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Keystone metabolites of crop rhizosphere microbiomes

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Abstract

The role of microbes in sustaining agricultural plant growth has great potential consequences for human prosperity. Yet we have an incomplete understanding of the basic function of rhizosphere microbial communities and how they may change under future stresses, let alone how these processes might be harnessed to sustain or improve crop yields. A reductionist approach may aid the generation and testing of hypotheses that can ultimately be translated to agricultural practices. With this in mind, we ask whether some rhizosphere microbial communities might be governed by “keystone metabolites”, envisioned here as microbially produced molecules that, through antibiotic and/or growth-promoting properties, may play an outsized role in shaping the development of the community spatiotemporally. To illustrate this point, we use the example of redox-active metabolites, and in particular phenazines, which are produced by many bacteria found in agricultural soils and have well-understood catalytic properties. Phenazines can act as potent antibiotics against a variety of cell types, yet they also can promote the acquisition of essential inorganic nutrients. In this essay, we suggest the ways these metabolites might affect microbial communities and ultimately agricultural productivity in two specific scenarios: i) biocontrol of beneficial and pathogenic fungi in increasingly arid crop soils and ii) promotion of phosphorus bioavailability and sustainable fertilizer use. We conclude with specific proposals for future research.

Introduction

The realization that resident microbes are intimately connected to human health is one of the most important conceptual awakenings that has swept the field of microbiology over the past two decades. Today, thousands of researchers are dedicated to studying various aspects of the human microbiome, and the potential for basic research to change disease treatment in our lifetimes is very real. While this progress is inspiring, we contend that studying rhizosphere microbiomes (*i.e.* microbial communities in soils in the vicinity of plant roots) is as important to sustaining global human health as studying the human gut microbiome.

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Though plant-microbe interactions is a venerable field, relative to their importance, studies of rhizosphere microbiomes have not received the attention they deserve. Our impetus to spotlight this area of research stems from the vital roles microbes play in agriculture and the uncertain future of food security in a changing climate [1, 2]. We approach this topic from the perspective of basic science, in particular microbiology, and propose that a fundamental understanding of the functioning of rhizosphere communities will be an essential part of predicting and responding to future agricultural stresses. Moreover, like animals, plants evolved in a microbial world (Figure 1A). Understanding how the rhizosphere microbiome can be tuned to sustain plant health in the face of future threats such as climate change and diminishing mineral nutrient reserves (Figure 1B,C) is a challenging and vital task.

How do we understand the functioning of any microbial community? Advances in sequencing technologies have revolutionized our ability to identify which microbes are present and which genes they are expressing in soils and other habitats, but our understanding of what drives their activities spatiotemporally is poor. While it is well-appreciated that diverse physical, chemical, and biological factors sustain and structure all microbial communities, we lack a framework that defines the importance of these factors at different stages in community development; defining such a hierarchy can be considered the grand challenge of the field. We propose that meeting this aspirational goal may benefit from a reductionist approach. Intuitively, not everything is likely to be equally important all the time; therefore, identifying the dominant factors that operate at different stages of microbial community development, under different environmental conditions, could lead us towards a predictive understanding. In this spirit, we introduce the concept of “keystone metabolites”. In loose analogy to the more familiar term “keystone species” from macroecology, where an individual species has a disproportionate impact on an ecosystem—such as the effect of starfish on the composition of the intertidal zone [3], we define keystone metabolites as microbially-produced molecules that play critical roles in shaping a microbial community. Our usage of “keystone” is more expansive than the classical definition: we define keystone metabolites as playing multiple roles in an ecosystem, not just one. While many keystone metabolites may exist, we posit that certain ones may have outsized effects in particular environments spatiotemporally, such that, if they were removed, the microbial community would look and behave completely differently.

How might we begin to test this idea? The first steps are to identify potential keystone metabolites of environmental importance, the interactions they mediate under different conditions, and experimental systems where their presence can be controlled and their impact measured. By way of illustration, for the remainder of this essay, we will discuss the potential for microbially-produced redox-active metabolites (RAMs) to function as keystone metabolites. RAMs fall under the broad categories of “natural products” or “secondary metabolites”, come in many different forms, and are produced by many different types of soil bacteria. Though produced and reactive intracellularly, RAMs are secreted and can also react extracellularly, exchanging electrons with different reaction partners. RAMs thus function as “electron shuttles”, accepting electrons from and donating electrons to diverse soil constituents (Figure 2A,B). For example, in oxic environments, reduced RAMs can react with molecular oxygen and generate toxic reactive oxygen species [4], whereas in anoxic environments, if ferric iron (Fe) minerals are present, reduced RAMs can reductively

liberate iron [5]. RAMs are attractive putative keystone metabolites precisely due to this versatile chemical reactivity: while potentially toxic, they can also play beneficial roles for their producers, including serving as signaling agents, supporting energy conservation under anoxia, and even facilitating nutrient acquisition—which of these roles dominates at any given instance can be predicted according to local environmental conditions [6]. Equally importantly, RAMs affect cells differently depending on their cell type and/or physiological state—for example, certain cells possess conserved machineries (transporters, oxidative stress defenses, repair enzymes, etc.) that defend them against RAM toxicity and/or are induced selectively [6].

Considering RAMs in crop soils provides a specific context wherein to develop our “keystone metabolite” hypothesis. Specifically, we examine how a diverse array of RAMs—exemplified by the well-studied and potentially agriculturally-important phenazines [7]—may be surprisingly relevant to two important challenges facing modern agriculture: modulating rhizosphere microbial community membership to sustain plant growth (*e.g.* controlling pathogenic vs. beneficial fungi; section 1) and improving phosphorus (P) retention and bioavailability (section 2). We conclude with a brief discussion of questions that could guide future research on these topics.

Section 1: RAMs as Microbial Interaction Mediators

The idea that RAMs can influence microbial communities is not new. Some of the best studied models are phenazines, colorful secondary metabolites that are made by many species of soil-dwelling *Actinobacteria* and *Proteobacteria*, including pseudomonads. Phenazines such as the environmentally relevant phenazine-1-carboxylic acid (PCA, Figure 2A) are well known to suppress or exclude a number of pathogens from agricultural crops, especially fungi [7]. Perhaps the best-known example of this is the requirement of PCA-production for pseudomonads that are capable of suppressing *Gaeumannomyces graminis* var. *tritici*, the fungal pathogen that causes take-all disease in wheat [8]. Other phenazines such as phenazine-1-carboxamide can also inhibit fungal pathogens, like *Fusarium oxysporum* f. sp. *radicis-lycopersici*, a cause of root rot in tomato plants [9]. This notion of fungal suppression by bacterially-produced phenazines extends into non-rhizosphere environments, such as phenazine-mediated suppression of *Candida albicans* and *Aspergillus fumigatus* in the context of the cystic fibrosis lung [10, 11]. The antimicrobial effects phenazines exert on fungi are thought to be linked to their redox activity, at least partially due to their ability to create reactive oxygen species and/or destabilize the electron transport chain, resulting in lowered ATP [10]. As soils become more arid and thus oxic for longer periods of time due to climate change, we propose these mechanisms will matter in considering how this class of RAMs may act as keystone metabolites.

Prior to elaborating on how phenazines may shape current and future rhizosphere microbial communities, let us consider how the microbiome may be tuned to promote plant health. Increasing temperatures represent a threat to farming, and warming in the period between 1981 and 2002 was estimated to have caused an annual combined loss of 40 mega-tons of primary food crops (wheat, maize and barley) worth \$5 billion per year as of 2002 [12]. One promising approach to boosting crop yields may be to apply beneficial microbes to the

rhizosphere that help plants withstand environmental stress. While nitrogen-fixing bacteria are well-known for their symbiotic relationships with legumes, here, we focus on the broader potential of fungi to help plants thrive. Despite the fact that certain fungal pathogens destroy a significant portion of food crops annually, there are many more beneficial fungi that promote tree and shrub growth by helping plants draw in water, scavenge nutrients from the soil, aid in stress tolerance and repel pathogens; indeed, almost all plant species have symbiotic relationships with fungi [13, 14]. Recent studies involving wheat, rice, and tomatoes indicate plant growth promoting fungi can also play a role in crop robustness [15–17]. These observations suggest that existing or added fungi merit consideration with respect to food security.

How are soil fungi likely to be impacted by an altered, drier climate? The results of environmental manipulation experiments have not yet reached a clear consensus, with increased warming only sometimes showing a change in fungal community composition or altered fungal abundance across a broad array of fungal taxa and niches [18, 19]. While some of this variation is likely attributable to the niche and host plant species studied, the wide range of findings is striking. Intriguingly, a global study of Earth's topsoils found that while environmental variables were strongly correlated with bacterial populations, they were only weakly correlated with fungal populations [20]. Yet for specific food crops, arbuscular mycorrhizal biomass was demonstrated to decrease under arid conditions [21]. Given the challenge of predicting the fate of soil-dwelling fungi in response to environmental variables, it is worth considering whether interactions with bacteria in the rhizosphere may be more important in driving fungal fate than changing abiotic variables *per se*.

Notably, fungal communities can be shaped by rhizosphere bacteria [20]. We can now ask, how might RAM producing bacteria fit into this picture? As noted above, it is well-established that RAMs such as phenazines can inhibit pathogenic fungi [7–9]; by extension, we would expect that beneficial soil fungi should also be susceptible to phenazine assault. It is therefore an intriguing possibility that RAM production may shape the rhizosphere community by acting on both pathogenic and beneficial fungi. There is significant indirect evidence supporting the hypothesis that bacteria help govern fungal activity geographically. Globally, one study of Earth's soils revealed an inverse relationship between trends in fungal and bacterial biomass across several climate zones [20]. Intriguingly, an inverse relationship in gene functional diversity between bacteria and fungi was seen in the same study, indicating that the genetic richness of bacteria and fungi do not peak concurrently even in environments that can support both types of organisms. Taken together, this suggests that inter-domain competition is prevalent in rhizosphere microbial communities.

Could RAMs such as phenazines shape future fungal-bacterial interactions as the climate changes? Intriguingly, bacterial populations in more arid environments exhibit lower taxonomic diversity and skew toward phenazine-producing clades such as *Actinobacteria* and certain *Proteobacteria* [22]; drier agricultural fields are enriched in phenazine-producing bacteria such as pseudomonads, while fields of the same soil type in the same geographic region that receive more moisture are not [23, 24]. Given that phenazine toxicity is linked to redox reactions with oxygen, and that drier soils are more permeable to oxygen, it stands to reason that the same concentration of soil phenazines may be more inhibitory to microbial

communities in areas that experience lower precipitation as a consequence of climate change. Therefore, fungal populations in drying agricultural regions may be endangered by increases in the abundance of phenazine-producing bacteria as well as the oxygen-enhanced potency of phenazines that they produce, a potential future consequence that requires awareness of how an endogenous bacterial metabolite can interact with changing abiotic, environmental variables (Figure 2B,C). That is, the abiotic stresses of climate change may be primarily translated into biotically-induced stressors for fungi.

While phenazine stress may increase in drier soils, the role of phenazines is likely to be more complex than simply killing competitors [6]. For instance, some studies have shown the minimal inhibitory concentration of PCA against certain filamentous fungi to be in the 10s or 100s of μM [25], far higher than their bulk concentration in soil (100s of nM range) [23]. While the true concentration may be higher in close proximity to phenazine producing bacteria, and the toxicity of PCA can be strongly altered by local pH (Figure 2A), bulk PCA concentrations suggest that some fungal or bacterial competitors may be merely slowed in their growth and not eliminated. Adding to the notion that the effects of phenazines are likely to vary spatiotemporally, it has been found that although phenazine production facilitates *Pseudomonas fluorescens* colonization of plant roots, the rest of the bacterial community was not significantly affected by phenazine production [26]. This points to phenazines serving multiple roles within rhizosphere microbiomes, as has been suggested for other antibiotics [27].

Because RAM activities are affected by oxygen, pH and water potential, RAMs may act as keystone metabolites for microbial communities by virtue of integrating abiotic environmental parameters and biotic interactions. The impact environmental variables can have on the toxicity of RAMs paints a nuanced picture of metabolites that may engage in a balancing act: some RAM stress may improve plant health by suppressing specific fungal pathogens, but too much may shift the microbial community away from an optimal composition of beneficial fungi. Several factors must be considered when predicting how keystone metabolites may shape microbial communities. For example, local microenvironmental gradients of moisture, pH and oxygen levels would be expected to influence the community's developmental outcome by impacting RAM abundance and toxicity, but so might the presence of protective bacteria. Strikingly, on a global level, fungal abundance positively correlates with the presence of bacterially-derived redox-stress tolerance genes in soil as well as bacterial populations that may produce phenazines [20]. Although it has been suggested these genes may be present to protect bacteria from fungally produced antibiotics, it is also possible that some bacteria housing these redox-stress tolerance genes may act as protective partners to members of the fungal community.

Given that many fungi in the rhizosphere are sensitive to RAM assault and yet are often found living in proximity to RAM producers, the hypothesis that protective partnerships exist is worth testing and may have relevance in applied agriculture. As depicted in Figure 2C, helper bacteria could provide protection to fungal partners, allowing them to enter the rhizosphere space even in communities that produce greater RAM stress due to increased abundance and toxicity of phenazines in drier soils. Depending on the nature of the fungus (pathogenic or beneficial) this might have very different outcomes for plant growth. Notably,

fungi can form associations with bacteria living on or in their hyphae, particularly with the *Burkholderiaceae* family [28]. Our preliminary findings suggest that some protective bacteria can exist in partnership with desirable fungal species, protecting them against RAM stress; whether such relationships have relevance in crop rhizospheres is an intriguing open question.

Section 2: RAMs as Weathering Molecules

Any gardener knows that, along with nitrogen, phosphorus is a key component of fertilizer. What is less well appreciated is that unlike nitrogen, which can be “fixed” through nitrogen fixation, P needs to be mined from phosphate rock. This can be either through the secretion of enzymes and metabolites by plants and microorganisms or industrial mining by humans for agricultural fertilizers. Due to this necessity for mineral extraction, P is often considered as the ultimate control on primary productivity over geologic time scales. On much shorter time scales, agriculture relies on a finite supply of phosphate rock, and while the topic is much debated, future shortages of phosphate fertilizers are predicted by some (Figure 1C, [29]). The severity of any future phosphate limitation will depend at least in part on our capacity for adaptation through more sustainable and efficient use of P fertilizers. One particular challenge is that phosphorus (usually in the form of phosphate) often adsorbs to iron minerals in soils, making it difficult to maintain P bioavailability. At the same time, attempts to combat low P bioavailability through excess fertilizer application can lead to P runoff and eutrophication, a process where excess nutrient inputs to water bodies stimulate algal blooms and subsequent heterotrophic consumption of these blooms draws down oxygen, ultimately harming aquatic life.

We contend that, in this context, RAMs and other secondary metabolites might increase P solubility, effectively serving as a catalyst for P weathering by reducing Fe minerals and liberating adsorbed P needed for plant and microbial growth. The proposal that RAMs have roles in P weathering is motivated by: i) evidence that redox-active ‘natural organic matter’ found in soils can solubilize mineral-adsorbed P by reductively dissolving Fe minerals, a feat that should also be achievable by many RAMs and ii) the intriguing finding that the production of some RAMs is increased under P limitation, suggesting the process may be used to alleviate microbial P stress. As we will discuss here, studying RAMs may allow for the controlled investigation and potentially the manipulation of chemical transformations that are important to soil function but technically challenging to study.

While a link between secondary metabolites and weathering may initially seem foreign, the idea is inspired by similarities between RAMs and compounds found in natural organic matter that are known to contribute to mineral weathering. Natural organic matter is a catchall term for the mix of molecules produced by plants and microbes as well as the degradation products that arise as biomass is slowly broken down in soils. Although the details remain mostly ambiguous, as a whole, the various acids, metal binding agents, and redox-active molecules in natural organic matter are thought to facilitate the weathering of soil minerals. Of particular relevance for this discussion are humic substances (humics), a loose category of redox-active molecules produced during the decomposition of biomass. Humics engage in electron transfer reactions and facilitate Fe mineral reduction [30]. It is

well understood by soil scientists that this reduction of Fe minerals can release adsorbed P through the processes of 'reductive dissolution' whereby ferric iron (Fe^{3+}) is reduced to ferrous iron (Fe^{2+}), thus dissolving the ferric mineral to which P is sorbed [31]. Additions of anthraquinone-2,6-disulfonate (AQDS, a molecule that is often used as an experimental model for humics and is structurally similar to phenazines [32]), have been shown to increase the amount of soluble phosphorus present in soils [33]. AQDS treatments also led to concomitant increases in Fe^{2+} , supporting the idea that AQDS solubilizes P via reductive dissolution.

Given the chemical similarities between RAMs and humics, could secondary metabolites such as phenazines facilitate P solubilization in the same way? Work in our lab has established that analogous to humics, phenazines can indeed reduce Fe minerals [5]. The capacity for phenazines to solubilize P still remains to be tested, but there is some evidence that Fe reduction by phenazines occurs in crop soils. Phenazine producing bacteria have been shown to alter Fe minerals in the wheat rhizosphere [34], raising the possibility that phenazine-facilitated reductive dissolution could be relevant for P solubilization in agricultural contexts. The potential link between P and phenazines is further strengthened by long-standing observations showing that pseudomonads, which are well studied phenazine producers, increase production of these RAMs in response to P limitation [35]. This correlation implies that microbes may use phenazines deliberately to obtain P under limiting conditions.

That RAM production can be regulated by environmental factors like P limitation is an important distinction between these molecules and others found in natural organic matter; one that has practical consequences. Unlike humics, where the source is unknown and innumerable structural variants exist, RAMs are produced by microbes that can be isolated and cultured in the lab. Many RAMs have well-studied biosynthetic pathways, the regulation of which can be queried directly using genetic techniques as well as by monitoring microbial metabolite production in response to environmental stimuli. Controlled biosynthetic regulation may also distinguish RAMs from other microbially secreted components of natural organic matter like organic acids (which can also aid in mineral solubilization) where regulation is not fully understood, and in some cases, production may be constitutive. Many metabolites with redox properties comparable to phenazines can be found in soils. It is our hope that by focusing on experimentally tractable examples like phenazines and studying the conditions that stimulate their production, we may be better able to decipher the processes governing chemical transformations in soils and potentially to harness them for agricultural productivity.

The weathering functions of RAMs, and other secondary metabolites, may be important to their putative role as keystone metabolites. If, as we suspect, RAMs truly increase P (and Fe) bioavailability in the environment, then this can be considered as a service that benefits the entire microbial community, not just the RAM producer. Additionally, the ability of RAMs to solubilize P depends on their redox activity (Figure 2B). In order to react with Fe minerals, RAMs must be in their reduced state. Redox reactions between reduced RAMs and Fe minerals yield oxidized RAMs that are no longer capable of solubilizing P. However, soil dwelling organisms are well known for their capacity to (re)-reduce a wide range of organic

molecules including humics as well as phenazines, a process often understood to be driven by the need for electron acceptors in the absence of oxygen [30, 32]. This raises the possibility that RAMs might be cycled by the microbial community. If true, relative to their concentration, RAMs may play an outsized role in nutrient solubilization: even a small concentration of these metabolites might be cycled many times. An open question is whether RAMs increase plant access to mineral nutrients like P and Fe, a function that might allow them to make even more drastic contributions to ecosystem structure.

Clearly more experiments are needed to determine whether RAMs and other secondary metabolites influence P bioavailability. However, at the very least, these observations warrant further studies focused on the biological basis for P regulation of these metabolites as well as their abiotic reactions with soil P sources.

Conclusions and Future Priorities

We have much to learn about how microbes organize themselves into productive communities in any environment. But challenges to food security in the context of climate change and potential mineral reserve depletion make it particularly important that we gain a mechanistic understanding of the rhizosphere microbiome. Microbes co-evolve with their environment, and can be expected to do so in the face of future challenges. If we can identify “keystone metabolites” that mediate this success and understand how they do so spatiotemporally, such insights may inform future, more sustainable, agricultural practices. Recognizing that RAMs are but one variable among many that shape rhizosphere ecology, we nevertheless believe focusing on these molecules may help us generate specific testable hypotheses about microbial interactions in the rhizosphere, including in an applied context.

An increased understanding of the ways RAMs impact plant colonization by beneficial and pathogenic fungi may allow us to conceive adaptive practices that support healthy rhizosphere communities. Such efforts should be informed by existing knowledge about the links between RAM toxicity and the abiotic and biotic microenvironment. As discussed in section 1, RAM producers appear more abundant on plant roots in drier conditions. If RAMs do not simply eliminate but instead modulate the growth and behavior of certain microbes as a function of local conditions, we would expect RAMs to exert very different effects on a rhizosphere microbiome depending on when in its development they are introduced and the accompanying microenvironmental conditions. For example, one possible intervention to foster the colonization of plant growth-promoting fungi might be to employ irrigation for a short period of time early in a plant’s life to inhibit RAM producers that could threaten fungi under an oxic environment, yet be neutral, or even beneficial to other members of the microbial community later in its development as certain microenvironments become anoxic, such as those within aggregate biofilms [6]. While this strategy may be naive, testing it is readily achievable with defined microbial communities and appropriately resolved spatiotemporal imaging studies for model plant systems.

Based on the mechanism for RAM solubilization of P introduced in section 2, might it also be possible to engineer chemical or microbial additives to foster P bioavailability and more sustainable use of P in agriculture? The most basic first set of experiments could focus on

abiotic studies of the reactions between RAMs and soil P species, followed by quantifying RAM P solubilization in soils and the effects on plant and microbial growth. The concept that soil microorganisms might be used to promote P bioavailability is well-established [36] and many bacterial soil isolates are known to secrete acids, a strategy that helps dissolve P-containing minerals such as calcium phosphate and hydroxyapatite rather than reductively liberating P that is adsorbed to iron minerals. Acid production is also one of the ways that fungi can increase plant P bioavailability. Revisiting this idea with P-regulated RAMs may allow us to gain a more detailed understanding of the timing of metabolite production as well as the solubilization of mineral-adsorbed vs mineral P sources. Potential synergies between these different P-solubilization strategies (acidification and RAM production) and different P-solubilizing organisms (fungi and bacteria) might also be explored. The fact that RAMs have well understood biosynthetic pathways allows relevant RAM producers to be easily identified from their genomes. Many RAM biosynthetic genes clusters are fairly modular and could potentially be mobilized, opening the possibility of extension to bioengineering.

We have highlighted diverse potential roles for RAMs in shaping crop rhizospheres. Understanding how these different functions fit together in complex soil environments is a formidable challenge. Regardless of whether RAMs turn out to be agriculturally important “keystone metabolites”, we hope this essay will stimulate future research into the mechanisms underpinning rhizosphere microbial interactions under changing environmental conditions. Such efforts should be prioritized to enable adaptive solutions to challenges facing agriculture in the coming decades.

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References

1. IPCC (2019). Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems. Shukla PR, Skea J, Calvo Buendia E, Masson-Delmotte V, Pörtner H-O, Roberts DC, Zhai P, Slade R, Connors S, van Diemen R, et al., eds.
2. Reid A, and Greene SE (2012). How microbes can help feed the world. Report on an American Academy of Microbiology Colloquium, Washington DC, 12 2012. (American Academy of Microbiology).
3. Yong E (2013). Bob Paine fathered an idea — and an academic family — that changed ecology. *Nature*, 286–289.
4. Hall S, McDermott C, Anoopkumar-Dukie S, McFarland A, Forbes A, Perkins A, Davey A, Chess-Williams R, Kiefel M, Arora D, et al. (2016). Cellular effects of pyocyanin, a secreted virulence factor of *Pseudomonas aeruginosa*. *Toxins* 8, 236–214.
5. Wang Y, and Newman DK (2008). Redox reactions of phenazine antibiotics with ferric (hydr)oxides and molecular oxygen. *Environmental Science & Technology* 42, 2380–2386. [PubMed: 18504969]

6. Grahl N, Kern SE, Newman DK, and Hogan DA (2013). The Yin and Yang of Phenazine Physiology. In *Microbial Phenazines*, Chincholkar S and Thomashow LS, eds. (Heidelberg: Springer, Berlin), pp. 43–69.
7. Chincholkar S, and Thomashow LS (2013). *Microbial Phenazines*, (Heidelberg: Springer, Berlin).
8. Thomashow LS, and Weller DM (1988). Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. *Journal of Bacteriology* 170, 3499–3508. [PubMed: 2841289]
9. Chin-A-Woeng TFC, Bloemberg GV, van der Bij AJ, van der Drift KMG, Schripsema J, Kroon B, Scheffer RJ, Keel C, Bakker PAHM, Tichy H-V, et al. (2007). Biocontrol by phenazine-1-carboxamide-producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Molecular Plant-Microbe Interactions* 11, 1069–1077.
10. Morales DK, Grahl N, Okegbe C, Dietrich LEP, Jacobs NJ, and Hogan DA (2013). Control of *Candida albicans* metabolism and biofilm formation by *Pseudomonas aeruginosa* phenazines. *mBio* 4, e00526–00512. [PubMed: 23362320]
11. Briard B, Bomme P, Lechner BE, Mislin GLA, Lair V, Prévost M-C, Latgé J-P, Haas H, and Beauvais A (2015). *Pseudomonas aeruginosa* manipulates redox and iron homeostasis of its microbiota partner *Aspergillus fumigatus* via phenazines. *Scientific reports* 5, 8220. [PubMed: 25665925]
12. Lobell DB, and Field CB (2007). Global scale climate–crop yield relationships and the impacts of recent warming. *Environmental Research Letters* 2, 014002.
13. Marjanovi Ž, and Nehls U (2008). Ectomycorrhiza and Water Transport. In *Mycorrhiza* Varma A, ed. (Berlin, Heidelberg: Springer), pp. 149–159.
14. Bonfante P, and Genre A (2010). Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. *Nature Communications* 1, 1–11.
15. Zhang S, Gan Y, and Xu B (2016). Application of plant-growth-promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. *Frontiers in Plant Science* 7, 306. [PubMed: 27014326]
16. Khadka RB, and Uphoff N (2019). Effects of *Trichoderma* seedling treatment with system of rice intensification management and with conventional management of transplanted rice. *PeerJ* 7, e5877. [PubMed: 30693151]
17. Yoo S-J, Shin DJ, Won HY, Song J, and Sang MK (2018). *Aspergillus terreus* JF27 promotes the growth of tomato plants and induces resistance against *Pseudomonas syringae* pv. *tomato*. *Mycobiology* 46, 147–153. [PubMed: 29963316]
18. Mucha J, Peay KG, Smith DP, Reich PB, Stefa ski A, and Hobbie SE (2018). Effect of simulated climate warming on the ectomycorrhizal fungal community of boreal and temperate host species growing near their shared ecotonal range limits. *Microbial Ecology* 75, 348–363. [PubMed: 28741266]
19. Castro HF, Classen AT, Austin EE, Norby RJ, and Schadt CW (2010). Soil microbial community responses to multiple experimental climate change drivers. *Applied and Environmental Microbiology* 76, 999–1007. [PubMed: 20023089]
20. Bahram M, Hildebrand F, Forslund SK, Anderson JL, Soudzilovskaia NA, Bodegom PM, Bengtsson-Palme J, Anslan S, Coelho LP, Harend H, et al. (2018). Structure and function of the global topsoil microbiome. *Nature* 560, 233–237. [PubMed: 30069051]
21. Varoquaux N, Cole B, Gao C, Pierroz G, Baker CR, Patel D, Madera M, Jeffers T, Hollingsworth J, Sievert J, et al. (2019). Transcriptomic analysis of field-droughted sorghum from seedling to maturity reveals biotic and metabolic responses. *Proceedings of the National Academy of Sciences* 116, 27124–27132.
22. Zeng Q, Dong Y, and An S (2016). Bacterial community responses to soils along a latitudinal and vegetation gradient on the Loess Plateau, China. *PLOS ONE* 11, e0152894. [PubMed: 27045518]
23. Mavrodi D, Mavrodi OV, Parejko JA, Bonsall RF, Kwak Y-S, Paulitz TC, Thomashow LS, and Weller DM (2012). Accumulation of the antibiotic phenazine-1-carboxylic acid in the rhizosphere of dryland cereals. *Applied and Environmental Microbiology* 78, 804–812. [PubMed: 22138981]

24. Mavrodi D, Mavrodi OV, Elbourne LDH, Tetu S, Bonsall RF, Parejko J, Yang M, Paulsen IT, Weller DM, and Thomashow LS (2018). Long-term irrigation affects the dynamics and activity of the wheat rhizosphere microbiome. *Frontiers in Plant Science* 9, 7279.
25. Simionato AS, Navarro MOP, de Jesus MLA, Barazetti AR, da Silva CS, Simões GC, Balbi-Peña MI, de Mello JCP, Panagio LA, de Almeida RSC, et al. (2017). The effect of phenazine-1-carboxylic acid on mycelial growth of *Botrytis cinerea* produced by *Pseudomonas aeruginosa* LV strain. *Frontiers in Microbiology* 8.
26. Bankhead SB, Landa BB, Lutton E, Weller DM, and Gardener BBM (2004). Minimal changes in rhizobacterial population structure following root colonization by wild type and transgenic biocontrol strains. *FEMS Microbiology Ecology* 49, 307–318. [PubMed: 19712423]
27. Davies J, and Ryan KS (2012). Introducing the parvome: bioactive compounds in the microbial world. *ACS chemical biology* 7, 252–259. [PubMed: 22074935]
28. Levy A, Merritt AJ, Mayo MJ, Chang BJ, Abbott LK, and Inglis TJJ (2009). Association between *Burkholderia* species and arbuscular mycorrhizal fungus spores in soil. *Soil Biology and Biochemistry* 41, 1757–1759.
29. Obersteiner M, Peñuelas J, Ciais P, van der Velde M, and Janssens IA (2013). The phosphorus trilemma. *Nature Geoscience* 6, 897–898.
30. Lovley DR, Coates JD, Blunt-Harris EL, Phillips EJP, and Woodward JC (1996). Humic substances as electron acceptors for microbial respiration. *Nature* 382, 445–448.
31. Stumm W, and Sulzberger B (1992). The cycling of iron in natural environments: considerations based on laboratory studies of heterogenous redox processes. *Geochimica et Cosmochimica Acta* 56, 3233–3257.
32. Hernandez ME, Kappler A, and Newman DK (2004). Phenazines and other redox-active antibiotics promote microbial mineral reduction. *Applied and Environmental Microbiology* 70, 921–928. [PubMed: 14766572]
33. Peretyazhko T, and Sposito G (2005). Iron(III) reduction and phosphorous solubilization in humid tropical forest soils. *Geochimica et Cosmochimica Acta* 69, 3643–3652.
34. LeTourneau MK, Marshall MJ, Grant M, Freeze PM, Strawn DG, Lai B, Dohnalkova AC, Harsh JB, Weller DM, and Thomashow LS (2019). Phenazine-1-carboxylic acid-producing bacteria enhance the reactivity of iron minerals in dryland and irrigated wheat rhizospheres. *Environmental Science & Technology* 53, 1–12. [PubMed: 30571107]
35. Whooley M, and McLoughlin A (1982). The regulation of pyocyanin production in *Pseudomonas aeruginosa*. *European Journal of Applied Biotechnology* 15, 161–166.
36. Goldstein AH (1986). Bacterial solubilization of mineral phosphates: Historical perspectives and future prospects. *American Journal of Alternative Agriculture* 1, 51–57.
37. Morris JL, Puttick MN, Clark JW, Edwards D, Kenrick P, Silvia P, Wellman CH, Yang Z, Schneider H, and Donoghue PC (2018). The timescale of early land plant evolution. *Proceedings of the National Academy of Sciences* 115, 1–10.
38. Gibson TM, Shih PM, Cumming VM, Fischer WW, Crockford PW, Hodgskiss MSW, Wörndle S, Creaser RA, Rainbird RH, Skulski TM, et al. (2017). Precise age of *Bangiomorpha pubescens* dates the origin of eukaryotic photosynthesis. *Geology* 46, 135–138.
39. Javaux EJ (2020). The Early Eukaryotic Fossil Record. In *Eukaryotic Membranes and Cytoskeleton*, Jékely G, ed., pp. 1–19.
40. Strullu-Derrien C, Selosse M-A, Kenrick P, and Martin FM (2018). The origin and evolution of mycorrhizal symbioses: from palaeomycology to phylogenomics. *The New Phytologist* 220, 1012–1030. [PubMed: 29573278]

Figure 1.

Plants evolved in a microbial world, interactions with microbes may shape their adaptations to future changes. A) Simplified timeline of plant and microbial evolution based on [37–40]. Figure is intended as a general summary, literature values can vary by several hundred million years from those shown and are constantly being refined. Viridiplantae refers to green algae, from which land plants (embryophytes) evolved. The figure interprets mycorrhiza broadly as any symbiotic relationship between a plant and a fungus. Fossil evidence of plant-fungal interactions can be found as early as 407 million years ago. Mycorrhizal fungi are proposed to have been integral to plant evolution and colonization of terrestrial environments (reviewed in [40]) and are placed at ~500 million years ago, concurrent with land plant evolution. B) Continued warming (dashed red line) and concomitant soil aridity may alter existing rhizosphere interactions between fungi and bacteria, possibly affecting plant health, as discussed in section 1. Graph shows global temperature anomaly for years 1994–2018, compared to baseline period of 1951–1980. Black line is global annual mean, grey shading represents annual uncertainty at a 95% confidence interval, solid red line is the 5-year lowess smooth. Full dataset and all analysis are from the National Aeronautics and Space Administration, data as well as details on uncertainty and smoothing can be found at: <https://data.giss.nasa.gov/gistemp/graphs>. C) Potential future shortages in the supply of phosphate rock (dashed blue line) used for fertilizers [29] may require more sustainable agricultural practices for P management as discussed in section 2. Global phosphate rock production as reported by the United States Geological Survey. Data were extracted from reports spanning the years 1994–2018: <https://www.usgs.gov/centers/nmic/phosphate-rock-statistics-and-information>.

Figure 2.

Roles for RAMs in promoting agricultural productivity. A) Phenazine-1-carboxylic acid (PCA), one of the primary RAMs produced by soil bacteria. The acid-base and redox chemistry of PCA are dictated by the chemical microenvironment and may affect PCA toxicity. At lower pH, PCA is uncharged and hence more cell permeable and harmful. When oxygen levels are high, PCA can react with oxygen to produce toxic reactive oxygen species (ROS). B) Phenazines such as PCA can react with diverse oxidants, including oxygen and ferric iron (Fe^{3+}) minerals. The reaction between oxygen and reduced phenazines produces toxic ROS such as O_2^- . In contrast, the reaction between ferric iron minerals and reduced phenazines produces ferrous iron (Fe^{2+}) and may also help solubilize mineral-adsorbed phosphorus (P, usually in the form of phosphate), through the process of reductive dissolution. The amount of oxygen in the environment dictates which of these reactions will proceed. Phenazine producing cells as well as non-producing microbial community members can re-reduce phenazines thus allowing these metabolites to be cycled. Phz_{ox} represents an oxidized phenazine, phz_{red} represents a reduced phenazine. C) Fungal organization in the rhizosphere may be partly determined by bacterially-produced RAMs. Both the concentration and redox state of RAMs (indicated by size and color, respectively of the orange/pink circle), will change with soil aridity. Left: Hypothetical RAM production and beneficial fungal colonization of a plant rhizosphere in modern conditions. Center:

Increased future soil aridity may favor RAM producing bacteria and increase the toxicity of RAMs due to higher oxygen penetration of the soil and concomitant ROS production, potentially excluding beneficial fungi. Right: Native or added helper bacterial partners (blue) that protect against RAMs may decrease RAM stress and allow for the establishment of beneficial fungi in drier soils. Note that a similar scenario involving pathogenic fungi is also possible, with the partner bacterium instead becoming an aid to plant pathogenesis rather than plant growth promotion.