Science and Justice 54 (2014) 238–244

Contents lists available at ScienceDirect

Science and Justice

journal homepage: www.elsevier.com/locate/scijus

Review

Limitations and recommendations for successful DNA extraction from forensic soil samples: A review

Jennifer M. Young *, Nicolas J. Rawlence [1], Laura S. Weyrich, Alan Cooper

Australian Centre for Ancient DNA, School of Earth and Environmental Sciences, University of Adelaide, Australia

ARTICLE INFO

Article history:
Received 30 August 2013
Received in revised form 12 January 2014
Accepted 18 February 2014

Keywords:
Soil
DNA
Extraction
Forensic
Metagenomics
HTS

ABSTRACT

Soil is commonly used in forensic casework to provide discriminatory power to link a suspect to a crime scene. Standard analyses examine the intrinsic properties of soils, including mineralogy, geophysics, texture and colour; however, soils can also support a vast amount of organisms, which can be examined using DNA fingerprinting techniques. Many previous genetic analyses have relied on patterns of fragment length variation produced by amplification of unidentified taxa in the soil extract. In contrast, the development of advanced DNA sequencing technologies now provides the ability to generate a detailed picture of soil microbial communities and the taxa present, allowing for improved discrimination between samples. However, DNA must be efficiently extracted from the complex soil matrix to achieve accurate and reproducible DNA sequencing results, and extraction efficacy is highly dependent on the soil type and method used. As a result, a consideration of soil properties is important when estimating the likelihood of successful DNA extraction. This would include a basic understanding of soil components, their interactions with DNA molecules and the factors that affect such interactions. This review highlights some important considerations required prior to DNA extraction and discusses the use of common chemical reagents in soil DNA extraction protocols to achieve maximum efficacy. Together, the information presented here is designed to facilitate informed decisions about the most appropriate sampling and extraction methodology, relevant both to the soil type and the details of a specific forensic case, to ensure sufficient DNA yield and enable successful analysis.

© 2014 Forensic Science Society. Published by Elsevier Ireland Ltd. All rights reserved.

Contents

* Corresponding author.
E-mail address: jennifer.young@adelaide.edu.au (J.M. Young).
[1] Present Address: Allan Wilson Centre for Molecular Ecology and Evolution, Department of Zoology, University of Otago, Dunedin, New Zealand.

http://dx.doi.org/10.1016/j.scijus.2014.02.006
1355-0306/© 2014 Forensic Science Society. Published by Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Forensic soil analysis is a multidisciplinary science that spans several fields of research because soils are highly individualistic and can be characterised using a number of techniques, including texture, mineralogy, consistency, particle size, pH and soil colour [1–4]. Contaminants and components imbedded within soil, such as pollen, fibres, glass or plant matter, provide valuable evidence in forensic investigations by linking particular suspects to specific sites [5–9]. In addition, soil DNA analysis also provides additional evidence, as microbial, plant, and human DNA within unknown forensic soil samples can be compared to that of reference samples [10,11]. For example, a major area of current forensic soil DNA research utilises Microbial Community Profiling (MCP) [12], including denaturing gradient gel electrophoresis (DGGE; [13]), amplicon-length heterogeneity PCR (LH-PCR; [14]), and terminal restriction fragment length polymorphism (T-RFLP; [15–17]). These methods all use DNA fragment length variation within different microbial species to produce a DNA fingerprint, but the results do not specify the individual taxa responsible for a given profile. In contrast, high-throughput sequencing (HTS) can produce thousands of sequencing reads per sample, providing a far more detailed picture of microbial communities present within the soil.

HTS combined with DNA metabarcoding [18] can be used to access both microbial and non-microbial DNA in soils. Typically, biological materials, such as plant roots, leaf fragments or insect larvae, found at a crime scene are often only present in trace amounts and cannot be identified by traditional morphological means, due to the absence of diagnostic morphological characters [19]. HTS provides the potential to utilise the DNA within the soil to identify these elements, and this technique has already shown considerable potential within forensic science [20]. For example, HTS analysis of blood-stains was able to conclusively identify individuals, when previous human-microsatellite typing failed [21]. Similarly, Fierer et al. demonstrated that microbial forensics can be used to discriminate between individuals, based on the microbial assemblage present on skin [22].

HTS techniques offer a wide range of applications for soil DNA profiling, through discriminating between criminal sites or linking a suspect and location. However, soil DNA profiling is problematic due to difficulties involved in recovering DNA from complex soil matrices, as well as temporal and seasonal variations in soil biota that may change during a forensic case. DNA profiles are strongly dependent on the extraction method used [23] and soil type examined (e.g. [24]). Consequently, DNA extraction forms a crucial step since incomplete lysis at this stage will transfer biases to downstream analyses. This review addresses common problems encountered during soil DNA extraction and discusses modifications which can be applied to overcome such difficulties and improve total DNA yield.

Soil DNA analysis also requires a basic knowledge of soil mineralogy and chemistry to interpret DNA interactions with soil particles and guide the use of common reagents in DNA extraction protocols. This review also provides a basic outline of soil components and describes how each interacts with DNA molecules to highlight the importance of soil properties on the retrieval of DNA from highly variable environmental samples.

2. Considerations prior to DNA extraction

In order to achieve reproducible, informative, and reliable results in soil forensic analyses, contamination must be closely monitored to accurately obtain a representative DNA extract of the soil sample under analysis. In addition to lab contamination, events at a scene which occur following a crime may also introduce contamination as a result of soil transfer effects and therefore must be taken into consideration. To ensure reproducible and accurate results it is necessary to ensure ample sample size and numbers during collection, to enable accurate identification of samples that do indeed originate from a common source. Finally, the time lapse between the crime and collection of reference samples must be accounted for since temporal and seasonal variations may influence results.

2.1. Sample contamination

Contamination is a major concern in any DNA analysis, especially in forensic science. Introduction of contaminant DNA from external sources can occur: 1) prior to collection by mixing with other sources of DNA, 2) during collection and storage, and/or 3) during laboratory analysis.

In many cases, the soil sample will have been removed from the environment by transfer to an object, or mixed with other soils during the crime [7]. This can make it difficult to separate the features of the contaminants from those of the original source. For example, layers of soils are commonly encountered on objects such as car tyres, so whenever possible layers should be sampled separately. The primary effects of soil transfer are particle size selective to some extent, and this varies depending on soil properties and mineralogy as well as the type of contact [25]. The coarse fraction of soil that adheres to objects is typically lost first, leaving fine soil particles available for analysis [26]. For this reason, Croft et al. [27] suggest that the fine soil fraction, comprised of particles <150 μm will provide the most accurate reflection of the original soil, as it excludes artefacts introduced by transfer effects. Thus, it may be beneficial to fractionate reference samples prior to extraction, in order to perform an analysis using only fine fractions for DNA profile comparisons. Fractionation of soil is commonly achieved using sieving methods [28]. Robertson et al. recommend the use of wet sieving over dry sieving to obtain more accurate and precise particle size distribution results [29]. However, water and equipment used in this step should be sterile (DNA free), as not to introduce DNA contamination at this stage.

Sample collection is a critical issue in forensic soil DNA analysis. Samples should ideally be recovered as soon as possible after a crime is identified, before contamination of the crime scene occurs. Forensic scientists should use the appropriate equipment, e.g. gloves and facemasks, to prevent introducing DNA from the scientist to the sample. In addition, sterile, air-tight containers should be used to prevent introduction of DNA from the laboratory equipment and the surrounding environment. Following collection, storage conditions and length of storage can influence DNA profiles [30,31]. This is of particular concern during microbial analysis, because microorganisms can continue to grow and divide within the soil after it has been collected. In general, fungal species can also survive longer and grow more efficiently in colder temperatures compared to bacterial species, ensuring that even refrigeration may not be an appropriate long term storage solution [32]. Ideally, DNA extractions should be carried out as soon as possible (within 24 h of collection) to minimise these effects. Where this is not possible, samples can be frozen to aid in preventing community change, or samples can be stored in protein inhibitors, such as RNAlater (Invitrogen) to prevent further microbial growth [33,34], although additional considerations prior to extraction would apply to ensure proper removal of protein inhibitors.

During laboratory analysis, it is critical to avoid introduction of DNA from the scientist, the reagents and the surrounding environment. DNA extractions, including extraction blanks, should be carried out in dedicated extraction hoods, and multiple no-template controls should be included in all DNA amplification steps to monitor contamination. The no-template controls should be processed and sequenced identically, in parallel to all samples. Any sequences obtained in no-template controls should be identified where possible, and discounted from sample sequences where necessary, using bioinformatics tools. Further, positive controls should be avoided where possible, to prevent the risk of within-experiment cross-contamination. It is critical to maintain the rigorous use of controls to monitor contamination at all stages of the experimental process. It is also important to note that clean no-template

controls, does not remove the possibility of either low concentration or stochastic contamination within reactions.

2.2. Changes in environmental conditions

The reproducibility of the soil profile is essential to accurately identify samples from a common source. Differences in environmental conditions need to be considered to assess the likelihood of successfully matching two samples, especially where temporal offsets are experienced between the time of transfer and retrieval of a forensic soil sample. The chemical properties of the soil matrix can be altered by environmental factors, such as rainfall, and as a result, the aerobic/anaerobic, oxidising/reducing conditions within a soil are also subject to change [35]. The effect of seasonal variation on the soil DNA profile obtained from forensic samples must also be considered. For example, soils collected monthly from five habitats over a one year period were used to assess seasonal variation of soil bacteria [15], and showed monthly fluctuations in community structure. Similarly, the effects of elevated temperature and the frequency of summer precipitation on soil fauna abundance were assessed over a two-year study in the Colorado Plateau desert. While microfauna experience seasonal fluctuations, the effect was not statistically significant for most groups, except amoebae [36]. Seasonal variations in soil DNA profiles could be useful in forensic analyses, as it provides a means to ascertain a timeline of events depending on the taxonomy detected. However, such variations could also prevent a match to reference samples if collected at a different time and/or under different environmental conditions.

The quality and length of the DNA fragments retrieved will depend on the environmental conditions, as well as the storage conditions of samples post-collection. Quantitative PCR (qPCR) can be used to estimate DNA degradation by quantifying the amount of DNA present at several fragment sizes within a sample [37–39]. This approach could be applied to evaluate the template quality and assist in determining the most appropriate DNA target based on the fragment length present in a forensic sample. If DNA extraction and/or PCR amplification success is uncertain as a result of poor quality DNA or biased microbial communities, then the soil sample may be better utilised for evidence by an alternative technique, which is not as susceptible to environmental change or organismal growth, such as phytolith or starch grain analysis [40].

3. Soil DNA extraction

The initial extraction step in the analysis of soil DNA is potentially the most crucial step. Incomplete cell lysis or DNA binding to soil components *in situ* will transfer bias to downstream analysis [41]. DNA can be extracted from soil either indirectly or directly [42,43]. Indirect extraction involves separation of cells from soil prior to lysis, whereas direct extraction cells are lysed in the presence of soil particles. Direct extraction is strongly favoured due to higher yields and reduced potential for contamination associated with fewer preparation steps. The initial DNA extraction step can be performed through physical disruption to disperse soil colloids (e.g. bead beating) or chemical disruption to lyse cell membranes (e.g. NaCl, SDS) and may include enzymatic disruption (e.g. Proteinase K, lysozyme). This is followed by DNA purification by various methods [42–45]. Many lab or user-specific protocols have been developed to improve soil DNA extraction efficiency however, the choice of soil DNA extraction method will influence the DNA profile obtained [46–49]. Different DNA extraction methods can result in an over- or under- representation of specific bacterial phyla [50]. These observations are further complicated because different DNA extraction techniques have been conducted only on single soil types, so extraction methods cannot be easily compared across different studies. This is a significant issue in forensic applications and standardisations and direct comparisons of protocols against multiple soil types will need to be conducted to ensure reliability of forensic soil analyses.

3.1. Commercial soil DNA extraction kits

Commercial kits offer a means for standardising soil DNA extraction, as the protocols can be easily implemented in any laboratory. Several biotechnology companies offer soil DNA extraction kits (summarised in Table 1) that are primarily favoured in forensic analysis over in-house protocols because of the ease of use. The most commonly used kit is the PowerSoil® DNA Isolation kit (MOBIO Lab Inc.), which is sufficient for most soil samples but becomes problematic for difficult samples such as those containing high levels of organics, clay and heavy metals. For such tough samples, alternative kits may be more efficient, as they have been developed to take into consideration the variability in soil type (e.g. NucleoSpin® Soil kit, Macherey-Nagel). Commercial kits are typically optimised for a maximum of 0.25 g soil; however, some allow larger volumes to be processed (e.g. PowerMax® DNA Isolation kit, MOBIO Lab Inc.). While the soil mass available for forensic analysis will vary, it is often limited. There have been mixed reports on the effect of soil mass on the reproducibility of soil DNA profiles [51–53]; however the effect of soil mass on the ability to differentiate between samples has not yet been examined.

Some manufacturers also offer extraction kits which use 96-well plates for high throughput of samples. There are now automated DNA extraction methods for soils, such as the Aurora (Boreal Genomics), which is based on an electrophoresis technology called SCODA (Synchronous Coefficient of Drag Alteration). This has been designed to allow sufficient extraction of high quality DNA from low template soils. However, there is a minimum fragment size limit of 300 bp, which may be problematic for many forensic soil samples containing degraded DNA.

3.2. Modifications to DNA extraction

Many modifications have been made to soil DNA extraction protocols, mainly by varying the physical treatment of material prior to lysis and/or by altering the chemical components incorporated in the extraction lysis buffer. To better understand the effect of these chemicals during extraction, it is vital to be aware of the soil components that interact with DNA molecules. Soils are composed of a solid phase comprising inorganic minerals and organic components, e.g. humic acids, as well as an aqueous phase of elements, inorganic/organic ions and molecules, that surrounds the solid particles. Each of these components plays a role in the retention of DNA molecules in soil and can affect the efficiency of DNA extraction. Previous DNA adsorption studies suggest stronger binding to the inorganic rather than organic components of soil [54–57]. However, organic components are problematic for PCR amplification if insufficiently removed during extraction.

3.2.1. Interactions of DNA and soil

This section describes the associations between DNA molecules and soil components to highlight the role and purpose of common chemical reagents for successful DNA extraction.

DNA commonly exists in soil in three forms: intracellular DNA deposited from living or deceased organisms; and extracellular DNA, either unbound in solution or physically bound via cation mediators [58]. Intracellular DNA can be released from cells using a physical pretreatment step in the presence of a lysis buffer. Extracellular DNA in aqueous solution interacts with the mineral surface based on polyelectrolyte adsorption, which is not restricted to oppositely charged components [59–62]. Therefore, double layer repulsion exerted between the negatively-charged mineral surface and negatively-charged phosphate group results in adsorbed and un-adsorbed portions along the length of the clay surface [59]. Consequently factors that influence this interaction, such as pH, presence of cations and soil dispersion [63–65], will affect adsorption of extracellular DNA onto the soil matrix and subsequently affect the efficiency of DNA extraction from soils.

Table 1
Details of common commercial soil DNA extraction kits.

Commercial kit	Soil mass	Automated (Y/N)	Cell lysis/physical treatment method	Comments
PowerSoil®DNA Isolation kit MOBIO	0.25 g	N	Beat-beating (different bead sizes and types available)	Does not work well with difficult samples, very gentle
PowerMax® DNA Isolation Kit MOBIO	Up to 10 g	N	Bead-beating (different bead sizes and types available)	Same protocol as PowerSoil® with larger volume
UltraClean® Soil DNA Isolation kit MOBIO	0.25 g	N	Bead-beating (ceramic or glass beads available)	96-well plate for high-throughput
SoilMaster™ DNA Extraction kit Epicentre®	0.10 g	N	Hot-detergent lysis Proteinase K	Targets high molecular weight DNA
Soil DNA Isolation kit NORGEN BIOTEK	0.25	N	Bead-beating	High humic acid content soils
NucleoSpin® Soil kit MACHERERY-NAGEL	<0.5 g	N	Offers two alternative lysis buffers to suit soil sample, bead-beating (ceramic beads)	Also an optional additive to enhance performance
SurePrep™ Soil DNA Isolation Kit Fischer BioReagents®	0.25	N	Bead-beating	Optimised for high water content soils
E.Z.N.A.® Soil DNA Kit OMEGA bio-tek	Up to 1.0 g	N	Incubation bead-beating (glass beads)	Soil in lysis buffer is incubated at 70 °C for 10 min
FavorPrep™ Soil DNA Isolation Kit (Mini/Midi) FAVORGEN® BIOTECH CORP	Mini: 0.2–1 g Midi: up to 10 g	N	Incubation, bead-beating (glass beads) Proteinase K	Soil in lysis buffer is incubate at 70 °C for 10 min
Maxwell® Research Systems PROMEGA	Process up to 16 samples	Y	Uses paramagnetic-particle technology	Maxwell ® 16 Forensic System available
Aurora System Boreal Gemonics	Up to 5 mL of lysed or prepared sample	Y	SCODA purification technology	High yield from low abundance samples, >300 bp fragments

3.2.2. Pre-treatment methods

The initial step in most commercial soil DNA extraction kits is physical disruption of the cells in a lysis buffer, most commonly by 'bead-beating,' which aims to release intracellular DNA into solution as well as homogenise the soil colloids. A variety of bead types (ceramic, glass, zirconium, metal) are used in commercial kits. The type and amount of beads used, the speed, and the temperature during bead beating have all been shown to have an effect on extraction efficiency [66]. Alternative physical treatments such as sonication, microwave, freeze-thaw and ultrasound have also been explored [67]. Subjecting samples to these treatments prior to extraction with the MoBio Powersoil® DNA Isolation kit has shown to improve DNA yields. However, inconsistent extraction efficiency remains an issue [23,68,69], and such treatments can result in fragmentation of DNA molecules [49]. Some protocols also include an incubation step at 55 °C and/or prolonged incubations to both increase the cell lysis and to aid soil dispersion of high clay content soils (e.g. E.Z.N.A.® Soil DNA kit, OMEGA bio-tek). Clearly, the necessity and effectiveness of different extraction pre-treatments will depend on the behaviour and properties of the soil sample.

3.2.3. Soil dispersion

Soils vary in their ability to disperse in a liquid. For example, saline or highly calcareous soils tend to aggregate clays in the soil and keep them flocculated. The dispersion properties may prevent the soil from being fully homogenous in the extraction buffer and subsequently influence the DNA extraction efficiency. For example, Ettenauer et al. [70] found that a finely ground sample powder strongly adsorbed the lysis buffer so that the mixture did not represent a homogenous liquid suspension. Soil dispersion is pH-dependent, and a soil subjected to a buffer with a high pH will result in better dispersion and more efficient DNA recovery. In addition, a high concentration of reagents such as tris, sodium dodecyl sulphate (SDS) and especially sodium chloride (NaCl) in extraction buffers can prevent soil dispersion by suppressing the charges on the clays. Ettenauer et al. [70] suggest that the ratio of soil mass to buffer volume may play a role in DNA extraction efficiency, as a result of the dispersion properties of the soil. If dispersion of soil in the lysis buffer is problematic, one option would be to reduce the sample size and combine multiple DNA extracts for downstream analysis.

3.2.4. Influence of pH

The pH of the soil and the lysis buffer are potentially the most influential factors in DNA adsorption to clay minerals, as pH affects the electrostatic property of both the clay mineral surface and the DNA phosphate. The phosphate group on DNA can exist in various oxidation states depending on pH: H_3PO_4, $H_2PO_4^-$ (monobasic), HPO_4^{2-} (bibasic) and PO_4^{3-} (tribasic) [35], which can all influence the strength of interaction towards the mineral surface. The electrostatic state of the clay mineral surface is dependent on the pH at which there is no net charge on the clay surface. This value is termed the Point Zero Charge (P_zC) and varies between clay types [65]. One study used 20 soil samples to show an increase in DNA yield with an increase in extraction buffer pH, with an optimum of pH 9 [67]. Additionally, numerous studies have shown an increase in DNA adsorption on soils with low pH [57,63,71]. For these soils, DNA released during the lysis step was potentially lost because it was more readily adsorbed onto the clay surface. For example, acidic clay mineral allophane can bind 95–99% of available DNA, with only 5% in an extractable form [71].

To prevent extracellular DNA from binding to the soil surface after lysis, sodium phosphate is commonly used in the lysis buffer. This competes with DNA phosphate groups to block binding sites on the clay surface, and thus increase DNA yield [71]. However, a small portion of DNA adsorb to soil at all phosphate concentrations [65]. Similarly, Taberlet et al. [51] suggest an extraction protocol based on a saturated phosphate buffer, which aims to block available binding sites on the clay mineral as well as displace DNA molecules already bound releasing them into solution. Alternatively, soil pre-treatment with RNA to saturate available adsorption sites has been shown to increase DNA yield from soils [63,67].

3.2.5. Influence of cations

DNA adsorption to clay minerals has also been shown to increase in the presence of cations in the extraction buffer [63,65,72]. Pastre et al. [62] report reduced DNA yields in the presence of high multi-valent salt concentration. This can be attributed to the effect of cation valency (the charge on the cation). DNA adsorption is enhanced by multivariant cations (positive charge greater than one) that act as bridges to tightly bind DNA molecules to the clay mineral surface [63,64,72]. Franchi et al. [58] show that, in the absence of cations, only 8.6% of DNA was adsorbed, whereas 96.2% was adsorbed in the presence of

4.0 nM Mg^{2+} cations. Furthermore, the effect of divalent cations has also been shown to be dependent on the species of cation, with a greater adsorption of DNA in the presence of Ca^{2+} compared to Mg^{2+} [65].

To prevent DNA adsorption via cations, reagents called chelating agents are often incorporated into the lysis buffer. Chelating agents remove metal ions from aqueous solution by forming stable water soluble complexes, thus preventing bridge formation between the DNA phosphates and the clay surface. The most commonly used chelating agent in DNA extraction protocols is ethylenediaminetetraacetic acid (EDTA) [13,68,73,74]. However, EDTA used at high concentrations can become problematic in PCR amplification reactions, as it also chelates cations such as Mg^{2+} necessary for polymerase activity in the PCR [49,75].

3.2.6. Removal of PCR inhibitors

In general, silica-based extraction methods have been shown to be more effective at removing PCR inhibitors than organic solvent-based methods (e.g. phenol/chloroform).

Soil organic matter, e.g. humic acids, can also become problematic in DNA analysis, as they can inhibit PCR amplification by suppressing the activity of the DNA polymerase and need to be removed during the extraction process [76]. Soil organic matter includes the compounds derived from living specimens during decomposition of cellular materials and biochemicals, such as carbohydrates, fats, waxes, lipids, alkanes, amino acids, proteins and organic acids, including DNA. High organic content soils are classically recognised by dark, brown to black soil colours. DNA adsorbed to organic molecules is not easily displaced and can be lost during DNA extraction [67,72].

To reduce PCR inhibition by humic substances, reagents are used to remove the inhibitor molecules, such as flocculants. Flocculants [77] have been shown to increase yields and reduce downstream PCR inhibition with high organic content soils [78]. Flocculant molecules reduce repulsion between adjacent inhibitor molecules causing aggregation, reducing solubility and allowing separation from the DNA extract [77]. Surfactants (amphiphilic organic molecules composed of a hydrophilic head and a hydrophobic tail) are known to disrupt cellular membrane bilayers but also contribute to the removal of PCR inhibitors during DNA extraction. Anionic surfactant SDS is most commonly used in soil DNA extraction buffers; however, some protocols substitute SDS for cationic surfactant cetyl trimethylammonium bromide (CTAB) [74,79,80] or N-lauryl sulphate, a molecular variation of SDS [81]. A wide-range of surfactants are available with different behaviour towards different soil types [82–84]. Similarly, polyvinylpolypyrrolidone (PVPP), a crosslinker that is insoluble in water, has been used to remove humic acids and other phenolic compounds by agglomeration [67,73,77].

DNA extraction from a soil sample with a high organic content or high heavy metal content may be improved by substituting SDS for an alternative surfactant, such as CTAB, or introducing flocculants to the lysis buffer. If a PCR reaction is unsuccessful, dilution of the DNA extract to reduce the impacts of inhibitors can also be tried [85]. Alternatively, a pre-lysis wash step can be included in the protocol for soils with high humic content or iron oxides to improve DNA extraction efficiency [86].

4. Standardisation of DNA extraction method

While there is much research currently underway to develop and improve the efficiency of DNA extraction from soils, the comparison of soil samples for forensics requires a standard protocol to ensure all samples are processed identically within a case. An ISO (International Organisation for Standardisation) standard has been published for soil microbial DNA extraction [87] to enable accurate data comparison between different groups of soil scientists. This standard is based on a publication by Martin-Laurent et al. and was evaluated across 13 laboratories worldwide using 12 soils, including those from arable forest and industrial sites [88,89]. However, it would also be beneficial to institute a standardised method across all forensic laboratories for comparative analyses as such a protocol has not yet been adopted.

In the event that reference samples are not available for comparison, retrieving maximum genetic information from the sample of interest would be preferable, warranting the use of tailored DNA extraction methods based on the specific soil properties, rather than following a standardised protocol.

5. Conclusions

The use of DNA metabarcoding and HTS technology to identify taxa from soil samples has the potential to provide a detailed picture of soil DNA communities, but is not without limitations. It is imperative that DNA extraction efficiency is maximised for forensic soil analyses because sample sizes are often limited and incomplete DNA extractions can introduce bias and therefore alter the interpretation of results. The reproducibility and optimal soil mass used in the extraction steps requires research, with conflicting reports about the ability to detect local endemics, rare species, and taxa with special habitat requirements. Due to the existence of a wide variety of soil DNA extraction protocols, there is currently no standardised extraction method used across forensic laboratories, potentially resulting in different profiles from the same sample. This may limit the potential to compare forensic samples across different forensic groups, countries, or even case studies.

The effect of environmental conditions on the DNA profile has potential to be useful for estimating the time of a crime, but can also complicate comparative analyses if soils are not collected simultaneously. Similarly, sample storage conditions following a crime will affect the DNA quality and type available for analysis, while DNA degradation rates in soils under different conditions remain largely unexplored. Similarly, how much bias is introduced by species growth during storage and the effects on identification is also currently unknown. All of these areas require considerable attention before forensic soil science can move forward. For example, if contamination is introduced there is the potential of obtaining a false positive result. Alternatively, inefficient DNA extraction and incomplete cell lysis could reduce genetic information and lead to a false negative result. Both of these outcomes would be detrimental to a case.

Assessment of soil properties prior to DNA extraction is necessary to enable use of the most appropriate protocol, exploiting knowledge of DNA adsorption to soil components and consideration of soil properties, such as pH, organic content, clay content and soil moisture. This assessment will provide an idea of the likely behaviour of the soil during DNA extraction and avoid potential losses of vital pieces of evidence. In addition to soil type, the most efficient DNA extraction protocol for forensic soil analysis will be highly dependent on the case, the availability of evidence, the value of that evidence, number of samples required and the cost of multiple extractions.

While DNA from soil samples recovered from a crime scene has the potential to revolutionise forensic research, considerable amounts of further research are required before soil DNA can be utilised as evidence in casework. Even before DNA is extracted, storage conditions can alter the DNA profile of soils; future research is needed to determine how this impacts the ability to discriminate between sites. Assessment of the effects of storage contamination on multiple different metabarcoding markers would determine which DNA targets are the most robust under different circumstances, i.e. which taxa should be sequenced if the samples have been stored at cool temperatures verses room temperature. Similarly, spatial variation within a single site may prevent accurate classification of unknown soils and also requires further investigation to survey site variation according to soil and habitat types. The resolution power of combining several different metabarcoding markers should also be tested, as this could provide greater discrimination between samples or sites. Lastly, standardisation of soil DNA forensic methods will need to be examined. When these issues are resolved, forensic scientists will be able to incorporate DNA soil analysis into commonly applied casework, providing robust, reliable evidence from soil DNA.

Acknowledgments

JMY is financially supported by an ARC Linkage Grant between the University of Adelaide and the Australian Federal Police. We thank the members of the Australian Centre for Ancient DNA for helpful comments on previous versions of the manuscript.

References

[1] A. Ruffell, Forensic pedology, forensic geology, forensic geoscience, geoforensics and soil forensics, Forensic Sci. Int. 202 (1–3) (2010) 9–12.
[2] K. Ritz, Lorna D., David Miller, Criminal and Environmental Soil Forensics, Criminal and Environmental Soil Forensics, Springer, 2009.
[3] K. Pye, Geological and soil evidence. Forensic applications, in: K. Pye (Ed.), Geological and soil evidence: forensic applications, xvii, 2007, p. 335.
[4] K. Pye, D.J. Croft, Forensic geoscience: introduction and overview, Geol. Soc. Lond., Spec. Publ. 232 (1) (2004) 1–5.
[5] D.C. Mildenhall, P.E.J. Wiltshire, V.M. Bryant, Forensic palynology: why do it and how it works, Forensic Sci. Int. 163 (3) (2006) 163–172.
[6] P.J. Wiltshire, Forensic ecology, botany, and palynology: some aspects of their role in criminal investigation, in: K. Ritz, L. Dawson, D. Miller (Eds.), Criminal and Environmental Soil Forensics, Springer, Netherlands, 2009, pp. 129–149.
[7] P.A. Bull, A. Parker, R.M. Morgan, The forensic analysis of soils and sediment taken from the cast of a footprint, Forensic Sci. Int. 162 (1–3) (2006) 6–12.
[8] C.A. Pounds, The recovery of fibres from the surface of clothing for forensic examinations, J. Forensic Sci. Soc. 15 (2) (1975) 127–132.
[9] C.A. Pounds, K.W. Smalldon, The transfer of fibres between clothing materials during simulated contacts and their persistence during wear: part I—fibre transference, J. Forensic Sci. Soc. 15 (1) (1975) 17–27.
[10] J. Horswell, Forensic comparison of soils by bacterial community DNA profiling, J. Forensic Sci. 47 (2) (2002) 350–353.
[11] L.M. MacDonald, et al., Microbial DNA profiling by multiplex terminal restriction fragment length polymorphism for forensic comparison of soil and the influence of sample condition, J. Appl. Microbiol. 105 (3) (2008) 813–821.
[12] G.F. Sensabaugh, Microbial community profiling for the characterisation of soil evidence: forensic considerations, in: K. Ritz, L. Dawson, D. Miller (Eds.), Criminal and Environmental Soil Forensics, Springer, Dordrecht, 2009, pp. 49–60.
[13] A. Lerner, et al., Can denaturing gradient gel electrophoresis (DGGE) analysis of amplified 16S rDNA of soil bacterial populations be used in forensic investigations? Soil Biol. Biochem. 38 (6) (2006) 118–1192.
[14] L.I. Moreno, et al., The application of amplicon length heterogeneity PCR (LH-PCR) for monitoring the dynamics of soil microbial communities associated with cadaver decomposition, J. Microbiol. Methods 84 (3) (2011) 388–393.
[15] M.S. Meyers, D.R. Foran, Spatial and temporal influences on bacterial profiling of forensic soil samples, J. Forensic Sci. (2008) 652–660.
[16] E.J. Lenz, D.R. Foran, Bacterial profiling of soil using genus-specific markers and multidimensional scaling, J. Forensic Sci. 55 (6) (2010) 1437–1442.
[17] C.A. MacDonald, Discrimination of soils at regional and local levels using bacterial and fungal t-RFLP profiling, J. Forensic Sci. 56 (1) (2011) 61–69.
[18] P. Taberlet, et al., Environmental DNA, Mol. Ecol. 21 (8) (2012) 1789–1793.
[19] J. Ward, et al., A grass molecular identification system for forensic botany: a critical evaluation of the strengths and limitations, J. Forensic Sci. 54 (6) (2009) 1251–1260.
[20] M.L. Metzker, Sequencing technologies—the next generation, Nat. Rev. Genet. 11 (1) (2010) 31–46.
[21] B. Brenig, J. Beck, E. Schütz, Shotgun metagenomics of biological stains using ultra-deep DNA sequencing, Forensic Sci. Int. Genet. 4 (4) (2010) 228–231.
[22] N. Fierer, et al., Forensic identification using skin bacterial communities, Proc. Natl. Acad. Sci. 107 (14) (2010) 6477–6481.
[23] S. Terrat, et al., Molecular biomass and meta taxogenomic assessment of soil microbial communities as influenced by soil DNA extraction procedure, Microb. Biotechnol. 5 (1) (2012) 135–141.
[24] C.L. Roose-Amsaleg, E. Garnier-Sillam, M. Harry, Extraction and purification of microbial DNA from soil and sediment samples, Appl. Soil Ecol. 18 (1) (2001) 47–60.
[25] K. Pye, S.J. Blott, Particle size analysis of sediments, soils and related particulate materials for forensic purposes using laser granulometry, Forensic Sci. Int. 144 (1) (2004) 19–27.
[26] V. Chazottes, C. Brocard, B. Peyrot, Particle size analysis of soils under simulated scene of crime conditions: the interest of multivariate analyses, Forensic Sci. Int. 140 (2–3) (2004) 159–166.
[27] D.J. Croft, K. Pye, Multi-technique comparison of source and primary transfer soil samples: an experimental investigation, Sci. Justice 44 (1) (2004) 21–28.
[28] R. Sugita, Y. Marumo, Screening of soil evidence by a combination of simple techniques: validity of particle size distribution, Forensic Sci. Int. 122 (2–3) (2001) 155–158.
[29] J. Robertson, et al., Particle size analysis of soils — a comparison of dry and wet sieving techniques, Forensic Sci. Int. 24 (3) (1984) 209–217.
[30] L.D. Bainard, J.N. Klironomos, M.M. Hart, Differential effect of sample preservation methods on plant and arbuscular mycorrhizal fungal DNA, J. Microbiol. Methods 82 (2) (2010) 124–130.
[31] Y.B. Lee, et al., Cold storage and pretreatment incubation effects on soil microbial properties, Soil Sci. Soc. Am. J. 71 (4) (2007) 1299–1305.
[32] M. Pesaro, et al., Effects of freeze–thaw stress during soil storage on microbial communities and methidathion degradation, Soil Biol. Biochem. 35 (8) (2003) 1049–1061.
[33] G. Mutter, et al., Comparison of frozen and RNALater solid tissue storage methods for use in RNA expression microarrays, BMC Genomics 5 (1) (2004) 88.
[34] J. Schnecker, et al., A field method to store samples from temperate mountain grassland soils for analysis of phospholipid fatty acids, Soil Biol. Biochem. 51 (2012) 81–83.
[35] A.R. Conklin, Introduction to Soil Chemistry: Analysis and Instrumentation, John Wiley and Sons, Inc., 2005
[36] B.J. Darby, et al., Few apparent short-term effects of elevated soil temperature and increased frequency of summer precipitation on the abundance and taxonomic diversity of desert soil micro- and meso-fauna, Soil Biol. Biochem. 43 (7) (2011) 1474–1481.
[37] B.E. Deagle, P.J. Eveson, S.N. Jarman, Quantification of damage in DNA recovered from highly degraded samples-a case study on DNA in faeces, Front. Zool. 3 (2006) 11.
[38] D.C. Murray, et al., DNA-based faecal dietary analysis: a comparison of qPCR and high throughput sequencing approaches, PLoS ONE 6 (10) (2011) e25776, http://dx.doi.org/10.1371/journal.pone.0025776.
[39] C. Gangneux, et al., Fungal, bacterial and plant dsDNA contributions to soil total DNA extracted from silty soils under different farming practices: relationships with chloroform-labile carbon, Soil Biol. Biochem. 43 (2) (2011) 431–437.
[40] M. Horrocks, A combined procedure for recovering phytoliths and starch residues from soils, sedimentary deposits and similar materials, J. Archaeol. Sci. 32 (8) (2005) 1169–1175.
[41] P.M. Hollingsworth, S.W. Graham, D.P. Little, Choosing and using a plant DNA barcode, PLoS ONE 6 (5) (2011) e19254, http://dx.doi.org/10.1371/journal.pone.0019254.
[42] P. Robe, et al., Extraction of DNA from soil, Eur. J. Soil Biol. 39 (4) (2003) 183–190.
[43] E.M. Gabor, E.J. de Vries, D.B. Janssen, Efficient recovery of environmental DNA for expression cloning by indirect extraction methods, FEMS Microbiol. Ecol. 44 (2) (2003) 153–163.
[44] J. Rajendhran, P. Gunasekaran, Strategies for accessing soil metagenome for desired applications, Biotechnol. Adv. 26 (6) (2008) 576–590.
[45] D.N. Miller, et al., Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples, Appl. Environ. Microbiol. 65 (11) (1999) 4715–4724.
[46] S. Knauth, H. Schmidt, R. Tippkötter, Comparison of commercial kits for the extraction of DNA from paddy soils, Lett. Appl. Microbiol. 56 (3) (2012) 222–228.
[47] A. Manjula, et al., Comparison of seven methods of DNA extraction from termitarium for functional metagenomic DNA library construction, J. Sci. Ind. Res. 70 (11) (2011) 945–951.
[48] X. Jia, et al., Comparisons of extraction and purification methods of soil microorganism DNA from rhizosphere soil, J. For. Res. 17 (1) (2006) 31–34.
[49] M. Krsek, E.M.H. Wellington, Comparison of different methods for the isolation and purification of total community DNA from soil, J. Microbiol. Methods 39 (1) (1999) 1–16.
[50] P.N. Holmsgaard, et al., Bias in bacterial diversity as a result of Nycodenz extraction from bulk soil, Soil Biol. Biochem. 43 (10) (2011) 2152–2159.
[51] P. Taberlet, et al., Soil sampling and isolation of extracellular DNA from large amount of starting material suitable for metabarcoding studies, Mol. Ecol. 21 (8) (2012) 1816–1820.
[52] S. Kang, A.L. Mills, The effect of sample size in studies of soil microbial community structure, J. Microbiol. Methods 66 (2) (2006) 242–250.
[53] L.G. Grundmann, F. Gourbiere, A micro-sampling approach to improve the inventory of bacterial diversity in soil, Appl. Soil Ecol. 13 (2) (1999) 123–126.
[54] P. Cai, et al., Microcalorimetric studies on the adsorption of DNA by soil colloidal particles, Colloids Surf. B: Biointerfaces 49 (1) (2006) 49–54.
[55] P. Cai, Q.Y. Huang, X.W. Zhang, Microcalorimetric studies of the effects of $MgCl_2$ concentrations and pH on the adsorption of DNA on montmorillonite, kaolinite and goethite, Appl. Clay Sci. 32 (1–2) (2006) 147–152.
[56] P. Cai, et al., Binding and transformation of extracellular DNA in soil, Pedosphere 15 (1) (2005) 16–23.
[57] P. Cai, et al., Adsorption of DNA on clay minerals and various colloidal particles from an Alfisol, Soil Biol. Biochem. 38 (3) (2006) 471–476.
[58] M. Franchi, J.P. Ferris, E. Gallori, Cations as mediators of the adsorption of nucleic acids on clay surfaces in prebiotic environments, Orig. Life Evol. Biosph. 33 (1) (2003) 1–16.
[59] D.J. Levy-Booth, et al., Cycling of extracellular DNA in the soil environment, Soil Biol. Biochem. 39 (12) (2007) 2977–2991.
[60] C.A.I. Goring, W.V. Bartholomew, Adsorption of mononucleotides, nucleic acids, and nucleoproteins by clays, Soil Sci. 74 (2) (1952) 149–164.
[61] G. Romanowski, M.G. Lorenz, W. Wackernagel, Adsorption of plasmid DNA to mineral surfaces and protection against DNase, Appl. Environ. Microbiol. 57 (4) (1991) 1057–1061.
[62] D. Pastre, et al., Specific DNA-protein interactions on mica investigated by atomic force microscopy, Langmuir 26 (4) (2010) 2618–2623.
[63] Y. Shen, et al., Influence of solution chemistry on the deposition and detachment kinetics of RNA on silica surfaces, Colloids Surf. B: Biointerfaces 82 (2) (2011) 443–449.
[64] T.H. Nguyen, M. Elimelech, Plasmid DNA adsorption on silica: kinetics and conformational changes in monovalent and divalent salts, Biomacromolecules 8 (1) (2007) 24–32.
[65] K. Saeki, T. Kunito, M. Sakai, Effects of pH, ionic strength, and solutes on DNA adsorption by andosols, Biol. Fertil. Soils 46 (5) (2010) 531–535.
[66] H. Bürgmann, et al., A strategy for optimizing quality and quantity of DNA extracted from soil, J. Microbiol. Methods 45 (1) (2001) 7–20.
[67] A. Frostegard, et al., Quantification of bias related to the extraction of DNA directly from soils, Appl. Environ. Microbiol. 65 (12) (1999) 5409–5420.

[68] Y.X. Jiang, et al., Integrated lysis procedures reduce extraction biases of microbial DNA from mangrove sediment, J. Biosci. Bioeng. 111 (2) (2011) 153–157.
[69] O. Inceoglu, et al., Effect of DNA extraction method on the apparent microbial diversity of soil, Appl. Environ. Microbiol. 76 (10) (2010) 3378–3382.
[70] J.D. Ettenauer, et al., Microbes on building materials—evaluation of DNA extraction protocols as common basis for molecular analysis, Sci. Total Environ. 439 (2012) 44–53.
[71] K. Saeki, M. Sakai, S.I. Wada, DNA adsorption on synthetic and natural allophanes, Appl. Clay Sci. 50 (4) (2010) 493–497.
[72] T.H. Nguyen, K.L. Chen, Role of divalent cations in plasmid DNA adsorption to natural organic matter-coated silica surface, Environ. Sci. Technol. 41 (15) (2007) 5370–5375.
[73] D. Zhang, et al., Evaluation of the impact of DNA extraction methods on BAC bacterial community composition measured by denaturing gradient gel electrophoresis, Lett. Appl. Microbiol. 53 (1) (2011) 44–49.
[74] D. Thakuria, et al., Importance of DNA quality in comparative soil microbial community structure analyses, Soil Biol. Biochem. 40 (6) (2008) 1390–1403.
[75] M. Brundin, et al., DNA binding to hydroxyapatite: a potential mechanism for preservation of microbial DNA, J. Endod. 39 (2) (2013) 211–216.
[76] R. Alaeddini, Forensic implications of PCR inhibition—a review, Forensic Sci. Int. Genet. 6 (3) (2012) 297–305.
[77] M.D. Braid, L.M. Daniels, C.L. Kitts, Removal of PCR inhibitors from soil DNA by chemical flocculation, J. Microbiol. Methods 52 (3) (2003) 389–393.
[78] L. Vanysacker, et al., Bacterial community analysis of activated sludge: an evaluation of four commonly used DNA extraction methods, Appl. Microbiol. Biotechnol. 88 (1) (2010) 299–307.
[79] S. Towe, et al., Improved protocol for the simultaneous extraction and column-based separation of DNA and RNA from different soils, J. Microbiol. Methods 84 (3) (2011) 406–412.
[80] R.I. Griffiths, et al., Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition, Appl. Environ. Microbiol. 66 (12) (2000) 5488–5491.
[81] J. Haile, Ancient DNA extraction from soils and sediments, Methods Mol. Biol. 840 (2012) 57–63.
[82] M.J. Rosen, F. Li, The adsorption of gemini and conventional surfactants onto some soil solids and the removal of 2-naphthol by the soil surfaces, J. Colloid Interface Sci. 234 (2) (2001) 418–424.
[83] C.N. Mulligan, R.N. Yong, B.F. Gibbs, Surfactant-enhanced remediation of contaminated soil: a review, Eng. Geol. 60 (1–4) (2001) 371–380.
[84] S. Paria, Surfactant-enhanced remediation of organic contaminated soil and water, Adv. Colloid Interf. Sci. 138 (1) (2008) 24–58.
[85] S.K. Juniper, et al., Extraction and purification of DNA from organic rich subsurface sediments (ODP Leg 169S), Mar. Geol. 174 (1–4) (2001) 241–247.
[86] J. He, Z. Xu, J. Hughes, Pre-lysis washing improves DNA extraction from a forest soil, Soil Biol. Biochem. 37 (12) (2005) 2337–2341.
[87] L. Philippot, et al., Soil microbial diversity: an ISO standard for soil DNA extraction, J. Soils Sediments 10 (7) (2010) 1344–1345.
[88] I. Petric, et al., Inter-laboratory evaluation of the ISO standard 11063 "Soil quality—method to directly extract DNA from soil samples", J. Microbiol. Methods 84 (3) (2011) 454–460.
[89] F. Martin-Laurent, et al., DNA extraction from soils: old bias for new microbial diversity analysis methods, Appl. Environ. Microbiol. 67 (5) (2001) 2354–2359.