Creating Woodland from Waste
The Healthy Soils Toolbox

FINAL REPORT
to
Cheshire County Council
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Executive Summary

This 12-month partnership project investigated the use of recycled greenwaste compost in remediation of brownfield land to soft end uses, compatible with the activities of the Mersey Forest. The work was a collaborative activity between Clean Merseyside Centre (CMC), The Mersey Forest, Ecological Restoration Consultants (ERC), Newlands and Liverpool John Moores University. The project was resourced with the support of a £400k grant from WRAP. A research component of the project, carried out at the university, employed two Post-Doctoral Researchers and a 0.6 Post-Graduate Research Technician.

During the year 20,000 tonnes of compost was recycled to brownfield sites. The largest single application (7,000 t) formed a component of a large-scale regeneration project at a former Royal Ordnance site at Chorley. Other sites between a peri-urban degraded agricultural field (a Landlife site, planted with native wild flower seeds), degraded land associated with the second runway development at Manchester Airport, urban allotments and brownfield sites (some of which were contaminated with metals and arsenic) that had been previously established and extensively studied as part of the Mersey Forest Brownfield Project. Compost was generally applied to 5cm depth (approximately 250t ha $^{-1}$) as a surface mulch. A Manchester Airport the compost was incorporated into existing soil. At the established experimental brownfield sites, compost was applied within an existing, multi-factorial replicated experimental design, allowing elucidation and more detailed study of the effects of compost application on soil processes. Site investigations were completed at all experimental sites.

Quality Assurance (QA) was the focus of the university research group, whose objectives were the accurate and practicable assessment of soil health. A comprehensive literature review was followed by an intensive evaluation and field-testing of more than 80 potential soil health assays. A soil health toolbox was devised and tested, containing 12 ‘tools’ that consisted of plant growth trials, soil macro- and micro-invertebrate sampling and analysis (including earthworm extraction, surface pitfall traps and Tullgren Funnel extraction), microbial assays (including microbial biomass and PCR techniques) and quantification of functional soil processes (including soil respiration, enzyme assays and ATP analysis). The devised toolbox was designed to be accessible to practitioners and commercial laboratories; all methodology has been fully described, catalogued and costed.

The period between compost application and toolbox testing (less than 6 months, largely during the winter period) meant it was difficult to show clear beneficial effects of compost application on soil health. However, there were some exceptions, for example at Manchester Airport sites where the compost had been incorporated into the soil in 2004. Other recent published studies have shown that a minimum period of 3 years is the necessary time to show clearer effects of compost applications, and further monitoring of the sites of the present project is required. Nevertheless, this scientific evaluation involving appraisal of current knowledge, the field study and direct experience of using compost has provided a strong argument in favour of using compost in brownfield land remediation. There are strong arguments that support compost applications to soil to improve soil characteristics, and to support biodiversity and soil functional processes. Recycling greenwaste compost to soil also supports other environmental agendas including carbon sequestration and flood risk prevention. Continued dissemination of the findings and further development of the work is an urgent priority.

1.0 Development of the Healthy Soils Toolbox

1.1 Introduction

Reclamation of contaminated land contributes to environmental health by improving the quality of soil and resolving contamination issues, but descriptors of soil health that can be used to recognise quality, identify problems and define endpoints are currently inadequate. There are limited guidelines as to what constitutes a healthy soil. Although the concept has been well discussed in the context of agricultural and forest soils, different indices are probably required for the environmental constraints associated with brownfield land remediation and the creation of new soils. Implicit to restored soil health is the existence of indicators or monitors of biodiversity, soil sustainability and acceptable risk management.

The project aimed to compile and provide a robust, relatively simple and practical toolbox of soil biological indicators appropriate for assessment of brownfield land, and particularly for brownfield land undergoing restoration to soft end-uses, for example to community forestry. The derived toolbox was to be tested on a range of established brownfield sites at different stages of restoration in NW England. This is a step towards providing guidance to environmental practitioners that can be used in conjunction with established site investigation methodologies. It was hoped this would also contribute to the broader national agendas concerned with healthy soils (Environment Agency, 2002; National Trust, 2003; DEFRA, 2004; European Commission, 2004).

1.2 Literature review

1.21 Assessment of soil health: biological requirements

A discussion of the rationale of the healthy soils toolbox and literature review where published during the year (Box, and appended article).

Abstract

Reclamation of contaminated land contributes to environmental health by improving the quality of soil and resolving contamination issues, but descriptors of soil health that can be used to recognise quality, identify problems and define endpoints are currently inadequate. There are no real guidelines as to what constitutes a healthy soil. Although the concept has been well discussed in the context of agricultural soils, different indices are probably required for the environmental constraints associated with brownfield land remediation and the creation of new soils. Implicit to restored soil health is the existence of indicators or monitors of biodiversity, soil sustainability and acceptable risk management. This paper considers the relevant descriptors of soil health in the search for well-defined and practicable measures of the functional integrity of soils that can be used for management of brownfield sites undergoing restoration to soft end-uses. A current project in Liverpool aims to provide a toolbox of robust descriptors of soil health for practitioners.

Dickinson, N.M. et al. (2005) Robust biological descriptors of soil health for use in reclamation of brownfield land. **Land Contamination and Reclamation**, **13**, 317-326

The assumption of the work was that the developed toolbox would be used in the future as part of Site Investigations. It was envisaged that the toolbox will be used subsequent to, and in combination with, analysis and evaluation of physico-chemical parameters of soil, following current best practice. The objectives were to support current site investigations by providing information about soil quality using evidence from a combination of plant growth trials, invertebrate, microbial and functional process assays (Figure 1).

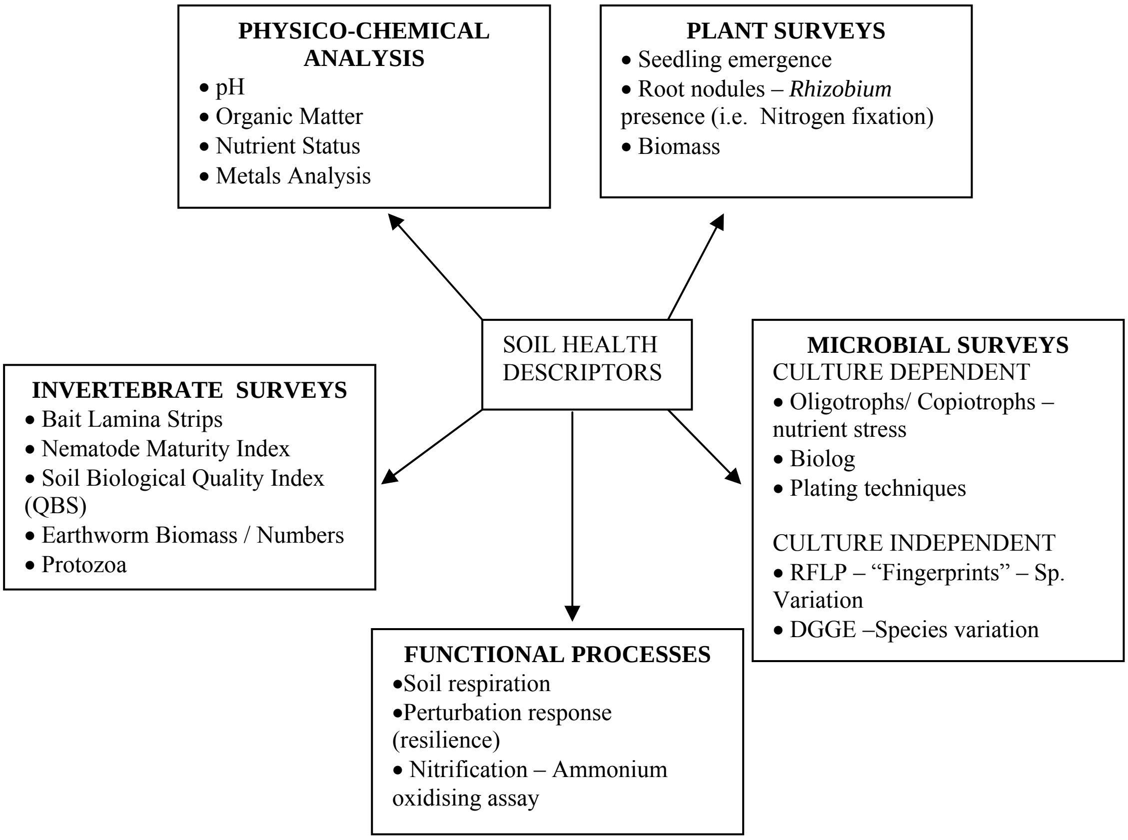


Figure 1. Examples of indicators that may be useful to assess the health of a soil.

2.0 Methodology

An extensive literature review was carried out to identify potentially practicable assays that could contribute to the toolbox. The resulting shortlist of assays was evaluated at five previously-studied brownfield sites.

2.1 Brownfield sites used to develop and test the toolbox

Soils from five sites were selected initially to develop the toolbox and protocols. Two of these sites already formed part of the Mersey Forest Brownfield Project: (i) Merton Bank (a former old-style sanitary landfill site in St. Helens used for chemical and alkali waste, closed in the 1970s, now managed as amenity grassland for public open space) and (ii) Cromdale Grove (also a former landfill site in St. Helens now used as amenity grassland). These sites were selected due to particular contamination issues and stress factors known to affect the sites (*see*: French, C.J., Dickinson, N.M. and Putwain, P.D. (2006) *Woody biomass phytoremediation of contaminated brownfield land*. Environmental Pollution, 141 (3), 387-389.

To provide good contrast, the other sites consisted of (iii) a peri-urban abandoned agricultural land site (New Pale Farm) and (iv) a productive brownfield woodland (Childwall Fields). The agricultural site had been heavily compacted by machinery and was of interest to Landlife National Wildflower Centre in Liverpool (http://www.landlife.org.uk) as a seed orchard. Childwall Fields (Site v) supports productive woodland and diverse ground flora and represents a well-developed healthy soil on brownfield land (Rawlinson, H. *et al*, 2004, *Woodland establishment on closed old-style landfill sites in N.W. England*. Forest Ecology and Management, 202, 265-280. Dickinson, N.M., *et al*, 2005, *Community forestry on old landfill sites.* Quarterly Journal of Forestry, 99, 263-270).

2.2 Assays for toolbox development

Selection of appropriate assays was predominantly based on the key features required for an effective bio-indicator. For inclusion in the toolbox, the assay required:

- Sensitivity to changes in pollution / disturbance / compaction etc.
- Correlation with practical soil functions
- Useful for clarifying ecosystem processes
- Identification is comprehensible to practitioners
- Low cost

Furthermore, a combination of assays was required in the toolbox that represented wide-ranging attributes associated with the perception of a healthy soil. Examples of the assays that were considered for inclusion in the toolbox, outline methodology and relative merits are shown in Table 1.

Technique	Method	Pros	Cons
CO_2 respiration	Respirometer- measures CO_2 evolution	1. Effective equipment available. Well studied	1. Not just microbial respiration, also invertebrates and plants 2. Costly equipment would be Required
Perturbation response	Any tools can be used following stressing of the soil	1. Potentially a highly meaningful assay	1. Time consuming
Seedling emergence & *Rhizobium* presence ISO 11269-2 Corncockle, Cornflower, Red clover, Ox eye daisy, Cornmarigold, Birds foot trefoil, Perennial rye grass	Native species, produces root nodules in association with *Rhizobium*. Grow 90 seeds in each soil type, count emergence and leave for 3 weeks then count root nodules- cut open to see if N fixing	1. Cheap 10 g seeds is £2 2. Easy	1. Requires glasshouse facility and maintenance during the trial
Measurement of inhibition of root growth ISO 11269-1	Growth of pre-germinated seeds under controlled conditions. Differences in the root lengths of seedlings grown in any test medium compared to controls are indicative of an effect.	1. Cheap 2. Easy	
Bait lamina strips	Cotton strips include bait suitable for invertebrates. Place in the ground and leave them for 1 week and count the number of piercings. The results indicate the amount of invertebrates in the soil.	1. Relatively cheap £100-200 for 100 2. Easy	1. Not extensively reported in the literature
Biological Index of Soil Quality (QBS)	Appoints scores (1-20) to soil arthropods according to their adaptations to the soil environment (Ecomorphological Index), QBS sums up these scores and thus characterises the study soil. Uses Tullgren funnels.	1. Cheap	1. Does not show why arthropods are not present or whether stressed 2. Identification may be time Consuming
Pitfall traps		1. Cheap	
Earthworm life history strategy	Separate out into the three feeding guilds	1. Gives indication as to what other organisms are in the soil i.e. if fungal feeding worms present, then fungus is present.	
Earthworm number and biomass	Count, wash and weigh	1. Cheap 2. Easy	
Nematode numbers and feeding guilds	Identify to feeding guilds, in order to give an indication of the amount of bacteria etc. present in the soil	1. Cheap 2. Fairly straightforward	1. Identification may be time consuming
Oribatid mite species	Extract with Tullgren funnels	1. Cheap	1. Identification is very hard 2. May be time consuming

Plate counts	Traditional method. Extract microbes from soil and spread them on agar, leave to incubate for several days.	1. Cheap 2. Fast 3. Easy	1. Biased towards fast growing species which produce lot of spores 2. Media selection 3. Colony spreading 4. Colony/colony inhibition 5. Growth conditions- temp., pH, light 6. Only detect 1-2 % community, that is dominant 7. Spores may become active on plates, that are not active in soil
Most Probable Number (MPN)	Uses substrates to test microbial degradation rates. Community level physiological profile	1. Choose own substrates 2. Fairly easy	1. Time required to decide on substrates 2. Equipment to analyse plates
Fungal slide technique	Traditional microbial assay	1. Cheap and easy	1. Uncertain reliability
Dehydrogenase enzyme	Uses substrates to test microbial degradation rates.	1. Easy	1. Equipment to analyse solution
Phosphomonoesterase enzyme	Uses substrates to test microbial degradation rates.	1. Easy	1. Equipment to analyse solution
Microbial carbon	Uses chloroform to destroy microbial cells, then measure the amount of carbon produced	1.Easy	1. Equipment to analyse solution
Adenosine TriPhosphate (ATP)	Microbial ATP from lysed cells	1. Simple and Easy	1. Specialist equipment required
Polymerase Chain Reaction (PCR)	State of the art / High Tech Analysis	1. Advanced technique for biodiversity	1. Requires high level skills
Protozoan identification	ID into the 4 major functional groups (flagellates, naked amoebae, testate amoebae, ciliates) or family	1. Easy to extract from soil 2. Cheap	1. Time consuming and difficult to identify

Table 1. Full list of assays short-listed for toolbox trialling

2.3 Sampling protocols

Site sampling followed guidelines set out in ISO 10381-1 and –2. Sampling was conducted using a non-systematic pattern (Figures 2 and 3), which was implemented in the central 50% of a site in order to introduce a buffer zone separating composted and non-composted areas of land, sampling information was also recorded (Table 2).

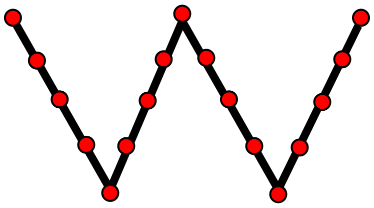


Figure 2. Non-systematic sampling pattern, where red dots indicate sampling points, the distance between sampling points is equal.

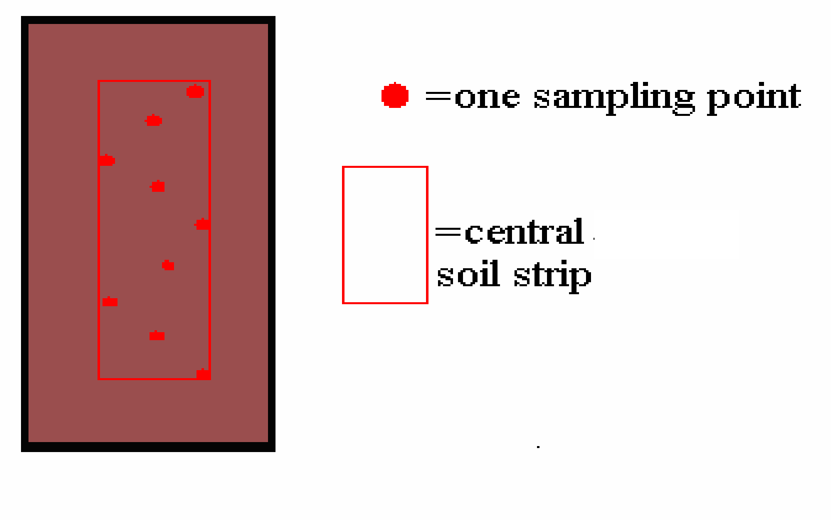


Figure 3. Example of sampling site where the central strip was used to conduct the non-systematic sampling.

Site:
Sampling dates:
Samplers:
Sampling tools:
Disturbed/undisturbed sample:
Depth of sample:
Volume of sample:
Single or composite sample (sample locations if composite):
Sample moisture status when collected:
Sample containers used:
Sample time and conditions of transport:
Sample time and conditions of storage:

Table 2. Information recorded at each site during the January 2006 sampling period.

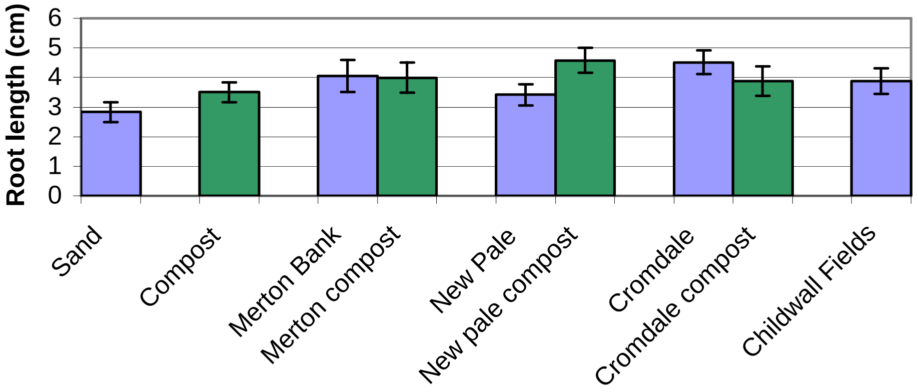
3.0 Results

This section contains the final assays selected for the toolbox, and provides a summary of the main findings for each assay. In most cases, data refer to site differences, sometimes with and without compost. The outcome of each assay (methodology and descriptors) is shown in template format in Appendix B.

3.1 Plant root length and biomass

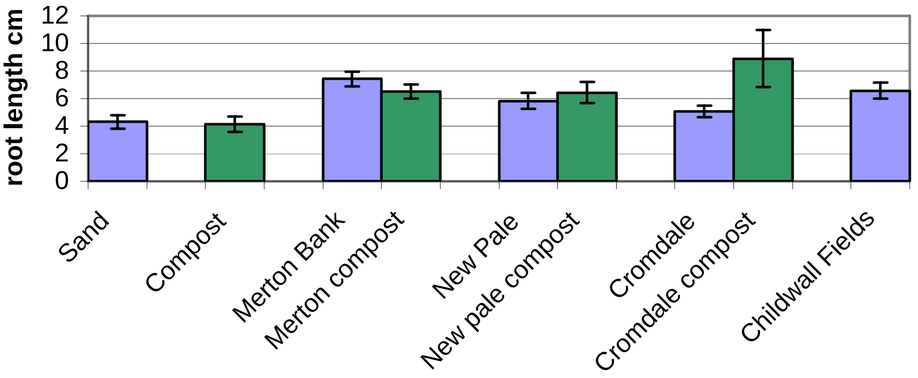
Compared to other species tested, Red Clover and Corncockle provided the most straightforward and readily measurable differences between treatments. Simple measures of root length provided a sufficiently reliable predictor of performance (Figures 6 and 7).

Figure 4. Mean root length (cm) of Red Clover planted in soil from experimental sites, with ± standard errors



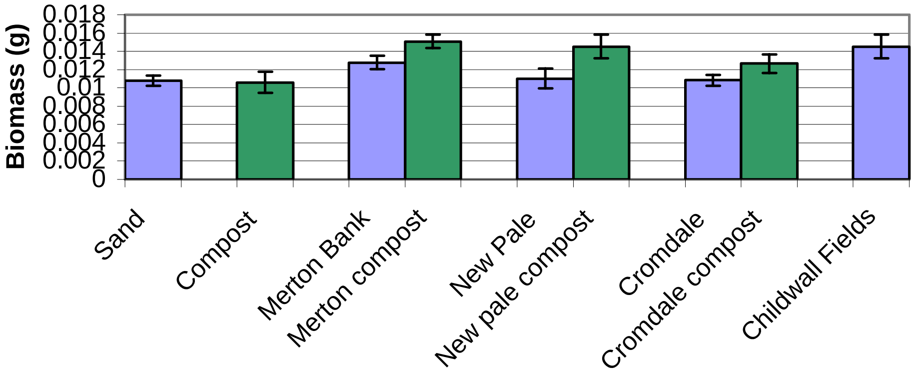
Red clover root length was greater in all the soils tested compared to the controls of sand and compost. New Pale soil beneath compost had longer Red clover roots compared to New Pale soil.

Figure 5. Mean root length (cm) of Corncockle planted in soil from experimental sites, with ± standard errors



Corncockle root length was greater in all the soils tested compared to the controls of sand and compost. Cromdale beneath compost had longer Corncockle roots compared to Cromdale soil.

Figure 6. Mean plant dry biomass (g) of Corncockle planted in soil from experimental sites, with ± standard errors

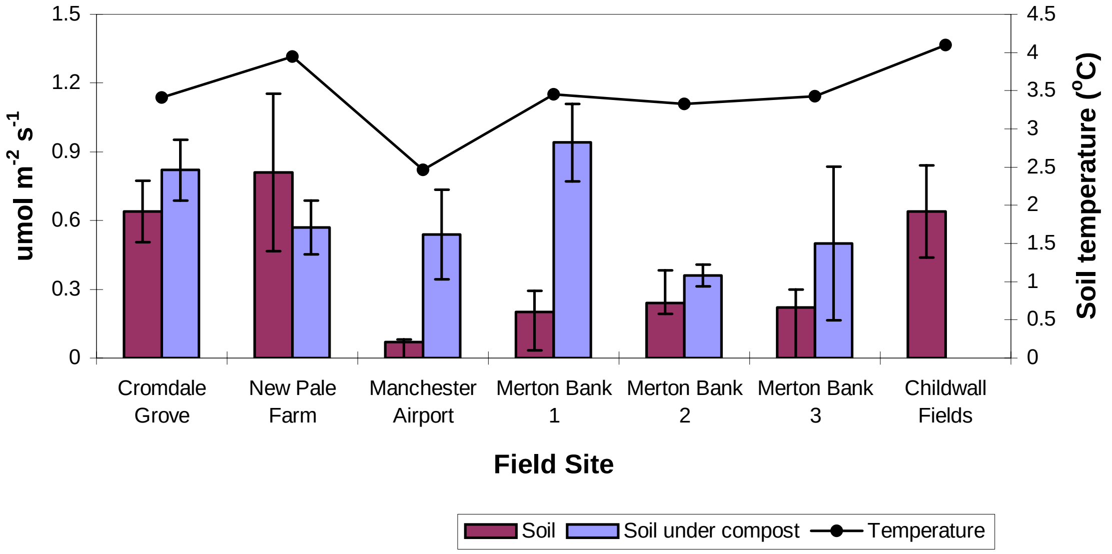


Corncockle dry biomass was greater from soil beneath the mulched compost than the soil without compost at all sites, this may be because the soil beneath the compost has a higher nutrient component due to leaching from the compost.

3.2 Basal soil respiration rate (Field Investigation)

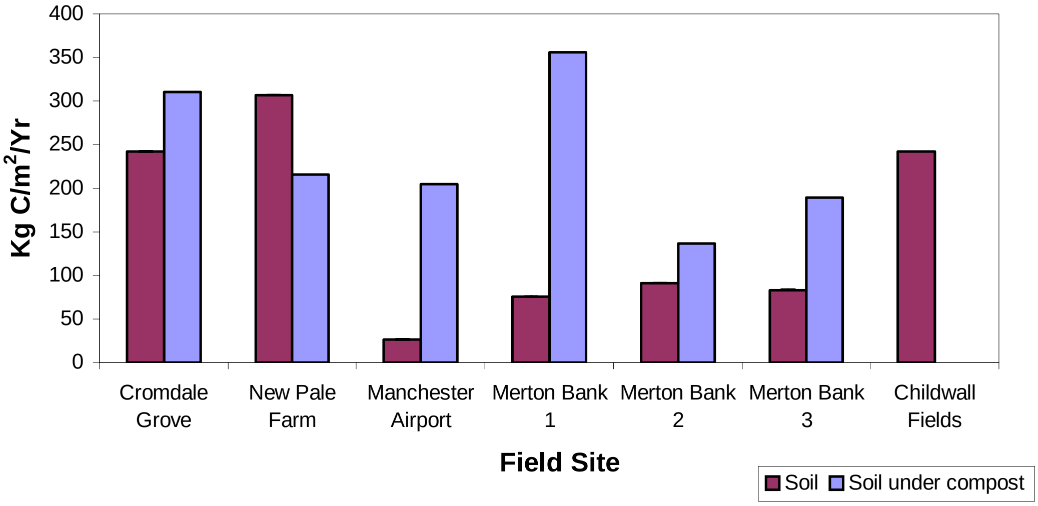
Detailed attention was given to measurement of soil respiration, which is one of the most obvious descriptors of biological functional activity in soil. Whilst primarily measuring microbial respiration, this is also a measure of plant root metabolism and is known to be strongly influenced by temperature and moisture status of the soil. Further detailed analysis of soil respiration was undertaken as an undergraduate honours project (L. Beesley, Appendix 2).

Figure 7. Net CO_2 Exchange Rate (NCER).



Carbon dioxide production was in all cases higher from soil under compost than soil alone. However it must be noted that the action of removing compost from the soil surface may have disturbed the microbes therefore causing them to respire more than usual. Respiration activity from soil without compost addition was greatest at Cromdale Grove and Childwall Fields, whilst Manchester Airport showed the lowest activity. The reduced microbial activity may be attributed to the lower soil temperature on that particular test day.

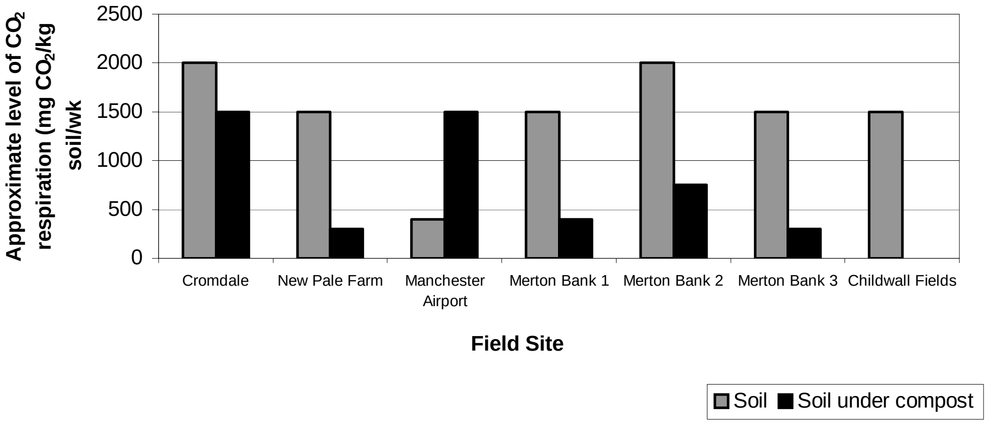
Figure 8. Estimated mean carbon production (kg) from composted and non-composted soils for a twelve month period.



With the exception of New Pale Farm, composted areas showed an estimated higher carbon production than non-composted soils over a twelve-month period.
At Childwall Fields (reference site) and New Pale Farm carbon production was greater than Merton Bank or Manchester airport sites for soil only.

3.3 Basal Soil Respiration Rate (Laboratory investigation)

Figure 9. Solvita Soil Respiration Laboratory Test based on the "soil quality curve" from gel indicators.

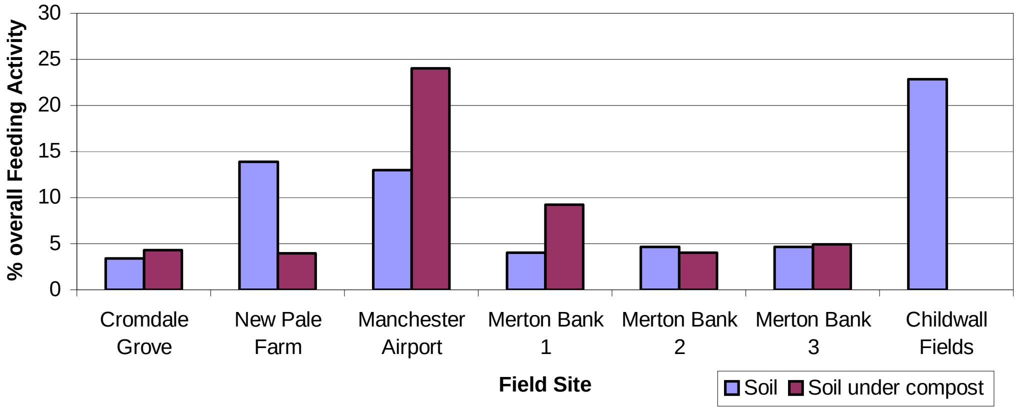


With the exception of Manchester airport, the laboratory soil respiration test showed that where compost had been applied to the soils, respiration rate was reduced considerably compared to non-composted areas. Manchester airport showed a higher respiration rate than non-composted soil, which may be a result of the compost being incorporated at this site.

3.4 Bait lamina strips

Bait lamina strips provide a measure of overall feeding activity in soil, without taking account of whether this is due to invertebrate or microbial activity. Manufactured strips contain a carbon and cellulose substrate and are inserted vertically into the soil for 7 days, after which the number of pierced baits in the strip is determined.

Figure 10. Variation in overall feeding activity (%) at field sites with and without compost application determined using bait lamina strips



Greatest overall feeding activity (OFA) was observed at Manchester airport were compost had been mixed with soil. Childwall Fields also showed a high level of feeding activity in the soil compared to the remaining sites. With the exception of plot 1 at Merton Bank, were feeding activity increased, compost had no positive effect at plots 2 and 3. New Pale Farm OFA decreased considerably in soil under compost. At Cromdale Grove there was a slight increase in OFA in soil under compost.

Figure 11. Mean feeding activity obtained at Cromdale Grove composted (n=4) and uncomposted (n=6) areas ± s.e.

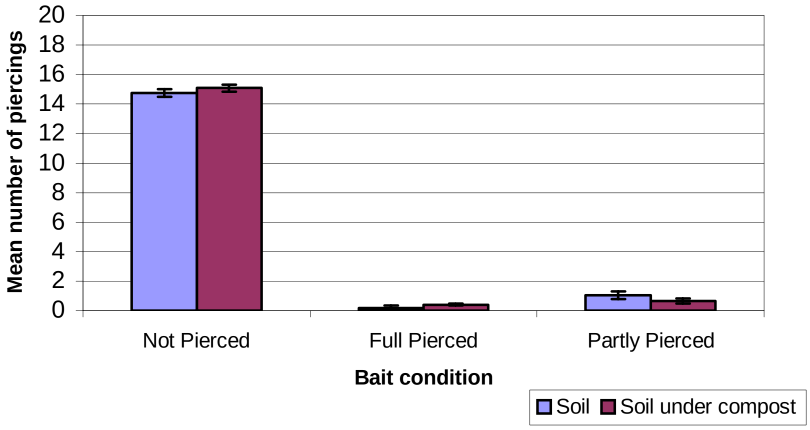


Figure 12. Mean feeding activity at New Pale Farm composted (n=4) and uncomposted (n=4) areas with ± standard errors.

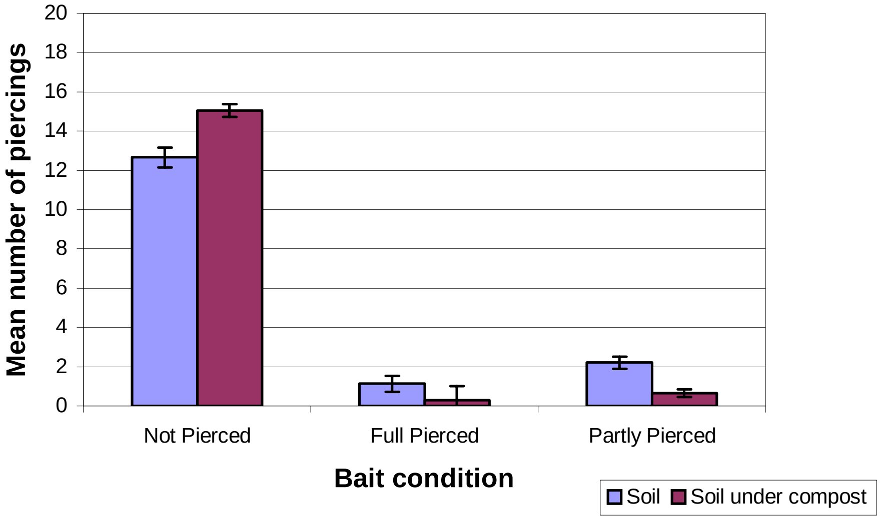


Figure 13. Mean feeding activity at Manchester Airport composted (n=3) and uncomposted (n=3) areas ± s.e.

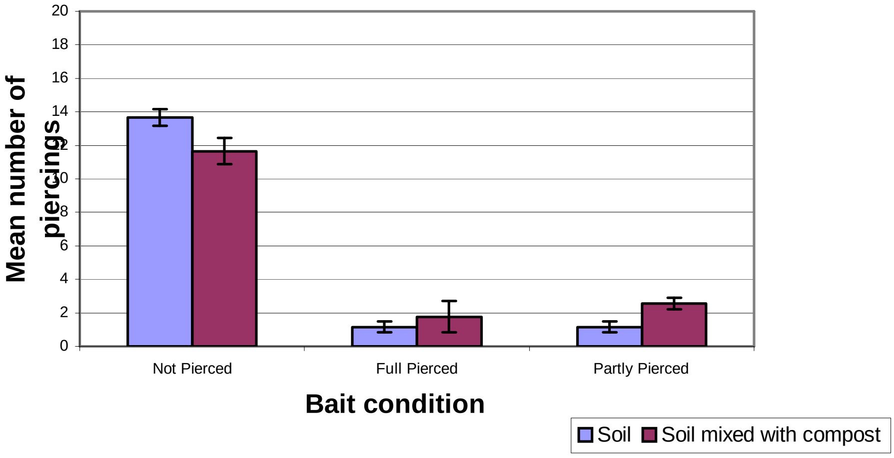


Figure 14. Mean feeding activity at Merton Bank 1 composted (n=3) and uncomposted (n=3) areas ± standard errors.

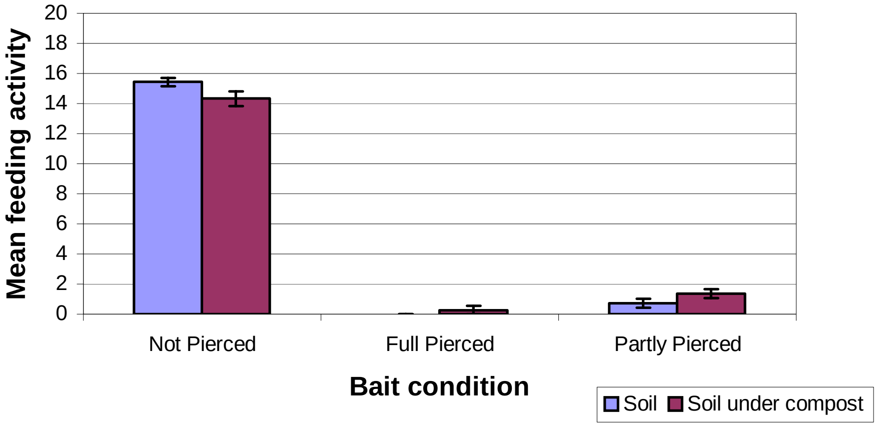


Figure 15. Mean feeding activity at Merton Bank 2 composted (n=3) and uncomposted (n=3) areas ± standard errors.

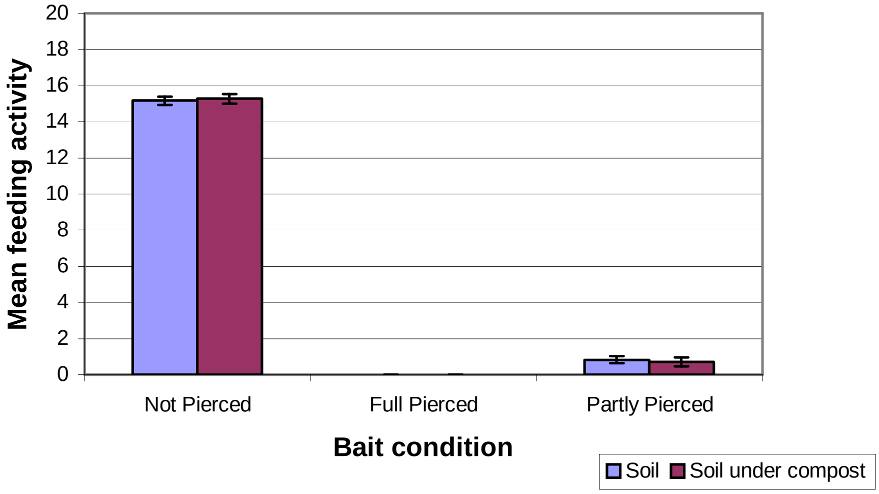


Figure 16. Mean feeding activity at Merton Bank 3 composted (n=3) and uncomposted (n=3) areas ± standard errors.

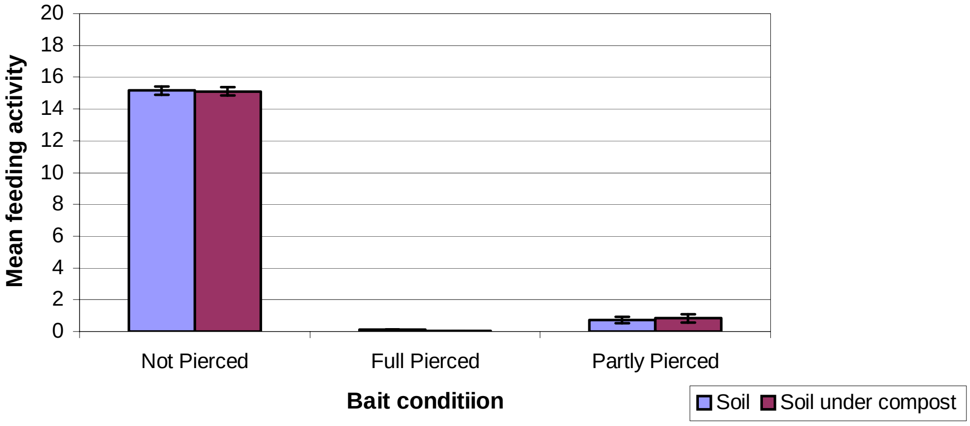
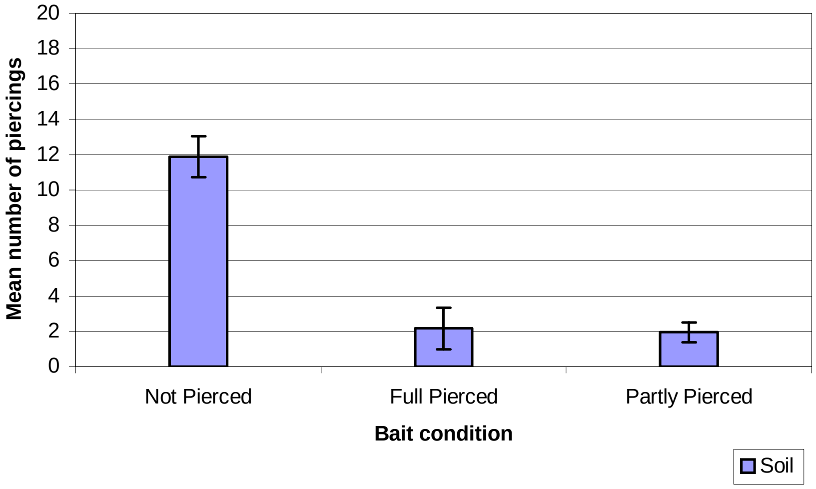


Figure 17. Mean feeding activity at Childwall Fields (n=3) ± standard errors.

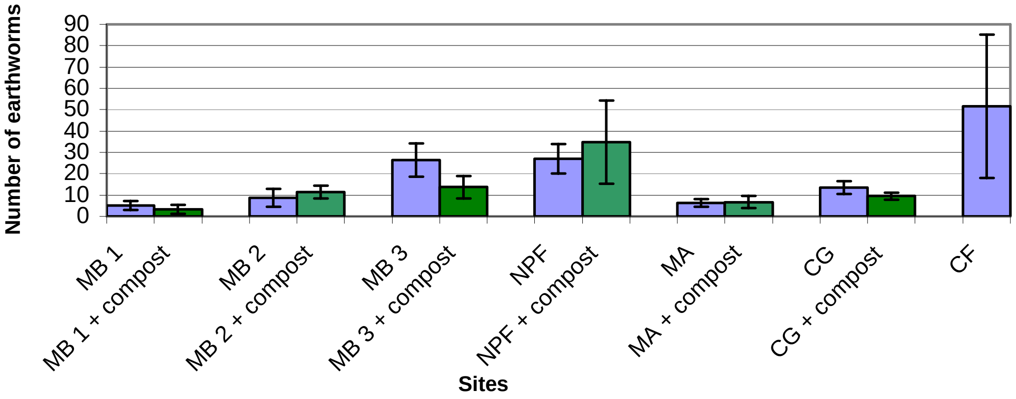


For all test soils, feeding activity was generally very low, with Childwall Fields having the highest mean number of piercings overall. At Manchester airport mean number of pierced baits were higher from soil mixed with compost than soil only. At New Pale Farm the reverse occurred, in that soil under compost showed a reduced mean number of pierced baits compared to soil only.

3.5 Earthworms (Numbers, Feeding Guilds and Biomass)

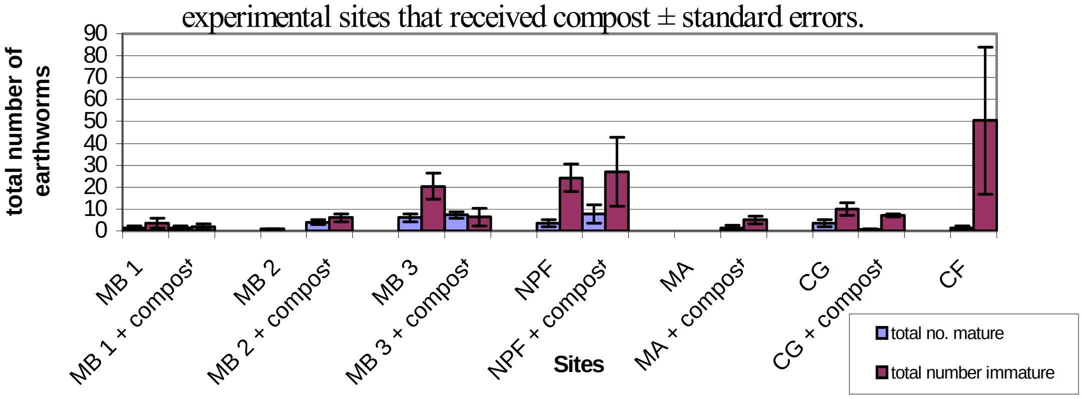
Earthworms are the most visible of the soil fauna and being relatively straightforward to sample they are amongst the more obvious biological indicators to be included in the toolbox. Accurate identification to species requires specialist knowledge, and attention was focussed on quantitative assessment of numbers, biomass and broad ecological groupings.

Figure 18. Mean number of earthworms from experimental sites that received compost, ± standard errors.



Overall, the number of earthworms at all sites was low compared to previous summer sampling, however once again Childwall Fields as the reference site, New Pale Farm and Merton Bank site 3 all have high numbers of worms present. The addition of compost to sites has had no significant effect on the number of earthworms present.

Figure 19. Mean number of mature and immature earthworms from experimental sites that received compost ± standard errors.



At Merton Bank 3, New Pale Farm, Manchester Airport, Cromdale Grove and Childwall Fields there were higher numbers of immature than mature earthworms.

Figure 20. Mean number of anecic earthworms from experimental sites that received compost, ± standard errors.

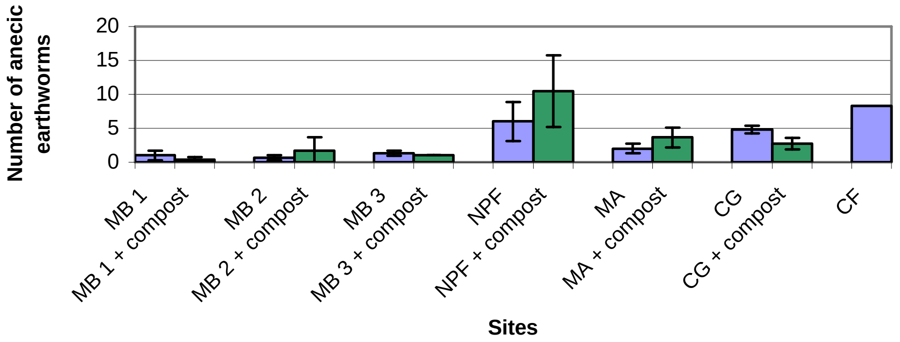


Figure 21. Mean number of endogeic earthworms at experimental sites that received compost, ± standard errors.

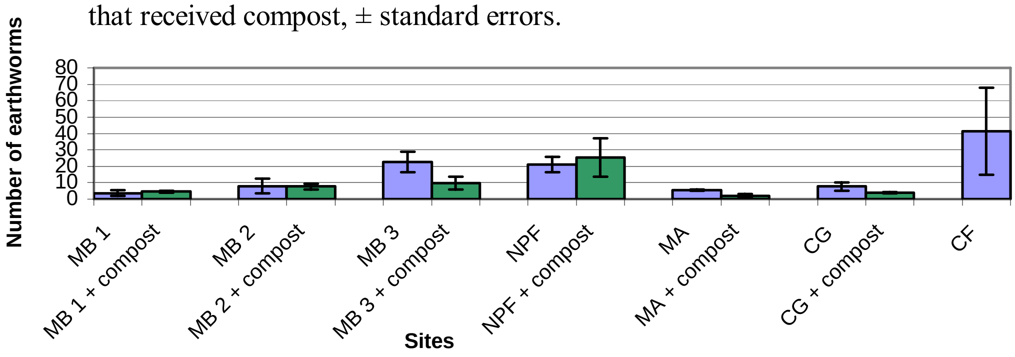


Figure 22. Mean number of epigeic earthworms from experimental sites that received compost, ± standard errors.

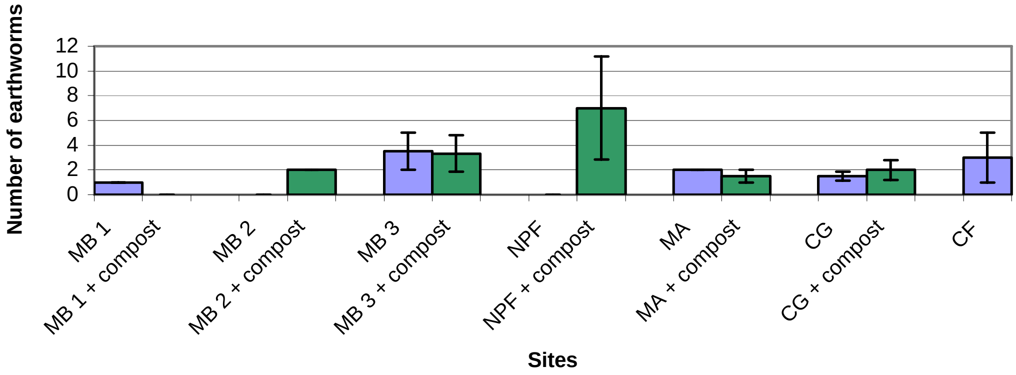
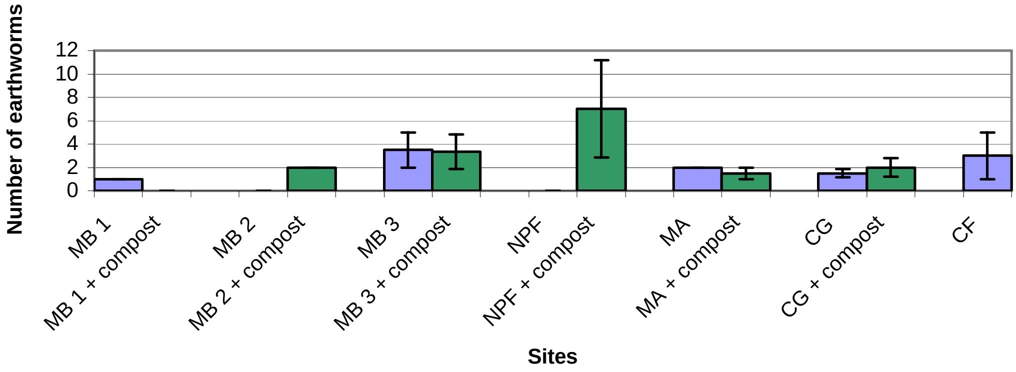


Figure 23. Mean number of epigeic earthworms at sites that received compost, ± standard errors.



The composted areas of New Pale Farm had higher numbers of both anecic and epigeic earthworms compared to the uncomposted areas. This may be because the compost acts as a soil insulator and prevents the topsoil from being frozen over the winter.; therefore the earthworms have a higher chance of survival if they remain in the soil beneath the compost layer. At all of the experimental sites there were greater numbers of endogeic than anecic and epigeic earthworms.

Figure 24. Mean wet weight of an anecic earthworm from experimental sites that received compost ± standard errors.

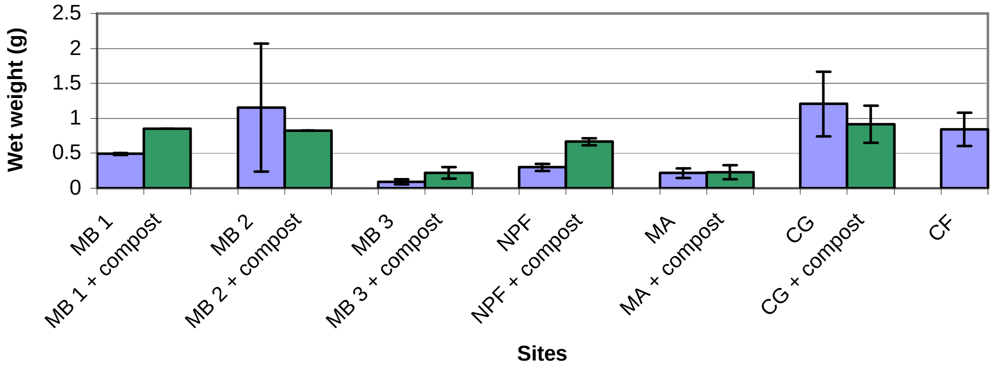


Figure 25. Mean wet weight of an endogeic worm at experimental sites that received compost, ± standard errors.

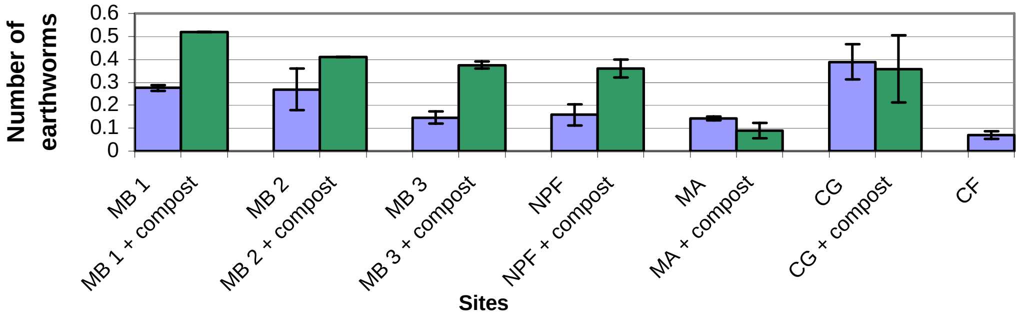
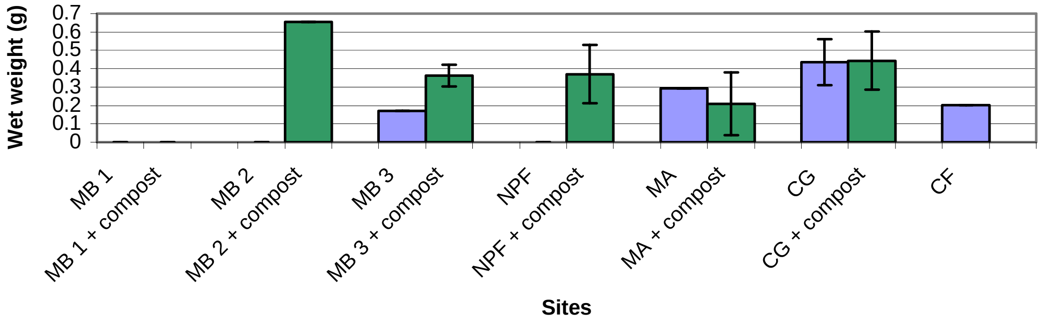


Figure 26. Mean wet weight of an epigeic earthworm at experimental sites that received compost, ± standard errors.



For the majority of sites the biomass of the earthworms was greater in the soil beneath the compost, this may be because there is a higher amount of their food source present.

Detailed site sampling of earthworms was carried out as part of an undergraduate honours project (J. Dodd, Appendix 2).

3.5.1 Effects of trial compost, sand and paper crumb mixtures

In the present section, the effects of compost, sand and paper crumb mixtures on earthworms is shown.

Figure 27. Mean biomass per earthworm at 0, 4, 8 and 12 weeks, ± standard errors

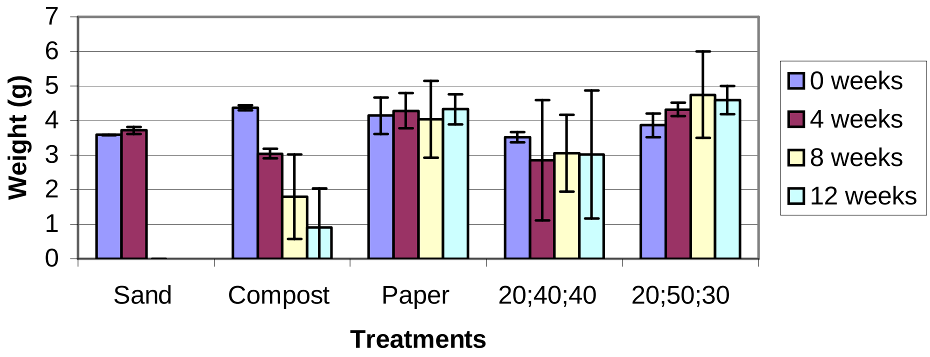
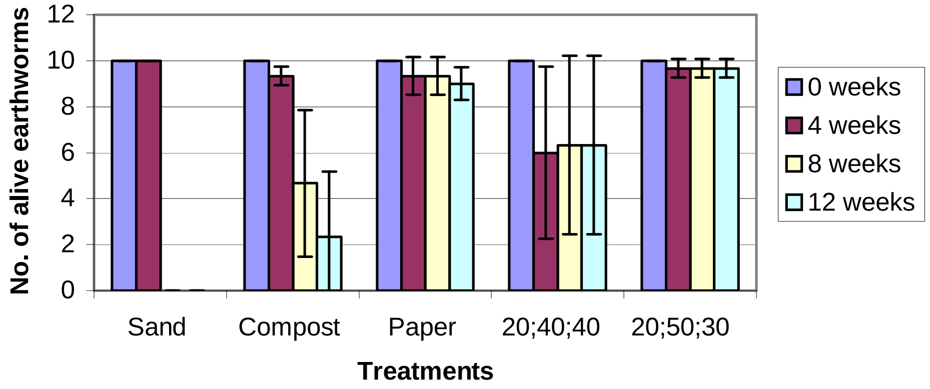


Figure 28. Mean number of alive earthworms at 0, 4, 8 and 12 weeks, ± standard errors

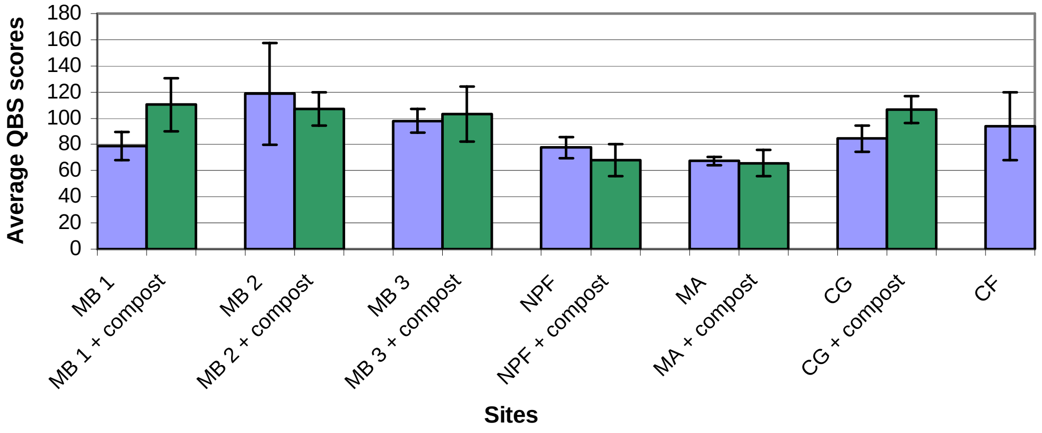


Earthworms in both sand and compost treatments had a significant reduction in both biomass and numbers over 12 weeks. Whereas, earthworms in paper crumb and both mixtures maintained both biomass and numbers over the 12 weeks.

3.6 Simplified QBS Technique

Carrying out quantititative analysis of soil invertebrate communities is restricted by difficulties with identification in the absence of advanced taxonomic skills. A lengthy learning process is not practicable for likely users of the toolbox. The emphasis of the present work was to minimize time spent at the microscope and to identify a practicable and meaningful assay. The QBS technique was considered to be the most likely current method that may serve this purpose.

Figure 29. Mean simplified QBS scores from experimental sites that received compost, ± standard errors.



The simplified QBS scoring systems uses simple invertebrate identification and cumulative scoring, the higher the score the 'healthier' the site is considered to be. Overall, New Pale Farm and Manchester Airport scored lower than the other sites, possibly due to poor soil structure from compaction and as a result waterlogging. The addition of compost as a layer over the soil has had no significant effect at any of the sites.

3.7 Pitfall traps

An assessment of soil fauna living primarily on the soil surface, where biodiversity is recognised to be particularly high, was considered to be a valuable component of the assessment of soil health. Pitfall traps were set up at the field sites and left for one week during the final testing of the toolbox methods in January 2006. After one week, the traps were collected and the contents were identified into the groups identified (Table 5).

	CF	**CG+ comp**	**CG**	**MA+ comp**	**MA**	**MB1 + comp**	**MB1**	**MB2 + comp**	**MB2**	**MB3 + comp**	**MB3**	**NP+ comp**	**NP**
Spiders	**3.67**	**4**	**3.17**	**1.67**	**5.67**	**0.5**	**4**	**3**	**1.5**	**3.5**	**5.5**	**15.25**	**22.25**
	0.41	*0.81*	*0.82*	*1.08*	*2.04*	*0.41*	*2.12*	*1.22*	*0.71*	*1.78*	*2.86*	*1.66*	*6.30*
Harvestman	**0**	**0**	**0.17**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	0	*0*	*0.18*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Earwigs	**0**	**0**	**0.5**	**0**	**0**	**0**	**0.33**	**0.33**	**0**	**0**	**0**	**0**	**0**
	0	*0*	*0.37*	*0*	*0*	*0*	*0.41*	*0.41*	*0*	*0*	*0*	*0*	*0*
Centipedes	**0**	**0**	**0**	**0**	**0**	**0**	**0.33**	**0**	**0**	**0.33**	**0**	**0**	**0**
	0	*0*	*0*	*0*	*0*	*0*	*0.41*	*0*	*0*	*0.41*	*0*	*0*	*0*
Millipedes	**0.33**	**0**	**0.67**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	0.41	*0*	*0.37*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Lepidoptera larvae	**0.33**	**0**	**0**	**0**	**0**	**0**	**0.33**	**0.33**	**0**	**0**	**0.33**	**0.25**	**0**
	0.41	*0*	*0*	*0*	*0*	*0*	*0.41*	*0.41*	*0*	*0*	*0.41*	*0.29*	*0*
Hemiptera	**0**	**0**	**0.5**	**0**	**1.33**	**0**	**0**	**0.33**	**0**	**0**	**0.33**	**0.5**	**0**
	0	*0*	*0.37*	*0*	*1.63*	*0*	*0*	*0.41*	*0*	*0*	*0.41*	*0.33*	*0*
Woodlice	**0**	**0.5**	**1.67**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	0	*0.58*	*0.88*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Coleoptera larvae	**2**	**0.5**	**0.5**	**3.33**	**2.67**	**1.33**	**0.33**	**1.33**	**0**	**1**	**0**	**3.75**	**2.25**
	0.71	*0.58*	*0.24*	*0.82*	*1.78*	*1.63*	*0.41*	*0.82*	*0*	*1.22*	*0*	*1.79*	*1.28*

Weevils	**0**	**0**	**0**	**0.33**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.75**	**0.5**
	0	*0*	*0*	*0.41*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0.55*	*0.33*
Flea Beetles	**0**	**0.75**	**0.33**	**0.67**	**0**	**0**	**0**	**0**	**0**	**0.33**	**0**	**0**	**0.5**
	0	*0.55*	*0.37*	*0.41*	*0*	*0*	*0*	*0*	*0*	*0.41*	*0*	*0*	*0.58*
Scarabaeidae	**0**	**0**	**0**	**0.67**	**1**	**0**	**0.33**	**0**	**0**	**0**	**0**	**0.75**	**0.25**
	0	*0*	*0*	*0.82*	*1*	*0*	*0.41*	*0*	*0*	*0*	*0*	*0.55*	*0.29*
Staphylinidae	**0.33**	**3**	**2.5**	**0.33**	**0**	**0**	**0.67**	**0.33**	**0**	**0.67**	**0**	**0.75**	**1**
	0.41	*1.05*	*0.62*	*0.41*	*0*	*0*	*0.41*	*0.41*	*0*	*0.82*	*0*	*0.55*	*0.47*
Ground Beetles	**0.33**	**0.5**	**0.83**	**0**	**0.33**	**0**	**0.67**	**0.33**	**0**	**0**	**0**	**1**	**0**
	0.41	*0.33*	*0.18*	*0*	*0.41*	*0*	*0.41*	*0.41*	*0*	*0*	*0*	*0.66*	*0*
Coleoptera (Others)	**0**	**0**	**0.33**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.5**
	0	*0*	*0.37*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0.58*

Table 5. Mean numbers (bold) and standard error (italics) of arthropod groups found at the study sites

	CF	**CG+ comp**	**CG**	**MA+ comp**	**MA**	**MB1 + comp**	**MB1**	**MB2 + comp**	**MB2**	**MB3 + comp**	**MB3**	**NP+ comp**	**NP**
Spiders	3.67	4	3.17	1.67	5.67	0.5	4	3	1.5	3.5	5.5	15.25	22.25
Harvestman	0	0	0.17	0	0	0	0	0	0	0	0	0	0
Centipedes	0	0	0	0	0	0	0.33	0	0	0.33	0	0	0
Staphylinidae	0.33	3	2.5	0.33	0	0	0.67	0.33	0	0.67	0	0.75	1
Ground Beetles	0.33	0.5	0.83	0	0.33	0	0.67	0.33	0	0	0	1	0
Number of groups represented	**3**	**3**	**4**	**2**	**2**	**1**	**4**	**3**	**1**	**3**	**1**	**3**	**2**

Table 6. Mean numbers of the main predator groups: Spiders (order: Araneae), Harvestmen (order: Opiliones), Centipedes (class: Chilopoda), Rove Beetles (family: Staphylinidae) and Ground Beetles (family: Carabidae)

The presence of the main predator groups indicates that their prey is also present and this suggests a greater species richness of soil arthropods. Generally the numbers of arthropods collected from pitfall traps at sites in January 2006 was much lower than during sampling in September 2005. This is probably due to seasonal variations and different life-cycle stages of the groups studied. The table above suggests that the areas at Cromdale Grove and Merton Bank 1 where compost had not been added were able to support a wider range of soil predators and both areas of New Pale Farm were able to support higher numbers of predators.

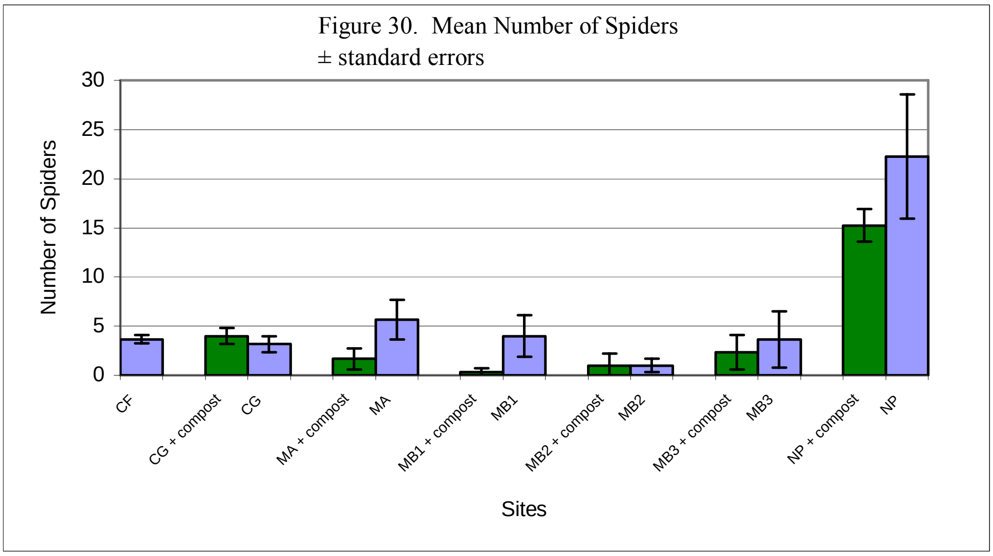


Figure 30. Mean Number of Spiders ± standard errors

The numbers of spiders were generally low at all sites except New Pale Farm where numbers were significantly higher on both the composted and uncomposted areas. The addition of compost at sites had no significant effect on the number or spiders present.

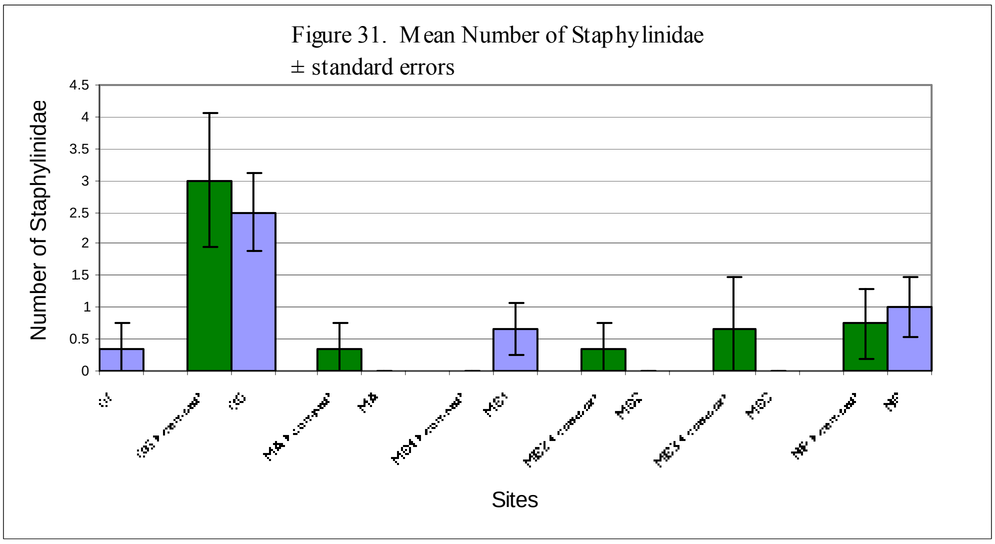
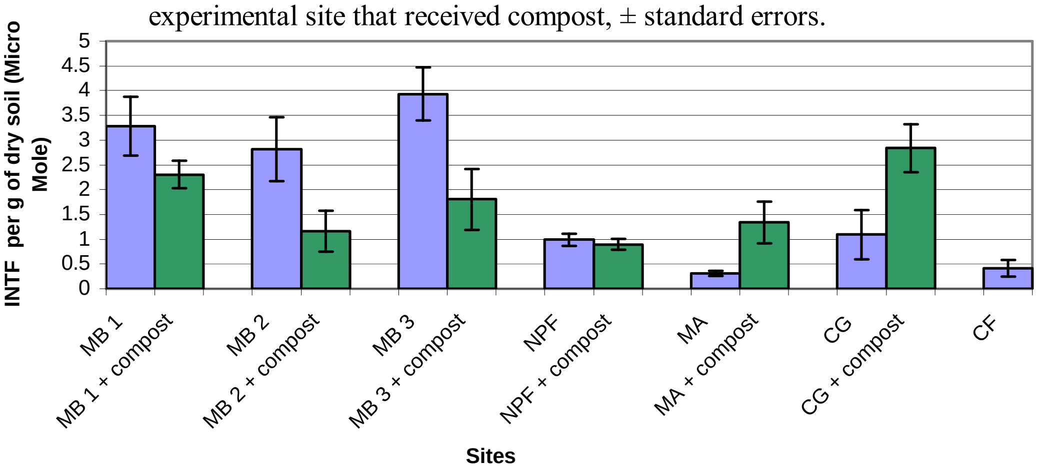


Figure 31. Mean Number of Staphylinidae ± standard errors

Higher numbers of rove beetles were found at Cromdale Grove than at the other sites where numbers were low or rove beetles were not found. No significant differences were observed between composted and uncomposted sites.

3.8 Microbial Dehydrogenase Enzyme Assay

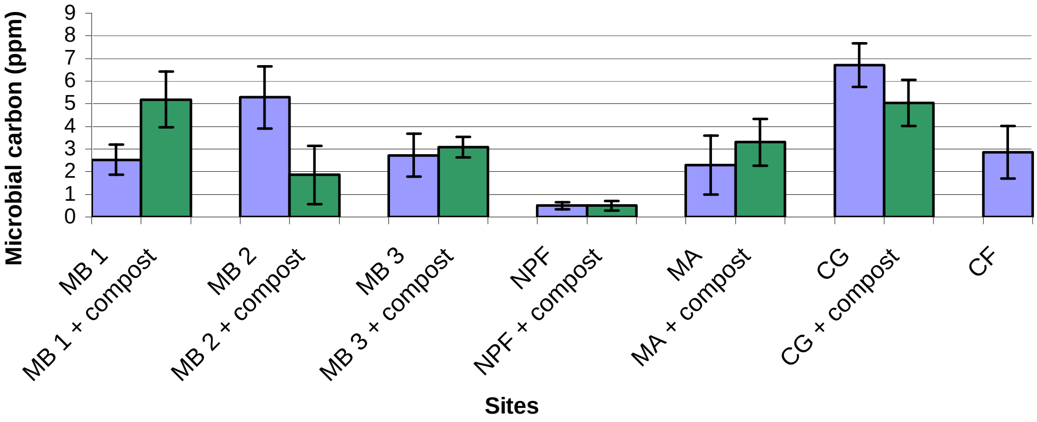
Figure 32. Mean micromoles of INTF per gram of dry soil from experimental site that received compost, ± standard errors.



Microbial dehydrogenase enzyme indirectly identifies the size of the active microbial population in a soil sample. Overall New Pale Farm, Manchester Airport and Childwall Fields all had lower activity than Merton Bank and Cromdale Grove, this may have been due to the soils being compacted, having a poor structure or being waterlogged. The addition of compost as a layer on top of the soil decreased the amount of microbial activity at Merton Bank, possibly due to re mobilising arsenic in the soil, and New Pale Farm where there is poor soil structure. Whereas, a layer of compost increased microbial activity in the soil beneath at Cromdale Grove and Manchester Airport where the compost has been incorporated into the topsoil.

3.9 Microbial Carbon

Figure 33. Mean microbial carbon content (ppm) from experimental sites that received compost, ± standard errors.

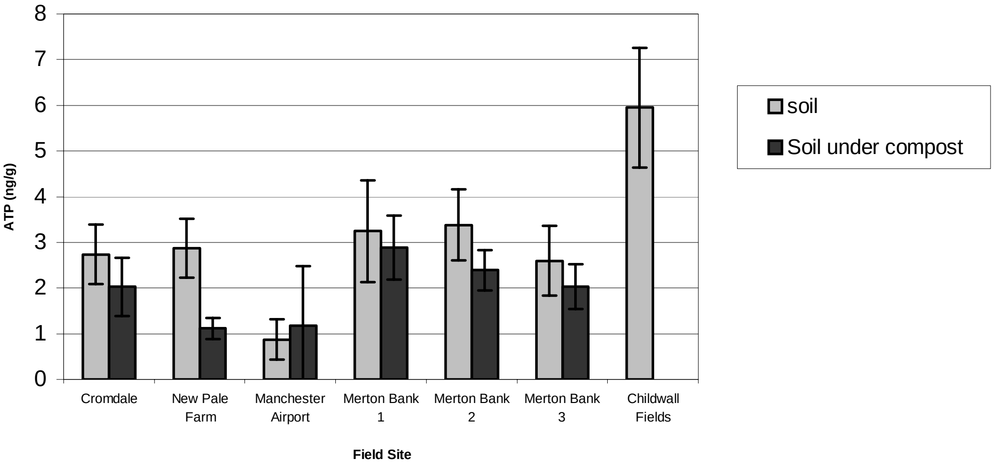


Microbial carbon content provides a broad descriptor that quantifies microbial biomass in the soil and is relatively straightforward to determine. Overall, New Pale Farm had the lowest microbial carbon content, probably due to poor soil structure (compaction), low organic matter content in the soil and low levels of nutrients. The addition of compost as a layer over the soil increased microbial carbon content at Merton Bank site 1, possibly due to an increase in organic matter. However, at Merton Bank site 2 compost decreased microbial carbon content which may be due to the re mobilisation of arsenic.

3.10 Microbial ATP

Luminometers are routinely used in medical laboratories and for food hygiene testing; existing, relatively low-priced and readily available instrumentation provides a potentially rapid technique to measure microbial activity. ATP content is determined by a luciferin-luciferase fluorescence reaction in lysed cells.

Figure 34. Combined 10cm soil core ATP (ng/g) concentrations from composted and uncomposted plots ± standard errors.



There was a reduction in ATP content in soils were compost had been surface spread (mulch). However, where it had been incorporated and mixed into the soil at Manchester airport, ATP increased slightly compared to non-composted areas. Childwall fields showed the highest level of ATP indicating a greater level of microbial activity at this site. Overall Manchester airport soil had the lowest ATP content indicating reduced microbial activity maybe due to the soil being compact and waterlogged therefore inducing anaerobic conditions.

Figure 35. Cromdale Grove ATP concentrations from composted (n=4) and uncomposted (n=6) plots ± s.e.

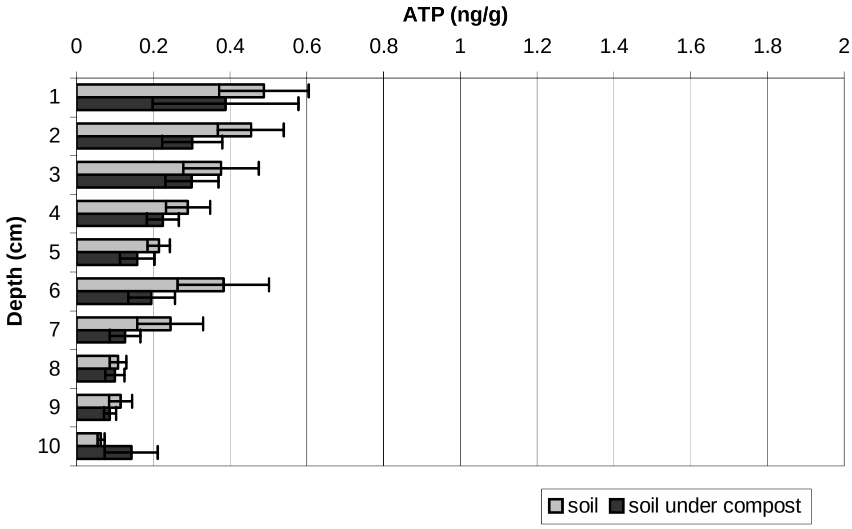


Figure 36. New Pale Farm ATP concentrations from composted (n=4) and uncomposted (n=4) plots ± standard errors.

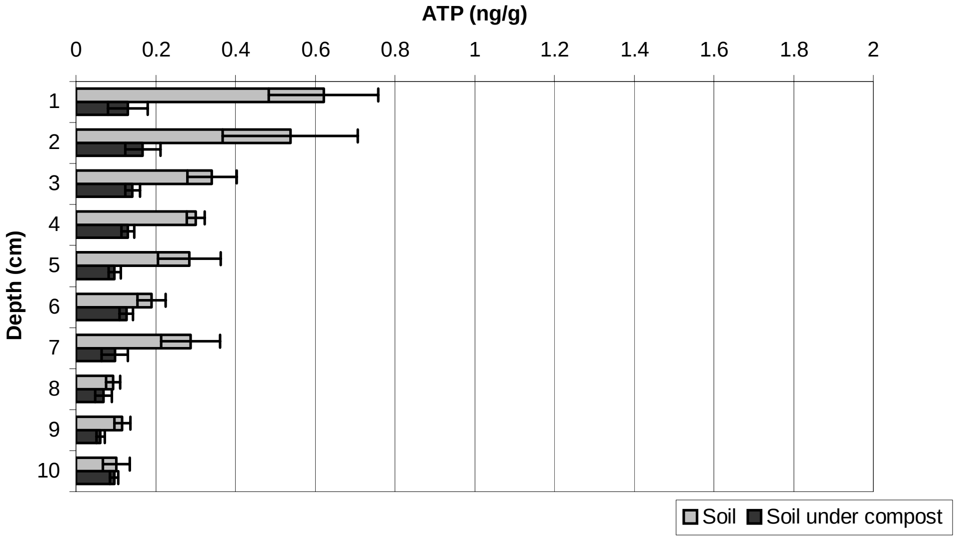


Figure 37. Manchester Airport ATP concentrations from composted (n=3) and uncomposted (n=3) plots, ± standard errors.

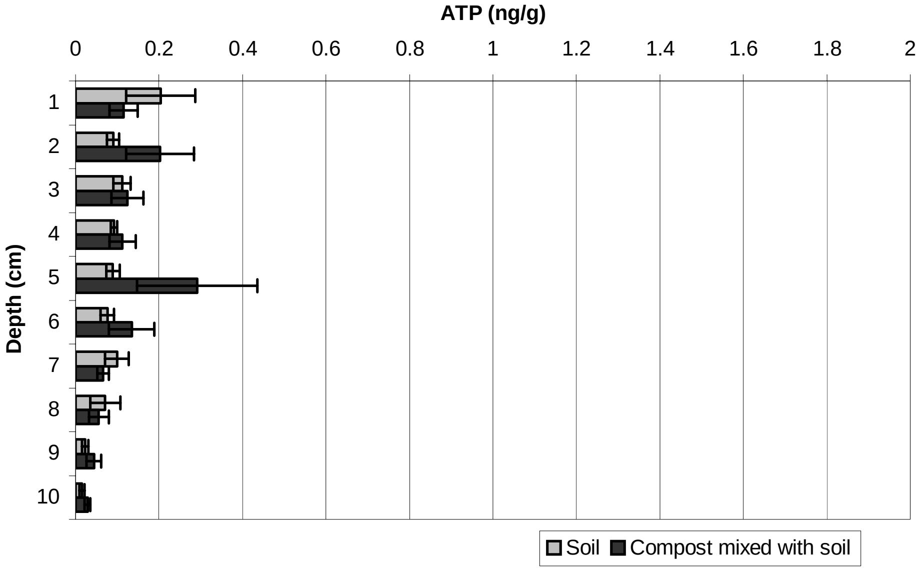


Figure 38. Childwall Fields ATP concentrations from soil plots only (n=3) ± standard errors.

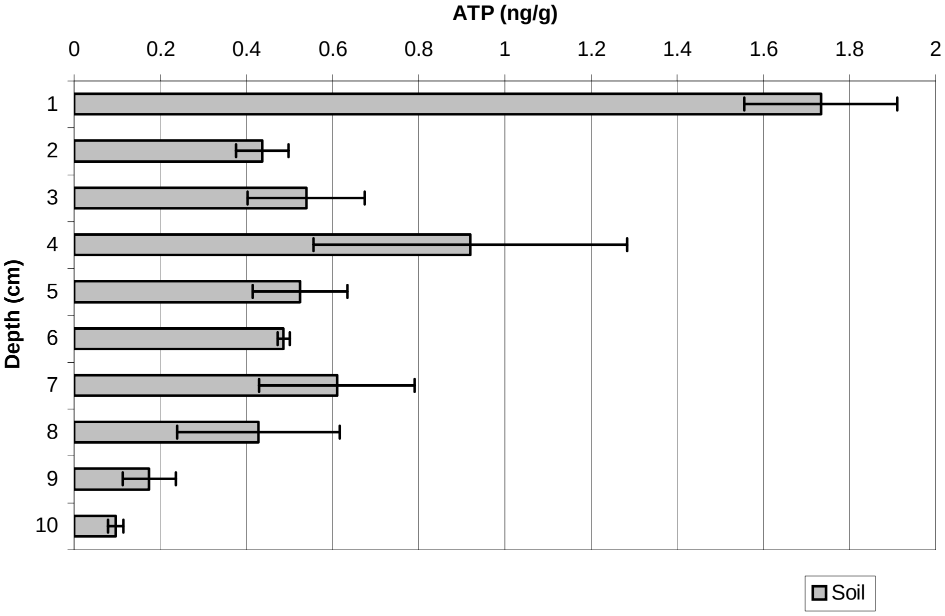


Figure 39. Merton Bank 1 ATP concentrations from composted (n=3) and uncomposted (n=3) plots ± standard errors.

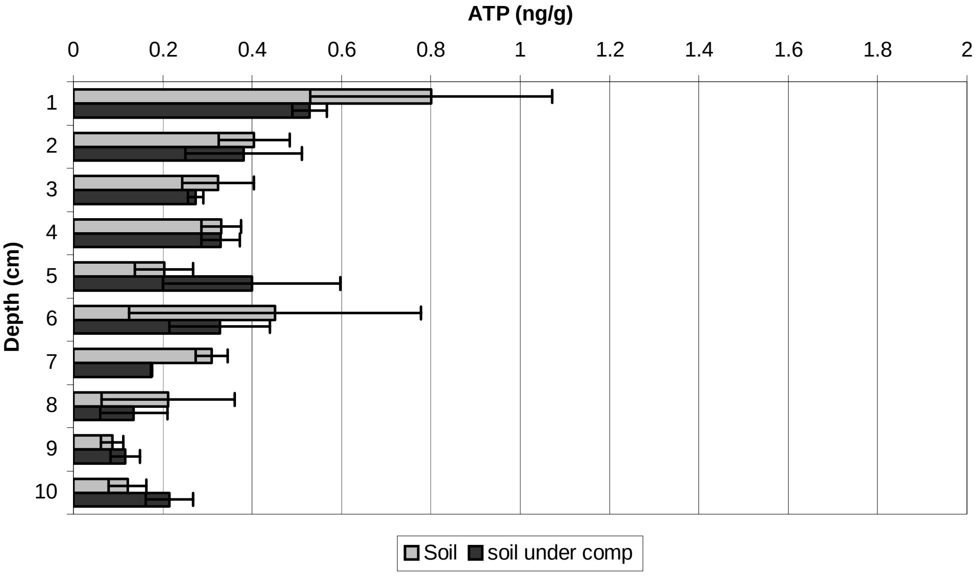


Figure 40. Merton Bank 2 ATP concentrations from composted (n=3) and uncomposted (n=3) plots ± standard errors.

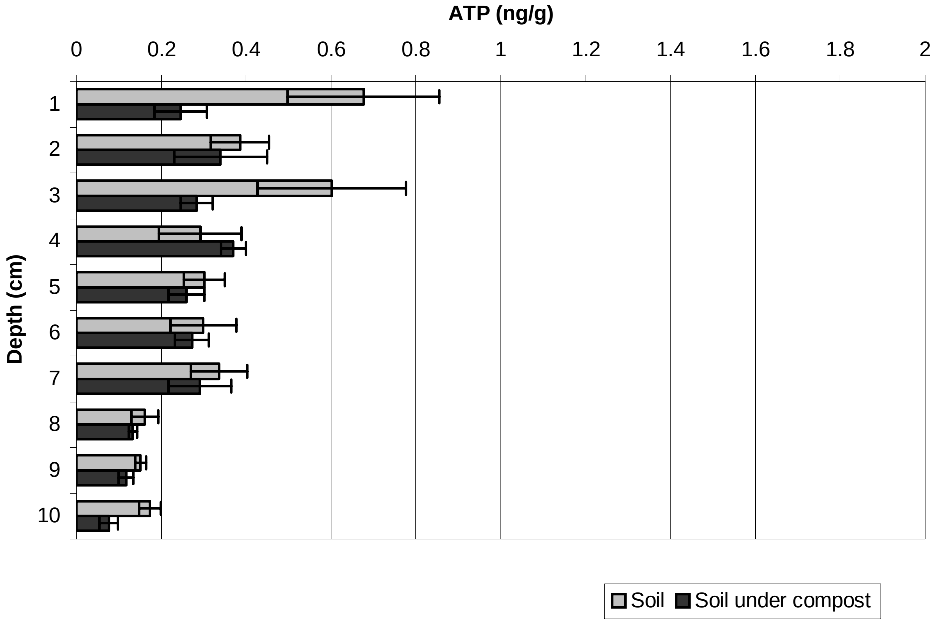
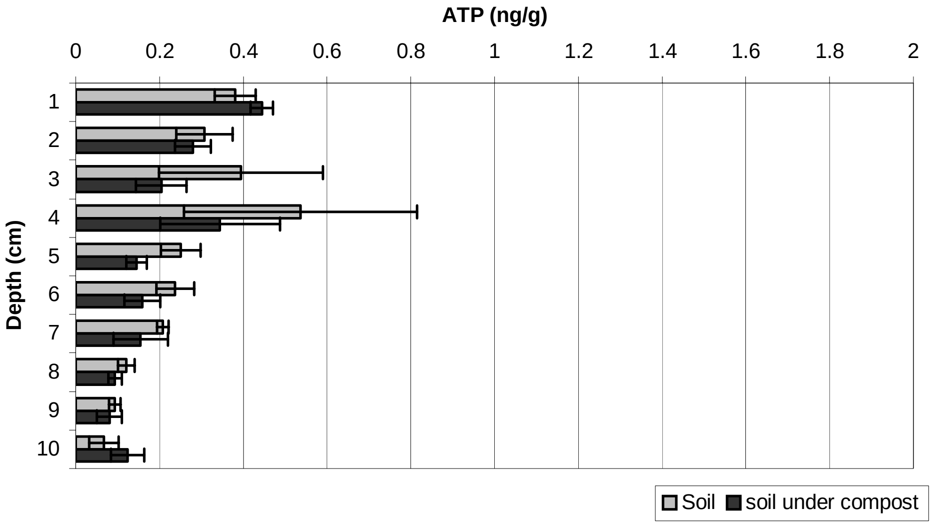


Figure 41. Merton Bank 3 ATP concentrations from composted (n=3) and uncomposted (n=3) plots ± standard errors.



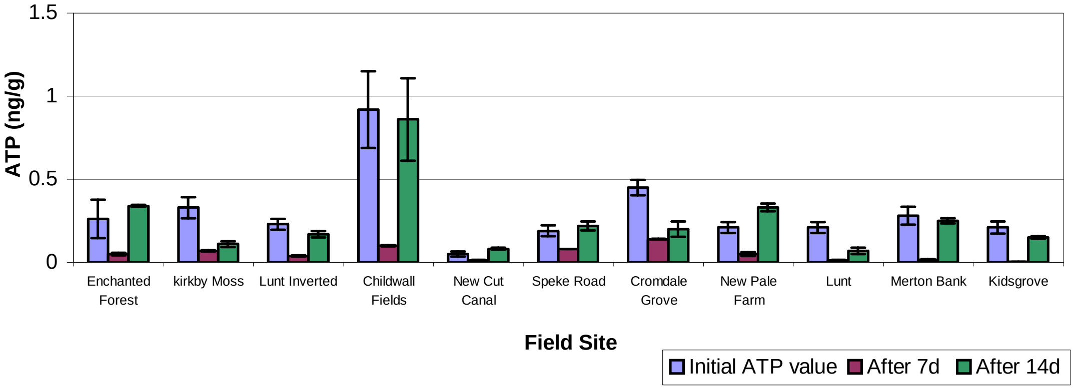
The depth profiles all show that in the top 5 cm of soil microbial activity is higher but decreases with depth to 10cm. This will be due to oxygen reduction in the soil with depth. However at Childwall Fields, ATP was 1.73 ng/g in the top 1cm fraction and over 0.4 ng/g at 8cm depth, therefore showing the greatest microbial activity compared to the other soils. A possible explanation could be that the soil does not suffer from contamination effects, poor structure or other stresses such as compaction.

3.10.1 Soil Resilience Testing

Microbial communities respond quickly to environmental changes, and there is an opportunity to impose environmental changes and to use the microbial response as a measure of soil health. Although time consuming and not considered to be an essential element of the toolbox, this does provide supportive information and is likely to contribute to the robustness of soil testing.

All soils showed varying degrees of recovery after the drought stress. Greatest recoveries were obtained from Enchanted forest, Childwall Fields, New Pale Farm, Merton Bank and Kidsgrove soils. This may indicate that the microbial populations present in these soils are more tolerant and robust to stresses imposed on them.

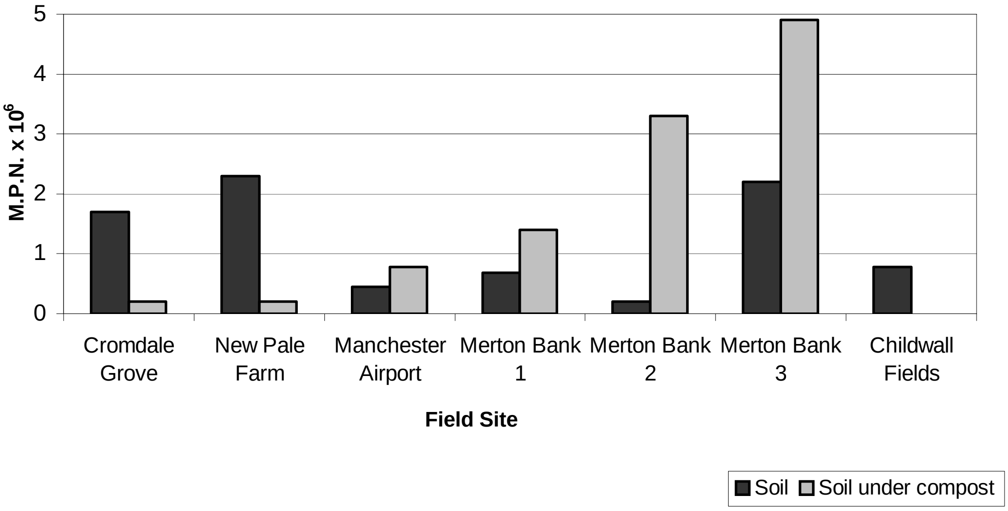
Figure 42. Soil resilience as depicted by changes in microbial biomass (ATP) before and after drought (35°C for 7 days). Values are the means (n=3) ± standard errors.



3.11 Microbial 'Most Probable Numbers'

Of standard microbial culturing techniques, Most Probable Numbers (MPN) is used routinely in the water testing industry and provides a broad measure of the presence of microorganisms. This technique is readily transferable to soils, but has limitations in that it may inaccurately reflect real conditions in the soil.

Figure 43. The 'most-probable-number' of bacteria present in soil and soil under compost.

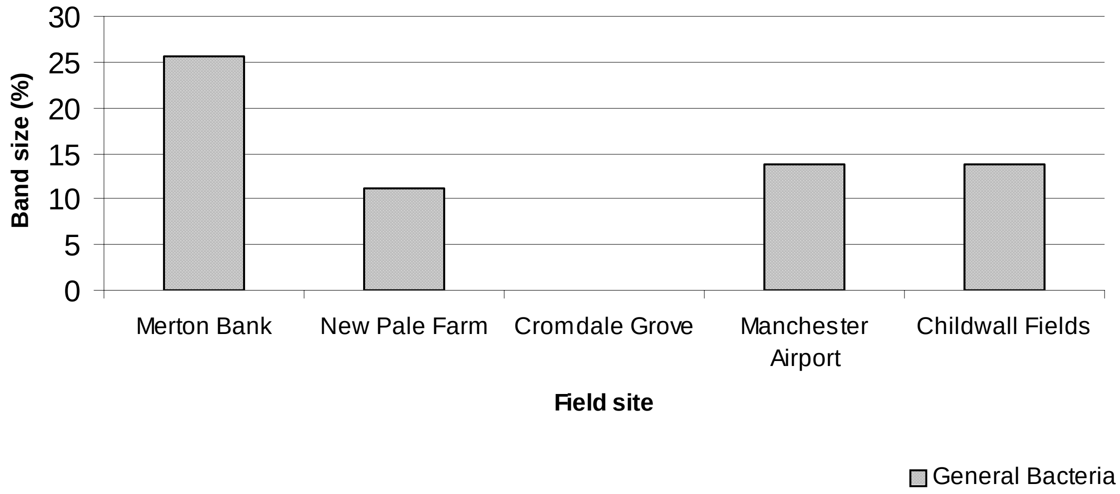


Cromdale Grove and New Pale Farm both showed higher numbers of bacteria present in the soils than in soil under compost. However the opposite occurred at Merton Bank and Manchester airport were bacterial numbers had increased in soil under compost. This test also showed that Childwall Fields had a reduced bacterial population compared to Cromdale, New Pale Farm and Merton Bank Plot 3.

3.12 Microbial PCR

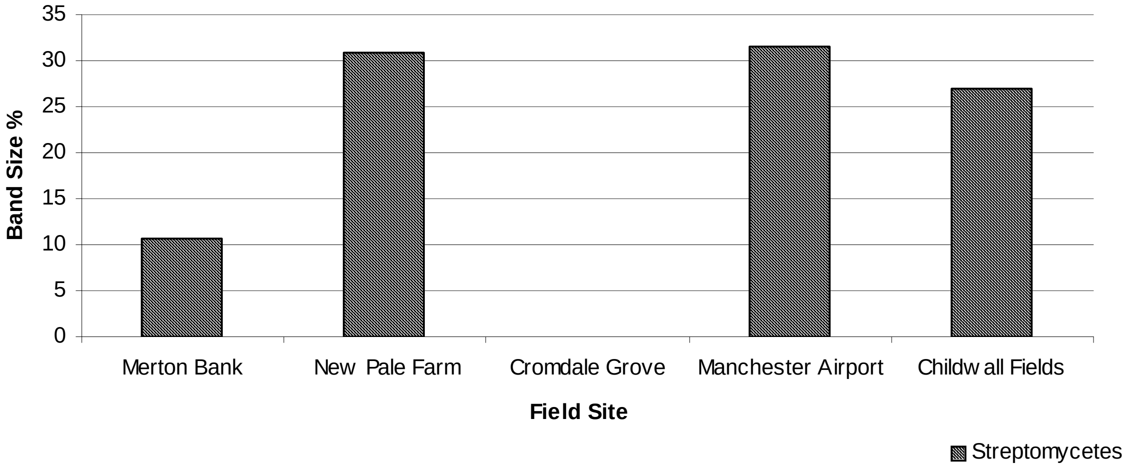
Microbial Polymerase Chain Reaction techniques provide a relatively advanced assessment of microbial biodiversity, and are becoming increasingly economic in their use with wider applications. The following graphs show differences in band widths obtained from electrophoresis gels.

Figure 44. Difference between gel electrophoresis band sizes (%) for the presence of General Bacteria



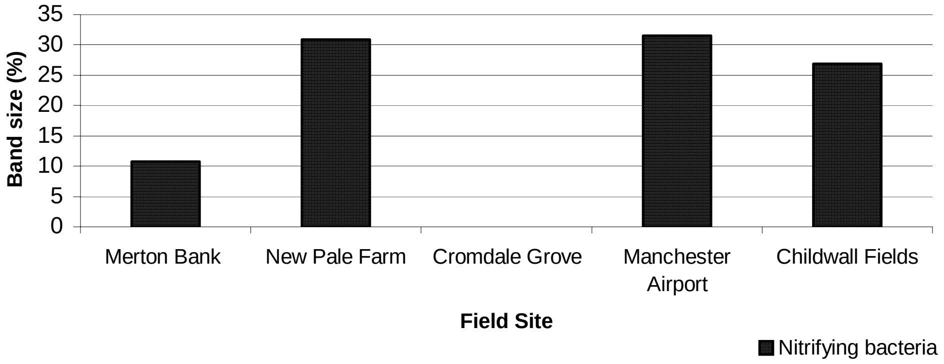
Merton Bank soil produced the largest percentage for general bacteria, whilst Cromdale Grove showed no bands. This may be due to extraction of the DNA from the soil.

Figure 45. Difference between gel electrophoresis band sizes (%) for the presence of Streptomycetes



Streptomycetes are found worldwide in soil, and are largely responsible, through the secretion of chemicals called geosmens, for the earthy smell of soil. Streptomycetes consequently play an important role in the degradation of organic matter, most commonly noted in compost heaps.

Figure 46. Difference between gel electrophoresis band sizes (%) for the presence of Nitrifiers



Nitrifying bacteria change ammonium (NH4+) to nitrite (NO2-) then to nitrate (NO3-) – a preferred form of nitrogen for grasses and most row crops. New Pale Farm, Manchester airport and Childwall Fields all showed the presence of nitrifying bacteria in the soil. Merton Bank soil showed a smaller concentration of nitrifiers.

Figure 47. Difference between gel electrophoresis band sizes (%) for the presence of fungi

Childwall Fields showed the largest percentage of fungi. This may be a possible reason why ATP concentrations are higher from this soil.

4.0 Assays to be included in the toolbox

4.1 Functional Process Assays

4.1.1 Basal field soil respiration rate

Rationale for inclusion in toolbox:	Carbon dioxide (CO_2) production is a measure of how biologically active a soil is.It is an index of microbial (and plant) activity.The more active a soil population is the more CO_2 is produced. Soil respiration is the second-largest factor in the flux of carbon between the earths ecosysyems and the atmosphere
Guidelines:	N/A
Estimated time taken to complete experiment	Collars are inserted into the soil and left undisturbed for at least an hour. Readings can then be taken within 5 minutes of the respirometer being turned on.
Monetary cost	: £10,000 for respirometer
Other considerations	

.

4.1.2 Basal laboratory soil respiration rate

Rationale for inclusion in toolbox:	See field respiration above
Guidelines:	N/A
Estimated time taken to complete experiment	24 hours
Monetary cost	Soil life test containers x 6, £45, Soil life test gel kit 12 tests, £30
Other considerations	Once filled with soil, the containers should be left undisturbed for 24 hours before commencement of the test

4.2 Plant Assays

Rationale for inclusion in toolbox:	The inhibition of root growth and plant biomass have been recognised internationally as being important for assessing brownfield soil quality to higher plant growth. Particularly as if plant growth is restricted at sites due to poor soil quality, there will be limited soil fauna at the site
Guidelines:	ISO 11269-1 Soil quality-Determination of the effects of pollutants in soil flora Part 1: Method for the measurement of inhibition of root growth. ISO 11269-2 Soil quality-Determination of the effects of pollutants in soil flora Part 2: Effects of chemicals on the emergence and growth of higher plants.

	Species: Corncockle (*Argrostemma githago*) and Red Clover (*Trifolium pratense*) Growth time: 2 weeks
Estimated time taken to complete experiment	8 h to sieve (4 mm) 10 pots of soil (depending on soil type), including time for setting up seed germination/seed planting and seedling harvest 8 h for 1 soil type and controls
Monetary cost	112 g of seed from The Organic Gardening Catalogue cost approximately £2. Plant pots approximately £1.50 each (13 cm width x 18 cm depth), Petri dishes, filter paper
Other considerations	Requires use of a greenhouse over winter

:

4.3 Invertebrates

4.3.1 Bait lamina strips

Rationale for inclusion in toolbox:	To measure functional integrity of a soil by assessing feeding activity of soil invertebrates. The method has been used to determine metal effects on soil invertebrate feeding
Guidelines:	N/A
Estimated time taken to complete experiment	4 hours for strip examination of 50 samples
Monetary cost	250 strips £395.06
Other considerations	Bait lamina strips are only produced by Terra Protecta GMBH in Germany and can take up to one month after ordering to arrive

.

4.3.2 Pitfall traps

Rationale for inclusion in toolbox:	Above-ground biodiversity and top carnivores
Guidelines:	N/A
Estimated time taken to complete experiment	Two hours per trap
Monetary cost	£1 per trap, Petri dishes
Other considerations	

4.3.3 Earthworms

Rationale for inclusion in toolbox:	Total numbers of earthworms is internationally recognised as being important biological indicators of soil quality
Guidelines:	ISO 11268-3 Effects of pollutants on earthworms- Guidance on the determination of effects in field situations
Estimated time taken to complete experiment	20 minutes per field quadrat; 5-10 minutes sorting and weighing earthworms depending on the number collected
Monetary cost	N/A
Other considerations	Requires use of a balance

4.3.4 Simplified Biological Index of Soil Quality (QBS)

Rationale for inclusion in toolbox:	Soil invertebrates are key in determining the soils turnover of nutrients, soil structure and microbiological status. Thus, the use of a simple key, that assigns a score to each invertebrate depending on its adaptations to living in the soil environment.
Guidelines:	N/A
Estimated time taken to complete experiment	20 minutes per sample
Monetary cost	Petri dishes
Other considerations	Requires the use of a Tullgren funnel set (6 for £1,000) and a microscope

4.4 Microbial Assays

The following microbial assays followed ISO 10381-6:1993. Whereby, samples were processed within 7 days of collecting the sample, soil was sieved (2 mm), and stored at 4°C in a loosely tied plastic bag in the dark, followed by a period of pre-incubation.

4.4.1 Dehydrogenase enzyme assay

Rationale for inclusion in toolbox:	Straightforward assay that reflects microbial processes
Guidelines:	Established protocols in literature (see Refs)
Estimated time taken to complete experiment	4 hours for 50 samples
Monetary cost	1 g of p-Iodonitrotetrazolium chloride (INT) costs £35.80 and 100 mg of INTF costs £6.75, filter paper, pipette tips, filter papers
Other considerations	Requires use of a spectrophotometer, funnels and a horizontal shaker

4.4.2 Microbial carbon analysis

Rationale for inclusion in toolbox:	Established technique for microbial biomass
Guidelines:	ISO 14240-2:1997 Soil quality- Part 4: Biological methods- Section 4.4 Effects of pollutants on microbes- Subsection 4.4.2 Determination of soil microbial biomass- Fumigation- extraction method
Estimated time taken to complete experiment	1 day for 15-20 samples
Monetary cost	1l of chloroform £14.50, pipette tips, filter papers

Other considerations	Requires the use of a balance, blacked out dessicator approximately (£100), vacuum pump, vortex and Organic Carbon analyser (approximately £10,000), funnels

4.4.3 Polymerase Chain Reaction (PCR) analysis

Rationale for inclusion in toolbox:	Due to the inability to culture most microorganisms from environmental samples, this posses an obstacle to understanding microbial ecology and diversity. PCR is a common method of creating copies of specific fragments of DNA. It can be used for the determination of particular species of bacteria / fungi present in a soil.
Guidelines:	N/A
Estimated time taken to complete experiment	8 hours (for nine different soil samples).
Monetary cost	100 units Failsafe enzyme £48, 8x primers £34.34, 2.5 ml Failsafe premix D £28, 100x pipette tips £7.50/£50.29, 100x tubes £57, 1x direct load wide range DNA marker £79.90, 1.5mM mastermix £18.25, Soilmaster DNA extraction kit (50 reactions) £154, 55 ml Inhibitor removal resin £39 and 50 spin columns £104, 100 g agarose £107.50
Other considerations	Requires the use of a Thermal cycler (£ 3000)

4.4.4 Adenosine Tri-Phosphate (ATP) analysis

Rationale for inclusion in toolbox:	Among the biological substances, ATP is considered to be suitable for the estimation of microbial activity because of its linear relationship between ATP content and microbial cell mass in soil
Guidelines:	N/A
Estimated time taken to complete experiment	36 samples per hour
Monetary cost	1 box x 100 Aqua trace water testing swabs £187, positive controls £14.49 and Uni-lite system £1,500, cores x10 £200, de-corer £1000, boxes x10 £10
Other considerations	

4.4.5 Microbial Most Probable Numbers (MPN)

Rationale for inclusion in toolbox:	MPN is a statistical multi-step fermentation assay technique which provides a statistical probability of the number of bacteria present in a sample.
Guidelines:	N/A
Estimated time taken to complete experiment	3 hours for 4 soils
Monetary cost	Nutrient broth, pipette tips
Other considerations	Clean lab or laminar flow cabinet required. It must be noted that certain bacterial species may not grow in the broth because of differing nutritional requirements, also other species may become dominant which were otherwise recessive in the soil due to the growth media

4.5 Toolbox Cost Analysis

A detailed costing of each tool in the toolbox was carried out (Table 5)

Assays	**Monetary cost for 9 samples- time and consumable (£)**	**Monetary cost for 16 samples- time and consumable (£)**	**Monetary cost for 25 samples- time and consumable (£)**	**Notes**
Functional process: Soil respiration (field test)	Time (45 mins)	Time (1.5h)	Time (2h 20mins)	Capital cost: **£10,900** (respirometer and 9 collars£100 each)
Soil respiration (laboratory test)	Soil life test gel kit, time (4h) **£22.50**	Soil life test gel kit, time (7h) **£30**	Soil life test gel kit, time (11h) **£62.50**	Capital cost: **£85** for 6 containers, sieve
Plants- Corncockle/Red Clover (1 ha bulked soil)	Seeds, Petri dishes, filter papers, time (6h) **£10**	Seeds, Petri dishes, filter papers, time (6h) **£20**	Seeds, Petri dishes, filter papers, time (6h) **£40**	Capital cost: **£500** (plant pots, balance, sieve, crucibles, soil shaker and sieves Greenhouse **£500**) heating and lighting over winter
Invertebrate feeding activity: Bait lamina strips	6 strips per sampling area=54, laboratory time (4h) **£86.40**	6 strips per sampling area=96, laboratory time (7h) **£153.60**	6 strips per sampling area=150, laboratory time (11h) **£240**	Need to return to site twice-set up and collection, takes 1 month for strips to arrive from Germany
Pitfall traps	cotton buds, Petri dishes, ethanol, time (5h) **£16**	cotton buds, Petri dishes, ethanol, time (8h) **£20**	cotton buds, Petri dishes, ethanol, time (13h) **£40**	Capital cost: **£230** (30 traps, microscope) Need to return to site twice-set up and collection,

Earthworm numbers and biomass	Weighing boats, time (7h) **£5**	Weighing boats, time (12h) **£5**	Weighing boats, time (19h) **£10**	Capital cost: **£210** (balance and quadrat)
Simplified QBS	Ethanol, Petri dishes, time (6h) **£5**	Ethanol, Petri dishes, time (11h) **£10**	Ethanol, Petri dishes, time (17h) **£20**	Capital cost: **£1,250** (6 Tullgren funnels and vials, microscope)
Microbial ATP content	Aqua trace water testing swabs, positive controls, boxes, time (15 mins) **£29**	Aqua trace water testing swabs, positive controls, boxes, time (30 mins) **£56**	Aqua trace water testing swabs, positive controls, boxes, time (45 mins) **£85**	Capital cost: **£2,750** (ATP machine, coresx10, decorer, and vials)
Microbial carbon	Chloroform, sulphuric acid, filter papers, time (6.5h) **£7.50**	Chloroform, sulphuric acid, filter papers, time (8h) **£15**	Chloroform, sulphuric acid, filter papers, time (9h) **£125**	Capital cost: **£12,390** (TOC analyser, dessicator, balance, vials, sieve and vacuum pump, funnels, fume hood)
Microbial dehydrogenase activity	INT, INTF, pipette tips, filter papers, time (45 mins) **£14**	INT, INTF, pipette tips, time (1h) **£15**	INT, INTF, pipette tips, time (2h) **£20**	Capital cost: **£8,500** (Horizontal shaker, balance, incubator at 15ºC, pipettes, sieve, plastic funnels and spectrophotometer)
Microbial MPN	Nutrient broth, pipette tips, time (16h) **£26.76**	Nutrient broth, pipette tips, time (29h) **£33.50**	Nutrient broth, pipette tips, time (45h) **£59.50**	Capital cost: **£14,600** (Autoclave, balance, vials, incubator at 19ºC, horizontal shaker, pipettes, lamina flow cabinet, Bunsen burner, sieve)
Microbial PCR	Pipette tips, microcentifuge tubes, Failsafe enzyme, primers, Failsafe premix D, tubes, direct load wide range DNA marker, mastermix, Soilmaster DNA extraction kit, Inhibitor removal resin and 50 spin columns, time (8h) **£75**	Time (8h) **£132**	Time (8h) **£207**	Capital cost: **£6,418** (PCR machine, balance, gel plate, pipettes, transluminator, centrifuge, sieve)
Totals	Consumables £297.16 Time 64 h 15 mins	Consumables £490.10 Time 99 h	Consumables £909 Time 144 h	Capital costs £58,333

Table5 Indication of the monetary cost (including an experts time at £15 per hour) to conduct each toolbox assay for 9, 18 and 25 sites on a one hectare site investigation. General costs: Spade, trowel,

auger, hammer, block of wood, polythene bags, canes, ruler, tape measures, elastic bands, labels, tweezers/forceps, boxes, permanent markers, disposable latex gloves, petrol, field work insurance and car hire. This table does not include time for going to the field and collecting the soil samples.

5.0 Discussion and Conclusion

The contribution of large-scale field experiments is required to provide vital supporting evidence of what constitutes a healthy soil in relation to biological or ecological indicators. The use of the derived index of health, of toolbox, should be used with caution. For example, contaminated or degraded sites may contain soils with highly elevated heavy metal concentrations may appear to be healthy; they may sustain a wide variety of edaphic organisms adapted to the toxin stress, different to those associated with cleaner soils, but would this indicate the soil is unhealthy? In the fut1re a definition such as "Statutory Healthy Soil" may be applied to Brownfield sites that have been remediated for community woodland purposes in order to differentiate from typically clean healthy ecosystems, for example Prairie grassland.

Guidelines may have to be introduced in order to determine land-use criteria. What constitutes a "healthy" soil to a farmer will be different to that of a forestry land manager, conservationist or housing developments. Baseline surveys should provide basic information about soil quality, for example pH, LOI, N, C and moisture content, but further to this a suite of bioindicators may be exploited depending on the intended final land-use in order to determine the soils health.

The present project has proposed 12 biological indicators of soil health that can be routinely measured, providing a quantifiable measure that can be understood and that potentially will provide meaningful information for practitioners involved in land reclamation and policy making. These simple indicators are required for this to be credible and realistic, in that identification to species level for invertebrate analysis, or state of the art microbial techniques may be somewhat out of reach in relation to time, money and expertise. Finally, the organism(s) must be easy and inexpensive to measure. Factors such as specific respiration (qCO_2) could be used as "baseline performance" guides to a soils microbial community, and research into site-specific baseline values are required in order to determine changes in the establishment of new microbial communities (Anderson, 2003). Similar site-specific baseline values could also be developed for invertebrate faunal communities. However any index of soil quality must consider soil function, and a soil that could be identified as high in quality for one function may not be for others (Nortcliff, 2002).

A tiered approach to the use of soil health criteria by practitioners is clearly required based on physico-chemical analysis, ecological surveys and bioassays, as has been used in sediment analysis (Chapman *et al.*, 2003). This approach may initially be used for preliminary investigations using physico-chemical analyses, bringing together a suite of tests ranging from pH to leaching procedures in order to establish a sites soil quality. In respect of this information, the sites fate in relation to further testing would then be determined. If a site were considered to have the potential to be developed by remediation, further investigations into its biological health could then be considered. This would allow practitioners to approach degraded / contaminated sites and following the different analyses and surveys determine the sites health.

Our recommendation is that the toolbox should always be used in comparison reference sites, measured at the same time to the brownfield site of interest. The reference sites preferably should be in the locality of the brownfield site and where medium- to long-term vegetation stability is known to exist. Without this comparison of a reference site, meaningful interpretation of data would be difficult or impossible. Finally, we propose that the 12 tools can be used selectively and that it may not be necessary to use all of these assays and analyses at all sites. Our hope is that this project has provided a significant step towards guidance for environmental practitioners to use in conjunction with established site investigation methodologies.

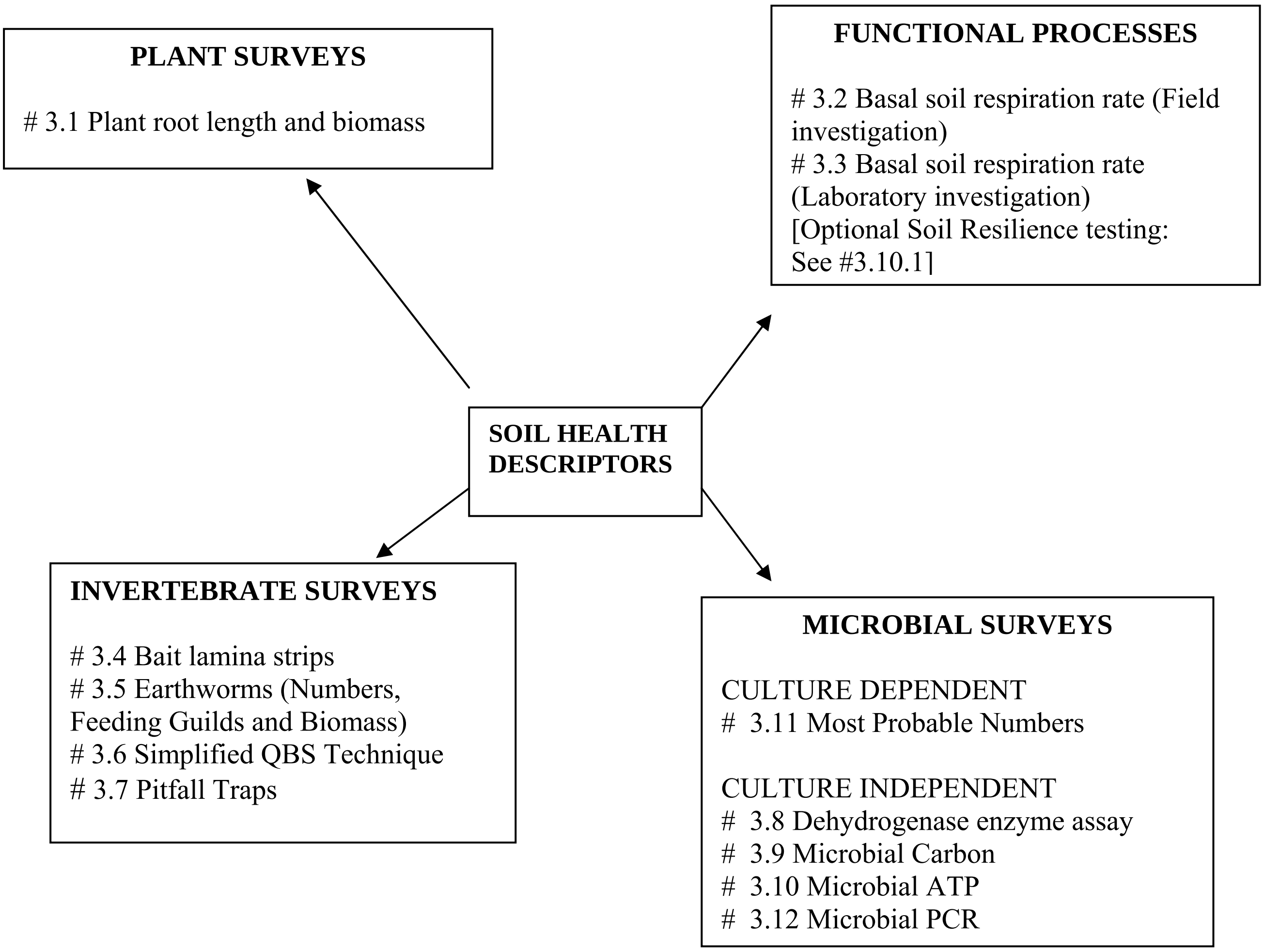


Figure 49. Final assay selection for bioindicator toolbox.

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Appendix A

Sampling information for experimental sites

Site:	**CG**	**NPF**	**MA**	**CF**	**MB1**	**MB2**	**MB3**
Sampling dates:	23/1/06	24/1/06	25/1/06	25/1/06	26/1/06	26/1/06	27/1/06
Samplers:	LU, WH, AP,RC	LU, WH, AP	LU, WH, AP,RC	LU, WH, AP,RC	LU, WH, RC	LU, WH, RC	LU, WH, AP,RC
Sampling tools:	Spade, auger, Tullgrens, cores	Spade, auger, Tullgrens, cores	Spade, auger, Tullgrens, cores	Spade, auger, Tullgrens, cores	Spade, auger, Tullgrens, cores	Spade, auger, Tullgrens, cores	Spade, auger, Tullgrens, cores
Disturbed / undisturbed sample:	Disturbed apart from ATP and QBS	Disturbed apart from ATP and QBS	Disturbed apart from ATP and QBS	Disturbed apart from ATP and QBS	Disturbed apart from ATP and QBS	Disturbed apart from ATP and QBS	Disturbed apart from ATP and QBS
Depth of sample:	10 cm	10 cm	10 cm	10 cm	10 cm	10 cm	10 cm
Volume of sample:	15-20 l	15-20 l	15-20 l	15-20 l	15-20 l	15-20 l	15-20 l
Single or composite sample:	Composite	Composite	Composite	Composite	Composite	Composite	Composite
Sample moisture status (%):	26-27	15-17	21-22	20	23-28	27-28	29
Sample containers used:	500 gage polythene bags, cores, Tullgrens	500 gage polythene bags, cores, Tullgrens	500 gage polythene bags, cores, Tullgrens	500 gage polythene bags, cores, Tullgrens	500 gage polythene bags, cores, Tullgrens	500 gage polythene bags, cores, Tullgrens	500 gage polythene bags, cores, Tullgrens
Sample time and conditions of transport:	10.30-5pm, 45 minutes at approx. 15ºC	10-4.30pm 45 minutes at approx. 15ºC	10.30-12pm 90 minutes at approx. 15ºC	1pm-4pm, 30 minutes at approx. 15ºC	9-11.30am, 45 minutes at approx. 15ºC	11.30-4.30pm, 45 minutes at approx. 15ºC	10-2pm, 45 minutes at approx. 15ºC
Sample conditions of storage:	Dark at 8ºC	Dark at 8ºC	Dark at 8ºC	Dark at 8ºC	Dark at 8ºC	Dark at 8ºC	Dark at 8ºC

LU= Louise Uffindell, WH= William Hartley, AP= Amanda Plumb, RC= Rafael Clemente

Appendix B

Templates detailing methods used in the toolbox

1. Title of the test:	Determination of the effects of pollutants on soil flora
2. Harmonisation	International
3. References	ISO 11269-1 and -2
4. Principle	Air dried biomass (Corncockle) and root length (Corncockle and Red Clover)
5. Test type	Subchronic, static
6. Test organism	Corncockle and Red Clover
Breeding stock	Seeds from the Wildflower centre and the Organic Gardening catalogue
Age	Seeds with radicle <2 mm
Feeding	N/A
7. Test substrate	Sieved soil (4 mm). Controls are compost and industrial sand
Volume	Approximately 500g/pot (DW)
8. Test conditions	Approximately 70 % WHC
Test chamber	Green house in winter and outside compound in summer
Temperature	Suitable for normal growth
pH	
Light intensity/quality	
Photoperiod	
Soil moisture	
9. No. Replicates	4
10. Test duration/incubation	2 weeks
11. Neg. control/dilution soil	N/A
12. Validity criteria	5 healthy seedlings per control pots
13. Pos. Control/reference toxicant Mean EC50, CV	N/A
14. Statistics	GLM
15. Testparameter(s)	Dry biomass (Corncockle) and root length (Corncockle and Red Clover)
16. Endpoints	2 weeks

Limitations/Comments:

The method is applicable to all soils, soil materials, waste or chemicals which may be applied to soil except where the contaminant is highly volatile or only affects photosynthesis. The method may be used to compare soils to monitor changes in their activity or to determine the effect of added substances. The method is not intended for use as a measure of the ability of the soil to support sustained plant growth. In the case of contaminated soil it may be necessary to dilute with uncontaminated soil or sand before testing.

As with other bioassays proposed, tests with higher plants are designed to consider the pollutant situation and bioavailability of pollutants that are not detected by chemical analysis. By applying a test period of at least 7 days, short-term changes in soil by the test plant itself are included.

The accumulation of pollutants in soils, their metabolism and effects on consumers are not investigated in the test. They also do not apply for assessment of soil fertility and productivity.

The requirements if the control compost must take into account the different soil uses and the type and origin of the soil (e.g. undisturbed soil, refilling material, excavated soil, remediated soil). Different soil compaction and nutrient deficiency as well as differences in the water-holding capacity and pore volume can cause differences in plant growth that need not necessarily be caused by the pollutant load and the hazard potential. Also available as test method: Standard Practice for Conducting Early Seedling Growth Tests (ASTM Designation: E 1598-94).

1.	**Title of the test:**	Earthworm numbers and biomass
2.	**Harmonisation**	
3.	**References**	Bouche, M.B. 1977. Strategies lombriciennes. In: Soil Organisms as Components of Ecosystems (eds. U. Lohm and T. Persson). Ecological Bulletins, Stockholm, pp. 122-132. Biology and Ecology of Earthworms. 3rd Ed. C.A. Edwards and P.J. Bohlen
4.	**Principle**	To determine the different ecological groups of earthworms present in a soil
5.	**Test type**	Numbers, guilds, maturity and biomass
6.	**Test organism**	Earthworm
7.	**Test substrate**	Soil
	Volume	0.5 m^2 quadrat and digging till you hit bedrock/ landfill clay cap or when earthworms cease to be present
8.	**Test conditions**	Using a 0.5 m^2 quadrat three random locations are selected at the field site. Each area delineated by the quadrat was dug until subsoil was located, usually to a depth of 0.15 m. The soil was then hand-sorted for the presence of earthworms.
9.	**No. Replicates**	3 quadrats per site investigated
10.	**Test duration/incubation**	NA
11.	**Neg. control/dilution soil**	NA
12.	**Validity criteria**	NA
13.	**Pos. Control/reference toxicant Mean EC50, CV**	NA
14.	**Statistics**	General linear models (GLM)
15.	**Test parameter(s)**	Numbers of earthworms counted and the community is divided into either surface active, non-pigmented species that are dark green in colour (epigeic), horizontal burrowers that are pale in colour (endogeic) and deep burrowers that are have dark pigmentation around the anterior and become paler towards the posterior (anecics).
16.	**Endpoints**	Earthworm numbers, guilds, maturity and biomass
17.	**Limitations/Comments:**	

1. Title of the test:	Simplified Biological Index of Soil Quality (QBS)
2. Harmonisation	
3. References	Microarthropod communities as a tool to assess soil quality and biodiversity: a new approach in Italy. Parisi, V et al. Agriculture, Ecosystems and Environment 105, pp 323-333, 2005
4. Principle	Appoints scores (1-20) to soil microarthropods according to their adaptations to the soil environment giving them an EMI (Eco-morphological Index). QBS sums up these scores and thus characterises the study soil. The higher the soil quality the higher will be the number of soil microarthropod groups well adapted to the soil habitat.
5. Test type	
6. Test organism	Soil microarthropod communities
Breeding stock	NA
Age	NA
Feeding	NA
7. Test substrate	Soil
Volume	
8. Test conditions	
Test chamber	Tullgren funnel – soil is added to the chamber and left for 7 days. The soil dries from the top due to the light bulb and the microarthropods move through the soil into a preservative (50% ethanol)
Temperature	Room temperature
pH	NA
Light intensity/quality	25 watt Bulb
Photoperiod	NA
Soil moisture	NA
9. No. Replicates	3 per soil
10. Test duration/incubation	7 days
11. Neg. control/dilution soil	NA
12. Validity criteria	NA
13. Pos. Control/reference toxicant Mean EC50, CV	NA
14. Statistics	GLM
15. Test parameter(s)	Presence of microarthropods
16. Endpoints	EMI Scores
17. Limitations/Comments: Does not require complex taxonomic identification and therefore non-specialists can use QBS. The QBS however does not show why certain arthropod species are not present, i.e. it does not indicate what stress is put on the community. Therefore this method must be accompanied with other indicators to determine the stress.	

An example of how the adapted QBS scoring system was implemented across sites

	Sample Site 1	**Sample Site 2**	**Sample Site 3**
20 points			
Protura			
Diplura	20	20	
Collembola no pigmentation etc.			
e.g.Neanuridae/Onychiuridae/Poduridae	20	20	20
e.g. Isotomidae		20	
e.g. Sminthuridae		20	20
Acari	20	20	20
Symphyla	20	20	20
Pauropoda			
Diplopoda <5 mm			
Chilopoda <5 mm poorly developed legs			
10 points			
Opiliones			
Isopoda	10		
Chilopoda >5 mm	10		10
Embioptera			
Diptera larvae			
Other holometabolous insect larvae	10		
8 points			
Collembola medium pigmentation etc.			
e.g.Neanuridae/Onychiuridae/Poduridae		8	
e.g.Isotomidae		8	
e.g. Sminthuridae			
5 points			
Aranea diameter <5mm			
Diplopoda >5mm			
Blatteria			
Hymenoptera: Ants			
1 point			
Aranea diameter >5mm			
Collembola dark pigmentation etc.			
e.g.Neanuridae/Onychiuridae/Poduridae			
e.g.Isotomidae			
e.g. Sminthuridae			
Coleoptera adults: adapted to soil surface	1		
Hymenoptera: excluding ants			
Orthoptera			
Psocoptera			
Thysanoptera			
Dermaptera			
Hemiptera	1		1
Diptera adults			
TOTAL	112	136	91
MEAN QBS	113		
STANDARD ERROR	**15.92**		

1. Title of the test:	Pitfall Traps
2. Harmonisation	
3. References	Work, T.T, Buddle, C.M., Korinus, L.M. and Spence, J.R. (2002) Pitfall Trap Size and Capture of Three Taxa of Litter-Dwelling Arthropods: Implications for Biodiversity Studies. Environmental Entomology, 31(3): 438-448
4. Principle	To measure the diversity of arthropods active at the soil surface
5. Test type	Group or species identification and numbers
6. Test organism	Soil surface dwelling arthropods
7. Test substrate	Soil or compost surface/leaf litter
Volume	
8. Test conditions	Traps consisted of 0.5litre, 11cm diameter glass containers buried so the lip of the container was flush with the soil surface. Traps contained 2-3cm of 50% alcohol solution as a preservative. Container lids were held 2-4cm above the traps using wooden supports to reduce the accumulation of leaf litter and rainwater. Traps were left in the field for a period of 1 week before collection for analysis.
9. No. Replicates	3 pitfalls traps per site investigated
10. Test duration/incubation	1 week
11. Neg. control/dilution soil	NA
12. Validity criteria	NA
13. Pos. Control/reference toxicant Mean EC50, CV	NA
14. Statistics	GLM
15. Test parameter(s)	Presence of arthropods
16. Endpoints	Species or groups present at each site and numbers
17. Limitations/Comments: Placement of traps in relation to other traps and vegetation, size and shape of trap, preservative type etc can all influence the number, type and relative abundance of species collected.	

1. Title of the test:	Feeding activity using bait lamina strips
2. Harmonisation	
3. References	Metal Effects on Soil Invertebrate Feeding: Measurements Using the Bait Lamina Method. Filzek, P.D.B. et al. Ecotoxicology, 13, 807-816, 2004.
4. Principle	To measure the Overall Feeding Activity (O.F.A) of soil organisms using a simple test.
5. Test type	
6. Test organism	Soil Fauna / micro-organisms
7. Test substrate	Soil
Volume	NA
8. Test conditions	Field conditions
9. No. Replicates	At each site 3 plots, 16 strips per plot
10. Test duration/incubation	Assay is left in situ for 7-14 days. After this period the strips are analysed using a light box / dissecting microscope for the determination of pierced baits
11. Neg. control/dilution soil	NA
12. Validity criteria	NA
13. Pos. Control/reference toxicant Mean EC50, CV	NA
14. Statistics	
15. Test parameter(s)	Feeding activity (not pierced, partly pierced and fully pierced)
16. Endpoints	Shows the overall feeding activity of soil fauna
17. Limitations/Comments: It is a simple and fast test, measuring the function of soil ecosystems, and so having high ecological relevance. A weakness of the test is that environmental and soil factors can influence its outcome. Studies at different times and locations make it difficult for comparisons. For example, season can influence feeding activity.	

1. Title of the test:	The "most-probable-number" method
2. Harmonisation	
3. References	Methods for Studying the Ecology of Soil Micro-organisms. Eds: Parkinson, D. Gray, T.R.G. & Williams, S.T. 1971.
4. Principle	Serial dilutions of soil are prepared (10^{-4} to 10^{-9}) and 1 cm^3 samples of these dilutions are transferred into 5 tubes of liquid nutrient broth. After incubation, the presence or absence of growth in the recovery cultures is recorded.
5. Test type	Micro-organisms
6. Test organism	Bacteria
Breeding stock	
Age	
Feeding	
7. Test substrate	Nutrient broth
Volume	
8. Test conditions	Laboratory incubator
Test chamber	Glass tubes
Temperature	20°C
pH	
Light intensity/quality	
Photoperiod	
Soil moisture	
9. No. Replicates	5 glass tubes per dilution
10. Test duration/incubation	7 – 14 days
11. Neg. control/dilution soil	Initial dilution 10^{-3}
12. Validity criteria	
13. Pos. Control/reference toxicant Mean EC50, CV	
14. Statistics	
15. Test parameter(s)	
16. Endpoints	Number of turbid tubes observed at three successive dilutions. The M.P.N. is the estimated mean viable count per inoculum taken from the most concentrated suspension.
17. Limitations/Comments: Not all bacteria will grow effectively in the medium	

1. Title of the test:	Microbial dehydrogenase activity
2. Harmonisation	N/A
3. References	PAGE, A.L., MILLER, R.H., KEENEY, D.R., METHODS OF SOIL ANALYSIS-CHEMICAL AND MICROBIOLOGICAL PROPERTIES ASA, INC. AND SSSA, INC., MADISON 1982 ED:2ND ED
4. Principle	Add 1 % 2,3,5- Triphenyltetrazolium chloride to soil samples incubate at 37ºC for 24 h and measure TPF formation spectrophotometrically
5. Test type	Dehydrogenase activity
6. Test organism	Bacteria and fungi
Breeding stock	N/A
Age	N/A
Feeding	N/A
7. Test substrate	Soil
Volume	5 g
8. Test conditions	Laboratory
Test chamber	Spectrophotometer
Temperature	37ºC
pH	N/A
Light intensity/quality	N/A
Photoperiod	N/A
Soil moisture	N/A
9. No. Replicates	3
10. Test duration/incubation	24 h
11. Neg. control/dilution soil	N/A
12. Validity criteria	N/A
13. Pos. Control/reference toxicant Mean EC50, CV	N/A
14. Statistics	General linear models
15. Test parameter(s)	Dehydrogenase activity
16. Endpoints	Determination of microbial dehydrogenase enzyme
17. Limitations/Comments:	

1. Title of the test:	Microbial biomass determination from chloroform fumigation extraction
2. Harmonisation	N/A
3. References	Dynamic response of microbial biomass, respiration rate and ATP to glucose additions Soil Biology and Biochemistry, 29 (8), 1997, 1249-1256 C.-S. Tsai, K. Killham and M. S. Cresser Leckie et al., Comparison of chloroform fumigation extraction, phospholipids fatty acid, and DNA methods to determine microbial biomss in forest humus. Soil Biology and Biochemistry 36 529-532
4. Principle	Initially the chloroform needs to be acid washed. Can extract chloroform labile carbon from soils and measure by using a spectrophotometer in order to determine microbial biomass.
5. Test type	Chloroform fumigation extraction
6. Test organism	Bacteria and fungi
Breeding stock	N/A
Age	N/A
Feeding	N/A
7. Test substrate	Soil
Volume	
8. Test conditions	Laboratory- University of Sheffield
Test chamber	Dessicator and Spectrophotometer
Temperature	<10ºC
pH	N/A
Light intensity/quality	Dark
Photoperiod	N/A
Soil moisture	Field conditions
9. No. Replicates	3
10. Test duration/incubation	24 h
11. Neg. control/dilution soil	Non fumigated soil
12. Validity criteria	N/A
13. Pos. Control/reference toxicant Mean EC50, CV	N/A
14. Statistics	General linear models
15. Test parameter(s)	N/A
16. Endpoints	Determination of microbial biomass
17. Limitations/Comments: Need to use a Labtoc for carbon quantification. Chloroform fumigation can only tell the amount of carbon in soil and not the species diversity.	

1. **Title of the test:**	Microbial biomass determination from ATP analysis using a luminometer
2. **Harmonisation**	
3. **References**	BIOTRACE International. Simplified method for estimation of microbial activity in compost by ATP analysis. Horiuchi, J.I, Ebie, K. Tada, K. Kobayashi, M. and Kanno, T. Bioresource Technology. 86: 95-98 (2003)
4. **Principle**	ATP can be detected with high sensitivity using the enzyme from fireflies called luciferase. Luciferase breaks down ATP and releases light. The light can be measured using a luminometer.
5. **Test type**	
6. **Test organism**	Microorganisms
Breeding stock	
Age	
Feeding	
7. **Test substrate**	Soil
Volume	Soil Cores taken to 15 cm depth
8. **Test conditions**	Laboratory
Test chamber	Luminometer cell
Temperature	Test swabs must be kept at between 2-8°C. Analysis however is at room temperature
pH	
Light intensity/quality	
Photoperiod	
Soil moisture	Fied capacity
9. **No. Replicates**	3 cores per site
10. **Test duration/incubation**	N/A
11. **Neg. control/dilution soil**	
12. **Validity criteria**	
13. **Pos. Control/reference toxicant Mean EC50, CV**	
14. **Statistics**	
15. **Test parameter(s)**	
16. **Endpoints**	Determination of microbial biomass
17. **Limitations/Comments:**	

1. Title of the test:	Basal Soil Respiration (CO_2): Field investigation
2. Harmonisation	
3. References	Fang, C & Moncrieff, J B. 2001. The dependence of soil CO2 efflux on temperature. *Soil Biology and Biochemistry* **33** 155-165 Bellamy, P. 2005. Carbon losses from all soils in England and Wales 1978-2003. *Nature* **437** 245-248 Detlef Schulze, E & Freibauer, A. 2005. Carbon unlocked form soils. *Nature* **437** 205-206
4. Principle	Measures CO_2 evolution from the soil
5. Test type	Micro-organisms
6. Test organism	Bacteria & fungi, but also takes into account plants and invertebrates
Breeding stock	
Age	
Feeding	
7. Test substrate	Soil
Volume	
8. Test conditions	Field conditions
Test chamber	Soil hood, consisting of a PVC pot with air stirrer fan and pressure equalisation vent. In addition a stainless steel “collar” to support the soil hood.
Temperature	NA
pH	NA
Light intensity/quality	NA
Photoperiod	NA
Soil moisture	Field capacity
9. No. Replicates	3
10. Test duration/incubation	Collars are to be left for a minimum of 1 hour before analysis
11. Dilution soil	
12. Validity criteria	
13. Pos. Control/reference toxicant Mean EC50, CV	
14. Statistics	
15. Test parameter(s)	
16. Endpoints	Determines how biologically active the soil is by how much CO_2 is evolved
17. Limitations/Comments: Not just microbial respiration, also invertebrates and plants . Costly equipment would is required	

1. Title of the test:	Basal Soil Respiration (CO_2) : Laboratory investigation
2. Harmonisation	
3. References	Fang, C & Moncrieff, J B. 2001. The dependence of soil CO2 efflux on temperature. *Soil Biology and Biochemistry* **33** 155-165 Bellamy, P. 2005. Carbon losses from all soils in England and Wales 1978-2003. *Nature* **437** 245-248 Detlef Schulze, E & Freibauer, A. 2005. Carbon unlocked form soils. *Nature* **437** 205-206
4. Principle	Measures CO_2 evolution from the soil using gel paddles which indicate CO_2 evolution by changing colour
5. Test type	Micro-organisms
6. Test organism	Bacteria & fungi, but also takes into account invertebrates
Breeding stock	
Age	
Feeding	
7. Test substrate	Soil
Volume	
8. Test conditions	Field conditions
Test chamber	Plastic pots filled with soil.
Temperature	NA
pH	NA
Light intensity/quality	NA
Photoperiod	NA
Soil moisture	Field capacity
9. No. Replicates	3
10. Test duration/incubation	Soil is added to the pot (test chamber) and left undisturbed for 24 hours. After this period a gel paddle is inserted into the soil and left for a further 24hrs. The colour is then determined.
11. Dilution soil	
12. Validity criteria	
13. Pos. Control/reference toxicant Mean EC50, CV	
14. Statistics	
15. Test parameter(s)	
16. Endpoints	Determines how biologically active the soil is by how much CO_2 is evolved
17. Limitations/Comments: Not just microbial respiration, also invertebrates and plants .	

1. Title of the test:	Polymerase Chain Reaction (PCR)
2. Harmonisation	
3. References	Application of real-time PCR to study effects of Ammonium on population size of ammonia-oxidising bacteria in soil. Okano et al., 2004. Applied and Environmental Microbiology
4. Principle	The purpose of a PCR is to make a huge number of copies of a gene.
5. Test type	Micro-organisms
6. Test organism	Bacteria / Fungi
Breeding stock	
Age	
Feeding	
7. Test substrate	Soil
Volume	0.1g
8. Test conditions	Determined by species of bacteria or fungi
Test chamber	PCR tube
Temperature	
pH	
Light intensity/quality	
Photoperiod	
Soil moisture	
9. No. Replicates	3
10. Test duration/incubation	Dependent upon PCR cycle
11. Dilution soil	
12. Validity criteria	
13. Pos. Control/reference toxicant Mean EC50, CV	
14. Statistics	
15. Test parameter(s)	
16. Endpoints	Determines the presence of a particular species of bacteria or fungi in the soil.
17. Limitations/Comments: The technique is valuable for determining the biodiversity of bacteria and fungi in soil. There are problems in extracting DNA if the soil has high levels of humic acids present. The technique can be expensive.	

Appendix C

Assay	Method	Why rejected	Reference
IndVal Index	Sites are grouped, and indicator species assessed	Fairly new method, only tested at metal polluted sites in France. Requires specialist knowledge	Nahmani et al. (2006) Soil Biol. & Biochem. 38, 385-396
Soil dwelling Diptera	Species, community analysis	Lack of knowledge available on their ecology and taxonomy. Difficult to identify.	Frouz (1999) Agric, Ecosy & Env 74, 167-186
Oribatid mite life history	Species, community analysis	Lack of knowledge available on their ecology and taxonomy. Requires specialist knowledge.	Migliorini et al., (2005) Pedobiologia 49, 1-13 Joehler, (1999) Agri. Ecosy. & Env. 74, 395-410
Collembolan species	Species, community analysis	Lack of knowledge available on their ecology and taxonomy. Difficult to identify.	Migliorini et al., (2005) Pedobiologia 49, 1-14
IndVal index	Species of various key invertebrates	Difficult to identify.	Nahmani and Rossi (2003) CR Biologies 326, 295-303
Folsomia candida reproduction test	Inhibition of the reproduction of collembolan by soil pollutants	Only useful when looking at high levels of contaminants. Unknown as to how sensitive the test is with a wide range of chemicals	Crouau and Moïa (2006) Ecotox & Env Safety
Enchytraeid reproduction test	Reproduction effects due to soil	Difficult to identify to species. Only survive in acidic soils-the experimental soils are alkaline. 6 week test duration.	Römbke and Moser (2002) Chemosphere 46, 1117-1140
Staphylinid beetles	Species diversity and specimen number	Provide no information about ecological characteristics. Difficult to identify to species.	Bohac (1999) Agric, Ecosy & Env 74, 357-372
Nematodes	PCR using nematode DNA to identify individuals to species	Expensive, time consuming, limited knowledge on nematode DNA	Floyd (2002) Molecuolar Ecology 11, 839-850
Nematodes: Maturity index	Classifies species to r or k strategists	Requires specialist knowledge.	Bongers (1990) Oeciologia 83, 14-19
Nematode (*Caenorhabditis elegans*)	Reproduction	Works best with contaminated soil	Bierkens et al. (1998) Chemosphere 37, 14-15
Woodlice	Biomass and species identification	Requires specialist knowledge.	Souty-Grosset et al., (2005) European Journal of Soil Biology 41,

			109-116
DNA adducts in earthworms	Count the number of adducts present	Only for use in assessing pollution.	Walsh et al., (1997) Soil Biology and Biochemistry 29, 721-724
Acute toxicity to earthworms	Place 10 worms in artificial soil spiked with test substance	Used for testing volatile substances	BS 7755-4.21:1994
Cricket (Gryllus bimaculatus)	LC 50 (i.e. 50 % death)	Unknown as to how sensitive the test is with a wide range of chemicals	Yoshimura, Endoh and Marada (2005) Ecological Indicators 5, 181-188
Gastropods	Inhibition of growth and reproduction	Only identifies heavy metal and pesticide pollution.	Cortet et al., (1999) Eur. J. Soil Biol.35, 115-134
Arachnids	Lethal and sublethal tests	Time consuming, difficult, only for use in assessing pollution.	Cortet et al., (1999) Eur. J. Soil Biol.35, 115-134
Isopods	Lethal tests	Only for use in assessing pollution.	Cortet et al., (1999) Eur. J. Soil Biol.35, 115-134
Ants	Species richness	Requires specialist knowledge	Lobry de bruyn (1999) Agri., Ecosy. Env 74, 425-441
Earthworm avoidance test	Avoidance to contaminated soil	Requires soil to be contaminated	Loureiro et al. (2005) Env. Poll. 138, 121-131
Isopod avoidance test	Avoidance to contaminated soil	Requires soil to be contaminated	Loureiro et al. (2005) Env. Poll. 138, 121-131
Maturity index for predatory mites	Classifies species to r or k strategists	Requires specialist knowledge	Ruf (1998) Applied Soil Eco. 9, 447-452
Acari : collembola ratio	Calculate total acari and collembolan and then use as a ratio	No difference between soils	Bachelier (1986) La vie animale dans le sol. ORSTOM, Paris, pp. 171–196.
Nematode family richness	Identify nematodes to family level	Requires specialist knowledge	Althoff and Thien (2005) J. of Teramechanics 42, 159-176
Plant parasitic nematode index	Identify number of nematodes that are plant parasitic	Requires specialist knowledge	Urzelai et al. (2000) The Sci. of the Total Env. 247, 253-261
Wasps	Accumulate metals as top of the food chain	Requires specialist knowledge, not directly in contact with soil	Urbini et al. (2006) Chemosphere In press
Vertebrate and invertebrate indicator species	Identify to species	Requires specialist knowledge, not all organisms directly in contact with soil, gives overall ecosystem health not just soil health	Hilty and Merenlender (2000) Biol. Conservation 92, 185-197

Plant species			
Astrantia major	Measure symptoms, intensity and appearance	Indicator of ozone pollution, species are not native to the UK ?	Manning and Godzik (2004) Environmental Pollution, 130, 33-39
Centuarea nigra	Measure symptoms, intensity and appearance	Indicator of ozone pollution, species are not native to the UK ?	Manning and Godzik (2004) Environmental Pollution, 130, 33-39
Centuarea scabiosa	Measure symptoms, intensity and appearance	Indicator of ozone pollution, species are not native to the UK ?	Manning and Godzik (2004) Environmental Pollution, 130, 33-39
Humulus lupulus	Measure symptoms, intensity and appearance	Indicator of ozone pollution, species are not native to the UK ?	Manning and Godzik (2004) Environmental Pollution, 130, 33-39
Hydrophytes	Uses species to quantify heavy metal pollution	Only show iron and manganese pollution, in wetland sediment	Demirezen and Aksoy (2006) Ecological Indicators 6, 388-393
Wheat	Seed germination, plant weight	Agricultural species not found on brownfield land	Dorn et al. (1998) Chemosphere 37, 845-860
Corn	Seed germination, plant weight	Agricultural species not found on brownfield land	Dorn et al. (1998) Chemosphere 37, 845-860
Wild oat	Seed germination, plant weight	Agricultural species not found on brownfield land	Dorn et al. (1998) Chemosphere 37, 845-860
Willow	Metal accumulator	Only indicator for metal pollution	Martens et al. (2006) Env. Poll. In press
Poplar	Metal accumulator	Only indicator for metal pollution	Martens et al. (2006) Env. Poll. In press
Rye	Seed germination, root elongation	Agricultural species not found on brownfield land	Plaza et al. (2005) Chemosphere 59, 389-396
Lettuce	Seed germination, root elongation	Agricultural species not found on brownfield land	Plaza et al. (2005) Chemosphere 59, 389-396
Maize	Seed germination, root elongation	Agricultural species not found on brownfield land	Plaza et al. (2005) Chemosphere 59, 389-396
Cress	Seed germination, root elongation	Agricultural species not found on brownfield land	Plaza et al. (2005) Chemosphere 59, 389-396
Wheat	Seed germination, root elongation	Agricultural species not found on brownfield land	Plaza et al. (2005) Chemosphere 59, 389-396
Cabbage	Seed germination, root elongation	Agricultural species not found on brownfield land	Plaza et al. (2005) Chemosphere 59, 389-396
Oat	Seed germination, shoot biomass	Agricultural species not found on brownfield land	Gorg et al. (2001) Chemosphere 44, 491-500
Turnip	Seed germination, shoot	Agricultural species not found on brownfield land	Gorg et al. (2001) Chemosphere 44,

	biomass		491-500
Broad bean	Genotoxicity	Works best with contaminated soil	Bierkens et al. (1998) Chemosphere 37, 14-15
Daubenmire technique	Use a quadrat to take visual estimates of the % bare soil at sites, grass and forbe dry biomass. Monitor over 1 y.	Brownfield sites have more than grass and forbes, therefore this technique does not take into account site use. Can not monitor site over 1 y.	Althoff and Thien (2005) J. of Teramechanics 42, 159-176
Microbes			
Algae	Species richness	Difficult to identify to species	Zancan, Trevisan and Paoletti (2006) Agric, Ecosy & Env112, 1-12
Biolog	Microbial degradation of 95 different carbon sources is measured	Very expensive, lag phase while cells grow may lead to false negatives. Substrates may be dominated by one species. Substrates may not be ecologically relevant.	Hill et al., (2000) Applied Soil Ecology 15, 25-36
Protozoa	Species richness	Requires specialist knowledge.	Wilkinson and Davis (2000) Acta Protozool 39, 23-26
Microbial phosphomonoesterase activity	Microbial activity	No difference between soil from contaminated and clean sites	De Mora et al. (2005) Applied Soil Ecol. 28, 125-137
Plate counting	Assess diversity and numbers	Culture 1 % microbes present in soil, favours fast growing species, may initiate the growth of spores.	Kirk et al., (2004) Journal of Microbiological Methods 58, 169-188
Buried glass slide technique	Percentage fungal growth (hyphae)	No difference between soils	Rossi et al. (1936) Soil Sci. 41, 53-66
Fatty Acid Methyl Ester Analysis (FAME)	Assess community diversity	Requires a high amount of material, results may be confounded by other micro organisms	Kirk et al., (2004) Journal of Microbiological Methods 58, 169-188
Ergosterol	Measure fungal biomass	Fairly limited knowledge about if it estimates the true fungal biomass	Ruzicka et al., (2000) Soil, Biology and Biochemistry 32, 989-1005
Copiotrophs:oligotrophs	Ration of fast to slow growing culturable microbes.	Results may be skewed by fast growing species. Can only culture 1 % of soil dwelling microbes.	Van Bruggen and Semenov (2000)
Mycorrhizal activity on roots	Species identification for arbuscular mycorrhizae and *Rhizobium*	Requires specialist knowledge	Schloter et al., (2003) Agri., Ecosyst., Environ. 98, 255-262

Ammonium oxidation	Measures potential nitrification and inhibition of nitrification	No difference between soils	ISO 15685
Microtox ® toxicity assay	Luminescent bacteria are used to measure toxicity	Works best with contaminated soil	Plaza et al. (2005) Chemosphere 59, 389-396
Spirotox test	Sublethal and lethal effects of soil of protozoa	Works best with contaminated soil	Plaza et al. (2005) Chemosphere 59, 389-396
Ostracodtoxkit ™	Uses neonates of the freshwater ostracod crustacean, lethal effects	Not very widely used for soil	Plaza et al. (2005) Chemosphere 59, 389-396
Umu test	Metabolic activation of the bacterium *S. typhimurium* measurement of DNA damage	Requires specialist knowledge	Oma et al. (1985) Mutat. Res. 147, 219-229
FDA hydrolysis	Hydrolysis of a compound by microbial enzymes	Lower limit of quantification of approximately 10^8 cells not practically useful.	Leon et al. (2006) Applied Soil Ecol. 31, 199-210
Microbial heterotrophic potential	Measure mineralisation of ^{14}C labelled organic substrate added to the soil	Expensive for compound and requires a specialist laboratory	Scour et al. (1986) Appl. Environ. Microbiol. 1028-1035
β glucosidase activity	Measures total microbial activity	Metabolisable organic carbon may interfere with the assay	Leon et al. (2006) Applied Soil Ecol. 31, 199-210
Arysulfatase activity	Measures total microbial activity	Can underestimate microbial activity	Leon et al. (2006) Applied Soil Ecol. 31, 199-210
Urease activity	Measures total microbial activity	Can underestimate microbial activity	Cepeda et al. (2000) Soil Biol. And Biochem. 32, 1867-1875
Substrate induced respiration	Add an isotope labelled glucose solution to soil and monitor respiration	May be expensive, and if radiolabelled requires specialist laboratories	Brohon et al. (2001) Soil Biol. And Biochem. 33, 883-891 ISO 14240-1
Fluorescein diacetate hydrolysis	Measures total microbial activity	High and low pHs interfere with the assay	Brohon et al. (2001) Soil Biol. And Biochem. 33, 883-891
Esterase inhibition	Uses *Raphidoceles subcapitata*	Works best with contaminated soil	Bierkens et al. (1998) Chemosphere 37, 14-15
Lumistox test	Luminescent bacteria are	Works best with contaminated soil	Brohon et al. (2001) Soil Biol. And

	used to measure toxicity		Biochem. 33, 883-891
Met Plate assay	Bacterial degradation of a substrate, resulting solution is filtered and analysed by a spectrophotometer	Measure heavy metal toxicity	Brohon et al. (2001) Soil Biol. And Biochem. 33, 883-891
ToxiChromo test	Tests for toxicants	Tests for toxicants/contamination	Juvonen et al. (2000) Ecotox. And Env. Safety 47, 156-166
BioTox test	Luminescent bacteria are used to measure toxicity	Works best with contaminated soil	Juvonen et al. (2000) Ecotox. And Env. Safety 47, 156-166
RET assay	Effects of toxic compounds on NAD reduction by sub mitochondrial particles	Requires specialised knowledge, outdated and can be expensive	Juvonen et al. (2000) Ecotox. And Env. Safety 47, 156-166
Mutatox test	Luminescent bacteria are used to measure toxicity	Works best with contaminated soil	Juvonen et al. (2000) Ecotox. and Env. Safety 47, 156-166
Alcaligenes eutrophus	Sensitive to metals	Works best with contaminated soil	Bierkens et al. (1998) Chemosphere 37, 14-15
Salmonella typhimurium	Genotoxicity sensor	Works best with contaminated soil	Bierkens et al. (1998) Chemosphere 37, 14-15
Photobacterium phosphoreum	Toxicity sensor	Works best with contaminated soil	Bierkens et al. (1998) Chemosphere 37, 14-15
Presence of *Rhizobium*	As a sensitive keystone species. E.g. microbial techniques or identification of root nodules	Formation of root nodules on plants takes a number of weeks. Microbial techniques for solely identifying *Rhizobium* are not widely used.	Van Bruggen and Semenov (2000) Applied Soil Ecol. 15, 13-24

APPENDIX C

Joshua Dodd

225169

Honours Project
2006

Variability in earthworm populations at a brownfield site, South Liverpool and the effects of soil compaction on *Lumbricus terrestris*.

Supervisor: Prof. Nick Dickinson

Abstract

A 22-day laboratory experiment investigating the effects of soil compaction on the behaviour of the earthworm *Lumbricus terrestris* was carried out alongside fieldwork examining the same subject and also the variability in earthworm population numbers on a brownfield site in South Liverpool. The lab work utilised sets of soils compacted to varying degrees with pre-weighed sets of earthworms added to them. The fieldwork was carried out during February 2006 and sampled earthworm numbers across an open, grassy area of land using a pocket penetrometer to measure soil compaction at each sample site. The laboratory experiment found *Lumbricus terrestris* to be negatively affected by compaction and in some cases caused *Lumbricus terrestris* to exclude itself from the soil choosing to live either on the surface of the soil of between the side of the container and the soil. The fieldwork uncovered little variability in population sizes but the results regarding soil compaction and earthworm numbers supported those obtained in the laboratory work.

Introduction

Earthworms are a key element in the functioning of a healthy soil system. They have many crucial roles including facilitating soil mixing and water infiltration and solute movement into the soil profile (Bastardie *et al.*, 2005). Earthworms also have strong influences over organic matter decomposition (Dunger and Voigtländer, 2005). In fact, worms have such serious applications in soil health that they can be considered one of, if not *the* most important invertebrate living in soils (Edwards and Bohlen, 1977).

Due to the pressures facing the Government regarding environmental issues it is becoming more and more commonplace for Brownfield land to be remediated, re - developed and used rather than sourcing previously undeveloped greenfield land. Brownfield land can be remediated for either hard (e.g. residential developments) or soft (e.g. recreation ground) end uses, but arguably the most important feature of a remediation project is sustainability. It is important for a remediation project not to require further attention after the project has finished as this can result in un – quantifiable costs being incurred. With respect to sustainable soil health, earthworms are a vital component. They perform constant ‘ecosystem services’ that are of considerable value (Doran and Zeiss, 2000).

For these reasons it is beneficial to encourage worms to live in a soil system. There are various features of a soil that have a direct effect on the presence or absence of earthworms. These factors include, but are not limited to, soil pH, organic matter content, presence/ absence of broadleaved trees (Ammer *et al*, 2005), soil temperature and moisture (Eriksen-Hamel and Whalen, 2006), soil nutrient content and level of soil compaction. In order to encourage the presence of earthworms knowledge of these factors and their influences is crucial. This report focuses on the impact soil compaction has on earthworms.

There is a variety of techniques that can be applied to earthworm sampling in the field. These techniques include earthworm collection using mustard and formalin solutions (Gunn, 1992; Zaborski, 2003; Chan *et al*, 2000) and standard digging and hand sorting. There is also literature to be found relating to time saving, ‘time – limited’ soil sorting (Schmidt, 2000). Although this method of soil sorting saves time it only provides an estimate of earthworm numbers. Since the fieldwork in this report only deals with relatively small numbers of earthworms, use of a time limited method is not relevant.

The aim of this work is to examine the spatial variability in earthworm populations and find how many samples are required in order to gain an accurate overview of the earthworm population of a given area. The method of sampling used in this work is a standard digging and hand sorting technique, chosen for its simplicity. This report also aims to find the effects of soil compaction on the behaviour of the earthworm *Lumbricus terrestris.*

Figure 1: Map with location of Childwall Fields marked by black arrow.

Methods and study site

Study site

Field sampling of various earthworm species took place across an 8.7 ha area of brownfield land in Childwall, South Liverpool. Liverpool City Council originally used the site for municipal landfill until the early 1970's when the depth of waste reached approximately 7m. The landfill was formed into three stepped, terraced tiers: top, middle and bottom. Since the landfill closed it has been remediated to a soft end use (recreation ground) by topping with clay rich material covered with a sandy loam topsoil. This was followed by the planting of various species of trees (Rawlinson, 2002). The vegetation cover is a combination of trees, shrubs and grasses. See Figure 1 for location.

Fieldwork

Fieldwork was carried out on two separate dates, preliminary work carried out on the 7th October 2005 and further work on the 11th February 2006 at Childwall Fields, Childwall, South Liverpool. On both occasions, worms were sampled by digging quarter meter square holes (measured with a quadrat) with a garden spade. The holes were

dug each time to a depth of 28 cm. This measurement was used for convenience, as it was the length of the spade head.

The objective of the preliminary work was to find the most successful sampling sites to use in the further work on the 11th February. For this reason, during the preliminary work, sampling of earthworms took place on all three of the tiers. 5 sample holes were dug on the middle tier, 3 on each of the top and bottom tiers. This inconsistent sampling pattern was used due to time limitations. Sampling was carried out at random across contrasting areas such as on bare soil, open grass and the base of trees. On the 11th February sampling took place on only the middle tier on randomly selected areas selected for their similarity in being open and grassy. The middle tier was used due to the greater range and volume of earthworm numbers recorded in the preliminary work. The open, grassy areas were selected for their ease of sampling.

The contents of each quarter metre square hole was placed straight from the hole onto a polythene sheet in order to prevent any worms escaping down into the ground or surrounding vegetation. This soil was then carefully hand sorted to remove all worms present. The worms collected from each hole were placed in labelled polythene bags and returned to the laboratory. During the fieldwork carried out on the 11th February measurements of compaction were taken of the surface of each square using a pocket penetrometer. The pattern of penetrometer readings for each hole is shown in figure 2.

In the laboratory the worms were divided into one of three classes: anecic (live in permanent, vertical burrows, these can be identified by their black head), epigeic (live in the top soil due to their poor burrowing capability, identified by their small size and deep pigmentation) and endogeic (live in complex, lateral burrows and identified by their pink colour). The number and weight of the worms in each group were recorded.

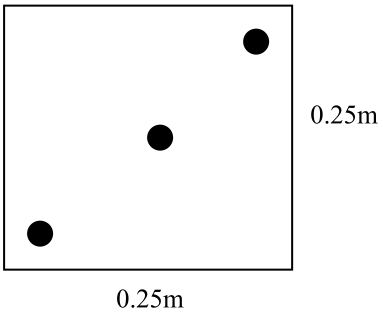


Figure 2: Diagram of penetrometer sampling pattern on 0.25m^2 earthworm sample holes. Black spots represent penetrometer sampling points.

Laboratory - based experiment

Soil collected at Childwall Fields was air-dried and sieved using a 4mm sieve. It was then spread thinly across three trays and moistened with a spray gun filled with de-ionised water. The contents of the three trays were then mixed and allowed to stand over night in a polythene bag in order to ensure consistent moisture levels throughout each box.

12 'Tupperware' style boxes with sealing lids measuring 18.5 x 14.5 x 10 (length x width x depth, cm) were washed with de-ionised water and each filled with 1500g of the moistened Childwall soil. These boxed soils were then compacted using a hydraulic press under the help and guidance of Clive Eyre, Faculty of Technology and Environment, to four different degrees of compaction with three repeats of each compaction. Counting the number of 'pumps' of the hydraulic press controlled the degree of compaction. The average soil volume before compaction was 1689.9cm^3. This un-compacted soil was denoted soil '4'. The average volume of the most compacted soil was 1242.15cm^3; this was denoted soil '1'. 22 pumps of the press were used to compact soil 1. The next soil up had an average volume of 1390.2cm^3 and was denoted soil '2'. 11 pumps of the hydraulic press were used to compact this soil. The average volume of soil '3' was 1527.1cm^3, 5 pumps were used to compact this soil. Each of the three repeats of soils 1 – 4 was labelled 'A', 'B' and 'C'. The maximum compaction was determined by compacting one soil until the box broke.

Single sets of 10 *Lumbricus terrestris* (anecic class) each were weighed and one set added to the top of each box. Three air holes were drilled in each lid and the lids were firmly placed on each box. The boxes were then placed in a cold room for a period of three weeks and one day.

Upon removal from the cold room the number of worms on the surface, in the soil and between the soil and the side of the box were separately recorded along with total weight of worms alive. A measure of soil disturbance was documented using a subjective scale of 1 – 5. 1 being undisturbed, 5 being maximum disturbance. Any other apparently significant observations or changes were also recorded.

Statistical analysis

The data were statistically analysed using the Minitab software package. All data were first tested for normality using the Anderson – Darling normality test. If the data were found to be non – normally distributed (non – parametric) then it was ranked and then had the Pearsons correlation test carried out on it. If the data was normally distributed (parametric) then it had the Spearmans correlation test

carried out on it. Correlation numbers nearest to 1 showed strong correlation, P – values below 0.05 showed the data to be statistically significant.

Results

The middle tier of the landfill presented the greatest range of earthworm species, the top tier presents the poorest range with no epigeic species found here at all. The largest number of any one species is found at ‘bottom, open grass’, where 50 endogeic earthworms were recorded. The middle tier has the greatest volume of earthworm numbers in comparison with the top and bottom tiers (Fig 3).

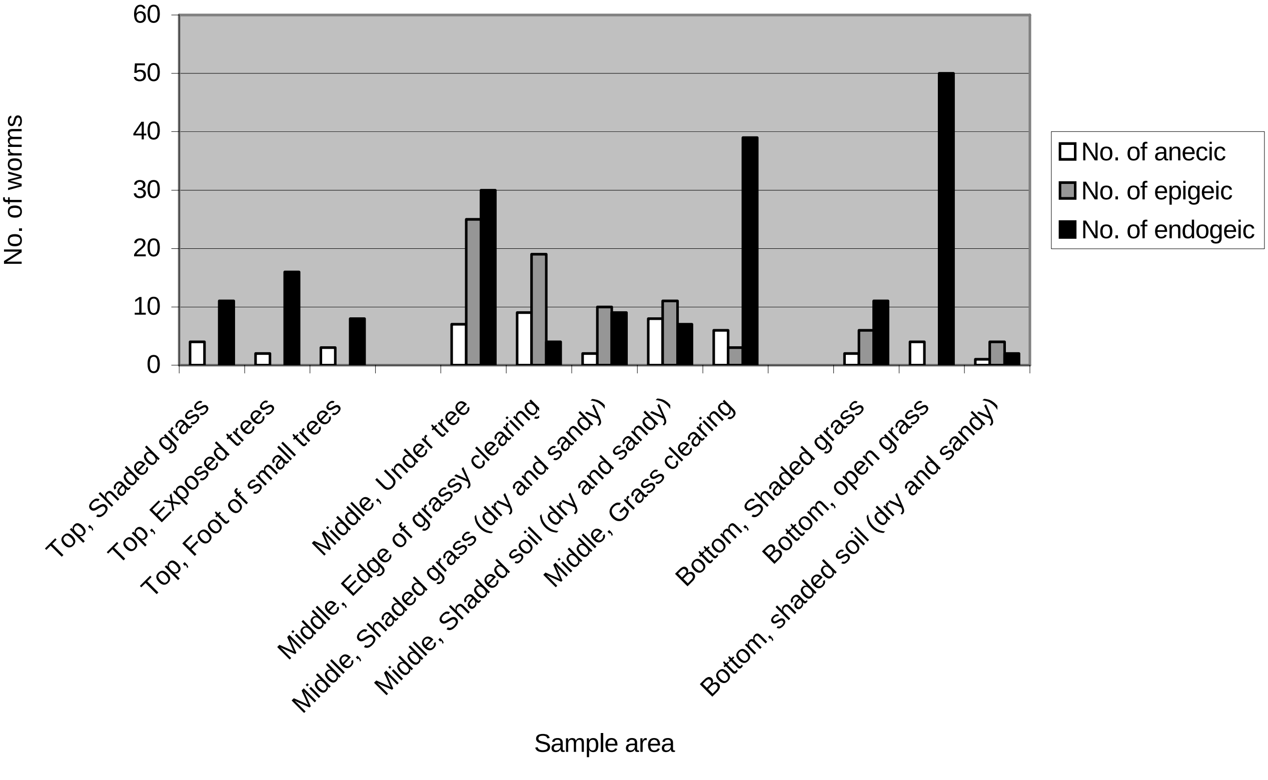


Figure 3: Data from preliminary fieldwork carried out on Childwall fields.

The highest penetrometer reading was found at sample 6 (2.97 Kg/ Sq cm). At this sample site no anecic earthworms were found. The lowest penetrometer reading was found at sample 1 (1.13 Kg/ Sq cm). There were 3 anecic earthworms found here. The range of number of earthworms between sample sites is 4 (Fig 4). There was a strong negative correlation between both number of anecic earthworms and weight of anecic earthworms with average penetrometer reading (Table 2). Both these correlation figures have P – values below 0.05 and so were significantly different.

There were 16 endogeic earthworms found at the lowest penetrometer reading (1.13 Kg/ Sq cm). At the highest penetrometer reading (2.97 Kg/ Sq cm) there were 15 endogeic earthworms found. The range of earthworm numbers between sample sites is 8 (Fig 5). The statistical data shows a strong negative correlation between both number and weight of endogeic earthworms and average penetrometer reading (Table 2). Although still strong correlations, these figures are comparatively lower than correlation figures found for the anecic earthworms. The P – values for both number and weight of the endogeic earthworms are below 0.05 and so are statistically significantly different.

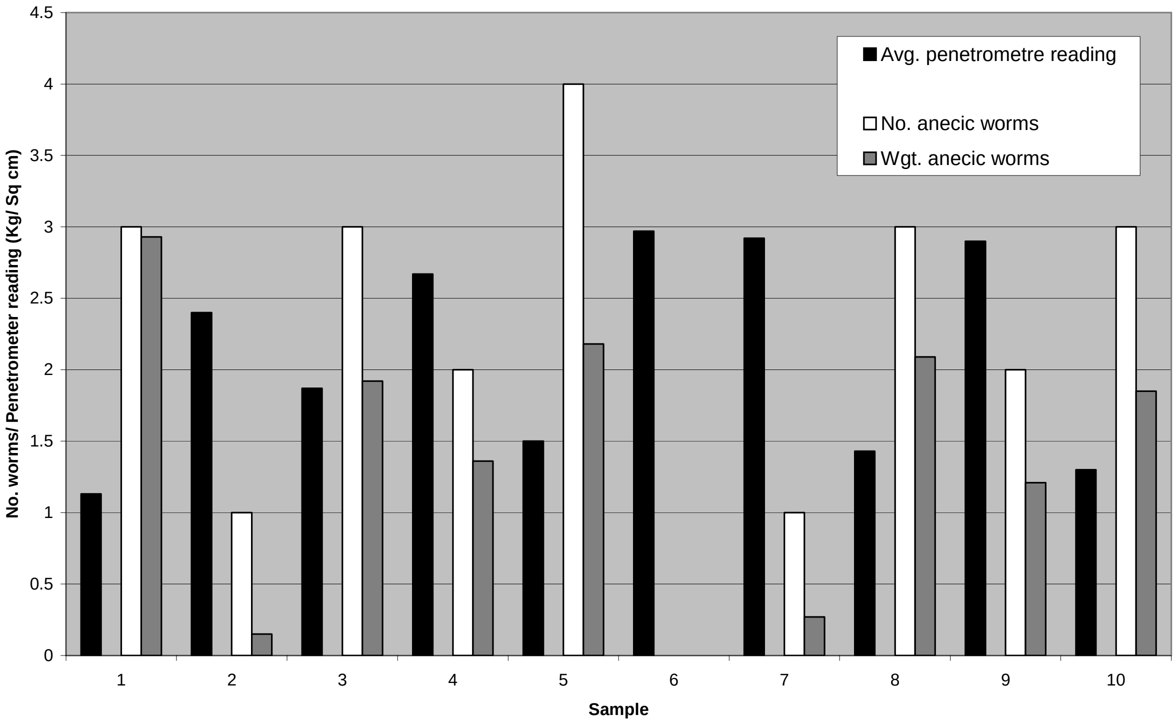


Figure 4: Anecic earthworm data coupled with penetrometer data collected during fieldwork at Childwall fields.

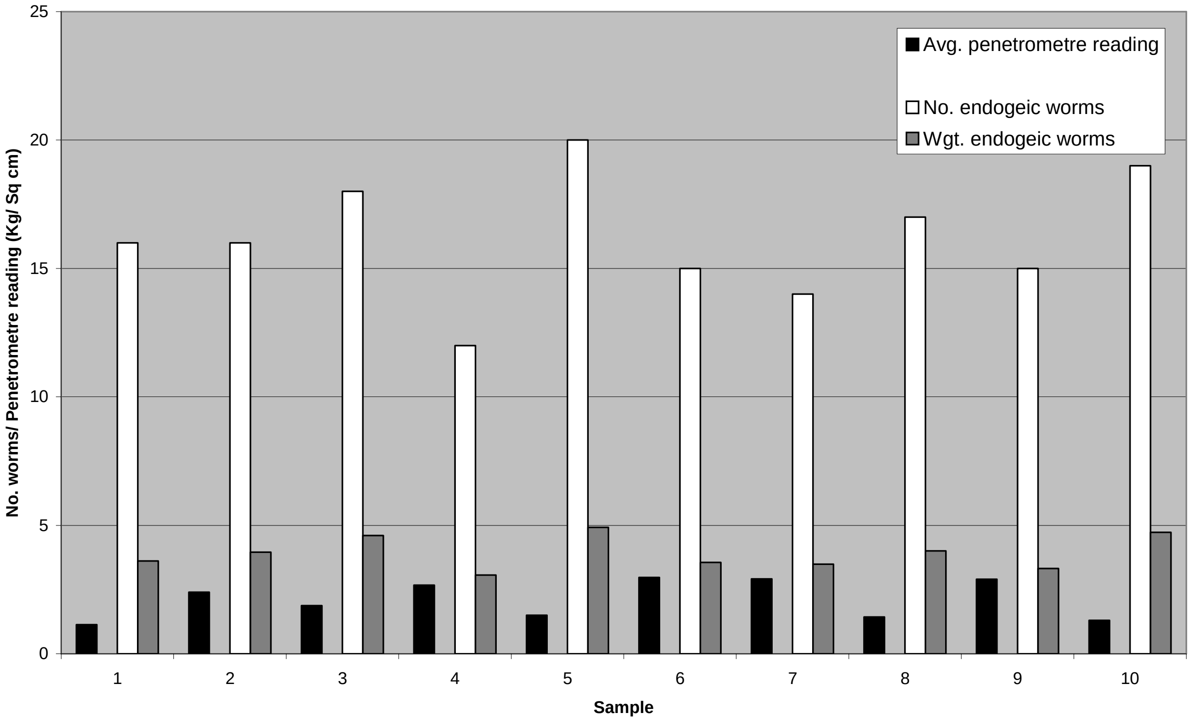


Figure 5: Endogeic earthworm data coupled with penetrometer data collected during fieldwork at Childwall fields.

There were considerably fewer epigeic earthworms recorded than anecic and endogeic. The highest and lowest penetrometer readings correspond to epigeic earthworm numbers of 0. The range of earthworm numbers between sample sites is 0 (Fig 6). The statistical results show weak positive correlations but also P – values greater than 0.05 meaning that this data cannot be considered statistically significant (Table 2).

100% of earthworms survived in all boxes with the exception of boxes 4B and 4C where 3 and 5 worms died respectively (Fig 7). There is a relatively strong positive correlation between number of earthworms alive and soil compaction but the P – value is above 0.05 and so the results are not statistically significant (Table 3).

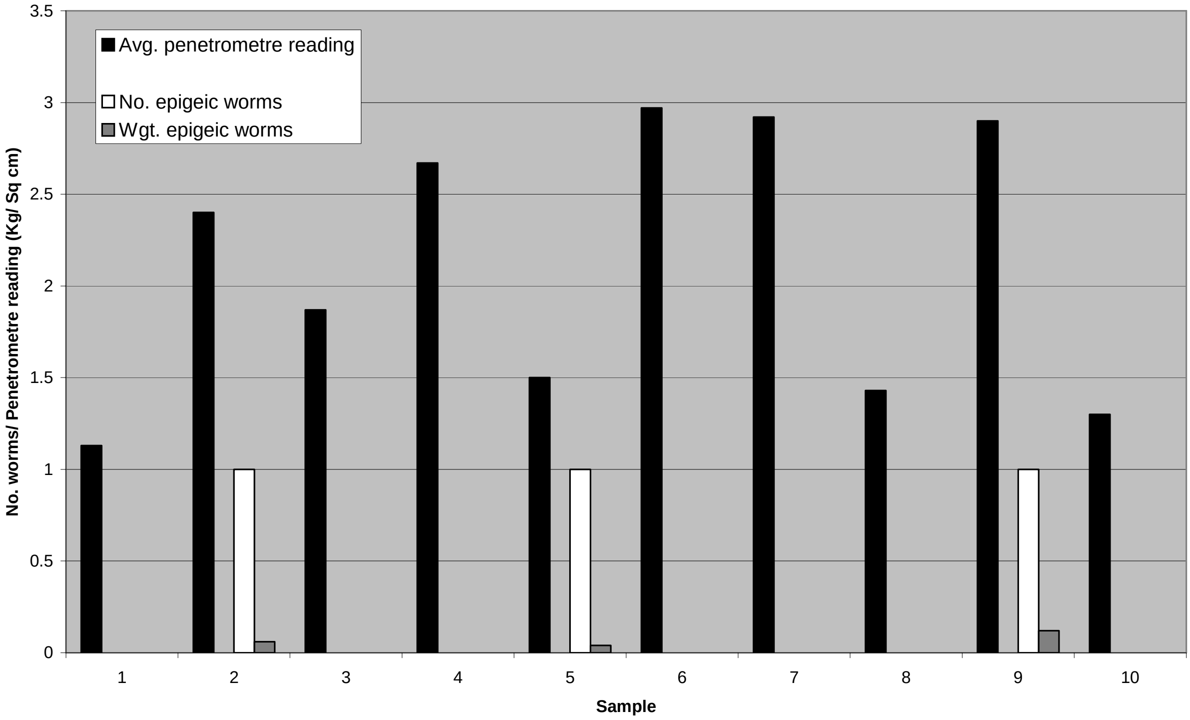
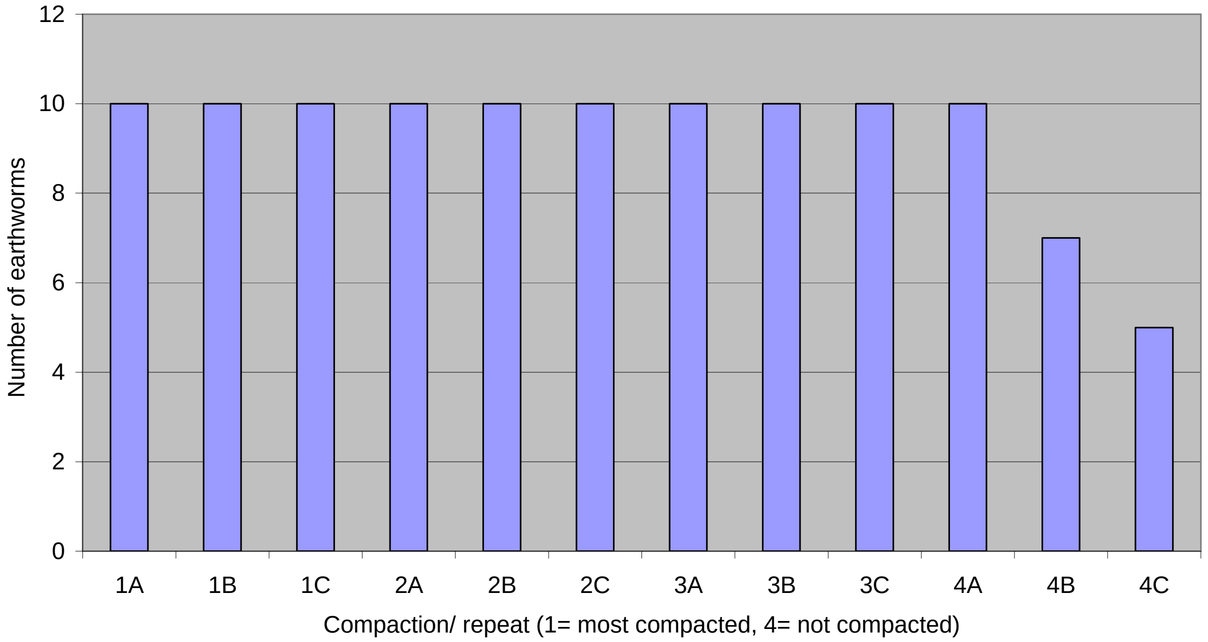


Figure 6: Epigeic earthworm data coupled with penetrometer data from fieldwork at Childwall fields.
Figure 7: Number of earthworms found alive in each box at end of 22-day lab based experiment.



There was a general trend of more worms found on the surface of the soil in the more compacted boxes. There were no worms found on the surface in boxes 3A and 4C. The greatest number of worms found on the surface is 3. These worms were found in box 1A, one of the three most compacted soils (Fig 8). A particularly strong positive correlation can be seen between worms found on the surface of the soil and compaction. The P – value shows these results are statistically significant (Table 3).

The greatest number of worms found in the soil were 9. These worms were found in box 4A, one of the three un compacted soils. The least worms were found in box 1A, the number of worms found here being 4; the soil in box 1A was one of 3 most compacted soils (Fig 9). There is a statistically insignificant negative correlation between worms found in soil and compaction (Table 3).

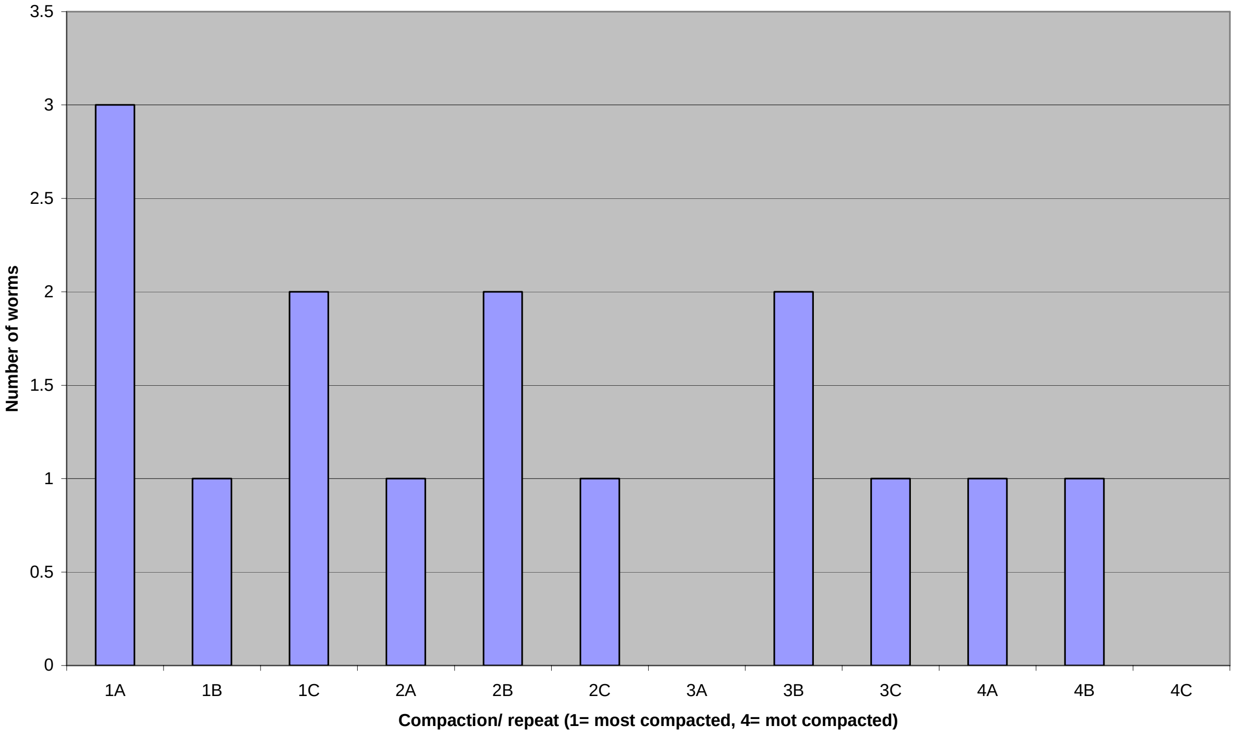


Figure 8: Number of earthworms found alive on surface of soil at end of 22-day lab based experiment.

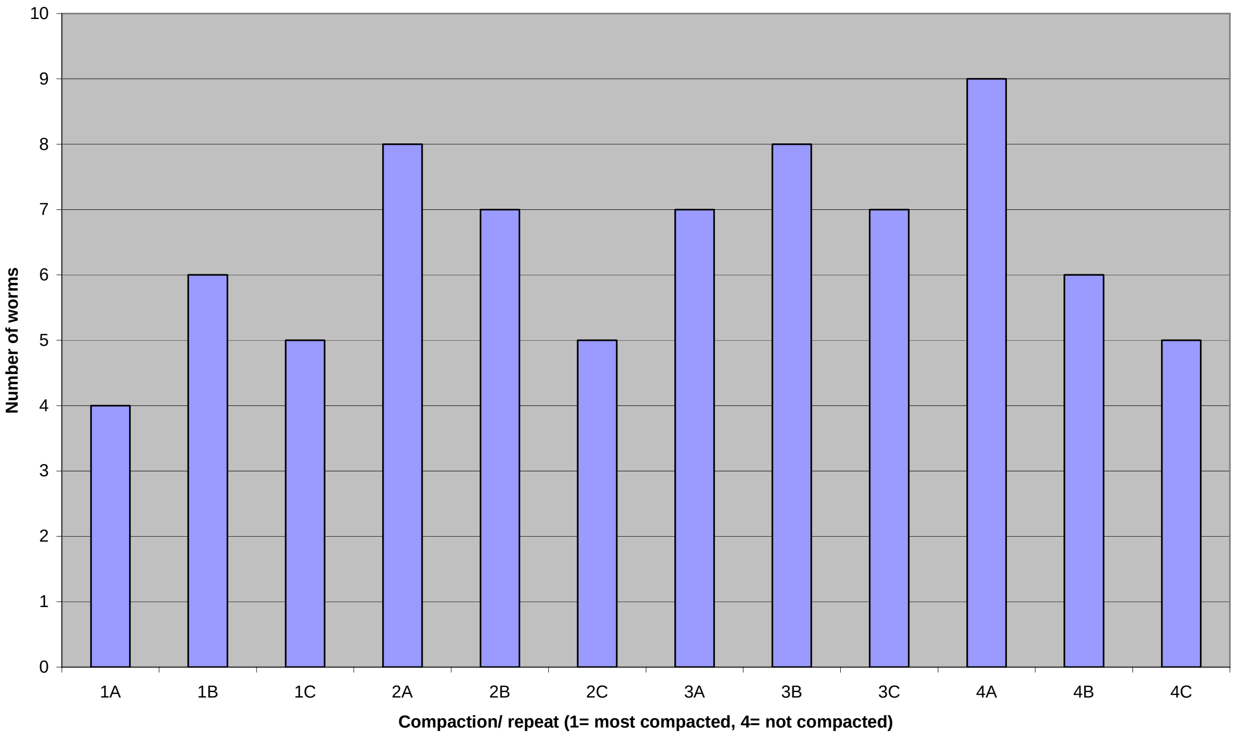


Figure 9: Number of earthworms found alive in soil at end of 22-day lab based experiment.

There were no worms found down the side of the box in the un compacted boxes (4A, 4B and 4C). The highest number of worms found down the side of a box was 4 in box 2C. Generally, more worms were found down the side of the box in the more compacted soils (Fig 10). The results from the statistical analysis show a strong statistically significant positive correlation between number of worms found down the side of the box and compaction (Table 3).

All three of boxes in sets 1 and 2 had negative weight changes and all three of the number 3 boxes had positive changes. Box 4A had a positive weight change, boxes 4B and 4C had negative weight changes. Generally – the more compacted soils show worm weight loss and the less compacted soils show worm weight gain with the exception of boxes 4B and 4C (Fig 11). There was a weak positive correlation between weight change and compaction that is statistically insignificant (Table 3).

With respect to soil surface disturbance, there was a pattern of more disturbance as soil compaction is increased. The more compacted boxes (boxes 1A – 1C and 2A – 2C) displayed smooth surface areas frequently interspersed with soil lumps and casts, the number 1 boxes having more lumps and casts than the number 2 boxes. All of the number 1 boxes had relatively clean lids with little soil spray present compared to boxes 2, 3 and 4. There was mould growth present in boxes 4B and 4C (Table 1).

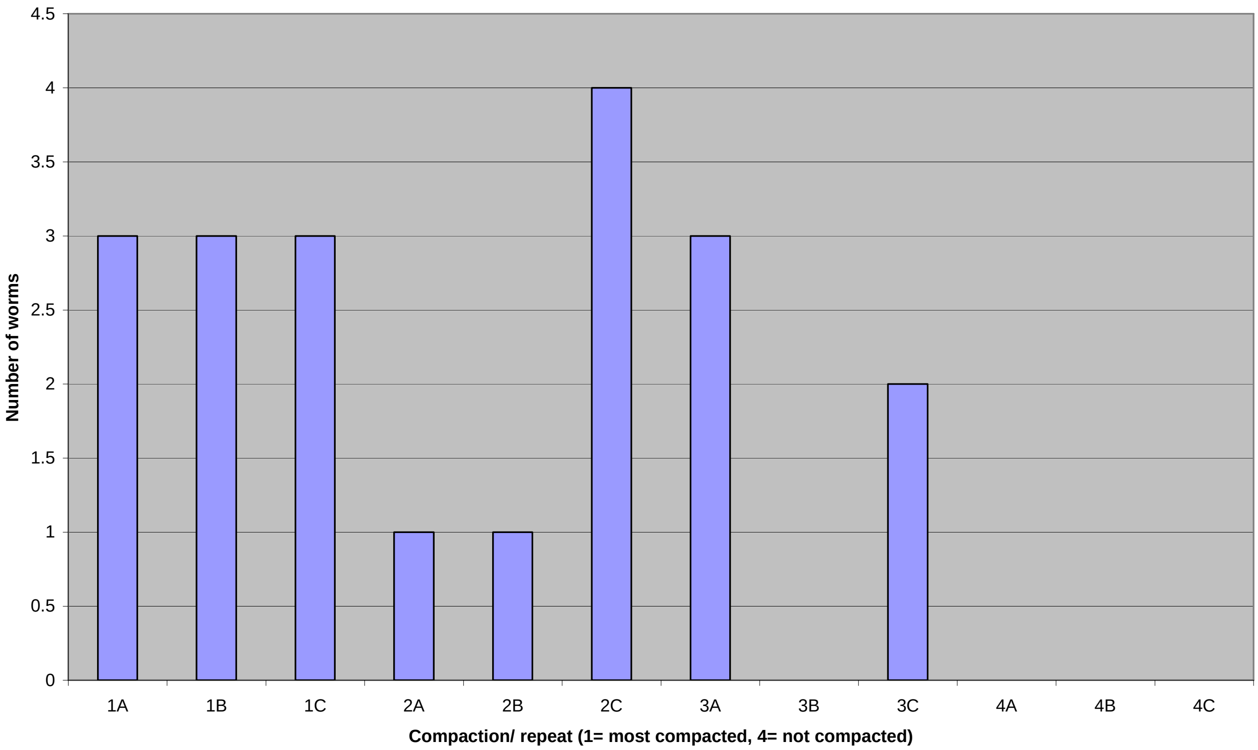


Figure 10: Number of earthworms found alive between side of box and soil at end of 22-day lab based experiment.

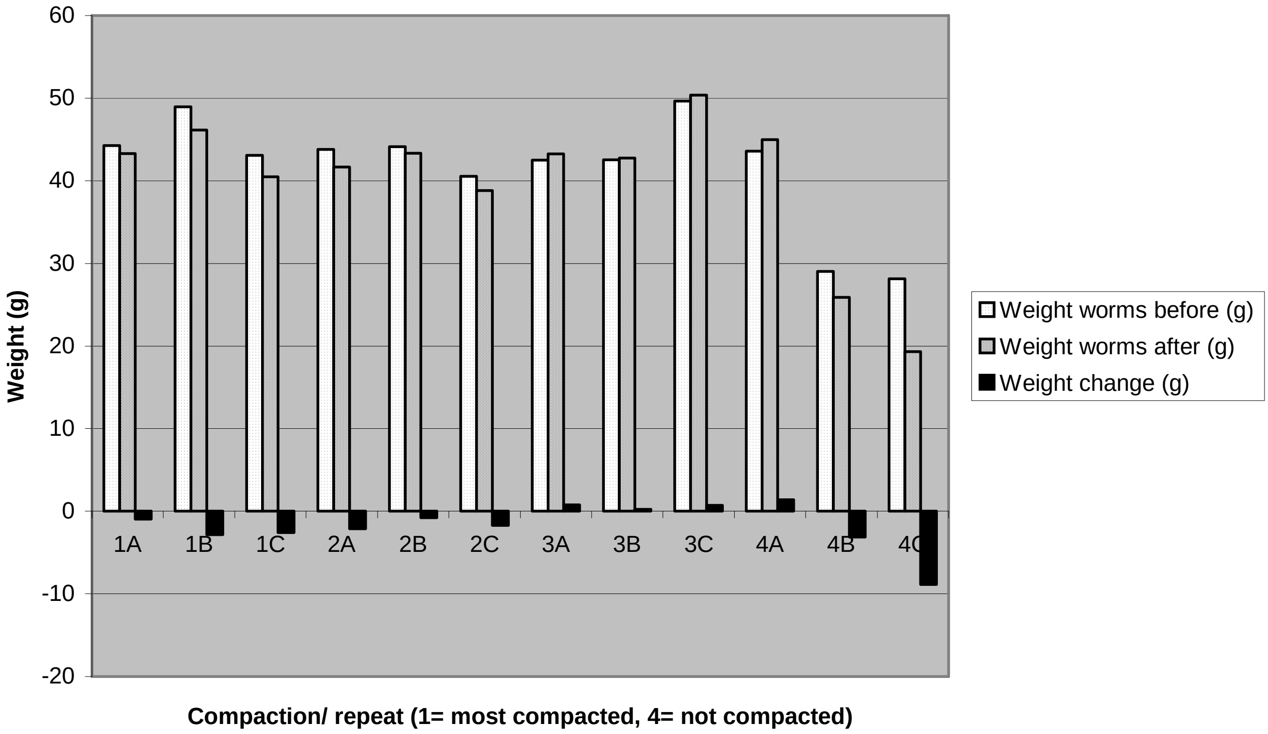


Figure 11: Graph of weight change of earthworms at end of 22-day lab based experiment.

Table 1: Interpreted soil surface disturbance from 22-day lab based experiment expressed numerically and qualitatively. Other significant notes about features of the soil and soil boxes also included.

Box number/ Repeat	***1A***	***1B***	***1C***	***2A***	***2B***	***2C***	***3A***	***3B***	***3C***	***4A***	***4B***	***4C***
Soil disturbance (1 = none, 5 = maximum)	*4* Areas of smooth compaction still present (more smooth area than in box 2). Less soil lumps, more casts than in box 2.	*4* ***Areas of smooth compaction still present (more smooth area than in box 2). Less soil lumps, more casts than in box 2.***	*4* ***Areas of smooth compaction still present (more smooth area than in box 2). Less soil lumps, more casts than in box 2.***	*4* Areas of smooth compaction still present.	*4* Areas of smooth compaction still present.	*4* Areas of smooth compaction still present.	*3* More casts and lumps of soil than in box 4.	*3* ***More casts and lumps of soil than in box 4.***	*3* ***More casts and lumps of soil than in box 4.***	*2* Fewer casts than in boxes 4B and 4C.	*3*	*3*
Other notes	***Inside of lids comparatively clean. Very little soil spray present.***	***Inside of lids comparatively clean. Very little soil spray present.***	***Inside of lids comparatively clean. Very little soil spray present.***	***Soil spray present on inside of lid.***	***Soil spray present on inside of lid.***	***Soil spray present on inside of lid.***	***Soil spray present on inside of lid.***	***Soil spray present on inside of lid.***	***Soil spray present on inside of lid.***	***4 seeds germinated on soil surface.*** Soil spray present on inside of lid.	***Mould growth present.*** Soil spray present on inside of lid.	***Mould growth present. 3 dead and rotting worms on surface.*** Soil spray present on inside of lid.

Statistical results

Table 2: Table of results showing correlation figures for number and weight of earthworm species compared to penetrometer readings from fieldwork. Numbers in bold ***= Pearson correlation value, numbers in*** **italics** ***= P – Value.***

	Avg. penetrometer reading
No. anecic worms	**- 0.824** *0.003*
Wt. anecic worms	**- 0.852** *0.002*
No. endogeic worms	**- 0.724** *0.018*
Wt. endogeic worms	**- 0.659** *0.038*
No. epigeic worms	**0.147** *0.685*
Wt. epigeic worms	**0.230** *0.523*

Table 3: Table of results showing correlation figures for lab based experiment results – numbers of worms and weight change, compared to compaction. Numbers in bold ***= Pearson correlation value, numbers in*** **italics** ***= P – Value.***

	Compaction
Avg. No. worms in soil at end	**- 0.742** *0.258*
Avg. No. worms on surface at end	**0.974** *0.026*
Avg. No. worms alive at end	**0.775** *0.225*
Avg. No. worms Down side of box at end	**0.963** *0.037*
Avg. weight change at end	**0.160** *0.840*

Discussion

Regarding the data collected from the laboratory based study (Figs 7 – 11 and Table 1); soil compaction does indeed have an impact on the behaviour of *Lumbricus terrestris*. Increased soil compaction has a negative impact on earthworm health. This is shown by the weight losses of the worms in all of boxes 1 and 2 (Fig 11). However, in boxes 4B and 4C where weight gains were expected, weight losses were recorded, disturbing the pattern. These weight losses are most probably the result of unhealthy worms from a different batch to those used for all the other boxes being mistakenly used. Although it is impossible to say with conviction without further work, it is predicted that, should healthy worms have been used in boxes 4B and 4C, positive weight changes would have been recorded in these boxes along with a strong negative correlation between weight change and compaction, as opposed to the weak positive correlation recorded (Table 3). The positive correlation recorded is statistically insignificant.

Increasing compaction also had a variety of other effects on earthworm behaviour. The more compacted soils had fewer earthworms incorporated within them (Figs 8 – 10). Instead, the earthworms were found either on the surface of the soil (Fig 8) or between the side of the box and the soil (Fig 10). Earthworm exclusion from compacted soils is a phenomenon that can be found in published literature (Birkas *et al*, 2004). Soil compaction also had an effect on earthworms in the field (Figs 4 - 6 and Table 2). Here soil compaction appeared to affect the different classes of earthworms differently. The anecic and endogeic earthworms were negatively affected, while the epigeic seemed to be positively affected. Although the positive correlation between penetrometer reading and number and weight of epigeic earthworms cannot be considered statistically significant in the present study (the P-value is above 0.05) it is possible that this result may be truthful. The manner in which epigeic earthworms live in the top section of the soil and possess only poor burrowing capabilities could mean that epigeic earthworms are largely unaffected by soil compaction, whereas the endogeic and anecic earthworms suffer, since they have to push and eat their way through the compressed soil profile (Hansen *et al*, 1999).

In the laboratory based experiment, differences were observed between the sets of boxes relating to the disturbance of the soil surface (Table 1). The un compacted soils (boxes 4A – 4C) showed little disturbance, with the soil surfaces looking almost exactly as they did when the boxes were sealed. The increased disturbance in the more compacted boxes was most likely the result of the increased burrowing effort required by the earthworms in the more compacted soils. Burrowing in the compacted soil would probably mean the earthworms had to expel the soil to the surface rather than just push the soil out the way as would be possible in the non-compacted soil.

There were also observed differences in the state of cleanliness of the inside of the box lids (Table 1). It appears from this data that the action of burrowing by the earthworms causes soil to be sprayed upwards; the soil on the insides of the lids suggests this. There was no water on the surface of the soils that might have been splashed upwards when the earthworms were placed in the boxes and the earthworms did not come into contact with the lids at this time, both of which might provide an explanation for soil on the lids. In boxes 1A – 1C however there was little soil on the inside of the lids. These observed differences suggest that burrowing in compacted soil might have an effect on some aspect of the earthworms burrowing mechanism. The effect and mechanism is not clear from these results

The presence of mould growth in boxes 4B and 4C is most probably linked to the death of earthworms in these boxes. Likewise, the reduction in number of casts in boxes 4B and 4C is also probably linked to the death of the earthworms – fewer earthworms equates to less burrowing activity.

When considering the variability in earthworm populations on the middle tier of the experimental site it is fair to say there is little variation. The greatest range between sampling sites is found with the endogeic earthworms (Fig 5), the range is 8. This suggests that an accurate picture of the earthworm population in open, grassy areas on the middle tier is obtained with few samples. However, it is not possible to say this with certainty without first taking a larger sample set and comparing it with the smaller one to see if there are any discrepancies. This sampling method does not account for variability between different types of habitat either. From the preliminary results (Fig 3) it is clear that there are differences between the sizes of earthworm populations found, for example, at the base of a tree and on bare exposed land.

It is extremely difficult to make a definite statement about the number of samples needed to give an accurate view of the earthworm population of a given area without first carrying out further investigative work. The data from Childwall fields proves this (Figs 3 – 6). The factors governing spatial distribution of earthworms are many and are highly likely to differ depending on the size of the site considered (Rossi, 2003).

Conclusion

Some clear patterns emerge from this work displaying the impacts of soil compaction on *Lumbricus terrestris*. *Lumbricus terrestris* is negatively impacted upon by soil compaction and often chooses to remove itself from a compacted soil profile. Because earthworms are an important part of a healthy soil system, this finding has implications in sustainable brownfield remediation. Soil compaction is an important consideration with respect to the presence and health of earthworm populations. Also, the action of earthworms burrowing into a compacted soil compared to a non-compacted soil results in greater disturbance of the soil surface. At Childwall Fields, South Liverpool, there is little variability in earthworm numbers across open and grassy areas.

Due to time constraints and some human error, there are irregularities in some patterns that warrant the need for further work. This further work should include: re-doing the laboratory based experiment with a greater number of repeats in order to clearly identify anomalies (such as earthworm deaths) and setting up the same laboratory based experiment using epigeic instead of anecic earthworms, to see the effect of compaction on this group of earthworms. The lack of a sound explanation for the absence of soil on the inside of box lids 1A – 1C means a deeper examination into the burrowing mechanism of *Lumbricus terrestris* is required if this observation is to be properly understood. A more detailed investigation into earthworm sampling variability incorporating multiple habitat types, not just open, grassy areas is also needed.

Acknowledgements

I would like to thank Prof. Nick Dickinson and Louise Uffindell for their continued support throughout this project, also Steven Taylor for his help in the field and Clive Erye from the Faculty of Technology and Environment for his guidance in the compaction of the soils for the laboratory based work. Finally, I would like to thank Dr Rowan Rothwell for her assistance in the proof reading of this report.

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APPENDIX D

Modelling the potential for enhancing Carbon Sequestration on brownfield soils using organic amendments by *in situ* soil and laboratory compost efflux measurement.

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Abstract

The addition of organic amendment to brownfield soils can significantly increase the carbon stored in these soils. Field based soil respiration measurements show that the addition of garden greenwaste could double the carbon stored in brownfield soils. Scaling up the measurements could predict regional and national carbon sequestration potential of brownfield land because both labile and resistant or recalcitrant carbon pools are accounted for. The advantages of this work to global climate change could be highly significant for future mitigation strategies. Further work is needed to build on the knowledge gained by this study to accurately model the carbon sequestration potential of brownfield land, especially at elevated soil temperatures and fluctuating soil moisture contents.

1. Introduction

Global climate change is one of the most prevalent subjects in current scientific literature; an internet search reveals over 100 million results for this subject. Carbon Dioxide is one of the major greenhouse gases known to contribute to climate change. Therefore mitigation measures are necessary to counter the damaging effects of the emission of CO_2 if this gas continues to be produced at current or even reduced levels. Carbon sequestration is the process by which Carbon from the atmosphere is stored in plant tissues, soils or the ocean, and can take two basic, terrestrial forms. Sequestration in plant tissues by the process of photosynthesis is the most publicised method but contains major limitations surrounding its permanence. The second method, and the one explored by this paper is sequestration in soils; specifically the potential to enhance the soil carbon pool by organic soil amendment. Soil organic matter has a favourable affect on the aeration of soils and serves as a source of energy for soil microorganisms (Stevenson & Cole, 1999) therefore could enhance the exchange of carbon between the atmosphere and the land. Breakdown of organic matter is dependant on soil temperature and moisture (Dickinson, 2003) meaning that soils of different regions may have very different organic matter breakdown patterns. Carbon sequestration is transboundary and has equal benefits to everyone, with one tonne of carbon locked up in Scotland having the same environmental value as one tonne in Wales (Brainard *et al*, 2003). It is estimated that the annual soil carbon loss during the period 1978 to 2003 in England and Wales totalled 13 million tonnes (Bellamy *et al*, 2005). This figure is equivalent to the entire UK reduction in carbon emissions between 1990 and 2002 (Detlef Schulze & Freibauer, 2005). Therefore and understanding of the processes involved and the potential for soil carbon sequestration is required. Smith (2005), in an overview of the permanence of soil organic carbon stocks suggests that, logically soils with a decreased carbon stock contain the best possibilities for sequestering carbon. Brownfield soils, by nature of their previous use are likely to be depleted in carbon due to sporadic disturbance, compaction, contamination and resultant lack of vegetation; especially so on former landfill sites that contain a shallow covering of topsoil giving the soil a poor physical structure (Dickinson, 2003). This means that their potential to sequester carbon is greater than, for example forest soils. This is a link explored by Bellamy *et al* (2005) who imply a relationship between initial soil carbon content and sequestration potential in a nationwide inventory. Conversely compaction causes pooling of organic matter in the upper horizons of soils limiting additions below those horizons (Brevik, 2002).

The aim of this research is to discover what effect, if any the addition of a layer of organic greenwaste amendment has on the potential of brownfield soils to sequester carbon. The implications of the findings are wide reaching both in social and economic terms as they could provide the basis on which to model future climate change predictions and also mitigating measures. Further to this the effects of organic amendments upon soil temperature can also be considered. This type of dynamic, *in-situ* carbon flux modelling has implications for both ecologists and economists as it represents a way to measure the offsetting effects of sequestration enhancing practices taking into account local climatic variation.

2. Materials and methods

The experimental application used for the present work is basic and utilises 3 brownfield sites located in the North West of England and 1 control site for comparison. The carbon flux is measured by means of an 'LC pro +' soil respirometer that uses an 'open chamber' system of analysis to compare a 'reference CO_2' value taken from an atmospheric receptor to an 'analysed CO_2' value taken via a soil hood placed on the soil (see Figure 1a and 1b). Analysis is dynamic meaning that the values taken are constantly compared against the mass airflow to produce a Net Carbon dioxide Exchange Rate (NCER) value (see Figure 1c). It is this value that is taken into consideration when exploring the potential for carbon sequestration. The NCER value is given as micro moles of CO_2 per square metre per second (μmol m^2 sec^{-1}). The unit also displays the soil temperature and atmospheric pressure.

Figure 1a. Components of the soil respirometer assembled in the field.

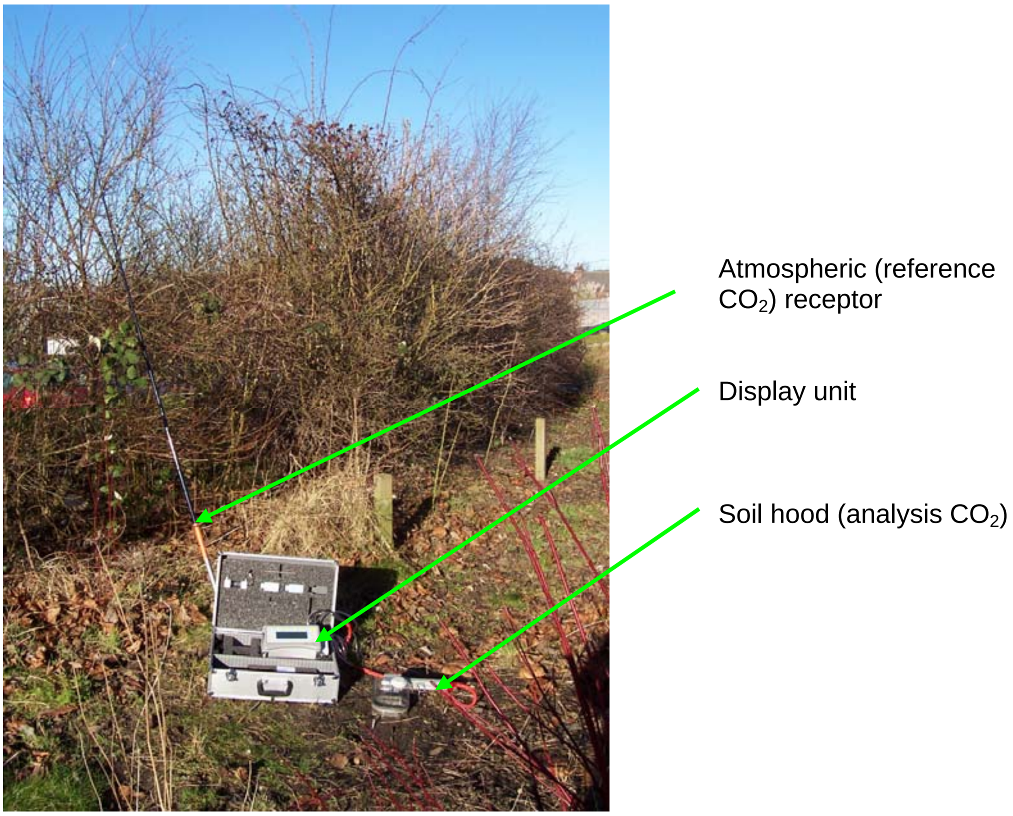


Figure 1b. Soil hood assembly as used in conjunction with the soil respirometer.

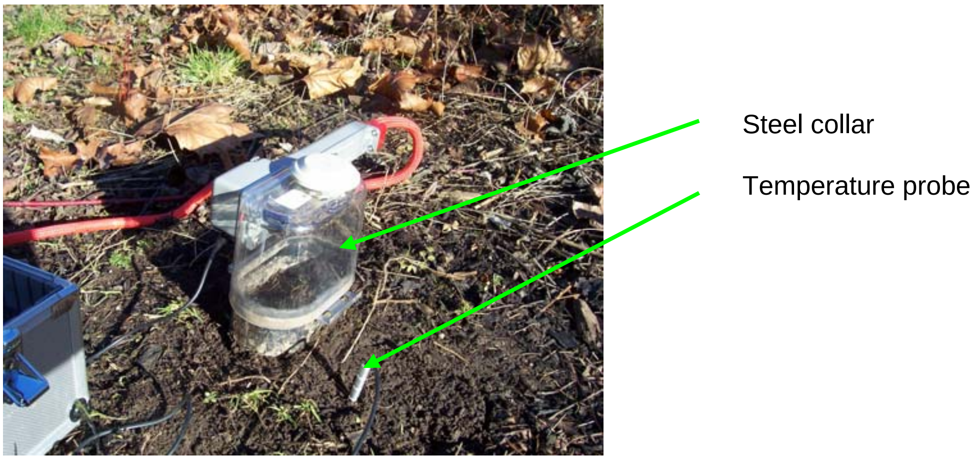
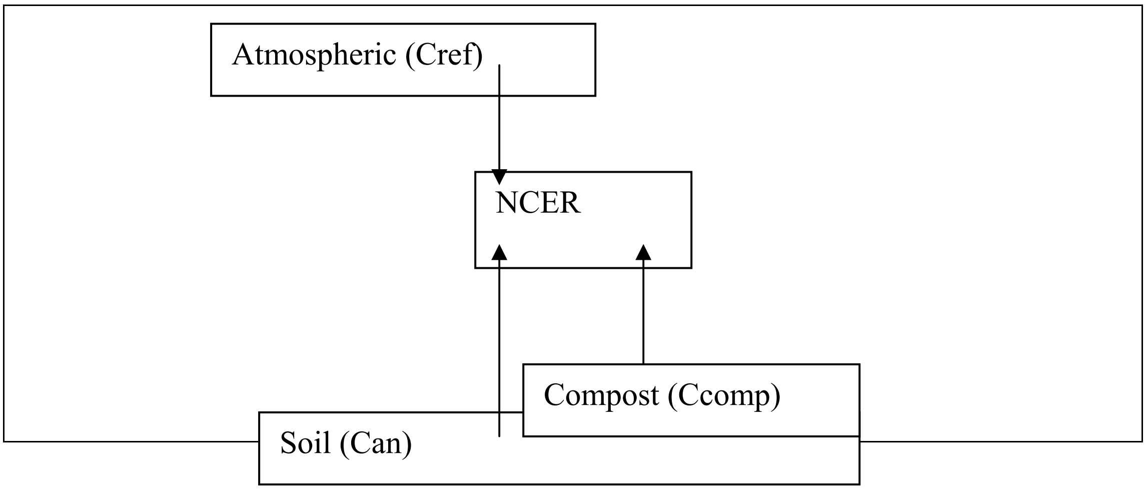


Figure 1c. Display unit showing NCER value and soil temperature (Tsoil m).

Figure 2. Diagrammatic representation of the soil respiration measurement process used in the present work.



Effectively there are 3 values derived in the calculation of carbon sequestration potential; Cref, Can and Ccomp. For this study Cref and Can are measured in the field and Ccomp is modelled in the laboratory.

2.1 Study sites

The four study sites are located within the Northwest of England which means that they are climatically similar.

Figure 3. Study site locations.

1. Childwall Woods, Liverpool. (SJ 415 887)

Childwall Woods is a former municipal landfill site where a large quantity of 'healthy' topsoil has been applied. This site is considered a 'control' site due to general health of the vegetation colonising it.

2. Cromdale Avenue, St. Helens. (SJ 531 946)
3. Merton Bank, St. Helens. (SJ 527 958)
4. New Pale Farm, Huyton. (SJ 452 889)

Sites 2, 3 and 4 are former municipal landfill and commercial waste disposal sites covered with topsoil.

2.2 Field soil respiration measurement

The correct use of the soil respirometer first required some preparation of the soil at each site. In order to gain an accurate NCER reading surface litter was cleared from the soil as this could increase the NCER value greatly. On clearing this litter disturbance of the soil was kept

to a minimum as this could enhance soil microbial activity leading to an inaccurate reading. At each of the 3 brownfield sites 10 random samples where taken; 5 on untreated soil and 5 on soil where organic amendment had been present. In each case the NCER of the soil was measured, that is to say the compost amendment was removed and the soil beneath was sampled. The mean depth of the compost was 10cm. Soil collars (as illustrated in Figure 1b) were used for each sample; these are inserted into the soil to a predetermined depth and effectively enclose an area of soil that is then sampled. Care was taken to insert the collars to a uniform depth for heterogeneity. After insertion into the soil each collar was left for 10 minutes to allow time for any slight soil disturbance to settle. Using the soil collars also ensure that a perfect seal is attained to prevent atmospheric (reference) CO_2 being analysed, which would greatly affect the NCER reading.
It was a requirement, before sampling began that the soil respirometer be correctly calibrated, which the unit performs using an internal canister of CO_2. A calibration was performed before sampling at each site for accuracy.
An accompanying soil temperature probe was inserted into the soil, also to a uniform depth adjacent to the soil collar to measure soil temperature (in degrees centigrade).

2.3 Laboratory measurement of compost NCER

Because our respiration measurements were taken on the soil with the compost removed the NCER value of the compost itself must be offset against the NCER value from the soil. The compost, when applied as an amendment layer replaces the soil at the interface between the atmosphere and the land. For the purpose of the present work this will be designated the suffix ‘Ccomp’. Modelling compost NCER in the laboratory used 3 replicate samples of compost at 4 temperatures; 0, 5, 10 and 15 degrees centigrade in incubators. These temperatures were used to facilitate an understanding of whether temperature has an effect on compost NCER values. Compost was placed into containers of 8cm depth in order to allow sufficient depth to sink a soil collar into each sample. Each sample was allowed 48 hours to reach its specified temperature and to settle after disturbance. To avoid moisture loss from the compost a lid was placed on each sample during the incubation stage. The NCER of each sample was measured using the same method as for soil respiration (as previously stated) leaving 24 hours to allow any additional disturbance resulting from the insertion of the soil collars to settle. Cref (atmospheric CO_2) values where obtained by placing the atmospheric receptor outside.

2.4 NCER results conversion

The soil respirometer provides NCER values in Micromoles per metre square per second (μmol m^2 sec^{-1}). For this study, to provide a site wide context the following scaling up has been applied:

- NCER (μmol m^2 sec^{-1}) x 0.01201 (μmol weight of carbon in grams) = NCER (g C m^2 sec^{-1})
- NCER (g C m^2 sec^{-1}) x 60 (g C m^2 min^{-1})
- NCER (g C m^2 min^{-1}) x 60 (g C m^2 hr^{-1})
- NCER (g C m^2 hr^{-1}) x 24 (g C m^2 day^{-1})
- NCER (g C m^2 day^{-1}) x 365 (g C m^2 yr^{-1})
- NCER (g C m^2 yr^{-1}) / 1000 (kg C m^2 yr^{-1})

Where C = carbon, g = grams, kg = kilograms, m^2 = square metres, sec^{-1} = seconds, min^{-1} = minutes, hr^{-1} = hours, day^{-1} = days, yr^{-1} = years.

3. Results

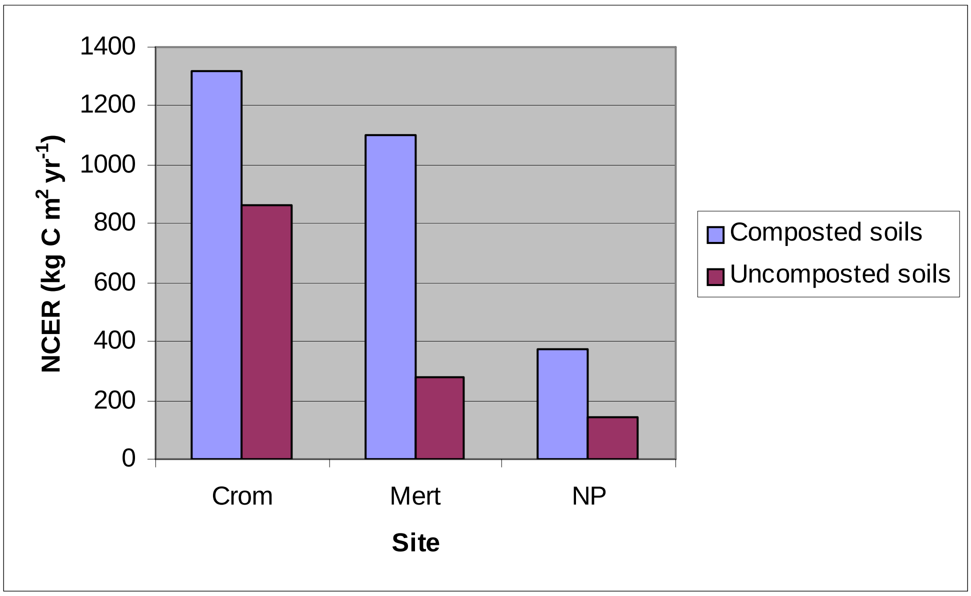
3.1 Field soil respiration measurements

All samples taken showed a marked difference in NCER between composted and uncomposted sites; in all cases the NCER value of the composted sites exceeded that of the uncomposted sites (see Table 1 and Figure 4).

Table 1. Mean NCER values for sampled sites showing the additional carbon sequestered by the soil under amendment (at a mean soil temperature of 5 ^{O}C).

Sample site	**Uncomposted soil NCER (kg C m^{2} yr^{-1})**	**Composted soil NCER (kg C m^{2} yr^{-1})**	**Addition by amendment (kg C m^{2} yr^{-1})**
Cromdale Av	863.5	1318	454.5
Merton Bank	276.5	1102	825.5
New Pale Farm	140	371	231
Average	**427**	**930**	**503**
Childwall control	531	N/A	N/A

Figure 4. Mean soil NCER values for the 3 study sites (5 replicates).

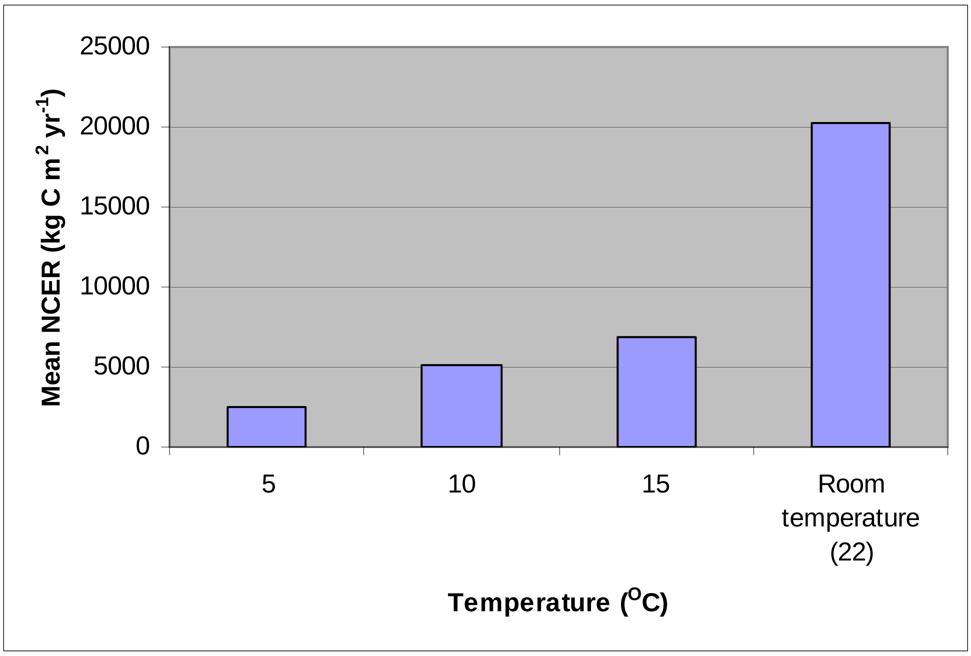


Compost NCER values increased exponentially with temperature when modelled in the laboratory.

Table 2. Laboratory compost respiration measurement values modelled under a range of temperatures.

Temperature (^{O}C)	**Mean compost NCER (kg C m^{2} yr^{-1})**
5	2541.4
10	5082.8
15	6881.8
Room temperature (22)	20191.1

Figure 5. Compost NCER changes with temperature comparison (3 replicates).



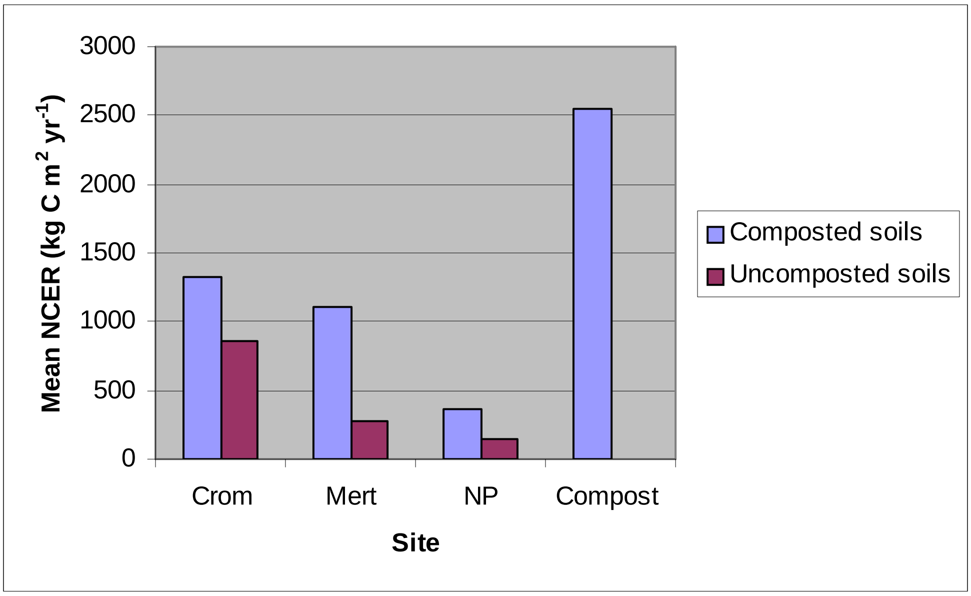
4. Discussion & Conclusions

Organic amendments, such as compost can add significant quantities of organic matter to the soils they are applied to. The soil NCER results show this to be the case on all sites, though the NCER value varies significantly from site to site, suggesting site specific limitations. It must be remembered that the values presented are a mean over the whole sample area so do not represent clearly the effect that, for example pooling of organic matter could have on the NCER values. As expected the contaminated sites showed a depleted mean level of soil carbon before amendment (except for Cromdale Avenue), probably due to disturbance and poor soil management in previous decades, though the results show site specificity and high levels of fluctuation when compared to the control site. Comparison to the control site also illustrates that significant additions of carbon can be obtained by amendment; the mean values show that this is in the order of 100% addition by weight. Table 2 demonstrates that compost temperature affects respiration rate when modelled in the laboratory. It is likely that field soil respiration rates will also demonstrate this trend, although the temperature differences are likely to be greatly reduced over the spatial area sampled for this study. A study by Fang & Moncrieff (2001) modelled the effects of soil temperature on respiration rates by using a range of temperature regimes between 10 and 40 ^{O}C. The results showed an exponential increase in soil respiration with temperature increases although there is no optimal temperature found. This supports the findings of the present work and well documented trends.

The advantage of exploring carbon sequestration by using an *in situ* respirometer is that both labile and resistant carbon pools are accounted for when sampling. It is likely that a high proportion of the organic carbon in the soils on the study sites exists in the labile, upper pool. This is due to the fact that the climatic conditions favour slower decomposition of organic matter. Therefore less carbon is stored in the lower horizons of these soils. Cromdale Avenue and Merton Bank sampling sites both supported some wayside vegetation, which could account for their higher initial soil NCER. New Pale farm was unvegetated. Fang *et al* (2005) find that, unlike previous suggestions the temperature sensitivity of this labile carbon pool may not be significantly higher that that of the resistant pool. This means that regional and global carbon sequestration may be able to be modelled more accurately than first thought based on the data from regional collection by *in situ* respiration measurements. Interestingly the soil temperature was 1.3 ^{O}C higher in the soil under compost amendment than the uncomposted soil, suggesting that the compost had the effect of stabilizing the soil temperature and preventing longwave heat losses at night for example. Therefore, using Fang & Moncrieffs (2001) 'exponential' model and the findings of the present work it is likely that this temperature difference accounts for at least some of the additional respiration by the amended soils. On a wider scale Smith (2005) presents the view that increased soil temperatures, as a result of climate change could increase or decrease soil carbon stocks. An extended growing season is cited as the mechanism by which increased carbon stocks could result from global warming. It is likely, especially on amended soils that increases in temperature could result in more rapid organic matter decomposition leading to possible decreases in soil carbon stocks, especially in the labile pool. At cooler temperatures slower decomposition would allow the organic matter time to penetrate to the resistant carbon pool, from where it is less likely to be lost back to the atmosphere by disturbance for example. This suggests that the UK climate could favour sequestration enhancement by organic amendment.

The carbon sequestration potential of brownfield land needs also to consider the respiration of the compost used to amend the soils (see Figure 2). The compost effectively replaces the soil it amends as the interface between the land and the atmosphere. The results presented in Table 2 show that compost respiration rates are greatly elevated compared to soil respiration rates suggesting that amendment by compost may have a negative effect on the short term levels of atmospheric CO_2 as implied by Smith (2005). Having said this, the additional carbon sequestered in the soils beneath the amendment may offset this over a given time period. Therefore future studies should monitor compost NCER over a yearlong basis. Figure 5 shows the difference in soil and compost respiration rates.

Figure 5. NCER values for soils and compost on samples brownfield sites.



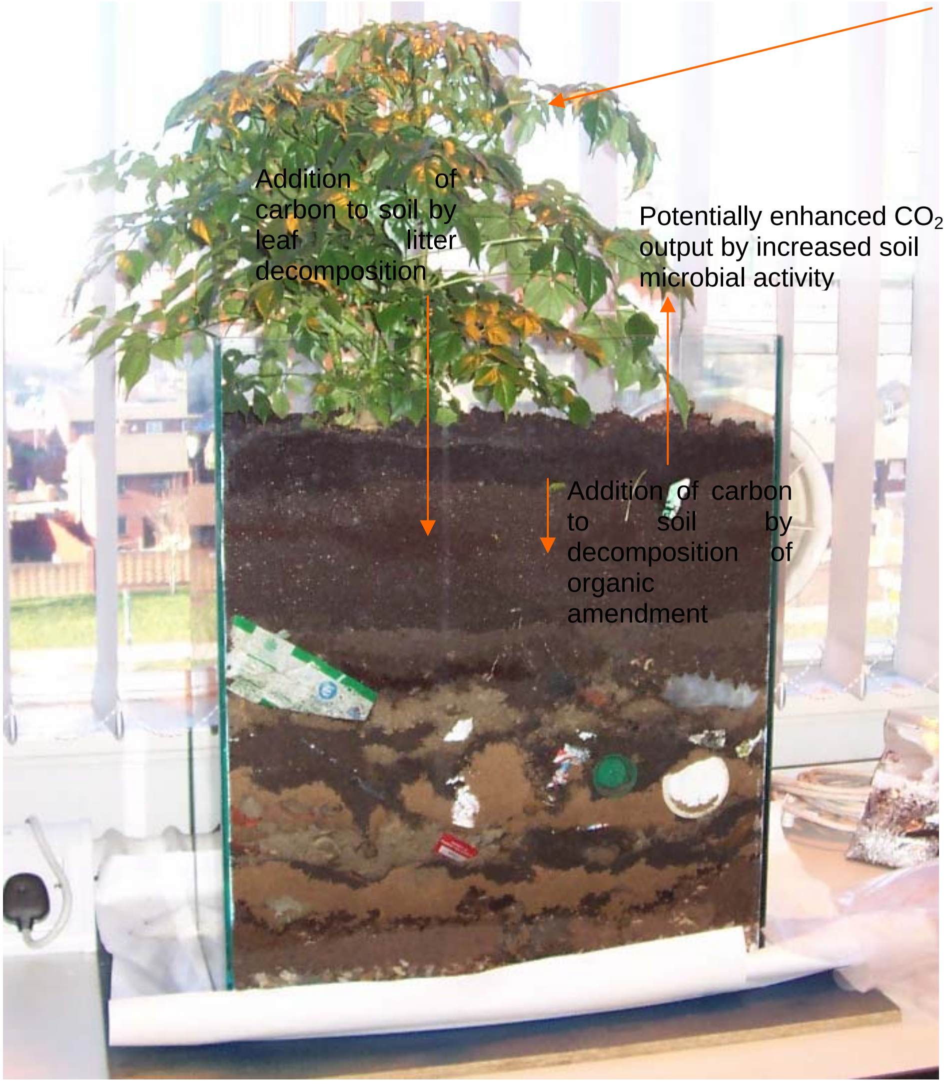
The results presented in Figure 5 suggest that carbon sequestration on brownfield land by using organic amendment could have negative implications for climate change (see also Figure 6). However it is important to stress that the methods used in this study make a number of assumptions. Firstly it is assumed that the soil NCER values are directly comparable between composted and uncomposted sites. It is undoubtedly the case that some disturbance due to removal of the compost layer will have occurred. To what extent this enhanced soil microbial activity and thus elevated the NCER reading is not known. As such the addition by amendment value may be higher than it would have been had the soil been left to settle for a longer time period. Also of note is the scale at which the results are considered. Murthy *et al* (2003) created a community level carbon dioxide efflux model to ascertain whether scaling up of small scale spatial soil respiration data could accurately predict a community level response. Their conclusions were that scaling up of small scale data, such as has been done for the data in the present work led to an overestimation of 36%

in soil respiration rates. One of the limitations of presenting the data as a yearly figure is that this assumes homogenous climatic conditions throughout the year. As has been discussed temperature is likely to have a significant effect on the soil respiration rate. Another climatic variable equally as important is precipitation and soil moisture content. Murthy *et al* (2003) conducted point soil respiration measurements on soils subject to moisture regimes. If the same scaling up of NCER values that is applied to the present work is applied to their findings then the wet NCER value is 1178 kg C m^2 yr^{-1} and the dry NCER value is 314 kg C m^2 yr^{-1}. It can be extrapolated that annual variations in soil moisture content could cause fluctuations in soil respiration rates. This also applies to regional variations in soil moisture content as a result of differing precipitation rates between the North and West of the UK and the South and East for example. Similarly the organic matter amendment is likely to retain moisture in the soil leading to higher soil moisture contents. This could go some way to explaining the elevated carbon content of the amended soils.

The model in figure 6 (overleaf) represents an urban soil profile, such as could be found on a brownfield site. As illustrated the amendment to the soil (upper dark brown horizon) enhances the addition of carbon to the soils below. However the enhanced microbial activity could lead to increased CO_2 production and, therefore could, potentially enhanced global warming.

Figure 6. Model of an urban soil profile illustrating carbon fluxes

CO_2 input via
photosynthesis



The interaction between the two climatic parameters considered here; soil temperature and moisture content, is explored by Smith (2005). Increases in temperature, by global warming for example will only increase soil respiration if the moisture content of the soil is sufficient to allow decomposition. Therefore moisture content becomes a limiting factor. Using organic amendments on soils to increase carbon storage needs therefore to consider the fact that the organic matter is likely to retain more moisture than the soil it amends and have a lower albedo. Therefore it is likely to be both warmer and moister favouring rapid decomposition and release of CO_2 to the atmosphere, possibly leading to a positive feedback loop and enhanced global warming. Smith *et al* (2003) suggest that during Autumn and Winter months soil respiration is controlled by temperature and during summer months soil moisture controls soil respiration. It is likely that organic amendments will create soil conditions that are more homogenous throughout the year meaning that sequestration fluctuations are not as substantial as on those soils not amended. Soil structure and texture are likely to have a significant effect on the speed at which organic amendments decompose and therefore the carbon sequestration potential. This study does not account for the changes in soil structure and texture but future studies may seek to classify soils on this basis. In retrospect, sampling of the composted soil should have been carried out with the compost *in situ* to avoid disturbance-induced errors. This would have accounted for the diffusion of CO_2 through the compost amendment layer better representing the true implications of this study for climate change. Data published by DEFRA on national emissions of CO_2 indicate that, for 2002 the annual total emissions of CO_2 in the UK were 551 Mt (551 million tonnes). Extrapolating the results of the present work indicates that 110 000 hectares of amended brownfield land would be required to offset this emissions figure. With more extensive sampling across a range of temperatures a quantifiable step could be made towards identifying the potential of amended brownfield land to offset CO_2 emissions and hence prevent further anthropogenically enhanced climate change.

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