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Review

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Defining the wheat microbiome: towards microbiome-facilitated crop production

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Abstract

Wheat is one of the world's most important crops, but its production relies heavily on agrochemical inputs which are notoriously harmful to the environment. It is well known that a multitude of microbes interact with eukaryotic organisms, including plants, and the sum of microbes and their functions associated with a given host is termed the microbiome. Plant-microbe interactions can be beneficial, neutral or harmful to the host plant. Over the last decade, with the development of next generation DNA sequencing technology, our understanding of the plant microbiome structure has dramatically increased. Considering that defining the wheat microbiome is key to leverage crop production in a sustainable way, here we describe how different factors drive microbiome assembly in wheat, including crop management, edaphic-environmental conditions and host selection. In addition, we highlight the benefits to take a multidisciplinary approach to define and explore the wheat core microbiome to generate solutions based on microbial (synthetic) communities or single inoculants. Advances in plant microbiome research will facilitate the development of microbial strategies to guarantee a sustainable intensification of crop production.

Keywords: wheat; rhizosphere; microbiome; sustainable intensification.

Declarations of interest: none.

1. Introduction – Wheat and agricultural intensification on a fast-growing world

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Wheat was one of the first domesticated crops, between 7,000 and 9,000 BC, and has undergone a process of expansion to global cultivation [1] (Bell, 1987). Bread wheat, *Triticum aestivum* L., is the most widely cultivated species, with more than 20,000 known varieties. It is one of the most important crops worldwide, occupying 17 percent of the total cultivated land in the world and providing the staple food for 35 percent of the world's population [2] (Laino et al. 2015). Between 10,000 and 4,000 years ago people began growing food, which led to the domestication of wild crops and the emergence of agriculture [3] (Taiz, 2013). Agricultural progress has supported population growth, which globally now is estimated to be 7.7 billion [4] (United Nations (UN), 2019). Wheat is a major world crop, but to meet the calorie requirement of an increasing world population, an 11% increase in wheat production is required by 2026 with just a 1.8% increase in cultivation area [5] (OECD/FAO, 2017). Furthermore, it is estimated that by 2050, population size will exceed 9.7 billion [4] (UN, 2019). A process of sustainable agricultural intensification must be implemented to make these crop productivity gains [6, 7] (Alexandratos and Bruinsma 2012; Davis et al. 2016) which will result in enhanced yield through increases in crop tolerance to biotic and abiotic stresses, improved nutrient use efficiency as well as the development of new bio-fertilizers [8, 9] (Dubey et al. 2020; Misra et al. 2020). It is well known that plants are colonized by microorganisms which can be beneficial to the host, and the potential of microbes to contribute to these sustainability goals has gained traction over the last years. A better understanding of patterns of microbiome assemblage is of fundamental importance as a prerequisite for the use of the microbiome in sustainable agriculture. In this review, we focus on factors driving the wheat microbiome assembly. Additionally, we highlight the gaps that need to be addressed towards a microbially-assisted sustainable intensification of wheat production. Finally, we briefly discuss the use of the microbiome as a source of microbial inoculants, through the application of synthetic communities (bioinoculants) and/or via optimization of agricultural practices to stimulate the beneficial indigenous microbial communities (biostimulation).

2. Factors affecting wheat microbiome structure and diversity

The advent of high throughput DNA sequencing technologies has facilitated amplicon sequencing-based research, metagenomics and metatranscriptomics to determine the composition and functions of microbial communities associated with different crops. This has allowed the

understanding of how different factors affect microbial communities associated with host plants in unprecedented detail in different niches in and around the host plant. Broadly speaking these can be divided into above-ground and below-ground niches. The phyllosphere [10] (Ruinen, 1956) refers to the above-ground parts of the plants, and most commonly to the leaves. The above-ground compartments comprise the leaves, stems (caulosphere) [11] (Compant et al. 2010), seeds and spikes or heads. In addition, we propose the term "*spicosphere*" as the niche comprised of wheat spikes, as it is an important reservoir for pathogenic and beneficial microorganisms living inside and on the surfaces of the rachis and spikelets (comprised of lemma, palea, glume, floret, awn and grain). Below-ground compartments can be divided into the rhizosphere [12] (Hiltner, 1904), the soil influenced by the host plant largely through root exudation, and the rhizoplane [13] (Clark, 1949), the surface of the root. In addition, microbes can reside within intercellular spaces (endosphere), either in above- or below-ground tissues as endophytes [14, 15] (Hallmann et al. 1997; Perotti, 1926) (Figure 1). Additionally, spermosphere is the term related to the dynamic zone surrounding germinating seeds [16, 17] (Nelson, 2004; Verona, 1958).

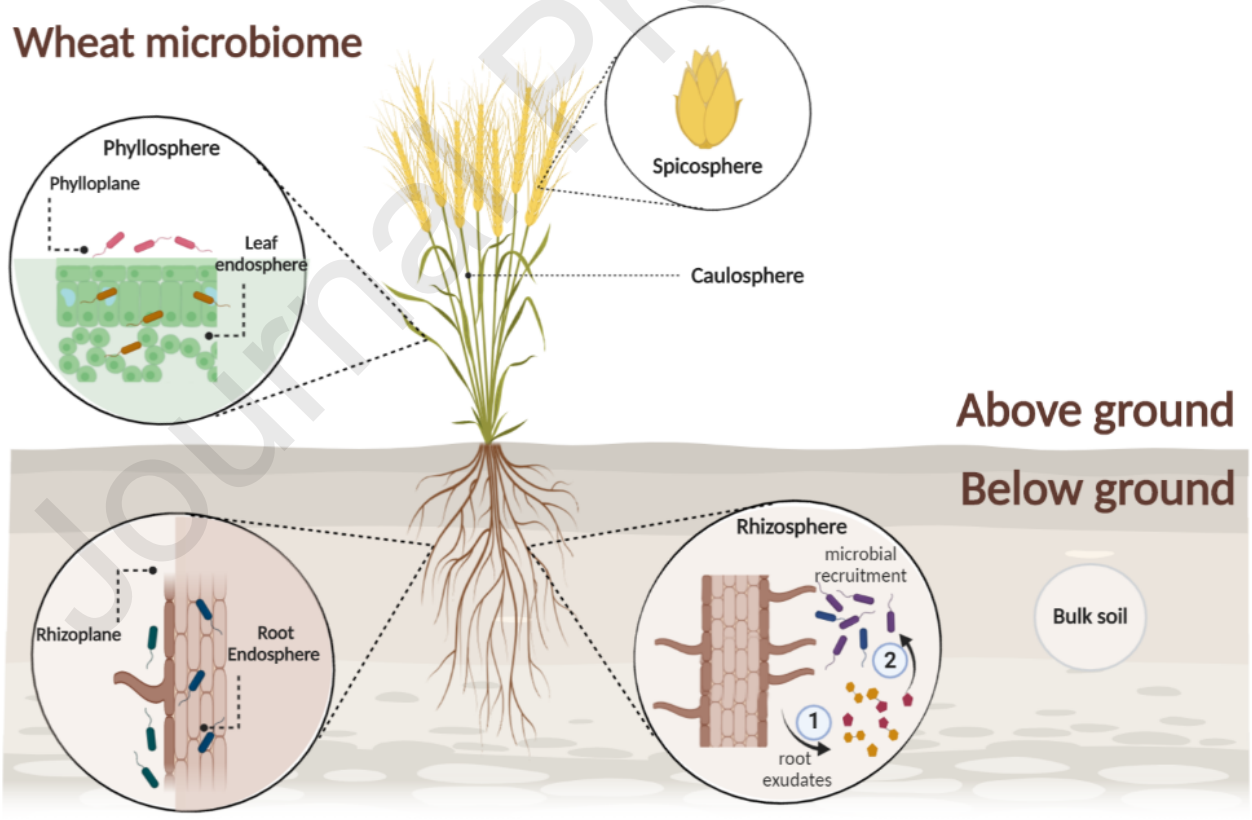

Figure 1. The wheat microbiome divided into above- and below-ground sections. The below-ground compartments are the rhizosphere and rhizoplane. The above-ground compartment is known as the phyllosphere, and subdivisions of this include the caulosphere and "*spicosphere*", with a detail of a spikelet. Created with BioRender.com

In addition to niche, many factors have been evaluated either alone or in combination to determine their influence on the wheat microbiome (Table 1). These include factors which are dependent on human interference (**anthropogenic**), soil-related factors (**edaphic**), **environmental**, which are related to natural conditions and **host** factors which are dependent on the plant species.

Table 1. Evaluation of factors to determine their influence on the wheat microbiome

Type	Factor	Reference
Anthropogenic	Exogenous compounds (fungicide)	[18, 19] Karlsson et al. (2014); Knorr et al. (2019)
	Exogenous compounds (glyphosate)	[20] Schlatter et al. (2017)
	Exogenous compounds (insecticides)	[21] Li et al. 2018
	Exogenous compounds (phosphine fumigation of stored wheat grains)	[22] Solanki et al. (2019)
	Exogenous compounds (plastic mulch film residues)	[23] Qi et al. (2020)
	Fertilization	[24, 25, 26, 27, 28, 29, 30, 31, 32, 33] Amadou et al. (2020); Chen et al. (2019); Illescas et al. (2020); Kavamura et al. (2018); Liu et al. (2020); Pagé et al. (2019); Robinson et al. (2016); Schmalenberger et al. (2009); Simonin et al. (2020); Yergeau et al. (2020)
	Inoculation of biocontrol agent	[26, 34, 35] Araujo et al. (2019; 2020); Illescas et al. (2020)
	Land use	[36-38] Kavamura et al. (2019); Rossmann et al. (2020); Schlatter et al. (2020a)

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	Management type	[39-41] Gdanetz; Trail (2017); Hartman et al. (2018); Ishaq et al. (2020)
	Overhead irrigation	[42] Mavrodi et al. (2018)
	Rotation	[43-48] Donn et al. (2014); Lupwayi et al. (1998); Mayer et al. (2019); Wen et al. (2016); Xiong et al. (2020); Yin et al. (2010)
	Tillage	[40, 41, 44, 48, 49] Hartman et al. (2018); Ishaq et al. (2020); Lupwayi et al. (1998); Yin et al. (2010); Yin et al. (2017)
Edaphic	Soil depth	[50, 51] Schlatter et al. (2020b); Uksa et al. (2017)
	Soil history	[52] Azarbad et al. (2020)
	Soil physicochemical characteristics	[24, 29, 50, 53-55] Amadou et al. (2020); Fan et al. (2017; 2018); Pagé et al. (2019); Schlatter et al. (2020b); Wolińska et al. (2020)
	Soil type	[32, 35, 56] Araujo et al. (2020); Schlatter et al. (2019); Simonin et al. (2020)
Environmental	Abiotic stresses (e.g. drought, humidity and temperature)	[42, 52, 57-60] Azarbad et al. (2020); Jochum et al. (2019); Latz et al. (2021); Mavrodi et al. (2018); Naylor et al. (2017); Stromberger et al. (2017)
	Biotic stresses (pathogens, weed)	[34, 35, 41, 61-66] Araujo et al. (2019; 2020); Hayden et al. 2018; Hu et al. (2019); Ishaq et al. (2020); Kerdraon et al. (2019); Rojas et al. (2020); Seybold et al. (2020); Yin et al. (2013)
	Geographical location	[32, 38, 43, 49, 53, 64, 67-70] Cordero et al. (2020); Donn et al. (2014); Fan et al. (2017); Latif et al. (2020); Mahoney et al. (2017); Rojas et al. (2020); Sapkota et al. (2017); Schlatter et al. (2020a); Simonin et al. (2020); Yin et al. (2017)
	Growing season	[38, 41, 56, 63, 67] Cordero et al. (2020); Ishaq et al. (2020); Kerdraon et al. (2019); Schlatter et al. (2019; 2020a)
Host	Breeding and domestication	[37, 71-76] Hassani et al. (2020); Kavamura et al. (2020); Kinnunen-Grubb et al. (2020); Rossmann et al. (2020); Sun et al. (2020); Tkacz et al. (2020); Valente et al. (2019)

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Genotype	[32, 33, 37, 43, 52, 55, 58, 60, 69, 70, 77, 78] Azarbad et al. (2020); Donn et al. (2014); Latz et al. (2021); Mahoney et al. (2017); Mauchline et al. (2015); Rossmann et al. (2020); Sapkota et al. (2017); Simonin et al. (2020); Stromberger et al. (2017); Wolińska et al. (2020); Yergeau et al. (2020); Zuo et al. (2014)
Growth stage	[25, 27, 30, 34, 35, 39, 43, 60, 64, 70] Araujo et al. (2019; 2020); Chen et al. (2019); Donn et al. (2014); Gdanetz; Trail (2017); Kavamura et al. (2018); Robinson et al. (2016); Rojas et al. (2020); Sapkota et al. (2017); Stromberger et al. (2017)
Leaf position	[70] Sapkota et al. (2017)
Niche	[26, 36, 38, 43, 44, 47, 49, 53, 54, 58, 67] Cordero et al. (2020); Donn et al. (2014); Fan et al. (2017; 2018); Illescas et al. (2020); Kavamura et al. (2019); Latz et al. (2021); Lupwayi et al. (1998); Schlatter et al. (2020a); Xiong et al. (2020); Yin et al. (2017)
Organs/Tissues	[24, 30, 35, 39, 58, 64, 79, 80] Amadou et al. (2020); Araujo et al. (2020); Gdanetz; Trail (2017); Huang et al. (2016); Kuźniar et al. (2020); Latz et al. (2021); Robinson et al. (2016); Rojas et al. (2020)
Plant hormones	[81, 82] Liu et al. (2017); Liu et al. (2018)

In the following sections, we focus on the different factors that affect the wheat microbiome structure, diversity and function. It is important to note that the factors discussed here are not exhaustive and exclusive, meaning there can be interactions of different factors accounting for changes in the wheat microbiome.

2.1. Anthropogenic factors driving microbiome assembly

2.1.1. Exogenous compounds

Current conventional agriculture relies heavily on the use of exogenous compounds which can be environmentally damaging as well as threatening to human health [83, 84] (Ansari et al. 2014; van Bruggen et al. 2018). These include the use of agrochemicals such as fertilizers, fungicides, insecticides and pesticides. However, research into the effect of the treatment of wheat

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seeds with neonicotinoid insecticides has revealed that they do not negatively impact wheat rhizosphere microbial communities [21] (Li et al. 2018). Similarly, the repeated pre-harvest application of glyphosate, the most widely used herbicide [85] (Malalgoda et al. 2020), had minimal impacts on soil and rhizosphere bacteria of wheat, with a small number of copiotrophic taxa benefiting from dying roots in the soil [20] (Schlatter et al. 2017). However, it's important to highlight that in-field applications of glyphosate can differ, thus in the later, the authors conducted a 3-year experiment in which glyphosate was applied at the end of six weeks, to simulate a pre-harvest application. Safer alternatives to these compounds could be the use of microbial-based natural products. The use of microorganisms as biological control agents is an environmentally benign alternative to pesticides [86] (Köhl et al. 2019), though a better understanding of these interactions is required to develop sustainable strategies to aid the establishment and persistence of beneficial microbes in agricultural systems. Besides, it is crucial to understand their impacts on indigenous soil microbial communities, given their role in the functioning of ecosystems. For example, Araujo et al. (2019; 2020) [34, 35] challenged soils infected with *Rhizoctonia solani and Pythium* sp. with biocontrol agents (*Paenibacillus fulvissimus* and *Streptomyces* spp.) to monitor changes in wheat microbial communities. Biocontrol isolates were able to modulate the endosphere and rhizosphere microbiomes, with generally low impact on indigenous microbial communities, as well as with a decrease in root disease and positive impacts on plant growth. The use of both low-density polyethylene (LDPE) and biodegradable plastic mulch films to increase crop productivity [23] (Qi et al. 2020) has been evaluated and the authors observed a significant effect of the residues on rhizosphere bacterial community composition and structure and volatiles emission, suggesting future efforts should concentrate at developing experiments to increase the understanding of these compounds on agroecosystems.

The impact of fertilizers on microbial communities is well studied. Application of high levels of inorganic nitrogen fertilizers reduced bacterial richness and diversity, leading to a less stable bacterial community structure, and this was exacerbated with increased crop maturity. Members of Acidobacteria and Planctomycetes were significantly depleted in treatments receiving inorganic N and 16S rRNA gene-predicted functional structure was also impacted [27] (Kavamura et al. 2018). In another study the use of organic amendments such as biochar and manure were compared to the use of mineral fertilization on above (spikelet) and belowground (rhizosphere and root) bacterial communities, with significant changes in their structure and diversity [24] (Amadou

et al. 2020). In addition, Chen et al. 2019 [25] found that nitrogen fertilization affected rhizosphere bacterial communities isolated from wheat plants during tillering but not during jointing and ripening.

2.1.2. Agricultural practices

Agricultural practices such as tillage and crop rotation can have detrimental effects on the environment, such as emissions of greenhouse gases (GHGs) [87] (Önder et al. 2011). No-tillage practices have been shown to reduced global warming potential when compared to conventional tillage [88] (Shakoor et al. 2021). The effect of tillage is stronger in the bulk soil than rhizosphere [49] (Yin et al. 2017). Similar findings were observed by Lupwayi et al. (1998) [44], in which the effect of tillage was more prominent in bulk soil than rhizosphere with significant decrease in bacterial diversity in the bulk soil.

Conventionally-tilled wheat monoculture and wheat-soybean rotation resulted in a lower bacterial diversity compared with the no-till treatment [48] (Yin et al. 2010). Hartman et al. 2018 [40] investigated the impact of common cropping practices (management type and tillage intensities) on bacterial and fungal communities in winter wheat. Root bacterial communities (rhizoplane or endosphere) were primarily affected by management type (conventional vs organic), whereas fungal communities were generally influenced by changes in tillage intensity.

Long-term monoculture can change soil properties, affecting bacterial diversity and this has been demonstrated by Mayer et al. (2019) [45]. Although they used maize monoculture, they were able to show that humus content was lower when compared to maize-wheat rotation, suggesting that lower concentrations of humus could decrease the amount of available nutrients for plant growth and decrease microbial richness. Some positive impacts of rotation of sunflower with wheat and maize on bacterial communities were observed, which could potentially alter plant productivity in agricultural systems [46] (Wen et al. 2016).

In a study conducted using samples from the Highfield experiment at the Rothamsted Research farm in Harpenden, Hertfordshire, UK [89] (Hirsch et al. 2017), conversion of grassland to an arable system resulted in a significant reduction in the abundance of OTUs assigned to specific bacterial taxa [36] (Kavamura et al. 2019). When comparing wheat grown in arable and

forest soil, Rossmann et al. (2020) [37] observed that the soil type had major impacts on bacterial and cercozoan rhizosphere communities and less influence on fungal community composition.

2.2. Edaphic conditions driving microbiome assembly

It is well known that differences in soil physical and chemical properties drive microbiome community structure in wheat. Amadou et al. (2020) [24] observed that the amendment of soil with biochar and manure as well as the addition of inorganic mineral fertilizers changed soil properties, in particular NH_4^+ content, and these impacted above (spikelet) and belowground (rhizosphere and root) bacterial community structure. Organic amendments can improve water retention and are associated with increased acid phosphatase, β-1,4-*N*-acetyl-glucosaminidase and phenol oxidase activity, whereas inorganic fertilizers lower the pH, increasing nutrient assimilability. Changes in chemical properties of rhizosphere soil, such as pH and nutrient availability which impact bacterial communities can also be attributed to root exudates [53] (Fan et al. 2017). Soil pH is the main driver of microbial community structure including archaeal, bacterial and fungal members [53, 54] (Fan et al. 2017; 2018). Soil texture has also been shown to be important in structuring microbial communities [56] (Schlatter et al. 2019).

Most soil microbial community structure studies have concentrated on the topsoil. However, [50] Schlatter et al. (2020b) and Uksa et al (2017) [51] have characterized the composition and diversity of bacterial communities across a wide range of soil depths. Both observed that Proteobacteriota are enriched in the topsoil, though the former also observed that Acidobacteria were more abundant at 10 cm, presumably because of soil acidification from fertilizer application. In addition, Uksa et al. (2017) [51] also observed that Firmicutes and Bacteroidota taxa were enriched in the subsoil.

2.3. Environmental factors driving microbiome assembly

2.3.1. Abiotic factors

In addition to soil properties, several abiotic factors can affect microbial communities. Latz et al. (2021) [58] observed location-dependent effects (in the glasshouse and outside the

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glasshouse) on wheat microbiome composition, which were likely a result from differences in the environmental conditions (temperature, humidity and precipitation). Water is one of the most limiting factors for plant development and agricultural losses due to drought are quite substantial. Azarbad et al. (2020) [52] investigated the influence of soil water stress history, wheat genotypes with differences in their drought tolerance, and short-term decrease in soil water content on microbial communities of wheat. Soil history, in this case, was soil from two fields which have been subjected to irrigation and no irrigation for almost 40 years. It was found that water regime was the main driver of bacterial and fungal community structure in the rhizosphere and root samples of wheat. Stromberger et al. (2017) [60] investigated the effect of different irrigation regimes on bacterial communities and observed an enrichment of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase bacteria in the rhizosphere of a drought tolerant cultivar, indicating that it either produces more ACC and ethylene or is more effective in recruiting ACC deaminase expressing bacteria into this niche. Mavrodi et al. (2018) [42] conducted a three-year field study on wheat grown in irrigated and non-irrigated plots to assess the effect of soil water status on bacterial communities. A decrease in the production of the antibiotic phenazine-1-carboxylic acid (PCA) and associated PCA producers (Phz+) *Pseudomonas* in the rhizosphere of irrigated plants was observed. They hypothesised that an increase in soil moisture perturbs interactions within the rhizosphere microbiome, altering the root exudation and soil properties.

2.3.2 Biotic factors

Biotic factors such as the presence of pathogens is another deterministic factor. Wheat residues can determine the epidemiology of Septoria tritici blotch as they support the growth of the causal fungal agent *Zymoseptoria tritici* [63] (Kerdraon et al. 2019). Their results show that pathogen infection dynamically changes bacterial and fungal interactions. In addition, it has become evident that soils inoculated with pathogens can become suppressive over time to specific pathogens [66] (Yin et al. 2013). Enrichment and activation of bespoke groups of microorganisms in soil can lead to microbial suppression of pathogens, however, the factors which contribute to the development of these systems are not yet fully understood [90, 91] (Chapelle et al. 2016; Raaijmakers and Mazzola, 2016). Yin et al (2013) [66] showed that *Chryseobacterium* and *Pseudomonas* became more prevalent in the rhizosphere over time after soil inoculation with

Rhizoctonia solani. These strains exhibited inhibitory activities against the fungus *in vitro* or reduced the infection in soils, indicating that they might play a role in the transition of conduciveness to suppressiveness. Hayden et al. (2018) [61] used a metatranscriptomics approach to characterize the active members and functions of the wheat rhizosphere microbiome in suppressive and conducive soil conditions to *Rhizoctonia solani.* They described the gene expression in the tri-trophic interaction and propose that this information can be used to direct management options to promote beneficial rhizosphere microbiota colonization and activity to reduce pathogen infection.

Similar to the gut microbiome, which is known to play an important role in host health [92] (Lamoureux et al. 2017), the microbiome of plants helps them tolerate biotic and abiotic stresses [93] (Vandenkoornhuyse et al. 2015). Thus, understanding the plant-microbiome interactions can be used to manage abiotic and/or biotic stresses. In addition, host defense mechanisms have an important role in structuring microbial communities [94, 95] (Jones et al. 2019; Teixeira et al. 2019). Teixeira et al. (2019) [95] proposed that the microbiome can protect the host against pathogens, directly via suppression with secondary metabolite production or through competition for resources; as well as indirectly, via the stimulation of the host's immune system. In other cases, pathogens have evolved mechanisms to overcome the immune defense. For example, the wheat pathogen *Zymoseptoria tritici* has been shown to induce systemic host susceptibility through altered plant metabolism and microbial community structure, making it more vulnerable to infection [65] (Seybold et al. 2020).

There are several other environmental factors that can contribute to differences in microbiome structure, diversity and function. Biogeographic studies aim to evaluate the distributions of soil microbial diversity, composition and functions over space and time from regional to global scales [96] (Chu et al. 2020). Fan et al. (2017) [53] studied nine wheat fields distributed across 800,000 km^2 to study the influence of geographical distance on bacterial communities from loosely and tightly bound rhizosphere soil, suggesting that geographic distance was the main driver of community distribution. Schlatter et al. (2020a) [38] explored bacterial and fungal communities of wheat grown in soil from four distinct locations, observing significant effects on the structure and composition of microbial communities which could be linked with differences in soil properties as previously discussed.

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Finally, seasonal changes can also account for differences in wheat microbiome. Schlatter et al. (2019) [56] observed significant effects of the growing season on bacterial and fungal community composition, however, richness and diversity were not affected.

2.4. Host microbiome selection

2.4.1. Niche, plant compartment and seed load

Niche plays an important role in shaping microbial communities. The root acts as a physical barrier and a subset of these bacteria can colonize the endosphere [36, 97] (Beckers et al. 2017; Kavamura et al. 2019). In addition to the bulk soil-derived microbial colonization of the plant host, the microbial seed load is also a source of microbes capable of colonizing the developing plant. Kavamura et al. (2019) [36] found using an embryo excision-based approach, that the seed-borne bacterial community was important for shaping the endosphere of wheat when plants were cultured in soil that was not adapted for wheat, whereas this was not the case for the rhizosphere community. In addition, Cordero et al. (2020) [67] demonstrated that when growing the same plant species on agricultural soils, variations between the endosphere and rhizosphere microbiome were observed, suggesting that the root microbiome is under a greater degree of host control. Specific phyla have been identified to be associated with different wheat compartments, with Proteobacteriota being the most abundant in the root endosphere, whereas Firmicutes and Actinobacteriota were more prevalent in the endosphere of leaves [30] (Robinson et al. 2016). To identify which factors contributed the most in shaping the fungal endosphere microbiome of different wheat compartments (roots, leaves and seeds), Latz et al. (2021) [58] analyzed ITS amplicon sequencing of wheat grown indoors and outdoors and concluded that environmental factors were more important for phyllosphere than rhizosphere and that airborne fungi are the main source of leaf and seed microbes. Donn et al. (2014) [43] performed a cross-year analysis of bacterial communities in an intensive wheat cropping system and observed changes over time in rhizosphere communities and those differences were not observed for bulk soil samples, suggesting they were plant instead of seasonally driven. In comparison to the bulk soil, rhizosphere microbial communities are less complex and more stable as demonstrated by co-occurrence networks [54] (Fan et al. 2018). In a more complete and recent study, Xiong et al. (2020) [47]

289 demonstrated the strong selection imposed by the host, showing a decrease in diversity and
290 complexity of bacterial communities from bulk soil > rhizosphere soil > rhizoplane > phylloplane
291 > root endosphere > leaf endosphere. Rhizosphere is the most studied niche, followed by the
292 phyllosphere. The microbiome of wheat spikes is less well documented; however, this niche is
293 important as some pathogens infect the spikes, such as *Fusarium graminearum* and *Magnaporthe*
294 *oryzae* pv. *Triticum* (MoT), causal agents of Fusarium head blight (FHB) and wheat blast,
295 respectively. However, it is known that bacterial diversity is lower in spikes than in the rhizosphere
296 [24] (Amadou et al. 2020). In addition, Rojas et al. (2020) [64] observed that when wheat is
297 infected by *Fusarium*, a shift in fungal endophytic community colonization dynamics occurs.
298 Furthermore, some genera (*Cladosporium, Itersonillia* and *Holtermanniella*) were found to
299 outcompete the pathogen, preventing the development of the disease. The bacterial endophytes of
300 wheat endosperm, germ, coleoptiles as well as roots and leaves were studied by Kuźniar et al.
301 (2020) [80]. They found several beneficial bacteria and *Pseudomonas* spp. was the only genus that
302 was detected in all samples. Vertical transmission of the wheat microbiome was assessed and taxa
303 belonging to *Erwinia,* Rhizobiales and fungal genus *Emericella* might be vertically transmitted
304 from seeds to sprouts [79] (Huang et al. 2016).
305
306 **2.4.2. Plant domestication, breeding and wheat genotype**
307
308 The introduction of reduced height (*Rht*) dwarfing genes into modern wheat cultivars
309 during the Green Revolution resulted in plants with increased yields when cultured with high
310 fertilization application, without productivity losses caused by lodging [98] (Hedden, 2003).
311 Consistent and continuing reductions in height with increases in yield were achieved worldwide
312 [99] (Law et al. 1978). Effectuated by breeding efforts, modern crops have diverged genetically
313 and phenotypically from their wild relatives. Selection for improved wheat varieties may have
314 resulted in changes to root architecture and physiology, which in turn might have affected
315 microbial communities [100, 101] (Bertin et al. 2003; Graaff et al. 2013). Wheat root-associated
316 microbiomes have dramatically changed through a transect of breeding history [73] (Kinnunen-
317 Grubb et al. 2020). Differential recruitment of bacterial communities in tall and semi-dwarf wheat
318 cultivars suggest breeding might have affected the ability of wheat to select and sustain a complex
319 bacterial community in the rhizosphere [72] (Kavamura et al. 2020), negatively impacting the

ability of modern plants to interact with plant growth-promoting rhizobacteria [76] (Valente et al. 2019). Similar findings were reported by Rossmann et al. (2020) [37], where the effect of wheat domestication on bacterial, fungal, and communities of cercozoa was evaluated. Both domestication and breeding affected network topology, with microbial co-occurrence networks from landraces and tall wheat cultivars being more connected, suggesting a reduced functional redundancy in the root microbiome of modern cultivars. Fungal endophyte communities in wild wheat are richer and more diverse than in cultivated wheat, representing a greater reservoir of potentially beneficial endophytes as a higher proportion of differentially abundant taxa was found [74] (Sun et al. 2020). The consequences of plant breeding for the associated microbiome are not yet fully understood, however, it has been proposed that domestication has disrupted selective processes in the assembly of the wheat microbiome [71] (Hassani et al. 2020). A synthetic hybrid hexaploid wheat was created to recapitulate the breeding history of wheat, suggesting that the D genome from *Ae. tauschii* (diploid) strongly select for Glomeromycetes and Nematoda. Besides, the ratio of eukaryotes to prokaryotes remains the same, likely due to a protective mechanism against soil-borne fungal diseases in wheat, which might be intrinsic to the wheat genome [75] (Tkacz et al. 2020).

The effect of different wheat genotypes has been thoroughly investigated [32, 33, 43, 52, 55, 58, 60, 69, 70, 77, 78] (Azarbad et al. 2020; Donn et al. 2014; Latz et al. 2021; Mahoney et al. 2017; Mauchline et al. 2015; Sapkota et al. 2017; Simonin et al. 2020; Stromberger et al. 2017; Wolińska et al. 2020; Yergeau et al. 2020; Zuo et al. 2014) and those differences could be attributed to the differential root exudate chemistry [60, 69, 78] (Mahoney et al. 2017; Stromberger et al. 2017; Zuo et al. 2014) and disease susceptibility [70, 77] (Mauchline et al. 2015; Sapkota et al. 2017). The use of genome-wide association studies (GWAS) will likely improve our understanding of the genetic basis of microbiome selection by host plants [58] (Latz et al. 2021).

2.4.3. Developmental stages

The plant microbiome structure dynamically changes over time from seed to the flowering stage. Donn et al. (2014) [43] demonstrated the evolution of bacterial communities within the rhizosphere, with an increased diversity with plant age and senescence. It appears that growth stage has a stronger influence on bacterial communities than on fungal community composition [25]

(Chen et al. 2019). Araujo et al. (2019) [34] observed that the diversity of bacterial genera increased over time, with some bacterial genera dominating the initial stages, such as *Agrobacterium*, *Bacillus*, *Flavobacterium*, *Rhizobium*, and *Rhodoplanes*, whereas other genera increased in the later stages, mainly *Actinoallomurus*, *Aminobacter* and *Mycobacterium*. Regarding fungal communities, *Alternaria*, *Fusarium*/*Gibberella*, and *Lewia* were common in the early stage and *Exophiala* at 12 weeks. The same trend in increased diversity over time was observed for endosphere communities. Gdanetz and Trail (2017) [39] observed an increase in both bacterial and fungal endosphere community diversity over time (vegetative, flowering and seed development stages) which could be explained by the ecological succession within the plant microbiome or a reflection of responses to metabolites produced by plant maturation. Sapkota et al. (2017) [70] studied the spatiotemporal variation in fungal communities within the wheat canopy at different growth stages, describing key fungal species in the phyllosphere and a general increase over time. However, Kavamura et al. (2018) [27] found that when comparing contrasting fertilization regimes, a reduction in bacterial richness was observed over time in the rhizosphere. It was also found that taxonomical diversity remained stable over time following high N application, although, a reduction was seen when N supply was suboptimal. In addition, Robinson et al. (2016) [30] when studying the root and leaf endosphere, a reduction in bacterial species richness with increased plant maturity regardless of fertilization regime was detected. As such, the relationship between microbial community composition and growth stage is complicated as it is influenced by many factors.

3. Core wheat bacterial communities

We have described the major drivers of microbiome structure in wheat. In addition, it is important to consider the core microbiome, members being consistent features of a dataset that are hypothesized to reflect underlying functional relationships with the host [102] (Shade and Stopnisek (2019). Different approaches have been used to determine the core microbiome of plants such as the use of a theoretical framework [103] (Toju et al. 2018), abundance-occupancy distribution [102] (Shade and Stopnisek, 2019), microbiome package in R [32, 104] (Lahti et al. 2017; Simonin et al. 2020), network analyses [105] (Cernava et al. 2019), DESeq2 [38] (Schlatter et al. 2020a), QIIME 2 [37, 106, 107] (Chopyk et al. 2020; Douglas et al. 2020; Rossmann et al.

2020). Although the term "core microbiome" has been widely used, there is disagreement surrounding its definition and to the method that should be deployed to define the core microbes which are associated with a given host [108] (Risely, 2020).

Attempts to define the core microbiome of wheat have utilized large datasets [38] (Schlatter et al. 2020a). One study identified a core microbiome of 30 bacterial, 24 fungal and 10 taxa assigned to protists by utilizing data from three wheat genotypes grown in eight contrasting soils from Europe and Africa [32] (Simonin et al. 2020). In another study, Rossmann et al. (2020) [37] identified 22 bacterial and 13 fungal taxa and 3 taxa assigned to protists corresponding to the core microbiome of modern wheat cultivars. However, only four bacterial genera (*Arthrobacter, Bradyrhizobium, Massilia* and *Nitrospira*), four fungal taxa (*Bionectria, Chaetomium, Exophiala* and *Fusarium*) and two protists (*Eocercomonas* and *Rhogostoma*) were common between the two studies (Figure 2, demonstrating that the determination of the core microbiome is challenging and that the most appropriate method to do this has not yet been identified. For example, networks have been used to identify keystones species of wheat [35, 69] (Araujo et al. 2020; Mahoney et al. 2017) and DESeq2 has been used as a tool to identify both the core and differentially abundant taxa within treatments [27, 36, 38, 42, 56, 72] (Kavamura et al. 2018; Kavamura et al. 2019; Kavamura et al. 2020; Schlatter et al. 2020a; Schlatter et al. 2019; Mavrodi et al. 2018) (Figure 2). No genus was found to be common among all these different studies. *Sphingomonas* was detected in 80% of the studies; *Bradyrhizobium* in 70%; *Massilia* and *Pseudomonas* in 60%; and *Arthrobacter, Chitinophaga, Flavobacterium, Mucilaginibacter, Pantoea, Pedobacter* and *Variovorax* in 50% of the studies. It is important to highlight that the list of genera observed in Figure 2 is not exhaustive, and the absence of other genera does not mean they are not present in those samples. It means that using the methods and tools available, these genera were found to be differentially abundant or were found to be keystone taxa when the different factors were considered.

With the definition of the core microbiome, it is possible to identify permanent community members as opposed to stochastic contributors for a given niche [109] (Berg et al. 2020). The recovery of representatives of such genera using culture-dependent methods and subsequent testing of their functional abilities both *in vitro* and *in planta* could be a strategy for the development of new inoculants. It follows that due to the phenomenon of functional redundancy, a true core microbiome based on taxonomy does not exist and that the core microbiome is a

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functional phenomenon, based on the presence of key genes which are not assessed in a taxonomical approach.

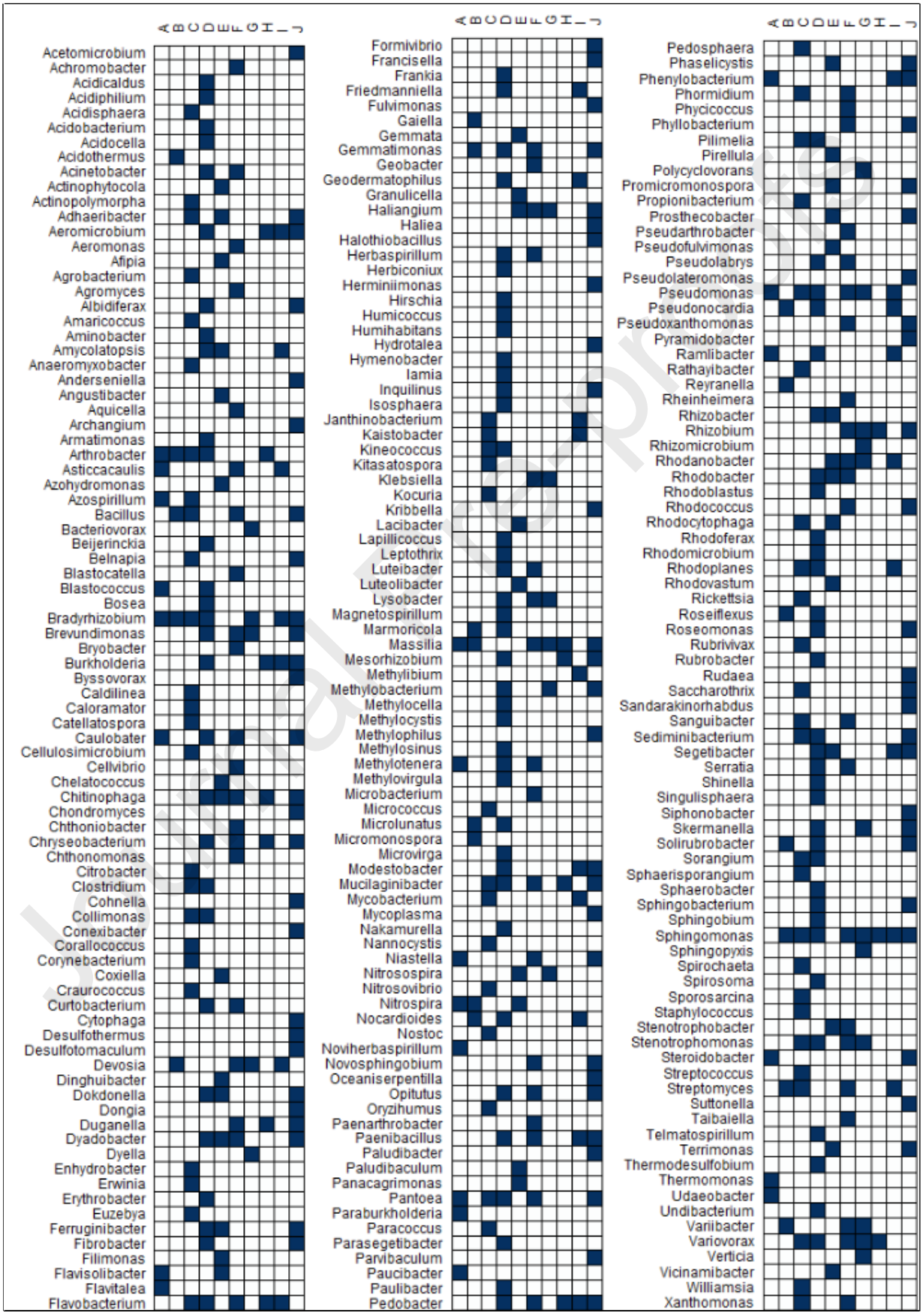

Figure 2. Correlation plot showing 256 bacterial genera commonly associated to wheat from ten studies (A-J) (A-Simonin et al. 2020 [32]; B- Rossmann et al. 2020 [37]; C- Araujo et al. 2020 [35]; D- Mahoney et al. 2017 [69]; E- Kavamura et al. 2018 [27]; F- Kavamura et al. 2019 [36]; G- Kavamura et al. 2020 [72]; H- Schlatter et al. 2020a [38]; I- Schlatter et al. 2019 [56]; J- Mavrodi et al. 2018 [42]). Studies A and B determined the core microbiome using R microbiome package and QIIME, respectively. Studies C and D used networks to identify keystone taxa. Studies E-J identified differentially abundant taxa using DESeq2.

3.1 Putative PGPR associated with wheat

Microbial communities in soil influence plant health, growth, and resource use efficiency, especially the subset that is selected by plants to form the root microbiome [110, 111] (Berendsen et al. 2012; Mendes et al. 2013). Bioprospecting microbes with plant growth-promoting (PGP) traits to increase productivity is a promising alternative to agrochemical application [112] (Nagargade et al. 2018). Plant growth-promoting rhizobacteria (PGPR) can influence plants through direct and indirect mechanisms [113] (Solano et al. 2008). Goswami et al. (2016) [114] define direct PGPR activity as any mechanism that directly enhances plant growth. Examples include phytohormone production such as abscisic acid, indole 3-acetic acid (IAA), gibberellin, cytokinin, and ethylene; nutrient (nitrogen, phosphorus, potassium and zinc) solubilization; nitrogen fixation, and siderophore production. Indirect mechanisms protect plants from infections and abiotic environmental stresses via the production of enzymes (cellulase, chitinase, protease), volatiles (ammonia, hydrogen cyanide), bioactive secondary metabolites, and osmolytes [115, 116] (Saraf et al. 2011; Tyc et al. 2017).

There is great potential for isolated bacteria to be used in improving wheat growth and many genera have been described in the literature as being capable of promoting plant growth. We searched the literature for specific PGP properties in bacterial genera commonly associated with wheat (Figure 2), with search results being displayed in Table 2.

Table 2. Bacterial genera frequently associated with wheat which have been found to demonstrate putative PGP functions

Phylum (Class)*	Genus	PGP

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		Function	Source
Actinobacteriota	*Aeromicrobium*	Phosphate solubilization, IAA and NH_3 production [117] (Yadav et al. 2014)	Cold desert [117] (Yadav et al. 2014)
	Arthrobacter	Phosphate solubilization, IAA, siderophore, NH_3 and GA production [117] (Yadav et al. 2014); Phosphate and zinc solubilization, IAA, siderophore, NH_3 and ACC production, nitrogen fixation and biocontrol of *Fusarium graminearum*, *Rhizoctonia solani* and *Macrophomina phaseolina* [118] (Verma et al. 2015); putative N_2 fixation [119] (Rilling et al. 2018)	Cold desert [117] (Yadav et al. 2014); wheat [118] (Verma et al. 2015); wheat rhizosphere[119] (Rilling et al. 2018)
	Streptomyces	Phosphate solubilization and siderophore, IAA and extracellular enzymes (chitinase, alkaline protease, phytase, cellulase) production [120] (Jog et al. 2012)	Wheat rhizosphere [120] (Jog et al. 2012)
Bacteroidota	*Chitinophaga*	Putative N_2 fixation [119] (Rilling et al. 2018)	Wheat rhizosphere and endosphere [119] (Rilling et al. 2018)
	Chryseobacterium	Phosphate, zinc and potassium solubilization, IAA, ACC, siderophore, NH_3, protease, cellulase and lipase production [121] (Gontia-Mishra et al. 2017)	Wheat rhizosphere [121] (Gontia-Mishra et al. 2017)
	Dyadobacter	Phosphate solubilization [122] (Zhang et al. 2012); nitrogen fixation [123] (Kumar et al. 2018)	Wheat rhizosphere [122] (Zhang et al. 2012); bulk soil [123] (Kumar et al. 2018)
	Flavobacterium	Phosphate and zinc solubilization, IAA, siderophore, HCN, NH_3 and ACC production [118] (Verma et al. 2015); Phosphate and zinc solubilization, IAA, ACC, siderophore and NH_3 production [121] (Gontia-Mishra et al. 2017)	Wheat [118] (Verma et al. 2015); wheat rhizosphere [121] (Gontia-Mishra et al. 2017)
	Mucilaginibacter	EPS production [124] (Han et al. 2012); IAA production [125] (Chimwamurombe et al. 2016)	Rhizoplane of Angelica sinensis [124] (Han et al. 2012); endosphere of *Tylosema esculentum* [125]

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			(Chimwamurombe et al. 2016)
	Segetibacter	Not available	Bulk soil from ginseng field [126] (An et al. 2007)
Firmicutes	*Bacillus*	Phosphate, potassium and zinc solubilization, IAA, siderophore, GA, HCN, NH_3 and ACC production, nitrogen fixation and biocontrol of *Fusarium graminearum*, *Rhizoctonia solani* and *Macrophomina phaseolina phaseolina* [118] (Verma et al. 2015); putative N_2 fixation [119] (Rilling et al. 2018); Zinc solubilization, IAA, ACC, NH_3, protease, and cellulase production [121] (Gontia-Mishra et al. 2017)	Wheat [118] (Verma et al. 2015], Wheat rhizosphere and endosphere [119] (Rilling et al. 2018); wheat rhizosphere [121] (Gontia-Mishra et al. 2017)
	Paenibacillus	Phosphate solubilization and NH_3 and IAA production [127] (Rana et al. 2011)	Wheat rhizosphere [127] (Rana et al. 2011)
Gemmatimonadota	*Gemmatimonas*	Not available	Anaerobic–aerobic sequential batch wastewater treatment reactor [128] (Zhang et al. 2003)
Myxococcota	*Haliangium*	Antifungal production [129] (Fudou et al. 2001)	Seaweed [129] (Fudou et al. 2001)
Proteobacteria (Alphaproteobacteria)	*Bradyrhizobium*	IAA production, protease and cellulolytic activity [130] (Masciarelli et al. 2014)	Seed endosphere of soybean [130] (Masciarelli et al. 2014)
	Brevundimonas	IAA, siderophore, GA and NH_3 production and biocontrol of *Fusarium graminearum*, *Rhizoctonia solani* and *Macrophomina phaseolina* [118] (Verma et al. 2015); NH3 and IAA production and phosphate solubilization [127] (Rana et al. 2011)	Wheat [118] (Verma et al. 2015); wheat rhizosphere [127] (Rana et al. 2011)

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	Caulobacter	IAA production and ARA [131] (Habibi et al. 2014); plant growth promotion [132] (Luo et al. 2019)	Rice endosphere [131] (Habibi et al. 2014); maize endosphere [132] (Luo et al. 2019)
	Devosia	Nitrogen fixation [133] (Rivas et al. 2002); biocontrol of *Fusarium graminearum* [134] (Sato et al. 2011)	Root nodules of *Neptunia natans* [133] (Rivas et al. 2002); wheat field soil [134] (Sato et al. 2011)
	Rhizobium	IAA, HCN and NH_3 production and heavy metal tolerance [135] (Singh and Lal 2016)	Wheat rhizosphere [135] (Singh and Lal 2016)
	Sphingomonas	Nitrogen fixation, phosphate solubilization, siderophore, IAA, and ACC deaminase production [136] (Correa-Galeote et al. 2018)	Maize endosphere [136] (Correa-Galeote et al. 2018)
Proteobacteria (Gammaproteobacteria)	*Burkholderia*	ACC deaminase and IAA production [137] (Shaharoona et al. 2007)	Wheat rhizosphere [137] (Shaharoona et al. 2007)

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Massilia	IAA, siderophore and protease production [125] (Chimwamurombe et al. 2016)	Endosphere of marama bean (*Tylosema esculentum*) [125] (Chimwamurombe et al. 2016)
Pantoea	Zinc solubilization, IAA, siderophore, GA, HCN, NH_3 and ACC production, nitrogen fixation and biocontrol of *Fusarium graminearum*, *Rhizoctonia solani* and *Macrophomina phaseolina* [118] (Verma et al. 2015)	Wheat [118] (Verma et al. 2015)
Pedobacter	IAA production [138 (Yuan et al. 2011)	Fertilized soil [138] (Yuan et al. 2011)
Pseudomonas	Phosphate and zinc solubilization, IAA, siderophore, GA, HCN, NH_3 and ACC production, nitrogen fixation and biocontrol of *Fusarium graminearum*, *Rhizoctonia solani* and *Macrophomina phaseolina* [118] (Verma et al. 2015); Phosphate, zinc and potassium solubilization, IAA, ACC, siderophore, NH_3, EPS, protease, and lipase production [121] (Gontia-Mishra et al. 2017); NH3, HCN and IAA production and antifungal activity against *Macrophomina phaseolina* [127] (Rana et al. 2011)	Wheat [118] (Verma et al. 2015); wheat rhizosphere [121] (Gontia-Mishra et al. 2017; Rana et al. 2011)
Rhodanobacter	IAA production, phosphate solubilization and antifungal activity against *Cylindrocarpon destructans* and *Fusarium solani*) [139] (Huo et al. 2020)	Ginseng rhizosphere [139] (Huo et al. 2020)

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Stenotrophomonas	Phosphate and potassium solubilization, IAA, siderophore, GA, HCN, NH_3 and ACC production, nitrogen fixation and biocontrol of *Fusarium graminearum*, *Rhizoctonia solani* and *Macrophomina phaseolina* [118] (Verma et al. 2015); Zinc solubilization, IAA, ACC, siderophore and NH_3 production [121] (Gontia-Mishra et al. 2017)	Wheat [118] (Verma et al. 2015); wheat rhizosphere [121] (Gontia-Mishra et al. 2017)
Variovorax	Inorganic phosphate solubilization [140] (Zheng et al. 2019); ACC deaminase, siderophore and IAA production and cadmium tolerance [141] (Belimov et al. 2005)	Bulk soil [140] (Zheng et al. 2019); indian mustard (*Brassica juncea*) rhizosphere [141] (Belimov et al. 2005)

*Taxonomy classification according to the Genome Taxonomy Database (GTDB) [142] (Parks et al. 2018).
ACC – 1-aminocyclopropane-1-carboxylate; ARA - acetylene reduction activity; EPS – exopolysaccharide; GA – gibberelic acid; HCN – hydrogen cyanide; IAA – indole 3-acetic acid; NH_3 – ammonia.

It should be noted that not all PGP functions described in Table 2 were observed in wheat. However, the fact that these bacteria are commonly associated with wheat does suggest that they could perform PGP activities in this crop. However, an important point is that the taxonomic affiliation of a bacterial isolate does not necessarily mean that it will perform a particular function. For example, *Rhizobium* spp. isolated in the UK are not able to fix nitrogen because they lack genes associated with this biosynthetic pathway [143] (Jones et al. 2016).

Another consideration for the use of PGP bacteria is their ease of culturability. Although Table 2 was based on PGP function in bacterial cultures, it should be noted that some genera are more difficult to culture than others. For example, *Segetibacter koreensis* has been isolated from soil from a ginseng field in South Korea [126] (An et al. 2007). Additionally, a *Gemmatimonas* strain was obtained from an anaerobic–aerobic sequential batch wastewater treatment reactor [128] (Zhang et al. 2003). Although widely spread in different environments, not many members of *Gemmatimonas* have been successfully cultivated [144] (Chee-Sanford et al. 2019). The genus *Haliangium* comprises myxobacteria with potential to produce bioactive secondary metabolites however, they are also hard to culture [145] (Mohr 2018). This highlights the need for improving and developing novel cultivation methods [146] (Busby et al. 2017).

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4. Gaps - How far are we from achieving a microbiome-facilitated sustainable agriculture?

The improvement of sequencing technologies has facilitated researchers to assess microbial communities in unprecedented detail. However, the deployment of microbes into agriculture has many challenges [147, 148] (Parnell et al. 2016; Sessitch et al. 2019). Some of these are related to the formulation of microbes, their susceptibility to stresses, and their ability to colonize different niches in the face of competition from indigenous microbes, as well as the in-field expression of the desirable function and warranty of their safety to native organisms and the environment. Sessitch et al. (2019) [148] highlighted that one of the main difficulties in moving towards field application is that trial screenings are performed in a way that does not mimic real conditions. Hu et al. (2019) [62] used a portable DNA sequencer to detect plant pathogens and analyze the microbiome of infected wheat. They suggest that a combination of on-site and centralized sequencing approaches would, in the future, revolutionize the management of agricultural biosecurity and reduce crop losses.

Other challenges, which will be explored in detail, in addition to improving the culturability of potential microbes, include combining different "omics" approaches towards a better understanding of the potential of microbiomes, the development of synthetic communities, and the identification of a global wheat core microbiome. These are important gaps that need to be addressed before microbiomes can be successfully and fully implemented in agriculture.

4.1 Multidisciplinary approach

It is well known that a great variety of microbes are associated with crop plants. Conventionally, this interaction has been studied with a culture-based approach, often with the inoculation of a single microbial species. A better understanding of patterns of microbiome assemblage and manipulation is of fundamental importance for microbiome utilization. However, as these sequencing approaches are correlative, there remains a dependency on culture-based techniques for the successful application of microbes to the environment. In addition, it is desirable to obtain a genome sequence of a microbe of interest, and this is best achieved from a pure culture of a given microbe, as opposed to the computational assembly from metagenomes, where it can be

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526 difficult to accurately associate core and accessory genetic elements to a particular genome. Until
527 recently only around 1% of bulk soil microbes and up to 10% of root-associated microbes were
528 amenable to culture. However, dilution-to-extinction [149] (Song et al. 2009), the development of
529 ichip [150] (Nichols et al. 2010), co-culturing, and other methods [151] (Stewart, 2012), have
530 improved culture-based recovery of the soil and root-associated microbiome dramatically, thus the
531 "1% culturability paradigm" needs to be revisited [152] (Martiny, 2019) and this is likely to
532 facilitate the isolation of new species with important functions to benefit the plant host. As
533 suggested by Schlaeppi and Bulgarelli (2015) [153], it might be useful to apply a combination of
534 both culture-independent methods with culture-dependent methods to enable the development of
535 inoculants towards a more reliable sustainable agriculture intensification. 16S rRNA gene and ITS
536 amplicon analysis, shotgun metagenomics or metatranscriptomics could be used to detect changes
537 in microbial communities, whereas cultivation techniques would be used to characterize the
538 physiological properties of microorganisms. Although cultivation-based techniques present some
539 limitations [36] (Kavamura et al. 2019), [154] Gutleben et al. (2018) suggest they are currently the
540 most reliable way to validate ecological hypotheses. The combination of different methods has
541 important implications for the field of microbial ecology [155] (VanInsberghe et al. 2013) and it
542 has been demonstrated by [156] Armanhi et al. (2018). The taxa identified in the previous section
543 could be used in the future for a targeted approach using culture-dependent methods coupled with
544 culture-independent methods to enable the characterization and isolation of promising
545 microorganisms for the development of synthetic communities (SynComs) will be further
546 discussed in Section 4.3.

547 Additionally, the functional screening of microbial isolates using traditional culture-based
548 methods focusing on the functions of single isolates are generally not high-throughput and have a
549 low resolution. To overcome this, next-generation physiology approaches on microbial ecology
550 studies to study the functions of microorganisms as communities in their native environment could
551 be applied [157] (Hatzenpichler et al. 2020). In addition, the culturability of "unculturable"
552 microbes must be improved either by developing new cultivation strategies or by refining the
553 existing ones.

554 Researchers should combine ecological studies, and database information on the
555 physiology and biochemistry of target isolates to efficiently uncover phylogenetically and
556 functionally new strains [158] Overmann et al. (2017). Data from amplicon and metagenomics

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sequencing are quite descriptive and should be combined with other "omics" data such as metatranscriptomics and metabolomics to obtain a holistic description of factors affecting the wheat microbiome. Additionally, as already discussed, culturomics [158] (Overmann et al. 2017) should be used to isolate potential microbial candidates, alongside with phenomics data [159] (Alcin-Albiac et al. 2020), where the metabolic and functional features of microbes are evaluated. Once isolates are obtained, single-cell genomics can be used for targeting genes of interest for classical genetics approaches, such as mutagenesis, deletion and complementation to prove the functional ability of the selected microbes. Finally, the effect of microbial inoculants on plants' performance can be verified through metaproteomics (host-level) or metabolomics in the rhizosphere (Figure 3). Understanding how plant's metabolites select different microbes is a field of research that has been receiving more attention. By identifying which root metabolites are responsible for the proliferation of specific microbes, root exudates can be purified or synthesized and used to increase the host's ability to recruit a beneficial microbiome [160] (Qiu et al. 2019). However, several bottlenecks have been identified by Reuben et al. (2008) [161], such as the cost and technical constraints to detect different metabolites, the absence of a well-curated database and chemoinformatics tools to enable analysis and interpretation of collected data. In the future, if limitations related to techniques, analyses, and integration with other mentioned "omics" sciences are overcome, incorporating metabolomics studies into microbiome studies would enable engineering of the native soil microbiome for increased plant growth and performance under bespoke conditions.

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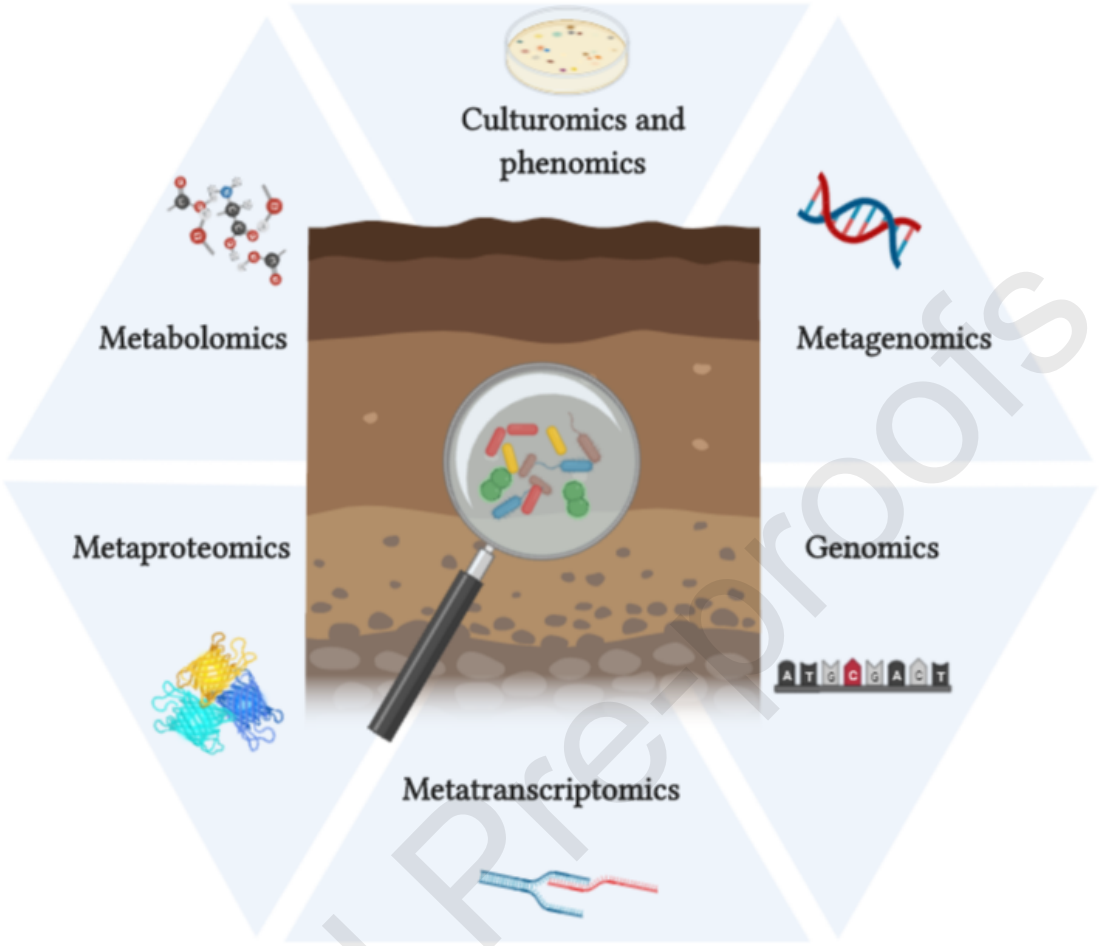

Figure 3. Proposed multidisciplinary framework for the successful use of microbiome in agriculture. Factors affecting the microbiome must be assessed through metagenomics (amplicon and shotgun), resulting in the description of the structure and diversity of microbial communities. Active microbial communities and genes should be assessed via metatranscriptomics. Additionally, culture-based methods should be used to recover isolates of interest (culturomics) and their functional and metabolic abilities evaluated by phenomics. Genomics can be used for targeting single cells or genes of interest using classical genetics approaches. And the effect of microbial inoculants on plant performance can be verified through metaproteomics (host-level) or metabolomics in the rhizosphere. Created with BioRender.com.

4.2. Identification of the real core microbiome

Describing the core microbiome of a healthy host would facilitate the design of synthetic microbial communities that are more likely to establish under specific conditions. However, translating our findings towards the development of new inoculants will require a further assessment of their culturability and functionality under desired conditions both in glasshouse and field trials. Additionally, future research should focus on a benchmarking of all publicly available wheat root microbiome datasets. This study would provide insights into the degree of microbial

functional redundancy in these systems and whether a taxonomically based global core wheat root microbiome exists, regardless of anthropogenic, edaphic, environmental and host-related factors.

4.3 Synthetic communities (SynComs) and the development of inoculants

The studies conducted on the wheat microbiome have highlighted which microbial communities are commonly associated with wheat and the factors responsible for the assembly of these communities. They might also offer hints to the identification of core representatives with possible plant growth-promoting traits, which could be used as inoculants or combined with other microbes into SynComs, which are artificially created by co-culturing two or more microbial strains in a specific medium [162] (Großkopf and Soyer 2014). Normally, they are designed for hypothesis testing and the selection of the members of these communities can be based on phylogeny, classification, networks or specific functions [163] (Vorholt et al. 2017), always taking into account the ecological interactions among the different taxa [162] (Großkopf and Soyer 2014). Microbial inoculants combine a native population of microbes with several kinds of compounds, such as plant hormones and growth regulators which are produced and released during fermentation [164] (Cassán et al. 2009). Ahemad and Khan (2011) [165] state that the exploitation of bacteria with multiple plant growth-promoting traits is beneficial, however, finding one bacterial strain with all desirable characteristics with the ability to colonize a variety of plant hosts and soil types is unlikely [166] (Kavamura et al. 2013), making the use of mixtures of microbes, also known as synthetic communities a good alternative. García-Jiménez et al. (2021) [167] point out there are important considerations when designing SynComs such as how the communities will be structured to ensure stability and the desired output. It is therefore essential to understand the compatibility among the different members of a given synthetic community so that when co-inoculated they benefit the host, are not antagonistic toward one another, and are resilient when challenged with biotic and/or abiotic stresses. Although several studies have demonstrated the potential of different microbes to improve plant performance under different conditions, others have shown microbial inoculants to give poor results. As such their successful deployment requires further methodological, technical, and theoretical advances before they can be considered as a reliable alternative to agrochemicals [160] (Qiu et al. 2019).

5. Summary and Outlook

Advances in the understanding of structure, diversity and functions of microbial communities associated with wheat and accompanying factors have been achieved in the last decades. We foresee great potential of microbiome manipulation for biostimulation of beneficial members of the indigenous microbiome to boost host performance under abiotic and biotic stresses. Identifying core microbiome function and the microbial genera responsible for these functions would reveal microbial targets for *in situ* manipulation. Alternatively, another approach would be the bioinoculation, addition of PGPR as microbial formulations (synthetic communities), however it is clear that a better understanding of bespoke conditions for successful establishment of inoculants is still required.

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AUTHOR STATEMENT

The authors state that the work described has not been published previously and that it is not under consideration for publication elsewhere.

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Declaration of interests

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☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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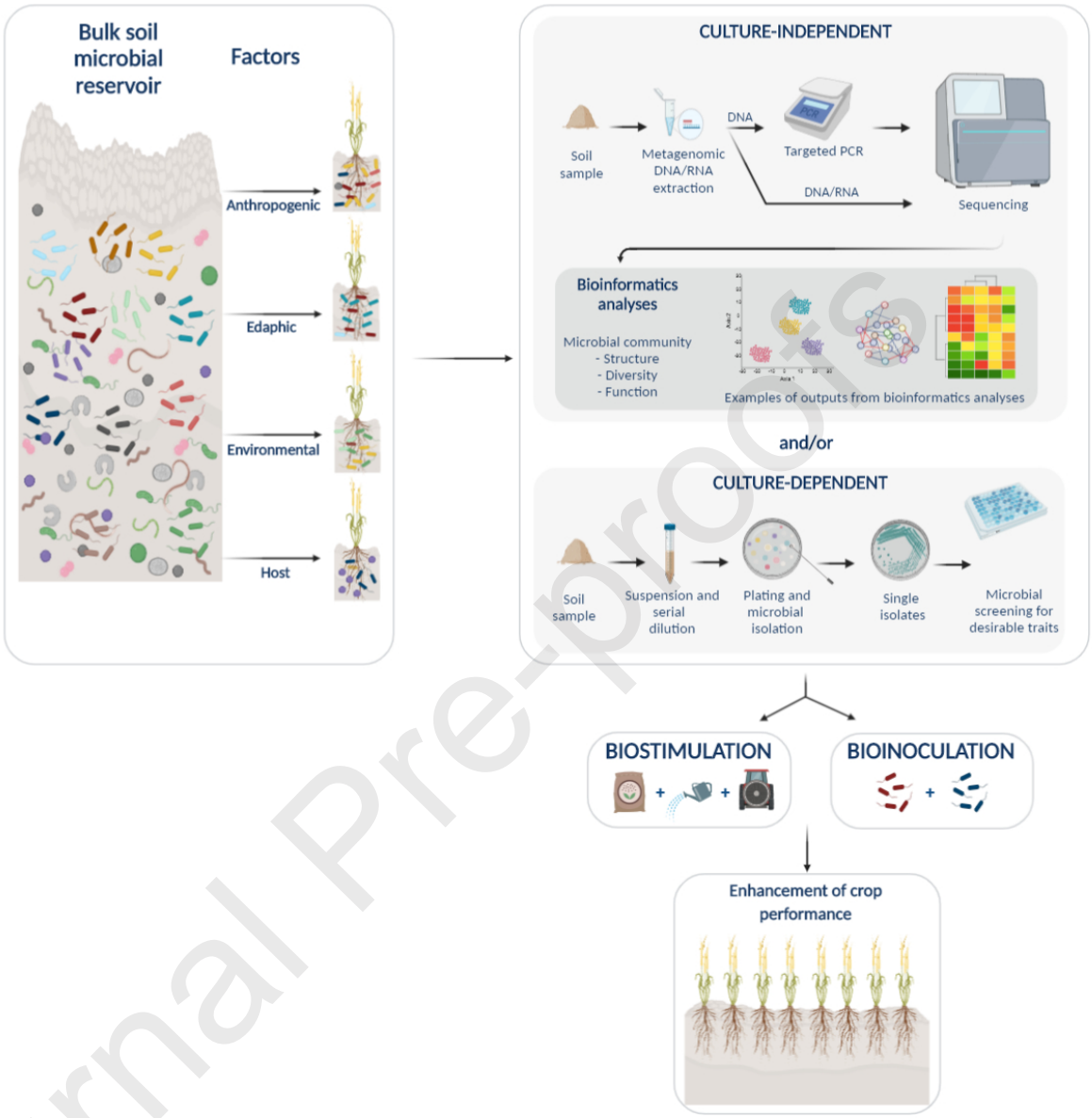

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