Ecosystem consequences of introducing plant growth promoting rhizobacteria to managed systems and potential legacy effects

Summary
The rapidly growing industry of crop biostimulants leverages the application of plant growth promoting rhizobacteria (PGPR) to promote plant growth and health. However, introducing nonnative rhizobacteria may impact other aspects of ecosystem functioning and have legacy effects; these potential consequences are largely unexplored. Nontarget consequences of PGPR may include changes in resident microbiomes, nutrient cycling, pollinator services, functioning of other herbivores, disease suppression, and organic matter persistence. Importantly, we lack knowledge of whether these ecosystem effects may manifest in adjacent ecosystems. The introduced PGPR can leave a functional legacy whether they persist in the community or not. Legacy effects include shifts in resident microbiomes and their temporal dynamics, horizontal transfer of genes from the PGPR to resident taxa, and changes in resident functional groups and interaction networks. Ecosystem functions may be affected by legacies PGPR leave following niche construction, such as when PGPR alter soil pH that in turn alters biogeochemical cycling rates. Here, we highlight new research directions necessary to improve predictions of the effects of biostimulants containing PGPR on ecosystem functions and their legacies (Box 1).

Ecosystem consequences of PGPR introductions
Biostimulant PGPR can have direct or indirect effects on ecosystem function (Fig. 1). Nutrient cycling can be altered directly by PGPR as they solubilize macronutrients, stimulate root nutrient uptake, and increase foliar nutrient content. Species of Bacillus, Pseudomonas, Aspergillus, and Flavobacterium produce phosphatases or organic acids that solubilize phosphorus from inorganic sources (De Pascale et al., 2018). Other PGPR can solubilize potassium (Han & Lee, 2005) and iron (Colla et al., 2015). Further, PGPR can indirectly influence solubility of macronutrients through shifting resident microbial interactions and community structure to a new state which could cascade to alter overall nutrient cycling (Nassal et al., 2018). Nutrient cycling is also modified as PGPR interact with roots. Nutrient uptake from organic fertilizers by roots can be stimulated and nutrient leaching from soil can be reduced by PGPR (Paungfoo-Lonhienne et al., 2019). With increased nutrient availability in soil and translocation by roots, foliar chemistry can concomitantly shift toward nitrogen enrichment (Larimi et al., 2014). Understanding the extent to which other soil macronutrient and micronutrient availability can be directly or indirectly increased by PGPR or their interactions with the resident community will be important to harness the potential for PGPR used as biofertilizers.

Crop yields depend on nutrient availability as well as interactions with invertebrates. The impact of PGPR on pollinating invertebrates are largely unknown. Plant–microbe interactions can expand the growing season for a crop (Panke-Buisse et al., 2015), thus influencing the timing of resource availability for migrating communities and ecosystem functions to new states (Zheng et al., 2018), and therefore we urge future research in these critical knowledge gaps.

While PGPR effects on host plants have been long studied, there is a dearth in research on their influence on ecosystem functions or legacies left in the resident community. A keyword search in the Web of Science found 313 articles describing biostimulants containing PGPR published since 1998 (‘biostimulant’ AND (‘rhizobacteria’ OR ‘bacteria’ OR ‘microb*’), webofscience.com). Interest in this research topic has expanded in recent years. Before 2015, fewer than 10 articles per year were published on this topic; the count jumped to 50+ per year since 2019. Despite increased interest in PGPR, only 12 of the 313 articles included the search term ‘ecosystem’ and one article included ‘legacy’. Here, we highlight potential effects of PGPR species introductions that may extend beyond the host plant to ecosystem functions that may persist longer than the PGPR populations (Fig. 1). We propose new research directions necessary to improve predictions of the effects of biostimulants containing PGPR on ecosystem functions and their legacies.

Introduction
Introduced species affect ecosystems in unpredictable ways because they are a new variable in a complex ecological network of existing interactions. Like other introduced species, plant growth promoting rhizobacteria (PGPR) have unpredictable ecosystem consequences and legacy effects; these outcomes are a current knowledge gap. Commercially available crop biostimulants sometimes contain PGPR that directly or indirectly increase plant productivity or crop yield, stimulate nutrient uptake, improve nutrient cycling efficiency, reduce pathogen loads, and increase plant tolerance to abiotic stress (Brown & Saa, 2015; Deng et al., 2019). Beyond the host plant effects, biostimulant PGPR may irreversibly shift...
pollinators and birds that feed on fruits, seeds, and pollinating insects. We postulate that as plants gain greater access to nutrients, the quality and quantity of nectar and pollen may subsequently increase and possibly increase pollinator abundance. Alternatively, plants interacting with PGPR may release larger volumes of volatile organic compounds (VOCs) that attract pollinators (Liu & Brettell, 2019). PGPR can also mediate plant–herbivore interactions. PGPR release VOCs that reduce herbivory by invertebrates (Mohanty et al., 2021). Induced systemic plant defenses against herbivores are triggered by PGPR activating the jasmonic acid immune signaling pathway (Hol et al., 2013). One reason we lack information about biostimulant PGPR effects on pollinators is that much PGPR research is glasshouse-based. Conducting field-based pollinator studies with controlled PGPR inoculations will elucidate how these microbes may affect pollinators. In this way, plant–pollinator interactions and the ecosystem service of pollination could potentially be altered by the introduction of novel microbes.

Another ecosystem consequence that affects crop yield is plant disease suppression. Many PGPR in biostimulants are added to crops for the purposes of reducing pathogen loads (Pieterre et al., 2014). For example, the commercial biostimulant Companion® Liquid Biological Fungicide (Douglas Plant Health, Liberty, MO, USA) contains Bacillus velezensis GB03. Application is intended to reduce pathogens such as those causing blight (Phytophthora, Sclerotinia), root rot (Pythium), and wilt (Fusarium). The hypothesized mechanism is that B. velezensis induces a systemic immune response across a wide range of plant hosts. Another potential mechanism is that the PGPR increases anti-fungal indigenous taxa (Xiong et al., 2017). Predictions of plant productivity following PGPR application may improve if we work to understand how plant disease suppression is related to interactions between PGPR and resident communities.

Microbial interactions affect organic matter persistence. Biostimulant PGPR may have indirect effects on ecosystem functions such as the accumulation and decomposition of organic matter through their interactions with the resident microbiome (Hellequin et al., 2019), with plants, or their individual activity. It is not well understood how PGPR may interact with key indigenous decomposer fungi, thus potentially altering decomposition rates and the release of plant-available nutrients (Kyker-Snowman et al., 2020). Interactions with plant roots may also contribute to organic matter accumulation. Metabolites, VOCs, or auxins released by
PGPR can affect root architecture, growth, and exudate production (Grover et al., 2021). These PGPR–root interactions may scale up to increase organic matter persistence, but more research is needed. PGPR may also contribute to soil organic matter accumulation through exuding biosynthesized compounds, such as extracellular enzymes, which can be long-lived in soil and sorb to minerals to produce mineral-associated organic matter (MaOM; Cotrufo et al., 2013). The interactions between PGPR microbes, plants, and resident fungi may have implications for rates of soil organic matter accumulation and decomposition.

**Legacy effects of PGPR introductions**

Introduction of new microbes could lead to genetic changes in the inoculant or community, yet the genetic impact of PGPR introductions remains largely unexplored. Any location with a high microbial density, such as the rhizosphere, can support rapid introductions of new microbes. Some research into nitrogen and potassium root acquisition and soil leaching currently exists. More research is needed to identify mobile genetic elements that may be transferred from PGPR to resident bacteria and whether they have lasting effects for community function. Further认识 of the mechanisms or relevant scale is still lacking, especially under field conditions.

1. **How do PGPR affect macronutrient and micronutrient cycling beyond host-plant acquisition?** PGPR may have traits to solubilize and mobilize macronutrients and micronutrients. Some research into nitrogen and potassium root acquisition and soil leaching currently exists. More research is needed to identify mobile genetic elements that may be transferred from PGPR to resident bacteria and whether they have lasting effects for community function. Further investigation because complete understanding of the mechanisms or relevant scale is still lacking, especially under field conditions.

2. **Do PGPR spread to other plant hosts in the ecosystem?** Host specificity is low among biostimulant microbes due to their application across various crops. Thus, escape from crops to adjacent ecosystems has a high potential, although persistence may be negligible. Mitigating effects caused by the escape of stimulated microbes is an area of active research (Jack et al., 2020; Kim & Lee, 2020; Stirling & Silver, 2020; Ke et al., 2021), but the likelihood and frequency that PGPR escape occurs is uncertain.

3. **How do PGPR affect organic matter persistence?** PGPR may affect the persistence and activity of key players in the resident microbiome. These changes in microbiome–microbe interactions could cascade to changes in soil properties mediated by microbes such as pH, erodibility, porosity, and water holding capacity of soil. Experimental applications of PGPR under field conditions that quantify soil parameters are needed.

4. **What are the multi-trophic consequences of introduced PGPR?** PGPR are known to release volatile organic compounds (VOCs) that can deter herbivores. Some VOCs attract pollinators, but whether PGPR VOCs attract pollinators is unexplored. Indirect multitrophic interactions, mediated by changes in plant phenology such as budbreak or flowering, may be spurred by PGPR and this remains a current knowledge gap. Additionally, PGPR may indirectly affect resident microbial abundances through antagonisms with bacterivores or fungivores.

5. **Do PGPR spread to other plant hosts in the ecosystem?** How long do legacy effects of PGPR inoculants last? After the inoculant ceases to persist in the community, they may have lasting effects on community composition and function (Mallon et al., 2018). Longitudinal studies that extend longer than the PGPR can be detected in the system are needed to address this knowledge gap. Changes in overall community function have been detected for weeks after an inoculant ceases to be detectable. It is possible the effects could last months or to the following growing season if the PGPR has caused community function to shift to an alternative stable state.

6. **How long do legacy effects of PGPR inoculants last?** PGPR may also contribute to soil organic matter accumulation through exuding biosynthesized compounds, such as extracellular enzymes, which can be long-lived in soil and sorb to minerals to produce mineral-associated organic matter (MaOM; Cotrufo et al., 2013). The interactions between PGPR microbes, plants, and resident fungi may have implications for rates of soil organic matter accumulation and decomposition.

7. **Does PGPR gene transfer by PGPR lead to changes in overall ecosystem function?** Ecosystem functions like nutrient cycling are emergent properties of individual microbial functions. If PGPR act as mobile genetic element donors, they could affect the total genetic capacity of the microbial community for ecosystem processes. More studies that focus on mobile genetic elements of PGPR and the likelihood they are incorporated into the resident microbiome are needed. The predictability of horizontal gene transfer from PGPR to resident bacteria and whether it has lasting effects for community function warrants further experimentation.
Displacement is predicted to be more common than augmentation and the likelihood of either outcome seems unrelated to the number of cells introduced (i.e. propagule pressure; Kurkjian et al., 2021). In either case, the persistence of PGPR has the potential to alter community function as PGPR interact with and change abundance of resident taxa (Kalam et al., 2017).

Plant growth promoting rhizobacteria that persist following introduction can leave a legacy by altering their microenvironment to promote their own fitness, through a process known as ‘niche construction’ (Callahan et al., 2014; McNally & Brown, 2015). Microbes living in soil alter their microenvironment by changing resource availability, pH, and soil structure through their activity and interactions (Thakur & Wright, 2017). Introduction of a new PGPR strain runs the risk of triggering an environmental change via niche construction that can then lead to further changes in community composition (Suez et al., 2018) and potentially alter ecosystem functions.

Plant growth promoting rhizobacteria introductions that do not persist in the community may still leave legacy effects (Mallon et al., 2018; Kurkjian et al., 2021). Resident microbial species respond to introduced competitors by shifting their realized niche breadth, and the degree to which niche breadth shifts depends on diversity of the resident microbiome. Less diverse resident microbiomes respond to inoculants by shifting their niche breadth more than more diverse resident microbiomes (Mallon et al., 2018). Inoculants may have legacy effects if they alter the abundance of key functional groups, change trait distributions or interaction networks (da Costa et al., 2020), or act as a resource that can stimulate growth of functional groups (Wei et al., 2015; Kurkjian et al., 2021). Even transient inoculants have been shown to induce shifts in resident microbial communities by altering the abiotic environment or disrupting species interactions and abundances (Amor et al., 2020). Transient inoculants may alter the resident community diversity and niche structure resulting in long-lasting consequences for plant health and soil processes. The legacies of introduced PGPR have received less attention than measuring the targeted outcome of plant growth, but understanding their legacies are critical to predicting and mitigating potential long-lasting effects.

A call for further research

Introducing PGPR may have unintended effects that cascade throughout the resident microbiome, adjacent plant communities, and the whole ecosystem (Box 1). We urge more research to elucidate how introducing PGPR impacts resident community structure and function, ecosystem function within the area of application (e.g. cropland), and the adjacent ecosystems. More research is needed on trophic cascades that microbial introductions may cause, especially those that could affect pollinator communities or soil organic matter persistence and thus directly influence plant health and productivity. The persistence of introduced PGPR over time as well as their legacy effects remain largely unknown. Engineering PGPR to persist longer may increase their effects for plant growth or health and this synthetic biology research is in progress (Haskett et al., 2021). Legacy effects may persist despite the introduced PGPR not remaining in the community. Longitudinal studies of introduced PGPR that measure microbial and ecosystem functions are needed. Our ability to predict establishment success of PGPR and the consequences for the resident microbiome, other trophic levels, and overall ecosystem function remain knowledge gaps in the rapidly expanding field of applied plant and microbial ecology.

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Author contributions

JAMM wrote the initial draft of this manuscript and conceived original ideas. JAMM, PEA, JKM, WM and MAC contributed to idea refinement, writing, and revising the manuscript.

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Data availability

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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