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BRIEF REPORT

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Interaction effects of pH and land cover on soil microbial diversity are climate-dependent

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Abstract

Factors regulating the diversity and composition of soil microbial communities include soil properties, land cover and climate. How these factors interact at large scale remains poorly investigated. Here, we used an extensive dataset including 715 locations from 24 European countries to investigate the interactive effects of climatic region, land cover and pH on soil bacteria and fungi. We found that differences in microbial diversity and community composition between land cover types depended on the climatic region. In Atlantic, Boreal and Continental regions, microbial richness was higher in croplands and grasslands than woodlands while richness in Mediterranean areas did not vary significantly among land cover types. These differences were further related to soil pH, as a driver of bacterial and fungal richness in most climatic regions, but the interaction of pH with land cover depended on the region. Microbial community composition differed the most between croplands and woodlands in all regions, mainly due to differences in pH. In the Mediterranean region, bacterial communities in woodlands and grasslands were the most similar, whereas in other regions, grassland and cropland-associated bacteria showed more similarity. Overall, we showed that key factors interact in shaping soil microbial communities in a climate-dependent way at large scale.

INTRODUCTION

Bacteria and fungi play essential roles in the distribution of plant communities and are central biotic actors of soil biogeochemical processes (Banerjee & van der Heijden, 2022; Delgado-Baquerizo et al., 2020). Soil bacterial and fungal communities are known to be shaped by several environmental factors, such as soil properties, land cover (i.e., vegetation type) and climate (Barnett et al., 2020; Labouyrie et al., 2023; Lauber et al., 2009; Mod et al., 2021). Various studies have demonstrated that the diversity and composition of soil bacterial communities is strongly linked to pH (Bahram et al., 2018; Constancias et al., 2015; Fierer & Jackson, 2006; Lauber et al., 2008; Rousk et al., 2010). Similarly, soil pH gradients shape fungal communities, although to a lower extent than bacterial assemblages (Bahram et al., 2018; Constancias et al., 2015; Lauber et al., 2008; Rousk et al., 2010; Tedersoo et al., 2020; Zhang et al., 2016). Several studies also showed that climate (e.g., temperature and humidity) and land cover influence soil microbial communities either directly or indirectly through biogeochemical processes and plant community composition changes (Bardgett et al., 2013; Classen et al., 2015; Tsiafouli et al., 2015). Although those drivers have been investigated in paired

interactions in the past (Barnett et al., 2020; Labouyrie et al., 2023; Lauber et al., 2009; Mod et al., 2021; Mukhtar et al., 2021), little is known about the three-way interactive effects of climate, pH and land cover on soil microbial communities at large spatial scale.

In earlier work, using the extensive LUCAS Soil Biodiversity dataset (715 locations across Europe [Orgiazzi et al., 2022]), we found that single factors and two-way interactions among soil properties, climate and vegetation shape microbial diversity and composition (Labouyrie et al., 2023). Here, we used the same dataset and specifically tested (i) whether three-way interactions among pH, land cover and climate better explain patterns in soil bacterial and fungal communities and (ii) whether the combined effects of land cover and pH on soil microbial richness, diversity and composition are climate-dependent. For that, we considered three dominant land cover types (cropland, grassland and woodland) and four European climatic regions (Atlantic, Boreal, Continental and Mediterranean; European Environmental Agency (EEA), 2017) (see Supplementary Material and Methods, Supplementary Tables S1 and S2, Supplementary Figure S1). We evaluated the relevance in terms of explanatory power of the three-way interaction compared to single and two-way interaction models, and further focused on the effect of pH and land cover on communities by climatic region.

First, we examined how bacterial and fungal α-diversity (measured by the observed [z]OTUs richness and Shannon diversity index) varied across different combinations of land cover types and climatic regions (Figure 1). We found that microbial richness and diversity significantly differed among land cover types for a given climatic region, and between regions for a given land cover type (Figure 1A, B, Supplementary

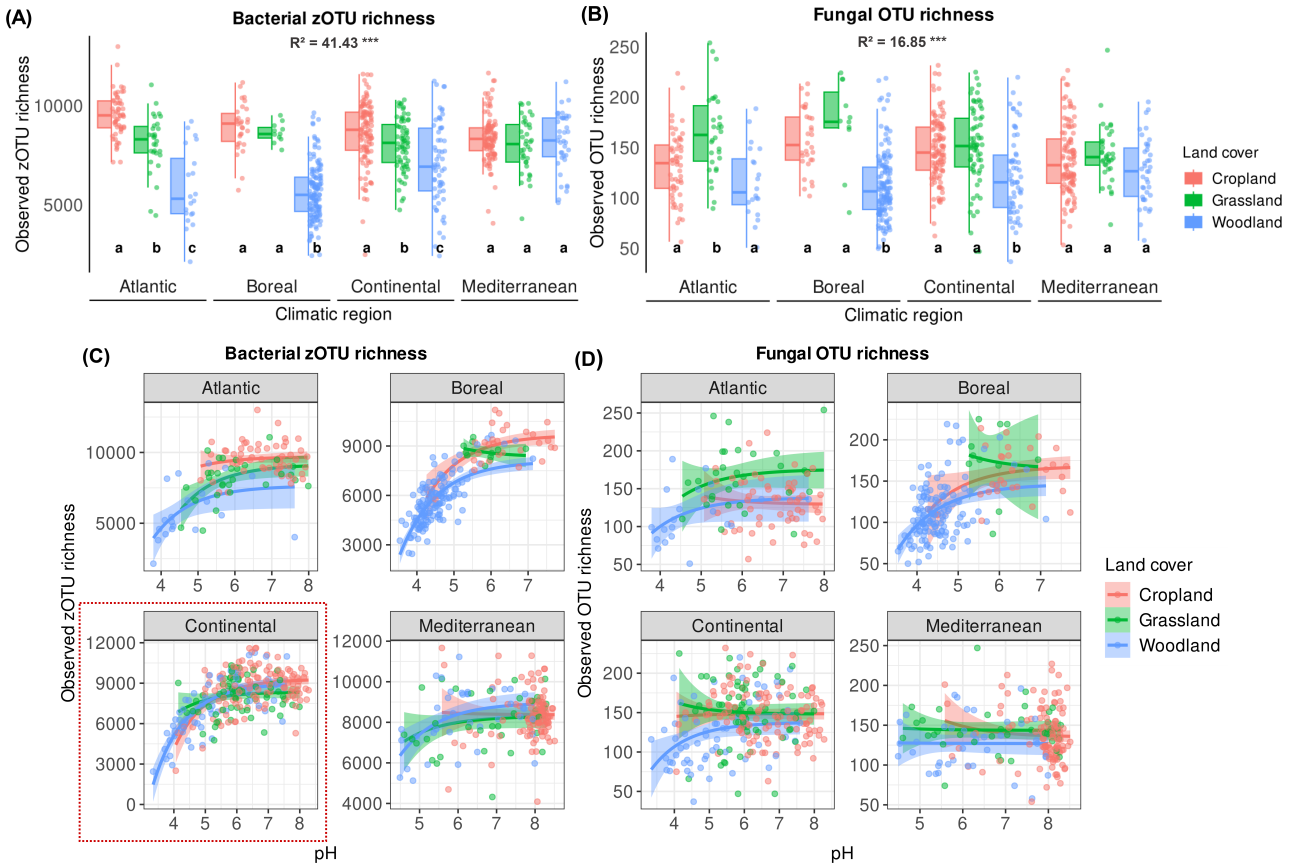

FIGURE 1 Effects of land cover and climatic region on bacterial (A) and fungal (B) observed (Z)OTU richness. The boxplot centre line indicates the median. The lower and upper hinges show the first- and third quartiles, respectively, and each whisker corresponds to 1.5 × the interquartile range from its respective hinge. R^2 values (%) on the boxplots correspond to the adjusted *R*-squared of the linear models testing the interaction between land cover and climatic region in explaining observed richness. Different letters represent significant differences among land cover types for each climatic region. The stars represent the level of significance of the *p*-value for the interaction term land cover: region in the associated Kruskal–Wallis test (***$p < 0.001$). Relationship between pH and bacterial (C) and fungal (D) observed (z)OTU richness for each land cover type by climatic region. Curves represent an inverse exponential relationship between observed (z)OTU richness and pH and are coloured by land cover type. Shaded areas represent the standard error. The red dotted square indicates that the interaction term between pH and land cover is significant in the associated model. In all plots, the number of sites per land cover-region combination is reported in Supplementary Table S2.

Figure S2, Supplementary Table S3). In three out of four climatic regions (i.e., Atlantic, Boreal, Continental), microbial α-diversity was on average lowest in woodlands compared to croplands and grasslands. In contrast, in the Mediterranean region, no differences in microbial richness across land cover types were found (Figure 1A, B), and differences in fungal diversity between croplands and woodlands were minor but significant (Supplementary Figure S2, Supplementary Table S3).

Considering then soil pH as an additional factor (Supplementary Figure S3 and Supplementary Table S4), we investigated how microbial richness and diversity varied with pH across land cover types under different climatic conditions (i.e., by climatic region). We found that pH and land cover explained microbial α-diversity in most climatic regions, but the interaction effect between pH and land cover varied across regions (Figure 1C, D, Supplementary Figure S2, Supplementary Tables S5 and S6). Accordingly, for the Atlantic, Boreal and Continental regions, bacterial and fungal richness and diversity were influenced by land cover and soil pH (Supplementary Table S6). However, the effect of pH on bacteria differed among land cover types only in the Continental region (three-way interaction effect); we found higher bacterial richness and diversity in croplands compared to grasslands under the same soil pH conditions, especially at high pH values (Figure 1C, Supplementary Figure S4 and Supplementary Table S6). In contrast, soil pH was found as the only relevant driver of bacterial α-diversity in the Mediterranean region (Supplementary Table S6). Despite significant variations in soil pH across diverse land cover types in this climatic region (Supplementary Figure S3), there was no clear link between fungal richness and diversity with pH levels (Supplementary Table S7).

While soil pH and land cover exert distinct influences on soil communities, both drivers also interplay in shaping them (Constancias et al., 2015). As soil pH is intricately tied to changes in land use, fully disentangling the effect of each driver on soil communities is not achievable. However, in our study, we used variation partitioning to attribute the part of model variance uniquely explained by soil pH and land cover in each climatic region, and shared by both factors. Such analysis showed that a high share of variance was commonly held by both pH and land cover together and could not be attributed to a specific driver (Supplementary Figure S5), confirming the intricate link existing between the two environmental factors in explaining microbial patterns. However, we also found that pH uniquely explained from 1.2% up to 31.2% of variations in microbial α-diversity, and that land cover uniquely explained from 0.4% up to 15.0% of these variations in the regions where both drivers were found significantly explaining soil community patterns (i.e., Atlantic, Boreal and Continental). We also found that in the Mediterranean region, the interconnection between soil pH and land cover in explaining microbial patterns was less important than in other regions (i.e., smaller shared variance, Supplementary Figure S5). In this region, variations in land cover types alone could not explain differences in bacterial α-diversity, while variations in pH alone could not explain differences in fungal α-diversity (Supplementary Figure S5). In addition, while land use types induced pH differences in all regions, the specific shift in pH between croplands and woodlands varied by region (Supplementary Table S2, Supplementary Figure S3) and was influenced by factors beyond land cover alone. Our results suggested that climate plays a significant role in this variation. We observed that soil pH ranges overlapped for the same land cover type across the Atlantic, Boreal and Continental regions, whereas Mediterranean soil consistently showed higher pH values (Supplementary Table S4, Supplementary Figure S3). This distinction suggested that the driving factor behind soil pH variations in Mediterranean soils was likely climatic, potentially attributable to drier conditions leading to limited leaching caused by minimal rainfall (Yaalon, 1997). In the Mediterranean region, bacterial richness and diversity may thus be impacted by variations in pH overruled by climate (Catania et al., 2022) rather than land cover. Also, different factors might play a more substantial role in influencing fungal diversity in this region, either related to land cover such as plant community richness and composition (Shihan et al., 2017), or impacting vegetation, such as climate, soil erosion or other soil properties (e.g., concentrations of macronutrients in soils [Goberna et al., 2012]). Such role of climate in modulating the effects of pH with land cover was supported by marginal effect plots, that showed differences in the contribution of pH to microbial α-diversity according to the combination of land cover type-climatic region considered (Supplementary Figure S6).

As of differences in soil community composition between sites (β-diversity), microbial community dissimilarity was highest between croplands and woodlands in all climatic regions. However, depending on the climatic region considered, the most similar communities were associated with distinct pairs of land cover types (Figure 2). For instance, bacterial communities from croplands and grasslands were the most similar in the Atlantic, Boreal and Continental regions, while grassland and woodland bacterial communities were the most similar in the Mediterranean region (Supplementary Table S7). While dissimilarities in microbial communities between croplands and woodlands could be attributed to their higher differences in associated soil pH (Supplementary Figure S3, [Labouyrie et al., 2023]), we argue that climate may play a role in the way pH and land cover interact in shaping soil community assemblies. The impact of soil

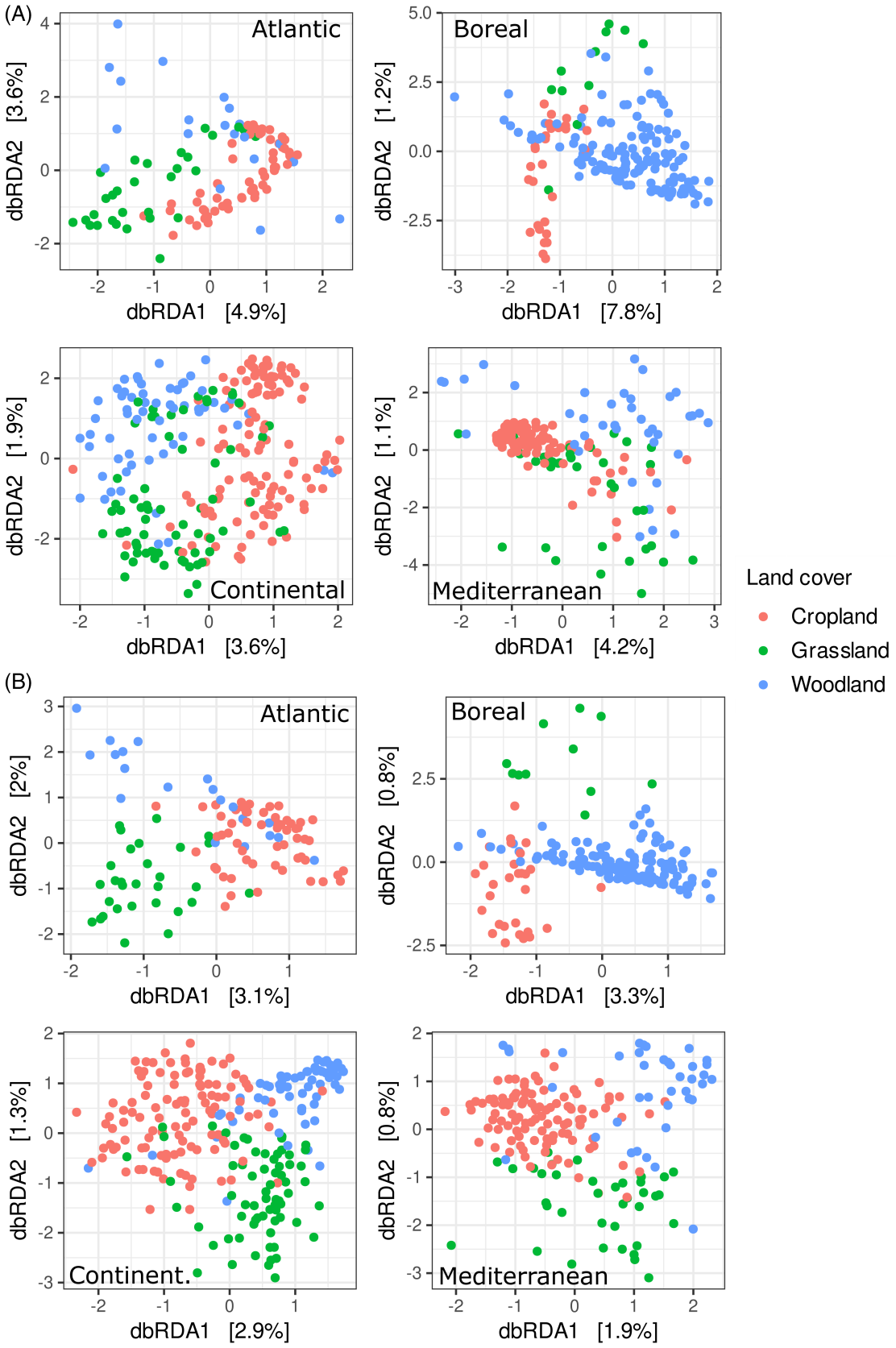

FIGURE 2 Ordination plots (dbRDA) after removing the pH gradient effect (i.e., partialling out pH), for the Bray–Curtis dissimilarity matrix calculated on the Hellinger-transformed sample-by-(z)OTU table, by climatic region, for bacteria (A) and fungi (B). In all plots, the number of sites per land cover-region combination is reported in Supplementary Table S2.

pH on community assembly varies geographically, influenced by the prevalence of neutral and acidic soils in each region (Barnett et al., 2020). This occurrence of different types of soil pH is also directly influenced by climatic factors (e.g., leaching and water availability), such that the intricate interplay between pH and land cover contributes to the context-specific nature of microbial community assembly in a climate-dependent way.

In terms of models' performance, bacterial and fungal α-diversity metrics were better explained when considering the interaction between land cover and climatic region rather than their single effects (Supplementary Table S5), suggesting that the impact of land cover on soil communities was climate-dependent. In addition, microbial community richness and diversity were best explained when adding soil pH as a third explanatory variable. Accounting for three-way interactions increased models' performance (higher explained variance and lower Akaike Information Criterion values), with up to 65.2% and 20.4% of total variation explained for bacterial and fungal α-diversity metrics, respectively. At the opposite, only 60.9%–14.0% of variance was explained when single variables were used as predictors (Supplementary Table S5). However, it is important to note that soil pH alone mostly explained the variance in bacterial communities, illustrating its prevalence in elucidating bacterial diversity patterns at large spatial scales (Labouyrie et al., 2023). Additionally, bacterial and fungal community composition (β-diversity) were influenced by three-way interactions among land cover, climatic region and soil pH, with up to 32.85% and 9.7% of total variation explained for the bacterial and fungal β-diversity, respectively. Only 16.6% and 4.2% of variance was explained when single variables were used as predictors (Supplementary Table S8).

Overall, our results highlighted the existence of climate-dependent interaction effects between land cover type and soil pH on bacterial and fungal communities at large scale. We showed that three-way interactions among key drivers best explained soil microbial community patterns. These findings are consistent with prior research highlighting the individual effects of each factor on soil microbes (Bahram et al., 2018; Fierer & Jackson, 2006; Mod et al., 2021), but the importance of their interactions had not been previously demonstrated on such a large geographical extent. Here, our approach represented a case study for delving into complex interactions within ecosystems. However, while the focus was deliberately limited to a reduced set of key drivers, it is important to recognize the broader range of environmental properties impacting soil communities, including soil organic matter content, nutrient availability, carbon-to-nitrogen ratio, soil texture, plant community composition, as well as anthropogenic disturbances (e.g., soil pollution and contamination) (de Vries et al., 2012; Galitskaya et al., 2021; Luo et al., 2022; Zheng et al., 2022). This was particularly true for fungal communities, for which a great proportion of the variability remained unexplained. Future research should focus on progressively building more complex interaction models, including specific combinations of vegetation, soil properties and other factors. To account for the impact of climate on environmental drivers of soil communities, such interaction models should take place under different current and projected climatic scenarios.

AUTHOR CONTRIBUTIONS

Maëva Labouyrie: Conceptualization; methodology; investigation; validation; visualization; formal analysis; writing – original draft; writing – review and editing; data curation. **Cristiano Ballabio:** Methodology; writing – review and editing; validation. **Ferran Romero:** Supervision; writing – review and editing; validation. **Panos Panagos:** Writing – review and editing; supervision; validation; resources; funding acquisition. **Arwyn Jones:** Supervision; writing – review and editing; project administration; resources; funding acquisition. **Leho Tedersoo:** Writing – review and editing; methodology; supervision; validation. **Marcel G. A. van der Heijden:** Conceptualization; methodology; validation; writing – review and editing; supervision; funding acquisition; resources; project administration. **Alberto Orgiazzi:** Supervision; conceptualization; methodology; validation; writing – review and editing; writing – original draft.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The raw data (DNA sequences) generated in this study have been deposited in the Sequence Read Archive (SRA) database under BioProject ID PRJNA952168. The sampling site metadata used in this study are available on the European Soil Data Centre (https://esdac.jrc.ec.europa.eu/content/soil-biodiversity-dna-bacteria-and-fungi).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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