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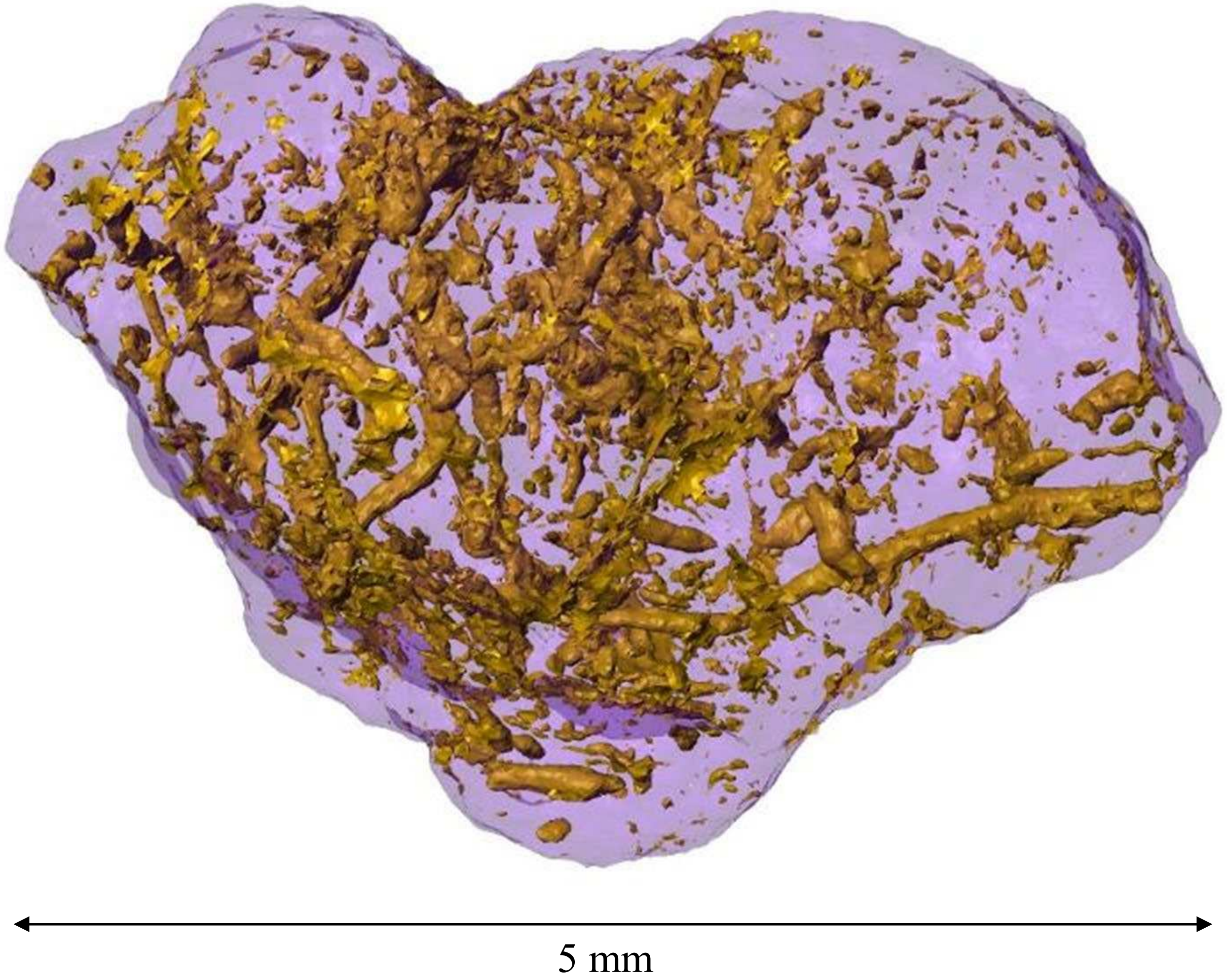

A 3D view of the pore system of a ~5 mm size aggregate grassland aggregate using X-ray microtomography at 5µm resolution. The pores are given in yellowish brown in the purple coloured aggregate solid matrix. This paper aims to characterise and quantify the pores in aggregates and establish its relevance to aggregate stability.

Running title: Aggregate pores and stability

Pore system characteristics of soil aggregates and their relevance to aggregate stability

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Abstract

Aggregates are the structural units of soils, and the physical stability is considered to be a keystone parameter of soil quality. However, little is known about the evolution of the pore system in aggregates and its importance in defining aggregate stability. In this paper, we investigated the pore system and stability of three dominant macroaggregate sizes (1-2, 2-5 5-10 mm) obtained from a fine sand-loamy Chernozem under three distinct land uses (arable, grassland and forest). We used non-invasive X-ray microtomography (XMT) in combination with pore network extraction to characterise PSD (pore size distribution) of aggregates and their potential changes upon continued submergence in water. We showed that smaller aggregates (1-2mm) have significantly higher total X-ray resolvable porosity than the medium (2-5 mm) and large (5-10 mm) aggregates. Also, using imaging tools, we demonstrated for the first time, that the pore system of stable aggregates from grassland and forest does not undergo significant changes upon continued submergence in water. It can be hypothesised that a physically stable pore structure allows the storage and transmission of water without a structural collapse, thereby contributing to aggregate stability. We found statistically significant positive correlations between different pore groups (closed pores, water holding pores and air space spores) and water stability of aggregates from all three land uses suggesting that pore system characteristics play a significant role in aggregate stability. Our results suggest that PSD is an important factor that determines the stability of soil aggregates.

Keywords: Imaging, Land Use, Aggregates, Pore network, Porosity, Tortuosity

Highlights

- The main aim is to establish links between aggregate pore system characteristics and stability.

- We analysed pore systems of macroaggregates from different land use using X-ray microtomography.
- There were no significant changes in the pore system in stable aggregates upon submergence.
- A stable pore system in aggregates is crucial for aggregate stability in water.

1.0 Introduction

Soil structure is a keystone indicator of soil quality, function and health (Kibblewhite et al., 2008). The stability of soil structure reflects the ability of soil to support soil flora and fauna through provision of void space as habitat, and the storage and transfer of water, gas and nutrients in soils (Utomo and Dexter, 1982; Amézketa, 1999; Bronick & Lal, 2005; Rabot et al., 2018; Banwart et al., 2012). In general, soil structure refers to the three-dimensional arrangement of soil voids within and between aggregates of primary soil particles whereby aggregates can be viewed as the structural units of soils. The development of aggregates is explained by the aggregate hierarchy model proposed by Tisdall & Oades (1982). Based on this conceptual model aggregates are sequentially formed through the action of organic (transient, temporary or persistent) binding agents leading to the formation of microaggregates (20-250 µm) and then to macroaggregates (>250 µm). It was also suggested that microaggregates could also be formed within macroaggregates due to the action of roots and microbiota (Oades, 1984) and aggregates also provide physical protection of organic carbon (Six et al., 2004).

Pores within aggregates (intraaggregate pores) can also be regarded as microsites for storage of air, water, nutrients and microbes which create localised pore-scale biogeochemical cycles. In unsaturated soils, all pores between 0.2 and 30µm retain water except the blocked pores with entrapped air (i.e. between -10 kPa and -1.5MPa matric potentials) and pores greater than 30µm in diameter typically filled with air (Dexter, 1988). Dexter (1988) also proposed the porosity exclusion principle, which states that each hierarchical order excludes the pores between the particles of the next higher order. Although this has not been verified experimentally, the theory suggested that smaller aggregates would have a denser packing (or lesser pore space) compared to larger aggregates. However, Lipiec et al. (2007) provided additional insights into the pore space within aggregate beds. In their investigation, they evaluated individual aggregate beds made of <0.25, 0.25-0.5, 0.5-1, 1-3, 3-5, and 5-10 mm sized aggregates for water retention and pore size distribution (PSD) and found that aggregate beds <1 mm exhibited bi-modal PSD associated with textural and structural domains whereas aggregate beds >1 mm produced tri-model PSD due to the additional macropore domain.

Non-destructive imaging tools such as X-ray microtomography (XMT) provide an alternative method to study PSD aggregates at a few micrometres resolution. For instance, Peth et al. (2008) used a high resolution (3.2-5.4 μm) XMT to image ~5 mm diameter aggregates from different land use (grass and conventionally-tilled). Based on the image analysis carried out using a small region of interest from near the centre of the aggregate, they found that the total porosities were higher for the conventionally-tilled (CT) aggregate (15.7%) compared with the grassland aggregate (11.1%). They also found higher relative proportions of smaller pores are observed in the former compared with the latter. Kravchenko et al (2011) reported that the aggregates under natural succession had more large pores (>97.5 μm) and small pores (<15 μm) than conventionally-tilled aggregates; whereas the medium size pores (37.5-97.5 μm) dominated in conventionally-tilled aggregates (Wang et al., 2012). A study by Zhou et al (2016) compared different fertiliser treatments on aggregate (3-5 mm) porosity in paddy soils using XMT and they found that total porosity of aggregates was higher when no fertiliser was applied in comparison to both fertiliser treatment (inorganic fertiliser with or without organic manure). Recently, (Bacq-Labreuil et al. 2018) showed the effect of land use (vegetation) on aggregates using XMT, and they found the total pore volume was influenced by vegetation as follows: grassland> arable> fallow.

According to Peth et al. (2008), further studies are required to link PSD with other soil properties, and there has been some progress in this direction; for example, Bailey et al. (2013) used imaging to understand the relationship between the porosity of 14 grassland aggregates of different sizes (0.250-0.425, 0.425-0.841, 0.841-1.0 mm) and microbial community composition and found no correlation between them. Similarly, the relationship between the aggregate PSD and stability was investigated by a few researchers. Soil structure stability is classically described by the mass distribution by particle size class of water stable aggregates (WSA as %) present in a soil sample. Note that several organic and inorganic binding agents contribute to the development of WSA (Tisdall & Oades, 1982; Amézketa, 1999; Bronick & Lal, 2005; Dal Ferro et al., 2012; Regelink et al., 2015; Rabot et al., 2018). Papadopoulos et al. (2009) examined 5 mm sized aggregates using XMT and found that aggregate porosity was not linked to stability; however, the authors reported that pore morphology might influence the stability of aggregates and the potential for slaking (i.e. the breakdown of macro-aggregates to microaggregates and primary textural units). However, using 1-2 mm sized aggregates Dal Ferro et al. (2012) established that aggregate stabilisation was strongly linked to the porosity.

If an aggregate is considered water stable, it must withstand the decrease in inter-particle cohesive forces within the aggregate imparted by wetting without a structural collapse through slaking, clay swelling or clay dispersion (Dexter, 1988). Conceptually, the presence of a stable macropore domain (including cracks and elongated pores) within aggregates may prevent trapping of air or build-up of air pressure in pores due to the entry of water. These stable pores may play an important role in transmitting water without disrupting the structure of aggregates and therefore could contribute to the stability of the aggregates (Lipiec et al., 2007; Papadopoulos et al., 2009). In other words, in a stable water aggregate, the macropore domain may remain stable when it is subjected to wetting, thus contributing to the stability. However, this concept requires experimental validation. Therefore, in this paper, we would like to test three important hypotheses, which are linked to pore system development in macroaggregates and their linkages to aggregate stability as below:

(1) The total XMT resolvable pore space in macroaggregates will increase with an increase in the size of the aggregates according to the porosity exclusion principle.
(2) Aggregates from less disturbed land use (e.g. grassland and forest) will have greater total porosity dominated by macropores compared to disturbed land use (e.g. arable).
(3) Stable aggregates will have a pore system that is resilient to changes during wetting.

The specific objectives are:

1. Describe XMT resolvable PSD in three different macroaggregate sizes (1-2; 2-5 and 5-10 mm) obtained from three different land uses (arable, grassland and forest) using XMT;
2. Examine the stability of aggregates during rigorous wetting regimes;
3. Evaluate the XMT resolvable PSD changes in stable aggregates before and after wetting

2.0 Methods

2.1 Site description and sampling

The samples were obtained from locations in the National Park Donau-Auen, which were developed on fine river sediments of the Danube River and part of the FM-CZO. The mean annual temperature in the area is about 9°C and mean annual precipitation ~550 mm with potential evapotranspiration of ~570 mm (Blaud et al., 2018). The soil is a fine sandy-loamy Haplic Chernozem (Mollic Fluvisols as per WRB) soils that are ~350 years old (Lair et al., 2009). Three contrasting land uses (arable, grassland and forest) were selected for soil sampling. The bulk soils characteristics at the time of sampling are given in Table 1, and more information about the site can be found in other references (Banwart et al., 2012; Regelink et al., 2015; Rousseva et al., 2017; van Leeuwen et al., 2017).

Aggregate sampling was carried in summer 2011 under dry soil conditions (pF 3.8 - 4.0) which enabled sieving and collection of aggregates in the field. Three sample locations were chosen as replicates within 30 m radius under each land use. The top 5 cm of the soil profile was scraped off to remove the surface leaf litter, earthworm castings and surface feeding roots in grassland and forest. The soil beneath (5-10cm) was then loosened using a spade and passed through a stack of sterilised sieves to collect different aggregate size classes (<0.25, 0.25-0.50, 1-2, 2-5 and 5-10mm) for various experiments including microbial diversity studies (e.g. Blaud et al., 2018). The soil aggregates were stored in plastic beakers and kept dry in the dark and cold room conditions (4ºC) for subsequent use. However, for this study, we used three dominant macroaggregate sizes which were 1-2, 2-5 and 5-10mm (see Table 1 for particle size distribution) which will be denoted by S (small), M (medium) and L (large), respectively, in this manuscript.

2.2 Experiments

The experiment included imaging of dry sieved aggregates collected from the field, followed using three wetting and drying cycles on major aggregate size fractions (S, M and L). Based on the water stability tests, we tested the effect of submergence on aggregates PSD through imaging as described below.

2.2.1 Microstructure measurements using XMT

Sixty-nine aggregates were scanned representing each size class (7 from S; 8 from M and L) from the three land uses. We used Skyscan 1172 XMT scanner available at the SKELETAL lab at the University of Sheffield with an effective pixel size of 10µm for L size and 5 µm for S and M size aggregates to achieve maximum resolution for a given aggregate size (See

Suppl. Material 1 for image acquisition settings) for this scanner. Individual aggregates were scanned by securely placing in them in Styrofoam (which does not appear in X-ray images) before fixing on the tomography stage to obtain 3D images of aggregates.

2.2.2 Aggregate stability using three wetting and drying cycles (WDC)

This experiment aimed to measure the amount of water stable macroaggregates (WSA) from S, M and L of each land use. The initial macroaggregate WSA (%) was measured using standard wet sieving procedure (method 1) with multiple sieves that were sequential (<0.25, 0.25-0.50, 1-2, 2-5 and 5-10mm) (Elliott, 1986). However, for the WDC experiment, a single 0.25mm sieve (method 2) was used to allow a simple and straightforward separation of water stable macro and microaggregates as both methods showed a strong positive and significant correlation (correlation coefficient= 0.99, not shown). Note that the sand content of the aggregates was checked using ultrasound stability tests (data not shown) before the wet sieving experiments to verify the need for sand correction. It was found that large sand (630-2000μm) and medium sand fractions (200-630μm) were negligible, and ~95% of sand particles were made of fine sand (63-200 μm). Based on this, the sand correction procedure was not followed for macroaggregates fractionation (>250μm).

Exposing aggregates to varying degrees of wetting and drying cycles (WDC) provided a better understanding of the structural resilience of aggregates; however, there is no consensus on number or duration of WDCs (Rabot *et al.*, 2018). Two WDCs were considered for S, M and L sized aggregates from all land use: (1) a short WDC with 2 hours of submergence followed by 22 hours drying at 25-35ºC in a laboratory (i.e. 24 hours per cycle); and (2) a long WDC, in which the aggregates were submerged for 24 hours followed by 24 hours drying (i.e. 48 hours per cycle). The short cycle was repeated four or nine times whereas the long cycle was repeated for four times only. Three replicates per treatment were used. We performed WSA analysis using method 2, as outlined earlier.

To investigate changes in soil aggregates due to processes such as slaking or expansion of clay, we performed additional imaging of individual aggregates after 24-hour continuous submergence in water to induce slaking. It was assumed that 24 hours were sufficient to fully saturate all pore spaces in aggregates and the water pressure on the pore walls could induce pore system instability and slaking. We used only a subset of three M, and L aggregates each from grassland and forest. In this experiment, selected aggregates were individually placed gently in a sterilised 50 ml beakers, and deionised water was added (~25 ml) along the side of the beakers until the aggregate to be completely submerged. The arable soil aggregates slaked and disintegrated rapidly within seconds after adding water; hence, they could not be included in this investigation. The samples were left for 24 hours in the laboratory conditions with a parafilm lid to prevent evaporation. After this, we syphoned the water out carefully without disturbing the aggregate, and any remaining water was left to dry naturally for approximately two days before imaging. Due to the fewer number of samples and better hardware availability, these aggregate images were processed at the original scanning resolution (5 and 10 μm for M and L) to study the PSD in detail.

2.3 Image processing and pore network extraction.

The working resolution was set at 20μm for L and 10μm for S and M due to a large number of samples and optimum hardware and software (Avizo 9.0.1) performance. All previously reported studies used a region of interest (ROI) while quantifying pore system in aggregates. While this is useful, it only will represent part of an aggregate, and the distribution of pores cannot be assumed spatially uniform throughout in an aggregate. Hence, we developed a new protocol for quantifying the total porosity in a given aggregate volume as described in

Supplementary Material 1, largely based on methods described in our previous publication (Menon et al., 2015). In general, the processing steps included image cropping using ImageJ to 8-bit JPEG files to reduce the computational burden of processing images in 3D using Avizo. The pores present after segmentation provided a total porosity of the image in 3D. For the segmentation, the solid particles were isolated using an image thresholding algorithm that uses a specified discrete attenuation value above which all pixels are considered as solid particles. To extract different types of pores from an image, a series of morphological filters were used as described in the Suppl. Material 1.

Based on our analysis, the porosity of each aggregate is presented, which is the proportion of total pores to the total volume of the aggregate. Further, we grouped the pores broadly into closed (or isolated air pockets in the structure), pores <50µm (water holding) and >50 µm (air space) to the total pore volume are also presented. Also, effective porosity (%) is presented, which is the proportion of the combined water holding and the air space to the total pore volume of each aggregate. Please note that the amount of resolvable pores in each category will depend on the resolution of the images.

The pore space can be segmented into its structural elements, including pores and throats (Dong & Blunt, 2007). Throats are the bottlenecks between each pair of connecting pores (Blunt, 2001). This simplified model allows us to simulate complicated processes in porous media within a computationally efficient framework. Thanks to their applicability and facilitated by computed tomography images, pore networks have been employed to model various porous material processes (Blunt, 2001; Valvatne et al., 2005; Dong & Blunt, 2007; Joekar-Niasar et al., 2008; Andrew et al., 2014). Inevitably, the applicability of such replicates of porous space is highly dependent on the porous media segmentation method used for pore network extraction. Thus, a more realistic network extraction requires less number of simplifications which will increase the accuracy by pore network modelling (Rabbani & Babaei, 2019).

Recently, watershed segmentation algorithm has been employed for pore network extraction from porous material images with complex geometries (Sheppard et al., 2004; Wildenschild & Sheppard, 2013; Rabbani et al., 2014, 2017b; Gostick, 2017; Rabbani & Salehi, 2017) which was used in this study (see Suppl. Material 1). This method utilises the geographical concept of watersheds to divide the pore-space of the porous material images into distinct pores and throats which could form an interconnected network of nodes and links (Rabbani et al., 2016, 2017a). Using the pore networks constructed, it will be possible to measure several properties of porous material (such as pore connectivity, pore radii, throat lengths and tortuosity) that provide quantified insights towards the changes of soil morphology when the aggregates are soaked in water. In this approach, it was assumed that throats are interfaces which connect adjacent pores, and they take up negligible volumes compared to pores (Rabbani & Babaei, 2019). To find the pore connectivity, which is the number of neighbour pore bodies connected to a single pore body, we scanned the segmented pore space image with a 3×3×3 sliding window. Each pore-body is labelled with a unique code, and from the codes of each neighbourhood voxels, the connectivity matrix (i.e. 1 for connected and 0 for no connection between pores) can be derived for an $N \times N$ matrix (N is the number of pores). A sample of extracted pore network image from aggregate from this study is provided in Suppl. Material 1.

Based on the definition of porous media tortuosity (Matyka et al., 2008), all path lengths are averaged and divided by the geometrical distance between the input and outlet set of pores. For defining inlet and outlet pores in the x-direction, we followed an ad hoc procedure. In x-direction, 5% of the pores with their first element of centre coordinates (positions along x-axis) smaller than the rest of pores are considered as inlet pores. Similarly, 5% of the pores with the first element of centre coordinates larger than the rest of the pores are considered as outlet pores. The same procedure was repeated for y and z directions. Knowing the positions of inlet and outlet pores, we can find the shortest pathways in the network that connect these pore bodies in each direction, thereby allowing to estimate directional tortuosity of networks by dividing the shortest path to the geometrical distance (Matyka et al., 2008).

2.4 Statistical analysis

All the statistical analyses were performed using R version 3.4.0 (R Foundation for Statistical Computing). The Post hoc Duncan test and the bootstrap correlations were performed using the "DescTools" and "boot" packages, respectively.

For the first and second experiment, the differences in pore characteristics due to land use and soil fractions was assessed by analysis of variance (ANOVA) coupled with Post Hoc Duncan test, with land use and soil fractions as factors. When the normality and or homoscedasticity of variances were not met, log transformation was applied. For the second experiment, the effect of WDC on WSA (%) was assessed by ANOVA test.

The effect of wetting on pores and pore network characteristics was investigated using paired Student's t-Test (as the same aggregate was measured before and after wetting). Spearman correlation and linear regression between WSA and pores characteristics including the three sizes of aggregates were performed for all or each land use. Due to the difference in the number of replicates between WSA (n = 3) and pores characteristics (n = 8), we used bootstrapping (a statistical procedure that resamples a single dataset to create thousands of simulated samples to derive sample statistics) on the correlations (5000 bootstrap replicates).

3. Results

3.1 PSD of Aggregates

The image processing protocols followed in the study allowed visualisation and distribution of different types of pores (closed, water holding and air space) within individual aggregates. In Figure 1 (a-c), we present cross-sectional views of representative aggregates with different types of pores (closed, water holding and air space pores) obtained from different land uses. Overall, effective porosity followed the same trend as total porosity, and the effective porosity was slightly smaller (1-2 %) than the total porosity as shown in Fig. 2 as it represented the percentage of pores occupied by water and air and does not include closed pore space. It was found that the total and effective porosities were significantly (P = < 0.05) affected by land use and aggregate sizes. Total aggregates porosity, in particular from M and L aggregates from the forest, was significantly higher (~4%) than the other two land uses. The data also showed that both total and effective porosities of S were greater than those compared to M and L and, were not influenced by the land use. In contrast to the total porosity, the effective porosity of S, M and L were not significantly different for grassland.

Further portioning of total pore volume to percentages of closed, water and air holding pores, showed closed pores tend to increase with the increase in aggregate size under arable land use whereas the opposite trend was found in grassland and forest. Notably, for the S size class, the percentage of closed pores was significantly higher in grassland and forest than in arable land (uppercase letters, Fig. 3).

On the other hand, closed pore space in the L aggregates from the forest was significantly lower than for the other land uses. Although there were no significant differences in the distribution of water holding pore volumes between land uses for any specific aggregate size class, in all cases, the proportion of water holding pores decreased with increasing aggregates size (lowercase letters Fig. 3). However, the proportion of air space pores showed an opposite trend to the water holding pores between aggregates size, with an increasing proportion of air space pores with increasing aggregate size (lowercase letters, Fig. 3). Significant differences in air space pore volume between all aggregate sizes were found for forest and grassland. The air space pores also showed little difference between land uses; only the L size class was significantly higher in forest land use compared to arable (uppercase letters, Fig. 3).

3.2 Water stability of aggregates

Overall, the land use had the strongest effect on WSA, with grassland showing the highest proportion (~90%), followed by forest (~80%), while arable land showed low WSA (~20%) (upper case italic in Fig. 4). It appears from the data that the stability of aggregates tends to increase with aggregate size. The increase in WDCs number had a significant impact on arable and forest aggregates, however, the stability of grassland aggregates did not change significantly (lowercase letters in Fig. 4). The number or cycles had a stronger effect than the duration of the wetting. In the arable, WSA after nine short WDCs was significantly lower than with four short or long WDCs regardless of the macroaggregates size. For the forest, each size of macroaggregate was affected slightly differently by WDCs, with 1-2 mm showing the lowest WSA proportion after nine long WDCs, while for 2-5 mm it was after four long WDCs.

3.3 Effect of submergence on PSD

To demonstrate the resilience and stability of pore systems, PSD was examined before and after 24-hour submergence in water for two size groups of aggregates from grassland and forest. In general, the wetting for 24 hours in water of aggregates sizes 2-5 mm and 5-10 mm, did not significantly change the proportion of porosity, effective porosity, closed pores, water holding pores and air space pores in both grassland and forest aggregates (Fig. 5, 6).

Furthermore, this data was further split into different pore size groups (<30, 30-100 and >100 um) for M and L aggregates (Fig. 7). The proportion of these pore size groups did not show any significant changes after 24 hours of wetting treatment (Fig. 7) for either M and L aggregates from both grassland and forest. The M aggregates were significantly dominated by 30-100 µm pores size, representing between 60-80 % of total pore space for grassland and forest (lowercase letter, Fig. 7) whereas pores> 100 µm dominated in L aggregates indicating a substantial macropore domain.

When we examined the changes in pore size, throat size, throat length, pore connectivity and tortuosity in x, y and z directions (Table 2) it was evident that no statistically significant changes occurred as a result of submergence, although the data showed that M samples had experienced more change compared to L from grassland and forest. Overall, for both sets of aggregate sizes, the changes of pore size, throat size and throat length remained below 10%.

Based on the PSD and WSA data obtained from these macroaggregates, we examined the relationship between stability (WSA%) and the pore space in aggregates using bootstrapped correlation (Spearman rank correlation, bootstrapped) and linear regression (Table 3 a & b). The analysis was performed on the data from all land uses as well as for each land use (Table 3a). The regression (linear) could be performed only for arable land use as the data from other land uses were not normally distributed (Table 3b). Values from these tables suggest that there is a significant positive correlation between aggregate stability and three different pore classes (closed, water and air holding) from individual land uses. When all data were pooled, this was also true except for water holding pores. The data also showed significant negative correlations between both porosity and effective porosity with stability for all land use combined and also for arable and grassland individually. The regression analysis revealed that for aggregates from arable soils, the pore system characteristics were significantly and linearly related to WSA as shown in Table 3b.

4. Discussion

We acknowledge the fact that not all soil pores can be resolvable due to XMT resolution used in this study. Also, it must be noted that we scanned and processed L aggregates with a different resolution, which was mainly due to the technical limitation of the scanner we used. Using a lower resolution (20µm) may have underestimated of porosities in L aggregates. Therefore results must be interpreted carefully while comparing different sizes of aggregates because S & M were processed at the same resolution (10µm). Nevertheless, this difference does not pose an issue while comparing different land-use types for each aggregate size class. It may be noted that some previous works suggested that changing the resolution had minimal impact on total porosity as previously shown by De- Ville (2017, p 170) & De-Ville et al. (2018a).

Despite a recent increase in articles using imaging to study soil structure, there are only a handful of studies that focussed on aggregates. These studies include observation of 4-6.0 mm diameter aggregates at 14.6µm resolution (Kravchenko et al., 2011; Wang et al., 2012; Ananyeva et al., 2013), 1-3 mm aggregates at 4.4µm (Nunan et al., 2006), ~5 mm diameter aggregates with 3.2-5.4 µm resolution (Peth et al., 2008) and multiple aggregate sizes (0.25-0.425, 0.425-0.841, and 841-1.0 mm with 1 µm resolution (Bailey et al., 2013). This current study, by comparison, draws on a relatively larger sample size (69 aggregates) covering three land uses with 7-8 replicates from each group. Whereas most of the previous studies looked at the influence of tillage (conventional vs grassland/natural succession) on the aggregate structure (Peth et al., 2008; Kravchenko et al., 2011; Ananyeva et al., 2013) while Bailey et al. (2013) focussed their study only on grassland. A recent high resolution (1.51 µm) XMT imaging study demonstrated that the total pore volume in aggregates (between 0.71 -2 mm) was highest in grassland, followed by arable and fallow, demonstrating the impact of land use (Bacq-Labreuil et al. 2018). The main difference is while processing the images, we used a complete aggregate volume rather than a region of interest (ROI) approach used in all previous studies. The problem of the ROI is it is user-defined (size, volume, position etc.) and it is most useful for materials with a relatively homogeneous structure, which is not the case for soil aggregates.

As Dexter (1988) proposed, each hierarchical order of aggregate excludes the pores between the particles of the next higher order. According to this hypothesis, the total porosity of aggregates will increase with an increase in size. Our data presented from three macroaggregates sizes suggest an opposite statistically-significant trend, especially for the arable land and grassland aggregate sizes S and M which were processed at the same resolution). Also, data from a previous study (Bailey et al., 2013), which used sub-millimetre sized aggregates (250-425; 425-841 and 841-1000 μm) did not reveal any particular trend between total pore volume (%) and aggregate size either. However, porosity obtained from these aggregates was much higher (17.2-54.9%) than we observed in our study (<10%) which is consistent with our results, which suggest that porosity may be likely to increase as the aggregate size decreases.

The characterisation of pore space also differed between studies. For instance, <15, 15-60 and >60μm pore size classifications were used to compare tillage systems in some studies (Kravchenko et al., 2011) whereas others used a simple histogram of pore volume distribution (Bailey et al., 2002; Peth et al., 2008). We have used three simple categories (closed, water holding and air spaces) of pores based on their potential role in water and air flow through the aggregates. The amount of resolvable pores in each category will depend on the image resolution. Closed pores or "blocked" pores (Dexter, 1988) which contain trapped air which will have no or limited contribution to the transport processes considered with our results show that such pores occupied approximately one-third of the total pore space available in aggregates. Hence, this pore volume was excluded for the calculation of effective porosity, explaining why it was always lower than the total porosity. The observed pattern suggests that in soils under arable land use, the closed pores increased with aggregate size contrasting with grassland and forest. The reasons for this pattern is likely due to the compaction from farm machinery because compaction leads to fragmentation of pores (i.e. macropores will be changed to micropores) as demonstrated by Menon et al. (2015).

In our recent investigations (De-Ville et al., 2017, 2018) 50 µm threshold was used to calculate water holding pores using XMT images based on the hypothesis proposed by Getter et al. (2007) to improve retention performance of green roofs substrates. Small and medium-sized aggregates in this study, for across the land uses contributed substantially (~30%) to the water storage compared to the large aggregates (~40%). The opposite was true for air space pores, which are critical for biota and drainage characteristics of the soils. Across the land uses, we could see an increasing trend in air pore volume with the size of aggregate, whereas the opposite was found for water holding pores. This is in agreement with the results from Lipiec et al. (2007) in which they showed the existence of a more complex PSD and a macropore domain in aggregates larger than >1 mm (Lipiec et al., 2007).

The breakdown of aggregates was explained by earlier studies (Yoder, 1936; Hénin S, 1938; Dexter, 1988) through air-trapping and breakdown (slaking) as a result of the entry of water into aggregates and it depends on the rate of wetting and water repellent properties of the aggregates (Chenu et al., 2000; Cosentino et al., 2006; Bartoli et al., 2016). A meaningful comparison of different wetting and drying cycles with previous studies is not very useful here due to inconsistencies in the methods used to characterised the soil structure (Rabot et al., 2018). Despite this, the impact on the WSA mass fraction of differently sized aggregates by different WDCs suggested that it can impact the stability of arable and forest soil aggregates compared to grassland aggregates, presumably due to higher SOC (Table 1) compared to the other land uses. This experiment also revealed that the stability increased with the size (except for grassland) and land use, regardless of the WDCs. Usually smaller aggregates are supposed to be denser and stronger as proposed by earlier studies (Kemper & Rosenau, 1984; Dexter, 1988; Elliott & Coleman, 1988; Oades, 1993; Fernández et al., 2010) and it is possible that the stability of the large aggregates is influenced by other factors such as Fe oxides and silt content, besides soil organic carbon (Regelink et al., 2015).

The relatively higher stability of grassland and forest aggregates motivated us further to the development of the third hypothesis on the stability of the pore system. Although some subtle changes in pore properties could be observed, in general, there was no statistically significant difference in total, and effective porosities and different pore groups obtained from M and L aggregates of forest and grassland suggested structurally resilient pore system in these aggregates. The only exception is the water holding pore space of forest L aggregates. This was not surprising because in our WDC experiment, we found some small decrease in the stability of that forest aggregates compared to grassland aggregates. It is also important to note that pore size, throat size and throat length are basic parameters and pore connectivity and tortuosity are higher level parameters. Logically, the slight changes in basic parameters could accumulate and lead to a more significant deviation in the higher level properties. For example, a single additional throat between a cluster of pores could lead to an increase in pore connectivity of all the cluster pores. That could be the reason behind the relatively greater changes observed for connectivity and tortuosity.

The data and the correlations from this study demonstrate that the pore system in stable aggregates undergoes relatively small and insignificant changes when submerged in water and may, therefore, explain aggregate stability. We hypothesise that the pore networks in stable aggregates act as conduits for transmission of fluids through without trapping the air and thereby suppressing the build-up of air pressure inside an aggregate preventing it from slaking, as previously proposed (Lipiec et al., 2007; Papadopoulos et al., 2009; Dal Ferro et al., 2012). However, it is important to further investigate the underlying mechanisms contributing to the stability of the pores in aggregates. Several biotic (organic matter, soil fauna, roots, microbes) and abiotic factors (particle size distribution, clay minerals, exchangeable cations and sesquioxides) influence aggregate stability (Tisdall & Oades, 1982; Le Bissonnais, 1996; Amézketa, 1999; Chenu et al., 2000; Márquez et al., 2004; Bronick & Lal, 2005; Abiven et al., 2009; Regelink et al., 2015). Among this, soil organic matter is most influenced by the land use and hence, can be a highly influential factor in determining the aggregate stability (Yvan et al., 2012). One of such possibilities is the increased carbon accumulation in these pores, as shown by Ananyeva et al. (2011). In their study, they found that larger pores (100µm) are associated with higher carbon accumulation. Therefore, it can be further hypothesised that the organic carbon accumulated in larger pores provides enhanced stability to the pore walls and prevent them from collapse when submerged.

5. Conclusions

The main aim of this paper was to establish links between aggregate pore system characteristics and aggregate stability. We described PSD, and their stability of three different macroaggregate sizes (1-2; 2-5 and 5-10 mm) obtained from three different land uses (arable, grassland and forest). To explain the stability of aggregates, we evaluated the PSD and pore network changes in water stable aggregates before and after wetting. Our results show that smaller (1-2mm) aggregates have a greater degree of X-ray resolvable porosity compared to 2-5 mm or 5-10 mm sized aggregates. We found a significant influence of land use on PSD and water stability, in particular, grassland and forest aggregates were more stable than the arable aggregates. Using data derived from X-ray microtomography images, we demonstrated that the pore system of stable aggregates does not undergo significant changes upon continued submergence in water, indicating that a stable pore system is crucial for aggregate stability. The stability of aggregates has been recognised as a keystone factor

(Abiven *et al*., 2009) for soil fertility and physical resilience to external forces such as wind or water; thus, this paper provides a new mechanistic understanding of WSA as an appropriate indicator for soil quality and health.

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References

Abiven, S., Menasseri, S. & Chenu, C. 2009. The effects of organic inputs over time on soil aggregate stability - A literature analysis. Soil Biology and Biochemistry, **41**, 1–12.

Amézketa, E. 1999. Soil Aggregate Stability : A Review. Journal of Sustainable Agriculture, **14**, 83–151.

Ananyeva, K., Wang, W., Smucker, A.J.M.J.M., Rivers, M.L.L. & Kravchenko, A.N.N. 2013. Can intra-aggregate pore structures affect the aggregate's effectiveness in protecting carbon? Soil Biology and Biochemistry, **57**, 868–875.

Andrew, M., Bijeljic, B. & Blunt, M.J. 2014. Pore-scale imaging of trapped supercritical carbon dioxide in sandstones and carbonates. International Journal of Greenhouse Gas Control, **22**, 1–14.

Bacq-Labreuil, A., Crawford, J., Mooney, S.J., Neal, A.L., Akkari, E., McAuliffe, C., Zhang, X., Redmile-Gordon, M. & Ritz K. 2018. Effects of cropping systems upon the three-dimensional architecture of soil systems are modulated by texture. Geoderma. 332, 73-83.

Bailey, V.L., McCue, L.A., Fansler, S.J., Boyanov, M.I., DeCarlo, F., Kemner, K.M. & Konopka, A. 2013. Micrometer-scale physical structure and microbial composition of soil macroaggregates. Soil Biology and Biochemistry, **65**, 60–68.

Bailey, V., Smith, J. & Bolton, H. 2002. Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. Soil Biology and Biochemistry, **34**, 997–1007.

Banwart, S., Menon, M., Bernasconi, S.M., Bloem, J., Blum, W.E.H., De Souza, D.M., Davidsdotir, B., Duffy, C., Lair, G.J., Kram, P., Lamacova, A., Lundin, L., Nikolaidis, N.P., Novak, M., Panagos, P., Ragnarsdottir, K.V., Reynolds, B., Robinson, D., Rousseva, S., de Ruiter, P., van Gaans, P., Weng, L., White, T. & Zhang, B. 2012. Soil processes and functions across an international network of Critical Zone Observatories: Introduction to experimental methods and initial results. Comptes Rendus - Geoscience, **344**, 758–772.

Bartoli, F., Hallett, P.D. & Cerdan, O. 2016. Le Bissonnais, Y. 1996. Aggregate stability and assessment of crustability and erodibility: 1. theory and methodology. European Journal of Soil Science , 47, 425-437. European Journal of Soil Science, **67**, 5–10.

Le Bissonnais, Y. 1996. Aggregate stability and assessment of soil crustability and erodibility : I . Theory and methodology. European Journal of Soil Science, **47**, 425–437.

Banwart S.A., Nikolaidis N.P., Zhu Y.-G., Peacock C.L. & Sparks D.L. 2019. Soil functions: connecting Earth's critical zone. Annual Review of Earth and Planetary Sciences (in Press).

Blaud, A., van der Zaan, B., Menon, M., Lair, G.J., Zhang, D., Huber, P., Schiefer, J., Blum, W.E.H., Kitzler, B., Wei, E.H., van Gaans, P. & Banwart, S. 2018. The abundance of nitrogen cycle genes and potential greenhouse gas fluxes depends on land use type and little on soil aggregate size. Applied Soil Ecology, **125**, 1–11.

Blunt, M.J. 2001. Flow in porous media - Pore-network models and multiphase flow. Current Opinion in Colloid and Interface Science, **6**, 197–207.

Bronick, C.J.J. & Lal, R. 2005. Soil structure and management: a review. Geoderma, **124**, 3–22.

Chenu, C., Le Bissonnais, Y. & Arrouays, D. 2000. Organic Matter Influence on Clay

Wettability and Soil Aggregate Stability. Soil Science Society of America Journal, **64**, 1479.

Cosentino, D., Chenu, C. & Le Bissonnais, Y. 2006. Aggregate stability and microbial community dynamics under drying–wetting cycles in a silt loam soil. Soil Biology and Biochemistry, **38**, 2053–2062.

Dal Ferro, N., Berti, A., Francioso, O., Ferrari, E., Matthews, G.P. & Morari, F. 2012. Investigating the effects of wettability and pore size distribution on aggregate stability: the role of soil organic matter and the humic fraction. European Journal of Soil Science, **63**, 152–164.

De-Ville. 2017. Hydrological Performance Evolution of Extensive Green Roof Systems. PhD thesis. University of Sheffield, U.K. Available online at http://etheses.whiterose.ac.uk/17718/

De-Ville, S., Menon, M., Jia, X., Reed, G. & Stovin, V. 2017. The impact of green roof ageing on substrate characteristics and hydrological performance. Journal of Hydrology, **547**, 332–344.

De-Ville, S., Menon, M., Jia, X. & Stovin, V. 2018. A longitudinal microcosm study on the effects of ageing on potential green roof hydrological performance. Water (Switzerland), **10**.

De-Ville, S., Demirci, E., Frost, M. & Pattison, I. 2018a. Non-Invasive Property Analysis of Heterogeneous Media Samples: How Small is Too Small?. EGU General Assembly 2018. Vienna, Austria, 8 – 13 April 2018.

Dexter, A.R. 1988. Advances in characterization of soil structure. Soil and Tillage Research, **11**, 199–238.

Dong, H. & Blunt, M.J. 2007. Micro-CT imaging and pore network extraction. Department of Earth Science and Engeneering, PhD Thesis Imperial College London.

Elliott, E.. 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Science Society of America Journal, **50**, 627–633..

Elliott, E.T. & Coleman, D.C. 1988. Let the soil work for us. Ecological Bulletins, 39:23-32.

Ferná ndez, R., Quiroga, A., Zorati, C., Noellemeyer, E., Argentina INTA, R.L.P., Covas, G. & Argentina, L.P. 2010. Carbon contents and respiration rates of aggregate size fractions under no-till and conventional tillage. Soil {&} Tillage Research, **109**, 103–109.

Getter, K.L., Rowe, D.B. & Andresen, J.A. 2007. Quantifying the effect of slope on extensive green roof stormwater retention. Ecological Engineering, **31**, 225-231.

Gostick, J.T. 2017. Versatile and efficient pore network extraction method using marker-based watershed segmentation. Physical Review E, **96**, 23307.

Hénin S. 1938. Etude physico-chimique de la stabilité structurale des terres. (At: https://tel.archives-ouvertes.fr/tel-00752081/document. Accessed: 17/6/2018).

Joekar-Niasar, V., Hassanizadeh, S.M. & Leijnse, A. 2008. Insights into the relationships among capillary pressure, saturation, interfacial area and relative permeability using pore-network modeling. Transport in Porous Media, **74,** 201–219.

Kravchenko, N., Wang, N.W., Smucker, J.M., Rivers, M.L., Kravchenko, A.N., Wang, A.N.W., Smucker, A.J.M. & Rivers, M.L. 2011. Long-term Differences in Tillage and Land Use Affect Intra-aggregate Pore Heterogeneity. Soil Science Society of America Journal, **75**, 1658.

Kemper, W.D., and R. Rosenau. 1984. Soil cohesion as affected by time and water content. Soil Science Society of America Journal, **48,**1001–1006

Kibblewhite, M.G., Ritz, K. and Swift, M.J., 2007. Soil health in agricultural systems. Philosophical Transactions of the Royal Society B: Biological Sciences, **363**, 685-701.

Lair, G.J., Zehetner, F., Hrachowitz, M., Franz, N., Maringer, F.-J. & Gerzabek, M.H. 2009. Dating of soil layers in a young floodplain using iron oxide crystallinity. Quaternary Geochronology, **4**, 260–266.

van Leeuwen, J.P., Djukic, I., Bloem, J., Lehtinen, T., Hemerik, L., de Ruiter, P.C. & Lair, G.J. 2017. Effects of land use on soil microbial biomass, activity and community structure at different soil depths in the Danube floodplain. European Journal of Soil Biology, **79**, 14–20.

Lipiec, J., Walczak, R., Witkowska-Walczak, B., Nosalewicz, A., Słowińska-Jurkiewicz, A. & Sławiński, C. 2007. The effect of aggregate size on water retention and pore structure of two silt loam soils of different genesis. Soil and Tillage Research, **97**, 239–246.

Márquez, C.O., Garcia, V.J., Cambardella, C.A., Schultz, R.C. & Isenhart, T.M. 2004. Aggregate-Size Stability Distribution and Soil Stability. Soil Science Society of America Journal, **68**, 725–735.

Matyka, M., Khalili, A. & Koza, Z. 2008. Tortuosity-porosity relation in porous media flow. Physical Review E - Statistical, Nonlinear, and Soft Matter Physics, **78**, 026306.

Menon, M., Jia, X., Lair, G.J., Faraj, P.H. & Blaud, a. 2015. Analysing the impact of compaction of soil aggregates using X-ray microtomography and water flow simulations. Soil and Tillage Research, **150**, 147–157.

Nunan, N., Ritz, K., Rivers, M., Feeney, D.S. & Young, I.M. 2006. Investigating microbial micro-habitat structure using X-ray computed tomography. Geoderma, **133**, 398–407.

Oades, J.M. 1984. Soil organic matter and structural stability: mechanisms and implications for management. Plant and Soil, **76**, 319–337.

Oades, J.M. 1993. The role of biology in the formation, stabilization and degradation of soil structure. Geoderma, **56**, 377–400.

Papadopoulos, A., Bird, N.R.A., Whitmore, A.P. & Mooney, S.J. 2009. Investigating the effects of organic and conventional management on soil aggregate stability using X-ray computed tomography. European Journal of Soil Science, **60**, 360–368.

Peth, S., Horn, R., Beckmann, F., Donath, T., Fischer, J. & Smucker, A.J.M. 2008. Three-Dimensional Quantification of Intra-Aggregate Pore-Space Features using Synchrotron-Radiation-Based Microtomography. Soil Science Society of America Journal, **72**, 897.

R Foundation for Statistical Computing. R Core Team.

Rabbani, A., Assadi, A., Kharrat, R., Dashti, N. & Ayatollahi, S. 2017a. Estimation of carbonates permeability using pore network parameters extracted from thin section images and comparison with experimental data. Journal of Natural Gas Science and Engineering, **42**, 85–98.

Rabbani, A., Ayatollahi, S., Kharrat, R. & Dashti, N. 2016. Estimation of 3-D pore network coordination number of rocks from watershed segmentation of a single 2-D image. Advances in Water Resources, **94**, 264–277.

Rabbani, A. & Babaei, M. 2019. Hybrid pore-network and Lattice-Boltzmann permeability modelling accelerated by machine learning. Advances in Water Resources, **126**, 116–128.

Rabbani, A., Baychev, T.G., Ayatollahi, S. & Jivkov, A.P. 2017b. Evolution of Pore-Scale Morphology of Oil Shale During Pyrolysis: A Quantitative Analysis. Transport in Porous Media, **119**, 143–162.

Rabbani, A., Jamshidi, S. & Salehi, S. 2014. An automated simple algorithm for realistic pore network extraction from micro-tomography images. Journal of Petroleum Science and Engineering, **123**, 164–171.

Rabbani, A. & Salehi, S. 2017. Dynamic modeling of the formation damage and mud cake deposition using filtration theories coupled with SEM image processing. Journal of Natural Gas Science and Engineering, **42**, 157–168.

Rabot, E., Wiesmeier, M., Schlüter, S. & Vogel, H.-J. 2018. Soil structure as an indicator of soil functions: A review. Geoderma, **314**, 122–137.

Regelink, I.C., Stoof, C.R., Rousseva, S., Weng, L., Lair, G.J., Kram, P., Nikolaidis, N.P., Kercheva, M., Banwart, S. & Comans, R.N.J.J. 2015. Linkages between aggregate formation, porosity and soil chemical properties. Geoderma, **247**–**248**, 24–37.

Rousseva, S., Kercheva, M., Shishkov, T., Lair, G.J., Nikolaidis, N.P., Moraetis, D., Kram, P., Bernasconi, S.M., Blum, W.E.H., Menon, M. & Banwart, S.A. 2017. Soil Water Characteristics of European SoilTrEC Critical Zone Observatories. Advances in Agronomy, **142**, 29–72.

Sheppard, A.P., Sok, R.M. & Averdunk, H. 2004. Techniques for image enhancement and segmentation of tomographic images of porous materials. In: Physica A: Statistical Mechanics and its Applications, **339**, 145–151.

Six, J., Bossuyt, H., Degryze, S., Denef, K., Six, □ J, Bossuyt, H., Degryze, S., Denef, K., Six, J., Bossuyt, H., Degryze, S. & Denef, K. 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil and Tillage Research, **79**, 7–31.

Tisdall, J. & Oades, J. 1982. Organic matter and water-stable aggregates in soils. Journal of soil science, **33**, 141–163.

Utomo, W. H., Dexter, a. R., UTOMO, W.H. & DEXTER, A.R. 1982. Changes in soil aggregate water stability induced by wetting and drying cycles in non-saturated soil. Journal of Soil Science, **33**, 623–637.

Valvatne, P.H., Piri, M., Lopez, X. & Blunt, M.J. 2005. Predictive pore-scale modeling of single and multiphase flow. In: Upscaling Multiphase Flow in Porous Media: From Pore to Core and Beyond, pp. 23–41.

Wang, W., Kravchenko, a. N., Smucker, a. J.M., Liang, W. & Rivers, M.L. 2012. Intra-aggregate Pore Characteristics: X-ray Computed Microtomography Analysis. Soil Science Society of America Journal, **76**, 1159.

Wildenschild, D. & Sheppard, A.P. 2013. X-ray imaging and analysis techniques for quantifying pore-scale structure and processes in subsurface porous medium systems. Advances in Water Resources, **51**, 217–246.

Yoder, R.E. 1936. A Direct Method of Aggregate Analysis of Soils and a Study of the Physical Nature of Erosion Losses1. Agronomy Journal, **28**, 337.

Yvan, C., Stéphane, S., Stéphane, C., Pierre, B., Guy, R. & Hubert, B. 2012. Role of earthworms in regenerating soil structure after compaction in reduced tillage systems. Soil Biology and Biochemistry, **55**, 93–103.

Zhou, H., Fang, H., Mooney, S. J., & Peng, X. 2016. Effects of long-term inorganic and organic fertilizations on the soil micro and macro structures of rice paddies. Geoderma, 266, 66-74.

Figures Captions

Fig. 1. (a-c). Example cross sectional view of (5-10 mm) aggregates from different land uses with different types of pores obtained by processing X-ray microtomography images (a. Arable, b. Grassland and c. Forest).

Fig. 2. Porosity and effective porosity (%) of soil aggregates sizes 1-2 (S), 2-5 (M) and 5-10 (L) mm from arable, forest and grassland soils. Mean and standard error are shown (n = 8, except for 1 – 2 mm where n = 7). Different lowercase letters (a, b or c) show significant ($P < 0.05$) differences between soil aggregates sizes for a specific land use. Different uppercase letters (A, B or C) show significant ($P < 0.05$) differences between land use for a specific soil aggregate size.

Fig. 3. Distribution of water holding, closed, and air space pores (%) of the total pore space of soil aggregates sizes 1-2 (S), 2-5 (M) and 5-10 (L) mm from arable, forest and grassland soils. Mean and standard error are shown (n = 8, except for 1 – 2 mm where n = 7). Different lowercase letters (a, b or c) show significant ($P < 0.05$) differences between soil aggregates sizes for a specific land use. Different uppercase letters (A, B or C) show significant ($P < 0.05$) differences between land use for a specific soil aggregate size.

Fig. 4. Distribution of water stable aggregates (%) of soil aggregates sizes 1-2 (S), 2-5 (M) and 5-10 (L) mm from arable, grassland and forest soils under 4 short, 4 long and 9 short wetting and drying cycles (WDCs). Mean and standard error are shown (n = 3). Different minuscule letters show significant ($P < 0.05$) differences between WDCs for a specific soil aggregate size and soil. Different capital letters show significant ($P < 0.05$) differences between soil aggregate sizes for a specific soil and WDC. Different italic capital letters show significant ($P < 0.05$) differences between soils for a specific soil aggregate size and WDC.

The minuscule and non-italic capital letters were not shown for grassland because no significant differences were found.

Fig. 5. Porosity and effective porosity (%) of soil aggregates size a) 2-5 (M) & b) 5-10 (L) mm from grassland and forest soils before and after 24h wetting in water. Mean and standard error are shown (n = 3). ** indicate significant ($P < 0.01$) difference between before and after wetting.

Fig. 6. Distribution of water holding, closed, and air space pores of soil aggregates size a) 2-5mm & b) 5-10 mm from forest and grassland soils before and after wetting 24h in water. Mean and standard error are shown (n = 3). *** indicate significant difference

Fig. 7. Distribution of pore sizes (%) < 30 μm, 30-100 μm and > 100 μm in aggregates 2-5 mm and 5-10 mm from forest and grassland soils before and after wetting 24h in water. Mean and standard error are shown (n = 3). Different lowercase letters (a, b or c) show significant ($P < 0.05$) differences between soil pore size for a specific aggregates size, wetting state and land use. Different uppercase letters (A, B or C) show significant ($P < 0.05$) differences between land use for a specific soil aggregate size, pore size and wetting state. No significant ($P > 0.05$) difference was found before and after wetting.

Table 1. Soil characteristics and soil aggregate size distribution of bulk soil samples on a dry mass basis at the time of sampling. Mean value ± one standard deviation (n = 3) are shown.

	Arable	Grassland	Forest
Location	48°09'N, 16°41'E	48°11'N, 16°44'E	48°08'N, 16°39'E
Water content (%)	11.3 ± 0.26	12.0 ± 0.26	17.1 ± 0.69
Soil pH (H_2O)	7.7 ± 0.14	7.4 ± 0.09	7.4 ± 0.17
Organic C (%)	2.4 ± 0.36	5.0 ± 0.60	3.8 ± 0.28
Total N (%)	0.13 ± 0.01	0.33 ± 0.04	0.25 ± 0.02
C_{org}/N	18.1 ± 1.83	15.0 ± 0.52	15.1 ± 1.02
$CaCO_3$ (%)	19.0 ± 1.90	21.1 ± 1.41	20.4 ± 0.62
Sand, 63-2000 µm (%)	32.7	8.2	22.5
Silt, 2-63 µm (%)	43.8	63.0	51.2
Clay, < 2 µm (%)	23.5	28.8	26.3

Soil Dry Aggregate Distribution (%)

> 10 mm	37.3 ± 9.1	7.9 ± 2.4	11.9 ± 4.4
5.0 - 10.0 mm	14.6 ± 2.4	21.5 ± 2.0	18.3 ± 2.7
2.0 - 5.0 mm	20.5 ± 4.0	37.8 ± 3.6	31.2 ± 2.2
1.0 - 2.0 mm	11.8 ± 2.4	14.5 ± 0.5	23.1 ± 8.4
0.5 - 1.0 mm	6.4 ± 3.5	5.2 ± 0.4	5.9 ± 1.7
0.25 - 0.5 mm	7.1 ± 4.6	6.9 ± 0.1	7.5 ± 2.7
< 0.25 mm	1.9 ± 1.3	6.1 ± 0.7	2.0 ± 0.8

Table 2. Changes in pore network characteristics in grassland and forest 2-5 (M) and 5-10 (L) mm aggregates after 24 hours submergence.

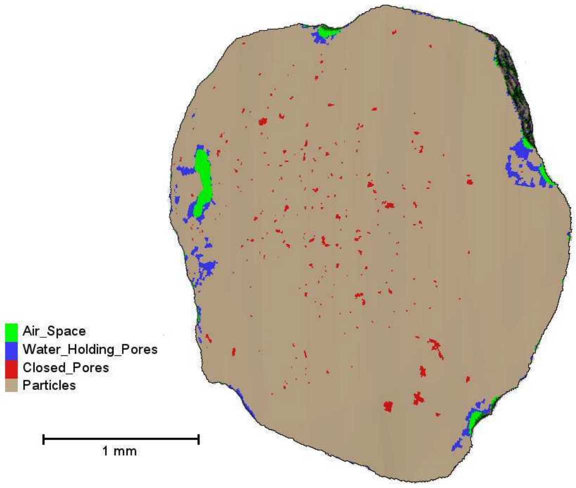
	Grassland Aggregates				Forest Aggregates			
	M (n=3)		L (n =3)		M (n=3)		L (n=3)	
	Before Wetting	After Wetting	Before Wetting	After Wetting	Before Wetting	After Wetting	Before Wetting	After Wetting
Pore Radius (µm)	48.59±2.84	45.44±1.91	95.74±1.75	96.3±1.73	46.14±1.04	44.80±1.54	94.91±3.39	94.62±2.85
Pore throat radius (µm)	16.99±1.17	19.23±2.71	36.41±1.38	34.54±1.56	17.33±0.32	18.55±1.25	37.01±2.96	36.16±3.31
Pore throat length(µm)	200.12±37.02	212.36±29.12	428.24±35.26	419.89±24.12	161.79±4.46	170.99±10.28	363.21±20.20	346.08±4.00
Average pore connectivity	7.29±0.61	3.18±0.47	4.17±0.31	5.02±1.11	7.47±0.68	5.07±2.33	5.19±0.49	6.07±0.63
Tortuosity (x)	1.39±0.09	1.78±0.25	1.59±0.01	1.45±0.10	1.32±0.09	1.58±0.30	1.73±0.17	1.51±0.04
Tortuosity (y)	1.33±0.04	2.18±0.57	1.63±0.08	1.61±0.09	1.35±0.12	1.63±0.32	1.45±0.02	1.46±0.13
Tortuosity (z)	1.44±0.06	2.07±0.50	1.50±0.08	1.61±0.11	1.36±0.04	1.50±0.20	1.41±0.03	1.41±0.08

Table 3 (a) Bootstrap statistics of Spearman correlation coefficient between WSA method 1 and pores characteristics. Significant correlation are shown in bold ($P < 0.001$).

Landuse	Bootstrap statistics	Porosity	Effective porosity	Closed pores	Water holding pores	Air space pores
All LU	Original value	**-0.25**	**-0.24**	**0.29**	0.10	**0.23**
	bias	0.0031	0.0011	0.0005	-0.0004	-0.0013
	Std error	0.080	0.076	0.076	0.073	0.070
	95% Conf Int	-0.40; -0.09	-0.38; 0.08	0.13; 0.43	-0.04; 0.25	0.08; 0.36
Arable	Original value	**-0.51**	**-0.51**	**0.77**	**0.65**	**0.75**
	bias	0.0079	0.0073	-0.0089	-0.010	-0.0098
	Std error	0.110	0.113	0.070	0.090	0.074
	95% Conf Int	-0.69; -0.25	-0.69; -0.24	0.59; 0.87	0.43; 0.79	0.56; 0.86
Grassland	Original value	**-0.46**	**-0.35**	**0.74**	**0.61**	**0.78**
	bias	0.0032	0.0032	-0.0060	-0.0077	-0.0066
	Std error	0.105	0.116	0.060	0.079	0.055
	95% Conf Int	-0.64; -0.22	-0.56; -0.09	0.59; 0.83	0.44; 0.75	0.63; 0.86
Forest	Original value	-0.07	0.08	**0.90**	**0.88**	**0.90**
	bias	-0.0017	-0.0002	-0.0082	-0.0091	-0.0090
	Std error	0.157	0.160	0.023	0.029	0.022
	95% Conf Int	-0.36; 0.25	-0.24; 0.39	0.86; 0.94	0.82; 0.93	0.86; 0.94

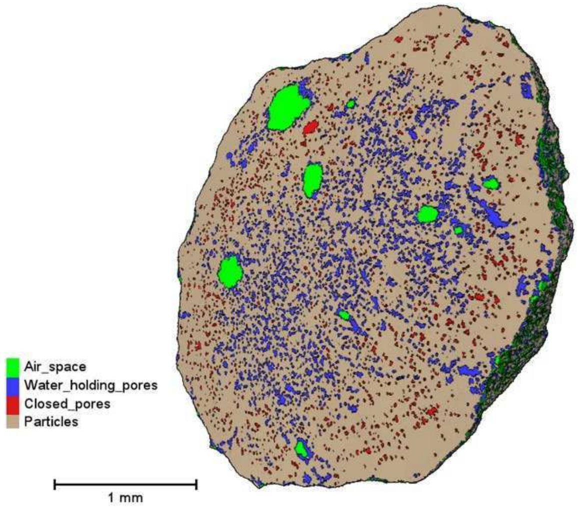
Table 3 (b). Bootstrap statistics of linear regression r^2 between WSA method 1 and pores characteristics. Significant correlation are shown in bold ($P < 0.001$).

	Bootstrap statistics	Porosity	Effective porosity	Closed pores	Water holding pores	Air space pores
Arable	Original value	**0.36**	**0.36**	**0.15**	**0.60**	**0.39**
	bias	0.0015	0.0016	0.012	-0.0019	0.0057
	Std error	0.088	0.088	0.086	0.080	0.112
	95% Conf Int	0.18; 0.52	0.18; 0.53	0.02; 0.34	0.42; 0.74	0.16; 0.60



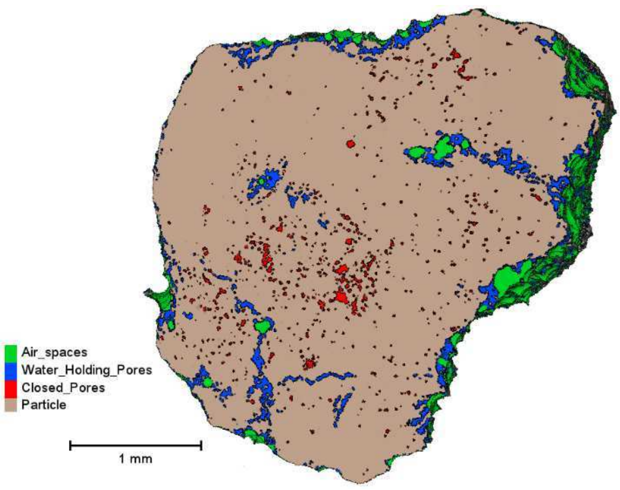
Air_Space
Water_Holding_Pores
Closed_Pores
Particles
1 mm

(a)



Air_space
Water_holding_pores
Closed_pores
Particles
1 mm

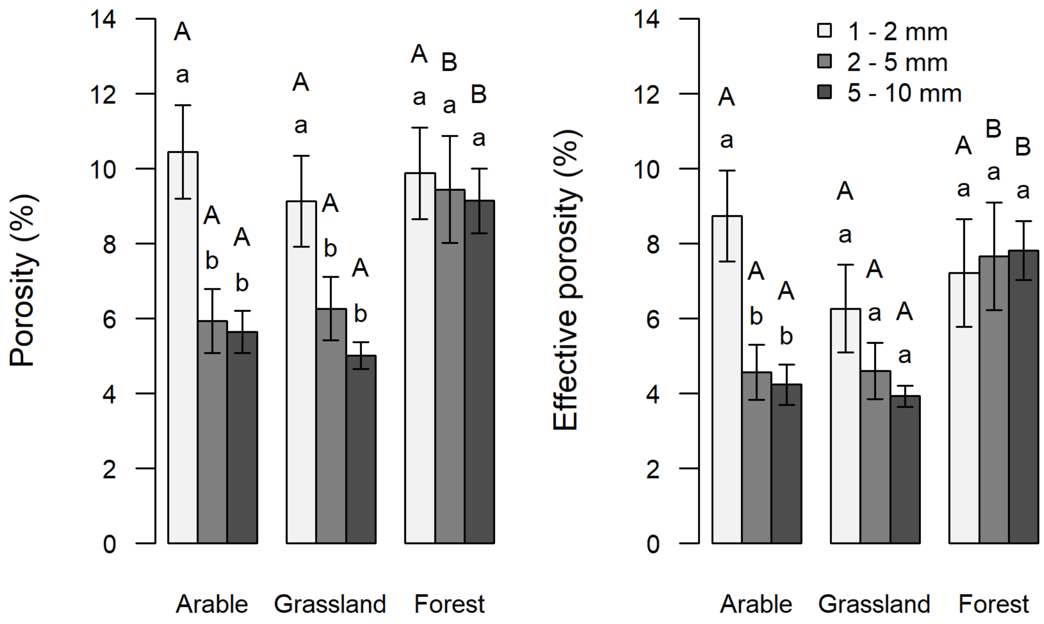
(b)



Air_spaces
Water_Holding_Pores
Closed_Pores
Particle
1 mm

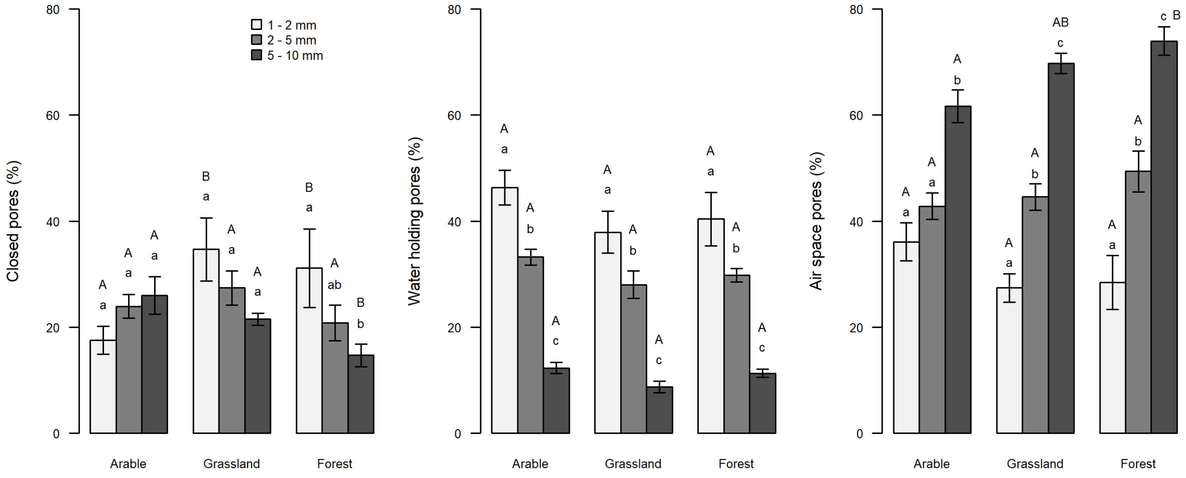
(c)

Figure 1.



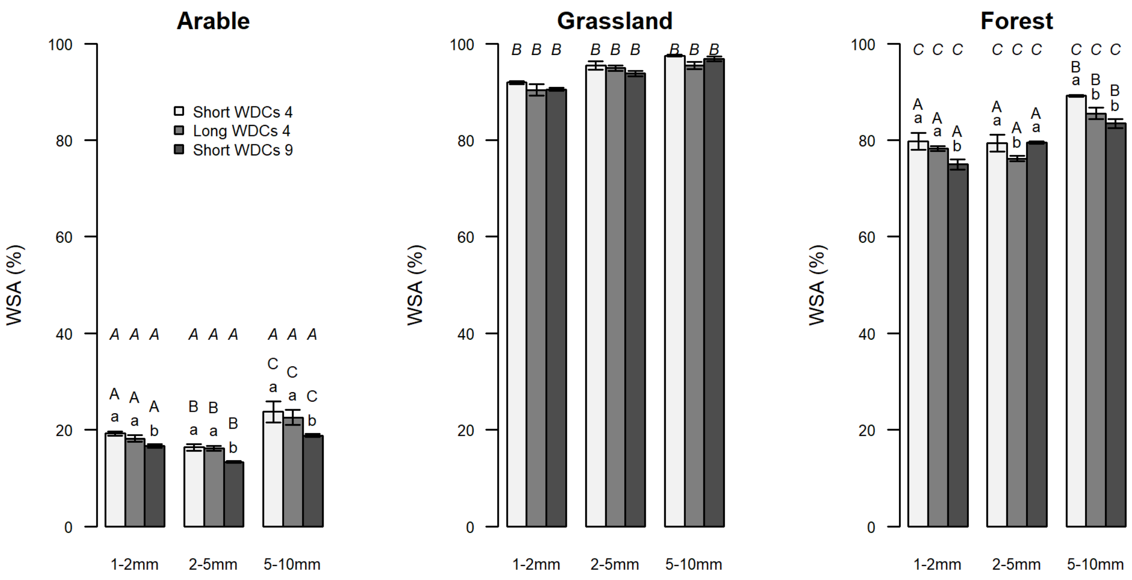
Porosity (%)
Effective porosity (%)
1 - 2 mm
2 - 5 mm
5 - 10 mm
Arable
Grassland
Forest

Fig. 2.



1 - 2 mm
2 - 5 mm
5 - 10 mm
Closed pores (%)
Water holding pores (%)
Air space pores (%)
Arable
Grassland
Forest

Fig. 3.



Arable
Grassland
Forest
Short WDCs 4
Long WDCs 4
Short WDCs 9
WSA (%)
0
20
40
60
80
100
1-2mm
2-5mm
5-10mm

Fig. 4.

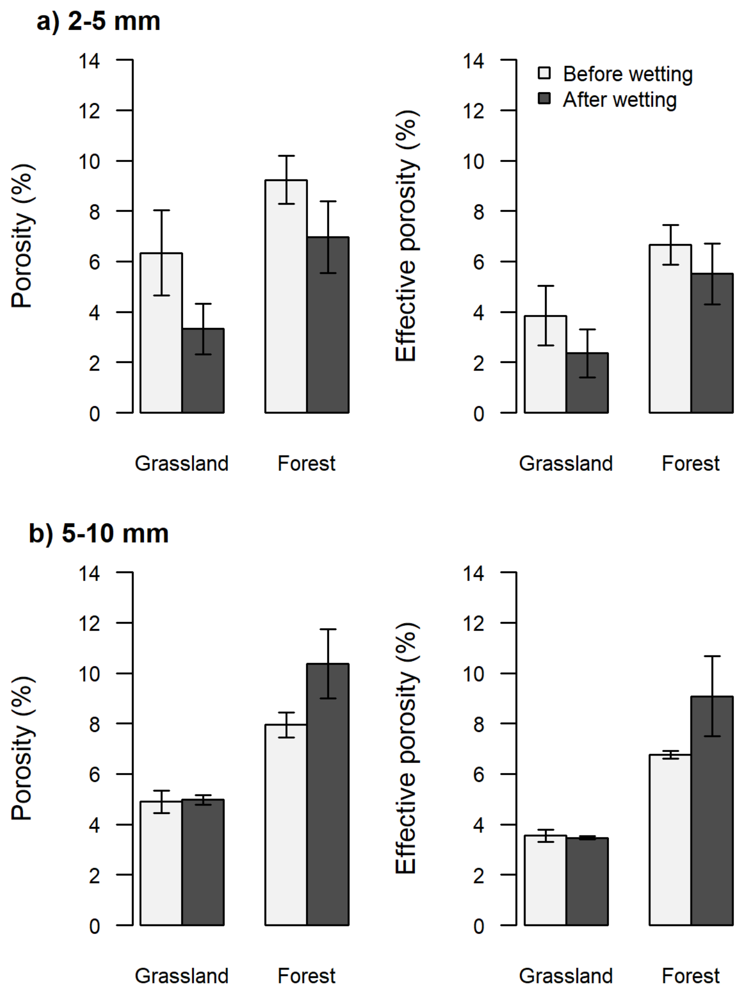
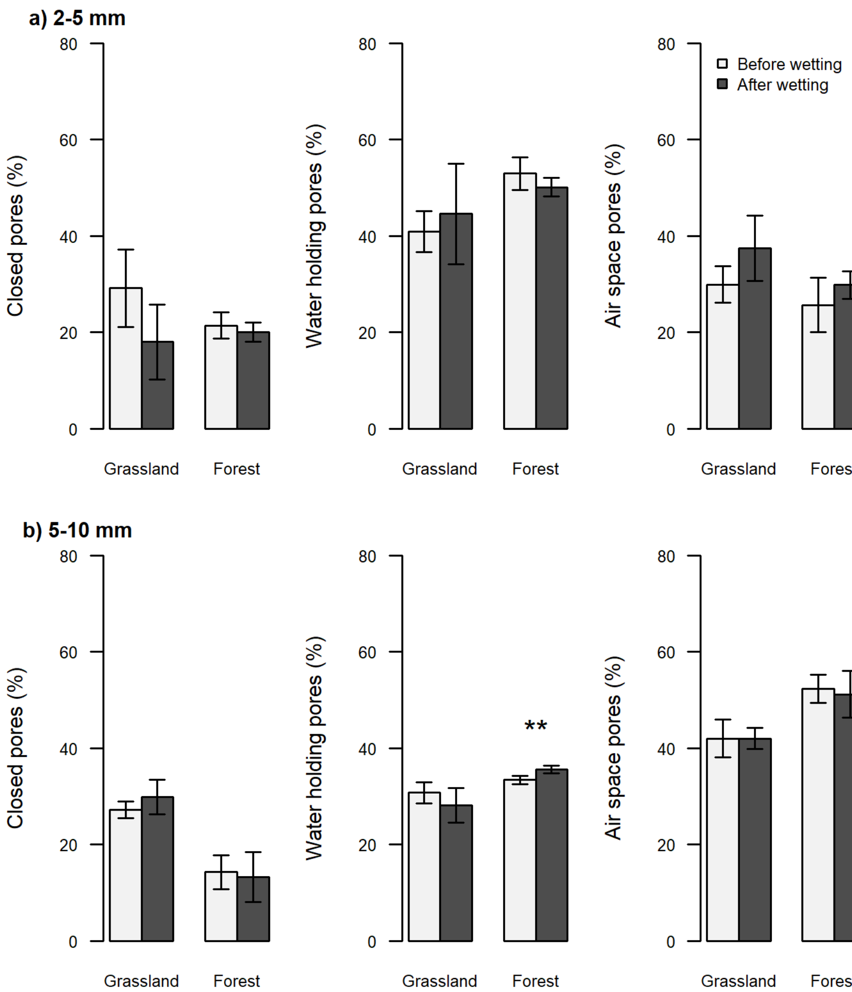
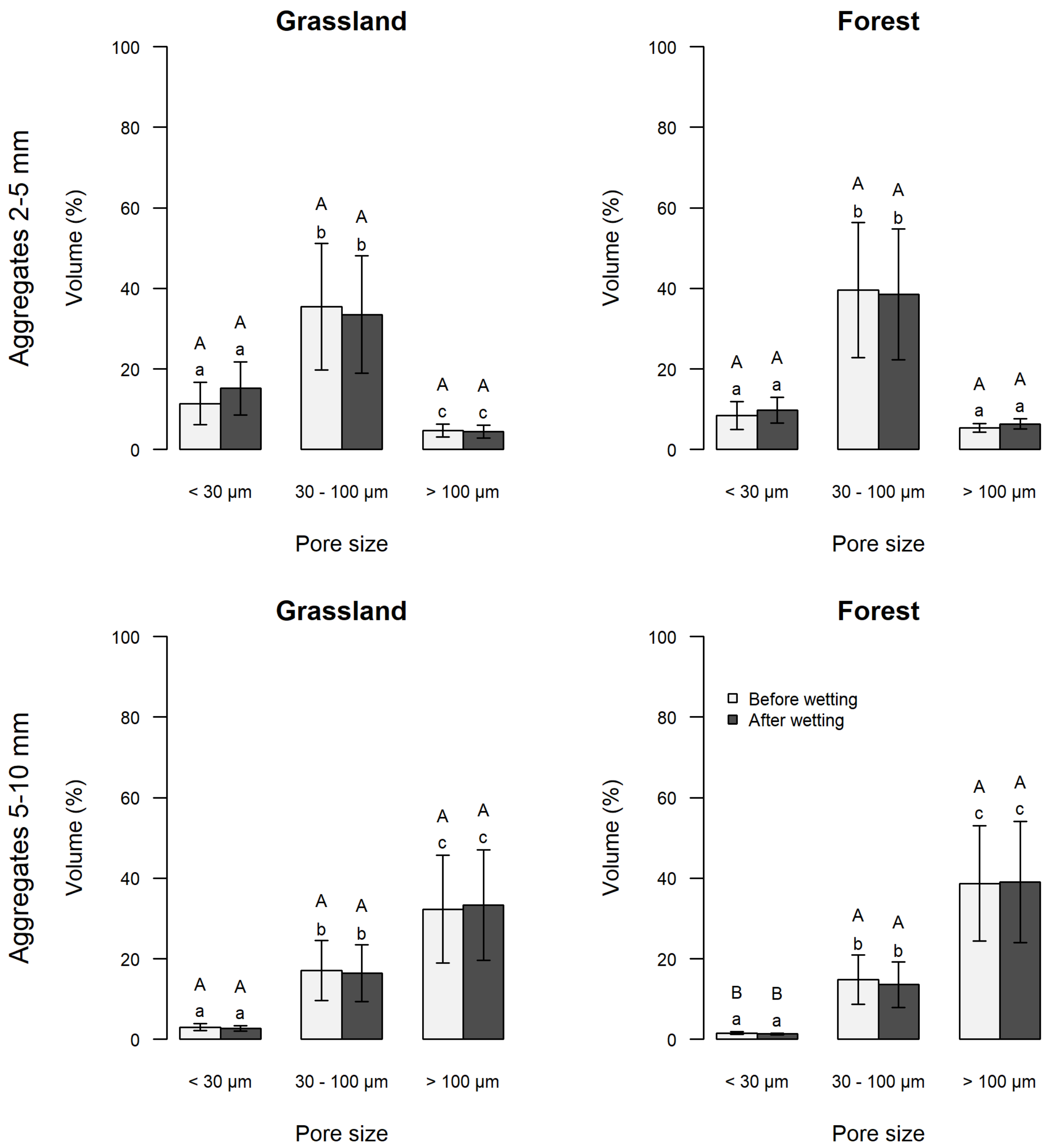

a) 2-5 mm
Porosity (%)
Effective porosity (%)
Before wetting
After wetting
Grassland
Forest
b) 5-10 mm
Porosity (%)
Effective porosity (%)
Grassland
Forest
0
2
4
6
8
10
12
14

Fig. 5.



a) 2-5 mm
b) 5-10 mm
Closed pores (%)
Water holding pores (%)
Air space pores (%)
Before wetting
After wetting
Grassland
Forest
**
0
20
40
60
80

Fig. 6.



Grassland
Forest
Aggregates 2-5 mm
Aggregates 5-10 mm
Volume (%)
0
20
40
60
80
100
A
B
a
b
c
< 30 µm
30 - 100 µm
> 100 µm
Pore size
Before wetting
After wetting

Fig. 7.

Supplementary Material 1 for image processing and pore network extraction

1. **Image acquisition settings**

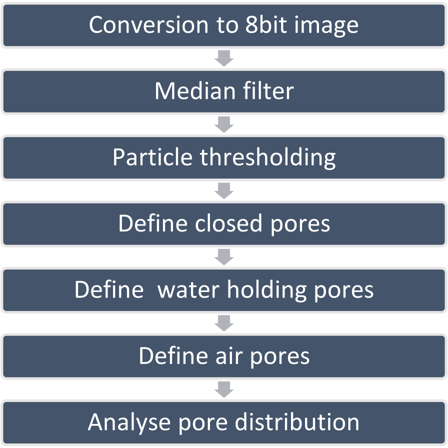
Beam hardening and partial volume effects can reduce the quality of the images. In order to avoid the beam hardening effect, metal filters are used in the micro-CT scanner to pre-harden the beam. Also, in order to reduce the partial volume effect, the rotation steps are selected to be as low as 0.700 degree. Other imaging settings are included in Table A.1.

Table A.1. Imaging settings used to scan aggregates of different sizes in this study

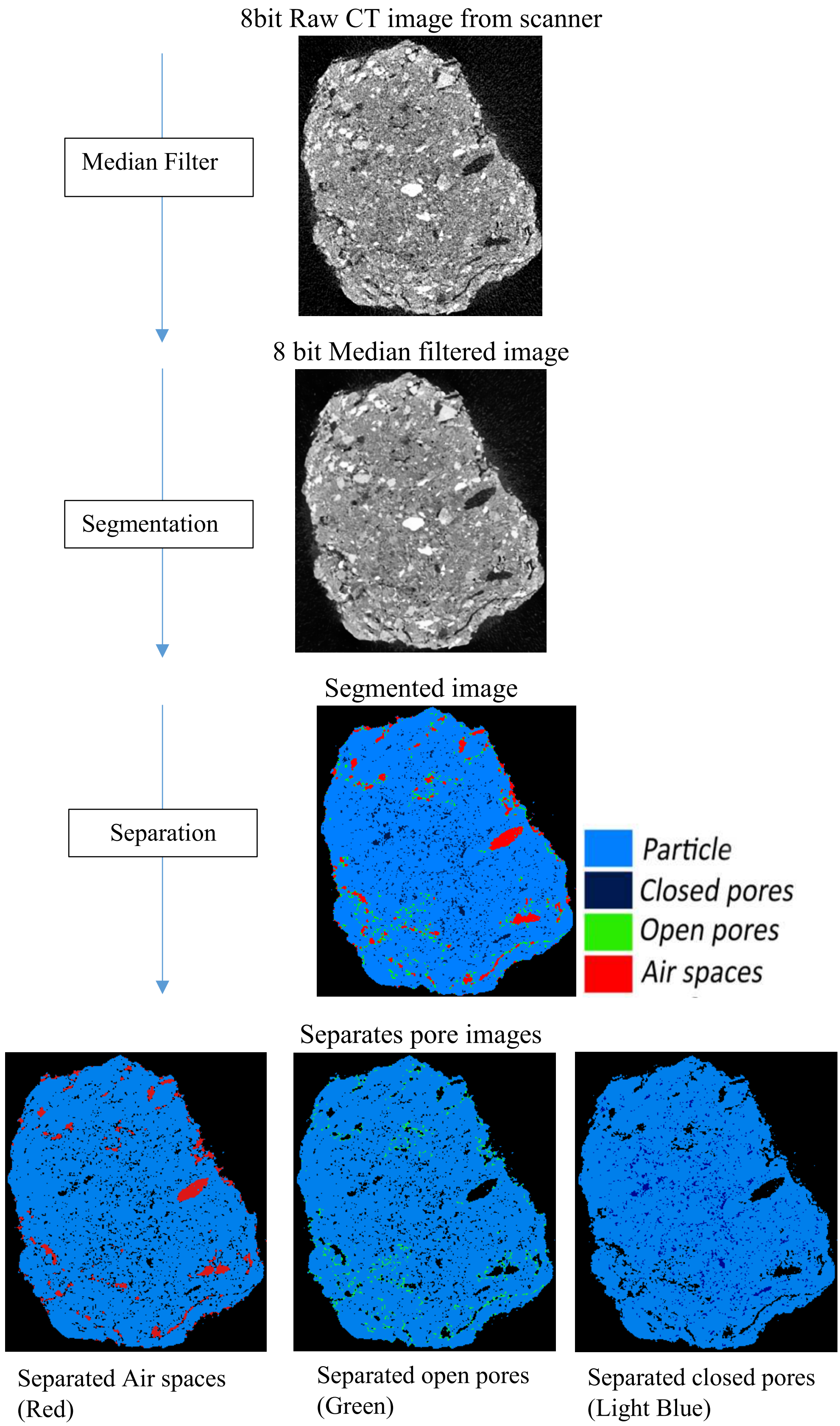
Image acquisition settings	Aggregate size (mm)	
	1-2 or 2-5	5-10
Source voltage (kV)	49	70
Source current (uA)	200	141
Camera pixels	1048 x 2000	
Imager rotation	0.32	
Pixel size	5.01	10.02
Object to source (mm)	46.755	93.465
Camera to source (mm)	214.136	
Al Filter 0.5 mm	Yes	
Image format	TIFF	
Exposure (ms)	295 or 590	
Rotation step (deg)	0.700	
360 rotation	Yes	
Median filter	On	
Flat field correction	On	
Geometrical correction	On	
Scanning trajectory	Round	

2. Image processing steps (Performed using Avizo 9.0.1)

Initially, 16bit Grayscale raw images obtained from the several different CT scans carried out were converted to 8bit images to reduce the computational requirements for processing. The resultant greyscale images obtained were then filtered using a 3D median filter (Avizo 9.0.1) with a neighbourhood of 6 and 1 iteration to reduce image noise. Next, a global thresholding algorithm based on the histogram of the greyscale image was applied to delineate soil mineral particles (higher attenuation values) from their corresponding background (lower attenuation values). Afterwards, to define the **closed pores** within aggregates, a 3D fill holes algorithm with a neighbourhood of 6 was applied which sealed off the isolated pores, these delineated pores were then delineated by subtracting the result from the defined particle mask. To define the **water holding pores**, a ball closing algorithm with a diameter of 50µm (equivalent number of pixels) was used to seal off all pores connected to the surface with a diameter smaller than 50µm. The resultant output mask was then subtracted from the particle mask with the defined closed pores being removed leaving only the required sized pores delineated. Lastly, to define the **air pores**, the particle mask was closed using a disc closing algorithm with a kernel size large enough to fully seal all the large and small internal pores (around 2.5% of the particle length). The disc instead of the ball was used as it reduced aggregate surface space inclusions by about 20%. The air pores were then delineated by subtracting particles, closed and water holding pores from the output mask leaving the air spaces. The steps followed for the above-described process are summarised in Supp. Figure 1 & 2 below.



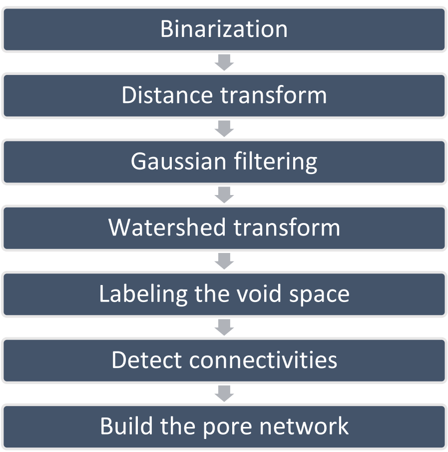
Supp. Fig. 1. Steps involved in image processing of aggregates



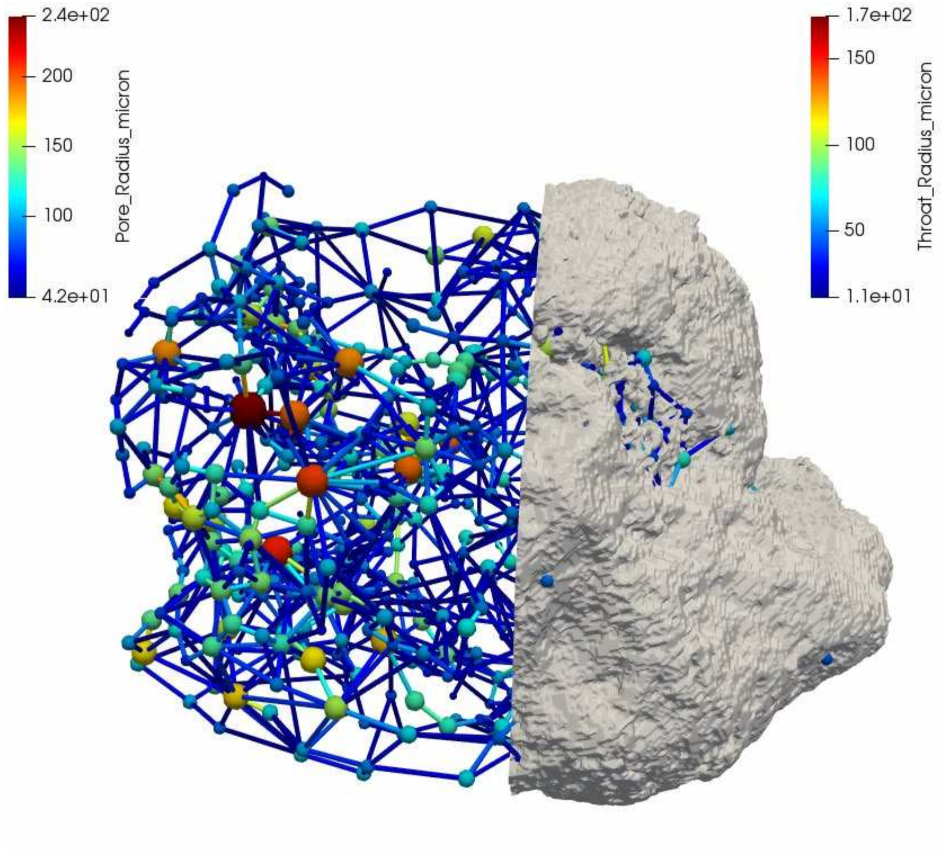
Supp. Fig. 2. Visualisation of major steps involved in image processing of aggregates

3. Pore Network Extraction steps

Binarised images of soil aggregates were analysed to extract the pore network model using watershed segmentation algorithm. We used an in-house code to define what part of the network is composed of pore-bodies and how they are connected to each other by pore-throats. In this approach, initially, we performed distance transform on 3-D binarised images and applied Gaussian filtering to avoid over-segmentation of the porous media. Then watershed transform was applied on the images that create several growing nuclei at the centre of the pores in which distance value is locally maximized. These nuclei keep growing based on the distance values until they touch a nucleus from the neighbouring pore. Then the touching voxels were recorded as pore-throats. The process continued until the whole volume of the void space be filled with the growing nuclei. Then, we subtracted the pore-throat voxels from the void geometry and applied morphological labelling to address each detected pore which was by then isolated from its neighbours. In the next step, with a 3×3×3 sliding window we browsed the whole labelled geometry to find and label the connections between each pair of pores as pore-throats. Finally, we measured the size of each pore-throat and thus, the network was fully extracted. See suppl. Figure 3 illustrates the steps in the described workflow and example pore network extracted is given in Suppl. Figure 4.



Suppl. Figure 3. Pore network extraction process using watershed segmentation algorithm



Suppl. Figure 4. Inside view of pore and throat sizes of an grassland aggregate (5-10 mm) based on the extracted pore network.