CRANFIELD UNIVERSITY

Laura Koester genannt Kurwald

Does Rewilding restore soil biodiversity and function?

School of Water, Energy and Environment
Environmental Engineering

MSc
Academic Year: 2017 - 2018

Supervisor: Prof Jim Harris
Associate Supervisor: Dr Mark Pawlett
September 2018

Word count: 7724

CRANFIELD UNIVERSITY

SCHOOL OF WATER, ENERGY AND ENVIRONMENT
Environmental Engineering

MSc

Academic Year 2017 - 2018

LAURA KOESTER GENANNT KURWALD

Does Rewilding restore soil biodiversity and function?

Supervisor: Prof Jim Harris
Associate Supervisor: Dr Mark Pawlett
September 2018

This thesis is submitted in partial fulfilment of the requirements for the degree of MSc

ABSTRACT

Rewilding aims to return managed land back to a wilder state and improve the soil quality. Spontaneous regeneration of nature provides opportunities for living systems to re-connect or establish a network of ecological functions. This research investigated the influence of a process-led, non-goal orientated ecological rewilding on nutrient cycling and soil microbial community characteristics. The project aims were to investigate the implications of rewilding (chronosequence since rewilding) on soil microbial community, composition and function, and to compare the results to disturbed agricultural land and undisturbed ancient woodland. A subset of four rewilded soils and two reference soils (arable land and ancient woodland) was analysed. Results showed that soil nutrients (total carbon, total organic carbon, total nitrogen, organic matter content, phosphorous), soil microbial community (microbial biomass) and respiration (multiple substrate induced respiration) showed significant increase over time since rewilding. Nutrients and microbial community were significant higher in rewilded soils in comparison to the reference arable soil and converged towards ancient woodland soil. The soil compaction (physical parameter) decreased with time since rewilding from heavily compacted to a loose uncondensed soil. Rewilded soil parameters diverged from disturbed arable soils and converged towards undisturbed woodland soil values. The biological, chemical and physical soil parameters were dependent on the environmental development. The environmental development from pasture to mixed bush, shrub, tree and grassland influenced the soil condition, nutrients increased and elevated the microbial community while soil compaction decreased. Rewilding appears to be influencing and improving soil quality. Further studies to identify the environmental influence and trophic-level interactions for rewilding on soil quality are recommended.

Keywords:

Rewilding, Soil quality, Nutrient cycling, Trophic-level interactions, Ecosystem functions

ACKNOWLEDGEMENTS

I would like to acknowledge my supervisors, Prof. Jim Harris and Dr Mark Pawlett as well as Knepp Estate, particularly Penny Green for their mentoring and support over the period of my thesis work. Also, I would like to thank the technician's team from Vincent Lab and Lab 244, especially Paul Barton, Maria Biskupska and Nuannat Simmons for their patience and attendance to teach me biological and chemical analysis. Many thanks to my thesis proof readers Anita and Laura and everybody who supported and cheered me during this period of time!

TABLE OF CONTENTS

ABSTRACT i
ACKNOWLEDGEMENTS ii
LIST OF FIGURES iv
LIST OF TABLES v
LIST OF ABBREVIATIONS vi
1 Introduction 1
2 Methodology 5
2.1 Soil sampling 5
2.2 Laboratory analyses 8
2.3 Statistical analysis 12
3 Results 13
4 Discussion 26
5 Conclusion 32
REFERENCES 33

LIST OF FIGURES

Figure 1: Soil microbiology, ecology and biochemistry. The different scales of biodiverstiy show the influence of flora and fauna on nutrient cycling, microbial biomass and microbial respiration (Paul, 2015). 3

Figure 2: Knepp Estate with highlighted sampling spots. Red highlighted are the spots inside the Estate (rewilded fields) the ancient woodland and the agricultural land next to the Estate are highlighted in black 6

Figure 3: Influence of chronosequence since rewilding on vegetation 13

Figure 4: Histograms over chronosequence since rewilding and depth profile with grouping (a-e) of chemical soil parameter: pH, OMC, EC, TOC, TC, TN, C:N ratio, P 17

Figure 5: Histograms over chronosequence since rewilding and depth profile of microbial biomass and mirco respiration..... 20

Figure 6: Histogram over chronosequence since rewilding and depth profile with grouping (a-e) of PLFA 23

Figure 7: PCA: Movement of microbial community structure depending on identified PLFA in topsoil 0 – 10 cm and depth 10 – 20 cm, 20 – 30 cm. .. 25

LIST OF TABLES

Table 1: Different rewilding types and description (Corlett, 2016) 1

Table 2: Field names and status of rewilding, sample details and Field description 7

Table 3: Soil analysation methods: biological and chemical..... 8

Table 4: Indicator PLFA for Bacteria and Fungi after (Pawlett et al., 2013)..... 9

Table 5: Compaction measurement 14

Table 6: Comparison of p-values of soil chemical parameters. Statistical analysis was carried out with one-way ANOVA (0 – 10, 10 – 20, 20 – 30 cm) for arable land, woodland and rewilded sites. 14

Table 7: Comparison of p- and F-values computed with one-way and factorial ANOVA. P- and F-values of analysed chemical soil parameters. The significant differences (SD) of p-values are grey highlighted consistent of SD are outlined. 18

Table 8: Comparison of p-values of biological soil parameters 18

LIST OF ABBREVIATIONS

C:N	Carbon to Nitrogen ratio
EC	Electrical conductivity
LoI	Loss on Ignition
MB	Microbial biomass
MB-C	Microbial biomass Carbon
MR	Microbial respiration
MRR	Microbial respiration rate
MSIR	Multiple substrate induced respiration
OM	Organic matter
OMC	Organic matter carbon
PC	Principal componment
PCA	Principle component analysis
pH	pH
PLFA	Phospholipid fatty acids
SD	Significant difference
SOM	Soil organic matter
TC	Total Carbon
TN	Total Nitrogen
TOC	Total organic Carbon

1 Introduction

Rewilding is large-scale restoration of ecosystems with spontaneous regeneration of nature. Rewilding provides opportunities for living systems to re-connect or establish a network of ecological functions (Rewilding Britain, 2018). In the late 1960s the American scientist Paul Martin first raised the idea of reintroducing primitive animals as proxies for their extinct ancestors to restore the lost ecosystem functions (Janzen and Martin, 1982). He presumed a restoration like this would bring back the natural ecological processes and benefits which changed due to land use over the time (Jordan, Gilpin and Aber, 1990). In the 1980's this idea of restoring ecological functions by reintroducing extant species was growing in Europe. The Dutch ecologist Frans Vera followed this vision and reintroduced primitive cattle and horse breeds as representatives for their extinct ancestors at a 6,000-hectare nature reservoir in east Amsterdam (Vera, 2000). The aim of re-establishing them is not to re-create the past, it is to return the natural dynamic processes into today's landscape (Hobbs et al., 2014; Marren, 2016).The term 'rewilding' was established in the 1990s and originally meant to protect habitats, create corridors for the animals to move and reintroduce predators instead of trying to recreate the Pleistocene Epoch (Carey, 2016). Nowadays, rewilding has become a broader term and means to turn a managed area back to wild or as close as possible (Corlett, 2016). There are four different types of rewilding, Table 1.

Table 1: Different rewilding types and description (Corlett, 2016)

Rewilding type	**Description**
Trophic rewilding	Outlines to restore top-down trophic interactions
Pleistocene rewilding	Bring back to a pre-human Pleistocene baseline
Ecological rewilding	Allowing the nature to reveal itself
Passive rewilding	Little or no human interference

England has three main rewilding projects – The River Wandle, Wild Ennerdale and the Knepp Estate (rewilding britain, 2018). Knepp Estate near Horsham

Sussex is UK's largest low-land rewilding project with 1,400 ha. It restores natural ecological processes and provides home for nationally important species by removing fences and giving animals free roam of the estate (Houses of Parliament, 2016; Tree, 2017). After generations of agricultural use, the land of Knepp farm was taken out of contract. Inspired by the Dutch ecologist Frans Vera (2000), a 'process-led', 'non-goal-orientated' ecological rewilding, where nature takes control of its own was introduced (Tree, 2017; Mills et al., 2017). The whole rewilding process started in 2003. From 2003 onwards to 2006 all fields in the Knepp Estate were taken out of agricultural production. In 2009 fences for the animals were put in around the Estate (Kernon Countryside Consultants and Land Use Consultants, 2007; Tree, 2018). Now, Knepp Estate effectively consists of three areas, the south, a middle area and the north. The south area is a spontaneous regeneration of vegetation, the middle area is re-seeded with a flower mix and the north is sowed with a grass seed mix (Burrell, 2002; rewilding britain, 2018).

The Knepp Wildland project achieved Higher-Level Stewardship funding from the Government 2010 and reintroduced pristine animals. Cattle, red deer, Exmoor ponies, Tamworth pigs and fallow deer with different feeding, grazing and browsing habitats and preferences were re-established to encourage the vegetation in different ways. Trophic-level interactions and diverse biodiversity are influenced by the re-establishing. Flora and fauna are determining the above-ground organic matter what influences the nutrient cycling, microbial biomass and micro respiration (Figure 1). Trophic-level interactions are in continuous development and the change of one member of the chain influences the whole organisation (Burrell, 2002; Rewilding Britain, 2018; Tree, 2018).

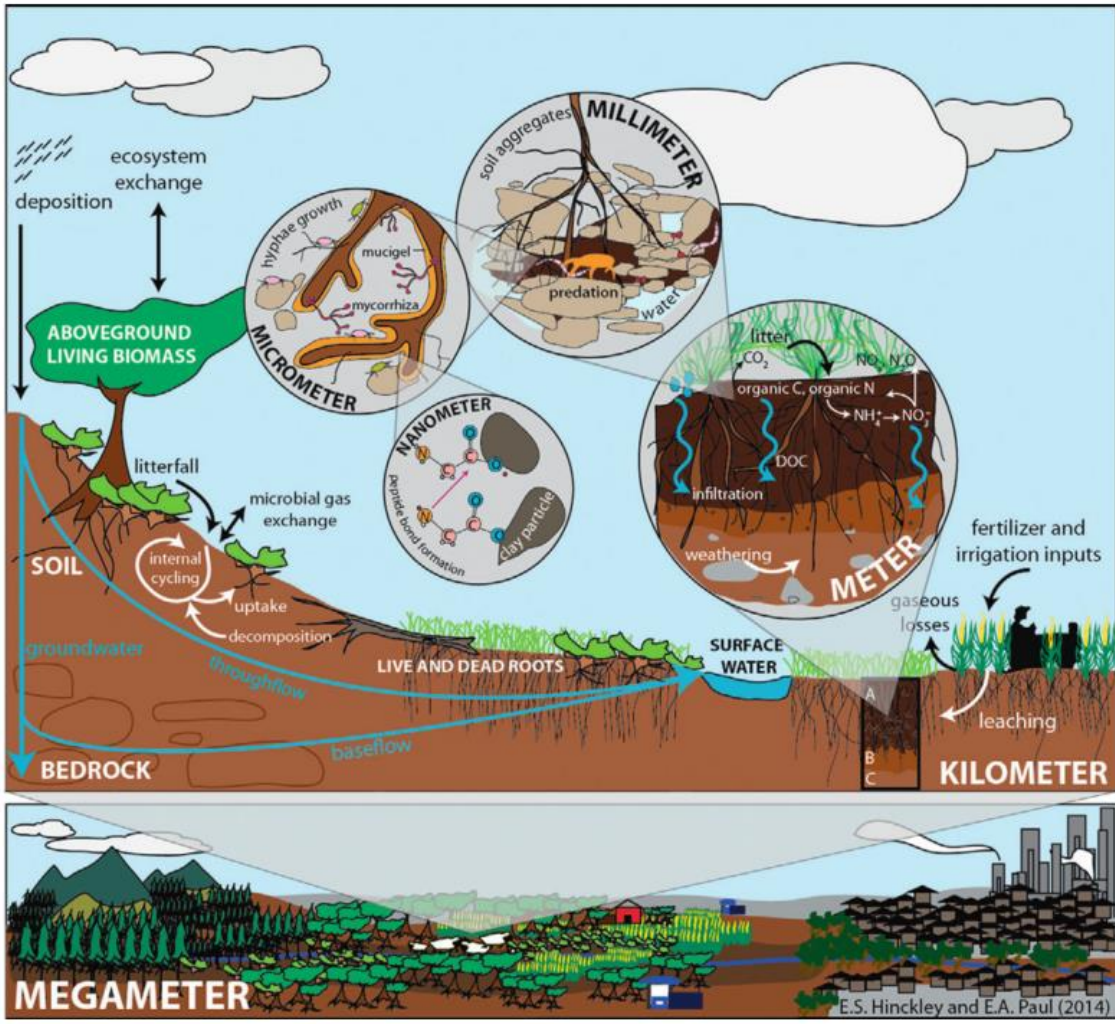


Figure 1: Soil microbiology, ecology and biochemistry. The different scales of biodiverstiy show the influence of flora and fauna on nutrient cycling, microbial biomass and microbial respiration (Paul, 2015).

Soil is composed of air, water and various microorganisms. It consists of inorganic compounds such as rocks and organic compounds of the flora and fauna that occupy in and around soil. The texture of soil can range from coarse sand to fine clay and the organic content varies between 5% to 80% by weight, depending on the area and the depth of the soil layer (Marshall, Holmes, 1988; Smith and Smith, 2009). Soil particles build the solid framework and are characterised depending on their diameter into smaller 2 µm, clay fraction, or bigger than 2 µm into silt, sand and gravel (Tan et al., 2007). Soil can be classified by its three properties, biological, chemical and physical. These three properties

determine the ecological function of the ecosystem and define the soil condition (Lal, 2015). The soil condition or soil quality can be measured by identification of microbial biomass and micro respiration, as well as biotic parameters (Doran and Zeiss, 2000). Biogeochemical processes in soil cause the storage, release and cycle of mainly nutrients as nitrogen, phosphorous, carbon and organic matter content. Nutrients as carbon, nitrogen and phosphorous can be retained in soil, transformed into plant available forms, or lost to air or water. Nutrient cycling can be measured through fertility indicators (mineral nitrogen, soil nitrate, soil phosphorous), organic matter indicators (C:N-ratio, microbial biomass carbon, organic matter, total organic carbon) and soil reaction indicators (pH) (Paul, 2015). Through photosynthesis plants convert atmospheric carbon dioxide into plant matter (carbohydrates, proteins, oils, fibres) made of organic carbon compounds. The organic matter passes into the soil system as soil carbon when plants and animals die and leave their remains on the soil. Soil organisms extract energy and nutrients out of organic matter and deallocate water, heat and CO2 back to the atmosphere (Kenrick, 2002; NRCS, 2011).

The project aimed to define the influence of rewilding on soil derived ecosystem functions and soil quality. The main objectives were:

1) To investigate implications of rewilding on soil microbial community composition (phenotypic profiles) and their function (catabolic profiles and nutrient cycling processes)

2) To compare rewilded soils to disturbed agricultural and undisturbed ancient woodland soil

3) To investigate implications of any effects of trophic-level interactions and ecosystem function on the microbial community

Hypothesis: Rewilding will restore soil quality (chemistry) and microbial community composition and function (biology). This restoration improves with time (artificial chronosequence) and throughout the depth profile.

2 Methodology

The present study examines the influence of rewilding on the soil condition. Soil biological and chemical parameters were determined (Table 3) at three depths (0–10 cm, 10–20 cm, 20–30 cm) within five areas of Knepp Estate (representing a chronosequence of time since rewilding) and compared to agricultural (disturbed) and ancient woodland (undisturbed) soil.

2.1 Soil sampling

The study was conducted on fields of the Knepp Estate near Horsham Sussex, England (grid reference TQ163209) and with agricultural land adjoining the Knepp Estate in the south. It is a low weald area with 300 m of clay over a bedrock of ironstone (Tree, 2017). The different sampling areas inside the Knepp Estate were chosen depending on their, time since rewilding was adopted as the management practice. The surrounding Wagstaffs Wood was set-aside in 1999 before the official rewilding process started and was detected as the oldest rewilded site. Sampling fields were elected to represent the process of rewilding and to constitute similar soil and environmental conditions. To fulfill similar soil and environmental conditions, such as soil structure/composition and weather/fauna, the decision was made to sample in only the north-east area of the Knepp Estate, Figure 2. Within this area, sampling spots of fields rewilded in different years (oldest 1999 to youngest 2006) were chosen. Next to the rewilded fields, ancient woodland was selected as a reference sampling area. This reference soil was designated to represent the ancient, initial soil conditions without human interference. The neighbouring arable land was part of the Knepp Estate until it was sold in 1983. The agriculture practice is still the same as it was 35 years ago under Knepp Estate. This arable soil was chosen as a reference to represent the initial state of the soil before rewilding started and since environmental conditions are the same. The fieldwork was carried out on the 11th of June 2018, temperature > 20°C, humidity >50% with light winds.

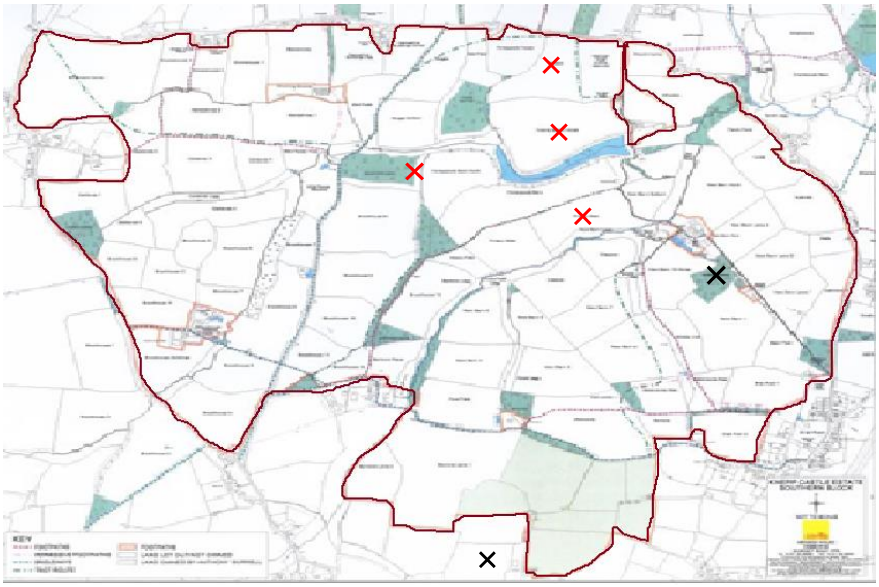


Figure 2: Knepp Estate with highlighted sampling spots. Red highlighted are the spots inside the Estate (rewilded fields) the ancient woodland and the agricultural land next to the Estate are highlighted in black

Four fields inside the Knepp Estate (representing a chrono sequence of time since rewilding), ancient woodland of Knepp Estate (undisturbed) and one agricultural (disturbed) soil were sampled at three depths (0–10 cm, 10–20 cm, 20–30 cm) according to the soil sampling plan. Three replicates of each field were collected. The samples were pulled out with a gouge auger at a depth of 0 – 10 cm, 10 – 20 cm and 20 – 30 cm and placed in self-sealing plastic bags. Also, the compaction of the sampled fields was measured. Compaction measurements were conducted with a penetrometer. The penetrometer was pressed in the soil until a pressure of 300 was achieved, with a maximum depth of 70 cm.Three replicate compaction tests (n=3) at two different spots within one field were representative of the soil compaction for the field.

Table 2: Field names and status of rewilding, sample details and Field description

Field name	Rewilded in	Number of samples	Field description
Agricultural land – Oakwood Farm	Still in agricultural use	Nine samples, three replicates 0 – 10 cm 10 – 20 cm 20 – 30 cm	Bought the land in 1983 from Knepp, is cultivating a rotation crop (beans – oat – wheat). Presently, beans as a recovery plant are cultivated. Very dry, clayey and partly heavily compacted soil
Ancient Woodland	-	Three samples, 0 – 10 cm	Original woodland. Very loose, soft soil (no compaction) with a dark brown colour. Layers of rotting leaves on top (Humus)
Surrounding Wagstaffs Wood	1999	Nine samples, three replicates 0 – 10 cm 10 – 20 cm 20 – 30 cm	Soil from two of the three sampling spots was very similar, the third one was darker, moister and less compacted. The surrounding area was dominated by trees and open grass land with bushes
Rainbow field	2003	Three samples, 0 – 10 cm	Small trees and high grass dominate the field. Soil is very sticky, dark brown clay and exhibits no compaction
Twenty-Seven Acres	2005	Nine samples, three replicates 0 – 10 cm 10 – 20 cm 20 – 30 cm	Lots of bushes and small trees are dominating the field. Very clayey dark soil with no colour change over depth profile, very soft soil shows no compaction
Fresco land	2006	Three samples, 0 – 10 cm	A very flat open field with mainly grass, flowers and a few bushes. The soil was solid and highly compacted

2.2 Laboratory analyses

In the laboratory, soil samples were analysed on their biological and chemical properties while physical properties were examined in the field. Different analytical methods (Table 3) are used to define the nutrient cycling processes, phenotypic and catabolic profiles.

Table 3: Soil analysation methods: biological and chemical

(Bartholomew, Clark and Scarsbrook, 1965; Carson, 2018; Cotching and Davies, 2015; Deparment of Geography, 2018; Fuhrman et al., 2005; Grisso et al., 2009; Lowery B., W.J. Hickey, M.A. Arshad, 1996; Microbial Insights, 2013; Pluske, Murphy and Sheppard, 1978; Quinlan, Richard; Wherrett, 2018; Regasamy, 2013)

Category	Method	Definition
Biological	Microbial Biomass (MB)	Is a measure of the mass of living component of soil organic matter (bacteria and fungi)
	Microbial Respiration (MicroResp, MR)	Is a measure of the respiration of the microbial community
	Phospholipid fatty acids (PLFA)	PLFA are a main component of the membrane of all microbes and provide information about the phenotype of the microbial community
Chemical	pH	pH is a measure of the concentration of hydrogen ions in the soil solution. It is divided into acidic, neutral and alkaline soils
	Electrical conductivity (EC)	Electrical conductivity or salinity is the ability of soil to transmit an electrical current
	Soluble Phosphorus (P)	Phosphorous is one of the most important nutrients for microbial acitiviy and plant production. It correlates to pH and is transported into the soil through rock and stone washouts and residues on soil surface.
	Total Carbon (TC)	Total carbon presents the sum of organic, elemental and inorganic carbonates

Category	Method	Definition
Chemical	Total organic Carbon (TOC)	TOC Presents the carbon contained within soil organic matter and influences many soil characteristics as colour, nutrient content, aeration and workability
	Total Nitrogen (TN)	Nitrogen in large quantities is needed for crop growth, it is essential for the production of biomass
	Loss of ignition (Lol)	Is a method to measure the organic matter content of the sediment

Laboratory analyses were carried out following British Standard Methods. Soil samples were fresh sieved (2 mm) and 300 g stored in a refrigerator (3°C).

Phospholipid fatty acids (PLFA) analysis, was performed on the fresh soil immediately after sampling and sieving. Approximately 10 g of fresh soil was extracted in a single-phase mixture using 0.8:1:2 citrate buffer; chloroform; methanol and fractionated into lipid classes by solid phase extraction. After, the phospholipid fraction was methylated, and fatty acids were cleaved from the phospholipid glycerol. In the last step, the fatty acid methyl esters were separated by gas chromatography (G.C. Agilent Technologies 6890N) and analysed using the G2070 ChemStation for G.C. systems software. The relative occurrence was calculated in mol%. By using a standard mixture of known PLFAs (SUPELCO, contains 26 fatty acid methyl esters) the main PLFAs in the soil samples were identified by comparison of the retention time and the concentration of each PLFA was determined Table 4.

Table 4: Indicator PLFA for Bacteria and Fungi after (Pawlett et al., 2013)

Category	**Organismal group**	**Indicator fatty acids**
Bacteria	Total bacteria	*i*15:0, *ai*15:0, 15:0, 16:1, *i*16:0, 16:1ω9, 16:1ω7*t*, *i*17:0, *ai*17:0, *cyc*-17:0, 17:0, *cyc*19:0

Category	Organismal group	Indicator fatty acids
Bacteria	Gram-positive bacteria	*i*15:0, *ai*15:0, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0
	Gram-negative bacteria	16:1, 16:1ω9, 16:1ω7*c*, 16:1ω7*t*,16:1ω5, 21:1
Fungi	Ectomycorrhizal	18:2ω6,9
	Arbuscular mycorrhizal	16:1ω5

Microbial biomass was determined using the fumigation-extraction method. The organic carbon from 15 g of moist, field soil sample was extracted with 0.5M potassium sulphate solution in fumigated and unfumigated samples. Fumigated samples were set in the cupboard for 24h ± 1, evacuated (6 times for 2 min) and shaken on a side-to-side shaker (set at $300\ min^{-1}$) for 30 min ± 1. After, both the fumigated and non-fumigated samples were filtered (filter paper Whatman #2) and stored in the fridge (4°C). The microbial carbon was estimated in the auto-analyser in comparison to the standard curve. The microbial biomass carbon was calculated by the increase in extracted organic carbon (difference of fumigated and non-fumigated) divided by the conversion factor 0.45.

Multiple substrate induced respiration profiles (MSIR) were determined using MicroResp™. Soil samples were incubated for seven days and detection plates as well as C-source stock were prepared in accordance with Campbell et al., (2003). The soil was dried to a range of 40 – 60% of its water holding capacity and added to the plates. Four replicates were set up for each soil substrate combination. A 25µL sample from each of the seven substrates (Gamma aminobutyric acid, alpha ketoglutaric, cirtic acid, l-malic, n-acetyl glucosamine, Galactose and Glucose) to analyse the substrate induced respiration, as well as distilled water for basal respiration was added to the soil. The deepwell plates were sealed with the detection plates and incubated for 5 h. After incubation, the detection plates were read at 570 nm with the microplate reader SoftMax Pro

software v5.4. (SMP 500-03517-XXXX). Identified data were transformed corresponding to Campbell et al. (2003) and the respiration rate was calculated.

Physiochemical analyses included the loss on ignition (LoI), pH, electrical conductivity (EC), available phosphorous (P), total carbon (TC), total organic carbon (TOC) and total nitrogen (TN). For the LoI (BS EN 13039:2000), the dehydrated soil was ashed in muffle furnace at 450°C for 4 hours ± 15 minutes and weighted. Soil pH was determined of air dried soil-samples in 1M KCl extract 1:5 mass to volume ratio (BS ISO 10390:2005). Electrical conductivity was extracted with water using an extraction ratio of 1:5 m/V to dissolve the electrolytes and measured with conductivity meter (BS 7755: Section 3.4:1994). To calculate the concentration of phosphorous soluble, the standard graph absorbance to concentration of phosphorous present was developed. After, phosphorus soluble was determined from air-dried soil samples treated with a 0.5M sodium hydrogen carbonate solution at pH 8.5 and then measured in spectrophotometer. The concentration of phosphorous soluble was calculated mathematically corresponding to the absorbance from the standard graph (BS 7755: Section 3.6:1995).

For total nitrogen (TN), total carbon (TC) and total organic carbon (TOC), the air-dried soil samples were grinded, sieved through a 0.2 µm sieve and transferred into temperature-resistant plastic bottles, which were placed in the oven for 2 hours at 105°C and cooled down in a desiccator. TN and TC were measured in the automatic sampler (elementar vario EL III) after being weighted (to 0.001 mg) and packed into aluminium-foil capsules. TOC samples were packed into silver foil capsules, weight to 0.001 mg and treated with 4M hydrochloric acid before drying at 90°C for 4 hours ±15 minutes. After, TOC samples were packed into larger aluminium-foil capsules and analysed in the automatic sampler (elementar vario EL III).

2.3 Statistical analysis

The experimental design comprised of two factors: chronosequence since rewilding (1) and soil depth (2) with triplicate of each. Data was determined for missing values and outliers. Outliers were verified on their reliability and deleted in case of misrepresentation. Statistical analysis was conducted with Statistica (Version 13.3) using $p< 0.05$ as the significant threshold value and graph were produced using Microsoft® Excel 2016. The biological and chemical soil properties were represented in histograms showing (1) and (2) using their relative frequency.

First, one-way ANOVA was used as a general linear model to identify the observed, weighted means of three representative samples (n=3) as well as standard error and significant differences between the soil samples. Second, Factorial ANOVA (general linear model) was applied to compare one dependent variable (biological or chemical property) over categories (1) chronosequence and (2) soil depth for a 95% confidence level. Post-hoc as Fisher LSD or Tukey HSD was conducted to categorise the homogeneous groups ($p< 0.05$). Histograms were created showing the mean, standard error and grouping for every single depth and trial. Results from the general linear models were gathered and modified in factor coordinates using principal component analysis (PCA). PCA, a dimensional reduction technique is used to identify potential groups, outliers, movements and correlations in the microbial community structure. The principle component scores (PC) were used to calculate and evaluate statistical differences and were presented in summary plots.

3 Results

External appearance of the fields

The rewilded sites were expected to visibly differ from each other regarding their vegetation. A spontaneous restoration with noticeable change over time was predicted. Investigation of the fields showed, that the vegetation changed with chronosequence since rewilding (Figure 3).

a) Arable land | **b)** Rewilded 2006 | **c)** Rewilded 2005 | **d)** Rewilded 2003 | **e)** Rewilded 1999

Figure 3: Influence of chronosequence since rewilding on vegetation

From arable land to oldest rewilded, the flora changed remarkably from open grass-land over shrub/bush-land to overgrown areas with pasture, shrubs and trees. Rewilded in 1999 (Figure 3e) showed a very developed environment with emerging forest and mature grown trees. Rewilding 2003 (Figure 3d) presented a mix of trees, bushes and grassland. Rewilded in 2005 (Figure 3c) was more spontaneous with a mix of grass- and bushland. The youngest rewilded field 2006 (Figure 3b), showed homogeneous vegetation cover with grass and scattered bushes. The arable land was dry with minimal vegetation sporadic on the soil (Figure 3a). There were significant visual differences between the rewilded soils and arable land. The differences related to the change of flora, and fauna and various recolonised animal species (Marren, 2016). Soil compaction of the different fields was not uniformly identified along the sites, Table 5.

Table 5: Compaction measurement

Field	**Depth until pushing 300**		**Compaction**	
Arable land	6 – 22 cm		Yes	
Rewilded 2006	52 – 67 cm		No	
Rewilded 2005	65 – 70 cm		No	
Rewilded 2003	63 – 70 cm		No	
Rewilded 1999	7 – 15 cm	70 cm	Yes	No
Ancient woodland	52 – 67 cm		No	

Soil analysis

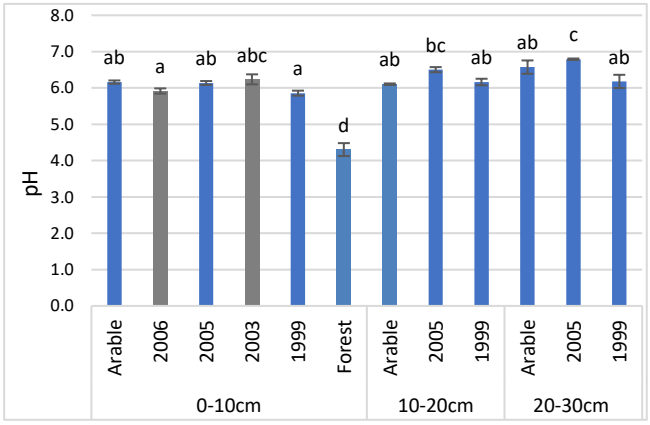
The soil was analysed on eight chemical soil parameters, which differed significantly between chronosequence since rewilding and over the depth. The p-value determined the significance of the results with $p < 0.05$ in all cases but EC (Table 6).

Table 6: Comparison of p-values of soil chemical parameters. Statistical analysis was carried out with one-way ANOVA (0 – 10, 10 – 20, 20 – 30 cm) for arable land, woodland and rewilded sites.

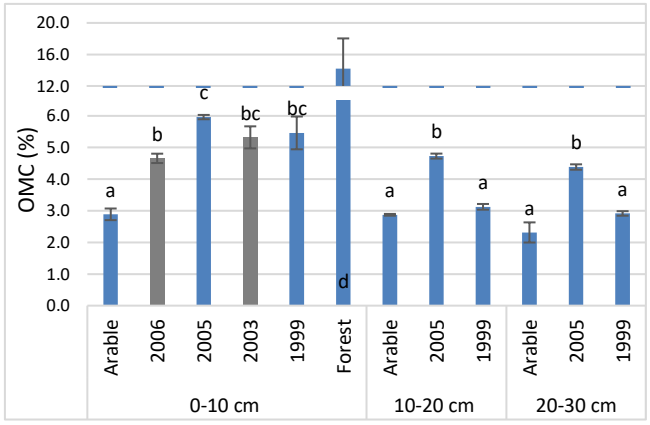
Chemical Parameter	pH	OMC	EC	TC	TN	C:N ratio	TOC	P
p-value	0.00000	0.00001	0.13001	0.00000	0.00000	.0.00000	0.00000	0.00000
SD	*	*		*	*	*	*	*

The examined soils were acidic ($pH < 7$), non-saline ($EC < 2$ dS/m), (Ullman, 2013)) and variable in soluble phosphorous over years and depth. There were slight variances in the pH between arable land and rewilded sites (mean

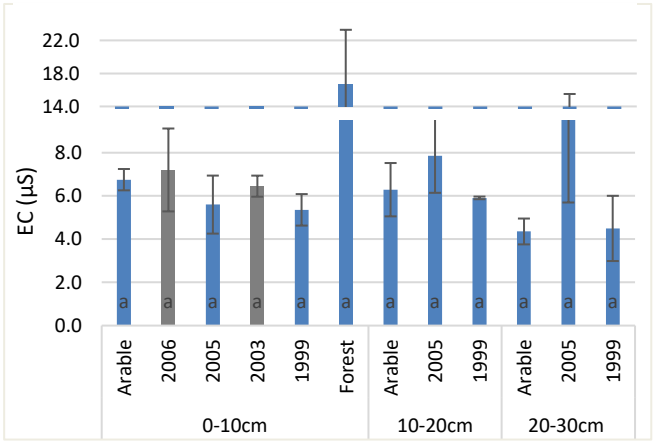
pH = 6.3). Forest pH was 32% lower than the mean, Figure 4a. The OMC increased 3.1% from arable land to rewilded in 1999 (over depth 0 – 10 cm). With depth, (from 0 – 10 cm to 20 – 30 cm) the OMC decreased, Figure 4b. The salinity (EC) increased from arable land to youngest rewilded and dropped out 25% to oldest rewilded site, Figure 4c. TOC, TC and TN behave similar, the amount of carbon and nitrogen raised from arable to youngest rewilded sites (2006, 2005), and slight dropped out by time of rewilding (2003,1999), Figure 4 d,e,f. The largest increase (arable to oldest rewilded) was found in sector 0 – 10 cm (TOC +1.1%, TC +1.2%, TN +0.1%) and lowest in sector 10 – 20 cm (TOC 1.1%, TC 1.2% TN 0.1%). Considerable fluctuation of soluble phosphorous was detected over depth 0 – 10 cm. Nevertheless, the amount of P declines within depths and increases from arable to rewilded before it decreases from youngest to oldest rewilded soil, Figure 4 h. The histograms show the combined results of the factorial and one-way ANOVA (one-way ANOVA: depth 0 – 10 cm for 2006, 2003 and woodland; grey arrow bars) as well as the Post-hoc grouping (a-e). P- and F-value were calculated using one-way ANOVA.



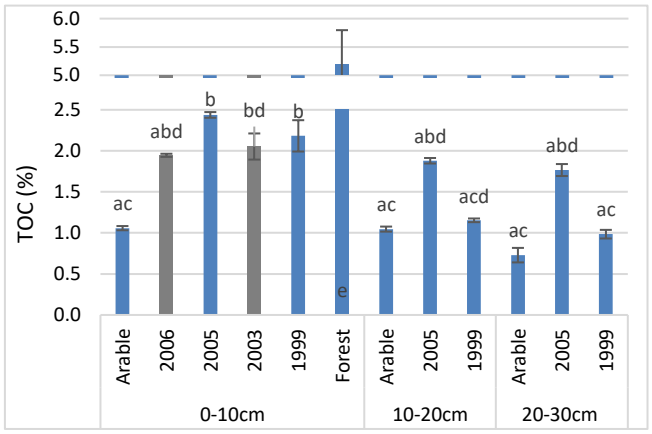
a) pH (mean ± standard error; n=3) of arable land, rewilded soils (rewilded in 2006, 2005, 2003, 1999) and ancient woodland (Forest) over depth 0 – 10; 10 – 20; 20 – 30 cm. There is a statistical significant difference between the groups at 5% confident level with ANOVA F(11,24)= 31.503, p= 0.00000, Posthoc TUKEY HSD.



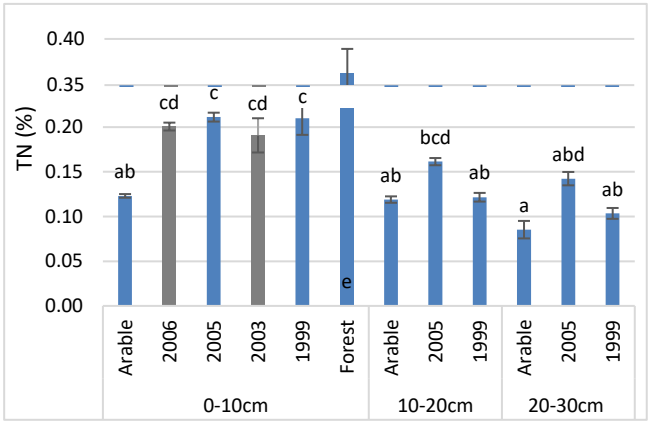
b) Figure b: Organic matter content in (%) (mean ± standard error; n=3) of arable soil and rewilded sites. Y-axis is broken (6-12%) as the high OMC content of the forest distorts the representation. There is a statistical significant difference between the groups at 5% confident level with ANOVA F(11,24)= 7.8198 p= 0.00001, Posthoc TUKEY HSD



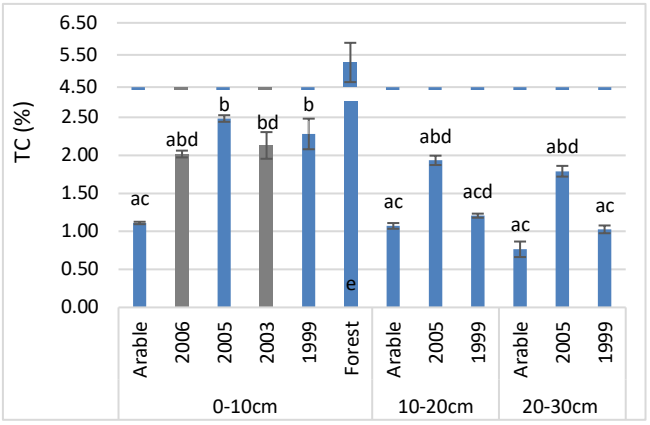
c) Figure c: Electronical conductivity in (µS) (mean ± standard error; n=3) of arable soil and rewilded sites. Y-axis was broken (8-14%) as the high salinity of the forest distorts the representation There is no statistical significant difference between the groups at 5% confident level with ANOVA F(11,24)= 1.7158 p= 0.13001, Posthoc Fisher LSD.



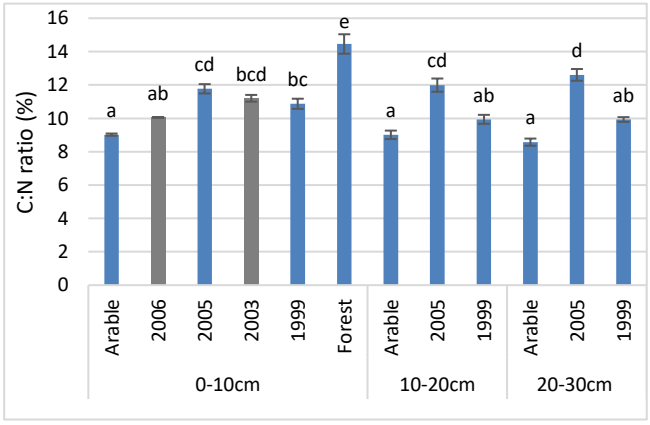
d) Figure d: Total organic Carbon in (%) (mean ± standard error; n=3) of arable soil and rewilded sites. Y-axis was broken (2.5-5%) as the high TOC content of the forest distorts the representation. There is a statistical significant difference between the groups at 5% confident level with ANOVA F(11,24)= 37.182 p= 0.00000, Posthoc TUKEY HSD.



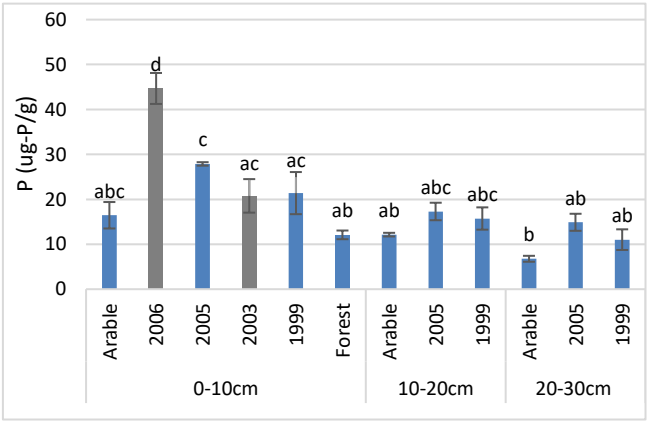
e) Figure e: Total Nitrogen in (%) (mean ± standard error; n=3) of arable soil and rewilded sites. Y-axis was broken (0.22 – 0.35%) as the high TN content of the forest distorts the representation of the other values. There is a statistical significant difference between the groups at 5% confident level with ANOVA F(11,24)= 38.582 p= 0.00000, Posthoc TUKEY HSD.



f) Figure f: Total Carbon in (%) (mean ± standard error; n=3) of arable soil and rewilded sites. Y-axis was broken (2.5 – 4.5%) as the TC of the Forest is 3% higher than 1999 0 – 10 cm and distorts the representation. There is a statistical significant difference between the groups at 5% confident level with ANOVA F(11,24)= 36.312 p= 0.00000, Posthoc TUKEY HSD.



g) C:N ratio in (%) (mean ± standard error; n=3) of arable soil and rewilded sites. Grouping shows the differences between arable, rewilded and ancient woodland. There is a statistical significant difference between the groups at 5% confident level with ANOVA F(11,24)= 34.202 p= 0.00000, Posthoc TUKEY HSD



h) Soluble Phosphorous in (ug-P/g) (mean ± standard error; n=3) of arable soil and rewilded sites. There is a statistical significant difference between investigated groups at 5% confident level with ANOVA F(11,24)= 15.425 p= 0.00000, Posthoc TUKEY HSD .

Figure 4: Histograms over chronosequence since rewilding and depth profile with grouping (a-e) of chemical soil parameter: pH, OMC, EC, TOC, TC, TN, C:N ratio, P

Further analysis showed, that there are differences between the p- and F-values of the one-way ANOVA and factorial ANOVA, Table 7. The woodland soil was identified as the main driver behind significant differences, as it never grouped together with arable or rewilded soils (but EC).

Table 7: Comparison of p- and F-values computed with one-way and factorial ANOVA. P- and F-values of analysed chemical soil parameters. The significant differences (SD) of p-values are grey highlighted consistent of SD are outlined.

	Parameter	pH	OMC	EC	TOC	TN	TC	C:N	P
One-way ANOVA	p-value	0.00000	0.00001	0.13001	0.00000	0.00000	.0.00000	0.00000	0.00000
	F-value F(11,24)	31.503	7.8198	1.7158	37.182	38.582	36.312	34.202	15.425
Factorial ANOVA	p-value	0.10772	0.00065	0.40761	0.00006	0.00103	0.00011	0.03093	0.74362
	F-value	2.2188	8.0855	1.0537	12.214	7.4168	10.946	3.3947	0.48923
	SD		*		*	*	*		

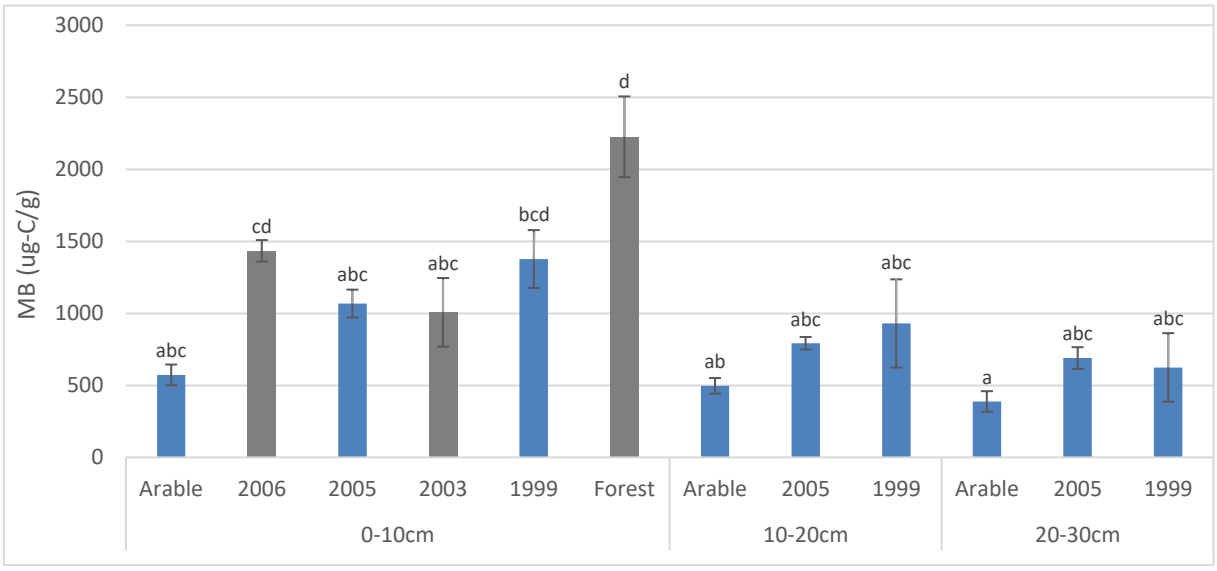
Furthermore, the soil was analysed on three biological soil parameters to examine the phenotypic functions, MB, MR and PLFA. There are some significant differences over depth and chronosequence since rewilding compared to arable land and ancient woodland. The significance of the results was indicated by the p-value ($p<0.05$ in all cases), Table 8.

Table 8: Comparison of p-values of biological soil parameters

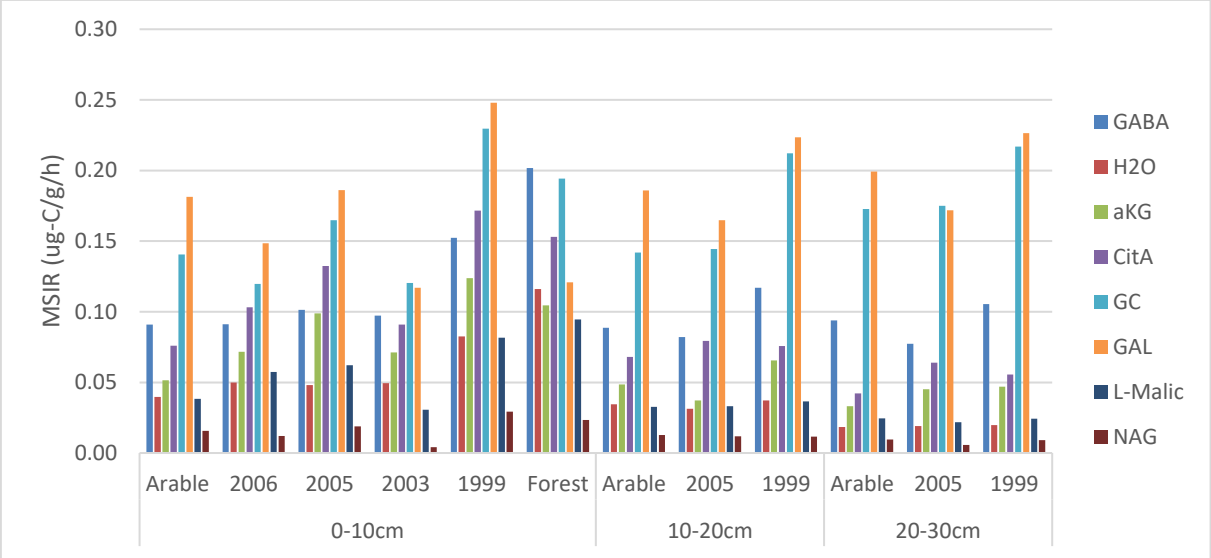
Biological parameter	MB	MR	PLFA
p-value	0.00001	0.00000	0.00022
SD	*	*	*

Microbial biomass, respiration and community were investigated between arable, rewilded and ancient woodland soils, Figure 5. The microbial biomass, analysed with one-way and factorial ANOVA, was shown to multiply over the time since

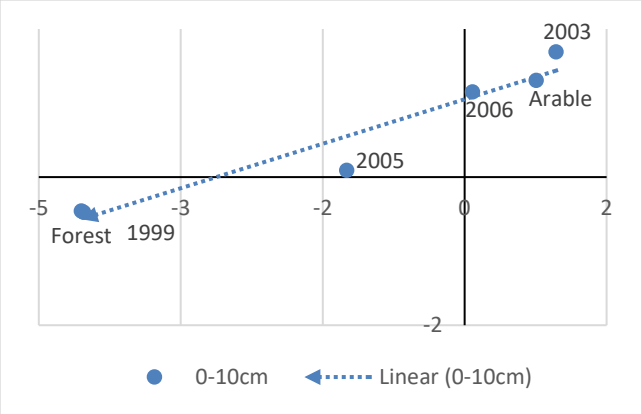
arable land was set aside. An increase by 140% in the microbial biomass carbon from arable to rewilded in 1999 (0 – 10 cm) was detected. In depth 10 – 20 cm microbial biomass carbon rose 87% from arable to oldest rewilded and 20 – 30 cm increased by 61% (Figure 5a). Multiple substrate induced respiration was quantified with one-way ANOVA and PCA (factor plane (1x2) with factor 1= 64.23% and factor 2= 22.18%). Factorial ANOVA performed on PCA scores showed a statistically significant difference between the groups. MSIR was measured after addition of chemicals to the soil. The use of different chemicals had a decisive influence on the respiration, highest respiration rate was measured from glucose and galactose treated soils (Figure 5b). Overall, the respiration rate changed with time of rewilding. The plot shows the transformation of respiration rate in topsoil which demonstrates a straight trend. Forest and oldest rewilded soil present a similar respiration rate (Forest (-4.05/-0.46), (1999(-4.03/-0.48)) (Figure 5c). In depth profiles of 10 – 20 cm and 20 – 30 cm, a similar change in respiration rate over time is displayed. There is a significant trendline from arable and youngest to oldest rewilded soil (Figure 5d).

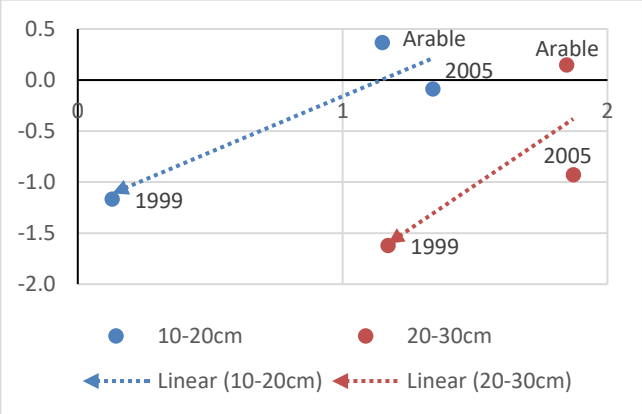


a) Microbial Biomass in (ug-C/g) (mean ± standard error; n=3) of arable soil, rewilded soil and ancient woodland. There is a statistically significant difference between the groups at 5% confident level with ANOVA F(11,24)= 8.7952 p= 0.00001, Posthoc TUKEY HSD



b) Multiple Substrate Induced Respiration in (ug-C/g/h) (mean; n=3) of arable soil, rewilded soil and ancient woodland. Microbial respiration was measured after addition of chemicals to the soil: Gamma aminobutyric acid, water, alpha ketoglutaric, citric acid, glucose, galactose, L-Malic, n-acetyl glucosamine. There is a statistical significant difference between the groups at 5% confident level with ANOVA Wilks lambda= 0.00029, F(88,120.94)= 3.3820 p=0.00000.





c) PCA of chronological change of MSIR rate in topsoil 0 – 10 cm. Factor coordinates generated with PCA (n=3, factor 1= 64.23 factor2= 22.18%) and ANOVA (mean, n= 3, p= 0.00000). Linear trend line shows the change of respiration.

d) PCA of chronological change of MSIR rate in 10 – 20 cm and 20 – 30 cm. Factor coordinates generated with PCA (n=3, factor 1= 64.23 factor 2= 22.18%) and ANOVA (mean, n= 3, p= 0.00000). Linear trend lines show the change of respiration for 10 – 20 cm and 20 – 30 cm.

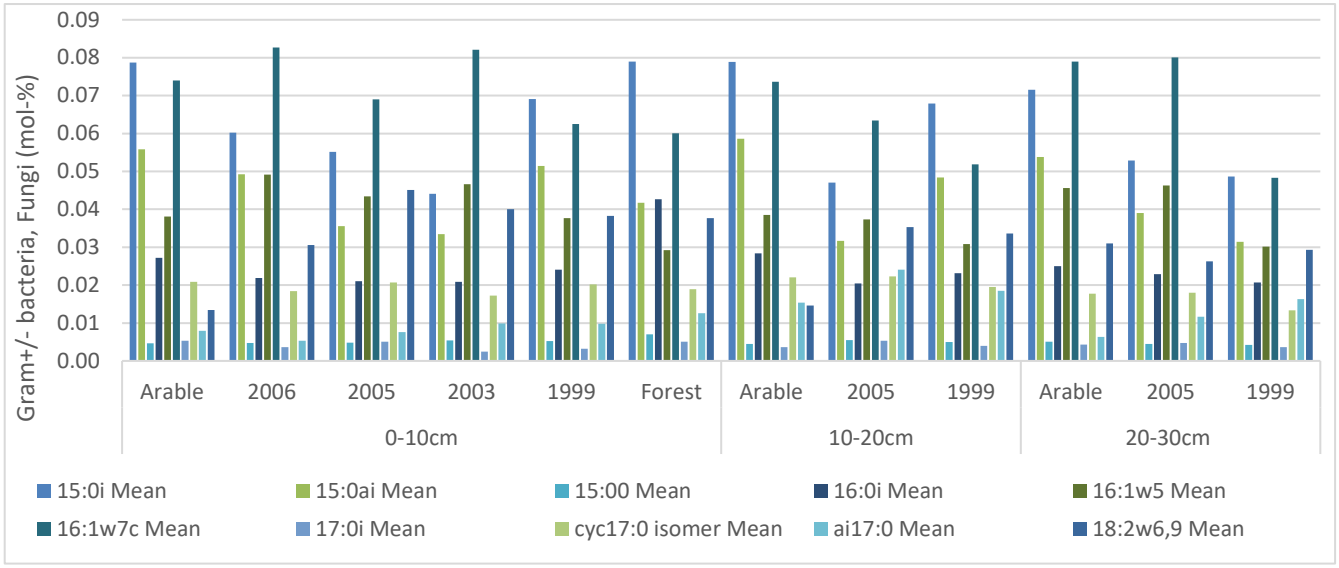
Figure 5: Histograms over chronosequence since rewilding and depth profile of microbial biomass and mirco respiration

Gram positive and negative bacteria as well as Fungi were identified on basis of their PLFAs. The PLFAs were investigated with ANOVA, a statistically significant difference between the groups was identified (Figure 6a), a deeper analysis with factorial ANOVA was carried out to outline differences in depths. Analysis of composition of microbial biomass showed development of gram positive (Figure 6b) and gram negative (Figure 6c) bacteria and fungi (Figure 6d). Gram-positive bacteria exhibit three different trends:

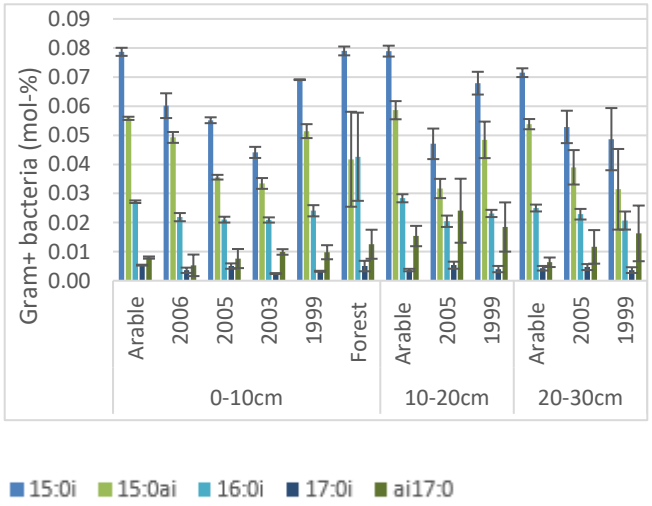
1) 15:0i, 15:0ai and 16:0 decreased from arable to rewilded in 2003 and increased from 2003 to forest soil. In depth 20 – 30 cm the number of bacteria is decreasing
2) 17:0i is very low in general existent but behaves similar to 15:0i/ai and16:0
3) 17:0ai is increasing since agriculture from low to medium proportion (compared to 15:0i) and grew within depth.

The gram-negative bacteria showed significant differences in their values (mol- %). Bacteria 16:1w5 fluctuates in the topsoil and slightly decreased from youngest to oldest rewilded soil. Forest soil demonstrated the smallest amount of gram negative bacteria in topsoil. In depth, the content of gram-negative bacteria shrank with time, whereas the quantity of bacteria for each field increased with depth.

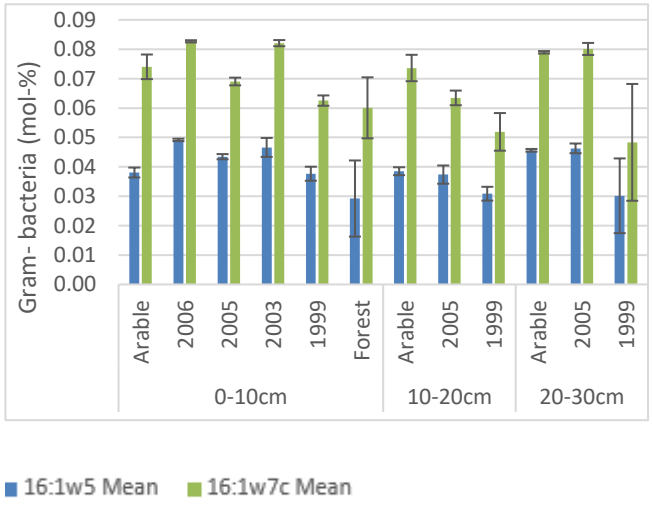
The fungi displayed significant differences between the two types 16:1w5 and 18:2w6,9 in arable soil. From arable soil to rewilded soil, both fungi types increased and stabilised over time of rewilding (0 – 20 cm). In depth 20 – 30 cm fungi are limited stable, great standard errors affect the analysis.



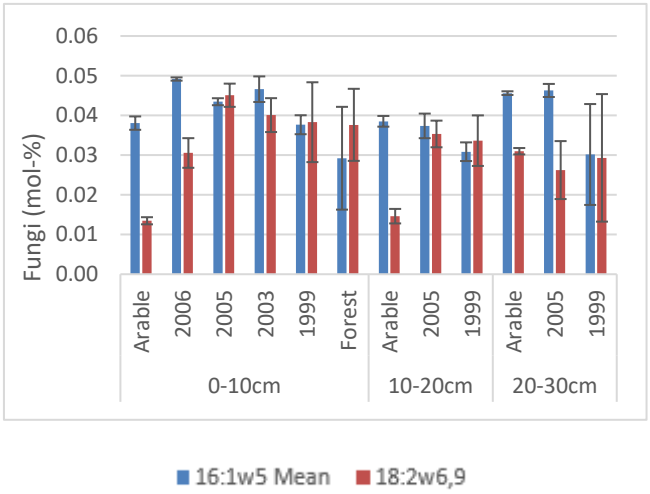
a) Phospholipid fatty acid indicators for gram+/- bacteria and fungi in (mol-%) (mean ± standard error; n=3) of arable soil, rewilded soil and ancient woodland. There is a statistical significant difference between the groups at 5% confident level with ANOVA Wilks lambda= 0.00000, F(253,38.86564)= 2.68 p-value 0.000217.



b) PLFA indicators for gram+ bacteria in (mol-%) (mean ± standard error) of arable soil, rewilded soil and ancient woodland. Only the gram-positive bacteria identified in the PLFA analysis are shown and compared.



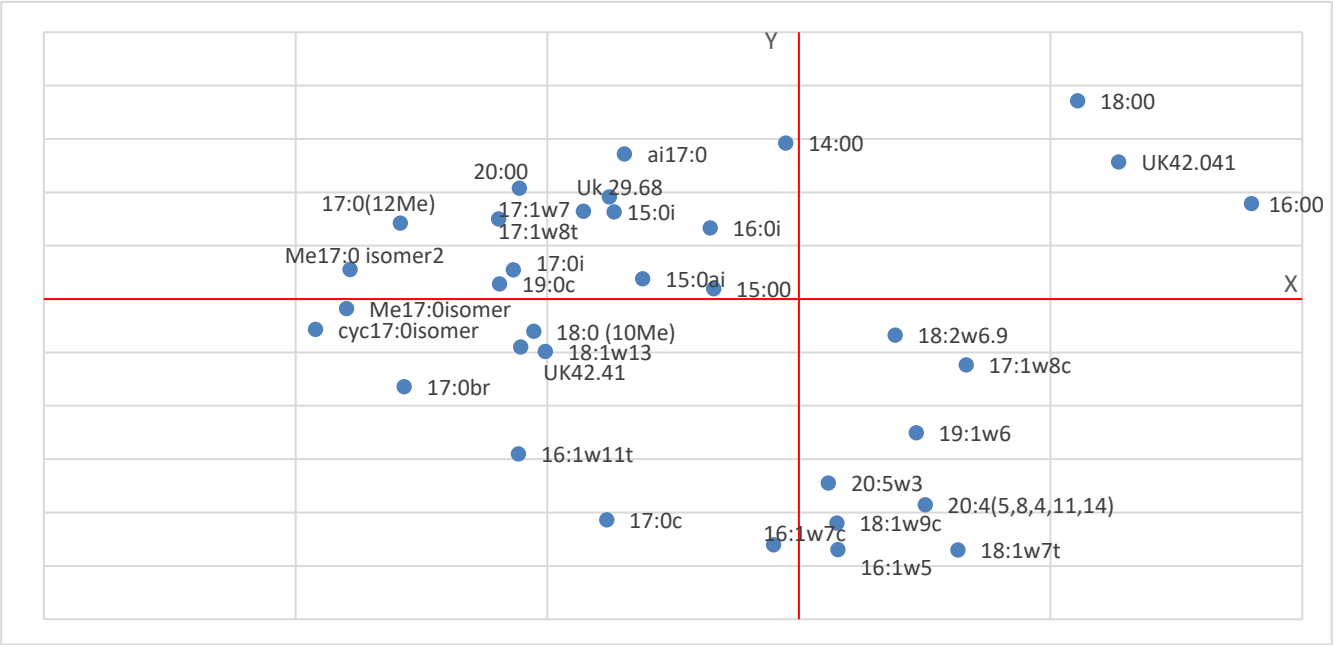
c) PLFA indicators for gram- bacteria in (mol - %) (mean ± standard error) of arable soil, rewilded soil and ancient woodland. Only gram-negative bacteria identified in the PLFA analysis are shown and compared.



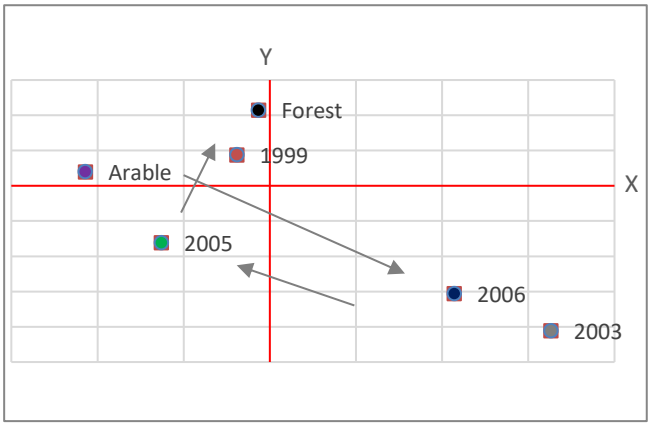
d) PLFA indicatiors for fungi in (mol-%) (mean ± standard error) of arable soil, rewilded soil and ancient woodland. Only the identified fungi of the PLFA analysis is presented

Figure 6: Histogram over chronosequence since rewilding and depth profile with grouping (a-e) of identified PLFA

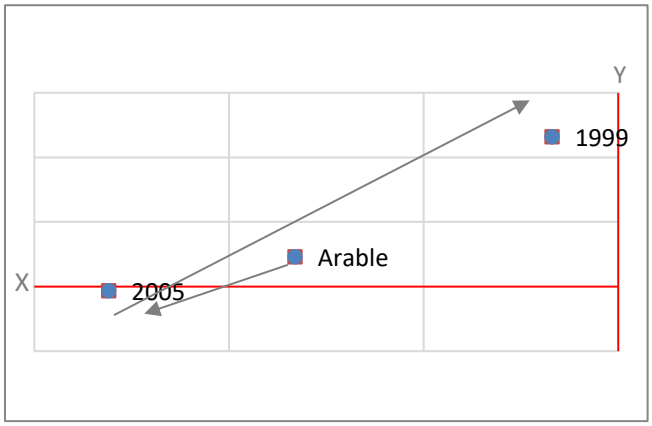
Change of microbial community structure was analysed between rewilded sites, arable land and ancient woodland over three depths, Figure 7.The orientation of PLFA (Figure 7a) is used to express the normal distribution. Depending on the normal distribution of PLFA, assertions of the movement of microbial community structure are made. The change of microbial community was identified for 0 – 10 cm depth (Figure 7b), 10 – 20 cm (Figure 7c) and 20 – 30 cm (Figure 7d). Greatest movement of microbial community was detected in the topsoil Figure 7b. Summary of the microbial movement over depth (Figure 7e) shows the significant differentiation of microbial community structure with time and depth.



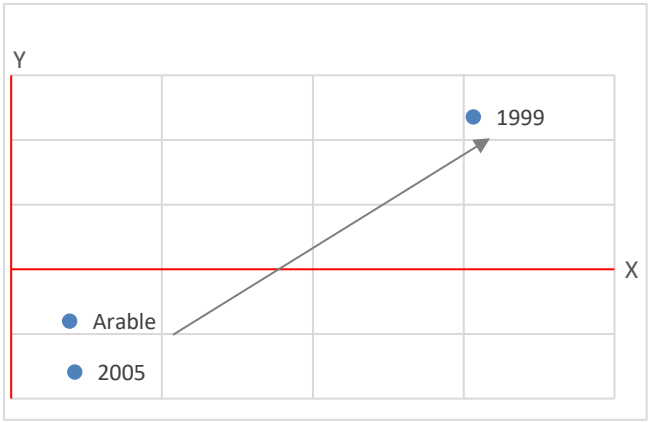
a) Principal component analysis of PLFA, shows the coordinates of the different identified PLFA. These orientations are used to determine the movement of the microbial community and their driver. PCA factor-plane (1x2) with factor 1= 26.57% and factor 2= 24.54%.

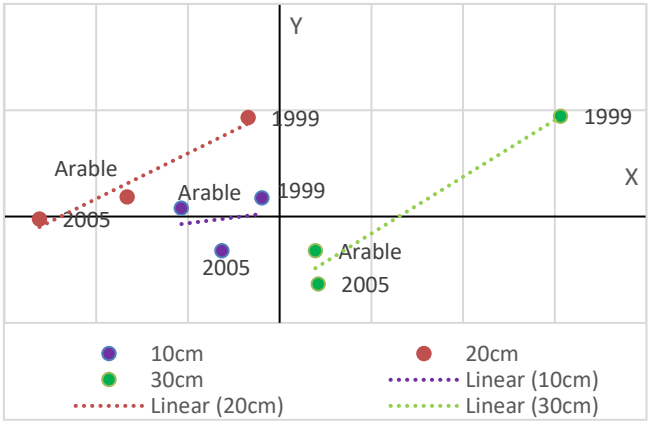


b) PCA of movement of microbial community in topsoil 0-10cm of arable soil, rewilded soil and ancient woodland. PCA shows projection of cases on factor-plane (1x2) cases with sum of cosine square >= 0.00 with factor 1= 26.57% and factor 2= 24.54%.



c) PCA of movement of microbial community in depth 10 – 20 cm of arable soil and soil rewilded in 2005 and 1999. PCA shows projection of cases on factor-plane (1x2), cases with sum of cosine square >= 0.00, with factor 1= 26.57% and factor 2= 24.54%.





d) PCA of movement of microbial community in depth 20 – 30 cm of arable soil and soil rewilded in 2005 and 1999. PCA shows projection of cases on factor-plane (1x2), cases with sum of cosine square >= 0.00, with factor 1= 26.57% and factor 2= 24.54%.

e) PCA summary of microbial community movement over depth with trend lines. Compares the movement of the microbial community over depth.

Figure 7: PCA of movement of microbial community structure depending on identified PLFA in topsoil 0 – 10 cm and depth 10 – 20 cm, 20 – 30 cm.

4 Discussion

Field and soil observation

Rewilded fields were expected to significant differ from each other as well as from arable land and ancient woodland. The spontaneous regeneration was great and development with chronosequence since rewilding was identified. The natural evolution from arable land to grassland over pasture and shrubs to trees was recognised. The external growth also influences the fauna and soil. Through the occurrence of new roam, new habitats were created, and various animals recolonised depending on their preferences. This finding is confirmed by the periodic animal stock valuation in Knepp Estate. In 2001/2, 188 fallow deer were found in the Estate. Four years later, in 2005/6 more than double the amount of deer was counted, furthermore Tamworth pigs, longhorn cattle and Exmore ponies were re-established. Since 2013, also red deer is recolonised. Also, Vera (2000) and Hobbs et al. (2014) designated the varying states of modification of complex ecosystems due to landscape restoration. However, the environmental trend leads to more complex root systems in the soil. The long and width roots of bushes, shrubs and trees tempt to loosen the soil and distribute nutrients in depth (Kenrick, 2002). The effect of the rooting system was confirmed by the results of the compaction test. Arable land was highly compacted, and the soil was very dry. With chronosequence since rewilding, the soil became looser and moister and no compaction was found in rewilded soils. Rewilded 1999 shows an exception, the first measured spot showed high compaction whereas the second spot did not show any compaction. There is no reasonable justification for the high compaction in this spot. It is possible, that this spot was used as a path for heavy machineries in the past. Another compaction measurement in this area would help to find the reason.

Soil chemistry

Rewilding was expected to restore soil quality (chemistry) and microbial community, composition and function with time (artificial chronosequence) and throughout the depth profile. The results of the study did partially support this hypothesis.

Soil EC was identified to be not saline in any of the soils. However, results of this study could not be compared to literature, as the results are compiled in dS and results in literature in $\frac{dS}{m}$ (Grisso et al., 2009). Another EC lab analysis using a different method should be carried out for a more meaningful comparison.

Arable and rewilded soils pH ratio was identified to be moderately acid (pH 6.1- 7) to acid (pH 5.1 – 6) by which the availability of nutrients is highest (Cookson, Murphy and Roper, 2008). Forest soil pH was characterised as very acid (pH= 4-5) which is a result of high ammonium content in soil. Reasons could be the high animal excretion (Bayrisches Landesamt für Umwelt, 2013) in forests as it is the main habitat and also the higher nitrogen amount in soil (ammonium is a product of nitrogen cycle).This would predict a drop of pH for old rewilded areas in future.

The very rapid increase of organic matter content from arable to soil is caused by the change of land management. Since the land management as fallowing and cultivation has stopped, soil organic matter (SOM) content regenerated. Zhao et al (2013) also identified the negative influence of agricultural management practices on SOM in soil due to residue removal and loss of initial carbon sources caused by uniform land management. With time since rewilding, the SOM increased and stabilised as a result of a balanced biodiverse vegetation and fauna. Plant residues and animal litter create a humus layer and nutrients as Nitrogen and Carbon trickle in the soil. Hobbs, Higgs and Harris (2009) acknowledge the SOM increase as a result of developing vegetation, flora and fauna. The SOM content of forest soil was identified to be 62% higher than rewilded 1999 content. High amount of plant litter is recognised to be the reason for the much higher SOM. Also Osman (2013) identified a 60% higher SOM content in forest soils compared to grassland and defined plant litter as its origin.

Highest concentration of SOM was found in topsoil since most OM occurs above-ground, and the drain of OM through soil layers reduced with depth (Kenrick, 2002; Zhao et al., 2013). SOM is determining plant nutrients (TN, P, TOC, TC) as it releases them during its decomposition and is correlated to soil fertility (Magdoff and Van Es, 2012).

The correlation between OMC and the nutrient cycling of TOC, TN, TC, P and C:N ratio was visible in the results. The change from crop rotation on arable land (beans, oat, wheat) to improved biodiversity leads to more complex root systems and stability in nutrient input (decomposition of SOM) and uptake, also identified by (Singh, J.S.; Raghubanshi, A.S.; Singh, R.S.; Srivastava, 1989)

Rewilded sites showed an increase of nutrients compared to arable soil. The developed vegetation creates more SOM which is degraded into nutrients and drained in the soil. Also, rewilded sites provide roaming and grazing areas for the animals whereby it comes to more animal residues on the soil (Vera, 2000).

Nutrients but P were found to be much greater in forest soils than arable and rewilded land. Forests serve to be habitat for most of the animal species whereby it comes to high amount of animal residues on forest soil, next to that the amount of plant litter on forest soil due to the great tree canopy is much higher. These facts lead to a high amount of OMC on the soil which in turn is responsible for the increase of nutrients and microbial biomass. Binkley and Fisher (2012) stated similar findings about nutrient cycling in forest soils.

Phosphorous only showed a very little movement in soil depth, which was also identified by Ludwick (1998) and a strong addiction to pH (Alt et al., 2011). The very low P-concentration in the forests topsoil is identified as a result of physical processes (Ludwick, 1998). Trees and bushes are protecting the ground from rainfall and create a humus layer on the soil, what impedes the release of P and further drain into/through the soil as well as increases the acid content of the soil. Further analysis of forest soil in depth could help to confirm this statement. If the forest soil is influenced by physical processes, all other nutrients will be low in depth as only low drainage of nutrients is possible. This also predicts a decrease in P-concentration and pH for old rewilded soils, as the vegetation is developing

with time and the spreading treetops are covering more soil. Additional nutrient analysis in the future are necessary to evaluate this statement.

C:N ratio is determined by carbon and nitrogen content in the soil. The ideal ratio for mineralisation and degradation processes ranges from 9 – 12% (Mooshammer et al., 2014). Similar values were found in rewilded soils (10 – 12% C:N) what leads to a great microbial activity, which in turn refers to a strong nutrient cycling in rewilded soils (Cookson, Murphy and Roper, 2008).

Microbial community structure

Microbial biomass is correlated with time and land-use. Especially the development of vegetation and change of biodiversity seems to influence the microbial community. The expected trajectory for microbial biomass in each depth within time and due to change of land-use is reflected and was also stated by Harris, (2009) who investigated the role of microorganisms in case of ecosystem restoration.

MB mainly consist of fungi and bacteria and is degrading the SOM (essential source for nutrients) which differs from site to site. The SOM of arable land was mainly crop residues and roots, rewilded sites developed with time and showed to have various sources of SOM as plant litters (from bush, shrubs, flowers, trees) and animal residues (from grazing and roaming animals). Forests SOM showed to mainly consist of tree and shrub litters and various animal residues as it is the main habitat for most of them. However, the microbial community changes depending on the composition and formation of SOM, as specific microorganisms prefer specific organic substances. Harris (2009), Hobbs, Higgs and Harris (2009) confirmed these findings with their studies about influence of changing landscapes on ecosystem functions and furthermore the reliance of microbial community on the above-ground community. Further analysis is needed to identify the specific development of MB-SOM correlation with rewilding.

With time since rewilding the microbial community increased. The general increase of MB in soil is a result of elevation and more variety in SOM above-

ground (Paul, 2015). In depth the amount of MB decreased as the quantity of plant and animal residues shrinks.

Analysis of the PLFA identifies, that the composition of microbial community changes with time and depth. Bacteria represented mainly by 15:0i, 15:0ai,16:0i and 16:1w7c showed a strong effect of time since rewilding in depth 20 – 30 cm. The number of bacteria showed to decrease in depth, what could be a result of impeded accessibility to nutrients, especially carbon. Fierer, Schimel and Holden (2003) analysed the abundance of microbial communities over depth and stated limited carbon amount in depth as its main driver.

Fungal indicators 16:1w5 and 18:2w6,9 showed significant differences in arable soil in all depth. The indicator 18:2w6,9 is substantial lower in arable soils than 16:1w5, but quickly recovers with rewilding. A reason for this enormous variance could be the agricultural treatment of plants and soil with pesticides/herbicides which encourage the decline of 18:2w6,9. Also, the uniform crop rotation could influence and lower the variety of fungi in soil. Further analysis is necessary as no evidence in literature could be found.

Microbial respiration

The substrate induced respiration rate showed to be significant stimulated by galactose and glucose. Respiration rate of galactose and glucose treated soils was about two to three times higher than the natural respiration rate (H2O). Microbial organisms need galactose and glucose for aerobic respiration processes. A higher amount of this substrates in soil leads to higher respiration rate and energy production (Merilä et al., 2010). Especially high was the natural and substrate induced respiration in 1999 topsoil. This indicates a great proportion of active microorganisms and conversion as well as degradation of nutrients to energy.

The PCA of MSIR change, shows distinct influence of time since rewilding and depth on microbial respiration. Significant is the change of MRR in topsoil. A straight trend line from arable over time since rewilding towards ancient woodland

is pictured and rewilded 2003 is identified as an outlier. The plot shows, that forest and rewilded 1999 are identified to have the same MRR what indicates very active microbial community with a great metabolic process. Interesting is the comparison of the microbial community and their respiration. Rewilded 1999 shows a lower MB than forest but the same MRR. Regarding the classification of microorganism's, the forest has a higher number of gram-positive bacteria and rewilded 1999 holds a higher number of gram-negative bacteria and fungi. This would indicate, that the respiration rate of gram-negative bacteria and fungi in soil rewilded 1999 is greater than the one of gram-positive bacteria in ancient woodland. Further analysis to confirm this statement are needed.

In conclusion, rewilding appears to be succeeding restoring vegetation, biodiversity and soil structure as well as function to a novel state which differs from the initial state and the past agricultural conditions. Hobbs, Higgs and Harris (2009) examined novel systems and stated a same finding, that characteristics of ecosystems can be retained but the composition and function lies outside the historic range.

5 Conclusion

The projects aim was to define the influence of rewilding on soil biodiversity and function by **1)** investigating the implications of rewilding on catabolic profiles and nutrient cycling as well as on phenotypic profiles, **2)** compare rewilded soils to disturbed agricultural soil and ancient woodland soil **3)** and investigate the effects of trophic-level interactions on microbial community. It was hypothesised that rewilding will restore soil quality and microbial community composition and function with time and throughout the depth profile. This hypothesis has been proved and supported by the results. Overall, the soil chemistry (nutrients) and biology (microbial biomass) increased due to rewilding (Aim 1). The catabolic profiles as well as phenotypic profiles stabilised with time of rewilding and increased in comparison to disturbed agricultural soil. The comparison of rewilded soil to disturbed agricultural soil showed an improvement in all cases, just pH and salinity did not show any modification (Aim 2). The comparison of rewilded soil to ancient woodland soil showed a strong convergence for nutrients, microbial biomass carbon and microbial respiration (Aim 2). The influence of rewilding over the depth profil was visible but due to the small amount of nutrients and microbial community in depth lower than in topsoil (0 – 10 cm). Nutrients, microbial biomass and respiration rate slightly increased also in depth. High impact of trophic-level interaction between the developing flora and fauna and nutrient cycling were determined (Objective 3). Soil nutrients developed together with the environment over chronosequence since rewilding and also the microbial community, composition and function evolved appropriate. The whole environment, flora, fauna and soil quality changed due to rewilding to a ‘wilder’ state. It was shown that rewilding has a strong effect on the soil quality as a result of environmental change and trophic-level interactions. Further investigation to analyse the change of soil quality in depth and a comparison between further rewilded soils should be done to make a more significant statement.

REFERENCES

Alt, F., Oelmann, Y., Herold, N., Schrumpf, M. and Wilcke, W. (2011) 'Phosphorus partitioning in grassland and forest soils of Germany as related to land-use type, management intensity, and land use-related pH', *Journal of Plant Nutrition and Soil Science*, 174(2), 195–209.

Bartholomew, W. V., Clark, F.E. and Scarsbrook, C.E. (1965) 'Nitrogen Availability', in *Soil Nitrogen*. American Society of Agronomy, 481–502. Available at: 10.2134/agronmonogr10.c13 (Accessed: 17 June 2018).

Bayrisches Landesamt für Umwelt (2013) 'Ammoniak und Ammonium.', *UmweltWissen*, 6(1), 1-12

Binkley, D. and Fisher, R. (2013) *Ecology and management of forest soils*.Fourth Edi. West Sussex: Wiley, Blackwell. Available at: 978-0-470-97946-4

Burrell, C. (2002) *Background — Knepp Wildland*. Available at: https://knepp.co.uk/background/ (Accessed: 28 May 2018).

Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S. and Potts, J.M. (2003) 'A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil', *Applied and Environmental Microbiology*, 69(6) American Society for Microbiology, 3593–3599. Available at: 10.1128/AEM.69.6.3593-3599.2003 (Accessed: 29 August 2018).

Carey, J. (2016) 'Rewilding.', *Proceedings of the National Academy of Sciences of the United States of America*, 113(4), 806–8.

Carson, J. (2018) 'Microbial Biomass.' *Soil Quality Pty Ltd, Australia, 1-2.* Available at: http://soilquality.org.au/factsheets/microbial-biomass

Cookson, W.R., Murphy, D. V. and Roper, M.M. (2008) 'Characterizing the relationships between soil organic matter components and microbial function and composition along a tillage disturbance gradient', *Soil Biology and Biochemistry*, 40(3), 763–777.

Corlett, R.T. (2016) ‘Restoration, Reintroduction, and Rewilding in a Changing World’, *Trends in Ecology and Evolution*, 31(6) Elsevier Ltd, 453–462.

Cotching, B. and Davies, S. (2015) ‘Soil Compaction’ *Soil Quality Pty Ltd, Australia,* 1-2. Available at: http://soilquality.org.au/factsheets/soil-compaction-tas (Accessed: 17 June 2018).

Deparment of Geography (2018) ‘Loss on Ignition. ’, *University College London,* 1-2. Available at: http://www.geog.ucl.ac.uk/resources/laboratory/laboratory-methods/lake-sediment-analysis/loss-on-ignition (Accessed: 17 June 2018).

Doran, J.W. and Zeiss, M.R. (2000) ‘Soil health and sustainability: managing the biotic component of soil quality’, *Applied Soil Ecology*, 15(1) Elsevier, 3–11. Available at: 10.1016/S0929-1393(00)00067-6 (Accessed: 6 June 2018).

Fierer, N., Schimel, J.P. and Holden, P.A. (2003) ‘Variations in microbial community composition through two soil depth profiles’, *Soil Biology and Biochemistry*, 35(1) Pergamon, 167–176. Available at: 10.1016/S0038-0717(02)00251-1 (Accessed: 31 August 2018).

Fuhrman, J.K., Zhang, H., Schroder, J.L., Davis, R.L. and Payton, M.E. (2005) ‘Water-soluble phosphorus as affected by soil to extractant ratios, extraction times, and electrolyte’, *Communications in Soil Science and Plant Analysis*, 36(7–8), 925–935. Available at: 10.1081/CSS-200049482 (Accessed: 27 August 2018)

Grisso, R., Alley, M., Holshouser, D. and Thomason, W. (2009) ‘Precision Farming Tools : Soil Electrical Conductivity. ’, *VCE Publications 442(442-508), 1-7.* Available at: http://pubs.ext.vt.edu/442/442-508/442-508.html (Accessed: 10 August 2018)

Harris, J. (2009) ‘Soil microbial communities and restoration ecology: Facilitators or followers?’, *Science*, 325(5940), 573–574.Available at: 10.1126/science.1172975 (Accessed: 17 August 2018)

Hobbs, R.J., Higgs, E., Hall, C.M., Bridgewater, P., Chapin, F.S., Ellis, E.C., Ewel, J.J., Hallett, L.M., Harris, J., Hulvey, K.B., Jackson, S.T., Kennedy, P.L., Kueffer,

C., Lach, L., Lantz, T.C., Lugo, A.E., Mascaro, J., Murphy, S.D., Nelson, C.R., Perring, M.P., Richardson, D.M., Seastedt, T.R., Standish, R.J., Starzomski, B.M., Suding, K.N., Tognetti, P.M., Yakob, L. and Yung, L. (2014) 'Managing the whole landscape: Historical, hybrid, and novel ecosystems', *Frontiers in Ecology and the Environment*, 12(10), 557–564. Available at: 10.1890/130300 (Accessed: 30 July 2018)

Hobbs, R.J., Higgs, E. and Harris, J.A. (2009) 'Novel ecosystems: implications for conservation and restoration', *Trends in Ecology and Evolution*, 24(11), 599–605.Available at: 10.1016/j.tree.2009.05.012 (Accessed: 27 August 2018)

Houses of Parliament (2016) 'Rewilding and ecosystem services | Farming Futures', *REWILDING and Ecosystem Services*, 537(1), 1–5.Available at: https://researchbriefings.parliament.uk/ResearchBriefing/Summary/POST-PN-0537 (Accessed: 13 June 2018)

Janzen, D.H. and Martin, P.S. (1982) 'Neotropical anachronisms: The fruits the gomphotheres ate' *Science*, 215(4528), 19-27 Available at: 10.1126/science.215.4528.19 (Accessed: 27 August 2018).

Jordan, W.R.I., Gilpin, M.E. and Aber, J.D. (1990) 'Restoration ecology: A Synthetic Approach to Ecological Research*.* ' *First Edit. The Quarterly Review of Biology 64(4),* 200-356. Available at: https://www.journals.uchicago.edu/doi/pdfplus/10.1086/416533 (Accessed: 28 August 2018).

Kenrick, P. (2002) 'The Origin of Roots', in Waisel, Yoav; Eshel, Amram; Kafkafi, U. (ed.) *Plant Roots - The Hidden Half*. Third Edit. New York: Marcel Dekker, 1–10.Available at: 0-8247-0631-5

Kernon Countryside Consultants and Land Use Consultants (2007) 'FEASIBILITY ASSESSMENT'. Sussex, UK, 1-92. Available at: https://static1.squarespace.com/static/595ca91bebbd1a1d0aaab285/t/5a50f941419202e32413820f/1515256141991/Knepp+Feasibility+Assessment+Final+2.3.07+compressed3.pdf

Lal, R. (2015) ‘Restoring Soil Quality to Mitigate Soil Degradation’, *Sustainability*, 7(5) Multidisciplinary Digital Publishing Institute, 5875–5895. Available at: 10.3390/su7055875 (Accessed: 6 June 2018).

Lowery B., W.J. Hickey, M.A. Arshad, R.L. (1996) ‘Soil Quality: Indicators: Infiltration. ’, *Soil water parameters and soil quality* Available at: http://soilquality.org/indicators/infiltration.html (Accessed: 17 June 2018).

Ludwick, A. (1998) ‘Phosphorus Mobility in Perspective’, *Potash&Phosphate Institute (PPI), Potash&Phosphate Institute of Canada (PPIC), (707), 1–2. Available at: www.ipni.net/ppiweb/ppinews.../98175-AEL%20P-mobility.pdf*

Magdoff, F. and Van Es, H. (2012) *Why is soil organic matter so important*., *Sustainable Agriculture Research&Education* Available at: 10.2134/cs2018.51.0205 (Accessed: 20 August 2018).

Marren, P. (2016) The great rewilding experiment at Knepp Castle, *British Wildlife*, 27(5), 333–339. Available at: 09580956

Marshall, Theo John; Holmes, J.W. (1988) *Soil physics*.Second Edi. University of Cambridge, 200-374. Available at: 978-0-521-35817-0

Merilä, P., Malmivaara-Lämsä, M., Spetz, P., Stark, S., Vierikko, K., Derome, J. and Fritze, H. (2010) ‘Soil organic matter quality as a link between microbial community structure and vegetation composition along a successional gradient in a boreal forest’, *Applied Soil Ecology*, 46(2) Elsevier, 259–267. Available at: 10.1016/J.APSOIL.2010.08.003 (Accessed: 31 August 2018).

Microbial Insights (2013) Phospholipid fatty acid analysis - interpretation guidelines, *Microbial Insights* 865, 1-6. Available at: https://www.microbe.com/plfa/ (Accessed: 17 June 2018).

Mills, J.G., Weinstein, P., Gellie, N.J.C., Weyrich, L.S., Lowe, A.J. and Breed, M.F. (2017) ‘Urban habitat restoration provides a human health benefit through microbiome rewilding: the Microbiome Rewilding Hypothesis’, *Restoration Ecology*, 25(6), 866–872.Available at: 10.1111/rec.12610

Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A., Schnecker, J., Takriti, M., Watzka, M., Wild, B., Keiblinger, K.M., Zechmeister-Boltenstern, S. and Richter, A. (2014) Adjustment of microbial nitrogen use efficiency to carbon:nitrogen imbalances regulates soil nitrogen cycling., *Nature communications*, 5, 1–7. Available at: 10.1038/ncomms4694 (Accessed: 26 August 2018).

NRCS, East National Technology Support Center. (2011) Soil Quality: Soil Functions: Nutrient Cycling. Available at: http://soilquality.org/functions/nutrient_cycling.html (Accessed: 2 August 2018).

Osman, K.T. (2013) 'Organic Matter of Forest Soils', in *Forest Soils*. Cham: Springer International Publishing, 63–76. Available at: 10.1007/978-3-319-02541-4_4 (Accessed: 21 August 2018).

Paul, E.A. (2015) *Soil microbiology, ecology, and biochemistry*.fourth.Paul, E. A. (ed.) Fort Collins, CO, USA: Natural Resource Ecology Laboratory and Department of Soil and Crop, Colorado State University. Available at: 9780124159556.

Pawlett, M., Ritz, K., Dorey, R.A., Rocks, S., Ramsden, J. and Harris, J.A. (2013) 'The impact of zero-valent iron nanoparticles upon soil microbial communities is context dependent', *Environmental Science and Pollution Research*, 20(2) Springer-Verlag, 1041–1049. Available at: 10.1007/s11356-012-1196-2 (Accessed: 2 July 2018).

Pluske, W., Murphy, D. and Sheppard, J. (1978) Total Organic Carbon, *Soil Quality Pty Ltd,* 1-2. Available at: http://www.soilquality.org.au/factsheets/organic-carbon (Accessed: 17 June 2018).

Quinlan, Richard; Wherrett, A. (2018) Nitrogen, *Soil Quality Pty Ltd*., Australia, 1-2. Available at: http://www.soilquality.org.au/factsheets/mineral-nitrogen (Accessed: 17 June 2018).

Regasamy, P. (2013) Soil pH, *Colorado State University Extension*, (base 10),

1–9. Available at: http://soilquality.org.au/factsheets/soil-ph-south-austral

Rewilding Britain (2018) *Knepp Estate - Rewilding Britain*., *Rewilding Britain* Available at: https://www.rewildingbritain.org.uk/rewilding/rewilding-projects/knepp-estate (Accessed: 29 May 2018).

Rewilding Britain (2017) *How Rewilding Reduces Flood Risk,* Rewilding Britain, West Sussex. Available at: https://www.rewildingbritain.org.uk/assets/uploads/files/publications/Final-flood-report/Rewilding-Britain-Flood-Report-Sep-6-16.pdf

Singh, J.S.; Raghubanshi, A.S.; Singh, R.S.; Srivastava, S.C. (1989) 'Microbial biomass acts as a source of plant nutrients in dry tropical forest and savnna', *Nature*, 338(6215), 499–500. Available at: https://doi.org/10.1038/338499a0

Smith, T.M. and Smith, R.L. (2009) *Ökologie.* München: Pearson Education. Available at: http://www.worldcat.org/title/okologie/oclc/254643067 (Accessed: 27 August 2018).

Tan, T.S., Phoon, K.K., Hight, D.W.. and Leroueil, S. (2007) *Characterisation and engineering properties of natural soils*. Taylor & Francis Group London. Available at: http://www.worldcat.org/title/characterisation-and-engineering-properties-of-natural-soils/oclc/929287920/viewport (Accessed: 27 August 2018).

Tree, I. (2017) 'The Knepp Wildland project', *Biodiversity*, 18(4) Taylor & Francis, 206–209. Available at: 10.1080/14888386.2017.1407258

Tree, I. (2018) *Wilding : the return of nature to an English farm*, 1-378. Available at: 9781509805099 (Accessed: 27 August 2018).

Ullman, J.L. (2013) *Soil Salinity in Agricultural Systems: The Basics* University of Florida,1-36. Available at: http://hos.ufl.edu/sites/default/files/faculty/gdliu/ullman.pdf

Vera, F.W.M. (2000) *GRAZING ECOLOGY AND FOREST HISTORY*.First Edit. Oxon, UK: CABI Publishing. Available at: 0851994423

Zhao, G., Bryan, B.A., King, D., Luo, Z., Wang, E., Song, X. and Yu, Q. (2013)

‘Impact of agricultural management practices on soil organic carbon: Simulation of Australian wheat systems’, *Global Change Biology*, 19(5), 1585–1597. Available at: 10.1111/gcb.12145