Microbial Biostimulants

A microbial plant biostimulant, according to the Reg.UE 2019/1009, consists of a microorganism or a consortium of microorganisms able to stimulate plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: (a) nutrient use efficiency; (b) tolerance to abiotic stress; (c) quality traits; (d) availability of confined nutrients in soil or rhizosphere. The allowed microorganisms are listed in the CMC-7 (Component Material Categories, number 7), which includes four different genera: Azotobacter spp., Mycorrhizal fungi, Rhizobium spp., and Azospirillum spp.

1. Introduction

Soil fertility, as defined by the Food and Agriculture Organization (FAO), is "the ability of a soil to sustain plant growth by providing essential plant nutrients and favorable chemical, physical, and biological characteristics as a habitat for plant growth" [1]. In recent years, it was observed that global agricultural soil has become seriously degraded. In particular, about 40% of the world's agricultural soil and 24% of the globe's productive areas have been subjected to a loss of fertility, production capacity, and biodiversity. These phenomena are mainly due to several different factors, including water and wind erosion, salinity, loss of organic matter, and environmental pollution [2][3].

In the last several years, research has strongly focused on the use of agro-ecological principles to minimize potentially harmful chemical inputs and manage ecological relationships and agro-biodiversity [4]. Agro-ecology is based on the conservation of biodiversity, on the strengthening of biological processes, and on the looping of biogeochemical cycles. Fitting with the agroecological principles, is the use of biostimulants, products that are able to not only act directly on plants, but also sustain productivity through the selection and stimulation of beneficial soil microorganisms [5].

Considering the environmental damage associated with current fertilization practices, a research priority is to optimize plant–microbe nutritional interactions for more sustainable agricultural systems [6]. Several plant growth-promoting rhizobacteria (PGPRs) were demonstrated as able to exert a beneficial effect on plant growth under nutritional and abiotic stress or during the restoration of polluted soils. Moreover, plants could also establish symbiosis with arbuscular mycorrhizal fungi (AMFs), which increases the root surface area for nutrient acquisition [7]. The number of research studies related to the beneficial use of microorganisms is increasing at an exponential rate due to the new technologies, which allow for an accurate selection and understanding of the added value of microbial consortia. The European Biostimulant Industry Council (EBIC), founded in 2011, is attempting to encourage the innovation in this field by requesting operational solutions for their harmonized regulatory treatment on the EU market, including safety requirements and an update of CMC-7 in the new legislation.

2. Plant Growth Promoting Microorganisms (PGPMs) and Their Biostimulant Activity

On the other hand, PGPR is a very heterogeneous group of endophytic bacteria, which includes the phyla Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes [8]. Among the innumerable genera, Aeromonas, Arthrobacter, Azospirillum, Azotobacter, Bacillus, Clostridium, Enterobacter, Gluconacetobacter, Klebsiella, Pseudomonas, Rhizobium , and Serratia are the most studied, mainly due to their wide diffusion [9]. PGPRs are found in the rhizosphere and are able to promote plant growth, with recent scientific evidence showing the importance of their role in enhancing soil productivity and tolerance to abiotic stress in plants [10].

Indole-3-acetic acid (IAA) is the major endogenous auxin in plants, and is able to regulate several cell processes, including cell elongation and division, root development, and root hair formation [11]. In this context, the relationship of PGPMs with auxins, especially with IAA, is clearer. Although an increase in IAA was observed in plant tissues grown in soils characterized by a lack of water or an excess of salt, a greater increase was recorded in plants that were further colonized by AMFs [12][13][14][15]. Moreover, in the same plants, variations of root morphology and architecture were also observed [13]. This evidence was additionally proven by Liu et al. who studied the auxin pathway in plants grown under drought stress and inoculated with Funneliformis mosseae. These authors observed the activation of auxin-related genes (PtYUC3 and PtYUC8), the up-regulation of auxin influx carriers (PtABCB19 and PtLAX2), and the down-regulation of auxin efflux carrier genes (PtPIN1 and PtPIN3) [14].

This peculiar ability to produce and secrete compounds that can be useful to the plant is not exclusively limited to the biosynthesis of IAA. Indeed, some PGPR strains are also capable of producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase, an enzyme that catalyzes the conversion of ACC, the precursor of ethylene, to α-ketobutyrate and ammonia. Consequently, the secretion of ACC-deaminase causes the decrease of ethylene level in both plant and soil, with a concomitant reduction of environmental stress effects on plants [16]. Finally, when PGPRs are able to secrete both IAA and synthesize ACC-deaminase, a cross-talk effect occurs. Indeed, IAA stimulates plant growth, meanwhile ACC-deaminase decreases plant ethylene levels [17].

On the other hand, non-enzymatic antioxidants, such as polyphenols, organic acids, vitamins, carotenoids, and glutathione, are also involved in the interaction of PGPMs and plants, influencing their responses to oxidative stress. For example, it was previously highlighted that the accumulation of proline and glycine-betaine may be useful for preventing cellular oxidative damage, in both AMF [18][19] and PGPR [16][20][21] inoculated plants. An exception was reported by Moreno-Galván et al., who described conflicting data regarding proline in PGPR-plant tissues when compared with non-inoculated drought stressed plants [22]. The authors suggested that inoculation could trigger an early proline accumulation in plants, probably by reducing the need for a late accumulation and supporting part of the plant adaptation to drought stress [22]. Moreover, in accordance with Tigka and Ipsilantis, Moreno-Galván et al. also assumed that the plant developmental stage played a key role in the proline accumulation [22][23].

3. Synergistic/Additive Effects Between Microbial Biostimulant Product Components

Consequently, several studies investigated the combined use of PGPRs and AMFs as biostimulant agents (**Table 1**). The biostimulant effectiveness of a great variety of consortia, containing both plant aiding bacteria and AMFs, was tested with different plants and growing conditions.

Table 1. AMF and PGPR consortia and their additive/synergistic biostimulant effects on inoculated plants.

AMFs/PGPRs Consortium	Host Plant	Abiotic Stress	Additive/Synergistic Effect	REF
Rhizophagus irregularis, *Funneliformis mosseae*, *Pseudomonas fluorescens*	*Cupressus arizonica* Green	Drought	Enhancement of plant growth parameter; increase of APX and GPX enzymes activities; decrease of H_2O_2 and lipid peroxidation; and alleviation of water-deficit damage and improvement of drought tolerance	[24]
Arthrobacter protophormia, *Rhizobium leguminosarum*, *Glomus mosseae*	*Pisum sativum* L.	Salt	Improvement of plant weight; decrease of proline content and lipid peroxidation; increase of pigment content; enhancement of nutrient uptake; alleviation of salt stress; and enhancement of AMF colonization and nodulation	[25]
Claroideoglomusetunicatum, *Acaulospora* sp., *Rhizobium* sp., *Burkholderia* sp.	*Schizolobium parahyba* var. *amazonicum*	-	Improvement of absorption of chemical fertilizers; and enhancement of wood yield	[26]
Rhizophagus irregularis, *Bacillus megaterium*, *Frateuria aurantia*	*Triticum aestivum* L.	-	Improvement of low-mobile nutrient uptake (Ca and Zn) and enhancement of nutrient uptake responses; increase of plant growth; enhancement of total microbial biomass and microbial metabolism; and increase in gluten quality	[27]
Pantoea agglomerans, *Bacillus* sp., *Rhizophagus fasciculatus*, *Rhizophagus aggregatum*	*Casuarina obesa* Miq.	Salt	Increase of survival rate of plants compared to control plants; improvement of frequency of mycorrhization; enhancement of chlorophyll and proline content; and higher resistance to salinity	[28]

AMFs/PGPRs Consortium	Host Plant	Abiotic Stress	Additive/Synergistic Effect	REF
Mesorhizobium sp., *Burkholderia* sp., *Pseudomonas* sp., *Rhizophagus irregularis*, *Funneliformis geosporum*, *Claroideoglomus claroideum*	*Cicer arietinum* L.	Drought	Increase of grain yield; and enhancement of crude protein content	[29]
Klebsiella variicola, *Glomus multisubtensum*, *Rhizophagus intraradices*	*Helianthus tuberosus* L.	-	Enhancement of plant growth and increase of tuber inulin content	[30]

An enhancement of plant biomass and yield was detected in Arizona cypress, chickpea, wheat, swamp oak, Jerusalem artichoke, and pea plants when inoculated with PGPRs and AMFs, often exhibiting better results than non-inoculated plants or plants inoculated with a single strain [29][28][24][27][25][30].

The application of an AMF microbial-based biostimulant containing R. intraradices and seaweed extracts on tomato plants positively stimulated plant growth and yield in a different, but complementary manner [31]. In this experiment, when AMF was inoculated alone, the treatment enhanced leaf development and early flowering, but also caused a decrease in protein biosynthesis, carbohydrates, and lipids. On the contrary, the application of seaweed extracts alone enhanced root development and protein content. Their combined application showed an additive effect (in leaf and root growth, and protein and carbohydrate content), but also a synergistic effect on tomato plants, resulting in an earlier flowering and AMF colonization when compared to single treatments [31]. Similar effects were observed when PGPRs (Bacillus licheniformis , Bacillus megatherium , Azotobacter sp., Azospirillum sp., and Herbaspirillum sp.) were inoculated with water algae (Chlorella vulgaris). In this study, Kopta et al. showed that bacterial-algal preparation significantly affected fresh weight, carotenoids, and total antioxidant capacity of lettuce plants under heat stress conditions in comparison to untreated control plants [32].

An additional strategy to improve plant growth and abiotic stress tolerance could be the combination of the PGPMs with their liquid culture in order to enhance PGPM field performance and extend their shelf-life in the soil [33]. For example, the combined application of Bradyrhizobium living cells with their cell-free culture supernatant (CFCS) and metabolite exopolysaccharides (EPS) were evaluated on pigeon pea [33]. Bradyrhizobium is known for its nitrogen fixation ability, phytohormone and siderophore production, phosphate solubilization capacity and exopolysaccharide synthesis [34]. Although, these features make this bacterium a good performer in terms of plant growth promotion, the best results were obtained with the mixture of the three components. The sole application of CFCS played a significant role in growth promotion through benzimidazole antioxidant activity and nodulation. Moreover, the increase of ascorbic acid, pantoic acid, and benzoic acid was recorded, suggesting that the application of CFCS made stronger the symbiotic association between plants and PGPR. The authors hypothesized that the positive effects exerted by EPS could be due to its functions as a protective coat for the inoculated PGPR and as carbon source useful for the improvement of root colonization, biofilm, and nodule formation. Based on the characteristics of each component, the best performance, in terms of plant growth, was observed when Bradyrhizobium was inoculated with CFCS and EPS. In particular, this formulation better contributed to supporting the growth of indigenous soil rhizobia then the sole inoculum [33].

4. Microbial Biostimulants as a Solution to Limit Land Degradation and Unsustainable Agriculture

Among the main causes of biological soil degradation, soil organic carbon pool impoverishment [35], soil pH [2][36][37], monocropping [2], and adverse climate conditions are the most important [38][39]. Indeed, the decrease of soil pH determines the minor microbial nutrient availability and the reduction of soil biological activity, leading to a decrease in the more sensitive and rare species, and limiting plant growth [2][36][37]. On the other hand, continuous monocropping causes soil depletion, leading to a decrease of beneficial microorganisms, an impoverishment of the soil microbial community structure, and an increase in pathogen presence [2]. Finally, adverse climate change, including enhanced rainfall, significantly reduces the species richness of soil bacteria and fungi [38][39]. Furthermore, erosion and variable warming reduce the network complexity of soil microbiomes [39][40]. This implies the reduction of decomposition activity and nutrient cycling, as well as resource availability. These factors limit the microbial resilience to environmental stresses by causing long-term adverse effects on soil functions [40].

Microbial functional diversity largely influences important soil processes (e.g., production of NO 3, and fluxes of N 2O and CH 4), and the loss of soil microbial diversity results in a decline of specialized soil functions followed by a decline in the important consequences of terrestrial ecosystems [41]. The importance of a highly diversified microbial component was

confirmed by its key role in the C cycle and in the development of soil organic matter (SOM). In fact, the microbial community and microbial byproducts are a strong driver of SOM production and heterogeneity [42][43], contributing to form more than half of the organic carbon in soil through the production of microbial necromass [44][45].

In addition to the role of soil microbiota in the cycling of elements, and in the stabilization of soil structure, elevated soil microbial activity is indispensable for efficient crop production, the ability to maintain healthy plants, and ensuring a good yield under different environmental conditions. Global environmental changes can compromise both plant and soil biodiversity, suggesting a complex feedback between plants and microorganisms under stressed environmental conditions [46][47]. Several studies demonstrated that climate change decreased plant diversity and yield, and a more negative effect was observed under reduced soil biodiversity [46]. This happens independently from plant genotypes, indicating that the negative effect of soil biodiversity loss could generally come from soil microbes [46][48]. In support of this evidence, plant growth under high microbial diversity displayed higher productivity and greater recovery under stress conditions. Moreover, the yield losses were mitigated in the presence of elevated soil microbial communities, suggesting their potential and crucial role as yield stabilizers after global change disturbances [48].

Furthermore, evidence highlights the importance of the microbial component and their diversity not only for crop management, but also as a promising biological tool to recover degraded soils and implement revegetation activities [49][50]. For example, a bacterial consortium (Azospirillum spp., Azoarcus spp., and Azorhizobium spp.) and two AMF-PGPR consortia (Rhizophagus irregularis and Azotobacter vinelandii , and R. irregularis , Bacillus megaterium , and Frateuria aurantia), inoculated wheat demonstrated a general increase in total microbial biomass and soil enzymatic activities. These findings suggest an enhanced microbial metabolism, mainly observed when the inoculum contained both PGPRs and AMFs [27]. In particular, the consortia composed of R. irregularis , B. megaterium , and F. aurantia , stimulated the cyanobacteria growth, which were then more able to produce a higher amount of plant growth-promoting substances. Similarly, the bacterial consortium stimulated the abundance of bacteria belonging to the Flavobacteriaceae family, which plays an important ecological function in terms of organic matter turnover [27]. Baldi et al. also reported on how AMF influenced soil biodiversity by enhancing soil microbial biomass up to 53% in an apricot orchard [51].

References

1. Stockdale, E.A.; Shepherd, M.A.; Fortune, S.; Cuttle, S.P. Soil fertility in organic farming systems–fundamentally different? Soil Use Manag. 2002, 18, 301–308.

2. Zhang, H.; Wang, R.; Chen, S.; Qi, G.; He, Z.; Zhao, X. Microbial taxa and functional genes shift in degraded soil with bacterial wilt. Sci. Rep. 2017, 7, 1–11.

3. Maximillian, J.; Brusseau, M.L.; Glenn, E.P.; Matthias, A.D. Pollution and Environmental Perturbations in the Global System. In Environmental and Pollution Science; Elsevier: Amsterdam, The Netherlands, 2019; pp. 457–476.

4. Le Mire, G.; Nguyen, M.L.; Fassotte, B.; Du Jardin, P.; Verheggen, F.; Delaplace, P.; Haissam Jijakli, M. Implementing plant biostimulants and biocontrol strategies in the agroecological management of cultivated ecosystems. A review. Biotechnol. Agron. Soc. Environ. 2016, 20, 299–313.

5. Hellequin, E.; Monard, C.; Chorin, M.; Le Bris, N.; Daburon, V.; Klarzynski, O.; Binet, F. Responses of active soil microorganisms facing to a soil biostimulant input compared to plant legacy effects. Sci. Rep. 2020, 10, 13727.

6. Jacoby, R.; Peukert, M.; Succurro, A.; Koprivova, A.; Kopriva, S. The role of soil microorganisms in plant mineral nutrition—Current knowledge and future directions. Front. Plant Sci. 2017, 8, 1–19.

7. Romano, I.; Ventorino, V.; Pepe, O. Effectiveness of Plant Beneficial Microbes: Overview of the Methodological Approaches for the Assessment of Root Colonization and Persistence. Front. Plant Sci. 2020, 11, 1–16.

8. Bulgarelli, D.; Schlaeppi, K.; Spaepen, S.; Van Themaat, E.V.L.; Schulze-Lefert, P. Structure and functions of the bacterial microbiota of plants. Annu. Rev. Plant Biol. 2013, 64, 807–838.

9. Pathania, P.; Rajta, A.; Singh, P.C.; Bhatia, R. Role of plant growth-promoting bacteria in sustainable agriculture. Biocatal. Agric. Biotechnol. 2020, 30, 101842.

10. Mannino, G.; Nerva, L.; Gritli, T.; Novero, M.; Fiorilli, V.; Bacem, M.; Bertea, C.M.; Lumini, E.; Chitarra, W.; Balestrini, R. Effects of Different Microbial Inocula on Tomato Tolerance to Water Deficit. Agronomy 2020, 10, 170.

11. Bhalerao, R.P.; Eklöf, J.; Ljung, K.; Marchant, A.; Bennett, M.; Sandberg, G. Shoot-derived auxin is essential for early lateral root emergence in Arabidopsis seedlings. Plant J. 2002, 29, 325–332.

12. Quiroga, G.; Erice, G.; Aroca, R.; Zamarreño, Á.M.; García-Mina, J.M.; Ruiz-Lozano, J.M. Arbuscular mycorrhizal symbiosis and salicylic acid regulate aquaporins and root hydraulic properties in maize plants subjected to drought.

Agric. Water Manag. 2018, 202, 271–284.

13. Zou, Y.N.; Wang, P.; Liu, C.Y.; Ni, Q.D.; Zhang, D.J.; Wu, Q.S. Mycorrhizal trifoliate orange has greater root adaptation of morphology and phytohormones in response to drought stress. Sci. Rep. 2017, 7, 1–10.

14. Liu, C.Y.; Zhang, F.; Zhang, D.J.; Srivastava, A.; Wu, Q.S.; Zou, Y.N. Mycorrhiza stimulates root-hair growth and IAA synthesis and transport in trifoliate orange under drought stress. Sci. Rep. 2018, 8, 1–9.

15. Abd-Allah, E.F.; Hashem, A.; Alqarawi, A.A.; Bahkali, A.H.; Alwhibi, M.S. Enhancing growth performance and systemic acquired resistance of medicinal plant Sesbania sesban (L.) Merr using arbuscular mycorrhizal fungi under salt stress. Saudi J. Biol. Sci. 2015, 22, 274–283.

16. Chandra, D.; Srivastava, R.; Glick, B.R.; Sharma, A.K. Drought-Tolerant Pseudomonas spp. Improve the Growth Performance of Finger Millet (Eleusine coracana (L.) Gaertn) under Non-Stressed and Drought-Stressed Conditions. Pedosphere 2018, 28, 227–240.

17. Glick, B.R. Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol. Res. 2014, 169, 30–39.

18. Hashem, A.; Alqarawi, A.A.; Radhakrishnan, R.; Al-Arjani, A.B.F.; Aldehaish, H.A.; Egamberdieva, D.; Abd Allah, E.F. Arbuscular mycorrhizal fungi regulate the oxidative system, hormones and ionic equilibrium to trigger salt stress tolerance in Cucumis sativus L. Saudi J. Biol. Sci. 2018, 25, 1102–1114.

19. Zheng, F.L.; Liang, S.M.; Chu, X.N.; Yang, Y.L.; Wu, Q.S. Mycorrhizal fungi enhance flooding tolerance of peach through inducing proline accumulation and improving root architecture. Plant Soil Environ. 2020, 66, 624–631.

20. Nawaz, A.; Shahbaz, M.; Asadullah, M.; Imran, A.; Marghoob, M.U.; Imtiaz, M.; Mubeen, F. Potential of Salt Tolerant PGPR in Growth and Yield Augmentation of Wheat (Triticum aestivum L.) under Saline Conditions. Front. Microbiol. 2020, 11.

21. Gou, W.; Tian, L.; Ruan, Z.; Zheng, P.; Chen, F.; Zhang, L.; Cui, Z.; Zheng, P.; Li, Z.; Gao, M.; et al. Accumulation of choline and glycinebetaine and drought stress tolerance induced in maize (Zea mays) by three plant growth promoting rhizobacteria (PGPR) strains. Pak. J. Bot. 2015, 47, 581–586.

22. Moreno-Galván, A.E.; Cortés-Patiño, S.; Romero-Perdomo, F.; Uribe-Vélez, D.; Bashan, Y.; Bonilla, R.R. Proline accumulation and glutathione reductase activity induced by drought-tolerant rhizobacteria as potential mechanisms to alleviate drought stress in Guinea grass. Appl. Soil Ecol. 2020, 147, 103367.

23. Tigka, T.; Ipsilantis, I. Effects of sand dune, desert and field arbuscular mycorrhizae on lettuce (Lactuca sativa, L.) growth in a natural saline soil. Sci. Hortic. 2020, 264, 109191.

24. Aalipour, H.; Nikbakht, A.; Etemadi, N.; Rejali, F.; Soleimani, M. Biochemical response and interactions between arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria during establishment and stimulating growth of Arizona cypress (Cupressus arizonica G.) under drought stress. Sci. Hortic. 2020, 261, 108923.

25. Barnawal, D.; Bharti, N.; Maji, D.; Chanotiya, C.S.; Kalra, A. ACC deaminase-containing Arthrobacter protophormiae induces NaCl stress tolerance through reduced ACC oxidase activity and ethylene production resulting in improved nodulation and mycorrhization in Pisum sativum. J. Plant Physiol. 2014, 171, 884–894.

26. Cely, M.V.T.; Siviero, M.A.; Emiliano, J.; Spago, F.R.; Freitas, V.F.; Barazetti, A.R.; Goya, E.T.; Lamberti, G.D.S.; Dos Santos, I.M.O.; De Oliveira, A.G.; et al. Inoculation of Schizolobium parahyba with mycorrhizal fungi and plant growth-promoting rhizobacteria increases wood yield under field conditions. Front. Plant Sci. 2016, 7, 1708.

27. Dal Cortivo, C.; Ferrari, M.; Visioli, G.; Lauro, M.; Fornasier, F.; Barion, G.; Panozzo, A.; Vamerali, T. Effects of Seed-Applied Biofertilizers on Rhizosphere Biodiversity and Growth of Common Wheat (Triticum aestivum L.) in the Field. Front. Plant Sci. 2020, 11, 1–14.

28. Diagne, N.; Ndour, M.; Djighaly, P.I.; Ngom, D.; Ngom, M.C.N.; Ndong, G.; Svistoonoff, S.; Cherif-Silini, H. Effect of Plant Growth Promoting Rhizobacteria (PGPR) and Arbuscular Mycorrhizal Fungi (AMF) on Salt Stress Tolerance of Casuarina obesa (Miq.). Front. Sustain. Food Syst. 2020, 4, 1–8.

29. Laranjeira, S.; Fernandes-Silva, A.; Reis, S.; Torcato, C.; Raimundo, F.; Ferreira, L.; Carnide, V.; Marques, G. Inoculation of plant growth promoting bacteria and arbuscular mycorrhizal fungi improve chickpea performance under water deficit conditions. Appl. Soil Ecol. 2021, 164, 103927.

30. Nacoon, S.; Jogloy, S.; Riddech, N.; Mongkolthanaruk, W.; Kuyper, T.W.; Boonlue, S. Interaction between Phosphate Solubilizing Bacteria and Arbuscular Mycorrhizal Fungi on Growth Promotion and Tuber Inulin Content of Helianthus tuberosus L. Sci. Rep. 2020, 10, 1–10.

31. González-González, M.F.; Ocampo-Alvarez, H.; Santacruz-Ruvalcaba, F.; Sánchez-Hernández, C.V.; Casarrubias-Castillo, K.; Becerril-Espinosa, A.; Castañeda-Nava, J.J.; Hernández-Herrera, R.M. Physiological, Ecological, and

Biochemical Implications in Tomato Plants of Two Plant Biostimulants: Arbuscular Mycorrhizal Fungi and Seaweed Extract. Front. Plant Sci. 2020, 11, 999.

32. Kopta, T.; Pavlíková, M.; Sękara, A.; Pokluda, R.; Maršálek, B. Effect of bacterial-algal biostimulant on the yield and internal quality of Lettuce (Lactuca sativa L.) produced for spring and summer crop. Not. Bot. Horti Agrobot. Cluj Napoca 2018, 46, 615–621.

33. Tewari, S.; Pooniya, V.; Sharma, S. Next generation bioformulation prepared by amalgamating Bradyrhizobium, cell free culture supernatant, and exopolysaccharides enhances the indigenous rhizospheric rhizobial population, nodulation, and productivity of pigeon pea. Appl. Soil Ecol. 2020, 147, 103363.

34. Ahmad, F.; Ahmad, I.; Khan, M.S. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol. Res. 2008, 163, 173–181.

35. Luján Soto, R.; Martínez-Mena, M.; Cuéllar Padilla, M.; de Vente, J. Restoring soil quality of woody agroecosystems in Mediterranean drylands through regenerative agriculture. Agric. Ecosyst. Environ. 2021, 306, 107191.

36. Wang, C.; Liu, D.; Bai, E. Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. Soil Biol. Biochem. 2018, 120, 126–133.

37. Zhou, Z.; Wang, C.; Luo, Y. Meta-analysis of the impacts of global change factors on soil microbial diversity and functionality. Nat. Commun. 2020, 11, 1–10.

38. Castro, H.F.; Classen, A.T.; Austin, E.E.; Norby, R.J.; Schadt, C.W. Soil microbial community responses to multiple experimental climate change drivers. Appl. Environ. Microbiol. 2010, 76, 999–1007.

39. Zhang, Y.; Dong, S.; Gao, Q.; Liu, S.; Zhou, H.; Ganjurjav, H.; Wang, X. Climate change and human activities altered the diversity and composition of soil microbial community in alpine grasslands of the Qinghai-Tibetan Plateau. Sci. Total Environ. 2016, 562, 353–363.

40. Qiu, L.; Zhang, Q.; Zhu, H.; Reich, P.B.; Banerjee, S.; van der Heijden, M.G.A.; Sadowsky, M.J.; Ishii, S.; Jia, X.; Shao, M.; et al. Erosion reduces soil microbial diversity, network complexity and multifunctionality. ISME J. 2021.

41. Trivedi, C.; Delgado-Baquerizo, M.; Hamonts, K.; Lai, K.; Reich, P.B.; Singh, B.K. Losses in microbial functional diversity reduce the rate of key soil processes. Soil Biol. Biochem. 2019, 135, 267–274.

42. Kallenbach, C.M.; Frey, S.D.; Grandy, A.S. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nat. Commun. 2016, 7, 1–10.

43. Zheng, T.; Miltner, A.; Liang, C.; Nowak, K.M.; Kästner, M. Turnover of gram-negative bacterial biomass-derived carbon through the microbial food web of an agricultural soil. Soil Biol. Biochem. 2021, 152, 108070.

44. Liang, C.; Amelung, W.; Lehmann, J.; Kästner, M. Quantitative assessment of microbial necromass contribution to soil organic matter. Glob. Chang. Biol. 2019, 25, 3578–3590.

45. Buckeridge, K.M.; Mason, K.E.; McNamara, N.P.; Ostle, N.; Puissant, J.; Goodall, T.; Griffiths, R.I.; Stott, A.W.; Whitaker, J. Environmental and microbial controls on microbial necromass recycling, an important precursor for soil carbon stabilization. Commun. Earth Environ. 2020, 1, 1–9.

46. Yang, G.; Roy, J.; Veresoglou, S.D.; Rillig, M.C. Soil biodiversity enhances the persistence of legumes under climate change. New Phytol. 2021, 229, 2945–2956.

47. De Vries, F.T.; Griffiths, R.I.; Knight, C.G.; Nicolitch, O.; Williams, A. Harnessing rhizosphere microbiomes for drought-resilient crop production. Science 2020, 368, 270–274.

48. Prudent, M.; Dequiedt, S.; Sorin, C.; Girodet, S.; Nowak, V.; Duc, G.; Salon, C.; Maron, P.A. The diversity of soil microbial communities matters when legumes face drought. Plant Cell Environ. 2020, 43, 1023–1035.

49. Caravaca, F.; Barea, J.M.; Palenzuela, J.; Figueroa, D.; Alguacil, M.M.; Roldán, A. Establishment of shrub species in a degraded semiarid site after inoculation with native or allochthonous arbuscular mycorrhizal fungi. Appl. Soil Ecol. 2003, 22, 103–111.

50. Diagne, N.; Baudoin, E.; Svistoonoff, S.; Ouattara, C.; Diouf, D.; Kane, A.; Ndiaye, C.; Noba, K.; Bogusz, D.; Franche, C.; et al. Effect of native and allochthonous arbuscular mycorrhizal fungi on Casuarina equisetifolia growth and its root bacterial community. Arid Land Res. Manag. 2018, 32, 212–228.

51. Baldi, E.; Gioacchini, P.; Montecchio, D.; Mocali, S.; Antonielli, L.; Masoero, G.; Toselli, M.; Sanjuan, S. Effect of Biofertilizers Application on Soil Biodiversity and Litter Degradation in a Commercial Apricot Orchard. Agronomy 2021, 11, 1116.