Different cover crop species have a contrasting impact upon soil structural genesis and microbial community phenotype

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Abstract

Cover crops (plants grown in an agricultural rotation between cash crops) can significantly improve soil quality via sequestering carbon, retaining nutrients, decreasing soil erosion, and maintaining belowground biodiversity. However, little is known about the effects of such plants upon soil structure. The aim of the study was to assess the impact of four species typically used as cover crops and which have contrasting root architecture (namely white clover, black oat, phacelia, tillage radish) on soil structural genesis and the associated modification of microbial community structure in a clay soil. The four plant species were grown in a replicated pot experiment with sieved clay soil(<2 mm), with unplanted soil as control for 8 weeks. X-ray Computed Tomography was used to quantify the formation of pore networks in 3D and phospholipid fatty acid analysis was performed to study the microbial community phenotype. Black oats developed a greater pore-connectivity than the other species throughout the growth period, whereas phacelia decreased the porosity and pore-connectivity. The microbial community phenotype under phacelia was notably different from the other species, with a greater proportion of fungal markers. Thus different plant species have differential effects upon soil

structural genesis and microbial community phenotype, which provides evidence that certain species may be more suitable as cover crops in terms of soil structural conditioning depending upon specific contexts.

Key words

Raphanus sativus L. cv. "Mimo", *Avena strigosa* L. cv. "Prate", *Trifolium repens* L. cv. "Galway", *Phacelia tanacetifolia* Benth. cv. "Angelia", X-ray Computed Tomography, soil structure, porosity, microbial community phenotype

Introduction

Soil structure is an important factor affecting crop production, primarily due to the characteristics of the soil pore network impacting on root growth, soil fauna, nutrient, water and gas exchanges[1]. Soil quality can be defined as the capacity of soil to maintain or enhance plant and organism development, air and water quality, and support human health and habitation[2,3]. Soil structure is considered an effective indicator of soil quality[1].

Moreover, plants are known to contribute to the structuring of soil via enmeshing and binding soil particles[4] and to break down larger aggregates via root penetration[5]. The genesis of soil structure is dynamic and requires energy, which can be provided by plant roots and fauna. Furthermore, root system architecture plays a critical role in soil structure formation. Plants producing large quantities of fine roots appear to be more effective in soil aggregate formation compared to fibrous roots which can be less effective in fracturing soil aggregates[6]. The presence of roots increases aggregate stability, the permeability of soil[7], soil porosity and pore connectivity[8], and asserts a great influence on microbial community structure in terms of both richness and diversity[9,10].

In agricultural systems, cover crops are increasingly sown between main crops[11] because they sequester carbon[3,12], decrease soil erosion[3], increase soil macro-porosity[13,14], and increase microbial diversity and richness[15,16]. Cover crops are also associated with increased abundance of saprophytic and mycorrhizal fungi in the microbial community structure[10,17,18]. However, the effect of cover crops on soil structural genesis is poorly understood. Most recent studies in this area have focused on the

role of cover crops in terms of remediating compacted soils or upon the soil microbial communities[9,12,14,16,18].

The aim of the study was to assess the impact of four species of plants commonly grown as cover crops in the UK (white clover, black oat, phacelia, tillage radish) on soil structural genesis and modification of microbial community structure. These plants were selected for their contrasting root morphologies in terms of tap root formation, vigorous deep-rooting and fibrous multi-branching root systems. We hypothesised that different root morphologies would influence soil structural genesis dependent upon the root phenotype: for example, tap root species may initiate concentric compaction surrounding the primary root growth which decreases porosity and diversity of pore sizes, compared to fibrous root species which may create a greater diversity of pore sizes positively impacting on porosity and pore-connectivity. X-ray Computed Tomography (CT) was used to quantify the formation of pore networks in 3D and phospholipid fatty acid (PLFA) analysis was performed to study the microbial community phenotype.

Results

Characteristics of the pore architecture

As hypothesized, the different plant species had contrasting affects on soil structure, as apparent from visual observation of the X-ray images (Fig. 1). Formal quantification of soil structural parameters confirmed this. There was a significant treatment x time interaction with respect to porosity (P<0.001; Fig. 2). The porosity of unplanted soil remained constant over the 8 weeks of the experiment with only slight increases at Weeks 2 and 6. In the presence of black oat, the porosity increased significantly at Weeks 2 and 6, but was similar to the control for Weeks 4 and 8. Whilst for clover, phacelia and radish, the porosity was essentially constant up to Week 6 and then decreased at Week 8 drastically (Fig. 2). There were significant treatment x time interactions with respect to cumulative pore size distribution at Week 8 (Fig. 3c), and pore-connectivity at Weeks 2 and 8 (Fig. 3e, f; P<0.001). At Weeks 0 and 2, the pore size distribution was essentially congruent for all treatments with approximately 50% of pore sizes <0.25 mm and 80% of pore sizes <0.4 mm (Fig. 3 a-c). At Week 8, phacelia increased the

proportion of the smaller pore sizes with approximately 50% of pore sizes <0.16 mm and 80% of pore sizes <0.31 mm (Fig. 3c).

At Week 0, the control columns had low pore connectivity displayed by a small Euler number for pores <0.09 mm (Fig. 3d). At Week 2, the overall pore connectivity had increased from Week 0. Soil planted with black oat had the greatest pore-connectivity, whilst the soil planted with phacelia was the least connected pore-system (Fig. 3e). At Week 8, the pore connectivity decreased for all treatments with the same pattern as Week 2: black oat soil had the greatest pore connectivity and phacelia the lowest pore-connectivity (Fig. 3f). There was a gradual evolution of pore size distribution and pore connectivity during the 8 weeks, and the data of Week 4 and 6 are shown in Supplementary Fig. 1 but omitted from Fig 3 for clarity. At the aggregate scale, there were no significant differences in porosity, pore size distribution and pore connectivity between any of the treatments (Supplementary Fig. 2).

Aggregate size distribution

At Week 0, the aggregate size distribution of the control showed an increase in size. This trend was not consistent for the size classes (425- 500 and 715-100 μm), where these sizes contained a significantly smaller proportion than neighbouring ones (Fig. 4a). This basic pattern persisted at Week 8 with a substantial increase in proportion of aggregates for the size class >2,000 μm. Planted soils with black oat and phacelia had a significantly greater proportion of aggregate between 2,000-300 μm than the control and the planted soil with clover and radish, but this trend was reversed for aggregates >2,000 μm (Fig. 4b).

Microbial phenotypic profiles

There were significant plant effects upon microbial community phenotypic structure with respect to PC1 and PC3 (both $P<0.001$), which collectively accounted for 61% of the variation (Fig. 5). Microbial community phenotype profiles differed significantly between both planted and unplanted soils, and between plant species. There was a significant effect of the plant species upon the microbial community phenotype apparent via PC1 and PC3 ($P < 0.001$ and $P = 0.012$ respectively) which together accounted for 61% of the variance. Community structure associated with phacelia was notably distinct from the other treatments apparent via PC1, and communities associated with black

oat distinct from those of clover, with black oat communities intermediate between these (Fig. 5a). PC3 discriminated communities associated at Week 0 (control) with those present at Week 8 in all cases (Fig. 5a). The loadings associated with PC1 were predominantly two fungal markers (18:2n6,9; 18:3n9) and one Gram negative marker (16:1n9; Fig. 5b). Markers associated with Gram positive bacteria contributed large loadings to PC3 (Fig. 5b).

The nature of the effect was due to an increase of the proportion of fungal markers and a decrease in the proportion of Gram positive bacterial markers for phacelia compared to the other treatments (Fig. 6).

Discussion

After 8 weeks of growth, black oat and phacelia columns contained substantial root materials, suggesting that visualisation of effects beyond this point would be inappropriate, and that any effects of the plants upon soil structure would be particularly manifest. The apparently limited effects on soil structure of white clover and tillage radish might have been due to a lesser development of root systems, as both plant species grew much more slowly than black oat and phacelia. After 8 weeks, the increase in proportion of the largest aggregates (>2,000 μm) for the control, the white clover and tillage radish might be caused by the high concentration of clay particles[4,19,20]. For black oat and phacelia treatments, the presence of plant roots decreased the proportion of the largest aggregates (>2,000 μm) by maintaining a greater proportion of aggregate sizes of 1,000-2,000 μm compared to the control. Therefore, the high proportion of roots decreased the proportion of larger aggregates (>2,000 μm), and increased the proportion of aggregate sizes from 1,000-2,000 μm (Fig. 4). The greater presence of roots in black oat and phacelia columns could have induced the breakdown of the larger aggregates, i.e. penetration roots through existing pores can destabilise macro-aggregate resulting in an increase in proportion of aggregate sizes from 2,000-1,000 μm[5,6].

The growth of Black oats did not significantly alter the soil porosity (Fig. 2). Between Week 2 and 8, black oat had decreased pore connectivity (Fig. 3b, c). However, for Week 2 and 8, black oat had the greatest pore connectivity compared to all treatments (Fig. 3b, c). Therefore, black oats maintained a greater porosity and pore-connectivity contrary to phacelia which significantly decreased porosity and

pore-connectivity at Week 8 (Fig. 2 and 3c) and increased the proportion of smaller pores compared to all treatments at Week 8 (Fig. 3f). This formation of pore sizes (between 0.05 to 0.16 mm) facilitates the flow path of water, gas and nutrients[21], however, the decrease in pore connectivity might counteract this shift in the pore sizes[22,23]. Thus, the formation of new pores, smaller than 0.3 mm, with a poor-connection might be involved in the creation of water storage pores[24,25]. At Week 8, black oat and phacelia columns were root bound, notwithstanding that the nature of inherent pore network was drastically different for both these treatments. Root biomass could not be determined since aggregate sampling precluded this. Thus, different modifications of pore networks were implemented by the inherent nature of root systems and not by the extent of rooting. Therefore, after 8 weeks of growth, black oat and phacelia showed evidence of impacting the pore network differently by modifying its characteristics in very different ways.

The non-significant impact of the plant at aggregate scale (Supplementary Fig. 2) might be influenced by the water regime, which was kept constant during the experiment. Wet and dry cycles are important for the modification of soil structure[24]. Notably by the disruption of aggregation due to clay particles swelling in the presence of water and the compression of trapped air in capillary pores which could lead to the creation of new pores[26,27]. The presence of plant applies local wet and dry cycles at the immediate root surface leading to a greater cohesion of root exudates and clay particles[28,29]. Hence the non-significant impact of plants on the micro-structure might be due to a lack of wet and dry cycles.

Principal component analysis (PCA) discriminated the microbial community at Week 0 compared to all the treatments at Week 8 along the *y*-axis, meaning microbial community evolved during the incubation period and was not discriminated by PC3 (Fig. 5a). This discrimination was associated with a shift of the bacterial community between both time points (Fig. 5b). Notwithstanding this, PCA distinctly discriminated phacelia in relation to PC1, which was associated with the saprophytic fungal marker 18:2n3,6 and 18:3n9 and Gram-negative bacteria 16:1n9 (Fig. 5)[30–33]. Moreover, phacelia showed a greater proportion of fungal marker compared to all treatments which revealed that phacelia increased the presence of saprophytic fungi (Fig. 6). Such microbes were likely utilising rhizodeposits as Gram-negative bacteria and fungi have been described to be involved in immediate assimilation of rhizodeposit carbon in grassland soils[33,34]. Another study showed that approximately two months after

sowing, fungi were an important factor, especially the non-mycorrhizal fungi, to discriminate microbial community structure between different cover crops[18]. Phacelia has been described as forming mycorrhizal associations[35–37], but there is no record in UK soils of mycorrhizal formation. A quantification of the mycorrhizal infection was performed on the phacelia root (Supplementary material and methods 2), which revealed no colonisation of roots by mycorrhizal hyphae (Supplementary Fig. 3). The discrimination of black oat and tillage radish via PC1 between control and clover treatments (Fig. 5a), showing that both plant species impacted slightly microbial community structure but not to the same extent as phacelia, which could be due to the nature of root characteristics[38].

Methods

Preparation of soil columns

Soil from the Worcester series, a clay soil (clay: 43.3%, silt: 28.4%, sand: 28.3%) was collected from 0-50 cm depth from an arable field situated at the University of Nottingham experimental farm in Bunny, Nottinghamshire, UK (52.52 °N, 1.07 °W). The soil was air-dried for 2 days, and passed through a 2 mm mesh sieve, to provide a homogenised structure. To re-activate the microbial community, the soil was re-wetted to 15% moisture content and incubated in bulk in black plastic bags slightly opened in a dark room at room temperature and then passed through a 10 mm sieve to ensure effective homogenisation. Polypropylene tubes (170 mm height x 68 mm diameter) with a 0.1 mm mesh adhered to the bottom were packed with the moist soil to a bulk density of 1 g cm^{-3}. Columns were saturated for 24 h and left to drain for 48 h to reach field moisture capacity (approximately 20%). Four cover crop species were selected for their contrasting root morphologies: a tap root species, tillage radish (*Raphanus sativus* L. cv. "Mimo"), a vigorous deep-rooting species, black oat (*Avena strigosa* L. cv. "Prate"), and two fibrous multi-branching species, white clover (*Trifolium repens* L. cv. "Galway") and phacelia (*Phacelia tanacetifolia* Benth. cv. "Angelia"). These species are commonly used as cover crops in arable systems. Pre-germinated seeds were sown into individual columns and adjusted to contain one emergent plant per column. Twenty replicates of each plant species, and of an unplanted (control) soil, were allocated in a random block design to allow for four replicates of each treatment to

198 be sampled after 0, 2, 4, 6 and 8 weeks. Columns were maintained in a growth chamber set at 16:8 h
199 light:dark cycle, 21:15 °C respectively and 70% humidity.
200
201 **X-ray Computed Tomography (CT)**
202 Homogenisation of column packing was checked by X-ray CT. Planted and unplanted columns were
203 scanned using Phoenix v|tome|x M scanner (GE Measurement and Control solution, Wunstorf,
204 Germany) set at a voltage of 180 kV with a current of 180 µA and at voxel resolution of 40 µm. A
205 multiple scan was performed for 1 h 29 s, with a total of 2160 projection images taken at a 250 ms
206 period using an averaging of 3 images and a skip of one. Longer scanning than might be routinely
207 employed was favoured to obtain the enhanced image contrast for thresholding. Columns were
208 destructively harvested after being scanned, and from the air-dried soil, three aggregates were
209 randomly selected per column (Supplementary Materials and Methods 1).
210 Scanned images were reconstructed using Phoenix datos|x2 rec reconstruction software. The scanned
211 images were optimised to correct for any sample movement during the scan and to reduce potential
212 noise using the beam hardening correction algorithm, set at 8. As a multi-scan routine was performed
213 on the soil column samples, VG StudioMax® 2.2 was used to merge the top, middle and bottom scans to
214 obtain a single 3D volume for the complete column. Image sequences of 40 x 40 x 120 mm were
215 extracted for image analysis from the columns.
216
217 **Image analysis**
218 Image manipulation was performed using Image J[39]. Quantification of 3D pore characteristics was
219 processed using QuantIm[40] following a standard method[8]. The 3D characteristics of pores quantified
220 were: (i) percentage of pores with a size greater than the scanning resolution (40 µm), hereafter
221 referred as porosity; (ii) pore size distribution, *viz.* the proportion of each pore size class within the
222 range 0.05 – 1.1 mm (for the columns) normalised by the total pore volume, expressed as a cumulative
223 value; (iii) pore-connectivity, determined by the Euler number normalized to the total volume[40]: the
224 more negative the Euler number, the greater the pore-connectivity.
225
226 **Sampling and measurement**

On each sampling occasion, the columns were scanned as above and then destructively harvested. Subsamples (c. 20 g) of the moist soil were stored at -82 °C and then freeze-dried; the rest of the soil was air-dried for further analysis. The freeze-dried and air-dried soils were stored in the dark at room temperature.

Aggregate size distribution

Aggregate size distributions were determined by passing 250 g of air-dried soil through a series of sieves sized 2000, 1000, 850, 500, 425, 300, 212 and 53 µm, via horizontal shaking for 3 minutes at 300 rotation.min^{-1} on a horizontal KS 500 shaker (Janke & Kunkel, Staufen, Germany). The mass of soil retained on each sieve was determined and normalized by the total mass of the sieved soil.

Microbial community phenotype profiling

The microbial community phenotypic community structure was profiled using the phospholipid fatty acid (PLFA) technique[41]. PLFA were extracted from 2 g of freeze-dried soil following a method derived from[41,42]. The lipid classes were separated using a solid phase extraction (SPE) column using Hypersep SPE column containing 50 mg of silica per 1 mL column. The extracted lipids were methyled via a transesterification process to convert them into dried fatty acid methyl ester. The fatty acids were disolved in 75 µL of hexane, for gas chromatography (GC) analysis. The GC analysis was performed using a GC and a DSQII mass spectrometer (Thermo Electron Corporation®), equipped with a Zebron capillary 'ZB-FFAP' column from Phenomex®. The dimensions of the column were 30 m length x 0.25 mm inner diameter x 0.25 µm film thickness. The method was 1 µL of the sample was injected in the column maintained at a constant temperature of 250 ºC, the carrier gas was helium set at 12.4 x 10^4 Pa. For each sample, a chromatogram was obtained with the retention time of each compound and the ion profile provided by the mass spectroscopy.

The markers were associated to different microbial groups as follows: Gram positives: i-15:00, a-15:00, i-16:00, a-17:00, 10me-16:00, 10me-18:00; Gram negatives: 16:1n9, 16:1n7, cy17:00, 18:1n7, cy19:00; saprophytic fungi: 18:1n9, 18:2n6,9, 18:3n9; and non-specific: 14:00, i-14:00, 16:00, 18:00, 18:1n16[30–32,43]. The percentage of the fatty acid indicators was used to analyse the proportion of microbial groups. The proportion of the microbial groups were calculated by the sum of all markers.

Statistical analysis

For the pore characteristics, two-way analysis of variance (ANOVA) was conducted using Genstat v 17.1 (VSN International Ltd 2014), performed on all primary variables using a split-plot design with the plant treatments and size classes of pores as factors and for the total porosity, time was added to the factor list.

PLFA profiles were analysed by principal component (PC) analysis and resultant PCs analysed by ANOVA.

Conclusions

These results revealed a contrasting effect of the root morphologies on the soil structure genesis, which validated our hypothesis. Vigorous deep-rooting species (represented by the black oats) maintained the porosity and pore connectivity whereas one of the fibrous multi-branching root species (phacelia) decreased porosity and pore-connectivity and enhanced the proportion of smaller pore (<0.31 mm). The tap root species (represented by tillage radish) and the second species of the fibrous multi-branching root species (white clover) decreased the porosity but had no significant impact on the pore connectivity. Therefore, the nature of the root architecture of these plant species likely modified the soil pore characteristics differently depending on the growth strategy of the plants. Moreover, the microbial community phenotype was also modified by the presence of plants.

These results confirmed that the diversity of root morphology and higher-order interactions between plant and soil biota impact soil structural genesis and dynamics[44]. This has practical and ecological implications since the nature of root morphology can have different effects upon soil structure. What is unclear is the extent to which such effects occur where plants are growing in combination, which occurs in natural systems, and can be prescribed in cover-crop mixtures. This warrants further investigation,

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Author contributions statement

A.B.L. led and conducted the experimental work, analysed the experimental results, drafted the manuscript and coordinated the revisions. K.R., S.J.M., and A.N. contributed advice on the analyses. All authors contributed to the revision of the manuscript. K.R. and S.J.M. supervised the overall project. All authors give final approval for publication.

Additional information

Supplementary information associated with this paper at …

Competing interests: The authors declare that they have no competing interests.

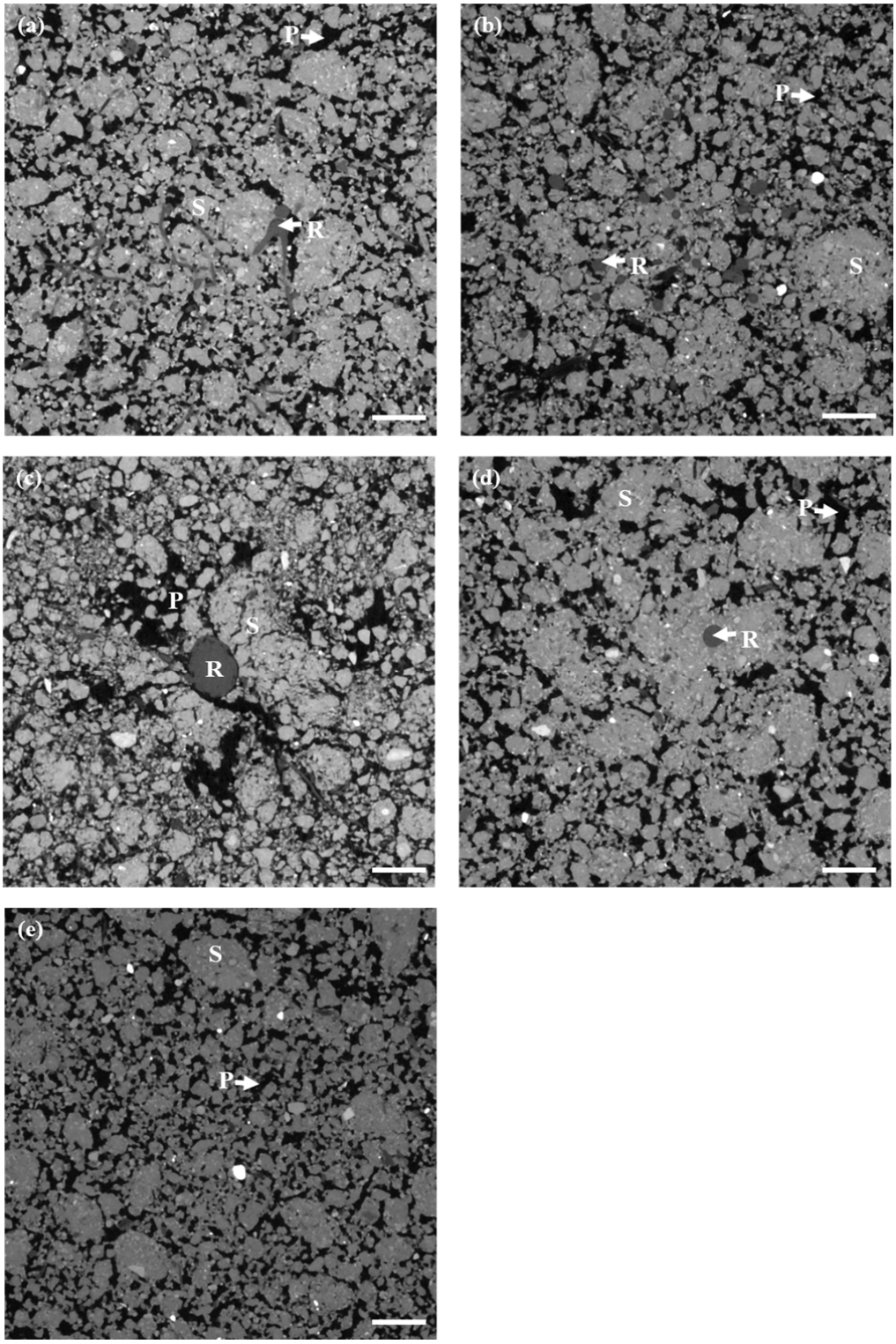


Figure 1: 2D images of cores (40 μm resolution) at Week 8, displayed as greyscale images denoting Hounsfield attenuation (darker shades relate to lower attenuation), region of interest: (a) white clover; (b) black oat; (c) Phacelia; (d) tillage radish; and (e) unplanted soil (S: soil matrix, P: pore, R: root).

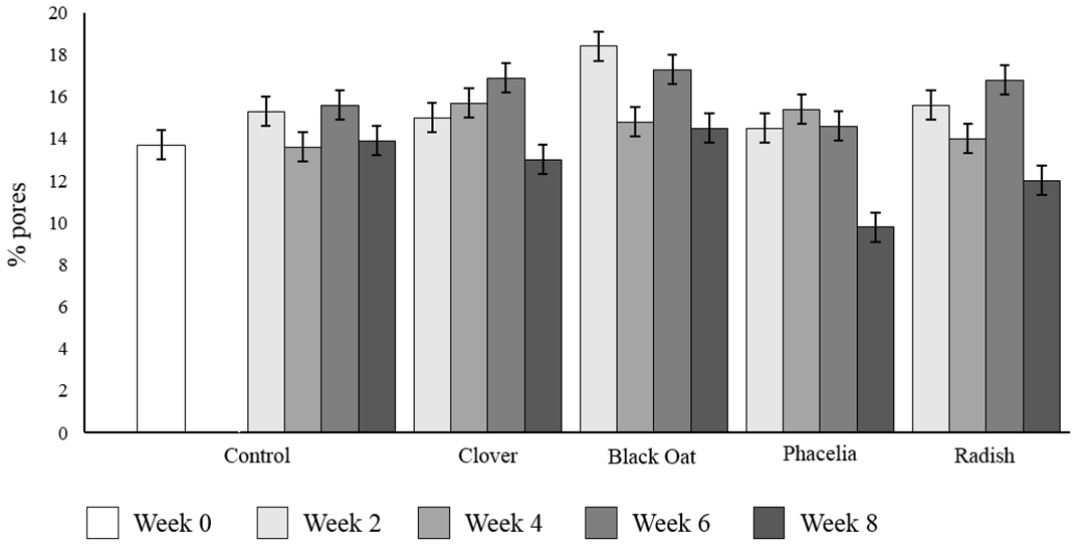
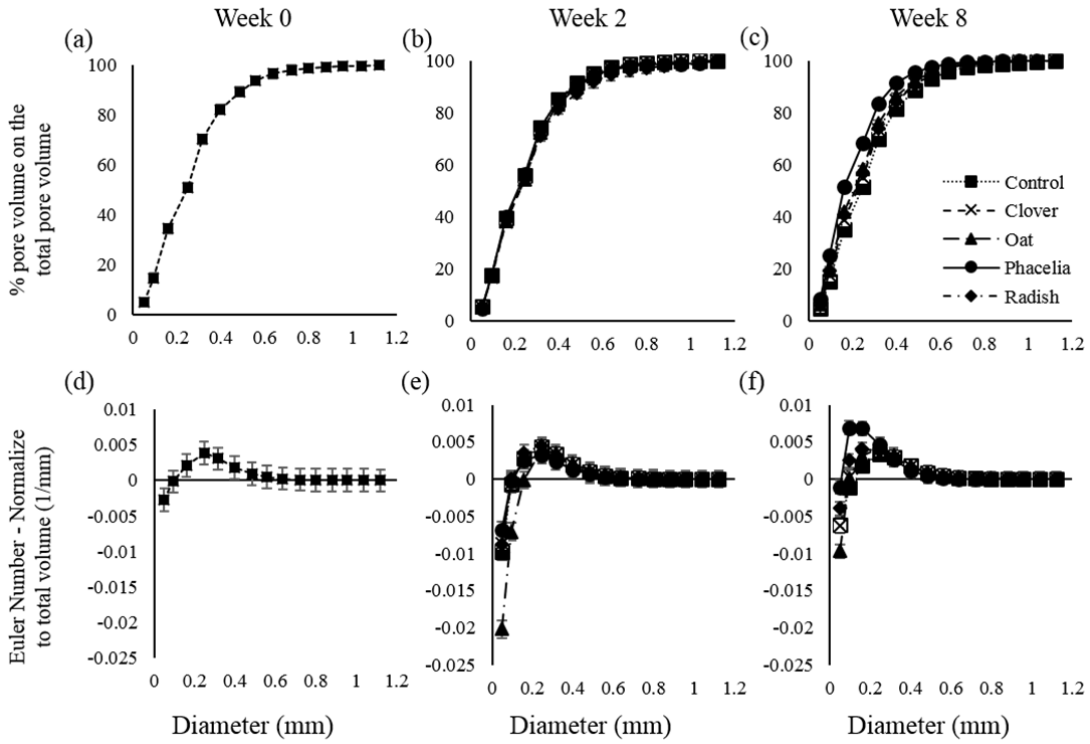


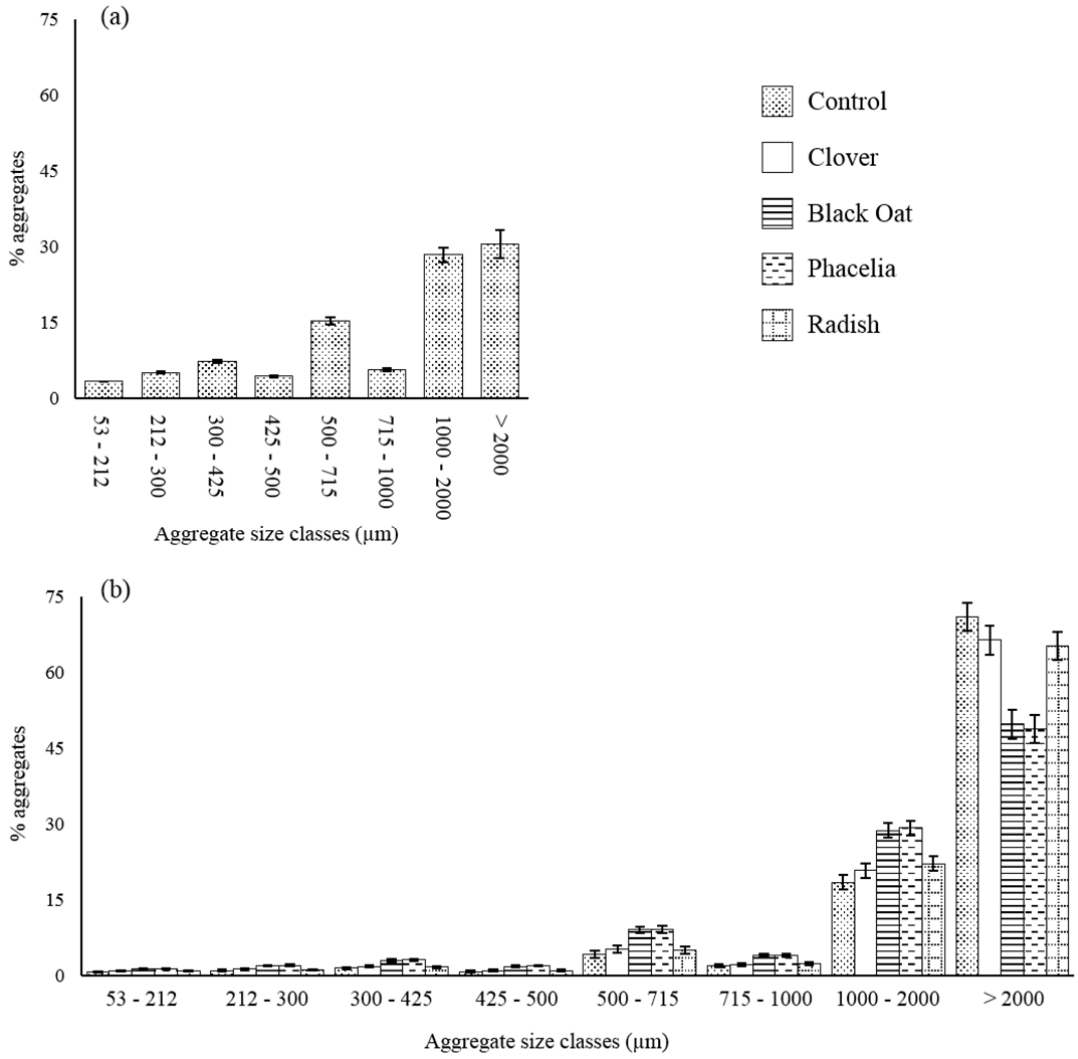
Figure 2: Porosity in relation to the planted treatment over the 8 weeks of growths expressed as percentage of relative pore to the total volume. Bars indicate means and whiskers denote pooled standard errors.



410

411 **Figure 3:** Minkowski functions of treatments at core scale (40 µm resolution) at three time points,
412 week 0 (a, d) week 2 (b, e) and week 8 (c, f): (a - c) cumulative pore size distribution; (d - f) pore-
413 connectivity of cores. Points show means, whiskers denote pooled s.e.

414



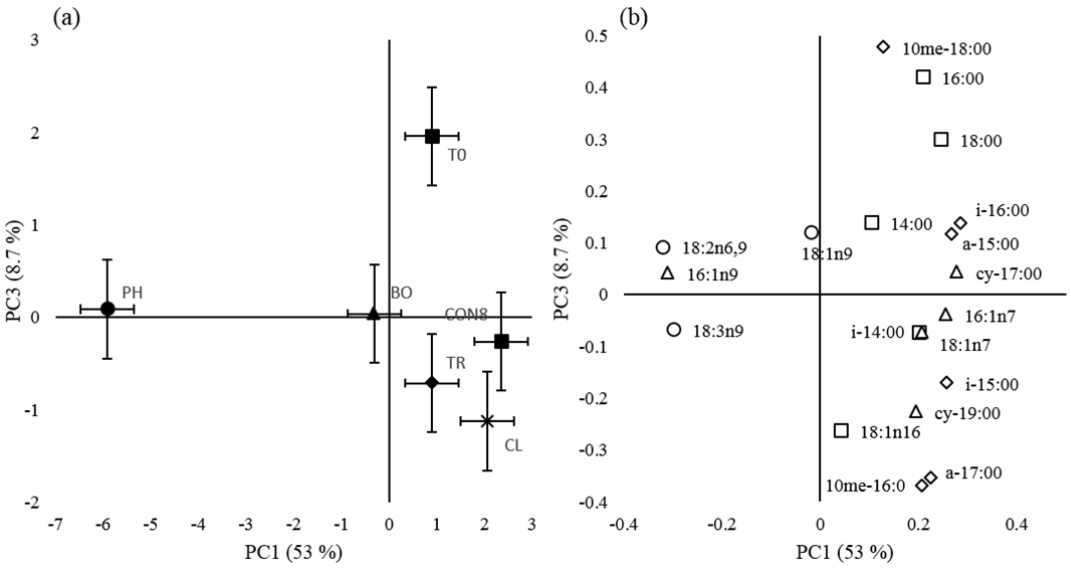
415

416 **Figure 4:** Aggregate size distribution displaying the starting condition at Week 0 (a) and the effect of

417 different plant species at Week 8. Bar charts represent means expressed as percentage of aggregates

418 relative to the total volume, and whiskers are pooled standard errors.

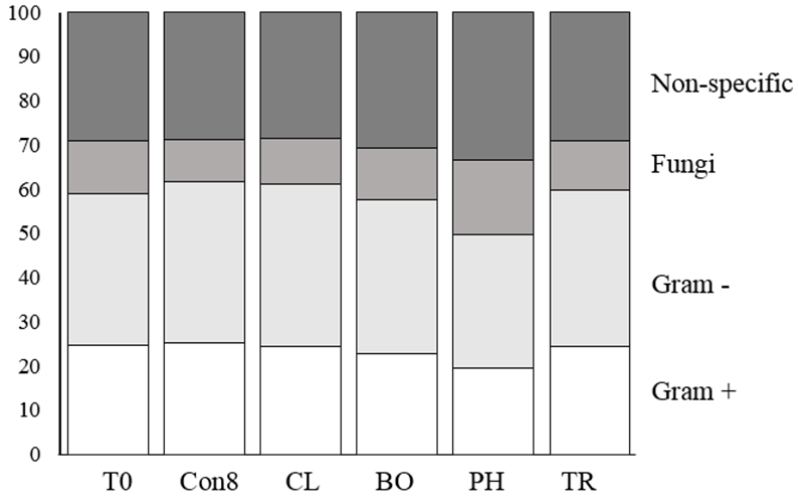
419



420

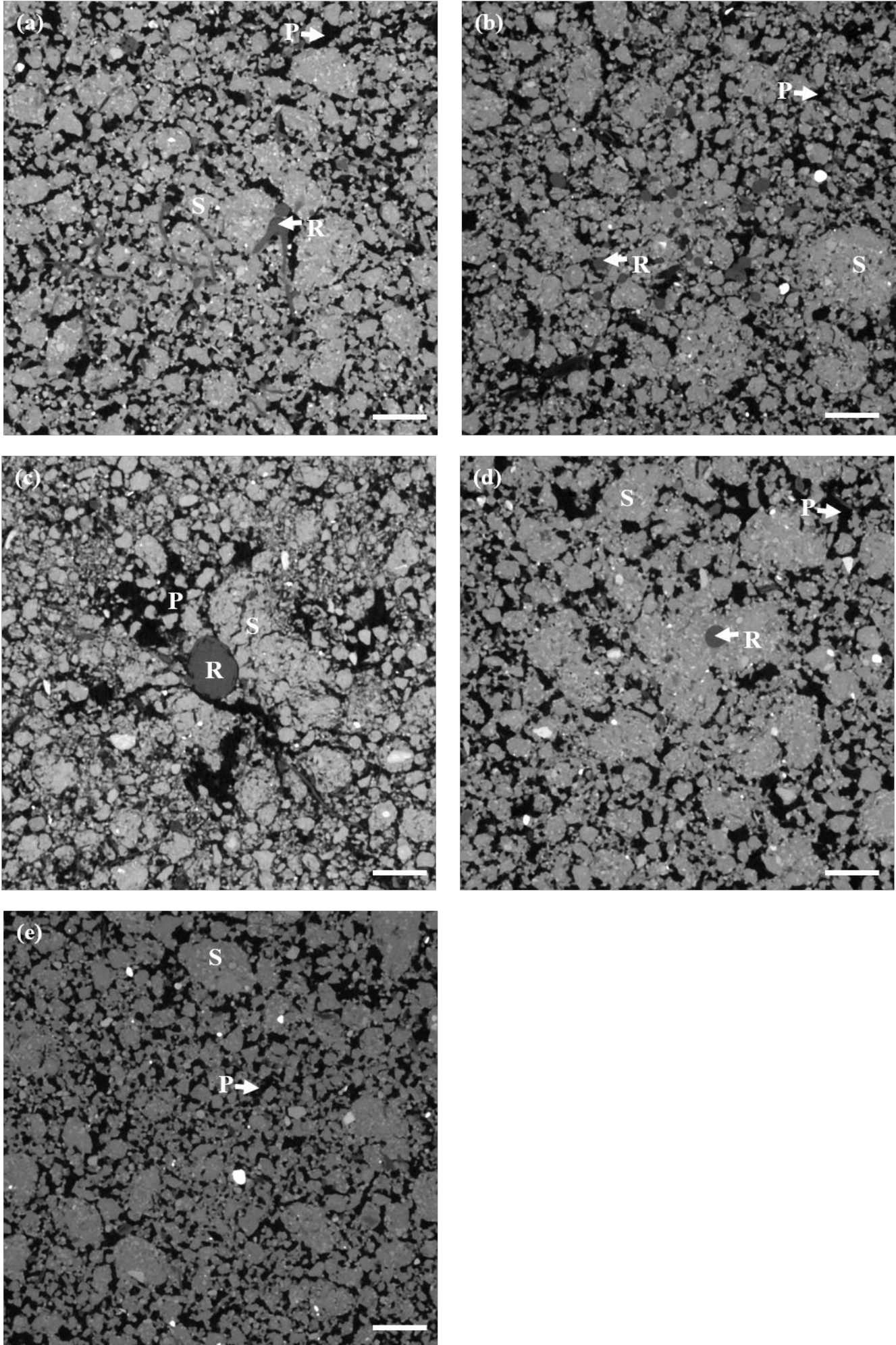
421 **Figure 5:** Effects of plant species upon microbial community phenotypes. (a) Principal component
422 (PC) ordination of first and third PCs (T0: control at week 0, CON8: control at week 8, CL: white clover,
423 BO: black oat, PH: Phacelia, TR: tillage radish; points show means, whiskers denote pooled s.e) and (b)
424 associated loading (◊ Gram positive Δ Gram negative ○ fungi □ non-specific markers).

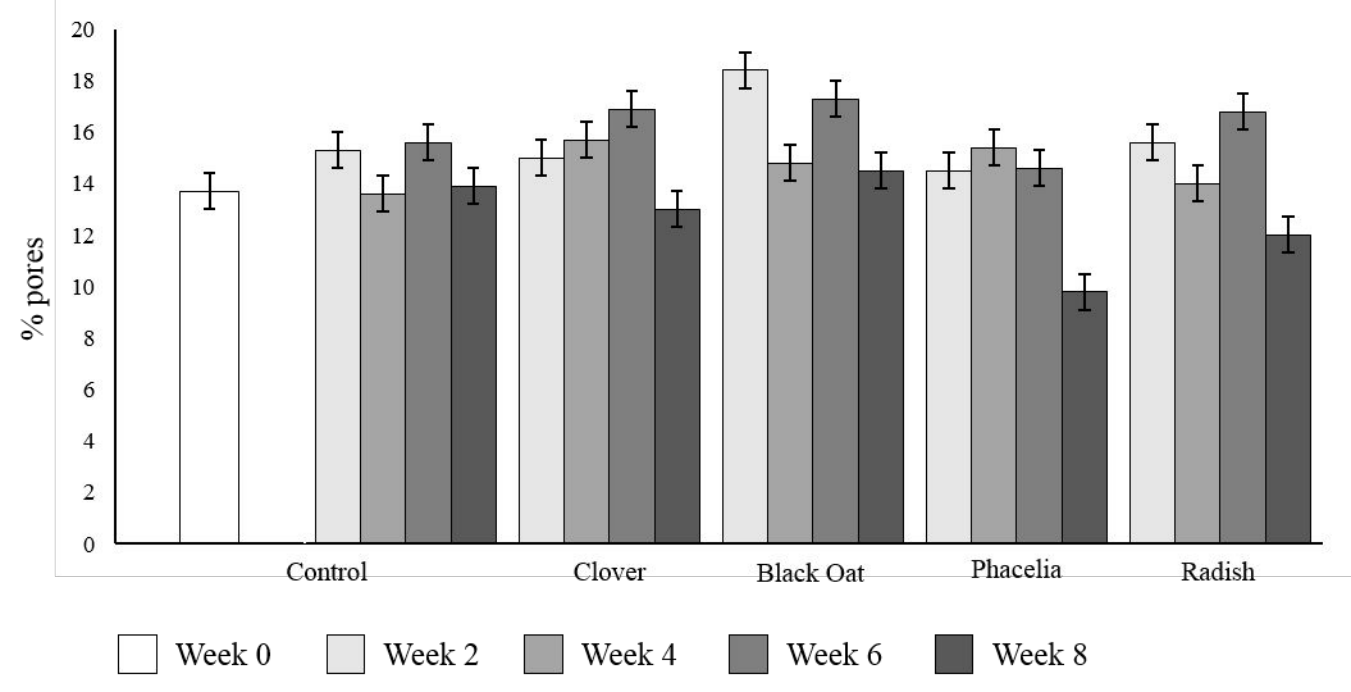
425

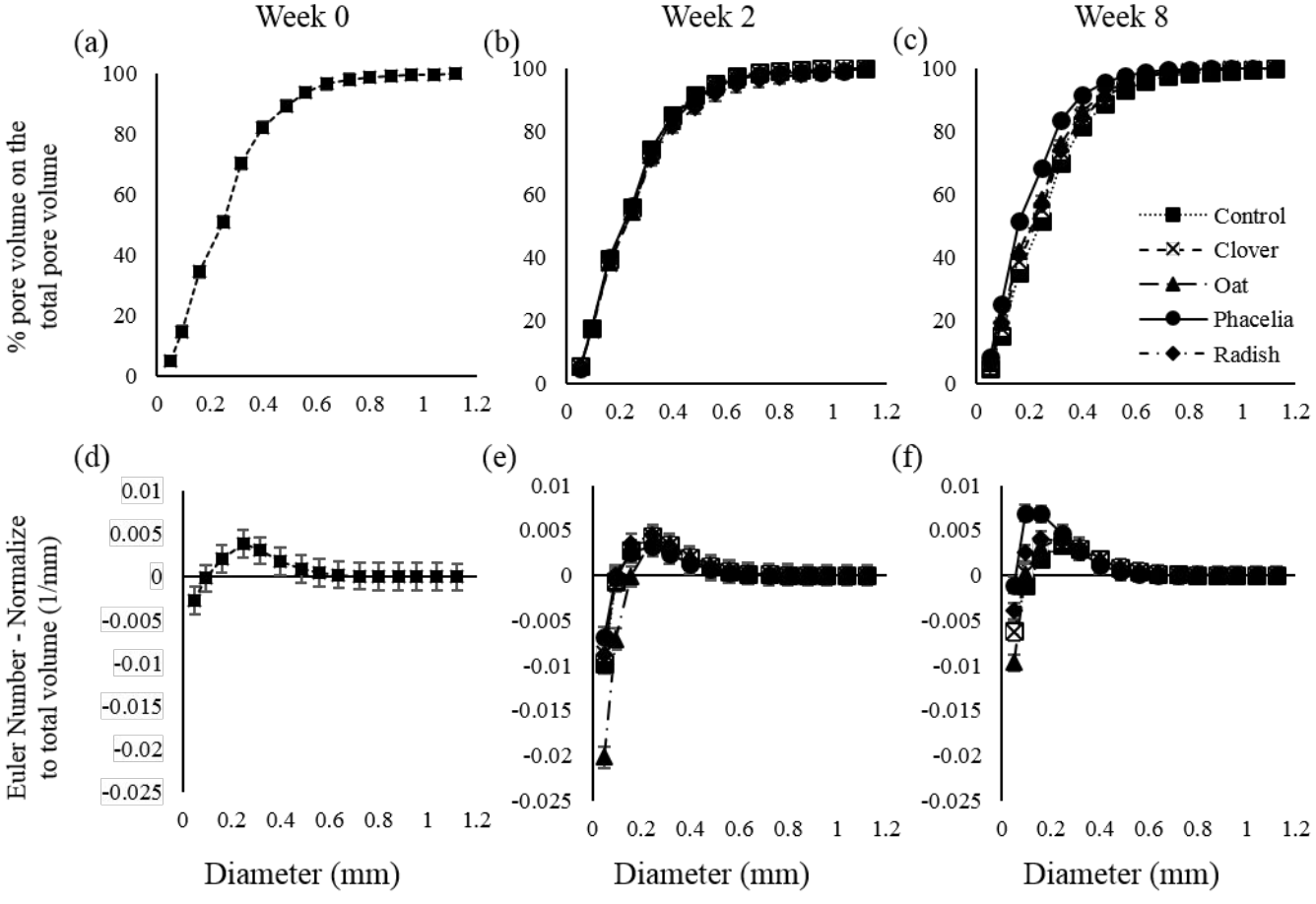


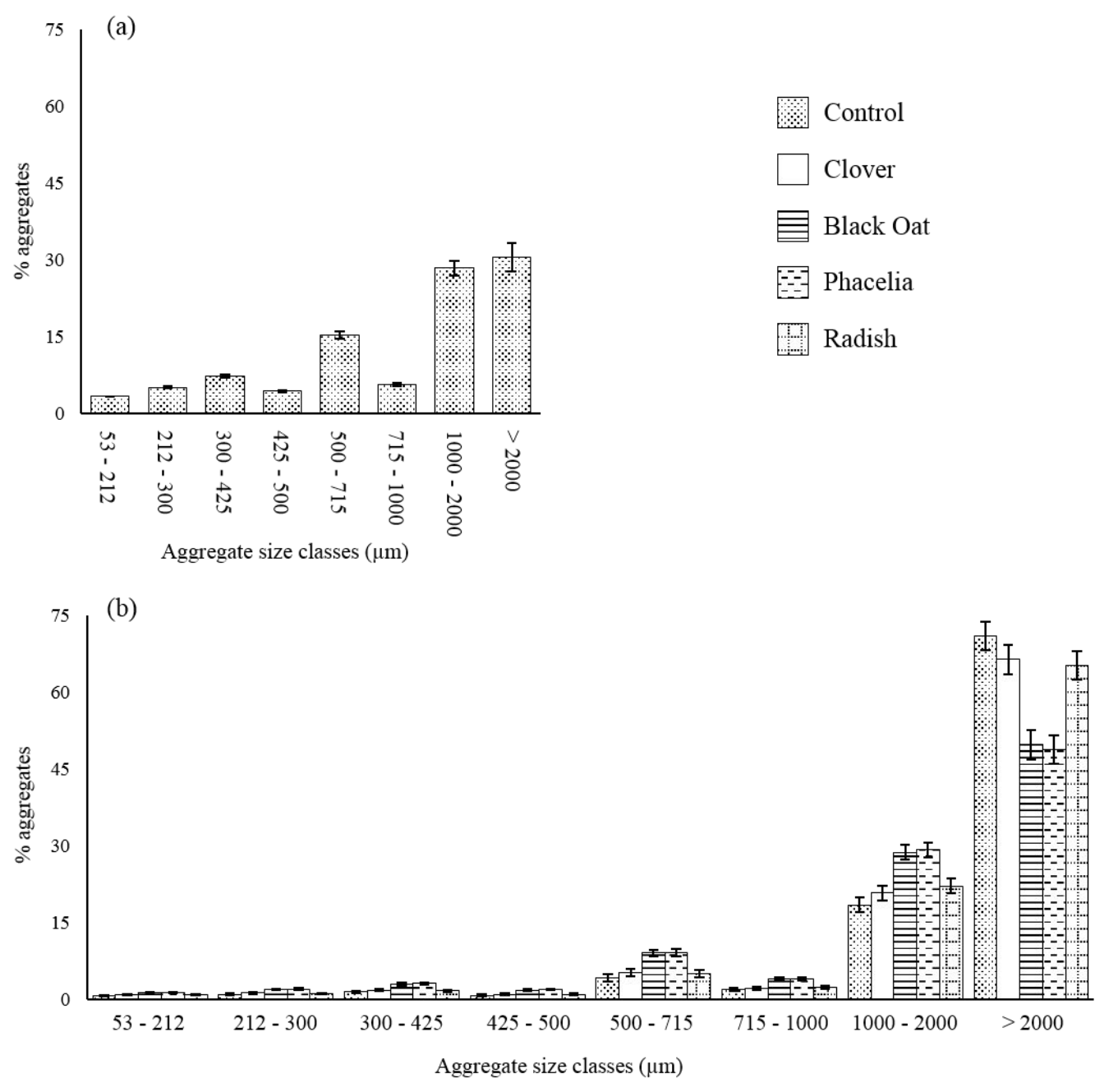
426

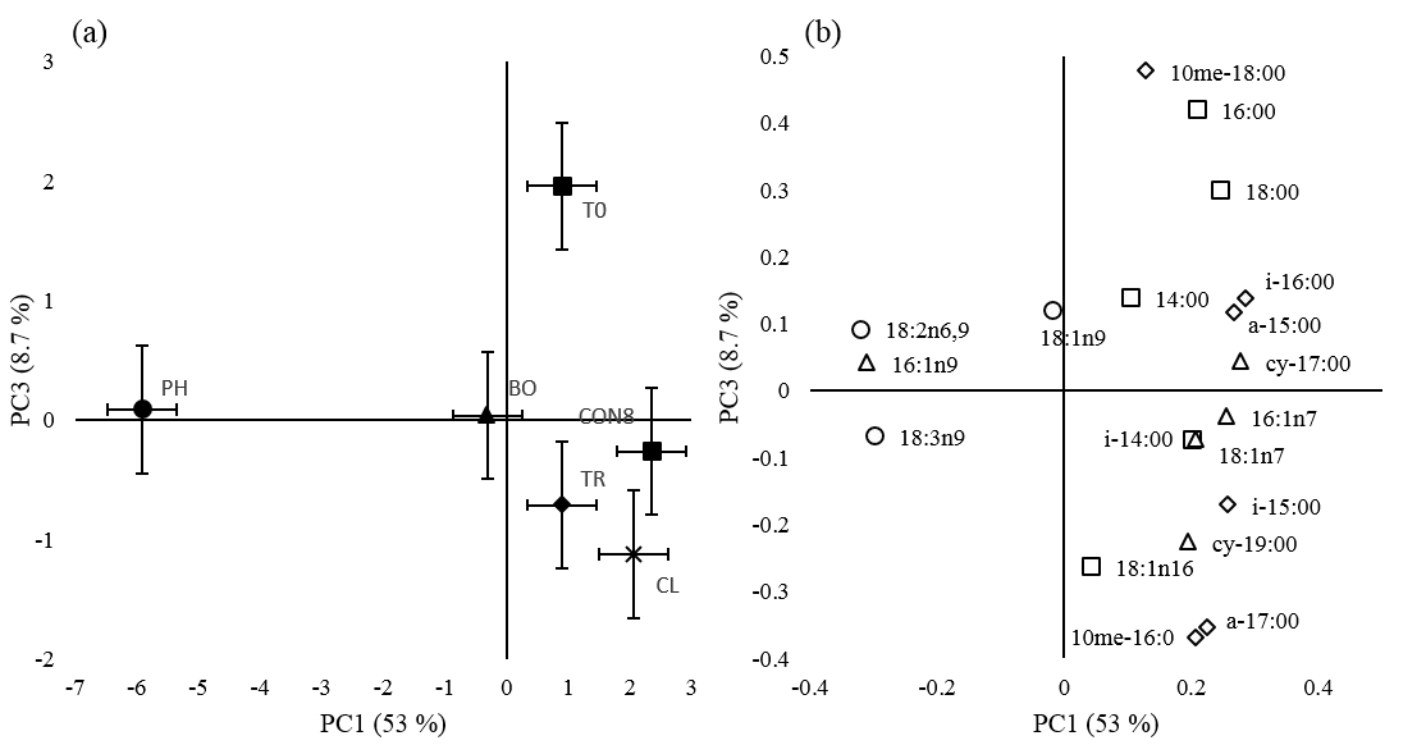
427 **Figure 6:** Proportion of PLFA divided per group of community: T0: control at week 0; after 8 weeks of
428 incubation: Con8: control; CL: clover; BO: Black oat; PH: phacelia and TR: Tillage radish

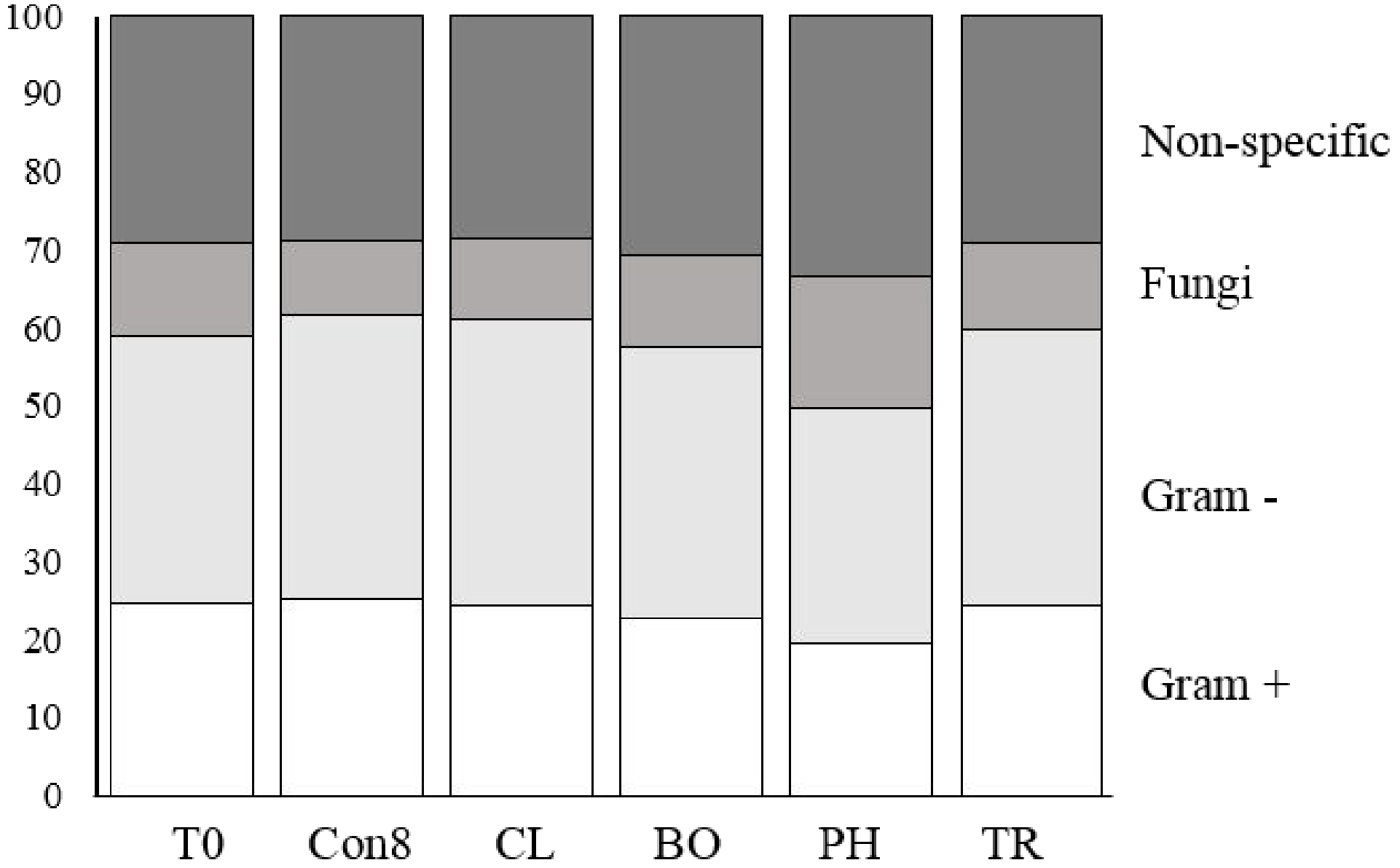

(a)
P
S
R
(b)
P
R
S
(c)
P
S
R
(d)
S
P
R
(e)
S
P


% pores
20
18
16
14
12
10
8
6
4
2
0
Control
Clover
Black Oat
Phacelia
Radish
Week 0
Week 2
Week 4
Week 6
Week 8


Week 0
Week 2
Week 8
(a)
(b)
(c)
(d)
(e)
(f)
% pore volume on the total pore volume
Euler Number - Normalize to total volume (1/mm)
Diameter (mm)
Diameter (mm)
Diameter (mm)
Control
Clover
Oat
Phacelia
Radish


(a)
75
60
45
30
15
0
% aggregates
Control
Clover
Black Oat
Phacelia
Radish
53 - 212
212 - 300
300 - 425
425 - 500
500 - 715
715 - 1000
1000 - 2000
> 2000
Aggregate size classes (µm)
(b)
75
60
45
30
15
0
% aggregates
53 - 212
212 - 300
300 - 425
425 - 500
500 - 715
715 - 1000
1000 - 2000
> 2000
Aggregate size classes (µm)


(a)
PC3 (8.7 %)
PC1 (53 %)
T0
PH
BO
CON8
TR
CL
(b)
PC3 (8.7 %)
PC1 (53 %)
10me-18:00
16:00
18:00
i-16:00
14:00
a-15:00
18:2n6,9
18:1n9
16:1n9
cy-17:00
16:1n7
18:3n9
i-14:00
18:1n7
i-15:00
cy-19:00
18:1n16
a-17:00
10me-16:0


100
90
80
70
60
50
40
30
20
10
0
T0
Con8
CL
BO
PH
TR
Non-specific
Fungi
Gram -
Gram +