

## The evolving copiotrophic/oligotrophic dichotomy: from Winogradsky to physiology and genomics

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**Abstract:**

Nearly 100 years ago, Winogradsky published a classic communication in which he described two groups of microbes, zymogenic and autochthonous. When organic matter penetrates the soil, zymogenic microbes quickly multiply and degrade it, then giving way to the slow combustion of autochthonous microbes. Although the text was originally written in French, it is often cited by English-speaking authors. We undertook a complete translation of the 1924 publication, which we provide as Supporting Information. Here we introduce the translation and describe how the zymogenic/autochthonous dichotomy shaped research questions in the study of microbial diversity and physiology. We also identify in the literature three additional and closely related dichotomies, which we propose to call exclusive copiotrophs/oligotrophs, coexisting copiotrophs/oligotrophs and fast/slow growing microbes. While Winogradsky focused on a successional view of microbial populations over time, the current discussion is focused on the differences in the specific growth rate of microbes as a function of the concentration of a given limiting substrate. In the future it will be relevant to keep in mind both nutrient-focused and time-focused microbial dichotomies and to design experiments with both isolated laboratory cultures and multi-species communities in the spirit of Winogradsky's direct method.

## Life and work of Sergei Winogradsky leading to the 1924 communication

Sergei Winogradsky was born in 1856 in Podolia, near Kiev, Ukraine, and died in Brie, France, in 1953. An extensive account of his life and works is given in (1, 2). During his undergraduate studies, Winogradsky participated in the Petersburg School of Plant Physiologists. As an apprentice of Andrei Famintsyn, he learned to view nature as a complex exchange of matter and energy. He also learned how to couple careful microscopic observations with nutritional and physiological experiments. The strong relationship between organism nutrition and physiology permeated a long career of investigations in Microbiology. In 1885 Winogradsky entered the laboratory of Anton deBary at the University of Strassburg. As a postdoc of sorts, he worked on the sulfur granules that accumulate in the filamentous bacterium *Beggiatoa* when in its natural environment. Using freshly isolated *Beggiatoa*, Winogradsky showed that the sulfur in these organisms is the sole energy source for respiration, and in that sense plays the same role as an external energy source in other organisms. This idea became the concept of chemolithotrophy and led eventually to the concept of sulfur and nitrogen cycles in Nature. Winogradsky's investigations of sulfur bacteria also left us with the Winogradsky column, essentially an ecosystem in a bottle. Winogradsky perfected his scientific training in Zurich at the Swiss Polytechnic Institute starting in 1888, mainly at the laboratory of Ernst Schultz. There, he showed that nitrification takes place in two steps, the conversion of ammonia to nitrite and of nitrite to nitrate, carried out by physiologically distinct groups of organisms. Thus, nitrification was another example of the chemolithotrophy he had described for sulfur bacteria. The ability of these organisms to couple the oxidation of an inorganic salt with the fixation of carbon dioxide, allowing for bacterial growth, also led to the idea of autotrophy and the cycles of nitrogen and sulfur. This makes Winogradsky indisputably one of the founders of Microbial Ecology. In 1891, Winogradsky accepted the directorship of the General Microbiology Service at the Institute of Experimental Medicine of St. Petersburg, where he would work for 15 years. There he was burdened by administrative tasks yet managed to report the first isolation of a free-living nitrogen fixer. In 1905, Winogradsky took early retirement for health reasons and practiced "scientific farming" to modernize the management of his familial estate in Gorodok, Ukraine. In 1921 he fled from Russia, returned to academic activity, and put his scientific vision to practical use in soil science at the Institut Pasteur, where he published his communication about zymogenic and autochthonous microbes.

## Winogradsky's direct method and the 1924 communication

Winogradsky viewed soil as a collective entity that respired, transformed organic and inorganic molecules and kept its components in a dynamically healthy balance (1, 2). He urged his colleagues to relinquish their devotion to pure culture methods and instead study the biological relationships that reign in the soils and regulate the fate of soil microbes. His "direct method" was an attempt to approach this problem (3). It is based on the microscopic and photographic study of soil samples or freshly isolated soil communities of microbes in conditions as natural as possible. Soil communities are studied as a whole because species interactions determine their roles in the natural community. Hypotheses about physiology and nutrition of the microbes in the community are used to design experiments in which specific nutrients are supplied in a controlled manner.

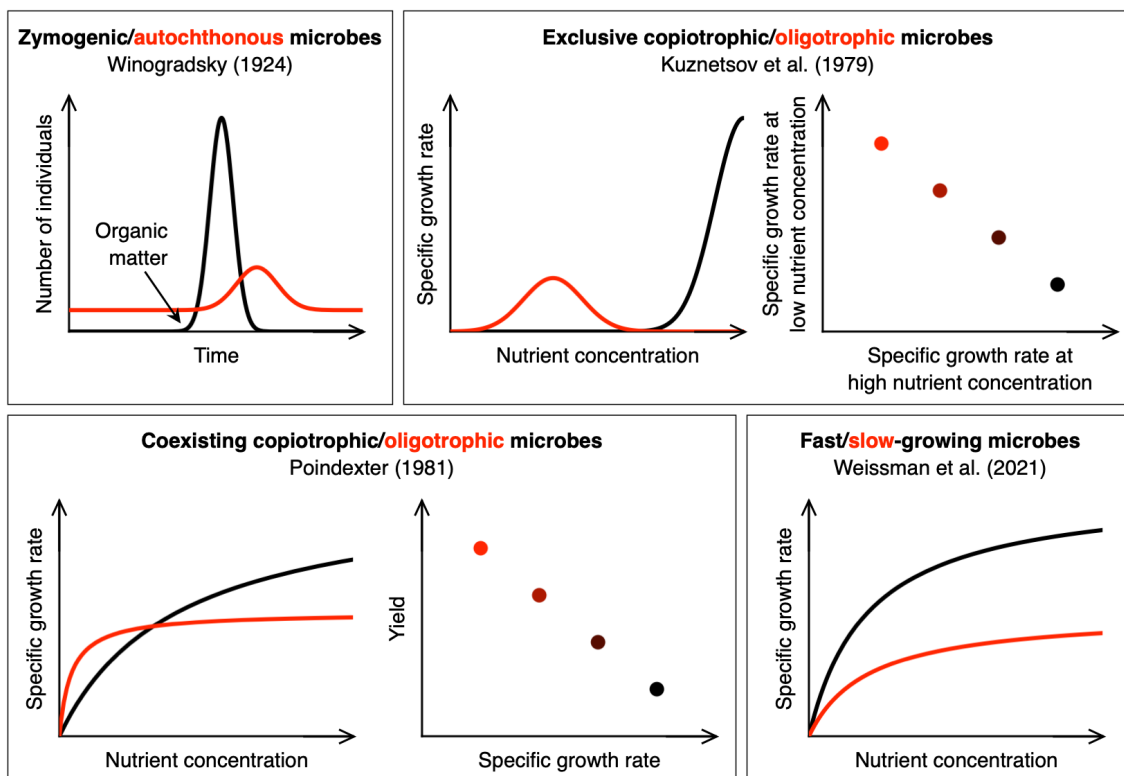
Winogradsky promoted his new approach in a variety of forums between 1923 and 1925. In 1924, he published the communication "*Sur la microflora autochtone de la terre arable*" in the journal *Comptes rendus hebdomadaires des séances de l'Académie des Sciences* (4), a scientific journal published in Paris by the French Academy of Sciences and originally created to briefly announce significant new results. Winogradsky's three-page text shortly describes in a colloquial style the successional observation of two "wide

groups of microbes” along the process of degradation of organic matter in the soil. These two groups of microbes were later termed zymogenic and autochthonous (5). There are no figures or tables, and the only reference is to a 1923 description of the author’s direct method for soil microbiology.

The observations that originate the definition of the zymogenic/autochthonous groups are qualitative (Figure 1, top left panel). It is implied throughout the text that these observations define a dichotomy, i.e., a given microbe is either zymogenic or else autochthonous, but not both. Zymogenic microbes penetrated into the soil from the outside with organic matter in the process of decomposition. This triggered a surge in the number of zymogenic microbes, which degraded this organic matter rapidly. Subsequent characterization of zymogenic microbes showed that they could easily be cultured in isolation on conventional media, while autochthonous microbes are not. A mixture of autochthonous microbes could be observed under the microscope after depositing on gelatinized media tiny particles of earth having received no manure for several years. This indigenous microflora of the arable land degraded nutrients in a “slow combustion”, could not be cultured in isolation at the time and were mostly cocci with a large cell size.

### **Further studies on zymogenic and autochthonous microbes**

Further studies defined zymogenic and autochthonous microbes in relation to environment-dependent differences in their spatial and temporal growth patterns and are diverse in terms of approach and methodology. Correspondingly, key experiments involve multiple species and are performed on environmental samples or laboratory setups designed to emulate natural sites, in the spirit of Winogradsky’s direct method. While the original terms were specifically defined for soil microbes, they have also been used for water dwelling microbes (6). Some reports focus on a successional view of the soil microbiome after the addition of organic matter, closely following Winogradsky’s proposal in that regard (7). Other successional studies address the effect of other environmental variables such as soil drying and rewetting (6) and plant diversity (8). In these three studies, the zymogenic/autochthonous dichotomy guided the interpretation of time courses for microbial diversity (6–8). A second class of studies deals with ecological variables, albeit not in a successional context. These variables include the spatial distribution of nutrients (9), plant-microbe interactions (10), and animal-microbe interactions (11). At this stage, the physiological traits associated with zymogenic/autochthonous microbes could be used to discuss the behavior of individual organisms (9–11). An additional line of work involves the cultivation of zymogenous consortia from polluted sites and their re-introduction to speed up bioremediation (12). This work uses the expected behavior of zymogenic microbes as the starting point of a biotechnological project (12).



**Figure 1. Quantitative representation of models for the zymogenic/autochthonous and copiotrophic/oligotrophic dichotomies from 1924 to the present day.** Top left: Successional view (number of individuals versus time) of zymogenic (red line) and autochthonous (black line) soil microbes in Winogradsky's original work (4, 5). Top right: Specific growth rates of exclusive copiotrophs/oligotrophs versus concentration of a limiting nutrient and the dominant underlying trade-off for this conceptualization, that between growth rate at low versus high nutrient concentrations. Growth of oligotrophs is inhibited by high nutrient concentration, while copiotrophs die out at low nutrient concentrations (13). Archetypal exclusive oligotrophs present a higher growth rate at low limiting nutrient concentrations, while archetypal exclusive copiotrophs present a higher growth rate at high limiting nutrient concentrations. Other microbes in the exclusive copiotrophic/oligotrophic spectrum present intermediate characteristics (13, 14) (see main text for discussion). Bottom left: Specific growth rates of coexisting copiotrophs/oligotrophs versus the dominant underlying trade-off for this conceptualization, that between microbial growth rate and yield. Coexisting copiotrophs have a higher maximal growth rate at saturating concentrations of the limiting nutrient than coexisting oligotrophs but attain it at higher nutrient concentrations (15, 16). At a given limiting nutrient concentration, archetypal coexisting oligotrophs grow slowly but with a high yield, while archetypal coexisting copiotrophs grow faster but at a lower yield. Other microbes in the coexisting copiotrophic/oligotrophic spectrum present intermediate characteristics (17) (see main text for discussion). Bottom right: Specific growth rates of fast-growing versus slow-growing microbes (18).

### From the zymogenic/autochthonous dichotomy to the concept of oligotrophic and copiotrophic microbes

In the decades following Winogradsky's communication, a parallel line of studies aimed at studying the isolated laboratory cultivation of zymogenic and autochthonous microbes in synthetic media. This enabled a detailed characterization of cell morphology, the dynamics and yield of nutrient utilization and the kinetics of growth in an isolated culture, often with species classification and naming as an end goal (9, 19). In this context, the zymogenic/autochthonous dichotomy was mainly defined in relation to the ability to grow



in media that are rich/poor in a growth-limiting nutrient and was gradually replaced in most works by the terms copiotrophic/oligotrophic (13–15). Since the amount of growth-limiting nutrient(s) became the main experimental variable, the species interactions and the temporal and spatial patterns of nutrient availability that Winogradsky focused on, gradually lost weight in the discussion. For example, it was reasoned that it is more appropriate to define an habitat in terms of the average flux of limiting nutrients rather than in terms of natural nutrient fluctuations (15).

The copiotroph/oligotroph dichotomy changed over time into three distinct conceptualizations. Here, we revisit each conceptualization and add new interpretations in the context of Monod curves. Oligotrophs were first defined in an operational manner as those water dwelling microbes that grow at low concentrations of growth-limiting nutrients (up to 10-15 milligrams of carbon per liter of culture medium) (14). Conversely, copiotrophs were understood to thrive at higher growth-limiting nutrient concentrations (14). Since nutrient availability can fluctuate in both nutrient-rich and nutrient-poor environments, copiotrophic microbes are said to live feast-and-famine cycles, while oligotrophic microbes are said to live fast-and-famine cycles (15). These definitions have been interpreted in three main ways, which we revisit here in the context of Monod curves, i.e the relationship between the specific growth rate (sometimes called relative growth rate) of a microbe during balanced growth of an isolated culture as a function of the concentration of a limiting nutrient (20). The specific growth rate of a microbe is normalized relative to biomass in the culture and can be used to compare the growth of different microbes.

The first interpretation, which we propose to call exclusive copiotrophs/oligotrophs, considers each class of microbes separately. For a given limiting nutrient, exclusive oligotrophs grow faster at low limiting nutrient concentrations, while higher nutrient concentrations inhibit their growth (13) (Figure 1, top right panels). In turn, exclusive copiotrophs grow well at high limiting nutrient concentrations and die out at lower nutrient concentrations. We understand the exclusive copiotrophic/oligotrophic dichotomy as a description of archetypal extremes of behavior rather than a description of the full spectrum of natural microbes (14, 21, 17). This dichotomy led to the proposal of a trade-off in microbial specific growth rate at low versus high substrate concentrations (13, 14). We choose to represent this trade-off as a straight line in the absence of strong evidence about its shape. In our view, we expect a specific microbe growing in a certain environment to be located at a specific point in the graph. This location may change in a different environment due to physiological plasticity. We represent this view in the figure by plotting an arbitrary number of points along the trade-off line.

The second interpretation, which we propose to call coexisting copiotrophs/oligotrophs, compares the two classes of microbes in the two nutrient regimes (16) using the dependence of specific growth rate on the concentration of a certain limiting nutrient measured for each organism in isolation (Figure 1, bottom left panels). In this competitive interpretation, both classes can grow in both nutrient-poor and nutrient-rich media (15), and their association to different nutrient regimes comes from the fact that coexisting copiotrophs have a higher maximal growth rate than coexisting oligotrophs but attain it at higher concentrations of growth-limiting nutrients. In this case, the dominant trade-off seems to be the one between specific growth rate and yield. In the extremes of the spectrum, oligotrophs grow slowly but use resources efficiently and copiotrophs grow fast at the expense of the efficiency of carbon use (17). In this sense, the coexisting copiotrophic/oligotrophic dichotomy would also be the extremes of a full spectrum, which we represent along a straight line. A specific microbe growing in a certain environment to be located at a specific location in the graph, which may change in different environments due to physiological plasticity.

We can derive a third definition for the copiotrophic/oligotrophic dichotomy from the correlation reported by Weissman and coworkers between maximal growth rate and genomic codon usage in highly expressed genes (18). Analysis of a diverse database of microbes led to the proposal that selection for rapid growth shapes the genomes for copiotrophs, but not oligotrophs, by optimizing codon usage for more efficient translation (18). As a result, codon usage patterns can be used to predict maximal growth rate without taking into account environmental conditions such as the availability of a given limiting nutrient (18). We propose that there is a plausible scenario in which optimization of codon usage for efficient translation affects growth regardless of limiting nutrient concentration. In this case, copiotrophs would grow faster than oligotrophs under all conditions in a Monod curve (Figure 1, bottom right panel). We may call these two groups fast- and slow-growing microbes.

The expected differences in physiology and genome evolution between oligotrophs and copiotrophs are different for exclusive copiotrophs/oligotrophs, coexisting copiotrophs/oligotrophs and fast/slow growing microbes. The growth of exclusive oligotrophs may be inhibited in nutrient-rich media due to, for example, a rising internal osmotic pressure due to increased transport or accumulation of toxic metabolites (13, 14). In turn, exclusive copiotrophs may die in nutrient-poor media because of high maintenance costs, synthesis reactions reversing their flow under energy starvation or other factors (13, 14). Then again, the proposal of coexisting copiotrophs/oligotrophs leads us to search for high-affinity nutrient uptake systems, a larger surface-to-volume ratio and streamlined information-processing machineries in oligotrophs compared to copiotrophs (14–17, 22, 23). Last, the dichotomy of fast- versus slow-growing microbes has been related to optimization of gene transcription and translation (18, 24).

It was soon noticed that no single interpretation of the copiotrophic/oligotrophic dichotomy is able to fully describe the complex behavior of natural communities. Some microbes are exclusive copiotrophs/oligotrophs, while others are coexisting (15). The proposed trade-off in microbial specific growth rate at low versus high substrate concentrations for exclusive copiotrophs/oligotrophs is not universally present (25), while the trade-off between growth rate and yield for coexisting copiotrophs/oligotrophs (17) remains to be tested extensively. In addition, optimization of translation efficiency alone is a poor predictor of the maximum specific growth rate of slow-growing microbes (18). Under this light, it is perhaps not surprising that the genomic traits reported by different studies to correlate with the copiotrophic/oligotrophic dichotomy are only partially overlapping (17, 22, 23, 26). Compounding the problem, we are not even considering the existence of multiple growth-limiting nutrients, interactions between species, how the behavior of microbes may vary according to physical variables such as temperature, pH or osmolarity and how these variables interplay with nutrient acquisition and metabolism.

## Outlook

The multiple meanings associated in the literature with the zymogenic/autochthonous and copiotrophic/oligotrophic dichotomies have been described as a “confusing” (27) and “murky” (18) situation. Some scenarios depicted in Figure 1 are indeed mutually incompatible. These dichotomies can be understood not only in terms of the traits associated to each category but also in terms of the relationship between the specific growth rate in isolated cultures and the concentration of one or more growth-limiting nutrients (Figure 1). This last approach can help us formulate models, deduce contrasting predictions for the dichotomies, and design future laboratory and field experiments using current techniques such as high-throughput growth measurements, metagenomics and meta-metabolomics. In turn, this may lead to a consensus for use of these terms and a deeper characterization of the diversity and biology of natural microbial communities (27).

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## **Conflict of Interest**

The authors have no conflict of interest to declare



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