CRANFIELD UNIVERSITY

SARAH DAVIDSON

REWILDING BENEFITS ECOSYSTEM SERVICES

SCHOOL OF WATER, ENERGY AND ENVIRONMENT
Land Reclamation and Restoration

MSc Thesis
Academic Year: 2018-2019

Supervisors: Jim Harris
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September, 2019

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Rewilding benefits ecosystem services

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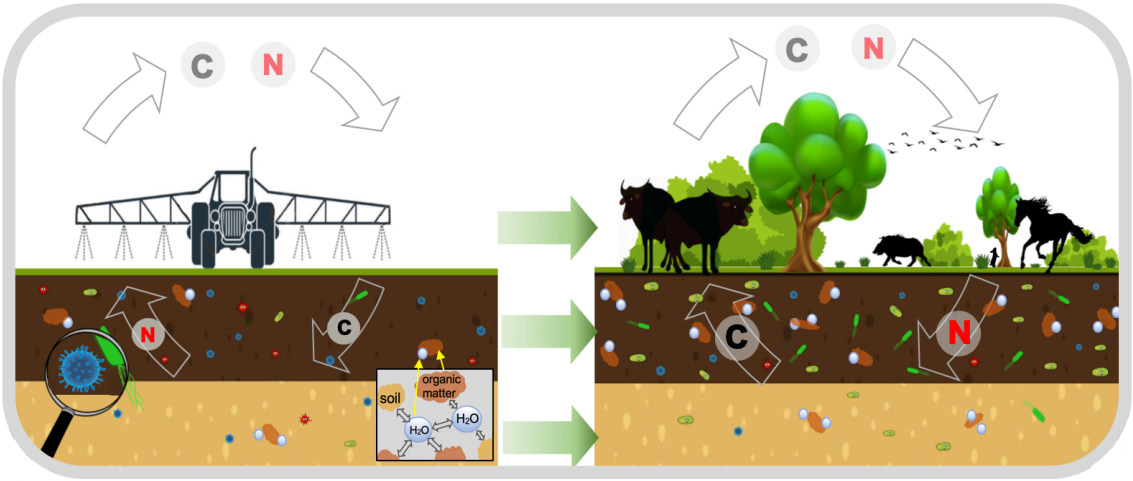
GRAPHICAL ABSTRACT



ABSTRACT

Intensive agriculture has led to degradation of soils and loss of biodiversity, resulting in a diminished capacity to deliver ecosystem services. Rewilding is believed to restore soil quality, but little is known about the effects on soil carbon and microbial communities. This study aimed to determine whether rewilding at the Knepp Estate had contributed to improved ecosystem services by investigating soil organic matter (SOM), total carbon (TC), total nitrogen (TN), and the soil microbial community by determining soil microbial biomass and phenotypic profiling by phospholipid fatty analysis (PLFA). Soils were collected at two depths (0-12 cm and 12-25 cm) from three rewilded locations. The locations were selected due to different rewilding conditions, and compared with soils from an arable site which had recently adopted less intensive methods of farming. Findings showed average increases of 125% Microbial Biomass C (MBC), 46% SOM, 65% TC and 53% TN in the top 12 cm of rewilded soils compared to the arable soil, as well as a shift in the microbial community profile, but changes were not significant at depth,

nor at all locations. Regression analysis showed an inverse relationship between the carbon/nitrogen (C:N) ratio and soil microbial communities. In conclusion, results indicate evidence of restoration of soils under rewilding which would benefit ecosystem services through gains in soil carbon stocks and microbial biomass, both of which increase nutrient cycling and soil biodiversity. The influence of the conditions of rewilding on the outcomes is uncertain as previous land use appears to have had a more dominant effect.

Keywords: Intensive agriculture, soil degradation, rewilding, soil organic matter, carbon, nitrogen, microbial biomass, PLFA, ecosystem services, Knepp.

Word count: 7,525

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
C:N	Carbon/nitrogen (ratio)
FAME	Fatty Acid Methyl Esther
HSD	Honestly Significant Difference
LOI	Loss on Ignition
LSD	Least Significant Difference
MBC	Microbial Biomass C
OMC	Organic Matter Content
PCA	Principal Components Analysis
PLFA	Phospholipid Fatty Acid
SOM	Soil Organic Matter
TC	Total Carbon
TCD	Thermal Conductivity Detector
TN	Total Nitrogen

INTRODUCTION

LAND USE CHANGES IN THE UK

Agriculture is the major land use in the UK, with approximately 70% of land being farmed (Parliament. Publications and Records, 2016). Traditionally, farming relied on the natural fertility-building properties of grasses, legumes and animals (Robinson and Sutherland, 2002), but since World War II many pasture-based systems have been turned over to arable use and farming practices have become increasingly intensive (The National Archives, 2019) with greater use of agrichemicals to maximise output (Robinson and Sutherland, 2002). Healthy functioning of above-ground ecosystems relies on biodiversity within the soil; however, the increased demand on land through intensive agriculture has resulted in reduced habitat and niche space, biodiversity, and biomass, with subsequent degradation to the underlying soils (Robinson and Sutherland, 2002; Breure, 2004) and at a considerable economic cost to the UK (Graves *et al*, 2014).

As soils form the basis of the supporting services upon which all other ecosystem services depend (see Fig. 1), the result has been a decline in the resilience of ecosystems and their ability to deliver ecosystem services (Blouin *et al*, 2013; Cerqueira *et al*, 2015). It is estimated that 60% of ecosystem services are currently affected due to the unsustainable use of soils under human activities, (Dewe and Senge, 2015).

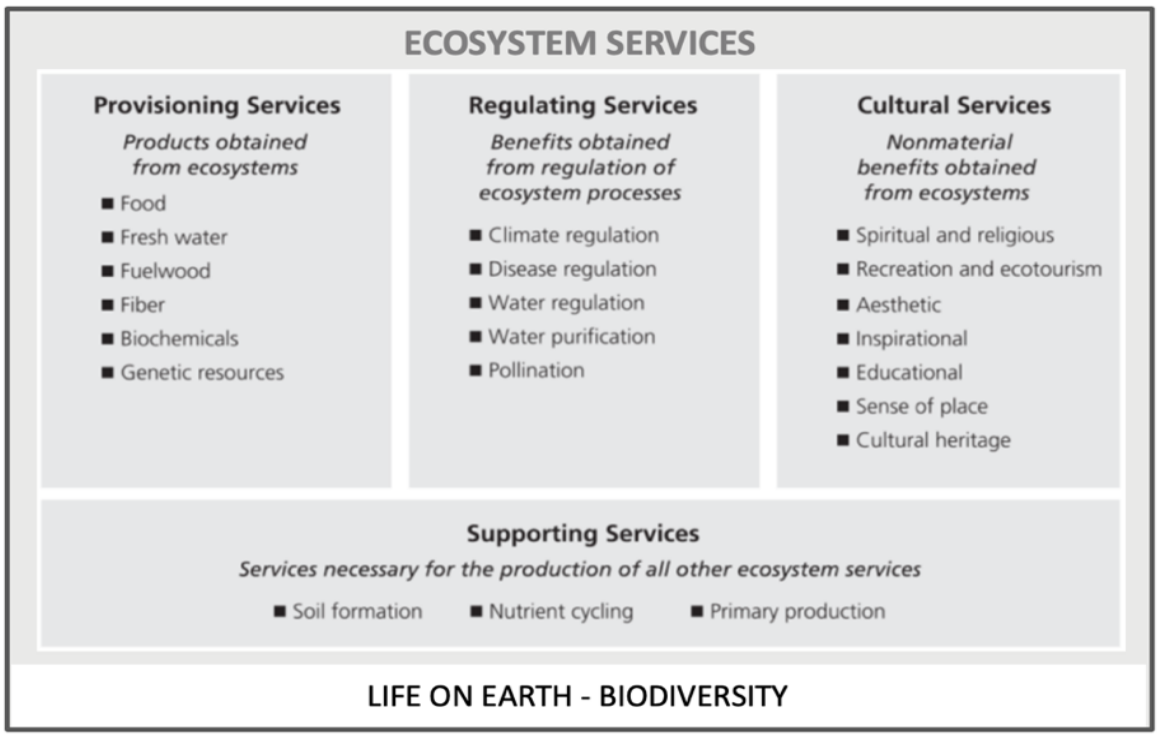

Figure 1: Ecosystem Services (*Adapted from: Millenium Ecosystem Assessment, 2005*).

ECOSYSTEM SERVICES AND SOILS

Ecosystem services derived from soils are a result of various soil properties and processes which work together and allow the soil to function as a complete system, and as such are not easily measured by a single parameter (Dominati, 2010). However, the loss of soil organic carbon and biodiversity, and increases of soil compaction and nutrient imbalances are thought to be some of the main threats to the healthy functioning of soils (FAO, 2015).

Soil organic matter (SOM) positively affects soil structure, as well as soil chemical and biological properties and processes, and is a useful measure of the healthy functioning of ecosystems (Panakoulia *et al*, 2017; Weil and Brady, 2017). More specifically, SOM plays an important role in the nutrient cycling processes within a soil, which affects primary production as well as modulating water storage and drainage, and therefore contributes to water purification and regulation (Weil and Brady, 2017; Dominati, 2010).

"The element carbon is the foundation of all life" (Weil and Brady, 2017). Soil carbon accounts for approximately 50% of the mass of SOM and quantities of carbon stored in soils are greater than in atmospheric CO_2 and the plant biomass on Earth combined, thus soil carbon contributes to primary production as well as playing a role in regulating the climate (de Graff *et al*, 2014; Weil and Brady, 2017). A significant proportion of the increase in atmospheric CO_2 is due to loss of organic matter from our soils (FAO, 2015).

Soil organisms constitute a major component of all soils, and are critical for most of the major functions provided by the soil in the form of ecosystem services (Breure, 2004; Skubala, 2013). One of the most important functions controlled by soil biology is the breakdown and cycling of SOM, and the total biomass of soil organisms is usually related to the quantity of SOM (Breure, 2004). Microorganism community structure has also been shown to play a role in the regulation of nutrient cycling, including carbon mineralisation and stabilisation (Xue *et al*, 2018). Thus microorganisms play a role in climate change as they act as sources and sinks of carbon, and the amount of microorganisms gives a good indication of the level of sustainability of soil functions and thus the stability of an ecosystem (Breure, 2004).

It is now recognised that by returning ecosystems to a more natural state, humanity will benefit from the services derived from the land (NERC, 2012). Rewilding is increasingly being seen as a cost-effective solution to reverse the damage caused to the soils under intensive agriculture (Parliament. Publications and Records, 2016), as well as improve biodiversity. Studies have shown that wild ecosystems are able to deliver higher quality

ecosystem services than other systems, for example higher carbon storage has been measured which improves biodiversity, water quality and flow regulation (Cerqueira *et al*, 2015). Certain ecosystem services, such as groundwater recharge and carbon sequestration, are higher on grassland than on agricultural land in the same area (Cerqueira *et al*, 2015), and different grazing regimes have been shown to affect the storage of carbon in the soil (Bagchi, Bhatnagar and Ritchie, 2012).

REWILDING

The original concept of rewilding was based on studies which showed that predators and large herbivores transform their environment, affecting the dynamics of ecosystems which can impact the entire food chain, and had three main requirements: 'top-down' trophic interactions; large areas of land for wide-ranging species; and connectivity of these areas to allow for movement and genetic diversity (Soulé and Noss, 1988), i.e. "3C rewilding" (Fig. 2) – cores, carnivores and corridors. Presently there are various interpretations of rewilding and the many rewilding projects around the world each have their own specific focus (Johns, 2019). However, they all aim to restore the dynamics within ecosystems in order that they may be resilient and self-sustaining (Cerqueira *et al*, 2015; Miller and Hobbs, 2019; Pettorelli, Duran and Toit, 2019). Additionally, they aim to have a continuous increase in 'wildness' where natural processes are in control and human intervention is minimised (Fig. 2) (Monbiot, 2013; Corlett, 2019, IUCN, 2019), although initially some human intervention may be required to reintroduce keystone species with specific functions (Parliament. Publications and Records, 2016). Nature-led changes result in undefined targets unlike traditional forms of conservation which often aim to preserve some defined pre-existing state, such as the restoration of a habitat or the protection of a single species (Pettorelli, Durant and Toit, 2019). Thus, nature is able to respond changes in the physical environment and reach new equilibriums as ecological system process are restored (Fraser, 2010; Miller and Hobbs, 2019). However, a return to the wilderness that existed prior to human intervention is not expected, as biodiversity loss, depleted soils and climate change mean that the ecosystems which will develop in the future will not be the same as those that existed in the past (Monbiot, 2013).

It is understood that biodiversity loss negatively affects ecosystem functions such as carbon cycling (de Graaff *et al*, 2014); therefore, trophic rewilding, which aims to restore biodiversity and biotic interactions, would be expected to restore these ecosystem functions (Figure 3). Research on rewilding specifically has largely focused on the interactions between reintroduced species and those already existing within the system, but has largely neglected the effects of rewilding on soils or the response of soil

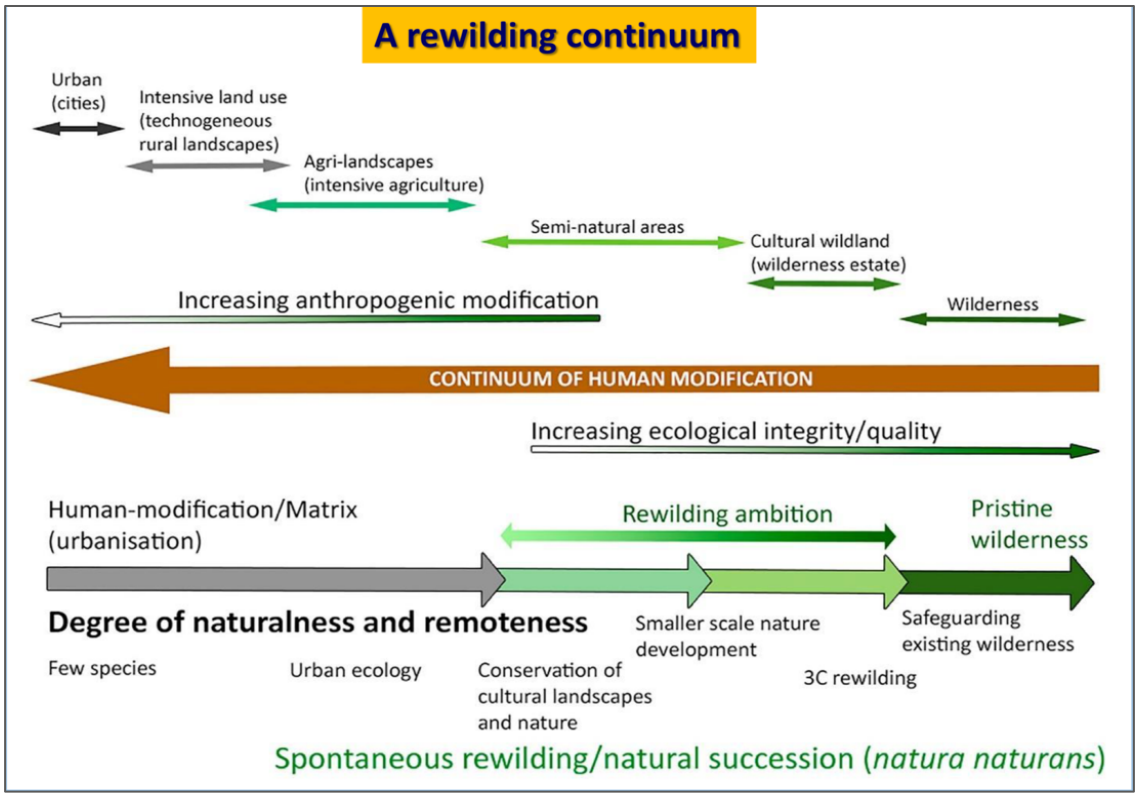

Figure 2. The direction rewilding projects aim to take (*Source: IUCN, 2019*).

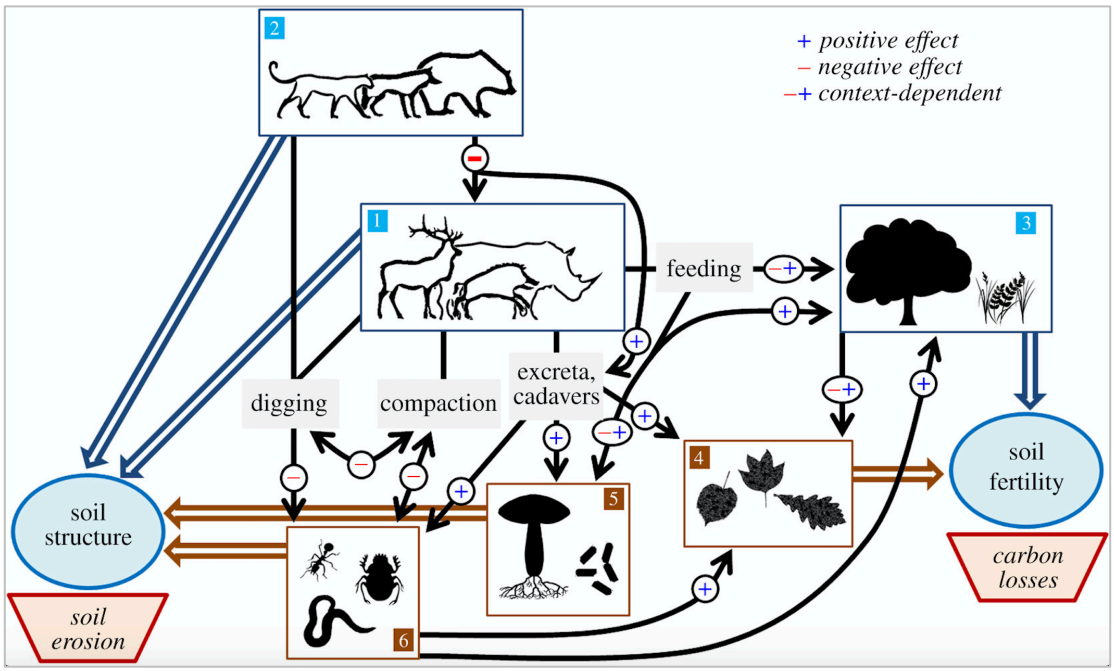

Figure 3. Interactions between main species present in trophic rewilding and main groups of soil biota, and the corresponding effects on plant-soil relationships *(Andriuzzi and Wall, 2018)*.

communities, making predictions about the impacts of rewilding on ecosystem functions uncertain, especially under changing climatic conditions (Andriuzzi and Wall, 2018).

The UK has a high population density, and thus faces significant land-use pressures which has resulted in a loss of certain keystone species historically (Sandom and Wynne-Jones, 2019), most notably large carnivores. The Knepp Wilding project therefore cannot be considered as rewilding in the truest sense of the word as humans play the role of large carnivores, and Knepp refer to their restoration process as 'wilding'. Nonetheless, as rewilding does not have targets and is simply a continuum of natural processes, such as vegetation succession and disturbance through interactions with wildlife (Sandom and Wynne-Jones, 2019), the project still fits into the 'rewilding ambition' referred to in Figure 2.

The Knepp Estate was previously under intensive agriculture and the aim of this investigation was to determine whether rewilding at the Knepp Estate has contributed to improved ecosystem services as a result of increased natural capital and processes within soils. This was achieved by comparing three rewilded sites with an agricultural site, specifically looking at amounts of SOM, TC and TN, as well as the microbial biomass and microbial community structure at two different depths within the soil. It is hypothesised that ecosystem services benefit from rewilding as soils will show evidence of restoration through increased SOM, carbon and microbial biomass at both depths, 0-12 and 12-25 cm, and that changes will also be reflected by a movement in the microbial community profile. It is conjectured that differences in the parameters will also be found between the soils from the three rewilded locations due to the different ways and times at which they were restored.

METHODOLOGY

STUDY AREA

The study location was the Knepp Estate in West Sussex, S.E. England. The area has a mean annual maximum and minimum temperature of 15.1°C and 6.2°C respectively, and 827 mm of precipitation (Met Office, 2019). The estate is 1,400 hectares in size and was previously under intensive agriculture. The estate is presently divided into three blocks, each having been taken out of intensive agriculture at different times and restored under different conditions (Fig. 4).

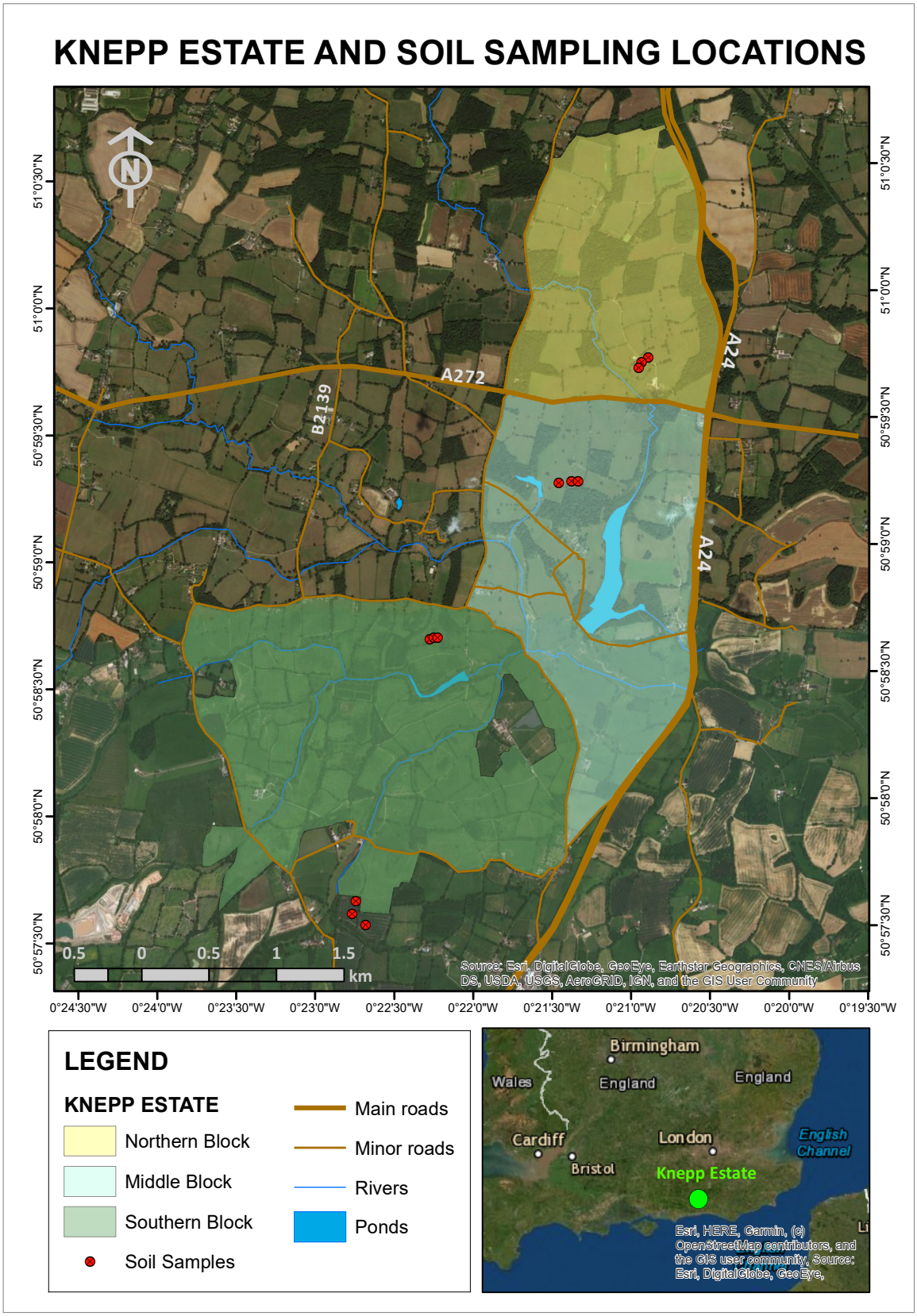

Figure 4. Coordinates of soil sampling locations (north to south): Northern Block: Lat. 50.996492. Long. -0.34881592; Middle Block: Lat. 50.987619, Long. -0.3553782; Southern Block: Lat. 50.977760; Long.-0.37009791; Agricultural site: Lat. 50.8755369, Long. -0.3799798.

Repton Park (within the Middle Block) was the first area to be restored in 2001. The land was treated with glyphosate and a wildflower mix planted; fallow deer were introduced in 2002, longhorn cattle a year later, followed by pigs and red deer**.** The Northern Block was sown with a Countryside Stewardship Scheme mix of eight species of native grass on areas not already under permanent pasture, and then cattle were brought in. In the Southern Block, fields were taken out of agricultural production over a period of years starting in 2001 (2003 for the fields sampled), with the least productive fields being set aside first (Tree, 2018), and left fallow with animals only being introduced in 2009. Longhorn cattle, Exmoor ponies, Tamworth pigs and fallow deer were introduced and later on, red deer (Tree, 2018) (Table 1).

Soil samples were taken from each of the three rewilded blocks at the Knepp Estate as well as from a neighbouring arable site for comparison (Figs. 4 and 5) to establish whether there were differences between the three rewilded soils. All sites historically belonged to Knepp and had been under permanent pasture until WWII when the land management practice changed to intensive arable production. The agricultural site continued under intensive agriculture until 2016 since when it has been farmed under a more conservative no-till regime. All areas sampled were on the same soil series, Wickham 1 (711e), which is characterised by slowly permeable, seasonally waterlogged soils which have a high clay content (NRSI, 2019). Soil samples from the rewilded blocks were taken from open, grassy areas to strive for similarity between sample types to allow comparisons to be made.

SOIL SAMPLE COLLECTION AND FIELD MEASUREMENTS

Sampling was conducted at the Knepp Estate on the 7th June 2019. The temperature ranged from 13-17°C during the sampling period. The weather was largely overcast, with some rain. However, the rain came following a long dry spell with the result that the clay soils were very hard making it impossible to take samples to 30 cm or to sample in as many locations as was initially intended. Sampling at Oakwood Farm, Shipley took place on 13 June 2019 with temperatures of 15°C, the weather was raining and overcast, but heavy rains over the previous week resulted in considerably wetter soils than at the Knepp Estate.

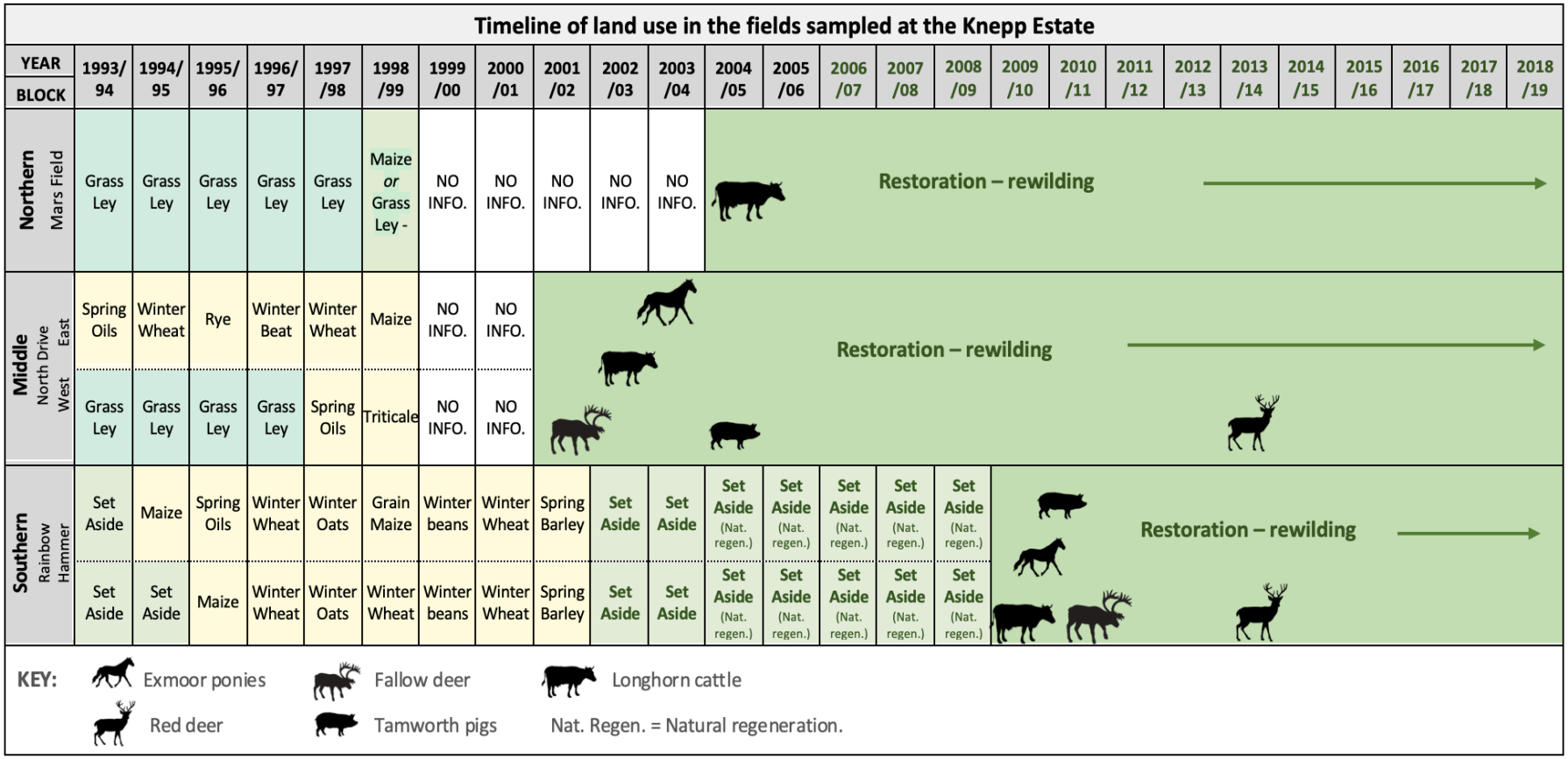

Table 1. Land use on the fields sampled at the Knepp Estate from 1993-2019. Location of animal symbols on the timeline indicate the time of their introduction.

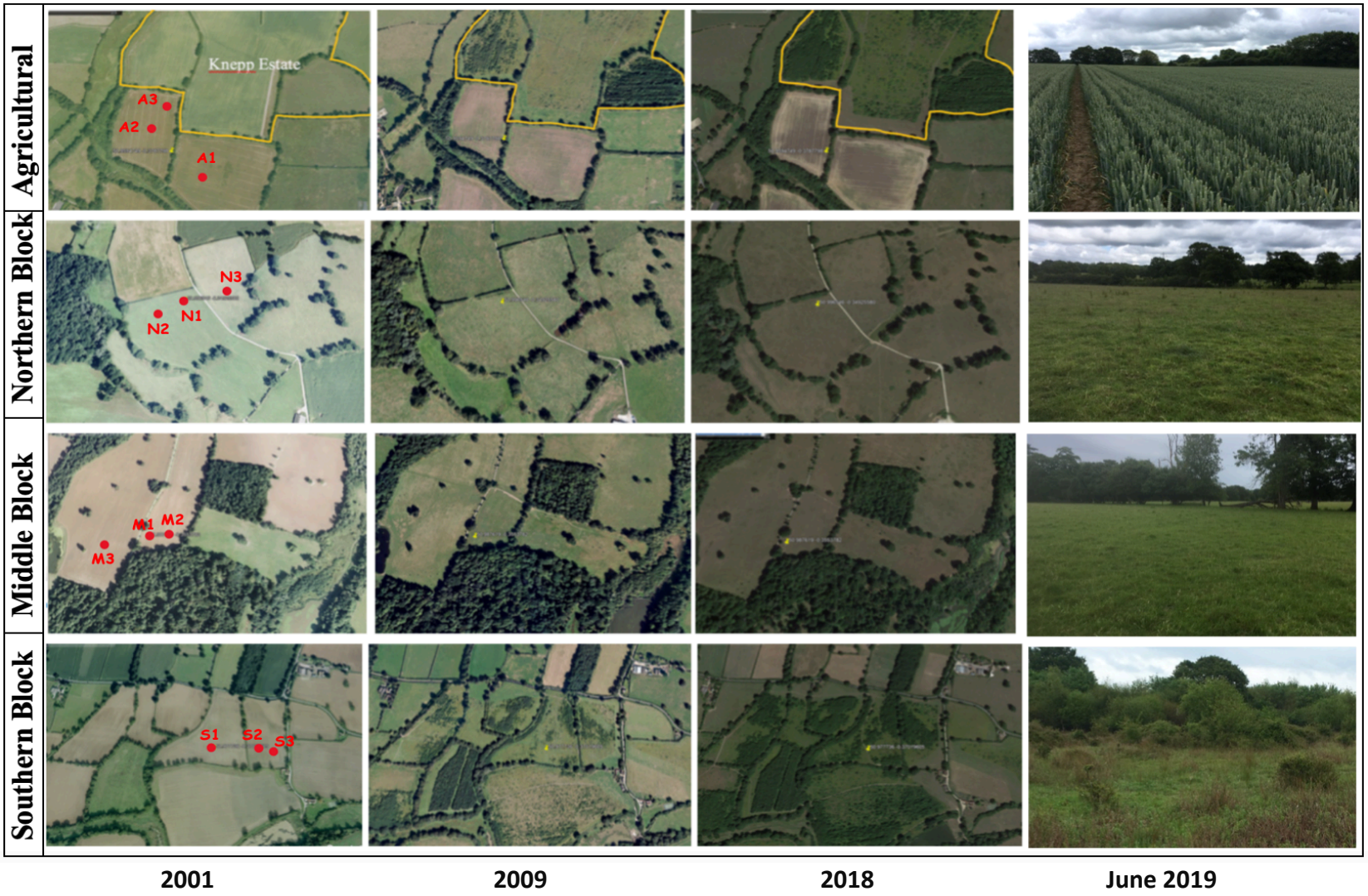

Figure 5. Appearance of sampling locations (in red) before and after rewilding *(Aerial images sourced from Google Earth)*

Soil samples were collected from random locations within each of the rewilding zones at the Knepp Estate and the agricultural site. Open areas away from trees, field margins and other areas with specific conditions were avoided to aim for an overall representation of the area (Soil Association, 2017). A gouge auger was used to take three replicates at each location using the 'W'-transect method with six individual cores to a depth of 25 cm. The Soil Association recommends sampling the top 10 cm if testing microbiology, i.e. where the soil is aerobic, and the top 15 cm for chemical analyses, and if both aspects are being tested then a point around 12 cm is ideal. The twelve samples were divided into two, top and bottom, giving a total of 24 samples. All samples were put into plastic bags, labelled and stored at 4°C until prepared for their final analysis.

Soil compaction was measured with a Dickey-John penetrometer to a maximum depth of 70 cm, or to 300 psi. 300 psi is taken to be the penetration resistance at which roots are almost unable to penetrate the soil (Duiker, 2002). Five readings were taken near each sampling location, and the mean value was taken. Soil moisture was tested with a Delta-T theta probe was used to test soil moisture. Five readings were taken, and the mean value was used for each location. A minidisk infiltrometer was used on a setting of 0.5 cm adjustable suction to measure unsaturated hydraulic conductivity. Infiltration rates are slow due to the clay soils, so time permitting, two readings were taken at each location. Air temperature readings were obtained from a local weather station.

LABORATORY ANALYSES

The fresh soil samples were sieved at 2mm and divided into three. One portion was stored at 4°C for MBC analysis, another was frozen at -85°C and freeze-dried for PLFA analysis, and the final subsample was air-dried and stored at room temperature for pH and loss on ignition (LOI), and a portion of the latter was fine ground for TC and TN.

Laboratory analyses were carried out using British Standards Institution (BSI) methods. Soil water content was determined by placing the fresh soil in an oven at 105°C for 24 hours and finding the difference in weight (BSI, 1994). LOI was used to determine SOM and was measured from a weighed sample of air-dried soil which was then dehydrated at 105°C for a minimum of 17 hours, then ashed in a muffle furnace at 450°C for four hours (BSI, 2000). The LOI which was calculated as the percentage of the dehydrated sample (BSI, 2005). Soil pH was measured using a pH meter in a 1:5 suspension of soil in deionised water. The pH meter determines the acidity of the soil by measuring the amount of ion exchange which occurs between the probe and the solution and can thus estimate the amount of hydrogen ions within the soil. TC (BSI, 1995) and TN (BSI, 2001) were measured from a finely-ground subsample of soil which had been dried in an oven

at 105°C for 2 hours. A sample of soil was packed into an aluminium foil capsule and was heated to 900°C in the presence of oxygen gas which oxidised the carbon to CO_2, and a thermal conductivity detector (TCD) then compared the CO_2 in the sample tested to a reference gas. The difference in thermal conductivity was assessed in order to determine the amount of carbon present. Under oxidation mineral and organic nitrogen compounds in the soil produce NO_X compounds as well as N_2. Copper was used to bind the excess oxygen and reduce the oxides to N_2. The TCD then measured the amount of nitrogen present.

Soil MBC estimates the mass of intact microbial cells from a measure of the carbon content of the cells. The fresh soil sample was divided into two and one part was fumigated with chloroform for 24 hours in order to destroy intact microbial cells. The organic carbon was then extracted from both the fumigated and non-fumigated samples by shaking the soil in a 50ml of 0.5 mol/l potassium sulphate solution, filtering the suspension and collecting the extracts. The organic carbon in the extracts was determined in the auto-analyser and the biomass carbon was calculated from the difference in the mean mass of organic carbon between the fumigated and non-fumigated samples and dividing the result by a conversion factor of 0.45 (BSI, 1997).

Phospholipid fatty acids are only found in the plasma membranes of living cells and phospholipid fatty acid (PLFA) analysis can be used to determine the structure of microbial communities in soils and it can easily be determined if a soil community has been affected by a change in conditions (Frostegård, Tunlid and Bååth, 2010). The organic portion of the soil was extracted using Bligh and Dyer (B&D) solvent, centrifuging and then pouring off the top, organic layer. The organic layer was then further separated by adding chloroform and a citrate buffer and centrifuging. The aqueous layer was removed, and the remaining lipid layer was dried under nitrogen to prevent oxidation. Fractionation of the lipid extract was done by reconstituting the extract using chloroform and then the sterols (neutral lipids) were washed out with chloroform, the glycolipids with acetone, finally, the phospholipids were eluted with methanol and collected and dried in an evaporator under nitrogen. Methylation was then used to separate the fatty acid from the glycerol part of the phospholipid and recover the fatty acid methyl esters (FAMEs). The phospholipid fraction was reconstituted using a toluene/methanol mix, then the lipids were hydrolysed using potassium hydroxide and incubated for 30 minutes at 37°C. The hydrolysis reaction was stopped by neutralising the pH using acetic acid, then a hexane/chloroform mix and deionised water were added and centrifuged to separate the two phases. The lower aqueous phase was discarded,

and the top organic phase was washed through sodium hydroxide with hexane to remove any unwanted components. The extract was dried and then reconstituted with 200 μl hexane and placed in a gas chromatograph vial for analysis. Gas chromatography identified the FAMEs using their retention times. Retention times are dependent on the length of the carbon chain in the fatty acid, and PLFAs are further separated depending on the position of the double bond as well as their branching configuration. The results obtained were converted to mol% to give the relative occurrence of the FAMEs. A total of 34 indicator fatty acids were identified and used as indicators of the presence of fungal and bacterial biomass. In order to estimate the fungal:bacterial ratio, 18:2ω6,9 was chosen to represent PLFAs of fungal origin and i15:0, ai15:0, 15:0, i16:0; i17:0, ai17:0, cyc17:0 and cyc19:0 (Frostegård and Bååth, 1996) and those PLFAs of uncertain origin or which were known to exist in both bacterial and fungal organisms were not used.

STATISTICAL ANALYSES

Statistica (Version 13.3, Tibco Software Co.) software was used to carry out the statistical analyses. Principal Components Analysis (PCA) was carried out on the PLFA results to emphasise variation and show any correlations between the data sets. One-way ANOVA was then conducted on the Factors 1 and 2 of the case variables to show the movement between the microbial communities. This was carried out for both depths together as well as separately. Factorial ANOVA was conducted to compare the effects of site and depth, as well as determine the effects of site and depth on pH, organic matter content (OMC), Total C and N, C:N ratio, MBC, PLFA fungal:bacterial ratio and PLFA fungal biomarker 18:2ω6,9. Post hoc Tukey HSD analyses were carried out on significant results to observe where variation amongst the means of the groups occurred. Post Hoc Tukey HSD was chosen above Fisher LSD as Tukey is less likely to result in a Type 1 error (Hilton and Armstrong, 2006). Statistical significance for all analyses was determined as $p<0.05$. PCA was also used to establish if any covariance existed between the data sets, and correlations between data sets were determined to see which variables were most related to each other and to establish whether chemical or biological parameters were responsible for any shift in the biology.

RESULTS

FIELD RESULTS

Due to the different weather conditions on sampling days, comparisons could not be made between compaction or infiltration at the arable site and the three rewilded locations. A distinct layer of compaction was observed at around 30-35 cm depth at the agricultural site, however, often, it wasn't possible to break through the surface layer at the rewilded sites, which was cemented due to the dry weather conditions, in order to establish if or where compaction existed at depth (see Appendix A for all field results).

LABORATORY RESULTS

Factorial ANOVA

The mean and standard error (n=3) of the parameters analysed are highlighted in Figure 6., with values given in Appendix B. Significant differences are those at $p<0.05$.

pH was in the range of 5.6-6.4 with no significant interaction between site and depth. Soils were significantly more acid at depth across the sites (mean pH = 6.3), than in the top 12 cm (mean pH=5.9). The agricultural site had the highest overall pH and the northern the lowest with a significant difference between the two.

Organic matter content was significantly higher in the rewilded soils in the top 12 cm compared to 12-25cm, with an average increase of 45%, whilst the arable soils only had an increase of 8%. The rewilded locations had on average 46% more OMC in the top 12 cm compared to the arable site, but the differences were only significant ($p<0.05$) at the Northern and Middle Blocks. Differences between the locations at depth are only observed at the Northern and Middle blocks.

Total C and N showed similar profiles to OMC. The rewilded sites had significantly higher levels of Total C (average 75%) and Total N (54%) at the surface than at depth, whereas the arable site only had a 13% and 10% increase in C and N respectively. In the top 12cm, the rewilded locations all had more total C (average 65%) and N (53%) than the arable site, although differences were only significant at the Northern and Middle Blocks. At depth the Northern Block had the highest levels of C and N, whilst the Southern Block had the lowest.

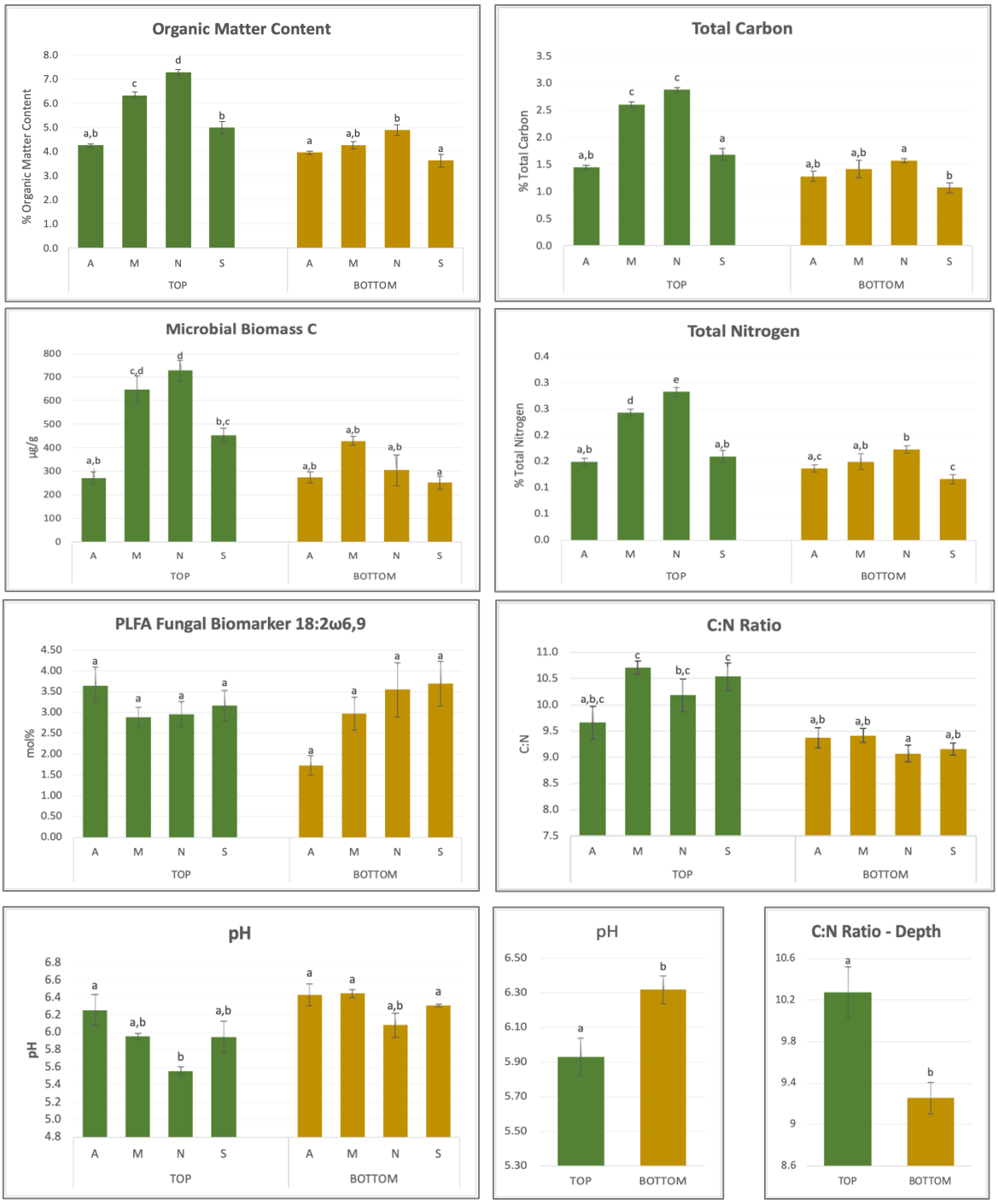

Figure 6. Mean values with standard error of soil properties for rewilded and arable soils at two soil depths at four locations (n=3): A = Agricultural; M = Middle Block; N = Northern Block; S = Southern Block. The effects of land use and depth upon soil properties as determined by Factorial ANOVA with Tukey Post-Hoc analysis. Different lowercase letters indicate significant differences ($p<0.05$). Where no significant difference between the groups at the 95% confidence level was found at site * depth, additional graphs for site and/or depth indicate where significant difference was found.

C:N ratio – The C:N ratios were all within the range of 9.1-10.7. There was a significant increase in the C:N ratio between the top 12 cm and depth in the rewilded soils, but not in the agricultural soils. The C:N ratio was higher at all rewilded sites compared to the agricultural site, although not at the 95% confidence level.

Microbial Biomass C - All of the rewilded sites had significantly more (average 90%) MBC in the top 12 cm compared to 12-25cm, whereas the arable site showed no change. In the top 12cm all rewilded soils had an average of 125% more MBC compared to the arable soils although this was only highly significant at the Northern and Middle Blocks.

PLFA – fungal biomarker, 18:2ω6,9, and the fungal:bacterial ratio displayed nearly identical profiles indicating that bacterial proportion of communities was relatively constant across all soils whilst the differences were in the fungal communities. Significant differences were not obtained at site or depth, with the biggest difference being seen at the agricultural site at 12-25 cm which had approximately 50% less than in the top 12 cm of both the arable and rewilded soils at depth.

Principal Components Analysis (PCA)

PLFA - The mean principal factors 1 and 2 of the PCA of the PLFA were plotted and together accounted for 47% of the variation (Fig. 7), which was not significant between all the soils when considering both depths together. However the movement of the microbial community profile between the top 12cm and 12-25cm on PC1 can be seen, and PC2 shows the shift of the microbial community from arable to rewilded soils.

In the top 12cm a movement on PC1 and PC2 can be seen between the arable and rewilded soils. At depth the movement between arable and rewilded soils is seen on PC1. However, on PC2 movement is only seen at the soils of the Southern Block.

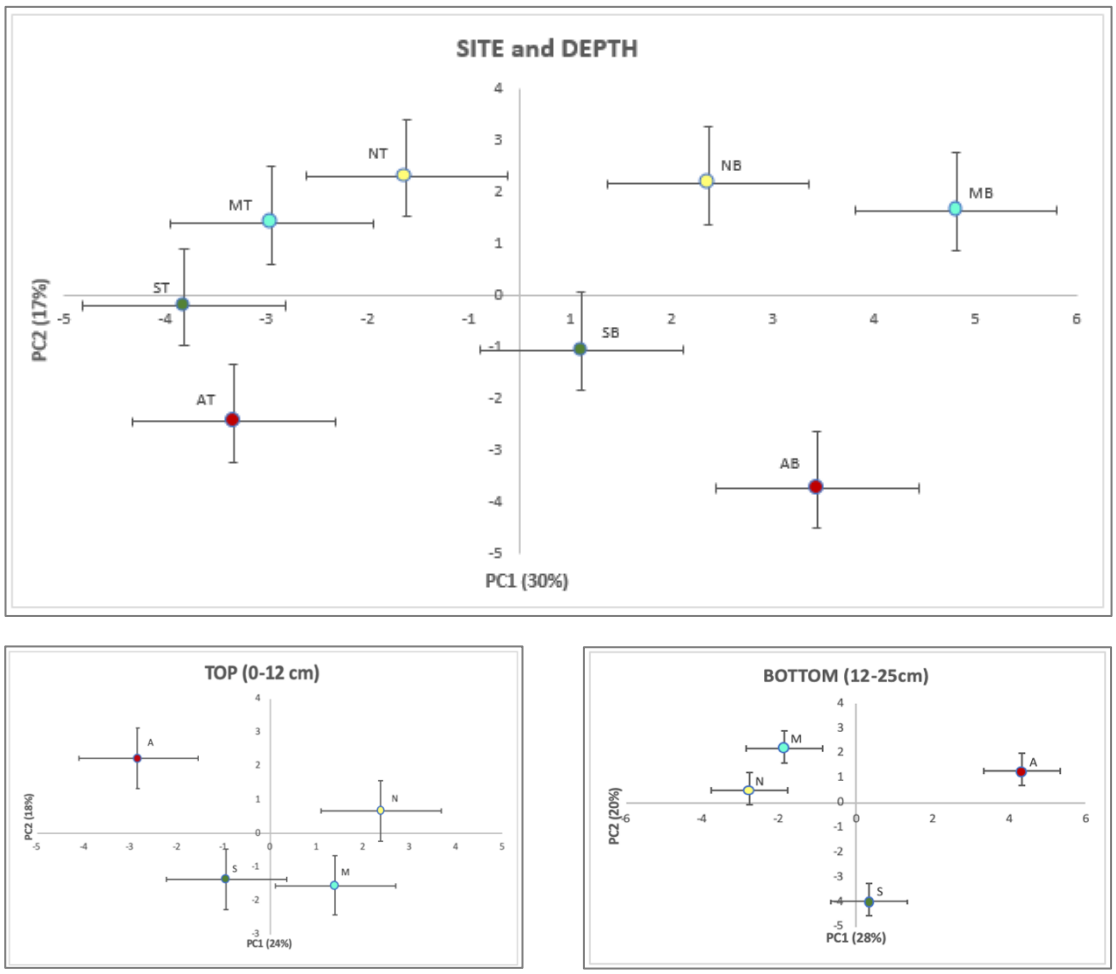

Figure 7. Principal Components Analysis of PLFA means ±standard error plotted to visualise the movement of the microbial communities under rewilding. Both depths (top); 0-12 cm (bottom left), 12-25 cm (bottom right)

Correlation and regression analyses

Regression analysis (Fig. 8) indicates increases in MBC as SOM content rises (r=0.89), and a decrease in pH with an increase in SOM (r=-0.78). pH was also found to be moderately inversely correlated with MBC (-0.61). An inverse relationship was found between the the C:N ratio and Factor 1 from the PLFA PCA (r=0.69).

Correlations were also run independently on soil parameters at 0-12 cm and 12-25 cm depth to observe any differences. In the top 12 cm, the most significant correlation was Factor 1 of PLFA with SOM (r=0.81), and moderately with pH (r=-0.66), but at depth, Factor 1 of PLFA negatively correlated with SOM (r=-0.59), but when the latter was plotted the data was clustered and was not included in the discussion (Appendix C). A complete table of correlation results can be found in Appendix B.

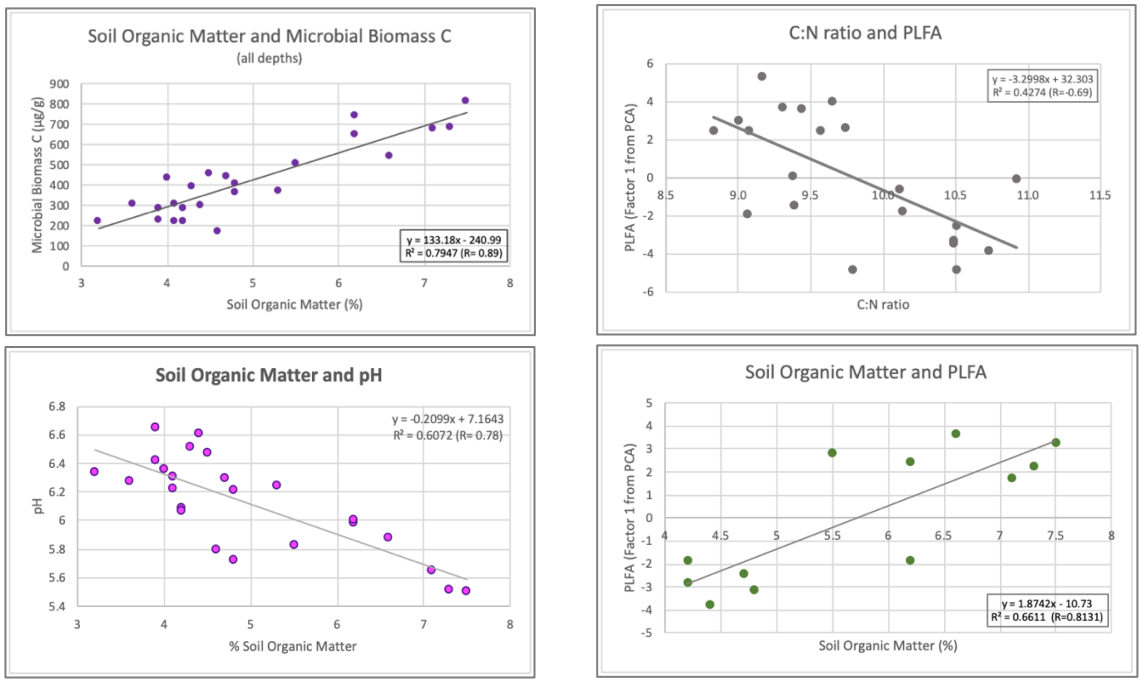

Figure 8. Regression analysis of SOM and MBC (top left), C:N ratio and Factor 1 of PCA of PLFA (top right), SOM and pH (bottom left) on both depths; regression analysis of SOM and PLFA factor 1 of PCA (bottom right) at 0-12 cm.

DISCUSSION

SUMMARY OF MAIN FINDINGS

Under rewilding, the top 12 cm of soils showed significant increases ($p<0.05$) in SOM, TC, TN and MBC compared with depth whereas the arable soil showed no significant change, and there was an inverse correlation between these parameters and pH. The differences between the rewilded and the arable soils in SOM, TC, TN and MBC were significant at the Northern and Middle Block, however, although increases were observed at the Southern Block, the results were not significantly different. At depth, any differences observed between the rewilded and arable soils were not significant, although increases in SOM, TC, TN and MBC were evident at the Middle and Northern Blocks. A shift in the microbial community profile was observed between the two depths along the PC1 axis of PCA and a movement along PC2 was observed between the rewilded and arable soils.

DISCUSSION OF RESULTS

Changes observed from agriculture to rewilding

The increases of SOM, TN (as SOM is a major source of nitrogen), and TC (which accounts for 50% of SOM on average (Weil and Brady, 2017)) found in the top 12 cm in the shift from agriculture to rewilding could be explained by increased litter input and root residues under permanent vegetation, as well as the effect of grazing herbivores. Grazing stimulates root growth and plant growth, drawing carbon down from the atmosphere and reducing nitrogen losses. Although carbon sequestration depends on various factors, grazing animals have been shown to increase carbon sequestration, as long as the grazing intensity ensures that the rate of grass consumption does not exceed the rate of plant growth (Garnett *et al*, 2017). At both depths, the strongly positive correlation of MBC with SOM (r=0.89) (and TC, r=0.90; TN r=0.89) is expected as microbial populations increase as more food becomes available.

The inverse correlations found between pH, and SOM, and thus TC, TN and MBC could occur for a variety of factors. The soils of the Wickham series are naturally acid, but base-rich (Cranfield University, 2019). Although increased SOM buffers soil pH, the increased acidity in the top 12 cm of the rewilded soils could be due to the accumulated SOM, increased microbial activity and root respiration, all of which can acidify soil (Weil and Brady, 2017), whilst the higher pH found at 0-12 cm in the arable soils could be a result of agricultural inputs, for which no information is available.

The shift in the microbial community structure observed along PC1 in the top 12 cm suggest that the changes in SOM, C and N and pH play a significant role in regulating the communities of microorganisms as the arable soils have the lowest amounts of SOM, C and N, and the highest pH, whilst the greatest shift observed was in the Northern and Middle Blocks which have the highest amounts of SOM, C and N. This is supported by the significant correlation of Factor 1 from the PCA of the PLFA analysis with SOM (r=0.81) in the top 12 cm and also with TC (r=0.73), TN (r=0.75) and pH (r=-0.66). The differences in these parameters between the two depths would also explain the change in microbial community structure observed between 0-12 cm and 12-25 cm. Correlations from both depths together show that Factor 1 from the PCA of PLFA inversely correlates with the C:N ratio. This supports other studies which show that the movement in the microbial community structure is influenced by fluctuations in C and N (Xue et al, 2018) as different microbes require varying proportions of C and N to thrive (Weil and Brady, 2017). The higher C:N ratio in the rewilded soils may result from greater organic matter input and an accumulation over time of the fraction of organic

matter which is difficult to break down. Fungi are known to prefer a higher C:N ratio, whilst bacteria favour a lower ratio, (Weil and Brady, 2017) so higher results in the fungal biomarker, 18:2ω6,9**,** would be expected in the rewilded soils in the top 12 cm. However the opposite was observed, but this may be due to grazing effects which have been shown to generally increase bacterial richness and reduce fungal richness (Eldridge et al, 2017).

The change from intensive agriculture to no-till

Although increases in SOM, TC and MBC were observed between the Southern Block and the arable soils, the results obtained were not significantly different at the 95% confidence level. The change three years ago from intensive arable agriculture to no-till on the arable soils sampled is likely to have resulted in a level of recovery from degradation under a previous, more intensive farming regime. Studies have shown that no-till soils have increased carbon storage (Smith *et al*, 2004), and higher microbial biomass than soils under more intensive forms of agriculture (Hsiao *et al*, 2019), and thus these increases could have partially eclipsed the results obtained from the rewilded soils. This hypothesis is based on what is found in the literature, but is not within the scope of this project as it cannot be said with all certainty that management of the arable soils under no-till has contributed to an increase in soil health.

Changes at depth

Increases of SOM were expected to be found at depth in the rewilded soils compared to arable soils, due to redistribution of SOM down the profile by soil organisms such as earthworms. The Northern and Middle Blocks have slightly more SOM at depth than the arable soils (+21%), but results were not significant, whilst the Southern Block shows the least amount of SOM, TC, TN and MBC at depth. This indicates that the soils of the Southern Block could have been the most degraded prior to restoration, having lost more of the SOM at this depth, which may have resulted in lower soil fauna populations, especially earthworms, which are responsible for much of the incorporation and mixing of SOM within a soil profile (Haygarth and Ritz, 2009). The results would also suggest that it takes significantly longer for organic matter to accumulate at depth in soils than at the surface

The only significant differences observed in the fungal biomarker, 18:2ω6,9, were between the top and bottom 12 cm of the arable soils. Herbicides are known to increase fungal biomass and reduce the bacterial biomass (Wang et al, 2018) and may have been

used under no-till resulting in the increase in the top 12cm, but it is not certain if this could result in the changes at depth.

Differences between the rewilded soils

The differences in SOM, and thus C, N and MBC between the rewilded soils could be for a variety of reasons. The land use prior to rewilding appears to have had the most significant effect. The soils sampled at the Northern Block had the highest levels of these parameters and were mostly under grass ley, where grass and crops or legumes are grown in rotation to increase soil fertility, prior to rewilding. The soils from the Middle Block, which yielded slightly lower results, had been under both arable and grass ley, while the soils at the Southern Block, which had the lowest levels of these parameters, had been under intensive arable agriculture during the same period. The fields sampled at the Southern Block were amongst the earliest to be taken out of production in that block, and as these were the least productive fields, and which further suggests that these soils could have been more degraded than the other soils.

The time when the different blocks were rewilded was considered as a contributing factor as carbon builds up slowly in the soil (Weil and Brady, 2017). However, the results from the top 12 cm do not indicate that time was a significant factor as the fields had been out of agricultural use for similar amounts of time (2001 - Middle, 2003 - Southern and 2004 – Northern), but perhaps the time when the herbivores were introduced contributed to the difference in results. The introduction of herbivores on the Southern Block occurred in 2009 as compared to 2001 (Middle) and 2004 (Northern). The fact that the Southern Block soils were grazed for a shorter period may also account for the lower levels of SOM, N, C and microbial biomass as grazing animals can contribute to soil carbon storage, but the effect of herbivores on the input and storage of carbon depends on a variety of factors such as the plant species and vegetative biomass, as well as the differences in grazing habits and dietary preferences of different herbivores as well as their stocking density, and the effects aren't yet fully understood (Chang *et al*, 2018).

The type of restoration was different at each location and may account for some differences in SOM, C, N and MBC. The Middle and Northern Blocks (if not under permanent pasture) had a wildflower mix and a grass mix sown as part of the restoration programme. In contrast, the Southern Block was set aside and this may have resulted in different species and structure of vegetation which would have affected the nature of the of the organic matter input into the soil which would have an effect on C, N and the microbial community structure. This would also have indirectly contributed to the

effects of grazing animals as, a difference in vegetation could also have influenced which plants the herbivores ate. The higher SOM levels in the soils of the Northern Block may be due to the different grazing regime at this block. The grazing density is lower (0.27 animals ha^{-1}, compared to 0.72 and 0.60 at the Middle and Southern Blocks respectively) and cattle are the only large herbivore present (Appendix D). Alternatively, while the Southern Block lay fallow and was allowed to regenerate naturally, this may have allowed more carbon to be locked up into the vegetative biomass (which is visibly different at the Southern Block) rather than the soil. During times of year when more organic matter is input into the system, microbes whose populations multiply quickly under optimal conditions (r-strategists) increase and break down the easily available carbon which is then used by plants (Weil and Brady, 2017). This leaves behind the harder to break down portion of SOM and the smaller populations of microbes which are niche specialists and whose populations tend to be smaller, but more stable (K-strategist microbes) which could account for the lower SOM and MBC at the Southern Block. If this was the case it would be expected that the SOM fraction of the soil was higher in complex compounds such as lignin, which are harder to break down, but this is outside the scope of this project.

The soils of the Northern and Middle blocks show the greatest movement along PC2 and the lack of grazing in the Southern Block fields as they lay fallow may have resulted in changes in vegetation which resulted in the different community structure observed here. Selective grazing can reduce the biomass of dominant plants allowing other species to move in and increase plant richness leading to more diverse conditions below ground and reducing domination of bacterial or fungal species and leading to a greater diversity in the microbial community (Eldridge et al, 2017). Further studies on both the vegetation community and structure and organic matter components in the soil and the grazing regimes of different herbivore communities would be required to establish connections.

BENEFITS TO ECOSYSTEM SERVICES.

Higher levels of SOM, carbon and nitrogen indicate improved nutrient cycling, carbon sequestration as well as primary production. Increases in SOM are known to improve the structure of clay soils (Weil and Brady, 2017) as well as improve their water-holding capacity, and augment their permeability which makes them less vulnerable to waterlogging. Increased SOM thus confers additional benefits of flood mitigation as well as making soils more resilient in times of drought. The higher levels of carbon sequestration in the rewilded soils indicate that rewilding can positively contribute to

climate regulation. Although CH_4 and CO_2 are also released to the atmosphere by herbivores, appropriate stocking rates have been shown to offset these losses (Garnett *et al*, 2017). The increase in soil microbial biomass will increase the rate of SOM decomposition, and therefore nitrogen mineralisation converting it into a form available to plants, and thus benefitting soil fertility, nutrient cycling and increasing primary production. Although soil microbes also release CO_2 as SOM is broken down, overall the net gain in soil carbon will counteract the losses. Indirect benefits to other ecosystem services also occur due to increased restoration of soils (Fig. 9).

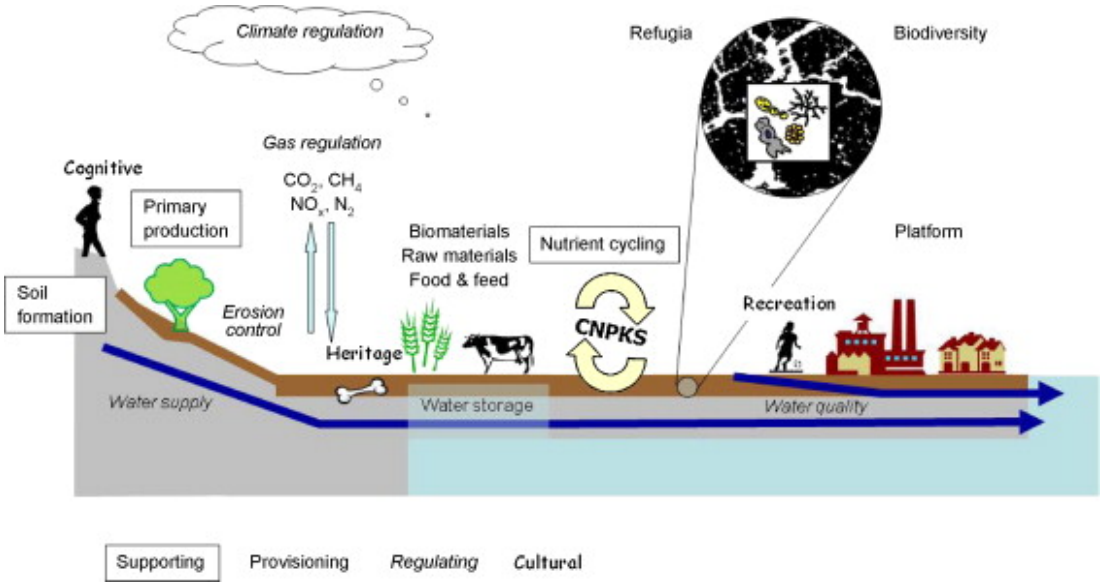

Figure 9. The key ecosystem goods and services provided by soil systems.
(Source: Haygarth and Ritz, 2009)

CONCLUSION

Under rewilding, greater inputs of SOM into the soil system are thought to be a result of permanent vegetation cover and the effects of grazing animals. The changes in SOM resulted in increased TC and TN, providing nutrients which allowed microbial populations to multiply. The shift in microbiology appeared to be due to changes in levels of carbon and nitrogen, and the high correlation with SOM in the top 12 cm suggests that organic matter could be used as an indicator of microbiology and could therefore be used to manipulate the microbial community. Significant restoration of the soils occurred only in the top 12 cm and changes below this depth may take

significantly longer than was thought and could be due to other factors such as changes in aeration and soil water conditions.

The influence of the way in which each of the rewilded blocks has had an effect on the soil is uncertain. Variations between the rewilded soils appeared to be mainly due to the intensity of land use prior to rewilding which would have affected the degree of degradation of the soils, but effects from different vegetation community structures as well as different grazing regimes on the soil properties cannot be discounted.

The change in land-use management at the arable site from intensive agriculture to no-till is believed to have buffered the difference in results between the arable and rewilded soils. Nonetheless, the results indicate that changes in soil properties and the soil microbiology directly benefit ecosystem services through SOM input, breakdown and accumulation; nutrient cycling and primary production, as well as indirectly benefitting other services such as climate regulation, water regulation and purification.

Further studies, which involve a more comprehensive soil sampling strategy within each rewilded block as well as between the blocks, would be recommended. This would provide more detailed information about the present properties of soils as a result of prior land use. In turn, this would allow more inferences to made on how the different methods of rewilding may have affected the outcomes and allow comparisons to be made between the blocks. Additionally, if possible, comparisons should be made against soils from the same area and of the same soil series which are still under intensive agriculture to give a better indication of the extent of the benefits to soils of rewilding.

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REFERENCES

Andriuzzi, W.S. and Wall, D.H. (2018) Soil biological responses to, and feedbacks on, trophic rewilding. 373. *Philosophical Transactions of the Royal Society B*. https://doi.org/10.1098/rstb.2017.0448

Bagchi, S., Bhatnagar, Y.V. and Ritchie, M.E. (2012) 'Comparing the effects of livestock and native herbivores on plant production and vegetation composition in the Trans-Himalayas', *Pastoralism: Research, Policy and Practice,* 20122:21.

Blouin, M., Hodson, M. E., Delgado, E. A., Baker, G., Brussaard, L., Butt, K. R., Dai, J., Dendooven, L., Peres, G., Tondoh, J. E., Cluzeau, D. and Brun, J. (2013) 'A review of earthworm impact on soil function and ecosystem services.' *European Journal of Soil Science*, 64: 161-182.

Breure A.M. (2004) 'Soil Biodiversity: Measurements, indicators, threats and soil functions', *First International Conference Soil and Compost Eco-Biology*. León, 83-96. Spain.

British Standards Institution (994). BS 7755-3.1:1994, ISO 11465:1993 Soil quality. Chemical methods. Determination of dry matter and water content on a mass basis by gravimetric method. London: BSI.

British Standards Institution (1995) BS 7755-3.8:1995, ISO 10694:1995 Soil quality. Chemical methods. Determination of organic and total carbon after dry combustion (elementary analysis) . London: BSI.

British Standards Institution (1997) BS EN ISO 14240-2:2011. Soil quality. Determination of soil microbial biomass. Fumigation-extraction method. London: BSI.

British Standards Institution (2000) BS EN 13039:2000 Soil improvers and growing media. Determination of organic matter content and ash. London: BSI.

British Standards Institution (2001) BS EN 13654-2:2001 Soil improvers and growing media. Determination of nitrogen. Dumas method. London: BSI.

British Standards Institution (2005) BS ISO 10390:2005 Soil quality. Determination of pH. London: BSI.

Cerqueira, Y., Navarro, L.J., Maes, J., Marta-Pedroso, C., Honrado, J.P. and Pereira, H.M. (2015) 'Ecosystem services: The opportunities of rewilding in Europe', in Pereira H.M. and Navarro L.M. (eds) *Rewilding European Landscapes*. Springer: Heidelberg, pp 47–64.

Chang, Q., Wang, L., Ding, S., Xu, T., Li, Z., Song, X., Zhao, X., Wang, D. and Pan, D. (2018) 'Grazer effects on soil carbon storage vary by herbivore assemblage in a semi-arid grassland'. *Journal of Applied Ecology.* September. Volume 55, Issue 5, pp 2517-2526.

Corlett, R.T. (2019) 'Auditing the wild: how do we assess if rewilding objectives are achieved?' in *Rewilding* Pettorelli, N., Durant, S.M. and du Toit, J.T. (eds), 2019 Cambridge: Cambridge University Press.

Dewe, W.S. and Senge, M. (2015) 'Earthworm Diversity and Ecosystem Services Under Threat*'. Reviews in Agricultural Science*, 3:25-35.

Dominati, E., Patterson, M. and Mackay, A. (2010). 'A framework for classifying and quantifying the natural capital and ecosystem services of soils'. *Ecological Economics, 69, 1858-1868.*

Duiker, S.W. (2002) *Agronomy Facts 63 – Diagnosing Soil Compaction Using a Penetrometer (Soil Compaction Tester).* Penn State College of Agricultural Sciences. Available at: https://extension.psu.edu/diagnosing-soil-compaction-using-a-penetrometer-soil-compaction-tester (Accessed: 22 May 2019)

Eldridge, D.J., Delgado-Baquerizo, M., Travers, S.K., Val, J., Oliver, I., Hamonts, K., and Singh, B,K. (2017) 'Competition drives the response of soil microbial diversity to increased grazing by vertebrate herbivores.' *Ecology*, 98(7), 2017, pp. 1922–1931

FAO (2015) '*Soils are endangered, but the degradation can be rolled back'*. 4 December. Available at: http://www.fao.org/news/story/en/item/357059/icode/ (Accessed: 20 July 2019)

Fraser, C. (2010) *Rewilding the World*. New York: Picador. ISBN: 9780312655419

Frostegård, Å. and Bååth, E. (1996) 'The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil.' *Biology and Fertility of Soils, 22, 59-65.*

Frostegård, Å., Tunlid, A. and Bååth, E. (2010) 'Use and misuse of PLFA measurements in soils.' *Soil Biology and Biochemistry*, Volume 43, Issue 8, August, pp. 1621-1625.

Garnett, T., Godde, C., Muller, A., Röös, E., Smith, P., de Boer, I.J.M., zu Ermgassen, E., Herrero, M., van Middelaar, C., Schader, C. and van Zanten, H. (2017). *Grazed and Confused? Ruminating on cattle, grazing systems, methane, nitrous oxide, the soil carbon sequestration question – and what it all means for greenhouse gas emissions.* FCRN, University of Oxford.

de Graaff, M.A., Adkins, J., Kardol, P. and Throop, H.L. (2014) 'A meta-analysis of soil biodiversity impacts on the carbon cycle.' *Soil.* 1, 257–271. DOI:10.5194/soil-1-257-2015

Graves, A.R., Morris, J., Deeks, L.K., Rickson, R.J. & Kibblewhite, M.G., Harris, J.A., Farewell, T. and Truckle, I. (2015) The total costs of soil degradation in England and Wales. *Ecological Economics*. 119. 399-413.

Haygarth, P.M. and Ritz, K. (2009) 'The future of soils and land use in the UK: Soil systems for the provision of land-based ecosystem services.' *Land Use Policy,* Volume 26, Supplement 1, December, pp S187-S197.

Hilton, A. and Armstrong, R. (2006) 'Statnote 6: post-hoc ANOVA tests.' *Microbiologist*, vol. 2006, pp. 34-36.

Hsiao, C. J.; Sassenrath, G. F.; Zeglin, L.; Hettiarachchi, G.; and Rice, C. (2019) 'Changes in Soil Microbiology Under Conventional and No-Till Production During Crop Rotation', *Kansas Agricultural Experiment Station Research Reports*: Vol. 5: Iss. 2. https://doi.org/10.4148/2378-5977.7746

IUCN (2019) *Commission on Ecosystem Management. Rewilding*. Available at: https://www.iucn.org/commissions/commission-ecosystem-management/our-work/cems-task-forces/rewilding (Accessed: 6 August 2019)

Johns, D. (2019) 'History of rewilding: ideas and practice' in *Rewilding* Pettorelli, N., Durant, S.M. and du Toit, J.T. (eds) Cambridge: Cambridge University Press.

Met Office (2019) *UK Climate Averages*. Available at: https://www.metoffice.gov.uk/research/climate/maps-and-data/uk-climate-averages/gcpffxw47 (Accessed: 8 July 2019).

Miller, J.R. and Hobbs, R.J. (2019) 'Rewilding and Restoration' in *Rewilding* Pettorelli, N., Durant, S.M. and du Toit, J.T. (eds) Cambridge: Cambridge University Press.

Monbiot, G. (2013). *Feral: searching for enchantment on the frontiers of rewilding*. London: Penguin. ISBN: 9780226325279.

National Soil Resources Institute (NRSI) (2019) Soils Site Report for location 514169E, 122278N, 5km x 5km, National Soil Resources Institute, Cranfield University. Accessed via: https://www.landis.org.uk/sitereporter

Soil Association, Parfey, S. (2017) '*Strategies for Soil Sampling'*. Available at: https://www.soilassociation.org/farmers-growers/farming-news/2017/strategies-for-soil-sampling/ (Accessed: 5 June 2019)

Panakoulia, S.K., Nikolaidis, N.P., Paranychianakis, N.V., Menon, M., Schiefer, J., Lair, G.J., Krám, P., and Banwart. S.A. (2017) 'Factors controlling soil structure dynamics and carbon sequestration across different climatic and lithological conditions', in

Advances in Agronomy, A.B. Steven, L.S. Donald (Eds.), Academic Press, Chapter 9, pp. 241-276

Pettorelli, N., Durant, S.M. and du Toit, J.T. (2019) 'Rewilding: a captivating, controversial, twenty-first-century concept to address ecological degradation in a changing world' in *Rewilding,* Pettorelli, N., Durant, S.M. and du Toit, J.T. (eds) Cambridge: Cambridge University Press

Robinson, R.A. and Sutherland, W.J. (2002) 'Post-war changes in arable farming and biodiversity in Great Britain. *Journal of Applied Ecology,* 39, pp. 157-176

The National Archives (2019). *The Cabinet Papers: The Farming and Agriculture Acts*. Available at: http://www.nationalarchives.gov.uk/cabinetpapers/themes/farming-agriculture-acts.htm (Accessed: 27 May 2019)

NERC (2012) *Valuing Ecosystem Services: Case Studies from Lowland England – Annex 4 Knepp Castle Estate Re-wilding: Sussex*. Natural England Commissioned Report NECR101, August.

Parliament. Publications and Records. (2016) *Rewilding and Ecoystem Services,* Postnote (537), September. Available at: https://www.parliament.uk/postnotes (Accessed: 27 May 2019).

Robinson, R.A. and Sutherland, W.J. (2002) 'Post-war changes in arable farming and biodiversity in Great Britain.' *Journal of Applied Ecology*, 39, 157-176.

Sandom C.J. and Wynne-Jones S. (2019) 'Rewilding a country: Britain as a study case' in *Rewilding* Pettorelli, N., Durant, S.M. and du Toit, J.T. (eds) Cambridge: Cambridge University Press

Smith, P., 2004. Carbon sequestration in croplands: the potential in Europe and the global context. European Journal of Agronomy. 20, 229–236

Soulé, M. and Noss, R. (1998), 'Rewilding and Biodiversity: Complementary Goals for Continental Conservation', *Wild Earth* 8, pp. 19–28, Fall.

Skubala, P. (2013) 'Biodiversity and ecosystem services in soil under threat.' *Journal of Pollution Effects and Control.* 1: e101.

Tree, I. (2018) *Wilding: The return of nature to a British farm*. London: Picador. ISBN 9781509805099.

Wang, B., Adachi, Y. and Sugiyama, S. (2018) 'Soil productivity and structure of bacterial and fungal communities in unfertilized arable soil.' PLoS ONE 13(9): e0204085. https://doi.org/10.1371/ journal.pone.0204085

Weil, R. and Brady, N. (2017) *The Nature and Properties of Soils.* Global Edition (p. 484). Harlow, England: Pearson Education Limited.

Xue, P.-P., Carrillo, Y., Pino, V., Minasny, B., and McBratney, A. B. (2018). 'Soil Properties Drive Microbial Community Structure in a Large Scale Transect in South Eastern Australia'. *Scientific Reports*, *8*(1), 11725. https://doi.org/10.1038/s41598-018-30005-8

Zhang, Q., Wu, J., Yang, F., Lei, Y., Zhang, Q., & Cheng, X. (2016). 'Alterations in soil microbial community composition and biomass following agricultural land use change.' *Scientific Reports*, *6*, 36587. https://doi.org/10.1038/srep36587

APPENDIX A – Field measurements of unsaturated hydraulic conductivity, soil moisture and compaction.

Site	Replicate	Infiltration (cm/s)	Soil Moisture (%)	Compaction (cm)
Agricultural	1	0.00007	69.78	33.6
"	2	0.00011	61.5	28.2
"	3	0.00014	86.32*	35.4
Northern	1	0.00015	25.38	14.8
"	2	-	22.64	5.2
"	3	0.00003	29.26	21.2
Middle	1	0.00008	16.38	2
"	2	-	23.02	3.2
"	3	-	16.46	11.6
Southern	1	0.00016	34.42	2.2
"	2		25.40	5.4
"	3	0.00044	20.68	3.6

** Some readings were given as 'above table' due to the wet conditions. Further readings were taken to enable an average to be reached.*

APPENDIX B – (i) Mean values and standard error for soil properties of rewilded and arable soils at two soil depths.

Parameter	Depth (cm)	Location				p		
		A	N	M	S	Site	Depth	Site*Depth
pH	0-12	6.3 ±0.2	5.6 ±0.0	6.0 ±0.0	6.0 ±0.2	0.002445	0.000195	0.428437
	12-25	6.4 ±0.1	6.1 ±0.1	6.4 ±0.0	6.3 ±0.0			
OMC (%)	0-12	4.3 ±0.1	7.3 ±0.1	6.3 ±0.1	5.0 ±0.3	0.000000	0.000000	0.000076
	12-25	4.0 ±0.1	4.9 ±0.2	4.3 ±0.1	3.6 ±0.3			
Total C (%)	0-12	1.4 ±0.0	2.9 ±0.0	2.6 ±0.0	1.7 ±0.1	0.000000	0.000000	0.000018
	12-25	1.3 ±0.1	1.6 ±0.0	1.4 ±0.2	1.1 ±0.1			
Total N (%)	0-12	0.15 ±0.01	0.28 ±0.01	0.24 ±0.01	0.16 ±0.01	0.000000	0.000000	0.000281
	12-25	0.14 ±0.01	0.17 ±0.01	0.15 ±0.02	0.12 ±0.01			
C:N	0-12	9.7 ±0.3	10.2 ±0.3	10.7 ±0.1	10.5 ±0.3	0.094752	0.000005	0.083028
	12-25	9.4 ±0.2	9.1 ±0.2	9.4 ±0.1	9.2 ±0.2			
Microbial biomass C (µg/g)	0-12	271 ±25	727 ±45	646 ±58	453 ±28	0.000009	0.000001	0.000744
	12-25	274 ±23	304 ±65	428 ±19	251 ±29			
PLFA fungal biomarker 18:2ω6,9 (mol%)	0-12	3.6 ±04	3.0 ±03	2.9 ±02	3.2 ±04	0.322192	0.555770	0.025953
	12-25	1.7 ±02	3.5 ±06	3.0 ±04	3.7 ±05			

(Top = 0-12 cm and Bottom = 12-25 cm) at four locations: A = Agricultural; N = Northern Block; M = Middle Block; S = Southern Block. The effects of land use and depth upon soil properties as determined by Factorial ANOVA are shown with statistically significant results ($p<0.05$) are shown in red.

APPENDIX B (ii) – Percentage differences between 0-12 cm and 12-25 cm and from arable to rewilded

	Site	LOI %	Total C %	Total N %	C:N	PLFA - 18:2w6,9	Microbial Biomass C
	PERCENTAGE INCREASES/DECREASES FROM TOP TO BOTTOM						
ARABLE	A	7.6	12.7	9.8	3.1	111.4	-1.1
REWILDED	M	48.4	83.8	62.2	13.7	-3.1	50.8
REWILDED	N	49.0	83.4	63.5	12.3	-16.7	139.4
REWILDED	S	37.6	57.3	37.1	15.0	-14.3	80.6
REWILDED SOILS MEAN		45	75	54	14	-11	90
	DIFFERENCE ARABLE TO REWILDED (PERCENTAGE INCREASE OR DECREASE)						
TOP		46	65	53	8	-18	125
BOTTOM		8	5	7	-2	97	20

APPENDIX B (iii) – Statistical results – ANOVA (top) and Tukey Post Hoc (bottom

%LOI

Univariate Tests of Significance for LOI % (Knepp Stats Lab.sta in Workbook1)
Sigma-restricted parameterization
Effective hypothesis decomposition

Effect	SS	Degr. of	MS	F	p
Intercept	590.0417	1	590.0417	6679.717	0.000000
Site	15.2417	3	5.0806	57.516	0.000000
depth	14.1067	1	14.1067	159.698	0.000000
Site*depth	3.8767	3	1.2922	14.629	0.000076
Error	1.4133	16	0.0883		

Tukey HSD test; variable LOI % (Knepp Stats Lab.sta in KneppStats.stw) Homo

Site	depth	LOI %	1	2	3	4
S	B	3.633333	****			
A	B	3.966667	****			
M	B	4.266667	****	****		
A	T	4.266667	****	****		
N	B	4.900000		****		
S	T	5.000000		****		
M	T	6.333333			****	
N	T	7.300000				****

Total C

Univariate Tests of Significance for Total C % (Knepp Sta

Effect	SS	Degr. of	MS	F	p
Intercept	73.01082	1	73.01082	3204.571	0.000000
Site	3.48415	3	1.16138	50.975	0.000000
depth	4.01802	1	4.01802	176.358	0.000000
Site*depth	1.27268	3	0.42423	18.620	0.000018
Error	0.36453	16	0.02278		

Tukey HSD test; variable Total C % (Knepp Stats Lab.sta in KneppS

Site	depth	Total C %	1	2	3
S	B	1.070000		****	
A	B	1.283333	****	****	
M	B	1.416667	****	****	
A	T	1.446667	****	****	
N	B	1.570000	****		
S	T	1.683333	****		
M	T	2.603333			****
N	T	2.880000			****

Total N

Univariate Tests of Significance for Total N % (Knepp Sta

Effect	SS	Degr. of	MS	F	p
Intercept	0.749067	1	0.749067	2899.613	0.000000
Site	0.033900	3	0.011300	43.742	0.000000
depth	0.025350	1	0.025350	98.129	0.000000
Site*depth	0.008950	3	0.002983	11.548	0.000281
Error	0.004133	16	0.000258		

Tukey HSD test; variable Total N % (Knepp Stats Lab.sta in KneppS

Site	depth	Total N %	1	2	3
S	B	0.116667	****		
A	B	0.136667	****	****	
M	B	0.150000	****	****	
A	T	0.150000	****	****	
S	T	0.160000	****	****	
N	B	0.173333		****	
M	T	0.243333			****
N	T	0.283333			****

C:N RATIO

Univariate Tests of Significance for C:N (Knepp Stats Lat

Effect	SS	Degr. of	MS	F	p
Intercept	2287.666	1	2287.666	16421.76	0.000000
Site	1.053	3	0.351	2.52	0.094752
depth	6.193	1	6.193	44.46	0.000005
Site*depth	1.114	3	0.371	2.66	0.083028
Error	2.229	16	0.139		

Tukey HSD test; variable C:N (Knepp Stats Lab.sta in KneppStats.st

Site	depth	C:N	1	2	3
N	B	9.1	****		
S	B	9.2	****	****	
A	B	9.4	****	****	
M	B	9.4	****	****	
A	T	9.7	****	****	****
N	T	10.2		****	****
S	T	10.5			****
M	T	10.7			****

MICROBIAL BIOMASS C

Univariate Tests of Significance for Microbial Biomass C (

Effect	SS	Degr. of	MS	F	p
Intercept	4220563	1	4220563	886.5958	0.000000
Site	295355	3	98452	20.6813	0.000009
depth	264988	1	264988	55.6650	0.000001
Site*depth	136570	3	45523	9.5629	0.000744
Error	76167	16	4760		

Tukey HSD test; variable Microbial Biomass C (KneppResults Working 1Aug in K

Site	depth	Microbial Biomass C	1	2	3	4
S	B	250.8974	****			
A	T	271.1338	****	****		
A	B	274.0654	****	****		
N	B	303.7155	****	****		
M	B	428.4250	****	****		
S	T	453.1401		****	****	
M	T	646.2210			****	****
N	T	727.2244				****

pH

Univariate Tests of Significance forpH (Knepp Stats Lab.sta in Workbook1)
Sigma-restricted parameterization
Effective hypothesis decomposition

Effect	SS	Degrees of Freedom	MS	F	P
Intercept	900.0075	1	900.0075	23037.74	0.000000
Site	0.8719	3	0.2906	7.44	0.002445
Depth	0.9009	1	0.9009	23.06	0.000195
Site*depth	0.1144	3	0.0381	0.98	0.428437
Error	0.6251	16	0.0391		

Tukey HSD test; variable pH (Knepp Stats Lab

Cell No.	Site	pH	1	2
2	N	5.821667		****
4	S	6.130000	****	****
3	M	6.200000	****	
1	A	6.343333	****	

PLFA - Fungal biomarker 18:2w6,9

Univariate Tests of Significance for 18:2w6,9 (Knepp Sta

Effect	SS	Degr. of	MS	F	p
Intercept	226.6498	1	226.6498	434.0269	0.000000
Site	1.9703	3	0.6568	1.2577	0.322192
depth	0.1891	1	0.1891	0.3621	0.555770
Site*depth	6.3133	3	2.1044	4.0299	0.025953
Error	8.3552	16	0.5222		

Tukey HSD test; variable 18:2w6,9 (Knepp St

Site	depth	18:2w6,9	1
A	B	1.726417	****
M	T	2.877980	****
N	T	2.955044	****
M	B	2.971503	****
S	T	3.164959	****
N	B	3.547471	****
A	T	3.649338	****
S	B	3.691825	****

FUNGAL:BACTERIAL RATIO

Univariate Tests of Significance for PLFA Fungal:bacteria

Effect	SS	Degr. of	MS	F	p
Intercept	0.323609	1	0.323609	338.3504	0.000000
Site	0.003952	3	0.001317	1.3772	0.285623
depth	0.001121	1	0.001121	1.1725	0.294946
Site*depth	0.011300	3	0.003767	3.9381	0.027936
Error	0.015303	16	0.000956		

Tukey HSD test; variable F:B ratio (

A	B	0.058631	****
M	B	0.103918	****
M	T	0.107025	****
N	T	0.113234	****
S	T	0.125610	****
N	B	0.133048	****
S	B	0.141540	****
A	T	0.145950	****

APPENDIX B (iv) – Statistical results – Correlations

R-values from correlations of soil parameters at both depths, 0-12 cm and 12-25 cm.

BOTH DEPTHS

Correlations (KneppResults Working 5 Aug in Workbook1)

Variable	pH	LOI %	Total C %	Total N %	C:N	Microbial Biomass	18:2w6,9	Factor 1	Factor 2
pH	1.00	-0.78	-0.77	-0.76	-0.54	-0.61	-0.04	0.38	-0.39
SOM %	-0.78	1.00	0.97	0.97	0.59	0.89	-0.06	-0.34	0.60
Total C %	-0.77	0.97	1.00	0.99	0.66	0.90	-0.11	-0.39	0.51
Total N %	-0.76	0.97	0.99	1.00	0.53	0.89	-0.10	-0.29	0.55
C:N	-0.54	0.59	0.66	0.53	1.00	0.58	-0.07	-0.69	0.15
Microbial B	-0.61	0.89	0.90	0.89	0.58	1.00	-0.10	-0.25	0.58
PLFA Fung	-0.05	-0.04	-0.09	-0.09	0.00	-0.10	0.99	-0.48	0.26
18:2w6,9	-0.04	-0.06	-0.11	-0.10	-0.07	-0.10	1.00	-0.37	0.32
Factor 1	0.38	-0.34	-0.39	-0.29	-0.69	-0.25	-0.37	1.00	0.00
Factor 2	-0.39	0.60	0.51	0.55	0.15	0.58	0.32	0.00	1.00

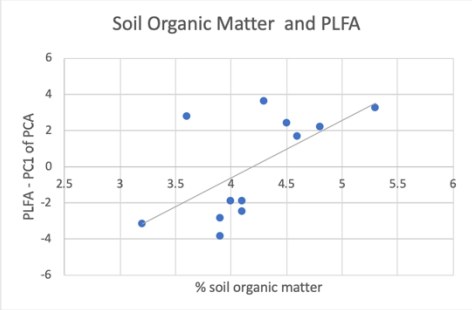
0-12 cm

	pH	LOI %	Total C %	Total N %	C:N	Microbial Biomass	18:2w6,9	Factor 1	Factor 2
pH	1.00	-0.75	-0.73	-0.74	-0.22	-0.65	0.70	-0.66	0.03
LOI %	-0.75	1.00	0.97	0.97	0.27	0.93	-0.49	0.81	-0.11
Total C %	-0.73	0.97	1.00	0.98	0.33	0.92	-0.52	0.73	-0.20
Total N %	-0.74	0.97	0.98	1.00	0.16	0.92	-0.47	0.75	-0.06
C:N	-0.22	0.27	0.33	0.16	1.00	0.32	-0.33	0.10	-0.69
Microbial B	-0.65	0.93	0.92	0.92	0.32	1.00	-0.34	0.74	-0.22
PLFA Fung	0.73	-0.53	-0.56	-0.52	-0.30	-0.39	0.98	-0.65	0.23
18:2w6,9	0.70	-0.49	-0.52	-0.47	-0.33	-0.34	1.00	-0.57	0.30
FACTOR 1	-0.66	0.81	0.73	0.75	0.10	0.74	-0.57	1.00	0.00
FACTOR 2	0.03	-0.11	-0.20	-0.06	-0.69	-0.22	0.30	0.00	1.00

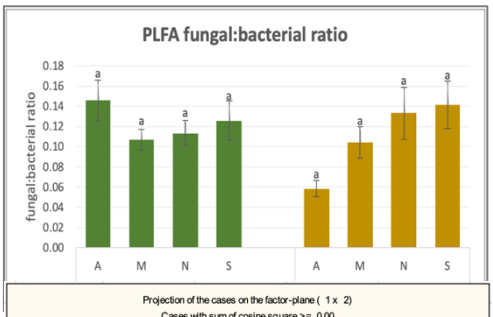
12-25 cm

	pH	LOI %	Total C %	Total N %	C:N	Microbial Biomass	18:2w6,9	Factor 1	Factor 2
pH	1.00	-0.33	-0.24	-0.25	0.01	0.41	-0.54	0.36	0.30
LOI %	-0.33	1.00	0.88	0.92	-0.05	0.37	0.13	-0.59	0.42
Total C %	-0.24	0.88	1.00	0.98	0.28	0.37	-0.02	-0.46	0.49
Total N %	-0.25	0.92	0.98	1.00	0.11	0.38	0.02	-0.51	0.48
C:N	0.01	-0.05	0.28	0.11	1.00	-0.03	-0.23	0.22	0.11
Microbial B	0.41	0.37	0.37	0.38	-0.03	1.00	-0.16	-0.43	0.61
PLFA Fung	-0.55	0.13	-0.03	0.01	-0.23	-0.21	1.00	-0.64	-0.59
18:2w6,9	-0.54	0.13	-0.02	0.02	-0.23	-0.16	1.00	-0.66	-0.53
Factor 1	0.36	-0.59	-0.46	-0.51	0.22	-0.43	-0.66	1.00	0.00
Factor 2	0.30	0.42	0.49	0.48	0.11	0.61	-0.53	0.00	1.00

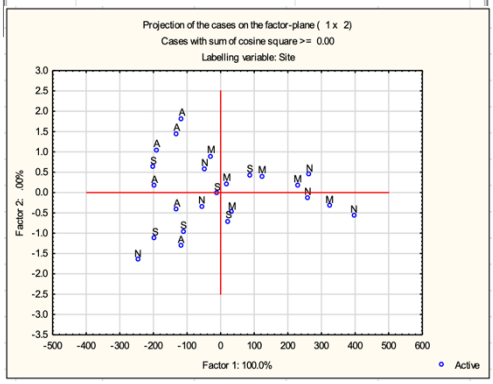
Appendix C. Results where no significant differences were found and were not included in the discussion



Clustering of data observed on scatterplot of SOM and PLFA. As data is not spread evenly along regression line of best fit, we cannot say that as SOM increases, so does the shift in PLFA. More samples would be required to give a clearer picture of what is going on here.

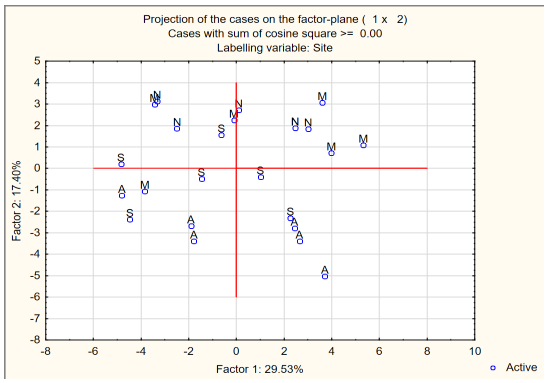


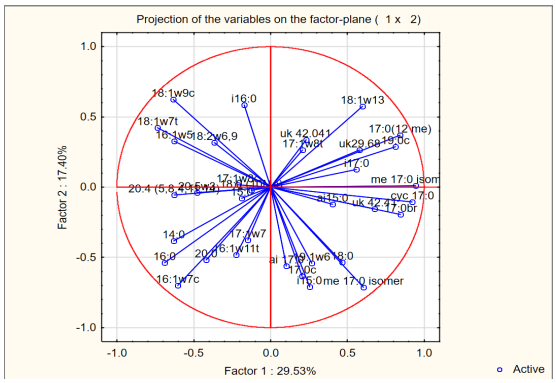
PLFA fungal/bacterial ratio: This was not discussed as further investigation of the literature revealed that there was even more doubt about the certainty of assigning a biomarker to bacterial or fungal than originally believed.



PCA of chemical and biological variables, (SOM, pH, C, N, C:N, 18:2w6,9; F:B; Microbial Biomass).

PCA of PLFA results – this was taken a step further and one-way ANOVA was carried out on the Factor 1 axis to show the movement of the communities.





APPENDIX D – Species, numbers and stocking density of grazing herbivores at the three blocks on the Knepp Estate.

Middle Block	2001-2	2002-3	2003-4	2004-5	2005-6	2006-7	2007-8	2008-9	2009-0	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16	2016-17	Mean ha^{-1} yr^{-1}	**MIDDLE**
Fallow Deer	188	242	397	493	431	350	275	365	280	170	190	260	300	300	425	220	0.53	Fallow Deer
Red Deer													17	17	44	34	0.05	Red Deer
Tamworth Pigs				10	7	7	18	14									0.02	Tamworth Pigs
Longhorn Cattle					39	30	48	62	58	60	67	58	68	85	90	81	0.11	Longhorn Cattle
Exmoor Ponies					7	10	14	18	13	16	1	0	5	5	4	6	0.01	Exmoor Ponies
																	0.72	***Mean stocking density***
Northern Block	2001-2	2002-3	2003-4	2004-5	2005-6	2006-7	2007-8	2008-9	2009-0	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16	2016-17	Mean ha^{-1} yr^{-1}	**NORTHERN**
Fallow Deer																	-	Fallow Deer
Red Deer																	-	Red Deer
Tamworth Pigs																	-	Tamworth Pigs
Longhorn Cattle						35	46	60	55	64	69	74	87	112	128	108	0.27	Longhorn Cattle
Exmoor Ponies																	-	Exmoor Ponies
																	0.27	***Mean stocking density***
Southern Block	2001-2	2002-3	2003-4	2004-5	2005-6	2006-7	2007-8	2008-9	2009-0	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16	2016-17	Mean ha^{-1} yr^{-1}	**SOUTHERN**
Fallow Deer									30	81	81	100	100	100	140	165	0.22	Fallow Deer
Red Deer													13	13	26	14	0.04	Red Deer
Tamworth Pigs									35	17	22	33	6	18	9	7	0.04	Tamworth Pigs
Longhorn Cattle									77	92	116	129	264	110	93	94	0.27	Longhorn Cattle
Exmoor Ponies									11	13	15	17	10	10	11	10	0.03	Exmoor Ponies
																	0.60	***Mean stocking density***

APPENDIX E – Raw data.

Site	% soil moisture (field moist to 105)		pH		LOI %		Total C %		Total N %		C:N		PLFA		Microbial Biomass C		PLFA F:B ratio	
	TOP	BOTTOM	TOP	BOTTOM	TOP	BOTTOM	TOP	BOTTOM	TOP	BOTTOM	TOP	BOTTOM	TOP	BOTTOM	TOP	BOTTOM	TOP	BOTTOM
A1	22.4	21.7	6.09	6.22	4.2	4.1	1.45	1.46	0.16	0.15	9.1	9.7	2.9579	1.3139	222.5	304.3	0.12	0.04
A2	20.4	20.6	6.61	6.65	4.4	3.9	1.37	1.18	0.14	0.13	9.8	9.1	4.4598	1.7511	301.8	288.6	0.18	0.06
A3	23.1	21.1	6.07	6.42	4.2	3.9	1.52	1.21	0.15	0.13	10.1	9.3	3.5303	2.1143	289.1	229.3	0.14	0.07
MEAN	21.98	21.13	6.26	6.43	4.27	3.97	1.45	1.28	0.15	0.14	9.66	9.37	3.65	1.73	271.13	274.07	0.15	0.06
SD	1.4	0.6	0.3	0.2	0.1	0.1	0.1	0.2	0.0	0.0	0.5	0.3	0.8	0.4	42.6	39.5	0.0	0.0
SE	0.8	0.3	0.18	0.12	0.1	0.1	0.0	0.1	0.0	0.0	0.3	0.2	0.4	0.2	24.6	22.8	0.0	0.0
N1	17.9	15.4	5.52	6.21	7.3	4.8	2.94	1.59	0.28	0.18	10.5	8.8	2.4073	3.4526	685.1	363.9	0.09	0.13
N2	16.3	13.9	5.65	5.8	7.1	4.6	2.83	1.5	0.27	0.16	10.5	9.4	3.0052	4.7100	679.6	174.0	0.12	0.18
N3	18.8	17.3	5.51	6.24	7.5	5.3	2.87	1.62	0.30	0.18	9.6	9.0	3.4526	2.4798	817.1	373.2	0.13	0.09
MEAN	17.7	15.5	5.6	6.1	7.3	4.9	2.9	1.6	0.28	0.17	10.2	9.1	3.0	3.5	727.2	303.7	0.11	0.13
SD	1.3	1.7	0.1	0.2	0.2	0.4	0.1	0.1	0.0	0.0	0.5	0.3	0.5	1.1	77.9	112.4	0.0	0.0
SE	0.7	1.0	0.0	0.1	0.1	0.2	0.0	0.0	0.0	0.0	0.3	0.2	0.3	0.6	44.9	64.9	0.0	0.0
M1	12.9	12.3	5.88	6.51	6.6	4.3	2.51	1.64	0.23	0.17	10.9	9.6	2.5027	2.2664	544.2	392.3	0.09	0.08
M2	13.4	11.9	5.98	6.36	6.2	4.0	2.68	1.1	0.25	0.12	10.7	9.2	2.7936	2.9935	649.8	438.4	0.11	0.10
M3	15.8	13.7	6.00	6.47	6.2	4.5	2.62	1.51	0.25	0.16	10.5	9.4	3.3376	3.6547	744.7	454.5	0.12	0.13
MEAN	14.1	12.6	6.0	6.4	6.3	4.3	2.6	1.4	0.24	0.15	10.7	9.4	2.9	3.0	646.2	428.4	0.11	0.10
SD	1.6	1.0	0.1	0.1	0.2	0.3	0.1	0.3	0.0	0.0	0.2	0.2	0.4	0.7	100.3	32.3	0.0	0.0
SE	0.9	0.6	0.0	0.0	0.1	0.1	0.0	0.2	0.0	0.0	0.1	0.1	0.2	0.4	57.9	18.7	0.0	0.0
S1	18.0	16.2	6.3	6.31	4.7	4.1	1.47	1.22	0.14	0.13	10.5	9.4	3.8263	4.2689	443.2	224.5	0.16	0.17
S2	15.6	12.4	5.83	6.28	5.5	3.6	1.82	1.08	0.18	0.12	10.1	9.0	2.5973	4.1916	506.4	308.7	0.09	0.16
S3	15.9	12.7	5.72	6.34	4.8	3.2	1.76	0.91	0.16	0.10	11.0	9.1	3.0713	2.6150	409.9	219.5	0.12	0.10
MEAN	16.5	13.8	6.0	6.3	5.0	3.6	1.7	1.1	0.16	0.12	10.5	9.2	3.2	3.7	453.1	250.9	0.13	0.14
SD	1.3	2.1	0.3	0.0	0.4	0.5	0.2	0.2	0.0	0.0	0.4	0.2	0.6	0.9	49.0	50.1	0.0	0.0
SE	0.8	1.2	0.2	0.0	0.3	0.3	0.1	0.1	0.0	0.0	0.3	0.1	0.4	0.5	28.3	28.9	0.0	0.0