

Chapter 1

Secondary Metabolites in Soil Ecology

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1.1 Introduction: Chemical Interactions in Soil

Interactions among organisms are central to understanding any ecosystem, perhaps with the exception of a short period when a newly created niche is colonized by its first inhabitants. Soil environment is not an exception, but biotic interactions dominating soil biology differ from those in other systems because of the dominating role of sessile organisms and the lack of autotrophy in soil (chemolithoautotrophs being an interesting but not significant exception). When chemical processes in soil are discussed, the traditional concept of food webs comes first to mind as a framework for the exchange of organic substances and flow of energy. Feeding, predation, degradation of macromolecular substrates and absorption of nutrients have dominated thinking about biogenic chemical processes in soil. The food web approach proved extremely fruitful in generating hypotheses and inspiring experimental approaches concerning the bulk transformation of organic matter, but it did not address phenomena related to chemical interactions which are more specific both on the chemical and on the taxonomical level and which cannot be adequately described in terms of energy flow and biomass transformation. These interactions involve compounds named secondary metabolites, which are not strictly needed for the survival and reproduction of their producers. Secondary metabolites are structurally highly diverse and each of them is produced only by a small number of species. They exert various biological effects, often at very low concentrations, and can be regarded as carriers of chemical communication among soil inhabitants.

The high complexity and heterogeneity of soil makes this matrix recalcitrant to chemical analysis. Methods for the determination of pesticides, polychlorinated biphenyls and other xenobiotics in soil have existed for a long time to monitor pollution of the environment, but it is only recently that dedicated analytical methods for natural metabolites in soil have been available (Mortensen et al. 2003). Apart

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from the complexity of soil matrix, analytical methods for secondary metabolites in the soil have to cope with the enormous diversity of the analyte itself. The resolution of current metabolomics approaches is far from adequate even for the metabolome of a single organism, let alone for systems orders of magnitude more complex. Adsorption phenomena, large differences in concentrations among metabolites and their heterogeneous distribution further complicate profiling of secondary metabolites in soil by current metabolomics techniques. We may need to focus on dominant metabolites and major effects first, gradually zooming into the system as the progress of analytical techniques allows us.

Most secondary metabolites produced by soil microbes appear to be secreted, an observation which corroborates their role in controlling biotic interactions. The research field addressing the role of secreted metabolites in an ecosystem is ecological chemistry. Concerning soil microorganisms, the antibiotics paradigm has dominated experimental approaches to the ecological role of secondary metabolites so far, followed by pathogenic interactions between microorganisms and plants. Other roles of secondary metabolites, such as facilitating symbiosis with insects, plants and higher animals, are documented but have rarely been addressed (Demain and Fang 2000; Sect. 1.6.3). For instance, it has been known since ancient times that fungal products may poison animals, but the idea that microbes produce toxins to protect their substrates from ingestion by animals did not surface until Janzen's pioneering paper was published in *The American Naturalist* (Janzen 1977). Even then, attempts to test this hypothesis experimentally have rarely been reported. The role of secondary metabolites in interactions among soil microorganisms or between a microorganism and a plant might appear to be easier to address, but rigorous testing of a working hypothesis in this area is tricky (see Sects. 1.6.2, 1.6.3, 1.7). Without the capability of manipulating secondary metabolite synthesis or their targets genetically, conclusive results are difficult if not impossible to obtain.

1.2 Should the Term “Secondary Metabolites” Be Abandoned?

More than a century ago, Kossel (1891) defined secondary metabolites by exclusion (compounds that do not belong to primary metabolites), provoking criticism which has never ceased. The current, generally accepted concept in line with Kossel's view is that primary metabolites are chemical components of living organisms that are vital for their normal functioning, while secondary metabolites are compounds which are dispensable. A distinguishing feature of secondary metabolites is that their production is limited to a group of species or genera and is rarely conserved over a wide taxonomical range, while primary metabolism is conserved among phyla and across kingdoms.

The specificity of secondary metabolism encouraged botanists and mycologists to use secondary metabolite production as a taxonomical characteristic in plants (Smith 1976) and fungi (Frisvad et al. 1998). Chemotaxonomy harbors risks, because on

the one hand a single-point mutation might block a whole biosynthetic pathway, and on the other hand there are indications that some gene clusters involved in secondary metabolite biosynthesis have been transmitted among species by a horizontal gene transfer. The use of chemotaxonomy for elucidating phylogenetic relationships was therefore limited, and it became obsolete with ready access to DNA sequences. However, chemotaxonomy has not lost its appeal as a rapid and inexpensive support for taxonomical classification of microbial isolates.

Many scientists studying secondary metabolites dislike the term, because it appears to imply an auxiliary importance of secondary metabolites compared with the importance of primary metabolites. Numerous attempts to replace “secondary metabolites” by other labels were undertaken without gaining wide acceptance. Several initiatives emphasized the biological role of these compounds. For example, the designation “ecological metabolites” stresses the role of secreted metabolites in interactions of their producers with other organisms. Similarly, Frisvad’s creation of “extrolites” (an outwardly producers directed chemically differentiated product of a living organism) is based on the notion that the function of many secondary metabolites is to control or modulate interactions with the environment. In the meantime the author has been using his term as a synonym for all secondary metabolites (Frisvad et al. 2004). The problem is that not all secondary metabolites fit his definition of extrolites, and for the majority of secondary metabolites we do not know whether they are “outwardly directed” or not. I suppose this is the reason why the term “extrolites” has not been embraced by the scientific community. In their recent review of fungal metabolomics, Frisvad’s colleagues abandoned the term “extrolites” completely, consistently using “secondary metabolites” (Smedsgaard and Nielsen 2005). Substitutes like the term “extrolites” will unlikely replace the established term “secondary metabolites” because their definitions do not cover the full range of natural products known as secondary metabolites, and because their applicability relies on information which is seldom available. Let us look at a couple of examples. Leaf-movement factors in nyctinastic plants are clearly secondary metabolites, but one would not call them ecological metabolites or extrolites. Sometimes both secondary and primary metabolites serve the same purpose, defeating any classification based on function. For instance, pyochelin is secreted by *Pseudomonas* sp. and citric acid is secreted by plant roots, both facilitating the uptake of mineral nutrients by their producers. A functional classification would blur the distinction between secondary metabolites (pyochelin as a nonribosomal peptide) and primary metabolites (citric acid as a member of the Krebs cycle), the preservation of which is desirable. From a practical point of view, the main problem with functional classifications is that for most newly described natural products we do not know anything beyond their structure and taxonomical affiliation of the producer, the latter information often being limited to a genus.

The traditional distinction between primary and secondary metabolism is straightforward and knowledge of the structure is usually sufficient for the assignment of a compound to primary or secondary metabolism. As useful as some of the suggested substitutes are in emphasizing functional aspects, terms like “extrolites,” “special metabolites” (Gottlieb 1990), “idiolites” (Demain 1986), “ecological