolysis and gasification, airborne and other pathogen disinfection and pollutant control, catalysis, non-thermal plasma, and nitrogen fixation, etc. He has received over 200 grants totaling over \$45 million in various funding for research, including major grants from USDA, DOE, DOT, DOD, LCCMR, and industries. He has served as guest editor or editorial board member of Bioresource Technology, Renewable Energy, Engineering, Applied Catalysis and Chemical Engineering, Journal of Food Process Engineering, The Open Plasma Physics Journal, and Associate Editor of Transactions of ASABE, Engineering Applications in Agriculture, and Transactions of CSAE, and Chairman of Editorial Board and Editor-in-Chief of International Journal of Agricultural and Biological Engineering, etc. He has supervised over 75 graduate students, 140 post-doctors, research fellows, and other engineers and scientists. He has given over 300 keynote lectures, invited symposium presentations, and short courses. His earlier LCCMR funded projects have resulted in several patented technologies which have been successfully licensed to the industry. He has the technical expertise and project management experience to ensure the execution of proposed projects.

Organization: U of MN - College of Food, Agricultural and Natural Resource Sciences

Organization Description:

The Center for Biorefining is a University of Minnesota research center affiliated with the College of Food, Agricultural and Natural Sciences and help coordinate the University efforts and resources to conduct exploratory fundamental and applied research and provide education on science and technology for environment protection and circular economy; stimulate collaboration among the University researchers, other public sector investigators, and private investigators involved in biobased production technology development; promote technology transfer to industries; and foster economic development in rural areas. The Center's research programs are founded by DOE, USDA, DOT, DOD, LCCMR, IREE, Xcel Energy, and other federal and state agencies, NGOs, and private companies. The Center is equipped with state of the arts analytical instruments, and processing facilities ranging from bench to pilot scale.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	\$ Amount
Personnel								
Professor/faculty		Primary Investigator - project lead, advises researchers, plans and directs research, oversees budget, monitors and reports progress			36.8%	0.03		\$8,274
Professor/faculty		Co-Primary Investigator - advises researchers, designs and directs experiments, conducts data analysis, writes reports and publications			36.8%	0.15		\$22,318
1 Graduate Research Assistant		Researcher - carries out experiments, collects and analyzes data, prepares reports and manuscripts, education			46.5%	3		\$162,681
							Sub Total	\$193,273
Contracts and Services								
							Sub Total	-
Equipment, Tools, and Supplies								
	Tools and Supplies	Purchase of lab and miscellaneous supplies, including algae strains, culture media supplements, chemicals, consumable supplies for analytical instruments, parts and components for fabricating experimental apparatuses	For running experiments and operating systems, chemical, physical, and biological analyses					\$6,117
							Sub Total	\$6,117
Capital Expenditures								
							Sub Total	-
Acquisitions and Stewardship								
							Sub Total	-
Travel In Minnesota								

	Miles/ Meals/ Lodging	8 one-day 1-person trips, 40 miles each round trip (\$0.655/mile), meals @\$50/person	Visits to CO2 emission sites, collect samples, conduct experiments on site.					\$610
							Sub Total	\$610
Travel Outside Minnesota								
							Sub Total	-
Printing and Publication								
							Sub Total	-
Other Expenses								
							Sub Total	-
							Grand Total	\$200,000

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
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Non ENRTF Funds

Category	Specific Source	Use	Status	Amount
State				
			State Sub Total	-
Non-State				
			Non State Sub Total	-
			Funds Total	-

Attachments

Required Attachments

Visual Component

File: [4f36b593-b61.pdf](#)

Alternate Text for Visual Component

Figure 1 shows how artificial algal biocrusts can restore soil functions quickly. Ideal algal species with survival and soil-restoring abilities will be identified using AI-based genomic comparison and virtual screening. The chosen algae will be grown under simulated microcosm conditions to assess their growth and restoration potential. Finally, the bio-ISRU...

Optional Attachments

Support Letter, Photos, Media, Other

Title	File
2022 Audit	2e409df0-fdc.pdf
Letter of Authorization	1fa5d6ee-041.pdf

Administrative Use

Does your project include restoration or acquisition of land rights?

No

Does your project have potential for royalties, copyrights, patents, or sale of products and assets?

Yes

Do you understand and acknowledge IP and revenue-return and sharing requirements in 116P.10?

Yes

Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF?

No

Does your project include original, hypothesis-driven research?

Yes

Does the organization have a fiscal agent for this project?

No

Does your project include the design, construction, or renovation of a building, trail, campground, or other capital asset costing \$10,000 or more?

No

Do you propose using an appropriation from the Environment and Natural Resources Trust Fund to conduct a project that provides children's services, as defined in Minnesota Statutes section 299C.61 Subd.7?

No

