This is the accepted manuscript version of the contribution published as:

Schlüter, S., Sammartino, S., Koestel, J. (2020): Exploring the relationship between soil structure and soil functions via pore-scale imaging *Geoderma* **370**, art. 114370

The publisher's version is available at:

http://dx.doi.org/10.1016/j.geoderma.2020.114370

*Exploring the relationship between soil structure and soil functions via pore-scale imaging*¹

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8 Abstract

9 Biogeochemical and structural heterogeneities at the pore-scale govern processes in soil in many 10 ways. They are therefore of key importance for understanding soil functioning. Prominent examples are 11 the stabilization of soil organic matter due to reduced bioavailability in aggregated soil structure, 12 preferential transport of nutrients and contaminants along macropores, highly localized greenhouse gas 13 emission around a few hotspots of microbial activity like particulate organic matter and the formation of 14 the rhizosphere as a complex system composed of plant roots, soil and associated microorganisms.

15 All of these processes have in common that the underlying relevant mechanisms are fairly well understood in artificial systems with reduced degrees of heterogeneity, like soil suspensions, glass 16 17 beads, micromodels with known structure and so on. However, the far more complex pore architecture of undisturbed soils leads to emergent system behavior which needs to be addressed when studying 18 19 these structure-mediated processes. The opaque nature of soils predestines the use of non-invasive 20 imaging techniques for exploring how biogeochemical and structural heterogeneities are shaping soil 21 functions. Such imaging techniques and subsequent image analyses are now widely used to study soils. 22 While previously many properties were defined only by averaged bulk parameters, pore-scale imaging 23 reveals details at smaller scales and provides spatial information, in two, three or even more dimensions 24 including time or multispectral data.

¹ This editorial contains parts of the habilitation thesis: Steffen Schlüter (2019): Exploring the relationship between soil structure and soil functions via pore-scale imaging", Martin Luther University Halle-Wittenberg, Germany.

This virtual special issue presents fourteen contributions that employ pore scale imaging in order to highlight the role of soil structure on soil functions or reversely the effect of soil processes on soil structure or report methodological advancements in pore scale imaging. In this editorial we briefly outline the different conceptions of soil structure, demonstrate the relevance of soil structure for various soil functions with a specific example and review the different avenues along which recent advances in pore scale imaging of soil have been made, before we briefly categorize and summarize the contributions to this virtual special issue.

32 **1. Introduction**

33 **1.1. Definition of Soil Structure**

34 Soil structure is the three-dimensional arrangement of solid soil constituents and voids across different 35 scales (Rabot et al., 2018), resulting from interactions of biotic and abiotic factors, including climate, 36 mineral composition, organic matter, roots, fungal hyphae, soil fauna, and tillage. The hierarchical 37 organization of soil structure is depicted in Figure 1, starting from soil horizons within a profile, over 38 structural features within a horizon like macropores and soil clods in a plowed horizon down to root 39 channels, cracks and spatial variability of matrix porosity within individual clods. Soil structure 40 constitutes the habitat for soil organisms, provides the paths for matter fluxes and the accessibility to the chemical interfaces for reactions in soil (Figure 1). At the same time, soil structure is shaped by 41 42 biological, chemical and physical processes and the feedbacks between them. It can thus evolve more or 43 less rapidly under these processes that are also influenced by seasonal variations and climatic events 44 such as long droughts and heavy rain. Essential ecosystem functions of soil like water storage and 45 transport, nutrient cycling, carbon storage, plant growth and the maintenance of biodiversity are to a 46 large degree governed by soil structure (Figure 1). In fact, soil structure can be interpreted as an integral 47 indicator of the soil ecological status, i.e., the capacity to host organisms, to sustain the production of 48 biomass, and to recover from external perturbation (Kibblewhite et al., 2008; Rabot et al., 2018; Young 49 and Crawford, 2004).



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52 Figure 1: Schematic of the hierarchical organization of soil structure, the various properties it fulfills and the soil 53 functions that are governed by it.

54 1.2. Two Perspectives on Soil Structure

There is a dichotomy in soil structure assessment through either the aggregate perspective or pore space perspective (Figure 2). A lively debate is currently ongoing in the soil science community especially about the usefulness of the aggregate perspective (Kravchenko et al., 2019a; Rabot et al., 2018; Wang et al., 2019; Yudina and Kuzyakov, 2019).

59 The traditional approach has always been to characterize soil structure through the size, shape, 60 grade and stability of soil aggregates (Ad-hoc-AG Boden, 2005; Jahn et al., 2006). This is standard procedure in soil surveys worldwide and common practice for farmers to evaluate the soil ecological 61 62 status from quick field observations. The fastest and cheapest soil structure assessment is achieved with drop-shatter tests, for which a spade-full of topsoil is dropped from a certain height, e.g 1 m, and a score 63 64 is derived from the size distribution, and shape of aggregates as well as earthworm and root abundance 65 (Ball et al., 2007; McKenzie, 2001; Shepherd et al., 2008). The scoring results have been shown to 66 correlate with soil compaction and the associated decrease in gas exchange, infiltration and agricultural 67 production (Guimarães et al., 2013; Mueller et al., 2009; Pulido Moncada et al., 2014; Shepherd, 2003). 68 However, scoring methods are rarely used in basic research because the assessment is somewhat subjective and the results depend on texture as well as time-variant soil moisture and biological activity 69 70 (Guimarães et al., 2011; Mueller et al., 2009; Newell-Price et al., 2013).

71 Soil structure can be measured more objectively with laboratory methods. The most common 72 method is to measure bulk density and derive porosity from the dry weight of undisturbed soil cores 73 with a given volume. The soil core extraction in the field, might not capture the field variability 74 representatively, is known to induce some disturbance along the wall and can become impossible for 75 high rock content and in the presence of woody roots (Page-Dumroese et al., 1999; Schlüter et al., 2011; 76 Vincent and Chadwick, 1994). Another common approach to characterize soil structure through 77 aggregate size distribution and stability according to various protocols grouped into wet-sieving and dry-78 sieving (Díaz-Zorita et al., 2002). The results are highly sensitive to specific details of each protocol 79 (energy, duration, repetition) and antecedent soil moisture (Almajmaie et al., 2017; Beare and Bruce, 80 1993). Despite these drawbacks aggregative sieving and stability tests remain being used as they can 81 inform about the susceptibility to slaking (wet-sieving) and resistance to mechanical disturbance (drysieving). They are also relatively easy to perform. Moreover the fragmentation of soil into different 82 83 aggregate size classes supports the concept of an aggregate hierarchy which assumes that 84 macroaggregates (>250 μm) form around particulate organic matter and microaggregates (<250 μm) are 85 released upon breakdown of macroaggregates (Angers et al., 1997; Tisdall and Oades, 1982). The 86 macroaggregates are supposed to be relatively short-lived as their binding agents are less persistent 87 than those within microaggregates.

88 This aggregate perspective on soil structure has frequently been criticized as the associated 89 methods rather aim at measuring the stability of soil structure than soil structure itself and the outcome 90 of these measurements highly depend on the applied energy (Baveye, 2006; Letey, 1991; Pagliai and 91 Vignozzi, 2002; Young et al., 2001). Moreover, it is the pore space and not the solid space that constitute 92 the spatial domain for water flow, matter fluxes and gas exchange, the habitat for soil biota and the 93 reactor of a multitude of reactions. Therefore, the characterization of pore space attributes in 94 undisturbed soil seems more promising to relate it to soil functions (Rabot et al., 2018). Methods for 95 pore space characterization can be roughly grouped into indirect methods and direct methods. Indirect 96 methods (e.g. mercury porosimetry, pressure chamber) derive pore attributes like the pore size 97 distribution from functional behavior like water retention curves. Direct observations of pore structure 98 are based on imaging (e.g. thin section microscopy, X-ray tomography). They allow for a qualitative 99 assessment of pore structure according to its formation (packing voids, microcracks, root channels, 100 earthworm burrows) as shown in Figure 2 and for a quantitative assessment through image analysis 101 resulting in properties like pore size distribution, pore connectivity or pore distances. Both direct and 102 indirect methods for pore structure assessment tend to be more time-consuming and labor-sensitive

103 than aggregate structure assessment. Imaging methods, in particular, suffer from limited access to the 104 required hardware and from some degree of subjectivity in the image processing protocols (Baveye et 105 al., 2010).

106 In summary, both approaches have their strengths and weaknesses and, more importantly, fulfill 107 different purposes and provide complementary information. Therefore, there is a clear merit in 108 combining both approaches for a more comprehensive picture on the links between soil structure and 109 soil functions.



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111 Figure 2: Summary of two competing views: the aggregate perspective and the pore space perspective. (a) Kühnfeld, 112 Halle, Germany (continuous maize, conventional tillage, 63% sand, 25% silt, 12% clay), (b) Hadera, Israel (orchard, 65% sand, 113 16% silt, 19% clay), (c) Bad Lauchstädt, Germany (grassland, 12% sand, 68% silt, 20% clay), (d) Garzweiler, Germany (crop 114

rotation, below plow layer, 5% sand, 81% silt, 14% clay); modified from (Rabot et al., 2018)

116 **1.3.** Relevance of soil structure for soil functioning

117 A comprehensive review of soil structure effects on these soil functions is beyond the scope of this 118 introduction, but can be found elsewhere (Kravchenko and Guber, 2017; Rabot et al., 2018). Instead we 119 use an illustrative example to demonstrate the role of soil structure for a few fundamental soil 120 processes and their implications for some of the abovementioned soil functions.



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Figure 3(a) shows the pore architecture of a 1 cm large, silt loam aggregate scanned with X-ray CT at a voxel resolution of 8 μm. The pore size distribution is obtained with the maximum inscribed sphere method and depicted from small (green) to large (red) diameters. This pore size information can be used to model water retention and the distribution of water and air at a certain matric potential by employing Young-Laplace law and the capillary rise equation that is derived from it:

$$h = \frac{2 \gamma \cos(\alpha)}{\varrho_w g r} \quad (1)$$

132 where h is the rise above a free water table in a cylindrical capillary with radius r, γ is interfacial 133 tension between water and air, α contact angle, ρ_w is the density of water and g is gravitational 134 acceleration. In hydraulic equilibrium this height above the free water table can be directly interpreted 135 as pressure head h_m (or capillary pressure P_c or matric potential ψ_m) in that soil depth. Assuming a 136 capillary bundle model, Eq. (1) can be recast to directly infer whether a pore with a certain radius will be 137 water or air-filled at a certain matric potential. This pore morphology based simulation of water 138 retention is done in Figure 3(b) for a matric potential of -30 hPa assuming pure water and perfect 139 wettability. Note that the capillary bundle assumption is a severe oversimplification, because in order to 140 drain a pore it is not only important whether its radius is large enough, but also whether there exists a 141 continuous path towards the atmosphere through which air can invade (Hazlett, 1995; Hilpert and 142 Miller, 2001). However, for the following example this difference is not important. Repeating this 143 analysis for decreasing matric potentials resembles a drainage process (Figure 2c-e). The soil moisture 144 characteristic (or water retention curve or moisture release curve or pF curve) could be directly 145 estimated from the water content at each drainage step. Larger packing pores and root channels are 146 drained first, whereas air invades microcracks and smaller intra-aggregate pores at a more negative 147 matric potential. At a matric potential of -300 cm \approx pF 2.5 all macropores (>50 μ m) and narrow 148 macropores (>10 µm) are drained and the soil has reached field capacity (Figure 2e). The unresolved 149 mesopores (>0.2 μ m) act as a reservoir for root water uptake as they hold the water against gravity by 150 capillary forces. The visible macropores, in turn, are essential for soil aeration at field capacity or for 151 preferential flow and solute transport when the soil is fully saturated (Rabot et al., 2018). Note that a 152 large part of the unresolved mesopores are textural pores between primary particles, whereas all visible 153 pores are structural pores.

154 In summary, water retention and soil aeration patterns are a direct imprint of the underlying pore 155 architecture. But this also has important ramifications into matter cycles as they govern diffusion 156 pathways, microhabitats and reaction patterns. This is demonstrated with contact distances to the 157 closest air-filled pore at different matric potentials (Figure 2f-h). At full saturation, air is only present 158 outside the aggregate so that on average dissolved oxygen has to diffuse 620 µm from the aggregate 159 boundary to reach any location within the soil matrix. For sake of simplicity this contact distance is 160 estimated by direct Euclidean distances, whereas pores are tortuos and real diffusion trajectories along 161 a concentration gradient are not straight but chaotic due to Brownian motion. When the aggregate is 162 drained this average air distance decreases substantially. At field capacity it already decreased by one 163 order of magnitude (70µm), which entails a much better supply with dissolved oxygen in the water-filled

164 soil matrix. This dissolved oxygen will be consumed through aerobic respiration in the soil matrix. If this 165 oxygen consumption exceeds the oxygen supply through diffusion along the oxygen gradient, then 166 anoxic zones may form in the aggregate center (Figure 2f-g). Their extent depends on local respiration 167 rates and contact distances. Hence, different microhabitats within short distances that allow for a high 168 microbial biodiversity and diverse functional behavior in soil are not just an imprint of the soil structure 169 per se, but evolve with the saturation-dependent distribution of air and water. Finally, plant roots and 170 soil macrofauna like earthworms are able to "engineer" their own pore space and thus modify aeration 171 and infiltration patterns in soil (Angers and Caron, 1998; Blouin et al., 2013; Bottinelli et al., 2015). They 172 are known to also be affected by soil structure and water distribution through the restrictions imposed 173 e.g. by mechanical resistivity and redox conditions (Bengough et al., 2011; Capowiez et al., 2009; Hamza 174 and Anderson, 2005; Whalley et al., 1995).

175 The implications of these micro-environmental conditions for carbon and nitrogen turnover are 176 manifold. Carbon mineralization rates through anaerobic respiration are about one order of magnitude 177 smaller than through aerobic respiration (Keiluweit et al., 2017). This leads to the well-known saturation 178 dependence of bulk soil respiration with an optimal respiration rate at intermediate soil moisture and a 179 decline towards full saturation and complete dryness (Moyano et al., 2013; Skopp et al., 1990). Under 180 very dry conditions a substrate diffusion limit arises, because the continuity in the water phase is lost 181 and microbes become separated from resources and may fall into metabolic arrest (Manzoni and Katul, 182 2014; Tecon and Or, 2017). Under very wet conditions an oxygen diffusion limit may arise, when oxygen 183 consumption exceeds the diffusive flux towards the location of oxygen consumption, so that anaerobic 184 respiration with alternative electron acceptors sets in (Linn and Doran, 1984). The susceptibility of 185 organic matter to mineralization therefore does not only depend on the chemical structure of the 186 organic compounds, which defines the electron donator-dependent energy gain, but also on the 187 moisture regime and resulting redox conditions which controls the electron acceptor-dependent energy 188 gain of the reaction (Keiluweit et al., 2016).

Soil structure does not only have an indirect effect carbon turnover through the regulation of water retention and soil aeration, but also exerts a direct control on carbon stabilization, when organic compounds are located in pores that are not accessible to microorganisms. In fact, this physical protection seems to be the main mechanism for long-term carbon storage next to protection in organomineral associations, whereas the importance of chemical recalcitrance might have been overstated in the past (Lehmann and Kleber, 2015; Schmidt et al., 2011). This physical protection might occur in pores that are smaller than a microorganism itself, or when they are separated by discontinuous water films, though this limitation is more relevant for bacteria than for fungi as they can overcome these barriers via hyphae (Ritz and Young, 2004). There are indications that this physical carbon protection is most relevant in fine-textured, structured soils, but less relevant in sandy soils (Christensen, 2001). There is an important feedback loop because soil structure does not only affect carbon turnover. Organic carbon is also a key driver in the formation of soil structure by acting directly as binding agent for mineral particles and indirectly by stimulating soil biota that modify soil structure (Rillig and Mummey, 2006; Six et al., 2004; Tisdall and Oades, 1982).

203 Soil structure is not only important for carbon turnover, but also for nutrient cycling, in particular 204 nitrogen. Reactive nitrogen exists in many soluble and gaseous forms in soil. Transformations between 205 them are regulated by environmental conditions (pH, temperature, moisture) and different 206 transformations may occur simultaneously in different niches and microsites in structured soil. This is 207 again demonstrated with the aggregate example in Figure 2. Denitrification, i.e. the reduction of nitrate 208 through anaerobic respiration, will occur in anoxic aggregate centers. Nitrification, i.e. the biological 209 oxidation of ammonium to nitrite and nitrate, in turn, is an aerobic process as it requires oxygen and will 210 therefore occur in direct vicinity to air-filled pores. The relative importance of both processes depends 211 on soil moisture. Nitrification rates increase with soil moisture in line with overall microbial activity until 212 it drops when aeration becomes a limiting factor. Denitrification emerges at saturations around 60-70% 213 and peaks around 90% (Linn and Doran, 1984). It does not only depend on the moisture-dependent 214 diffusion distances in soil, but also on the distribution of organic carbon in soil. In a seminal study Parkin 215 (1987) demonstrated that 25-85% of denitrification activity was associated with particulate organic 216 matter that comprised less than 1% of the soil volume. The concentration of microbial activity in 217 microbial hotspots is therefore not only immanent to carbon cycling but also to nitrogen cycling (Kuzyakov and Blagodatskaya, 2015) and their distribution in space is controlled by soil structure. 218

219 **1.4.** Recent Advances in Pore-Scale Imaging of Soil Systems

Pore-scale imaging studies like the example above have developed into a powerful tool to gain more insights into the complex interactions between soil structure and soil functions in intact soils. Recent advances in pore scale imaging of soils have been achieved along four major avenues that we briefly outline here without any claim to cover the recent literature completely.

1.4.1. Development of new imaging protocols

In addition to continuous progresses in imaging hardware, new image processing protocols are also 225 226 being developed constantly to improve the robustness, objectivity or accuracy in microstructure 227 characterization. The best combination of methods for image enhancement, image segmentation and 228 image analysis needs to be found again and again for each study in order to best extract the relevant 229 structural information from the raw data. It is still common practice to segment a raw (or filtered) gray 230 scale image into foreground and background with histogram-based thresholding methods even though 231 it has been shown frequently that locally-adaptive methods that take some neighborhood information 232 into account are often superior (lassonov et al., 2009; Schlüter et al., 2014). For some imaging tasks like 233 root detection it is helpful to extract features based on their shape in addition to gray values (Gao et al., 234 2019; Schulz et al., 2013), as the cylindrical shape of roots is unique among all materials in soil, whereas 235 the gray value is not. Other useful properties for feature extraction may include texture and local 236 heterogeneity of materials (Andrew, 2018; Schweizer et al., 2018). With density calibrated images, a 237 mixing law has been used to determine the thresholds for void segmentation (Sammartino et al., 2012)

The next revolution in image segmentation and feature extraction is expected to be brought about by machine learning and deep learning techniques. This class of supervised techniques require some level of user input in terms of training data but may lead to superior results by finding for every targeted material some characteristic patterns in a higher-dimensional feature space constituted by the gray value (and other properties) at the original resolution (and other hierarchy levels). Some current examples include root segmentation (Koebernick et al., 2017; Soltaninejad et al., 2019) and pore segmentation in natural porous media (Chauhan et al., 2016; Wang et al., 2020).

245 Some progress in studying dynamic processes has been achieved by repetitive imaging of the same 246 sample at different temporal scales e.g. to study the evolution of an infiltration front or solute plume 247 within hours (Haber-Pohlmeier et al., 2010; Koestel and Larsbo, 2014; Luo et al., 2008; Sammartino et 248 al., 2015; Tötzke et al., 2017) or the soil structure dynamics under natural conditions over years 249 (Garbout et al., 2013; Koestel and Schlüter, 2019). Depending on the research question, image 250 registration of subsequent scans might be required for a perfect spatial alignment of consecutive 251 images, as the sample is typically moved in between image acquisitions. This is the case when soil 252 deformation due to compaction (Peth et al., 2010; Schlüter et al., 2016; Watanabe et al., 2012) or due to 253 bioturbation (Koestel and Schlüter, 2019) is of interest.

The appeal of a method does not only depend on its performance but also on its availability. Therefore, the merit of developing and maintaining publicly available plugins and packages (Koestel, 2018; Legland et al., 2016; van der Walt et al., 2014) for popular imaging software like ImageJ or scikitimage cannot be appreciated enough.

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1.4.2. Linking different techniques with correlative imaging

259 Any imaging technique is selective in the local property that is mapped and thus the kind of 260 information which can be retrieved. For a more comprehensive view on microenvironments in soil it is 261 appealing to combine various imaging methods through a correlative imaging approach. For instance, 262 plant-soil interactions can be understood better with a combination of X-ray tomography to map the 263 spatial distribution of solids and pores, neutron tomography to map water content and water uptake 264 and magnetic resonance imaging to detect the release of gel-like root exudates in the rhizosphere 265 (Haber-Pohlmeier et al., 2019). The method by which all images are spatially aligned with each other is 266 called image registration or co-registration. The combination of two-dimensional microscopy data 267 through image registration, also denoted as correlative microscopy, facilitates the combination of 268 biological and chemical information from a whole suite of microscopic and micro-spectroscopic 269 techniques (Baveye et al., 2018). The next frontier is to combine this biochemical information retrieved 270 from exposed surfaces with three-dimensional, structural information of the intact sample though 2D-271 3D image registration (Juyal et al., 2019; Kravchenko et al., 2019b; Schlüter et al., 2019b). The required 272 image registration is challenging due to different dimensionality and scale but also very different image 273 content. Nevertheless, this methodological approach will progress quickly as it enables a more holistic 274 view on microenvironments in soil that combines biotic with abiotic information.

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1.4.3. Relating microstructure to soil functioning

276 Pore scale imaging and image analysis are now being routinely used to study a wide range of soil 277 properties for a better understanding of soil functions summarized in Figure 1. To this category we 278 would also add studies that investigate reverse effects, i.e. changes in soil structure due to a specific 279 process, be it natural or management induced. Meanwhile, there is a large number of respective studies 280 published in peer-reviewed, scientific literature. These pore-scale imaging studies are mostly carried out 281 on targeted laboratory experiments or field sampling campaigns from selected sites in order to address 282 a specific research question with a limited set of samples. This is a valid approach for gaining basic 283 process understanding, but generalizations beyond the specific experimental setup are often not 284 warranted, i.e. different behavior for different soil type, land use or environmental conditions.

285 In general, there is huge potential in collecting data from many studies with similar objectives in 286 large databases to reveal general trends, dependencies or tipping points (Jorda et al., 2015; Rillig et al., 287 2019). Based on regression analyses with a multitude of samples so-called pedo-transfer functions can 288 be developed to estimate some emergent soil behavior such as hydraulic conductivity (Van Looy et al., 289 2017) or soil respiration (Moyano et al., 2012) from a set of readily available parameters (e.g. bulk 290 density, soil organic matter content, clay content, etc). Image-derived pore space properties have so far 291 not been incorporated systematically for this kind of data mining due to the limited number of studies in 292 which the targeted emergent behavior and pore space properties are measured jointly. The need to 293 establish such databases which include microstructural properties has recently been stressed (Fatichi et 294 al., 2020; Rabot et al., 2018; Zhang and Schaap, 2019) and the studies that investigate e.g. the impact of 295 macropore structure on soil hydraulic conductivity are converging towards a small set of promising 296 image-derived pore metrics that best predict flow (Koestel et al., 2018; Müller et al., 2018; Paradelo et 297 al., 2016; Schlüter et al., 2019a; Zhang et al., 2019).

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1.4.4. Combine pore scale imaging with modelling

There are two major avenues along which the combination of pore scale imaging with modelling can be beneficial.

301 First, a pore scale image can directly serve as a model domain for spatially resolved models. The 302 modeling result can then be compared directly to measurements, when carried out on the same 303 domain, e.g. saturated and near-saturated flow (Schlüter et al., 2019a), to assess measurement artifacts 304 or the validity of assumption made during modelling. More often, direct measurements are impossible 305 to obtain due to the small size of investigated volumes or a general lack of suitable techniques to probe 306 a given property. In that case, image-based models are still extremely useful for explorative modelling. 307 With this type of "what if" modelling, different scenarios of a property that cannot be readily measured 308 are constructed and the effect on emergent system behavior is explored. Some recent examples for this 309 hypothesis testing comprise the effect of substrate distribution within image-derived pore space 310 geometries on biodegradation and biodiversity (Portell et al., 2018) or the effect of root hairs and local 311 water saturation in the rhizosphere on root water uptake (Daly et al., 2016).

Second, image-derived parameters can help to constrain parameters of continuum-scale models operating on larger scales. The success of this approach depends on whether the often empirical parameters actually depend on these independently measured, microstructural properties. Sammartino 315 et al. (2015) derived the active macropore network of a macroporous soil using analysis of CT-images 316 and a validation by brilliant blue staining of the preferential flow pathways. They demonstrated the 317 strong link between the active macropore network and the percolating backbone. In a similar approach Gerke (2012) estimated the interface between macropores and the soil matrix using X-ray CT-images. 318 319 Indeed, this inter-domain area is a very important parameter as it controls most of the mass exchange 320 processes during infiltration. With some exceptions also in this virtual special issue (Haas et al., 2020; Soto-Gómez et al., 2020), analyses of CT images towards geometric parameters associated with the 321 322 active macropore network has not been adopted much, but are considered very promising to make 323 models less empirical and more physically-based.

324 2. Contents of the Virtual Special Issue

In the introduction we have outlined how soil structure governs soil functions and the potential of pore scale imaging to explore this relationship. This virtual special issue entitled "Recent advances in pore scale imaging of soil systems" presents fourteen contributions that applied pore scale imaging for a whole range of objectives. They can be structured into three thematic blocks.

329 **2.1.** Method development

One block of contributions comprises studies on methodological aspects of pore scale imaging. Torre et al. (2020) generated more realistic, synthetic test images with the truncated multi-fractal method that share common properties with X-ray CT images of real soil. Therefore, these test images do not only possess ground truth information required to validate the performance of segmentation methods per se, they are also more likely to indicate the failure of a segmentation method when X-ray CT scans of real soil are segmented. This is demonstrated by superior segmentation results of a locallyadaptive method over a global thresholding method.

Pot et al. (2020) explored the impact of choices made during different image processing steps with respect to modeled saturated hydraulic conductivity (K_{sat}) obtained with image-based Lattice-Boltzmann simulations. They showed that K_{sat} can change by two orders of magnitude, which is mainly dependent on how pore necks are segmented. Moreover, the Kozeny Carman equation based on image-derived properties was able to explain the variation in K_{sat}. Haas et al. (2020) developed a method that is potentially able to derive the mass exchange parameter in two-domain solute transport models from independent, image-derived metrics based on the small-scale distribution of a fluorescent dye mapped on a 2-D cross-section of a soil aggregates, using digital photography in the visible light range.. The dye redistribution was traced with time-lapse imaging which serves as a proxy for the movement of adsorbing chemicals.

347 **2.2.** Soil structure dynamics

348 The second block of papers looks into soil structure dynamics in the laboratory or under natural 349 conditions induced by various structure-forming processes.

Diel et al. (2019) investigated pore structure changes through crack formation and closure during wetting/drying cycles for repacked soils with different texture, organic matter content and clay mineralogy. A higher proportion of clay minerals with swell/shrinking capacity induced stronger crack formation, whereas a higher organic matter content lead to a higher density of cracks with smaller aperture. By means of a structure labeling approach with small garnet particles they showed that thorough soil structure dynamics cannot be induced by wetting/drying cycles as the cracks that are formed in the first cycles are reused in subsequent cycles.

Pires et al. (2020) also looked into soil structure changes induced by wetting/drying cycles and their effect on water retention curves measured with suction tables; an often overlooked effect that may have a considerable impact on the actual measurement. Measured water retention curves were affected by a series of up to twelve wetting/drying cycles which was explained by internal pore structure changes in terms of pore tortuosity and pore connectivity especially in the hydraulic contact region between the samples and the suction table.

Koestel and Schlüter (2019) investigated soil structure dynamics under natural conditions by time lapse imaging of an in-growth core that was repeatedly reburied in a garden soil. With this proof-ofconcept study they demonstrated the temporal variability of pore space metrics induced by different biotic and abiotic process like soil settling, root growth or faunal activity. Through deformation analysis they showed the much larger extend of lateral compaction by taproot growth as compared to macrofaunal burrowing. Lucas et al. (2019) examined soil structure formation in a 24 year "space-for-time" agricultural chronosequence established for reclamation of a lignite mining area. With a new biopore segmentation method they were able to distinguish root channels from other structural pores. Biopore density had reached equilibrium after twelve years underneath the plow layer, but only 10% of these channels are actually filled with roots each year. This study shows how fast plant roots create a stable and connected biopore system and how this is disrupted by soil tillage, which produces completely contrasting pore characteristics.

Ferreira et al. (2019) scrutinized the effect of liming on soil aggregation and pore structure in an acidic silty-clay, no-till topsoil 30 months after lime application. Only in those layers where liming affected chemical soil properties (pH, base saturation) could an increase in macroporosity with more elongated and better connected pores be observed. Increased biopore formation was observed as a secondary effect of liming.

381 **2.3.** Relationship between soil structure and soil functions

382 Schlüter et al. (2020) compared topsoil samples under conventional tillage and no-till with respect to 383 saturated and near-saturated hydraulic conductivity measured with hood infiltrometers in the field, 384 tension disk infiltrometers on intact soil cores and simulated with 3D image-based flow simulations. No-385 till soils exhibit higher, lower or indifferent hydraulic conductivities depending on the tension and 386 technique. Moreover, the pore metrics that best predicted conductivity also changed with tension and 387 technique. The critical pore diameter had very high predictive power on saturated hydraulic conductivity 388 when simulated on no-till soils with large earthworm burrows in an otherwise compact soil matrix. In 389 plowed soils macroporosity and pore connectivity best describe flow through the loosened soil matrix.

390 Piccoli et al. (2019) explored the effect of soil structure on gas transport properties with a special 391 focus on scale effects. They showed that the bigger the volume of intact samples, the more dominant 392 are large, continuous biopores that evoke more efficient gas transport. The smallest considered sample 393 size (4.7 cm³) the pore network was dominated by small isolated air-filled pores that obstruct gas 394 diffusion at field capacity (-100 hPa). While some microstructural properties showed indications for scale 395 invariance in the investigated range of sample sizes and image resolutions, emergent system behavior in 396 terms of gas transport is clearly not. Thus, sample size needs to be accounted for when comparing gas 397 transport properties between studies.

398 Koestel et al. (2020) evaluated representative elementary volumes (REVs) for the X-ray-derived 399 porosity and pore-connectivity in undisturbed soil columns (67 mm diameter, 60 mm height). These 400 pore-space features are intrinsically linked to water retention and soil hydraulic properties. The 401 existence of such REVs would support the adequacy of respective continuum-scale models. Koestel et al. 402 (2020) detected a range of observation scales, between 15 and 65 mm, for which the mean porosity was 403 scale-independent. Due to a lack in statistical homogeneity, it remained however unclear whether 404 porosity REVs existed. The authors found no potential REVs for the pore-network connectivity. They 405 recommend future studies on larger soil samples and a re-evaluation of the required of REV criteria in a 406 continuum modelling context.

Soto-Gómez et al. (2020) investigated solute transport and colloid transport in intact soil cores and were able to show correlations between transport properties and some metrics of the percolating pore network. Moreover, the area of pore walls stained by fluorescent colloids correlated with the imagederived metrics of the percolating pore space. The morphology of the percolating backbone showed huge differences between no-till, shallow till and organic plots with high and low earthworm activity on a sandy loam.

Mawodza et al. (2020) applied neutron tomography to visualize wheat root growth and soil moisture distribution in aggregated soil. They demonstrated that in heterogeneous soil the emergence and growth of lateral roots depends on local soil structures. Moreover, local water contents were higher in larger soil aggregates suggesting that these may be able to provide the plants with water during periodic dry spells.

Menon et al. (2020) explored links between the pore space properties and the stability of soil aggregates. They could show that land use has a significant influence on both the pore size distribution and the fraction of water-stable aggregates. The pore system of stable aggregates did not undergo significant changes upon continued submergence in water, indicating that a stable pore system is crucial for aggregate stability. Supposedly, a stable pore system facilitates the transmission of fluids without trapping the air and thereby suppressing the build-up of air pressure inside an aggregate preventing it from slaking.

426 Acknowledgements

427 The guest editors like to thank Geoderma, especially Yvan Capowiez and Jan Willem van Groenigen,

428 for the invitation to manage this virtual special issue and express their deep gratitude to all contributing

429 authors and anonymous reviewers for their share in producing a very interesting selection of papers.

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