Programme Area: Bioenergy

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Title: Chronosequence Methodology Approaches in Literature, Including Specific Recommendations for Sampling for ELUM

Abstract:

This deliverable presents a critical review of paired site and chronosequence approaches in the literature and provides specific sampling recommendations and a UK sampling roadmap for the Ecosystem Land-Use Modelling project (ELUM). It looks beyond bioenergy plantations to uncover the best practices to determine the effects of Land Use Change (LUC) on soil carbon stocks. Work Package 2 (WP2) will adopt these approaches in the context of the most relevant bioenergy transitions across the UK. The findings from this review will inform the development of a statistically robust sampling framework to meet the assumptions of paired site and chronosequence approaches.

Context:

The ELUM project has studied the impact of bioenergy crop land-use changes on soil carbon stocks and greenhouse gas emissions. It developed a model to quantitatively assess changes in levels of soil carbon, combined with the greenhouse gas flux which results from the conversion of land to bioenergy in the UK. The categorisation and mapping of these data using geographical information systems allows recommendations to be made on the most sustainable land use transition from a soil carbon and GHG perspective.

Some information and/or data points will have been superseded by later peer review, please refer to updated papers published via www.elum.ac.uk

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Chronosequence methodology approaches in literature, including specific recommendations for sampling for ELUM

REPORT

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<u>Aidan M. Keith</u>[1], Emily Bottoms[1], Pete Henrys[1], Jonathan Oxley[1,4], Kim Parmar[1,2,] Mike Perks[3], Rebecca Rowe[1,2], Saran Sohi[2], Elena Vanguelova[3] and Niall P. McNamara[1]

[1]Centre for Ecology & Hydrology, Lancaster, LA1 4AP.

[2]School of GeoSciences, University of Edinburgh, EH9 3JN.

[3]Forest Research, Northern Research Station, Roslin, EH25 9SY.

[4] Energy Technologies Institute, Loughborough, LE11 3UZ.

EXECUTIVE SUMMARY

This report presents a critical review of paired site and chronosequence approaches in the literature and provides specific sampling recommendations and a UK sampling roadmap for the Ecosystem Land-Use Modelling project (ELUM). It looks beyond bioenergy plantations to uncover the best practices to determine the effects of Land Use Change (LUC) on soil carbon stocks.

Work Package 2 (WP2) will adopt these approaches in the context of the most relevant bioenergy transitions across the UK. Consequently, there is a need to consider the criteria for achieving valid and robust comparisons of LUC, changes in the spatial variability of soil carbon and other soil properties during LUC, and how these relate to UK-relevant bioenergy transitions. The findings from this review will inform the development of a statistically robust sampling framework to meet the assumptions of paired site and chronosequence approaches.

Paired site and chronosequence soil sampling will now commence on the range of identified fieldsites across the UK in line with our sampling roadmap and developed methodologies.

The review begins with a brief background to the project, including an overview of the importance of LUC to soil carbon and the sustainability of bioenergy transitions, and the specific objectives of Work Package 2 (Section 1).

The assumptions behind paired site and chronosequence approaches and the need to represent key soil types and climatic zones are then discussed in the context of achieving valid and robust LUC comparisons (Section 2). Paired site and chronosequence approaches have been used extensively by others to examine changes in soil carbon, particularly when considering longer-term changes.

An overview of technical issues associated with changes in the spatial variability of soil properties across LUC transitions is presented (Section 3). Firstly, it deals with issues associated with horizontal variability and discusses sampling strategies that may be used to account for such differences between bioenergy land uses. Secondly, it examines issues associated with changes in vertical strata and sampling depth, and in particular, appraises several methods available to account for bulk density changes and their effect on soil carbon measurements. A summary of the sampling approaches used by others is provided in two graphs.

Section 4 considers the potential bioenergy LUC transitions that may occur in the UK and outlines soil changes likely to be important in those transitions most relevant in the UK.

A summary of the key findings from the review and associated specific recommendations for WP2 sampling is provided in Sections 5 and 6, respectively. The most important of these is the need to adopt a paired site and chronosequence approach to examine transitions to bioenergy crops. Furthermore, these approaches need to adopt a hierarchical spatial soil sampling strategy, must include samples up to 1 m depth, and must quantify soil carbon stocks on a cumulative and/or equivalent soil mass per unit area basis. These key findings and recommendations are presented in the context of a pilot study which was conducted during Q3 2011 to test different spatial sampling strategies (Appendix I).

Finally, in Appendix II we provide an overview of our year 1 sampling roadmap for Work Package 2.

CONTENTS

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1. INTRODUCTION

Soils contain as much as three times the quantity of carbon (C) as the atmosphere at a global scale (Batjes, 1996; Lal, 2004). This includes around 1500 Gigatons of C to a depth of 1m (Smith, 2004); the upper 1m of soil in the UK is estimated to contain around 4.6 Gt (Bradley *et al.*, 2005) equivalent to nearly 8 times the UK total greenhouse gases (GHG) emissions in 2010 (DECC, 2011). Soils have the potential to act both as sources or sinks for C and GHGs and therefore can play a pivotal role in the mitigation of climate change. Land use and land cover are dominant factors which influence soil C and GHG dynamics over the long term. Conversion of natural habitats to agricultural land use may severely deplete soil C within only a few years (Davidson & Ackerman, 1993; Murty *et al.*, 2002; Don *et al.*, 2011) while it can take decades to recover former levels of soil C following reversion of agricultural to extensive land use (Paul *et al.*, 2002; Laganière *et al.*, 2010; Poeplau *et al.*, 2011). Land Use Change (LUC) is second only to fossil fuel combustion as a source of GHGs (IPCC, 2007). Consequently, LUC and land management will be critically important in determining the balance of C between soil and the atmosphere. Understanding these effects is vital to be able to model and predict the outcomes of future scenarios of LUC and climate change on soil C stocks and GHG emissions.

Increasing demand for bioenergy crops is expected in order to meet commitments to reduce carbon emissions and improve energy security, and this is likely to have a substantial impact on LUC in Europe (Smith *et al.*, 2000; Rowe *et al.*, 2009; Hastings *et al.*, 2009; Don *et al.*, in press). Modelling work does suggest that bioenergy has the potential to mitigate GHG emissions in Europe by providing a substantial fraction of Europe's energy demand (Hastings *et al.*, 2009; Rowe *et al.*, 2009). For example, Hastings *et al.* (2009) estimated that within predicted future yield and climatic constraints the perennial grass *Miscanthus* could provide up to 12% of Europe's energy needs by 2050; though this would come at the expense of 35% arable land. Smith *et al.* (2000) examined European policy options and the potential carbon mitigation of different agricultural land-management strategies and found that, while surplus arable land was the most important resource, bioenergy crops showed greatest potential for C mitigation. Woodland regeneration and bioenergy plantation exhibited greatest maximum yearly C mitigation potential (Smith *et al.*, 2000). Biomass energy sources are seen as a key resource if targets for renewable energy production in the UK are to be met (Grogan & Matthews, 2001; Ostle *et al.*, 2009; Rowe *et al.*, 2009; Aylott *et al.*, 2010).

Bioenergy land use has the potential to offset use of fossil fuels, increase soil organic carbon (SOC) stocks, reduce GHG emissions and bring wider environmental benefits, but there still remain concerns about its sustainability (Cowie *et al.*, 2006; Field *et al.*, 2008; Rowe *et al.*, 2009). While it may have a negative indirect effect on food production and food security via competition for land it is also evident that planting bioenergy crops may in some contexts result in a decline in soil C stocks (Cowie *et al.*, 2006). There is a risk therefore that LUC to bioenergy crops could result in a depletion of soil C stocks compared with conventional agricultural and forestry (Cowie *et al.*, 2006). As bioenergy crops are not necessarily C neutral, with pre-harvest emissions potentially offsetting any potential C savings via reduced fossil fuel use, any loss of soil C could increase the risk of bioenergy crops becoming carbon positive ((Don *et al.*, in press). Past land use will undoubtedly have an important influence on the C balance following plantation of bioenergy crops (St Clair *et al.*, 2009; Laganière *et al.*, 2010). Clearly, there is a need to better quantify effects of LUC associated with dedicated energy crops on soil C and the GHG balance (Don *et al.*, in press). However, there are few, if any, long-term data sets on soil C changes after LUC to bioenergy cropping.

To fill this gap the ELUM project aims to produce a dataset of soil C for major UK bioenergy land use transitions using paired and chronosequence soil sampling approaches. This review provides a critical assessment of soil sampling methods for examining soil C change using these approaches. It examines the existing methods used in published studies, addresses a range of technical issues and looks beyond bioenergy plantations to uncover the best practices to determine the effects of LUC on soil C stocks. In conjunction with our review we have carried out additional pilot field trials (Appendix I) to facilitate the production of a statistically robust sampling framework for use on the ELUM project. The acceptance criteria for this review (Deliverable D2.1) are:

"A written review of existing chronosequence and paired site sampling methods for comparing LUC effects applicable to soil carbon LUC studies. The report will review methods employed to achieve valid LUC comparisons; cover the range of methods available to correct for bulk density variation effects on soil carbon values; and identify existing sampling strategies for encapsulating soil variability. Specific recommendations for the ELUM project will be made. An appendix will detail the Year 1 chronosequence sampling strategy."

2. ASSESSING EFFECTS OF LAND USE CHANGE (LUC)

2.1 Literature search

The main objective of this literature search was to identify the most appropriate methods for measuring soil C changes after LUC rather than reviewing and analysing the magnitude of change in the C stock itself after LUC. However, to provide some context we highlight some of the scientific outcomes from a number of key reviews in Section 2.2.

Our literature search was primarily made using the Web of Knowledge database (http://wok.mimas.ac.uk) and aimed to find relevant publications that have examined the effects of LUC on *soil C* using paired site and chronosequence approaches. Web of Knowledge encapsulates a range of databases which index published papers, conference and workshop proceedings. This search was made by publication title and used the terms 'soil carbon' and 'sampling' or 'methods' or 'bulk density' or 'change' or 'land use change' or 'paired-site' or 'chronosequence' or 'biofuel' or 'bioenergy' or 'miscanthus' or 'SRC' or 'willow' or 'rotation forestry' or 'coppice'. The bioenergy search terms were included to ensure that we captured all these highly relevant studies; however, the overall search was not constrained to bioenergy. The reference list of recent key papers (Anderson-Texeira *et al.*, 2009; Laganière *et al.*, 2010; Don *et al.*, 2011; Poeplau *et al.*, 2011) identified in this initial search (e.g. reviews and meta-analyses) were also scrutinised to retrieve further relevant publications. As an additional check we queried the publications which had cited these key papers (i.e. Guo & Gifford, 2002). Overall our approach allowed us to rapidly identify the key literature that would direct our soil sampling strategy.

2.2 Key reviews of LUC on soil C stocks

A number of meta-analyses on LUC and soil C have been published in the last decade and the work by Guo & Gifford (2002) may be considered a seminal paper, given the range of transitions it examines. The others since consisted of more specific reviews and meta-analyses on particular land use transitions, for example, from forest to agricultural land use (Murty *et al.*, 2002), afforestation of agricultural land (Paul *et al.*, 2002; Laganière *et al.*, 2010), and biofuels (Anderson-Texeira *et al.*, 2009). More recently there have also been meta-analyses examining the effects of land use change on soil C within particular climatic zones e.g. tropical (Don *et al.*, 2011) and temperate (Poeplau *et al.*, 2011). The paired site approach tends to dominate the studies used in these meta-analyses.

The meta-analysis by Guo and Gifford (2002) looked at the effects of LUC on soil C using 74 primary studies from across 16 countries. Increases in soil C were shown for LUC from forest to pasture (+8%), crop to pasture (+19%), crop to plantation forest (+18%) and crop to secondary forest (+53%), while decreases were shown for pasture to crop (-59%), forest to crop (-42%), forest to plantation (-13%), and pasture to plantation (-10%) (Guo & Gifford, 2002). This again highlights the importance of previous land use to soil C changes and also that the greatest potential for soil C gains is in the conversion from crop to other land uses (Laganière *et al.*, 2010). Differences depending on specific factors (e.g., tree type) have also been shown within these relatively broad transitions (Guo & Gifford, 2002). For example, while broadleaf plantation had little influence on soil C change, the plantation of conifers, in particular pine, reduced soil C by 12% (Guo & Gifford, 2002). A recent meta-analysis by Laganière *et al.* (2010) supported these earlier findings with broadleaved trees having the greatest effect on soil C following afforestation of agricultural land compared to Eucalyptus and pine. The fact that plantation forestry may have a limited or even negative impact on soil C change, and the differences between tree type and tree species, will have implications highly relevant to bioenergy transitions.

These previous reviews highlighted broad differences in C change between different LUC transitions but they lack detailed examination of temporal dynamics. An earlier review on C change examining the LUC from agricultural use to both permanent pasture and secondary forest estimated mean rates of C accumulation per year in a range of transitions (Post & Kwon, 2000). Poeplau *et al.* (2011) has taken the temporal aspect a step further by using data from the pool of relevant studies to model C response functions across transitions. Furthermore, different regression models have the best fit against the different LUC transitions (Poeplau *et al.*, 2011). If sites covering a broad range of ages are sampled then it would be possible to calculate such C response functions for bioenergy transitions in the UK.

2.3 Achieving valid and robust LUC comparisons

2.3.1 Assumptions of the paired site/chronosequence approaches.

There are generally three types of approach to examine effects of LUC, 1) a retrospective design, 2) paired sites and 3) a chronosequence (Paul *et al.*, 2002; Laganière *et al.*, 2010). A retrospective design makes repeated measurements in the same plots over time and this offers the most reliable design as it removes variability associated with comparing sites (Laganière *et al.*, 2010). Changes in soil C may be best obtained by these repeated measurements but the rate of change is slow and a considerable number of years may elapse before significant changes can be detected. Therefore, retrospective sampling over long time scales is at odds with the nature of scientific funding streams (3-5 year programs) and does not always provide data at the time it is required. These general issues are reflected in the lesser proportion of LUC studies utilising the retrospective or repeated measures approach (Figure 1). To circumvent these issues it is necessary to use a space-for-time substitution and this includes both paired site and chronosequence designs.

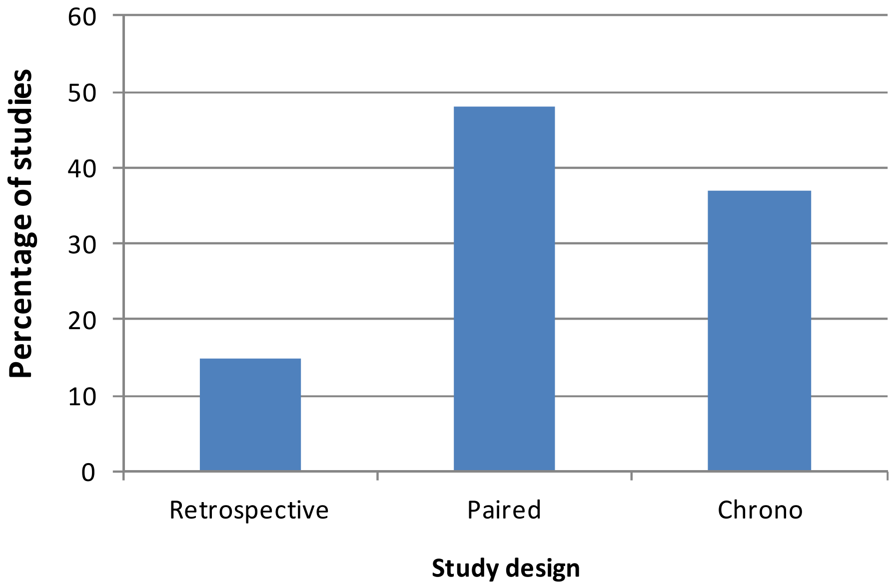

Figure 1. Relative proportions of different approaches used in studies examining land use change effects on soil C. Data extracted from selected published papers identified in literature search (n=54).

A paired site approach compares the new planted system which has undergone transition to an adjacent piece of land. The adjacent pair is the natural or original vegetation which is the baseline representing the steady state (Cambardella & Elliot, 1992; 1993; 1994). Making the comparison reliable is very important since errors in the results will occur if the initial C stock prior to land use change was not similar for both pairs (which is usually not known). Thus care is required in selecting sites to represent the pre-LUC situation to minimise the impact of this variation, and in turn, it is important that examined sites have a clear management or experimental history.

A chronosequence approach is essentially a group of sites with an extending range of ages. The chronosequence approach has been used to investigate soil change over timescales from centuries to as long as millennia (e.g. Thompson, 1981; Davis & Condron, 2002; Wardle *et al.*, 2004). Examining the effects of bioenergy LUC transitions on soil C generally concerns timescales from a few to over a hundred years (Martens *et al.*, 2004).

Paired site and chronosequence approaches therefore seem an appropriate and necessary tool to address questions on soil C changes across the lifespan of bioenergy transitions. The proportion of LUC studies utilising the paired site and chronosequence approaches is relative high (Figure 1). Nevertheless, a number of assumptions must be satisfied for these approaches to be validly applied. The overall assumption is that each site only differs in its age (or for example, time since disturbance) and has the same biotic and abiotic history (Laganière *et al.*, 2010). For example, a chronosequence or artificial time series may be established using fields of similar soil type in similar climates that have been under the management practice of interest for differing periods of time. Walker *et al.* (2010) indicates that the most suitable chronosequences have a predictable and convergent trajectory like organic matter accumulation. Replication within stages and checking that multiple stand characteristics vary across stages provide further verification of the suitability of a chronosequence (Walker *et al.*, 2010). The paired site and chronosequence approaches have been called into question as a valid approach to examine change over time since studies often fail to validate the basic assumptions (Johnson & Miyanishi, 2008). However if used appropriately, paired site and chronosequence approaches are important tools which can generate invaluable insights into temporal changes in soil that could not be achieved otherwise (Walker *et al.*, 2010)

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2.2.2. Representation of key climatic zones and soil types

It is clear that the effects of bioenergy transitions on soil C and GHGs may be dependent on the environmental context (Hastings *et al.*, 2009; Hillier *et al.*, 2009). In their meta-analysis, Laganière *et al.* (2010) showed that climatic zone had a significant effect on change in SOC following afforestation of agricultural land. The greatest increase occurred in temperate maritime climates, followed by temperate continental, sub-tropical and tropical, and no change in Boreal climates (Laganière *et al.*, 2010). Although at this level, the whole of the UK would generally be considered to have a temperate maritime climate, there are still climatic differences that would impact yields and potential soil changes (Aylott *et al.*, 2010). Indeed, Berthrong *et al.* (in press) demonstrated that change in SOC depended on annual precipitation, with wetter sites losing and drier sites gaining SOC following afforestation.

Similarly, soil types may modify the impact of bioenergy transitions on soil C dynamics. Laganière *et al.* (2010) showed differences in SOC change under different soil conditions with SOC having a greater increase after afforestation of agricultural land in soils with a high clay content (>33%) and with a high pH. Soils with high clay content had around 25% more C than soils with low clay content after afforestation of agricultural land (Laganière *et al.*, 2010). Paul *et al*. (2002) also noted the clay content of soil can influence changes in soil C following afforestation. Don & Schulze (2008) found that retention of dissolved organic carbon was related to soil texture.

It is possible that looking at a narrow range of sites may bias the generality of any significant effects. Wellock *et al*. (2011) found no significant effect of afforestation on SOC in contrast to two other Irish chronosequence studies that did detect differences. Wellock *et al*. (2011) suggested that this was due to the fact that the variables under investigation in the other studies were limited to age only, since tree species and soil type were held constant over a narrow topographical range. However, it may also be that there is an inherent geographic bias due to particular combinations of climatic and soil conditions favouring particular bioenergy transitions (Powers *et al.*, 2011). It is therefore important that sites are replicated across these climatic gradients and soil types as far as possible.

3. DEALING WITH SPATIAL VARIABILITY ACROSS LUC TRANSITIONS

3.1 Horizontal variability

3.1.1 Key issues associated with horizontal variability

'Inherent' variability

The ability to detect changes in soil properties such as SOC and bulk density (BD) will be influenced in part by their natural variability across the landscape. Soil horizontal variability can have 'inherent' structure due to micro-topography, historical land use and biological activity (Boone *et al.*, 1999; Klironomos *et al.*, 1999; Fraterrigo *et al.*, 2005; Wei *et al.*, 2008). Even apparently homogeneous arable fields can have high variability in SOC (Robertson *et al.*, 1997; Conant *et al.*, 2002; Zhang *et al.*, 2011). The use of geostatistical techniques can be used to uncover spatial dependencies between samples and inform subsequent sampling designs; however, these approaches generally require that hundreds of samples are taken across a site and so their application has been limited to one or two sites (Klironomos *et al.*, 1999; Liu *et al.*, 2011).

Differences in variability across and between bioenergy transitions
Different bioenergy crops are associated with different pre-planting levels of disturbance and this may influence the variability of soil properties. For example, sites will generally be ploughed before planting SRC/SRF (Rowe *et al.*, 2009; Brogan & Matthews, 2001). Many studies have demonstrated that woody and perennial plants increase spatial variability of soil properties (e.g. Jackson & Caldwell, 1993; Fraterrigo *et al.*, 2005; Fearnside & Barbosa, 1998; Conant *et al.*, 2003; Pärtel & Helm, 2007). In particular, horizontal variability may be high over short distances with forest sites. Pärtel & Helm (2007) showed that while mean SOC and moisture was lower in forest relative to grassland plots, the variability was greater (as measured by the coefficient of variation). Fraterrigo *et al.* (2005) also highlighted differences in between-site, within-site and within-plot variance between pasture, logged and reference (old-growth forest) land uses. The pasture land had the lowest levels and reference forest the greatest levels of within-site and within-plot variability for soil C (Fraterrigo *et al.,* 2005). Variability in soil properties may increase over the lifespan of 2nd generation bioenergy crops like SRC willow and *Miscanthus,* and consequently, any sampling strategy utilising the paired site or chronosequence approach must be able to handle differences in variability.

The planting, management and harvesting of 2nd generation bioenergy crops will promote spatial structure in soil properties. For example, linear features such as track and crop rows in willow or *Miscanthus* plantations will develop and these may have very different SOC contents or bulk densities (see Appendix I for differences in BD between track and row in willow SRC highlighted in the pilot study). Conen *et al.* (2005) found differences in soil C concentration between furrow, ridge and undisturbed strata that had been created following ploughing and planting of forestry. Therefore, a horizontal sampling strategy also needs to be able to deal with such linear features.

3.1.2 Sampling strategies for encapsulating soil variability

Design-based sampling and model-based sampling are two broad approaches in sampling design to deal with structure in spatial variability (Allen *et al.,* 2010). Design-based sampling and model-based sampling should not be confused with design-based and model-based inference, respectively. Design-based sampling is intended to produce unbiased estimates on the basis of random sampling. A systematic grid can also be used as part of a design-based or random sampling scheme provided that its origin is randomly positioned (Allen *et al.*, 2010). Model-based sampling aims to optimise spatial coverage of sampling locations and may involve stratification and proportional sampling. Fraterrigo *et al.* (2005) used a cyclic sampling design derived from time-series analysis. This enables detection of spatial structure at several multiples of the smallest lag (Fraterrigo *et al.,* 2005). Conen *et al.* (2005) used stratified sampling in a forest system to account for furrow, ridge and undisturbed strata. Zhang *et al.* (2011) suggested that stratified sampling could reduce the coefficient of variation of SOC. Although sample sizes and type II error rates can be reduced using a stratified design (Klironomos *et al.*, 1999), it also requires prior knowledge of the spatial structure. Consequently, the model-based approach is simply unfeasible across a large-scale study covering a range of different types of land use.

The design-based approach is therefore perhaps more suited to examine changes in SOC since it will produce unbiased average values at a given site (Allen *et al.*, 2010). However, it is also recognised that in bioenergy plantations such a random approach using a grid may be susceptible to spatial bias concerning the linear features as mentioned previously. One option to overcome this may be to use a hierarchical sampling strategy. This involves cores sampled from microplots within a site (*sensu* Conant & Paustian, 2002; Conant *et al.,* 2003). Using a simulated sampling, Conant & Paustian (2002) tested the effect of different potential sampling combinations of microplots and soil cores from a total of 18 cores (e.g. 2×9, 3×6, 6×3, 9×2) on different components of variance. Within-microplot variability was generally greater under the 2×9 and 3×6 configurations and lesser under the 9×2 configuration. Conant *et al.* (2003) also examined sampling strategy for soil C stock in cultivated and forest soils; variability was greater in forest compared to cultivated meaning that more micro-plots would be needed to detect change in forest. Therefore the number of microplots chosen should be guided by the most variable land use types.

Note: We undertook a pilot study to assess spatial variability of soil C and nitrogen with 1) different bioenergy crops at the same site and 2) different configurations of number of microplots and numbers of soil cores per microplot (see Appendix I). Our simulated sampling suggested that a combination of 4 or 5 microplots each with 3 cores would have the least variability per land use of those configurations assessed (see Appendix I).

3.2 Vertical variability

3.2.1 Key issues associated with vertical variability

Inclusion of organic layer

There are often questions as to how the forest floor (i.e., litter and organic layers) are dealt with in soil sampling (Boone *et al.*, 1999). Poeplau *et al.* (2011) states that the forest floor is an integral component of the soil and must be considered when sampling forest soils. Indeed, in studies involving forest soils, C in the organic and mineral horizons is often examined separately (e.g. Conen *et al.*, 2005; Vesterdal *et al.*, 2008; Karhu *et al.*, 2011).

In a meta analysis by Poeplau *et al.*, (2011), grassland afforestation resulted in a positive C balance, but only when forest floor material was included. Laganiere *et al.* (2009) also tested the effect of inclusion of organic layer on soil C change in their meta-analysis of LUC effects and found that this may be partly responsible for the different outcomes of similar studies. Consequently, Laganiere *et al.* (2009) recommended that the organic layer should be included, but separately, and that the 0cm point set at the interface of the organic and mineral horizons for other sampling. Across a variety of bioenergy transitions, dealing with the organic layer separately is likely to present difficulties for ready comparison. These difficulties may be one of the reasons that national-scale soil surveys do not separate these layers and remove only the litter and vegetative material (e.g. Emmett *et al.*, 2008; Bellamy *et al.*, 2005). However, Tremblay *et al.* (2006) sampled the L and F horizons together and included the H layer of the forest floor in the 0-10cm sampled depth where it was present.

We recommend that the L and F layer is sampled and recorded and that soil cores are taken from the H layer.

Maximum sampling depth

Soil C in deeper soil layers may be affected by bioenergy crops, particularly woody species or those with deep root systems i.e., *Miscanthus* (see Kell, 2011). It cannot be assumed that the deep soil layers remain unchanged if looking at SOC over time (Harrison *et al.*, 2011). Shallow soil sampling may vastly underestimate carbon pools and therefore bias findings. For instance, Guo & Gifford (2002) showed that the magnitude of soil C change depended on the sampling depth. Forest to cropland studies sampling 30cm or less found a large reduction in soil C, whereas those studies sampling to greater than 60cm found no overall change. Conversely, in cropland to pasture transitions, SOC increased by around 30% when studies sampled to 20cm or less, whereas in those sampling between 60 and 100cm SOC increased by only 15% (Guo & Gifford, 2002). Liebig *et al.* (2005) found in a paired site study that differences in SOC between switchgrass stands and cultivated cropland were most pronounced at 30-60cm and 60-90cm depths. Kravchenko and Robertson (2011) insist that trends in soil C change should be examined in horizons separately. Don *et al.* (2011) highlight that the impact of land-use change on SOC was not restricted to the surface soil, but that relative changes were equally high in the subsoil, thus stressing the importance of sufficiently deep sampling.

Just under half (44%) of our selected publications examining effects of LUC on soil C sampled to a depth of 30cm or less (Figure 2). The mean depth of sampling was 34.2cm in the meta-analysis of 33 studies on the effects on SOC of afforestation on agricultural land (Laganière *et al.*, 2010). Poeplau *et al.* (2011) calculated the mean depth of sampling for different LUC transitions. For example, studies sampled to average depths of 23.5cm in transitions from cropland to grassland, 28cm in transitions from cropland to forest (including the forest floor) and 38.9cm in transitions from grassland to forest (including the forest floor) (Poeplau *et al.*, 2011). Sampling to shallow depths (e.g. 30cm) may provide only a partial account of soil C stocks and overlook potential differences in deeper layers (Jobbagy & Jackson, 2000).

We recommend that soil sampling is to 1m wherever it is technologically and practically achievable. Currently, in WP2 we have resources for one 1m core per field.

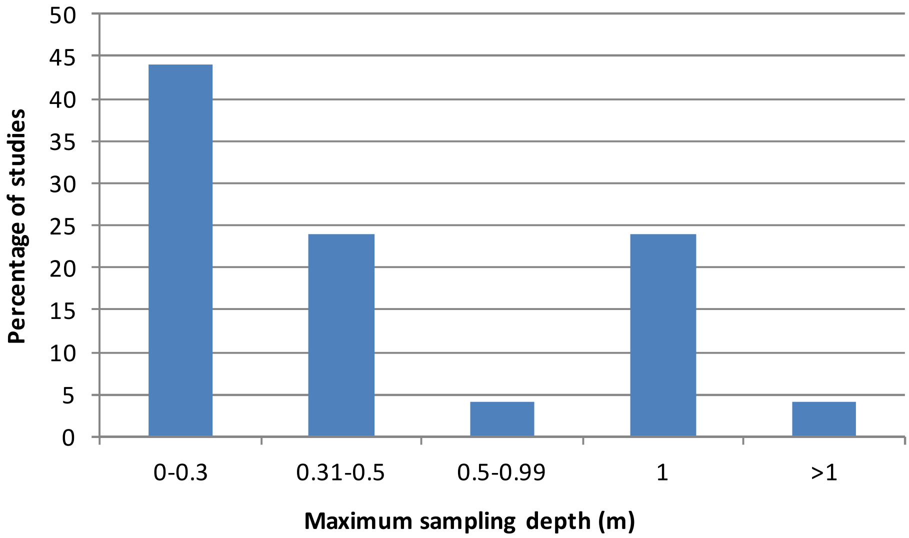

Figure 2. Relative proportions of maximum depths sampled in studies examining land use change effects on soil C. Data extracted from selected published papers identified in literature search (n=50).

Bulk density measurement

BD is the mass of soil per unit volume and a key measure of soil physical structure and pore space (Elliot *et al.*, 1999). It may change with moisture content in soils with high clay content, due to compaction by cultivation machinery and cattle, and by changes in land use (Gifford & Roderick, 2003). It is very important to be able to estimate changes in SOC stock changes (Gifford & Roderick, 2003; Don *et al.*, 2011).

Standard measurements involve removing an intact core of at least 5 cm in diameter or if the soil is not suitable to obtain an intact and uncompacted core (e.g., very sandy or a heavy clay), then different methods may have to be used, for example, the pit method (Elliot *et al.*, 1999). In some cases BD cores may be taken at depth from the side-wall of a hand-dug soil pit. Cores are then oven-dried at 105°C and stones removed for accurate assessment of soil mass and volume. The measurement of BD is frequently made on dedicated cores, but it is preferable however, to measure BD and C content on the same sample so that accurate measures of C stock can be calculated (Hamburg, 2000).

The UK Countryside Survey (CEH) conducted a pilot study to compare different protocols to estimate BD in different soil types (Emmett *et al.*, 2008), including coring and the pit method. Five different types of cores were tested: 10 cm long x 5 cm diameter round core, 15 cm x 6.4 cm round core, 10 cm long x 5 cm square metal core, 10 cm long x 8 cm square metal core, 8 cm long x 4 cm diameter round core. The soil pit method involved digging a pit, then filling the resulting hole with a plastic bag and using water to measure the volume. The different soil types tested were: clayey soil, sandy soil, peaty soil, stony soil and a woodland loam. In the laboratory, samples were weighed, separated out onto a tray and dried at 105°C. Once dry, the soils were sieved and stones and soil separated. All components were weighed and the BD calculated. The values of BD estimated from cores and pits were similar, and were within the ranges of typical values expected for each of the soil types (Emmett *et al.*, 2008). The recommendations were to use the 15 cm long x 5 cm diameter core, which was to be hammered into the ground, and removed using pliers. Importantly, the same core was used for BD, soil C, total N and pH determinations.

We recommend this integrated approach, utilising the same cores for BD and SOC, to provide accurate data and allow efficient processing.

3.2.2 Accounting for bulk density changes

Our selected publications highlight that almost three-quarters of studies examining SOC change during LUC do not account for differences in BD and compare different land uses on an equivalent soil mass (Figure 3). This proportion is very much in agreement with Poeplau *et al.* (2011) who found that in only 15% of studies examined had a mass correction been applied. Although in some studies it is acknowledged that no correction was necessary, since no significant BD changes had taken place (Farley *et al.*, 2004; Breuer *et al.*, 2006; Tremblay *et al.*, 2006; Guo *et al.*, 2007; Schipper *et al.*, 2007; Parfitt & Ross, 2011; Potvin *et al.*, 2011). Don *et al.* (2011) showed that only 52% of the studies they examined reported BD and that without correcting for BD changes and comparing an equivalent soil mass that the LUC effects would have been underestimated by 28%. This indicates that correcting for BD changes is an important step in comparing the effects of LUC on soil C.

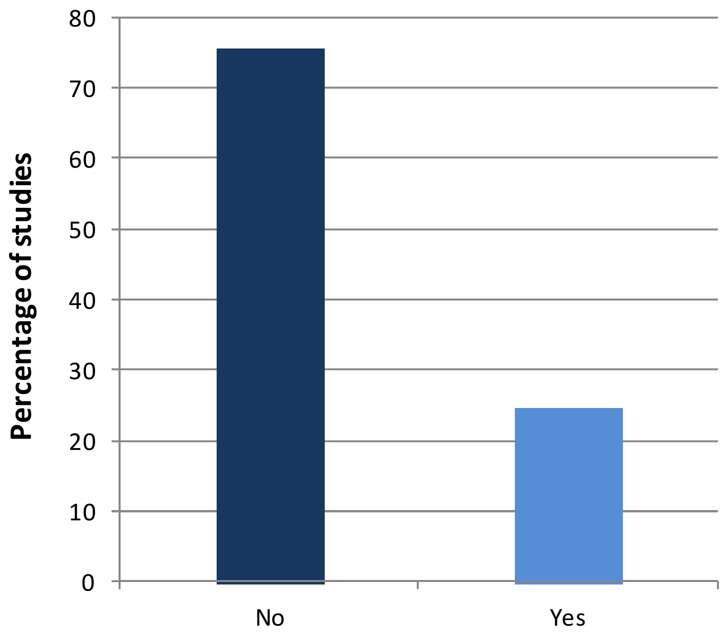

Figure 3. Do studies examining land use change effects on soil C correct the data to account for differences in dry soil mass? Data extracted from selected published papers identified in literature search (n=50).

Detailed here are several related approaches that have been used post-hoc in soil C calculations to overcome potential problems associated with fixed depth sampling when BD changes following LUC. Adjusting for changes in BD ensures that soil C is assessed across LUC based on the same mass of soil per unit area (Ellert & Bettany, 1995; Gifford and Roderick, 2003; VandenBygaart & Angers, 2006; Lee *et al.*, 2009). Two broadly distinct approaches based on equivalent soil mass and cumulative soil mass are being used to reduce the sampling error associated with concomitant changes in soil C concentration and BD. Both have merits and potential limitations and there has been debate in the literature as to which approach may be optimum (Lee *et al.*, 2009; McBratney & Minasny, 2010; Lee & Six, 2010).

Equivalent Soil Mass
Early efforts to deal with the problem of changing BD involved taking a sample 5cm deeper where BD was lowest, separating this extra 5cm depth and, once soil mass was known for each segment, physically adding sufficient sieved and moist soil from the bottom 5cm sample to make it comparable to the denser soil (Jenkinson, 1971). This is clearly a complex and time-consuming process that would not be feasible on a large-scale and it is not without its inaccuracies (Jenkinson, 1971). Ellert & Bettany (1995) developed the equivalent soil mass (ESM) approach using genetic soil horizons as datums for sampling depth across land uses. Zan *et al.* (2001) then replaced the use of genetic horizons with the use of depth increments.

The ESM method specifies a reference for each soil depth sampled from which ESM is derived. This is typically the initial sampling or, in the case of LUC transitions, the original land use. The ESM is defined as the soil mass per unit area for the chosen reference layer and equivalent C is the C mass stored in that chosen reference layer (Lee *et al.*, 2009). If BD has decreased in the reference layer due to LUC, then a hypothetical portion of the soil mass from the layer below is added so that the same mass of soil is compared in the final land use (or bioenergy crop). This mass is removed from the second layer and these calculations continue down the depth increments. The same corrections for soil mass are made where BD increases, but in the opposite direction (Lee *et al.*, 2009). A correction for the bottom depth increment is impossible if BD decreases and a portion of this soil remains unaccounted for. Lee *et al.* (2009) developed the maximum ESM and minimum ESM methods to deal with situations where the original reference soil mass is not known.

Cumulative Soil Mass

Measuring soil C using the soil surface as a datum can present significant problems because surface elevation can change when soils are compacted or expanded (Gifford & Roderick, 2003; Wuest, 2009). As mentioned, sampling to a fixed depth across a LUC transition where changes in BD occur will result in different soil mass being compared. The cumulative soil mass approach accomodates compaction and expansion of the soil profile because it is based upon mass coordinates rather than spatial coordinates (Gifford & Roderick, 2003; Wuest 2009).

To measure soil C using the cumulative mass approach a core is simply divided into several segments (or depths) and the dry soil mass and soil C are measured on each individual segment. The cumulative soil C data are then interpolated between each depth and plotted against cumulative dry soil mass. Cumulative soil C can then be compared at a standard dry soil mass. Gifford & Roderick (2003) recommend as standard references using a soil dry mass of 0.4 t m^{-2} for sampled depths of around 30 cm and 1.2 t m^{-2} for sampled depths of 1m. Although this approach does not actually require BD to be estimated, Gifford & Roderick (2003) suggest that it should still be reported for interpretation and modelling purposes. This straightforward and practical approach could be utilised to provide standard comparisons of soil C across bioenergy transitions. A comparison of soil carbon profiles from the pilot study using both cumulative depth (spatial coordinates) and cumulative mass is presented in Appendix I.

4. POTENTIAL BIOENERGY LUC TRANSITIONS IN THE UK

Potential transitions

There is potential for 18 different bioenergy LUC transition scenarios for the UK (Table 1). These cover transitions from arable, grassland and forestry to wheat, oil seed rape, sugar beet, *Miscanthus*, Short rotation coppice (SRC) and Short rotation forestry (SRF). These transitions include the main 1st and 2nd generation energy crops that are being considered in the UK and encapsulate a range of different management scenarios e.g. N-application, rate/harvest type and date. A detailed review of these transitions and their impacts will be provided by WP1. This following text provides an explanatory overview which aids the design of our sampling roadmap.

The likelihood of certain transitions and our knowledge of the likely impact vary (Table 1). For example transitions from forestry to bioenergy crops seem unlikely. While transitions to first generation crops are being increasingly challenged on grounds of sustainability (Gomez *et al.*, 2008; Hill, 2007) and may therefore become less common in the future. Indeed recommendations by Gallagher on sustainable biofuels (RFA, 2008) suggest that it is unlikely that food crops will have any significant role in the UK post 2020 for the supply of bioenergy. This reduction in the use of 1st generation crops is in part expected as a result of developments in the new methods to produce transport fuels form 2nd generation lignocellulosic biomass (Heaton *et al.* 2008; Rowe *et al.* 2009). This shift from 1st to 2nd generation crops may in turn lead to an increased demand for these 2nd generation bioenergy crops, and thus an increase in the land area devoted to them (Hill, 2007; Rowe *et al.* 2009)

Our knowledge of the potential impact of these transitions on soil C and other soil properties also varied between the transitions (Table 1). Overall, there are more uncertainties in LUC transitions to 2nd generation bioenergy crops compared to 1st generation crops (i.e., Anderson-Texeira *et al.*, 2006; Dawson & Smith 2007; Post & Kwon 2000; Guo & Gifford, 2002). This high level of uncertainty is due to two main factors:

- Novelty of 2nd generation crops, which at least within the UK and with in many other temperate regions have only recently become widely grown at commercial scales.

- Large change in cultivation practice that these crops represent, meaning that current well developed soil models for other arable crops may not easily be applied to these crops.

Table 1 provides a summary of the likelihood and expected trend of LUC and our level of knowledge of the potential impact of soil properties of the 18 potential transitions within the within the UK. This clearly highlights both the increasing importance of 2nd generation crops within the UK and our current limited knowledge of the potential effect.

Table 1: Summary table of possible bioenergy land use transition in the UK, the likelihood of certain transitions both currently and in the future and our knowledge of the possible effects on soil properties.

Bioenergy crop		Original land use								
		Grass *to*			Arable *to*			Forest *to*		
		Likelihood Now	Future Likelihood	Current Knowledge	Likelihood Now	Future Likelihood	Current Knowledge	Likelihood Now	Future Likelihood	Current Knowledge
1st gen	**Sugar Beet**	Low-Med	↓	Good	V. High	↓	Good	Low	↓	Good
	Wheat	Low-Med	↓	Good	V. High	↓	Good	Low	↓	Good
	Oil Seed Rape	Low-Med	↓	Good	V. High	↓	Good	Low	↓	Good
2nd gen.	**Miscanthus**	Med	↑	Poor	High	↑	Poor	Low	↑	Poor
	SRC	Med	↑	Poor	High	↑	Poor	Low	↑	Poor
	SRF	Med	↑	Poor	Med	↑	Poor	Med	↑	Poor

Likelihood refers to the likelihood of a given transition to bioenergy happening now. Arrows under future likelihood simply indicate direction of change, not the magnitude of direction (Anderson-Texeira *et al*., 2006; Dawson and Smith 2007; Gomez *et al*., 2008; Guo and Gifford, 2002; Heaton *et al.* 2008; Hill 2007; Post and Kwon 2000; RFA, 2008; Rowe *et al*., 2009).

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Potential effects of UK Bioenergy transitions on soil properties

Although our knowledge of the effects on soil properties is limited for some transitions, initial studies on 1st and increasingly 2nd generation crops do provide some insight on which we can base hypothesis of the likely impacts. Changes in soil C are of primary interest in the context of these bioenergy transitions. The effects of individual transitions have been shown to be very site specific with variation in soil texture, climate and original C stocks having a marked impact on the change in soil C (Anderson-Texeira *et al*., 2009; Rowe *et al*., 2009; Wilson *et al*., In press). Overall patterns of change in SOC however, are become increasingly apparent as the number of research papers in this area is increasing.

Table 2 provides an indication of the expected changes in soil C based on current literature. For 1st generation crops, the magnitude of changes in land use from current arable practices are expected to remain relatively low. As a result impacts on soil C are expected to be minimal (Rowe *et al.,* 2009). Conversion of grassland or forest to 1st generation crops requires a larger change in practices. Historically conversion of grassland and forest to arable production has been common practice and therefore is relatively well studied (Del Galdo *et al*., 2003; Grünzweig *et al*., 2004; Martens *et al*., 2004; Murty *et al*., 2002). Although not all these conversion were specifically for bioenergy production, the similarity between current arable production and 1st generation crops means that these studies do provide a strong indication of the likely impact of these conversions. As noted, the effects may still be affected by site-specific factors, but in general such conversions have in most cases been found to lead to a decrease in soil carbon (Murty *et al*., 2002; Del Galdo *et al*., 2003; Grünzweig *et al*., 2004; Martens *et al*., 2004).

Fewer studies have been conducted on 2nd generation energy crops, and as a result the pattern of change in soil carbon with land use transitions is less clear (Anderson-Texeira *et al*., 2009; Rowe *et al*., 2009; Vanguelova *et al*., 2011; Zimmerman *et al*., in press). Changes in soil carbon can also take some time to reach a steady state; thus due to the novelty of these crops, data on the long term effect of these crops on soil carbon is also limited (Anderson-Texeira *et al*., 2009; Rowe *et al*., 2009; Zimmerman *et al*., in press). Based on current studies, expected patterns in changes in soil carbon are given in Table 2 but it is clear that more information is needed before firm conclusions can be drawn.

Table 2. Expected changes soil C across relevant bioenergy LUC transitions.

Bioenergy crop		**Original land use**		
		Grass	**Arable**	**Forest**
1st gen.	**Sugar Beet**	↓	SAME	↓
	Wheat			
	Oil Seed Rape			
2nd gen.	**Miscanthus**	↓ or ↑	SAME or ↑	↓
	Short Rotation Coppice	↓ o or ↑	SAME or ↑	↓ or SAME
	Short Rotation Forestry	↓ or SAME or ↑	SAME or ↑	SAME

(Murty *et al*., 2002; Del Galdo *et al*., 2003; Martens *et al*., 2004; Anderson-Texeira *et al.*, 2009; Dondini *et al.,* 2009; Grünzweig *et al*., 2004; Rowe *et al*., 2009, Zimmerman *et al*., in press).

5. KEY FINDINGS

- Paired site and chronosequence approaches are the most suitable ways to examine transitions to bioenergy crops. (Section 2.3.1, p6)

- A hierarchical sampling strategy (i.e., microplot design) that can accommodate variability across different spatial scales is necessary, given the range of bioenergy transitions to be examined and with diverse changes in soil properties taking place during these different transitions. (Section 3.1.2, p9)

- Sampling including the organic layer and to a depth of 1m is important for the most complete and accurate assessment of soil C change across bioenergy transitions. (Section 3.2.1, p10)

- Bulk density can be estimated with the same cores used to measure soil C. (Section 3.2.1, p12)

- The measurement of bulk density and the need to derive soil C estimates based on the same soil mass per unit area is essential for valid assessment of LUC effects on soil C. (Section 3.2.2. p13)

These key findings echo Hamburg (2000) who suggested three rules to ensure that soil sampling is adequate to reach valid conclusions. These were that all soil horizons must be considered (e.g. mineral and organic), that soils must be considered to at least a depth of 1m (or the top of the C horizon), and that measurements of soil bulk density and carbon concentrations must be from the same samples.

6. SPECIFIC RECOMMENDATIONS FOR THE ELUM PROJECT

Based on the critical review of paired and chronosequence approaches in the published literature, discussion of the associated technical issues and the results from a pilot study, we present the following recommendations for the Work Package 2 soil sampling. Some of these recommendations (1-4) reconfirm our initial strategy as outlined in our original proposal while others (5-6) are specific outcomes from this review:

1. Only sites with a clear management or experimental history will be considered for sampling using paired and chronosequence approaches to ensure reliable assessment of bioenergy transitions.

2. Identified sites should be selected for sampling so that they are replicated and distributed across a UK climatic gradient where possible.

3. Sampling should comprise a combination of short cores (0-30cm) and deep cores (0-100cm).

4. Soil samples should be divided into 0-15 and 15-30cm sections to allow comparison with existing national-scale soil monitoring schemes (e.g., Countryside Survey and National Soil Institute survey, et al.)

5. In each land use in a transition, the sample distribution should consist of a hierarchical design with randomly distributed plots and a number of micro-plots at increasing distances (e.g. 1m, 1.5m, 2m).

6. Two post-hoc methods which account for bulk density differences, equivalent soil mass (ESM) and cumulative soil mass are recommended.

Next steps

The output from 2.1 will feed directly into the WP2 field trial soil sampling campaign, which in turn will be reported in the year one Chronosequence Report (D2.2). These same approaches, unless revised after year one experience, will be reflected in the year two chronosequence report (D2.3) and the final detailed report on soil C and bioenergy LUC for the UK (D2.4).

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APPENDIX I – PILOT STUDY

Methods

Site description
The pilot sampling was conducted at the Brattleby Bioenergy Network Site, Lincolnshire using adjacent fields of an Oil Seed Rape (OSR)/Wheat rotation and Willow Short Rotation Coppice (SRC).

Approach and sampling strategy
The objectives of the pilot sampling were to assess spatial variability of soil C and nitrogen with 1) different bioenergy crops at the same site and 2) different configurations of numbers of microplots and soil cores.

Semi-variograms were constructed with soil C data from an earlier study at the Brattleby site (Unpublished data) using the *geoR* package (Ribeiro Jr. & Diggle, 2001) in the R statistical environment (R Development Core Team, 2011). These data indicated that sample total C was independent at a distance of approximately 25m. A square 6x6 grid of 25m x 25m cells (36 cells and 49 intersects in total) was mapped on to each bioenergy crop, leaving a 25m buffer between the grid and the field boundaries. In each grid eight intersects were randomly selected as sample points. At the first four sample points four micro-plots were sampled; one at the intersect, one each at a distance of 1m, 1.5m and 2m from the intersect in. At the second four sample points two micro-plots were sampled, one at the intersect and one at a distance of 1m from the intersect in a random compass direction. The differing numbers of soil cores sampled within microplots minimised the total number of soil cores to be taken while still allowing various combinations of microplots and cores to be examined.

Soil sampling and processing
Soil sampling took place between 10 and 12 August 2011. Cores were taken to 30cm depth using a soil corer with a 5cm diameter. A total of 24 soil cores were taken in both the OSR/Wheat and Willow fields according to the sampling strategy. For samples taken in the Willow SRC a record was made on the location of the sample i.e. crop row, edge or track.

In the laboratory soil cores were divided into 0-15cm and 15-30cm depths. Cores were sliced lengthways and a quarter weighed before freezing, ensuring that it contained no stones or large piece of litter. The remaining portion of each soil core was placed in a foil tray and air-dried at 25°C for at least 10 days before being crushed and sieved to 2mm. Stones and debris retained on the sieve were weighed and their volume estimated by displacement of water. A 10 g sub-sample of the air-dried, sieved soil was oven-dried at 105°C for 24hr to determine moisture content. Moisture content of this sub-sample was back-calculated so that original dry mass of the whole soil core could be estimated. Bulk density (BD) was estimated using all these mass measurements following the protocol used in the Countryside Survey (Emmett *et al.*, 2008). Oven-dry soil was ground to a fine powder in a ball mill and 10mg analysed for total C and N in an elemental analyser.

Statistical analyses and simulated sampling

Differences in soil BD, carbon concentration and carbon density between the OSR/Wheat field and Willow SRC were tested using a mixed model with microplot nested within plot as a random effect.

Soil C profiles were calculated using both a cumulative depth and cumulative soil mass.

The soil C and N data were used to test different configurations of the numbers of microplots and soil cores on spatial variability using simulated sampling. This was carried out using a bespoke function in the R statistical environment (R Development Core Team, 2011).

Results and Discussion

Differences between bioenergy crops

Soil BD was greater in Willow SRC than OSR at 0-15cm depth but there was no difference at 15-30cm (Table 1). In addition, those samples located on track in Willow SRC generally had a greater BD (Figure 1). C concentration was greater in the Willow SRC compared to OSR/Wheat at 0-15cm but this was not significantly different (Table 1).

A comparison of soil C in the OSR/Wheat and Willow SRC using cumulative depth (Figure 2a) and cumulative soil mass (Figure 2b) highlights how using spatial coordinates as the datum may overestimate differences between land uses.

Alternative spatial sampling configurations

The simulated sampling of soil C in the OSR field suggested that spatial variability was relatively high and this may be because the field had been ploughed in recent weeks. Both the 0-15cm (Figure 3a) and 15-30cm (Figure 3b) carbon data suggested that at least five microplots with three or four soil cores per microplot would be needed to reduce variability. Simulated sampling of soil C in the Willow SRC field was far more clearcut with all soil variables having a decrease in variability with three cores per microplot for all numbers of microplot (Figure 4). Simulations using soil C density gave similar results in the Willow SRC. Though a combination of five microplots each with three soil cores could not be simulated the data suggest that this would be an suitable sampling strategy to reduce variability in the estimates of soil C and N in bioenergy transitions.

Table 1. Mean values of soil properties under OSR and Willow at 0-15cm and 15-30cm depths.

Depth (cm)	Crop	Bulk density (g cm^{-3})	C conc (%)	C dens (kg m^{-2})	N conc (%)
0-15	OSR	0.96	1.59	2.0	0.24
	Willow	1.37	1.76	3.4	0.24
15-30	OSR	1.32	1.38	2.6	0.22
	Willow	1.44	1.22	2.5	0.19

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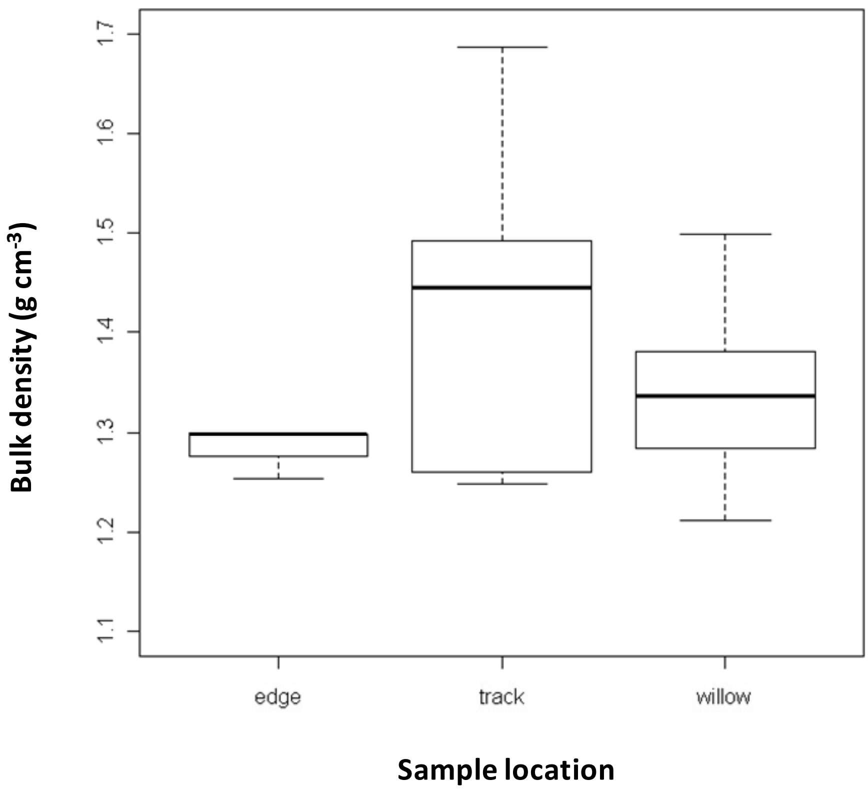

Figure 1. Differences in soil bulk density between crop row (willow SRC), track and edge locations in the SRC willow bioenergy crop at Brattleby.

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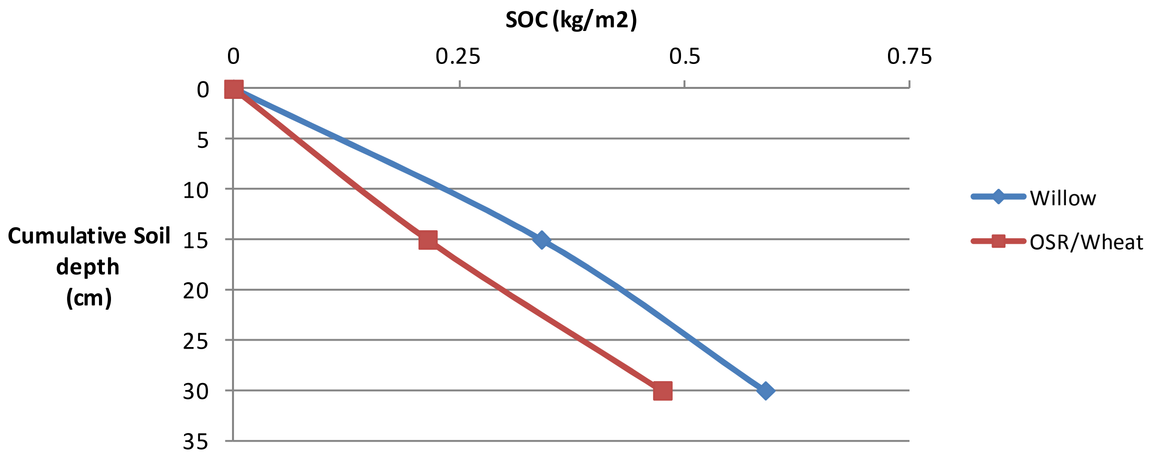
a)



b)

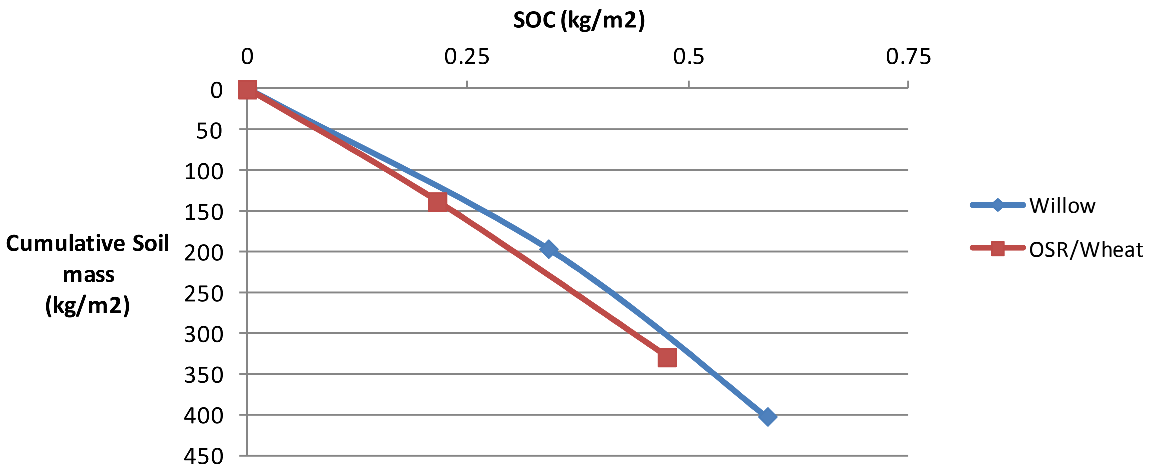


Figure 2. Soil C profile using a) cumulative depth and b) cumulative soil mass approaches for the Willow SRC and OSR/Wheat land-uses at Brattleby.

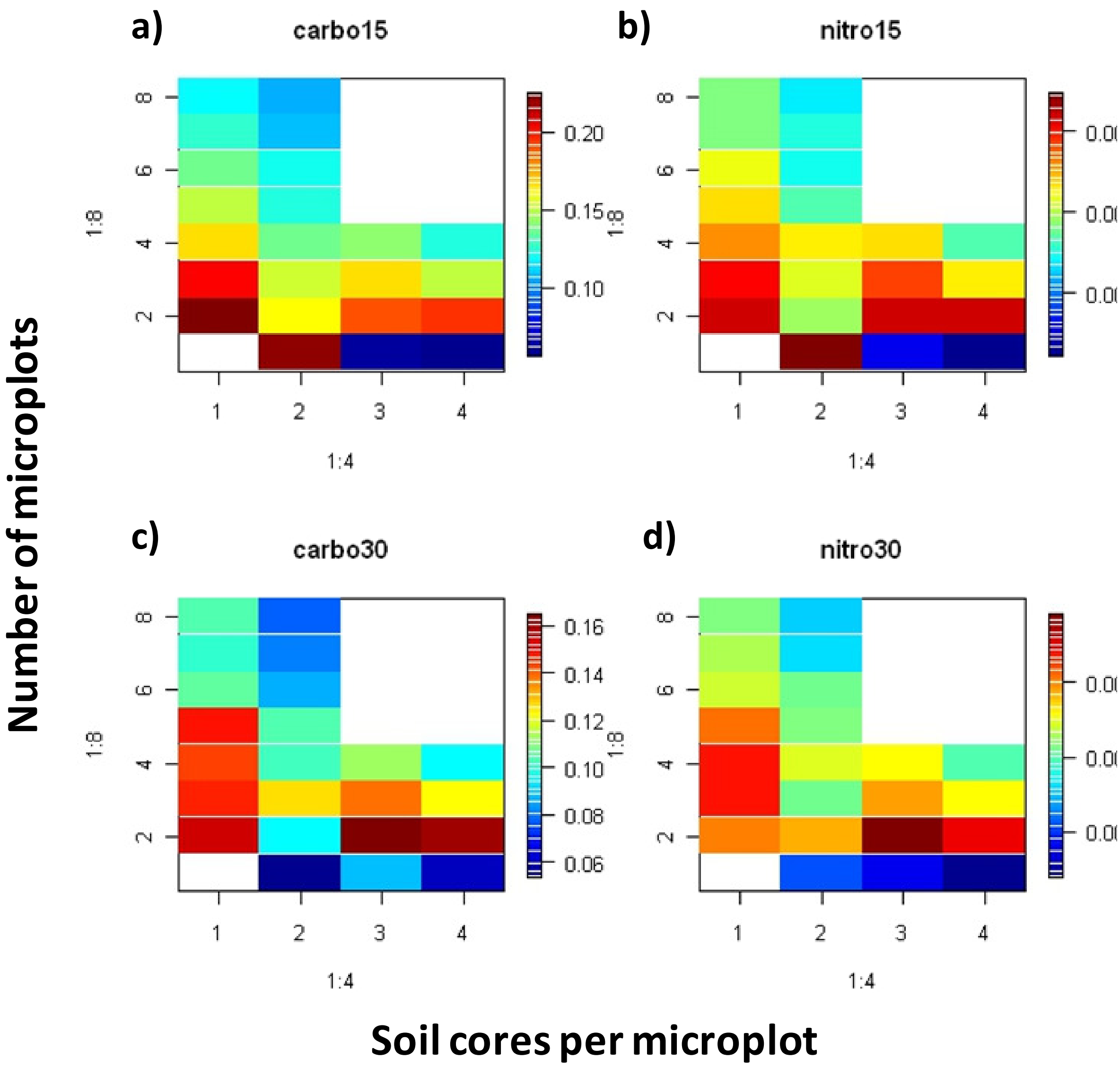


Figure 3. Variability in soil C (a,c) and soil N (b,d) concentration at depths of 0-15cm (a,b) and 15-30cm (c,d) in the OSR field with different combinations of number of microplots and soil cores per microplot. Colours represent total variability (between and within microplots). Blank combinations could not be assessed.

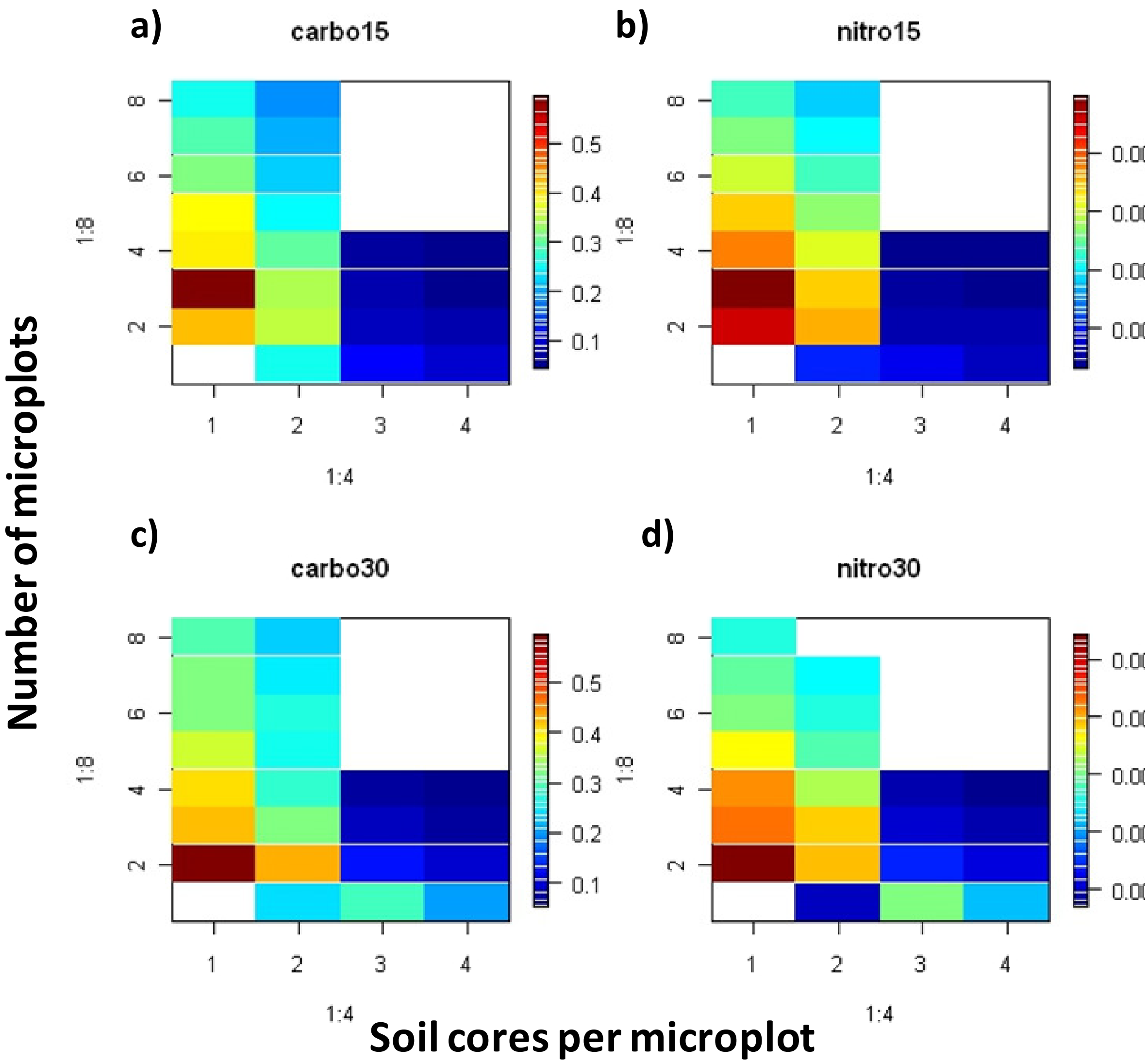


Figure 4. Variability in soil C (a,c) and soil N (b,d) concentration at depths of 0-15cm (a,b) and 15-30cm (c,d) in the Willow SRC field with different combinations of number of microplots and soil cores per microplot. Colours represent total variability (between and within microplots). Blank combinations could not be assessed.

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APPENDIX II – SAMPLING ROADMAP FOR ELUM DETAILING 1 YEAR SAMPLING ROADMAP

Our year one field campaign will primarily on focus on Short Rotation Forestry (SRF) sites where ready access to the locations of plantations with good management history is available. Due to the time constraints in year one, SRF was deemed the most appropriate means of ensuring we sampled from a sufficient number of fieldsites in line with our project plan. The locations highlighted in Table A2.1 demonstrate that we have a good geographical diversity encapsulating a wide range of SRF species. SRF species have been grouped into 4 categories, namely, Coniferous, High Productivity, Non native and Broadleaf. Through the Forest Research Database, additional SRF locations are available if we deem that further sampling is necessary. In year 3 we plan to sample some additional SRF locations which have been recently planted (2010/11) as part of the FR DECC field trials.

Table A2.1 *Sampling roadmap for year 1*

Location			Coniferous (CON)		High Productivity SRF (HP)		Non Native (NN)		Broadleaf (BL)		
	Grass	Arable	Scots Pine	Sitka Spruce	Poplar	Alder	Eucalyptus	Nothofagus	Birch	Sycamore	Cherry
Gisburn Forest, Yorkshire	X		X	X		X					
Hartwood, Lanarkshire	X	X		X	X						X
Glensaugh, Kincardineshire	X									X	
Broxa 98,N. Yorkshire	X		X			X		X			
Broxa 80, N. Yorkshire	X		X X X						X		X
Alcan, Northumberland	X						X				
Henfaes, Gwynedd	X					X				X	
Bronydd Mawr	X									X	
Brattleby, Lincolnshire	X	X			X						

Forward look: In year two sampling will be focused on transitions from grassland and arable crops to willow SRC and Miscanthus. Information on a minimum of 100 (50 willow SRC and 50 Miscanthus) potential sites will have been collected by December 2011 through our collaboration with the CarboBiocrop (CBC) project (data on 88 sites is already available to WP2). This information includes data on the availability of paired land use (adjacent arable and grassland fields), age of bioenergy plantations, soil type, location and crop condition. It is expected that early in year two additional data on soil clay content and soil carbon will be available from the initial sampling conducted with CBC. This will enable a more targeted site selection using the CBC generated datasets.