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Quantitative characterization of soil micro-aggregates: New opportunities from sub-micron resolution synchrotron X-ray microtomography

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Abstract

Soil microaggregates are the fundamental building block, at the micron scale, of the highly hierarchical structure of soils, and can exert a significant control on the local biological metabolism and microbial community partitioning. In this study we propose an analysis protocol for the morphometric characterization of complete soil microaggregates based on sub-micron resolution synchrotron X-ray microtomography. A comprehensive characterization of the aggregate morphology is the first step towards a complete characterization of the soil microaggregates, when trying to correlate morphometric parameters with physical and/or biological properties, or when building models (e.g., effective diffusivity, microbial distribution, etc.). We demonstrate our characterization approach on two single microaggregate samples from dramatically different soil environments: one from Kansas, primarily composed by inorganic particles, and one from Barrow (Alaska) dominated by plant fragments. A series of state-of-the-art morphometric analysis techniques have been employed providing quantitative results highlighting specific differences of the two samples. The role of the microstructure in a scenario microbial population development has been discussed and it has been found that the Barrow microaggregate seems to be more favorable, from a purely geometrical point of view, as also confirmed by a simple model presented in this work. The potential of this approach, when coupled with chemical and biological analysis for a fully comprehensive characterization of soil aggregates in the larger picture of enhanced biological activity, is evident.

Keywords	Synchrotron X-Ray microCT; Soil microaggregate; Quantitative image analysis; Modeling.
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Suggested reviewers	Marco Keiluweit, Francesco De Carlo, John Crawford, Mark Rivers, Hu Zhou

Submission Files Included in this PDF

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Dear Editor,

please find the manuscript submitted for publication in "Geoderma" with the title:

Quantitative characterization of soil micro-aggregates: new opportunities from sub-micron resolution synchrotron X-ray microtomography

by

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The manuscript submitted illustrates the analysis of two different (in origin, composition, and morphology) soil microaggregates via synchrotron X-ray microtomography. While this technique has been already employed in the past for similar purposes, there are many significant novel aspects in the present work:

- 1) Data quality: with the 325 nm per px of theoretical resolution of the reconstructed datasets, coupled with novel reconstruction techniques such as phase retrieval algorithms, the quality of the data is unprecedentedly high. This allows more precise analysis compared to most of the data present in literature.
- 2) Quantitative morphometric analysis completeness: no other soil dataset present in literature has been characterized in a quantitative fashion to this extent. This completeness helps with a better characterization, but also in finding the key morphological parameters and their relation with the sample properties.
- 3) Having measured (and analyzed) the *whole* soil microaggregate, and not a cropped volume as typically done, opened opportunities for novel analysis strategies, e.g. the calculation of the sample outer surface and the measurement of the openings of the microaggregate. A characterization of the interface with the outer world is of paramount importance to better understand the dynamic equilibrium of the aggregate with the surrounding environment, and this characterization is not present in literature.
- 4) The points above can lead to a model aimed at calculating the pore space accessible to different classes of microorganisms (from a purely geometrical point of view). While simplified, such a model can potentially highlight (coupled with the analysis in #2) different physical properties (diffusion, microbial populations, etc.) in aggregates with different morphological characteristics.

Even if this work is mostly on the technical side, we find that the results can be of interest to the community of the soil scientists. The improved data collection/reconstruction procedure, coupled with the most advanced morphometric characterization, can provide the soil scientists novel analysis tools and procedures to better understand the complexity of soils; or to be coupled with local biological information; or to be used as a realistic

starting point for modeling. Also for this reason we plan to make the datasets (including surfaces, skeletons, etc.) available to the public on an online database, after publication, given its broad interest, difficulty of execution (a correct skeleton, a proper outer surface, etc. are not just a click on a software GUI, unfortunately), and general interest.

We hope you find the present work interesting and worth of publication in Geoderma, we have checked publications on this journal concerning X-ray microCT and we find this work could be a proper addition in that area, and could be of general interest to the scientist interested in better understanding the microstructure of soils at the nano- and micro- scale.

If suggestions for possible referees were welcome, I'd consider:

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Sincerely

Marco Voltolini

Dear Editor,

Please find below the answers to the comments from the referee point by point (our answers in blue)

The authors present a method for the high-resolution scanning of microaggregates (<250 micrometers) using SXR-CT and associated calculation and display of summary metrics and networks derived from this scanning. The method is certainly of interest to the soil science community and the authors do a nice job of justifying their work. The manuscript is generally well written and was a pleasure to read. There are several points of clarification and organization, however, that I urge the authors to address as well as grammatical and cosmetic changes that should improve the flow and clarification of the manuscript.

I recommend moving the discussion of the sphere-normalized surface-to-area volume ratio in L649-658, the Minkowski functionals in L664-684, and the fractal dimension in L695-701 to the methods section as these describe the metrics used in the manuscript.

We understand the suggestion of moving the introduction of those analysis tools in a separate section, but we think that those paragraphs are belonging to the dedicated morphometric analysis section, where each technique is introduced and then the specific results shown. It keeps the flow of concepts more uniform and tidy, without the need of going back-and-forth mentally.

Sample choice -> Measurement -> Data optimization and cleaning -> Measurements on the data divided by type and their results -> Final Discussion.

The biggest limitation to the proposed method is a lack of discussion of error or uncertainty with the resulting metrics. For example, the statement on L1280-1282 that “it is clear that the microaggregate richer in organic matter (Barrow) is about twice as porous as the predominantly inorganic one (Kansas)” assumes that the two aggregates chosen represent the two environments from which the sample was taken. Can any conclusions really be made between two aggregates less than 250 micrometers in diameter separated by 3000 mi? What is the variability of the various resulting metrics determined from the scanning for each site?

Although the authors insert a statement in L1294 that “...a statistically valid generalization from one small sample set is not possible...” they do not attempt to deal with this very real problem. Can the authors shed light on how many samples, for example, need to be taken to appropriately characterize the variability of the results? It seems to me that the smaller the unit of examination (which the SXR-CT enables) the more important the need for replication and an understanding of variability.

This is indeed a very important issue, but a comprehensive discussion about representativeness would require an effort beyond the scope of this work. As we claim, this is not about comparing two localities, but two microaggregates. “Kansas” and “Barrow” are intended as labels for the microaggregates, not for the localities.

Ideally, the only way to answer would be to measure a large amount of single microaggregates and calculate the heterogeneity among aggregates, for each morphometric analysis procedure. If we consider the single microaggregate as an object of the “microaggregate” class, a typical number of measurements would typically vary from hundreds to thousands objects (these are typical numbers for shape preferred orientation analysis, as in Voltolini et al., 2011 cited in the text). But the number of microaggregates needed for reaching statistical meaningfulness could also depend on the single parameters considered (e.g., the distribution of the porosity could be much sharper than the aspect-ratio). This is beyond the scope of this work, which is focused on finding the parameters that can better characterize differences in single microaggregates, not a comparison between “all” Kansas and Barrow microaggregates. We only wanted two markedly different (in texture/composition) aggregates to check which are the parameters that can better describe their difference (and their impact on some of the microaggregate physical properties). Actually, if the focus would be the comparisons of two locales, the best approach would likely be a multi-resolution one, where info at different scales and resolutions are combined, since a large number of measurements (and analyses!) on single microaggregates would require a huge and at present unrealistic amount of time and effort, which a multi-scale approach (where in low-resolution scans different classes of microaggregates *could* be identified and labeled) could -in theory- reduce.

We have added in the text some comments in the discussion section addressing this issue, as properly suggested by the reviewer.

There are multiple problems with punctuation in this manuscript especially with the use of commas. For instance, all uses of “e.g.” require an immediate subsequent comma (e.g., L130). Uses of a conjunctive adverb (e.g., “thus”) should be preceded and followed by commas where appropriate (e.g., L141).

Corrected

There are several times where the authors utilize the abbreviation “e.g.” outside of a parenthetical statement (e.g., L218). In these cases, the “e.g.” should be spelled out as “for example” with a subsequent comma.

Corrected for all the occurrences in the text

In multiple places, the authors have used a colon where a semicolon is actually appropriate (e.g., L218, 305, etc.). These colons should be changed throughout the manuscript where appropriate.

Corrected as suggested where needed.

Other detailed changes include:

L262 Change “scientists” to “scientist.” +

L305 Change “somehow” to “somewhat.” +

L313 Replace “techniques example on” with “of.” +

L344 The phrase “in the scenario of the origin of ‘hot spots’ in soils” is unclear. Please reword to clarify your use of the term “hot spots.” +

L361 Add “Biological Station” after “Konza Prairie.” +

L367 Change “amongst” to “among.” +

L382 Change “prior the” to “prior to the.” +

L384 Change “is” to “was.” +

L527 Change “int” to “into.” +

L610 Change “of” to “between” and “chosen” to “made.” Remove “the one.” +

L619 Replace “allowing us” with “the ability.” +

L622 Change “but smaller features, e.g. single clay minerals platelets, cannot be resolved” to “but without the ability to resolve (e.g., single clay mineral platelets).” +

L645 Make “Surface Area” lowercase. +

L649-651 Make “Sphere-Normalized Surface-to-Area Volume Ratio” lowercase. +

L666-676 Make “Integral Mean Curvature” lowercase. +

L680 Make “Characteristic” lowercase and remove “instead.” +

L695 Make “Fractal Dimension” lowercase. +

L732 Change “previously” to “in section 2.3.” Change “Local Thickness” to “LT” since the abbreviation is already defined in L546. +

L744 Add “of” after “modeling.” +

L807 Change “will be further discussed later” to “are discussed in section 4.” +

L826 Make “Connected Component Labeling” lowercase. +

L865-867 Change “While an approximation based” to “Although this approximation is based.” +

L867-869 The phrase “...such calculations allow hypotheses to be generated regarding what regions...” is awkward and unclear. Please reword. +

L1072 Add “sample” after “Kansas.” +

L1143 Make “Star Length Distribution” lowercase. Since many readers will likely be unfamiliar with this method, I recommend adding a brief sentence describing it in general. +

L1162 Make “Pole Figures” lowercase. +

L1180 Should “distribution” be changed to “density?” “Density” and “Distribution” are both correct and used in general texture analysis. In all my previous publications involving texture analysis I’ve used “Distribution” and I’d like to keep that definition for consistency. Interestingly enough, a similar issue exists for the Orientation Density/Distribution Function (ODF), where “distribution” seems to be slightly preferred. But they are interchangeable.

L1182-1184 What about values less than one? +

L1184 I’m unclear what the authors mean by “whole particle.” +

L1183 How is the shape of the particle elongated vertically shown in the figure? +

L1195 Change “constituents” to “constituents.” +

L1203-1205 Change “Hints about the relationship of the PF’s with the sample” to “Relationships between PFs and the sample.” +

L1207 Change “PF’s” to “PFs.” +

L1209 Change “PF’s” to “PFs.” +

L1255 Change “such e.g. a Lattice” to “such as the Lattice.” +

L1576 Change “scientists” to “scientist.” +

L1636 Remove the comma after the second dash. +

The specific issues listed above marked with a “+” have been corrected as suggested.

Other minor changes have been done to improve the readability of the manuscript.

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4 ***Quantitative characterization of soil micro-aggregates:***
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7 ***new opportunities from sub-micron resolution***
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10 ***synchrotron X-ray microtomography***
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32 ***Abstract***
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36 Soil microaggregates are the fundamental building block, at the micron scale, of the
37
38 highly hierarchical structure of soils, and can exert a significant control on the local
39
40 biological metabolism and microbial community partitioning. In this study we propose an
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42 analysis protocol for the morphometric characterization of complete soil microaggregates
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44 based on sub-micron resolution synchrotron X-ray microtomography. A comprehensive
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46 characterization of the aggregate morphology is the first step towards a
47
48 **comprehensivecomplete** characterization of the soil microaggregates, when trying to
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50 correlate morphometric parameters with physical and/or biological properties, or when
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59 building models (~~e.g.e.g.~~, effective diffusivity, microbial distribution, etc.). We
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61
62 demonstrate our characterization approach on two single microaggregate samples from
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64 dramatically different soil environments: one from Kansas, primarily composed by
65
66 inorganic particles, and one from Barrow (Alaska) dominated by plant fragments. A
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68 series of state-of-the-art morphometric analysis techniques have been employed
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70 providing quantitative results highlighting specific differences of the two samples. The
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72 role of the microstructure in a scenario microbial population development has been
73
74 discussed and it has been found that the Barrow microaggregate seems to be more
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76 favorable, from a purely geometrical point of view, as also confirmed by a simple model
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78 presented in this work. The potential of this approach, when coupled with chemical and
79
80 biological analysis for a fully comprehensive characterization of soil aggregates in the
81
82 larger picture of enhanced biological activity, is evident.
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86
87 *Keywords: Synchrotron X-Ray microCT; Soil microaggregate; Quantitative image*
88
89 *analysis; Modeling.*
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91

92 93 **1. Introduction** 94 95 96

97
98 Soil is perhaps the ultimate “complex system”, with physical, chemical and biological
99
100 components interacting in a non-linear manner yet displaying clear properties of co-
101
102 evolution and self-organization (Young and Crawford, 2004). The physical structure of
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104 soil acts in a deterministic manner to regulate the assembly and activity of soil biota,
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106 including microorganisms, and through their activity, soil biota continuously shape and
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115 re-form both the macroscale and microscale structure of soil that in turn poses new
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117 constraints on biological activity. This pattern of co-evolution has led to the concept of
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119 soils being considered as ‘extended composite phenotypes’, (Phillips, 2009) where soils
120
121 themselves “*are an expression of the cumulative impacts of the biosphere on surface*
122
123 *processes*”. As the fundamental units of soil, aggregates and their physical, chemical, and
124
125 biological properties could be considered an extended soil phenotype.
126
127

128 The aggregation properties of soil constituents lead to a classification of aggregate
129
130 forms, primarily based on size and basic physical properties (*e.g.e.g.*, stability in water,
131
132 Six et al., 2000). Microaggregates, those less than 250 μm in diameter (Edwards and
133
134 Bremner, 1967) are critical for the sequestration of carbon in soil (*e.g.e.g.*, Six et al.,
135
136 2000, 2004, Vogel et al., 2014). Within microaggregates, pore structural properties that
137
138 evolve during aggregate formation and stabilization result in organic matter being
139
140 encapsulated within submicron pores (McCarthy et al., 2008) and, thus, protected from
141
142 microbial decomposition due to kinetic and spatial constraints. Given its importance, the
143
144 relationship between pore space geometry on the diversity and activity of soil
145
146 microorganisms has been a topic of notable interest (reviewed in Or et al., 2007).
147
148 Significant fractions of soil pore space can be inaccessible to soil bacteria (Chenu and
149
150 Stotzky, 2002), and observations of bacteria confined within pore spaces are common
151
152 (Foster et al., 1988), while predators such as protozoa can be isolated from their bacterial
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154 prey due to the interaction between pore geometry and the larger cell dimensions of
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156 protozoa (Vargas & Hattori, 1986, Wright et al., 1995).
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159

160 Perhaps the strongest constraints on microbial metabolism within microaggregates
161
162 are those of gas, moisture, and solute transport. Diffusive gas transport in particular is
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171 limited by the aperture and connectivity of exterior pores to deeper environments as well
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173 as pore hydration state. In the near-surface, anoxic microenvironments can develop in the
174
175 interior of microaggregates due to O₂ diffusion limitations as well as aerobic
176
177 communities on aggregate surfaces. Due to the difficulties in measuring spatial
178
179 concentrations gradients within microaggregates, many studies exploring these
180
181 constraints focus on modeling approaches (Currie, 1962; Smith, 1980; Renault & Stengel.
182
183 1994; Ebrahimi & Or, 2015). Currie (1962) and Smith (1980) provide 1D continuum
184
185 analytical models for such processes which are strongly dependent on an averaged D_{eff},
186
187 the effective gas diffusivity of the aggregate. Aggregate-specific D_{eff} values are difficult
188
189 to estimate, particularly for partial saturation states where capillary-bound water impacts
190
191 gas transport. Ebrahimi and Or (2015) present a pore-resolved numerical model of gas
192
193 and nutrient diffusion in microbially active aggregates and demonstrate that aerobic and
194
195 anaerobic microenvironments quickly develop; such spatial gradients in turn drive
196
197 community partitioning, particularly in wetter environments. A common limitation~~s~~ of all
198
199 such studies is the absence of realistically parameterized pore geometries, particularly for
200
201 microbially “remodeled” aggregates which are unlikely to have homogeneous network
202
203 structures as shown in prior imaging studies (~~e.g.e.g.~~, Peth et. al. 2008, Alba-Tercedor et.
204
205 al. 2015).

206
207
208
209 Given the importance of soil pore geometry and pore network connectivity for
210
211 biodiversity and biogeochemical cycling, it is critical to develop quantitative
212
213 measurement approaches to obtain soil aggregate microstructure at the relevant length
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215 scales (i.e. sub-micron). The microstructure of these fundamental soil building blocks
216
217 will likely have a significant impact on the ability to host specific microorganisms.∴
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226
227 e-g-for example large pores with many small apertures can provide a safe environment for
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229 specific microorganism colonies to thrive. The direct correlation of microstructure and
230
231 microorganism distribution is beyond the scope of this work, which focuses in on
232
233 development of morphometric parameters and analysis strategies to obtain a
234
235 comprehensive description of the microstructure of soil microaggregates. Our results do,
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237 however, provide insight into prior experimental studies examining the role of
238
239 microaggregates in disrupting predation (Wright et al. 1995, Vargas and Hattori, 1986).
240
241

242 A first attempt at correlating Synchrotron X-Ray micro-Computed Tomography
243
244 (SXR- μ CT) data with microbial populations obtained via 16S rRNA pyrosequencing has
245
246 been attempted in Bailey et al. (2013), where no clear correlation of microbial
247
248 populations with microstructure has been found. While that work is valid, the lack of an
249
250 advanced analysis of the SXR- μ CT data (only proxies of pore size distributions and
251
252 surface areas have been considered as descriptors for the microstructure) might have
253
254 neglected important hints that could have been successful in finding an eventual
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256 correlation. In this context, we are offering an example of a state-of-the-art measurement
257
258 and data analysis that can be used for a complete characterization of *whole* (in contrast
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260 with cropped subvolumes, as usually found in literature) soil microaggregates at the
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262 nanoscale, providing the soil scientists community a series of tools that can be used in
263
264 future works where a comprehensive *quantitative* characterization of soil
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266 microaggregates is needed.
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269 XR-CT (both using conventional and unconventional radiation sources, at
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271 different scales) has been successfully used for soil characterization in the past, given its
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273 ability to provide 3D volumes describing the structure soil samples. One of the main
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283 advantages of this technique is its non-destructive nature, which allows the observation of
284
285 undisturbed samples, such as roots in soils (Heerman et al., 1997; Mooney et al., 2013),
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287 macropores in large (~20 cm) soil cores (Pierret et al., 2002), and characterization of pore
288
289 space in ~3 mm aggregates (Nunan et al., 2006; Peth et al., 2008). More specific works,
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291 focusing on the characterization of the pore networks in soils, at different scales, can be
292
293 found in Petrovic et al. (1982), and Anderson et al. (1990) where the main focus was the
294
295 estimate of the density of the soil samples. At the mm scale, XR-CT has also been used to
296
297 locate layering in soil samples as presented by Macedo et al. (1998). Other studies
298
299 included porosity and flow properties in measured soils, as presented in Peyton et al.
300
301 (1992), Heijs et al. (1995), Clausnitzer and Hopmans (2000). Fractal properties in soils
302
303 have been studied although their calculation and practical implications have been
304
305 some ~~what~~ controversial. Examples can be found in: Perfect et al., (1992), McBratney
306
307 (1993), Peyton et al., (1994), Anderson and McBratney (2005), Gimenez et al., (1997),
308
309 Gibson et al. (2006).

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311
312 In this work we present state-of-the-art data processing and morphometric analysis
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314 ~~techniques example on~~ two different soil microaggregates (slightly smaller than 250
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316 μm) to quantify microstructural attributes. The purpose is to show an analytical protocol
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318 that can be used to describe the microstructure of the soil microaggregates in a
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320 quantitative fashion, and to provide the scientific community (~~e.g.e.g.~~ modelers) with
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322 datasets of *complete* microaggregate particles with sub-micron resolution to be used as a
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324 realistic starting point and/or validating dataset for synthetic soil models. To validate this
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326 approach, ~~w~~We have chosen two markedly different soil microaggregates, both in
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328 composition and texture, the first rich in inorganic material and the second rich in organic
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339 material (plant fragments) to better monitor which parameters can describe those
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341 differences. This quantitative description of the soil microaggregates is fundamental for a
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343 complete study complementing the role of the soil microaggregates microstructure,
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345 chemical heterogeneities, and microorganism distributions in ~~the scenario of the~~ research
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347 about the origin and development ~~origin~~ of “hot spots” in soils, and to contribute to our
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349 understanding of the functional stability and spatial variability within soils in general.
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356 ***2. Materials and Methods***

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360 *2.1 Sample choice and preparation*

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365 Two distinct soils were selected for our study: one from the Konza Prairie Biological
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367 Station, Kansas, and a second from Barrow Experimental Observatory (BEO), Alaska.
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369 The two samples selected for this evaluation varied in bulk density, organic matter
370
371 content, and mineralogy, among~~st~~ other parameters. Microaggregates were obtained via
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373 manual dissection under a microscope, where the sorting was based on size (200 to 250
374
375 μm diameter), and on the ability to remain coherent under mechanical stimulation.
376
377 Preparation of the aggregates for analysis involved inserting the microaggregates into a
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379 thin walled 300 μm borosilicate glass capillary (Charles Supper); the capillary tubes were
380
381 immediately sealed using hard wax to maintain the original humidity as much as possible.
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383 The capillary tube was used to assist sample mounting and to maintain sample integrity at
384
385 proper moisture conditions. Sealing and acclimation (~1 hour) at the synchrotron
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beamline experimental hutch prior to the experiment is fundamental to keep the soil aggregate at proper conditions and avoid motion artifacts due to variations in humidity during the scan. The very high resolution of the measurement makes the dataset prone to motion artifacts, so a nearly perfectly stable sample was a necessity.

2.2 SXR- μ CT measurement and data reconstruction

The SXR- μ CT experiments were carried out at X-ray Imaging Beamline 8.3.2 (BL 8.3.2) at the Advanced Light Source (ALS) at the Lawrence Berkeley National Laboratory (LBNL). For the tomographic measurement, a 11 keV monochromatic beam was selected via a multilayer monochromator. The glass capillary containing the individual aggregates was mounted on a rotating stage, ~ 8 mm from the scintillator of the detector system; a short distance was selected to avoid strong phase-contrast effects due to free space propagation of the highly coherent XR beam. The detector system consisted of a LuAG 20 μ m thick scintillator mounted in front of a 20~~x~~ objective lens; the visible light signal obtained from the scintillator was recorded on a CCD camera (Optique Peter, Lentilly, France) obtaining a dataset with a resulting voxel size of 325 nm. 1800 projections were recorded for each sample over a 180° rotation, and the exposure time was 1 second per projection.

Before reconstruction of tomographic 2D slices, a single-distance phase retrieval algorithm was applied to the projection datasets. Phase-retrieval is beneficial in this kind of datasets since it improves the contrast between the different phases and makes the phase-contrast artifacts less pronounced, both these characteristics are of great help with

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450
451 the segmentation via thresholding process. After a conventional flat-field correction of
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453 the single projection, we applied the Paganin single-distance phase retrieval algorithm
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455 (Paganin et al, 2002) as implemented in the ImageJ plugin ANKAPhase (Weitkamp et al.,
456
457 2011), which proved to be effective (e.g.e.g., ~~Volto~~Volto~~lini~~lini ~~Arzilli~~Arzilli et al., 2015) with datasets
458
459 collected in the near-field Fresnel diffraction region (Bronnikov, 2002). After phase-
460
461 retrieval the slices were reconstructed with a conventional filtered back-projection
462
463 algorithm (Kak and Slaney, 1987).
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465

466
467 Once the stacks of slices were reconstructed, the datasets were manually cropped
468
469 to isolate the soil microaggregate, separating it from the glass capillary, to obtain an 8 bit
470
471 grayscale volume used as a starting point for subsequent analysis. The volume rendering
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473 of the two particles, whole and with a virtual vertical cut to show the internal structure,
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475 are shown in Figure 1. It is immediately possible to qualitatively appreciate the
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477 microstructural differences and identify in the “Barrow” microaggregate a large amount
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479 of material originating from plant debris, visible as a cellular texture with large interior
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481 pores (right panel). In contrast, the microaggregate obtained from the Kansas prairie site
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483 (left panel) appears to be an aggregate of mineral particles with smaller interior pore
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485 spaces, displaying a more interstitial type of pore space.
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489 *2.3 Data treatment and separation*

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494 To allow analysis of pore-space morphologies, the first step in our analytical approach is
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496 segmentation, aimed at separating the phases of interest into binary volumes. A simple
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498 manual thresholding procedure was possible due to the strong contrast between the solid
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507 and voids voxels in the samples obtained after the phase retrieval procedure. However,
508
509 the analysis of microaggregates requires a deviation from classical approaches for
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511 segmentation commonly applied to XR-CT datasets. In most situations, the image
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513 volume is cropped and only a subsection of the system is segmented and analyzed. In our
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515 case we wanted to capture the entire particle and determine the properties of the
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517 aggregate exterior surface. This boundary forms the interface between the aggregate and
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519 the outside world and is key to imposing boundary conditions when looking for
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521 interactions of the outside with the microaggregate pore space, ~~e.g. for example for in~~ gas
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523 transport modeling, predation, etc. This approach comes with a challenge, mainly the
524
525 problem of separating the air outside the microaggregate from the air in the voids inside
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527 the sample despite the fact that the phases are continuous. This cannot be done ~~with via~~
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529 thresholding procedures, since the air inside and outside the sample has of course the
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531 same XR attenuation values (translated ~~into~~ the same grayscale value, in the tomographic
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533 dataset), thus the necessity of morphology-based methods.
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536
537 We have developed an iterative procedure using the Fiji software framework
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539 (Schindelin et al., 2012) that allows the calculation of such an external surface and
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541 provides a separation of the pore space from the air outside the sample. This procedure
542
543 can produce results similar in concept to the snake-based “active contour” segmentation
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545 algorithms used in the medical imaging field (~~e.g.e.g.~~, Yushkevich et al., 2006). The
546
547 procedure ~~we applied also functions works well~~ in 3D and can be applied to systems
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549 where large objects need to be extracted and segmented. The procedure consists of a
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551 series of operations involving local thickness (LT) analysis of the voids using the plugin
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553 described in Dougherty and Kunzelmann (2007). LT analysis is used to obtain a first,
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rough, outer surface and a closing procedure is used to close the smaller outer pores. 3D void filling is then used to fill the largest internal voids, and finally masking is utilized to preserve the small features that are commonly erased by the morphological operators such as eroding and dilating processes. The filled soil aggregate particle obtained is then used to obtain a “sample surface” again through morphological operators of the binary erosion/dilation class coupled with Boolean operations. This outer surface obtained with the procedure described above is shown in Figure 2 rendered in red and superimposed on a rendering of the Barrow sample. The virtual cuts reveal how the surface calculation effectively “wraps” the aggregate exterior without penetrating into the interior pore spaces, thus providing information on the interface between the aggregate and the surrounding environment. The procedure of calculating a surface separating the pore space from the outer air is the starting point for subsequent analyses on the two datasets.

Results

3.1 Morphometric analysis: Basic analysis

The procedure described above generates two base binary volumes for the morphometric analysis: a volume for inner voids (the pore space) and a volume of the solid phase. This allows a variety of analytical techniques to be carried out in order to obtain quantitative information describing the two different phases. We stress that all the morphometric analysis needs to be considered with the actual resolution of the measurement in mind.

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619 some values (such as surface area values) are very sensitive to image resolution and the
620 value listed must be read with this intrinsic limitation in mind. For SXR- μ CT analysis, a
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624 compromise ~~between~~ field of view and resolution needs to be ~~chosen~~~~made~~; we have
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626 chosen ~~the one~~ the setup with the ability allowing us to image ~~the~~ whole soil
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628 microaggregate with the highest resolution possible; With the chosen setup the resolution
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630 was very high, especially when compared to similar measurements presented in literature,
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632 but still without the ability to resolve the ~~but~~ smallest ~~st~~ features; (e.g.e.g., single clay
633
634 minerals platelets)~~, cannot be resolved~~. We present a comparison of a range of classical
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636 morphometric metrics applied to our two samples, including porosity, surface area,
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638 sphere-normalized surface-area-to-volume-ratio, Minkowski functionals, and fractal
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640 dimension metrics; such metrics provide a first step in grouping aggregate properties.
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642
643 The first analysis carried out was to calculate the basic properties of the
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645 aggregates, such as their volumes and porosities. In Table 1, a summary of the
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647 morphometric analyses carried out on the two samples is listed. From the volumetric
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649 analysis, we can see how the Barrow sample is ~22% larger in volume than the Kansas
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651 one. The cellular texture and interior pores seen in the Barrow sample results in a high
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653 porosity (81%) while the Kansas aggregate has a porosity more typical of granular
654
655 composites (43%). The ~~s~~Surface ~~a~~Area (SA) calculation also shows a higher value for the
656
657 Barrow sample but the impact of sample size makes such a non-normalized SA
658
659 problematic to interpret. To overcome this problem a ~~s~~Sphere-~~n~~Normalized ~~s~~Surface-to-
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661 ~~a~~Area ~~v~~Volume ~~r~~Ratio (SNSVR) was calculated with SNSVR defined as SA_{obj}/SA_{sph} .
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663 SA_{obj} is the object surface area, and SA_{sph} is the surface area of a sphere with the same
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665 volume of the object. The farther the object surface is from a spherical surface, the higher
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675 the SNSVR value (1 being the value for a perfect sphere). After this correction, the
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677 Barrow sample still shows a larger area available per volume units, but both the
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679 microaggregate particles display values related to a markedly complex surface.
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681
682 The Minkowski functionals (see ~~e.g.e.g.~~, Ohser and Muecklich, 2000) can be used
683
684 as topological descriptors of a binarized volume. More specifically the ~~i~~Integral ~~m~~Mean
685
686 ~~c~~Curvature (IMC) (Russ and DeHoff, 2012) is a value related to the concavity/convexity
687
688 (depending on the sign of the IMC) of the surfaces present in the sample. The Euler
689
690 ~~c~~Characteristic (EC) (Odgaard and Gundersen, 1996) is ~~instead~~ a value related to the
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692 connectivity of the objects in the volume: positive values mean isolated objects, while
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694 connected networks generally display negative values. The values measured for the IMC
695
696 of the framework of solids and the EC of the pore space highlight a highly connected
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698 network dominated by concave shapes of the solid framework, with the Barrow sample
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700 being slightly more interconnected and with a slightly weaker dominance of convex
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702 surfaces.
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705
706 Another frequently used parameter for texture analysis is the ~~f~~Fractal ~~d~~Dimension
707
708 (FD), which has been applied to the solids in the aggregate, providing an index of self-
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710 similarity of the microstructural features at different scales. The FD has been calculated
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712 using the box-counting method (Liebovitch and Toth, 1989) and values obtained show
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714 that the Barrow aggregate (FD = 2.401) is slightly more self-similar than Kansas sample
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716 (FD = 2.353). Both values are relatively low (for volume data $2 \leq FD \leq 3$) highlighting
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718 moderate fractal properties, at the considered resolution and sample size. Prior studies
719
720 examining the fractal dimension of micro-aggregates using destructive mass/radius
721
722 analysis have yielded slightly higher values (FD = 2.75 to 2.93, Young & Crawford,
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731 1991), the differences in sample origin and measurement approach make comparisons
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733 challenging.
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735 736 737 738 *3.2 Local thickness (LT) analysis*

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742 As discussed ~~previously~~ in section 2.3, ~~Local Thickness (LT)~~ analysis is an extremely
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744 useful approach for porous material characterization borrowed from the bone scientists
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746 community, where it is generally used for the analysis of cancellous bone, measuring
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748 parameters such as the “trabecular thickness” and “trabecular separation” (Parfitt et al.,
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750 1987; Simmons and Hipp, 1997; Accardo et al., 2005). The same algorithm has been later
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752 adopted in different contexts e.g. for example as part of multiphase flow modeling
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754 approaches by Silin et al. (2010) under the name “maximum inscribed spheres” and is
755
756 finding increased use in modeling of geologic samples. The term “local thickness” for a
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758 voxel is used to mean the diameter of the maximum inscribed sphere in the structure that
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760 contains the voxel. In Table 1., where the LT results are summarized, we use more
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762 generic terms, where “structure separation” is the LT analysis calculated on the pore
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764 space volume, while with “structure thickness” we mean the LT analysis carried out on
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766 the solid framework. The analysis has been performed using the Fiji plugin from
767
768 Dougherty and Kunzelmann (2007), and graphical results are shown in Figure 3 for both
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770 the migroaggregates.
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774 In Figure 3 a vertically cut rendering is superimposed with the LT-labeled volume
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776 of the pore space, and a volume rendering of the cut LT volume itself. The image clearly
777
778 displays the thickness variations of the pore space within the volume, with the Kansas
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787 sample showing interstitial voids created by the aggregation of the silt particles, while in
788
789 Barrow the larger, smoother, voids due to the presence the plant fragments and their
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791 cellular structures are evident. A larger variation of LT values is also immediately
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793 observable from the renderings in figure. A quantification of the LT analysis was
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795 performed and the results are presented again in Table 1. The mean of the structure
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797 separation (mean LT of the pore space) is larger in the Barrow sample, but also the
798
799 standard deviation is significantly larger and the maximum LT value of voids is present
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801 in the Barrow sample as well, this again because of the presence of the plant fragments
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803 with large voids surrounded by small ones. This variability is recognizable when looking
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805 at more detailed data than the one summarized in the Table 1. In Figure 4 we show a plot
806
807 of the LT values distribution in the pore space for both Kansas and barrow. The higher
808
809 variability suggested by the summarizing values here becomes even more evident with
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811 the two distributions being markedly different, with values of higher LT values being
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813 more frequent in the Barrow sample. The smallest LT values are also more frequent in
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815 the Barrow sample, while the small LT values are generally more frequent in the Kansas
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817 microaggregate, showing a sharper LT distribution curve. This feature and its
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819 implications ~~will be~~ are discussed in section 4~~further-discussed-later~~.
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824 Concerning the structure LT analysis (“structure thickness”) values for Barrow
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826 are generally slightly larger, with a much larger standard deviation value and especially a
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828 larger maximum. This because in the Barrow sample a ~50 µm large single mineral
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830 particle is present (top of the sample).
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833 834 *3.3 Geometrical accessibility analysis* 835 836 837 838

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845 The concept of LT, combined with the outer surface calculated via the procedure
846 described above, and the connected component Ilabeling (CCL, see e.g.e.g., Hu et al.
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850 2005) can be used to calculate the parts of the sample accessible from the outside by
851 objects with different sizes. This class of analysis has obvious applications as a simple
852 model able to provide insights about pore-size constrained microbial colonization of
853 aggregates, as well as the spatial limits of predation by larger organisms. The procedure
854 is straightforward: a threshold value (corresponding to the size of the structuring element
855 -spherical- considered for accessibility) is applied to the LT volume. A 5 voxel thick
856 outer surface is added to the volume and a CCL procedure is initiated starting from the
857 outer surface; this will find all the parts of the sample accessible from the outside. The
858 outer surface is then removed using a masking procedure with the pore space binary
859 volume. The volume left is the pore space geometrically accessible from the outside by a
860 spherical element with the value corresponding to the threshold value used.
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873 This procedure can be used to detect the parts of the pore space theoretically
874 accessible to microorganisms of known size, using only geometric parameters. This
875 isolates the volumes of the samples accessible by the different structuring elements from
876 the outside, along pathways with throats larger than the structuring element. Although
877 this approximation is based~~While an approximation based~~ solely on geometry, such
878 calculations allow to obtain some information about which parts of the sample could be
879 accessible to different classes of microorganisms, segmented by their characteristic size.
880 hypotheses to be generated regarding what regions of the aggregate are accessible to
881 different classes of microorganisms. Bearing in mind the resolution of the measurements,
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899 we considered three different classes of microorganisms based on their size: 0.65 μm for
900 average microbial cells, 2 μm for large microbes, and 10 μm for protozoa. Aggregate
901 microarchitecture can be expected to influence the distribution and activity of
902 microorganisms, for example microaggregates possessing large internal chambers with
903 entrances small enough to selectively exclude larger competitors and/or predators would
904 represent potential activity hotspots and refuges for select portions of the community.
905 Results of the analysis are summarized again in Table 1. From this set of calculations, it
906 is possible to see that both microaggregate samples are close to totally accessible to
907 objects .65 μm large, while 2 μm structuring elements can still enter the majority of the
908 pore-space in both the Barrow (86%) and Kansas (76%) samples. The largest (10 μm)
909 structuring element, the size of small protozoa, cannot enter any of the pore space of the
910 Kansas, inorganic-rich, microaggregate, while a small part of the sample (3%) is
911 accessible in the Barrow one.
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927 The potentially accessible parts of the pore space are displayed, superimposed to
928 the 8bit volume rendering for all the particles in Figure 5. In this figure it is possible to
929 observe the parts of the sample accessible to microbes of different sizes; for example the
930 top lobe of the Kansas sample can be fully colonized by .65 μm microorganisms but has
931 limited pore-space available to 2 μm microbes. Neither aggregate, with the exception of a
932 single large pore in the Barrow sample, has a pores/throats system of sufficient size to
933 accommodate 10 μm microorganisms, suggesting that both microaggregates could
934 provide protection to internal communities from predation by protozoa. Previous
935 microcosm studies have demonstrated that aggregate microstructure protects microbial
936 communities from such predation (e.g.e.g., Vargas and Hattori, 1986, Wright et al. 1995)
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955 but prior studies could not characterize the internal aggregate structure which confers this
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957 effect.
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959 960 961 962 *3.4 Skeleton analysis* 963

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966 Another important descriptor of pore network topology is the pore space skeleton. A
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968 skeleton is a 1D topological descriptor of the 3D pore space which captures network
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970 connectivity in a simplified form, suitable for discrete models of flow and transport. In
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972 this study we used the “thinning” algorithm to efficiently compute the medial axes of
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974 both aggregate pore spaces (Lee et al., 1994; Lindquist et al., 1996; Palágyi and Kuba,
975
976 1999). The skeleton of the pore space of the connected network was calculated and the
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978 skeleton voxels were also labeled to identify branches, joints and end points in the
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980 framework.
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983 The results of the skeleton analysis are summarized in Table 2. The network
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985 statistics indicates that the Barrow sample has the greater pore network complexity; this
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987 sample displays a significantly larger number of branches and junctions while the number
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989 of end points and the average branch length are very similar. This feature highlights a
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991 similar, basic, accessibility from the outside to two networks markedly different in
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993 complexity. This topic will be further analyzed and discussed, when another concept of
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995 “accessibility”, based on the analysis of the size of the openings of the microaggregates
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997 facing the outside will be introduced. In Figure 4 a frequency distribution plot of the
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999 branch lengths in the two microaggregates is shown: the Barrow sample displays a wider
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1001 distribution, with a larger number of the smallest and larger branch lengths, while the
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Kansas sample has a larger amount of the smaller (but not the smallest) branches. This, again, highlights the differences of the two pore network topologies, with the Barrow being the more complex.

The renderings of the two samples with the calculated thinning skeletons, labeled with respect to the LT value of each voxel to introduce pore diameter information in the skeleton, are shown in Figure 6. From the colors of the skeleton it is possible to see how in the Barrow sample the blue colors (extremely small values of LT) and the hotter colors (higher values of LT) are more frequent than in the Kansas sample, where small and moderately small (blue and greens) values are visibly more frequent. This is in accordance with the LT analysis discussed previously.

The network renderings also provide a qualitative representation of the differences in skeleton architecture between the two samples; the Kansas sample exhibits an interstitial skeleton, typical of the pore space generated from granular materials. In contrast, the Barrow sample shows more complicated structures, including components with many short branches and small LT values as well as parts with single long branches following the medial axes of the largest structures. These features appear to be generated by the interconnected cellular texture of the plant fragments incorporated into the aggregate.

3.5 The interface to the outer world: openings analysis

The outer surface of the aggregate is a critical interface linking it to the exterior environment, mediating gas and solute transport as well as microbial colonization. A key

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numerical task is, thus, extracting the apertures which exist on the aggregate exterior; these components are the required boundary condition for pore-scale modeling of diffusion and reaction within the single microaggregate system. As discussed in previous sections, our processing flow has already extracted (a) the exterior bounding surface and (b) the skeleton and LT map for the aggregate pore space. By selecting the skeleton endpoints which terminate within close proximity (5 voxel lengths) of the outer surface and then labeling them with the LT thickness we can generate a map of aggregate openings with the appropriate dimensions. The lower panel of figure 4 shows a histogram of the size of open pores on the aggregate surface for both samples considered. As can be seen, the slopes of the opening size distribution curves are markedly different, being the Barrow one steeper, highlighting a higher amount of small apertures, and a higher small-to-large apertures ratio. This is again a difference due to the Kansas being an aggregate of particles with an interstitial kind of pore space, while the Barrow is made of mainly small plant fragments with ~~either~~ very small openings with a the few extremely large ones, where the biggest sects of the plant structure are broken and exposed to the surface. This results in the markedly different distribution of the openings.

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Figure 7 provides a more graphical representation of the opening calculations showing grayscale volume renderings of the two microaggregates with opening pores marked in color. The paired figures show the same openings superimposed ~~toen~~ the internal network structure (the skeleton in white). As can be seen, both samples are dominated by small exterior pores with the Barrow sample having smaller opening dimensions, indicated by the cooler colors (blue). The large exterior pores (orange/red) are relatively rare features on both aggregate surfaces.

3.6 Anisotropy analysis

Soil microaggregates can be formed by different components with varying shapes including rounded mineral particles, clay platelets, and fibrous/cellular organic materials as well as bioproducts. The shape of these constituent materials can control the shape of the microaggregate and the presence of anisotropic components can influence soil aggregate properties (e.g.e.g., Emerson, 1959). Considering that existing continuum models of gas diffusion in aggregates assume isotropic effective diffusivities, detection of strong pore-space anisotropy is a useful tool for evaluating the applicability of such models.

Fabric anisotropy can be measured directly from high quality tomographic datasets. One such approach is described in Voltolini et al. (2011), however it requires the separation of each single object in the dataset, a difficult constraint for aggregate characterization. When interior object separation is not possible, different approaches can be used. The most widely utilized technique is the mMean iIntercept lLength (MIL) method (Witthehouse, 1974), but this shows some limitations since it ideally requires spherical cropping and is prone to artifacts in datasets with objects described by small numbers of voxels. To minimize these issues we decided to use the sStar lLength dDistribution (SLD) method (Odgaard et al., 1997), which measures the mean object lengths for all orientations, using the implementation present in Quant3D (Ketcham and Ryan, 2004) for the first calculation, then-finally a series of Matlab® scripts based on the

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1179 MTEX toolbox (Bachmann et al., 2010) were used for data handling, corrections,
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1181 normalization and plotting.
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1184 To study the anisotropy of the two microaggregates we applied the SLD method
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1186 to the solids and on the filled shape of the whole microaggregates. The former provides
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1188 the quantification of the anisotropy of the internal microaggregate structure (solids),
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1190 while the latter describes the anisotropy of the shape of the microaggregate itself. A
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1192 comparison of the two ~~pPole f~~Figures (PFs) obtained from this analysis highlights any
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1194 relationship of the microstructure with the shape of the microaggregate. In Figure 8 the
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1196 PFs describing the Kansas and Barrow microaggregates for the solids and the whole
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1198 microaggregates are plotted. In the Kansas sample (top) it can be seen that the structure is
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1200 made by isotropic/randomly oriented components, since the PF displays values extremely
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1202 close to one along all directions. Values in PFs are in multiples of random distribution
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1204 (m.r.d.), where 1 is the value of a perfectly isotropic object and higher values towards $+\infty$
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1206 imply progressively stronger anisotropy. The orientation space with values <1 imply an
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1208 orientation density smaller than a random (i.e. isotropic, uniform) distribution, and in
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1210 addition to the maximum value, the minimum value in the PFs is an important parameter
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1212 as well, representing the percentage of objects in the sample that can build a random
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1214 distribution, thus giving additional information about the sharpness of the texture. The PF
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1216 for the Kansas whole ~~micropartiele-aggregate~~ denotes that some anisotropy is present;
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1218 this is clearly visible from the renderings where the shape of the particle is elongated
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1220 vertically and slightly flat, similarly to a 3-axes ellipsoid. This result shows that even if
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1222 the microaggregate particle is elongated, there is no internal anisotropy present, in its
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1235 components. This result is in line with the qualitative observation of the microaggregate
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1237 constituents, where many rounded silt particles are recognizable.
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1239 The Barrow microaggregate is different; the PF of the whole particle suggests a
1240 slightly platy morphology, but the PF of the microstructure of the solids clearly shows a
1241 more fiber-like texture with the elongation axis in the platelet plane. The texture is weak
1242 (max at 1.25 m.r.d.), but anisotropy is clearly present. Relationships between PFs and the
1243 sample ~~Hints about the relationship of the PF's with the sample~~ can be seen at the bottom
1244 of Figure 8 where the renderings of the soil microaggregates, with the same orientation as
1245 the PF's are displayed. The virtual cut plane of the aggregates corresponds to the plane of
1246 the PF's as well. This analysis confirms that an accurate quantification of the anisotropy
1247 in single soil microaggregates is achievable using the methods described above.
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1263 ***Discussion***

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1267 Two soil microaggregates of different origins and internal structures have been analyzed
1268 via sub-micron resolution SXR- μ CT. A variety of techniques to analyze different
1269 microstructural parameters have been applied to provide a description of the different
1270 features of the aggregate microarchitecture in a descriptive fashion. This class of
1271 approaches are increasingly used in analysis of soil systems (e.g.e.g., De Gryze et al.,
1272 2006; Peth et al., 2008a,b; Zhou et al., 2013; Ma et al., 2015; Peth et al., 2015). The
1273 potential flexibility of the resolution/FOV ratio, with both conventional and
1274 unconventional X-ray sources, allows scans within a large range of scales (Sleutel et al.,
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1291 2008). Synchrotron radiation, given its high flux, monochromaticity, and spatial
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1293 coherence, has been recognized as a very important tool for the soil scientist since the
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1295 first 3D imaging beamlines were developed (Spanne et al., 1994). Microtomographic data
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1297 are also used for modeling more complex physical properties of soils and rocks, such
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1299 ~~ase.g. a the~~ Lattice Boltzmann approach for evaluating permeability (Menon et al., 2011;
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1301 Khan et al., 2012) or direct numerical simulations of pore-scale reactive chemistry
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1303 (Molins et al. 2012).
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1306 In this work we focus mainly on the geometrical differences of single
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1308 microaggregates and exploit the high flux and resolution of BL 8.3.2 to provide a more
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1310 detailed structural description. The new tools make it possible to develop the analysis of
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1312 the entirety of microaggregates at sub-micron resolution, overcoming the need to crop
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1314 subvolumes. This is of great importance since we were able to study the interface of the
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1316 single microaggregates with the external world, whereas cropped volumes would make
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1318 this kind of study impossible, and would not provide correct boundary conditions when
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1320 used for modeling.
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1323 The size of the two microaggregate studied is similar. From the analysis of the
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1325 internal porosity, it is clear that the microaggregate richer in organic matter (Barrow) is
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1327 about twice as porous as the predominantly inorganic one (Kansas). This increased
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1329 porosity appears to be the result of large pores with a cellular texture, as visible in Figure
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1331 2b. While a statistically valid generalization from our small sample set is not possible,
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1333 the results from the Barrow aggregate suggest that detrital plant matter has an important
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1335 role in controlling internal porosity. Prior studies of microaggregate structure have noted
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1337 that formation often initiates around a “core” of plant debris (Oades and Waters 1991,
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1347 Golchin et al. 1994); our results suggest that this core material may also provide a unique
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1349 structural environment for microbial activity distinct from microaggregates which are
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1351 primarily granular in texture. It is important to remark that in this work we are only
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1353 comparing two aggregates with different texture and composition to find the
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1355 morphometric parameters that would better describe those differences, and the links of
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1357 those parameters with specific properties of the aggregate; we are not comparing two
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1359 specific environments. The latter task would require a much larger number of samples,
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1361 and likely a different approach, combining multi-resolution measurements, where the
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1363 large FOV would be used to identify the different type/classes of microaggregates present
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1365 in each locale (and quantify their distribution), while the high resolution would target the
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1367 specific single microaggregates (as shown in this work), representative for each class, to
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1369 fully characterize them. Such an approach would likely allow reaching an acceptable
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1371 statistical meaningfulness for each site, as needed to take into account some of the intra-
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1373 site variability of microaggregates, but without measuring an unrealistically high number
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1375 of single microaggregates and run a full analysis on all of them.
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1379 The internal aggregate surface area is another important parameter due to both its
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1381 role in reaction kinetics as well as a microbial growth substrate. Concerning the surface
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1383 area of the two microaggregates, the Barrow sample shows a larger surface area with a
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1385 slightly higher complexity than the Kansas sample. This is likely due to the rough surface
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1387 present in the Kansas sample, composed mainly of poorly sorted silt/clay particles, and
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1389 the smoother surfaces present in the Barrow sample. However, the complexity of the
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1391 pores space in the Barrow sample ultimately generates a higher surface area per volume.
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1403 The Minkowski functionals, as expected, describe a complex structure with a
1404 tightly interconnected pore space. This is also confirmed by the skeleton analysis,
1405 highlighting again the extensive complexity of the soil microaggregates at this scale. The
1406 fractal analysis shows a moderate fractal behavior for both the microaggregates, with no
1407 significant differences between the inorganic- and organic- based particles. Independent
1408 of use as a complexity measure, the fractal dimension of aggregates has been linked to
1409 measures of erodability in past studies (e.g.e.g., Ahmadi et al. 2011), hence it may such
1410 measurements may provide insight to microaggregate evolution over time.
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1420 The LT analysis discussed previously provides detailed information on pore space
1421 statistics across the aggregate; the distribution of voxel LT values provides useful
1422 statistical constraints including the aperture variance, a key parameter in stochastic
1423 network models of soil structure which is often guessed at. The LT distribution in the
1424 Kansas sample is clearly sharper than the Barrow aggregate, which exhibits a higher
1425 variance in pore sizes and apertures. This statistical difference is due to the plant
1426 fragments: the large voids present in these structures, coupled with small voids generated
1427 by clay particle aggregation, generated a broader distribution of pores. These
1428 observations suggest that the Barrow microaggregate might provide a better host for
1429 microbial activity due to the combination of a large internal porosity and a broad size
1430 distribution of internal microenvironments.
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1443 The novel strategy for soil particles analysis presented also allows the calculation
1444 of pore space accessibility metrics for single aggregates, potentially a key control on
1445 protection of aggregate microbial communities from predation by larger organisms
1446 (e.g.e.g., protozoa). It is worth remarking that this is a theoretical accessibility based
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1459 purely on the geometry of the pore space: ~~characteristics~~ features such as characteristic
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1461 microorganism shape, biological needs, reproduction rates, etc. are not considered. The
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1463 resulting accessibility metrics assume a rigid spherical body to determine the ideal
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1465 potential access. We have chosen three different sizes, compatible with characteristic
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1467 organism sizes and image resolution. First we have performed the accessibility analysis
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1469 for objects .65 μm large, a typical mid-size bacterial cell. The theoretical accessibility for
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1471 a virtual microorganism of this size from outside the sample is very large: 95% for the
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1473 Kansas sample and 98% for the Barrow aggregate. This is not surprising since this value
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1475 is close to the resolution of the measurement and the pore space is strongly
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1477 interconnected. More interesting is the accessibility for objects with the size comparable
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1479 to large bacteria strains: 2 μm . The differences here are more marked since some parts of
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1481 the Kansas sample, more complex and with small, not well connected, pores are present
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1483 and therefore they are non-accessible to the 2 μm virtual bacteria. In the Barrow sample
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1485 the accessibility is still very high (86%, compared with the 76% of Kansas), this is due to
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1487 the fact that the size of the object is still smaller than the size of a significant number of
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1489 openings on the surface and because of the very high connectivity (and throat sizes) of
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1491 the pore space, allowing the objects to move rather freely once entered the
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1493 microaggregate pore space.
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1498 As a last test, we considered objects 10 μm large: this is the size of small
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1500 protozoa, an active bacterial predator. Prior experimental studies (Wright et al. 1995)
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1502 have utilized even larger protozoa with mean sizes in the 20-30 μm range (*C. steinii*) to
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1504 study the protective nature of aggregates. A microaggregate largely accessible to
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1506 protozoa would be potentially unsafe for the internal microbial community. In the two
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1515 microaggregates examined, the virtual protozoa cannot enter any pore space in the
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1517 Kansas sample, and can only enter a small single portion of the pore space in the Barrow
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1519 one, highlighting how these microaggregates can in theory provide a protective
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1521 environment for bacterial communities. Our imaging study is largely consistent with prior
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1523 experiments documenting this phenomenon (Vargas and Hattori, 1986, Wright et al.
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1525 1995). Given the protective role of microaggregates and the availability of a diverse set
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1527 of associated microenvironments (Ranjard and Richaume, 2001), the role of aggregate
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1529 pore morphology in controlling community structure might provide a promising path
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1531 towards understanding the biogeochemical response of such systems (Remenant et al.
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1533 2009).
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1537 In addition to bulk accessibility metrics, we quantified the aperture dimensions of
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1539 the outer surface of each aggregate sample, a metric useful in defining outer boundary
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1541 conditions and flux limitations for gas transport. In the Kansas sample the exterior
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1543 aperture sizes are generally larger than the Barrow sample as can be seen in the color map
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1545 used in Figure 7. The Barrow sample does however have a small number of large open
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1547 exterior pores generated by open tubular structures present in the detrital plant
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1549 components. This analysis provides a quantitative approach to estimating the unoccluded
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1551 surface/total volume ratio for the aggregate, a parameter required when modeling oxygen
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1553 diffusion and consumption in aggregated soils (e.g.e.g., Renault and Stengel, 1994).
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1557 The analysis of anisotropy revealed that the Kansas aggregate is effectively
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1559 isotropic in terms of microstructure despite an elongated shape. In contrast, the Barrow
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1561 aggregate was anisotropic on the pore scale due to the presence of aligned pores in the
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1563 detrital plant fragment. While we did not numerically compute effective diffusivity
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1571 coefficients for the two aggregate samples, the lower isotropy index for the Barrow
1572 sample (0.763) suggest that preferential diffusion along the axis of the aligned pores
1573 could significantly impact gas and solute transport.
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1578 Figure 9 provides a graphical summary of the analysis suite displayed for a thin
1579 (~20 μm) horizontal slice in each aggregate. Each image shows the typical characteristics
1580 discussed in this section and it is possible to better understand the differences in LT,
1581 skeleton and openings in the two microaggregates. In Figure 9a the 8bit rendering of the
1582 solids is superimposed with the LT volume: the differences in porosity and pore size
1583 distributions are immediately visible, as in Figure 9b (LT volume alone). In Figure 9c the
1584 8bit volume is superimposed with the skeleton (labeled with the LT values) and the
1585 openings (labeled with a color corresponding to their diameter). It is possible to
1586 appreciate how well the skeleton fits the pore space and the role of the bigger chambers
1587 due to the presence of the plant fragments in the Barrow microaggregate. In Figure 9d the
1588 8-bit volume is removed to highlight the features of the skeletons and of the openings;
1589 we see a more complex pattern of the skeleton in the barrow samples with shorter and
1590 smaller branching linking the outside of the particle with the pore space, while the
1591 Kansas particle displays classic interstitial pore space features in both the skeleton and
1592 the openings.
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1610 A significant potential use of the detailed structural analysis presented is for the
1611 direct numerical modeling of pore-scale biogeochemical processes in microaggregates.
1612 The recent study of Ebrahimi and Or (2015) presents an elegant network modeling
1613 approach capable of capturing the boundary of aerobic activity and community
1614 partitioning within a single aggregate. The network architecture used in the modeling,
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1627 however, was a theoretical regular framework generated to match capillary
1628 pressure/matric potential data on an aggregate collection and did not contain the detail
1629 present in our direct imaging study. We believe that high quality SXR- μ CT can fill an
1630 important gap in such modeling studies by providing an appropriate network, aperture
1631 distribution, and set of boundary conditions to realistically capture biogeochemical
1632 processes at the aggregate scale. This interaction between experimentalists providing the
1633 modelers realistic starting points and validation datasets is bound to become more and
1634 more important in many fields. The new direction of building online experimental data
1635 repositories (e.g.e.g., <https://www.digitalrockportal.org/>) will also have an increasingly
1636 important role in connecting experimental and modeling groups, including of course the
1637 soil scientists community.

1655 ***Conclusions***

1659 SXR- μ CT measurements on single soil microaggregates, coupled with advanced analysis
1660 techniques have significant potential to improve the characterization of this unique
1661 microbial environment. The suite of tools we present may aid future studies seeking to
1662 correlate aggregate microstructure with microbial community structure and function. In
1663 this study two markedly different microaggregates have been analyzed and the results
1664 show how soil microstructures can be quantified and potentially linked back to biological
1665 processes. Prior work has demonstrated a direct impact for different processes such as the
1666 protection from predators (Griffiths and Young, 1994; Young and Ritz, 1998), the

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1683 distribution of nutrients (Chenu et al., 2001), or for environmental issues such as local
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1685 variations in heavy metal concentrations (Ranjard et al., 2000).
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1688 In the two samples we have shown the Kansas microaggregate, mostly inorganic
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1690 in nature, displays a typical interstitial pore space, created by the aggregation of rounded
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1692 mineral particles and aggregates. A more complex microstructure is present in the
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1694 Barrow microaggregate, with a strong organic component, discernible also from the XR
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1696 attenuation values, due to the high percentage of plant fragments. This microaggregate
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1698 shows a significantly larger amount of pore space potentially available to bacteria, and
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1700 this pore space is accessible only to small- to medium- sized microorganisms. Following
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1702 the geometrical concept alone the Barrow microaggregate provides a better environment
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1704 for the potential development of bacteria colonies, providing a larger and well protected
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1706 space to the microorganisms.
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1709 The quantitative microstructural characterization -aim of the present work-, albeit
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1711 fundamental, is only a single ~~factor~~element for a truly complete characterization of soil
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1713 microaggregates. The distribution of the chemical compounds needed for the
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1715 development of the microorganisms, and of microorganisms themselves, in the
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1717 microaggregates also play a key role and a comprehensive study about the role of
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1719 microaggregates in the development of spots of highly increased biological activity in
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1721 soils. Future improvements in X-ray imaging techniques, with both conventional and
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1723 unconventional sources, and further improvements and automation of the analysis part
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1725 will play an important role in achieving a better knowledge of the mechanisms related to
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1727 soil microaggregates, especially when coupled with techniques aimed at describing the
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1729 distribution of the different microbial communities.
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2579 **Figure Captions**
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2584 Figure 1.

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2586 Whole and vertically cut volume renderings of the Kansas and Barrow soil
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2588 microaggregates.
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2592 Figure 2.

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2594 Barrow microaggregate showing the calculated “outer surface” (displayed in red), in a
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2596 vertically cut sample with a partial covering of the surface (a), and a thin slice with the
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2598 surface following the outer border (b).
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2600
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2603 Figure 3.

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2605 The two microaggregates showing the local thickness volume superimposed to the 8bit
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2607 volume rendering and alone. Volumes are cut to better show the internal features.
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2611 Figure 4.

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2613 Frequency plots showing the distribution of (from top to bottom): local thickness voxels,
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2615 skeleton branch lengths, surface openings.
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2620 Figure 5.

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2622 Pore space accessibility from the outside for spherical structuring elements of different
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2624 sizes: .65 μm (yellow), 2 μm (green), 10 μm (red).
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2635 Figure 6.
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2637 Renderings of the skeletons, displayed superimposed both with a cut 8bit volume and
2638 alone. The skeleton is labeled with the local thickness values for each voxel.
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2644 Figure 7.

2645 Analysis of openings: the rendering on the left for the two microaggregates shows the
2646 8bit rendering with the openings marked by cubes labeled with respect their size. On the
2647 right the skeleton and the openings alone are plotted.
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2654 Figure 8.

2655 Star Length Distribution analysis for anisotropy characterization. The SLD analysis has
2656 been carried out on both the solids of the microaggregates (PF's on the left) and the
2657 whole completely filled aggregate (PF's on the right). Values are in multiples of random
2658 distribution, PF's are in equal area projection, upper hemisphere. The bottom of the
2659 figure shows the whole and horizontally sectioned microaggregates oriented as the PF's.
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2669 Figure 9.

2670 Thin horizontal slice of the two samples showing in more details and summarizing the
2671 main analyses carried out on the microaggregates.
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Table 1. Morphometric analysis.

	<i>Kansas</i>	<i>Barrow</i>
<i>Volumetric Analysis</i>		
Total Aggregate Volume [μm^3]	2.819E+06	3.619E+06
Total Volume of solids [μm^3]	1.970E+06	1.998E+06
Total Volume of voids [μm^3]	8.486E+05	1.621E+06
Porosity [%]	43.1	81.1
<i>Surface Area Analysis</i>		
Surface Area [μm^2]	3.505E+06	4.850E+06
SNSVR	11.8	13.8
<i>Minkowski Functionals and Fractal Analysis</i>		
Integral of mean curvature (solids) [μm^{-2}]	-45197.6	-21798.8
Euler Characteristic (voids) [μm^{-3}]	-312.6	-410.6
Fractal dimension 3D (voids)	2.353	2.401
<i>Local Thickness Analysis</i>		
Structure Separation Mean [μm]	3.13	5.32
Structure Separation σ [μm]	1.89	4.13
Structure Separation Max [μm]	12.77	24.74
Structure Thickness Mean [μm]	6.46	7.45
Structure Thickness σ [μm]	5.44	11.44
Structure Thickness Max [μm]	28.66	47.07
<i>Geometrical Accessibility Analysis</i>		
0.65 μm elements accessibility [% of voids]	95.5	98.0
2 μm elements accessibility [% of voids]	76.0	86.2
10 μm elements accessibility [% of voids]	0	3.2
<i>Anisotropy analysis SLD -solids-</i>		
Isotropy index (I)	0.937	0.763
Elongation index (E)	0.019	0.153

Table 2. Skeleton analysis

	<i>Kansas</i>	<i>Barrow</i>
Number of branches	54550	81012
Number of true junctions	29752	44202
Number of end points	11143	13552
Number of triple points	23113	33105
Number of quadruple points	4953	7713
Average branch length [μm]	3.41	3.40

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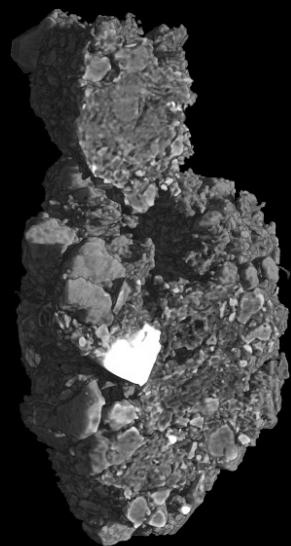
Max branch length [μm]	24.54	46.07
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3 **Highlights**
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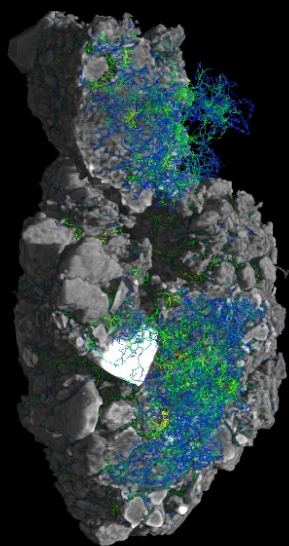
- 5 - Synchrotron X-ray tomography of whole soil aggregates at unprecedented
6 resolution.
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10 - Advanced image processing for quantitative soil microaggregates
11 characterization.
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14 - Modeling of the morphological accessibility for ideal microorganisms.
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Volume rendering



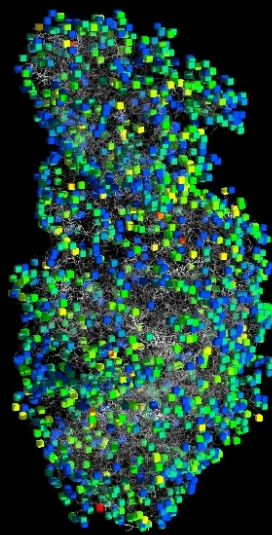
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Pores space skeleton analysis



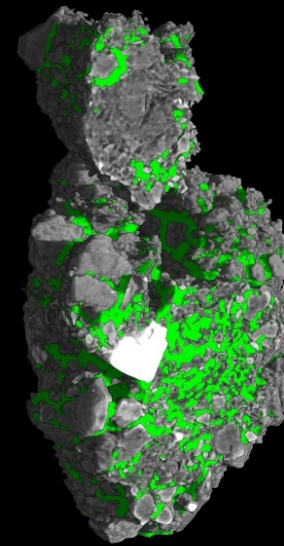
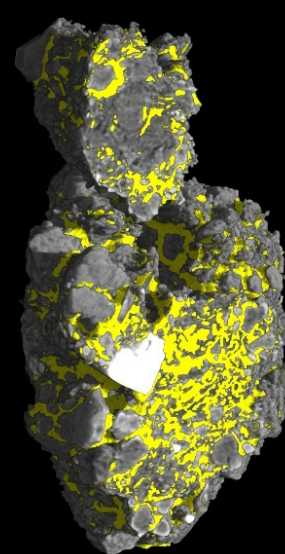
.65 [μm] 13

Openings analysis



.65 [μm] 6

Geometrical accessibility



Microorganism size:

● 0.65 μm
● 2 μm

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4 ***Quantitative characterization of soil micro-aggregates:***
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7 ***new opportunities from sub-micron resolution***
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10 ***synchrotron X-ray microtomography***
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17 Franklin¹
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25 California, Berkeley, CA 94720.
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32 ***Abstract***
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36 Soil microaggregates are the fundamental building block, at the micron scale, of the
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38 highly hierarchical structure of soils, and can exert a significant control on the local
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40 biological metabolism and microbial community partitioning. In this study we propose an
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42 analysis protocol for the morphometric characterization of complete soil microaggregates
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44 based on sub-micron resolution synchrotron X-ray microtomography. A comprehensive
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46 characterization of the aggregate morphology is the first step towards a complete
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48 characterization of the soil microaggregates, when trying to correlate morphometric
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50 parameters with physical and/or biological properties, or when building models (e.g.,
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59 effective diffusivity, microbial distribution, etc.). We demonstrate our characterization
60 approach on two single microaggregate samples from dramatically different soil
61 environments: one from Kansas, primarily composed by inorganic particles, and one
62 from Barrow (Alaska) dominated by plant fragments. A series of state-of-the-art
63 morphometric analysis techniques have been employed providing quantitative results
64 highlighting specific differences of the two samples. The role of the microstructure in a
65 scenario microbial population development has been discussed and it has been found that
66 the Barrow microaggregate seems to be more favorable, from a purely geometrical point
67 of view, as also confirmed by a simple model presented in this work. The potential of this
68 approach, when coupled with chemical and biological analysis for a fully comprehensive
69 characterization of soil aggregates in the larger picture of enhanced biological activity, is
70 evident.
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87 *Keywords: Synchrotron X-Ray microCT; Soil microaggregate; Quantitative image*
88 *analysis; Modeling.*
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93 **1. Introduction**

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98 Soil is perhaps the ultimate “complex system”, with physical, chemical and biological
99 components interacting in a non-linear manner yet displaying clear properties of co-
100 evolution and self-organization (Young and Crawford, 2004). The physical structure of
101 soil acts in a deterministic manner to regulate the assembly and activity of soil biota,
102 including microorganisms, and through their activity, soil biota continuously shape and
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115 re-form both the macroscale and microscale structure of soil that in turn poses new
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117 constraints on biological activity. This pattern of co-evolution has led to the concept of
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119 soils being considered as ‘extended composite phenotypes’, (Phillips, 2009) where soils
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121 themselves “*are an expression of the cumulative impacts of the biosphere on surface*
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123 *processes*”. As the fundamental units of soil, aggregates and their physical, chemical, and
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125 biological properties could be considered an extended soil phenotype.
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129 The aggregation properties of soil constituents lead to a classification of aggregate
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131 forms, primarily based on size and basic physical properties (e.g., stability in water, Six
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133 et al., 2000). Microaggregates, those less than 250 μm in diameter (Edwards and
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135 Bremner, 1967) are critical for the sequestration of carbon in soil (e.g., Six et al., 2000,
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137 2004, Vogel et al., 2014). Within microaggregates, pore structural properties that evolve
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139 during aggregate formation and stabilization result in organic matter being encapsulated
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141 within submicron pores (McCarthy et al., 2008) and, thus, protected from microbial
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143 decomposition due to kinetic and spatial constraints. Given its importance, the
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145 relationship between pore space geometry on the diversity and activity of soil
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147 microorganisms has been a topic of notable interest (reviewed in Or et al., 2007).
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149 Significant fractions of soil pore space can be inaccessible to soil bacteria (Chenu and
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151 Stotzky, 2002), and observations of bacteria confined within pore spaces are common
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153 (Foster et al., 1988), while predators such as protozoa can be isolated from their bacterial
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155 prey due to the interaction between pore geometry and the larger cell dimensions of
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157 protozoa (Vargas & Hattori, 1986, Wright et al., 1995).
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161 Perhaps the strongest constraints on microbial metabolism within microaggregates
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163 are those of gas, moisture, and solute transport. Diffusive gas transport in particular is
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171 limited by the aperture and connectivity of exterior pores to deeper environments as well
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173 as pore hydration state. In the near-surface, anoxic microenvironments can develop in the
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175 interior of microaggregates due to O₂ diffusion limitations as well as aerobic
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177 communities on aggregate surfaces. Due to the difficulties in measuring spatial
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179 concentrations gradients within microaggregates, many studies exploring these
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181 constraints focus on modeling approaches (Currie, 1962; Smith, 1980; Renault & Stengel.
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183 1994; Ebrahimi & Or, 2015). Currie (1962) and Smith (1980) provide 1D continuum
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185 analytical models for such processes which are strongly dependent on an averaged D_{eff},
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187 the effective gas diffusivity of the aggregate. Aggregate-specific D_{eff} values are difficult
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189 to estimate, particularly for partial saturation states where capillary-bound water impacts
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191 gas transport. Ebrahimi and Or (2015) present a pore-resolved numerical model of gas
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193 and nutrient diffusion in microbially active aggregates and demonstrate that aerobic and
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195 anaerobic microenvironments quickly develop; such spatial gradients in turn drive
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197 community partitioning, particularly in wetter environments. A common limitation of all
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199 such studies is the absence of realistically parameterized pore geometries, particularly for
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201 microbially “remodeled” aggregates which are unlikely to have homogeneous network
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203 structures as shown in prior imaging studies (e.g., Peth et al. 2008, Alba-Tercedor et al.
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205 2015).

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209 Given the importance of soil pore geometry and pore network connectivity for
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211 biodiversity and biogeochemical cycling, it is critical to develop quantitative
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213 measurement approaches to obtain soil aggregate microstructure at the relevant length
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215 scales (i.e. sub-micron). The microstructure of these fundamental soil building blocks
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217 will likely have a significant impact on the ability to host specific microorganisms; for
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227 example large pores with many small apertures can provide a safe environment for
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229 specific microorganism colonies to thrive. The direct correlation of microstructure and
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231 microorganism distribution is beyond the scope of this work, which focuses in on
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233 development of morphometric parameters and analysis strategies to obtain a
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235 comprehensive description of the microstructure of soil microaggregates. Our results do,
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237 however, provide insight into prior experimental studies examining the role of
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239 microaggregates in disrupting predation (Wright et al. 1995, Vargas and Hattori, 1986).
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242 A first attempt at correlating Synchrotron X-Ray micro-Computed Tomography
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244 (SXR- μ CT) data with microbial populations obtained via 16S rRNA pyrosequencing has
245
246 been attempted in Bailey et al. (2013), where no clear correlation of microbial
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248 populations with microstructure has been found. While that work is valid, the lack of an
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250 advanced analysis of the SXR- μ CT data (only proxies of pore size distributions and
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252 surface areas have been considered as descriptors for the microstructure) might have
253
254 neglected important hints that could have been successful in finding an eventual
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256 correlation. In this context, we are offering an example of a state-of-the-art measurement
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258 and data analysis that can be used for a complete characterization of *whole* (in contrast
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260 with cropped subvolumes, as usually found in literature) soil microaggregates at the
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262 nanoscale, providing the soil scientist community a series of tools that can be used in
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264 future works where a comprehensive *quantitative* characterization of soil
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266 microaggregates is needed.
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269 XR-CT (both using conventional and unconventional radiation sources, at
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271 different scales) has been successfully used for soil characterization in the past, given its
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273 ability to provide 3D volumes describing the structure soil samples. One of the main
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283 advantages of this technique is its non-destructive nature, which allows the observation of
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285 undisturbed samples, such as roots in soils (Heerman et al., 1997; Mooney et al., 2013),
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287 macropores in large (~20 cm) soil cores (Pierret et al., 2002), and characterization of pore
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289 space in ~3 mm aggregates (Nunan et al., 2006; Peth et al., 2008). More specific works,
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291 focusing on the characterization of the pore networks in soils, at different scales, can be
292
293 found in Petrovic et al. (1982), and Anderson et al. (1990) where the main focus was the
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295 estimate of the density of the soil samples. At the mm scale, XR-CT has also been used to
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297 locate layering in soil samples as presented by Macedo et al. (1998). Other studies
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299 included porosity and flow properties in measured soils, as presented in Peyton et al.
300
301 (1992), Heijs et al. (1995), Clausnitzer and Hopmans (2000). Fractal properties in soils
302
303 have been studied although their calculation and practical implications have been
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305 somewhat controversial. Examples can be found in: Perfect et al., (1992), McBratney
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307 (1993), Peyton et al., (1994), Anderson and McBratney (2005), Gimenez et al., (1997),
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309 Gibson et al. (2006).

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313 In this work we present state-of-the-art data processing and morphometric analysis of two
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315 different soil microaggregates (slightly smaller than 250 μm) to quantify microstructural
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317 attributes. The purpose is to show an analytical protocol that can be used to describe the
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319 microstructure of the soil microaggregates in a quantitative fashion, and to provide the
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321 scientific community (e.g., modelers) with datasets of *complete* microaggregate particles
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323 with sub-micron resolution to be used as a realistic starting point and/or validating dataset
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325 for synthetic soil models. To validate this approach, we have chosen two markedly
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327 different soil microaggregates, both in composition and texture, the first rich in inorganic
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329 material and the second rich in organic material (plant fragments) to better monitor which
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339 parameters can describe those differences. This quantitative description of the soil
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341 microaggregates is fundamental for a complete study complementing the role of the soil
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343 microaggregates microstructure, chemical heterogeneities, and microorganism
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345 distributions in the research about the origin and development of “hot spots” in soils, and
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347 to contribute to our understanding of the functional stability and spatial variability within
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349 soils in general.
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351 352 353 354 355 356 **2. Materials and Methods** 357

358 359 360 *2.1 Sample choice and preparation* 361

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365 Two distinct soils were selected for our study: one from the Konza Prairie Biological
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367 Station, Kansas, and a second from Barrow Experimental Observatory (BEO), Alaska.
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369 The two samples selected for this evaluation varied in bulk density, organic matter
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371 content, and mineralogy, among other parameters. Microaggregates were obtained via
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373 manual dissection under a microscope, where the sorting was based on size (200 to 250
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375 μm diameter), and on the ability to remain coherent under mechanical stimulation.
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377 Preparation of the aggregates for analysis involved inserting the microaggregates into a
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379 thin walled 300 μm borosilicate glass capillary (Charles Supper); the capillary tubes were
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381 immediately sealed using hard wax to maintain the original humidity as much as possible.
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383 The capillary tube was used to assist sample mounting and to maintain sample integrity at
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385 proper moisture conditions. Sealing and acclimation (~1 hour) at the synchrotron
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395 beamline experimental hutch prior to the experiment is fundamental to keep the soil
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397 aggregate at proper conditions and avoid motion artifacts due to variations in humidity
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399 during the scan. The very high resolution of the measurement makes the dataset prone to
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401 motion artifacts, so a nearly perfectly stable sample was a necessity.
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404 405 406 *2.2 SXR- μ CT measurement and data reconstruction* 407

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410 The SXR- μ CT experiments were carried out at X-ray Imaging Beamline 8.3.2 (BL 8.3.2)
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412 at the Advanced Light Source (ALS) at the Lawrence Berkeley National Laboratory
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414 (LBNL). For the tomographic measurement, a 11 keV monochromatic beam was selected
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416 via a multilayer monochromator. The glass capillary containing the individual aggregates
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418 was mounted on a rotating stage, ~ 8 mm from the scintillator of the detector system; a
419
420 short distance was selected to avoid strong phase-contrast effects due to free space
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422 propagation of the highly coherent XR beam. The detector system consisted of a LuAG
423
424 20 μ m thick scintillator mounted in front of a 20 \times objective lens; the visible light signal
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426 obtained from the scintillator was recorded on a CCD camera (Optique Peter, Lentilly,
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428 France) obtaining a dataset with a resulting voxel size of 325 nm. 1800 projections were
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430 recorded for each sample over a 180 $^\circ$ rotation, and the exposure time was 1 second per
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432 projection.
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436 Before reconstruction of tomographic 2D slices, a single-distance phase retrieval
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438 algorithm was applied to the projection datasets. Phase-retrieval is beneficial in this kind
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440 of datasets since it improves the contrast between the different phases and makes the
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442 phase-contrast artifacts less pronounced, both these characteristics are of great help with
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451 the segmentation via thresholding process. After a conventional flat-field correction of
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453 the single projection, we applied the Paganin single-distance phase retrieval algorithm
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455 (Paganin et al, 2002) as implemented in the ImageJ plugin ANKAPhase (Weitkamp et al.,
456
457 2011), which proved to be effective (e.g., Arzilli et al., 2015) with datasets collected in
458
459 the near-field Fresnel diffraction region (Bronnikov, 2002). After phase-retrieval the slices
460
461 were reconstructed with a conventional filtered back-projection algorithm (Kak and
462
463 Slaney, 1987).
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465
466 Once the stacks of slices were reconstructed, the datasets were manually cropped
467
468 to isolate the soil microaggregate, separating it from the glass capillary, to obtain an 8 bit
469
470 grayscale volume used as a starting point for subsequent analysis. The volume rendering
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472 of the two particles, whole and with a virtual vertical cut to show the internal structure,
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474 are shown in Figure 1. It is immediately possible to qualitatively appreciate the
475
476 microstructural differences and identify in the “Barrow” microaggregate a large amount
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478 of material originating from plant debris, visible as a cellular texture with large interior
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480 pores (right panel). In contrast, the microaggregate obtained from the Kansas prairie site
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482 (left panel) appears to be an aggregate of mineral particles with smaller interior pore
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484 spaces, displaying a more interstitial type of pore space.
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489 *2.3 Data treatment and separation*

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494 To allow analysis of pore-space morphologies, the first step in our analytical approach is
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496 segmentation, aimed at separating the phases of interest into binary volumes. A simple
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498 manual thresholding procedure was possible due to the strong contrast between the solid
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507 and voids voxels in the samples obtained after the phase retrieval procedure. However,
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509 the analysis of microaggregates requires a deviation from classical approaches for
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511 segmentation commonly applied to XR-CT datasets. In most situations, the image
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513 volume is cropped and only a subsection of the system is segmented and analyzed. In our
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515 case we wanted to capture the entire particle and determine the properties of the
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517 aggregate exterior surface. This boundary forms the interface between the aggregate and
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519 the outside world and is key to imposing boundary conditions when looking for
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521 interactions of the outside with the microaggregate pore space, for example in gas
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523 transport modeling, predation, etc. This approach comes with a challenge, mainly the
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525 problem of separating the air outside the microaggregate from the air in the voids inside
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527 the sample despite the fact that the phases are continuous. This cannot be done via
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529 thresholding procedures, since the air inside and outside the sample has of course the
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531 same XR attenuation values (translated into the same grayscale value, in the tomographic
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533 dataset), thus the necessity of morphology-based methods.
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537 We have developed an iterative procedure using the Fiji software framework
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539 (Schindelin et al., 2012) that allows the calculation of such an external surface and
540
541 provides a separation of the pore space from the air outside the sample. This procedure
542
543 can produce results similar in concept to the snake-based “active contour” segmentation
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545 algorithms used in the medical imaging field (e.g., Yushkevich et al., 2006). The
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547 procedure we applied works well in 3D and can be applied to systems where large objects
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549 need to be extracted and segmented. The procedure consists of a series of operations
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551 involving local thickness (LT) analysis of the voids using the plugin described in
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553 Dougherty and Kunzelmann (2007). LT analysis is used to obtain a first, rough, outer
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563 surface and a closing procedure is used to close the smaller outer pores. 3D void filling is
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565 then used to fill the largest internal voids, and finally masking is utilized to preserve the
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567 small features that are commonly erased by the morphological operators such as eroding
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569 and dilating processes. The filled soil aggregate particle obtained is then used to obtain a
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571 “sample surface” again through morphological operators of the binary erosion/dilation
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573 class coupled with Boolean operations. This outer surface obtained with the procedure
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575 described above is shown in Figure 2 rendered in red and superimposed on a rendering of
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577 the Barrow sample. The virtual cuts reveal how the surface calculation effectively
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579 “wraps” the aggregate exterior without penetrating into the interior pore spaces, thus
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581 providing information on the interface between the aggregate and the surrounding
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583 environment. The procedure of calculating a surface separating the pore space from the
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585 outer air is the starting point for subsequent analyses on the two datasets.
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593 ***Results***

594 595 596 597 *3.1 Morphometric analysis: Basic analysis*

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601 The procedure described above generates two base binary volumes for the morphometric
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603 analysis: a volume for inner voids (the pore space) and a volume of the solid phase. This
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605 allows a variety of analytical techniques to be carried out in order to obtain quantitative
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607 information describing the two different phases. We stress that all the morphometric
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609 analysis needs to be considered with the actual resolution of the measurement in mind;
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619 some values (such as surface area values) are very sensitive to image resolution and the
620 value listed must be read with this intrinsic limitation in mind. For SXR- μ CT analysis, a
621 value listed must be read with this intrinsic limitation in mind. For SXR- μ CT analysis, a
622 value listed must be read with this intrinsic limitation in mind. For SXR- μ CT analysis, a
623 value listed must be read with this intrinsic limitation in mind. For SXR- μ CT analysis, a
624 compromise between field of view and resolution needs to be made; we have chosen the
625 setup with the ability to image the whole soil microaggregate with the highest resolution
626 possible. With the chosen setup the resolution was very high, especially when compared
627 to similar measurements presented in literature, but still without the ability to resolve the
628 smallest features (e.g., single clay minerals platelets). We present a comparison of a
629 range of classical morphometric metrics applied to our two samples, including porosity,
630 surface area, sphere-normalized surface-area-to-volume-ratio, Minkowski functionals,
631 and fractal dimension metrics; such metrics provide a first step in grouping aggregate
632 properties.
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643 The first analysis carried out was to calculate the basic properties of the
644 aggregates, such as their volumes and porosities. In Table 1, a summary of the
645 morphometric analyses carried out on the two samples is listed. From the volumetric
646 analysis, we can see how the Barrow sample is ~22% larger in volume than the Kansas
647 one. The cellular texture and interior pores seen in the Barrow sample results in a high
648 porosity (81%) while the Kansas aggregate has a porosity more typical of granular
649 composites (43%). The surface area (SA) calculation also shows a higher value for the
650 Barrow sample but the impact of sample size makes such a non-normalized SA
651 problematic to interpret. To overcome this problem a sphere-normalized surface-to-area
652 volume ratio (SNSVR) was calculated with SNSVR defined as SA_{obj}/SA_{sph} . SA_{obj} is the
653 object surface area, and SA_{sph} is the surface area of a sphere with the same volume of the
654 object. The farther the object surface is from a spherical surface, the higher the SNSVR
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675 value (1 being the value for a perfect sphere). After this correction, the Barrow sample
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677 still shows a larger area available per volume units, but both the microaggregate particles
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679 display values related to a markedly complex surface.
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682 The Minkowski functionals (see e.g., Ohser and Muecklich, 2000) can be used as
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684 topological descriptors of a binarized volume. More specifically the integral mean
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686 curvature (IMC) (Russ and DeHoff, 2012) is a value related to the concavity/convexity
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688 (depending on the sign of the IMC) of the surfaces present in the sample. The Euler
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690 characteristic (EC) (Odgaard and Gundersen, 1996) is a value related to the connectivity
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692 of the objects in the volume: positive values mean isolated objects, while connected
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694 networks generally display negative values. The values measured for the IMC of the
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696 framework of solids and the EC of the pore space highlight a highly connected network
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698 dominated by concave shapes of the solid framework, with the Barrow sample being
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700 slightly more interconnected and with a slightly weaker dominance of convex surfaces.
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703 Another frequently used parameter for texture analysis is the fractal dimension
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705 (FD), which has been applied to the solids in the aggregate, providing an index of self-
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707 similarity of the microstructural features at different scales. The FD has been calculated
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709 using the box-counting method (Liebovitch and Toth, 1989) and values obtained show
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711 that the Barrow aggregate (FD = 2.401) is slightly more self-similar than Kansas sample
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713 (FD = 2.353). Both values are relatively low (for volume data $2 \leq \text{FD} \leq 3$) highlighting
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715 moderate fractal properties, at the considered resolution and sample size. Prior studies
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717 examining the fractal dimension of micro-aggregates using destructive mass/radius
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719 analysis have yielded slightly higher values (FD = 2.75 to 2.93, Young & Crawford,
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731 1991), the differences in sample origin and measurement approach make comparisons
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733 challenging.
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735 736 737 738 *3.2 Local thickness (LT) analysis* 739

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742 As discussed in section 2.3, LT analysis is an extremely useful approach for porous
743 material characterization borrowed from the bone scientist community, where it is
744 generally used for the analysis of cancellous bone, measuring parameters such as the
745 “trabecular thickness” and “trabecular separation” (Parfitt et al., 1987; Simmons and
746 Hipp, 1997; Accardo et al., 2005). The same algorithm has been later adopted in different
747 contexts for example as part of multiphase flow modeling approaches by Silin et al.
748 (2010) under the name “maximum inscribed spheres” and is finding increased use in
749 modeling of geologic samples. The term “local thickness” for a voxel is used to mean the
750 diameter of the maximum inscribed sphere in the structure that contains the voxel. In
751 Table 1., where the LT results are summarized, we use more generic terms, where
752 “structure separation” is the LT analysis calculated on the pore space volume, while with
753 “structure thickness” we mean the LT analysis carried out on the solid framework. The
754 analysis has been performed using the Fiji plugin from Dougherty and Kunzelmann
755 (2007), and graphical results are shown in Figure 3 for both the migroaggregates.
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772 In Figure 3 a vertically cut rendering is superimposed with the LT-labeled volume
773 of the pore space, and a volume rendering of the cut LT volume itself. The image clearly
774 displays the thickness variations of the pore space within the volume, with the Kansas
775 sample showing interstitial voids created by the aggregation of the silt particles, while in
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787 Barrow the larger, smoother, voids due to the presence the plant fragments and their
788 cellular structures are evident. A larger variation of LT values is also immediately
789 observable from the renderings in figure. A quantification of the LT analysis was
790 performed and the results are presented again in Table 1. The mean of the structure
791 separation (mean LT of the pore space) is larger in the Barrow sample, but also the
792 standard deviation is significantly larger and the maximum LT value of voids is present
793 in the Barrow sample as well, this again because of the presence of the plant fragments
794 with large voids surrounded by small ones. This variability is recognizable when looking
795 at more detailed data than the one summarized in the Table 1. In Figure 4 we show a plot
796 of the LT values distribution in the pore space for both Kansas and barrow. The higher
797 variability suggested by the summarizing values here becomes even more evident with
798 the two distributions being markedly different, with values of higher LT values being
799 more frequent in the Barrow sample. The smallest LT values are also more frequent in
800 the Barrow sample, while the small LT values are generally more frequent in the Kansas
801 microaggregate, showing a sharper LT distribution curve. This feature and its
802 implications are discussed in section 4.

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821 Concerning the structure LT analysis (“structure thickness”) values for Barrow
822 are generally slightly larger, with a much larger standard deviation value and especially a
823 larger maximum. This because in the Barrow sample a ~50 μm large single mineral
824 particle is present (top of the sample).

825 826 827 828 829 830 831 832 *3.3 Geometrical accessibility analysis*

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843 The concept of LT, combined with the outer surface calculated via the procedure
844 described above, and the connected component labeling (CCL, see e.g., Hu et al. 2005)
845 can be used to calculate the parts of the sample accessible from the outside by objects
846 with different sizes. This class of analysis has obvious applications as a simple model
847 able to provide insights about pore-size constrained microbial colonization of aggregates,
848 as well as the spatial limits of predation by larger organisms. The procedure is
849 straightforward: a threshold value (corresponding to the size of the structuring element -
850 spherical- considered for accessibility) is applied to the LT volume. A 5 voxel thick outer
851 surface is added to the volume and a CCL procedure is initiated starting from the outer
852 surface; this will find all the parts of the sample accessible from the outside. The outer
853 surface is then removed using a masking procedure with the pore space binary volume.
854 The volume left is the pore space geometrically accessible from the outside by a spherical
855 element with the value corresponding to the threshold value used.
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871 This procedure can be used to detect the parts of the pore space theoretically
872 accessible to microorganisms of known size, using only geometric parameters. This
873 isolates the volumes of the samples accessible by the different structuring elements from
874 the outside, along pathways with throats larger than the structuring element. Although
875 this approximation is based solely on geometry, such calculations allow to obtain some
876 information about which parts of the sample could be accessible to different classes of
877 microorganisms, segmented by their characteristic size. Bearing in mind the resolution of
878 the measurements, we considered three different classes of microorganisms based on
879 their size: 0.65 μm for average microbial cells, 2 μm for large microbes, and 10 μm for
880 protozoa. Aggregate microarchitecture can be expected to influence the distribution and
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899 activity of microorganisms, for example microaggregates possessing large internal
900 chambers with entrances small enough to selectively exclude larger competitors and/or
901 predators would represent potential activity hotspots and refuges for select portions of the
902 community. Results of the analysis are summarized again in Table 1. From this set of
903 calculations, it is possible to see that both microaggregate samples are close to totally
904 accessible to objects .65 μm large, while 2 μm structuring elements can still enter the
905 majority of the pore-space in both the Barrow (86%) and Kansas (76%) samples. The
906 largest (10 μm) structuring element, the size of small protozoa, cannot enter any of the
907 pore space of the Kansas, inorganic-rich, microaggregate, while a small part of the
908 sample (3%) is accessible in the Barrow one.

920 The potentially accessible parts of the pore space are displayed, superimposed to
921 the 8bit volume rendering for all the particles in Figure 5. In this figure it is possible to
922 observe the parts of the sample accessible to microbes of different sizes; for example the
923 top lobe of the Kansas sample can be fully colonized by .65 μm microorganisms but has
924 limited pore-space available to 2 μm microbes. Neither aggregate, with the exception of a
925 single large pore in the Barrow sample, has a pores/throats system of sufficient size to
926 accommodate 10 μm microorganisms, suggesting that both microaggregates could
927 provide protection to internal communities from predation by protozoa. Previous
928 microcosm studies have demonstrated that aggregate microstructure protects microbial
929 communities from such predation (e.g., Vargas and Hattori, 1986, Wright et al. 1995) but
930 prior studies could not characterize the internal aggregate structure which confers this
931 effect.

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955 *3.4 Skeleton analysis*
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959 Another important descriptor of pore network topology is the pore space skeleton. A
960 skeleton is a 1D topological descriptor of the 3D pore space which captures network
961 connectivity in a simplified form, suitable for discrete models of flow and transport. In
962 this study we used the “thinning” algorithm to efficiently compute the medial axes of
963 both aggregate pore spaces (Lee et al., 1994; Lindquist et al., 1996; Palágyi and Kuba,
964 1999). The skeleton of the pore space of the connected network was calculated and the
965 skeleton voxels were also labeled to identify branches, joints and end points in the
966 framework.
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976 The results of the skeleton analysis are summarized in Table 2. The network
977 statistics indicates that the Barrow sample has the greater pore network complexity; this
978 sample displays a significantly larger number of branches and junctions while the number
979 of end points and the average branch length are very similar. This feature highlights a
980 similar, basic, accessibility from the outside to two networks markedly different in
981 complexity. This topic will be further analyzed and discussed, when another concept of
982 “accessibility”, based on the analysis of the size of the openings of the microaggregates
983 facing the outside will be introduced. In Figure 4 a frequency distribution plot of the
984 branch lengths in the two microaggregates is shown: the Barrow sample displays a wider
985 distribution, with a larger number of the smallest and larger branch lengths, while the
986 Kansas sample has a larger amount of the smaller (but not the smallest) branches. This,
987 again, highlights the differences of the two pore network topologies, with the Barrow
988 being the more complex.
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1012 The renderings of the two samples with the calculated thinning skeletons, labeled
1013 with respect to the LT value of each voxel to introduce pore diameter information in the
1014 skeleton, are shown in Figure 6. From the colors of the skeleton it is possible to see how
1015 in the Barrow sample the blue colors (extremely small values of LT) and the hotter colors
1016 (higher values of LT) are more frequent than in the Kansas sample, where small and
1017 moderately small (blue and greens) values are visibly more frequent. This is in
1018 accordance with the LT analysis discussed previously.
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1026 The network renderings also provide a qualitative representation of the
1027 differences in skeleton architecture between the two samples; the Kansas sample exhibits
1028 an interstitial skeleton, typical of the pore space generated from granular materials. In
1029 contrast, the Barrow sample shows more complicated structures, including components
1030 with many short branches and small LT values as well as parts with single long branches
1031 following the medial axes of the largest structures. These features appear to be generated
1032 by the interconnected cellular texture of the plant fragments incorporated into the
1033 aggregate.
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1045 *3.5 The interface to the outer world: openings analysis*

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1049 The outer surface of the aggregate is a critical interface linking it to the exterior
1050 environment, mediating gas and solute transport as well as microbial colonization. A key
1051 numerical task is, thus, extracting the apertures which exist on the aggregate exterior;
1052 these components are the required boundary condition for pore-scale modeling of
1053 diffusion and reaction within the single microaggregate system. As discussed in previous
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1067 sections, our processing flow has already extracted (a) the exterior bounding surface and
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1069 (b) the skeleton and LT map for the aggregate pore space. By selecting the skeleton end-
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1071 points which terminate within close proximity (5 voxel lengths) of the outer surface and
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1073 then labeling them with the LT thickness we can generate a map of aggregate openings
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1075 with the appropriate dimensions. The lower panel of figure 4 shows a histogram of the
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1077 size of open pores on the aggregate surface for both samples considered. As can be seen,
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1079 the slopes of the opening size distribution curves are markedly different, being the
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1081 Barrow one steeper, highlighting a higher amount of small apertures, and a higher small-
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1083 to-large apertures ratio. This is again a difference due to the Kansas being an aggregate of
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1085 particles with an interstitial kind of pore space, while the Barrow is made of mainly small
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1087 plant fragments with very small openings with a the few extremely large ones, where the
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1089 biggest sects of the plant structure are broken and exposed to the surface. This results in
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1091 the markedly different distribution of the openings.
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1095 Figure 7 provides a more graphical representation of the opening calculations
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1097 showing grayscale volume renderings of the two microaggregates with opening pores
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1099 marked in color. The paired figures show the same openings superimposed to the internal
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1101 network structure (the skeleton in white). As can be seen, both samples are dominated by
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1103 small exterior pores with the Barrow sample having smaller opening dimensions,
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1105 indicated by the cooler colors (blue). The large exterior pores (orange/red) are relatively
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1107 rare features on both aggregate surfaces.
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1112 *3.6 Anisotropy analysis*

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1123 Soil microaggregates can be formed by different components with varying shapes
1124 including rounded mineral particles, clay platelets, and fibrous/cellular organic materials
1125 as well as bioproducts. The shape of these constituent materials can control the shape of
1126 the microaggregate and the presence of anisotropic components can influence soil
1127 aggregate properties (e.g., Emerson, 1959). Considering that existing continuum models
1128 of gas diffusion in aggregates assume isotropic effective diffusivities, detection of strong
1129 pore-space anisotropy is a useful tool for evaluating the applicability of such models.
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1138 Fabric anisotropy can be measured directly from high quality tomographic
1139 datasets. One such approach is described in Voltolini et al. (2011), however it requires
1140 the separation of each single object in the dataset, a difficult constraint for aggregate
1141 characterization. When interior object separation is not possible, different approaches can
1142 be used. The most widely utilized technique is the mean intercept length (MIL) method
1143 (Witthehouse, 1974), but this shows some limitations since it ideally requires spherical
1144 cropping and is prone to artifacts in datasets with objects described by small numbers of
1145 voxels. To minimize these issues we decided to use the star length distribution (SLD)
1146 method (Odgaard et al., 1997), which measures the mean object lengths for all
1147 orientations, using the implementation present in Quant3D (Ketcham and Ryan, 2004) for
1148 the first calculation, finally a series of Matlab[®] scripts based on the MTEX toolbox
1149 (Bachmann et al., 2010) were used for data handling, corrections, normalization and
1150 plotting.
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1166 To study the anisotropy of the two microaggregates we applied the SLD method
1167 to the solids and on the filled shape of the whole microaggregates. The former provides
1168 the quantification of the anisotropy of the internal microaggregate structure (solids),
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1179 while the latter describes the anisotropy of the shape of the microaggregate itself. A
1180 comparison of the two pole figures (PFs) obtained from this analysis highlights any
1181 relationship of the microstructure with the shape of the microaggregate. In Figure 8 the
1182 PFs describing the Kansas and Barrow microaggregates for the solids and the whole
1183 microaggregates are plotted. In the Kansas sample (top) it can be seen that the structure is
1184 made by isotropic/randomly oriented components, since the PF displays values extremely
1185 close to one along all directions. Values in PFs are in multiples of random distribution
1186 (m.r.d.), where 1 is the value of a perfectly isotropic object and higher values towards $+\infty$
1187 imply progressively stronger anisotropy. The orientation space with values <1 imply an
1188 orientation density smaller than a random (i.e. isotropic, uniform) distribution, and in
1189 addition to the maximum value, the minimum value in the PFs is an important parameter
1190 as well, representing the percentage of objects in the sample that can build a random
1191 distribution, thus giving additional information about the sharpness of the texture. The PF
1192 for the Kansas whole microaggregate denotes that some anisotropy is present; this is
1193 clearly visible from the renderings where the shape of the particle is elongated vertically
1194 and slightly flat, similarly to a 3-axes ellipsoid. This result shows that even if the
1195 microaggregate particle is elongated, there is no internal anisotropy present, in its
1196 components. This result is in line with the qualitative observation of the microaggregate
1197 constituents, where many rounded silt particles are recognizable.

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1220 The Barrow microaggregate is different; the PF of the whole particle suggests a
1221 slightly platy morphology, but the PF of the microstructure of the solids clearly shows a
1222 more fiber-like texture with the elongation axis in the platelet plane. The texture is weak
1223 (max at 1.25 m.r.d.), but anisotropy is clearly present. Relationships between PFs and the
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1235 sample can be seen at the bottom of Figure 8 where the renderings of the soil
1236 microaggregates, with the same orientation as the PFs are displayed. The virtual cut plane
1237 of the aggregates corresponds to the plane of the PFs as well. This analysis confirms that
1238 an accurate quantification of the anisotropy in single soil microaggregates is achievable
1239 using the methods described above.
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1250 *Discussion*

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1254 Two soil microaggregates of different origins and internal structures have been analyzed
1255 via sub-micron resolution SXR- μ CT. A variety of techniques to analyze different
1256 microstructural parameters have been applied to provide a description of the different
1257 features of the aggregate microarchitecture in a descriptive fashion. This class of
1258 approaches are increasingly used in analysis of soil systems (e.g., De Gryze et al., 2006;
1259 Peth et al., 2008a,b; Zhou et al., 2013; Ma et al., 2015; Peth et al., 2015). The potential
1260 flexibility of the resolution/FOV ratio, with both conventional and unconventional X-ray
1261 sources, allows scans within a large range of scales (Sleutel et al., 2008). Synchrotron
1262 radiation, given its high flux, monochromaticity, and spatial coherence, has been
1263 recognized as a very important tool for the soil scientist since the first 3D imaging
1264 beamlines were developed (Spanne et al., 1994). Microtomographic data are also used for
1265 modeling more complex physical properties of soils and rocks, such as the Lattice
1266 Boltzmann approach for evaluating permeability (Menon et al., 2011; Khan et al., 2012)
1267 or direct numerical simulations of pore-scale reactive chemistry (Molins et al. 2012).
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1291 In this work we focus mainly on the geometrical differences of single
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1293 microaggregates and exploit the high flux and resolution of BL 8.3.2 to provide a more
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1295 detailed structural description. The new tools make it possible to develop the analysis of
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1297 the entirety of microaggregates at sub-micron resolution, overcoming the need to crop
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1299 subvolumes. This is of great importance since we were able to study the interface of the
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1301 single microaggregates with the external world, whereas cropped volumes would make
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1303 this kind of study impossible, and would not provide correct boundary conditions when
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1305 used for modeling.
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1308 The size of the two microaggregate studied is similar. From the analysis of the
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1310 internal porosity, it is clear that the microaggregate richer in organic matter (Barrow) is
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1312 about twice as porous as the predominantly inorganic one (Kansas). This increased
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1314 porosity appears to be the result of large pores with a cellular texture, as visible in Figure
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1316 2b. While a statistically valid generalization from our small sample set is not possible, the
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1318 results from the Barrow aggregate suggest that detrital plant matter has an important role
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1320 in controlling internal porosity. Prior studies of microaggregate structure have noted that
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1322 formation often initiates around a “core” of plant debris (Oades and Waters 1991,
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1324 Golchin et al. 1994); our results suggest that this core material may also provide a unique
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1326 structural environment for microbial activity distinct from microaggregates which are
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1328 primarily granular in texture. It is important to remark that in this work we are only
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1330 comparing two aggregates with different texture and composition to find the
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1332 morphometric parameters that would better describe those differences, and the links of
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1334 those parameters with specific properties of the aggregate; we are not comparing two
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1336 specific environments. The latter task would require a much larger number of samples,
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1347 and likely a different approach, combining multi-resolution measurements, where the
1348 large FOV would be used to identify the different type/classes of microaggregates present
1349 in each locale (and quantify their distribution), while the high resolution would target the
1350 specific single microaggregates (as shown in this work), representative for each class, to
1351 fully characterize them. Such an approach would likely allow reaching an acceptable
1352 statistical meaningfulness for each site, as needed to take into account some of the intra-
1353 site variability of microaggregates, but without measuring an unrealistically high number
1354 of single microaggregates and run a full analysis on all of them.
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1364 The internal aggregate surface area is another important parameter due to both its
1365 role in reaction kinetics as well as a microbial growth substrate. Concerning the surface
1366 area of the two microaggregates, the Barrow sample shows a larger surface area with a
1367 slightly higher complexity than the Kansas sample. This is likely due to the rough surface
1368 present in the Kansas sample, composed mainly of poorly sorted silt/clay particles, and
1369 the smoother surfaces present in the Barrow sample. However, the complexity of the
1370 pores space in the Barrow sample ultimately generates a higher surface area per volume.
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1379 The Minkowski functionals, as expected, describe a complex structure with a
1380 tightly interconnected pore space. This is also confirmed by the skeleton analysis,
1381 highlighting again the extensive complexity of the soil microaggregates at this scale. The
1382 fractal analysis shows a moderate fractal behavior for both the microaggregates, with no
1383 significant differences between the inorganic- and organic- based particles. Independent
1384 of use as a complexity measure, the fractal dimension of aggregates has been linked to
1385 measures of erodability in past studies (e.g., Ahmadi et al. 2011), hence it may such
1386 measurements may provide insight to microaggregate evolution over time.
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1403 The LT analysis discussed previously provides detailed information on pore space
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1405 statistics across the aggregate; the distribution of voxel LT values provides useful
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1407 statistical constraints including the aperture variance, a key parameter in stochastic
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1409 network models of soil structure which is often guessed at. The LT distribution in the
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1411 Kansas sample is clearly sharper than the Barrow aggregate, which exhibits a higher
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1413 variance in pore sizes and apertures. This statistical difference is due to the plant
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1415 fragments: the large voids present in these structures, coupled with small voids generated
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1417 by clay particle aggregation, generated a broader distribution of pores. These
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1419 observations suggest that the Barrow microaggregate might provide a better host for
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1421 microbial activity due to the combination of a large internal porosity and a broad size
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1423 distribution of internal microenvironments.
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1426 The novel strategy for soil particles analysis presented also allows the calculation
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1428 of pore space accessibility metrics for single aggregates, potentially a key control on
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1430 protection of aggregate microbial communities from predation by larger organisms (e.g.,
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1432 protozoa). It is worth remarking that this is a theoretical accessibility based purely on the
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1434 geometry of the pore space: features such as characteristic microorganism shape,
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1436 biological needs, reproduction rates, etc. are not considered. The resulting accessibility
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1438 metrics assume a rigid spherical body to determine the ideal potential access. We have
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1440 chosen three different sizes, compatible with characteristic organism sizes and image
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1442 resolution. First we have performed the accessibility analysis for objects .65 μm large, a
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1444 typical mid-size bacterial cell. The theoretical accessibility for a virtual microorganism of
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1446 this size from outside the sample is very large: 95% for the Kansas sample and 98% for
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1448 the Barrow aggregate. This is not surprising since this value is close to the resolution of
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1459 the measurement and the pore space is strongly interconnected. More interesting is the
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1461 accessibility for objects with the size comparable to large bacteria strains: 2 μm . The
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1463 differences here are more marked since some parts of the Kansas sample, more complex
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1465 and with small, not well connected, pores are present and therefore they are non-
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1467 accessible to the 2 μm virtual bacteria. In the Barrow sample the accessibility is still very
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1469 high (86%, compared with the 76% of Kansas), this is due to the fact that the size of the
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1471 object is still smaller than the size of a significant number of openings on the surface and
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1473 because of the very high connectivity (and throat sizes) of the pore space, allowing the
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1475 objects to move rather freely once entered the microaggregate pore space.
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1479 As a last test, we considered objects 10 μm large: this is the size of small
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1481 protozoa, an active bacterial predator. Prior experimental studies (Wright et al. 1995)
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1483 have utilized even larger protozoa with mean sizes in the 20-30 μm range (*C. steinii*) to
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1485 study the protective nature of aggregates. A microaggregate largely accessible to
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1487 protozoa would be potentially unsafe for the internal microbial community. In the two
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1489 microaggregates examined, the virtual protozoa cannot enter any pore space in the
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1491 Kansas sample, and can only enter a small single portion of the pore space in the Barrow
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1493 one, highlighting how these microaggregates can in theory provide a protective
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1495 environment for bacterial communities. Our imaging study is largely consistent with prior
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1497 experiments documenting this phenomenon (Vargas and Hattori, 1986, Wright et al.
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1499 1995). Given the protective role of microaggregates and the availability of a diverse set
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1501 of associated microenvironments (Ranjard and Richaume, 2001), the role of aggregate
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1503 pore morphology in controlling community structure might provide a promising path
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1515 towards understanding the biogeochemical response of such systems (Remenant et al.
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1517 2009).

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1519 In addition to bulk accessibility metrics, we quantified the aperture dimensions of
1520 the outer surface of each aggregate sample, a metric useful in defining outer boundary
1521 conditions and flux limitations for gas transport. In the Kansas sample the exterior
1522 aperture sizes are generally larger than the Barrow sample as can be seen in the color map
1523 used in Figure 7. The Barrow sample does however have a small number of large open
1524 exterior pores generated by open tubular structures present in the detrital plant
1525 components. This analysis provides a quantitative approach to estimating the unoccluded
1526 surface/total volume ratio for the aggregate, a parameter required when modeling oxygen
1527 diffusion and consumption in aggregated soils (e.g., Renault and Stengel, 1994).
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1539 The analysis of anisotropy revealed that the Kansas aggregate is effectively
1540 isotropic in terms of microstructure despite an elongated shape. In contrast, the Barrow
1541 aggregate was anisotropic on the pore scale due to the presence of aligned pores in the
1542 detrital plant fragment. While we did not numerically compute effective diffusivity
1543 coefficients for the two aggregate samples, the lower isotropy index for the Barrow
1544 sample (0.763) suggest that preferential diffusion along the axis of the aligned pores
1545 could significantly impact gas and solute transport.
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1554 Figure 9 provides a graphical summary of the analysis suite displayed for a thin
1555 (~20 μm) horizontal slice in each aggregate. Each image shows the typical characteristics
1556 discussed in this section and it is possible to better understand the differences in LT,
1557 skeleton and openings in the two microaggregates. In Figure 9a the 8bit rendering of the
1558 solids is superimposed with the LT volume: the differences in porosity and pore size
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1571 distributions are immediately visible, as in Figure 9b (LT volume alone). In Figure 9c the
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1573 8bit volume is superimposed with the skeleton (labeled with the LT values) and the
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1575 openings (labeled with a color corresponding to their diameter). It is possible to
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1577 appreciate how well the skeleton fits the pore space and the role of the bigger chambers
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1579 due to the presence of the plant fragments in the Barrow microaggregate. In Figure 9d the
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1581 8-bit volume is removed to highlight the features of the skeletons and of the openings; we
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1583 see a more complex pattern of the skeleton in the barrow samples with shorter and
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1585 smaller branching linking the outside of the particle with the pore space, while the
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1587 Kansas particle displays classic interstitial pore space features in both the skeleton and
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1589 the openings.
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1593 A significant potential use of the detailed structural analysis presented is for the
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1595 direct numerical modeling of pore-scale biogeochemical processes in microaggregates.
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1597 The recent study of Ebrahimi and Or (2015) presents an elegant network modeling
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1599 approach capable of capturing the boundary of aerobic activity and community
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1601 partitioning within a single aggregate. The network architecture used in the modeling,
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1603 however, was a theoretical regular framework generated to match capillary
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1605 pressure/matric potential data on an aggregate collection and did not contain the detail
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1607 present in our direct imaging study. We believe that high quality SXR- μ CT can fill an
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1609 important gap in such modeling studies by providing an appropriate network, aperture
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1611 distribution, and set of boundary conditions to realistically capture biogeochemical
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1613 processes at the aggregate scale. This interaction between experimentalists providing the
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1615 modelers realistic starting points and validation datasets is bound to become more and
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1617 more important in many fields. The new direction of building online experimental data
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1627 repositories (e.g., <https://www.digitalrockportal.org/>) will also have an increasingly
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1629 important role in connecting experimental and modeling groups, including of course the
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1631 soil scientist community.
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1638 *Conclusions*

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1642 SXR- μ CT measurements on single soil microaggregates, coupled with advanced analysis
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1644 techniques have significant potential to improve the characterization of this unique
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1646 microbial environment. The suite of tools we present may aid future studies seeking to
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1648 correlate aggregate microstructure with microbial community structure and function. In
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1650 this study two markedly different microaggregates have been analyzed and the results
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1652 show how soil microstructures can be quantified and potentially linked back to biological
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1654 processes. Prior work has demonstrated a direct impact for different processes such as the
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1656 protection from predators (Griffiths and Young, 1994; Young and Ritz, 1998), the
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1658 distribution of nutrients (Chenu et al., 2001), or for environmental issues such as local
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1660 variations in heavy metal concentrations (Ranjard et al., 2000).
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1663 In the two samples we have shown the Kansas microaggregate, mostly inorganic
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1665 in nature, displays a typical interstitial pore space, created by the aggregation of rounded
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1667 mineral particles and aggregates. A more complex microstructure is present in the
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1669 Barrow microaggregate, with a strong organic component, discernible also from the XR
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1671 attenuation values, due to the high percentage of plant fragments. This microaggregate
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1673 shows a significantly larger amount of pore space potentially available to bacteria, and
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1683 this pore space is accessible only to small- to medium- sized microorganisms. Following
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1685 the geometrical concept alone the Barrow microaggregate provides a better environment
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1687 for the potential development of bacteria colonies, providing a larger and well protected
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1689 space to the microorganisms.
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1692 The quantitative microstructural characterization -aim of the present work- albeit
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1694 fundamental, is only a single element for a truly complete characterization of soil
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1696 microaggregates. The distribution of the chemical compounds needed for the
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1698 development of the microorganisms, and of microorganisms themselves, in the
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1700 microaggregates also play a key role and a comprehensive study about the role of
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1702 microaggregates in the development of spots of highly increased biological activity in
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1704 soils. Future improvements in X-ray imaging techniques, with both conventional and
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1706 unconventional sources, and further improvements and automation of the analysis part
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1708 will play an important role in achieving a better knowledge of the mechanisms related to
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1710 soil microaggregates, especially when coupled with techniques aimed at describing the
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1712 distribution of the different microbial communities.
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2523 **Figure Captions**
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2528 Figure 1.

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2530 Whole and vertically cut volume renderings of the Kansas and Barrow soil
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2532 microaggregates.
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2536 Figure 2.

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2538 Barrow microaggregate showing the calculated “outer surface” (displayed in red), in a
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2540 vertically cut sample with a partial covering of the surface (a), and a thin slice with the
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2542 surface following the outer border (b).
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2546 Figure 3.

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2548 The two microaggregates showing the local thickness volume superimposed to the 8bit
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2550 volume rendering and alone. Volumes are cut to better show the internal features.
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2555 Figure 4.

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2557 Frequency plots showing the distribution of (from top to bottom): local thickness voxels,
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2559 skeleton branch lengths, surface openings.
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2564 Figure 5.

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2566 Pore space accessibility from the outside for spherical structuring elements of different
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2568 sizes: .65 μm (yellow), 2 μm (green), 10 μm (red).
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2579 Figure 6.
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2581 Renderings of the skeletons, displayed superimposed both with a cut 8bit volume and
2582 alone. The skeleton is labeled with the local thickness values for each voxel.
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2588 Figure 7.
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2590 Analysis of openings: the rendering on the left for the two microaggregates shows the
2591 8bit rendering with the openings marked by cubes labeled with respect their size. On the
2592 right the skeleton and the openings alone are plotted.
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2598 Figure 8.
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2600 Star Length Distribution analysis for anisotropy characterization. The SLD analysis has
2601 been carried out on both the solids of the microaggregates (PF's on the left) and the
2602 whole completely filled aggregate (PF's on the right). Values are in multiples of random
2603 distribution, PF's are in equal area projection, upper hemisphere. The bottom of the
2604 figure shows the whole and horizontally sectioned microaggregates oriented as the PF's.
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2613 Figure 9.
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2615 Thin horizontal slice of the two samples showing in more details and summarizing the
2616 main analyses carried out on the microaggregates.
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Table 1. Morphometric analysis.

	<i>Kansas</i>	<i>Barrow</i>
<i>Volumetric Analysis</i>		
Total Aggregate Volume [μm^3]	2.819E+06	3.619E+06
Total Volume of solids [μm^3]	1.970E+06	1.998E+06
Total Volume of voids [μm^3]	8.486E+05	1.621E+06
Porosity [%]	43.1	81.1
<i>Surface Area Analysis</i>		
Surface Area [μm^2]	3.505E+06	4.850E+06
SNSVR	11.8	13.8
<i>Minkowski Functionals and Fractal Analysis</i>		
Integral of mean curvature (solids) [μm^{-2}]	-45197.6	-21798.8
Euler Characteristic (voids) [μm^{-3}]	-312.6	-410.6
Fractal dimension 3D (voids)	2.353	2.401
<i>Local Thickness Analysis</i>		
Structure Separation Mean [μm]	3.13	5.32
Structure Separation σ [μm]	1.89	4.13
Structure Separation Max [μm]	12.77	24.74
Structure Thickness Mean [μm]	6.46	7.45
Structure Thickness σ [μm]	5.44	11.44
Structure Thickness Max [μm]	28.66	47.07
<i>Geometrical Accessibility Analysis</i>		
0.65 μm elements accessibility [% of voids]	95.5	98.0
2 μm elements accessibility [% of voids]	76.0	86.2
10 μm elements accessibility [% of voids]	0	3.2
<i>Anisotropy analysis SLD -solids-</i>		
Isotropy index (I)	0.937	0.763
Elongation index (E)	0.019	0.153

Table 2. Skeleton analysis

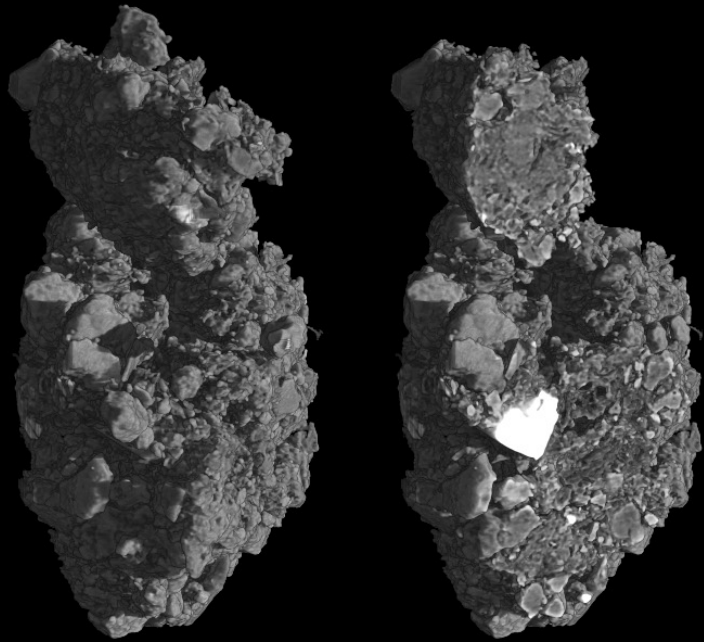
	<i>Kansas</i>	<i>Barrow</i>
Number of branches	54550	81012
Number of true junctions	29752	44202
Number of end points	11143	13552
Number of triple points	23113	33105
Number of quadruple points	4953	7713
Average branch length [μm]	3.41	3.40

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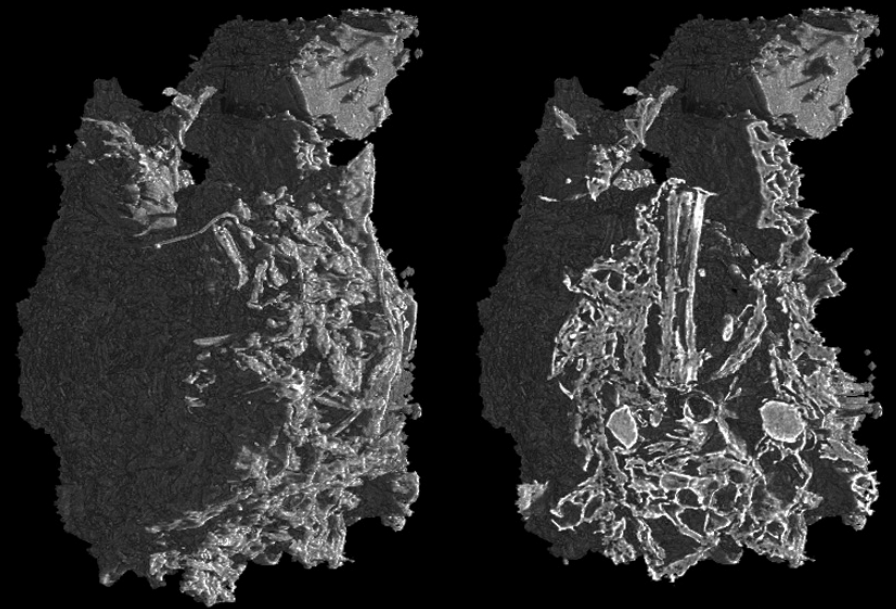
Max branch length [μm]	24.54	46.07
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2799
2800

Kansas



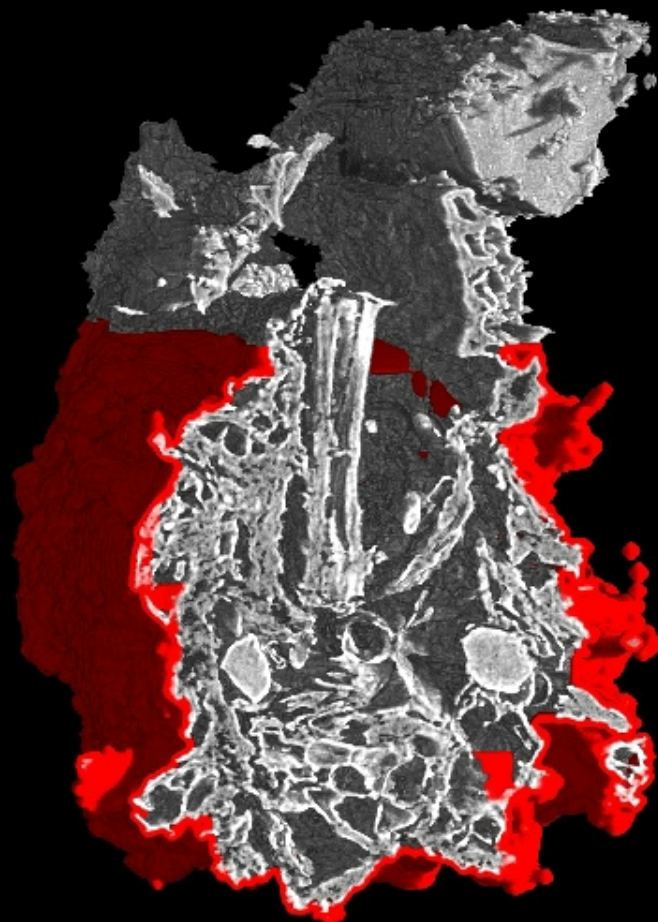
Barrow



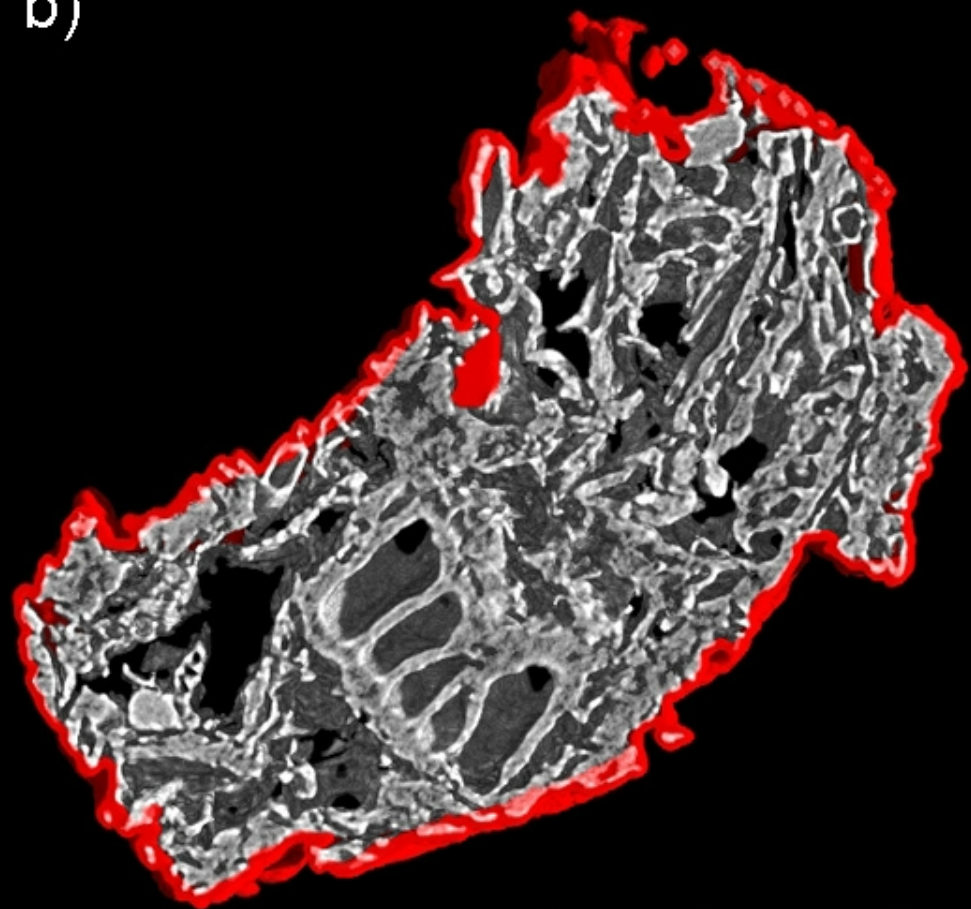
50 μm

Calculating the outer surface on the Barrow sample

a)



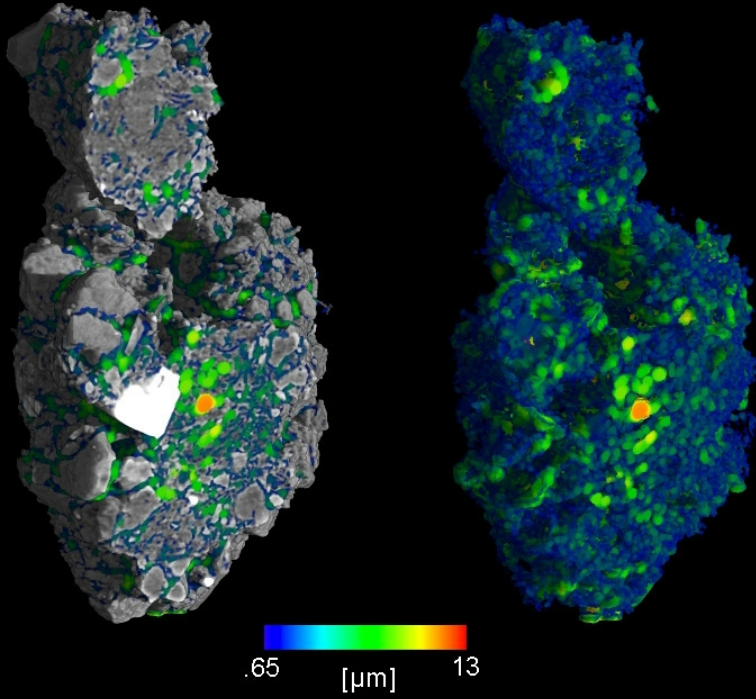
b)



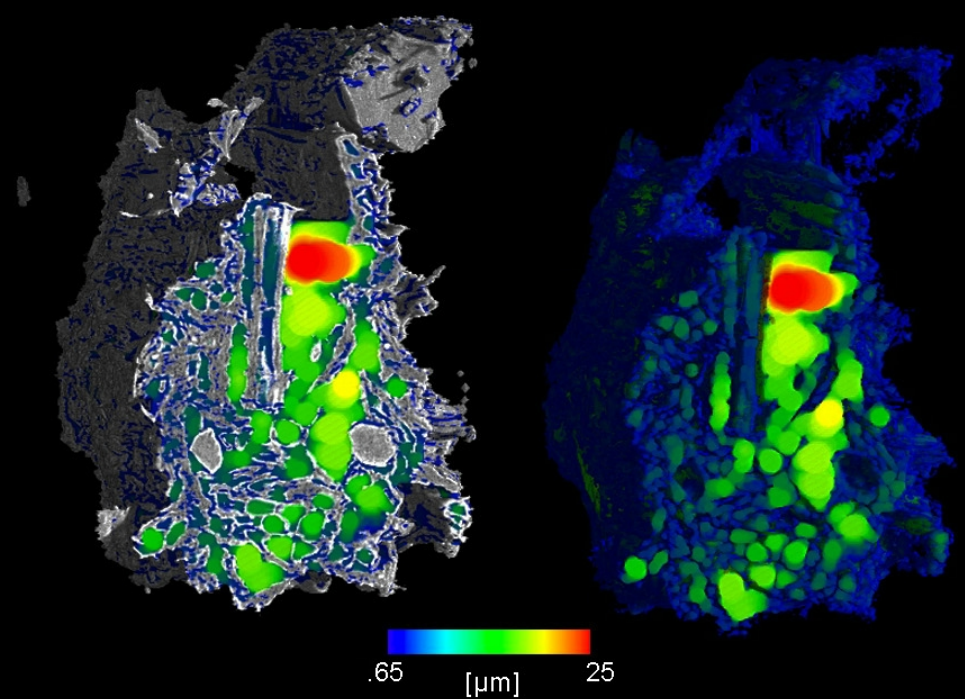
● Outer surface

Local thickness analysis

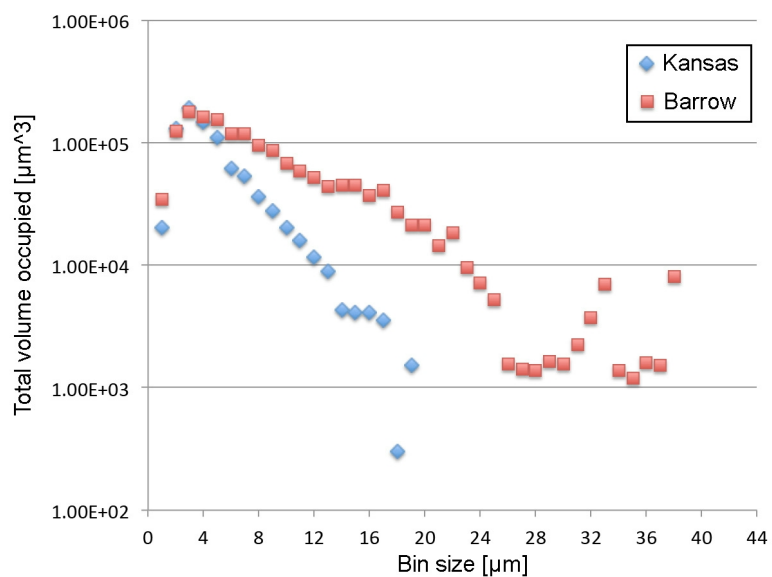
Kansas



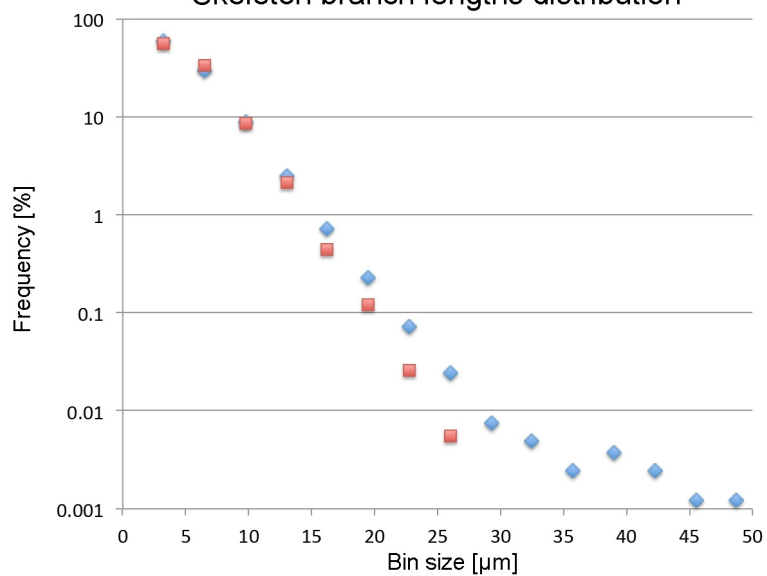
Barrow



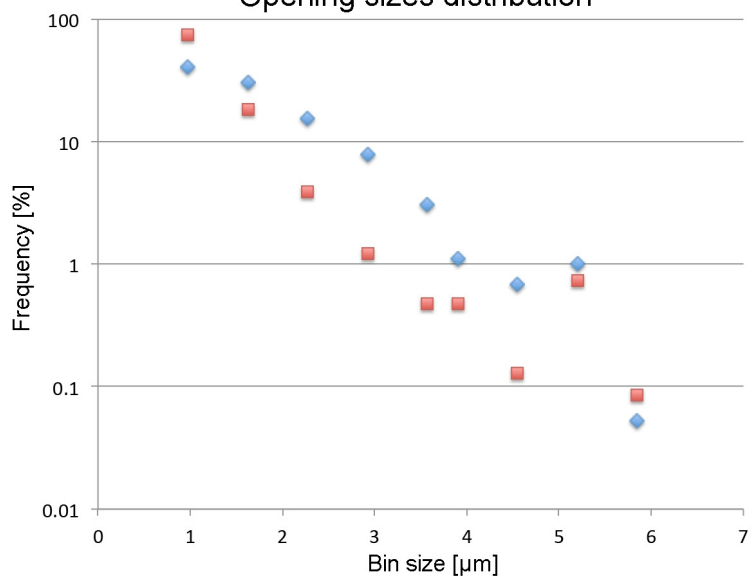
Local thickness distribution



Skeleton branch lengths distribution



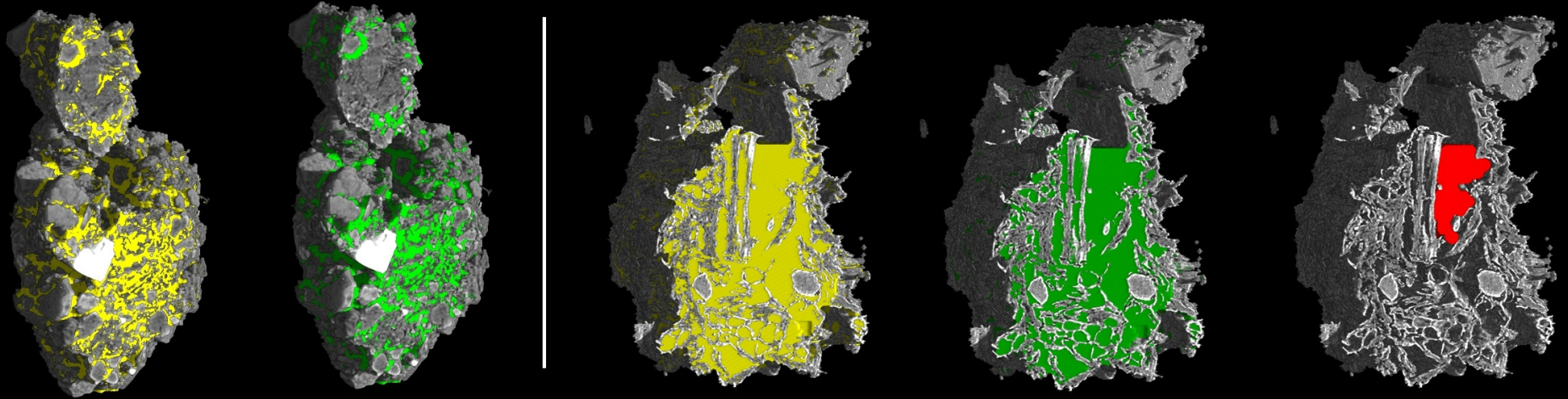
Opening sizes distribution



Geometrical accessibiity analysis

Kansas

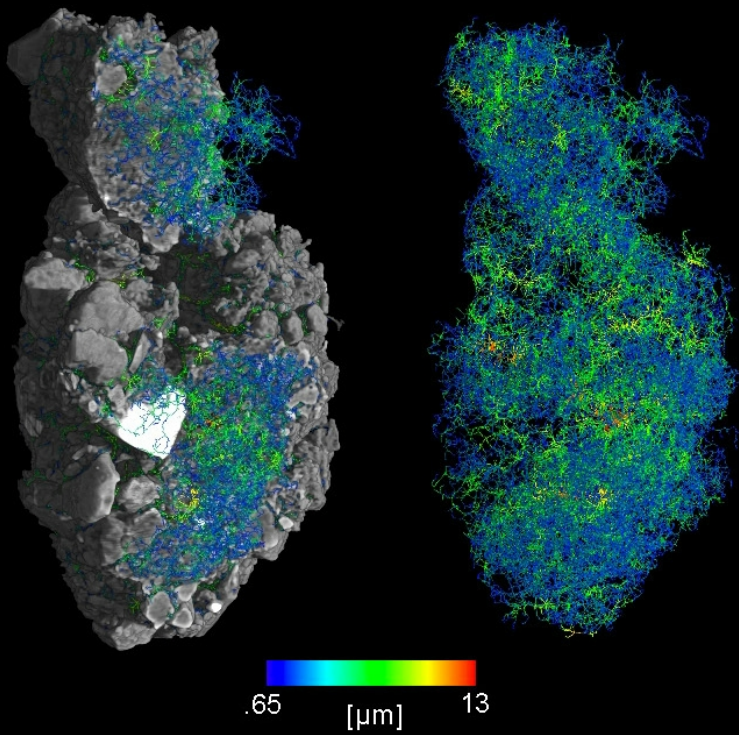
Barrow



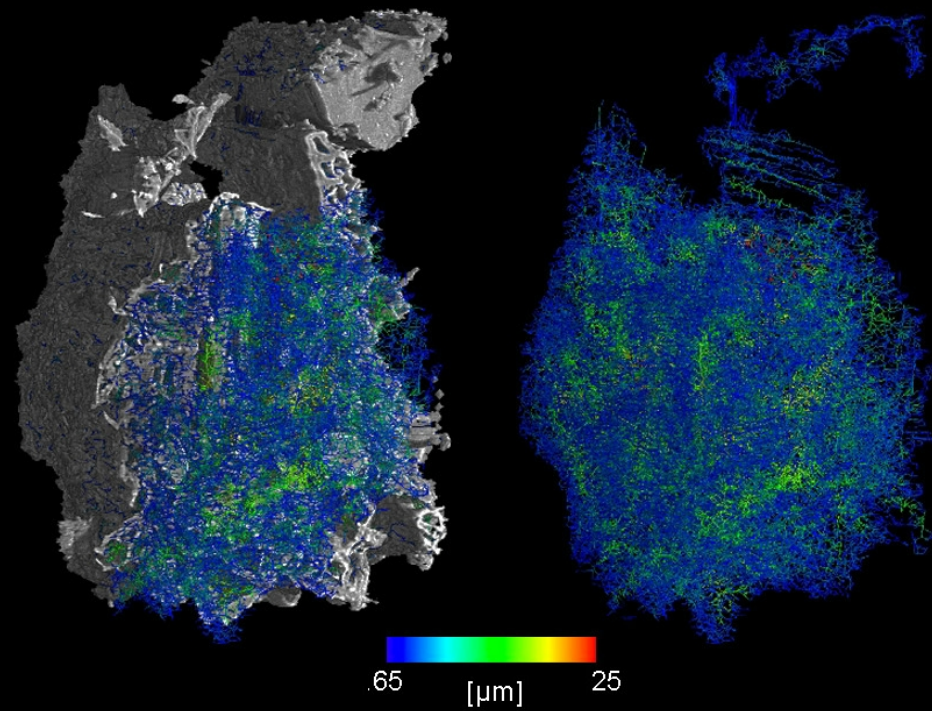
Spherical structuring element size: ● 0.65 μm ● 2 μm ● 10 μm

Skeleton analysis

Kansas

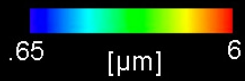
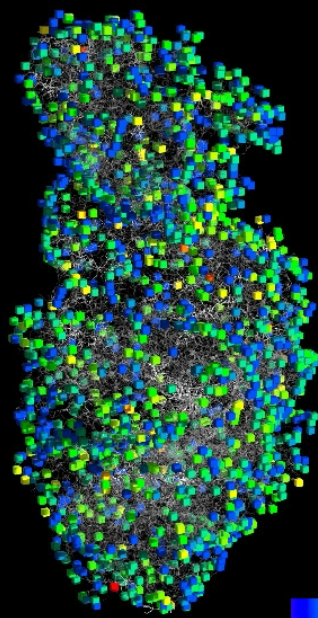
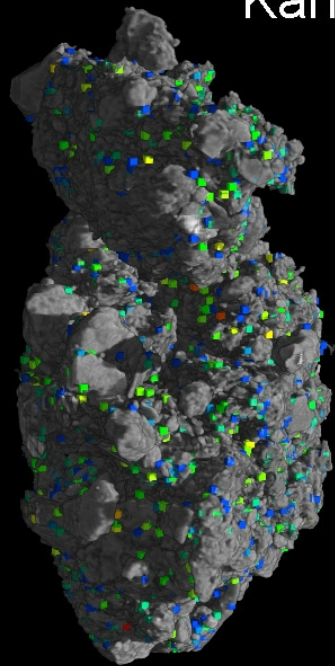


Barrow

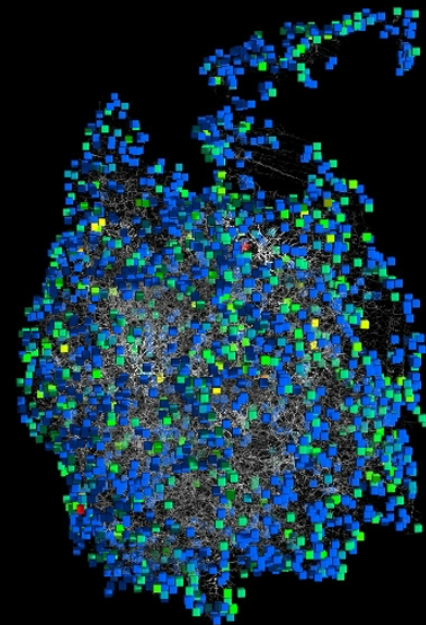
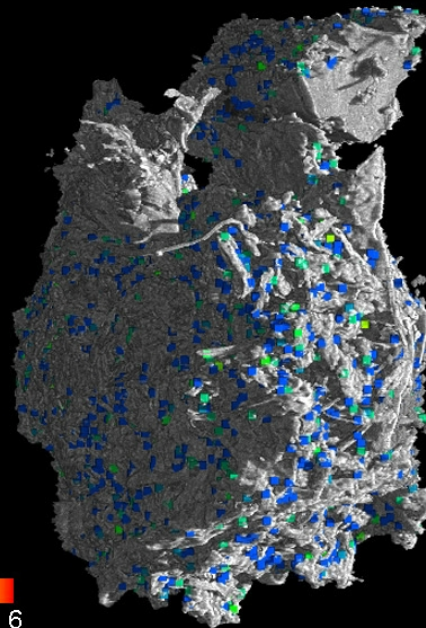


Openings analysis

Kansas



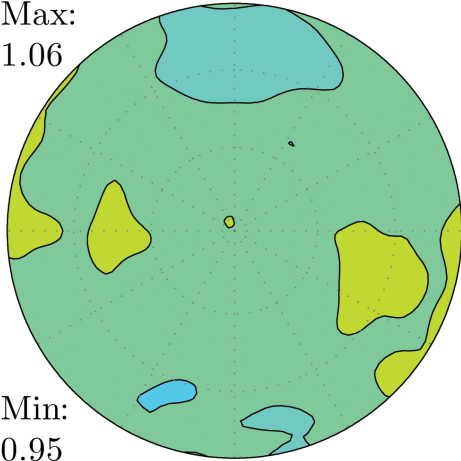
Barrow



Anisotropy analysis: Star Length Distributions

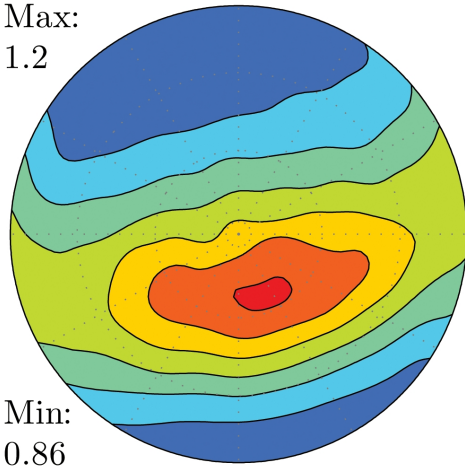
Kansas

Max:
1.06

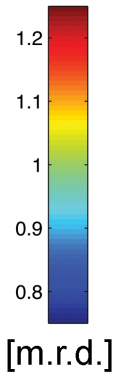


Min:
0.95

Max:
1.2

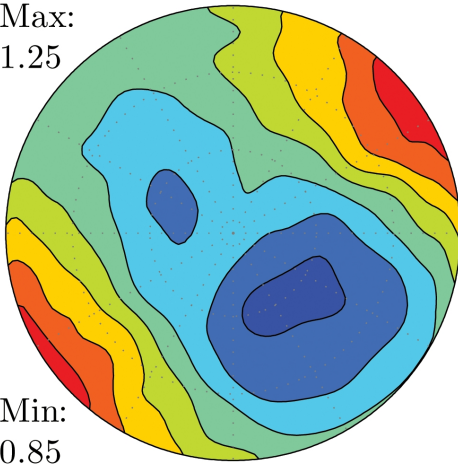


Min:
0.86



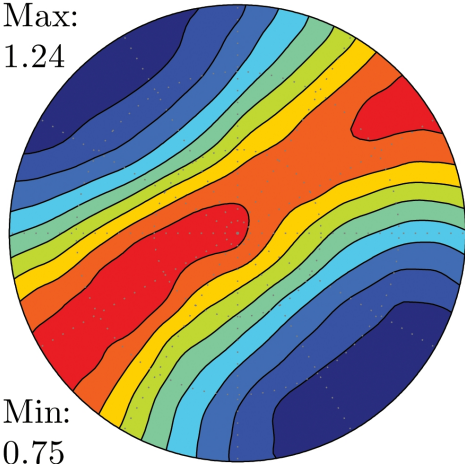
Barrow

Max:
1.25



Min:
0.85

Max:
1.24



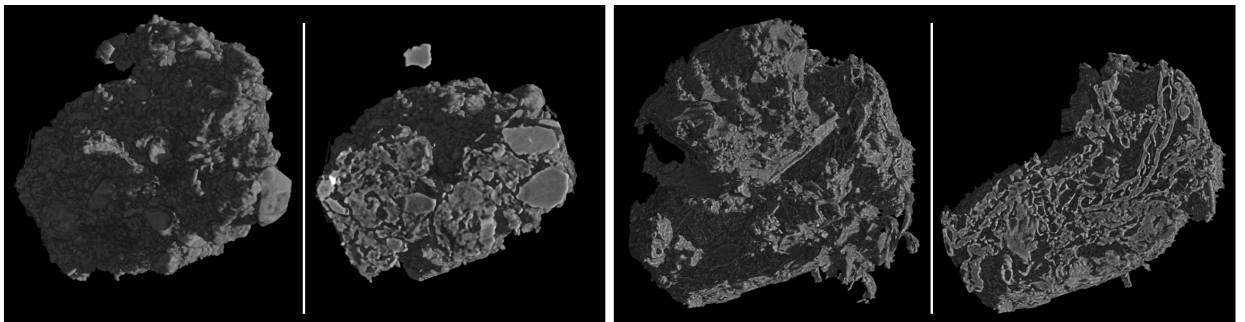
Min:
0.75

SLD solids

SLD microaggregate shape

Kansas

Barrow

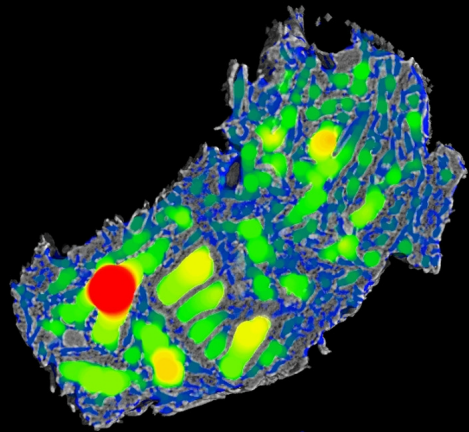
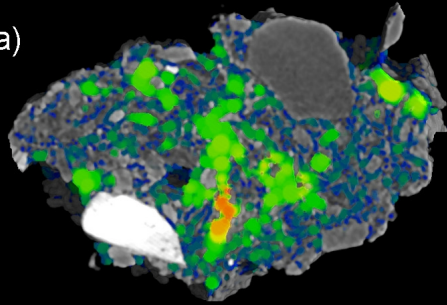


Local thickness, skeleton, and openings analysis on a thin horizontal slice

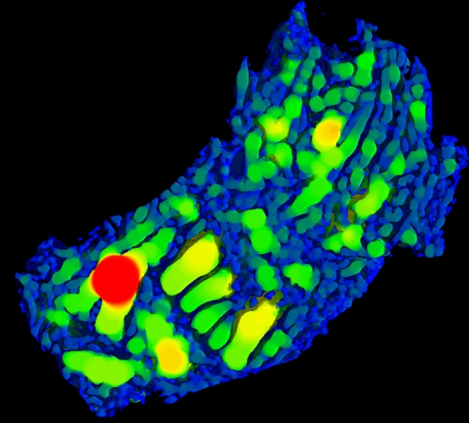
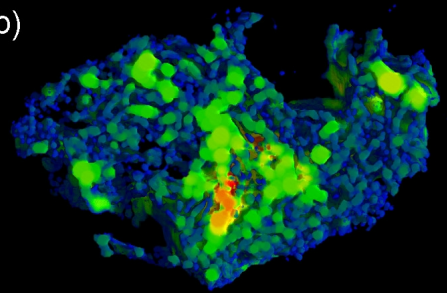
Kansas

Barrow

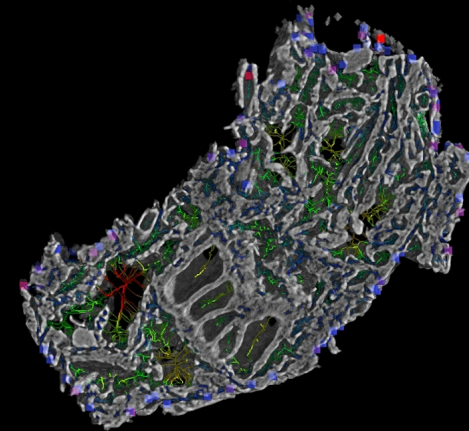
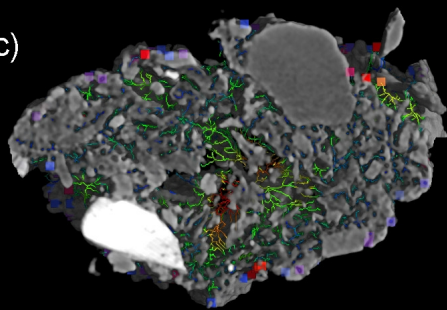
a)



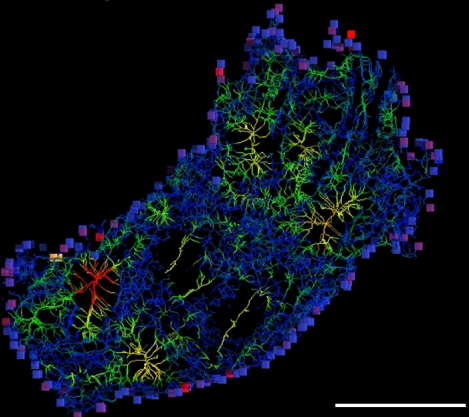
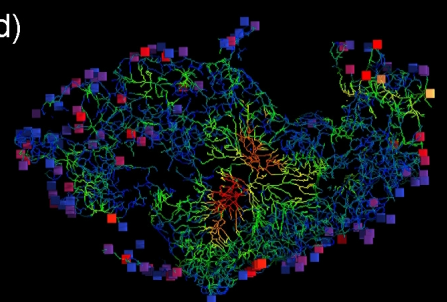
b)



c)



d)



Skeleton: .65 [μm] 12

Openings: .65 [μm] 5

50 μm