



The role of abiotic factors modulating the plant-microbe-soil interactions: toward sustainable agriculture. A review

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Abstract

Microbial soil communities are active players in the biogeochemical cycles, impacting soil fertility and interacting with aboveground organisms. Although soil microbial diversity has been studied in good detail, the factors that modulate its structure are still relatively unclear, especially the environmental factors. Several abiotic elements may play a key role in modulating the diversity of soil microbes, including those inhabiting the rhizosphere (known as the rhizosphere microbiome). This review summarizes relevant and recent studies that have investigated the abiotic factors at different scales, such as pH, temperature, soil type, and geographic and climatic conditions, that modulate the bulk soil and rhizosphere microbiome, as well as their indirect effects on plant health and development. The plant-microbiome interactions and potential benefits of plant growth-promoting rhizobacteria are also discussed. In the last part of this review, we highlight the impact of climate change on soil microorganisms via global temperature changes and increases in ultraviolet radiation and CO₂ production. Finally, we propose the need to understand the function of soil and rhizospheric ecosystems in greater detail, in order to effectively manipulate or engineer the rhizosphere microbiome to improve plant growth in agricultural production.

Additional key words: abiotic interactions; plant growth-promoting rhizobacteria; rhizosphere microbiome; soil.

Abbreviations used: DGGE (Denaturing Gradient Gel Electrophoresis); PGPR (Plant Growth-Promoting Rhizobacteria); UVR (Ultraviolet Radiation)

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Introduction

An ecosystem is made up of a community of living organisms interacting with each other and their environment, also known as the abiotic environment or environmental factors. However, gaining a detailed understanding of an ecosystem can be quite difficult due to the potential complexity of multiple and often multifactorial ecological interactions (Chapin *et al.*, 2011). Determining the effects of various components on an ecosystem, including both biotic and abiotic factors, is highly relevant to understanding how an ecosystem works as a whole. Indeed, studies on multi-trophic interactions and multi-communities have shown that the biota of the surface of the soil can affect the biota beneath soil (Wardle *et al.*, 2004).

The part of the soil that is influenced by the roots of plants is known as the rhizosphere (Hiltner, 1904). This micro-ecosystem is composed of a biota (mainly microorganisms) that inhabits the soil and can form complex communities that interact in beneficial, harmful, or neutral way with plants. Beneficial plant-microbe interactions are of particular interest, since we can take advantage of these functions in order to improve and promote the growth, development, and health of plants for several applications, including agricultural purposes (Santoyo *et al.*, 2012; Glick, 2014). Therefore, the microbial communities inhabiting the rhizosphere, also known as the rhizosphere microbiome, have been studied for decades and recognized as an important factor influencing the physiology and development of plants;

some have even gone so far to define the rhizosphere biome as a second plant genome (Berendsen *et al.*, 2012). Similarly, these microorganisms receive benefits from plants that select them and promote their development in several ways, either by increasing their survival under stress conditions, or by producing hormones that stimulate microbial growth and/or eliminate pathogens in the soil (Berendsen *et al.*, 2012; Santoyo *et al.*, 2012; Hernández-León *et al.*, 2015); this aspect of the interaction will be discussed in more detail below. Moreover, neither plants nor rhizosphere microorganisms can be considered separately from the abiotic components of the ecosystem.

Several studies have emphasized the importance of differentiating between the influence of abiotic and biotic factors on the generation and maintenance of microbial diversity in the rhizosphere. According to the theory of Bass-Becking who stated that “Everything is everywhere, but the environment selects” (Wit & Bouvier, 2006), many researchers have attempted to identify the specific factors that determine microbial biodiversity and their particular

influence on an ecosystem, using various theoretical and experimental approaches, both in the laboratory and the field.

From this ecological perspective, Wardle (2006) reviewed the influence of several sources of biotic origin on soil microbial diversity, including plant species, interactions between organisms within and beyond the rhizosphere, and animal and human activities. In the present review, we focus on the influence of abiotic factors on the diversity of soil microbes, especially those inhabiting the rhizosphere, that may be relevant to plant development, including type of soil, pH, temperature, and geographical and other environmental characteristics (Figure 1).

Moreover, increasing evidence demonstrates the impacts of climate change on the biota of the planet, and life in the soil is no exception. From this perspective, we propose various scenarios in which the rhizosphere microbiome can be used to improve plant growth, with particular focus on agricultural crops, to alleviate the negative consequences of climate change on agricultural productivity.

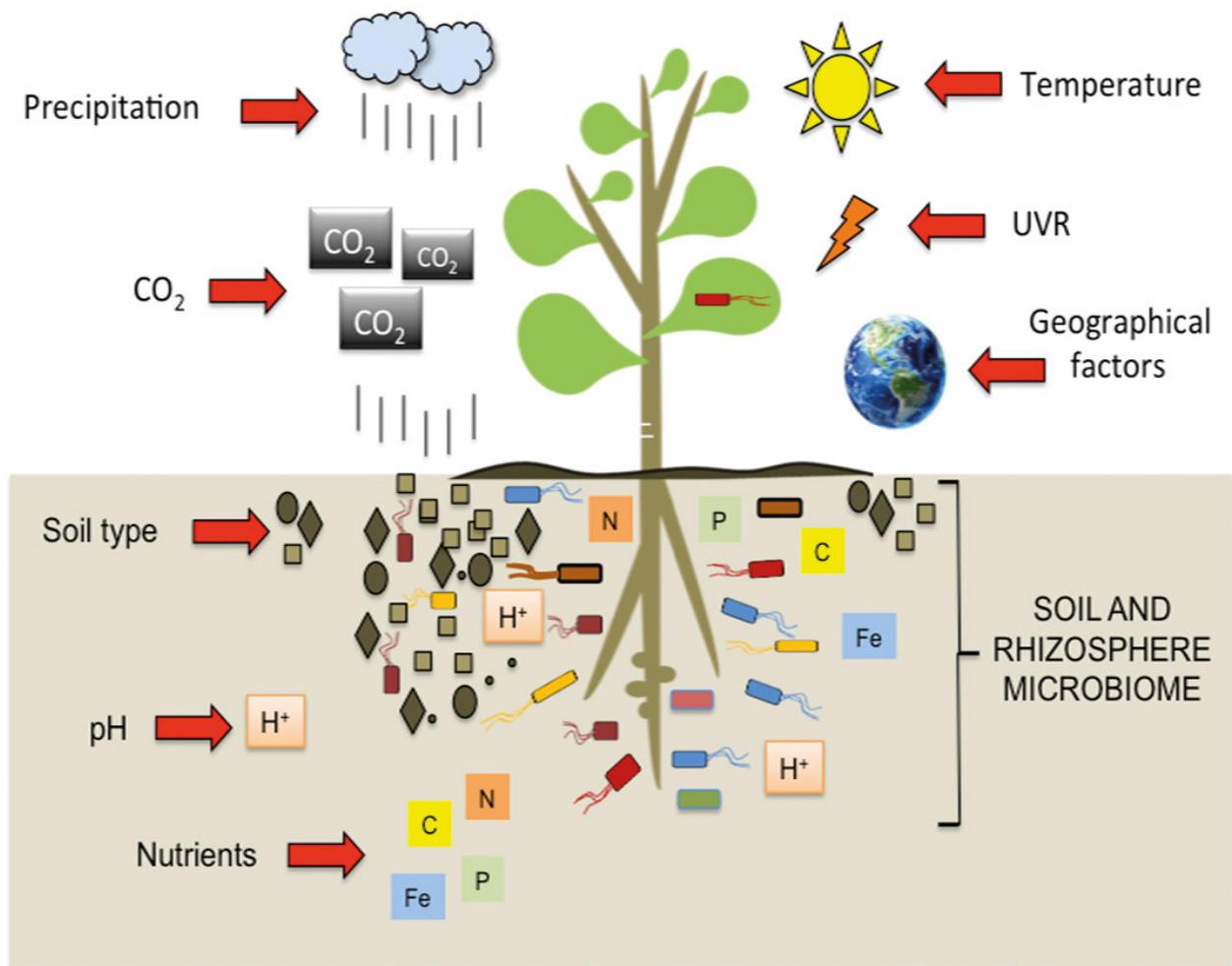


Figure 1. Abiotic factors modulating the soil and rhizosphere microbiome. See text for details.

Plant-microbiome beneficial interactions and biotechnology potential

For more than a century, the rhizosphere has been recognized as a microenvironment where microorganisms inhabit and have a great influence on the development and health of plants. The rhizosphere is influenced by the exudates excreted from the plant roots, which are secondary metabolites that can have various functions in the rhizosphere. For example, exudates such as carbohydrates, organic acids, vitamins, or amino acids may be chemo-attractants to microorganisms, which can metabolize the nutrients and allow for population growth (Bertin *et al.*, 2003; Bais *et al.*, 2006). Other exudates such as phenolic derivatives, in particular flavonoids, specifically attract rhizobia bacteria, a heterogeneous group of bacteria that includes the genera *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, and *Allorhizobium* (Hernández-Salmerón *et al.*, 2013). In general, rhizobia can form symbiotic associations with legumes and fix atmospheric nitrogen to convert it into ammonium, thereby making nitrogen available for the plant (Biate *et al.*, 2015). Moreover, some exudates are part of the plant defence system, such as phenolic compounds and terpenoids, which have efficient antibacterial and antifungal capacity. In addition, some volatile compounds emitted by the root were recently identified as part of the plant defence system (Niederbacher *et al.*, 2015).

The rhizosphere is an ecosystem with particularly high microbial diversity and is thus a great source for discovering new taxa and genetic material with high biotechnological potential, including for agrotechnology (Escalante-Lozada *et al.*, 2004; Handelsman, 2004; Hernández-León *et al.*, 2010). The proliferation of beneficial microorganisms, particularly bacteria in the rhizosphere, is of particular interest for generating new bioinoculants or biopromoters that do not have negative impacts on the environment or human and animal health (Santoyo *et al.*, 2012; Owen *et al.*, 2015). It has been proposed that plants can interact with plant growth-promoting bacteria, better known as plant growth-promoting rhizobacteria (PGPR). PGPR-relevant genera include the nitrogen-fixing rhizobia *Bacillus*, *Pseudomonas*, *Arthrobacter*, *Erwinia*, *Serratia*, *Azotobacter*, *Azospirillum*, *Burkholderia*, *Caulobacter*, and *Chromobacterium* (Bhattacharyya & Jha, 2012; Santoyo *et al.*, 2012). Several studies have demonstrated the important contributions of PGPR to plants, implicating both direct and indirect mechanisms (Glick, 2012), including conferring resistance to different stresses such as drought (Rolli *et al.*, 2014), temperature (Alexandre & Oliveira, 2013), salinity

(Kang *et al.*, 2014), tolerance to heavy metals (Glick, 2014), and biocontrol pathogens (Martinez-Absalón *et al.*, 2014; Hernandez Leon *et al.*, 2015). Therefore, the rhizosphere microbiome can significantly influence the development, health, and survival of plants in unfavourable conditions; in this way, the rhizosphere biota can be considered as a type of intrinsic mechanism of plant survival, and has thus coevolved with plants.

The importance of the composition of microorganisms in the rhizosphere is well-established, as well as their influence on the specific types of ecological functions that can be performed to benefit plants. These interactions can either have a moderate influence or be crucial and indispensable for plant survival under particularly adverse or stressful situations. The rhizosphere microbiome may benefit the plant through various mechanisms, such as the promotion of plant growth (Lugtenberg & Kamilova, 2009), symbiosis (Gage, 2004; Koch *et al.*, 2010), provision of nutrients through nitrogen fixation (Van Rhijn & Vanderleyden, 1995; Raymond *et al.*, 2004), solubilization of phosphate (Vassilev *et al.*, 2006), soil contaminants removal (Kuiper *et al.*, 2004) and biocontrol of phytopathogens (Compant *et al.*, 2005; Zhuang *et al.*, 2007).

The rhizobial microbiome is also recognized as a potential source for the entry of bacteria into the plant roots (Marquez-Santacruz *et al.*, 2010). Bacteria with capacity to colonize and survive within the internal tissues of a plant are collectively known as bacterial endophytes. According to Kado (1992), endophytic bacteria inhabiting the interior of diverse plant tissues should not trigger any harm to the host plant. The mechanisms by which bacterial endophytes promote plant growth are similar to those of rhizospheric bacteria, and have been classified into direct and indirect mechanisms (see Glick, 2012). Some researchers have ranked the importance of bacterial endophytes beyond that of rhizospheric bacteria for plants, since they are in closest contact with the plant and may therefore be better able to exert their beneficial effects on plant health (Rashid *et al.*, 2012). Indeed, endophytic bacteria have been considered as a plant's internal microbiome (Gaiero *et al.*, 2013), and their potential for improving plant growth and other biotechnological applications such as phytoremediation have been widely recognized (Newman & Reynolds, 2005; Gaiero *et al.*, 2013; Brader *et al.*, 2014).

However, research into the factors responsible for the selection and structure of the rhizosphere microbiome is still in the early phases, and thus forms the focus of this review to highlight the work carried out to date, potential of these findings, and remaining questions to be tackled. Other excellent reviews are available for the

reader that describe the detailed mechanisms or potential of PGPR for reducing environmental stresses or other more general areas such as agriculture (Bhattacharyya & Jha, 2012; Santoyo *et al.*, 2012; Glick, 2014).

Abiotic factors affecting the soil and rhizosphere microbiome

The soil and rhizosphere ecosystems are affected and modulated by several environmental factors. Since an ecosystem is composed of multiple interacting biological and non-biological elements, it is hard to classify them and study them as separate pieces. Indeed, in several cases, the soil or rhizosphere microbiome is affected by two or more abiotic factors, thereby complicating the analysis of the specific effects of single factors (Liu *et al.*, 2000; Drenovsky *et al.*, 2004; Fierer & Jackson, 2006; Cleveland *et al.*, 2007; Roesch *et al.*, 2007; Beauregard *et al.*, 2010; Castro *et al.*, 2010; Rousk *et al.*, 2010; Das *et al.*, 2011; Andrew *et al.*, 2012; Stomeo *et al.*, 2012; Van Horn *et al.*, 2013). Nevertheless, we have tried to summarize these individual effects as much as possible, and have classified them according to our best criteria in the following sections. Table 1 resumes relevant works of diverse abiotic factors modulating the microbial diversity.

Structure and soil type

The soil is considered to be a complex environment, whose origin is derived from the mixture of minerals, gases, liquids, organic matter, and living organisms that sustain plant growth (Bronick & Lal, 2005). The main mineral constituents of the soil are sand, silt, and clay. The specific mineral composition defines the soil type, because the mineral content will influence the porosity and soil moisture; for example, fertile soil requires a porosity of 50%, in which half the soil is occupied by gas and the other half by liquids. These interfaces open up the possibility of forming gradients of nutrients, pH, and gases, leading to innumerable microenvironments that offer distinct ecological niches. The organisms living in the soil, including bacteria, fungi, and viruses (Hättenschwiler *et al.*, 2005), may have important ecological roles because of their participation in the cycle of nutrients through the degradation of organic matter and minerals, which in turn provides essential nutrients for plant growth (Uroz *et al.*, 2009).

It has been argued that the physiological effects of plants should be considered to have equal importance to any other abiotic factor for the soil microbiome, because of the consequent effects on the functions of the organisms living in the soil ecosystem (Verville *et*

al., 1998). The presence of a plant not only promotes the growth of soil microbial communities directly but also influences the abiotic properties that influence their growth indirectly (Singh *et al.*, 2009). Other studies have suggested that soil characteristics (Girvan *et al.*, 2003) and the geographic factors are the most important factors in shaping the structure of the soil microbial communities; however, soil microorganisms can also have a significant effect on the formation of soil aggregates (Tisdall, 1996; Bronick & Lal, 2005). In particular, soil moisture content shows the strongest impact on the microbial community structure among other factors, even greater than the effect of nutrients in the soil (Singh *et al.*, 2009). This has been documented in research conducted on soils under extreme environmental conditions such as in the tundra of the Canadian Arctic (Chu *et al.*, 2011), Antarctic soils (Yergeau *et al.*, 2007), and the Tibetan permafrost soil (Zhang *et al.*, 2013). In an analysis of soil bacteria diversity, Zhang *et al.* (2013) found that *Proteobacteria* was the dominant group and was significantly associated with the amount of soil moisture. Geyer *et al.* (2014) investigated the association between the type of soil and bacterial diversity in Polar desert soils, and also found that moisture content was closely related to the abundance of several bacterial genera.

In one particularly interesting study, the ability of microorganisms to move according to the amount of soil moisture was analysed (Bashan *et al.*, 1996). Mobility is important for bacteria to colonize other soil or rhizosphere spaces that are closest to the plant root exudates or nutrients. For example, the genus *Azospirillum* was found to travel a distance of 40–60 mm in 96 hours in a sandy soil with a moisture content of 16%; however, in soils with only 10% moisture, displacement was reduced to 20 mm over the same time period (Bashan *et al.*, 1996). These findings indicated that soil moisture is directly related to an organism's ability to colonize the rhizosphere. Precipitation is closely associated with the formation of the specific type and structure of soils, especially arid or semiarid soils. In this regard, Bachar *et al.* (2010) studied the effect of rainfall in arid and semi-arid soils, noting that the abundance of bacteria decreased with respect to precipitation; however, bacterial diversity was independent of the precipitation gradient.

Soil aggregates have also been shown to be an important element allowing for the selection or survival of certain microbial groups; for example, the division *Acidobacteria* is often found in soil macroaggregates but not soil microaggregates (Mummey *et al.*, 2006). Moreover, communities can also vary according to the size of the pore dwellings (Ruamps *et al.*, 2011), which influences carbon mineralization (Ruamps *et al.*, 2013).

Table 1. Relevant examples of abiotic factors modulating the rhizosphere microbiome in different places of study

Abiotic modulating factor	Plant rhizosphere/ type of soil	Microbiome profiling technique	Modulated or found taxa, general features	Place of study	Reference
K, C, Ca	McMurdo Dry Valleys soils	Denaturing Gradient Gel Electrophoresis (DGGE), Terminal Restriction Fragment Length Polymorphism (T-RFLP) and 16S rRNA gene clone library construction	<i>Proteobacteria</i> , <i>Actinobacteria</i> and <i>Firmicutes</i> were dominant in all horizons. <i>Acidobacteria</i> , <i>Actinobacteria</i> , <i>Bacteroidetes</i> and <i>Gammaproteobacteria</i> were mainly found in permafrost interface	Antarctica	Stomeo <i>et al.</i> , 2012
pH, C	Cacti rhizosphere (<i>Carnegiea gigantea</i> and <i>Pachycereus pringlei</i>) and bulk soil	Multiplexed pyrosequencing of the 16S rRNA genes	Family <i>Desulfurococcaceae</i> was correlated with carbon and several classes of the phylum <i>Acidobacteria</i> with pH	Sonoran desert, AZ, USA	Andrew <i>et al.</i> , 2012
Soil water content, C	Rice and tomato/ Yolo silt loam soil	Phospholipid Fatty Acid (PLFA) profiles	Species not detected. Soil water content and organic carbon availability are major determinants of the general microbial community composition	California, USA	Drenovsky <i>et al.</i> , 2004
DOM (Dissolved organic matter)	Tropical rain forest soil	Libraries of small-subunit ribosomal RNA genes (SSU rRNA)	<i>Gammaproteobacteria</i> and <i>Firmicutes</i> groups were increased while <i>Acidobacteria</i> were reduced	Costa Rica	Cleveland <i>et al.</i> , 2007
Agricultural practices disturbances (Intense grazing, seasonal drought and fire)	Desert grassland	Carbon substrate utilization patterns in Biolog plates. Soil enzyme activity.	Species not specified. Fire and summer drought reduced soil microbial substrate utilization and enzyme activities. Winter drought, increased soil microbial diversity and activity.	Chihuahua, Mexico	Liu <i>et al.</i> , 2000
Temperature	Acid mine drainage (AMD) biofilms	FISH and Tandem Mass Tag (TMT)-based proteomics	<i>Leptospirillum</i> group III decreased with increasing temperature	Richmond Mine, CA, USA	Mosier <i>et al.</i> , 2015
Temperature, atmospheric CO ₂ and precipitation	Captina silt loam soil	Ribosomal DNA quantitative PCR (qPCR)	The relative abundance of <i>Proteobacteria</i> was greater in the wet soil. <i>Acidobacteria</i> abundance was greater in dry treatments. Fungal abundance increased in warm treatments	National Ecological Research Park, Oak Ridge, TN, USA	Castro <i>et al.</i> , 2010
Type of soil	Maize, sugarcane and Morrow Plots /three agricultural and boreal forest soils	DNA pyrosequencing	The most abundant bacterial groups in all four soils were the <i>Bacteroidetes</i> , <i>Betaproteobacteria</i> and <i>Alphaproteobacteria</i> . Forest soil is a rich phylum but less diverse of Archaeal species compared to the three agricultural soils	Brazil, USA (Florida, Illinois) and Canada	Roesch <i>et al.</i> , 2007
pH	Typic Paleudalf soil	qPCR and bar-coded pyrosequencing	Relative abundance and diversity of bacteria were positively related to pH. The abundance of fungi was unaffected or weakly modulated by pH	Hoosfield acid strip (Rothamsted Research, UK)	Rousk <i>et al.</i> , 2010
Soil moisture, pH, electrical conductivity, soil organic matter, major nutrients and ions.	McMurdo Dry Valleys soils	Pyrosequencing of the 16S rRNA gene	<i>Acidobacteria</i> and <i>Actinobacteria</i> were prevalent at the organic carbon rich, mesic and low elevation sites, while <i>Firmicutes</i> and <i>Proteobacteria</i> were dominant at the high elevation, low moisture and biomass sites	Taylor and Wright Valleys (Antarctica)	Van Horn <i>et al.</i> , 2013
pH	Multiple soil types	T-RFLP	Bacterial diversity was higher in neutral soils and lower in acidic soils, highly correlated with soil pH	North and South America	Fierer & Jackson, 2006
Moisture	Herbaceous species and pasture/Grassland	T-RFLP	Moisture had a comparatively higher impact on bacterial community, on fungal community soil N and C had a stronger effect	Scotland, UK	Singh <i>et al.</i> , 2009
Phosphorus fertilization	Alfalfa/ loamy clay soil	DGGE and PLFA	The application of fertilizer was associated with shifts in the composition of fungal and bacterial communities without affecting their richness	Saskatchewan, Canada	Beauregard <i>et al.</i> , 2010
CO ₂ and temperature	Rice/ tropical soil	Measurement of microbial biomass-C and soil enzyme activities	Elevated CO ₂ significantly increased the mean microbial biomass carbon (MBC) content and soil enzyme activities and temperature	India	Das <i>et al.</i> , 2011

For example, in forest soils, species richness was shown to be modified according to the soil horizon, which promotes an organic layer for bacteria and a mineral layer that is mostly inhabited by Archaea (Uroz *et al.*, 2013).

Furthermore, the effectiveness of bacterial communities in promoting plant growth has been investigated in different soils. Egamberdiyeva (2007) observed that genera, including *Pseudomonas*, *Bacillus*, and *Mycobacterium*, that are more efficient in stimulating the uptake of N, P, and K in corn plants were more likely to grow in nutrient-deficient soils compared to nutrient-rich soils. Moreover, apparent changes in the bacterial communities in the rhizosphere were observed in an experiment where microbial diversity was compared in three soil types with three types of plants using independent molecular techniques such as 16S ribosomal gene sequencing and denaturing gradient gel electrophoresis (DGGE) (Marschner *et al.*, 2004). Several factors were found to contribute to the species composition in the rhizosphere, but the plant roots, through excretion of exudates, showed a highly selective effect that was comparable to the influence of soil type (Marschner *et al.*, 2004). Therefore, although much has been discovered about the influence of the soil type as an abiotic modulating factor of the soil microbiome, more work is needed to gain a detailed picture of the relative effect of soil type on modulating the rhizosphere microbiome. The development of novel *in vitro* methods could help to unveil these gaps in knowledge, since an open system can be hard to control for single-factor evaluations. This type of research is currently lacking.

Soil pH

The pH indicates the concentration of hydronium ions $[H_3O]^+$ present in the soil (or another system), and therefore determines the acidity or alkalinity of the soil; thus, pH is a key factor in many soil science studies. Soil pH is also regarded as one of the main elements defining the structure of microbiome communities (Lauber *et al.*, 2009; Andrew *et al.*, 2012; Zhalnina *et al.*, 2014). Soil pH varies substantially from the regional to the global scale, and therefore can affect microbial communities, as soil microbes show a wide range of optimal pH tolerance. Several studies have focused on the effect of pH at different scales. For example, a continent-wide study clearly showed an association between soil pH and the presence of certain microbial communities (Fierer & Jackson, 2006; Lauber *et al.*, 2009), demonstrating that pH was the main factor responsible for this variation. A pioneering study in biogeography of soil microbial diversity at the

continental scale was carried out by Fierer & Jackson in 2006. The authors collected 98 soil samples from North and South America, and characterized and compared the bacterial community composition using the ribosomal DNA-fingerprinting method. Their results showed that bacterial diversity was unrelated to site temperature, latitude, and other variables, and the community composition was instead largely independent of geographic distance. Interestingly, the diversity and richness of the soil bacterial communities differed by ecosystem type, which the authors mainly attributed to differences in the soil pH. Similarly, other researchers have studied the effect of soil pH at the regional level and found an association of microbial diversity and soil pH and/or pH as a key modulating factor for variation in community composition (Rousk *et al.*, 2010).

Furthermore, pH was recently proposed to be the best predictor of microbial diversity at the phylum level (Geyer *et al.*, 2014), which is consistent with a recent study of the presence of *Acidobacteria* along an elevational gradient (Zhang *et al.*, 2014). *Acidobacteria* is one of the most dominant soil genera, which could reflect its metabolic plasticity. The authors selected four elevation gradients (from 1000 to 2800 m) of a mountain in central China. Interestingly, they observed a significant single-peak distribution pattern between the OTU number and elevation. Their Jaccard and Bray–Curtis index analyses showed that the *Acidobacteria* compositional similarity significantly decreased with an increase in elevation distance. Finally, the authors concluded that soil pH, soil temperature, and plant diversity may be the key factors shaping the soil *Acidobacteria* community.

Soil pH is directly related to the availability of nutrients for plants by controlling the chemical forms of the soil compounds. This has also been suggested to be an indirect limiting factor for microbial soil communities (Zhalnina *et al.*, 2014). Neutral soils generally harbour a greater microbial or bacterial diversity, while acidic soils tend to show lower diversity indices (Fierer & Jackson, 2006; Lauber *et al.*, 2009; Rousk *et al.*, 2010). Note that soil pH would only affect the survival of certain microbial species and is not a general factor for all species.

On the other hand, other studies have found no association between soil pH and the bacterial diversity of the ecosystem. For example, in a biogeographic study of the nitrogen-fixing rhizobacterium *Sinorhizobium meliloti* across several regions of Croatia, various abiotic factors were analysed, including the soil pH; however, only soil type and other geographical factors appeared to be responsible for shaping the genetic diversity of the 128 isolates analysed (Donnarumma *et al.*, 2014). Likewise, in a separate study conducted over

a mountain range in China from tropical rainforests to boreal coniferous forests across various altitudes, climates, and soils, pH was not always identified as the most important factor contributing to the composition and diversity of soil microbial communities, which were associated with multiple factors (*e.g.*, nutrient variability, temperature, altitude) that vary in each region (Angel *et al.*, 2010; Singh *et al.*, 2013). Therefore, based on the evidence collected to date, pH appears to be an important agent that can influence soil microbial diversity; however, this is not a generality.

Soil nutrients

The effect of nutrients in the soil and their impacts on plants, as well as agricultural production has been extensively studied in various regions worldwide (Ryan & Sommer, 2012). In agricultural soils, one of the major constraints to production is an infertile soil, determined by the three main nutrients: nitrogen (N), carbon (C), and phosphorus (P) (Reich & Oleksyn, 2004; Ryan & Sommer, 2012). Other nutritional factors such as iron can also affect the abundance of the rhizosphere microbiome, particularly bacteria (Yang & Crowley, 2000). Nutrient limitation problems are usually readily solved immediately with the application of chemical fertilizers, which have negative effects for the environment and for human and animal health (Geiger *et al.*, 2010). In addition, this is only a short-term solution to infertile soil and is not sustainable. The fertility of a soil is the product of complex biotic and abiotic interactions in which soil microorganisms play a major role in the decomposition of organic matter, generating available nutrients for plants. In turn, the improved plant growth allows for exploration of the roots for nutrient acquisition, thereby allowing the soil microbes to adhere to and inhabit the roots, and thus the rhizosphere. Therefore, soil nutrients and their bioavailability have both direct and indirect (through plants) effects on the diversity and abundance of the rhizosphere microbiome (Berendsen *et al.*, 2012). Furthermore, an imbalance in the proportions of nutrients in the soil can impact biodiversity in many ways through different processes that change the characteristics of terrestrial environments. Similarly, the function and land use can be affected by cultural practices that in turn affect the soil microbial community (Joergensen & Emmerling, 2006).

Nitrogen enrichment is a predominant factor in some soil types that can have substantial effects on both plant productivity and the composition of bacterial communities (Turner *et al.*, 1997; Clark *et al.*, 2007). In some cases, experimental enrichment of nitrogen was found to result in an increase in plant

productivity, but at the expense of lower plant and bacterial community species richness and diversity (Suding *et al.*, 2005). Levels of nitrogen enrichment in these experimental studies often exceed the current rates of the atmospheric deposition of N; however, even relatively low levels of chronic nitrogen enrichment are a threat to the conservation of different environments such as grasslands (Clark & Tilman, 2008).

Carbon is also one of the main determinants of the structure and function of microbial communities in the soil (Degens *et al.*, 2000; Drenovsky *et al.*, 2004; Ahmed *et al.*, 2008). Degens *et al.* (2000) used the microbial catabolic evenness as a measure of soil microbial diversity in soils with different organic C pools; for example, total organic C, microbial biomass C, and potentially mineralizable C. Their results showed that land use was significantly associated with microbial catabolic evenness, since certain practices deplete organic C stocks in soils, which may cause declines in the catabolic diversity of soil microbial communities.

Another important soil nutrient is phosphorus, which is also a modulating factor of the rhizosphere microbiome. For example, Beauregard *et al.* (2010) analysed the effect of P application for eight years on the soil microbial diversity of alfalfa monocultures, using the microbial diversity profile of phospholipid fatty acids and DGGE. Their results showed that application of P modified the structure of the communities of fungi and bacteria, but did not influence species richness. Coolon *et al.* (2013) reported that anthropogenic activities such as burning of grasslands could increase the availability of nutrients such as N or P, and analysed the effects of N and P enrichment in grasslands in North America on the structure of bacterial populations. To detect changes in diversity, they sequenced the V3 region of the 16S ribosomal gene. Their results showed that the enrichment of soil N, but P, significantly altered the bacterial community diversity, structure, and abundance of individual taxa. This study has important implications for the management of rangeland ecosystems and further highlighted the modulating effects of N and P as significant nutrient factors in natural ecosystems.

Geographical factors: Altitude, latitude, and longitude

There have been a breadth of studies conducted on the influence of geographical factors such as altitude (defined as the height of a point on the Earth's surface above sea level), latitude (the angular distance between the equator and a given point on Earth), and longitude (angular distance between a given point and the Greenwich meridian or prime meridian, which is

measured from 0° to 180° East or West), on species distributions and diversity; however, research on their influence on microorganisms is extremely scarce to non-existent. Recently, Van Horn *et al.* (2013) published a paper on the influence of certain abiotic factors (pH, sulphates, organic matter) that control the biodiversity of soils in Antarctica. The authors found that in sites rich in organic carbon, with a low elevation, members of the phyla *Actinobacteria* and *Acidobacteria* were frequent, whereas *Firmicutes* and *Proteobacteria* were dominant at sites of high elevation and low humidity. Of note, the microbial parameters were significantly related to soil water content and soil characteristics, including soil pH, organic matter, and sulphates. However, the magnitude and even the direction of these relationships varied among basins, and application of models showed evidence of significant contextual effects at the local and regional levels. The authors thus concluded that their study demonstrated the importance of geographical scale sampling to determine the specific geographical elements controlling the characteristics of the soil microbial community.

In this sense, it is widely accepted that microbial diversity is affected by multiple factors, since both abiotic and biotic elements form pieces of the same puzzle (*i.e.*, the ecosystem). For example, some studies have shown a pattern of decline, both with respect to the richness and diversity of bacteria along an elevation gradient; *i.e.*, lower species richness and diversity is observed at higher elevations (Kerkhoff *et al.*, 2008; Wang *et al.*, 2014). However, these studies also suggest that altitude, longitude, and latitude are not acting as unique modulating factors, but rather it is the combination of different abiotic elements such as atmospheric pressure, temperature, solar radiation, and the ultraviolet-B (UV-B) radiation fraction, that is responsible for this biogeographical phenomenon. However, this effect cannot be generalized for microorganisms. As one example, Fierer & Jackson (2006) provided evidence that bacterial diversity cannot be clearly defined in terms of latitude in analysis throughout the American continent; they concluded that although there was a trend of diversity change along latitude, this was not a significant determining factor of bacterial biodiversity. Other studies showed that the soil bacterial diversity in the Arctic was not fundamentally different from that found in other biomes (Chu *et al.*, 2010). Therefore, more large-scale studies on this topic are clearly needed to clarify these relationships.

Global climate change: effects of increased UV radiation, CO₂ and temperature

Climate change is defined as a dramatic and constant change in the global climate. This drastic

and fast change in climate could result in an increase in temperature, causing frequent droughts and atypical rains in several regions of the planet. Although the causes may be different, there is clear evidence that climate change affects life on earth and its biological processes. Heterotrophic microorganisms are not protected from these consequences, since they are part of biogeochemical processes such as C and N cycles.

Furthermore, climate change has great impacts on plant biology, which can lead to consequent changes in the associated rhizosphere microbiome. The interaction between the root exudates and rhizosphere microbiome is the result of a long co-evolutionary process (Badri & Vivanco, 2009). Recently, different chemical compositions of root exudates from *Arabidopsis* were found to be selected by various specific microbial communities (Badri *et al.*, 2013) that respond to environmental changes. Therefore, temporal changes in root exudates appear to show great potential to affect the microbial community in response to climate change.

Bardgett *et al.* (2013) documented the effect of elevated concentrations of CO₂ generated through root exudates of the plant on modulating the structure and function of the rhizospheric soil microbial community. Likewise, the presence and expression of functional genes in soil microbial communities have been shown to change in response to climate change disturbances. For example, bacteria with spore-forming ability are expected to predominant and withstand adverse conditions such as a drought season better than others; this mechanism allows these bacteria to survive for long periods of stress, but is not generally applicable for all species such as gram-negative bacteria (Drenovsky *et al.*, 2004), which hypothetically would not be able to survive as well in the face of drastic environmental changes over either the short or long term.

UV radiation (UVR)

Approximately 3.2% of the total solar energy is in the UV range from 290 to 320 nm (Cutchis, 1974). Photons of these wavelengths are sufficient to cause direct DNA damage, thereby exerting damage to organisms with high or prolonged UV exposure (Sundin & Jacobs, 1999). The main damage includes thymine and cytosine dimer formation and single- or double-stranded breaks, leading to mutations or loss of genetic information generated during the DNA repair processes, ultimately affecting cell viability (Santoyo & Romero, 2005).

The depletion of the stratospheric ozone layer, in part due to the accumulation of chlorofluorocarbons and accumulation of CO₂, has allowed for an increase in the flow of solar UV-B, in the range of 280–320 nm (Müller *et al.*, 1997). These effects are stronger in the

Polar regions (Caldwell *et al.*, 1982) where the ozone layer has thinned, and ecosystems are particularly sensitive to disturbance (Callaghan & Jonasson, 1995). Polar plant communities highly depend on the nutrient cycling carried out by soil microorganisms. In this sense, UVR is an abiotic factor that has direct effects on soil microorganisms (Formánek *et al.*, 2014). UV-B radiation has direct effects on soil microorganisms, including a change in pigment content, growth, and induction of carbon assimilation in amino acid synthesis (Sinha *et al.*, 1999).

The rhizosphere is a habitat primarily influenced by the carbon sources and nutrients released by plants; in response to stress, plants can alter metabolism of the roots, which can have clear consequences for the selection of different bacterial communities. This is because the rhizosphere microbial composition is largely influenced by plants, and therefore the composition of root exudates may serve as the main energy and carbon sources for fungi and bacteria, which have different requirements (Dohrmann & Tebbe, 2005).

The phyllosphere (plant surface) community is relatively more exposed to the effects of UVR (and other environmental factors) compared to the soil or rhizospheric community. The phyllosphere microbiome is therefore dominated by only a few taxa compared to communities in the root zone. This observed reduction of diversity in the phyllosphere community is attributed to the drastic and more frequent changes in the environment of the phyllosphere such as temperature, relative humidity, and solar radiation (Lynch, 1990; Dohrmann & Tebbe, 2005); thus, selection for tolerant taxa is stronger at this level.

Despite the fact that soil microbial communities are generally more abundant than phyllosphere communities, they are also more sensitive to environmental factors such as elevated levels of UV-B (Johnson *et al.*, 2002); however, some species, including those that are part of the rhizosphere microbiome, differ in their sensitivity to UV-B radiation-induced damage (Arrage *et al.*, 1993). There appear to be diverse mechanisms for UVR tolerance in bacterial species. For example, UVR tolerance in the plant pathogenic bacteria *Pseudomonas syringae* is conferred by the plasmid encoding the *ruLAB* operon involved in DNA repair (Cazorla *et al.*, 2008). The production of an extracellular polysaccharide that can absorb UVR has been implicated in conferring UVR tolerance *Xanthomonas campestris* (Hugenholz *et al.*, 1998). The pigmentation of phyllospheric bacteria is another reported UV protection mechanism, specifically for exposure to UV-A radiation (320–400 nm). Most isolates of the bacterial phyllosphere community have been shown to produce pigments, which suggests that UVR protection is conferred by pigments important

for survival in the phyllosphere. Indeed, the carotenoid compounds produced by *Erwinia herbicola* have been shown to play an important role in cellular protection against UV-A radiation (Whipps *et al.*, 2008).

Non-motile gram-positive bacteria isolated from Antarctic soil were found to be tolerant to UVR owing to a melanin synthesis-protective mechanism (Bhattacharyya & Jha, 2012). The domain Archaea is also resistant to UV-B (Thummes *et al.*, 2007), and there is a general trend of increased species diversity in environments with higher levels of UV-B exposure (Robson *et al.*, 2005). The gram-negative bacterial community in Arctic soils experiences particular environmental stresses and nutrient limitations (Rinnan *et al.*, 2005). Avery *et al.* (2003) conducted a study of the response of the rhizosphere microbial communities associated with populations of *Deschampsia antarctica* (a native vascular plant from Antarctica), which were exposed to UV-B, and found that radiation not only modified the overall growth of the plant but also the production of secondary metabolites. Furthermore, alterations in root metabolism may have an influence on the selection of different bacterial communities. Sundin & Jacobs (1999) found that most of the bacterial strains identified in the phyllosphere in *Arachis hypogaea* were gram-positive, with the genus *Bacillus* dominant, and showed good tolerance to UVR based on comparison to two control species, *Pseudomonas aeruginosa* and *Pseudomonas syringae*, which are UV-sensitive and UV-tolerant, respectively.

While light does not penetrate easily into the soil, particularly at greater depths; however, UV-B radiation induces changes in soil microbial communities and biomass, as well as alters the populations of small invertebrates, and these changes have important implications for the soil nutrient cycle (Caldwell *et al.*, 2007) given that rhizosphere microorganisms are needed to provide nutrients for plants and that microbial growth in turn depends on the plant root exudates. In summary, UVR exposure reduces the biomass of the roots resulting in less colonization of the microbial community, which in turn leads to low amounts of nutrients in the soil; thus, UVR has potential to disturb the soil community.

CO₂

Atmospheric CO₂ enrichment produces severe effects on terrestrial ecosystems and also interacts with the carbon cycle below ground. The main cause of these effects is the change in organic carbon dynamics. Previous studies have demonstrated reductions in pasture microbial decomposition rates after exposure to high concentrations of CO₂ (Van Ginkel *et al.*, 2000;

Hu *et al.*, 2001). Hu *et al.* (2001) also suggested that a high level of CO₂ would result in decreased amounts of available N for microorganisms due to the consequent improvement of plant growth, thus reducing the degradation ability of the microorganisms. These effects of elevated atmospheric CO₂ concentrations on the dynamics of soil organic matter lead to indirect effects on the soil structure. The response of plants to elevated atmospheric CO₂ concentration has been well studied across diverse systems. Atmospheric CO₂ is closely associated with C availability in the soil; therefore, the detailed effects of this abiotic factor on microbial soil communities are the same as those discussed above in the section soil nutrients.

Temperature

Global warming decreases the moisture content in the soil, which limits the ability of microbial organisms to disperse, survive, and colonize soil spaces (Carson *et al.*, 2010). Similarly, an increase in ambient temperature results in heating of the soil, which can modify the structure of the rhizosphere microbiome that established from interactions with a plant. Zogg *et al.* (1997) studied this effect *in vitro* by analysing the microbial communities through bacterial phospholipid profiles in soils subjected to prolonged changes in temperature from 5 to 25°C. Interestingly, both the kinetics of microbial respiration and the community structure varied across this wide range of temperatures. The authors suggested that changes in the composition of the microbial community following seasonal variations in soil temperature or smaller annual increases associated with global climate change have the potential to alter the decomposition of organic matter, which would in turn affect the bioavailability of carbon.

Mosier *et al.* (2015) recently investigated the effect of high temperatures on the expression of proteins using tandem mass tag technology-based proteomics in a microbial community located in an acidic mine drainage. Remarkably, a very clear correlation was observed between the different temperatures and the expression profile of proteins; in particular, those proteins involved in carbon use were repressed in two genotypes of *Leptospirillum*. Although this study was not conducted in a rhizosphere microbial community, it nonetheless demonstrates the importance of analyzing the effect of an abiotic factor such as temperature on community composition. Furthermore, this study indicates the value of adopting proteomic techniques to evaluate the expression pattern of proteins in other microbial communities with respect to the other main environmental parameters described herein.

Conclusions and perspectives: Toward sustainable agriculture

The rhizosphere microbiome consists largely of bacteria that benefit plant growth, which are better known as PGPR. Multiple mechanisms of plant growth promotion have been proposed based on PGPR, whether direct or indirect, including the production of siderophores, phytohormones, volatile compounds, or 1-aminocyclopropane-1-carboxylate deaminase, in addition to their capabilities of biocontrol and antagonism to plant pathogens (Santoyo *et al.*, 2012). Therefore, the potential of PGPR to the benefit of agricultural production has been amply demonstrated, but has not been sufficiently exploited and implemented, particularly in developing countries. Currently, pesticides, nitrogen fertilizers, and other chemicals are being overused and abused to enhance agricultural production. The long-term effect of fertilizers can produce positive effects on microbial soil and rhizosphere populations (Shi *et al.*, 2010), while other drastically opposite to their use in agriculture (Compant *et al.*, 2005). As the human population continues to grow, so will the demand for food. Currently, there are more than 7000 million people inhabiting our planet, and it is expected that the demand for food will double by 2050 (Tilman *et al.*, 2002).

It is therefore essential to understand the abiotic and biotic interactions to best exploit the rhizosphere microbiome to benefit agricultural production. Likewise, understanding of the continuous environmental changes and impacts of anthropogenic effects on the environment and climate must be taken into account for future agricultural practices.

Some authors have proposed the need to manipulate the rhizosphere by generating transgenic plants that modulate signalling between plant root exudates and the rhizosphere microorganisms (Ryan *et al.*, 2009; Chaparro *et al.*, 2012), since the roots exudates are important to attract certain microbial species in the rhizosphere. However, this approach would require the release of a genetically modified organism, which is currently restricted by law in several countries. Another interesting option would be the generation of a microbiome-based bioinoculant, perhaps by selecting dozens or hundreds of bacterial and/or fungal species with proven plant growth-promoting and biocontrol activities. This bioinoculant could be employed by direct application to agriculture crops (such as other bioinoculants based on single or a few mixed species that are already in use and commercialized), in order to improve soil fertility and, consequently, crop production. Such an approach would avoid the use of chemicals that harm the environment and human and animal health, or

genetically modified organisms, which are under a high level of scrutiny by some sectors of society and are thus tightly regulated. Finally, it is imperative to take the next steps as soon as possible to avoid food shortages, especially in the face of a changing climate. Toward this end, we need to take full advantage of the knowledge generated thus far and continue studying the abiotic factors affecting agriculture in order to effectively predict the consequences of environmental changes on productivity. In particular, the beneficial interactions between plants and the rhizosphere microbiome should be further exploited by conducting basic research with the aim of achieving more sustainable global agriculture.

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