DOCTOR OF PHILOSOPHY

Role of conventional soil classification in the prediction of soil quality indicatord

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Award date:
2010

Awarding institution:
Bangor University

Role of conventional soil classification in the prediction of soil quality indicators

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A thesis submitted in candidature to the University of Wales, Bangor for the degree of Doctor of Philosophy

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August 2010

SUMMARY

Soil surveys and soil classification, are based on a static view of soil properties (subsoil properties) that tend not to change significantly on the human time scale; however, most growers and land managers identify soils as dynamic systems (topsoil properties). The current conventional soil classifications therefore, fall short of describing the dynamic/functional behaviour of soils or the soil quality indicators, which is of most interest to land managers. The scope of this thesis is to investigate whether broad soil types defined by traditional soil classification, can be used to predict the soil quality indicators (SQIs) and whether the SQIs can be used to classify soils which can predict soil function and bacterial biodiversity. In addition, we investigated whether other factors (e.g. vegetation classes) regulate the SQIs and whether there are critical limits in the SQI in different soil types or vegetation types (AVCs). To achieve this, we monitored (1) Carbon turnover rates monitored over a (i) 90 day and (ii) 1.5 y period using ^{14}C-labelled artificial root exudates or ^{14}C-labelled plant leaves, (2) soil quality factors and the dominant attributes in the factors, (3) Soil respiration, mineralisation and biodiversity, in the different soil types and AVCs. Results from several statistical methods employed on these SQIs revealed significant differences between soil types or AVCs, however, the differences were small. In most cases only the Peat or Pelosol soils were distinctly different from the rest of the soils or the Heath and Bogs, Moorland and Grass Mosaic, and Upland Wooded from the rest of the rest of habitats. The definition of the class limits remained ambiguous, as exclusive reference values for each soil type or AVCs could not be established due to overlaps in SQI ranges. Statistical soil classification by cluster analysis based on selected soil physico-chemical properties did not improve its predictability of the soil function and diversity. We conclude that conventional soil classification provides a poor predictor of most SQIs. We further conclude that long-term laboratory mineralisations in soils at constant temperature failed to reveal major differences between soil types and that laboratory mineralization studies may provide a poor proxy for predicting soil C sequestration potential. We ascribe this to the inability of short term biological assays to represent pedogenic processes which have taken ca. 10,000 y to become manifest.

***Key words*:** soil classification, soil quality (indicator), C sequestration, soil function, biodiversity

Dedication

A dedication to my wife Kamo and my cute boys- Mute and Temwani

ACKNOWLEDGEMENTS

I would like to thank my supervisors Prof. David L. Jones, Prof. Douglas L. Godbold and Prof. Bridget Emmett of CEH, Bangor, for their invaluable time, expertise, advice, support and guidance, without which, I would not have completed this thesis. I owe many thanks to Dr. Paul W. Hill for helpful critical reading of chapters and discussions, practical guidance and technical assistance in the setting up and monitoring of the experiments.

I acknowledge Commonwealth Fellowship Programme and DEFRA for funding my studentship.

I acknowledge Centre for Ecology and Hydrology (CEH) for allowing me to use soil samples and data from the 2007 Countryside Survey (CS). CEH staff especially field staff, Steve Hughes and Annie Britain for collection, sorting and preparing of samples for my analyses. Thanks to Claire Wood in Lancaster for her efficient delivery of data to me whenever I needed it.

Thanks to Gareth Williams for his technical assistance with the use of radioactive materials; Jonathan Roberts, Gordon and Paula Roberts for practical assistance in their laboratories. Thanks to Rob I. Griffiths and Robert T.E. Mills for sharing their data with me (bacterial diversity and SR data respectively).

I would like to thank my wife Kamo M. Simfukwe for her huge love, patience, consideration, assurance and encouragement, without which I could not have gone through this challenging experience. "Thanks 'baby' for the good meals in spite your busy schedules". Thanks to our children Mute and Temwani for their love and provision of light moments at home thereby providing an incentive to finish so that we could have more time together. I owe many thanks also to my extended family and friends for their encouragement, love and moral support.

My pastor David Finney and his wife Kyung-Ju deserve an equally huge thank you for their love, practical support and spiritual guidance during our studentship in Bangor. Thanks to Ebenezer Evangelical Church membership for their love and support.

Lastly I acknowledge my many colleagues in the SENRGY group for their companionship

Above all I owe everything to the Almighty God for giving me life and saving love, together with everything I needed. Glory be to God and to Him alone- amen.

'...You are my servant; I have chosen you and have not rejected you. So do not fear, for I am with you; do not be dismayed, for I am your God. I will strengthen and help you; I will uphold you with my righteous right hand.' *(Isaiah 41:9, 10)*

List of abreviations

Abbreviation	Description
^{14}C	Carbon 14 isotope
a_1	Fast phase substrate allocation
a_2	Slow phase substrate allocation
ANOVA	Analysis of variance
AVC	Average vegetation class
BQ	Biophysical quotient
HL_1_l	Fast phase half life for labile substrate
HL_1_p	Rapid phase half life for plant material substrate
HL_2_l	Slow phase half life for labile substrate
HL_2_p	Slow phase half life for plant material substrate
k_1	Fast phase mineralisation rate constant
k_2	Slow phase mineralisation rate constant
LMW	Low molecular weight
NH_4^+	Ammonium
NO_3^-	Nitrate
OM	Organic Matter
SBQI	Soil biological quality indicator
SEM	Standard error mean
SOC	Soil organic Carbon
SOM	Soil organic matter
SR	Soil respiration
SQ	Soil quality
SQI	Soil quality indicator
SQF	Soil quality factor
TRFLP	Terminal Restriction Fragment Length Polymorphism
WRB	World Reference Base

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Chapter 1

Overview of thesis

1.1 General Introduction

Soil classification is intended to organize our knowledge on soils so that their properties may be remembered and their relationships be understood most easily for a specific objective (Rossiter, 2001). The process therefore involves formation of classes by grouping the soil on the basis of their common properties. These classes can be either natural i.e. based on their inherent properties or technical based on selected characteristics to serve a specific objective (Rossiter, 2001; Palm, 2007). Soil classification can enable generalisations and predictive statements to be made about soils and their properties. The soil types defined by traditional soil classification systems are based on the subsoil and do not pay as much attention to the topsoil which is the most important part of the soil for food production, for soil management and for degradation control (FAO, 1998). In other words, classic taxonomic keys consider only static properties of the soils and ignore the dynamic soil properties (also called soil quality indicators; SQI) and yet the understanding of the processes and factors relevant to the status and change of the dynamic properties in the topsoil contributes to recommendations for sustainable land management practices. Soil quality can be defined as the capacity of the soil to function within the natural or

managed ecosystem boundaries, to sustain plant or animal health and productivity, to maintain or enhance air and environmental quality and to support human health and habitation (Karlen, 1997). Soil quality can best be monitored using soil quality indicators (such as SOC stocks, pH, Bulk density (compaction) or accumulation of contaminants) that can indicate changes occurring in the quality of the soil under a management system. These soil quality indicators are mostly associated with the topsoil which is ignored in the classic taxonomies. Therefore, in general there is lack of a direct link between the soil classifications systems and the many dynamic soil quality indicators. This lack of direct link between the traditional soil classification and the dynamic topsoil characteristics led to the development of the "Fertility Capability Classification system" (FCC) in 1975 and the "Topsoil Characterisation for Sustainable Land Management" in 1998, in order to bridge the gap between classic soil classification and soil fertility (Sanchez et al., 1982). These classifications were intended to complement the traditional soil classifications (especially the FAO led classification system e.g. WRB).

In spite of the recent enhanced interest in the characterisation of topsoils and measurement of soil quality indicators for soil quality monitoring; there is little effort in establishing relationships between the soil types defined by conventional classification and the dynamic soil quality indicators. In order to meet the needs of growers and land managers there is a need to bridge this gap effectively or there must be a paradigm shift in the way we view soils, from static to dynamic. Investigation and building reliable predictive relationships between the classic soil classifications and the soil quality indicators will greatly increase the value of soil maps and classifications. This thesis explores these relationships using various approaches. Consequently, the main aim of this thesis was to determine relationships between soil quality indicators (SQIs) and key soil types to ascertain:

(1) Whether broad soil types defined by traditional soil classification systems can be used to predict SQI,

(2) Whether these SQIs can be used for defing soil types that can prdict soil soil function and biodiversity

(3) The extent to which vegetation and/or soil type are the major regulators of soil biological indicators,

(4) Whether there are critical limits in SQIs with respect to different soil types and vegetation types.

1.2 Aims and objectives

1. To build relationships between the soil types defined by traditional soil claffication or physiochemical properties and the SQIs and determine whether these soil types can be used to predict the SQIs.
2. To assess whether soil types defined by cluster analysis are a better predictor of soil function and biodiversity
3. To determine which SQIs are better at discriminating between soil types and or AVCs.
4. To examine and compare the effect of soil types and AVCs on SQI variability.
5. To investigate critical limits in SQIs within different soil types and/AVCs.

1.3 The plan of thesis

The thesis is divided into eight chapters as detailed below:

Chapter 2 is the literature review, detailing (1) soil classification systems and use, (2) soil quality monitoring approaches worldwide, and its relationship to policy, and finally, (3) the role

of role organic matter cycling in relation to (1) and (2) and to this thesis.

The experimental work is presented as separate scientific papers in Chapters 4-7. With this approach of thesis writing, repetitions in the introductory material, methods and references were inevitable.

Chapter 3 is a brief chapter describing the study area.

Chapters 4 and 5 were incubation experiments where carbon turnover in each soil type was studied over time (0-3 months and 0-18 months period respectively) by monitoring the mineralization of either a labile (^{14}C-labelled artificial root exudates) or more recalcitrant C source (^{14}C-labelled plant leaves) in soil held at field capacity at 10°C.

Chapter 6 aims to identify soil quality factors/indicators and key attributes, assess their variability within and between the soil types and vegetation types (AVCs) and select which soil attributes within these factors can be used as soil quality indicators. The selected soil quality indicators were in turn tested against the overarching objectives of this thesis.

Chapter 7 reports the findings of the analysis of the relationships between soil function and diversity and key soil types, soil parameters, and AVCs. We sought to establish the factors that correlated and split (regulated) the soil biological quality indicators (SBQIs) of microbial function and bacterial diversity into homogenous groups using regression (multivariate) tree analysis. Furthermore, cluster analysis on selected physico-chemical characteristics was used to group the soils. The new groups were tested whether they were a better soil classification than conventionally classified soil types in predicting the soil function and biodiversity.

Chapter 8 represents a general discussion of the results from all the experimental chapters, highlights the key conclusions and identifies areas for further work.

The last part is the **appendices** which includes the land classification key, additional pictures, tables, and figures of all the experiments which could not be included in the chapters of the thesis.

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Chapter 2

Literature review

2.1 Soil classification

2.1.1 Introduction

In the natural environment, soil changes in its characteristics vertically, from the surface downwards to the rock and laterally from one place to another within and among continents, regions, landscapes, catchments and plots (Courtney and Trudgill, 1976; Wander et al., 2002). Studies by Beckett and Webster (1971) in the lateral changes in soil variability show that as much as half of the total variation in some properties can occur within a horizontal distance of one meter. A variety of classification systems have been developed to describe the soil groups. Soil classification is the process of grouping soil individuals into more or less homogeneous groups with respect to defined objectives (Rossiter, 2007). The homogeneity within the groups is with respect to soil properties and functions. There are two main purposes of soil classification. The first is to enable generalised information to be transmitted from one individual to another and secondly is to make the generalisations and predictive statements about what will happen in a particular circumstance (Courtney and Trudgill, 1976; Avery 1990; Rossiter, 2007). Soils are classified and mapped according to either the natural or technical/artificial approaches described

below. Many countries have developed their own classification systems, depending on the needs and soils of the country and have proved to be useful tools for correlation and mapping. The USDA Soil Taxonomy and the WRB are the most widely known and used soil classification systems.

Closely related terms to soil classification are soil taxonomy and soil mapping. Soil taxonomy is the practice of describing, categorizing, and naming soils, intended for easier communication of information about different kinds of soils, how they are used, their properties, and where they are found (Smith, 2010). Soil mapping on the other hand involves locating and identifying the different soils that occur, collecting information about their location, nature, properties and potential use, and recording this information on maps and in supporting documents to show the spatial distribution of every soil (Soil Survey Staff, 1999; Cranfield University, 2010). Soil classification systems are used to map and identify different types.

2.1.2 Different approaches worldwide

Soils are classified and mapped according to either the natural/systematic or technical/artificial classification approaches. The natural approach classifies soils based on the entire set of its characteristics to understand their mutual relationship and natural coherence while the technical approach classifies soils by grouping individuals that are alike in selected characteristics to serve a specific purpose (Muir, 1962; Palm et al., 2007). In other words, the natural approaches characterize and classify soils as they exist by themselves, without reference to use, while the technical approaches classify soils according to their suitability for specific uses. Examples of the technical approached include: Hydrologic response, suitability classes

(FAO Framework for Land Evaluation), Land Use Capability (USDA, LCC) and Fertility Capability Classification.

Many national and international soil classification systems use the natural approach. The US and the FAO (world reference base) systems in particular were both designed to accommodate all known kinds of major types of soils and are therefore useful for international reference purposes (Avery, 1990). However, national classification systems have been retained for internal use in most countries because scientists and the governing bodies concerned prefer to classify soils of their own countries as conveniently and meaningfully as possible and to avoid frequent changes likely to cause confusion (Avery, 1990). Moreover, these systems were not meant to replace national soil classification systems, but be a tool for better correlation between national systems (FAO, 1998b).

2.1.3 Soil classification in the England and Wales: past and present

The first significant attempts at soil classifications were in Russia led by Dokuchaiev which were published in the 1880s and were largely based on climate and natural vegetation (Courtney and Trudgill, 1976; Curtis et al., 1976). The United States and the Netherlands followed but their classifications were based on reasonably easily defined soil characteristic measurable in the field or the laboratory (Curtis et al., 1976). In Britain, although detailed mapping started in the 1920s, considerable advances in soil classification were made after the Second World War (Curtis et al., 1976). According to Courtney and Trudgill (1976), by 1940 enough information had been gathered to enable a detailed classification to become possible. Six main soil groups were proposed in the scheme namely: (i) brown earths, (ii) podzols, (iii) gley soils, (iv) calcareous soils, (v) organic soils and (vi) undifferentiated alluvium. These groups

were derived from Dokuchaiev's system but were used with minor modifications and were used until 1974.

The new British classification of 1973, revised in 1980 and 1990 which is still in use in England and Wales is mainly the work of B.W. Avery. Avery devised a comprehensive system for soil mapping in England and Wales embodying some principles from the USDA and the Netherlands classification systems with modifications (Curtis et al., 1976). The new system is based on the classification of soil profiles (vertical soil sections) rather than the *pedon/polypedon* used in the USDA system (Avery, 1990; Curtis et al., 1976). The soil is classified based on properties that affect land use capability (Avery, 1990). These properties considered are those that are relatively permanent and can be observed or measured in the field, or inferred within limits from field examination by comparison with analysed samples.

In summary, there are two fold primary divisions in this classification: the organic (peaty) soils and the mineral soils. At the secondary level, there are six major groups of mineral soils and one of organic soil. Each major group is further divided into groups, sub-groups and series, giving a hierarchical system defined at four successive categories. The three higher categories are divided based on the composition of the material and partly on the presence or absence of major diagnostic horizons that have an important agronomic, hydrological, ecological or engineering significance (Rudeforth et al., 1984; Avery, 1990). The soil groups and subgroups are subdivisions based on general properties inherited from the soil parent material. These subdivisions are intended to reveal broad soil behavioural differences within the major soil groups. The lowest category is a soil series level. Soil series are a subdivision of the subgroup with narrowly defined diagnostic properties based on lithological differentiation including stoniness, mode of origin, texture and mineralogy of the soil that are not considered on a higher

level (Avery, 1973; Avery, 1990). Usually, for ease communication, soil series are named from the place where they were first described though each now has standardised definitions based on diagnostic properties. For a detailed description of the British classification scheme, readers are referred to the book by Avery (1990) called *Soils of the British Isles*.

2.1.4 Other international Soil classification systems

2.1.4.1 US Soil Taxonomy

Soil Taxonomy was developed by the United States Department of Agriculture and is one of most widely used soil classification systems. Soil Taxonomy is a hierarchical system classifying soils at six levels or categories based on diagnostic soil horizons and soil climatic conditions. The six categorical levels allows a very precise classification of a pedon/soil (pedon: - smallest volume unit of soil body; Soil Survey Staff, 1999). The broadest category is Order. Lower categories, in which classes are successively more narrowly defined, are the Suborder, Great Group, Subgroup, Family, and Series. The diagnostic soil horizons, measured properties and materials that define the different classes are quantitatively defined.

The Soil Taxonomy key counts 12 Soil orders representing the major soil regions of the USA. In addition to diagnostic horizons Suborders take into account soil moisture and temperature regimes in the classification of soils. Great groups represent subdivisions of Suborders based on similar kind, arrangement, and degree of expression of horizons. Subgroup names are Great group names modified by one or more adjectives which denote how soils differ from the typical concept of the Great group. Families and Series levels serve purposes that are largely pragmatic (Soil Survey Staff, 1999). The family level represents groups of soils that have a similar crop response to management while the series level is a subdivision of the families with

narrower ranges of properties permitted (Soil Survey Staff, 1999). The series name is abstract, usually place or people's names.

The system uses taxonomic names that are embedded with information about the soils being classified. The taxonomic name is a useful code that defines the soil in quantitative terms; though, its use is often hampered by the seeming complexity of the nomenclature (Palm et al., 2007). But once this classification system is understood, the name imparts much about the characteristics of the soil. The USDA soil taxonomy is fully described in Soil Survey Staff (1999) Agriculture handbook number 436 and is available on the USDA NRCS website.

2.1.4.2 World Reference Base (WRB)

The world reference base (WRB) is a FAO led soil classification system with a modular (building-block) structure (Deckers et al., 2003) defined at two levels of abstraction: (1) the thirty-two (32) Soil Reference Groups (RSG), and (2) a list of the qualifiers. RSG are differentiated based on morphology and/or pedogenesis, and/or analytical criteria (Deckers et al., 2003; Rossiter, 2007). The taxonomic units are defined in terms of measurable and observable diagnostic horizons, which are the basic identifiers in soil classification. The diagnostic horizons are defined by a combination of characteristic soil properties and/or soil materials.

The soil belongs to the first RSG in the list, for which it meets all specified requirements. Once the RSG is identified, applicable qualifiers (detailed soil properties) are used as prefixes and suffixes to define individual soil units/profiles. Prefix qualifiers are those that are typically associated to the RSG while all other qualifiers are listed as suffixes (FAO, 2006). For a detailed description of the WRB classification system, readers are referred to FAO World Soil Resources Report 103, available from the FAO and ISRIC websites.

According to IUSS Working Group WRB, FAO (2006), the WRB was not meant to substitute national soil classification systems but rather to serve as a common denominator for communication at an international level. However, lower levels emphasize soil features that are important for land use and management making it relevant for local diversity at country level.

2.1.4.3 Topsoil and soil fertility capability soil classifications

Topsoil characterization has recently received increased attention, particularly for soil quality monitoring. Conventional national and international soil classification systems hardly consider the topsoil in spite of its importance for soil quality. The Fertility Capability Classification (FCC) system is one of the very few systems which attempts to bridge the gap between soil classification and soil fertility constraints (FAO, 1998a). The system is intended to interpret Soil Taxonomy or the WRB and use additional soil attributes in a way that is directly relevant to plant growth (Sanchez et al., 1982). It emphasizes the topsoil properties because of their relation to fertility and management. The FCC system groups soils according to their fertility constraints in a quantitative manner.

The FAO 'Topsoil Characterization for Sustainable Land Management" is based on the FCC but expands the number of topsoil influencing features, such as organic matter status, land use and erosion/land degradation, to make it even more practical and widely applicable (FAO, 1998a). It was developed to be used additionally for describing topsoils and to combine it with the "World Reference Base for Soil Resources" (WRB) (Broll et al., 2006). Thus, Sanchez et al. (2003) acknowledged that integrating the quantitative topsoil attributes with FCC and Soil Taxonomy maybe the best way of measuring soil quality.

The topsoil lower limit is set at 30 cm depth, or at a root growth inhibiting layer, whichever is shallower. The topsoils are grouped by texture, organic material, organic matter status, physical,

chemical and biological features, drainage features, land use, erosion or degradation, external physical conditions, and slope class. The topsoil classification and the FCC are described in detail by FAO (1998a) and Sanchez et al. (2003) respectively.

2.1.5 Statistical approaches

2.1.5.1 Numerical classification

The emergence, development and access to computers have encouraged the use of multivariate techniques in the classification of soils. The techniques that have been employed include ordination, construction of hierarchical systems by numerical means, and methods of analysing the dispersion of a population which can be used to improve or optimise pre-existing classifications (Webster, 1977). Numerical classifications were originally developed for studying plant communities by ecologists; however, Rayner (1966) adopted these methods to classify soils from Glamorganshire by taking twenty-three profile descriptions and the results of laboratory measurements on soil samples of ninety-one horizons (Curtis and Courtney, 1976). Since then many soil scientists have used these methods to classify soils at various levels. Numerical taxonomies may be used to reduce the subjectivity inherent in many soil classification schemes (Avery 1990).

Webster and Oliver (1990) describe a simple numerical classification of soils using numerical values they called dissection, where the measured range of properties of interest is divided at certain critical or convenient points. If there are two or three properties that are considered as important then all the scales can be divided to produce a classification that is still manageable with a few groups. However, when many properties are relevant, then the

classification becomes complex and necessitates the need for an alternative method (Webster and Oliver, 1990). Multivariate grouping has been one of the alternatives.

2.1.5.2 Multivariate classification

The concept of multivariate grouping is one in which the individuals share many attributes, but for which no single attribute is either sufficient or necessary to confer class membership (Webster and Oliver, 1990). The degree of resemblance between individuals or classes is computed and the resulting similarity matrix is used to construct a hierarchy or dendrogram. Hierarchical algorithms can be agglomerative, grouping individuals by similar properties, working bottom-up from a set of individuals, to a set of classes, and then grouping the classes into super-classes, or they can be divisive working top-down from a single super class splitting down to individuals (Rossiter, 2001). Examples of the multivariate methods used in soil classification include, cluster analysis, discriminant analysis, multivariate regression tree and ordination methods.

Webster and Oliver (1990), cite two disadvantages of numerical classifications. Firstly, though the groups may be generally useful, they cannot be expected to be most suitable for any particular purpose; indeed, they might not be useful for any desired purposes. Secondly, it may be very difficult to create keys for identifying their members and for allocation of new members. In other words, these groups though they are 'natural' in respect to individuals and properties included in the original study, allocation of new members is not a simple matter and it can be very difficult to construct an identical key. The third disadvantage cited by Avery (1990) is the problem of dealing with the inherent vertical variations. In addressing this problem, some scientists have initially treated horizons recognised in the field as separate entities and then calculated similarities between all of them as the basis for comparing the profiles to which they

belong, while other scientists have described or measured the properties at specific depths and compared the profiles accordingly (Avery, 1990).

According to Sokal and Snearth (1963), a 'natural' hierarchy can only be constructed if the population exhibits 'nested' clustering, however, soil populations rarely do that. Webster and Oliver (1990) favour a non-hierarchical numerical classification as an alternative. A non-hierarchical classification according to Webster and Oliver (1990) subdivides a set of individuals on which several properties have been measured into two or more disjoint groups. Each of these individuals belongs to one and only one group which is partitioned. In this method, any clusters that are present in the population will optimize the subdivision in some sense. Classes will be created within which there is minimum variation, and between which the differences are maximised. This may indeed be a better classification especially in populations which may lack inherent hierarchical structure.

2.1.5.3 Ordination methods

As opposed to classification where units are arranged in discrete classes, ordination methods arrange units in a uni- or multi-dimensional order called "ordination space" (Gauch, 1982). The distances between points on the ordination space are measures of their degree of similarity (Gauch, 1982; Palmer, 1993). Ordination is a data reduction method that summarizes information in a simpler, more space-efficient, more visual means, relating the axes to environmental gradients (Gauch, 1982; Jongman et al., 1995; Cleland and Ramm, 2010). Ecologists use ordination methods to investigate the relationships between samples and species in a low-dimensional space (Palmer, 1993; 2010). Ordination methods in soil classification have been used mainly to analyse relationships between individuals in a reduced multidimentional space to manageable proportions (Avery 1990; Rudney 1976). Ordination *per se* does not

provide a classification, but does reveal relationships between individuals and groups when presented in one, two, three or several dimensional scatter (Rudney, 1976). Principal component analysis (PCA), correspondence analysis (CA), Redundancy Analysis (RDA), Canonical correspondence analysis (CCA) Detrended correspondence analysis (DCA) are among the most commonly used ordination methods. These methods are described in detail elsewhere (e.g. ter Braak and Verdonschot, 1995; McGarigal et al., 2000; Tabachnick and Fidell, 1995).

2.1.5.4 Multivariate regression tree analysis

Multivariate regression tree (MRT) is a technique that can be used to explore, describe, and predict relationships between multiple response variables and predictor variables (De'ath, 2002). Rather than trying to model the general relationship between the response variable and the predictor variables, the MRT recursively partition the multidimensional space defined by the predictor variables into clusters that are homogeneous with respect to response variables (Vayssieres et al., 2000). MRT form clusters of samples by repeated splitting of the data, with each split defined by a few simple if-then conditions based on predictor variables (De'ath, 2002). The splits are chosen to minimize the dissimilarity of samples within clusters, with each cluster representing an assemblage of response variables and predictor variables.

Whereas the MRT attempts to predict the values of a continuous response variable from one or more continuous and/or categorical predictor variables, Classification-Trees attempt to predict values of a categorical response variable (class, group membership, etc.) from one or more continuous and/or categorical predictor variables. The MRT have the advantage of ease and robustness of construction; ease of interpretation and ability to handle skewed distributions and missing values in both response and predictor variables and therefore represents an alternative technique to many traditional statistical approaches (De'ath and Fabricius, 2000). The issue of

over-fitting can be resolved by cross-validation, where a grown tree is 'pruning back to an honest tree' (Vayssieres et al., 2000), by elimination of superfluous branches.

2.2 The soil quality concept

2.2.1 Introduction

A precise definition of soil quality (SQ) has proved to be elusive, probably due to the innate difficulty in defining soil itself and to the multifaceted nature (i.e., scientific, personal, and social) of environmental concerns (Carter, 2002). A short and comprehensive definition of soil quality has been proposed by Doran and Safley (1997) as: "The capacity of a specific soil to function as a vital living system, within natural or managed ecosystem boundaries, to sustain plant and animal health and productivity, maintain or enhance quality of air and water environments, and support human health and habitation". It is a suite of the soil's physical, chemical and biological properties to perform the above functions (Elliott, 1997; Winding et al., 2005). Larson and Pierce (1994) defined it most simply as the 'fitness for use'. The quality of soil has an impact on soil productivity, food quality and safety, human and animal health, and environmental quality (Parr et al., 1992). However, the SQ concept has been strongly associated with efforts to address agricultural sustainability (Parr et al., 1992; Wander et al., 2002), but soil quality can be judged by any or all of these functions. It is clearly not an inherent soil property (like pH) but a value judgement based on human needs, i.e. placing a value on soil in regard to a specific function, purpose, or use.

Soil quality is considered to consist of three interrelated aspects: physical, chemical, and biological. For instance, soil biological communities are sustained across the entire range of

chemical and physical conditions within which life can exist and function (Doran and Safely, 1997; Sina, 2003). At the same time, the soil constituents are continually modified by chemical and biological products of their associated life forms (i.e. plants, animals and microbes; Tate, 2000). Some soil properties change quickly and are highly variable (e.g. soil respiration), while others can take decades to change (e.g. soil carbon).

2.2.2 Past trends - the changing concept of soil quality

Current efforts to define soil quality and develop multi-factor assessment protocols can be traced to publications from the 1970s (Karlen et al., 2008). The foundation of the soil quality concept stems from the basic idea of fitness for use in regard to agricultural use of soil, which was reflected in early and ongoing attempts at classifying soil suitability or land capability (Karlen et al., 2008). During the mid-to late 1980's, attention began to shift from erosion and production agriculture to sustainable agriculture, environmental health, and preservation of the soil resource through sustainable soil management (Weinhold et al., 2002; Karlen et al., 2008). Over the next several years the soil quality concept was further developed through symposia and workshops that resulted in a number of books and proceedings being published. The USDA has played a major role in promoting the SQ concept within the USA and across the world through creation of several institutions including the USDA Soil Quality Institute, publications of books and journals, development and promotion of user-oriented soil quality scorecards and test kits and several symposia (Karlen et al., 2008).

Since the soil quality concept was suggested in the early 1990s (Karlen et al., 1997), various perceptions have emerged. In its infant stage, soil quality meant suitability or limitation of a soil for particular use (Seybold et al., 1998). The concept has often been related to the

quantity of crops produced, but recently has also been related to the quality of crops which can be related to human health (Warkentin, 1995). The definitions of the concept have been changing to incorporate the new understanding gained of the soil resource over time. Recently, there has been an emphasis to include various biological parameters in the definition of soil quality because soils are a habitat for a wide diversity of biota (OECD, 2004; Winding et al., 2005). However, one of the main problems in the use of most biological indicators for soil quality estimation is interpreting the results (Palojarvi and Nuutinen, 2002). Furthermore, modern definitions of soil quality have included the use of the various functions that the soils perform in ecosystems, such as recycling of nutrients, partitioning of water and solutes, supporting human health and habitation (Karlen et al., 1997; Seybold et al., 1998). Consideration of the changes in the understanding of the soil quality concept with time allows for putting the present concerns in the context of other ideas in the bid to reverse soil degradation and improve soil quality (Warkentin, 1995).

2.2.3 Soil quality and Soil function

From the definition above, soils serve various ecological functions: they sustain biological production, preserve environmental quality, and ensure health of living organisms. More specifically, we shall make reference to the five soil function described by Seybold (1998) which are:

1. Sustain biological activity, diversity, and productivity.
2. Provide support for socioeconomic structures and protection for archaeological treasures and associated human habitation.
3. Regulation and partitioning of water and solute flow.

4. Filtering, buffering, degrading, immobilising, and detoxifying organic and inorganic materials including industrial and municipal by-products and atmospheric deposition.
5. Storing and cycling nutrient and other elements within the earth's biosphere.

Therefore the quality of a soil can be adequately defined and measured only within the context of the function/s ascribed to the soil (Olson, 1990). The soil functions are assessed using suitable attributes or indicators. Reference values for indicators can be defined, indicating good or poor soil functioning. Several researchers have used soil quality index schemes in an attempt to integrate information from multiple indicators in the assessment of SQ.

2.2.4 Soil quality indicators

There are several ways employed to evaluate soil quality and none are universally accepted. Most approaches rely on a set of soil quality indicators (SQIs). A SQI can be defined as a measurable surrogate for environmental process that collectively tells us whether the soil is functioning normally (Acton and Padbury, 1993; Punkhurst et al., 1997). Indicators can be sensory, physical, chemical and biological (Granatstein, 2002). Soil quality assessments often rely on indicators from several of these categories, and one of the challenges is how to integrate them together (Granatstein, 2002; Pathak et al., 2005). The use of a quantitative index is a common approach used to circumnavigate this problem. The dynamic soil qualities or indicators are most useful when they indicate or measure change in the attribute (Carter, 2002). Soil quality has been related to single soil variables such as soil organic matter (Allison, 1973), the ratio of microbial biomass C to soil organic C content (Sparling, 1992), or the ratio of soil C to N (Yamakura and Sahunalu, 1990). It is however, improbable that soil quality can be represented by any single property (Yakovchenko et al., 1996; Shaxson, 1998).

If a soil indicator is used to describe more than one soil function it is likely that its interpretation will be different for each function (Harris et al., 1996). For example, high nitrate concentrations are good for crop production but bad for groundwater protection. Thus a soil that may be considered to be of high quality for one function may not be so for other functions.

Soil quality indicators can range from highly qualitative descriptive assessments to highly quantitative analytical assessments. Liebig and Doran (1999) categorized four assessment approaches, namely: (i) farmers' perceptions, based on observational field experiences using organoleptic tests, i.e. information based on our senses of sight, touch, taste and smell, and using words as descriptors; (ii) field-descriptive, relying upon visual and tactile observations but following specific classifying methods; (iii) field-analytical, allowing for quantitative, on-site assessment of soil quality indicators, and; (iv) laboratory-analytical, based on well established protocols and often considered as standards to which other assessments are compared.

2.2.5 Biological indicators

Generally, biological properties have received less emphasis than chemical and physical properties in characterizing soil quality because their effects are difficult to measure or predict (Parr et al., 1992). Recently, there is an increasing interest in using biological properties because of their great potential to be used as soil quality indicators. A microbial indicator can be defined as a microbial parameter that represents properties of the environment (state variables) or impacts to the environment, which can be interpreted beyond the information that the measured or observed parameter represents by itself (Nielsen and Winding, 2002). OECD (2004) suggests that, microbial indicators should account for differences and complexity of soils across landscapes, climates, historic and present land use and farm management practices.

Microorganisms manifest varying life forms from autotrophic, lithotrophic to heterotrophic and serve as food for many other soil organisms (Winding et al., 2005). They make up the largest part of the total biomass in the soil and are responsible for the turnover of soil organic matter (Granatsten, 2002; Winding et al., 2005). In addition, microorganisms affect the physical properties of soil by production of exudates and debris which helps in the building and maintaining of soil structure as these materials function as glue that stabilises soil aggregates (Winding et al., 2005). Furthermore, microorganisms affect water-holding capacity, infiltration rate, crusting, erodibility, and susceptibility to compaction (Elliott et al., 1996). Microbiological properties are the second most important soil biological agents after plants (Yakovchenko et al., 1996). Thus the biological components of soils have considerable potential as integral indicators of soil quality because of their intimate relationship with the surroundings coupled with their strength to respond to a variety of land management practices across plant species, soil types, and seasons (Neher et al., 1995: OECD, 2004; Winding et al., 2005).

However, Palojärvi and Nuutinen (2002) mention two major obstacles in the application of microbial SQI. Firstly, no clear relationship has been established between soil organisms and arable soil quality. Secondly, and perhaps more importantly, many biological soil properties are sensitive to changes in environmental conditions in short timescales making their use as indicators more difficult.

2.2.6 Soil quality monitoring

Since soil degradation rarely leads to an immediate system failure, cultures and civilizations often ignore its gradual decline. Consequently, numerous civilizations have collapsed or relocated due to severe degradation and destruction of the soil on which they

depended (Granatstein, 2002; Wienhold, 2004). With this historical background we can take steps to protect and enhance the finite soil resource, in the face of large expansions of farm production and intensification of land management. The application of various natural resource management (NRM) interventions have necessitated the need to address the problem of soil degradation worldwide (Anderson, 2003; Sahrawat, 2010). To diagnose and quantify the impacts of various NRM interventions, appropriate, measurable and reliable soil quality indicators are necessary (Karlen, 1997; Pathak et al., 2005). Impact assessment is essential for the development of suitable management strategies for soil quality and to maximise productivity and sustainability for the benefits of society (Karlen, 1997; Pathak et al., 2005; Sahrawat, 2010).

Various combinations of physical, chemical, biological and visual attributes have been proposed (e.g. Shaxson, 1998; Arshad and Coen, 1992; Karlen et al., 1997; Granatstein, 2002; Pathak et al., 2005) to serve as indicators of a change in soil quality depending on the functions for which assessment is being made under particular agroclimatic conditions. A trade-off among the nearly infinite list of parameters can be made. For example, table 2.1 shows a list of the most common parameters and their rationale in their selection, for the function of various agricultural and natural resource management interventions. For specific soil function like regulation of the water cycle, specific parameters of bulk density, texture and organic matter can be selected.

In general, the physical and physico-chemical parameters are of little use as they are mainly static, unless a soil undergoes a really drastic change (Gil-Sotres et al., 2005). According to Karlen et al. (1997), monitoring of the inherent SQI can enable us to determine the natural ability of soil to function i.e. the assessment of soil parameter values that reflect the full potential of a soil to perform a specific function. On the contrary, the biological and biochemical parameters are sensitive to the slight modifications that the soil can undergo in the presence of

any degrading agent (Yakovchenko et al., 1996; Nannipieri et al., 1990) and therefore must be included in the evaluation of total sustainability of soil natural functions and its different uses (Gil-Sotres et al., 2005). The dynamic SQI can enable us to determine the condition or 'health' of the soil; where the soil is assumed to be excellent quality if it is functioning at full potential at the 'best management practices' or poor quality if it is below (Karlen et al., 1997). This requires a comparison of the current state of indicator values to the original values or the desired (known) values (Karlen et al., 1997; Seybold, 1997; Wienhold et al., 2004). This approach can also be used to follow temporal trends associated with specific land-use decisions and management interventions (Karlen et al., 1997; Seybold, 1998). Monitoring of SQ trends require establishment of baseline values for various indicators and measuring change in those indicators over time. The SQ is regarded as improving if the change in SQI is positive or declining if it is negative or sustaining if there is no net change (see Fig. 2.1) (Seybold, 1998). For example if we consider total C as the target indicator for soil quality in arable land use, where more total C is associatedwith better soil quality than less, monitoring the amount of tatal C in the soils over time can reveal whether soil quality is declining, stable or increasing.

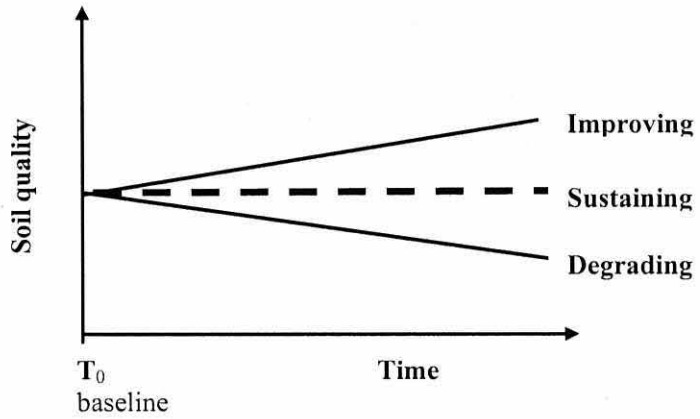

Figure 2.1. Soil quality trend monitoring from time (T_0) can result in improving, sustaining or degrading soil conditions (adapted from Karlen et al., 2008)

2.2.7 Minimum data set

Given a large number of soil properties that may be determined, it is not feasible to include all known indicators in the determination of soil quality. On the other hand, no single indicator is able to reflect the complex nature of soil. Therefore, identifying key soil attributes that are sensitive to soil functions allows the establishment of minimum data sets (MDS). The MDS will provide a practical assessment of one or several soil processes of importance for a specific soil function (Larson and Pierce, 1991, 1994; Nielsen and Winding, 2002). Generally, indicators of a MDS should be selected on the basis of their ease of measurements, reproducibility, and their sensitivity towards key variables controlling soil quality (Larson et al., 1994).

Critical limits or threshold values must be determined for the proposed soil indicators. Critical limits are the desirable range of values for a selected soil indicator that must be maintained for normal functioning and preservation of soil ecosystem health (Arshad and Martin, 2002). These critical limits help to monitor changes and determine trends in the improvement or deterioration of soil quality (Palojärvi and Nuutinen, 2002; Arshad and Martin, 2002). A minimum number of indicators (minimum data set) need to be measured to evaluate the changes in soil quality resulting from various management systems. Table 2.1 lists the commonly used key indicators for SQ monitoring for Agricultural and natural resources management interventions.

Selected indicator	Rationale for selection
Physical	
Texture	Retention and transport of water and chemicals; modelling use, soil erosion and variability estimate
Depth of soil, topsoil, and rooting	Estimate rooting volume for crop production and erosion
Infiltration	Potential for runoff, leaching, and erosivity
Bulk density (BD)	Plant root penetration, porosity, adjust analyses to volumetric basis
Water holding capacity	Related to water retention, transport, and erosivity; available H_2O; calculate from BD, texture, and OM
Chemical	
Soil organic matter (OM)	Defines soil fertility, stability, and erosion extent; pesticide and water retention, and use in process models
pH	Defines nutrient availability, pesticide absorption and mobility, process models
Electrical conductivity	Defines crop growth, salinity, soil structure, water infiltration; presently lacking in most process models
Extractable N, P, and K	Capacity to support plant growth, environmental quality indicator
Biological	
Microbial biomass C and N	Microbial catalytic potential and repository for C and N; modelling: early warning of management effects on OM
Mineralizable N	Soil productivity and N supplying and leaching potential; mineralization/immobilization rates; process modelling
Soil respiration	Microbial activity measure (in some cases plants), process modelling; estimate of biomass activity

Table 2.1 Proposed key soil indicators (MDS) for soil quality assessment (adapted from Arshad and Martin, 2002).

2.2.8 Different approaches in SQ intergration,

There are three main approaches regarding the use of general and specific parameters to estimate soil quality: (1) the use of individual properties; (2) the use of simple indexes; or (3) the use of complex indexes derived from combinations of different properties or deduced on the basis of statistical procedures.

2.2.8.1 Use of soil quality index

Individual soil properties, in isolation, may not be sufficient to quantify changing soil conditions. Soil organic matter, for example, is often used as an indicator of soil quality. But research has shown that significant biological, chemical, and physical differences can exist between two soils with the same organic matter (Granatstein and Bezdicek, 1992).

Researchers have proposed various soil quality index schemes in an attempt to integrate information from multiple indicators. Indexing is about defining a single integrated soil quality index from specific SQ indicators (Granatstein, 2002). There are several approaches that are used to develop and integrate SQIs into an overall SQ index. They range from a simple scorecard, farmer-base subjective ratings to more objective and complex frameworks such as multi-objective analysis principle of system engineering developed by Karlen and Scott (1994). Seybold et al. (1998) and Bastida et al. (2008) give a summary of a few approaches that are commonly used. However, there is still a large degree of subjectivity that goes into creating a quantitative index. For example, the index will depend on the choice of the functions, which indicators to include, what scoring functions and the weightings to applied (Granatstein, 2002).

Wienhold et al. (2004) describe an approach to index soil quality indicators for the purposes of comparing them among the sites or treatment. They propose that SQI values need to

be normalised using mathematical scoring curves that are developed to describe the relationships between an indicator value and a specific soil process. Indicator selection for a particular process or function can be done using expert opinion or a statistical procedure such as principle component analysis. The scoring curves can be constructed to account for the effects that inherent soil properties and climate have on the indicator being evaluated. Indicators for a MDS can then be quantified for soils under a range of management systems and the indicators can be scored using the curves. The scored values are then combined in some way (additive, multiplied, or weighted) to form an index value for that management system.

These indexing procedures can be easily modified for different soils and can be used to enumerate dynamic soil quality ratings, determine trends in those ratings, and thus be used to quantify long-term effects of alternate land uses or soil management decisions (Karlen et al., 2003). Index values created in a similar way can then be compared among management systems or over time for a particular management system (Wienhold et al., 2004).

2.2.8.2 Farmer score card

This is one of the qualitative measures of soil quality. They tend to be more subjective in their measurements, but can be more easily and some times more informative to the land managers (Seybold et al., 1998). According to Seybold et al. (1998), the scorecard is a farmer-based subjective rating system (the farmers' perception of soil quality) based on sensory observations such as look, feel and smell, and places indicators into rating scales of healthy, impaired and unhealthy. The system uses 43 SQI or soil attributes that integrate observations throughout the growing season, but does not recognise the relative importance of these indicators. This system was developed for cropping systems in Wisconsin, USA. Score cards have been adapted to cover other regions and farming systems.

2.2.8.3 Multiparametric quality indices

According to Bastida et al., (2008), Karlen et al. (1994) were probably the first to establish a multiparametric index for soil quality. Their framework normalised scoring functions established by Karlen and Stott (1994), for evaluating a production system's effect on soil quality in Lancaster (WI, USA). They developed an integrative equation based on selected soil functions which are weighted and presented by Bastida et al., (2008) as follows:

$$\text{Soil Quality} = q_{we}(wt) + q_{wma}(wt) + q_{rd}(wt) + q_{fqp}(wt) \qquad \text{Eq [2.1]}$$

Where:

q_{we} is the rating for the soil's ability to accommodate water entry,

q_{wma} is the rating for the soil's ability to facilitate water transfer and absorption,

q_{rd} is the rating for the soil's ability to resist degradation,

q_{fqp} is the rating for the soil's ability to sustain plant growth, and

wt is the numerical weight for each soil function.

The, overall soil quality score is the sum of all function scores.

One of the downsides in using their framework is that their weighting methods are subjective and not based on mathematics or statistics (Bastda et al., 2008). The weights reflect the importance of a soil function in fulfilling the overall goals of maintaining soil quality under specific conditions or purposes (Bastida et al., 2008). This framework has been adapted and used by other researchers (e.g. Andrews et al., 2002; Sharma et al., 2005). Other frameworks have also been developed to evaluate non-agricultural soils which have been widely used (e.g. Doran and Parkin, 1994; Trasar-Cepeda et al., 1998).

2.2.9 Soil quality in relationship to policy

Soil quality is evaluated mainly to provide farmers and advisors with a soil management tool and to monitor the sustainability of arable land use (Palojarvi and Nuutinen, 2002). Soil quality has been used as an educational concept to make students, policymakers, producers, and the public more aware of the essential processes soils perform (Wienhold, 2002).

In relation to policy, concerns over the decline in soil quality, and in making attempts to reverse these trends, policy makers have worked towards making laws to protect the soil resources. Thus, the soil quality concept provides a foundation for national policy to protect the environment (Seybold et al., 1998). For example, Dennis Keeney, director of the Leopold Center for Sustainable Agriculture (IO, USA), called for an enactment of a national soil quality act, similar to the water and air quality (Arshad and Martin, 2002).

In Europe, a threat to soil health has been reported since the 1970s, but there was also a strong belief in a self-remediation capacity of soil (Filip, 2002). In the 1980s, there were first moves towards the development of a well-aimed soil protection by Germany and The Netherlands, and later also in the European Community (Filip, 2002). Finally, on 1 March 1999 a Federal Soil Protection Act was put in force in Germany. However, the practical application of these policies requires well-justified standards and reliable monitoring methods to be available (Filip, 2002). Subsequently, in 2006 the European Union created and adopted a Soil Thematic Strategy (STS) (COM(2006) 231) and has proposed a Soil Framework Directive (SFD) (COM(2006) 232) with the objective to protect soils across the EU in a coordinated way. The strategy and the proposal have been sent to the other European Institutions for the further steps in the decision-making process and full ratification by Member States is still pending. The proposed STS and SFD includes: The establishment of a common framework to protect soil on the basis of the principles

of preservation of soil functions, prevention of soil degradation, mitigation of its effects, restoration of degraded soils and integration in other sectoral policies. The requirement to identify, describe and assess the impact of some sectoral policies on soil degradation processes with a view to protect soil functions. The requirement for land users to take precautionary measures when their use of the soil can be expected to significantly hamper soil functions.

The rationale is to ensure a more rational use of land in accordance with Article 174 of the EC Treaty and to maintain as many soil functions as possible. This requires identification of areas at risk of erosion, organic matter decline, salinisation, compaction and landslides, and establishment of national programmes of measures and the need to assess the extent of the areas at risk of these threats. To ensure a coherent and comparable approach, in this exercise common elements (parameters) which are known to be driving forces for the different threat are to be used with risk reduction targets and programmes to be adopted by member countries. Furthermore limits were set to the amounts of dangerous substances that could be introduced into the soil, to avoid accumulation that would hamper soil functions and create a risk to human health and the environment. In addition the member countries are to setup a mechanism to fund remediation of the contaminated ophan sites that are identified

2.2.10 Reservations to the concept

The soil quality paradigm has received several criticisms within the soil science community, because many believe that the concept generally lack sufficient quantification and scientific rigour, has generalized and oversimplified the collective knowledge and wisdom developed through several centuries of intensive, in-depth, global studies of soil resources (Sojka and Upchurch, 1999; Herrick, 2002; Letey et al., 2003; Sojka et al., 2003). More specifically,

these authors' criticisms include the following among others: (i) The definition of soil quality is elusive, ambiguous and value-laden. The SQ definition is confounded by countless circumstance-specific, function-dependent scenarios and therefore ultimately too complex to define and creates almost unimaginable indexing complexity. Additionally, implicitly included in the definition are social, economic, biological and other value judgments which have great potential for disagreement. (ii) Indexing of soil status is often times misleading and carries risks to the scientific assessment process, and to the scientist's role as a data interpreter and science mediator. Moreover, the functional relationship between SQ and SQIs cannot always be established empirically. (iii) The concept has policy overtones, and yet fails to reconcile conceptual contradictions and offer no practical means to manage conflicting, and often contradictory soil management requirements for the multiple functions of soil that occur simultaneously. For example, in soil function as a filter, soil quality is high when it has a high capacity to sink toxins before threatening soil-borne organisms or the safety of food crops. On the other hand, making a soil unclean by adding "toxic" herbicides and pesticides improves soil quality for crop production by suppressing target organisms.

Therefore these authors believe that emphasis should be directed towards using available technical information to motivate and educate farmers on 'quality soil management' involving management practices that optimise the combined goals of high crop production, low environmental degradation, and sustained resource use rather than 'soil quality management' (Sojka et al., 2003).

However, several other scientists (e.g. Doran et al., 1994; Carter et al., 1997; Karlen et al., 1997; Karlen et al., 2001; Carter, 2002; Karlen et al., 2008) believe that with further refinement, soil quality indicators could provide a more useful tool for assessing soil quality and solving

problems especially in intensive soil resource use. It may be useful to note that indicators for monitoring soil quality could also help towards developing quality soil management as it can enable users to determine the sustainability of soil and land management systems over time (Palojarvi and Nuutinen, 2002). In addition, the assessments are viewed as tools intended to alert users, in a manner analogous to a "consumer price index," that soil resource problems have or may be occurring (Karlen et al., 2008).

2.2.11 Future perspectives

The evaluation of the applicability of physical and biological soil properties in soil quality assessment is an important challenge for the future. There is a need for basic research to select and develop proper indicators, that would be applicable at different scales (i.e. farm, landscape/regional, national and international scales) (Palojarvi and Nuutinen, 2002). There is also a need for the development of robust tools for integrating the information gained with the various soil quality indicators. For example, Karlen et al. (2001) suggested the development of appropriate indexing frameworks. The rating of indicators in these frameworks will also need the application of reliable mathematical and statistical tools including multivariate methods such as the principle component analysis in dealing with interpretation of the multiple indictors (Palojarvi and Nuutinen, 2002).

Furthermore, more powerful, quicker and yet accurate tools for assessment of soil quality are needed. Infrared spectroscopy is becoming a really powerful tool for soil quality assessment. For example, Shepherd and Walsh (2002) developed and used soil spectral libraries for rapid characterisation of soil properties using the diffuse reflectance spectroscopy. They successfully correlated the reflectance of the soils with the soil functional properties in order to predict the

functional attributes of new samples. Thus infrared spectroscopy could hold a key to a new inexpensive, rapid and accuracy tool for assessing soil quality.

Biological soil quality has been focused on indicators such as respiration, microbial biomass, enzyme activities, nematodes etc (Bastida et al., 2008). Recently, there has been a growing interest in the use of molecular methods to measure soil quality indicators (e.g. Puglisi et al., 2005). However, at the moment these indicators are difficult to measure and are overly sensitive (Bastida et al., 2008) and therefore unsuitable for the purpose.

Overall, the soil quality concept is here to stay, however, new and improved tools will be needed to guide sustainable land use and soil management decisions in the 21st century (Karlen et al., 2008)

2.3 The role of organic matter

2.3.1 The role of organic matter in the soil quality concept

Soil organic matter (SOM) can be defined as 'the organic fraction of the soil exclusive of undecayed plant and animal residues' (Reeves, 1997). Soil organic matter content is the balance between the addition of organic inputs to the soil and decomposition by soil biota. SOM is widely regarded as a vital component of a healthy soil. It is an important part of soil physical, chemical and biological fertility. Total SOM influences soil compactibility, friability, and soil water-holding capacity while aggregated SOM has major implications for the functioning of soil in regulating air and water infiltration, conserving nutrients, and influencing soil permeability and erodibility (Carter, 2002). More specifically, SOM affects the soil's capacity to retain and release nutrients for plant growth by contributing to its cation exchange capacity and through the

mineralization of organic N, phosphorus, and sulphur (Palm, 2002). Furthermore, soil carbon serves as the energy and substrate source for microbial processes; respiration and nutrient storage and turnover (Reeves, 1997). Thus, it has a great deal of control on many of the key soil functions and perhaps the most important indicator of soil quality because of its impact on other physical, chemical and biological indicators of soil quality (Reeves, 1997; Franzluebbers, 2002). Consequently, it is the most consistently reported soil attribute from long-term studies and is a keystone soil quality indicator because of its pervasive role in promoting soil ecosystem functions (Weil and Magdoff, 2004).

Almost all soil and crop management practices have implications for SOM, therefore the changes in the total amount of SOM as well as in the labile carbon fractions and microbial biomass have been used as criteria to evaluate practices and thereby monitor soil quality and health (Weil and Magdoff, 2004). Thus in many soil quality monitoring programs SOM has been included as part of the minimum data set.

2.3.2 The role organic matter in soil classification

As noted above, the SOM fraction in soil is believed to give control to many soil properties. Therefore, many soil-classification systems take into account SOM content in their classification schemes. Nearly all soils contain more than traces of both mineral and organic components in some horizons, but most soils are dominated by one or the other (Soil Survey Staff, 1999). Each system defines the criteria for classifying soils as either mineral or organic. For example, the USDA Soil Taxonomy, generally classifies a soil as an organic soil (Histosol) if more than half of the upper 80 cm of the soil is organic or if organic soil material of any thickness rests on rock or on fragmental material having interstices filled with organic materials

(Soil Survey Staff, 1999). The British classification system defines organic soils as those having more than 40 cm of organic material within the upper 80 cm, or more than 30 cm or organic material resting on the bedrock or extremely stony material. SOM is also used in the classifications to identify some diagnostic horizons such as the mollic, melanic and histic horizons (FAO WRB, 2006). SOM is even more important in topsoil classification systems where organic material, organic matter status and biological features are used to group topsoils. The topsoil classification emphasises a pragmatic soil management approach. Being inextricably linked to many other soil attributes, SOM is considered an integral aspect in the topsoil classification system.

2.3.3 Soil organic matter in this thesis

Numerous studies suggest that SOM cycling is dependent on soil type. For example, the total amount of SOC in the soil profile as well as its distribution with depth is dependent on soil types among other factors (Krull et al., 2004). Saggar et al (1996 and 1999) in their research showed that SOM decomposition and resident time in the soil is influenced by (1) soil texture, which affects the surface area of the soil, and (2) by mineralogy, which affects the nature of organo-mineral complexes. Studies have further shown that different soil types with different clay contents reach different SOC equilibria (Krull et al., 2004). On the other hand, SOM is known to play important roles in the maintenance as well as improvement of many soil properties. SOM is integrally tied to many soil quality indicators and is arguably the most significant single indicator of soil quality and productivity (Revees, 1997). In this thesis we explore the relationships that the soil types share with several dynamic soil quality indicators (including SOM storage and cycling). The laboratory determination of microbial process-level

indicators like SOM decomposition rates, microbial biomass, which could rapidly assess changes in soil quality, was also investigated for predictive relationships with soil types and vegetation classes.

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Chapter 3

Description of the study area and the Countryside Survey programme

3.1 Study area and climate

Soils were collected by the countryside survey 2007, from all over Great Britain representing the main types of landscape and soil groups. The countryside survey was part of the several surveys of the UK countryside aimed at monitoring changes that may be taking place in the countryside, and is briefly described in section 3.6. The general climate is temperate with the mean annual temperature ranging from 7.5°C in North Scotland and 10.6°C in South East and Central England. The mean annual rainfall ranges from 650 mm in East Anglia to 1700 mm in Western Scotland, and the mean annual soil temperature is 10°C at 10 cm depth (Matthew, 2006).

3.2 Soils of the British Isles

The soils in the British Isles are quite varied, both vertically and laterally. These variations are both in morphology and composition and this variation can be attributed to differences in

climate, vegetation and associated organisms, relief, parent material, and age of land form (Avery, 1990). According to Curtis et al. (1976), in the British environment, a distinction can be made between lowland and the upland Britain with regard to soil forming factors. In lowland Britain the soils are essentially man-made due to long periods of agriculture. It was originally covered by deciduous forests which were cleared for land use (Rudeforth et al., 1984). The natural soil forming factors are largely masked by the effects of cultivation, drainage and fertilizing practices resulting in soils that are often termed 'agricultural brown earths', (Curtis et al., 1976; Rudeforth et al., 1984). However, even though the ploughing and use of machinery, draining, marling and use of lime and fertilizers has partly changed the soil profile features, natural relationships between different soils and their environment are still clear, (Rudeforth et al., 1984).

In upland Britain, the soil forming factors have been in operation on relatively unaltered, natural and semi-natural soils. Soil formation in the upland climates are characterised by high rainfall amounts with low temperatures, low solar radiation due to cloud cover and dramatically reduced evapotranspiration resulting in high leaching and water logging on poorly drained surfaces (Curtis et al., 1976). Due to lower temperatures, there is reduced biological activity and slower rates of decompositions, resulting in the formation of organic horizons and peat (Curtis et al., 1976). There is also higher frost incidence at higher elevations causing ice formation and thawing which in turn cause disruptions of structures in the surface layers (Rudeforth et al., 1984).

3.3 Major soils

Soils in this study were classified at the major group level yielding six classes we called "soil types" namely: Lithomorphic, Brown earths, Gley soils, Podzolic, Peats (organic soils) and Pelosols which are briefly defined and sumarised from Avery (1990):

3.3.1 Lithomorphic soils

Lithomorphic soils are immature soils with a bed rock or little altered regolith at shallow depth. They have no diagnostic B horizon or a prominent (cumulic) A horizon (usually less than 10 cm thick), no gleyed or hydrocalsic subsurface horizon, no organic surface layer. They are comparable to most Entisol, a few Inceptisol, and Mollisol orders in US Soil Taxonomy. In the WRB (2006) systems, they are comparable to Leptosols and some Regosols.

3.3.2 Brown soils

These are usually well drained soils with a weathered or argillic B horizon and no gleyed subsurface horizon within 40 cm depth. They are usually brown or reddish in colour. They have a prominent cumulic A horizon and a few lack a distinct B horizon. The US Soil Taxonomy classifies them as Alfisols while the WRB classifies them as Luvisols, Acrisols and Cambisols.

3.3.3 Gley soils

The Gleys are soils with a gleyed or hydrocalcic subsurface horizon that starts within 40 cm depth. They are periodically or permanently saturated with water or formed under wet

conditions. The group is divided into two major groups: the Surface-water gleys and the Ground-water gleys depending on the source of the logging water.

(a) Surface-water gleys are formed by impeded drainage cause by an impermeable layer within the profile so that water logging occurs in the upper horizons of the profile.

(b) Ground-water gleys are characterised by shallow fluctuating ground water table causing water logging in lower part of the profile.

The US Soil Taxonomy classifies them as Entisols, Inceptisols, Mollisols and Alfisols with aquic moisture regimes, while the WRB classifies them as Gleysols, Stagnosols, and some Fluvisols, Luvisols, Acrisols and Planosols

3.3.4 Podzolic soils

These are described by mineral soils with an albic E horizon over a podzolic B horizon (Bh and/or Bs), a thin iron pan (Bf) or both. They have a characteristic feature of a bleached (ashy-grey) upper subsurface horizon associated with a loss of sesquioxides and organic matter to the lower illuvial horizon in the profile. The US Soil Taxonomy classifies them as Spodosols and some Inceptisols while in the WRB they are correlated to Podzols.

3.3.5 Peat soils

These soils have more than 40 cm organic matter (OM) within the upper 80 cm excluding fresh litter or at least 30 cm OM resting directly on top of bedrock or skeletal materials and has no mineral horizon of colour value more than 4. They are also referred to as organic soils. They are correlated to Histosols in both the US Soil Taxonomy and the WRB classifications.

3.3.6 Pelosol soils

Pelosols are slowly permeable non-alluvial clay soils with distinct top soil, weathered or argillic B horizon and a non-calcareous gleyed subsurface horizon within 40 cm depth. These soils crack deeply in dry seasons with block or prismatic subsurface horizons. Pelosols are correlated to Vertisols in both the US Soil Taxonomy and the WRB classifications.

3.4 Distribution of major soil in the British Isles

A 1:1,000,000 scale generalized soil map of the EEC countries was produced showing the distribution of 15 broad soil associations. The soil associations were defined based on dominant soil groups or subgroups and in one case by thermal regime (Avery, 1990). The schematic soil map (Fig 3.1) gives a general distribution of soil associations in the British Isles. More detailed soil surveys have been done at different times and in different parts of the British Isles. For instance, in 1947 a 1:63,360 scale soil maps for parts of England and Wales were produced and a national map at 1:250,000 scale in 1979.

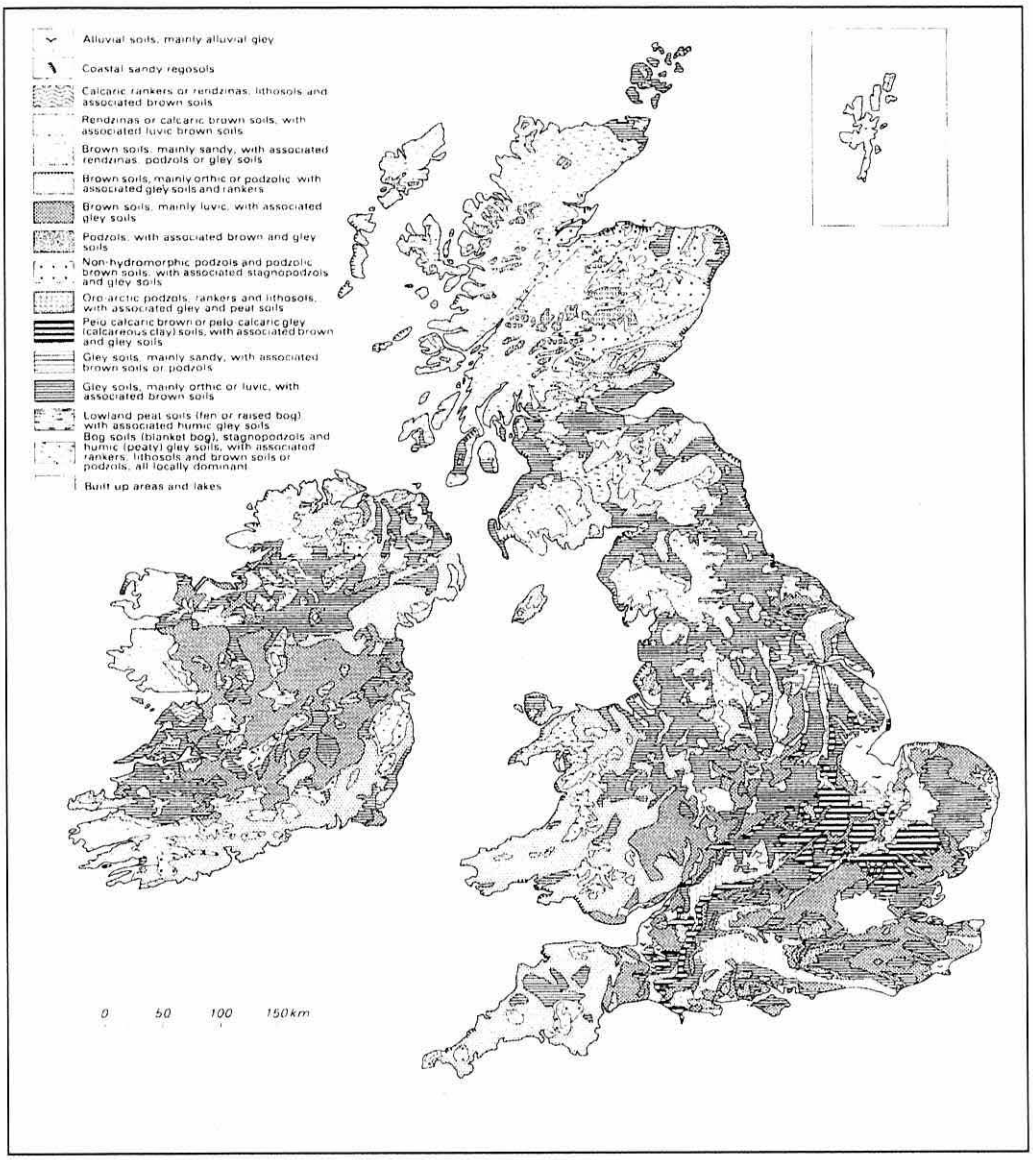


Figure 3. 1. Schematic soil map of the British Isles showing soil associations defined based on dominant soil groups or subgroups (Avery, 1990).

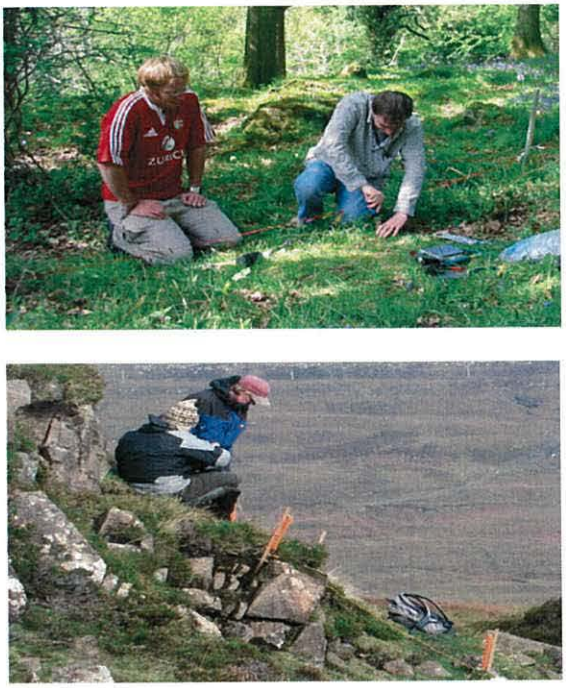
3.5 Geology

The parent materials in the uplands of Britain are mainly derived from hard and resistant Palaeozoic rocks and are of low base content with major amendments of the periglacial processes (Curtis et al., 1976). However, for both low and upland parent material, quaternary sediments of

glacial, aeolian, alluvial, colluvial or biogenic origin, which were laid down at various stages in the evolution of the present landscape form part of the parent materials of soil in many places in Britain (Avery, 1990).

3.6 Countryside Survey

The 2007 Countryside Survey (CS) is summarised in the 'UK Headline Messages from 2007' by Carey et al. (2007). The 2007 CS was a UK Government programme undertaken to assess the status of natural resources in the UK countryside. This was part of the several surveys carried out in the past to study the natural resources of the UK countryside. The previous surveys took place in 1978, 1984, 1990 and 1998. In each of these surveys, the countryside was sampled and studied using rigorous scientific methods, so that the results at these intervals could be compared so that the gradual and subtle changes that occur in the UK countryside could be studied over time. The survey used the CEH Land Cover Map coupled with the field surveys. The Land Cover Map uses data from satellites to form a digital map of the different types of land over across the UK while the field surveys involved an in-depth study of a sample of 591 individual 1 km ×1 km squares across Great Britain (Emmett et al., 2010).



Collecting soil samples. Source: Carey et al., 2008, CEH

Samples being prepared for anlysis: Carey et al., (2008), CEH

Figure 3. 2 Sample collection and preparations

of sampling 'plots' within each square, soil samples were collected, vegetation, freshwaters and other landscape features were studied in detail (see Emmett et al., 2010 for details). The soils were taken to the laboratory for various tests (e.g. Olsen P, pH, EC, loss-on-ignition, percentage C and N, mineralisable N among other attributes). The methods used to carry out the field and laboratory tests are detailed in the CS Technical reports 1-9/07 available on the CEH website for CS. The results were compared with findings from previous CS, to measure and analyse change in the countryside (Carey et al 2008). The map (Fig3.4) shows the general distribution of samples across the GB.

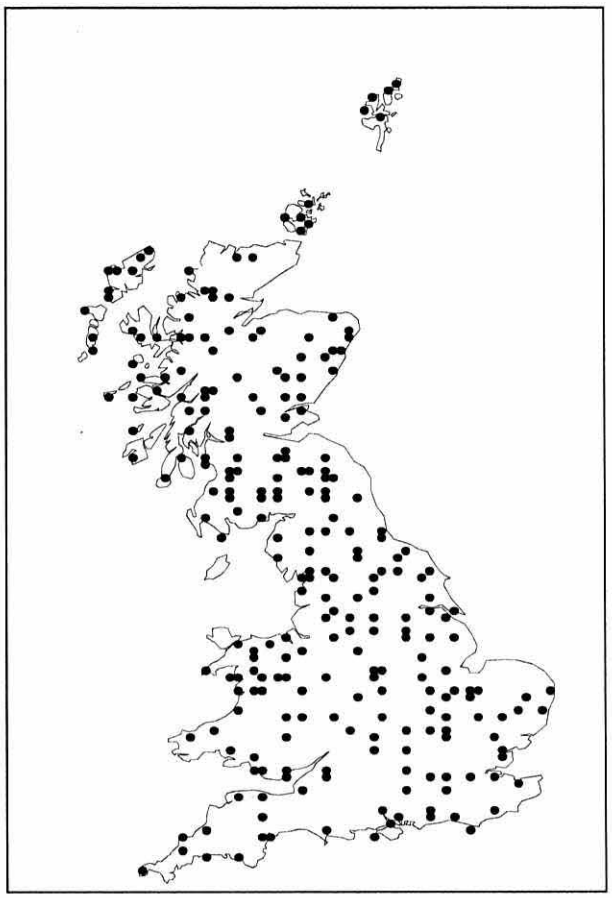


Figure 3.3 Map showing the general location of the Countryside Survey sample squares across the GB (created from the map template and the 10 km^2 coordinate locations of samples)

3.6.1 Soil classification method

The soils were classified during the 1978 and 1990 countryside surveys using the British soil classification system of Avery (1973 and 1980). In 1978, pits were dug at each site of the selected plots in the 1km squares; soils were sampled and classified to the sub group level. In 1990, the Institute of Terrestrial Ecology (ITE/CEH) commissioned a soil survey, to coincide with the main Countryside Survey to map the soil. The Macaulay Land Use Research Institute carried out the survey in Scottish squares, whilst Soil Survey and Land Research Centre, now NSRI dealt with English and Welsh squares. The survey was mapped at 1:25 000. The Major soil

groups level used here were a product of a rigorous comparison process between 1978 data and 1990 maps. The description was derived and allocated manually and therefore the product was a more accurate classification than either the 1978 or the 1990 classifications taken in isolation.

3.6.2 Policy applications of Countryside Survey

Carey et al. (2008) briefly outlines the many potential policy application of the Countryside Survey results as follows:

- Biodiversity: assessment of status and trends in Broad and Priority Habitats, measuring progress towards the 2010 target of halting biodiversity loss;
- Natural environment: measurement and improved understanding of ecosystem goods and services;
- Sustainable agriculture and agri-environment schemes: understanding effects of agricultural policy on the natural environment, including assessment of farmland habitats such as grasslands, hedges and cereal field margins;
- Water resources: context and baseline assessment for the EU Water Framework Directive, especially for headwater streams and ponds;
- Soil protection: measurement of long term trends in soil quality, including soil carbon;
- Sustainable forestry: information on isolated trees and plant diversity within woodlands, to supplement the National Inventory of Woodlands and Trees;
- Urban development: estimates of areas of habitat affected by urban development;
- Air quality: assessment of impacts of air pollution on terrestrial habitats, soils and headwater streams;

- Climate change: provide information to help estimate carbon emissions from land cover change and soils, and to detect impacts of climate change in the countryside.

In this thesis, we took advantage of the rich sample size and data set from the Countryside Survey to test the soil quality concept on the traditional soil classification. In addition, we measured the following soil properties to enrich our data set: microbial biomass C and N, substrate mineralisation in different soils types, phenolics, humics, amino acids, TOC, TN, nitrates and ammonium. We used various statistical methods to analyse the data in order to assess relationships that may occur between the soil types and soil quality indicators.

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Chapter 4: Article I

Major soil types provides a poor indicator of carbon turnover rates in soil

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This paper has not been submitted to any Journal

ABSTRACT

Most soil surveys and classifications are based on soil geomorphic, physical and chemical properties. Typically, microbial properties of the soil (e.g. biomass and functional diversity) or soil biological quality indicators (SBQIs) are not directly considered in soil taxonomic keys, yet soil classification schemes are often used to infer soil biological function relating to policy (e.g. soil pollution attenuation, climate change mitigation). To critically address this, our aim was to assess whether rates of carbon turnover in a diverse range of soils ($n > 500$) could effectively be described and sub-divided according to broadly defined soil types by conventional soil classification schemes. Carbon turnover in each soil over a 90 d period was assessed by monitoring the mineralization of either a labile (^{14}C-labelled artificial root exudates) or more recalcitrant C source (^{14}C-labelled plant leaves) in soil held at field capacity at 10°C. A double exponential first order kinetic model was then fitted to the mineralization profile for each individual substrate and soil. ANOVA of the modelled rate constants and pool sizes revealed significant differences between soil types; however, these differences were small regardless of substrate type. Principle component and cluster analysis further separated some soil types or groups; however, the definition of the class limits remained ambiguous, as exclusive reference values for each soil type could not be established since the model parameter ranges greatly overlapped. We conclude that broadly defined soil types using conventional soil classification provide a poor predictor of C mineralization at least over short time periods. We ascribe this to the high degree of microbial functional redundancy in soil combined with the inability of short term biological assays to represent pedogenic processes which have taken ca. 10,000 y to become manifest.

Keywords: C substrate; C residence time; Nutrient cycling; OM Decomposition; Low molecular weight and High molecular weight Carbon.

4.1 Introduction

Conventional soil surveys and classifications are typically based on soil geomorphic, physical and chemical properties in which the microbial properties or soil biological quality indicators (SBQIs) are not directly considered. Yet, SBQIs can provide a greater indication of key processes operating in soils at the present time (Parr et al., 1992). In many instances it is this classification information that is required by policymakers when devising strategies for soil protection and evaluating ecosystem service provision. One example of this is the potential role that different soil types have to play in the sequestration of C within policy-relevant timescales (i.e. <30 years). This may be a feature more related to SBQIs rather than traditional soil classes which may reflect processes that dominated thousands of years ago.

Approximately 80% of global, terrestrial biosphere C storage occurs in soil, however, the distribution of this C varies significantly between soil types (IPCC, 2001). In some cases this C may be very old and may provide a false impression of C sequestration potential in the short term (Paul et al., 1997). Given current concerns about increasing concentrations of CO_2 in the atmosphere and their potential effects on global climate, it is of the utmost importance that the factors controlling soil C storage in contrasting soils are understood. Empirically, the quantity of C stored in any soil is determined by the difference between rates of organic matter input and rates of organic matter loss. Most inputs of organic matter to soils are from plants, and most losses are due to decomposition of organic matter C by soil microbes and subsequent return to the atmosphere as respired CO_2. To a first approximation, the rate of decomposition of organic matter in different soil types is determined by four factors: environmental conditions (e.g. climate, drainage and land management), the quality of the organic matter (e.g. C:N:P:polyphenol ratios), soil mineralogy (e.g. clay content and type) and existing C content

(Anderson and Domsch, 1990; Chotte et al., 1998; von Lützow et al., 2006; Ananyeva et al., 2008; Marschner et al., 2008; Smith, 2008).

Organic matter inputs can be broadly characterised into two pools (van Hees et al., 2005). Pool 1 contains highly bioavailable, low molecular weight (MW) compounds (e.g. sugars, organic acids and amino acids) which have a turnover time in soil of hours, while Pool 2 contains more recalcitrant plant polymers (e.g. cellulose, lignin and some proteins) which break down over days-to-months timescale. Low MW compounds are continually released to the soil through root exudation as well as when cells are lysed. Structural polymers are delivered at times of cell death (Nguyen, 2003; Jones et al., 2004). The decomposition of low MW compounds and that of more recalcitrant polymers has often been attributed to different taxa of soil microorganisms (McGill et al., 1981; Henriksen and Breland, 1999; Ingwersen et al., 2007; Ekschmitt et al., 2008; Poll, et al., 2008).

Association of organic matter with soil minerals has often been linked to changes in its residence time in soils (Torn et al., 1997; Paul et al., 2008; Kögel-Knabner et al., 2008). In particular, longer C residence times have been attributed to organic matter protection by association with clay minerals (Saggar et al., 1996, 1999; von Lützow et al., 2006). Further, the capacity for protection of organic matter by association with minerals is dependent on the availability of mineral surfaces (von Lützow et al., 2006; Kögel-Knabner et al., 2008). Consequently, given similar mineralogy, younger soils with lower C contents may have a higher capacity for the protection of organic matter than soils with higher pre-existing C contents. Currently, the exact mechanisms controlling the residence time of organic matter in soil are not fully understood, and a variety of other factors such as soil pH and presence/availability of elements other than C, modify rates of C residence in individual soils (Kuzyakov et al., 2007;

Rillig et al., 2007; Paul et al., 2008).

Organic matter turnover rates in soil have frequently been measured by the addition of isotopically-labelled substrates to soil and measuring mineralisation rates by capturing evolved CO_2 (Nguyen and Guckert, 2001; Perelo and Munch, 2005; Boddy et al., 2007; Hill et al., 2008). In this investigation we used this approach to examine the variation of substrate mineralisation rates across a range of UK soil classes. Environmental conditions and C substrate quantity and quality were controlled. Thus, we were able to directly investigate the effect of soil type on the turnover of organic matter. In addition to the specialisation of different microbial taxa on different qualities of organic matter decomposition, their distribution is in the soil is also dependent upon soil structure and mineralogy (Ekschmitt et al., 2008; Kögel-Knabner et al., 2008). Consequently, the decomposition of C substrates also shows some specificity to soil type (Kögel-Knabner et al., 2008). Thus, we hypothesised that measurements of the mineralisation rate of organic matter of more than one quality would increase the capacity of this SBQI to resolve differences in soils. This is in addition to the more fundamental importance of evaluating residence times of organic matter in different ecosystems.

4.2 Material and methods

4.2.1 Soil Sampling and preparation

To encompass all the major soil and land use types, a total of 524 soil samples were collected throughout the UK, according to a 15 km square grid laid across the country as described by Scott (2008). Fig. 4.1 shows the general location and distribution of samples across the UK.

Figure 4. 1 Map showing the location of soil samples used in this study

At each grid intersection, a 1 km^2 sample area was selected. Within the 1 km^2 sample area, 3 plots (5 × 5 m^2) were randomly located and a single 15 cm long × 4 cm diameter soil sample was collected from each of the plots. Additional information about vegetation and soils were also collected from the same plots. The 1 km^2 areas were stratified within 45 Land Classes (see Appendix 1). Across all land use categories, the dominant soil types (% of total) were: Brown soils (31%), Surface water gleys (18%), Podzolic soils (15%), Peats (13%), Groundwater gleys (12%), Lithomorphic soils (8%), and Pelosols (3%). All the sites were characterised by a temperate climate with a North-South mean annual temperature range of 7.5 to 10.6°C and East-West mean annual rainfall range from 650 to 1700 mm (Mathew, 2006).

To normalize for soil moisture and ensure all soils were at field capacity, artificial rainfall (125 μM NaCl, 15.7 μM $CaCl_2$, 1.3 μM $CaSO_4$, 15.3 μM $MgSO_4$, 12.3 μM H_2SO_4 was applied to each soil core (10°C) until 150 ml of leachate had been collected according to the protocol described by Emmett et al. (2008). The soils were then incubated at 10°C for 28 d to equilibrate, after which the samples were broken up, mixed by hand, and visible roots/stones removed.

4.2.2 Soil classification

Soils were classified according to the England and Wales Soil Classification system (Avery, 1990). The system is hierarchical, defined at four successive categorical levels, with classes termed major soil groups, soil groups, soil subgroup and soil series. Soils were classified to one of the six major soil groups namely; Lithomorphic, Brown, Surface-water Gleys, Ground-water Gleys, Podzol, Peat and Pelosol soils (see page 56 for the method of classification).

Major soil type	Abbreviation	World Reference Base
Brown (163)	Browns	Cambisols, and some Luvisols, Acrisols
Lithomorphic (42)	Lithom	Leptosols and some Regosols
Gley (63+94)	GWGs & SWGs	Gleysols, Planosols & some Fluvisols/Luvisols
Podzolic (79)	Podzol	Podzols
Peat (68)	Peat	Histosols
Pelosols (15)	Pelosol	Vertisols
N=524		

Table 4.1 shows conceptually comparable classification of the soils in the World reference base (WRB) Classification. Number in brackets indicates the number of samples for that soil type

4.2.3 Mineralisation of substrates

A simple or complex ^{14}C-isotopically labelled C substrate was used to estimate mineralisation rates in soil. The simple C substrate was chosen to reflect low molecular weight root exudates and comprised a solution of ^{14}C-glucose (50 mM), ^{14}C-citrate (10 mM), ^{14}C-fructose (5 mM), ^{14}C-malate (5 mM), ^{14}C-sucrose (5 mM) and ^{14}C-succinate (2 mM) and possessed a specific activity of 8.4 Bq μmol^{-1} C. The complex C substrate consisted of ^{14}C-labelled shoots of *Lolium perenne* (L.) with a specific activity of 12.3 kBq g^{-1}. The ^{14}C-enrichment of *Lolium perenne* plant material was performed by pulse labelling with $^{14}CO_2$ at a constant specific activity according to Hill et al. (2007). The *Lolium perenne* plant material was used because of its ecological siginificance in British grassiland soils.

To characterise the ^{14}C label in the plant material, a sequential chemical fractionation was performed according to Jones and Darrah (1994). Briefly, 50 mg of finely ground plant material was sequentially extracted in 8 ml deionised water for 30 min at 85°C, 8 ml 20% ethanol for 30 min at 80°C, 5 ml 0.3% HCl for 3 h at 95°C and 5 ml 1 M NaOH for 1 h at 95°C. After each extraction step, the sample was centrifuged (5000 *g*, 15 min), the supernatant removed and its ^{14}C content determined using Optiphase 3® Scintillation fluid (PerkinElmer, Waltham, MA) and a Wallac 1404 Liquid Scintillation Counter (PerkinElmer, Waltham, MA).

For each soil, 10 cm^3 was placed into a sterile 50 cm^3 polypropylene container. Either 0.5 ml of the ^{14}C-labelled simple C substrate (artificial root exudates) or 100 mg of the ^{14}C-labelled complex C substrate (*Lolium perenne* shoots) was then added to the soil. A further 0.5 ml of distilled water was added to the soil receiving the complex C substrate to maintain the same moisture content in both treatments. A vial containing 1 M NaOH (1 ml) was then placed above the soil and the polypropylene containers hermetically sealed. The $^{14}CO_2$ capture efficiency of the NaOH traps was >95%. The soils were then placed in the dark in a climate-controlled room (10°C) and the NaOH traps exchanged after 0.5 h, 1 d, 7 d, 14 d, 28 d and 90 d. The $^{14}CO_2$ in the NaOH traps was determined by liquid scintillation counting as described above.

4.2.4 Mineralisation kinetics

A double first order kinetic model was fitted to the experimental data using Sigmaplot v10.0 using a least squares minimization routine (SPSS Inc., Chicago, IL) where.

$$Y = [a_1 \times \exp(-k_1 t)] + [a_2 \times \exp(-k_2 t)] \qquad \textbf{[Eq. 4.1]}$$

Where Y represents the amount of ^{14}C remaining in the soil, a_1 and a_2 describe the size of the two organic matter pools in the model at time 0, k_1 and k_2 are the exponential coefficients describing the rate of turnover of pools a_1 and a_2 respectively, and t is time after substrate addition.

For the simple C substrate, pool a_1 is attributable to the rapid use of substrate in catabolic processes while pool a_2 is attributable to the slower turnover of C incorporated into the microbial biomass via anabolic processes (Paul and Clark, 1989; Boddy et al., 2007, 2008). For the complex C substrate, pool a_1 is attributable to the rapid use of labile C (e.g. simple sugars, proteins, amino acids), while pool a_2 is attributable to the slower turnover of both the C incorporated into the microbial biomass via anabolic processes and the plant structural C (e.g. cellulose, hemicelluloses and lignin) (Ingwersen et al., 2007).

The half life (HL_1) of the substrate pool (a_1) was calculated as follows:

$$\boldsymbol{HL_1 = \frac{\ln(2)}{k_1}} \qquad \textbf{[Eq. 4.2]}$$

When ^{14}C is transformed by microbial processes, a proportion of it remains in the soil and so may enter and re-enter the biomass repeatedly (Kouno et al., 2001). Consequently, due to the uncertainty of connectivity between pools a_1 and a_2 we did not calculate the half life for pool a_2. The stabilisation of organic matter C in soils has been linked to the soil mineralogy, and especially to clay and silt content (Six et al., 2002; Paul et al., 2008; Stewart et al., 2009). As an index of the stabilisation of C in soils we calculated the Biophysical Quotient (BQ; Bradbury et al., 1993; Saggar et al., 1994; Saggar et al., 1999) where

$$\boldsymbol{BQ = \frac{Respired\ ^{14}C}{Residual\ ^{14}C}} \qquad \textbf{[Eq. 4.3]}$$

According to these authors, high BQ values indicate that more C is respired than is retained (i.e. low stabilisation effect of organic matter), while low BQ values indicate that more C retained than is respired (i.e. a higher C stabilisation effect).

4.2.5 Statistical analysis

Mineralisation rates in the seven soil types were compared using a one way ANOVA using SPSS v14.0 (SPSS Inc.). Post hoc multiple comparisons (pairwise) tests were made using Gabriel test where homogeneity of variance was assumed and Games-Howell procedure where unequal variance was assumed to identify significant differences among specific group pairs. We accepted $P \leq 0.05$ as an indication of statistical significance.

A principal component analysis (PCA) and a cluster analysis were carried out using Minitab v15 (Minitab Inc., State College, PA) to explore the interrelationships between soil types and the model parameters. For the cluster analysis, the average linkage method and a squared Euclidean distance measure were used with the similarity level measured on the vertical axis. The variables were standardised to minimize the effect of scale differences since the variables were in different units.

4.3 Results

4.3.1 Substrates mineralisation

Following addition of the ^{14}C-labeled substrates (both simple C and complex substrates) to the soil, there was an initial rapid phase of $^{14}CO_2$ evolution followed by a secondary slower phase of evolution (Fig. 4.2). The double exponential decay equation gave a good fit to the

biphasic experimental data for both substrate forms (R^2 > 0.9984 ± 0.0002 and 0.9994 ± 0.0001 for simple and complex C substrates respectively; n = 524; Fig. 4.2). The exponential decay coefficients and half-lives (*HL*) describing the mineralization of both substrate are presented in Table 4.2. The amount of ^{14}C recovered and the calculated biophysical quotient (*BQ*) in the seven soil major soil groups are shown in Table 4.3.

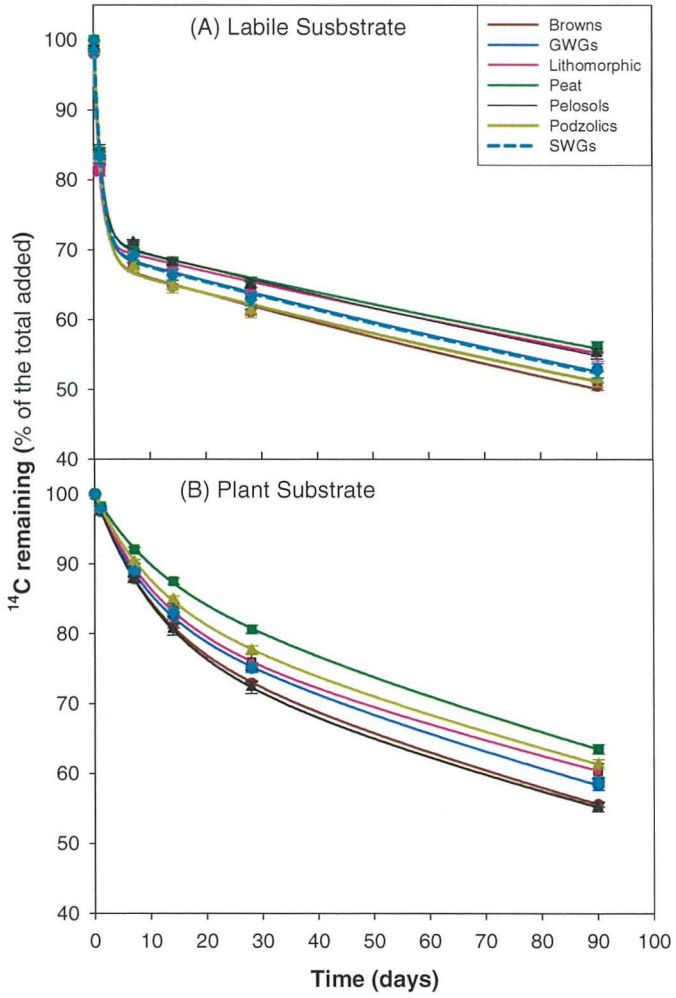

Figure 4.2 The amount of ^{14}C remaining in different soil types after the addition of a simple C substrate (labile; Panel A) and a more complex C substrate (plant material; Panel B). The curves represent fits of a double first order decay model to the experimental data. Values are means ± SEM, n=524.

(a) Labile substrate					
Soil type	a_1	k_1	HL_1	a_2	k_2
Browns	32.5 ± 0.5	0.74 ± 0.03[a]	1.15 ± 0.05[a]	67.0 ± 0.1	0.0035 ± 0.0001[a]
GWGs	31.1 ± 1.2	0.80 ± 0.04[b]	1.06 ± 0.07[a]	68.3 ± 1.2	0.0032 ± 0.0002[b]
Lithomorphics	29.9 ± 1.2	0.96 ± 0.05[c]	0.82 ± 0.05[b]	69.7 ± 1.2	0.0027 ± 0.0001[c]
Peats	30.0 ± 0.8	0.78 ± 0.03[b]	1.00 ± 0.05[a]	69.5 ± 0.8	0.0028 ± 0.0001[c]
Pelosols	28.2 ± 1.2	0.81 ± 0.10[b]	1.02 ± 0.12[a]	71.5 ± 1.2	0.0028 ± 0.0002[c]
Podzolics	31.3 ± 0.8	0.76 ± 0.03[a]	1.10 ± 0.07[a]	68.2 ± 0.8	0.0031 ± 0.0001[c]
SWGs	29.2 ± 0.8	0.81 ± 0.04[b]	1.00 ± 0.05[a]	70.3 ± 0.8	0.0031 ± 0.0001[c]
ANOVA	NS	*	*	NS	***

(b) Plant substrate					
Soil type	a_1	k_1	HL_1	a_2	k_2
Browns	19.8 ± 0.4[a]	0.097 ± 0.002	7.7 ± 0.2	80.2 ± 0.4[a]	0.0039 ± 0.0001[a]
GWGs	18.7 ± 0.8[a]	0.108 ± 0.004	7.0 ± 0.3	81.2 ± 0.8[a]	0.0036 ± 0.0001[b]
Lithomorphics	16.9 ± 1.2[a]	0.099 ± 0.006	7.8 ± 0.5	83.0 ± 1.2[a]	0.0034 ± 0.0001[c]
Peats	12.6 ± 0.7[b]	0.097 ± 0.005	8.2 ± 0.4	87.3 ± 0.7[b]	0.0034 ± 0.0001[c]
Pelosols	20.4 ± 1.5[a]	0.095 ± 0.005	7.5 ± 0.4	79.6 ± 1.5[a]	0.0039 ± 0.0003[a]
Podzolics	15.0 ± 0.7[c]	0.102 ± 0.004	7.7 ± 0.3	84.9 ± 0.7[c]	0.0034 ± 0.0001[c]
SWGs	18.9 ± 0.7[a]	0.093 ± 0.004	8.3 ± 0.3	81.0 ± 0.7[a]	0.0033 ± 0.0001[c]
ANOVA	***	*NS*	*NS*	***	***

Table 4.2. Coefficients for the first order decomposition model describing the turnover of a simple C substrate (a) and a more complex C substrate (b) in a range of soil types. The pool size and the mineralisation rate constant for the fast and slow phases of the kinetics model are represented by a_1 and a_2, and k_1 and k_2 respectively. The half times for the respective pools were defined by $0.693/k$. Values represent means ± SEM, n=54. *NS* indicates not significant between groups ($P>0.05$) while *, ** and *** indicate significant differences at the $P < 0.05$, $P < 0.01$ and $P < 0.001$ levels respectively. Statistical differences between soil types is shown by subscript letters at $P<0.05$.

Soil type	Labile substrate (% total ^{14}C added)			Plant substrate (% total ^{14}C added)		
	Respired C	Residual C	*BQ*	Respired C	Residual C	*BQ*
Browns	50.2	49.8 ± 0.7	1.03 ± 0.03^{a}	43.5	56.5 ± 0.4	0.78 ± 0.01^{a}
GWGs	48.9	51.1 ± 1.7	0.95 ± 0.06^{a}	40.9	59.1 ± 0.6	0.70 ± 0.02^{b}
Lithomorphic	45.8	54.2 ± 1.7	0.81 ± 0.04^{b}	39	61.0 ± 0.9	0.65 ± 0.02^{c}
Peats	45.8	54.2 ± 1	0.86 ± 0.03^{c}	35.6	64.4 ± 0.6	0.56 ± 0.01^{d}
Pelosol	44.1	55.9 ± 1.8	0.81 ± 0.05^{b}	43.8	56.2 ± 1.6	0.83 ± 0.03^{a}
Podzolic	47.9	52.1 ± 1.1	0.97 ± 0.04^{a}	37.4	62.6 ± 0.6	0.61 ± 0.02^{e}
SWGs	45.8	54.2 ± 1.0	0.94 ± 0.05^{a}	39.6	60.4 ± 0.6	0.67 ± 0.02^{c}
ANOVA			***			***

Table 4.3. Total ^{14}C recovered (as $^{14}CO_2$) and the calculated biophysical quotient (*BQ*) in the seven soil types for the simple C substrate (labile) and the complex C substrate (plant substrate). *BQ* is the ratio of the respired and residual ^{14}C in the soil. Values represent means ± SEM, n=54. *** indicate significant differences at $P < 0.001$ levels. Statistical differences between soil types is shown by subscript letters at $P<0.05$.

4.3.2 *Distribution of ^{14}C in plant material*

Of the total ^{14}C contained in the plant material and subsequently added to soil 32.9 ± 1.5% was extractable by water, 4.2 ± 0.2% by ethanol, 16.8 ± 0.6% by HCl, 27.5 ± 0.4% by NaOH and 18.5 ± 2.2% was insoluble residue (see distribution chart in appendix 2). These components approximately correspond to readily decomposable or neutral-detergent soluble C (water and ethanol soluble), cellulose and hemicellulose (HCl soluble) and lignin (NaOH soluble and insoluble-humus) fractions of organic matter respectively (Domisch et al., 1998; Moorhead and Sinsabaugh, 2006; Ekschmitt et al., 2008).

4.3.3 *Dependence of ^{14}C mineralisation on substrate type*

Following additions of substrates to the soils, the loss of ^{14}C from soil showed an initial rapid phase followed by a secondary slower phase (Fig. 4.2). The pattern was similar for all soils types. The recovery of $^{14}CO_2$ was greater and faster ($P \leq 0.05$) from soils with labile substrate than from those with the plant substrate. By day 7, the recovery of $^{14}CO_2$ in labile substrate amended soils represented 29 to 32% of the total ^{14}C added. An additional 15 to 17% was recovered over the remaining 83 d. In contrast, when the more complex plant substrate was added to soils, only 8-12% was recovered as $^{14}CO_2$ in 7 d and another 29-33% over the remaining 83 d. The rate of mineralisation in the labile C amended soils decreased sharply at about day 7, compared to that of the plant amended soils whose decrease was more gradual.

The half-lives calculated from k_1 for the complex plant C substrate were approximately 7 to 10-fold greater ($P < 0.001$) than the half lives calculated for the labile substrate amended soils (Table 4.2). In contrast, the k_2 rate constant describing the mineralisation of pool a_2 was 6 to 39%

greater for the more complex C substrate (yielding shorter half-times if calculated) than those for the simple C substrate ($P < 0.001$).

4.3.4 Dependence of ^{14}C mineralisation on soil type

4.3.4.1 Simple C substrate

The allocation of the simple C substrate ^{14}C to the rapidly-respired pool (a_1) was non-significant ($P>0.05$) among the soil types (Table 4.2a). The substrate ^{14}C allocation in the Brown soils (highest) was only 13% more than in Pelosols (lowest). Although the half-time of this pool (HL_1), derived from exponential coefficient k_1, show significant soil type differences ($P<0.05$), only Lithomorphic soils were different from the rest. The exponential coefficient for the slower phase of mineralisation (k_2) separated three significantly ($P<0.001$) different groups being: Browns (1) > GWGs (2) > Lithomorphics, Peats, Pelosols, Podzols and SWGs (3) (Numbers in brakets are group numbers). While the biophysical quotient (BQ) separated the Browns, GWGs, Podzols and SWGs (1) > Peat (2) > Lothomorphics and Pelosol (3) at $P<0.001$ level of significance

4.3.4.2 Complex C substrate

Microbial allocation of the ^{14}C derived from the labelled plant material to the rapidly respired pool (a_1) was significantly different among the soil types showing 25-38% lower in the Peat soils than for all the other soils ($P < 0.001$) except for the Podzols with 16% bigger a_1 ($P <0.01$; Table 4.2b). Allocation of ^{14}C to pool a_1 was 24-26% lower ($P<0.001$) for the Podzols than for the Brown SWG, GWGs and Pelosol soils. There were no significant differences ($P>0.05$) among the soil types with respect to the half-time of pool a_1 (HL_1) with the shortest half life being only *ca.* 15.5% shorter than the longest. The exponential coefficient for the slower

phase of the mineralisation of ^{14}C from plant material (k_2) showed significant differences ($P<0.001$) among the soil types with 15-18% larger for Brown and Pelosol soils than for the Lithomorphics, Podzolics, Peats or than for SWGs. The biophysical quotient (BQ) was equally significantly different ($P<0.001$) among soil types and separated the Browns and Pelosols>GWGs>SWGs and Lithomorphics>Podzolics>Peat.

4.3.5 Correlation between exponential coefficients

There was no significant relationship ($P > 0.05$) between the rate of C cycling through the pools a_1 and a_2, and no correlation was observed in the relationships of k_1 and k_2 values between and within the two substrate types.

4.3.6 Multivariate analyses

Principal component analysis (PCA) biplot and the cluster analysis dendrogram are shown in Fig. 4.3 and Fig. 4.4 respectively to illustrate the interrelationships among the model parameters and soil types. The PCA yielded a clear separation of some soil types with respect to several principal components. The first two principle component accounted for 76% (47 and 29% respectively) of the total variance observed in the variables, and therefore, represents the two most important uncorrelated components/axes for our data set to explain differences in the soil types. In other words, the maximum variance in soil types is projected or "extracted" along the first axis, and the maximum variation uncorrelated with axis 1 is projected on the second axis. The first axis represents a gradient from variables on Browns and GWGs (on the right) separated from the Lithomorphics GWGs and Peats (on the left) with the Pelosols, and the Podzolics (in the middle) being intermediate soils. The first axis is a half life gradient with HL_2_1

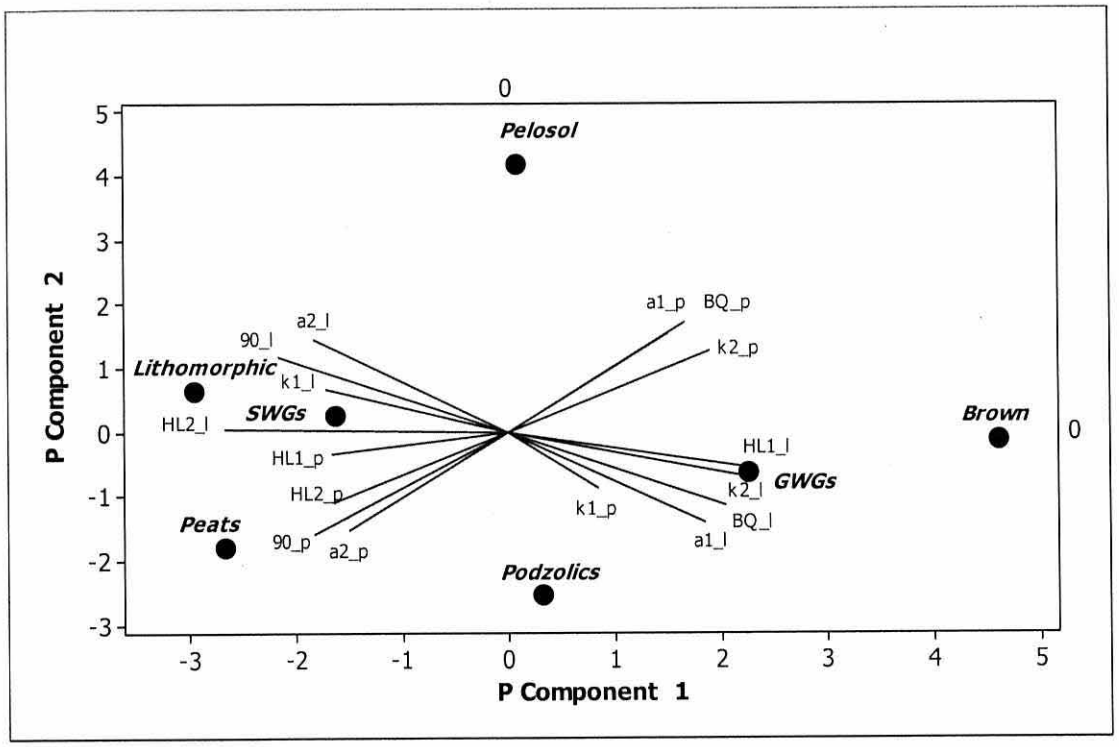

Figure 4.3. A PCA biplot of model parameters and the soil types. The parameters are represented by lines and the abbreviation of the parameter and the soils by dots and soil names abbreviations. The suffix or prefix _l and _p represent simple (labile) and complex (plant) substrates respectively

and HL_1_p scoring high on soil types on the left and HL_1_l and k_2_l on soil types on the right (compare Fig. 4.3 and Table 4.2). The second axis separated the Pelosols (on top) from the Peats and Podzolic soils (on the bottom) and all others being the intermediate soils. However, the PCA suffers from the horse shoe effect and therefore we cannot easily tell whether the Pelosols are at one end of a secondary gradient, or if its position at the end of axis 2 is merely a distortion. The Horseshoe Effect is an artifact of the PCA in which the second axis is curved and twisted relative to the first, and does not represent a true secondary gradient (Palmer, 2010).

The direction of the variable arrows indicates the greatest change in magnitude of the variable, whereas its length may be related to the rate of change (Ramette, 2007). Angles between variable arrows reflect their correlations, e.g. putative interactions between variables (Ramette, 2007).

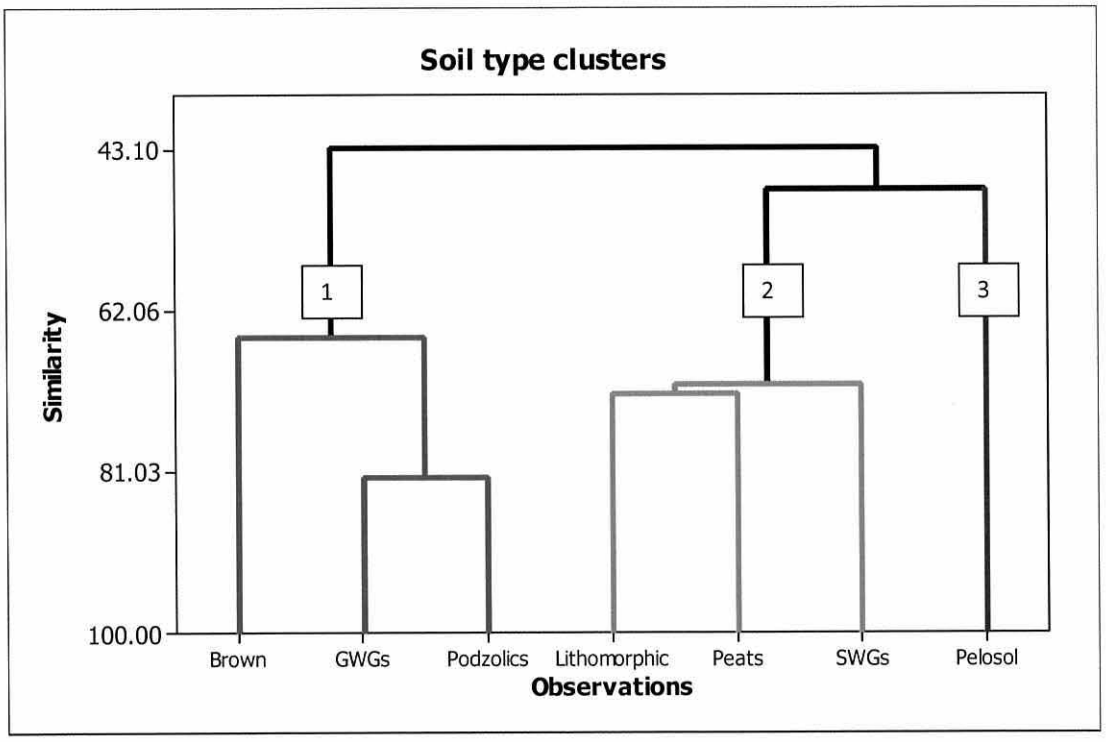

Figure 4.4. Cluster analysis tree diagram (dendrogram) showing three different soil groups at about < 50% similarity level in the model parameter means.

The dendrogram (Fig. 4.4) from the cluster analysis also shows the relationship amongst the soils. Upon examination of the similarity and distance levels, the final partitioning was set to identify three soil groups which were distinctly different from each other but also share common characteristics within themselves. The groups formed were: group 1- comprising Browns, GWGs, and Podzolics; group 2 – comprising lithomorphic, Peats and SWGs and group 3- comprising Pelosol.

4.4 Discussion

4.4.1 Dependence of mineralization on substrate type

Both allocation to the rapidly respired pool, a_1 and the rate at which this pool was mineralized to $^{14}CO_2$ were considerably reduced in the plant material amended soils as compared with the soils amended with the labile substrate. On average, at 7d, the total labile substrate ^{14}C being mineralized to $^{14}CO_2$ was 33% while that from plant material was only 10.5%. This resulted in approximately 3 times as much of the labile substrate ^{14}C being mineralized to $^{14}CO_2$ in the first 7 days as the plant material ^{14}C. However, as the water-soluble and alcohol soluble (and presumably more available) portion of the plant material ^{14}C represented only 37% of the total ^{14}C activity, the initial mineralization rate is as would be expected if the same mechanism of microbial decomposition were operating for both substrates. In contrast to soils amended with the labile substrate, soils amended with plant material maintained a comparatively high rate of ^{14}C mineralization and k_2 in the longer term. The more gradual decline in the mineralization rate can be seen in the shape of the curves in Fig. 4.2. Thus, it was clear that the rate of microbial mineralisation was dependent upon the quality of the available C substrate. We suggest that this difference in dynamics between the two substrates results from the second phase of plant material decomposition being dominated by the slower mineralization of more resistant substances, rather than by $^{14}CO_2$ being evolved during cycling of ^{14}C in the microbial biomass. As different soil microbiota are adapted to decompose different substrate types (Jenkinson, 1977; Henriksen and Breland, 1999; Ekschmitt et al., 2008), the use of the two substrates here provides information on the activity of different functional groups of microbes and how this may differ between soil types.

4.4.2 *Dependence of ^{14}C mineralisation on soil type and implications for soil C sequestration*

The rate of organic matter decomposition to CO_2 in soils is affected by many factors including temperature, moisture content and substrate availability (Fromm et al., 1993; Cavigelli et al., 2005; Kuzyakov et al., 2007; Ananyeva et al., 2008). In this investigation all these factors were kept the same across all soils, implying that differences in soil $^{14}CO_2$ evolution patterns were wholly dependent upon soil type.

Capture of $^{14}CO_2$ from the soils with the overall lowest mineralisation rates was only 12 and 19% (labile and plant material, respectively) less than that from the soils with the overall highest mineralisation rates. Thus, in general, the effect of soil type on the decomposition rate of organic matter was minor. Given that such a wide range of soil types from a similarly wide range of ecosystems were investigated, it is surprising that the capacity of soil microbes to take up and utilize the added organic matter varied so little. Consequently, the capacity for C storage in soils was also largely independent of soil type. Correspondingly, Ananyeva et al. (2008) also found no essential difference in microbial efficiency (qCO_2) in soil types from different climatic regions across European Russia. However, despite the similarities there were some soil type-dependent differences. Most notably, microbes in Peat soils tended to allocate the lowest proportion of the complex substrate to the rapidly-respired pool a_1, and had relatively low k_2 values for both substrates. Thus, as would be expected from the large existing C stores (IPCC, 2001), Peat soils had the lowest decomposition rates and, given equal input rates, would have the greatest capacity for soil C sequestration, regardless of the substrate type. This tendency for Peat soils to allocate more C to the slow-respired pool a_2 and low decomposition rates was more apparent with the plant substrate. In addition, the lowest values of BQ for the plant substrate support this

assumption. Conversely, Brown soils had a relatively high allocation to pool a_1, high k_2 values and high BQ values for both substrates, leading to a lower C sequestration rate per unit of added C than most soils. The accumulation of plant C in peat soils is generally attributed to the slowing of decomposition due to anaerobic conditions. However, the difference in decomposition rates between Peat and Brown soils under the same physical conditions shows a more fundamental difference in the capacity of the soil microorganisms to decompose added C. The structure of the Peat soil matrix consisting of a high proportion of small pores and a very heterogeneous pore structure, provide a conducive environment for C retention, very different from that of a granular porous structure of many mineral soils (Domisch et al., 1998). The greater allocation of C to pool a_2, may indicate that more added C was allocated to microbial growth, and if favorable conditions were to be maintained Peat soil microbiota ultimately develop the same capacity for C mineralisation as microbes in the other soils.

In contrast to the Peat or clayey soil such as Pelosol soils, where the residence time of C in the soil is largely attributed to environmental conditions and peat matrix (Domisch et al., 1998) or soil textural/mineralogical composition (Saggar et al., 1999) respectively, relatively high capacity for soil C storage has been linked to geological and soil forming factors such as age. High capacity to store new C has often been attributed to young, low C soils such as Lithomorphic soils where sites for organic matter protection are unoccupied i.e. has high C saturation deficit (von Lützow et al., 2006; Paul et al., 2008; Yang et al., 2008; Stewart et al., 2009). As the projected enhancement of the C storage capacity is due to an intrinsic quality of the soil, the potential for C storage in these soils can be tested more directly, than in the Peat soils which are a product of external factors (e.g. water logging and pH). Broadly, relatively higher C sequestration rates due to geological factors appeared to be borne out by our

measurements. Despite having a relatively short HL_1 for the labile substrate showing an active microbial biomass, Lithomorphic soils had smaller k_2 values for both substrates than most soils, showing a longer residence time for most of the added C regardless of the form of C (not shown). Pelosols also appeared to have a relatively long residence time for C. For the labile substrate they had relatively high allocation to a_2, low k_2 and low BQ values. In contrast, the capacity of Pelosols to sequester C derived from plant litter appeared to be relatively low and similar to that of Brown soils. This suggests that protection of microbes, and microbially modified C by clay particles was significant, whilst the decomposition rate of unmodified plant material was not significantly altered by clay content.

4.4.3 Inter relationships among variables

The PCA and the cluster analysis revealed some distinct groups of soil types with respect to the first and second principal components used. Although some soil types were clearly distinct from others (e.g. Peats versus Brown), other soils were not (e.g. Podzolics versus SWG) making it difficult to separate them (Figs 4.3 and 4.4). Cutting the dendrogram at 50% similarity level yielded three distinct soil groups (Fig. 4.4) with the Browns, GWGs, Podzolics forming group 1; the Lithomorphics Peats and SWG in group 2 and the Pelosols forming group 3. Elevating the final partitioning to a higher similarity level, could result in splitting Browns from the GWGs and Podzolics in Group 1 making four overall soil groups. In theory we could even separate all groups into individual soil types at a higher level of similarity. However, this partitioning at a higher level is of no practical significant since the soil types in these groups are very similar and exhibit non significant differentiations with respect to the parameters under consideration.

Classification of soils by clustering and other numeric methods presents problems of subjectivity and the results obtained may be affected by the selection and weighting of properties on which measurement of similarity are based (Avery 1990). Other concerns include the choice of the clustering method and the need to include a large numbers of both individuals and attributes to achieve a fairly unbiased result (Avery, 1990). In addition, the use of several or all possible attributes as the basis for each subdivision of a sample population implies that no single attribute is either sufficient or necessary to bestow class membership. Thus, allocation of new members is not a simple matter and it can be very difficult to construct an identical key (Avery, 1990). Consequently, although the cluster analysis produced three reasonable 'natural' groups with respect to attributes included in the study, the practical implications of defining characteristic property ranges for each soil group and much less the soil type was still ambiguous, as the actual property ranges of the parameters under consideration overlapped considerably. The desirable feature would be that the membership of a soil type to a class should be to the exclusion of all others. The ranges could involve usage of one or a combination of a few parameters which would distinguish the soil type from all others. Therefore, there is still need to continue searching for other soil biological quality indicators which can define soil types.

4.4.4 Tentative soil classification key

Figure 4.5 provides an overview and logic for the sequence of the major soils groups in the tentative soil classification key based on the kinetic modelling of C turnover presented here. The soils are allocated to sets or groups on the basis of dominant identifiers, i.e. the parameters whose property ranges are uniquely characteristic to that soil or group.

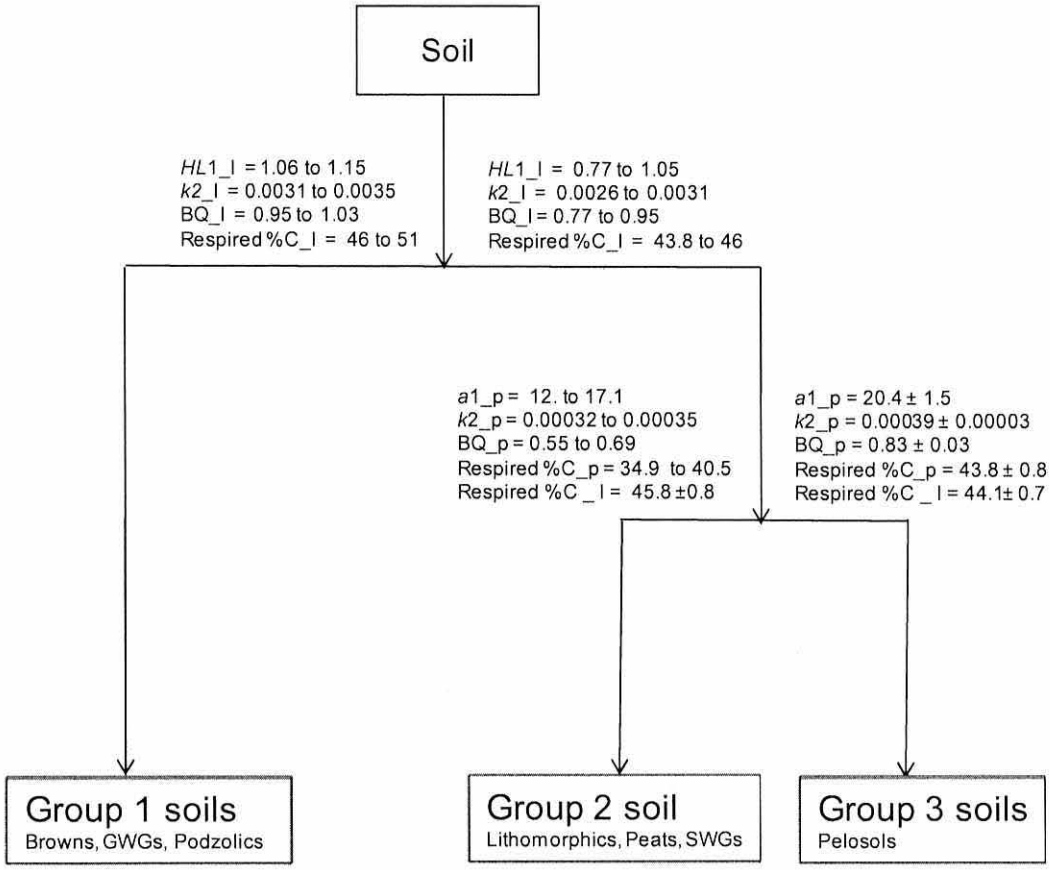

Figure 4.5. A tentative soil classification key based on the model parameters (means ± SEM)

The three soil groups are formed by two hierarchical splits. The first split was based on the half life (HL_1_l) of the simple C substrate, the decomposition rate constant (k_2_l), biophysical quotient (BQ_l) and percentage C respired. The second split was based mainly on the plant substrate mineralization parameters a_1_p, k_2_p, BQ_p and the total percentage of respired C. The first split separated the Browns, GWGs and Podzolics on the left (group 1 soils) and the rest on the right. The second split on the right separated the Pelosols (group 3 soils) from the Lithomorphics, Peats and the SWGs (group 2 soils).

Though in theory the classification key seem quite well laid out, practically we are likely to find it difficult to classify the soils (new members). Most soils would be misclassified or even fall out of the ranges stipulated in the key as there was a great amount of variability within and similarities between the soil groups in the group means of most properties.

4.5 Conclusion

Although the rate of decomposition of two contrasting C substrates varied by soil type, very few soils showed significant soil type effect on the rate of decomposition. Overall, most model parameters proved similar across soil types, possibly because soil moisture was optimal for microbiological activity and growth in these soils. The capture of ^{14}C evolved from the soils with the lowest mineralisation rate was only 12 and 19% less than those with the highest mineralisation rate for labile and plant material respectively. The differences among the soils were exhibited mainly in either more of the C was being respired rapidly and/or in the amount of C allocated to a pool. The differences observed were related to the BQ value i.e. the soil C protective capacity of silt and clay content, which correlated strongly with the mineralisation rate constant in the slow phase regardless of substrate type. These results suggest that the soil types derived from soil surveying classified at major group level may not be at an adequate resolution to allow predictions of variation in the substrate mineralisation rates across the GB soils. Or it could be that the parameters under consideration are by their nature incapable of differentiating the soil types. This is owing to the multiple overlaps in the ranges defined by mean ± SEM of model parameters, making it impossible to establish exclusive reference values for each soil type.

Therefore, we conclude that broadly defined soil types by conventional soil classification provide a poor predictor of C mineralization at least over short time periods. We ascribe this to the high degree of microbial functional redundancy in soil combined with the inability of short term biological assays to represent pedogenic processes which have taken ca. 10,000 y to become manifest. Notwithstanding this failure, the findings of this study can add valuable information to characterise soil types or groups, thereby providing insight about SOM cycling in different soil types. Further investigation may need to consider finer resolution of soil types to reveal soil type effect on substrate mineralisations rates.

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Chapter 5: Article II

Stabilization of root exudate and plant residue-derived ^{14}C during long term incubation in six major soil types

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This paper has not been submitted to any Journal

ABSTRACT

Stabilization of plant-derived carbon (C) in different soil types is influenced by vegetation quality and a wide range of soil physical (e.g. texture, structure), biological (e.g. size, structure and biomass of the microbial community) and chemical characteristics (e.g. pH, C saturation deficit and C input levels) alongside edaphic and management factors (e.g. climate, tillage regime). Understanding the impact of soil type on C sequestration is important for the effective design of large scale greenhouse gas mitigation strategies, optimal provision of ecosystem services and the promotion of sustainable agricultural systems. In this study, we investigated the effect of soil type on long term rates of C cycling using a simple (artificial root exudates) or complex (plant leaves) ^{14}C-labelled substrate. Briefly, topsoils from six major soil types were collected from around the GB, brought to field capacity, and the microbial mineralization of each ^{14}C-labelled substrate monitored in each soil over a 1.5 year period at 10°C. A double first order exponential kinetic decay model, with rate constants of k_1 (for C pool 1) and k_2 (for C pool 2), was then fitted to the experimental ^{14}C mineralization data to estimate C residence time in soil. The results showed that the low molecular weight (MW) root exudate-C was respired much more rapidly than the complex higher MW plant litter derived-C (i.e. higher k_1 values). In contrast, the second slower phase of mineralization (C pool 2) was similar for both C substrates (i.e. similar k_2 values). Overall, soil type-dependent differences in the capacity for C storage were evident in the exudate-C amended soil only, where the Brown soils and Lithomorphic soils tended to respire relatively more C than the ground- and surface-water gley soils. The initial soil levels of acidity, soluble N, humic/phenolic substances, microbial biomass, biodiversity, eco-physiological quotients, and C:N:P ratio did not correlate with the mineralization rate of either substrate. We conclude that long term incubations of soil at constant temperature failed to reveal major differences between soil types and that laboratory mineralization studies may provide a poor proxy for predicting soil C sequestration potential.

Keywords: Carbon cycling, Mineralisation, Soil classification, Soil organic matter turnover

5.1 Introduction

Soil types classified at the major soil group level show major differences based on their predominant pedogenic characteristics within the soil profile as well as differences in the composition or origin of the material within the soil profile (Avery, 1990). Within the UK, most soils have been evolving for more than 10,000 years and clear differences in the amount and spatial distribution of C accumulation have occurred within the soil profile during this time. Within a shorter policy-relevant time scale (ca. 5-50 years) these diverse soil types might present different capacities for rates of C cycling and subsequent stabilization, due to differences in their intrinsic physical, chemical and biological properties. The mechanisms for C stabilization in non-agricultural soils are still poorly understood and consequently the potential for C stabilization at the landscape scale is often difficult to predict (von Lutzow et al., 2006). Thus, studies of C cycling in different soil types at the landscape/regional level are rare.

The content of SOC in soil ranges from less than 1% in sandy soils to almost 100% in Peat soils. In addition, relatively high capacity to store SOC has also been linked to geologic and soil forming factors of age, where young soils such as Lithomorphic soils have a relatively high capacity to store SOC due to its high C saturation deficit (von Lutzow et al., 2006; Stewart et al., 2009). It is generally suggested that there are three main mechanisms responsible for the stabilization of soil organic C (SOC) in soil; (1) biochemical stabilization by selective preservation of certain recalcitrant compounds; (2) inter-molecular interactions between organic matter (SOM) and either minerals or other SOM and; (3) physical protection of SOM from biological attack (Sollins et al., 1996; Baldock et al., 2004; von Lutzow et al., 2006). It seems apparent however, that in the whole soil and even in individual soil horizons that several mechanisms of SOM stabilization may be operating simultaneously, but to different degrees (von

Lutzow et al., 2006). Generally, the stabilization of residue-derived C in the whole soil is mainly influenced by its inherent physico-chemical characteristics such as soil texture, mineralogy and pH (Six et al., 2002; Stewart et al., 2008), climate, the balance between the C input and output levels, C saturation deficit (i.e. how far a soil is from its saturation level; Stewart et al., 2008; 2009) and the decomposition rate (von Lutzow et al., 2006). Soil textural effects are caused by the stabilizing properties that clay and silt particles have on SOM. SOM is either physically trapped in the very small spaces between clay/silt particles or chemically protected through adsorption onto clay surfaces, both of which prevents SOC from being mineralized by soil microbes (Milne and Heimsath, 2008). Soils with high clay/silt content therefore have a tendency to possess higher SOC than soils with low clay content under similar land use and climate conditions (Six et al., 2002).

A range of factors have been implicated in regulating rates of C cycling and residence time in soil including: pH, soluble N content, humic/phenolic substances, microbial biomass, microbial diversity and soil C:N and N:P ratio. Low pH reduces C turnover rates by reducing microbial activity and nutrient turnover in soil. In addition, enzyme denaturation may also occur at extreme pHs and soluble forms of Al^{3+} at low pH may form protective complexes with SOM leading to reduced rates of SOC turnover (Zunino et al., 1982; Kaiser and Guggenberger, 2000; Mayer and Xing, 2001). The priming of SOM turnover by living roots due to rhizodeposition (e.g. root exudation of sugars, amino acids and organic acids) has also been documented by various authors, for example, Kuzyakov et al (2000), Kuzyakov, (2002) and Gärdenäs et al., (2010). High concentrations of humic substances (HS) and soluble phenolics are known to exhibit an inhibitory effect on SOC degradation by a range of mechanisms. For example, HS and phenolics are microbially refractory and are powerful substrate, enzyme and metal ion

complexing agents resulting in a decrease in substrate bioavailability (Freeman et al., 1990; Keum and Li, 2004; Grinhut et al., 2007). They can also be toxic to plant and microbial growth, indirectly reducing rates of SOC cycling (Fernando and Robert, 1976; Magharaj et al., 1986).

It is generally accepted that organic matter inputs can increase soil microbial biomass and activity due to an increase in energy availability (Fontaine et al., 2004; Jin et al., 2010). The soil microbial biomass in itself can provide a labile source and sink of C, N, P and S (Dalal, 1998). Therefore the size of the microbial biomass may provide a proxy of microbial activity and consequently C turnover in soil. On one hand, the relationship between microbial diversity and function in soil is largely unknown, but biodiversity has been assumed to influence ecosystem stability, productivity and resilience towards stress and disturbance (Torsvik and Ovreas, 2002). Since key nutrient transformations are perform by specialized microbes (McGuire and Treseder, 2009), C cycling in soil is likely to depend on its microbial diversity. However, studies have shown that soil is characterized by functional redundancy such that no evidence indicates existence of any relationship between microbial diversity and decomposition rates of organic matter (Nannipieri, 2003). Functional redundancy means that different species perform the same functional role in ecosystems so that changes in species diversity does not affect ecosystem functioning (Nannipieri, 2003).

The C:N:P ratios of soil and plant litter can provide a reliable indicator of substrate quality and lability (Yamakura and Sahunalu, 1990; Hazelton and Murphy, 2007). Frequently, C turnover in soil is limited by the availability of nutrients (e.g., N and P; Cleveland et al., 2006), especially in the early stages of OM breakdown (Berg, 2000). Nutrient ratios lower than 25 and 16 for C:N and N:P respectively generally indicate that N and P are not limiting OM decomposition in soil. However, the late phase of OM decomposition is characterised by an

increase in the concentrations of lignin and N, P and S nutrients (Berg, 2000; Lorenz et al., 2004). During these late stages, there is a negative relationship between N concentration and lignin mass-loss rate as well as between N concentration and litter mass-loss rate (Berg et al., 1982; Berg, 2000). The rate-suppressing influence of N has been attributed to (i) repression of lignolytic enzyme production in fungi, and (ii) the reaction of lignin products with ammonium or amino acids to form recalcitrant SOM complexes (Berg, 2000).

The aim of this study was to test the hypothesis that different soil types would exhibit significantly different rates of SOM turnover over an annual timescale. Further, we hypothesized that these soil type driven differences would be apparent with a more complex high molecular weight (MW) C substrate in comparison to a simpler low MW C substrate. Lastly we hypothesized that measures of soil quality (e.g. pH, EC, C:N ratio, N:P ratio, soluble N, soluble phenolics etc) would correlate with, and help to explain, the observed differences in rates of C turnover.

5.2 Materials and methods

5.2.1 Field sites and climate

Soils (n = 54) from the major classes were collected from around the GB. Soils were classified according to the British Soil Classification system (Avery, 1990) and classified into one of six major soil groups namely; Brown (n = 16), Surface Water Gley (SWG, n = 12), Ground Water Gley (GWG, n = 11), Podzol (n = 8), Peat (n = 7) and Lithomorphic (n = 1), The equivalent FAO soil classes are presented in Table 4.1 in chapter 4 article I of this thesis. The 54 soils were selected randomly from a larger set of samples (n = 524) collected under the CEH

Countryside Survey 2007 programme. The map (Fig.1) shows the distribution of the sample sites

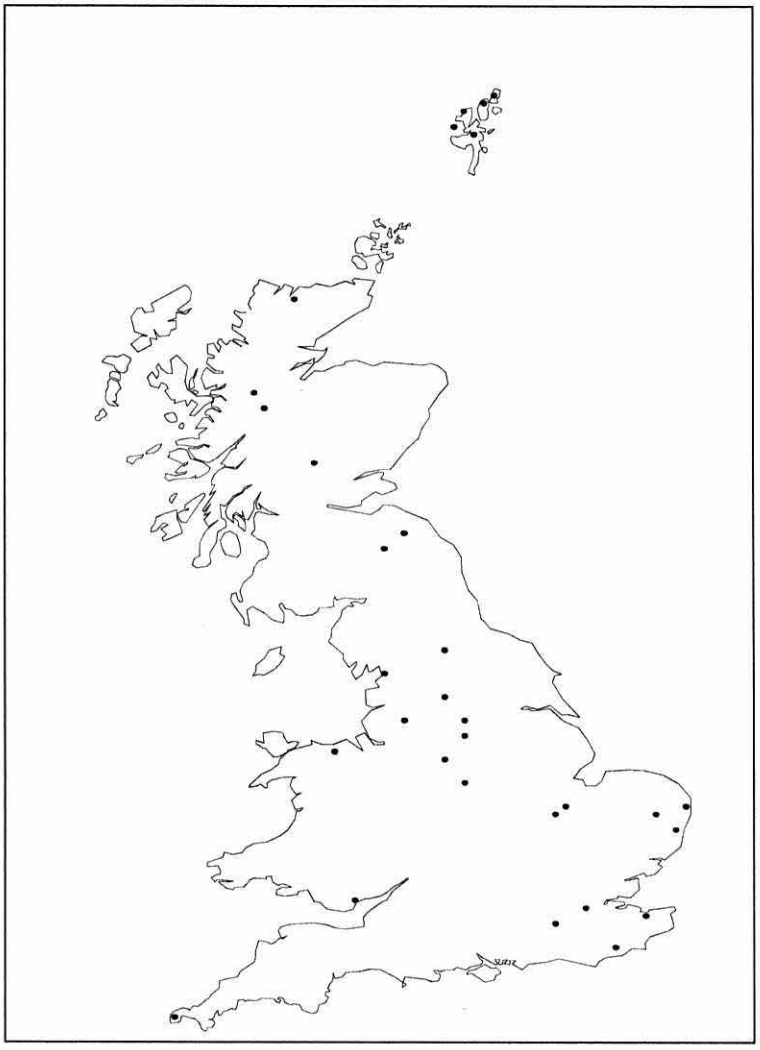

Figure 5. 1 The map showing the distribution of the sample sites across GB

The general climate is classified as temperate with a mean annual temperature ranging from 7.5-10.6°C along a North-South gradient and mean annual rainfall ranging from 650-1700 mm along an East-West gradient. Overall, the mean annual soil temperature was 10°C at 10 cm depth (Matthew, 2006).

5.2.2 Mineralisation of substrates

A simple or complex ^{14}C-isotopically labelled C substrate was used to estimate mineralisation rates in soil. The simple C substrate was chosen to reflect low molecular weight root exudates and comprised a solution of ^{14}C-glucose (50 mM), ^{14}C-fructose (5 mM), ^{14}C-sucrose (5 mM), ^{14}C-citrate (10 mM), ^{14}C-malate (5 mM) and ^{14}C-succinate (2 mM) and possessed a specific activity of 8.4 Bq μmol^{-1} C. The complex C substrate consisted of ^{14}C-labelled shoots of *Lolium perenne* (L.) with a specific activity of 12.3 kBq g^{-1}. The ^{14}C-enrichment of *Lolium perenne* plant material was performed by pulse labelling with $^{14}CO_2$ at a constant specific activity according to Hill et al. (2007).

To characterise the ^{14}C label in the plant material, a sequential chemical fractionation was performed according to Jones and Darrah (1994b), briefly described in chapter 4: article 1 (see appendix 2 for the distribution of the fractions in the plant material.

For each soil, 10 cm^3 was placed into a sterile 50 cm^3 polypropylene container. Either 0.5 ml of the ^{14}C-labelled simple C substrate (artificial root exudates) or 100 mg of the ^{14}C-labelled complex C substrate (*Lolium perenne* shoots) was then added to the soil. A further 0.5 ml of distilled water was added to the soil receiving the complex C substrate to maintain the same moisture content in both treatments. A vial containing 1 M NaOH (1 ml) was then placed above the soil and the polypropylene containers hermetically sealed. The $^{14}CO_2$ capture efficiency of the NaOH traps was >95%. The soils were then placed in the dark in a climate-controlled room (10°C) and the NaOH traps exchanged after 0.5 h, 1 d, 7 d, 14 d, 28 d and 90 d and thereafter every 34 days until a total of 518 d. The $^{14}CO_2$ in the NaOH traps was determined using Optiphase 3® Scintillation fluid (PerkinElmer, Waltham, MA) and a Wallac 1404 Liquid Scintillation Counter (PerkinElmer, Waltham, MA).

5.2.3 Mineralisation kinetics

A double first order kinetic model was fitted to the experimental data using Sigmaplot v10.0 using a least squares minimization routine (SPSS Inc., Chicago, IL) where.

$$Y = [a_1 \times \exp(-k_1 t)] + [a_2 \times \exp(-k_2 t)] \qquad \textbf{Eq. [5.1]}$$

Where Y represents the amount of ^{14}C remaining in the soil, a_1 and a_2 describe the size of the two organic matter pools in the model at time 0, k_1 and k_2 are the exponential coefficients describing the rate of turnover of pools a_1 and a_2 respectively, and t is time after substrate addition.

For the simple C substrate, pool a_1 is attributable to the rapid use of substrate in catabolic processes while pool a_2 is attributable to the slower turnover of C incorporated into the microbial biomass via anabolic processes (Paul and Clark, 1989; Boddy et al., 2007, 2008). For the complex C substrate, pool a_1 is attributable to the rapid use of labile C (e.g. simple sugars, proteins, amino acids), while pool a_2 is attributable to the slower turnover of both the C incorporated into the microbial biomass via anabolic processes and the plant structural C (e.g. cellulose, hemicelluloses and lignin) (Ingwersen et al., 2007). When ^{14}C is transformed by microbial processes, a proportion of it remains in the soil and so may enter and re-enter the biomass repeatedly (Kouno et al., 2001). In spite of the uncertainty of connectivity of substrate pools between a_1 and a_2 (Boddy et al., 2007; Oburger and Jones, 2009), we still calculated the a_2 pool half-life to give the reader some idea of residence times.

The half life (HL_2) of the substrate pool (a_2) was calculated as follows

$$\boldsymbol{HL_2} = \frac{\ln(2)}{k_2} \qquad \textbf{Eq [5.2]}$$

5.2.4 Soil analyses

All soils were analysed as follows: The pH in water was measured using 10 g of field-moist soil in a 50 ml plastic beaker to which 25 ml of deionised water was added giving a ratio of soil to water of 1:2.5 (w/v). The suspension was stirred thoroughly and left to stand for 30 minutes after which time the pH electrode was inserted into the suspension and a reading taken after a further 30 s. Moisture content was determined by weight loss after oven drying 10 g soil at 105°C overnight (16 h). Soil moisture at field capacity was estimated by saturating the soil followed by measuring the soil water retained at -33 kPa suction pressure. Loss on ignition (LOI) was the weight loss measured on 10 g soil (pre dried at 105°C) when heated at 375°C for 16 h. Total soil C and N were measured at CEH Lancaster using UKAS accredited method SOP3102 (on an Elementar Vario-EL analyser; Elementaranalysensysteme GmbH, Hanau, Germany) detailed in Emmett et al. (2008). Olsen P was determined by Olsen P method detailed in Emmett et al., (2010). Briefly, Olsen P was measured on a 5 g air dried and sieved (< 2mm) soil sample. The soil was extracted with 100 ml of 0.5 M sodium bicarbonate at pH 8.5. The phosphorus in the extract was determined colorimetrically using a continuous flow analyser. The analyser method used molybdenum blue at 880nm with the addition of a dialysis step to overcome the effect of the Olsen's reagent. Soil bulk density was determined (mass/volume) after removing stones (> 2 mm). Details of these methods are given in full in Emmett et al. (2008).

Microbial C and N were determined on a 10 g soil sample using the chloroform-fumigation-extraction (CFE) method of Vance et al. (1987). For each soil sample, the microbial C and N in the control and the fumigated subsamples were extracted with 100 ml of 1 M KCl and measured using a TOC-VCSH/CSN total organic C analyzer (Shimadzu, Kyoto, Japan). To

account for incomplete extractability, correction factors of 0.45 and 0.54 were used for microbial C and N respectively. Microbial C = (TOC in fumigated samples - TOC in control samples).

Soil electrical conductivity was determined in the soil using a 4520 conductivity meter (Jenway Ltd, Essex, UK). Soluble phenolics in leachates were determined using the modified method of Box (1983) and Ohno and First (1998). Soluble humic substances in leachates were estimated by measuring the absorbance of solutions at 254 and 400 nm using a PowerWave XS scanning microplate spectrophotometer (BioTek® Instrument, Winooski, VT). Amino acids in soil leachates were determined fluorometrically by the OPAME procedure of Jones et al. (2002). Total soluble organic C and N in leachates were determined using a TOC-VCSH/CSN analyzer (Shimadzu Corp., Kyoto, Japan). Ammonium and nitrate in the leachates were determined using a Skalar SAN^{++} segmented-flow autoanalyzer (Skalar, Brede, Netherlands). The soil C:N and N:P ratios were calculated from the analyses made above. Soil diversity was determined using molecular profiles (TRFLPs) of total bacterial communities on soil cores according to Griffiths et al. (2000) and as described more fully in Article 4 of this thesis. The Shannon index (H) of microbial diversity was calculated using $H = - \Sigma p_i \, ln \, (p_i)$ where p_i is the relative abundance of each TRFLP peak within each sample.

5.2.5 Statistical analysis

The six soil types were compared using a one way ANOVA (using SPSS version 14.0; SPSS Inc., Chicago, IL). Post hoc multiple comparisons (pairwise) tests were made using Gabriel test where homogeneity of variance was assumed and Games-Howell procedure where unequal variance was assumed to identify significant differences among specific group pairs. We accepted $P \leq 0.05$ as an indication of statistical significance.

5.3 Results

5.3.1 Substrate mineralization kinetics

The mineralization of the ^{14}C-labelled substrates within all the different soil types followed a biphasic pattern with an initial rapid phase of $^{14}CO_2$ evolution followed by a secondary slower phase (Fig. 5.2). Overall, a double exponential decay model gave a good fit to the experimental data (r^2 values >0.97 in all cases). The turnover rate (k_1) of exudate-C in the first phase (k_1 = 0.316 ± 0.081 d^{-1}) was significantly greater ($P < 0.01$) than for the more complex plant-derived C (k_1 = 0.031±0.002 d^{-1}). In contrast, the rate constant describing the second mineralization phase (k_2) was similar ($P > 0.05$) for both substrates (k_2 = 0.00067 ± 0.00005 d^{-1} for the simple exudate-C and 0.00061 ± 0.00003 d^{-1} for the more complex plant-derived C). In the case of the simple C this second mineralization pool reflects exudates C taken up into the microbial biomass and which is subsequently turned over (i.e. microbial biomass-C turnover). In the case of the ^{14}C-plant material it reflects a combination of turnover of C incorporated into the microbial biomass and turnover of recalcitrant plant polymers.

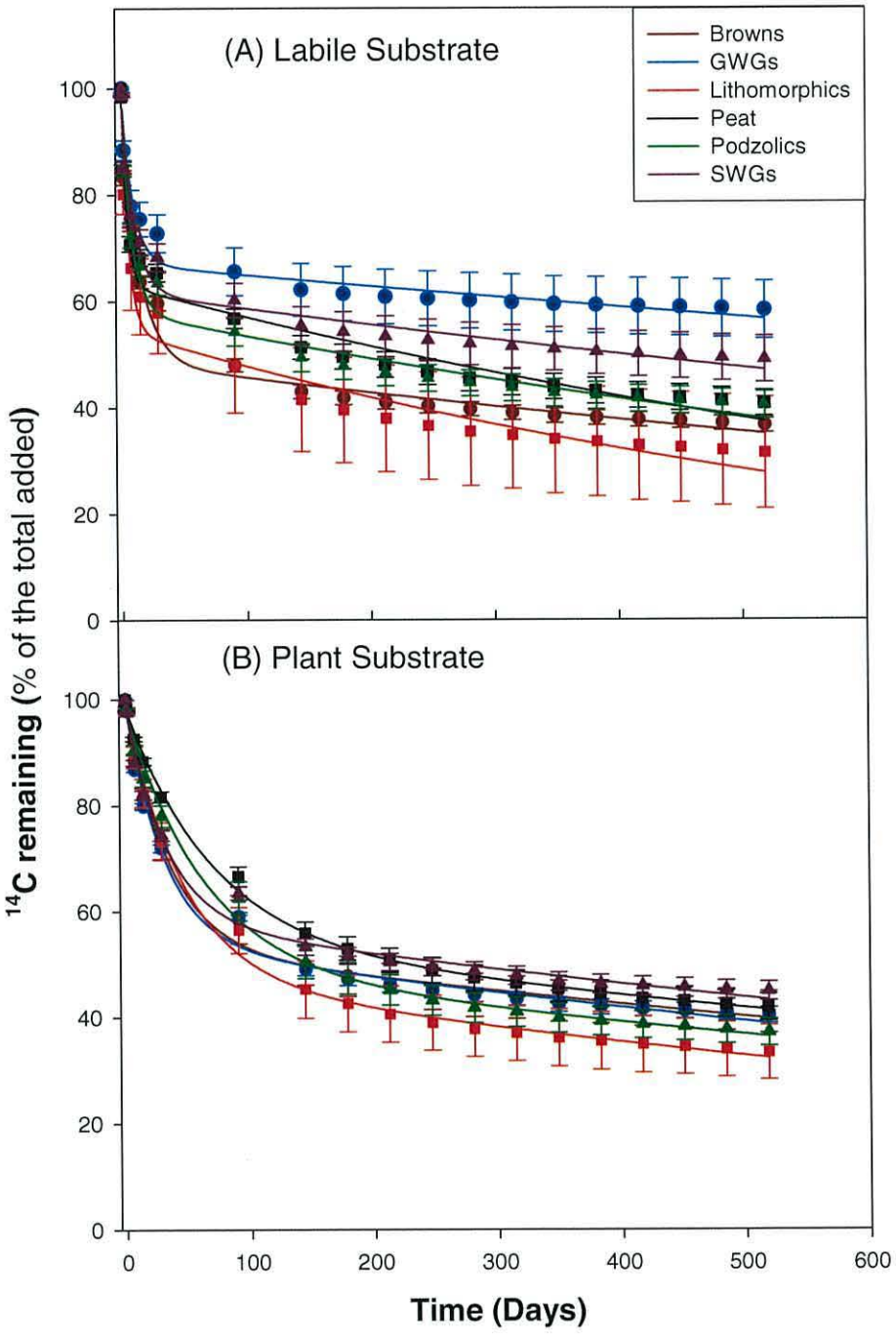

Figure 5.2. Amount of ^{14}C remaining in different soil types after the addition of a labile (panel A) or complex (panel B) ^{14}C-labelled substrate. Symbols represent experimental data points whilst lines represent fits to a double exponential kinetic decay model. Values represent means ± SEM, n =54

Soil type	^{14}C-labelled exudates			^{14}C-labelled plant material		
	Allocation to C pool 2 (a_2) [%]	Rate constant (k_2) for pool 2 [day^{-1}]	^{14}C remaining in soil at end [%]	Allocation to C pool 2 (a_2) [%]	Rate constant (k_2) for pool 2 [day^{-1}]	^{14}C remaining in soil at end [%]
Brown (n=16)	50 ± 1 [a]	0.00073 ± 0.00009 [a]	37 ± 2 [a]	50 ± 3 [a]	0.00061 ± 0.00006 [a]	40 ± 4 [a]
SWG (n=12)	66 ± 3 [b]	0.00045 ± 0.00007 [b]	51 ± 4 [b]	56 ± 2 [a]	0.00060 ± 0.00005 [a]	45 ± 2 [a]
GWG (n=11)	64 ± 3 [b]	0.00050 ± 0.00011 [b]	53 ± 5 [b]	53 ± 1 [a]	0.00065 ± 0.00008 [a]	40 ± 2 [a]
Lithom (n=1)	51 [a]	0.001 [c]	31 [c]	37 [b]	0.00098 [b]	33 [b]
Peat (n=7)	64 ± 2 [b]	0.00099 ± 0.00014 [c]	41 ± 2 [a]	51 ± 5 [a]	0.00042 ± 0.00005 [c]	41 ± 2 [a]
Podzolics (n=8)	59 ± 5 [b]	0.00076 ± 0.00011 [a]	40 ± 3 [a]	47 ± 4 [a]	0.00070 ± 0.00011 [a]	37 ± 2 [a]
Average	**59 ± 1**	**0.00067 ± 0.00005**	**43 ± 2**	**52 ± 1**	**0.00061 ± 0.00003**	**40 ± 1**
ANOVA	**	***	***	**	**	**

Table 5.1. Kinetic model parameters describing the turnover of a simple (root exudates) or complex (plant litter) ^{14}C-labelled C substrate through model pool 2 in a range of different soil types. Values represent means ± SEM, n=54. Superscript letters indicate significant differences ($P < 0.01$) between individual soil types. The ANOVA *, ** and *** indicate significant difference at the $P < 0.05$, $P < 0.01$ and $P < 0.001$ level respectively. Lithom, SWG and GWG represent Lithomorphics, surface and groundwater gley soils respectively.

The substrate C mineralization graphs shown in Figure 5.2 together with the results presented in Table 5.1 generally indicate that the type of C substrate was a significant factor affecting the rate of C cycling in the different soil types. On average, however, the overall differences in the ^{14}C remaining in the exudate-amended soils at the end of the 1.5 y incubation period was only 3% more than that which remained in plant substrate amended soils. The amount of ^{14}C remaining for both the exudates and plant-derived ^{14}C was lowest in the lithomorphic soils and highest in the surface- and ground-water gley soils. More specifically, the exudate-amended soils showed significant ($P < 0.01$) soil type differences in the percentage ^{14}C remaining after the 1.5 y incubation period where, three significantly different groups were observed namely; (1) Brown, Peats and Podzolic soils (2) GWGs and SWGs soils, and (3) Lithomorphic soils. Similarly, the plant substrate amended soils also showed significant soil type differences ($P < 0.01$) in the percentage ^{14}C remaining, however, this lacked statistical power as only one Lithomorphic soil sample was considered. The allocation of substrate-C to the 2nd mineralization pool (pool a_2) was similar among all soil types for both ^{14}C-labelled substrates with the exception of the Brown and Lithomorphic soils with the ^{14}C-exudates and the Lithomorphic soil with the ^{14}C-plant substrate.

5.3.2 Relationship between soil pH and C sequestration

Ultimately, it is the amount of C allocated to model Pool 2 and its subsequent rate of turnover that determines how much C is stored in soil from the substrates added here. The relationship between soil pH and the amount of C allocated to pool 2 (a_2) and its subsequent rate of turnover (k_2) for both the simple and complex ^{14}C-labelled substrates is shown in Fig. 5.3 (panels A-F). Overall, pH exerted little inhibitory effect of on substrate decomposition for both substrate types (as evidenced by poor correlation, $r < 0.3$ and low R^2 values). Less than 5% of the differences in the amount of ^{14}C remaining in the soil after 1.5 y, the size of pool a_2

or its rate of turnover (k_2) could be explained by differences in pH for both substrate types. The only exception was for the exudate k_2 values, where 15.9% of its variability could be explained by pH, however, the observed relationship (r = -0.40; Pearson correlation), though significant ($P < 0.05$), had very little predictive value (nearly horizontal to the pH axis). It was also contrary to our hypothesis that low pH would inhibit the rate of decomposition due to reduced microbial activity and rate of nutrient turnover.

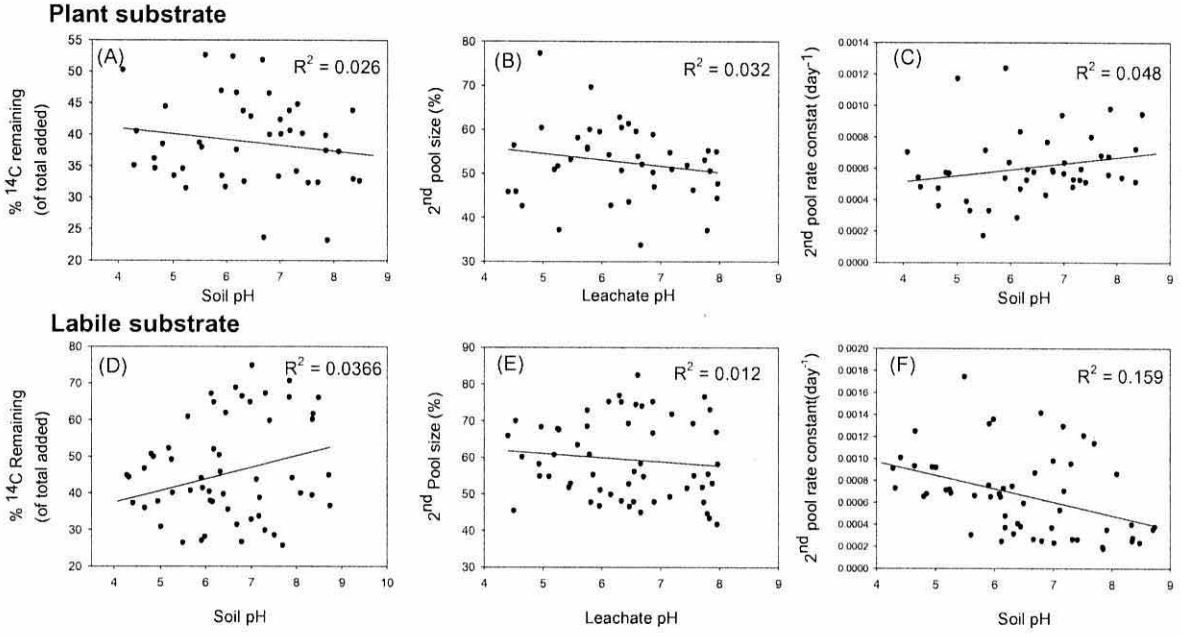

Figure 5.3. The relationship between soil pH and the residual ^{14}C remaining in the soil after 1.5y (panels A, D), the percentage allocation of substrate-C to the second mineralization pool a_2 (panels B, E), and the rate constant (k_2) describing the turnover of pool a_2 (panels C, F). The relationships are presented for a complex C substrate in panels A-C and for a simple C substrate in panels D-F.

5.3.3 Relationship between soluble humic substances, phenolics and amino acids in relation to C sequestration

The relationship between humic substances (mainly composed of polyphenolic organic moieties) and substrate turnover in soil is shown in Fig. 5.4. Overall, humic substances appeared to have a very slight inhibitory effect on C sequestration (Fig. 5.4 panels (A) and (B)). To the contrary, the scatter plot of the 2nd rate constant with the humic substances in panel (C) and (D) showed a weak positive relationship indicating that ^{14}C residence time reduced with increasing humic substance concentrations, contrary to our expectation. Similarly, in panels (E) and (G), the phenolics concentrations exhibited a very weak inverse relationship with percentage ^{14}C remaining in soil after 1.5 y ($R < 0.09$), again this is opposite to our expectation. The amino acids had an equally slight and positive correlation with the percentage ^{14}C remaining (panel (H)) and the 2nd pool size substrate allocation (panel (I)). The relation depicted in panel (H) implies an inhibition effect on C turnover of the ^{14}C-labelled exudate mixture in soils. The graph shown in panel (F) indicates no predictive value even though it has an R^2=0.157 as the line of best fit is almost horizontal.

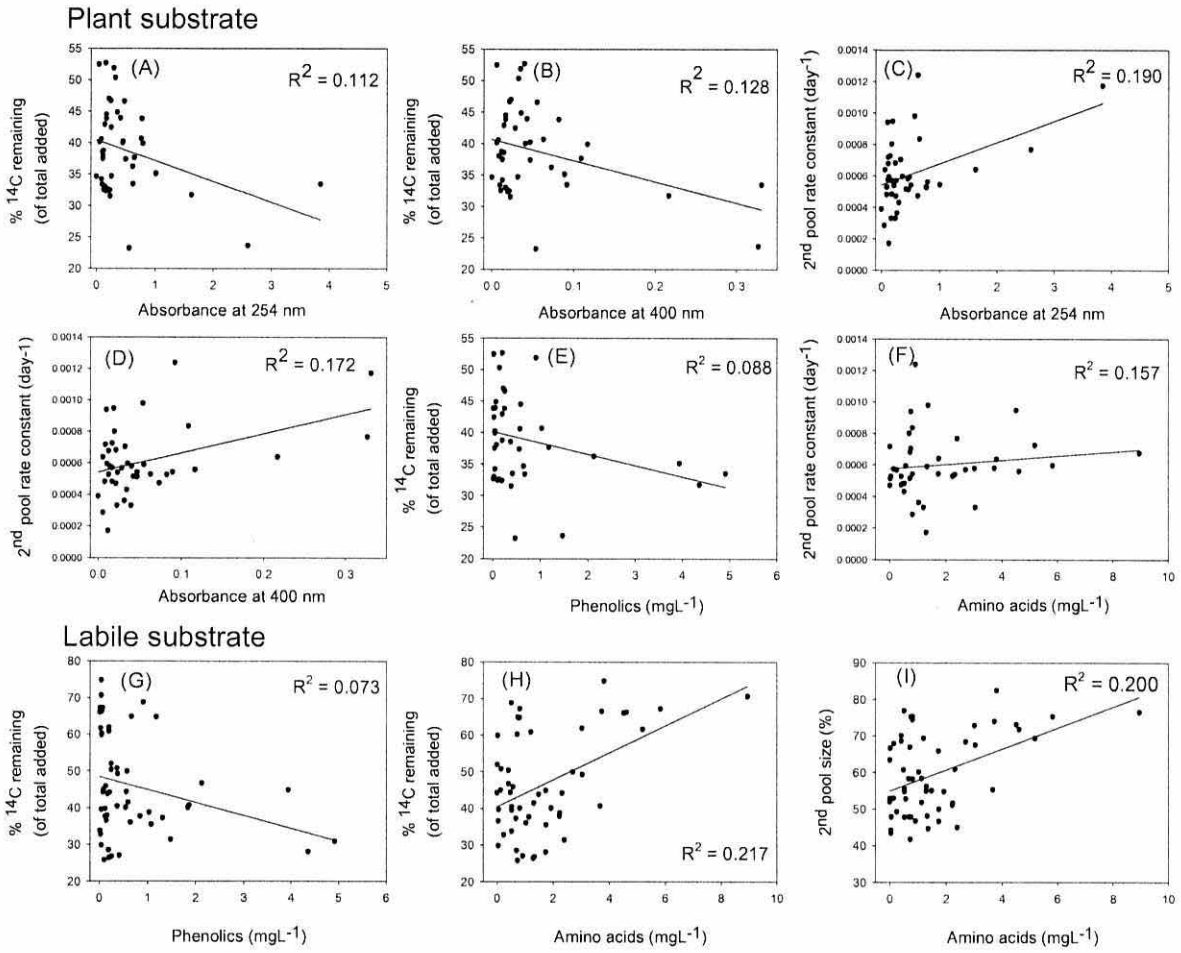

Figure 5.4. The relationship between humic substance concentration in solution and the ^{14}C are remaining after 1.5 y (panels A, B), or and the 2nd rate pool rate constant (panels C, D); soluble phenolics (mg L^{-1}) and ^{14}C are remaining after 1.5 y (panels E and G); amino acids (mg L^{-1}) and the 2nd pool rate constant (day^{-1}; panel F and H) or and 2nd pool substrate allocation size (panel I) (n = 54).

5.3.4 Relationship between microbial biomass, microbial quotient and metabolic quotient in relation to C sequestration

The microbial biomass had a weak relation (r=0.30; P<0.05) with the exudates rate constant (k_2) and the residual ^{14}C percentage in the soil after 1.5 y of incubation. The corresponding coefficient of determination was equally low (R^2 ≤0.04; Fig. 5.5 panel (D)). The calculated microbial and metabolic quotients (i.e. microbial biomass/organic matter and soil respiration/microbial biomass) showed no significant relationships (r<0.27) with the

substrates rate constant (k_2) and the residual ^{14}C percentage from both substrate types. Moreover, the relationships in panels (C, E) were ambiguous as they showed that the increase in microbial and metabolic quotients had an inhibitory effect in the rate of substrate decomposition contrary to our hypothesis.

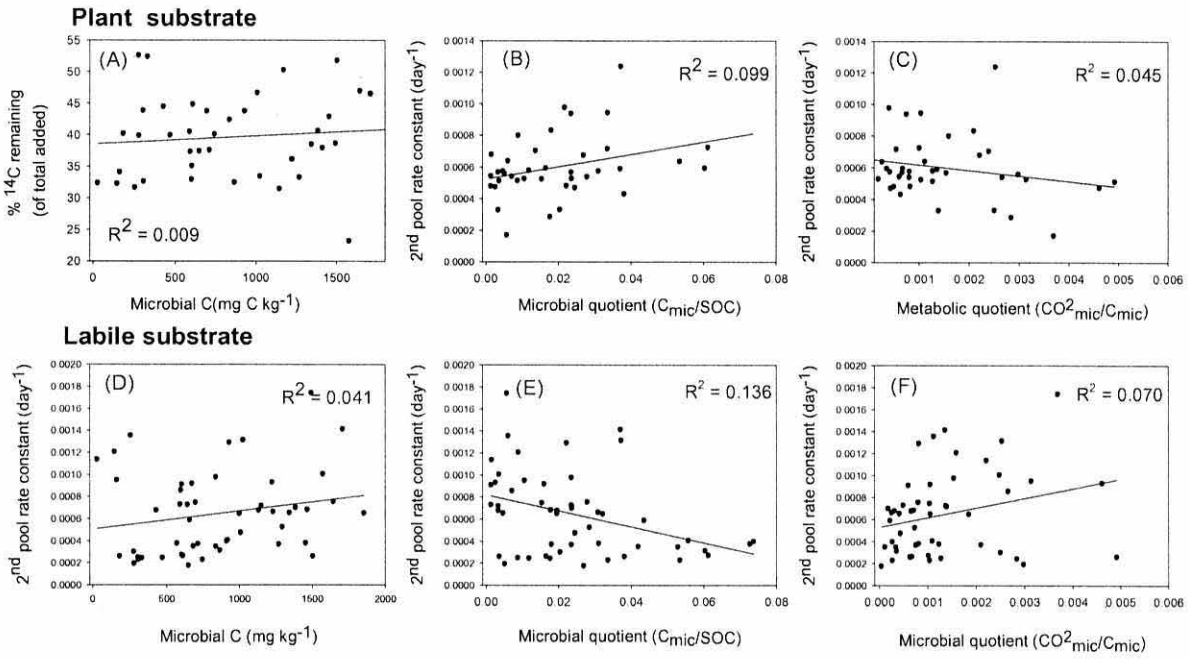

Figure 5.5. Relationship between microbial biomass (mg kg^{-1}) and the amount of plant-derived ^{14}C remaining in the soil after 1.5 y (panel A). Relationship between microbial biomass, microbial and metabolic quotients and the rate constant (k_2) describing the turnover of pool a_2 (day^{-1}; panels B-F) (n = 54).

5.3.5 Effects of bacterial biodiversity on C sequestration in soil

The bacterial biodiversity (shannon index) exhibited a weak correlation with the percentage ^{14}C remaining and the 2nd pool rate of ^{14}C turnover in soils with the ^{14}C-labelled plant substrate (r = 0.26; $P < 0.05$; k_2, r = 0.31, $P < 0.05$ respectively). In the ^{14}C-labelled exudates amended soils, the bacterial diversity had an equally weak correlation with the percentage ^{14}C remaining and the 2nd pool rate of ^{14}C turnover ($r < 0.25$; $P<0.05$). Generally,

the rate of plant substrate decomposition increased with bacterial biodiversity (Fig. 5.6 (A, B)), and consequently more C was mineralised in soil with higher bacterial biodiversity. In the exudate treatment (Fig. 5.6 (C, D)), the opposite trend appeared to be apparent. However, both the scatter plots and the correlation coefficients show that the relationships between diversity and C turnover were very weak ($R^2 \leq 0.16$).

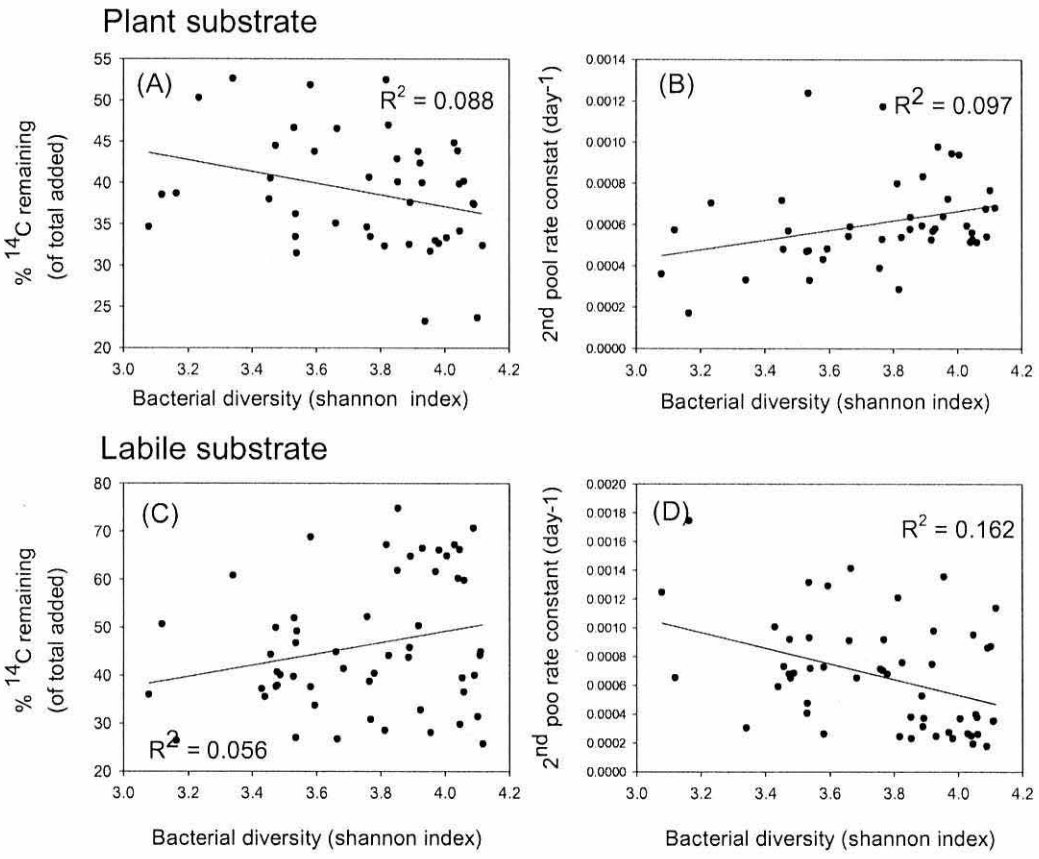

Figure 5.6. Relationship between soil bacterial diversity and the rate of C turnover of either complex (plant litter) or a simple (root exudates) C substrate in soils. The measures of soil C turnover included the percentage amount of ^{14}C remaining in the soil after 1.5 y (panel A, C) and the rate constant (k_2) describing the turnover of pool a_2 (day^{-1}; panels B, D).

5.3.6 *Relationship between soil C-to-N and N-to-P ratio and C sequestration*

Across all samples, soil C:N and N:P ratio showed only a very weak relationship and effect on the rate of C turnover of the ^{14}C-labelled simple (exudates) and complex (plant litter) substrates (Fig. 5.7; $r < -0.35$; $R^2 < 0.04$).

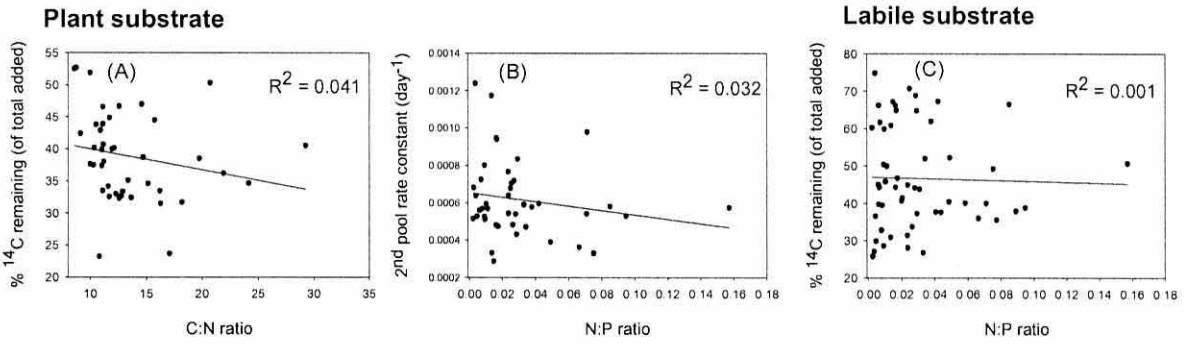

Figure 5.7. Relationship between soil C:N and N:P ratio and the rate of C turnover of either a simple (root exudates) or complex (plant litter) C substrate in soil. The measures of soil C turnover included the percentage amount of ^{14}C remaining in the soil after 1.5 y (panel B, C) and the rate constant (k_2) describing the turnover of pool a_2 (day^{-1}; panel A), (n = 54).

5.4 Discussion

5.4.1 *Substrate quality and mineralization across soil types*

Overall, the experimental data from all soil types fitted well to a double exponential decay model indicating that there was a rapidly mineralized substrate-C pool and a slowly processed substrate-C pool. In no cases was a lag phase in mineralization observed indicating that the internal and external enzymes and transporters required to process the added C were already present in the soil and microbial community. This is supportive of the idea that significant functional redundancy exists in soil for common substrates such as those used here. In general, there was a difference in partitioning of added substrate within the soil

microbial community between soils amended with ^{14}C-labelled exudates and those with plant material. For instance, the parameters obtained by fitting the double exponential decay model to the experimental data show that at an intermediate incubation period (ca. 90 d), the allocation of added substrate-C to the second mineralization phase (pool a_2) was greater for the plant material treatment than in the exudate amended soils (82 versus 70% respectively; data not presented). However, on extending the incubation period to 1.5 y, the amount of substrate-C allocated to pool a_2 was more in exudates than in plant material amended soils (59 versus 52% respectively). This highlights the need for long-term incubations as opposed to the short term assays used in most experiments (ca. 1-14 d).

As expected, the rate of substrate mineralization was dependent upon the quality of the available C substrate. This is exemplified by the slower rate of ^{14}C depletion of plant-derived C compared to the rapid depletion of the exudate-derived ^{14}C in the first mineralization phase (k_1, pool a_1). Soil type-dependent differences in the capacity for long term C storage was evident in the root exudate amended soil only, where the Brown and the Lithomorphic soils respired significantly more C than the gley soils.

5.4.2 The fate of added substrates at long term incubation

The decomposition of exudates was soil dependent; however, generally the decomposition process was very rapid in comparison to the more complex plant substrate. By day 7, approximately 30% of the exudate-C had been respired in comparison to only 10% of the plant C. This difference in C usage reflects the fact that in contrast to plant-C, exudates-C require no extracellular enzymes prior to transport into the microbial cells (at least not in the short term; Jones et al., 1994; Jones, 1999). Jones (1999) found very little ^{14}C-label (<4%) in the soil solution and exchangeable phases after 96 h of amino acids addition, meaning that 96% was either respired or incorporated into the new cell biomass. Contrary to our

expectation, the total $^{14}CO_2$ emissions after long-term incubation were greater for the plant material amended soils than those amended with exudates although the differences were small (57±2% of the exudates-C and 60±1% of the plant-C). This is contrary to expectation where it was expected that the plant material C would have a much longer residence time than exudates C in soil. However, it is clear that C in the microbial biomass also has a long residence time in soil. It is known that when substrate C is incorporated into microbial cells, a small amount (15-40%) of the original substrate-C can remain in soil after 1 y while the majority is transformed into necromass (Zunino et al., 1982). This necromass must also have a relatively short residence time (<1 y), otherwise it would massively increase in soil over pedogenic time scales. Correspondingly, Fröberg et al. (2006) reported a substantial exchange between the soil matrix DOC and added DOC. In another study, Marx et al. (2009) found that, although the newly built microbial biomass after the addition of exudates, consisted to a large extent of exudate-C, the proportion of soil-derived C in the microbial biomass of exudate-treated soil was greater than the control microbial biomass-C, further confirming the operation of these exchange processes. Considering, such a substantial amount of exudate (43%) and plant material (40%) C remains in soil after 1.5 y and has a pool half-life of 4.0±0.3 y and 3.6±0.2 y respectively (calculated from the 2nd pool rate constants), we speculate that it is possible that the exchange processes and other C stabilization mechanisms were operational in the soils in addition to incorporation into new microbial biomass. Since the labile portions of plant substrate and the exudates are utilised quite rapidly (within a few day) by the microbes, apart from utilization of labile C in the production of new cell biomass (more recalcitrant form of C), it is possible that a portion of the microbially processed substrate was also stabilized directly into the soil by sorption–desorption reactions. This mechanism maybe responsible for the observed low rates of mineralization and consequently long half lives in the 2nd pool (approx. > 4yrs; calculated from 2nd pool mineralization half

times). It is also possible that steric inhibition prevented microbial access to physically occluded substrates.

5.4.3 Effect of pH on SOM cycling

It is well known that low pH adversely affects plant enzymes and the availability of nutrients to plants, thereby reducing plant productivity. Apart from reducing the quality and quantity of C input, low pH is also known to reduce C turnover rates by reducing microbial activity and nutrient turnover in the soil. Microbial activity is optimal in the range pH 6 to 8 within which range most cellular and extra-cellular enzymes are also most active for N and C cycling (Dalal, 2001). However, microbial activity as well as nutrient turnover is only significantly affected at pH < 4.5 from the combined impact of H^+, Al^{3+} and possibly Mn (Santa, 2000; Dalal, 2001). At low pH, Al^{3+} forms Al-OM complexes and other organomineral aggregates which can prevent access by enzymes and consequently reduce C turnover (Zunino et al., 1982; Kaiser and Guggenberger, 2000; Mayer and Xing, 2001).

In this study, most of the soils were above pH 4.5 and therefore excess acidity did not exert a significant effect on C cycling (Fig 1). Furthermore, the addition of the substrates could have increased the pH of the soil thereby improving the conditions of soils to a level where the pH had no significant adverse impact on the decomposer organisms and/or OM decomposition rates. Yan et al. (1996) and Tang et al. (1999) found that additions of malate, citrate, glycine and glucose to the soil resulted in a net pH increase due to the dissociation of carboxylic groups and the subsequent decarboxylations processes during their decomposition. Therefore, although pH can be a major driver of SOC cycling, there was no evidence that the initial pH was a significant factor influencing C turnover rates in our soils over long time periods.

5.4.4 *Effects of soluble amino acids, humic substances and phenolics on C sequestration*

Addition of amino acids to the soils is known to cause priming effect on the decomposition of the soil organic matter (Kuzyakov, 2007). The results from the long term incubation experiment showed no evidence of the priming effect to the decomposition of the substrates added. Several experiments have shown that amino acids in soil solution are extremely transient, turning over approximately 20 times per day depending on the soil type and environmental conditions (Jones, 1999; Jones and Kielland, 2002). Amino acids like most low MW organic compounds within SOM are predominantly used for soil microbial respiration rather than incorporation into structural components (e.g. cell walls; van Hees et al., 2005; Oburger and Jones, 2009). Most amino acids are weakly sorbed to the soil's solid phase and no extracellular enzymes are required prior to transport into the microbial cells (Jones et al., 1994; Jones, 1999). On the other hand, amino acids may form complexes with humic materials present in the soil leading to their decreased bioavailability and therefore, prolonging their resident time. In view of their fast turnover rates and probably their low concentrations (0.002-5 μM) in this experiment, amino acids were therefore not expected to cause a priming effect on the long term C cycling in the soil.

In general, a range of ecosystem processes, particularly biodegradation pathways, are known to be hampered in the presence of humic substances (Wetzel, 1995). With respect to direct microbial degradation, humic substances are thought to be recalcitrant (Schlesinger, 1977; Wetzel, 1995; Thomas, 1997). Humic substances have been shown to exhibit inhibitory effect on the degradation of SOM in various ways. For example, (1) adsorption of substrates on humic acid and the concomitant decrease of bioavailability, (2) preferential reaction (and complexation) between enzyme (e.g. laccase) and humic acid over the substrate, (3) a chemical reaction between enzyme (e.g. laccase) and humic acid leading to subsequent

denaturation, resulting in a decreased affinity for substrate (Keum and Li, 2004), and (4) adsorption of the enzyme-substrate complex onto the humic substance (Bums, 1986; Vuorinen and Saharinen, 1996; Grinhut et al., 2007). Keum and Li (2004) observed a very strong inhibition of laccase by HA at high concentrations (150 mM) during the degradation of polychlorobiphenyls (PCBs).

Similar to humic substances, soluble phenolics (a component of humic substances) are potent inhibitors of enzymes responsible for SOM decomposition (Painter, 1991; Wetzel, 1992; Appel, 1993, Pind et al., 1994). Phenol-containing compounds are both microbially refractory and powerful metal ion complexing and chelating agents resulting in a decrease in availability of metal ions to microbes (Freeman et al., 1990). Phenolics, oxidized by microbial enzymes or mineral catalysts (quinones), covalently bind with amino acids, sugars, and minerals to form a matrix recalcitrant to microbial digestion (Appel, 1993). Phenolics' inhibitory actions are especially important in peat which is renowned for containing high concentrations of soluble phenolics coupled with the low rates of organic matter decomposition (Freeman et al., 2004). The anoxic conditions and low pH in peat severely restrict the scope of microbial activity (Painter, 1991) and the phenol oxidase activity allowing accumulation of phenolics, which in turn inhibit and thus down-regulate the activity of other enzymes (e.g. hydrolase) that are responsible for decomposition of organic matter components (Freeman et al., 2004). These mechanisms may have been taking place in the Peat soils in our experiment. The soils amended with the plant substrate had the longest C residence in Peat soils [i.e. lowest 2^{nd} phase mineralization rates (k_2); 40% lower than the Podzolic soils (highest); $P<0.01$] and subsequently had greater amounts of the substrate remaining in the soil at the end of the 1.5yr incubation period. However, examining the effect of soluble phenolics and humic substances across all soils, revealed no evidence that they had any inhibitory effect on C cycling. We speculate that the lack of inhibitory effect of these

substances on the long term C cycling was due to their relatively low concentrations. Furthermore, the concentrations of humic substances and phenolics could have changed greatly over time in the experiment due to altered rates of production and decomposition (Kästner et al., 1999).

5.4.5 Effect of microbial biomass and diversity on C sequestration

Soil microbial biomass typically comprises <5% of SOM yet performs a critical function in C cycling in soil (Sparling, 1992; Dalal ,1998; Nannipieri, 2003). Generally microbial biomass is an indicator of metabolic activity (Maier et al., 2009) and controls SOM decomposition and nutrient cycling in soil (Scow, 1997; Dalal, 1998; Broos et al., 2007; McGuire and Treseder, 2009). It has been generally assumed that decomposition rate is proportional to the growth rate of the decomposers and therefore microbial biomass represents an important component in most decomposition models (McGuire and Treseder, 2009). In our experiment, the non significant correlation of microbial biomass with C cycling indicates that the initial size of microbial biomass was not an important factor regulating SOM decomposition. Since the measurements were done in the laboratory, factors of soil moisture and temperature were controlled, and therefore the activity of microorganisms and the microbial biomass in the soil were mainly influenced by the availability (quality and quantity) of substrate (Domisch et al., 1998; Kurzatkowski et al., 2004; Okpokwasili and Nweke, 2005; Marschner et al., 2008). Many studies have shown a flush in microbial biomass following the incorporation of easily degradable organic residues. For example, Fosu et al. (2007) measured up to a 250% increase in microbial biomass in C substrate-amended soils compared to the control after 4 d of incubation. Therefore it is not surprising that the initial biomass content did not have a significant effect on the subsequent C mineralization rates and amounts since it depended on the availability of intrinsic C stocks.

Similarly, microbial biodiversity did not significantly influence the C cycling in our soils. This is partially contrary to the view of McGuire and Treseder (2009) who reported that key nutrient transformations are performed by specialised microbes. According to them, there are no generalist microbes capable of conducting all the nutrient transformations required to maintain ecosystem function across a broad range of environmental conditions. Therefore, microbial biodiversity should correlate to ecosystem function. Notwithstanding, the results are consistent with robust empirical evidence indicating that soil is characterized by functional redundancy to the extent that, there has been no evidence showing a relationship existing between microbial diversity and decomposition rates of organic matter (Nannipieri, 2003). A reduction in biodiversity (for example when there is a reduction in organic C stocks; Degens, 2000) has little effect on overall processes in soil because other microorganisms can take on the functions of the missing species (Nannipieri, 2003). Though, this be the case, a minimum number of species remain essential for ecosystem functioning, while a high species diversity is essential for both functioning and maintenance under changing conditions (Loreau et al., 2001; Nannipieri, 2003). The above result therefore indicates that below-ground biodiversity was above the minimum for ecosystem functioning.

Microbial quotient (microbial biomass-to-SOC ratio) or qMic provides a measure of soil organic C dynamics and can be used as an indicator of net C loss or accumulation (Nielsen and Winding, 2002). The microbial metabolic quotient (respiration-to-biomass ratio) or qCO_2, is used as a measure of microbial efficiency and as an index of ecosystem development (during which it supposedly declines) and disturbance or stress (during which it supposedly increases) (Wardle and Ghani, 1995; Yan et al., 2003). There was no significant relationship between the qCO_2 or qMic and the second pool rate constants k_2 for both the exudate and the plant substrate. The effect of disturbance (e.g. substrate additions) can have a

very quick recovery (Turbe et al., 2010), in which case, the long term C cycling may have no correlation with the initial qCO_2.

5.4.6 Effects of C:N and C:P ratio on C sequestration

The C and N cycles in soils are linked through the processes of N assimilation, N mineralization, denitrification and the decomposition of SOM by microorganism (Yano et al., 2000). The C:N ratio provides an indicator of quality rather than quantity of SOM (Yamakura and Sahunalu, 1990; Hazelton and Murphy, 2007). It is related to the speed of decomposition and the rate at which organic nutrients are mineralised and become available for re-absorption into plants (Yamakura and Sahunalu, 1990). Soil C:N ratios <25 indicate that decomposition may proceed at a maximum possible rate depending on the environmental conditions, while those > 25 indicate slow decomposition needing N addition (Stevenson and Cole, 1999; Hazelton and Murphy, 2007). The N:P ratio has been used as an indicator to determine nitrogen or phosphorus limitation (Reddy and DeLaune, 2008).

The soil C:N ratio was generally <25 in soil although we could not tell how much was freely available for uptake by microbes. The lack of correlation between mineralization rates and C:N:P ratios indicated that neither N nor P were limiting ^{14}C-substrate decomposition both in the early and late stages of decomposition. As the soils were not leached during the 1.5 y incubation period and there was no plant nutrient sink it is likely that the concentrations of NO_3^- and PO_4^{3-} would be much higher at the end of the experiment than at the start since there was no uptake by pland or leaching (Davidson et al., 1990). However, during the later stages, there was no evidence of a negative relationship between N concentration and SOM mass-loss rates. The high N concentration at the late stages is known to cause a rate-supressing influence on lignin and litter mass-loss due to: (i) repression of lignolytic enzyme

production in fungi, and (ii) the reaction of lignin products with ammonium or amino acids to form recalcitrant SOM complexes (Berg, 2000).

5.5 Conclusion

It is indeed intriguing that the initial pH, amino acids, humics, phenolics, microbial biomass, bacterial biodiversity, eco-physiological quotients, and C:N:P ratio had no significant effect on the long term C sequestration across a broad range of soil types. It is possible that the addition of the labile substrates may have changed the bio-chemical conditions and the composition of the residue C in the soils over time (e.g. pH, EC and concentrations of substances e.g. cations (Yan et al., 1996) so that the initial physiochemical and biological status of soil solution had very little bearing on the long term C cycling in soil. The added substrates decomposed in the manner described by first order kinetics and therefore, it can be concluded that the most important factor for C mineralization and nutrient release from substrates was simply the amount and quality of the substrates being decomposed. The substrate quality effect was mostly evident in the initial stage where, the exudates mineralized more rapidly than the plant material. For example, 25-43% C was respired in 28 d in exudates while in plant substrates, similar amounts were respired over 90 d across the soil types. However, it would have been interesting to have measured the above parameters at the end of the experiment to investigate how the final concentration levels could have changed and be related with C cycling parameters. This perhaps could be a consideration for future research.

Importantly, this study has revealed that this sort of long-term laboratory incubation provides a poor indicator of C storage potential in soil. It is therefore advised that future studies should be performed in the field and with a greater diversity of substrates differing in quality. It is also clear that microbial diversity had little bearing on the overall rate of C

turnover in soil. This implies that regulatory authorities should not invest resources in the routine monitoring of below-ground diversity, at least not for assessing C cycling in semi-natural and agricultural ecosystems.

5.6 References

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Chapter 6: Article III

Identification of soil quality factors and indicators and their predictability using seven major soil types

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This paper has not been submitted to any Journal

ABSTRACT

Five major, external factors of soil formation (climate, organisms, relief, parent material and time), and several smaller, less identifiable ones, drive pedogenic processes and create soil patterns. However, biological indicators of soil quality play no direct role in traditional soil classification and surveys. To support their inclusion in classification schemes, previous studies have shown that soil type is a key factor determining microbial community composition in arable soils. This suggests that soil type could be used as proxy for soil biological function and vice versa. In this study we assessed the relationship between soil biological indicators with either vegetation cover or soil type. A wide range of soil attributes were measured on soil from across the GB to investigate whether; (1) appropriate soil quality factors (SQFs) and indicators (SQIs) can be identified, (2) soil classification can predict SQIs; (3) which soil quality indicators were more effectively predicted by soil types, and (4) to what extent do aggregate vegetation classes (AVCs) act as major regulators of SQIs. Factor analysis was used to group 20 soil attributes into six SQFs namely; *Soil organic matter*, *Organic matter humification*, *Soluble nitrogen*, *Microbial biomass*, *Reduced nitrogen* and *Soil humification index*. *Soil organic matter* was identified as the most important SQF in the discrimination of both soil types and AVCs. Among the measured soil attributes constituting the *Soil organic matter* factor were, microbial quotient and bulk density were the most important attributes for the discrimination of both individual soil types and AVCs. The *Soil organic matter* factor discriminated three soil type groupings and four aggregate vegetation class groupings. Only the Peat soil and Heath and bog AVC were distinctly discriminated from other groups. All other groups overlapped with one another, making it practically impossible to define reference values for each soil type or AVC. Comparatively, AVCs were a better predictor of the SQIs than the soil types. We conclude that conventionally classified soil types at major group level provides a poor predictor of routinely measured SQIs (and/or SQFs) however, SQIs can be used to characterise the conventionally classified soil types.

Keywords: Soil quality, Multivariate classification, Discriminant analysis, Cluster analysis

6.1 Introduction

The multiple roles and functions of soil have resulted in several broad definitions of soil quality. One of the most recent definitions for soil quality (SQ) was proposed by a committee for the Soil Science Society of America (chaired by Karlen) as: "the capacity of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation" (Sparling, 1997; Arshad and Martin, 2002; Winding et al., 2005). The quality of any soil has two parts: (1) the natural or inherent quality which is based on the parent geological material and soil-state-factors and is rather static, and (2) the dynamic soil quality which encompasses those soil properties that can change over relatively short time periods in response to human use and management (Carter, 2002; Fließbach et al., 2007). In contrast to the inherent SQ, the dynamic SQ can be used to monitor temporal trends on the same soil. There is no universally applicable set of inherent SQ criteria and optimum values (Carter, 2002) because soils with differences in the soil forming factors have different absolute capabilities (Seybold et al., 1998; Karlen et al., 2001). Therefore, soil quality and indicators have been defined by very different criteria and approaches dependent on the various functions the soil performs (Rapport et al., 1997; Carter, 2002). In spite of the lack of standard methodology and "critical limits", it is possible to develop SQ ranges for specific soils evaluated with regard to specific land use and management regimes.

Soil quality is evaluated in terms of measurable soil attributes that measure specific physical, chemical, and biological properties; also known as soil quality indicators (SQIs; Shukla et al., 2006). Many of these properties are interrelated and the best SQIs are those that integrate and have the combined effect of several properties or processes. SQIs should generally be linked and/or correlated with ecosystem processes and functions and should be responsive to variations in management and climate on an appropriate time scale (Doran and

Safley, 1997). The SQIs which respond over the medium term i.e. those that are sensitive over years and decades, may be the most useful for indicating soil quality changes as opposed to those which change either very rapidly (e.g. seasonally) or very slowly (e.g. over centuries) (Rapport et al., 1997). Thus, measurement of key SQIs over time can be used to establish whether the quality of a soil under a given land use and management system is improving, declining or stable (Karlen et al., 2001; Shukla et al., 2006).

Soil types are known to be inextricably determined by the physical, chemical and biological processes operat ing in soil, yet the biological indicators are rarely used in traditional soil classification and surveys (Cavigelli et al., 2005). Studies conducted by a number of researchers, such as Parkin (1993), Buyer et al. (2002), Girvan et al. (2003) and Ulrich and Becker (2006), have shown that soil type is a key factor determining microbial community composition in arable soils. Furthermore, Rapport et al. (1997) and Lagomarsino et al. (2009) reported that microorganisms and microbial communities can provide an integrated measure of soil quality; an aspect that cannot always be obtained with physical and chemical measures and/or analyses of higher organisms. Bioindicators of SQ generally include microbial indicators such as microbial biomass, activity and biodiversity (Rapport et al., 1997; Nielsen and Winding, 2002). The quotients of *microbial respiration-C-to-microbial biomass-C* (qCO_2) and the *microbial biomass-C-to-organic matter-C* ratio (qMic) avoids the problems of comparing trends in soils with different organic matter or microbial biomass content and appears to provide a more sensitive indicator of soil changes than either activity or population measurements alone (Lagomarsino et al, 2009). However, there is scant information available on the relationships of these SQIs with conventionally classified soil types. Therefore in this study, we use multivariate statistical methods to explore these relationships using 20 physico-chemical and biological soil properties. Using factor analysis the 20 correlated variables were reduced to 6 uncorrelated factors (also called soil quality

factors (SQFs)) that were linear functions of the original variables. The main questions addressed in this study were: (1) Can appropriate soil quality factors (SQFs) and indicators (SQIs) be identified? (2) Can major soil types be used to predict SQFs and SQIs? (3) Which SQFs and SQIs correlate with soil type in GB soils? (4) To what extent do AVCs act as major regulators of SQFs or SQIs?

6.2 Materials and methods

6.2.1 Soil sampling and preparation

To encompass all the major soil and land use types, a total of 304 soil samples were collected throughout GB, according to a 15 km square grid laid across the country as described by Scott (2008). Figure 6.1 shows the general location and distribution of samples across the GB.

The sample collection strategy, sample treatment and soil classification are detailed in chapter 4 of this thesis. Similarly, leachate collection and the normalisation of soil moisture using artificial rainfall to ensure all soils were at field capacity have also been described previously. 50 ml of the unfiltered leachate was collected and stored at -18°C for further analysis.

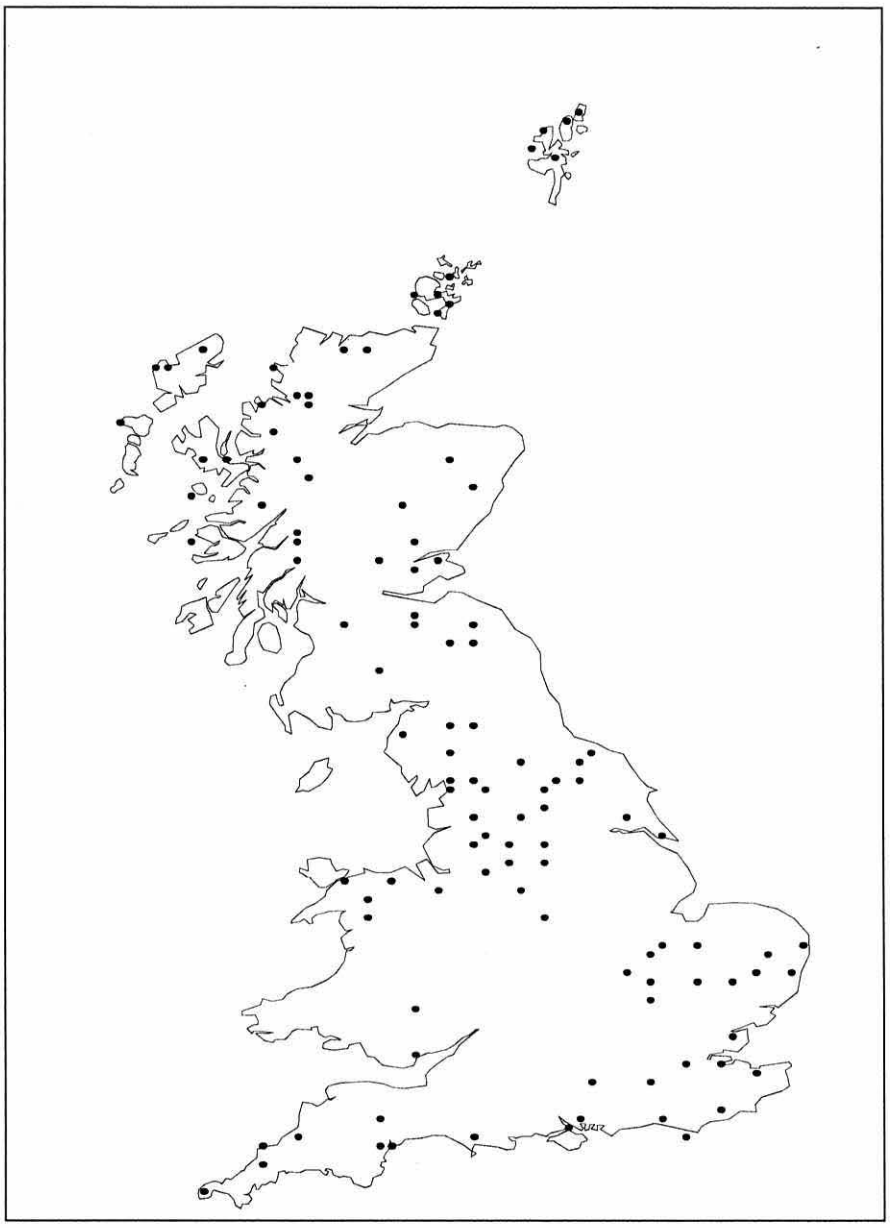


Figure 6.1. The map showing the general distribution of the sample points used in the study

Across all land uses (appendix 1) and aggregate vegetation class (AVC) categories, the dominant soil types (% of total) were: Brown soils (32%), Surface water gleys (19%), Peats (15%), Groundwater gleys (13%), Podzolic soils (11%), Lithomorphic soils (8 %) and Pelosols (2%). See table 5.1 in chapter 5 for their equivalents in the WRB classification. All the sites were characterised by a temperate climate with a North-South mean annual temperature range of 7.5 to 10.6°C and East-West mean annual rainfall range of 650 to 1700 mm (Mathew, 2006).

6.2.2 Aggregate vegetation classes

The vegetation data from the plots were analysed using the classification by Aggregate Classes (ACs) or Aggregate Vegetation Classes (AVCs). The AVCs were the vegetation types produced from a quantitative hierarchical classification of the different species found in sample plots. The eight AVCs used for assessing vegetation condition are listed in Table 6.1. Across all the soils sampled, the AVCs represented (% of the total) were 22% Heath and bogs, 20% Infertile grasslands, 18% Crop and weeds, 17% Fertile grasslands, 10% Moorland grass mosaics, 7% Upland wooded, 4% Tall grass and herbs and 2% Lowland wooded.

Aggregate vegetation class (AVC) +(abrev)	Description
1. Crops and weeds (Craw)	Weedy communities of cultivated and disturbed ground, including species-poor arable and horticultural crops.
2. Tall grass and herbs (Tgah)	Less intensively managed tall herbaceous vegetation typical of field edges, roadside verges, stream sides and hedge bottoms.
3. Fertile grassland (Frtg)	Agriculturally improved or semi improved grassland. Often intensively managed agricultural swards with moderate to high abundance of perennial rye grass.
4. Infertile grassland (Infg)	Less-productive, unimproved and often species rich grasslands in a wide range of wet to dry and acid to basic situations.
5. Lowland wooded (Lwlw)	Vegetation dominated by shrubs and trees in neutral or basic situations, generally in lowland Britain. Includes many hedgerows.
6. Upland wooded (Uplw)	Vegetation of broadleaved and conifer woodland often in more acidic situations, generally in upland Britain.
7. Moorland grass mosaics (Mrgm)	Extensive, often unenclosed and sheep grazed hill pastures throughout Britain.
8. Heath and bog (Htab)	Vegetation dominated by heathers. Includes drier heaths as well as bog. Mostly in the uplands.

Table 6.1. The Aggregate vegetation classes (AVCs) used for assessment of vegetation condition. The brackets indicate the abbreviation of the vegetation class (adapted from Smart et al., 2003).

6.2.3 Soil analysis

Soil pH was determined in soil-distilled water extracts (1:2.5 w/v soil to water soil ratio) on a 10 g field moist soil using a glass electrode (Gelplas general purpose electrode, BDH) and HI-209 pH meter (Orion research, Boston, MA, USA). Soil moisture was

determined by weight loss after oven drying at 105°C overnight (>16hrs). Water content at field capacity was estimated by saturating the soil followed by measuring the water retained in the soil at -33 kPa. Bulk density was calculated (mass/volume) after removal of stones (>2 mm in diameter). Loss on ignition (LOI) was undertaken at 375°C for 16 h. Soil organic carbon (SOC) was calculated according to the method of Ball (1964) where

$$SOC = ((0.458 \times LOI) - 0.4) \qquad [Eq. 6.1]$$

Phosphorus was determined by the Olsen P method. Total C and N were determined using UKAS accredited method SOP3102 and an Elementar Vario-EL elemental analyser (Elementaranalysensysteme GmbH, Hanau, Germany) detailed in Emmett et al., 2008. All the assays undertaken above are detailed in Emmett et al. (2008 and 2010).

Soil respiration (SR) was determined on a 15 cm long, 2.5 cm diameter soil cores with a 1250 cm^3 head space. The soils were incubated at 10°C for 1 h (at which linearity was established). Subsequently, the head space gas was analysed for CO_2 concentration using a Clarus 500 Gas Chromatograph (Perkin Elmer Corp., Beverley, MA). The CO_2 flux was established by comparing the CO_2 concentration before and after incubation. Soil microbial biomass C and N were estimated on moist soil samples using the modified chloroform-fumigation-extraction (CFE) method of Vance et al. (1987). For each soil 10g of the control and the fumigated samples were extracted with 1 M KCl. The TOC and TN in the 1 M KCl extracts was determined using a TOC-VCSH/CSN analyzer (Shimadzu Corp., Kyoto, Japan). Incomplete extractability correction factors of 0.45 and 0.54 were used for microbial C and N respectively (Joergensen and Mueller, 1996a; 1996b; Fließbach et al., 2006). Soil microbial biomass was therefore calculated according to the formula: $C_{mic} = EC/kEC$, where EC = (TOC in fumigated samples - TOC in control samples) and $kEC = 0.45$, and $N_{mic} = EN/kEN$,

where EN = (total N in fumigated samples – total N in control samples) and kEN = 0.54. The microbial C:N ratios were subsequently calculated from these values.

The metabolic and microbial quotients were calculated indices. The metabolic quotient or coefficient was calculated as the ratio between the CO_2-C from basal respiration and the microbial biomass-C (CO_2-C_{resp}-to-C_{mic}), expressed as μg CO_2-C mg^{-1} biomass-C h^{-1}. It is also known as the specific respiration rate (qCO_2) (Anderson and Domsch, 1993). The microbial quotient was calculated as the ratio between the microbial biomass-C-to-total organic C (C_{mic}-to-C_{org}).

6.2.4 Leachate analysis

Leachate TOC (total organic C) and total organic N (TN) were measured using a TOC-VCSH/CSN analyzer (Shimadzu Corp., Kyoto, Japan) and the DOC:TN ratio subsequently calculated. Nitrate and ammonium concentrations were measured with a Skalar SAN^{++} segmented-flow autoanalyser (Skalar, Breda, Netherlands), based on the cadmium (Cd) reduction method (Maynard and Kalra, 1993; Griffin, et al., 1995) and the modified Berthelot reaction (Searie, 1984) respectively. Electrical conductivity (EC) was measured with a standard platinum 1 cm electrode on a 4520-EC meter (Jenway Ltd, Dunmow, Essex, UK). pH was measured using a glass electrode (Gelplas general purpose electrode, BDH) on a HI-209 pH meter (Orion research, Boston, MA, USA). Total free amino acids were determined using the fluorometric OPAME procedure of Jones et al. (2002) and a Cary Eclipse Fluorescence Spectrophotometer (Varian Inc., Australia) using a leucine standard. Humic substances were determined by measuring the absorbance of 350 μL of leachate at 254 and 400 nm (UV and visible range respectively) on a PowerWave XS scanning microplate spectrophotometer (BioTek® Instrument, Winooski, VT). The absorbance of deionised water was used as a control. A humification index (HIX) was calculated by

dividing the absorbance at 254 nm by the absorbance at 400 nm (Zsolnay et al., 1999; Embachar et al., 2007). Soluble phenolic concentrations were assayed using a modification of the method of Box (1983) and Ohno and First (1998) using Na_2CO_3 (1.9 M) and the Folin-Ciocalteu reagent (Sigma-Aldrich, Poole, Dorset) (DeForest et al., 2005). The blue-coloured phenolics were measured at 750 nm using a PowerWave XS scanning microplate spectrophotometer (BioTek® Instrument, Winooski, VT). The

6.2.5 Statistical analyses

ANOVA, Factor, Discriminant and Cluster analyses were all determined using SPSS version 15.0 (SPSS Inc., Chicago, IL) and GenStat version 8 (VSN international Ltd, Hemel Hempstead, UK). They were used to analyse the measured attributes to investigate the effect of soil types and AVCs on the SQIs identified. To identify significant differences between treatments, post hoc multiple comparison (pair-wise) tests were made using the Gabriel test where homogeneity of variance was assumed and Games-Howell procedure where unequal variance occurred. Some variables were clearly not normally distributed judging from the Q-Q plots (data not presented); however, all the factors (SQFs) from factor analysis and discriminant analysis were normally distributed.

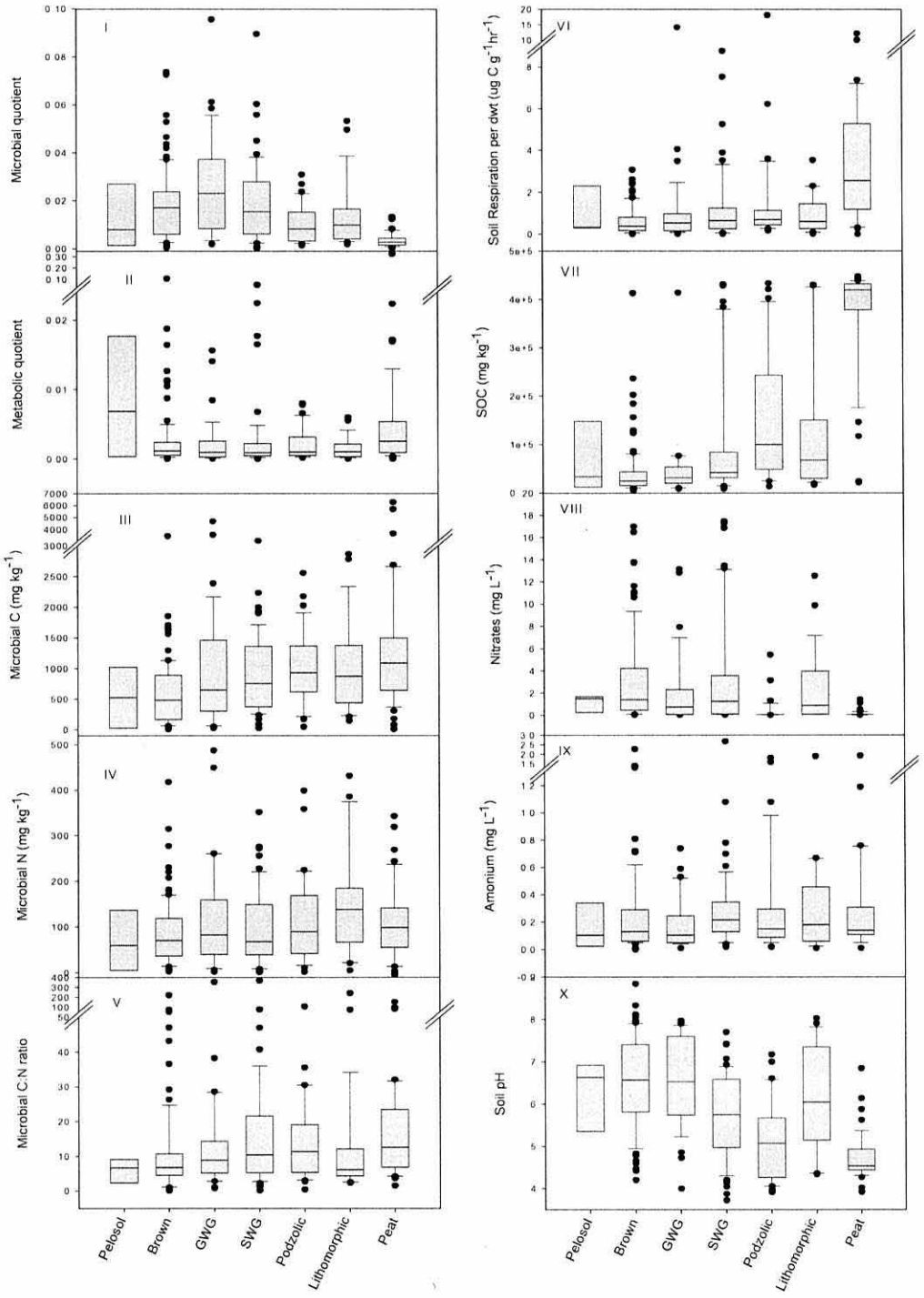
For the cluster analysis, the average linkage method and a squared Euclidean distance measure were used with a rescaled distance cluster combined measure on the similarity axis. The variables were standardized to minimize the effect of scale differences since the variables possessed different units.

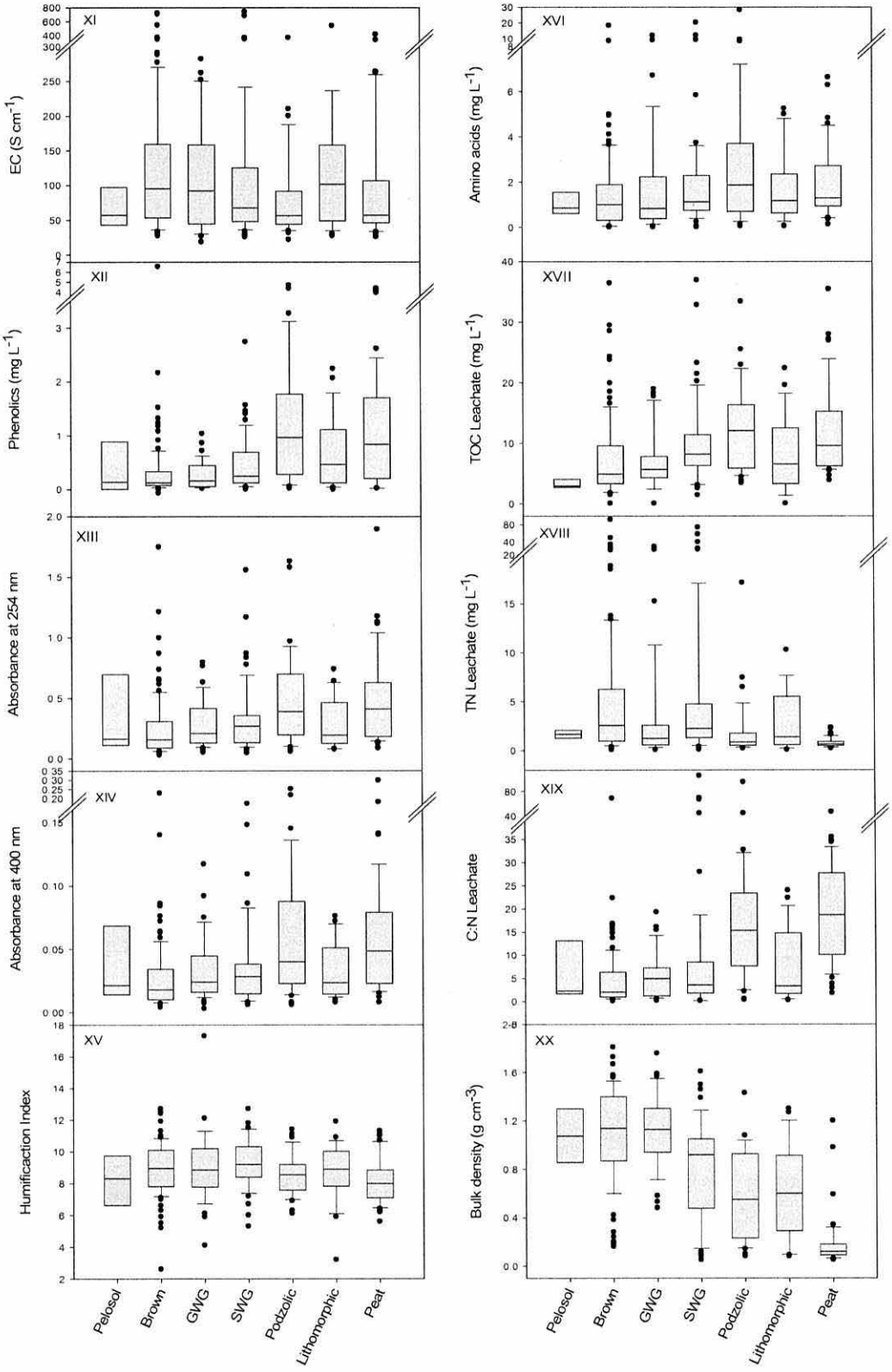
6.3 Results

6.3.1 Biological, physical and chemical properties of soils

The box and whisker plots in fig 6.2 graphically display each variable's location and spread within each soil type, plotted side-by-side on the same graph to enable a comparison of the data set. The plots also show the data's symmetry and skewness. The summary statistics include 10^{th}, 25^{th} (lower quartile, Q1), 50^{th}, (median, Q2) 75^{th}, (upper quartile, Q3), and 90^{th} percentiles. Outliers are shown by filled circles outside the ends of the whiskers. The end of the whiskers can either be a minimum or an adjusted value depending on the presence of the outlier values.

Figure 6.2. Box plots showing the spread of each measured soil property for each soil type. The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median (50th percentile), and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers below and above the box indicate the 10th and 90th percentiles where outliers are present.




XI
EC (S cm-1)
XII
Phenolics (mg L-1)
XIII
Absorbance at 254 nm
XIV
Absorbance at 400 nm
XV
Humification Index
XVI
Amino acids (mg L-1)
XVII
TOC Leachate (mg L-1)
XVIII
TN Leachate (mg L-1)
XIX
C:N Leachate
XX
Bulk density (g cm-3)
Pelosol
Brown
GWG
SWG
Podzolic
Lithomorphic
Peat

6.3.2 Relationships among soil properties

Correlation analysis of the 20 soil attributes representing soil biological, physical and chemical properties resulted in significant correlation ($P < 0.05$) in 112 of the 190 soil attribute pairs (Table 6.2). Of these, the highest significant ($P<0.01$) positive correlations was between humic substances at 254 nm versus those at 400 nm ($r = 0.97$). Other highly significant ($P<0.01$) positive correlations were between the absorbance at 254 nm or 400 nm versus DOC ($r = 0.78$ and $r = 0.71$ respectively); leachate TN versus NO_3^- ($r = 0.78$), and bulk density versus pH ($r = 0.70$). Other notable significant ($P<0.01$) positive correlations ($r > 0.50$) were between: microbial-N versus microbial-C, SOC versus soil respiration, the leachate C:N ratio versus SOC, electrical conductivity versus both nitrate and TON, phenolics versus absorbance at 254 nm and TOC versus absorbance at 400 nm. The highest significant ($P<0.01$) negative correlation was between bulk density versus SOC ($r = - 0.83$) Other notable significant ($P<0.01$) negative correlations were between: bulk density versus either microbial-C ($r = -0.42$), soil respiration ($r = -0.51$) or the leachate C:N ratio ($r = -0.47$); SOC versus qMic ($r = -0.47$) and pH versus either SOC ($r = -0.66$), absorbance at 400 nm ($r = -0.42$), leachate TOC ($r = -0.40$) or leachate C:N ratio ($r = -0.47$).

Table 6.2. Correlations among physical, chemical and biological soil attributes using Pearson correlation methods

Variable	qMic	qCO2	Mic C	Mic N	Mic CN	SR	SOC	Nitrate	NH4	pH	EC	Phenols	Abs@ 254	Abs@ 400	HIX	Am acids	TOC_L	TN_L	CN_L	BD
*q*Mic	1																			
qCO_2	-0.07	1																		
Mic C	0.23(**)	-0.07	1																	
Mic N	0.17(**)	-0.05	0.63(**)	1																
Mic C:N	0.18(**)	-0.02	0.03	-0.24(**)	1															
SR	-0.26(**)	-0.01	0.31(**)	0.09	-0.02	1														
SOC	-0.47(**)	-0.04	0.39(**)	0.09	0.04	0.61(**)	1													
Nitrate	0.21(**)	-0.01	-0.15(**)	-0.12(*)	0.20(**)	-0.22(**)	-0.33(**)	1												
NH4	-0.05	-0.04	0.08	0.06	-0.03	0.02	0.04	0.06	1											
pH	0.35(**)	0.08	-0.31(**)	0.00	-0.11(*)	-0.39(**)	-0.66(**)	0.25(**)	-0.18(**)	1										
EC	0.03	0.06	-0.09	0.01	0.17(**)	-0.08	-0.03	0.59(**)	0.03	0.12(*)	1									
Phenols	-0.23(**)	-0.01	0.19(**)	0.08	-0.01	0.27(**)	0.39(**)	-0.19(**)	0.38(**)	-0.36(**)	0.04	1								
Abs@ 254	-0.24(**)	-0.01	0.10(*)	-0.03	0.05	0.22(**)	0.34(**)	-0.19(**)	0.23(**)	-0.42(**)	-0.04	0.58(**)	1							
Abs@ 400	-0.23(**)	-0.01	0.10(*)	-0.06	0.05	0.21(**)	0.35(**)	-0.19(**)	0.23(**)	-0.42(**)	-0.08	0.60(**)	0.97(**)	1						
HIX	0.06	0.02	0.00	0.11(*)	0.13(**)	-0.07	-0.14(**)	0.24(**)	0.01	0.10(*)	0.37(**)	-0.09	-0.04	-0.22(**)	1					
Am acids	-0.04	-0.03	0.09	-0.04	0.28(**)	0.03	0.11(*)	-0.02	0.48(**)	-0.15(**)	-0.01	0.23(**)	0.09	0.09	0.11(*)	1				
TOC_L	-020(**)	0.01	0.12(*)	-0.02	0.06	0.29(**)	0.35(**)	-0.18(**)	0.32(**)	-0.40(**)	0.01	0.56(**)	0.78(**)	0.71(**)	0.08	0.23(**)	1			
TON_L	0.18(**)	0.02	-0.08	-0.05	0.24(**)	-0.14(**)	-0.21(**)	0.78(**)	0.11 (*)	0.09	0.66(**)	-0.08	-0.13(**)	-0.14(**)	0.31(**)	0.05	-0.05	1		
C:N_L	-0.25 (**)	-0.04	0.16(**)	-0.02	0.03	0.33(**)	0.50(**)	-0.33(**)	-0.04	-0.47(**)	-0.05	0.34(**)	0.38(**)	0.37(**)	-0.06	0.02	0.38(**)	-0.25(**)	1	
BD	0.46(**)	0.05	-0.42(**)	-0.22(**)	-0.07	-0.51(**)	-0.83(**)	0.35(**)	-0.14(**)	0.70(**)	0.10(*)	-0.38(**)	-0.35(**)	-0.33(**)	0.04	-0.17(**)	-0.37(**)	0.21(**)	-0.47(**)	1
Variable	qMic	qCO2	Mic C	Mic N	Mic CN	SR	SOC	Nitrate	NH4	pH	EC	Phenols	Abs@ 254	Abs@ 400	HIX	Am acids	TOC_L	TN_L	CN_L	BD

*Correlation is significant at $P < 0.05$ level, and ** at the $P < 0.01$ level; n=304

*q*Mic, microbial quotient; qCO_2, metabolic quotient; Mic C, microbial carbon (mg C/kg); Mic N, microbial nitrogen (mg C/kg); Mic C:N, microbial C:N ratio; SR, soil respiration (mg/kg/hr); SOC, soil organic carbon (mg/kg); nitrate (mg/L); NH4, ammonium (mg/L); EC, ($\mu S\ cm^{-1}$); Phenols, Soluble phenolics (mg/L); Abs @ 254 and 400, absorbance at 254 and 400 nm; HIX, humification index; Am acids, Free amino acids (μM); TOC/TN_L, total organic carbon/nitrogen in leachate (mg/L); BD, bulk density.

Due to differences in the units of individual variables, Factor Analysis (FA) was performed using a correlation matrix on the standardised values of the measured 20 attributes. The generalised least-squares method was used to extract factors because it is robust and requires no assumptions of sample coming from a multivariate normal distribution (SPSS 15.0 online help, 2006). The first six factors with eigenvalues > 1 were retained for interpretation, whilst factors with eigenvalues < 1 explained less total variation than individual soil attributes (Brejda et al., 2000). The retained factors accounted for > 61% of the total variance in the measured attributes (Table 6.3). The retained factors were subjected to a varimax rotation. A varimax rotation redistributes the variance of significant factors and minimizes the number of variables that have high loadings on each factor, thereby simplifying the interpretation of the factors (SPSS 15.0 online help, 2006).

Factors	Initial eigenvalues			Extraction sums of squared loadings			Rotation sum of squared loadings		
	Total	Proportion of variance (%)	Cumulative variance (%)	Total	Proportion of variance (%)	Cumulative variance (%)	Total	Proportion of variance (%)	Cumulative variance (%)
Factor 1	5.31	26.6	26.6	3.60	18.0	18.0	3.35	16.7	16.7
Factor 2	2.64	13.2	39.8	3.22	16.1	34.1	2.96	14.8	31.5
Factor 3	2.03	10.1	49.9	2.14	10.7	44.8	2.28	11.4	42.9
Factor 4	1.73	8.7	58.6	1.56	7.8	52.6	1.65	8.3	51.2
Factor 5	1.31	6.6	65.1	0.65	3.3	55.9	1.32	6.6	57.8
Factor 6	1.18	5.9	71.1	1.15	5.7	61.6	0.76	3.8	61.6

Table 6.3. Total variance (Eigenvalue), proportion and cumulative variance explained by factor analysis using correlation matrix (standardized data) on the measured attributes.

The relative importance of each soil attribute, in terms of its contribution to all of the factors, was judged by its communality value (Field, 2005; Ayoubi and Khormali, 2008) and is shown in Table 6.4. The six factors explained > 90% variance in absorbance @ 254 and 400 (absb@254 and 400), microbial carbon (Mic C), and soil organic carbon (SOC); > 80% in total organic nitrogen in leachate (TN_L) and bulk density (BD); > 70% in microbial nitrogen (Mic N), Nitrate, Ammonium, electrical conductivity (EC), and total organic carbon in leachate (TOC_L); > 60 % in microbial quotient (*q*Mic), pH and humification index (HIX); > 50 % microbial C/N ratio (Mic CN), soil respiration (SR), and phenolics; and < 50 % C/N ratio of the leachate (CN_L) and microbial metabolic quotient (qCO_2) (Table 6.4). Attributes with the low communality estimates (e.g. qCO_2 and leachate C:N) were the least important for interpreting factors. The magnitudes of the loadings were used as a criterion for interpreting the relationship between the soil attributes and the factors. Soil attributes were assigned to the factor for which the loadings were highest.

Variable	Factor1	Factor2	Factor3	Factor4	Factor5	Factor6	Communality extraction
Bulk density	-0.86	-0.19	0.13	-0.18	-0.11	-0.05	0.87
pH	-0.68	-0.28	0.02	-0.06	-0.18	0.05	0.68
***q*Mic**	-0.54	-0.13	0.12	0.45	-0.01	-0.07	0.67
Soil organic C	0.92	0.16	-0.08	0.08	0.01	-0.06	0.91
Soil respiration	0.61	0.06	-0.07	0.09	0.03	-0.06	0.50
Microbial-C	0.29	0.03	-0.04	0.89	0.09	-0.03	0.90
qCO_2	0.05	-0.05	-0.05	-0.18	0.01	0.00	0.10
Microbial-N	0.05	-0.04	-0.08	0.75	-0.02	0.14	0.73
Microbial C:N	0.07	0.05	0.30	-0.03	0.11	0.01	0.51
Nitrate-N	-0.27	-0.09	0.81	-0.04	0.01	0.04	0.77
Ammonium-N	0.01	0.23	0.05	0.05	0.78	0.01	0.72
Elec. conductivity	0.03	0.00	0.74	-0.03	-0.05	0.22	0.70
Soluble phenolics	0.29	0.52	-0.03	0.06	0.32	-0.10	0.55
Absorb@ 254 nm	0.17	0.98	-0.06	-0.01	0.04	0.03	1.00
Absorb@ 400 nm	0.17	0.96	-0.05	-0.02	0.04	-0.20	0.99
HIX	-0.06	-0.06	0.25	0.07	0.05	0.76	0.69
Amino acids	0.11	0.06	0.02	0.01	0.66	0.05	0.56
TOC (leachate)	0.24	0.71	-0.02	0.00	0.29	0.18	0.73
TN (leachate)	-0.12	-0.06	0.91	0.01	0.09	0.07	0.87
C:N (leachate)	0.47	0.26	-0.17	-0.02	-0.06	0.02	0.42

Table 6.4. Proportion of variance (loadings) using varimax rotation and communality estimates for soil attributes of the retained factors.

The first factor explained 17 % (see Table 6.3) of the total variance. It was named *soil organic matter (SOM)* because it had high positive loading for SOC (0.92), soil respiration (0.61) and leachate C:N ratio (47), a high negative loadings for bulk density (-0.86), pH (-0.68) and moderately on *q*Mic (-0.54). Grouping *q*Mic with the *SOM* factor rather than factor 4 was as a result of its stronger correlation with attributes constituting the *SOM* factor namely, soil respiration (r = -0.26), SOC (r = -0.47) and bulk density (r = 0.46) rather than with Microbial-C (r = 0.23) and Microbial-N (r = 0.17) of factor 4 (Table 6.3). The second factor explained 15 % of the total variance with a high positive loading for soluble phenolics

(0.52), leachate absorbance at 254 nm (0.98), 400 nm (0.96) and leachate TOC (0.71) and consequently, was termed *OM humification.* The third factor explained 11 % of the total variance with high positive loadings for nitrate (0.81), leachate TN (0.91) and electrical conductivity (0.74) and was therefore termed *soluble nitrogen* factor. The fourth factor explained 8 % of total variance and had positive loadings for Microbial-C (0.89), Microbial-N (0.75) and a moderately high loading for *q*Mic (0.45), and was termed *microbial biomass.* The fifth factor explained 7 % of total variance and had positive loading for ammonium (0.78) and amino acids (0.66) and was termed *reduced N.* The sixth factor explained only 4 % of the total variance and had a high positive loading for HIX (0.76) and was termed *soil humification index.*

6.3.3 Effect of soil types on attribute means and factor scores

One way ANOVA revealed that most of the soil attributes and factor scores varied significantly with soil types (Table 6.5). However, pairwise comparison showed that the effect of soil types on most attribute was very small.

Soil attributes	Soil types							SEM	ANOVA
	Brown	Groundwater gley	Lithomorphic	Peat	Pelosol	Podzolic	Surfacewater gley		
Bulky density	1.10 ^{a}	1.11 ^{a}	0.63 ^{b}	0.19 ^{c}	1.08 ^{a}	0.58 ^{b}	0.81 ^{b}	0.06	0.00
pH	6.55 ^{a}	6.56 ^{a}	6.24 ^{ac}	4.71 ^{b}	6.18 ^{ac}	5.08 ^{b}	5.73 ^{c}	0.2	0.00
Microbial quotient	0.018 ^{a}	0.026 ^{a}	0.014 ^{ac}	0.003 ^{b}	0.014 ^{abc}	0.010 ^{c}	0.018 ^{a}	0.003	0.00
SOC (g/kg)	42 ^{a}	45 ^{a}	132 ^{b}	377 ^{c}	92 ^{ab}	151 ^{b}	98 ^{b}	23	0.00
Soil Respiration (mg/kg/hr)	0.63 ^{a}	1.10 ^{a}	0.93 ^{a}	3.35 ^{b}	1.63 ^{ab}	1.58 ^{ab}	1.18 ^{a}	0.45	0.00
Microbial C (g/kg)	0.59 ^{a}	1.00 ^{ab}	1.03 ^{ab}	1.37 ^{b}	0.54 ^{a}	1.02 ^{ab}	0.89 ^{ab}	0.13	0.00
***q*CO2**	0.073	0.002	0.001	0.011	0.01	0.002	0.003	0.012	NS
Microbial N (mg/kg)	85 ^{a}	119 ^{ab}	148 ^{b}	113 ^{ab}	71 ^{ab}	111 ^{ab}	99 ^{ab}	16	0.03
C:N (Microbial)	12.4	19.6	18.9	19.7	36.3	29.9	33.2	12	NS
Nitrate N (mg/L)	3.00 ^{a}	2.04 ^{ac}	2.32 ^{ac}	0.13 ^{b}	1.13 ^{c}	0.37 ^{bc}	3.08 ^{a}	0.39	0.00
Amonium N (mg/L)	0.25	0.18	0.3	0.27	0.17	0.31	0.3	0.05	NS
Ec (uS cm^{-1})	129	107	124	99	74	81	116	16	NS
Soluble phenolics (mg/L)	0.33 ^{ac}	0.26 ^{a}	0.68 ^{bc}	1.10 ^{b}	0.56 ^{abc}	1.20 ^{b}	0.46 ^{c}	0.16	0.00
Absob @ 254 nm	0.25 ^{a}	0.28 ^{a}	0.29 ^{ab}	0.47 ^{b}	0.45 ^{ab}	0.48 ^{b}	0.32 ^{ab}	0.48	0.00
Absob @ 400 nm	0.028 ^{a}	0.033 ^{a}	0.032 ^{ab}	0.061 ^{b}	0.047 ^{ab}	0.061 ^{b}	0.036 ^{ab}	0.009	0.00
HIX	9.0 ^{ab}	9.0 ^{ab}	8.7 ^{ab}	8.2 ^{a}	8.3 ^{ab}	8.6 ^{ab}	9.3 ^{b}	0.3	0.03
Free amino acids (µM)	1.52	1.83	1.67	1.95	1.15	3.1	2.08	0.4	NS
Leachate TOC (mg/L)	7.5 ^{a}	6.9 ^{a}	8.2 ^{ab}	12.0 ^{b}	12.8 ^{ab}	12.3 ^{b}	9.8 ^{ab}	2.2	0.00
Leachate TON (mg/L)	5.82 ^{a}	3.47 ^{ac}	3.16 ^{ac}	0.78 ^{b}	1.62 ^{c}	1.81 ^{bc}	6.69 ^{a}	0.8	0.01
Leachate C:N	4.6 ^{a}	5.5 ^{a}	7.2 ^{a}	19.0 ^{b}	9.1 ^{ab}	17.5 ^{b}	9.7 ^{a}	2.4	0.00
Factors	**Factor scores**								
Factor 1	-0.52 ^{a}	-0.63 ^{a}	0.15 ^{b}	1.58 ^{c}	-0.59 ^{a}	0.2 ^{b}	-0.07 ^{b}	0.12	0.00
Factor 2	-0.17	-0.05	-0.13	0.22	-0.46	0.44	0	0.15	NS
Factor 3	0.09	-0.1	-0.06	-0.13	-0.4	-0.28	0.23	0.11	NS
Factor 4	-0.24 ^{a}	0.36 ^{b}	0.30 ^{b}	0.03 ^{ab}	-0.21 ^{ab}	0.01 ^{ab}	0.02 ^{ab}	0.19	0.04
Factor 5	-0.05	-0.22	-0.03	-0.11	-0.2	0.36	0.14	0.17	NS
Factor 6	0.06	-0.1	0.17	-0.3	-0.29	-0.2	0.23	0.18	NS

Table 6.5. Soil attribute means and factor scores in the different soil types. The first 5 variables are most important for discrimination soil types. Statistical significant difference between soil types are shown by different subscript letters ($P<0.05$) in the attribute and factors; n =304.

In most cases, only the Peat soils were clearly significantly ($P < 0.01$) different from all the other soil types. Only *SOM* and *microbial biomass* factors (Factors 1 and 4 respectively) varied significantly ($P < 0.05$) with soil type. *SOM* factor mean scores were negative for Brown, GWG, SWG and Pelosol soils and positive for Lithomorphic, Peat and Podzolic soils. Peats had the highest score and were significantly different from all other soil types on the *SOM* factor. Furthermore, Peat soils had the highest SOC content to which the analysis also confirmed. The mean scores for *SOM* factor did not vary significantly ($p > 0.05$) within Browns, GWGs and Pelosols nor did it do so among the Lithomorphic, Podzolic and SWG soils. The *Microbial biomass* factor varied significantly ($P < 0.05$) between Brown versus GWG soil types and Lithomorphics only. Mean scores for *OM humification*, *soluble N*, *reduced N* and *humification index* did not vary significantly ($P > 0.05$) among all soil types.

6.3.4 Soil quality indicators across soil types

Discriminant analysis of the six statistical factors in relation to soil types, indicated that the *SOM* was the most powerful in discriminating among the seven soil type groups based on the magnitude of their discriminant coefficients (Eq. (6.2)). The first canonical discriminant function explained 90 % of the total variance based on Wilks's Lambda, ($P < 0.001$) (table not shown) and therefore was the most important canonical discriminant function for discriminating soil types using the soil quality factors identified. Although the second canonical discriminant function was also significant ($P = 0.03$) based on Wilks's Lambda, it only accounted for 4 % of the total variance and therefore was not used.

$$Y_1 = 1.43\ (SOM) + 0.29\ (OM\ humification) - 0.14\ (soluble\ N) + 0.08\ (microbial\ biomass) + 0.03\ (reduced\ N) - 0.22\ (HIX) \qquad \textbf{[Eq. 6.2]}$$

Therefore the group differences across soil types shown by ANOVA can be explained in terms of *SOM*, judging from the discriminant coefficient which was five-fold larger than the coefficient for the *OM humification* factor and several fold greater than the rest of the factors. Discriminant analysis of the measured attributes constituting *SOM* (i.e. *q*Mic, soil respiration (SR), soil organic C (SOC), pH and bulk density (BD)) indicated that microbial quotient (*q*Mic) was the most powerful attribute discriminating the soil types (Eq. 6.3).

$$Y_2 = 8.75 \times 10^{-6} (SOC) - 1.99 (qMic) - 0.50 (BD) - 0.04 (pH) - 0.05 (SR) \quad \textbf{[Eq. 6.3]}$$

The discriminant coefficient for *q*Mic was four-fold larger than the coefficient for bulk density and more than 40-fold for the rest. The *q*Mic was significantly correlated with bulk density (0.46**), soil organic C (-0.47**), pH (0.35**) and soil respiration (-0.26**) while bulk density was significantly correlated with soil organic C (-0.83**), pH (0.70**) and soil respiration (-0.53**) meaning that *q*Mic and bulk density, though correlated, were the most important and dominant attributes for assessing soil quality across soil types. The mean comparisons using the Games-Howell approach indicated that the bulk density had similar discriminating power as the *SOM* factor among the soil types. *q*Mic mean values varied significantly with soil types separating Peat < Podzols < Browns, GWGs and SWGs soils in increasing order (Table 6.5).

6.3.5 Effect of aggregate vegetation class on factor scores

Aggregate vegetation class (AVC) showed more effects on factor scores than the soil types. The significant effects were observed in *SOM*, *OM humification*, *microbial biomass* and *humification index*. The *soluble N* and *reduced N* factors showed no significant variation among the AVCs (Table 6.6). The *SOM* factor had the highest factor scores ($P < 0.001$) in

Heath and Bog. Mean scores between Moorland Grass Mosaics and Upland Woodland did not vary significantly ($P > 0.05$); nor among Fertile Grasslands, Infertile Grassland, Lowland Wooded and Tall Grass Mosaic. The mean scores were lowest in Crop and Weeds and were significantly different ($P < 0.001$) from all other AVCs except in Tall Grass and Herbs.

Means scores for *OM humification* factor varied significantly ($P < 0.001$) between Crop and Weeds versus Herb and Bog, and Infertile Grasslands; all other pairs did not vary significantly. For *microbial biomass* factor, Crop and Weeds and Tall Grass and Herbs varied significantly ($P < 0.001$) against the Fertile Grassland, Infertile Grasslands, Heath and Bog, and Moorland Grass Mosaics, while all other pairs were not significantly different ($P > 0.05$). The *humification index* factor showed that the mean scores varied significantly ($P < 0.001$) among Crop and Weeds versus Infertile Grassland and Moorland Grass Mosaics versus Lowland Wooded only.

Average vegetation class mean factor scores										
Factors	Crop & weeds	Fertile grasslands	Heath & bog	Infertile grassland	Lowland wooded	Moorland grass mosaics	Tall grass & herbs	Upland wooded	SEM	ANOVA
Factor 1	-0.80^{a}	-0.54^{b}	1.43^{c}	-0.50^{b}	-0.40^{b}	0.62^{d}	-0.64^{ab}	0.20^{bd}	0.10	0.00
Factor 2	-0.40^{a}	-0.11^{ab}	0.30^{b}	0.02^{b}	0.41^{ab}	-0.11^{ab}	-0.06^{ab}	0.51^{ab}	0.19	0.00
Factor 3	0.34	0.07	-0.09	-0.03	0.05	-0.34	0.12	-0.28	0.14	NS
Factor 4	-0.49^{a}	0.16^{b}	0.07^{b}	0.27^{b}	-0.19^{ab}	0.28^{b}	-0.61^{a}	-0.21^{ab}	0.16	0.00
Factor 5	-0.39	0.09	0.13	0.02	-0.12	0.22	-0.20	0.18	0.14	NS
Factor 6	-0.29^{a}	0.04^{ab}	-0.35^{ab}	0.13^{b}	1.15^{c}	0.18^{b}	0.38^{bc}	0.63^{bc}	0.17	0.00
Soil attributes	**Soil attribute mean values**									
Soil resp	0.29^{a}	1.00^{b}	3.22^{c}	0.77^{b}	0.67^{ab}	1.44^{b}	0.43^{ab}	1.41^{b}	0.23	0.000
Soil organic C	16.7^{a}	43.6^{b}	350.2^{c}	43.8^{b}	46.4^{b}	185.6^{c}	25.0^{ab}	119.8^{c}	11.2	0.000
pH	7.3^{a}	6.4^{b}	4.6^{c}	6.3^{b}	6.2^{abd}	5.2^{d}	6.6^{ab}	4.7^{dc}	0.2	0.000
Bulk density	1.37^{a}	1.06^{b}	0.21^{c}	0.95^{b}	0.89^{b}	0.41^{d}	1.22^{ab}	0.48^{d}	0.05	0.000
***q*Mic**	0.021^{a}	0.023^{a}	0.005^{b}	0.021^{a}	0.015^{ab}	0.009^{b}	0.015^{ab}	0.010^{ab}	0.003	0.000

Table 6.6. Effect of Aggregate vegetation class on factor scores and soil attribute means. Soil resp=soil respiration; *q*Mic= microbial quotient. Statistical significant difference between vegetation classes are identified by different subscript letters ($P<0.05$) in the attribute and factor means; n =304.

6.3.6 Soil quality indicators across Aggregate Vegetation Classes (AVC)

The first canonical discriminant function of the discriminant analysis of the six factors across the AVCs explained 94% of the total variance (Wilks's Lambda, $P < 0.001$) whose coefficients were used in the equation below:

$$Y_3 = 2.12\ (SOM\ ) + 0.49\ (OM\ humification) - 0.35\ (soluble\ N) + 0.30\ (microbial\ biomass) + 0.36\ (reduced\ N) - 0.20\ (soil\ HIX) \quad \textbf{[Eq. 6.4]}$$

From the discriminant coefficients in Eq. 6.4, *SOM* factor was the most powerful discriminating among the eight different AVCs. The *SOM* factor was more than four-fold larger than the coefficients of all others soil quality factors under consideration.

The discriminant analysis of the measured attributes constituting the *SOM* factor showed that BD and *q*Mic were the most powerful discriminating soil attributes among the seven habitats (AVCs) (Eq. 6.5).

$$Y_4 = 3.27\ (BD) - 2.45\ (qMic) - 2.75 \times 10^{-6}\ (SOC) + 0.70\ (pH) + 0.08\ (SR) \quad \textbf{[Eq. 6.5]}$$

Bulk density possessed similar discriminating power as the *SOM* factor among the AVCs. Bulk density values were significantly different ($P < 0.001$) among AVCs with the lowest mean values in Heath and Bog (0.21 g cm^{-3}) < Upland Wooded (0.48 g cm^{-3}) and Moorland Grass Mosaic (0.41 g cm^{-3}) < Fertile Grass (1.06 g cm^{-3}), Infertile Grass (0.95 g cm^{-3}), Lowland Wooded (0.89 g cm^{-3}) < Tall Grass and Herbs (1.21 g cm^{-3}) and Crop and Weeds (1.37 g cm^{-3}; Table 7.6).

6.3.7 Main and interactions effect of soil types and AVCs

The results of the two-way ANOVA on the first canonical discriminant function on all 20 variables showed significant ($P < 0.01$) main and interaction effects. The main effect of soil types and the effect of soil types * AVCs interaction on the attribute's scores was very small (Partial Eta Square = 0.09 and 0.16 respectively), while the main effect of the AVCs was large (Partial Eta Square = 0.42; Table. 6.7).

Source	Type IV sum of squares	Df	Mean Square	F	Sig.	Partial eta squared
Corrected model	553.14	38	14.56	36.36	0.001	0.844
Intercept	3.42	1	3.416	8.532	0.004	0.032
Soil Type * AVC_Desc	**18.98**	**25**	**0.759**	**1.896**	**0.008**	**0.157**
Soil Type	**10.36**	**6**	**1.726**	**4.311**	**0.001**	**0.092**
AVC_Desc	**73.97**	**7**	**10.57**	**26.39**	**0.001**	**0.420**
Error	102.09	255	0.400			
Total	655.24	294				
Corrected Total	655.24	293				

Table 6.7. Tests of between-subjects effects; Dependent variable: 1st Canonical Discriminant function scores from of all the soil attributes measured. AVC_desc means AVC description; Soil Type*AVC_Desc means the interaction between the soil type and AVC effects.

The cross tabulation of AVCs versus soil types (appendix 3), showed that 27 out of 56 combinations or cells, the soil types were sampled less than the calculated expected counts in the AVCs. In 16 combinations, the soil types were not at all represented in the AVCs. The most affected were the Lowland Wooded and Tall Grass and Herbs where, only Brown and SWGs were samples in the former and only Browns, GWG and SWGs in the latter.

6.4 Discussion

6.4.1 Effect of soil types and AVCs on the soil quality factors and/or indicators

A set of 20 correlated soil attributes were grouped into six factors uncorrelated called soil quality factors, using factor analysis. The factors identified contribute to one or more key soil functions proposed by Larson and Pierce (1991) and therefore could be considered soil quality indicators (Brejda et al., 2000). Since the soil quality factors cannot be measured directly (Elliott, 1997; Brejda et al., 2000), the effect of soil types and the AVCs on these factors were inferred by monitoring soil attributes that comprised them.

Not all the soil quality factors varied significantly with soil types or with AVCs. Only *SOM* and *microbial biomass* factors varied significantly ($P < 0.001$) by soil types. *SOM* was able to discriminate the highest number of soil groups, separating the Peats (group 1) with the highest scores, from (group 2) Lithomorphics, Podzols, and SWGs with intermediate scores, and from (group 3) Browns, GWGs and Pelosols with the lowest scores (Table. 6.5), thus rendering three distinct soil type groupings. The *microbial biomass* factor had a minor effect, discriminating the Browns from GWGs and Lithomorphics only. The soil attributes constituting these soil quality factors (Soil respiration, SOC, pH, bulk density, qMic, Microbial-C and -N) showed significant ($P < 0.01$) variations discriminating at most three groups of soil types. In all the attributes considered, Browns, GWGs and Pelosols were grouped together. *SOM* factor, SOC and bulk density attributes separated the Peats as a unique soil group from all other soil types, which is not entirely a surprising result, since the peats are highly organic in nature with low BD as opposed to mineral soils with low OM content and higher bulk densities. Thus, the most important soil quality indicator associated with specific soil types or groups was the *SOM* factor with qMic > bulk density as the most important attributes.

Similarly, the most important SQF differentiating the AVCs across the GB was *SOM* factor with bulk density > *q*Mic attributes being the most important attributes. Four distinct AVC groups were separated based on *SOM* factor and BD. Heath and bog was exclusively separated as one group (1). Other groups were: (2) Crop and weeds with Tall grass and herb; (3) Fertile grassland, Infertile grassland, Lowland wooded, Tall grass and herbs, and Upland wooded; (4) Moorland grassland mosaic with Upland wooded. The Upland wooded and Tall grass and herbs were intermediate habitats classifying in more than one of these groups. The rest of the factors and attributes discriminated three or less groups. The soil attributes were generally better in discriminating the AVCs than the SQF (Table 6.6)

Since *q*Mic and bulk density were moderately correlated (r=0.46**), they may be redundant as indicators to be used together. If only one attribute were to be used to monitor soil quality in soil types and AVCs, *q*Mic and BD respectively seems to offers the greatest potential judging from their high weights on the respective prediction models. However the *q*Mic (microbial biomass/SOC) may be a 'MUST be included' soil attribute in the minimum data set, due to its important role in several soil functions, being a fraction of soil carbon. Soil carbon influences a wide range of soil functions including bulk density, infiltration, pesticide buffering, aeration, aggregate formation, pH, buffer capacity, cation-exchange properties, mineralization, and the activity of soil organisms (Larson and Pierce, 1991; Seybold et al., 1997). However, since the measurement of bulk density is reasonably easy to obtain, it is therefore reasonable to consider it together with SOC, microbial and biomass C as minimum data set for assessing soil quality across vegetation classes in the study area.

Pedogenesis has taken place over thousands of years in the UK. During this period there has been a range of climate change related vegetation colonization phases starting from tundra heath and cycling through a range of forest types (Fitzpatrick, 1980). During this period parent material/topography, climate and vegetation would have been stable for long

periods of time leading to the differentiation of soils. This was followed by progressive forest clearance which started approximately 1000-3000 years ago with vegetation cover becoming more grassland and heathland dominated. The last 200 years, however, has seen intense management of these soils with the addition of fertilisers, lime and organic wastes combined with mechanical mixing of the soils which has reversed centuries of acidification and soil horizon development. This homogenisation of the soil has led to shifts between soil types even over short timescales (e.g. humic-podzolic to brown soils on improved upland grasslands) and the loss of peat soils in intensive agricultural areas (e.g. East Anglia). One key question is therefore whether it is historical soil type or current vegetation that is more important in driving soil processes in the short term (e.g. over a 10-25 year timescale)? Here we found that more soil quality factors showed an AVC effect rather than a soil type effect. All soil quality factors varied significantly ($P < 0.01$) by AVCs except *soluble N* and *reduced N* factors though none discriminated more than four groups. It is possible that some of the soil quality factors that were insensitive to vegetation may represent inherent soil qualities that are controlled by other key factors of soil formation (e.g. parent material/topography), while those which significantly varied by AVCs may represent dynamic soil qualities, possessing great potential for assessing management practices on soil quality (Jenny, 1994; Soybold et al., 1997; Brejda et al., 2000; Buyer et al., 2002).

Most indicators available in literature have not been validated nor their sensitivity tested in a wide range of situations (Velasquez et al., 2007). Some of the attributes measured and the soil quality indicators identified in this work are not usually used in the monitoring of soil quality, but are candidates for potential alternatives.

6.4.2 Prediction of SQF and SQI by soil type or AVC

The clusters from multivariate classification are "natural" groups, which uses the "minimum-variance" solution; where a population is partitioned into cluster subsets by minimizing the total within group variation while maximising between groups variance (Wishart, 1968). The groups/cluster formed from the multivariate analysis need to have no significant overall spread. The clusters therefore, should correspond to data modes (distinct modes). However, most of our cluster modes defined by soil types were not always distinct. Most of them were separated from each other by significant "noise" data, making it impossible to resolve all the clusters. Thus, the definition of the reference values for each soil type or AVC was ambiguous, since most soils types or AVC groups could not be differentiated (Fig. 6.3). Forming, describing and defining the groups could involve the use all measured attributes even though only a few could be differentiating (Soil survey staff, 1999). Even when the soil quality factors/indicators and attributes were used in combination, some groups/clusters could still not be resolved. Therefore, the soil quality indicators and attributes identified in this study can only be used to characterise soil types and AVCs groups rather than for prediction or classification. From the discriminant plots and the dendrogram in Fig. 6.3 three groups can be defined in soil types and four groups in the AVC.

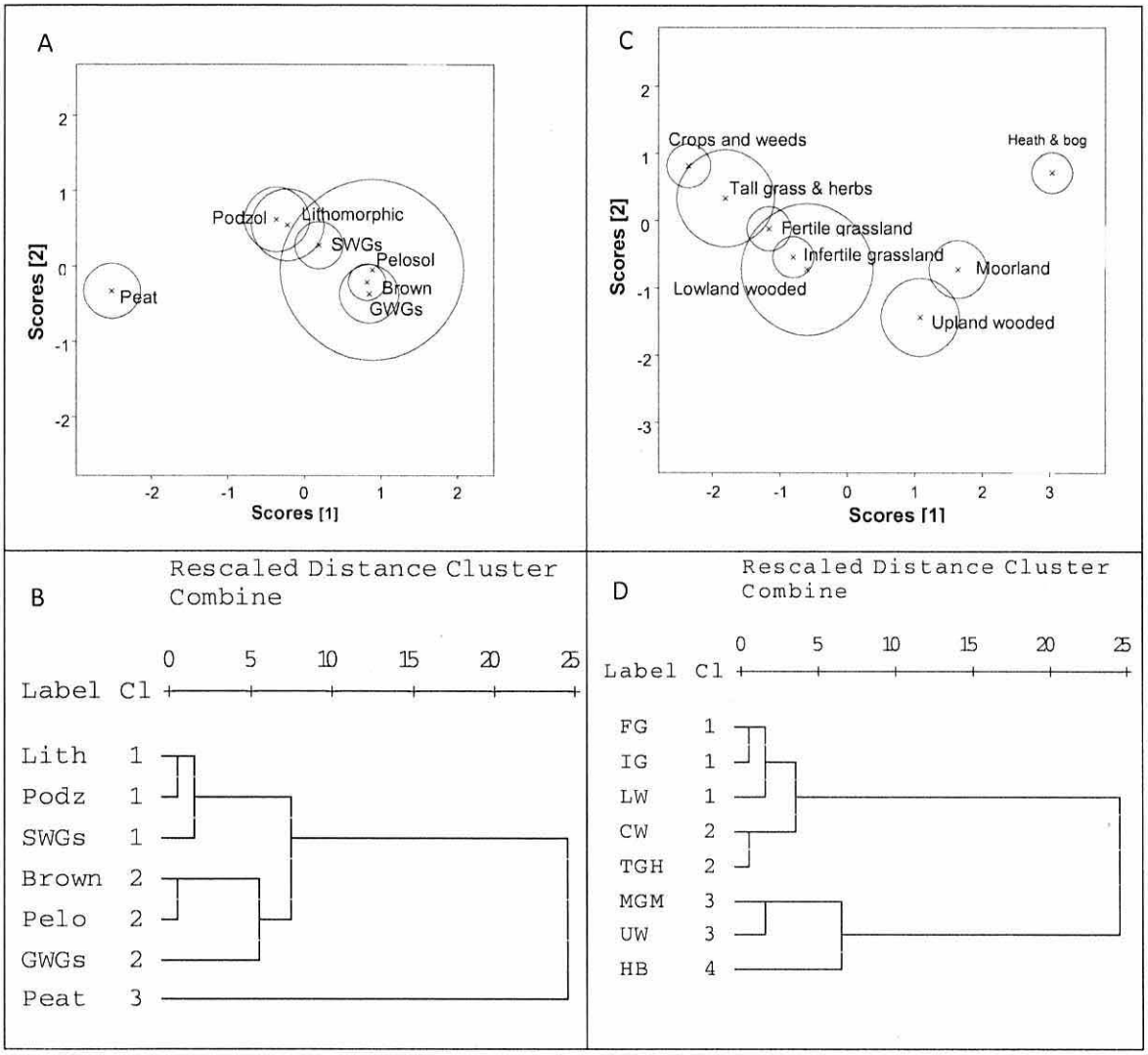


Figure 6.3. Discrimination plots showing 95% confidence circles around the means for soil types (Panel A) and AVCs (Panel B). Panels B and D are the respective cluster analyses dendrograms using a complete linkage method. The soil and AVCs groups were based on the most important attributes (*q*Mic, SOC, Microbial-C and bulk density). The clusters are shown by the numbers at the end nodes. Abbreviations: Lith, Lithomorphic; Podz, Podzolic; SWGs, surface water gleys; GWGs, Groundwater gleys; Pelo, Pelosol; FG, Fertile grassland; IG, infertile grassland; LW, Lowlamd wooded; CW, Crop and weeds; TGH, Tall grass and herbs, MGM, Moorland mosaic; UW, Upland wooded; HB, Heath and bog

Defining differentiating criteria for these groups in the soil types could involve the use of bulk density attribute to define unique property ranges for the first groups, a combination of soil respiration and SOC attributes for the second group, and a combination of *q*Mic, soil respiration, SOC, pH and bulk density attributes for the third group. The Pelosols were the most dispersed and unreliable group for the purpose of attribute membership prediction, probably due to the fact that they were under sampled, considering that only six samples were included in the analysis. The classification of the AVCs using discriminate and cluster analyses on key attributes yielded four clusters. Defining differentiating criteria for these groups could involve the use of a combination of soil respiration, SOC, pH attributes to define property ranges for the first, second and fourth groups and bulk density attribute for the third group. Tall Grass and Herbs and Lowland Wooded were under sampled (with 11 and 6 samples respectively; appendix 3) which greatly compromised their predictive accuracy as can be observed from the large 95% confidence circles which overlapped with other AVC groups.

6.4.3 To what extent do soil types and/ or AVC act as major regulators of SQI?

The two-way ANOVA and the tests of between-subjects effects on the first canonical discriminant (CD) function from the discriminant analysis (DA) of the 20 physical, chemical and biological properties showed significant differences between groups (soil types and AVCs) as well as significant differences in the effect of soil type on the soil attributes between the AVC (significant interaction of soil type × AVC; Table 6.7). The 'practical' significance of each term from Partial Eta Square values indicates that AVCs (with a large Partial Eta Square = 0.42), were a better regulator of the SQIs than soil types (with a weak Partial Eta Square = 0.09). The effect size for the interaction was equally relatively weak (Partial Eta Square = 0.16). The conclusion of the significant ($P < 0.01$) interaction effect of

soil type × AVC is that the soil type differences in the first CD function (or attributes) partly depended on the AVCs where the soil was sampled. A multiple comparison of all soil type groups in each AVC group would be required to draw specific conclusions regarding the interaction effects, which is quite complex and is beyond the scope of this thesis. Suffice to say that there was a partial and varied soil type × AVC interaction across all levels. These interactions confirm Jenny's (1994) theory that the biotic factor (of which vegetation plays a major role) is amongst others an important soil forming factor. However, the results from the cross tabulation indicated that not all soil types were well represented in the AVCs in going by the calculated expected counts. In some cases soil types were not at all represented (see appendix 3). This problem can contribute to the complexity and accuracy in the interpretation of the interaction effect observed above.

6.5 Conclusions

The dominant SQFs/Is and attributes varied by both soil type and AVC. The *SOM* factor was the most discriminating factor for both soil types and AVCs with microbial quotient and bulk density as the most discriminating measured attributes. The discriminant analysis on the important measured attributes comprising the *SOM* factor produced three fairly homogenous groups for soil types and four groups for AVCs. It was however, impossible to define reference values in the SQF/I or attributes for separate individual soil types or AVCs, as property ranges greatly overlapped due to large between group variability (probably due to integrating large spatial areas). Some of the differences observed in soil types with regard to soil attributes were in part dependent on the AVCs differences.

Therefore, whether SQIs can be predicted by soil types remains an open question. This study has shown that soil types or AVCs are poor predictors for SQF and indicators derived from factor analysis. However, different sets of SQIs and attributes for different regions have been

used in the past in different studies suggesting that there may not be a universal optimum set of indicators for use across different regions of differing climatic conditions. Therefore, the search for SQIs which can be predicted by soil types continues.

Future work

For future work it might be worthwhile to make special consideration for the climatic, spatial and parent material variability in the sampling designs in addition to the inclusion of other promising soil attributes. Finer resolution of soil types should be used instead of the broad soil types. Management factors should also be included (e.g. fertilizer regime). A further consideration should be in the sampling design, to ensure equal and adequate representation of soil types in the aggregate vegetation classes in order to accurately capture the interaction effect.

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Chapter 7: Article IV

Prediction of soil function and bacterial biodiversity using major soil types, topsoil characteristics or vegetation type

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This paper has not been submitted to any journal

ABSTRACT

Soils are a geologic, climatic and vegetative legacy that produces heterogeneity in soil biodiversity and function at the landscape scale. The activity, diversity, structure and abundance of microbial communities will therefore reflect soil type and its characteristics; yet, these aspects are not used in traditional soil classification. Recent concern about climate change has increased the need to establish predictive relationships between soil properties and soil processes to improve our understanding and enable management of ecosystems to mitigate the effect of these changes. This study therefore aimed to explore the relationships between soil biodiversity and function of >500 topsoils (0-15 cm) collected across a broad range of soil types and vegetation classes (AVCs) in the UK. Measurement of soil function included laboratory-based mineralisation of ^{14}C-labelled plant material and artificial root exudates over 90 d, basal soil respiration (SR) and potential mineralisable nitrogen from intact soil cores held at field capacity. Soil bacterial biodiversity was evaluated using the Shannon's diversity index calculated from molecular fingerprints of bacterial communities. Results from multivariate regression tree analysis and ordination methods revealed that AVCs > NO_3^- > moisture content at field capacity (MCFC) > Olsen P determined the grouping splits in the laboratory substrate-induced mineralisation assays. The grouping splits in basal soil respiration were largely based on soil organic matter, the grouping split in mineralisable N was based on NO_3^- > AVC/MCFC, while the grouping splits in biodiversity were based on soil pH and C:N ratio. The combined analysis of soil function and diversity created groupings split by AVCs > pH > NO_3^- with biodiversity, slow phase substrate allocation and half lives (i.e. key responses) highly characterising the groups. We conclude that major soil type classes, soil function and bacterial biodiversity are largely uncorrelated and therefore major soil types cannot effectively be used to predict soil function and bacterial biodiversity. The soil function and bacterial biodiversity are mainly determined by a few specific but contrasting soil properties such as soil pH, C concentration, nutrient availability, and moisture content, rather than the broad soil types. We, however, suggest that the multivariate descriptions of soil types based on specific soil physical and chemical properties may be better predictors of soil function and biodiversity than are the univariate analyses or the conventional broad soil type classes.

Key words: biological soil quality indicators; soil function; soil biodiversity; soil respiration; nitrogen mineralisation.

7.1 Introduction

Traditional national and international soil taxonomies are often defined on subsoil properties and are frequently poor at describing the many inherent and dynamic attributes of topsoils such as soil microbial diversity and function, even though these attributes play a critical role in determining soil processes and quality (Panikov, 1999; Sanchez et al., 2003; Turbe et al., 2010). Consequently, the characterization of top soils (0-20 cm) has gained enhanced attention in recent years, particularly for environmental monitoring purposes and for defining and profiling soil quality indicators (FAO, 1998). More generally, top soils harbour most of the soil biomass and dominate functions that influence the provision of supportive and regulatory ecosystem services (Turbe et al., 2010).

One of the most important purposes of a soil classification scheme is for the prediction of soil properties across a range of geographical scales. This is of particular interest to policymakers who wish to understand the consequences of implementing policies which promote alternative land uses and soil management regimes (e.g. agri-environment schemes, climate change mitigation, protection of water quality). For this approach to be successful requires that the general tenet *that soils in different locations but with the same classification will respond in the same way*, holds true. There are a number of assumptions that need to be critically evaluated before accepting this statement such as the consideration that some national classifications were carried out more than 50 years ago when the land use regime, vegetation cover and climatic variables (e.g. N and S deposition) may have been significantly different (Vitharana et al., 2008). Further, the scale and accuracy to which soils have been mapped at the landscape level will also be a critical determinant of the reliability of soil maps (Butler, 1980; Vitharana et al., 2008; Borujeni et al., 2009). Specifically, this relates to the potential for abrupt transitions in soil type, which are unrealistic in landscapes where lateral changes in soil are gradual, and that maps essentially ignore spatial variation in soil

properties within mapping units (Kempen et al., 2010). This is exemplified by Marsman and De Gruijter (1986) who found that within-map unit variation of soil properties ranged between 65 and 80% of the total variation in a 1600 ha sandy area in the Netherlands whilst Vitharana et al. (2008) also reported that traditional soil maps were a poor predictor of soil chemical properties. Similarly, Jones et al. (2009) found few differences in soil function in relation to dissolved organic nitrogen cycling over a global latitudinal gradient that encompassed a huge variation in soil type. They ascribed this lack of difference to the large amount of functional redundancy in soil microbial communities suggesting that only some soil processes are highly soil type dependent (i.e. keystone processes).

Under anticipated global changes in climate and land use there has been a growing interest in the prediction of soil microbial processes and particularly greenhouse gas (GHG) emissions at a regional and national scale for inventory purposes (Palm et al., 2007). In many countries, models such as DNDC are being used to facilitate calculation of country-specific GHG emissions to meet the IPCC Tier II reporting requirements (Smith et al., 2010; Zhang et al., 2010). The underlying data used to drive these models is typically derived from national soil inventories and associated maps (Brown et al., 2004). It is therefore vital that these maps accurately reflect the temporal and spatial variability in soil biological processes. The relationship between microbial diversity and function within and between a wide range of soil types, is largely unknown (Wall et al., 2005). Despite this poor understanding, below-ground biodiversity has been assumed to influence ecosystem stability, productivity and resilience towards stress and disturbance (Bengtsson, 1998; Torsvik and Ovreas, 2002; Nannipieri et al., 2003).

To date, few studies have examined soil functioning from a broad geographical viewpoint (i.e. comparing many contrasting soil types at a landscape scale). Most knowledge about soil functioning is based on individual soil types factored by management systems at

farm or catchment level. Therefore, the aim of this study was to explore the relationships that exist between soil function and diversity in relation to soil type and vegetation cover in topsoils (0-15 cm) collected from across the UK. Using multivariate regression tree analysis and ordination methods, discriminant and clusters analysis, we investigated: (1) the explanatory variables (soil attributes) which best describe the response variables of soil microbial function and bacterial biodiversity (2) Which soil function and diversity variables best split the response variables and (3) whether soil types defined by cluster analysis of soil attributes can better predict function and diversity compared to conventionally defined major soil types.

7.2 Materials and methods

7.2.1 Field site, soil sampling and preparation

To encompass all the major soil and land use types, a total of 624 soil samples were collected throughout the UK, according to a 15 km square grid laid across the country as described by Scott (2008). The general distribution of the samples is shown in Figs 3.3 and 4.1 of this thesis. At each grid intersection, a 1 km^2 sample area was selected. Within the 1 km^2 sample area, 3 plots (5 × 5 m^2) were randomly located and a single 15 cm long × 4 cm diameter soil sample was collected from each of the plots. Additional information about vegetation and soils were also collected from the same plots. The 1 km^2 areas were stratified within 45 Land Classes (see Appendix 1). All the sites were characterised by a temperate climate with a North-South mean annual temperature range of 7.5 to 10.6°C and East-West mean annual rainfall range from 650 to 1700 mm (Matthew, 2006).

Across all land use and vegetation class categories, the dominant soil types (% of total) were: Brown soils (33%), Surface water gley soils (19%), Podzolic soils (14%), Peat

soils (12%), Groundwater gley soils (11%), Lithomorphic soils (8%), and Pelosol soils (3%). See Table 5.1 for their equivalents in the World Reference Base (WRB) soil classification system. Across all land uses and soil types, eight aggregate vegetation classes (AVCs) were identified as (abbreviation and % of the total land area): Infertile Grassland (Infg; 21%), Heath and Bog (Htab; 20%), Fertile Grassland (Frtg; 19%), Crops and Weeds (Craw; 14%), Moorland Grass Mosaics (Mrgm; 11%), Upland Woodland (Uplw; 8%), Tall Grass and Herb (Tgah; 4%), Lowland Woodland (Lwlw; 3%). For details of AVC classification see Table 6.1 in chapter 6 of this thesis.

To normalize for soil moisture and ensure all soils were at field capacity, artificial rainfall (125 μM NaCl, 15.7 μM $CaCl_2$, 1.3 μM $CaSO_4$, 15.3 μM $MgSO_4$, 12.3 μM H_2SO_4; Emmett et al., 2008) was applied to each soil core (10°C) until 150 ml of leachate had been collected according to the protocol described by Emmett et al. (2008). The soils were then incubated at 10°C for 28 d to equilibrate, after which the samples were broken up, mixed by hand, and visible roots/stones removed.

7.2.2 Soil analyses

Analyses on the soils of mineralisable N, mineralisable ammonium-N and nitrate-N, total C and N, Olsen P, bulk density, pH (water), soil moisture, soil moisture at field capacity, loss-on-ignition (LOI) were analyses according to Emmet et al., (2010); The soil leachate measurements of: electrical conductivity, pH, soluble phenolics, humic substances, free amino acids, total dissolved C and N, nitrate and ammonium and C/N ratio were performed as described in Chapter 6 of this thesis. Soil respiration (SR) was determined on a 15 cm long, 2.5 cm diameter soil cores with a 1250 cm^3 head space, incubated at 10°C for 1 h, using climate controlled-incubators briefly described in chapter 6 of this thesis.

Soil diversity was determined using molecular profiles of total bacterial communities on soil cores according to Griffiths et al., (2000). Briefly, a separate adjacent soil core was taken and homogenised under sterile conditions. Total nucleic acids were extracted from 0.25 g of soil using a previously described method (Lane, 1991), modified to include a 30 min hexadecyltrimethylammonium bromide (CTAB) freeze-thaw, soft-lysis stage. TRFLP analysis of 16S rRNA genes was performed using forward primer 63F 5'-CAGGCCTAACACATGCAAGTC-3' labelled at the 5' end with 6FAM fluorescent dye (Sigma Genosys) and reverse primer, 519R (Lane, 1991) 5'- GTATTACCGCGGCTGCTG – 3' (MWG operon) modified as detailed by Thomson et al. (2010). Amplicons were purified using the PureLink PCR purification kit (Invitrogen Corp., Carlsbad, CA), then digested using restriction endonuclease MspI (Promega, Madison, WI). Fragment analysis was performed using a 3730 DNA analyser (Applied Biosystems, Life Technologies Corporation, Carlsbad, CA) and individual TRFs were binned manually using Genemarker software (SoftGenetics LLC, State College, PA). Prior to statistical analyses the intensity of each fragment was converted to a proportional abundance, by dividing by the total intensity of all detected fragments. The Shannon index (H) of diversity was then calculated using $H = - \Sigma p_i \ ln\ (p_i)$ where p_i is the relative abundance of each TRFLP peak within each sample.

7.2.3 Substrate mineralisation in soils

A simple or complex ^{14}C-isotopically labelled C substrate was used to estimate mineralisation rates in soil. The simple C substrate was chosen to reflect low molecular weight root exudates and comprised a solution of ^{14}C-glucose (50 mM), ^{14}C-fructose (5 mM), ^{14}C-sucrose (5 mM), ^{14}C-citrate (10 mM), ^{14}C-malate (5 mM) and ^{14}C-succinate (2 mM) and possessed a specific activity of 8.4 Bq μmol^{-1} C. The complex C substrate consisted of ^{14}C-labelled shoots of *Lolium perenne* L. with a specific activity of 12.3 kBq g^{-1}. The ^{14}C-

enrichment of *Lolium perenne* was achieved by pulse labelling with $^{14}CO_2$ at onstant specific activity according to Hill et al. (2007). To characterise the ^{14}C label in the plant material, a sequential chemical fractionation was performed according to Jones and Darrah (1994), briefly described in Chapter 4 of this thesis.

For each soil, 10 cm^3 was placed into a sterile 50 cm^3 polypropylene container. Either 0.5 ml of the ^{14}C-labelled simple C substrate (artificial root exudates) or 100 mg of the ^{14}C-labelled complex C substrate (*Lolium perenne* shoots) was then added to the soil. A further 0.5 ml of distilled water was added to the soil receiving the complex C substrate to ensure the same moisture content in both treatments. A vial containing 1 M NaOH (1 ml) was then placed above the soil and the polypropylene containers hermetically sealed. The $^{14}CO_2$ capture efficiency of the NaOH traps was >95%. The soils were then placed in the dark in a climate-controlled room (10°C) and the NaOH traps exchanged after 0.5 h, 1 d, 7 d, 14 d, 28 d and 90 d. The $^{14}CO_2$ in the NaOH traps was determined using Optiphase 3® Scintillation fluid (PerkinElmer, Waltham, MA) and a Wallac 1404 Liquid Scintillation Counter (PerkinElmer, Waltham, MA).

7.2.4 Decomposition model

The depletion of ^{14}C from the soil samples after the addition of the substrates was described by a double first order kinetic decay model fitted to the data using SigmaPlot 8.0 (SPSS Inc, Chicago, IL) as follows:

$$Y = [a_1 \times \exp(-k_1 t)] + [a_2 \times \exp(-k_2 t)] \qquad \text{Eq [7.1]}$$

Where Y is the concentration of ^{14}C remaining in the soil samples; a_1 and a_2 describe the size of the substrate pools at time 0; k_1 and k_2 are the respective exponential coefficients describing the first and second mineralisation phases; t is time after the substrate addition.

Pool a_1 is attributable to the rapid use of substrate ^{14}C as respiratory after uptake by soil microbes (Boddy et al., 2007, 2008). The half life (HL_1) of the substrate pool (a_1) was calculated as follows

$$HL_1 = \frac{\ln(2)}{k_1} \qquad \text{Eq [7.2]}$$

The slower second mineralisation phase $^{14}CO_2$ evolution has been attributed to microbial turnover within soils (Paul and Clark, 1989; Boddy et al., 2007). When ^{14}C is transformed by microbial processes, it remains in the soil and so may enter and re-enter the biomass repeatedly (Kouno et al., 2001). Due to the uncertainty of connectivity of substrate pools between a_1 and a_2 (Boddy et al., 2007; Oburger and Jones, 2009), we did not calculate the half life for pool a_2.

7.2.5 Statistical analysis

The statistics programme *R* was used to perform both univariate and multivariate regression tree analyses to identify and differentiate classes or groups. Canoco for windows v4.54 programme (Biometrics, Wageningen, Netherlands) was used to generate the *Canonical* Correlation Analysis (CCA) biplot of all soil functional measures and boidiversity as response variables with all the physical, chemical and environmental variables as explanatory variables. The X-Y plots of selected variables were done using Sigma plot 10.0 (SPSS Inc, Chicago, IL). SPSS 16.0 (SPSS Inc, Chicago, IL) and GenStat 8.0 (VSN International, Oxford, UK) were used to classify the soil by two-step cluster analysis and discriminant analysis respectively. Observations were classified using a two-step cluster analysis based on selected physico-chemical variables, with soil types as grouping variable. We created the cluster membership variables for the 'new' soil types (soil clusters) in the

working data file for further analysis. The discriminant analysis was used to (i) investigate how far samples belonging to different soil classes could be correctly classified, and (ii) to determine the 95% confidence interval circles around the group means for the seven soil types, using selected combination of physico-chemical variables measured on them. The selected physico-chemical variables for the soils are presented in Table 7.1.

Variable description	**Units**	**Abbreviation**
Soil pH measured in water		Soil pH
Soil moisture content at field capacity	% dry weight	MCFC_dwt
Soil carbon content	% dry weight	% C
Soil nitrogen content	% dry weight	% N
C/N ratio	Ratio	C/N ratio
Soil Olsen P	mg kg^{-1} dry weight	OLSEN_P
Soil loss on ignition (LOI)	% dry weight	LOI
Nitrate in leachate	mgL^{-1}	NO3_L
Ammonium in leachate	mgL^{-1}	NH4_L
Phenols in leachate	mgL^{-1}	Phenols
Amino acids in leachate	mgL^{-1}	A.acids
Soil bulk density	g cm^{-3}	BD
Soil type		
Aggregate vegetation class		AVC

Table 7.1. Selected soil physico-chemical variables used in the multivariate analysis.

Variable	Units/description	Abbreviation
Shannon entropy	Bacterial diversity Shannon index	Shannon
Soil respiration per LOI (SR)	$\mu g\ C\ g\ LOI^{-1}\ hr^{-1}$	SR_LOI
Soil respiration per dwt (SRdwt)	$\mu g\ C\ g\ dwt^{-1}\ hr^{-1}$	SR_dwt
Soil respiration per sqm (SR/m^2)	$\mu g\ C\ g\ dwt^{-1}\ hr^{-1} m^{-2}$	SR_sqM
Soil mineral N per SR per LOI (N/SR/LOI)	$mg\ N\ mg\ C^{-1}\ /gC\ g\ LOI^{-1}$	N_SR_LOI
Soil mineral N per SR per dwt (N/SRdwt)	$mg\ N\ mg\ C^{-1}\ /gC\ g\ dwt^{-1}$	N_SRdwt
Soil mineral N per g LOI (N/LOI)	$mg\ N\ g\ LOI^{-1}$	Nmin_LOI
Nitrate N per LOI	Nitrate portion of mineralisable nitrogen per LOI	NO3N_LOI
Nitrate N per dwt	Nitrate portion of mineralisable nitrogen per dry weight soil	NO3N_dwt
Ammonium N per LOI	Ammonium portion of mineralisable nitrogen per LOI;	NH4N_LOI
Ammonium N per dwt	Ammonium portion of mineralisable N per dry weight	NH4N_dwt
Labile substrate decay parameters		
a_1l	Fast dacay pool partition	a_1_l
HL-l	Fast decay half life	HL_1_l
k_2l	Slow decay rate constant	k_2_l
90l	Percent C remaining after 90 days incubation	90_l
Plant substrate decay parameters		
a_1p	Fast decay pool partition	a_1_p
HL-p	Fast decay half life	HL_1_p
k_2p	Slow decay rate constat	k_2_p
90p	Percent C remaining after 90 days incubation	90_p

Table 7.2. The list of all the soil function and biodiversity variables measured on the soil samples.

7.3 Results

7.3.1 Relationships between soil function/diversity, soil types, soil parameters and AVCs

The *canonical* correlation analysis (CCA) plot (Fig. 7.1) represents the soil function and diversity (as response variables represented as point) versus the physico-chemical and environmental variables (as explanatory variables represented as arrows) relationships in a two-dimensional space. CCA biplot visualises the correlation between response variables and explanatory variables. The direction of the axes (e.g. left vs. right; up vs. down) is arbitrary and should not affect the interpretation, but axis-1 is more important than axis-2. Points in the same direction with arrows indicate that the corresponding response variables and explanatory variables are correlated with each other. Points and arrows in opposite directions are negatively correlated. Long arrows and points further from the origin are more important than the short lines or points near the origin of the axis. Points and lines at 90° angles indicate that the two corresponding variables are uncorrelated.

The CCA biplot of the soil function measures and diversity data as response variables and physical, chemical and environmental variables as explanatory variables (Fig. 7.1) revealed that axis-1 was predominantly a mineral soil and pH versus organic soils, nitrogen and C:N ratio gradient as shown by the density/pH versus SOM, % N and C:N plots. In contrast, axis 2 was identified as a 'nutrient' gradient as indicated by NO_3^- and Olsen-P, though % N is more related to axis-1, but axis-2 is an unimportant axis as it explained only 5% of the total variance.

Soil respiration (SR) per m^2 was essentially related to bulk density. In contrast, soil respiration per gram dry weight, together with ammonium N (NH_4^+-N) per dwt and the plant diversity measure (DCA1) were correlated with the amount of organic C (as shown by

association with LOI, AVCs, percentage total C). SR per LOI (SR_LOI) was not strongly correlated to any physical, chemical and environmental variable being near the origin of the axes of the biplot. The response variable of mineralisable N per SR per gram dwt (N_SRdwt), mineralisable N per SR per LOI (N_SR_LOI) and nitrate proportion of mineralisable N per dwt (NO3N_dwt) were related to the amount of nitrate in the leachate (NO3_L) and Olsen P.

The substrate-induced respiration measures and the biodiversity measures (Shannon and DCAs) were all grouped together near the origin of the axes, limiting their interpretability. However, they were all positively related to the amount and quality of soil organic matter (LOI, total C and N, C:N ratio), vegetation cover type (AVC) and soil type (4 cluster soil classification by cluster analysis; TSC4), but negatively related to pH and bulk density.

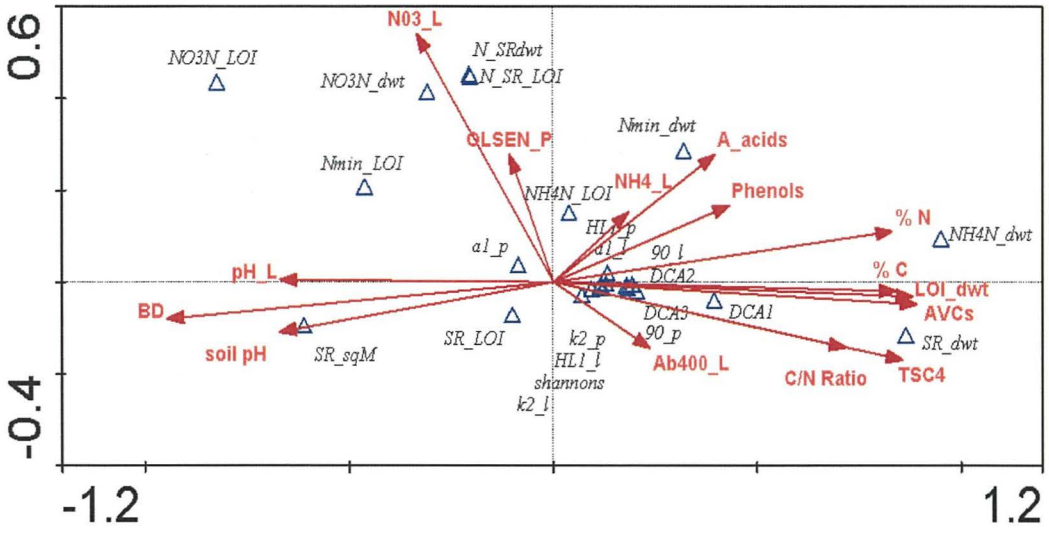

Figure 7.1. CCA plot of the functional and diversity data as response variables and the best 15 physical, chemical and environmental variables as explanatory variables; n=624.

Response variables: N_SRdwt, mineralisable nitrogen per SR per dry weight; SR_dwt, soil respiration per dry weight; SR_sqM, SR per square meter; SR per LOI; NO3N_LOI, nitrate portion of mineralisable nitrogen per LOI; NO3N_dwt, nitrate portion of mineralisable nitrogen per dry weight soil; NH4_LOI, ammonium portion of mineralisable nitrogen per LOI; NH4_dwt, ammonium portion of mineralisable nitrogen per dry weight; Shannon, bacterial diversity Shannon index; DCA1-3, detrended correspondence analysis axis 1-3 of plant diversity; a_1_p and a_1_l, substrate allocation to the rapid mineralisation phase; HL_1_p and HL_1_l, rapid mineralisation half life; k_2_p and k_2_l, the slow mineralisation rate constants and; 90_p and 90_l the ^{14}C remaining after 90d. p and l stands for plant and labile substrates.

The explanatory variables were: Soil pH, pH_L is leachate pH; ab400_L, leachate absorbance at 400 nm; HIX, humification index; AVCs, aggregate vegetation classes; Soil types; TSC4, two step cluster analysis with 4 groups; MCFC, soil moisture content at filed capacity; % C, soil carbon content; % N, soil nitrogen content; C/N ratio; LOI, loss on ignition; Olsen P, soil Olsen P; NO3_L, leachate nitrates; NH4_L, leachate ammonium; phenols, phenolics; A.acids, leachates amino acids; BD, soil bulk density;

Correlation table (not shown) and plots of response variables against explanatory variables showed that only a few pairs were significantly correlated ($P < 0.05$). pH emerged as the main gradient correlating with most functions. For instance, Fig 7.2 panel (A), shows that the allocation of percentage C to the slow phase decreased with increasing pH; panel (C) shows that more C was mineralized at high pH than at low pH; panel (D) shows that there was more SOM accumulation at low pH than at high pH; panel (E) shows that the quality of SOM increasing acididty; and panel (F) shows that high pH favours a higher bacterial diversity than at low soil pH values; and panel (G) shows that more SOM is mineralized in soils with a high bacterial diversity compared to those with low diversity. All other scatter plots of function/diversity with the physic-chemical properties were indeterminate with R^2 values < 0.005.

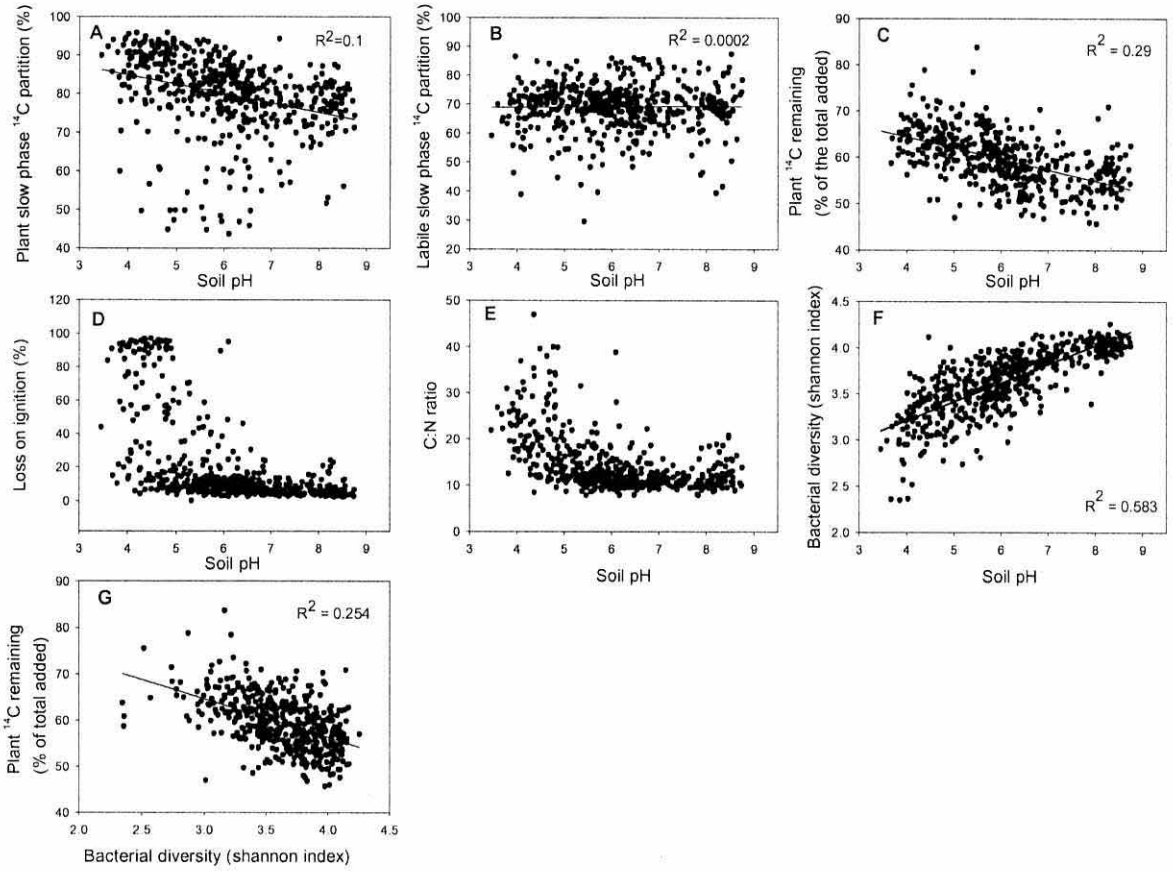

Figure 7.2. Plots of soil pH against various soil properties across a broad range of soils from throughout the UK (panels A-F). Panel G shows the bacterial diversity Shannon index (Shannon index) plotted against the amount of ^{14}C-plant substrate remaining after 90 days of incubation.

7.3.2 Substrate-induced respiration variables

A multivariate regression tree (MRT) of all substrate-induced functional measures (model parameters of a_1, HL_1, k_2 and ^{14}C remaining after 90 d for both labile and plant substrate; Table 7.4) as response variables with all the physical, chemical and environmental data as explanatory variables, highlights the AVCs as the dominant splitting factor followed by MCFC and nitrate in the leachate. Heath and bogs (Htab), Moorland grass mosaic (Mrgm) and Upland woodland (Uplw) on the right were separated from the rest of the habitats on the left (being Crop and weed (Craw), Fertile grassland (Frtg), Infertile grassland (Infg),

Lowland wooded (Lwlw) and Talgrass and herbs (Tgah)). The AVCs on the left were split into group 1 with MCFC per dwt < 47.49 % against groups 2 and 3 with MCFC per dwt ≥ 47.49 %. Groups 2 and 3 were further split based on Olsen P (mg kg^{-1}), group 2 being soil with high to low Olsen P (P = 2.7-36.5 mg kg^{-1}) and group 3 with very high Olsen P concentrations (36.6-191 mg kg^{-1}). The habitats on the right were defined by low nitrate concentrations of < 0.195 mg L^{-1} (group 4) or moderate concentrations ≥ 0.195 mg L^{-1} (group 5; see Fig. 7.3).

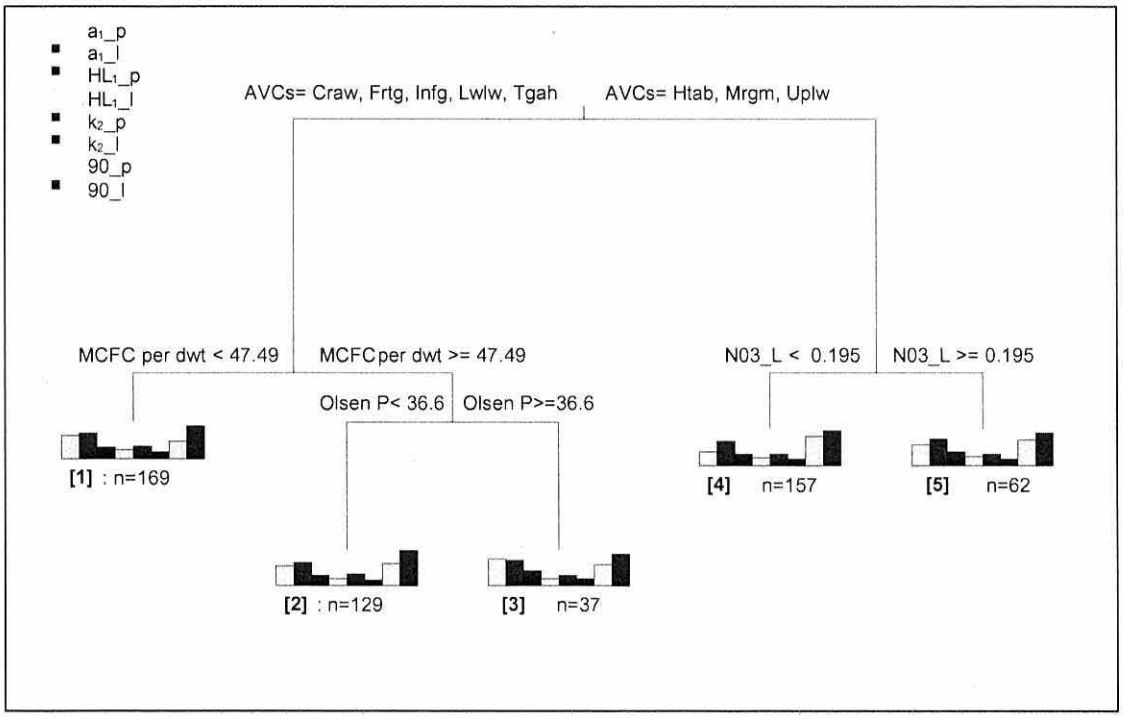

Figure 7.3. MRT of percentage substrate allocation to the rapid pool (a_1_p and a_1_l), half lives for rapid decomposition phase (HL_1_p and HL_1_l), slow phase mineralisation rate constants (k_2_p and k_2_l) and the percentage of C remaining after 90 day incubations (90_p and 90_l) as response variables. The suffixes _p and _l stands for the ^{14}C-labelled plant and labile substrate parameters respectively. The explanatory variables included all physical, chemical and environmental variables listed in the legend of Fig. 7.1.

Abbreviations: Craw, Crop and weeds; Frtg, fertile grassland; Infg, infertile grassland; Lwlw, Lowland wooded; Tgah, Tall grass and herb; Htag, Heath and bogs; Mrgm, Moorland grass mosaic; Uplw, upland wooded. MCFC per dwt, moisture content at field capacity per dry weight, NO3_L, leachate nitrates (mgL).

7.3.3 Soil respiration on soil cores

Soil respiration (SR) measured on the intact soil cores, however, showed a different picture to substrate-induced respiration. The MRT splits in SR were based on organic matter (i.e. LOI percent per dwt) throughout the major splitting levels. Fig 7.4 shows a four leaf tree split based on LOI percent per dwt throughout the splitting levels. The soils on the left hand side of the tree form groups 1 and 2 and were characterised by moderately high (10.5-31.4% LOI) and very high organic matter contents (≥31.4% LOI). In contrast, groups 3 and 4 on the right hand side were characterised by moderate and low organic matter contents (4.3-10.5% and <4.3% LOI respectively).

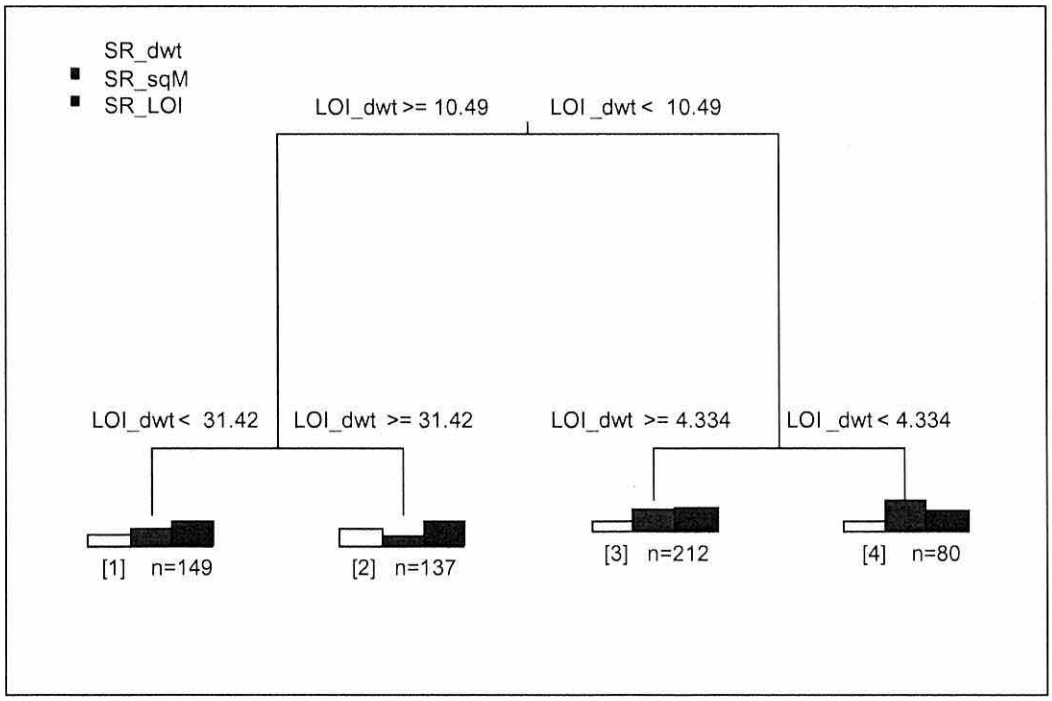

Figure 7.4. MRT of soil respiration per dry weight (SR_dwt), soil respiration per square metre (SR_sqM) and soil respiration per loss on ignition (SR_LOI) as response variables. The explanatory variables included all physical, chemical and environmental variables listed in the legend of Fig. 7.1.

The univariate regression trees (URT) analysis revealed: (1) splits based on pH of SR per LOI; (2) Splits in the mineralisable N per LOI was based on leachate nitrate and; (3) splits in mineralisable N per SR per LOI based on leachate nitrates and to a less extent MCFC and AVC. The URT analysis of the rapid half lives (HL_1), decomposition rate constants (k_2) for both plant and labile substrates produced 1 node trees judging from the cross validation plots. Each tree was split by different explanatory variables. The URT analysis of percentage C remaining after 90 days incubation for the plant substrate produced a 3-node tree with AVCs, pH, and MCFC as splitting variables (figures not shown).

7.3.4 Bacterial biodiversity

The splits in the MRT on microbial diversity were mainly based on pH and soil C:N ratio. This scenario concurs with the CCA plot presented in Figure 7.1. The analysis produced a five-leaf tree as shown in Fig. 7.5. The five associated soil groupings were simply defined by moderate to low pH soils on the right (pH < 5.27) or by moderate to high pH soils on the left (pH ≥ 5.27). The soils on left were defined by three increasing pH levels namely; pH 5.27 - 5.80 (group 2), pH 5.81- 6.86 (group 1) and pH ≥ 6.87 (group 3). On the other hand, the soils on the right were further split based on the C:N ratio of soil organic matter with group 4 characterised by a C:N ratio ≥16.57 and group 5 by C:N ratio of <16.57.

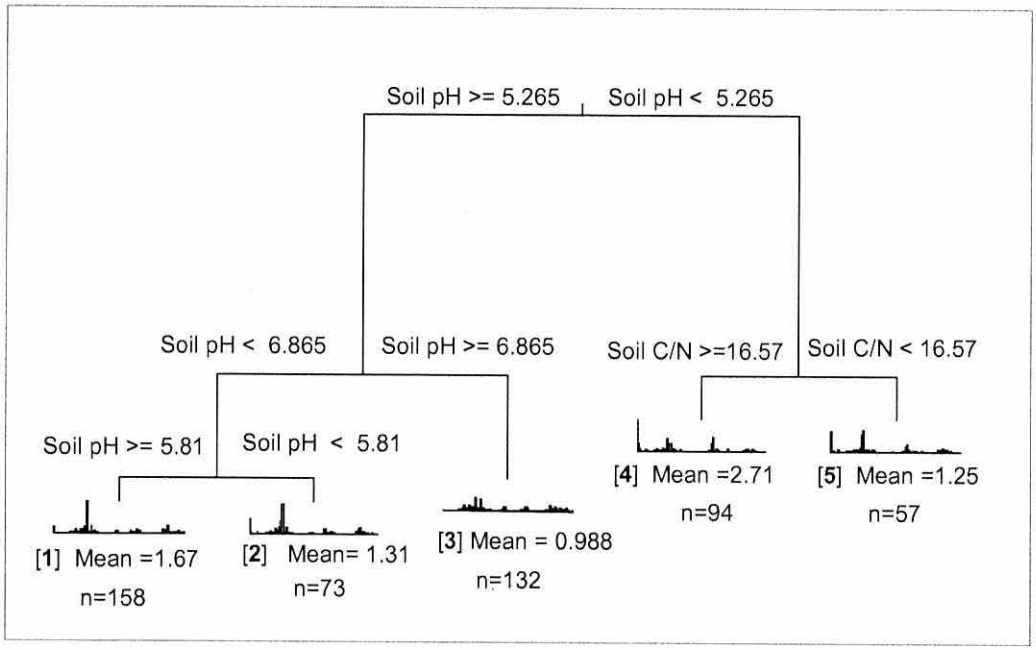

Figure 7.5. MRT of bacterial species abundance as response variables with all the physical, chemical and environmental variables as explanatory variables (the explanatory variables are listed in the legend of Fig.7.1).

7.3.5 Combined MRT for soil function and bacterial diversity measures

The MRT of all the soil functional and diversity measures combined yielded a three node tree as the best predictive tree (Fig 7.6), with major splits made based on AVCs and pH. Overgrowing the tree to a five-node tree revealed further splits in the habitats (Htab, Mrgm and uplw) on the left. The splits were based on NO_3^- and total organic C (mg L^{-1}) in the leachate to yield groups 1-3. The habitats on the right were characterised by moderate to low pH (<6.605, group 4) and those with high pH (≥6.605, group 5; Fig 7.6).

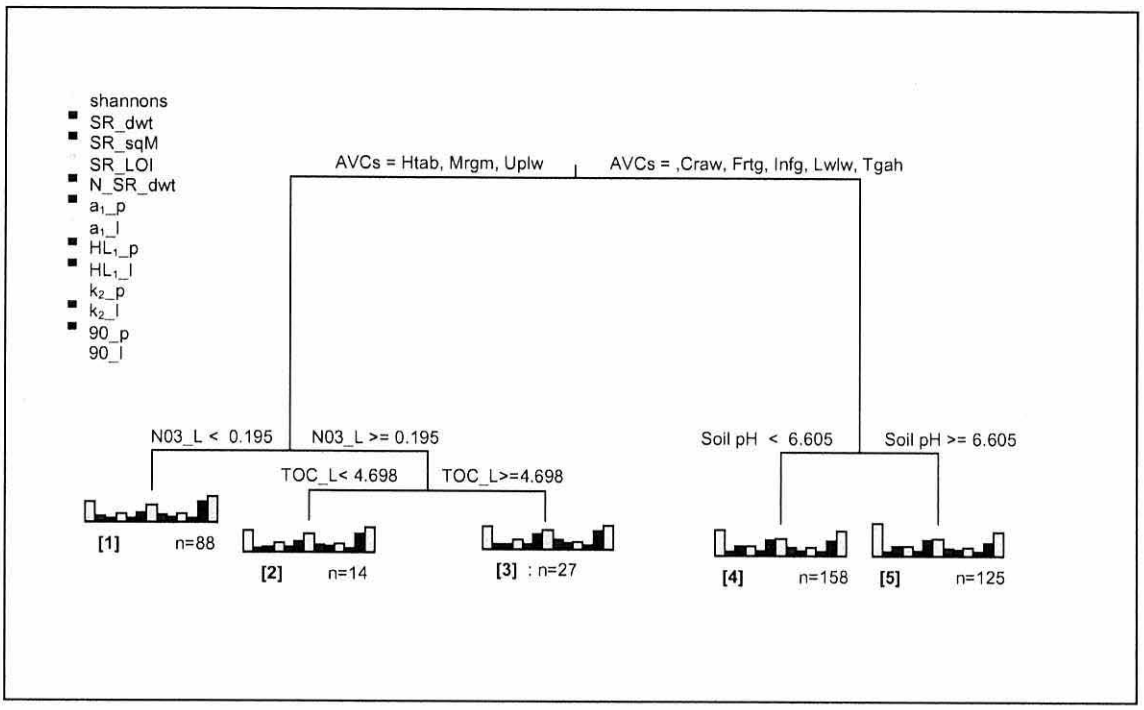

Figure 7.6. MRT analysis of the combined soil function and diversity measures as response variables versus physical, chemical and environmental variables as explanatory. The explanatory variables are listed in the legend of fig 7.1. The response variables were: Bacterial diversity Shannon index (shannons), soil respiration per dry weight (SR_dwt), SR_sqM; SR_LOI; mineralisable N per SR per dwt (N_SR_dwt); substrate allocation to the rapid mineralisation phase (a1_p and a1_l); rapid mineralisation half life (HL1_p and HL1_l); the slow mineralisation rate constants (k2_p and k2_l) and the ^{14}C remaining after 90d (90_p and 90_l). Suffixes p and l stands for plant and labile substrates.

Overall, judging from the CCA plot and the MRTs presented above, the effective environmental factors defining clusters in the soil biological quality indicators of microbial soil function and diversity are: AVCs, LOI, pH, C/N ratio, MCFC and NO_3^- and to a lesser extent Olsen P. The size of the tree was selected by cross–validation. The three-leaf tree was clearly identified as having the smallest estimated predictive error (Fig. 7.7).

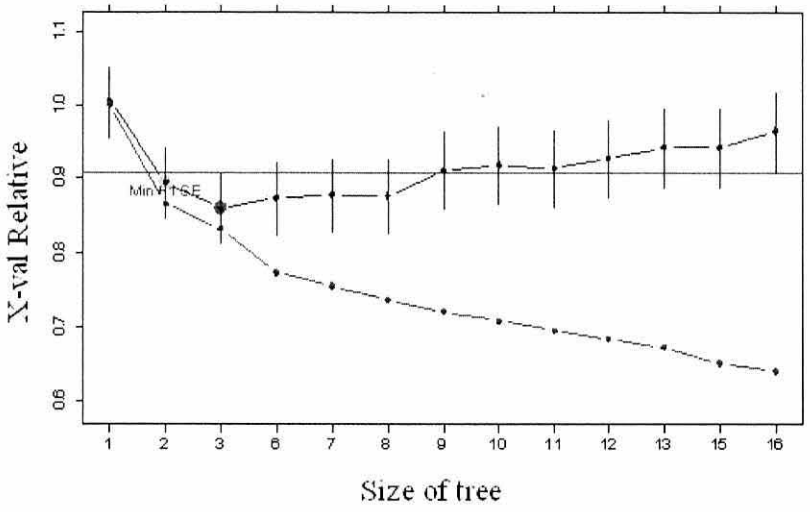

Figure 7.7. Cross-validation of the overgrown tree indicates a reduced tree size to three nodes. The relative error (line without standard error bars) decreases with tree size, whereas the cross-validated relative error (line with error bars) decreases to a minimum for a tree size of three nodes, and then increases, showing that the best predictive tree is a three node tree. The vertical bars indicate one standard error for the cross-validated relative error, and the solid line across indicates one standard error above the minimum cross-validated relative error and suggests a tree size of three leaves.

7.3.6 Relationship between physico-chemical variables, soil function and diversity

An examination of both the CCA plot, URTs and the MRTs portray an unclear link between biodiversity and function. The 'substrate-induced' function and bacterial diversity index being near the origin of the biplot posed a great challenge for meaningful interpretation of their relationships, both with the environmental variables and with each other. However, they both appear to be related based on low pH and high SOM, AVCs, percentage C and N and C:N ratio. There may be a relationship between biodiversity and the substrate-induced respiration as they were both plotted near each other (clustered together) on the ordination plane, though they were both close to the origin of the axes. There was no relationship between biodiversity and SR per LOI as they were at right angles to each other about the

origin (Fig 7.1). Furthermore, SR on intact soil core was correlated with different physico-chemical variables from the substrate induced respiration. The strongest association was that of SR per dry weight with the amount of SOM, the scenario depicted also in the MRT presented in Fig 7.4 (splits based on LOI). However, none of these measures were depicted according to the broad soil types defined by the conventional classification as a splitting variable of the function or bacterial diversity.

7.3.7 Multivariate soil classification using physico-chemical variables

An initial exploratory search for clusters in the data together suggested 3 to 4 clusters of soil types could be identified based on physico-chemical variables. The nature of these clusters was investigated using the cluster analysis using variables presented in Tables 7.3. Figure 7.8 visually suggests a good discrimination between Peats, Podzolic and the rest of the soils in the 95% confidence circles around the group means.

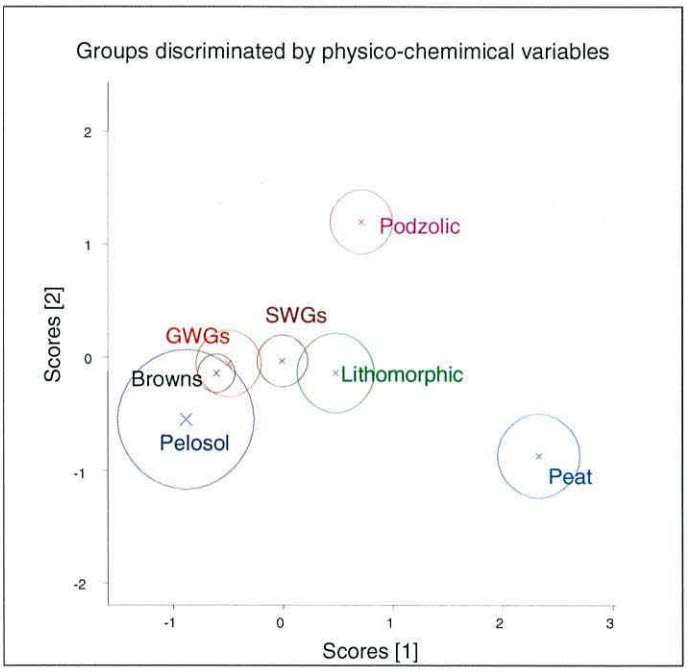

Figure 7.8. 95% confidence circles around the group means for the seven soil types defined by physico-chemical variables determined using discriminant analysis. The coloured spots represent individual soils of a soil type.

The four cluster classification based on physicochemical properties (Table 7.3) shows that cluster 1 and 3 are mainly Brown soils/ SWGs/ GWGs with a few inclusions from other soil groups. Cluster 2 is mainly a Podzolic/Lithomorphic soils combination with high inclusions from SWGs and Browns. Cluster 3 is a Browns/GWG/SWGs/Pelosols group with few inclusions from other soil groups. Cluster 4 is mainly Peat with high inclusions from Lithomorphics and GWGs.

Clusters	Soil type						
	Brown	GWGs	SWGs	Peat	Pelosol	Podzol	Lithomorphic
Cluster 1	68	14	19	0	3	3	6
Cluster 2	14	7	18	5	0	49	17
Cluster 3	83	35	43	1	11	2	5
Cluster 4	4	2	15	35	0	9	11
Combined	169	58	95	41	14	63	39

Table 7.3. Classification of soil samples based on the four clusters derived from cluster analysis of physico-chemical variables with respect to the soil types.

The three cluster classification (Table 7.4) show that cluster 1 is mainly Browns/GWGs/Pelosol and SWGs soils group with inclusions from the lithomorphic and podzolic soils. The dominant soil group in cluster 2 were podzolic soils with high inclusions from SWG, and lithomorphics, while cluster 3 contained mainly peat soil with inclusions from SWG and lithomorphics.

Clusters	Soil type						
	Brown	GWGs	SWGs	Peat	Pelosol	Podzol	Lithomorphic
Cluster 1	149	47	59	0	14	5	11
Cluster 2	16	9	21	6	0	49	17
Cluster 3	4	2	15	35	0	9	11
Combined	169	58	95	41	14	63	39

Table 7.4. Classification of soil samples based on the three clusters derived from cluster analysis of physico-chemical variables with respect to soil types.

Although the inclusion of the statistical soil classification groups contributed moderately to the creation of axis 1 of the CCA biplot (TSC4;- four soil types; Fig. 7.1), the new soil groups failed to predict the soil function or diversity using the MRT analysis. The 3 cluster classification based on the physico-chemical did not emerge as part of the best 15 explanatory variables strongly correlated to the soil function and diversity.

7.4 Discussion

7.4.1 Factors splitting soil function and diversity

Multivariate regression trees are well suited to situations where we wish to identify key factors and their levels that maximize the homogeneity in the response variables within the resulting two nodes, while maximising the deviance between the split nodes (Larsen and Speckman, 2004). The terminal nodes represent the groups/clusters of data formed by the tree, and are also called the leaves of the tree. The split is defined usually based on one explanatory variable. The MRT analysis identifies "splitting" variables based on an exhaustive and robust search of all possibilities in the response variables (Vignon and Sasal, 2010). The MRT methods do not require any assumptions about the relationships between observations and explicative variables (De'ath, 2002).

Our MRTs relate soil functional measures and bacterial biodiversity to different soil types and selected physico-chemical and environmental characteristics of UK soils. We performed the multivariate regression trees on basal soil respiration, substrate-induced respiration (substrate mineralisation) and biodiversity separately, assuming that these separate measures were independent. N mineralisation was the subject of a different study and is only used as a possible explanatory factor for the purposes of this study. This assumption was due to the fact that the treatment of the samples for each of the soil functional measures and

bacterial biodiversity were very different. The soil respiration measures were undertaken on intact "undisturbed" soil cores whose CO_2 flux included excised plant root respiration, while the substrate-induced mineralisation was mainly the result of the "potential" microbial respiration from highly disturbed soils samples and mainly depended on the quantity and quality of the added substrates in addition to the physiochemical status of the soil. We however performed the MRT on all the soil function and diversity data together (as response variables; Fig. 7.6) in order to: (1) allow a visual interpretation of the relative importance of factors that defined the splits at multi-scales, and (2) to identify the response variables that most strongly determine the splits when considered simultaneously.

The environmental factors defining splits in the substrate-induced soil functions were vegetation cover class, NO_3^- and moisture content at field capacity (MCFC). The splits in basal soil respiration on the other hand were entirely based on the amount of organic matter (LOI). The differences in the splitting variables observed between the two soil function measures is most likely due to the differences in the substrate quality in the treatments. Additions of the labile substrates or fresh litter to the soil stimulate microbial activity and increases decomposition rates. With easily decomposable substrates, where the effect of N addition is positive, the variability in N content may correlate with the rate of decomposition (Fog, 1988). Hence the observed correlation of substrate-induced respiration with vegetation cover class and NO_3^-, which may both be thought of being surrogates of nitrogen supply and availability in the soil, respectively. Furthermore, the moisture content at field capacity may be considered as an indicator of soil textural differences and moisture availability in the soils. The textural differences of organic versus mineral soils and sandy versus clayey soils can cause differences in the respiration rates. But even more differences can be seen between intact soils versus mixed soils as mixing soil can cause aeration and accessibility of substrate by microbes thereby enhancing rates of mineralisation. Thus the MCFC was depicted as an

important factor splitting the substrate-induced respirations. However, the results showing correlation of basal soil respiration with the organic matter (LOI) concur with previous studies showing that soil function is mainly dependent on C availability (Rousk et al., 2009). Thus organic matter content (LOI) was the predominant splitting variable of basal soil respiration.

Bacterial biodiversity on the other hand, was mainly correlated with soil pH, which again is a documented assumption (Rousk et al., 2009). Different combinations of the response variables including single variables were investigated and all produced plausible trees. However, the structures and the order of the variables splitting them varied with each combination. None of the combinations were able to depict the soil types as a major factor correlating with the soil function or diversity. Correspondingly, combining all the soil function and the biodiversity data as response variables in a single MRT analysis revealed that the vegetation cover type was the principle factor determining the splits in the combined response variables (Fig. 7.6). The next split was based on soil pH as the second most important factor followed by nitrate. Total organic carbon in the leachate (TOC_L) was depicted but only as a minor factor.

The combined MRT analysis reveals the relative sensitivity of response variables to several physical, chemical and environmental variables considered simultaneously. In almost all the nodes, the relative response of the bacterial diversity Shannon index, the amount of rapidly mineralized substrate allocated to Pool 1 for both substrates (a_1), and the percentage (of the total) ^{14}C remaining after 90 days incubation of labile and plant substrates (90_l and 90_p respectively) were predominant (i.e. dominant functions determining the splits; see bar charts at the end nodes (Fig. 7.6)). More specifically, the Shannon index was more predominant on more or less managed habitats on the right (i.e. Crop and weed, Fertile grasslands, Infertile grassland Lowland wooded and Tall grass and herbs) with splits based on

pH, while a_1 and 90_l and 90_p were predominant on the semi-natural habitats on the left (i.e. Heath and bog Moorland grass mosaic and Upland wooded) with nitrate and TOC as splitting factors. Basal soil respiration on soil cores and the rest of substrate-induced respiration parameters for both substrates appeared to be among the least sensitive response variables to several predictors.

A comparison of explanatory variables at work in the combined MRT and the separate MRTs of soil function and biodiversity responses (Figs 7.3, 7.4 and 7.5) reveals that the first level split in the combined MRT was influenced by substrate-induced mineralisation variables (with AVC as a splitting factor). The second level splits were by bacterial biodiversity (Shannon) response variable (with pH as a splitting factor) and substrate-induced mineralisation again (as seen from leachate NO_3^- being the splitting factor). The combined MRT in a single tree was easy to interpret compared to the multiple trees of the URTs analysis, when examining the factors affecting the co-occurrence of all the soil function and biodiversity measures together. Thus, the MRT has the potential to both generate a simple descriptive model and also to accurately predict the response variables, using a single analysis.

7.4.2 Effect of pH on soil function and diversity

The constrained CCA biplot depicts an inverse association of pH with the functional/diversity measures. The bacterial diversity Shannon index and almost all the substrate-induced mineralisation parameters were associated with low pH (directly opposite the pH arrow). However, the bacterial diversity Shannon index and the substrate-induced mineralisation parameters were all close to the origin thereby limiting their interpretability. Even so, the slow pool substrate mineralisation is thought to be mainly the decomposition of the more recalcitrant C forms (Boddy et al., 2007) carried out predominantly by fungi which

thrives better at low pH in comparison to bacteria which are more dominant at higher soil pHs (Fig. 7.2 panel g) (Bååth and Anderson, 2003; Kemmitt et al., 2006). More specifically, the X-Y plots (Fig. 7.2) illustrate the important relationships of pH with various variables. Soil pH is known to have a direct control of biomass composition of fungi and bacteria and microbial biodiversity (panel C and F) (Rousk et al., 2009). In addition, soil pH has a strong influence on carbon availability (panel D), nutrient availability (panel E) and solubility of metals (Kemmitt et al., 2006; Aciego-Pietri and Brookes, 2008; Rousk et al., 2009). Moreover, pH is known for its varied influence on multiple parameters (Rousk et al., 2009). It is therefore not unexpected that the pH had such a strong influence on bacterial diversity and ultimately the combined response variables. On the other hand, substrate-induced respiration and basal respiration are mostly substrate type, amount and nutrient dependent, and thus AVCs, LOI and nitrate were depicted as strong factors, apart from the moisture content at field capacity which also relates to the physical characteristic of the soil.

7.4.3 Do soil types defined by cluster analysis better predict soil function and biodiversity?

Despite inclusion of the statistically generated soil types (clusters) in the analysis, the new soil types did not sufficiently improve the prediction of soil function and biodiversity using the MRT analysis. The link between soil function and biodiversity with respect to soil types or other routinely measured soil attributes was unclear since both measures, except the SR per dwt and SR per dwt per m^{-2} on soil cores, were near the centre of the biplot. Separate MRTs of each functional and biodiversity measures in complementation with the ordination biplot depicted different explanatory variables as splitting factors.

A lack of predictability of soil biological quality indicators (soil function and biodiversity) by soil types may be indicative of a more fundamental difference in the nature

of the two categories of soil properties. The former being the product of the short term (<1 y) soil mineral–soil biota interaction (Sanchez, 2003) while the latter being the product of long term (usually >1000 y) soil forming processes (Jenny, 1994). Consequently, the variations in the more permanent soil properties that define the soil types may not necessarily correlate to the variations in the more transient soil properties (i.e. the soil function and diversity). Moreover, the soil type describes the whole soil profile – whilst the soil functions we have measured are for the topsoil (0-15 cm) only. Thus, broad soil types defined by traditional (soil profile) classification can encompass very wide and overlapping ranges in soil properties. Contrarily, soil function and biodiversity are sensitive to specific soil properties such as pH, nutrient availability, temperature, and moisture content (Stotzky, 1997; Florinsky et al., 2004; Kurola, 2006; Kemmitt et al., 2006; Aciego-Pietri and Brookes, 2008; Rousk et al., 2009). Cavigelli et al. (2005) also investigated soil type effects on microbial properties on the landscape level. They found that there were fewer soil type effects than the specific soil physical and chemical properties effects on the microbial properties. They further found that soil type effects on microbial properties were highly dependent on how soil type was defined. It is therefore, not surprising that MRTs failed to pick the soil types among several physico-chemical parameters as an important factor in splitting soils based on soil function and biodiversity measures. This is due to the fact that the splitting variable is selected among all possibilities, also bearing in mind that the selection and choice of soil properties interjects considerable bias into the analysis (Buol et al., 2003).

Since soil function and diversity are influenced by multiple environmental factors, we suggest that the multivariate descriptions of soil types based on specific soil physical and chemical properties are better predictors of soil function and biodiversity than are the broad soil types or the univariate analyses. Evidence supporting this position is the fact that the CCA (Fig 7.1) depicts the soil types defined by cluster analysis of selected physico-chemical

variables (TSC4) as one of the important variable defining the 1st axis alongside AVCs and LOI. Therefore, a careful selection of key physical and chemical soil properties to generate the soil types by multivariate statistical methods may effectively predict soil function and biodiversity. The general lack of correlation of soil type or properties with soil functional measurements and biodiversity, coupled with the overriding correlation of soil function to AVCs may well be an indication that there may be soil characteristics which we do not understand or measure which determines soil function and biodiversity.

7.5 Conclusions

Using a *canonical* correlation analysis (CCA) approach, it was apparent that the substrate-induced mineralisation, biodiversity and basal soil respiration (per LOI) were clustered together near the centre of the axes (limiting the importance of these variables for their interpretation) but were positively related to vegetation cover type, total C and N content but negatively related to pH. Similarly, a multivariate regression tree (MRT) approach depicted pH as the main splitting factor for biodiversity, vegetation cover type and nitrate as the main factors for substrate-induced mineralisation (apart from moisture content at field capacity) and SOM for basal soil respiration on the intact soil cores. The factors defining splits in the combined soil function and diversity were vegetation cover type > pH > nitrate, highlighting the biodiversity index, ^{14}C-substrate partitioning to microbial pool, and the percentage ^{14}C remaining after 90 days incubation as the most sensitive response variable to these predictors. The broad soil types, both conventionally classified or groups obtained via cluster analysis, were not a major factor in predicting the soil function or diversity. Furthermore, these results support previous studies showing that microbial properties are mainly dependent on pH, carbon, nutrient availability and environmental variables such as temperature and moisture rather than broad soil types. We however suggest that multivariate

descriptions of soil types based on a well informed selection of specific soil physical, chemical and biological properties may provide a better predictor of soil function and biodiversity than are the broad soil types or the univariate analyses. Moreover, the general lack of correlation of the soil properties with the function and biodiversity measurements we chose, coupled with the dominant correlation of soil function to vegetation cover type indicates that there may be other soil characteristics which are unknown to us which largely determine soil function and biodiversity.

Future work

Further investigations are also needed to explore inclution of factors such as the soil Ca:Al ratio, P saturation of exchange surfaces, specific mineralogical components (e.g. clay type) or the concentration of specific humic substances that regulate (via inhibition) enzyme activity in soil. Furthermore, chemical fingerprinting of soils might reveal unique 'function indicating' peaks (e.g. infra red analysis of soils; Igne et al., 2010). For the soil types, a finer resolution may be better than major soil types, therefore soil types defined at detailed level of soil classification (e.g. soil series level or topsoil texture classification, drainage class or other soil property measures) may improve predictions of soil function and biodiversity.

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Chapter 8

General conclusions

8.1 Introduction

This thesis describes fundamental research in the relationship between the major soil types defined by traditional soil classification and the soil quality concept. The methodologies and results from experimental work have been presented and discussed individually in Chapters 4, 5, 6 and 7. This chapter serves to summarise and integrate the individual results, leading to conclusions by answering the initial questions raised by the aims of this thesis, which were:

To determine relationships between soil quality indicators (SQIs) and key soil types to ascertain:

(1) Whether broad soil types defined by traditional soil classification systems can be used to predict SQI,

(2) Whether soil physico-chemical properties can be used to define soil types that can better predict soil function and biodiversity,

(3) The extent to which vegetation and/or soil type are the major regulators of soil quality indicators,

(4) Whether there are critical limits in SQIs with respect to the different major soil types.

8.2 Determination of relationships between SQIs and key soil types

The relationships between SQIs and the soil types and soil physico-chemical and biological properties were investigated using various statistical methods which included: ANOVA, Cluster analysis, Discriminant analysis, Principle component analysis, Canonical correspondence analysis, Factor analysis, Pearson correlation matrix, X-Y plots, Univariate and Multiple regression trees (URT and MRT). The soil quality indicators considered were: C cycling or substrate mineralisation at 0-3 months and 0-18 month years, soil respiration, microbial biomass, microbial and metabolic quotients, soil biodiversity, soil pH and EC, SOM, mineralisable-N, humic substances, soluble phenolics, TOC and TON. There were several significantly ($P<0.05$) correlated relationships between the soil types or soil attributes or vegetation classes with the soil quality indicators.

8.3 Can major soil types predict soil quality indicators?

Soil quality indicators and soil quality factors were monitored in different major soil types defined by conventional soil classification methods to ascertain whether the differences were soil type specific. The laboratory mineralisations of the labile ^{14}C-labelled artificial root exudates and the recalcitrant ^{14}C-labelled plant leaves in both short and long-term incubation showed minor differences in their variability across all soil types regardless of substrate type. Cluster analysis and principle component analysis performed on the modelled rate constants and pool sizes revealed three cluster groupings namely; (i) Brown, GWGs and Podzolic soils; (ii) Lithomorphic, Peat and SWGs soils; and (iii) Pelosol soils. Contrary to our expectations, no single or group of the model parameters describing the C cycling in the soils were able to adequately separate the soil types.

Factor analysis of 20 soil attributes were condensed into six factors also called soil quality factors (SQF)) namely; *Soil organic matter, Organic matter humification, Soluble*

nitrogen, Microbial biomass, Reduced nitrogen and *Soil humification index. Soil organic matter* was identified as the most important SQF in the discrimination of both soil types and AVCs. Among the measured soil attributes constituting the *Soil organic matter* factor, microbial quotient and bulk density were the most important attributes for discrimination between soil types. The *Soil organic matter* factor along with the dominant attributes discriminated three soil type groups namely; (i) Brown, GWG and Pelosol soils; (ii) Lithomorphics, Podzolic and SWG soils; and (iii) Peat soils. Peat soils were most distinctly discriminated from others, making a clear division of mineral soils versus organic soils.

The soil biodiversity and soil functional measures of soil respiration on intact soil cores and substrate respiration in each soil type did not show characteristic trends in each soil type either. The CCA and the MRTs with biodiversity and function as response variables and the physico-chemical variables as explanatory variables revealed that the most dominant factors splitting microbial diversity and function were AVC>pH>NO_3^-. Again, soil types were not among the important factors splitting biodiversity or function.

We conclude that major soil types defined by conventional soil classification provides a poor predictor of soil biodiversity, soil function of soil respiration, N and C mineralization at least over short time periods (i.e. < 2 y). We furthermore, long term incubations of soil at constant temperature failed to reveal major differences between soil types and that laboratory mineralization studies may provide a poor proxy for predicting soil C sequestration potential in soils. The soil quality factors (SQF) extracted from 20 routinely measured soil attributes, along with soil attributes constituting the dominant SQF were equally poorly predicted by soil types. The Vegetation classes were a better predictor of SQIs than the soil types. A lack of predictability of soil biological quality indicators (soil function and biodiversity) and the SQFs and SQIs by major soil types may be indicative of a more fundamental difference in the nature of the two categories of soil properties. The SQIs are mainly a product of the short

term (<5 years) soil mineral–soil biota interaction and mostly measured from the top soil (Sanchez, 2003) while the broad conventional classified soil types are a product of long term (usually >5,000 years) soil forming processes (Jenny, 1994) measured from subsoils. Consequently, the variations in the more permanent soil properties that define the soil types may not necessarily correlate with the variations in the more transient soil properties. Moreover, broad soil types defined by traditional classification can encompass very wide and overlapping ranges in soil properties. To the contrary, SQIs are sensitive to specific soil properties; for example soil function and diversity are sensitive to pH, nutrient availability, temperature, and moisture content.

8.4 Are soil types defined by physico-chemical properties a better predictor of SQIs?

Cluster analysis was performed on selected physico–chemical properties to obtain clusters or groups of soils. These clusters memberships were used in the CCA and the MRTs analyses to investigate whether they were important factors in splitting the soil function or diversity. The new classification did not adequately improve the prediction of the soil function or biodiversity.

However, since soil function and diversity are influenced by multiple physico-chemical and environmental factors, we suggest that the multivariate descriptions of soil types based on specific soil physical and chemical properties may be better predictors of soil function and biodiversity than are the broad soil types or the univariate analyses. Therefore we hypothesize that a careful choice of soil properties based on experience and expert opinion, using statistical methods, can produce soil clusters that can better predict the SQIs.

8.5 To what extent do soil types and/ or AVC act as major regulators of SQI?

The two-way ANOVA and the tests of between-subjects effects on the first canonical discriminant (CD) function from the canonical discriminant analysis (CDA) of the 20 physical, chemical and biological properties and the 6 factors showed significant main and interaction effect of soil type × AVC. The AVCs were a better regulator of the SQIs than soil types with partial eta squared of 0.42 versus 0.09 respectively. The partial eta squared is a measure of the effect size for each independent variable on the dependant variable. Larger values of partial eta squared indicate a greater amount of variation accounted for by the model term, to a maximum of 1. Soil type therefore did not have a great effect on the response variable compared to AVCs. Similarly, MRTs also revealed that AVCs were a superior factor in splitting the soil functions and biodiversity. The soil functions and biodiversity were split by AVCs>pH>NO_3^-/SOM in the order of their importance, the soil type was not depicted as an important splitting variable. This is in congruence with most studies which have shown that soil function is mostly dependent on nutrient availability, substrate quality and amount, which AVCs can be considered a remote proxy rather than soil types.

8.6 Definition of class limits of SQIs in soil type

Identifying critical limits in SQIs involves the determination of the desirable ranges of values for selected soil indicators that are identified and can be used to monitor functioning of soil ecosystem quality/health. Within this critical range, the soil performs its specific functions in natural ecosystems. However, defining critical limits in the SQIs with respect to soil types seeks to find the natural ranges of selected SQIs in each soil type. Ranges of values in SQIs defined by mean ± SEM were not mutually exclusive for each soil type. A few were able to differentiate groups of soils. In a few cases, either the Pelosol soils or the Peat soils

were differentiated from the rest of the soil types. The observed distinction of the two soil types from the rest may have been due to either to their generally high content of clay in Pelosol soils or high content of SOM and moisture in Peat soils, both, which elicit high C stabilisation in soil. In AVCs; Heath and Bogs, Moorland Grassland Mosaics and Upland Wooded were mostly grouped together and were differentiated from the rest of the habitats. Again these are habitats associated with the Peaty or high C stabilisation environmental conditions, more or less semi-natural habitats versus intensively managed habitats respectively. Therefore, ranges in the first order kinetic model rate constants and pool sizes were incapable of differentiating individual soil type or AVCs owing to the multiple overlaps in the ranges defined by mean ± SEM of model parameters. We ascribe this to the high degree of microbial functional redundancy in soil combined with the inability of short term biological assays or the dynamic topsoil properties to represent pedogenic subsoil processes which have taken ca. 10,000 y to become manifest. Equally, ranges in the SQ factors and indicators and their constituent soil properties (including; the microbial biomass, microbial diversity index (Shannon index), SOC, Bulk density pH) heavily overlapped among the soil types or AVCs, and no exclusive ranges could be defined. However, the soil quality indicators can be used to characterise the soil types or groups.

8.7 Overall conclusions

Broadly defined soil types using traditional soil classifications fall short of describing most of the soil quality indicators and the dynamic/functional behaviour of soils. The traditional soil classification is based on the static view of soil properties, mostly those in the subsoil. The SQI concept is based on the dynamic view of soil properties, mostly measured in the topsoil. The results of the analysis in this thesis have show that the two concepts are worlds apart. Soil types defined at a broad or major group level did not exhibit predictive relationships with

the SQIs measured in this thesis. Thus, the SQIs are unable to represent the pedogenic processes which have taken millennia to become manifest. However, the biological soil quality indicators were related to specific soil physico-chemical properties. For example the soil function and biodiversity were significantly ($P < 0.01$) correlated to the pH or C content. Furthermore, long term incubations of soil at constant temperature failed to reveal major differences between soil types and that laboratory mineralization studies may provide a poor proxy for predicting soil C sequestration potential.

Therefore, soil types defined by a carefully selected univariate or multivariate physico-chemical soil properties that underpin SQIs and processes may be a better approach to relate the two concepts.Overall, the AVCs were a better regulator of SQIs than the soil types and provided a significant interaction effect on the SQIs in soil types. Class limits for SQIs in both the soil types or the AVCs, however, could not be defined because their ranges (defined by mean ± SEM) greatly overlapped.

8.8 Future work

Future work considerations have been stated at the end of some of the experimental chapters and here we provide the summary:

1. Further investigation may need to consider finer resolution of soil types i.e. detailed soil classification level such as soil series level or the top soil classification, texture or drainage class soil types to investigate their predictive power over the soil quality indicators of soil C storage and cycling, soil biodiversity and other soil functions.
2. Other special considerations can include climatic, spatial and parent material variability in the sampling designs, including ensuring equal and/or adequate representation of soil types in the aggregate vegetation classes in order to accurately capture the interaction effect.
3. Management factors can also be included (e.g. lime/fertilizer regimes).
4. The statistical soil classification soil types (univariate or multivariate) should include physico-chemical attributes that underpin the soil processes including Ca/Al ratio and pH which are thought to be a major factor controlling soil function and biodiversity.
5. Key soil quality indicators can also include measures of key soil enzymes (e.g. cellulase, protease, phosphatase, sulfatase), their potential to release N_2O and CH_4.

References

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Appendices

9.1 Appendix 1

Land class classification

LAND CLASS: ONE

Geography:	S. Wales, S.W. England, S. England.
Land form:	Alluvial plains, low ridges, or plateaus with little surface drainage.
Topography:	Gently rolling country or almost flat country mainly at medium to low altitude.
Landscape:	Varied lowland landscapes with hedges, trees and farm buildings.
Land use:	Cereals, good grasslands and limited native vegetation.
Soils:	Mainly brown earths but also gleys.
Vegetation:	Limited but grassland where present.

LAND CLASS: TWO

Geography:	S. England, S.W. Midlands.
Land form:	Downland summits and scarps, low ridges or occasionally alluvial plains.
Topography:	Sweeping curves or smooth slopes with land at medium low or low altitudes.
Landscape:	Mainly open or wooded downland with few hedges and scattered farmhouses,
Land use:	Mainly good grassland but extensive cereals and built upland.
Soils:	Brown earths or calcareous brown earths.
Vegetation:	Rough grassland or bracken where present.

LAND CLASS: THREE

Geography:	E. Anglia, S.E. England.
Land form:	Alluvial plains or shallow river valleys with low broad ridges.
Topography:	Flat or almost flat with virtually all land at low altitude.
Landscape:	Prairie type lowlands with intensive agriculture and declining hedges,
Land use:	Cereals, other crops and short term grassland.
Soils:	Gleys, calcareous brown earths and brown earths.
Vegetation:	Virtually absent.

LAND CLASS: FOUR

Geography:	E. Anglia margins, S. England, S. Midlands.
Land form:	Fenland or flood plains with intricate drainage patterns.
Topography:	Flat or virtually flat, almost entirely at low altitude.

Landscape: Intensively farmed lowlands often under urban pressure.

Land use: Arable, with cereals and other crops, good grassland and urban.

Soils: Gleys with some calcareous brown earths.

Vegetation: Virtually absent.

LAND CLASS: FIVE

Geography: S. England, S.W. England, S.W. Midlands, S. Wales.

Land form: Variable from scarpland to downland and valley floors.

Topography: Uniform gentle slopes or smooth outlines mostly at low altitude.

Landscape: Varied lowlands with many natural features.

Land use: Mixed farmland although predominantly good grass; much urban,

Soils: Gleys and brown earths predominate.

Vegetation: Limited but varied where present from bracken to rushes.

LAND CLASS: SIX

Geography: S.W. England, S. Wales and S.W Midlands.

Land form: Dissected tablelands and plateaus with many small rivers.

Topography: Complex with many broad even slopes and the majority of land at medium/low altitude

Landscape: Intricate with small fields enclosed by hedges on banks with small woodlands.

Land use: Mainly good grassland but with some barley.

Soils: Brown earths and gleys predominate.

Vegetation: Limited to small areas.

LAND CLASS: SEVEN

Geography: S. England, S.W. England and Wales coasts.

Land form: Variable coastal morphology, mainly cliffs cut into tablelands.

Topography: Usually coastal cliffs, rarely estuarine, most land low altitude.

Landscape: Varied coasts backed by lowland farmland with farm houses.

Land use: Mainly pasture with some arable and good grass.

Soils: Brown earths but also other types.

Vegetation: Limited, but varied particularly moorland and grassland types.

LAND CLASS: EIGHT

Geography: E. Anglia, S. England, Wales, N.W. England coasts.

Land form: Marine alluvial plains bordering estuaries or rarely rocky coasts.

Topography: Mainly flat hut with some steeper coasts, most land low altitude.

Landscape: Usually flat coasts backed by good farmland effected by urban development.

Land use: Mainly pasture but some arable, extensive mudflats and urban development.

Soils: Gleys and brown earths.

Vegetation: Limited, but rough grassland where present.

LAND CLASS: NINE

Geography: N. Midlands, N.E. England, and S.E. Scotland.

Land form: Mainly valley floors and flood plains of large rivers together with bluffs.

Topography: Almost flat or gently rolling, most land medium/low altitude,

Landscape: Open lowland country often with declining hedges, intensive agriculture.

Land use: Mixture of good grass and arable with many urban areas.

Soils: Brown earths, gleyed brown earths and gleys.

Vegetation: Very limited, bracken or rough grassland where present.

LAND CLASS: TEN

Geography: N. Midlands, N.E. England, and S.E. Scotland.

Land form: Mainly valley floors or alluvial plains often with moderate scarps on margins.

Topography: Gentle slopes, often long with the majority of land medium/low but also low altitude.

Landscape: Well-farmed lowland country with many hedgerows and small woods.

Land use: Mainly arable but with good grassland and pasture also widespread.

Soils: Gleys with some brown earths.

Vegetation: Very restricted.

LAND CLASS: ELEVEN

Geography: E. and C. Midlands.

Land form: Alluvial plains or low broad ridges drained by small streams.

Topography: Very gradual slopes or flat with almost all land at low altitude.

Landscape: Open landscapes with large fields and declining hedgerows.

Land use: Arable predominates particularly wheat with good grassland and urban.

Soils: Gleys and brown earths.

Vegetation: Very restricted,

LAND CLASS: TWELVE

Geography: E. Midlands and Fens.

Land form: Mainly fens or flood plains and large rivers otherwise graded ridges.

Topography: Flat or almost flat entirely at low altitude.

Landscape: Prairie landscapes with derelict hedges and urban development.

Land use: Arable, mainly wheat with limited good grassland and urban.

Soils: Gleys and brown earths.

Vegetation: Virtually absent.

LAND CLASS: THIRTEEN

Geography:	N. Wales, N.W. England, S.W. Scotland.
Land form:	Heterogeneous, from low ridges in alluvial plains to scarps and river valleys.
Topography:	Smooth slopes, rarely steeper almost entirely at low altitudes.
Landscape:	Varied lowland landscapes with hedged small fields often affected by urban.
Land use:	Usually mixtures of arable and good grassland but also variety of other uses.
Soils:	Gleys and brown earths predominate but other types often present.
Vegetation:	Bracken and rough grassland, but also some moorland.

LAND CLASS: FOURTEEN

Geography:	N.W. and N.E. England, S.W Scotland.
Land form:	Mainly marine or alluvial flood plains bordering estuaries, rarely rocky coasts.
Topography:	Fiat or gently sloping with the majority of land at low altitude.
Landscape:	Prairie landscapes with fences or neglected hedges much affected by urban development.
Land use:	Mainly arable but also good grassland and much urban.
Soils:	Gleys, gleyed brown earths and brown earths.
Vegetation:	Very little present.

LAND CLASS: FIFTEEN

Geography:	Wales, N. England.
Land form:	Variable from dissected plateaus to valley floors bordered by escarpments,
Topography:	Complex with shallow or occasionally steep slopes, flat land almost entirely medium/low altitude.
Landscape:	Intricate lowland landscapes with many natural features.
Land use:	Mainly pasture mixed with good land and arable.
Soils:	Brown earths, gleys and some brown podzolics.
Vegetation:	Restricted but mainly rough grassland and some bracken.

LAND CLASS: SIXTEEN

Geography:	N. England, S.W, Scotland.
Land form:	Flood plains or valley floors with escarpments or gently folded.
Topography:	Mainly undulating land with some flat areas mainly at low altitudes.
Landscape:	Varied lowland, well-farmed landscapes with many hedges.
Land use:	Varied with mixtures of arable pasture and good grassland.
Soils:	Brown earths and gleys.
Vegetation:	Varied but with grassland types predominating and some moorland.

LAND CLASS: SEVENTEEN

Geography: S.W. England, Wales, N. England.

Land form: Plateaus or tablelands, with scarps often dissected by small rivers.

Topography: Some gentle slopes, but mainly quite steep hillsides at medium/high altitude.

Landscape: Open or enclosed marginal uplands with walls, fences and occasional farmhouses,

Land use: Mainly pastures with some good grassland.

Soils: Brown earths and brown podsolics but a range of other soils.

Vegetation: Mainly rough grassland types but also some moorland.

LAND CLASS: EIGHTEEN

Geography: Wales, N. England, W. Scotland.

Land form: Glaciated river valleys with steep scarps backing onto tablelands or distinct mountains.

Topography: Steep hillsides predominate with some more moderate slopes mainly at medium high altitudes.

Landscape: Mainly open, rugged uplands but with some areas transitional to enclosed land.

Land use: Predominantly rough grazing with some limited pasture land.

Soils: Brown podsolics, brown rankers, peats and other upland types.

Vegetation: Mainly moorland with extensive peatland and montane grassland,

LAND CLASS: NINETEEN

Geography: N. England, S. Scotland.

Landform: Broad ridges or flat topped or rounded summits with small rivers with flat floor.

Topography: Mainly moderately steep slopes but also some rather steep hillsides at medium high altitudes.

Landscape: A mixture of enclosed upland but also open mountains often afforested.

Land use: Mainly rough grazing or forest but some pasture.

Soils: Varied upland type but brown earths, podsols and peats the most abundant.

Vegetation: Mainly moorland but also mountain grass and peat types,

LAND CLASS: TWENTY

Geography: N. England, S. Scotland.

Land form: River valleys often with subsidiaries and scarps backing onto rounded hills.

Topography: Often complex including steep hillsides and more moderate gradients at medium/high altitudes.

Landscape: Mixtures of upland and marginal lowland with fences and walls.

Land use: Much pasture but some good grassland and occasional crops.

Soils: Gleys and brown earths with some other upland types.

Vegetation: Mainly rough grassland types but some peatland also.

LAND CLASS: TWENTY-ONE

Geography: C. and N. Scotland.

Land form: Peneplain surfaces with complex drainage or broad ridge with indistinct summits.

Topography: Predominantly quite steep hillsides but also some more moderate slopes

Landscape: Bleak upland landscapes, sometimes enclosed by walls or fences and afforested.

Land use: Open range grazing or forest.

Soils: Peats, peaty gleys or podsols.

Vegetation: Moorland or peatland types with some rough grassland.

LAND CLASS: TWENTY-TWO

Geography: N. England, S., C. and N. Scotland.

Land form: Dip slopes of plateaus or broad glacial valleys leading to rounded summits.

Topography: Slopes of variable gradient from steep to moderate and almost entirely at medium/ high altitudes.

Landscape: Mainly high moors but sometimes enclosed or afforested.

Land use: Mainly rough grazing but also woodland and occasional crops.

Soils: Peaty gleys,peaty podsols and peats but also other upland soils.

Vegetation:

LAND CLASS: TWENTY-THREE

Geography: N. England, C. and N. Scotland.

Land form: Ridges, scarps and corries leading to mountain Summits or rarely glaciated valleys

Topography: Extremely steep hillsides, sometimes less so, with the land at high altitudes.

Landscape: Open mountainous landscapes with wide vistas.

Land use: Limited open range grazing.

Soils: Peats, peaty podsols, podsols and brown rankers.

Vegetation: Mainly moorland types but also mountain grassland and peatland types.

LAND CLASS: TWENTY-FOUR

Geography: C. and W. Scotland.

Land form: Glaciated valley sides often reaching from base to rocky summits sometimes peaks emergent from peneplains.

Topography Precipitous and extremely steep slopes with land at high altitude.

Landscape: Rugged mountain scenery often rocky with fast flowing streams.

Land use: Limited open range grazing.

Soils: Brown rankers peats or peaty podsols, some peaty gleys.

Vegetation: Mainly peatland types but also mountain grassland and moorland.

LAND CLASS: TWENTY-FIVE

Geography: N.E. England, S. E., C. and N.E. Scotland.

Land form: Alluvial flood plains and morraines of glacial origin.

Topography: Virtually flat or gently rolling land mainly at low altitudes.

Landscape: Intensively farmed lowlands with fences and scattered farmhouses.

Land use: Mainly barley but with much good grassland.

Soils: Brown earths, gleys and gleyed brown earths.

Vegetation: Restricted to a few grassland types.

LAND CLASS: TWENTY-SIX

Geography: N.E. England, C. and E. Scotland.

Land form: Valley floors and coastal plains of glacial origin, sometimes with' emergent outcrops.

Topography: Undulating or smooth slopes mainly at low altitudes.

Landscape: Rather mixed lowland landscapes often affected by urban development.

Land use: Mainly good grassland but also much barley and pasture.

Soils: Brown earths and gleys.

Vegetation: Limited but mainly moorland types where present.

LAND CLASS: TWENTY-SEVEN

Geography: N. England, C., E. and N.E. Scotland.

Land form: Varied but mainly valley floors and bluffs occasionally with ridges and scarps.

Topography: Variable from mixtures of gentle and steep slopes to uniform moderate gradients mainly at medium low or low altitudes.

Landscape: Mainly well fenced lowlands, often mixed with woodland.

Land use: Arable, particularly barley but also much pasture and good grassland.

Soils: Brown earths and gleys.

Vegetation: Restricted but some grassland and moorland types.

LAND CLASS: TWENTY-EIGHT

Geography: N. England, S. and N.E. Scotland.

Land form: Heterogeneous from meandering riversides to peneplains or alluvial plains.

Topography: Mainly virtually flat but some gentle gradients at medium/low altitudes.

Landscape: Heterogeneous from enclosed farmed landscapes to open moorland.

Land use: Pasture or rough grazing predominate but some good grasslands also.

Soils: Variable but mainly gleys brown earths or peats.

Vegetation: Mainly peatland types where present but also grassland and moorland.

LAND CLASS: TWENTY-NINE

Geography: W. Scotland.

Land form: Indented coastlines with more cut platforms and raised beaches.

Topography: Uneven topography, usually with easy slopes but some steeper areas at low or medium/low altitudes.

Landscape: Complex scenery containing many contrasting elements.

Land use: Mainly open range grazing but also some crofting.

Soils: Mainly peats but also rankers and brown earths.

Vegetation: Mainly peatland and moorland types but also some bracken.

LAND CLASS: THIRTY

Geography: Extreme W. Scotland.

Land form: Mainly peneplains with meandering streams sometimes with low hills,

Topography: Variable from complex to almost flat at medium low extending to medium high altitudes.

Landscape: Open moorlands near to the sea with rocky outcrops and lochs.

Land use: Open range grazing and crofting.

Soils: Mainly peats with some peaty podsols.

Vegetation: Mainly peatland with some moorland types.

LAND CLASS: THIRTY-ONE

Geography: N. Scotland and Isles.

Landform: indented with some coastal plains backed by low hills.

Topography: Mainly broad gentle curved outlines and some steeper areas mainly at low/medium altitudes.

Landscape: Windswept, exposed coasts with the enclosed land divided into small fields.

Land use: Mainly rough grazing but some good grassland and pasture with crofting.

Soils: Brown earths peats and some podsols.

Vegetation: Mainly moorland but also some peatland and grassland types.

LAND CLASS: THIRTY-TWO

Geography: N.W. Scotland and Isles.

Land form: Peneplain surfaces or low ridges, sometimes coastal.

Topography: Variable from complex to even rounded slopes mainly at medium/low altitudes.

Landscape: Bleak moorlands often with scattered lochs and eroding peat hags.

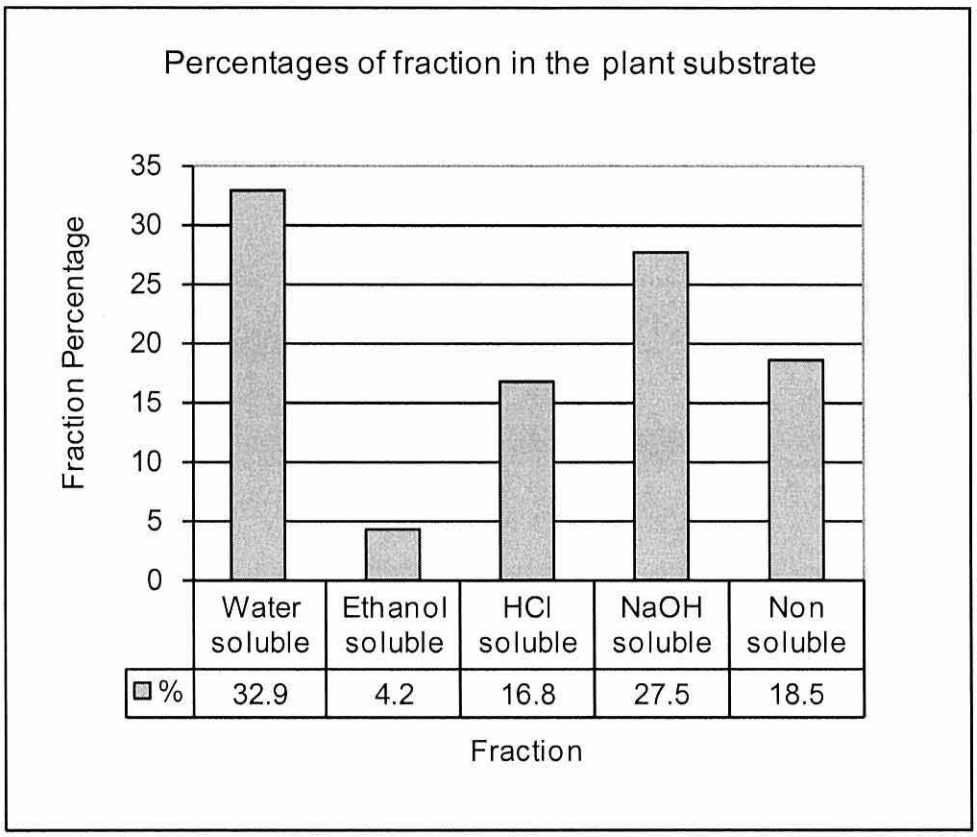
Land use: Mainly open range grazing but some pasture.

Soils: Mainly peats but some rankers.

Vegetation: Predominantly peatland types but also some moorland.

9.2 Appendix **2**

The composition of the plant substrate added to the soil.



9.3 Appendix 3

The cross tabulation table of AVCs versus soil types. The * shows AVCs that were under-sampled with respect to the soil type

	Aggregate Veg class * Soil description Cross tabulation								
		Soil description							Total
		Browns	GWGs	Lithom	Peat	Pelosol	Podzol	SWGs	
Crop and weeds	Count	34	8	5	0*	0*	2*	5*	54
	Expected Count	17	7	5	8	1	6	10	54
Fertile grasslands	Count	17	15	2*	2*	2*	1*	14	53
	Expected Count	17	7	5	8	1	6	10	53
Heath and bog	Count	2*	0*	7	35	0*	11	11*	66
	Expected Count	21	8	6	10	1	7	13	66
Infertile grassland	Count	24	10	7	1*	2*	6*	12	62
	Expected Count	20	8	5	9	1	7	12	62
Lowland wooded	Count	4	0*	0*	0*	0	0*	2	6
	Expected Count	2	1	1	1	0	1	1	6
Moorland grass mosaics	Count	5*	0*	3	8	0*	9	6	31
	Expected Count	10	4	3	5	1	3	6	31
Tall grass and herbs	Count	6	3	0*	0*	0	0*	2	11
	Expected Count	3	1	1	2	0	1	2	11
Upland wooded	Count	4*	2*	2	0*	2	5	6	21
	Expected Count	7	3	2	3	0	2	4	21
Total	Count	96	38	26	46	6	34	58	304

The end