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Effect of Precision Nitrogen Fertilization of Grassland on Soil Microbial Structure

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Abstract: The synergistic application of advanced technologies enables precise determination of plant growth, health, and nutritional requirements. However, despite the widespread use of modern technologies, the microbial status of the soil is often neglected, even though it significantly impacts soil productivity. Soil microbial activity serves as a crucial indicator of site-specific soil conditions. This article presents efforts to explore the quantitative and qualitative relationships between identified actinomycetes and soil nitrate content, as well as their distribution within the soil profile. Field data analysis facilitated the assessment of nitrate concentrations and the evaluation of the quantitative and species composition of actinomycetes in the soil profile at depths ranging from 0.05 to 0.35 m. The highest nitrate concentration (22 mg/100 g of soil) and actinomycete abundance (1076 CFU/g of soil) were observed in the topsoil layer. Additionally, spatial correlations between these parameters were analyzed for each soil layer. The correlation coefficients were approximately −0.6, indicating an inverse relationship. Areas with low nitrogen content corresponded to reduced microbial abundance within the soil profile, as supported by the spatial correlation data. These findings demonstrate the potential to predict actinomycete abundance in the soil profile based on nitrate content, offering valuable insights into soil health and productivity.

Keywords: soil microorganisms; spatial distribution; soil profile; nitrogen fertilization; grassland

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1. Introduction

Soil is increasingly regarded as a non-renewable resource from the perspective of a human lifetime, as its regeneration process after degradation occurs extremely slowly. Due to the crucial importance of soil for plant and animal production, maintaining it in good condition is of utmost importance [1]. Modern agriculture, especially in developed countries, is characterized by its high intensity. During the growing season, heavy machinery is often used for cultivation, planting, and harvesting, and crops are intensively fertilized and treated with pesticides to maximize yields [2]. Agricultural management plays a key role in shaping soil processes and functions. However, due to the biophysical limitations of soil and the ongoing technological advancements, new methods for assessing it are emerging, the impact of which on soil processes remains largely unknown. This significantly hinders the analysis of the potential of these practices of sustainable intensification, developed in response to the growing demand for food and non-food products [3].

The growing demand for food resulting from population growth is the main reason for the intensification of agricultural production. The drive to increase yields on existing agricultural lands has led to the widespread use of fertilization as the primary method to achieve this goal [4]. The overuse of fertilizers has become a common practice worldwide, often resulting in negative impacts on crop productivity [5,6]. Modern intensive agriculture is based on the assumption that short-term land management can significantly affect the long-term sustainability of food production [7]. A lack of understanding of fertilization principles and improper management have adverse effects on both the environment and the economics of production [8]. Precision agriculture offers a solution in the form of optimal nutrient distribution, tailored to the specific needs of individual crops [9]. This method allows for the maximization of the production potential of each plant within a given agricultural plot, treating it as a system with high variability in local parameters [10,11]. This makes it possible to balance nutrients in the soil, ensuring optimal crop development across the entire plot [12]. A key element of this technology is the collection of spatial data specific to local application zones, which serve as the basis for creating application maps [13]. Soil sampling data are usually in a point format, so spatial prediction methods are used to estimate values for areas not covered by sampling [14]. Interpolation, one of the basic tools in Geographic Information Systems (GIS) and geostatistics, enables forecasting values based on georeferenced samples and calculating a continuous surface [15]. Data from soil chemical analysis and GNSS observations are combined to create spatial maps of nutrient distribution, allowing for the observation of local variability and precise fertilization management [16].

Soil condition plays a key role in environmental protection, biodiversity conservation, and the implementation of appropriate agricultural practices [17–19]. Agricultural activities significantly affect the composition of soil microorganisms, altering their diversity and activity both quantitatively and qualitatively. The intensification of agriculture, including the use of plant protection products, can disrupt soil ecosystem functions, negatively impacting its stability, as well as the diversity and productivity of plants [20–22]. Improving soil productivity to increase yields requires considering its physicochemical and microbiological characteristics. Effective agricultural management should be based on a comprehensive assessment of soil potential, taking into account the limited availability of easily accessible substrates that could support the long-term growth of microorganisms [23]. However, assessing soil quality is a challenge, as it is strongly dependent on land use and the available resources of the agroecosystem [24]. Approaches to soil quality assessment can be either qualitative or quantitative. Soil quality indicators include a combination of physical, chemical, and biological properties [25]. Among the physical indicators, texture, bulk density, water retention capacity, retention characteristics, porosity, aggregate stability, soil depth, pH, electrical conductivity, and cation exchange capacity are distinguished. Biological indicators, on the other hand, include the abundance, activity, and diversity of soil organisms, as well as enzyme activity [26–28]. Microorganisms are a particularly sensitive measure of soil quality, which cannot always be assessed based on physical or chemical indicators. They play a crucial role in biogeochemical cycles, soil structure formation, and pollutant degradation [29]. Due to their short generation time and close interaction with the environment, microorganisms respond quickly to environmental changes and disturbances. Changes in their abundance and activity can be used as indicators of soil condition and the qualitative changes occurring within it [30–32].

Plants obtain nutrients from the products of microbial activity because soil microorganisms constantly oxidize dead plant residues, converting them into nitrogen and mineral compounds that are available to plants and essential for their growth. Soil microorganisms are divided into main groups such as bacteria, actinomycetes, fungi, and algae. Actino-

mycetes are a large and widely distributed group of microorganisms in the soil, with their population estimated at 10^4–10^8 individuals per gram of soil. They are particularly sensitive to low pH (optimal pH ranges from 6.5 to 8.0) and excessive soil moisture. The highest population of actinomycetes occurs in the surface layer of the soil, decreasing with depth. However, individual strains can be found in all soil layers [33]. Typical filamentous actinomycetes primarily belong to the genera Streptomyces and Micromonospora. They are responsible for recycling agricultural waste, utilizing carbon and nitrogen compounds such as cellulose, hemicellulose, proteins, and lignin [34]. Most actinomycetes are aerobic organisms, although some exhibit limited nitrate-reducing capabilities. In the process of organic matter decomposition, actinomycetes act at a later stage, after bacteria and fungi, which are the first to attack fresh organic residues. Actinomycetes are involved in the breakdown of more resistant, harder-to-decompose material, producing dark pigments that affect the color of humus. They are also responsible for the further decomposition of humus, contributing to the formation of more stable forms of organic matter in the soil.

The Green Revolution contributed to the widespread use of mineral fertilizers, which significantly increased crop yields but also led to changes in soil microorganism communities. In the short term, the added nutrients stimulate microbial activity, particularly of bacteria, which quickly utilize them [35]. However, the intensive use of these resources can lead to rapid depletion of organic carbon in the soil, and consequently, a reduction in microbial diversity over the long term [36]. Such changes have significant implications for soil health. To reconcile the short-term benefits of fertilizer application with their long-term impact, it is necessary to precisely adjust nutrient doses and develop new technologies that support sustainable agriculture. Long-term use of mineral fertilizers significantly alters the structure of soil bacterial communities, affecting both their abundance and species diversity. Although initial fertilizer applications increase bacterial populations due to the added nutrients [37], species diversity often decreases. This reduction in diversity limits the soil's resilience to disturbances [38]. Changes also occur in the functional groups of bacteria. Nitrogen fertilizers promote the growth of bacteria that use inorganic nitrogen while reducing the abundance of nitrogen-fixing bacteria, which naturally replenish nitrogen levels in the soil [39]. These shifts affect other critical soil processes, such as phosphorus solubilization and organic matter decomposition. Moreover, long-term fertilizer use stimulates denitrifying bacteria, which may lead to increased greenhouse gas emissions, threatening both climate stability and soil fertility [40]. These changes highlight the need to consider trade-offs between immediate agricultural benefits and the long-term health of the soil and ecosystem balance. Land management affects the structure of microbial communities by altering nutrient levels, which can lead to shifts in the dominant microbial groups, such as from bacteria to fungi. Actinomycetes play a special role in the soil ecosystem because they can form symbioses with non-leguminous plants, fixing atmospheric nitrogen. This nitrogen becomes available to both the host plants and other plants in their vicinity. Since nitrogen is one of the key limiting factors for plant growth in the environment, its fixation is crucial for ecosystem productivity. It is estimated that about 15% of globally fixed nitrogen comes from the symbiotic interactions between actinomycetes of the Frankia family and their host plants. Actinomycetes also contribute to improving the availability of nutrients and minerals, synthesizing plant growth regulators, and demonstrating the ability to inhibit phytopathogens [41]. Thanks to these properties, they not only support plant growth but also protect them from diseases. Moreover, actinomycetes operate in an environmentally friendly manner, supporting the biotic balance of the soil by participating in the nutrient cycling process. These actions contribute to the improvement of soil quality and increased crop productivity [42,43]. Given the crucial role of actinomycetes in soil

ecosystem functioning, they can be considered potential indicators of fertilizer efficiency and soil health [44].

Understanding the impact of precise nitrogen fertilization on the microbiological structure of soil is crucial for improving its quality and increasing agricultural production efficiency. Proper nitrogen fertilization management supports microbial activity, which positively influences nitrogen cycling and soil health. In recent decades, numerous studies have been conducted on the impact of various agronomic practices on the abundance, diversity, and activity of soil microorganisms [45–55]. As Maurer et al. [56] point out, long-term monitoring of microbiological parameters, such as microorganism biomass or metabolic rate, allows for a better characterization of the soil's productive potential and the observation of its changes over time. However, reports on the spatial assessment of mineral components in soil based on microorganism abundance are extremely rare, and there is still a lack of studies in this field. Consequently, spatial methods for assessing soil productivity based on these indicators are also non-existent, which was a key motivation for undertaking the research described in this article. The aim of the study was to quantitatively and qualitatively determine the relationship between the presence of actinomycetes and nitrate content in the soil, as well as to analyze the distribution of these microorganisms in the soil profile.

2. Materials and Methods

2.1. Experiment Detail

The study site where various experimental tests were conducted was located at a family farm in Zędowice, Poland, with a total agricultural land area of 25 hectares (coordinates of the test site—50°34′47.7″ N 18°30′32.9″ E). The structure of the farmland was characterized as a single-purpose farm focused on crop production. Of the total land, approximately 15 hectares was arable land, where cereals, corn, and alfalfa were cultivated, while 10 hectares consisted of grassland. The farm's soils are predominantly podzolic and pseudo-podzolic, with smaller areas of proper chernozem and a weak rye complex.

For the selection of the experimental plots, data archived at the farm were utilized, assuming that the soil tillage practices had been consistent for at least five years, and the degree of implementation of Agriculture 4.0 systems was well understood. Additionally, the CORINE Land Cover database, the Copernicus Program, and the SatAgro service were employed to aid in selecting plots from which laboratory samples were taken. Existing databases on agrotechnical practices, soil chemistry, and crop yields at the farm were also used to identify plots with notable production potential.

A training ground was selected (Figure 1a,b), where no soil tillage had been performed but precision fertilization treatments had been applied using a parallel driving system. The soil profile in this area exhibited significant variation in the vertical plane (Figure 1c). The number of measurement points depended on the initial measurements, but there was no fewer than one measurement point per hectare of the experimental field. Samples were collected after the harvest, and the processes were repeated over three consecutive cycles.

Weather conditions were recorded by WH 2600 weather stations (Hirschau, Germany), which are equipped with an internet protocol that allows real-time data visualization, archiving, and export in a selected frequency regime. These stations were equipped with a wind vane, wind speed sensor, solar panel, temperature and humidity sensors, a UV sensor, a light sensor, and a rain sensor. The technical specification of the station included: transmission range in open space up to 100 m, frequencies of 433 MHz/868 MHz/915 MHz, temperature range from −40 °C to 60 °C (accuracy ±1 °C, resolution 0.1 °C), relative humidity measurement in the range of 1% to 99% (accuracy ±5%), rainfall measurement in the range of 0–9999 mm (accuracy ±10%, resolution 0.3 mm for rainfall <1000 mm

and 1 mm for rainfall >1000 mm), wind speed of 0–50 m/s (accuracy ±1 m/s for wind speed <5 m/s, ±10 m/s for wind speed >5 m/s), and light measurement in the range of 0–400 kLux (accuracy ±15%). During the study, the average temperature was 14.4 °C, the average dew point temperature was 8.2 °C, the average air movement speed was 4.9 km/h, the average humidity was 67.8%, the average solar radiation was 164.6 W/m^2, and the average rainfall was 3.2 mm. The pH value remained in the range of 5.4–6.2 at the studied depths of the soil profile.

(**a**) (**b**) (**c**) (**d**)

Figure 1. Testing ground: (**a**) research site; (**b**) sampling points; (**c**) soil profiles; (**d**) electrical conductivity.

2.2. Identification of Sampling Locations

The choice of sampling locations within the test site was based on the available conductivity map of the farmland (Figure 1d). This selection was made with the understanding that precise identification of the structure of plant nutrients, both within the soil profile and at field scales, is particularly important in the context of the qualitative and quantitative attributes of microorganisms (Figure 1d).

2.3. Microbiological Analysis

2.3.1. Collection and Preparation of Samples for Microbiological Analysis

Soil samples for microbial isolation were collected using a microbiological knife under microbiologically sterile conditions and placed in sterile containers, in accordance with PN-ISO 10381-6:1998 [57]. In the laboratory, the soil samples were spread in a thin layer on cardboard trays and left to air-dry for two days. The drying area was kept free from dust, gases, and vapors. Periodically, the soil was stirred, and larger lumps were broken up. Once air-dried, the soil was placed into labeled containers (including the date of collection, sample number, and depth of collection) and sealed. Before the sample was prepared for analysis, it was weighed, and impurities such as pebbles and plant roots were removed. The impurity-free soil was then sieved through a sieve with a 1 mm mesh diameter.

2.3.2. Preparation of Culture Media for Isolation of Microorganisms

Gauss medium was used for the isolation of actinomycetes. The media were prepared immediately before isolation in sterile conical flasks. A 0.85% (*w*/*v*) was used to prepare the dilutions. Each ingredient was measured using a calibrated analytical laboratory balance with an accuracy of 0.0001 g, from Radwag (Radom, Poland), model AS 220.R2, and quantitatively transferred into glass bottles. The ingredients were then dissolved in an appropriate amount of demineralized water and stirred until fully dissolved. The pH was measured using a pH meter from Mettler Toledo (Columbus, OH, USA), model MA235. The prepared media were sterilized in an autoclave for 15 min at a temperature of 121 °C and a pressure of 1 bar.

2.3.3. Microorganism Isolation

The isolation of microorganisms was performed using the serial dilution method according to Koch (Wichita, KS, USA) [58]. This method involves making successive

10-fold dilutions of the test sample to obtain single colonies of microorganisms or to ensure their absence in the final dilution. For each test, 10 g of soil samples were transferred into flasks containing 90 mL of saline solution. Six successive 10-fold dilutions of the test samples were then prepared, as illustrated in Figure 2. Petri dish cultures were prepared by transferring 1 cm^3 of the dilution onto a plate and pouring cooled medium over it. The medium and suspension were thoroughly mixed by moving the plate in a figure-eight motion. Actinomycetes were cultured for 120 h.

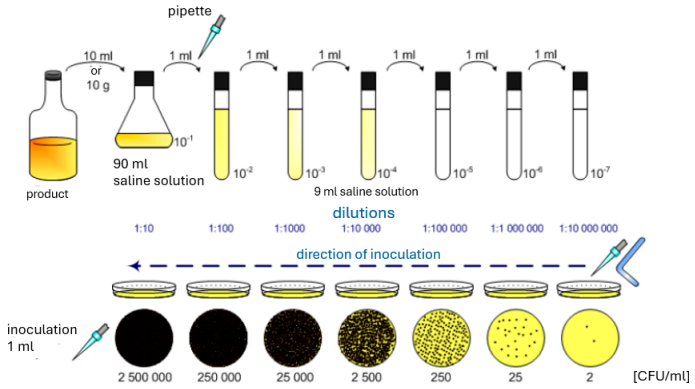


Figure 2. Scheme for the preparation of 10-fold dilutions [59].

2.3.4. Species Identification of Microorganisms by MALD-TOF MS Technique

To perform MALDI-TOF analysis (Figure 3), microorganisms are placed on a target plate, along with a matrix solution that crystallizes with the sample and causes cell lysis. The plate is then placed in an instrument where a laser transforms the bacterial components (mainly ribosomal protein molecules) into gas-phase ions, which are separated and identified according to their mass-to-charge ratio. The mass spectrometer produces a spectral "fingerprint" unique to the microorganism being analyzed. The organism is then identified by comparing its spectral profile with a reference database. Correlations in peak position and intensity between the experimental and database spectra are used to generate a matching score. This score reflects the level of confidence that the unknown isolate matches the candidate microorganism from the database. Protein mass pattern spectra can be used to identify bacteria at the genus, species, or even subspecies level [60]. The identification of microorganisms isolated during the study was carried out at the Microbiological Laboratory of the Jagiellonian Centre of Innovation in Krakow, Poland.

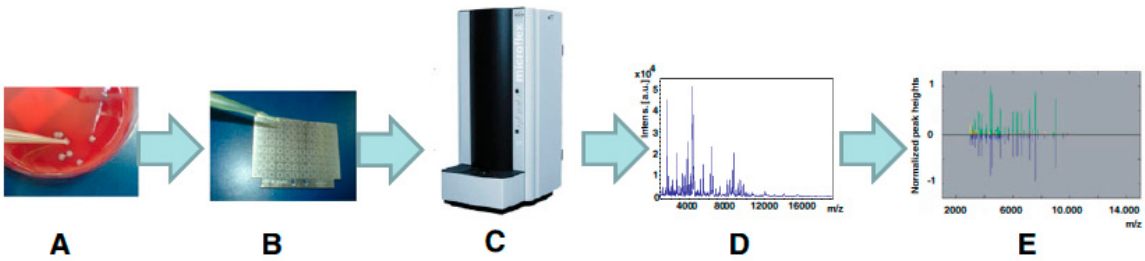


Figure 3. MALDI-TOF MS procedure (**A**) colony collection, (**B**) samples are applied to a matrix and air-dried, (**C**) MALDI-TOF MS measurement, (**D**) analyte-specific mass spectrum, (**E**) analyte identification by automatically matching the generated mass spectrum with spectra in the database [60].

2.4. Determination of Nitrate Content by Colorimetric Method

The determination of nitrate (NO_3^-) in the samples was conducted in accordance with PN-R-04028:1997 "Soil Chemical and Agricultural Analysis, Sampling and Determination

of Nitrogen and Ammonium Ions in Mineral Soils" [61]. The research was performed by the District Chemical and Agricultural Station in Krakow.

2.5. Creating Digital Maps of Spatial Variation of Measured Quantities

The Inverse Distance Weighted (IDW) method, which does not require modeling and is one of the simplest interpolation techniques [62], was used to interpolate the measured quantities treated as deterministic variables. The advantage of this approach is its ability to capture local variation in the attributes of the interpolated surface. In the calculations, only a limited number of measurement points located within a specified neighborhood of the interpolation point are used, allowing the algorithm to process large datasets efficiently—Equation (1) [63].

$$Z_j = \frac{\sum_{i=1}^{n} \frac{Z_i}{h_{ij}^{\beta}}}{\sum_{i=1}^{n} \frac{1}{h_{ij}^{\beta}}} \quad (1)$$

where:

Z_j—the value of the feature Z estimated at the point j,
Z_i—the value of feature Z measured at point i (one of the n data points in the environment),
h_{ij}—effective distance between points i and j,
β—power exponent–weight of distance.

ESRI ArcView GIS 3.3 software was used to interpolate point data, visualize vector and raster maps, perform map operations such as logical queries and spatial data selections, and overlay interdependent data from individual maps. The interpolation was performed with a resolution of 10 m. When mapping the spatial variation of measured quantities, the same interpolation parameters were applied: a weight of 2, with the number of neighboring points set to 12 [64].

2.6. Statistics

Statistical analyses were performed using the Statistica 13 statistical package (StatSoft, Inc., Tulsa, OK, USA). The normality of the distribution of the studied parameters was analyzed using the Kolmogorov–Smirnov test.

To account for the spatial nature of the parameters under study, the spatial correlation method was employed as an example of a geostatistical method [65]. Spatial correlation coefficients were determined using the calculation package of ESRI ArcView GIS 3.3 software.

3. Results and Discussion

3.1. Determination of Nitrate Content

As the depth of the soil profile increased, there was a decrease in nitrate content. In the soil layers at 0.15 m and 0.25 m, the decrease in nitrate values was 7.83 mg NO_3^-/100 g of soil, with low coefficients of variation of 17% (0.15 m) and 15% (0.25 m). In contrast, a much lower decrease in nitrate content was observed in the deeper soil layers (between 0.25 m and 0.35 m)—only 0.68 mg NO_3^-/100 g of soil. The largest variation (12.02 mg NO_3^-/100 g) in nitrate content was recorded at depths between 0.05 m and 0.35 m, with a coefficient of variation of no more than 13%. A graphical display of the nitrate content in the soil at a specific point is shown in Figure 4. To determine the spatial distribution of nitrate content in the soil at all analyzed depths, their spatial variation was mapped (Figures 5–8).

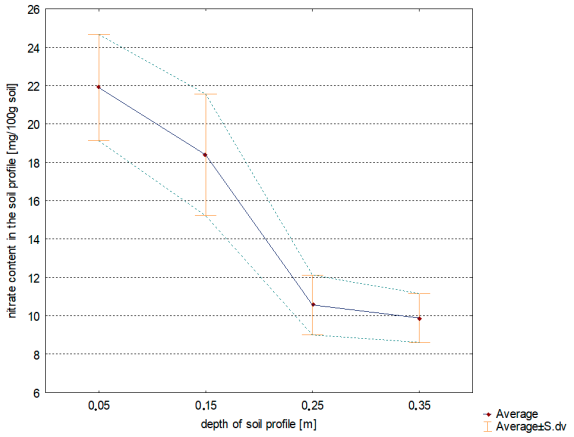


Figure 4. Nitrate content in the soil profile.

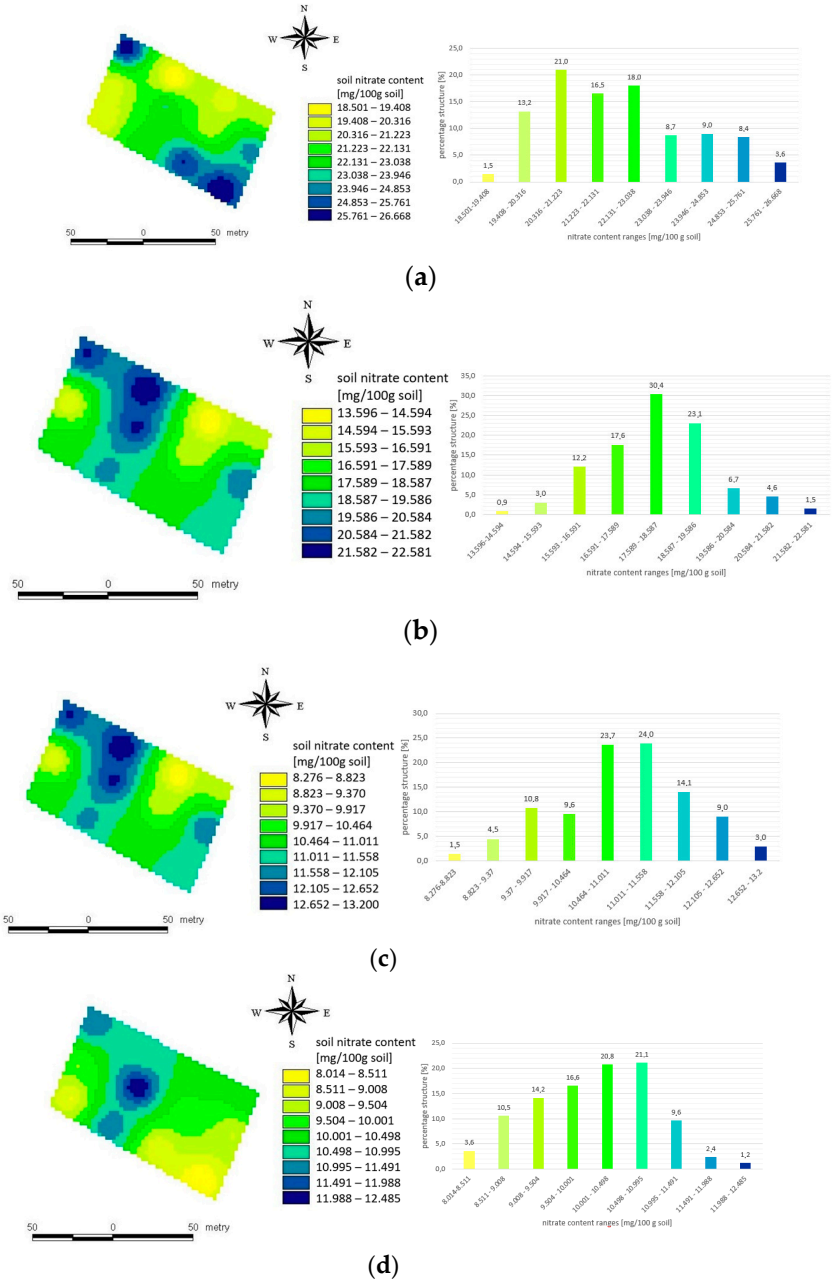


Figure 5. Spatial variation of soil nitrate content and its percentage structure in the field; (**a**) at a depth of 0.05 m; (**b**) at a depth of 0.15 m; (**c**) at a depth of 0.25 m; (**d**) at a depth of 0.35 m.

The field area with the highest soil nitrate content at a depth of 0.05 m (ranging from 25.762 to 26.668 mg/100 g of soil) accounted for 3.6% of the total area (Figure 5a). A small portion, about 1.5% of the total investigated area, depicted a zone of lowest soil nitrate content (18.501 to 19.408 mg NO_3^-/100 g). In an area covering 21% of the field, soil nitrate content was observed to be in the range of 20.317 to 21.223 mg NO_3^-/100 g of soil. This area was located in the western and northeastern parts of the field. For soil nitrate content recorded at a depth of 0.15 m, the highest nitrate content was within the range of 21.583 to 22.581 mg NO_3^-/100 g of soil, occupying 1.5% of the area (Figure 5b). Soil nitrate content in the range of 17.59 to 18.587 mg NO_3^-/100 g of soil characterized an area accounting for as much as 30.4%. This area included the western and eastern parts of the field. The field area with the highest soil nitrate content at a depth of 0.25 m (ranging from 12.653 to 13.2 mg NO_3^-/100 g of soil) accounted for 3% of the total area (Figure 5c). The areas with the lowest soil nitrate content, ranging from 8.276 to 8.823 mg/100 g of soil, accounted for twice the size of the aforementioned area. This region included the western and northeastern parts of the field. Similarly, in an area covering about 24% of the tested field at the same depth of 0.25 m, the nitrate content of the soil was observed to be in the range of 11.012 to 11.558 mg NO_3^-/100 g of soil. For soil nitrate content recorded at a depth of 0.35 m, the highest nitrate content ranged between 11.989 and 12.485 mg NO_3^-/100 g of soil (Figure 5d).

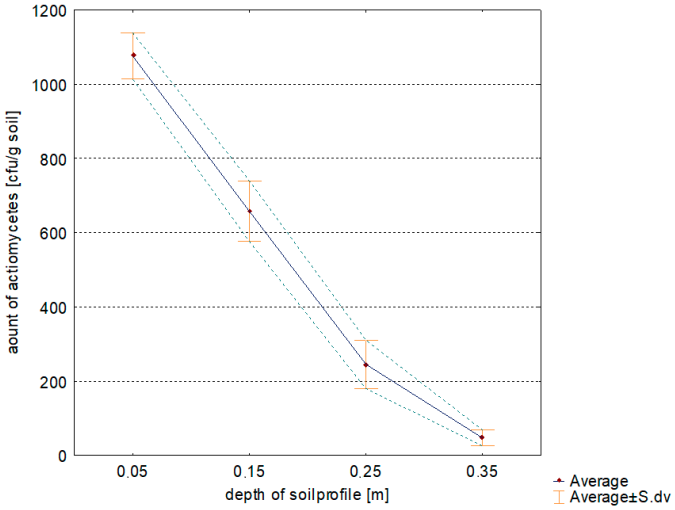


Figure 6. Amount of actinomycetes in the soil profile.

Based on the collected data, there is a clear downward trend in soil nitrate content as the depth of the soil profile increases. The decrease is particularly significant in the shallower soil layers (0.15 m and 0.25 m), where it reached an average reduction of 7.83 mg NO_3^-/100 g of soil, with low coefficients of variation of 17% and 15%, respectively. In contrast, a smaller decrease of only 0.68 mg NO_3^-/100 g of soil was observed in the deeper soil layers (0.25 m and 0.35 m). The results also highlight the spatial variation of soil nitrate content across different layers of the soil profile. In all layers, the area with the lowest nitrogen content occupied up to 1.5% of the study area, while the area with the highest nitrogen content comprised up to 3.6%. This aligns with observations by Małecka et al. [66], who noted a similar trend for both conventional and reduced tillage. However, exceptions to the general downward trend were identified by Blecharczyk et al. [67]. Specifically, in conventionally tilled soil, there was an increase in nitrogen content in the soil profile, ranging from 91 to 93 mg/100 g of soil at a depth of 0.2 m. Conversely, other tillage methods, such as shallow plowing, disc harrowing, and the use of a stubble unit, exhibited a decrease in soil nitrogen content. Research examining the effects of different tillage practices on

nitrogen content in agricultural soils and the analysis of changes in soils under various types of land use reveal important aspects related to nitrogen cycling in agricultural and natural environments [68–70]. The manipulation of crop residues and vegetation, as well as the conversion of forests to cropland, has a significant impact on soil nitrogen availability. Notably, no-till practices may promote the accumulation of organic matter in the surface layers of the soil, which can influence nitrogen cycling. Furthermore, findings regarding the relationship between microbial enzyme activity and soil nitrogen content underscore the necessity of considering these factors in modeling and planning agrotechnical measures. The conclusions drawn from these observations highlight the importance of monitoring soil nitrate content and suggest that selecting appropriate tillage methods and considering plowing depth can significantly influence soil nitrate levels.

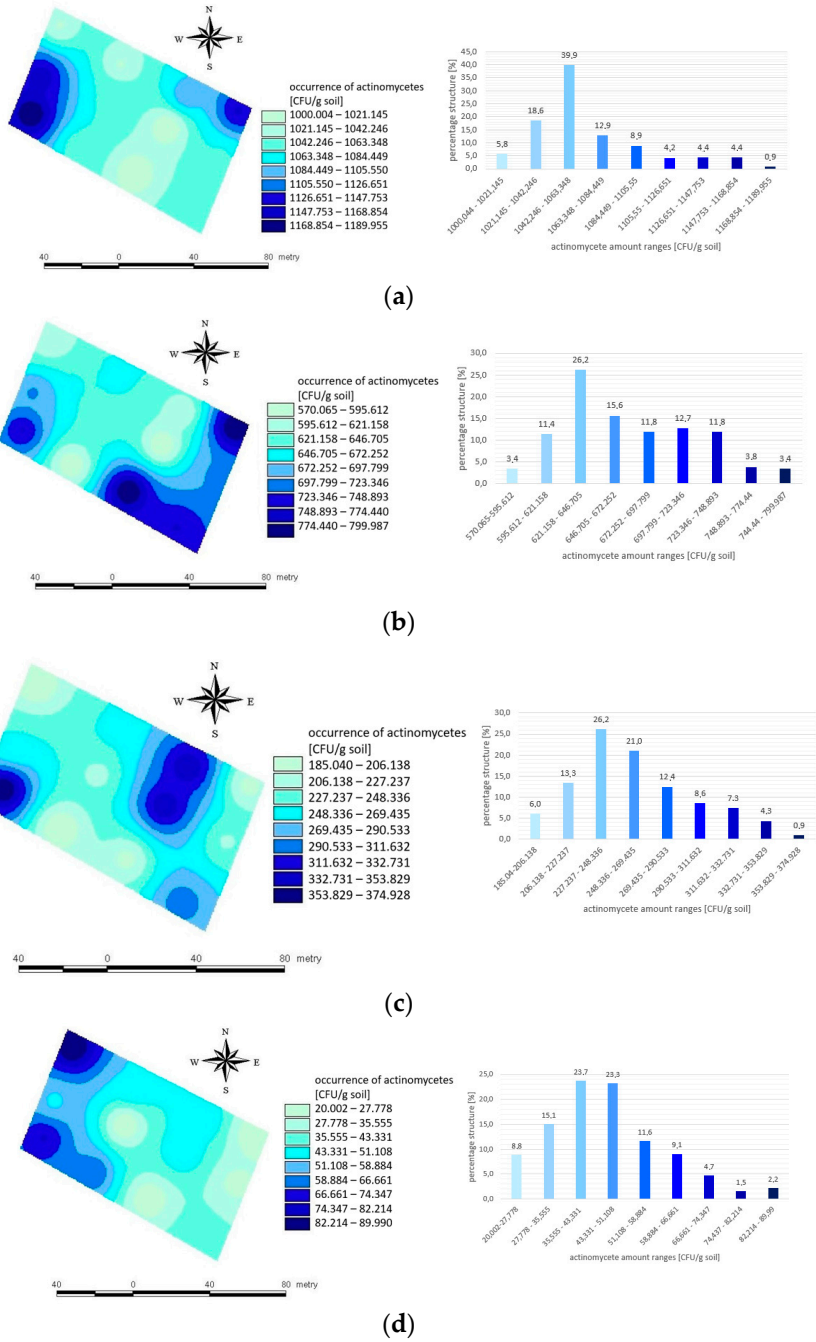


Figure 7. Spatial variation of occurrence of actinomycetes in the soil profile; (**a**) at a depth of 0.05 m; (**b**) at a depth of 0.15 m; (**c**) at a depth of 0.25 m; (**d**) at a depth of 0.35 m.

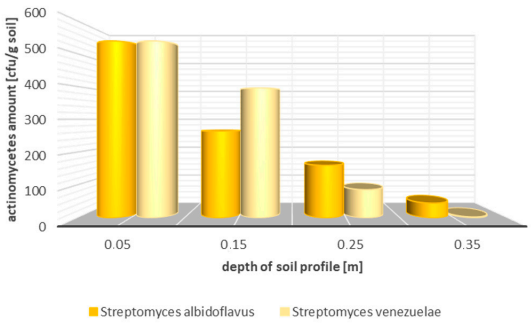


Figure 8. Average amount of individual strains of actinomycetes in the soil profile.

3.2. Quantitative and Qualitative Characteristics of Actinomycetes in the Soil Profile

The lowest mean value of actinomycete abundance was obtained at a depth of 0.35 m, while the highest was recorded at 0.05 m. These values were 46.9 CFU/g of soil and 1075.6 CFU/g of soil, respectively, with coefficients of variation of 46% and 6%. The observed values of actinomycete abundance were significantly different at different depths of the soil profile. Figure 6 illustrates the abundance of actinomycetes at various levels of the soil profile.

The area of the field characterized by the highest abundance of actinomycetes in the soil at a depth of 0.05 m (ranging from 1168.855 to 1189.955 CFU/g of soil) accounted for only 0.9% of the total area. An area comprising 5.8% of the tested area had the lowest abundance of actinomycetes, with values ranging from 1000.044 to 1021.145 CFU/g of soil. In an area covering as much as 39.9% of the field, actinomycete values were observed to fall within the range of 1042.247 to 1063.348 CFU/g of soil, corresponding to the central part of the study area (Figure 7a). Regarding the abundance of actinomycetes in the soil at a depth of 0.15 m, the highest abundance was recorded in an area occupying 3.4% (ranging from 744.441 to 799.987 CFU/g of soil). The same area (3.4%) was also occupied by regions with the lowest abundance of actinomycetes. An area of 26.2% was occupied by regions located in the central part of the field (Figure 7b), where the abundance of actinomycetes fell within the range of 621.159 to 646.705 CFU/g of soil. In the lower parts of the soil profile at a depth of 0.25 m, the highest abundance of actinomycetes was recorded in an area located mainly at the southwestern end of the field, covering 0.9% of the total area. The lowest abundance was recorded at the eastern end and in the central part of the field, accounting for 6%. An area covering 26.2% of the field had an abundance of actinomycetes falling within the range of 227.238 to 248.336 CFU/g of soil (Figure 7c). At a depth of 0.35 m, the highest abundance of actinomycetes, ranging from 82.215 to 89.99 CFU/g of soil, was recorded in an area occupying 2.2% of the field. The lowest abundance of actinomycetes, with values ranging from 20.002 to 27.778 CFU/g of soil, was found in a plot covering 8.8% of the field. A significant abundance of microbes was characterized by an area occupying as much as 23.7% of the field, located in the eastern and central parts (Figure 7d).

Two strains were selected for identification: *Streptomyces albidoflavus* (Figure 9) and *Streptomyces venezuelae* (Figure 10). The number of ray species in each layer of the soil profile is shown in Figure 8. The presence of *Streptomyces albidoflavus* and *Streptomyces venezuelae* strains was recorded at all depths within the analyzed interval (0.05–0.35 m). It can be observed that the abundance of both species decreased with depth. In the topsoil layer, the average abundance of *Streptomyces albidoflavus* and *Streptomyces venezuelae* was 538 CFU/g of soil for each species. However, in the layer at a depth of 0.15 m, the abundance of *Streptomyces venezuelae* exceeded that of *Streptomyces albidoflavus* by an average of 131 CFU/g of soil. In the deeper soil layers (0.25–0.35 m), the abundance of

Streptomyces albidoflavus was higher, averaging 160 CFU/g of soil at a depth of 0.25 m and 45 CFU/g of soil at a depth of 0.35 m.

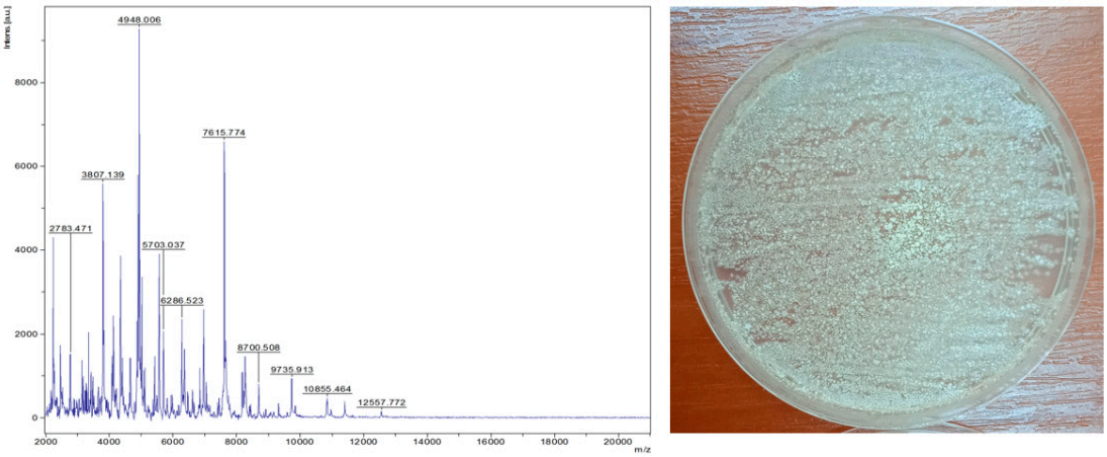


Figure 9. Result of species identification of *Streptomyces albidoflavus*: (**a**) Mass spectrum; (**b**) Macroscopic appearance of the colony.

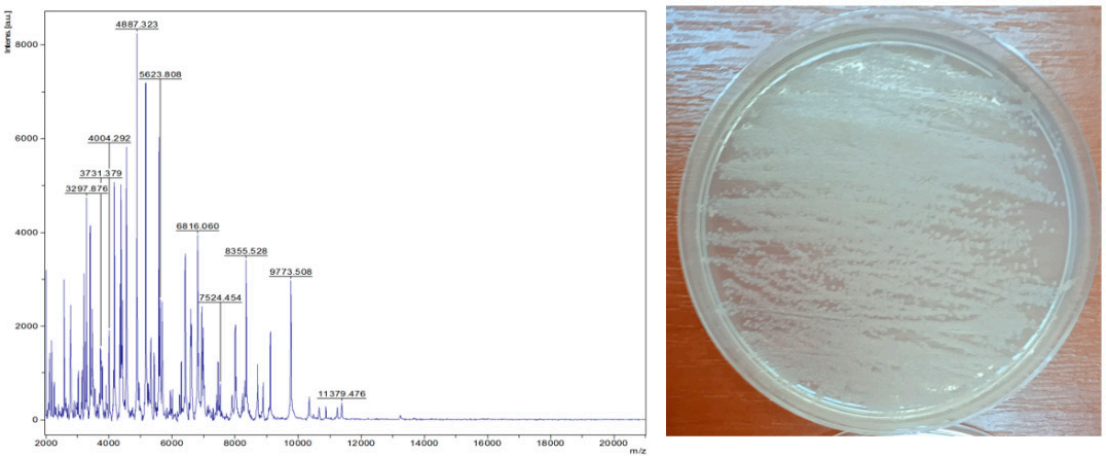
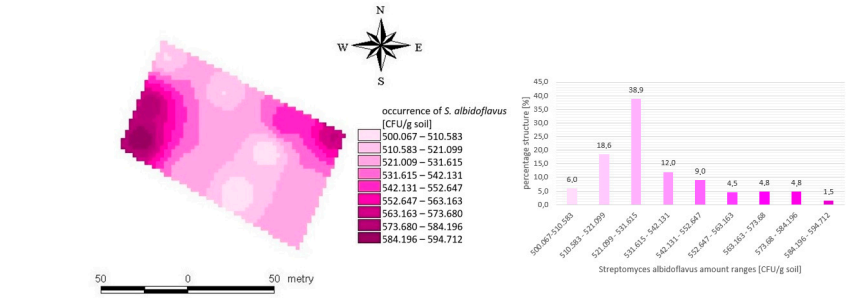


Figure 10. Result of species identification of *Streptomyces venezuelae*: (**a**) Mass spectrum; (**b**) Macroscopic appearance of the colony.

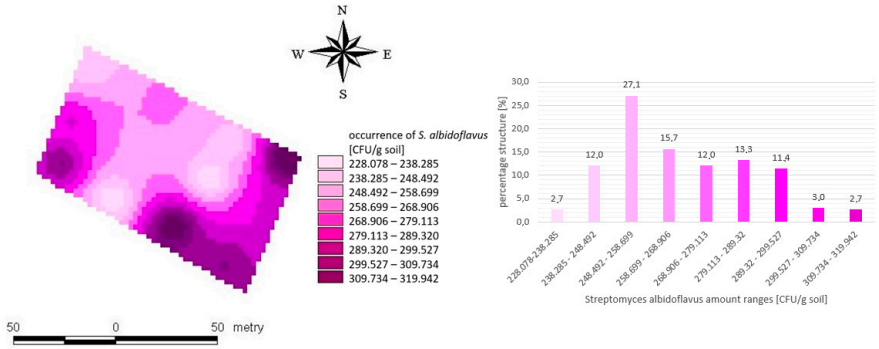
The obtained mass spectra were compared with the mass spectra exhibiting the highest similarity index found in Bruker's MALDI Biotyper database (Figures 9a and 10a).

In the soil profile at a depth of 0.05 m, two species of actinomycetes were identified: *Streptomyces albidoflavus* and *Streptomyces venezuelae*. In the case of *Streptomyces albidoflavus*, the highest abundance was found within the range of 584.196 to 594.712 CFU/g of soil in an area occupying 1.5% of the total field. This area was located in the northeastern and southwestern parts of the field (Figure 11a). The area characterized by the lowest abundance of this species (range 500.067 to 510.583 CFU/g of soil) was found in the central part of the field, representing about 6% of the total tested area. This central area had a significant abundance, accounting for as much as 38.9%. At a depth of 0.15 m, *Streptomyces albidoflavus*, the highest abundance, falling within the range of 309.734 to 319.942 CFU/g of soil, was found in an area occupying 2.7% of the total field. This area was located in the southern and northeastern parts of the field (Figure 11b). The area characterized by the lowest abundance of this species (range 228.078 to 238.285 CFU/g of soil) included a consolidated area (2.7%) in the central part of the field, where a significant abundance was

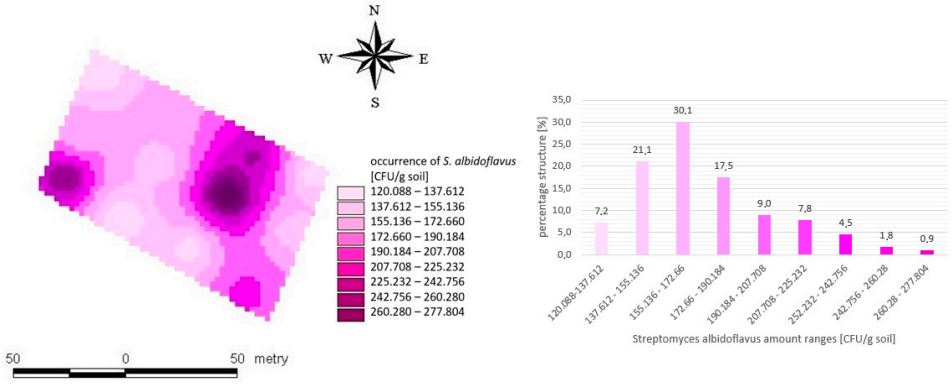
found (27.1%). In the soil profile at a depth of 0.25 m, the species *Streptomyces albidoflavus* exhibited the highest abundance, falling within the range of 260.28 to 277.804 CFU/g of soil, with the area occupying only 0.9%. This area was located in the southwestern and eastern parts of the field (Figure 11c). The area characterized by the lowest abundance of this species (range 120.088 to 137.612 CFU/g of soil) covered a larger area (7.2%), consolidated in the southern and eastern parts of the field. The central part of the field (30.1%) exhibited a significant abundance of *Streptomyces albidoflavus*. In the soil profile at a depth of 0.35 m, *Streptomyces albidoflavus* again showed the highest abundance, falling within the range of 78.371 to 85.79 CFU/g of soil, with the area occupying 2.7%. This area was located in the northwestern part of the field (Figure 11d). The area characterized by the lowest abundance of this species (range 19.02 to 26.439 CFU/g of soil) included an area (8.3%) consolidated in the eastern part of the field. The eastern part of the field (23.2%) also exhibited a significant abundance.



(**a**)

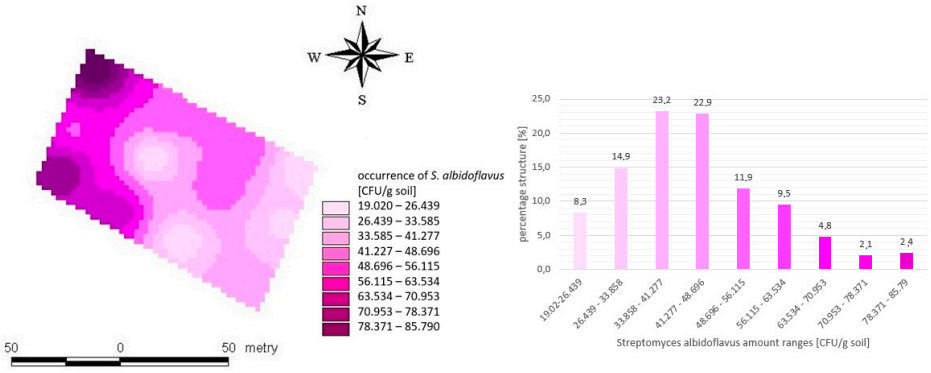


(**b**)



(**c**)

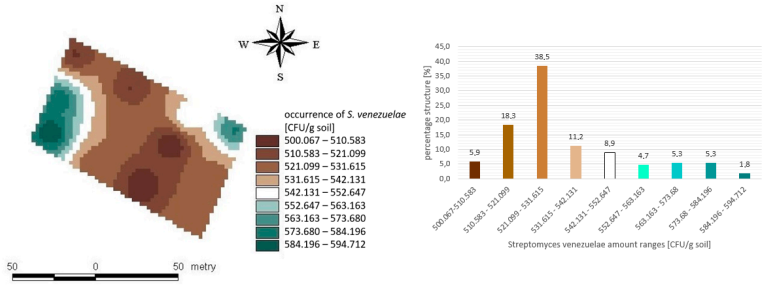
Figure 11. *Cont.*



(**d**)

Figure 11. Spatial variation of occurrence of *Streptomyces albidoflavus* in the soil profile; (**a**) at a depth of 0.05 m; (**b**) at a depth of 0.15 m; (**c**) at a depth of 0.25 m; (**d**) at a depth of 0.35 m.

The highest abundance of *Streptomyces venezuelae* fell within the range of 584.196 to 594.712 CFU/g of soil, characterized by an area occupying 1.8% of the field. This area was also located in the northeastern and southwestern parts of the field (Figure 12a). The area with the lowest abundance of this species (range 500.067 to 510.583 CFU/g of soil) included an area (5.9%) consolidated in the central part of the field. The significant abundance of this species was mainly located in the western and eastern parts of the field, accounting for as much as 38.5%. In the case of *Streptomyces venezuelae* at a depth of 0.15 m in the soil profile, the highest abundance, falling within the range of 464.602 to 479.912 CFU/g of soil, was characterized by an area occupying only 5.2%. This area was located in the eastern part of the field (Figure 12b). The area with the lowest abundance of this species (range 342.117 to 357.428 CFU/g of soil) included a consolidated area (2.9%) in the southern and eastern parts of the field. The area characterized by significant abundance was mainly located in the western part of the field, accounting for 29.1%. *Streptomyces venezuelae*, the highest abundance at a depth of 0.25 m in the soil profile, falling within the range of 123.389 to 130.682 CFU/g of soil, was characterized by an area occupying 1.2%. This area was located in the southern and northern parts of the field (Figure 12c). The area characterized by the lowest abundance of this species (range 65.044 to 72.337 CFU/g of soil) included an area (6%) consolidated in the western and southeastern parts of the field. The central part of the field was characterized by a significant abundance (25.7%). For *Streptomyces venezuelae* at a depth of 0.25 m in the soil profile, the highest abundance, falling within the range of 4.545 to 4.988 CFU/g of soil, was characterized by an area occupying 1.8%. This area was also located in the northwestern part of the field (Figure 12d). The area characterized by the lowest abundance of this species (range 1.001 to 1.444 CFU/g of soil) included an area (6.3%) consolidated in the central and northeastern parts of the field. A significant abundance was primarily located in the central part of the field (27.5%).



(**a**)

Figure 12. *Cont.*

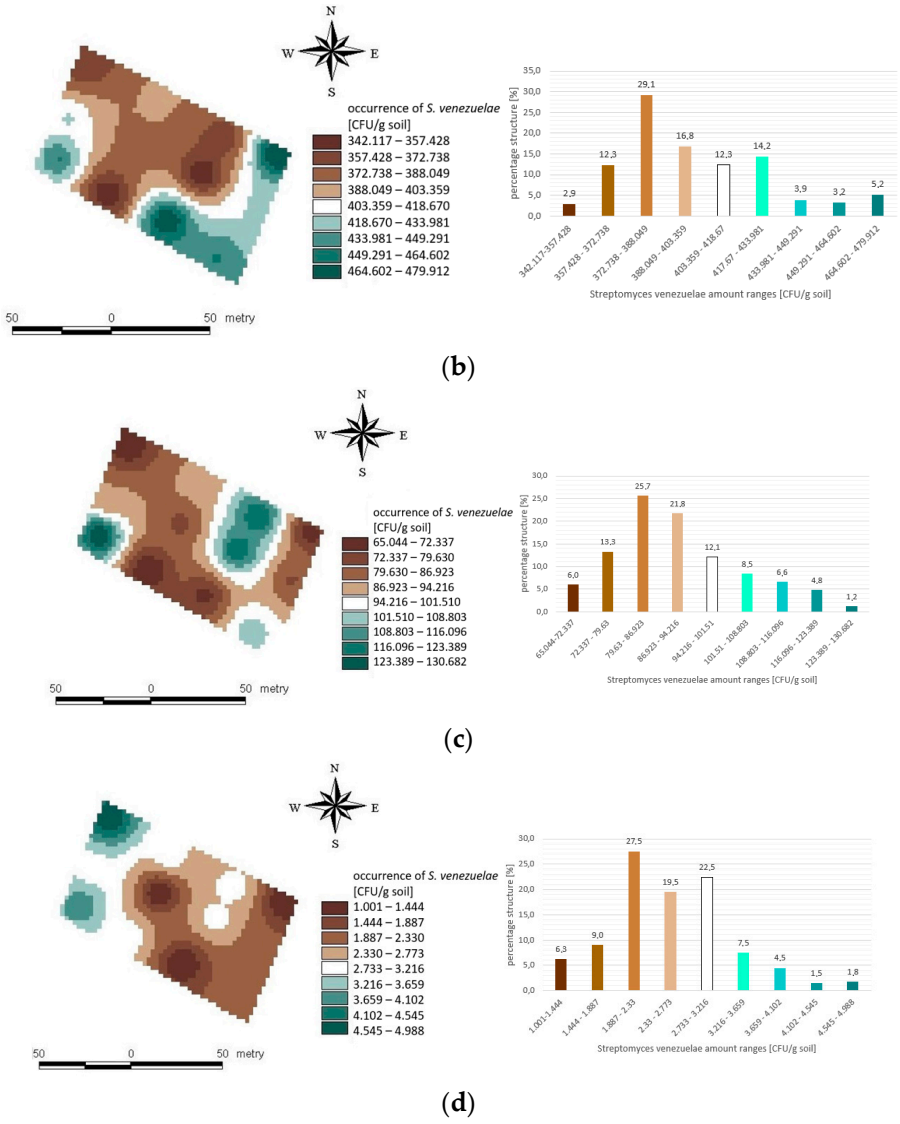


Figure 12. Spatial variation of occurrence of *Streptomyces venezuelae* in the soil profile; (**a**) at a depth of 0.05 m; (**b**) at a depth of 0.15 m; (**c**) at a depth of 0.25 m; (**d**) at a depth of 0.35 m.

The analysis of data regarding the abundance of actinomycetes in the soil profile offers valuable insights into the diversity of these organisms in the study area. The results reveal significant variations in the average number of actinomycetes depending on soil depth. The average abundance was lowest at a depth of 0.35 m, with only 46.9 CFU/g of soil, while it peaked at 0.05 m, reaching as high as 1075.6 CFU/g of soil. Interestingly, the areas with the highest and lowest actinomycete abundance accounted for just 0.9% and 5.8% of the field area, respectively. Similar findings were reported by Małecka [71] and Kiełbasa [72]. Notably, the presence of *Streptomyces albidoflavus* and *Streptomyces venezuelae* strains across all depths of the soil profile suggests their widespread distribution in the study area. Ingram et al. [73] and Liu et al. [74] highlighted an important observation regarding microbial distribution in soil: they found that the topsoil layer (0–5 cm) harbored a higher number of microorganisms (including actinomycetes) compared to the deeper layer (5–15 cm). This difference is likely due to the higher density of roots in the topsoil, which provides essential nutrients. The supply of water-soluble compounds makes microbial activity in the root zone particularly dynamic. Moreover, actinomycetes were observed to be the second most abundant group of microorganisms in the soil, regardless of the intensity of grassland use. Musiał et al. [75] noted the significant influence of agricultural

practices and atmospheric conditions on soil microbial composition. They found that actinomycetes responded most markedly to vegetation management practices. For instance, higher populations of actinomycetes were recorded in mowed areas, especially during the last mowing cycle, when their numbers reached 102.4 CFU/g dry mass of soil in mowed meadows, compared to 43.9 CFU/g dry mass of soil in pastures. Additionally, a high abundance of actinomycetes was observed during dry periods. The authors also emphasized that actinomycetes significantly influence the inorganic nitrogen content of the soil, irrespective of agricultural practices, underscoring the importance of this group of microorganisms in the soil nitrogen cycle.

3.3. Spatial Correlation Coefficients Between Isolated Actinomycetes Species and Soil Nitrate Content

To account for the spatial nature of the parameters studied, the spatial correlation method was employed. For the superficial soil layer (0.05 m), a spatial correlation coefficient of −0.52 was obtained (Table 1). Similarly, for the soil stratum at a depth of 0.15 m, a spatial correlation coefficient of 0.19 was found between the abundance of *Streptomyces albidoflavus* and *Streptomyces venezuelae* and the nitrate content of the soil profile. A high value of the spatial correlation coefficient was recorded in the soil layer at a depth of 0.25 m, where it was −0.59 for *Streptomyces albidoflavus* and −0.57 for *Streptomyces venezuelae*. In the case of the deepest soil layer (0.35 m), a lower spatial correlation coefficient was observed, with a value of 0.26 for *Streptomyces albidoflavus* and 0.22 for *Streptomyces venezuelae*.

Table 1. Spatial correlation coefficients between isolated actinomycetes species and soil nitrate content.

		***Streptomyces albidoflavus* [cfu/g Soil]**				***Streptomyces venezuelae* [cfu/g Soil]**			
	Depth [m]	**0.05**	**0.15**	**0.25**	**0.35**	**0.05**	**0.15**	**0.25**	**0.35**
Nitrate content [mg/100 g soil]	0.05	−0.52				−0.52			
	0.15		0.19				0.19		
	0.25			−0.59				−0.57	
	0.35				0.26				0.22

Calculations made at the level of significance $\alpha = 0.05$.

The analysis of the spatial nature of the studied parameters using the spatial correlation method offers valuable insights into the relationship between actinomycetes abundance and nitrate content in the soil profile. The results reveal variations based on the depth of the soil layer examined. For the surface soil layer (0.05 m) and the layer at a depth of 0.15 m, similar spatial correlation coefficients were observed: −0.52 for the abundance of *Streptomyces albidoflavus* and 0.19 for *Streptomyces venezuelae* in relation to nitrate content. In contrast, a higher spatial correlation coefficient of −0.59 for *Streptomyces albidoflavus* and −0.57 for *Streptomyces venezuelae* was recorded in the soil layer at a depth of 0.25 m. These results suggest a stronger relationship between the abundance of these actinomycetes and the nitrate content in this specific soil layer. In the deepest soil layer (0.35 m), lower spatial correlation coefficients were obtained, measuring 0.26 for *Streptomyces albidoflavus* and 0.22 for *Streptomyces venezuelae*. This indicates a reduced influence of nitrate content on the abundance of these actinomycetes in the deeper soil layer. Overall, the analysis of spatial correlation coefficients suggests that the abundance of actinomycetes is related to nitrate content in the various layers of the soil profile within the study area. Research by Liu et al. [76] indicates that total nitrogen content in the soil can significantly impact microbial biomass and activity. Their findings demonstrate that the structure and distri-

bution of actinomycetes communities are closely tied to the availability and quality of total nitrogen. Furthermore, studies by Liu et al. [77] and Sul et al. [78] have identified clear positive correlations between total nitrogen content and actinomycetes abundance, underscoring the importance of this nutrient in shaping soil microbial composition. Additionally, changes in total nitrogen content have been shown to affect microbial composition variation, as confirmed by Ramirez et al. [79]. Their study highlighted an upward trend in the abundance of Actinobacteria in soil in response to nitrogen addition, suggesting that alterations in nitrogen content can lead to shifts in the functioning and metabolism of soil microorganisms.

4. Conclusions

Knowledge of nitrate content allows for the inference of actinomycetes abundance in the soil profile. Areas with low nitrogen levels typically exhibit lower microbial abundance, as confirmed by the spatial correlation coefficients. Furthermore, specific species of actinomycetes present in the soil profile can be identified, providing valuable insights into the soil's productive suitability and its nitrogen compound richness. The findings from this study establish a foundation for developing methodological guidelines for cultivation technology that incorporate information about actinomycetes. This approach can serve as a determinant for fertilization practices, promoting more effective management of soil health and fertility.

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