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ORIGINAL ARTICLE

Soil chemical markers distinguishing human and pig decomposition islands: a preliminary study

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Abstract

The decomposition of vertebrate cadavers on the soil surface produces nutrient-rich fluids that enter the soil profile, leaving clear evidence of the presence of a cadaver decomposition island. Few studies, however, have described soil physicochemistry under human cadavers, or compared the soil between human and non-human animal models. In this study, we sampled soil to 5 cm depth at distances of 0 cm and 30 cm from cadavers, as well as from control sites 90 cm distant, from five human and three pig cadavers at the Australian Facility for Taphonomic Experimental Research (AFTER). We found that soil moisture, electrical conductivity, nitrate, ammonium, and total phosphorus were higher in soil directly under cadavers (0 cm), with very limited lateral spread beyond 30 cm. These patterns lasted up to 700 days, indicating that key soil nutrients might be useful markers of the location of the decomposition island for up to 2 years. Soil phosphorus was always higher under pigs than humans, suggesting a possible difference in the decomposition and soil processes under these two cadaver types. Our preliminary study highlights the need for further experimental and replicated research to quantify variability in soil properties, and to identify when non-human animals are suitable analogues.

Keywords Decay · Necrobiome · Phosphorus · Nitrogen · Soil · Physicochemistry · Australian facility for Taphonomic experimental research

Introduction

The decomposition of human remains involves the breakdown of organs and tissues, and the release of fluids that transfers a variety of organic compounds and inorganic salts into the soil profile [1, 2]. The fluids and nutrients that enter the soil form a 'cadaver decomposition island' (CDI) that is biologically enriched relative to nearby soil [3]. Such trace evidence can be useful for investigations into the location or timing of death of individuals [2, 4].

Soil changes under decomposing human and other animal remains are typically ephemeral, but include elevated concentrations of macronutrients (e.g. C, N, P) and increases in physicochemical properties such as electrical conductivity and pH [1, 2, 5–7]. Other soil properties can also change and be useful indicators of cadaver decomposition, including elevated levels of lipids and cholesterol [8], and soil microbial communities that respond to nutrient inputs [9, 10]. A complex range of variables are therefore available to measure when examining soil responses to decomposition.

Insights gained from studies of soil and human decomposition include the extent of lateral movements of cadaver nutrients [11] and the timing of soil chemistry changes during different phases of decomposition as an indication of the post-mortem interval [4]. The different soil properties that change under decomposing cadavers therefore display important

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differential temporal and spatial dynamics [12]. This reflects progression through decomposition stages and soil microbial processing of the nutrient inputs [6, 10, 13]. Improved understanding of the dynamics of soil changes occurring during cadaver decomposition can provide an important backdrop to forensic investigations by demonstrating the magnitude of changes that might be expected, and their longevity, each of which might inform estimations of the location or timing of death of an individual.

The study of human cadavers is restricted in most countries of the world, resulting in most decomposition studies occurring in licensed decomposition facilities in the USA [14]. This means that soil researchers elsewhere have had to use non-human animal carcasses as 'analogues' or 'proxies' for human cadavers [e.g. 5, 7]. The use of such animal models has demonstrated many critically important patterns and processes occurring in soil chemistry, including the timing of peak release of specific elements [7, 12, 15]. Nevertheless, explicit comparison of soil elemental and physicochemical properties between human and other animal remains is lacking, and it is still unknown if other animals are suitable proxies for humans. Evidence is emerging of differences in the decomposition patterns, and timing and composition of volatile organic compounds and insect fauna at human and pig cadavers [16–18]. These studies raise questions about what other differences might be apparent between humans and other animals, including differences in soil chemistry, and if pigs are reliable models for forensic research.

In this study, we examined a suite of soil physicochemical markers at human cadavers and pig carcasses (hereafter cadavers) at the Australian Facility for Taphonomic Experimental Research (AFTER) [14]. We took samples at increasing distances from the lateral side of each cadaver. Cadavers had undergone between approximately 80 to 700 days of decomposition, and this provided a wide temporal range to examine the longevity of soil changes. As is the case with most human decomposition studies, all cadavers had different starting conditions and backgrounds, preventing true replication and detailed statistical analysis. Nevertheless, we were able to examine our data to answer the following questions: (i) Which soil markers are elevated under a cadaver? (ii) Do soil markers show any lateral spread from a cadaver? (iii) How long do changes in soil persist? (iv) Do any soil markers distinguish cadaver type (human vs pig)?

Methods

The site of our study, the Australian Facility for Taphonomic Experimental Research, is a human decomposition research facility operated by the University of Technology Sydney. The 4.86 ha site is situated in the Hawkesbury region of western Sydney (33° 38′ S, 150° 39′ E), and is characterized by dry sclerophyll *Eucalyptus* forest [8]. Ambient temperatures at AFTER were recorded every 15 min at the site using a HOBO MX2302 Ext temperature and relative humidity data logger (Onset Computer Corporation, Bourne, Massachusetts, USA) protected by a solar radiation shield.

Previous characterization of the soil in our study area was performed by Luong et al. [8], and was described as consisting of a dark brown organic-rich topsoil (the O and A1 horizons) to a depth of up to 15 cm. The A2 and B horizons underlying the soil consist of predominantly sands with some silts and minor clays, colored in shades of yellow, brown and grey, and extending to depths below ground of approximately 30 cm and 80 cm, respectively [8].

Human and pig cadavers

We sampled soils from the decomposition islands of five human cadavers (located inside AFTER) and three pig cadavers (located approximately 100-150 m outside AFTER) (Table 1). All human and pig cadavers were part of previous research projects at AFTER, and we took advantage of these sites to examine soil physicochemical properties. The human cadavers were delivered through the UTS body donation program, approved by the UTS Human Research Ethics Committee Program Approval (UTS HREC REF No. ETH15–0029). The human donors were delivered to AFTER within 48 h of death and placed directly onto the ground on their backs in a designated 5 × 5 m plot within the facility. The domestic pigs (*Sus scrofa*) were purchased post-mortem from a licensed abattoir, therefore requiring no ethics approval in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004). Pigs were killed by a captive head bolt and transported to AFTER within 1 h of death, then placed on their sides. Both human and pig cadavers were placed on flat ground that had been mown or cleared of vegetation, although small grass clumps were present. A scavenger-proof cage was placed over all pigs and humans to prevent disturbance of the remains.

Soil sampling

We collected soil samples at 0 cm, 30 cm, and 90 cm from the lateral side of each of the five human and three pig cadavers (Fig. 1a). We considered samples taken at 90 cm to be *outside* the visible decomposition island, and were treated as 'controls' for comparison with 0 cm and 30 cm samples that were *within* the visible decomposition island. We used bulk density rings of 42 mm diameter and 52 mm depth (94.1 cm^3 volume) to take the samples. The rings were placed over the center of the marked distance and hit into the ground with a rubber mallet (Fig. 1b). We ensured that soil samples were taken from areas that were flat and avoided any small hollows or nearby vegetation. All soil samples were transferred to sealed plastic

Table 1 Summary of the duration of decomposition (days) and corresponding accumulated degree days (ADD) for each of the five human (H) and three pig (P) cadavers. Soil samples were taken once only from cadavers spanning a range of decomposition stages

Cadaver Type	Days of decomposition (ADD)						
	85 (982)	89 (1049)	227 (3915)	231 (3958)	415 (7226)	471 (7843)	761 (13609)
Human	H1	H2	–	H3	H4	H5	–
Pig	–	P1	P2	–	–	–	P3

bags and kept cool in an insulated container while on-site, and then transferred to a freezer (−20 °C) at our laboratory for storage within 8 h of sampling.

Laboratory processing of soil samples

Prior to laboratory analysis, we dried our soil samples in an oven at 45 °C for 4 days until the mass of samples was stable. We re-weighed the samples to give dry mass and calculate gravimetric water content and bulk density [19]. Soil samples were then homogenized by light grinding with a mortar and pestle to reduce the size of aggregates, and passed through a 2 mm sieve to remove extraneous organic matter such as plant roots and invertebrates. We analyzed the soil samples to quantify key physicochemical properties shown in Table S1 (see Supplementary Electronic Material). We determined electrical conductivity (EC) and pH on 1:5 soil to water extracts using a TPS WP-81 m. Total carbon (C) and total nitrogen (N) were determined with Dumas dry combustion and conductometric analysis (Vario Max CNS, Elementar, Germany) [20]. Total phosphorus (P) was determined after Kjeldahl digestion at 350 °C. Ammonium (as NH_4-N) and nitrate (as NO_3-N) were determined following a 1:10 soil to 2 M potassium chloride extraction. Phosphate (as PO_4-P) was determined following a 1:40 soil to 0.5 M sodium bicarbonate (pH 8.5) extraction. Analyte concentrations were determined calorimetrically using flow injection autoanalysis (Lachat Instruments, Milwaukee, Wisconsin, USA) [21].

This study was preliminary with no statistical analysis completed except for the control soils. The eight control soil samples taken at 90 cm distance from each cadaver were used to calculate a mean and standard error for each of the physico-chemical markers we examined.

Results

We found that several soil properties had higher measures directly under each cadaver at 0 cm relative to samples taken at 30 cm or at the controls (Figs. 2, 3 and 4, Table S1). Soil moisture, conductivity, and pH (Fig. 2) were most clearly elevated up to approximately 200 days of decomposition and directly under the cadavers, although human cadavers had higher soil moisture during this time than pigs (Fig. 2a), but pigs had slightly higher pH than humans (Fig. 2c). Of note was the absence of elevated soil moisture, EC, or pH at human 1 (85 days of decomposition) compared with the very high measures obtained from under human 2 (89 days of

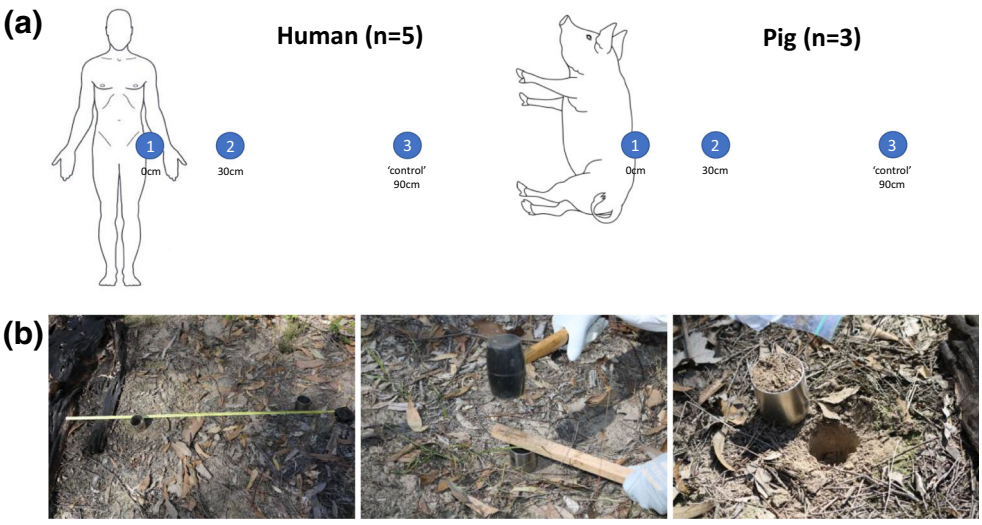

Fig. 1 **a** Soil sampling design from human and pig cadavers, with soil cores taken at 0, 30, and 90 cm from the lateral side. Cores taken from 90 cm were treated as 'controls' as they were outside the visible decomposition island. **b** Soil cores were 48 mm diameter × 52 mm depth and were taken with a bulk density ring and rubber mallet

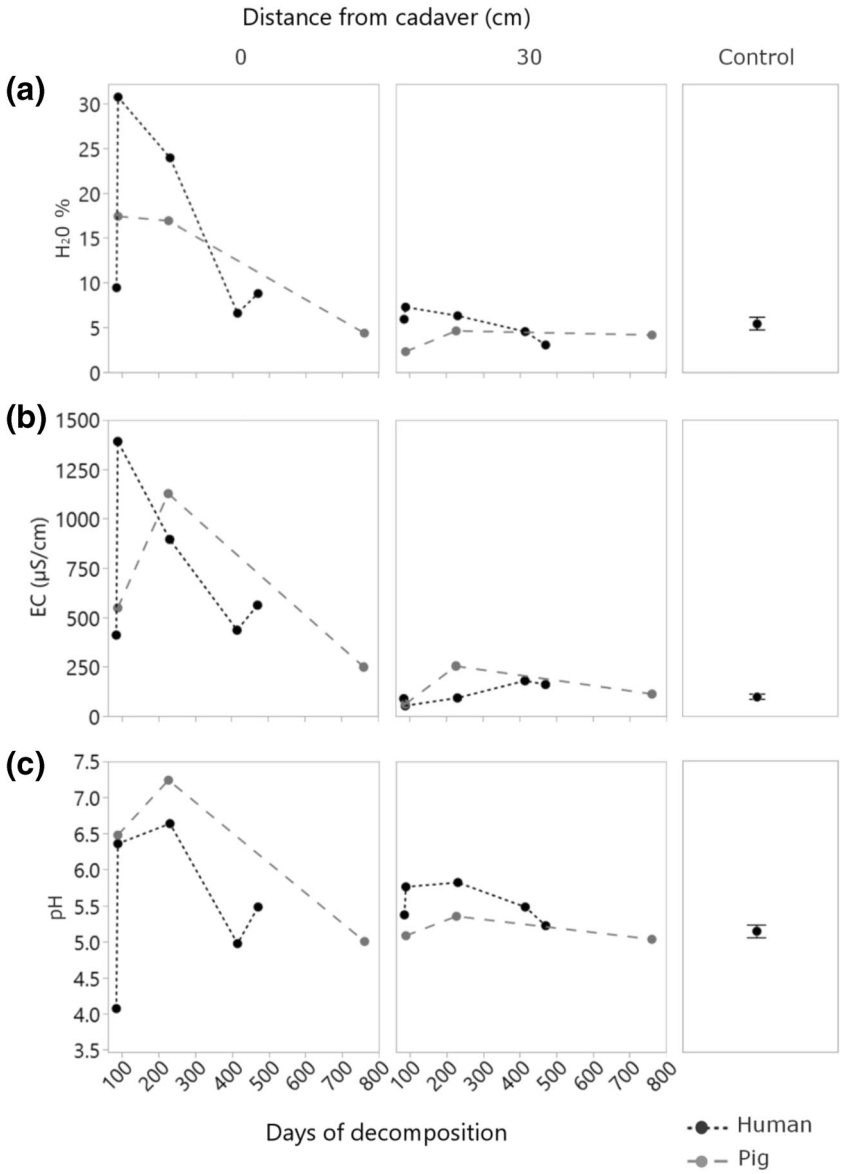

Fig. 2 Soil **a** moisture, **b** electrical conductivity, and **c** pH at two distances (0 cm, 30 cm), from different human and pig cadavers (human = black dots, pig = grey dots). The third panel shows the mean and standard error derived from all samples (pig and human) collected at 90 cm, which acted as controls. Cadavers are ordered by days of decomposition, and lines joining data points denote groupings of cadaver type only

decomposition). The only direct comparison between cadavers was for human 2 and pig 1 (both at 89 days decomposition), with human 2 having notably higher soil EC and H_20 content (Fig. 2).

Total carbon was variable and showed no clear pattern in the soil at different distances, or time of decomposition (Fig. 3a). Total phosphorus was highest at 0 cm distant from human and pig cadavers (except for human 1), including pig 3 that had undergone 761 days of decomposition (Fig. 3b). Notably, soil phosphate was higher at pig than human cadavers at 0 and 30 cm distant (Fig. 3c), was consistently high under all pigs and at all distances, and remained elevated right through to 761 days of decomposition (Fig. 3c).

Total nitrogen (Fig. 4a), nitrate (Fig. 4b) and ammonium (Fig. 4c) all showed their highest concentrations directly under both human and pig cadavers (0 cm). Nitrate was elevated for pigs from day 89 but soil for humans did not display higher nitrate levels until day 415 (Fig. 4b). Ammonium levels at 0 cm were over 20 times those of samples from controls 90 cm away, peaked at approximately 89–231 days, and then declined (Fig. 4c).

Discussion

We set out to quantify soil physicochemical properties that characterized cadaver decomposition islands, and to identify any key differences in the soil between humans and pigs. Our findings showed that some soil properties were substantially elevated directly under cadavers, and less so at 30 cm

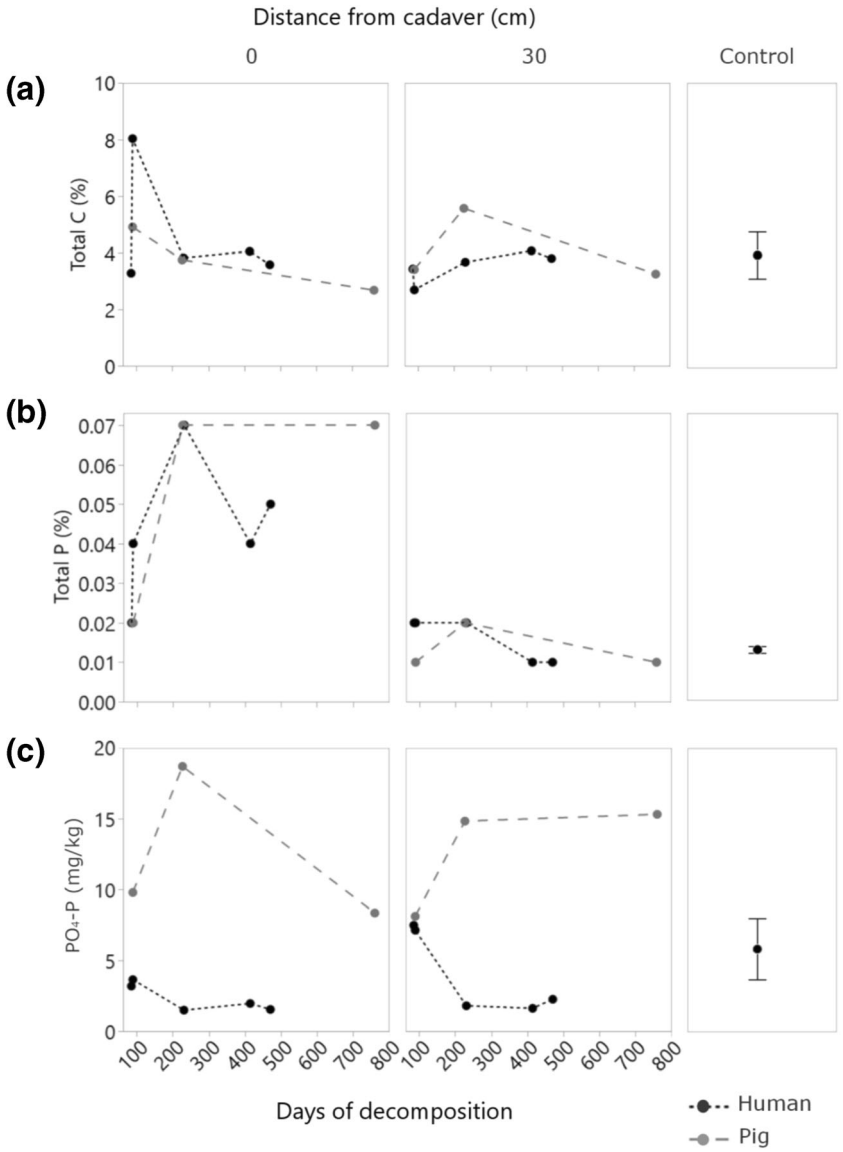

Fig. 3 Soil **a** total carbon, **b** total phosphorus, and **c** phosphate (PO_4-P) concentrations at two distances (0 cm, 30 cm), from different human and pig cadavers (human = black dots, pig = grey dots). The third panel shows the mean and standard error derived from all samples (pig and human) collected at 90 cm, which acted as controls. Cadavers are ordered by days of decomposition, and lines joining data points denote groupings of cadaver type only

compared to our controls. Further, some changes in the soil lasted beyond 700 days of decomposition. Our study provides new information about the spatial and temporal boundaries defining the cadaver decomposition islands of humans and pigs.

Spatial extent of the decomposition island

We found that the highest concentrations of soil nutrients, pH or EC, relative to control samples, was always directly under each cadaver. The lateral spread of these soil markers was limited, although there was some evidence of changes extending to 30 cm around each cadaver. In future work, additional and closely spaced samples would help to confirm the patterns of lateral spread more accurately [c.f. 11]. Lateral spread of decomposition products has been shown to be quite substantial at our research site (AFTER), with cholesterol detected as far away as 2.5 m at a depth of 0.49 m after only 14 days of decomposition [8]. Our study, however, showed that the concentrations of shallow soil nutrients (< 5 cm) were very localized and much higher than measures taken at controls. Our cadavers were sampled between 85 and 760 days after death, and were not moved or scavenged as might occur under more 'natural' circumstances, and so this likely limited our detection of the lateral spread of organic materials over the soil surface. Scavenging by animals can potentially move body parts many meters away [22], and therefore increase the diameter of the decomposition island considerably. Our study shows the limited spatial movement of decomposition products in the top 5 cm of soil in the absence of scavenging or disturbance.

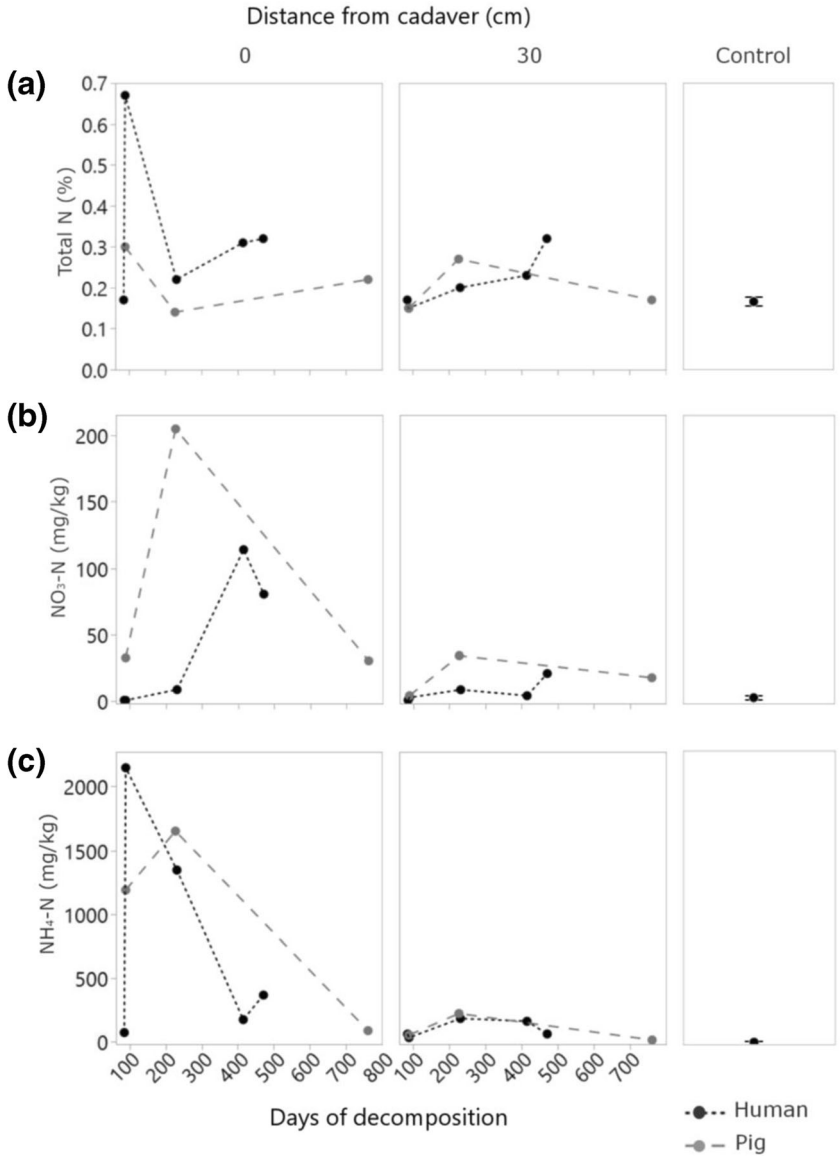

Fig. 4 Soil **a** total nitrogen, **b** nitrate (NO_3-N), and **c** ammonium (NH_4-N) concentrations at two distances (0 cm, 30 cm), from different human and pig cadavers (human = black dots, pig = grey dots). The third panel shows the mean and standard error derived from all samples (pig and human) collected at 90 cm, which acted as controls. Cadavers are ordered by days of decomposition, and lines joining data points denote groupings of cadaver type only

The values of our soil measures of the decomposition island were broadly similar to those reported by other studies using human cadavers. For example, we recorded maximum pH values up to 6.6, whereas Fancher et al. [4] reported a maximum of 6.5, and Aitkenhead et al. [11] reported values up to 6.4. A notable exception was ammonium, which reached a concentration of 2147 mg/kg at human 2 in our study, which was 10 times higher than reported by Aitkenhead et al. [11] and four times higher than reported by Fancher et al. [4]. This would suggest that comparing absolute values across studies is difficult when soils are from different biomes and cadavers at different post-mortem intervals. Additionally, soils were extracted with water in the aforementioned studies, whereas we used KCl to extract ammonium, which displaces ammonium from exchange sites on clays and may lead to higher values than if using water alone.

Temporal longevity of the decomposition island

The longevity of the different elevated soil physicochemical markers is noteworthy. We found that ammonium and nitrate levels peaked between 89 and 230 days and declined thereafter. A similar time window was identified for elevated soil moisture and pH (see Fig. 2). The decline in pH is likely due to the gradual increase in soil organic acids, including amino acids [13] and fatty acids [2]. By contrast, phosphorus (total and as orthophosphate) remained elevated at 700 days and appeared likely to remain elevated for considerably more time. Such profound changes in soil phosphorus have been detected previously at vertebrate carcasses [23, 24], and will affect nearby plant growth, which could lead to favorable conditions for nutrient-tolerant or weedy species [24]. We identified

different timing of elemental inputs, including nitrogen inputs during active and advanced decay (approximately 80–200 days in our study) and phosphorus inputs during dry decay and skeletonization (> 400 days in our study). This pattern has also be found in carcasses of other vertebrate species such as rabbits, beavers, and rats [6, 7, 15]. Our study supports the idea that soil chemistry might be useful for estimation of post-mortem intervals over extended time frames of several months [4].

Pigs as human analogues for soil forensic research

At the outset of this study, we did not expect any differences in soil properties between pigs and humans, and this proved to be true for most soil markers we measured. The chemical composition of human and pig cadavers is broadly similar [3], and so the inputs might also be expected to be similar. However, we found that soil phosphorus (as orthophosphate) was always higher under pigs than humans, and also that nitrate peaked earlier at pigs than at humans. The mechanism causing earlier nitrate peaks at pigs is unclear, but might be related to differences in the relative availability of microbial resources (e.g. dissolved organic carbon) and the utilization of ammonium. Comparing soil microbial communities and the nitrification process under pigs and humans will help address this knowledge gap. The differences in soil phosphorus between the two cadaver types suggests there might also be fundamental differences in the decomposition process. Visual observations of decomposition have indicated desiccation and drying of human remains much more so than for pigs, which have tended to undergo more rapid decomposition and skeletonize more readily [16, 18]. The warm and relatively dry environment of the research facility might be one reason for the drying generally, but other biological mechanisms might also be at play. For example, the microbial community on the human donor bodies is likely to have been very different from the pigs, and the role of these microbes in the breakdown of tissues or attraction of blowflies may therefore have been suppressed. The medical history, cause of death, diet, or body composition of the human cadavers also might have affected their internal and external microbiome and other decomposition processes. It is worth noting the relatively low values of soil moisture and accompanying nutrients under human 1 compared with human 2, despite both being at similar stages of decomposition (85 and 89 days, respectively, see Table 1). Human 1 (>100 kg) was substantially larger in size than human 2 (approx. 55 kg), suggesting that body fat may have perhaps interfered with the movement of decomposition fluids into the soil profile. Importantly, this observation shows that considerable variation in soil markers can occur among human cadavers, as well as between human and animal cadavers. Although we cannot discount a possible role of diet, we suggest that the more complete decomposition of the pigs, and their skeletonization, is a likely contributor to their elevated soil phosphorus. We postulate that the more rapid exposure of the internal skeletal remains of pigs has meant that the phosphorus-rich bones can come into contact with the soil interface, allowing weathering processes to slowly disintegrate the bone, and leaching of the phosphorus into the soil.

Conclusion

Our study was preliminary but has shed new light on the spatial and temporal patterns of soil physicochemical responses to human and pig cadaver decomposition in an Australian environment. We were able to show that all markers of soil chemistry changed most strongly directly under the cadavers, with limited lateral spread, and that these changes were present for approximately 700 days. However, we also identified potential differences in human and pig decomposition processes at the soil interface leading to greater soil phosphorus levels under pigs. Our study was constrained by the lack of replication of cadavers of similar decomposition age, and we could not apply statistical methods or quantify variation in soil properties. Regardless, given the growing interest in potential decomposition and entomological differences between humans and pigs [16, 18, 25], our study further underscores the question of whether the carcasses of non-human animals are reliable analogues for human bodies. Further research is needed to address this important problem, and requires a multidisciplinary, replicated, and experimental approach.

Key points

1. Soil physicochemical properties under decomposing human and pig cadavers were examined.
2. Soil moisture, electrical conductivity, and concentrations of nitrate, ammonium, and orthophosphate were higher directly under cadavers compared with controls outside the visible decomposition island.
3. Evidence of elevated soil nutrients lasted up to 700 days.
4. Soil phosphorus was higher under pigs than humans, suggesting a possible difference in the decomposition processes under these two cadaver types.

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Statement of author contributions PB, CS, BD, MU and JW contributed to the study conception and design. Material preparation, data collection and laboratory and data analysis were performed by PB, AR, and BD. PB wrote the first draft of the manuscript and all authors commented on versions of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no potential conflicts of interest. Research described in this manuscript involved deceased humans and animals. The human cadavers were delivered through the UTS body donation program, approved by the UTS Human Research Ethics Committee Program Approval (UTS HREC REF No. ETH15–0029). Pigs were purchased post-mortem from a licensed abattoir, therefore requiring no ethics approval in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

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