

Elsevier Editorial System(tm) for Forensic Science International
Manuscript Draft

Manuscript Number:

Title: Preliminary soilwater conductivity analysis to date clandestine burials of homicide victims

Article Type: Original Research Paper

Section/Category: Questioned Documents and Physical Science

Keywords: forensic geoscience; conductivity; PMI; PBI; clandestine grave

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Abstract: This study reports on a new geoscientific method to estimate the post-burial interval (PBI) and potential post-mortem interval (PMI) date of homicide victims in clandestine graves by measuring decomposition fluid conductivities. Soil water analysis from a simulated clandestine grave (which contained a domestic pig carcass) in a semi-rural environment had significantly elevated conductivity measurements when compared to background values. A temporal rapid increase of the conductivity of burial fluids was observed until one year post burial, after this values slowly increased until two years (end of the study period). Conversion of x-axis from post-burial days to 'accumulated degree days' (ADD) resulted in an improved fit for multiple linear regression analyses. ADD correction allowed comparison with two other conductivity grave studies and demonstrated it was possible to date a discovered grave and hence the victim if past local temperatures could be obtained and burial depth measured. Contemporary soil moisture sampling was also undertaken to quantify this potential variable. Research also has implications to time illegal animal burials. Further research is required to extend the monitoring period, to use human cadavers and investigate other soil types and depositional environments.

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4th December 2009

Dear Sir/Madam,

Please find accompanying this letter the files for our submitted article, entitled “preliminary soilwater conductivity analysis to date clandestine burials of homicide victims”.

Determining PMI of recently deceased individuals have been extensively researched using a variety of methods and revisions to said methods. However, dating PMI over a longer time period or importantly the Post-Burial Interval (PBI) emplacement within a burial site is more uncertain and lacking a simple technique.

This manuscript is the first to document a new and simple technique to date PMI or estimate how long a body may have been buried for using the conductivity of the decomposition fluids. Our previous FSI paper (Jervis et al. 2009) has suggested that these fluids are the primary reason why electrical resistivity surveys work to detect the positions of clandestine burials of homicide victims. Our new suggested PBI/PMI technique, based on a two year monitoring study, is to simply measure the conductivity of decomposing fluids as we have found this has a two-step, linear relationship. Results are reinforced by comparison to another study as well as to a simulated ‘discovered’ burial, where a ~16% error was found between the conductivity estimated date compared to the true burial date. We have also converted post-burial days into accumulated degree days (ADD) which improved the date mis-match error (to ~12%) and will importantly allow comparison with other studies and cases. Finally we have included the raw data in tabulated form as supplementary material for others to use for comparison purposes. We believe that the paper will be of great interest to forensic pathologists and other law enforcement professionals.

The corresponding author is Jamie Pringle. Contact details are given above and also in the manuscript. Contact details for my co-authors are in the manuscript.

The submitted work has not been published previously and is not under consideration for publication elsewhere. Part of Figures 1 and 2a have been adapted from the Jervis et al. (2009) FSI paper entitled “Time-lapse resistivity surveys over simulated clandestine burials”, although this paper was focused on geophysical surveys over the simulated clandestine burials.. Initial results were discussed at the EAGE Near Surface Geophysics 2009 meeting, held on the 8-9 September 2009 in Trinity College, Dublin, Ireland.

All authors contributed to the research project and the manuscript. Jamie Pringle took part in the design of the study, helped create the experimental site, collected some samples, analysed the data and wrote the majority of the manuscript. John Jervis took part in the design of the study, helped create the experimental site, collected samples, took part in the interpretation of the data and helped in the writing of the manuscript. John Cassella took part in the design of the study, took part in the interpretation of the data and helped in the writing of the manuscript. All authors have approved the final version of the manuscript.

We have provided black-and-white figures for the print version and 2 colour figures (Figures 1 & 5) for the electronic (on-line) version. We have finally included two e-file supplementary material raw data tables for interested readers to download and perhaps compare to their studies.

Yours sincerely,

A handwritten signature in black ink, appearing to read 'J. Pringle'. The signature is written in a cursive, flowing style with a large initial 'J' and 'P'.

Jamie Pringle & co-authors.

Preliminary soilwater conductivity analysis to date clandestine burials of homicide victims

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Abstract

This study reports on a new geoscientific method to estimate the post-burial interval (PBI) and potential post-mortem interval (PMI) date of homicide victims in clandestine graves by measuring decomposition fluid conductivities. Soil water analysis from a simulated clandestine grave (which contained a domestic pig carcass) in a semi-rural environment had significantly elevated conductivity measurements when compared to background values. A temporal rapid increase of the conductivity of burial fluids was observed until one year post burial, after this values slowly increased until two years (end of the study period). Conversion of x-axis from post-burial days to 'accumulated degree days' (ADD) resulted in an improved fit for multiple linear regression analyses. ADD correction allowed comparison with two other conductivity grave studies and demonstrated it was possible to date a discovered grave and hence the victim if past local temperatures could be obtained and burial depth measured. Contemporary soil moisture sampling was also undertaken to quantify this potential variable. Research also has implications to time illegal animal burials. Further research is required to extend the monitoring period, to use human cadavers and investigate other soil types and depositional environments.

Keywords: forensic geoscience; conductivity; PBI; PMI; clandestine grave

1. Introduction

The use of geoscientific techniques in forensic investigations are broadly divided into laboratory- and field-based [1]. Field-based geoscientists have been employed to locate homicide victim's graves, weapons and other buried or concealed objects using a variety of techniques. The search methods include remote sensing [2-3], cadaver dogs [4], methane [5] and soil probes [6-7], near-surface geophysics, which includes metal detectors [8-10], geochemical surveys [5] and mass excavations [11]. Laboratory-based techniques often use trace evidence from a discovered burial to link a suspect to the crime scene [1] although there have been reliability issues [12].

There has been extensive taphonomy research on estimating the PMI of very recently deceased above-ground discovered individuals, for example, using body temperatures [13-15], cadaver entomology [16-17] and entomofauna [18], vitreous potassium [19], serum sodium:potassium concentration ratios [20], cardiac troponin [21] and thanatochemistry [22], etc. For longer deceased individuals, there is more date uncertainty; analysis of decomposition tissue stages [23-24] and indeed entomology have shown promise [25], and for skeletal remains, there are a variety of morphological, chemical, physical, immunological and histological PMI methods suggested [26-30], odontology and tooth loss [31] as well as radionuclides and trace elements [32-33].

With discovered buried individuals, the discussed PMI methods may also provide a Post-Burial Interval (PBI) date, although this may be significantly different from the PMI [30], but determination of both PMI and PBI may be critically important for forensic case investigators. There are significant decreased decomposition rates observed between surface and buried individuals respectively [24,34-35], with a decomposition rate difference of up to eight times being suggested [36].

Researchers have generally suggested three major site contributing factors for this difference which are; organic content, environmental factors and organism accessibility [37-38]. Environmental factors include pH [39], redox conditions [38], ambient temperatures [40-42], and hence associated decomposition rate changes [43-44], seasonality, time of burial [45] and depth below ground level [46], soil type and texture [34,44, 47] and moisture content [37,44], local land use and environment [48-49].

The presence of a decomposing cadaver can have a significant affect on the surrounding surface soil or indeed buried 'grave soil'; for example elevated levels of elements with respect to background values [27,38,47,50-51], including phosphate and nitrates [52], total carbon [53-54], potassium, magnesium, sodium and iron [55-56] and ninhydrin reactive Nitrogen [35,57]. Elevated levels of volatile organic compounds [58-60] and pH [52,54,61] have also been recorded. These affects have been suggested to cause cadaver-associated clothing and textile degradation which have also been suggested to estimate a PBI [49,62].

Although poorly understood, 'grave soil' has been shown to be able to be detected electrically by resistivity surveys in criminal investigations [10], graveyards [52,63] and controlled experiments [64-65]. Successful target detection has been found to be predominantly due to elevated conductivity levels relative to background values [52,66]. Elevated conductivity levels downstream of murder victim deposition site(s) have also been reported [67].

The aim of this study was *firstly* to regularly extract and quantify fluid conductivities from both a shallow buried pig (*Sus scrofa*) carcass *in situ* and background soilwater, in order to create base-line temporal data over a two year period. The *second* aim was to determine if taking soil samples around a discovered shallow burial could assist in determining the approximate PBI. The *third* aim was to simultaneously collect appropriate site data (rainfall, soil moisture and temperature) so that results could be compared to other studies and applied to criminal investigations. The *fourth and final* aim was to compare the results to other simulated studies to determine the validity of the method and if data analysis agreed with burial dates.

2. Materials and Methods

2a. Study Site

The chosen controlled test site was located on Keele University campus, ~200 m above sea level, close to the town of Newcastle-under-Lyme in Staffordshire, U.K. (Fig. 1a). The study site and simulated clandestine grave was the same one used for geophysical investigations [66]. The test site is located ~200 m away from the Keele University weather observation station, which continually measures daily rainfall and air/ground temperatures as well as having soil temperature probes at 0.1 m, 0.3 m and 1.0 m depths below ground level (bgl). Figure 2 shows summary rainfall and relevant temperature data over the monitoring period. The local climate is temperate, which is typical for the UK. The study site was a grassed, small rectangular area (~25 m x ~25 m), surrounded by small deciduous trees. This study site is therefore representative of a semi-rural environment. Nearby borehole records show the Carboniferous (Westphalian) Butterson Sandstone bedrock geology is present ~2.6 m bgl. Local soil maps, however, designated this area as made ground, due to now-demolished greenhouses being onsite. Initial soil sampling indicated a vertical site succession of a shallow (0.01 m) organic-rich, top soil (Munsell colour chart colour (Mccc): 5yr/2/2.5), with underlying 'A' Horizon (Mccc: 5yr/3/3) comprising predominantly of a natural sandy loam that contained ~5 % of isolated brick and coal fragments. The natural ground 'B' Horizon was

encountered at ~0.45 m bgl, dominated by sandstone fragments from the underlying bedrock.

2b. Simulated Graves

The Human Tissue Act (2004) prevents human cadavers from being used for experiments in the UK. A proxy domestic pig (*Sus scrofa*) carcass, sourced from the local abattoir, was therefore used instead to simulate a homicide victim, after following the necessary permissions from the Department for Environment, Food and Rural Affairs (DEFRA) had been obtained. Pigs are commonly used as they comprise similar chemical compositions, size and tissue:body fat ratios [43,65]. A simulated 'grave' ~2 m x ~0.5 m was hand-excavated to 0.5 m bgl on the 7th December, 2007. The pig cadaver, which weighed ~80 Kg, was placed in the grave (Fig. 1b). The pig had been dead for less than 5 h at the time of burial, having been killed with an abattoir bolt gun. Within the grave, a soil water sample lysimeter was placed between the carcass and the grave wall (Fig. 1b). The porous end cap of a model 1900 (SoilMoisture Equipment Corporation™) soil water sample lysimeter was then vertically inserted into a mixture of excavated soil and water. A 'slurry' was made and applied to the buried end of the lysimeter to ensure a good hydraulic conductivity between the ground and the lysimeter [68]. The simulated grave was backfilled to ground level with the excavated ground material and the 'grave' had the overlying grass sods carefully replaced. An empty grave was also dug nearby following the same procedure (Fig. 1a). A control site lysimeter was installed ~10 m

from the grave by digging a narrow hole (~ 0.3 m × 0.3 m) to ~0.6 m depth bgl. The same lysimeter emplacement procedure as detailed previously was followed. Once installed, the exposed ends of the lysimeters were sealed with a rubber stopper and a vacuum pump used to generate a lysimeter suction of 65 kPa, in order for the instruments to draw fluid from the soil.

2c. Sample Collection & Measurements

Two days before any lysimeter sample was extracted, the rubber stopper was removed from each lysimeter and any fluid present was extracted using a plastic syringe with a narrow tube attachment, before being resealed and the vacuum pump employed to maintain vacuum pressure. On the day of sampling, the extraction procedure was repeated and the sample placed in a labelled plastic sample bottle (Fig. 1c). A portable WTW™ Instrument Multi-line P4 conductivity meter was immediately placed within the sample bottle, and a reading was taken after the conductivity values and temperature readings had equilibrated. Measured conductivity values were automatically corrected by the conductivity meter to a reference temperature (25°C) and are 0.1°C accurate. This measurement procedure was repeated to check reading value repeatability and reliability. The collected samples were then frozen. Finally conductivity and pH values were also measured on defrosted samples.

Volumetric moisture content and porosity were measured for grave soil samples (collected from the empty grave) and site soil samples (collected from a control location within the site). Soil samples were collected using augers, and subsequently oven dried to give moisture content and porosity measurements (see [66] for further details).

3. Results

Field soil porosity measurements averaged 59.2% (2.14 SD with 54.3% to 63.0% range) and 55.0% (1.98 SD with 49.9% to 58.5% range) for the empty grave and background site samples respectively. Field volumetric moisture content measurements averaged 31.5% (2.42 SD with 24.7% to 35.9% range) and 29.9% (3.12 SD with 19.7% to 34.5% range) for the empty grave and background samples respectively (see eTable 1 for raw data and [66] Jervis et al. 2009b for one year soil graph results). As would be expected in a Northern hemisphere seasonal climate, highest moisture content values were in the late summer months. Note it was unseasonally dry in the autumn of year two but also the wettest early winter on UK records.

Field soilwater measurements (eTable 1) demonstrated that background soilwater conductivity values were consistent over the two year survey period (averaging 482 mS/cm \pm 0.1), the grave leachate conductivity values increased throughout the survey period, from 266 \pm 0.1 (12 days) to 31,400 \pm 0.1 mS/cm (638 days post-burial)

respectively (Fig. 3a). Note the last reading (727 days post-burial) had a significantly reduced conductivity of 24,600 mS/cm. Conductivity changes during the first 364 post-burial days were reported in Jervis et al. 2009b [66]. The 'grave' conductivity values were double the background values after only two weeks of post burial. Leachate values could be grouped into three linear regressions, 0 – 150, 150 – 307 and 307 – 720 post-burial days respectively (cf Fig. 3a). The first two regressions had a good fit with the collected data (R^2 values of 0.9662 and 0.9915 respectively), with the third regression line demonstrating less confidence (R^2 values of 0.7189). The second linear regression represents the highest period of conductivity increase, increasing by $\sim 145 \mu\text{S}/\text{cm}/\text{d}$ every 14 days post-burial. This could be related to daily temperatures rapidly warming during spring to early summer (see Fig. 2). Site temperature variation could be removed from raw conductivity values by weighting each day by its average daily temperature and then giving each day post-burial an Accumulated Degree Day (ADD) following standard methods [27]. This study had the advantage of having temperature probe measurement data available from the actual mid-cadaver depth (0.3 m bgl) from the nearby meteorological weather station, instead of using average air temperatures (Fig. 2). This allowed a data reduction to only two linear regressions being needed, with an improved correlation for the first 307 days of burial (R^2 value of 0.9932, see Fig. 3b). The temperature of soilwater samples was also measured immediately upon extraction; leachate soilwaters averaged $0.8^\circ\text{C} \pm 0.1$ warmer than the background soilwater (eTable 1). Laboratory pH measurements on defrosted samples showed significant variations between successive samples of both

background and leachate soilwaters, thus not suggestive of confidence in the pH method to determine a burial date (eTable 1).

4. Discussion

Every suspected burial site is unique, having different soil types with varying proportions of natural and anthropogenic materials, varying soil porosities and textures, micro-climate and associated temperature regimes, vegetation type, burial environment, etc. which all affect the potential PBI and/or the PMI determination. Many authors have attempted to quantify these as previously mentioned. The target body will also be highly variable from case to case, including target size and organic content, depth of burial and time of deposition. Burial depth is important as this will affect temperature and the associated rate of decomposition [36,43]. The time of burial will be important, especially in seasonal climates, for example, if death occurs in the summer season the decomposition would occur more rapidly than if it occurred during the winter [45].

However, as demonstrated by this study in a semi-rural environment with a sandy loam soil, elevated soilwater conductivity values with respect to background values still looks to be a robust method to date a discovered burial. Other research [43,69] have suggested below-ground decomposition rates follow a sigmoidal pattern rather than an above-ground linear one once local site temperatures are taken into account, but this research suggests a two-stage linear relationship between

conductivity and ADD during the first two years of burial. The first 307 post-burial days also showed a high degree of correlation (Fig. 3b). This study has the advantage over other decomposition studies (e.g. [35]) in that only one pig is sampled and the 'grave' does not need to be repeatedly excavated and refilled; therefore the leachate fluids remain *in situ and in context*, albeit extracted by the lysimeter. Study results show there would be greater degree of confidence with dating a burial in the first 307 post-burial days or 3,257 ADD due to the comparatively steep linear regression, as compared to the second year or 6,973 ADD. Note that leachate conductivity values were already double that of background values after only 14 days post burial and continued to increase until the penultimate sample (Fig. 3 and eTable 1). There was a significant decrease in conductivity in the 727 post-burial days / 7,533 ADD sample which does not follow the trend of the rest of the sampled data. This could be due to the unusually high rainfall during the last month of monitoring or the start of a new conductivity trend.

Obtaining an accurate date of a discovered burial would depend upon the forensic search investigators being able to quantify the major site variables already discussed. From this study, it is suggested that the two most important factors are *daily site temperatures* and *depth of the discovered burial*. Local temperature data from a nearby meteorological weather station would be required to convert ADD back to post-burial days. Average daily temperatures could be summed-back from the discovered average daily temperature to the date of burial as previously described. If only daily air temperatures are available, these can be corrected for

the depth of discovered burial using the method of [70]. The accumulated degree days can then be corrected so that their measured conductivity linearly correlates.

This study also suggests that two of the other important site variables (apart from temperature and depth of burial) would be the local soil type and burial environment as suggested in previous reports [34,44,48,71]. To address these issues, this study compared current study results with a previous conductivity study from a simulated clandestine grave, from an urban depositional environment [65]. Once the 154 days post-burial data were corrected for ADD at the depth of burial and background soil water conductivity subtracted from 'grave' leachate conductivity values for both studies, the resulting datasets could then be compared over the same time period (Fig. 4a and eTable 2). The linear best-fit lines showed very similar gradients ($\sim 9.3 \mu\text{S}/\text{cm}/\text{d}$ and $\sim 10.6 \mu\text{S}/\text{cm}/\text{d}$ for this and the [66] study respectively), although there is almost a doubling of conductivity values. This was possibly due to a different soil type (sandy loam versus made ground) and a contrasting environment of deposition (semi-rural versus urban) for this study and the [65] study respectively. The urban study also used a 31 Kg pig, in contrast to the 80 Kg pig used in this study. However, correcting for the contrasting carcass weights, using the method by [27], which involved multiplying measured conductivities by 0.5 and 1.1677 respectively, did not improve the data match (Fig. 4b). Indeed, [14] found dating PMI using temperature did not need adjustments for body weight.

An experimental limitation with this study is that lysimeters will not be available to obtain samples within a discovered burial. To therefore test this method on a 'discovered burial', a separate simulated grave, ~1 m x ~0.5 m, was hand-excavated to 0.5 m bgl on the Keele test site on the 21st October 2008. A ~25 Kg pig carcass was deposited before the 'grave' was refilled and grass sods replaced, following the described methodology. This grave was then 'discovered' and hand-excavated on 30th July 2009, with the observed leachate fluids being measured by insertion of the calibrated conductivity meter (Fig. 5). Once the 19,780 mS/cm conductivity value was plotted (Figure 3), a burial date mis-match of 45 days was observed between the burial date estimated from the conductivity measurement and the *actual* date, which was a 15.7% error over the burial time period. Once corrected for ADD the estimated 288 ADD mis-match gave an improved 11.5% error. Where a discovered burial did not have observed leachate, it would then be necessary to collect soil within the burial and then centrifuge samples to extract soilwater, following [57] methodology.

There are still potential unknowns for burial dating purposes in casework. One potential unknown would be the determination of the time gap (if any) between death and burial. This could be determined using other methods as described in the introduction section. It is also interesting to note that freezing of skeletal muscle tissue (such as refrigerator storage of a body before burial) does not effect the subsequent decomposition rate [72]. A second unknown would be determining if the body had been moved and re-buried. This would affect this dating method as fluids

would not be present from the first burial and thus a conductivity burial date would not be accurate. Other research has also found repeated burial affects tissue decomposition rates [73], although bone surface analysis looks promising for clarifying more complex burial histories [74].

To detect a suspected burial location rather than date a discovered one, elevated conductivity levels downstream of both murder victim deposition sites [67] and cemeteries [52] have been previously measured, and compared to upstream values. It would be difficult to apply this presumptive detection test on land, although again centrifuging collected soil samples to extract soilwater and obtain a conductivity value [35,57], would be suggested around a relatively small area which had *a priori* information. The authors would not recommend freezing field samples for later analysis, as comparisons between field and laboratory-derived conductivities from the first 223 post-burial days demonstrated that defrosted sample values were, on average, 40% lower from those measured in the field (see eTable 1).

Further work should *firstly* be to continue site monitoring, in order to extend base-line data and determine if conductivity values from the grave continue, over time post-burial, to slowly increase, stabilise or reduce back to background values. If conductivity values begin to decrease over longer time frames, this will give two potential burial times, rather than the one currently suggested by this study. *Secondly*, it is important that the experiment is replicated in other soil types, for example, sandy and chalky soils. Other studies have shown this can be important,

for example, recording significant pH variations [39] and soil textures [44] that affect conductivity. *Thirdly*, different burial environments from the semi-rural and urban environments detailed in this study should be investigated. Two obvious potential burial environments would be woodland and moorland environments. *Fourthly*, analytical chemical techniques should be utilised to examine the soilwater samples. This would hopefully clarify why there was a clearly observed change in conductivity versus time after a year of burial. It may also determine if elements, compounds or acids could be used as a complimentary dating mechanism. For example, elevated levels of nitrates around buried pigs, total carbon & pH [54], total nitrogen, phosphorus and lipid-Phosphorus [61] should be measured. [58-59] found specific volatile organic compounds (VOCs) to be both diagnostic of human burials and concentrations varied over post-burial time.

5. Conclusions

Base-line temporal data collected over a simulated clandestine grave containing a pig cadaver found increasing conductivity levels with respect to background levels until the end of the two year study period. Site temperatures and burial depth were used to convert post-burial days to accumulated degree days, which allowed an improved fit of linear regression lines. From this study, there would be a good confidence in this method up to 307 days post-burial or 3,315 ADD; after this period the comparatively shallow linear regression would make dating a discovered burial more problematic. Comparisons with a previous 154 days post-burial or 2,040 ADD,

demonstrated similar linear trends but with a doubling of conductivity values that may be related to contrasting soil types and depositional environments. Conductivity measurements from a final simulated 'discovered burial' showed a 15.7% burial date and an 11.5% ADD burial date mis-match respectively. This method could be applied to time burial of other material, e.g. illegal animal burials.

6. Acknowledgements

A 2007 Keele University Innovation in Teaching Award (No. 49) partly funded this research. John Jervis is jointly funded by an Engineering and Physical Sciences Research Council (EPSRC) and RSK Stats Ltd. CASE PhD grant. We acknowledge the following members of staff from Keele University; Tim Millington and Malcolm Wright for assistance in creating the study site, Ian Wilshaw for assistance in installing and maintaining the lysimeters. The authors also wish to thank Amy Cowles and Vicky Hill from Keele University for logistical and technical support and both Keele and Staffordshire University forensic science undergraduates for undertaking pilot investigative projects.

7. Role of the funding source

The EPSRC and RSK Stats Ltd. had no involvement with the project.

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9. Figure Captions:

Fig. 1. (A) Study site photograph with location (inset); (B) Annotated photograph of simulated clandestine 'grave'; (C) Background lysimeter (to act as control) and fluid measuring accessories (see text).

Fig. 2. Summary site statistics of total rainfall (bars) and average temperature (lines) data (bgl = below ground level), measured over the two year study period (see text).

Fig. 3. (A) Measured pig leachate (grave), and background (control) soilwater fluid conductivity values, modified from Jervis *et al.* (2009b). (B) Corrected conductivity versus accumulated degree day plot produced from (A) by summing average daily 0.3m bgl post-burial temperatures (see text).

Fig. 4. (A) Measured study site conductivities from this study (Keele grave) to a previous study (CSH grave) over a six month period. Note post-burial days have been converted to accumulated degree days and background conductivity values have been subtracted from pig leachate values for comparative purposes (see text). (B) Conductivity values have also been corrected for pig carcass weight (see text). Modified from Jervis *et al.* (2009a,b).

Fig. 5. Annotated photograph of the simulated 'discovered' burial and the observed pig leachate that was sampled and measured.

10. Supplementary eMaterial Captions:

eTable 1. List of groundwater and soil data measurements derived during this study at the primary Keele test site. Note ADD is for 0.3 m bgl (see text).

eTable 2. List of field-measured conductivities and temperatures from the secondary Crime Scene House test site (see Jervis et al. 2009a for details).

Figure 1 colour (web)

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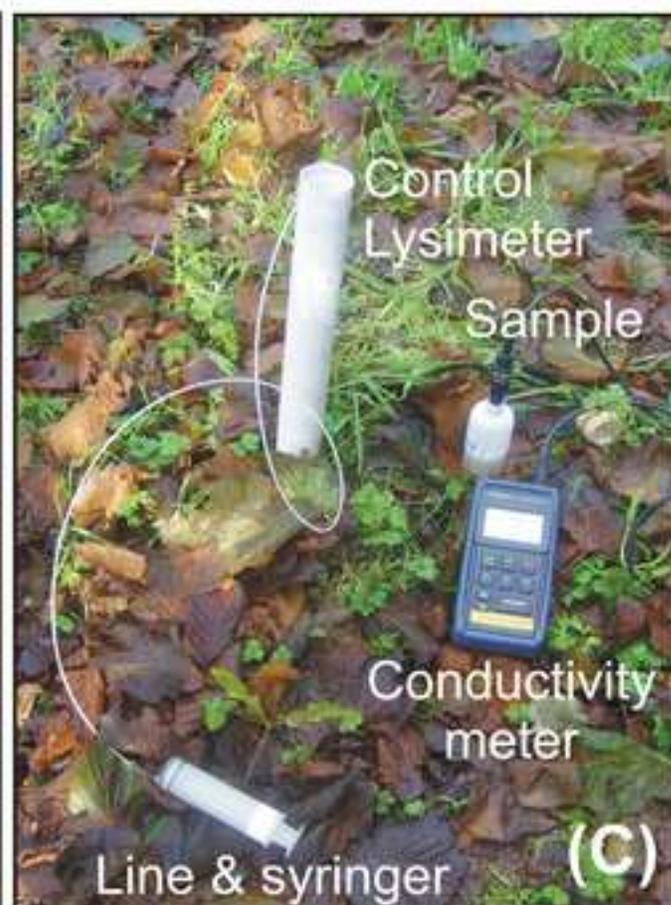
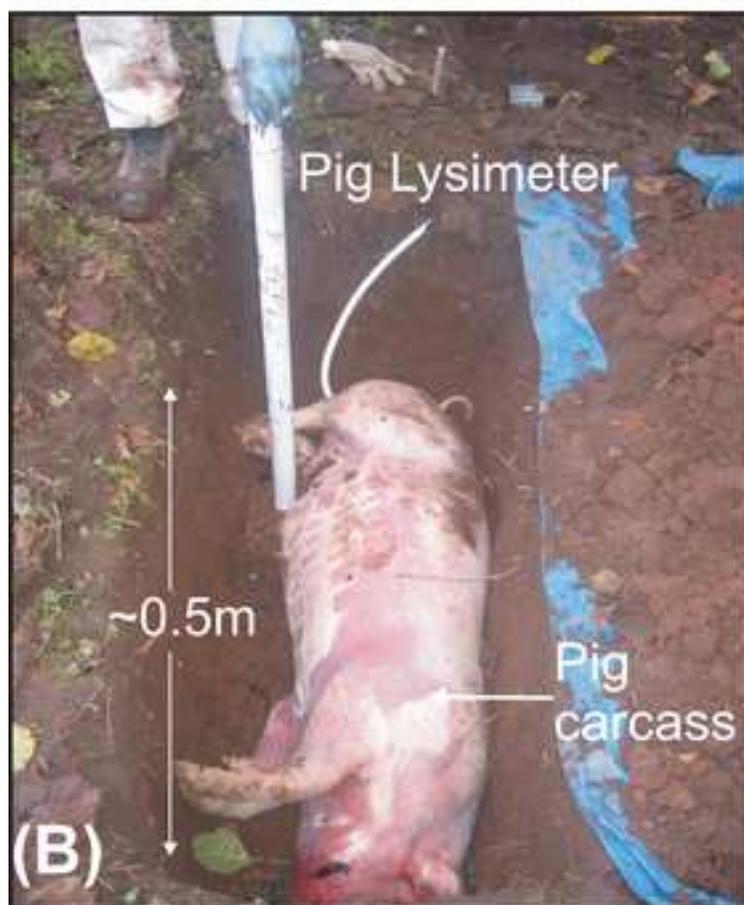
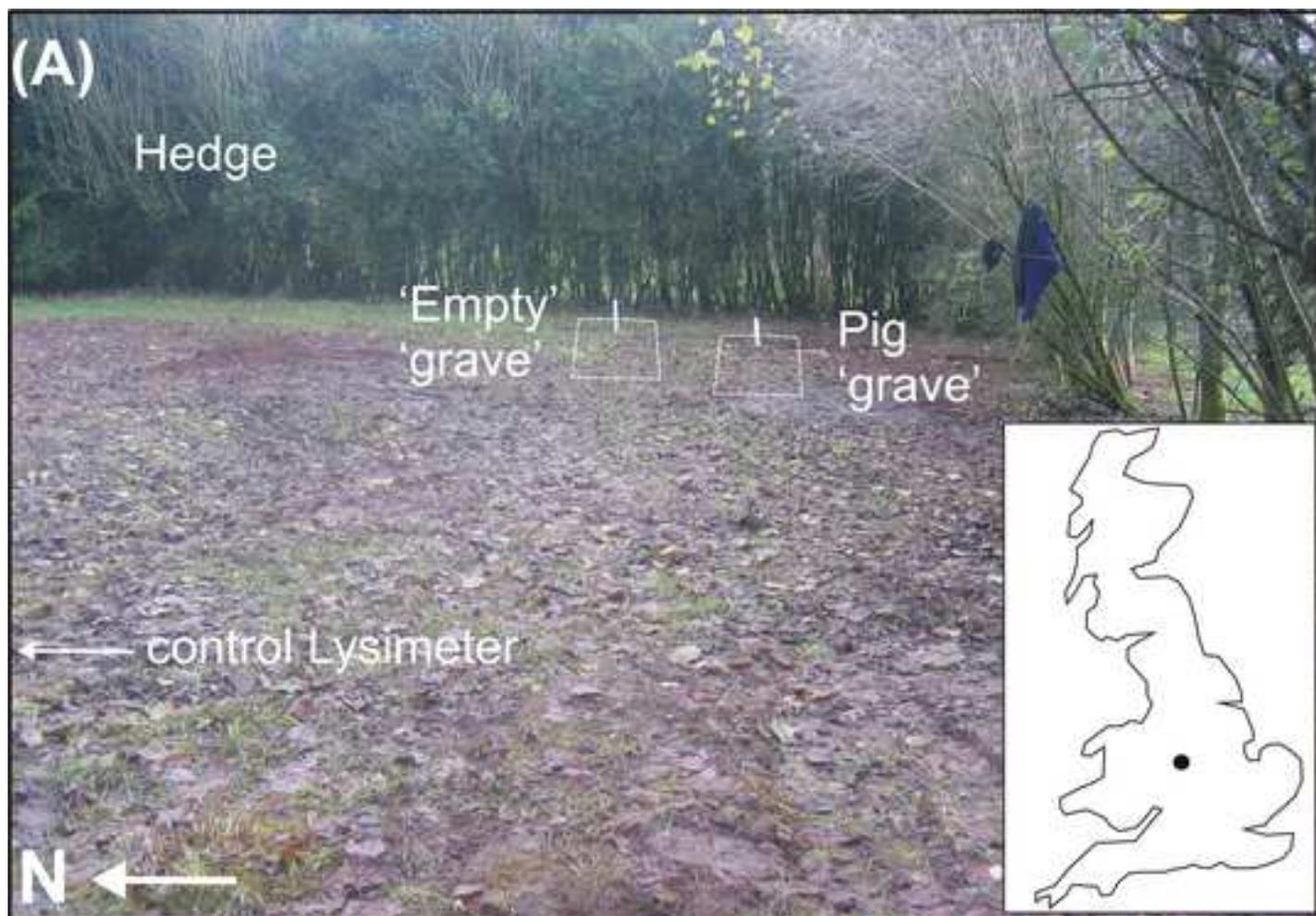


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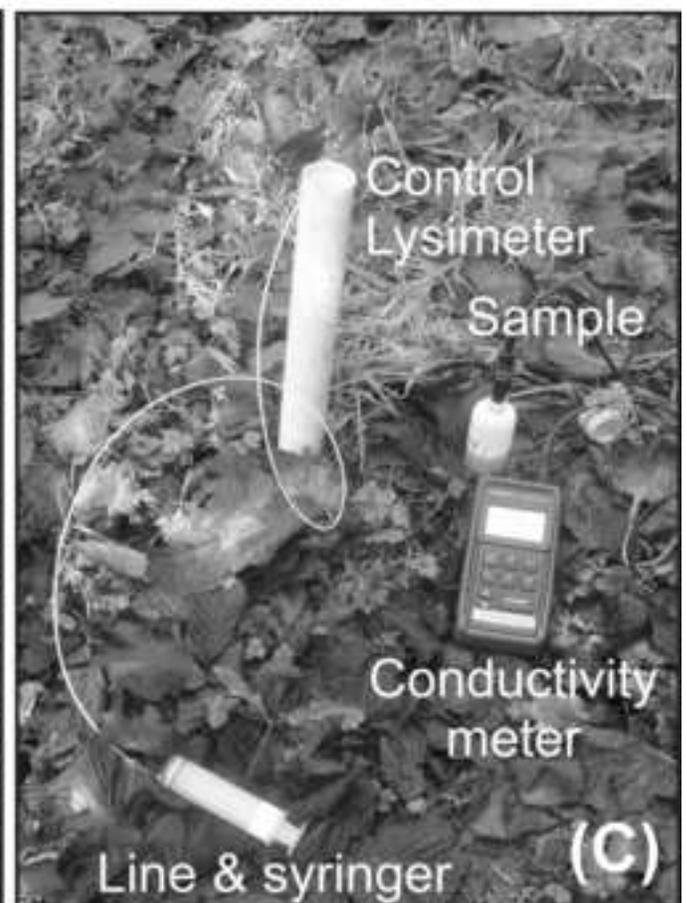
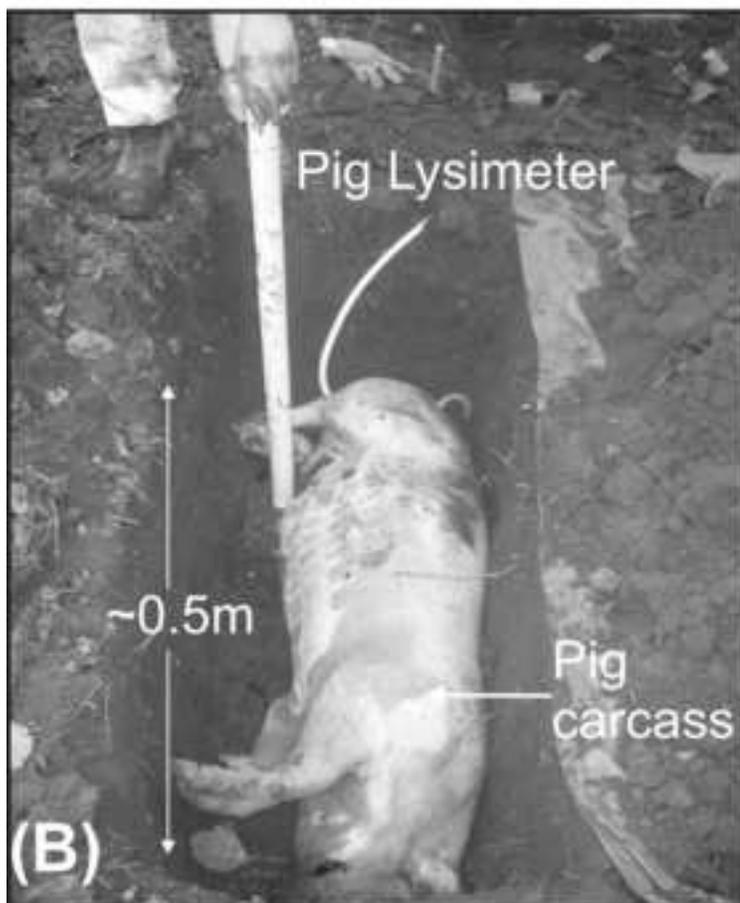
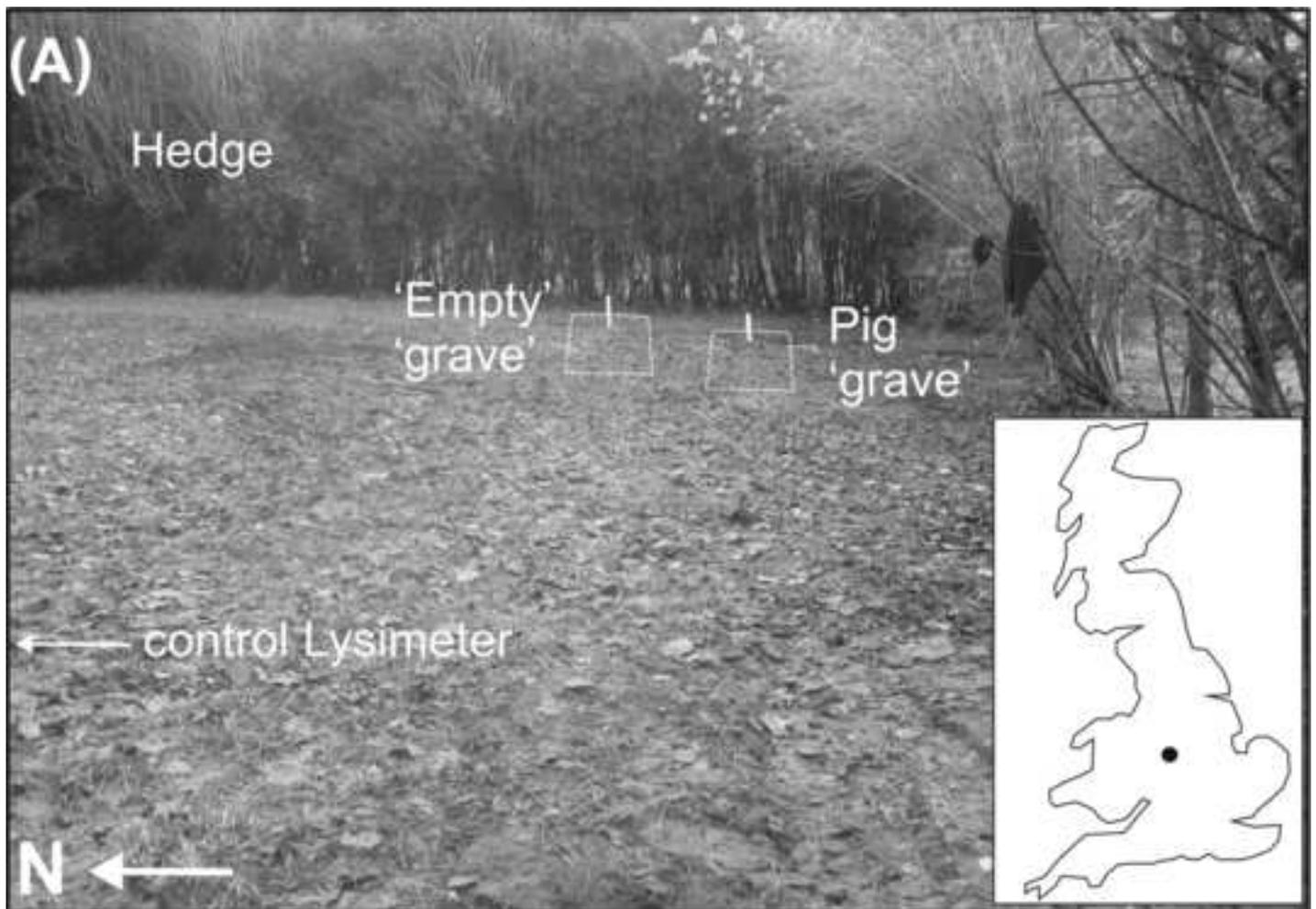


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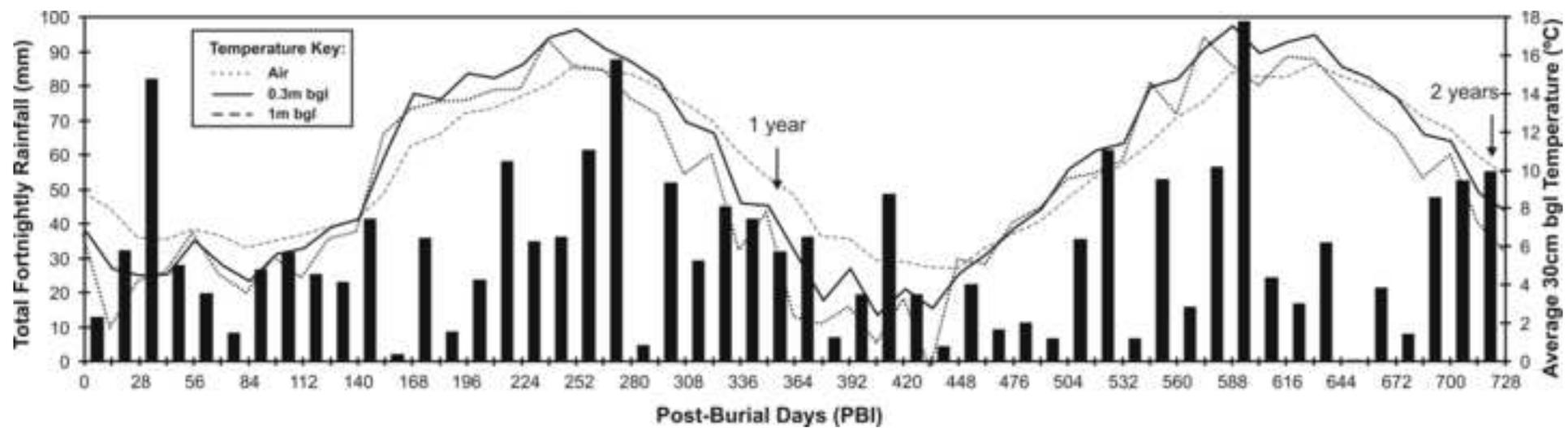


Figure 3

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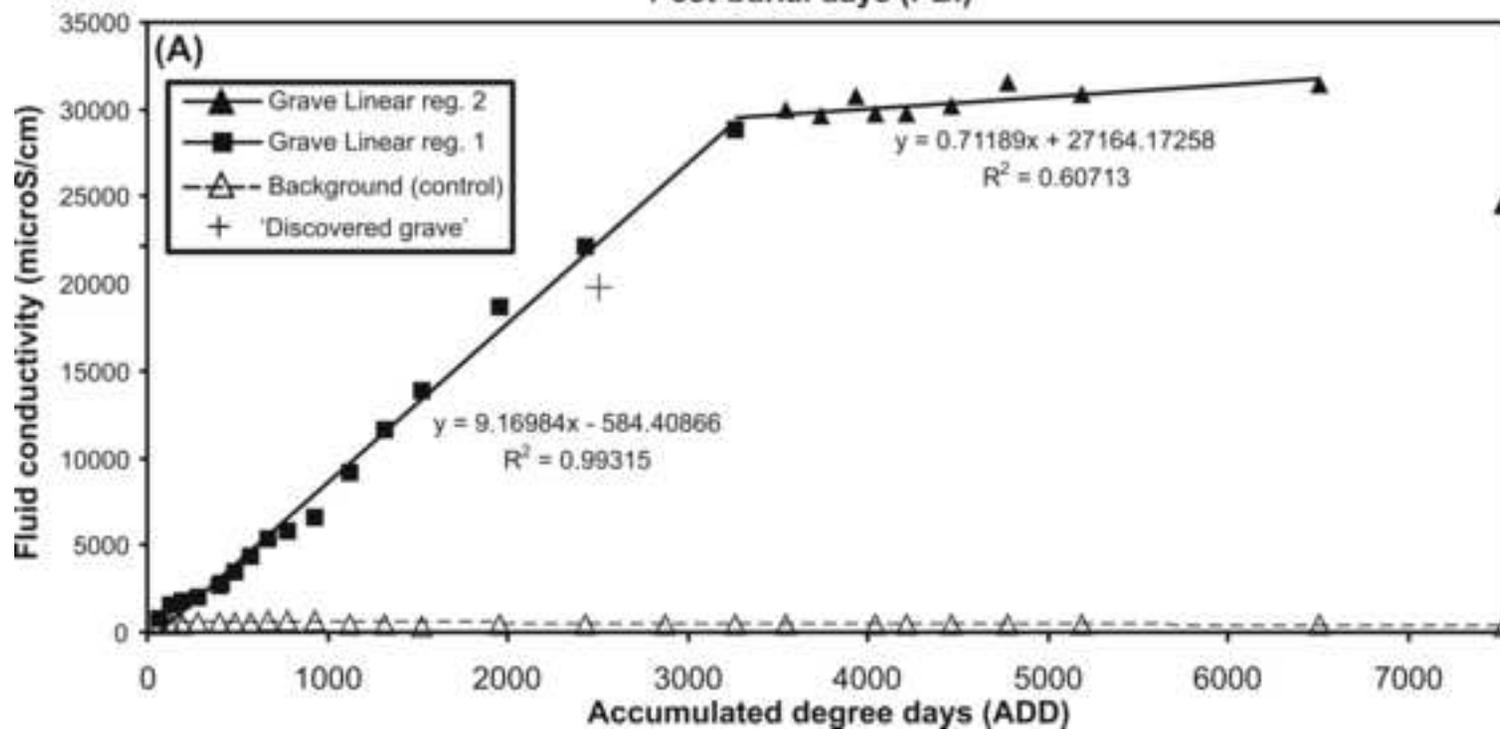
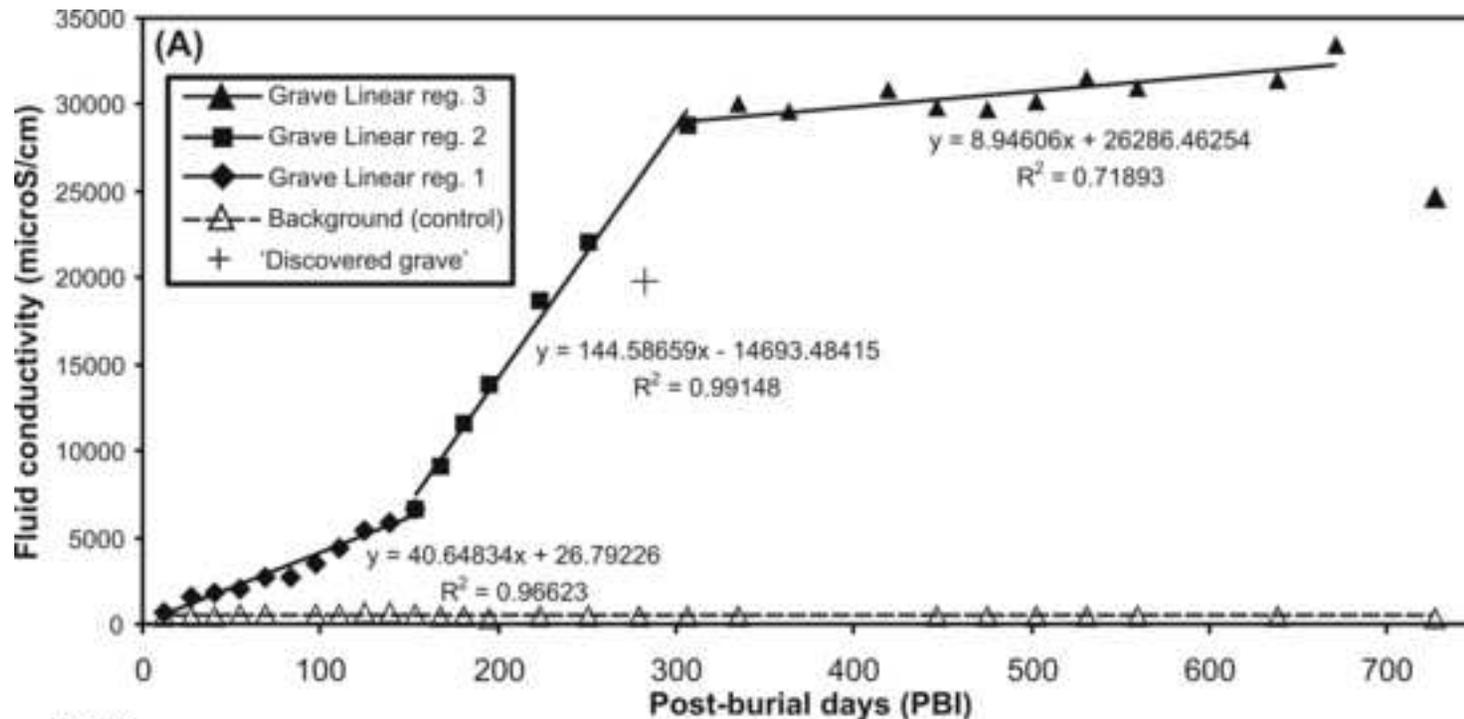


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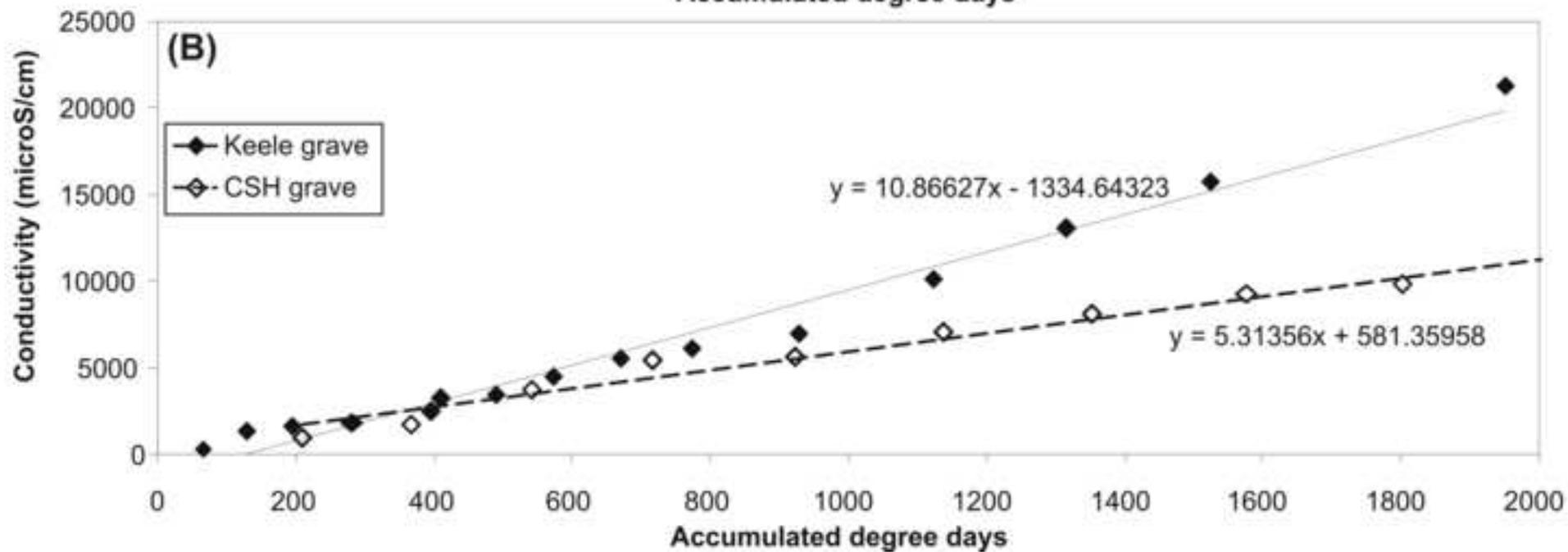
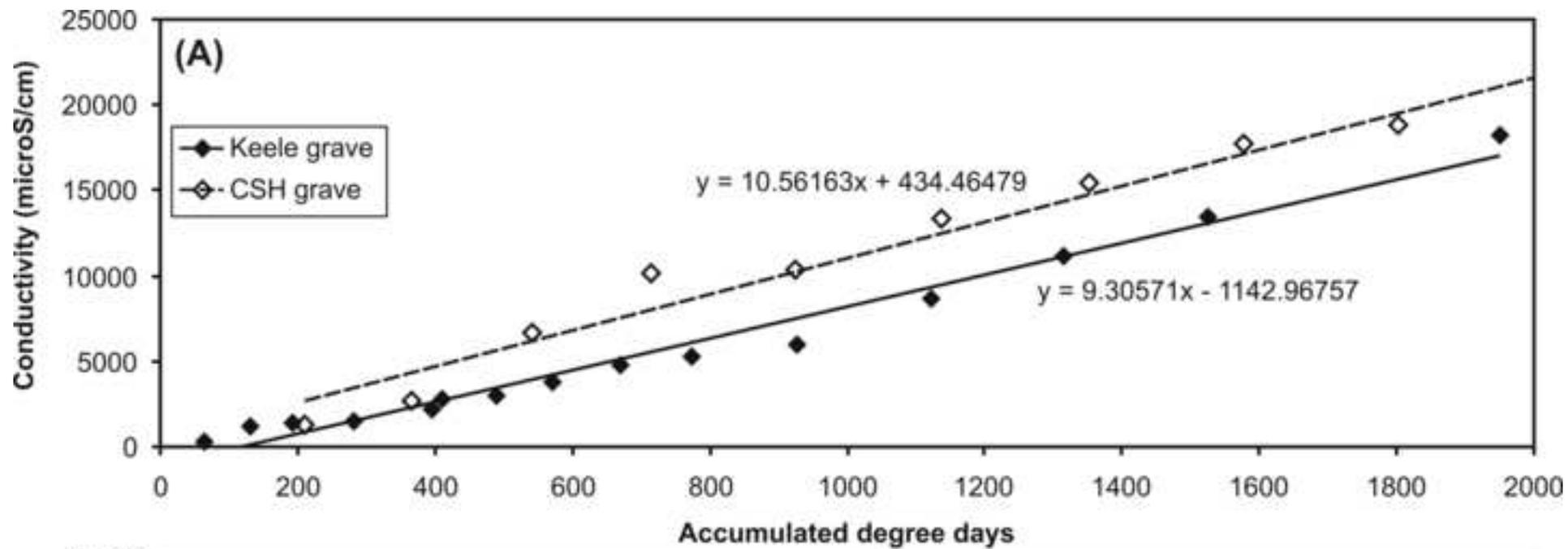


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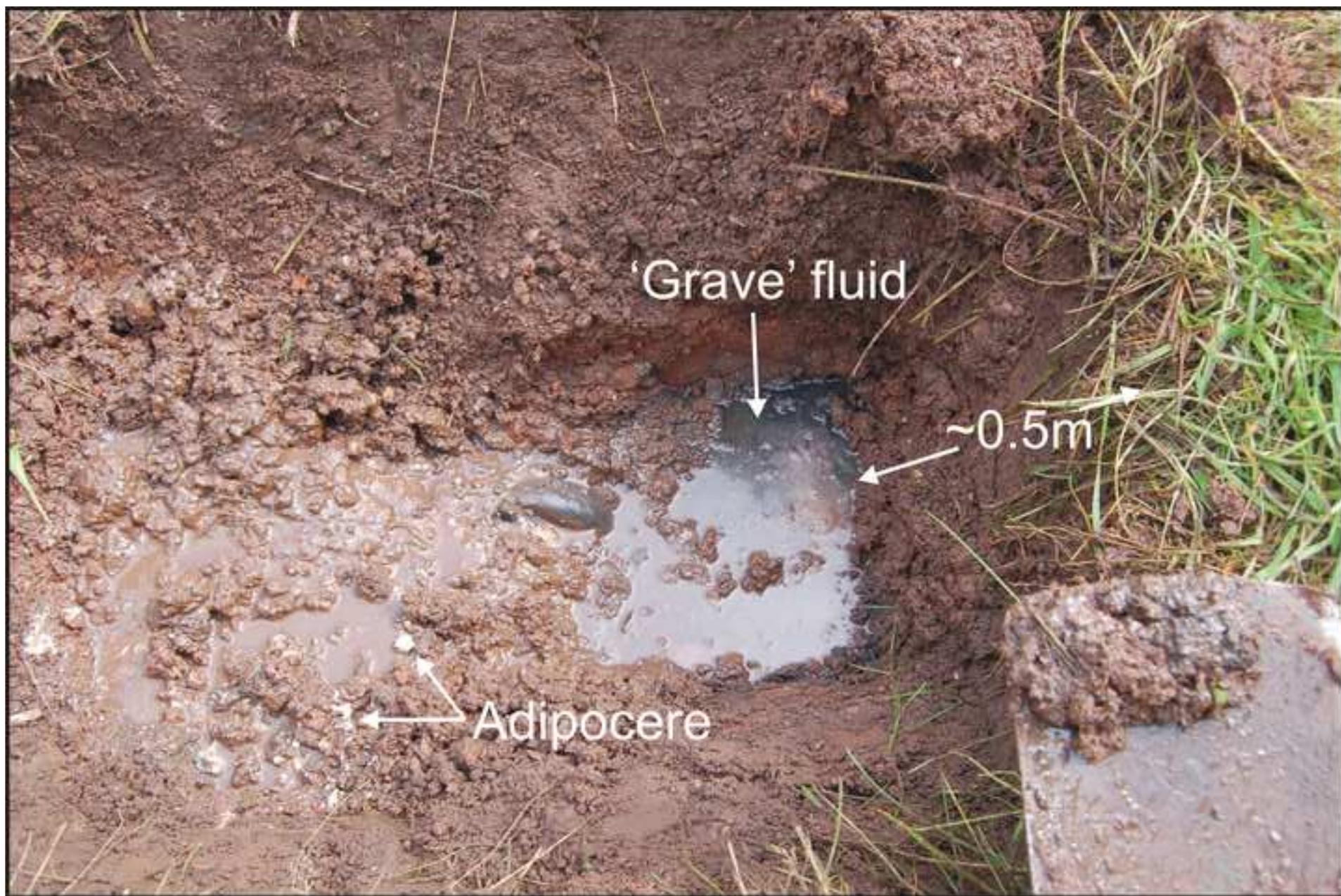
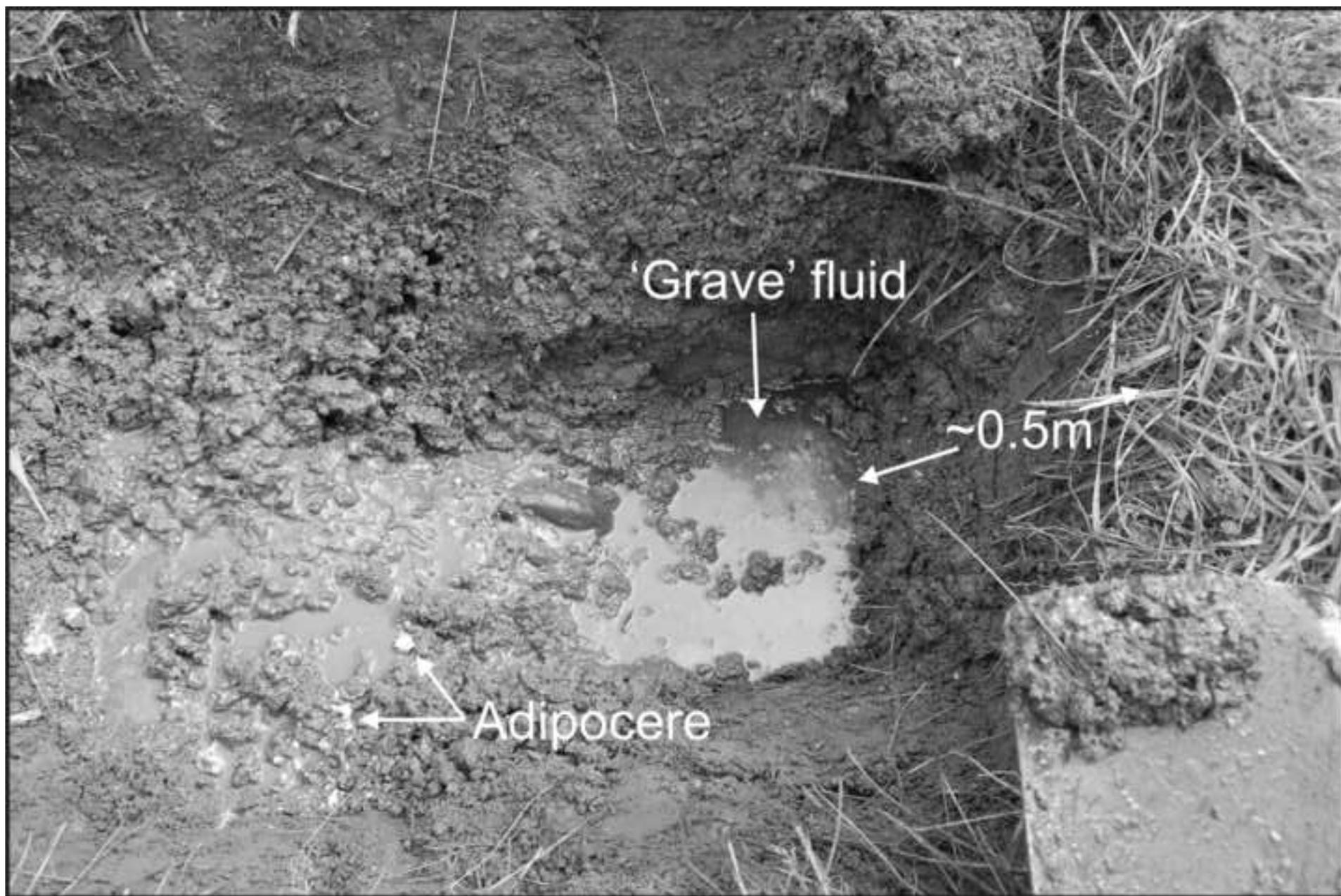


Figure 5 greyscale (print)
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