Land-use- and climate-mediated variations in soil bacterial and fungal biomass across Europe and their driving factors

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ABSTRACT

Elucidating contents and drivers of soil bacterial and fungal biomass in contrasting land uses and climates at European scale is useful to define appropriate policies for the conservation of the ecosystem services that soil microorganisms provide. Here, we aimed to (i) quantify and compare bacterial and fungal biomass in 513 European soils collected from three different land uses (forests, grasslands, and croplands) and climates (arid, temperate, and cold) through analysis of fatty acid methyl esters; (ii) model the factors controlling soil bacterial and fungal biomass; and (iii) investigating levels of bacterial and fungal biomass in cropland soils cultivated with three important crop types in Europe: cereals, oil-producing crops, and orchards. Bacterial biomass decreased with land use in the following order: grasslands > croplands > forests and was found to be the highest in temperate environments. Similar patterns were found for biomass of Gram-positive and Gram-negative bacteria and Actinobacteria. Soil fungal biomass was greater in forests than in croplands and grasslands and was favoured by colder environments. The fungi to bacteria ratio (F/B) decreased as follows: forests > croplands > grasslands, with soils in colder climates showing greater F/B ratios in croplands and forests. Soil texture, soil organic carbon, and nitrogen were shown to directly drive bacterial and fungal biomass. The biomass of the different microbial groups was not influenced by the crop type when only croplands were considered. Fungi appear to be more susceptible to agricultural soil use than bacteria. Moreover, agricultural use of soil seems to buffer the effect of harsh climatic conditions on soil bacterial biomass. The present study improves our understanding of the combined effects of land use and climate on soil bacterial and fungal biomass across Europe.

1. Introduction

Soil microorganisms are a key component of terrestrial ecosystems (Fierer, 2017). They mediate processes that are critical for land productivity, soil fertility, and delivering other ecosystem services such as nutrient cycling, degradation of contaminants, pathogen control, and climate regulation (Delgado-Baquerizo et al., 2016b; Köninger et al., 2022). In the last few decades, the practices related to intensive agriculture (e.g., land conversion, continuous cropping cycles,

indiscriminate use of fertilizers and pesticides, excessive tillage, etc.) have led to land degradation and soil health loss and have negatively impacted soil microbial diversity (Pulleman et al., 2022; Tilman et al., 2002). In this scenario, new European agro-environmental policies are being developed under the auspices of the "Common Agricultural Policy post-2020" (Pe'er et al., 2019). These new regulations aim to move towards more sustainable food systems, with emphasis in the restoration and conservation of soil biota, in general, and soil microbial communities, in particular (Guerra et al., 2021; Šumrada et al., 2020; Zeiss

et al., 2022). Both the correct development and implementation of these policies need a deep understanding of the microbial communities under changing environments (Smith et al., 2021).

Land-use type determines important changes in plant cover, soil organic matter (SOM), soil structure, and quantity and quality of nutrients for microorganisms, influencing soil microbial communities in terms of diversity, composition, functionality, and biomass (Dequiedt et al., 2011). Cropland soils usually represent a dynamic environment for bacteria and fungi, with frequent fertilization, intercropping, irrigation, as well as homogenization and microhabitat destruction due to tillage (Szoboszlay et al., 2017). Instead, grassland and forest soils are usually non-tilled, present a more preserved and less dynamic structure, and contain higher contents of soil organic carbon (SOC, Baldrian et al., 2016; Szoboszlay et al., 2017). Land-use type is thus a potential key driver of soil bacterial and fungal biomass that must be studied and modeled (Smith et al., 2021). However, the effects of land use on soil microbial biomass do not stand alone. Climate, by influencing temperature, precipitation, and sunlight hours, can counter or exacerbate the influence of land use on soil microbial biomass (Trivedi et al., 2016; Wan et al., 2021). Therefore, a more comprehensive understanding of the impact of land use on soil bacterial and fungal biomass can be achieved when climate-related variations are also considered (Kostin et al., 2021).

Predictors of soil total microbial biomass under changing land uses and climates have shown to be soil pH, texture, nutrient contents, and moisture availability at different geographical scales (Dequiedt et al., 2011; Hu et al., 2014; Wang et al., 2022b). However, the explanatory power of these factors tends to weaken as geographical scale expands, and inconsistence between works has been reported (Guerra et al., 2021; Hendershot et al., 2017; Serna-Chavez et al., 2013). Furthermore, most studies analyzing soil microbial biomass at broad geographical scales are focused on total microbial biomass (i.e., microbial biomass C), while much less is known about patterns and predictors of bacterial and fungal biomass separately (Wan et al., 2021). Bacteria and fungi vary in a multitude of physiological and life-history traits, which make them to differently respond to changing environmental conditions (Bahram et al., 2018; Waring et al., 2013). Compared to bacteria, fungi generally have slower growth and turnover rates, greater C to nitrogen (N) stoichiometry, increased capacity to degrade a wider range of substrates, and higher C use efficiency (Yu et al., 2022). In comparison with fungi, bacteria tend to dominate in locations with high soil nutrient contents, warmer temperatures, and in frequently disturbed soils (Brito et al., 2021; Fierer et al., 2009; Yu et al., 2022). Therefore, the direction and extent of the responses of soil bacterial and fungal biomass to changes in land use and climate are expected to be not concomitant at broad geographical scales.

At the European Union scale, works comprehensively investigating how land use (by comparing croplands, grasslands, and forests) and climate (cold, temperate, and arid) mediate changes in soil bacterial and fungal biomass are useful for policy-making purposes and the development of predictive models for soil biodiversity conservation (Köninger et al., 2022; Zeiss et al., 2022). A recent study, using the same collection of soils as the one used here, has evaluated the changes in soil microbial biomass (measured as substrate-induced respiration) under different land uses and climatic conditions across Europe and has identified the predictors of such shifts (Smith et al., 2021). Therefore, in the present work, we aimed to go beyond the aforementioned study by (i) comparing bacterial and fungal biomass (and not only soil total microbial biomass through indirect methods) in soils from croplands, grasslands, and forests located across Europe and belonging to three broad climate types: cold, temperate, and arid; (ii) modeling the factors controlling soil bacterial and fungal biomass under contrasting land uses and climates; and (iii) investigating levels of bacterial and fungal biomass in cropland soils cultivated with three important crop types in Europe: cereals, oil-producing crops, and woody orchards. To do this, biomass of soil bacteria (including Gram-positive (GP) and Gram-negative (GN) bacteria and Actinobacteria) and fungi was determined

across 513 sites in Europe through fatty acid methyl ester (FAME) analysis. We hypothesized that both land use and climate would significantly affect soil bacterial and fungal biomass, in concordance with the results of Smith et al. (2021). In general, decreased bacterial and fungal biomass was hypothesized to characterize croplands in comparison with the other more natural land uses (forests and grasslands). This effect was expected to be more evident in fungi than in bacteria due to the higher susceptibility of fungi to intensive farming practices (Clocchiatti et al., 2020; Yu et al., 2022). We also hypothesized that soils in cold climates would have greater fungal biomass than those in temperate or arid environments, while bacterial biomass would benefit from temperate climate conditions (Yu et al., 2022). On the other hand, irrespectively of the land use, we expected to find the lowest bacterial and fungal biomass in arid environments since they are characterized by low SOC contents and high erosion rates due to climatic limitations (a low ratio of mean annual precipitation (MAP) to potential evapotranspiration) and a more limited vegetation cover in comparison with temperate and cold climates (Plaza-Bonilla et al., 2015). Crop type was also hypothesized to influence soil microbial biomass in croplands. Soils under woody orchards were hypothesized to harbor higher bacterial and fungal biomass since the agricultural practices associated to these agricultural systems were expected to be less detrimental than those applied to cereals or oil-producing crops.

2. Materials and methods

2.1. Soil sampling

Soil samples were collected from 513 locations in 24 European Union member states and the United Kingdom under the auspices of the 2018 "Land Use/Cover Area frame statistical Survey" –LUCAS– (Orgiazzi et al., 2018) in the period from April to December 2018 (Fig. 1). Soils were collected from 327 croplands, 107 grasslands, and 79 forests, reflecting the dominant land-use types across the focus countries (Supplementary Dataset 1). To assess the effect of climate and its interaction with land use on soil bacterial and fungal biomass, locations were classified as belonging to three broad climates according to the Koppen-Geiger climate classification: arid, $MAP < 10 \times P_{threshold}$; temperate, $T_{hot} > 10$ and $0 < T_{cold} < 18$; and cold, $T_{hot} > 10$ and $T_{cold} \leq 0$. MAP stands for mean annual precipitation. The meaning of $P_{threshold}$ varies according to the following rules: if 70 % of MAP occurs in winter then $P_{threshold} = 2 \times MAT$, if 70 % of MAP occurs in summer then $P_{threshold} = 2 \times MAT + 28$, otherwise $P_{threshold} = 2 \times MAT + 14$. T_{hot} stands for temperature of the hottest month, and T_{cold} stands for temperature of the coldest month (Peel et al., 2007). In this way, 79 of the soils were collected from arid climates, 216 from temperate, and 218 from cold. Mean annual temperature (MAT) ranged between -0.6 and $19.4\ °C$, MAP between 341 and 1694 mm, and the aridity index (AI) between 0.19 and 2.34. Further, since we were also interested in investigating how crop type may impact soil microbial biomass, soils under three dominant types of crops were selected and compared: cereals (CER, barley, maize, oats, and wheat, among others; 186 soil samples), oil-producing crops (OIL, rape, soya, and sunflower; 46 soil samples), and woody orchard (ORC, olive groves, pear and apple orchards, and vineyards; 48 soil samples). Details about location, land use, predominant vegetation, and climate of each sampling site are presented in Supplementary Dataset 1. Each sample was a composite of five subsamples from the top 20 cm of soil: four subsamples orthogonally collected in a 2-m radius from a central subsample. After sampling, the soils were sieved (2 mm) and stored at $-20\ °C$ until further processing for FAME analysis. More details about the soil sampling strategy and the LUCAS initiative can be found in Orgiazzi et al. (2018).

2.2. Environmental and physicochemical characterization of soil samples

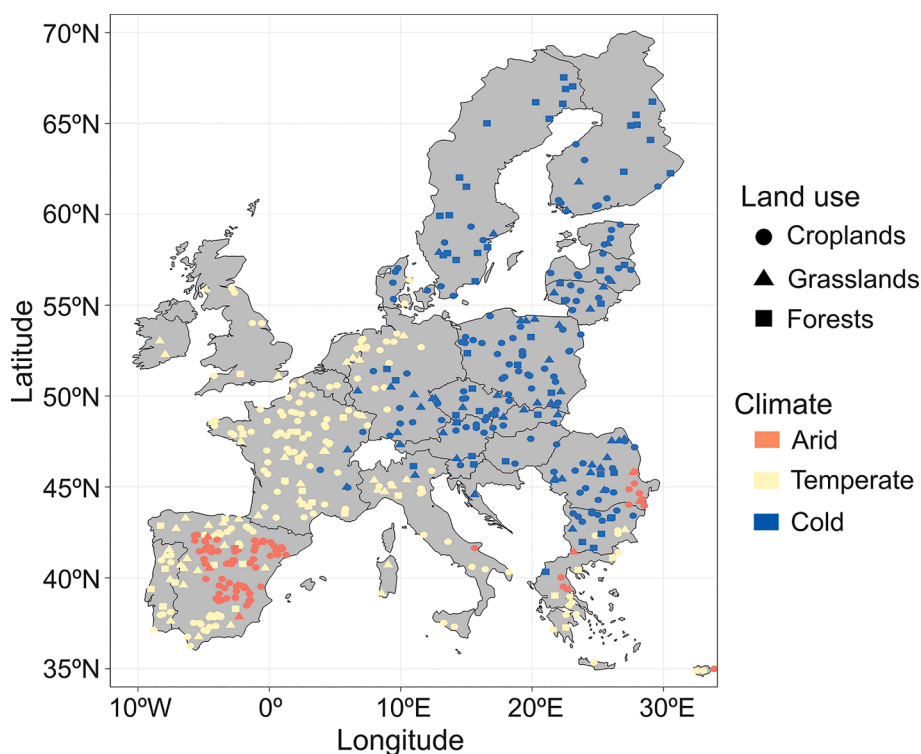Physicochemical characterization of soil samples included texture

Fig. 1. Location, land-use type, and climate characterizing the 513 soils included in the study.

(percentages of sand, silt, and clay), pH (in H_2O), electrical conductivity (EC), and contents of SOC, total N, total phosphorus (P), and extractable potassium (K). These characterizations were done following standard methods (Orgiazzi et al., 2018). Data on bulk density (BD, top 15 cm soil) were collected from SoilGrids (Hengl et al., 2017), net primary productivity (NPP) from MODIS (moderate resolution imaging spectroradiometer) aboard the NASA's Terra satellite (https://modis.gsfc.nasa.gov), MAT and MAP from WorldClim 2 (Fick and Hijmans, 2017), and AI from CGIAR (Trabucco and Zomer, 2018).

2.3. Determination of fatty acid methyl esters

Analysis of FAME, hereafter called fatty acids, was used to assess the biomass of soil bacteria, GP and GN bacteria, Actinobacteria, and fungi (Schutter and Dick, 2000). We are aware that fatty acid analysis only represents a proxy of the actual soil microbial biomass present in a given sample. However, data obtained through this method have been shown to correlate well with absolute measurements of microbial biomass (i.e., amounts of microbial C per g soil) (Lee et al., 2007) and be more reliable than those obtained through other techniques, such as the quantification of target genes in soil-extracted DNA (Baldrian et al., 2013; Lee et al., 2007). Since there are uncertainties on the reliability of the conversion factors transforming fatty acid contents into biomass (Bastida et al., 2021; Frostegård et al., 2011), we decided not to apply them, and use instead the amounts of fatty acids as a measurement of soil microbial biomass.

Contents of fatty acids in the 513 samples comprising the present study were measured according to the method described by Schutter and Dick (2000). Briefly, fatty acids were extracted from microbial cells and released as methyl esters by incubating 3 g soil of each sample with 0.2 M methanolic KOH during 1 h at 37 °C under periodic shaking. Afterwards, samples underwent pH neutralization with 1 M acetic acid. Fatty acids were then partitioned into an organic phase by adding hexane, followed by centrifugation (480 × g for 10 min) and evaporation of the hexane in a SpeedVac (Labogene). Fatty acids were finally dissolved in isooctane and analyzed with a Trace Ultra, Thermo Scientific gas

chromatograph fitted with a 60-m capillary column (SGE Analytical Science, BPX70, 60 m × 0.25 mm ID × 0.25 μm film), using Helium as the carrier gas and a flame ionization detector. The chromatographic conditions were as follows: (i) an initial temperature of 120 °C for 0.5 min, increased to 140 °C with a ramp of $1\,°C\,min^{-1}$, (ii) then to 170 °C at $2\,°C\,min^{-1}$, (iii) and finally to 210 °C at $2\,°C\,min^{-1}$. Fatty acids were identified and their absolute amounts in each sample (nmol g^{-1} soil) were calculated using commercially available FAME mixes (Sigma-Aldrich).

Fatty acids were described by standard nomenclature. Each fatty acid was thus named using the total number of C atoms:number of double bonds, followed by additional information about the position of the terminal double bond (ω). Other notations are "Me" for methyl, "cy" for cyclopropane, and the prefixes "i" and "a" for iso and anteiso-branched fatty acids, respectively. The fatty acids i15:0, a15:0, i16:0, i17:0, 16:1w9, cy17:0, cy19:0, 10Me16:0, and 10Me18:0 were used as representatives of bacteria (Dungait et al., 2011; Frostegård et al., 1993). The GP representative fatty acids were i15:0, a15:0, i16:0, i17:0, 10Me16:0, and 10Me18:0; and the GN ones were 16:1ω9, cy17:0 and cy19:0 (Dungait et al., 2011; Frostegård et al., 1993). The fatty acids 10Me16:0 and 10Me18:0 were used as actinobacterial markers. The fatty acids 18:2w6,9t and 18:2w6,9c were representative of the fungal biomass (Brant et al., 2006; Rinnan and Bååth, 2009). Ratios between fatty acid contents of GP and GN bacteria (GP/GN) and fungi and bacteria (F/B) were calculated.

2.4. Statistical analyses

Non-parametric statistics were used to assess the significance of land use, crop type, and climate on environmental, physicochemical, and bacterial and fungal biomass data since they did not follow a normal distribution even after their transformation. Two-way univariate PERMANOVA (Permutational Analysis of Variance) was used to check whether environmental and physicochemical variables and microbial biomass measurements were significantly affected by land use or crop type and climate and their interactions by using the *adonis* function in

the R package *vegan* (Oksanen et al., 2013). Pairwise permutation tests were further used to compare land uses, crop types, and climates by taking advantage of the *pairwisePermutationMatrix* function in the R package *rcompanion* (Mangiafico, 2017).

Random forest analysis was used to identify the most important predictors of the different microbial groups' biomass. The list of predictors included land use (a categorical variable including the levels croplands, grasslands, and forests), environmental conditions (MAP, MAT, AI, and NPP), and soil physicochemical properties (sand, silt, and clay proportions, BD, EC, pH, and contents of SOC, N, P, and K). The importance (i.e., % increase in mean squared error, MSE) of each predictor and its significance were computed with the R packages *RandomForest* and *rfPermute,* using 999 trees and *nrep* = 120 (Archer, 2022). The performance of each random forest was evaluated using the R package *rfUtilities* (Evans and Murphy, 2019). Further, we used linear and quadratic functions to evaluate the direction and shape of the relationship between the significant predictors and biomass of the different microbial groups. The best model fit was selected by identifying the regression with the lowest Akaike information criterion values (Delgado-Baquerizo et al., 2017b). Regressions were calculated using the R package *stats* (R Core Team). A correlation heatmap was generated using the *Hmisc* (Harrell Jr, 2019) and *corrplot* (Wei et al., 2017) packages in R.

Structural equation modeling (SEM) was used to build a detailed system-level understanding of the major direct and indirect effects of land use, climate, and soil physicochemical properties on the biomass of bacteria and fungi, as well as the F/B ratio. SEMs were not constructed for GP and GN bacteria and Actinobacteria since these fractions are included in the group bacteria. A priori SEMs were constructed taking into consideration current knowledge on interactions between land use, climate, and soil physicochemical properties as well as the results of the random forest modeling (Fig. S1). In this way, those variables identified as significant by random forest analyses and land use (as categorical variable) were hypothesized to have a direct effect on soil microbial biomass, and the rest of the model was constructed around this assumption. This approach was used on the basis of previous studies (Delgado-Baquerizo et al., 2016a). Among the climate-related variables, MAT showed the highest mean predictor importance in random forest modeling and was thus selected to be included in the SEMs (Fig. 3). Both SOC and N were shown to be significant predictors of bacterial and fungal biomasses; however, only N (which showed a higher mean predictor importance in random forest modeling than SOC) was included in the models to avoid multicollinearity, since both parameters were highly correlated ($\rho = 0.87$; $P < 0.001$) (Fig. S3) (Bastida et al., 2019). The R package *lavaan* (Rosseel et al., 2017) was used to test whether our log-transformed data fitted our a priori models. Four metrics were used to quantify the goodness of fit of our models: (i) the χ^2 test, (ii) the Bollen-Stine bootstrap test, (iii) the root mean square error of approximation (RMSEA), and (iv) the comparative fit index (CIF) (Delgado-Baquerizo et al., 2017a; Schermelleh-Engel et al., 2003). Since some of the variables were not normally distributed, the probability that a path coefficient differs from zero was tested using bootstrap tests. Bootstrapping tests in such cases do not assume that the data match a particular theoretical distribution.

Data visualizations were performed using the R package *ggplot2* (Wickham, 2016) and CorelDRAW ver. 2020.

3. Results

3.1. Soil physicochemical properties across land uses and climates

All the studied environmental and soil physicochemical parameters significantly varied with land use and climate (Fig. S2 and Table S1). NPP in grasslands and forests was similar, but higher than that in croplands, and decreased with climate in the following order: temperate > cold > arid. Soil texture changed with land use and climate; forest and

cold environments had the highest soil sand contents. BD showed decreased values in forests and in colder environments. Soil pH decreased with land use as follows: croplands > grasslands > forests, and with climate in the following order: arid > temperate > cold. EC in soils from croplands and grasslands and from arid and temperate climates was similar and higher than that of forests and cold climates, respectively. SOC and N contents were higher in the more natural ecosystems (forests and grasslands) in comparison with croplands, while the opposite was observed for soil P and K contents. Soils in temperate and cold climates contained more SOC, N, and P, while the highest K contents were detected in arid climates.

3.2. Soil microbial biomass across land uses and climates

Across the 513 soils studied, bacterial fatty acid contents ranged between 12 and 353 nmol g^{-1} soil and varied significantly with land use and climate (Fig. 2 and Table S2). Irrespectively of the climate, bacterial biomass significantly decreased with land use in the following order: grasslands > croplands > forests, and with climate as follows: temperate > cold > arid (Table S2). The same pattern was observed for GP. GN bacterial pools were lower in forest soils and cold climates in comparison with those in cropland and grassland soils and in the other two climates, respectively. The GP/GN ratio was found to be lower in croplands and arid climates in comparison with grasslands and forests and with temperate and cold climates, respectively (Table S2). Forest and grassland soils presented similar contents of actinobacterial fatty acids and higher than those in cropland soils. Actinobacterial fatty acid content significantly decreased in arid climates (Table S2). The analysis of the interaction between land use and climate proved that in cropland soils, the biomass of bacteria, GP, and Actinobacteria did not vary among climates; however, grassland and forest soils in temperate and cold climates harbored more biomass of these microbial groups than arid climates (Fig. 2). In temperate climates, bacterial biomass did not differ among land uses, while the opposite was observed for arid and cold climates (Fig. 2). Biomass of GP and GN bacteria and Actinobacteria in the soils of the three land uses changed with climate. The GP/GN ratio was not dependent on land use in arid climates, but it was higher in temperate and cold climates than in arid ones in croplands and grasslands. Except for the GP/GN ratio, the interaction land use × climate was found to be not significant.

Fungal fatty acid contents ranged between 2 and 57 nmol g^{-1} soil and were significantly influenced by land use and climate, but not by their interaction (Fig. 2 and Table S2). Soils in forests and in temperate and cold climates harbored higher fungal biomass. Regarding the interaction between land use and climate, amounts of fungal fatty acids did not differ among climates in cropland and grassland soils, but they did so in forests, with temperate and cold climates showing increased fungal biomass. In temperate and cold climates, fungal fatty acid contents were independent of land use, but, in arid climates, lower soil fungal biomass was detected in forests in comparison with grasslands and croplands (Fig. 2). The F/B ratio decreased with land use in the following order: forests > croplands > grasslands, with temperate and cold climates presenting increased F/B ratios in croplands and forests (Fig. 2 and Table S2). Instead, in grasslands, the highest F/B ratios were detected in arid climates. These findings along with the significant variations in F/B ratios with land use within each climate would explain the significant interaction effect of land use × climate on this ratio.

3.3. Predictors of soil microbial biomass across Europe

Random forest analyses showed that SOC, N, and sand (i.e., soil texture) contents were the most important (and significant) predictors of land-use- and climate-driven changes in bacterial, GP, and GN biomass across Europe (Fig. 3). In the case of fungi and Actinobacteria, SOC and N were identified as the significant predictors. The GP/GN ratio, besides SOC and N, was driven by NPP, soil P content, and land use (as
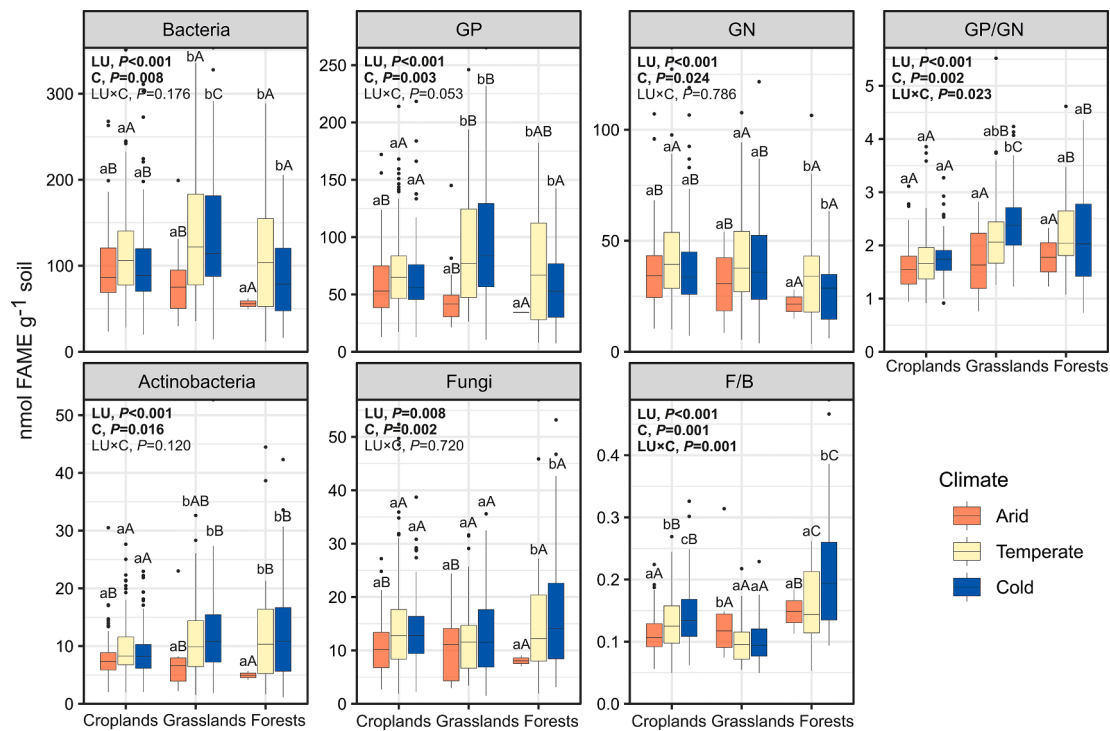
Fig. 2. Box plots comparing biomass (measured as fatty acid content) of soil bacteria, Gram-positive (GP) and Gram-negative (GN) bacteria, and Actinobacteria, as well as the Gram-positive/Gram-negative (GP/GN) and fungi/bacteria (F/B) ratios in croplands, grasslands, and forests under arid, temperate, and cold climates. *P*-values of two-way PERMANOVA for the factors land use (LU) and climate (C), and their interaction are shown at the top of each figure. Different lowercase letters above each box denote significant differences among climates within each land use, and different capital letters denote significant differences among land uses within each climate according to pairwise permutation tests. The boxes represent the interquartile range (IQR) between the first and third quartiles (25th and 75th percentiles, respectively), and the vertical line inside the box defines the median. Whiskers represent the lowest and highest values within 1.5 times the IQR from the first and third quartiles, respectively. Dots represent outliers.
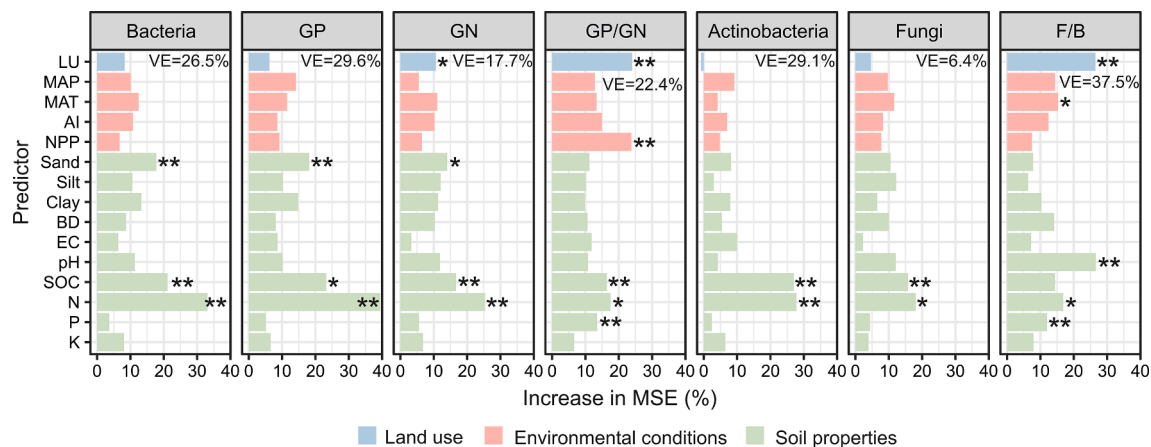

Fig. 3. Random forest mean predictor importance (% increase in MSE (mean square error)) of the studied variables as predictors of biomass of soil bacteria, Gram-positive (GP) and Gram-negative (GN) bacteria, and Actinobacteria, as well as the Gram-positive/Gram-negative (GP/GN) and fungi/bacteria (F/B) ratios. Significance levels are shown at *P < 0.05 and **P ≤ 0.001. Predictors belonging to the same category were represented with the same color according to the legend. VE = variance explained (%). LU = land use. MAP = mean annual precipitation. MAT = mean annual temperature. AI = aridity index. NPP = net primary production. Sand, silt, and clay = soil sand, silt, and clay contents, respectively. BD = bulk density. EC = electrical conductivity. SOC = soil organic carbon. N and P = soil total nitrogen and phosphorus, respectively. K = extractable potassium.

categorical variable). Significant variables explaining variations in the F/B ratio were land use, pH, soil N and P contents, and MAT (Fig. 3). These results were confirmed by regression and correlation analyses (Fig. 4 and Fig. S3 and S4). The relationships between bacterial (and GP and GN bacteria) biomass and SOC and N as well as between fungal biomass and SOC were fitted to the quadratic function. The other analyzed relationships were identified as linear (Fig. 4 and Fig. S4).

Further, SEMs were constructed for bacteria, fungi, and F/B to understand how the factors identified by random forest as significant and hypothesized to exert a direct effect on microbial biomass were influenced by land use, climate (MAT), and plant cover (NPP) (Fig. 5). Bacterial SEM (explaining 32 % of bacterial biomass variability) confirmed that this group's biomass is positively and negatively controlled by N and sand (texture), respectively. N contents were negatively affected by
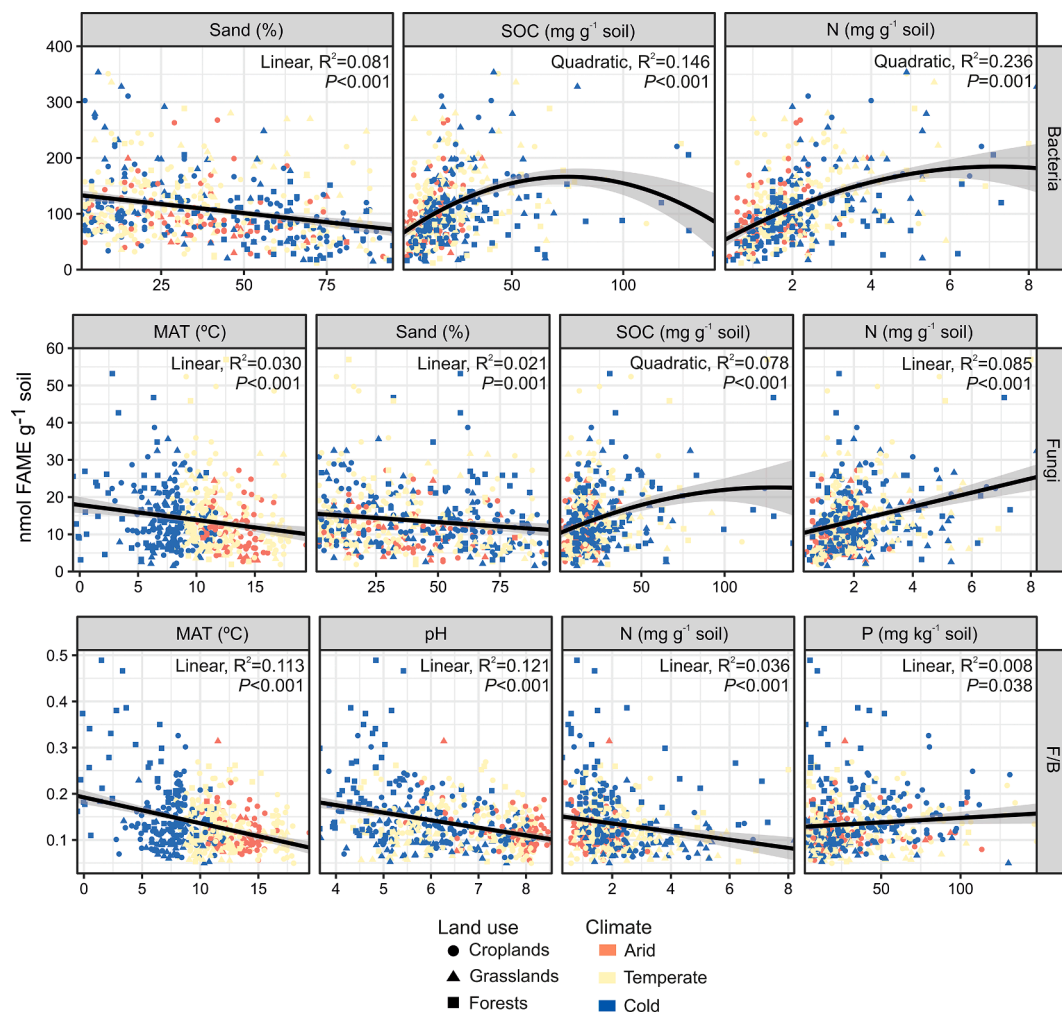
Fig. 4. Dependences of soil bacterial and fungal biomass and the fungi/bacteria (F/B) ratio on selected environmental variables evaluated by regression analyses. The best model (linear or quadratic) fitting each regression is indicated at the top of each figure. Shaded areas represent 95 % confidence intervals for the regression line. R^2 and *P*-values are shown for each regression analysis. Sand = soil sand content. SOC = soil organic carbon. N and P = soil total nitrogen and phosphorus, respectively. MAT = mean annual temperature.

land use, MAT, and sand, and positively by NPP. Unexpectedly, MAT and soil texture did not exert a direct influence on NPP, which was regulated by land use. Our fungal SEM included N and land use as the only factors having a direct effect on this group biomass. However, the model did not fit our data (Fig. S5), and a second SEM was developed, where, besides N and land use, MAT and sand were also hypothesized to have a direct effect on fungal biomass (Fig. 5). This model fitted well our data and explained 12 % of the variation in fungal biomass. As for bacteria, land use directly regulated fungal biomass but also indirectly by inducing changes in N and NPP. Up to 29 % of the variability in F/B ratio was explained by our SEM (Fig. 5). Land use and P exerted positive direct effects on F/B, while those exerted by pH, N, and MAT were negative. The three soil physicochemical properties (pH, N, and P) were influenced by land use, NPP, MAT, and sand; NPP, in turn, was also influenced by land use (Fig. 5). Therefore, our models show that soil bacterial and fungal biomass and the F/B ratio are controlled to a higher extent by soil texture and soil physicochemical properties. Land use and climate exerted direct effects on these groups but also indirectly by affecting nutrient pools and plant cover (NPP).

3.4. Soil microbial biomass across contrasting crop types

Soil texture and all the analyzed soil physicochemical properties changed with crop type (i.e., cereals vs oil-producing crops vs orchards),

with the exception of SOC (Table S3 and Fig. S6). However, the biomass of the different microbial groups and the GP/GN and F/B ratios did not significantly change with crop type (Table S4 and Fig. 6). In general, temperate environments harbored higher soil microbial biomass than the other climates in the studied croplands. Climate was a significant factor for the fatty acid contents of the soils under the three crop types studied. The interaction crop type × climate was found to be not significant for neither of the microbial groups nor the microbial ratios.

4. Discussion

The biogeographic distribution of soil microbial biomass and its drivers have previously been studied at regional, continental, and global scales (Serna-Chavez et al., 2013; Szoboszlay et al., 2017; Wang et al., 2022b; Yang et al., 2022). Despite these works, our study represents a significant step forward on this topic by (i) focusing on the effect of land use on soil bacterial and fungal biomass under contrasting climatic conditions at the European scale and (ii) quantifying soil bacterial and fungal biomass in a comprehensive number of samples through the application of the same analytical approach (i.e., fatty acid analysis). Such a standardized approach has benefits over *meta*-analyses working on data from studies that may not always be comparable (Perveen et al., 2019). Furthermore, the application of fatty acid analysis allowed us to study the biomass patterns of different soil microbial groups, which is
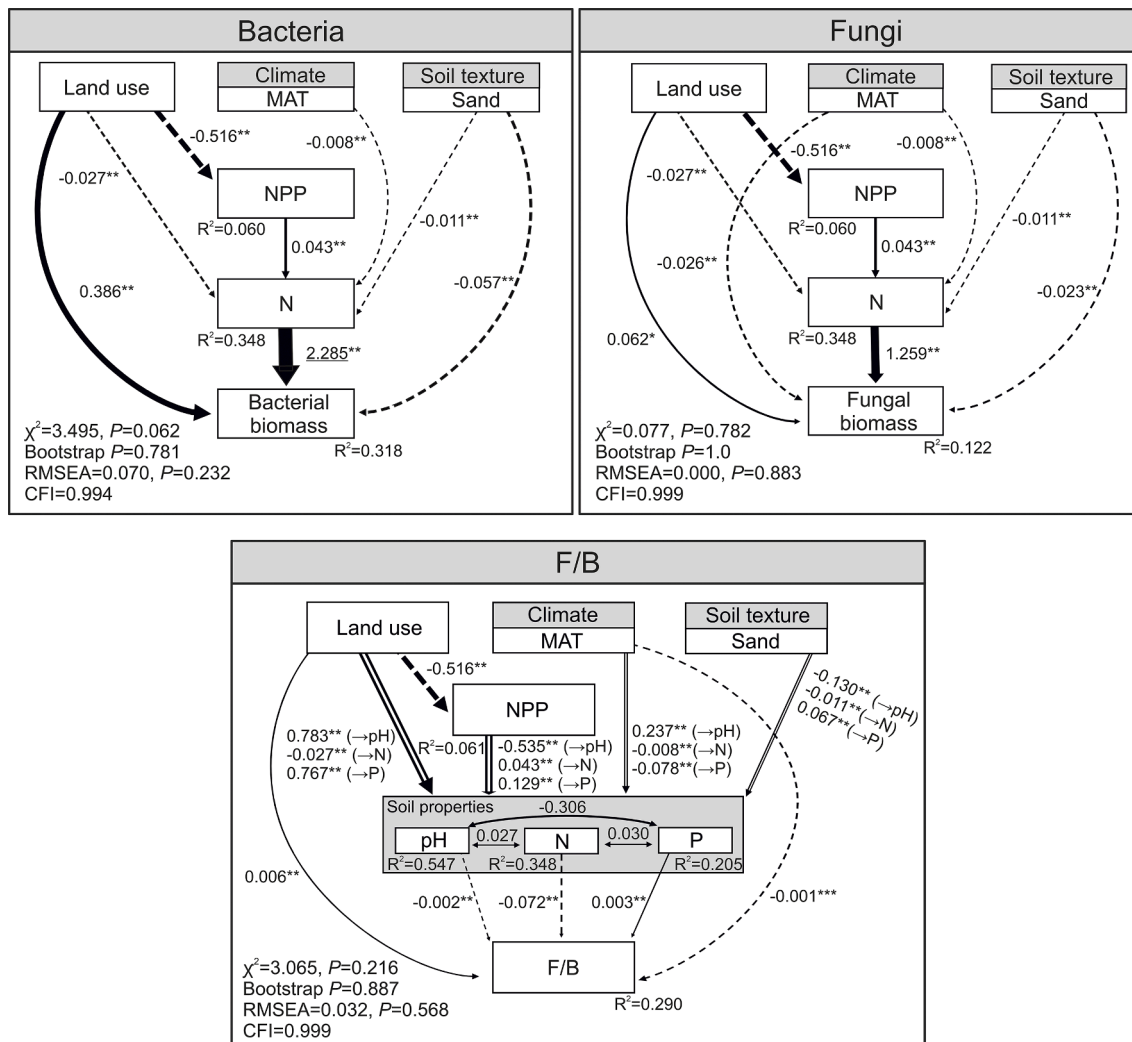
Fig. 5. SEM (structural equation modeling) assessing the direct and indirect effects of selected factors on soil bacterial and fungal biomass and the fungi/bacteria (F/B) ratio. Numbers adjacent to arrows are standardized path coefficients and are indicative of the effect size. Only significant effects ($P < 0.05$) are indicated, and significance levels are shown at *$P < 0.05$ and **$P \leq 0.001$. Continuous, dashed, and double-lined arrows indicate positive, negative, and mixed relationships, respectively. Underlined path coefficients indicate quadratic relationships. In the SEM on F/B, double-headed arrows represent covariance between variables. R^2 denotes the proportion of variance explained for every response variable by the model. The models were satisfactorily fitted to data, as suggested by non-significant χ^2 values and non-parametric bootstrap, and by values of RMSEA (root mean square error of approximation) and CIF (comparative fit index). MAT = mean annual temperature. NPP = net primary production. AI = aridity index. Sand = soil sand content. N and P = soil total nitrogen and phosphorus, respectively.

more informative than the quantification of total microbial biomass C (Wan et al., 2021).

4.1. Bacterial biomass and its driving factors across Europe

Contrary to our initial hypothesis, soil bacterial biomass was not the lowest in croplands, but in forests. This pattern was especially evident in arid and cold environments. This result demonstrates that agricultural land use may not always involve a reduction in bacterial biomass, at least at broad geographical scales. Our initial expectations were based on the detrimental effects that some agricultural practices (intensive cropping, tillage, agrochemical application, machine operations, or irrigation, which leads to frequent dry/wet cycles) have on soil bacterial biomass and the strong resource extraction from the ecosystem that agriculture involves (Scotti et al., 2015; Tsiafouli et al., 2015). In concordance, the lowest contents of SOC and N were detected in croplands. However, croplands contained higher contents of soil P and K than forests and grasslands, which is seen in relation to the common and continued application of fertilizers to agricultural soils (Ballabio et al., 2019). Independently of the land use, the highest contents of bacterial

fatty acids were found in temperate environments. This demonstrates that cold and, especially, arid environments favor to a lesser extent soil bacterial biomass (He et al., 2020). However, we found that soils under croplands and grasslands had higher contents of bacterial biomass than those under forests in arid climates and that croplands in the three climates harbor similar contents of bacteria. These results suggest that agricultural practices do not only have a homogenization effect on soil bacterial diversity (Wang et al., 2022a), but also on biomass. This result may also indicate that soil agricultural management across Europe tends to buffer the negative impact of unfavorable climatic conditions on bacterial biomass (Siebert et al., 2019), especially in arid environments. Unfortunately, the European collection of soils used in the present study (the LUCAS survey) does not contain information on the agricultural practices being applied to each studied cropland. This prevented us from digging deeper into the changes in bacterial biomass with the type of agricultural practice. Despite this, in arid climates, we detected that soil P contents in forests were extremely low and lower than those of grasslands and croplands (Fig. S2), ecosystems which were probably subjected to fertilization. This would explain why bacterial biomass was the lowest in forest soils in arid environments and would be suggesting
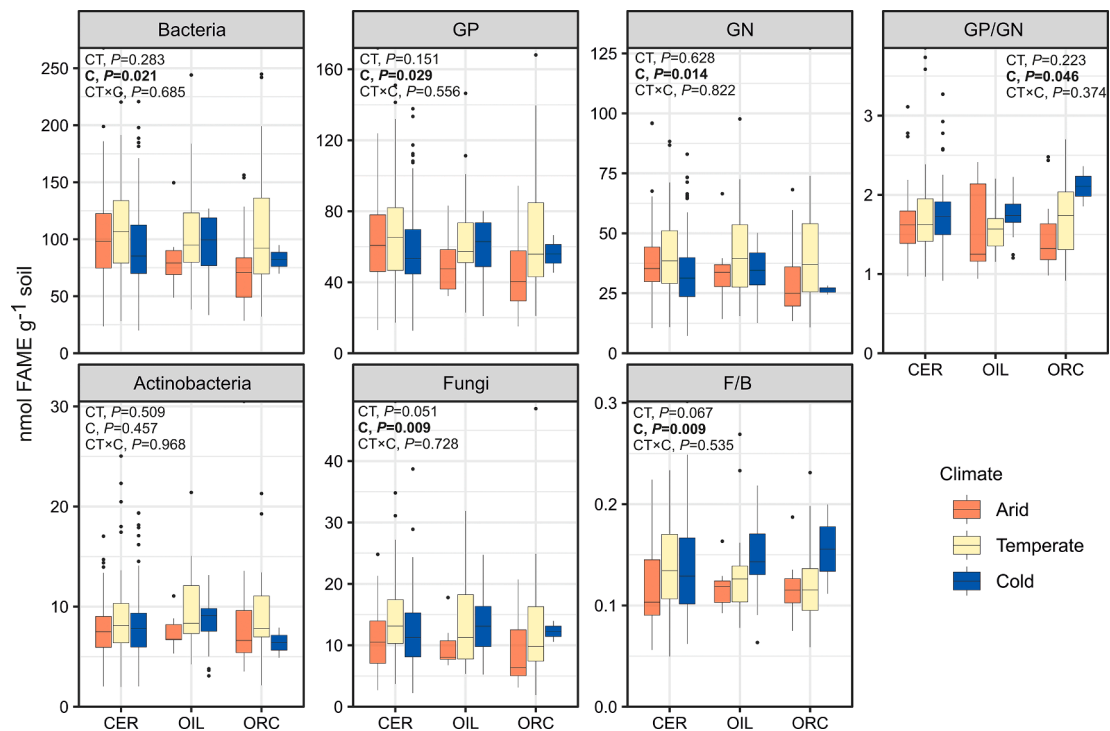
Fig. 6. Box plots comparing biomass (measured as fatty acid content) of soil bacteria, Gram-positive (GP) and Gram-negative (GN) bacteria, and Actinobacteria, as well as the Gram-positive/Gram-negative (GP/GN) and fungi/bacteria (F/B) ratios in cropland soils cultivated with cereals (CER), oil-producing crops (OIL), and orchards (ORC) under arid, temperate, and cold climates. *P*-values of two-way PERMANOVA for the factors crop type (CT) and climate (C), and their interaction are shown at the top of each figure. The boxes represent the interquartile range (IQR) between the first and third quartiles (25th and 75th percentiles, respectively) and the vertical line inside the box defines the median. Whiskers represent the lowest and highest values within 1.5 times the IQR from the first and third quartiles, respectively. Dots represent outliers.

that bacteria are P-limited in these ecosystems.

Our modeling approaches evidenced that the contents of sand, SOC, and N in soil were the main drivers of bacterial biomass, which is in concordance with previous studies at broad geographical scales (He et al., 2020; Wan et al., 2021; Yang et al., 2022). Bacterial biomass increased with decreasing sand content and increasing SOC and N levels. The quadratic relationship found between bacterial biomass and contents of SOC and N may indicate that bacterial biomass increases with SOC and N until a peak is reached, after which some other biotic factors such as competition may impede bacterial growth although these nutrient levels increase (Bastida et al., 2021). Grasslands harbored the highest levels of bacterial biomass despite the highest SOC contents were observed in forests. Bacteria in grasslands drive rapid nutrient cycling, which promotes the existence of fast-growing, nutrient-rich plant species (Crowther et al., 2019). This fact, along with the lack of significant differences in N contents between grasslands and forests, might explain why soil N content explained a higher proportion of bacterial biomass variation than SOC. Bacterial biomass in forest soils could be restricted by the cold temperatures dominating most of them (a high number of the studied forests were from Scandinavia or from high elevations) and the higher contents in sand. Fine-textured soils represent a more favorable habitat for bacterial growth than sandy ones by offering better protection from desiccation, toxic exogenous compounds, and predation by protozoa (Dequiedt et al., 2011; Ranjard and Richaume, 2001). Further, SEM complemented the data obtained by random forest by showing that land use and climate influence bacterial biomass via changes in N (and in SOC since these two parameters were highly correlated in our dataset). This concurs with the findings of the global *meta*-study by Wan et al. (2021). NPP also showed to indirectly control bacterial biomass through changes in N, which is in line with the assumption that soil physical and chemical parameters serve as a bridge to link the aboveground and belowground communities (Siles and Margesin, 2017; Wardle et al.,

2004).

GP and GN bacteria and Actinobacteria followed similar patterns to those described for total bacterial biomass; and contents of SOC, N, and sand (for GP and GN) were also identified as drivers of their biomass. These results are contrary to our initial expectations, since GN bacteria have been identified as r-strategists (copiotroph) and GP as K-strategists (oligotroph) (De Vries and Shade, 2013). Therefore, a positive correlation between K-strategists and SOC and N was not expected. This suggests that the general classification of broad bacterial groups as copiotroph or oligotroph may be an oversimplification of large variations in ecological attributes and microbial lifestyles (Ding et al., 2015).

4.2. Fungal biomass and its driving factors across Europe

Fungal biomass was detected to be the greatest in forests and colder environments. This result was initially expected since fungi have been associated with ecosystems containing more chemically recalcitrant organic matter (such as that of forests) and increased SOC and N contents (Bahram et al., 2018). Cold conditions (such as those predominating in many of our forest soils) promote SOM accumulation by slowing down the metabolic activity of heterotrophic organisms (Crowther et al., 2019). In this way, fungal biomass has already been positively linked to latitude (He et al., 2020). Furthermore, fungi have been shown to be even more susceptible to intensive farming practices than bacteria (Clocchiatti et al., 2020; Yu et al., 2022). For example, tillage greatly disrupts soil fungal mycelia, and fungi are more affected than bacteria by dry/wet cycles associated with irrigation since they reside in larger pores (Brito et al., 2021; Six et al., 2006). Our data evidenced that cropland and grassland soils across Europe harbor similar contents of fungal biomass and lower than those of forests. This indicates that a more intense land use, as that applied to many croplands and some grasslands, leads to decreased fungal biomass. In a global

meta-study, He et al. (2020) also reported that contents of fungal biomass in cropland and grassland soils were similar, but lower in comparison to those in forests in tropical and boreal biomes. In our study, fungal fatty acid contents did not vary among land uses in temperate and cold habitats; this lack of significance is seen in relation to the high variability of data.

Random forest analysis identified SOC and N as main drivers of fungal biomass across Europe. Complementary, SEM evidenced that MAT and sand content not only influence fungal biomass directly, but also indirectly by inducing changes in N (due to the high correlation between N and SOC, this effect is extrapolated to SOC). The direct effects of soil texture on N and SOC can be mediated by lower stability and high susceptibility to mineralization of SOM in sandy soils in comparison to fine-textured ones (Hartley et al., 2021). As in the case of bacterial communities, land use directly and indirectly (via N and NPP) controls fungal biomass, evidencing the complex relationships between below- and above-ground organisms through soil physical and chemical properties (Mitchell et al., 2010). The positive link between SOC and N and fungal biomass has previously been reported and supports our findings (Bastida et al., 2021; He et al., 2020). However, it is worthy to note the low explanatory power of our models. This could be a consequence of the aforementioned high variability of the data within each land use and climate or because our models did not account for some critical predictor of soil fungal biomass. For example, there might be uncaptured variations in soil moisture (Wan et al., 2021) or soil disturbance level with changing land use or agricultural practices. Moreover, we were not able to consider the management and disturbance history of the sampling sites (Allan et al., 2014; de Vries et al., 2012) as well as the potential effects of the surrounding landscape (Le Provost et al., 2021).

4.3. F/B ratio and its driving factors across Europe

Bacteria and fungi differ in a multitude of physiological and life-history traits with significant implications for nutrient cycling and ecosystem functioning (de Vries et al., 2013; Fierer, 2017). From a simplified perspective, bacteria are assumed to be strongly associated with the fast soil energy channel, which supports fast turnover of easily available organic substrates. Fungi, in turn, are believed to have a preponderant role in the slow soil energy channel, which sustains slower decomposition of more complex organic matter and retention of nutrients (Malik et al., 2016; Pulleman et al., 2022). Increased F/B ratios have been proposed to be indicative of healthy soils, since they are normally high in natural ecosystems and have been related to a more conservative C and nutrient turnover (Six et al., 2006). In fact, the idea that a high F/B ratio is a desirable property of agroecosystems is commonly used (Pulleman et al., 2022). Our study found the highest F/B ratios in forest soils and the lowest in grasslands, and not in croplands, as we initially expected. This is explained by the higher absolute fatty acid contents of grasslands in comparison with croplands (Smith et al., 2021). Importantly, the same pattern was observed under the three climates, suggesting general relevance of our findings. Wan et al. (2021) reported decreased F/B ratios with land-use intensification at global scale, especially in temperate and boreal climates. These findings suggest that more sustainable agricultural practices should aim at increasing F/B ratios in soil, in order to favor soil C storage and nutrient conservation (de Vries et al., 2013). However, this assumption has recently been questioned (Fierer et al., 2021; Pulleman et al., 2022). Cycling of C and other nutrients in cropland soils not only depends on bacteria and fungi and their relationships, but also on complex and multitrophic food webs, which we know very little about (Pulleman et al., 2022). On the other hand, increased abundances of saprotrophic fungi in comparison to bacteria in cropland soils can also indicate improved capabilities for soil structure formation and disease suppression (Clocchiatti et al., 2020).

Our models identified MAT, pH, as well as N and P contents as significant predictors of the F/B ratio across Europe. In a recent global

meta-study, Yu et al. (2022) identified MAT and NPP as strong determinants of fungal dominance over bacteria. Although our random forest modeling did not identify NPP as a significant predictor, SEM showed that NPP, along with MAT and soil texture, indirectly influence the F/B ratio by inducing changes in pH, N, and P contents. The same authors found a convex relationship between the F/B ratio and soil pH, with fungi dominating only within a range of 5 to 6. He et al. (2020) observed an inverse unimodal relationship between F/B ratio and soil pH also at the global scale, with the lowest F/B ratio at pH 6.3. However, soils analyzed in the context of the present study ranged in pH between 3.7 and 8.5, and we found a negative linear relationship between the F/B ratio and soil pH. These discrepancies may result from differences in the spatial scale, land uses considered, and range of soil pH. Interestingly, we also found that soil P content is a significant predictor of the F/B ratio at European scale. The positive relationship between F/B ratio and soil P content could indicate that fungal productivity will become P rather than N limited under the current N deposition levels, especially in ecosystems without P fertilization such as forests (Odriozola et al., 2021).

4.4. Crop type does not influence soil bacterial and fungal biomass

In comparison with grasslands and forests, the ecological relationships between below- and above-ground organisms are less understood in croplands (Dequiedt et al., 2011). To address this gap, we also investigated differences in soil microbial biomass across crop types. The type of crop and agricultural practices are expected to influence soil physical and chemical properties, and ultimately soil microbial biomass, by affecting soil aggregates, root density, amount and composition of root exudates, soil oxygen diffusion rates, and nutrient contents, among others (Li et al., 2021; Lohila et al., 2003; Martins et al., 2009). For example, tillage is usually more frequently applied in cereal lands than in monoculture orchards. On the contrary, organic amendments are expected to be more common in orchards than in soils under cereals. In line with this argumentation, we expected to find differences in soil properties in concomitance with variations in soil microbial biomass across crop types (cereals, oil-producing crops, and orchards). However, despite all the evaluated soil physicochemical properties, except SOC, varied among the studied crop types, the biomass of the different soil microbial groups and the GP/GN and F/B ratios did not differ. This points towards the crucial importance of SOC as a driver of microbial biomass in croplands (Smith et al., 2021). Microbial diversity (Tardy et al., 2015) and even the microbial diversity/biomass relationships (Bastida et al., 2021) have also been shown to be dependent on SOC in croplands, which evidences the importance that needs to be given to this soil pool during the development and implementation of more sustainable agricultural practices. In addition, these results highlight the large heterogeneity entailing agriculture across continental scales (crop species, irrigation factors, applied fertilizers, tillage type, pesticides, etc.), which may blur statistical differences across crop types.

5. Conclusions

Biomass of soil bacteria and fungi as well as the F/B ratio were influenced by land use and climate across Europe. Bacteria were more abundant in grasslands and temperate environments, while fungi dominated in forests and cold climates. Fungal biomass was more susceptible to soil agricultural management than bacterial biomass. In fact, the agricultural use of soil seems to favor bacterial biomass by buffering the negative impact of harsh climatic conditions. Soil texture, SOC, and N were shown to be the main factors directly driving bacterial and fungal biomass. SOC and N, in turn, are regulated by land use, climate, and plant cover. Therefore, the development of more sustainable agricultural policies in Europe to restore and conserve soil microbial biomass should consider these complex relationships between below- and above-ground communities and environmental conditions, through

variations in soil physical and chemical properties. Bacterial and fungal biomass in croplands were not influenced by crop type, which was explained by the absence of significance variations in SOC contents among crops. Further research is needed to identify specific agricultural practices for multifunctional soil microbial communities in croplands.

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.

Data availability

All the data used in the present study are available in Supplementary Dataset 1.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2023.116474.

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