Master's thesis

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The influence of reduced tillage on soil fertility indicators in two long-term organic field experiments

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Abstract

Conventional agriculture calls for global change towards more sustainable agrosystems. Reduced tillage (RT) presents an interesting alternative to conventional tillage (CT) in order to reduce soil degradation while keeping control of weed pressure. However, research gaps on soil quality assessment in long-term organic farming under RT need to be filled, especially for different soil textures. . The aim of this work was to study the effects of RT on soil fertility indicators in two organic long-term field experiments (LTEs). The study compared the effects of RT (chisel plow to 10 cm) and CT (traditional moldboard ploughing 15-20 cm) on two LTEs from FiBL in Northern Switzerland differentiated by high clay (Frick, AG) and silt-loam (Aesch, BL) textures, including two associated untilled grasslands as a reference. To assess soil fertility, physical (bulk density), chemical (pH, SOC, TN, C_{mic} and N_{mic}, micronutrients) and biological (bacteria, fungi, earthworms) soil properties were measured at three depths (0-10 cm, 10-20 cm, 20-30 cm). Additional effort was brought to the in-depth analysis of microbial communities using next generation sequencing technique. Statistical analysis included ANOVA, PCA, RDA and Mantel tests to evaluate the significant effect of tillage, depth and sites on soil parameters and microbial populations. The results showed that RT enhanced most of the soil fertility indicators in both sites compared to CT. The positive effect of RT was more pronounced in the clayey soil of Frick for chemical parameters (SOC stocks, TN, micronutrients), with a visible stratification of nutrients with depth, thus resembling to the natural grassland (G) plots, while CT lead to more homogenous layers. Fungi copies were also enhanced in the Frick soil under RT. The biological indicators (total soil DNA, bacteria, and earthworms) surprisingly seemed to be more enhanced by RT in the silty soil of Aesch. RT could significantly decrease bulk density in the top layer of Frick site, and only minorly in Aesch site, while it increased compared to CT in the second layers of both sites. Biomolecular samples were composed of 60'000 sequences each, and the data obtained by sequencing showed an excellent correlation with all environmental variables ($r=0.5853$, $p=0.001^{***}$). PCA on microbial community showed a clear distinction between both sites and between the natural G plots and the cultivated RT and CT plots, especially in Frick. In Aesch, microbial community showed significantly different structures linked with depth and without tillage differentiation, while in Frick the microbial community was very similar regardless of tillage treatment, and minorly with of depth. Nitrifiers included few *Archaea* (Gen. *Nitrosopumilus*), and *Bacteria* (Phylum *Nitrospirae* with Gen. *Nitrospira*, Class α- and β-*Proteobacteria* with Gen. *Nitrobacter*, *Nitrosomonas*, *Nitrosovibrio* and *Nitrospira*). Microbial diversity indices showed that the diversity was greater in Aesch than in Frick, greatest in the top layers, and lower in the G plots than in the RT and CT plots. This work enabled to confirm enhanced soil fertility indicators with RT practices under two organic LTEs, and especially in the high SOC-sequestering clay soil, while the biomolecular analysis surprisingly showed enhanced biological parameters in the silty soil. The underlying processes regarding site specific changes still need to be untangled through deeper analyses, including specific target on soil physical parameters under different tillage conditions and for different soil textures, and extended knowledge on the microbial community behavior at a functional level.

Keywords: organic agriculture, reduced tillage, long-term experiment, soil fertility, soil texture, microbial community, earthworms

Résumé

L'agriculture d'aujourd'hui est amenée à se tourner vers des pratiques plus durables pour palier au changement climatique. Le travail réduit du sol (RT) présente une alternative intéressante au travail conventionnel du sol (CT) afin de réduire sa dégradation tout en gardant le contrôle sur les adventices. Cependant, les recherches sur la qualité du sol en RT en agriculture biologique (AB) à long terme est limitée, et en particulier pour différentes textures de sol. L'objectif de ce travail est d'étudier les effets du RT sur les indicateurs de fertilité du sol dans deux essais de longue durée en AB. L'étude a comparé les effets du RT sur deux essais du FiBL au nord de la Suisse différenciés par deux textures : argileux (Frick, AG) et limoneux (Aesch, BL) ; avec deux prairies non labourées comme référence. Pour évaluer la fertilité du sol, les propriétés physiques (densité), chimiques (pH, carbone organique du sol, azote total, C_{mic} et N_{mic}, micronutriments) et biologiques (bactéries, champignons, vers de terre) du sol ont été mesurées à trois profondeurs (0-10 cm, 10-20 cm, 20-30 cm). Un travail supplémentaire d'analyse approfondie des communautés microbiennes a été fait en utilisant la technique de séquençage massif. L'analyse statistique comprenait des tests ANOVA, PCA, RDA et Mantel pour évaluer l'effet du travail du sol, de la profondeur et des sites, sur les paramètres du sol et les populations microbiennes. Les résultats ont montré que le RT a amélioré la plupart des indicateurs de fertilité du sol dans les deux sites par rapport au CT. L'effet positif du RT était plus prononcé dans le sol argileux de Frick pour les paramètres chimiques (stocks de carbone organique, azote total, micronutriments), avec une stratification visible des nutriments en profondeur, ressemblant ainsi aux parcelles de prairies naturelles (G), alors que le CT démontrait des couches plus homogènes. Les champignons sont davantage améliorés dans le sol de Frick. Les indicateurs biologiques (ADN total du sol, bactéries, vers de terre) ont étonnamment semblé être davantage améliorés par le RT dans le sol limoneux d'Aesch. Le RT a pu diminuer significativement en superficie la densité apparente sur le site de Frick, mais de façon moindre à Aesch. Les échantillons biomoléculaires étaient composés de 60'000 séquences chacun, et les données obtenues par séquençage ont montré une excellente corrélation avec toutes les variables environnementales ($r=0.5853$, $p=0.001$***). L'ACP sur la communauté microbienne a montré une distinction claire entre les deux sites et entre les parcelles naturelles G et les parcelles cultivées RT et CT, en particulier à Frick. A Aesch, les communautés microbiennes ont montré des structures significativement différentes selon la profondeur et sans différenciation du travail du sol, alors qu'à Frick les communautés microbiennes étaient très similaires indépendamment du traitement du travail du sol, et de façon mineure selon la profondeur. Les nitrifiantes comprenaient quelques *Archaea* (Gen. *Nitrosopumilus*), et *Bacteria* (Phylum *Nitrospirae* avec Gen. *Nitrospira*, Classe α- and β-*Proteobacteria* avec Gen. *Nitrobacter*, *Nitrosomonas*, *Nitrosovibrio* and *Nitrospira*). Les indices de diversité microbienne ont montré que la diversité était plus grande à Aesch qu'à Frick, plus grande dans les couches supérieures, et plus faible dans les parcelles G que dans les parcelles RT et CT. Ce travail a permis de confirmer une amélioration des indicateurs de fertilité en RT dans les deux essais, et en particulier dans le sol argileux, tandis que l'analyse biomoléculaire a identifié une amélioration des paramètres biologiques dans le sol limoneux. Les processus sous-jacents de ces changements spécifiques doivent être approfondis, par un focus sur paramètres physiques pour des sols à textures différentes, et par une analyse poussée sur la communauté microbienne à un niveau fonctionnel.

Mots clés : agriculture biologique, travail réduit du sol, essais de longue durée, fertilité du sol, texture du sol, communauté microbienne, vers de terre.

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Abbreviations

ANOVA	Analysis of variance
B	Boron
BD	Bulk density
C	Carbon
Ca	Calcium
Carb	Carbonates
CFE	Chloroform fumigation extraction
C_{mic}	Microbial carbon
CT	Conventional tillage
Cu	Copper
DM	Dry matter
DNA	Deoxyribonucleic acid
Fe	Iron
FiBL	Research Institute of Organic Agriculture
G	Grassland
K	Potassium
LTE	Long-term field experiment
Mg	Magnesium
Mn	Manganese
mWHC	Maximum water holding capacity
N	Nitrogen
N_{mic}	Microbial nitrogen
N_{min}	Mineral nitrogen
N_{org}	Organic nitrogen
NPOC	Non-purgeable organic carbon
P	Phosphorus
PCA	Principal components analysis
qPCR	Quantitative polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
RT	Reduced tillage
SOC	Soil organic carbon
SOM	Soil organic matter
TC	Total carbon
TN	Total nitrogen
TNb	Total nitrogen bound
WC	Water content

1 Introduction

Agriculture is one of the many sources of anthropogenic activities contributing to greenhouse gas emissions, deterioration of the environment and human health, thus forming a pillar of global challenges regarding climate change mitigation and biodiversity conservation. Conventional agriculture has numerous negative environmental impacts such as soil and water pollution, loss of biodiversity, and land degradation, which calls for efforts towards more sustainable agricultural practices (Francaviglia et al., 2019; Hartman et al., 2018; Pimentel et al., 1995).

Among the widely used agricultural practices is tillage, which is traditionally done by ploughing. Ploughing enables soil preparation and incorporation of fertilizer before seeding, and is a key practice for weed control (Peigné et al., 2018). However, it also creates a physical disruption of top soil by full-inversion, which results in a homogeneous layer of soil having uniform physicochemical characteristics. Conventional tillage (CT) by ploughing has been shown responsible for several decades to have pronounced negative effects on soil erosion (Seitz et al., 2018), loss of soil organic matter (Baker et al., 2007; Berner et al., 2008), degradation of soil structure (Araya et al., 2022; Chivenge et al., 2007), and of soil biota (Ferrara et al., 2022; Kraut-Cohen et al., 2020). While numerous agricultural trials focusing on no-till practices, particularly in Northern America, have shown promising results in maintaining soil integrity, concerns have arisen about weed pressure and related yield reductions (Mäder and Berner, 2012), particularly in organic farming. Indeed, weed elimination in conventional no-till systems is often done with herbicides, but in organic farming, such synthetic inputs are not permitted (Armengot et al., 2015; Cooper et al., 2016; Peigné et al., 2018). Alternative tillage practices were thus developed in order to maintain soil structure and keep partial control on weeds, while still enabling soil preparation before cultivation. In Europe, methods based on reducing tillage intensity, rather than no-till, are currently being developed and tested (Carr et al., 2012). Reduced tillage (RT) practices in organic farming refer to "*the reduction of plowing depth but also non-inverting, less-invasive soil loosening*" as given by Mäder and Berner (2012). It is part of the so-called "conservation agriculture" practices, which includes superficial reduced tillage, permanent soil cover and diverse crop rotation practices, with the desire to preserve the integrity of soil, and therefore, its suitability of soil for agricultural production (Govaerts∗ et al., 2009; Sommer et al., 2007).

The evaluation of agricultural soils is usually gathered around the "fertility" terminology, as explained by Patzel, Sticher, and Karlen (2000) and Bünemann et al. (2018). Moreover, in the review from Bünemann et al. (2018) about soil quality assessment, the definition of a fertile soil is given by "[a soil which] provides essential nutrients for crop plant growth, supports a diverse and active biotic community, exhibits a typical soil structure and allows for an undisturbed decomposition". By this definition, we can understand

that soil provides specific functions for agriculture, for which conservation practices are orientated towards (de Santiago et al., 2019; Peigné et al., 2018). The evaluation of soil fertility is a vast field of research among the scientific community. The evaluation of soil fertility relies on the assessment of soil parameters from three categories: physical properties, chemical properties, and biological properties. Physical properties include soil texture and density, as well as water holding capacity or aggregate formation and stability. Several researches are specifically focused on the role of aggregates, and point out their importance in controlling water and gas exchange but also organic carbon sequestration (Six et al., 2004, 2000). Chemical parameters, such as pH, nutrients load and carbon fractions, give an insight of nutrients cycle and their availability for crops (Bongiorno et al., 2019; Fließbach et al., 2007). Thirdly, biological parameters encompass the study of plant development and rooting, but also soil biota, including macroorganisms such as earthworms and decomposers, and microorganism populations including bacteria, protozoa and fungi (Denier et al., 2022; Stone et al., 2016). This vast diversity of organisms all play a crucial role in soil structure maintenance, nutrient cycling such as nitrogen and phosphorus and organic matter decomposition, which in turns provides elements for plant growth (Hartman et al., 2018; Lori et al., 2017; Mirzavand et al., 2022).

In this perspective, comparison of soil fertility parameters in controlled long-term experiments (LTE) provides a method to study soil response to RT compared to CT and thus assess its sustainability with respect to soil health (Nunes et al., 2020). Several worldwide studies showed beneficial effects of RT in arable cropping systems, as improved soil physical, chemical and biological properties. Maintenance of soil integrity by non-inversion tillage in RT enables protection of soil aggregates (Balesdent et al., 2000; Loaiza Puerta et al., 2018; Six et al., 2004), which enhances hydrological and physical soil properties compared to CT (Araya et al., 2022; Crittenden et al., 2015; Fontana et al., 2015; Seitz et al., 2018). Chemical parameters were also enhanced with RT, mostly carbon and nitrogen (Berner et al., 2008; Doran, 1980; Gadermaier et al., 2012; Hu et al., 2020; Tully and McAskill, 2020), as well as other soil nutrients (de Santiago et al., 2019). Numerous studies focused on the beneficial effects of RT on soil organic carbon contents, particularly in the soil top layers, as RT practices could potentially serve as a carbon sink for climate change mitigation (Cooper et al., 2016; Francaviglia et al., 2019; Krauss et al., 2022, 2022; Peigné et al., 2007; Schulz et al., 2014). Finally, biological parameters were greatly improved by reduced tillage practices, such as microbial biomass content and enzymatic activity (Berner et al., 2008; Domnariu et al., 2022; Krauss et al., 2020; Mirzavand et al., 2022; Zuber and Villamil, 2016) or earthworm biomass (De Notaris et al., 2021; Denier et al., 2022; Kuntz et al., 2013; Moos et al., 2016). The study from Peigné et al. (2018) assesses the evolution of soil fertility indices (chemical, physical, biological including earthworms) in a 10-year French organic farming trial with RT, showing increased superficial organic carbon and nitrogen contents, but no improvement of physical nor biological parameters. Thus,

research about RT practices in organic farming using long-term field trials show promising results regarding the fertility of soils in several regions of the world.

Research still needs to unravel the effects of RT on soil properties and functions, and in particular for different pedoclimatic conditions. Indeed, several publications studied the response of a given soil to tillage practices, but a deeper comprehension of the effects of reduced tillage practices on different soil textures is called, to evaluate the long-term feasibility of RT (Gadermaier et al., 2012). Works from Six et al. (2000) and a study of Chivenge et al. (2007) hypothesized that RT has only minimal effect in sandy soils, because of their low ability to form aggregates and to protect soil organic matter (SOM) compared to clayey soils, and was also found by the study from Parajuli et al. (2021). In this paper, we want to study effect of RT in two LTEs of the FiBL characterized by different soil textures, i.e. high-clayey and silty loam, which adds experimental input on the site-specifics effect of RT. Indeed, if the difference between clayey and sandy soil seems clear, the distinct effect between clayey and silty soil is still reported to date.

Moreover, the use of DNA sequencing methods in agricultural soils form opportunities the investigate beneficial effect of RT on soil microbial community, but the recent application of such methods restricts the lack of data to evaluate the effects of RT (Epp Schmidt et al., 2022; Morugán-Coronado et al., 2022; Nunes et al., 2020). In some publications, microbial diversity was decreased in two silty soils (Degrune et al., 2016; Sengupta and Dick, 2015), while others showed non-significant changes in microbial richness with RT practices (Frøslev et al., 2022; Hartman et al., 2018; Kraut-Cohen et al., 2020; Wagg et al., 2018; Xia et al., 2019). Also, studies of specific bacterial community in the N soil cycling, such as nitrifiers, showed contradictory results. While the study from Doran (1980) showed increased nitrification in ploughed soils, other showed increased nitrifiers in RT soils (Krauss et al., 2017a; Legrand et al., 2018). Thus, we consider in this work the potential of modern DNA sequencing methods to study how microbial community is affected by RT in the two LTEs of interest, for which, to date, no biomolecular approach has been implemented.

In this study, two LTEs from the FiBL were used: a first long-term trial in Frick (AG), which has already demonstrated the numerous beneficial effects of RT in a high-clayey soil, as summarized in a recent review from Krauss et al. (2020). Another LTE, aged twelve-year-old and situated in Aesch (BL), aims at testing the effects of reduced tillage practices on a silty soil, with a similar approach regarding tillage. The study will be conducted at three different depths to account for potential depth effects, and with three tillage treatments: conventional tillage (CT), reduced tillage (RT), untilled soil (permanent grassland, G). The effect of RT in the two soils will be assessed through three working hypotheses:

1) The increasing gradient of soil quality indicators from ploughed soil (CT) to reduced soil (RT) is more pronounced on a clay soil compared to a silty soil.

2) There is a clear distinction for both sites between the amount of nitrifiers from undisturbed soil (G) – reduced soil (RT) – ploughed soil (CT), with more nitrifiers in ploughed soil.

3) SOC stocks and microbial communities diversity increase with decreasing soil disturbance intensity.

2 Materials and Methods

2.1 Sites description

Two LTEs with organic farming practices have been conducted by the FiBL in Aesch (BL) since 2010, and in Frick (AG) since 2002 to study the impact of different tillage and fertilization systems on field crops. (Berner et al., 2008; Gadermaier et al., 2012; Krauss et al., 2017a). In this study, the tillage conditions only (RT *vs* CT) are considered. Additionally, grasslands (G) associated with both study sites were considered as a reference, to assess "untilled" condition plots. The grassland sites were chosen to be untilled for the longest time known (from technical personal and surrounding farmers knowledge), as close as possible to the LTEs, and with similar slopes.

The Aesch LTE shown in Figure 1 is located next to a farm called Schlatthof (47°28'N 7°34'E, 351 m) since 2010. The mean annual precipitation is 990 mm and the mean temperature is 10.5 °C (Krauss et al., 2022). The soil type is a Haplic Luvisol with a silty-loam texture (20% clay, 52% silt, 28% sand) (Krauss et al., 2022). The studied grassland is located at the North-East of the LTE (47°28'N, 7°34'E, 352 m), as shown in Figure 2.

Figure 1: Aesch LTE of soil sampling day, 14th of March.

Figure 2: Grassland plot in Aesch, 18th of March.

The Frick LTE seen in Figure 3 is located next to the FiBL (47°51'N 8°02', 350 m) since 2002. The climatic characteristics are a mean annual precipitation of 1030 mm, and a mean temperature of 10.4 °C (Krauss et al., 2022). The soil type is a very clayey Vertic Cambisol (46.5% clay, 24.5% silt, and 29.0% sand (Cania et al., 2020)). The studied grassland is located at the East of the LTE (47°30'N, 8°01'E, 349 m), as shown in Figure 4. In addition, the meteorological data from Aesch during the 2010-2021 period and from Frick in the 2002-2021 period are shown in Appendix 8.1.

Figure 3: Frick LTE on soil sampling day, 24th March.

Figure 4: Grassland plot in Frick, 29th of March.

The crops of both LTEs are grown according to specific rotations. In Frick, since 2014, the rotation scheme started with winter wheat (*Triticum aestivum* L., cv. Titlis), followed by silage maize (*Zea mays* L., cv. Amadeo), spelt (*Triticum spelta* L., cv. Ostro), and a 2-year grass-clover ley mixture (*Trifolium pratense* L., *Trifolium repens* L., *Lolium perenne* L., *Festuca pratensis* Huds., *Dactylus glomerata* L., *Phleum pratense* L.). In Aesch, the rotation scheme is composed of silage maize (cv. Fabregas), field bean (*Vicia faba*, cv. Olan), winter wheat (cv. Runal (2017) and Wiwa (2020)) and grass-clover (same mixture as in Frick). During the March-September 2022 period, grass-clover mixture was cultivated on both LTEs, as seen in Figure 5. The LTE have different fertilization schemes (but the fertilizer effect is no studied here). In the Aesch LTE, the fertilization treatments are organic slurry *vs* mineral (non-organic) *vs* no fertilization, while in Frick the fertilization conditions are slurry *vs* slurry + composted manure. For this work, the plots of the Aesch LTE under "Slurry 2 -120 kg Nt" are considered, and "Slurry without biodynamic preparation" for Frick LTE, such that the fertilization systems are the most similar as possible.

The fertilizer application of this year (2022) was done after the soil sampling, in late March 2022). In the Aesch LTE, liquid cattle slurry was applied, and in Frick, liquid dairy cattle slurry was applied, with an application volume of 40 m^3/ha. A detailed view of the two crops management for the 5 previous years is presented in the Table 1 below.

In the Aesch grassland plots, main botanical species were several grasses (*Poaceae*), buttercups (*Ranunculus repens* L.), clover (*Trifolium* L.), veronica (*Veronica officinalis* L.*),* plantain (*Plantago lanceolata L.*), dandelion (*Taraxacum officinale)* and purple dead nettle (*Lamium purpureum* L.).

In the Frick LTE grassland plots, flora was composed of several grasses (*Poaceae*), dandelion (*Taraxacum officinale)* buttercups (*Ranunculus repens* L.), plantain (*Plantago lanceolata* L.), clover (*Trifolium* L.), veronica (*Veronica officinalis* L.*),* meadow salsify *(Tragopogon pratensis* L.).

Figure 5: Close-up on the grass-clover in Frick LTE, 5th of March.

Table 1: Timetable of soil culture and tillage in Aesch and Frick LTEs in the past 5 years.

Aesch		
Date	**Crop**	**Tillage**
2018	Silage maize: 05.2018 – 09.2018	CT: plough + rotary harrow RT: rotary harrow
2019	Winter wheat: 10.2018-07.2019	CT: plough RT: WecoDyn chisel
2020	Cover crop: 08.2019-08.2020	CT: chisel RT: chisel
2021	Grass-clover: 08.2020-now	CT: rotary hoe RT: rotary hoe
2022	Grass-clover	4-5 cuttings in total (10.05.2022)
Frick		
Date	**Crop**	**Tillage**
2018	Grass-clover: 09.2016 – 10-2018 (CT) / 08.2018 (RT)	CT: chisel RT: chisel + rototiller 9 cuttings in total
2019	Winter wheat: 11.2018-07.2019 Intercrop oat-winter vetch-crimson-clover: 08.2019-04.2020	CT: plough + rotary harrow RT: WecoDyn chisel + rotary harrow CT: chisel RT: chisel

2020	Silage Maize: 04.2020-08.2020	CT: plough + rotary harrow RT: WecoDyn chisel + rotary harrow
2021	Spelt: 10.2020 – 08.2021	CT: plough + rototiller RT: chisel plough + rototiller
2022	Grass-clover: 09.2021 - now	CT: chisel + rotary harrow + rototiller RT: chisel + rotary harrow + rototiller Cutting 1 (02.02.2022) Cutting 2 (21.06.2022)

In conventional tillage conditions, the ploughing is done with a moldboard plough that annually inverted the soil in the 18 cm of the upper soil layer, as presented in Figure 6. Additionally, a rotary harrow was used for seedbed preparation.

Figure 6: Moldboard plough commonly used for conventional tillage.
Photo: Alfred Berner, FiBL

In reduced tillage condition, the soil is managed with a chisel plough or a skim plough working in the first 5-10 cm of the upper soil layer, as shown in Figure 7 and Figure 8, and a rotary harrow for seedbed preparation. Chisel plough annually can undercut weeds at 5 cm below the soil surface or loosen the soil at 10 cm depth, depending on the equipped blades. Skim plough or stubble cleaner allows a shallow inversion in the 5 cm of the top soil and is used for superficial mulch incorporation and grass-clover ley termination.

Figure 7: Chisel plough on the Ecodyn cultivator. Photo: Hansueli Dierauer, FiBL.

Figure 8: Skim plough (Stoppelhobel). Photo: Alfred Berner, FiBL.

2.2 Experimental design and layout

The experimental factors of the study sites are the soil management (RT *vs* CT *vs* G), and the fertilization, (the latter not considered for this work). Both LTEs are arranged in a strip-split-plot design, with four replicates for each tillage (RT or G), giving a set of 8 plots from each LTE, plus 4 grassland replicates in the vicinity of the LTEs. In Aesch, the plots are 17 m x 12 m wide, and in Frick 12 m x 12 m, so that typical farming engines can go on. Detailed maps of the field setups are presented in the Appendix 8.2.

Four sampling sites (three only for biomolecular analysis) were considered for each LTE, to imitate the four replicates of each tillage condition. Thus, an overall of 12 plots per LTE is studied. In the Figure 9 and Figure 10 presented below, the location of the LTE plots and the grassland plots are shown.

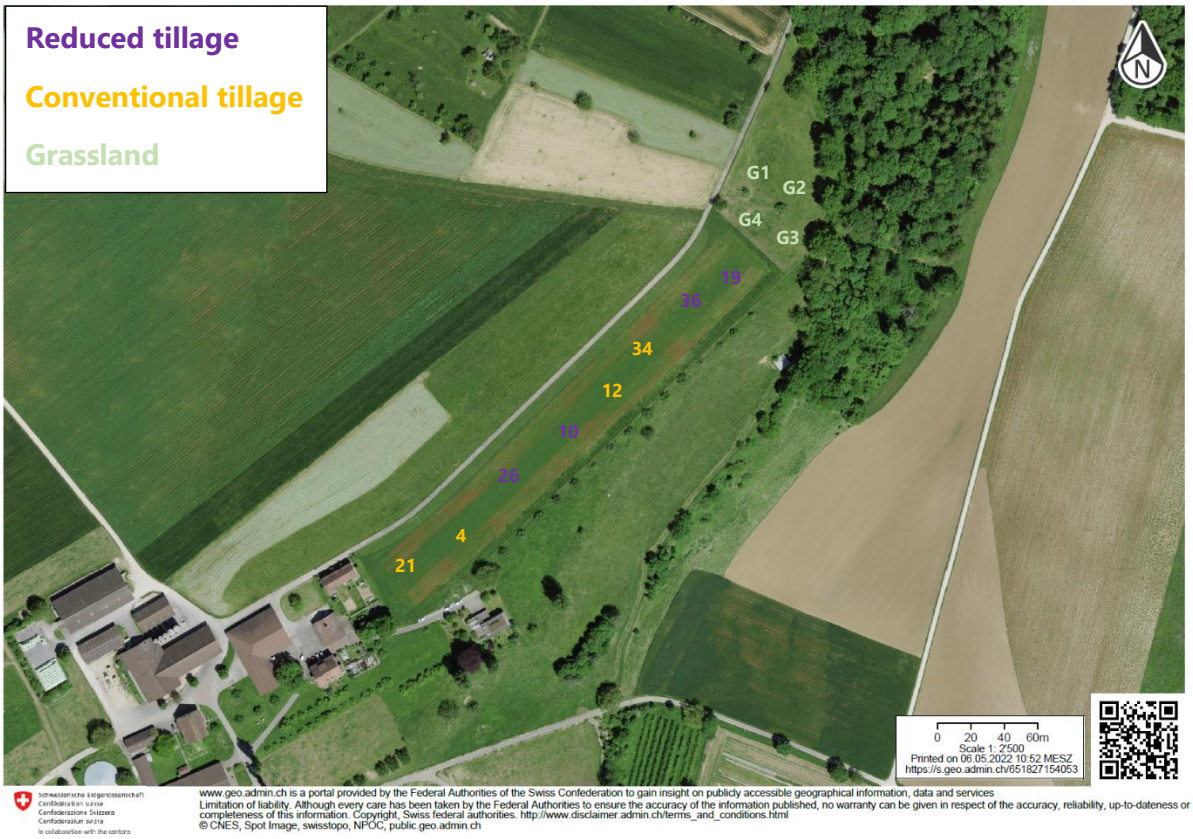

Figure 9: Location of the LTE and the sampling plots in Aesch.

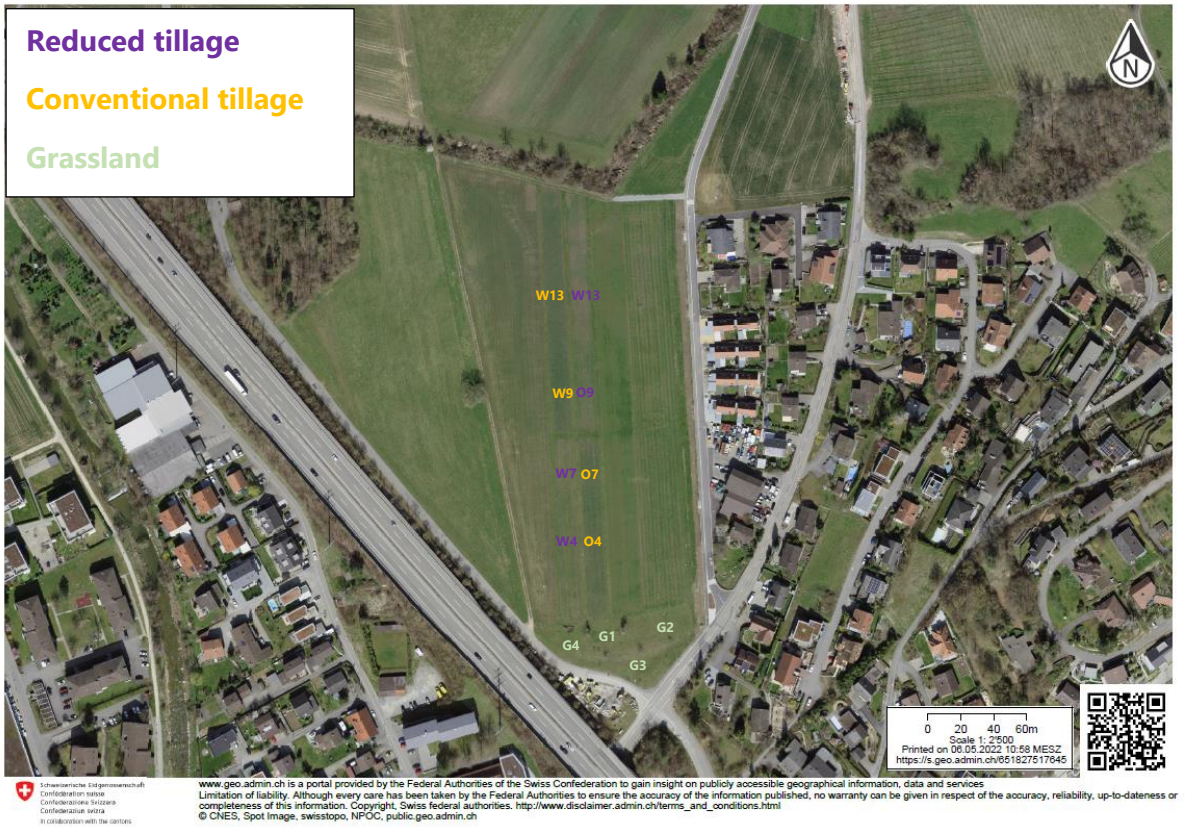

Figure 10: Location of the LTE and the sampling plots in Frick.

2.3 Soil sampling

Bulk soil samples were collected in Aesch on the 14th,15th, and 18th of March and on the 24th of March in Frick LTE.

Soil samples were taken at the three different layers using a soil auger with a diameter of 2.5 cm, also using a hammer for the Frick sampling where the soil was more resistant. A series of about ten cores were collected for each plot, separating the layers in three different bags, to get approximately 700 g of homogenized soil per selected depth (0-10 cm, 10-20 cm, 20-30 cm). At the same time, approximately 10 g of soil was collected in Falcon tubes and immediately frozen in a cooler for DNA analysis. During the sampling, gloves were used and the sampling material (augers, knifes) was washed between each plot sampling. Figure 11, Figure 12, Figure 13 and Figure 14 show the sampling procedure and examples of soil cores from both LTEs.

Figure 11: Soil sampling setup in Aesch, 14th of March.

Figure 12: Soil sampling setup in Aesch, 18th of March.

Figure 13: An example of a core extraction from the grassland plot G3 in Aesch LTE, 18th of March.
One can see at a depth of 5-7 cm a zone with a low soil density, which was repeatly noticed in the Aesch grassland plots.

Figure 14: An example of a core extraction from the grassland plot G1 in Frick LTE, 24th of March.
One can see a clear difference in texture compared to Aesch soil. A piece of anthropogenic red brick is visible.

For bulk density measurement, specific soil samples were collected for bulk density determination using calibrated cylinders of about 100 cm^3, special augers and hammers (Ø 0.05 m, Sample ring kit C, Eijkelkamp, NL). The cylinders were collected on the 28th and 29th of March in the Frick LTE, and on the 31st of March in Aesch LTE. Several pictures are shown below (Figure 15, Figure 16, Figure 17, Figure 18).

Figure 15: Use of the special auger for bulk density determination.

Figure 16: Example of a cylinder core extracted.

Figure 17: Example of cylinder collected in the top layer of grassland in Frick, 28th of March.

Figure 18: Example of cylinder collected in the bottom layer of a grassland in Frick, 28th of March.

The fresh soil samples were sieved at 5 mm in the next 2-3 days (Figure 19, Figure 20), and divided in two subsamples: about 400 g was kept fresh and stored at 4 °C in plastic bags for N_{mic} and C_{mic} analyses, and the remaining was air-dried for various chemical analysis.

The soil samples for DNA analysis were frozen quickly at -18 °C (no sieving) and sent for processing to the EPFL CG-CEL, as shown in Figure 21.

Figure 19: Soil sieving procedure. A sample of Aesch soil is shown.

Figure 20: A sample from Frick which clearly shows the clayey texture.

Figure 21: Soil samples for DNA extraction.

Three layers were considered for the soil parameters: Layer 1 (0-10 cm), Layer 2 (10-20 cm), and Layer 3 (20-30 cm). The first layer corresponded to the depth of the reduced tillage, the second layer corresponded to the additional depth subject to ploughing, and the third layer corresponded to the layer under tillage, also where plant roots take up nutrients. In total, 36 samples per LTE were collected, summing to 72 samples (2 sites x 3 tillage conditions x 4 replicates x 3 layers).

2.4 Soil analyses

The Agroscope methods are standards Swiss protocols applied at FiBL ("Development and validation of reference methods of the Swiss federal agricultural research stations for analysis of soils and fertilizers").

2.4.1 Physical parameters

2.4.1.1 Bulk density

Bulk density was measured following the PYZYL-PN, PYZYL-PA and PYZYL-D methods. At each plot, a set of 3 cylinders per depth were collected, by pressing with the hammer the cylinder-carrying auger within the appropriate layer depth (Figure 15), obtaining a soil sample where the topping edges were properly cut with a sharp blade, as illustrated in Figure 16, Figure 17 and Figure 18. On the following days, the full cylinders were weighted at the soil laboratory, emptied and weighted for tare. The soil contained in the cylinders was then placed in small tared aluminum trays, weighted, dried at 105°C for 24 h, and weighted again in order to determine the dry matter content.

The dry matter content was calculated as follows:

$$DM\ [\%] = \frac{DW}{FW} \times 100 \qquad (1)$$

With:

Tare: Weight of the empty Cylinder/Drying tray [g]

DW: Gross weight of the of the dry soil sample - Tara [g]

FW: Gross weight of the naturally moist sample - Tara [g]

The soil bulk density was calculated as follows:

$$BD\ [g \cdot cm^{-3}] = \frac{DW\ [g]}{Ring\ volume\ [cm^3]} \qquad (2)$$

And corrected for the presence of stones and earthworms as follows:

$$BD\ [g \cdot cm^{-3}] = \frac{DW\ [g] - Stone\ weight\ [g] - Earthworm\ weight\ [g]}{Ring\ volume\ [cm^3] - \frac{Stone\ weight\ [g]}{Stone\ density\ [g \cdot cm^{-3}]} - \frac{Earthworm\ weight\ [g]}{Earthworm\ density\ [g \cdot cm^{-3}]}} \qquad (3)$$

With:

Stone density: Approximation of rock density = 2.46 g/cm^3

Earthworm: Approximation of Earthworm density = 0.96 g/cm^3

2.4.1.2 Water holding capacity

The maximum water holding capacity (mWHC) was measured according to the B-WHC method from Agroscope ("Development and validation of reference methods of the Swiss federal agricultural research stations for analysis of soils and fertilizers") and adapted by FiBL.

Maximum water holding capacity was determined for a subset of the samples only, to verify a sufficient soil water content, corresponding to 40–50% maximum water holding capacity, necessary to the incubation of soil sample preceding the microbial biomass determination with chloroform fumigation extraction (CFE). For the Aesch samples, one replicate per tillage condition only was analyzed for water holding capacity (plots 4, 10, G1; considering the 3 layers). The Frick samples were already evaluated, by visual assessment and high clay content, to be wet enough for incubation, thus the procedure was not repeated.

Field-moist soil samples were weighted and placed in standardized cylinders with porous bottom, and progressively saturated with water by capillary force, as seen in Figure 22. When full saturation was reached (approximately one hour), the cylinders were placed for approximately four hours in a water-saturated sand bath illustrated in Figure 23, letting the exceeding soil water to flow towards the sand bath that was regularly emptied of the surplus water. The water-saturated samples were emptied in aluminum tray, weighted, dried at 105°C for 24 h and weighted again.

Figure 22: Soil samples progressively saturated with water for mWHC determination.

Figure 23: Soil samples in the sand bath for mWHC determination.

The maximum water holding capacity was determined according to the following equation:

$$mWHC\ [g] = \frac{AW-(FW\times\frac{DM}{100})}{FW\times\frac{DM}{100}} \qquad (4)$$

With:

Tara: Weight of the empty Cylinder/Drying tray [g]

AW: Gross weight of the soil sample at max. WHK (g) – Tara [g]

FW: Gross weight of the naturally moist soil sample (g) – Tara [g]

DM: Dry matter of the naturally moist soil sample [%]

The water content related to the naturally moist soil was calculated as follows:

$$WC\ [\%] = (1-\frac{DM}{100})\times 100 \qquad (5)$$

2.4.2 Chemical parameters

2.4.2.1 pH – H_2O

The pH of oven-dried samples was measured according to the pH method from Agroscope and adapted by FiBL.

A soil suspension made of 20 g and 50 mL demineralized water (1:10, w/v) was shaken and stand overnight before measurement with pH-Meter (WTW InoLab pH Level 1) and associated pH-Electrode (WTW SenTix 81), in a continuously stirred soil solution. The Figure 24 below illustrates the pH measurement.

Figure 24: Measurement of the pH on a stirring platform.

2.4.2.2 Carbon content: TC, Carbonates, SOC, SOC_{stock}

Total carbon (TC) and soil organic carbon (SOC) contents from oven-dried samples were determined according to the FiBL methods based on a single-run dual temperature combustion method (Bisutti et al., 2007).

TC was determined by direct combustion of the oven-dried soil samples at 900°C on Elementar Analyser Vario Max Cube C/N (Elementar Analyse system GmbH D-63505 Langenselbold), by thermal conductivity detection. In-organic carbon (or mineral carbon, later called Carbonates (Carb)) was determined by combustion of a second soil sample at 500°C in muffle furnace, with subsequent combustion with the same analyzer. The determination of total and organic carbon was done by the laboratory technicians.

The organic matter content was derived from total carbon and mineral carbon contents:

$$SOC[\%] = TC[\%] - Carb[\%] \qquad (6)$$

Additionally, the organic carbon stocks can be calculated as following:

$$SOC_{stock}\,[g \cdot m^{-2}] = SOC\,[\%] \times Depth_{soil\ layer}\,[m] \times Area\,[m^2] \times BD\,[g \cdot cm^{-3}] \times 10^6\,[cm^3 \cdot m^{-3}] \qquad (7)$$

2.4.2.3 Soil nutrients

Total Nitrogen (TN) was simultaneously determined with TC from the oven-dried soil samples with 900°C direct combustion by Elementar Analyser Vario Max Cube.

Soil micronutrients (P, K, Mg, Ca, Cu, Fe, Mn, B) were analyzed by an external laboratory (LBU Labor für Boden- und Umweltanalytik, Eric Schweizer SA) using 1:10 NH_4-Acetat-EDTA. Soil samples used for these analyses were grinded and sieved at 2 mm before shipment.

2.4.3 Biological parameters

2.4.3.1 Soil microbial biomass C (C_{mic}) and N (N_{mic})

Soil microbial biomass was measured by Chloroform-Fumigation-Extraction (CFE) from the method B-BM-FE of Agroscope ("Development and validation of reference methods of the Swiss federal agricultural research stations for analysis of soils and fertilizers"), developed by Vance et al. (1987) and Brookes et al. (1985). The principle consists in treating soil samples with chloroform gas, which leads to the disruption of the microbial cells, while the remaining organic matter of the soil is not altered. After extraction with a K_2SO_4 solution, according to the method B-CN-EX, non-purgeable organic carbon (NPOC) and total nitrogen bound (TNb) contents are analyzed, according to the methods B-OC-E and B-NT-E respectively. By comparing non-purgeable organic carbon (NPOC) and total nitrogen bound (TNb) contents of the non-fumigated and the fumigated soil samples, the microbial biomass can be determined. The analysis of NPOC and TNb was done by direct 800 °C combustion using the Multi N/C S100S analyzer (Analytik Jena GmbH, D-07745 Jena). In this study, the determination of microbial biomass by CFE was done with only one measurement per sample (unlike other methods where it was done with three replicates per sample). In addition to the 36 samples from each site, three replicates of a reference soil and three blank solutions of K_2PO_4 solution was used for analyses.

Field-moist soil samples were used, as their water content was sufficient for soil biological measures (See Section Water holding capacity). The analysis began with the incubation of half of the fresh soil samples at 25°C for one week. A mass corresponding to 20 g of dry soil matter was weighted two times, for non-fumigated samples and fumigated samples. In parallel, the dry matter content for the samples was determined by weighting and drying at 105°C. The extraction for NPOC and TNb analysis in the non-fumigated samples was done by placing the soil in 250 mL bottles filled with 80 mL of a K_2PO_4 (0.5 M) solution, stirring (90 min, 250 rpm), and filtration with paper filters (MN615¼, Macherey-Nagel, Düren, Germany) placed on funnels, as illustrated in Figure 26. The first 10 mL of filtrate was thrown away, and approximately 30 mL of filtrate was extracted in Falcon tubes and immediately frozen until analysis.

The chloroform fumigation was done using a desiccator as illustrated in Figure 25. Previously weighted soil samples were placed in small beakers and placed in the desiccator whose walls were kept wet using moist paper. In the middle of the desiccator, a beaker filled with 30 mL chloroform was placed, and the desiccator was air-sealed using grease. The desiccator was connected to the vacuum and brought to an absolute pressure of 100 mm Hg for chloroform boiling for two minutes. After a 24 h incubation in the dark, the chloroform was removed, and the remaining vapors were removed from the desiccator with repeated aeration and vacuum washes. The fumigated soil samples were transferred to glass bottles, and the identical determinations of NPOC and TNb by filtration and a K_2PO_4 solution were performed.

Figure 25: The desiccator used for chloroform fumigation. Here, the desiccator is shown after the 24 h incubation, connected to the vacuum for aeration.

Figure 26: Extraction of the organic contents by filtration for microbial biomass determination.

Soil microbial biomass was then calculated according with the following formulas:

$$C_{mic} = \frac{EC}{kEC} \qquad (8)$$

$$N_{mic} = \frac{EN}{kEN} \qquad (9)$$

With

$EC = NPOC_{non-fumigated} - NPOC_{fumigated}$

kEC = 0.45 (Joergensen, 1996)

$EN = TNb_{non-fumigated} - TNb_{fumigated}$

kEN = 0.54 (Joergensen and Mueller, 1996)

2.4.3.2 Earthworm sampling

The earthworm sampling was done according to the method B-RW-E from Agroscope and adapted by FiBL ("Development and validation of reference methods of the Swiss federal agricultural research stations for analysis of soils and fertilizers").

The sampling of earthworms was spread over two weeks because of the time-consuming procedure and the number of people needed. The Aesch sampling was done between the 12th and the 13th of April, while the Frick sampling lasted the following week, as illustrated in Table 2 below. The sunny and warm weather conditions that led to soil drying during the sampling timelapse is an important factor to consider and will be discussed later.

Table 2: Time-lapse of Earthworm sampling.

Site	**Aesch**	**Aesch**	**Frick**	**Frick**	**Frick**	**Frick**	**Frick**	**Frick**
Date	12/04	13/04	14/04	15/04	19/04	20/04	21/04	22/04
Earthworm Sampling plots	4 10 12 19 21 26 34 36	G1 G2 G3 G4	W13	O13	W9	O9 W7 O7	W3 O3 G4	G2 G3 G1

At each sampling plot, a square of 30 cm x 30 cm was dig down to 15-20 cm using a spade and a fork (Figure 27, Figure 28). The pile of the soil was broken up by hands at about 1 cm resolution in order to gather the earthworms. Several example of earthworms are illustrated in Figure 29, Figure 30, Figure 31, Figure 32. In order to get the deep-hidden individuals, a method using a mustard solution is commonly used, as the sulfuric compounds in mustard are skin-irritant to earthworms and causes them to crawl to the surface. A mustard solution made of 30 g of mustard powder in 5 L water was poured in the hole, so that after 20 min waiting, the earthworms coming up to the surface were collected.

Figure 27: Sampling area definition for earthworm sampling.

Figure 28: Hole of 20 cm deep in Frick LTE.

Figure 29: Earthworm found in Aesch soil.

Figure 30: Earthworm found in Frick soil.

Figure 31: Earthworm found in Frick soil.

Figure 32: Earthworm found in Frick soil.

The collected earthworms were kept in water. They were then sorted by maturity state, differentiating juveniles (J), adults (A), and sections of earthworms (O), and weighted. Adults are recognizable by the presence of their sexual organ called clitellum. Several illustrative pictures are shown in Figure 33, Figure 34, Figure 35, Figure 36, below.

Figure 33: A plentiful earthworm sample from Aesch LTE.

Figure 34: Example of earthworm sample from Aesch. Adults are on the top, juveniles on the bottom.

Figure 35: Example of Earthworm sample for Frick LTE.

Figure 36: Comparison between CT (on the left) and RT (on the right) samples from Frick.

2.4.3.3 DNA analyses: extraction and amplification by qPCR

DNA extraction and amplification were done at the EPFL Central Environmental Laboratory (GR-CEL) using the frozen soil samples. Soil samples, as well as extractions of positive control (ZymoBIOMICS Microbial Community Standard, Zymo, USA) and negative controls (sterile water, one control per extraction cycle) were processed using the DNeasy PowerSoil Pro kit on a QiaCube robot (all Qiagen, USA), according to the manufacturer's instructions. Prior to automated extraction, initial extraction buffer and samples were heated to 60°C for 5 min and processed with a Precellys bead beating system (2x 15 s, at 5500 rpm with a 20 s interval) (Bertin Instruments, France). 600 µL of the extraction solution was recovered by centrifugation (1 min, 12'000 ×g) and loaded into the QiaCube robot. Finally, DNA was eluted in 100 µL of final C6 extraction buffers and quantified using a NanoDrop OneC (Thermo, USA).

Quantification of the bacteria16S rRNA genes was performed in triplicate using the primers 338f (5′-ACTCCTACGGGAGGCAGCAG-3′) and 520r (5′-ATTACCGCGGCTGCTGG-3′) in 10 µL reactions with a MIC qPCR Cycler (BioMolecular Systems, Australia), as follows: 2.5 mL template DNA, 2.1 µL water, 0.2 µL of each primer (10 µM stock) and 5 µL of 2× SensiFAST SYBR® No-ROX Kit (Bioline, England). Samples were cycled (40 cycles) at 95°C for 5 s, followed by extension at 62°C for 15 s and acquisition at 72°C for 15 s. The final melting step was performed from 72°C to 95°C, at a rate of 0.1°C/s.

Fungal species were quantified by targeting a 351-bp region in the fungal 18S rRNA gene using the FungiQuant primer set (5′-GGRAAACTCACCAGGTCCAG-3′ and 5′-GSWCTATCCCCAKCACGA-3′)(Liu et al., 2012), This analysis was conducted as follows: 2.5 mL template DNA, 0.5 µL water, 1 µL of each primer (10 µM stock) and 5 µL of 2× SensiFAST SYBR® No-ROX Kit (Bioline, England). Samples were similarly cycled (40 cycles) at 95°C for 5 s, followed by extension at 60°C for 15 s and acquisition at 72°C for 15 s. The final melting step was carried out from 72°C to 95°C, at a rate of 0.1°C/s.

Analysis of the results was performed using the integrated analytical software (micPCR, BioMolecular Systems, Australia). On average, efficiency (0.88 – 101.2%) and r^2 values (> 0.995) were determined from eight points of serial dilutions (10^8–10^1 copies) of each target gene. Based on the calibration curves, Ct values were used to calculate the gene copy numbers, which were normalized to the mass (ng) of extracted DNA. For practical machine manipulation reasons, only 11 out of the 12 samples per LTE and depth were extracted, without using the G4 samples.

2.4.3.4 DNA analyses: microbial community sequencing

Microbial community analyses were performed using the Quick16S NGS Library Prep Kit (Zymo Research, USA) targeting the microbial 16S ribosomal gene, according manufacturer's instructions. Four replicates per depth and per site for the RT and CT samples, and three replicates per depth and per site for G samples were used. In addition, various negative and positive controls (ZymoBIOMICS Microbial standards, Zymo, USA) were used, for a total of 72 samples. Sequencing was performed at the Lausanne

Genomic Technologies Facility (GTF) of the University of Lausanne, by means of pair-end 300 on an Illumina platform. A little more than 4,2 million pair-end reads were retained after processing on Mothur (Schloss, 2020), including the removal of low quality reads in order to correct possible errors induced by the library production and sequencing protocols. The mapping on Mothur was done using the version on the Greengenes database (gg_13_8_99), with the script available in Appendix 8.3, and database available on https://greengenes.secondgenome.com/.

Lastly, 60'000 sequences were retained per sample for the statistical multivariate analyses.

From this dataset, the proportion of nitrifiers to the overall microbial community was calculated as a percentage. To do so, entire set of sequences affiliated to the microbial genus were cumulated, obtaining between 76 (Frick, G, 20-30 cm) et 682 (Aesch, RT, 10-20 cm) sequences, out of a total of 60'000 sequences per sample.

Hereunder, Table 3 summarizes the sampling and analyzes procedures for each parameter.

Table 3: Summary of analyses.
**standard Swiss protocols.*

Sampling date	Analysis date	Lab	Soil manipulation	Parameter	Method
March	May	Frick	Special cylinder cores	Bulk density	PYZYL-D*
March	May	Frick	5 mm sieved, 105°C dried	pH H_2O	pH*
March	May	Frick	5 mm sieved, 105°C dried	TC, SOC, TN	Bisutti et al. 2007
March	July	LBU	2 mm sieved, 105°C dried	Soil micronutrients (P, K, Mg, Ca, Cu, Fe, Mn, B)	External laboratory
April	April	Frick	In field	Earthworm biomass (total/ juveniles /adults) in top layer only	B-RW-E*
March	April	Frick	5 mm sieved, 4°C stored	Microbial Biomass (C_{mic}, N_{mic})	B-BM-FE*, B-CN-EX*, B-OC-E*, B-T-E*
March	June	EPFL	Stored at -18°C	Total soil DNA extracted	DNA sequencing
March	June	EPFL	Stored at -18°C	Copies of bacteria genes	16S rRNA by qPCR
March	June	EPFL	Stored at -18°C	Copies of fungi genes	18S rRNA by qPCR
March	July	EPFL	Stored at -18°C	Nitrifiers proportion in the bacterial community	16S rRNA by qPCR
March	July	GTF Lausanne	Stored at -18°C	Microbial community sequencing	16S rRNA by qPCR

2.5 Data analyses

From the 72 samples, the averages and standard deviations of the four replicates per plot were computed on Excel using Pivot table, thus differentiating LTE, depth and tillage condition in 18 values (2 sites x 3 depths x 3 tillage conditions) in order to create a data table. For the rest of the statistical analyses, the initial data considering 72 samples with the four replicates were considered.

A mixed model analysis of variance (ANOVA) was first used to identify the statistical significance of the experiment factors on the different soil parameters. The factors Site, Tillage and their interactions were defined as fixed factors. The Site factor represents the soil texture difference. Significant differences between the two factors and their interaction were depicted. The analysis was done for each of the three soil layers. The ANOVA analysis was performed with R version 2.14.1 using the *nlme* package (R Core Team, 2012). C_{mic} and N_{mic} in 10-20 cm and 20-30 cm depth layers were square root transformed. A Tukey post hoc test was also run per soil depth in order to show the significant differences between the parameter values in each site and tillage condition. To visually address the differences in LTEs and tillage conditions, boxplots of the variables were created with the *ggplot* function on R. For each parameter, the data from the three layers were separated into three figures, except for earthworms, that were determined for the top layer only. The R codes for ANOVA analysis and boxplotting are available in Appendix 8.4 and Appendix 8.5 respectively.

Secondly, an exploratory data analysis was conducted on the environmental parameters gathering all replicates separately, in order to investigate how the samples between both LTEs, the three tillage conditions and three depths were distributed. A clustering technique based on minimum variance was implemented by using the Ward algorithm with the *hclust* function, after standardization using *decostand* and *vegdist* from the *vegan* package. In the dataset, the chemical and physical parameters were all included. In the biological dataset, only C_{mic}, N_{mic} and total extracted DNA were considered. The number of copies of bacteria and fungi collected by qPCR were removed from the dataset for this first analysis, because different species from the two different sites may be subject to different replication effectiveness in the qPCR, resulting in a possible bias in the number of copies extracted for each site (Azarbad et al., 2022; Fierer et al., 2005). Moreover, the earthworm's parameters were removed as they did not have any values for the second and third layers, and will be treated separately. The microbial community data was not used at this point of the analysis.

As a strong site differentiation was confirmed by the clustering analysis, PCA was conducted for each LTE separately, in order to compare the influence of the soil physical and chemical parameters, later called the environmental parameters, and of the biological parameters, with bacteria and fungi copies

re-introduced, between the two LTEs. Earthworms were included in the 0-10 cm depth, but not in the 10-20 cm and 20-30 cm depths. Microbial community structure data was not considered at this point of the analysis. Also, as bacteria and fungi data from qPCR extraction was done for the first three grassland replicates only, all the environmental data from G4 replicates was removed for the analysis. Moreover, the missing bulk density values for the plot W9 of Frick were replaced by the mean of the three corresponding replicates (O4, O7 and W13). The PCAs, calculations of eigenvalues and variances percentages, scaling 2 PCAs biplots were carried out with the *vegan* and *stats* package. In scaling 2 biplot, the angles between parameters reflect their correlations (0° positively correlated; 90° no correlation; 180° negatively correlated) Furthermore, correlation between variables were calculated using Spearman algorithm, with the function *cor(method="Spearman")* and *corrplot* from the *stats* and *corrplot* packages.

Thirdly, clustering technique, PCA and Mantel tests on microbial community data (from sequencing analysis) with a posteriori projection and interpretation of environmental factors were equally carried out on R with the *vegan* packages as shown in Coral et al. (2018), with separated procedure for the two sites. A second analysis was conducted on the dataset of the 0-10 cm layer, with the inclusion of the earthworms data. Permutations and ANOVA tests were run on the environmental parameters combined with microbial community structure. The R codes for the clustering technique, PCAs and statistical tests are available in Appendix 8.6.

Lastly, diversity indices were calculated from the detailed data produced by massive sequencing of microbial communities. In this work, these indices were calculated at the phylogenetic level of Genus. It was the lowest phylogenetic level at which it was possible to work with precision, as the sequences obtained, of the order of 550 bp, did not allow a precise analysis at the species level.
A diversity index is a quantitative measure that reflects the number of different types in a community, and that can simultaneously consider phylogenetic relationships between individuals such as richness or evenness. The indices chosen are classical in microbial ecology. First of all, the Genus Richness (S) reports all the genera encountered in each sample. Fisher's alpha is an indicator of biodiversity independent from the size of the sampling, whereas chao1 and ACE (Abundance-based coverage estimators) indexes are measures of diversity (abundance) which, beside richness, consider the ratio of rare individuals. For all three cases, the higher the value, the greater the intrinsic richness (alpha diversity) in the sample. Another calculated index was Pielou's evenness, which allows to calculate the regularity of species, and refers to the degree of relatedness of the number of each species in an environment. A calculated value of Pielou's evenness varies from 0 (no evenness) to 1 (complete evenness).

3 Results

3.1 Environmental parameters

The ANOVA results are presented in the Table 4 hereunder with the corresponding p-values for environmental parameters and microbial DNA parameters. The detailed table is presented in Appendix 8.7. Additionally, a synthetic table of the data is presented in Appendix 8.8, with colored formatting. The boxplots of soil environmental parameters are presented below.

Table 4: Results (p-values) of the ANOVA assessing the effects of Site (Trial), Tillage (Tillage) and their interaction on the environmental and biomolecular parameters.

Parameter	**Bulk density**		**Soil Water Content**		**pH**		**SOC**		**SOC stock**	
Depth	Fixed	p-value	Fixed	p-value	Fixed	p-value	Fixed	p-value	Fixed	p-value
0-10 cm	Trial	0.696	Trial	**0.000**	Trial	**0.045**	Trial	**0.000**	Trial	**0.000**
	Tillage	**0.000**	Tillage	**0.019**	Tillage	0.220	Tillage	**0.000**	Tillage	**0.000**
	Trial:Tillage	0.229	Trial:Tillage	0.110	Trial:Tillage	0.290	Trial:Tillage	0.101	Trial:Tillage	0.277
10-20 cm	Trial	**0.020**	Trial	**0.000**	Trial	0.317	Trial	**0.001**	Trial	**0.000**
	Tillage	**0.028**	Tillage	0.135	Tillage	0.921	Tillage	0.993	Tillage	0.762
	Trial:Tillage	**0.007**	Trial:Tillage	**0.000**	Trial:Tillage	0.791	Trial:Tillage	0.535	Trial:Tillage	0.727
20-30 cm	Trial	**0.027**	Trial	**0.000**	Trial	**0.000**	Trial	**0.000**	Trial	**0.000**
	Tillage	0.841	Tillage	0.789	Tillage	0.379	Tillage	0.305	Tillage	0.282
	Trial:Tillage	0.232	Trial:Tillage	**0.032**	Trial:Tillage	0.651	Trial:Tillage	0.379	Trial:Tillage	0.161

Parameter	**Soil TN**		**P**		**K**		**Mg**		**Ca**	
Depth	Fixed	p-value	Fixed	p-value	Fixed	p-value	Fixed	p-value	Fixed	p-value
0-10 cm	Trial	**0.000**	Trial	0.711	Trial	**0.000**	Trial	**0.000**	Trial	**0.000**
	Tillage	**0.000**	Tillage	0.150	Tillage	**0.000**	Tillage	0.911	Tillage	0.757
	Trial:Tillage	0.107	Trial:Tillage	0.272	Trial:Tillage	**0.000**	Trial:Tillage	0.830	Trial:Tillage	0.556
10-20 cm	Trial	**0.001**	Trial	0.891	Trial	**0.000**	Trial	**0.000**	Trial	**0.000**
	Tillage	0.595	Tillage	0.062	Tillage	**0.000**	Tillage	0.933	Tillage	0.644
	Trial:Tillage	0.227	Trial:Tillage	0.321	Trial:Tillage	**0.000**	Trial:Tillage	0.917	Trial:Tillage	0.354
20-30 cm	Trial	**0.000**	Trial	0.412	Trial	**0.000**	Trial	**0.000**	Trial	**0.000**
	Tillage	0.270	Tillage	**0.005**	Tillage	**0.000**	Tillage	0.843	Tillage	0.456
	Trial:Tillage	0.394	Trial:Tillage	0.379	Trial:Tillage	**0.000**	Trial:Tillage	0.847	Trial:Tillage	0.251

Parameter	Cu		Fe		Mn		B		Nitrifiers	
Depth	Fixed	p-value	Fixed	p-value	Fixed	p-value	Fixed	p-value	Fixed	p-value
0-10 cm	Trial	0.444	Trial	0.619	Trial	0.766	Trial	**0.000**	Trial	**0.009**
	Tillage	0.602	Tillage	0.412	Tillage	0.538	Tillage	0.171	Tillage	0.673
	Trial:Tillage	0.128	Trial:Tillage	**0.004**	Trial:Tillage	0.523	Trial:Tillage	0.472	Trial:Tillage	**0.011**
10-20 cm	Trial	0.695	Trial	**0.044**	Trial	0.934	Trial	**0.000**	Trial	**0.000**
	Tillage	0.327	Tillage	0.120	Tillage	0.542	Tillage	**0.004**	Tillage	**0.035**
	Trial:Tillage	**0.042**	Trial:Tillage	**0.001**	Trial:Tillage	0.669	Trial:Tillage	0.399	Trial:Tillage	**0.001**
20-30 cm	Trial	0.401	Trial	**0.003**	Trial	0.486	Trial	**0.000**	Trial	**0.000**
	Tillage	0.070	Tillage	**0.020**	Tillage	0.402	Tillage	**0.002**	Tillage	0.479
	Trial:Tillage	**0.035**	Trial:Tillage	0.074	Trial:Tillage	0.345	Trial:Tillage	0.305	Trial:Tillage	**0.000**

Parameter	Nb of adults		Nb of juveniles		Biom. of adults		Biom. of juv.	
Depth	Fixed	p-value	Fixed	p-value	Fixed	p-value	Fixed	p-value
0-10 cm	Trial	0.980	Trial	0.924	Trial	**0.000**	Trial	**0.005**
	Tillage	0.126	Tillage	**0.002**	Tillage	**0.027**	Tillage	0.318
	Trial:Tillage	0.297	Trial:Tillage	0.249	Trial:Tillage	**0.004**	Trial:Tillage	0.287

Parameter	Soil C_{mic}		Soil N_{mic}		Total soil DNA		Bacteria		Fungi	
Depth	Fixed	p-value	Fixed	p-value	Fixed	p-value	Fixed	p-value	Fixed	p-value
0-10 cm	Trial	**0.000**	Trial	**0.002**	Trial	0.153	Trial	0.300	Trial	**0.000**
	Tillage	**0.000**	Tillage	**0.000**	Tillage	**0.001**	Tillage	**0.000**	Tillage	0.555
	Trial:Tillage	0.918	Trial:Tillage	0.219	Trial:Tillage	0.252	Trial:Tillage	**0.003**	Trial:Tillage	0.905
10-20 cm	Trial	**0.000**	Trial	**0.000**	Trial	0.206	Trial	0.106	Trial	**0.000**
	Tillage	0.118	Tillage	0.183	Tillage	0.070	Tillage	0.214	Tillage	0.815
	Trial:Tillage	0.269	Trial:Tillage	0.731	Trial:Tillage	0.902	Trial:Tillage	0.745	Trial:Tillage	0.591
20-30 cm	Trial	**0.008**	Trial	**0.050**	Trial	0.184	Trial	**0.000**	Trial	**0.000**
	Tillage	0.107	Tillage	0.173	Tillage	0.991	Tillage	0.491	Tillage	0.194
	Trial:Tillage	0.185	Trial:Tillage	0.347	Trial:Tillage	0.351	Trial:Tillage	0.718	Trial:Tillage	0.482

3.1.1 Bulk density

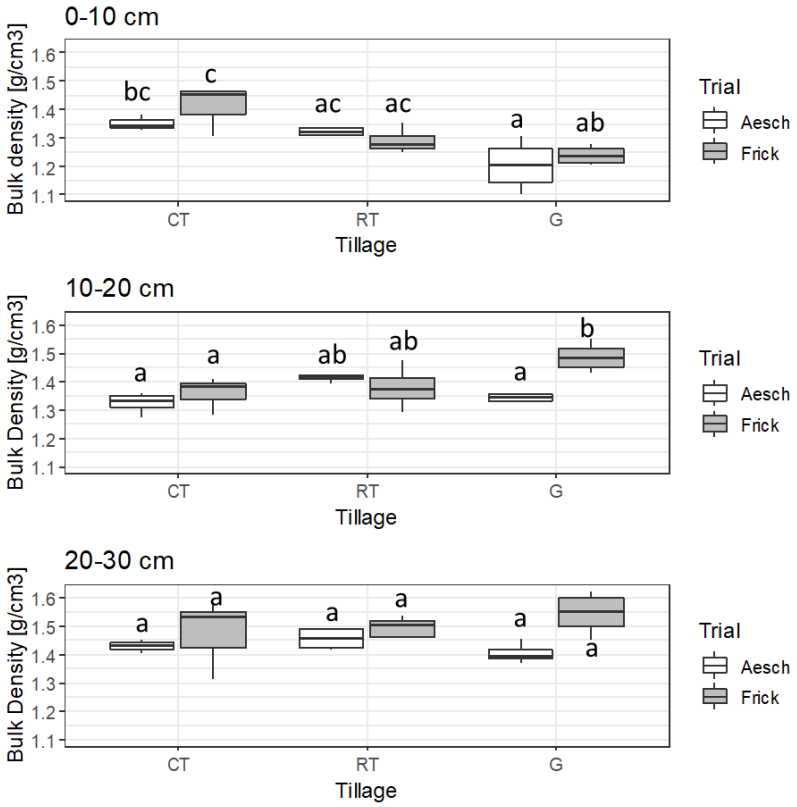

Figure 37: Bulk density [g/cm^3] of the three tillage conditions at the sites of Aesch and Frick and for the three sampling depths.
The letters represent the results of the Tukey post hoc test run for each layer.

The boxplot of bulk densities with the addition of the results obtained after Tukey post hoc test is shown in Figure 37.

In the 0-10 cm layer and for both sites, a tillage effect was clearly marked ($p<0.001$), with decreasing bulk densities from CT to RT, and from RT to G. Significant differences were observed between G and the two other tillage conditions for both sites. It was visible that the difference between CT and RT was more pronounced for Frick soil than for Aesch soil.

In the second layer (10-20 cm), an interaction effect between site and tillage was recorded ($p=0.007$): the difference between tillage conditions was not significant for Aesch, while in Frick, bulk density was significantly lower in RT and CT than in G.

Below the ploughing depth, in the layer 20-30 cm, no tillage effect was reported, but bulk density was higher in Frick than in Aesch ($p=0.027$). Note that bulk density in both LTEs increased compared to the two layers above.

Tillage treatment impacted bulk density differently between the different layers: In the top layer, CT conditions lead to higher bulk density than G and RT, whereas in the second and third layers, the RT condition lead to higher bulk densities than CT.

The effect of tillage was also different for the two sites in the first and second layers: bulk density under CT conditions was lower in Aesch than in Frick, while under RT condition, bulk density was lower in Frick than in Aesch.

3.1.2 Water content

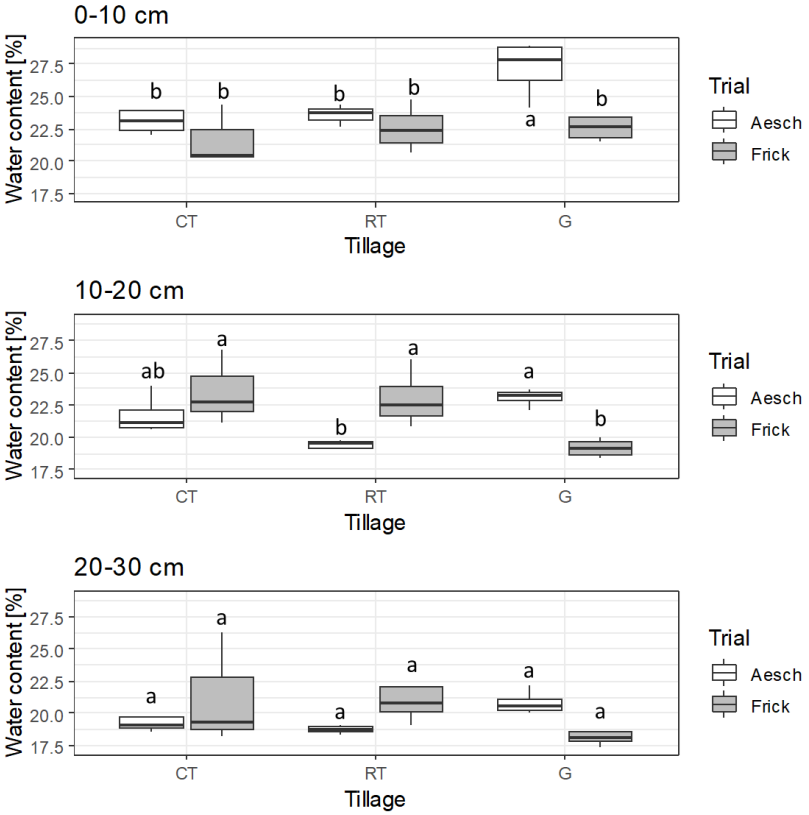

Figure 38: Water content [%] of the three tillage conditions at the sites of Aesch and Frick and for the three sampling depths.
The letters represent the results of the Tukey post hoc test run for each layer.

The boxplot of water contents is shown in Figure 38.

In the first layer, no effect of RT compared to CT was observed at any sites. The grassland plot of Aesch was significantly higher from the RT plot. WC was higher in Aesch than in Frick for RT ($p<0.001$).

In the second layer, RT did not affect WC compared to CT. An effect of combined tillage and site was recorded ($p<0.001$): in Aesch, WC was significantly higher in the grassland than in RT; in Frick, WC was significantly lower in the grassland than in the RT and CT plots. WC was higher in Frick than in Aesch in cropped plots, and was higher in Aesch than in Frick in grassland plots ($p<0.001$).

In the third layer, no tillage effect was recorded, but a site effect was reported, with higher values in the cropped plots of Frick than Aesch, and a higher value in Aesch grassland than in Frick grassland (p=0.032).

Note that in the first layer, WC was higher in Aesch than in Frick for both CT and RT, while in the second and third layers, WC was higher in Frick than in Aesch (p<0.001). Also, WC increased from first to second layer in Frick, while in Aesch, WC decreased from first to second layer. In the third layer, WC decreased for both sites compared to the second layer.

3.1.3 pH

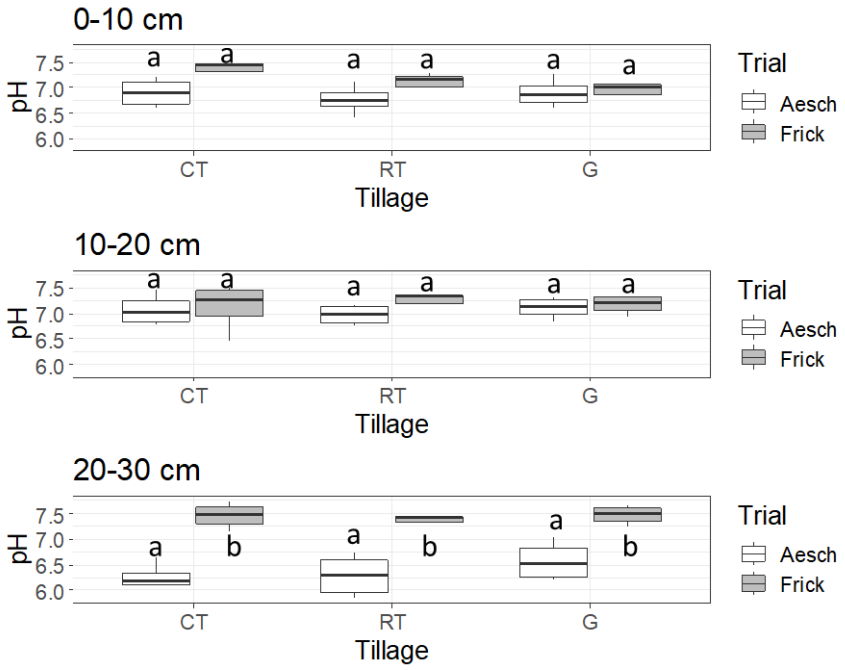

Figure 39: pH [-] of the three tillage conditions at the sites of Aesch and Frick and for the three sampling depths. The letters represent the results of the Tukey post hoc test run for each layer.

The boxplot of pH is shown in Figure 39.

The first layer showed a small but not significant tillage effect on the Frick site, with a decrease of pH from CT to RT to G, from 7.5 to 7. The first layer showed a small site effect (p=0.045), with higher pH values in Frick than in Aesch and with the most visible difference for CT.

In the second layer, no site or tillage effect was reported.

In the third layer, pH sharply decreased of one unit in the Aesch (p<0.001), but without tillage effect.

Thus, in the three layers, the effect of tillage on pH was not significant at each site. The pH did not significantly change between the layers except for the decrease in the third layer of Aesch as mentioned before.

3.1.4 Organic carbon and nutrients

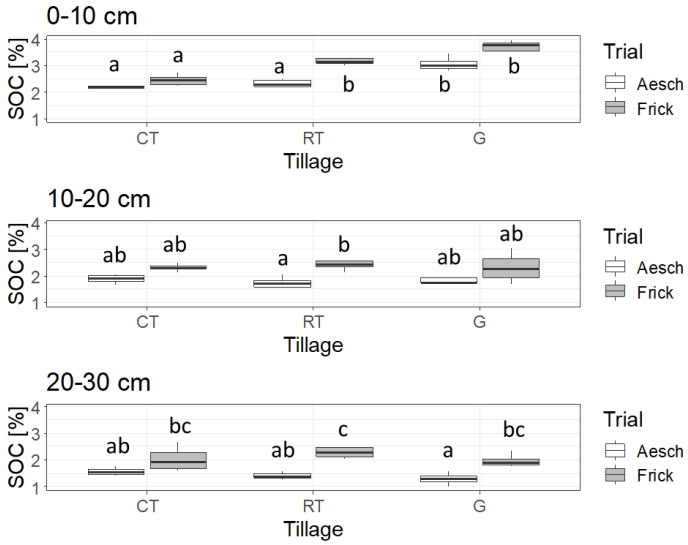

Figure 40: SOC [%] of the three tillage conditions at the sites of Aesch and Frick and for the three sampling depths. The letters represent the results of the Tukey post hoc test run for each layer.

The boxplot of SOC contents is shown in Figure 40.

The SOC content was higher in Frick than in Aesch for every depth and tillage condition ($p<0.001$).

The first layer exhibited an effect of tillage ($p<0.001$), with higher SOC content from CT to RT and from RT to G. The increase in SOC under RT was significant in Frick but not in Aesch. Also, in Frick, the SOC value for RT was similar to the grassland value. In Aesch, CT and RT were not significantly different, but both were significantly lower than G.

The second and third layers only demonstrated a site effect ($p<0.001$) and no tillage effect. The site effect showed a higher SOC in Frick compared to Aesch, with a greater gap between the sites under RT.

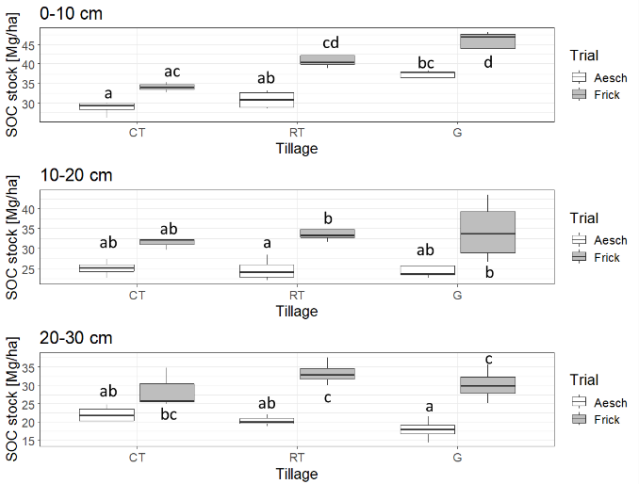

Figure 41: SOC stock [Mg/ha] of the three tillage conditions at the sites of Aesch and Frick and for the three sampling depths.
The letters represent the results of the Tukey post hoc test run for each layer.

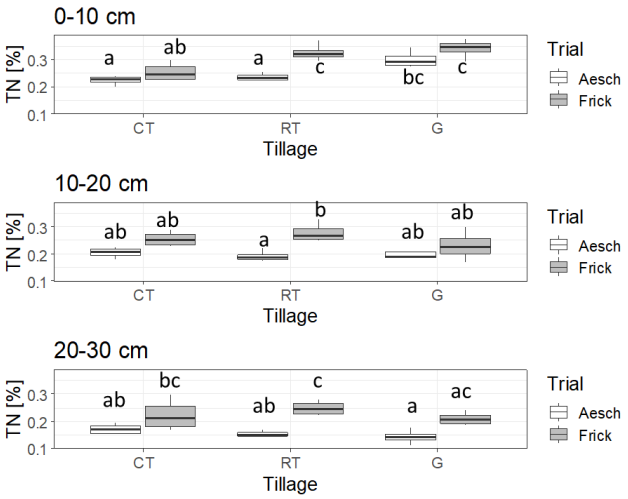

Figure 42: TN [%] of the three tillage conditions at the sites of Aesch and Frick and for the three sampling depths.
The letters represent the results of the Tukey post hoc test run for each layer.

The boxplot of SOC stocks and TN are shown in Figure 41 and Figure 42.

SOC stocks and TN content showed similar pattern than SOC: higher contents in Frick than in Aesch, no tillage effect in Aesch and a significant increase in RT to meet G level in the first layer in Frick, and no tillage effect in second and third layers.

3.1.5 Micronutrients

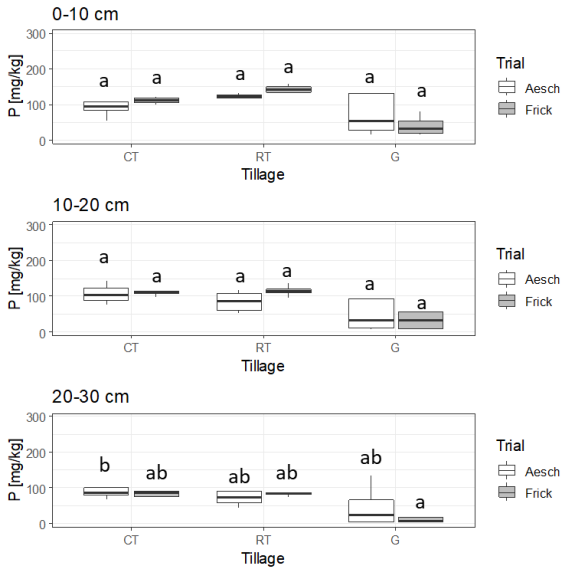

Figure 43: P [mg/kg] of the three tillage conditions at the sites of Aesch and Frick and for the three sampling depths.
The letters represent the results of the Tukey post hoc test run for each layer.

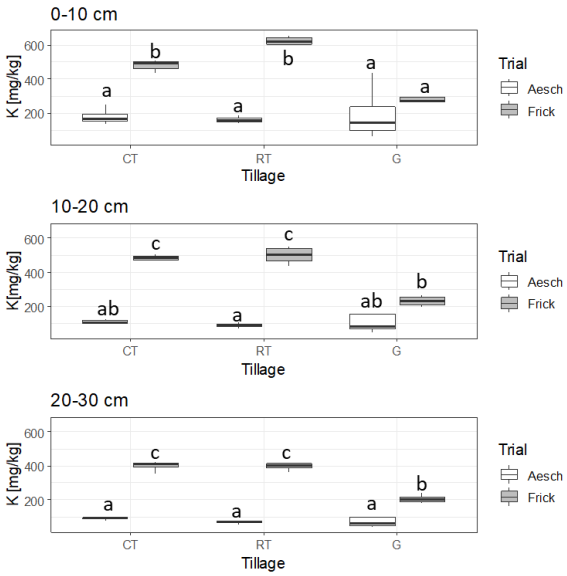

Figure 44: K [mg/kg] of the three tillage conditions at the sites of Aesch and Frick and for the three sampling depths.
The letters represent the results of the Tukey post hoc test run for each layer.

The boxplot of P contents is shown in Figure 43.

In the first and second layers, the P content was not influenced by site or tillage. In the third layer, A small tillage effect was observed with lower concentrations in the grasslands than in the cropped plots, but without significant difference between RT and CT.

The boxplot of K contents is shown in Figure 44.

In the three layers, the K concentrations were higher in Frick site than in Aesch site, with significant difference between sites in RT and CT plots but not in the grassland plots (interaction effect: p_value < 0.001). The concentrations between RT and CT did not significantly differ.

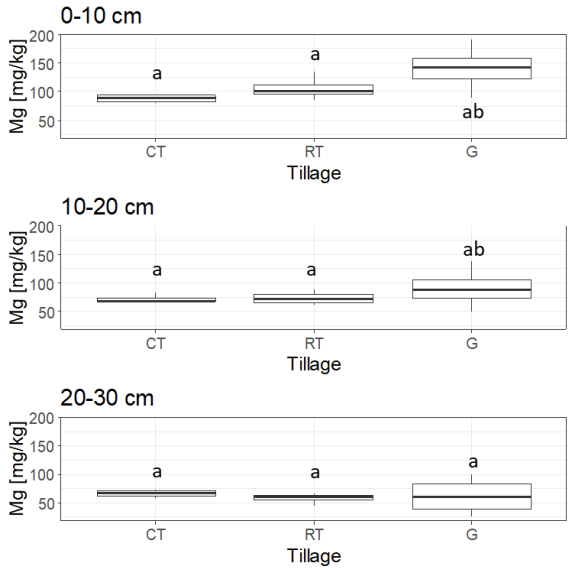

Figure 45: Mg [mg/kg] of the three tillage conditions at the site of Aesch for the three sampling depths. The letters represent the results of the Tukey post hoc test run for each layer.

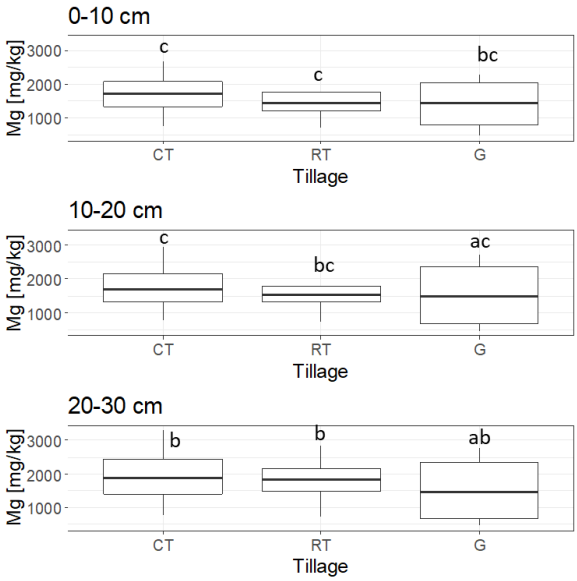

Figure 46: Mg [mg/kg] of the three tillage conditions at the site of Frick for the three sampling depths. The letters represent the results of the Tukey post hoc test run for each layer.

The boxplot of Mg contents is shown separately for both sites, in Figure 45 and Figure 46.

The Mg concentrations were 10 times higher in Frick than in Aesch (site effect: p_value < 0.001), but the tillage effect was not significant.

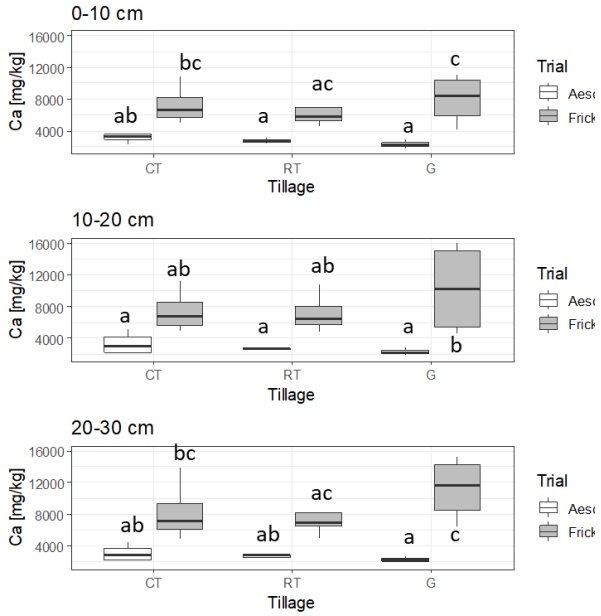

Figure 47: Ca [mg/kg] of the three tillage conditions at the sites of Aesch and Frick and for the three sampling depths.
The letters represent the results of the Tukey post hoc test run for each layer.

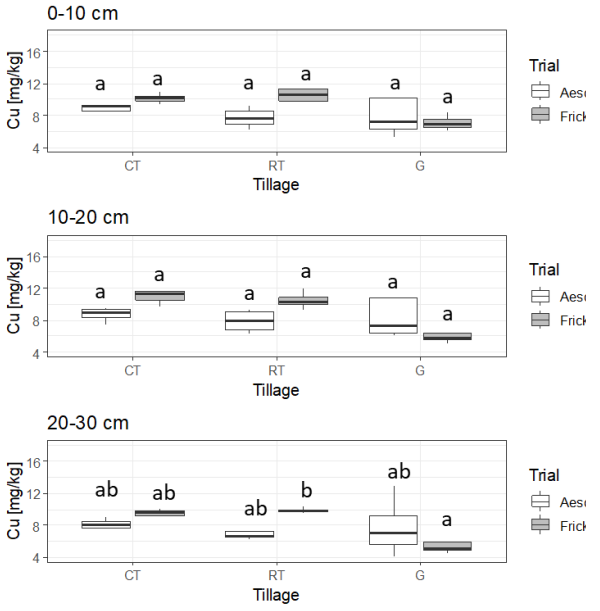

Figure 48: Cu [mg/kg] of the three tillage conditions at the sites of Aesch and Frick and for the three sampling depths.
The letters represent the results of the Tukey post hoc test run for each layer.

The boxplot of Ca contents is shown in Figure 47.

In the three layers, the tillage did not significantly affect the Ca concentrations. A site effect was observed as higher values were measured in Frick site than in Aesch site ($p < 0.001$).

The boxplot of Cu contents is shown in Figure 48.

In the first and second layers, there was no significant tillage or site effect. We could see that the concentrations were slightly higher in Frick than in Aesch in the cultivated plots, while no difference was seen in the G plots.

In the third layer, an interaction effect between site and tillage was observed ($p=0.035$) with increased concentrations in RT plot compared to G in Frick, and no tillage difference in Aesch.

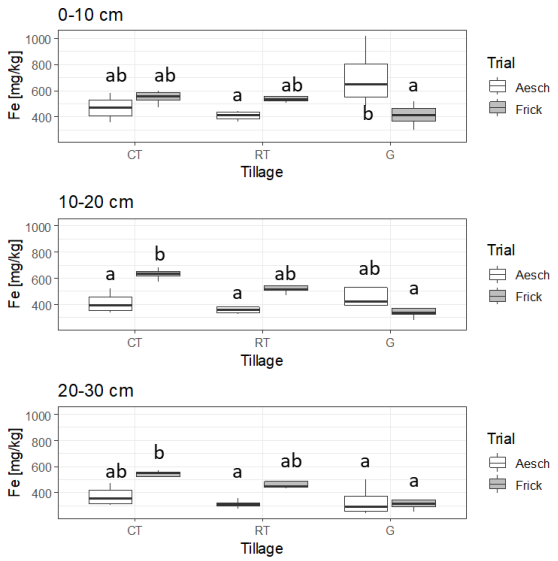

Figure 49: Fe [mg/kg] of the three tillage conditions at the sites of Aesch and Frick and for the three sampling depths. The letters represent the results of the Tukey post hoc test run for each layer.

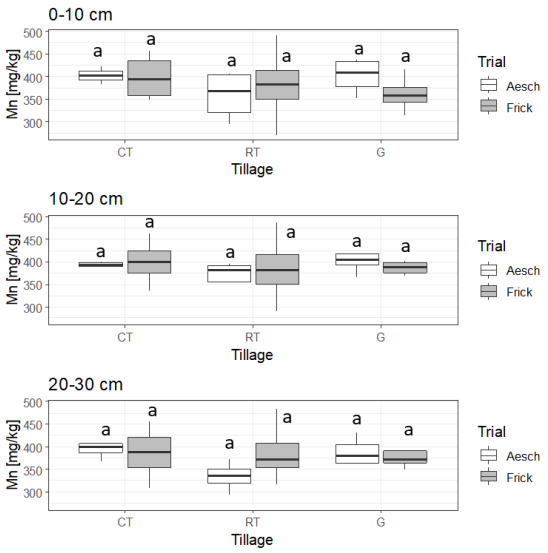

Figure 50: Mn [mg/kg] of the three tillage conditions at the sites of Aesch and Frick and for the three sampling depths.
The letters represent the results of the Tukey post hoc test run for each layer.

The boxplot of Fe contents is shown in Figure 49.

In the first layer, an interaction effect was observed: in RT and CT plots, the Fe concentrations were higher in Frick than in Aesch, and the Fe concentrations were higher in Aesch than in Frick in the G plots. However, the difference between CT/RT and G plots was not significant.

In the second layer, the site difference was significant for CT plots but not for RT or G plots. Similar to the first layer, the concentrations were higher in the cultivated plots in Frick site.

In the third layer, there was no significant site or tillage difference reported.

The boxplot of Mn contents is shown in Figure 50.

The Manganese concentrations were not influenced by site or tillage ($p>0.001$).

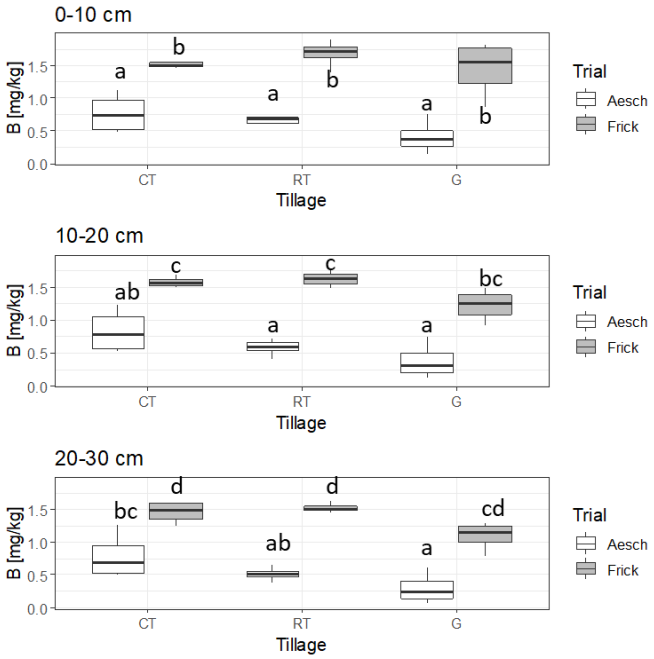

Figure 51: B [mg/kg] of the three tillage conditions at the sites of Aesch and Frick and for the three sampling depths. The letters represent the results of the Tukey post hoc test run for each layer.

The boxplot of B contents is shown in Figure 51.

In the three layers, the concentrations of Boron were significantly higher in Frick than in Aesch ($p<0.001$), without any significant tillage difference.

3.1.6 Earthworms

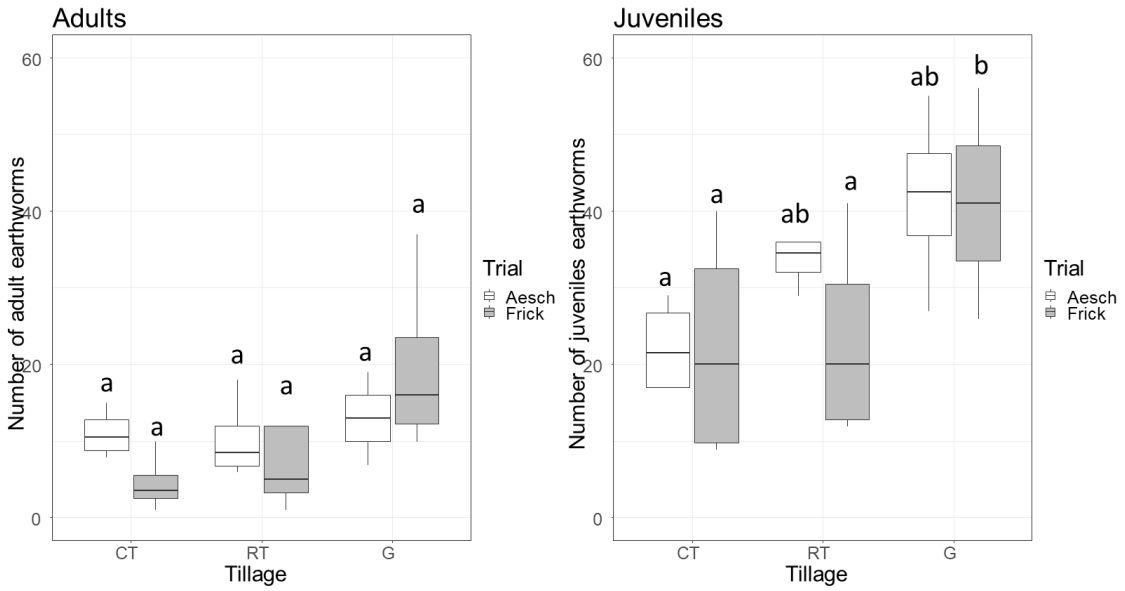

Figure 52: Number of adults (on the left) and juveniles (on the right) in Aesch and Frick sites. The letters represent the results of the Tukey post hoc test.

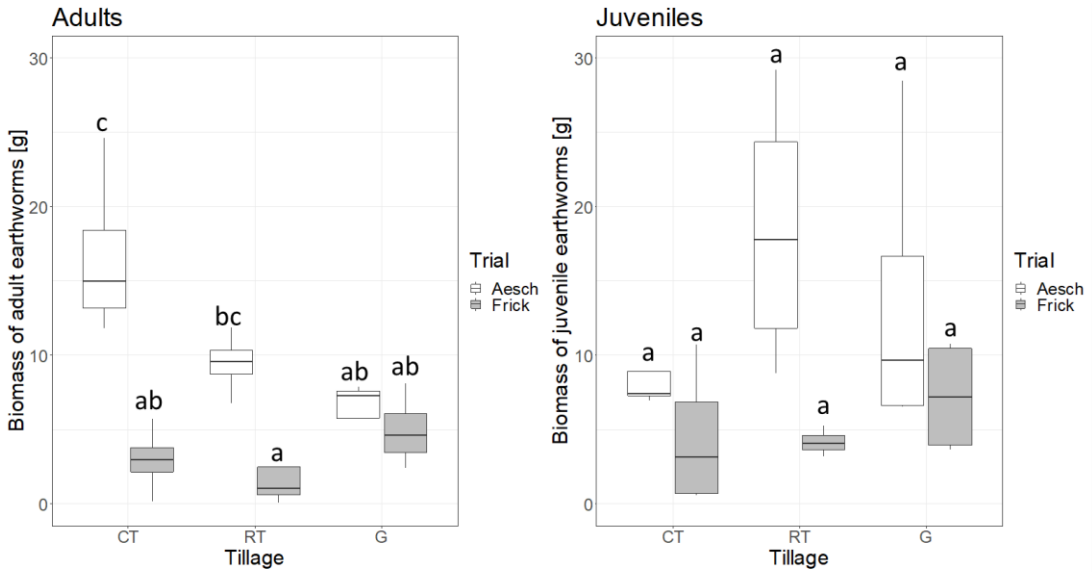

Figure 53: Biomass [g] of adults (on the left) and juveniles (on the right) in Aesch and Frick sites. The letters represent the results of the Tukey post hoc test.

The boxplots of earthworm numbers are presented in Figure 52, and the biomasses in Figure 53. The data on the sections of earthworms (O) was not considered because considered non-valuable. The number of adult and juvenile earthworms did not have any site effect. While no tillage effect was observed on the number of adult earthworms, a significant effect of tillage between CT and RT was noticeable in the counting of juveniles (p-value = 0.002): in Aesch, more juvenile individuals seemed to be observed in RT than in CT, and even more in the grassland sites than in the cropping sites (but showed n.s.). In Frick, no difference between RT and CT was visible, and were both significantly lower than G.

On the opposite, adult and juvenile biomasses were greater in Aesch than in Frick (p-value<0.001 and p-value=0.005 respectively). The biomass of adults showed an interaction effect between site and tillage (p-value = 0.004)). In Frick, biomasses seem to be (n.s.) greater in G than in CT and than RT. In Aesch, biomasses were surprisingly higher in CT than in RT, and even higher than G plots.

Noteworthy, these results about number and biomasses of earthworms are probably biased by sampling and weighting issues as discussed later, which is also visible by the high standard deviations of the parameters values.

3.2 Microbial population

3.2.1 Microbial biomass

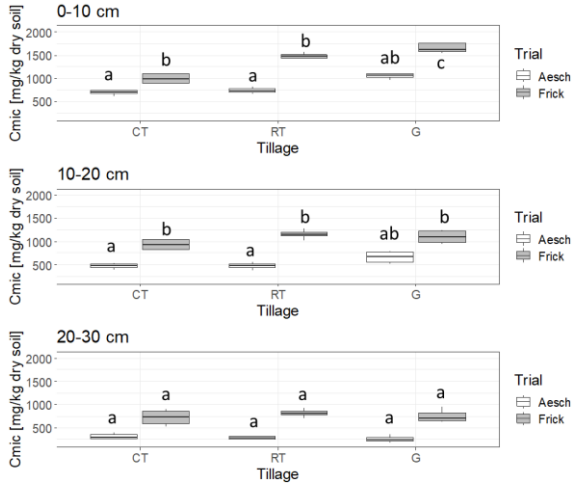

Figure 54: C_{mic} [mg/kg dry soil] of the three tillage conditions at the sites of Aesch and Frick and for the three sampling depths.
The letters represent the results of the Tukey post hoc test run for each layer.

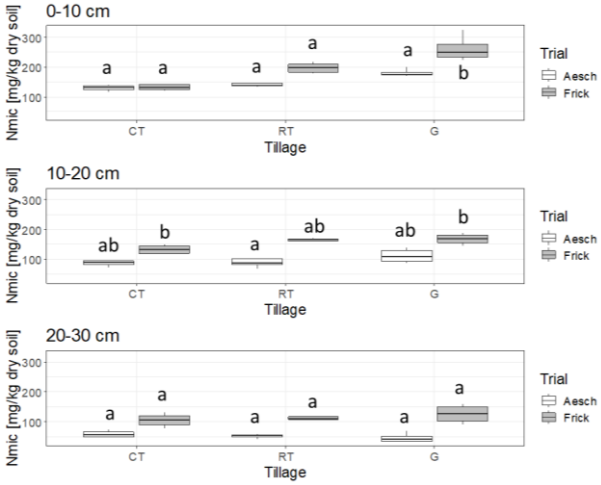

Figure 55: N_{mic} [mg/kg dry soil] of the three tillage conditions at the sites of Aesch and Frick and for the three sampling depths.
The letters represent the results of the Tukey post hoc test run for each layer.

The boxplot of C_{mic} and N_{mic} are shown in Figure 54 and Figure 55.

In the first layer, carbon microbial biomass did not show an effect of RT compared to CT in Aesch. In Frick, C_{mic} was higher than in Aesch for the three tillage conditions. We could observe a significant effect between tillage and untilled soils (p<0.001).

In the second and third layers, no tillage effect was observed, and the values were also significantly higher in Frick than in Aesch.

N_{mic} showed similar pattern than C_{mic}: a small increase in RT compared to CT in the first layer of Frick, and no tillage effect elsewhere. The increase in RT compared to CT in Frick was less pronounced than with C_{mic} values. Also, the site difference (higher values in Frick than in Aesch) was less pronounced than for C_{mic} values.

The higher contents in grassland compared to cultivated plots was less visible for microbial biomasses (C_{mic} and N_{mic}) than for nutrients (SOC and TN).

3.2.2 DNA extracted

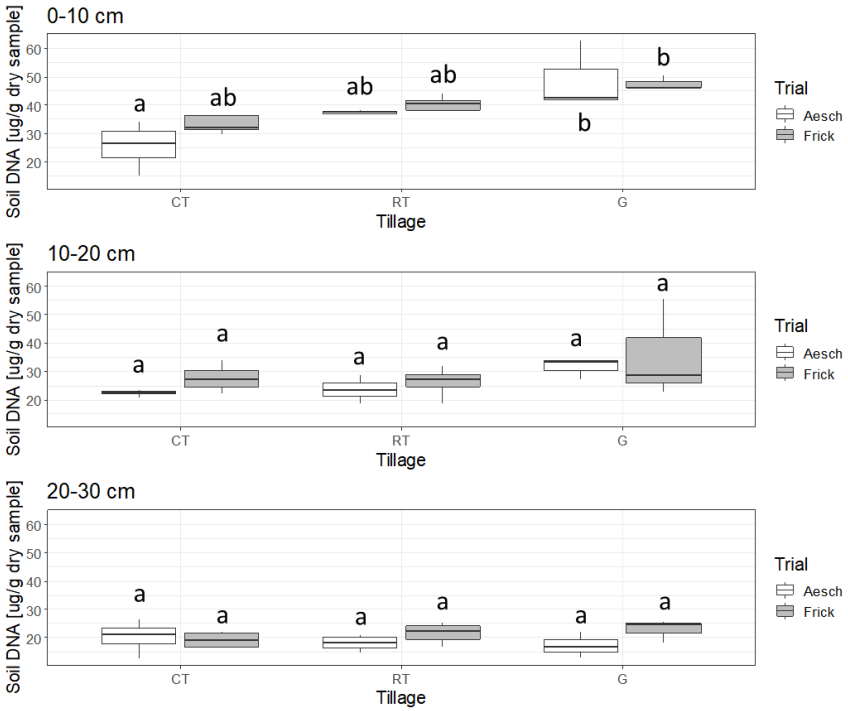

Figure 56: Soil DNA extracted [ug/g dry soil] of the three tillage conditions at the sites of Aesch and Frick and for the three sampling depths.
The letters represent the results of the Tukey post hoc test run for each layer.

The boxplot of the quantities of DNA extracted in soil samples is shown in Figure 56.

In the first layer, the ANOVA reported only a tillage effect (p=0.001). An increasing gradient of DNA was observed for CT-RT-G conditions for both LTEs, with slightly higher values in Frick than in Aesch. The difference between RT and CT was more pronounced in Aesch than in Frick.

In the second and third layers, no effect of tillage or site was significant.

A decrease in soil DNA was visible in the second and third layers compared to the first layer. Interestingly, even if the amount of extracted DNA was higher in RT than in CT for the first layer, the gap between the two tillage conditions closes as the soil DNA decreased in the lower layers.

3.2.3 Bacterial DNA quantitation

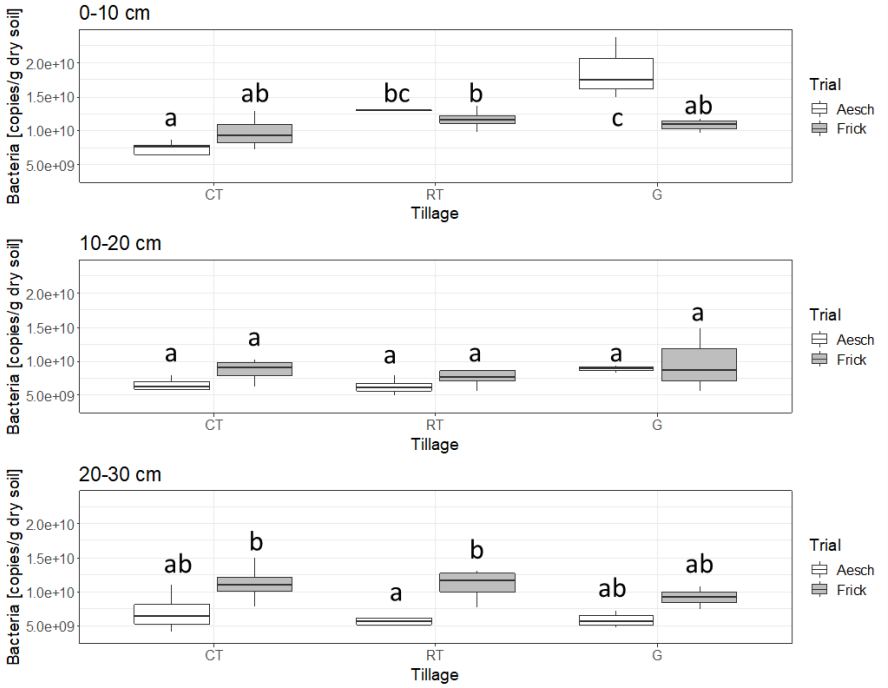

Figure 57: Bacteria [copies/g dry soil] of the three tillage conditions at the sites of Aesch and Frick and for the three sampling depths.
The letters represent the results of the Tukey post hoc test run for each layer.

The boxplot of the numbers of copies of quantified bacteria is shown in Figure 57.

In the first layer, the tillage effect was different for both LTEs (interaction: p=0.003). In Aesch, the number of bacterial copies was significantly higher and closer to G in RT than in CT. In Frick, the number of bacterial copies was considered equivalent for the three tillage conditions.

In the second layer, no effect of tillage or site was significant. We could observe slightly higher values in Frick site.

In the third layer, bacterial copies were always higher in Frick than in Aesch (p<0.001), without any tillage effect.

3.2.4 Fungal DNA quantitation

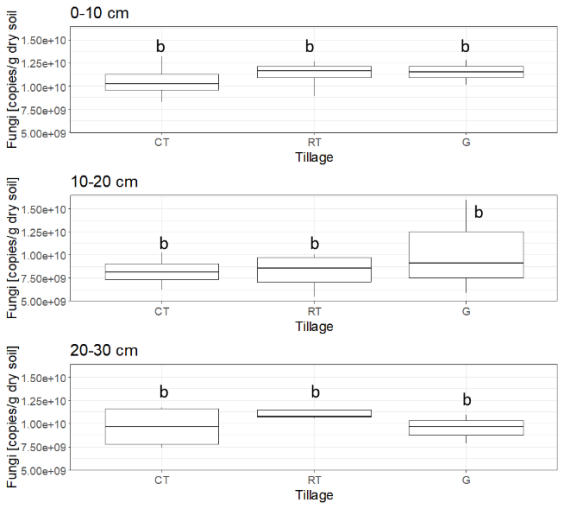

Figure 58: Fungi [copies/g dry soil] of the three tillage conditions in Aesch and for the three sampling depths. The letters represent the results of the Tukey post hoc test run for each layer.

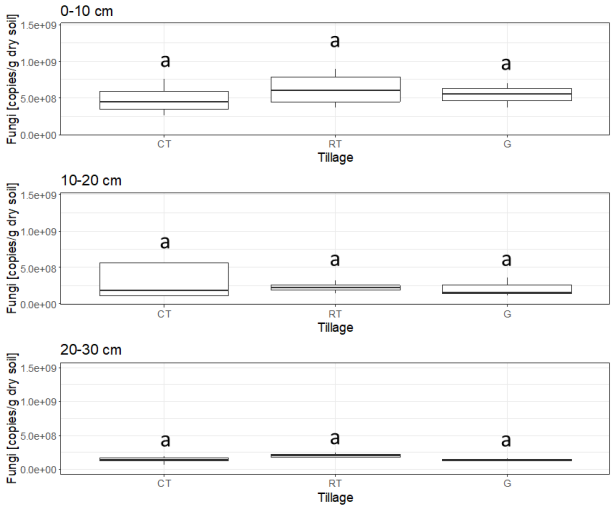

Figure 59: Fungi [copies/g dry soil] of the three tillage conditions in Frick and for the three sampling depths. The letters represent the results of the Tukey post hoc test run for each layer.

As a major differentiation in fungi number of copies was visible between both sites, with values 10 times higher in Aesch than in Frick ($p<0.001$), two boxplots were separately created. The boxplots of the numbers of copies of sequenced fungi of Aesch and Frick sites are shown *with different scales* in Figure 58, and in Figure 59 respectively. In the table of data in Appendix 8.8, the data conditional formatting was separated for both sites.

In Aesch, fungi copies were higher in RT than in CT (n.s.) in the three layers. Also, the amount of fungi copies was higher in the first layer than in the second and third layers.

In Frick, the RT effect on fungi increase seemed to be more pronounced than in Aesch in the first layer. Also, the number of copies of fungi sharply decreased in the second and third layers.

The different effects of tillage in the two sites is more visible in the table of data, in the Appendix 8.8, rather than in the boxplot. In the top layer, the fungi copies range from 1.05E10 under CT to 1.13E10 in RT to 1.15E10 in G in Aesch, while they range from 4.79E8 in CT to 6.10E8 in RT to 5.43E8 in G in Frick.

3.2.5 Nitrifiers

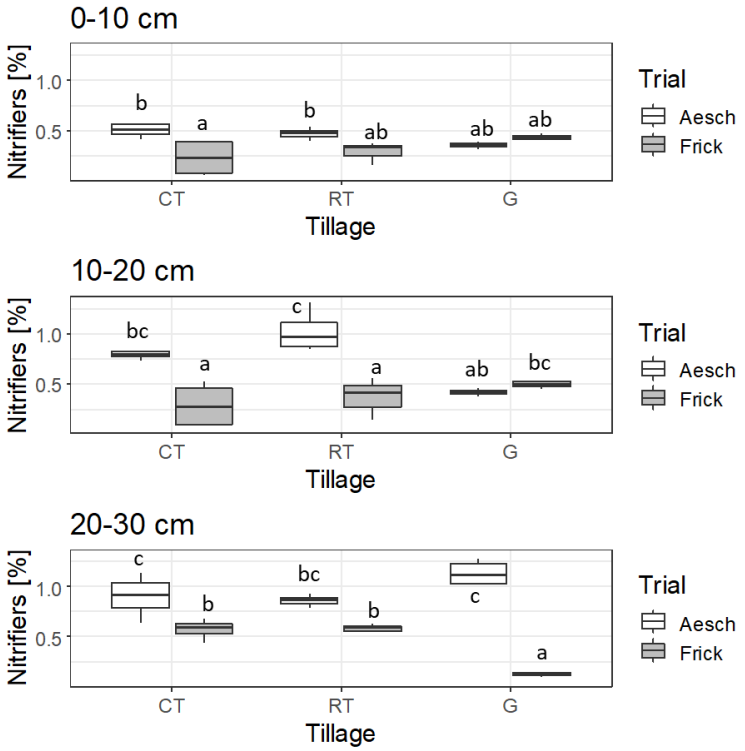

Figure 60: Nitrifiers proportion [%] of the three tillage conditions at the sites of Aesch and Frick and for the three sampling depths.
The letters represent the results of the Tukey post hoc test run for each layer.

Biomolecular analysis showed that in this study, the nitrifying *Archaea* represent only a negligible fraction of the analyzed sequences (> 0.01%), with some representatives of the genus *Nitrosopumilus*.

For the *Bacteria* kingdom, the nitrifiers are essentially represented by the phylum *Nitrospirae* and its genus *Nitrospira*.

Smaller quantities of nitrifying bacteria were detected among the two classes alpha and beta *Proteobacteria*, with notably the genera *Nitrobacter*, *Nitrosomonas*, *Nitrosovibrio* and *Nitrospira*.

The boxplot of the proportions of nitrifiers in the microbial community is shown in Figure 60.

In the first layer, there was an interaction effect between site and tillage ($p=0.011$): In Aesch the effect of tillage was not significant and seemed to decrease from CT to RT to G, while in Frick, the proportion of nitrifiers seemed to increase from CT to RT to G, but also without significant difference. In RT and CT systems, the proportion of nitrifiers was higher in Aesch than in Frick.

In the second layer, there was a site effect ($p<0.001$) in CT and RT plots, with greater nitrifiers proportions in Aesch than in Frick. The effect of tillage was different at the two sites: in Frick CT and RT conditions showed decreased nitrifiers proportion compared to G; in Aesch, the RT conditions lead to significantly higher nitrifiers proportion than in CT and G.

In the third layer, there was significantly more nitrifiers in Aesch than in Frick ($p<0.001$). The effect of tillage was non-significant in Aesch, while in Frick G has significantly less nitrifiers than in CT and RT.

3.3 Site differentiation by clustering

The clustering analysis is presented by a dendrogram in Figure 61 below. The samples are labelled as follows: The first letter corresponds to the site (*A/F* for Aesch / Frick), the second letter to the tillage treatment (*C/R/G* for CT / RT/ G), the third character to the plot number, and the final number following the underscore to the depth (*10/20/30* for 0-10 cm / 10-20 cm / 20-30 cm).

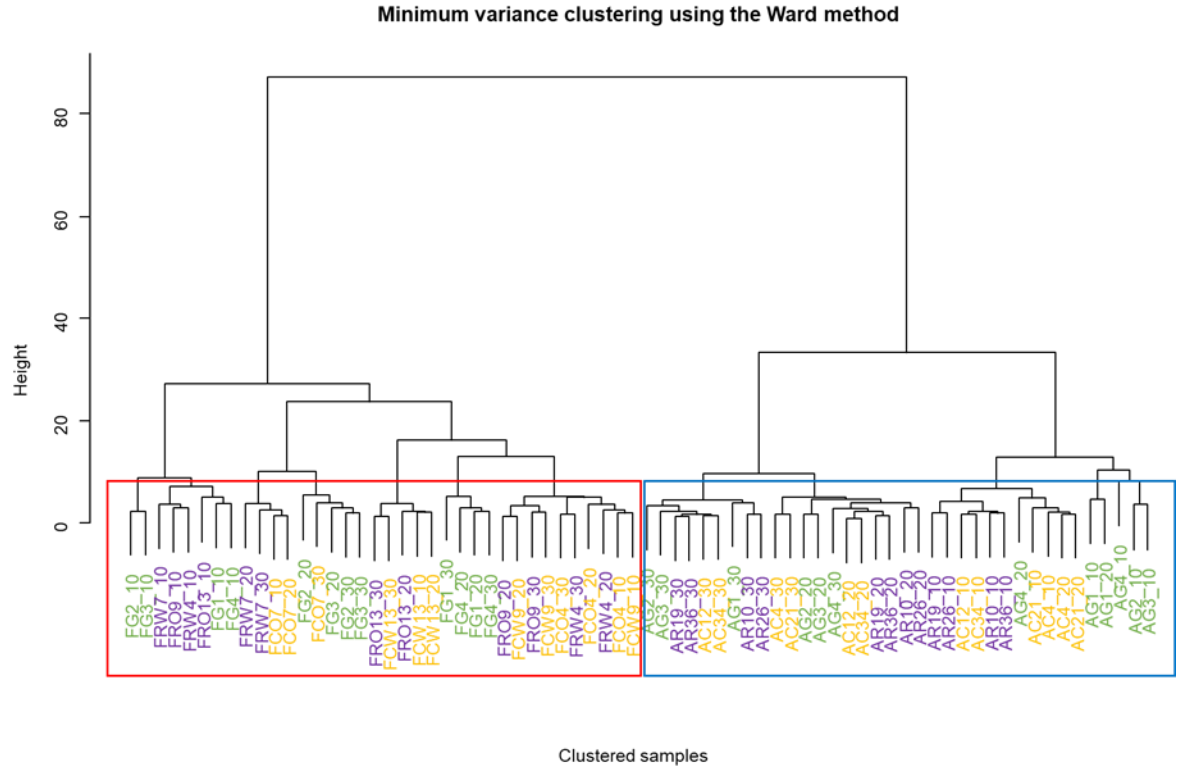

Figure 61: Clustering on the environmental parameters of all samples from the Aesch and Frick sites using the Ward method after the Hellinger transformation.
The clusters are delimited by colored rectangles.

The clustering technique showed a clear split between the samples from Frick (red cluster, on the left in the Figure above) and Aesch (blue cluster, on the right).

Within the blue Aesch cluster, samples were divided into two subclusters that separated samples from the 0-10 cm depth and the ones collected from the 10-20 cm and 20-30 cm depths, but without a clear differentiation induced by the tillage method.

Within the red Frick cluster, the four grassland samples from the Frick top layer are grouped with the four samples of the Frick top layer in reduced tillage condition at the extreme left of the Figure. Other clustering patterns among Frick samples regarding depth or the tillage method were less distinct.

3.4 PCA on environmental dataset

In the Figure 62 and Figure 63 hereunder, the PCA of Aesch and Frick are shown. The correlation plots are also presented in Figure 64 and Figure 65. The Spearman correlation matrices are available in the Appendix 8.9.

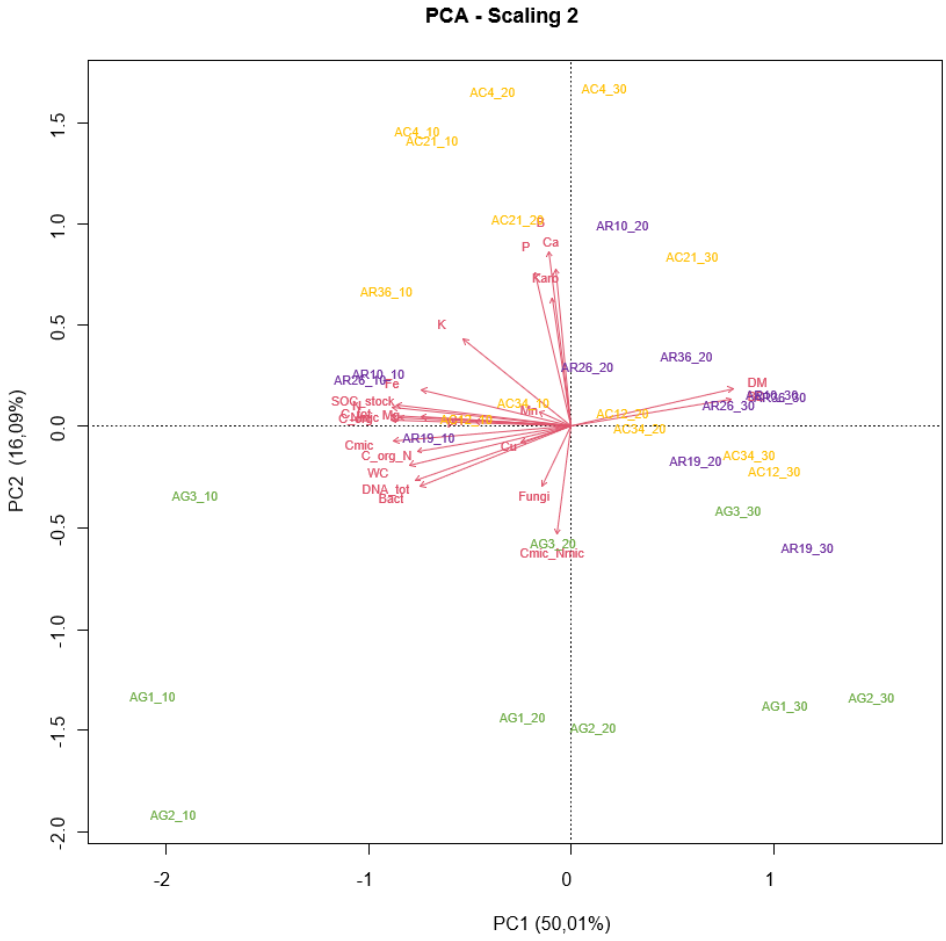

Figure 62: Principal component analysis (PCA) for tillage conditions and sampling depths for Aesch site. Arrows on the correlation biplot show the environmental and biomolecular soil parameters.The angles between descriptors in the biplot reflect their correlations (0° positively correlated, 90° no correlation, 180° negatively correlated).

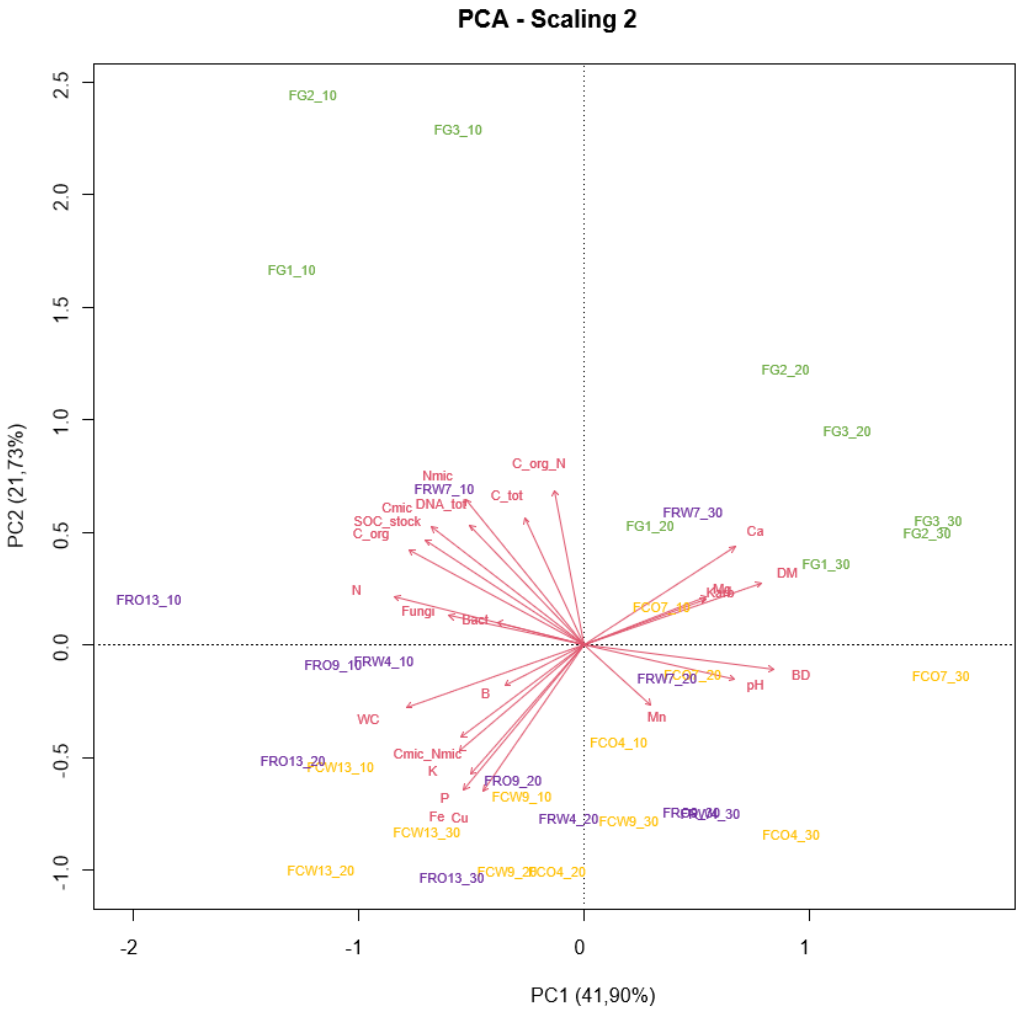

Figure 63: Principal component analysis (PCA) for tillage conditions and sampling depths for Frick site.
Arrows on the correlation biplot show the environmental and biomolecular soil parameters.The angles between descriptors in the biplot reflect their correlations (0° positively correlated, 90° no correlation, 180° negatively correlated).

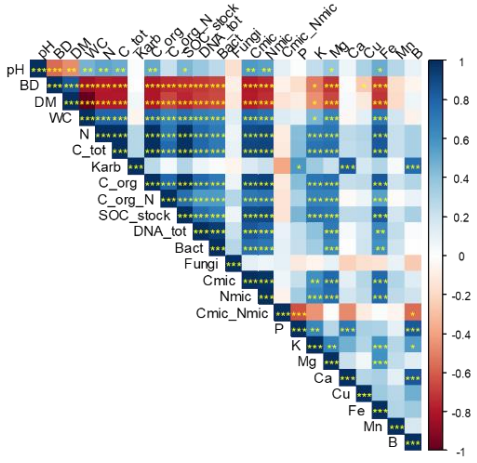

Figure 64: Correlation plot of the environmental and biomolecular parameters in Aesch site.
The color ramp shows the Spearman rho values, and the stars show the significance (<0.05, ** <0.01, *** <0.001).*

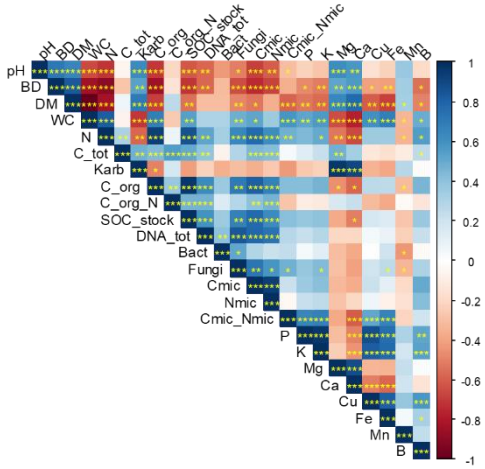

Figure 65: Correlation plot of the environmental and biomolecular parameters in Frick site.
The color ramp shows the Spearman rho values, and the stars show the significance (<0.05, ** <0.01, *** <0.001).*

In Aesch, PC1 explained 50,01% of variance and PC2 explained 16,09% of the variance. The first axis was mostly composed of the carbon (SOC, SOC_stocks) and nutrients (Fe) contents, WC as well as bacteria and total soil DNA. The majority of the variance on the first axis was explained by depth, with visible graduation from the top layer to the second and third layers (from left to right. DM and BD were negatively correlated with carbon contents and bacteria and soil DNA (***), meaning that the top layers that were richer in organic matter and in bacteria were more wet, and the deeper sampler were less rich but drier. The second axis was composed of P, Ca, B. The correlation matrix showed that the chemicals parameters describing soil nutrients were strongly positively correlated (SOC, microbial biomass, DNA, micronutrients). BD was strongly negatively correlated with SOC, N, Mg, Fe, and bacteria. Fungi was not significantly correlated to another parameter.

In Frick, the first axis explained less variance (41,90%), and the second axis explained more variance (21,73%) compared to Aesch. The parameters were more spread in all directions, indicating that the parameters represented well the samples. A depth gradient was visible from the first layer to the third layer from top-left hand corner to bottom-right corner. Carbon parameters were positively correlated with each other, and negatively correlated with BD, similarly to Aesch. pH was negatively correlated with the carbon and nutrients contents, bacteria (r=-0.338) and fungi (r=-0.398*). Interestingly, fungi and bacteria were strongly positively correlated (r=0.382*).

Separate PCAs were conducted for the 0-10 cm, 10-20 cm and 20-30 cm layers and are presented in Figure 66 to Figure 77 below.

For the 0-10 cm layer, data from earthworm were included in the PCA.

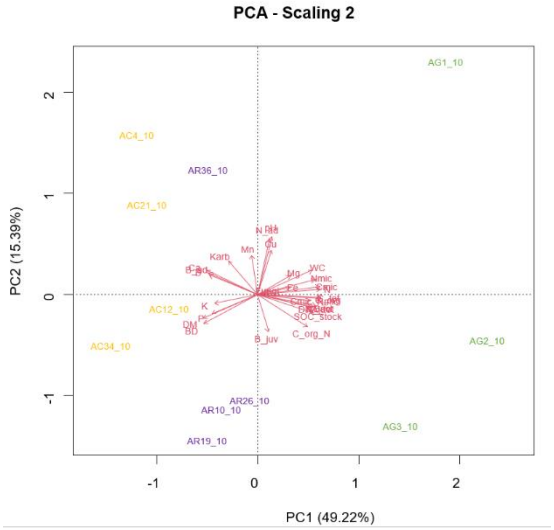

Figure 66: Principal component analysis (PCA) for tillage conditions for 0-10 cm samples of Aesch site.
Arrows on the correlation biplot show the environmental and biomolecular soil parameters.The angles between descriptors in the biplot reflect their correlations (0° positively correlated, 90° no correlation, 180° negatively correlated).

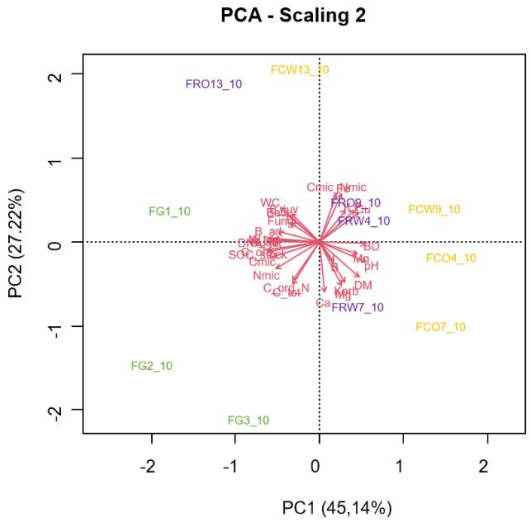

Figure 67: Principal component analysis (PCA) for tillage conditions for 0-10 cm samples of Frick site.
Arrows on the correlation biplot show the environmental and biomolecular soil parameters.The angles between descriptors in the biplot reflect their correlations (0° positively correlated, 90° no correlation, 180° negatively correlated).

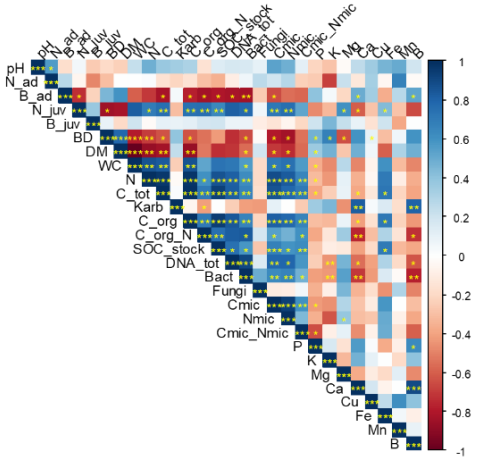

Figure 68: Correlation plot of the environmental and biomolecular parameters in 0-10 cm sample of Aesch site.
The color ramp shows the Spearman rho values, and the stars show the significance (<0.05, ** <0.01, *** <0.001).*

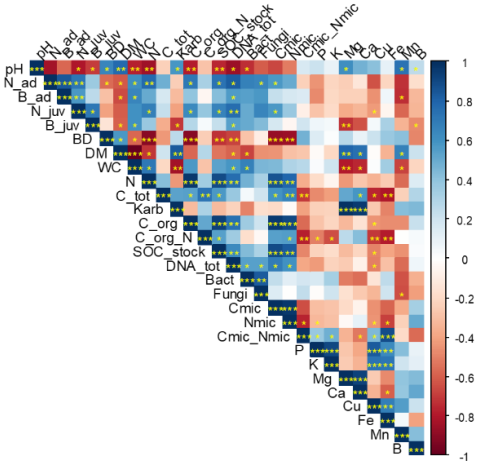

Figure 69: Correlation plot of the environmental and biomolecular parameters in 0-10 cm sample of Frick site.
The color ramp shows the Spearman rho values, and the stars show the significance (<0.05, ** <0.01, *** <0.001).*

In the first layer of Aesch site (Figure 66, Figure 68), the samples were separated along PC1 (49,22%), with carbon and nutrients contents increasing and DM and BD decreasing from CT to RT to G. The second axis explained 15,39% of variance, and was contained by earthworm and pH values. BD and DM were negatively correlated with organic carbon contents, TN, DNA and bacteria, but not with fungi. The

biomass of adult earthworms was surprisingly negatively correlated with SOC (r=-0.677*), while the number of juveniles was positively correlated with SOC (r=0.811*).

In Frick (Figure 67 and Figure 69), the same gradient from CT to RT to G was visible (PC1: 45,14%; PC2: 27,22%), with increasing SOC and decreasing BD, but also with decreasing pH. pH was negatively correlated with SOC (r=-0.772*) and biological parameters, i.e. DNA, fungi and bacteria.

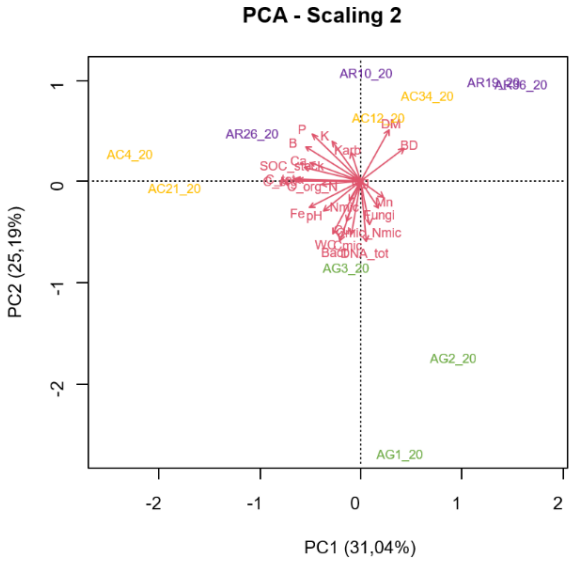

Figure 70: Principal component analysis (PCA) for tillage conditions for 10-20 cm samples of Aesch site.
Arrows on the correlation biplot show the environmental and biomolecular soil parameters.The angles between descriptors in the biplot reflect their correlations (0° positively correlated, 90° no correlation, 180° negatively correlated).

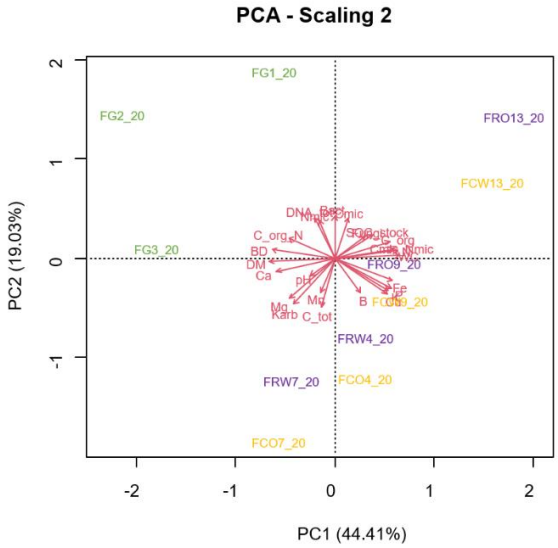

Figure 71: Principal component analysis (PCA) for tillage conditions for 10-20 cm samples of Frick site.
Arrows on the correlation biplot show the environmental and biomolecular soil parameters.The angles between descriptors in the biplot reflect their correlations (0° positively correlated, 90° no correlation, 180° negatively correlated).

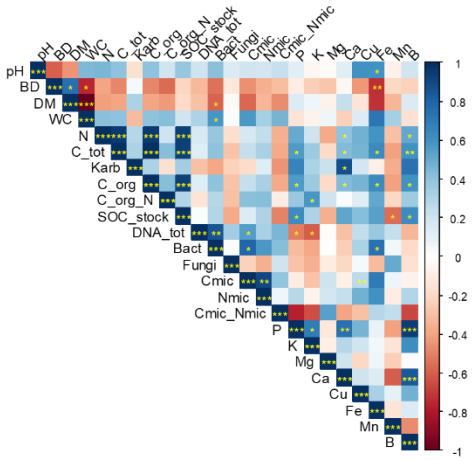

Figure 72: Correlation plot of the environmental and biomolecular parameters in 10-20 cm sample of Aesch site.
The color ramp shows the Spearman rho values, and the stars show the significance (<0.05, ** <0.01, *** <0.001).*

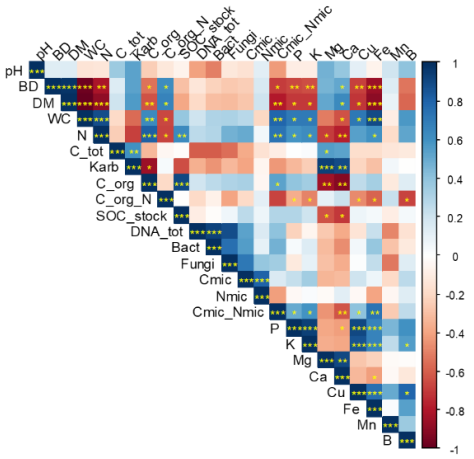

Figure 73: Correlation plot of the environmental and biomolecular parameters in 10-20 cm sample of Frick site.
The color ramp shows the Spearman rho values, and the stars show the significance (<0.05, ** <0.01, *** <0.001).*

In the second layer of Aesch site (Figure 70, Figure 72), PC1 explained 31,04% of variance and PC2 explained 25,19% of variance. The gradient here was from G to CT and to RT (from bottom to top). The gradient from G to RT to CT was visible, but differentiation between RT and CT was less pronounced than in the first layer. The grassland samples were more clustered along the second axis compared to the samples from CT and RT. The correlations between the parameters were not significant. Here also, the differentiation from RT and CT was not clearly visible, but both RT and CT were clearly separated from G samples.

In Frick (Figure 71, Figure 73), PC1 explained 44,41% of variance and PC2 explained 19.03% of variance. DM and BD were strongly negatively correlated with Iron contents. K and Ng positively correlated with Carbonates contents and negatively correlated with SOC.

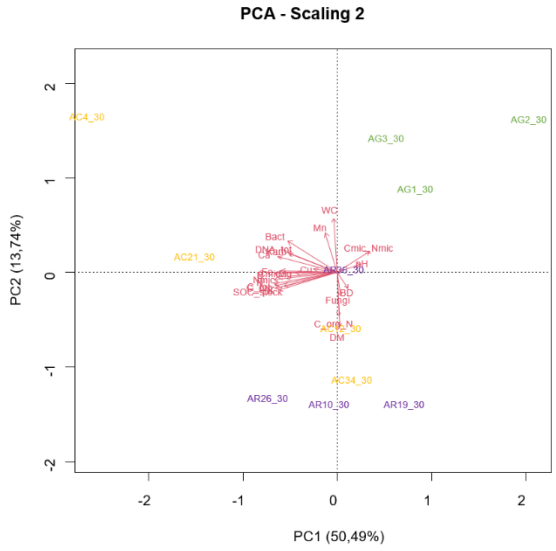

Figure 74: Principal component analysis (PCA) for tillage conditions for 20-30 cm samples of Aesch site. Arrows on the correlation biplot show the environmental and biomolecular soil parameters.The angles between descriptors in the biplot reflect their correlations (0° positively correlated, 90° no correlation, 180° negatively correlated).

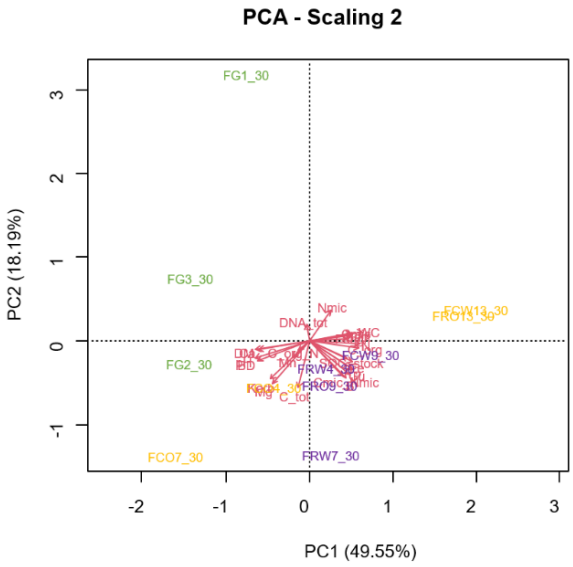

Figure 75: Principal component analysis (PCA) for tillage conditions for 20-30 cm samples of Frick site. Arrows on the correlation biplot show the environmental and biomolecular soil parameters.The angles between descriptors in the biplot reflect their correlations (0° positively correlated, 90° no correlation, 180° negatively correlated).

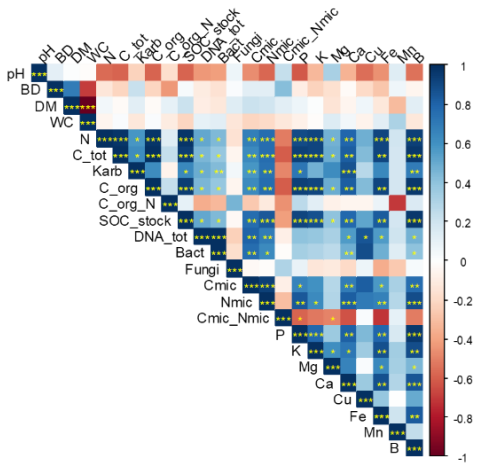

Figure 76: Correlation plot of the environmental and biomolecular parameters in 20-30 cm sample of Aesch site.
The color ramp shows the Spearman rho values, and the stars show the significance (<0.05, ** <0.01, *** <0.001).*

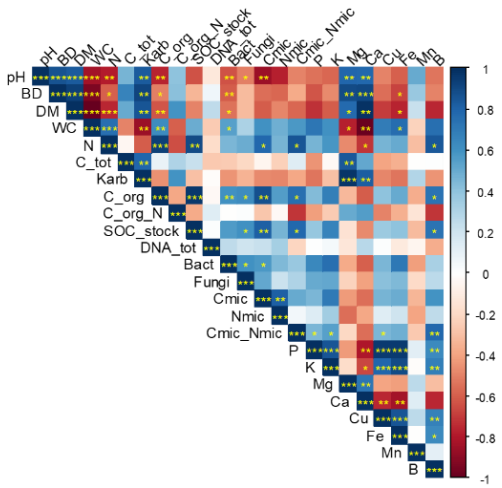

Figure 77: Correlation plot of the environmental and biomolecular parameters in 20-30 cm sample of Frick site. The color ramp shows the Spearman rho values, and the stars show the significance (<0.05, ** <0.01, *** <0.001).*

In the third layer of Aesch site (Figure 74, Figure 76), the first axis explained 50,49% of the variance while the second axis explained 13,74 % of the variance. The three grassland plots were directed towards high pH. Bacteria were slightly correlated with organic contents (SOC and bacteria: r=0.628*), while fungi did not show any significant correlation. Organic contents were correlated with concentrations of P, K, Ca, Fe and B.

In Frick (PC1: 49,55%, PC2: 19,19%) (Figure 75, Figure 77), a gradient from G to CT to RT is visible. Bacteria and fungi negatively correlated with pH. Carbonates were correlated with Mg and Ca. Ca was negatively correlated with Cu and Fe, but P and K was positively correlated with Cu and Fe.

3.5 PCA on microbial community

3.5.1 Microbial community analysis

The microbial community analysis was done based on the microbial 16S RNA gene sequences, including both *Archaea* and *Bacteria*. The results showed a predominance of *Bacteria* forming 90% of the sequences. *Archaea* (including possible nitrifiers) thus constitute only a minor fraction of the microbial community and will not be discussed in this work.

Each sample collected at the two sites of Aesch and Frick was composed of 60'000 sequences, of which a very small proportion could not be correctly affiliated to recognized sequences of the Greengenes database. This large number ensured the robustness of the analysis and an excellent representativity of the species. A Mantel correlation test performed between the environmental data and the data obtained by sequencing showed an excellent correlation ($r=0.5853$, $p=0.001$***), which allowed to validate the quality of the data produced to date. The table of the microbial community phyla is presented in Appendix 8.10.

Due to time constraints, the statistical analysis conducted on this dataset was done at the Phylum level, i.e. the highest level of phylogeny. Various studies conducted on microbial habitats showed important similarities when lower phylogenetic levels, from class to genus, were considered (P. Rossi, pers. comm.). Future analyses could allow to refine the models presented here, by considering more detailed phylogenetic levels.

3.5.2 Clustering and PCA

The microbial community analysis was performed first on the entire set with the three depths. Then, as the data collected on earthworms were only for the top layer and were not included in the first analysis, a second analysis enabled to consider earthworm data, and was performed on all samples collected at 10 cm depth (22 samples, 2x 4 replicates for CT and RT and 2x 3 replicates for G).

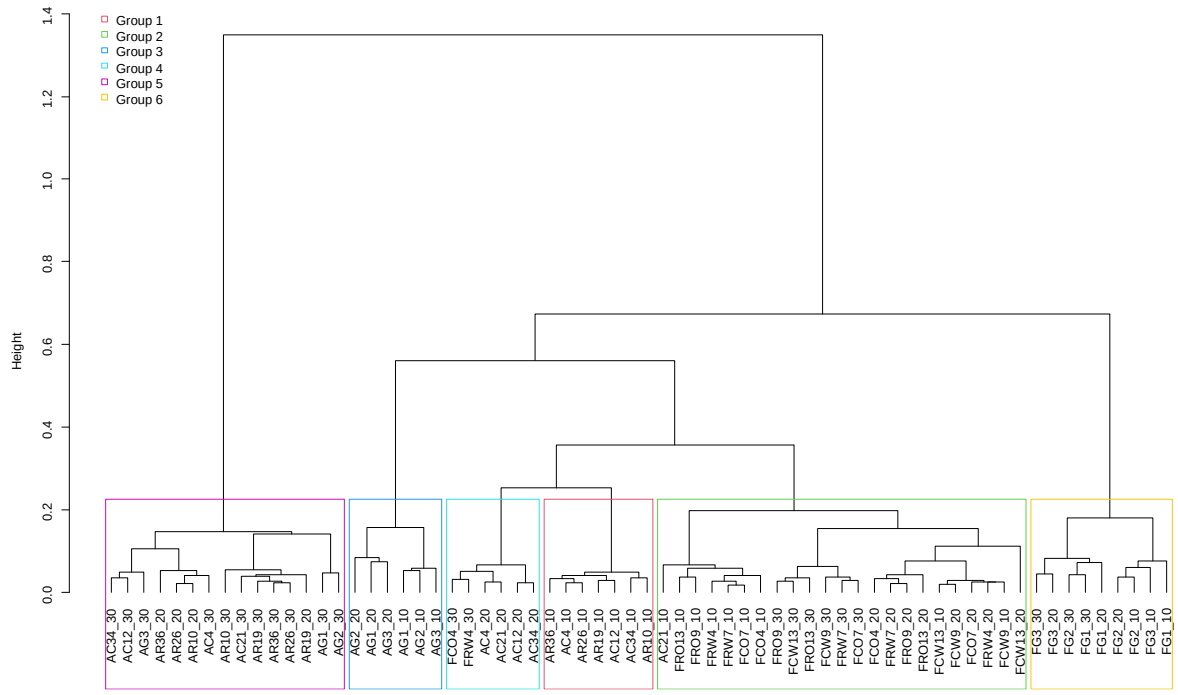

Figure 78: Clustering on the microbial community data of all samples from the Aesch and Frick sites using the Ward method after the Hellinger transformation.
The clusters are delimited by colored rectangles.

The results of the clustering technique presented in Figure 78 showed a strong influence of the sites on the microbial structures. As for the environmental analyses, a strong disparity between the samples from the two sites was highly visible. Here, the biomolecular analysis allowed to go further, with the visible formation of six logically and easily delimited clusters. Only a few samples were "misplaced" with respect to their origin, which indicates a noteworthy formation of gradients within the communities. The clusters are formed in order (from left to right):

- Purple cluster: all Aesch samples from 10-20 cm and 20-30 cm, regardless of tillage treatment.
- Dark blue cluster: all Aesch G samples, at 0-10 cm and 10-20 cm depths.
- Light blue cluster: The CT Aesch samples from 10-20 cm depth. Here, two exceptional outliers from Frick were present.
- Red cluster: all Aesch samples from 0-10 cm, for both CT and RT. Similarly to the purple cluster, this indicated a convergence of the communities regardless of the tillage treatment applied for Aesch soil.
- Green cluster: the largest of all, which included all the Frick samples, for both treatments CT and RT, and for the three depths. Note that this cluster could theoretically be subdivided into three sub-clusters according to the sampling depths. Here again, only one Aesch outlier was present.
- Yellow cluster: Strongly separated from all the others, with all the samples from the Frick G, at all depths, indicating a very strong specific identity.

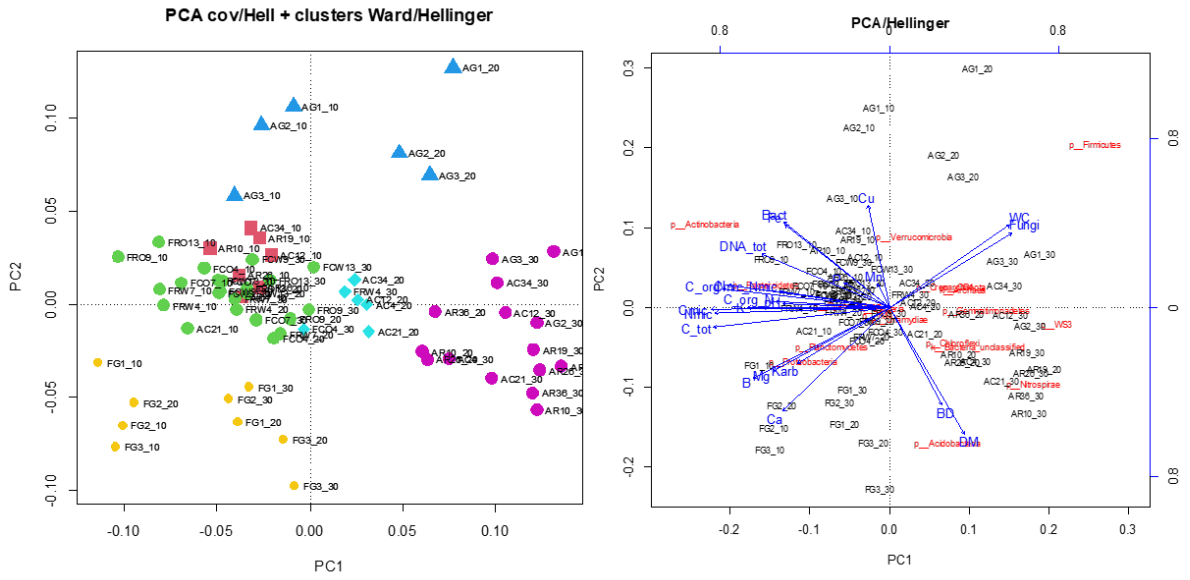

Figure 79: PCA of microbial community data with data from both sites and three layers.
Left: PCA on covariance matrix and Ward/Hellinger clustering of microbial community data. Right: PCA with a posteriori presentation of the environmental variables, and the presentation of the main microbial Phyla. The Phyla accounting for more than 0.01% of the total amount of sequences for all samples are displayed only. The PC1 and PC2 axes represent 48% and 19% of the observed variance respectively.

The six clusters obtained by Ward's method are presented in the form of a PCA in Figure 79 (left) with the same colors. Three clusters of them (purple, dark blue and yellow) were well delimited and present in the margin of the diagram. In the same Figure 79 (right), the environmental variables (represented by blue vectors) best able to explain their respective position were clearly established. The purple cluster (Aesch deep layers, on the right in the PCA) was mainly represented by low amounts of nitrogen and carbon. Interestingly, the position of the phylum *Nitrospira* (composed of nitrifying bacteria) seemed to be predominant here. The samples of the dark blue cluster (Aesch G at 0-10 and 10-20 cm) seemed to be those with the lowest concentrations of Carb, Mg, B and Ca, and with the lower BD. The samples were also more dispersed. Contrary to the dark blue cluster, the yellow cluster (Frick G samples, on the bottom-right hand corner) seemed to be characterized by the highest concentrations of Carb, Mg, B and Ca, and the lowest presence of fungi. The position of both Phyla *Proteobacteria* (including bacteria often associated with plant roots) and *Planctomycetes* (including terrestrial bacteria playing an important role in the nitrogen cycling anammox process) seemed to indicate the presence of significant amounts of rhizosphere-related bacteria. However, only refined phylogenetic studies will corroborate this hypothesis. The three other clusters, i.e. the red cluster (Aesch samples from 0-10 cm), the green cluster (Frick CT and RT), and the light blue cluster (The CT Aesch samples from 10-20 cm depth) formed more compact clusters and were located towards the center of the diagram, indicating a possible positive evolution towards more balanced microbial communities. For instance, the red cluster of top Aesch samples seemed to be richer in C and N contents than the purple deep layers of Aesch, and more

equilibrated in than the G Aesch samples lacking in Mg, B and Ca. Similarly, the green cluster of Frick seemed to be, regardless of tillage, more equilibrated in than the G Aesch samples with extensive amounts of Mg, B and Ca, and lacking fungi.

The microbial community analysis in the first layer, considering earthworms data, is presented hereunder.

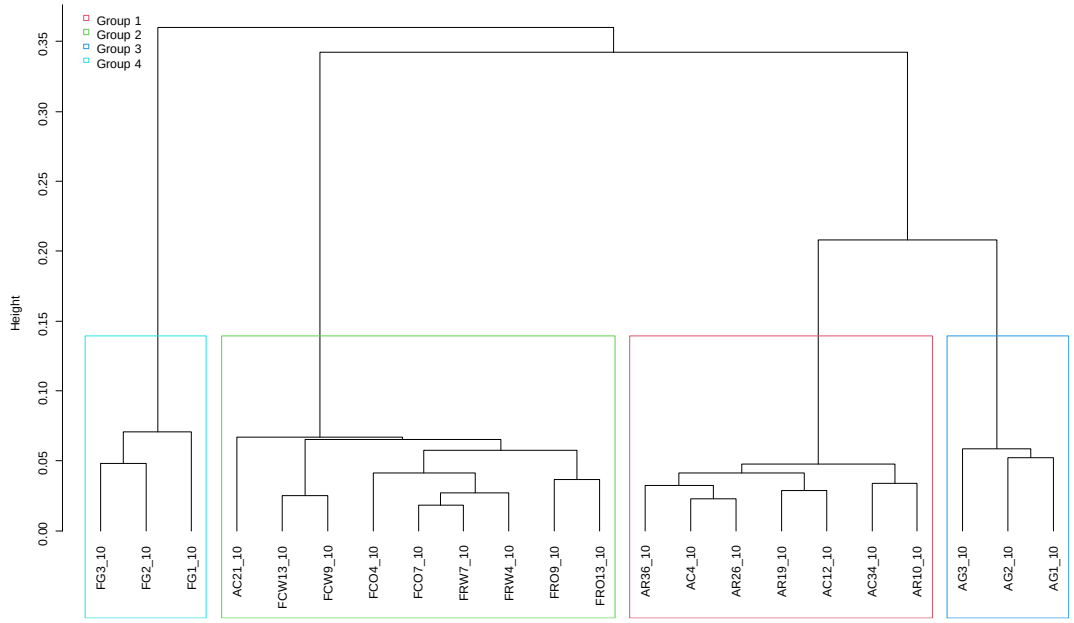

Figure 80: Clustering technique data of all samples taken at the Aesch and Frick sites at a depth of 0-10 cm using the Ward method after the Hellinger transformation.
The clusters are delimited by colored rectangles.

The dendrogram of the clustering technique is presented in Figure 80. This second analysis showed the formation of four clusters which are distinguished according to the site and the tillage conditions, with only one sample as an "outlier". The list of clusters is as follows, from left to right:

- Light blue cluster: The three G samples from Frick, standing out clearly from the other three.
- Green cluster: All other Frick samples, including the outlier from Aesch, and regardless of the tillage treatment applied.
- Red cluster: all samples from Aesch under RT or CT.
- Dark blue cluster: the three G samples from Aesch.

This 0-10 cm single depth analysis confirmed the strong dependence of the microbial communities on the sites considered. Moreover, and for both sites, no clear distinction can be made between the two tillage conditions. Also, the grassland samples of both are clearly distinguishable from the cultivated plot samples.

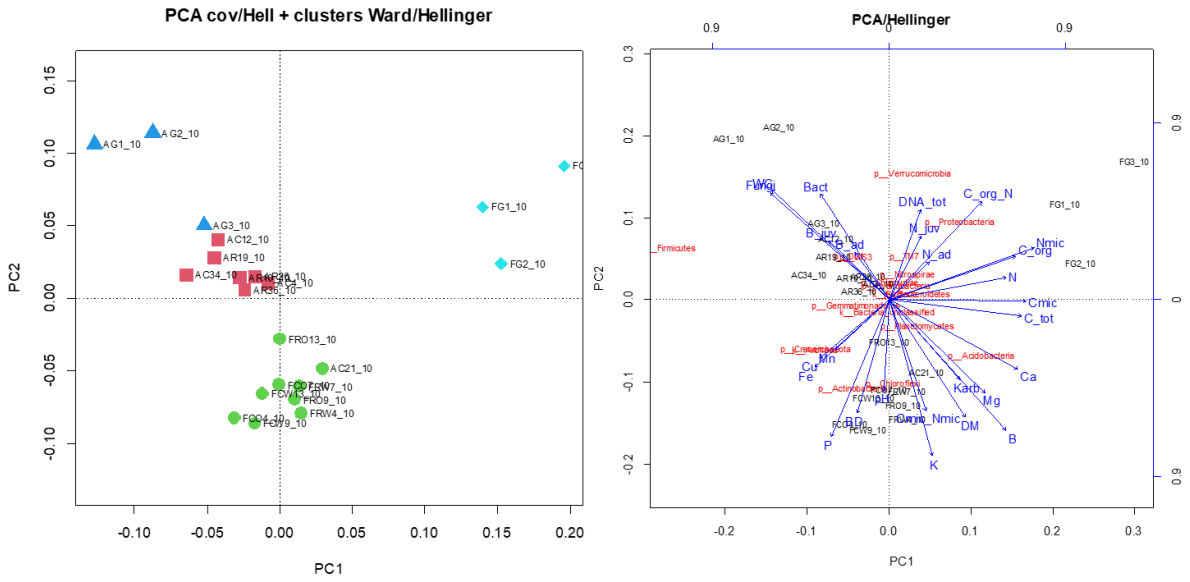

Figure 81: PCA of microbial community data with first layer data from both sites and with earthworms. Left: PCA on covariance matrix and Ward/Hellinger clustering of microbial community data. Right: PCA with a posteriori presentation of the environmental variables, and the presentation of the main microbial Phyla. The Phyla accounting for more than 0.01% of the total amount of sequences for all samples are displayed only. The PC1 and PC2 axes represent 49% and 29% of the observed variance respectively.

The four clusters obtained by Ward's method are presented in the form of a PCA in Figure 81 (left) with the same colors. The four clusters are clearly distinguished, with similar influence of environmental parameters as described above: Aesch G with lower Carb, Mg, B, Ca, BD and Frick G with higher Carb, Mg, B, Ca, BD; and Aesch RT and CT with lower carbon and nitrogen contents. Additionally, we could observe that the Frick RT and CT samples had the highest BD, P and K. Interestingly, there was a large distance between the G samples and those of RT and CT at the Frick site, while this distance was smaller for the Aesch site, confirming an important site effect. Paradoxically, the two tillage conditions RT and CT are closer for both sites, which would significate a development of very similar microbial communities.

Lastly, a 1000-permutation test followed by an ANOVA performed on the PCA was conducted to study the correlation between the environmental parameters and the microbial community data, and is visible in Table 5.

Table 5: Results of the permutation tests (1000 permutations) and ANOVA on each environmental parameter used for a posteriori interpretation of the PCA analysis.
*The significant codes are: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' '.*

Parameter	r^2	Pr(>r)	Sign.	Parameter	r^2	Pr(>r)	Sign.
pH	0.2186	0.076923	.	Bacteria	0.4130	0.009990	**
Nb of adults	0.0796	0.446553		Fungi	0.6647	0.000999	***
Biomass of adults	0.0882	0.416583		C_{mic}	0.4903	0.003996	**
Nb of juveniles	0.1335	0.251748		N_{mic}	0.6162	0.001998	**
Biomass of juveniles	0.1848	0.129870		C_{mic}/N_{mic}	0.3550	0.021978	*
BD	0.3589	0.010989	*	P	0.5696	0.000999	***
DM	0.5074	0.006993	**	K	0.6768	0.000999	***
WC	0.6716	0.000999	***	Mg	0.4632	0.003996	**
TN	0.3696	0.011988	*	Ca	0.5562	0.001998	**
C_{tot}	0.4613	0.000999	***	Cu	0.2126	0.113886	
Carb	0.2934	0.033966	*	Fe	0.2605	0.052947	.
SOC	0.4646	0.004995	**	Mn	0.1384	0.213786	
SOC/N	0.4735	0.003996	**	B	0.7948	0.000999	***
DNA_{tot}	0.2377	0.070929	.				

From the permutation and ANOVA test, we could see that some of the environmental parameters did not correlate with the microbial community structures. This was particularly the case for all the variables concerning earthworms, the latter having therefore no influence on the structuring of the communities. This was also the case for total soil DNA, Cu, Fe and Mn. Surprisingly, pH was also uncorrelated with microbial community structures, whereas this variable is known to have an important influence on communities. In this, the low variability of pH in the samples as described before probably explains this result. The variables having the higher correlations with microbial community structures were total C, P, K and B. SOC contents, Mg and Ca had lower correlation intensities.

3.6 Microbial community diversity

In Table 6 below, the five calculated indices are presented.

Table 6: Diversity and regularity indices calculated on the basis of all samples taken from the Aesch and Frick sites. These indices are calculated at the phylogenetic level of the genus. The color ramp shows the highest (green) and lowest (red) values.

Trial	Depth	Tillage	Genus Richness S		Fisher's alpha		Pielou's evenness		chao1		ACE	
			mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
Aesch	0-10 cm	CT	687.50	14.89	108.90	2.80	0.77	0.01	793.75	54.63	776.16	41.08
		RT	675.00	23.62	106.56	4.42	0.77	0.00	783.45	42.51	761.02	32.19
		G	609.33	18.58	94.38	3.40	0.74	0.01	686.55	17.27	679.96	20.16
	10-20 cm	CT	649.50	18.95	101.80	3.52	0.75	0.00	724.17	34.41	718.76	28.71
		RT	620.25	22.16	96.39	4.09	0.75	0.01	700.92	39.22	683.59	35.05
		G	596.33	50.50	92.06	9.25	0.72	0.01	679.24	79.81	667.38	65.96
	20-30 cm	CT	665.50	7.68	104.77	1.43	0.75	0.01	768.87	43.97	759.12	28.01
		RT	625.25	17.84	97.31	3.28	0.74	0.00	712.24	35.71	694.07	31.95
		G	548.67	72.27	83.50	12.88	0.73	0.01	606.12	94.45	599.56	92.55
Frick	0-10 cm	CT	599.50	42.72	92.62	7.76	0.75	0.00	661.05	61.43	662.48	63.47
		RT	593.00	27.75	91.41	5.06	0.76	0.00	682.54	56.12	664.31	46.44
		G	605.00	13.45	93.59	2.46	0.75	0.10	707.5	45.94	689.41	21.66
	10-20 cm	CT	619.00	6.98	96.15	1.28	0.74	0.01	697.59	32.53	693.97	26.18
		RT	588.75	71.94	90.75	12.91	0.75	0.01	672.04	122.85	663.97	115.62
		G	585.33	21.22	90.01	3.85	0.74	0.02	676.54	38.37	674.48	39.00
	20-30 cm	CT	619.75	19.17	96.30	3.52	0.75	0.00	709.46	34.68	694.51	32.39
		RT	598.75	3.86	92.44	0.71	0.76	0.01	668.97	17.43	666.77	14.87
		G	566.67	7.09	86.62	1.28	0.75	0.00	632.33	19.47	632.51	13.61

The first four diversity indices showed an excellent agreement: for each sample: S, Fisher's alpha, chao1 and ACE were systematically in phase and indicated the same trend.

The results showed an almost similar Pielou's regularity for all samples (0,72 – 0,77), with maximum values for the samples from Aesch top layer (0,77) and minimum values for the samples of deep (20-30 cm) G plots of the same site. There were only minimal variations between the two sites, and a slightly greater regularity for the Frick site than for the Aesch site.

In Aesch, the top layers had the highest microbial diversity in RT and CT, without clear distinction between both treatments. We could observe slightly higher diversity values in CT than in RT in the 10-20 cm and 20-30 cm layers. Surprisingly, the lowest diversity of all samples was measured in the deep 20-30 cm layer for the grassland samples. We could see a high depth effect, with higher diversity in the top layers than in the bottom layers.

The Frick site showed a different picture, with less pronounced variations with depth and globally lower diversity indices than in Aesch. In the top layer, the G plots had the greatest diversity while at 10-20 cm and 20-30 cm, it was the samples under CT that were having the greatest diversity.

4 Discussion

4.1 Enhanced soil fertility indicators in different soils textures under RT

In this study, we wanted first to investigate the effect of RT compared to CT on two different LTEs, in terms of physical, chemical and biological soil indicators that enable to give an evaluation of soil fertility as described by Bünemann et al. (2018). This work aims to provide a deeper knowledge on the effect of RT systems for soils with different textures.

According to our results, RT enhanced most of soil parameters in the top layer. More precisely, RT significantly enhanced SOC stocks and TN in Frick, and bacterial copies in Aesch, enhanced but not significantly BD and microbial biomass in Frick, soil total DNA and earthworms in Aesch, but did not affect WC nor pH. In the second and third layers, RT did not significantly enhance the soil parameters compared to CT in neither site. Hereunder, we are discussing the effect of RT in the top layer of the two sites, for a set of 8 parameters retained for their classical importance in assessing soil fertility.

4.1.1 Bulk density

The only parameter measuring physical state of the soil in this study was bulk density. Although non-significant, RT seemed to decrease BD in the top layer of both sites, which corresponds to conclusions drawn by Veenstra et al. (2006) and D'Haene et al. (2008).

Several sampling issues for cylinders extraction were encountered. The day of sampling at Aesch LTE was rainy, with a peak of precipitation between 8 and 9 am (1 mm) that delayed the start of sampling, and another precipitation between 12 and 1 pm (1 mm). The top soil was visually more humid due to the rainfall, such that the top soil water contents are significantly higher in Aesch than in Frick (Figure 38). Sampling in Frick was subject to a significant amount of "failed" cylinders sampling, because the soil easily broke within the cylinders, which also reduces the representativity of the data.

A more pronounced decrease in BD under RT was noticed for the clayey Frick site, such that RT appeared more advantageous in the dense clay soil of Frick than the lighter silty soil of Aesch. Thus, the hypothesis is confirmed for bulk density. However, in the second layers, the bulk densities were found lower in CT than for RT, and especially in Aesch soil. This illustrates the decompaction effect of ploughing until the 18 cm depth, compared to the RT system which does not reach this depth. In the third layer, the bulk densities were increased in both sites and independently of tillage regime, due to the absence of tillage engine.

4.1.2 pH

The pH did not show significant changes regarding tillage system at both sites. Thus, any affirmative conclusion can be drawn on the effect of pH in tillage systems. We could observe in Aesch an acidification in the third layer in the three tillage conditions, which describes an effect that is not due to

the soil cultivation of the LTE plots, because grasslands were also affected. In general, such soil acidification is due to fertilizer leaching (Lesturgez et al., 2006; Tang et al., 2013). It seems that the soil itself is subject to a decrease in pH at lower depth, but appropriate conclusions cannot be drawn. This drop of soil pH may not be a direct effect of land-use practices, but rather a site-specific effect.

4.1.3 Soil organic matter (SOC) stocks

RT increased SOC stocks in the top layer compared to CT, and lowered with increasing depth, while CT showed a more homogenous SOC content across the two first layers. This stratification effect in RT was described by Baker et al. (2007) and Luo, Wang, and Sun (2010), highlighting that the distribution of carbon stocks changes with RT practices, with higher SOC in the top layers and decreasing SOC in the bottom layers. Publications similarly describe stratification effect in RT systems compared to CT systems, such as in the Frick LTE (Fontana et al., 2015; Gadermaier et al., 2012; Krauss et al., 2020, 2017b) and other experimental fields (Loaiza Puerta et al., 2018; Parajuli et al., 2021; Peigné et al., 2018).

In the Frick site, the increase in SOC stocks under RT compared to CT was clearly marked and could reach SOC contents measured in the natural grassland, thus demonstrating the positive effects of reduced tillage on soil quality. RT enables to keep a top organic carbon accumulation and visible stratification as it can be found in natural ecosystems (Bausenwein et al., 2008). In Aesch, the stratification in SOC stocks was not observed. The high clay content in Frick soil is a possible factor of this enhanced effect of RT, because of the clay role in the binding of organic matter (Bausenwein et al., 2008; Franzluebbers and Arshad, 1997). Formation of SOC binding complexes with clay particles promotes the structuration of soil in aggregates, a process described as the "physical protection" of SOC with aggregates by Balesdent, Chenu, and Balabane (2000). With reduced soil-disturbing process in clayey soils, soil aggregates are better preserved, which enables a better retention of SOC in the soil. This retention capacity was described by as a "protective effect of clay" by several authors (Cania et al., 2020; Chivenge et al., 2007; Franzluebbers and Arshad, 1997).

From there, we can propose that the choice of tillage practice should be thought with consideration of the inherent soil texture, whose structure and capacity to protect organic matter is a critical aspect in order to maintain a good soil quality (Cooper et al., 2016). In clayey soils, RT practices can enable a soil decompaction in superficial layers and favor the formation of SOC sequestering aggregates, increasing SOC stocks and decreasing bulk density (Balesdent et al., 2000). In silty soils, because of minimal impacts on SOC changes, a focus on enrichment in SOC should be done with another solution than RT only such as with fertilizer application (Berner et al., 2008; Krauss et al., 2020; Loaiza Puerta et al., 2018), or by considering cover crops as it is common in conservation agriculture practices (Chivenge et al., 2007).

Importantly, it should be noted that the two long-term experiments are not aged the same. The LTE in Frick is 10 years older than the one of Aesch, so a bigger gap between two tillage systems could also be

explained by age rather than soil texture only. But, our findings support the hypothesis that clay soil increases the SOC stock gradient between RT and CT compared to silty soil.

4.1.4 Microbial biomass (C_{mic}, N_{mic})

The microbial biomasses measured by C_{mic} and N_{mic} showed consistent results with the SOC stocks distribution. A stratification of bacterial biomasses was observed and goes in line with results from Cania et al. (2019) and Martín-Lammerding et al. (2015). We could observe that the Frick soil had enhanced microbial biomass with RT compared to CT, but the effect was not significant. These results are in line with the numerous studies showing that RT increases microbial biomass compared to CT (Berner et al., 2008; Cania et al., 2019; Fontana et al., 2015; Gadermaier et al., 2012; Krauss et al., 2020; Kuntz et al., 2013). Additionally, the results showed a high site effect, with higher biomass in the clay soil of Frick. Thus, a tillage effect was more pronounced in Frick than in Aesch for microbial biomass, which validates the hypothesis statement for this parameter. Reduced fluctuation in soil water content in the clayey soil of Frick may furnish more stable conditions for microbial growth (von Lützow et al., 2002), but additional evaluation of the water holding capacity could be investigated in the two soils to confirm this hypothesis.

4.1.5 Soil DNA

The extracted soil DNA did not show significant changes between RT and CT at neither sites, and did not show any site effect. However, we could notice an increase in soil DNA due to RT conditions, and especially in Aesch LTE. Thus, the measure of microbial biomasses and DNA showed contradictory results: the microbial biomass was higher in Frick soil, but the extracted DNA was more responsive to RT in Aesch soil. This difference is probably due to the two different measuring techniques for the two parameters. In Frick, a high SOC was observed compared to Aesch, and the method used to extract DNA is possibly impacted by the richness in organic matter in the soil. In the SOC rich soil of Frick, the resulting DNA contents are thus lowered more importantly.

4.1.6 Bacteria

The increasing effect of RT compared to CT on bacteria was more significant and more pronounced in Aesch than in Frick in the first layer. In the second and third layers, there was no tillage difference, but rather a site effect, with higher bacteria counts in Frick. In line with the results of extracted DNA, the effect of RT is not as expected: the Aesch soil enables a greater enhancement of bacteria counts under RT, rather than the clay soil of Frick, which rejects the hypothesis.

The evaluation of RT on microbial community is still an important topic of research with contradictory conclusions: increased bacterial abundance under RT were found by several authors (Hartman et al., 2018; Krauss et al., 2020, p. 20; Morugán-Coronado et al., 2022), while decreased bacterial abundance were observed by other authors (de Graaff et al., 2019; Degrune et al., 2016). Krauss et al. (2020) worked on the clay soil of Frick while Degrune et al. (2016) worked on a Belgian silty loam, giving that the

conclusions of the literature are the opposite of our results. Hartman et al. (2018) observed increased bacteria under RT in a loam soil, going towards the Aesch soil texture. However, the studies were based on different measurement methods, which can also question with the comparability of the results. Thus, the study of bacteria changes in different sites under RT is further needed, also considering systematic measurement methods. The biomolecular analyses discussed later offers an interesting alternative to better understand which specific bacteria are affected by tillage practices.

4.1.7 Fungi

The number of fungi was impressively higher in Aesch soil than in Frick soil but the effect of tillage was not significant in the statistical ANOVA tests, because of the high range difference between both sites. However, from the numbers, we could observe a higher increase in fungi copies with RT in the Frick soil (+27.3%) than in the Aesch soil (+7.6%). With separated tests, a significant effect of RT would have probably been reported.

In comparison, studies on Frick soil showed that CT system were more prone to destroy the fungi hyphae system (Carr et al., 2012), so that RT system lead to more fungi abundance and more fungal-decomposition pathway (Gadermaier et al., 2012; Krauss et al., 2022; Kuntz et al., 2013; Säle et al., 2015) than bacterial pathway. In a Swiss loam soil, studies showed increased increasing activity of fungi under RT system (Hartman et al., 2018; Wagg et al., 2018). In other sites, Domnariu et al. (2022) found evidence of increased fungal abundance in no-till systems in a clay loam soil with ergosterol measurements, while the review from Morugán-Coronado et al. (2022) highlights the general positive effect of RT on fungi abundance based on PLFA measurements, without any site-specific effect. In our study, if we could observe a higher effect in Frick than in Aesch, the hypothesis seems valid for fungi abundance. However, the lack of research on fungi abundance with comparable methods calls for more studies.

4.1.8 Earthworms

The results regarding earthworm abundance and biomass were surprising and largely inconsistent. In order to understand the possible biases for earthworm sampling, a brief description of earthworm biology is first given. Three taxonomic groups are distinguished in earthworm community. Anecic earthworms are long, dark-pigmented earthworms living in a single vertical tunnel, travelling up and down to feed from surface organic matter. Endogenic earthworms are unpigmented, living in the top 20 cm, where they horizontally move and feed from SOC. Epigenic earthworms are pigmented, living in the surface soil and decaying organic matter usually found in compost piles.

In this study, the earthworm counting was delicate and more time-consuming than expected, which probably impacted the final results. Indeed, a high timeframe between the sampling from the two sites hinders an accurate comparison between the two sites. Moreover, the weather conditions got the soil drier each day, stepping back the earthworms from surface. It was visually evident during the manual

soil breaking that the soil from Aesch contained more endogenic earthworms, especially in the upper layer within the roots, compared to Frick soil which was already dry and contained very few earthworms in the dry rooted upper layer. Also, the extraction of anecic earthworms with the mustard solution was not successful, so the genuine number of earthworms must be underestimated and not comparable between sites, and even between plots within a site. This mustard extraction method was already tested by FiBL in previous studies and was also surprisingly unsuccessful, which raises issues about the protocol, yet well established and used in the scientific framework, or about the mustard solution itself used by the institute. Thus, drying weather conditions unfavored the endogenic earthworms extraction, while the escape of an important part of the anecic earthworms in the down tunnels could not be properly balanced with the an effective mustard solution extraction.

Precedent publications showed that RT increased earthworm population (Anken et al., 2004; Briones and Schmidt, 2017; De Notaris et al., 2021; Kuntz et al., 2013; Lefèvre, 2009), and also in reviews from several authors (Briones and Schmidt, 2017; D'Hose et al., 2018). In our study, the effect of RT is not well reported. Still, we can see that Aesch LTE gathered in general more earthworms than in Frick. This tendency could be explained by the dry soil sampling bias in Frick explained above, but could also be explained by two different soil textures. We would have expected more earthworms in the soil of Frick, because of its high SOC content acting as a food source. However, as mentioned in the study from Holmstrup et al. (2011), the effect of soil texture of earthworm population is not confirmed from actual research and is still poorly studied. Also, we would have expected more earthworms under RT conditions compared to CT, due to the high SOC contents in the top layers. We suppose that the effect of tillage is more important than soil texture, but from this unique set of data which must be biased, the hypothesis of increased earthworm communities in the clay soil of Frick compared to the silty soil of Aesch is not accepted.

1.1.9 Conclusive statements

To summarize the hypothesis assessment, we can confirm that the effect of tillage was different between the two sites: chemical parameters, such as SOC stocks (and micronutrients), showed a greater effect of RT in the clayey soil of Frick. On the other hand, the biological parameters (quantity of extracted DNA, bacteria and earthworms), were improved in the Aesch soil. Fungi copies were improved in Frick soil. Physical parameter, represented here only by bulk density, was improved by RT in the Frick soil, but was adversely affected in Aesch soil.

The Table 7 hereunder gives a qualitative evaluation of the potential of RT to enhance the above-mentioned soil fertility indicators. Additionally, the indicators regarding the nitrifying population and the microbial community diversity were added, and are discussed in the second and third chapters of this discussion.

Table 7: Effect of RT on the selection of soil fertility indicators in the sites of Aesch and Frick.

Parameter		Aesch	Frick
Physical	Bulk density	+	++
	RT could lower bulk density in the clay soil of Frick. In the silty soil of Aesch, CT rather than RT was more prone to help decrease bulk density.		
Chemical	pH	0	0
	SOC stocks	+	+++
	RT was able to increase SOC stocks in the clay soil of Frick. In the silty soil of Aesch, the effect of RT was not visible. The pH was not influenced by tillage practices.		
Biological	Microbial biomass	+	++
	Extracted soil DNA	+	+
	Bacteria	++	+
	Fungi	+	++
	Microbial diversity	+	-
	Nitrifiers	-	+
	Earthworms	+	+
	RT lead to increased biological parameters in the silty soil of Aesch but only marginally in the clay soil of Frick.		

4.2 Site-specific response of nitrifying bacteria to tillage intensity

The second aim of this work was to examine the population of nitrifying bacteria, with the proposal that a ploughing tillage regime would increase the nitrifiers. The latter play a crucial role in the cycling of N in the soil: their ability to oxidize ammonium (NH_3) to nitrite (NO_2^-) and nitrate (NO_3^-) in aerobic conditions enables the soil plants to take up nitrogen. However, as nitrate is highly subject to leaching, the rate of nitrification must be controlled in cultivated plots. Moreover, nitrate can be further denitrified and lost in gaseous nitrogen (N_2O and N_2) in anaerobic conditions. The formation of N_2O gases from microbial activity in agricultural soils are largely contributing to climate change, and a deeper knowledge on how nitrifiers behave in agricultural fields, and in particular how they are affected by tillage practices was called out by Krauss et al. (2017). Indeed, as the RT practices induce changes in soil environmental, as shown in the first part of this discussion, the microbial community growth is affected (Harder and Dijkhuizen, 1983). In particular, studies reported reduced or delayed mineralization with RT practices. This adversely affects the release of nitrogen in available forms for plant growth by the nitrifiers, so deeper knowledge on the underlying processes are of major importance (Mäder and Berner, 2012; Peigné et al., 2007)

In this study, the tillage effect in the proportion of nitrifiers measured by 16S gene sequencing was non-significant at any sites (Figure 60). However, we could possibly observe in the top layer a gradient from CT to RT to G in both sites, in two opposite directions: In Aesch, the proportion of nitrifiers seemed to

decrease with decreasing tillage management, while in Frick, the proportion of nitrifiers seemed to increase with decreasing tillage management. In the second layer, the difference between RT an CT was non-significant fron the ANOVA tests for both sites, but because a high site differenciation was dominant. However, we could observe, with distinction from the two sites, that RT seemed to increase the proportion of nitrifiers compared to CT: As we expected significant and unidirectional gradient for the two sites, the results go towards a rejection of the initial hypothesis.

In comparison to our results, the study from Krauss et al. (2017) showed that in the Frick soil, nitrifiers also had increased activity in RT soils. Doran (1980) similarly showed increased nitrifiers in RT soils. However, the studies rely on different measuring techniques based on enzymatic measurements, while in the given study we used 16S sequencing methods. DNA based research from Liu et al. (2022) showed that RT increased nitrifiers abundance, which also contradicts our initial hypothesis. The method in this study only measure the presence of species, and not their activity. A specific set of nitrifier species was used to count their relative proportions, and certainly does not include all the actual nitrifiying population of the soil. Thus, an assessment of how the nitrifiers evolve in RT conditions compared to CT is hardly confident with our dataset. Moreover, the one-time soil sampling in this study forms a limit to the understanding of ongoing soils processes. The soil sampling in early March, during an early warm spring must have favored an early microbal activity in the soils, and decrease a possible tillage difference. A time-dependant analysis could provide more accurate monitoring of the nitrifiers activity, rather than focusing on a single-time sampling.

We could observe a significant site effect, with more nitrifiers in the silty soil of Aesch than in the clayey soil of Frick. We can hypothesize that the soil in Aesch has improved growing conditions for nitrifiers, that are aerobic chemoautotrophs, thus growing with oxygen and carbon dioxide as a carbon source. The results on bulk density do not prove a better aeration in soils of Aesch, while the SOC stocks were showed to be lower in Aesch site compared to Frick site. Thus, the high load of SOC in clayey site seems to act as an inhibitor for the nitrifying activity, while soils with lower SOC contents could increase nitrifiers activity. Additionally, we could observe lower contents of TN in the silty soil of Aesch, which could also demonstrate a higher N cycling in Aesch soil. In this perspective, we could expect to find more nitrifiers activity in a lighter soil under CT conditions, where the SOC contents are lower than in a clay-rich and RT soil, but further investigation is needed. As mentioned before, a focused monitoring of N cycling population, including enzymes, and ammonium, nitrite and nitrate concentrations, could provide a better understanding of the drivers of nitrification changes under RT in both sites.

With the given data from with year and conditions, we reported an effect of soil texture, but a RT effect which was controversial to our initial hypothesis, i.e. increased in ploughed soils. More advanced, dynamic and systematic evaluation of the nitrifiers in other soils are required at this point in order to gain knowledge in the effects of RT in nitrifying population, such as through specific activity monitoring.

4.3 Mixed effects of soil disturbance intensity on SOC stocks and microbial diversity

Thirdly, we wanted to investigate to which extent do SOC stocks and microbial communities diversity increased with decreasing soil disturbance intensity.

SOC is a major parameter in the evaluation of a soil quality, because of its role on soil biota energy source and on soil structure (Bausenwein et al., 2008; Six et al., 2004; Wiesmeier et al., 2019). In particular, SOC acts as a feeding source for soil microbial populations, promoting their development and activity to fulfill their ecosystem functions (Bausenwein et al., 2008; Lori et al., 2017). They participate in the soil biochemical health, by maintaining organic matter and nutrients cycling and acting in pest and disease control, but also in the soil physical structure, by producing particle-binding agents for the formation of an aggregated, well-aerated soil, which beneficially promotes nutrients availability and colonization sites (Cania et al., 2019). Thus, the understanding of their reactivity to different soil management systems are of high importance for the development of alternative agricultural practices. In particular, RT, which decreases the physical disruption compared to ploughing techniques, participates in the formation of a stable and stratified environment for microorganisms (Kraut-Cohen et al., 2020), similar to natural ecosystems, promoting the growth and activity of microbiota.

In our study, we could clearly show that the SOC stocks were enhanced with RT compared to CT, with a net stratification effect, thus accumulating carbon in the top layers. Moreover, the perennial grasslands had the highest SOC stocks, especially in the top layers, showing a clear increasing SOC gradient with decreasing tillage. Reduced tillage is presented here as an effective soil-preservative method to meet a natural stratified environment, which participates in enhanced SOC accumulation and aggregates formation by microbial activity. However, the microbial community diversity analysis gave opposite results: the site of Frick, which had significantly higher SOC levels, showed lower diversity indices compared to Aesch. Also surprisingly, we found the highest diversity indices in the CT plots but not in the RT plots through the whole soil profile, while the CT plots were having higher contents in SOC. Further, the grassland plots were showing the lowest microbial diversity indices, especially in the third layer at both sites.

Thus, a tillage effect on microbial diversity was indeed observed in our study, but not in the direction as expected, because samples with higher SOC contents had the lower microbial diversity scores. In the previous works, studies on the effect of RT on microbial communities diversity showed contradictory results: Legrand et al. (2018) and Frøslev et al. (2022) found that RT increases species diversity (number and proportions of individuals), but not richness (the number of species without proportions),

suggesting that RT would change the community structure only, but not promoting additional microbial individuals. In contradiction, the study from Degrune et al. (2016) showed decreased diversity indices under RT in a silty soil. The latter proposed that soil breakdown by ploughing could form numerous small colonization spaces for bacteria development, and particularly for minor bacteria species, thus promoting bacterial growth and increasing the observed richness compared to RT. However, Sengupta and Dick (2015) found that intense ploughing decreased bacterial richness in a silt-loam soil. Further, several authors (Hartman et al., 2018; Kraut-Cohen et al., 2020; Wagg et al., 2018; Xia et al., 2019) all showed non-significant changes in microbial richness with RT practices but microbial structure changes. Overall, an effective change in microbial composition is noticed with RT practices compared to ploughing, as mentioned by the reviews from Gupta et al. (2022) and Morugán-Coronado et al. (2022), but the effects seem site-specific. In the Frick soil, no increase in microbial richness was observed despite the positive effect of RT on SOC stocks. In the Aesch soil, the microbial diversity seemed fundamentally higher than in the Frick site, and also unfavored by RT. Still, for both sites, microbial richness was not higher under RT, and much less in natural grasslands, but rather favored in CT conditions. We did not expect to have such lower diversity indices in the site of Frick compared to the site of Aesch. Hypothetically, we consider high site-specific effects. A possible explanation would be the higher bulk densities found in the Frick site, inhibiting colonization sites for microbial communities, or the nutrient balance or availabilities. Also, bacteria community is certainly very sensitive to dynamic soil properties, such as its pore water and air contents, loads in micronutrients, so numerous factors are to be considered when looking at microbial communities patterns (Bünemann et al., 2006), such as the fertilizer applications and the meteorological factors, and more specifically in a time-dependant way.

Underlying processes in microbial community changes with tillage practices are not clear yet, and within the timeframe of this work, could not be further investigated. Deeper microbial community analyses could be conducted with the set of data produced for this study, with an evaluation at deeper phylogenetic level, so as to achieve comparison with other authors studies produced so far.
However, an important aspect to keep in mind in our research is the agricultural framework of the targeted effects on microbial community, which implies crop production. In this perspective, more than only looking at the microbial community populations, an assessment on the microbial functional diversity could target their role and ecosystem services in agricultural soil, with combined analysis of the yields. A focus on specific microorganisms roles, such as N cycling (given the recently discoveries on the potential greenhouse emissions in RT systems), or their resilience to particular events, such as droughts, form more opportunities regarding the characterization of microbial population in agricultural management.

5 Conclusions

As environmentally friendly agriculture becomes an increasingly important issue in climate change mitigation, alternative practices are called upon to diminish the negative effects of conventional agro-systems - soil depletion and erosion, loss of biodiversity, and pollution at all scales of ecosystems. Organic farming fixes the objectives to suppress the use of synthetic fertilizers and pesticides, whose effects on human and ecosystem health, but also on greenhouse gases emissions are adversely recognized. Moreover, conservation agriculture forms a set of agricultural methods including cropping rotations, cover crops and reduced soil disturbance that aims at preserving soil integrity and inherent soil functions for sustainable arable cropping.

In this work, we wanted to contribute to the actual scientific research regarding reduced tillage (RT) practices at the Swiss Research Institute of Organic Agriculture (FiBL). If RT is already known to have positive impacts on soil structure and fertility, some gaps regarding the effects in different soil textures still needs to be closed. In addition, the knowledge regarding microbial community behaviors in RT systems is still poorly developed and needs modern DNA sequencing research support. In this perspective, two long-term experiments (LTE) of two soil textures, silty (Aesch, BL) and clayey (Frick, AG), were sampled in reduced tillage (RT) *vs* conventional tillage (CT) *vs* grassland (G) conditions according to depth (0-10 cm, 10-20 cm,20-30 cm). The effects of RT on the two soils were investigated in light of the concept of soil fertility concept, the definition and evaluation of which are based on a set of physical, chemical and biological parameters, and analyzed using ANOVA, PCA and correlation tests tools. Then, we performed an extended analysis of microbial community with DNA sequencing methods at the phylum and genus levels, in order to examine the changes in microbial community structures and diversity in RT systems. In this work, we were also interested on nitrifying populations, because of their major role in soil nitrogen cycle.

In agreement with previous studies, we were able to confirm in this study that RT improved soil fertility compared to CT, and that RT, by decreasing soil disturbance and mixing, promotes SOC and nutrients stratification similar to the untilled natural grasslands. Notably, clustering techniques highlighted high differentiation between the samples from the two sites, which demonstrates a probable site-specific impact of RT system and depending on the type of parameters. Indeed, in the clayey soil of Frick, the stratification effect of RT was particularly high regarding chemical parameters (SOC stocks, TN, micronutrients), because of the high C and nutrients physical binding property of clay. Regarding physical parameters, the bulk density was decreased under RT in the top layer in Frick LTE, because the high clay content could probably promote a well-structured soil by aggregate formation. We clearly noticed that the biological indicators (total soil DNA, bacteria, and earthworms) were in turn more enhanced by RT in the silty soil of Aesch compared to the clayey soil of Frick in the top layers, but not

the fungi, which raises new research questions about the role of soil texture in RT system. Notably, the earthworms sampling was subject to experimental biases, whose mitigated conclusions calls for repetitive procedure. Biomolecular samples analysis by DNA sequencing produced a large set of data that showed once again a clear distinction between both sites on the microbial community. However, the effect of tillage methods was not significant, as the populations were more structured within a depth gradient and more responsive to the differences in environmental parameters between both sites. We could identify nitrifying populations, whose response was not significant in the direction of tillage methods but rather oriented towards site differences, implying that further investigations must be conducted, possibly through an analysis of their functional activity. Finally, microbial diversity indices lead to surprising conclusions, showing that the microbial diversity was found the lowest in the natural grasslands, and higher in the CT treatments and in the silty soil of Aesch. These findings support that the microbial community was probably influenced by specific factors to be further investigated, and shows that the RT systems do not form a single argument in microbial biodiversity enhancement, and that future studies could study the effects of RT through a functional diversity lens.

To conclude, while this work enabled to corroborate an enhanced soil quality with RT practices under two organic LTEs, and especially in a high SOC-sequestering clay soil, the underlying processes regarding site specific changes still need to be deepened. In particular, the physical quality assessment was rather poor because only represented by bulk density, and calls for in-depth study, for instance at the mineralogical level. Inherent soil texture has major impacts on soil structure, thus changes in specific physical parameters, such as soil aggregation, penetration resistance or water dynamics could be investigated under different tillage regimes in a similar study framework. Coupled with a complete evaluation of the fine particles contents, these works would aim to decipher the response of tillage practices in different soil types. Notably, the two LTEs were different in soil texture but also in implementation ages, which possibly acts as a factor in the observed effect of RT. With this statement, a long-term assessment of the parameters should be closely monitored, also considering the effect of rotational cropping applied in the LTEs, in order to reveal possible dynamics and compare them with the works from other authors. Finally, this study was gathering a lot of data and aimed at untangling the effect of several factors (tillage, soil texture, depth), which are already separately complex. Due to time constraints, we minorly considered the depth effect and the comparison of the cultivated plots with the undisturbed grassland, which could be more specifically studied in further studies. As a supplementary effort towards the comprehension of RT systems, an advanced microbial community structure determination was conducted for the first time in the two LTEs of this study, which forms promising study opportunities. The growing interest in DNA-based methods is justified by their potential to understand biotope response to specific agronomic practices aimed at improving the biodiversity of

agricultural soils. Within the timeframe of this study, the analysis was conducted at high phylogenetic levels and rather superficially, but extended competent analysis could include the study of targeted bacterial groups because of their functional role in agricultural soils. Thus, with deeper knowledge on the role of soil texture and on microbial community changes in RT systems, optimized and site-specific soil fertility improvements could be applied in farms, supporting the much-needed implementation of effective, and sustainable, agronomic management strategies.

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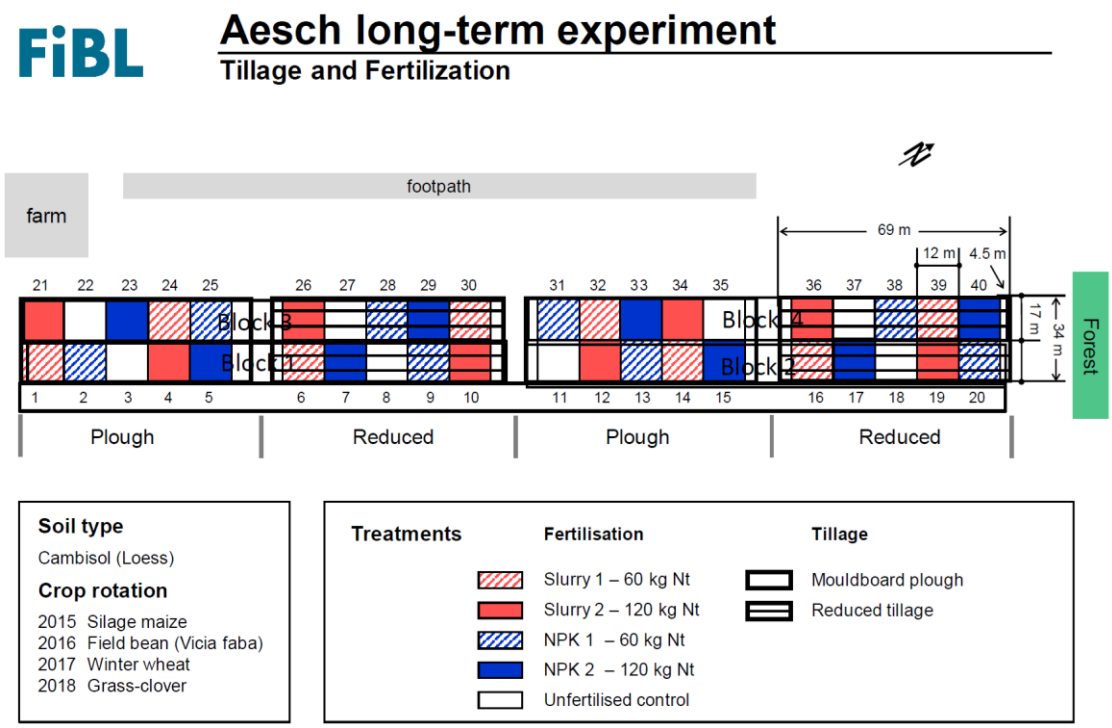
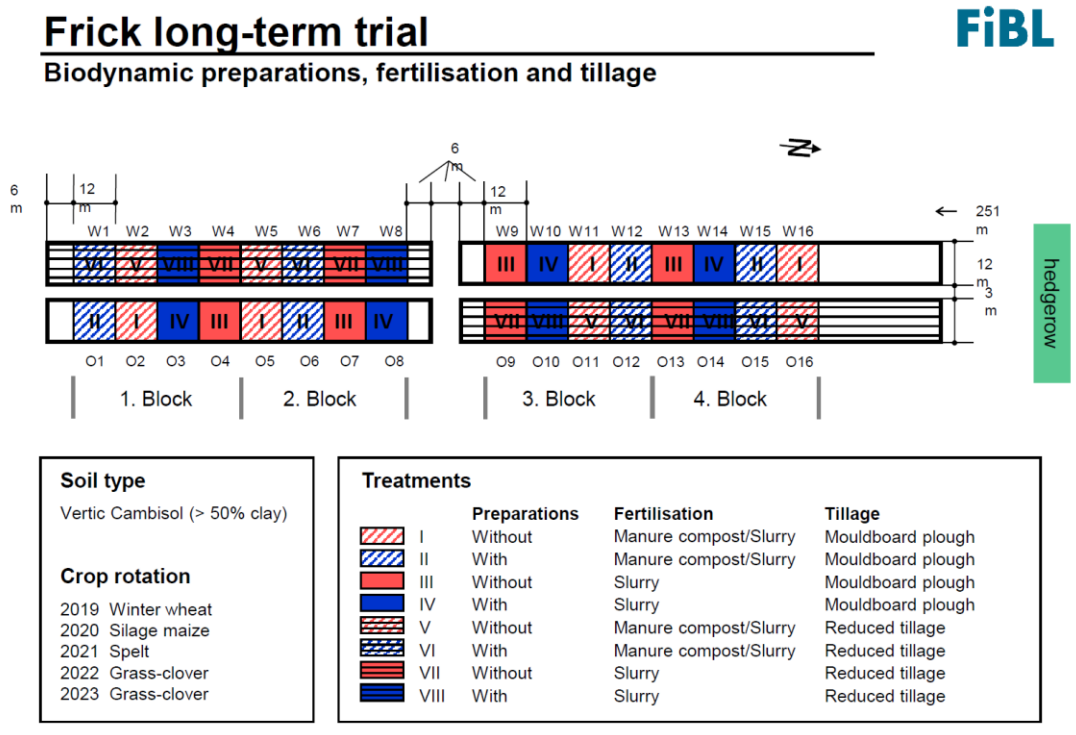
8 Appendix

8.1 Appendix 1: Meteorological data (source: https://www.agrometeo.ch/fr)

8.2 Appendix 2: Detailed design of Aesch and Frick LTEs





8.3 Appendix 3: Mothur script for the microbial community mapping

```
########################################
#                                      #
#  ILLUMINA DATA ANALYSIS PIPELINE     #
#                                      #
#  Master Sophie Caquot EPFL-FiBL      #
#                                      #
#  July 2022                           #
########################################
# Pierre Rossi @ EPFL - GR-CEL Vers. 2022/07
# Datasets provided by GTF LIMS based on the ZYMO 16S Kit
#################
# INITIALISATION
# Locate fastq file [namefile].fastq and place a copy in /Desktop/[DedicatedFolder]
# Open Terminal

cd Desktop/[DedicatedFolder]
mothur

# Assemble forard and reverse reads:
make.contigs(ffastq=[NameFile].fastq, rfastq=[NameFile].fastq, processors=6)

Output File Names:
[NameFile]_R1.trim.contigs.fasta
[NameFile]_R1.scrap.contigs.fasta
[NameFile]_R1.trim.contigs.qual
[NameFile]_R1.scrap.contigs.qual
[NameFile]_R1.contigs.report

###################################
# TRIMMING FOR QUALITY AND LENGTH

trim.seqs(fasta=[NameFile].trim.contigs.fasta, qfile=[NameFile].trim.contigs.qual, qaverage=25, maxambig=0, maxhomop=8,
minlength=350, maxlength=500, flip=T, processors=6)

# Output File Names:
[NameFile].trim.contigs.trim.fasta
[NameFile].trim.contigs.scrap.fasta
```

[NameFile].trim.contigs.trim.qual

[NameFile].trim.contigs.scrap.qual

summary.seqs(fasta=current)

#####################################

RE-SAMPLE

sub.sample(fasta=[NameFile].trim.contigs.trim.fasta, size=80000)

Output File Names:

[NameFile].trim.contigs.trim.subsample.fasta

summary.seqs(fasta=current)

############################

UNIQUE, ALIGN AND FILTER

Alignment made using the Greengenes database

Be sure to use the most recent one (see http://www.mothur.org/wiki/Greengenes-formatted_databases)

unique.seqs(fasta=[namefile].trim.contigs.trim.subsample.fasta)

Output File Names:

[NameFile].trim.contigs.trim.subsample.names

[NameFile].trim.contigs.trim.subsample.unique.fasta

summary.seqs(fasta=current, name=current)

align.seqs(candidate=[NameFile].trim.contigs.trim.subsample.unique.fasta, template=gg_13_8_99.refalign, flip=t, search=kmer, ksize=9, align=needleman, gapopen=-1, processors=6)

Output File Names:

[NameFile].trim.contigs.trim.subsample.unique.align

[NameFile].trim.contigs.trim.subsample.unique.align.report

filter.seqs(fasta=[namefile].trim.contigs.trim.subsample.unique.align, vertical=T)

Output File Names:

[NameFile].filter

[NameFile].trim.contigs.trim.subsample.unique.filter.fasta

Let's re-run the unique.seqs command making sure to use the name option

unique.seqs(fasta=[NameFile].trim.contigs.trim.subsample.unique.filter.fasta,
name=[NameFile].trim.contigs.trim.subsample.names)

Output File Names:

[NameFile].trim.contigs.trim.subsample.unique.filter.names

[NameFile].trim.contigs.trim.subsample.unique.filter.unique.fasta

##

DENOISING: Single Linkage Preclustering method

pre.cluster(fasta=[NameFile].trim.contigs.trim.subsample.unique.filter.unique.fasta,
name=[NameFile].trim.contigs.trim.subsample.unique.filter.names, diffs=2)

Takes time...

Output File Names:

[NameFile].trim.contigs.trim.subsample.unique.filter.count_table

[NameFile].trim.contigs.trim.subsample.unique.filter.unique.precluster.fasta

[NameFile].trim.contigs.trim.subsample.unique.filter.unique.precluster.count_table

[NameFile].trim.contigs.trim.subsample.unique.filter.unique.precluster.map

##

DE-UNIQUE, RE-SAMPLE AND CHANGE NAMES

deunique.seqs(fasta=[NameFile].trim.contigs.trim.subsample.unique.filter.unique.precluster.fasta,
name=[NameFile].trim.contigs.trim.subsample.unique.filter.names)

Output File Names:

[NameFile].trim.contigs.trim.subsample.unique.filter.unique.precluster.redundant.fasta

summary.seqs(fasta=current)

sub.sample(fasta=[NameFile].trim.contigs.trim.subsample.unique.filter.unique.precluster.redundant.fasta, size=60000)

Output File Names:

[NameFile].trim.contigs.trim.subsample.unique.filter.unique.precluster.redundant.subsample.fasta

In a terminal, remove gaps and non coding characters (. and -)

tr -d ".-" < [NameFile].trim.contigs.trim.subsample.unique.filter.unique.precluster.redundant.subsample.fasta > [NameFile].fasta

##

STATISTICAL ANALYSIS: PHYLOGENY ON GROUP FILE USING MOTHUR

Make a group from all samples:

Following the next exemple...

make.group(fasta=b1.clean.redundant.fasta-b2.clean.redundant.fasta-b3.clean.redundant.fasta, groups=b1-b2-b3)

Pour samples de Aesch

make.group(fasta=So1.fasta-So2.fasta-So3.fasta-So4.fasta-So5.fasta-So6.fasta-So7.fasta-So8.fasta-So9.fasta-So10.fasta-So11.fasta-So14.fasta-So15.fasta-So16.fasta-So17.fasta-So18.fasta-So19.fasta-So20.fasta-So21b.fasta-So22b.fasta-So23b.fasta-So24b.fasta-So27.fasta-So28.fasta-So29.fasta-So30.fasta-So31.fasta-So32.fasta-So33.fasta-So34.fasta-So35.fasta-So36.fasta-So37.fasta-Pos.fasta-NTC.fasta, groups=So1-So2-So3-So4-So5-SO6-So7-So8-So9-So10-So11-So14-So15-So16-So17-So18-So19-So20-So21b-So22b-So23b-So24b-So27-So28-So29-So30-So31-So32-So33-So34-So35-So36-So37-Pos-NTC)

Output File Names: mergegroups, change name to AllSamples.groups

Merge all ".fasta" files:

merge.files(input=b1.clean.redundant.fasta-b2.clean.redundant.fasta-b3.clean.redundant.fasta, output=AllSamples.fasta)

merge.files(input=So1.fasta-So2.fasta-So3.fasta-So4.fasta-So5.fasta-So6.fasta-So7.fasta-So8.fasta-So9.fasta-So10.fasta-So11.fasta-So14.fasta-So15.fasta-So16.fasta-So17.fasta-So18.fasta-So19.fasta-So20.fasta-So21b.fasta-So22b.fasta-So23b.fasta-So24b.fasta-So27.fasta-So28.fasta-So29.fasta-So30.fasta-So31.fasta-So32.fasta-So33.fasta-So34.fasta-So35.fasta-So36.fasta-So37.fasta-Pos.fasta-NTC.fasta, output=AllSamples.fasta)

Output File Names: AllSamples.fasta

summary.seqs(fasta=AllSamples.fasta)

Pour samples de Frick

make.group(fasta=So40b.fasta-So41b.fasta-So42b.fasta-So43b.fasta-So44b.fasta-So45b.fasta-So46b.fasta-So47b.fasta-So48b.fasta-So49b.fasta-So50b.fasta-So53b.fasta-So54b.fasta-So55b.fasta-So56b.fasta-So57b.fasta-So58b.fasta-So59b.fasta-So60b.fasta-So61b.fasta-So62.fasta-So63.fasta-So66.fasta-So67.fasta-So68.fasta-So69.fasta-So70.fasta-So71.fasta-So72.fasta-So73.fasta-So74.fasta-So75.fasta-So76.fasta-So516477.fasta-So122538.fasta, groups=So40b-So41b-So42b-So43b-So44b-SO45b-So46b-So47b-So48b-So49b-So50b-So53b-So54b-So55b-So56b-So57b-So58b-So59b-So60b-So61b-So62-So63-So66-So67-So68-So69-So70-So71-So72-So73-So74-So75-So66-So516477-So122538)

Output File Names: mergegroups, change name to AllSamples.groups

Merge all ".fasta" files:

merge.files(input=b1.clean.redundant.fasta-b2.clean.redundant.fasta-b3.clean.redundant.fasta, output=AllSamples.fasta)

merge.files(input=So40b.fasta-So41b.fasta-So42b.fasta-So43b.fasta-So44b.fasta-So45b.fasta-So46b.fasta-So47b.fasta-So48b.fasta-So49b.fasta-So50b.fasta-So53b.fasta-So54b.fasta-So55b.fasta-So56b.fasta-So57b.fasta-So58b.fasta-So59b.fasta-So60b.fasta-So61b.fasta-So62.fasta-So63.fasta-So66.fasta-So67.fasta-So68.fasta-So69.fasta-So70.fasta-So71.fasta-So72.fasta-So73.fasta-So74.fasta-So75.fasta-So76.fasta-So516477.fasta-So122538.fasta, output=AllSamples.fasta)

Output File Names: AllSamples.fasta

summary.seqs(fasta=AllSamples.fasta)

Pour tous les samples de Aesch ET de Frick

make.group(fasta=So1.fasta-So2.fasta-So3.fasta-So4.fasta-So5.fasta-So6.fasta-So7.fasta-So8.fasta-So9.fasta-So10.fasta-So11.fasta-So14.fasta-So15.fasta-So16.fasta-So17.fasta-So18.fasta-So19.fasta-So20.fasta-So21b.fasta-So22b.fasta-So23b.fasta-So24b.fasta-So27.fasta-So28.fasta-So29.fasta-So30.fasta-So31.fasta-So32.fasta-So33.fasta-So34.fasta-So35.fasta-So36.fasta-So37.fasta-Pos.fasta-NTC.fasta-So40b.fasta-So41b.fasta-So42b.fasta-So43b.fasta-So44b.fasta-So45b.fasta-So46b.fasta-So47b.fasta-So48b.fasta-So49b.fasta-So50b.fasta-So53b.fasta-So54b.fasta-So55b.fasta-So56b.fasta-So57b.fasta-So58b.fasta-So59b.fasta-So60b.fasta-So61b.fasta-So62.fasta-So63.fasta-So66.fasta-So67.fasta-So68.fasta-So69.fasta-So70.fasta-So71.fasta-So72.fasta-So73.fasta-So74.fasta-So75.fasta-So76.fasta-So516477.fasta-So122538.fasta, groups=So1-So2-So3-So4-So5-So6-So7-So8-So9-So10-So11-So14-So15-So16-So17-So18-So19-So20-So21b-So22b-So23b-So24b-So27-So28-So29-So30-So31-So32-So33-So34-So35-So36-So37-Pos-NTC-So40b-So41b-So42b-So43b-So44b-SO45b-So46b-So47b-So48b-So49b-So50b-So53b-So54b-So55b-So56b-So57b-So58b-So59b-So60b-So61b-So62-So63-So66-So67-So68-So69-So70-So71-So72-So73-So74-So75-So76-So516477-So122538)

merge.files(input=So1.fasta-So2.fasta-So3.fasta-So4.fasta-So5.fasta-So6.fasta-So7.fasta-So8.fasta-So9.fasta-So10.fasta-So11.fasta-So14.fasta-So15.fasta-So16.fasta-So17.fasta-So18.fasta-So19.fasta-So20.fasta-So21b.fasta-So22b.fasta-So23b.fasta-So24b.fasta-So27.fasta-So28.fasta-So29.fasta-So30.fasta-So31.fasta-So32.fasta-So33.fasta-So34.fasta-So35.fasta-So36.fasta-So37.fasta-Pos.fasta-NTC.fasta-So40b.fasta-So41b.fasta-So42b.fasta-So43b.fasta-So44b.fasta-So45b.fasta-So46b.fasta-So47b.fasta-So48b.fasta-So49b.fasta-So50b.fasta-So53b.fasta-So54b.fasta-So55b.fasta-So56b.fasta-So57b.fasta-So58b.fasta-So59b.fasta-So60b.fasta-So61b.fasta-So62.fasta-So63.fasta-So66.fasta-So67.fasta-So68.fasta-So69.fasta-So70.fasta-So71.fasta-So72.fasta-So73.fasta-So74.fasta-So75.fasta-So76.fasta-So516477.fasta-So122538.fasta, output=AllSamples.fasta)

summary.seqs(fasta=AllSamples.fasta)

Create the corresponding count file to save up memory:

unique.seqs(fasta=AllSamples.fasta) #Avec name file

unique.seqs(fasta=AllSamples.fasta, format=count) @Avec count file

Décider si un count file vaut mieux que un name file !!

Output File Names:

AllSamples.count_table

AllSamples.unique.fasta

Create a taxonomy file (names file):

classify.seqs(fasta=AllSamples.unique.fasta, group=AllSamples.groups, name=AllSamples.names, template=gg_13_8_99.fasta, taxonomy=gg_13_8_99.gg.tax, cutoff=80, probs=F, processors=30)

Output File Names:

AllSamples.gg.wang.taxonomy

AllSamples.gg.wang.tax.summary

AllSamples.gg.wang.flip.accnos

###

STOP OPTION: taxonomic assignment for figures and files for statistic using R

###

8.4 Appendix 4: R code for ANOVA tests

```
################################################################
# ANOVA
#
# Sophie Caquot & Maike Krauss, FiBL, JUNE 2022
################################################################

# Select your favourite working folder. It must contain all data files and libraries
#setwd("C:/Users/pierre/Desktop/R-Statistics")
setwd("C:/Users/sophie.caquot/OneDrive - Forschungsinstitut für biologischen Landbau FiBL/Documents/R")
#setwd("C:/Users/maike.krauss/Desktop/Caquot")

# Effacer tous les objets en m?moire (global environment)
rm(list=ls())
# Effacer les commandes ant?rieures dans la console
cat("\014")

# Load the required packages (after installation)
library(nlme)
library("multcomp")
library("ggplot2")
library("reshape2")
library("lsmeans")
source("C:/Users/sophie.caquot/OneDrive - Forschungsinstitut für biologischen Landbau FiBL/Documents/R/Summary-SE.R") #
function summarySE()
source("C:/Users/sophie.caquot/OneDrive - Forschungsinstitut für biologischen Landbau FiBL/Documents/R/function_Maike.R") #
function summarySE()

# Load dataset
data = read.csv2("env_tot.csv",dec=",")
data$TrialTillage=paste0(data$Trial, data$Tillage)
data[,c(1:4,33)] <- lapply(data[,c(1:4,33)], as.factor) # Transformation zu Faktoren (wichtig f?r ANOVA)
# log transformation
#data2=cbind(data,log(data[,c(8,12,14,16:18)]),sqrt(data[,c(8,12,14,16:18)]))

layer=c("A","B","C")
var=colnames(data)
# data selection
untreated=c(5:7,9:11,13,19:28)
loga=c(8,12)
sroot=c(14:18)
earth=c(29:32)
```

```
#############################################################################################################
#
# ANOVA mit stetigen Daten
# ANOVA create output file
summary=as.data.frame(matrix(NA,nrow=1,ncol=17))
colnames(summary)=c("Variable","Trial","Tillage","Depth","N","mean","median","sd","se","ci","tukey",
                    "Fixed","DF","SumSq","MeanSq","F-value","p-value")
j <- 1

# untreated Variables
for (i in 1:17) { # Variable loop
  df=data[,c(1:4,33,untreated[i])]
  colnames(df)=c("ID","Trial","Tillage","Depth","TrialTillage","var")

  for(m in 1:3){ #Loop soil layers
    #### ANOVA ######################
    df2=df[which(df$Depth == layer[m]),]

  tab=summarySE(df2, measurevar="var", groupvars=c("Trial","Tillage","Depth"),na.rm=TRUE) #Mittelwerte etc. berechnen
  tab[,c(1:3)] <- lapply(tab[,c(1:3)], as.character) # Transformation zu Faktoren (wichtig f?r ANOVA)

  M=lm(var ~ Trial*Tillage, data=df2)

  # Ancova-Result
  aov=as.data.frame(anova(M))
  aov$Fixed=row.names(aov)
  aov=aov[,c(6,1:5)]
  aov[5:6,]=NA

  ## Tukey Test
  M1=lm(var ~ TrialTillage, data=df2)
  tuk=cld(glht(M1, linfct=mcp(TrialTillage="Tukey")))   # Tukey-test, letter-based display
  tab$tukey=as.data.frame(tuk$mcletters$Letters)[[1]] # Umwandlung in Tabelle

  n <- length(tab[,1]) # Anzahl Zeilen in tab
  k <- j+n-1           # Zeilen in summary Ende

  # Summarytabelle zusammenf?hren
  summary[j:k,1] <- var[untreated[i]]
  summary[j:k,2:11] <- tab
  summary[j:k,12:18] <- aov
  j <- j+n           # Zeilen in summary Start
```

```
  ### Bilderoutput  ################################################
  E <- residuals(M)
  Fit <- fitted(M)
  plot(E~Fit)
  title(paste0(var[untreated[i]],"-Depth:",layer[m],": NV =", signif(shapiro.test(E)$p.value,2)))
  dev.copy(pdf,paste0("Diagnostic-Development",var[i],".pdf"))
  dev.off()
  } #end loop soil layer
} # Ende Variablen Schleife

#############################################################################################################
############
# log transformed variables
# untreated Variables
for (i in 1:2) { # Variable loop
  df=data[,c(1:4,33,loga[i])]
  colnames(df)=c("ID","Trial","Tillage","Depth","TrialTillage","var")

  for(m in 1:3){ #Loop soil layers
    #### ANOVA ######################
    df2=df[which(df$Depth == layer[m]),]

    tab=summarySE(df2, measurevar="var", groupvars=c("Trial","Tillage","Depth"),na.rm=TRUE) #Mittelwerte etc. berechnen
    tab[,c(1:3)] <- lapply(tab[,c(1:3)], as.character) # Transformation zu Faktoren (wichtig f?r ANOVA)

    M=lm(log(var) ~ Trial*Tillage, data=df2)

    # Ancova-Result
    aov=as.data.frame(anova(M))
    aov$Fixed=row.names(aov)
    aov=aov[,c(6,1:5)]
    aov[5:6,]=NA

    ## Tukey Test
    M1=lm(log(var) ~ TrialTillage, data=df2)
    tuk=cld(glht(M1, linfct=mcp(TrialTillage="Tukey")))   # Tukey-test, letter-based display
    tab$tukey=as.data.frame(tuk$mcletters$Letters)[[1]] # Umwandlung in Tabelle

    n <- length(tab[,1]) # Anzahl Zeilen in tab
    k <- j+n-1           # Zeilen in summary Ende

    # Summarytabelle zusammenf?hren
    summary[j:k,1] <- var[loga[i]]
    summary[j:k,2:11] <- tab
```

```
   summary[j:k,12:18] <- aov
   j <- j+n          # Zeilen in summary Start

   ### Bilderoutput  ################################################
   E <- residuals(M)
   Fit <- fitted(M)
   plot(E~Fit)
   title(paste0("Log transformed: ",var[loga[i]],"-Depth:",layer[m],": NV =", signif(shapiro.test(E)$p.value,2)))
   dev.copy(pdf,paste0("Diagnostic-Development",var[i],".pdf"))
   dev.off()
  } #end loop soil layer
} # Ende Variablen Schleife

#########################################################################################################
############
# sqrt transformed variables
# untreated Variables
for (i in 1:5) { # Variable loop
  df=data[,c(1:4,33,sroot[i])]
  colnames(df)=c("ID","Trial","Tillage","Depth","TrialTillage","var")

  for(m in 1:3){ #Loop soil layers
   #### ANOVA ######################
   df2=df[which(df$Depth == layer[m]),]

   tab=summarySE(df2, measurevar="var", groupvars=c("Trial","Tillage","Depth"),na.rm=TRUE) #Mittelwerte etc. berechnen
   tab[,c(1:3)] <- lapply(tab[,c(1:3)], as.character) # Transformation zu Faktoren (wichtig f?r ANOVA)

   M=lm(sqrt(var) ~ Trial*Tillage, data=df2)

   # Ancova-Result
   aov=as.data.frame(anova(M))
   aov$Fixed=row.names(aov)
   aov=aov[,c(6,1:5)]
   aov[5:6,]=NA

   ## Tukey Test
   M1=lm(sqrt(var) ~ TrialTillage, data=df2)
   tuk=cld(glht(M1, linfct=mcp(TrialTillage="Tukey")))   # Tukey-test, letter-based display
   tab$tukey=as.data.frame(tuk$mcletters$Letters)[[1]] # Umwandlung in Tabelle

   n <- length(tab[,1]) # Anzahl Zeilen in tab
   k <- j+n-1          # Zeilen in summary Ende
```

```
   # Summarytabelle zusammenf?hren
   summary[j:k,1] <- var[sroot[i]]
   summary[j:k,2:11] <- tab
   summary[j:k,12:18] <- aov
   j <- j+n          # Zeilen in summary Start

   ### Bilderoutput  ################################################
   E <- residuals(M)
   Fit <- fitted(M)
   plot(E~Fit)
   title(paste0("Sqrt transformed: ",var[sroot[i]],"-Depth:",layer[m],": NV =", signif(shapiro.test(E)$p.value,2)))
   dev.copy(pdf,paste0("Diagnostic-Development",var[i],".pdf"))
   dev.off()
  } #end loop soil layer
} # Ende Variablen Schleife

#########################################################################################################
############
# Earthworm data

for (i in 1:4) { # Variable loop
  df=data[,c(1:4,33,earth[i])]
  colnames(df)=c("ID","Trial","Tillage","Depth","TrialTillage","var")
  df2=df[complete.cases(df),]  # exclude NA

   tab=summarySE(df2, measurevar="var", groupvars=c("Trial","Tillage","Depth"),na.rm=TRUE) #Mittelwerte etc. berechnen
   tab[,c(1:3)] <- lapply(tab[,c(1:3)], as.character) # Transformation zu Faktoren (wichtig f?r ANOVA)

   M=lm(var ~ Trial*Tillage, data=df2)

   # Ancova-Result
   aov=as.data.frame(anova(M))
   aov$Fixed=row.names(aov)
   aov=aov[,c(6,1:5)]
   aov[5:6,]=NA

   ## Tukey Test
   M1=lm(var ~ TrialTillage, data=df2)
   tuk=cld(glht(M1, linfct=mcp(TrialTillage="Tukey")))   # Tukey-test, letter-based display
   tab$tukey=as.data.frame(tuk$mcletters$Letters)[[1]] # Umwandlung in Tabelle

   n <- length(tab[,1]) # Anzahl Zeilen in tab
   k <- j+n-1          # Zeilen in summary Ende
```

```
  # Summarytabelle zusammenf?hren
  summary[j:k,1] <- var[earth[i]]
  summary[j:k,2:11] <- tab
  summary[j:k,12:18] <- aov
  j <- j+n          # Zeilen in summary Start

  ### Bilderoutput  ################################################
  E <- residuals(M)
  Fit <- fitted(M)
  plot(E~Fit)
  title(paste0(var[earth[i]],": NV =", signif(shapiro.test(E)$p.value,2)))
  dev.copy(pdf,paste0("Diagnostic-Development",var[i],".pdf"))
  dev.off()
} # Ende Variablen Schleife

#########################################################################################################
####################
# write table
write.table(summary,"220809_Stats_Sophie.csv", sep=";",row.names=F, col.names=T, append=F)
```

8.5 Appendix 5: R code for plotting boxplots

```
# Sophie Caquot, EPFL, JUNE 2022

#################################################################

# Select your favourite working folder. It must contain all data files and libraries

#setwd("C:/Users/pierre/Desktop/R-Statistics")

setwd("C:/Users/sophie.caquot/OneDrive - Forschungsinstitut für biologischen Landbau FiBL/Documents/R")


# Effacer tous les objets en mémoire (global environment)

rm(list=ls())

# Effacer les commandes antérieures dans la console

cat("\014")


# Load the required packages (after installation)

# vegan must be loaded after ade4 to avoid some conflicts

library(ade4)

library(vegan)

library(FactoMineR)

library("factoextra")

library(cluster)

library(gclus)

library(Hmisc)

library(corrplot)

library(gplots)

library(ggplot2)

library(ggpubr)


# Additional functions required for the following operations

source("coldiss.R")

source("panelutils.R")

source("evplot.R")

source("rquery_cormat.r")

# Function to compute a binary distance matrix from groups

source("grpdist.R")

# Function to draw ordered dendrograms with groups

source("hcoplot.R")
```

```
#################################################################
###### 1. Import data sets
# Environmental dataset (total)
env = read.csv2("env_tot_plot.csv", dec=",")
dim(env)
head(env)

env_10 <- subset(env, Depth == "A")
env_20 <- subset(env, Depth == "B")
env_30 <- subset(env, Depth == "C")
env_Aesch <- subset(env, Trial == "Aesch")
env_Aesch_10 <- subset(env_Aesch, Depth == "A")
env_Aesch_20 <- subset(env_Aesch, Depth == "B")
env_Aesch_30 <- subset(env_Aesch, Depth == "C")
env_Frick <- subset(env, Trial == "Frick")
env_Frick_10 <- subset(env_Frick, Depth == "A")
env_Frick_20 <- subset(env_Frick, Depth == "B")
env_Frick_30 <- subset(env_Frick, Depth == "C")
#################################################################
####### 2. Boxplot with tillage differentation
#BD, can be used for any other parameter
graph_10 <- ggplot(env_10, aes(x=Tillage, y=BD, fill=Trial)) +
 geom_boxplot(outlier.shape = NA) +
 scale_fill_manual(values=c("Aesch"="white", "Frick"="grey")) +
 scale_x_discrete(limits = c("CT", "RT", "G")) +
 theme_bw() +
 labs(title="0-10 cm") +
 ylab("Bulk density [g/cm3]") +
 ylim(min(env$BD,na.rm=TRUE),max(env$BD,na.rm=TRUE))+
 theme(text = element_text(size = 12))
graph_20 <- ggplot(env_20, aes(x=Tillage, y=BD, fill=Trial)) +
 geom_boxplot(outlier.shape = NA) +
 scale_fill_manual(values=c("Aesch"="white", "Frick"="grey")) +
 scale_x_discrete(limits = c("CT", "RT", "G")) +
 theme_bw() +
 labs(title="10-20 cm") +
```

```
  ylab("Bulk Density [g/cm3]") +
  ylim(min(env$BD,na.rm=TRUE),max(env$BD,na.rm=TRUE))+
  theme(text = element_text(size = 12))
graph_30 <- ggplot(env_30, aes(x=Tillage, y=BD, fill=Trial)) +
  geom_boxplot(outlier.shape = NA) +
  scale_fill_manual(values=c("Aesch"="white", "Frick"="grey")) +
  scale_x_discrete(limits = c("CT", "RT", "G")) +
  theme_bw() +
  labs(title="20-30 cm")+
  ylab("Bulk Density [g/cm3]") +
  ylim(min(env$BD,na.rm=TRUE),max(env$BD,na.rm=TRUE))+
  theme(text = element_text(size = 12))
windows()
ggarrange(graph_10, graph_20, graph_30, ncol = 1, nrow = 3)

##Fungi in Aesch, can be used for Fungi in Frick
graph_10 <- ggplot(env_Aesch_10, aes(x=Tillage, y=Fungi)) +
  geom_boxplot(outlier.shape = NA) +
  scale_fill_manual(values=c("Aesch"="white", "Frick"="grey")) +
  scale_x_discrete(limits = c("CT", "RT", "G")) +
  theme_bw() +
  theme(text = element_text(size = 15))+
  labs(title="0-10 cm") +
  ylab("Fungi [copies/g dry soil]") +
  ylim(min(env_Aesch$Fungi,na.rm=TRUE),max(env_Aesch$Fungi,na.rm=TRUE))
graph_20 <- ggplot(env_Aesch_20, aes(x=Tillage, y=Fungi)) +
  geom_boxplot(outlier.shape = NA) +
  scale_fill_manual(values=c("Aesch"="white", "Frick"="grey")) +
  scale_x_discrete(limits = c("CT", "RT", "G")) +
  theme_bw() +
  theme(text = element_text(size = 15))+
  labs(title="10-20 cm") +
  ylab("Fungi [copies/g dry soil]") +
  ylim(min(env_Aesch$Fungi,na.rm=TRUE),max(env_Aesch$Fungi,na.rm=TRUE))
graph_30 <- ggplot(env_Aesch_30, aes(x=Tillage, y=Fungi)) +
  geom_boxplot(outlier.shape = NA) +
```

```
  scale_fill_manual(values=c("Aesch"="white", "Frick"="grey")) +
  scale_x_discrete(limits = c("CT", "RT", "G")) +
  theme_bw() +
  theme(text = element_text(size = 15))+
  labs(title="20-30 cm")+
  ylab("Fungi [copies/g dry soil]") +
  ylim(min(env_Aesch$Fungi,na.rm=TRUE),max(env_Aesch$Fungi,na.rm=TRUE))
windows()
ggarrange(graph_10, graph_20, graph_30, ncol = 1, nrow = 3)

##Eartworm number, can be used for earthworm biomass
plot_adults <- ggplot(env, aes(x=Tillage, y=N_ad, fill=Trial)) +
  geom_boxplot(outlier.shape = NA) +
  scale_fill_manual(values=c("Aesch"="white", "Frick"="grey")) +
  scale_x_discrete(limits = c("CT", "RT", "G")) +
  theme_bw() +
  theme(text = element_text(size = 30))+
  ylab("Number of adult earthworms") +
  ylim(0,60)+
  labs(title="Adults")
plot_juveniles <- ggplot(env, aes(x=Tillage, y=N_juv, fill=Trial)) +
  geom_boxplot(outlier.shape = NA) +
  scale_fill_manual(values=c("Aesch"="white", "Frick"="grey")) +
  scale_x_discrete(limits = c("CT", "RT", "G")) +
  theme_bw() +
  theme(text = element_text(size = 30))+
  ylab("Number of juveniles earthworms")  +
  ylim(0,60) +
  labs(title="Juveniles")
windows()
ggarrange(plot_adults,plot_juveniles, ncol = 2, nrow = 1)
```

8.6 Appendix 6: R code for statistical analysis

```
################################################################
# Analysis of spe/env data sets in environmental microbiology
#
# Modified from: D. Borcard & F. Gillet
# Multivariate Analysis in Community Ecology: Constrained ordination and other analysis
#
# For Sophie Caquot - EPFL/FiBL
#
# Pierre Rossi, CEL-EPFL, MAY 2022
################################################################

## Script to compute Mantel tests
# for evaluation of the spe and env data derived from agricultural soils

# Select your favourite working folder. It must contain all data files and libraries
setwd("C:/Users/sophie.caquot/OneDrive - Forschungsinstitut für biologischen Landbau FiBL/Documents/R")

# Effacer tous les objets en m?moire (global environment)
rm(list=ls())
# Effacer les commandes ant?rieures dans la console
cat("\014")

# Load the required packages (after installation)
# vegan must be loaded after ade4 to avoid some conflicts
library(ade4)
library(vegan)
library(FactoMineR)
library("factoextra")
library(cluster)
library(gclus)
library(Hmisc)
library(corrplot)
library(gplots)

# Additional functions required for the following operations
```

```
source("coldiss.R")
source("panelutils.R")
source("evplot.R")
source("rquery_cormat.r")
# Function to compute a binary distance matrix from groups
source("grpdist.R")
# Function to draw ordered dendrograms with groups
source("hcoplot.R")

#################################################################
# 1. Import data sets
#################################################################

# Bacterial community structures dataset (Illumina)
spe <- read.csv2("So-spe-genus.csv", dec=",", row.names=1)
dim(spe)
summary(spe)
head(spe)

# Si besoin, charger des taxon sp?cifiques
Comma <- data.frame(spe[,6])
# On peut aussi faire comme ceci:
# Comma <- data.frame(spe["Comamonadaceae"])
dim(Comma)

# Bacterial community structures selected (Illumina)
spe <- read.csv2("spe_select.csv", dec=".", row.names=1)
dim(spe)
summary(spe)
head(spe)

# Environmental dataset (total)
env = read.csv2("env_quant_tot_aesch.csv", dec=",", row.names=1)
env = read.csv2("env_quant.csv", dec=",", row.names=1)
dim(env)
head(env)
```

```
summary(env)



# Environmental dataset (nominatives)
env_nom = read.csv2("env_nom.csv", row.names=1, colClasses = "factor")
dim(env_nom)
head(env_nom)
summary(env_nom)
?daisy

# Environmental dataset (quantitatives)
env_quant = read.csv2("env_quant_tot_frick_30.csv", dec=",", row.names=1)
dim(env_quant)
head(env_quant)

# Environmental data splits in quantitative data sets for Mantel
# Une ligne de code pour chaque variable est n?cessaire.

# Dans ce cas, un fichier par variable est cr?e et charg?
NH4PO4_in = read.csv2("NH4PO4_in.csv", dec=".", row.names=1)
dim(NH4PO4_in)

# Ici, la variable est reprise de la matrice env g?n?rale:
sCODNH4_in <- data.frame(env["sCODNH4_in"])
dim(sCODNH4_in)

# If necessary, remove sites or explanatory variables
# env <- env[, -c(13)] # Enl?ve une colonne
# env <- env[, -c(5:7)] # Enl?ve plusieurs colonnes
# spe <- spe[-33,]  # Enl?ve une ligne
# env <- env[-33,]  # Enl?ve une ligne

# If necessary, set aside a variable
# var <- env[,1]
# Remove this variable from the env matrix
# env2 <- env[, -c(33)]
```

```
# Check data
# Il ne doit y a voir que des "TRUE"
row.names(spe)%in%row.names(env)
row.names(env)%in%row.names(spe)

#################################################################
# 2. Clustering techniques on environmental data
#################################################################

env.z = decostand(env_quant, "standardize")
env.de = vegdist(env.z, "euc")

# Ward algorithm
env.dw = hclust(env.de, "ward")             # Minimum variance clustering
# Complete linkage method
env.dc = hclust(env.de, "complete")

# Plot dendrograms of clustering
windows(12,8)
plot(env.dw, main="Minimum variance clustering using the Ward method", xlab="Clustered samples", sub="")

# Fusion levels
windows(8,8)
par(mfrow=c(1,1))
plot(env.dw$height, nrow(env_quant):2, type="S", main="Ward/Euclidean",
    ylab="k (number of clusters)", xlab="h (node height)", col="grey")
text(env.dw$height, nrow(env_quant):2, nrow(env_quant):2, col="red", cex=0.6)

#################################################################
# 3. PCA on environmental data
#################################################################

# S?lection de variable explicatives
# Pour Quelle
# env.select <- env[,c(1,2,25,13,14,11,8,12,18,17)]
```

```
# Pour Forder
# env.s <- env2[,c(13,19,1,12,17,16,21,20,3,23)]
# env2<-env.s

env.pca <- rda(env_quant, scale=TRUE)
# TRUE calls for a standardization of the variables

env.pca
summary(env.pca)                                    # Default scaling 2
summary(env.pca, scaling=1)

# Eigenvalues
windows()
ev <- env.pca$CA$eig
evplot(ev)

# Percentage of variance for each axis
perax <-100*ev/sum(ev)
perax
write.table(perax, file = "PercentageAxes.csv", sep = ";", col.names = NA)

# Apply Kaiser-Guttman criterion to select axes
ev[ev > mean(ev)]

# plot PCA using biplot.rda
windows(24,12)
par(mfrow=c(1,2))
biplot(env.pca, scaling=1, main="PCA - Scaling 1")
biplot(env.pca, scaling=2, main="PCA - Scaling 2")

# Notes:
# Scaling 1: The angles among descriptors are meaningless !
# Variables that have vectors longer than the radius of the circle make a higher contribution than average and can be interpretd with confidence
# Scaling 2: Correlation biplot - the angles between descriptors in the biplot reflect their correlations
# Borcard et al Numerical Ecology with R, page 120-...
```

```
#############################################################

# 4. Correlations between environmental data

#############################################################


# Analyse de la colin?arit? des variables

windows()

heatmap(abs(cor(env_quant)), # corr?lation de Pearson (note: ce sont des valeurs absolues!)

        col = rev(heat.colors(6)),

        Colv = NA, Rowv = NA)

legend("topright",

       title = "R de Pearson",

       legend =  round(seq(0,1, length.out = 6),1),

       y.intersp = 0.7, bty = "n",

       fill = rev(heat.colors(6)))


# Les statistiques du rho de Spearman sont utilis?es pour estimer le coefficient de corr?lation bas? sur le rang. Ce sont des tests statistiques dits robustes car ils ne d?pendent pas de la distribution des donn?es. Le test de corr?lation de Spearman est recommand? lorsque les variables ne suivent pas une loi normale.


env.spearman <- cor(env_quant, method="spearman")

round(env.spearman, 2)


# Reorder the variables before plotting

# Source "panelutils.R if not done already


env.o <- order.single(env.spearman)

op <- par(mfrow=c(1,1), pty="s")

windows()

pairs(env_quant[,env.o], lower.panel=panel.smooth, upper.panel=panel.cor, diag.panel=panel.hist, main="Spearman Correlation Matrix")


corr.spearman <- symnum(env.spearman, abbr.colnames=FALSE)

corr.spearman

write.table(corr.spearman, file = "Correlation-Spearman.csv", sep = ";", col.names = NA)


rho.spearman <- rcorr(as.matrix(env_quant), type=c("spearman"))

rho.spearman

write.table(rho.spearman, file = "Rho-Spearman.csv", sep = ";", col.names = NA)
```

```
windows()

corrplot(env.spearman, type="upper", order="hclust", tl.col="black", tl.srt=45)

windows()

corrplot(env.spearman, type="upper", order="hclust", method="number", tl.col="black", tl.srt=45, p.mat = rho.spearman,
sig.level = 0.01)

p.env <- cor.mtest(env_quant, conf.level = 0.95)


windows()

corrplot(env.spearman, type="upper", tl.col="black", method = "color",

p.mat = p.env$p, sig.level = c(0.001, 0.01, 0.05), insig = 'label_sig',  pch.cex = 0.9,  tl.srt= 45,  pch.col = 'yellow')


# Les corr?lations positives sont affich?es en bleu et les corr?lations n?gatives en rouge. L?intensit? de la couleur et la taille des
cercles sont proportionnelles aux coefficients de corr?lation. A droite du corr?logramme, la l?gende de couleurs montre les
coefficients de corr?lation et les couleurs correspondantes




#################################################################

# 5. Tests de Mantel

# Corr?lations entre matrice spe et variables

#################################################################


spe <- read.csv2("So-spe-vdt-phylum.csv", dec=",", row.names=1)

dim(spe)

head(spe)


# Transform the species dataset, indispensable pour travailler avec la matrice esp?ce

spe.hell = decostand(spe, "hellinger")


# Compute a Hellinger dissimilarity matrix from the transformed species data

spe.dh = vegdist(spe.hell, "euclidean")


# Environmental dataset (total)

env_quant = read.csv2("So-env-vdt.csv", dec=",", row.names=1)

dim(env_quant)

head(env_quant)

summary(env_quant)
```

```
# Compute a Gower's dissimilarity matrix from ALL kind of environmental data types

# require(cluster) if not done already

env_quant.gower <- daisy(env_quant, metric = c("gower"))


# Pour un test avec une seule variable env, chacune doit ?tre transform?e individuellement

# NH4PO4_in.gower <- daisy(NH4PO4_in, metric = c("gower"))


# Mantel tests

# Test unique entre toutes les variables env et toutes les esp?ces:

mantel(spe.dh, env_quant.gower)


# Test entre toutes les esp?ces et une seule variable (exemple)

mantel(spe.d, NH4PO4_in.gower)


# Les test identiques sont men?s comme ci-dessus

# L'hypoth?se nulle est l'absence d'une corr?lation, en cas de R ?lev? et de p bas, on doit rejeter l'hypoth?se et accepter la pr?sence d'une corr?lation


# ajout d'une 3e matrice pour enlever son effet sp?cifique

mantel.partial(spe.d,env_nom.gower,env_quant.gower)


# ****************************************

# Analyses de variance (ANOVA) et test de Tuckey entre bact?ries/fungi totales et donn?es nominales


spe <- read.csv2("spe_q_aesch.csv", dec=",", row.names=1)

dim(spe)


env_nom = read.csv2("env_nom_aesch.csv", row.names=1, colClasses = "factor")

dim(env_nom)


# Type de cultures et bact?ries

windows()

boxplot(spe$Bact ~ env_nom$Type, xlab="Type",ylab="Bacteria")

lm.Type <- aov(spe$Bact~as.factor(env_nom$Type))

summary(lm.Type)

TukeyHSD(lm.Type, "as.factor(env_nom$Type)")

windows()
```

```
plot(TukeyHSD(lm.Type, "as.factor(env_nom$Type)"))

# Type de cultures et Fungi
windows()
boxplot(spe$Fungi ~ env_nom$Type, xlab="Type",ylab="Fungi")
lm.Type <- aov(spe$Fungi~as.factor(env_nom$Type))
summary(lm.Type)
TukeyHSD(lm.Type, "as.factor(env_nom$Type)")
windows()
plot(TukeyHSD(lm.Type, "as.factor(env_nom$Type)"))

#################################################################
# 6. Heatmap
# Corr?lations entre matrice spe et variables
#################################################################

# Transform the species dataset
spe.hell = decostand(spe, "hellinger")
env.z <- as.data.frame(scale(env_quant))

### Pairwise correlation between spe and env
pwpc <- cor(spe.hell, env.z, use = "everything", method = c("spearman"))
windows(12,6)
heatmap.2(pwpc, col=bluered, trace="none")
windows(10,6)
heatmap.2(pwpc, col=rev(heat.colors(8)), trace="none")


#################################################################
# 7. Analyse de la matrice spe
# Principal Component Analysis - PCA
#################################################################

# Import datasets
spe = read.csv2("So-spe-phylum.csv", dec=".", row.names=1)
dim(spe)
```

```
head(spe)

# Transform the species dataset
spe.hell = decostand(spe, "hellinger")

# Compute dissimilarity and distance matrices (Q mode)



# On the Hellinger distance matrix, compute the Ward clustering method
spe.dh = vegdist(spe.hell, "euclidean")
spe.hw = hclust(spe.dh, "ward")              # Minimum variance clustering

# Plot dendrograms of Hellinger distance based clusterings
windows(8,6)
plot(spe.hw, main="Ward method", xlab="", sub="")

# Fusion levels
# Hellinger distance based clustering
windows(8,8)
plot(spe.hw$height, nrow(spe):2, type="S", main="Ward/Hellinger",
    ylab="k (number of clusters)", xlab="h (node height)", col="grey")
text(spe.hw$height, nrow(spe):2, nrow(spe):2, col="red", cex=0.6)

# Average silhouette width (Rousseeuw internal quality index)
windows(16,8)
par(mfrow=c(1,2))

# Hellinger/Ward
Si = numeric(nrow(spe))
for (k in 2:(nrow(spe)-1)) {
       sil = silhouette(cutree(spe.hw, k=k), spe.dh)
       Si[k] = summary(sil)$avg.width
}
k.best = which.max(Si)
plot(1:nrow(spe), Si, type="h", main="Silhouette-optimal number of clusters - Hellinger/Ward",
    xlab="k (number of groups)", ylab="Average silhouette width")
```

```
axis(1, k.best, paste("optimum",k.best,sep="\n"), col="red", col.axis="red")

# Spearman's rank correlations
# Ward/Hellinger
kt = data.frame(k=1:nrow(spe), r=0)
for (i in 2:(nrow(spe)-1)) {
        gr = cutree(spe.hw, i)
        distgr = grpdist(gr)
        kt$r[i] = cor(spe.dh, distgr, method="spearman")
}
kt
k.best = which.max(kt$r)
plot(kt$k, kt$r, type="h", main="Spearman-optimal number of clusters - Ward/Hellinger",
    xlab="k (number of groups)", ylab="Spearman's rank correlation")
axis(1, k.best, paste("optimum",k.best,sep="\n"), col="red",
    col.axis="red")

k = 4 #set here manually the optimal group number !

# Silhouette plot of Ward/Hellinger
spehw.g = cutree(spe.hw, k)
sil = silhouette(spehw.g, spe.dh)
silo = sortSilhouette(sil)
rownames(silo) = row.names(spe)[attr(silo,"iOrd")]
windows()
plot(silo, main="Silhouette plot - Ward/Hellinger", cex.names=0.5,
    col=spehw.g+1, nmax.lab=100)

# Dendrogram with rectangles delimiting the X clusters
# Ward/Hellinger
# Re-order the dendrogram
windows(30,20)
spe.hwo = reorder.hclust(spe.hw, spe.dh)
plot(spe.hwo, hang=-1, xlab="83 samples", sub="8 groups",
    ylab="Height (Hellinger distance)",
    main="Minimum variance clustering (reordered)")
```

```
so = spehw.g[spe.hwo$order]
gro = numeric(k)
for (i in 1:k) {
       gro[i] = so[1]
       if (i<k) so = so[so!=gro[i]]
}
rect.hclust(spe.hwo, k=k, border=gro+1, cluster=spehw.g)
legend("topright", paste("Group", 1:k),
      pch=22, col=2:(k+1), bty="n")
hcoplot(spe.hw, spe.dh, k)

# PCA on covariance matrix (Hellinger distances), with k = X groups
k = 6
spe.pca = rda(spe.hell)
spe.pca
windows()
plot (spe.pca)

# With clustering
gr = cutree(spe.hw, k)

# Plot the sites with cluster symbols
k = length(levels(factor(gr)))
sit.sc = scores(spe.pca, choices = c(1,2), display="wa", scaling=1)
windows()
pl = plot(spe.pca, display="sites", type="n", scaling=1,
         main="PCA cov/Hell + clusters Ward/Hellinger")

# Plot the points with different symbols and colors
points(sit.sc, cex=2, col=1+c(1:k)[gr], pch=14+c(1:k)[gr])
text(sit.sc, rownames(spe), pos=4, cex=.7)

# Add clustering dendrogram if required
ordicluster(pl, spe.hw, col="dark grey")

# Add a legend for groups
```

```
# (click at the place on the plot you wish to draw the legend)
legend(locator(1), paste("Group",c(1:k)), pch=14+c(1:k), col=1+c(1:k), pt.cex=2)



##################################################################
# 8. Species PCA on covariance matrix
# A posteriori interpretation of the species by significative # environmental variables
##################################################################

# Transform the species dataset
spe.hell = decostand(spe, "hellinger")

# PCA on a covariance matrix (default scale=FALSE)
spe.pca = rda(spe.hell)
spe.pca
windows()
plot (spe.pca)
summary(spe.pca)

# Selection of the significant variables
windows(8,8)
par(mfrow=c(1,1))
fit = envfit(spe.pca, env_quant, perm=1000)
fit
plot(spe.pca, type="t", main=paste("PCA/Hellinger"))
plot(fit, axis=T)

# Eigenvalues
ev = spe.pca$CA$eig
ev
# Percentage of variance for each axis
100*ev/sum(ev)
# Apply Kaiser's rule to select axes
ev[ev > mean(ev)]
# Broken stick model (MacArthur 1957)
n = length(ev)
```

```
bsm = data.frame(j=seq(1:n), p=0)
bsm$p[1] = 1/n
for (i in 2:n) {
        bsm$p[i] = bsm$p[i-1] + (1/(n + 1 - i))
}
bsm$p = 100*bsm$p/n
bsm

# Plot eigenvalues and % of variance for each axis
windows()
par(mfrow=c(2,1))
barplot(ev, main="Eigenvalues", col="bisque", las=2)
abline(h=mean(ev), col="red") # average eigenvalue
legend("topright", "Average eigenvalue", lwd=1, col=2, bty="n")
barplot(t(cbind(100*ev/sum(ev),bsm$p[n:1])), beside=T,
        main="% variance", col=c("bisque",2), las=2)
legend("topright", c("% eigenvalue", "Broken stick model"),
       pch=15, col=c("bisque",2), bty="n")



########################################
# 9. Compute diversity indices
########################################

N0 <- rowSums(spe > 0)                          # Species richness S
H <- diversity(spe)                             # Shannon H
N1 <- exp(H)                                    # Shannon diversity number
N2 <- diversity(spe, "inv")                     # Inverse Simpson diversity number
J <- H/log(N0)                                  # Pielou's Evenness
E1 <- N1/N0                                     # Shannon Evenness (Hill's ratio)
E2 <- N2/N0                                     # Simpson Evenness (Hill's ratio)
alpha <- fisher.alpha(spe)                      # Fisher's Alpha diversity index

div <- data.frame(N0, H, N1, N2, E1, E2, J, alpha)
div
write.table(div, file="Sophie_Estimators-I_genus-lvl.csv", sep=";")
```

```
# Accumulation model

estimate <- estimateR(spe)

write.table(estimate, file="Sophie_Estimators_II_genus-lvl.csv", sep=";")


##### END #####
```

8.7 Appendix 7: Table of ANOVA results

Parameter	Bulk density						Soil Dry matter						Soil Water Content						pH					
Depth	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value
0-10 cm	Trial	1	0.00	0.00	0.16	0.696	Trial	1	30.21	30.21	12.13	**0.003**	Trial	1	126.85	126.85	28698.58	**0.000**	Trial	1	0.36	0.36	4.65	**0.045**
	Tillage	2	0.09	0.05	13.98	**0.000**	Tillage	2	27.02	13.51	5.43	**0.015**	Tillage	2	0.04	0.02	5.02	**0.019**	Tillage	2	0.26	0.13	1.65	0.220
	Trial:Tillage	2	0.01	0.01	1.61	0.229	Trial:Tillage	2	15.56	7.78	3.13	0.070	Trial:Tillage	2	0.02	0.01	2.52	0.110	Trial:Tillage	2	0.21	0.10	1.33	0.290
	Residuals	17	0.06	0.00			Residuals	17	42.32	2.49			Residuals	17	0.08	0.00			Residuals	18	1.39	0.08		
10-20 cm	Trial	1	0.02	0.02	6.65	**0.020**	Trial	1	1.05	1.05	0.41	0.532	Trial	1	120.83	120.83	24538.12	**0.000**	Trial	1	0.09	0.09	1.06	0.317
	Tillage	2	0.02	0.01	4.42	**0.028**	Tillage	2	10.15	5.07	1.96	0.171	Tillage	2	0.02	0.01	2.26	0.135	Tillage	2	0.01	0.01	0.08	0.921
	Trial:Tillage	2	0.03	0.02	6.73	**0.007**	Trial:Tillage	2	63.82	31.91	12.34	**0.000**	Trial:Tillage	2	0.14	0.07	14.29	**0.000**	Trial:Tillage	2	0.04	0.02	0.24	0.791
	Residuals	17	0.04	0.00			Residuals	17	43.96	2.59			Residuals	17	0.08	0.00			Residuals	18	1.55	0.09		
20-30 cm	Trial	1	0.03	0.03	5.89	**0.027**	Trial	1	1.62	1.62	0.40	0.536	Trial	1	120.66	120.66	14017.47	**0.000**	Trial	1	6.68	6.68	76.90	**0.000**
	Tillage	2	0.00	0.00	0.17	0.841	Tillage	2	2.14	1.07	0.26	0.772	Tillage	2	0.00	0.00	0.24	0.789	Tillage	2	0.18	0.09	1.02	0.379
	Trial:Tillage	2	0.02	0.01	1.60	0.232	Trial:Tillage	2	30.21	15.11	3.71	**0.046**	Trial:Tillage	2	0.07	0.04	4.23	**0.032**	Trial:Tillage	2	0.08	0.04	0.44	0.651
	Residuals	17	0.08	0.00			Residuals	17	69.20	4.07			Residuals	17	0.15	0.01			Residuals	18	1.56	0.09		
Parameter	**Soil TC**						**SOC**						**SOC stock**						**Soil TN**					
Depth	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value
0-10 cm	Trial	1	8.54	8.54	51.15	**0.000**	Trial	1	2.15	2.15	31.93	**0.000**	Trial	1	428.58	428.58	43.64	**0.000**	Trial	1	0.02	0.02	20.75	**0.000**
	Tillage	2	3.28	1.64	9.83	**0.001**	Tillage	2	4.37	2.19	32.56	**0.000**	Tillage	2	312.57	156.28	15.91	**0.000**	Tillage	2	0.03	0.01	15.76	**0.000**
	Trial:Tillage	2	0.24	0.12	0.72	0.498	Trial:Tillage	2	0.35	0.18	2.61	0.101	Trial:Tillage	2	27.23	13.61	1.39	0.277	Trial:Tillage	2	0.00	0.00	2.54	0.107
	Residuals	18	3.01	0.17			Residuals	18	1.21	0.07			Residuals	17	166.96	9.82			Residuals	18	0.02	0.00		
10-20 cm	Trial	1	8.44	8.44	61.91	**0.000**	Trial	1	1.70	1.70	15.64	**0.001**	Trial	1	410.16	410.16	23.10	**0.000**	Trial	1	0.02	0.02	14.68	**0.001**
	Tillage	2	0.03	0.01	0.10	0.905	Tillage	2	0.00	0.00	0.01	0.993	Tillage	2	9.82	4.91	0.28	0.762	Tillage	2	0.00	0.00	0.53	0.595
	Trial:Tillage	2	0.17	0.08	0.62	0.548	Trial:Tillage	2	0.14	0.07	0.65	0.535	Trial:Tillage	2	11.52	5.76	0.32	0.727	Trial:Tillage	2	0.00	0.00	1.61	0.227
	Residuals	18	2.45	0.14			Residuals	18	1.96	0.11			Residuals	17	301.78	17.75			Residuals	18	0.02	0.00		
20-30 cm	Trial	1	11.42	11.42	64.71	**0.000**	Trial	1	2.65	2.65	34.08	**0.000**	Trial	1	661.55	661.55	57.35	**0.000**	Trial	1	0.03	0.03	30.46	**0.000**
	Tillage	2	0.36	0.18	1.03	0.376	Tillage	2	0.20	0.10	1.27	0.305	Tillage	2	31.49	15.74	1.36	0.282	Tillage	2	0.00	0.00	1.41	0.270
	Trial:Tillage	2	0.11	0.05	0.30	0.742	Trial:Tillage	2	0.16	0.08	1.02	0.379	Trial:Tillage	2	47.08	23.54	2.04	0.161	Trial:Tillage	2	0.00	0.00	0.98	0.394
	Residuals	18	3.18	0.18			Residuals	18	1.40	0.08			Residuals	17	196.09	11.53			Residuals	18	0.02	0.00		
Parameter	**SOC/N**						**Soil Cmic**						**Soil Nmic**						**Soil Cmic/Nmic**					
Depth	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value
0-10 cm	Trial	1	0.00	0.00	3.20	0.090	Trial	1	413.32	413.32	54.36	**0.000**	Trial	1	15.85	15.85	12.47	**0.002**	Trial	1	17.61	17.61	130.46	**0.000**
	Tillage	2	0.03	0.01	15.66	**0.000**	Tillage	2	193.32	96.66	12.71	**0.000**	Tillage	2	35.68	17.84	14.03	**0.000**	Tillage	2	0.25	0.13	0.94	0.409
	Trial:Tillage	2	0.00	0.00	0.97	0.397	Trial:Tillage	2	1.31	0.66	0.09	0.918	Trial:Tillage	2	4.20	2.10	1.65	0.219	Trial:Tillage	2	3.24	1.62	12.02	**0.000**
	Residuals	18	0.02	0.00			Residuals	18	136.86	7.60			Residuals	18	22.89	1.27			Residuals	18	2.43	0.13		
10-20 cm	Trial	1	0.00	0.00	1.75	0.202	Trial	1	686.92	686.92	46.44	**0.000**	Trial	1	54.96	54.96	18.82	**0.000**	Trial	1	12.91	12.91	57.67	**0.000**
	Tillage	2	0.02	0.01	6.69	**0.007**	Tillage	2	71.28	35.64	2.41	0.118	Tillage	2	10.90	5.45	1.87	0.183	Tillage	2	0.09	0.04	0.19	0.827
	Trial:Tillage	2	0.02	0.01	5.34	**0.015**	Trial:Tillage	2	41.86	20.93	1.42	0.269	Trial:Tillage	2	1.86	0.93	0.32	0.731	Trial:Tillage	2	2.52	1.26	5.63	**0.013**
	Residuals	18	0.03	0.00			Residuals	18	266.24	14.79			Residuals	18	52.57	2.92			Residuals	18	4.03	0.22		
20-30 cm	Trial	1	0.00	0.00	0.17	0.683	Trial	1	288.14	288.14	9.04	**0.008**	Trial	1	24.20	24.20	4.47	**0.050**	Trial	1	8.14	8.14	59.24	**0.000**
	Tillage	2	0.00	0.00	0.05	0.956	Tillage	2	162.95	81.48	2.56	0.107	Tillage	2	21.10	10.55	1.95	0.173	Tillage	2	0.06	0.03	0.22	0.801
	Trial:Tillage	2	0.00	0.00	0.26	0.773	Trial:Tillage	2	119.15	59.57	1.87	0.185	Trial:Tillage	2	12.18	6.09	1.13	0.347	Trial:Tillage	2	0.93	0.46	3.38	0.058
	Residuals	18	0.06	0.00			Residuals	17	541.70	31.86			Residuals	17	91.99	5.41			Residuals	17	2.34	0.14		

Parameter	Total soil DNA						Bacteria						Fungi						P					
Depth	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value
0-10 cm	Trial	1	0.75	0.75	2.25	0.153	Trial	1	1.38E+08	1.38E+08	1.15	0.300	Trial	1	3.69E+10	3.69E+10	796.52	**0.000**	Trial	1	4.60E+02	4.60E+02	0.14	0.711
	Tillage	2	7.29	3.64	10.99	**0.001**	Tillage	2	3.49E+09	1.74E+09	14.50	**0.000**	Tillage	2	5.66E+07	2.83E+07	0.61	0.555	Tillage	2	1.37E+04	6.86E+03	2.11	0.150
	Trial:Tillage	2	1.00	0.50	1.50	0.252	Trial:Tillage	2	2.05E+09	1.03E+09	8.53	**0.003**	Trial:Tillage	2	9.29E+06	4.65E+06	0.10	0.905	Trial:Tillage	2	9.10E+03	4.55E+03	1.40	0.272
	Residuals	16	5.31	0.33			Residuals	16	1.92E+09	1.20E+08			Residuals	16	7.41E+08	4.63E+07			Residuals	18	5.85E+04	3.25E+03		
10-20 cm	Trial	1	0.68	0.68	1.74	0.206	Trial	1	4.01E+08	4.01E+08	2.94	0.106	Trial	1	3.22E+10	3.22E+10	204.88	**0.000**	Trial	1	4.20E+01	4.20E+01	0.02	0.891
	Tillage	2	2.48	1.24	3.16	0.070	Tillage	2	4.64E+08	2.32E+08	1.70	0.214	Tillage	2	6.50E+07	3.25E+07	0.21	0.815	Tillage	2	1.42E+04	7.10E+03	3.25	0.062
	Trial:Tillage	2	0.08	0.04	0.10	0.902	Trial:Tillage	2	8.19E+07	4.09E+07	0.30	0.745	Trial:Tillage	2	1.71E+08	8.53E+07	0.54	0.591	Trial:Tillage	2	5.28E+03	2.64E+03	1.21	0.321
	Residuals	16	6.28	0.39			Residuals	16	2.18E+09	1.37E+08			Residuals	16	2.51E+09	1.57E+08			Residuals	18	3.93E+04	2.18E+03		
20-30 cm	Trial	1	0.42	0.42	1.93	0.184	Trial	1	3.20E+09	3.20E+09	22.33	**0.000**	Trial	1	4.31E+10	4.31E+10	959.40	**0.000**	Trial	1	6.88E+02	6.88E+02	0.71	0.412
	Tillage	2	0.00	0.00	0.01	0.991	Tillage	2	2.13E+08	1.07E+08	0.74	0.491	Tillage	2	1.63E+08	8.16E+07	1.82	0.194	Tillage	2	1.41E+04	7.06E+03	7.24	**0.005**
	Trial:Tillage	2	0.49	0.24	1.12	0.351	Trial:Tillage	2	9.70E+07	4.85E+07	0.34	0.718	Trial:Tillage	2	6.85E+07	3.43E+07	0.76	0.482	Trial:Tillage	2	2.00E+03	9.99E+02	1.02	0.379
	Residuals	16	3.50	0.22			Residuals	16	2.29E+09	1.43E+08			Residuals	16	7.18E+08	4.49E+07			Residuals	18	1.76E+04	9.76E+02		
Parameter	K						Mg						Ca						Cu					
Depth	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value
0-10 cm	Trial	1	497019.09	497019.09	88.88	**0.000**	Trial	1	1.24E+07	1.24E+07	37.52	**0.000**	Trial	1	1.21E+08	1.21E+08	32.17	**0.000**	Trial	1	3.57E+00	3.57E+00	0.61	0.444
	Tillage	2	93634.51	46817.26	8.37	**0.000**	Tillage	2	6.23E+04	3.11E+04	0.09	0.911	Tillage	2	2.12E+06	1.06E+06	0.28	0.757	Tillage	2	6.07E+00	3.04E+00	0.52	0.602
	Trial:Tillage	2	137247.46	68623.73	12.27	**0.000**	Trial:Tillage	2	1.25E+05	6.24E+04	0.19	0.830	Trial:Tillage	2	4.56E+06	2.28E+06	0.61	0.556	Trial:Tillage	2	2.69E+01	1.34E+01	2.31	0.128
	Residuals	18	100652.35	5591.80			Residuals	18	5.95E+06	3.31E+05			Residuals	18	6.76E+07	3.76E+06			Residuals	18	1.05E+02	5.81E+00		
10-20 cm	Trial	1	508845.79	508845.79	133.35	**0.000**	Trial	1	1.46E+07	1.46E+07	32.79	**0.000**	Trial	1	1.81E+08	1.81E+08	21.21	**0.000**	Trial	1	1.00E+00	1.00E+00	0.16	0.695
	Tillage	2	64693.41	32346.71	8.48	**0.000**	Tillage	2	6.21E+04	3.10E+04	0.07	0.933	Tillage	2	7.71E+06	3.85E+06	0.45	0.644	Tillage	2	1.50E+01	7.49E+00	1.19	0.327
	Trial:Tillage	2	120929.11	60464.56	15.85	**0.000**	Trial:Tillage	2	7.76E+04	3.88E+04	0.09	0.917	Trial:Tillage	2	1.88E+07	9.41E+06	1.10	0.354	Trial:Tillage	2	4.76E+01	2.38E+01	3.79	**0.042**
	Residuals	18	68683.28	3815.74			Residuals	18	8.02E+06	4.45E+05			Residuals	18	1.54E+08	8.54E+06			Residuals	18	1.13E+02	6.28E+00		
20-30 cm	Trial	1	381369.32	381369.32	307.63	**0.000**	Trial	1	1.75E+07	1.75E+07	33.89	**0.000**	Trial	1	2.43E+08	2.43E+08	35.37	**0.000**	Trial	1	2.36E+00	2.36E+00	0.74	0.401
	Tillage	2	43003.84	21501.92	17.34	**0.000**	Tillage	2	1.78E+05	8.90E+04	0.17	0.843	Tillage	2	1.13E+07	5.64E+06	0.82	0.456	Tillage	2	1.98E+01	9.88E+00	3.10	0.070
	Trial:Tillage	2	54092.44	27046.22	21.82	**0.000**	Trial:Tillage	2	1.73E+05	8.64E+04	0.17	0.847	Trial:Tillage	2	2.05E+07	1.03E+07	1.49	0.251	Trial:Tillage	2	2.60E+01	1.30E+01	4.08	**0.035**
	Residuals	18	22314.53	1239.70			Residuals	18	9.29E+06	5.16E+05			Residuals	18	1.24E+08	6.88E+06			Residuals	18	5.74E+01	3.19E+00		
Parameter	Fe						Mn						B						Nitrifiers					
Depth	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value
0-10 cm	Trial	1	3.53E+03	3.53E+03	0.26	0.619	Trial	1	2.69E+02	2.69E+02	0.09	0.766	Trial	1	5.36E+00	5.36E+00	77.31	**0.000**	Trial	1	8.93E-02	8.93E-02	8.96	**0.009**
	Tillage	2	2.57E+04	1.29E+04	0.93	0.412	Tillage	2	3.77E+03	1.89E+03	0.64	0.538	Tillage	2	2.71E-01	1.35E-01	1.95	0.171	Tillage	2	8.09E-03	4.05E-03	0.41	0.673
	Trial:Tillage	2	2.16E+05	1.08E+05	7.83	**0.004**	Trial:Tillage	2	3.95E+03	1.97E+03	0.67	0.523	Trial:Tillage	2	1.08E-01	5.42E-02	0.78	0.472	Trial:Tillage	2	1.22E-01	6.08E-02	6.10	**0.011**
	Residuals	18	2.49E+05	1.38E+04			Residuals	18	5.29E+04	2.94E+03			Residuals	18	1.25E+00	6.93E-02			Residuals	16	1.60E-01	9.97E-03		
10-20 cm	Trial	1	4.04E+04	4.04E+04	4.68	**0.044**	Trial	1	1.52E+01	1.52E+01	0.01	0.934	Trial	1	4.67E+00	4.67E+00	95.43	**0.000**	Trial	1	8.31E-01	8.31E-01	33.96	**0.000**
	Tillage	2	4.12E+04	2.06E+04	2.39	0.120	Tillage	2	2.77E+03	1.38E+03	0.63	0.542	Tillage	2	7.28E-01	3.64E-01	7.43	**0.004**	Tillage	2	2.04E-01	1.02E-01	4.17	**0.035**
	Trial:Tillage	2	1.67E+05	8.33E+04	9.64	**0.001**	Trial:Tillage	2	1.80E+03	9.00E+02	0.41	0.669	Trial:Tillage	2	9.47E-02	4.73E-02	0.97	0.399	Trial:Tillage	2	5.15E-01	2.57E-01	10.52	**0.001**
	Residuals	18	1.55E+05	8.63E+03			Residuals	18	3.94E+04	2.19E+03			Residuals	18	8.81E-01	4.90E-02			Residuals	16	3.92E-01	2.45E-02		
20-30 cm	Trial	1	6.62E+04	6.62E+04	11.49	**0.003**	Trial	1	1.06E+03	1.06E+03	0.51	0.486	Trial	1	4.08E+00	4.08E+00	87.32	**0.000**	Trial	1	1.53E+00	1.53E+00	102.33	**0.000**
	Tillage	2	5.68E+04	2.84E+04	4.93	**0.020**	Tillage	2	4.01E+03	2.00E+03	0.96	0.402	Tillage	2	7.97E-01	3.98E-01	8.53	**0.002**	Tillage	2	2.30E-02	1.15E-02	0.77	0.479
	Trial:Tillage	2	3.49E+04	1.74E+04	3.02	0.074	Trial:Tillage	2	4.72E+03	2.36E+03	1.13	0.345	Trial:Tillage	2	1.18E-01	5.92E-02	1.27	0.305	Trial:Tillage	2	5.72E-01	2.86E-01	19.16	**0.000**
	Residuals	18	1.04E+05	5.76E+03			Residuals	18	3.76E+04	2.09E+03			Residuals	18	8.40E-01	4.67E-02			Residuals	16	2.39E-01	1.49E-02		

Parameter	Nb of adults						Biomass of adults						Nb of juveniles						Biomass of juveniles					
Depth	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value	Fixed	DF	SumSq	MeanSq	F-value	p-value
0-10 cm	Trial	1	0.04	0.04	0.00	0.980	Trial	1	328.86	328.86	31.49	**0.000**	Trial	1	1.50	1.50	0.01	0.924	Trial	1	417.33	417.33	10.36	**0.005**
	Tillage	2	315.08	157.54	2.33	0.126	Tillage	2	92.65	46.33	4.44	**0.027**	Tillage	2	2805.25	1402.63	8.71	**0.002**	Tillage	2	98.47	49.24	1.22	0.318
	Trial:Tillage	2	175.58	87.79	1.30	0.297	Trial:Tillage	2	156.51	78.25	7.49	**0.004**	Trial:Tillage	2	484.75	242.38	1.50	0.249	Trial:Tillage	2	107.96	53.98	1.34	0.287
	Residuals	18	1215.25	67.51			Residuals	18	187.98	10.44			Residuals	18	2899.00	161.06			Residuals	18	724.88	40.27		

8.8 Appendix 8: Table of data

Trial	Depth	Tillage	Bulk density [g/cm3]		Soil Dry matter [%]		Soil Water Content [%]		pH		Soil TC [%]		Carbonates [%]		SOC [%]		SOC stock [Mg/ha]		Soil TN [%]		SOC/N [-]	
			mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
Aesch	0-10 cm	CT	1.35	0.02	76.94	1.00	23.06	1.00	6.90	0.30	2.16	0.17	0.03	0.02	2.13	0.15	28.73	1.72	0.22	0.02	9.52	0.23
		RT	1.33	0.03	76.40	0.73	23.60	0.73	6.77	0.29	2.34	0.14	0.02	0.02	2.32	0.15	30.87	2.33	0.24	0.01	9.85	0.22
		G	1.20	0.09	72.78	2.20	27.22	2.20	6.90	0.29	3.07	0.28	0.01	0.00	3.05	0.28	36.66	2.81	0.30	0.03	10.18	0.31
	10-20 cm	CT	1.33	0.04	78.28	1.56	21.72	1.56	7.07	0.31	1.92	0.18	0.02	0.01	1.90	0.18	25.12	1.96	0.20	0.02	9.26	0.08
		RT	1.41	0.01	80.80	0.84	19.20	0.84	6.97	0.21	1.77	0.21	0.02	0.02	1.75	0.22	24.69	2.83	0.19	0.02	9.07	0.18
		G	1.35	0.02	76.95	0.70	23.05	0.70	7.11	0.21	1.90	0.31	0.01	0.00	1.89	0.31	25.50	4.30	0.21	0.03	9.20	0.21
	20-30 cm	CT	1.43	0.02	80.44	1.38	19.56	1.38	6.28	0.25	1.58	0.18	0.03	0.02	1.55	0.16	22.18	2.26	0.17	0.02	9.08	0.20
		RT	1.45	0.04	81.25	0.36	18.75	0.36	6.29	0.43	1.42	0.12	0.02	0.01	1.40	0.13	20.36	1.40	0.15	0.01	9.14	0.19
		G	1.40	0.04	79.14	0.94	20.86	0.94	6.57	0.39	1.30	0.24	0.01	0.00	1.29	0.24	17.98	2.93	0.14	0.03	9.00	0.10
Frick	0-10 cm	CT	1.41	0.09	78.31	2.29	21.69	2.38	7.34	0.20	3.22	0.55	0.77	0.73	2.45	0.21	34.05	1.35	0.25	0.03	9.67	0.47
		RT	1.29	0.05	77.44	1.80	22.73	1.86	7.06	0.29	3.82	0.38	0.59	0.57	3.23	0.30	41.62	3.47	0.33	0.03	9.90	0.22
		G	1.24	0.03	77.42	0.99	22.56	1.33	6.89	0.30	4.11	0.65	0.50	0.41	3.62	0.38	44.85	5.11	0.34	0.04	10.64	0.18
	10-20 cm	CT	1.36	0.06	76.44	2.86	23.12	2.33	7.13	0.47	3.05	0.58	0.74	0.65	2.31	0.15	31.36	1.54	0.25	0.03	9.11	0.65
		RT	1.38	0.08	76.99	2.24	22.73	1.96	7.21	0.27	3.18	0.45	0.68	0.71	2.50	0.36	34.29	3.05	0.28	0.04	8.96	0.40
		G	1.49	0.05	80.85	0.74	19.20	1.06	7.18	0.18	2.91	0.32	0.59	0.52	2.33	0.58	34.48	7.71	0.23	0.06	10.09	0.26
	20-30 cm	CT	1.47	0.14	78.72	4.34	21.19	3.66	7.47	0.26	2.85	0.41	0.83	0.77	2.01	0.49	28.57	5.44	0.22	0.06	9.08	0.27
		RT	1.48	0.07	78.59	2.56	21.19	2.30	7.36	0.15	2.99	0.68	0.73	0.62	2.26	0.23	33.39	3.29	0.25	0.03	9.16	1.15
		G	1.54	0.08	81.67	1.00	18.28	1.12	7.48	0.19	2.60	0.56	0.64	0.56	1.96	0.28	30.18	4.38	0.21	0.02	9.31	0.40

Trial	Depth	Tillage	P [mg/kg]		K [mg/kg]		Mg [mg/kg]		Ca [mg/kg]		Cu [mg/kg]		Fe [mg/kg]		Mn [mg/kg]		B [mg/kg]	
			mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
Aesch	0-10 cm	CT	97.34	37.79	179.29	48.60	88.40	7.73	3167.75	627.27	8.55	1.24	467.63	99.77	402.65	17.09	0.77	0.31
		RT	119.27	14.75	160.89	19.27	106.04	21.04	2711.5	341.75	7.66	1.28	406.20	36.69	359.05	55.06	0.64	0.12
		G	104.33	129.44	193.41	166.38	140.98	42.61	2296	456.56	9.24	5.44	700.48	238.82	401.43	40.45	0.41	0.26
	10-20 cm	CT	106.77	29.05	110.18	11.55	72.18	7.59	3384.75	1420.48	8.74	0.94	412.43	83.82	393.78	5.27	0.83	0.34
		RT	84.30	30.58	88.40	12.74	73.88	12.26	2719.75	105.11	7.91	1.46	357.40	25.60	364.33	45.83	0.58	0.13
		G	73.65	101.25	138.56	133.57	91.14	36.27	2315.25	357.53	9.73	5.57	504.43	181.89	408.28	38.27	0.37	0.28
	20-30 cm	CT	94.05	28.50	88.37	10.19	65.82	7.07	3104.25	1103.09	7.98	0.98	372.73	77.43	393.80	17.63	0.79	0.36
		RT	71.60	22.47	67.27	7.78	57.60	8.96	2620.5	356.72	7.13	1.19	313.35	34.36	334.48	33.18	0.52	0.11
		G	46.89	61.15	88.84	70.78	61.49	33.33	2271.5	354.47	7.75	3.78	332.35	118.59	389.00	31.66	0.29	0.24
Frick	0-10 cm	CT	111.04	9.18	480.77	32.41	1704.80	796.93	7257.75	2601.81	10.14	0.60	546.48	56.39	399.15	51.93	1.52	0.08
		RT	142.83	11.80	626.72	25.24	1539.95	777.66	6389.5	2141.09	10.52	0.89	545.10	48.74	382.05	90.67	1.68	0.21
		G	40.81	29.49	289.54	38.04	1404.08	860.95	7994.25	3236.15	7.11	0.99	410.00	94.72	361.85	41.83	1.44	0.43
	10-20 cm	CT	109.88	7.61	479.39	30.59	1788.90	914.52	7430.75	2760.00	10.96	0.94	634.70	45.87	399.93	52.48	1.58	0.08
		RT	114.76	15.40	500.84	53.39	1586.58	767.30	7171.75	2577.84	10.48	1.12	533.35	61.34	384.48	80.83	1.63	0.13
		G	32.15	27.61	230.57	31.16	1541.68	1115.94	10305.5	5903.08	6.16	1.25	352.40	71.95	386.75	15.56	1.22	0.25
	20-30 cm	CT	79.25	18.14	397.15	31.20	1955.30	1055.75	8244.75	3949.23	9.30	0.95	527.55	56.05	386.40	61.99	1.45	0.17
		RT	85.00	9.95	396.48	25.77	1809.68	862.85	7598.75	2735.70	9.89	0.34	476.33	62.22	387.40	69.81	1.52	0.08
		G	16.15	19.20	207.20	25.02	1542.53	1112.14	11252	4087.62	5.55	1.22	329.73	79.58	383.33	37.46	1.10	0.22

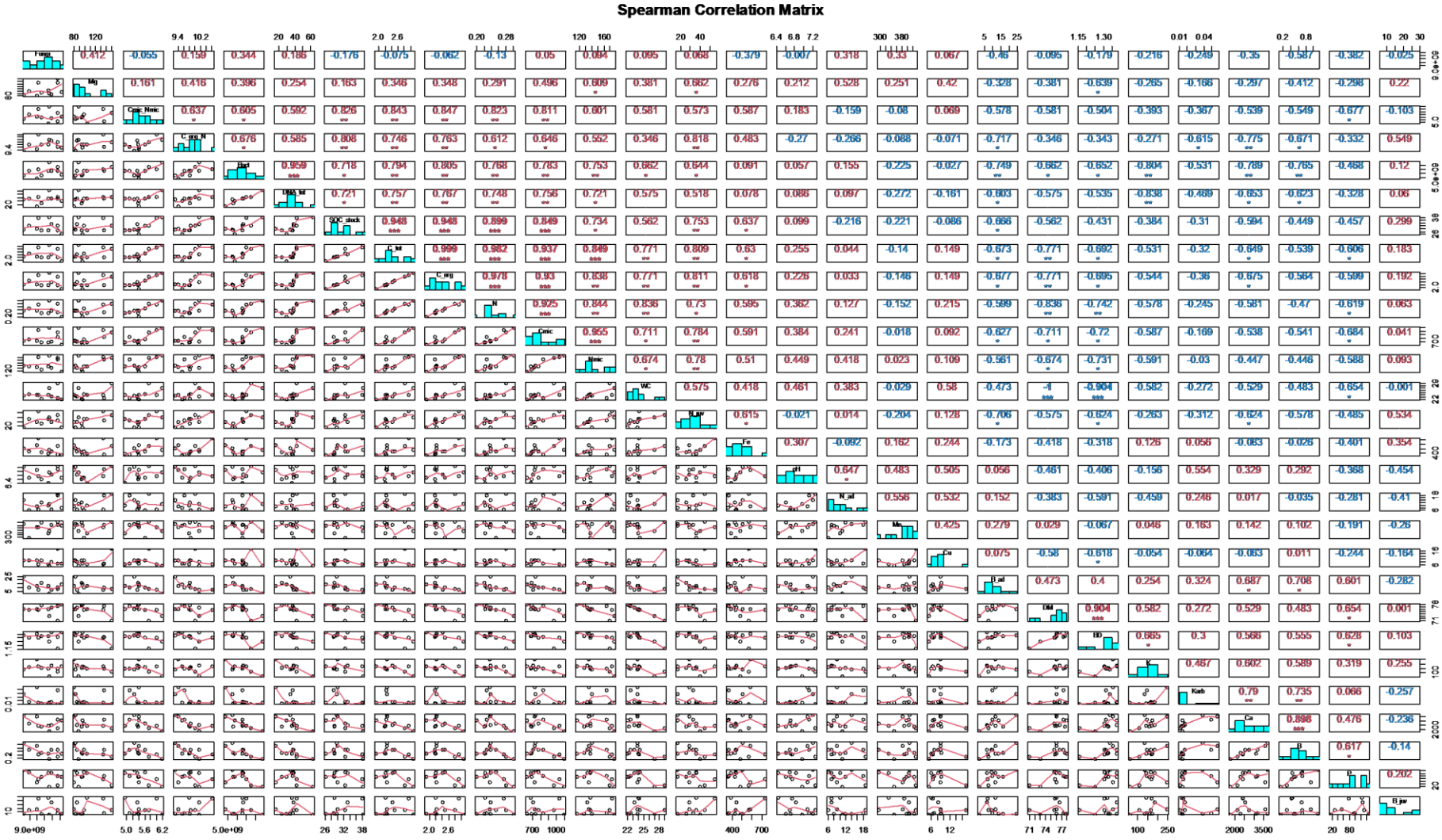
Trial	Tillage	Nb of adults		Biomass of adults [g]		Nb of juveniles		Biomass of juveniles [g]	
		mean	sd	mean	sd	mean	sd	mean	sd
Aesch	CT	11.00	3.16	16.62	5.64	22.25	6.18	8.81	3.10
	RT	10.25	5.44	9.47	2.10	33.50	3.32	18.39	9.30
	G	13.00	5.16	6.08	2.87	41.75	11.64	13.60	10.32
Frick	CT	4.50	3.87	2.98	2.27	22.25	15.28	4.41	4.80
	RT	10.25	13.33	2.03	2.65	23.25	13.67	4.17	0.89
	G	19.75	12.09	4.95	2.45	53.50	19.00	7.21	3.89

Trial	Depth	Tillage	Soil Cmic [mg/kg dry soil]		Soil Nmic [mg/kg dry soil]		Soil Cmic/Nmic		Soil DNA [g/g dry soil]		Bacteria [copies/g dry soil]		Fungi [copies/g dry soil]		Nitrifiers [%]	
			mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
Aesch	0-10 cm	CT	693.84	51.46	129.32	10.85	5.37	0.07	25.66	8.15	6.84E+09	2.32E+09	1.05E+10	2.05E+09	0.50	0.08
		RT	748.05	71.30	142.74	14.28	5.25	0.29	37.44	0.73	1.31E+10	2.27E+08	1.13E+10	1.65E+09	0.47	0.06
		G	1051.35	63.20	179.63	13.45	5.86	0.27	48.95	11.86	1.88E+10	4.53E+09	1.15E+10	1.32E+09	0.36	0.04
	10-20 cm	CT	475.95	63.48	86.97	10.03	5.46	0.13	22.34	1.19	6.61E+09	1.01E+09	8.22E+09	1.69E+09	0.79	0.04
		RT	478.49	229.56	97.00	55.54	5.15	0.90	23.56	4.25	6.33E+09	1.29E+09	8.19E+09	2.10E+09	1.02	0.21
		G	660.46	136.83	111.62	24.52	5.93	0.32	31.42	3.73	8.92E+09	4.89E+08	1.03E+10	5.12E+09	0.42	0.04
	20-30 cm	CT	319.31	63.17	58.16	11.82	5.50	0.12	20.38	5.77	7.02E+09	2.99E+09	9.63E+09	2.30E+09	0.98	0.12
		RT	283.26	39.69	52.20	7.34	5.43	0.29	18.05	2.87	5.67E+09	6.30E+08	1.14E+10	1.39E+09	1.14	0.13
		G	257.39	73.21	45.73	16.73	5.67	0.44	17.42	4.42	5.90E+09	1.31E+09	9.48E+09	1.60E+09	0.76	0.10
Frick	0-10 cm	CT	1000.39	283.94	130.82	42.71	7.63	0.47	35.51	8.31	9.80E+09	2.49E+09	4.79E+08	2.19E+08	0.44	0.03
		RT	1455.61	293.89	196.57	38.63	7.43	0.50	39.44	4.63	1.17E+10	1.57E+09	6.19E+08	2.46E+08	0.38	0.04
		G	1707.45	228.92	261.60	43.99	6.56	0.42	47.43	2.50	1.09E+10	1.03E+09	5.43E+08	1.69E+08	0.10	0.05
	10-20 cm	CT	929.89	158.72	131.47	23.38	7.06	0.27	27.61	4.99	8.70E+09	1.78E+09	4.83E+08	6.49E+08	0.49	0.04
		RT	1157.39	189.30	162.11	20.32	7.14	0.37	26.22	5.43	8.07E+09	2.24E+09	2.30E+08	7.62E+07	0.48	0.07
		G	1105.90	153.90	167.69	18.68	6.59	0.45	35.64	17.22	9.74E+09	4.71E+09	2.13E+08	1.30E+08	0.10	0.03
	20-30 cm	CT	718.21	138.67	105.48	17.22	6.78	0.26	19.26	2.99	1.12E+10	2.90E+09	1.36E+08	5.72E+07	0.57	0.10
		RT	819.17	141.50	116.11	19.64	6.96	0.51	21.59	3.79	1.10E+10	2.50E+09	1.89E+08	6.61E+07	0.57	0.02
		G	754.18	454.20	125.69	75.94	6.11	0.47	22.90	4.07	9.17E+09	1.72E+09	1.38E+08	2.71E+07	0.13	0.07

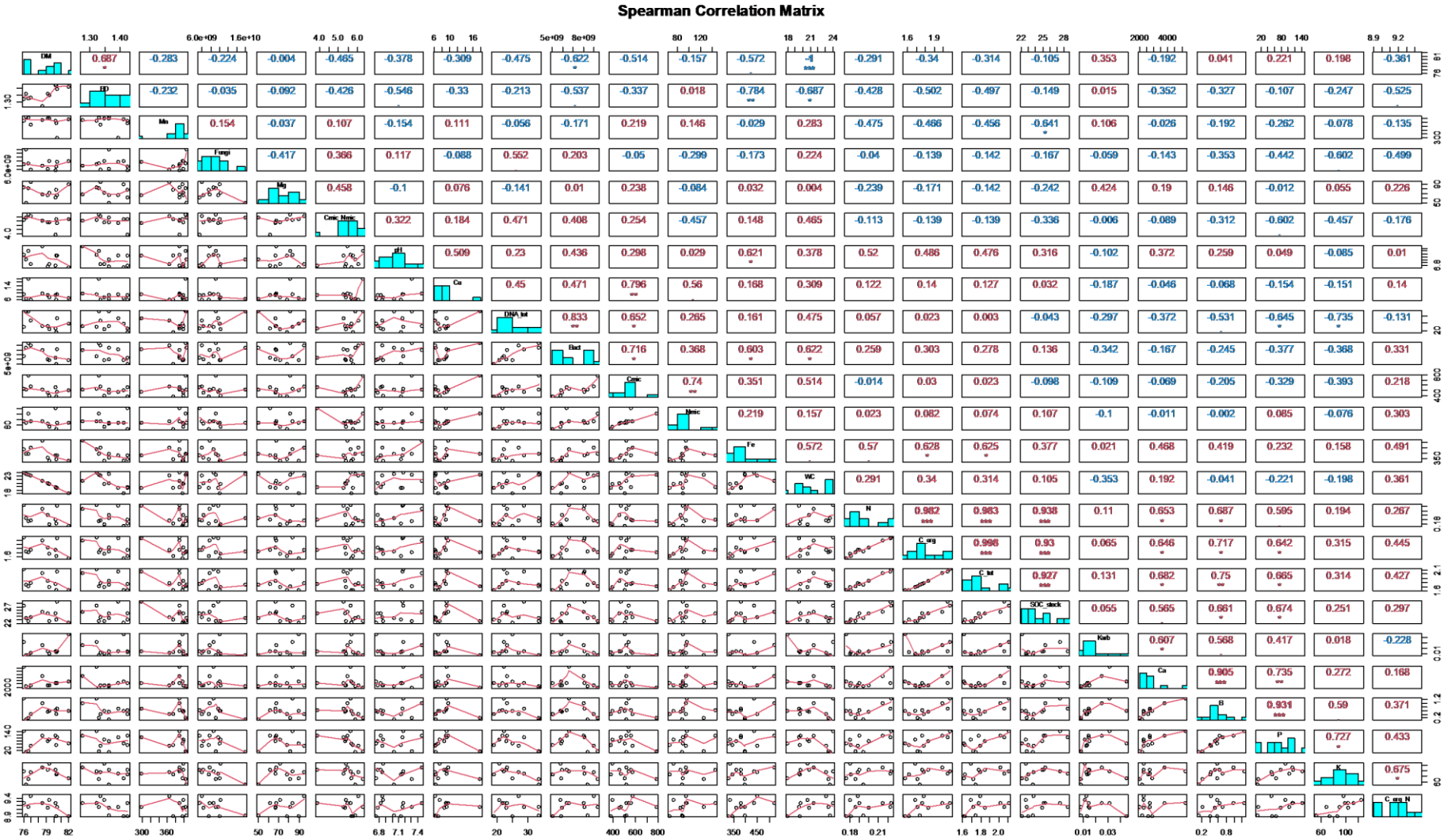
8.9 Appendix 9: Spearman Correlation matrices of environmental parameters

Aesch Entire dataset

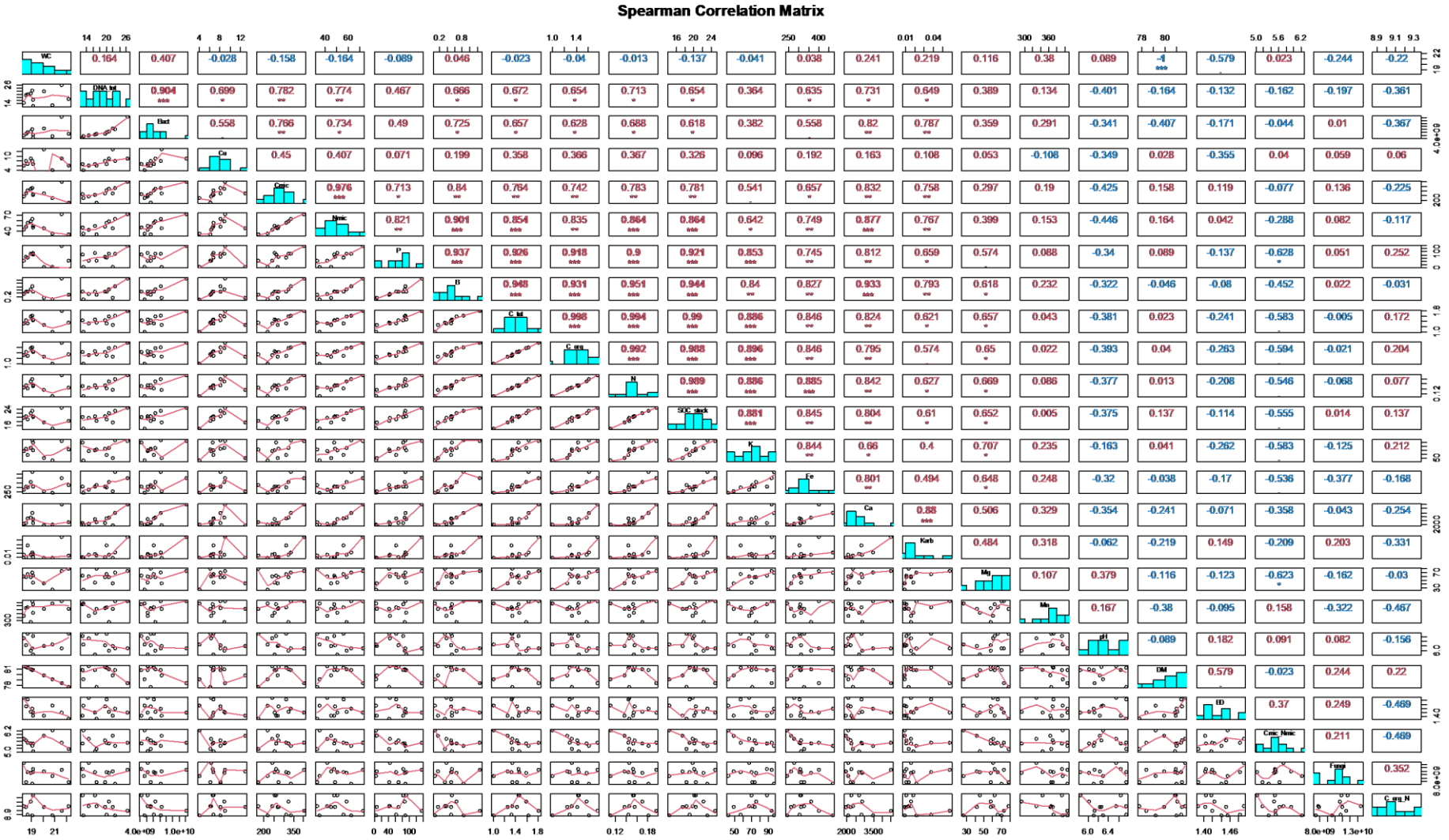
Aesch 0-10 cm



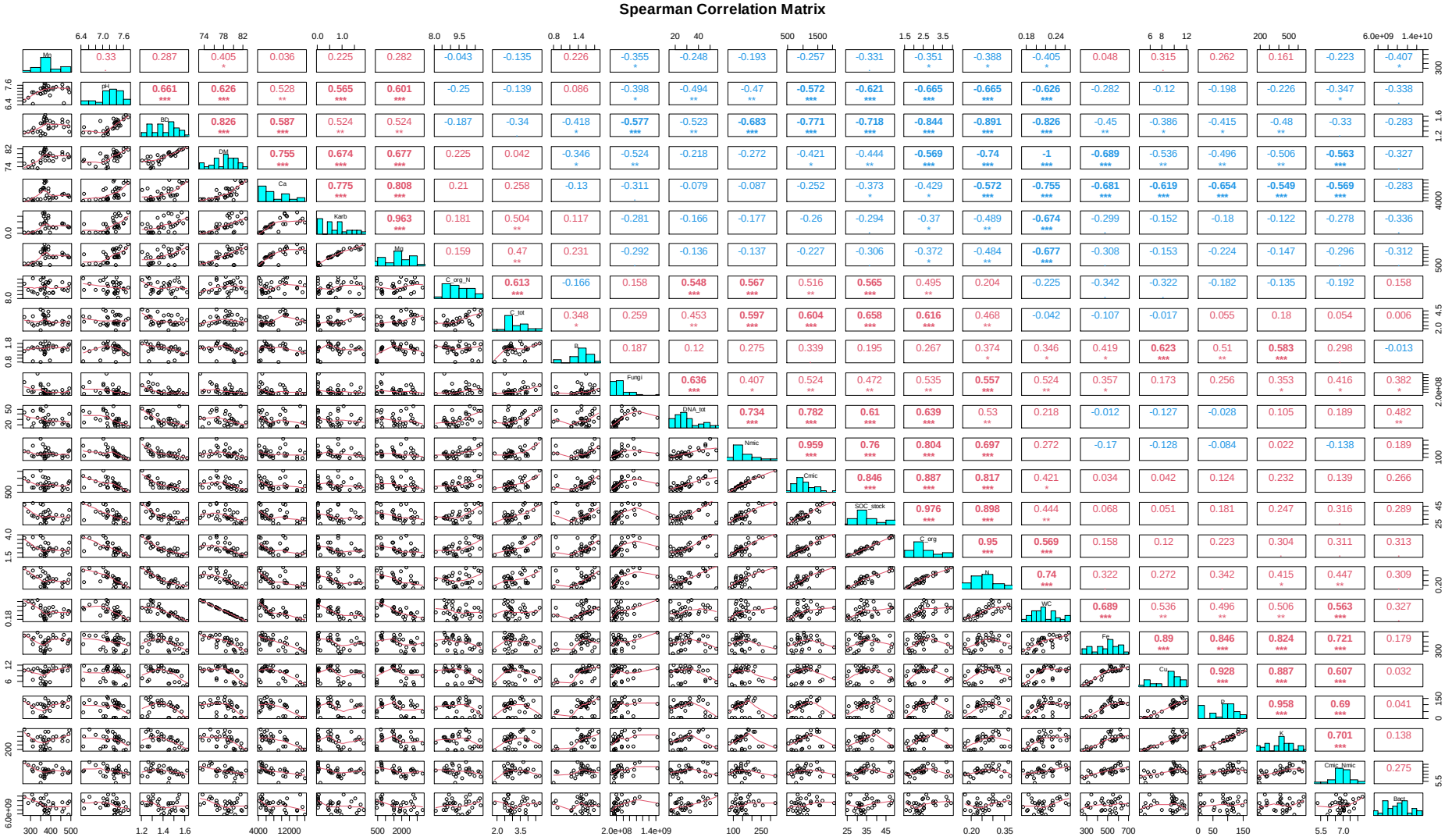
Aesch 10-20 cm



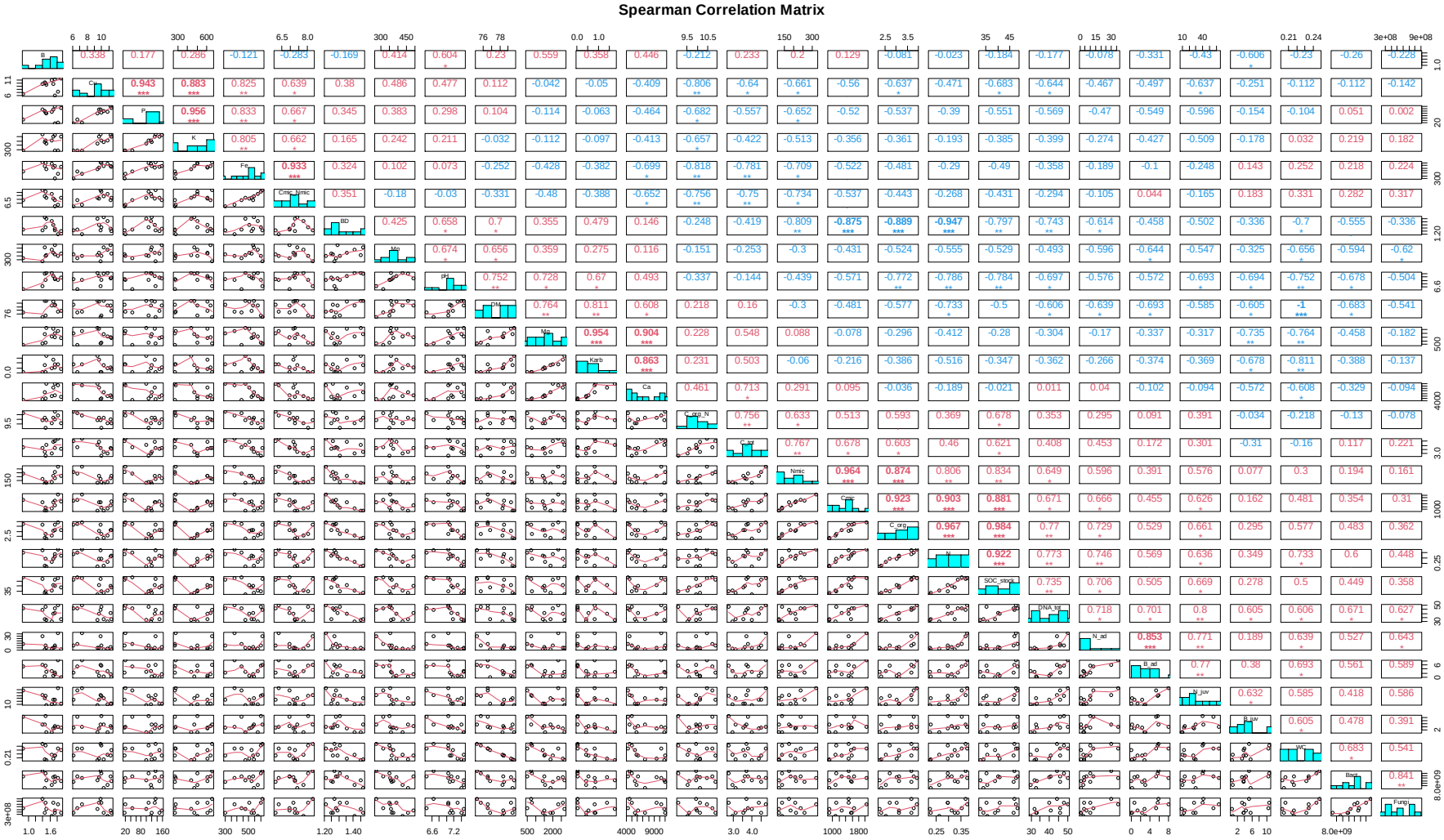
Aesch 20-30 cm



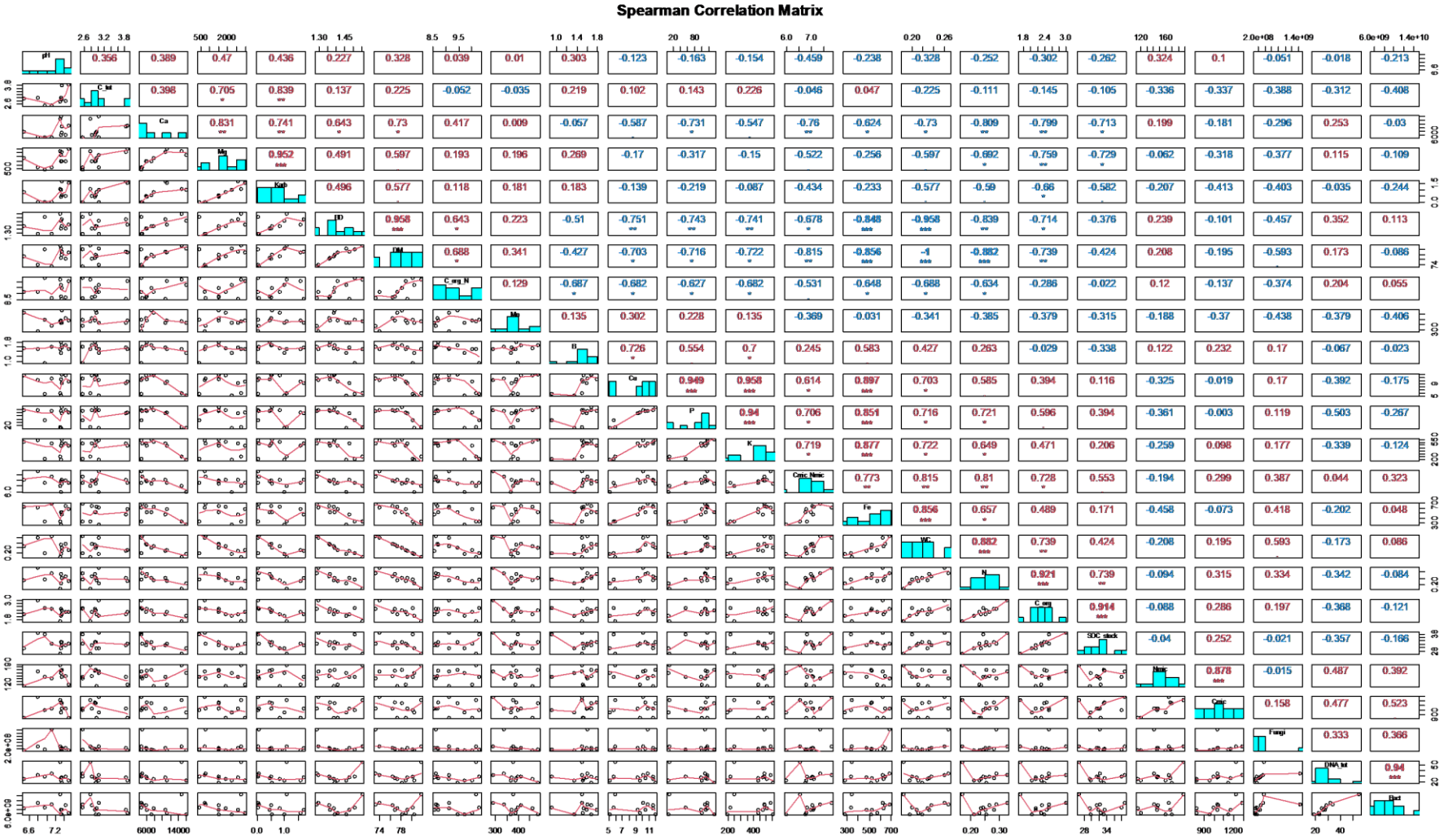
Frick Entire Dataset



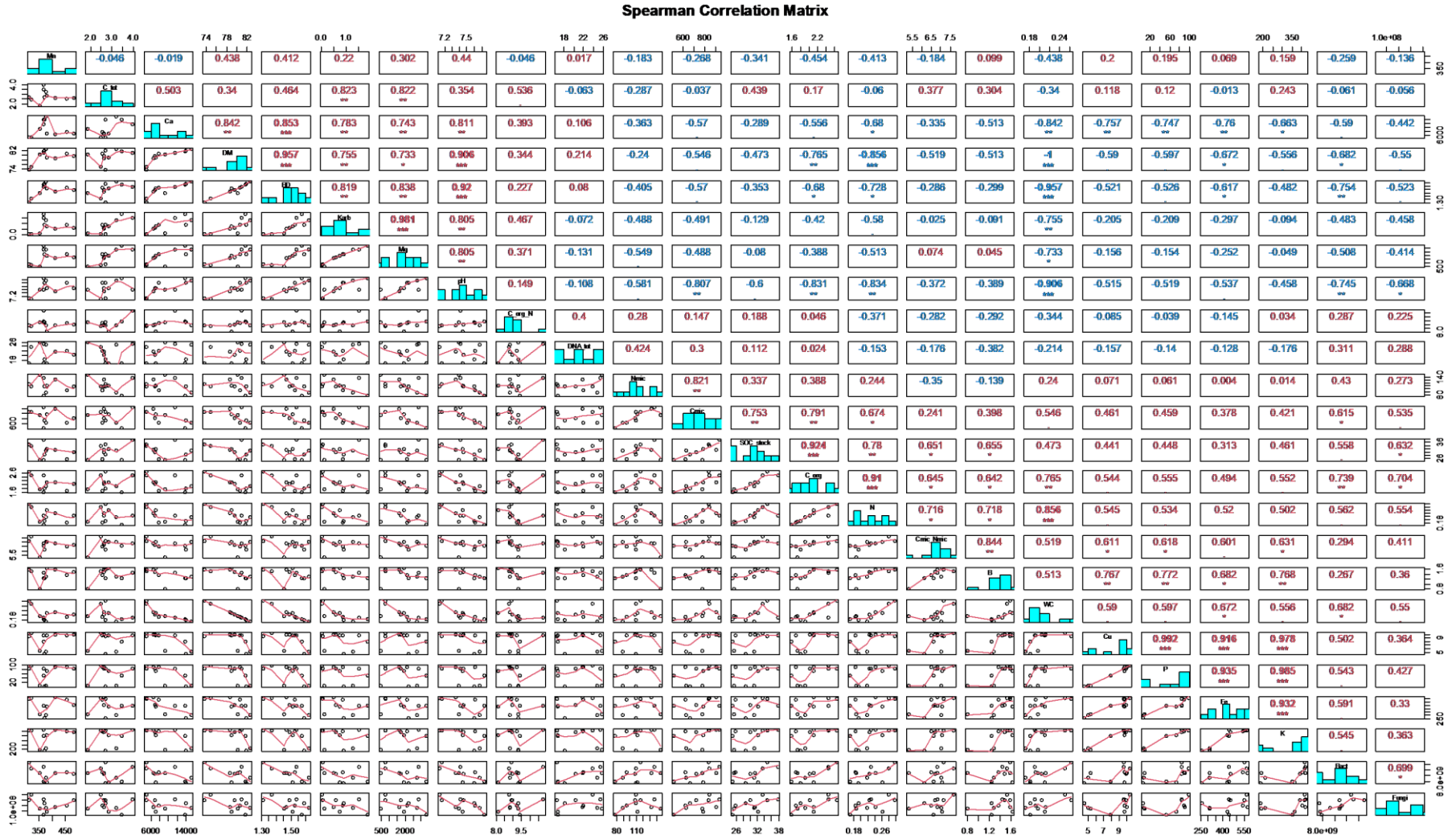
Frick 0-10 cm



Frick 10-20 cm



Frick 20-30 cm



8.10 Appendix 10: Table of microbial community phyla

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	Crenarchaeota	*Bacteria_unclass*	*Acidobacteria*	*Actinobacteria*	*Bacteroidetes*	*Chlamydiae*	*Chloroflexi*	*Cyanobacteria*	*Firmicutes*	*Gemmatimonadetes*	*Nitrospirae*	*OD1*	*Planctomycetes*	*Proteobacteria*	*TM7*	*Verrucomicrobia*	*WS3*
AC4_10	797	642	7162	17041	2077	58	3586	364	4575	689	763	87	2838	16449	151	2108	279
AC12_10	809	630	6816	16670	2168	127	3143	341	5462	973	752	126	2719	16061	174	2390	266
AC21_10	479	761	7363	19814	1299	34	4061	145	3578	663	614	50	3353	15413	110	1783	197
AC34_10	912	852	6264	18069	1950	102	3000	355	5588	834	554	125	2579	16087	167	1995	215
AR10_10	767	892	6145	18463	2578	112	3015	320	4789	587	641	90	2896	15892	117	2125	258
AR19_10	795	628	6761	18003	1721	113	3206	283	5243	870	587	100	2765	15467	105	2733	313
AR26_10	803	738	6930	17936	2139	102	3286	255	4539	717	705	106	2819	15710	142	2409	325
AR36_10	984	748	7448	17397	1989	98	3323	594	4567	741	705	138	2671	15549	132	2223	356
AG1_10	557	465	5627	17825	1402	106	2272	174	8588	568	553	125	2573	15045	127	3470	190
AG2_10	647	606	5184	18997	1088	116	2188	118	6559	409	562	100	2451	15641	74	4622	278
AG3_10	620	560	6163	19380	1557	120	2776	116	5627	621	633	80	2424	15564	132	3100	214
AC4_20	935	889	9064	15664	1373	89	3604	151	6459	719	972	87	2573	14877	80	1743	395
AC12_20	1120	945	8194	16315	1445	132	3475	208	5739	906	1188	81	2551	14853	71	2029	404
AC21_20	816	782	9941	15034	1345	87	3519	201	6062	768	1044	72	2443	15041	62	2048	431
AC34_20	1060	1049	7636	16913	1278	128	3356	177	5884	1024	1046	158	2258	15331	97	1858	361
AR10_20	1043	1084	9665	14241	1183	107	3840	121	6016	833	1370	95	2653	14745	63	2012	589
AR19_20	1265	1535	10283	12788	565	168	4160	98	6922	1216	2120	154	2198	13103	34	2064	850
AR26_20	1073	999	9233	14049	1222	104	4206	173	5668	878	1687	82	2802	14547	56	2245	629
AR36_20	888	1091	9003	13991	1057	95	3653	127	6489	927	1376	79	2261	15039	43	3013	576
AG1_20	576	843	5835	14236	1159	71	2445	88	11218	658	823	146	2062	13549	52	5496	435
AG2_20	805	820	6520	15091	958	82	2659	60	7140	566	895	109	1918	14492	32	6979	521
AG3_20	651	790	7718	15484	1140	91	3394	93	8984	980	857	127	1852	13500	110	3510	441
AC4_30	1263	1198	8857	14401	914	102	4103	114	5985	1066	1623	113	2767	14936	43	1474	651
AC12_30	873	1359	9225	13484	815	123	4330	139	6955	1332	1419	289	2269	13182	196	2753	693
AC21_30	773	1454	9718	13443	758	79	4375	105	6180	1205	1725	84	2479	14221	41	2050	952
AC34_30	955	1100	8274	13643	940	158	3972	124	7692	1622	1311	275	2475	13864	218	2188	713

AR10_30	1045	1569	9168	12740	645	164	4914	91	5652	1588	2179	141	2515	14100	50	1658	1119
AR19_30	887	1557	9815	12894	666	165	4150	70	6807	1191	1792	174	2412	13293	39	2722	896
AR26_30	1054	1298	10378	12196	872	129	4178	79	6774	1156	1956	121	2610	13231	49	2458	993
AR36_30	999	1628	10277	12342	700	137	4473	97	6080	1215	1795	117	2650	13523	40	2443	1041
AG1_30	1246	1353	7872	14312	570	116	3783	31	7609	1050	1945	165	1961	12577	64	3781	1169
AG2_30	1338	1318	8625	14177	558	105	4602	37	5512	988	2119	144	1948	12427	55	4175	1343
AG3_30	1103	1022	8949	14951	794	98	4241	127	7972	1035	1351	146	2211	12435	57	2576	645

Frick

	Crenarchaeota	Bacteria_unclassified	Acidobacteria	Actinobacteria	Bacteroidetes	Chlamydiae	Chloroflexi	Cyanobacteria	Firmicutes	Gemmatimonadetes	Nitrospirae	OD1	Planctomycetes	Proteobacteria	TM7	Verrucomicrobia	WS3
FCO4_10	1057	716	7438	19970	1696	103	3884	338	4525	588	549	10	3299	13690	42	1831	75
FCO7_10	840	672	7614	19996	1769	75	3658	250	4052	543	512	15	3052	14527	51	2090	95
FCW9_10	873	629	8582	20201	1381	74	3813	135	4461	598	576	7	2922	13590	21	1845	123
FCW13_10	710	653	8765	19867	1480	45	3799	137	4681	662	547	11	2433	13779	34	2115	58
FRW4_10	892	639	7734	20833	1492	94	3535	123	3486	494	526	18	3312	14597	68	1887	89
FRW7_10	698	677	7659	20312	1835	82	3515	146	3889	519	463	14	3146	14709	56	1983	70
FRO9_10	796	607	6842	21671	2112	96	3372	146	3576	519	372	1	2879	14560	35	2158	34
FRO13_10	546	571	7351	20654	2060	79	3360	157	4347	636	390	18	2524	14392	56	2565	65
FG1_10	307	507	8098	17262	3151	86	2571	139	2313	514	648	22	2642	17607	215	3606	100
FG2_10	268	708	8836	17521	1655	111	2964	296	2133	365	806	14	3253	17801	115	2792	137
FG3_10	186	609	8884	15314	1635	150	2751	396	1828	370	807	14	3191	19347	204	3986	88
FCO4_20	861	981	9439	18380	1207	87	3867	84	4608	633	771	17	2594	14432	22	1645	162
FCO7_20	885	724	7966	19297	1668	81	3855	128	4448	633	550	13	2705	14443	35	2221	129
FCW9_20	805	716	8425	19670	1319	64	3701	171	4423	586	633	4	2585	14457	32	2131	79
FCW13_20	733	733	9064	18186	1174	82	3656	1231	4676	626	649	4	2324	13739	22	2766	122
FRW4_20	920	743	8944	18927	1390	96	3508	121	4397	514	639	9	2952	14245	38	2179	145
FRW7_20	808	911	9588	17832	1271	89	3529	92	4633	512	854	13	2470	14641	49	2195	236
FRO9_20	910	810	9555	17552	1146	111	3703	123	4870	573	821	4	2627	14190	34	2561	173
FRO13_20	692	860	8733	18659	1294	70	3289	140	4701	620	668	8	2415	14612	33	2885	120

FG1_20	432	906	10703	16104	1388	108	2548	77	2877	547	1301	20	2198	16721	73	3649	156
FG2_20	394	619	8114	16583	1611	140	3021	177	2611	329	668	15	3964	18645	122	2651	113
FG3_20	371	1102	9712	14020	889	147	2876	88	3886	561	1283	23	3586	18302	200	2542	199
FCO4_30	1040	825	9350	16624	1447	107	3878	208	5145	618	904	15	2654	14740	58	1965	185
FCO7_30	975	841	8049	18782	1229	112	3692	139	4512	627	849	20	3128	14979	45	1596	190
FCW9_30	1311	719	7689	18907	1379	92	3591	84	5470	533	554	15	3206	14504	40	1602	82
FCW13_30	1221	819	8263	17586	1205	158	3469	115	5951	711	828	13	3217	13798	36	2256	143
FRW4_30	1047	863	9389	16563	1138	87	3532	135	6352	562	977	12	2349	14411	39	2123	189
FRW7_30	1280	993	7557	19140	1303	124	3287	126	4468	491	655	12	3290	15257	39	1578	134
FRO9_30	1401	784	8816	17533	1117	121	3357	78	5169	587	937	9	3350	14281	39	1961	214
FRO13_30	1228	628	7960	17440	1489	149	3148	157	5201	587	757	17	3248	15311	25	2272	166
FG1_30	826	731	9585	15691	1615	145	2283	69	3674	481	1204	37	2811	17683	83	2664	179
FG2_30	614	839	8619	16233	1163	134	2776	110	3563	446	1047	32	3393	18362	159	2058	199
FG3_30	457	967	11101	12998	1346	170	3322	218	3239	597	1412	44	3482	17229	178	2637	284