Quantifying the impact of microbes on soil structural development and behaviour in wet soils

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Abstract

There is evidence that microbial populations play an important role in altering soil pore geometry, but a full understanding of how this affects subsequent soil behaviour and function is still unclear. In particular the role of microorganisms in soil structural evolution and its consequence for pore morphological development is lacking. Using a combination of bio-chemical measurements and X-ray Computed Tomography (CT) imaging, a temporal comparison of microscale soil structural development in contrasting soil environments was made. The aim was to quantify the effect of microbial activity in the absence of other features likely to cause soil deformation (e.g. earthworms, roots etc.) on soil structural development in wet soils, defined by changes in the soil porous architecture i.e. pore connectivity, pore shape and pore volume during a 24 week period. Three contrasting soil textures were examined and changes compared between field soil, sterilised soil and a glucose enhanced soil treatment. Our results indicate that soil biota can significantly alter their microhabitat by changing soil pore geometry and connectivity, primarily through localised gaseous release. This demonstrates the ability of microorganisms to modify soil structure, and may help reveal the scope by which the microbial-rich rhizosphere can locally influence water and nutrient delivery to plant roots.

1. Introduction

Our understanding of the spatiotemporal nature of soil structure remains limited, despite its importance for nutrient availability to plant roots, gaseous exchange through the soil profile and movement of solutes through pore networks (Hinsinger et al., 2009). It has long been acknowledged that soil microbial activity and function are closely linked to structure (Crawford et al., 2012; Oades, 1984; Six et al., 2004), with processes related to soil biological activity such as: i) the microbial exudation of secondary metabolites and binding agents (Bossuyt et al., 2001; Six et al., 2006); and ii) particle enmeshment by fungal communities (Moreno-Espíndola et al., 2007; Tisdall, 1994) both increasing adhesion between soil particles and generating particle aggregation. However, the rate, nature and scale by which changes occur remains uncertain.

Soil microstructure is known to regulate microbial function (Young and Ritz, 2000), largely through influencing the spatial distribution of soil biota (Nunan et al., 2006). Distinct local microenvironments associated with microbial communities cause highly variable biological activity throughout the soil matrix (Fenchel, 2003), with evidence for microorganism patchiness confined to specific niches recorded at hundred μm scales (e.g. Nunan et al. (2003)). The consequence of microorganism patchiness in altering the functional response of soil systems has helped to elucidate driving factors responsible for ecological community development (Parkin, 1993). For example, enrichment of substrate through organic matter at the soil surface enables microbial proliferation of the upper soil horizons, inducing enhanced soil aggregate size and stability (Bossuyt et al., 2001; De Gryze et al., 2006a). However, knowledge regarding the degree and timescales over which soil structural development takes place in terms of aggregation and pore evolution is still unclear, mainly due to the issue of scale and access to appropriate technology to probe this. The majority of previous work in this area has

71 focused on soil structural development at the macroscale (>150 μm) largely due to the
72 resolution of the equipment available at the time (e.g. Atkinson et al. (2009)).
73 Macroaggregate formation and re-stabilisation rates have been correlated with microbial
74 activity, increasing with the addition of microbial substrate and decreasing rapidly as
75 the substrate is utilised (De Gryze et al., 2006b). More recent investigations have
76 successfully quantified the influence of microbial activity on soil aggregate formation
77 for various mycorrhizal fungal treatments (Hallett et al., 2009), roots (Martin et al.,
78 2012) and clay mineralogy (Barré and Hallett, 2009), demonstrating the diverse nature
79 of microbe-soil-plant interactions. However, macroscale quantification of soil structure
80 is perhaps not the most relevant scale for investigations of the soil microbial
81 community. It follows that when investigating soil aggregate and subsequent pore
82 formation, quantification at the microscale can provide a more rigorous assessment of
83 microbial-induced aggregation due to the microscopic changes to structure anticipated,
84 that may be missed at coarser resolutions. At this smaller scale, changes to pore size
85 distributions within individual aggregates (Feeney et al., 2006) and aggregate formation
86 under wheat residue addition (De Gryze et al., 2005) indicate the potential for microbial
87 driven structural changes that can occur in just a few weeks. Likewise Crawford et al.
88 (2012) demonstrated an increase in porosity and surface connectivity in samples
89 harbouring fungal activity over a 25 day incubation, but no significant effect of bacterial
90 activity in isolation or bacteria and fungi combined on soil structure at a resolution of 53
91 μm. However, quantification of the microbial impact on soil morphological
92 development at smaller pore scales, its impact over longer timeframes, and subsequent
93 consequences for the overall connectivity of pore systems, remains unknown.
94
95 Previous research exploring the impact of microorganisms in the evolution of soil
96 structure has been limited by our inability to non-invasively visualise the soil

environment. Advances in industrial X-ray micro CT (Computed Tomography) in the last five years, in particular, provide a higher image quality and resolution, with shorter scan times resulting in more samples analysed in a given time period. In this paper, we present a fully replicated experiment with multiple time points from three contrasting microbial treatments. Three different soil types per microbial treatment were each held at a water content higher than the air entry potential, simulating soil water contents found in moist field soils. The aims were to quantify the temporal influence of microbial activity on soil structure and pore morphology development (defined by changes in porosity, pore connectivity, volume, and shape during the incubation period), by selectively inhibiting and promoting microbial growth. We examined the potential for soil microorganisms to i) alter the physical architecture of soil over time in the absence of other biotic features liable to cause soil deformation (e.g. earthworms, roots); ii) influence the surface connected pore architecture of initially unconnected pore space in homogenised soil; iii) alter the size, shape and nature of pore space; and iv) alter soil structure in the absence of abiotic factors e.g. localised wetting and drying cycles. We hypothesise that microbial populations in carbon enhanced soils will have significantly higher biomass (Joergensen and Raubuch, 2002) and activities (Bossuyt et al., 2001; Steinbeiss et al., 2009) than those in field soil, which will alter the physical soil matrix to a more porous state.

2. Methods

2.1. Soil Column Preparation and Sampling

A loamy sand soil from the Newport series (sand 83.2%, silt 4.7%, and clay 12.1%; pH 6.35; organic matter 2.93%; FAO Brown Soil) taken from the University of Nottingham farm at Bunny, Nottinghamshire, UK (52.8586°, -1.1280°), a silty clay loam soil from the Batcome series (sand 18.3%, silt 49.0%, and clay 32.7%; pH 5.76; organic matter

7.02%; FAO Chromic Luvisol) taken from Rothamsted Highfield Arable plot, UK (51.8047°, -0.3628°), and a clay loam soil from the Worcester series (sand 35.6%, silt 31.5%, and clay 32.9%; pH 6.50; organic matter 5.19%; FAO Argillic Pelosol) also taken from the University of Nottingham farm at Bunny, were sieved at field moisture to <2 mm. A sub-section of each soil texture was γ-irradiated at 27 kGy (Isotron, Daventry, UK) to sterilise the soil. Irradiation at this level is reported to eliminate all fungi, actinomycetes, algae, invertebrates and bacteria from the soil (McNamara et al., 2003). Four sterile replicate columns (25 mm diameter x 70 mm height) for each soil texture were uniformly packed to a bulk density of 1.2 Mg m^{-3} for three microbial treatments: i) Unaltered 'field' soil; ii) γ-irradiated 'sterile' soil; and iii) Glucose amended 'enhanced activity' soil . Columns were packed in four layers, and the surface of each layer scarified prior to additional layers being added, in order to prevent compaction layers being produced (confirmed later by X-ray imaging). All columns were saturated with sterilised (autoclaved) deionised water and left to drain for three days to reach a notional field capacity (confirmed with water release curve data, Figure S1). Micropore tape was placed over the macrocosms to reduce water loss and prevent sample contamination, whilst still enabling gaseous exchange. Macrocosms were incubated for 24 weeks in a glass fronted incubator at 16°C. The water status of the columns was maintained (determined by weight) by addition of sterile deionised water every 1-2 days in equal amounts to the top and bottom of the columns. To the glucose enhanced soil, glucose was added in equal measures to the top and bottom of the macrocosm every 2 days totalling 0.05 mg C g^{-1} soil day^{-1}, a rate which is reported to be similar to rhizodeposition (Preston et al., 2001). A sterile control was used solely to ascertain the influence of the watering regime on the resultant temporal changes to soil porosity.

149 *2.2. X-ray Computed Tomography (CT)*

150 CT scanning was conducted using a Phoenix Nanotom 180NF scanner (GE Sensing and
151 Inspection Technologies, Wunstorf, Germany) set at 120 kV and 100 µA, with a 0.1
152 mm Cu filter. The voxel size was set at 12.38 µm, with the centre of the macrocosm 55
153 mm from the X-ray source and each scan taking 33 minutes to complete. The total
154 number of projection images for each column was 720 per scan at a detector size of
155 2304 x 2304 pixels, creating *ca.* 5 GB file sizes when reconstructed as 8-bit volumes.
156 Four replicate columns per soil texture per microbial treatment (36 in total) were
157 repeatedly scanned at weeks 0, 2, 4, 8, 16 and 24 of incubation, generating a total of 180
158 scans over the course of the investigation (the scanner was unavaliable for week 16 of
159 the glucose treatment). The precise positioning of the scanner manipulator stage and
160 reconstructed region in all subsequent scans ensured that each column was repeatedly
161 scanned and analysed in exactly the same location throughout the investigation,
162 enabling direct comparisons of porosity and pore characteristics to be made over time.
163 Samples were scanned in a random order at each sampling interval to minimise the
164 influence of short-term diurnal changes in pore development. The dose to the centre of
165 each macrocosm was calculated at 3.9 Gy for each scan, equating to a total of 23.1 Gy
166 over the course of the 24 week investigation. This is well below the limit of ~10 kGy
167 reported to cause damage to soil-borne microbial populations (McNamara et al., 2003;
168 Zappala et al., 2013).

169

170 *2.3. Image Processing, Segmentation and Analysis*

171 Raw grey-scale X-ray CT images were processed using ImageJ 1.44
172 (http://rsbweb.nih.gov/ij/). A uniform contrast enhancement was applied to 1% of
173 saturated pixels in order to improve image clarity, followed by a Median filter of radius
174 1.5 pixels to remove noise in the data but maintain feature borders. To separate pores

from the surrounding soil matrix, the *Li* global automatic threshold algorithm was used (Li and Tam, 1998). This uses an iterative method based on selecting the threshold which minimises cross entropy between the segmented and original image. Prior to this, different manual and automatic thresholds were compared on a subset of images to assess the optimum global thresholding technique and to optimise the image analysis routine. The chosen routine was representative of soil porosity across all soil textures throughout the soil profiles as illustrated by manual comparison of raw to binarised images at multiple depths along each macrocosm. A 14.36 x 14.36 x 24.76 mm (1160 x 1160 x 2000 pixels) region of interest was cropped to exclude the area outside of the soil column and to reduce edge effects, before bright and dark pixels of radius 1.0 pixels were removed using the *Remove Outliers* tool to convert selected features to the opposite phase in the binary image and thus exclude any remaining noise. The resulting binary images were analysed using the *Analyse Particles* tool, which scans the binarized pores in 2D image slices until it finds their edge, measures their individual area and circularity, makes it invisible, and then resumes scanning of the next pore. In this way information for the total porosity for each individual image (2000 images in total per scan), and the pore shape (circularity) for each individual pore within each image (*ca.* ~3 million pores per image stack) were calculated. Pores less than 2 pixels in diameter were excluded from the analyses, as the minimum object size accurately detectable is often viewed as twice the scanning resolution (Rogasik et al., 2003). Therefore, the imaging resolution in this study was 24.76 μm.

Surface pore connectivity was assessed for each sample using a novel flood-fill principal available as a tool in VG Studio Max® 2.0 software (Figure 1). Briefly, a black image slice representing absolute pore space was manually inserted into the top of the image stack (Figure 1b), and used as a seedbed for the *Region Growing* selection tool.

This selects pore space directly connected to the adjacent image above, effectively 'tracking' pore space with a direct connection to the soil surface down through the soil profile. At the point at which connection to the surface is lost, the measurement ceases. By isolating the surface connected pore space as a region of interest and removing the original artificial pore slice from the selection, the method gives a visual representation of surface connected pore development with time (Figure 1c). By comparing the temporal ratio of connected pore volume to total sample porosity, the technique provides a means of quantifying the spatial evolution of the pore network over time and allows changes in microscale surface-connected pore architecture to be mapped.

2.4. Microbial Biomass and Activity

Microbial biomass C and biomass N were estimated by fumigation extraction (modified from Vance et al. (1987)). Briefly, two sub-samples of 12.5 g fresh weight soil were destructively harvested from the macrocosms previously used for microbial respiration analysis. One sub-sample was fumigated for 24 h at 25°C with ethanol-free $CHCl_3$. Following fumigation the soil was extracted with 50 ml 0.5 M K_2SO_4 by 1 hour of orbital shaking, and filtered. The non-fumigated sub-sample was extracted similarly at the time fumigation began. Extracts were frozen at -20°C until analysis, and then defrosted and analysed on a Shimadzu TOC-V CPH with TNM-1 total nitrogen analyser. A value of 0.45 was selected as the coefficient to convert 'chloroform-labile' carbon to microbial biomass carbon (Jenkinson et al., 2004).

Microbial respired carbon dioxide was used to provide an indication of microbial activity at each sampling time point. Respiration rates from four replicate columns per soil texture and per microbial treatment were ascertained using a gas chromatograph (Shimadzu GC-2014 Gas Chromatograph) at weeks 0, 2, 4, 8, 16 and 24 of incubation.

227 At each sampling point, single soil columns were sealed in a 250 ml flask, which were
228 over pressurised with 80 ml ambient air. 20 ml of gas headspace was analysed at 40
229 minute intervals for 120 minutes, allowing a flux of respired carbon dioxide to be
230 calculated. Laboratory air was analysed in the same way to correct for ambient carbon
231 dioxide concentration.
232
233 *2.5. Statistical Analyses*
234 Four replicate samples per microbial treatment per soil texture (36 samples in total)
235 were randomly assigned as 'scanned' samples at the beginning of the investigation, and
236 a repeated measurement multi-variate mixed model (REML) was performed in GenStat
237 Release 15.1 (VSN International) to isolate the effects of sampling period on X-ray CT
238 measures of porosity, pore size and pore connectivity over the 24 week incubation. A
239 separate sub-set of samples were randomly assigned to biological analysis (4 replicate
240 samples per microbial treatment per soil texture for each sampling interval – 216
241 samples in total), and the respiration rate non-destructively ascertained before a
242 destructive measure of biomass for the same samples quantified at each sampling
243 interval. A total of 36 samples was used to calculate respiration rates and then
244 destructively sampled at each sampling interval. Each column was analysed individually
245 using ANOVA, with all interaction of the explanatory variables. Standardised residual
246 plots were examined in GenStat to check data normality, and where variation was high
247 with high mean values, the data were $\log_{10}$ transformed. Comparison of means was
248 based on least significant differences (L.S.D.) at the $P = 0.05$ probability level.
249

3. Results

3.1. The effect of microbial treatments on soil structure

The visual impact of biota on soil structure could be seen after 2 weeks in the loamy sand texture under glucose enhancement, with the formation of large air-filled gaps/pores during a dramatic swelling of the soil matrix highlighting the potential for rapid and large-scale alterations under simple carbohydrate addition (Figure 2a). After 2 weeks of incubation there was a visual accumulation of fungal hyphae on the soil surface of glucose enhanced loamy sand columns (Figure 2a) and in the bottom half of the silty clay loam glucose enhanced soil column at 24 weeks (Figure 2b). Fungal growth was visually absent throughout the 24 week incubation in the glucose enhanced clay loam, all field and all sterilised soil treatments. Fungal growth could not be quantified in this instance by X-ray CT due to its low density in comparison to the surrounding soil particles. As such it was classified as pore space in the resulting analysis.

Microcracks began to develop in the glucose enhanced soils almost immediately (2 weeks), whilst cracks in the field soils were only observed after 8 weeks of incubation. The addition of glucose caused an increase in individual pore volumes across all soil textures ($P < 0.05$; Figure 3; Video S2). After 24 weeks of incubation, mean porosities of the soil under glucose enhancement at the resolution used had risen from 37.8 and 9.3% to 54.4 and 24.0% in the loamy sand and clay loam soils respectively ($P < 0.05$; Figure 4c). This accounts for the increase in sample volume during the investigation observed in Figure 2b and c. In comparison a decrease in porosity was observed in the sterile and field treatment up to week 8 of incubation ($P < 0.05$), most likely due to slumping under self-weight (Figure 4a, b). Following this, mean porosities in the field

treatment increased from 36.5 to 44.0% in the loamy sand texture ($P < 0.05$; Figure 4b) and from 14.1 to 17.1% in the clay loam soil ($P < 0.05$; Figure 4b), demonstrating the role that the resident microbial populations play in increasing pore size in the absence of further environmental perturbations between 8 and 24 weeks of incubation. Variability (standard error) in X-ray CT-derived porosities between samples of the same soil texture was low across all microbial treatments but increased with time (Figure 4). The temporal differences for all textures under 'field' treatment were small compared to those from the glucose enhanced soil.

There was a significant degree of slumping (i.e. a decrease in sample porosity to a more stable state) during the 24 week incubation in the sterilised loamy sand and silty clay loam treatments ($P < 0.05$; Figure 4a), but no significant change in the clay loam texture. During the first 8 weeks of incubation the degree of slumping in the sterile loamy sand treatment was greater than that observed in the field soil (a decrease in total porosity of 9.9 and 6.6% respectively; $P < 0.05$), suggesting that even at this early stage, microbial populations in the field soil treatment were able to actively manipulate the soil porous architecture and counteract the effects of particle slumping. There was no significant increase in porosity over the 24 week incubation across any texture in the sterile treatment, indicating that localised wetting and drying cycles created by the watering regime in this study had no significant impact on pore enlargement or microcrack formation.

Pore shape (circularity) under the glucose enhancement was variable between soil textures, but was generally uniform in distribution throughout the macrocosms. Pore shape was significantly affected during 24 weeks of incubation for loamy sand and silty clay loam textures ($P < 0.01$; Figure 5a, b). In the loamy sand treatment there were

fewer irregular pores and a greater number of rounded pores at week 24 ($P < 0.05$; Figure 5a). In the silty clay loam soil, pores became more irregular with time, with a two-fold increase in the number of pores in the lower circularity range (0-0.2) and fewer regular shaped pores over the incubation period. There was no change in the circularity of pores in the clay loam treatment during incubation ($P > 0.05$).

Increases in the porosity of the soil matrix during incubation had a significant impact on the surface connected pore architecture (i.e. porosity with a continuous connection to the surface), resulting in a more complex pore network in the upper part of the soil columns (Figure 6) and significantly altering the continuity of the surface connections of pores in all soils over time ($P = 0.05$; Figure 7). Under glucose enhancement pre-existing surface pore connections grew larger in diameter and volume (Video S3). Furthermore new crack development in the upper soil regions was rapid, increasing in size and becoming more elongated with time. Throughout the incubation the surface connected porosity accounted for > 99% of total sample porosity for the loamy sand texture (Figure 7a), demonstrating the highly connected nature of the pore network in coarser textured soils (at the resolution explored here). Mean surface connected porosities in silty clay loam and clay loam increased by 24.2 and 45.6% to 96.8 and 52.9% respectively by week 24 (Figure 7a; $P < 0.05$), but there was no significant change to mean surface connected porosity in the loamy sand texture. There was no significant increase in the mean surface connected porosity in the field and sterile treatments.

3.2. The effect of microbial treatments on microbial population respiration and biomass

Respiration rate declined across all soil textures with time in the sterile and field treatments ($P < 0.05$; Figure 8). Respiration under glucose enhancement was variable, but generally increased with time and was *ca.* 50 times greater than those observed in field soil ($P < 0.05$; Figure 8). After 24 weeks of incubation all treatments had significantly different respiration rates ($P < 0.05$), with the largest difference between textures within the glucose treatment. The clay loam texture systematically exhibited the highest respiration rate, with loamy sand and silty clay loam being similar throughout. The cumulative respiration of sterile and field treatments tended to plateau after the 24 week incubation across all soil textures. The order of peak cumulative respiration followed clay loam > silty clay loam > loamy sand in the sterile and glucose treatment (Figure 8d and f), but clay loam > loamy sand > silty clay loam in the field soil (Figure 8e). By the end of the 24 week incubation cumulative respiration was still rising in all treatments (Figure 8d, e, f), indicating the nutrient resource in all treatments, including the glucose addition, did not become limiting to growth as microbial populations were still actively proliferating.

Microbial biomass carbon (chloroform-labile C) was significantly altered by the interaction of microbial treatment and soil texture ($P < 0.01$; Figure 9). Microbial biomass showed a 4-fold difference between sterile and field treatments, and a 10-fold increase under glucose enhancement ($P < 0.05$; Figure 9). Difference within the biomass measurements was high in the glucose treatment and increased with time, both between and within sample replicates (Figure 9c). The sterile and field treatments had a higher biomass by week 24, with a decline after week 8.

4. Discussion

4.1. Implications of substrate addition for soil structural development

This investigation allowed us to quantify the physical changes to soil structure in soils at moisture contents relevant to conditions in the region of the capillary fringe. The glucose treatment allowed us to directly compare soil structure alterations due to carbon input under controlled conditions, at a similar rate to which roots exude carbon into the rhizosphere. Whilst the addition of glucose has limitations over using true exudates (i.e. the composition of the carbon source does not account for the range of amino acids and sugar sources in root exudates, nor the influence of mucilage on altering the water holding capacity of the soil), it does provide a first step to understand the coupling of microbial activity and soil physical behaviour at the microscale.

The rate at which soil porosity increased in the sandy loam and clay loam soils was at its maximum between weeks two and four of incubation (Figure 4c), presumably because an initial homogeneous soil structure became more heterogeneous with time. Fracturing of the soil matrix was apparent along distinct horizontal planes (Figure 2), possibly corresponding to unavoidable zones of weakness created during the packing process. Vinther et al. (1999) indicated microbial biomass, number and processes (e.g. denitrification) were higher in agricultural and forest field samples adjacent to macropores than matrix samples, due to increased substrate supply and bacterial cell transport through the macropores. The soil fracturing forced the translocation of soil up through the macrocosm, as indicated by an alteration in height between initial packing conditions. We can attribute this to the gaseous release by soil microorganisms, as this was not apparent in either the field or sterile treatments. Pagliai et al. (1981) suggested the possibility of large pore formation during carbon dioxide liberation through organic matter decomposition in peat samples. Thus, the potential for soil microorganisms to

377 alter structure in this way, aside from other known mechanisms of extracellular
378 polysaccharide production, filamentous structures (e.g. fungal hyphae) and extruded
379 biopolymer-induced aggregation (Oades, 1993), has been speculated for some time.
380 However, a lack of structural change in the sterile treatment negates the role of
381 decomposing soil organic matter for carbon dioxide liberation in this investigation.
382 Emerson and McGarry (2003) hypothesised that an increase in soil porosity with
383 increasing carbon contents was derived not only due to the pores created following
384 organic matter decomposition, but due to fungal mycelium invading the pore space
385 surrounding organic matter. A clear accumulation of fungal hyphae in the upper and
386 lower regions of the loamy sand and silty clay loam glucose enhanced soils may
387 therefore have played a key role in altering the soil porous architecture, although could
388 not directly be quantified using X-ray CT. De Gryze et al. (2005) also observed
389 extensive fungal hyphae proliferation surrounding decaying organic residue, with
390 reported differences in pore size distributions consistent with those observed in this
391 investigation. De Gryze et al. (2006a) suggests there is an intriguing feedback between
392 microbial activity and soil pore structure, with its understanding being essential to
393 improving knowledge of phenomena such as gaseous release. The direct observation of
394 gaseous release manipulating soil structure and pore geometry, highlights this feedback,
395 although here microbial proliferation and activity was a cause for and not as a
396 consequence of soil structural evolution. Furthermore, the role that the microbial
397 populations play in altering the surface connected porosity of the samples through
398 bridging pore gaps and creating an overall more porous and better connected network to
399 the surface will have implications for the flow of solutes and water through the soil
400 profile as well. Thus, conceptual models of soil aggregation and associated resource
401 flows based purely on: 1) the microbial secretion of metabolic products; and 2) the
402 enmeshment of soil particles by filamentous organisms, may not fully capture the

403 complexity of the rhizosphere by neglecting the role that gaseous release can play in the
404 temporal dynamics of microscale transport processes.
405
406 *4.2. Relationship between pore functioning and microbial communities*
407 Respiration rates increased at week 24 in the loamy sand and clay loam soils under
408 glucose addition, but the total microbial biomass decreased over the same period
409 (Figure 8c and, Figure 9c). Furthermore, under field soil treatments biomass fluctuated
410 throughout the investigation, despite respiration continually falling (Figure 8b, Figure
411 9b). The degree of fluctuation in activity accounts for the changes in observed soil
412 porosities (i.e. increases in soil porosity as microbial activity increased), but does not
413 account for alterations to biomass. Due to differences in soil chemical properties and
414 microbial respiration rates between textures, population differences may occur with
415 time as well as with depth. Likewise, increased biomass in the field and sterile
416 treatments at the beginning of incubation relates to the flush in activity observed by
417 Franzluebbers et al. (2000) as packing-induced disturbance and soil re-wetting favours
418 short-term microbial proliferation (as observed in Figure 8a and b). Clearly the
419 microbial community plays a key role in soil pore development. However, further
420 investigation of community structure development over a timeframe longer than
421 previously used is needed to provide insight into the communities responsible for the
422 structural changes observed in this study.
423
424 The largest soil structural changes occurred when soil respiration was at a maximum.
425 We hypothesised that as microbial exudates act as binding agents for aggregate
426 formation, this process itself would alter soil microporosity which could then be
427 detected using non-invasive imaging. However we suspected that binding agents would
428 only be produced in sufficient quantity to alter the physical soil structure after a period

of months. Due to the simultaneous nature of processes occurring we are unable to differentiate whether microbial released binding agents or carbon dioxide production are responsible for driving the structural changes observed. The maximum respiration in the glucose enhanced clay loam soil occurred at 4 weeks of incubation, with the largest change increase in porosity occurring between weeks 2 and 4 (Figure 4c and Figure 8c). Bossuyt et al. (2001) found similar results although at a different temporal scale, reporting maximum aggregation occurred 10 days after peak microbial activity and thus providing evidence for large-scale structural alterations in terms of pore geometry over short time intervals.

4.3. The relationship between soil structure, texture and organic matter

Changes to soil porosity depended on soil texture. Oades (1993) described the cohesive properties of clay and the key role they play in governing structure formation in field soils. Generally, the rank order porosity change was loamy sand > clay loam > silty clay loam (Figure 4) regardless of microbial treatment. However, there was a clear difference in the degree of structural change observed in contrasting soil textures in the field soil treatment. Mean macrocosm porosities increased 7.5% between weeks 8 and 24 of incubation in the loamy sand columns, compared to increases of only 3.0% and 1.5% in the silty clay loam and clay loam respectively. This suggests the microbial influence is largely related to: i) the cohesive strength of soil particles; ii) the inherent particle sizes creating altered pore morphologies during artificial packing; iii) the organic content of the soil; and iv) variations in nutrient availability in different soil textures. We suggest that when substrate input is uniform, solute mobility is lower in finer textured soils known to display high tortuosity (Moldrup et al., 2001), although this is matric potential dependent. We hypothesise that this may confine microbial growth to ‘pockets’ in which substrate is accessible. Conversely, in coarser textured soils we suggest there is

455 an improvement in the movement of metabolites towards sites containing microbial
456 populations, enabling their rapid proliferation. It is known that the natural spatial
457 heterogeneity of soil harbours increased microbial populations in distinct microsites
458 (Nunan et al., 2006), although they were unable to demonstrate whether this variability
459 resulted in changes to the physical soil microenvironment. Our work demonstrates the
460 impact of this increased activity on pore function, with an enhanced potential for
461 gaseous exchange created by a more porous system increasing microbial proliferation in
462 localised regions.
463
464 Using a different sampling approach, Nunan et al. (2003) observed a decreased
465 structural variation in subsoil samples where the soil nutrient status was rate-limiting.
466 They observed evidence for patchiness of bacterial densities in all samples of high
467 fertility, with gradients of bacterial growth highest in regions of soluble substrate. This
468 supports our findings where fracturing of the loamy sand soils at distinct locations under
469 substrate addition is thought to follow increased microbial activity in these regions. In
470 this current investigation the formation of microcracks in the field soils towards the end
471 of the incubation period may be attributed to microbial-derived processes such as
472 particle aggregation, but also due to carbon dioxide evolution breaching pore gaps and
473 leading to an increase in the overall connectivity of the pore system (Kettridge and
474 Binley, 2011; Figure 6). One potential hypothesis is that high concentrations of carbon
475 dioxide are evolved at sites of high microbial activity, and subsequently transported and
476 trapped in distinct soil locations thus leading to fracturing of the soil matrix. This is
477 particularly important for the relatively moist soils used in this investigation, as carbon
478 dioxide evolution exceeded the dissolution potential in the surrounding pore water,
479 creating the fractures observed in Figure 2. As no cracks were observed in the sterile
480 treatment across all soil textures, the potential effect of daily wetting/drying cycles can

481 be discounted. Velde (2001) and De Gryze et al. (2006a) observed cracks of 100 µm
482 and 27-67 µm respectively during drying of soil columns and incubation. Higher
483 increases in porosity in the loamy sand samples compared to silty clay loam (and clay
484 loam) textures parallels results obtained by De Gryze et al. (2006a) during incubation of
485 sand and silty clay loam aggregates.
486
487 Differences in porosity may also reflect the contrasting organic matter levels in the soils
488 (loamy sand – 2.9%, silty clay loam – 7.0%, clay loam – 5.2%). High organic matter is
489 known to contribute to enhanced aggregate stability (Tisdall and Oades, 1982), so it is
490 possible the coarser textured treatments with lower organic matter contents had
491 weakened bonds between soil particles and hence soil porosities influenced more by
492 microbial populations over the timeframe of the treatment application. Furthermore,
493 organic matter has different shrink/swelling and water retention characteristics to that of
494 soil (Garnier et al., 2004), with the stabilization of pore structure due to organic
495 composition thought to further enhance cracking through the uneven dissipation of
496 forces originating from wetting/drying cycles (De Gryze et al., 2006a). Whilst no
497 increase in porosity through wetting and drying of the soil matrix itself was observed in
498 any soil texture studied, we suspect that decomposition of the organic constituents,
499 coupled with the application of glucose in the glucose enhanced treatment, may have
500 contributed to the small increases in porosity observed throughout the incubation in the
501 clay loam and silty clay loam textures of the field and glucose enhanced treatments. Soil
502 cracks are thought to develop in hexagonal, squared (Velde, 1999) or triangular
503 (Kodikara et al., 2000) patterns, which may account for the shift to a greater number of
504 irregular shaped pores at the end of the incubation period in clay loam and silty clay
505 loam soils containing higher organic contents (Figure 5). Conversely, the loamy sand
506 treatment, with a lower organic matter content to the soil, had a temporal shift to a

507 greater number of ‘regular’ shaped pores through increased pore volume due to gaseous
508 release.
509
510 **5. Conclusions**
511 Soil-borne microbial populations rapidly create highly connected soil pore networks due
512 to evolution of carbon dioxide by microbial respiration within soil, significantly
513 deforming the soil matrix and having important consequences for pore architecture.
514 During the structural reorganization of these relatively moist soils, increases in porosity
515 under carbon enhanced conditions coupled with changes to pore surface area are likely
516 to be beneficial for root aeration and water flow, at least for aggregated soils with a low
517 to medium bulk density. The activity of soil microorganisms is governed by gaseous
518 exchange and the availability of substrate and water, so there is a key relationship
519 between localised soil porous architecture and microbial populations. Increased porosity
520 in coarser textured soils corresponded to a large net respiration demand, with
521 subsequent gaseous release fracturing the soil matrix and increasing not only pore
522 volume and shape, but also altering the surface connectivity of the pore network. Hence,
523 there lies a key feedback between soil microbial populations and structural evolution,
524 with activity being both the cause for and response to altered structural states through
525 improved gas transport networks. It is clear from this and other work that previous
526 investigations at the macroscale may have overlooked the implications for soil flow
527 properties by missing alterations to pore architecture at this scale (Taina et al., 2008;
528 Wildenschild et al., 2002). However, the microscale resolution achievable in modern-
529 day non-destructive imaging approaches opens avenues for the future quantification of
530 the role that specific microbial communities play in the development of soil structure, so
531 that resulting soil microheterogeneity can be better understood and incorporated into
532 hydrological models.

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List of Figures

687 Figure 5 – The influence of soil texture on pore shape (circularity) at the beginning and
688 end of incubation for the glucose enhanced soil. Values are means (n=4), error bars
689 denote double s.e.. Standard errors of the difference of means are shown for (1)
690 Sampling week, (2) Circularity range, (3) Soil texture.
691
692 Figure 6 - Visual representation of changes to surface pore connectivity in silty clay
693 loam after: a) 0 weeks; b) 2 weeks; c) 4 weeks; d) 8 weeks; e) 24 weeks of incubation,
694 with associated surface connected porosities of the whole macrocosm. All images are of
695 a subsection of sample with dimensions 14.38 x 11.88 x 0.74 mm (1160 x 960 x 60
696 pixels), for ease of visualisation.
697
698 Figure 7 – The influence of soil texture on surface pore connectivity throughout
699 incubation for the glucose enhanced soil as a function of: a) total volume of pore space;
700 b) total sample volume. Values are means (n=4), error bars denote double s.e.. Standard
701 errors of the difference of means are shown for (1) Sampling week, (2) Soil texture.
702
703 Figure 8 – Respiration during 24 weeks of incubation: a-c - respiration rate; d-f -
704 cumulative respiration from the soils for three microbial treatments of a, d – gamma-
705 irradiated sterile soil; b, e – unaltered field soil; c, f after addition of glucose (0.05 mg C
706 g-1 soil), for three contrasting soil textures. Values are means (n=4), double error bars
707 denote s.e. Note different scales used on y-axis. Standard errors of the difference of
708 means are shown for (1) Sampling week, (2) Microbial treatment, (3) Soil texture.
709
710 Figure 9 – Effect of various treatments on the soil microbial biomass during 24 weeks of
711 incubation for three contrasting soil textures: a) Gamma-irradiated sterile soil; b)
712 Unaltered field soil; c) Glucose enhanced soil (0.05 mg C g-1 soil). Values are means

713 (n=4), error bars denote double s.e. Standard errors of the difference of means are
714 shown for (1) Sampling week, (2) Microbial treatment, (3) Soil texture.
715

List of Supplementary Data

Figure S1 – Water release characteristics of the loamy sand, clay loam and silty clay loam soil textures used in this investigation. The water release characteristic was measured using pressure chamber apparatus, and fitted to the van Genuchten-Mualem model (van Genuchten, 1980).

Video S2 – Changes to the morphology of an individually isolated pore for each sampling interval throughout the incubation period, in the clay loam soil texture under glucose enhancement.

Video S3 – Changes to the porous connectivity of the silty clay loam texture under glucose enhancement at each sampling interval, as shown by porosity with a continuous connection to the soil surface.