SASHYA: Preservation of God's Gold Devising of Seed Bank for the preservation of Indigenous Albumen

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Abstract:
Each crop variety has immeasurable impacts not only on agriculture but also on biodiversity. To prevent the extinction of some rare and historic plant varieties, we have started a project to preserve the seeds of these plants and establish a seed bank. Seed banks store rare commercial seeds for cultivation or further study and research. The Seed Bank focuses on protecting indigenous crop varieties and minimizing the risk of extinction. In general, there are two types of seeds, "Orthodox seeds" and "Recalcitrant Seeds" which are considered in reference to seed bank storage. It focuses primarily on orthodox seed species to withstand drought and / or freeze during ex situ conservation. To complete this project, we have performed the following steps:

- **Collection of seeds: we collected healthy, ripe, indigenous seeds from the farmers door step.**
- **Preparation of Seeds for preservation: Separate and clean them followed by drying the seeds for better storage life.**
- **Packaging of the seeds: We packed the seeds in an air tight container with an arid condition.**
- **Storage of the Seeds: Then packaged seeds were preserved in suitable environment.**
- **Periodic germination test of the seeds: To prevent deterioration problem of seed we have germinated them from time to time.**

Besides that, we did some statistical analysis of seeds as well as cotyledons on the basis of several parameters to check the quality of seeds for further biotechnological applications like Tissue culture, Protoplast fusion etc. Till now we have stored more than 40 types of indigenous variety of different grains, pulses and oil seeds in previous 45 days with their proper database.

Introduction:

Across the tree of life, populations have evolved the capability to traumatize suboptimal conditions by participating in dormancy, whereby people enter a reversible state of reduced metabolic activity. The ensuing seed banks are complex, storing info and conveyance memory that offers rise to multi-scale structures and networks spanning collections of cells to entire ecosystems. we have a tendency to define the elemental attributes and emerging phenomena related to dormancy and seed banks, with the vision for a unifying and mathematically primarily based framework that may address issues within the life sciences, starting from world modification to cancer biology. Seed banks are reservoirs of metabolically inactive individuals. Collectively, the genetic, phenotypic, and practical diversity of those dormant individuals will influence the behaviour of biological systems in necessary and wide-ranging ways. most biological entities can generate a seed bank, however maybe the foremost painting and well-studied examples come back from plants. Thousands of years ago, major transformations in culture and society arose once humans began to harness the range and longevity of plant seeds[1]. A lot of later, Darwin's curiosity was piqued by seed banks when over five hundred forms of plants germinated from solely 3 tablespoons of mud collected from the margins of an English pond. This semiconductor diode him to cogitate the ways that during which animals would possibly promote the movement of dormant propagules, and the way this successively might have an effect on the geographic distribution of species[2]. Since then, a chic body of theoretical, comparative, and experimental work has provided vital insight into the stabilising role of seed banks for the range and eco-evolutionary dynamics of plant populations and communities[3-7]. Impressed by the observations in natural systems, seed-banking vaults are designed round the world to assist preserve diversity and guarantee food security through the semipermanent storage of rare plant cultivars that are in danger of extinction because of rising temperatures, sickness outbreaks, and different natural disasters[8]. Yet, seed banks are by no means that restricted to the planet of plants. they're created once people transition into a dormant state, a method that has severally evolved varied times throughout Earth's history and is even thought of in some origins-of-life models[9-10]. Seed banks are important buffers that impart resiliency on biological systems. Dormancy provides protection from inhospitable conditions can otherwise cut back an individual's fitness. once lucky, a minimum of a number of these people will awake within the future when the surroundings is a lot of contributively for growth and reproduction. Given its consequences for evolution and ecology, substantial effort has been devoted towards characterizing the mechanisms that management seed-bank dynamics. In some populations, the success of dormancy is achieved by responsive transitioning between metabolic states wherever the finely tuned regulation of signals, hormones, and even neural circuits[11] permits organisms to interpret, integrate, and reply to info regarding their internal and external surroundings in an exceedingly settled fashion. For people in different populations, the power to enter dormancy and delay reproduction, despite biological process preparedness, involves random bet-hedging, that is effective at increasing mean fitness in unpredictable environments, so reducing the chance of extinction[12]. Whereas the underlying details may be vital for an in depth understanding of however dormancy operates at intervals a specific organism or population, it's not our objective to produce a comprehensive description of the molecular and physiological factors underlying dormancy among broad lineages of plants, animals, and microorganisms. Rather, our goal is to achieve new insight into seed banks by characteristic common options and important variables that are amenable to systems theory and mathematical modelling. Approaches that embrace random processes and nonlinear interactions in massive systems are significantly well poised for understanding the emergence of complicated seed-bank patterns across scales. Our hope is that such a framework won't solely result in generalizable and prophetical knowledge, however additionally foster cross-disciplinary efforts by assuaging confusion stemming from the lexicon that's usually related to dormancy and seed banking[13]. It's been over a time period since theoreticians took the initial steps to formalize a seed-bank theory[14-15]. Motivated by observations in nature, macroscale phenomena have typically been sculptural as high-octane systems, providing insight into the adaptiveness of seed banks and optimality of dormancy in unsteady environments, we constructed the Seed Bank, named 'SASHYA' for the preservation of indigenous varieties of crops. Taken together, these complementary views gift a chance for unification and universality, while additionally raising intrinsic and difficult issues for mathematicians. As such, the target of our paper is to introduce and outline the core seed-bank attributes and dormancy-related processes that may be studied and sculptural across systems and scales. We have a tendency to explore the implications of seed banks for key biological phenomena, together with biological processes, population dynamics, species interactions, and system functioning. we have a tendency to conclude with a developing on the frontiers and challenges of the seed-bank framework for sensible problems within the life sciences, including aspects of human health and world modification biology.

Fundamentals of our Seed Bank Theory:

We followed the centre attributes and techniques which can be vital for organising a preferred framework for seed banks and their dynamics. We emphasize that seed banks have multiscale residences. For example, metabolic transitions arise on time scales spanning seconds to millennia and perform throughout ranges of organic organization, starting from genes inside people to electricity waft inside meals webs. While a few seed bank residences are tractable to empirical measurement, others may be extra tough to describe, which creates possibilities for investigations that leverage idea and modelling.

Physical features of our seed bank:

The bodily manifestation of SASHYA has critical implications for knowledge dormancy dynamics and emergent phenomena. Compared to contributors of the energetic pool, dormant individual regularly tackle specific sizes, densities, and motilities. These morphological differences can facilitate the shipping of propagules to new environments, mainly while dormant people act as marketers of dispersal for destiny generations, as in flora and zooplankton. Although it's far properly documented that maximum offspring will broaden inside near region to their natal site, dormancy has the capability to amplify the dispersal kernel and the geographic variety of a few species[16]. In many systems, however, energetic and dormant people have comparable morphological functions and occupy the identical habitat. For example, withinside the floor waters of lakes and oceans, phytoplankton are on the mercy of currents, that may result in the homogenization of people inside water masses, regardless of their metabolic status[17]. In such environments, if energetic and dormant individuals cannot with no trouble be distinguished, then eco-evolutionary styles can be obscured or in any other case move undetected, which probably ends in biased interpretation of empirical observations[18]. Yet in different systems, seed banks play an critical function withinside the improvement and creation of biophysical systems. For example, dormant people are incorporated into the corporation of multicellular biofilms and tumors. In such systems, the aggregate of energetic and dormant cells creates systems that adjust fluid dynamics, oxygen availability, and the era of heat, which in flip can impact toxin production, conversation networks, or even the lateral switch of DNA[19,20]. In this way, variant withinside the metabolic interest of neighbouring people can impact the spatial configuration of SASHYA and generate comments that affects system behaviour.

SASHYA and emergent phenomena:

Once established, SASHYA can result in the emergence of thrilling styles and dynamics that don't always align with expectancies from a few classical theories that don't comprise dormancy. This is due to the fact SASHYA include structure, which imparts reminiscence at the system. Through the method of not on time resuscitation, people from the beyond can stand up withinside the destiny and have an effect on behaviour, along with equilibria and stability, however additionally multiscale organisation and feedback. In this section, we define a number of the essential procedures which are changed via way of means of seed banks and talk how dormancy impacts essential evolutionary and ecological styles.

Population dynamics with a SASHYA:

Seed banks have vital implications for the dynamics and long-time period balance of populations made of people belonging to the equal species. The mixed use of fashions and empirical information has verified that populace dynamics are higher anticipated while dormancy is included, although tiers and transitions are every now and then tough to quantify in nature[21]. For example, through the usage of stochastic projection matrix fashions, it's been proven that species with longer existence spans are much less touchy to variability in critical costs resulting from fluctuations in environmental situations[22]. Recent research has begun to leverage the strength of different modelling tactics, just like the coalescent, together with whole-genome information to deduce the significance of dormancy at the demographic records of populations. Together, those efforts have brought about the winning view that, through briefly postponing metabolic sports and dispensing reproductive output over longer durations of time, seed banks lessen the chance that people succumb to destructive situations. As a consequence, vital demographic functions of a populace, consisting of its length and age shape, are encouraged through seed bank dynamics[21,23]. The populace-stage results of seed banks are touchy to the quantity of time that a character spends in a metabolically inactive state. While dormancy enables people preserve energy, there are nonetheless different basal metabolic needs that need to be met, consisting of fees related to mobile homeostasis and the restore of macromolecular damage, that could make contributions to dwindled health related to aging[24]. Other elements affecting the house time of a seed financial institution consist of the compounded hazard of intake through predators, contamination through pathogens, and bodily burial[25]. Because of those elements, a few seed banks turnover extra hastily than others. For example, much less than 10 % of seeds belonging to an annual plant species withinside the Sonoran Desert persevered past 5 years[23], at the same time as radiometric courting discovered that diapausing zooplankton can hatch from lake sediments after extra than one hundred years[26]. Astonishingly, possible microorganisms may be recovered from historic substances which can be one hundred million years old[27].

Types of Seed Banks[28]

Table 1. Subordinate seed bank categories and terms proposed for distinction.

limiting criteria	'seed bank'		
medium (spatial allocation)	seeds stored in the soil or on the soil surface **'soil seed bank'**	seeds floating in or drifted by water **'aquatic seed bank'**	seeds held by above-ground parts of plants, (also includes passive storage, e.g. in bird nests **'aerial seed bank'** (**'canopy-stored seed bank'** for serotinous woody plants)
seed longevity	survival time is up to 1 year **'transient seed bank'**	survival time from 1 year to 5 years **'short-term persistent seed bank'**	survival time longer than 5 years **'long-term persistent seed bank'**
biological organiza-tion level	a single species studied **'specific seed bank'**	phytosociologically undefined units (e.g. ploughed lands, flower beds, etc.) with respect of total seed pool **'seed bank assemblage'***	natural, semi-natural vegetation units (asso-ciations) studied with respect of total seed pool **'seed bank community'**

* 'Seed bank assemblage' is suggested also to name subsets (e.g. grasses *vs.* herbs or annuals *vs.* perennials, etc.) of a total species pool of either natural or man-made habitats.

Fig 1: Table of seed bank types

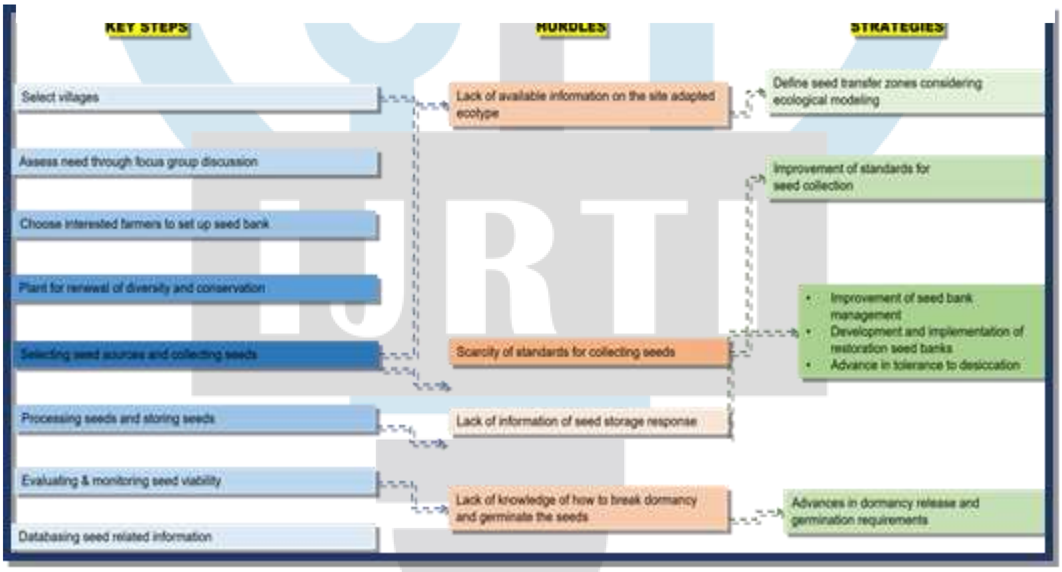

Fig 2: SOP for developing SASHYA

Mechanism of Working:

Each crop variety has immeasurable impacts not only on agriculture but also on biodiversity. That's why to prevent the extinction of some rare and historic plant varieties, we have started a project to preserve the seeds of these plants and establish a seed bank and named it 'SASHYA: Preservation of God's Gold'. It stores rare commercial seeds of many indigenous varieties of plants found in West Bengal, for their cultivation or further study and research. The Seed Bank focuses on protecting indigenous crop varieties and minimizing the risk of their extinction. In general, there are two types of seeds, "Orthodox seeds" and "Recalcitrant Seeds" which are considered in reference to seed bank storage.

1. **Orthodox Seeds:**

Orthodox seeds are long-lived seeds and can be successfully dried to moisture contents as low as 5% without injury and are able to tolerate freezing. Orthodox seeds are therefore, also termed as desiccation tolerant seeds. In-fact, the life span of orthodox seeds can be prolonged with low moisture content and freezing temperatures. Ex-situ conservation of orthodox seeds is therefore, not problematic. Orthodox seeds are exemplified by most annual and biennial crops and Agroforestry species which are relatively small-seeded (in comparison to unorthodox seeds). Orthodox seeds include for example, Citrus aurantifolia, Capsicum annum, Hamelia patens, Lantana camera, guava (Psidium guajava), Cashew (Anacardium occidentale) and most grains and legume types. The longevity or life span of orthodox seeds may vary from over a year to many hundred years depending upon the particular species and storage conditions. A notable example of a long-lived orthodox seed which survived accidental storage followed by controlled germination as mentioned earlier, is the case of 2000 years old Judean date palm seed which was successfully sprouted in 2005. However, the upper survival time limit of properly stored orthodox seeds remains unknown.

2. Recalcitrant Seeds (Unorthodox Seeds):

Fig 3: Packaging of the seeds

Recalcitrant seeds are remarkably short-lived which cannot be dried to moisture content below 20-30% without injury and are unable to tolerate freezing. Recalcitrant seeds are therefore, also termed as desiccation sensitive seeds. Recalcitrant seeds are difficult to be successfully stored and their ex-situ conservation is problematic. It is because of their high moisture content that encourages microbial contamination and results in more rapid seed deterioration. Secondly, storage of recalcitrant seeds at freezing temperatures causes the formation of ice-crystals which disrupt cell membranes and causes freezing injury. Therefore, the plants that produce recalcitrant seeds must be stored in growing phase (i.e., as growing plants) rather than as seeds and propagated vegetatively. Recalcitrant species belong to trees and shrubs of mostly tropics and also of temperate areas which are moist and some plants which grow in aquatic environment. Some common examples of plants that produce recalcitrant seeds (which are generally larger than orthodox seeds) include, avocado, cacao, coconut, jackfruit, lychee, mango, rubber, tea, some horticultural trees, and several plants used in traditional medicine.

We focuses primarily on orthodox seed species to withstand drought and / or freeze during ex situ conservation.

To establish a seed bank there are several steps and protocol, and making a standard SOP for establishment of a seed bank is not only important but also necessary[Fig 2]. To complete this project, we have performed the following steps:

- **Collection of seeds:** This is the first and most important step of making a seed bank, as the future of the seed bank depends on this step. We collected ripe and indigenous seeds in their native form, from different farmers across west Bengal.

- **Preparation of Seeds for preservation**: After the collection of seeds, the second most important step is to prepare them for storing. To prepare the seeds we first segregated the good and bad seeds. Then discarded the bad or damaged seeds as they will not be able to germinate in future. After the segregation, the good seeds were properly dehydrated in broad sunlight to vapourised the moisture of the seeds. Hydrated seeds will bring microbial contamination and aren't suitable for long term storage, that's why this step is essential.

- **Packaging of the seeds:** The storage of prepared seeds were a little bit challenging for us, as we have to find such packaging techniques, so that the seeds must not bring microbial contamination as well as it's germination power should not be hampered. Finding the way out, we packed the seeds into a air tight container with a non-hazardous inorganic substance called Slaked Lime or Calcium Hydroxide [$Ca(OH)_2$] in it. Slaked lime can soak the moisture inside the container which will provide a dehydrated condition for the seeds inside the container. Besides that Slaked lime can prevent attacks of Aphid and many other insects. Slaked Lime is ideal for packaging of seeds as it is non-hazardous and inert with respect to seeds[Fig 3].

- **Storage of the Seeds:** Then packaged seeds were preserved in suitable environment. As seeds can be damaged in both very high and very low temperature, so we stored them in room temperature (27° c), into aseptic conditions. This specific conditions helps the seed to resides in their native form, which is very much essential for long term storage [Fig 4].

Fig 4: Storage of seeds

- **Periodic germination test of the seeds:** To prevent deterioration problem of seeds, we have germinated them from time to time in MS media and grow cotyledons. As well as we did some biotechnological experiments like Plant tissue with the seeds to check their dormancy and viability [Fig 5].

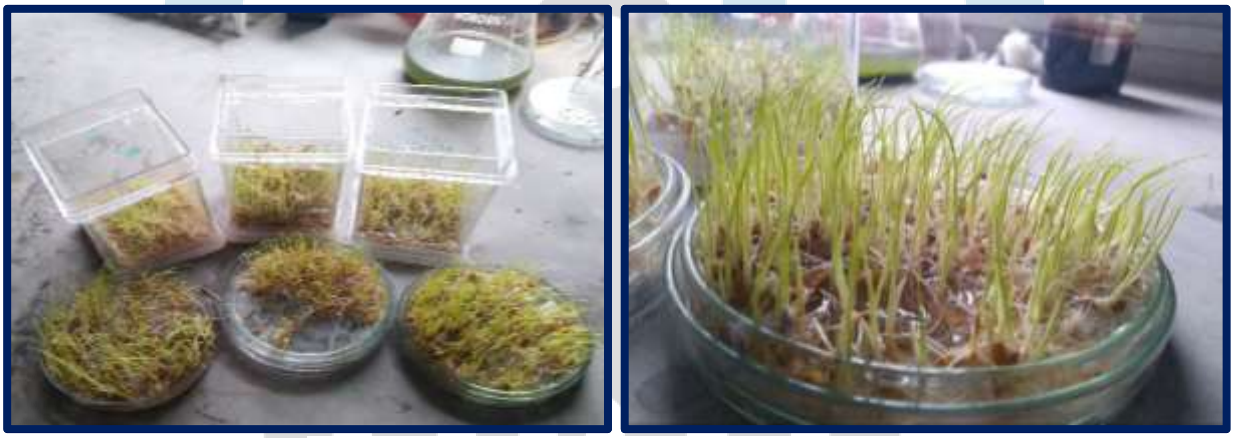

Fig 5: Results of periodic germination

- **Making of database**: Along with all this steps we also gathered important data like nutritional values, special requirement for grow, special features of the plant, geographical and biological aspects seasonal requirements, ecological importance etc. for each and every seed and made a database. On a regular basis the database is updating, as every week we collect different indigenous variety of crops, pulses and oil seeds etc. Till now we have stored more than 40 types of indigenous variety of different grains, pulses and oil seeds in previous 45 days with their proper database.

- **List of collected seeds:** Chaitali, Kabiraj, Shyamasree, Santoshi, Rohini, Deush, Marishal, Dudheswar, Danaguri, Dudh kalma, Kalam kathi, Kerala Sundari, Barsha, Sitasaal, Gobindo bhog, Chamarmani/Raniakondo, Lebusaal, Sabitapatnai, Bhadoi, Lal Swarna, Hutra/Ghutra, Lolat, Mali sorshe, Shhet, Lal tisi(mosnya), Sada bokhla, Kalo Bokla(Baklosh), Jara moosoori, sada moosori, Laldana (chola dal), Kali kolai, Chandra mukhi (kalo jeera), Ghuni matar, Sonamukhi dhaniya, Konika, Amrit, Karai khesari etc.
  Here is the hyperlink of our Database:

  https://docs.google.com/spreadsheets/d/1b3-knPwmf8JVJtjzgywkqa1HMYbMqTZMfCC7o4eZOUs/edit#gid=1922252928

Conclusion:
We make the case that seed banks play a crucial role in determining the behaviour observed in a variety of biological systems, spanning collections of cells to entire ecosystems. Seed banks come with different attributes and physical features, each of which can affect the transitions into and out of dormancy in complex ways, giving rise to a multitude of emergent phenomena. The mathematical description of seed banks is in many areas still in its infancy, but various new techniques are being developed and new challenges are being identified. We argue in favour of a systems theory perspective, in which different lines of research and

different viewpoints are combined to facilitate further progress. Seed banks represent a paradigm that will keep us busy for decades to come.

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References:

1. Smith, B. D. Documenting plant domestication: The consilience of biological and archaeological approaches. Proc. Natl Acad. Sci. USA 98, 1324–1326 (2001).
2. Darwin, C. R. On the Origins of the Species. (John Murray, 1859).
3. Venable, D. L. & Lawlor, L. Delayed germination and dispersal in desert annuals: escape in space and time. Oecologia 46, 272–282 (1980).
4. Ellner, S. ESS germination strategies in randomly varying environments.1. Logist.Type models Theor. Popul. Biol. 28, 50–79 (1985).
5. Levin, D. A. Seed bank as a source of genetic novelty in plants. Am. Nat. 135, 563–572 (1990).
6. Evans, M. E. K., Ferriere, R., Kane, M. J. & Venable, D. L. Bet hedging via seed banking in desert evening primroses (Oenothera, Onagraceae): demographic evidence from natural populations. Am. Nat. 169, 84–94 (2007). Simulations and field data support bet-hedging via dormancy.
7. Kortessis, N. & Chesson, P. Germination variation facilitates the evolution of seed dormancy when coupled with seedling competition. Theor. Popul. Biol. 130, 60–73 (2019).
8. Peres, S. Saving the gene pool for the future: Seed banks as archives. Stud. Hist. Philos. Sci. Part C. Stud. Hist. Philos. Biol. Biomed. Sci. 55, 96–104 (2016).
9. Tocheva, E. I., Ortega, D. R. & Jensen, G. J. Sporulation, bacterial cell envelopes and the origin of life. Nat. Rev. Microbiol. 14, 535–542 (2016).
10. Ginsburg, I., Lingam, M. & Loeb, A. Galactic Panspermia. Astrophys. J. Lett. 868 (2018).
11. Takahashi, T. M. et al. A discrete neuronal circuit induces a hibernation-like state in rodents. Nature 583, 109-114 (2020).
12. Seger, J. & Brockmann, J. H. What is bet-hedging? In Oxford Surveys in Evolutionary Biology (eds Harvey P. H. & Partridge L.) Vol. 4, 182–211 (Oxford University Press, 1987). Comprehensive review of bet-hedging in population biology.
13. Considine, M. J. & Considine, J. A. On the language and physiology of dormancy and quiescence in plants. J. Exp. Bot. 67, 3189–3203 (2016).
14. Cohen, D. Optimizing reproduction in a randomly varying environment. Theor. Biol. 12, 119–129 (1966). Among the first mathematical models describing the benefits of delayed seed germination.
15. Amen, R. D. A model of seed dormancy. Bot. Rev. 34, 1–31 (1968).
16. Wisnoski, N. I., Leibold, M. A. & Lennon, J. T. Dormancy in metacommunities. Am. Nat. 194, 135–151 (2019).
17. Jones, S. E. & Lennon, J. T. Dormancy contributes to the maintenance of microbial diversity. Proc. Natl Acad. Sci. USA 107, 5881–5886 (2010).
18. Locey, K. J. et al. Dormancy dampens the microbial distance-decay relationship. Philos. Trans. R. Soc. B Biol. Sci. 375, 20190243 (2020). Combined field and modeling approach demonstrating that dormancy can alter biogeographic patterns.
19. Chihara, K., Matsumoto, S., Kagawa, Y. & Tsuneda, S. Mathematical modeling of dormant cell formation in growing biofilm. Front. Microbiol. 6, 534 (2015).
20. Frank, S. A. Metabolic heat in microbial conflict and cooperation. Front. Ecol. Evolution 8, 275 (2020).
21. Kalisz, S. & McPeek, M. A. Demography of an age-structured annual: resampled projection matrices, elasticity analyses, and seed bank effects. Ecology 73, 1082–1093 (1992).
22. Morris, W. F. et al. Longevity can buffer plant and animal populations against changing climatic variability. Ecology 89, 19–25 (2008).
23. Moriuchi, K. S., Venable, D. L., Pake, C. E. & Lange, T. Direct measurement of the seed bank age structure of a Sonoran desert annual plant. Ecology 81, 1133–1138 (2000).
24. Moger-Reischer, R. Z. & Lennon, J. T. Microbial ageing and longevity. Nat. Rev. Microbiol. 17, 679–690 (2019).
25. Dalling, J. W., Davis, A. S., Schutte, B. J. & Arnold, A. E. Seed survival in soil: interacting effects of predation, dormancy and the soil microbial community. J. Ecol. 99, 89–95 (2011).
26. Hairston, N. G. & Kearns, C. M. Temporal dispersal: ecological and evolutionary aspects of zooplankton egg banks and the role of sediment mixing. Integr. Comp. Biol. 42, 481–491 (2002).
27. Morono, Y. et al. Aerobic microbial life persists in oxic marine sediment as old as 101.5 million years. Nat. Commun. 11, 3626 (2020).
28. P.Csontos, Seed banks: ecological definitions and sampling considerations, Community Ecology.76 (June 2007).