


COMMENTARY

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Creating pathways for collaboration between Argentina and the UK to utilise microbial biofilms in sustainable agriculture

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Abstract

Microbial biofilms are complex communities of microorganisms that exist in various environments, including natural and human-built systems and have a significant economic impact on a global scale. In addition to their detrimental impacts, biofilms have been extensively studied for their potential benefits. In agriculture, biofilms are considered key organisational structures of microbes, exploited as biofertilisers, biostimulants, and biocontrol agents, with the potential to enhance soil health and plant growth. Despite ongoing research, there are still large knowledge gaps in the understanding of the mechanisms governing interaction between biofilms and plants, and how these can be manipulated to promote sustainable agriculture. The UK's National Biofilms Innovation Centre [NBIC] and Argentine researchers have established a dialogue aimed at addressing these gaps and improving agricultural productivity through the integration of new technologies that can promote soil health whilst reducing environmental impact. Future research collaborations between the two countries in this area could have significant benefits for global agricultural innovation and the development of sustainable food systems. This publication takes on a 'white paper' format, consolidating complex discussions from a workshop between NBIC and Argentine researchers. It offers a comprehensive summary encompassing the insights, perspectives, and outcomes generated during the discussions among the participants, pinpointing three key priority areas for collaborative activities that were identified: (1) Using plant root biofilm composition as a sensor for soil health and to optimise interventions, (2) Biofilms and soil health resilience in a changing environment, (3) Intelligent seeds and innovative / automated large-scale monitoring systems. For the three identified priority areas, the early engagement of end-users [farmers] will be paramount to maximise technology adoption. Commitment from the governments and support from funding bodies in both countries will be essential for the establishment of robust research programmes and long-term successful collaborations between researchers, industry and end users.

Keywords Biofilm, Soil health, Climate change, Root biofilm, Microbiota, Agricultural innovation, Seed coating, Research agenda

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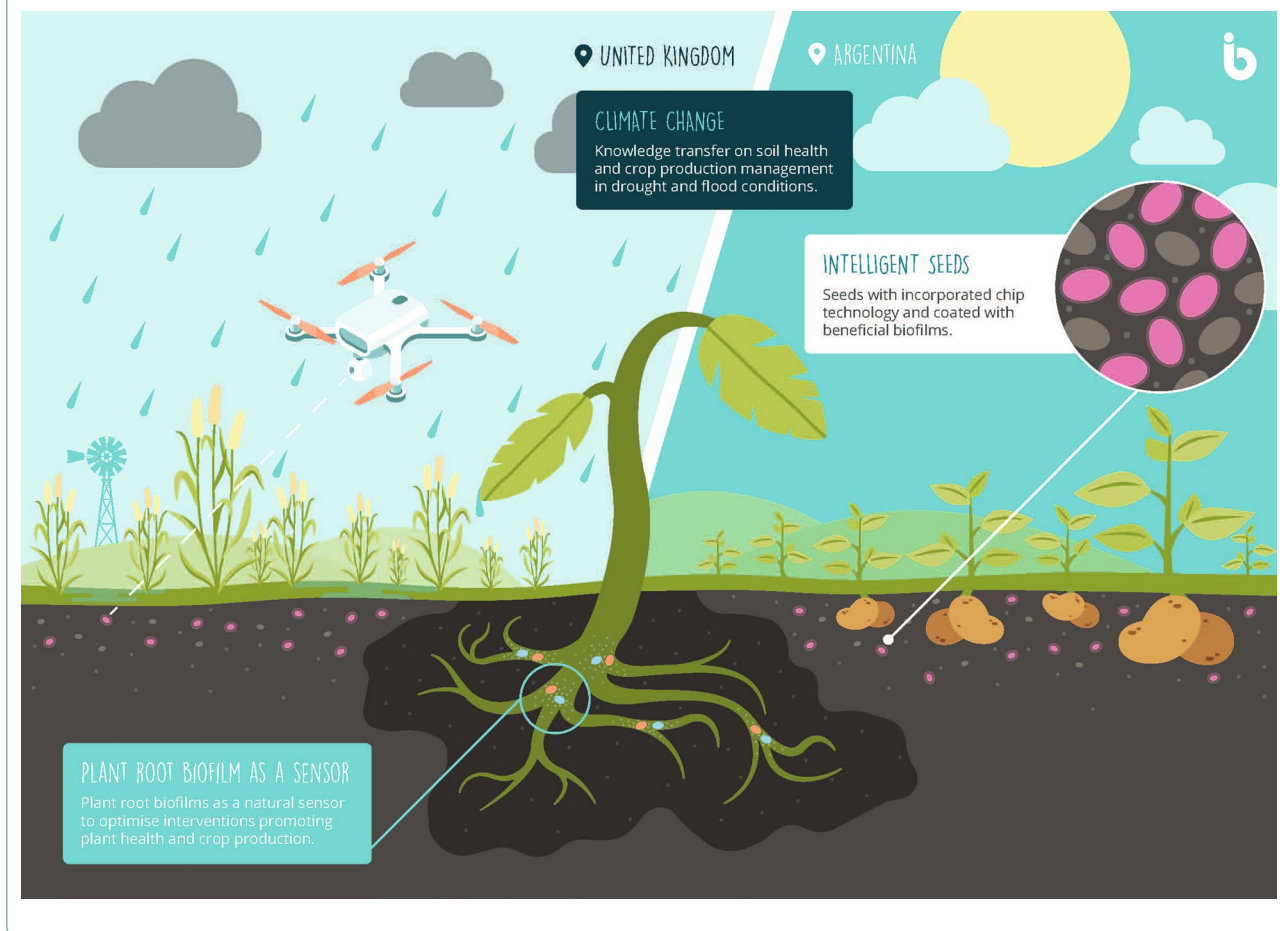
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Graphical Abstract



Background

Microbial biofilms are interactive communities of interface-associated microbial cells, enclosed in an extracellular polymeric matrix [EPM] made of compounds such as polysaccharides, proteins, extracellular DNA, and lipids. While relatively unknown to the general public, they are ubiquitous in nature and human-built environments generating an estimated US \$5 trillion impact on global economic activity. (Cámara et al. 2022) The general perception of biofilms tends to be often negative due to their association with persistent infections or biofouling in industrial settings. However, it is important to acknowledge the advantageous aspects associated with biofilms. These complex microbial assemblies have been utilised in a wide array of applications, spanning from wastewater treatment and bioremediation (Verma et al. 2023; Mishra et al. 2022) to food fermentation, (Yao et al. 2022; Rahman et al. 2023) enhancement of agricultural soil health, (Seneviratne et al. 2017) as well as the development of

biological sensors for environmental monitoring. (Prévost and Rabaey 2017) The engineering and management of biofilms hold significant potential benefits including pollutant removal capabilities (Arindam and Suman 2016) or the possibility of pharmaceutical production. (Hayta et al. 2021; Patwardhan et al. 2022).

In agriculture, biofilms have the potential to be used as biofertilisers, biostimulants, soil bioremediators and biocontrol agents. Assembling biofilms in the rhizosphere can provide many benefits from improved plant growth and health to contributing to the overall health and stability of soil ecosystems. (Ajijah et al. 2023) Biofilms can help to increase the availability of nutrients and their uptake by plants by facilitating root nodulation and nitrogen fixation. (Poole et al. 2018) As an example, the use of rhizobia biofilms provides an important aspect of sustainable agriculture practice, especially in growing legumes, utilising the symbiotic relationship between rhizobia and plants to enhance nitrogen availability in

the soil whilst reducing the need for synthetic nitrogen fertilisers. (Carareto Alves et al. 2014; Terpolilli et al. 2012) Numerous microorganisms, including *Pseudomonas*, *Bacillus*, *Rhizobium*, *Xanthobacter*, *Enterobacter*, to name a few, play a pivotal role in the breakdown of organic matter around roots and in the solubilisation and uptake of minerals such as iron, zinc, manganese, chlorine, silicone, copper, and others, essential for processes necessary for plant development and health such as photosynthesis, respiration, or protection against oxidative stress. (Mitter et al. 2021; Salas-González et al. 2021) Biofilms have also been shown to protect plants from pathogens by providing a physical barrier and through the production of antimicrobial compounds. (Berendsen et al. 2018; Salomon et al. 2017; Berlanga-Clavero et al. 2022) A notable example of such protection from pathogens has been described by Snathanam et al. (Santhanam et al. 2015), who demonstrated through large-scale field trials, that inoculation of soil with a mixture of native bacterial isolates protected tobacco plants [*Nicotiana attenuate*], significantly reducing the incidence and mortality caused by fungal infection responsible for wilt disease. Another area where biofilms play a role is in regulating the levels of phytohormones in the rhizosphere, which can help to promote plant growth and development, and to regulate responses to environmental stresses, (Orozco-Mosqueda et al. 2023; Finkel et al. 2020) as demonstrated, for example by Finkel et al. (Finkel et al. 2020) who showed that *Variovorax* bacteria, by manipulating plant hormone levels, have the ability to reverse inhibition of *Arabidopsis* plants root growth, induced by other bacterial strains. Furthermore, biofilms help plants to cope with abiotic environmental stresses such as drought or salinity, by producing compounds that improve water retention and ion uptake. (Bhagat et al. 2021; Mathur and Roy 2021) Biofilms can contribute to the formation of soil aggregates, which can improve soil structure and stability, and increase water infiltration and retention. (Bhagat et al. 2021; Costa et al. 2018) In summary, microbial biofilms can promote the growth of a diverse range of plant species by creating a more favourable rhizosphere environment.

Investigation into the manipulation, control and exploitation of biofilms has gained momentum and seen an increase in research activity and funding in the last decade. However, there are still many knowledge gaps with regards to the mechanisms of interaction between natural polymicrobial biofilms and plants, and how to exploit these interactions to promote plant growth and prevent crop diseases. Improved understanding of biofilms, in this context, is key to discovering, controlling and directing the behaviour of microbial communities to create a sustainable environment. International collaboration

in this field is essential to advance global agricultural innovation and develop more sustainable food systems. Driven by this incentive, the UK's National Biofilms Innovation Centre [NBIC], in collaboration with leading Argentine researchers, organised a workshop within the framework of the SAMIGE [Argentinean Society for General Microbiology] annual congress, to establish a dialogue between researchers and industry representatives from both countries (NBIC 2023). This workshop brought together complementary expertise on exploitation of biofilms in agriculture with the aim of addressing knowledge gaps, improving agricultural productivity through the uptake of new technologies in both countries and tackling the global issue of climate change by promoting soil health, reducing the use of chemical treatments and their environmental impact. This publication, in its 'white paper' form, encapsulates the diverse discussions that occurred during the workshop and provides a comprehensive overview of the insights, perspectives, and outcomes that arose from the cooperative dialogue between the workshop participants. Three priority areas, that would benefit greatly from the future research collaborations between the UK and Argentina, emerged from the discussions and are described here. They are accompanied by proposed specific research activities that could be incorporated into future collaborative projects to address these priority areas. It is important to recognise that although the topics outlined below would be applicable to partnerships spanning various countries and regions globally, the primary focus of this white paper is not to offer an exhaustive review of these areas but to document the discussions held between these two countries.

Priority areas for UK-Argentina collaboration

Plant root biofilm composition as a sensor to optimise interventions

Root microbiomes play a key role in plant health. (Custódio et al. 2022; Finkel et al. 2017) They provide essential functions to enhance plant nutrition and deliver protection against biotic and abiotic stressors. (Custódio et al. 2022; Finkel et al. 2017) The interactions of soil microbiome with plant roots are complex and enable the formation of biofilms on roots, which assist with nutrient acquisition, growth proliferation, removal of contaminants, protection against plant pathogens, and promoting the establishment of other beneficial microorganisms in the soil. As [i] a wide variety of environmental stressors affect microbial adhesion, microbe-microbe and root-microbe interactions; [ii] the root biofilm provides a physical barrier to the diffusion of modulatory molecules from the root; and [iii] protects the root against abiotic and biotic stresses such as pH changes, desiccation,

osmotic stress, and pathogen attack, (Bogino et al. 2013) it is expected that the analysis of changes in the composition and properties of the root biofilm in response to environmental fluctuations can inform the status of the roots in response to these stresses. Therefore, by understanding the relationships between the properties and composition of root biofilms and the resistance / sensitivity of roots to biotic and abiotic stresses, we propose to use plant root biofilms, forming part of the wider microbiome, as a natural sensor to optimise interventions which promote plant health and crop production.

The objective of this proposed area of research is to further our understanding on the role of root biofilms in promoting plant health and resilience to fluctuations in environmental conditions. This will unveil how these biofilms contribute to the resistance of plant roots to various biotic and abiotic stresses and enable the design of bespoke inoculants which bypass the negative impact of these.

Comparison between environments in the UK and Argentina would enable the establishment of core differences and similarities between the two countries regarding healthy vs unhealthy root microbiota in a wider range of environmental conditions as the climate in the UK and Argentina differs significantly due to their geographical locations and topography. Additionally, it is proposed to focus on wheat and potatoes as two of the main crops grown in both countries, with similar climatic conditions for cropping but counter-seasonal growing. These two crops are proposed as they represent two different plant groups, one being a monocot [wheat] and the other dicot [potato] and having different characteristics and growth habits that influence how they are cultivated, propagated, and used. They also present two different scenarios of farming: extensive and intensive, respectively.

To accomplish the outlined objectives aimed at addressing this priority area, the following collaborative activities are proposed:

- Establishing the microbial composition of biofilms in roots from wheat and potatoes grown in rich and poor soils from different environments in the UK and Argentina, utilising advanced multi-omics technologies and developing, where possible, new methods for biofilm studies under agriculturally relevant conditions.
- Using multi-omics approaches to establish correlations between plant sensitivity and resistance to biotic and abiotic stresses [pathogens, drought, salinity, extreme temperatures] and the composition of microorganisms in the root biofilms, including determination of changes in root structure and healthy growth parameters.

- Testing the main hypotheses, derived from correlation studies, by using microbial collections available from *in vitro* recolonisation experiments. This will advance the discovery of new methods to study root biofilm and facilitate the establishment of causality between biofilm properties and composition and relevant plant phenotypes that could be extrapolated to more relevant agricultural conditions.
- Development of databases to store data generated above and use of artificial intelligence to analyse these data, establishing correlations and allowing predictions to be made on plant health and crop yield based on the composition of plant-associated microbiomes.
- Design of strategies, based on the devised predictions, to manipulate soil and root biofilms / microbiomes to restore healthy soil biodiversity and aid crop production under adverse conditions. This could be achieved using bespoke seed inoculants with the appropriate healthy microbial composition, as proposed in 'Priority Area 3' of this paper.

Targeting climate change and soil health

Soil is a natural resource for global food production but also provides an essential environment for ecosystems, biodiversity, carbon sequestrations and water availability. (Eckardt et al. 2022) Climate change can have a major impact on soil and vice-versa, the changes in the use of land and soil can drastically influence climate change. (Eckardt et al. 2022) The increasing human population and demand for food and land use have put pressure on soils in recent decades. It has been estimated that a third (Global Land Outlook 2022) of global soils is now degraded. There is a complex relationship between climate change and soil health, influenced by plant-microbiota interactions. (Omae and Tsuda 2022; Trivedi et al. 2022) Climate change-induced shifts in temperature, precipitation, and extreme events impact microbial communities in the soil, affecting nutrient circulation and overall soil health. These changes also cause abiotic stressors like drought or heat, activating crucial responses in plant-microbiota symbioses that contribute to plant resilience. Microbial mechanisms in the rhizosphere related to improving abiotic stress tolerance in plants include the production of biopolymers and biofilms, which provide a protective matrix around roots and increase resilience of plants to these factors. (Trivedi et al. 2020) For example, beneficial interactions, such as mycorrhizal associations and nitrogen-fixing microorganisms, are triggered enhancing nutrient availability. (Omae and Tsuda 2022; Trivedi et al. 2022) Unravelling the dynamics

of plant-microbiota interactions and the adaptation strategies employed by plants and microbes to cope with changing environmental conditions is essential for developing sustainable agricultural practices. This knowledge is needed to understand the pivotal role of these interactions in maintaining soil health under the pressures of a changing climate. Furthermore, to design faster and environmentally friendly strategies, the principles governing the interaction between soil and climate changes must be understood. This would contribute to exploration of innovative approaches such as utilising biofilm-forming microbiota to preserve natural resources and develop more resilient crops, contributing to the advancement of environmentally friendly agriculture. (Custódio et al. 2022)

Acknowledging the intricate connections between soil health, climate change, and the complex plant-microbiota interactions, targeting soil health and climate change has been identified as one of the priority areas, where the UK-Argentina collaboration could bring complementary knowledge, expertise, and know-how from and to both countries to address the shared challenges posed by global warming and environmental variability in both countries. As an example, global warming signs can be seen in the UK in recent years, with extreme weather leading to droughts and flooding that are becoming a problem in crop production. The climate in Argentina is hotter in general, with the predictions by the Intergovernmental Panel on Climate Change [IPCC] suggesting that this situation is likely to get aggravated with the addition of increased localised torrential rainfalls in some areas. (IPCC 2022) The abiotic stress conditions such as drought, excess water and increasing temperatures in soils, are considered the most significant factors reducing agricultural productivity nowadays. Therefore, knowledge on how to manage soil health and crop production under increasing temperatures, droughts or excess water conditions could be transferred between the UK and Argentina.

The aim of this proposed research area is to investigate and understand the dynamics of the relations between soil health, climate change and the adaptation strategies used by plants and microbes to cope with shifting environmental conditions. The ultimate goal will be to use this knowledge to maintain soil health and enhance agricultural productivity.

This area interlinks strongly with the actions proposed in the 'Priority Area 1' and the proposed collaborative activities include:

- Maintaining focus on two different crop types, which are common to both regions: wheat [grain] and potatoes [root].
- Sampling and sequencing the microbiota from biofilms commonly associated with potatoes and wheat roots in both countries under different environmental conditions [temperatures, rain fall, and compaction], and build collections of fully sequenced microbial isolates that contain the main determined taxonomic categories of microbes.
- Identification of environment-specific beneficial microbiota across the two countries, followed by validation, under controlled conditions and with the use designed microbial synthetic communities, of the importance of the identified microbes for crops performance under stress conditions.
- Optimisation of microbial combinations to be exploited in agricultural practices to provide protection to crops against droughts, floods and soil compaction. These microbial combinations should be designed to also contain beneficial traits associated with increased carbon sequestration in the roots, produce stable organic matter, and protect against plant pathogens. These approaches should lead to sustained agricultural production under changing environmental conditions.

Intelligent seeds

For many important crops, the cultivation cycle begins with a direct sowing of seeds. The use of seed coating technologies enables uniform distribution of inoculants, including plant beneficial microbes, (Custódio et al. 2022; Simonin et al. 2022) to the surface of the seeds. This maximises stable crop production while reducing the use of agrochemicals.

The concept of "intelligent seed" has not yet been validated and forms a central focus of the proposed collaborative activities. The term 'intelligent seed' can encompass various perspectives but most of them are centred around tracing plant processes from the seed. One proposed strategy involves utilising high biofilm-producing microorganisms to enhance microbial survival in both seeds and plants through the colonisation in the rhizosphere. It has to be noted that the coating of seeds with biofilm may not necessarily be the method for introducing microorganisms into the seeds and rhizosphere. Sandhya et al. (Sandhya et al. 2009) demonstrated that inoculation of seed in a culture of biofilm-producing *Pseudomonas putida*, yielding a high content of exopolysaccharide, significantly increased the survival and biomass of *Helianthus annuus* [sunflower] under drought stress, highlighting the protective role of biofilms on root surfaces. For insights into the formulation of seed coatings as tools for delivering beneficial microbes to agricultural crops, we refer readers to Rocha et al. (Rocha

et al. 2019) There is a growing need for approaches which would facilitate the use of customised microbial consortia. There is also an increasing demand for tailored formulations to enhance the flow and plantability of film-coated crops in the open field environment.

The area of research proposed here aims to establish a correlation between the biofilm production capacity of various microorganisms, their multiplication in fermenters [liquid or solid], their survival capacity on seeds and in the rhizosphere post-inoculation, followed by the monitoring of plant development and health.

Argentina has been developing state-of-the-art multipurpose seed coatings, which can incorporate both chemicals and digital technologies [seed-chips] providing real-time data on the impact of these chemicals on plant health parameters. These coatings have the potential to incorporate microbes which can form beneficial biofilms on plant roots upon germination. This would enable real-time monitoring of the impact of different inoculants on plant health. Currently, these technologies are only used in small field trials and would require suitable monitoring systems for large scale trials. To address the need for the development and implementation of technologies which enable large scale field monitoring, the UK has been developing advanced soil and growth monitoring technologies such as drone-based field monitoring and ground penetrating radars [GPR] which are increasingly applied by farmers for scouting and logistics in large fields. In addition, it has also been developing the next generation of sensor capabilities to monitor soil environmental parameters. The scale of arable cropping in Argentina provides an opportunity to gather large datasets, facilitating the development of crop modelling. These models could, subsequently, be extrapolated to inform the crop farming under the UK climate. In the UK, the models could be validated and refined using in-field sensors and regular UAV imaging, incorporating technologies such as LIDAR, hyperspectral and multi-spectral imaging.

Based on the above complementary capabilities between Argentina and the UK the following collaborative activities are proposed to develop intelligent seeds and innovative monitoring systems, which would enable agile optimisation of inoculants based on their impact on plant health and soil parameters:

- Incorporation of beneficial biofilm-forming microbes in seed coatings containing the chip technology developed in Argentina, using already tested and well-performing microbial inoculants to provide a solid starting point.
- Development of drone-based monitoring systems in the UK for real-time reading of chips used in seeds.
- Development of pilot green-house experiments to enable monitoring of the effectiveness of drones in recording plant / soil health parameters induced by coated seeds related to plant infection control, followed by performance assessment of the system.
- Implementation of large field trials using optimised ground penetrating radars on fields seeded with intelligent coated seeds with different inoculants, containing biofilm-forming microorganisms, to compare their performance via monitoring plant / soil health parameters. Standardised databases would be built from the collected data, to inform performance optimisation of seed biofilm-forming inoculants.

Conclusions

International collaboration is essential for advancing global agricultural innovation and establishing sustainable food systems. Here, we describe the outcomes of discussions held between the UK and Argentina, highlighting three priority areas that could profit from future research collaborations between the two countries, while recognising that the topics described in this 'white paper' would be applicable to partnerships and cooperations between different countries and regions globally. For the three priority areas identified, early engagement of end users [farmers] will be paramount to maximise technology adoption. Business models of long-term cooperation will need to be established to maximise impact. There are many benefits that would come from building networks and collaborations between the UK and Argentina, yet the commitment from governments and support from funding bodies will be essential for the establishment of robust research programmes and long-term successful collaborations between researchers, industry and end users across both countries.

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Author contributions

PDR, MC and GC wrote the manuscript. All the authors took part in the discussions leading to this paper. All authors reviewed and approved the final manuscript.

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Availability of data and materials

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

Authors declare no competing interests.

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