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Human Cadaver Burial Depth Affects Soil Microbial and Nutrient Status

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Shallow burial (c. <0.3 m) of human cadavers provides an alternative to standard burial depth (c. 1.0 m) as this can enhance the natural recycling of nutrients to the soil through improved interactions between the corpse and the soil ecosystem. However, there is a paucity of knowledge describing the interactions between the human cadaver and soil microbiology at any depth. The effects of shallow were compared to standard burial depth on soil chemical (available nitrogen and phosphorus, and organic matter) and microbial (total biomass and activity, fungal biomass, and microbial community composition) characteristics in two soil types (sandy loam and clay). Measurements were taken six and eight weeks after the burial of fresh pork ribs (used as a substitute for the human cadaver). Quantities of plant available nitrogen, as both ammonium-N (clay soil) and nitrate-N (both soil types), were greater where the pork was shallow buried. In addition, there was a shift in the composition of the bacterial component of the soil microbial community where the pork was shallow buried compared to deep burial (sandy loam soil only). There were no differences between the two burial depths (both soil types) in soil organic matter, available phosphorus, total microbial biomass or activity, or the proportion of fungi within the microbial community. The differences in available nitrogen and the lack of differences in the bacterial community composition between the two depths for the clay soils is likely to be due to reduced pore space and hence reduced oxygen at depth, which would dominate any response of the microbial community to the decomposing meat.

Introduction

Natural burial (internment of the cadaver to soil without hindering or enhancing decomposition processes) often includes shallow burial to improve cadaver exposure to the soil microbial community, thus promoting the recycling of nutrients to the soil.

Keywords: cadaver decomposition, microbiology, soil depth, soil texture

Corpse decomposition, mediated by microbial processes on the surface of and within the cadaver, can provide large amounts of nutrients to the soil (Carter *et al.* 2007). This change in soil nutrient content can alter the microbial community composition of the soil environment (Finley *et al.* 2014). However, at depth there will be less oxygen available for microbial decomposition, thus it is likely that cadaver/soil interactions will change through the soil profile. Soil textural properties may compound this effect, as finer textured clay soils will have less pore space and so less oxygen available for microbial decomposition compared to coarser sandy soils. However, there is little knowledge regarding the effects of human decomposition processes on soil microbial properties (Finley *et al.* 2015; Hopkins *et al.* 2000) at any depth.

The aim of this study was to compare the effects of the decomposing cadaver (using pork meat as a human cadaver substitute) on soil properties at two burial depths (0.3 m and 1.0 m depth, representing shallow and standard burial practice respectively), within two soils of different textures (sandy loam and clay). The hypotheses were that a) where shallow buried, the soil microbiology will play a greater part in nutrient cycling processes compared to standard burial depth, and b) soil texture affects this contribution. These hypotheses were investigated through observations of changes in the soil organic matter content, microbial biomass, activity, and community composition, and plant available nutrients (nitrogen and phosphorus) over time (eight weeks).

Materials and methods

Individual fresh pork ribs (450 g) were buried in either a sandy loam (80% sand, 13% silt and 7% clay) or a clay (34% sand, 32% silt and 34%) soil, at a depth of either 0.3 m (shallow burial) or 1 m (standard burial). As such the experimental design comprised of: Soil Texture (2) X Burial Depth (2) with three replicates. The soil and buried pork meat were contained in PVC pipes (50 cm diameter), of which there were 12 in total. Soil was analysed prior to pork burial to ascertain baseline conditions, and then twice after experiment initiation (6 and 8 weeks after burial) to ensure that the analysis corresponded to later stages of cadaver decomposition whereby nutrients are purges into the soil ecosystem. Soils were sampled from the immediate vicinity (2 cm) of the pork, through a side window, pre-cut in the PVC pipe to minimise soil/ meat disturbance. Soils were then analysed according to standard procedures and included: organic matter (British Standards, 2000), plant available nitrogen (as ammonium NH_4^+-N and nitrate NO_3^-N) (MAFF method 53: 1986), plant available phosphorus (British Standards, 1995), microbial biomass (an estimate of the total living microbial population) by the fumigation extraction technique (Vance *et al.* 1987), microbial community (phenotypic) composition by phospholipid fatty acid analysis (PLFA) (Frostegård *et al.* 1991; Pawlett *et al.* 2013) using the PLFA signature biomarker 18:2ω6 as an indicator of fungal biomass (Frostegård *et al.* 1991), and soil respiration rate to indicate microbial activity (Pawlett *et al.* 2013). The soil temperature was an average of 19.2 °C (±0.3 °C) throughout the experiment.

Data was analysed using a factorial ANOVA (LSD): soil depths (2) x soil textures (2) using a Repeated Measures (RM) design (at 6 and 8 weeks). PLFA data was analysed using Principal Component Analysis (PCA) followed by RM- ANOVA of the PCA factor

scores. Statistics were performed using Statsoft, Inc. (2012) STATISTICA version 11 (data analysis software system), with an alpha value of 0.05.

Results and discussion

The first stage of the nitrogen cycle involves the mineralisation of organic forms of nitrogen to plant available N as ammonium (NH_4-N). In our experiment, NH_4-N (Figure 1a) was greater (almost x 3) where the pork was shallow buried in the clay soil; however, there was no difference in NH_4-N concentrations at the two burial depths for the sandy loam soil. This suggests that shallow burial will increase mineralisation processes compared to deep burial in the clay but not in the sandy soil. Following the conversion of organic N to NH_4-N, the next stage in the nitrogen cycle is its oxidisation to NO_3-N (nitrification) by aerobic nitrifying bacteria. NO_3-N (Figure 1b) was greater where the pork was shallow buried, for both the sandy loam and clay soil; however, this reduction was much greater in the clay soil. Indeed the mean NO_3-N at the depth of 1.0m in the clay soil was 2 mg/kg, compared to 178 mg/kg in the shallow soil, suggesting greater nitrification where the pork was shallow buried. Microorganisms that mineralize nitrogen require oxygen, which would be more available in shallow compared to the deeper soil. In addition, clay soil would have reduced pore space and so less available oxygen compared to the sandy loam, and so reduced

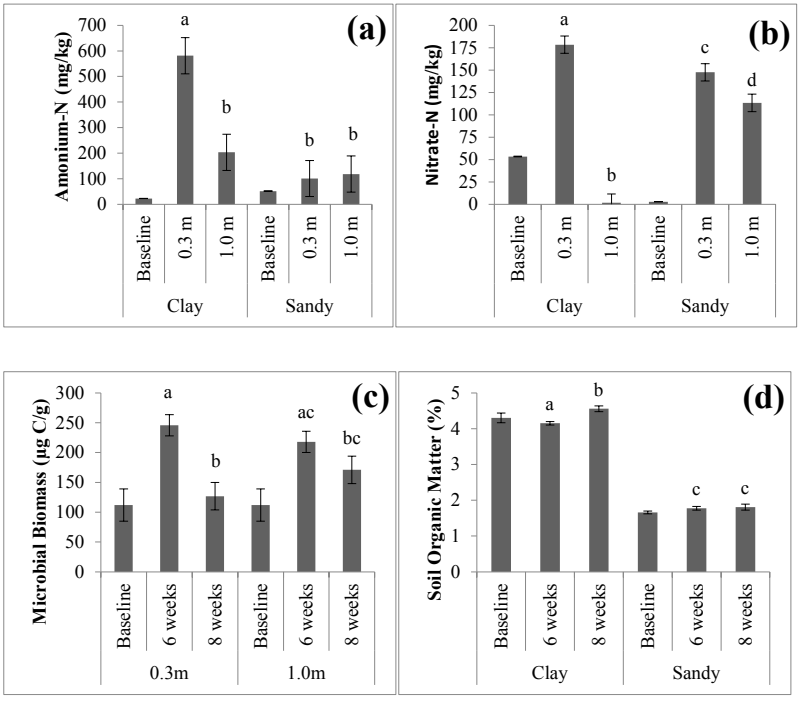

Figure 1. The effects of experimental treatments on: (a) ammonium-N (b) nitrate-N (c) microbial biomass (d) organic matter. Data are means (with SE). Letters above the bars signify homogenous groups (p>0.05). Baseline represents the soil prior to the addition of pork meat.

nitrification. Both NH_4-N and NO_3-N are more available to plants than organic forms of N. Increased available nitrogen with shallow burial is likely to benefit plants; however, it is also important to consider that plant available forms of nitrogen can be detrimental to the environment as they are more soluble and so are easily leached to groundwater. Although burial depth affected quantities of plant available nitrogen, neither soil depth nor soil texture affected phosphorus availability, which was 43 mg/kg (mean ± SD 6 mg/kg) for both soils, irrespective of experimental treatments.

There was a reduction in microbial biomass between sampling times in both the shallow and deep burial treatments, but there was no significant effect of either soil depth or soil texture within either of the sampling times (Figure 1c). For microbial activity, there were differences between soil textures, but no effect of burial depth. Microbial activity was greater in the clay soil compared to the sandy loam soil, with the mean for both burial depths and times of analysis being 7.8 µg C-CO_2/g soil/h (± SE 0.7) and 3.0 µg µg C-CO_2/g soil/h (± SE 0.7) respectively. Hopkins *et al.* (2000) reported increased microbial biomass and activity in grave soils; however, burial depth in that study was 10cm and soil sampling occurred 430 days after burial compared to shallow burial of 30cm and 8 weeks post burial soil sampling in our research. Although soil depth did not affect microbial biomass or activity in our study, there was a shift in the microbial community composition (PC2: $p<0.001$) in the sandy loam, but not for the clay soil (Figure 2). Microbial fatty acids that contributed to the PC1 loadings included negative (<-0.8) contributions from 17:0i, cyc17:0, ai17:0, 17:0br, 17:1ω8t, 17:1ω7, 17:0 (Me), 18:0 (Me) and positive (>0.8) contributions from 16:1ω11t, 16:0, and 18:1ω7t. Only two fatty acids contributed to the loadings on PC2, which were 20:4 (<-0.8) and 16:0i (>0.8). The shift in the microbial community corresponds to time on PC1 and soil depth on PC2 (for the sandy loam). This shift was due to the bacterial component of the soil microbial community as the proportion of fungi (as indicated by the PLFA biomarker 18:2ω6, 9) was not affected by burial depth (and did not contribute to PCA loadings). However, the clay soil had greater fungal biomass compared to the sandy loam soil, with 9.6 mol% (± SE 0.7) compared to 5.8 mol% (± SE 0.7) respectively (means for both burial depths and times of analysis). The lack of an effect of burial depth in the clay soil is likely to be due to a greater influence of water stress and oxygen demand on the microbial community for the finer textured soil. These environmental factors are likely to dominate any other effects and thus the microbial community composition in the clay soils was normalised (for instance, it was similar at both depths). In the sandy loam soil, the relatively larger pore spaces compared to the clay soil will result in greater oxygen availability, which is likely to promote microbial community development and activity. Extrapolation of PCA data in Figure 2 may suggest that the shift in bacterial community composition with soil depth may increase in time, however this would need to be validated with further analysis. With reduced levels of available oxygen in the clay soils, it is likely that an anaerobic bacterial community is developing, which can be detrimental to healthy plant root development.

As with the fungi results, burial depth did not affect quantities of soil organic matter; however there were differences between soil textures, with clay soil having greater baseline organic matter content compared to the sandy loam soil (Figure 1d).

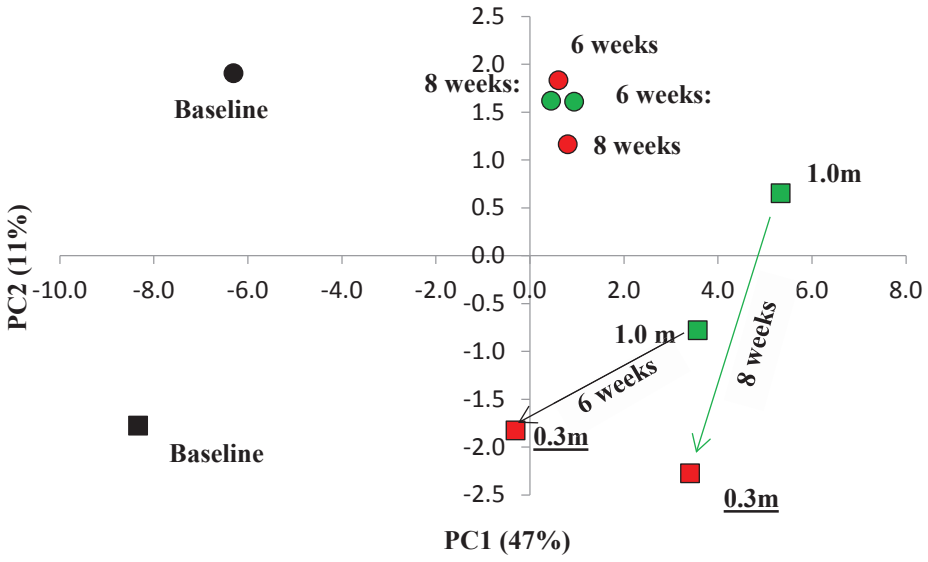

Figure 2. Microbial community composition (PLFA: PC means). Circle data points = clay, square data point = sandy loam, red = shallow, green = deep, black data points represent the baseline soil conditions prior to pork meat burial.

Fungi are critical primary decomposers of organic matter in many ecosystems, with complex enzyme systems and physical (hyphal) capability of degrading a wide range of recalcitrant forms of organic matter. Thereby as burial depth did not affect the soils fungal biomass, it may not be surprising that burial depth did not affect soil organic matter content.

It is important to consider that this experiment was conducted without plants and in isolation from larger soil organisms, such as earthworms. Both plant roots and earthworms may interact with the decomposing meat, thus aiding the transfer of organic constituents and microbiology from the meat to the wider soil environment (and vice versa). In addition, the non-significant results may be due to sampling timings. (Benninger *et al.* 2008) recorded a peak of soil phosphorus availability two weeks after leaving a pig carcass on the soil surface. Sampling within the present experiment occurred six and eight weeks after burial, therefore any initial peak in nutrients or microbiology shortly after burial would not have been identified within the present investigation. However, soil phosphorus in Brenninger's *et al.* (2008) research was still greater than the control after 100 days suggesting that if a peak in soil phosphorus did occur within our experiment it would have be detected. The difference may be that the extractable soil phosphorus in Brenninger's *et al.* (2008) research was extremely low (<0.05 µg /g) and so increases are more likely to be identified, whereas in our study available phosphorus was 43 µg /g (mean).

A waxy material (known as adipocere) forms on the decaying cadaver in oxygen-limited environments (Fiedler *et al.* 2012). This adipocere formation is likely to affect cadaver/soil interactions. Visual examination upon excavating the soil/meat cores at the end of the experiment identified adipocere formation in all of the samples,

which suggests low oxygen conditions (and/or high water contents) (Carter *et al*. 2010; Fiedler *et al*. 2012), and thus a barrier to cadaver/soil interactions, especially relating to soil (micro)biological processes.

Conclusions

The effects of burial depth on available nitrogen (increase) and soil microbial community (bacterial) composition were demonstrated experimentally, and these effects are specific to soil type. Others (for example, Hopkins *et al*. 2000) have also observed increased available nitrogen, microbial biomass and activity following cadaver burial, however previous studies did not observe the effect of burial depth or interactions of the buried cadaver with soil microbial community composition. Effects identified are likely to be due to altered oxygen availability with soil depth and between soil textural types. The shift in microbial community composition in the sandy loam soil is likely to promote cadaver decomposition and thereby nutrient cycling in the longer term. This finding requires verification and development through: increasing the length of time of the experiment; increasing the frequency of sampling; including any physico-biochemical processes associated with vegetation, and investigating effects in a wider range of soil types. Wrapping the meat in cloth will provide more realistic representation of natural burial practice. Results attained may also be beneficial in a forensic context for the use of soil microorganisms to increase the accuracy of extended Post-Mortem Interval (PMI) estimations (time since death) as few studies have focused on the use of microbial signatures to estimate PMI.

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