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# Soil bacteria respond to regional edapho-climatic conditions while soil fungi respond to management intensity in grasslands along a European transect

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ABSTRACT

Soil microbial community structure is determined by environmental conditions and influenced by other factors, such as the intensity of the land use management. Studies addressing the effect of environmental factors and management on grassland soil microbial communities at the continental scale are missing, and the wide range of ecosystem services provided by these ecosystems are thus also wanting. To address this knowledge gap, this study presents data on grassland soil microbial communities along a pan-European agro-ecological gradient. The transect included five geographical locations (Sweden, Germany, Switzerland, Portugal mainland, Portugal Azores). At each location, soils were collected in two regions characterized by favourable and less favourable conditions for plant growth. In each of these ten regions, grasslands along a gradient of management intensity were selected, i.e. grassland under intensive, less intensive and extensive management. Phospholipid fatty acid analysis (PLFA) was used to characterize the microbial community structure (PLFA pattern) in relation to climatic and soil properties. Over the whole geographical range, the environmental properties determined the soil microbial community structure. In Sweden and Switzerland, the regional growth conditions had the strongest influence on the soil microbial communities, while in Germany, Portugal mainland and Azores the management intensity was more important. Splitting up this whole community response into individual groups reveals that, in general, saprotrophic fungal biomarkers were highest in extensively managed grasslands while bacterial biomarkers differed mainly between the regions. We conclude that at the transect level, climate and soil properties were the most important factors influencing soil bacterial community structure, while soil fungal groups were more responsive to grassland management intensity. Overall agricultural sustainability could benefit from informed soil health promoting management practices, and this study contributes to such knowledge, showing the importance of management for the soil microbial biomass and community structure.

## 1. Introduction

Grasslands are among the world's largest biomes which, together with other grass-dominated habitats (e.g., savannah, shrubland and tundra), cover about 30–40% of the Earth's terrestrial surface (Blair et al., 2014), with 316 million ha on the European continent (area from MODIS, FAOSTAT, 2015). However, continuous expansion of agricultural land, climate change, grazing and other management strategies are

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important issues that affect the survival of semi-natural grasslands across Europe (Veen et al., 2009), and these threatening processes are expected to continue during the next decades (Millennium Ecosystem Assessment, 2005). Preservation of grasslands ecosystems is an important aim of the Common Agricultural Policy (CAP) of the European Union, with maintenance of permanent grasslands being a compulsory task under the greening measures for direct payments to farmers (European Commission, 2018).

Grassland ecosystems can be described by their age and continuity such as annual, cultivated, permanent, temporary, naturalized, semi-natural and natural grasslands (Allen et al., 2011). All, excluding the natural one, can be defined as anthropogenic grasslands, as they are maintained by diverse management strategies, from extensive grazing and annual cutting to intensive farming practices associated with intensive grazing, frequent cutting, ploughing, sowing and/or fertilization (Yuan et al., 2016). In addition to providing forage for livestock or biomass, grasslands also provide various cultural, regulating and supporting ecosystem services, e.g., landscape aesthetics, belowground carbon (C) sequestration, primary production, regulation of biogeochemical cycles and biodiversity conservation (Lemaire et al., 2011; Pommier et al., 2017).

Low intensive grassland management (i.e. low cutting frequency/grazing intensity and low fertilization levels) can lead to long-term increases in biodiversity. If species richness in the original grassland was low and if a seed bank or neighbouring habitats are present, these can colonize the field with new plant species (Marriott et al., 2004; Heinsoo et al., 2020). In addition, reducing the management intensity increases the amount of soil organic matter, acting as a C sink (Conant et al., 2001; De Deyn et al., 2011a). On the contrary, agricultural intensification, or intensive management practices, generally leads to a loss of both plant and animal biodiversity in grasslands (Soussana and Duru, 2007), affecting C cycling by decreasing C sequestration and soil C stocks (Soussana et al., 2004). In addition, agricultural intensification has also been shown to modify soil microbial community structure and species composition due to modification in the plant cover and nutrient inputs (Ahemad et al., 2009; Xu et al., 2017).

Biomass, structure and activity of the soil microbial community determines the functioning and long-term sustainability of terrestrial ecosystems, including grasslands (Bender et al., 2016). Both arbuscular mycorrhizal fungi (AMF) and saprotrophic fungi (SF) play central roles by improving soil structure due to the effects of hyphal entanglement and the exudation of different polysaccharides and glycoproteins with adhesive properties which stabilize soil components (Ritz and Young, 2004; Mardhiah et al., 2014). AMF colonize the roots of a wide range of plant species, enhancing plant nutrient and water uptake due to the increased volume of soil that is in contact with the hyphae as compared with the plant roots (Clark and Zeto, 2000; Chen et al., 2018). Saprotrophic fungi contribute to nutrient cycling by retention of nutrients in their hyphae, and decomposition of polymeric C substrates by synthesizing extracellular oxidative enzymes (Koranda et al., 2014).

At a global scale and through different land use types, soil properties and climate are the main drivers of the community structure and functioning of soil microorganisms (Bahram et al., 2018). At the regional scale, the soil microbial community of grasslands responds to the current land-use intensity (Steenwerth et al., 2002) as well as to increased management intensity (Attard et al., 2010; Xu et al., 2017) but it is still not clear whether this holds over a larger geographical range. This lack of clarity arises from both the lack of studies and from the fact that management methods are highly context dependent. At the local scale, agricultural management practices can have a strong impact on soil microorganisms (Manoharan et al., 2017; Babin et al., 2019), with soil tillage, fertilization strategy and pesticide application being found to alter the soil microbial communities (Ahemad et al., 2009; Sun et al., 2016) as well as by the inclusion of perennial vegetation into crop rotations (Alagele et al., 2020).

Edaphic and environmental variables have been reported as constraining and driving the distribution of soil bacteria (Bahram et al., 2018), while fungal distribution is limited by the availability of resources and is favoured instead of bacteria in non-managed grasslands (Grayston et al., 2001; Xu et al., 2017). Both saprotrophic and mycorrhizal fungi are sensitive to changes in the local environment, responding with reduced biomass to mechanical disturbance, such as soil preparation in agricultural systems. On the other hand, several studies have described strong effects of management on soil nutrient availability and enzyme activities, but without a major effect on soil microbial communities (Bowles et al., 2014), which has been attributed to the complexity and site-specific characteristics of the soil ecosystem (Bünemann et al., 2018).

The soil microbial communities of grasslands have been studied in detail at the plot scale (Bittman et al., 2005; De Deyn et al., 2011b; Dietrich et al., 2017), while beyond the regional scale, studies specifically focused on grasslands are not only scarce but mainly focus on soil chemical properties (van Leeuwen et al., 2017). Other studies in grasslands along a European gradient have focused on plant diversity (Kirwan et al., 2007; Scimone et al., 2007), plant yield (Finn et al., 2013), weed suppression (Connolly et al., 2018), species dynamics (Brophy et al., 2017) and plant functional trait signatures (Pakeman et al., 2009). For the characterization of soil microbial communities of grasslands, phospholipid fatty acid (PLFA) and neutral lipid fatty acids (NLFA) analysis has been extensively used as biomarkers for soil microbial biomass and community structure to identify environmental drivers (Yao et al., 2018), and assess management strategies impacts such as fertilizer addition (Canarini et al., 2016; Wang et al., 2017a).

This study was carried out within the BIOINVENT project, using a pan-European transect from Sweden in the north to Azores (Portugal) in the south. The knowledge gap is an integrative view on several environmental components, i.e. climate, soil physicochemical properties, and soil biology in grasslands at the large scale. The objective was to relate soil microbial biomass and community structure (based on the PLFA and NLFA biomarkers) to soil properties, climatic conditions and agricultural management intensity. Considering the aim of our study and the above cited literature, we pose the following hypotheses:

1) The total soil microbial community structure is driven by interrelated climatic and soil physicochemical properties at the survey level (i.e. pan-European geographical scale), and by management intensity at the regional level.
2) The soil bacterial biomass differs along the survey responding to the climatic and soil physicochemical properties, but it remains stable across management intensities.
3) Irrespective of region, saprotrophic fungal biomass is favoured in extensively managed grasslands as compared to grasslands under intensive management practices, while the impact on the arbuscular mycorrhizal fungi will be interesting in itself on a descriptive level, as currently little is known.

## 2. Materials and methods

### 2.1. Site descriptions and experimental design

A pan-European field survey was carried out in ten regions. This was based on the five geographic locations, Sweden (SE), Germany (DE), Switzerland (CH), Portugal mainland (PT), and Portugal Azores (AZ) (Fig. 1), hereafter referred to as countries for simplicity. Within each country, two regions with distinct conditions for plant growth were sampled, one favourable (F) and one less favourable (LF), based on agro-climatic conditions (Table 1, supplementary Figs. 1 and 2). The F and LF regions differed in each country and were represented by lowlands and highlands in DE and CH while represented by the south and north areas of SE and AZ (i.e. São Miguel Island), respectively. For PT, the northern region represented the F region and the southern represented the LF region. The favourability of regions were based on altitude, temperature

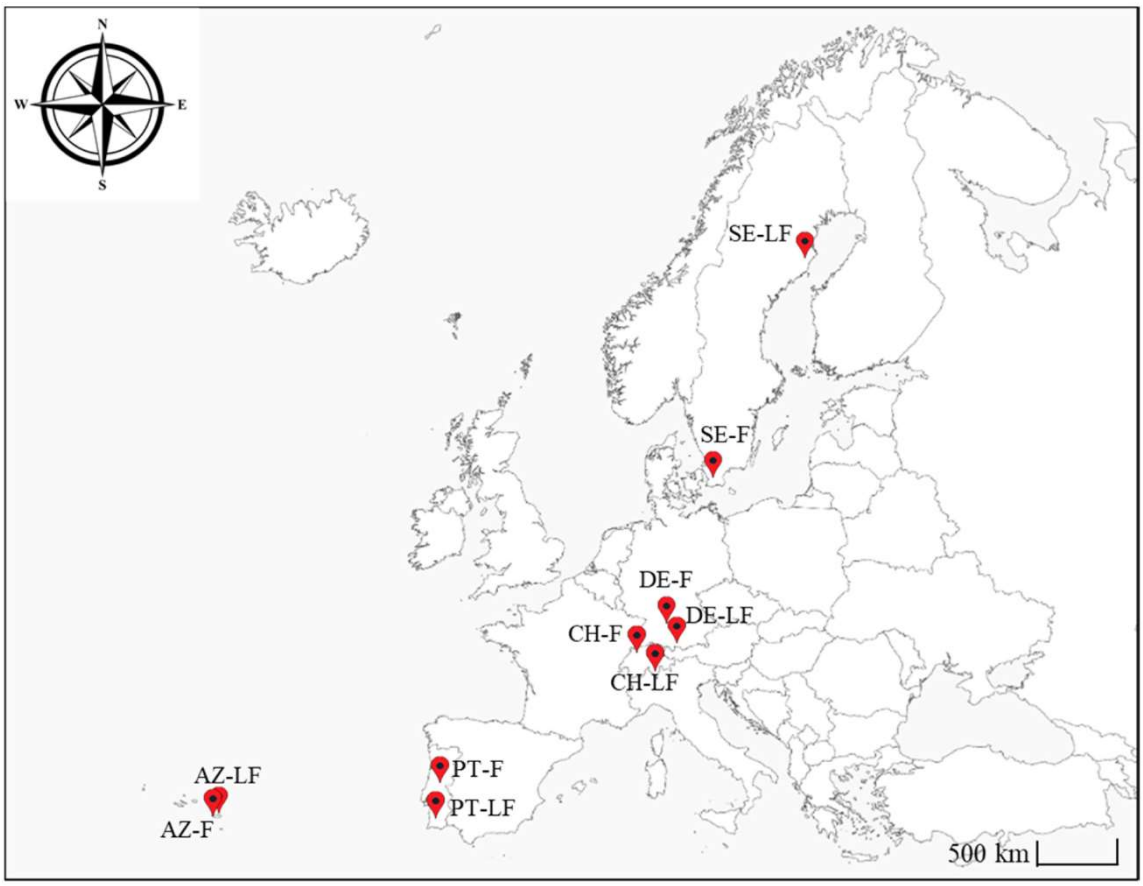


**Fig. 1.** Map indicating the ten regions included in the survey. Abbreviations: SE = Sweden, DE = Germany, CH = Switzerland, PT = Portugal mainland, AZ = Portugal Azores, F = Favourable region, and LF = Less favourable region.

**Table 1**
The annual mean temperature (Temp), precipitation (Precip), mean minimum (Min.Temp) and maximum (Max.Temp) temperature and altitude (Alt) for each agro-climatic region included in the sampling for this survey (average ± SD). For other abbreviations see Fig. 1.

| Country | Region | Temp (°C) | | | Precip (mm) | | | Min.Temp (°C) | | | Max.Temp (°C) | | | Alt (masl) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SE | F | 7.6 | ± | 0.3 | 703 | ± | 43 | −2.7 | ± | 0.7 | 19.6 | ± | 0.5 | 99.4 | ± | 45 |
| | LF | 3.1 | ± | 0.4 | 602 | ± | 26 | −10.7 | ± | 0.5 | 19.5 | ± | 0.2 | 112 | ± | 47 |
| DE | F | 8.0 | ± | 0.3 | 1332 | ± | 210 | −5.0 | ± | 0.3 | 22.0 | ± | 0.3 | 757 | ± | 47 |
| | LF | 6.3 | ± | 1.0 | 1594 | ± | 263 | −6.7 | ± | 1.0 | 20.3 | ± | 1.0 | 1077 | ± | 165 |
| CH | F | 9.8 | ± | 0.5 | 1088 | ± | 153 | −2.5 | ± | 0.5 | 23.8 | ± | 0.5 | 493 | ± | 76 |
| | LF | 5.7 | ± | 1.2 | 864 | ± | 263 | −6.9 | ± | 1.2 | 19.9 | ± | 1.3 | 1298 | ± | 214 |
| PT | F | 16.0 | ± | 1.1 | 798 | ± | 124 | 5.4 | ± | 2.4 | 29.4 | ± | 1.5 | 228 | ± | 179 |
| | LF | 16.6 | ± | 0.6 | 662 | ± | 50 | 7.3 | ± | 2.2 | 28.3 | ± | 2.3 | 182 | ± | 105 |
| AZ | F | 14.7 | ± | 1.1 | 1296 | ± | 103 | 10.8 | ± | 1.1 | 19.8 | ± | 1.1 | 552 | ± | 188 |
| | LF | 14.7 | ± | 1.1 | 1250 | ± | 116 | 10.8 | ± | 1.1 | 19.8 | ± | 1.0 | 569 | ± | 185 |

and precipitation, and in AZ also on north/south facing slopes. In SE, the F and LF regions were geographically distant (~1200 km), while in the other countries their respective F and LF regions were geographically closer (ranging from ~20 to ~400 km) (Fig. 1). In each of the ten regions (5 countries × 2 regions per country), grassland fields were selected to best represent the following grassland categories: intensively managed grasslands with high nutrient input and frequent utilizations (i.e., cutting and grazing events) (INT), less intensively managed grasslands with less nutrient input and low cutting frequency or grazing (LI), and finally grasslands under extensive management, corresponding to semi-natural grasslands (EXT). These definitions were chosen even though the exact levels of fertilization, cutting frequency and grazing intensity differed among the countries, due to management regulations explicit for each country (Supplementary Table 1). In the three grassland types, 12 grasslands of each intensity level were sampled in each region in SE, DE and CH. In the F region of PT, the sampling included 8 INT, 14 LI and 14 EXT managed grasslands, while the LF region included 16 INT, 10 LI and 10 EXT managed grasslands. In the F region of AZ, the sampling included 12 INT, 11 LI and 6 EXT managed grasslands, while the LF region included 10 INT, 13 LI and 8 EXT managed grasslands. In total, 348 fields were sampled (72 in SE, DE, CH and PT and 60 in AZ).

### 2.2. Soil sampling

In each field, a standardized study unit with four quadratic sub-plots (each sub-plot measuring 2 × 2 m; Supplementary Fig. 3) was established to provide a structured sampling design representative of the sampled field. In each corner of the sub-plots, the top 20 cm of soil was sampled with a soil auger of Ø 2.5 cm. The 16 soil cores were thoroughly mixed to obtain a composite sample per field to be used for the

determination of general and microbial soil properties. The soil samples were placed in cool boxes and transported to the laboratory, where they were sieved (2 mm) and homogenized within 24 h after sampling. Thereafter a subsample of 50 g was immediately stored at −20 °C and later freeze-dried, for the microbial analysis, and another subsample of 200 g was air-dried.

In addition, in the centre of each subplot, the plant cover was cut to the soil surface level without disturbing the soil and avoiding compaction, and thereafter a soil sample was carefully taken using a metal cylinder (10 cm in height, Ø 10 cm) for determination of the soil bulk density, providing four subsamples per field.

### 2.3. Soil properties

The analysis of the general soil properties for the whole survey was performed with the air-dried soil samples. For calculation of soil bulk density (ISO 11272:2017), the net weight of fresh and dry soil was determined before and after drying (24 h, 105 °C) and subtracting the weight of the cylinder. The bulk density (g $cm^{-3}$) was calculated as the ratio of net weight of the dry soil and volume of soil sample. For soil texture, particle size distribution was determined by the sieving-sedimentation method (ISO 11277:2020). Soil pH was measured in a suspension with distilled water (1:5) (ISO 10390:2005). For determining soil organic matter content, 300 mg of air-dried and milled (<2 mm, IKA A10) soil was burned in a muffle kiln (550 °C, 3 h, Nabertherm B400) in nickel capsules, and the total soil organic matter (SOM) content was determined by loss on ignition (CEN - EN 15935). For measurement of total phosphorus ($P_{tot}$), the remaining ash from the 300 mg burned soil used for the SOM measurement, was digested in 10 ml 13% $HNO_3$ (1:4) (ISO 54321:2020) and analysed using ICP-OES. Available phosphorus ($P_{av}$) in soil was determined calorimetrically after extraction in ammonium lactate and acetic acid (Egner et al., 1960). Total nitrogen (N) and carbon (C) concentrations were measured in air-dried milled soil (<2 mm, IKA A10), using a FLASH 2000 elemental analyser (Thermo Scientific) (ISO 10694:1995 for total C and ISO 13878:1998 for total N). Soil water content (WC) was determined by drying the soil for 24 h at 105 °C (ISO 11465:1993).

### 2.4. Microbial community structure

The soil microbial community was analysed by PLFA (phospholipid fatty acids) and NLFA (neutral lipid fatty acids) analysis. Fatty acids were extracted from freeze-dried soil samples, sent from all the partner countries, following the method described by Frostegård et al. (1993). Briefly, lipids were extracted from soil samples with a chloroform: methanol:citrate buffer (1:2:0.8 *v*/v/v) and separated into neutral lipids, glycolipids and phospholipids using a pre-packed silica column and elution chloroform, acetone and methanol, respectively. Phospholipids and neutral lipids were methylated, and fatty acid methyl esters were identified by gas chromatography with a flame ionization detector (GC-17A, Shimadzu). An internal standard (19:0) was added to the samples, for quantification of fatty acids. Based on the relative retention time, 26 different fatty acids were identified and 13 were considered to be of bacterial origin (i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7t, 17:1ω8, i17:0, a17.0, 17:0, cy17:0, 18:1ω7, and cy19:0) (Barreiro et al., 2015). The abundance of the 18:2ω6 PLFA was used as a biomarker for the non-mycorrhizal or saprotrophic fungi (SF), which can appear in plants as well, but the negligible impact of plant roots on the amount of this fatty acid (Kaiser et al., 2010) support the use of 18:2ω6 as a fungal indicator in soil. The 12 remaining PLFA are present in both bacterial and fungal organisms (Frostegård et al., 2011) and are thus indicative of soil microbial organisms in general. The relative abundance (%) of all PLFAs present in the sample, including the general ones that are not group specific, was used for the analysis of the PLFA patterns. For the specific estimation of the AMF biomarker, the 16:1ω5 NLFA was used (Kundel et al., 2020), due to the high correlation between the NLFA quantity and AMF spore counts (Sharma and Buyer, 2015). This method is a sensitive technique that can be used for microbial biomass estimation, albeit with some limitations (Frostegård et al., 2011).

### 2.5. Climatic data

Values for climatic data were modelled using the CHELSA (Climatologies at high resolution for the earth's land surface areas), which is a high resolution (30 arc *sec*) climate data set for the earth's land surface areas currently hosted by the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL). It includes temperature and precipitation patterns for various time periods. CHELSA is based on the output of a quasi-mechanistic statistical downscaling global reanalysis and global circulation model output (Karger et al., 2017). The annual mean temperature (Temp) and the annual mean precipitation (Precip) were used for the analysis, and the annual mean minimum temperature (Min. Temp) and annual mean maximum temperature (Max.Temp) for climate description.

### 2.6. Statistical analysis

The relative abundance of individual PLFAs (% of each fatty acid of the total of 26 considered) were subjected to a nested PERMANOVA analysis, using a Bray-Curtis dissimilarity matrix, and to a non-metric multidimensional scaling (NMDS) to identify the main differences in their patterns. The soil microbial biomarker quantity data (nmol per gram soil, PLFA for bacteria and SF, NLFA for AMF) was subjected to a nested ANOVA analysis; while associations between these biomarkers, physicochemical soil properties and climatic variables were analysed using general linear models (GLM). For the GLM analysis, Log was used as a link function and country, region, management, and environmental properties were modelled as fixed factors. The overall best models were selected based on the following criteria: (i) a maximum likelihood approach based on Akaike's Information Criterion (AIC, Akaike, 1973) corrected for small sample size (AICc) and on the respective AIC weights; (ii) goodness-of-fit of the tested models, calculated as the percentage of the sum of squares explained by the model (Zuur et al., 2007); and (iii) models only selected where all the regression coefficients were significantly different from zero. The model with the lowest AICc is considered to best fit the data. We considered all models with a ΔAICc value <2 (i.e., the difference between the model's AICc and the lowest AICc value) as with similar levels of importance, therefore all such models were included in the equally parsimonious group of “best models”. Principal component analysis (PCA) was used to reduce the information in the PLFA data set and redundancy analysis (RDA) analysis was used to explain those values with environmental variables. The statistical analyses were performed using the R software package (R studio, version 3.6.1) and the PRIMER-E software for the PERMANOVA analysis (version 7, Clarke and Warwock, 2001).

## 3. Results

### 3.1. Soil microbial community structure at the survey level

The nested PERMANOVA analysis of the whole dataset (Table 2) showed that the country, the growth conditions (F and LF) and the management intensity (INT, LI, EXT), affected the PLFA pattern ($p < 0.001$). The within-country nested PERMANOVA exhibited an effect of growth conditions on the PLFA pattern in SE ($p < 0.001$) and CH ($p < 0.01$), but not in the other countries ($p > 0.05$) (Table 2). The country-wise nested PERMANOVA also indicated an effect of management intensity on the PLFA pattern in all countries ($p < 0.001$ for SE, CH, PT, AZ and $p < 0.05$ for DE) (Table 2). At the country level, the square root of the component of variation values showed a large effect of region (i.e. grass growth conditions) and a smaller impact of management in SE; very small effects of region and management in DE; an intermediate

**Table 2**
Nested PERMANOVA analysis of the whole PLFA (%) data set. Abbreviations: ALL = all the countries, each country (see Fig. 1), Co = Country, Gr = Growing conditions, Ma = Management. The sign | indicates that the different levels are nested in each other.

| | | df | SS | MSS | ECV | Sq.root | ECV% | PseudoF | P(perm) | Perms | *p*-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ALL | Co | 4 | 1.583 | 0.396 | 0.005 | 0.071 | 0.465 | 6.826 | 0.000 | 7678 | 0.0001 |
| | Co \| Gr | 5 | 0.290 | 0.058 | 0.001 | 0.037 | 0.125 | 4.653 | 0.000 | 9920 | 0.0001 |
| | Co \| Gr \| Ma | 20 | 0.252 | 0.013 | 0.001 | 0.028 | 0.073 | 3.499 | 0.000 | 9829 | 0.0001 |
| | Residues | 318 | 1.145 | 0.004 | 0.004 | 0.060 | 0.336 | | | | |
| | Total | 347 | 3.329 | | | | | | | | |
| SE | Gr | 1 | 0.218 | 0.218 | 0.006 | 0.076 | 0.577 | 23.078 | 0.000 | 10 | 0.0001 |
| | Gr \| Ma | 4 | 0.038 | 0.009 | 0.000 | 0.022 | 0.047 | 2.503 | 0.000 | 9903 | 0.0008 |
| | Residues | 66 | 0.249 | 0.004 | 0.004 | 0.061 | 0.376 | | | | |
| | Total | 71 | 0.505 | | | | | | | | |
| DE | Gr | 1 | 0.019 | 0.019 | 0.000 | 0.018 | 0.081 | 2.557 | 0.000 | 10 | 0.0795 |
| | Gr \| Ma | 4 | 0.030 | 0.008 | 0.000 | 0.019 | 0.087 | 2.26 | 0.010 | 9925 | 0.0121 |
| | Residues | 66 | 0.221 | 0.003 | 0.003 | 0.058 | 0.831 | | | | |
| | Total | 71 | 0.271 | | | | | | | | |
| CH | Gr | 1 | 0.043 | 0.043 | 0.001 | 0.031 | 0.242 | 5.753 | 0.000 | 10 | 0.0021 |
| | Gr \| Ma | 4 | 0.030 | 0.007 | 0.000 | 0.020 | 0.098 | 2.771 | 0.000 | 9901 | 0.0007 |
| | Residues | 66 | 0.178 | 0.003 | 0.003 | 0.052 | 0.661 | | | | |
| | Total | 71 | 0.251 | | | | | | | | |
| PT | Gr | 1 | 0.007 | 0.007 | 0.000 | 0.018 | 0.052 | 0.373 | 0.519 | 180 | 0.8729 |
| | Gr \|Ma | 4 | 0.072 | 0.018 | 0.001 | 0.034 | 0.180 | 3.743 | 0.000 | 9911 | 0.0001 |
| | Residues | 66 | 0.316 | 0.005 | 0.005 | 0.069 | 0.768 | | | | |
| | Total | 71 | 0.395 | | | | | | | | |
| AZ | Gr | 1 | 0.003 | 0.003 | 0.001 | 0.024 | 0.104 | 0.164 | 0.598 | 720 | 0.9436 |
| | Gr \| Ma | 4 | 0.082 | 0.021 | 0.002 | 0.042 | 0.310 | 6.148 | 0.000 | 9903 | 0.0001 |
| | Residues | 54 | 0.181 | 0.003 | 0.003 | 0.058 | 0.586 | | | | |
| | Total | 59 | 0.267 | | | | | | | | |

situation in CH, with an effect of region, followed by management. Only an effect of management was detected in PT and AZ, with the strongest impact in AZ, while no impact of growing conditions was detected (Table 2). The betadisper analysis, based on Bray-Curtis distances, showed that the dispersion within each region of the survey was not homogeneous ($p < 0.001$), therefore a NMDS plot including only the

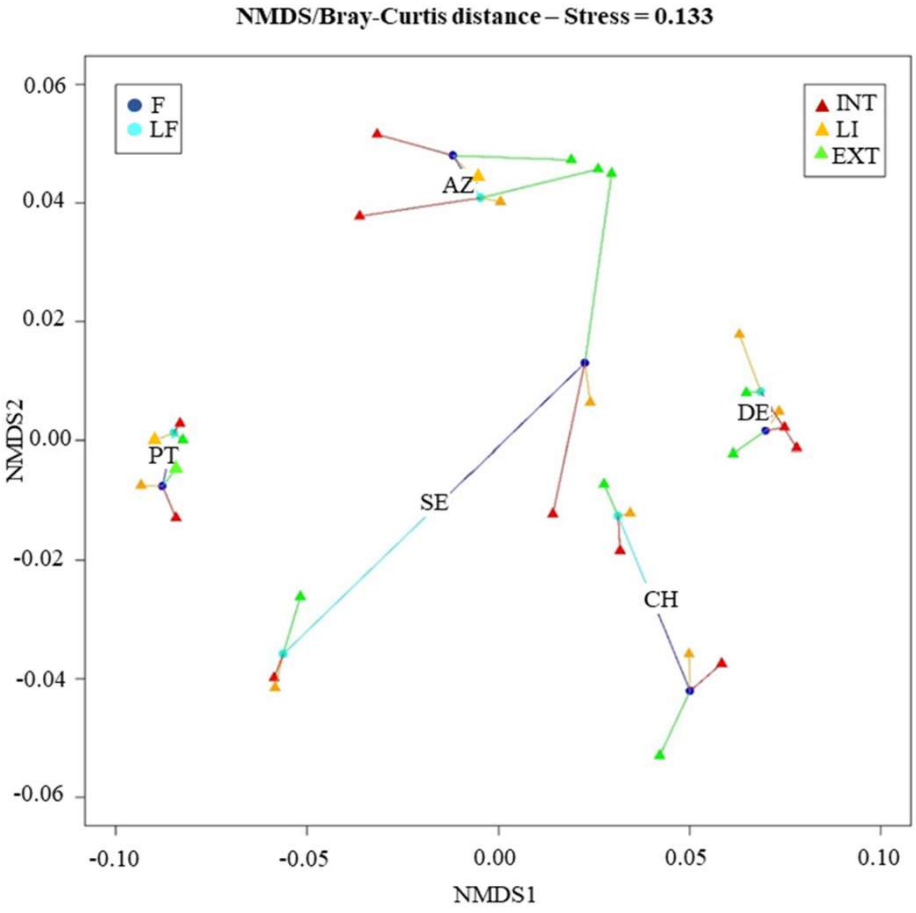


**Fig. 2.** Distance between centroids of the different regions in multi-dimensional Euclidean space. Management type: INT = Intensive, LI = Less Intensive, EXT = Extensive. For more abbreviations see Fig. 1.

centroids was performed, confirming the PERMANOVA result, that countries were well separated based on the PLFA profiles (Fig. 2, Supplementary Table 2). SE showed the largest differentiation between regions, i.e. centroids distance (cd), of all the countries in the survey (cd = 0.125), with the variation due to management being much larger in the F than LF region (Fig. 2, Supplementary Table 2). The variation in the PLFA profiles was much lower in DE between F and LF (cd = 0.068), while CH showed a clear distance between regions (cd = 0.077) and some differentiation between management intensities (Fig. 2, Supplementary Table 2). In PT, a reduced variation in the PLFA profiles was observed, with the smallest difference between regions (cd = 0.063), and in AZ two parallel management gradients at F and LF regions (cd = 0.067) were apparent (Fig. 2, Supplementary Table 2).

The PCA applied to the PLFA data differentiated between four (broken stick model) and six (average eigenvalue criterion) main components, explaining 62% to 74% of the variance, respectively. Ordination based on the first two components showed that the geographic distance (country) was the most distinctive factor for the microbial community structure, even though there were also overlaps between countries (Fig. 3), and the first axis of the PCA clearly separated PT from the other countries.

Following the correlation among several PLFAs (Supplementary Fig. 4) and aiming to analyze the effect of environmental variables on the microbial community, we performed RDA on the first four principal components constrained by the environmental variables (Fig. 4). The first axis of the RDA analysis (RDA 1, Fig. 4) separated PT from the other countries, with the samples from PT having on average a significantly lower amount of SOM, N, C and $P_{tot}$ (Supplementary Table 3). The second factor was also associated with Temp (positive values), and Precip (negative values) and separated a sequence of countries – AZ and SE, DE and CH. The factor most associated with the second axis was soil pH, which appears to be associated with the geographical location starting in AZ and SE and going up to DE and CH (RDA 2, Fig. 4, Supplementary Table 3).

### 3.2. Fatty acid biomarkers concentration differentiation along the survey

The average sum of the PLFAs bacterial biomarkers (Fig. 5) was lower in PT (40 nmol $g^{-1}$) ($p < 0.001$), followed by SE, CH and AZ with similar values (149, 156 and 163 nmol $g^{-1}$, respectively), and highest in DE (236 nmol $g^{-1}$) ($p < 0.01$). In SE, the values were higher in the F region (197 nmol $g^{-1}$) than in the LF (101 nmol $g^{-1}$) ($p < 0.01$), while no significant differences were detected between F and LF in the other countries (Supplementary Table 4). Significantly higher values for the average sum of the PLFAs bacterial biomarkers were found under EXT management in LF growth conditions in AZ (199 nmol $g^{-1}$) ($p < 0.05$), and under EXT management in F growth conditions in SE (287 nmol $g^{-1}$) ($p < 0.001$), both compared to the respective INT management (131 and 156 nmol $g^{-1}$ respectively) (Fig. 5, Supplementary Table 4).

The concentration of the PLFA 18:2ω6, biomarker for saprotrophic fungi (Fig. 5), was lower in the samples from PT, AZ and DE (3, 6 and 7 nmol $g^{-1}$, respectively) and higher in CH and SE (10 nmol $g^{-1}$) ($p < 0.01$). In DE, the values for this fatty acid were higher in the LF region (9 nmol $g^{-1}$) than in the F region (5 nmol $g^{-1}$) ($p < 0.01$), while no significant differences between F and LF were detected in the other countries (Supplementary Table 5). Furthermore, 18:2ω6 values were higher in the samples under EXT management as compared to the INT management, in the F region in SE (16 and 9 nmol $g^{-1}$, respectively) ($p < 0.001$), in the LF region in DE (12 and 8 nmol $g^{-1}$, respectively) ($p < 0.01$), and in both the F and LF regions in both CH (12 vs. 6 nmol $g^{-1}$ and 13 vs. 8 nmol $g^{-1}$ respectively, $p < 0.01$) and AZ (7 and 4 nmol $g^{-1}$, respectively, $p < 0.05$). No differences were detected in PT ($p > 0.05$)

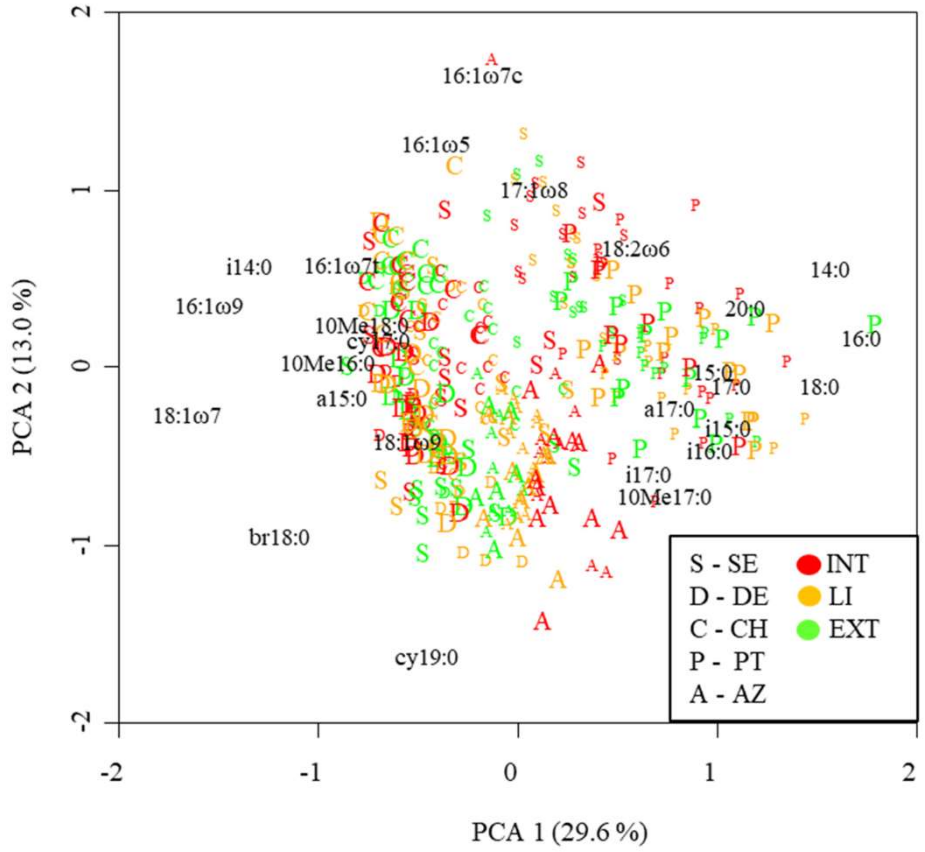


**Fig. 3.** Biplot showing the results of the principal component analysis applied to the 26 PLFAs data set (relative abundance in %). Identification of the PLFAs in black. Colored letters indicate sites, with large letters for F and small letters for LF region, and colors for the management type. For abbreviations see Figs. 1 and 2.

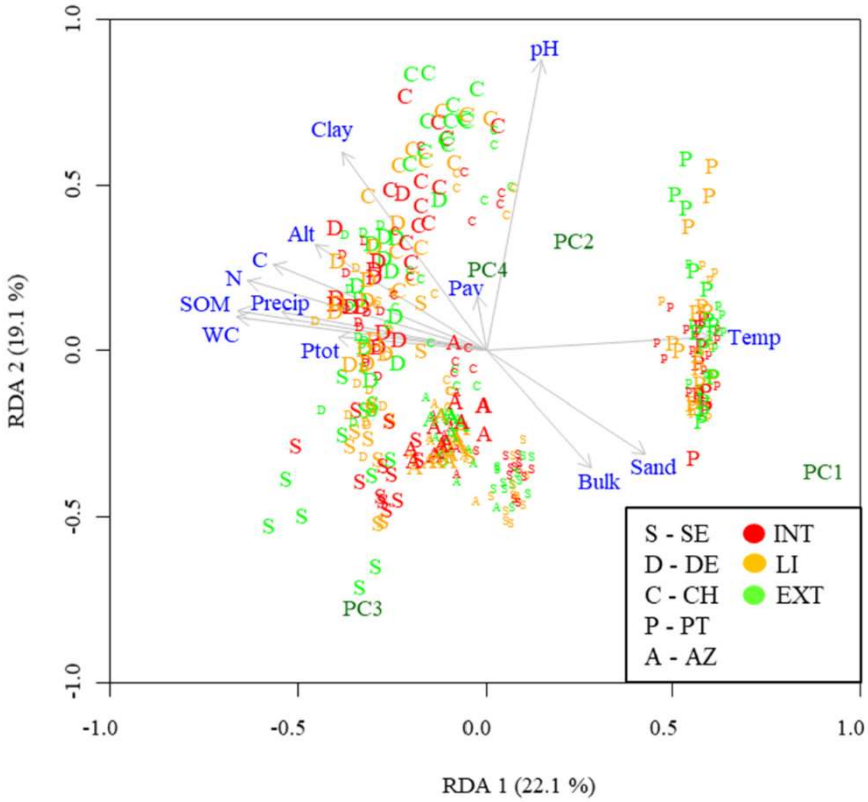


**Fig. 4.** Triplot showing the results of an RDA applied to the four components extracted by the PCA applied to the PLFAs data set, and to the soil and climate variables. Identification of the four components in dark green. Colored letters indicate sites, with large letters for F and small letters for LF region, and colors for the management type (for abbreviations see Figs. 1 and 2). Blue text with arrows refers to the soil and climate predictors: pH, soil organic matter (SOM), total C (C), total N (N), total P ($P_{tot}$), available P ($P_{av}$), proportion of sand, silt and clay, soil water content (WC), bulk density (Bulk), average annual precipitation (Precip), average annual temperature (Temp), and altitude (Alt). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 5, Supplementary Table 5).

The concentration of NLFA 16:1ω5, biomarker for arbuscular mycorrhizal fungi, was lower in DE, SE, AZ and PT (19, 20, 25 and 30 nmol $g^{-1}$ respectively), than in CH (39 nmol $g^{-1}$) ($p < 0.05$) (Fig. 5). There were no significant differences in this NLFA under different growth conditions in any country (Supplementary Table 6). With regard to management, 16:1ω5 values showed a non-uniform response, varying among regions, with higher values in the LI than in the INT managed grasslands in the F region both in SE ($p < 0.05$) and AZ ($p < 0.001$), and under EXT management as compared with the INT management in the F region both in CH ($p < 0.001$) and PT ($p < 0.05$) (Fig. 5; Supplementary Table 6).

### 3.3. Association between the microbial biomarkers and environmental properties

Different general linear models (GLM) were explored to detect linkages between fatty acids and environmental properties (data not shown), both at the survey and country level. For all the biomarkers (bacterial, SF and AMF), the nested model including several environmental properties had the lowest akaike information criterion (AIC) values, indicating a good fit of the model (Table 3). The sum of bacterial biomarkers showed a positive association with soil organic matter (SOM), total soil phosphorus ($P_{tot}$) and soil water content (WC) (Table 3). For the SF and AMF biomarkers, there was a positive effect of SOM and elevation (Alt) and pH, respectively (Table 3). Negative associations between soil bulk density and total carbon (C) with the bacterial biomarkers, % of sand and silt and bulk density with the SF biomarker, and precipitation (Precip) with the AMF biomarker, were detected (Table 3).

Different variables drove soil microbial community structure in each country depending on the specific soil properties and climatic conditions. In SE, similar to the general survey, the nested model including several environmental factors showed the lowest AIC for the bacterial, SF and AMF biomarkers (Supplementary Table 7). The sum of the bacterial biomarkers showed significant, positive associations with $P_{tot}$ and WC and negative associations with available soil phosphorus ($P_{av}$), less favourable region (LF) and less intensive (LI) and intensive (INT) management. Regarding the SF biomarker, the model included significant, positive associations with C and $P_{tot}$, and negative associations with total soil nitrogen (N), SOM, $P_{av}$, % of sand and silt, bulk density, LF, LI and INT. For the AMF biomarker, the model included significant, positive associations with less intensive management in both favourable and less favourable regions (F-LI and LF-LI), and negative associations with $P_{av}$, and % of sand and silt (Supplementary Table 7).

In DE, for the sum of bacterial biomarkers, the model with the lowest AIC included region and soil factors, with a significant positive effect of SOM, $P_{tot}$ and LF. Regarding the SF biomarker, the model with the lowest AIC included management with significant, positive associations with C, % of clay and Alt, and negative associations with LI and INT. For the AMF biomarker the model with better adjustment also included management with significant, positive associations with pH and C and negative associations with LI, INT, $P_{av}$, % of silt and soil bulk density (Supplementary Table 7).

In CH, for the sum of the bacterial biomarkers, the model with the lowest AIC included a positive effect of SOM and $P_{av}$, and a negative effect of pH and LF. For the SF biomarker, the model with the better adjustment included management with a significant positive association with SOM, and negative associations with INT, LI, soil bulk density and WC. Regarding the AMF biomarker, the nested model included a significant positive association with pH and a negative association with F-INT (Supplementary Table 7).

In PT, for the sum of bacterial biomarkers, the nested model included significant, positive associations with LF-INT, $P_{av}$, % of silt and clay, and negative associations with LF, F-INT, bulk density and average temperature (Temp). Regarding the SF biomarker, the model included significant, positive associations with % of clay and Alt, but with a very low adjusted $R^2$. Regarding the AMF biomarker, the model included

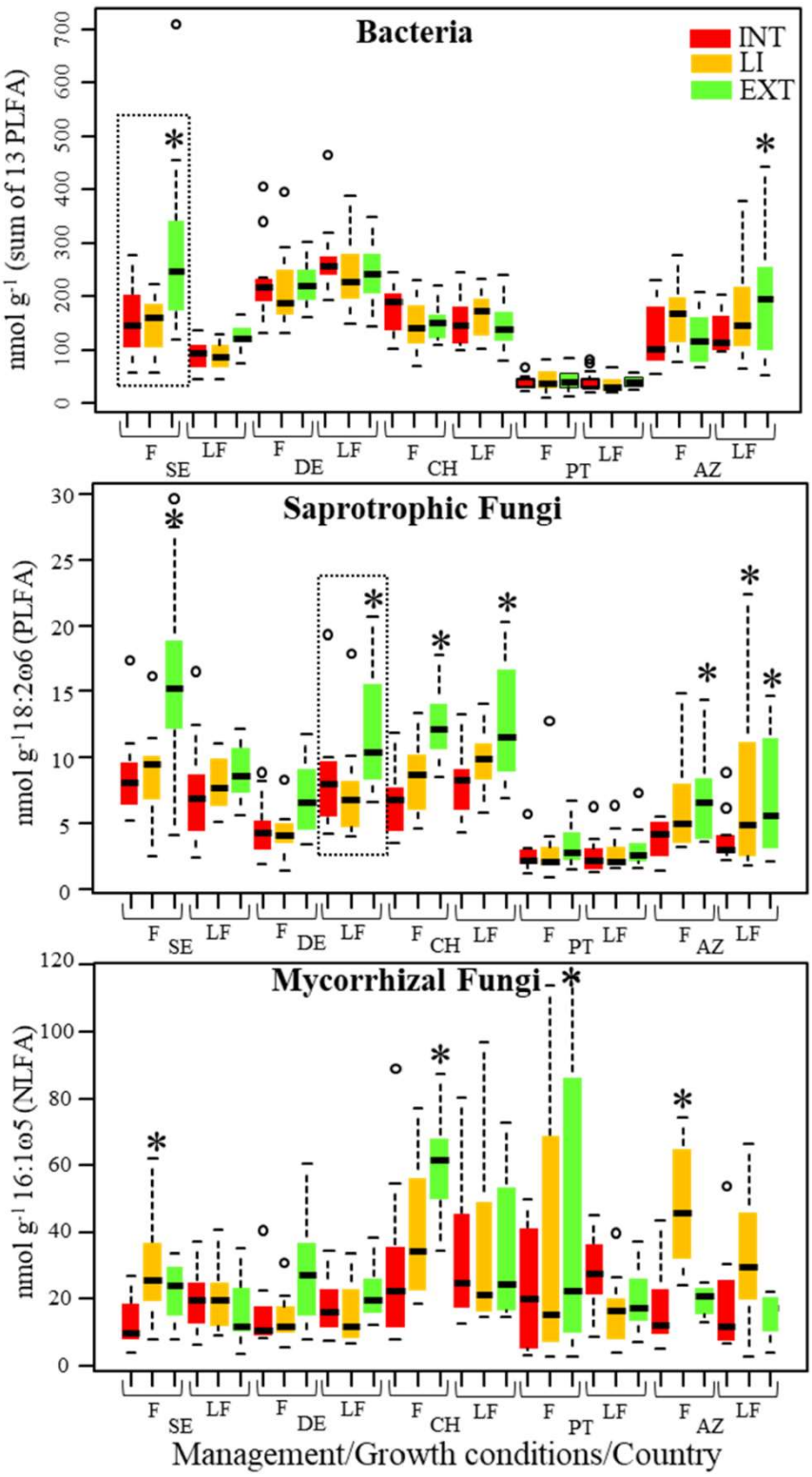


**Fig. 5.** Measured values of the fatty acids biomarkers of bacteria and saprotrophic and mycorrhizal fungi, of the samples from the ten regions of the study. The dotted boxes represent the growth conditions and the asterisks represent the management practices, compared with intensive ones, with a significant effect ($p < 0.05$), according to the nested ANOVA. The notches in the boxes represent the median and the circles on top of the boxes represent outliers. For abbreviations see Figures 1and 2.

significant, positive associations with Temp, Alt and % of clay, and negative associations with LF, WC and % of sand and silt. (Supplementary Table 7).

In AZ, for the sum of the bacterial biomarkers, the model included only significant, positive associations with N and WC. Regarding the SF biomarker, the model also included significant, positive association with N but negative associations with SOM and $P_{tot}$. Finally, for the AMF biomarker the model shows a significant, positive association with LI (Supplementary Table 7).

## 4. Discussion

### *4.1. Soil microbial community determined by environmental factors at the survey level and by management intensity at the regional level*

In agreement with the first part of our first hypothesis, the main

**Table 3**
GLM between the environmental properties and the fatty acid values (nmol $g^{-1}$) for the sum of the 13 PLFA bacterial biomarkers, only PLFA 18:ω6 (the saprotrophic fungal biomarker) and only NLFA 16:1ω5 (the arbuscular mycorrhizal fungal biomarker). The data comes from all the samples of the survey. For abbreviations see Fig. 4 and Table 2. The sign | indicates that the different levels are nested in each other.

| Fatty acids | Models | Association | AIC | Adjusted $R^2$ |
|---|---|---|---|---|
| Sum PLFA bacteria | Co\| Gr \| Ma (SOM + $P_{tot}$ + WC) | + | 3705 | 0.77 |
| | Co\| Gr \| Ma (C + Bulk) | − | | |
| PLFA 18:2ω6 (Saprotrophic fungi) | Co \| Gr \| Ma (SOM + Alt) | + | 1817 | 0.604 |
| | Co \| Gr \| Ma (Sand + Silt + Bulk) | − | | |
| NLFA 16:1ω5 (Mycorrhizal fungi) | Co \| Gr \| Ma (pH) | + | 2992 | 0.403 |
| | Co \| Gr \| Ma (Precip) | − | | |

differences in soil microbial community structure (as indicated by PLFA patterns) along the European transect (survey level) were determined by the climatic and physicochemical soil properties, i.e. precipitation and soil pH. Regarding the second part of the hypothesis, the PLFA discriminated among management intensities, but the response differed in each country. This agrees with Szukics et al. (2019), who have found that the abundance of N transforming microorganisms differs along management transects in mountain grasslands, but with specific responses varying between different countries. Indeed, as grasslands have been reported to be characterized by unique biotic and abiotic conditions, the effects of management practices on fungal and bacterial biomass have been shown to differ worldwide and to be context dependent (McTavish et al., 2021).

Our results are in agreement with the recent literature, identifying environmental variables (climatic factors and soil properties) as the main drivers of niche differentiation of bacteria and fungi in a global topsoil survey (Bahram et al., 2018). Climatic gradients have long been identified to play a key role in determining soil microbial community structure in areas with large variation in temperature (Staddon et al., 1998), while soil physicochemical properties are determinant, even over large distances, if the fluctuations in terms of precipitation are small (Xue et al., 2018). In the transect studied here, the pattern of the fatty acids differed mainly between countries, confirming that it may be a useful tool for the discrimination of different biogeographical zones (Francisco et al., 2016).

The environmental properties, as previously stated, largely determined the soil microbial community structure, but these properties were also subject to the impact of management. A review of global data sets by Conant et al. (2001), revealed that grassland management may modify soil organic C stocks, probably as an indirect effect of the change in plant species composition (Loiseau et al., 2005). Similarly, restoration practices that increase plant diversity can favour C sequestration (De Deyn et al., 2011a). The lack of robust correlations of soil C and extensive management in our survey, even though a positive tendency was observed, may have arisen from the difficulty to detect changes in response to management and land use since the total C pool is large (Haynes, 2005). Another soil property clearly indicative of management is the amount of P in the soil, as P fertilization is a common grassland management practice worldwide. The higher $P_{av}$ in the F regions of SE and CH is most likely related to fertilization practices. The south of Sweden (F region) was subjected to agricultural intensification in the period 1950–1980, when agricultural soils were loaded with large quantities of inorganic P fertilizer to improve crop production (Ulén et al., 2007). This past fertilization could be a possible explanation for the observed high levels of available P, even though currently the ratio of organic (with high amounts of P) to mineral fertilizer is much larger in the north than in the south of SE. A similar situation could have occurred in CH, although in this country policies to reduce P fertilization have been in place since 1989 (Mehr et al., 2018).

Our results indicated a distinct difference in the soil microbial community structure between the F and LF region in SE and CH, likely related to the regional climate, i.e. temperate vs. subarctic climate in SE, and moderate continental vs. alpine climate in CH. On the other hand, these differences might be influenced as well by the higher values of $P_{av}$ in the F region comparing with the LF region in both countries, which may result in shifts in the soil microbial community structure through lower AMF biomass as well as microbial biomass in general (Cruz et al., 2009). Low availability of P limits vegetation development, forcing the plants to rely more on AMF symbiosis (Jansa et al., 2011; Ma et al., 2021), whereas the contrary will down-regulate the carbon investments made from the plant to the fungal symbiont (Hammer et al., 2011). In the rest of the countries of the survey the difference in the environmental conditions (climate and soil properties) between the F and LF regions were smaller, or almost non-existent, compared to SE and CH. In DE, PT and AZ the impact of management was bigger than the regional differentiation, while in SE and CH the management impact was significant at the regional level.

### *4.2. Soil microbial biomass in relation to the climatic and soil physicochemical properties*

Our second hypothesis was confirmed in terms of the positive relationship between the bacterial biomarkers and soil organic matter (SOM) together with soil total C and N content. Additionally, the regions of our study under more rainy climatic conditions (DE, CH and AZ) had a higher abundance of bacterial biomarkers, suggesting more favourable bacterial conditions due to the indirect positive effect of precipitation on the build-up of SOM (Derner and Schuman, 2007). We did not detect an impact of the management on the bacterial biomass, even though other authors have found impacts of different management strategies, like positive effects of organic fertilization on bacterial biomass (Faust et al., 2017).

The SF biomarker responded to the latitudinal gradient with a decrease in abundance going southward, i.e. from SE to PT and AZ, which likewise has been previously described by Bahram et al. (2018) and He et al. (2020). The reasons for this decrease are not fully elucidated yet, but fungi are better adapted to the lower temperatures inherent of higher latitudes than bacteria (Pietikäinen et al., 2005). The expected positive association between the SF biomarker and SOM was not evident, and moreover, SF was negatively associated with SOM at the country level in SE and AZ. These negative associations may be an indirect effect of competitive interactions between SF and bacteria (Bahram et al., 2018), even though SOM quality largely determines the bacterial community structure (Millard and Singh, 2010), while SF are mainly influenced by litter input. Regarding the AMF, the negative association between the AMF biomarker and precipitation contradict other studies (Chen et al., 2017). Plants may, however, enhance AMF colonization to alleviate drought impact and improve their growth and reproductive response to water stress (Jayne and Quigley, 2014). Nevertheless, this may only be true under moderate water stress, such as in the dry Mediterranean conditions of PT. AMF communities are more influenced by plant diversity than by SOM (Millard and Singh, 2010), but environments with high levels of available P can affect them negatively (Wang et al., 2017b), similarly to what was evident in SE and DE. AMF dynamics can be indirectly affected by pH (Dumbrell et al., 2010), with optimum values of soil pH for the availability of P in the range of pH 6–7 (Conyers and Moody, 2009) and with immobilization processes above pH 8, due to the precipitation of calcium phosphates, and iron phosphates below pH 6 (Penn and Camberato, 2019). The pH values from CH showed a large variability, but for most of the samples the values were not within the P immobilization limits (values between 6 and 8), suggesting that other factors rather than $P_{av}$ and pH, for example

the plant species richness (De Deyn et al., 2011b), may have determined the high occurrence of AMF in CH.

The larger relative concentration of the AMF biomarker in comparison to the SF biomarker might be related to the sampling time. Previous studies have found larger SF biomass in early spring while bigger abundance of AMF biomass have been detected in early summer (Birgander et al., 2014), when our sampling took place for most of the regions.

### 4.3. Impact of management intensity at the regional level on the soil fungi

In our study, we observed a positive response of SF in extensively managed grasslands, thus confirming our third hypothesis. A recent study across different long-term experiments in Europe, showed that the management impact in the soil fungal communities differs between sites, soils and crops species (Hannula et al., 2021).

The increase in the fungal biomarker under extensive management detected in our study can be used as an indicator of enhanced efficiency of nutrient cycling and decomposition processes (Bardgett and McAlister, 1999). This suggests that, in our study, fungi rather than bacteria were more sensitive to intensive management. Lemanski and Scheu (2015) have shown that fungal but not bacterial phospholipid fatty acid biomarkers varied with plant composition, indicating fungi respond more sensitively than bacteria to changes in the grassland management. In addition, practices such as an increased ploughing frequency can impact the soil microbial community by decreasing microbial biomass (Mathew et al., 2012) or modified microbial diversity, with a large impact, for example, of conservation tillage practices on fungal diversity (Wang et al., 2016). Chemical fertilizer input is another agricultural practice with a great impact on the soil microbiota, decreasing microbial diversity (Manoharan et al., 2017), and biomass (Geisseler et al., 2016). In grasslands, fungal abundance has been reported to be negatively affected by fertilizer application (Bittman et al., 2005), while Ryan and Graham (2018) report that ploughing alters the mycorrhizal fungal community, but may not reduce abundance and diversity of AMF. This difference in the management impact on AMF may have been due to the differences in soil and climatic conditions, which have been shown to confound comparisons and generalizations of results in a meta-analysis (Jansa et al., 2006). In our survey, results of AMF abundance were inconclusive, similar to what has been previously described in temperate grasslands (Geisseler et al., 2016). Other management practices reported to influence AMF biomass are irrigation (Zhang et al., 2018) and P fertilization (Collins and Foster, 2009), the latter causing a decrease in AMF biomass. In our study, irrigation and P mineral fertilization were applied solely in the intensive grasslands of PT, which in addition showed the lower amount of soil organic matter of the whole survey. Even though the soils from PT were fertilized with P, their values of total P were the lowest of the whole survey, suggesting that these soils are generally P-deficient. The higher AMF biomass, as compared to the other microbial groups, in the PT grasslands was likely the consequence of the plants forming AMF symbiosis to compensate for this P deficiency (Ma et al., 2021).

## 5. Conclusions

At the spatial scale considered in this pan-European survey, soil microbial community structure, as indicated by the PLFA patterns, differed between geographical regions, since the soil and climatic properties influence the soil microbial community to a greater extent than management. To elucidate the effects of management, a regional perspective was needed. The amount of bacterial biomass differed along the geographic gradient, according to differences in climate and soil properties, while the fungal biomass was more responsive to management intensity. We conclude that extensive grassland management provided a better habitat for fungal colonization, within the management intensity gradient, irrespective of geographic location. This may have implications for improved sustainability in agricultural soil management, where soil fungi are allowed to contribute to improved soil structure and the resulting improved growth conditions.

## 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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2021.104264.

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