Soil metabarcoding identifies season indicators and differentiators of pig and *Agrostis/Festuca* spp decomposition

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Highlights

- NGS identified 16S taxonomic shifts in soil during subsurface decomposition.
- *Arthrobacter*, *Sphingobacterium* and *Pedobacter* identified as microbial clock and season indicators for pig.
- *Sphingobacterium* differentiated between pig and plant litter soil necrobiomes.
- *Devosia* differentiated between control and treatment soil community compositions.

ABSTRACT

To gain a better understanding of how environmental microbiota respond to cadaver decomposition, a forensic ecogenomic study was made with soil only control and 4 g each of *S. scrofa domesticus* and plant litter (*Agrostis/Festuca* spp) buried individually in a sandy clay loam (80 g) in sealed but perforated triplicate microcosms. The next-generation sequencing (Illumina Miseq) of the soil bacteria (16S rRNA gene) clade revealed seasonal taxomonic shifts at genus-level for the pig and plant litter microcosms compared to the non-burial controls. In particular, numerical abundances of *Sphingobacterium* (5.9%) and *Pedobacter* (24.1%) for the pig microcosms, and *Rhodanobacter* (18.1%) and *Shinella* (4.6%) for the plant litter microcosms, identified bacterial genera that could be tracked to establish a (seasonal) subsurface postmortem microbial clock. Also, family-level resolution revealed members that were unique to the control, grass and pig soils after 365 days.

Keywords: Cadaver; Forensic ecogenomics; Sus scrofa domesticus; Plant litter; Illumina Miseq

INTRODUCTION

Characterization of soil microbial communities by culture-independent techniques has indicated their potential forensic applicability in estimating postmortem interval (PMI). Nevertheless, accurate PMI estimation still poses a challenge since soil is a complex heterogeneous habitat with diverse microbial communities. Thus, next-generation sequencing techniques such as 454-pyrosequencing, Illumina and Ion Torrent [1–5] have identified likely microbial taxa that are involved in and/or affected by cadaver decomposition [4,6–9]. Consequently, terms such as "microbiome", "necrobiome" and "thanatomicrobiome" [1,2,10,11] have been applied and coined to describe microbial communities associated with decomposition. For example, Lauber et al. [2] reported changes in the necrobiome of mice (*Mus musculus*) during active and advanced decay with an increased decomposition rate in non-sterile soil. On a seasonal basis, Carter et al. [12] recorded variations in microbial communities from soil beneath a decomposing *Sus scrofa domesticus* and emphasised the importance of seasonality in PMI estimation. Also, Hyde et al. [5] observed changes in bacterial

community composition with decomposition stages where *Ignatzschineria*/*Wohlfahrtimonas* and *Acinetobacter* spp were predominant during the bloat/purge and skeletonization phases, respectively. Similarly, Pechal et al. [13] used swine carcasses and reported changes in bacterial communities at phylum and family taxonomic levels and suggested that the shifts could be used to estimate and define unique decomposition intervals. Thus, Metcalf et al. [1] and Pechal et al. [13] advocated the utility of the microbial community as a potential "postmortem microbial clock".

Although these studies highlighted the potential and relevance of forensic ecogenomics, more investigations are needed to elucidate fully the complex interactions between cadaver decomposition and the impacted surrounding soil ecology. Towards this, microbial community dynamics of soil only (control), *S. scrofa domesticus* and plant litter (*Agrostis/Festuca* spp) decompositions were characterized in a 365-day (Study II) microcosm study. The experimental design facilitated specific research objectives to:

(i) Identify the 16S bacterial and 18S eukaryote soil community compositions during decomposition;
(ii) Determine taxa that reflect seasonal changes; and
(iii) Measure the predominance of (similar) taxa as a result of *S. scrofa domesticus* and plant litter (*Agrostis/Festuca* spp) decomposition.

MATERIALS AND METHODS

Soil collection and processing. As detailed previously by Olakanye et al. [14], sandy clay loam was collected from a site at Bishop Burton College of Agriculture, Lincolnshire, U.K. (Lat. 53.27°N, Long. 0.52°W) for Study II. The soil was milled thoroughly (Retsch SM 100, Retsch, Haan, Germany) and sieved (ASTM - standard soil sieve No 10; 2 mm mesh; sterilized by autoclaving at 120°C, 15 psi for 20 min) to ensure homogeneity. The microcosms were filled with the fine soil fraction and maintained outdoors (Teesside University, Middlesbrough, U.K.; Lat. 54.5722° N, Long. 1.2349° W).

Experimental design and sampling. As reported previously [14], triplicate microcosms (polyethylene, 127 ml, 50 x 70 mm; VWR, Lutterworth, U.K.) were established

for each of the control (80 g soil), *S. scrofa domesticus* (soil + 4 g pig) and *Agrostis/Festuca* spp (soil + 4 g plant litter) to enable destructive sampling on days 7, 14, 28, 60, 120, 180, 300 and 365 between July 2013 and July 2014. These microcosms were perforated every 7 mm (width) x 35 mm (height) for hygiene maintenance, aeration and moisture migration. The soil nutrient properties, pH and temperature measurements are presented in Olakanye et al. [14].

DNA extraction and next-generation sequencing. Soil DNA was extracted from the control, plant litter and pig microcosms as described previously [8] with FastDNA®Spin Kit for Soil (MP Biomedicals, U.K.) according to the manufacturer's instructions and stored at -20°C until needed. Triplicate extracts from days 0, 28, 180 and 365, representing the months of July 2013 (summer), January 2014 (winter) and July 2014 (summer), were pooled for the control, plant litter (*Agrostis/Festuca* spp) and *S. scrofa domesticus* soils and used for 16S rRNA gene analysis.

All samples were sequenced with Illumina Miseq platform (Research and Testing Laboratory, Lubbock, Texas, U.S.A.) with the primer sets 28F/519R (16S bacterial gene; V1 – V3 region; 5'-GAGTTTGATCNTGGCTCAG-3'/5'-GTNTTACNGCGGCKGCTG-3') [15]. The raw sequences were processed in FASTQ format, merged with PEAR Illumina paired-end read merger and converted into FASTA formatted sequences for quality checking and filtering. Operational taxonomic unit selection was made with UPARSE and Chimera checking was performed using UCHIME executed in *de novo* mode. Taxonomy was assigned using USEARCH global search algorithm (http://drive5.com/usearch/), while the phylogenetic tree was constructed using MUSCLE (www.researchandtesting.com/"version 2.2.4").

Data analyses. The relative abundances (%) of microbial taxa were determined as the number of operational taxonomic units (OTUs) reads relative to the total number of OTUs reads for all the samples [16]. Unclassified OTUs were filtered out and only OTUs >3% (relative abundance) were plotted. Alpha diversity was estimated with Shannon diversity [17]. The phylogenetic distance matrices were analysed using Bray-Curtis dissimilarity with nonmetric dimensional scaling (NMDS). A heatmap was constructed for family relative abundances (>0.3 %) using the gplots package (R version 3.3.2, gplots version 3.0.1; R Core Team) with increasing colour codes from grey to yellow. Differences at family-level taxonomic

resolution between control and treatments (plant litter and pig) were analysed by PERMANOVA (PAST 3.10, 2015). Pair wise multiple comparisons after a multi-way ANOVA with Tukey (HSD) *post hoc* tests ($p < 0.05$) were used to analyse significant differences in OTUs between the control, treatments and seasons [4,16,18,19].

RESULTS

16S taxonomic resolution. The control, *Agrostis/Festuca* spp and *S. scrofa domesticus* microcosms recorded a total of 212 248 sequences for days 0, 28, 180 and 365 combined (Table S1). On day 0, Proteobacteria accounted for ≥ 50% of the total community with *Hyphomicrobium*, a genus consisting of chemoorganotrophic rod-shaped denitrifying bacteria [20], belonging to Alphaproteobacteria, dominant. Overall, four phyla, Actinobacteria, Proteobacteria, Bacteroidetes and Firmicutes, were numerically dominant across all three microcosms with Bacteroidetes recording increased abundance in the pig microcosms on day 365 (summer 2014) (Table S1).

Analysis of dominant taxa at family-level, including Micromonosporaceae, Rhizobiaceae, Planococcaceae, Xanthomonadaceae, Hyphomicrobiaceae and Sphingobacteriaceae, highlighted temporal and seasonal 16S community composition shifts with decomposition as revealed by the heatmap (Fig. 1). For example on day 28, Rhizobiaceae showed differences between all three microcosms with, generally, increased relative abundance in the presence of *Agrostis/Festuca* spp. Likewise, Planococcaceae and Micromonosporaceae showed increases on day 28 for the pig treatment compared to both the control and plant litter microcosms. On day 180 (winter 2013), the pig microcosm recorded Staphylococcaceae as the only taxon shift. Unique taxa shifts were recorded exclusively for the control, plant litter and pig microcosms on day 365 (summer 2014). For example, Norcardioidaceae, Comamonadaceae, Alicyclobacillaceae and Bradyrhizobiaceae characterised the control while Nocardiaceae, Alcaligenaceae, Micrococcaceae and Hyphomicrobiaceae were unique to the pig soils. Finally, Xanthomonadaceae and Sphingobacteriaceae were the only families that recorded increased numerical abundances that were distinctive to the plant litter and pig soils, respectively. The PERMANOVA analysis between the control and treatments showed no significant difference ($p = 0.055$).

Pairwise comparison with Turkey *post hoc* identified OTUs that recorded statistically significant differences at family-level resolution between the control and treatment soil samples (Table 1), and due to seasonal differences (Table 2). For example, Micrococcaceae, Gram-positive aerobic bacteria, Sphingobacteriaceae, Staphylococcaceae, Gram-positive facultative anaerobic bacteria [21], and Alcaligenaceae, aerobic Gram-negative rod or coccobacilli chemoorganotrophic bacteria [22], which was associated with advanced stages of decomposition [1], were statistically significantly different ($p < 0.05$) for the pig interment when compared to both the control and *Agrostis/Festuca* spp litter microcosms (Table 1). While Alicyclobacillaceae, aerobic Gram-positive chemoorganotrophic bacteria, and Comamonadaceae, aerobic Gram-negative bacteria commonly found in soil and water habitats [23], were significantly different ($p < 0.05$) for the control microcosm, only Xanthomonadaceae, aerobic Gram-negative straight rod obligate bacteria [24], were significantly different ($p < 0.05$) for the plant litter microcosms. Also, Alicyclobacillaceae recorded a statistically significant difference for the control compared to the plant litter and pig treatments ($p = 0.001$).

Seasonal taxa differences (Table 2) were recorded mostly during summer 2014 with clades such as Micrococcaceae, Sphingobacteriaceae, Alicyclobacillaceae, Comamonadaceae, Xanthomonadaceae and Alcaligenaceae significantly different ($p < 0.05$) when compared to summer and autumn 2013. Only Staphylococcaceae was significantly different ($p < 0.05$) during winter 2013. Uniquely, Micrococcaceae, Caulobacteraceae and Alcaligenaceae recorded statistically significantly different abundances for the pig compared to the control and plant litter microcosms, and then between summer 2014 and the other two seasons.

The Shannon index plot (Fig. 2) showed no statistically significant differences ($p > 0.05$). However, the two-dimensional NMDS (stress = 0.15) of the bacterial community structure at genus-level identified temporal differences with days 28 (summer 2013) and 180 (winter 2014) significantly different from day 365 (summer 2014) (Fig. 3). For example, *Hyphomicrobium* and *Solirubrobacter* decreased from 6.78% and 2.89% on day 0 to <1% and below detection on day 365, respectively (Fig. 4). Also, *Kribella* was detected (5.45% – 10.84%) until day 180 but not on day 365. In contrast, dominances of *Rhodanobacter* (18.12%)

and *Dyella* (3.04%) were recorded only on day 365 for the *Agrostis/Festuca* spp microcosm. While *Rhodanobacter* was dominant at the same sampling time from the pig microcosm, *Dyella* was not detected.

Other taxonomic shifts included increases in the relative abundances of *Shinella* (4.63%), aerobic Gram-negative nitrogen-fixing symbiotic bacteria [25] and aerobic Gram-positive bacteria [21,26] *Micromonospora* (16.27%) and *Sporosarcina* (3.72%) on day 28 for the plant litter and *S. scrofa domesticus* treatments, respectively. This contrasted the dominance of *Actinoplanes* (3.24%), Gram-positive spore-forming mycelium aerobic bacteria [26], and *Solirubrobacter* (8.37%), Gram-positive aerobic bacteria [26], for the control microcosms. In addition, a seasonal shift from summer to winter between days 28 and 180 resulted in the dominance of *Bacillus* for the control and treatments.

Together with overall community structure differences with time, taxa abundances were examined further to identify treatment-specific indicators, particularly on day 365. Thus, the subsequent seasonal change from winter to summer resulted in a dominance of Proteobacteria for both the control and plant litter microcosms, which contrasted a dominance of Bacteriodetes in the *S. scrofa domesticus* treatment and a specifc predominance of Firmicutes in the control soil. Variations at the genus-level characterized the three microcosm types with the presence of *Alicyclobacillus* and *Tumebacillus*, aerobic Gram-positive chemoorganotrophic bacteria from the family Alicyclobacillaceae [21], and chemolithoautotophic *Bradyrhizobium* [27] for the control and *Rhodanobacter* for the *Agrostis/Festuca* spp treatment. Also, increases in *Rhizobium*, Gram-negative symbiotic bacteria of the phylum Proteobacteria involved in nitrogen fixation, deamination, ammonification and denitrification [25], were recorded for the control and plant litter microcosms. Likewise, the pig microcosms were characterized by *Rhodococcus*, *Arthrobacter*, *Pedobacter*, *Devosia* and *Sphingobacterium*, aerobic Gram-negative bacteria from the family Sphingobacteriaceae that contain sphingophospholipid and ceramides in their cell membranes [28]. Specifically, the *S. scrofa domesticus* microcosms recorded dominances of *Pedobacter* (24.14%) and *Devosia* (6.31%) compared to the plant litter (1.31%; 1.78%) and control (0.01%; 0.15%) (Fig. 4). Also, *Sphingobacterium* was absent in the plant litter microcosm but recorded

a 5.92% dominance in the *S. scrofa domesticus* treatment. Similarly, *Arthrobacter* and *Rhodococcus* were not recorded for the control but were present at 4.85% and 3.53% abundances, respectively, in the pig treatment.

DISCUSSION

Cadaver decomposition is a complex process that affects the soil microbiota. The ability to identify components that are involved actively in and impacted by decomposition, irrespective of the cadaver microbiome, justifies further the relevance and applicability of cadaver/soil ecology interaction analyses for postmortem interval and time-since-burial estimations. The use of next-generation sequencing techniques in necrobiome studies as a potential PMI tool has aided the identification of microbial taxa that are involved in decomposition [1,2,5]. According to Metcalf et al. [29], approximately 40% of the total soil microbiota are involved at the onset of decomposition at low relative abundance.

To better understand key temporal and seasonal interactions between soil ecology and cadaver decomposition, we compared microbial community dynamics in the presence of two different carbon sources: *S. scrofa domesticus*, as a human taphonomic proxy; and *Agrostis/Festuca* spp, as a non-animal organic material. Overall, the 16S community structure varied in the presence of pig and plant litter. Although common to most soils, dominances of Proteobacteria; Actinobacteria; Bacteroidetes and Firmicutes as also reported in forensic contexts [4,5,10], were consistent with this study where they accounted for approximately 90% of the bacterial phyla. Specifically, the phylum-level taxonomic community shifts resulted with decreases in Proteobacteria and increases in Actinobacteria and Firmicutes as reported previously [5,7,13], but on day 28 for this study. Characteristically, these taxa have been associated with soil microbiota, the human microbiome and meat spoilage [30].

Ubiquitous families such as Micromonosporaceae and Bacillaceae were recorded for the entire decomposition timeline but with the former, in particular, recording highly consistent relative abundances throughout the trial. The taxon would, therefore, be an identifier for this soil type but neither a target for pig/plant litter decomposition nor a microbial clock indicator. In contrast, Staphylococcaceae was recorded solely for the *S. scrofa domesticus* treatment after 180 days with no specific differentiators for the control and *Agrostis/Festuca* spp soils, making

this family a decomposition indicator for the human analogue and winter season. Further to this, new families that were often exclusive of one treatment or the control highlighted temporal divergences, which were considerably pronounced on day 365. Thus repeat studies on this soil type, using the same decomposition substrates and timeline, could target the temporally unique families towards understanding the dynamic processes within the soil necrobiome community.

While Cobaugh et al. [4] observed the presence of *Shinella* in the advanced stage of human cadaver decomposition, we observed its dominance in the plant litter microcosm only, and during early decomposition. The dominance of *Solirubrobacter* on day 28 (summer 2013) for the control soil microcosm contrasted the work of Carter et al. [12] who reported increased abundance of Solirubrobacterales in the winter period. Additionally, the presence of *Staphylococcus*, Gram-positive facultative anaerobic bacteria [21], for the *S. scrofa domesticus* microcosms on day 180 identified a useful community temporal and seasonal indicator for winter in this soil type. Although increases in the relative abundance of Sphingobacteriaceae (Bacteroidetes phylum) have been reported previously during decomposition [1,12], we observed its increase in the pig microcosm particularly on day 365 during summer 2014.

Taxa analyses, which emphasized dominance rather than presence/absence, suggested that Micromonosporaceae/*Micromonospora* were likely early (day 28) PMI indicators of pig decomposition at family/genus-level. *Devosia,* Sphingobacteriaceae/*Sphingobacterium*, *Pedobacter* and Xanthomonadaceae/*Rhodanobacter* were then seasonal (summer) PMI markers that also differentiated between pig and plant litter during late (day 365) decomposition. Furthermore, while Metcalf et al. [1] associated a predominance of the Xanthomonadaceae with mouse decomposition, its decrease in the *S. scrofa domesticus* microcosms aligned our findings to the work of Hyde et al. [5] and Pechal et al. [13] who used human cadaver and swine carcass as the animal model. In particular, the heatmap identified Sphingobacteriaceae as a key family indicator for summer and 365 days since interment. Also, *Arthrobacter* and *Rhodococcus* were ideal genus-level microbial clocks to differentiate between control and pig for the sandy clay soil.

Some bacterial (*Bacillus*) members dominanted the soil controls in comparison with the *S. scrofa domesticus* burial soils and so excluded these genera as microbial clock indicators

for this soil type. Notwithstanding this, shifts in clades such as *Sphingobacterium* and *Rhodanobacter* provided evidence for 16S-based divergences between pig and vegetation decomposition. In particular, the sole incidence of *Sphingobacterium* and the marked dominance increase of *Pedobacter* in the presence of the mammalian taphonomic proxy, concomitant with the absence of the former in the control and plant litter micrososms, identified them as (seasonal) microbial clock indicators for the sandy clay soil. Similarly, some families were recorded exclusivly for the control, pig or plant litter soils at the end of the study. These genera/families could, therefore, be targeted to identify/and or predict the presence of a body, 365 days after interment.

Generally, the recorded divergences identified families and genera that could be useful microbial indicators in subsurface decomposition studies, for which there is considerable paucity. Notwithstanding this, the effects of time and season must be explored comprehensively and separately. They, therefore, mandate further robust and protracted experimental designs that consider: (i) different start (D0) dates, possibly from different seasons during the same calendar year; and (ii) the same start and end dates during similar seasons but in consecutive/different years. Thus the identification/differentiation of consistent (non-transient) temporal and seasonal microbial clocks for the same soil type and animal model would be attained.

Initiatives such as the human microbiome [31] and thanatomicrobiome [19] projects aimed to identify and differentiate core and transient members that can be used to establish health and disease states, and be adopted as microbial clock indicators for enhanced PMI determinations, respectively. In parallel, and although outwith the forensic context, multi-team studies (e.g. Stone et al. [32]) have illustrated the utility of analysing microbial ecological dynamics of soils under similar and different management regimen, between similar soil types, and/or across geographical locations with different climatic conditions. Similarly, the effects of soil depth (surface, subsurface) and season (summer, winter) on microbial community size, structure and function were exemplified by Blume et al. [33] who recorded sesonal effects on subsurface population structure and activity. Notwithstanding this, changes and similarities in

soil ecology are typically dependent on several factors including experimental design, analytical method(s), site, soil type, depth, vegetation cover, etc. Therefore, concerted efforts are required for soil necrobiome profiling both aboveground and in the subsurface to establish the applicability of temporal- and seasonal-focused ecogenomic analyses in forensic scenarios. Ultimately, it is essential to study site-specific non-burial controls in protracted decomposition-based investigations. This will elucidate the occurrences of both unique and universal soil microbial taxa within each site.

CONCLUSIONS

In summary, next-generation sequencing of pooled DNA samples for the forensic ecogenomics study identified taxonomic changes at both family and genus levels due to the presence of decomposing material, particularly *S. scrofa domesticus,* with temporal effects determined for the bacterial communities. This is the first research where microbial decomposer communities of two C/N sources were compared in a forensic ecogenomics context to further knowledge of necrobiomes. As reported in Olakanye et al. [8,14], DGGE-based profiling identified spatio-temporal shifts in ecological indices between the burial and non-burial soils, generally, and relative to *S. scrofa domesticus* burial depth. The current trial reflects next-generation sequencing analysis where pooled DNA samples provided a preliminary investigation of bulk microbial taxonomic differentiation between the presence and absence of a decomposing human cadaver analogue, and in comparison to plant litter. The approach models real cases more closely where soil microbial community analyses, from trace evidence and often with no opportunities for replicates, would parallel other forensic intelligence gathering for suspected crime locations. Our results suggested that non-burial and gravesoils can be differentiated at genus-level with the possibility of a bacterial clock for estimating postmortem interval/time-since-burial.

While this study is novel and provided further insight of the soil necrobiome community, it was made with processed soils for maxima of 365 days. Therefore, we recommend more *in situ* investigations with unprocessed soils of different types, whole cadavers/mammalian proxies, various plant litters, different start dates within the same year and same start dates/seasons across different years, with relevant attendant

mathematical/statistical analyses, for comprehensive subsurface postmortem necrobiome community analyses.

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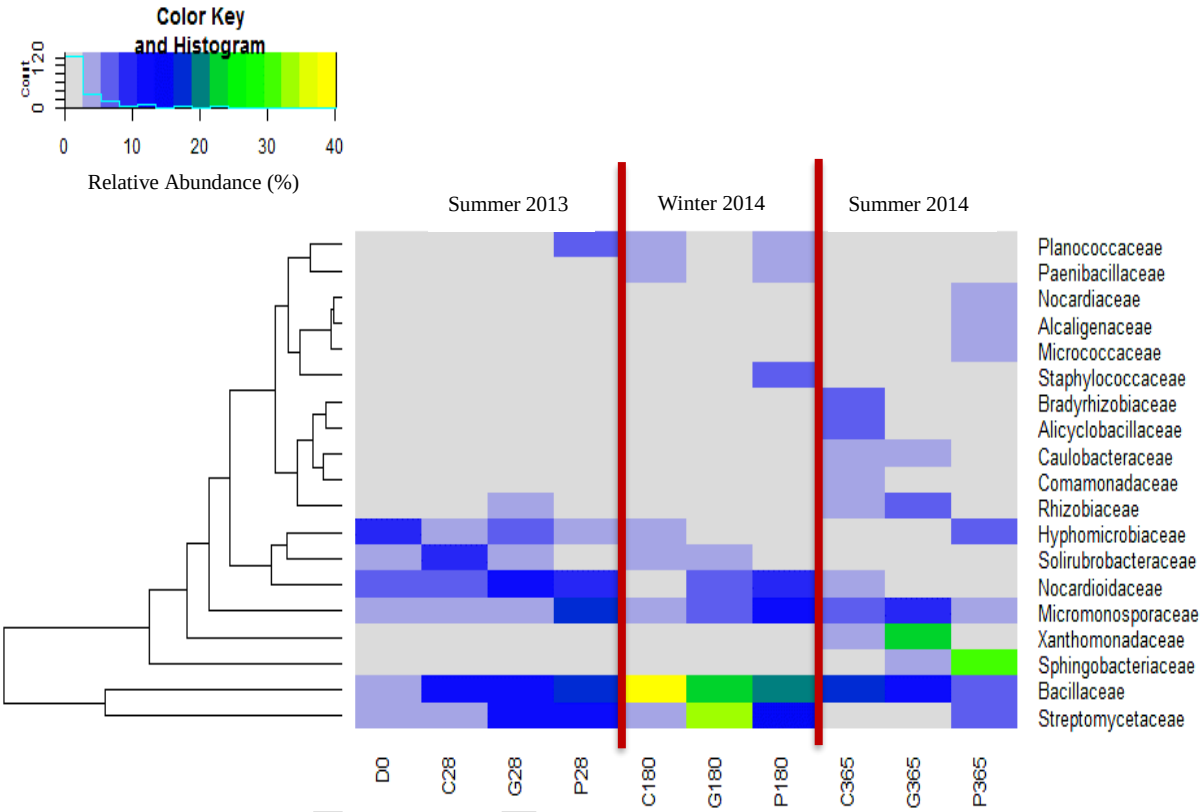

FIG 1 Heatmap to visualize the relative abundances (%) of the most predominant bacterial families (>0.3%) for the control (C), plant litter (*Agrostis/Festuca* spp; G) and pig (*Sus scrofa domesticus*; P) microcosms on days 0 (D0), 28, 180 and 365.

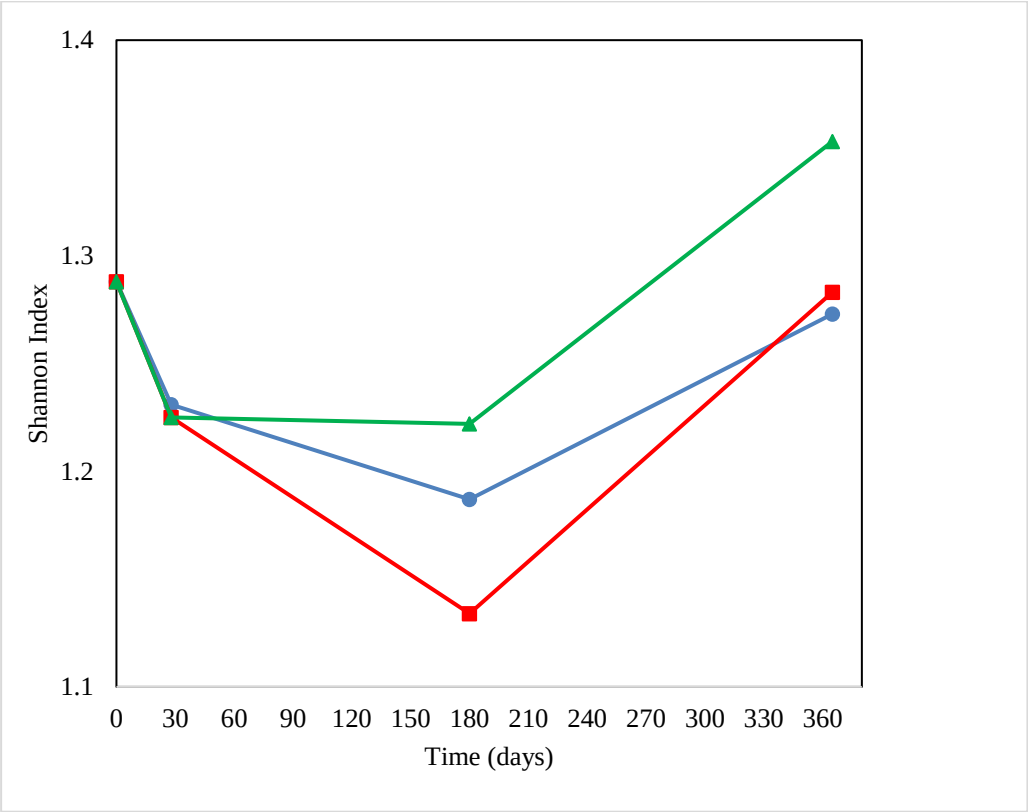

FIG 2 16S bacterial taxa Shannon index diversity plot of pooled DNA samples for the control (C; ●), plant litter (G; ▲) and pig (P; ■) microcosms on days 0, 28, 180 and 365.

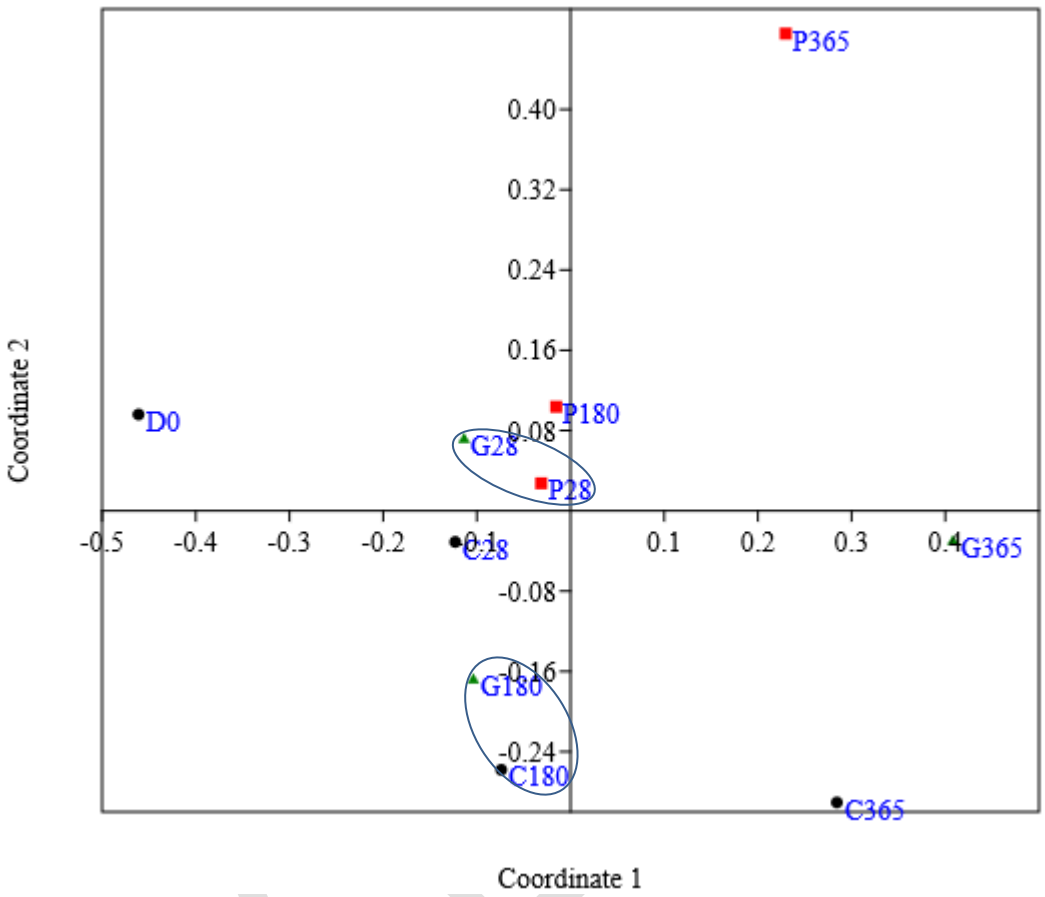

FIG 3 NMDS (stress = 0.15) plot for 16S bacteria community at genus level for the control (C; ●), plant litter (G; ▲) and pig (P; ■) microcosms on days 0, 28, 180 and 365.

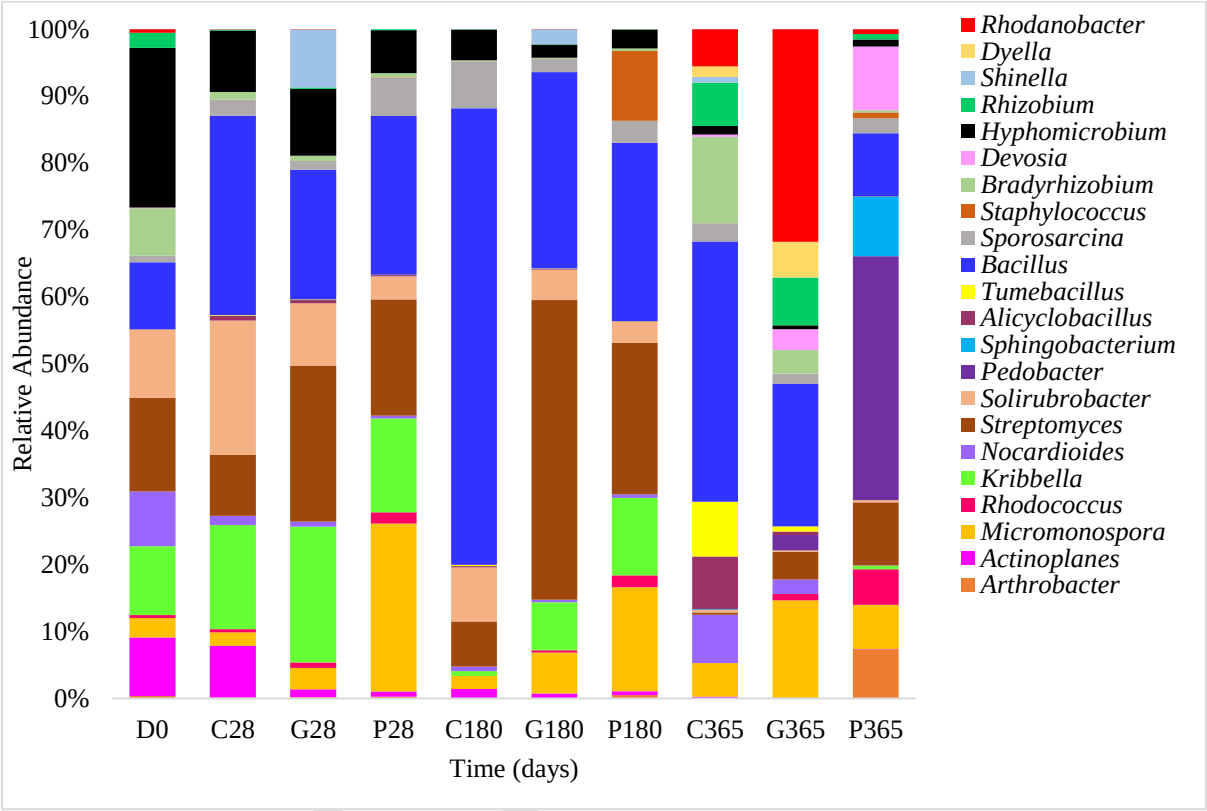

FIG 4 Bacterial taxa (genus) resolution of pooled DNA samples for the control (C), plant litter (*Agrostis/Festuca* spp, G) and pig (*Sus scrofa domesticus*, P) microcosms on days 0 (D0), 28, 180 and 365.

Table 1 Family-level OTUs that are statistically significantly different ($p < 0.05$) between the control and treatments soils according to the least squares means (LS-means). Combinations sharing the same letter are not significantly different while those with no letter in common are significantly different (a, b, c) as calculated by multi-way ANOVA with Tukey (HSD) *post hoc* tests.

OTUs (family)	Control	Plant Litter	Pig	$p < 0.05$
Micrococcaceae	0.027 b	0.036 b	1.778 a	0.000
Sphingobacteriaceae	0.112 c	1.458 b	10.034 a	0.000
Alicyclobacillaceae	2.574 a	0.360 b	0.041 b	0.001
Staphylococcaceae	0.000 b	0.017 b	2.495 a	0.000
Caulobacteraceae	1.435 a	1.767 a	0.319 b	0.002
Alcaligenaceae	0.097 b	0.324 b	1.125 a	0.003
Comamonadaceae	1.157 a	0.603 b	0.089 c	0.002
Xanthomonadaceae	1.241 b	7.688 a	0.471 c	0.000

Table 2 Family-level OTUs that are statistically significantly different ($p < 0.05$) between seasons according to the LS-means. Combinations sharing the same letter are not significantly different while those with no letter in common are significantly different (a, b, c) as calculated by multi-way ANOVA with Tukey (HSD) *post hoc* tests.

OTUs (family)	Summer 2013	Winter 2013	Summer 2014	$p < 0.05$
Micrococcaceae	0.108 b	0.115 b	1.619 a	0.000
Sphingobacteriaceae	0.014 b	0.003 b	11.588 a	0.000
Alicyclobacillaceae	0.134 b	0.144 b	2.697 a	0.001
Staphylococcaceae	0.003 c	2.317 a	0.191 b	0.000
Caulobacteraceae	0.246 b	0.043 b	3.232 a	0.002
Alcaligenaceae	0.161 b	0.040 b	1.346 a	0.003
Comamonadaceae	0.125 b	0.015 b	1.709 a	0.002
Xanthomonadaceae	0.289 b	0.018 b	9.093 a	0.000