

Special Issue on ‘Hide and Seek of Soil Microbes—Who Is Where with Whom and Why?’

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1. Introduction

Our question posed for and used as title of the special issue ‘*Hide and Seek of Soil Microbes*’—*Who is Where with Whom and Why?* can be considered as the central paradigm of soil microbial ecology, covering and embracing all relevant aspects and topics.

- Soil is life!
- Soil, the solid matrix of all terrestrial ecosystems: the most complex, diverse and heterogeneous ecosystem, harboring plenty of micro-niches and hot spots for microorganisms (*Who is Where?*)
- Soil, the source of life, which is subjected to environmental stressors, especially within the context of anthropogenic-driven challenges. Soil microbes, the microbial inhabitants of soil, live in complex, diverse consortia. They drive all biogeochemical processes, and they are involved in all nutrient cycles (*Who is with Whom and Why?*)

Although technical innovations boost soil scientists’ opportunities to assess soil ecosystems and ask big questions, fundamental focus points remain and require attention more than ever:

- **Experimental design and sampling strategy.** Although addressed previously, there are things to remember and far more things to uncover and to take home. Spatial heterogeneity in particular is still rarely considered in the set-up of scientific experiments.
- **Microbial diversity.** Ecological hypotheses involve the observation of species number. The current methodology does not necessarily provide this information. Likewise, the controversially discussed analysis of relative abundance data needs validation and scientific awareness.
- **Functions.** Omics benefit soil science. However, understanding the soil ecosystem from a mechanistic point of view differs from what recent research has termed ‘function’. Functioning implies malfunctioning, thereby reducing the soil ecosystem to a system providing a service. For comprehension, coherent and precise definitions are indispensable. In addition, the precious information provided by sophisticated methods warrants critical discussion to draw relevant conclusions.

2. ‘Hide and Seek of Soil Microbes—Who Is Where with Whom and Why?’

In light of the above, this special issue was introduced with the aim of opening a vivid discussion on the following topics:

- Adequate experimental design for representative study of soil ecosystems;
- Possibilities and limitations of observing microbial diversity;
- Possibilities and limitations of data analysis and their impact on ecological conclusions;
- Microbial spatial heterogeneity across different scales;

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- How to observe microbial niche partitioning and occupation;
- Soil ecosystems, soil microbial communities, and their ‘function’. In what sense can microbes, microbial communities and ecosystems have a function?

This special issue represents a concise and strong scientific reaction to our questions posed, with three Regular Full Length Research Articles ([1] cited by 1, viewed by 474; [2] cited by 1, viewed by 878, and [3] cited by 28, viewed by 2772), and two Review Articles ([4] viewed by 911; and [5] cited by 3, viewed by 1038). Various topics related to our posed questions—in general or in particular—have been addressed.

The first paper, authored by M. Zotte, J. Mayerhofer, H. Embleton, K. Wechselberger, J. Enkerli, and H. Strasser [1], presents an impressive and promising example of biological pest control of *Diabrotica* populations (corn rootworm, *Diabrotica v. virgifera*-Coleoptera, Chrysomelidae) using inundative mass application of *Metarhizium brunneum* BIPESCO 5 (Hypocreales, Clavicipitaceae). This case study points out the importance of a strong experimental design (long-term field studies with different cultivation techniques and infestation rates) and concludes, supported by the obtained data, that crop rotation remains the option of choice for rapid pest population decline at high pest densities [1].

In the second paper, the authors S.D. Veresoglou, L. Grünfeld, and M. Mola, [2] address how soil spatial structures in plant mesocosms, i.e., habitat connectance and habitat quality, alter the predictability/stochasticity of the community composition of arbuscular mycorrhizal fungi (AMF). This is one of very few studies experimentally quantifying how micro-landscape structures increase the stochasticity of AMF communities. Thus, micro-landscape structures could support the persistence of less competitive species in the ecosystem. This is particularly meaningful in the case of AMF, which are poorly researched in this regard, as their presence/absence might determine the establishment of plant individuals in the ecosystem. Overall, the authors provided evidence that the community structure of AMF is less responsive to spatiotemporal manipulations than root colonization rates, which is a facet of the symbiosis that is currently poorly understood.

The third paper, authored by C. Sansupa, S.F.M. Wahdan, S. Hossen, T. Disayathanoowat, T. Wubet, and W. Purahong [3], presents, for the first time, the successful application of the FAPROTAX database, originally developed for marine ecosystems, to the soil ecosystem, providing evidence about its potential as a powerful tool for predicting ecological relevant functions of soil bacterial and archaeal taxa derived from 16S rRNA amplicon sequencing. The authors conclude that although FAPROTAX cannot predict the function of all detected taxa, it is capable of a fast-functional screening or grouping of 16S bacterial data derived from any ecosystem. It was suggested that additional datasets of both the taxonomy and functional references could further improve the database, thereby increasing the number of functionally assigned OTUs derived from 16S rRNA. The innovative aspect and scientific relevance of this study [3] is confirmed by the huge number of records (28 citations; 2772 views) within the first months after the release of our special issue.

In this special issue, next to the beforementioned case studies, there are two Review Articles, one critically discussing ‘Methods for Studying Bacterial–Fungal Interactions in the Microenvironments of Soil’ [4], and the other focusing on ‘Thermodynamics of Soil Microbial Metabolism: Applications and Functions’ [5].

The review by E. Mandolini, M. Probst and U. Peintner [4] perfectly matches with one of the Guest Editors’ desired expectations, being among the driving forces for editing this special issue. The review focused on microscale variations in soil properties constraining the distribution of fungi and bacteria, and to the extent of their interactions and consequent behavior and ecological roles. The review points out that a realistic assessment and understanding of bacterial–fungal interactions is only possible by considering the spatiotemporal complexity of their microenvironments. The authors succeeded in further raising awareness of this important aspect by critically and extensively discussing possible methodologies, embracing culture-dependent and culture-independent tools along

with suggesting new applications of current technologies to answer newly formulated research questions, in order to better glimpse the intricate lives of microorganisms.

Furthermore, the second review, authored by N. Barros [5], perfectly satisfies the Guest Editors' expectations by introducing an innovative approach for studying soil microbiota. The review presents the state of the art of the very intriguing and promising approach of characterizing the thermodynamics of soil microbial metabolism as a potential tool for the in-depth assessment and comprehension of their strategies for survival, as well as defining their evolutionary state. The author pinpoints the fact that the still unexplored role of microbial diversity—using the energy from the soil organic substrates, and, therefore, the who, where, with whom, and why of managing that energy—could be assessed by unraveling the nature of the soil organic substrates and by monitoring the energy released by the soil microbial metabolism (decomposition vs. assimilation of soil organic substrates). Moreover, the author is right that soil organic content/matter needs differentiation in order to explain the soil carbon cycle in a more appropriate, meaningful and detailed manner.

3. Conclusions and Outlook

There is still a long way to go, and a lot of research to do, so as to adequately—if it is possible at all (?)—answer the fundamental questions posed in the present special issue. As partially shown in our special issue (recent case studies and reviews), molecular microbial ecology, i.e., its available tools and those continuously evolving, no doubt has the potential to further enlighten the (still) black box, soil. In this regard, e.g., the discriminatory study of the extracellular (exDNA) and intracellular (iDNA) fractions of the total environmental DNA pool (eDNA), might be a promising approach to (i) further increase the overall information stored about microbiota in soil, e.g., [6], and other environments [7], including specific habitats such as deadwood [8], and (ii) to correctly interpret, critically discuss, and draw relevant conclusions of DNA-based results. Moreover, in the era of culture-independent high-throughput molecular analyses coupled with advanced ecological networking via bioinformatics, the basic, defining steps of any experiment must be taken seriously and correctly, i.e., the experimental design including sampling strategy, soil storage and DNA extraction methods (reviewed by [9,10]). Furthermore, culture-dependent methods (classical microbiology) must not be neglected, and efforts have to be made to increase the number of cultivable microorganisms for further characterization. In fact, the combination of culture-dependent and culture-independent analyses of soil microbiota, and that of all other ecosystems, including aquatic systems, is recommended now more than ever, and can be considered key to the in-depth answer to the question: *Who is Where with Whom and Why?*

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