Physical and microbiological aspects of the soil seed bed in the early formation of the barley root system (*Hordeum vulgare* L.): tomographic studies

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Abstract. Barley seeds (Mikhailovsky sort) in a model physical experiment with a two-layer density soil bed (the range of soil density from 0.7 to 1.2 g/cm³) of silty loam arable soil (Albic Glossic Retisols (Lomic, Cutanic), Moscow Region) were placed for germination at the boundary of different-density layers at optimum moisture content. During the period from planting to 7-10 days, the dynamics of the root system development was studied using an x-ray microtomograph "Bruker SkyScan 1172G" (Belgium) while simultaneously studying the composition of soil biota, which was reconstructed from microbial markers (fatty acids and their derivatives). The roots of the germinating seed mastered the entire pore space of the soil, regardless of the density zones studied in experiments. The number of bacteria increases by the fifth day with a noticeable dominance of actinobacteria (aerobacteria *Actinobacteria* spp., *Rhodococcus equi*) and Firmicutes (anaerobes *Ruminococcus* sp.) due to the destruction of the seed coat with its subsequent decrease on the seventh day.

1. Introduction
The complex soil-root-micro-organism consortium differs from the entire soil biome due to specific trophic chains, moisture potential distribution, and redox reactions. Activity and functioning, localization within the root system of rhizosphere biota are diverse and dynamic. It is known that groups of microorganisms can differentially populate the root system [1-2] and are distributed differently around the root. How micro-organisms soil biota are changed as the plant grows and the root system forms, is an extremely complex task, especially if it is solved in a regular dynamic aspect. The root system performs the mechanical function of fixing, absorbing moisture, nutrients, and interacting with the environment, which is determined by the morphology of the root system. Root morphology also affects yield, stress tolerance to factors such as drought, soil compaction, and nutrient deficiencies. [3] Plant roots have a great influence on the physical and biochemical properties of the surrounding soil. They stimulate the growth and activity of microorganisms and, as a result, affect the regulation of decomposition of soil organic matter [4]. The spatial and temporal dynamics of processes occurring underground in real time is complicated by both the opacity of the soil and the sensitivity of roots to extraction from the soil, damage to small lateral roots. All these properties have made the rhizosphere the subject of many studies involving non-invasive methods of volumetric visualization [5-7]. It is believed that the architecture of the root system is the result of the interaction of the genotype and growth
conditions. For example, it was found that rice has a growth-specific response to changes in soil structure [8]. A non-invasive computed tomography method was used in combination with microdialysis to determine the absorption characteristics of nitrogen forms in the rhizosphere.

This combination of complementary methods provides a unique ability to measure the nutrients available to roots without destruction and almost in real time [9].

Studies using computed tomography still only inspire hope for conducting quantitative experiments and obtaining stable relationships, in particular, the use of non-destructive methods of x-ray tomography [10-13]. At the moment, there are practically no non-destructive methods for simultaneous research of the formation of root systems, rhizosphere and microbiota composition in the zone of the emerging root system of plants.

The aim of this study: to study the root system of barley seedlings in the early stages of development, while using x-ray computed tomography methods and studying groups of microorganisms in the basal biota.

2. Objects and methods

Soil aggregates 3-5 mm of silty loam arable soil (Albic Glossic Retisols (Lomic, Cutanic), Moscow Region, Eldigino village were used in the experiment. 56°08' 023" North latitude, 37°048'116" East longitude) obtained by dry sifting. Basic properties Albic Glossic Retisols is shown in table 1.

Table 1. Some physical properties of arable horizon Albic Glossic Retisols (Lomic, Cutanic) (Moscow region)

| Depth, cm | Texture of soil, % mass | Field capacity,% | Saturation conductivity, cm/day | C_org |
|-----------|-------------------------|------------------|-------------------------------|-------|
|           | <0.002 | 0.002–0.05 | >0.05 |                               |       |
| 0–10      | 10.50  | 84.78       | 4.72  | 30.1                          | 21.6  | 1.23 |

The model seedbed was created in roentgen-transparent plastic cylinder with a diameter of 1.3 cm, height 4 cm There was a filter paper drain at the bottom. The bottom layer is compacted soil from sifted aggregates up to a density of 1.2 g / cm³. The top layer consisted of separate aggregates of 3-5 mm, the bulk density of this layer is 0.7 g / cm³. Between the layers was placed barley seed (Hordeum vulgare L.). The average values for the studied object for germination energy were 92%, and for germination-61%. The seedlings were grown for 7 days starting from the day of planting, in complete darkness, at a constant air temperature of 26°C.

Watering was performed once at the beginning of the experiment. Water saturated the soil and excess water flowed freely through the drainage. At the beginning of the experiment, the soil moisture content was close to the field capacity (30%), but at the end, on the 7th day, the humidity was near 14%.

The granulometric composition of the studied objects was determined by laser diffraction using a laser particle size analyzer "Analysette 22 comfort" (FRITCH, Germany). Determination of the carbon content in soil samples was carried out by dry combustion in the air stream using the Express analyzer "AN-7529 "(manufacturer "Meter", Gomel) [14]. Soil density, the soil water field capacity, and filtration coefficient were determined by traditional classical methods described in the literature [15].

2.1 Tomographic survey

Tomographic studies were performed using the equipment of the center for collective use of scientific equipment "Functions and properties of soils and soil cover" V. V. Dokuchaev Soil Science Institute. An x-ray microtomograph "Bruker SkyScan 1172G" (Belgium) was used for tomography. Tomographic survey was performed on the 1, 3, 4, 5, 6, 7th days at a resolution of 16.1 µm.
The study parameters, such as sample size and shooting settings, were repeatedly worked out in other studies [16-21]. In this experiment, the priority was the speed of shooting, because due to the rapid growth of roots, there is a risk of getting a distorted image. Tomography of one segment (out of five in the sample) at optimal settings took about 40-60 minutes, but in our case we managed to reduce the time to 10 minutes. As a result of computer tomography data processing, a volume structure with clearly distinguishable x-ray contrast phases (soil, pore space, grain with sprouts and roots) was obtained, but complicated by high image noise. Automatic phase segmentation (Otsu method) [22] is partially functional in such conditions, and it reliably allows segmenting only the mineral phase (soil). In preparation for the analysis, manual threshold and Median filter, Despeckle were used, which made it possible to separate the pore space and the organic matter of the germinating seed with a high degree of confidence.

The calculation of seed and root volumes was performed using Bruker CT analyzer (CTan) software during all study periods. A tool in CTan – Structure separation was used to calculate the pore size. This tool is a method for calculating the local thickness of an object by filling it with virtual spheres from the inside [23].

2.2 Microbiological research.
The composition of the microbial community was reconstructed from microbial markers (fatty acids and their derivatives - fatty _hydroxyl acids and aldehydes), which were determined after acid methanolysis of soil samples by the molecular method of gas chromatography – mass spectrometry (GC-MS). The analysis was performed on the GC-MS system “HP-5973 Agilent Technologies” (USA). The analysis method is described in detail in these sources [24-25]. Samples were prepared for analysis: control-the initial soil in which no barley was grown; soil from samples with sprouting barley seed on the 5th and 7th days. Measurements were performed in three-fold repetition.

3. Results and Discussion
Figure 1 shows tomographic horizontal sections of soil in the box for underground and aboveground layers. It is clearly morphologically seen that subseminal layer had fine pores mainly with a diameter near 0.02 mm, while for above seminal layer the individual units and interaggregate pores size with the diameter up to 0.6-0.8 mm are characteristic, facilitating good aeration above seed layer.

![Figure 1. Tomographic horizontal sections of soil in the subseminal (left) and supraseminal (right) layers of soil (solid phase gray, pores – black). 3 day of the experiment. d=1.3 cm.](image)
Figure 2 shows the tomographic data on pore distribution in layers allowed us to identify the main pore ranges. In the subseminal layer compacted to 1.2 g/cm$^3$, the pore diameter did not exceed 2 mm, while the largest volume of pore space (71%) comes to the pores up to 0.17 mm, which may be due to the destruction of aggregates. In the bulk layer of 3-5 mm aggregates, 52% of the pore space is occupied by pores up to 1 mm, 38% is occupied by pores of 1-2, 2 m in size.

Using tomographic imaging, we observed a decrease in the volume of seed for the period from 3 to 7 days from 50% to 36% of the total volume of the plant located in the survey zone. At the same time, the change in the share of the volume occupied by the root mass was from 22% to 61% over the same period.

Volume calculations were performed based on the volume structure corresponding to the sprouted seed. The calculation tool in CTan – Structure separation is used to calculate the pore size and thickness of seedlings.

The composition of the microbial community reconstructed from the fatty acid analysis of bacterial membrane structures showed the presence of 38 species of microorganisms belonging to 5 phylums, and a species of mycorrhizal fungi Gigaspora was also isolated. The experiment was repeated three times. Table 2 shows the result.

Among the microorganisms, 3 phylum Actinobacteria, Proteobacteria and Firmicutes dominated, the other two – Bacteroidetes and Cyanobacteria were represented in a relatively small number and small variety of species.

Table 2. Total abundance of soil microbial species of the model seedbed at different stages of barley germination, control-without planting

| Composition of soil biota, cells $\times 10^6$/g | Control | 5 day average | 7 day average |
|-----------------------------------------------|---------|---------------|--------------|
| **Actinobacteria**                            |         |               |              |
| Actinobacteria spp.                           | 43.8    | 382.6         | 63.7         |
| Arthrobacter sp.                              | 12.6    | 10.6          | 12.3         |
| Cellulomonas sp.                              | 11.3    | 19.1          | 7.9          |
| Corynebacterium sp.                           | 3.2     | 2.0           | 3.0          |
| Propionibacterium sp.                         | 8.7     | 4.8           | 9.0          |
| Mycobacterium sp.                             | 3.5     | 4.9           | 5.6          |
| Rhodococcus equi                              | 31.3    | 29.6          | 27.3         |
| Rhodococcus terrae                            | 0.6     | 0.9           | 0.0          |
| Pseudonocardia sp.                            | 3.0     | 4.4           | 2.5          |
| Streptomyces-Nocardiosis                      | 14.2    | 9.2           | 13.2         |
| Nocardia carnea                               | 2.8     | 2.7           | 3.5          |
| Actinomadura roseola                          | 0.6     | 0.3           | 1.2          |

Figure 2. Pores for subseminal layer in volumes of pores (mm$^3$-1) and in % of the total porosity (2), for aboveseminal layer (3 and 4, respectively).
| Phylum          | Genus and Species                  | day 1 | day 3 | day 5 |
|-----------------|-----------------------------------|------|------|------|
| **Bacteroidetes** | Sphingobacterium spiritovorum     | 1.7  | 1.5  | 1.8  |
|                 | Bacteroides hypermegas             | 0.2  | 0.1  | 0.2  |
|                 | Bacteroides ruminicola             | 1.3  | 1.1  | 1.5  |
|                 | Cytophaga sp.                      | 2.4  | 2.1  | 2.2  |
|                 | Riemirella sp.                     | 1.5  | 1.3  | 1.2  |
| **Firmicutes**   | Bacillus subtilis                  | 5.1  | 5.8  | 4.6  |
|                 | Bacillus sp.                       | 0.0  | 0.9  | 0.0  |
|                 | C.pasteurianum                     | 19.9 | 49.6 | 11.0 |
|                 | C.perfringens                      | 0.1  | 0.2  | 0.1  |
|                 | Clostridium OPA*                   | 7.3  | 17.7 | 3.9  |
|                 | Ruminococcus sp.***               | 81.9 | 107.2 | 43.5 |
| **Proteobacteria** | Acetobacter sp.                   | 9.0  | 6.8  | 7.4  |
|                  | Agrobacterium radiobacter          | 2.5  | 0.4  | 0.9  |
|                  | Pseudomonas fluorescens           | 3.8  | 4.2  | 7.6  |
|                  | P.putida                          | 0.9  | 0.6  | 2.0  |
|                  | P. vesicularis                    | 1.4  | 1.2  | 1.6  |
|                  | Sphingomonas adgesiva              | 1.0  | 1.5  | 2.0  |
|                  | Sphingomonas capsulata            | 2.0  | 2.1  | 3.0  |
|                  | Xanthomonas sp.                   | 4.7  | 3.6  | 4.9  |
|                  | Enterobacteriaceae                | 5.7  | 2.0  | 13.5 |
|                  | Aeromonas hydrophila              | 7.8  | 9.8  | 6.5  |
|                  | WARB***                           | 3.0  | 2.7  | 2.5  |
|                  | Desulfovibrio sp.                 | 6.1  | 5.9  | 5.3  |
|                  | Nitrobacter sp.                   | 17.4 | 25.5 | 13.3 |
|                  | Caulobacter sp.                   | 5.0  | 4.0  | 6.1  |
| **Cyanobacteria** | Anabaena (cyanobacteria)           | 6.5  | 6.0  | 9.0  |

The phylum Actinobacteria dominated throughout the experiment, the peak number was reached on day 5, and then the number of microorganisms approached the original, but still remained higher. This trend was observed in aerobic species Actinobacteria spp. The peak number on day 5 and its decrease by day 7 was observed in Cellulomonas sp, Pseudonocardia sp., while the number of Rhodococcus equi decreased gradually, which is probably due to the destruction of the seed shell. The decrease in seed volume was observed for the period from 3 to 7 days from 50% to 36% of the total volume of the plant located in the survey area, as can be seen from the results of tomography. These species have an active complex of cellulolytic enzymes that decompose complex organic compounds (the seed coat) under aerobic conditions. Products of cellulose hydrolysis can be carbon dioxide, water. The number of Streptomyces-Nocardiosis decreased by 7 days. This may indicate that only certain types of actinobacteria in the soil near the germinating seed participate in the hydrolysis of the seed coat.

The General trend within the phylum of Proteobacteria is expressed by the peak number on the 5th day, with a gradual decrease in the number, which by the 7th day became lower compared to the control sample. This applies to the anaerobic hydrolytic Ruminococcus sp and the aerobic nitirifier Nitrobacter sp.. The increase in these species indicated the initial destruction of the cellulose shell of the grain and the processes of fixation and conversion of nitrogen in the microbiota of the germinating seed, necessary
for the formation of a certain C/N ratio. Abundance of anaerobic \textit{Ruminococcus} sp. both types of iron reducers can be explained by the presence of Fe(OH)$_3$ in the soil absorbing complex, which is restored by anaerobic iron reducers. As with other anaerobes, their numbers decrease by the 7th day. These species in the process of their anaerobic metabolism form such gaseous components of soil air as hydrogen, carbon dioxide, oxygen.

A gradual increase in numbers by the 7th day was observed in aerobic species such as \textit{Pseudomonas fluorescens}, \textit{Pseudomonas putida}, \textit{Pseudomonas vesicularis}, and \textit{Sphingomonas adgesiva}. This also indirectly indicates a change in the gas composition towards aerobic conditions. As the gas regime changed, the number of aerobic and anaerobic microorganisms changed, respectively.

The total number of phylum \textit{Firmicutes} species decreases by 7 days, passing through the peak number on the 5th day. This is the case with the number of \textit{Clostridium pasteurianum} (which is presumably related to the desiccation of the soil and the change of conditions towards aerobic) and facultative anaerobes \textit{Bacillus subtilis}, which participate in the processes of nitrogen transformation (denitrification and ammonification) and stimulate the activity of rhizospheric nitrogen fixators [26].

Among the fungal microbiota, the mycorrhizal species \textit{Gigaspora} stood out mainly, the content of which in the initial period (during seed planting) reached an average of 5.3 mg per gram of soil.

Then it increased to 8.3 mg/g of soil, and then it decreased to 3.4 mg/g of soil by the 7th day. Apparently, the decrease in the mass of fungi was primarily affected by a decrease in soil moisture, which at the end of the experiment was 14.4% (about 50% of the lowest moisture content). In addition, mycorrhizal fungus to the seventh day passes, apparently, from the rhizosphere state to the rhizoplane of the developing root system of the seed.

In the chosen method, along with the obvious and undeniable advantage of being able to observe changes in the architecture and growth of the root system in the entire volume of the sample without destruction, there are also disadvantages. For example, the complexity and subjectivity of the process of manual segmentation of tomograms, the restriction on the size of the sample. The small volume of the studied model of the seedbed allows us to study the earliest processes of formation of underground organs and better use the advantages of the tomographic method. There is a belief that the morphological features and" behavioral " differences of roots found in seedlings will be inherent in the adult plant [27], while attention is drawn to the fact that it is often the physical, biological and chemical environment of the soil that largely has a dominant influence on the growth and development of young roots [27].

**Conclusion**

Thus, the number and composition of soil biota undergoes dynamic changes at the initial stage of seed germination. These processes are physiologically consistent with the growth of the root system and changes in the seed. So, it is noticeable that the desiccation of the soil leads to an increase in the number of aerobic species by the 7th day and a decrease in anaerobic species. The decrease in the volume of the seed shown on the tomographic survey for the period from 3 to 7 days from 50% to 36% of the total volume of the plant occurs when the biota is constantly dominated by actinobacteria (aerobacteria \textit{Actinobacteria} spp., \textit{Rhodococcus equi}) - "active hydrolytics" that decompose complex organic compounds (the seed shell) in aerobic conditions. At the same time, the total number of bacteria increases by the 5th day. In the next 2 days, it decreases (the number of actinobacteria decreases), which is probably due to the destruction of the seed coat.

The interrelatedness of the methods of visual research results of computed tomography and quantitative research method molecular complex of microorganisms around germinating seed overlap in the visual field determine the dynamics of its hydration and the formation of pores filled with water and air when changing water, air and gas regimes and the accumulation of metabolic products of aerobic and anaerobic species of microorganisms.

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References

[1] Marilley L and Aragno M 1999 Applied soil ecology 13 127–36
[2] Yang C H and Crowley D E 2000 Applied and environmental microbiology 66 345–51
[3] Metzner, R, van Dusschoten D, Bühler J, Schurr U and Jahnke S Front Plant Sci 5 469 https://doi.org/10.3389/fpls.2014.00469
[4] Gregory P 2006 European Journal of Soil Science 57 2–12 https://doi.org/10.1111/j.1365-2389.2005.00778.x
[5] Lafond J, Han L, Dutilleul P 2015 Front Plant Sci 6 1111 doi:10.3389/fpls.2015.01111
[6] Zappala S, Mairhofer S, Tracy S et al 2013 Plant Soil 370 35–45 https://doi.org/10.1007/s11104-013-1596-1
[7] Mooney S, Pridmore T, Helliwell J et al 2012 Plant Soil 352 1–22 https://doi.org/10.1007/s11104-011-1039-9
[8] Rogers E, Monaenkova D, Mijar M, Nori A, Goldman D and Philip N 2016 Benfey Plant Physiol 2028–40 doi: 10.1104/pp.16.00397
[9] Linek J, Han L, Dutilleul P 2015 Front Plant Sci 6 1111 doi:10.3389/fpls.2015.01111
[10] Lafond J, Han L, Dutilleul P 2015 Front Plant Sci 6 1111 doi:10.3389/fpls.2015.01111
[11] Gerke K, Skvortsova E and Korost D 2012 Eurasian Soil Sci 45 700–9 doi:10.1134/S1064229312070034
[12] Ivanov A, Shein E and Skvortsova E Eurasian Soil Sci 2019 52 50–7 doi:10.1134/S106422931901006X
[13] Jiang Z, van Dijke M I J, Geiger S, Ma J, Couples G D and Li X 2017 Advances in Water Resources 107 280–9 doi:10.1016/j.advwatres.2017.06.025
[14] Shein E V, Milanovskii E Ju, Khaydapova D D, Pozdnjakov A I, Tjugai Z N, Pochatkova T N, Demboveckii A V 2017 Workshop on solid phase physics of soils: tutorial (Moscow: Buki-Vedi) p 119
[15] Theories and Methods of Soil Physics. Edited by Shein E V, Karpachevskiy L.O.1 2007 (Moscow: Grif and K) p 616 (in Russian)
[16] Skvortsova E, Shein E, Abrosimov K, Romanenko K, Yudina A, Klyueva V, Khaidapova D and Rogov 2018 Eurasian Soil Science 51 190–9 doi: 10.1134/S1064229318020102
[17] Ivanov A, Shein E and Skvortsova E 2019 Eurasian Soil Science 52 50–7 doi:10.1134/S106422931901006X
[18] Müller K, Kauwalu S, Young I, McLeod M, Moldrup P, de Jonge L W and Clothier B 2018 Geoderma 313 82–9 doi:10.1016/j.geoderma.2017.10.020
[19] Helliwell J R, Sturrock C J, Grayling K M, Tracy S R, Flavel R J, Young I M, Whalley W R and Mooney S J 2013 European Journal of Soil Science 64 279–97 doi:10.1111/ejss.12028
[20] Borges J A, Pires L F, Cássaro F A, Roque W L, Heck R J, Rosa J A and Wolf F 2018 Soil Tillage Research 182 112–2 doi: 10.1016/j.still.2018.05.004
[21] Wildenschild D and Sheppard A P 2013 Advances in Water Resources 51 217–46 doi: 10.1016/j.advwatres.2012.07.018
[22] Otsu N 1979 IEEE Trans Sys. Man. Cyber : journal 9 62—6 doi: 10.1109/TSMC.1979.4310076
[23] Yudina A. Romanenko, K 2019 Mechanistic Understanding of Soil Hierarchical Structure. EGU General Assembly 2019, Conference poster DOI:10.13140/RG.2.2.26167.16809
[24] Verkhovtseva N V and Osipov G A 2002 *Microbiology of composting, Springer-Verlag Berlin Heidelberg* 99–108 doi: 10.1007/978-3-662-08724-4_8 (http://www.scirp.org/journal/abb/)

[25] Shekhovtsova N V, Marakaev O A, Pervushina K A, Osipov G A Advances 2013 *Biosci Biotechnol* 4 35–42

[26] Pishchik V N, Vorobyev N I, Moisseev K G, Sviridova O V and Surin V G 2015 *Eurasian Soil Science* 48 77-84 https://doi.org/10.1134/S1064229315010135

[27] Liao H and Yan XL *Acta Bot Sin* 43 1161–6

[28] Hargreaves C E, Gregory P J and Bengough A G 2009 *Plant Soil* 316 285–297 https://doi.org/10.1007/s11104-008-9780-4