

The Role of Microbes in Agriculture: Sergei Vinogradskii's Discovery and Investigation of Chemosynthesis, 1880–1910

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Abstract. In 1890, Sergei Nikolaevich Vinogradskii (Winogradsky) proposed a novel life process called chemosynthesis. His discovery that some microbes could live solely on inorganic matter emerged during his physiological research in 1880s in Strassburg and Zurich on sulfur, iron, and nitrogen bacteria. In his nitrification research, Vinogradskii first embraced the idea that microbiology could have great bearing on agricultural problems. His critique of agricultural chemists and Kochian-style bacteriologists brought this message to the broader agricultural community, resulting in an heightened interest in biological, rather than chemical methods to investigate soil processes. From 1891 to 1910, he directed the microbiological laboratory at the Imperial Institute of Experimental Medicine in St. Petersburg, Russia, where he expanded his chemosynthesis research to a broad investigation of the manifold significance of autotrophic organisms in soil processes. This work and that of his students attracted the serious attention of agricultural chemists and soil scientists in Russia and abroad, changing essentially the way they understood and investigated the role of microbes in the soil. His student, Vasilii Omelianskii, effectively integrated Vinogradskii's approach into Russian and Soviet, and international agricultural microbiology. Vinogradskii's activities in the late 19th century reflect the changes occurring more broadly in science. At that time, microbiologists such as Louis Pasteur, Eugenius Warming, and Martianus Beijerinck were contributing new laboratory methods and theoretical perspectives to incipient disciplines closely related to agriculture: ecology, soil science, and soil microbiology.

Keywords: agricultural chemistry, autotrophism, chemosynthesis, microbiology, nitrification, soil science, Sergei Vinogradskii, Vasilii Omelianskii

Intended especially for the theoretical study of [nitrogen] fixation
these biological tests thus have an interesting agricultural

application: being very simple and sensitive, they can provide the agronomist with information that he finds impossible to collect by chemical analysis.

(To the Seventh Congress of Industrial Chemistry, Paris.)
Sergei Vinogradskii, 1927¹

Introduction

In 1890, Vinogradskii (1856–1953) solved one of the pressing mysteries of agricultural science. Drawing on his skills in plant physiology and microbiology, he teased apart the complex biological processes involved in nitrification – the conversion of ammonia to nitrates and nitrites in the soil. Agricultural chemists had proposed various chemical explanations for how nitrogen was converted from a free state in the atmosphere into a useable form for plant nutrition. That Vinogradskii's explanation superseded those of the chemists, reflected an important movement in the agricultural sciences toward reliance on biological investigations and marked a new level of experimental sophistication in solving agricultural questions.

Nitrification had attracted Vinogradskii's attention during his investigation of autotrophic microorganisms and discovery of chemosynthesis.² In 1890, he described chemosynthesis as a new feature of life processes – just as energy entered the “cycle of life” through photosynthesis, so it was produced from inorganic matter by chemosynthesis. He arrived at this conclusion by bringing together an original constellation of scientific techniques. His discovery captured the attention of the world scientific community, and especially that of agricultural scientists. He thus became a representative of the generation of plant physiologists, who in 1880s – pursuing the lines of investigation recently opened by Louis Pasteur – were developing microbiology into an agricultural discipline.

¹ Winogradsky, 1927, p. 10.

² By 1894, Vinogradskii had accepted the term “chemosynthesis” (coined by Pfeffer) in place of his original “anorgoxydantism.”

From Europe's Bread Basket to the Music of Science

Vinogradskii's roots were in Russian agriculture. His father had amassed substantial wealth and property as president of the first Bank of Commerce in Kiev, which placed the family in the upper crust of the landed gentry. The family also enjoyed the prestige and wealth of his mother's Skorpanskii lineage, which had held an eminent position in the region for centuries. They lived on a sprawling, 5000 acre estate near Kiev in the Ukraine, the heart of Russia's "black-earth" region and Europe's renowned 'bread basket.' As part of the agricultural community, the family estate also harvested lumber, bred horses, and helped develop Russia's newest industry – sugar beets. Vinogradskii maintained a lifelong interest in the family estate and problems of agriculture, but was uninspired by the prospect of life as a landed gentryman. Upon graduation from gymnasium at 17, he "looked down the long empty road of his future" and struggled to choose between three possible lives: as *pomeshchik* (landed-gentry 'farmer'), a virtuoso pianist, or a 'professor' in the natural sciences.³

His dilemma exemplified that of many other gentrymen in post-Emancipation Russia, where a new social structure was emerging. The gentry was in decline and adolescents, like Vinogradskii, were searching for new professional niches.⁴ Gymnasium had left him with "no interests, no strivings" and had aroused "no vocations."⁵ After a series of disappointments – Law was "deadly boring," the natural sciences enjoyed little prestige in his provincial society, and his musical genius failed to impress anyone at the St. Petersburg Conservatory – he eventually entered the Department of Natural Sciences at St. Petersburg University. There he found his place. After studying with Dmitri Mendeleev – famous for creating the periodic system of elements, but in Vinogradskii's experience a poor lecturer – he walked into Andrei Famintsyn's plant physiology laboratory. Famintsyn's charismatic personality, lively lecture style, and exciting combination

³ Vinogradskii, (Undated). When Vinogradskii referred to "professor" here he was thinking along the lines of a Dmitri Mendeleev – a professor who taught courses, and conducted research in their own laboratory. Biographical treatments of Vinogradskii include: Omelianskii, 1927 and Waksman, 1953. Recently a new work appeared containing the above works by Omelianskii and Waksman; Mazing, et al., 2002.

⁴ These changes were the subject of Turgenev's, *Father's and Sons*, which is often taken as a reflection of the divide in generations over the place of science in Russian society. Bailes, 1990, pp. 1–5; Miliukov, 1962, pp. 270–273.

⁵ Vinogradskii, 1949, p. 4.

of natural history and laboratory investigation renewed Vinogradskii's interest in science.⁶

Vinogradskii's First Investigation: An Agricultural Problem?

Vinogradskii's agricultural background suited him well as a student in the Department of Natural Sciences at the St. Petersburg University. When he entered the university in the late 1870s, the Tsarist government was increasing its support for soils studies in the face of massive and destructive droughts in Southern Russia. He also soon learned that agriculture was a prominent theme in his chosen fields – chemistry, plant physiology, and microbiology – all of which were undergoing dramatic changes. For example, the Russian botanists with whom he trained, like their Western European counterparts, were expanding botany to include the study of plant nutrition and physiological laboratory techniques. Moreover, Famintsyn, who was Russia's leading plant physiologist, encouraged Vinogradskii to pursue microbiology – a field that Pasteur had recently raised to a new level of methodological sophistication. This combination of plant physiology and microbiology provided Vinogradskii the technical skills and theoretical orientation that would lead him to discover chemosynthesis.

Famintsyn founded his physiological approach on the idea that microbial nutrition is a thermodynamic process. Vinogradskii adopted both Famintsyn's perspective and his novel combination of observational skills from natural history and experimental techniques from physiology.⁷ Famintsyn applied the thermodynamic approach in his own research, using artificial light produced by kerosene lamps

⁶ Later Vinogradskii would add that "since the experimental sciences are completely based on observations and experiments, the main method to introduce beginners in any of the sciences is ... only by way of observations and experiments, which should be carried out in parallel with theory, and best of all if a little bit beforehand; this is in order that the participant can familiarize himself, if only a little, with the materials before hearing in detail the theory of a given field." Ibid.

⁷ Famintsyn had initiated this research in mid-1860s at the same time as other early plant physiologists such as Wilhelm Pfeffer and Julius Sachs. Later Vinogradskii would add that "since the experimental sciences are completely based on observations and experiments, the main method to introduce beginners in any of the sciences is ... only by way of observations and experiments, which should be carried out in parallel with theory, and best of all if a little bit beforehand; this is in order that the participant can familiarize himself, if only a little, with the materials before hearing in detail the theory of a given field." Vinogradskii, (Undated), p. 9.

(necessitated by the rarity of sunlight during St. Petersburg's dark winters) to measure precisely the amount of energy needed by plants.⁸ That is, he could study a plant's nutrition thermodynamically by comparing the energy input (from light) to output (plant growth). This thermodynamic view lay at the core of his teaching and laboratory-based investigations, and it also provided him a theme by which to organize all of nature: "[t]he exchange of matter and the transformation of energy," united the living world into a holistic entity and "are among the main functions of every living being; inseparably connected to them are all life functions, not only in the animal, but also in the plant organism."⁹ Vinogradskii imbibed this view and Famintsyn's practical lessons at the laboratory bench, and applied them to his other primary scientific interest, microbiology. Employing a thermodynamic view of life to investigate the organisms of Pasteurian microbiology prepared Vinogradskii to imagine the existence of life based solely on inorganic matter.

A second important dimension to Vinogradskii's research style came not from his mentors in the university, but rather from foreign scientific literature.¹⁰ Where the former awoke his interest in Pasteurian microbiology, the latter revealed a world rich in alternative research techniques and instruments.¹¹ The highlights of his readings were Pasteur's investigations of microbial agency in nature's most curious phenomena: fermentation and decomposition.¹² Pasteur's *Etudes sur la vinaigre*, *Etudes sur la vin*, and especially his *Etudes sur la biere* became Vinogradskii's primary reference books. In these, Vinogradskii encountered not only an array of laboratory apparatus that facilitated his investigation of microbial nutrition – and which he could purchase at a fancy downtown glassware store – but also the organism upon which he focused his first extensive investigation. Here he also encountered Pasteur's notion that fermentation might extend to soil processes.

⁸ Famintsyn, 1865, pp. 39–56; Senchenkova, 1981, pp. 86–109; on Sachs and Famintsyn's race see idem., pp. 88–92; and on the kerosene lamp work see idem., pp. 91–94 and Strogonov, 1996, pp. 39–41.

⁹ Famintsyn, 1883, p. 10.

¹⁰ As a wealthy student, he could afford to build an extensive personal library and collection of laboratory instruments.

¹¹ Pasteur had become a hero in Russia for his well-publicized rabies treatment. In this account, he had administered his treatment successfully to a group of Russian peasants sent to Paris by the Tsar. Geison, 1995.

¹² "It is no wonder," he wrote, "that Pasteur's works occupied the pivotal spot in my library," which "were at the time still novel." Vinogradskii, (Undated), p. 10.

Pasteur's initial microbiological work, which he conducted, in part, to improve France's wine, vinegar, and beer fermentation industries, demonstrated the agricultural importance of that science. In 1870s, these investigations entered the scope of agriculture as possible explanations of chemical soil processes, such as the formation of nitrogen and ammonia. European agricultural chemists and plant physiologists soon came to investigate these soil processes not as chemical transformations, but as biological phenomena. In their research, they increasingly viewed nature's elements as nutrients that operated in a circulatory exchange of matter and energy. The historian of science and medicine, Andrew Mendelsohn has recently demonstrated the inherently "ecological" approach of the Pasteur school, as well as the thoroughly agricultural context in which Pasteur's microbiology flourished.¹³

In 1881, Vinogradskii began a 2 year study that would serve as his Master's degree research project. Here his agricultural experiences as a young man in Kiev resurfaced and stimulated his choice of experimental organism. Pasteur had described the activities of numerous microorganisms, but Vinogradskii was already well-acquainted with one, *Mycoderma vini*, a common yeast that was a nuisance to the sugar beet industry.¹⁴ Although Vinogradskii would soon study other non-agricultural organisms, the experimental skills that he employed to investigate the physiology of this yeast and the theory by which he interpreted his results, would provide the framework for his later contributions to agriculture. In this investigation, he applied Famintsyn's laboratory regimen for studying plant nutrition thermodynamically and employed the equipment used so effectively by Pasteur. Specifically, Vinogradskii synthesized the thermodynamic vision with a laboratory-based experimental program organized around a strong inventory of flexible techniques and a conceptual and rhetorical strategy of direct observation. Here he eschewed and criticized the suspect, deductive reasoning of previous researchers. For example, Karl Nägeli had initially impressed Famintsyn and Vinogradskii with an extensive array of parallel experiments designed to determine the relationship between a microbe's morphology and its nutrition. Nägeli lost the confidence of his

¹³ Mendelsohn, 1996.

¹⁴ The details of his first research project fall out of the bounds of this essay. For an analysis of this investigation, see Ackert, 2004, pp. 50–55. It is significant, however, that by selecting this organism, he could apply the new laboratory skills he was learning to an agriculturally significant problem; *M. vini* also caused disease in sugar beets. Although his research offered no application for alleviating this problem, it demonstrated that from the outset Vinogradskii related his scientific research to agricultural questions.

Russian colleagues, however, because he failed to control his cultures for contamination.¹⁵ In St. Petersburg, Vinogradskii pursued this thermodynamic explanation only at the level of the internal workings of an organism. During the next stage of his career he would explore its full ramifications by investigating the ‘thermodynamics of life’ in its global, ecological context.

Exotic Physiological Types: Vinogradskii’s research with Anton de Bary

After earning his Master’s degree in 1884, Vinogradskii strove to expand the horizons both of his professional career and his scientific investigations.¹⁶ Beginning in 1887, he began a 2 year internship at the University of Strassburg with Anton de Bary, whose reputation as a pioneer of the new botany was attracting many foreign students to his courses and laboratory.¹⁷ In St. Petersburg, Vinogradskii – constrained by what he came to see as the rudimentary conditions of Famintsyn’s laboratory, and the mundane physiology of *M. vini* – had investigated the thermodynamics of microbial life only as a laboratory phenomenon. In Strassburg, he employed the same microcultural methods with which he had studied *Mycoderma* nutrition. He now used them, however, to explore a new kind of experimental object suggested to him by De Bary – a genus of bacteria known as *Beggiatoa*. Where *Mycoderma* was a species of common cultivated yeast, *Beggiatoa* inhabited one of nature’s most exotic places – sulfur springs. Drawing Vinogradskii out of the physical

¹⁵ In this research, Nägeli offered a new version of Liebig’s mechanistic theory of fermentation that reconsidered the role of fungi in the process. For Nägeli, the fermenting fungus’ “living plasma” acts directly on its surrounding environment by transferring “the molecular vibrations that are inherent in its atoms and molecules.” These vibrations then produce “shakings” that cause sugar particles to decompose into smaller compounds. Famintsyn, 1884, pp 228–230; Nägeli, 1879, p. 174.

¹⁶ When Vinogradskii completed his Master’s thesis in botany in the fall of 1884, he decided, like many of his generation, that to promote his career, he needed the professional credentials of a Western European education. In part, he studied in Western Europe and not in St Petersburg to avoid the harsh climate of the northern city and because his extensive personal wealth afforded him the ability to. Vinogradskii, (Undated), p. 13. It also was common for students (Russian, American, or other) at his stage of development to spend a couple of years training in Western Europe.

¹⁷ His specific choice of the University of Strassburg no doubt owed much to Famintsyn, who had himself studied with De Bary in the late 1850s. Vinogradskii might also have worked with Simon Schwendener at the University of Berlin, Gottlieb Haberlandt in Tübingen, and Eduard Strasburger in Bonn, to name only a few (see Cittadino, 1990 for a discussion of these schools).

confines of the laboratory, his experiences with *Beggiatoa* taught him to acknowledge and then exploit the interrelationship between laboratory investigations and free nature. This perspective would play a crucial role later, when he would apply microbiology to agricultural questions.

At De Bary's request, Vinogradskii set out to investigate recent challenges to his new mentor's assertion that a class of microscopic fungi (including *Beggiatoa*) maintained constant life cycles. If Vinogradskii could corroborate De Bary's claim, the next task would be to establish a new classification system for these organisms. Vinogradskii's assigned project was central to De Bary's own interest in investigating the stability of microorganism species. Vinogradskii was intimately familiar with this question, and no doubt consented to pursue it because it formed the core issue in a long-standing debate over the fundamental nature of microbes.

The microbiologists who participated in this dispute are as difficult to classify, perhaps, as the microorganisms they studied, but they can be generally divided into two groups – the monomorphists and pleomorphists. Tending to see nature as a stable collection of species, monomorphists identified species of microorganisms according to the mature stage of their relatively regular life cycles. They thus classified the extraordinary variety of microbial life much like the higher plants and animals – separated into numerous species. Pleomorphists, on the other hand, accounted for this variation in microbial shape by postulating the extreme plasticity of only a few species. Pleomorphists believed that these few species (some proposed that only two existed) changed shape in relation to the conditions of existence; consequently, for them any microbial taxonomy was at best provisional. Vinogradskii's own views and practices intersected these debates only tangentially.¹⁸ Rhetorically he supported a staunch monomorphist position – advocating for the views of Ferdinand Cohn and De Bary against those of the pleomor-

¹⁸ With few exceptions, previous portrayals of Vinogradskii's discovery of new physiological types and later chemosynthesis have relied too heavily on his own portrayal of the events, especially as related in his autobiographical remarks. Most historians have drawn primarily on *Microbiologiia Pochvy* (the Russian translation of the French compendium of his life's work), or his own account of this period in other autobiographical writings. Few, it seems have spent time with the original German and French published reports which contain a wealth of information, relative both to his scientific efforts and personal history. It is difficult to tease out the multiple influences on his research during this period. On the monomorphism–pleomorphism debate see Ackert, 2004, pp. 73–87; Amsterdamska, 1988; Mazumdar, 1995, pp. 46–59; Penn and Dworkin, 1976; Strick, 2000, pp. 123–128; and Vinogradskii, 1937.

phists, as expressed primarily by Ray Lankester, Eugenius Warming, Karl Nägeli, and Wilhelm Zopf.¹⁹

Initially adhering to the prescribed methods based on Robert Koch's postulates, Vinogradskii tried to cultivate the wild *Beggiatoa* samples he had collected in a series of artificial solutions.²⁰ Frustrated, however, by *Beggiatoa*'s inability to grow in these standard cultures he decided to investigate their nutritional requirements.²¹ As these experiments progressed he relied increasingly on the repertoire of techniques he had developed in Famintsyn's physiological laboratory – e.g. growing cultures on microscope slides, in retorts, and in parallel trials. His research led him to the surprising conclusion that *Beggiatoa*'s most intriguing characteristic – the appearance and disappearance of sulfur granules in their filamental bodies – was determined by its nutritional process. That is, where others considered the sulfur a coincidental morphological characteristic, he viewed it as the organism's defining physiological trait – *Beggiatoa* could convert poisonous hydrogen sulfide into food.²²

In the process of this research, Vinogradskii came to believe that he was uncovering a fundamentally novel phenomenon – the existence of autotrophic physiological types. Encouraged by the success of his methods, he applied them to other organisms, subsequently finding that iron bacteria and nitrogen bacteria also possessed the ability to live on inorganic matter (ferrous oxide and nitrates respectively). Investigating the role of these organisms in nature led him to define “a new kind of life,” what he called “anorgoxydantism,” by which

¹⁹ Whether it was solely due to De Bary's encouragement or whether the nature of the debate itself suggested it, Vinogradskii focused his attacks primarily on Zopf, one of the most outspoken pleomorphists and an outspoken critic of De Bary.

²⁰ Winogradsky, 1887, p. 569. Post-Kochian bacteriology mandated that a successful morphological investigation of bacteria required the use of the pure liquid and gel cultures that had become standard in 1880s.

²¹ Ibid. In his published account, Vinogradskii claimed that he was forced to adopt a physiological approach at this juncture in his investigation. It is clear, however, that his investigation, since its inception, was at its core physiological.

²² The “extraordinary speed and constancy with which *Beggiatoa* stored sulfur” led Vinogradskii to conclude “that the production of sulfur granules occurred only through the oxidation of hydrogen sulfide.” Winogradsky, 1887, p. 505. Unlike in his St. Petersburg investigation, Vinogradskii had the experiences of observing the behavior of his organism in its natural environment. He correlated his laboratory findings with *Beggiatoa*'s growth patterns in its indigenous settings – swamps, marshes, bogs, and sulfur springs on steep Alpine slopes. The organisms lived in these exotic places because there they found the sulfur they needed to live. For him this was no mere coincidence. *Beggiatoa* were fulfilling their specific role in nature – the decomposition of sulfur.

organisms could oxidize inorganic matter unassisted by the energy of either light or organic material. For him, these autotrophic organisms were the biological agents that transferred matter between nature's inorganic and organic realms – that is, his new “physiological types” were essentially direct links between nature's biological and mineral realms. It was one thing to understand the general roles these microbes played in nature, and quite another to suggest that these roles might be used to manage specific environments, such as agricultural soils. Vinogradskii would make that leap the following year, when, upon De Bary's death, he moved to that great center of agricultural chemistry, Zurich, Switzerland. His frustrations and ultimate successes during his Strassburg research had taught him one great lesson: to bring a thermodynamic perspective to microbial physiology he would need to improve his chemistry skills.²³

Chemosynthesis as an Agricultural Method

Chemosynthetic organisms dominated Vinogradskii's research for the next decade, during which he underwent three dramatic transformations: his research interests shifted from plant physiology to agricultural microbiology, he developed his taxonomically oriented concept of autotrophic physiology into a new definition of life itself – as “chemosynthesis;” and his career emerged from relative obscurity into the scientific limelight. These changes were set into motion primarily by his work in Zurich, where he focused his efforts on the nitrogen bacteria and their significance for agricultural soil processes.

Vinogradskii's work in Zurich's extensive scientific community changed his research priorities: he expanded the physiological approach he had learned in St. Petersburg – that is, correlating natural chemical processes with microbial agents – to include organic and physical chemistry. At the Swiss Polytechnic Institute he worked with two chemists who, like himself, would fundamentally change the nature of nitrification research.²⁴ Arthur Hantzsch – who would become one of

²³ Zavarzin, 1989, pp. 5–21.

²⁴ Although he later denied that the directors of these laboratories influenced his work in any significant way, it seems very likely that Vinogradskii's increased sophistication in chemistry can be attributed only to this Zurich experience. His disavowal can be ascribed to his claims in priority for discovering chemical autotrophism. Much of his scientific legacy – especially for later founding an ecological microbiology – was based on his 1890s papers.

Europe's top analytical chemists – taught Vinogradskii to challenge the strict demarcation of the inorganic and organic realms.²⁵ Hantzsch developed this perspective in his stereochemical investigation of organic nitrogen compounds, and Vinogradskii adapted it to microbiology. The physical chemist, Ernst Schulze trained Vinogradskii to study nitrification more effectively using physiological and agricultural chemistry, and to consider bacterial nutrition as an agricultural question.²⁶

The Background to Vinogradskii's Nitrification Studies

Over the course of his nitrification work in Zurich, Vinogradskii transitioned from plant physiology to an agriculturally informed soil microbiology. Nitrification – the study of how ammonia is transformed (oxidized) into nitrates and nitrites in the soil – became a pressing topic of scientific research in the late 19th century, especially after it had been shown to be a biological phenomenon. When Vinogradskii took up this subject, however, scientists had yet to reach any consensus about the nature of this phenomenon.²⁷ The various schools of agricultural chemistry, plant physiology, and microbiology differed in terms of disciplinary criteria and methodologies, essentially debating whether to prioritize the chemical, biological, or physical dimensions of their investigations. Vinogradskii's survey of this literature, turned up a number of recent investigations that considered the biological nature of nitrification.

²⁵ Hantzsch's studies of the stereochemistry of organic nitrogen compounds and the integration of physico-chemical methods of measurement in analytical chemistry contributed to a new understanding of the relationship between inorganic and organic compounds. On Hantzsch's life and work see Stocklöv, 1998, pp. 18–52. One of the works that led to his wide influence on the history of chemistry was Hantzsch, 1890.

²⁶ Zavarzin, 1989, p. 13. Schulze was primarily concerned with understanding how and in which phases organic substances are formed from carbonic acid, ammonia, and water. His work on the production of nitrogenous compounds during the seed germination provided helped found phyto-chemistry. Meyer, 1898, p. 536. On Vinogradskii in Ernst Schulze's laboratory see Zavarzin, 1989, pp. 13–14. Schulze applied physical chemistry methods to investigate agricultural problems. He published several articles on a variety of subjects: Schulze, 1888, p. 525; idem, 1895, pp. 394–396.

²⁷ The source of Vinogradskii's initial interest in nitrification is unclear. Possibly, as Zavarzin thought, it was likely that Vinogradskii's interest was aroused by the polemics surrounding Guyon and Dupetit's denitrification work. These French agricultural chemists succeeded in isolating the organisms responsible for denitrification – a process that many feared might lead to soil infertility during the application of fertilizers, since this process converts soil nitrogen compounds back to elemental nitrogen gas, which then escapes to the atmosphere. Vinogradskii might have thought that a better understanding of the reverse process, nitrification, would prove helpful. Zavarzin, 1989, p. 12.

In 1870s, Jean Jacques Schloesing and Müntz were the first to investigate nitrification as a biological phenomenon.²⁸ As Professor of Agricultural Chemistry at the newly founded Institute National Agronomique in 1875, Schloesing was assigned to determine the feasibility of transporting sewage from Paris to the French countryside for irrigation and fertilization purposes.²⁹ With Müntz, he set out to investigate whether the presence of humus (organic matter consisting primarily of decaying plants) in the soil was necessary to purify the sewage water.³⁰ Defining purification as complete nitrification, they measured how long it took for all the ammonia to disappear. Surprised by the long-delay before oxidation even began (20 days) they surmised that nitrification was not caused merely by access to “active oxygen” (a chemical explanation) but rather by the action of intermediary “organized ferments.”³¹ To test the biological agency, they used boiling temperatures and chloroform gas to create laboratory conditions deadly to living beings – nitrification did not occur.³² They concluded their first nitrification report with the simple statement that it only remained “to discover and isolate the nitrifying organisms.” Their best efforts over the next year using Pasteur’s methods, however, succeeded in describing these organisms only by the vague term “*ferment nitrifique*.”³³

Between 1879 and 1881, Robert Warington, an agricultural chemist at the Rothamsted Agricultural Experiment Station in England, approached nitrification as an agricultural problem. He confirmed the conclusions of Schloesing and Müntz, but went a step further. He established that soil nitrification was a two-stage process in which bacteria first convert ammonia into nitrites and subsequently into nitrates.³⁴ Although Warington had studied bacteriological techniques with the Austrian bacteriologist Eduard Emanuel at London’s Brown Institution, he failed to isolate the nitrifying organisms.³⁵

²⁸ In 1877, Schloesing and Müntz demonstrated that nitrification is a biological process by using chloroform vapors to inhibit the production of nitrates. Schloesing and Müntz, 1877–1879. On the contributions of Schloesing and Müntz to the history of microbiology, see: Doetsch, 1960, pp. 103–107, and Waksman, 1932, pp. 62–63. For a discussion of the relationship between soil chemistry and plant growth see Ihde, 1964, pp. 420–426.

²⁹ Doetsch, 1960, p. 103.

³⁰ Schloesing and Müntz, 1877, 84(7): 302–303.

³¹ Ibid., p. 302.

³² Ibid., p. 303.

³³ Idem., 89(21): 892.

³⁴ Waksman, 1932, p. 63.

³⁵ Doetsch, 1960, pp. 156–157.

Hereaus took up this line of research and in 1886 claimed that he had succeeded in isolating the organisms.³⁶ In soil, water, and air cultures rich in ammonia and organic nitrogen, he succeeded in observing nitrification. Using standard pure culture methods, he isolated the bacterial species living in his cultures, two of which, he found, produced intensive nitrification. Vinogradskii, however, questioned the sterility of Hereaus's cultures and pointed out that the nitrification Hereaus had detected was most likely due to "an inadvertent contamination of sulfates and sulfites, which float in the air at all times."³⁷ Although he was critical of the methods and conclusions of this literature, it taught Vinogradskii that nitrification was primarily a biological and agricultural problem, a lesson he confirmed in Schultze's laboratory. He became so devoted to this view, that he criticized even the biologically minded "French-chemists" for relegating biology to mere handmaiden status. He was also dismayed by microbiologists for too strictly adhering to the standardized "approved methods."³⁸ Thus, the biological understanding of nitrification was limited to the claims of a few researchers who accepted that microbes of some kind caused nitrification in the soil. The nature of these organisms remained obscure.

For Vinogradskii, these previous investigations failed to satisfy his most important criterion – to identify the precise organism physiologically responsible for nitrification.³⁹ With the experience of his sulfur and iron bacteria research, his new chemical training in Zurich, and his background in Russian plant physiology he engaged the question from a novel perspective. Synthesizing the "thermodynamics of life" approach that he learned from Famintsyn with the advanced chemistry he studied at Zurich, Vinogradskii set out to explain the mystery of nitrification according to the vital activity (nutrition and respiration) of specific microbiological agents.

A New Method: "Elective Cultures"

Investigating nitrification led Vinogradskii to develop the "elective culture" method; specific yet flexible, it would become the workhorse of his technical repertoire. He devised elective cultures in order to isolate

³⁶ Hereaus, 1886, pp. 193–234.

³⁷ Winogradsky, 1890, 4: pp. 215–216.

³⁸ Ibid., p. 220.

³⁹ Ibid., pp. 215–231, 257–275, 760–811, and idem., 1891, 5: 92–100, 577–616. He also abstracted these articles for presentations to the French Academy of Sciences: Winogradsky, 1890–1891. See also Koch, 1891, pp. 669–672, 680–685, 698–701.

nitrifying organisms, a goal that continually had frustrated him. Like the investigators mentioned above, he initially relied on standardized bacteriological methods and laboratory-grown stocks, and like them, he failed to induce strong nitrification.⁴⁰ His previous experience growing successful cultures of sulfur and iron bacteria inspired him to try a new approach – one guided by his view that laboratory investigations had to be correlated with an organism's role in nature. He designed a protocol for exploiting the tight physiological relationship he believed organisms shared with their nutrient environment. First, he would search for the liquid culture most favorable to nitrification. Second, he would strictly maintain these cultural conditions while conducting a series of transplants designed to eliminate the species not adapted to those conditions. Third, having reduced the number of species in the culture as much as possible while maintaining an intense level of nitrification, he then could isolate the single or multiple nitrifying species. Subsequently, he would be able to test the nitrifying abilities of each isolated species in pure cultures.⁴¹ Following this careful plan he attempted cultivations in several solutions; yet still he failed to achieve significant nitrification.

The solution came when Vinogradskii combined his Strassburg physiological research with his Zurich chemistry training – a synthesis that marked a key event in his development as an agricultural microbiologist. Strassburg had taught him to shun the laboratory's stores for fresh samples of Zurich soils. To these little clumps of free nature, he applied his new skills in analytical and physical chemistry. After numerous attempts to create a "liquid nutritive environment" suitable for nitrification, he finally succeeded, but only inadequately.⁴² In a breakthrough moment conditioned by his previous research "the idea came to [him], to eliminate all organic matter" from his culture, which immediately produced intensive nitrification.⁴³ In this event, the lessons learned in Strassburg and Zurich coalesced in a new understanding of nature's life processes. To oxidize all three materials – hydrogen sulfide, ferrous oxide, and ammonia – bacteria, he concluded, required no organic matter, which, in fact, it actually impeded it. Henceforth, he grew his nitrogen bacteria cultures without organic matter – on a simple mixture of "very pure water" and inorganic ammonia salts.⁴⁴ What previously seemed like abnormal and artificial growth conditions, in the

⁴⁰ Winogradsky, 1890, 4: 220–221.

⁴¹ *Ibid.*, p. 222.

⁴² He did succeed in producing faint nitrification. *Ibid.*

⁴³ *Ibid.*

⁴⁴ He procured his water from Lake Zurich. *Ibid.*, p. 223

light of his work on other exotic microorganisms, now seemed natural – he had simply refined the media to its most basic and necessary contents. This discovery vindicated Vinogradskii's strategy in coming to Zurich for advanced schooling in chemistry.

The key to solving the enigma of nitrification would be his skills in biology. The keen observational abilities he had developed in his physiological and microbiological research would reveal to him previously undiscovered details of bacterial activity. To explore how nitrogen bacteria negotiated their inorganic environment he juxtaposed macroscopic observations of landscapes with microscopic observations of communities and individuals. He had noticed with unaided eyes that the retorts were divided into distinct areas: at the surface a cloud formed, and on the bottom where the ammonia salts collected there were two fine layers: a darker one and a perfectly white one.⁴⁵ Over time, this white layer “changed strangely,” turning grayish in color and gelatinous in consistency.⁴⁶ He sought an explanation for this geographical variation at the microscope level. The example of his *M. vini* investigation suggested that the organisms responsible for oxidizing ammonia would exist only in the top layer.⁴⁷ None of the microbes he found there, however, were able to provoke nitrification on their own. Then, his eyes drawn to a peculiar layer at the bottom of the retort, he noticed transparent lumps of ammonia salt covered with “thick groups of beautiful oval bacteria.”⁴⁸ He strongly suspected that he had found his agent of nitrification.

Proof, however, was not in seeing, but in correlating biological isolation and chemical activity. His task was to eliminate the four additional species that were living in his culture, and thus, that might play a role in nitrification. To “elect” the oval bacteria from the others, he needed to determine the cultural medium in which only they could live. Then, if nitrification still occurred, he had his proof. Using tediously purified retorts, inorganic salts, and water, he made an environment rich in ammonia salts and no organic matter. Introducing his “beautiful ovals” and unavoidably some of the other organisms, he produced conditions in which the ovals had an advantage over the other species. The ovals (which he would call *Nitrosomonas*) quickly dominated the culture and eliminated all of the ammonia. Now convinced of the effectiveness of his elective culture method, he made it the prototype for

⁴⁵ Ibid., p. 226.

⁴⁶ Ibid.

⁴⁷ Ibid., p. 225.

⁴⁸ Ibid.

all of his subsequent research. Using it he had proved that the vital activity of the *Nitrosomonas* required nitrogen-related material as their sole food source.

The nitrification research provided Vinogradskii with his third example of an inorganically based respiration from which he divined what he called a new physiological fact – the fact of chemosynthesis. His experiments had shown “that living beings could accomplish a complete synthesis of organic matter on our planet independent of the solar rays.”⁴⁹ That is, since they contained no chlorophyll, these organisms could not obtain energy from light to drive their oxidation processes.⁵⁰ Chemosynthesis, then, was the process by which organisms developed in a purely mineral environment, acquiring their only source of nutrition from a single inorganic material.

Vinogradskii extrapolated from his 3 year’s of laboratory experiments to challenge fundamental assumptions in agricultural chemistry and general physiology. Although his predecessors Schloesing and Müntz had found a special organism related to nitrification, Vinogradskii considered their term for it, “*ferment nitrifique*,” much too general.”⁵¹ He thought it improbable that a single organism could be responsible for “exercising [nitrification] over the entire surface of the globe.” He proposed instead using a broader classification based on “physiological type” under which one could classify numerous species or varieties.⁵² Thus, for him, nitrifiers represented an entire cast of nitrifying organisms that existed not only in Zurich’s soils but also in all soils on the Earth.⁵³

⁴⁹ Ibid., pp. 269 and 275.

⁵⁰ Ibid., p. 275. Although he did not apply the term chemosynthesis to this phenomenon until later, the concept – that a general physiological condition existed, in which organisms did not need light or oxygen to live – was implicit in his description.

⁵¹ Ibid., pp. 230–231. He did eventually use this term after he isolated the two species of microbes that participated symbiotically in nitrification – calling them *ferment nitreux* and *ferment nitriques*. Idem., 1891, vol. 5.

⁵² Idem., 1890, 4: 230; idem., 1891, p. 593.

⁵³ In order to refute the doctrine “held by physiologists world round,” that organisms can survive without organic nutrients, one would have to provide very weighty proof. For 3 months, he successfully cultivated them in a solution, which was excruciatingly prepared to be devoid of organic matter (including boiling all his glassware twice in sulfuric acid). To prove definitively the nature of nutrition he measured the amount of organic carbon in the sediment of his cultures. If carbon appeared during the experiment, only *Nitrosomonas* could have produced it. He varied the cultures by adding different forms of nitrogen to them. In all cases, carbon appeared in the test cultures, proving, he thought, that *Nitrosomonas* possessed the ability to assimilate carbon from carbon dioxide.

Vinogradskii's global perspective was no mere rhetorical flourish – it allowed him to comprehend the significance of his laboratory research for processes occurring in natural conditions. His commitment to the concept of the “cycle of life,” thus, required him study microbial nitrification as a universal or global phenomenon. Drawing on his international contacts, he requested soils from Europe and other “exotic sources” such as Africa, Asia, South America, and Australia.⁵⁴ After isolating the nitrifying organisms from these samples, he compared their ability to oxidize ammonia in his elective culture and in their original soils. Even though these organisms came from different environments, he found that they oxidized ammonia at the same rate. This proved to him that nitrification was a universal, biological phenomenon that transcended local conditions.⁵⁵ At Zurich, then, his investigation of nitrification taught him that biology, microbiology specifically, had agricultural applications; that lesson would guide his career over the next five decades.

In the above investigations, he accumulated information and experiences, which led him to develop his idea of chemosynthesis. For each organism that he studied he generalized to a broader classification; from *Beggiatoa* to sulfur bacteria, from *Leptothrix* to iron bacteria, and from *Nitrosomonas* to *Azotobacter*, or nitrogen bacteria. During the nitrogen studies, he became convinced that the analogy he recognized between these three physiological types could be extended to all of microbiology. A substantial number of species did not require what most physiologists considered the necessary ingredients for life. Not only did new organic matter arise from the life processes of living beings through photosynthesis, he argued, but also as a result of chemosynthesis. This notion swept through the scientific community and was adopted by scientists in a broad spectrum of disciplines including plant physiology, soil science, and eventually ecology.⁵⁶ It also had the immediate effect of launching Vinogradskii on a career in microbiology.

⁵⁴ He requested samples from Emil Duclaux, Cramer (Zurich), Treub (Buitenzorg), and Cavalcanti (Campinas). From Europe he used soils from Zurich, Gennevilliers, Kazan, and his home town of Podolia (He had investigated these two Russian soils previously, in the winter of 1889–1890.) He received his African soils from La Reghaia, Rouiba, Mitidja, and Tunis. The Asian soils were sent from Buitenzorg, Java, and Tokyo, Japan. The American soils were from Campinas, Brazil, and Quito, Ecuador. The Australian soil came from Melbourne. *Idem.*, 1891, p. 581.

⁵⁵ *Ibid.*, p. 593.

⁵⁶ Today, nitrogen availability is understood to be one of the crucial bottlenecks in ecological, and hence, agricultural process.

His research altered fundamentally the way many scientists understood the history of the earth, focusing their attention on the role the smallest living beings played in forming the environment.⁵⁷ This new understanding of microbial nutrition contributed a significant new perspective to a number of scientific disciplines that formed or expanded at the end of the 19th century, including microbiology, soil science and geobotany (the foundation of Russian plant ecology), and agricultural chemistry. Vinogradskii himself would take his new perspective to his next assignment at the Imperial Institute of Experimental Medicine, where he organized one of the first programs of soil and agricultural microbiology.

Soil Microbiology at the Imperial Institute of Experimental Medicine, 1890–1905

The widespread acceptance of chemosynthesis earned Vinogradskii a number of job offers from prestigious institutions. The president of the Zurich Polytechnic Institute invited him to deliver a course of lectures as a privatdozent. In 1891, the Russian immunologist Elie Metchnikov brought Vinogradskii an invitation from Louis Pasteur to set up a bacteriological laboratory at the newly founded Pasteur Institute. In addition, Vinogradskii received a comparable offer from the newly established Imperial Institute of Experimental Medicine (IEM) in St. Petersburg.⁵⁸ After long deliberation, he accepted the Russian offer and in 1891 moved there to head the laboratory of general microbiology. Even though he worked in a medical research institute – and supported their mission – he had the freedom to apply his laboratory research on chemosynthetic organisms to agricultural problems.

By 1893, Vinogradskii had committed himself to soil microbiology. His nitrification research had “opened a very wide field” and he was getting a flood of ideas.⁵⁹ He also had hired a very capable assistant in

⁵⁷ For example, the plant physiologist, Wilhelm Pfeffer, incorporated Vinogradskii’s chemosynthesis research in his discussion of plant nutrition. Pfeffer discussed Vinogradskii’s work in his section on “The Production of Organic Substances through the Assimilation of Carbonic Acid,” which he subdivided into: (A) Photosynthetic Assimilation and, (B) Chemosynthetic Assimilation. Pfeffer, 1897, pp. 346–349; 385, 547–548.

⁵⁸ For a recent discussion of the conditions at the IEM during Vinogradskii’s tenure, see Todes, 2002, pp. 20–40.

⁵⁹ Letter from Vinogradskii to Elie Metchnikov dated February 9, 1894, Reikhberg, 1931, p. 167.

Vasilii Omelianskii, who was a student of Vinogradskii's old chemistry professor Menschutkin. With this optimistic outlook, a well-appointed laboratory, and a devoted collaborator, Vinogradskii pursued a series of topics that bridged general microbiology and soil science, and provided the foundation for his program in soil microbiology.

The ultimate direction of his research during 1890s reflected his interest in one of Russia's newest disciplines – soil science. His research in Zurich had convinced him that soil microbiology could be applied to practical questions in sanitation and agriculture. He now drew on that experience to aid soil scientists, whom the Tsarist government had commissioned to save Russian agriculture. It was unclear to him at this time, however, how his microbiology could practically assist the soil scientist in the field. Following his preference for laboratory research he contributed, thus, by developing methods for investigating soil formation processes, such as cellulose decomposition and nitrogen fixation. These methods and the research he conducted with them became increasingly valuable to Russian soil scientists.⁶⁰ To understand these developments – and especially the connection between soil science and agriculture – we must briefly review the history Russian soil science during this period.

In 1890s, Russian soil science was experiencing a period of great expansion, characterized by the ascendancy of the Dokuchaev school, the formation of new institutes, and the organization of large research expeditions.⁶¹ During this period, soil scientists were staking out their disciplinary boundaries and striving to balance their commitments to natural history with a growing interest in experimentation and laboratory-based investigations. They were attracted to Vinogradskii's concept of autotrophism and nitrification research, which appeared in the early 1890s, seeing it as a way to make their science more experimental and to fulfill the central tasks of understanding the formation of soil.

Kostychev (1845–1895) was the first to introduce microbiology into soil science. From 1870 to 1893, he wielded considerable authority in soil science – teaching courses in agriculture and soil science at the

⁶⁰ During his tenure at the IEM, Vinogradskii also applied his microbiological research skills to medical and health questions. In particular, he investigated the nature of a plague that had appeared in south eastern Russia. I do not treat this story in full because it does not relate directly to Vinogradskii's development as an ecologist. It may, however, help to understand how ecological microbiology relates to epidemiology and medical bacteriology. On the influence of the post-1861 reforms on soil science, see Krupenikov, 1981, pp. 155–156.

⁶¹ On the history of soil science: Ivanov, 2003; Krupenikov, 1981; and Yaalon and Berkowitz, eds., 1997.

Forestry Institute; and at times at the St. Petersburg University. Between 1883 and 1891, he held administrative positions of agriculture inspector (1883–1891) and then director (1893–1895) in the Ministry of Agriculture and State Property.⁶² In the middle of his career, the government dispatched him and the founder of Russian microbiology, L. S. Tsenkovskii, to Europe to study at the laboratories of Pasteur and Koch. Sent there to study the production of anthrax vaccines, Kostychev took an interest in Pasteur's work on soil microbes. Upon his return to Russia, Kostychev initiated some of the first investigations of soil microbiology in Russia.⁶³ His research provided the first experimental proof that microscopic fungi contributed to the formation of humus in the soil and asserted that the formation of "chernozem (black earth) is a botanical issue."⁶⁴ Soil, for him resulted from the accumulation and cycling of humus (decaying organic matter), a decomposition process, in which bacteria and fungi played the central role.⁶⁵ By creating a place for microbiology in soil science during its formative period, Kostychev prepared the way for Vinogradskii's soil microbiology in the 1890s.

Dokuchaev (1846–1903) contributed most to the founding of scientific soil science.⁶⁶ He was the first to conceptualize the soil as "an independent natural-historical body," to create methods for studying it, and to develop the first system of laws for understanding the organization and formation of soil "zones."⁶⁷ He outlined the theoretical and practical significance of soil science and trained two generations of soil scientists and geobotanists. He established the discipline of soil science through his teaching, publications, expeditions, not only in Russia, but around the world. Dokuchaev's ideas were also seminal in leading to the "Nature Protection Network" and its concept that nature was organized into bounded, compact ecological communities existing in balance with one another.⁶⁸

⁶² Ivanov, 2003, p. 97; Krupenikov, 1981, p. 175.

⁶³ Ivanov, 2003, pp. 97, 99. For an example of Kostychev's work in this area, see Kostychev, 1951, pp. 363–366.

⁶⁴ Ivanov, 2003, p. 99; Sibirtsev, 1949, pp. 193–197; and Kossovich, 1949, pp. 201–202.

⁶⁵ Ivanov, 2003, p. 177.

⁶⁶ For a comprehensive list of work on Dokuchaev's life and scientific activity, see Krupenikov, 1981, pp. 151–153.

⁶⁷ Ivanov, 2003, pp. 46–49.

⁶⁸ Weiner, 1988, pp. 12–13; and Idem, 1999, p. 261. It is to these developments that one can trace the origin of a Russian ecology, distinct from the British and American varieties, to these developments. Worster, 1994, pp. 195–220.

During his trips to investigate Russia's chernozem (black earth) regions in 1870s, Dokuchaev had developed a natural historical conception of the soil.⁶⁹ In 1879, he had defined the soil as "the surface mineral-organic formation ... that originates due to the interaction of living and dead organisms, the matrix [or gangue] of rock, the climate, and the topography (*rel'ef*) of the terrain."⁷⁰ By 1886, he expanded his definition to include the idea that the soil forms from rock "naturally changed by the reciprocal action (*vzaimnoe vliianie*) of water, air, and various types of organisms, living and dead, which have a definite composition, structure, and color."⁷¹ Prior to Vinogradskii's discovery of autotrophic microorganisms, Dokuchaev relegated microorganisms and animals to a role secondary to that of plants, which were able to "synthesize organic matter."⁷² In 1890s, Vinogradskii's research convinced Dokuchaev that autotrophic microorganisms were the primary synthesizers in soil formation.⁷³

Dokuchaev envisioned a place for microbiology in his reform of soil science training. In 1891, he participated in a commission that studied higher education in agriculture. He found the only active organization – the Novo-Aleksandriiskii Institute of Agriculture and Forestry – to be "in a condition of complete disorganization."⁷⁴ After he became director of that institute the following year he introduced educational reforms that included teaching physics, chemistry, geology, soil science, and plant physiology.⁷⁵ These courses produced Russia's first, well-trained specialists in the areas of agriculture and forestry.⁷⁶ In an attempt to extend his reforms to the university system, where agriculture was not taught, Dokuchaev recommended that the government establish chairs in soil science and microbiology.⁷⁷

For Dokuchaev, Vinogradskii's research represented a significant advance in applying bacteriology to agriculture and soil science. Following the work of Pasteur and the German microbiologist, Ferdinand Cohn, Vinogradskii (and a number of other microbiologists) had developed new methods to investigate geological processes in terms of

⁶⁹ Ivanov, 2003, p. 48.

⁷⁰ Kir'ianov, 1966, p. 39; Ivanov, 2003, pp. 59–61.

⁷¹ Krupenikov, 1981, pp. 161–162.

⁷² Ivanov, 2003, p. 48.

⁷³ Ibid.

⁷⁴ Kir'ianov, 1966, p. 63.

⁷⁵ Krupenikov, 1981, p. 165.

⁷⁶ Kir'ianov, 1966, p. 64.

⁷⁷ Dokuchaev, 1895.

the vital activity of bacteria.⁷⁸ Especially important, Dokuchaev thought, was the role of bacteria in the nitrogen cycle. Referring to Vinogradskii's ongoing research at the IEM on nitrification, Dokuchaev felt confident that "without bacteria, plants would not be able to use the scanty nitrogen reserves in the soil."⁷⁹ Vinogradskii's work also appeared prominently in Dokuchaev's plan for a new university course in microbiology.⁸⁰ At the top of Dokuchaev's list of the most important "physiological bacterial types," he placed: the nitrifying organisms, sulfur bacteria, iron bacteria, and nitrogen fixing bacteria – the past and present targets of Vinogradskii's research.

Dokuchaev taught his holistic soil science to a generation of Russian scientists – and through them to many in the world scientific community. Vernadsky (1863–1945), one of his best known students, would later develop Dokuchaev's system into an expansive program encompassing the entire realm of the Earth. Vernadsky later founded geochemistry and its related discipline biogeochemistry, and is best known in the West for his concept of the "biosphere" (the area on the Earth where life existed or could exist).⁸¹ Although there is no evidence that Vernadsky and Vinogradskii were in direct communication at this time, it is clear that they shared an appreciation of Dokuchaev's ecological approach to nutrient cycles. Acknowledging their common interests, Vernadsky later taught Vinogradskii's work to his geology and geography students.

Vernadsky incorporated Vinogradskii's concept of autotrophism into his understanding of the geochemical evolution of the environment.⁸² Autotrophism – the ability of organisms to "live independent of the energy of light, because they get the energy for their vital processes from minerals" – would become a crucial component of Vernadsky's concept of the biosphere.⁸³ In particular, Vinogradskii aided Vernadsky

⁷⁸ Ibid., pp. 49–54.

⁷⁹ Ibid., 51. Here Dokuchaev drew on Vinogradskii's description of nitrification's two stages of conversion: from ammonia to nitrite, and nitrite to nitrate.

⁸⁰ Ibid., pp. 56–61. Although he admitted getting the program for this course only from an unspecified "well-known specialist in the given branch of natural science," it is probable that he received it from Vinogradskii.

⁸¹ Vernadsky first made public his biosphere concept in a series of lectures at Kiev University (1918–1919), the Academy of Sciences in St. Petersburg (1921), at the Petrograd Agricultural Institute (1921–1922), and finally, while on temporary assignment to the Curie laboratory at the Sorbonne in Paris (1923–1924). He published his lectures in 1924 as *Essay on Geochemistry* and in 1926 as *La Biosphere*. On Vernadsky see Bailes, 1990.

⁸² Vernadsky, 1994, pp. 98–101; and idem, 1930, p. 88.

⁸³ Vernadsky, 1930, p. 89.

in portraying the “geochemical history of living matter,” by providing him a mechanism – autotrophic bacteria – for understanding how “living matter transports chemical elements through the biosphere.”⁸⁴ For Vernadsky, Vinogradskii’s discovery of autotrophism and his array of laboratory techniques made it possible to investigate the migration of chemical elements throughout the biosphere. Synthesizing Vinogradskii’s approach with his own mathematical and chemical techniques, Vernadsky could now investigate how autotrophic organisms concentrated various elements in nature. Moreover, it provided him another way for understanding the dynamics between the inorganic and organic realms of the biosphere.

The growth of Russian soil science – especially the Dokuchaev school – occurred during a period characterized by the rapid growth of capitalism, including in the agricultural sector.⁸⁵ The 1861 reforms brought increased commercial development, which included a dramatic increase in the country’s reliance on its agriculturally rich southern “black earth” region. Due to droughts and poor land management, Russian agriculturalists failed to keep pace with these new demands, leading the government to seek new solutions.⁸⁶ Drawing on the scientific community that had become such an important part of Russian society, the government organized numerous expeditions to various areas of the country to collect data on soil and botanical resources. Organized by Russia’s soil scientists, these expeditionary experiences led to new understanding of the interrelationship between soil and vegetation. Specifically, soil scientists came to view vegetation as an important factor influencing the formation and development of the soil. The close ties between soil science and the Russian government’s agricultural

⁸⁴ Ibid.

⁸⁵ Trass, 1976, p. 91.

⁸⁶ The strife during Russia’s “Hungry Years, 1891–1892,” reached its highest level during the summer of 1891, which experienced “the most serious crop failure since the 1830s” leaving its “great, black earth granary ... stand[ing] empty.” There were two general causes of the famine. Meteorological conditions – an early onset of winter, a summer drought – had led to bleak harvests. In addition, government policies in place since the emancipation act of 1861 – perhaps exacerbated by Vysnegradskii’s 1887 reforms – had left the peasant population living in chronic poverty and misery. Especially hard hit were the black earth regions, where peasants responded to the failed harvests with radical measures – devoting more of their fallow and hay fields to crop production, developing a “mania for chopping down trees,” and reducing the size of their cattle herds. These actions proved disastrous for Russian agriculture, leading, respectively, to soil exhaustion, the loss of natural windbreaks that lessened the effects of drought, and the loss of much needed fertilizers. Robbins, 1975, pp. 1–10.

programs would prove to be long-lasting; in 1890s, it provided the context for Vinogradskii's soil microbiology.

Glimpses of a 'Soil Microbiology'

Although he was director of the general microbiology laboratory at a prestigious medical institute, the bulk of Vinogradskii's research was in soil microbiology. Upon his return to St. Petersburg, he reconnected with his teachers and colleagues and, for the first time, mentored a few students of his own. Soil science was a popular new science and he no doubt learned much about it and Dokuchaev's expeditions at the meetings of the St. Petersburg Society of Naturalists, of which he was a member.⁸⁷ And although he did not participate in the expeditions himself, he became familiar with their goals, organizational structure, and methodologies.⁸⁸ In addition, he developed strong contacts with experts at the Forestry Institute, seeking their advice on managing the forests of his Ukrainian estate.⁸⁹ These interests led him to envisage the agricultural applications of his microbiological research.

Vinogradskii found it difficult to bring microbiology to soil science. In his own independent work, he continued to apply his elective culture method to investigate the role of nitrogen bacteria in the soil. In 1893, he confirmed the biological nature of the nitrogen fixing; in 1895, he isolated *Clostridium pasteurianum*, the first non-symbiotic nitrogen-fixing bacteria;⁹⁰ and in 1902, he published an extensive morphological and physiological taxonomy of these species.⁹¹ Throughout these investigations, he developed the elective culture method into a versatile tool for studying nearly any microbial process occurring in natural soil

⁸⁷ Vinogradskii had been a member of the St. Peterburgskoe Obshchestvo Estestvoispytatelei since 1881. The reports from the expedition appeared in each of the sections of the society and were published in its journal. For a review of the activities of the society between 1888 and 1893, see St. Peterburgskogo Obshchestva Estestvoispytatelei, 1893, Vol. XXV.

⁸⁸ During his years at St. Petersburg University's botanical department, he had studied with Beketov. Vinogradskii may also have participated in the local excursions Beketov organized. Ackert, 2004, Chapter 1.

⁸⁹ Waksman, 1953, pp. 32–33.

⁹⁰ Beijerinck had recently discovered bacteria living in symbiosis with leguminous plants in their root nodules. Beijerinck, 1888, pp. 769–771.

⁹¹ Winogradsky, 1893, 116(24): 1385–1388; Idem., 1893, 118(7): 353–355.

or water environments.⁹² Although these investigations found an appreciative and international audience, his greatest impact on Russian soil science and agriculture would be through the students he mentored in this research.

Vinogradskii assigned his students research topics that combined microbiology and soil scientists and agriculture. Although he had only a dozen students from 1891 to 1910, many of them became some of Russia's leading scientists. Three of these collaborated with Vinogradskii on what were some of the earliest investigations of soil microbiology: V. V. Polovtsev [his *praktikant* (research assistant)]; Fribes (a member of the laboratory's staff); Vasilii Omelianskii (his assistant director). In 1892, Vinogradskii directed Polovtsev to investigate a question central to soil science – whether nitrogen bacteria could decompose organic matter.⁹³ Vinogradskii organized this project to defend his theory of chemosynthesis, proving again that nitrogen bacteria could survive only on inorganic matter. That same year, he assigned Fribes to investigate the role of microbes in flax retting – the process of soaking flax to soften and separate the fibers by partial decomposition.⁹⁴ Fribes' project not only continued Vinogradskii's efforts to improve the elective culture method, it also had practical significance related to the production of textiles. Fribes, who was an official in the Ministry of Agriculture, essentially bridged the Government and Vinogradskii's laboratory. His close relationship with Vinogradskii exemplified the close ties between microbiology and the interests of both the Imperial Free Economic Society – the same institution that had commissioned Dokuchaev to organize his expeditions – and the Ministry of Agriculture.⁹⁵

Vinogradskii's First Student, V. L. Omelianskii: Microbes as "Living Reactives"

Of all his students, Omelianskii did the most to extend Vinogradskii's legacy in agricultural microbiology. Omelianskii not only adopted and developed Vinogradskii's methods in his own research, but he also

⁹² Ibid. Throughout this process, he continued his general program of physiological research, by striving to create cultures that would provide environmental conditions favorable for a single vital function.

⁹³ IIEM, 1892, pp. 24 ob. –25 ob.

⁹⁴ Ibid. Fribes applied Vinogradskii's elective culture method to isolate the bacteria responsible for decomposing certain kinds of plant fibers. Fribes, 1895, p. 742.

⁹⁵ Fribes presented his results "On the Retting of Flax" at the society's annual meeting in 1896. IIEM, 1896, pp. 37–37ob.

passed this training on to new generations of Russian microbiologists, physicians, and soil scientists.⁹⁶ Through Omelianskii's publications, courses, and institutional organizing, Vinogradskii's soil microbiology became integrated into agriculture in Russia and the international community.⁹⁷

Vinogradskii took a risk when he hired Omelianskii to be his first laboratory assistant in 1893; Omelianskii had little or no experience in microbiology. After earning a BA in chemistry from St. Petersburg University in 1890 under D. I. Menshutkin, he decided not to pursue a doctorate and, instead, went to work in a metallurgy factory.⁹⁸ While working in industry, Omelianskii kept alive his interest in chemistry and independently studied of its application in soil science.⁹⁹ Menshutkin noticed, however, that Omelianskii possessed considerable talent in chemistry, enough to recommend him to be Vinogradskii's assistant. Vinogradskii "had grown accustomed to respecting [Menshutkin's advice] since his own student days" and his old chemistry professor's sanction in this matter was more than adequate.¹⁰⁰

⁹⁶ The list of praktikanty and visitors to Vinogradskii's Department of General Microbiology at the IEM was not as long as for other departments. For example, during this period the physiologist, Ivan Pavlov, could count on the assistance of dozens of praktikanty. The few that worked with Vinogradskii had extraordinarily successful career Russian science. On the history of the Department of General Microbiology at the IEM; Isachenko, 1941, p. 96.

⁹⁷ Omelianskii later directed the work of M. N. Rubel' on the question of nitrification in biological filters (1913), E. I. Nikolaevna in her investigations of actinomycetes, S. N. Bukhbinder on pigment formation in colorless bacteria, N. M. Perepelitsina on the use of triptophan reactions in bacteriology, I. A. Makrinov on the aerobic ferments of nicotinic materials, O. M. Bogoliubov on the bacteriology of lactobacillus, L. D. Shturm on sapropel (an aquatic sludge rich in organic matter), Germanov on methods for investigating the soil microflora, and V. P. Neelov on the chemistry of the nitrification process (1922). Isachenko, 1941, p. 96.

⁹⁸ Razumovskaia, 1960, p. 17.

⁹⁹ In 1891, Omelianskii took copious notes on two important Russian agrochemical works: Grando's, "Novaia mineral'no-gumusovaia teoria pitaniia rastenii," published in *Sel'skoe Xosiatvo i Lesovodstvo*, (1872, 1873); and G. G. Gustavson's, "20 Lektsii Agronomicheskoi Khimii." Gustavson worked at the Moscow Agricultural Institute at the end of the nineteenth century, where he developed chemical investigations related to Dokuchaev's "concept of the soil as a natural body." Ivanov, 2003; on Gustavson at the Moscow Agricultural Institute, see p. 137. Omelianskii had also read the works of the founding fathers of Russian soil science including numerous articles and books by Dokuchaev, including his "Kratkaia programma dlia izsledovaniia pochva, in which Dokuchaev proposed methods for investigating soils as organisms; and Kostychev's, "Pochvy chernosemnoi oblasti Rossii, ix proiskhozhdenie, sostav i svoistva," (St. Petersburg, 1886). Omelianskii, Undated notebooks, 1–62 ob.

¹⁰⁰ Mazing et al., 2002, pp. 306–309; see p. 306 for quote.

Vinogradskii assigned his new assistant practical work, believing it to be the fastest rout to technical proficiency. When he arrived in Vinogradskii's laboratory in 1893, Omelianskii possessed an extensive knowledge of agricultural chemistry, but a limited understanding of the biology of the soil.¹⁰¹ He recalled that he "quickly and imperceptibly mastered the bacteriological techniques" and that his "knowledge of chemistry provided a good basis for investigating microbial physiology."¹⁰² It seemed to him that during this training, "a 'microbiological wisdom (*mudrost'*)' arose on its own".¹⁰³ It is clear, however, that he did not assimilate his wisdom from some mysterious atmosphere – Omelianskii had adopted Vinogradskii's approach.

Omelianskii's participation in Vinogradskii's ongoing investigations gave him not only the opportunity to practice microbiological techniques, but also framed his future scientific interests. He joined the laboratory when Vinogradskii was investigating the assimilation of gaseous nitrogen by microbes. It was during this research, that Vinogradskii was using the elective culture method to isolate the first free-living nitrogen-fixing organism, *C. pasteurianum*. The questions that drove this research and the methods by which they were explored were Vinogradskii's. Omelianskii, however, conducted the bulk of the analyses.¹⁰⁴ In the process, he learned first-hand Vinogradskii's method for developing laboratory techniques to explore the role of microbes in the nature. By the end of the century, Omelianskii was publishing independent articles, yet these still reflected the laboratory's direction. He collaborated with Vinogradskii to promote the elective culture method for investigating nitrification and isolating nitrifying microbes from the soil.¹⁰⁵

Through his institutional activities, mentoring, and publications, Omelianskii continued Vinogradskii's efforts to expand microbiology's place in Russian science. In the first decades of the 20th century, Vinogradskii moved increasingly into retirement leaving Omelianskii to assume his duties. After 1906, for example, Omelianskii served temporarily as director of the IEM's Department of General Microbiology (a position that became permanent upon Vinogradskii's resignation in

¹⁰¹ The early soil science literature largely ignored the biological components of the soil and only very rarely discussed any investigations into it. Most of these works cited the work of Pasteur and Charles Darwin on the formation of soil.

¹⁰² Ibid.

¹⁰³ Ibid., 18.

¹⁰⁴ Vinogradskii, 1895, pp. 293–351.

¹⁰⁵ Omelianski, 1899a, b; Vinogradsky und Omelianski, 1899.

1912). During this period, Omelianskii “preserved a great scientific heritage;” through his numerous investigations and “outstanding” teaching, he exerted “a great influence on the development of microbiological sciences in [Russia].”¹⁰⁶ In 1906, he accepted an invitation to teach microbiology at the newly organized Lokhvitskoi–Skalon advanced natural history courses for women.¹⁰⁷ In 1909, he published the first Russian textbook on general microbiology, *The Fundamentals of Microbiology* (*Osnovy Mikrobiologii*), which went through nine (including several posthumous) editions.

Omelianskii’s experience in Vinogradskii’s laboratory during the 1890s taught him how to apply microbiology to soil science.¹⁰⁸ Reflecting on this training, in 1918 he outlined “The Goals of Soil Microbiology.”¹⁰⁹ Placing their science firmly in the discipline of soil science, at the top of his list Omelianskii cited the mantra of the Dokuchaev school: “The soil as a natural-historical body. Soil formation.”¹¹⁰ Soil microbiologists, Omelianskii taught, would approach this topic by investigating “the disintegration and destruction of minerals and rock under the influence of microorganisms, as an essential factor in soil formation.”¹¹¹ Using Vinogradskii’s elective culture method, soil microbiologists would “reveal the content of microbes in the soil, which provoke defined biochemical transformations,” including ammonia fixation, nitrogen fixation, and cellulose decomposition.¹¹² In a perfect synthesis of Vinogradskii’s microbiology and Dokuchaev’s soil science, Omelianskii trained soil microbiologists to study how the soil microflora changed under the influence of the various conditions,” including the content of organic matter, the absorption ability and structure of the soil, temperature, aeration and humidity, and the distribution and mixture of nutritive materials.¹¹³ From the first investigation he conducted with Vinogradskii in 1893 to the biography he wrote on his

¹⁰⁶ Razumovskaia, 1960, pp. 18, 24. In 1909, for example, Omelianskii taught the Vinogradskii approach to Nikolai Kholodnyi.

¹⁰⁷ Ibid., p. 24.

¹⁰⁸ Vinogradskii’s faith in his student’s ability was so strong that in 1899, when he fell seriously ill and returned to Kiev to convalesce, he left his department in Omelianskii’s excellent care. Upon Vinogradskii’s retirement in 1912, Omelianskii assumed directorship of the department and laboratory, where he developed a comprehensive program in soil microbiology.

¹⁰⁹ Omelianskii, Undated, p. 1.

¹¹⁰ Ibid.

¹¹¹ Ibid.

¹¹² Ibid.

¹¹³ Ibid. 2–3

mentor in 1927, Omelianskii extended, and further developed, Vinogradskii's microbiological approach to soil science and agriculture.

Scientific Agriculture at Home: Vinogradskii Retires to Gorodok

In St. Petersburg, Vinogradskii confined his interest in soil science to the laboratory investigations of nitrification and related microbial soil processes. On his Gorodok estates, however, he concentrated his scientific efforts to practical agricultural problems: improving his estate's forests, greenhouses, and crops. A serious case of nephritis in 1898–1899, gave him the unexpected opportunity to explore more fully one of the careers he had abandoned for science – that of a *pomeshchik* or large-scale landowner. He could not have returned, however, to the kind of life lived by his father – Vinogradskii had been transformed by his scientific education and career. When not busy with his institutional, society, or research duties in St. Petersburg, he devoted his time to “scientific farming” on his large estate, which included nearly 5000 acres of old growth forest, horse stables, an extensive beet-sugar factory, and several flour mills. Drawing on the expertise he had developed in St. Petersburg and the assistance of experts from the Forestry Institute of Kiev, he decided which trees to cull and which to leave, and established an experimental nursery. In addition, he organized a model dairy farm and an orchard. These activities suggest that Vinogradskii's interests in farming had shifted from that simply of a *pomeshchik*, or landlord, to include those of an agriculturalist and soil scientist.

Conclusions: Vinogradskii's Extended Legacy in Agricultural Microbiology

The history of Vinogradskii's discovery of microbial autotrophism and chemosynthesis is an example of the introduction of microbiology into agricultural research. His step-wise transformation from plant physiologist to soil microbiologist was predicated on the fulfillment of three goals. In his research from 1882 to 1900, he carried out Famintsyn's project to extend physiological thermodynamics to the study of microorganisms. In the process he brought Pasteurian microbiology to a new level of technical sophistication. Finally, having gleaned in Zurich the power of microbiology for investigating and solving agricultural questions, in Russia he organized a multifaceted research project along those lines. His work during this period represents a new phase in the history of agricultural science. Microbiologists would now play a major

role in developing the laboratory methods and theoretical perspectives on which were founded incipient disciplines closely related to agriculture: ecology, soil science, and soil microbiology. Vinogradskii, however, did not leave this work to his students.

In a strange twist of fate, after a decade of retirement, Vinogradskii would reemerge in 1922 to secure and extend his legacy in the agricultural microbiology. In 1917, it seemed that his final publication would be his contribution to Lafar's *Technical Handbook* (1904) and that his retirement and quiet withdrawal to Gorodok had been permanent.¹¹⁴ In his mid-sixties, Vinogradskii no doubt planned to live out his life as a *pomeshchik*. In 1918, however, the upheaval caused by the October Revolution and the Russian Civil War – which were especially destructive in the Kiev area – forced him and his family to flee for their lives. Brought on by the Bolsheviks he despised, this hardship awakened him from his latent life and opened a new chapter in his career.

Drawing on his scientific credibility – his most valuable remaining asset – he resurrected his career, founded a new institution, and stimulated a new movement in science. After spending a year at the Agriculture Department of the University of Belgrade, where he taught courses in agricultural microbiology, Vinogradskii sought a new place with better resources for research. His friend and colleague, Emil Roux, director of the Pasteur Institute in Paris, used a recent donation to create a place for Vinogradskii – a new laboratory for agricultural microbiology. Working for a new patron Vinogradskii now applied his three decades of experience in microbial physiology and soil science to practical use in agriculture. In the laboratory, he adapted the “elective culture” method he had developed in 1890s to help microbiologists apply their soil science research to practical questions in agriculture. He also brought his research and the message that biology had much to offer agriculturalists to numerous French agriculture societies.

Attracted to the language of ecology that was emerging as a powerful force in the scientific community in the early 20th century, he translated his experimental program into an ecological research program. By 1930s, Vinogradskii considered not only his current investigations, but also all of his previous work to be ecology. His first investigations on *M. vini*, his discovery of chemosynthesis, and his extensive investigations of the nitrogen cycle – all, he now thought, expressed the ideals of 20th century ecology. Remaking himself as an ecologist, he transformed the biological approach he had developed over four decades of physiological and microbiological investigations into a novel research program

¹¹⁴ Lafar, 1904.

that excited a new generation of microbiologist, medical bacteriologists, and soil scientists. For Vinogradskii, a Pasteurian microbiologist steeped in Russian soil science – an *ecological* approach meant investigating a microbe's role in nature physiologically, that is, exploring its nutrition and respiration as a natural process mediated by the plenum of environmental conditions.

From a variety of platforms – articles in professional scientific journals and applied science reports, speeches at conferences, his correspondence with an international network, and mentoring at the Pasteur Institute – he simultaneously promoted his own career, his methods, and the development of new scientific fields. Through these efforts, his vision of an ecological soil microbiology radiated through a community of agricultural researchers spread around the world in agricultural experiment stations and bacteriological and microbiological institutes. By adopting his methods these scientists translated Vinogradskii's approach into their ongoing research programs, making it available for new generations of scientists.

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