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Microbial functional genes influenced by short-term experimental drought across European agricultural fields

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ABSTRACT

Agricultural intensification and extreme weather events can represent considerable stress to soil microorganisms and their functions by influencing the key players behind the degradation of soil organic matter. A better understanding of the diversity and abundance of microbial functional genes that predict the functional potential of soils, can link the microbial communities to their key ecosystem functions. As there are still gaps in understanding how the functional genetic diversity behind microbial extracellular enzymes is influenced by events like drought and soil carbon management, an agricultural experiment over a range of different climatic conditions and soil properties was set-up across Europe. In Sweden, Germany and Spain, fields with varying levels of soil organic carbon were subjected to a short-term experimental drought. The diversity and composition of genes encoding for carbohydrate-related extracellular enzymes were determined using a 'captured metagenomics' technique. Functional gene diversity differed among the European regions and to a range of soil factors such as organic carbon and water content. The functional and taxonomic gene composition significantly differed between the climatic regions, while an effect of short-term drought was only observed in Germany. The results indicate that some soil microbial communities and their functional genes displayed a certain degree of resistance. The results suggest that soil microbial communities respond differently to short-term drought mainly due to regional adaptations to already dry environments and differences in their soil physicochemical properties.

1. Introduction

Agricultural intensification and extreme weather events such as changes in precipitation represent considerable stress to soil microorganisms, their functional activities and influence the key players behind the degradation of soil organic matter (Geisen et al., 2019; Jansson and Hofmockel, 2020). Climate models forecast an increase in the frequency and intensity of extreme weather events such as extreme drought (Pachauri et al., 2014; Iglesias and Garrote, 2015; Spinoni et al., 2015). In many European regions extreme droughts events are projected to become shorter, but more frequent and severe, particularly during the crop growing season (Spinoni et al., 2018; Vogel et al., 2019). This can lead to shifts in microbial communities and their functions (Hueso et al., 2012; Ochoa-Hueso et al., 2018; Schimel, 2018), including their extracellular enzyme activities that are responsible for soil organic matter degradation (Burns et al., 2013; Gao et al., 2021). The drought effects on soil microbial communities largely depend on the duration, frequency and intensity of drought events or the historical precipitation regimes (Hoover and Rogers, 2016; Meisner et al., 2018; Preece et al., 2019). Intensification of agriculture and consequent losses of soil organic carbon (SOC) can magnify the consequences of drought effects on microbial communities (de Graaff et al., 2019), however careful agricultural management practices have the potential to promote SOC levels (Lessmann et al., 2021). SOC can increase the resistance of microbial communities to drought, mitigate the negative effects of drought on crop yields and improve soil properties such as water holding capacity (Lal et al., 2011; Manns and Martin, 2018; Droste et al., 2020; Naylor et al., 2020).

It has been difficult to link specific taxa to ecosystem processes and to consequently predict functional responses of soil microorganisms (Fierer, 2017). Thus, a better understanding of the diversity and abundance of microbial functional genes that predict the functional potential of soil microorganisms, can link the knowledge of microbial communities to their key ecosystem functions (Torsvik and Øvreås, 2002; Zhang

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et al., 2015). In contrast to taxonomic information and inferring functions from taxonomy, the advantage of studying functional genes can enable the prediction of soil functions in the ecosystem (Escalas et al., 2019). Soil microbial genes coding for specific enzymes related to particular ecosystem processes can help us to establish link between genetic diversity, community structure and further to ecosystem functions, such as carbon cycling through studying carbohydrate-active enzymes (CAZy) (Cantarel et al., 2009; Yang et al., 2014; Manoharan et al., 2017). The CAZy database covers the majority of the enzyme groups involved in the different stages of the plant biomass degradation and many other related processes (Peng et al., 2018; Graham and Hofmockel, 2022). However, there are still many unknown enzymes and mechanisms involved in the soil organic matter degradation. Furthermore, this can help us to better understand the shifts in microbial communities and their diversity of functional genes due to environmental changes they are exposed to (Wang et al., 2017).

Recently developed methodologies such as 'captured metagenomics' allow us to identify and characterize genes that are responsible for key ecosystem processes carried out by soil microorganisms and can enhance our understanding in describing the microbial functional potentials in soils (Manoharan et al., 2017; Dai et al., 2021). This approach targets genes encoding for enzymes related to specific functions from different microorganisms. Using the 'captured metagenomics' technique, we can target genes of interest related to soil organic matter decomposition, that are present in low abundance in a total genome pool, while avoiding the massive sequencing efforts required of a large-scale metagenomic study. The use of 'captured metagenomics' technique allows linking of taxonomic and functional diversity of soil microorganisms, which can be connected to different drivers (agricultural management, extreme weather events or soil properties) that are influencing the functional potential of soil microbial communities that degrade soil organic matter.

Since little is known of how the soil microbial community composition and its diversity at a functional genetic level are affected by drought events, we designed a study implementing a short-term experimental drought in agricultural soils with varying levels of SOC across Europe. In order to determine the functional genetic diversity of soil microorganisms in these agricultural soils we applied the 'captured metagenomics' technique (Manoharan et al., 2015) on genes coding for extracellular enzymes involved in the carbon cycling. We addressed the following questions: I) How can extreme weather events, specifically short-term experimental drought, influence the diversity and composition of functional genes related to soil organic matter degradation? II) What soil factors influence the functional gene composition in European agricultural fields? III) Is there a link between the taxonomic and functional composition of captured genes in response to experimental drought?

2. Materials and methods

2.1. Experimental design and soil collection

This study was a part of the ERA-Net Biodiversa project 'SOILCLIM' and performed in three agricultural areas across Europe; in Southern Sweden (region Scania: SE), Northwestern Germany (region Lower Saxony: DE) and Southeastern Spain (region Almería: ES). In each region, five agricultural sites with winter wheat (*Triticum aestivum* L., cv. Wiwa) were selected and each included two fields (within a close distance) with contrasting levels of SOC content (i.e., "low" and "high" categories). These contrasting levels of SOC content are the consequence of different agricultural management practices applied in these agricultural sites. The locations of the agricultural sites, their climatic conditions and soil properties are available in the Supplementary Table 1. The experiment was set up in spring 2018 with rainout-shelters to impose drought by reducing the ambient precipitation throughout the spring and summer winter wheat growing season (Kundel et al., 2018). To control for potential roof effects by the shelters the drought treatments consisted of I) a rainout-shelter reducing 65% of the precipitation (Roof treatment, R), II) a control treatment for potential rainout-shelters artefacts where the rainout-shelter was present but did not reduce the precipitation (Roof-Control treatment, RC), and III) an unmanipulated control without a rainout-shelter (Control treatment, C). The entire experimental design resulted in 90 plots (3 regions × 5 sites × 2 fields with contrasting SOC levels × 3 drought treatments).

Soil samples from all sites were collected in 2018 during the experimental time. To ensure similar phenological status of the winter wheat the date of sampling differed between the three climatic regions (Supplementary Table 2). Methodology for collecting and analysing soil properties and vegetation data is described (Supplementary data) and data is available in the Supplementary Table 1.

2.2. DNA extractions and captured metagenomics

To characterize the diversity and composition of functional genes degrading soil organic matter, multiple soil cores (~1 kg of bulk soil, 3 cm in diameter, 20 cm depth) from each plot were collected and pooled. The samples were transported in cooled boxes, sieved (2 mm) and stored at −20 °C for subsequent DNA extraction. Total genomic DNA was extracted from 500 mg of each soil sample using the NucleoSpin Soil DNA extraction kit (Macherey-Nagel, Duren, Germany). The quality and the quantity of the extracted DNA were determined using NanoDrop 2000 (NanoDrop Technologies, Wilmington, Delaware, USA).

The 'captured metagenomics' technique was applied on the extracted DNA where so called functional genes encoding for extracellular enzymes responsible for soil organic matter degradation were enriched and sequenced (Kushwaha et al., 2015; Manoharan et al., 2015). The enrichment used unique oligonucleotide probes that were designed based on the genes coding for carbohydrate-active enzymes (CAZymes) covering all three domains of life (Bacteria, Eukaryota and Archaea). For the detection of excretory signal peptides, these genes were analysed with TargetP v1.1 (Emanuelsson et al., 2000) or SignalP v4.1 (Nielsen et al., 1997). Selected sequences with extracellular signal were further processed through the MetCap pipeline (Kushwaha et al., 2015) for design of probes (Supplementary Table 3). These custom-designed oligonucleotide probes (Table 1) were produced by Roche NimbleGen, Inc., compatible with NimbleGen SeqCap EZ protocol (Roche NimbleGen, California, USA). A database of these selected sequences coding for extracellular enzymes that were used for probe generation was set-up (here called exTDB: extracellular targeted database) and later used for the evaluation of functional genetic diversity and composition.

The soil DNA libraries were hybridized according to the SeqCapEZ protocol with the custom-designed oligonucleotide probes (Manoharan et al., 2017). The captured DNA libraries were prepared and sequenced on one lane with Illumina HiSeq 4000 system in paired-end mode (2 × 150 bp), at the Centre for Genomic Research, University of Liverpool. The raw sequencing data (fastq files) is publicly available at the EMBL-ENA database (http://www.ebi.ac.uk) under the project ID PRJEB38716.

2.3. Bioinformatics and data analysis

The analysis of data and statistics was conducted in R v4.0.5 (R Core Team, 2021) within RStudio (RStudio Team, 2021). The graphical visualization of results was performed using the *ggplot2* package (Wickham, 2016). The detailed documentation of sequence data processing, data analysis and associated commands are available https://github.com/KatjaKo/FunMetGen.

2.3.1. Soil water analyses

The effects of the experimental drought, climatic region, their interaction and effects of SOC content on the soil water content were assessed using a linear mixed-effect model (*lme4* package; Bates et al.

Table 1
The number of enzyme families and their sequences used for probe generation (Supplementary Table S3) along with their respective numbers in the captured sequence libraries. Abbreviations: auxiliary activities (AA), carbohydrate-binding modules (CBM), carbohydrate esterases (CE), glycoside hydrolases (GH), glycosyl transferases (GT), and polysaccharide lyases (PL), CAZy (a database of Carbohydrate-Active enZYmes), exTDB (extracellular targeted database).

Enzyme class	Number of enzyme families in the databases		Number of captured enzyme families	Total number of nucleotide coding sequences	Total number of generated probes	Number of captured sequences
	CAZy	exTDB				
AA	17	14	14	5096	6765	537,778
CBM	86	79	65	20,404	25,202	11,283,275
CE	17	16	16	18,989	19,241	1,968,913
GH	166	137	126	33,271	41,400	10,041,000
GT	110	94	65	9210	18,154	3,968,465
PL	38	27	23	6979	9714	1,442,096
Total	434	367	309	93,949	120,476	29,241,527

(2015)). Climatic region and drought treatment were fixed factors, SOC content as covariate and field site (i.e., five agricultural sites in each region) as a random factor. The SOC content was taken as a covariate because of varying SOC levels between the sites and the fields (low and high SOC categories, Supplementary Fig. 1). If a significant effect of a fixed factor was observed, multiple comparisons (*emmeans* package; Lenth (2021)) were performed.

2.3.2. Functional genetic diversity and composition

The captured sequences were quality filtered, trimmed and merged as described in the Supplementary data. The resulting sequences were functionally annotated by aligning them to the exTDB using a DIAMOND blastx alignment (Buchfink et al., 2015). The functional genetic diversity was measured as the number of unique enzyme IDs assigned from the exTDB of each sample. The rarefaction curves showed that the sequencing depth was sufficient for most samples, except for two samples in Spain, thus these two samples were excluded from further analyses. Rare genes (less than 1%) were filtered out from the counts of unique enzyme IDs (*edgeR* package; Robinson et al. (2010)). The remaining counts were then normalized by a variance stabilizing transformation (Love et al., 2014). The effects of the experimental drought, climatic region and their interactive effect on the whole dataset were tested with a permutational multivariate analysis of variance (PERMANOVA, function *'adonis()'*) on the normalized data from all samples in this study. In addition, the effect of experimental drought on the whole dataset was also tested with field sites nested using the *'strata'* argument in the *'adonis()'* function. A post-hoc test was applied on significant factors (*'pairwise.adonis()'* function). A principal component analysis (PCA) was used to visualize the functional composition of the carbohydrate-related extracellular enzymes in the whole dataset. The relationships between soil and plant properties to the functional gene composition on the whole dataset was assessed using the *'envfit()'* function, where the selected significant variables ($p < 0.05$) were fitted onto the ordination as vectors.

As the climatic regions caused the most significant variation on the functional gene composition in the whole dataset, the composition within each climatic region was further explored. When evaluating the effects of drought and agricultural intensification, i.e., carbon intensity ("low" and "high" SOC categories) on the functional gene composition in each region separately, the batch-effect correction (*'limma::removeBatchEffect()'*) was applied at the field site level. SOC was taken as a category ("low" and "high"), and the effects were evaluated using the permutational multivariate analysis of variance (PERMANOVA, function *'adonis()'*). Additionally, effects of SOC content on the functional gene composition were also analysed as a continuous variable using the *'envfit()'* function within each climatic region. Similar to the analysis on the whole dataset, a principal component analysis (PCA) was used to visualize the functional composition in each region.

Taxonomic assignments of the sequences were done using the contigtax tool (https://github.com/NBISweden/contigtax) in combination with exTDB. The taxonomic data was transformed to relative abundances for each sample and further the differences between samples were calculated based on the Bray-Curtis dissimilarity metric. PERMANOVA and post-hoc tests were performed on the taxonomy data similar to the functional composition as explained above. The taxonomic composition was visualized using a principal coordinate analysis (PCoA).

2.3.3. Differential abundance analysis

Differential abundance analysis of the carbohydrate-related extracellular enzymes was done using the limma-voom pipeline v 3.48.3 (Law et al., 2014; Ritchie et al., 2015). The differentially abundant genes for the different factors in each comparison were calculated using the *'lmFit()'* with linear models and normalized log2 CPM for each gene. For the whole dataset the regions were compared along with the drought treatments and for the region-specific datasets the treatments were compared. Finally, the empirical Bayes smoothing was applied to the standard errors along with the Benjamini-Hochberg method (Benjamini and Hochberg, 1995) for adjusting the p-values for multiple testing. Significant differentially abundant genes (padj <0.05) were sorted by the average abundance and evaluated at a log2 fold-change. The correlation of significantly abundant genes (padj <0.05) on the enzyme family level from the whole dataset to selected soil properties (SOC, soil pH and soil water content) was evaluated using a linear model in limma (Ritchie et al., 2015).

3. Results

3.1. Functional genetic diversity

In total, >240 million reads were obtained from the 90 samples, and these covered 309 of the 367 CAZy families targeted in the exTDB (Table 1). They belong to six different enzyme classes (auxiliary activities (AA), carbohydrate-binding modules (CBM), carbohydrate esterases (CE), glycoside hydrolases (GH), glycosyl transferases (GT), and polysaccharide lyases (PL)). The percentage of the captured reads matching the exTDB was on average 12.2% (overall), for Sweden (10.6%), Germany (8.9%), and Spain (16.5%). The captured functional genetic diversity was slightly higher in Spain, whereas in Sweden and Germany similar levels of the diversity were reached (Supplementary Fig. 2).

3.2. Effects of the experimental drought on soil water content

The soil water content in the experimental plots was significantly affected by the experimental drought, the SOC content and the climatic region (Fig. 1, Supplementary Table 4). In addition, at the time of sampling, soils in Sweden were generally drier than the soils in Germany and Spain. The soil water content in Sweden in all drought treatments was consistently lower compared to the other two regions. The soil

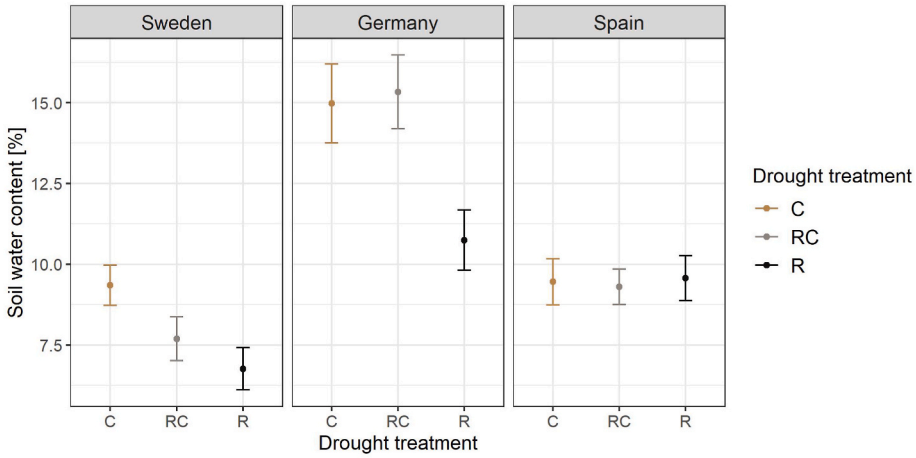

Fig. 1. Soil water content (g water/g soil) in the experimental plots of winter wheat in the three climatic regions (Sweden, Germany and Spain) and three drought treatments (C – Control, RC – Roof-Control, R – Roof). Points with error bars represent means and standard errors of the soil water content (n = 10).

water content was positively influenced by SOC content in Sweden and Germany, but not in Spain as determined by the linear mixed effect model (Supplementary Fig. 3).

3.3. Functional gene composition

There were large differences in the composition of the functional genes among the three climatic regions (Fig. 2). The composition of the functional genes displayed higher similarity between Sweden and Germany, compared to Spain, which clearly differed from the other two climatic regions. These observations were confirmed by a significant difference in the composition of captured functional genes between the three climatic regions ($p = 0.001$, Table 2). Detailed statistics is available in the Supplementary Table 5. The functional gene composition was significantly affected by soil and plant properties across all regions (Fig. 2, Supplementary Table 6). The SOC content was significantly

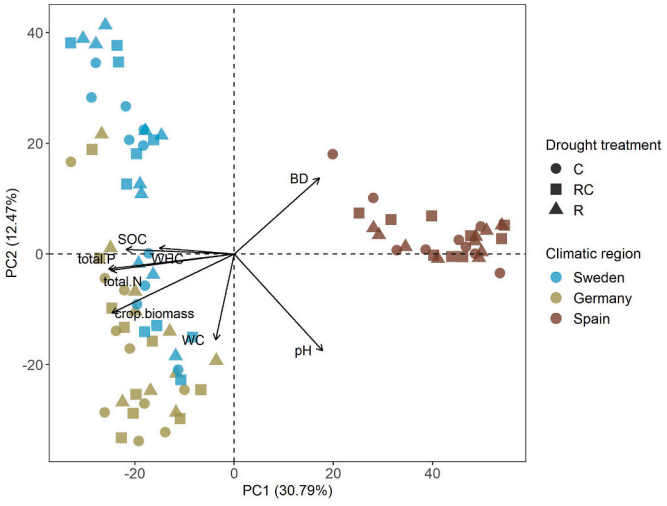

Fig. 2. Principal component analysis (PCA) plot of the normalized functional gene abundances on unique enzyme ID level. Coloured markers indicate Sweden (blue), Germany (green) and Spain (brown). Shapes represent the drought treatments (circle: C – Control, square: RC – Roof-Control, triangle: R – Roof). The arrows represent the vectors of the selected significant soil and plant properties to the PCA ordination. Abbreviations: WHC, water holding capacity; BD, bulk density; total P, total soil phosphorus; total N, total soil nitrogen; WC, soil water content (g water/g soil); SOC, soil organic carbon content. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Results from a PERMANOVA assessing effects of the drought treatment, climatic region and their interaction on the functional gene composition. Significant p-values are indicated in bold (***$p < 0.001$).

Effect	R^2	F-value	p-value
Drought treatment	0.011	0.738	0.769
Region	0.384	25.962	**0.001*****
Drought treatment x Region	0.020	0.683	0.931

correlated to the functional composition in Germany and Sweden.

In total, 1129 differentially abundant genes were found when comparing Germany and Spain, 634 between Sweden and Germany, and 1104 between Spain and Sweden. These genes belonged to 116 different enzyme families and the majority of them to the CBM and GH enzyme classes. Moreover, the differentially abundant genes also showed that Spain was very different compared to Sweden and Germany in all enzyme classes (Fig. 3). The abundances of the GT and CE families were higher in soils from Sweden, whereas the AA enzymes in Spain were almost absent (Fig. 3). Finally, no differentially abundant genes for the experimental drought were detected across the three climatic regions.

Across the three climatic regions, there were positive correlations between SOC and two genes: CE1 and CE4, and negative correlations between SOC and GT51 and CBM50 (Supplementary Fig. 4a). Positive correlations were detected between soil pH and significant genes: CBM2, PL11, CBM13 and GH23 (Supplementary Fig. 4b). Correlations between soil water content and GH67, GH3, PL10 and GH2 genes were also positive (Supplementary Fig. 4c).

While looking into the functional gene composition within each climatic region, it was affected by the experimental drought ($p = 0.036$, Table 3a) only in Germany. PC2 (Fig. 4a) separated most of the Roof treatment samples from Control and Roof-Control treatment samples. A differential abundance analysis revealed one (ADJ45077.1) out of 1443 genes that was significantly (padj <0.05) more abundant in the Roof than in the Control treatment. The functional composition was affected by carbon intensity both in Germany ($p = 0.001$, Table 3a) and in Sweden ($p = 0.01$, Table 3b). The SOC content (continuous variable) also affected the functional gene composition in both regions (Germany: $p < 0.01$ and Sweden: $p < 0.05$). The differences in functional gene composition in both regions between and within field sites due to the contrasting SOC levels were observed (Fig. 4).

3.4. Taxonomic composition and functional genes

The taxonomic annotations revealed that most of the captured

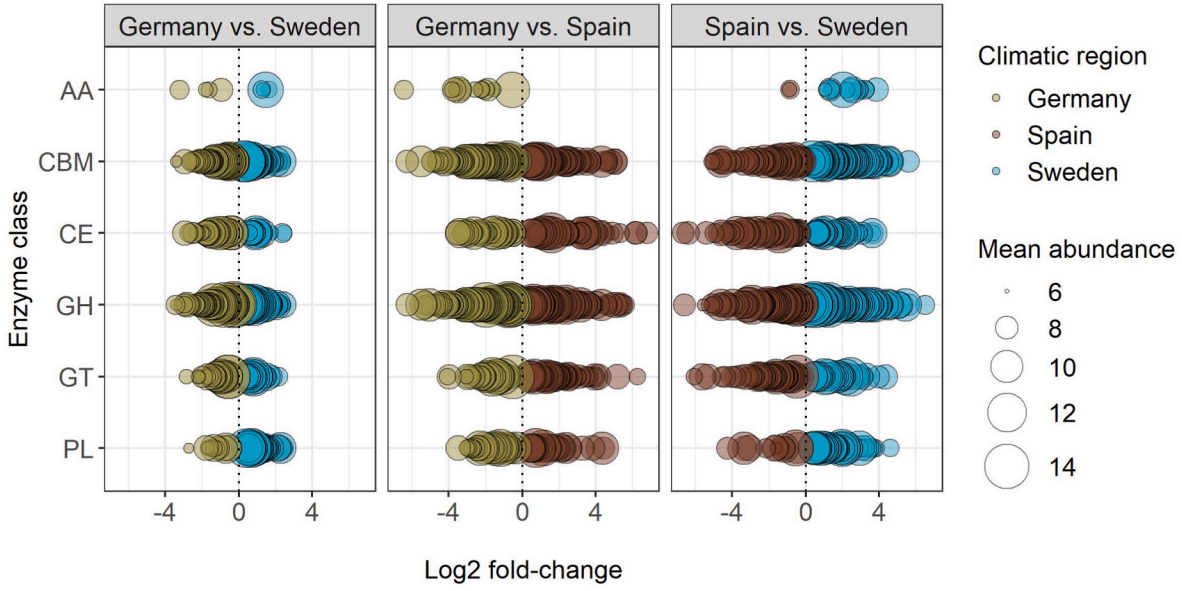

Fig. 3. Differential abundance analysis of the functional genes for the pairwise comparison between Sweden (blue), Germany (green) and Spain (brown) that were statistically significant (padj <0.05). Each dot represents a unique enzyme ID identified as significantly different. The area of each dot is average abundance across all samples, in log2 counts per million reads (CPM), here named as mean abundance and its position according to the log2 fold-change. Abbreviations: auxiliary activities (AA), carbohydrate-binding modules (CBM), carbohydrate esterases (CE), glycoside hydrolases (GH), glycosyl transferases (GT), and polysaccharide lyases (PL). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3a
Results from a PERMANOVA assessing effects of the drought treatment and carbon category on the functional gene composition in Germany. Significant p-values are indicated in bold (*p < 0.05, ***p < 0.001).

Effect	R^2	F-value	p-value
Drought treatment	0.085	1.302	**0.036***
SOC category	0.063	1.917	**0.001*****

* Results of post-hoc test for drought treatment displayed significant differences between C and R (p = 0.022).

Table 3b
Results from a PERMANOVA assessing effects of the drought treatment and carbon category on the functional gene composition in Sweden. Significant p-values are indicated in bold (***p < 0.001).

Effect	R^2	F-value	p-value
Drought treatment	0.071	1.096	0.291
SOC category	0.086	2.660	**0.001*****

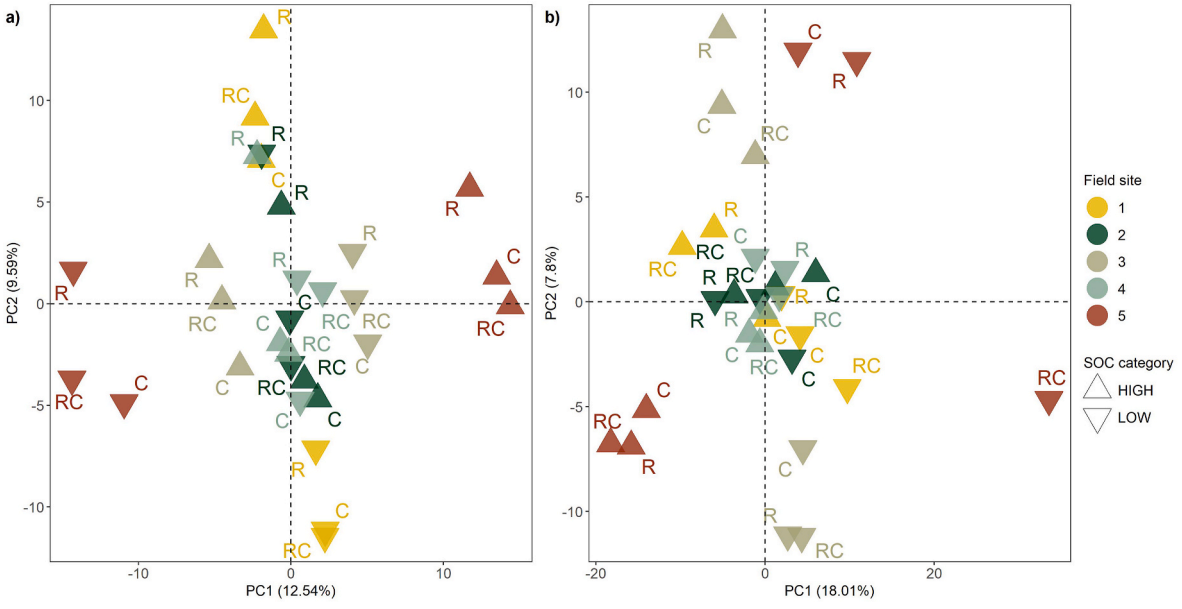

Fig. 4. Principal component analysis (PCA) plot of the normalized functional gene abundances in (a) Germany and (b) Sweden based on the unique enzyme ID level. The colours represent the five agricultural field sites within each region (1–5), two different shapes represent the SOC categories (triangle up – "high" and triangle down – "low"), and the letters represent the drought treatments (C – Control, RC – Roof-Control, R – Roof). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

sequences across the three regions belonged to Bacteria (89%) and Eukaryota (2%) and the rest remained taxonomically unassigned (9%). Regardless of the climatic region and the drought treatment, bacterial communities were dominated by Actinobacteria in Spain and Proteobacteria in Germany and Sweden (Supplementary Fig. 5). Acidobacteria was only found in Sweden and Germany while Bacteroidetes was found in all three regions. Climatic region was the main responsible factor for the taxonomic composition of functional genes, whereas experimental drought did not have an impact (Supplementary Table 7).

To understand the links between functions and taxonomy at a higher resolution, sequences belonging to bacterial phyla (abundance >1%) were selected along with their corresponding CAZy family. In total, there was one family belonging to AA, eight belonged to CBM, four to CE, seven to GH, three to GT and two to PL (Fig. 5). Actinobacteria had the highest number of the CAZymes, followed by Proteobacteria. Sweden and Germany showed similar profiles, the most pronounced differences were observed in families CBM50 and GH23. In contrast, Spain displayed a slightly different profile with families CBM13, CBM2, CBM50 and GH13, GH23 showing higher abundance of Actinobacteria.

4. Discussion

4.1. Effects of short-term drought on functional gene composition

Overall, we observed effects of the short-term experimental drought on the soil water content in the experimental plots. Despite the general drought in Europe (Di Liberto, 2018) the drought produced by the rainout-shelters was amplified and led to significant differences in the soil water content in the experimental plots. The largest differences between control and drought experimental plots were found in Sweden and Germany, where also soil carbon reduced the water losses. However, such a trend was not observed in Spain, where all drought treatments contained similar soil water content. In general, arid and semiarid regions, such as Mediterranean regions are characterised by low levels of SOC and contain higher amounts of soil inorganic carbon, up to 90% (Eswaran et al., 2000; Plaza et al., 2018). Despite the importance of soil inorganic carbon in the carbon cycling in arid and semiarid areas (Plaza-Bonilla et al., 2015), it is not fully understood if soil inorganic carbon has a potential to mitigate effects of drought. Specifically, it remains to be explored how soil inorganic carbon may alter the functioning of the SOC content in this respect and further its ability to buffer drought effects on taxonomic and functional characteristics of soil microbial communities.

The reduction in soil water in the experimental plots achieved with rainout-shelters excluding 65% of ambient precipitation, was not enough to affect the functional gene composition in two of the three regions. Short-term experimental drought however significantly affected functional gene composition in Germany. There was one gene that was more abundant in the drought treatment compared to the control treatment, i.e., ADJ45077.1. This protein belongs to the SGNH hydrolase superfamily that contains enzymes with varying hydrolytic activities, such as proteases, lipases and esterases (Akoh et al., 2004). In our exTDB, this protein was found in two enzyme families, CBM2 and CE3. CE3 has been previously found in rhizosphere soils (Bi et al., 2021) and CBM2 can among others bind celluloses and hemicelluloses (Lopez-Mondejar et al., 2016). Besides, the majority of representatives in CBM2 are cellulases of bacterial origin (Sidar et al., 2020). Additionally, this protein has been primarily found in Actinobacteria, which are known to be highly abundant under drought (Acosta-Martínez et al., 2014; Ochoa-Hueso et al., 2018; Canarini et al., 2021). Whether this specific protein is resistant or somehow linked to drought conditions remains to be explored under more severe and frequent drought events and in an extended range of physicochemical properties in agricultural soils.

The functional genetic composition in Swedish and Spanish soils was resistant to the short-term experimental drought. Characteristics of the experimental year, particularly wet conditions in the Southwestern part of Europe (Copernicus, 2018) could explain why functional genetic composition in Spain in contrast to Germany was not affected by the experimental drought. More specifically, in contrast with much of Central and Northern Europe that were characterized with the extremely dry summer (Di Liberto, 2018), the south of Europe experienced one of the wettest springs, with short and heavy rainfalls (Copernicus, 2018). Another possible explanation is that soil microbial communities in Spain are already adapted to survive drought periods. Generally, soil microorganisms in these environments (arid and semiarid) can withstand extreme dry conditions as they have the ability to adapt to changes in water potential and low resource conditions, microbial cells have thicker walls, and some microorganisms have high resistance to desiccation and the ability to form spores (Sharma and Gobi, 2016; Schimel,

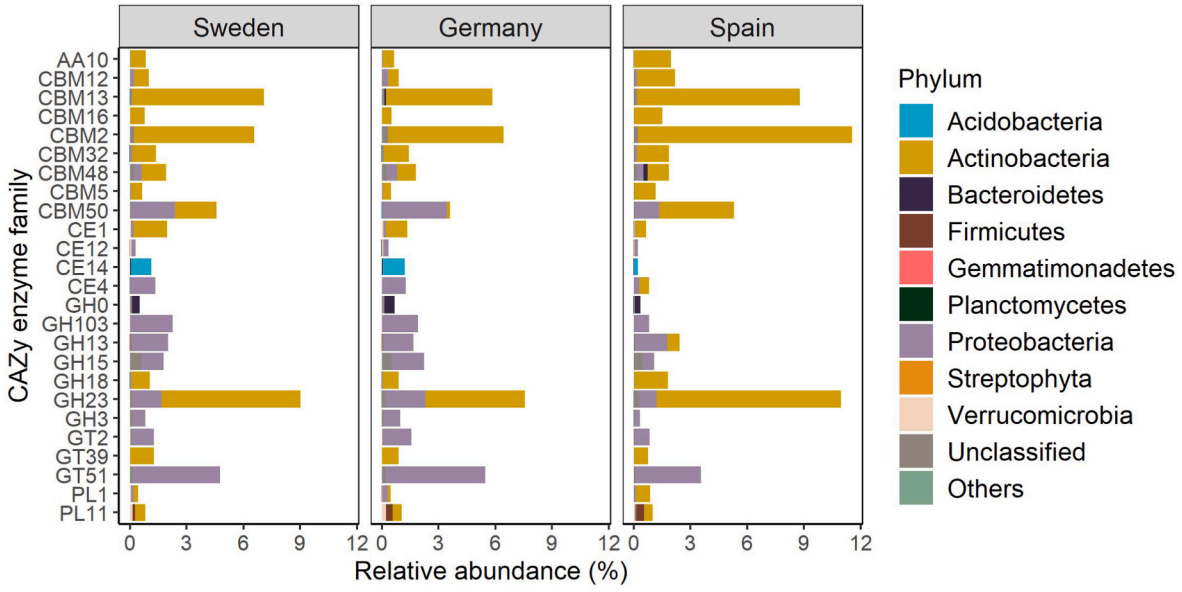

Fig. 5. The bars show the relative abundance (>1%) of CAZy enzyme families of the dominant bacterial phyla in the three climatic regions (Sweden, Germany and Spain). Abbreviations: auxiliary activities (AA), carbohydrate-binding modules (CBM), carbohydrate esterases (CE), glycoside hydrolases (GH), glycosyl transferases (GT), and polysaccharide lyases (PL).

2018). For example, members of Actinobacteria are generally more adapted to drier soils (Acosta-Martínez et al., 2014; Mohammadipanah and Wink, 2016). While dry climate adaptation of microorganisms in Spanish soils may explain the resistance to short-term drought, the resistance in Swedish soils may be explained by the extreme drought of the experimental year, and the potential buffering ability of SOC. In comparison to the previous years, field sites in Sweden received less rainfalls during the experimental period. The rainout-shelters application led to a further reduction in soil water content, from already low levels of soil water. We hypothesize that the effect of experimental drought on functional gene composition in Sweden might be overshadowed by the dry conditions of the experimental year and the higher SOC contents in these soils. Enhanced levels of SOC benefits the water holding capacity (Rawls et al., 2003; Droste et al., 2020), and thus we speculate that the SOC in the Swedish soils in contrast to German soils, buffered the soil water levels to a degree that made the composition of functional genes resistant to water shortage. The absolute effect of SOC on soil water levels are complex, and also depend on other physicochemical soil properties and agricultural management practices that affect the SOC content (Jiao et al., 2020). Therefore, a better understanding of relationships between carbon management practices, soil properties and their effects on SOC and its direct buffering ability on the resistance of soil microbial communities and their functioning could be better understood.

4.2. Effects of soil properties and location on functional gene composition

To the best of our knowledge, the diversity of functional genes encoding for carbohydrate-related extracellular enzymes in European agricultural soils, or their responses to extreme weather has until now not been extensively investigated. On a local scale, Manoharan et al. (2017) showed that land-use management affects functional genetic diversity of microbial communities. Our results show that the functional gene composition of soil microorganisms was significantly affected by the climatic region on a European scale, but the effect of a short-term drought was only present in one region. The diversity of the functional genes was similar in Sweden and Germany compared to Spain, where the enzyme families CE and GT had high abundances in comparison to Swedish and German fields, whereas the AA family was very rare. Genes from the CBM family that are often attached to specific GH families and are important for the degradation of cellulose and hemicellulose (Agbor et al., 2014; Sidar et al., 2020), were slightly more abundant in Germany. CBMs display high functional diversity and substrate specificity, and can be found in different taxonomic groups such as bacteria and fungi (Sidar et al., 2020). A widespread group of enzymes from class GH that contains cellulolytic and hemicellulolytic enzymes (Henrissat and Davies, 1997; Lopez-Mondejar et al., 2016; López-Mondéjar et al., 2016) was more abundant in Sweden and Germany. This is in accordance with Manoharan et al. (2017) that soils with higher organic matter content (Sweden and Germany) tend to harbour more enzymes with cellulolytic and hemicellulolytic activities.

Soils in these three regions across Europe are exposed to different climatic conditions (temperature, precipitation), which may lead to changes of soil physicochemical characteristics and can influence the microbial communities (Griffiths et al., 2011; Ma et al., 2016). Our results also show significant correlations of specific functional genes towards key soil properties, particularly SOC content, soil pH and soil water content. In contrast to Swedish and German soils, the Spanish soils were characterized with lower amount of SOC, high amount of soil inorganic carbon and high pH.

SOC is recognized as a major potential factor for microbial activity and functional diversity in soils (Lagomarsino et al., 2012; Manoharan et al., 2017) and was positively correlated with CE1 and CE4 families. The CE1 family, known as one of the most diverse and CE4 as one of the most abundant families of CE (Nakamura et al., 2017) is encoding for enzymes that act on xylan (Lopez-Mondejar et al., 2016) and are involved in the degradation of hemicellulose (López-Mondéjar et al., 2019). The genes GT51 and CBM50 were negatively correlated with SOC content, with GT51 predicted to have hemicellulolytic activities (López-Mondéjar et al., 2019). In line with previous studies (Fierer and Jackson, 2006; Ma et al., 2016; Bahram et al., 2018), soil pH affected the functional composition with enzyme families: CBM2, PL11, CBM13 and GH23 that were positively correlated to the soil pH. GH23 contains a cellulose-binding domain with cellulolytic and hemicellulolytic potential (Lopez-Mondejar et al., 2016; Bi et al., 2021). In addition, soil water is an important factor regulating the activity of soil extracellular enzymes (Gao et al., 2021).

Changes in soil microbial carbohydrate-related extracellular enzymes could happen due to direct effects of drought on soil water content or indirectly through changes in soil properties such as pH (Henry, 2012; Xiao et al., 2018). We found the enzyme families GH67, GH3, PL10 and GH2 to be positively correlated to the soil water content. However, further work would be needed to understand the relation of these enzyme families to the changes in soil water content.

4.3. Linking functional genes and taxonomy

The annotated taxonomic groups were mainly of bacterial origin, with a particularly high abundance of Actinobacteria and Proteobacteria that are commonly found in agricultural soils (Delgado-Baquerizo et al., 2018; Ochoa-Hueso et al., 2018). Previous studies have shown an increase in the abundance of Actinobacteria in soils exposed to drought (Bouskill et al., 2013; Canarini et al., 2021) or in drylands (Maestre et al., 2015). Therefore, it is not surprising that compared to Sweden and Germany, Actinobacteria was highly abundant in Spain. This bacterial phylum is diverse, resistant to drought, highly abundant in arid soils and adapted to soils with poor nutrient availability (Mohammadipanah and Wink, 2016). Actinobacteria are typically linked to the degradation of polysaccharides, hemicellulose and cellulose (Větrovský et al., 2014). The phylum Bacteroidetes was slightly more abundant in Germany and is one of the most common phyla in soils. Moreover, Bacteroidetes in soils are important carbohydrate degraders, producing many different CAZymes (Larsbrink and McKee, 2020). Acidobacteria, one of the most abundant phyla in soils such as grasslands, forests and pasture soils (Kalam et al., 2020) was found in Sweden and Germany, but not in Spain which could also be explained by SOC. Links found between microbial taxonomic groups and functional genes helped to better understand the microbial mechanisms behind the organic matter degradation. Enzymes in class AA, a key class for the decomposition of lignin by oxidative enzymes, like AA10 are involved in the decomposition of recalcitrant carbon and found more in traditionally drier and low SOC containing soils of Spain. Similarly, enzymes GH18 and GH23 that were found more in Spain are involved in chitin degradation (Liao et al., 2019). GH13 has been found in high abundance in agricultural soils with winter wheat and grass management in Southern Sweden (Manoharan et al., 2017) and on the other hand in low abundance in agricultural soils in Southwestern China (Bi et al., 2021).

5. Conclusions

Our study provides novel experimental evidence on how short-term experimental drought in summer months in European agricultural soils can affect the functional genes of microorganisms responsible for soil organic matter degradation. The functional genetic diversity across a range of climatic conditions significantly differed from Atlantic and Central to Mediterranean Europe. In response to a short-term drought, the functional gene composition changed in Germany but not in Sweden or Spain. Our results suggest that soil microbial communities respond differently to short-term drought events due to I) regional adaptations to survive harsh conditions in dry environments like selecting for microorganisms with thicker cell walls and II) the differences in their soil physicochemical properties, such as SOC and soil pH. To be able to

predict the impact of drought events more accurately on soil microbial communities more attention should be paid towards their interactions with carbon management practices. Moreover, with studies of the actively transcribed genes we would be able to detect how above-mentioned effects alter the diversity more precisely and functioning of soil microbial communities.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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