SOILGUARD

Sustainable soil management to unleash soil biodiversity potential and increase environmental, economic and social well being

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D 3.2 Report on the region and biome-specific impact of climate stressors on soil biodiversity status and cascading effects on soil multifunctionality under different types of soil management

WP	WP3 The impact of climate stressors on soil biodiversity and soil multifunctionality			
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1. Document History

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2. Content

3. List of Figures

4. List of Tables

5. List of Abbreviations

Abbreviation	Meaning
DNA	desoxyribonucleic acid
ASV	amplicon sequence variant
DS	drought severity
DT	differential temperature (referring to under the heaters vs out of the heaters)
BE	Belgium
DK	Denmark
FGCZ	Functional Genomics Center Zurich (Zurich, Switzerland)
FI	Finland
HU	Hungary
IE	Ireland
LV	Latvia
ES	Spain
NCP	nature contributions to people
IPBES	Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services
IÖW	Institut für Ökologische Wirtschaftsforschung
LSTs	local sampling teams (partners in each region with a WP3 field experiments)
ROS	rain-out shelters
PLFA	phospholipid-derived fatty acids
QC	quality control

6. Summary

Sustainable farming systems are assumed to better buffer the detrimental impacts of climate change compared to conventional systems, by promoting soil biodiversity. Studies focusing on the effect of multiple climate change stressors (e.g. droughts, and heatwaves) on biodiversity and multifunctionality are still rare and might show yet unpredictable consequences when combined. In this deliverable we present the results corresponding to the effects of on-field climate change simulations and soil management on soil biodiversity and multifunctionality for 7 locations in Europe, representing 3 biomes (cropland, grassland, and forests) and 5 biogeographic regions (Mediterranean, Atlantic, Continental, Pannonian, and Boreal). Specific climate change conditions to be simulated were obtained from region-specific climate models (D3.1).

In task 3.3, our results of the soil biodiversity analyses show that the seven different sites harbor a unique soil biodiversity in terms of prokaryotes, fungi, and eukaryotes, with the factor site explaining 54-75% of the variance in the biodiversity data. Management effects were highly site-dependent (interaction term site x management explaining around 10% of the variability). Drought effects on soil biodiversity were small, varied with the region studied, and were often masked by other drivers. However, using constrained statistics and analyses separated by site, it was possible to show some effects of drought.

Regarding the effects of climate change and soil management on soil multifunctionality, the results were also region-specific. In general, alternative[1] management regimes enhanced several of the 13 ecosystem indicators measured. Alternative management had a positive, although marginal effect, on biodiversity, and particularly evident in sites with comparatively low soil organic carbon (i.e. sites in Belgium, Denmark, Spain and Hungary). Contrary to our expectations, we found relatively weak support for the buffering impact of alternative soil management to mitigate the negative impacts of future climate scenarios. All soil functions studied showed significant interactions involving soil biodiversity, management regime, and/or simulated drought. The latter shows the prevalent role that soil biota plays in mediating the response of soil functioning to management or climate.

With this deliverable, we aim to illustrate the type of results that our consortium will produce. Most analyses are being conducted at writing, but the preliminary results presented promise to become important tools to give recommendations and predictions regarding the biodiversity-mediated response of soil multifunctionality to future climate scenarios under contrasting management regimes.

7. Introduction

Assessing soil biodiversity is a key challenge that needs to be addressed. SOILGUARD co-creates a conceptual and analytical framework with the potential to become the global standard for future assessments of soil biodiversity status and its contribution to soil multifunctionality and human wellbeing. With WP3 we attempt to implement an experimental design, combining multiple study sites across biomes with in-situ climate change simulations, with the goal to study the impacts of climate change on soil biodiversity and multifunctionality in soils with contrasting management regimes. We will later provide information to the other WPs of SOILGUARD, to then create evidence to fill the gaps of knowledge and quantify the environmental, economic and social consequences of climate change and unsustainable soil management.

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[1] In this document, *alternative* refers to the opposite to *conventional*. The differences between *conventional* and *alternative* management regimes are provided in **Table 2**. The word "*sustainable*" may be used in this document to refer also to *alternative* management, especially in a general context.

We completed task 3.1 (*"Future region-specific climate challenges*") in 2022 (as reported in D3.1.), and we have successfully completed task 3.2 *("On-field climate simulations set up")*. Infrastructure to impose the projected drought and heatwave conditions were installed in the seven sites in 2022 and 2023. Adjacent fields managed either conventionally (i.e., conventionally managed cropland, mono-species high-input grasslands, and clear-cut forests) or alternatively (i.e. organically managed cropland, mixed-species low-input grasslands, and continuous cover forests) were located at each region. Drought simulations using rain-out shelters (ROS) in combination with manual irrigation to reach realistic drought scenarios as indicated by the climate projections, were performed for both management regimes (conventional and alternative) at each site in 2022 and 2023. Heatwaves were simulated in 2023. Here, we report on the infrastructure installation of 2023 and on the first results of soil biodiversity and soil multifunctionality of one sampling point of 2022.

With the objective of determining the effect of combined climate stressors on soil biodiversity under different management regimes we have initiated and are currently working on task 3.3 *("Effects of increasing droughts and heat waves on soil biodiversity")*. Further, with the objective of correlating predicted soil biodiversity dynamics with fluctuations in soil multifunctionality, we are working on task 3.4 *("Region-specific cascading effects of future soil biodiversity dynamics on soil multifunctionality")*. The biodiversity and multifunctionality of samples collected in one sampling point in 2022 and largely analyzed in 2023 are presented in this report. Samples collected in a second sampling point in 2022, and during the drought and heatwave simulations in 2023 are currently being processed and will be included in the Deliverable D3.3. A detailed list of samples generated during the field simulations are presented in Table 1. To further clarify, we have included in this deliverable, results of T3.2 (section 8), T3.3 (section 9) and T3.4 (section 10) from the first sampling timepoint. The other timepoints, including year 2 (2023) will be added to the deliverable 3.3, which is the final version of D3.2. This is in accord with the grant agreement (see page 107 GA): "D3.2 : Report on the region and biome-specific impact of climate stressors on soil biodiversity status and cascading effects on soil multifunctionality under different types of soil management [30] Results of T3.2, T3.3 and T3.4." ; and "D3.3 : Report on the region and biome-specific impact of climate stressors on soil biodiversity status and cascading effects on soil multifunctionality under different types of soil management - Final [42]".

Table 1. Description of soil samples collected during the two field simulations. † T2 were not collected in DK or BE because the soil was tilled and mixed and no longer the same after the recovery phase. See abbreviations for countries in the table at the beginning of this document.

Year	Sampling point	Time point description	Countries	country x climate treatment x management x replicates x soil horizon	Total samples
2022	T1	at the end of drought simulation	BE, DK, HU, LV, IE, ES	6*2*2*3*1	72
	T1	at the end of drought simulation	FI	1*2*4*3*2	48
	T2	two months after drought simulation	HU, LV, IE, ES†	4*2*2*3*1	48
2023	T0	right before the drought simulation	BE, DK, HU, LV, IE, ES	6*2*2*3*1	72
	T1	at the end of drought simulation	BE, DK, HU, LV, IE, ES	6*3*2*3*1	108
	T1	at the end of drought simulation	FI	1*2*4*3*2	24
	T2	two months after drought simulation	BE, DK, HU, LV, IE, ES	6*3*2*3*1	108
Total soil samples					480

8. Task 3.2. Climate simulations at the different experimental sites.

The objective of this task was to set up the infrastructure to correctly simulate the projections developed in task 3.1. We have successfully simulated future climate conditions with respect to the occurrence of drought and heatwaves for 7 EU NUTS-2 regions, each represented by one country and one experimental site. The experimental sites were in three different biomes, i.e. cropland (Belgium, Denmark, Hungary, Latvia, Spain), grassland (Ireland) and forest (Finland). Each site featured one conventional and one alternative management regimes, except Finland, which featured four different management regimes (Table 2, Appendix H).

Table 2. Experimental factors for drought simulations in 2023 across different countries, representing each of the seven EU NUTS-2 regions in Belgium, Denmark, Finland, Hungary, Ireland, Latvia, Spain. A factorial combination of the management x drought x heatwave was installed at the different experimental sites. For the forests in Finland, the alternative regime corresponds to the continuous forest treatment (CCF40) whereas the conventional management regime corresponds to a clear-cut forest (T12)[2]. In all cases alternative management was selected based on the EU regulation[3].

Biome	Levels	Countries (NUTS-2 region)	Description
Farmland	Conventional	Belgium (West Flanders), Denmark (Northeastern Denmark), Hungary (Western Transdanubia), Latvia (Latvia), Spain (Murcia Region)	Follows conventional agricultural management practices according to EC guidelines
	Alternative		Follows organic agricultural management practices according to EC guidelines
Grassland	Conventional	Ireland (Southern Ireland)	Low-diversity grassland (monoculture ryegrass) with high-fertilizer input
	Alternative		Diverse mixed-species grassland (6 species) with low-fertilizer input
Forest	CCF40	Finland (South Finland)	Uneven aged, continuous cover forest with dominant trees ca. 40 years old
	T40		Even aged, thinned from below forest with dominant trees ca. 40 years old
	TSW12		Even aged, shelter-wood felling forest including mounding, seedlings planted 12 years ago
	T12		Even aged, clear-cut forest including mounding, seedlings planted 12 years ago

[2] The other two forest management regimes in Finland represent intermediate treatments, and for the moment, are excluded from the analyses presented in this deliverable. All the forest management will be included in the analyses of D3.3

[3] (2018/848 of the European Parliament and of the Council of 30 May 2018 on organic production and labelling of organic products and repealing Council Regulation (EC) No 834/2007:
Article 3 Definitions: 'organic production' means the use, including during the conversion period referred to in Article 10, of production methods that comply with this Regulation at all stages of production, preparation and distribution.
Article 5 General Principles: respect for nature's systems and cycles and the sustainment and enhancement of the state of the soil, the water and the air, of the health of plants and animals, and of the balance between them.
Article 12: Plant Production Rules: Operators that produce plants or plant products shall comply with the detailed rules set out in Part I of Annex II.
Annex II Part I: 1.1. Organic crops, except those which are naturally grown in water, shall be produced in living soil, or in living soil mixed or fertilized with materials and products allowed in organic production, in connection with the subsoil and bedrock; 1.7. For plants and plant products to be considered as organic products, the production rules laid down in this Regulation shall have been applied with respect to the parcels during a conversion period of at least two years before sowing. 1.9. only fertilizers and soil conditioners that have been authorized pursuant to Article 24 for use in organic production shall be used, and only to the extent necessary.

Drought simulations were performed for two consecutive years, between June and August of 2022, and June and September of 2023, while heatwaves were simulated in 2023. In 2023 we added the new experimental condition in the fields, to study the combined effects of drought and heatwaves. Heatwave simulations were not possible in 2022 because the protocols and infrastructure required to accurately simulate heatwaves were not yet available. Specifically, the soil sampling protocol was finalized during summer of 2022, and the prototype for heatwaves was validated during 2022, only available to be implemented at the end of the year 2022. Additionally, given the differences in crops and climatic regions of the different experimental sites, the droughts were imposed at slightly different times during spring and summer of each year. A detailed timeline for 2023 is presented in Table 3 and Figure 1.

Additionally, as explained in the technical report of the second reporting period of SOILGUARD (RP2), it was not possible to collect samples at T0 (baseline, right at simulation starting point) in 2022 because we did not have a harmonized soil sampling protocol when the simulations were starting (June 2022). Additionally, we were focused on defining the fields to sample, which caused logistic delays to install the rainout shelters. The protocol was finalized, with contributions from all partners, in August 2022, and implemented first in T1, 2022 (at the end of the simulation with the rainout shelters). Additionally, the field sites used for the simulations in Belgium and Denmark were tilled after the crop was harvested, and soil from other points in the field was then mixed with the soil that had been subjected to drought. Therefore, the T2 sampling point (two months after the simulation) could not be sampled in these two countries. In 2023, we did collect samples at T0, T1 and T2 in all croplands and the grassland, considering the learning experience from 2022

In our perspective missing T0 for 2022 is not a major drawback because T0 is generally not comparable with T1 or T2, since the biological activity and diversity of the soil changes with seasonal succession and continued management interventions. The validity of the results of T1 and T2 should therefore not be compromised, as each sampling point would stand by itself when comparing management and drought effects within each sampling point (see for example Santos-Medellín et al., 2021). In other words, the sampling points can be analyzed independently.

Furthermore, we decided to drop the T2 sampling point for the forest. Specifically for these forest soils, by analyzing T0, T1 and T2 we wouldn't have obtained meaningful results as then there had been the possibility to sample only two treatments. However, it is important to consider different forest ages, i.e. different time points along the rotation time of forest trees (80 years rotation compared to 2 months for crops). Therefore, to understand the impact of supposedly more sustainable practices such as continuous cover forestry – which includes a mixture of differently aged trees – we need to compare it to conventional even-aged forestry systems at different stages, e.g. young seedling stage, middle -aged forest, old forest. Moreover, we feel that the lack of T0 and T2 is not a problem (Santalahti et al. 2016). In fact, the greatest change in forest soil communities happens during the snow melting after winter when decomposers peak compared to symbiotrophs, but during the summer months the communities are rather stable. Furthermore, sampling depth in the proposal was 10 cm, but an organic layer in these forest regions is mostly less than 10 cm. Thus, forest soils are very different from ploughed agricultural soils (which are vertically homogenized until 20-30 cm depth) and sampling should consider these different layers. The organic overlay and the underlaying mineral soil are fundamentally different in terms of carbon stock and other soil chemical properties that drive soil biodiversity. Thus, we decided to analyze the organic and mineral layers separately in the second year. The relevance of soil stratification in forests under different management practices for soil biodiversity remains poorly understood. We expect to shed light on this issue. Additional explanations of the reasons to dropping T2 in 2022 for Belgium and Denmark are provided in Table 1 and in the paragraphs above.

Table 3. Schedule for drought and heatwave simulations at the different field sites in 2023. Activities are indicated as colors provided in the legend below the table. For 2022, the schedule was provided in D3.1.

Country	February	March	April	May	June	July	August	September	October	November	Crop
Spain											oats
Hungary											winter wheat
Belgium											grass-clover
Ireland											grass-clover and other species
Latvia											wheat
Finland											spruce
Denmark											conv: spring barley; org: horsebeans

	Field preparations		Heatwave simulations
	Drought simulations		Collection of last sample

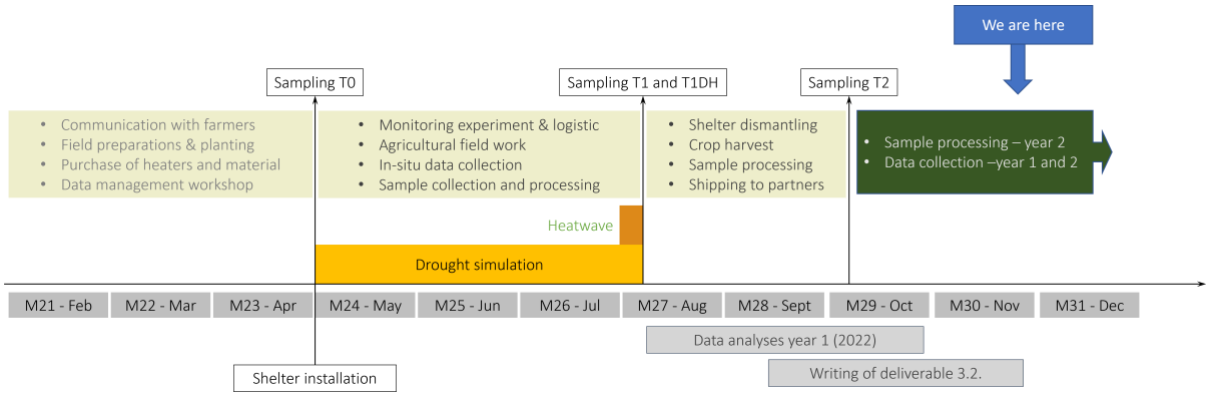

Figure 1. *Timeline of activities related to the field simulations of drought and heatwaves for 2023.*

In 2023, drought simulations were performed, similarly to 2022, by controlling irrigation according to the projections (see Table 3 in D3.1) in the sheltered plots; in contrast to 2022, the percent reduction in precipitation in 2023 was applied in comparison to the *actual* precipitation during the simulation period, rather than in relation to the *expected* precipitation as done in 2022. This allowed us to reach the projections more precisely. Heatwaves in Belgium, Denmark, Hungary, Latvia, and Ireland were imposed with infrared heaters, using the prototype described in D3.1, and as described in **Figure 2**. Given that heatwaves occur naturally in Spain, the simulations there were performed differently, by mitigating the natural heatwave instead of imposing an artificial heatwave. The method is described in **Appendix A** and consists of covering an area of 1.5 m² under the unsheltered control with a shade net during a heatwave period. Heatwaves were not simulated in Finland after a thorough assessment of safety by the local team. The necessity to bring power generators to the boreal forest was considered very high fire risk, therefore they did not simulate heatwaves. Some images showing the installation of the ROS and the infrared heaters, or the shading nets, in 2023 in the fields are provided in **Figure 3**. Please refer to the last part of this section for a detailed description of the measures we took to compensate for the reduced number of climate simulations in Finland (“*Lack of heatwave simulations in Finland and compensation by extending management regimes in the forest”)*.

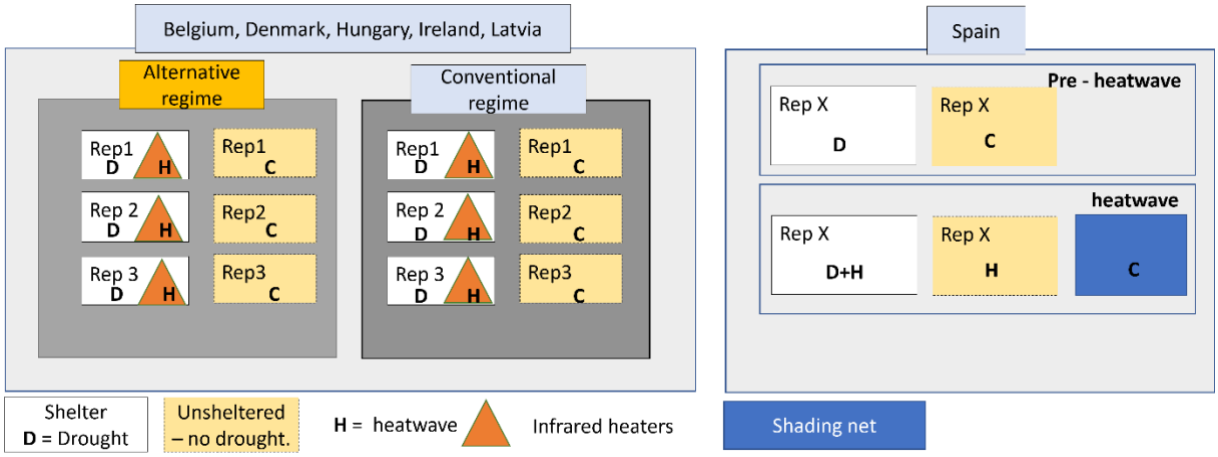

Figure 2. Experimental design of the agricultural and grassland systems. We show treatment distribution with either infrared heaters, or shading nets for the heatwave simulations. All experimental sites had shelters, including Finland. However, note that heatwaves were not simulated in Finland. C = un-sheltered control. Rain-out (ROS) shelters were randomly located in the available area for the experiment, but the controls were always located next to the ROS, which corresponds to a nested design (drought nested into fields). This has been considered in the models used for statistical analyses. The figure intends to give an idea of the treatments but does not represent the true spatial configuration in the field.

Figure 3. Experimental set up of climate simulations in 2023. A. Field manager Helle Hestbjerg installing soil temperature sensors in the Danish experimental site. B. Controlled irrigation under the shelters by a field technician in the Belgian experimental site. Shading nets to mitigate heatwaves in the experimental site of Spain. C. Infrared set up to simulate heatwaves in the grassland site of Ireland. D. Power generators to feed the infrared heaters in the Irish site. E. Shelter installation in the experimental site of the boreal forest in Finland. Images provided by the field managers.

In relation to sampling timepoints, in 2022, soil samples were collected at the end of the drought simulation experiments to assess the initial impact of drought (T1), and two months later to assess the recovery from drought (T2). Soils were not sampled at the beginning of the drought simulations in 2022 because the sampling protocol was written during the summer of 2022. In 2023, we studied more in-depth the soil status by sampling before drought and heatwaves were imposed, thus, adding a third sampling timepoint. So, in 2023, the first soil sampling took place before the drought simulation at shelter installation to measure the baseline (T0), and again at the end of the drought and heatwave simulation (T1) as well as two months after shelter dismantling (T2). Again, only in 2023, T1

was divided into two scenarios, depending on the climate simulation: T1D samples were collected in the sheltered area on the side where no heatwaves were simulated and thus only received the drought treatment, whereas T1DH samples were collected in the sheltered areas where the heatwaves were simulated and thus experienced the combination of drought and heatwave (see **Table 1**). The samples collected in task 3.2. were used to measure soil biodiversity with metabarcoding in task 3.3, and to measure soil properties and to link local information to measure soil multifunctionality in task 3.4. A map of the soil sample processing with details on the progress to mid-November 2023, is presented in **Figure 4**.

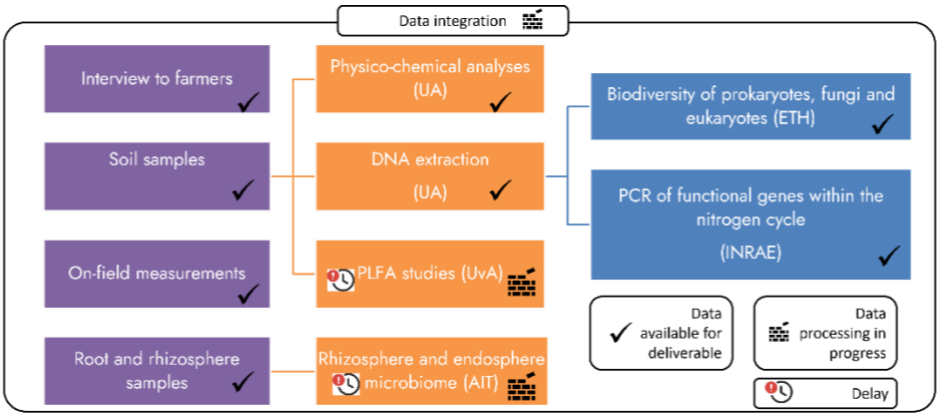

Figure 4. Pathway of soil samples within the WP3 partners and progress of sample processing and data analyses. Delays happened due to technical issues with analytical equipment at UvA, and from the facility performing sequencing for AIT.

Lack of heatwave simulations in Finland and compensation by extending management regimes in the forest

Our partners in Finland (LUKE) were not allowed to perform heatwaves in their experimental fields due to the risk of fire, but they continued to simulate droughts under the four management systems (compared to two management systems at all other sites). Additionally, they have sampled two soil horizons (organic and mineral). With these two additional modules, the Finish team has been compensating for the missed heatwave simulation. Furthermore, we have decided to include the temporal dimension of forest in the way of treatments, as described in detail in **Appendix H**. Briefly, instead of sampling before, right after, and two months after the drought simulations, as performed for croplands and for the grassland, we have sampled only right after the drought simulations in the forest but using three different managements treatments with varying ages in the succession of forest. We consider this sampling scheme more meaningful for forests, given the slower pace at which changes occur in these ecosystems.

For the sake of consistency with other countries, and to facilitate the first statistical analyses performed for D3.2, we have included only two of the management treatments evaluated in Finland (one conventional, one alternative). However, finally, the analyses for Finland must be done separately, and the results will be included in D3.3. This had been disclosed in foot note #2 of this deliverable.

9. Task 3.3. Effects of increasing droughts and heat waves on soil biodiversity

The objective of task 3.3 is to increase our understanding of the future effects of climate stressors on soil biodiversity. In this report we show the results of the bulk soil (section 9.1), and root and rhizosphere (section 9.2) biodiversity, using metabarcoding of taxonomy markers. We present also qPCR (quantitative PCR) of nitrogen cycling guilds. All these results were obtained from T1 soil samples

of 2022 (see **Table 1**). Biodiversity measured with PLFAs and NFLAs, as well as food-web and co-occurrence networks will be presented in D3.3. Although the samples of T2 from 2022 have been analyzed by the respective partners (see **Figure 4**), some data were not available to be presented in this deliverable, and therefore will be presented in D3.3. Metagenomics will be performed after all the metabarcoding has been finalized, and the most interesting contrasts among the different treatments are selected, in 2024. Similarly, food-web and co-occurrence networks will be analyzed after the data from 2023. A general overview of all sites' effects is provided in the main text of this deliverable, and detailed methods, as well as analyses by specific sites are presented in **Appendixes B - F**.

For the metabarcoding analyses, we aimed to cover most of the soil biodiversity by targeting three biological groups, namely **prokaryotes, fungi, and eukaryotes**. Prokaryotes, comprising bacteria and archaea; and fungi, are the chemical engineers of soil processes (Hartmann and Six, 2022), and are therefore presented first in our analyses. Eukaryotes include not only fungi, but also protists, nematodes, arthropods, and earthworms and are presented after prokaryotes and eukaryotes, given that they comprise a higher level in the trophic network in soils, modulating soil functions using different process, when compared to prokaryotes and fungi. As described in detail below, and in **Appendix C**, we used molecular detection of specific genes that are unique to each of these groups to taxonomically classify and measure their abundance in the soils collected at the different experimental sites. This method, named metabarcoding, is largely used to perform high-throughput surveys of microbial communities in environmental samples, and is commonly used in soil science to describe its biodiversity.

9.1. Prokaryotic, fungal and eukaryotic soil biodiversity

9.1.1. Methods

A general overview of the workflow followed in presented in **Figure 5**. Soils samples collected at the end of the drought simulation (T1) in 2022 were processed by UA as detailed in **Appendix B**. Briefly, 0.25 g of freeze-dried soils were used to extract DNA by the team at UA. The extracted DNA was shipped to ETH, where amplicon libraries for each of the three organism groups, namely prokaryotes, fungi, and eukaryotes were prepared (**Appendix C**), and sent for sequencing to the Functional Genomics Center Zurich (FGCZ, Zurich, Switzerland). Two months after, the sequencing data were received and processed with a bioinformatic customized procedure (see details in **Appendix C**) to obtain taxonomically annotated ASVs (amplicon sequence variants, approximations of species) and their abundance per sample (sample x observation matrix). With these datasets, basic quality control (QC) and community level biodiversity analyses were performed (see details in Appendix A).

First, we analyzed alpha diversity, which measures the average species diversity per sample and per experimental factor. We used a total of three indexes for alpha diversity: observed richness (number of ASVs), evenness (equality of ASV distribution), and Shannon diversity (a combination of the two previous). Second, we analyzed beta diversity, which measures the extent of change in community composition, or degree of community differentiation, in relation to different experimental factors (Whittaker, 1960). For measuring beta diversity, we used the Bray-Curtis dissimilarity of the normalized abundances of the different taxa per sample to calculate the effects of the different experimental factors with permutational multivariate analysis of variance (PERMANOVA). Then, we used two different ordination methods to visualize differences in beta diversity between management regimes and in response to the climate stressors, first the unconstraint method, principal coordinate analyses (PCoA), and the constraint ordination method, canonical analyses of principal coordinates (CAP) applied on the Bray-Curtis dissimilarity measure. Here, we present Shannon and PERMANOVAs of Shannon for alpha diversity, and PERMANOVAS and CAP for beta diversity (Bray-Curtis dissimilarity measure). We also provide the percentage of variability of the diversity metrics explained by the experimental factors in the text. To keep the reader's focus on the key results, we present result of

Shannon, PERMANOVAS and CAPs because these metrics show the best differentiation of samples by experimental factors and illustrate the results clearly.

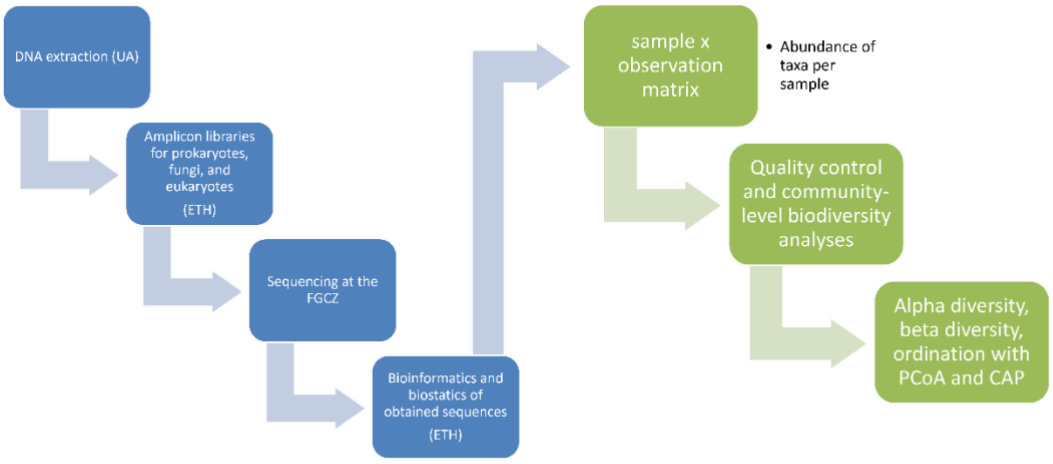

Figure 5. *Overview of the workflow followed to measure biodiversity in soil samples. PCoA: Principal coordinate analyses. CAP: Canonical analyses of principal coordinates. FGCZ: Functional genomics center Zurich.*

9.1.2. Results

In Spain, we have obtained very low DNA concentrations (average ± standard deviation = 1.7 ± 0.96 ng/µL, range 0.00 – 3.1 ng/µL) despite trying different extraction methods, e.g. even by extracting DNA from 10 g of soil. This corresponds to the extremely low microbial biomass, which was the lowest across countries and management based on the PLFA analysis (58 ± 20 compared to the average of 415 ± 108 nmol PLFA g-1 soil for all samples – complete PLFA results will be presented in D3.3). However, we were able to extract enough DNA from the organic fields from Spain for further analysis. Therefore, the analyses presented below, for Spain, show only the soil biodiversity results from organically managed fields (alternative, ALT).

For the rest of the soil's samples, corresponding to the experimental sites in the other 6 NUTS-2 regions, we obtained high-quality reads from the three biological groups across both management types, and the sequencing effort was sufficient to capture a large part of biodiversity (Appendix C). Here, we present results of soil biodiversity analyses of all the experimental sites and treatments. Region-specific analyses of the metabarcoding data are presented in Appendix C.

The field sites in Latvia, Belgium and Denmark showed a higher **prokaryotic** biodiversity when compared to the sites in Spain, Hungary, Ireland and Finland (**Figure 6)**. Drought treatments generally showed slightly lower biodiversity values, except under conventional management in the boreal forest of Finland (**Figure 6**). However, these differences were not significant. Site explained 85 and 75% of the variability in alpha diversity of prokaryotes and other eukaryotes respectively, while a smaller proportion of the variability (~1%) was explained by management, which was site-dependent for the prokaryotes (**Table 4**). The drought treatment had a small and only marginally significant influence on prokaryotes and fungi, but not on other eukaryotes (**Table 4**). Noticeably, neither site, nor management were significant for fungi, while drought was significant, explaining ~2% of the variability. Significant interactions between management and drought for the prokaryotic diversity contrast with the lack of interaction of these two factors for fungal and other eukaryotic communities.

Table 4. Effects of experimental factors on alpha diversity of prokaryotes, fungi, and eukaryotes through PERMANOVA analyses of results of the Shannon diversity index. F, P and R^2 values are presented, and bolded when significant or italicized when marginally significant.

Factor	Prokaryotes F(P)	R^2	Fungi F(P)	R^2	Eukaryotes F(P)	R^2
Site (S)	**163.8 (0.001)**	**0.850**	1.9 (0.110)	0.138	**21.1 (0.001)**	**0.653**
Management (M)	**9.2 (0.006)**	**0.008**	0.2 (0.655)	0.003	*3.6 (0.069)*	*0.019*
Drought (D)	**4.8 (0.024)**	**0.004**	**5.4 (0.028)**	**0.067**	1.4 (0.229)	0.006
S × M	**17.1 (0.001)**	**0.074**	0.6 (0.746)	0.035	0.4 (0.827)	0.014
S × D	1.5 (0.199)	0.008	0.7 (0.683)	0.051	0.3 (0.937)	0.010
M × D	**4.8 (0.042)**	**0.004**	0.5 (0.512)	0.006	0.5 (0.467)	0.004
S × M × D	1.8 (0.161)	0.008	0.9 (0.522)	0.053	1.2 (0.315)	0.034

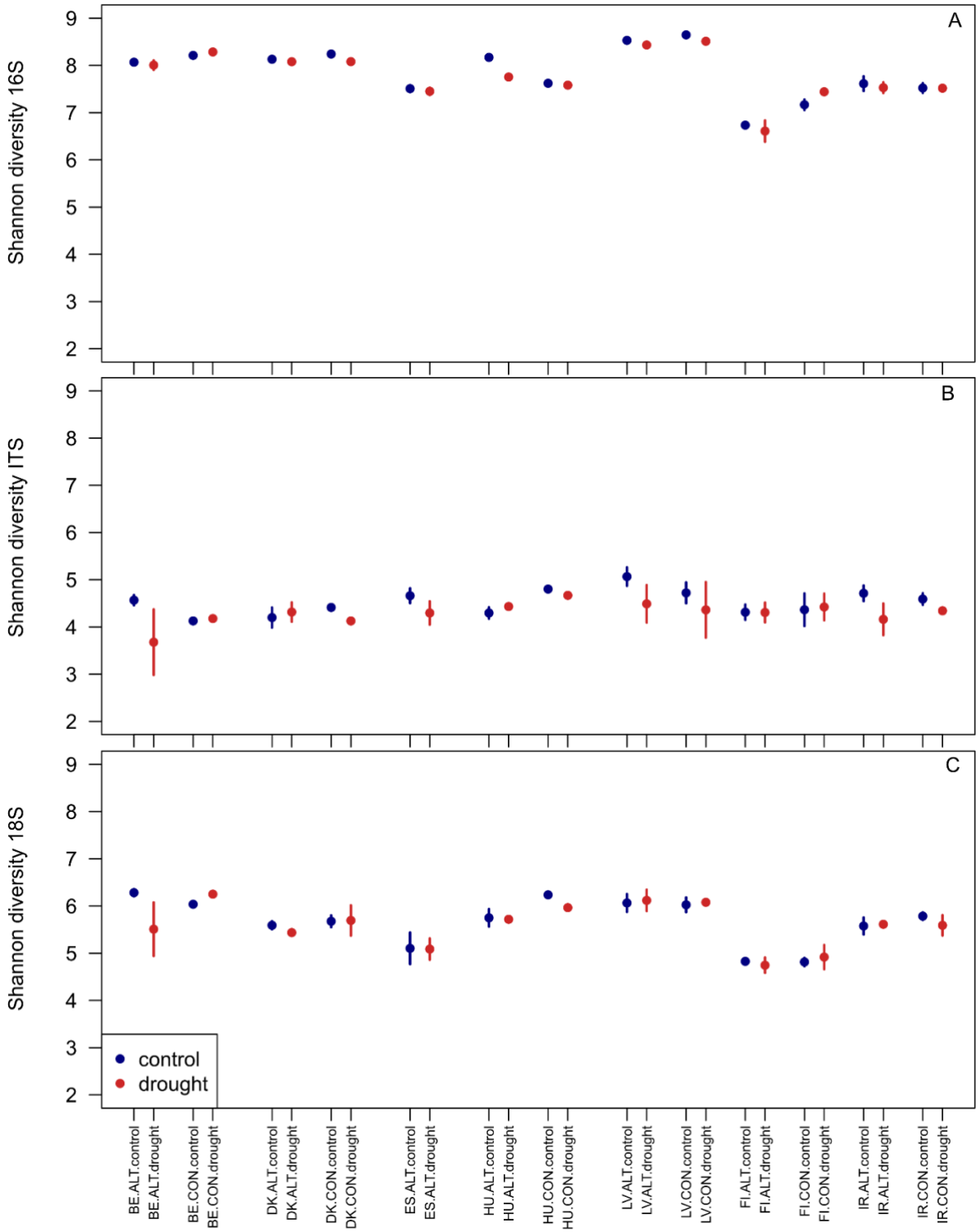

Figure 6. Alpha diversity of the sampled soils, measured with the Shannon diversity index, of prokaryotic (A), fungal (B) and eukaryotic (C) communities across the different field sites, color-coded by drought treatment, and split by management regime (ALT or CON). Individual values for each biological replicate (circles) and the average value across replicated (vertical line) are provided. ALT: alternative management, including organic farming in croplands (BE, DK, ES, HU, LV), mixed-species low-input systems in grasslands (IE), and continuous cover regimes in forests (FI). CON: conventional management, including conventional farming systems (BE, DK, ES, HU, LV), mono-species high-input grasslands (IE), and clearcut forests (FI).

Prokaryotic beta diversity showed a clear distinction by site (Figure 7)Figure 7Error! Reference source not found., with this factor explaining 75% of the variability (Table 5). However, a constrained analyses

where the drought treatment was used as constraining factor revealed separation by drought and by management (Figure 8). The management regime (conventional versus alternative) explained only 2% of variability but with the effect showing a strong dependency on the site (interaction term site x management explaining 10% of the variability, Table 5). Overall, the drought treatment did not significantly affect beta diversity of prokaryotes (Table 5).

Differences in prokaryotic beta diversity resulted in clear taxonomic patterns driven by site and management, with less variation across replicated samples (Figure 9 and Figure 10). The drought treatments had no clear general impact at phylum and genus level. Specifically, at the phylum level, Planctomycetota, Verrucomicrobiota, Acidobacteria, and Proteobacteria were the four most abundant phyla across all samples. There was a reduction in Firmicutes, Crenarchaeota and Chloroflexi in the forest (Figure 9). At the genus level, relative abundances are visibly changed with management, and the soil profiles of the sites in Latvia, Finland and Ireland had unique compositions compared to the other field sites (Figure 10).

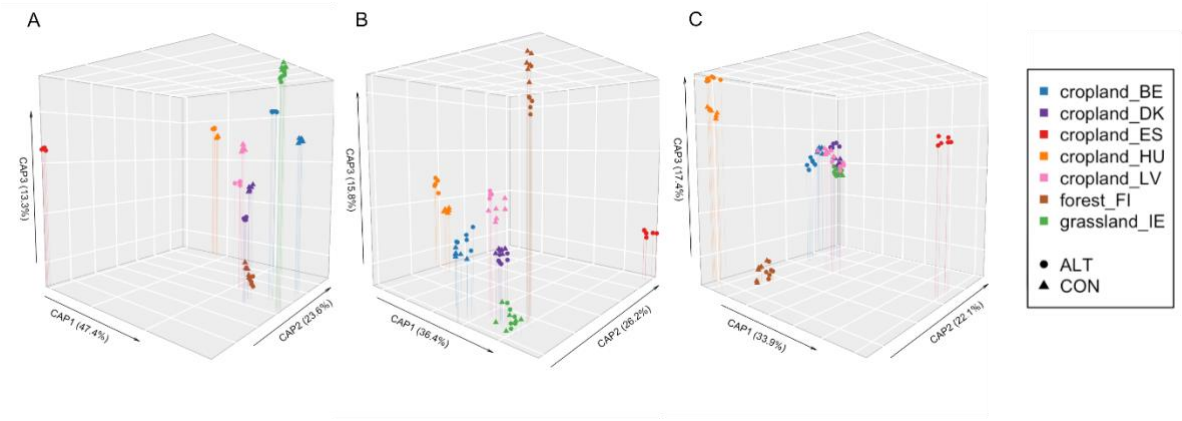

Figure 7. Beta diversity of sampled soils through canonical analysis of principal coordinates of prokaryotes (A), fungi (B), and eukaryotes (C) Bray-Curtis dissimilarity measure. The dots represent individual samples, comprising three biological replicates per treatment combination. Colors correspond to countries, and shapes to management. ALT: alternative management, including organic farming in croplands (BE, DK, ES, HU, LV), mixed-species low-input systems in grasslands (IE), and continuous cover regimes in forests (FI). CON: conventional management, including conventional farming systems (BE, DK, ES, HU, LV), mono-species high-input grasslands (IE), and clearcut forests (FI).

Table 5. Effects of the experimental factors on beta diversity of prokaryotes, fungi, and eukaryotes through PERMANOVA analyses of the Bray-Curtis dissimilarity measure. F, P and R^2 values are presented, and bolded when significant or italicized when marginally significant.

Factor	Prokaryotes F(P)	R^2	Fungi F(P)	R^2	Eukaryotes F(P)	R^2
Site (S)	**66.3 (0.0001)**	0.754	**22.4 (0.0001)**	0.575	**18.4 (0.0001)**	0.540
Management (M)	**9.5 (0.0001)**	0.018	5.9 (0.0001)	0.025	**4.7 (0.0001)**	0.023
Drought (D)	1.1 (0.3402)	0.002	1.4 (0.1241)	0.006	*1.6 (0.0562)*	*0.008*
S × M	**10.8 (0.0001)**	0.103	5.0 (0.0001)	0.107	**4.1 (0.0001)**	0.100
S × D	1.0 (0.4672)	0.011	*1.3 (0.0596)*	*0.032*	**1.4 (0.0097)**	0.040
M × D	1.0 (0.3833)	0.002	1.1 (0.3065)	0.005	1.2 (0.2496)	0.006
S × M × D	1.2 (0.2041)	0.011	*1.2 (0.0995)*	*0.026*	**1.3 (0.0370)**	0.032

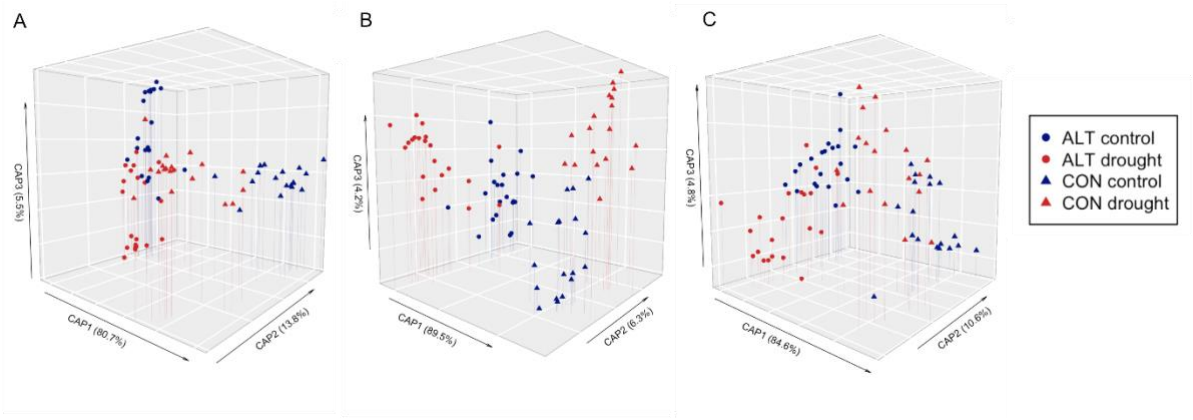

Figure 8. Beta diversity of sampled soils through canonical analysis of principal coordinates of prokaryotes (A), fungi (B), and eukaryotes (C) Bray-Curtis dissimilarity measure. The dots represent individual samples, comprising three biological replicates per treatment combination. Colors correspond to drought treatments, and shapes to management. ALT: alternative management, including organic farming in croplands (BE, DK, ES, HU, LV), mixed-species low-input systems in grasslands (IE), and continuous cover regimes in forests (FI). CON: conventional management, including conventional farming systems (BE, DK, ES, HU, LV), mono-species high-input grasslands (IE), and clearcut forests (FI).

Fungal alpha biodiversity was little affected by the examined factors, with substantial variability among replicated plots (**Figure 6**). Site had significant effect on fungal observed richness, but not Pielou's

evenness or Shannon diversity (Table 4). The management regime had no effects on alpha diversity, although there were some site-specific effects of management (see Appendix C). The drought treatment slightly reduced Shannon diversity (Figure 6).

Fungal beta diversity was affected by site (explaining 58% of the variability) and management (explaining 2%), with the management effect being site-dependent (explaining 3% by management, and 11% for the interaction of management with site). The drought treatment had no significant effects on the fungal beta diversity (Table 5). Despite some variability across samples, there are specific taxonomic patterns across the different sites, management systems and drought treatments, particularly visible at the genus level. Notably, Latvia and Ireland had a greater relative abundance of Glomeromycota, and Basidiomycota was much more abundant in the forest (Finland). In the arable fields, Chytridiomycota was more prominent compared to the grasslands. Evident changes at the genus level as response to drought such as decreased abundance of Pitoderma and Trichosporon under drought were obtained for Hungary and Finland, respectively. As mentioned above, a constrained analysis showed that beta diversity of fungi was influenced by drought and management (Figure 8).

Eukaryotic alpha diversity was affected by site (explaining 65% of the variability) and management (explaining 2% of its variability) (Table 4). Spain and Finland had the smallest Shannon indexes (Figure 6). The drought treatment reduced the observed richness (and explained 7% of its variability), but not the other alpha diversity metrics (Figure 6). Beta diversity was affected by site (explaining 54% of the variability) and management (explaining 2%), with the management effect being site-dependent (interaction explaining 10% of the variability). The drought treatment had a marginally significant influence, but with a significant site-dependency (4% variability explained by the interaction) (Table 5). Specific taxonomic patterns emerged between sites. Although no clear visual differences between management systems and drought treatments were found as shown by the relative abundances of genera and phyla (Figure 9 and Figure 10). A constraint analysis of beta diversity showed that eukaryotic communities did separate by drought and management (Figure 8).

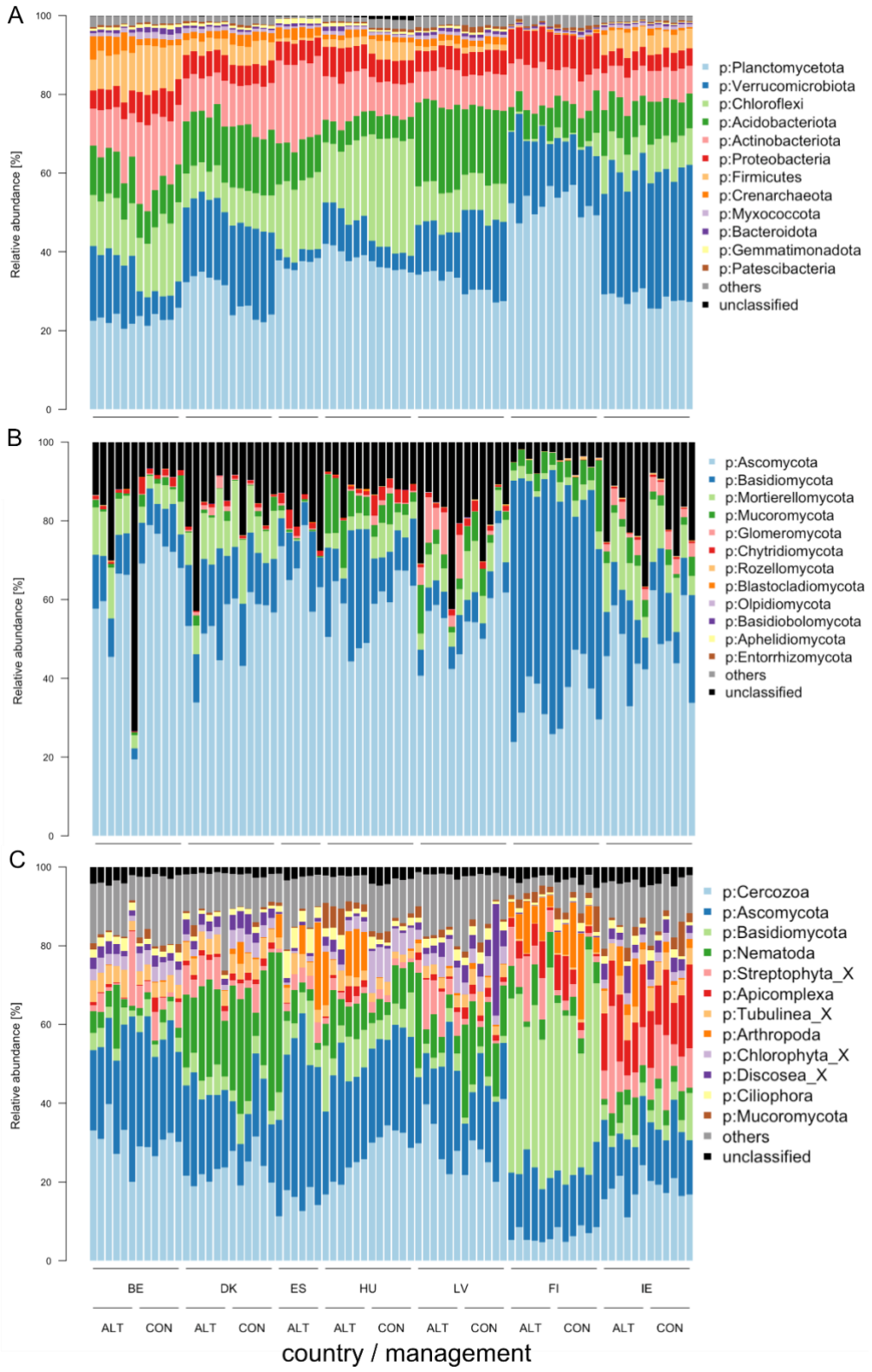

Figure 9. Relative abundance of phyla of prokaryotes (A), fungi (B,) and eukaryotes (C) across the samples, grouped by country and management. ALT: alternative management, including organic farming in croplands (BE, DK, ES, HU, LV), mixed-species low-input systems in grasslands (IE), and continuous cover regimes in forests (FI). CON: conventional management, including conventional farming systems (BE, DK, ES, HU, LV), mono-species high-input grasslands (IE), and clearcut forests (FI). The three first samples (from left to right) within a given country x management combination correspond to control, and the next three, to drought

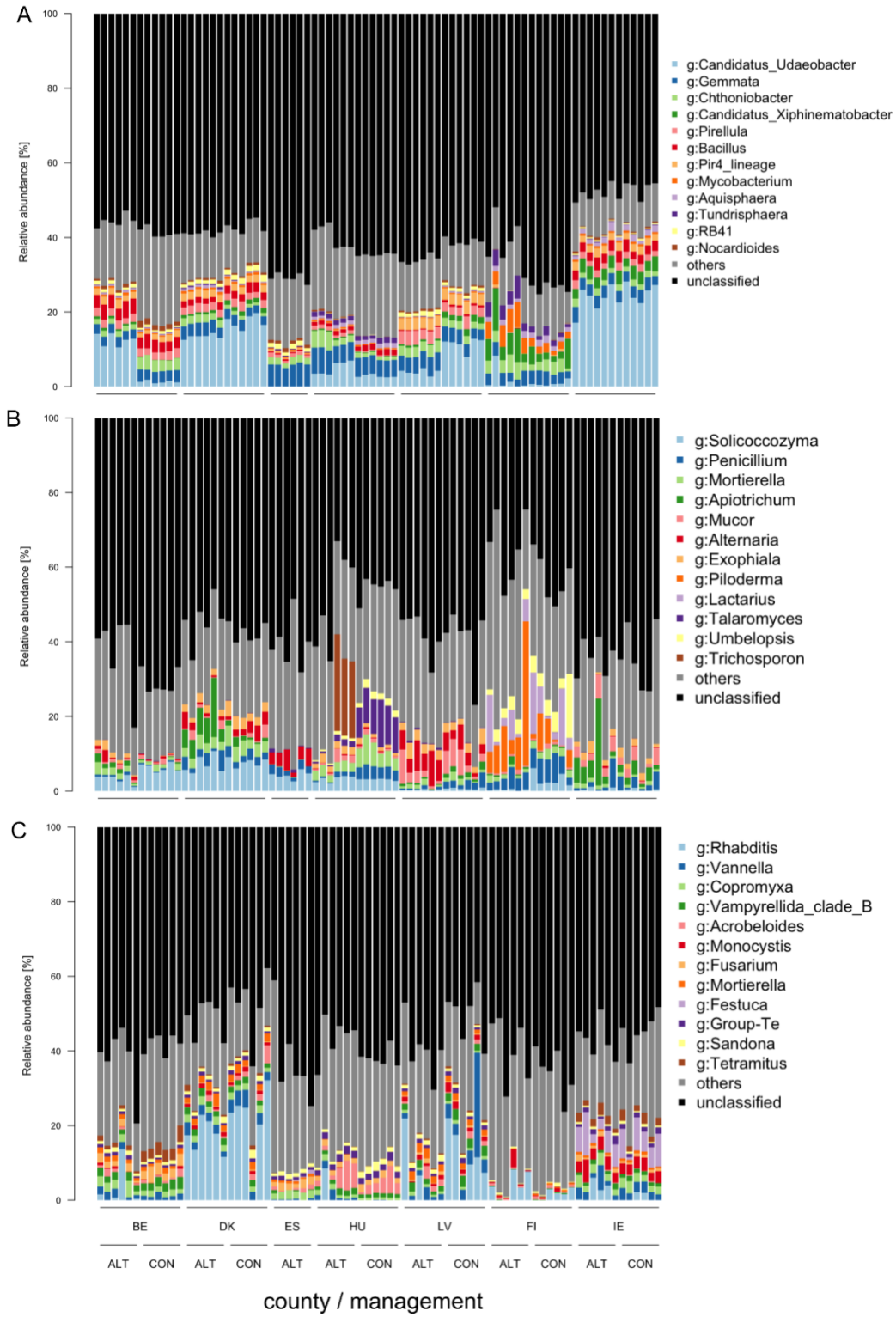

Figure 10. Relative abundance of the most abundant genera of prokaryotes (A), order of fungi (B) and orders of eukaryotes (C) across the samples, grouped by country and management. ALT: alternative management, including organic farming in croplands (BE, DK, ES, HU, LV), mixed-species low-input systems in grasslands (IE), and continuous cover regimes in forests (FI). CON: conventional management, including conventional farming systems (BE, DK, ES, HU, LV), mono-species high-input grasslands (IE), and clearcut forests (FI). The three first samples (from left to right) within a given country x management combination correspond to control, and the next three, to drought.

9.1.3. Discussion and preliminary conclusions from Task 3.3.

In this first assessment of the variability of soil biodiversity in the experimental sites we found that the different sites harbor a unique soil biodiversity, with the factor site explaining 13-75% of the variance in the data of alpha and beta soil diversity. Also, management effects significantly influenced all three groups of the soil biota but explained only around 2% of the variability. However, these management effects were highly site-specific, with the interaction between site and management explaining around 10% of the variability. Drought effects were small, occasionally site-specific, and often masked by other drivers. Constrained statistics and analyses separated by site were able to reveal these effects.

Fungal and eukaryotic communities seem to be less responsive than prokaryotes to site, management and drought, while showing effects of specific groups. Further analyses would entail the research of possible conditions favoring these specific groups using a taxonomic-level analyses. These results, together with the prokaryotic biodiversity, indicate that more in-depth analyses are needed to identify taxonomic groups that respond to the combined effects of management and drought. It is important to note that depending on the taxonomic rank, we have covered varying percentages of the biodiversity of the collected soils with the metabarcoding analyses. For example, prokaryotes, eukaryotes and fungi were represented in ~100, ~90, and ~80% respectively at the phylum level (Figure 9). However, only ~50% of the known genera were detected with this approach (Figure 10).

Overall, we show that each site should be taken individually to assess the effects of climate change and management on soil biodiversity, revealing conditions under which climate change might have impact on the biodiversity.

9.1.4. Next steps for Task 3.3.

Given that we have reported on the general patterns of soil biodiversity changes at T1 for 2022, we will continue analyzing the metabarcoding data of 2022 to include the recovery phase, T2, and compare the two sampling points. We will then further analyze data from 2023 and compare the different sampling points and the two years. After having received a comprehensive overview of the results, we will select samples from the combination of treatments that have shown the most significant effects on soil biodiversity for metagenome sequencing to get a better understanding of changes in the underlying functional potential of these communities.

9.2. Plant-soil interactions

Data for rhizosphere and root endosphere samples from T1 (drought period) in the first year (2022) are presented. Data from the second year (2023) of simulation are currently queued for sequencing.

9.2.1. Material and Methods

A detailed description of sampling, sample preparation and laboratory analyses is presented in Appendix D. Briefly, oven-dried root and rhizosphere samples were shipped to the labs in AIT for processing and rhizosphere soil collection, and separation of root tissue. A PCR protocol was developed to prepare the libraries, and then the sequencing was outsourced to LGC (Berlin, Germany).

9.2.2. Results

Most samples yielded sufficient reads for microbial community analyses, but some samples had to be excluded from further analyses due to low read numbers. Therefore, data from nearly all Finish and Hungarian root endosphere samples are missing for both, the prokaryotic and the fungal communities. Furthermore, in the Belgian samples, roots were broken into small pieces, most likely because of the drying process. A proper separation of rhizosphere soil and root material was therefore difficult.

Alpha diversity

Alpha diversity measured by the Shannon Diversity Index was significantly higher in the rhizosphere than in the root endosphere for both prokaryotes and fungi in most countries where sufficient data were available for both compartments (Figure 11). No comparison between the rhizosphere and endosphere compartments was possible for Hungary and Finland due to missing endosphere samples. Additionally, no difference in prokaryotic alpha diversity could be observed for the Belgian samples, most likely due to incomplete separation of endosphere and rhizosphere material during sample preparation for DNA isolation. Interestingly, this mixing effect was not observed for the fungal alpha diversity.

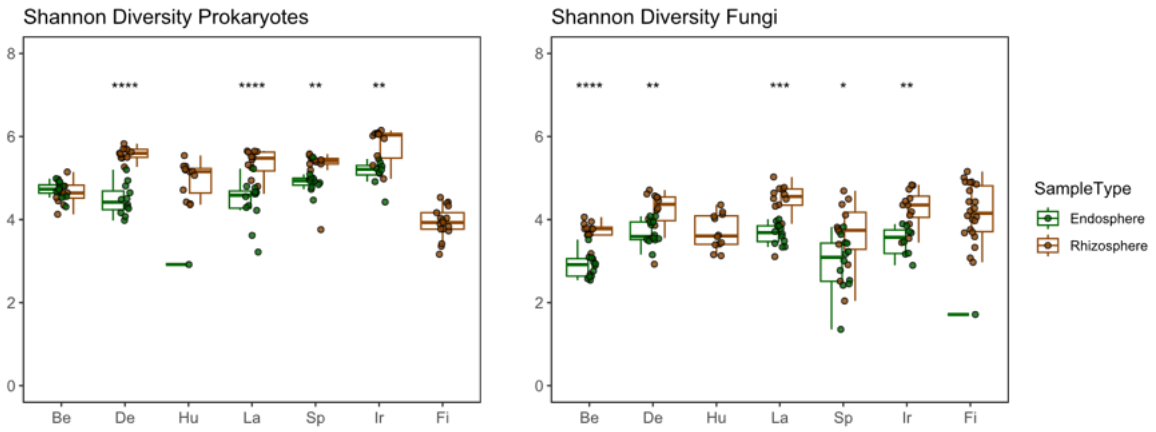

Figure 11. Alpha diversity (Shannon Diversity Index) of prokaryotes (left panel) and fungi (right panel) in root endosphere (green) and rhizosphere (brown). Individual values for each sample (circle) are shown together with boxplots by compartments (endosphere vs. rhizosphere). Data from different management regimes and drought treatments were combined. Significant differences (Wilcoxon test) between compartments are indicated by asterisks (p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001, **** p ≤ 0.0001).*

Differences in alpha diversity were statistically significant for the factor site for prokaryotes and fungi in both root compartments (Table 6). The difference between alternatively managed soils (organic croplands, mixed-species grasslands, continuous-cover forests) and conventionally managed soils (conventional croplands, mono-species grasslands, clear-cut forests) was only significant for prokaryotic alpha diversity in the root endosphere, showing slightly higher values in the alternatively managed soils. A slightly increased prokaryotic alpha diversity in the root endosphere under alternative management regimes was found in Belgium, Latvia and Spain (Figure 12). In the rhizosphere, no global management effect was observed; however, a significantly higher prokaryotic alpha diversity was observed in the alternatively managed soils in Belgium, Denmark, Latvia and Finland, while the opposite was true for Hungary and Ireland. No significant effects of management on fungal alpha diversity in endosphere and rhizosphere could be observed (Figure 12). Drought effects on alpha diversity were generally small, but became visible in the root endosphere in Denmark for prokaryotes and to a lesser degree also for fungi (Figure 12).

Table 6. Effects of experimental factors on prokaryotic and fungal alpha diversity in the root endosphere and rhizosphere as assessed by ANOVA of the Shannon diversity index. Values represent F-ratios and P-values, in bold when significant (p < 0.05).

Factor	Root endosphere		Rhizosphere	
	Prokaryotes F(P)	Fungi F(P)	Prokaryotes F(P)	Fungi F(P)
Site (S)	**16.1 (<0.001)**	**12.4 (<0.001)**	**49.3 (<0.001)**	**6.4 (<0.001)**
Management (M)	**9.5(0.004)**	1.8 (0.193)	0.2 (0.626)	0.0 (0.850)
Drought (D)	0.4 (0.518)	2.4 (0.131)	0.2 (0.693)	0.0 (0.859)
S × M	2.5 (0.058)	1.0 (0.415)	**9.1 (<0.001)**	**5.1 (<0.001)**
S × D	**3.2 (0.023)**	0.7 (0.605)	1.4 (0.240)	**2.8 (0.019)**
M × D	1.5 (0.234)	**5.0 (0.032)**	2.3 (0.137)	**5.4 (0.024)**
S × M × D	2.0 (0.130)	1.5 (0.233)	1.5 (0.207)	0.6 (0.675)

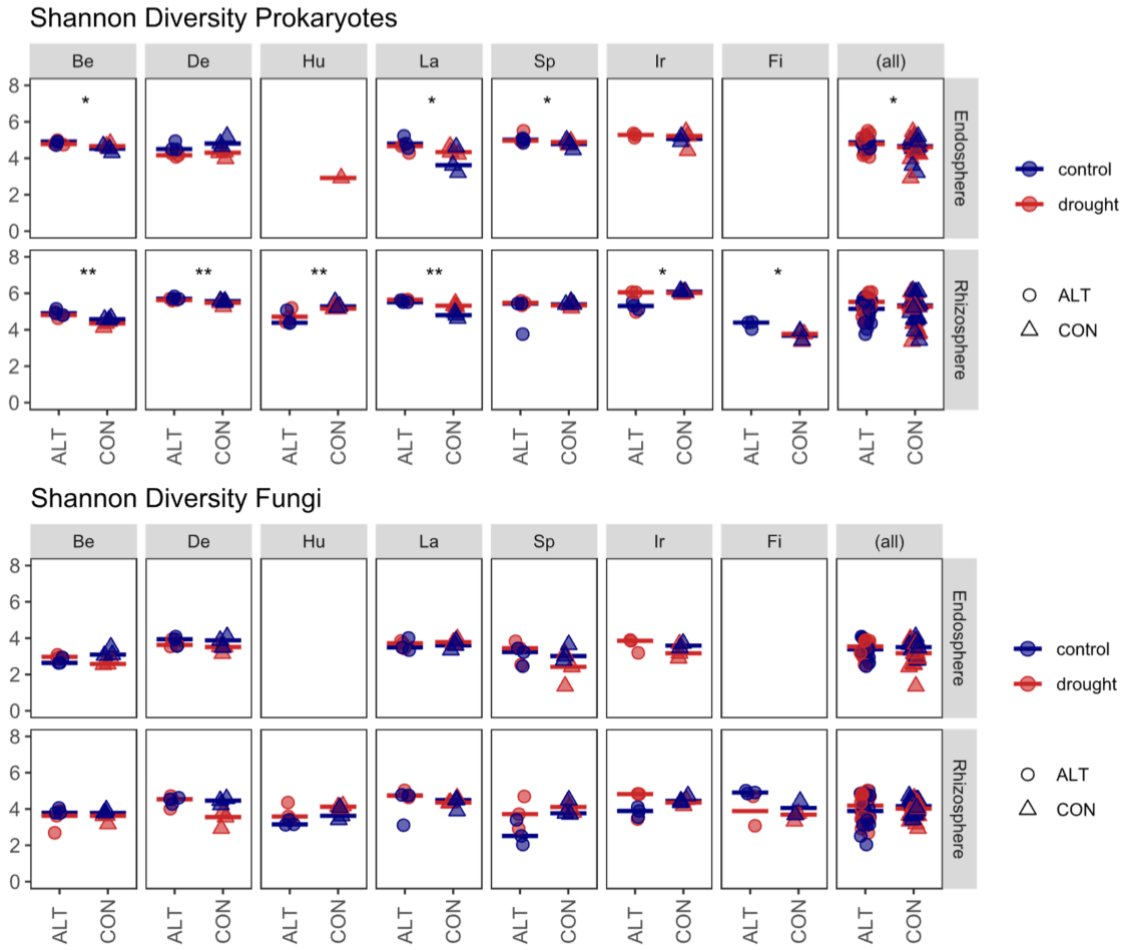

Figure 12. Alpha diversity (Shannon Diversity Index) of prokaryotes (upper panel) and fungi (lower panel) in root endosphere (upper row in each panel) and rhizosphere (lower row in each panel). Individual values for each sample are shown together with the median for control (blue) and drought (red) treatment for each management regime (alternatively managed soils: circle; conventionally managed soils: triangle) separated by country. Right margins show values for all countries. Statistically significant differences (Wilcoxon test) between management regimes are indicated by asterisks. Significant differences (Wilcoxon test) between management regimes are indicated by asterisks (p ≤ 0.05; ** p ≤ 0.01).*

Beta diversity

Differences in microbial community composition between root compartments (endosphere vs. rhizosphere) were statistically significant but explained only 5.5% and 3.2% of the variance for the prokaryotic and fungal communities, respectively (Table 7). For each compartment, site was the most important factor and explained around 50% and 35% of the variance for the prokaryotic and fungal communities, respectively. Therefore, a strong clustering of the communities by site was observed in the PCoA (Figure 13). An additional 8-12% of the variance was be attributed to the interaction between site and management (S × M).

Table 7. Effects of experimental factors on prokaryotic and fungal beta diversity in the root endosphere and rhizosphere as assessed by PERMANOVA of the Bray-Curtis dissimilarities. Values represent F-ratios and P-values, in bold when significant (p < 0.05).

Factor	Root endosphere		Rhizosphere	
	Prokaryotes F(P)	Fungi F(P)	Prokaryotes F(P)	Fungi F(P)
Site (S)	**14.3(0.001)**	**9.1(0.001)**	**17.4(0.001)**	**7.4(0.001)**
Management (M)	**3.7(0.001)**	**1.8(0.014)**	**3.4(0.001)**	**1.9(0.004)**
Drought (D)	**2.4(0.006)**	1.5(0.054)	**1.7(0.042)**	1.4(0.072)
S × M	**4.1(0.001)**	**1.9(0.001)**	**4.1(0.001)**	**2.4(0.001)**
S × D	**2.2(0.001)**	**1.4(0.023)**	**1.6(0.002)**	**1.6(0.001)**
M × D	1.3(0.163)	1.0(0.447)	1.4(0.132)	1.3(0.092)
S × M × D	1.3(0.102)	0.9(0.677)	**1.6(0.003)**	**1.3(0.002)**

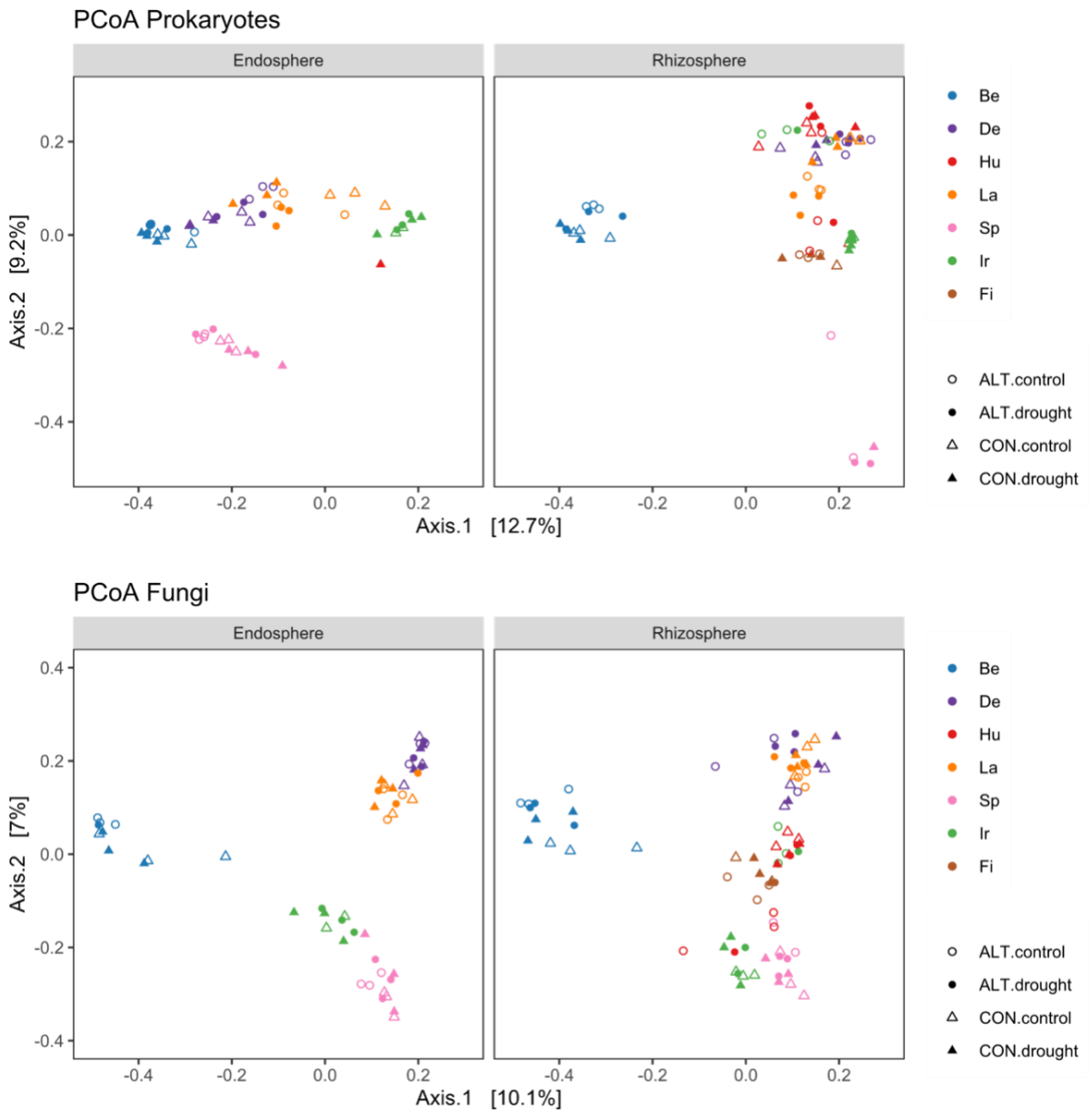

Figure 13. Beta diversity of prokaryotic (upper panels) and fungal (lower panels) communities in the root endosphere (left panels) and rhizosphere (right panels) samples visualized by principal coordinate analysis (PCoA). Dots represent individual samples, comprising three biological replicates per treatment combination. Colors correspond to countries, shapes to management and filling to drought treatment.

When each country was analyzed separately, separation of root endosphere and rhizosphere samples became clearly visible for both prokaryotic and fungal communities, respectively (**Figure 14**). For Belgium, there was, however, an overlap between the two root compartments for prokaryotes, as it was expected based on the difficulties in separating root fragments and rhizosphere soil. For fungal communities, this effect was not observed.

In Belgium and Denmark, a separation of communities from alternatively and conventionally managed soils were clearly visible, especially for the prokaryotes, but also for fungi. To a lesser extent, this was also the case in Latvia. In Ireland, the separation of management regimes was only evident in the rhizosphere communities but interestingly not in the root endosphere communities. No management effect was observed for the Spanish samples.

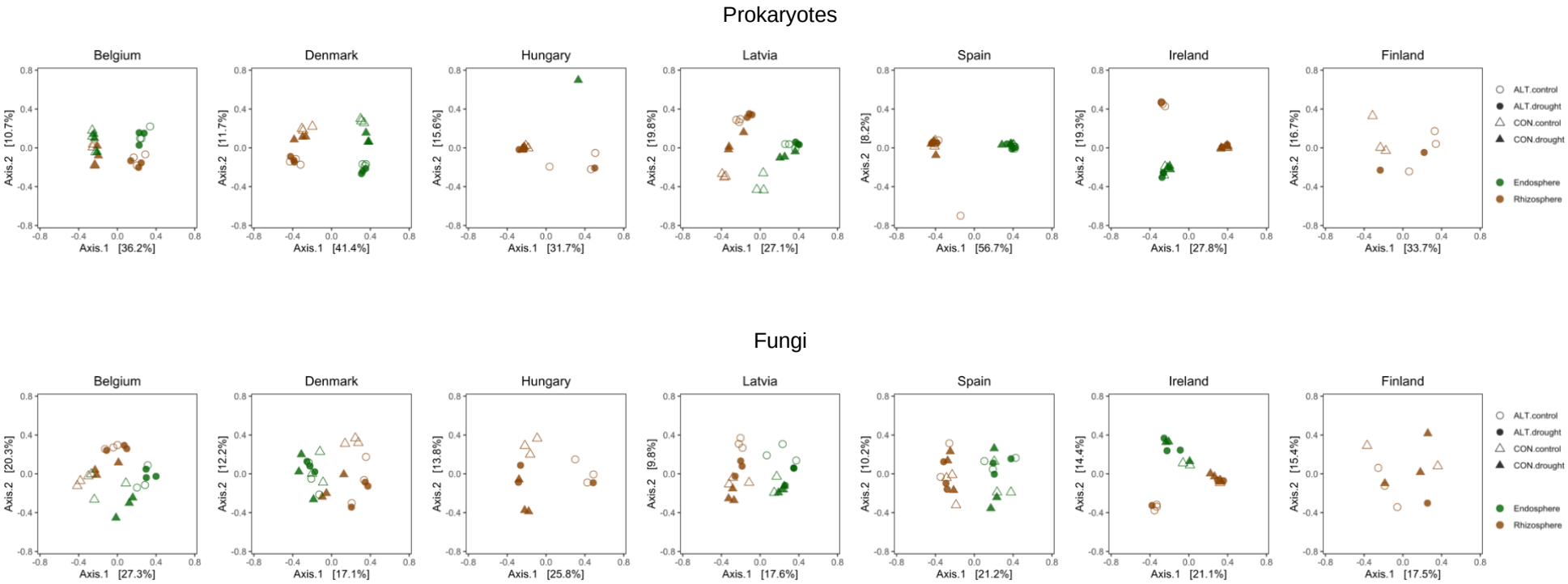

Figure 14. Beta diversity of prokaryotic and fungal communities in root endosphere (green) and rhizosphere (brown) separately shown for each site. Dots represent individual samples, comprising three biological replicates per treatment combination. Shapes correspond to management and filling to drought treatment.

Taxonomic composition of root endosphere and rhizosphere communities

Archaea, which were partially covered by the prokaryote-specific primer pair, were essentially absent from all root endosphere samples, but reached relative abundances up to ca. 5% in rhizosphere samples from Denmark, Latvia and Spain (**Figure 15**). Archaea showed low relative abundance in Ireland, Belgium and Hungary, and were essentially absent in Finland.

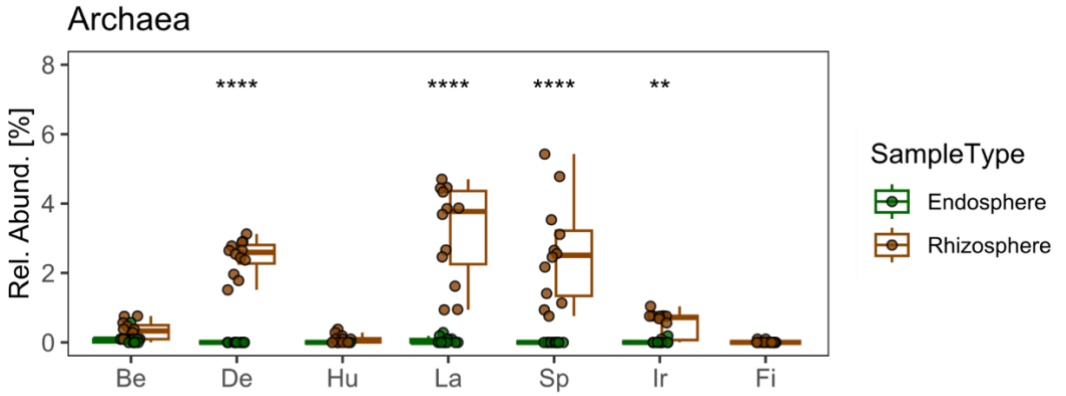

*Figure 15. Relative abundance of Archaea in root endosphere (green) and rhizosphere (brown) samples across the different sites. Individual values for each sample (circle) are shown together with boxplots by compartment (endosphere vs. rhizosphere). Data from different management regimes and drought treatments were combined. Significant differences (Wilcoxon test) between compartments are indicated by asterisks (** $p \leq 0.01$; **** $p \leq 0.0001$).*

The six most abundant prokaryotic phyla showed significant differences in relative abundance between the root compartments (endosphere vs. rhizosphere) across all countries, management types and drought treatments (**Figure 16**). Actinobacteriota, Proteobacteria and Bacteroidota showed higher relative abundance in the root endosphere, whereas Planctomycetota, Firmicutes and Chloroflexi showed higher relative abundance in the rhizosphere.

The research leading to these results has received funding from the European Union Horizon 2020 Research & Innovation programme under the Grant Agreement no. 101000371.

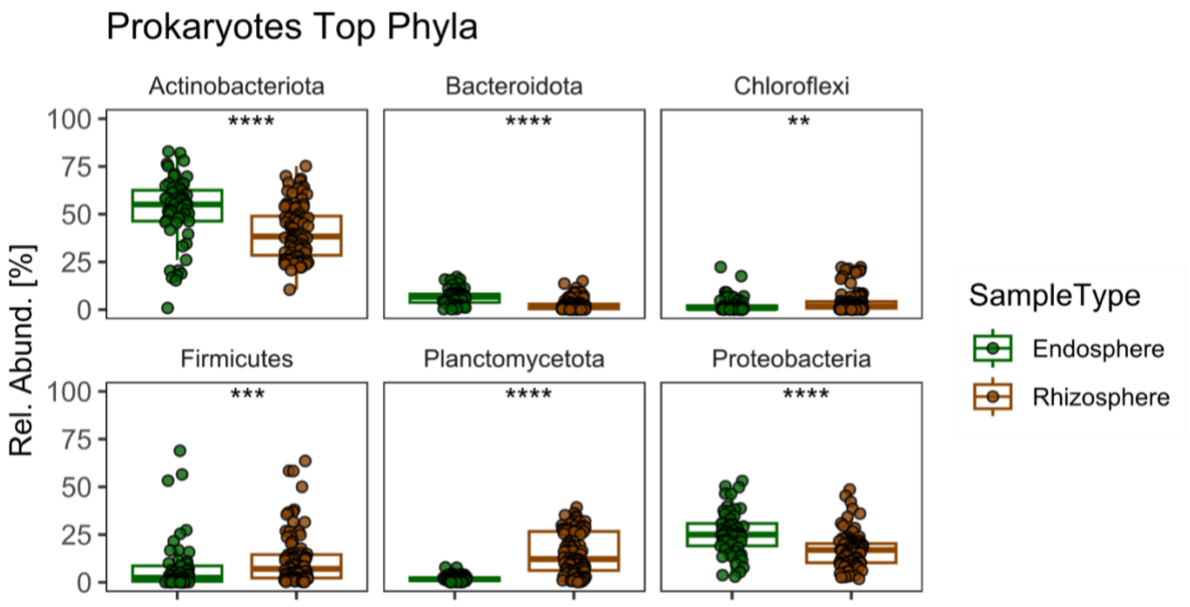

*Figure 16. Relative abundance of the topmost abundant prokaryotic phyla in root endosphere (green) and rhizosphere (brown) samples. Individual values for each sample (circle) are shown together with boxplots by compartment (endosphere vs. rhizosphere). Data from different countries, management regimes and drought treatments were combined. Significant differences (Wilcoxon test) between compartments are indicated by asterisks (** p ≤ 0.01; *** p ≤ 0.001; **** p ≤ 0.0001).*

No consistent management and drought effects on abundant prokaryotic phyla were detected. In some cases, statistically significant effects were seen at some sites, but patterns were not consistent across all sites. Relative abundances of the group Planctomycetota in the rhizosphere across the different management regimes and drought treatments at each site are shown exemplary in **Figure 17**.

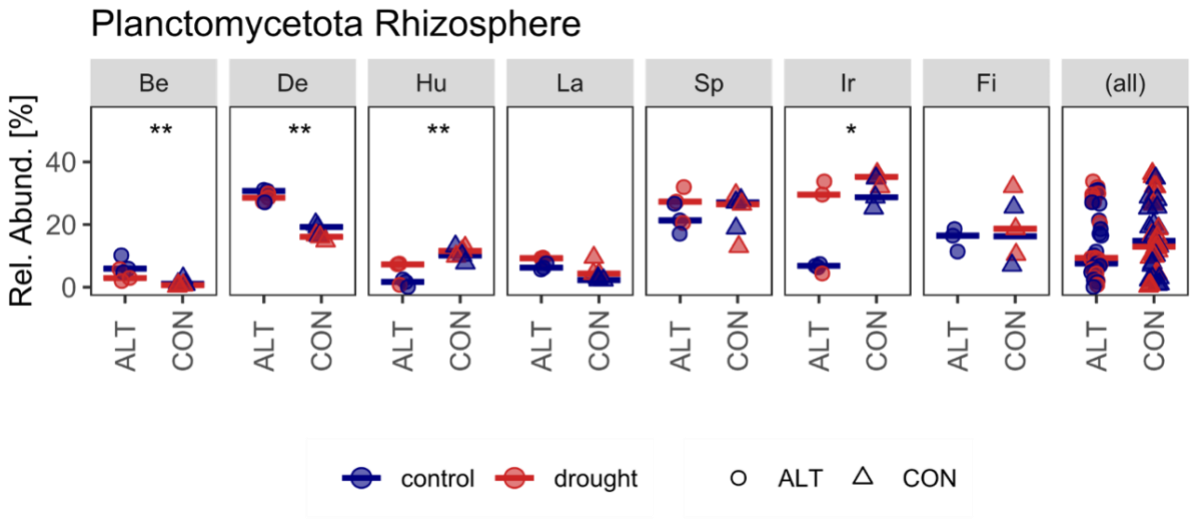

Figure 17.. Relative abundance of Planctomycetota in the rhizosphere. Individual values for each sample are shown together with the median for control (blue) and drought (red) treatment for each management regime (alternatively managed soils:

The research leading to these results has received funding from the European Union Horizon 2020 Research & Innovation programme under the Grant Agreement no. 101000371.

circle; conventionally managed soils: triangle) separated by country. Right margin shows values for all countries. Significant differences (Wilcoxon test) between management regimes are indicated by asterisks (p ≤ 0.05; ** p ≤ 0.01).*

For fungi, two fungal orders were identified that showed differences in relative abundance between the two different root compartments. Capnodiales had higher relative abundance in the rhizosphere, whereas Xylariales had higher relative abundances in the endosphere (**Figure 18**). Members of the Xylariales are often found as endophytes in different plant tissues (Becker and Stadler, 2021).

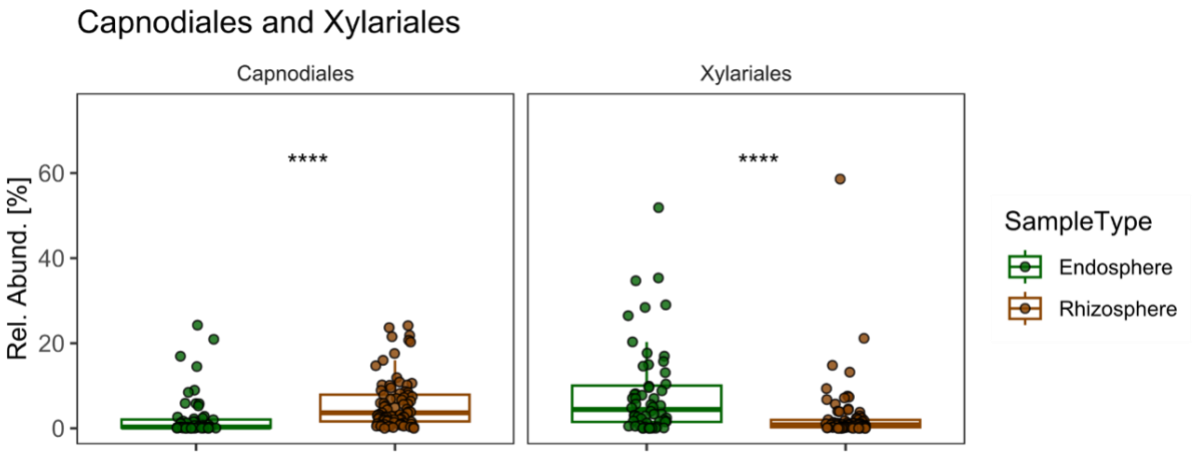

Figure 18. *Relative abundance of the fungal orders Capnodiales and Xylariales in root endosphere (green) and rhizosphere (brown) samples. Individual values for each sample (circle) are shown together with boxplots by compartment (endosphere vs. rhizosphere). Data from different sites, management regimes and drought treatments were combined. Significant differences (Wilcoxon test) between compartments are indicated by asterisks (**** p ≤ 0.0001).*

9.2.3. Preliminary conclusions

Compartment (root endosphere vs. rhizosphere) had a strong impact on alpha and beta diversity, and strong differences in community composition were observed across the different sites. Management regimes and drought treatment were of lower importance and showed site-dependent effects. Changes in root endosphere and rhizosphere microbial communities largely correlate with the changes observed in the bulk soils.

9.2.4. Next steps

Analyses of the samples from year two (2023) are currently in progress. Combined analysis of datasets from bulk soil (ETH), root endosphere and rhizosphere (AIT) are planned and will be discussed in detail in an upcoming data workshop (September 2024 in Barcelona).

9.3. Nitrogen-cycling guilds

9.3.1. Methods

DNA extracted from bulk soil at University of Alicante was shipped to INRAE to quantify total prokaryotic abundance and N cycling genes. Here, we present results of 2022, sampling point T1, right at the end of the simulation. The abundance of the total bacterial community was quantified using 16S rRNA gene primer-based real-time quantitative PCR (qPCR) assays (Muyzer et al., 1993). The *amoA* gene was used as molecular marker to quantify the bacterial and archaeal ammonia-oxidizers (AOB

The research leading to these results has received funding from the European Union Horizon 2020 Research & Innovation programme under the Grant Agreement no. 101000371.

and AOA, respectively, (Rotthauwe et al., 1997, Bru et al., 2011) as well as the comammox *amoA* targeting clade A and B from the *Nitrospira* genus (abbreviated *comaA* and *comaB* respectively, Pjevac et al., 2017). The *nirK* and *nirS* genes were used to quantify the denitrifiers (Henry et al. 2004; Throbäck et al. 2004), and the N_2O-reducers were quantified using the *nosZI* and *nosZII* genes (Henry et al., 2006; Jones et al., 2013).

9.3.2. Results

A synthesis of the results is presented in **Table 8**. Detailed results are presented in Appendix F. In general, 16S rRNA gene abundance was not significantly affected by any of the experimental treatments in any of the countries, except for Hungarian soils. In Hungary, the abundances of all targeted N-cycling microbial communities also changed in response to drought and/or management treatments. In contrast no effects of the latter were detected in Ireland. The bacterial ammonia oxidizers (AOB) to archaeal ammonia oxidizers (AOA) ratio, both involved in nitrification, was significantly influenced by the management treatment in Belgium, Denmark, Latvia and Hungary. Yet, the proportion AOB in the total bacterial community showed opposite trends in response to management. The relative abundances of bacteria performing the complete nitrification (comammox clade A and comammox clade B), were affected by drought in Latvia, and by management in Hungary and Finland. The ratio of comammox clade B over comammox clade also significantly changed in response to management in Latvia and Hungary. The nirK and nirS gene relative abundances, corresponding to the denitrifiers capacity, as well as their ratio, were significantly influenced by management in Belgium, Hungary and Finland. In Hungary, significant interactions of the management and drought on nirK and nirS relative abundances were detected. The relative abundances of the nosZI and nosZII genes, corresponding to the bacteria capable to reduce the GHG N2O, were affected by management in Latvia and Hungary. In Belgium, Denmark and Hungary, the nosZII to nosZI ratio was affected by drought, and this effect was dependent on management in Denmark, as shown by a significant interaction of drought and management treatments.

The research leading to these results has received funding from the European Union Horizon 2020 Research & Innovation programme under the Grant Agreement no. 101000371.

*Table 8. Summary of the effects of management (M), drought (D) and their interaction on the N-cycling guilds (D*M), and the associated related processes. Conv and Alt stand for conventional and alternative management respectively, and > and < symbols for significantly higher or lower mean in the different treatments (Tukey's test p<0.05). Only significant effects are shown.*

		Belgium	Denmark	Finland	Hungary	Ireland	Latvia
Total community	16S (copies/ng DNA)				D (Control<Drought)		
Nitrification	AOB/16S		M (Conv<Alt)		M (Conv<Alt)		M (Conv>Alt)
	AOA/AOB	M (Conv<Alt)	M (Conv>Alt)		M (Conv>Alt)		M (Conv<Alt)
Denitrification	nirK/16S	M (Conv<Alt)		M (Conv>Alt)	D*M		
	NirS/16S				M (Conv>Alt), D*M		
	NirK/NirS	M (Conv<Alt)		M (Conv>Alt)	M (Conv<Alt)		
N2O reduction	NosZ1/16S				M (Conv>Alt)		
	NosZII/16S				M (Conv<Alt)		M (Conv>Alt)
	NosZII/NosZI	D (Control<Drought)	D*M, D (Control>Drought)		D (Control>Drought)		
Complete nitrification	ComaA/16S			M (Conv>Alt)	M (Conv<Alt)		D (Control>Drought)
	ComaB/16S				M (Conv<Alt)		D (Control>Drought)
	ComaB/ComaA				M (Conv<Alt)		M (Conv>Alt)

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9.3.3. Next steps

Analyses of the second timepoint in 2022 (two months after the simulation), as well as the sampling points of 2023 (T0, T1 and T2) are in progress. Once we have all the results compiled, we will integrate them into the multifunctionality results to try to find possible explanatory variables of the observed differences in the nitrogen cycle guilds.

9.4. Progress report on metagenomics, PLFAs, NLFAs and food-web and co-occurrence networks

Metagenome sequencing: These analyses will be done once we have identified the factor combination with the most interesting and contrasting communities in the metabarcoding data. Therefore, this will be performed after samples from 2023 (year 2) are analyzed with metabarcoding.

PFLAs and NFLAs: Soils were collected across the LTEs by each local sampling team at T1 and T2 of 2022, and T0, T1, and T2 of 2023. Soils for the analysis of phospholipid-derived fatty acids (PLFAs) and neutral lipid-derived fatty acids (NLFAs) were frozen, freeze-dried and shipped with cooling packs to UvA. There, each soil sample was gently crushed, then it was sieved through a 1-mm mesh to eliminate most root fragments, which could otherwise interfere with the measurement of fungal PLFAs. After that, samples were stored cold until analysis. PLFAs and NLFAs were extracted from all arable soils, grassland soils, and from the organic layer of forest soils. The results of these analyses are not available for this version of the deliverable but will be included in D3.3. A detailed explanation is provided in **Appendix G**.

Food-web and co-occurrence networks analyses for each LTE and climate scenario: These analyses will be done once we have the entire dataset, including data from 2022 and 2023.

10. Task 3.4 Region-specific cascading effects of future soil biodiversity dynamics on soil multifunctionality

The main aim of this task is to understand the effect of sustainable management (vs conventional) and the climate change treatment posed (rainout shelter during this first year) on ecosystem functioning. To do so, we have quantified the impact of climate stressors and soil management on soil functions (*Table 9*) for each of the seven experimental sites. Later, we pooled together all our cropland sites (five out of seven experimental sites) to further analyze the influence of soil management and climate stress on the synergies and trade-offs between each pair of functions, as well as to better understand the role that soil biodiversity has in determining the response of soil functioning to our treatments.

10.1. Region and biome-specific impact of climate stressors on soil biodiversity and multifunctionality status under different types of soil management

10.1.1. Methods

We have measured 14 out of the 18 ecosystem indicators planned for WP3 (*Table 9*). All soil variables are being measured following standard protocols (Appendix E) and, whenever possible, adjusted to

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methodological guidelines by SOILBON (Guerra et al. 2021) and JRC (https://esdac.jrc.ec.europa.eu/ESDB_Archive/eusoils_docs/other/EUR26102EN.pdf). The latter includes the soil depth at which samples were taken (0-10 cm), the DNA extraction kit used, and the measurements of bulk density, litter decomposition, soil enzymatic activities, soil aggregate stability and nutrient cycling.

For illustrative purposes only, the nature contribution to people (NCP) to which each soil analysis relates to is provided in Table 5. This NCP classification is based on IPBES terminology and the valuation (between 0-100% for each NCP, adding up to 100% in total) comes from the interviews to stakeholders of three of the experimental sites, performed by WP4. While this NCP classification and its measure of relative importance is not used in the analyses presented in this deliverable, these will be considered when calculating multifunctionality metrics in Deliverable 3.3. Detailed descriptions of the methods to acquire the different variables as well as the statistical procedures and models are provided in Appendices E to G. These ecosystem indicators (*Table 9*) were quantified in parallel to soil biodiversity (including the data presented in task 3.3). The ecosystem indicators are measured on samples from first year, 2022 (year 1), at the two sampling points: T1 [resistance phase, during the climatic treatment] and T2 [recovery phase, after the climatic treatment]. For simplicity, however, only results for the resistance phase are presented in this deliverable. To produce region-specific evaluations of the impact of climate stressors and soil management on soil functioning, we used two-way ANOVAs (considering soil management, climatic treatment, and their interaction). These analyses were performed for each ecosystem indicator and experimental site separately.

Table 9. Soil physico-chemical analyses and other soil functions, organized by their relationship with nature's contributions to people (NCPs). NCPs follow the IPBES terminology and have the valuation of their relative importance for local stakeholders (adding up to 100%), according to results from WP4, averaged across the different regions. Specific measurements and units are provided, together with the status of those measurements for WP3 (first year sampling; second year are all in progress) and the partner producing this information. Justification for the relationship with NCPs or other additional information is provided as "comments". LSTs = local sampling teams (partners of each region where we have field experiments), rest of acronyms at the beginning of this document. Different colors are added for visualization purposes (to differentiate between different categories of Nature contributions to people [NCPs])

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Related NCP	Measurement	Units	Status	Comments
None, basic physicochemical attributes	Texture	% sand, clay and silt	Done (**UA**)	General soil characterization, not directly related to NCPs, but important to interpret results
	Bulk density	kg soil/m3 volume	Done (LSTs, UA)	
	pH	Unitless	Done (**UA**)	
	Electric conductivity	µS/cm	Done (**UA**)	
Food production (32.1%; range 18.6-44.5%)	Crop yield	Kg/Ha	In progress (**LSTs**)	Obtained directly from the farmers or measured in situ by LSTs
Soil formation and protection (22.6%; range 13.8-27.4%)	Soil aggregates stability	Semi-quantitative (scores 1 to 12)	Done (**UA**)	Related to resistance to further erosion
	Available P	mg P/kg soil	In progress (**UA**)	Plant-available nutrients
	Available N	mg NO3-/kg soil + mg NH4-/kg soil	Done (**UA**)	
	Litter decomposition (tea bag index)	% weight loss/day	Done (LSTs, UA)	Capacity to degrade litter
	Soil enzymatic activities	nano-mols of MUF (methylumbelliferyl)/g. dry soil · hour	Done (**UA**)	Potential capacity of the soil to cycle amino acids, carbohydrates, and mineral (P) resources.
	Potential N mineralization	mg N/kg soil · day	Done (**UA**)	Potential rates of different steps within the N cycle (ammonification, nitrification, depolymerization)
	Degradation C sources (MicroResp)	µg/g · h of CO_2-C	In progress (**UA**)	Potential capacity of the soil to degrade multiple C sources.
	N cycle guilds	gene copies/ng of DNA	Done (**INRAE**)	Abundance of 8 genes related to ammonia

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				oxidizing bacteria (AOB) archaea (AOA), and Nitrospira (ComaA, ComaB), denitrifiers (NirK, NirS), and nitrous oxide reducers (NosZ1, NosZ2). Quantifies different pathways of the N cycle
	Amount of mycorrhizal fungi	Estimated biomass of AMF, based on NLFAs	In progress (**UvA**)	Mycorrhizal fungi aids crop growth, mainly under nutrient-limiting conditions
Climate regulation (21.6%; range 14.7-34.5%)	Soil organic C	g. organic C/kg soil	Done (**UA**)	Will be combined with bulk density to obtain soil C stocks
Regulation of hazards and extreme events (6.1%; range 1.1-10.3%)	Water infiltration	Amount of time in infiltrating 50% of the 10 ml added	Done (**UA**)	Helps with flood and drought regulation
	Water holding capacity	% (g. water retained/g. dry soil)	Done (**UA**)	
Regulation of detrimental organisms (5.3%; range 1.9-10.6%)	Leaf damage	% of leaf surface damaged by pathogenic fungi or herbivorous insects	In progress (**LSTs, UA**)	Estimated from leaf pictures

10.1.2. Results

As in Task 3.3, our results regarding soil functioning are strongly region-specific. However, taken collectively two general patterns can be drawn from our two-way ANOVAs. First, sustainable soil management generally benefits soil functioning, with more limited effects on soil biota (section 9.1;**Figure 19**; Appendices E and F). Sustainable soil management benefited a substantial proportion of the ecosystem indicators measured in four of the seven study sites, all croplands. The experimental sites with the highest soil organic C levels (Finland, Ireland, Latvia) showed the least positive effects of sustainable soil management (**Figure 19**). The latter result supports the notion that organic agriculture and other soil sustainable management techniques may be more beneficial in places with relatively low organic carbon levels and therefore with a stronger potential to enhance soil carbon storage (reviewed in Rehberger et al. 2022).

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Second, the positive effect of sustainable management generally weakens under drought conditions (Figure 19Figure 19; Appendix E). In four out of the seven experimental sites (Denmark, Hungary, Ireland, Latvia) we found significant Management x Drought interactions on several of the ecosystem indicators measured (detailed results in Appendix E). This generally shows that the benefits of sustainable management are more limited under drought conditions.

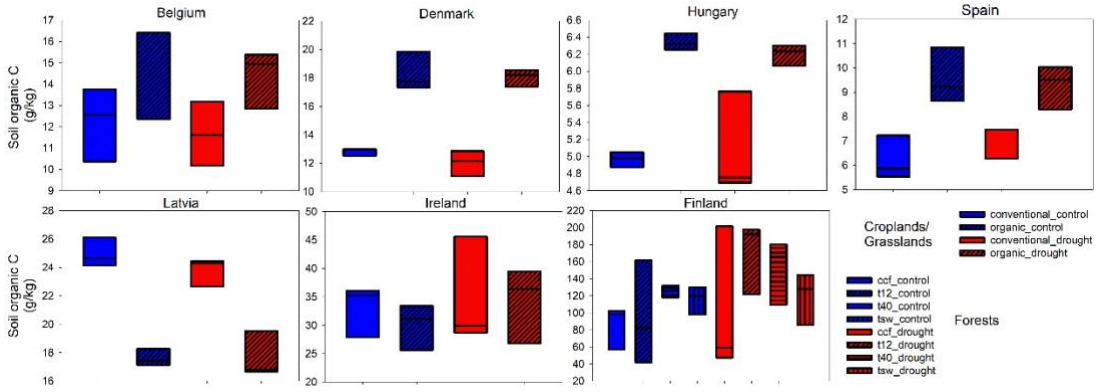

Figure 19. Results for soil organic carbon quantification (resistance phase) across the different sites and treatments considered. Panels above reflect the more general results of: i) enhanced soil properties under organic farming (dashed vs empty boxes), and ii) dampening of such positive effects under drought (red vs blue columns). Panels below are those showing the opposite (conventional > organic, Latvia) or different (weaker management effects; Finland, Ireland). Two legends are shown, one for Finish forests (4 management treatments) and one for the rest (grasslands mono- and polycultures are coded as "conventional" and "organic", respectively, for simplicity). N = 3 for all treatment combinations.

Taken collectively, our preliminary results suggest strong benefits of shifting from conventional to organic agriculture in croplands, with little evidence in favor of, or against, similar conversions on forests or grasslands. We found relatively weak support for the buffering impact of organic management to mitigate the negative impacts of future climatic scenarios on soil functioning. Hence, our results so far suggest that conventional to organic management conversions could be more beneficial if focusing on those regions that are expected to suffer less from a drier climate in the future.

10.2. Soil biodiversity as mediator of the impacts of climate and management on soil functioning

10.2.1. Methods

In this subtask, we investigated the role of soil biodiversity (as measured in Task 3.3) on the response to soil management and climatic stressors of the ecosystem indicators detailed above (*Table 9*). To gain statistical power and be able to include more predictors in our analyses, we pooled all our cropland sites together for this subtask. This allowed us to estimate how soil biodiversity modulates the response of soil functioning to management and climate. To do so, we performed linear models with soil management, drought, soil biodiversity (Shannon´s index of the 16S, 18S and ITS regions), and the local climate (aridity index) of each experimental site as predictors, considering interactions between them (

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Table 10). Furthermore, we considered the influence of soil characteristics (texture, pH, EC) as additional predictors in our models, which we performed for each ecosystem indicator separately.

To perform these analyses, we used site-based standardizations using Z-scores (details in Appendix E), so all variables across regions become comparable. We also focused only on cropland sites (five out of seven) to reduce experimental noise including different biomes.

10.2.2. Results

The results from the linear models show that all measured soil functions responded significantly to at least one interaction between soil biota and either drought, management and/or aridity (

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Table 10). Our linear models explained a substantial variation in our data (R^2 range 0.26-0.71), with more frequent significant effects for sand content, management, or fungal diversity (

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Table 10).

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Table 10. Summary statistics of the linear models performed for the resistance phase (T1) in all cropland experimental sites, across regions, collectively (Belgium, Denmark, Hungary, Latvia, Spain). T-values are shown for those predictors selected in the most parsimonious models for each variable (according to their AIC), highlighting in bold those that are significant (P < 0.05) and in italics those that are marginally significant (P < 0.10). To ease visualization of those results related to the modulating effect of soil biota, interactions between our proxies for soil Prokaryote, Eukaryote, and Fungal diversity (all based on soil DNA sequencing) and the treatments evaluated and/or local aridity levels are highlighted. Water hold. = water holding capacity, avail. N = total soil available nitrogen; B-gluco. = B- glucosidase enzymatic activity; Xylanase = xylanase enzymatic activity; Phosp. = phosphatase enzymatic activity; N-A-glu.= N-acetyl glucosaminidase enzymatic activity; N min. = potential N mineralization.

	Organic C	Water hold.	Infil.	Avail. N	B-gluco.	Xylanase	Phosp.	N-A-glu.	N min.
Management (M)	**-2.63**	**-2.75**	*-1.88*	*-1.92*	-0.73	0.90	-1.23	-0.33	**3.35**
Drought (D)	-1.33	-1.04	-0.93	**-2.85**	1.29	1.69	-0.64	-1.07	-0.04
Prokaryote (Bc)	-0.59	-1.50	**-2.09**	0.40		-0.16	-0.13	-0.45	**-2.65**
Metazoan (Eu)	0.52	0.03	0.99	0.09	0.28	-0.17	-1.28	**2.35**	-0.55
Fungi (Fu)	**-2.29**	*-1.97*	*-1.77*	*-1.97*	-0.52	*1.94*	-1.55	-0.47	1.25
Aridity (A)	-0.21	**-2.51**	-1.53	*-1.73*	-0.66	0.72	0.32	**2.49**	**2.54**
pH	**-3.53**	**-2.13**	*-1.81*	**-4.07**		-1.09	*-1.79*		
Sand content (%)	**-2.48**	**-3.33**	**2.29**	1.70	**-3.07**	**-2.33**	**-3.76**		
Electric conduc.				**3.34**	**2.08**	*1.83*	**4.11**		
M x D	1.28			**2.18**	-0.90	*-1.82*	-0.58	**2.09**	-1.47
M x A	*1.99*	**2.54**	1.68	*1.79*	*1.86*	0.36	0.87		**-2.93**
M x Bc	1.36	*1.93*	**2.04**	0.04		-1.25	-1.41	**2.43**	0.87
M x Eu	**3.16**	**2.19**			-0.24	0.61	*2.01*		**-2.12**
M x Fu				0.96	1.29		**2.35**		0.11
D x Bc				**2.93**		-0.61	-0.33		-0.47
D x Eu	**-2.23**	*-1.80*	*-1.80*	**-2.23**	-1.58	-0.88			1.14
D x Fu	**2.22**	*1.91*	*1.85*	**3.83**		*-2.02*			*-1.90*
A x Bc				-1.43		*1.87*	*1.81*		**3.77**
A x Eu	**-2.87**			-1.55	1.06	0.62	0.74	**-2.09**	-1.68
A x Fu				1.14	*-1.94*	**-2.72**	**-2.41**	-1.54	**-2.07**
M x D x Bc				**-2.80**		1.70	1.52		*1.74*
M x D x Eu					1.57	1.47			1.23
M x D x Fu									
M x A x Bc				1.40					**-3.51**
M x A x Eu					-1.24	-1.25	*-1.90*		1.58
M x A x Fu				-1.16					*1.74*
Adjusted R^2	**0.44**	**0.45**	**0.26**	**0.62**	**0.48**	**0.41**	**0.60**	**0.48**	**0.71**

The diversities of all organisms considered in our analyses (prokaryotes, eukaryotes and fungi) were strong modulators of the impacts of climate and management on soil functioning (

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Table 10). In general, the relationships between soil biodiversity and functioning became more positive under drought than control treatments (generally positive Drought x Soil biota interactions; see Figure 20 for an example). This was especially true for prokaryotes and fungi. Conversely, relationships between soil biodiversity and soil functioning became generally more negative in more arid regions (negative Aridity x Soil biota interactions; Table 10).

Significant interactions of soil biota with management were obtained for organic carbon, water holding capacity, infiltration, phosphorus content, N-acetyl-glucosaminidase activity, and mineral nitrogen content. These indicate that soil biodiversity also plays an important role in modulating the responses to soil functioning to shifting agricultural management.

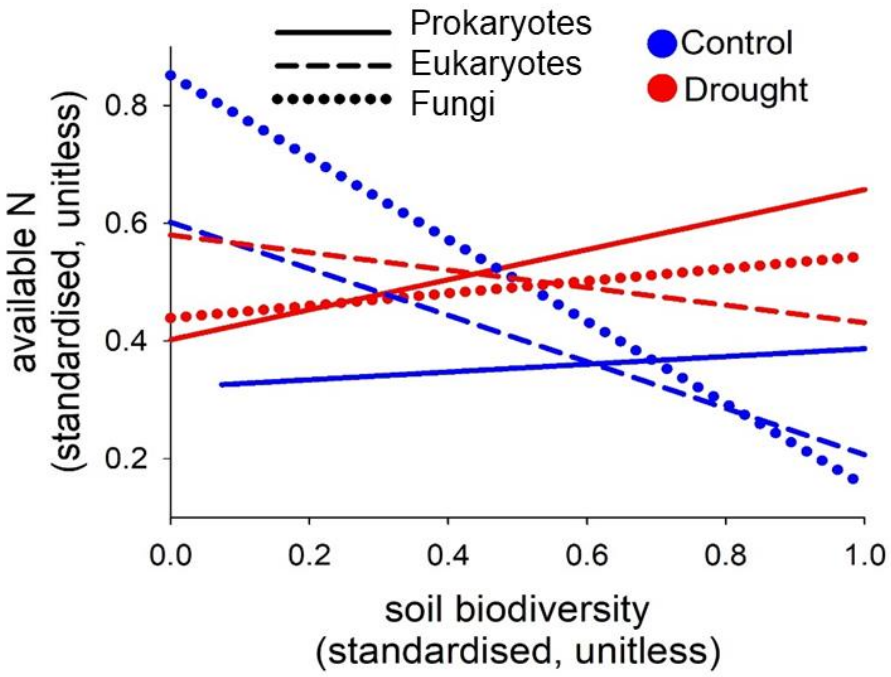

Figure 20. Changing influence of soil biota by ecosystem functioning in drought vs control conditions. Soil biota become more positive under our drought treatment than in the control plots. Data from different types of soil management and experimental sites across regions are pooled together. Only one functional indicator (available N) is shown, to ease visualization and for simplicity.

10.3. Synergies and trade-offs

10.3.1. Methods

To evaluate potential changes in synergies and trade-offs in between ecosystem indicators, we calculated the Pearson´s correlations between each pair of them. If a correlation between a given pair of ecosystem indicators is large and positive, this is interpreted as a "synergy" between them, meaning that win-win scenarios to maximize both are possible. If a correlation between a given pair of

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ecosystem indicators is large and negative, this is interpreted as a “trade-off”, meaning that if we want to maximize one it will likely come at the cost of the other.

To evaluate how contrasting management and climatic scenarios affected these synergies and trade-offs, we compared their Pearson´s correlations before (raw data, independent of conditions) and after filtering by management and drought treatments via partial correlations. As in the previous subtask (10.2), we pooled all our cropland sites together and used site-level standardizations (**Table 10**)

10.3.2. Results

Most functions evaluated were positively correlated between each other when analyzing data across management and drought treatments collectively. These functional synergies (positive correlations) between ecosystem indicators remained relatively consistent even when imposing the experimental drought (see “filtered by drought” in **Table 11**). However, a very large proportion of these synergies disappeared when filtering by the effect of Management, suggesting that multiple ecosystem functions can be more difficult to maintain simultaneously at high levels within a given agricultural management (either sustainable only, or conventional only). The only positive synergies that persisted when accounting for the influence of management in these correlations were those involving soil enzymatic activities.

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Table 11. Synergies and trade-offs (as in positive and negative Pearson´s correlations) for all pairs of functions evaluated. We report correlations between the raw data (site-level standardizations, all sites [resistance phase] included), and partial correlations after filtering by Management, Drought and both of our treatments. N = 60 in all cases excepting those involving litter decomposition (N= 20). *To ease visualization, correlations stronger than |0.3| are shaded (blue for positive, orange for negative).*

RAW DATA

	water_h	infiltration	litter_dec.	beta_glu	xylan	phosph.	N-A-glucos.	available_N	N_miner.
organicC	0.73	0.10	0.48	0.62	0.51	0.64	0.45	0.49	0.31
water_h		0.11	0.57	0.58	0.54	0.62	0.44	0.41	0.22
infiltration			-0.02	-0.04	0.08	0.03	-0.04	0.20	-0.23
litter_dec.				0.38	0.24	0.47	0.27	0.34	0.65
beta_glu					0.79	0.74	0.70	0.47	0.28
xylan						0.82	0.67	0.33	0.23
phosph.							0.59	0.49	0.31
N-A-glucos.								0.37	0.25
available_N									0.05
N_miner.									

FILTERED BY MANAGEMENT

	water_h	infiltration	litter_dec.	beta_glu	xylan	phosph.	N-A-glucos.	available_N	N_miner.
organicC	0.21	0.01	-0.16	0.19	-0.16	-0.19	0.08	-0.09	0.23
water_h		0.15	0.04	-0.20	0.17	0.16	0.05	-0.37	-0.02
infiltration			0.10	-0.30	0.12	0.07	0.02	-0.10	0.26
litter_dec.				-0.37	-0.48	-0.18	-0.43	0.10	0.23
beta_glu					0.14	0.04	0.54	0.13	0.00
xylan						0.70	0.78	-0.21	-0.18
phosph.							0.50	0.00	-0.33
N-A-glucos.								-0.11	-0.08
available_N									-0.14
N_miner.									

FILTERED BY DROUGHT

	water_h	infiltration	litter_dec.	beta_glu	xylan	phosph.	N-A-glucos.	available_N	N_miner.
organicC	0.75	-0.17	0.48	0.76	0.55	0.62	0.64	0.40	0.77
water_h		-0.13	0.56	0.65	0.69	0.74	0.64	0.29	0.69
infiltration			-0.06	-0.28	-0.05	-0.11	-0.11	0.03	-0.05
litter_dec.				0.39	0.24	0.46	0.27	0.43	0.65
beta_glu					0.66	0.70	0.82	0.42	0.72
xylan						0.88	0.90	0.22	0.57
phosph.							0.80	0.42	0.60
N-A-glucos.								0.27	0.60
available_N									0.40
N_miner.									

FILTERED BY MANAGEMENT & DROUGHT

	water_h	infiltration	litter_dec.	beta_glu	xylan	phosph.	N-A-glucos.	available_N	N_miner.
organicC	0.15	-0.07	-0.19	0.22	-0.17	-0.22	0.08	0.03	0.19
water_h		0.01	0.00	-0.16	0.17	0.12	0.05	-0.23	-0.14
infiltration			0.06	-0.27	0.11	0.03	0.01	0.12	0.18
litter_dec.				-0.36	-0.49	-0.20	-0.43	0.19	0.20
beta_glu					0.14	0.06	0.55	0.08	0.04
xylan						0.70	0.79	-0.22	-0.20
phosph.							0.51	0.07	-0.38
N-A-glucos.								-0.13	-0.08
available_N									0.00
N_miner.									

10.4. Discussion and preliminary conclusions from Task 3.4

Sustainable management showed strong benefits for ecosystem functioning. These benefits were particularly pronounced in our cropland sites with generally low initial soil organic carbon.

Sustainable management may buffer only partly the negative impacts of ongoing climate change in European soils. These results are in accordance with previous reports of the beneficial effects of organic agricultural management for soil biodiversity and functioning (e.g., Barral et al. 2015; Gong et

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al. 2022), but also to their higher vulnerability under drier or more uncertain climatic conditions (e.g., Knapp & Van der Heijden 2018).

The numerous significant Management x Soil biota interactions we found indicate that soil biodiversity also plays an important role in modulating the responses to soil functioning to shifting agricultural management. Most of these interactions were positive, suggesting that organic farming has more positive effects on functioning if coupled with an enhanced soil biota than alone.

The results presented above show the potential of SOILGUARD´s findings to provide sound recommendations and predictions regarding the response of soil multifunctionality to future climatic scenarios under contrasting management approaches. However, we must advise strong caution regarding our preliminary conclusions, as these are drawn using only part of WP3´s first year results. Yet, we hope these serve to illustrate the type of results that our Consortium will produce and their implications.

10.5. Next steps for Task 3.4

We will finish measuring the ecosystem indicators planned for WP3, including remaining variables in Table 5, and sampling times (year 2). This work is advancing according to schedule.

Once gathering all ecosystem indicators, we will calculate multifunctionality metrics, using an equal weighting for all indicators (standard procedure) and a stakeholder-oriented valuation, using results from WP4.

Results presented in Table 6 are still over-parameterized. To solve this, we will: i) include data from the rest of sampling periods to gain statistical power, and ii) consider a simpler set of predictors (e.g., study site as random factor instead of aridity and the interactions involving it, soil multiversity as a single soil biodiversity metric).

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11. Appendix A. Method to mitigate heatwaves at the Spanish field site

The field managers in Murcia used shadow nettings to mitigate the effects of natural heatwaves instead of using heaters to simulate one. This was decided after observing a total of three heatwaves in 2022 with temperatures raising up to 44 °C. Pilot tests were performed during Spring 2023 in the Province of Alicante to ensure heat differences resembling heatwave model predictions (5 days with temperature difference of 6 °C). We found that two layers of a Raschel white shadow netting (50%, Macoglass, Spain) placed 35 cm from the ground, caused a soil temperature reduction of 5.5 - 0.5 °C for most of the time in each day (Table A). Therefore, during the last part of the drought simulation, we placed these shadow nets covering an area of 1.5 m² inside each ROS, by attaching them with a polyester rope to the ROS structure, 35 cm from the surface (Figure A.1). TOMST moisture and temperature sensors were installed under the different combinations of conditions as depicted in Figure 2. With this experimental set up, the treatments during the heatwave reduction period were slightly different from the other countries. In Spain, the control treatment was also affected by the heatwave period; the heatwave with accumulated drought treatment was under the ROS, and the accumulated drought with no heatwave was the area under the shadow netting (Figure A.2).

Table A.1. Pilot experiment for heatwave reduction results. Δ indicates change.

TOMST sensor	Day* (Δ°C)	Night* (Δ°C)	%Day* with 5.5<Δ<7.5°C
Air: 12 cm	4.1	-0.5	42%
Surface: 0 cm	8.3	-1.5	65%
Soil: -6 cm	7.8	2.5	78%

Figure A.1. Shadow netting placement during the heatwave reduction period.

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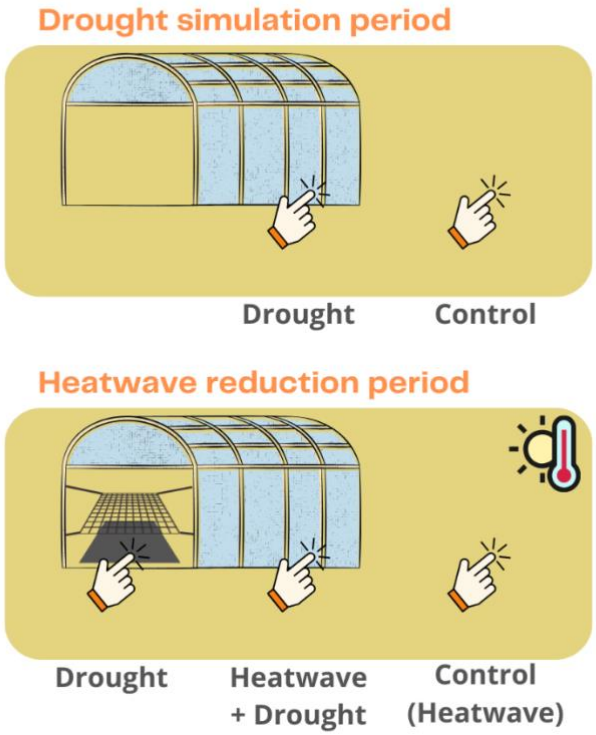

Figure A.2. Sampling design diagram for heatwave reduction experiments.

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12. Appendix B. Soil DNA extraction method followed by University of Alicante

Freeze-dried soils were received from each local sampling team, and their DNA was extracted using 0.25 gr of soil and the DNeasy PowerSoil HTP 96 Kit (Qiagen Inc., Valencia, CA, USA). At the beginning of the project, it was recommended by the Advisory Board to extract the DNA from a larger amount of soil (10 g) using the DNeasy Powermax soil Kit (from the same company), to be able to extract more information of the largest soil organisms. However, repeated trials showed that the quality of the DNA concentration extracted was not good enough for sequencing. This was perhaps due to the homogenizer used, although we followed the instructions from the company in this regard and other colleagues within the Consortium later expressed issues when using this kit too. In any case, after a meeting between the soil DNA sequencing experts from SOILGUARD (ETH, LEITAT, INRAE, UA, UvA) on the 28th of February 2023, it was decided to keep the PowerSoil kit, which is also the one recommended and used in SOILBON (Guerra et al. 2021). Results with this kit were more satisfactory and the DNA concentrations were, in most cases (except Spain T1 in the conventional fields), enough to amplify and sequence the marker genes. Samples were sent to ETH and INRAE for sequencing and qPCR analysis on the 30th of March 2023. To try to obtain a better quality for the Spanish samples, these were repeatedly extracted by triplicate, but this was not enough to obtain enough amount of viable DNA, so these were discarded for further sequencing.

12.1. References appendix B

Guerra, CA et al. (2021). Tracking, targeting, and conserving soil biodiversity. Science 371, 239-241. DOI: 10.1126/science.abd7926

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13. Appendix C. Metabarcoding of soil DNA to track biodiversity of prokaryotes, eukaryotes, and fungi.

13.1. Methods

Amplicon library preparation

Extracted DNA was received from UA in 1.5 mL vials. Concentration was measured via UV/VIS spectrophotometry with the QIAxpert system (QIAGEN) and normalized to a concentration of 10 ng μL^{-1} using a QIAgility liquid handling station (QIAGEN). Amplicon sequencing libraries were prepared using primers for prokaryotes (16S ribosomal RNA genes), eukaryotes (18S ribosomal RNA genes), and fungi (ribosomal internal transcribed spacer, ITS) as detailed in Table 1, which in addition included the sequencing primer sites of the Nextera Illumina adapters 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3' and 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3' (Illumina Inc., San Diego, CA, USA). A total volume of 25 µL was used to perform PCR amplification, containing 40 ng of template DNA, 1 x GoTaq® Colorless Master Mix, 2.5 to 3.0 mM MgCl2, 0.4 µM for 16S and ITS primers, and 0.4 µM of Euk575Fngs and 0.8 µM of Euk895Rngs (mixing by QIAgility, QIAGEN). The PCR plates were then transferred to a C1000™ Touch Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA) for amplification with separate programs for each amplicon: 16S (initial denaturation 95°C x 5 min; 30 cycles of denaturation 95°C x 40 sec, annealing 58°C x 40 sec, elongation 72°C x 1 min; final elongation 72°C x 10 min), ITS (initial denaturation 95°C x 5 min; 35 cycles of denaturation 95°C x 40 sec, primers annealing 58°C x 40 sec, elongation 72°C x 1 min; final elongation 72°C x 10 min), and 18S (initial denaturation 95°C x 5 min; 35 cycles of denaturation 95°C x 40 sec, primers annealing 55°C x 40 sec, elongation 72°C x 1 min; final elongation 72°C x 10 min). Amplification quality was analyzed via capillary electrophoresis on a QIAxcel Advanced system (QIAGEN). Three technical replicates were amplified for each sample and pooled after quality check. For indexing PCR, the pooled DNA samples were sent to the Functional Genomics Center Zurich (FGCZ, Zurich, Switzerland). Before paired-end sequencing on the Illumina NextSeq 2000 platform (Illumina Inc., San Diego, CA, USA), indexed PCR products were purified, quantified, and pooled in equimolar ratios.

Table C.1. Information of the primers used to produce amplicon libraries for metabarcoding of genes of three biological groups used to study soil biodiversity.

Targeted group	Amplified region	Name and sequence	Reference
Bacteria/Archaea	16S V3-V4	341F (5'–CCTAYGGGDBGCWSCAG-3') 806R (5'-GGACTACNVGGGTHTCTAAT-3')	Frey et al., 2016
Eukaryotes	18S V4	Euk575Fngs (5'-ASCYGYGGTAAYWCCAGC-3') and Euk895Rngs (5'-TCHNHGNATTTCACCNCT-3')	Guerra et al., 2021
Fungi	ITS2	ITS3ngs (5'-CANCGATGAAGAACGYRG-3') and ITS4ngs (5'-CCTSCSCTTANTDATATGC-3')	Tedersoo and Lindahl, 2016

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Bioinformatics

Processing of paired-end Illumina reads was done using a customized pipeline largely based on VSEARCH (Rognes et al., 2016) as previously described (Longepierre et al., 2021). In brief, the pipeline included removal of PhiX control sequences using Bowtie2 (Langmead & Salzberg, 2012); trimming of PCR primers (Martin, 2011); merging of paired-end reads (VSEARCH); quality filtering by maximum expected error (VSEARCH); delineation of sequences into amplicon sequence variants (ASVs) (VSEARCH) with *minsize* of 8; removal of chimeras (VSEARCH, Edgar, 2016); target verification using Metaxa2 (Bengtsson et al., 2015), for the 16S and 18S rRNA genes and ITSx (Bengtsson et al., 2013) for the ITS2 sequences. Unsupported sequences were discarded. The final sample x observation table was obtained by mapping the quality filtered reads of each sample against the verified ASV sequences using VSEARCH with settings, maxaccepts 100, maxhits 1, and a minimum identity of 97%. Taxonomic classification of each verified ASV sequence was performed by running the SINTAX algorithm implemented in VSEARCH against the SILVA v.138 database (Pruesse et al., 2007) for the 16S rRNA gene sequences (bacteria and archaea), against the UNITE v.8.3 database (Abarenkov et al., 2010) for the ITS2 sequences (fungi), and against the PR2 v5.0 database for 18S rRNA gene sequences using a bootstrap cutoff of 0.8.

Biostatistics

All statistics were performed in R v.4.3.1. We normalized the read numbers of all samples in the three groups using an iterative subsampling procedure to perform analyses of biodiversity (Schloss, 2023). Alpha-diversity was assessed by calculating observed richness, Shannon diversity index and Pielou's evenness of ASVs based on the mean of 100-fold subsampled ASV matrices using the functions *rarefy*, *specnumber*, and *diversity* in the R package *vegan* (Oksanen et al., 2020). Beta-diversity was assessed based on the mean Bray-Curtis dissimilarities calculated from the 100-fold subsampled ASV-matrices using the function vegdist in vegan. Effects of experimental factors (site, management, drought) on alpha- and beta-diversity were assessed using permutational multivariate analysis of variance (PERMANOVA, Anderson et al. (2001) with 9999 permutations. Differences in alpha-diversity were examined by mean strip plots, differences in beta-diversity were examined by principal coordinate ordinations (PCoA Gower (1996) using the *cmdscale* function in *vegan*, and by canonical analysis of principal coordinates (CAP, Anderson and Willis (3003) using the *CAPdiscrim* function in the R package *BiodiversityR* (Kindt and Coe, 2005).

In terms of statistical analyses, the setup in the forest with the four management systems and the two soil horizons is entirely different from the other six systems. Therefore, the data from Finland will need to be analyzed separately, thereby also solving the issues with the unbalanced design. If unbalanced designs exist, e.g. due to missing values, we use different resemblance metrics to compare if the results and conclusions change in comparison to Bray-Curtis. We want to note here that Bray-Curtis is an important metric for ecological data because it does not consider double absences as contributing to similarity between samples.

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13.2. Supplementary results

QC of sequences

We obtained high-quality sequences from the three biological groups. This was verified with the quality profile of the sequences, that was above 30 and close to 40. Also, the length distribution of the reads was among the expected values for the targeted amplicons. Additionally, we obtained evenly distributed number of reads across the different samples specially for the prokaryotes and fungi. Only one and three samples had considerably higher read numbers for bacteria and fungi, respectively; and several samples had greater number of reads than the average for the Eukaryotes. However, no inherent bias in read numbers across the different factor levels was found for either of the three groups. We nevertheless normalized the read numbers of all samples in the three groups using iterative subsampling to the minimum number of reads per sample to perform analyses of biodiversity. The number of sequences and ASVs (proxy of species) and the total number of reads across all the samples prior to normalization are presented in Table C.2.

Table C.2. Number of sequences and ASVs (proxy of species) and the total number of reads across all the samples.

Group	Total number of reads	Total number of ASVs
Prokaryotes	46280779	106611
Eukaryotes	38267917	37551
Fungi	30868310	12752

Region-specific analyses of soil biodiversity

Belgium

Conventional management had significantly greater **prokaryotic** alpha diversity in **Belgium** (**Figure 6**, Table C.3.). Although drought effects were masked by the effect of management, drought separated prokaryotic communities in the organic fields as shown by beta diversity analyses (Figure C.1.). Specifically, the relative abundance of Proteobacteria increased, and Verrucomicrobia decreased in the conventional field (Figure C.2.).

Fungal alpha diversity was not significantly affected by management or drought in the analyzed Belgian soils, but beta diversity was affected (Table C.4.). Opposite to prokaryotes that separated well by drought only under organic management, fungal communities separated by drought in the conventional field (Figure C.3.). Relative abundances of fungal phyla Chytridiomycota decreased, and Mortierellomycota increased in the conventional fields, compared to the alternative fields. Management had a significant effect on the **eukaryotic** observed richness in Belgium, as well as on the beta diversity (Table C.5.). Drought separated the eukaryotic communities only in the organic field, like the trend observed for prokaryotes (Figure C.5). The relative abundance of the phylum Discoba was visually increased in the conventional field, compared to the organic field.

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Denmark

Both management and drought significantly affected the **prokaryotic** alpha diversity (as measured by observed richness and evenness, Table C.3). In general, observed richness values were smaller in the organic fields. Management was significant also for prokaryotic beta diversity (Table C.3., Figure C.1.), showing a separation of the communities by drought treatment in both the organic, and conventional fields, but with larger distances among drought levels in the conventional field compared to the organic field. The taxonomic composition in the two levels of drought and management are not evident at the phylum level (Figure C.2.).

Fungal observed richness was significantly greater in the conventional fields, regardless of the drought treatment in the Danish soils (Table C.4). No significant effects of management or drought treatment were detected on beta diversity (Table C.4.), but the communities do show separation by drought in the conventional fields (Figure C.3.). No evident changes in phyla relative abundances were found across the different drought or management (Figure C.4.).

Eukaryotic observed richness was significantly greater in the organic fields and smaller under drought (Table C.5.). Like prokaryotic and fungal beta diversity, the eukaryotic communities separated by drought under the conventional management (Figure C.5.). The phylum Chlorophyta seems to be slightly favored in the conventional fields compared to the organic fields (Figure C.6.).

Hungary

Both drought, and management had significant effects on alpha and beta **prokaryotic** diversity in Hungary (Table C.3.). Conventional fields had significantly smaller Shannon values, as well as fields treated with drought (**Figure 6**). Communities separated better in the organic fields than in the conventional fields (Figure C.1.). Phyla Verrucomicrobia, Acidobacteriota, and Crenarchaeota had greater, and Actinobacteria and Chloroflexi had reduced relative abundances compared to the organic fields.

Management was significant to the **fungal** alpha and beta diversity, and drought was significant only to the beta diversity (Table C.4.). The conventional fields had greater Shannon index (**Figure 6**). Communities clearly separated by management regime and drought treatments (Figure C.3.). The relative abundance of the phyla Glomeromycota was increased, and phyla Basidiomycota was reduced in the conventional field (Figure C.6.).

Eukaryotic alpha and beta diversity were significantly affected by both, management and drought (Table C.5.). Shannon index values were greater in the conventional field compared to the organic fields, and in the control, plots compared to the drought plots (**Figure 6**). Sample separation was very similar to fungal communities, with a clear separation by management regime and drought treatments (Figure 5.C.). Relative abundances of the phyla Streptophyta and Discosea were increased in the conventional fields compared to the organic fields. Opisthokonta-Metazoa seems to be increased in the drought plots of the organic field (Figure C.6.)

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Latvia

Prokaryotic alpha diversity was significantly affected by management and drought in Latvia (Table C.3.), with organic and control plots having greater Shannon diversity index values (Figure 6). Samples separated well by both, management and drought treatment (Figure C.1.). The relative abundances of the phyla Verrucomicrobia, Chloroflexi, and Firmicutes were increased in the conventional fields compared to the organic fields. Drought increased slightly the relative abundance of the phyla Proteobacteria in the organic fields (Figure C.2.).

Fungal observed richness in Latvia was significantly decreased in the conventional field (Table C.4.), but no effects were observed on Shannon index (Figure 6, Table C.1.). Beta diversity was significantly different at each management regime (Table C.1.), and the communities separated well by management and drought (Figure C.3.). The relative abundances of the phyla Mortierellomycota, Glomeromycota, and slightly Ascomycota, were increased, and Basidiomycota and Mucoromycota were decreased in the conventional fields compared to the organic fields (Figure C.4.).

Eukaryotic observed richness in Latvia had significantly greater values in the conventional and control plots than the organic and drought plots (Table C.4.). Beta diversity index was significantly different between management regimes and drought treatments (Table C.4.), and the communities separated well by management and drought (Figure C.5.). The relative abundances of the phyla Rhizaria-Cercozoa, Streptophyta, Alveolata-Apicomplexa, Tubulinea, Chlorophyta, Discosea, and Discoba, were increased, and Opisthokonta -Fungi and Opisthokonta-Metazoa were decreased in the conventional fields compared to the organic fields (Figure C.6.).

Spain

Only organic fields yielded enough DNA for downstream analyses at T1 in 2022 in Spain. No significant differences were found between the drought treatments in alpha or beta prokaryotic biodiversity using PERMANOVAS (Table C.3.), but the constraint analyses show good separation of samples by drought (Figure C.1.). No evident changes were found at the phylum level in the taxonomic composition (Figure C.2.). Fungal and eukaryotic diversity showed similar patterns to the prokaryotic diversity in Spain (Figure C.6.).

Ireland

No significant differences were found between the drought treatments in alpha or beta prokaryotic biodiversity using PERMANOVAS (Table C.3.) in Ireland. However, the CAP analyses show separation of the prokaryotic communities by management and drought (Figure C.1.). The relative abundance of the phyla, Planctomycetota, slightly Actinobacteriota, and Proteobacteria were increased, and Chloroflexi Firmicutes, Crenarchaeota, Acidobacteria, Firmicutes, Crenarchaeota and Myxococcota were reduced in in the mono-species grassland compared to the multispecies grassland (Figure C.4.).

Marginal effects of drought were found on the alpha and beta diversity of fungal communities in Ireland (Table C.4.), with drought treatments having slightly smaller values, especially in the multispecies grassland (Figure 6). Fungal communities separated well by either drought treatment or management regime (Figure C.3.). The relative abundances of the phyla Ascomycota,

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Mortierellomycota, and Chytridiomycota were reduced, and Basidiomycota were increased in the mono-species grassland compared to the multispecies grassland (Figure C.3.). The phylum Mucoromycota was decreased in the drought compared to the control treatment of the multispecies grassland (Figure C.3.).

Like the prokaryotic biodiversity, no effects of management or drought were detected on **eukaryotic** communities with PERMANOVAS (Table C.5.). However, the samples show separation by drought and management (Figure C.5.). The relative abundances of Opisthokonta-Fungi, Streptophyta-Streptophyta-X, Alveolata-Apicomplexa increased, and Rhizaria-Cercozoa, Tubulinea, Chlorophyta, Discosea, Alveolata-Ciliophora, Stramenopiles-Gyrista, and Discoba decreased in the mono-species grasslands compared to the multispecies (Figure C.6.).

Finland

Alpha diversity of **prokaryotic** communities was significantly greater in the conventional plots compared to the alternative plots, but not by drought (Table C.3., **Figure 4**). Drought separated the communities more clearly in the conventional than in the alternative plots (Figure C.1.). The relative abundances of the phyla Verrucomicrobia and Firmicutes increased, and slightly Acidobacteriota, Actinobacteriota, and Crenarchaeota decreased in the conventional compared to the alternative plots (**Figure C.2.**). The relative abundance of Myxococcota and Bacteroidota were decreased in the drought plots of the conventional regime compared to the control plots of this regime (Figure C.4.).

In Finland, **fungal** alpha diversity was not significantly different by management or drought. However, fungal beta diversity was significantly different by management regimes (Table C.4., Figure C.3.). The relative abundance of the phyla Mucoromycota and Mortierellomycota increased, and Chytridiomycota decreased in the conventional fields compared to the alternative fields. In the alternative management, Chytridiomycota decreased under drought conditions compared to the control plots (Figure C.4.).

Alpha **eukaryotic** biodiversity was not significantly different by management or drought. However, fungal beta diversity was significantly different by management regimes, and communities separated well by management and drought (Table C.5., Figure C.5.). The relative abundance of the phyla Alveolata-Apicomplexa and Chlorophyta-Chlorophyta-X increased, and Evosea-Evosea-X increased in the conventional regime, compared to the alternative (Figure C.6.). In the conventional fields, Alveolata-Ciliophora decreased, and Chlorophyta increased under drought compared to the control (Figure C.6.).

The research leading to these results has received funding from the European Union Horizon 2020 Research & Innovation programme under the Grant Agreement no. 101000371.

Table C.3. PERMANOVAs results of alpha and beta diversity of prokaryotes by field site. F values are followed by P values in parentheses of each metric. Significant or marginally significant effects of management, drought treatment, or the interactions are bolded.

Diversity	Metric	Management (M)	Drought (D)	M×D
Belgium				
Alpha diversity	Observed richness	0.4(0.5394)	0.0(0.9246)	**4.3(0.0729)**
	Shannon diversity index	**12.1(0.0083)**	0.0(0.9299)	1.2(0.3047)
	Pielou's evenness	**27.0(0.0008)**	0.0(0.9578)	0.1(0.7287)
Beta diversity	Bray-Curtis dissimilarity	**20.0(0.0050)**	0.8(0.5750)	0.9(0.5210)
Denmark				
Alpha diversity	Observed richness	**71.4(0.0000)**	7.3(0.0269)	1.9(0.2021)
	Shannon diversity index	1.5(0.2597)	**5.3(0.0497)**	1.4(0.2765)
	Pielou's evenness	**5.1(0.0542)**	**3.9(0.0847)**	1.1(0.3266)
Beta diversity	Bray-Curtis dissimilarity	**24.0(0.0010)**	1.5(0.1290)	1.4(0.1560)
Hungary				
Alpha diversity	Observed richness	**515.6(0.0000)**	**111.3(0.0000)**	**107.1(0.0000)**
	Shannon diversity index	**903.0(0.0000)**	**359.7(0.0000)**	**245.1(0.0000)**
	Pielou's evenness	**593.0(0.0000)**	**319.4(0.0000)**	**189.7(0.0000)**
Beta diversity	Bray-Curtis dissimilarity	**0.0(0.0010)**	**0.0(0.0030)**	**0.0(0.0030)**
Latvia				
Alpha diversity	Observed richness	**38.1(0.0003)**	**9.3(0.0494)**	0.3(0.5300)
	Shannon diversity index	**6.6(0.0335)**	**9.3(0.0157)**	0.3(0.6214)
	Pielou's evenness	1.8(0.2149)	**6.4(0.0355)**	0.1(0.7355)
Beta diversity	Bray-Curtis dissimilarity	**0.0(0.0010)**	0.0(0.1900)	0.0(0.1990)
Spain				
Alpha diversity	Observed richness	-	0.4(0.7910)	-
	Shannon diversity index	-	0.0(0.6290)	-
	Pielou's evenness	-	0.1(0.1620)	-
Beta diversity	Bray-Curtis dissimilarity	-	0.5(0.7000)	-
Ireland				
Alpha diversity	Observed richness	0.4(0.5390)	0.1(0.7420)	0.1(0.7710)
	Shannon diversity index	0.2(0.6610)	0.2(0.7070)	0.1(0.7240)
	Pielou's evenness	0.6(0.4580)	0.4(0.5540)	0.1(0.7420)
Beta diversity	Bray-Curtis dissimilarity	64.1(0.6140)	8.9(0.9270)	10.1(0.9390)
Finland				
Alpha diversity	Observed richness	**4.2(0.0741)**	0.7(0.4308)	0.3(0.6141)
	Shannon diversity index	**22.7(0.0014)**	0.3(0.5877)	2.3(0.1686)
	Pielou's evenness	**22.3(0.0015)**	0.1(0.8046)	2.7(0.1422)
Beta diversity	Bray-Curtis dissimilarity	**21.0(0.0040)**	1.7(0.7120)	1.7(0.8150)

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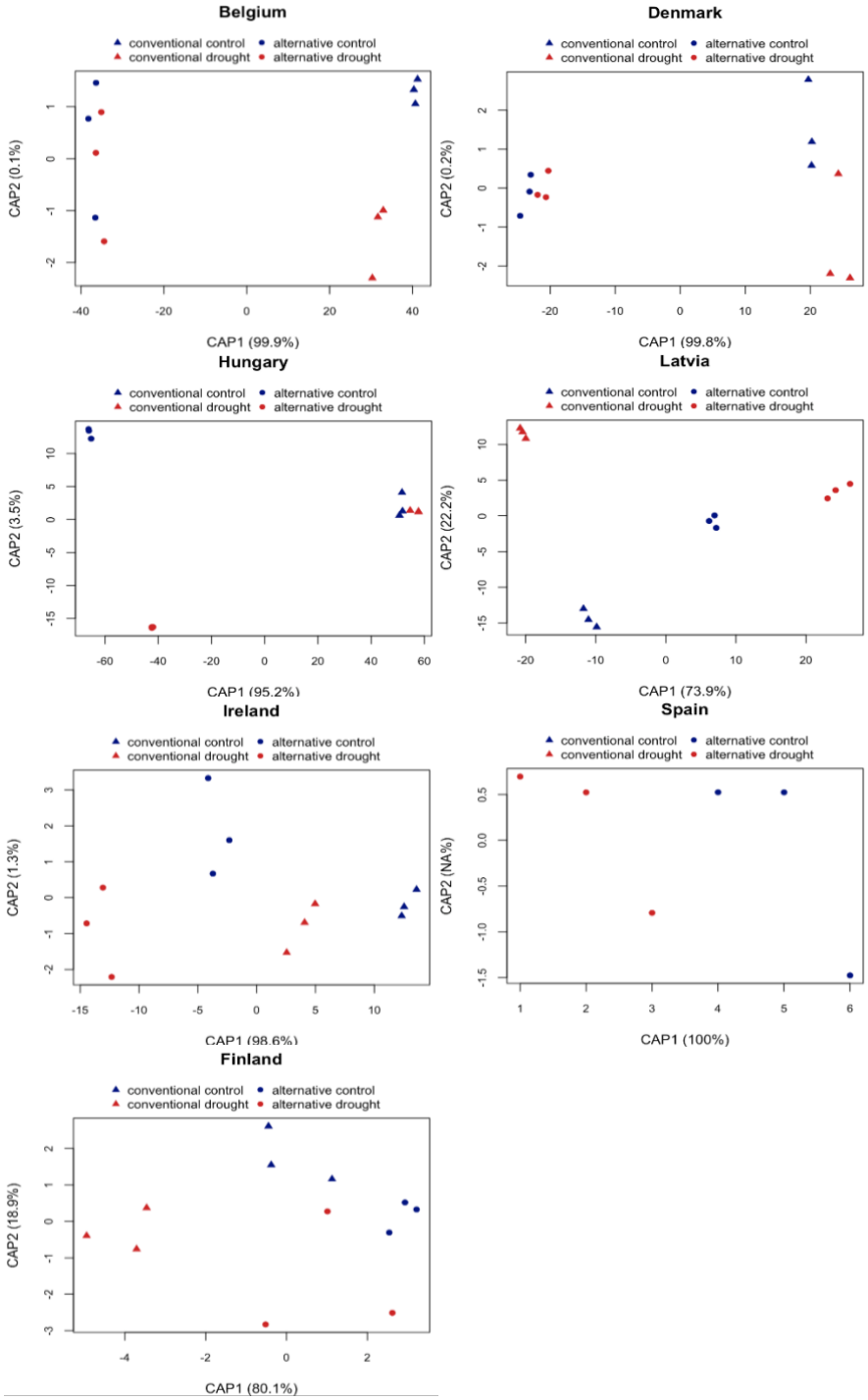


*Figure C.1. Beta diversity of sampled soils. Canonical analysis of principal coordinates of Bray distances of iterative subsampled datasets of **prokaryotes** by field site. The dots represent individual samples, comprising three biological replicates per treatment combination. The percent success of classification is indicated in each axis. Alternative management includes organic in agricultural lands (BE, DK, ES, HU, LV), grassland mixture in IE, and continuous forest in FI. Conventional management includes conventional agriculture (BE, DK, ES, HU, LV), mono-species grasslands in IE, and clearcut young, generated forest in FI.*

The research leading to these results has received funding from the European Union Horizon 2020 Research & Innovation programme under the Grant Agreement no. 101000371.

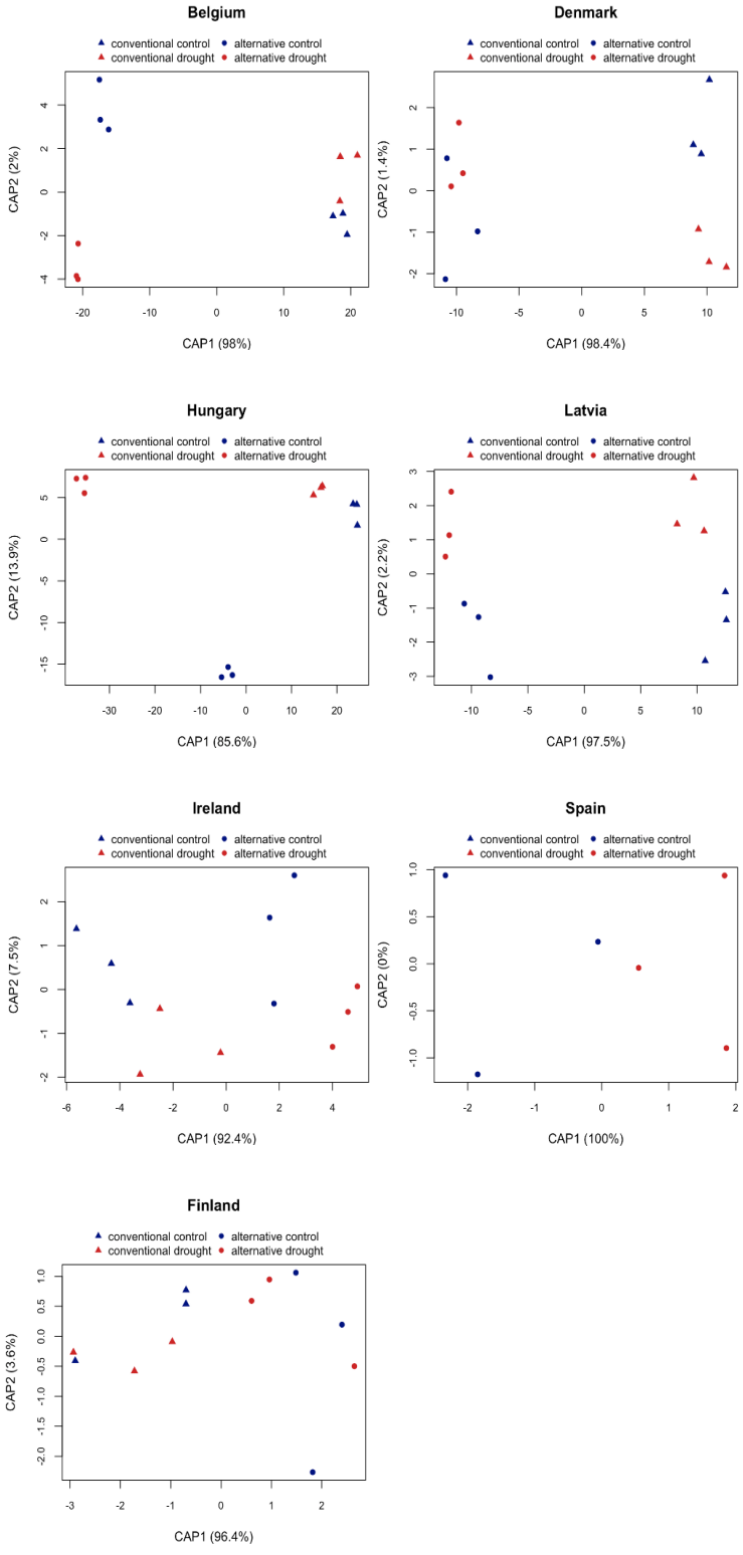
Figure C.2. Relative abundance of the most abundant prokaryotic phyla by field site Alternative management includes organic in agricultural lands (BE, DK, ES, HU, LV), grassland mixture in IE, and continuous forest in FI. Conventional management includes conventional agriculture (BE, DK, ES, HU, LV), mono-species grasslands in IE, and clearcut young, generated forest in FI.

The research leading to these results has received funding from the European Union Horizon 2020 Research & Innovation programme under the Grant Agreement no. 101000371.

*Table C.3. PERMANOVAs results of alpha and beta diversity of **fungi** by field site. F values are followed by P values in parentheses of each metric. Significant or marginally significant effects of management, drought treatment, or the interactions are bolded.*

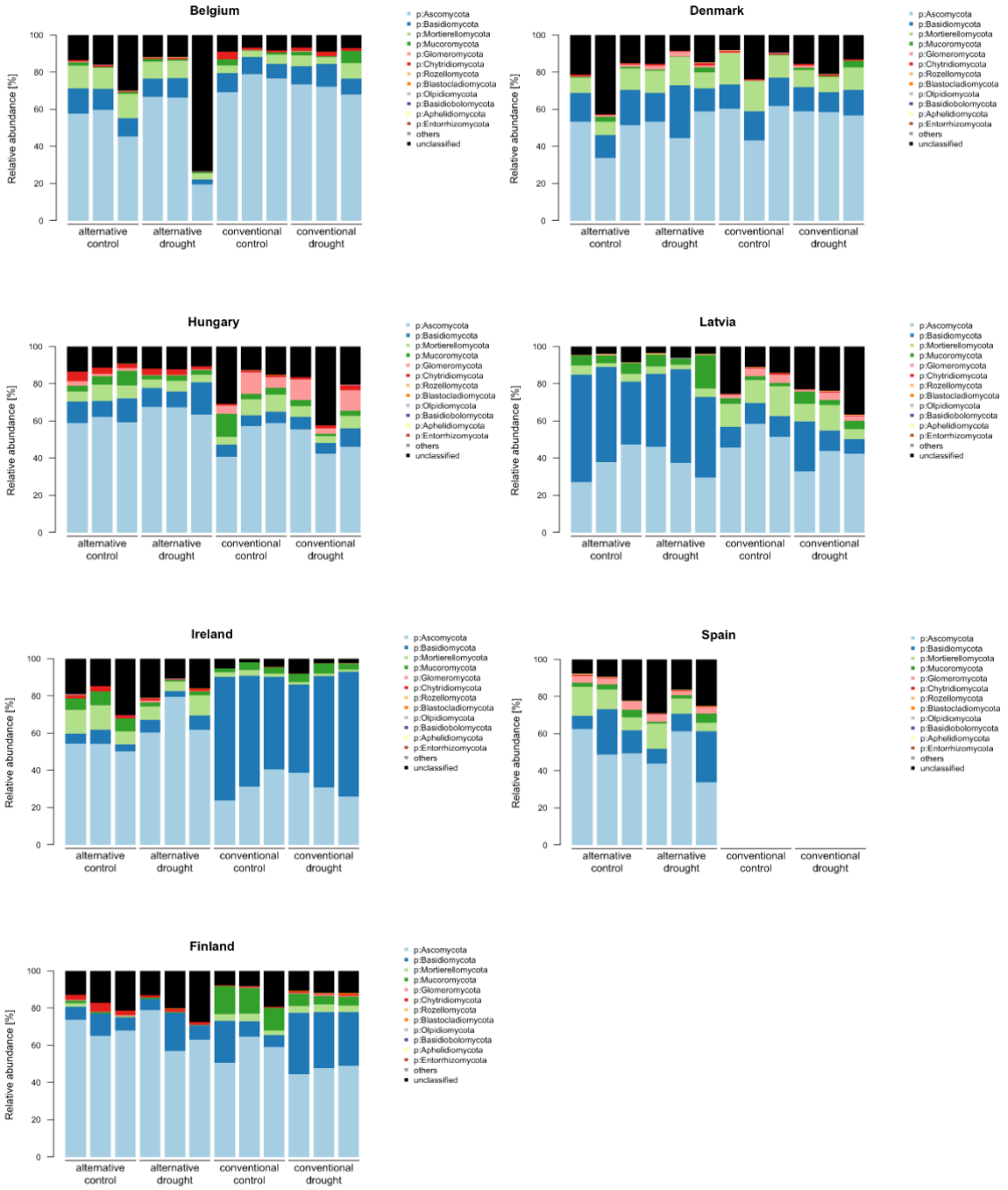
Diversity	Metric	Management (M)	Drought (D)	M×D
Belgium				
Alpha diversity	Observed richness	2.1(0.1854)	0.8(0.4074)	**5.2(0.0527)**
	Shannon diversity index	0.0(0.9340)	1.4(0.2700)	1.8(0.2220)
	Pielou's evenness	0.0(0.9860)	1.4(0.2730)	1.5(0.2530)
Beta diversity	Bray-Curtis dissimilarity	**9.4(0.0020)**	0.6(0.7380)	0.9(0.5190)
Denmark				
Alpha diversity	Observed richness	**23.9(0.0012)**	0.1(0.7184)	0.1(0.7094)
	Shannon diversity index	0.0(0.9290)	0.3(0.5870)	1.8(0.2180)
	Pielou's evenness	1.1(0.3310)	0.5(0.4960)	2.1(0.1850)
Beta diversity	Bray-Curtis dissimilarity	**10.5(0.0010)**	1.0(0.3810)	**2.5(0.0680)**
Hungary				
Alpha diversity	Observed richness	**67.1(0.0000)**	2.0(0.1930)	1.9(0.2030)
	Shannon diversity index	**21.0(0.0018)**	0.0(0.9769)	2.8(0.1347)
	Pielou's evenness	**32.0(0.0005)**	0.0(0.8917)	3.6(0.0931)
Beta diversity	Bray-Curtis dissimilarity	**31.9(0.0010)**	**9.0(0.0030)**	**8.1(0.0040)**
Latvia				
Alpha diversity	Observed richness	**7.3(0.0269)**	0.3(0.6103)	0.7(0.4317)
	Shannon diversity index	0.4(0.5560)	1.5(0.2580)	0.1(0.7840)
	Pielou's evenness	0.2(0.6660)	1.6(0.2430)	0.1(0.8290)
Beta diversity	Bray-Curtis dissimilarity	**4.8(0.0010)**	1.7(0.0950)	1.2(0.2050)
Spain				
Alpha diversity	Observed richness	-	0.0(0.9250)	-
	Shannon diversity index	-	1.5(0.2830)	-
	Pielou's evenness	-	4.1(0.1140)	-
Beta diversity	Bray-Curtis dissimilarity	-	0.7(1.0000)	-
Ireland				
Alpha diversity	Observed richness	0.2(0.6660)	0.0(0.9990)	0.0(0.9450)
	Shannon diversity index	0.0(0.8870)	**4.0(0.0820)**	0.6(0.4730)
	Pielou's evenness	0.0(0.9344)	**4.5(0.0665)**	0.7(0.4417)
Beta diversity	Bray-Curtis dissimilarity	1.1(0.2970)	**1.5(0.0880)**	0.5(0.9900)
Finland				
Alpha diversity	Observed richness	1.5(0.2580)	0.1(0.7220)	0.3(0.6130)
	Shannon diversity index	0.1(0.7550)	0.0(0.9210)	0.0(0.9040)
	Pielou's evenness	0.0(0.9100)	0.0(0.8400)	0.1(0.8100)
Beta diversity	Bray-Curtis dissimilarity	**1.9(0.0080)**	0.7(0.9810)	0.7(0.9510)

The research leading to these results has received funding from the European Union Horizon 2020 Research & Innovation programme under the Grant Agreement no. 101000371.



The research leading to these results has received funding from the European Union Horizon 2020 Research & Innovation programme under the Grant Agreement no. 101000371.

*Figure C.3. Beta diversity of sampled soils. Canonical analysis of principal coordinates of Bray distances of iterative subsampled datasets of **fungi** by field site. The dots represent individual samples, comprising three biological replicates per treatment combination. The percent success of classification is indicated in each axis. Alternative management includes organic in agricultural lands (BE, DK, ES, HU, LV), grassland mixture in IE, and continuous forest in FI. Conventional management includes conventional agriculture (BE, DK, ES, HU, LV), mono-species grasslands in IE, and clearcut young, generated forest in FI..*



The research leading to these results has received funding from the European Union Horizon 2020 Research & Innovation programme under the Grant Agreement no. 101000371.

*Figure C.4 Relative abundance of the most abundant **fungal** phyla by field site Alternative management includes organic in agricultural lands (BE, DK, ES, HU, LV), grassland mixture in IE, and continuous forest in FI. Conventional management includes conventional agriculture (BE, DK, ES, HU, LV), mono-species grasslands in IE, and clearcut young, generated forest in FI.*

The research leading to these results has received funding from the European Union Horizon 2020 Research & Innovation programme under the Grant Agreement no. 101000371.

*Table C.4. PERMANOVAs results of alpha and beta diversity of **eukaryotes** by field site. F values are followed by P values in parentheses of each metric. Significant or marginally significant effects of management, drought treatment, or the interactions are bolded.*

Diversity	Metric	Management (M)	Drought (D)	M×D
Belgium				
Alpha diversity	Observed richness	**6.1(0.0389)**	1.1(0.3275)	6.4(0.0350)
	Shannon diversity index	1.0(0.3540)	0.8(0.4090)	2.4(0.1630)
	Pielou's evenness	1.5(0.2510)	0.7(0.4420)	1.8(0.2160)
Beta diversity	Bray-Curtis dissimilarity	**6.0(0.0020)**	0.8(0.6630)	0.9(0.5580)
Denmark				
Alpha diversity	Observed richness	**9.2(0.0161)**	**6.6(0.0334)**	1.6(0.2371)
	Shannon diversity index	0.8(0.3860)	0.1(0.7140)	0.2(0.6600)
	Pielou's evenness	1.6(0.2390)	0.0(0.9180)	0.4(0.5520)
Beta diversity	Bray-Curtis dissimilarity	**5.7(0.0020)**	1.4(0.1070)	1.2(0.2030)
Hungary				
Alpha diversity	Observed richness	**27.0(0.0008)**	**5.5(0.0475)**	0.0(0.1429)
	Shannon diversity index	**7.5(0.0252)**	**3.9(0.0832)**	2.6(0.1459)
	Pielou's evenness	**16.2(0.0038)**	3.3(0.1085)	**4.3(0.0716)**
Beta diversity	Bray-Curtis dissimilarity	**15.8(0.0010)**	**6.2(0.0030)**	**5.5(0.0010)**
Latvia				
Alpha diversity	Observed richness	**43.6(0.0002)**	**20.5(0.0019)**	0.1(0.7535)
	Shannon diversity index	0.0(0.8330)	0.1(0.7490)	0.0(0.9940)
	Pielou's evenness	0.9(0.3650)	0.7(0.4270)	0.0(0.9830)
Beta diversity	Bray-Curtis dissimilarity	**4.3(0.0010)**	**1.8(0.0630)**	**1.8(0.0610)**
Spain				
Alpha diversity	Observed richness	-	0.2(0.7040)	-
	Shannon diversity index	-	0.0(0.9680)	-
	Pielou's evenness	-	0.1(0.7690)	-
Beta diversity	Bray-Curtis dissimilarity	-	0.8(0.9000)	-
Ireland				
Alpha diversity	Observed richness	0.3(0.6240)	0.1(0.8200)	0.4(0.5240)
	Shannon diversity index	0.4(0.5520)	0.3(0.6100)	0.6(0.4600)
	Pielou's evenness	0.5(0.4840)	0.3(0.5840)	0.3(0.5830)
Beta diversity	Bray-Curtis dissimilarity	1.1(0.3170)	1.1(0.2950)	0.6(0.9760)
Finland				
Alpha diversity	Observed richness	0.8(0.3950)	0.1(0.8270)	0.0(0.9500)
	Shannon diversity index	0.3(0.5710)	0.0(0.9020)	0.4(0.5340)
	Pielou's evenness	0.2(0.6680)	0.0(0.9360)	0.6(0.4730)
Beta diversity	Bray-Curtis dissimilarity	**2.4(0.0040)**	0.9(0.6130)	0.8(0.7030)

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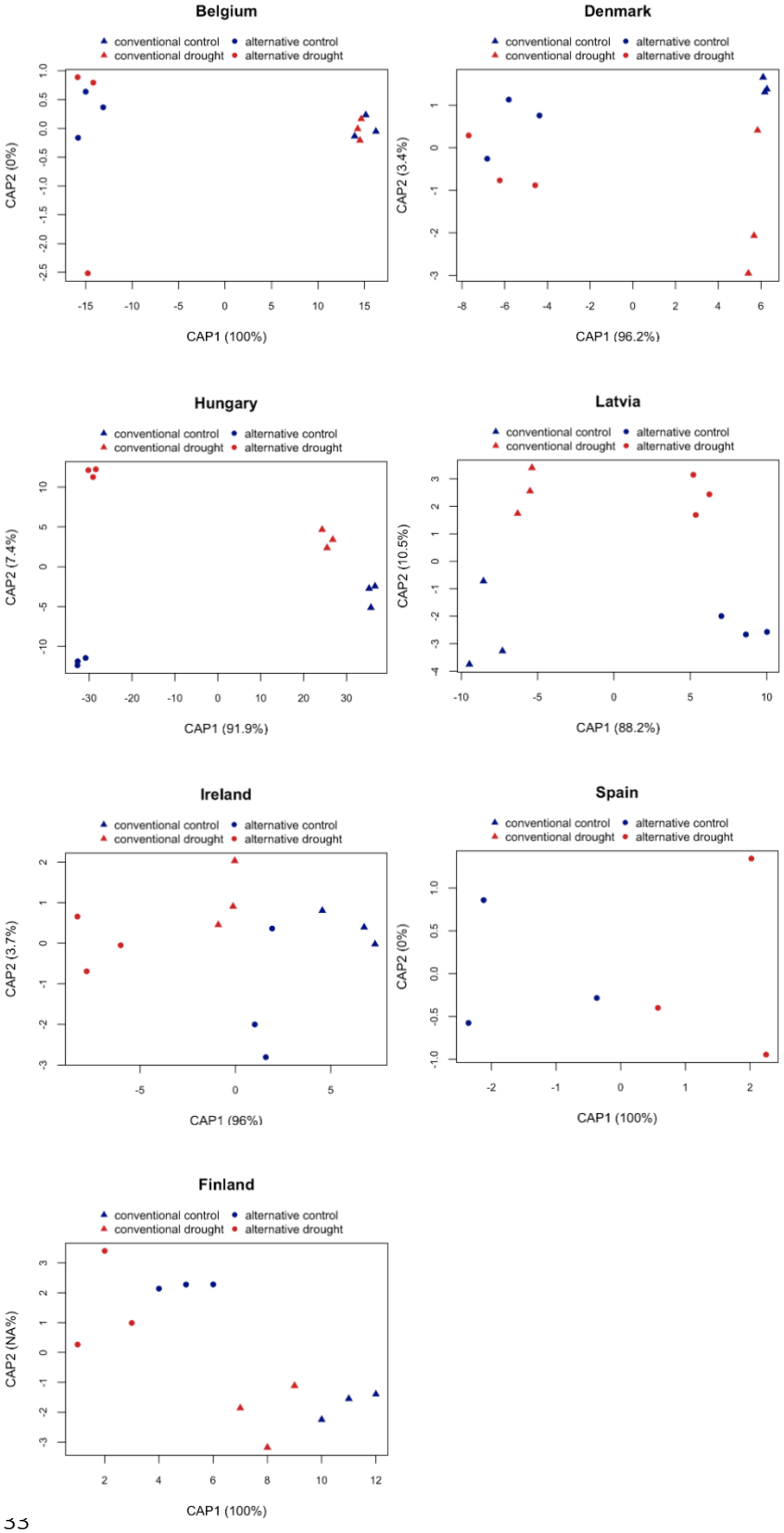

The research leading to these results has received funding from the European Union Horizon 2020 Research & Innovation programme under the Grant Agreement no. 101000371.

*Figure C.5. Beta diversity of sampled soils. Canonical analysis of principal coordinates of Bray distances of iterative subsampled datasets of **eukaryotes** by field site. The dots represent individual samples, comprising three biological replicates per treatment combination. The percent success of classification is indicated in each axis. Alternative management includes organic in agricultural lands (BE, DK, ES, HU, LV), grassland mixture in IE, and continuous forest in FI. Conventional management includes conventional agriculture (BE, DK, ES, HU, LV), mono-species grasslands in IE, and clearcut young, generated forest in FI.*

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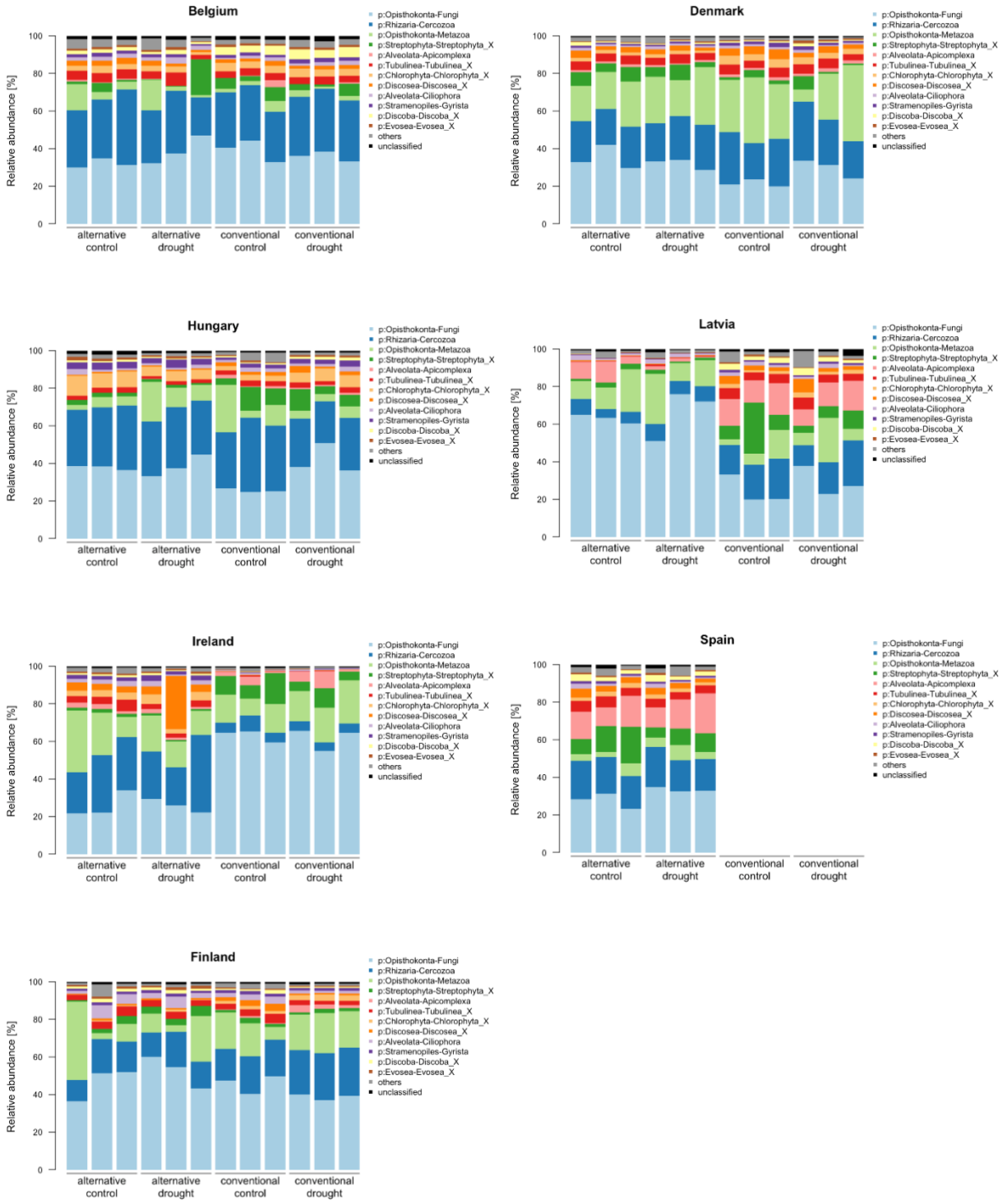

*Figure C.6 Relative abundance of the most abundant **eukaryotic** phyla by field site Alternative management includes organic in agricultural lands (BE, DK, ES, HU, LV), grassland mixture in IE, and continuous forest in FI. Conventional management includes conventional agriculture (BE, DK, ES, HU, LV), mono-species grasslands in IE, and clearcut young, generated forest in FI.*

The research leading to these results has received funding from the European Union Horizon 2020 Research & Innovation programme under the Grant Agreement no. 101000371.

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14. Appendix D. Metabarcoding of plant-microbiome interactions.

14.1. Methods

Sampling of rhizosphere and roots

Cropland – maize, wheat barley: Three plants representative for the plot in height and health status were selected close to the sampling points 1, 2 and 4 (see soil sampling protocol). Plants were excavated together with the root system down to a depth of 10 cm.

Cropland – potatoes: Three representative plants were selected on the ridge between the rows for bulk soil sampling. Plants were excavated together with the root system down to a depth of 10 cm. Tubers, stolons and bigger roots were removed.

Grassland: Three soil cores with a representative plant composition were collected close to soil sampling position 1, 2 and 4 (see soil sampling protocol). The same corer as used for bulk soil sampling was used and samples were taken down to a depth of 10 cm.

Forest: Three replicates of outplanted *Picea abies* seedling per treatment where sampled.

From all samples, bulk soil and stones were removed by gentle shaking or by rubbing if soil was too loamy. The combined roots together with the adhering rhizosphere soil from three plants per sampling area were transported to the local laboratories and kept cooled (without freezing) until further processing. Subsequently, samples were spread on an appropriate heat-stable tray and dried for 2-3 h at 85 °C. After drying, a representative subsample of an estimated 100-200 g containing both, rhizosphere and roots was placed in appropriate containers (plastic bags or plastic buckets) together with a clean silica bag. Where root development was extremely poor (e.g. samples from Spain), less sample was taken. Containers were tightly closed to avoid rewetting during shipment. Containers were correctly labeled and shipped to the AIT laboratory in Tulln (Austria).

DNA extraction

Due to the drying step nearly no rhizosphere soil remained attached to the root but accumulated in the container. Further washing of the roots in PBST according to the protocol did not result in recovery of substantial amounts of rhizosphere soil. Therefore, the soil present in the container was taken as the rhizosphere soil. Roots were washed, surface sterilized and dried. Fine roots were selected from the sample and ground to a fine powder with steel balls in a bead beater. DNA from rhizosphere soil and root powder was isolated with the MagAttract PowerSoil Pro kit (Qiagen) according to manufacturer's protocols. Establishment of amplification and sequencing protocols

All library preparations and sequencings are done externally at LGC (Berlin, Germany). To avoid co-amplification of mitochondrial and plastid sequences with the 16S rRNA gene primers for bacteria and archaea, the PNA-clamp method (Lundberg et al., 2013) was applied. During library preparation for high-throughput sequencing, a PNA clamp is added to the PCR mixture, that specifically blocks amplification of 16S rRNA gene sequences from mitochondria and chloroplasts. This method had to be established at LGC before analyzing the SOILGUARD samples. Tests were done with seven soil and seven wheat root samples in the presence and absence of PNA clamps and in the presence and

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absence of LGC-specific additives in all combinations. LGC-specific additives increase efficiency and lower bias during library preparation. Bioinformatic analyses for the test data set were done with the packages phyloseq (McMurdie and Holmes, 2013) and vegan (Oksanen et al., 2018). Bray-Curtis dissimilarities were used for calculation of PERMANOVA.

High-throughput sequencing

All DNA from rhizosphere and root samples are currently with LGC for MiSeq library preparation. Bacterial and archaeal 16S rRNA genes are amplified with primers 16S0341MF (CCTAYGGGDBGCWSCAG) and 16S0806MR (GGACTACNVGGGTHTCTAAT). PNA-clamps were added to root samples. The fungal ITS2 region was amplified in a two-step protocol with a preamplification with primer pair FungiQuant-F (GGRAAACTCACCAGGTCCAG) and TW13 (GGTCCGTGTTTCAAGACG) and a final amplification of the ITS2 region with primer pair ITS3ngs (CANCGATGAAGAACGYRG) and ITS4ngs (CCTSCSCTTANTDATATGC). Amplification protocols were harmonized with protocols used for microbial community analyses in soil samples from WP2 and WP3. Raw data from sequencing of rhizosphere and root samples are expected by the end of October 2023. Bioinformatic analyses will be done as outlined in Perazzolli et al. (2022).

Establishment of the PNA clamp method for the analysis of bacterial and archaeal communities in root samples

Bacterial communities in the seven soil samples were highly similar in the presence and absence of PNA clamps during the library preparation step ($p = 0.841$; PERMANOVA). The presence of the LGC specific additive, on the other hand, had a strong impact on the community ($p = 0.001$; PERMANOVA). It lowered the bacterial diversity and changed the community composition. The LGC specific additive increased the relative abundance of Verrucomicrobiota and Actinobacteriota, and decreased the relative abundance of Chloroflexi, Patescibacteria, WPS-2 and Gemmatimonadota. According to information from LGC, communities in the presence of their specific additive more closely resemble the original community as deduced from results with mock-communities. In root samples, addition of the PNA clamps reduced plastid reads from ca. 70% to less than 5% of total reads.

A combination of PNA clamps and LGC-specific additive for 16S rRNA gene library preparation from SOILGUARD root samples will therefore be used. By this approach we expect efficient amplification of bacterial and archaeal partial 16S rRNA gene sequences with little contamination from mitochondrial and plastid sequences and little bias for subsequent sequencing on the MiSeq platform.

14.2. References Appendix D

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15. Appendix E. Region-specific cascading effects of future soil biodiversity dynamics on soil multifunctionality – detailed methods and supporting information.

15.1. Detailed methods

Soil physical-chemical analyses and other ecosystem processes: Soil texture was measured following Kettler et al. (2001), and pH and electric conductivity by using a 1:1 (soil:water) dissolution, agitated during 30 minutes, following standard soil protocols (e.g., Kalra et al. 1995). Similarly, water holding capacity was estimated by saturating 20 g of dry soil with water and letting it rest for 24 hours covered in parafilm, to avoid evaporation (e.g., Grizzetti et al. 2016). After this period, we used a modified version of the infiltration protocol by Mills et al. (2009) to obtain an estimate of soil water infiltration capacity. Bulk density was measured *in situ*, according to the protocol detailed in the SOILGUARD soil sampling protocol (e.g., Arshad et al. 1997). Crop yield and leaf damage information was obtained in situ by the different local sampling teams, and then treated and curated by UA. Crop yield is based on landowner estimates (or estimated in situ under the climate change treatments by the local sampling teams). Leaf damage estimates used high quality images and followed the BugNet protocol (https://www.bug-net.org/), providing two measurements: insect and fungal pathogen damage (as % of leaf surface). Soil nutrients (N and P) were estimated by colorimetry using chemical extractants appropriate for each one (Olsen & Sommers 1982; Allen et al. 1986). Soil organic carbon was obtained by ^{13}C isotopic analyses after acid fumigation (Harris et al. 2001), which avoid the use of the highly pollutant Potassium dichromate and adheres to EU´s green card standards. Litter decomposition and soil enzymatic activities followed the tea bag (Keuskamp et al. 2013) and fluorometry (Dick et al. 2018) methodologies, respectively, also recommended by SoilBON (Guerra et al. 2021). Soil aggregate stability was evaluated by combining the slake and remould tests (Field & McEnzie 1997), as similar water-based resistance methodologies are also used by well-established organizations such as the United States Geological Survey (USGS) and the Commonwealth Scientific and Industrial Research Organization (CSIRO) and provide good proxies of soil mechanical and chemical resistance to erosion.

The only issues we have experienced regarding this part of the methodology were: i) soil aggregates could not be measured for some samples, as large enough particles were not possible to obtain, ii) we repeated all N-related measurements due to a problematic reactive, which rendered suspicious N transformation rates, and iii) some of the leaf damage and litter decomposition data were not judged reliable due to lack of quality in the pictures or missing field data, so these were coded as NAs in the database. The database is periodically (every 2 months) shared amongst those partners in charge of curating or using it (ETH, UA, UKCEH, ICT), or whoever member of the SOILGUARD consortium requesting it.

Statistical analyses: Two sets of analyses have been performed (Table E.1.). The first one is a site-level analyses were the effects of our treatments: management (conventional vs alternative), drought (rain-out shelter vs control), and their interactions are evaluated through a two-way ANOVA. This was repeated for all soil functioning and biodiversity indicators.

The second set of analyses tried to better understand the mediating effect of soil biota on the response of soil functioning to drought and management. These were performed including multiple

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regions together, to allow for a more complex set of predictors. To do so, we focused only on croplands (5 out of 7 study sites), and used site-based standardizations. This standardization, using Z-scores and the mean and standard deviation across all sites for each region, effectively removes a large part of the statistical noise derived from studying different sites, crop types, and climatic contexts in our results, allowing us for a more solid assessment of the effects of our treatments. Furthermore, in these analyses we also included aridity (as obtained from Trabucco & Zomer 2019), soil pH, electrical conductivity and texture (% sand) as additional predictors, to account for the influence of local climate and soil conditions. With this approach, we can produce more general and sound conclusions. Finally, to evaluate potential changes in synergies and trade-offs in between functions across contrasting management and climatic scenarios, we compared their Pearson´s correlations before (raw data, independent of conditions) and after filtering by management and drought treatments via partial correlations.

While we have already measured multiple functions, multifunctionality indices are sensitive to the amount and type of functions included (reviewed in Manning et al. 2018). To avoid potential confusion from a yet incomplete set of functions, therefore, we only report here analyses on individual functions. We will report on multifunctionality metrics in D3.3 once we have the complete set of functions measured.

Table E.1. *Summary of the statistical analyses presented in this report. Two levels of organization are considered (site-level, multiple cropland regions together), and the specific analyses performed in each case are detailed.*

Level	Statistical analysis	Summary
Site	Two-way ANOVA: management × drought	Site-specific response of soil biodiversity and functioning to drought under different soil managements.
Multiple regions (general)	Linear models: function = management × drought × aridity × soil_biodiversity + pH + EC + sand_content	Uses site-level standardizations to allow analyzing multiple regions together. Stronger statistical power allows for more complex tests and evaluate modulating effects of soil biodiversity and current climate (aridity).
	Pearson´s correlations between pairs of functions	Quantify synergies (positive correlations) and trade-offs (negative correlations) overall (region-based standardizations). By using partial correlations, we filter out the effect of management and drought on these synergies and trade-offs.

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15.2. Region and biome-specific impact of climate stressors on soil biodiversity and multifunctionality status under different types of soil management

Belgium did not show any significant Management x Drought interactions, suggesting that soil management has generally positive effects on soil biodiversity and functioning (Table E.2, all but one significantly positive Management effects), independently of the climatic conditions. Similar results were found for the N-cycle genes (Appendix F). The drought treatment had weak effects on the response variables measured so far, with only two negative effects registered for soil water infiltration capacity and available N.

As with Belgium, **Denmark**´s soils showed a generally positive and significant response to alternative vs conventional management (Table E.3; Appendix F). This was true for all but two of the variables analyzed (water infiltration capacity and aggregate stability). Four out of 11 variables (36%) showed significant Management x Drought interactions, although their directions were different. For soil aggregate stability and β-glucosidase enzymatic activity, the positive effects of alternative vs conventional management became *stronger* under drought. However, for litter decomposition and xylanase enzymatic activity, the positive effects of the alternative organic management were masked by the drought treatment.

In **Finland**, and contrary to most other regions, the drought treatment had similarly stronger effects than the contrasting forest managements considered. Drought significantly reduced β-glucosidase and phosphatase enzymatic activities, and water infiltration capacity (Table E.4). Forest management showed little promise to buffer these negative impacts, as we did not find any significant Management x Drought interactions. Forest management effects were highly idiosyncratic, with positive effects of the "alternative" managements (continuous cover forest, 40 years-old: CCF40; more sustainable clear-cut practices: TSW12) for soil phosphatase and glucosaminidase activities, but with higher values of the Prokaryote diversity and the abundance of denitrifier genes (Appendix F) in the conventional practices (clear-cut forests, 12-years old: T12).

The site in **Hungary** is where we found the largest proportion of significant Management and Drought effects differences. For all but two of the variables analyzed, including the N-cycling guilds (Appendix F), we found either a significant Management, Drought, or Management x Drought interaction (Table E.5.). In all cases where Management and Drought interacted, the positive effects of the alternative management declined under drought conditions. As found with the complementary analyses presented in 9.1 above, soil biota -specifically eukaryotes, including fungi- was in general less responsible than soil functioning to agricultural management or drought. The latter only responded negatively to organic farming in Hungary (Table E.5), but not in the other sites.

Ireland´s grasslands showed by far the weakest effects of our treatments across all regions. We did not find any management (monoculture vs mixture), or drought, effects in any of the response variables considered. The only three exceptions to this pattern were: i) litter decomposition (Table E.6.) which followed the cross-regional patterns of higher functioning level under the alternative management (mixture) that weakens under drought (significant Management x Drought interaction), ii) lower bacterial abundance (not richness), and higher nosZII N2O-reducers in monoculture grasslands under

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drought relative to mixture grasslands (Appendix F), and iii) lower fungal diversity under drought, independently of grassland management (Table E.6).

Latvia, together with Ireland, provided an exception to the general positive effects of the alternative management on soil properties (Table E.7.). In eight out of nine significant Management effects, we found declines in soil biota or functioning under organic vs conventional farming, and this was true also for bacterial nitrifier abundance (Appendix F). These negative effects of organic farming were generally more detrimental under drought conditions.

Finally, the site in Spain followed the general trends observed for other sites (Table E.8.). We found positive effects of organic farming in six out of 11 functional variables studied, like results observed in Belgium, Denmark or Hungary. These positive effects also extended to soil biota (here measured as soil DNA concentration, as the low amounts encountered prevented us from sequencing it).

Overall, the sites with the highest soil organic C levels (Finland, Ireland, Latvia) showed the least positive effects of sustainable soil management (Figure 20). The latter result supports the notion that organic agriculture and other soil sustainable management techniques may be more beneficial in places with relatively low organic carbon levels and therefore with a stronger potential to enhance soil carbon storage (reviewed in Rehberger et al. 2022).

Taken collectively, our preliminary results suggest strong benefits of shifting from conventional to organic agriculture in croplands, with little evidence in favor of or against similar conversions on forests or grasslands. We found relatively weak support for the buffering impact of the alternative management to mitigate the negative impacts of future climatic scenarios. Hence, our results so far suggest that conventional to organic management conversions could be more beneficial if focusing on those sites that are expected to suffer less from a drier climate in the future.

*Table E2. Summary statistics for **Belgium**. Fischer´s F and their associated P-values (***< 0.001; ** < 0.01, * < 0.05, º < 0.10) are shown for each response variable analyzed (rows) and treatment (columns). Significant results are highlighted in red, to ease visualization. The last column summarizes the direction of the effect. Alt > Conv means that the alternative soil management [Alt] show larger numbers than conventional [Conv] farming; and Alt < Conv means the opposite. Dr < Co means that the drought [Dr] treatment diminished that variable regarding our climatic control [Co], and Dr > Co means the opposite.*

Response Variable	Management(M)	Drought(D)	M x D	Effect
Litter decomposition	**6.57***	1.31	2.93	Alt. > Conv.
β-glucosidase	**11.13***	0.09	1.68	Alt. > Conv.
Xylanase	**6.56***	1.31	2.93	Alt. > Conv.
N-A-glucosaminidase	**16.19****	0.44	1.97	Alt. > Conv.
Phosphatase	**57.16*****	0.29	2.43	Alt. > Conv.
Available N	1.77	**10.60****	0.00	Dr < Co
N mineralization	**9.47***	0.44	0.42	Alt. > Conv.
Aggregate stability	0.00	0.47	1.06	
Organic carbon	**7.41***	0.37	0.00	Alt. > Conv.
Water holding capacity	**26.6*****	0.32	0.05	Alt. > Conv.
Water infiltration	0.21	**6.94***	0.60	Dr < Co
Prokaryotes	**11.66****	0.03	1.17	Alt. < Conv.
Eukaryotes	0.98	0.74	2.32	
Fungi	0.00	1.42	1.75	

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*Table E.3. Summary statistics for **Denmark**. Fischer´s F and their associated P-values (***< 0.001; ** < 0.01, * < 0.05, º < 0.10) are shown for each response variable analyzed (rows) and treatment (columns). Significant results are highlighted in red, to ease visualization. The last column summarizes the direction of the effect. Alt > Conv means that the alternative soil management [Alt] show larger numbers than conventional [Conv] farming; and Alt < Conv means the opposite. Dr < Co means that the drought [Dr] treatment diminished that variable regarding our climatic control [Co], and Dr > Co means the opposite.*

Response Variable	Management(M)	Drought(D)	M x D	Effect
Litter decomposition	**40.02*** **	0.20	**11.10***	Alt. > Conv., weaker under drought
β-glucosidase	**67.13*** **	0.51	**5.72***	Alt. > Conv., even more under drought
Xylanase	**40.02*** **	0.20	**11.10***	Alt. > Conv., weaker under drought
N-A-glucosaminidase	**10.23***	0.14	0.16	Alt. > Conv.
Phosphatase	**11.79****	1.24	0.45	Alt. > Conv.
Available N	**7.15***	1.13	0.90	Alt. > Conv.
N mineralization	**284.36*** **	**5.38***	0.99	Alt. > Conv.; Co < Dr
Aggregate stability	**7.36***	2.27	**7.36***	Alt. < Conv., only under drought
Organic carbon	**132.67*** **	1.10	0.27	Alt. > Conv.
Water holding capacity	**19.19****	**3.18º**	0.02	Alt. > Conv.; Dr < Co
Water infiltration	1.49	0.17	0.00	
Prokaryotes	1.50	**5.66***	1.17	Co > Dr
Eukaryotes	0.81	0.15	0.20	
Fungi	0.00	0.32	1.79	

*Table E.4. Summary statistics for **Finland**. Fischer´s F and their associated P-values (***< 0.001; ** < 0.01, * < 0.05, º < 0.10) are shown for each response variable analyzed (rows) and treatment (columns). Significant results are highlighted in red, to ease visualization. The last column summarizes the direction of the effect. Alt > Conv means that the alternative soil management [Alt] show larger numbers than conventional [Conv] farming; and Alt < Conv means the opposite. Dr < Co means that the drought [Dr] treatment diminished that variable regarding our climatic control [Co], and Dr > Co means the opposite.*

Response Variable	Management(M)	Drought(D)	M x D	Effect
Litter decomposition	0.50	0.00	0.70	
β-glucosidase	1.23	**8.23***	0.18	Dr < Co
Xylanase	0.50	0.00	0.70	
N-A-glucosaminidase	**6.14****	0.48	0.57	TSW> T12 > T40 > CCF
Phosphatase	**2.99º**	**7.99***	1.26	Dr > Co CCF > TSW12 > T40 > T12
Available N	1.42	0.23	0.45	
N mineralization	2.55	2.94	0.24	
Organic carbon	1.19	2.73	0.73	
Water holding capacity	1.19	0	1.71	
Water infiltration	0.54	**5.27***	0.07	Dr < Co
Prokaryotes	**11.19*** **	0.00	1.36	T12 = TSW12 > T40 = CCF40
Eukaryotes	1.91	2.32	2.84	
Fungi	0.58	0.62	0.19	

*Table E.5. Summary statistics for **Hungary**. Fischer´s F and their associated P-values (***< 0.001; ** < 0.01, * < 0.05, º < 0.10) are shown for each response variable analyzed (rows) and treatment (columns). Significant results are highlighted in red, to ease visualization. The last column summarizes the direction of the effect. Alt > Conv means that the alternative soil management [Alt] show larger numbers than conventional [Conv] farming; and Alt < Conv means the opposite. Dr < Co means that the drought [Dr] treatment diminished that variable regarding our climatic control [Co], and Dr > Co means the opposite.*

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Response Variable	Management(M)	Drought(D)	M x D	Effect
β-glucosidase	**141.51*** **	2.18	1.58	Alt. > Conv.
Xylanase	**27.76*** **	0.71	**0.03***	Alt. > Conv., weaker under drought
N-A-glucosaminidase	**86.52*** **	**8.51***	**6.64***	Alt. > Conv., weaker under drought
Phosphatase	**77.58*** **	**6.95***	**14.67****	Alt. > Conv., weaker under drought
Available N	**25.11*** **	7.17	**33.31*** **	Alt. > Conv., weaker under drought
N mineralization	3.25	0.23	1.46	
Organic carbon	**47.8*** **	0.01	0.44	Alt. > Conv.
Water holding capacity	**150.2*** **	2.46	0.51	Alt. > Conv.
Water infiltration	1.92	0.69	0.69	
Prokaryotes	**798.1*** **	**318.1*** **	**208.8*** **	Alt. > Conv., weaker under drought
Eukaryotes	**7.54***	3.85	2.56	Alt. < Conv.
Fungi	**21.1****	0.00	2.74	Alt. < Conv.

*Table E.6. Summary statistics for **Ireland**. Fischer´s F and their associated P-values (***< 0.001; ** < 0.01, * < 0.05, º < 0.10) are shown for each response variable analyzed (rows) and treatment (columns). Significant results are highlighted in red, to ease visualization. The last column summarizes the direction of the effect. Alt > Conv means that the alternative soil management [Alt] show larger numbers than conventional [Conv] farming; and Alt < Conv means the opposite. Dr < Co means that the drought [Dr] treatment diminished that variable regarding our climatic control [Co], and Dr > Co means the opposite.*

Response Variable	Management(M)	Drought(D)	M x D	Effect
Litter decomposition	**8.16***	**33.99****	**12.33***	Alt. > Conv., but only with NO drought
β-glucosidase	0.00	1.83	0.67	
Xylanase	0.10	1.09	0.01	
N-A-glucosaminidase	0.73	0.48	0.00	
Phosphatase	1.36	2.36	1.16	
Available N	0.00	0.18	0.06	
N mineralization	0.16	2.64	0.00	
Aggregate stability	0.25	0.25	0.25	
Organic carbon	0.33	0.36	0.44	
Water holding capacity	0.05	0.36	1.51	
Water infiltration	0.14	0.34	0.04	
Prokaryotes	0.19	0.16	0.14	
Eukaryotes	0.38	0.27	0.59	
Fungi	0.02	**3.93***	0.57	Co > Dr

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*Table E.7. Summary statistics for **Latvia**. Fischer´s F and their associated P-values (***< 0.001; ** < 0.01, * < 0.05, º < 0.10) are shown for each response variable analyzed (rows) and treatment (columns). Significant results are highlighted in red, to ease visualization. Significant results are highlighted in red, to ease visualization. The last column summarizes the direction of the effect. Alt > Conv means that the alternative soil management [Alt] show larger numbers than conventional [Conv] farming; and Alt < Conv means the opposite. Dr < Co means that the drought [Dr] treatment diminished that variable regarding our climatic control [Co], and Dr > Co means the opposite.*

Response Variable	Management(M)	Drought(D)	M x D	Effect
β-glucosidase	**5.93***	**8.43****	**3.51º**	Alt. < Conv., even more under drought
Xylanase	1.97	2.94	**15.16****	Alt. < Conv., but only under drought
N-A-glucosaminidase	1.66	0.74	0.29	
Phosphatase	**28.5*****	**6.83***	**3.82º**	Alt. < Conv., even more under drought
Available N	8.78**	5.73*	3.28	Alt. < Conv., Dr > Co
N mineralization	9.75**	21.76***	3.49	Alt. > Conv., Co > Dr
Organic carbon	**110.2*****	0.75	0.88	Alt. < Conv.
Water holding capacity	**60.5*****	1.54	**20.8*****	Alt. < Conv., even more under drought
Water infiltration	**5.9***	2.64	**3.5º**	Alt. < Conv., even more under drought
Prokaryotes	**6.44***	**8.86***	0.28	Alt. < Conv., Co > Dr
Eukaryotes	0.05	0.12	0.00	
Fungi	0.38	1.48	0.08	

*Table E.8 Summary statistics for **Spain.** Fischer´s F and their associated P-values (***< 0.001; ** < 0.01, * < 0.05, º < 0.10) are shown for each response variable analyzed (rows) and treatment (columns). Significant results are highlighted in red, to ease visualization. The last column summarizes the direction of the effect. Alt > Conv means that the alternative soil management [Alt] show larger numbers than conventional [Conv] farming; and Alt < Conv means the opposite. Dr < Co means that the drought [Dr] treatment diminished that variable regarding our climatic control [Co], and Dr > Co means the opposite. Since DNA could not be sequenced for some of the samples in Spain, here soil DNA concentration is used as an indicator of soil biota.*

Response Variable	Management(M)	Drought(D)	M x D	Effect
Litter decomposition	0.50	0.00	0.70	
β-glucosidase	**16.9****	1.40	0.24	Alt. > Conv.
Xylanase	**14.7****	3.35	1.42	Alt. > Conv.
N-A-glucosaminidase	3.25	1.92	0.59	
Phosphatase	**5.93***	1.41	0.04	Alt. > Conv.
Available N	**11.80****	0.02	2.23	Alt. > Conv.
N mineralization	0.26	0.05	0.62	
Aggregate stability	1.67	1.67	0.60	
Organic carbon	**27.81*****	0.29	1.17	Alt. > Conv.
Water holding capacity	0.44	0.39	0.62	
Water infiltration	**6.49***	0.06	0.17	Alt. > Conv.
Soil DNA	**14.9*****	2.71	0.07	Alt. > Conv.

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16. Appendix F. Quantification of nitrogen-cycling guilds.

16.1. DNA amplification

Quantitative PCR reactions were carried out in a ViiA7 (Life Technologies, United States) in a 15 µl reaction volume containing 7.5 µL of Takyon MasterMix (Eurogentec, France), 1–2 µM of each primer, 250 ng of T4 gene 32 (MP Biomedicals, France), and 4.5 ng of DNA. Standard curves were obtained using serial dilutions of linearized plasmids containing appropriated cloned targeted genes from bacterial strains or environmental clones. No template controls gave null or negligible values. The presence of PCR inhibitors in DNA extracted from soil was estimated by mixing a known amount of standard DNA with soil DNA extract prior to qPCR. No inhibition was detected in any case. Two to four independent runs were performed for each gene. The relative abundances of the N-cycling microbial communities were calculated based on the ratio of the functional gene copy numbers to the total 16S rRNA gene copy numbers, yielding a percentage of the abundance of the studied microbial communities relative to the total bacterial community abundance. Archaeal nitrifier abundance was analyzed relatively to the bacterial nitrifier abundance. The ratios of *nosZII* to *nosZI*, *nirK* to *nirS*, and *comaA* to *comaB* were also analyzed. The results from the qPCR assays of DNA samples from Spain have been excluded from the following analysis because of the small amount of DNA available, which affected the robustness of the analyses.

16.2. Statistics

Statistical analyses were conducted using R statistical software version 4.2.1 (R Core Team, 2022) and focused on the region level and aimed at evaluating the effects of the drought treatment, the management type (conventional versus alternative) on the N-cycling potentials and the total bacterial community abundance, in each country. Two-way ANOVA was used to test the interaction of management and drought, followed by Tukey's tests for mean comparisons. Effects of management and drought taken independently were also tested using one-way ANOVA, followed by Tukey's tests for mean comparisons. For the forest samples from Finland, alternative management corresponds to continuous forest and conventional to clear cut. For grassland samples from Ireland, alternative management corresponds to mixture grassland and conventional to monoculture grassland.

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16.3. Region-specific results

Belgium

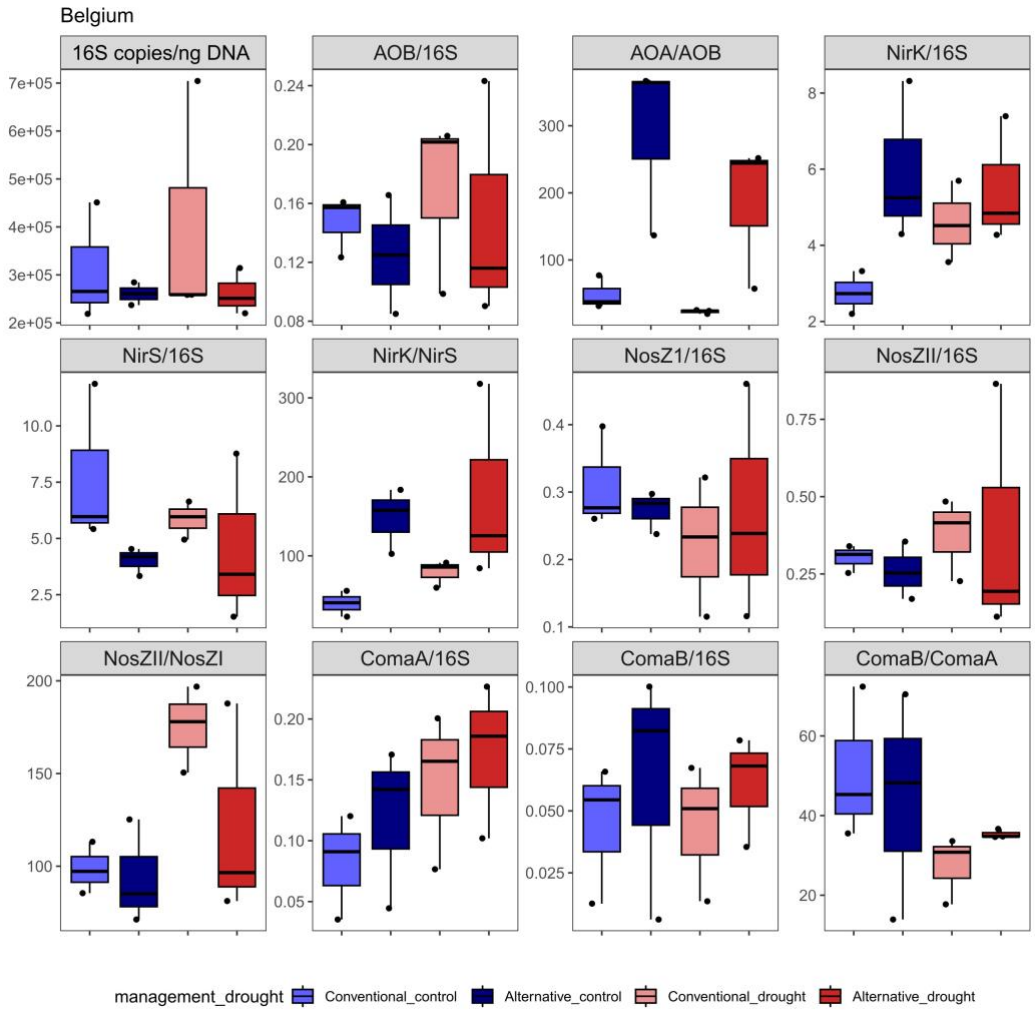

Figure F.1. Total bacterial community abundance (16S rRNA gene copies per ng of DNA), relative abundances in the bacterial community of bacterial amoA nitrifiers (AOB/16S), archaeal amoA nitrifiers abundance relative to AOB (AOA/AOB), denitrifiers (nirK/16S and nirS/16S) and their relative proportion (nirK/nirS), N_2O-reducers (nosZI/16S and nosZII/16S) and their relative proportion (nosZI/nosZII), complete amoA nitrifiers clade A and B (comaA/16S and comaB/16S), and their relative proportion (comaA/comaB), in Belgian soils across management type and drought treatment. Alternative management in this case is organic management. Boxes show the inter-quartile range between the 1st and 3rd quartiles, with median indicated by the line and whiskers indicate the maximum and minimum of the inter-quartile range. Control samples are depicted in blue shades, drought samples by red shades, lighter shades depict conventional management and darker shades organic management.

In Belgian soils (Figure F.1), the relative abundance of archaeal nitrifiers (AOA/AOB) was significantly affected by management (Two-way ANOVA, $p<0.01$), with much lower proportions in conventional soils compared to organic ones (Tukey's test, $p<0.05$). The *nirK* denitrifier relative abundance, as well

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as the *nirK*/*nirS* ratio were also significantly reduced in conventionally managed soils (Tukey's test, $p<0.05$). Drought only affected the proportion of clade II *nosZ* N_2O reducers relative to the clade I abundance, which was larger with drought (Tukey's test, $p<0.05$), especially in conventionally managed soils.

Denmark

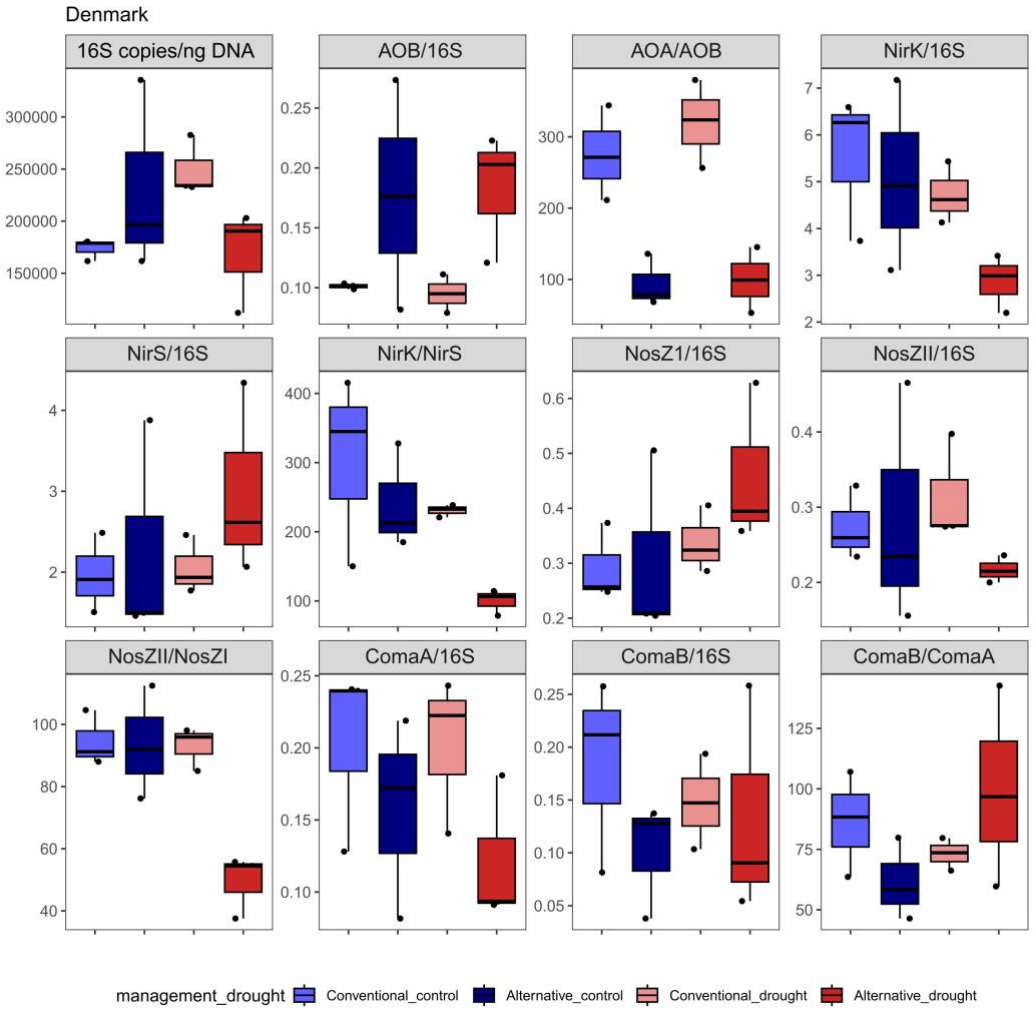

Figure F.2. Total bacterial community abundance (16S rRNA gene copies per ng of DNA), relative abundances in the bacterial community of bacterial amoA nitrifiers (AOB/16S), archaeal amoA nitrifiers abundance relative to AOB (AOA/AOB), denitrifiers (nirK/16S and nirS/16S) and their relative proportion (nirK/nirS), N_2O-reducers (nosZI/16S and nosZII/16S) and their relative proportion (nosZI/nosZII), complete amoA nitrifiers clade A and B (comaA/16S and comaB/16S), and their relative proportion (comaA/comaB), in Danish soils across management type and drought treatment. Alternative management in this case is organic management. Boxes show the inter-quartile range between the 1st and 3rd quartiles, with median indicated by the line and whiskers indicate the maximum and minimum of the inter-quartile range. Control samples are depicted in blue shades, drought samples by red shades, lighter shades depict conventional management and darker shades organic management.

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In Danish soils (Figure F.2.), the relative abundance of bacterial nitrifiers (AOB/16S) was significantly higher in organic soils (Tukey's test, $p<0.05$). Concomitantly, the proportion of archaeal nitrifiers relative to the bacterial ones (AOA/AOB) was lower in organic soils (Tukey's test, $p<0.001$). The relative proportion of clade II to clade I *nosZ* N_2O reducers was significantly reduced with drought (Tukey's test, $p<0.001$), especially in organic soils, with a significant interaction of management and drought (Two-way ANOVA, $p<0.01$).

Finland

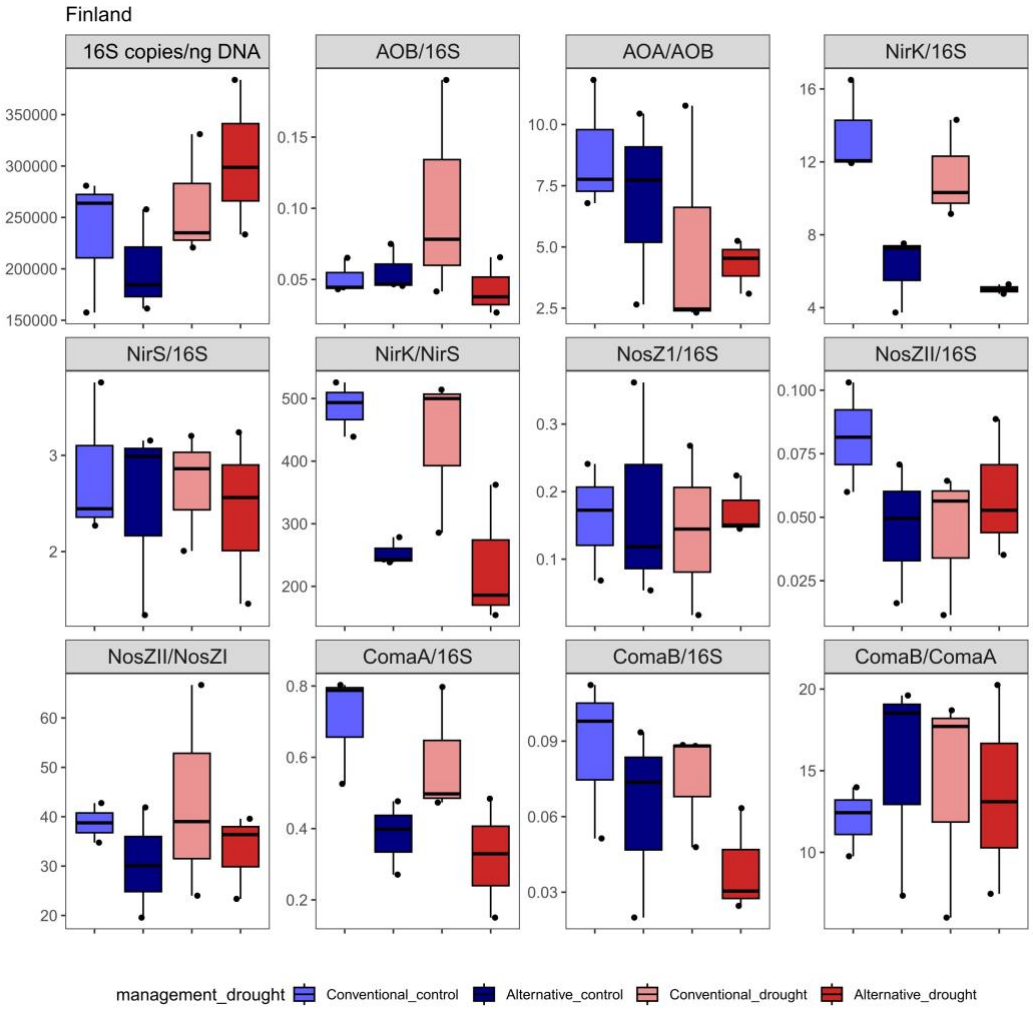

Figure F.3. Total bacterial community abundance (16S rRNA gene copies per ng of DNA), relative abundances in the bacterial community of bacterial amoA nitrifiers (AOB/16S), archaeal amoA nitrifiers abundance relative to AOB (AOA/AOB), denitrifiers (nirK/16S and nirS/16S) and their relative proportion (nirK/nirS), N_2O-reducers (nosZI/16S and nosZII/16S) and their relative proportion (nosZI/nosZII), complete amoA nitrifiers clade A and B (comaA/16S and comaB/16S), and their relative proportion (comaA/comaB), in Finnish soils across management type and drought treatment. Conventional management stands for clearcut and alternative management for continuous forest. Boxes show the inter-quartile range between the 1st and 3rd quartiles, with median indicated by the line and whiskers

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indicate the maximum and minimum of the inter-quartile range. Control samples are depicted in blue shades, drought samples by red shades, lighter shades depict conventional management and darker shades organic management.

In the Finnish forest soils (Figure F.3.), the clearcut treatment (i.e. conventional) significantly increased the *nirK* denitrifier abundance and its relative proportion to the *nirS* denitrifiers, in comparison to the continuous forest treatment, whatever the drought treatment (Tukey's test, $p<0.01$ for both). The relative abundance of comaA complete nitrifiers followed the same trend (Tukey's test, $p<0.05$).

Hungary

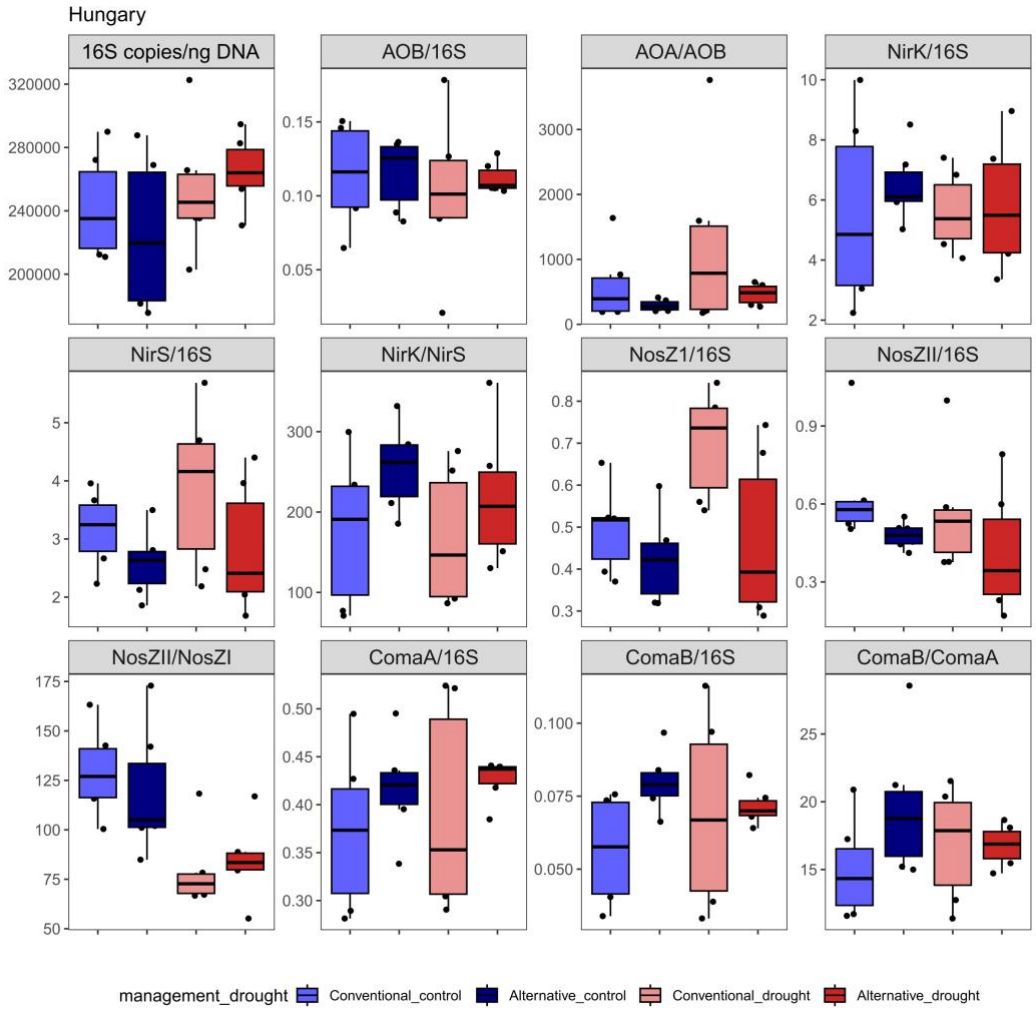

Figure F.4. *Total bacterial community abundance (16S rRNA gene copies per ng of DNA), relative abundances in the bacterial community of bacterial amoA nitrifiers (AOB/16S), archaeal amoA nitrifiers abundance relative to AOB (AOA/AOB), denitrifiers (nirK/16S and nirS/16S) and their relative proportion (nirK/nirS), N_2O-reducers (nosZI/16S and nosZII/16S) and their relative proportion (nosZI/nosZII), complete amoA nitrifiers clade A and B (comaA/16S and comaB/16S), and their relative proportion (comaA/comaB), in Hungarian soils across management type and drought treatment. Alternative management in this case is organic management. Boxes show the inter-quartile range between the 1st*

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and 3rd quartiles, with median indicated by the line and whiskers indicate the maximum and minimum of the inter-quartile range. Control samples are depicted in blue shades, drought samples by red shades, lighter shades depict conventional management and darker shades organic management.

In Hungarian soils (Figure F.4.), the management type significantly affected all N-cycling guilds, except the relative proportion of clade II to clade I *nosZ* N_2O reducers. A significant increase in organic soils was observed for the AOB relative abundance, the *nirK*/*nirS* ratio, and the *nosZ*II relative abundance (Tukey's test, $p<0.05$). In contrast, the AOA relative abundance to AOB, the *nirS* and the *nosZ*I relative abundances significantly decreased in organic soils compared to conventionally managed soils (Tukey's test, $p<0.05$). ComaA and comaB relative abundances, and their ratio, despite high variability in conventional soils, were all higher in organic soils (Tukey's test, $p<0.01$). Drought affected positively the total abundance of the bacterial community (Tukey's test, $p<0.05$), and negatively the nosZII/nosZI ratio (Tukey's test, $p<0.05$). Significant interaction effects of management and drought were found on the *nirK* relative abundance (Two-way ANOVA, $p<0.001$), and the *nirS* relative abundance (Two-way ANOVA, $p<0.05$)

Ireland

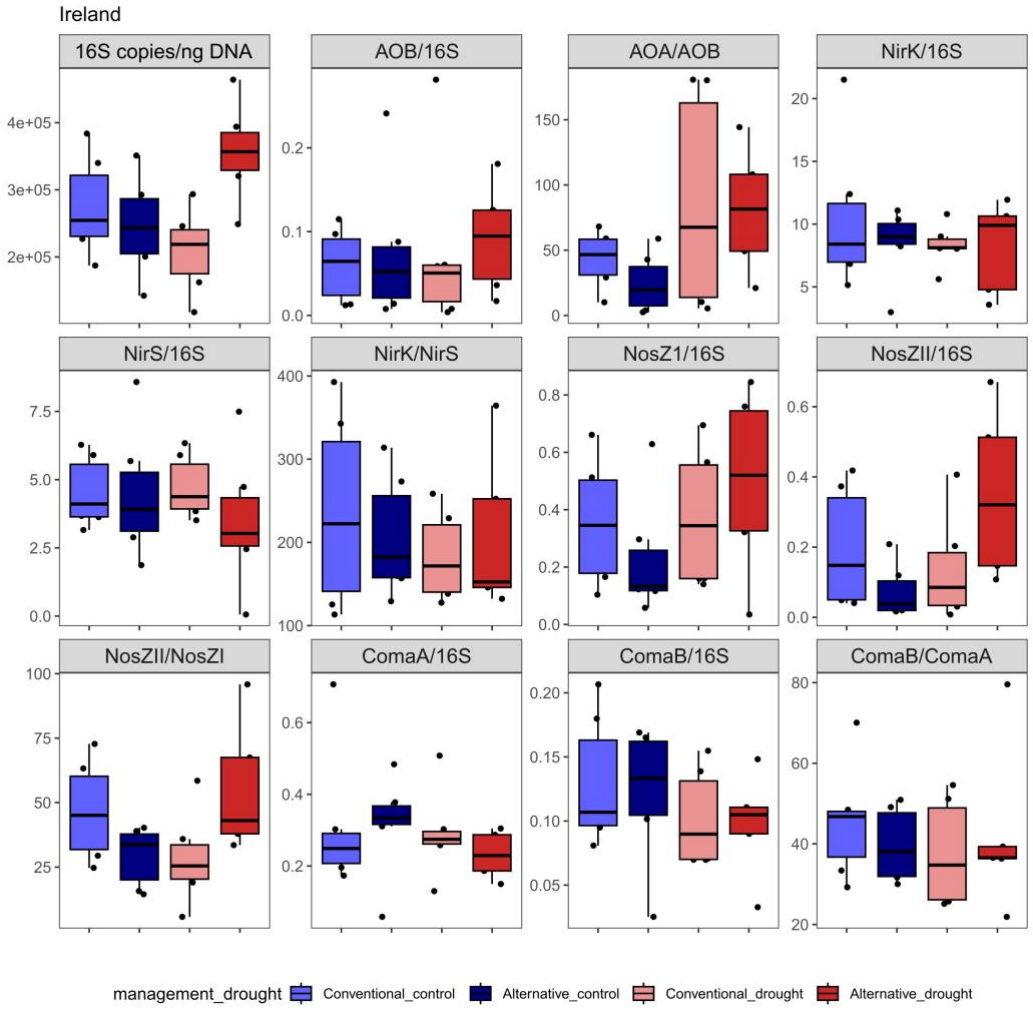

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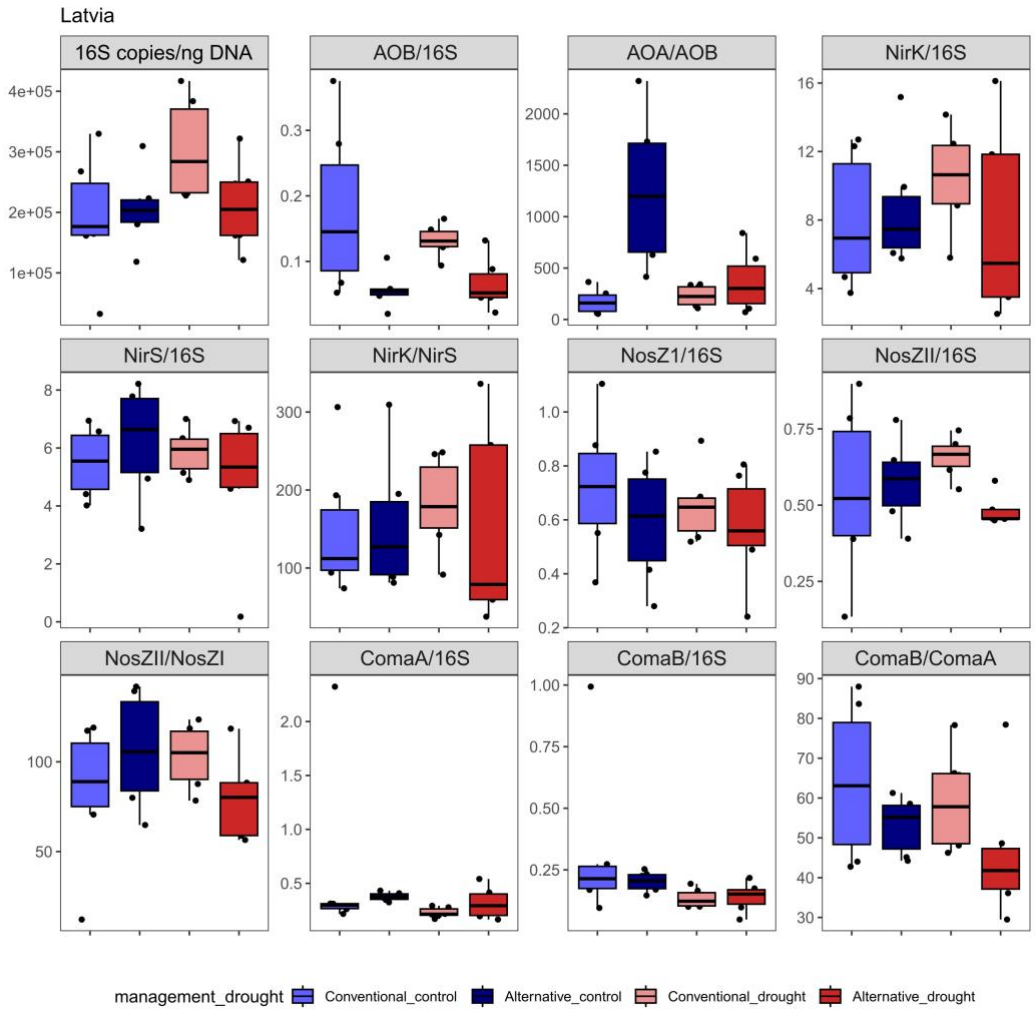
Figure F.5. Total bacterial community abundance (16S rRNA gene copies per ng of DNA), relative abundances in the bacterial community of bacterial amoA nitrifiers (AOB/16S), archaeal amoA nitrifiers abundance relative to AOB (AOA/AOB), denitrifiers (nirK/16S and nirS/16S) and their relative proportion (nirK/nirS), N_2O-reducers (nosZI/16S and nosZII/16S) and their relative proportion (nosZI/nosZII), complete amoA nitrifiers clade A and B (comaA/16S and comaB/16S), and their relative proportion (comaA/comaB), in Irish soils across management type and drought treatment. Conventional management stands for monoculture and alternative management for mixture culture. Boxes show the inter-quartile range between the 1st and 3rd quartiles, with median indicated by the line and whiskers indicate the maximum and minimum of the inter-quartile range. Control samples are depicted in blue shades, drought samples by red shades, lighter shades depict conventional management and darker shades organic management.

No significant difference was detected in the Irish pasture soils, whatever the N-cycling guilds, drought treatment and management type (Figure F.5., Tukey's test, $p>0.05$).

Latvia



The research leading to these results has received funding from the European Union Horizon 2020 Research & Innovation programme under the Grant Agreement no. 101000371.

Figure F.6. Total bacterial community abundance (16S rRNA gene copies per ng of DNA), relative abundances in the bacterial community of bacterial amoA nitrifiers (AOB/16S), archaeal amoA nitrifiers abundance relative to AOB (AOA/AOB), denitrifiers (nirK/16S and nirS/16S) and their relative proportion (nirK/nirS), N_2O-reducers (nosZI/16S and nosZII/16S) and their relative proportion (nosZI/nosZII), complete amoA nitrifiers clade A and B (comaA/16S and comaB/16S), and their relative proportion (comaA/comaB), in Latvian soils across management type and drought treatment. Alternative management in this case is organic management. Boxes show the inter-quartile range between the 1st and 3rd quartiles, with median indicated by the line and whiskers indicate the maximum and minimum of the inter-quartile range. Control samples are depicted in blue shades, drought samples by red shades, lighter shades depict conventional management and darker shades organic management.

In Latvian soils (Figure F.6.), bacterial nitrifier abundance (AOB/16S) was with significantly higher in conventional soils (Tukey's test, $p< 0.05$), while archaeal nitrifiers (AOA/AOB) were significantly lower (Tukey's test, $p< 0.05$). Clade II *nosZ* N_2O reducers abundance and the *comaB*/*comaA* proportion were also higher in conventional soils (Tukey's test, $p< 0.05$), at a larger extent in organic soils. Yet no significant effect of the management-drought interaction was detected. Both clade A and B of complete nitrifiers had decreased abundance under drought (Tukey's test, $p< 0.05$).

The research leading to these results has received funding from the European Union Horizon 2020 Research & Innovation programme under the Grant Agreement no. 101000371.

16.4. References Appendix F

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17. Appendix G. Measurement of PLFAs and NLFAs in soil

17.1. Methods

For the extraction, 1 g of soil was used for arable and grassland soils, and 0.5 g were used for forest organic soils. The extraction method followed the method of Bligh and Dyer (1959), with the modifications introduced by Buyer and Sasser (2012) and (Ellis and Ritz (2018), which allow for a higher throughput of the method.

Briefly, lipids were extracted with a mixture of 1:2:0.8 chloroform, methanol and pH 7.4-phosphate buffer. The lipid extract was then evaporated, resuspended in chloroform and fractionated into neutral lipids and phospholipids with solid phase extraction columns containing 50 mg of silica each (Bond Elut-SI 12102068, Agilent Technologies, Santa Clara, California, US). Neutral lipids were eluted in chloroform, while phospholipids were eluted with a 5:5:1 chloroform, methanol and water solution. Solvents were evaporated, after that, both neutral lipids and phospholipids were subjected to a transesterification reaction by adding toluene, methanolic 0.1 M KOH, and by applying heat (37°C). A mixture of fatty acids was thus obtained for each sample and lipid fraction. PLFAs and NLFAs were then re-extracted from the reaction mix by using chloroform and 0.075 M acetic acid. After evaporating chloroform, the extracts were resuspended in hexane.

PLFA and NLFA extracts were measured with gas chromatography (Agilent Technologies) coupled with both a mass spectrometry detector and a flame ionization detector. The mass spectra were used for confirming the identity of the detected compounds, while the data collected with FID were used for compound quantification. Quantification was performed with the open-source software OpenChrom 1.5.0 (Lablicate GmbH, Hamburg, Germany). Compound quantification relied on the use of two external standards (BAME 26 component mix Supelco, and FAME 37 component mix Supelco, Merk, Darmstadt, Germany), three additional external standards (CAS 822-05-9, 2490-51-9, and 14101-91-8, Larodan AB, Solna, Sweden), and three internal standards. 10 nmol of 1,2-Dinonadecanoyl-sn-glycero-3-phosphocholine (CAS 95416-27-6) and 6.7 nmol of tritridecanoin (CAS 26536-12-9) were added at the beginning of the extraction for quantifying the extraction efficiency for PLFA and NLFA, respectively. 9.3 nmol of methyl laurate (CAS 111-82-0) were added to each extract, to correct for system variations during the measurement.

This measurement quantified the concentration in soil of 31 fatty acids (TableG.1.). Each compound was interpreted as a marker for either bacteria, actinobacteria, methanotrophic bacteria, fungi, arbuscular mycorrhizal fungi, or as a general marker for microbes or microbial storage (Table G.1.) (Ratledge, 2008; Frostegård et al., 2011; Joergensen, 2022; Mason-Jones et al., 2023). The concentrations of compounds assigned to the same type of organism were summed up, obtaining a proxy for their biomass in soil. All PLFA markers were summed up, as an estimation of the abundance of all microbial cells, while the sum of all NLFA was considered as a proxy for the abundance of intracellular microbial storage.

Table G.1. Fatty acids derived from phospholipids and neutral lipids, as extracted and analyzed in soils of the SOILGUARD network of sites.

Fatty acid	PLFA interpretation	NLFA interpretation
C10:0	general (microbes)	general (microbial storage structures)

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C14:0	general (microbes)	general (microbial storage structures)
iC15:0	**bacteria (gram +)**	general (microbial storage structures)
aiC15:0	**bacteria (gram +)**	general (microbial storage structures)
C15:0	general (microbes)	general (microbial storage structures)
iC16:0	**bacteria (gram +)**	general (microbial storage structures)
C16:1ω7c	**bacteria (gram -)**	general (microbial storage structures)
C16:1ω6c	**bacteria (gram -)**	general (microbial storage structures)
C16:1ω5c	general (microbes)	**arbuscular mycorrhizal fungi (spores in soil)**
C16:0	general (microbes)	general (microbial storage structures)
10MeC16:0	**actinobacteria**	general (microbial storage structures)
iC17:0	**bacteria (gram +)**	general (microbial storage structures)
aiC17:0	**bacteria (gram +)**	general (microbial storage structures)
aiC17:1ω5c	**bacteria (gram +)**	general (microbial storage structures)
C17:1ω8c	**bacteria (gram -)**	general (microbial storage structures)
C17:1ω7c	general (microbes)	general (microbial storage structures)
C17:0	general (microbes)	general (microbial storage structures)
10MeC17:0	**actinobacteria**	general (microbial storage structures)
C18:3ω6c	**fungi**	general (microbial storage structures)
C18:2ω6c	**fungi (best marker for fungi)**	general (microbial storage structures)
C18:1ω9c	general (microbes)	general (microbial storage structures)
C18:1ω9t	**fungi**	general (microbial storage structures)
C18:1ω8c	**methanotrophic bacteria**	general (microbial storage structures)
C18:0	general (microbes)	general (microbial storage structures)
10MeC18:0	**actinobacteria**	general (microbial storage structures)
cyC19:0	**bacteria (gram -)**	general (microbial storage structures)
aiC19:0	**bacteria (gram +)**	general (microbial storage structures)
C20:4ω6c	**algae**	general (microbial storage structures)
C20:1ω9c	**algae**	general (microbial storage structures)
C20:0	general (microbes)	general (microbial storage structures)
C22:6ω3c	**algae**	general (microbial storage structures)

17.2. Progress

For this methodology, we had problems with the delivery and functioning of a centrifugal evaporator (miVac Quattro, Genevac Ltd, Ipswich, UK). This machine is essential for fast evaporation of solvents during the extraction, and it was purchased for this project on 14th July 2022. The delivery of the machine and its components, however, was postponed by the vendor from early 12th October 2022 to 10th January 2023 (centrifugal evaporator) and 8th March 2023 (rotor). This caused a delay in testing and establishing the technique at UvA: we had a fully operational protocol in May 2023. After that, the samples coming from European sites were extracted and quantified. Unfortunately, on 6th July 2023 a component of the miVac evaporator had a failure and it could not be used for extractions until reparation or replacement. As per today, this process is still ongoing. To complete the data collection, we changed the evaporation method by drying samples under a N2 flux. This was used for the remaining samples, which include samples of WP3 and from international sites of WP2. Using N2 flux allows us to collect the data, however the method takes twice as much time compared with the evaporator. For this reason, PLFA/NLFA data for WP3 will be available not sooner than late October 2023.

17.3. References Appendix G

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18. Appendix H. Detailed management of the different fields

18.1. Croplands

18.1.1. Belgium

*Table H1. Detailed management of the fields used for the experiments in Belgium. alt = alternative management, which refers here to organic; con = conventional management. *This refers to the yield obtained in the field containing the plots, not to the yield of the experimental plot itself. Information provided by the field manager Thomas Van den Sante.*

descriptor	2022		2023	
	alt	con	alt	con
crop	Potatoes var. Nirvana	Potatoes var. Carolus	40 kg/ha (10 kg crossbred ryegrass; 6 kg English ryegrass tetra MT; 6 kg English ryegrass diploid MT; 2,5 timothee; 2 kg beemdlangbloemgras, 5 kg rietzwenkgras, 8 kg red clover, 2 kg white clover, 1 kg incarnaatclover)	40 kg/ha (10 kg crossbred ryegrass; 6 kg English ryegrass tetra MT; 6 kg English ryegrass diploid MT; 2,5 timothee; 2 kg beemdlangbloemgras, 5 kg rietzwenkgras, 8 kg red clover, 2 kg white clover, 1 kg incarnaatclover)
fertilizer source	Solid stable manure at the end of 2021, liquid cow slurry in 2022, shortly before planting.	Ammoniumnitrate 27%	Pig slurry	ammoniumnitrate (AN) 27%
amount fertilizer applied to experimental field	Stable manure 2021 : 24 t/ha (mainly contributes to higher N mineraisation in 2022). Pig slurry 2022: 40 t/ha (3,3 kg Total N/t, about 60% of the aplied N is plant available)	150 kg N/ha (555 kg ammoniumnitrate/ha)	(20 t/ha) 80kg N/ha plant available	100 kg N/ha (370 kg AN/ha)
pest control method	none	Chemical, about weekly treatments with (alternating) ; Caligula, Infinito, Ranman Top, Shirlan, Spotligt Plus, axidor, zorvec endavia, Cypomur and Gozai.	none	none
weed control method	Weed contol was done mechanically. The potatoes were planted on a level field and when the plant is large enough a bit of ground is heaped up against the planting row. This was done 3 times during the season.	17/05/2022:0,25 L Centium + 2,5 L Stomp Aqua + 2 L Proman	none	none
soil preparation method	The catch crop (rye) was mowed and removed, the stubbs werecut loose two or tree times using a supperficial (5 cm) cutting cultivator. Deep tillage : non inversed tillage (Dent michel -> working depth, about 20 cm, there is no plough pan on the field. Field preparation : rotary harrow (done at the same time as the rows were created and the tubers planted).	Ploughing (25 cm) + rotary harrow (intensive) and ridge creation at planting (the field has a plough pan)	Non inversing tillage 20-25 cm (Dent Michel) followed by field preparation with rotary harrow and sowing (in 2022, no tillage in 2023)	Ploughing 25 cm + rotary harrow followed by sowing (in 2022, in 2023 no tillage)
irrigation method	rainfall simulator (designed for erosion measurements) 32 - 33.5 L/m², two weeks later than the conventional field	rainfall simulator (designed for erosion measurements) 25-35 L/m² in the sheltered plots. Control plots received no irrigation	sprinkler 35 L/m² in the control watered plots	sprinkler 35 L/m² in the control watered plots
field yield*	4000 kg/ha	about 3500 kg/ha	1000-2000 kg/ha	1000-2000 kg/ha

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18.1.2. Denmark

*Table H2. Detailed management of the fields used for the experiments in Denmark. alt = alternative management, which refers here to organic; con = conventional management. *This refers to the yield obtained in the field containing the plots, not to the yield of the experimental plot. Information provided by the field manager Helle Hestbjerg.*

descriptor	2022		2023	
	alt	**con**	**alt**	**con**
crop	Spring barley	Spring barley	Faba beans	Spring barley
fertilizer source	organic	Inorganic + oganic	Organic	Inorganic + organic
amount fertilizer applied to experimental field	25 t pig slurry	150 kg inorganic + 36 t pig slurry, Mangannitrate 1 kg	none	80 kg inorganic + 30 t pig slurry
pest control method	none	1/6: Comet Pro 0,2 L.	none	20/6: Pirimor 0.05 L, Lamdex 0.04 L, Pictor Actine 0.1 L.
weed control method	none	5/4: Legacy 0,13 l. 19/5: Trimmer 7g, Mustang Forte 0,25 L, Pxixaro 0,15 L.	none	4/5: Lecacy 0.12L. 27/5: Pixxaro 0.15 L, Trimmerr 6.2 g, Zypar 0.25 L.
soil preparation method	Plowing, harrowing	Plowing, harrowing	Harrowing	Harrowing
irrigation method	none			
field yield*	5100 kg/ha	5300 kg/ha	1100 kg/ha	4200 kg/ha

18.1.3. Hungary

*Table H3. Detailed management of the fields used for the experiments in Latvia. alt = alternative management, which refers here to organic; con = conventional management. *This refers to the yield obtained in the field containing the plots, not to the yield of the experimental plot. Information provided by the field manager Tóth Zoltán.*

descriptor	2022		2023	
	alt	**con**	**alt**	**con**
crop	maize	maize	winter wheat	winter wheat
fertilizer source	Molasses fermentation residue	NPK 10:20:20 + CAN	Molasses fermentation residue	NPK 10:20:20 + CAN
amount fertilizer applied to experimental field	2 tons/ha, 80kg N, 0,8kg P, 132kg K plant available	128 kg/ha N, 40 kg/ha P2O5, 40 kg/ha K2O	2 tons/ha, 80kg N, 0,8kg P, 132kg K plant available	128 kg/ha N, 40 kg/ha P2O5, 40 kg/ha K2O
pest control method	no	no	no	Fungicide: Prosaro 1L/ha (prothioconazole + tebuconazole), Insecticide: Karate Zeon 0,2L/ha (lambda-cyhalothrin)
weed control method	mechanical	Lumax (chemical)	mechanical weed comb	Bizon 1L/ha (diflufenican + florasulam + penoxsulam)
soil preparation method	ploughing (25-28cm)	ploughing (25-28cm)	disk tiller combined with seeder	ploughing (25-28cm) + seedbed preparation with harrow
irrigation method	none			
field yield*	5500 kg/ha	7000 kg/ha	3000 kg/ha	6500 kg/ha

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18.1.4. Latvia

*Table H4. Detailed management of the fields used for the experiments in Latvia. alt = alternative management, which refers here to organic; con = conventional management. *This refers to the yield obtained in the field containing the plots, not to the yield of the experimental plot. Information provided by the field manager Ina Alsina.*

descriptor	2022		2023	
	alt	**con**	**alt**	**con**
crop	winter wheat	winter wheat	spring wheat	spring wheat
fertilizer source	none	ammonium nitrate 34% N; ammonium sulphate, 21% N, 24% S	none	Basic fertilizer: NPK 15:15:15, Additional fertilizers: ammonium nitrate 34% N; ammonium sulphate, 21% N, 24% S
amount fertilizer applied to experimental field	none	In spring, after resumption of vegetative growth - ammonium nitrate 200 kg ha; In the second top-dressing (GS 31– 32) ammonium sulphate 150 kg ha, in the heading (GS 50-53) - ammonium nitrate 100 kg ha	none	Basic fertilization - 300 kg ha, Additional fertilization: GS 31-32 - ammonium nitrate 200 kg ha; in the heading (GS 50-53) - ammonium sulphate 100 kg ha
pest control method	none			
weed control method	harrowing	Herbicide: Biathlon 4D (tritosulfuron 714 g/ha, florasulam 54 g/ha) 0.06 kg/ha	harrowing	herbicide: Biathlon 4D (tritosulfuron 714 g/ha, florasulam 54 g/ha) 0.06 kg/ha
soil preparation method	ploughing 22-24 cm	ploughing 22-24 cm	ploughing 22-24 cm	ploughing 22-24 cm
irrigation method	none			
field yield*	1950 kg/ha	3860 kg/ha	1800 kg/ha	3700 kg/ha

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18.1.5. Spain

*Table H5. Detailed management of the fields used for the experiments in Spain. alt = alternative management, which refers here to organic; con = conventional management. *This refers to the yield obtained in the field containing the plots, not to the yield of the experimental plot. Information provided by the field manager Luis Daniel Olivares.*

descriptor	2022		2023	
	alt	**con**	**alt**	**con**
crop	wheat with mixed	oatmeal	oatmeal	oatmeal
fertilizer source	organic manure applied in previous seasons	inorganic fertilization applied in previous seasons	organic manure applied in previous seasons	inorganic fertilization applied in previous seasons
amount fertilizer applied to experimental field	none			
pest control method	none			
weed control method	none			
soil preparation method	crop residues maintaining, year crop rotation	Intensive tillage	crop residues maintaining, year crop rotation	Intensive tillage
irrigation method	none			
field yield*	300 kg/ha	900 kg/ha	none	none

The research leading to these results has received funding from the European Union Horizon 2020 Research & Innovation programme under the Grant Agreement no. 101000371.

18.2. Grassland

18.2.1. Ireland

*Table H6. Detailed management of the fields used for the experiments in Ireland. alt = alternative management, which refers here to organic; con = conventional management. *This refers to the yield obtained in the field containing the plots, not to the yield of the experimental plot. Information provided by the field manager Kerry Ryan.*

descriptor	2022		2023	
	alt	**con**	**alt**	**con**
crop	Multispecies grass	Grass-clover grassland	Multispecies grassla	Grass-clover grassland
fertilizer source	none	46% K AN and 7% S	none	none
amount fertilizer applied to experimental field	none	83 kg N/ha	none	none
pest control method	none			
weed control method	none			
soil preparation method	none			
irrigation method	Manual - 150 L (8.6 mm) added during drought treatment on 16th August 2022	Manual - 150 L (8.6 mm) added during drought treatment on 16th August 2022	Ambient rainfall - 41.2 mm during drought treatment, 11th – 17th July 2023	Ambient rainfall - 41.2 mm during drought treatment, 11th – 17th July 2023
field yield *	13563 kg DM/ha	12225 kg DM/ha	10172 kg DM/ha	11158 kg DM/ha

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18.3. Forest

18.3.1. Finland

*Table H7. Detailed management of the fields used for the experiments in Finland. alt = alternative management, which refers here to organic; con = conventional management. *This refers to the yield obtained in the field containing the plots, not to the yield of the experimental plot. Information provided by the field manager Taina Pennanen. ** Alternative forest management is Continuous-Cover Forestry (CCF) system. The main feature of CFF is the permanent retention of a high degree of canopy cover throughout management and especially in partial harvesting entries such as single-tree selection. *** Conventional forest management is clearcutting-based Rotation Forestry (RF). RF is based on rotational management of tree crops where clearcutting and artificial regeneration recurs with 60–100-year intervals.*

descriptor	2022		2023	
	alt**	**con*****	**alt****	**con*****
crop	Norway spruce (*Picea abies*)			
fertilizer source	none			
amount fertilizer applied to experimental field	none			
pest control method	none			
weed control method	none			
soil preparation method	none	mounding after clear-cutting	none	mounding after clear-cutting
irrigation method	manual			
field yield*	yearly yield can not be estimated. Standing stock 194 m^3/ha	yearly yield can not be estimated. Standing stock vary acccording to the rotational stage; 33-314 m^3/ha	yearly yield can not be estimated. Standing stock 194 m^3/ha	yearly yield can not be estimated. Standing stock vary acccording to the rotational stage; 33-314 m^3/ha

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