AGROCHIMICA

International Journal of Plant Chemistry,
Soil Science and Plant Nutrition
of the University of Pisa

Vol. 67 - Special Issue (2023)

Sustainable development and climate change: 30 years after the *Honoris Causa* degree in Agricultural Sciences awarded to Lester Russell Brown

Proceeding of the Conference held in Pisa
on October 20, 2022

Edited by Lorenzo Cotrozzi and Marco Landi

PISA
UNIVERSITY
PRESS

Agrochimica, Vol. 67 - Special Issue (2023)

Beneficial soil microbiota as drivers of sustainable crop production

Gergely Ujvári[1], Alessandra Turrini[1], Luciano Avio[1], Monica Agnolucci[1,*]
[1] Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy

Keywords: arbuscular mycorrhizal fungi, bacterial endophytes, biological soil fertility, plant growth promoting bacteria

Abstract: The risks of rapidly growing human population, unsustainable environmental destruction, consequent food scarcity and destabilization of the global geopolitics were early recognized by Prof. Lester R. Brown, an environmentalist and enthusiast raising voice for future generations and emphasizing the costs, consequences and realities of our modern lifestyle. This short review was written to honour his lifelong work and determination to a great cause, discussing recent achievements of agricultural microbiology and how they may contribute to the goal of sustainable agricultural development.

Introduction

As global population is expected to approach 10 billion people by 2050 (UN, 2022), modern day agricultural sector has to face various challenges in order to satisfy persistently growing world needs (Brown, 2012). Indeed, Lester R. Brown, a pioneer environmentalist, often referenced food as the "weak link" in the endeavour of saving our civilization. His complex view on food scarcity and its origins shed light on global environmental risks greatly induced by human activities, such as soil erosion, the depletion of underground water resources and the consequences of climatic changes (Brown, 2012). In the same period, other concerns have been raised on the environmental costs and limits of conventional agriculture. The revolution of easily available chemical fertilizers led to an estimated consumption of 191.98 million tonnes worldwide in 2019 (expressed as N, P_2O_5 and K_2O), showing continuously increasing tendencies with an annual average growth of 1.5% until 2022 (FAO, 2019). Although such fertilizers boosted agricultural productivity on the short term, their excessive use was found to increase N_2O and NO_x greenhouse-gas emissions (Robertson and Vitousek, 2009) and to negatively impact soil health and long-term soil fertility, contributing to diversity reduction in native microbial communities (Sun et al., 2015).

[*] Corresponding author: monica.agnolucci@unipi.it.

DOI 10.12871/000218572023011

Unsustainable agricultural practices may lead to soil degradation and yield drops in vulnerable regions, as it occurred, e.g., in Lesotho (Mbata, 2001), in Kazakhstan (Almaganbetov and Grigoruk, 2008), and as it is expected to unfold in Amazonia (Oliveira et al., 2013). Therefore, in past decades, serious scientific efforts were done for the progression of agriculture in order to reach the goal of sustainable agricultural intensification (Foley et al., 2011). Some focused on cultivating cover crops (Njeru et al., 2014), applying green manures (Cherr et al., 2006), introducing organic or integrated soil fertilizing regimes (Diacono and Montemurro, 2011) and evaluating rhizosphere-related beneficial traits and improved nutrient-use efficiency of new plant varieties (Schmidt et al., 2016), while others investigated on the possible beneficial traits of soil microbes and their adequate management in agricultural systems. In the latter process, a wide variety of soil microorganisms have been selected for their plant growth promoting potentials, and microbial inoculants have been developed to be used as biofertilizers, biostimulants and biopesticides (Menendez and Garcia-Fraile, 2017).

The rhizosphere represents a promising frontier for research and development in agronomic sciences. This fraction of soil in the vicinity of plant roots functions as a metabolically active interface connecting the belowground to the aboveground biota, having crucial impacts on biodiversity and ecosystem function (Philippot et al., 2013). The rhizosphere is enriched with sugars, amino acids and organic acids deposited by the plant roots, providing nourishment and recruitment signals to large and diverse selected microbial communities that have principal roles in plant growth, nutrition and health (Philippot et al., 2013). Although their importance seems indisputable, a better understanding of the dynamics of these plant-associated microbial communities would be crucial for advancing agricultural sustainability in the terms of facilitating the selection and development of future microbial inocula, monitoring the effects of conventional and novel agronomic practices and evaluating agro-ecosystem functions.

The role of arbuscular mycorrhizal fungi

Among soil microorganisms, arbuscular mycorrhizal fungi (AMF, phylum Glomeromycota) represent an important group due to their symbiotic nature and to their key role in the enhancement of plant nutrition, health and crop quality. AMF appear to be ubiquitous in all ecosystems (Smith and Read, 2008) and establish mutualistic symbioses with ca.

80% of land plants, including the major food crops, such as cereals, pulses, fruit trees and vegetables, but also industrial crops like cotton, sunflower and oil palm, while they contribute to soil stability, too (Smith and Read, 2008).

After the establishment of the symbiotic connection, AMF obtain photosynthetic carbon compounds from the host plant, and develop an extraradical mycelium (ERM) reaching out from the root cortex to the rhizosphere and beyond. Given its high surface-to-volume ratio, its advanced depletion range respect to that of the plant roots alone and its specific nutrient transporters, the ERM functions as a highly efficient auxiliary absorption system, facilitating the uptake and transport of water and mineral nutrients, such as P, N, S, K, Ca, Cu and Zn into the roots (Smith and Read, 2008). Interestingly, once the symbiosis is formed, the viability and functionality of AMF hyphae may be uncoupled from host plant lifespan (Pepe et al., 2018). Recent studies on ERM suggested that the assessment of the diversity and regulation of fungal nutrient transport genes may allow a better selection for best performing AMF isolates (Giovannini et al., 2020, 2022).

However, AMF do not only provide nourishment to their partners; they also improve plant performance and health by increasing tolerance to biotic and abiotic stresses (Bitterlich et al., 2018) and induce changes in the secondary metabolism of plants stimulating the biosynthesis of health-promoting phytochemicals (Avio et al., 2018). Overall, their multiple beneficial traits make AMF a promising source of microbial inocula to be used as biostimulants, biofertilizers and bioenhancers in sustainable agriculture (Rouphael et al., 2015).

Over the years, scientific knowledge accumulated on the complex implications of the management of AMF communities in agronomic fields. As it was evidenced by Turrini et al. (2018b), interactions can occur between AMF identity and crop identity, ultimately expressing significant effects on plant productivity. In particular, diverse native assemblages of AMF from an agronomic field had contrasting effects on the growth of onion, pepper and lettuce plants with a maximum increase of 119% in the shoot dry weight of lettuce, while plant mineral nutrition for both N and P was affected only in pepper and lettuce. On the other hand, DNA sequencing revealed that the field inoculum performing best in all crops anchored a more diverse AMF community respect to inocula with a poor performance. Moreover, the highly different assemblages of AMF communities actively colonizing the roots of the three crop species suggested that the plants were rewarding their best fitting symbiotic partners. Similar results were obtained by Turrini et al. (2016) in organic maize fields, revealing that the composition of root colonizing AMF communities depended more on the host plant identity than on different cover crop treatments. These findings show that host preference may represent a main driver of AMF community dynamics in agroecosystems, regulating the abundance of different AMF partners according to their functional significance. In another study concerning the application of cover crops in organic maize cultivation, major influences of cover crop species on field mycorrhizal inoculum potential and subsequent maize growth were reported (Njeru et al., 2014).

AMF-associated bacterial communities

AMF live associated with large and diverse microbial communities which may colonize mycorrhizal roots, spores, peridial and extraradical hyphae, originating a complex and metabolically active environment called mycorrhizosphere (Rambelli, 1973). While many of these microorganisms are supposed to feed on fungal biopolymers and hyphal exudates (Roesti et al., 2005), members of the mycorrhizosphere microbiota also showed multiple interesting functional activities ranging from the role of “mycorrhiza helper” to that of “plant growth promoter”. Mycorrhiza helper bacteria (MHB) can promote spore germination, mycelial growth and mycorrhizal establishment via, e.g., growth factor production and detoxification of antagonistic substances (Sangwan and Prasanna, 2022), while plant growth promoting bacteria (PGPB) have the ability to enhance plant growth, nutrition and health via, e.g., N^2-fixation, P-solubilization, hormones, siderophores and antibiotics production (Philippot et al., 2013). However, bacteria possessing both beneficial functional traits have also been described (Xavier and Germida, 2003), suggesting that MHB and PGPB may act synergistically with AMF, leading to enhanced performance of the host plants, and therefore making them a promising target for agricultural research and sustainable development (Turrini et al., 2018a).

Studies investigating the diversity and community composition of AMF spore-associated microbiota with culture-independent molecular approaches reported the presence of diverse bacterial communities in strict intimacy with AMF spores (Agnolucci et al., 2015). For a broader taxonomic view, metagenomic analysis of the bacterial communities associated with a commercial inoculum of *Rhizoglomus irregulare*

BEG72 revealed a remarkable biodiversity represented by 276 bacterial genera and 165 bacterial families, mostly belonging to Proteobacteria and Bacteroidetes (Agnolucci et al., 2019a). Moreover, six and five isolates demonstrated considerable indole-acetic acid (IAA) or siderophore production, respectively, while three of them exhibited both beneficial traits. In a similar assessment of the functional diversity of AMF-associated bacterial communities, Battini et al. (2016) isolated 374 bacterial strains from the spores of *Rhizoglomus irregulare* IMA6, many of them able to solubilize P and to produce siderophores and IAA. Some of such isolates positively affected plant growth and nutrition by increasing hyphal length density and hyphal length specific P-uptake in mycorrhizal maize plants (Battini et al., 2017).

Multipartite interactions at the root-soil interface

Emerging scientific evidence suggests that multipartite interactions in the rhizosphere should be strongly considered during the development and adequate implementation of innovative microbial inocula for sustainable and organic agriculture. Tripartite mutualistic networks were discovered in different combinations of rhizosphere bacteria, AM fungi and host plants, such as *Bacillus* sp. PIS7 and a mix of AMF isolates, which not only formed a mutually beneficial association with wheat and maize plants, but the efficiency of their symbiotic network even exceeded that of the inorganic fertilization treatments in the terms of grain yield (Wahid et al., 2020). Similar phenomena were reported in leguminous plants, such as cowpea (Omirou et al., 2016), soybean (Meghvansi et al., 2008) and *Acacia gerrardii* (Hashem et al., 2016).

Interestingly, some studies reported increases in the root nodulation and rhizobial colonization of mycorrhizal plants, which may significantly improve plant nutrition (Meghvansi et al., 2008; Tajini et al., 2011). Recently, an elegant *in vivo* experiment allowed for the first time to get an insight into AMF/rhizobia interaction, revealing that AMF hyphae may act as "fungal highways" for the bacteria, as *gfp*-tagged *Bradyrhizobium diazoefficiens* was not only able to tightly adhere to the surfaces of *Glomus formosanum*, but also to transfer along the hyphae more efficiently than in substrate without AMF (de Novais et al., 2020).

In our laboratory, researches are underway, aiming at investigating the role played by AMF in shaping root endophytic bacterial communities (Ujvári et al., 2021), following the results obtained by PCR-DGGE and metagenomic analyses showing that the colonization of two cultivars of durum wheat by *Funneliformis mosseae* IMA1 caused major shifts in the composition of root endophytic bacterial communities (Agnolucci et al., 2019b). Further mechanistic and systematic studies on the multipartite plant-AMF-bacteria interactions at the root-soil interface are still needed in order to implement composite microbial inocula in sustainable and organic agriculture, where the functioning of such multipartite associations may be crucial for crop production.

Conclusions and future prospects

The continuous growth of global population and the unfolding negative impacts of human activities on soil fertility and soil health pose multiple challenges to modern agriculture. Soil microbiota, a functionally and taxonomically rich community of microorganisms, may hold a lot of potential to unlock in order to reduce chemical inputs in crop production. Numerous studies pointed out the beneficial effects of AMF on plant growth, nutrition and health, which may be dependent on their associated microbiota, too, as bacteria thriving in the mycorrhizosphere demonstrated multiple plant growth promoting and mycorrhiza helper traits. Moreover, multipartite microbial interactions at the root-soil interface may further increase crop yield and environmental benefits, thus their implementation in organic and low-input agriculture should be considered.

Although worldwide scientific efforts aimed the quest for agricultural sustainability in the past decades, our knowledge on the topic is still limited. In the years to come, a series of key questions remain to be answered by targeted research on root-associated microbial communities, concerning, e.g., the untangling of complex microbial interactions in the rhizosphere, the development of adequate microbial inocula for different crops and environmental conditions, as well as the efficient introduction of sustainable practices in agriculture preserving soil health.

Acknowledgements

This work was supported by the University of Pisa (Fondi di Ateneo) and is part of G.U. doctoral thesis work at the University of Pisa.

REFERENCES

Agnolucci M., Avio L., Pepe A., Turrini A., Cristani C., Bonini P. et al. (2019a). Bacteria associated with a commercial mycorrhizal inoculum: Community composition and multifunctional activity as assessed by Illumina sequencing and culture-dependent tools. Front. Plant Sci. 9, 1956.

Agnolucci M., Battini F., Cristani C., Giovannetti M. (2015). Diverse bacterial communities are recruited on spores of different arbuscular mycorrhizal fungal isolates. Biol Fertil Soils 51, 379-389.

Agnolucci M., Palla M., Cristani C., Cavallo N., Giovannetti M., De Angelis M. et al. (2019b). Beneficial plant microorganisms affect the endophytic bacterial communities of durum wheat roots as detected by different molecular approaches. Front. Microbiol. 10, 2500.

Almaganbetov N., Grigoruk V. (2008) Degradation of soil in Kazakhstan: Problems and challenges. In: *Soil chemical pollution, risk assessment, remediation and security* (Simeonov L., Sargsyan V., eds.). Springer, Dordrecht, The Netherlands, 309-320.

Avio L., Turrini A., Giovannetti M., Sbrana C. (2018). Designing the ideotype mycorrhizal symbionts for the production of healthy food. Front. Plant Sci. 9, 1089.

Battini F., Cristani C., Giovannetti M., Agnolucci M. (2016). Multifunctionality and diversity of culturable bacterial communities strictly associated with spores of the plant beneficial symbiont *Rhizophagus intraradices*. Microbiol. Res. 183, 68-79.

Battini F., Grønlund M., Agnolucci M., Giovannetti M., Jakobsen I. (2017). Facilitation of phosphorus uptake in maize plants by mycorrhizosphere bacteria. Sci. Rep. 7, 4686.

Bitterlich M., Rouphael Y., Graefe J., Franken P. (2018). Arbuscular mycorrhizas: A promising component of plant production systems provided favorable conditions for their growth. Front. Plant Sci. 9, 1329.

Brown L.R. (2012). *Full planet, empty plates: The new geopolitics of food scarcity*. W.W. Norton & Company, New York, NY, USA.

Cherr C.M., Scholberg J.M.S., McSorley R. (2006) Green manure approaches to crop production: A synthesis. Agron. J. 98, 302-319.

de Novais C.B., Sbrana C., da Conceição Jesus E., Rouws L.F.M, Giovannetti M., Avio L. (2020). Mycorrhizal networks facilitate the colonization of legume roots by a symbiotic nitrogen-fixing bacterium. Mycorrhiza 30, 389-396.

Diacono M., Montemurro F. (2011). Long-term effects of organic amendments on soil fertility. In: *Sustainable Agriculture Volume 2* (Lichtfouse E., Hamelin M., Navarrete M., Debaeke P., eds.). Springer, Dordrecht, The Netherlands, 761-786.

FAO (2019) *World fertilizer trends and outlook to 2022*. Food & Agriculture Organization of United Nations, Rome, Italy.

Foley J.A., Ramankutty N., Brauman K.A. et al. (2011). Solutions for a cultivated planet. Nature 478, 337-342.

Giovannini L., Sbrana C., Avio L., Turrini A. (2020). Diversity of a phosphate transporter gene among species and isolates of arbuscular mycorrhizal fungi. FEMS Microbiol. Lett. 367.

Giovannini L., Sbrana C., Giovannetti M., Avio L., Lanubile A., Marocco A., Turrini A. (2022). Diverse mycorrhizal maize inbred lines differentially modulate mycelial traits and the expression of plant and fungal phosphate transporters. Sci. Rep. 12, 21279.

Hashem A., Abd_Allah E.F., Alqarawi A.A., Al-Huqail A.A., Wirth S., Egamberdieva D. (2016). The interaction between arbuscular mycorrhizal fungi and endophytic bacteria enhances plant growth of *Acacia gerrardii* under salt stress. Front. Microbiol. 7, 1089.

Mbata J.N. (2001). Land use practices in Lesotho: Implications for sustainability in agricultural production. J. Sustain. Agric. 18, 5-24.

Meghvansi M.K., Prasad K., Harwani D., Mahna S.K. (2008). Response of soybean cultivars toward inoculation with three arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* in the alluvial soil. Eur. J. Soil Biol. 44, 316-323.

Menendez E., Garcia-Fraile P. (2017). Plant probiotic bacteria: Solutions to feed the world. AIMS Microbiol. 3, 502-524.

Njeru E.M., Avio L., Sbrana C., Turrini A., Bocci G., Bàrberi P., Giovannetti M. (2014). First evidence for a major cover crop effect on arbuscular mycorrhizal fungi and organic maize growth. Agron. Sustain. Dev. 34, 841-848.

Oliveira L.J.C., Costa M.H., Soares-Filho B.S., Coe M.T. (2013). Large-scale expansion of agriculture in Amazonia may be a no-win scenario. Environ. Res. Lett. 8, 024021.

Omirou M., Fasoula D.A., Ioannides I.M. (2016). *Bradyrhizobium* inoculation alters indigenous AMF community assemblages and interacts positively with AMF inoculum to improve cowpea performance. Appl. Soil Ecol. 108, 381-389.

Pepe A., Giovannetti M., Sbrana C. (2018). Lifespan and functionality of mycorrhizal fungal mycelium are uncoupled from host plant lifespan. Sci. Rep. 8, 10235.

Philippot L., Raaijmakers J.M., Lemanceau P., van der Putten W.H. (2013). Going back to the roots: The microbial ecology of the rhizosphere. Nat. Rev. Microbiol. 11, 789-799.

Rambelli A. (1973). The rhizosphere of mycorrhizae. In: *Ectomycorrhizae* (Marks G.C., Kozlowski T.T., eds.). Academic Press, London, UK, 299-349, 1st ed.

Robertson G.P., Vitousek P.M. (2009). Nitrogen in agriculture: Balancing the cost of an essential resource. Annu. Rev. Environ. Resour. 34, 97-125.

Rouphael Y., Franken P., Schneider C., Schwarz D., Giovannetti M., Agnolucci M. et al. (2015). Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. Sci. Hortic. 196, 91-108.

Sangwan S., Prasanna R. (2022). Mycorrhizae helper bacteria: Unlocking their potential as bioenhancers of plant arbuscular mycorrhizal fungal associations. Microb. Ecol. 84, 1-10.

Schmidt J.E., Bowles T.M., Gaudin A.C.M. (2016). Using ancient traits to convert soil health into crop yield: Impact of selection on maize root and rhizosphere function. Front. Plant Sci. 7, 373.

Smith S.E., Read D.J. (2008) *Mycorrhizal symbiosis*. Academic Press, London, UK, 3rd ed.

Sun R., Zhang X.X., Guo X., Wang D., Chu H. (2015). Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. Soil Biol. Biochem. 88, 9-18.

Tajini F., Trabelsi M., Drevon J.J. (2011). Co-inoculation with *Glomus intraradices* and *Rhizobium tropici* CIAT899 increases P use efficiency for N_2 fixation in the common bean (*Phaseolus vulgaris* L.) under P deficiency in hydroaeroponic culture. Symbiosis 53, 123-129.

Turrini A., Avio L., Giovannetti M., Agnolucci M. (2018a). Functional complementarity of arbuscular mycorrhizal fungi and associated microbiota: The challenge of translational research. Front. Plant Sci. 9, 1407.

Turrini A., Bedini A., Loor M.B., Santini G., Sbrana C., Giovannetti M., Avio L. (2018b). Local diversity of native arbuscular mycorrhizal symbionts differentially affects growth and nutrition of three crop plant species. Biol. Fertil. Soils 54, 203-217.

Turrini A., Sbrana C., Avio L., Njeru E.M., Bocci G., Bàrberi P., Giovannetti M. (2016). Changes in the composition of native root arbuscular mycorrhizal fungal communities during a short-term cover crop-maize succession. Biol. Fertil. Soils 52, 643-653.

Ujvári G., Turrini A., Avio L., Agnolucci M. (2021). Possible role of arbuscular mycorrhizal fungi and associated bacteria in the recruitment of endophytic bacterial communities by plant roots. Mycorrhiza 31, 527-544.

United Nations (2022). *World Population Prospects 2022*. Department of Economic and Social Affairs of United Nations, New York, NY, USA.

Wahid F., Fahad S., Danish S., Adnan M., Yue Z., Saud S. et al. (2020). Sustainable management with mycorrhizae and phosphate solubilizing bacteria for enhanced phosphorus uptake in calcareous soils. Agriculture 10, 334.

Xavier L.J.C., Germida J.J. (2003). Bacteria associated with *Glomus clarum* spores influence mycorrhizal activity. Soil Biol. Biochem. 35, 471-478.